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Simply the equivalent of Penelope's weaving and unweaving process?

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Habituation and dehabituation to dichlobenil

Simply the equivalent of Penélope's weaving and unweaving process?

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The habituation of cell cultures to cellulose biosynthesis inhibitors constitutes a valuable method for learning more about the plasticity of plant cell wall composition and structure. The subculture of habituated cells in the absence of an inhibitor (dehabituation) offers complementary information: some habituation-associated modifications revert, whereas others remain, even after long-term (3–5 years) dehabituation processes. However, is dehabituation simply the opposite to the process of habituation, in the same way that the cloth woven by Penélope during the day was unwoven during the night? Principal Component Analysis applied to Fourier Transformed Infrared (FTIR) spectra of cell walls from dichlobenil-habituated and dehabituated bean cell lines has shown that dehabituation follows a different pathway to that of habituation. Principal component loadings show that dehabituated cells have more pectins, but that these display a lower degree of methyl-esterification, than those of habituated ones. Further analysis of cell walls focusing on the first steps of habituation would serve to identify which specific modifications in pectins are responsible to the fine modulation of cell wall architecture observed during the habituation/dehabituation process.

Key words: cell-wall, cellulose, dichlobenil, habituation-dehabituation, Fourier transform infrared spectroscopy, principal component analysis

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The habituation of cell cultures to the presence of lethal concentrations of cellulose biosynthesis inhibitors illustrates the ability of cells to survive with a modified cell wall and is therefore a valuable experimental technique for gaining an insight into the plasticity of plant cell wall composition and structure. Dichlobenil-habituated cultures usually display some common features: slower growth rates, irregularly shaped cells, a trend to grow in clumps when cultured in suspension and compensation of reduced cell wall cellulose content with other cell wall components.[1-3]

Most of the cell wall changes induced during the habituation to dichlobenil reverted when cells were dehabituated by culturing them in an inhibitor-free medium.[4-7] However, even in long term (3–5 years) dehabituated cell cultures, some habituation-induced cell wall modifications remain, such as altered extractability of pectins, a decrease in arabinogalactan proteins and hydroxyproline-rich glycoproteins epitopes, and the presence of a soluble β-(1,4)-glucan, although cellulose levels were restored.[5-7] Most remarkably, in addition to these stable changes in cell wall architecture, bean dehabituated cells retained a high capacity to cope with lethal concentrations of dichlobenil, as dehabituated cells were forty times more tolerant to dichlobenil than non-habituated cells.[5] In an attempt to explain the dichlobenil resistance of dehabituated cells it was found that they had a constitutively increased peroxidase activity, indicating a positive relationship between habituation to dichlobenil and antioxidant capacity.[7]

If most of the cell wall modifications induced during the habituation to dichlobenil eventually revert to those of non-habituated cells during the dehabituation process, a question arises: is dehabituation simply the inverse of habituation, in the same way that the cloth woven by Penelope during the day was unwoven during the night, as narrated in Homer's *The Odyssey*?

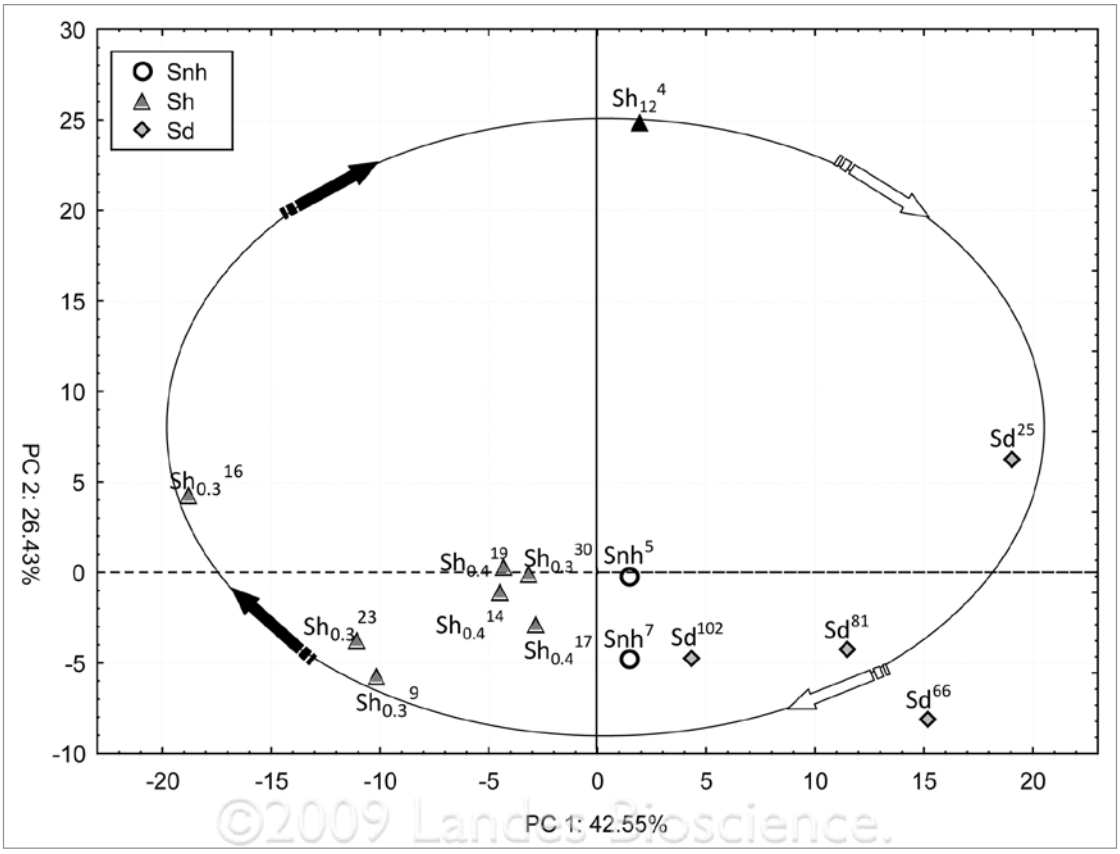

Figure 1. Principal Component Analysis of spectra of cell walls from different calluses. A plot of the first two Principal Components scores is represented based on the FTIR spectra of cell walls from non-habituated cells (Snh, ○), cells habituated to different dichlobenil concentrations (Sh, ▲), and cells previously habituated to 12 μM dichlobenil, with a different number of subcultures in the absence of the herbicide (Sd, ◆). Subindexes indicate dichlobenil concentrations in the growth media of habituated cells (0.3, 0.4 or 12 μM); superindexes indicate number of subcultures in the same media. Arrows indicate the different pathways followed by dichlobenil habituation and dehabituation: black arrows, from non-habituated to habituated cells (habituation), and white arrows, from habituated to non-habituated cells (dehabituation).

Principal Component Analysis applied to Fourier Transformed InfraRed spectra of cell walls has been demonstrated to be a powerful technique for conducting comparative analysis of a wide range of cell wall samples.[3,8] Therefore, a suitable approach to answering this question consists in comparison of cell walls from dichlobenil-habituated and dehabituated bean cell lines using this technique.

Clearly, FTIR spectra of cell walls from dehabituated cells with few subcultures in the absence of the herbicide resemble those from cultures habituated to high dichlobenil concentrations.[5] However, the spectra from cells habituated to low inhibitor concentrations and from cells dehabituated for long periods of time[7] were more similar to those from non-habituated ones. In fact, when Principal Component Analysis is applied to the entire range, Principal Component 2 (PC2) discriminates between Sh_{12} (corresponding to cells habituated to high dichlobenil concentration) and the rest of the spectra, which is indicative of the above-mentioned similarity (**Fig. 1**). Nevertheless, PC1 clearly discriminates between spectra from long-term dehabituated cell walls (located at the positive side) and those from cells habituated to low dichlobenil concentrations (at the negative side). This indicates that progression towards dehabituation follows a different path to that of habituation.

With the aim of identifying those factors which determine this different pathway, PC1 and PC2 loading factors were analyzed (**Fig. 2**). This analysis indicated that PC2 (explaining 26.4% of total variance) has a positive correlation with wavenumbers attributed to uronic acids (1,420 and 1,600 cm^{-1}) and galactose (950 cm^{-1}), and a negative correlation with wavenumbers associated with cellulose (1,040, 1,060, 1,175, 1,320 and 1,370 cm^{-1}) and xyloglucan (1,125 cm^{-1}). Thus, Sh_{12} cell walls (clearly located at the positive side of PC2) are pectin enriched and cellulose/xyloglucan impoverished. As explained above, PC1 discriminates between cell walls from dehabituated cell lines and those from cells habituated to low concentrations of dichlobenil. PC1 (accounting for 42.55% of total variance) has a negative correlation with wavenumbers associated with methyl-ester groups (negative peaks at 1,250 and 1,720 cm^{-1}), and a positive correlation with the so called "fingerprint" region (980–1,200 cm^{-1}). Therefore, cell walls from dehabituated cells (those located at the positive side of PC1) would have lower methyl-esterified pectins when compared with cells habituated to low concentrations of dichlobenil.

Previous results had revealed that dichlobenil habituated cells experienced a

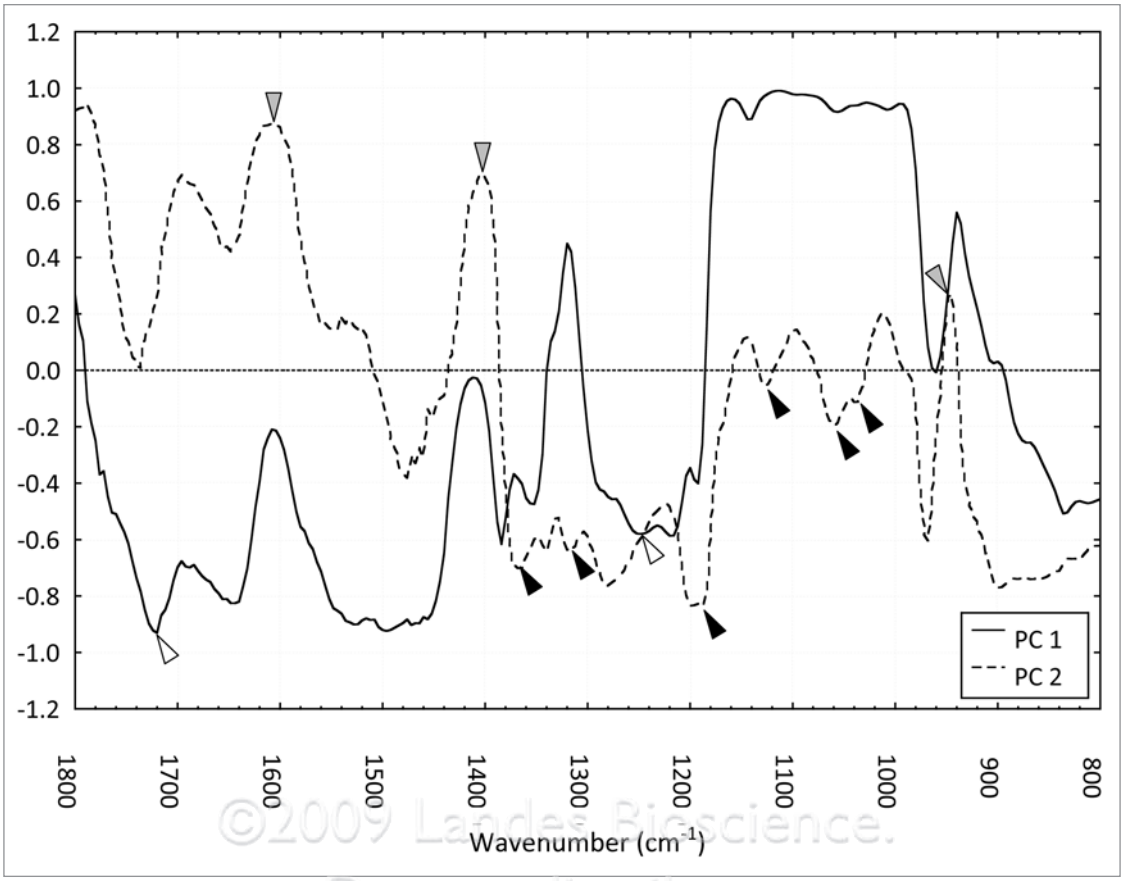

Figure 2. Loadings for PC1 and PC2 corresponding to Figure 1. White arrowheads point wavenumbers associated with methyl-esterification; black arrowheads, those associated with cellulose and hemicelluloses, and grey arrowheads indicate wavenumbers associated with uronic acids and galactose.

progressive reversion in their cell wall composition when they were subcultured in an inhibitor-free medium, gradually increasing their xyloglucan and cellulose content,[5,6] and that both dichlobenil habituated and dehabituated cells showed changes in the distribution of pectin among cell wall fractions: cell suspensions with a low habituation level had cell walls with a higher amount of pectins, and these were more methyl-esterified.[6]

Now, FTIR spectroscopy in association to Principal Component Analysis has shown that, although some of the changes observed in the first steps of habituation and in the last steps of dehabituation are common (i.e., reversion of cellulose content), some other changes affect habituated and dehabituated cells differently, and that these changes involve mainly pectin composition and organization. A more detailed analysis of cell walls focusing on the first steps of habituation will serve to identify which specific modifications are responsible for the differences observed in the pectic component and, consequently, responsible for the fine modulation of cell wall architecture.

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