The xyloglucans : are they new elicitors of Arabidopsis thaliana immunity?

Justine Claverie, Christelle Guillier, Daphnée Brulé, Marie-Claire Heloir, Benoît Darblade, Xavier Daire, Benoît Poinssot

The xyloglucans: are they new elicitors of *Arabidopsis thaliana* immunity?

Justine Claverie, Christelle Guillier, Daphnée Brulé, Marie-Claire Héloir, Benoit Darblade, Xavier Daire and Benoit Poinssot

First, second, third, fourth, sixth and seventh authors: UMR Agroécologie 1347, INRA/Université de Bourgogne/Agrosup Dijon/CNRS, 17 rue Sully, BP 86510, 21000 Dijon, France; fifth author: Elicityl, 176 avenue Ambroise Croizat, 38920 Crolles, France
E-mail address: justine.claverie@inra.fr

Highlights

- Fragments derived from plant cell wall xyloglucans induce *Arabidopsis thaliana* defence responses and protection against *Botrytis cinerea*
- Xyloglucan-triggered immunity against *B. cinerea* requires the phytoalexin, ethylene and jasmonic acid-dependent pathways

Introduction

Plant resistance is based on their ability to perceive microorganisms and induce immune responses to stop their invasion. This recognition is possible via the perception of eliciting molecules released during the plant/pathogen interaction. These elicitors, called PAMPs (Pathogen-Associated Molecular Patterns), gather conserved molecular patterns such as bacterial flagellin or fungal chitin and activate a set of defence-associated responses termed PAMP-triggered immunity (PTI; Newman et al., 2013). Plants are also able to distinguish fragments from plant cell wall such as oligogalacturonides (OGs; Ferrari et al., 2013) commonly called DAMPs (Damage-Associated Molecular Patterns).

Xyloglucans (Xh) are the main component of hemicellulose in eudicot primary cell walls and are composed of a β-1-4-glucan backbone with side chains of xylose, fucose or galactose. The first aim of this study was to investigate if Xh were new DAMPs of Arabidopsis immunity and characterised their mode of action.

Material and methods

Arabidopsis seeds of the WT Columbia (Col-0) and mutants in the same background were obtained from the Nottingham Arabidopsis Stock Center (NASC). Plants were grown in a controlled growth chamber for 4 weeks. Arabidopsis cells were cultivated as previously described (Trdá et al., 2014). Cells or plants were treated with water, Xh or OG taken as a positive control (both used at 1 g/l for defence responses and 2.5 g/l for protection assays). In cell suspensions, H_2O_2 production was determined using the chemiluminescence of luminol (Dubreuil-Maurizi et al., 2011). Cytosolic Ca^{2+} variation ($[Ca^{2+}]$cyt) measurements were carried out on Arabidopsis transformed plant expressing apoaequorin according to Manzoor et al. (2013). Trdà et al. (2014) previously described protein extraction, SDS-PAGE and western blotting for MAPK phosphorylation analysis. RNA extraction and quantitative real-time PCR reactions were performed as proposed by Manzoor et al. (2013) using primers for the amplification of defence marker genes (*PR-1*, *PAD3*, *ICS1* and *LOX3*). Callose deposition was revealed by aniline blue staining. Two days after treatment, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis* infections were performed according to Manzoor et al. (2013).

Results and discussion

Xh treatment induced a dose-dependent MAPK phosphorylation in Arabidopsis cell suspensions. From 5 to 60 min, Xh treatment induced a rapid phosphorylation of two MAPKs with relative molecular masses of 43 and 47 kDa. Treatment with Xh did not induce any free $[Ca^{2+}]$cyt variations whereas OG treatment induced a rapid and transient increase in free $[Ca^{2+}]$cyt that peaked after 30 sec. Xh did not trigger any H_2O_2 production, as observed in control cells but OG treatment induced an oxidative burst with maximal H_2O_2 production detected at 10 min. To investigate late defence responses, we analysed callose deposition at the site of infection by *B. cinerea* after elicitor treatments. Xh and OG-treatment resulted in a significant increase of callose production 3 days post infection with the pathogen. The expression of different defence genes was analysed by qPCR. Xh triggered the accumulation of *PR-1*, *PAD3*, *LOX3* and *ICS1* transcripts. To further investigate the efficacy of xyloglucans to induce resistance, we performed protection assays against the necrotrophic fungi *B. cinerea* and the biotrophic oomycete *H. arabidopsidis*. Xh treatment applied 48 h before pathogen infection significantly reduced both the *B. cinerea* lesion diameter and the *H. arabidopsidis* sporulation on Arabidopsis leaves. Together, these results suggest that Xh are new elicitors of Arabidopsis immunity. Interestingly, some defence responses triggered by Xh are different from those induced by OG. As Arabidopsis responds to Xh treatment, we aimed to identify some signalling components. By using a genetic approach with T-DNA mutants in different defence responses, our data indicated that the Xh-triggered immunity against *B. cinerea* requires the phytoalexin (*cyp71A13*, *pad3*, *pad2*), ethylene (*etr1*, *ein2*) and jasmonic acid-dependent pathways (*dde2*, *lox3*, *coi1*).These results show that Xh are recognised by Arabidopsis. In order to identify a receptor involved in Xh perception or signalling, knock-out mutants of previously known *A. thaliana* receptors or candidate receptors up-regulated in microarray analysis have been tested. All these mutants will be tested by analysing MAPK activation assays after Xh treatment.

Acknowledgements

This work was financially supported by the Conseil Régional de Bourgogne, INRA and the ANR Plant KBBE project PATRIC (Grant ANR-13-KBBE-0001-01; BP, DB).

References

Dubreuil-Maurizi C et al. 2011. Glutathione deficiency of the Arabidopsis mutant *pad2-1* affects oxidative stress-related events, defense gene expression, and the hypersensitive response. Plant Physiology 157: 2000-2012.

Ferrari S et al. 2013. Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth development. Frontiers in Plant Science 4: 49.

Manzoor H et al. 2013. Involvement of the glutamate receptor AtGLR3.3 in plant defence signaling and resistance to Hyaloperonospora arabidopsidis. Plant Journal 76:466-480.

Newman MA et al. 2013. MAMP (microbe-associated molecular pattern) triggered immunity in plants. Frontiers in Plant Science 4: 139.

Trdà L et al. 2014. The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. New Phytologist 201: 1371-1384.