Physiological and Molecular Plant Pathology 79 (2012) 31–39

Contents lists available at SciVerse ScienceDirect

Physiological and Molecular Plant Pathology

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

Oligo-carrageenans induce a long-term and broad-range protection against pathogens in tobacco plants (var. Xanthi)

Jeannette Vera, Jorge Castro, Rodrigo A. Contreras, Alberto González, Alejandra Moenne*

Faculty of Chemistry and Biology, University of Santiago of Chile, Casilla 40, Correo 33, Santiago, Chile

ARTICLE INFO

Article history:
Accepted 27 March 2012

Keywords:
Oligo-carrageenans
Defense responses
Tobacco mosaic virus
Botrytis cinerea
Pectobacterium carotovorum
PAL
Nicotiana tabacum

ABSTRACT

Here, we analyzed the effect of oligo-carrageenans kappa, lambda and iota on protection against tobacco mosaic virus (TMV), *Botrytis cinerea* Whetzel and *Pectobacterium carotovorum* Waldee and suppression of infections in tobacco plants. Treatment with increasing concentrations of oligo-carrageenans, increasing number of treatments and increasing time after treatment enhanced protection against TMV indicating a long-term protection that mimics vaccination, mainly oligo-carrageenan lambda. In addition, oligo-carrageenans induced protection against *P. carotovorum* with similar efficiencies and against *B. cinerea,* mainly oligo-carrageenans lambda and iota. Moreover, oligo-carrageenans induced a partial suppression of TMV and *P. carotovorum* infections and a complete suppression of *B. cinerea* infection at systemic level. Furthermore, oligo-carrageenans induced a sustained activation of phenylalanine ammonia lyase activity and the accumulation of phenylpropanoid compounds with potential antimicrobial activities suggesting these compounds are involved in protection and suppression effects.

1. Introduction

The interaction between a plant and a pathogen can induce the release of oligosaccharides derived from plant and/or pathogen cell walls which can act as elicitors or suppressors of plant defense responses [1,2]. The best characterized oligosaccharide elicitors are oligo-galacturonides derived from plant pectin, chito-oligosaccharides derived from fungal cell walls, hepta-β-glucosides derived from *Phytophtora sojae* cell walls, lipochito-oligosaccharides derived from Gram-negative bacteria and rhamnolipids derived from *Pseudomonas aeruginosa* cell walls [3–6]. These and other plant oligosaccharides such as cellodextrins derived from cellulose and a fructo-oligosaccharide obtained from white burdock roots activate defense responses in plants leading to protection against plant pathogens [7–9]. It is now clearly established that plant and pathogen-derived oligosaccharides interact with specific receptors located in the plasma membrane triggering signaling pathways [10]. In particular, receptors that bind lipochito-oligosaccharides, hepta-β-glucosides and chito-oligosaccharides have been recently cloned and they showed a LysM domain resembling the peptidoglycan-binding domain present in Toll-like receptor of mammalian cells coupled to a kinase domain that activate signal transduction [11–14]. In addition, it was recently determined that oligo-galacturonides binds to Wall-Associated Kinase-1 (WAK-1) receptor which contains an extracellular domain having several Epidermal Growth Factor (EGF)-like repeats and an N-terminal and non-EGF domain that binds oligo-galacturonides [15] which is coupled to an intracellular Ser/thr kinase domain that activate signal transduction [16,17].

It has also been shown that seaweed (marine macroalgae) polysaccharides such as laminarin, sulphated laminarin, fucans and lambda carrageenan and oligosaccharides such as oligo-carrageenan kappa, the oligo-alginate Poly-Ma and the oligo-sulphated-galactan Poly-Ga also stimulate defense responses in plants inducing protection against bacteria, fungi and viruses [18–25]. Infiltrating the native carrageenan lambda to tobacco leaves induced the stimulation of defense responses such as the increase in the level of transcripts encoding a sesquiterpene cyclase, a chitinase and a proteinase inhibitor [23]. In addition, carrageenan kappa, depolymerized by enzymatic hydrolysis, elicited the activity of β-1,3 glucanase in *Rubus fruticosus* protoplasts and cells [24]. However, in the latter cases the effective protection against pathogens was not determined.

It was recently shown that the seaweed oligo-sulphated-galactan Poly-Ga induced protection against tobacco mosaic virus (TMV) in tobacco plants. This protection was dose-dependent, treatment number-dependent and increased with time after treatment indicating a long-term protection that mimics vaccination [26]. The

Abbreviations: B. cinerea, Botrytis cinerea; *P. carotovorum, Pectobacterium carotovorum*; LOX, lipoxygenase; PAL, phenylalanine ammonia lyase; tobacco mosaic virus, TMV.

* Corresponding author. Tel.: +56 2 7181106.
E-mail address: alejandra.moenne@usach.cl (A. Moenne).

0885-5765/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pmpp.2012.03.005

oligo-sulphated-galactan Poly-Ga was obtained by acid hydrolysis of the native sulphated galactan of the red seaweed *Schyzimenia binderi* and showed a molecular weight of 8.5 kDa [25]. Poly-Ga is constituted by 20 units of sulphated galactose linked by alternate β,1–3 and α,1–4 glycosidic bonds with sulphate groups in positions 2, 3 and 4 of the galactose ring (Fig. 1A). Poly-Ga induced a sustained increase in phenylalanine ammonia lyase (PAL) activity and the accumulation of several free and conjugated phenylpropanoid compounds with potential antimicrobial activities in tobacco plants [25,26]. Interestingly, the increase in PAL activity linearly correlated with the decrease in the number of necrotic lesions induced by TMV infection indicating the increase in PAL activity and the concomitant accumulation of phenylpropanoid compounds may determine, at least in part, the long- term protection against TMV in tobacco plants [26].

However, the sulphated-galactan required to prepare Poly-Ga is not easy to obtain because *S. binderi* is a very low abundant marine macroalga. In order to avoid this problem, we prepared oligo-carrageenans by acid hydrolysis of commercial pure carrageenans kappa 2, lambda and iota having an average molecular weight of 10 kDa [27]. Oligo-carrageenans kappa 2, lambda and iota consist of approximately 20 units of sulphated galactose linked by alternate β-1,4 and α-1,3 glycosidic bonds. Sulphate groups are located in positions 2, 4 and 6 of the galactose ring with anhydrogalactose units present in some cases (Fig. 1B–D). Specifically, oligo-carrageenan kappa has one sulphate group per disaccharide unit and an anhydrogalactose residue, oligo-carrageenan lambda has three sulfate groups per disaccharide unit and oligo-carrageenan iota presents two sulphates groups per disaccaharide unit and an anhydrogalactose residue (Fig. 1B–D). Thus, the degree of sulphatation increase in oligo-carrageenans lambda, iota and kappa and only oligo-carrageenans kappa and iota have anhydrogalactose residues. In particular, the structure of oligo-carrageenan lambda is more closely-related to that of Poly-Ga. Thus, Poly-Ga and oligo-carrageenans clearly differ in structure suggesting that these oligosaccharides may bind to specific plasma membrane receptors triggering different signal transduction pathways in plants.

In addition to eliciting defense responses and resistance, plant and seaweed oligosaccharides can stimulate or inhibit plant growth and development [9,25–30]. In particular, oligo-galacturonides and galactoglucomannans inhibit plant growth by interfering with auxin-induced effects [9,28,29] whereas the oligo-alginate Poly-Ma, the oligo-sulphated-galactan Poly-Ga and oligo-carrageenans kappa, lambda and iota stimulate plant growth [25,27,30]. In particular, it was determined that oligo-carrageenans stimulate growth in tobacco plants by enhancing photosynthesis, basal metabolism and cell division, mainly oligo-carrageenan kappa and iota [30].

In this work, we analyzed the ability of oligo-carageenans kappa, lambda and iota to induce protection against pathogens and suppression of infections in tobacco plants. To this end, we initially determined the effect of increasing concentrations, increasing number of treatments and increasing time after treatment on protection against TMV infection in tobacco plants. In addition, we analyzed the ability of oligo-carrageenans to induce protection against the fungus *Botrytis cinerea* and the bacteria *Pectobacterium carotovorum* as well as to induce suppression of the viral, fungal and bacterial infections at systemic level. Furthermore, we analyzed the effect of oligo-carrageenans on PAL and lipoxygenase (LOX) defense enzyme activities as well as on the level of phenylpropanoid compounds with potential antimicrobial activities.

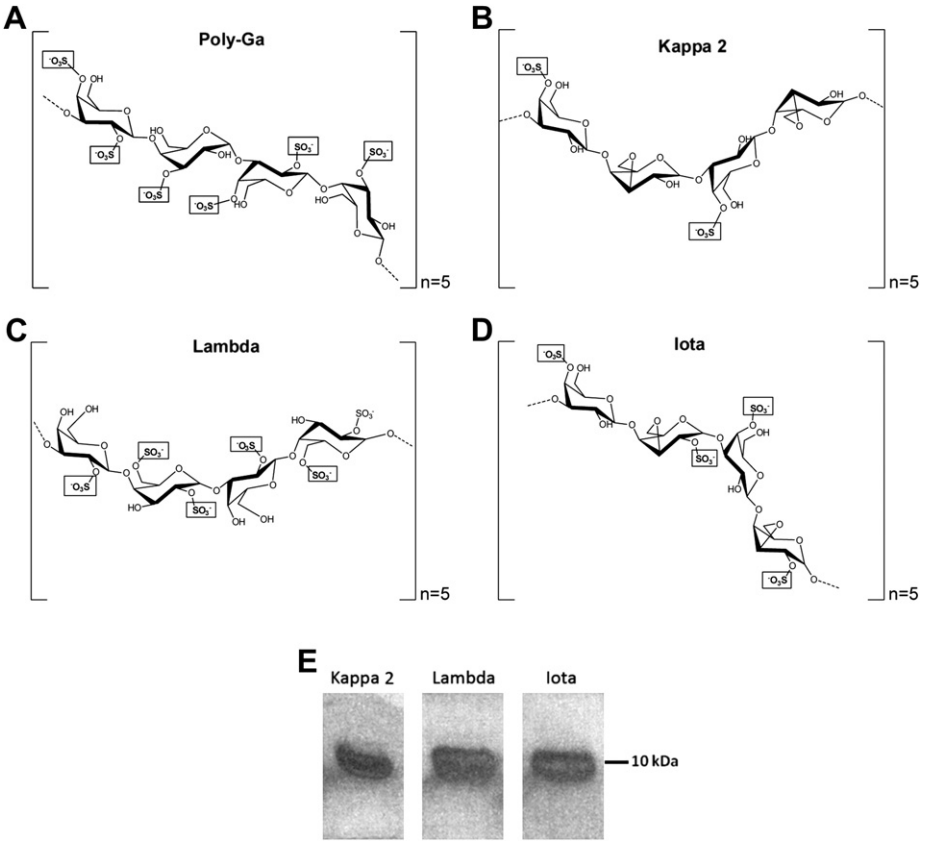

Fig. 1. Structure of Poly-Ga (A) and oligo-carrageenans kappa 2 (B), lambda (C) and iota (D). Size of oligo-carragenans kappa 2, lambda and iota (10 kDa) is indicated by a hyphen (E).