Phytochemistry 72 (2011) 365–371

Contents lists available at ScienceDirect

Phytochemistry

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

Cell wall composition as a maize defense mechanism against corn borers

Jaime Barros-Rios [a,*], Rosa A. Malvar [a], Hans-Joachim G. Jung [b], Rogelio Santiago [a]

[a] Misión Biológica de Galicia (CSIC), Apartado 28, E-36080 Pontevedra, Spain
[b] USDA-Agricultural Research Service, Plant Science Research Unit and Univ. of Minnesota, Dept. of Agronomy and Plant Genetics, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

ARTICLE INFO

Article history:
Received 30 April 2010
Received in revised form 13 December 2010
Available online 1 February 2011

Keywords:
Zea mays
Ostrinia nubilalis
Sesamia nonagrioides
Resistance
Hydroxycinnamates
Polysaccharides
Lignin
Pith
Rind

ABSTRACT

European and Mediterranean corn borers are two of the most economically important insect pests of maize (*Zea mays* L.) in North America and southern Europe, respectively. Cell wall structure and composition were evaluated in pith and rind tissues of resistant and susceptible inbred lines as possible corn borer resistance traits. Composition of cell wall polysaccharides, lignin concentration and composition, and cell wall bound forms of hydroxycinnamic acids were measured. As expected, most of the cell wall components were found at higher concentrations in the rind than in the pith tissues, with the exception of galactose and total diferulate esters. Pith of resistant inbred lines had significantly higher concentrations of total cell wall material than susceptible inbred lines, indicating that the thickness of cell walls could be the initial barrier against corn borer larvae attack. Higher concentrations of cell wall xylose and 8-*O*-4-coupled diferulate were found in resistant inbreds. Stem tunneling by corn borers was negatively correlated with concentrations of total diferulates, 8-5-diferulate and *p*-coumarate esters. Higher total cell wall, xylose, and 8-coupled diferulates concentrations appear to be possible mechanisms of corn borer resistance.

1. Introduction

Maize (*Zea mays* L.) resistance to European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and Mediterranean corn borer (MCB), *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) has been extensively evaluated because these are two of the most economically important insect pests of maize production in North America and southern Europe, respectively (Gianessi et al., 2003; Velasco et al., 2007). Several physical and biochemical characteristics (general plant traits, antibiotic compounds, repellent or attractant metabolites, etc.) have been studied as constitutive resistance mechanisms to corn borers (Malvar et al., 2008). There has also been significant research directed toward cell wall concentration, composition, and structure as possible resistance traits to corn borers (Buendgen et al., 1990; Santiago et al., 2006a). Resistance to ECB has been related to cell wall polysaccharide and lignin content of maize stalks (Ostrander and Coors, 1997; Martin et al., 2004).

Cell wall bound forms of hydroxycinnamic acids in cereals consist largely of *p*-coumaric (*p*-CA) and ferulic (FA) acids (Hartley and Jones, 1978). All FA is ester linked to arabinoxylan and some of these FA molecules form additional covalent linkages to lignin (Ralph et al., 1992). While some *p*-CA is similarly esterified to arabinoxylan, the majority of *p*-CA is esterified to syringyl units of lignin (Ralph et al., 1994). Formation of diferulates (DFA) and higher oligomers of FA has been shown to cross link arabinoxylan chains (Bunzel, 2010). The deposition of DFAs in various tissues (kernel, leaf, pith, rind and nodes) has been shown to be associated with resistance to pests such as ECB (Bergvinson et al., 1997), Southwestern corn borer (*Diatraea grandiosella* Dyar) and sugarcane borer (*Diatraea saccharalis* Fabricius) (Ramputh, 2002), maize weevil (*Sitophilus zeamais* Motschulsky) (García-Lara et al., 2004), MCB (Santiago et al., 2006a,b, 2008) and diseases such as *Gibberella* stalk and ear rot (*Fusarium graminearum* Schwabe) (Bily et al., 2003; Santiago et al., 2007). Several quantitative trait loci (QTL) for concentrations of cell wall esterified *p*-CA, esterified and etherified FA, and esterified 5-5 DFA and 8-*O*-4 DFA that were identified by Barrière et al. (2008) also co-localized with QTLs identified in other studies for ECB damage (Cardinal et al., 2001).

Previous work of our group identified sources of maize resistance to corn borer (Butrón et al., 1999; Ordás et al., 2002). We have shown that resistant inbred lines contained significantly higher concentrations of DFA than susceptible lines (Santiago et al., 2006a,b), however, it is unknown if other changes in cell wall concentration or composition are associated with corn borer resistance of these maize lines. Our current objectives were (i) to determine the concentration of the cell wall polysaccharide components, lignin concentration and composition, and hydroxycinnamates (*p*-CA, FA, and DFA) in pith and rind tissues of resistant

* Corresponding author. Tel.: +34 986 854 800; fax: +34 986 841 362.
E-mail address: jbarros@mbg.cesga.es (J. Barros-Rios).

0031-9422/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2011.01.004

and susceptible inbred lines of maize, and (ii) to examine the role of these cell wall components as maize constitutive defense mechanisms against corn borers.

2. Results and discussion

2.1. Pith vs. rind cell-wall composition

Pith and rind tissues were both analyzed in order to gather comprehensive data regarding maize stalk cell walls, however, the following data presentation is focused primarily on pith tissue because that is the tissue where corn borer larvae tunnel and feed. It has been assumed that the cell wall polysaccharides are indigestible to Lepidopterans larvae, which utilize mainly soluble carbohydrates and proteins as nutrients (Terra et al., 1987). As expected, most of the cell wall components were found at higher concentration when calculated on a dry matter (DM) basis in rind than pith tissues with the exception of galactose, uronic acids, and total DFAs (Supplemental Table S1). Compositional analysis showed that cell wall components accounted for 320 g kg^{-1} DM in pith tissue and 580 g kg^{-1} DM in rind tissue (Supplemental Table S1), indicating higher concentrations of cytoplasmic components (proteins, lipids, ash, organic acids, etc.) in pith tissue. This greater concentration of total cell wall material in rind may explain why corn borer larvae, particularly MCB larvae, enter the stem through the base of the internode where the intercalary meristem is located and the cells are the least developed (Santiago et al., 2003; Barros et al., 2010). While total cell wall concentration was numerically higher in resistant lines for both tissues, the statistical contrast of resistant vs. susceptible lines was only significant for pith tissues. All inbred lines had higher rind cell wall concentrations than the cell wall concentration in pith tissue of resistant lines.

In pith tissues glucose was the predominant constituent of the cell wall polysaccharides (58%), followed by xylose (27%), uronic acids (6%), arabinose (5%), mannose (2%), and galactose (2%) (Supplemental Table S2). Arabinoxylan is the major hemicellulose component in most cereal cell walls, although there are large differences in the degree of arabinose substitution among tissues (Hazen et al., 2003). Therefore, the high values for xylose and arabinose probably represent a high content of arabinose-substituted xylan (arabinoxylan), although some arabinose may also stem from pectic arabinan side-chains. Lignin content represented 17.8% of the total cell wall mass (Supplemental Table S2). Syringyl units (S) were more common than guaiacyl units (G) in lignin. Pith and rind tissues had approximately the same S/G ratio (1.48 and 1.51, respectively), which was similar to previous data for mature maize stalks (Lapierre, 1993).

Hydroxycinnamates such as FA and *p*-CA are minor components in plant cell walls (Bunzel, 2010). Our analyses found that ester bound *p*-CA was the most abundant hydroxycinnamic acid detected in maize pith and rind tissues (2.1% and 2.7% of total cell wall, respectively), with FA (ester plus ether bound) also present in significant quantities (1.4% and 1.5% of total cell wall). Together these monomeric phenolics accounted less than the 5% of the total cell wall in both tissues (Supplemental Table S2). Three different DFAs were identified and quantified: 8-5-DFA, 8-*O*-4-DFA, and 5-5-DFA. The 8-5-DFA was calculated as the sum of 8-5-open or non cyclic and 8-5-benzofuran or cyclic forms, because it has been reported that 8-5-non-cyclic form may be a product after alkaline hydrolysis of the cyclic form, the only naturally occurring in plant cell walls (Ralph et al., 1994), The dimers in order of abundance for pith tissue were 8-5-DFA (52% of total DFAs measured), 8-*O*-4-DFA (35%), and 5-5-DFA (13%). Rind tissue differed with the most abundant dimer being 5-5-DFA (41%), followed closely by 8-5-DFA (39%), and 8-*O*-4-DFA (20%) present in lower proportion (Supplemental Table S2). Overall, DFAs only accounted for 0.1% and 0.0002% of mature maize pith and rind tissue cell walls, respectively.

A higher lignin concentration was observed in rind than pith tissue (70% higher) (Supplemental Table S1). This fact may partially account for lower concentrations of esterified DFAs in the rind than pith (92% less) because some DFA molecules become cross liked to lignin through ether and other covalent linkages, such as occurs for FA (Ralph et al., 1992). Any DFAs that were ether linked to lignin could not be determined by the alkaline hydrolysis method employed in the current study.

2.2. Environmental effects

Concentrations on a DM basis of most pith cell wall components were significantly different between locations, except for *p*-CA, FA ethers, and total DFA (data not shown). These differences in cell wall component abundance were probably due to the different growth environments at the two locations. Usually Zaragoza is warmer and drier than Pontevedra during the growing season. Although temperature profiles in 2008 were similar for the locations, Pontevedra had greater precipitation overall (data not shown). Pith tissue at Zaragoza had higher total cell wall and individual cell wall component concentrations, except Klason lignin and 5-5-DFA which were not different between locations (data not shown). As total cell wall concentration differed between locations, we examined the data on a cell wall basis to determine if composition of the walls varied independently of total wall accumulation. Zaragoza still had higher concentrations of uronic acids, arabinose, galactose, and glucose on a cell wall basis, however, Klason lignin was greatest at Pontevedra (data not shown). Lower stem internodes should have completed cell wall development by 30-d post-flowering (Jung, 2003), therefore, differences between locations suggest that growing conditions affected cell wall development.

Based on biomass productivity, which did not differ based on measured of plant height, neither location was more stressful than the other (data not shown). Corn borer tem tunneling was different between the two locations, with MCB showing larger tunnels at Pontevedra than ECB tunneling at Zaragoza (Table 2). However, this difference in tunneling could reflect differences between the damage potential of these two corn borer species rather than environmental impacts on cell wall development.

Although differences for locations were confirmed for most cell wall components, a genotype × location interaction was only significant for uronic acid concentration. The interaction was due to the EP42 inbred line which had higher uronic acid concentration at Pontevedra than at Zaragoza (data not shown). Also, the A509 inbred line had the second highest uronic acid concentration at Zaragoza but the lowest concentration at Pontevedra.

2.3. Genotypic effects

Analysis by location for stem tunneling data for corn borer damage showed that resistant and susceptible lines differed for length

Table 1
Pedigree and resistance classification of four maize inbred lines used in the current study.

Genotypes	Pedigree	Group[a]
A509	A78 × A109	R
EP39	Fino	R
EP42	Tomiño	S
EP47	(EP4 × A239) $EP4^2$	S

[a] R, resistant; S, susceptible. Resistance classification based on tunneling by ECB and MCB across multiple evaluations (Butrón et al., 1999; Ordás et al., 2002).