

## Future agriculture systems

UDC 631.461.52:581.557.2:577.175.19

doi: 10.15389/agrobiol.2018.1.3eng

doi: 10.15389/agrobiol.2018.1.3rus

### **NEGATIVE HORMONAL REGULATION OF SYMBIOTIC NODULE DEVELOPMENT. II. SALICYLIC, JASMONIC AND ABSCISIC ACIDS** (review)

**A.V. TSYGANOVA, V.E. TSYGANOV**

All-Russian Research Institute for Agricultural Microbiology, Federal Agency for Scientific Organizations, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail [tsyganov@arriam.spb.ru](mailto:tsyganov@arriam.spb.ru) (✉ corresponding author)

ORCID:

Tsyganova A.V. [orcid.org/0000-0003-3505-4298](https://orcid.org/0000-0003-3505-4298)

Tsyganov V.E. [orcid.org/0000-0003-3105-8689](https://orcid.org/0000-0003-3105-8689)

The author declares no conflict of interests

Acknowledgements:

Supported financially by Russian Science Foundation (project № 14-24-00135)

Received September 5, 2016

c

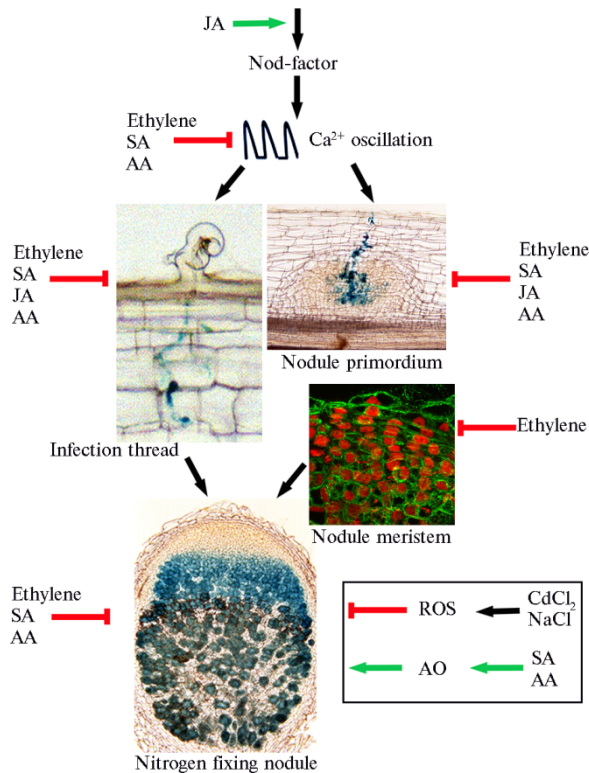
### Abstract

As a result of interaction with rhizobia, legume plants are able to fix atmospheric nitrogen in symbiotic nodules. Development and functioning of symbiotic nodules are under strong host plant control, including phytohormonal regulation (B.J. Ferguson et al., 2014). Due to the fact that nodule formation is a highly energy-consuming process, nodule number is restricted by plant. The negative regulation of nodulation involves, along with ethylene (A.V. Tsyganova et al., 2015), also salicylic (P.C. Van Spronsen et al., 2003; G. Stacey et al., 2006), jasmonic (Sun et al., 2006) and abscisic (Ding et al., 2008) acids. It is important to note that all listed phytohormones act at the different stages of development and functioning of symbiotic nodules. The first negative effects of jasmonic and abscisic acids are related to the blocking of calcium oscillations (J. Sun et al., 2006; Y. Ding et al., 2008), induced by Nod factors (lipochitooligosaccharides synthesized by rhizobia and activating a program for the development of infection and nodule organogenesis). Calcium oscillations are also blocked by ethylene (G.E. Oldroyd et al., 2001). Salicylic, jasmonic and abscisic acids influence the further development of symbiosis, blocking both the growth of infection threads (through which the rhizobia penetrate into the root), and the formation of nodule primordia (T. Nakagawa et al., 2006; J. Sun et al., 2006; Y. Ding et al., 2008). For abscisic acid it was shown that its negative effect on the development of nodule primordia is mediated by the influence on cytokinin signal transduction pathway (Y. Ding et al., 2008). Salicylic, jasmonic and abscisic acids also negatively affect the nitrogen-fixing activity of the nodules, and for abscisic acid it has been shown that the negative effect is associated with the activation of the production of nitrogen monoxide NO (A. Tominaga et al., 2010). Nevertheless, all these phytohormones can have a positive effect on the formation and functioning of nodules. For example, jasmonic acid activates the expression of rhizobial *nod* genes that control the synthesis of Nod factors (F. Maboob et al., 2006). It is interesting to note that for salicylic and abscisic acids a positive role in activating the defense mechanisms in plants under the action of stress factors has been shown, which leads to a decrease in their negative effect on the functioning of the nodules (F. Palma et al., 2013, 2014). Future studies of the interaction of ethylene, salicylic, jasmonic and abscisic acids in the negative regulation of the formation of nitrogen-fixing nodules are of great interest. Such studies should shed light on why several phytohormones are involved in negative regulation and what the specificity of each of them is. It is important to study the possibility of practical use of mutants with a lower level of any of the phytohormones (ethylene, salicylic, jasmonic and abscisic acids). Therefore, it seems promising to study the mutant *enf1* (*enhanced nitrogen fixation1*), obtained on the model legume *Lotus japonicus* and characterized by an increased level of nitrogen fixation (A. Tominaga et al., 2009). At the same time, it should be considered that a change in the level of a certain phytohormone can have a negative impact both on the development of the plant and its response to abiotic and biotic stresses.

Keywords: plant-microbe interactions, legume-rhizobial symbiosis, symbiotic nodule, phytohormones

An adaptive capacity for biological fixation of nitrogen is characteristic feature of legumes as a result of interaction with soil rhizobia [1]. During the in-

teraction, specialized organs are formed on the legume roots, called symbiotic nodules; rhizobia in them are differentiated into bacteroids, which are able to fix atmospheric nitrogen [2]. The symbiotic nodule development is based on the exchange of signaling molecules [3]. An important role in signaling belongs to phytohormones such as jasmonic acid [4], cytokinins and auxins [5, 6], gibberellins [7], ethylene [8, 9], abscisic acid [10], salicylic acid [11], strigalactones, and brassinosteroids [12-14]. The formation of a nitrogen-fixing nodule is very energy-consuming, that is why the nodule number is strictly regulated by the plant. We have previously reviewed the negative regulation of the development and functioning of symbiotic nodules by ethylene (Fig.).



**Effects of ethylene, salicylic, jasmonic and abscisic acids on the development and functioning of a symbiotic nodule.** The black arrows are subsequent stages of nodule development, the green arrows are the positive regulation, and the red arrows are the negative regulation. The box shows the regulation under the action of stress factors. JA — jasmonic acid, SA — salicylic acid, AA — abscisic acid, ROS — reactive oxygen species, AO antioxidants.

mutualistic legume-rhizobial symbiosis is particularly studied.

One of the first publications reported on the effect of different concentrations of salicylic acid on nodulation during the pre-treatment of *Vigna mungo* (L.) Hepper T-9 variety seeds. A decrease in nitrogenase activity was detected at all studied concentrations. At the same time, 10  $\mu$ M concentration stimulated nodulation, whereas at higher concentrations (100  $\mu$ M and 1 mM), the effect was negative [16]. In another study, the role of Nod factors in inhibiting salicylic acid-mediated defense responses in legumes was studied. Inoculation of alfalfa (*Medicago sativa* L.) by *NodC*<sup>-</sup> rhizobia mutant unable to synthesize Nod factors, as well as by the incompatible strain of *Rhizobium leguminosarum* bv. *trifolii* resulted in a signifi-

This review's focus is to discuss the role of salicylic, jasmonic and abscisic acids in the development and functioning of nodules of legumes. Certain specificity has been shown for these three phytohormones, although all of them affect negatively the development and functioning of nodules. In addition, jasmonic acid positively influences the very early stages of nodule development, and salicylic and abscisic acids have adaptive effect on the functioning of nodules under stress.

Salicylic acid is important in flowering, aging, resistance to pathogens and abiotic stresses [14]. The most studied role of salicylic acid in the induction of plant immunity when attacking pathogens. It is manifested in the activation of PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI), effector-triggered immunity (ETI), and in systemic acquired resistance (SAR) [15]. In recent years, the role of salicylic acid in the mu-

cant increase in salicylic acid accumulation compared to plants inoculated with a wild type strain [17]. Salicylic acid at 25  $\mu\text{M}$  concentration, having no visible effect on plant development, caused a delay in the formation of nodules and a decrease in their number when used exogenously for pre-treatment of alfalfa plants before inoculation with rhizobia. Simultaneous addition of  $10^{-7}$  M of Nod factor of *R. meliloti* and 25  $\mu\text{M}$  salicylic acid to the nutrient medium reduced the number of formed primordia by 75 %. Consequently, Nod factors along with the induction of infection and nodule organogenesis are involved in suppressing the accumulation of salicylic acid, which is necessary for the successful development of nodule primordium [17].

Study of Frisson variety of pea (*Pisum sativum* L.) wild type plants and of P2 mutant (*sym30*) unable to form symbiotic nodules and arbuscular mycorrhiza showed salicylic acid accumulation in P2 plants inoculated with *R. leguminosarum* bv. *viciae* wild strain or the *NodC* mutant [18]. Inoculation with a strain of mycorrhizal fungus *Glomus mosseae* (Nico. & Gerd.) Gerd. and Trappe also led to a greater accumulation of salicylic acid in P2 plants compared to the wild type plants. In inoculation with a pathogenic *Pseudomonas syringae* ssp. *syringae* strain there were no differences in the amount of salicylic acid between the mutant and wild type plants. Perhaps the *sym30* mutation causes specific resistance to symbiotic microorganisms, which is associated with the accumulation of salicylic acid [18].

The negative effect of salicylic acid was also described for legume plants with determinate type nodules with limited activity of the nodule meristem. The treatment of soybean (*Glycine max* (L.) Merr.) seedling roots with 1 mM or 4 mM salicylic acid significantly reduced the number of nodules formed [19]. Treatment of the leaves with 1 mM salicylic acid, on the contrary, increased the nodule number. T. Sato et al. (20) compared the effect of salicylic acid on nodulation in the parental Williams soybean variety and in two NOD1-3 (*rj7*) and NOD2-4 (*rj8*) hypernodulating mutants forming increased nodule number. A 5-day-old seedlings were pre-treated with 100  $\mu\text{M}$  salicylic acid for 5 days, after which they were inoculated with *Bradyrhizobium japonicum* USDA110 strain. There was a significant decrease in the number of nodules on the roots of the parental variety, and they were not capable of nitrogen fixation. At the same time, in both hypernodulating mutants, the nodule number was reduced much lesser. These results suggest that salicylic acid is involved in the autoregulation of nodulation [20].

The effect of salicylic acid was investigated in *Vicia sativa* L. plants inoculated by *R. leguminosarum* bv. *viciae* RBL 5523 and 248 strains. Salicylic acid at 100  $\mu\text{M}$  concentration added to the nutrient solution completely inhibited nodule formation [21]. However, a 5  $\mu\text{M}$  salicylic acid for strain RBL 5523 and 0.5  $\mu\text{M}$  for strain 248 stimulated nodulation. In *Lotus japonicus* (Regel) K. Larsen plants inoculated with TONO, R7A and E1R strains and treated with 100  $\mu\text{M}$  salicylic acid, nodulation was not inhibited. A negative effect of treatment with 100  $\mu\text{M}$  salicylic acid was observed for pea plants of the parental variety Frisson and the supernodulating mutant P88 inoculated with *R. leguminosarum* bv. *viciae* RBL 248, for alfalfa (*M. sativa* L.) plants inoculated with *Sinorhizobium meliloti* RCR 2011, and for clover (*Trifolium repens* L.) inoculated with *R. leguminosarum* bv. *trifolii* ANU 843. At the same time, treatment with 100  $\mu\text{M}$  salicylic acid did not inhibit formation of determinate nodules on bean (*Phaseolus vulgaris* L.) Negro Jamapa variety plants inoculated with *R. elti* CE3 and soybean plants (*Glycine soja* Siebold & Zucc.) inoculated with *S. fredii* HNO1 [21].

Subsequently, it was found that the negative effect of salicylic acid on *V. sativa* is manifested in the first 72 hours after plant inoculation. Pre-treatment for 24 hours did not affect the nodule number, and the addition of salicylic acid after 72 hours reduced their number by only 50 %. Salicylic acid has been shown to affect the association of rhizobia with the root surface, since curled root hairs and infection threads have not been detected. At the same time, root treatment with purified Nod factors caused deformation of root hairs under the addition of 100  $\mu$ M salicylic acid, while cell divisions in the inner root cortex were blocked [21].

In further experiments, the transgenic *L. japonicus* and *Medicago truncatula* Gaertn. plants with bacterial *nahG* gene encoding salicylate hydroxylase were used [22]. Transgenic *L. japonicus* lines having one or two copies of the *nahG* gene had a decreased content of salicylic acid, which correlated with an increased number of nodules and infection threads in root hairs. Transgenic plants showed a significant increase in the root length. The authors did not note the increase in the number of infection threads per centimeter of the root compared to wild type plants. In experiments with spot inoculation of roots with rhizobia, which made it possible to level the effect of root elongation, it was shown that the nodule number increases in plants expressing *nahG*. Similar results were obtained with another model legume, *M. truncatula*. Inoculation of composite plants expressing *nahG* resulted in a 2-fold increase in the number of infection threads and nodules compared to the control, with no visible effect on root growth [22]. Probably, salicylic acid may be involved in plant autoregulation of the nodule number at the stage of infection thread formation through the activation of defense reactions [22] (see Fig.).

The role of salicylic acid in the nodule formation was also studied in cell cultures of wild type *L. japonicus* plants and the Nod<sup>-</sup> mutant *LjSYM4-2* carrying a mutation in the *CASTOR* gene which encodes protein of nuclear membrane ion channel. This channel is involved in calcium oscillations induced by Nod factors [23]. The *LjSYM4-2* mutant is also unable to form arbuscules when inoculated with mycorrhizal fungus, as the fungal infection is aborted in the colonized epidermal cells in which the program of premature cell death is activated [24]. The mutant cell culture showed increased sensitivity to 0.5 and 1 mM salicylic acid, which was manifested in a sharp increase in the number of dead cells compared to that in the wild type cell culture [25]. A two-peak increase in the content of hydrogen peroxide was observed in the mutant culture, which is characteristic of the cellular response to the pathogenic attack [26]. Increased production of hydrogen peroxide preceded the activation of the production of nitrogen monoxide NO. That is, the content of salicylic acid, which wild type cells perceive as the physiological norm, in a mutant culture becomes a signal for the launch of cell death. This culture also revealed a constitutive increase in the expression of the *LjPR1* gene [25]. In composite plants, in the roots of which the *nahG* gene was expressed, no nodules were formed. This confirms that the altered sensitivity to salicylic acid in the mutant *LjSYM4-2* does not cause the Nod<sup>-</sup> phenotype.

Two-day pre-treatment with 0.1 and 0.5 mM salicylic acid positively influenced nitrogenase activity and nodule biomass in 49-day-old alfalfa (*M. sativa*) plants which were subsequently exposed to 200 mM NaCl for 12 days [27]. It is suggested that this effect is associated with the antioxidant metabolism activation by salicylic acid (see Fig.). A positive effect was also observed when 10<sup>-5</sup> M salicylic acid were used to treat aboveground part of 30-day-old chickpea (*Cicer arietinum* L.) variety Avarodhi plants which grew in soil contaminated with cadmium (25 mg) [28]. An increase in the nodule number, nitrogenase ac-

tivity, leghemoglobin content, as well as enzymes involved in nitrogen assimilation was observed in the control 90-day-old plants and those grown in polluted soil [28].

The study of *R. leguminosarum* bv. *viciae* 3148 strain revealed the presence in rhizobia of two systems of active efflux of substances from the cell (efflux pump) of the MFS (major facilitator superfamily) type controlled by the *salRAB* and *rmrA* genes activated by salicylic acid [29]. Mutation in the *sala* gene led to a significant inhibition of rhizobia growth in the presence of 2 mM salicylic acid, whereas alteration in the *rmrA* gene had no such effect. Both mutations did not affect the ability of rhizobia to form symbiotic nodules and their nitrogen-fixing activity. These results can be explained by the presence in the rhizobia genome of additional systems of active efflux of substances from the cell which compensate for the loss of the functional product of the *salRAB* and *rmrA* genes [29].

Jasmonic acid is involved in the regulation of various processes of plant development during biotic and abiotic stresses including legume-rhizobial symbiosis (30).

It was assumed that not only flavonoids, but also other substances associated with the phenylpropanoid pathway may be involved in activation of the rhizobia *nod* genes expression [31]. It has been shown that jasmonic acid and methyl jasmonate can induce the expression of *nod* genes in some strains of rhizobia, and jasmonic acid together with naringenin, a natural flavonoid inducer, have a synergistic effect [31]. Later, the positive effect of jasmonic acid and methyl jasmonate on the production of Nod factors (see Fig.) was confirmed for *B. japonicum* 532C and USDA3 strains [32]. The assumption that jasmonic acid, along with flavonoids, may be involved in the induction of rhizobia *nod* genes is also confirmed by the fact that its high content was observed in the root tips of soybean (*G. max* (L.) Merr.) seedlings of Williams variety [33]. It was also shown that jasmonic acid participates in the induction of the biosynthesis of flavonoids, since the treatment of *M. truncatula* seedlings with methyl jasmonate led to the induction of the *MtFNSII-2* gene encoding flavon synthase II [34].

However, in *M. truncatula* plants grown on a medium containing jasmonic acid the nodule number decreased. The effect was observed already at 0.1  $\mu$ M concentration, and 10  $\mu$ M concentration completely inhibited nodulation. However, the presence of 10  $\mu$ M jasmonic acid in the culture medium did not affect the development of *S. meliloti*, that is, jasmonic acid primarily prevents the infection of host plant with rhizobia (see Fig.) [35]. Jasmonic acid has been found to suppress the expression of the *ENOD11* and *RIP1* genes that are activated at the initial stages of symbiosis, as well as calcium oscillations caused by the Nod factor [35] (see Fig.). It was previously shown that ethylene also blocks calcium oscillations [36] (see Fig.). High concentrations of jasmonic acid (100  $\mu$ M) completely suppress calcium oscillations, while lower amounts change oscillation frequency [35]. In *sickle*, an ethylene-insensitive mutant, the frequency of calcium oscillations decreased at lower levels of jasmonic acid compared to the wild type, that is, ethylene inhibits the effect of jasmonic acid during the development of nodules and these hormones act as antagonists in the regulation of calcium oscillations induced by Nod factors [35]. Adding aminoethoxyvinylglycine, the ethylene inhibitor, to the wild type plants or using the *sickle* mutant significantly reduced negative impact on nodulation, indicating a synergistic effect of jasmonic acid and ethylene in regulation of nodulation (see Fig.).

Spraying *L. japonicus* shoots with  $10^{-4}$ - $10^{-3}$  M methyl jasmonate resulted in significant suppression of nodulation in wild type plants and the *har1-4* hypernodulating mutant. When plants were treated with lower concentrations of

methyl jasmonate ( $10^{-5}$ - $10^{-6}$  M), a stronger inhibition was observed for the *har1-4* mutant, which may be explained by the higher effect of low concentrations of methyl jasmonate on the number of nodules formed in the mutant lacking the ability perceive the signal of autoregulation [37]. Methyl jasmonate has a negative effect on the nodule number, blocking the root hair curling, the growth of infection threads and the formation of nodule primordia [37] (see Fig.).

Jasmonic acid activates expression of *vspA*, *vspB* and *Lox2* genes in the hyper-nodulating soybean (*G. max* (L.) Merr.) SS2-2 mutant carrying a mutation in *NTS/GmNARK* gene which encodes a serine-threonine receptor protein kinase similar to CLAVATA1 [38]. In contrast, expression of the *PR1* gene controlling the response to salicylic acid was declined. The mutant also showed a 2-fold increase in the content of jasmonic acid in the leaves compared to the wild type. Treatment of plants with methyl jasmonate resulted in a decrease in the nodule number; however, in *har1-4* mutant the nodule number decreased to a greater extent than in the wild type plants [37], and in the SS2-2 mutant—to a lesser extent [38]. In not inoculated mutant plants, there was a higher expression of jasmonic acid response genes in the leaves but not in the roots compared to the wild type plants. *NTS/GmNARK*, as a negative regulator of jasmonic acid synthesis in the leaves, probably participates in a defense mechanism dependent on jasmonic acid, along with participation in the autoregulation of the nodule number [38].

In nodules of *M. truncatula*, allen oxide cyclase, an enzyme involved in jasmonic acid biosynthesis, is localized in plastids of uninfected cells in the nitrogen fixation zone, as well as in nodule cortex cells [39]. The pattern of distribution of allen oxide cyclase did not differ in nodules formed by effective and ineffective strains. Measurement of jasmonic acid level did not reveal a significant difference between the roots and nodules. In transgenic roots of the composite *M. truncatula* plants in which the *MtAOC1* gene was turned off by RNA interference (RNAi), there were no changes in nodule development, that is why the authors concluded that jasmonic acid does not participate in the regulation of this process [39].

However, jasmonic acid has positive effect on the formation of nodules in *L. japonicus*. The phytochrome B mutant (*phyB*) had a reduced content of photosynthesis products, as well as jasmonoyl-isoleucine conjugate, the active jasmonic acid derivative [40]. This mutant formed a smaller number of nodules, and 0.1  $\mu$ M jasmonic acid increased their number, as compared to the wild type plants. In wild type plants grown at low the red/far red light ratio, the nodule number was also reduced, and jasmonic acid treatment increased this number. These data indicate that jasmonic acid is involved in the photomorphogenetic regulation of nodule formation through plant perception of the red/far red light ratio [40]. Incubation of 3-week-old soybean (*G. max* (L.) Merr.) nodules of the Don Mario variety for 5 days in solutions containing jasmonic or 12-oxophytodienic acid, a precursor in the biosynthesis of jasmonic acid, led to an increase in the number and size of cells in nodules [41].

Absciscic acid is an important plant hormone involved in the adaptation to various stresses such as drought, cooling, and salinity [42].

Treatment of pea roots with absciscic acid (see Fig.) inhibits nodulation and block cell divisions in the root cortex which are activated during the nodule formation [43]. The negative effect of absciscic acid has been shown for soybean (*G. max* (L.) Merr.) wild type plants and for hypernodulating mutant NOD1-3 [44, 45], as well as for the clover (*T. repens*) and *L. japonicus* plants [46]. Treatment of the *L. japonicus* plants with 1 and 10  $\mu$ M abamine, the inhibitor of 9-

cis-epoxy-carotenoid dioxygenase which is involved in the biosynthesis of abscisic acid, resulted in a lower level of endogenous abscisic acid and an increase in the nodule number [46]. It was shown that abscisic acid significantly reduces the number of curled root hairs and hairs with infection threads [46] (see Fig.). Later, the negative effect of abscisic acid on nitrogen fixation was revealed. In pea plants, daily treatment of 3-week-old nodules with 100  $\mu\text{M}$  abscisic acid for 9 days resulted in a significant decrease in nitrogen fixation [47]. The treatment of bean (*P. vulgaris* L.) seedlings with 1 and 10  $\mu\text{M}$  abscisic acid also reduced nitrogen fixation. When 100 mM NaCl was added to the plants at 1  $\mu\text{M}$  abscisic acid, nitrogen fixation was reduced to a lesser extent than without treatment [48]. Pre-treatment of alfalfa (*M. sativa* L.) plants with 10  $\mu\text{M}$  abscisic acid reduced nitrogen fixation. Similarly to bean plants, treatment with abscisic acid reduced the negative effect of 200 mM NaCl on the nitrogen fixing activity of nodules [49]. The authors associate this effect with the activation of the antioxidant defense system in nodules by abscisic acid (see Fig.).

Experiments on *M. truncatula* plants revealed the dose-dependent negative impact of abscisic acid on the number of nodules and infection threads [50]. The negative effect was confirmed when analyzing the expression of two marker genes that are activated in the early stages of symbiosis, *RIP1* and *ENOD11*. Abscisic acid, like jasmonic acid [35] and ethylene [36], affects calcium oscillations induced by Nod factors (see Fig.). The 1 mM abscisic acid completely blocked calcium oscillations, whereas lower concentrations altered their frequency and amplitude [50]. It was established that at high concentrations of Nod factors, the negative effect of abscisic acid can be leveled, that is, during nodule development, the quantitative balance between Nod factor and abscisic acid is important [50].

Importantly, abscisic acid and ethylene regulate the development of nodules independently. In *M. truncatula* roots, the overexpression of the *abi1-1* mutant allele of the *ABSCISIC ACID INSENSITIVE1* gene of *Arabidopsis thaliana* (L.) Heynh. encoding nonfunctional protein phosphatase 2C, results in blocking the abscisic acid signaling pathway and an increase in the nodule number [50]. Another *sta-1* mutant for the *SENSITIVITY TO ABA* gene (*STA*) induced directly in *M. truncatula* formed decreased nodule number. At the same time, the sensitivity of formation of nodule primordia to abscisic acid was increased in the mutant, while the root hair deformation and curling under the action of Nod factors became less susceptible to abscisic acid [50]. It has also been shown that abscisic acid reduces the expression of *ENOD40* and *NIN* genes that is activated in the inner layers of the root during the formation of nodule primordium under the influence of cytokinin [50]. In *L. japonicus snf2* plants carrying a mutation with loss of function in *LOTUS HISTIDINE KINASE 1* (*LHK1*) gene which encodes a cytokinin receptor, abscisic acid suppresses the formation of spontaneous nodules. The obtained data confirmed the assumption that the abscisic acid blocks the development of nodule primordia by affecting the cytokinin signaling cascade [43, 50]. It was previously shown that the abscisic acid/cytokinin ratio is lower in the supernodulating soybean mutant *nts382* compared to the wild type [51].

Screening of *L. japonicus* mutants insensitive to abscisic acid revealed *enf1* (enhanced nitrogen fixation 1) variant with a 1.7-fold number of nodules and higher nitrogen fixation [52]. This mutant also produced more infection threads. It was shown that the mutant phenotype is determined by a reduced content of abscisic acid. Treatment of wild type plants with abamine led to higher nitrogen fixation accompanying a decreased amount of abscisic acid. The *enf1* mutation did not affect expression of genes involved in nitrogen fixation, but

caused a decrease in the content of nitrogen oxide NO [52, 53], the inhibitor of nitrogen fixation [54].

Thus, the negative effect of salicylic acid on the development of symbiotic nodules was revealed both during exogenous treatment and in transgenic plants when endogenous level of salicylic acid altered. The negative effect was observed in plants with determinate nodules with limited activity of the meristem and in plants forming indeterminate ones with long activity of the meristem. Salicylic acid also leads to a decrease in nitrogen fixation. It should be noted that under salt stress, the nitrogen fixation decreased to a lesser extent in plants subjected to salicylic acid pre-treatment, which is probably due to the activation of the antioxidant defense system when exposed to salicylic acid. The positive effect of salicylic acid on nodule formation and nitrogen fixing activity has been recently described in chickpea plants grown both in the presence and in the absence of cadmium salts in the soil. It was shown that exogenous jasmonic acid serves as a negative regulator of nodulation, and also can play positive role in induction of rhizobia *nod*-genes. However, endogenous jasmonic acid does not participate in symbiotic nodule development. Negative regulation of nodule development has also been demonstrated for abscisic acid using both exogenous treatments and mutants with an altered level of endogenous abscisic acid. Pre-treatment with abscisic acid reduced the negative effect of salt stress, probably due to the activation of antioxidant defense.

Thus, in general, salicylic, jasmonic and abscisic acids, like ethylene, are negative regulators of the development and functioning of symbiotic nodules. Regulation occurs at different stages of nodule development. However, these phytohormones may be also involved in positive regulation, especially under stresses. Of great interest are further studies to describe the interaction between ethylene, salicylic, jasmonic and abscisic acids in regulation of legume-rhizobial symbiosis and to detect the peculiarities of the action of each phytohormone.

## REFERENCES

1. Tsyganova V.A., Tsyganov V.E. *Uspekhi sovremennoi biologii*, 2012, 132(2): 211-222 (in Russ.).
2. Tsyganova A.V., Kitaeva A.B., Brevin N.Dzh., Tsyganov V.E. [Cellular mechanisms of nodule development in legume plants. *Sel'skokhozyaistvennaya Biologiya [Agricultural Biology]*, 2011, 3: 34-40 (in Russ.).
3. Oldroyd G.E. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.*, 2013, 11(4): 252-263 (doi: 10.1038/nrmicro2990).
4. Hause B., Schaarschmidt S. The role of jasmonates in mutualistic symbioses between plants and soil-born microorganisms. *Phytochemistry*, 2009, 70(13): 1589-1599 (doi: 10.1016/j.phytochem.2009.07.003).
5. Desbrosses G.J., Stougaard J. Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. *Cell Host Microbe*, 2011, 10(4): 348-358 (doi: 10.1016/j.chom.2011.09.005).
6. Suzaki T., Ito M., Kawaguchi M. Genetic basis of cytokinin and auxin functions during root nodule development. *Front. Plant Sci.*, 2013, 4: 42 (doi: 10.3389/fpls.2013.00042).
7. Hayashi S., Gresshoff P.M., Ferguson B.J. Mechanistic action of gibberellins in legume nodulation. *J. Integr. Plant Biol.*, 2014, 56(10): 971-978 (doi: 10.1111/jipb.12201).
8. Guinel F.C. Ethylene, a hormone at the center-stage of nodulation. *Front. Plant Sci.*, 2015, 6: 1121 (doi: 10.3389/fpls.2015.01121).
9. Tsyganova A.V., Tsyganov V.E. Negative hormonal regulation of symbiotic nodule development. I. Ethylene (review). *Agricultural Biology*, 2015, 50(3): 267-277 (doi: 10.15389/agrobiology.2015.3.267eng).
10. Stec N., Banasiak J., Jasiński M. Abscisic acid-an overlooked player in plant-microbe symbioses formation? *Acta Biochim. Pol.*, 2016, 63(1): 53-58 (doi: 10.18388/abp.2015\_1210).
11. Rivas-San Vicente M., Plasencia J. Salicylic acid beyond defense: its role in plant growth and development. *J. Exp. Bot.*, 2011, 62(10): 3321-3338 (doi: 10.1093/jxb/err031).
12. Ryu H., Cho H., Choi D., Hwang I. Plant hormonal regulation of nitrogen-fixing nodule organogenesis. *Mol. Cells*, 2012, 34(2): 117-126 (doi: 10.1007/s10059-012-0131-1).
13. Nagata M., Suzuki A. Effects of phytohormones on nodulation and nitrogen fixation in legumi-



- nous plants. In: *Advances in biology and ecology of nitrogen fixation*. T. Ohyama (ed.). InTech, Rijeka, Croatia, 2014: 111-128 (doi: 10.5772/57267).
14. Ferguson B.J., Mathesius U. Phytohormone regulation of legume-rhizobia interactions *J. Chem. Ecol.*, 2014, 40(7): 770-790 (doi: 10.1007/s10886-014-0472-7).
  15. Vlot A.C., Dempsey D.M.A., Klessig D.F. Salicylic acid, a multifaceted hormone to combat disease. *Annu. Rev. Phytopathol.*, 2009, 47: 177-206 (doi: 10.1146/annurev.phyto.050908.135202).
  16. Ramanujam M.P., Jaleel V.A., Kumaravelu G. Effect of salicylic acid on nodulation, nitrogenous compounds and related enzymes of *Vigna mungo*. *Biologia Plantarum*, 1998, 41(2): 307-311 (doi: 10.1023/A:1001859824008).
  17. Martinez-Abarca F., Herrera-Cervera J.A., Bueno P., Sanjuan J., Bisseling T., Olivares J. Involvement of salicylic acid in the establishment of the *Rhizobium meliloti*-alfalfa symbiosis. *Mol. Plant Microbe In.*, 1998, 11(2): 153-155 (doi: 10.1094/MPMI.1998.11.2.153).
  18. Blilou I., Ocampo J.A., García-Garrido J.M. Resistance of pea roots to endomycorrhizal fungus or *Rhizobium* correlates with enhanced levels of endogenous salicylic acid. *J. Exp. Bot.*, 1999, 50(340): 1663-1668 (doi: 10.1093/jxb/50.340.1663).
  19. Lian B., Zhou X., Miransari M., Smith D.L. Effects of salicylic acid on the development and root nodulation of soybean seedlings. *J. Agron. Crop Sci.*, 2000, 185(3): 187-192 (doi: 10.1046/j.1439-037x.2000.00419.x).
  20. Sato T., Fujikake H., Ohtake N., Sueyoshi K., Takahashi T., Sato A., Ohyama T. Effect of exogenous salicylic acid supply on nodule formation of hypernodulating mutant and wild type of soybean. *Soil Sci. Plant Nutr.*, 2002, 48(3): 413-420 (doi: 10.1080/00380768.2002.10409219).
  21. van Spronsen P.C., Tak T., Rood A.M., van Brussel A.A., Kijne J.W., Boot K.J. Salicylic acid inhibits indeterminate-type nodulation but not determinate-type nodulation. *Mol. Plant Microbe In.*, 2003, 16(1): 83-91 (doi: 10.1094/MPMI.2003.16.1.83).
  22. Stacey G., McAlvin C.B., Kim S.Y., Olivares J., Soto M.J. Effects of endogenous salicylic acid on nodulation in the model legumes *Lotus japonicus* and *Medicago truncatula*. *Plant Physiol.*, 2006, 141(4): 1473-1481 (doi: 10.1104/pp.106.080986).
  23. Riely B.K., Loughon G., Ané J.-M., Cook D.R. The symbiotic ion channel homolog DMI1 is localized in the nuclear membrane of *Medicago truncatula* roots. *Plant J.*, 2007, 49(2): 208-216 (doi: 10.1111/j.1365-313X.2006.02957.x).
  24. Bonfante P., Genre A., Faccio A., Martini I., Schauser L., Stougaard J., Webb J., Parniske M. The *Lotus japonicus* *LjSym4* gene is required for the successful symbiotic infection of root epidermal cells. *Mol. Plant Microbe In.*, 2000 13(10): 1109-1120 (doi: 10.1094/MPMI.2000.13.10.1109).
  25. Bastianelli F., Costa A., Vescovi M., D'Apuzzo E., Zottini M., Chiurazzi M., Schiavo F.L. Salicylic acid differentially affects suspension cell cultures of *Lotus japonicus* and one of its non-symbiotic mutants. *Plant Mol. Biol.*, 2010, 72(4-5): 469-483 (doi: 10.1007/s11103-009-9585-8).
  26. Levine A., Tenhaken R., Dixon R., Lamb C. H<sub>2</sub>O<sub>2</sub> from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, 1994, 94(4): 491-501 (doi: 10.1016/0092-8674(94)90544-4).
  27. Palma F., Lypez-Gymez M., Tejera N.A., Lluch, C. Salicylic acid improves the salinity tolerance of *Medicago sativa* in symbiosis with *Sinorhizobium meliloti* by preventing nitrogen fixation inhibition. *Plant Sci.*, 2013, 208: 75-82 (doi: 10.1016/j.plantsci.2013.03.015).
  28. Hayat S., Hayat Q., Alyemeni M.N., Ahmad A. Salicylic acid enhances the efficiency of nitrogen fixation and assimilation in *Cicer arietinum* plants grown under cadmium stress. *J. Plant Interact.*, 2014, 9(1): 35-42 (doi: 10.1080/17429145.2012.751635).
  29. Tett A.J., Karunakaran R., Poole P.S. Characterisation of SalRAB a salicylic acid inducible regulatively regulated efflux system of *Rhizobium leguminosarum* by *viciae* 3841. *PLoS ONE*, 2014, 9(8): e103647 (doi: 10.1371/journal.pone.0103647).
  30. Wasternack C., Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany*, 2013, 111(6): 1021-1058 (doi: 10.1093/aob/mct067).
  31. Rosas S., Soria R., Correa N., Abdala G. Jasmonic acid stimulates the expression of nod genes in *Rhizobium*. *Plant Mol. Biol.*, 1998, 38(6): 1161-1168 (doi: 10.1023/A:1006064807870).
  32. Mabood F., Souleimanov A., Khan W., Smith D.L. Jasmonates induce Nod factor production by *Bradyrhizobium japonicum*. *Plant Physiol. Bioch.*, 2006, 44(11): 759-765 (doi: 10.1016/j.plaphy.2006.10.025).
  33. Creelman R.A., Mullet J.E. Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *PNAS USA*, 1995, 92(10): 4114-4119 (doi: 10.1073/pnas.92.10.4114).
  34. Zhang J., Subramanian S., Zhang Y., Yu O. Flavone synthases from *Medicago truncatula* are flavanone-2-hydroxylases and are important for nodulation. *Plant Physiol.*, 2007, 144(2): 741-751 (doi: 10.1104/pp.106.095018).
  35. Sun J., Cardoza V., Mitchell D.M., Bright L., Oldroyd G., Harris J.M. Crosstalk between jasmonic acid, ethylene and Nod factor signaling allows integration of diverse inputs for regulation of nodulation. *Plant J.*, 2006, 46(6): 961-970 (doi: 10.1111/j.1365-313X.2006.02751.x).
  36. Oldroyd G.E.D., Engstrom E.M., Long S.R. Ethylene inhibits the Nod factor signal transduc-

- tion pathway of *Medicago truncatula*. *Plant Cell*, 2001, 13(8): 1835-1849 (doi: 10.2307/3871322).
37. Nakagawa T., Kawaguchi M. Shoot-applied MeJA suppresses root nodulation in *Lotus japonicus*. *Plant Cell Physiol.*, 2006, 47(1): 176-180 (doi: 10.1093/pcp/pci222).
  38. Seo H.S., Li J., Lee S.-Y., Yu J.-W., Kim K.-H., Lee S.-H., Lee I.-J., Paek N.-C. The hyper-nodulating *nts* mutation induces jasmonate synthetic pathway in soybean leaves. *Mol. Cells*, 2007, 24(2): 185.
  39. Zdyb A., Demchenko K., Heumann J., Mrosk C., Grzeganeck P., Göbel C., Feussner I., Pawlowski K., Hause B. Jasmonate biosynthesis in legume and actinorhizal nodules. *New Phytol.*, 2011, 189(2): 568-579 (doi: 10.1111/j.1469-8137.2010.03504.x).
  40. Suzuki A., Suriyagoda L., Shigeyama T., Tominaga A., Sasaki M., Hiratsuka Y., Yoshinaga A., Arima S., Agarie S., Sakai T., Inada S., Jikumaru Y., Kamiya Y., Uchiumi T., Abe M., Hashiguchi M., Akashi R., Sato S., Kaneko T., Tabata S., Hirsch A.M. *Lotus japonicus* nodulation is photomorphogenetically controlled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. *PNAS USA*, 2011, 108(40): 16837-16842 (doi: 10.1073/pnas.1105892108).
  41. Costanzo M.E., Andrade A., del Carmen Tordable M., Cassán F., Abdala G. Production and function of jasmonates in nodulated roots of soybean plants inoculated with *Bradyrhizobium japonicum*. *Arch. Microbiol.*, 2012, 194(10): 837-845 (doi: 10.1007/s00203-012-0817-y).
  42. Umezawa T., Nakashima K., Miyakawa T., Kuromori T., Tanokura M., Shinozaki K., Yamaguchi-Shinozaki K. Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. *Plant Cell Physiol.*, 2010, 51(11): 1821-1839 (doi: 10.1093/pcp/pcq156).
  43. Phillips D.A. Abscisic acid inhibition of root nodule initiation in *Pisum sativum*. *Planta*, 1971, 100(3): 181-190 (doi: 10.1007/BF00387034).
  44. Cho M.J., Harper J.E. Effect of abscisic acid application on root isoflavonoid concentration and nodulation of wild-type and nodulation-mutant soybean plants. *Plant Soil*, 1993, 153(1): 145-149 (doi: 10.1007/BF00010552).
  45. Bano A., Harper J.E., Augé R.M., Neuman D.S. Changes in phytohormone levels following inoculation of two soybean lines differing in nodulation. *Funct. Plant Biol.*, 2002, 29(8): 965-974 (doi: 10.1071/PP01166).
  46. Suzuki A., Akune M., Kogiso M., Imagama Y., Osuki K., Uchiumi T., Higashi S., Han S.Y., Yoshida S., Asami T., Abe M. Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. *Plant Cell Physiol.*, 2004, 45(7): 914-922 (doi: 10.1093/pcp/pch107).
  47. González E.M., Gálvez L., Arrese-Igor C. Abscisic acid induces a decline in nitrogen fixation that involves leghaemoglobin, but is independent of sucrose synthase activity. *J. Exp. Bot.*, 2001, 52(355): 285-293 (doi: 10.1093/jexbot/52.355.285).
  48. Khadri M., Tejera N.A., Lluch C. Alleviation of salt stress in common bean (*Phaseolus vulgaris*) by exogenous abscisic acid supply. *J. Plant Growth Regul.*, 2006, 25(2): 110-119 (doi: 10.1007/s00344-005-0004-3).
  49. Palma F., Lypez-Gymez M., Tejera N.A., Lluch C. Involvement of abscisic acid in the response of *Medicago sativa* plants in symbiosis with *Sinorhizobium meliloti* to salinity. *Plant Sci.*, 2014, 223: 16-24 (doi: 10.1016/j.plantsci.2014.02.005).
  50. Ding Y., Kalo P., Yendrek C., Sun J., Liang Y., Marsh J.F., Harris J.M., Oldroyd G.E. Abscisic acid coordinates nod factor and cytokinin signaling during the regulation of nodulation in *Medicago truncatula*. *Plant Cell*, 2008, 20(10): 2681-2695 (doi: 10.1105/tpc.108.061739).
  51. Caba J.M., Centeno M.L., Fernández B., Gresshoff P.M., Ligerio F. Inoculation and nitrate alter phytohormone levels in soybean roots: differences between a supernodulating mutant and the wild type. *Planta*, 2000, 211(1): 98-104 (doi: 10.1007/s004250000265).
  52. Tominaga A., Nagata M., Futsuki K., Abe H., Uchiumi T., Abe M., Kucho K., Hashiguchi M., Akashi R., Hirsch A.M., Arima S., Suzuki A. Enhanced nodulation and nitrogen fixation in the abscisic acid low-sensitive mutant *enhanced nitrogen fixation1* of *Lotus japonicus*. *Plant Physiol.*, 2009, 151(4): 1965-1976 (doi: 10.1104/pp.109.142638).
  53. Tominaga A., Nagata M., Futsuki K., Abe H., Uchiumi T., Abe M., Kucho K., Hashiguchi M., Akashi R., Hirsch A., Arima S., Suzuki A. Effect of abscisic acid on symbiotic nitrogen fixation activity in the root nodules of *Lotus japonicus*. *Plant Signaling & Behavior*, 2010, 5(4): 440-443 (doi: 10.4161/psb.5.4.10849).
  54. Shimoda Y., Shimoda-Sasakura F., Kucho K., Kanamori N., Nagata M., Suzuki A., Abe M., Higashi S., Uchiumi T. Overexpression of class 1 plant hemoglobin genes enhances symbiotic nitrogen fixation activity between *Mesorhizobium loti* and *Lotus japonicus*. *Plant J.*, 2009, 57(2): 254-263 (doi: 10.1111/j.1365-313X.2008.03689.x).