Biochemical composition of the pericarp cell wall of popcorn inbred lines with different popping expansion

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ABSTRACT

The popping expansion is a characteristic that is positively related with the quality of popcorn. A positive correlation between the volume of expansion and the thickness of the pericarp, and between the proportion of the opaque/shiny endosperm and the grain weight and volume, were postulated. However, there are no reports in the literature that address the importance of cell wall components in the popping expansion. Here, we investigate the biochemical composition of the pericarp cell walls of three inbred lines of popcorn with different popping expansion. Inbred lines GP12 (expansion volume >40 mL g^{-1}), P11 (expansion volume 30 mL g^{-1}) and P16 (expansion volume 14 mL g^{-1}) were used for the analysis and quantification of monosaccharides by HPAEC-PAD, and ferulic and *p*-coumaric acids and lignin by HPLC. Our hypothesis is that the biochemical composition of the pericarp cell walls may be related to greater or lesser popping expansion. Our data suggest that the lignin content and composition contribute to popping expansion. The highest concentration of lignin (129.74 µg mg^{-1}; 12.97%) was detected in the pericarp cell wall of the GP12 inbred line with extremely high popping expansion, and the lowest concentration (113.52 µg mg^{-1}; 11.35%) was observed in the P16 inbred line with low popping expansion. These findings may contribute to indicating the quantitative trait locus for breeding programs and to developing other methods to improve the popping expansion of popcorn.

1. Introduction

Popcorn [*Zea mays* var. *everta* (Sturtev) L. H. Bailey], classified as a special type of common corn *Zea mays* L., is less studied than some other types of corn. Popcorn is one of the oldest and most popular snacks in the world and is consumed in homes, offices, parks, and social events (Ulloa et al., 2010; Monitor Mercantil, 2019).

The main characteristic that differentiates popcorn from other types of corn is the grain. The pericarp of popcorn has greater thickness and mechanical strength, approximately 1.4 times greater than that of common corn (Hoseney et al., 1983). The heat transfer coefficient in the popcorn kernel is approximately 1.9 times higher than in the common corn kernel, providing a more efficient heat transfer into the grain, so

that the internal temperature increases quickly (Silva et al., 1993). Sweley et al. (2013) suggested that the cell wall matrix of popcorn is more orderly than that of common corn. The grain expands when subjected to temperatures around 180 °C for a short period (Hoseney et al., 1983; Silva et al., 1993; Mishra et al., 2014). In the expansion process, the endosperm becomes gelatinized, subsequently inflated, and becomes spongy due to the internal pressure of the vapor, which can reach 930.8 kPa or 10 atm (Hoseney et al., 1983; Silva et al., 1993). When the pericarp ruptures, the gelatinized endosperm overflows, which solidifies, thus forming the popcorn flower (Hoseney et al., 1983).

The popping expansion is positively related with the quality of the popcorn. The removal of micrometric layers from the pericarp leads to a decrease in the expansion of the grains. Absence of pericarp leads to a

90.4% decrease in grain burst (Hoseney et al., 1983). A positive correlation between the volume of expansion and the thickness of the pericarp, and between the proportion of the opaque/shiny endosperm and the grain weight and volume, were postulated (Silva et al., 1993).

Plant cell walls may present very distinct properties, depending on composition and architecture. Cellulose microfibrils provide strength to support osmotic tension, which controls cell expansion. Hemicelluloses cross-link cellulose microfibrils, intermittently interacting with them (Scheller and Ulvskov, 2010; McFarlane et al., 2014).

In addition to the polysaccharide moiety, the second most abundant component of the cell wall is lignin. It results from the oxidative polymerization of three cannonical phenolic alcohols. Lignin appears after the primary growth and provides the cell wall with hydrophobicity and mechanical strength (Moreira-Vilar et al., 2014). In tissues such as vessels and fibers, the impregnation of lignin seals the cell wall hermetically killing the cell that produced it. Lignin is anchored to the polysaccharides through ferulic acid, a phenolic acid that can polymerize with lignin and ester-link to arabinose branching of arabinoxylans (Oliveira et al., 2015).

The least abundant polymeric component of the type II cell wall is the structural proteins. Their role is not yet clearly established. Hydroxyproline- and proline-rich proteins are the main representatives. They also cross-link with other polymers of the cell wall and are thought to work as a scaffold during the formation of the wall plate and perhaps throughout all the cell's development. Structural proteins also perform a role in cell defense and in controlling cell growth (Lamport et al., 2011).

In common corn, the pericarp represents approximately 5–6% of the dry mass of the grain, and it is composed of hemicellulose (~67%), cellulose (~23%) and lignin (~0.1%) (Paes, 2006). Xylose is the monosaccharide with the highest percentage (50.9%), followed by arabinose (35.6%), galactose (8.2%), glucose (3.5%) and mannose (1.9%) (Yoshida et al., 2014). Hydroxyproline-rich glycoproteins (Hood et al., 1991a; b), proline-rich protein (Vignols et al., 1999) and ferulic acid (Bento-Silva et al., 2018) have also been identified as acting directly on the formation and composition of the cell walls of the common corn pericarp. In popcorn, it was determined that the cellulose matrix increased crystallinity during microwave heating and is responsible for the development of exothermic events (Tandjung et al., 2005). However, there are no other records in the literature that address the importance of cell wall components in the expansion volume of popcorn kernels.

Here, we have investigated the biochemical composition of the pericarp cell wall of three inbred lines of popcorn with different popping expansion. Our hypothesis was that the biochemical composition of the pericarp cell walls may be related to greater or lesser popping expansion of popcorn.

2. Material and methods

2.1. Popcorn inbred lines

Three inbred lines of popcorn (early cycle) with different popping expansion, GP12 (expansion volume >40 mL g^{-1}), P11 (expansion volume 30 mL g^{-1}) and P16 (expansion volume 14 mL g^{-1}) were used for the analysis and quantification of monosaccharides, ferulic and *p*-coumaric acids and lignin.

The experiment was carried out and maintained in a greenhouse (23° 25′S; 51° 57′ W; 550 m). The seeds were planted in polyethylene pots (11 L), containing MECPLANT substrate (MAPA PR09549 10001-D), class "F" soil conditioner, composed of pine bark, vermiculite, a corrective of acidity and macronutrients. Three parts of earth for one part of substrate were used, and three seeds per pot were planted. Thinning was carried out afterwards, maintaining only two plants. The cover fertilization took place in post-emergence with complex fertilizer. Two daily irrigations were made, and the pots were kept clean, with the manual removal of any weeds and insect pests. The experimental design

was completely randomized, formed by 3 lines x 1 collection time (mature) x 5 repetitions (one ear corresponds to one repetition), making a total of 15 treatments. After 33 days of planting, each inbred line was self-pollinated. The ears were collected when they reached physiological maturity (from the 30th day after pollination).

The grains of each inbred line were individually extracted from the ears, and the pericarp from each grain was removed manually, lyophilized in a Christ Alpha 2–4 LD Plus, at −51 °C for 24 horas, and crushed in a AltMix with microspheres to obtain a fine powder. The pericarps were stored in individual polypropylene microtubes (2 mL) and stored in a freezer.

2.2. Analysis and quantification of monosaccharides

The monosaccharides were extracted from the pericarp cell wall following the protocols designed by Gorshkova et al. (1996). The cell wall was prepared with 5.0 mg of powdered pericarp placed in microtubes (2 mL), to which was added 1.5 ml of 80% ethanol; the mixture was kept in a water bath at 80 °C for 20 min. After that period, the microtubes were centrifuged for 10 min at 20,800×*g*. Extraction with 80% ethanol was carried out four times. The supernatants were discarded to remove soluble sugars and other soluble compounds. The precipitate obtained with the alcohol-insoluble residue (AIR) of the cell wall was washed with distilled water and dried at 60 °C for 24 h. The residue cell wall AIR (2 mg) was placed in microtubes (2 ml), and 1.0 mL of 2 N trifluoroacetic acid (TFA) was added for hydrolysis of the cell wall. The mixture was kept for 1 h at 100 °C with gentle agitation. Then, the mixture was kept at room temperature and dried in speed-vac.

The hydrolyzed dry pericarp was dissolved in 1 mL of MilliQ water and filtered through a Millipore filter (20 μm mesh). The monosaccharide solution was analyzed in a chromatograph (HPAEC-PAD) on a CarboPac SA10 column (ICS 5000, Dionex-Thermo®) using a mixture of 99.2% water and 0.8% (v/v) 150 mM NaOH as eluent (1.0 mL min^{-1}). Monosaccharides were detected with a post-column addition of 500 mM NaOH (1.0 mL min^{-1}).

2.3. Analysis and quantification of ferulic and p-coumaric acids

The analyses of ferulic and *p*-coumaric acids were carried out following the protocol designed by Ascensao and Dubery (2003). The pericarp flour of the corn kernels (0.1 g) was macerated with 4 ml of 50% methanol. Then, the samples were kept in a water bath at 80 °C for 90 min and vortexed after 30 min. The samples were cooled in an ice bath and centrifuged at 1000×*g* for 15 min. The supernatant was discarded, and 1 ml of 50% methanol was added to the samples, which were centrifuged again under the same conditions. The supernatant was again discarded, and 1 ml of 50% methanol was added to the samples, which were centrifuged at 1000×*g* for 15 min. The supernatant was discarded and the precipitate was dried at 60 °C for 24 h. After that, 5 mL of 0.5 M NaOH was added to the precipitate. The samples were kept at 96 °C for 120 min and cooled at room temperature in an ice bath for 20–30 min.

The acidification of the samples was done with 5 M HCl until reaching pH 2.0. Then, the samples were centrifuged at 1000×*g* for 15 min and 6 ml of ethyl ether was added, followed by vigorous stirring of the samples. After phase separation, the ether fraction was removed, which was dried and resuspended in a mobile phase. The samples were filtered through a Millipore filter (20 μm mesh) and analyzed by Shimadzu® high performance liquid chromatography (HPLC). The components were separated on a C18 column (250 mm × 4.6 mm, 5 mm; Supelco Discovery®). The mobile phase used was methanol/acetic acid 4% in water (30/70, v/v), with a flow of 1.0 mL min^{-1} for 20 min with the oven at 40 °C. The quantification of ferulic acid and *p*-coumaric acid was performed at 322 nm and 309 nm, respectively.

2.4. Analysis and quantification of lignin

Quantification of lignin was performed after successive washes of the cell wall as described by Ferrarese et al. (2002). The ground pericarp (300 mg in 5 ml Falcon tubes) was washed three times with 50 mM phosphate buffer pH 7.0. After each wash, centrifugation was carried out at $1400 \times g$ for 10 min. The supernatant was discarded and the precipitate washed three more times with Triton® X-100 1% (v/v) in 50 mM phosphate buffer pH 7.0. After each wash, centrifugation was carried out at $1400 \times g$ for 10 min. Then, two washes were done with 1 M NaCl in pH 7.0 buffer and, after each wash, centrifugation at $1400 \times g$ was performed for 10 min. After centrifugation, two more successive washes were done with distilled water (7 mL) followed by centrifugation at $1400 \times g$ for 10 min. Finally, two washes with acetone (P.A.) were performed, followed by centrifugation at $1400 \times g$ for 10 min. The precipitate (protein-free fraction of the cell wall) was dried at 60 °C for 24 h.

Acetyl bromide 25% diluted in cold acetic acid was added (0.5 mL) in 20 mg of the protein-free wall fraction. The mixture was incubated in a water bath for 30 min at 70 °C. After incubation, the samples were quickly cooled in an ice bath and 0.9 mL of 2 M NaOH was added to stop the reaction. Then, 0.1 mL of hydroxylamine-HCl (7.5 M) and 5 mL of cold acetic acid (P.A.) were added. The samples were centrifuged at $1400 \times g$ for 5 min, and the supernatants were analyzed by spectrophotometry at 280 nm.

The monomers of lignin were analyzed employing the nitrobenzene oxidation method, according to Scalbert and Monties (1986). The protein-free fraction of the cell wall (50 mg) was placed in a stainless-steel container containing 0.9 mL of 2 M NaOH and 100 µL of nitrobenzene and maintained at 170 °C for 150 min. After oxidation, the samples were kept at room temperature and were washed (twice) with deionized water, and the solution was poured into a separating flask. After that, 5 mL of chloroform was added and the samples were manually agitated and acidified with 350 µL of 5 M HCl. The mixture was homogenized and the ether phase containing the organic extracts was removed. The chloroform was added twice more (5 mL per extraction) and the ether phase containing the organic extracts was collected. The organic extracts were combined, dried, and resuspended in methanol.

The organic extracts suspended in methanol were filtered in a Millipore filter (20 µm mesh) and analyzed by Shimadzu® high performance liquid chromatography (HPLC). The components were separated by a C18 column (250 mm × 4.6 mm, 5 mm; Supelco Discovery®). The mobile phase used was methanol/acetic acid 4% in water (20/80, v/v), with a flow of 1.2 mL min^{-1} for 20 min. The quantifications of the p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monomers were performed at 290 nm using the corresponding standards. The results were expressed as the percentage of the sum of the monolignols.

2.5. Data analysis

We performed the analysis of variance and one way Tukey test using SASM-Agri software (Canteri et al., 2001) to compare the data of the quantification of monosaccharides, ferulic and p-coumaric acids, and lignin.

3. Results and discussion

Xylose was the monosaccharide detected in the highest concentration in the pericarp cell wall in the three inbred lines of popcorn, GP12, P11, and P16, while rhamnose was detected in the lowest concentration (Table 1). However, no significant difference was observed in the concentrations of the seven identified monosaccharides (xylose, arabinose, galactose, glucose, mannose, fructose, and rhamnose) between inbred lines GP12, P11, and P16 with different expansion volume (Table 1Supplentary). The types and amounts of monosaccharides that make up hemicellulose and pectin in the pericarp cell wall are not different in inbred lines with different popping expansion. On the other

Table 1

Monosaccharides obtained by TFA (trifluoroacetic acid) from the pericarp cell wall in the three lineages of popcorn (GP12, P11, and P16) with different expansion capacities (EC).

Monosaccharides (µg·mg^{-1})	Lineages		
	GP12	P11	P16
Xylose	165.61 ± 4.62	155.85 ± 9.68	175.72 ± 10.90
Arabinose	98.92 ± 3.32	94.02 ± 5.73	101.82 ± 4.55
Galactose	43.27 ± 2.65	44.38 ± 2,93	43.57 ± 2.45
Glucose	9.18 ± 1.19	12.26 ± 0,87	11.88 ± 1.36
Mannose	1.80 ± 0.27	1.97 ± 0.22	2.12 ± 0.15
Fucose	1.13 ± 0.13	1.10 ± 0.09	1.35 ± 0.15
Rhamnose	1.02 ± 0.13	0.91 ± 0.08	0.87 ± 0.08

GP12: very high EC; P11: high EC; P16: low EC. Statistical analysis was conducted for each row with SASM-Agri software (Canteri et al., 2001) for analysis of variance and subsequent one-way Tukey test.

hand, our study showed that the concentrations of xylose, arabinose, and glucose in the pericarp cell wall in the three inbred lines of popcorn are three or more times greater than in the pericarp of common maize reported by Yoshida et al. (2014). Besides, the galactose concentration in the pericarp cell wall in the three inbred lines of popcorn is five times greater than the concentration of galactose in the pericarp of common maize, while the concentration of mannose was similar in popcorn and common maize (Yoshida et al., 2014).

The concentration of ester-linked ferulic acid was higher in the P16 inbred line with low popping expansion than in the P11 inbred line with high popping expansion (Table 2). However, it was not higher than the concentration of ferulic acid detected in the GP12 inbred line, which is the one that presents the highest popping expansion. Ferulic acid promotes tissue cohesion, restricts cell expansion, contributes to the mechanical properties of mature tissues, and protects plants against pathogens (Tandjung et al., 2005; Chateigner-Boutin et al., 2016; Lin et al., 2017; Zhang et al., 2019). Ferulic acid acts as a hub connecting the polysaccharide moiety (where it is ester-linked) to lignin, where it forms C–C and ether-bonds (Hassan and Burton, 2018). The higher concentration of ferulic acid in the P16 inbred line, therefore, seems to be related to its lower content of lignin. When less lignin is available to polymerize with ferulic acid, more ferulic acid can be extracted by saponification.

The p-coumaric acid was found in a lower concentration than ferulic acid in the three popcorn inbred lines (Table 2). The highest concentration of p-coumaric acid (4.75 mg g^{-1}) was detected in the pericarp cell wall of the GP12 inbred line with the highest popping expansion, and the lowest concentration (2.98 mg g^{-1}) was observed in the P16 inbred line with low popping expansion. Concentrations of p-coumaric acid between 3.5 and 3.3 mg g^{-1} have been reported in common corn bran (Buranov and Mazza, 2009). Monolignols has been described as exported to the apoplast ester-linked p-coumaric acid as a means to reduce its reactivity. As a consequence, lignified cell walls of maize can

Table 2

Ferulic and p-coumaric acid content (mg·g^{-1}) found in the pericarp cell walls of the popcorn lineages (GP12, P11 and P16) with different expansion capacities (EC).

Acid	Lineages		
	GP12	P11	P16
Ferulic	15.06 ± 1.40[ab]	13.71 ± 1.04[b]	18.07 ± 0.57[a]
p-coumaric	4.75 ± 0.38[a]	3.95 ± 0.35[ab]	2.98 ± 0.17[b]

GP12: very high EC; P11: high EC; P16: low EC. Means followed by the same lowercase letters on the same line do not differ at 5% probability, by the Tukey test. Statistical analysis was conducted for each row with SASM-Agri software (Canteri et al., 2001) for analysis of variance and subsequent one-way Tukey test. [Ferulic acid MS = 199.96*; p < 0.05 by F test; CV = 13.6%. p-coumaric acid MS = 125.33*; p < 0.05 by F test; CV = 16.2%].

contain up to 3% of *p*-coumarate (Schäfer et al., 2019) and, in shoots of adult common maize, *p*-coumaric acid constitutes 15–18% of lignin (Hatfield et al., 2008). Therefore, the variation in *p*-coumaric acid content in the inbred lines must reflect the variations in lignin. A thicker and more integral pericarp has been described as essential for the popcorn grain burst (Hoseney et al., 1983; Silva et al., 1993). The concentration of *p*-coumaric acid is, thus, positively related with the popping expansion of popcorn.

The highest concentration of lignin (129.74 μg mg^{-1}; 12.97%) was also detected in the pericarp cell wall of the GP12 inbred line with extremely high popping expansion, and the lowest concentration (113.52 μg mg^{-1}; 11.35%) was observed in the P16 inbred line with low popping expansion (Fig. 1). A higher amount of lignin in the pericarp may indicate greater resistance and confer greater capacity for expansion of the grain (Hoseney et al., 1983; Silva et al., 1993; Mishra et al., 2014).

The percentage of the *p*-hydroxyphenyl (H) and guaiacyl (G) monomers was higher in the pericarp cell wall of the GP12 inbred line than in the pericarp cell wall of P11 and P16 inbred lines. The higher percentage of syringyl (S) monomer was observed in the P11 inbred line (Table 3). The S/G ratio was lower in the GP12 inbred line, while similar ratios were observed in the P11 and P16 inbred lines. The proportion of lignin S and G subunits determines the degree and nature of the polymeric crosslink. The monomer G can perform up to three parallel linkages and, therefore, form branching lignin filaments. In turn, S monomer can only form two linkages and tends to form only linear lignin filaments. Although the more filamentous lignin is associated with hardwood, an increase in G monomers leads to a highly cross-linked lignin (Ferrer et al., 2008) that presents greater thermal stability (Poletto, 2016). The lowest S/G ratio detected in the pericarp cell wall of the GP12 inbred line indicates that this greater thermal stability can contribute to the popping expansion, by supporting heat for more time before the grains burst.

4. Conclusion

Our data strongly suggest that besides the thickness of the endocarp, the lignin content and composition contribute to the popping expansion. These findings confirm our hypothesis that the biochemical composition of the pericarp cell walls is related to greater popping expansion of popcorn. This may contribute to suggesting a QTL for breeding programs as well as developing other methods to improve the popping expansion of popcorn.

Funding

CRediT authorship contribution statement

Clério Valentin Damasceno Junior: Conceptualization, Formal analysis, conducted experiments of analysis and quantification of monosaccharides, conducted experiments of analysis and quantification of ferulic and p-coumaric acids, and lignin, Funding acquisition, CAS developed the three popcorn inbred lines with different popping expansion: very high (GP12), high (P11) and low (P16) popping expansion. conducted the planting and maintenance of popcorn inbred lines, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Samantha Godoy:** Formal analysis, conducted experiments of analysis and quantification of monosaccharides, conducted experiments of analysis and quantification of ferulic and p-coumaric acids, and lignin, CAS developed the three popcorn inbred lines with different popping expansion: very high (GP12), high (P11) and low (P16) popping expansion. conducted the planting and
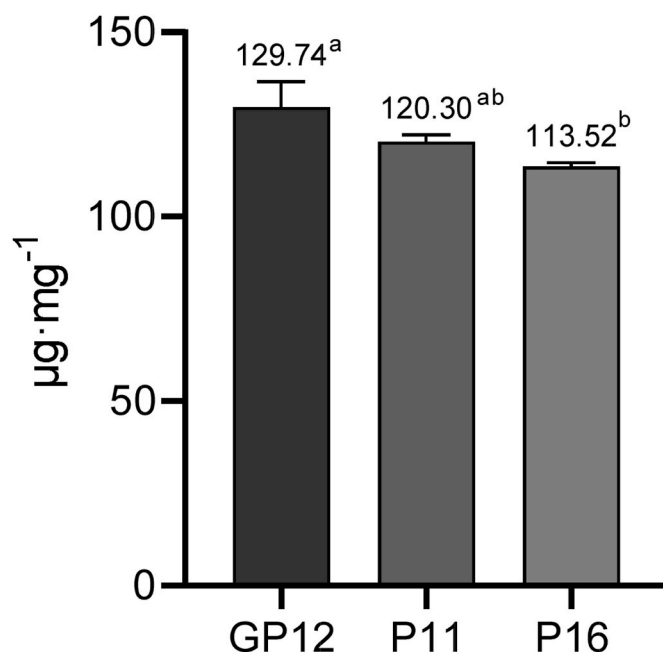

Fig. 1. Concentration of lignin from the pericarp of the popcorn maize lineages GP12 (very high expansion volume), P11 (high expansion volume) and P16 (low expansion volume) by 5% Tukey test. Statistical analysis was conducted for each row with SASM-Agri software (Canteri et al., 2001) for analysis of variance and subsequent one-way Tukey test. [MS = 331.75*; p < 0.05 by F test; CV = 7.58%].

Table 3
Percentage of lignin monomers (S: syringyl; G: guaiacyl; H: *p*-hydroxyphenyl) obtained from the pericarp cell walls of popcorn lineages GP12 (very high expansion capacity) P11 (high expansion capacity) and P16 (low-capacity expansion).

Lineages	Monomers (%)			
	H	G	S	S/G
GP12	9.0	81.0	10.0	0.12
P11	7.0	71.0	22.0	0.30
P16	7.0	72.0	21.0	0.29

maintenance of popcorn inbred lines, Software, Visualization. **Adriana Gonela:** Conceptualization, Data curation, Formal analysis, Funding acquisition, conducted experiments of analysis and quantification of monosaccharides, Project administration, Resources, Supervision, Validation, Visualization. **Carlos Alberto Scapim:** Visualization. **Adriana Grandis:** Data curation, Project administration, Supervision, Validation, Visualization. **Wanderley D. dos Santos:** Project administration, Supervision, Validation, Visualization. **Claudete Aparecida Mangolin:** conducted experiments of analysis and quantification of ferulic and p-coumaric acids, and lignin, Software, Visualization. **Marcos S. Buckeridge:** Formal analysis, conducted experiments of analysis and quantification of monosaccharides, Visualization. **Maria de Fátima P.S. Machado:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.crfs.2021.12.011.

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