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Published version:

DOI:10.1080/15592324.2016.1165379

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Belmondo, Simone; Calcagno, Cristina; Genre, Andrea; Puppo, Alain; Pauly, Nicolas; Lanfranco, Luisa. NADPH oxidases in the arbuscular mycorrhizal symbiosis. PLANT SIGNALING & BEHAVIOR. 11 (4) pp: 1165379-1165381.
DOI: 10.1080/15592324.2016.1165379

Plant Signaling & Behavior

ISSN: (Print) 1559-2324 (Online) Journal homepage: http://www.tandfonline.com/loi/kpsb20

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To cite this article: Simone Belmondo, Cristina Calcagno, Andrea Genre, Alain Puppo, Nicolas Pauly & Luisa Lanfranco (2016): NADPH oxidases in the arbuscular mycorrhizal symbiosis, Plant Signaling & Behavior, DOI: 10.1080/15592324.2016.1165379

To link to this article: http://dx.doi.org/10.1080/15592324.2016.1165379

Accepted author version posted online: 28 Mar 2016.

Short Communication

NADPH oxidases in the arbuscular mycorrhizal symbiosis

Simone Belmondo[1], Cristina Calcagno[1], Andrea Genre[1], Alain Puppo[2], Nicolas Pauly[2], Luisa Lanfranco[1]

[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Via Accademia Albertina 13, 10123 Torino, Italy

[2] INRA, Université de Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France.

Keywords

Arbuscular mycorrhizal symbiosis, NADPH oxidase, *Medicago truncatula*, spatio-temporal gene expression, reactive oxygen species (ROS), respiratory burst oxidase homolog (RBOH)

Correspondence to: Luisa Lanfranco; Tel: 00390116705969, Fax: 00390116705962

Email: luisa.lanfranco@unito.it

Abstract

Plant NADPH oxidases are the major source of reactive oxygen species (ROS) that plays key roles as both signal and stressor in several plant processes, including defense responses against pathogens. ROS accumulation in root cells during arbuscular mycorrhiza (AM) development has raised the interest in understanding how ROS-mediated defense programs are modulated during the establishment of this mutualistic interaction. We have recently analyzed the expression pattern of five NADPH oxidase (also called RBOH) encoding genes in *Medicago truncatula*,

showing that only one of them (*MtRbohE*) is specifically upregulated in arbuscule-containing cells. In line with this result, RNAi silencing of *MtRbohE* generated a strong alteration in root colonization, with a significant reduction in the number of arbusculated cells. On this basis, we propose that MtRBOHE-mediated ROS production plays a crucial role in the intracellular accommodation of arbuscules.

TEXT

Roots of ~80% of plant species in natural and agricultural systems are colonized by arbuscular mycorrhizal (AM) fungi, a crucial component of the plant microbiota.[1] In this mutualistic symbiosis the fungus delivers to the plant mineral nutrients, mainly phosphorus and nitrogen, in exchange for carbon.[2] Besides promoting plant growth, AM fungi sustain other important functions such as soil aggregation and water retention, tolerance to biotic and abiotic stresses and increase in plant biodiversity.[3] The clear ecological and economic importance of this symbiosis has strongly boosted the interest of the scientific community.

This very ancient and intimate plant-fungus association is thought to rely on a rigorous colonization program that leads the plant cell to accommodate intracellular fungal structures, including hyphae and highly branched arbuscules.[4] Root colonization is associated with massive rewiring of nutrient fluxes that guarantee reciprocal benefits to both the host plant and the fungus.[5] Important advances have been achieved in the last years on the molecular mechanisms governing the symbiosis; however, the way this mutualistic interaction overtakes plant defence remain largely obscure. In this frame, a key role is emerging for fungal effector proteins as communication factors, in analogy to several plant pathogenic interactions.[6,7] The SP7 secreted protein from *Rhizophagus irregularis* was indeed shown to interfere with the expression of plant defence genes.[6]

On the plants side, a conserved defense response to pathogens is the production of Reactive Oxygen Species (ROS) which play a pivotal role in regulating numerous responses to biotic and abiotic stresses in plants. The complexity in ROS responses to diverse stimuli has been proposed to rely on the multiple regulatory mechanisms of ROS production *via* NADPH oxidases, one of the primary sources of ROS.[8] NADPH oxidases, known in plants as RBOH (respiratory burst oxidase homolog) catalyze the production of superoxide by transferring electrons from NADPH to molecular oxygen, with secondary generation of H_2O_2. They are encoded by a multigene family, with up to 10 different members in the model plant *Arabidopsis thaliana*.[9,10] Complex mechanisms of RBOH regulation, from transcriptional to post-translational level, occur and contribute to RBOH expression and function in an array of tissue types and developmental stages under various environmental conditions. [8,11]

The activation of specific RBOH isoforms is responsible for ROS accumulation in several plant-pathogen interactions[12,13] and in the symbiotic interaction between legumes and nitrogen-fixing rhizobia.[14,15,16]

H_2O_2 has been detected in root cells colonized by AM fungi.[17,18,19] Interestingly, the up-regulation of fungal genes implicated in oxidative stress defense has also been reported in mature mycorrhizas[19,20] suggesting that protection against localized ROS-based host defense responses may be involved in arbuscule formation and/or maintenance.

Starting from the hypothesis that plant RBOH could be good candidates for H_2O_2 production in arbuscular mycorrhizas, we have analysed in a recent publication the spatio-temporal expression profiles of five *Rboh* genes from the model legume *Medicago truncatula* (*MtRbohA*, *MtRbohB*, *MtRbohE*, *MtRbohG*, *MtRbohF*) during the establishment of the AM symbiosis.[21] *MtRboh* transcript levels did not drastically change in total RNA extractions from whole mycorrhizal and non mycorrhizal roots in a time course experiment of root colonization (7, 14, 28 and 60 days post-inoculation), with the highest expression level always observed for *MtRbohG*. This is not surprising, because AM colonization is an asyncronous process and plant responses often develop on a small local scale in mycorrhizal roots. To achieve a more detailed view of gene expression pattern we used a complementary cellular and molecular approach that allowed transcript localization in different cell types. The analysis of *Agrobacterium rhizogenes*-transformed roots expressing a GUS transcriptional fusion construct with *MtRboh* promoters showed that all genes are expressed in the central cylinder (in both mycorrhizal and non mycorrhizal conditions), underlying the importance of RBOH and ROS in cell wall metabolism.[22] This approach also highlighted the expression of *MtRbohE* in cells containing arbuscules.

The laser microdissection technique clearly showed the expression of two genes, *MtRbohG* and *MtRbohE*, in cortical cells, whether or not they were colonized by fungal hyphae. Thus, this technique turned out to be more sensitive than the GUS histochemical assay since the *MtRbohG* promoter activity was never observed in cortical cells. Remarkably, *MtRbohE* transcripts appeared more abundant in arbuscule-containing cells compared to adjacent non colonized cells, supporting the results obtained with the GUS assay. A summary of the expression pattern of the five analysed *MtRboh* genes in a mycorrhizal root is shown in Figure 1.

To further clarify the role of *MtRbohE*, we generated RNAi lines. While *RbohE*-silenced plants showed a normal nodulation phenotype, an altered AM colonization pattern was observed in the root cortex, with fewer arbuscules and more abundant intercellular hyphae, compared to control roots (Fig. 2). Altogether our data indicate the transient up-regulation of *MtRbohE* expression in arbusculated cells and suggest a role for *MtRbohE* in arbuscule accommodation within cortical cells.

Our results integrate those from Arthikala and colleagues who recently characterized RBOH in arbuscular mycorrhizas of another legume plant: the *PvRbohB* gene from *Phaseolus vulgaris* (homolog of *MtRbohG*) turned out to act as a negative regulator of the AM symbiosis while it is required for root infection by rhizobia.[23,24]

Although only two *Rboh* genes from two different legumes have been characterized so far [10,14,23,24] these findings suggest that different gene members of the RBOH family play distinct functions in the AM symbiosis; moreover, some of them may have even have opposite functions (promotion *versus* inhibition) in the two types of root symbioses. Interestingly, in the case of *MtRbohE*, no phenotype has been observed during rhizobial symbiosis.[21] The temporal and spatial fine tuning of RBOH-derived ROS, seem therefore to contribute to the establishment of fully functional interactions in these plant-microbe associations. The AM symbiont may also participate to ROS production by specific fungal NADPH oxidases (also known as Nox). Nox, which also belong to a gene family with up to three (A, B and C) classes, play a key role in fungal cellular differentiation and development.[25] Interestingly, a NoxA gene was shown to be critical for maintaining a mutualistic symbiosis between the fungal endophyte *Epichloe festuca* and its host plant *Lolium perenne*.[26,27] Molecular and *in silico* analyses revealed that the AM fungus *Rhizophagus irregularis* possess Nox genes, belonging to class A and B, which are expressed in arbuscule-containing cells (Fiorilli and Lanfranco, unpublished results). A fungal contribution to NADPH-oxidases-related processes in the *in planta* phase can thus be envisaged.

Future challenges will be to decipher how the NADPH-oxidases activities not only from the plant but also from the fungal partner exert their control over the AM colonization process eventually interacting with other signals.

Aknowledgments

Research was funded by the BIOBIT-Converging Technology project (WP2) to LL, the University grant (60%) to LL and AG, the BLAN07-2_182872 ANR research program to AP.

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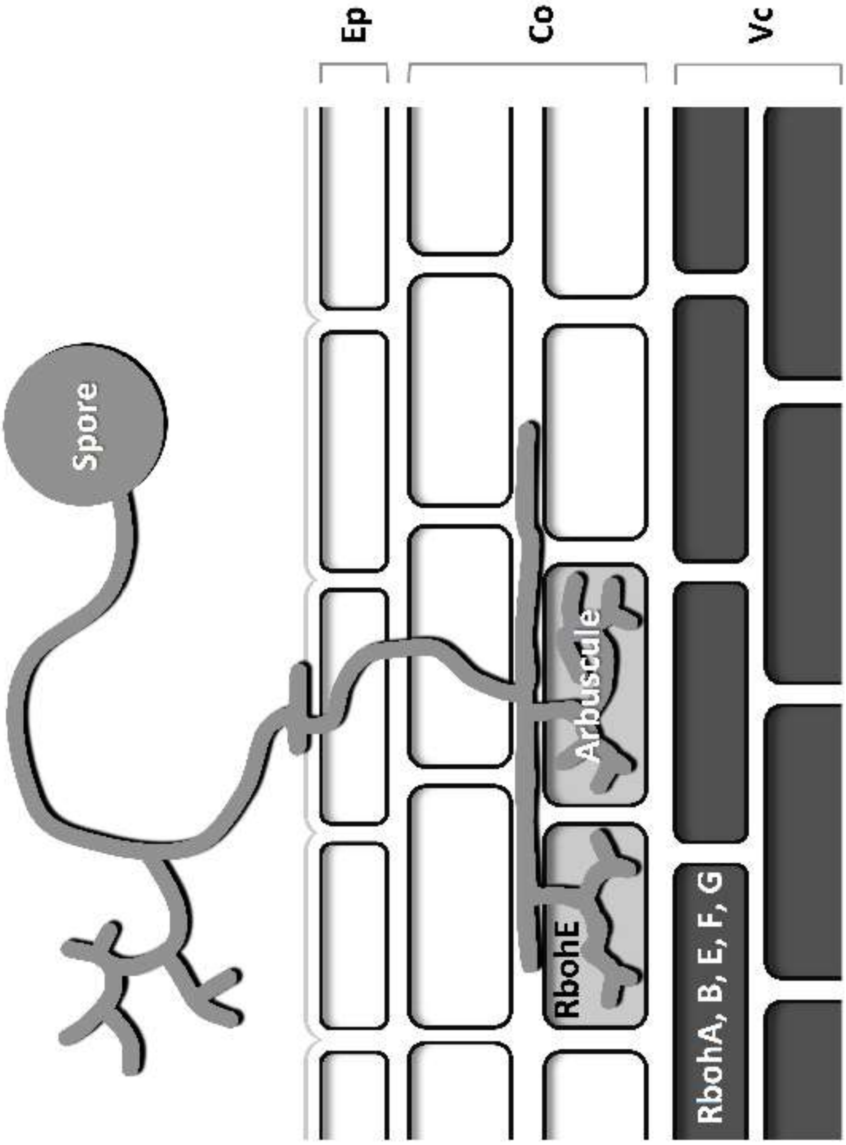

Figure 1 Scheme of the expression profiles of the investigated *MtRboh* genes in a mycorrhizal root. Based on histochemical GUS assays *MtRbohA, B, E, F* and *G* are expressed in the vascular cylinder while *MtRbohE* is also expressed in arbuscule-containing cells. Ep: epidermis; Co: cortex; Vc: vascular cylinder.

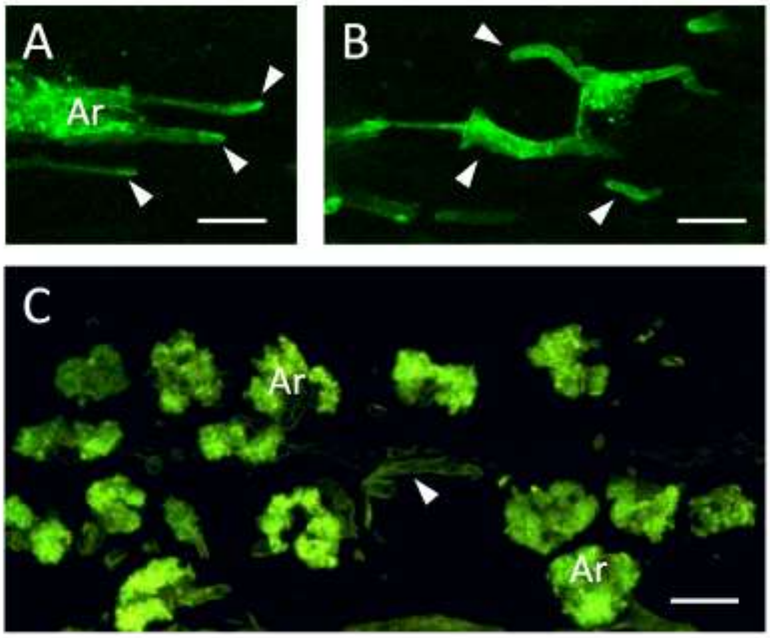

Figure 2 Altered arbuscular mycorrhizal colonization in *MtRbohE* RNAi-silenced lines of *Medicago truncatula*. *MtRbohE*-silenced plants generated an abnormal colonization pattern (A, B), with fewer arbuscules (Ar) and more frequent intercellular hyphae (arrowheads), compared to wild type roots (C). Confocal images of WGA-FITC stained fungal structures in root longitudinal sections. Bars = 20µm.