Leaf tensity: a method for rapid determination of water requirement information in *Brassica napus* L.

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**ABSTRACT**

Water regulation caused by enzymes, such as carbonic anhydrase (CA), changes the water status, making it difficult to diagnose water deficit using leaf water potential ($\psi_f$) or stomatal conductance ($g_s$). Therefore, new methods for timely and accurately determining plant water status should be established. In this study, CA activity, $\psi_f$, leaf tensity ($T_d$), photosynthetic characteristics and plant growth of *Brassica napus* L. seedlings under drought and subsequent rewatering were analysed. Results indicated that $T_d$ could reflect the plant water status better than $\psi_f$ or $g_s$ and played an important role in the photosynthesis of *B.* *napus*. *B.* *napus* exhibited good restorability at the 40 g L$^{-1}$ polyethylene glycol level. The rewatering strategy for *B.* *napus* was excellent at 40 g L$^{-1}$ (−0.15 MPa)→20 g L$^{-1}$ (−0.11 MPa). $T_d$ could be used for the rapid determination of water requirement information in *B.* *napus* during winter drought period.

**1. Introduction**

Rape is an important oil crop in China and other countries. This crop is mainly planted in southern China and used as a material for edible oil extraction or feedstock for fuel extraction, offering great economic values (Bhardwaj et al. 2015). In the karst regions of southwestern China, the hot and humid climate and widely developed karst environment are conducive for the formation of loam, which can be used to cultivate plants. For example, the soil used for cultivation in Puding County, Guizhou Province, is mainly loam (Wang et al. 2010). However, the plants in these regions usually suffer from frequent temporary water deficiency because of a considerably shallow soil with low water-holding capacity. Moreover, the agricultural water resource in these regions is very scarce, especially during the winter drought period. As a result, crop production is low and local economic development is impeded (Zhu 1997). *Brassica* *napus* L., a commonly cultivated type of rape, is characterized by high grain yield and is therefore suitable for widespread cultivation in the karst regions (Qaderi et al. 2007). In addition, correct timing of *B.* *napus* irrigation helps to reduce agricultural irrigation costs and ensure good crop yield.

Carbonic anhydrase (CA, EC 4.2.1.1) are zinc-containing metalloenzymes that catalyse the reversible conversion of CO$_2$ to bicarbonate. CA are widely distributed and involved in diverse physiological processes in animals, plants, archaea and eubacteria (Hu et al. 2011). Under drought stress conditions, CA activity in plants is activated. CA then catalyses the conversion of intracellular bicarbonate into H$_2$O and CO$_2$, changes cell water status and delays the water requirement in plants (Fernández et al. 2015). The instantaneous indicators, such as leaf wilting degree, predawn leaf water potential ($\psi_f$), stem water potential, stomatal conductance ($g_s$), transpiration rate ($E$), leaf water content (WC) and stem diameter, are traditionally considered as the indirect indexes for determining water status in plant (Gallardo et al. 2006; Zhang et al. 2011; Pathan et al. 2014; Gaudin et al. 2017; Milliron et al. 2018). However, water regulation caused by enzymes, such as CA in plants, changes the water status and volume of cells, making it difficult to diagnose water deficit using $\psi_f$, $g_s$ or other indirect indexes. Therefore, new methods or indicators for timely and accurately determining plant water deficit status and physiological drought resistance should be established to predict the appropriate irrigation time.

Water status is significantly related to cell turgidity or shrinkage in plant leaves, which are composed of numerous cells. The variation of cell volume is caused by cell turgidity or shrinkage (Turner and Burch 1983). Variations in cell volume and cell sap concentration are indicated by leaf tensity ($T_d$). The physiological capacitance ($C_p$) and $\psi_f$ are related to the cell sap concentration. Leaf $C_p$ is associated with the effective thickness ($d$) and area ($A$) of leaves in contact with capacitor plates. The ratio of $A$ and $d$ is defined as leaf tensity ($T_d = A/d$) (Zhang et al. 2015). Furthermore, $T_d$ exhibits a better relationship with net photosynthetic rate than $\psi_f$; therefore, changes in $T_d$ can reflect the leaf water status (Turner and Burch 1983; Irigoien et al. 1992; Wu et al. 2015). Photosynthetic activity is always inhibited when plants suffered from drought stress (Batra et al. 2014), which causes serious yield and economic losses (Kleiber et al. 2017). The rapid and accurate prediction of photosynthesis provides methods for determining the physiological drought resistance threshold. Irrigation based on this threshold may prevent the excessive decline of crop yield. However, studies have found indirect relationships between photosynthesis and water status and obtained results based on the irreversible damages.
found in the plants (Xing and Wu 2012; Wu et al. 2016; Kleiber et al. 2017). Thus, direct relationships between photosynthesis and \( t_d \) combined with the latter’s rapid determination can help to quickly determine the physiological drought resistance threshold and predict the appropriate irrigation time.

In this study, *B. napus* was selected as the experimental material. The effects of drought and subsequent rewatering on CA activity, \( \psi_t \), C\(_P\), photosynthetic traits, leaf area (A) and plant dry weight of seedlings were analysed. Variations in \( t_d \) and water requirement information in *B. napus* were also investigated. The irrigation time of *B. napus* was then determined. The results could provide a new method for rapidly determining the appropriate irrigation time of *B. napus* during winter drought period using \( t_d \).

2. Materials and methods

2.1. Plant growth and treatment

The new hybrid rapeseed Qianyou No. 17 was used. It exhibits high yield, strong resistance and good quality in *B. napus* commonly planted in the karst area of southwest China. The seedlings and plants of *B. napus* were germinated, cultivated and treated according to Xing et al. (2016) in a growth chamber at the Institute of Agricultural Engineering, Jiangsu University, Jiangsu Province, China (32.2° N, 119.45° E). The *B. napus* plants were sown in 12-hole trays containing quartz sand and grown in a growth chamber with 12 h photoperiods [300 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) Photosynthetic Photon Flux Density (PPFD)], day/night temperatures of 28°C/20°C and relative air moisture level of 70%.

After 2 months of growth, four drought stress levels were created by preparing 4 concentrations of poly-ethylene glycol (PEG) 6000 (i.e. 0, 20, 40 and 80 g L\(^{-1}\)) with corresponding water potentials of −0.08, −0.11, −0.15 and −0.22 MPa (Michel and Kaufmann 1973). Rewatering was conducted on day 8 from the onset of the drought stress treatment. The seedlings, which grew in 0, 20, 40 and 80 g L\(^{-1}\) PEG treatment solutions, were transferred into 0, 10, 20 and 40 g L\(^{-1}\) PEG treatment solutions with corresponding −0.08, −0.09, −0.11 and −0.15 MPa water potentials, respectively. The rewatering phase lasted for 4 days. The solution was changed with a new batch of mixed solution every other day during the treatments. The fourth and fifth youngest fully expanded leaves from the top (three plants from each treatment group) were chosen for measurement. Determination was conducted on day 8 from the onset of the drought stress treatment and on day 4 from the onset of the rewatering treatment.

2.2. Determination of CA activity, photosynthesis and chlorophyll-a fluorescence parameters

The fourth and fifth youngest fully expanded leaves from the top were chosen for CA activity measurement. Three plants from each treatment group were used for the measurement. Leaf tissues (0.3–0.8 g) were quickly frozen in liquid nitrogen and ground with 3 mL extraction buffer (0.01 mol L\(^{-1}\) barbitone sodium with 0.05 mol L\(^{-1}\) mercaptoethanol, pH 8.3). The homogenate was centrifuged at 13,000 r min\(^{-1}\) and 0°C for 5 min and then placed on ice for 20 min. CA activity was determined with the electrometrical method of Wilbur and Anderson (1948) with modifications (Xing and Wu 2012). In brief, CA activity was assayed at 0–2 °C in a mixture containing 4.5 mL 0.02 mol L\(^{-1}\) barbitone buffer (5, 5-diethylbarbituric acid; pH 8.3), 0.4 mL sample and 3 mL CO\(_2\)-saturated water. CA activity was expressed in Wilbur and Anderson (WA) units as WA = (t\(_0\)/t)−1, where \( t_0 \) and \( t \) were the time (second) measured for the pH change (8.2–7.2) with buffer alone (\( t_0 \)) and with sample (\( t \)).

The net CO\(_2\) assimilation rate \( (A_m, \mu\text{mol (CO}_2\text{)} m^{-2} s^{-1}) \) and stomatal conductance \( (g_s, \text{mmol} m^{-2} s^{-1}) \) were measured with the method described by Xing and Wu (2012). Chlorophyll-a fluorescence (ChlF) was measured with a pulse amplitude modulated ChlF imaging system (IMAGING-PAM, Heinz Walz GmbH, Effeltrich, Germany). Before the measurements, the leaves were dark-adapted for 30 min to ensure complete relaxation of all reaction centers. The minimum chlorophyll fluorescence \( (F_o) \) was determined using a measuring beam, whereas the maximum chlorophyll fluorescence \( (F_m) \) was recorded after a 0.8 s saturating light pulse (6000 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)). The maximum quantum yield of photosystem II \( (F_v/F_m) \) was calculated using the following equation: \( F_v/F_m = (F_m - F_o)/F_m \).

2.3. Determination of leaf water content, water potential, physiological capacitance and leaf tensile force

The leaf was dried in an oven at 80°C. The fresh and dry weights were measured using an electronic analytical balance (B5A124S, Sartorius, Gottingen, Germany), and WC was calculated according to the difference between the fresh and dry weights of leaf. CP was measured using an LCR tester (model 3532-50, Hioki, Nagano, Japan) with a frequency and voltage of 3 kHz and 1 V, respectively. The leaf was clipped in a custom-made parallel-plate capacitor (Zhang et al. 2015). Using a dew point microvoltmeter in a universal sample room (C-52-SF, Psysro, Wescor, Logan, Utah), \( \psi_t \) was measured at the same position of the leaves with \( CP \) testing. Moreover, \( t_d \) was calculated according to Equation (1).

\[
T_d = \frac{A}{d} = \frac{C_P}{e_o} \left( \frac{1000iRT}{81000iRT + (81 - a)M\psi_t} \right)
\]

where \( A \) is the effective area of the leaf in contact with the capacitor plates expressed in cm\(^2\), \( d \) is the leaf effective thickness expressed in cm, \( i \) is the dissociation coefficient with a value of 1, \( R \) is the gas constant with a value of 0.0083 L MPa mol\(^{-1}\) K\(^{-1}\), \( T \) is the thermodynamic temperature (\( T = 273 + t°C \)) expressed in K, \( e_o \) is the vacuum dielectric constant with a value of 8.854 × 10\(^{-12}\) F m\(^{-1}\), \( a \) is the relative dielectric constant of cytosol solute, \( M \) is the relative molecular mass of the cytosol solute expressed in g mol\(^{-1}\), and the value 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 3.3, \( M \) was 342 g mol\(^{-1}\), and the temperature was 20°C. Equation 1 could be rewritten as follows:

\[
T_d = \frac{C_P}{(717.17 + 96.75\psi_t)}
\]
2.4. Mathematical model between net CO₂ assimilation rate and leaf tensity

The $A_n - C_i$ response curves are fitted using the following equation:

$$A_n = \frac{CE \times C_i \times A_{max}}{CE \times C_i + A_{max}} - R_{exp}$$  \hspace{1cm} (3)

where $A_n$ is the net CO₂ assimilation rate [$\mu$mol (CO₂) m⁻² s⁻¹], $CE$ is the carboxylation efficiency [$\mu$mol (CO₂) m⁻² s⁻¹], $C_i$ is the intracellular CO₂ concentration (μmol mol⁻¹), $A_{max}$ is the net CO₂ assimilation rate at CO₂ saturation [$\mu$mol (CO₂) m⁻² s⁻¹], and $R_{exp}$ is the photorespiration rate [$\mu$mol (CO₂) m⁻² s⁻¹].

Incorporating $a = A_{max}$, $b = A_{max}/CE$ and $Y_0 = -R_{exp}$ into Equation 3, we can obtain the following expression:

$$Y = Y_0 + \frac{aX}{b + X}$$  \hspace{1cm} (4)

where $Y$ is defined as the net H₂O assimilation rate [$\mu$mol (H₂O) m⁻² s⁻¹], $X$ is the leaf tensity ($T_d$ cm), $-Y_0$ is the physiological water loss rate [i.e. transpiration and guttation, μmol (H₂O) m⁻² s⁻¹]; $a$ is the net H₂O assimilation rate when the intracellular water is sufficient [$\mu$mol (H₂O) m⁻² s⁻¹], and $a/b$ is the hydration efficiency expressed as UT m⁻³ s⁻¹, where UT is equal to 10² μmol.

CO₂ and H₂O are both substrates for photosynthesis, and the net assimilation rate for H₂O is similar to that for CO₂. Moreover, the plant leaf is composed of a large number of cells, and the variation of cell sap concentration and cell volume can be reflected by $T_d$ which can indicate plant water status. Therefore, the relationship between $A_n$ and $T_d$ can also be fitted using Equation (4).

2.5. Measurement of chlorophyll content, leaf area and plant dry weight

The chlorophyll content (SPAD) was measured using a chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan). Leaf area ($A$) was measured using a portable leaf area meter (AM-200, ADC, UK), from the onset of the drought stress treatment to day 18. Five plants from each treatment group were selected and dried in an oven at 80°C at the end of the drought and subsequent rewatering treatments. Dry weight per plant (g) was measured using an electronic analytical balance (BSA124S, Sartorius, Gottingen, Germany).

2.6. Leaf area growth model

Given that the growth rate of $A$ can reflect the plant growth status, the relationship between $A$ and time (D) can be fitted using a four-parameter logistic equation (Equation 5).

$$A = A_0 + \frac{a}{1 + (D/D_0)^b}$$  \hspace{1cm} (5)

where $A_0$ is the initial leaf area during logarithmic growth phase, $a$ is the upper limit of the leaf area, $D_0$ is the number of days when the leaf area reaches half of the maximum value during the logarithmic growth phase, and $b$ is a constant.

The duration from the onset of observation to the logarithmic growth phase is calculated as follows:

$$DT_t = D_0 + \frac{2D_0}{b}$$  \hspace{1cm} (6)

The duration of the logarithmic growth phase is calculated as follows:

$$DT_{log} = \frac{-4D_0}{b}$$  \hspace{1cm} (7)

Then, the relative time required when the growth rate of the leaf area reaches the maximum value ($RT_{GRM}$) is calculated as follows:

$$RT_{GRM} = \frac{D_0 - DT_t}{DT_{log} + DT_t}$$  \hspace{1cm} (8)

The lower the $RT_{GRM}$ value, the faster the growth rate of the leaf area reaches the maximum value.

2.7. Statistical analysis

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

3. Results

3.1. CA activity

During the drought phase, the CA activity at 20 and 40 g L⁻¹ PEG levels was the highest (Figure 1). The CA activities at 0 and 40 g L⁻¹ PEG levels were significantly reduced after rewatering compared with those in the drought phase. By comparison, the CA activity at 20 g L⁻¹ PEG level increased after rewatering compared with that in the drought phase. CA activities at 20 and 40 g L⁻¹ PEG levels were all higher than those at 0 g L⁻¹ PEG level during drought and rewatering phases.

3.2. Leaf water content, water potential and leaf tensity

A significantly lower value of WC was observed at 80 g L⁻¹ PEG level compared with that at 0 g L⁻¹ PEG level during the drought phase. Moreover, significant decreases of WC values were found at 40 and 80 g L⁻¹ PEG levels compared
with that at 0 g L⁻¹ PEG level during the rewatering phase (Table 1). However, the value of WC at each treatment level during the drought phase showed no significant difference compared with that during the rewatering phase. During the drought phase, ψᵣ values at 40 and 80 g L⁻¹ PEG levels were significantly lower than those of the other two levels. The ψᵣ value at 80 g L⁻¹ PEG level was the lowest, and the values at 0 and 20 g L⁻¹ PEG levels showed no significant difference (Table 1). The ψᵣ values at 40 and 80 g L⁻¹ PEG levels increased after rewatering compared with those in the drought phase. By contrast, the values at 0 g L⁻¹ PEG level decreased after rewatering. The ψᵣ values at 20 and 40 g L⁻¹ PEG levels were significantly higher than those of the other two levels during the rewatering phase. Low Tₕ values were associated with increasing drought stress (Table 1). Tₕ values at 0 and 20 g L⁻¹ PEG levels decreased after rewatering compared with those in the drought phase. In addition, Tₕ values at 40 and 80 g L⁻¹ PEG levels exhibited minor increase after rewatering compared with those in the drought phase.

### 3.3. Photosynthetic characteristics

Low Aₙ values were associated with increasing drought stress during the drought phase (Figure 2(A)). The Aₙ values at 0 and 20 g L⁻¹ PEG levels showed no significant difference during the drought phase. Those at 40 and 80 g L⁻¹ PEG levels during the rewatering phase exhibited no significant difference compared with those during the drought phase. The Aₙ values at 20, 40 and 80 g L⁻¹ PEG levels were 97%, 78% and 63% of that at 0 g L⁻¹ PEG level during the drought phase. Moreover, the values at 20, 40 and 80 g L⁻¹ PEG levels were 82%, 84% and 65% of that at 0 g L⁻¹ PEG level during the rewatering phase. During the drought phase, gₑ values at 0, 20 and 40 g L⁻¹ PEG levels showed no significant difference (Figure 2(B)), whereas that at 80 g L⁻¹ PEG level decreased significantly. The gₑ values at 20 and 40 g L⁻¹ PEG levels increased after rewatering, whereas those at 0 and 80 g L⁻¹ PEG levels exhibited no significant difference after rewatering compared with those in the drought phase.

### 3.4. Correlation of leaf tensity, water content, water potential, stomatal conductance and net CO₂ assimilation rate

The Pearson correlation coefficients for the relationship of Tₕ, WC, ψᵣ, gₑ, and Aₙ are shown in Table 2. Tₕ was significantly correlated with WC, ψᵣ, and Aₙ. Aₙ was significantly correlated with Tₕ, WC, ψᵣ, and gₑ. However, gₑ exhibited no significant relationship with Tₕ and WC. WC showed no significant relationship with ψᵣ. (Table 1)

### 3.5. Relationship between leaf tensity and net CO₂ assimilation rate

The relationship between Aₙ and Tₕ displayed a good correlation and could be fitted well by the rectangular hyperbola equation $Y = -3.91 + (17.95X/0.91 + X)$, $R^2 = 0.84$, $n = 24$, $P < 0.0001$ (Figure 3). In other words, an increase in Tₕ was correlated with an increase in Aₙ during drought and subsequent rewatering phases. Moreover, the parameters a, b and Tₑ could be estimated using Equation (2). The physiological water loss rate, net H₂O assimilation rate when the intracellular water was sufficient and hydration efficiency were 3.91 μmol (H₂O) m⁻² s⁻¹, 17.95 μmol (H₂O) m⁻² s⁻¹ and 19.73 UT m⁻³ s⁻¹, respectively.

### 3.6. Chlorophyll content, leaf area and plant dry weight

The chlorophyll content at 80 g L⁻¹ PEG level was higher than those at 0, 20 and 40 g L⁻¹ PEG levels during drought phase (Table 3). High chlorophyll contents were associated with increasing drought stress at 0–40 g L⁻¹ PEG levels during the rewatering phase. During the drought phase, the chlorophyll contents at 20, 40 and 80 g L⁻¹ PEG levels showed no significant difference compared with those during the rewatering phase. Fitting curves of the relationship between A and D are shown in Figure 4, and the corresponding fitting equations are shown in Table 4. The relationship between A and D at 0, 20 and 40 g L⁻¹ PEG levels displayed good correlations. However, the negative value ($a = -1.12$) of the upper limit of leaf area at 80 g L⁻¹ PEG level indicated that the plant ceased growing and the leaf wilted. RTGRM values of A at 0, 20 and 40 g L⁻¹ PEG levels were 0.50, 0.36 and 0.33, respectively. Low values of A were associated with increasing drought stress, whereas fast growth rate for the leaf area to reach its maximum value was associated with increasing drought stress during the drought and rewatering phases. The plant dry weight at 40 g L⁻¹ PEG level did not significantly differ from that at

### Table 1. Effect of drought and subsequent rewatering on leaf water content (WC, %), water potential (ψᵣ, MPa) and leaf tensity (Tₕ, cm²).

| PEG concentrations (g L⁻¹) | WC     | ψᵣ   | Tₕ  | PEG concentrations (g L⁻¹) | WC     | ψᵣ   | Tₕ  |
|----------------------------|--------|------|-----|----------------------------|--------|------|-----|
| 0                          | 88.51  | -0.74| 4.66| 0                          | 87.01  | -1.18| 3.11|
| (0.04)                     | (0.04)| (0.05)|     | (0.04)                     | (0.04)| (0.04)|     |
| 20                         | 86.34  | -0.72| 3.89| 20                         | 84.18  | -0.75| 2.11|
| (0.02)                     | (0.04)| (0.19)|     | (0.02)                     | (0.02)| (0.06)|     |
| 40                         | 85.66  | -2.20| 1.77| 40                         | 82.44  | -0.77| 1.87|
| (0.03)                     | (0.03)| (0.01)|     | (0.03)                     | (0.03)| (0.10)|     |
| 80                         | 85.35  | -2.80| 1.33| 80                         | 82.47  | -1.12| 1.55|
| (0.01)                     | (0.01)| (0.01)|     | (0.03)                     | (0.06)| (0.02)|     |

*Means in the same column followed by different letters differ significantly at p ≤ 0.05, according to one-way ANOVA and t-test (standard errors shown in parentheses).

*Arrows (→) indicate that the plants were transferred from one treatment solution into another.
intracellular bicarbonate into $\text{H}_2\text{O}$ and $\text{CO}_2$ and altered the leaf water status under drought conditions (Wu and Xing 2012). Water regulation caused by enzymes, such as CA in plants, prevented the reduction in $\psi_L$ at 20 g L$^{-1}$ PEG level. The value of $\psi_L$ was mainly influenced by vacuolar concentration (Porcel and Ruiz-Lozano 2004). The stable value of $\psi_L$ at 20 g L$^{-1}$ PEG level indicated that the vacuolar concentration did not vary significantly. As drought stress increased, higher CA activity at 40 g L$^{-1}$ PEG level meant a stronger water regulation capacity, which provided more water in the cytosol of cells, and prevented the reduction of WC.

The increase of $g_s$ at 20 and 40 g L$^{-1}$ PEG levels after the rewathering phase indicated that water deficiency in $B. napus$ was prevented, and $A_n$ demonstrated better stability at 40 g L$^{-1}$ PEG level during the drought and subsequent rewathering phases compared with that at 20 g L$^{-1}$ PEG level. However, the increase of vacuolar concentration and decrease of vacuole volume were still caused by increasing drought stress. As a result, $T_d$ could respond sensitively to the drought condition. By compressing the vacuoles in cells with the reduction of $T_d$, water in vacuoles could then efficiently enter into the cytosol of cells, thereby improving the water supply efficiency for photosynthesis. $T_d$ could represent the variation of water status in the cytosol, which could also be analysed according to the movement of water in the cells (Figure 6).

Furthermore, $T_d$ exhibited good correlations with $A_n$, WC and $\psi_L$ in this study. $T_d$ could reflect the plant water status better than $\psi_L$ or $g_s$ and played an important role in the photosynthesis of $B. napus$.

4.2. Physiological tolerance threshold

The rectangular hyperbolic equation is derived from the Michaelis–Menten equation. The value of $K$ in the Michaelis–Menten equation indicates the substrate concentration when the corresponding velocity is half-maximal. Similarly, the value of $b$ in the rectangular hyperbolic equation (Equation 4) indicates the value of $T_d$ when $A_n$ is half-maximal (Dowd and Riggs 1965). A whole rectangular hyperbolic
Table 3. Effect of drought and subsequent rewatering on chlorophyll Content (SPAD)*.

| Drought Phase | Chlorophyll Content* (SPAD) | Watering Phase | Chlorophyll Content* (SPAD) | Increment in Chlorophyll Content During Re-watering Phase |
|---------------|-----------------------------|----------------|-----------------------------|--------------------------------------------------------|
| PEG concentrations (g L⁻¹) | 0.00 | 0.00 | 0.00 | 0.00 |
| 0 | 35.93 ± 2.53 ab | 0–0 | 38.37 ± 0.78 c | 2.43 |
| 20 | 32.93 ± 0.73 b | 20–20 | 44.70 ± 0.44 b | 11.77 |
| 40 | 37.73 ± 0.99 ab | 40–40 | 50.67 ± 2.47 a | 12.93 |
| 80 | 39.90 ± 1.81 a | 80–40 | 44.83 ± 2.53 ab | 4.93 |

*Means ± SE. Values in the same column followed by different letters differ significantly at p ≤ 0.05 according to one-way ANOVA and t-test.

Figure 4. Fitting curves of the relationship between leaf area (A, cm²) and time (D, Days).

The photochemical apparatus of *B. napus* to increasing drought stress also indicated slight damage to the PSII reaction centers under drought stress (Wu and Xing 2012). Moreover, plasmolysis in cells occurred at the point of threshold $\psi_p$ under drought stress conditions (Oppenheimer and Jacoby 1963). The cell volume varied correspondingly. As drought stress increased, leaf cells could no longer shrink at 80 g L⁻¹ PEG level. No further considerable decrease in $T_d$ at 80 g L⁻¹ PEG level indicated that plasmolysis occurred and that the leaf cells were possibly damaged. Water status in the plants was characterized by $T_d$, and the variation of $T_d$ could reflect the plant drought resistance (Wu et al. 2015). Stable $T_d$ value at 40 g L⁻¹ PEG level implied high water regulation ability of CA in the plant during drought and subsequent rewatering phases. *B. napus* exhibited good restorability at 40 g L⁻¹ PEG level. These results indicated that the physiological tolerance threshold of *B. napus* to drought stress was 40 g L⁻¹.

Therefore, as a rapid non-destructive measurement parameter, $T_d$ could be used to determine the physiological tolerance threshold of *B. napus* to drought stress and analyse its restorability under drought stress conditions. Moreover,

Table 4. Fitting equations of the relationship between leaf area (A, cm²) and time (D, Days).

| PEG concentration (g L⁻¹) | Fitting equations | r² | n | p |
|---------------------------|-------------------|----|---|---|
| 0–0 | $A = 19.83 + \frac{52.26}{1 + \left(\frac{D}{6.90}\right)^{0.37}}$ | 0.9977 | 18 | <0.0001 |
| 20–10 | $A = 22.48 + \frac{23.57}{1 + \left(\frac{D}{4.57}\right)^{0.37}}$ | 0.9930 | 18 | <0.0001 |
| 40–20 | $A = 28.52 + \frac{9.12}{1 + \left(\frac{D}{3.32}\right)^{0.37}}$ | 0.9413 | 18 | <0.0001 |
| 80–40 | $A = 28.28 + \frac{-1.12}{1 + \left(\frac{D}{8.71}\right)^{0.37}}$ | 0.5069 | 18 | <0.0001 |

*Indicate that the plant ceased growing and the leaf wilted at 80–40 g L⁻¹.
the prediction accuracy of physiological tolerance threshold by using \( T_d \) could be validated using the growth status of \( B. napus \).

### 4.3. Growth status and evaluation of rewatering strategy

For many plants, leaf expansion is more sensitive to water stress than leaf abscission (Muchow 1985). The leaf is the main organ for organic matter production. Therefore, a small average leaf size will affect photosynthesis and growth. Lower transpiration can also decrease the water loss in a single plant. In some plants, having a large number of small leaves is a strategy for adapting to drought conditions (Lopez et al. 1997). However, a high leaf area growth rate comparatively offsets the photosynthetic product loss caused by leaf area decline under water stress. Therefore, the growth status of plants can be evaluated by the relative time required when the growth rate of the leaf area reaches the maximum value (\( RT_{GRM} \)). The lower the \( RT_{GRM} \) value, the faster the growth rate of the leaf area reaches the maximum value.

Five rewatering strategies were set up in this study, including 80–40 g L\(^{-1}\), 40–20 g L\(^{-1}\), 20–10 g L\(^{-1}\) and 0–0 g L\(^{-1}\). The rewatering strategy for \( B. napus \) was excellent at 40–20 g L\(^{-1}\). Moreover, the chlorophyll content of \( B. napus \) at 40 g L\(^{-1}\) PEG level exhibited significantly better restorability than that at 0 g L\(^{-1}\) PEG level after rewatering. The reduction in plant dry weight at 20 and 40 g L\(^{-1}\) PEG levels was prevented compared with that at 0 g L\(^{-1}\) PEG level.

The results were consistent with the prediction of physiological tolerance threshold by using \( T_d \).

### 4.4. Irrigation strategy for \( B. napus \)

Irrigation of \( B. napus \) was implemented at 40 g L\(^{-1}\) (–0.15 MPa) and terminated at 20 g L\(^{-1}\) (–0.11 MPa). The \( \psi_L \) values of \( B. napus \) at 40 and 20 g L\(^{-1}\) PEG levels were –2.20 and –0.72 MPa, respectively. According to our previous research (Hu 2016), the same \( \psi_L \) values of \( B. napus \) were also observed at 10.5% (\( P_1 \)) and 18.4% (\( P_2 \)) soil water content (\( P \)) when cultivated in loam. For example, given that the saturated soil moisture of loam (\( P_a \)) did not exceed 20%, the ratio (\( r \)) of the deficit irrigation (DI) volume to the sufficient irrigation volume (\( Q \)) of loam could be calculated as follows: \( r = (P_2 - P_1)/P_a - P_1 \). Therefore, \( V \) of loam was 83.2% Q. DI scheduling could be implemented on \( B. napus \) when cultivated in loam during the winter drought period.

### 5. Conclusions

Water regulation caused by enzymes, such as CA in plants, changed the variation of \( \psi_L \) and \( g_s \), stabilized the photosynthetic capacity and delayed the water requirement in \( B. napus \). \( T_d \) could reflect the plant water status better than \( \psi_L \) or \( g_s \) and played an important role in the photosynthesis of \( B. napus \). No further considerable decrease in \( T_d \) at 80 g L\(^{-1}\) PEG level indicated that plasmolysis occurred and that leaf cells were possibly damaged. \( B. napus \) exhibited good restorability at 40 g L\(^{-1}\) PEG level. As a rapid non-destructive measurement parameter, \( T_d \) could be used to determine the physiological tolerance threshold of \( B. napus \) to drought stress and analyse its restorability under drought stress conditions. The results of chlorophyll content, plant dry weight and \( RT_{GRM} \) values also indicated the excellent rewatering strategy for \( B. napus \) at 40–20 g L\(^{-1}\). Therefore, irrigation of \( B. napus \) was implemented at 40 g L\(^{-1}\) (–0.15 MPa) and terminated at 20 g L\(^{-1}\) (–0.11 MPa). Considering the same influences of drought stress on \( \psi_L \) of \( B. napus \), the corresponding \( P \) values of loam were 10.5% and 18.4%, respectively. By using loam as an example, the ratio of the DI volume to the Q of loam was 83.2%. \( T_d \) could be used for the rapid determination of water requirement information in \( B. napus \) during winter drought period.

### Disclosure statement

No potential conflict of interest was reported by the authors.
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