Carbohydrate Polymers 164 (2017) 31–41

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Two banana cultivars differ in composition of potentially immunomodulatory mannan and arabinogalactan

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ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form 10 January 2017
Accepted 21 January 2017
Available online 22 January 2017

Keywords:
Banana
Polysaccharides
Musa sp.
Mannan
Arabinogalactan
Cell wall
Polysaccharide structure
Bioactive polysaccharides

ABSTRACT

Banana (*Musa acuminata* and *M. acuminata* x *M. balbisiana*) fruit cell walls are rich in mannans, homogalacturonans and xylogalacturonan, rhamnogalacturonan-I, and arabinogalactans, certain forms of which is considered to have immunomodulatory activity. The cultivars Nanicão and Thap Maeo represent two widely variants with respect to compositional differences in the forms of these polysaccharides. Nanicão has low amounts of mannan in the water-insoluble and water-soluble fraction. Both cultivars have high amounts of water-soluble arabinogalactan. These commelinoid monocots lack the (1 → 3),(1 → 4)-β-D-glucans of grasses, but Thap Maeo has higher amounts of non-starch glucans associated with wild species than does Nanicão. High amount of callose was found in both cultivars. As immunomodulatory activity is associated with the fine structure and interaction of these polysaccharides, breeding programs to introgress disease resistance from wild species must account for these special structural features in retaining fruit quality and beneficial properties.

1. Introduction

Fruits are rich in soluble and insoluble fiber, comprising complex homo- and heteropolymers, such as pectins and hemicelluloses. These polysaccharides are widely studied because their breakdown and solubilization during fruit ripening yield softness and juiciness to the pulp. Moreover, there is a growing interest in the biological activity of its polysaccharides due to their potential beneficial effect to the human health. Banana fruits contain various compounds, such as lectin, polysaccharides, flavonoids, alkaloids, steroids and glycosides that may produce physiological effects (Onyenekwe, Okereke, & Owolewa, 2013; Scarminio, Fruet, Witaicenis, Rall, & Di Stasi, 2012; Sansone, Sansone, Dias, Shiga, & Nascimento, 2016).

The commercially available bananas are diploids or triploids of *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), or are hybrids of the two species produced with their crossbreeding, resulting in classification groups AAA, AA, AB, AAB and ABB. Cultivar Thap Maeo is a variant of cultivar Mysore and belongs to AAB genomic group, whilst Nanicão belongs to AAA genomic group. Thap Maeo and Mysore cultivars are replacing traditional commercial cultivars because to their higher resistance to Black Sigatoka (black leaf-streak disease), a serious leaf spot disease of bananas that results in yield losses of 33–50% (Mobambo et al., 1993). Although resistance to Black Sigatoka is observed primarily in leaves, changes in the cell-wall composition related to disease resistance might be observed in the fruits, and these differences might impact fruit quality. For instance, cultivar Mysore has anthocyanidin conjugates with cell-wall polymers (regarded to possess strengthening and defense functions against pathogens) whilst, Nanicão lacks of anthocyanidins linked to the cell wall and has higher susceptibility to disease (Bennett et al., 2010).

Chemical composition and structure of the cell wall components are the principal determinants of fruit texture, so characterization of newly introduced cultivars for disease resistance is essential to assure texture is not lost. Polysaccharides for the human health are highly dependent on their structure (degree of branching, molecule size and monosaccharide composition) that confers water

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http://dx.doi.org/10.1016/j.carbpol.2017.01.079
0144-8617/

holding properties, solubility and availability for fermentation by colonic bacteria, and immunomodulatory effects (Blackwood, Salter, Dettmar, & Chaplin, 2000).

In this work, we evaluated the composition and linkage structure of banana fruit wall polysaccharides to assess the impact of introgression of a diverse germplasm to increase disease resistance on the fine structure of wall polysaccharides that impart proper pulp softening and beneficial properties for human health.

2. Material and methods

2.1. Material

Banana fruits *Musa acuminata* cv. Nanicão (genomic group AAA) were purchased from CEAGESP (São Paulo State, Brazil) and *M. acuminata* x *M. balbisiana* cv. Thap Maeo (genomic group AAB) were harvested in the plantation located in Itapetininga (São Paulo State, Brazil). Fruits were classified as ripe, based on ethylene and respiration levels, and amounts of starch and soluble sugars. Ripe banana fruits were peeled, frozen in liquid N_2 and freeze-dried. The freeze-dried materials were ground in mortar and boiled at 70–80 °C in methanol: chloroform (1:1, v/v) for 30 min to remove fat, pigments and to inactivate enzymes. The suspensions were filtered in sintered glass funnel, and the residues washed extensively with acetone to remove pigments. The residues were dried at ambient temperature and kept in a desiccator until use. The defatted material was used to cell wall polysaccharide extraction, enzymatic assay and β-glucan determination.

2.2. Extraction of cell wall polysaccharides

About 1 g of fruit pulp was soaked in 50 mL of 0.08 M sodium phosphate, pH 6.0. Exactly 100 μL of heat-stable α-amylase from *B. licheniformis* (Megazyme International Ireland, EC 3.2.1.1) was added, and the mixture was incubated at 40 °C for 2 h. The pH was then adjusted to 4.5 with HCl, and 200 μL of amyloglucosidase from *A. niger* (Megazyme International Ireland Limited, Ireland, EC 3.2.1.3) was added. The mixture was incubated at 35 °C for 2 h, then adjusted to pH 7.5 with NaOH, and 100 μL of protease of *B. licheniformis* (Megazyme International Ireland Limited, Ireland, EC 3.4.21.14) was added, and the mixture was incubated at 35 °C for an additional hour. The hydrolysate was centrifuged at 9000 *g*, and the pellet was washed extensively with 3 vol. of 50 mL of deionized water. About 100 mL of DMSO (90% in water, v/v) was added to the residue, and the mixture was sonicated in ultra-sound bath for 1 h (2x) and centrifuged at 10,000*g* for 10 min, and the supernatant was discarded. The pellet was washed 3 times with 90% DMSO and then washed exhaustively with water. The residue was re-suspended in water, and freeze-dried and called water-insoluble polysaccharides (WIP).

The aqueous supernatant (hydrolysis buffer) and the wash solutions were combined and brought to 80% EtOH (v/v). The ethanolic mixture was heated to 70 °C for 15 min and ice-cooled to precipitate the polysaccharides and other material, which were pelleted at 9000 *g* and washed extensively with chilled 80% EtOH. The ethanol-insoluble material was re-suspended in water, frozen in liquid N_2 and freeze-dried. This fraction was named water-soluble polysaccharide (WSP).

2.3. Monosaccharide determination and linkage analysis

2.3.1. Carboxyl reduction of uronosyl residues

Duplicate samples of water-soluble and insoluble banana cell wall materials were carboxyl-reduced with $NaBD_4$ after activation with a water-soluble carbodiimide, as described by Kim and Carpita (1992) and modified by Carpita and McCann (1996). A colorimetric assay for uronic acids in the presence of neutral sugars (Filisetti-Cozzi & Carpita, 1991) was used to confirm that the reduction of the carboxyl groups was 95% or greater. For each of sets of materials, two samples of each were used for monosaccharide and linkage analysis.

2.3.2. Monosaccharide distribution

Monosaccharides were obtained from uronosyl-reduced cell wall material (1–2 mg) by hydrolysis in 1 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 90 min. One-half millilitre of *t*-butyl alcohol was added, and the TFA-alcohol mixtures were evaporated in a stream of nitrogen, reduced with $NaBH_4$, and alditol acetates were prepared according to Gibeaut and Carpita (1991). Derivatives were separated by gas-liquid chromatography (GLC) on a 0.25 mm × 30 m column of SP-2330 (Supelco, Bellefonte, PA). Temperature was held at 80 °C during injection then rapidly ramped to 170 °C at 25 °C min^{-1}, and then to 240 °C at 5 °C min^{-1} with a 10 min hold at the upper temperature. Helium flow was 1 mL min^{-1} with split-less injection. The electron impact mass spectrometry (EIMS) was performed with a Hewlett-Packard MSD at 70 eV and a source temperature of 250 °C. The proportion of 6,6-dideuteriogalactosyl was calculated using pairs of diagnostic fragments *m*/*z* 187/189, 217/219 and 289/291 according to the equation described in Kim and Carpita (1992) that accounts for spillover of ^{13}C.

2.3.3. Linkage analyses

For linkage analysis, about 1 mg of polysaccharides dried in a vacuum desiccator over P_2O_5 were suspended in 2 mL of dry DMSO (Pierce silylation grade) were per-*O*-methylated with Li^+ methylsulfinylmethanide, prepared by adding 1.25 mL of 2.5 M *n*-butyllithium in hexanes (Aldrich) to the polysaccharides purged by Argon. Upon evaporation of the hexanes and formation of Li^+ alkoxide ions, methyl iodide (Aldrich) was added according to Gibeaut and Carpita (1991). The per-*O*-methylated polymers were recovered after addition of water to the mixture and partitioning into chloroform. The chloroform extracts were washed five times with a three-fold excess of water each, and the chloroform was evaporated in a stream of nitrogen gas. The partly methylated polymers were hydrolyzed in 2 M TFA for 90 min at 120 °C. One-half millilitre of *t*-butyl alcohol was added, the TFA-alcohol mixture was evaporated in a stream of nitrogen gas, and the sugars were reduced with $NaBD_4$ and acetylated. The partly methylated alditol acetates were separated on the same column used to alditol acetates. After a hold at 80 °C for 1 min during injection, the derivatives were separated in a temperature program of 160 °C to 210 °C at 2 °C min^{-1}, then to 240 °C at 5 °C min^{-1}, with a hold of 5 min at the upper temperature. All derivative structures were confirmed by electron-impact mass spectrometry (Carpita & Shea, 1989).

2.4. Enzymatic digestion of cell wall polysaccharides

2.4.1. Cell wall polysaccharides composition

Suspensions containing 10 mg mL^{-1} of water-soluble and water-insoluble polysaccharides in buffer were incubated with the following glycosidases at 40 °C for 2 h. A blank without enzyme were also carried out in parallel.

2.4.1.1. Xyloglucan determination. Polysaccharide suspensions were hydrolyzed using 10 U mL^{-1} of xyloglucanase from *Paenibacillus* sp. (E.C. 3.2.1.151, E-XEGP, Megazyme International Ireland, Wicklow, Ireland) in 100 mM sodium acetate buffer, pH 5.5.

2.4.1.2. Mannan determination. Polysaccharide suspensions were hydrolyzed using 3.9 U mL^{-1} of *endo*-(1 → 4)-β-D-mannanase from