POLYAMINES AND LEGUMES: JOINT STORIES OF STRESS, NITROGEN FIXATION 
AND ENVIRONMENT

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Abstract:

Polyamines (PAs) are natural aliphatic amines involved in many physiological processes in almost all living organisms, including responses to abiotic stresses and microbial interactions. This review presents the profuse evidence that relates changes in polyamines levels during responses to biotic and abiotic stresses in model and cultivable species within Leguminosae, and examines their potential roles in the functioning of symbiotic interactions with nitrogen fixing bacteria and arbuscular mycorrhizae. The family Leguminosae constitutes an economically and ecologically key botanical group for humans, being also regarded as the most important protein source for livestock. The ability of legumes to establish symbiotic interactions with nitrogen fixing bacteria and arbuscular mycorrhizae fungi, gives to some legume species that are able to exploit their molecular machinery “pioneer” attributes, with better competition in nutrient-poor soils and higher adaptation to restricted environments. However, many legume crops may be affected by climate change-derived environmental stresses, whereby maintaining their yields safe from adverse environmental conditions is probably one of the biggest challenge facing modern agriculture. Therefore, the obtaining of vigorous genotypes with higher tolerance to abiotic and biotic stressors has turned an increasingly important biotechnological target. At this scenario, PAs can play an important role, and genetic manipulation of crop plants with genes encoding polyamine PA biosynthetic pathway enzymes is envisioned as a strategy to achieve plants with improved stress tolerance and symbiotic performance. As linking plant physiological behavior with "big data" available in "omics" is an essential step to improve our understanding of legumes responses to global change, we also examined integrative MultiOmics approaches available to decrypt the interface legumes-PAs-abiotic and biotic stress or interactions. These approaches are expected to accelerate the
identification of stress tolerant phenotypes and the design of new biotechnological strategies to increase their yield and adaptation to marginal environments, making a better use of available plant genetic resources.
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1. Introduction

Polyamines (PAs) are organic polycations, acknowledged as regulators of plant growth, development and stress responses, being putrescine (Put), spermidine (Spd) and spermine (Spm) the most related to this physiological role (Cohen, 1998). A high number of metabolites and enzymes participate in PAs metabolism (see (Calzadilla et al., 2014) for an extensive description). The diamine Put can be synthesized directly from ornithine by the enzyme ornithine de-carboxylase (ODC, EC 4.1.1.17) or indirectly, via a series of intermediates following decarboxylation of arginine by arginine decarboxylase (ADC, EC 4.1.1.19) (Fig. 1). In turn, Spd and Spm are synthesized from Put by successive additions of aminopropyl groups provided by decarboxylated S-adenosylmethionine (SAM), a metabolite synthesized by the enzyme S-adenosylmethioninedecarboxylase (SAMDC, EC 4.1.1.50). The aminopropyl additions to Put are catalyzed by the aminopropyl-transferases Spd (EC 2.5.1.16) and Spm synthases (EC 2.5.1.22). Both ADC and ODC pathways are present in higher plants and bacteria. (Fig. 1)

PAs are present in cells as free and bound forms, in variable amounts, depending on the species and developmental stage (Alcázar et al., 2010; Hussain et al., 2011; Jiménez-Bremont et al., 2007). The free forms of PAs show water-soluble properties and therefore, are easily translocated within cells. They may cause conformational stabilization/destabilization of DNA, RNA, chromatin, and proteins due to their ability to form electrostatic linkages with negatively charged molecules (Alcázar et al., 2010; Wimalasekera et al., 2011). PAs can stabilize membranes or nucleic acids, binding to their negative surfaces (Galston and Sawhney, 1990; Kusano et al., 2008). Although H₂O₂ derived from PAs catabolism contributes to reactive oxygen species (ROS) (Gonzalez et al., 2011), PAs can also act as ROS scavengers and activate antioxidant enzymes.
In addition, PAs display effects on vacuolar channels in plants (Pottosin and Muñiz, 2002), particularly on the plant plasma membrane ion channels and pumps (Pottosin and Shabala, 2014). Notwithstanding the precise molecular mechanisms by which PAs control plant responses to abiotic stress remain unknown, several aspects about their apparently clashing roles in the development have been reviewed the last years, including the involvement of PAs signaling in direct interactions with different metabolic routes and intricate hormonal cross-talks, nitric oxide formation, modulation of ion channel activities and Ca\(^{2+}\) homeostasis (Alcázar et al., 2010; Bitrián et al., 2012; Minocha et al., 2014; Podlešáková et al., 2018; Singh et al., 2018; Tun et al., 2006; Yamasaki and Cohen, 2006).

The family Leguminosae, containing close to 770 genera and over 19,500 species (LPWG, 2017), is the third largest Angiosperms family in terms of species numbers after Asteraceae and Orchidaceae. A considerable number of features make legumes an excellent model system to study the different aspects of PAs metabolism. Legumes are key components of natural and agricultural ecosystems (Escaray et al., 2012). Certainly, some legumes, such as soybean (Glycine max L. Merr.) and peanut (Arachis hypogaea), are food crops of major economic importance for livestock and human consumption, as their seeds are rich in proteins, carbohydrates, and oils (Duranti and Gius, 1997; Graham and Vance, 2003). In addition, legumes are characterized by their ability to establish symbiotic interactions with nitrogen fixation bacteria (NFB) and arbuscular mycorrhizal fungi (AMF), allowing their adaptation to soils with diverse environmental constraints (Escaray et al., 2012; Gibson et al., 2008). Several biotic and abiotic stresses affect legume crop productivity throughout the world. Therefore, under the context of a continue global population growth and climate change (Tubiello et al., 2007), using crop and theoretical models to study PAs metabolism, with views to modify traits of agronomic importance such as higher stress tolerance or higher crops yields is probably one of the biggest challenge facing modern agriculture. Three subfamilies
integrate the legumes group, being Papilionoideae the largest one, which contains most of the crop and theoretical model species used to study different aspects of plant biology. The most frequent legume models are *Lotus* (*L. japonicus*) and *Medicago* (*M. truncatula*). As the last two species come from the same cool season legume clade (Galegoid), common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) have been proposed as legume models from the tropical season legume clade (Phaseolid) (Straub et al., 2006), being the last species considered as the most important in the United States (Gepts et al., 2005). Regarding research on PAs, the legume species considered model for their study are not well defined. At first, investigations on plant PAs biosynthesis and regulation were initiated with *Lathyrus sativus* because their seeds are rich as source of L-homoarginine (Adiga and Prasad, 1985). However, most of the published PAs research in legumes was carried out on the first four species.

In order to support legume crops yields and understand their limitations, biological, physiological and diverse omics studies have been carried out in the last 80 years. The recent development of genomic tools, such as genome sequences, ESTs, oligonucleotide and cDNA microarrays, and comprehensive databases, such as the Legume Information System (http://www.comparative-legumes.org), make legumes an excellent model to study PAs metabolism. In particular, genomic sequencing of *M. truncatula* (Young et al., 2011) and *L. japonicus* (Sato et al., 2008), differing in their different patterns of root nodule development (Barker et al., 1990; Handberg and Stougaard, 1992), and of various legume crops such as soybean (Schmutz et al., 2010), pigeonpea (Varshney et al., 2012) and chickpea (Varshney et al., 2013) have provided a solid framework to explore PA metabolism for legume crop improvement.

Legumes are fundamental to sustain food security at a global level. However, the current paradigm in plant science is characterized by a disconnection of ecophysiology and 'omics', which have been established in parallel, with only exceptional cross-talks for the past 20 years. A novel
field has been proposed in order to capitalize the concurrent advances of both areas into a single discipline: ‘ecophysiomics’ (Flexas and Gago, 2018). This multidisciplinary approach would require joining forces, equipment and abilities in the context of user-friendly integrative bioinformatics resources and concurrent shared research protocols. Classic ecophysiology methodologies will require being adapted to massive high-throughput studies: the emerging phenomics field. This utopic collaborative environment is essential to advance in the understanding of legumes at levels ranging from cellular to agroecosystem scales. Then we may be able to use this understanding and translate it into practices or biotechnological tools.

This review explores the contribution made by studies on legume species on the basic knowledge of PAs metabolism, their role in tolerance to biotic and abiotic stresses and the establishment of mutualistic relationships relevant to the physiology of plants and the environment. As major future challenges are the development and implementation of progressive methods of genetic improvement oriented to develop varieties of legumes that have genetic recovery capacity against environmental stresses, we also get a glimpse of the state of the art toolkit landscape in legume research.

2. Polyamines and abiotic stress in legumes

2.1. Drought stress

Huge variations in rainfall distribution patterns and frequency, as a result of the climate change, reduce the amount of available water for crops, causing extensive periods of drought (Trenberth, 2011). Additionally, the amount of water supply for irrigation is expected to decrease or at best, to be maintained as a result of many aquifers depletion (Wheeler and Von Braun, 2013). In this scenario, a considerable effort is required to increase production of rainfed legumes, via the
adaptation of cropping systems and the design of new plant varieties (Tester and Langridge, 2010; Varshney et al., 2018). The water deficit affects a broad spectrum of plant functions such as transpiration, photosynthesis, leaf and root growth, and reproductive development (Chaves et al., 2003; Fuad-Hassan et al., 2008), impacting on cell division, hydraulics, cell wall mechanics, primary and secondary metabolism and ROS detoxification.

Several pieces of evidence suggest that PAs would be involved in the regulation of abiotic drought stress response. Among them, the enhanced stress tolerance from either the exogenous PAs application or the overexpression of biosynthesis-related genes resulting in enhanced stress tolerance, while a decrease in the PAs endogenous level has the opposite effect (Liu et al., 2015). In addition, PAs strongly promote the expression of functional and regulatory genes involved in water stress tolerance (Gill and Tuteja, 2010a). Besides, several studies have also confirmed that PAs play a role in plant stress tolerance by modulating reactive ROS homeostasis and photosynthesis during stress. In addition, PAs strongly promote the expression of functional and regulatory genes involved in water stress tolerance (Gill and Tuteja, 2010a).

Drought perturbs photosynthesis due to CO₂ limitations resulting from stomatal closure and biochemical restrictions associated with reducing power accumulation (Pinheiro and Chaves, 2010). To cope with energy imbalances and prevent ROS formation, the chloroplasts employs several strategies which were recently reviewed (Vanlerberghe et al., 2016). In such circumstances, PAs play an essential role on the protection of the photosynthetic apparatus (Hamdani et al., 2011) by interaction with photosystem II and light harvesting complex proteins, preserving the integrity of the thylakoid membranes structure and microsome. PAs application reduces the microsomal membrane fluidity in primary leaves of *P. vulgaris*, being Spd and Spm more effective than Put (Roberts et al., 1986). In *Lupinus luteus*, a drought-tolerant legume growing for 24 h on salt or sorbitol led to a higher level of PAs bound to microsomal membranes (Legocka and Kluk, 2005).
PAs most likely rigidify microsomal membrane surfaces, stabilizing them against NaCl and osmotic stress damage as part of an adaptive mechanism to salt and osmotic stress of drought-tolerant lupine plants, authors concluded. Inversely, a significant decrease in the contents of all PAs associated with the microsomes was observed in *P. vulgaris* (sensitive to water deficit and salinity) growing in sorbitol and salt conditions (Legocka and Sobieszczuk-Nowicka, 2012). These results suggested that microsome- and thylakoid-associated PAs could be good markers of plant stress tolerance. In a similar way, drought induces oxidative stress due to an imbalance between ROS formation and scavenging that results from the disturbance of prooxidant-antioxidant balance in the cell, in favor of the former (Fariduddin et al., 2013). ROS accumulation causes damage to membrane lipids, raising the level of malondialdehyde (MDA). This, in turn, decreases the membrane fluidity and damages membrane proteins inactivating the related receptors, enzymes, and ion channels (Gill and Tuteja, 2010b). PAs, mainly Put and Spm, are responsible for the scavenging of ROS and can indirectly affect the activities of the involved enzymes including catalase, peroxidases, and superoxide dismutase (Alcázar et al., 2010; Minocha et al., 2014; Sánchez-Rodríguez et al., 2016). In fact, in *G. max* (Radhakrishnan and Lee, 2013) and *Trifolium repens* plants subjected to drought (Li et al., 2014b), the exogenous application of Spm and Spd regulates antioxidant defense system by increasing the reduced glutathione concentration or catalase activity, and by reducing MDA levels.

In the current scenario of climate change-derived limitation of water availability, PAs metabolism manipulation to increase tolerance to drought stress in genetically modified legumes is envisioned as a source of benefits for the world population, projected to reach 9 billion in 2050. The constitutively overexpression of oat arginine decarboxylase 2 gene in *M. truncatula* (Duque et al., 2016), and driven by the stress-inducible RD29A promotor in *L. tenuis* (Espasandin et al., 2014), improved the physiological responses to water deficit of drought-stress plants (Espasandin et
al., 2014), also demonstrating that Put controls the abscisic acid (ABA) content in response to drought, by modulating the expression of 9-cis-epoxycarotenoid dioxygenase (NCED, which catalyzes a regulatory step in ABA biosynthesis). Last result is part of a bulk of evidence supporting the theory that Put and ABA are assimilated in a positive feedback loop, in which ABA and Put communally promote each other’s biosynthesis in response to abiotic stress (see (Singh et al., 2018)).

As the intensity of stress increases, PAs promotes osmoregulation and preserves the leaf relative water content. This effect is due to accumulation of either, proline (Cicer arietinum, G. max, L. tenuis) or γ-aminobutyric acid (GABA) (T. repens) at the cellular level (Espasandin et al., 2014; Nayyar et al., 2005; Yong et al., 2017). Likewise, the exogenous application of Spd plays a vital role in accelerating starch metabolism by increasing α- and β-amylase activities during the germination process of T. repens seeds, under low soil water content (Li et al., 2014a), and leads to the accumulation sucrose, fructose, sorbitol, and dehydrins in leaves (Li et al., 2015) during water deprivation. In addition, PAs support the induced-root growth required to utilize soil water resources. In effect, the rise in Put level either by overexpression of related biosynthetic genes or exogenous application promotes root development in L. tenuis (Espasandin et al., 2014), C. arietinum, G. max (Nayyar et al., 2005; Su et al., 2006) and M. sativa (Zeid and Shedeed, 2006).

Drought also alters PAs metabolism in nodules. Particularly, it decreased S-adenosylmethionine synthase (a PAs precursor) in soybean (Alam et al., 2010; Gil-Quintana et al., 2015), M. truncatula (Larrainzar et al., 2007) and pea (Irar et al., 2014).

2.2. Salt stress
Salinity is a severe problem for crops worldwide (Flowers, 2004), affecting around 800 million ha. (FAO, 2008). Salt stress disturbs plants in two-phases way; the first characterized by a reduction of shoot growth due to of the osmotic stress caused by salt concentration in the rhizosphere, and the second provoked by the toxic effect of ions when they are absorbed (Munns and Tester, 2008). Generally, plant responses to salt stress and drought are closely related, but the direct targets and receptors of osmotic signals, which induce PA accumulation, are not known.

Some studies have addressed the osmotic component of salt stress. Proline is an amino acid playing a highly beneficial role in plants exposed to osmotic stress conditions (Hayat et al., 2012). It is well-known that both, PAs and proline, possess a common precursor: glutamate. Glutamate can be directly converted to proline through glutamate Δ1-pyrroline-5-carboxylate pathway or indirectly to PAs via its acetylation in ornithine and arginine (Hayat et al., 2012). In two-week-old soybean seedlings subjected to 0, 50, 100 and 150 mM NaCl for seven days, Su and Bai (2008) (Su and Bai, 2008) found a negative correlation between proline accumulation and endogenous Put content and estimated the quantitative contribution of Put degradation to proline formation in 15 to 20%. However, Sanchez et al., (2005) (Sanchez et al., 2005) found that PAs did not always parallel proline accumulation in *L. glaber* (actually *L. tenuis*) under long-term salt stress. On other hand, studies on *Pisum sativum* and *Vicia faba* have contributed to reveal the beneficial role of PAs on ionic homeostasis regulation. It is well known that non-selective cation channels are PAs targets (Liu et al., 2000) and in *P. sativum*, channel blockage by PAs led to the prevention of K⁺ efflux from mesophyll cells induced by salt stress (Shabala et al., 2007). PAs alter intracellular Ca²⁺ homeostasis by modulating both, Ca²⁺ influx and efflux transport systems at the *P. sativum* root cell plasma membrane (Zepeda-Jazo et al., 2011), as well as influences nitric oxide formation. In *V. faba*, exogenous ABA stimulated apoplast copper amine oxidase (CuAO) activity, increased hydrogen peroxide (H₂O₂) production and Ca²⁺(cys) levels in guard cells, and induced stomatal
closure (An et al., 2008). Exogenous Put application exhibited similar effects than ABA on the regulation of cytosolic free Ca\(^{2+}\) level, which resulted in stomatal closure (An et al., 2008).

In 2005, Sánchez et al., (Sanchez et al., 2005) used *L. tenuis* (moderately salt tolerant glycophyte) as a model to test the hypothesis that free Spd and Spm are biochemical indicators of salt stress response. These authors evaluated PAs content in three different long-term salt stress approaches: germinating and growing seedlings under salinity, salinization of growing plants and imposition of salt stress to clone stem cuttings obtained from plants recovered from saline lowlands. Results from these experiments showed a similar trend concerning higher PAs content i.e., a salt induced a decrease of free Spd and an increase of free Spm, in line with the idea that PAs are biochemical indicators of salt stress. Authors stated that their results argued against a relationship between the salt induced reduction of growth rate and Spd content, and hypothesized that during plant response to salinity, Spm might be related to stress signaling.

In NaCl-treated *Prosopis strombulifera*, Put accumulation was related to the antioxidant defense system in this species (Reginato et al., 2012). Also, GABA is an important intermediate involved in ROS scavenging under abiotic stress and has been proposed that it contributes to stress protection (Bouche and Fromm, 2004; Liu et al., 2011). GABA accumulation mainly proceeds through the GABA shunt pathway, and alternatively by the PAs degradation pathway, since it is a product of Put catabolism by diamine oxidase (DAO) and terminal catabolism of Spd (Xing et al., 2007). NaCl (100 mM) stress induced higher GABA accumulation in *G. max* through stimulating the activity of DAOs. The measurement of DAO activity in soybean roots showed that its activity greatly increased when salinity was raised, whereas GABA levels were reduced concomitantly to PAs increment during stress recovery.

Several studies using “omics” techniques have revealed that salinity modulates the expression of genes involved in PAs metabolism. Using transcriptomic, Liu et al., (2019) (Liu et
al., 2019) confirmed previous results (Xing et al., 2007) showing the occurrence of the alternative pathway for GABA accumulation, that is the expression of PAs oxidase in the PAs degradation pathway, in *G. max* plants exposed to saline stress. The same phenomenon had been reported previously for *V. faba* plants grown under hypoxia (Yang et al., 2013). In *C. arietinum* (chickpea) roots, a transcriptomic study showed the salt-induced up-regulation of ADC and SAMDC (Molina, 2008). In 2011, Sánchez et al. (Sanchez et al., 2011) compared the physiological and molecular responses to salt stress of six *Lotus* species, three of them (*L. japonicus*, *L. filicaulis*, and *L. burtii*), used as models for legume genetics (Kawaguchi et al., 2005; Perry et al., 2003), and the other three species (*L. corniculatus*, *L. tenuis* and *L. uliginosus*) used in world agriculture as forages. Their comparative ionomic, transcriptomic and metabolomic analyses of *Lotus* genotypes revealed conserved and divergent system responses to salinity within this genus. In particular, several known salt-induced genes coding for enzymes of PAs metabolism, that were common to all sensitive genotypes were also found to be responsive in the more tolerant genotypes. These shared transcripts included many genes previously implicated in plant stress such as enzymes of PAs biosynthesis and catabolism, like proline oxidase, polyamine oxidase (PAO, E.C. 1.5.3.11), SAMDC, and Spm synthase.

Also, some recent metabolomic studies indicated that salinity modulates the content of different free PAs in legumes, some of them uncommon, and generally increasing their contents (e.g.: cadaverine (Cad) in *Pr. strombulifera*, (Llanes et al., 2016). In a salt tolerant soybean, neutral salt-induced stress increases in GS/GOGAT cycle, proline synthesis pathway and amino acid metabolism, suggesting that these plants could synthesize more nitrogen-containing compounds including PAs (Jiao et al., 2018).

Using quantitative profiling, Dias et al., (2015) (Dias et al., 2015) detected a decrease in the Put concentration in two chickpea genotypes, both presenting contrasting tolerance to salinity,
during their response to stress. Interestingly, the sensitive genotype also had reduced GABA. These results, along with those reported for *G. max* by Xing et al., (2007) (Xing et al., 2007) suggest that PAs are likely to exert its effect in legume salt tolerance through GABA formation. Hence, sustaining GABA levels could be a strategy to cope with salinity in legumes.

Most of the above mentioned studies focused on effects caused by salt on plants after hours or days of stress. However, it has been suggested that fast adjustments (responses evolving within the first 1.5 h upon stress treatment) might be relevant for an initial stress perception and subsequent systemic propagation of the information from stressed to non-stressed parts of the plant. In 2015, Geilfus et al., (Geilfus et al., 2015) performed an extensive metabolomic analysis of the fast responses to moderate NaCl stress in *V. faba*. The metabolite profile revealed a rapid reduction in the content of leaf Spd, a PA that is especially relevant for H_{2}O_{2} production during its catabolism by the enzyme PAO. This fast reduction of leaf Spd was suggested to contribute to the excessive ROS production observed in these plants, which started simultaneously 45 min after NaCl treatment. However, authors did not report whether that early oxidative burst served as a beneficial event under NaCl stress or caused oxidative damage.

The action of ROS in the apoplast may involve a delicate balance between cleavage and cross-linking activities (Cosgrove, 1999) and several authors have reported that apoplastic ROS promote cellular elongation (Rodríguez et al., 2009; Schopfer, 2001). In 7 day-old soybean seedlings treated with NaCl, salinity caused a substantial reduction in Put and Spd levels in soybean hypocotyls, being Put almost undetectable under stress conditions (Campestre et al., 2011). In contrast, important increases of Spm and Cad (the preferred substrate for the copper-containing amine oxidase (CuAO)) levels were originated by the saline treatment. Authors demonstrated that ROS generated as consequence of PAs catabolism participate of the hypocotyl elongation of salinized soybean plants.
A significant number of additional works have analyzed the salt-induced changes in the PAs profiles of different legumes like soybean (Zhang et al., 2014), bean (López-Gómez et al., 2014a, 2016; Shevyakova et al., 2013; Talaat, 2015; Zapata et al., 2008), *L. tenuis* (Maiale et al., 2004; Sanchez et al., 2005; Sannazzaro et al., 2007). Taken together, these works do not show a congruent pattern of PAs changes due to saline treatment, not even within the same plant species, whereby it is not possible to extract a unique conclusion on PAs physiological role on plant tolerance to salinity.

### 2.3. Extreme environments and heavy metals.

Evolution has allowed plants to adapt to extreme environments, including severe cold, high salinity, drought conditions, intense heat, acid soils and desert environments (Oh et al., 2013). Plants that inhabit those environments and can grow optimally at, or near those extreme ranges are usually called extremophiles, and harbor a range of mechanisms that allow them to withstand these extreme environments. Among plant species adapted to live in such conditions are some legumes, whose PAs are thought to be involved in their adaptation to extreme environments. Heat stress induced accumulation of PAs in heat-tolerant ground nut (*A. hypogaea*), a phenomenon already observed on cell cultures of heat tolerant plants and absent on susceptible ones (Königshofer and Lechner, 2002; Raval et al., 2018). A protective role of PAs in this condition has also been demonstrated in mung bean (*Vigna radiata*) and pigeon pea (*Cajanus cajan*) under heat stress. Application of exogenous Put, Spd or Spm resulted in the enhancement of the thermal protection response, improving seedling growth (Basra et al., 1997; da Silva et al., 2015). Also, the protective role of the *de novo* PAs synthesis was revealed by the treatment with inhibitors of PAs biosynthesis, which rendered the seedlings vulnerable to heat-shock, being the inhibitory effect reversed by Put addition (Basra et al., 1997).
Heavy metal toxicity may cause chlorosis, necrosis and several alterations in plant phenotype (Benzarti et al., 2008). One of the symptoms of metal phytotoxicity is oxidative stress, so plant defense system includes a battery of diverse antioxidants (Hossain et al., 2012). Literature on PAs metabolism in legumes exposed to metal stress is limited. Some works have shown that the presence of heavy metals such as Zn, Cu, Cd, Mn, Pb, and Fe may induce PA biosynthesis in plants (Franchin et al., 2007; Groppa et al., 2007), but whether they play a role as plant protectors against heavy metals or they are a reflection of metal toxicity is under debate (Lin and Kao, 1999). Heavy metal stress induced the accumulation of Spd in pigeon pea (Radadiya et al., 2016) and of Put, Spm and Spd in mung bean, with PAs catabolism as mediators of last accumulations (Choudhary and Singh, 2000). Also in mung bean, exogenous Spm application reduced Cd content, accumulation, and translocation to different plant organs, which consequently reduce ROS production and oxidative damage, preventing chlorophyll degradation (Nahar et al., 2016).

Soil acidification is a natural process which occurs mostly in tropical and subtropical regions like the rainforest of Brazil (Rahman et al., 2018), leading to Al solubilization (Hoekenga et al., 2003), resulting toxic for plants and limiting crop production (Kochian et al., 2004). Few works have addressed the role of PAs metabolism during legume plant response to Al toxicity. For instance, P. vulgaris inoculated with Rhizobium leguminosarum bv. phaseoli strongly reduced the nodule number when cultivated at pH 4.5, whereas PAs concentration was 50-fold higher than at pH 5.5 (Wolff et al., 1995). Rhizobial growth may also be hampered by high Al concentrations in soil. However, the microsymbiont and the infection process were shown to be less sensitive to Al toxicity than host plant growth (Arora et al., 2010; Kopittke et al., 2015). In the fast-growing R. fredii P220, homospermidine (Hspd) content increased with the lowering of the medium pH (Fujihara and Yoneyama, 1993). Although there is no well-defined mechanism reported for acid-
tolerant in bacteria yet, PA accumulation has been proposed as one of their mechanisms of tolerance to Al (Ferreira et al., 2012; Fujihara and Yoneyama, 1993).

3. PAs and biotic interactions in legumes

3.1. Plant pathogens

PAs metabolism is tightly regulated during plant-pathogenic interactions (Jiménez Bremont et al., 2014). Accumulating evidence indicates that PAs play an essential role in maintaining cell viability during biotic stress, but they also participate in the elicitation of plant defense responses, either functioning as signaling molecules or rather enabling the generation of ROS through their oxidation by PAOs. Part of the information supporting the important role played by PA oxidation during plant response to microbes has emerged from studies using legume species. In chickpea, Rea et al., (2002) observed induced DAO activity during the interaction with the nechrotrophic pathogen Ascochyta rabiei, and consequently, the inhibition of such activity by 2-bromoethylamine increases plant susceptibility. Put accumulated in common bean after treatment with elicitors isolated from the fungus Fusarium oxysporum (Broetto et al., 2005), even though the activity of DAOs was not investigated. These studies suggest that Put oxidation might play an important role in plant defense against fungal pathogens.

Transcriptomic data indicates that the regulation of PAs metabolism occurring upon pathogen recognition by legume plants is an intricate and complex process that obeys to genotype, plant growth-stage and to the kind of pathogen involved. For instance in a resistant line of P. vulgaris, Spm synthase and SAMDC were downregulated in the first stages of infection by the hemibiotrophic fungus Colletotrichum lindemuthianum, compared to a susceptible line (Padder et al., 2016). However, in later stages ADC, ODC and a Spd/Spm synthase transcripts were
upregulated, while those encoding hydroxycinnamoil transferases and N-acetyl transferases were downregulated, indicating that PAs conjugation has no relation to plant resistance. Nevertheless, the PAs metabolism is not always directly linked to plant resistance. Thus, PAs synthesis and degradation were equally upregulated in tolerant and susceptible lines. In tolerant and susceptible lines of *L. japonicus* and *M. truncatula* confronted with the bacteria *Pseudomonas syringae*, PAs synthesis and degradation were equally up-regulated (Bordenave et al., 2013; Nemchinov et al., 2017). These discrepancies make difficult to shed light on the real contribution of PAs to plant resistance. For instance, Spd and Spm synthase activities were also up-regulated in soybean against the cyst nematode *Heterodera glycines* (Wan et al., 2015). SAMDC and ADC transcripts were downregulated, and the PAO gene family was upregulated in response to the Asian soybean rust *Phakopsora pachyrhizi* Sydow (Panthee et al., 2009). In turn, downregulation of ODC and Spm synthase were reported in *L. sativus* in response to *A. lathyri* (Almeida et al., 2015) and in the *M. truncatula–Phymatotrichopsis omnivore* interaction (Uppalapati et al., 2009), respectively. Thus, further research is required for a deeper understanding of the connection between the regulation of PAs homeostasis and plant biotic stress tolerance.

It is known that defense mechanisms deployed by plants against pathogens depend on the coordinated activation of signaling pathways involving the production of hormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (Et). In addition, some reports have demonstrated a clear connection between PAs and hormone metabolism. For instance, Ozawa et al. (2009) (Ozawa et al., 2009) demonstrated that the treatment of lima bean (*P. lunatus*) with PAs (particularly Spm), led to an increment on JA levels, which in turn promote the production of volatile terpenoids capable of protecting plants against herbivores. Moreover, co-treatment with Spm and JA led to a higher terpenoids production, which has a high potential as a strategy for herbivores control. JA is also produced as a consequence of tissue damage and the attack of
pathogenic fungi. Chickpea plants treated with JA provoked a remarkable induction in DAO expression and conversely, antagonists of JA (such as SA and ABA) repressed the expression of this gene (Rea et al., 2002). In turn, chickpea plants treated with inhibitors of the Spd synthesis (such as cycloheximide) showed higher levels of Et, which seems to be a consequence of the accelerated SAM production and induction of enzymes participating in Et biosynthesis (Gallardo et al., 1994, 1995). These data demonstrate a bidirectional relationship between PAs and defense signaling pathways mediated by hormones.

3.2 Root symbiosis

3.2.1 Interaction with rhizobia.

Legumes may establish symbiotic associations with 98 species of NFB (Weir, 2011). During the interaction a new plant organ, the symbiotic root nodule is formed (Brewin, 1991; Hadri et al., 1998), which hosts bacteria in an optimized environment for fixing atmospheric dinitrogen (Wagner, 2012). The first step in the establishment of the symbiosis is the recognition and invasion of the appropriate legume by the bacteria. Bacterial entry proceeds through newly formed tubular structures called infection threads; the bacterial entry induces cortical cells to active cell division leading to nodule formation; the branching infection thread guides rhizobia to the underlying nodule cells, within which bacteria are released, then differentiating into nitrogen-fixing bacteroid; the nitrogenase enzyme synthesized by the bacteroids converts atmospheric molecular nitrogen to ammonia (Jones et al., 2007).

First studies in nitrogen-fixing systems on PAs may be traced back to the identification of the bacterial PA sym-Hspd as the main PA in root nodules of lupine, broad bean, runner bean (P. coccineus) and pea (P. sativum), and in R. leguminosarum and R. phaseoli (Smith, 1977). Since
then, reports related to nitrogen-fixing systems in legumes have been mainly addressed at studying the PAs composition in various plant-rhizobia symbiotic associations and the role of PAs metabolism on the establishment and functional regulation of plant-rhizobia symbiosis.

3.2.1.1 PAs composition.

Compared with shoots and roots, legume nodules contain high concentrations of free PAs, most of them appearing to be in the cytosol (Fujihara et al., 1994). Besides the main PAs (Put, Spd and Spm), sym-Hspd is widespread among Bacteria and Archaea (Shaw et al., 2010), and relatively high concentrations of this PA were observed in nodules of all the leguminous plants (Fujihara et al., 1995; Lin et al., 2015). Pathways for Hspd biosynthesis in rhizobia have been reviewed by (Fujihara, 2008). In addition to Hspd, nodules may contain a variety of biogenic amines, most of them nodule-specific, for example: 4-aminobuty1-Cad (Fujihara et al., 1995); β-Phenethylamine (β-PEA; (Fujihara et al., 2002)), and alkenyl analogs of Spd and Hspd (Fujihara, 1999). Interestingly, these unsaturated PAs were not present in neither cultured rhizobial cells nor uninfected host tissues, suggesting that their production relays on the particular environmental conditions that prevail within the nodule. In contrast, canavalmine appears to be of plant cell origin (Fujihara et al., 1986). In the M. sativa-Sinorhizobium meliloti symbiosis, most of the PAs detected in the nodule were produced by the bacteroids, with Hspd as the most abundant PA, being these levels lower to those detected in the free-living bacteria (López-Gómez et al., 2014b). The last was proposed to be related with their ability to adapt to different environmental conditions.
3.2.1.2 Role of PAs metabolism on the establishment and functional regulation of plant-rhizobia symbiosis

PAs levels in root nodules are five to ten times higher than those in other plant organs (Fujihara et al., 1995; Ozawa and Tsuji, 1993; Whitehead et al., 2001). However, little is known about the functional role of PAs within the root nodules. Arginine is a metabolic precursor in the biosynthetic pathway of Put, Spd, and Spm. The response of *P. vulgaris* roots to the inoculation with an argC mutant strain of *R. etli* (unable to grow with ammonium as the sole nitrogen source) revealed that the early root responses such as root-hair curling, mitotic activity of root cells (from the outer cortex), formation of infection threads, and nodules were absent in the arginine auxotrophous, but they were observed in the wild-type parent (Ferraioli et al., 2001). These results constituted a hint that PAs could be involved in the nodulation ability of *R. etli*. However, several experimental results advocate for a dual role of PAs, as stimulators or inhibitors on nodule development and functioning. Functionally, nodule senescence is defined as a decline in the whole plant nitrogen fixation (Wagner and Sarath, 1987), which starts soon after flowering (Hungria and Franco, 1988). In the *Vigna mungo-Rhizobium* nitrogen fixation system, Put, Spd, Spm, N$_2$-ase activity and leghemoglobin reached a peak during flowering (Stage 2) and then began to decline (Lahiri et al., 2004). In contrast, Spm treatment considerably delayed the senescence-induced decrease of N$_2$-ase activity (primary determinant of nodule senescence; (Sutton, 1983; Wagner and Sarath, 1987)). Authors suggested that if senescence could be delayed, it would be possible to improve the nitrogen gain during the period of seed growth.

In the *Galega orientalis–R. galegae* nitrogen-fixing system, 10 and 50 μM of various PAs incremented nodule formation ability, nodule biomass accumulation and nitrogenase activity of nodulated roots (Vassileva and Ignatov, 1999). However, at 100 μM a negative effect was observed. In this host-bacteria system, diamine concentrations 0.01 to 1.0 mM decreased poly-b-hydroxybutyrate
(PHB) accumulation in bacteroids and increased the oxygen consumption by these bacteroids, when isolated, whereas concentrations between 1.0 and 2.0 mM increased the PHB accumulation, decreased oxygen consumption and nitrogenase activity (Vassileva and Ignatov, 2002). These results point out to a concentration-dependent effect of PAs on symbiosis development.

Other studies have provided some evidence that PAs metabolism is involved in the plant-mediated control of root colonization by rhizobia. Wisniewski and collaborators (2000) (Wisniewski et al., 2000) postulated that within the lumen of the infection threads, hardening may result from inter-protein cross-linking driven by H$_2$O$_2$ produced after DAO oxidizes Put and Cad (Smith and Barker, 1988). Transformed pea (P. sativum) lines showing deficiency of DAO activity were less sensitive to the inhibitory effects of exogenous Put and less active in the crosslinking of matrix glycoprotein, indicating that Put derived products of DAO activity could retard nodule development (Wisniewski and Brewin, 2000).

Real-time reverse-transcription-polymerase chain reaction showed the expression of genes encoding ADC and ODC (LjADC1 and LjODC) genes in L. japonicus nodules during their development, being maximal at 10 days post infection (preceding nitrogen fixation), and declining after 28 days post infection (Flemetakis et al., 2004). The expression profile of these genes correlated well with the expression patterns of genes involved in cell division and expansion (such as L. japonicus Cyclin D3 and an $\alpha$-expansin gene) suggesting that PAs are primarily involved in nodule development. A similar expression pattern was observed for the expression of genes encoding Spd and Spm synthase (LjSPDS, LjSPMS; (Efrose et al., 2008). However, PAs continued accumulating throughout nodule development to maturity and senescence, indicating that these compounds may have additional roles during the symbiotic nitrogen fixation (SNF). Besides temporally, LjSPDS, LjSPMS, LDC and jODC expressions were also spatially coordinated, since it occurred mainly in the nodule parenchyma and vascular bundles (Efrose et al., 2008; Flemetakis et
New methodologies like the matrix-assisted laser desorption/ ionization mass spectrometry imaging (MALDI-MSI, Veličković et al., 2018) could be promising for going deeper into the spatial and temporal metabolic nodule complexity of PAs distribution during the infection and nodule formation. SAM donates its n-propylamine group in the biosynthesis of Spd and Spm from Put (Roje, 2006). SAM and Spm distributions among soybean nodule compartments were recently visualized using MALDI-MSI (Veličković et al., 2018).

Nodule formation demands for photosynthetic products, so legumes limit nodulation to levels that are strictly necessary to maintain the nitrogen-fixing activity. PAs have been shown to be involved in a self-regulatory mechanism in soybean. In nodules of this plant species, addition of 200 μM Spd and Put inhibited by 37 and 54% the H⁺-ATPase activity, and both inward and outward ammonium channels, showing that high PAs levels have potential to reduce nitrogen supply to the plant in vivo (Whitehead et al., 2001). Also in soybean, foliar Spd and Spm/Put ratios are always lower in the super-nodulating soybean mutant En6500, compared with its parent Enrei (Terakado et al., 2006). Foliar Spd or Spm applications led to a reduction of both nodule numbers in both mutant and parental lines, whereas the foliar treatment with a specific inhibitor of Spd increased the root nodule number in the parental line. These results suggested that high Spd levels in shoots might suppress the root nodule formation. However, experiments applying 15N-labeled Put to soybean leaves and recording the time course of 15N incorporation into PA molecules in different organs, strongly indicated that foliar applied polyamine and its metabolites are only slightly transported to the roots (Terakado and Fujihara, 2008). The foliar application of brassinazole, an inhibitor of BR synthesis, increased the nodule number in the wild-type Enrei and Spd biosynthesis from Put is suppressed in En6500, probably due to a deficiency in the brassinosteroids synthesis. Thus, nodulation appears to be affected also through a brassinosteroids-induced alteration of the relative PAs concentrations (Terakado et al., 2006).
Previously, we reviewed available information on the effect of PAs on legume-rhizobia symbiosis establishment. Other authors have addressed the inverse question of how the symbiosis affects PAs levels in the host. For example, inoculating *M. sativa* plants with *R. meliloti* maintained higher free Spd and Spm concentrations in roots and shoots than non-symbiotic ones under saline stress (Goicoechea et al., 1998). Also, *L. tenuis* plants inoculated with *Mesorhizobium tianshanense* presented higher Spm concentration and growth than uninoculated ones, even under saline condition, suggesting the involvement of this polyamine in the tolerance of *L. tenuis* to that stress (Echeverria et al., 2013).

The relevancy of PAs (particularly Hspd) for normal bacterial proliferation and for mechanisms of adaptation to environmental stress, such as acid, saline or osmotic stresses has been reviewed by (Fujihara, 2008). Also, a polyamine-transporting ATPase-encoding gene was induced at both 4 and 10 °C acclimation of *Mesorhizobium* strain N33, as revealed by a metabolomics analysis (Ghobakhloo et al., 2015). Plants of *P. vulgaris* treated with 100 mM NaCl and inoculated with a *R. tropici* mutant strain, impaired in the synthesis of Hspd, had a lower nodule fresh weight than the corresponding plants inoculated with the wild type strain (López-Gómez et al., 2016). In soybean, addition of a moderate concentration (50μM) Cd increased Put in both, nodules and roots, due to induced ADC and ODC, and reduced DAO activities (Balestrasse et al., 2005). Last authors proposed that increased Put level could be an alternative strategy of the plant to maintain a nitrogen reservoir in roots and nodules for further surviving metal stress.

### 3.2.2 Interactions with arbuscular mycorrhizal (AM) fungi

Most lineages of terrestrial plants form symbiotic associations with fungi belonging to the Div. Glomeromycota, called arbuscular mycorrhizae (Bonfante and Genre, 2008; Wang et al.,
This symbiosis facilitates the incorporation of water and nutrients from the soil to the root, increasing plant tolerance against various types of biotic and abiotic stresses. The origin of this symbiotic association probably dates back to the very beginning of the colonization of the earth by photosynthetic organisms, about 350-400 million years ago (Selosse et al., 2015; Strullu-Derrien et al., 2014). Although plants can grow in the absence of AM fungi, fungi are obligate biotrophs. The colonization of roots by fungi requires the mutual recognition of both components of the symbiosis (Gadkar et al., 2001; Vierheilig and Piche, 2002), the adhesion of the fungal hyphae to the root surface, their penetration, and inter-cellular proliferation among cortical parenchymatic cells, and the invasion of these cells to form arbuscules: highly branched fungal structures that facilitate the exchange of nutrients between symbionts (Gutjahr and Parniske, 2013).

Despite the view that this association with fungi was determinant for the success of land colonization and true root development, the amount of information regarding PAs roles on the establishment and functional regulation of this symbiosis, and on the physiology of mycorrhizal legume plants is very scarce. This information is presented below.

In *P. sativum*, exogenously applied PAs increased the colonization frequency in pea roots of plants inoculated with *Glomus intraradices* (El-Ghachtouli et al., 1995). In the last plant species, a similar role for PAs was proposed on basis of results showing that difluoromethylornithine (DFMO, an irreversible inhibitor of Put synthesis) application inhibited colonization by *Gl. mosseae* of *P. sativum* roots, while this effect was reverted by simultaneous application of Put (El-Ghachtouli et al., 1996a). It was also suggested that PAs would interact with pectinases, increasing adhesion or penetration of the fungi through the plant cell wall, enhancing contact points with roots (El-Ghachtouli et al., 1995). Moreover, a positive correlation was found between polyamine chain length and their stimulation of fungal development. Besides the effect on initial AM infection events by acting directly on mycelial growth of an AM fungus, PAs may act through the inhibition
of Et production by roots, shown to have negatively affects AM infection in soybean (Morandi, 1989) and in *M. sativa* (Azcon-Aguilar et al., 1981).

In soybean, AM colonization increased plant PAs levels and in parallel, the silencing of arginine decarboxylase gene (*GmADC*) had a negative effect on mycorrhizal colonization and also affected the normal development of the plant (Salloum et al., 2018). Interestingly, the silencing of *G. max* *GmDAO* gene in the same experimental system, promoted arbuscule formation (Salloum et al., 2018). All these studies successfully demonstrated the importance of PAs in the stimulation of mycorrhizal growth.

A few works have addressed the role played by PAs on the AM-induced mitigation of plant abiotic stress in legumes. In mycorrhizal *Trigonella foenum-graecum*, a reduction of salt-induced damage of the cell membrane ultrastructure was attributed to a higher PAs (and osmolyte) concentration (Evelin et al., 2013). The inoculation with selected AM fungi *Funneliformis mosseae* (syn. *Gl. mosseae*); *Rhizophagus intraradices* (syn. *Gl. intraradices*), and *Claroideoglomus etunicatum* (syn. *Gl. etunicatum*)] caused amelioration of the negative effects produced by NaCl in *V. faba* (Abeer et al., 2014). In the last work, the fact that AMF-induced increases in PAs occurred in a higher extent to that induced by NaCl itself, was interpreted by authors as a proof of the protective role of these phytoconstituents against salt stress. Likewise, in *L. tenuis* plants mycorrhized with *Gl. intraradices*, a higher content of total free PAs, compared to non-AM ones was reported by Sannazzaro et al., (2007) (Sannazzaro et al., 2007). Since PAs have been proposed as candidates for the regulation of root development under saline situations, authors suggested that the better shape to cope with salt stress displayed by AM *L. tenuis* plants was related to the higher polyamine levels registered.

Two studies have addressed the PAs metabolism in pre-symbiotic structures of AM fungi. In ungerminated spores of *Gl. mosseae*, Put and Spd were the main free PAs identified, being their
endogenous concentrations suggested as limiting factors for fungal growth (El-Ghachtouli et al., 1996b). Moreover, when polyamine biosynthesis via ODC was blocked by DFMO, a specific irreversible ODC inhibitor, spore germination and hyphal growth were greatly reduced, while simultaneous treatment with DFMO and Put attenuated the inhibitory DFMO effect. On the other hand, Spd and Spm were measured in spores of the AM fungus *Gigaspora rosea*, in presence or absence of different Put biosynthesis inhibitors (Sannazzaro et al., 2004). Results from the last work demonstrated that both arginine and ornithine decarboxylase pathways participate in Put biosynthesis in this fungal species. These results suggested that PAs are also involved in regulating the earliest developmental stages of AM symbiosis.

4. Integrative tools at the spotlight: MultiOmics approaches to decrypt the interface legumes-PAs-abiotic and biotic stress or interactions.

Mayor future challenges are the development and implementation of progressive methods of genetic improvement oriented to develop varieties of legumes that have genetic recovery capacity against environmental stresses. Nowadays, high-throughput sequencing technologies are efficiently applied for massive mapping and concurrent identification of quantitative trait loci (QTL) and candidate genes associated to yield and biomass phenotypes, as well as both biotic and abiotic stresses resistance-linked promising traits in (wild) legumes. As for both proteomics and metabolomics, massive phenotyping (phenomics) tools needed to elucidate the emergent relations of genetic architecture and traits are scarce (Abdelrahman et al., 2018), asymmetrically implemented, and frequently inaccessible to plant scientist from low-income countries. These limitations are even more pronounced when phenomics is intended to be applied in the fields, where target crop context, weather, or geographic cues might interfere with proper sensor accuracy.
during data acquisition. As a spin of phenomics, Burridge et al., (2016) (Burridge et al., 2016) introduced the concept of “shovelomics” as a procedure integrating manual measures, image analysis and scoring during the selection of optimal root architecture of cowpea and common bean in response to phosphate deficiency and drought stress. There is opportunity to enhance traditional phenotyping tools into integrated multisensory system, combining ultrasonic and normalized vegetation index sensors, thermal radiometers, portable spectrometers, and RGB cameras to measure diverse traits in the field, as has been implemented by Bai et al. (2016) (Bai et al., 2016) for soybean canopy traits, at early and late growing seasons.

We are at the verge of a post-genomic era, where whole genome sequencing and assembly is becoming a relatively feasible research output in a significant share of the plant science community. This emerging commodity, which has bypassed from model plants to any crop of interest, is valuable only in the context of interactive databases that facilitate the eventual linking of different levels of information attained by diverse platforms (Thompson et al., 2009). Needless to say, the path from predicted genes in a genome to agronomic traits is dependent of the appropriate tools to manage, link and exploit large quantities of heterogeneous data.

4.1 Towards the integration of metabolomics, transcriptomics and phenomics

Oomics such as transcriptomics and metabolomics, have had essential roles in identifying how plant-microbe associations could deviate from classical outcomes in specific conditions (Romero et al., 2017; Rosenberg and Zilber-Rosenberg, 2018). Metabolomics studies have provided a profound knowledge of the metabolite landscape of plants, mostly biased to the level of single individuals as experimental units. For instance, an emerging role of PAs in transcriptional regulation and translational modulation of the stress response has been proposed, based on high-throughput sequencing technologies (discussed in (Tiburcio et al., 2014)). On the other hand, these
techniques have had limited engagement at the scale of complex agricultural systems, which are multifaceted structures significantly more challenging to assess, nonetheless at the same time being the precise target were translational research should be focused in order to improve crops (Alseekh et al., 2018). In addition, the selected experimental units have been pervasively dominated by model plant species, which raises the question of whether the obtained results could be extrapolated to crops. For instance, in legumes, metabolomics has been applied mostly to *M. truncatula* and *L. japonicus*. In the former, the effect of rhizobial node factor has been associated to the level of oxylipins, which could induce Nod factor signaling in early symbiosis (Zhang et al., 2012). Whether this effect is analogous in *M. sativa* remains unknown. Perhaps it is also worth mentioning some intrinsic limitations associated to the current state of metabolomics from a technological perspective. In the context of nucleic acid omics, the relatively power of metabolism assessment instruments is certainly low. While the number of metabolites estimated in plants is ca. 200,000 (Bino et al., 2004), we are able to measure only a couple of hundreds. It is evident that among omics, both proteomic and metabolomics platforms are lagging well behind the impressive growing outputs of nucleic sequencing infrastructure. The use of omics is essential to address key questions, such as the specific role of PAs in signaling during abiotic stress (Pál et al., 2015). Transcriptomic assessment of gene expression, perhaps the most prevalent approach to investigate the specific effects of plant coping with stress or development, should not only be restricted to mRNA. This technique has been extensively used to assess the global transcriptional response of PAs transgenic over-expressors during abiotic stress (reviewed in (Marco et al., 2011)). Other RNA species, such as small RNAs have been reported to play important roles in legume symbiosis, nitrogen fixation, and general plant development. In addition, microRNAs could have specific effects associated to modulation of transcription factors via translational arrest. In this scenario, mRNA transcriptomic would fail to identify biological effects of miRNAs (Hussain et al., 2018). The development of
gene networks based on transcriptomics data has scaled reductionist approaches associated to target sequencing traditional practices. Gene networks based predictions have successfully been applied to survey the uptake, translocation, remobilization and general regulation of N metabolism in model and crop species (Fukushima and Kusano, 2014). Predictions from transcriptomic data could be weighed with additional complementing technologies. For instance, in soybean differentially regulated proteins were identified by integrated proteomics and metabolomics during hormone treatment presenting a supported model of altered flavonoid and isoflavonoid metabolism upon Et and ABA treatment (Gupta et al., 2018). Another exciting example of complementation of transcriptomics and metabolomics data in legumes was reported during phosphate deficiency in different plant organs (reviewed in Abdelrahman et al., 2018). It is interesting to point out that several of the databases used to assess legume data are restricted to model species. For instance, MedicCyc, which includes over 250 pathways, associated metabolites and enzymes of *M. truncatula*, or SoyKB (Soybean Knowledge Base), the most extended resource for soybean translational genomics. There is a need to extend these resources to additional crops, which are valuable tools to evaluate in-depth omics data (Bagati et al., 2018). In this context, the envisioning of legume molecular targets to be used in biotechnological applications would be more realistic, and the role of PAS maybe, deeply understood.

### 4.2 Introducing physiomics in the post-genomic era.

With the objective to reduce the necessary inputs for cultivation and to develop environmentally and sustainable crops, there is an urgent need to combine innovative experimental designs and technologies which could lead to the understanding of a continuum of processes which delineate the plant biome from molecules to ecosystems. Translational plant biology is at the interface of environmental impacts and the genetic bases of crop improvement. A plethora of tools
and practices such as precision agriculture, molecular breeding by marker-assisted selection and QTLs, balanced energy and carbon management, fertilizer and irrigation use are dependent on a better understanding of the intrinsic physiomics of our crops. Multidisciplinary approaches integrating from basic genetic information to systems biology will lead the following generation of crops (Jez et al., 2016). Novel genome editing tools are introducing significant advances in order to apply surgical changes on crops genomes based on the new wave of integrative omics information. Computational models derived from genome networks, signaling and transcriptional pathways would serve as input for interpreting (and confirming) the eventual effect of simultaneous perturbation of multiple editing events resulting from genome editing techniques oriented to fine-tuning biochemical processes in crops. These strategies are paving the way to a future where metabolic engineering of crops would be an avenue to both augment profitable margins of commodities and limit the ecological footprints associated to crop practices (Jez et al., 2016). Multiplatform physiological data provides the basis for phenomic networks (Kumar et al., 2015). Numerous examples of insights from relatively large phenotype datasets from legumes are available. For instance, phenomic networks derived from the assessment of parallel measures of traits such as dry mass, shoot and root morphology, root aerenchyma, chlorophyll levels, dark-adapted Fv/Fm, and stomatal conductance from QTL mapped recombinant inbred lines of *L. japonicus* were analyzed in the context of waterlogging. The integration of these data points suggested that asymmetrical adjustments of shoots and roots are associated to this stress (Striker et al., 2014). A prevalent question is how we integrate large extents of variables, both quantitative and qualitative, which may have *a priori* non-linear relationships between them. Some resources are emerging which provide insights into complex relationships derived from highly heterogeneous datasets (including the capacity to assess not only omics data but also morpho-agronomic traits).
such as Cluster-match which has promising features in next generation agronomic data-mining (Pividori et al., 2018).

While we are dissecting legumes genomes with magnifying glasses, we should not lose perspective. We have to understand the interplay of legumes, bacteria, fungi and viruses. We cannot keep ignoring the emergent effects of every player in complex biological systems, only because simplistic approaches are more easily assessed. The future is not only drones and satellites; the future is understanding the inherent features of the crop holobiome.

4.3 Systems biology through the magnifying lens: the concept of holobiome in plant experimental biology.

The holobiont is composed by both the host (plant) and its associated microbiome. This complex association function as a different and multifaceted biological entity, with a supplementary organismal state to the one derived of its parts (Rosenberg and Zilber-Rosenberg, 2018). Natural (and artificial) selection appears to be operating at the holobiome level and thus the separation of its components in reductionist scientific approaches, which are the state of the art in most research fields, may have had appalling effects in the literature and our conception of plants as living organisms. In this scenario, recent reports indicate that host genomes are highly conserved and more resilient to change due to genetic mutations occurring relatively slow. On the other hand, the microbiome is dynamic and prone to change rapidly under environmental perturbations, and thus provide a means for swift adaptation to perturbations (stress). This can be achieved by shifting levels/presence of particular microbes, gaining of additional microbes, mutation and horizontal gene transfer (Rosenberg and Zilber-Rosenberg, 2018). Thus, microbes provide the needed plasticity of the host to a dynamic context. It is intriguing how this concept, which appears to be so obvious when described, has been ignored by most experimental studies crops in the last 50 years.
of plant physiology literature, where associated microbes (excluding known pathogens or symbionts) have been systematically ignored in the experimental design. The foundational bases of the paradigm of plant health are being redefined by novel studies. For instance, recently by in planta community perturbation experiments, an emergent role of bacterial commensals in plant survival and protection against root-derived filamentous eukaryotes from the root microbiome has been debunked, evidencing a striking interkingdom balance of microbial diversity (Durán et al., 2018). Needless to say, the impressive cutting-edge Arabidopsis technological toolkit applied there is far from being available for crops or even other plant models.

### 4.4 Current challenges and future perspectives

In the last few years, a shift in the prototypical host-microbe paradigm has arisen, redefining the concept of symbiont and pathogen. Insights from reassessment of well-established microbes associated with beneficial interactions, which in certain conditions have been reported to increase the ability of the host to gain nutrients were found to be in certain conditions also associated with disease. Moreover, common disease associated agents have been also found to have no impacts on their target plants in diverse contexts.

There is a significant amount of data derived from multiple platforms obtained in the most diverse treatments of legumes (Fig. 2). However, the lack of homogeneity in experimental settings and conditions, data acquisition, sampling process, data handling among others limits the potential of extrapolation/integration or meta-analysis (Bukhari et al., 2015). We call to revisit a long standing proposal, a renewal of the PA research landscape based on holistic approaches such as system biology (Montanez et al., 2007). There is a need to generate additional crop specific databases, harboring re-usable data, well established protocols and integrative tools, which if adopted by the legume scientific community could lead to a timelier and sustainable advance in the
field. Holistic system biology approaches will pave the way to the understanding of the gene-to-
metabolite networks that define legume and PAs metabolisms interrelationships.
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6. Figures

Figure 1. Polyamine metabolism. Biosynthetic pathways for PAs and related metabolites are indicated by continuous lines. Dashed lines show the PAs catabolism reactions. Biogenic Polyamines biosynthetic pathways in nodules are represented inside a pink area. Abbreviations: Put, putrescine; Cad, Cadaverine; Spd, spermidine; Spm, spermine; tSpm, thermospermine; sym-Hspd: sym-Homospermidine; 4AB-Cad: 4-aminobutyl-Cadaverine; SAM, S-adenosylmethionine; dcSAM, decarboxylated S-adenosylmethionine; ACC, aminocyclopropane carboxylic acid. Numbers refer to enzymes: 1, arginine decarboxylase (ADC, EC 4.1.1.19); 2, agmatine iminohydrolase (EC 3.5.3.12); 3, N-carbamoylputrescine amido-hydrolase (EC 3.5.1.53); 4, ornithine decarboxylase (ODC, EC 4.1.1.17); 5, spermidine synthase (SPDS, EC 2.5.1.16); 6, spermine synthase (SPMS, EC 2.5.1.22); 7, thermospermine synthase; 8, L-lysine decarboxylase (EC 4.1.1.18); 9, SAM synthetase; 10, SAM decarboxylase (SAMDC, EC 4.1.1.50); 11, ACC synthase (EC 4.4.1.14); 12, ACC...
oxidase (EC 1.14.17.4); 12, back-conversion polyamine oxidase (non-specific polyamine oxidase, EC 1.5.3.17); 13, terminal catabolism polyamine oxidase (propane-1,3-diamine-forming, EC 1.5.3.14); 14, diamine oxidase (DAO, EC 1.4.3.6); 15, homospermidine synthase (ammonia-forming; EC 2.5.1.45); 16, homospermidine synthase (propane-1,3-diamine-forming; EC 2.5.1.44); 17, phenylalanine decarboxylase (EC 4.1.1.53).
Figure 2. Envisioning multi-omics integrations to decrypt the interface legumes-PAs-abiotic and biotic stress and PAs roles in the holobiome interactions.