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Rapid Measurement of Drought Resistance in Plants Based on Electrophysiological Properties

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ABSTRACT. Water loss in plant leaves causes mesophyll cells and their cell walls to shrink; thus, the cell volume becomes smaller. When leaf cells absorb water and expand, the cell volume becomes larger. The characteristic of water retention for cells is related to this expansion and contraction and is expressed as leaf tensity. In this study, leaves of *Broussonetia papyrifera* and *Morus alba* were used to examine the physiological capacitance,

water potential, minimal fluorescence, and maximal photochemical efficiency of photosystem II (PS II) before and after water loss. The measured physiological capacitance value and water potential were used to calculate the relative tensity of leaves. The values of relative tensity in *B. papyrifera* and *M. alba* were 3.965 and 2.624, respectively. By measuring the minimum chlorophyll fluorescence and maximal photochemical efficiency of PS II in the leaves, the relative minimal fluorescence and maximal photochemical efficiency were calculated; the measured minimal fluorescence and maximal photochemical efficiency were 5.496 and 7.640 for *B. papyrifera* and 6.577 and 5.359 for *M. alba*, respectively. Results of the two methods showed that the drought-resistance ability of *B. papyrifera* was greater than that of *M. alba*. The electrophysiological characteristics of the plants reflected their ability to resist drought.

Keywords. Chlorophyll fluorescence, Drought resistance, Electrophysiological characteristics, Physiological capacity, Water potential.

Plants are easily affected by drought stress, which causes growth inhibition. A timely and effective determination of drought stress in plants ensures that plant damage is prevented. This aspect is explored in the present study. Plants survive under water-deficient conditions using a series of physiological and cellular processes culminating in stress tolerance, as described by Shinozaki and Yamaguchi-Shinozaki (2007). Farooq et al. (2009) described that plants exhibit a range of mechanisms, which include reduction of water loss by increased diffusive resistance and enhanced water uptake in abundant deep root systems, to withstand drought stress. Transgenic maize plants with increased ZmNF-YB2 gene expression exhibited tolerance to drought based on their response to a number of stress-related parameters, including chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis (Nelson et al., 2007). Therefore, drought resistance in plants can be determined through their photosynthetic and physiological characteristics.

Chlorophyll fluorescence (ChlF) analysis is a rapid, non-destructive diagnostic tool for the photosynthetic apparatus and overall health of plant tissue, as described by Roháček and Bartáček (1999). Changes in ChlF emissions caused by PS II provide information on almost all aspects of photosynthetic activity; thus, Panda et al. (2008) supposed that this parameter indicates the tolerance of plants to environmental stresses, including drought. ChlF imaging is an alternative method to accurately determine the freezing tolerance of leaves. This process is rapid, inexpensive, and can be used in large-scale screening; thus, Ehlert and Hinch (2008) presented a new approach to elucidate freeze tolerance of plants. ChlF measurements of a sensitive rice cultivar (IR29) under salt stress were done by Moradi and Ismail (2007). They revealed that the plants exhibited increased non-photochemical quenching and decreased electron transport rate. The ratio of steady-state chlorophyll fluorescence (F_s) and minimum chlorophyll fluorescence (F_o), i.e., F_s/F_o , which was measured by Flexas et al. (2002) with a portable instrument (PAM-2000), is a good method to promptly detect water stress. This technique may become a useful guide to determine irrigation requirements. However, the determination of ChlF parameters, especially F_o and maximal chlorophyll fluorescence (F_m), must be performed in a special environment. Moreover, the process is time consuming, and the instrument used for determination is extremely expensive. Therefore, a convenient and low-cost device or method to determine drought resistance in plants should be developed.

Water deficiency in plants can also be determined using their electrophysiological properties. A miniaturized, non-destructive sensor that employs a microwave microstrip ring resonator (MRR) was developed to estimate the water content in a single wheat grain. The resonant frequency,

bandwidth, and quality factor of the MRR were calibrated against water content, as described by Abegaonkar et al. (1999). Water content in a peanut kernel was determined by measuring the shift in resonant frequency and change in cavity transmission characteristics when a peanut was inserted in a cavity. The water content obtained in this process was independent of the mass of the kernel and peanut type, as described by Kraszewski and Nelson (1993). Noble et al. (2000) observed the amount of water applied per day, leaf-air temperature, and soil moisture content based on electrical resistance. The water potential of a plant was correlated with the concentration in the plant components, which were reflected by hydraulic resistance and capacitance, as discovered by Koide et al. (1991). A pressure chamber was used by Turner et al. (1988) to determine water potential and to establish membrane damage of plants at various points in the xylem. Meinzer et al. (2003) used the physiological capacitance of sapwood to scale several whole-tree water transport properties. These properties were determined from measurements of an upper branch and basal sap flow, branch water potential, and axial and radial movements of deuterated water (D_2O) injected into the trunk base as tracer. Water content, physiological capacitance, and water potential in leaves decreased when drought stress increased. A significant relationship was observed between physiological capacitance and water content or potential. Therefore, the status of water in leaves may be obtained from the variations of physiological resistance and capacitance.

The status of water in plants is characterized by the tensity of the plant cells. Leaves of *Broussonetia papyrifera* and *Morus alba* were used as materials in this study. The physiological capacitance and water potential of the leaves were determined to calculate the relative tensity of the leaves. The leaf tensity reflects the drought resistance of plants. Meanwhile, the drought resistance in the plants was also analyzed using the ChlF parameters of the leaves. Comparison of the two methods was performed to validate whether the relative tensity of leaves reflects the drought resistance of plants. The results of this study may be used to develop a more convenient and inexpensive method of drought-resistance determination in plants. This method can be used to obtain precise information on the water requirement in leaves.

Materials and Methods

Experimental Materials

The experiment was conducted in a growth chamber at the Institute of Agricultural Engineering, Jiangsu University, Jiangsu Province, China (32.20° N, 119.45° E). Fifty leaves from *B. papyrifera* and *M. alba* plants on the campus of Jiangsu University were selected as the experimental materials. About 0.6 m long branches from the two plants were picked. Leaves of similar size and growth were taken from the third, fourth, and fifth leaf positions of each branch. The fresh leaves were placed in water immediately after they were removed from the trees and were soaked for 30 min. After soaking, water on the surface of the leaves was removed. Finally, the leaves were placed on a dry ventilated desktop for 5 h. Measurements were done in triplicate at 0 (baseline), 1, 2, 3, 4 and 5 h after water loss.

Determination of Chlorophyll Fluorescence

ChlF was measured using a pulse amplitude modulated (PAM) ChlF imaging system (IMAGING-PAM, Heinz Walz GmbH, Effeltrich, Germany), as described by Calatayud et al. (2006). The leaves were placed in a dark area for 30 min. This process was performed to ensure the complete relaxation of all reaction centers in the leaves before measurements were conducted. The minimum

chlorophyll fluorescence (F_o , the initial fluorescence level) was determined using a measuring beam, whereas the maximum chlorophyll fluorescence (F_m , the maximum fluorescence level) was obtained after a 0.8 s saturating-light pulse ($6000 \mu\text{mol m}^{-2} \text{s}^{-1}$). The maximum quantum yield of PS II (F_v/F_m , the maximum quantum yield of photosystem II) in the leaves was calculated using: $F_v/F_m = (F_m - F_o)/F_m$, where F_v is the variable fluorescence, which was described by Panda et al. (2006), Thomas and Turner (2001), and Kooten and Snel (1990). The relative fluorescence value obtained at the other time periods was calculated using: $RCF_i = CF_i/CF_0$, where CF is the chlorophyll fluorescence parameters F_o or F_v/F_m , and i is the time period (i.e., 0, 1, 2, 3, 4, and 5 h). The sum of the t values of the relative fluorescence parameters (T_{RCF}) of the plants was calculated using: $T_{RCF} = \sum RCF_i$.

Water Potential and Physiological Capacitance

With a dew point microvoltmeter in a universal sample room (C-52-SF, Psypro, Wescor, Logan, Utah), the water potential (W) of the leaves was measured as described by Kim et al. (2003). The leaf physiological capacitance (C) was measured using an LCR tester (model 3532-50, Hioki, Nagano, Japan). The frequency and voltage used were 3 kHz and 1 V, respectively, as chosen by Ksenzhek et al. (2004) and Michalov (1983). The leaf was clipped in a custom-made parallel-plate capacitor (Kandala et al., 2007; Kandala and Sundaram, 2010) with a diameter of 7 mm (figs. 1 and 2). The mean value was obtained from the determination on the leaf physiological capacitance of ten points on each leaf.

Calculation of Relative Cell Tensity

The relationship between the plant tissue water potential (W) and [cytosol](#) solute concentration is:

$$W = -iCRT \quad (1)$$

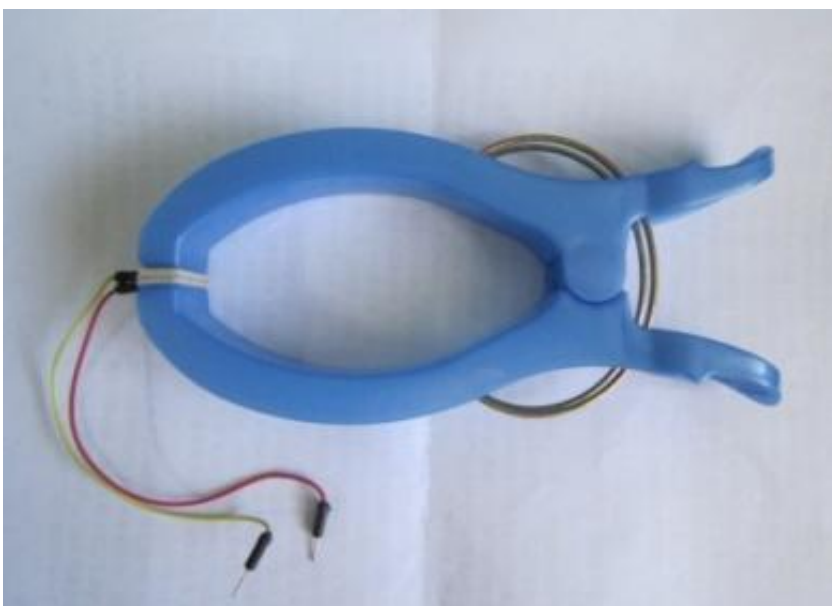


Figure 1. Parallel-plate capacitor used in the study.

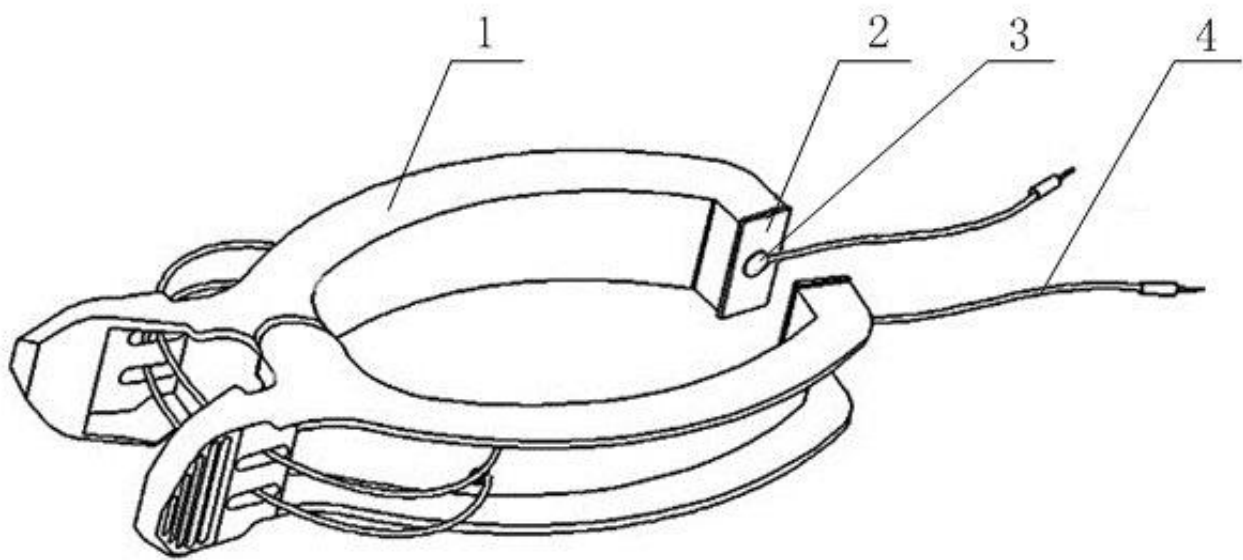


Figure 2. Schematic of the parallel-plate capacitor: 1 = plastic clip, 2 = foam board, 3 = electrode, and 4 = wire.

where

W = plant tissue water potential (MPa)

i = dissociation coefficient (1)

Q = cytosol concentration of solute (mol L^{-1})

R = gas constant ($0.0083 \text{ L Pa mol}^{-1} \text{ K}^{-1}$)

T = thermodynamic temperature (K, $T = 273 + t$ °C).

The cytosol solute of the leaf was obtained as a dielectric. The leaf was clipped between the two electrodes of the parallel-plate capacitor, which formed a parallel-plate capacitor sensor. The water potential of the leaf varied with the cytosol solute concentration in the leaf. This behavior caused a change in the dielectric constant of the cytosol solute in the leaf tissue that was between the two electrodes. In effect, the physiological capacitance (C) of the leaf was affected. The C of the leaf is expressed using equation 2:

$$C = \frac{\epsilon_0 \epsilon_r A}{d} \quad (2)$$

where

C = physiological capacitance of leaf (pF)

ϵ_0 = vacuum dielectric constant ($8.854 \times 10^{-12} \text{ F m}^{-1}$)

ϵ_r = relative dielectric constant of cytosol solute

A = effective area of leaf that is in contact with capacitor plates (m^2)

d = effective thickness of leaf (m).

The cytosol of the leaf was divided into water and solute. The proportion of solute in the total cytosol of the leaf was P , and the proportion of water was $1-P$. The relative dielectric constant of water at normal temperature is 81. Meanwhile, the relative dielectric constant of the **cytosol** solute in the leaf was defined as a . The relative dielectric constant of the **cytosol** solute of the leaf was expressed using equation 3:

$$\varepsilon_r = (1-P) \times 81 + P \cdot a = [81 - (81-a)P] \quad (3)$$

Incorporating equation 3 into equation 2, equation 2 was rewritten as equation 4:

$$C = \frac{\varepsilon \cdot [81 - (81-a)P] \cdot A}{d} \quad (4)$$

The relationship between the proportion of solute in the total cytosol of the leaf (P) and the solute concentration (Q) was $Q = 1000P/M$, where M is the relative molecular mass of the cytosol solute.

Comparing the relationship between P and Q , equation 4 can be rewritten as equation 5:

$$C = \frac{\varepsilon_0 A \cdot \left[81 - \frac{(81-a)MQ}{1000} \right]}{d} \quad (5)$$

Incorporating equation 1 into equation 5, equation 5 was rewritten as equation 6:

$$C = \frac{\varepsilon_0 A \cdot \left[81 + \frac{(81-a)MW}{1000 iRT} \right]}{d} \quad (6)$$

$$\text{Then } \frac{d}{A} = \frac{\varepsilon_0 \left[81 + \frac{(81-a)MW}{1000 iRT} \right]}{C} \quad (7)$$

$$\text{and } y = \frac{d}{A} = \frac{\varepsilon_0}{C} \left[81 + \frac{(81-a)MW}{1000 iRT} \right] \quad (8)$$

where y represents d/A , and $1/y$ is defined as the leaf tensity of the plant.

For a given material, the relative dielectric constant (a) and molecular mass (M) of the **cytosol** solute have assigned values. In this study, the sugar $C_{12}H_{22}O_{11}$ was identified as the solute in the cytosol; therefore, a was 3.3 and M was 342. The leaf tensity of the plants ($Td_i = 1/y$, where $i = 0, 1, 2, 3, 4, 5$) was determined to indicate the water status in the leaves. The relative tensity of the leaves after water loss was obtained using: $RTd_i = Td_i/Td_0$, where $i = 0, 1, 2, 3, 4, 5$. The sum of the relative tensity values ($SRTd_i$, where $i = 0, 1, 2, 3, 4$, and 5 h) was defined as the drought resistance of the plant (RDC).

Analysis of the Reliability of the Method

The following function was used to check the reliability of determining the drought resistance of plants via the relative tensity of the leaves: $y' = f(x_1, x_2)$, where y' refers to the combined effect of the relative chlorophyll fluorescence indexes (F_o and F_v/F_m), x_1 is F_o , and x_2 is F_v/F_m . The relative

tensity of the leaves (RTd) has a functional relationship with y' .

We used SPSS (ver. 17.0, IBM) to perform the statistical analysis. The Pearson correlation coefficient (r) between the relative tensity and the combined effect of the relative chlorophyll fluorescence indexes of the leaf, as well as the significance level (p -value), were determined. The numerical values of r and p were used to measure the degree of correlation between RTd and y' , and the reliability of

determining the drought resistance of plants by the relative tensity of leaves was tested.

Results

Physiological Capacitance, Water Potential, and Chlorophyll Fluorescence

The physiological capacitance (PC) of *B. papyrifera* and *M. alba* increased significantly after 1 h and decreased after 2 h (table 1). The PC values of *B. papyrifera* and *M. alba* after 3, 4, and 5 h were all lower than their baseline values. However, the PC of *B. papyrifera* exhibited a significant increase after 5 h compared with the values obtained after 3 or 4 h. The water potential of *B. papyrifera* increased after 1 h but significantly decreased after 2 h when compared with the baseline value, and further decreases were not significant. Meanwhile, the water potential of *M. alba* decreased when water was lost. The minimum chlorophyll fluorescence (F_o) of *B. papyrifera* and *M. alba* decreased and increased, respectively, when the leaves underwent water loss (table 2). The maximum quantum yield of PS II (F_v/F_m) in *B. papyrifera* and *M. alba* significantly increased and decreased, respectively, when the leaves underwent water loss.

Time (h)	Physiological Capacitance (pF)		Water Potential (MPa)	
	<i>B. papyrifera</i>	<i>M. alba</i>	<i>B. papyrifera</i>	<i>M. alba</i>
0	574.305 b ?43.500	647.961 b ?40.440	-1.833 bc ?0.098	-1.677 a ?0.041
1	790.003 a ?74.020	810.027 a ?48.140	-1.417 a ?0.058	-1.977 c ?0.033
2	448.183 c ?33.330	92.418 c ?8.450	-1.560 a ?0.055	-1.983 c ?0.035
3	184.729 e ?7.740	40.453 c ?3.690	-1.787 b ?0.027	-1.817 b ?0.048

4	123.351 e ?8.380	34.418 c ?1.430	-1.967 c ?0.046	-2.023 c ?0.007
5	228.365 d ?13.960	22.110 c ?0.570	-1.990 c ?0.010	-1.777 ab ? 0.022

[a] 賡煖alues are means 盪D. Means in the same column followed by the same letter are not significantly different.

Table 2. Chlorophyll fluorescence parameters of *B. papyrifera* and *M. alba* after water loss.^[a]

Time (h)	Minimum Chlorophyll Fluorescence		Maximum Quantum Yield of PS II	
	<i>B. papyrifera</i>	<i>M. alba</i>	<i>B. papyrifera</i>	<i>M. alba</i>
0	0.123 a ?0.006	0.100 d ?0.003	0.654 c ?0.022	0.813 a ?0.002
1	0.109 b ?0.006	0.154 a ?0.005	0.754 a ?0.009	0.692 c ?0.004
2	0.129 a ?0.005	0.137 b ?0.005	0.728 ab ?0.004	0.715 bc ?0.006
3	0.102 b ?0.004	0.118 c ?0.003	0.713 b ?0.003	0.705 c ?0.009
4	0.108 b ?0.003	0.106 d ?0.006	0.740 ab ?0.004	0.711 bc ?0.003
5	0.105 b ?0.004	0.149 ab ?0.004	0.712 b ?0.003	0.721 b ?0.004

[a] 賡煖alues are means 盪D. Means in the same column followed by the same letter are not significantly different.

Relative Tensity, Chlorophyll Fluorescence, and Drought Resistance

The leaf tensions of *B. papyrifera* and *M. alba* were calculated with equation 8 and $Td_i = 1/y$, using the values of PC and water potential in table 1. The temperature was 20°C. The leaf tensions of *B. papyrifera* and *M. alba* both increased after 1 h and decreased significantly after 2 h (table 3). Compared with the leaf tension of *B. papyrifera*, that of *M. alba* exhibited a significant decrease after 2 h. The leaf tension of *B. papyrifera* and *M. alba* after 3, 4, and 5 h were all lower compared with that obtained at baseline; however, the value of *B. papyrifera* after 5 h exhibited some increase compared with the leaf tension values after 3 and 4 h.

Relative leaf tension was defined as $RTd_i = Td_i/Td_0$. The sum of the RTd_i results ($i = 0, 1, 2, 3, 4$, and 5 h) was defined as the RDC. As shown in table 4, the drought resistance of *B. papyrifera* was better than that of *M. alba*.

The relative chlorophyll fluorescence values ($RCF_i = CF_i/CF_0$) and their sum ($T_{RCF} = SRCF_i$) were defined and calculated, as shown in table 5. The relative initial fluorescence of *B. papyrifera* was lower than that of *M. alba*, which illustrated that the output of fluorescence in *B. papyrifera* was lower than that in *M. alba*. Meanwhile, the relative maximum photochemical quantum yield of PS II in *B. papyrifera* was higher than that of *M. alba* when the reaction center opened.

Table 3. Leaf tension of <i>B. papyrifera</i> and <i>M. alba</i> after water loss.		
Time (h)	<i>B. papyrifera</i>	<i>M. alba</i>
0	1.066	1.170
1	1.364	1.543
2	0.793	0.176
3	0.334	0.075
4	0.235	0.066
5	0.435	0.041

Table 4. Relative leaf tension and drought-resistance ability of <i>B. papyrifera</i> and <i>M. alba</i> . RDC = drought resistance of plant.		
Relative Tensity	<i>B. papyrifera</i>	<i>M. alba</i>
RTd_0	1	1
RTd_1	1.280	1.319
RTd_2	0.744	0.150
RTd_3	0.313	0.064
RTd_4	0.220	0.056
RTd_5	0.408	0.035
RDC	3.965	2.624

Time (h)	Relative Minimum Chlorophyll Fluorescence		Relative Maximum Quantum Yield of PS II	
	<i>B. papyrifera</i>	<i>M. alba</i>	<i>B. papyrifera</i>	<i>M. alba</i>
0	1	1	1	1
1	0.886	1.540	1.154	0.851
2	1.049	1.370	1.113	0.879
3	0.829	1.180	1.090	0.867
4	0.878	1.060	1.131	0.875
5	0.854	1.490	1.089	0.887
T_{RCF}	5.496	7.640	6.577	5.359

Correlation between RTd and y'

Using SPSS 17.0 to perform the statistical analysis, the correlation between the relative leaf tensity and the combined effect of the relative chlorophyll fluorescence indexes of the two plants was analyzed. The correlation coefficient (r) between the relative leaf tensity and the combined effect of the relative chlorophyll fluorescence indexes for *B. papyrifera* was 0.957 ($p = 0.001$), and the r for *M. alba* was 0.848 ($p = 0.016$). The testing results indicate that there was a good correlation between the relative leaf tensity and the combined effect of the relative chlorophyll fluorescence indexes in the two plants. The relative leaf tensity may be used to determine drought resistance of plants.

Discussion

Plants differ in their physiological capacitance and leaf water potential response to water stress. The physiological capacitance of *B. papyrifera* leaves increased, and the cells expanded after 1 h loss of water, which caused a decrease of solute concentration in the cytosol, and the water potential of *B. papyrifera* increased simultaneously. At the same time, the physiological capacitance of *M. alba* leaves increased, and the water potential decreased. The water potential of *B. papyrifera* became independent of water after 5 h loss of water, compared to 4 h for *M. alba*, while the physiological capacitance increased, which indicates that the concentration of solute in the cytosol was not affected, and the leaf cells expanded in response to the continuing loss of water. However, the water potential of *M. alba* increased, and the physiological capacitance of the leaves decreased, which indicates that the concentration of solute in the cytosol could have a threshold value for dysfunction of cells, and the increasing water potential was the plants' method for survival when the loss of water continued. As a whole, neither the leaf water potential nor the physiological capacitance could accurately reflect the leaf water status of plants when they were determined separately. The synergistic changes in leaf water potential and physiological capacitance allow the plants to cope with water stress.

Bai and Zhang (2013) found that water has the ability to sustain tension of cells in leaves and other tissues to maintain the inherent state of the plants. Cell tensity can be used to characterize the water

status of plants, and changes in tension can reflect the speed of water loss and the ability to hold water, as described by Irigoyen et al. (1992). The ability to hold water was associated with the drought resistance of plants (Hurd, 1974). The results in table 3 show that the leaf tensity of *B. papyrifera* changed and the ability of the plant to hold water was better, while the leaf tensity of *M. alba* decreased sharply and its ability to hold water was not good. *B. papyrifera* exhibited better ability to resist drought stress than *M. alba*, which is the same as the result obtained by Wu et al. (2011).

F_o is the fluorescence yield when the PS II reaction centers are completely activated. An increase in F_o indicates that adversity has produced damage that is not easy to reverse, or reversible inactivation of the PS II reaction centers of crop leaves, as discovered by Song et al. (2009). The F_v/F_m value is the conversion efficiency of light energy of PS II, as described by Schreiber et al. (1995). A decrease in F_v/F_m shows that the maximum conversion efficiency of light energy of PS II is restrained (Woo et al., 2008) and the activity centers of PS II are damaged (Osmond et al., 1993). The data in table 5 show that the PS II reaction centers of *B. papyrifera* were not restrained by light and were not damaged during water loss. However, the PS II reaction centers of *M. alba* were restrained by light and were damaged. The maximum conversion efficiency of light energy of PS II of *B. papyrifera* was not restrained, and the activity centers of PS II were not damaged, whereas *M. alba* was restrained and damaged. Therefore, *B. papyrifera* showed better drought resistance than *M. alba*.

Moreover, the results showed that the leaf tensity of the plants was associated with the activation of PS II reaction centers and with the conversion efficiency of the light energy of PS II. This phenomenon was due to the water holding ability of the leaves. This ability maintained the leaf tensity and prevented PS II damage; thus, the degree of inhibition of the PS II reaction centers and the maximum conversion efficiency of the light energy of PS II was small.

The two methods have the ability to compare drought resistance between different varieties of plants and within the same species at different ages. Leaf tensity directly reflects the plant's ability to resist drought in terms of water loss, whereas chlorophyll fluorescence parameters indirectly reflect the plant's ability to resist drought based on the change in the reaction centers after dehydration. Both methods are feasible. Chlorophyll fluorescence can be used to indicate the drought resistance of plants. In the absence of chlorophyll fluorescence measurement, the electrophysiological properties (i.e., leaf tensity) of the leaves may be used to indicate the drought resistance of plants.

Conclusion

High light energy conversion efficiency and stable PS II reaction centers caused *B. papyrifera* to exhibit stronger drought resistance than *M. alba*. This finding was based on the chlorophyll fluorescence parameters. In the absence of chlorophyll fluorescence measurement, the variation of the relative leaf tensity may be used to determine the drought resistance of plants. This parameter is based on the physiological capacitance and tissue water potential of the plant. The two methods are able to compare the drought resistance between different varieties of plants and within the same species at different ages. The methods are simple and rapid, provide scientific data for drought-tolerant cultivar breeding and precise irrigation, and have an important theoretical and practical meaning for precise prediction of plant water requirements.

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