Genetic manipulation engineers the elasticity of arabidopsis thaliana

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We estimated ultrasonically the stiffness in Arabidopsis inflorescence stems in the radial direction for two commonly used ecotypes (*Columbia* and *Landsberg erecta*). Plants were grown in laboratory conditions for 16 days and 20 stems per ecotype were measured *in vivo* applying 4MHz ultrasonic longitudinal tone bursts propagating along the radial direction of the plants. The effects of AtCAD1 (*Arabidopsis thaliana Coumaryl Alcohol Dehydrogenase 1*) gene silencing in *Col* ecotype were tested using the same method for a small set of plants grown in greenhouse conditions. We recorded significantly higher relative stiffening in the Landsberg variant (39% over 16 days, p = 0.002) compared to the Col ecotype, and 10% higher end-point values (p = 0.03) at the base of the stem. The results are the first ultrasonically measured sound velocity values for this commonly used plant model species, creating a quantitative mechanical measure for the effects of wood composition modification.

1   Introduction

Arabidopsis thaliana is a widely used model species in plant research. It is a fast growing, annual plant, whose genome is relatively small (33,282 genes) and well studied [1, 2, 3]. Consequently, it is ideal for molecular biology research and both ecological and evolutional studies [4]. However, few studies report on the mechanical properties of these plants, and existing has been destructive [5, 6].

Studying mechanical parameters *in vivo* offers information about both gene functions and about the developmental mechanics of such plants. We herein characterize mechanical properties of living plants *in vivo* and follow their alteration during growth.

2   Methods

The samples comprised 20 3 weeks old *Arabidopsis thaliana* plants, 10 of both ecotypes *Columbia* (Col) and *Landsberg erecta* (Ler). The plants were grown in laboratory conditions (variable R_H, 22 °C) where they received indirect sunlight for 12 hours a day on average. The plants received water twice a week on set days.

The velocity of 4 MHz 10 V_{PP} 5 cycle longitudinal ultrasound bursts propagating in the radial direction was measured from each stem daily using a custom built setup comprising of two ultrasonic transducers, aluminum cone-shaped waveguides (tip diameter 2.2 mm), a precision scale
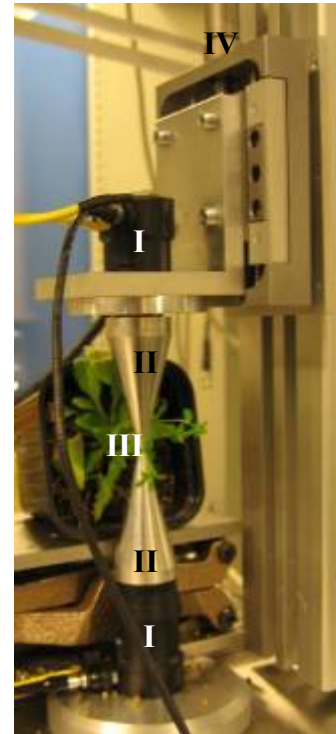

Fig.1 Measurement setup featuring transducers (I), waveguides(II) dry-coupled to the sample (III) and a micrometer measuring the distance between the waveguide tips(IV).

and a micrometer, cfr. Fig. 1. The TX transducer (S12 HB 1-8, 1.4-7.7 MHz -3 dB) was coupled to the TX waveguide using ultrasonic gel, and both of the waveguides (TX and RX) were dry-coupled to the sample. The through-sample

Fig. 2 Growth of a single plant (Landsberg type) during the measurement: first measurement day (leftmost), 7th day (middle) and the 15th day (rightmost). The circles mark the points where the measurements were done; as the plant grew taller, more points were added at 3 cm intervals.

propagated signals were then detected using the RX transducer (S12 W4, 3.1-4.9 MHz -3dB) gel-coupled to the waveguide and digitized using an oscilloscope which transferred the data over a GPIB bus to the measurement computer. The received signals were amplified 60 dB and 0.5 kHz high-pass filtered to remove low frequency noise. The variation in the time-of-flights through the waveguides was 1‰, indicating that the system was stable.

The TOF was determined by comparing the time-of-arrival (TOA) to the trigger instant of the signal transmitted through the waveguides alone and for the waveguides and sample. The TOA was determined as the instant when the first cycle reached its peak value. To improve the temporal resolution, a fourth-order polynomial was automatically fitted to the received signal. This was done by identifying the first amplitude value that exceeded a set threshold value (e.g. 2% of the maximum). The time instant of the peak value was determined from the fitted data as the temporal position of the maximum of the fitted polynomial (cfr. Fig. 3).
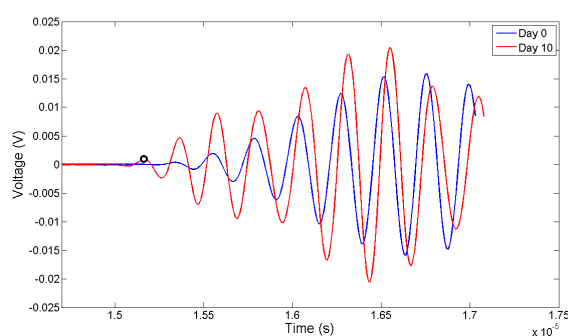

Fig. 3 Signals transmitted through a Columbia ecotype plant on days 0 and 10. The detected TOA on day 10 is marked with a circle.

The precision scale was used to measure the contact pressure (5±0.5 kPa) between the waveguides and the sample. The distance between the transducers was measured while under pressure using the built-in micrometer to avoid measurement errors arising from the elastic recovery of the samples.

Each plant was measured at different heights, starting from 1 cm from the base of the stem, and then at 3 cm intervals as the plants gained height, cfr. Fig. 2. Each measurement was repeated three times at each point to remove effects of bad coupling and to ensure sufficient statistics for error analysis. In addition, the time-of-flight through the waveguides alone was measured before each measurement to quantify the error arising from the waveguide-transducer coupling. After the point where no significant daily growth was detected, the plants were measured only every second day.

The measurement was done as a blind test: The samples were randomly numbered from 1-20, hiding the ecotype identification until the end of the data analysis. In addition, the data analysis and measurements were performed by separate persons.

In addition to the 20 samples measured during their growth, a small series was performed on three inflorescence stems of Columbia, and 6 inflorescence stems of Columbia plants with silenced *AtCAD1* gene expression. The plants were ~10 cm tall, and were grown in greenhouse conditions. The measurements were performed at 1 cm from the base of the stem.

3   Results

The measured sound velocities ranged from 1700±20 to 3100±90 m/s, yielding an elastic modulus of 1.4-4.8 GPa (assuming the plant density to be 500 kg/m^3, which was the average measured value). At 1 cm height, a 8% average relative increase for the Columbia- and 39% for the Landsberg variant in the sound velocity was detected over 16 days (Fig. 4 and 5).
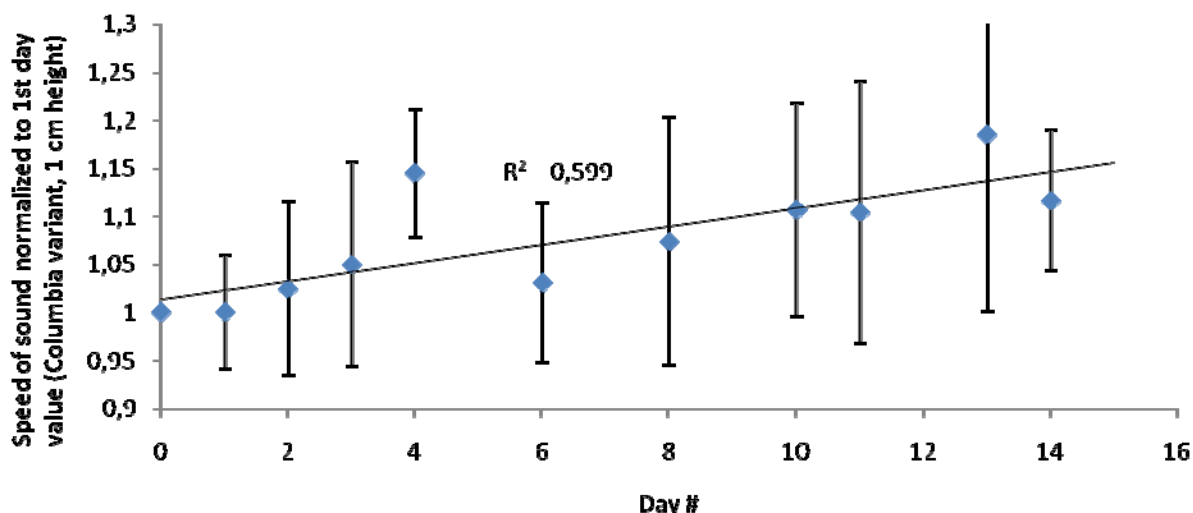

Fig. 4 Development of the 20% trimmed average longitudinal speed of sound in radial stem direction relative to the 1st day value for the Columbia variant. A linear increase in the sound velocity is detected.
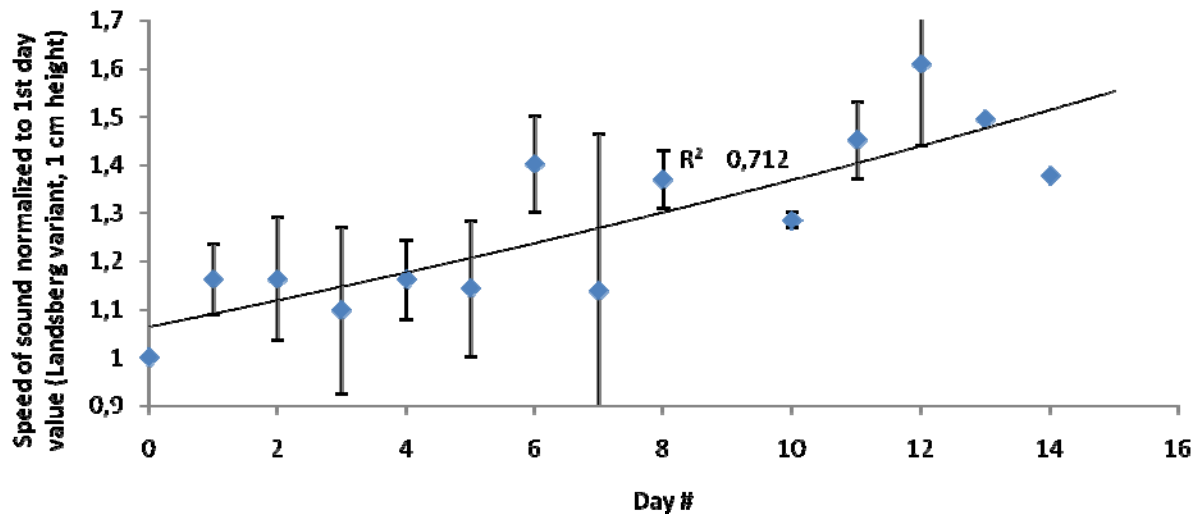
Fig. 5 Development of the 20% trimmed average longitudinal speed of sound in radial stem direction relative to the 1st day value for the Landsberg variant. An exponential increase in the sound velocity is detected.

The mean biological variance (defined as the standard deviation of the average value of all velocity values on the last measurement day) in the sound velocities for the entire population was 10%.

An example of a single plant of the ecotype Landsberg is shown in Fig. 6. The average velocities at 1 cm height on the last day of the measurements differed between the ecotypes by 10% (p = 0.03, N=15), the Landsberg variant having a higher mean velocity.
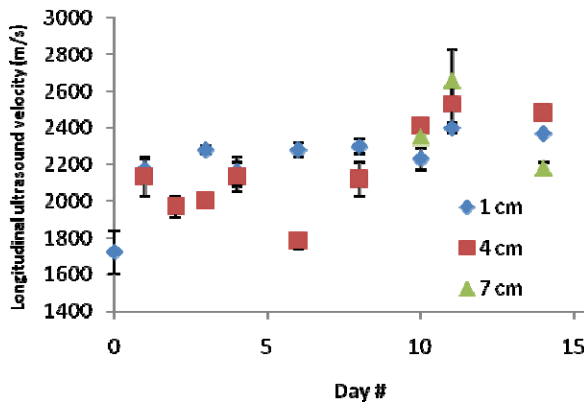

Fig. 6 Development of the longitudinal sound velocity in the radial direction for a Landsberg ecotype plant as a function time for three different heights.

In the small series performed on the greenhouse grown plants the mean velocity was notably smaller (400 m/s). Preliminary results showed a minor (10%) decrease in the sound velocity for the *AtCAD1* silenced plants (p = 0.1).

4   Discussion

The results show that 1) we were able to *in vivo* measure the mechanical changes in the stems during growth, and 2) that were able to differentiate between the two ecotypes. A clear increase in the sound velocity was detected at 1 cm height, whereas only a slight increase at 4 cm height. This was expected, since the lower parts of the *Arabidopsis thaliana* stem are more lignified and stronger than the upper

parts[7], and since it carries the weight of the plant, it should be the stiffest [8]. With the assumption of a 500 kg/m^3 density the obtained modulus values match with earlier measured bending modulus results[9].

The *Landsberg* variant (Ler) expressed slightly higher sound velocities than *Columbia* variant, which indicates a higher stiffness. It also expressed significantly higher relative stiffening between days 0 and 15, 39% vs. 8% (p=0.002) than the Col variant. This corresponds well with the fact that Ler inflorescence stem is thicker than Col[10]. In the preliminary experiments using a small series a small difference between the *AtCAD1* gene silenced and Columbia wild type was detected. A more extensive study is required to confirm this difference.

In the future, a new measurement series applying a controlled growth environment (growth cabinet) will be made. In addition, a third series with *AtCAD1* silenced variants will be measured in addition to the two wildtypes for a longer period of time in order to detect possible tree-like structure development. In addition, development of method to measure localized density *in vivo* is crucial, since it allows determining quantitative mechanical properties.

5   Conclusion

An *in vivo* experiment was performed over 16 days for wildtype *Arabidopsis thaliana* var. Columbia and var. Landsberg erecta. Changes in the sound velocity (and thus stiffness) were detected as a function of growth for both ecotypes.

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