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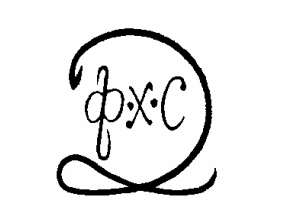

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FTIR ANALYSIS OF XYLEM VESSEL CELL WALLS IN TWINING STEM OF *Dioscorea balcanica*

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ABSTRACT
Using stem cross sections of *Dioscorea balcanica*, as a model, we detected changes in anatomy and structural organization of xylem vessel cell walls (CWs) linked to stem twining in liana plants. UV microscopy, scanning electron microscopy and Fourier transform infrared (FTIR) microspectrometry were used. Different microfibrils orientation in vessel CWs of twisted compared to straight internodes, revealed by histological examination, coincide with the lower lignin content, the lower amount of xylan and cellulose, and the higher amount of xyloglucan, showed by FTIR. Xylem vessels resist high mechanical strain developed in twisted internodes by decreased CW rigidity (lower lignin content) and extensibility (higher xyloglucan content), and increased elasticity (lower xylan content).

INTRODUCTION
In liana plants anatomical and morphological adaptations to mechanical strain involve high flexibility of the stem tissue structures, achieved by structural changes in the cell walls (CWs) of different tissues: fibers, xylem (vessels and tracheids) and parenchyma [1].
Xylem vessels are elongated hollow cells subjected to high pressure of surrounding tissues resisted by their highly lignified walls to prevent cell collapse. This could be particularly relevant for herbaceous perennial lianas [2], such as *D. balcanica*.

We used UV microscopy, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) microspectrometry to analyze structural changes in vessel CWs of liana plant *D. balcanica* related to stem twining.

METHODS
Plant material: *Dioscorea balcanica* Košanin, family Dioscoreaceae, is an herbaceous monocotyledonous dioecious tuberous perennial liana, an endemic, endangered species and a Tertiary relict of Balkan Peninsula [3]. Stem cross sections (free-hand sectioning with a razor blade) of straight and twisted internodes (**Figure 1A**) were used.
Microscopy analysis: For SEM: dried free-hand stem sections were coated with gold, analyzed, and photographed with a TESCAN VEGA 3 SB at 20 kV (TESCAN, Brno, Czech Republic). For fluorescence microscopy (UV 358 nm - excited autofluorescence): unstained stem sections were mounted in glycerol, observed, and photographed by light-fluorescence Zeiss Axiovert microscope (Carl Zeiss GmbH, Gottingen, Germany).
Fourier transform infrared (FTIR) microspectrometry: An area of 50 μm x 50 μm in the vessel CWs was selected. Spectra were collected using a ThermoNicolet Nexus spectrometer (Madison, WI) with a Continuum microscope accessory; 20 spectra for each internode in transmission mode with 4cm^{-1} resolution. Spectra were baselined and normalized [4]. The spectra of corresponding internodes from 2 plants were similar, so here we present the spectra of selected internodes.

Statistical analysis: Student's t-test for independent samples was used to determine the significance of the difference (at level 0.05) between average values for every individual wavenumber of the FTIR spectra, as described previously [5]. The obtained t-values (Y-axis) were plotted against the wavenumbers of the spectrum (X-axis).

RESULTS AND DISCUSSION

Using stem cross sections of *Dioscorea balcanica*, as a model, we detected changes in anatomy and structural organization in xylem vessel CWs linked to stem twining in liana plants. Our previous results obtained by light microscopy revealed no differences either in the structure or in lignification of, both, sclerenchyma or parenchyma cells between straight and twisted internodes. The difference was visible only by SEM, and FTIR microspectrometry [6]. Changes in parenchyma CW structure related to stem twining include: lower amount of xyloglucan and cellulose, and higher amount of xylan and lignin (with modified organization) [6].

In the present study, UV microscopy showed no difference in lignification of vessel CWs between straight and twisted internodes (**Figure 1C,** and **Figure 1E**), but SEM images indicate the difference in microfibrils orientation – vessel CWs in twisted internodes (tension side) showed cellulose microfibrils oriented almost parallel to the stem axis (**Figure 1B**), while in **Figure 1D**, in straight internodes cellulose microfibrils are oriented at very high microfibril angle to the stem axis.

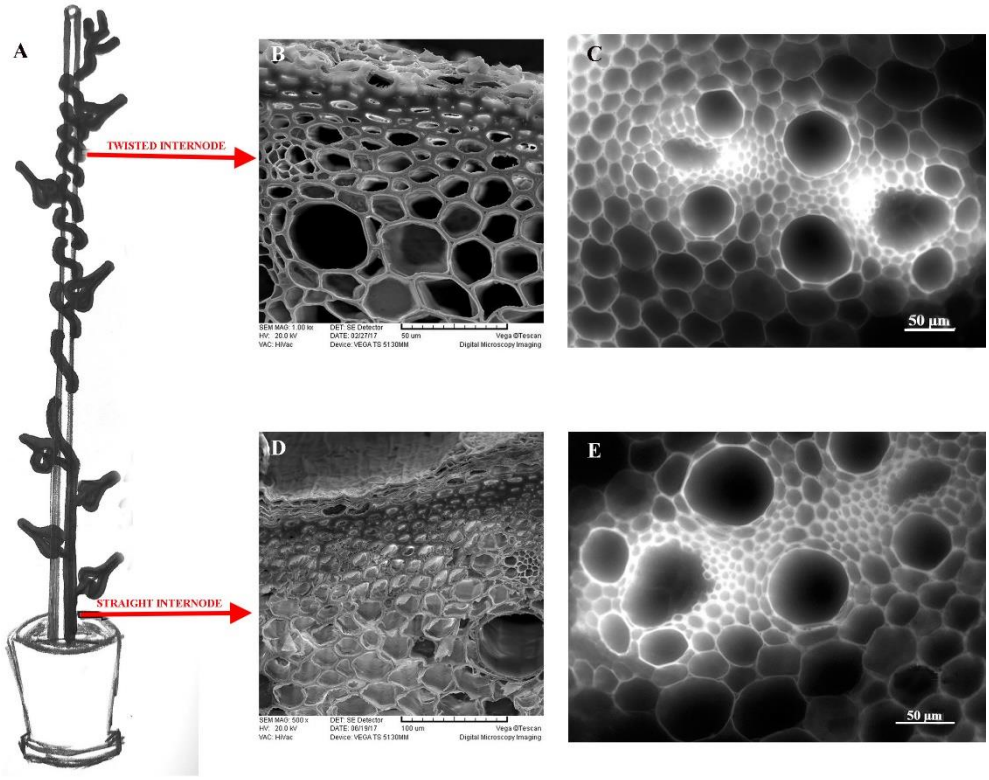

Figure 1. Sheme of experimental plant (**A**); SEM micrographs of twisted (**B**) and straight (**D**) internode; UV – excited autofluorescence of twisted (**C**) and straight (**E**) internode, n=8.

Figure 2 shows overlaid FTIR spectra of CWs of vessels of the straight and twisted internodes in the region 800–1800 cm^{-1}. The band at 896 cm^{-1} is characteristic for anomeric b-linkage of glucose in xyloglucan [7]. The region from 900 - 1100 cm^{-1} is specific for polysaccharides (xyloglucan). The interaction of cellulose fibrils and a matrix are mediated by changes in the amount of non-cellulosic polysaccharides and lignin [8]. Hemicelluloses interact with cellulose providing CW structural strength: xyloglucan binds to the surface of cellulose tethering cellulose microfibrils together [9]

controlling cell wall extensibility [10], while xylan and cellulose fibrils bonding affects CW elasticity [11].

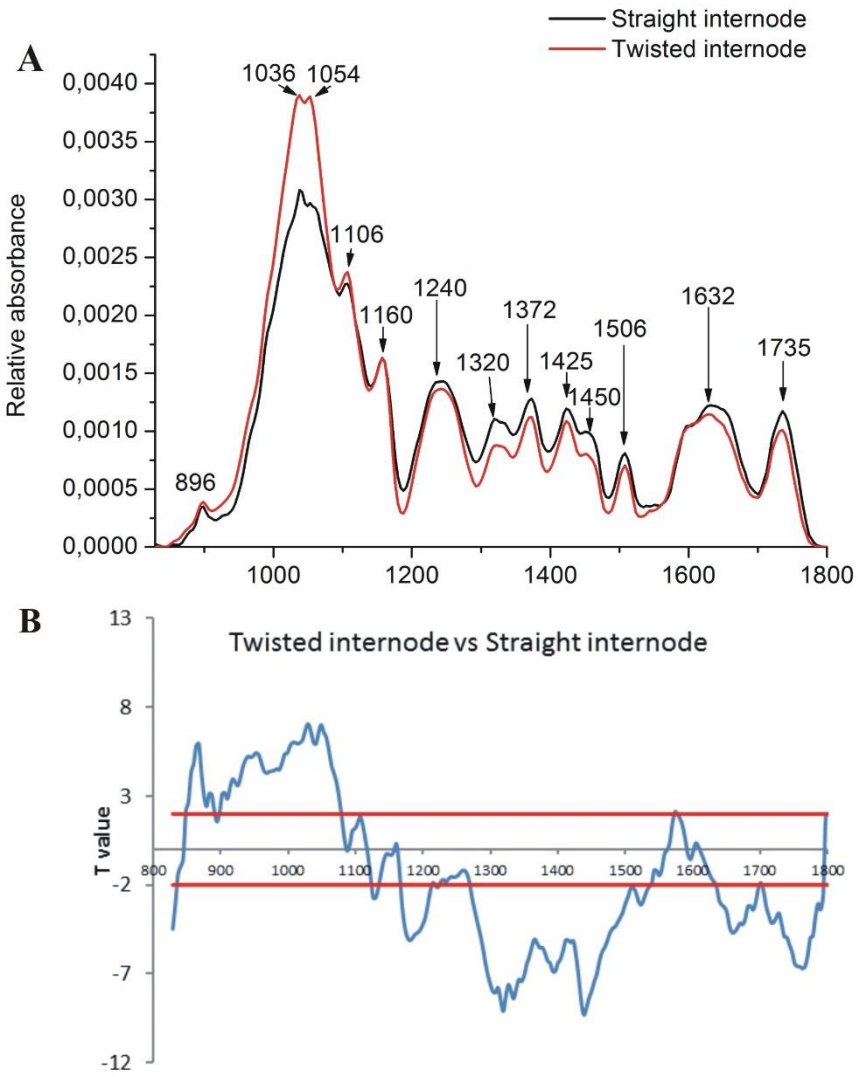

Figure 2. A) overlaid FTIR spectra of vessel CWs of straight and twisted internodes in the region 800–1800 cm^{-1}; **B)** t test for twisted versus straight internodes (horizontal red lines— 5% significance threshold)

Four distinct vibration bands that were related to cellulose, viz. the antisymmetric C–O–C bridge stretching vibration at 1160 cm^{-1}, CH_2 wagging vibration at 1320 cm^{-1}, the C–H bending vibration at 1372 cm^{-1} and the C–OH bending vibration of the CH_2–OH group at 1425 cm^{-1} were found [11-13]. The bands at 1240 cm^{-1} (the C–O stretching in the O=C–O group), 1451 cm^{-1} (CH_2 symmetric bending on the xylose ring) and 1735 cm^{-1} (carbonyl group vibration, the C=O stretching vibrations in the O=C– OH group of the glucuronic acid units) are characteristic for xylan [12-14]. The bands at 1506 cm^{-1} (aromatic skeletal vibrations) and 1596 cm^{-1} (C=C aromatic ring vibrations plus C=O stretch) are characteristic for guaiacyl and syringyl lignin [15, 16]. The 1632 cm^{-1} band is characteristic of C= bond in the lignin monomer side chain [17, 18].

T test (**Figure 2B**, right y-axis, black line) was used for the evaluation of differences in the mean of absorbances at specific wavelengths between vessels of twisted versus straight internode. The amount of xylan (absorbances at 1735, 1451, 1240 cm^{-1}) and cellulose are higher in vessels CWs of

straight internodes (**Figure 2B**). Contrary, the amount of xyloglucan (from 900 to 1100 cm^{-1}) is higher in vessels CWs of twisted internodes (**Figure 2B**). Lignin content is lower (wavenumber at 1506cm^{-1}) in vessel CWs of twisted internodes.

CONCLUSION

According to the results obtained by UV microscopy and SEM, as well as FTIR microspectroscopy, different cell types (xylem vessels, and parenchyma, as cells with different shape/structure/function ratio) developed different CW structural changes to support a twisting force and resist mechanical strain established in twisted internodes. Opposite to parenchyma cells, xylem vessels showed lower lignin, xylan and cellulose content, but higher xyloglucan content, resulting in decreased CW rigidity and extensibility but increased elasticity.

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