Journal of Plant Physiology 179 (2015) 35–39

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Short communication

Molecular cloning of the *BLADE-ON-PETIOLE* gene and expression analyses during nodule development in *Lupinus luteus*

Kamil Frankowski[a], Emilia Wilmowicz[a,c,*], Agata Kućko[a,c], Agnieszka Zienkiewicz[a,d], Krzysztof Zienkiewicz[b,d], Jan Kopcewicz[a]

[a] Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, 1 Lwowska Street, 87-100 Toruń, Poland
[b] Department of Cell Biology, Nicolaus Copernicus University, 1 Lwowska Street, 87-100 Toruń, Poland
[c] Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, 4 Wileńska Street, 87-100 Toruń, Poland
[d] Department of Biochemistry, Cellular and Molecular Biology of Plants, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Profesor Albareda 1, 18008, Granada, Spain

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 23 January 2015
Accepted 26 January 2015
Available online 12 March 2015

Keywords:
BLADE-ON-PETIOLE
Leghemoglobin
Lupinus luteus
Nodules

ABSTRACT

The *BLADE-ON-PETIOLE* (*BOP*) genes have been recently shown to play an essential role in many physiological processes, including embryogenesis, meristem determinacy, leaf patterning and nodule development. In our research we used *Lupinus luteus*, a plant with great agronomic potential due to its high protein content and nitrogen fixation ability. In this work, *LlBOP* in *L. luteus* was identified for the first time and its expression during nodule development was analyzed. The high expression levels of *LlBOP* and *LlLbI* (*LEGHEMOGLOBIN*), essential to nitrogen-fixing symbiosis, were noted in the developing root nodules and were correlated with the occurrence of leghemoglobin. All of these data indicate that *LlBOP* is an important regulator of root nodule formation and functioning in *L. luteus*.

Introduction

Lupinus luteus (*Fabaceae*), a widely cultivated species in Poland, Australia and Mediterranean countries, is characterized by a high protein content of its seeds and has a positive effect on soil fertility, which are key factors for subsequent crops. The ability of lupine to fix nitrogen in symbiosis with bacteria of the *Bradyrhizobium* genus has been known from more than a century. This process is carried out in nodules, structures forming from root cortical cells in response to an external signal from free-living rhizobacteria (Fernández-Pascual et al., 2007). Nitrogenase, the enzymatic complex responsible for nitrogen fixation, is rapidly inactivated by oxygen, but at the same time, oxygen is needed for the bacteroids to obtain energy necessary for reducing nitrogen to ammonia. This contradiction, the "oxygen paradox" was explained by the functioning of leghemoglobin (Lb), a monomeric protein that exhibits a high affinity to oxygen (Rajasekaran et al., 2011). Lb effectively transports O_2 from plant tissues to the nodule cells and maintains its low but stable concentration, allowing for the simultaneous occurrence of nitrogenase activity and bacteroid respiration (Becana et al., 2010). Bacteroid action provides ammonia, which is incorporated by the plant into essential biomolecules, such as proteins and nucleic acids, contributing to yield increase (Gresshoff et al., 2014). Interestingly, recent work has indicated that BOPs coordinate many other plant growth and development processes: organ abscission embryogenesis, meristem determinacy, leaf patterning, inflorescence architecture as well as flower and nodule development (Khan et al., 2014). A mutation in the *NODULE ROOT* and *COCHLEATA* genes, orthologs of *AtBOPs* from *Medicago truncatula* and *Pisum sativum*, respectively, results in abnormalities such as roots developing from the apical part of the nodule (Couzigou et al., 2012).

In this paper, for the first time, we report the isolation and molecular characterization of the *BLADE-ON-PETIOLE* gene from *Lupinus luteus* (*LlBOP*). The *LlBOP* expression pattern was characterized during nodule development. Accordingly, the ability of nodules to fix nitrogen was assayed by quantifying the *LlLbI* mRNA. The total biological nitrogen fixation rate at given developmental stages was measured by the LlLbI content – an indirect method for nitrogenase activity determination.

Abbreviations: BOP, BLADE-ON-PETIOLE; Lb, leghemoglobin.

* Corresponding author at: Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, 1 Lwowska, 87-100 Toruń, Poland. Tel.: +48 566114461; fax: +48 566114440.
E-mail addresses: kfrank@o2.pl (K. Frankowski), emwil@umk.pl (E. Wilmowicz), kuckoa@poczta.onet.pl (A. Kućko), agazet@umk.pl (A. Zienkiewicz), kzienk@umk.pl (K. Zienkiewicz), kopcew@umk.pl (J. Kopcewicz).

http://dx.doi.org/10.1016/j.jplph.2015.01.019
0176-1617/

Material and methods

Plant material

The commercial epigonal cultivar Taper of yellow lupine (*Lupinus luteus*) was used in the study. The *L. luteus* seeds (Poznań Plant Breeding Tulce, Wiatrowo, Poland) were treated with Sarfun (250 cm^3/100 kg seeds) (*Organika-Sarzyna S.A.*, Nowa Sarzyna, Poland), inoculated with *Bradyrhizobium lupini* (Nitragina 3 g/kg seeds, "*BIOFOOD S.C*", Wałcz, Poland) for 2 h and subsequently sown in 11 dm^3 pots (5 seeds per pot, with a spacing of 0.02 m) filled with class V soil material. The seeds were planted at a depth of 0.03–0.04 m. The lupine was grown in a cultivated chamber at a temperature of $22 \pm 1^{\circ}C$ under long day conditions (110 $\mu mol\ m^{-2}\ s^{-1}$, cool white fluorescent tubes by *Polam*, Warsaw, Poland).

In the experiments examining *LlBOP* and *LlLbI* expression during nodule development, roots from plants were harvested at days 27 and 34 (vegetative stages), as well as 48 and 55 (generative stages) after sowing (Fig. 1). Roots were divided into two parts: the upper parts, containing nodules (Fig. 1A–D) and the lower ones without nodules (Fig. 1 A1–D1).

Plant material for expression analyses was frozen in liquid nitrogen and stored at −80 °C until RNA isolation procedures. All experiments were designed in three independent biological replications.

Molecular cloning of LlACT *and* LlBOP *cDNAs*

The tissue of *L. luteus* (1.0–1.5 g) was homogenized in a sterile chilled mortar with a pestle. Total RNA was isolated with the NucleoSpin RNA Plant, *MACHEREY-NAGEL GmbH & Co. KG* (Düren, Germany) according to the manufacturer's instructions. All of the primers used in PCR reactions were synthesized by "*Genomed S.A.*" (Warsaw, Poland). One μg of total RNA primed with anchored oligo$(dT)_{18}$ primers was used for first strand synthesis with the Transcriptor Reverse Transcriptase, Transcriptor First Strand cDNA Synthesis Kit (*ROCHE Diagnostics GmbH*, Germany) according to the instructions of the manufacturer. PCR reactions, using degenerated primers for *LlACT* (5′-CCGAACMASTTYCCCTGYAA-3′ (forward) and 5′-GRATTTTCACCAAGTACTCYG-3′ (reverse)) and *LlBOP* (5′-CGTTGATCTTGCTCTTGAYACTCTC-3′ (forward) and 5′-ATTGAGKCCTTCTCCCATTACCAT-3′ (reverse)) constructed on the basis of conserved sequences encoding *ACTs* in *Cicer arietinum* (GenBank acc. no. XM_004485477.1), *Glycine max* (GenBank

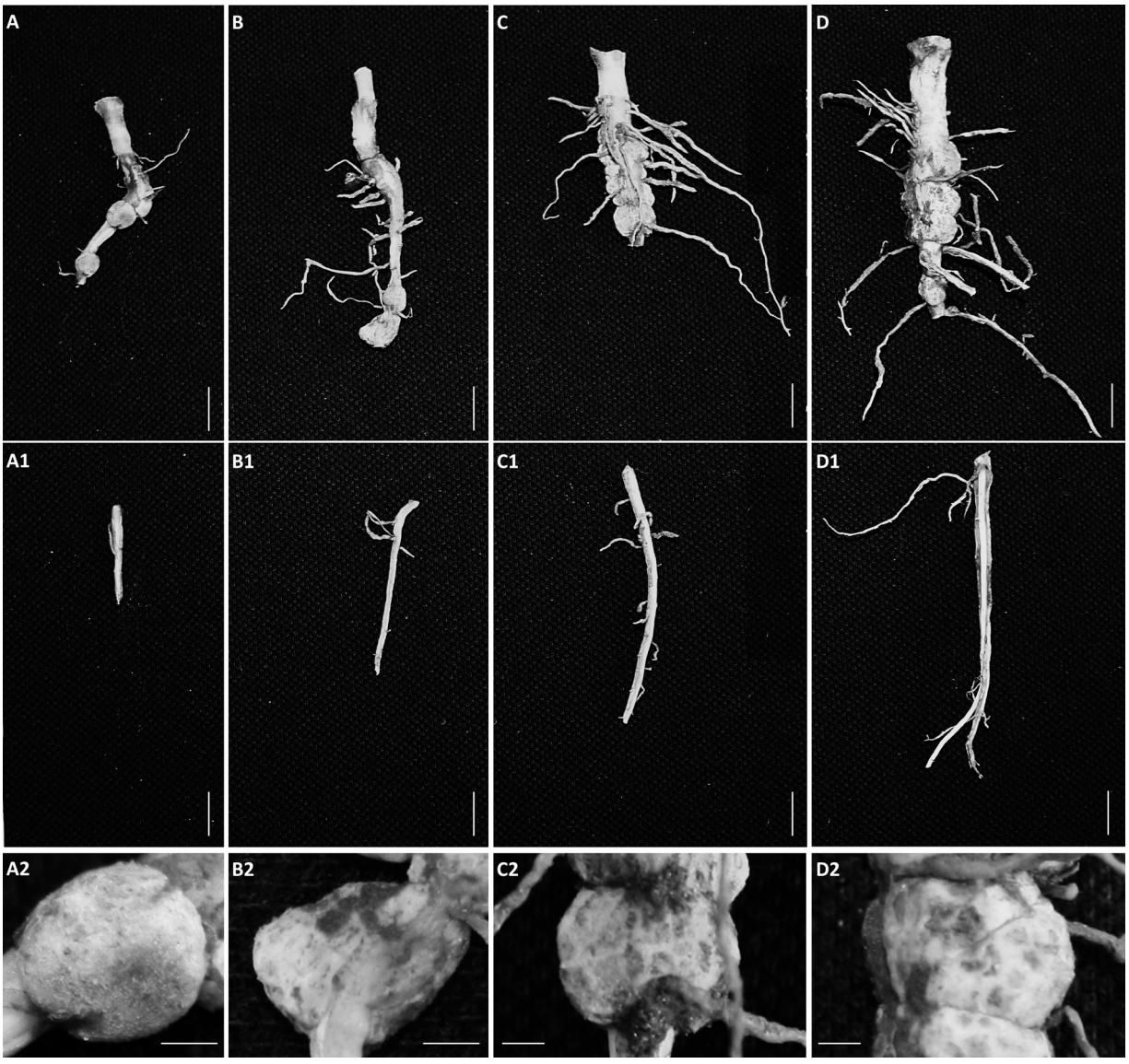

Fig. 1. Root development and nodulation stages in *Lupinus luteus*. Roots were harvested from 27-, 34-, 48- and 55-d-old plants and divided into sections containing (A-D) and not containing (A1-D1) nodules. Enlarged images of the nodules (A2-D2). Bars = 1 cm in (A-D) and (A1-D1); 2 mm in (A2-D2).