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Plant Physiology and Biochemistry 44 (2006) 759–765
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Jasmonates induce Nod factor production by *Bradyrhizobium japonicum*

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Received 10 January 2006; accepted 10 October 2006
Available online 02 November 2006

Abstract

Jasmonates are signaling molecules involved in induced systemic resistance, wounding and stress responses of plants. We have previously demonstrated that jasmonates can induce *nod* genes of *Bradyrhizobium japonicum* when measured by β-galactosidase activity. In order to test whether jasmonates can effectively induce the production and secretion of Nod factors (lipo-chitooligosaccharides, LCOs) from *B. japonicum*, we induced two *B. japonicum* strains, 532C and USDA3, with jasmonic acid (JA), methyl jasmonate (MeJA) and genistein (Ge). As genistein is well characterized as an inducer of *nod* genes it was used a positive control. The high-performance liquid chromatography (HPLC) profile of LCOs isolated following treatment with jasmonates or genistein showed that both JA and MeJA effectively induced *nod* genes and caused production of LCOs from bacterial cultures. JA and MeJA are more efficacious inducers of LCO production than genistein. Genistein plus JA or MeJA resulted in greater LCO production than either alone. A soybean root hair deformation assay showed that jasmonate induced LCOs were as effective as those induced by genistein. This is the first report that jasmonates induce Nod factor production by *B. japonicum*. This report establishes the role of jasmonates as a new class of signaling molecules in the *Bradyrhizobium*–soybean symbiosis.

Keywords: *Bradyrhizobium japonicum*; Genistein; Jasmonates; Nod factor; *nod* gene induction

1. Introduction

The early events that lead to successful formation of nitrogen fixing nodules constitute a multi-step interaction between rhizobia and their host plants. During the initial stages of this complex inter-organismal interaction, legume plant roots synthesize and exude a diverse array of compounds into the rhizosphere, including the well characterized flavonoid signals [24]. These compounds serve as chemoattractants for rhizobia, influence bacterial growth and selectively induce the expression of nodulation genes of symbiotic rhizobia [4,10,21]. Soybean roots exude isoflavonoids, primarily genistein and diadzein, which are effective inducers of the *nod* genes in *Bradyrhizobium japonicum* [17].

The transcriptional regulation of *nod* genes is complex and varies from species to species and even from strain to strain. However, in all cases, this process seems to require the regulatory protein nodD which, after interaction with appropriate flavonoid signal(s) from plants, binds with the *nod* box, a conserved, *cis*-acting promotor element that induces the expression of the *nod* genes. The DNA helix in the vicinity of the *nod* box is deformed so that the adjacent *nod* genes can be transcribed [12]. The result of the *nod* gene induction is the biosynthesis of Nod factors—structurally known as lipo-chitooligosaccharides (LCOs) [5]. These Nod factors are exported from rhizobial cells and act as return bacteria-to-plant signals; they stimulate root hair curling (HAC), infection thread formation and nodule morphogenesis in the host plants. The bacteria reside inside these nodules as bacteroids and fix atmospheric dinitrogen, which is supplied to the plants in exchange of organic acids [33].

Nod factors are composed of three to five 1–4 β-linked *N*-acetyl glucosamine units with the *N*-acetyl group of the terminal non-reducing sugar replaced by an acyl chain with 16 or 18 carbons and with varying degrees of saturation. Various "decorations" at both reducing and non-reducing termini of the chitin backbone are possible, such as methylation, acetylation, carbamoylation or sulfation. Thus, the resulting Nod factors have high degree of diversity. Nod factors are structurally

Abbreviations: HPLC, high-performance liquid chromatography; JA, jasmonic acid; LCO, lipo-chitooligosaccharide; MeJA, methyl jasmonate.

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doi:10.1016/j.plaphy.2006.10.025

diverse and a single rhizobial strain may produce a range of metabolites [32]. Research efforts during the last decade have made possible the structural identification of many Nod factors from a wide range of rhizobia. These LCOs show specificity in their signal activity and contribute to host specificity in the rhizobia–legume nodulation process [13,31,33].

Jasmonic acid (JA) is a derivative of linolenic acid, and consists of a cyclopentanoic ring where a pentenyl side chain and an acetic acid are attached (Fig. 1). It is biosynthesized in plants via the octadecanoid pathway [34]. JA occurs ubiquitously in plants and plays a central role in internal signaling pathways leading to the activation of defense responses to insect feeding, pathogen infection and plant responses to environmental stresses [7,35]. The role of JA in multiple aspects of plant growth and physiology has been an attractive area of research. JA also plays key roles in plant development [7], and touch perception by plants (mechanoperception) [36]. It is recognized as a major intermediate in the wound signal transduction cascade [11]. JA induces the production of phytoalexins [2] in plants and it has been suggested that JA works as a signal in elicitor-induced biosynthesis of phytoalexins by inducing transcription of the genes coding for phenylalanine ammonia lyase (PAL) [15] and chalcone synthase (CHS) [27].

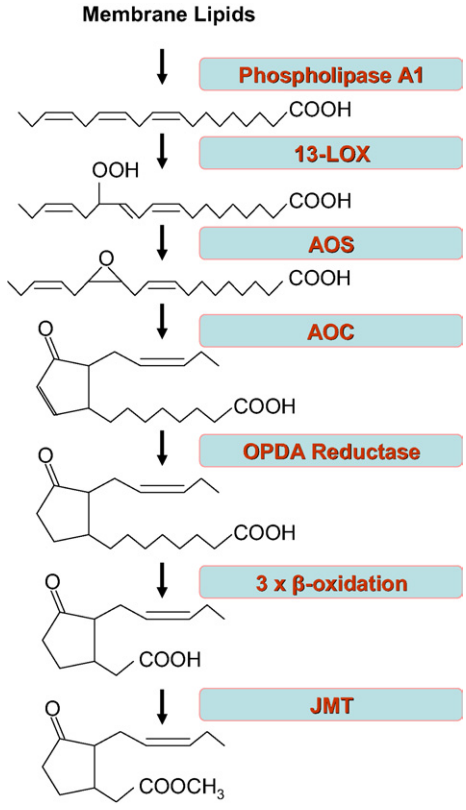

Fig. 1. The biosynthesis of jasmonic acid in plants. The biosynthesis of jasmonates begins with fatty acid peroxidation of linolenic acid, which is believed to be produced from the plasma membrane via the action of phospholipase A1. The pathway is generally referred to as the octadecanoid pathway due to its 18 carbon precursor, linolenic acid. The names of the enzymes involved in this pathway are 13 lipoxygenase (13-LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), 12-oxophytodienoic acid (OPDA) reductase, and jasmonic acid methyl transferase (JMT) (adapted from Cheong and Choi [6]).

Both legume and non-legume roots have been shown to synthesize JA. Dathe et al. [9] reported that wheat seedling roots biosynthesize JA, which is then exuded into the rhizosphere. Germinating soybean seeds and seedling roots synthesize large quantities of JA, with maximum concentration being observed in the seedling axis, stem hook and plumule 12-h after the onset of germination [8]. Previous studies have shown that jasmonates can act as signal molecules in the early stages of legume–rhizobia symbioses by inducing the expression of the *nod* genes of *B. japonicum* [20] and *Rhizobium* [28]. Since *nod* genes are responsible for the production of Nod factors, we conducted this study to test the hypothesis that induction of *B. japonicum* with jasmonates actually causes the production and excretion of Nod factors (LCOs).

2. Results

2.1. Jasmonates induce LCOs production by B. japonicum

The high-performance liquid chromatography (HPLC) profile of LCO extract from induced bacterial cultures of both strains showed that jasmonates and genistein induced LCO production from *B. japonicum* cells. *B. japonicum* produces a number of structurally diverse LCOs [32]; however, in this study, we quantified the four LCOs from the induced cultures (Fig. 2) that *B. japonicum* cells produce in the greatest abundance. We found that both jasmonates (JA and methyl jasmonate (MeJA)) and genistein induced all the four LCOs from induced bacterial cultures (Fig. 3). However, there was variation among the yields of individual LCOs following induction with JA, MeJA or genistein. Jasmonates (JA and MeJA) preferentially induced LCO (Nod BjV (Ac, $C_{16:0}$, MeFuc; RT 32.24 min) as compared to genistein induced cultures. Genistein induced cultures showed low levels of this LCO (Fig. 3). The two strains produced different amounts of LCOs. Strain USDA3 was more sensitive to inducer molecules; induced cultures of USDA3 produced more LCO than induced cultures of 532C (Fig. 4).

2.2. Synergism between genistein and jasmonates

Since the Nod factor production profile of jasmonate induced cultures was different from that of genistein induced cultures, and given that jasmonates and flavonoids are very different chemically (flavonoids and lipids, respectively), we also investigated the Nod factor production pattern of the tested strains following exposure to genistein and jasmonates in combination. When the inducer molecules were added to bacterial cultures together, at optimum concentrations for each (5 μM for genistein and 50 μM for JA and MeJA), Nod factor production was greater than when either was used alone. Again strain USDA3 yielded more LCOs than strain 532C (Fig. 4).