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ORIGINAL PAPER

Production and function of jasmonates in nodulated roots of soybean plants inoculated with *Bradyrhizobium japonicum*

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Abstract Little is known regarding production and function of endogenous jasmonates (JAs) in root nodules of soybean plants inoculated with *Bradyrhizobium japonicum*. We investigated (1) production of jasmonic acid (JA) and 12-oxophytodienoic acid (OPDA) in roots of control and inoculated plants and in isolated nodules; (2) correlations between JAs levels, nodule number, and plant growth during the symbiotic process; and (3) effects of exogenous JA and OPDA on nodule cell number and size. In roots of control plants, JA and OPDA levels reached a maximum at day 18 after inoculation; OPDA level was 1.24 times that of JA. In roots of inoculated plants, OPDA peaked at day 15, whereas JA level did not change appreciably. Shoot dry matter of inoculated plants was higher than that of control at day 21. Chlorophyll *a* decreased more abruptly in control plants than in inoculated plants, whereas *b* decreased gradually in both cases. Exogenous JA or OPDA changed number and size of nodule central cells and peripheral cells. Findings from this and previous studies suggest that increased levels of JA and OPDA in control plants are related to senescence induced by nutritional stress. OPDA accumulation in nodulated roots suggests its involvement in "autoregulation of nodulation."

Keywords *Bradyrhizobium* · Jasmonates · Soybean

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Abbreviations

AOC	Allene oxide cyclase
AON	Autoregulation of nodulation
AOS	Allene oxide synthase
DM	Dry matter
JA	Jasmonic acid
JAs	Jasmonates
LOX	Lipoxygenase
MeJA	Methyl-jasmonate
OPDA	12-Oxophytodienoic acid

Introduction

The Gram-negative soil bacterium *Bradyrhizobium japonicum* induces root nodulation and nitrogen (N_2) fixation in soybean [*Glycine max* (L.) Merrill], its principal leguminous host. Formation of an N_2-fixing root nodule is a complex developmental event that depends on a specific chemical "dialogue" between a prokaryote (microsymbiont) and a eukaryote (macrosymbiont). Various phytohormones (auxins, cytokinins, gibberellins) have long been known as "dialogue molecules" or "key signals" for nodule development. The roles of molecules other than these "traditional phytohormones" in legume nodule development have received increasing attention during the last two decades. In particular, the group of lipid hormones known as jasmonates (JAs) has been found to be involved in several aspects of soybean–bacteria interaction and proposed as a new class of naturally occurring inducers of Nod factor production, one of the first steps in the interaction between root cells and rhizobial bacteria (Rosas et al. 1998; Hause and Schaarschmidt 2009; Ferguson et al. 2010).

JAs belong to a family of oxygenated fatty acid derivatives, collectively termed oxylipins, that are produced via

oxidative metabolism of polyunsaturated fatty acids. Synthesis and signal transduction pathways of JAs were recently reviewed (Wasternack and Kombrink 2010). The initial substrates are α-linolenic acid (α-LeA; C18:3) or hexadecatrienoic acid (C16:3) released from plastidial galactolipids by phospholipases. Following oxidation of α-LeA by lipoxygenase (LOX) to 13(S)-hydroperoxyoctadecatrienoic acid (13(S)-HPOT), the first committed step of jasmonic acid (JA) biosynthesis is conversion of the LOX product to the allene oxide 12,13(S)-epoxyoctadecatrienoic acid (12,13(S)-EOT) by allene oxide synthase (AOS). This unstable compound is enzymatically cyclized by allene oxide cyclase (AOC) to *cis*-(+)-12-oxophytodienoic acid ((9S,13S)-OPDA), which is the end-product of the plastid-localized part of the JA biosynthesis pathway, and has the same stereochemical configuration as naturally occurring (+)-7-*iso*-JA. Translocation of 12-oxophytodienoic acid (OPDA) into peroxisomes, where the subsequent part of the JA biosynthesis pathway occurs, is mediated by the ABC transporter COMATOSE and/or an ion-trapping mechanism (Theodoulou et al. 2005). The final step is reduction of the cyclopentenone ring, catalyzed by a peroxisomal OPDA reductase (OPR), to yield JA.

After JA is formed, methylation by a JA-specific methyl transferase produces methyl-jasmonate (MeJA) (Seo et al. 2001).

JAs affect nodulation in legume–bacteria interactions in several ways. Rosas et al. (1998) found that exogenous application of JA caused induction of *nod* gene in *Rhizobium leguminosarum*. Mabood and Smith (2005) showed that JA and methyl-jasmonate (MeJA) induced expression of nodulation genes in *B. japonicum* and that pre-incubation with JAs enhanced root nodulation, N_2 fixation, and plant growth in soybean under controlled environmental conditions. Mabood et al. (2006a) found subsequently that inoculation of soybean plants with *B. japonicum* cells incubated with MeJA alone, or in combination with genistein (GE), caused increases in nodule number, nodule dry matter (DM) per plant, and seasonal N_2 fixation, in comparison with control cells. JAs also induced production and secretion of lipo-chitooligosaccharides (LCOs), a Nod factor. JA and MeJA were more effective inducers of LCO production than GE, and JA or MeJA plus GE was more effective than JA or MeJA alone (Mabood et al. 2006b). Pre-incubation of *B. japonicum* with MeJA increased plant growth, DM accumulation, and grain yield of soybean under short-season field conditions (Mabood et al. 2006c). Findings by other groups suggest that JA regulates Nod factor-induced signaling and nodulation in both synergistic and antagonistic manners with others regulators, *for example*, ethylene (Sun et al. 2006; Zhao and Qi 2008). Aside from these studies of early Nod factor responses, however, little is known about the role of JAs in nodule development and function in legumes.

The present study was designed to: (1) measure and compare production of JA, and its precursor OPDA, in control versus nodulated roots from soybean plants inoculated with *B. japonicum* and in isolated nodules; (2) establish correlations between JAs production, nodule number, and plant growth during legume–bacteria symbiosis; and (3) quantify the effects of exogenously applied JA and OPDA on nodule cell number and size.

Materials and methods

Plant material

Soybean seeds [*G. max* (L.) Merr.] cv. Don Mario, with high purity and 95 % germination capacity according to the International Seed Test Association (ISTA), were inoculated with agronomic dose (3 ml kg^{-1}) of *B. japonicum* E109 culture, in early stationary growth phase containing a titer of 5×10^9 cfu ml^{-1}. *B. japonicum* E109 (formerly USDA138 of the NRS collection, USA) provided by Ing. Agr. Alejandro Perticari, Instituto de Microbiología y Zoología Agrícola, Instituto Nacional de Tecnología Agropecuaria, Castelar, Argentina (Perticari et al. 1996), is one of the strains most commonly used for soybean inoculation in Argentina. Bacterial cultures were grown at 28 ± 1 °C in 250-ml flasks containing 100 ml yeast extract mannitol (YEM) medium (mannitol 10 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NaCl 0.1 g, yeast extract 0.4 g, distilled water 1,000 ml, pH 6.8), on a Orbital Shaker OS-10 (CK-Tech-UE) (150 rpm), in the dark.

Plant growth conditions

Inoculated and non-inoculated soybean seeds were grown in plastic pots (10 liter volume) containing washed and sterile sand as inert support. Substrate field capacity was pre-adjusted to 60 % with nitrogen-deficient sterile 25 % (v/v) Hoagland's solution and maintained until the end of the experiment. Seedlings were grown for 21 days in a growth chamber with photoperiod 16-h light (30 °C)/8-h dark (20 °C), at 80 % RH, to obtain the maximal and constant number of nodules per plant according to standard Burton's test (Burton et al. 1972) for inoculated legume seeds. Root samples from control and inoculated plants were harvested at 6, 9, 12, 15, 18, and 21 days after *B. japonicum* inoculation. Nodules were excised from plants at day 9 due to macroscopic occurrence of nodule and day 15 due to maximum nodule number on roots. The experiments were performed in triplicate.

Extraction, purification, and estimation of JA and OPDA

JA and OPDA were extracted and pre-purified as described by Andrade et al. (2005). Control roots, nodulated roots, and isolated nodules (200 mg DM) were homogenized with 10 ml methanol, 50 ng [$^2H_6$]jasmonic acid [($^2H_6$] JA], and 100 ng [$^2H_5$]12-oxo-phytodienoic acid [($^2H_5$)OPDA] as internal standards. The homogenate was filtered under vacuum on a column with cellulose filter. The extract was dried, dissolved with 10 ml methanol, and loaded on a column filled with 3 ml DEAE-Sephadex A25 (Amersham Pharmacia Biotech AB, Sweden) (Ac^--form, methanol). The column was washed with 3 ml methanol and then with 3 ml 0.1 N acetic acid in methanol. Eluents with 3 ml of 1 N acetic acid in methanol, and 3 ml of 1.5 N acetic acid in methanol, were collected, evaporated, and analyzed by liquid chromatography-electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) as described below. Measurements of JAs in control and nodulated roots were taken in triplicate. For the measurement of JAs in isolated nodules, a combined sample of 30 nodules was obtained randomly from 3 plants.

Liquid chromatography-electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS)

Mass spectrometric analysis was performed on a quadruple tandem mass spectrometer (MS–MS, Quattro Ultima, Micromass, Manchester, UK) fitted with an electrospray ion source (ESI). A mixture of all unlabeled compounds and internal standards was separated by reversed-phase high-performance liquid chromatography (HPLC) and analyzed by tandem mass spectrometry with multiple reaction monitoring (MRM) to determine retention times for all compounds. The spectrometer software used was MassLynx™ v. 4.1 (Micromass, Manchester, UK). Response was calculated as product ion peak area x (IS concentration/IS product ion peak area), where IS concentration is the amount of internal standard added. JA and OPDA were separated from tissues by HPLC. An Alliance 2695 separation module (Waters, Milford, MA, USA) equipped with a 100 × 2.1 mm, 3-µm RESTEK C_{18} column was used to maintain performance of the analytical column. Fractions were separated using a gradient of increasing methanol concentration, constant glacial acetic acid concentration 0.2 % in water, and initial flow rate 0.2 ml min^{-1}. The gradient was increased linearly from 40 % methanol/60 % water–acetic acid at 25 min to 80 % methanol/20 % water–acetic acid. After 1 min, initial conditions were restored, and the system was allowed to equilibrate for 7 min. MRM mode was used for the determination of JA and OPDA. These compounds were monitored at *m*/*z* transitions of 210/59 and 292/165, with retention times of 14.20 and 18.70 min, respectively. Collision energies used were 20 electron volts (eV) for JA and 30 eV for OPDA, and cone voltage was 35 V.

Growth parameters and pigment analysis

Four control and 4 inoculated plants were separated into roots and shoots, and fresh matter (FM) mass and length were recorded. Samples were dried in an oven at 60 °C until constant dry matter (DM) mass was obtained. FM and DM were expressed as g $plant^{-1}$. Chlorophyll *a* and *b* were extracted from 100 mg FM of unifoliate leaves and estimated as described by Porra (2002). For this, leaves were homogenized in a mortar with 10 ml acetone 80 %, centrifuged 5 min at 5,000 rpm, and filtered. Pigment levels were measured using a spectrophotometer, with wavelengths of 646.6 and 663.6 nm corresponding to chlorophyll *a* and *b*, respectively. Acetone–water (80:20) was used as a blank control. Results were quantified as described by MacKinney (1941) and Vernon (1960). Experiments were performed in triplicate.

Exogenous application of JA and OPDA

Nodules were chosen at the similar developmental stage. Nodules size was measured with a caliber (2 mm), excised from nodulated roots at day 21 after inoculation, washed in distilled water, and placed in 25-ml Erlenmeyer flasks containing JA [(±)-1α-2β-3-oxo-2-[cis-2-pentenyl] cyclopentaneacetic acid], 99 % purity, or OPDA [12-oxo-phytodienoic acid], 95 % purity, kindly provided by Dr. O. Miersch, Institute of Plant Biochemistry, Halle, Germany. Based on previous reports on exogenous application of MeJA (Yoon et al. 2009) or OPDA (Fliegmann et al. 2010) to soybean seedlings or cell suspension cultures, we used concentrations of 10^{-4}, 10^{-6}, and 10^{-8} M for JA, and 10^{-6} and 10^{-8} M for OPDA, applied in water solutions. Water alone was used as a control. Flasks containing two nodules were incubated for 5 days at 25 °C, in the dark, and the material was then processed for histological studies. Experiments were performed in duplicate; results presented are means of four subsamples of four nodules.

Nodule histological studies

Nodules were fixed in FAA (ethanol–water–formaldehyde–acetic acid, 50:35:10:5), dehydrate in a graded series of ethanol and xylol, starting with alcohol 70 %, and embedded in Histowax™. Longitudinal and transverse serial sections of the whole nodule, ranging from 8 to 10 µm in thickness, were cut with a rotary microtome. Sections were stained with hematoxylin-safranin-Fast Green and mounted in Depex™ (Johansen 1940; O'Brien and McCully 1981).

Photomicrographs were taken with an Axiophot Carl Zeiss microscope equipped with AxioCam HRC camera, computer image capture, and digitization by AxioVision 4.3 program. Size and number of cells in the infected area were measured. Longitudinal and transverse sections with diameter ~2 mm were obtained from each nodule. For the determination of cell number, 10 sections from each nodule were analyzed. In the central region of the nodule, two zones were defined: Zone I, central area; Zone II, peripheral area adjacent to cortex. Areas studied were ~62,000 μm^2. Results were expressed as means of cell number recorded for Zone I versus Zone II and for various treatments. For the determination of cell size, four sections of the central region were observed for each nodule. In each section, twenty infected cells were selected at random and the long axis was measured.

Statistical analysis

Shoot DM was analyzed by nonparametric Kruskal–Wallis test, with a posteriori Dunn test; $p < 0.05$ considered significant. Results from chlorophyll quantification, and exogenous application of OPDA and JA to nodules, were subjected to ANOVA analysis, with a posteriori Tukey's test.

Results

Endogenous jasmonates

OPDA and JA were detected in roots of control plants, in roots nodulated by *B. japonicum*, and in isolated nodules. Levels of both compounds differed between control versus nodulated roots.

In control roots, an abrupt peak of OPDA was observed at day 18. In nodulated roots, OPDA level reached a maximum at day 15. OPDA peaked earlier in nodulated roots (day 15) than in control roots (day 18, Fig. 1a).

JA in control roots also peaked at day 18. Level of endogenous JA was low and did not show significant variation in nodulated roots during the experiment (Fig. 1b).

In nodulated roots, the magnitude of OPDA increase (Fig. 1a) was greater than that of JA, for example, 5.5 times at day 15 (Fig. 1b), whereas in control roots, OPDA level was 1.24 times that of JA at day 18.

Nodule formation was evident in taproot at day 9 and in lateral root at day 12. Total nodule number showed a steady increase until day 15, when it reached its maximal value of 13 nodules plant^{-1} (Fig. 2). The maximal nodule number, occurring when the nodulation system was well established,

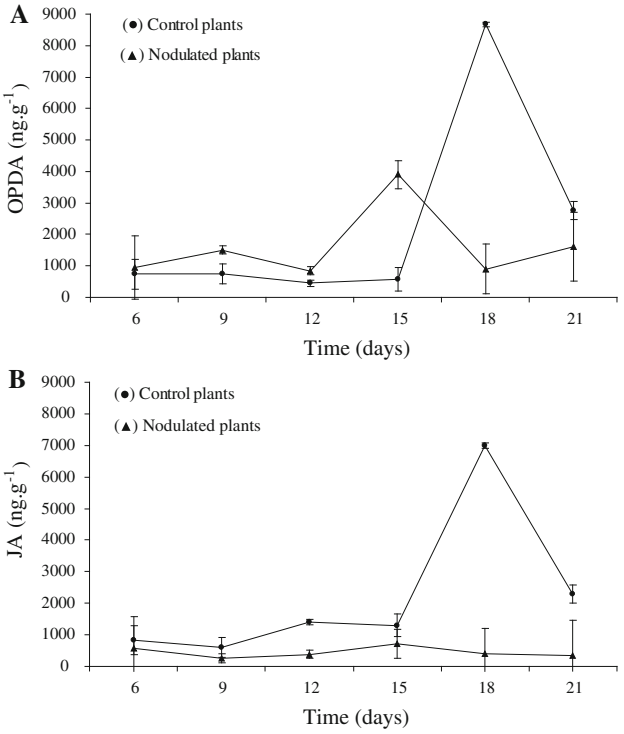

Fig. 1 Levels of 12-oxophytodienoic acid (OPDA) and jasmonic acid (JA) in roots of control soybean plants and plants inoculated with *B. japonicum* strain E109, at various times (days) after inoculation. **a** OPDA; **b** JA. Values shown are mean ± SD ($n = 3$)

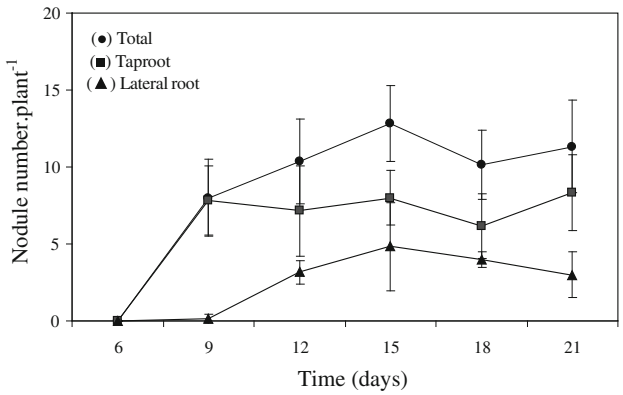

Fig. 2 Mean numbers of nodules in taproot, lateral root, and total nodules in inoculated plants at various times (days) after inoculation. Mean ± SD ($n = 3$)

coincided with the OPDA peak recorded in nodulated roots at day 15 (Fig. 1a).

In isolated nodules, OPDA level was very high (31.484 ng g^{-1}) at day 9, when nodule formation was evident in taproot, and much lower (2.998 ng g^{-1}) at day 15, when total nodule number was maximal. In contrast, JA level was high at day 9 (14.443 ng g^{-1}) and relatively constant until day 15 (12.123 ng g^{-1}).

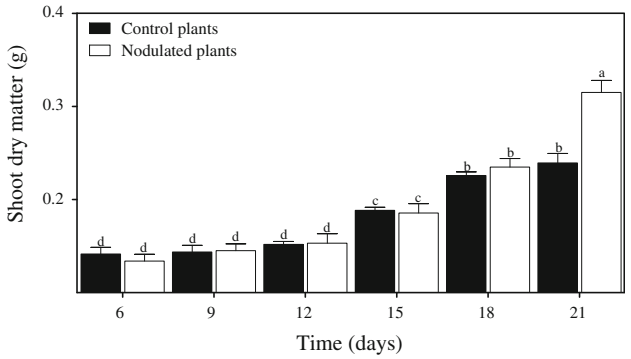

Fig. 3 Shoot dry matter (g) of control plants (*black bars*) and nodulated plants (*white bars*) at various times (days) after inoculation. Mean ± SD ($n = 3$). Values indicated by different letters are significantly different at ($p < 0.05$)

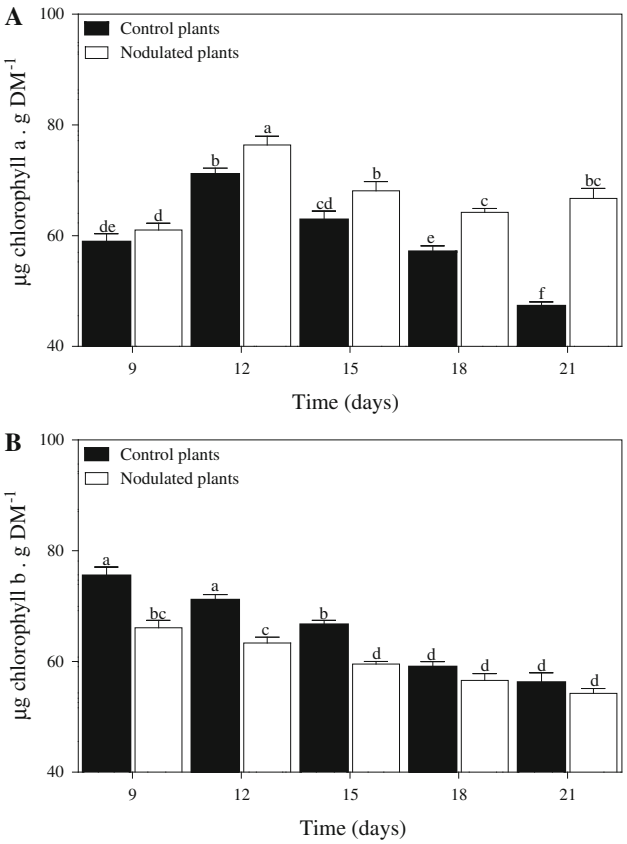

Fig. 4 Levels of chlorophylls in control plants and nodulated plants at various times (days) after inoculation. **a** chlorophyll *a*; **b** chlorophyll *b*. Mean ± SD ($n = 3$). Values indicated by different letters are significantly different at $p < 0.001$

Growth of shoots, as DM, increased throughout the experiment for both control and nodulated plants. Shoot DM value at day 21 was significantly higher for nodulated than for control plants (Fig. 3). Shoot length, root length, and root DM were not affected by *B. japonicum* inoculation (data not shown).

Chlorophyll *a* level in control plants increased from day 9 to 12 and then decreased steadily through day 21. In nodulated plants, chlorophyll *a* level was higher (1.4 times) than in control plants at day 21 (Fig. 4a). Chlorophyll *b* level decreased steadily throughout the experiment in both control and nodulated plants (Fig. 4b).

Nitrogen (N) status was assessed based on biomass production (shoot DM) and chlorophyll concentration in shoots and leaves, two plant life cycle parameters that are highly sensitive to presence/absence of N. Chlorophylls in particular are considered direct "N status markers," whose levels reflect early N deficiency. For example, Evans (1989) reported that total chlorophyll content is closely related to leaf N in C_3 species and that photosynthetic machinery accounts for >50 % of leaf N content.

Effect of exogenous jasmonates (JAs)

In view of the effect of JAs on cell expansion in various plant tissues, and the high levels of JAs observed inside nodules, we studied the effect of exogenously applied OPDA and JA on isolated nodules. We used 10^{-4}, 10^{-6}, and 10^{-8} M for JA and 10^{-6} and 10^{-8} M for OPDA, based on the reports of Yoon et al. (2009) and Fliegmann et al. (2010) about JAs exogenous application to soybean seedlings or cell suspension cultures. Both number and size of central and peripheral cells were altered by these treatments. JA at 10^{-4} M concentration increased central and peripheral cell number dramatically (Fig. 5a-b) and cell size to a lesser extent (Fig. 5c). JA at lower concentrations (10^{-6} and 10^{-8} M) affected number of peripheral cells and cell size (Fig. 5b-c), but not number of central cells (Fig. 5a). OPDA at 10^{-6} M caused significant increase in number of peripheral cells (Fig. 5b), but did not affect cell size, or number of central cells (Fig. 5a-c). OPDA at lower concentration (10^{-8} M) enhanced all three parameters (Fig. 5a-c); central cell number increased 128 %, and cell size increased 110 % relative to control.

Effects of 10^{-6} M JA and 10^{-6} M OPDA on the above parameters were further studied by photomicrography. General sections from cortical and central regions of nodules are shown in Fig. 6a, c, e. Central region Zones I and II were used for analysis. Detailed views of central regions are shown in Fig. 6b, d, f. Infected cells, containing symbiosomes, display dense cytoplasm and disappearance of the central vacuole. Uninfected cells retain a typical vacuole throughout nodule development.

Discussion

Many plant species have developed mutualistic interactions with nitrogen-fixing bacteria and/or arbuscular mycorrhizal

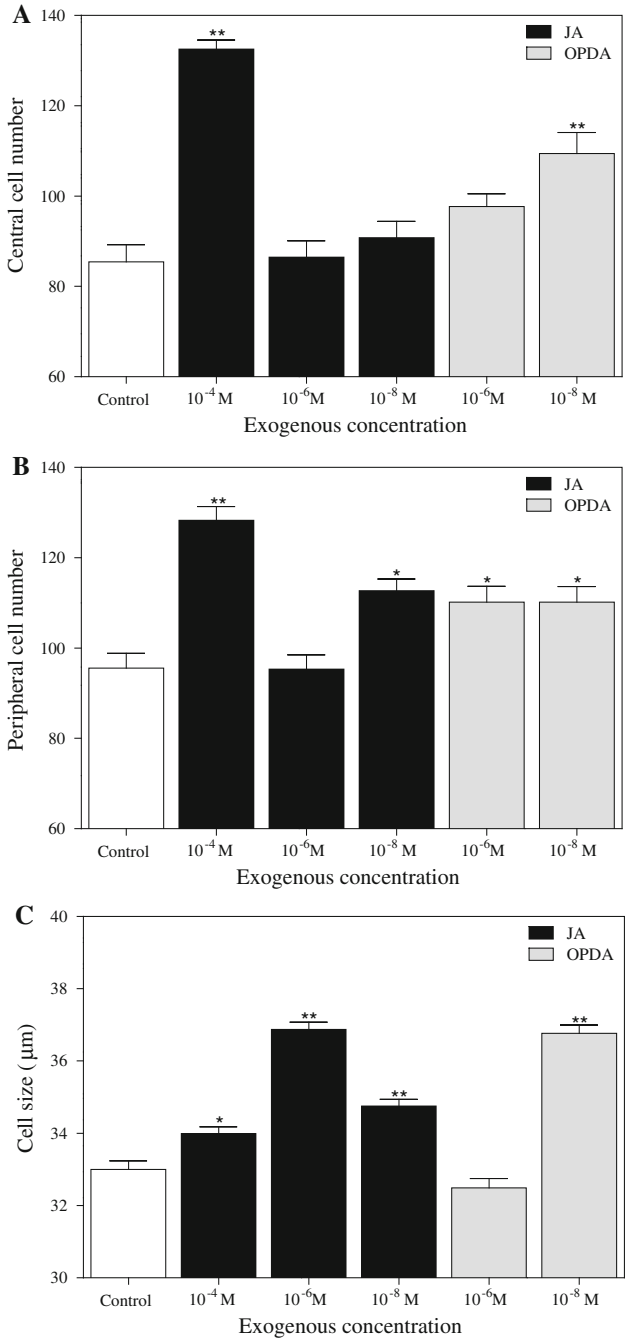

Fig. 5 Effect of exogenously applied JA and OPDA on number and size of cells of nodules. **a** number of central cells (mean ± SD, $n = 20$). **b** number of peripheral cells (mean ± SD, $n = 20$). **c** cell size (mean ± SD, $n = 80$). * $p < 0.05$, ** $p < 0.001$

fungi. These relationships are based on mutual recognition and a high degree of coordination that depends on activity of a number of signaling molecules, including JAs. In the *Bradyrhizobium*–soybean symbiosis, JAs induce transcription of nodulation genes (Mabood and Smith 2005).

Studies so far are limited to effects of exogenous application of JAs, particularly JA and MeJA, on early events of nodulation, N_2 fixation, and N partitioning (Rosas et al. 1998; Rossato et al. 2002; Mabood and Smith 2005; Mabood et al. 2006a, b). In cultured soybean cells (Fliegman et al. 2010), JA, OPDA, and coronalon (a synthetic jasmonate analogue) all induced accumulation of 7,4′-dihydroxyflavone, a compound involved in plant–microbe interactions (Martens and Mithöfer 2005). There are limited data regarding endogenous levels of JAs in nodules and nodulated roots, and the role of JAs in nodule formation and function during *Bradyrhizobium*–soybean interaction.

The senescence visually observed in control plants by day 18 may be associated with accumulation of OPDA and JA, and with N starvation caused by absence of *Bradyrhizobium* and loss of chlorophyll, particularly chlorophyll *a*. Low N availability may trigger production of JAs in roots and consequent senescence process in leaves. JA biosynthesis is up-regulated at the transcriptional level following nutrient or mineral deprivation (Pawuels et al. 2009). Transcription of genes for JA biosynthetic enzymes (*e.g.*, LOX2, AOS, AOC) is enhanced by K^+ starvation (Armengaud et al. 2004) and by sulfur starvation (Hirai et al. 2003; Nikiforova et al. 2003), indicating a role of JA in plant mineral nutrition.

A role of JAs in initiation and progression of leaf senescence, although still controversial, has been suggested for many plant species (Sembdner and Parthier 1993; He et al. 2001; Seltmann et al. 2010). In monocarps, such as soybean, senescence is a highly regulated process, involving decreased photosynthesis, loss of chlorophyll, and breakdown of CO_2-fixing enzymes such as ribulose bisphosphate carboxylase oxygenase (Rubisco).

In the present study, high levels of JAs in control plants were correlated with reduced chlorophyll levels and N starvation, indicating a connection between JAs and senescence. Seltmann et al. (2010) demonstrated a similar decline in total chlorophyll in *Arabidopsis thaliana* leaves during senescence, coinciding with increased levels of JA and OPDA. In *Brassica napus* L., addition of MeJA to nutrient solution reduced photosynthetic activity, chlorophyll content, and nitrate uptake (Rossato et al. 2002); the latter effect was partially reversed when MeJA was removed.

In the present study, in contrast to control plants, inoculated plants showed increase of shoot DM at day 21 and did not show symptoms of senescence until the end of the experiment.

Nodule development is an energetically expensive process, and the number of nodules is typically controlled by the host plant, through an "autoregulation of nodulation (AON)" process involving long-distance signaling (Ferguson et al. 2010). In studies of *M. truncatula*, reduction of JA levels seems to affect arbuscular mycorrhization, but not nodulation (Zdyb et al. 2011). The different reaction

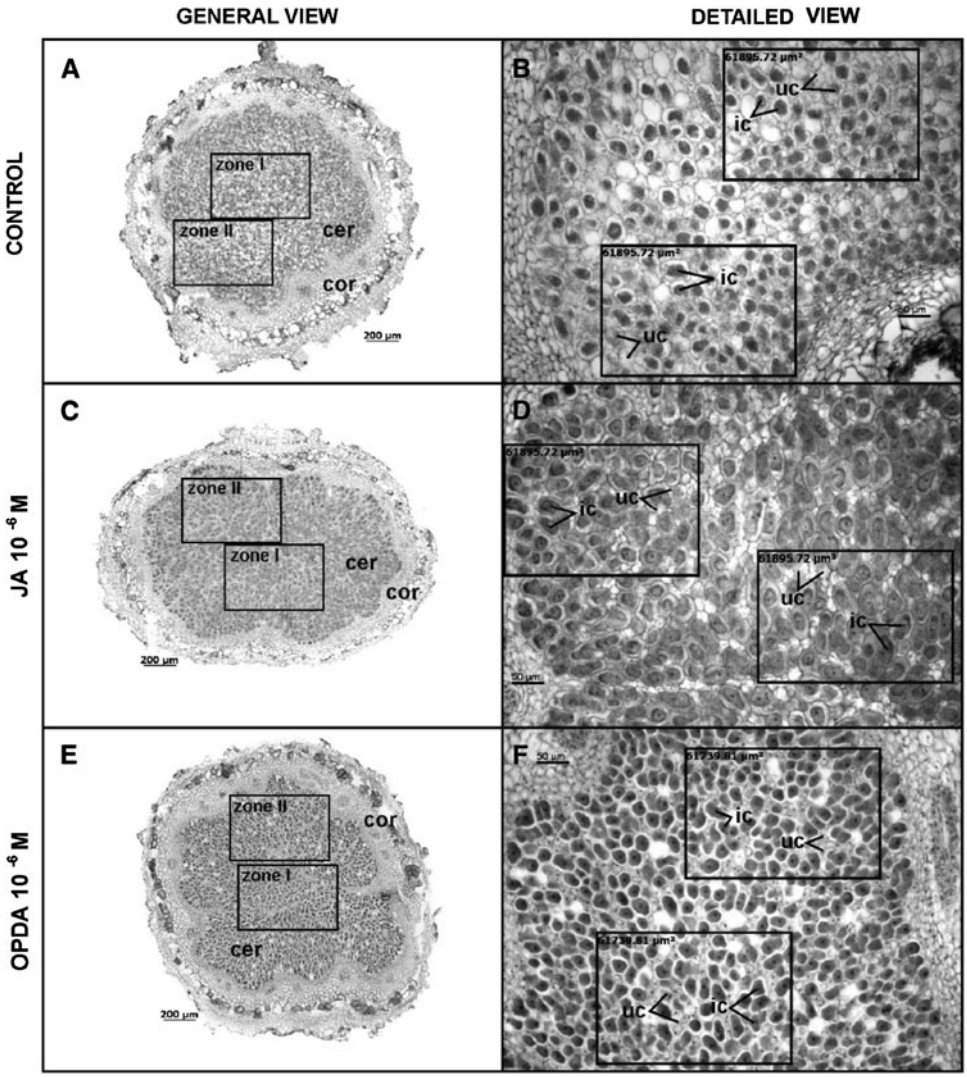

Fig. 6 Photomicrographs of longitudinal and transversal sections of nodules. **a** General view: control, **b** detailed view: control, **c** general view: JA 10^{-6} M, **d** detailed view: JA 10^{-6} M, **e** general view: OPDA 10^{-6} M, and **f** detailed view: OPDA 10^{-6} M. Central zone (Zone I) and peripheral zone (Zone II) are marked with black. Area shown ~62,000 µm^2. *cer* central region, *cor* cortical region, ***ic*** infected cell, *uc* uninfected cell

of soybean may be due to the difference between the induction of indeterminated (*M. truncatula*) versus determinated (soybean) nodules.

Nevertheless, JA appeared to act as a negative regulator of nodulation, by inhibiting expression of early nodulation genes (Sun et al. 2006). There is some recent evidence that JA level is positively correlated with nodulation in soybean (Seo et al. 2007; Kinkema and Gresshof 2008). In contrast, Nakagawa and Kawaguchi (2006) reported that shoot-applied MeJA exhibited a strong inhibitory effect on nodulation in the wild type and even in the hypernodulating phenotype of the *L. japonicus* har1-4 mutant. However, several studies gave conflicting results about JAs participation in nodulation.

We propose that JAs function as part of a hormonal network to control nodule development through AON, consistent with the findings of Kinkema and Gresshoff (2008). The accumulation of OPDA we observed at day 15, coinciding with maximal nodule number in nodulated roots, suggests that OPDA is involved in AON as a signal molecule, independent of JA.

The high levels of OPDA and JA found in isolated nodules suggest that these compounds play a greater role in *Bradyrhizobium*–soybean symbiosis than previously suspected. Consistent with our findings, Mohammadi et al. (2003) observed activity of LOX enzymes in effective nodules induced by *B. japonicum* strains 2122 and 2143 in soybean. LOX1-specific activity was several-fold higher in nodules than in adjacent root tissue and appeared to be a result of plant–symbiont interaction. Using microarray analysis, Hayashi et al. (2008) identified genes associated with nodule development in soybean. LOX expression during nodulation was relatively complex; at least eight different LOX genes were expressed in nodules, some of which were probably involved in nodule development. The high LOX activity and high level of JAs in nodules confirm participation of these partner components in steps of *Bradyrhizobium*–soybean interaction.

Levels of JAs inside nodules are high, and previous studies have shown that JA and MeJA induce cell expansion in potato tubers (Takahashi et al. 1994), corpus cells of tuber buds (Castro et al. 1999), and meristematic regions of stolons (Cenzano et al. 2003). We therefore examined effects of exogenous OPDA and JA in soybean nodules and found that these compounds increased number and size of cells to various degrees, depending on concentration. Levels of JAs during early stages of nodule development appear to affect cell growth processes. Along this line, Hause and Schaarschmidt (2009) proposed that JAs affect nodule cell growth, and the aging process, by influencing antioxidant metabolism. Our knowledge of the function of JAs in mutualistic symbioses in general remains highly fragmentary, but the present findings support their involvement in *Bradyrhizobium*–soybean interaction.

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