

## Abscisic acid — an overlooked player in plant-microbe symbioses formation?

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**Abscisic acid (ABA) is an ubiquitous plant hormone and one of the foremost signalling molecules, controlling plants' growth and development, as well as their response to environmental stresses. To date, the function of ABA has been extensively investigated as an abiotic stress molecule which regulates the plants' water status. However, in the context of symbiotic associations, ABA is less recognized. In contrast to well-described auxin/cytokinin and gibberellin/strigolactone involvement in symbioses, ABA has long been underestimated. Interestingly, ABA emerges as an important player in arbuscular mycorrhiza and legume-rhizobium symbiosis. The plant's use of stress hormones like ABA in regulation of those interactions directly links the efficiency of these processes to the environmental status of the plant, notably during drought stress. Here we provide an overview of ABA interplay in beneficial associations of plants with microorganisms and propose ABA as a potential factor determining whether the investment in establishing the interaction is higher than the profit coming from it.**

**Key words:** abscisic acid, symbioses, nodulation, arbuscular mycorrhiza

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### INTRODUCTION

Plants are known for their ability to establish and maintain sophisticated interactions with microorganisms such as fungi and nitrogen-fixing bacteria. It is a feature which positively affects plant's life not only at optimal environmental conditions but more importantly upon nutrition/water shortage. The best studied symbiotic associations include the legume-rhizobia symbiosis (LRS) between legumes and nitrogen-fixing bacteria, which are collectively called rhizobia, as well as arbuscular mycorrhiza (AM) between most terrestrial plants and fungi of the phylum *Glomeromycota* (Oldroyd, 2013). Whereas AM fungi can colonize the majority of land plants, root nodule symbiosis with rhizobia almost exclusively involves legumes (*Fabaceae*). The molecular basis of them, still not fully deciphered, hides plenty of potential agricultural resolutions.

Regarding AM interaction, the main purpose of its establishment is to increase plant water and mineral conductance in exchange for carbohydrates (Harrison, 2012). AM is the essential way of compensating phosphorus deficiency (Yoneyama, 2012) however it is worth remembering that >20% of total photosynthesis-derived sugars are being transferred to the microbial symbiotic

partner (Bago *et al.*, 2003). The moment of first mycorrhizal interaction is thought to be a breakthrough point in plants evolution from aquatic to terrestrial environment (Gutjahr & Parniske, 2013).

The plant promotes mycorrhiza by exudation of carotene derivatives, class of plant hormones, strigolactones (Isayenkow *et al.*, 2005; Landgraf, 2012). In response to strigolactone presence in the rhizosphere, fungus starts to exudate its own lipochitooligosaccharides (LCOs) and chitooligosaccharides, called Myc factors (Maillet *et al.*, 2011). Perception of Myc factors triggers a chain of cytological changes, such as nucleus displacement and stimulation of calcium spiking exclusively in the cells vulnerable to accommodate the fungus (Gutjahr & Parniske, 2013). Calcium ion is thought to be a secondary messenger universal for all eukaryotic cells. Variation in intracellular free Ca<sup>2+</sup> concentration, called the aforementioned calcium spiking, is one of the crucial steps in signalling pathway activated in plant upon symbiotic partner presence (Navazio & Mariani, 2008). Fluctuations of calcium spiking frequency lead to the formation of pre-penetration apparatus (PPA) (Sieberer *et al.*, 2012; Gutjahr & Parniske, 2013). These actions enable the fungal infection hyphae to penetrate through the root rhizodermis to the outer cortex (Chabaud *et al.*, 2011; Maillet *et al.*, 2011). Eventually, the mature, tree-shaped structures called arbuscules are formed in inner cortex as a main place of nutrient and water exchange between partners.

Legume-rhizobia symbiosis, on the other hand, is an intimate relation between legumes and nitrogen-fixing rhizobial bacteria that leads to the formation of unique lateral organs called nodules. In exchange for an available nitrogen, host plant provides bacteria with a photo-synthetically fixed carbon as well as the accommodation and suitable conditions for the nitrogen fixation inside nodules. Establishment of LRS is highly energy-consuming process for the plant, the cost of which is estimated at 12–17 grams of carbon per gram of nitrogen (Crawford *et al.*, 2000). It is postulated that evolutionarily younger LRS symbiosis stems from AM and recruited some of its genetic program in terms of early signalling events (Kistner & Parniske, 2002).

The first step of LRS is represented by a so called “chemical dialogue” between symbionts. Plants under nitrate limitation, release to the rhizosphere (iso)flavonoids that act as chemoattractants and induce expression of bacterial nod genes. Following the perception of bacterial

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**Abbreviations:** ABA, abscisic acid; AM, arbuscular mycorrhiza; AMF, arbuscular mycorrhiza fungi; LRS, legume-rhizobia symbiosis; NF, Nod factor

Nod factor (NF), a cascade of action is initiated in plant roots, including curling of the root hairs and formation of infection threads. Concomitantly in the root cortex a nodule primordium is developed where bacteria are delivered by the infection thread and are subsequently taken up into the nodule cells. The aforementioned events are triggered by signalling pathway, including kinase receptor activity, calcium spiking and alteration of nodulation-related genes expression (Oldroyd *et al.*, 2013). After accommodation, the rhizobia differentiate into bacteroids and fix atmospheric nitrogen inside organelle-like structure called symbiosome, within the nodule. Nutrient flow between partners takes place across the aforementioned symbiosome membrane (Clarke *et al.*, 2014).

Symbiotic associations are largely controlled by a proper balance of a wide set of plant hormones. For instance, coordinated action of auxin and cytokinin is necessary for nodule primordia formation in the inner cortex of the root upon rhizobial infection (Miri *et al.*, 2015; Ferguson & Mathesius, 2014). Alongside, gibberellins act as negative regulators of arbuscule formation (Foo *et al.*, 2013) and strigolactones are considered as signalling molecules stimulating hyphae branching (Kretzschmar *et al.*, 2012). For years abscisic acid (ABA) has been regarded as a plant hormone with multiple functions regulating plant physiology and reacting to various abiotic and biotic stresses. ABA is synthesized from precursors, mainly carotenoids and xanthophylls found in plastids. Synthesis enzymes are present mainly in veins of vegetative tissues, guard cells, maternal tissues and embryo (Boursiac *et al.*, 2013). ABA is widely known for its role in response to drought and being accumulated in guard cells what results in stomatal closure and avoidance of transpirational water loss. Its participation in salt stress, pathogen invasion resistance as well as in developmental processes, such as seed dormancy and germination is well documented (Goggin *et al.*, 2009; Rodriguez-Gacio *et al.*, 2009).

Symbiosis establishment is energetically expensive at standard circumstances and requires far higher “investment” while stress conditions appear. Moreover, it is

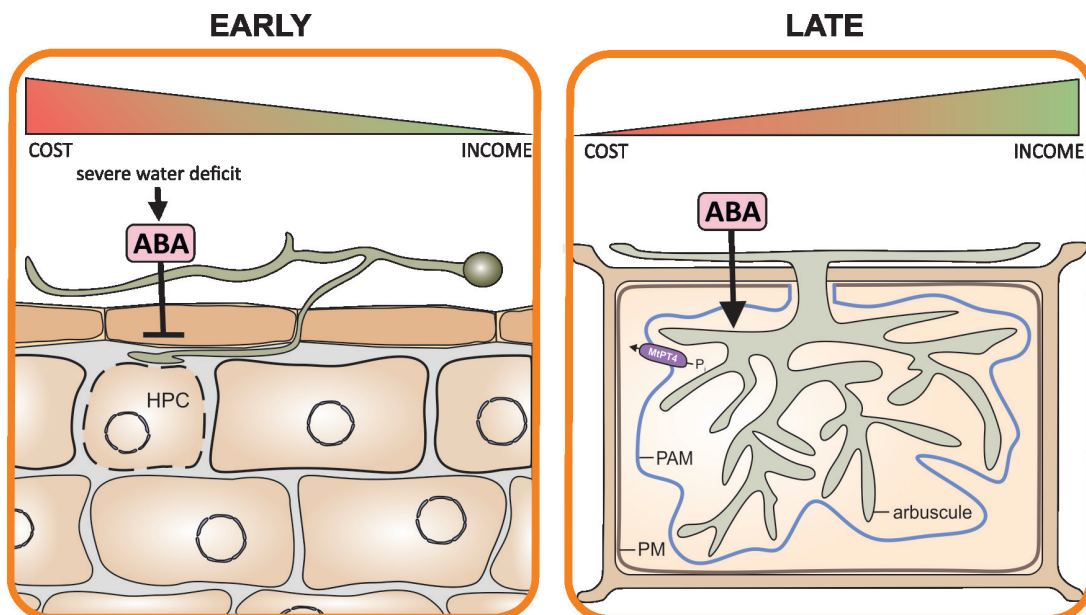
important to differentiate the costs of enrolment from the costs of functional maintenance. Bearing in mind the knowledge about pleiotropic role of ABA in perception/reaction to various environmental stimuli we here present ABA also as a plant hormone modulating the establishment of beneficial plant-microbe interactions.

#### MYCORRHIZATION EFFICIENCY DEPENDS ON ABSICISIC ACID

The research conducted on tomato suggests that ABA is the crucial mediator in plant-microbe interactions such as AM. It was shown that colonization rate of the root in ABA-treated wild plants is 10% higher than in those never exposed to ABA. Additionally, histological observations of tomato *sitiens* mutant with reduced endogenous ABA concentration show significantly lower level of arbuscular mycorrhizal fungi (AMF) colonization (Herrera-Medina *et al.*, 2007; Martin-Rodriguez *et al.*, 2010, 2011). Moreover, observations of fungal alkaline phosphatase activity, a marker enzyme of fungal infection efficiency, in the same study indicate that the deficiency in AMF colonization in *sitiens* plants can be restored by exogenous ABA application thus ABA seems to make plants more susceptible to mycorrhizal fungi infections (Herrera-Medina *et al.*, 2007).

Colonization, however, is not the only stage of mycorrhiza that ABA appears to be involved in. The previously mentioned tomato *sitiens* mutant has nearly 35% lower arbuscule content compared to wild type plants. Interestingly though, the percentage of arbuscules at first morphological stages of arbuscule formation in *sitiens* is over 2 times higher than in WT and then drastically drops down by the aforementioned 35% (Herrera-Medina *et al.*, 2007).

A phosphate transporter PT4 is present exclusively in the arbuscules. Research conducted on tomato shows that *LePT4* transcript accumulation is clearly associated with the presence of ABA as ABA-deficient *sitiens* mutant shows significantly reduced amount of *LePT4* mRNA than WT. What is more, the relative *LePT4* ex-



**Figure 1. ABA impact on arbuscular mycorrhiza considering early and late stages as well as costs/incomes of symbiosis establishment and maintenance upon drought stress**

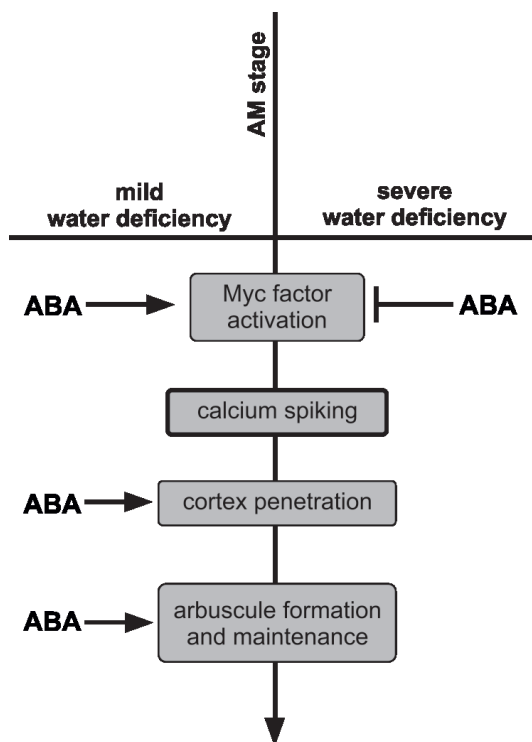
ABA negatively affects arbuscular fungi colonization upon severe water deficiency and stimulates the maintenance of already formed mature arbuscules. HPC, hypodermal passage cell; PM, plasma membrane; PAM, periarbuscular membrane.

pression in this mutant does not raise throughout arbuscule development stages, like it does in wild type plants (Martin-Rodriguez *et al.*, 2010). The data indicates that ABA plays a role in AM establishment as well as in arbuscule development and maintenance (Fig. 1).

Perception of ABA appears crucial for modulation of AM. As ABA signalling pathway is concerned, a protein phosphatase 2A (PP2A) is strongly emphasized. This particular phosphatase with its associated proteins is reported to be involved in ABA signalling (Cutler *et al.*, 2010; Hu *et al.*, 2014). Interestingly, PP2A signalling role takes place downstream of ABA core signalling pathway, in contrast to PP2C which acts within this pathway (Santiago *et al.*, 2012). PP2A is a holoenzyme consisting of three subunits: the scaffolding subunit A, the regulatory subunit B and the catalytic subunit C. The B subunits which are further classified into B, B' and B'' subfamilies, are believed to determine the substrate specificity and the subcellular localization of the PP2A holoenzymes (Matre *et al.*, 2009). The PP2AB'1 subunit is reported to be engaged in ABA signalling during AM. Mutation in PP2AB'1 results in a reduced level of AM colonization. Interestingly, the effect cannot be rescued by permissive ABA application. It has been postulated that the gene encoding PP2AB'1 is transcriptionally active upon ABA presence, especially during AM (Charpentier *et al.*, 2014). It has been proposed that the action of PP2AB'1 takes place after the hyphae enters the root cortex, representing another stage of AM formation that ABA is involved in.

#### THE ABA IMPACT DEPENDS ON THE CONCENTRATION

ABA controls the establishment of arbuscular mycorrhiza, however the effect of ABA depends also on its concentration. Data obtained for *Medicago truncatula*, a



**Figure 2. General overview of ABA impact on different AM stages depending on water deficit endurance**  
ABA may act above and/or below calcium spiking, which is a central point in early signalling pathway during AM.

model legume plant, indicates that ABA acts as a positive regulator promoting AM by increased fungal colonization at low concentrations. Increase of ABA concentration eventually results in an impairment of AM establishment. In wild-type plants, 5  $\mu$ M ABA significantly intensifies average percentage of AM colonization, while 50  $\mu$ M ABA-treated plants manifest AM level reduced by 45% compared to 5  $\mu$ M ABA treatment. It has been suggested that application of high ABA concentrations impairs Myc factor induced calcium oscillations by the suppression of non-sulfated LCOs activation (Charpentier *et al.*, 2014). The results seem well-reasoned when interrelated to abscisic acid role in drought stress perception. Low concentration of ABA simulates first stages of water shortage recognition. At this stage, AM appears to be a perfect tool to overcome the potential drought stress by improvement of water uptake and conductance, without the energetic overinvestment that could disrupt plant physiology. Later on, during severe water deficiency, high ABA level acts as a brake signal, suggesting the AM establishment would be too expensive energetically. At this point, even the abundance of fungi colonizing the plant would not be sufficient enough to compensate the effect of stress endurance (Fig. 2).

#### AN INTIMATE CROSS-TALK BETWEEN PLANT HORMONES TUNES THE INTERACTION

ABA is not the only hormone influencing AM. The other main player seems to be ethylene. The antagonistic interaction between the hormones' signalling pathways based on a negative feedback is thought to be one of the essential factors in modulating plant development (Beaudoin *et al.*, 2000). It has been proposed that ethylene has more to do with calcium spiking at the early stages of AM establishment (Martin-Rodriguez *et al.*, 2010). It has recently been reported that tomato ABA-deficient mutants have tendency for ethylene accumulation which results in less intensive AM colonization (Martin-Rodriguez *et al.*, 2011). Tomato *siliens* plants, treated with 0.3 mM silver thiosulfate (STS) to block ethylene perception, exhibit the exact effect as wild type plants supplied with ABA (Herrera-Medina *et al.*, 2007). Whether that means that ethylene perception is just as important as ABA level, it remains unknown for now. Nevertheless, it can be stated that ethylene negatively regulates ABA biosynthesis (Martin-Rodriguez *et al.*, 2010).

The other highly interesting relation appears between ABA and aforementioned strigolactones, important, if not crucial, to pre-symbiotic AM signalling stages. Strigolactones are also considered to be responsible for further hypopodium and arbuscule formation (Akiyama *et al.*, 2005). The interplay between these two hormones starts at the very core of their biosynthesis pathway, as they are both derived from carotenoids (Booker *et al.*, 2005). Experimental data from comparison studies on three different tomato ABA-deficient mutant lines shows a strong correlation between the reduction of ABA and strigolactone content in roots (mutant compared to its corresponding wild type) (Lopez-Raez *et al.*, 2010).

#### ABA AS A NEGATIVE REGULATOR OF NODULATION

It is a well-known fact that nodulation efficiency is strongly dependent on environmental circumstances and remarkably slowdowns upon abiotic stresses such as drought (Marino *et al.*, 2007; Gil-Quintana *et al.*, 2013). It is therefore not surprising that major drought sensing and



response hormone — ABA — was recognized long time ago as a negative regulator of nodulation in many legume plants. Numerous research groups showed that external application of ABA suppresses root nodule formation, for example in *Pisum sativum* (Philips, 1971), *Glycine max* (Bano & Harper, 2002), *Lotus japonicus* (Suzuki *et al.*, 2004), *Trifolium repens* (Suzuki *et al.*, 2004), *Phaseolus vulgaris* (Khadri *et al.*, 2006) and *Medicago truncatula* (Ding *et al.*, 2008). Conversely, treatment with abamine, specific ABA biosynthesis inhibitor, causes increased both nodule number and enhanced nitrogen fixation along with decline of endogenous ABA content in *L. japonicus* (Suzuki *et al.*, 2004; Tominaga *et al.*, 2009). Finally, several legume mutants exhibiting both altered symbiotic phenotypes and altered endogenous ABA level or sensitivity have been identified (Ding *et al.*, 2008; Tominaga *et al.*, 2009; 2010). Based on the currently available data, ABA emerges as a modulator of nodule formation that affects numerous signalling pathways at different stages of symbiotic interaction.

### EARLY STAGES OF LRS ARE AFFECTED BY ABA

The link between ABA and early phases of nodulation was provided by Ding and coworkers. They showed that ABA pretreatment abolishes induction of early nodulin genes in *Medicago* (*Rip1*, *ENOD11*) in response to NF and *Sinorhizobium meliloti* and affects the calcium spiking. Moreover, they observed that ABA application dramatically reduces infection events in the plant epidermis which manifests by decreased number of visible infection threads and bacteria microcolonies in the region of root hair curl (Ding *et al.*, 2008). These findings are consistent with the observation that ABA is able to block the root hair deformation and inhibit growth of infection threads in *T. repens* (Suzuki *et al.*, 2004). The role of ABA in nodulation suppression is additionally supported by the analysis of transgenic *M. truncatula* expressing Arabidopsis-derived *abi1.1* (*abscisic acid insensitive 1*). The *abi1.1* is a mutated allele encoding dysfunctional type 2C protein phosphatase (PP2C) and blocks ABA signalling in transformed plants. It was observed that *Medicago* roots expressing *abi1.1* exhibit hypernodulation phenotype and are more sensitive to NF-dependent induction of *ENOD11* (Ding *et al.*, 2008).

What is more, it was demonstrated that ABA influences the cytokinin-dependent cell division in inner cortex which gives rise to a new nodule. Mentioned above research group revealed that ABA treatment can suppress *ENOD40* and *NIN* induction by exogenous cytokinin addition. It also arrests spontaneous root nodule formation in the *L. japonicus snf2* mutant, carrying gain-of-function mutation in the cytokinin receptor LHK1. They also found that *sta1* (*sensitive to ABA 1*) mutant of *M. truncatula* with reduced nodule numbers, is hypersensitive to ABA in case of nodule primordium formation and concomitantly exhibits low sensitivity to ABA at epidermal level. Thereby they provided evidence supporting thesis proposed in 1971 by Philips about the role of abscisic acid in inhibition of nodule primordium formation through interfering with cytokinin pathway (Philips, 1971; Ding *et al.*, 2008). The additional clue pointing to relation between ABA and cytokinin in nodulation was given by Caba and coworkers. They revealed that supernodulating phenotype of soybean *nts382* mutant is caused by lower than in wild type ABA/cytokinin ratio (Caba *et al.*, 2000).

Summing up, Ding with colleagues eventually proved that ABA controls as a negative regulator both: i) the in-

itiation of bacterial infection in the epidermis, controlled by NF and ii) the promotion of cortical cell division to form nodule primordia *via* cytokinin signalling (Ding *et al.*, 2008). Based on aforementioned data authors state the hypothesis that ABA coordinates the infection process and nodule organogenesis, being two spatially separated yet simultaneous events, making ABA an indispensable factor altering nodulation properties according to the environmental and developmental status of the plant (Ding *et al.*, 2008; Ding & Oldroyd, 2009) (Fig. 3).

### ABA AFFECTS NITROGEN FIXATION EFFICIENCY

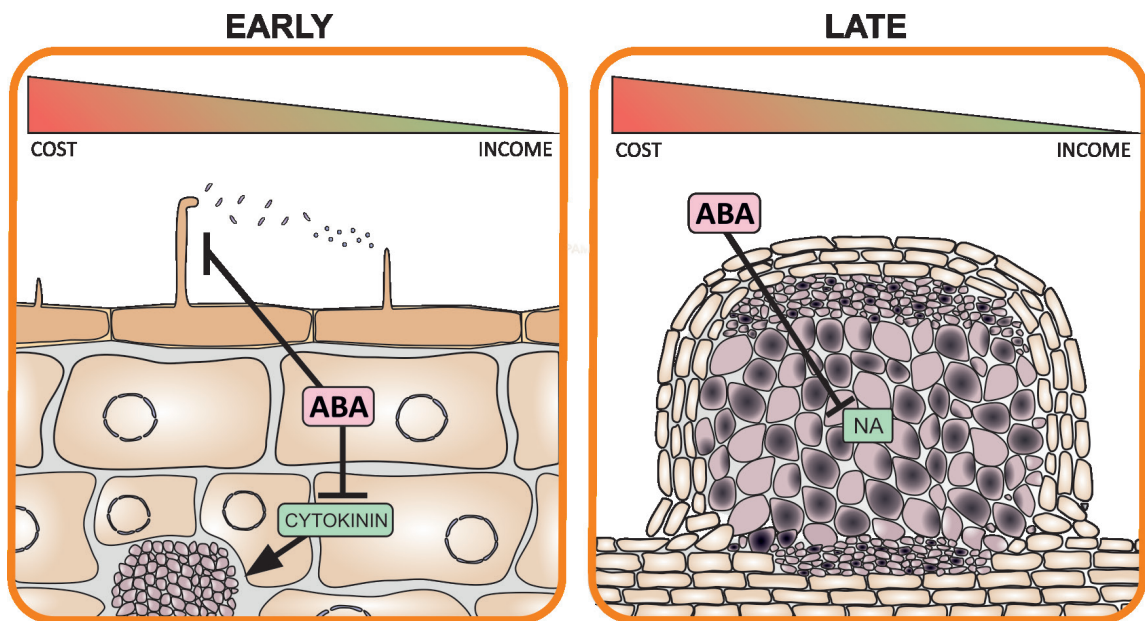
The role of ABA is not limited to affecting early stages of nodule formation. It was revealed that ABA can trigger changes in mature pea nodules metabolism. It was observed that the level of leghemoglobin which buffers free oxygen inside nodules, largely declines after external addition of ABA. Subsequently, this causes decrease of symbiotic-nitrogen fixation provided by the enzyme complex nitrogenase, extremely sensitive to O<sub>2</sub> (Gonzales *et al.*, 2001). Other research group isolated and characterised *L. japonicus* mutant, called *enhanced nitrogen fixation 1* (*enf1*), exhibiting higher activity of nitrogenase compared to the wild type. Authors found that *enf1* mutant accumulates lower amount of endogenous ABA and therefore is less sensitive to ABA-mediated suppression of nitrogenase. Deeper studies led to the observation that elevated nitrogenase activity in *enf1* mutant is associated with reduced production of nitric oxide (NO) (Tominaga *et al.*, 2009; 2010). It is worth pointing out that nitric oxide is well known inhibitor of nitrogen fixation process (Hichri *et al.*, 2015) (Fig. 3).

### ABA CONTROLS NODULATION LOCALLY

Nodulation is an expensive process therefore must be strictly regulated to ensure the appropriate level of nitrogen fixation without excessive depletion of carbon pool. Otherwise, as it is clearly shown in case of hyper- or super nodulating mutants, it can have a detrimental effect on plant growth and development. The level of nodulation is negatively regulated by a combination of local and systemic pathways. The latter one, termed autoregulation of nodulation (AON), is responsible for inhibition of future nodules development through long distance signalling *via* root-shoot-root loop (Kassaw *et al.*, 2015). Although, abscisic acid was suggested to be involved in AON (Caba *et al.*, 2000; Bano *et al.*, 2002), Biswas with coworkers revealed that ABA acts locally. Based on split root experiments on *L. japonicus* they demonstrated that exogenously added ABA suppresses nodulation only on the ABA-treated side, while other part of the root remains not affected (Biswas *et al.*, 2009). Along with ABA also ethylene and jasmonic acid (JA) were recognised as negative regulators of nodule formation, acting locally. All three phytohormones suppress Nod Factor signalling, however unlike JA and ethylene that function synergistically, ABA seems to act independently or with little crosstalk with ethylene pathway (Gresshoff *et al.*, 2009; Ding *et al.*, 2008).

### LEGUME-RHIZOBIA SYMBIOSIS UPON DROUGHT AND SALINITY STRESSES — COSTS EXCEED INCOMES

New organs development and afterward biological nitrogen fixation in mature nodules entail high energy con-



**Figure 3. ABA impact on legume-rhizobia symbiosis considering early and late stages as well as costs/incomes of symbiosis establishment and maintenance upon drought stress**

ABA acts as a negative controller of both rhizobial infection and primordium formation, as well as the nitrogenase activity (NA) in mature nodules.

sumption by the host. Therefore plant deciding about LRS establishment has to balance between its nutritional needs and extra costs incurrence, especially in hostile environmental conditions. Among deleterious circumstances, drought and salinity appear as major factors that adversely affect not only plants' growth and development but also suppress root nodule formation and reduce nitrogen-fixation capacity. Many lines of evidence point to these stressful conditions' influence on LRS in different legume species, e.g. *P. sativum* (Marino *et al.*, 2007), *G. max* (Serraj, 2003; Miao *et al.*, 2012; Gil-Quintana *et al.*, 2013), *Cicer arietinum* (Nasr-Esfahani *et al.*, 2014) and *P. vulgaris* (Faghire *et al.*, 2011). It is worth recalling that both salinity and drought engage ABA-mediated response, thus observed nodulation inhibition could in some range depend on ABA pathway.

## CONCLUSIONS

Bearing the already gained knowledge in mind, there is still a lot to discover in the matter of plant-microbe interactions being controlled by phytohormones and small signalling molecules which are still not fully deciphered. Regarding symbioses efficiency upon stressful conditions, the review of currently available data facilitates a comprehension of a dual role of ABA. In contrast to generally positive role during AM colonization, ABA acts as a negative regulator in legume-rhizobia symbiosis. It is worth to underline that AM and LRS share a common signalling pathway, however the opposite regulation by ABA implies different effects on symbiosis-specific signalling cascade and/or developmental processes.

It is tempting to hypothesise that there has to be a factor determining whether the investment in establishing a particular interaction is higher than the profit coming from it. The role of ABA in interpreting environmental conditions is undeniable. Does fluctuation of ABA level work as an alert system that calculates a ratio between costs and incomes, especially upon environ-

mental stresses? Understanding the complexity of such responses is the main challenge for the upcoming years.

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