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The use of FTIR spectroscopy to monitor modifications in plant cell wall architecture caused by cellulose biosynthesis inhibitors

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Fourier Transform InfraRed (FTIR) spectroscopy is a powerful and rapid technique for analyzing cell wall components and putative cross-links, which is able to non-destructively recognize polymers and functional groups and provide abundant information about their in muro organization. FTIR spectroscopy has been reported to be a useful tool for monitoring cell wall changes occurring in muro as a result of various factors, such as growth and development processes, mutations or biotic and abiotic stresses. This mini-review examines the use of FTIR spectroscopy in conjunction with multivariate analyses to monitor cell wall changes related to (1) the exposure of diverse plant materials to cellulose biosynthesis inhibitors (CBIs) and (2) the habituation/dehabituation of plant cell cultures to this kind of herbicides. The spectra analyses show differences not only regarding the inhibitor, but also regarding how long cells have been growing in its presence.

Why Study Cell Walls?

Plant cell walls constitute the outermost layer of plant cells, providing a protective physical barrier against harsh biotic and abiotic stresses. They determine the shape and the size of the cells, as well as other important properties such as texture, mechanical strength, resistance to pathogenic microorganisms, and the capacity to bind and sequester toxic ions and molecules. The presence of cell walls is a differential feature of plant cells compared with animal cells. Cell walls are determinant constituents of plant cell differentiation and they play an essential role in cell function, controlling cell growth and fate, and providing useful molecules in signal transduction in the case of pathogen attack and other environmental stresses.[1,2]

The study of this structure is of great interest due to its multiple applications, whether considering it as a complete structure or attending its components. Plant cell walls form a part of our daily lives, as timber, paper and the five "F"s: fabric, feed, fibers, food and fuel.

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Cell walls exhibit a certain degree of compositional and structural plasticity, and their components can be reorganized in diverse ways. One important goal for various industries is to obtain engineered plants whose cell walls can be modified in different ways, for example, cell walls that can be more efficiently broken down whilst maintaining their mechanical properties and their capacity to cope with pathogens, for use as improved materials for biofuels.

Prior in-depth knowledge of cell wall designs, their heterogeneity throughout the plant kingdom, and the compositional and structural plasticity of their architecture is required in order to obtain redesigned walls. These chemical and structural features have traditionally been characterized by fractionation of isolated cell walls, followed by different chromatographical techniques, such as thin layer chromatography, paper chromatography, HPLC or more frequently, gas chromatography of the derivatized sugars in each fraction. These analytical methods are time consuming and require relatively large amounts of sample. Furthermore, the solvents and conditions needed to extract and solubilize polysaccharides from the cell wall may produce undesirable reactions or change the structure of the polysaccharides (i.e., remove ester-linked groups). Another disadvantage associated with these methods is loss of information about the environment in which the extracted components were originally located, that is, the fine structure of the cell wall. Different microscopy techniques have been used to avoid these problems, but nevertheless, part of the information about different components is still lost. Nowadays, immunocytochemical techniques constitute a good approach for analyzing cell walls, as an increasing number of antibodies able to recognize significant epitopes has been raised.[3]

The Advantages of FTIR Spectroscopy in Cell Wall Analysis

In contrast to fractionation and related analytical methods, FTIR spectroscopy offers many advantages: (1) the method is a rapid and easy technique for analyzing cell wall components; (2) it requires small amounts of sample; (3) it non-destructively recognizes polymers and functional groups (summarized in **Table 1**); (4) as a consequence, it provides ample information about changes in cell wall components and putative cross-links therein; (5) it is able to provide abundant information about

Table 1. A summary of wavenumbers obtained by FTIR spectroscopy of cell walls and their assignment to main cell wall components

Assigned cell wall component	Wavenumber (cm^{-1})	References
Cellulose	900, 1040, 1060, 1160, 1320, 1367	7, 22, 44
Pectin	952, 1014, 1097, 1104, 1146, 1243	44, 45
Non-esterified uronic acid	1420, 1600–1630	12
Esterified uronic acid	1740	9
Xyloglucan	1041, 1078, 1120, 1317, 1371	44, 45
Arabinose	975	46
Galactose	945	46
Amide I (protein)	1550, 1650	10
Phenolic ring	1515, 1630	7
Phenol	1430	10
Phenolic ester	1720	7, 10

their in muro organization; and (6) it is possible to combine with microscopy and analyze the heterogeneity of different zones of an organ or areas of a cell.

Together, these advantages render FTIR spectroscopy a suitable method to initially characterize or compare a large quantity of cell wall samples. Despite the improvements this technique contributes to cell wall analysis procedures, it should be borne in mind that FTIR methodologies also have their limitations, such as the semiquantitative nature of the analyses, the intricacy and complexity of cell wall spectra and the appreciable overlap in absorption and vibrational coupling between chemical bonds corresponding to different cell wall polymers.

Taking into account that the information that FTIR methodologies provide is incomplete, it should be complemented by the use of other techniques. In this sense some emerging tools to analyze cell walls have been developed in the last years, such as the so-called "high-throughput" platforms. These include nuclear resonance magnetic (NMR), oligosaccharide mass profiling (OLIMP), polyacrylamide gel electrophoresis analysis of oligosaccharides, cell wall binding arrays and comprehensive microarray polymer profiling (CoMPP).[4]

Initially, the application of FTIR methodology was also limited by the large amount of data obtained and by overlapping band components. However, the development of computerized systems and the use of statistical tools have enabled easier processing of the information obtained from the spectra, thus increasing the interest and usefulness of these spectroscopic techniques. Principal components analysis (PCA) has been used to reduce the high dimensionality of the data from the several hundred data points of a spectral set to a lower number of dimensions, facilitating an exploratory analysis of the bulk of data in order to detect internal groupings.[5] The variability within each spectrum in relation to the mean of the population is then represented as a smaller set of values (axes) termed principal components (PC). It is possible to derive a spectrum related to the PC score (named a PC loading) that represents an independent source of spectral variability with respect to all the data. The analysis of loading factors can be used to identify the molecular factors that underlie the grouping or for discrimination of the original data.[6]

The use of PCA in conjunction with FTIR spectroscopy has proved to be an important tool in the study of cell wall structure and composition heterogeneity in a species,[7,8] or among different species,[5,9-11] as well as in the identification and classification of mutants with altered cell wall compositions.[6,12-14] This approach has been also very useful in the characterization of the modifications appearing in this kind of mutants[15-20] or in transgenic plants with altered cell walls[21] and in the modifications to cell wall composition induced by different biotic or abiotic stress factors.[22,23]

FTIR Spectroscopy and Cellulose Biosynthesis Inhibitors (CBIs)

Although different compounds which act as CBIs have been described, to date little is known about their effect on the structure and composition of cell wall polysaccharides.[24] FTIR spectra from cell wall preparations have been used to identify a set of cell wall modifications provoked by different CBIs. In particular, FTIR spectroscopy used in conjunction with multivariate analyses has been very useful in order to characterize cell wall modifications associated with (1) the exposure of cells or complete plants to CBIs and (2) the habituation/dehabituation of plant cell cultures to this kind of herbicides. In this review, we will focus on the advances made in both fields, mainly in recent years.

Using FTIR to Monitor the Effects of CBIs

Based on FTIR microscopy of hypocotyls of etiolated plants, Mouille et al.[12] developed a procedure for the rapid classification and identification of Arabidopsis cell wall mutants and wild type plants treated with several CBIs. Cluster analysis of cell walls from hypocotyls treated with dichlobenil (DCB, 1 and 5 μM) and isoxaben (2 and 4 nM) showed that both inhibitors were grouped together with well known cellulose deficient mutants such as *PRC1*, *KOB1* and *RSW1*. Later, it was confirmed that FTIR spectra from the cell walls of Arabidopsis hypocotyls treated with 4 nM flupoxam also grouped in this cluster.[25] The spectral differences indicated that cell walls from these mutants and CBI-treated seedlings contained less cellulose and more esterified pectic polysaccharides. However, when seedlings were treated with low concentrations of isoxaben (0.1 and 0.05 nM), their cell wall spectra grouped together with wild type and isoxaben resistant mutants (*IXR*).[12,25]

The method developed by Mouille et al.[12] was used to obtain additional evidence on thaxtomin A and ancymidol as CBIs. Cell walls from hypocotyls of plants treated with 50 to 200 μM thaxtomin A were isolated and their spectra clustered with those of hypocotyls treated with high concentrations of isoxaben, DCB and flupoxam, and those of cellulose-deficient mutants.[25-27] Later, ancymidol, previously described as a plant growth retardant primarily affecting gibberellin biosynthesis, was also reported to be capable of inhibiting cellulose synthesis.[28] The action of ancymidol as a CBI was further confirmed using the same procedure:

plants treated with ancymidol and gibberellin clustered with mutants known to be defective in cellulose synthesis or with wild-type plants treated with isoxaben or DCB.[28]

In order to obtain a global overview of the effects of CBIs, bean calluses have been cultured in the presence of different putative or recognized CBIs, at concentrations equal to their respective I_{50} value (concentration of herbicide required to inhibit the increase in dry weight by 50%) (Garcia-Angulo P, et al. unpublished). The herbicides studied were DCB, isoxaben, AE F150944, flupoxam, triazofenamide, compound 1, CGA 325'615, oxaziclomefone and quinclorac. After 30 d, cellulose content was assayed, cell walls were isolated, and their FTIR-spectra obtained and analyzed (García-Angulo P, et al. unpublished). Overall results revealed differences between herbicides. Isoxaben was the only inhibitor that provoked a decrease in the amount of cellulose in the cell wall after such exposure, an effect which was also seen in the PCA applied to the cell wall FTIR spectra, where isoxaben was separated from the rest of the inhibitors and located towards the negative side of PC1 (this PC loading had a strong positive correlation with peaks attributed to cellulose).

Lastly, FTIR has also been used to monitor changes to cell walls in different bean seedling regions (root apical, root differentiation and hypocotyl) after treatment with DCB and isoxaben. (García-Angulo P, et al. unpublished). When digital subtraction of spectra was used, it was observed that both inhibitors provoked similar effects on root differentiation and hypocotyl regions, but different effects on the root apical region. In differentiating and hypocotyl regions, both inhibitors provoked an increase in xyloglucan and uronic acid wavenumbers, and a decrease in cellulose and galactose ones (see **Table 1**). However, in the apical region, spectra from DCB-treated seedlings showed a decrease in cellulose-associated peaks and an increase in uronic acids and xyloglucan wavenumbers, whereas those spectra from isoxaben-treated seedlings showed a decrease in uronic acid peaks, and an increase in galactose, arabinose and xyloglucan signals. These results confirm that the use of CBIs is a valuable tool in understanding different cell wall-related processes, and that FTIR in combination with PCA is a suitable and rapid method to monitor and initially characterize the modifications induced by these CBIs.

FTIR as a tool to analyze cell wall modifications during habituation and dehabituation to CBIs. FTIR has frequently been used in the analysis of plant cell cultures habituated to grow in the presence of lethal concentrations of CBIs. One of the earliest studies was carried out on suspension-cultured tomato cells habituated to grow on 12 μM DCB.[29] In this study, the spectra of non-habituated and habituated cells were compared and it was observed that non-habituated spectra were similar to those obtained from onion parenchyma, while spectra from habituated cells resembled the spectra from polygalacturonic acid. The FTIR results coincided with chemical analyses, both indicating a higher proportion of uronic acids in habituated cells.[29]

Later, the process of habituation of bean callus cells was analyzed, comparing the spectra from non-habituated callus cell walls with those from habituated to different DCB concentrations up to 12 μM callus cell walls.[30] The results obtained for FTIR spectra from habituated cells showed that as the concentration of herbicide increased, so did the peaks corresponding to uronic acids, free carboxylic acid groups and ester links.

Subsequently, a study of DCB habituation and dehabituation (DCB-habituated cells repeatedly subcultured in absence of the inhibitor) of bean cell suspensions was conducted by means of FTIR spectroscopy.[31,32] As in DCB-habituated bean calluses, FTIR spectra from habituated suspension-cultured cells showed increased peaks corresponding to uronic acids, ester linkages and carboxylic linkages, indicating a progressive enrichment in pectins as the level of habituation to the inhibitor increased. Throughout the dehabituation process, peaks assigned to pectins gradually decreased, in such a way that FTIR spectra from long-term dehabituated cell walls resembled the spectra from non-habituated cells. However, even after a long period in absence of the inhibitor (up to 100 subcultures, approximately 4–5 years), spectra from dehabituated cell walls maintained some features which differed from those of non-habituated cells, i.e., lower intensity of some cellulose/hemicellulose related peaks.[32]

Recently, FTIR spectroscopy in conjunction with PCA has also been used to study whether or not DCB habituation and dehabituation processes followed inverse paths, using bean cell suspensions.[33] An interesting feature emerged in this study: although the spectra from cells habituated to high concentrations of DCB were clearly different from all other spectra, the group of spectra from cells habituated to low concentrations of DCB, and the spectra from dehabituated cells which had been grown in the absence of the herbicide for a long time were similar to those from non-habituated cells, but clearly differed between them, indicating that the progression towards habituation followed a different path than that towards dehabituation. Differences seemed to reside in the methyl-esterification degree of pectins, being lower in dehabituated cells than in cells habituated to low DCB concentrations.[33]

As multivariate analysis has proved to be a useful tool for gaining a clearer understanding of differences between collections of FTIR spectra, this approach was also used successfully in bean callus-cultured cells to extensively monitor the DCB-habituation process with regard to differences in DCB concentration and DCB exposure-time.[34] Both PCA and cluster analysis revealed that spectra could be classified into three groups corresponding to different levels of habituation: (1) non-habituated and low level of habituation, (2) intermediate level of habituation and (3) high level of habituation. This grouping is strongly correlated with cellulose content of cell walls. An interesting feature revealed in this study was the fact that modifications to cell wall structure caused by the inhibitor were not only dependant on DCB concentration, but also in how long cells had been growing on the same DCB concentration.

Subsequently, the combination of FTIR spectra and multivariate analysis was used to monitor the habituation process of bean callus-cultured cells to quinclorac.[35] In this case, the habituation was carried out to determine whether quinclorac was a CBI, since previous results on the activity of this herbicide were contradictory

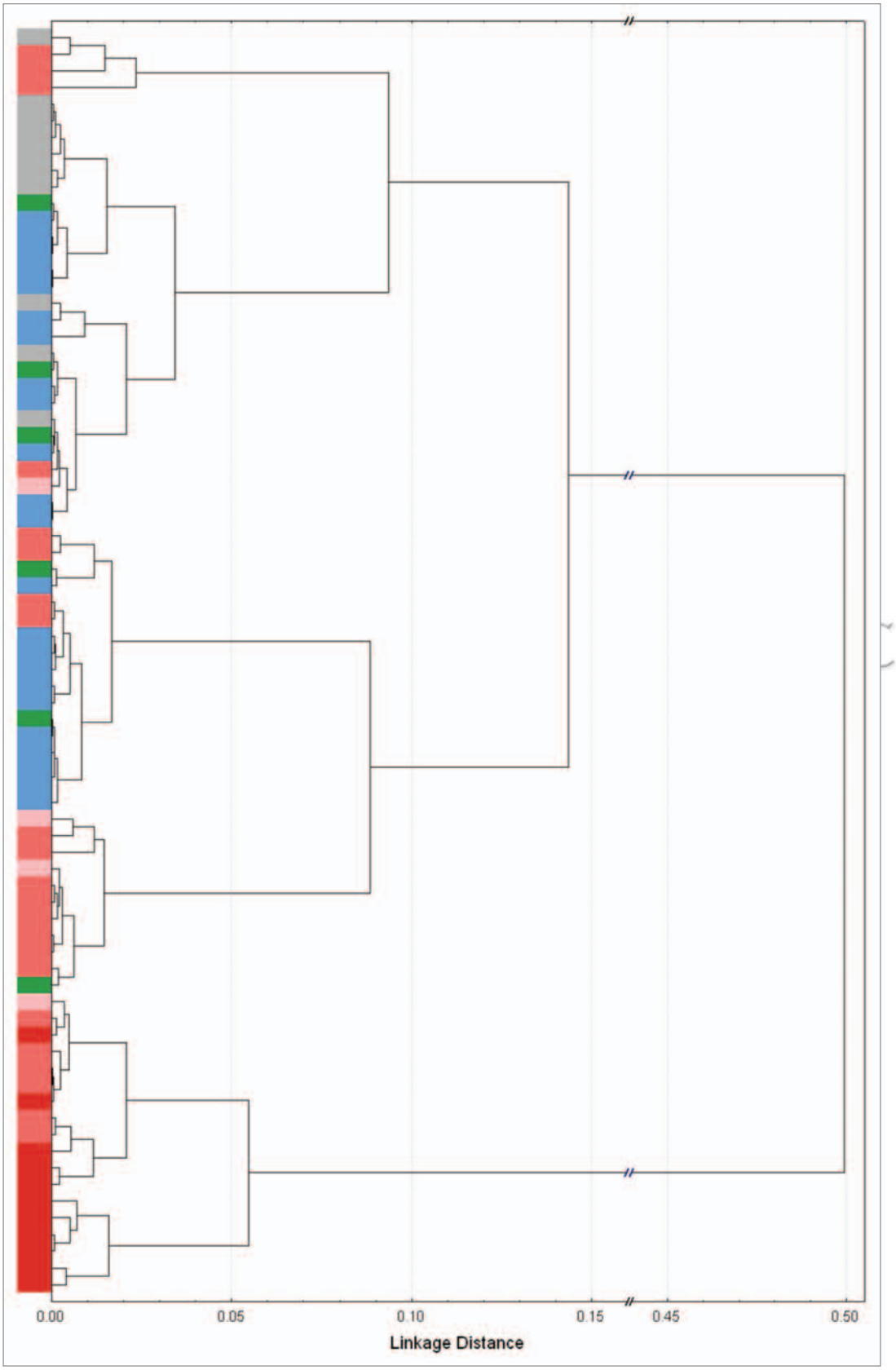

Figure 1. Cluster analysis of spectra from bean cells habituated to different herbicides. For clearer presentation of the results, each type of cell is represented by a different color. ■: Non-habituated cells; ■: Low level of habituation to DCB (0.5 μM for up to 7 subcultures); ■: Intermediate level of habituation to DCB (0.5 μM with more than 7 subcultures, to 4 μM for the first subculture); ■: High level of habituation to DCB (4 μM with more than one subculture, to 12 μM); ■: Quinclorac-habituated cells (10–30 μM); ■: Isoxaben-habituated cells (0.05–0.3 μM).

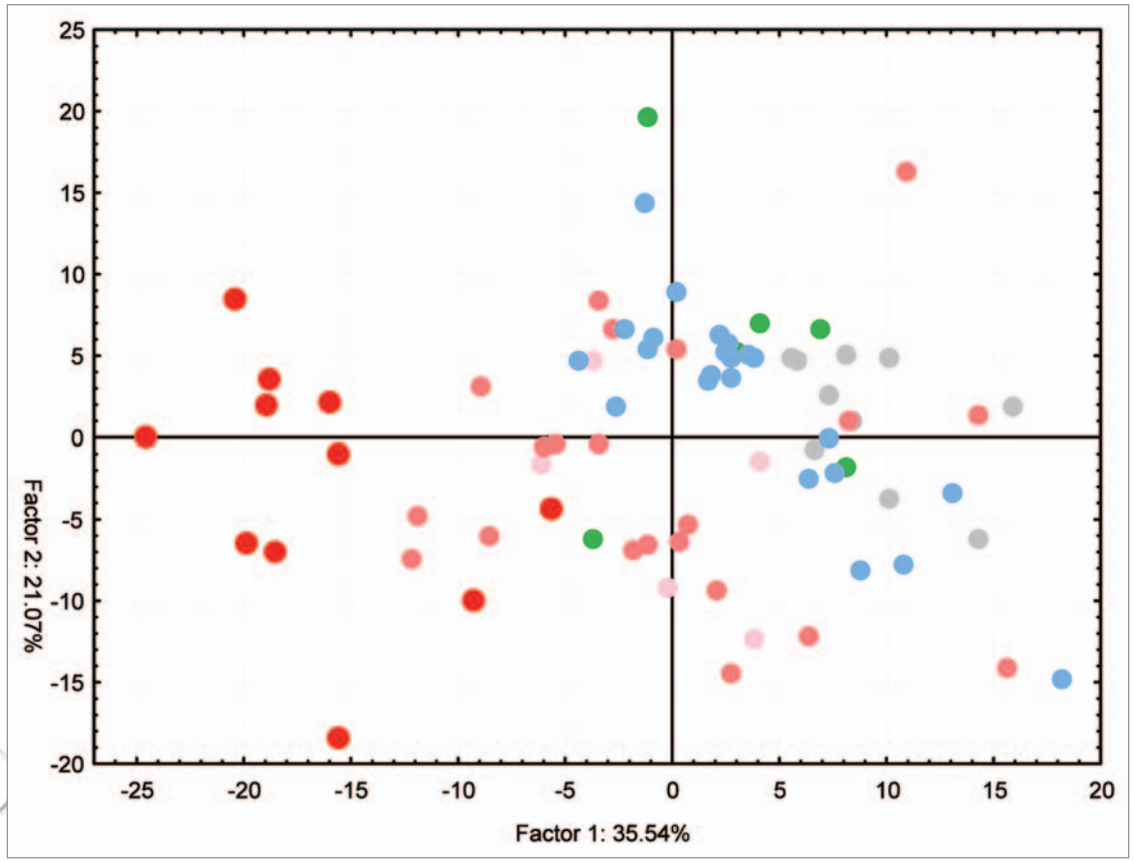

Figure 2. Principal Component Analysis of FTIR spectra from bean cells habituated to different herbicides. ●: Non-habituated cells; ●: Low level of habituation to DCB; ●: Intermediate level of habituation to DCB; ●: High level of habituation to DCB; ●: Quinclorac-habituated cells; ●: Isoxaben-habituated cells. Conditions of habituation are as described in the legend of **Figure 1.**

in this respect.[36-39] If quinclorac inhibited cellulose biosynthesis, it would be likely for quinclorac-habituated cells to present reduced amounts of this polymer and an increase in pectic polysaccharides, in a similar manner to that shown by isoxaben-habituated and DCB-habituated bean cells.[30,31,40] However, the spectra and multivariate analyses performed on cell walls from quinclorac-habituated cells did not detect these features. When compared with non-habituated cells, the main difference was due to a decrease in peaks related to pectins and an enhancement in those assigned to proteins. No significant difference was detected in those wavenumbers related to cellulose and/or hemicelluloses. Chemical analyses confirmed these results, as no significant modification in cellulose content of quinclorac-habituated cells was found. However, the herbicide seemed to affect plant cell walls by decreasing the pectin methyl-esterification degree and, as a consequence, probably disrupting the integration of newly secreted pectins into the cell wall. This proved that quinclorac was not a CBI.[35]

Finally, all these spectra from non-habituated, DCB-, isoxaben- and quinclorac-habituated bean callus-cultured cells were analyzed by means of multivariate analysis. Cluster analysis (**Fig. 1**) divided the spectra into two groups, separated by 0.5 units; the lower group included all the spectra from cells with a high level of habituation to DCB and some spectra from cells with a medium level of habituation to this herbicide. The remaining DCB-habituated cells (medium and low-level of habituation) were grouped together with isoxaben-habituated cells. It is noteworthy that almost all non-habituated cells were grouped together in a single sub-branch in the top section of the cluster. In agreement with the suggestion that quinclorac is not a CBI and provokes a different kind of cell wall modification, no trends similar to those of DCB- and isoxaben-habituated cells were observed in spectra from quinclorac-habituated cells, which were dispersed in all branches of the cluster.

When a PCA was performed on this same group of spectra, a clear gradient was defined along PC1, with low levels of herbicide habituation on the positive side and high levels of habituation on the negative side (**Fig. 2**). This gradient correlated to cellulose/xyloglucan and pectin content, as determined by PC1 and PC2 loadings (**Fig. 3**). It was found that PC1 positively correlated with wavenumbers associated with cellulose/xyloglucan and negatively correlated with those assigned to pectins. Therefore, spectra from cell walls with a higher content in cellulose-hemicelluloses were located on the positive side of PC1, namely, non-habituated cells, isoxaben- and quinclorac-habituated cells and some cells with low or medium levels of habituation to DCB. In contrast, those spectra from cell walls with a higher content of pectic polysaccharides were situated on the negative side of PC1, that is, cells with a high level of habituation to DCB and some with a medium level.

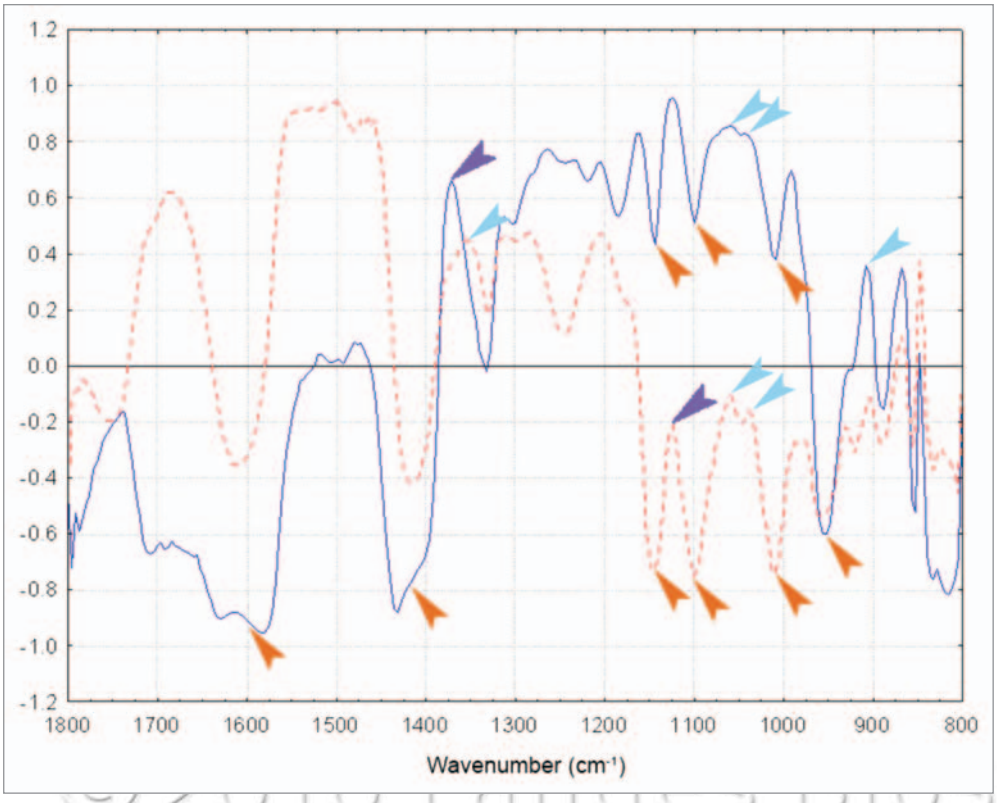

Figure 3. Loadings for PC1 and PC2 corresponding to Principal Component Analysis presented in Figure 2. — PC1; – – – PC2. Arrowheads point to wavenumbers corresponding to pectins (■), cellulose (■) and xyloglucan (■).

Lastly, maize cells have also recently been habituated to grow in the presence of lethal concentrations of DCB.[41] In this case, FTIR monitoring together with multivariate analysis showed not only a reduction in cellulose content in the cell walls of habituated cells, but also an increase in the signals corresponding to aromatic rings. These results were confirmed by other chemical analyses, such as gas and liquid chromatography,[41-43] showing a higher proportion of arabinoxylans cross-linked by phenolics in habituated cells. The analysis also showed that the modifications occurred gradually over the habituation process.

Conclusions

The results described here show that FTIR spectroscopy is an efficient and rapid method for identifying a broad range of structural modifications in cell walls that appear as a consequence of the exposure of cells or complete plants to CBIs and as a result of the habituation/dehabituation of cells to this kind of compounds.

We also have illustrated how the combination of multivariate analyses and FTIR spectra provides complementary information which enables us to establish the different contributions of various components during habituation/dehabituation to CBIs. Thus, it may be concluded that multivariate analysis together with chemical characterization of cell walls is a valid tool for monitoring and analyzing the changes occurring in cell wall composition and architecture associated with the use of CBIs, and could be used in the future for monitoring and identifying cell wall changes related to other abiotic or biotic stresses.

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References

1. Fry SC. Plant Cell Walls. In: Robert K, Ed. Handbook of Plant Science. Chichester, UK: Wiley Interscience 2007:266-76.
2. Seifert GJ, Blaukopf C. Irritable walls: The plant extracellular matrix and signaling. Plant Physiol 2010; 153:467-78.
3. Lee KJD, Marcus SE, Knox JP. Cell wall biology: perspectives from cell wall imaging. Mol Plant 2011; 4:212-9.
4. Persson S, Sørensen I, Moller I, Willats W, Pauly M. Dissection of Plant Cell Walls by High-Throughput Methods. In: Ulvskov P, Ed. Annual Plant Reviews: Plant Polysaccharides, Biosynthesis and Bioengineering. Oxford, UK: Wiley-Blackwell 2011:43-64.
5. McCann MC, Chen L, Roberts K, Kemsley EK, Séné S, Carpita NC, et al. Infrared microspectroscopy: sampling heterogeneity in plant cell wall composition and architecture. Physiol Plant 1997; 100:729-38.
6. Chen L, Carpita NC, Reiter WD, Wilson RH, Jeffries C, McCann MC. A rapid method to screen for cell-wall mutants using discriminant analysis of Fourier transform infrared spectra. Plant J 1998; 16:385-92.
7. Carpita NC, Defernez M, Findlay K, Wells B, Shoue DA, Catchpole G, et al. Cell wall architecture of the elongating maize coleptile. Plant Physiol 2001; 127:551-65.
8. McCann MC, Defernez M, Urbanowicz BR, Tewari JC, Langewisch T, Olek A, et al. Neural network analyses of infrared spectra for classifying cell wall architectures. Plant Physiol 2007; 143:1314-26.
9. McCann MC, Hammouri M, Wilson R, Belton P, Roberts K. Fourier Transform Infrared Microspectroscopy is a new way to look at plant cell walls. Plant Physiol 1992; 100:1940-7.
10. Séné CFB, McCann MC, Wilson RH, Grinter R. Fourier-Transform Raman and Fourier-Transform Infrared Spectroscopy; an investigation of five higher plant cell walls and their components. Plant Physiol 1994; 106:1623-31.
11. Dokken KM, Davis LC, Marinkovic NS. Use of infrared microspectroscopy in plant growth and development. Appl Spectr Rev 2005; 40:301-26.
12. Mouille G, Robin S, Lecomte M, Pagant S, Höfte H. Classification and identification of Arabidopsis cell wall mutants using Fourier-Transform Infrared (FT-IR) microspectroscopy. Plant J 2003; 35:393-404.
13. Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, et al. Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 2007; 104:15572-7.
14. Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, et al. A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis. Curr Biol 2007; 17:922-31.
15. Fagard M, Desnos T, Desprez T, Goubet F, Refregier G, Mouille G, et al. *PROCUSTE1* encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of Arabidopsis. Plant Cell 2000; 12:2409-23.

16. Schindelman G, Morikami A, Jung J, Baskin TI, Carpita NC, Derbyshire P, et al. COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in Arabidopsis. Genes Develop 2001; 15:1115-27.
17. Brown M, Goubet F, Wong FW, Goodacre R, Stephens E, Dupree P, et al. Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. Plant J 2007; 52:1154-68.
18. Daras G, Rigas S, Penning B, Milioni D, McCann MC, Carpita NC, et al. The *thanatos* mutation in *Arabidopsis thaliana* cellulose synthase 3 (*AtCesA3*) has a dominant-negative effect on cellulose synthesis and plant growth. New Phytol 2009; 184:114-26.
19. Bischoff V, Nita S, Neumetzler L, Schindelasch D, Urbain A, Eshed R, et al. *TRICHOME BIREFRINGENCE* and its homolog *AT5G01360* encode plant-specific DUF231 proteins required for cellulose biosynthesis in Arabidopsis. Plant Physiol 2010; 153:590-602.
20. Feraru E, Feraru MI, Kleine-Vehn J, Martinière A, Mouille G, Vanneste S, et al. PIN polarity maintenance by the cell wall in Arabidopsis. Curr Biol 2011; 21:338-43.
21. Zenoni S, Reale L, Tornielli GB, Lanfaloni L, Porceddu A, Ferrarini A, et al. Downregulation of the *Petunia hybrida* α-expansin gene PhEXP1 reduces the amount of crystalline cellulose in cell walls and leads to phenotypic changes in petal limbs. Plant Cell 2004; 16:295-308.
22. Wilson RH, Smith AC, Kacuráková M, Saunders PK, Wellner N, Waldron KW. The mechanical properties and molecular dynamics of plant cell wall polysaccharides studied by Fourier-Transform Infrared Spectroscopy. Plant Physiol 2000; 124:397-406.
23. Martín JA, Solla A, Woodward S, Gil L. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. Tree Physiol 2005; 25:1331-8.
24. Acebes JL, Encina A, García-Angulo P, Alonso-Simón A, Mélida H, Álvarez JM. Cellulose biosynthesis inhibitors: their uses as potential herbicides and as tools in cellulose and cell wall structural plasticity research. In: Lejeune A, Deprez T, Eds. Cellulose: Structure and Properties, Derivatives and Industrial Uses. New York: Nova Publishers 2010; 39-73.
25. Robert S, Mouille G, Höfte H. The mechanism and regulation of cellulose synthesis in primary walls: lessons from cellulose-deficient Arabidopsis mutants. Cellulose 2004; 11:351-64.
26. Scheible W, Fry B, Kochevenko A, Schindelasch D, Zimmerli L, Somerville S, et al. An Arabidopsis mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from Streptomyces species. Plant Cell 2003; 15:1781-94.
27. Bischoff V, Cookson SJ, Wu S, Scheible WR. Thaxtomin A affects CESA-complex density, expression of cell wall genes, cell wall composition, and causes ectopic lignification in *Arabidopsis thaliana* seedlings. J Exp Bot 2009; 60:955-65.
28. Hofmannová J, Schwarzerová K, Havelková L, Boriková P, Petrásek J, Opatrny Z. A novel, cellulose synthesis inhibitory action of ancymidol impairs plant cell expansion. J Exp Bot 2008; 59:3963-74.
29. Wells B, McCann MC, Shedletzky E, Delmer D, Roberts K. Structural features of cell walls from tomato cells adapted to grow on the herbicide 2,6-dichlorobenzonitrile. J Microsc 1994; 173:155-64.
30. Encina A, Moral RM, Acebes JL, Álvarez JM. Characterization of cell walls in bean (*Phaseolus vulgaris* L.) callus cultures tolerant to dichlobenil. Plant Sci 2001; 160:331-9.
31. Encina A, Sevillano JM, Acebes JL, Álvarez J. Cell wall modifications of bean (*Phaseolus vulgaris*) cell suspensions during habituation and dehabituation to dichlobenil. Physiol Plant 2002; 114:182-91.
32. García-Angulo P, Alonso-Simón A, Mélida H, Encina A, Acebes JL, Álvarez JM. High peroxidase activity and stable changes in the cell wall are related to dichlobenil tolerance. J Plant Physiol 2009; 166:1229-40.
33. García-Angulo P, Alonso-Simón A, Mélida H, Encina A, Álvarez JM, Acebes JL. Habituation and dehabituation to dichlobenil. Simply the equivalent of Penelope's weaving and unweaving process? Plant Signal Behav 2009; 4:1069-71.
34. Alonso-Simón A, Encina AE, García-Angulo P, Álvarez JM, Acebes JL. FTIR spectroscopy monitoring of cell wall modifications during the habituation of bean (*Phaseolus vulgaris* L.) callus cultures to dichlobenil. Plant Sci 2004; 167:1273-81.
35. Alonso-Simón A, García-Angulo P, Encina A, Acebes JL, Álvarez J. Habituation of bean (*Phaseolus vulgaris*) cell cultures to quinclorac and analysis of the subsequent cell wall modifications. Ann Bot 2008; 101:1329-39.
36. Koo SJ, Neal JC, DiTomaso JM. 3,7-dichloroquinolinecarboxylic acid inhibits cell-wall biosynthesis in maize roots. Plant Physiol 1996; 112:1383-9.
37. Koo SJ, Neal JC, DiTomaso JM. Mechanism of action and selectivity of quinclorac in grass roots. Pestic Biochem Physiol 1997; 57:44-53.
38. Grossmann K, Kwiatkowski J. The mechanism of quinclorac selectivity in grasses. Pestic Biochem Physiol 2000; 66:83-91.
39. Tresch S, Grossmann K. Quinclorac does not inhibit cellulose (cell wall) biosynthesis in sensitive barnyard grass and maize roots. Pestic Biochem Physiol 2003; 75:73-8.
40. Díaz-Cacho P, Moral R, Encina A, Acebes JL, Álvarez J. Cell wall modifications in bean (*Phaseolus vulgaris*) callus cultures tolerant to isoxaben. Physiol Plant 1999; 107:54-9.
41. Mélida H, García-Angulo P, Alonso-Simón A, Encina A, Álvarez J, Acebes JL. Novel type II cell wall architecture in dichlobenil-habituated maize calluses. Planta 2009; 229:617-31.
42. Mélida H, García-Angulo P, Alonso-Simón A, Álvarez JM, Acebes JL, Encina A. The phenolic profile of maize primary cell wall changes in cellulose-deficient cell cultures. Phytochemistry 2010; 71:1684-9.
43. Mélida H, Álvarez J, Acebes JL, Encina A, Fry SC. Changes in cinnamic acid derivatives 1 associated with the habituation of maize cells to dichlobenil. Mol Plant 2011; In press.
44. Kacuráková M, Capek P, Sasinkova V, Weller N, Ebringerova A. (2000) FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydr Polym 2000; 43:195-203.
45. Coimbra MA, Barros A, Rutledge DN, Delgadillo I. FTIR spectroscopy as a tool for the analysis of olive pulp cell-wall polysaccharide extracts. Carbohydr Res 1999; 317:145-54.
46. Coimbra MA, Barros A, Barros M, Rutledge DN, Delgadillo I. Multivariate analysis of uronic acid and neutral sugars in whole pectic samples by FT-IR spectroscopy. Carbohydr Polym 1998; 37:241-8.