Non-Destructive Measurement of Vascular Tissue Development in Stems of Miniature Tomato Using Acoustic Method*

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Abstract

The guided wave effect resembling that of annual rings found in woods and the cortical region of bones is believed to be observable in vascular tissues of herbaceous plants. The properties of acoustic waves traveling through the vascular tissue in the stem of a miniature tomato were measured using a piezoelectric pulser and receiver. The thickness of the vascular tissues and the stem’s water content were measured. The detected acoustic waves showed a guided wave effect. The apparent sound velocity, \( v_a \), was related to the vascular tissue thickness, \( t_v \). These results reveal that the detected acoustic waves traveled along the vascular tissues in stems. The maximum peak intensity of the detected acoustic waves, \( I_{\text{max}} \) was also related to \( t_v \). Furthermore, wilting of the examined plants decreased the \( I_{\text{max}} \), although \( v_a \) was not changed. The decrease in \( I_{\text{max}} \) might result from cavitations and embolisms with a subsequent increase in air pores in xylem tissues. These results demonstrate that the measurement of acoustic waves traveling through vascular tissue is a useful tool for the non-destructive evaluation of vascular tissue development and embolism density in xylem tissues.

Keywords: Ultrasonic Testing, Sound Velocity, Tomato, Herbaceous Plants, Vascular Tissues

1. Introduction

For decades, acoustic measurements have been used for inspecting wood quality\(^{(1)}\). In particular, ultrasonic parameters - sound velocity and attenuation - are strongly influenced by the structural anisotropy of woods\(^{(1)}\). Such anisotropy is closely associated with annual rings, grain orientation, and wood knots. The annual rings consist of porous structures (earlywood) and dense structures (latewood). Thus, the velocity of acoustic waves traveling circumferentially around a tree trunk is related to guided wave (Lamb wave) effects induced in the latewood, which has thick walls and high density\(^{(2)}\). Lamb waves are known to be generated with multiple reflections and interference in thin structures. The generation and detection of Lamb waves in pipes and plates has been studied for a long time\(^{(3)}\). The velocity of ultrasonic waves through isotropic media can usually be described by the elastic modulus and density of the medium. Furthermore, Lamb-wave propagation is influenced by geometrical border conditions. Several wave modes appear with geometrical dispersion of velocities\(^{(4)}\). A popular mode of Lamb waves is the zero anti-symmetric (A0) Lamb wave. These A0 Lamb waves are generated when the
The wavelength is much larger than the pipe or plate thickness. The velocity depends on the ratio of the thickness and the wavelength. Consequently, the velocity of ultrasonic waves becomes that of a Rayleigh wave (surface waves) as the thickness increases \(^{(4)}\). Tatarinov et al. measured the propagation of these waves in long bone and found that the wave velocity in bone had a similar dependence on thickness as the A0 Lamb wave in plates at the frequency of 100 kHz\(^{(5)}\). In a simplified model of the bone cortical region presented as a plane layer, the A0 Lamb wave can be generated at frequencies that correspond to wavelengths that are much greater than the cortical thickness. Bucur also showed that the guided wave (Lamb wave) effect in spruce and the velocity of the Lamb waves traveling along the annual rings increased concomitantly with the width of annual rings when the width was much smaller than the wavelength\(^{(2)}\). This behavior in the velocity of Lamb waves against the width is also similar to A0 mode waves and is believed to be observable when vascular tissues of herbaceous plants are the subject of analysis. Figure 1 presents a cross-section of a miniature tomato stem. Vascular tissues have a higher density than the other structures and are distributed circumferentially in stems like annual rings in trees or cortical regions in bones. For that reason, it is expected that the A0 Lamb wave travels along the vascular tissues, the thickness of which also influences the Lamb wave velocity: the measurement of the Lamb wave velocity might affect the development of vascular tissues in herbaceous plants. Estimation of development of vascular tissues in living plants can be useful for diagnosing drought resistance and growth of plants. Plants transport water from the soil to their leaves through xylem conduits in vascular tissues\(^{(7)}\). The transported water is evaporated from leaves during the day because stomata open for cells to obtain CO\(_2\) for photosynthesis. Such water evaporation at leaves is called transpiration. It generates negative pressure (water stress) in the xylem to pull water up from the soil\(^{(6)}\). The ability to transport water, i.e., the resistance against drought stress, is therefore deeply related to the development of xylem conduits. On the other hand, phloems are included in vascular tissues as well as xylem conduits. They transport the production of the photosynthesis reaction from leaves to other elements: the development of phloems is apparently related to the growth of the plants because they require production of the photosynthesis reaction for their growth.

For this study, using a piezoelectric pulser and receiver, we measured the velocity of acoustic waves traveling through vascular tissues in stems of a miniature tomato plant. The thickness of the vascular tissues and the stem water content were also measured and the relation between the measured parameters was investigated. Subsequently, the possibility of non-destructive evaluation of vascular tissues and water status in living miniature tomato stems was discussed.
Nomenclature

A0: zero anti-symmetric mode
S0: zero symmetric mode
\( \bar{d} \): average diameter of stem
\( \bar{t}_v \): average thickness of vascular tissues
\( v_a \): apparent sound velocity
\( I_{\text{max}} \): maximum intensity of waveform
\( t_k \): rising time (\( k \) represents sample name)
\( P_k \): peak position of wavelet spectrum (\( k \) represents sample name)
\( f_w \): relative water content of stem

2. Experimental Procedures

2.1 Materials

Potted miniature dwarf tomato (Lycopersicon esculentum Mill.) plants were used for the experiments. Pots 12 cm in height and 9 cm in diameter were filled with a soil mixture of peat moss, perlite, ash, and fertilizer. Seedlings were transplanted into the soil. The plants had been grown with an artificial light irradiance from a high-pressure sodium lamp. The lighting intensity was set to 120 µmol·m\(^{-2}\)·s\(^{-1}\) at the base of the shoot and the lighting cycle provided 12 hours of light per day. The temperature was set at 20–30°C and the pot was irrigated once a day. A month after seedling transplantation the plants that had grown well (30-50 cm in height) were examined using acoustic testing. Nine well irrigated samples and four unwatered samples were examined. Acoustic measurements of the unwatered samples were done when wilting was first observed. Furthermore, seven fruit bearing samples that had been well irrigated were also examined.

2.2 Measurements

For acoustic testing, a 12.5-mm-diameter piezoelectric vibration plate (EFBS12D43; Panasonic Inc.) and a piezoelectric AE sensor with a 3-mm-diameter head amplifier (M304A; Fuji Ceramics Corp.) were used as the acoustic pulser and receiver respectively. The pulser and sensor were bonded on the main stem of the examined plant using acrylic instant adhesive and fixed face to face, as shown in Figs. 2 and 3. An electric burst wave of
100–200 mV amplitude and 400 ns rise time was input to the pulser to generate the acoustic waves. Applying a voltage between the electrodes of the piezoelectric plate causes mechanical distortion due to the piezoelectric effect. The distortion of the piezoelectric plate results in compression waves, which are transmitted through the stem and are detected by the receiver. The resonant frequency of the piezoelectric plate was 12 kHz according to the manufacturer’s catalog. However, the actual resonant frequency of the generated waves was unknown because it was influenced by the stem on which the pulser was bonded. The pairs of pulsers and receivers were attached on 3–8 points of the main stem on each shoot, and the acoustic waves detected by the receiver at each point were recorded using a personal computer via an A/D converter. The AE sensor used in this study had a resonance frequency of 510 kHz\(^{(8)}\). The frequency analyzed in this study was less than 100 kHz and thus the frequency response of the AE sensor had no peak.

After acoustic measurements, two 1-cm-long test pieces were cut from each measuring point. Freehand cross-sections were made from one of the pieces and observed using stereomicroscopy. The stem diameter and the thickness of the vascular tissues were measured randomly 10 times for each test piece from the observed images. Using these measurements, the average stem diameter, \(d\), and the average thickness of the vascular tissues, \(l_v\), were calculated. The water content of the remaining test pieces was then measured. The mass percentage of relative water content, \(f_w\), of the tested stem was determined from the fresh weight and the oven dry weight of the pieces.

3. Results and Discussion

3.1 Relation between the Vascular Tissue Thickness and the Stem Diameter

Vascular tissues are expected to thicken concomitantly with the stem diameter because plant stems thicken with growth. Figure 4 shows that the miniature tomato used for this study showed this tendency but had wide scattering. Examples of stereomicroscopic observation of the cross-sections of stems are presented in Fig. 5. The diameters of both stems in Fig. 5 are almost equal, but a significant difference of \(l_v\) exists; the vascular tissues of sample A are over three times thicker than those of sample B. Consequently, it is quite difficult to evaluate the thickness of vascular tissues from the stem diameter.
3.2 Measurement of the Acoustic Wave in Vascular Tissue

The detected acoustic waves of both samples in Fig. 5 at the acoustic measurements are depicted in Fig. 6. The signal maximum intensity, $I_{\text{max}}$ of sample A is about twice that of sample B: as thicker vascular tissue, a bigger acoustic wave was received when the stem diameter was almost constant. Figure 6(b) also reveals that the arrival time of the acoustic wave propagated in sample A was shorter than that in sample B. Assuming that the acoustic waves travel along the vascular tissues and that the apparent velocity of the acoustic wave is half of the circumference of the stem divided by the traveling time, the apparent sound velocity in sample A was higher than that in sample B. Figure 7 presents the results of wavelet analysis of the waveforms in Fig. 6(a). The wavelet spectrum in Fig. 7 includes several peaks. Shifting of a few peaks is visible, although most peaks are mutually indistinguishable. The position of peak $P_A$ in Fig. 7(a) in the wavelet spectrum of sample A was shifted to a longer time with increased frequency. This tendency is observable in S0 mode Lamb waves. On the other hand, the shifting of peak $P_B$ in Fig. 7(b) in sample B shows an opposite tendency from that found in A0 mode Lamb waves. Figure 7 reveals that the detected acoustic waves might include Lamb waves with several modes, although it was difficult to evaluate the wave velocity of each mode because the waves overlapped.
In this study, the apparent sound velocity, $v_a$, was determined from the rising time when the detected signal crossed over the threshold level, 0.5% at normal intensity, as depicted in Fig. 6(b) in which $t_A$ and $t_B$ signify the rising times for samples A and B respectively. It is therefore necessary to discuss the propagation route of the first arrival wave with the velocity of the $v_a$. The acoustic waves which travel in a straight line from the pulser and the sensor apparently have lower velocity and higher attenuation in the pith than in the vascular tissues because the pith has a coarser and more porous structure than the vascular tissues. Hence, if the route of the first arrival wave was a straight line and the vascular tissues was constant, the $v_a$ would decrease as the diameter of the stem and the pith increased experimental results did not show such a behavior. For example, the $v_a$ of the stem which had a diameter of 3.6 mm and a vascular tissues thickness of 0.4 mm was 690 m/s. On the other hand, the $v_a$ of another stem which had a diameter of 6.1 mm and the same thickness of vascular tissue as the former was 670 m/s. Thus the change of the $v_a$ was negligible compared to $v_a$ behavior related to the vascular tissues thickness, as described later. Consequently, it can be concluded that the route of the first arrival waves was via the vascular tissues though further investigation is necessary to clarify whether the first arrival waves are Lamb waves.

Furthermore, the peak intensity, $I_{\text{max}}$, was determined from the maximum value of the signal, as depicted in Fig. 6(a) to investigate the behavior of the acoustic attenuation with vascular tissue thickness. Figure 8 shows the Fourier spectrum of the waveforms in Fig. 6(a). The spectrum peaks of 20–40 kHz of sample B are apparently smaller than those of sample A. Thus the difference in $I_{\text{max}}$ between sample A and B was closely related to the acoustic waves of 20–40 kHz. Then a ninth-order band-pass filter was processed using software that simulated characteristics of an RC filter to extract the waveforms of 20–40 kHz. The filtered waveforms presented in Fig. 9 reveal that the acoustic waves of 20–40 kHz in sample A are faster than that in sample B in contrast to the rising time of whole waves, as depicted in Fig. 6(b). This tendency reveals that the filtered waves might be S0 mode Lamb waves because the wave velocity is higher for thinner vascular tissues.

![Fig. 8. Frequency spectra of waveforms in Fig. 6(a).](image1)

![Fig. 9. Filtered waveforms in Fig. 6(a) using 9th RC filter at 20–40 kHz.](image2)

### 3.3 Relation between Vascular Tissue Thickness and Acoustic Properties

Figure 10 shows the relation between $d$ and $v_a$ and $I_{\text{max}}$. The $v_a$ has a weak dependency on the stem diameter as well as the peak intensity. On the other hand, both acoustic parameters, $v_a$ and $I_{\text{max}}$, showed a strong dependency on the $l_v$, with which they were increased linearly, as depicted in Fig. 11. Consequently, $v_a$ and $I_{\text{max}}$ are more useful to evaluate, nondestructively, the development of vascular tissue than the stem diameter. Actually, $v_a$ might be dominated by the A0 mode Lamb waves because it increased concomitantly with $l_v$, although the respective modes of the detected waves were indistinguishable. Long-range measurements are effective in identifying the existence
of several modes. In this study, the pulser and the receiver were installed on the same clamp, for the convenience of measurements. In future studies, the sensor and the pulser will be installed separately in the axial direction of the stem to measure acoustic waves traveling a much longer distance than those in this study.

As presented in Fig. 10(b), the acoustic signals detected by the receiver showed no attenuation attributable to the increase in the traveling distance because of short-range measurement; $I_{\text{max}}$ was not decreased when $d$ increased. In contrast, $l_v$ was closely related to $I_{\text{max}}$, as shown in Fig. 11(b); the attenuation decreased with increased $l_v$. The acoustic waves of 20-40 kHz are deeply influenced on $I_{\text{max}}$ and might be S0 mode Lamb waves, as mentioned in the previous section. The attenuation of S0 mode waves is less for thicker plates or pipes when the frequency is constant \(^9\). This behavior can be also explained according to the relation between $I_{\text{max}}$ and $l_v$ in Fig. 11(b).

![Fig. 10. Relation between $d$ and (a) $v_a$ and (b) $I_{\text{max}}$. Each point represents each measured value of all samples.](image)

![Fig. 11. Dependency of (a) $v_a$ and (b) $I_{\text{max}}$ on $l_v$. Least-squares fitting lines are also shown. Each point represents each measured value of all samples.](image)

### 3.4 Influence of Drought Stress on Acoustic Properties of Vascular Tissue

Velocity and attenuation of acoustic waves traveling along the vascular tissues might be influenced by other factors, e.g., water, soil, and growing status, aside from the vascular tissue thickness. Figure 12 portrays a comparison of the respective values of $v_a$ and $I_{\text{max}}$ of the waveforms of well-irrigated, unwatered, and fruit-bearing (well irrigated) samples. The $v_a$ shows almost perfect linearity against $l_v$, even if the samples suffered from being unwatered or were fruit bearing. On the other hand, $I_{\text{max}}$ of the unwatered samples was apparently lower than that of the other samples, and the attenuation increased because of drought stress.
In addition, the unwatered samples had reduced relative water content in the stem, as presented in Fig. 13. Reportedly, the negative pressure of water in the xylem (water stress) was increased and the turgor pressure in cells was decreased when the stem water potential was decreased because of lack of water\(^{6}\). Consequently, the samples that were unwatered showed stem shrinkage, with a subsequent decrease of the relative water content of the stem. Such a drought condition also decreased the elastic modulus of the plant through degradation of the cell turgor pressure\(^{10}\). The velocity of the acoustic bulk wave is well known to be directly related to \(\sqrt{E/\rho}\), where \(E\) and \(\rho\) respectively denote the elastic modulus and the density of the medium \(^{7}\). Consequently, the decrease in the stem’s elastic modulus attributable to the unwatered condition is expected to reflect a decrease in the sound velocity, but Fig. 12(a) shows no such behavior. Figure 12(a) reveals that the decrease in the density of the stem might also occur under unwatered conditions. Such a decrease in the stem density is explainable by the theory of cavitation and embolism in stems\(^{11}\). Because the drought stress to the plants is increased, cavitation occurs in xylem elements as tiny air bubbles in the element suddenly expand. Eventually, the element becomes filled with air where cavitation occurs. Such a phenomenon is called an embolism. The number of embolisms increases concomitantly with drought stress. Thus, the ability of water transportation decreases greatly and finally, the plant begins wilting. The increase in the number of embolized elements decreases the stem density and the stem porosity is increased. Van Lepereen et al. observed embolisms in Chrysanthemum using Cryo-SEM; their results support the theory that the decrease in water content generates many air pores in the xylem\(^{12}\). Furthermore, the generation of air pores in the xylem because of embolisms can explain the decrease in \(I_{\text{max}}\) (the increase in attenuation) in the unwatered condition, as presented in Fig. 12(b) because such air pores are believed to be a scattering source in vascular tissues as a result of the large difference of acoustic impedance between air and water. The generation of embolisms also decreases the density resulting in the acoustic impedance in vascular tissues. Eventually the difference in acoustic impedance between the vascular tissues and the pith becomes smaller as the embolism density increases, although the acoustic impedance of the pith is much lower than that of the vascular tissues because it
has coarse and porous structures. Thus, the increase in embolism density decreases the reflection of the acoustic waves at the interface between the vascular tissues and the pith, and the scattering of the acoustic waves traveling in the vascular tissues is increased. Such a decrease in the degree of the reflection may be another reason for the increase in acoustic attenuation due with the embolism. The influence of embolisms on the acoustic attenuation of acoustic waves in vascular tissues must be studied further, although Cryo-SEM is necessary to observe the air-pore distribution in stems. Despite the existence of many techniques to diagnose the water status in plants, no technique exists for non-destructive and quantitative evaluation of embolisms in living plants. The results of this study suggest that measurement of acoustic waves traveling through vascular tissues is a useful tool for the non-destructive evaluation of development of vascular tissues and embolism density in xylem tissues.

4. Conclusions

Acoustic waves propagating in stems of miniature tomato plants were measured using a piezoelectric pulser and receiver. Results of acoustic measurements were compared for the stem diameter, vascular tissue thickness, and stem water content. The influence of drought stress on the acoustic parameter was investigated.

(1) The detected acoustic waves showed the guided wave effect; the peak frequency shift was observed in the wavelet spectrum and the apparent sound velocity, $v_a$, was related to the vascular tissue thickness, $l_v$. These results reveal that the detected acoustic waves traveled along the vascular tissues in the stem. Actually, $v_a$ showed a stronger correlation with $l_v$ and was more useful than the stem diameter for non-destructive evaluation of vascular tissue development.

(2) The maximum peak intensity of the detected acoustic waves, $I_{\text{max}}$, was also found to be related to $l_v$. Furthermore, wilting of the examined plants decreased $I_{\text{max}}$, although $v_a$ was not changed. The decrease in $I_{\text{max}}$ might result from cavitations and embolisms, with subsequent increases in the number of air pores in xylem tissues.

References

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