Leaf stiffness of two Moraceae species based on leaf tensity determined by compressing different external gripping forces under dehydration stress

Deke Xing, Xiaole Chen, Yanyou Wu, Qian Chen, Lin Li, Weiguo Fu and Yu Shu

ABSTRACT
Leaf water status determination based on mechanical and electrophysiological properties helps determine the inherent as well as instantaneous leaf dehydration tolerance synchronously. The leaf water potential ($\Psi_w$), physiological capacitance (CP) and gripping force ($F$) were determined with leaves of Broussonetia papyrifera (L.) Vent. and Morus alba L. Real-time leaf stiffness (LCS$_{rt}$) and maximum leaf stiffness (LCS$_{max}$) were investigated by compressing a leaf with external gripping force. Results indicated that LT displayed good correlation with $F$. Compared to M. alba, a better instantaneous dehydration tolerance or pressure resistance in B. papyrifera was correlated to its persistent stronger LCS$_{rt}$ or LCS$_{max}$ respectively. B. papyrifera showed better flexibility and tolerance to wider range of pressure than M. alba. The higher leaf mechanical strength helped to maintain a higher outward pulling force of cell walls; thus, the subsequent negative pressure effectively inhibited cellular water loss. B. papyrifera exhibited better drought resistance than M. alba.

Introduction
Water maintains cell tension and helps branches and leaves stand upright. Furthermore, it generates turgor that contributes to the movement of stoma or other plant structures and cellular growth (Kroeger et al. 2011). Plant growth is often affected by biotic or abiotic stresses, one of which is drought (Grossi et al. 2016). However, drought resistance differs amongst plant species, and the rapid determination of plant drought resistance helps in the implementation of appropriate irrigation strategies on the plant (Ega et al. 2017). The assessment of leaf traits is one of the methods for studying plant drought resistance (Petrov et al. 2018), a slower water loss rate in the leaf is associated with better drought resistance of plants (Zhou et al. 2018).

Leaf water status could be rapidly determined by analyzing the variation of leaf tensity (LT) during the water loss process (Zhang et al. 2015). Calculated according to the coupling relationship between leaf water potential ($\Psi_w$) and physiological capacitance (CP), LT reflected water status better than $\Psi_w$, and $\Psi_w$ was easily affected by the environment (Javed et al. 2017). The online monitoring and prediction of leaf water status can be realized through the determination of LT. However, pressure variation caused by the gripping force (which was used for clamping leaf during the CP determination) changes the concentration of the cytosol solute and the elasticity and plasticity of the cells in the leaf, which causes dielectric constant variation in the cytosol solute. Thus, the values of LT change (Zhang et al. 2015). Therefore, the subsequently assessed plant drought resistance varies as gripping force changes. As such, the plant drought resistance determined by using a specific gripping force was defined as instantaneous drought resistance. Measurements of CP should be conducted under the same gripping force, in order to make the comparison of drought resistance among different plant species credible. However, it is difficult to keep the gripping force consistent at each measurement (Xing et al. 2018). Therefore, it is unreasonable to compare the drought resistance among different plant species only based on the electrophysiological properties.

Mechanical properties, which are closely related to the internal architectures, proved to be another useful tool to investigate drought resistance of plants (Balsamo et al. 2015). Leaf mechanical properties were affected by water status, and a positive correlation between leaf internal architecture, tensile strength and tolerance to dehydration for grasses were observed (Balsamo et al. 2006; Rascio et al. 2015). Plant cells are composed of the cell wall and intracavitary substances (mainly protoplasm), and cell turgor plays an important role in the mechanical stability (Malgat et al. 2016). The wall, located outside the membrane, is a thick and tough layer with a slight elasticity. It not only forms a strong network that functions like a boundary, preventing unconstrained flow of water or nutrition and maintaining internal pressures of cells but also plays key roles in bearing external load and maintaining the mechanical strength of the plant body (Cosgrove 2016). Lignin, which fills in the cellulose cytoskeleton, is a necessary element for the cell walls of all vascular plants. Lignin strengthens the hardness of the cell walls and enhances the mechanical support and compressive strength of the cells (Boerjan et al. 2003). Generally, plants with higher drought resistance could enhance the mechanical strength of cell...
walls by rapidly improving lignin biosynthesis under drought stress conditions (Fan et al. 2006; Yin et al. 2017). High stiffness is correlated to higher mechanical strength of the wall, which subsequently yields negative pressure and effectively inhibits water loss (Deng and Zhang 1998; Charrier et al. 2016). However, mechanical properties are always measured at the point of failure load. Therefore, tissue water status determined based on mechanical properties is an inherent value and implies the inherent drought resistance of plants. Nevertheless, the plant tissues will be damaged irreversibly during the measurement. Therefore, it is hard to continuously monitor plant drought resistance through mechanical properties.

Considering the above drawbacks, the aims of this study were to investigate the coupling relationship between the gripping force and electrophysiological parameters, synchronously determine and assess the inherent and instantaneous plant drought resistances, and combine with the variability of their difference, then comprehensively compare the drought resistance among different plant species. As such, the continuous monitoring of drought resistance of plants can be realized and the influence of inconsistent gripping force on leaf water determining can be reduced or avoided.

Broussonetia papyrifera (L.) Vent. and Morus alba L., which belong to the Moraceae family, are characterized by a rapid growth rate and greater adaptability to adversities than other members of the family (Wu et al. 2009). These species are always cultivated as medicinal or ornamental plants. Researches revealed that B. papyrifera exhibited better drought resistance than M. alba due to its higher bicarbonate use capacity and better water status (Wu and Xing 2012). The latter maintained the LT of different plant species. As such, the continuous monitoring of drought resistance of plants could be determined and the influence of inconsistent gripping force on leaf water determining can be reduced or avoided.

In this study, B. papyrifera and M. alba were selected as experimental materials, researches on the behaviors of leaf mechanical combined with electrophysiological properties under dehydration stress help understand the biomechanics mechanisms of plants in adapting to drought adversity. And a method for rapidly and comprehensively determining plant drought resistance based on the mechanical and electrophysiological properties could be developed.

Materials and methods

Plant materials

The experiment was performed on the campus of Jiangsu University, Jiangsu Province, China (32.20°N, 119.45°E). Eight years old B. papyrifera and M. alba plants which grew in the yellow-brown soil on a sunny slope were selected as the experimental materials. The area receives a mean annual precipitation of approximate 1100 mm and has a mean temperature of about 15.6°C. Fresh branches from the two plants were picked in July. Leaves growing uniformly were taken from the fourth and fifth leaf positions of each branch. The fresh leaves removed from the branches were placed in double distilled water immediately and soaked for 30 min in order to keep all the leaves in the same initial state (water-saturated), which could make the comparison of drought resistance among different plant species credible. After soaking, water on the surface of the leaves was removed. Finally, the detached leaves were placed on a dry ventilated desktop in the laboratory for 5 h, the temperature was 26°C. Photosynthetic Photon Flux Density (PPFD) was 160 μmol m−2 s−1, and relative air humidity was 40%. Measurements were done in triplicate at 0 (baseline), 1, 2, 3, 4 and 5 h after water loss.

Methods

Physical model of LT and F

The gravity equation is:

\[ F = (M_i + m)g \]  

where \( F \) is the gravity (gripping force; unit: N), \( M_i \) is the mass of iron (unit: kg), \( m \) is the mass of the foam board and electrode (unit: kg), and \( g \) is the acceleration of gravity with a value of 9.80 N kg⁻¹.

Cytosol solute in the leaf was taken as the dielectric. The leaf was clipped between the two electrodes of the parallel-plate capacitor, which formed a parallel-plate capacitor sensor. LT of the plant under different \( F \) could be determined by changing the mass of iron in the parallel-plate capacitor (Figure 1(a)). Pressure variation changed the concentration of the cytosol solute and the elasticity and plasticity of the cells in the leaf, which caused dielectric constant variation in the cytosol solute and leaf effective thickness (\( d_L \)) between the two electrodes of the parallel-plate capacitor. Thus, the LT changed.

The elasticity of the cell in the leaf was correlated to the water content in the cells. LT differed with plant species under the special \( F \).

The equation for Gibbs’s free energy is:

\[ \Delta G = \Delta H + PV \]

The equation for the energy of the capacitor is:

\[ W_C = \frac{1}{2} U^2 CP \]

where \( W_C \) is the energy of the capacitor, which is equal to the work converted from Gibbs’s free energy (\( \Delta G \); \( W_C = \Delta G \)); \( \Delta H \) is the internal energy of the system composed of cells in the plant leaves; \( P \) is the pressure imposed on the plant cells, \( V \) is the volume of plant cells; and \( U \) is the test voltage.

\( P \) can be calculated using the following equation:

\[ P = \frac{F}{ACP} \]

The CP of the leaf was expressed using Equation (5):

\[ CP = \frac{\varepsilon_0 R A_{CP}}{d_L} \]

where \( \varepsilon_0 \) is the vacuum dielectric constant with a value of 8.854 × 10⁻¹² F m⁻¹ and \( \varepsilon_r \) is the relative dielectric constant of the cytosol solute.

LT was calculated according to Equation (6). The unit was cm² cm⁻¹ (Zhang et al. 2015):

\[ LT = \frac{ACP}{d_L} = \frac{CP}{\varepsilon_0} \left[ \frac{1000iRT}{81000iRT + (81 - a)M_i} \right] \]

where \( A_{CP} \) is the effective area of the leaf in contact with the capacitor plates (unit: cm²); \( d_L \) is the leaf effective thickness (unit: cm); \( i \) is the dissociation coefficient (with value of 1); \( R \) is the gas constant (with value of 8.30 × 10⁻³ L MPa mol⁻¹ K⁻¹); \( T \) is the thermodynamic temperature (\( T = 273 + t \) °C, JOURNAL OF PLANT INTERACTIONS 611).
unit: K); $\varepsilon_0$ is the vacuum dielectric constant (with value of $8.854 \times 10^{-12}$ F m$^{-1}$); $a$ is the relative dielectric constant of the cytosol solute; $M$ is the relative molecular mass of the cytosol solute (unit: g mol$^{-1}$); and 81 is the relative dielectric constant of water at normal temperature. In this study, the sugar C$_{12}$H$_{22}$O$_{11}$ was identified as the solute in the cytosol; therefore, $a$ was 3.30, $M$ was 342 g mol$^{-1}$, and $t$ was 20°C. Equation (6) could be rewritten as:

$$\text{LT} = \frac{A_{CP}}{d_{L^t}} = \frac{\text{CP}}{717.17 + 96.75 \Psi_L} \quad (7)$$

According to Equations (2), (3), (4), (5) and (7), the relationship between LT and $F$ could be expressed as follows:

$$\text{LT} = \frac{2\Delta H}{\varepsilon_0 \varepsilon_r U^2} + \frac{2V}{\varepsilon_0 \varepsilon_r A_{CP} U^2} F \quad (8)$$

Incorporating $\frac{2\Delta H}{\varepsilon_0 \varepsilon_r U^2} = y_0$ and $\frac{2V}{\varepsilon_0 \varepsilon_r A_{CP} U^2} = k$ into Equation (8) changes this equation to:

$$\text{LT} = y_0 + kF \quad (9)$$

where $y_0$ and $k$ are the model parameters.

Determination of LT under different $F$ was conducted on fresh leaves taken from fresh branches. The CP of these leaves was measured using an LCR tester (model 3532-50, Hioki, Nagano, Japan). The frequency and voltage used were 3 kHz and 1 V, respectively. Each leaf was clipped onto the custom-made parallel-plate capacitor (a) with a diameter of 10 mm (Figure 1(a)). With a dew point microvoltmeter in a universal sample room (C-52-SF, Psypro, Wescor, Logan, Utah), $\Psi_L$ was measured at the same position of the leaves with the above CP testing.

The relationship curve between LT and $F$ for *B. papyrifera* or *M. alba* was established using SigmaPlot (ver. 12.5, Systat Software, Inc., San Jose, Cal.). The relationship between LT and $F$ was fitted, respectively. The model parameters $y_0$ and $k$ of *B. papyrifera* or *M. alba* were estimated, respectively.

### Calculation of leaf stiffness

The effective thickness per leaf area ($d_{LA}$, cm cm$^{-2}$) could be calculated using Equation (10):

$$d_{LA} = \frac{d_l}{A_{CP}} = \frac{1}{\text{LT}} \quad (10)$$

During the process where the leaf was compressed by an external force, the moving distance of the force was defined as $d_m$ (unit: cm cm$^{-2}$). According to Equations (9) and (10), $d_m$ could be calculated as follows:

$$d_m = d_{LA0} - d_{LA} = \frac{kF}{y_0 \times (y_0 + kF)} \quad (11)$$

where $d_{LA0}$ is $d_{LA}$ under 0 N external force and $d_{LA}$ is $d_{LA}$ under F N external force.

Meanwhile, as the leaf was compressed by an external force, pressure per leaf area was presented as stress ($\sigma$, N mm$^{-2}$). $\sigma$ was calculated as follows:

$$\sigma = \frac{F}{A_F} \quad (12)$$

where $A_F$ is the area of the leaf in contact with the probe implementing the gripping force.

According to Equations (11) and (12), leaf stiffness (LCS) was calculated as follows:

$$\text{LCS} = \frac{\sigma}{d_{m}} \times 10 = \frac{10 \times y_0 \times (y_0 + kF)}{k \times A_F} \quad (13)$$

The unit of LCS was N mm$^{-1}$.

### Determination of real-time leaf stiffness

LT and $F$ at each moment of water loss were defined as real-time LT (LT$_{rt}$) and real-time $F$ (Fr$_{rt}$), respectively. The CP of leaves at each moment of water loss was measured using the LCR tester with a frequency and voltage of 3 kHz and 1 V, respectively. The leaf was clipped onto the custom-made parallel-plate capacitor (b) with a diameter of 10 mm (Figure 1(b)). With the same dew point microvoltmeter in a universal sample room, $\Psi_L$ was also measured at the same moment of the leaves with the above CP testing. LT$_{rt}$ was calculated according to Equation (7) based on the above values of CP and $\Psi_L$, and Fr$_{rt}$ was calculated according to Equation (9).

**Figure 1.** Schematic of the parallel-plate capacitor (a) and (b): 1 = bracket; 2 = foam board; 3 = electrode; 4 = wire; 5 = iron; 6 = plastic bar; 7 = fixation clamp; 8 = plastic clip.
AF was calculated as follows:

\[
AF = \frac{10 \times y_0 \times (y_0 + kF_{\text{max}})}{k \times \frac{\pi}{2}}
\]

**Determination of maximum leaf stiffness**

The maximum gripping force \(F_{\text{max}}\) of leaf at each moment of water loss was measured with the texture analyzer TA.XT-Plus (Stable Micro System, United Kingdom) using the P/2n probe with a diameter of 2 mm. The instrument working parameters were determined by the test mode compression; with pretest speed at 2 mm s\(^{-1}\), test speed at 1 mm s\(^{-1}\), post-test speed at 2 mm s\(^{-1}\) and trigger force at 100 N (ensure that the leaf is crushed and the cell is broken up).

The diameter of the probe implementing \(F_{\text{max}}\) was 2 mm, \(A_p\) was calculated as follows: \(A_p = \pi \times \frac{\text{diameter}^2}{4}\), where the value of \(\pi\) was 3.14. Then \(LCS_{\text{max}}\) was calculated by using the following equations:

\[
LCS_{\text{max}} = \frac{10 \times y_0 \times (y_0 + kF_{\text{max}})}{k \times \frac{\pi}{2}}
\]

**Statistical analysis**

All collected data were analyzed using SPSS software (version 13.0, SPSS Inc., New York). The differences between the stress levels were assessed using the least significance difference post-hoc test at 5% significance level \((P \leq 0.05)\). The data were shown as the means ± standard errors determined using the one-sample T-test. The confidence interval was 95%.

**Results**

**Relationship between LT and F**

The model parameters \(y_0\) and \(k\) of \(B.\ papyrifera\) and \(M.\ alba\) were estimated using Equation (9). The relationship curves (Figure 2) between LT and F for \(B.\ papyrifera\) and \(M.\ alba\) was obtained using SigmaPlot (ver. 12.5, Systat Software, Inc., San Jose, Calif.). The relationship between LT and F was fitted. The fitting equations between LT and F for \(B.\ papyrifera\) and \(M.\ alba\) were obtained using SPSS software (version 13.0, SPSS Inc., New York). The difference between the stress levels was assessed using the least significance difference post-hoc test at 5% significance level \((P \leq 0.05)\). The data were shown as the means ± standard errors determined using the one-sample T-test. The confidence interval was 95%.

**Table 1.** Leaf water potential \((\Psi_L, \text{MPa})\) and real-time leaf tensity \((LTrt, \text{cm}^2\ \text{cm}^{-1})\) of \(B.\ papyrifera\) and \(M.\ alba\).

| Time (h) | \(B.\ papyrifera\) | \(M.\ alba\) | \(B.\ papyrifera\) | \(M.\ alba\) |
|---------|------------------|--------------|------------------|--------------|
|         | \(\Psi_L\) (MPa) | \(LTrt\) (cm\(^2\) cm\(^{-1}\)) | \(\Psi_L\) (MPa) | \(LTrt\) (cm\(^2\) cm\(^{-1}\)) |
| 0       | 1.12 ± 0.03ab    | 1.26 ± 0.01a  | 1.12 ± 0.03ab    | 1.26 ± 0.01a  |
| 1       | 1.71 ± 0.07a     | 1.49 ± 0.10b  | 1.44 ± 0.10a     | 1.22 ± 0.11a  |
| 2       | 1.40 ± 0.11b     | 1.37 ± 0.08b  | 1.14 ± 0.08ab    | 0.31 ± 0.02b  |
| 3       | 1.48 ± 0.18b     | 2.57 ± 0.13c  | 0.98 ± 0.01b     | 0.27 ± 0.01b  |
| 4       | 2.32 ± 0.11c     | 0.81 ± 0.11b  | 0.20 ± 0.05c     |               |
| 5       | 2.78 ± 0.06d     | 3.18 ± 0.10d  | 0.51 ± 0.02c     | 0.33 ± 0.01d  |

Note: The mean ± SE \((n = 5)\) followed by different letters in the same column differ significantly at \(P \leq 0.05\), according to one-way ANOVA and t-test.

**Table 2.** Real-time gripping force \((F_{\text{rt}}, \text{N})\) and real-time leaf tensity \((LCS_{\text{rt}}, \text{N} \text{cm}^{-2})\) of \(B.\ papyrifera\) and \(M.\ alba\).

| Time (h) | \(B.\ papyrifera\) | \(M.\ alba\) | \(B.\ papyrifera\) | \(M.\ alba\) |
|---------|------------------|--------------|------------------|--------------|
|         | \(F_{\text{rt}}\) (N) | \(LCS_{\text{rt}}\) (N cm\(^{-2}\)) | \(F_{\text{rt}}\) (N) | \(LCS_{\text{rt}}\) (N cm\(^{-2}\)) |
| 0       | 1.81 ± 0.02a     | 0.31 ± 0.01a | 2.02 ± 0.19a     | 0.06 ± 0.01a  |
| 1       | 1.71 ± 0.24a     | 0.30 ± 0.03a | 1.37 ± 0.10b     | 0.05 ± 0.01b  |
| 2       | 1.54 ± 0.17ab    | 0.28 ± 0.02a | 1.30 ± 0.10b     | 0.04 ± 0.01b  |
| 3       | 1.21 ± 0.03b     | 0.25 ± 0.01b | 1.12 ± 0.02b     | 0.04 ± 0.00b  |
| 4       | 0.83 ± 0.25b     | 0.20 ± 0.03b | 0.76 ± 0.15c     | 0.03 ± 0.01c  |
| 5       | 0.16 ± 0.04c     | 0.13 ± 0.01c | 0.35 ± 0.04d     | 0.02 ± 0.00d  |

Notes: The mean ± SE \((n = 5)\) followed by different letters in the same column differ significantly at \(P \leq 0.05\), according to one-way ANOVA and t-test.

*This column stands for the percent value after water loss treatment with reference to that of the 0 h.
Table 3. Maximum gripping force (F_{max}) and maximum leaf stiffness (LCS_{max}, N mm^{-1}) of B. papyrifera and M. alba at each water loss moment.

| Time (h) | B. papyrifera | M. alba |
|----------|----------------|---------|
|          | F_{max} (N)    | LCS_{max} (N mm^{-1}) | [%] | F_{max} (N) | LCS_{max} (N mm^{-1}) | [%] |
| 0        | 15.89 ± 0.26f | 227.12 ± 3.56f | 100 | 19.35 ± 0.26f | 375.05 ± 5.56f | 100 |
| 1        | 18.54 ± 0.43e | 262.90 ± 5.80e | 116 | 21.45 ± 0.45c | 375.01 ± 7.05c | 132 |
| 2        | 21.05 ± 0.65d | 296.48 ± 8.77d | 131 | 23.49 ± 0.64b | 456.19 ± 1.21b | 123 |
| 3        | 23.35 ± 0.58c | 327.85 ± 7.77c | 144 | 25.64 ± 0.52b | 489.1 ± 0.98b | 132 |
| 4        | 27.17 ± 0.83b | 379.43 ± 11.16b | 167 | 28.54 ± 0.84a | 56.24 ± 1.59a | 152 |
| 5        | 30.01 ± 1.00a | 417.82 ± 13.52a | 184 | 31.54 ± 0.84 | 61.24 ± 1.59 | 152 |

Notes: The mean ± SE (n = 5) followed by different letters in the same column differ significantly at P ≤ 0.05, according to one-way ANOVA and t test.

This column stands for the percent value after water loss treatment with reference to that of the 0 h.

Table 4. Difference between LCS_{rc} and LCS_{max} (LCS_{rc}, N mm^{-1}) and variability of LCS_{rc} in B. papyrifera and M. alba at each water loss moment.

| Time (h) | B. papyrifera | M. alba |
|----------|----------------|---------|
|          | LCS_{rc} (N mm^{-1}) [%] | Variability | LCS_{rc} (N mm^{-1}) [%] | Variability |
| 0        | 226.81 ± 1.00 | 40 ± 24 | 37.04 ± 1.00 | 24 |
| 1        | 262.60 ± 116 | 24 | 41.01 ± 111 | 13 |
| 2        | 296.60 ± 131 | 9 | 45.69 ± 123 | 1 |
| 3        | 337.60 ± 144 | 4 | 46.58 ± 126 | 2 |
| 4        | 379.22 ± 167 | 27 | 48.90 ± 132 | 8 |
| 5        | 417.69 ± 184 | 44 | 56.24 ± 152 | 28 |

Note: LCS_{rc} represents the difference between real-time leaf stiffness (LCS_{r}) and maximum leaf stiffness (LCS_{max}) at each water loss moment.

This column stands for the percent value after water loss treatment with reference to that of the 0 h.

This column stands for the absolute value of the difference between the percent value at each water loss moment and the average of percent values among six water loss moments.

Maximum leaf stiffness

Table 3 shows the values of F_{max} and LCS_{max} at each moment of water loss. High values of F_{max} in B. papyrifera and M. alba were correlated with increasing water loss moment. The increase in B. papyrifera was more significant than that in M. alba. F_{max} of M. alba at 2, 3 and 4 h exhibited no significant difference. LCS_{max} exhibited the same variation with F_{max} within the same plant species. Value of LCS_{max} in B. papyrifera at 2 h was already 131% of that at 0 h, while the value of LCS_{max} in M. alba at 4 h increased to 132% of that at 0 h. Values of LCS_{max} in B. papyrifera were significantly higher than that in M. alba at each water loss moment.

Variability of the difference between LCS_{rt} and LCS_{max}

High values of LCS_{rc} in B. papyrifera and M. alba were correlated with increasing water loss moment (Table 4). The increase in B. papyrifera was more significant than that in M. alba. B. papyrifera exhibited higher average variability of LCS_{rc} (24.67) than M. alba (12.67) during the water loss treatment period.

Discussion

The water loss rate can be used to characterize the dehydration resistance of a detached leaf. Water regulation caused by enzymes, such as carbonic anhydrases (CAs, EC 4.2.1.1), thickening (Eynck et al. 2012), which provide mechanical support for the wall and prevents the cell wall from collapsing effectively inhibited, thus decreasing the water loss rate (Deng and Zhang 1998). When the gripping force implemented on leaves was high enough (equal to failure load), leaf cells could no longer shrink, and F_{max} was mainly influenced by the substance composition of leaf cells (Figure 3). Since lignin synthesis and cell wall thickening of plants could be provoked by water deficit, F_{max} increased when the leaves suffered continuous water loss. As a result, the calculated values of LT_{max} and LCS_{max} increased correspondingly. A stronger B. papyrifera could help improve water absorption capacity and reduce natural water loss in a short time. This result was consistent with the research by Ren et al. (2015) in rice.
lignin synthesis and cell-wall thickening capacity in drought-tolerant plants caused more significant increase of LCS\textsubscript{max} in B. papyrifera than that in M. alba. Higher LCS\textsubscript{max} values at each water loss moment and more significant increase of LCS\textsubscript{max} with increasing water loss time indicated that B. papyrifera exhibited better pressure resistance under dehydration conditions. Besides, the above response trait could also be used for evaluating the quality of B. papyrifera tissues when they were used as raw materials for paper-making. However, since the LCS\textsubscript{max} needed to be measured at the point of failure load, the plant tissues would be damaged irreversibly during the determination, therefore, it became hard to realize the online monitoring of plant drought resistance.

The difference between LCS\textsubscript{max} and LCS\textsubscript{rt} was defined as leaf stiffness variable capacity (LCS\textsubscript{vc}) in this study. The average variability of LCS\textsubscript{vc} during the water loss treatment period could represent the flexibility and range of external forces tolerated by leaf. The average variability of LCS\textsubscript{vc} in B. papyrifera was higher than that in M. alba, which indicated that the leaves of B. papyrifera showed better flexibility and tolerance to a wider range of external forces under dehydration conditions. The higher leaf mechanical strength in B. papyrifera helped to maintain a higher outward pulling force of cell walls in the leaf; thus, the subsequent negative pressure could effectively inhibit cellular water loss. Therefore, B. papyrifera exhibited better drought resistance than M. alba. This result is consistent with the results of the studies by Wu et al. (2009) and Zhang et al. (2015).

**Conclusions**

Leaf water potential could not completely reflect the plant water status due to the influences of the surroundings and water regulation caused by enzymes, such as CA in plants. Although the determination of LT was less influenced by the surrounding, the results were easily affected by the area of the leaf in contact with the plates of custom-made parallel-plate capacitor or the gripping force. In this study, the variations of leaf mechanical and electrophysiological properties were synchronously analyzed by determining LT together with \( F \); thus, LCS\textsubscript{rt}, LCS\textsubscript{max} and the variability of LCS\textsubscript{vc} were investigated to analyze the leaf dehydration tolerance. The influence caused by the variation of the leaf area in contact with the plates of the custom-made parallel-plate capacitor and inconsistent gripping force could be reduced or avoided.

LT displayed good correlation with \( F \). The persistent stronger LCS\textsubscript{rt}, more significant increase in LCS\textsubscript{max} together with the higher average variability of LCS\textsubscript{vc} indicated that B. papyrifera exhibited better pressure resistance and better flexibility and tolerance to wider range of external forces under dehydration conditions. The higher leaf mechanical strength in B. papyrifera helped to maintain a higher outward pulling force of cell walls in the leaf; thus, the subsequent negative pressure could effectively inhibit cellular water loss. Therefore, B. papyrifera exhibited better drought resistance than M. alba. The synchronous investigation of LCS\textsubscript{rt}, LCS\textsubscript{max} and the variability of LCS\textsubscript{vc} could not only realize the online monitoring of plant drought resistance but also reduce the influence of inconsistent gripping force on leaf water determination. The above-mentioned method could also be used for detecting the plant responses to other environmental stresses.

**Disclosure statement**

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References

Balsamo R, Boak M, Nagle K, Peethambaran B, Layton B. 2015. Leaf biochemical properties in Arabidopsis thaliana polysaccharide mutants affect drought survival. J Biomech. 48(15):4124–4129. DOI:10.1016/j.jbiomech.2015.10.016

Balsamo RA, Willgen CV, Bauer AM, Farrant J. 2006. Drought tolerance and cell wall plasticity in maize. Ann Bot. 97:895–911. DOI:10.1093/aob/mcl068

Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. Annu Rev Plant Biol. 54:519–546. DOI:10.1146/annurev.arplant.54.031902.134938

Charrier G, Torres-Ruiz JM, Badel E, Burlett R, Chotard B, Cochard H, Delmas CE, Domene JC, Jansen S, King A, et al. 2016. Evidence for hydraulic vulnerability segmentation and lack of xylem refilling under tension. Plant Physiol. 172(3):1657–1668. DOI:10.1104/pp.16.01079

Cosgrove DJ. 2016. Plant cell wall extensibility: connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. J Exp Bot. 67(2):463–476. DOI:10.1093/jxb/erv411

Deng WS, Zhang DP. 1998. Water relation-related mechanism of the changes in the sensitivity of the growth of grape berry to drought during the berry development. Acta Hortic Sin. 25(2):123–128. In Chinese. http://www.cqvip.com/read/read.aspx?id=3030441

Egea G, Fernández JE, Alcon F. 2017. Financial assessment of adopting irrigation technology for plant-based regulated deficit irrigation scheduling in super high-density olive orchards. Agr Water Manage. 187:47–56. DOI:10.1016/j.agwat.2017.03.008

Eynck C, Ségui-Swartz G, Clarke WE, Parkin IAP. 2012. Monolignol biosynthesis is associated with resistance to Sclerotinia sclerotiorum in Camelina sativa. Mol Plant Pathol. 13:887–899. DOI:10.1111/j.1364-3703.2012.0798x

Fan L, Linker R, Gepstein S, Tanimoto E, Yamamoto R, Neumann PM. 2006. Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulation of cell wall phenolics. Plant Physiol. 140(2):603–612. DOI:10.1104/pp.105.073130

Grossi D, Rustioni L, Lorenzo GSD, Fallica O, Brancadoro L. 2016. Water deficit effects on grapevine woody tissue pigmentation. Hortic Sci. 43:188–194. DOI:10.17221/186/2015-HORTSCI

Javed Q, Wu YY, Azeeem A, Ullah I. 2017. Evaluation of irrigation effects using dilute salted water based on electrophysiological properties of plants. J Plant Interact. 12(1):219–227. DOI:10.1080/17429145.2017.1319501

Kroeger JH, Zerzour R, Geitmann A. 2011. Regulator or driving force? The role of turgor pressure in oscillatory plant cell growth. PLOS ONE. 6(4):e18549. DOI:10.1371/journal.pone.0018549

Lewis NG, Yamamoto E. 1990. Lignin: occurrence, biogenesis and biodegradation. Ann Rev Plant Physiol Plant Mol Biol. 41(1):455–496. DOI:10.1146/annurev.arplant.41.1.455

Malga R, Faure F, Boudaoud A. 2016. A mechanical model to interpret cell-scale indentation experiments on plant tissues in terms of cell wall elasticity and turgor pressure. Front Plant Sci. 7:1–11. DOI:10.3389/fpls.2016.01351

Petrop V, Petrova A, Dimitrov I, Tashiev T, Olsoska K, Brestic M, Misheva S. 2018. Relationships between leaf morpho-anatomy, water status and cell membrane stability in leaves of wheat seedlings subjected to severe soil drought. J Agron Crop Sci. 204(3):219–227. DOI:10.1111/jac.12255

Rascio A, Rascio N, Rinaldi M, Valentini M. 2015. Functional, histological and biomechanical characterization of wheat water-mutant leaves. Physiol Plantarum. 154(2):210–222. DOI:10.1111/ppl.12280

Ren BB, Wang M, Chen YP, Sun GM, Li Y, Shen QR, Guo SW. 2015. Water absorption is affected by the nitrogen supply to rice plants. Plant Soil. 396(1–2):397–410. DOI:10.1007/s11104-015-2603-5

Wu YY, Liu CQ, Li PP, Wang JZ, Xing DK, Wang BL. 2009. Photosynthetic characteristics involved in adaptability to Karst soil and alien invasion of paper mulberry (Broussonetia papyrifera (L.) vent.) in comparison with mulberry (Morus alba L.). Photosynthetica. 47(1):155–160. DOI:10.1111/j.1099-0095.2006-0026-3

Wu YY, Xing DK. 2012. Effect of bicarbonate treatment on photosynthetic assimilation of inorganic carbon in two plant species of Moraceae. Photosynthetica. 50(4):578–594. DOI:10.1111/j.1364-3703.2012-01145.x

Yin NW, Li JN, Liu X, Lian JP, Fu C, Li W, Jiang JY, Xue YF, Wang J, Chai YR. 2017. Lignification response and the difference between stem and root of Brassica napus under heat and drought compound stress. Acta Agron Sin. 43(11):1689–1695. In Chinese. DOI:10.3724/ SP.J.1006.2017.01689

Zhang MM, Wu YX, Xing DK, Zhao K, Yu R. 2015. Rapid measurement of drought resistance in plants based on electrophysiological properties. T ASABE. 58(6):1441–1446. DOI:10.13031/tras.58.11022

Zhou AM, Liu EH, Liu J, Feng S, Gong SF, Wang JG. 2018. Characterization of increased cuticular wax mutant and analysis of genes involved in wax biosynthesis in Diarthus spicatus. Hortic Res. 5:1–9. DOI:10.1038/s41438-018-0044-z