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Screening of New *Dendrobium officinale* Strains Adapted to Karst Forest Environmental Stress Based on Electrophysiological Detection Method

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Abstract: At present, the main methods of breeding Dendrobium officinale (*D. officinale*) include introduction and domestication, selective breeding, hybrid breeding, and mutation breeding. In the process, traditional methods of field investigation of agronomic traits are often used to select resistant varieties. Although these breeding methods are effective, they have a certain degree of subjectivity and empiricism, and the breeding cycle is long. Electrophysiological instruments were used in this experiment to test the material transport and metabolic capacity of *D. officinale* in a karst forest epiphytic environment and to quickly evaluate the suitability of different strains of *D. officinale* in a drought environment. These instruments detected the data on electrophysiological information of leaves of different strains of *D. officinale* under long-term drought conditions, providing immediate access to the inherent electrophysiological information of the leaves of these strains. Based on the electrophysiological parameters of *D. officinale* leaves as defined by the inherent electrophysiological information of plants, the water metabolism, nutrient transport, and metabolic capacity in different leaves were evaluated. The key electrophysiological indexes were verified by combining the results of chlorophyll fluorescence and chlorophyll content. The results indicate the following: (1) Parameters defined based on electrophysiological information effectively characterized the differences in intracellular water utilization, the nutrient transport status, and the metabolic capacity of different *D. officinale* strains. (2) The intrinsic physiological resistance, intrinsic physiological reactance, active nutrient transport capacity, and passive nutrient transport capacity were closely related to the growth status and chlorophyll function of *D. officinale* leaves. These electrophysiological parameters could serve as critical indicators for evaluating the drought resistance of *D. officinale*. (3) Under severe drought stress, strain LH1 exhibited less leaf damage, adequate water/nutrient supply, vigorous life activities, and excellent drought resistance. We found that strain LH1 demonstrates better adaptation to the arid environment of karst forest lands. The electrophysiological detection method employed in this study offers a new technique for screening wild-cultivated *D. officinale* resistance strains. The results indicate that the real-time online leaf electrophysiological information measured by the method in this study can characterize the energy and material metabolism of crops, greatly improving the efficiency of crop-variety selection and reducing costs. These conclusions can be used to obtain real-time information on the transmission of water and nutrients within plant cells and can provide theoretical support for studying the adaptation mechanisms of crops to adverse environments.

Keywords: chlorophyll fluorescence; drought stress; electrophysiological information; resistant strain screening

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1. Introduction

Due to the harsh growth environment of wild Dendrobium and the over-exploitation of its resources, wild Dendrobium resources are on the verge of exhaustion. Recently, cultivation techniques for *Dendrobium officinale* (*D. officinale*) have been explored and implemented in various provinces of China, including Zhejiang, Yunnan, Guangxi, Guizhou, Jiangxi, and Hunan. These provinces have established ecological cultivation systems in the main producing areas of *D. officinale*, and the cultivation techniques have been developed and improved [1,2]. Planting on stones and trees are two types of *Dendrobium* field planting in Guizhou. Among these, the planting-on-stones area of *Dendrobium nobile* is about 6000 hectares, the planting-on-trees area of *D. officinale* is about 3266.67 hectares, and there is also a small amount of *Dendrobium fimbriae*, *Dendrobium denneanum*, and *Dendrobium loddigesii*. As of 2019, the total output value is CNY 3.6 billion [3]. However, the global climate has led to gradual warming, resulting in uneven seasonal precipitation and abnormal climates worldwide. The karst area of southwest China has been experiencing rare droughts and high temperatures. Planting *D. officinale* in the wild often faces the challenge of water scarcity, resulting in slow growth and low yields. Under the premise of decreasing rainfall and limited agricultural water use, there is an urgent need to improve the yield of simulated wild *D. officinale*.

Drought stress directly disrupts the water balance of plant cells, leading to nutrient and water deficits, metabolic disorders, damage to the light system, and chlorophyll degradation. These effects, in turn, impact the photosynthetic process by reducing the energy supply and metabolic accumulation in plants. Furthermore, drought stress can cause an excessive accumulation of reactive oxygen species and the enhancement of membrane lipid peroxidation, resulting in damage to the photosynthetic performance of plants, reduced biomass, and growth stagnation, which have a serious impact on plant yield and quality [4,5]. Plants also have different response mechanisms to drought stress due to different durations and degrees of drought stress. Studies have shown that drought stress can limit the normal physiological metabolism of plants, and severe stress levels can lead to plant death [6]. In addition, plants can maintain cell structure and photosynthesis through osmotic regulation and can delay leaf senescence and death in severe water scarcity [7,8]. The long-term exposure of plant leaves to the external environment is the most direct organ in response to external environmental conditions, and their structure may better reflect the impact of drought than other organs [9,10]. Plant photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, play a crucial role in capturing light energy. Their function directly influences the capacity of the light energy utilization of plants and, consequently, the overall photosynthetic efficiency.

Related studies have shown that drought affects the photosynthetic efficiency of plants, damages cell membranes, alters membrane permeability, and increases the electrical conductivity of plants. To protect themselves from direct stress, plants utilize inorganic ions such as K^+ and Cl^- and certain small organic molecules to regulate the balance of osmotic potential inside and outside the cell, thereby stabilizing enzyme conformation within cells [11,12]. When plant cells experience stress-induced damage, alterations in their structure, composition, and ion permeability also result in changes in their electrical properties. Therefore, by detecting abnormal electrical signals in plants and monitoring related electrophysiological parameters, we can quickly and quantitatively access the physiological growth status of plants under stress [13]. From September 2022 to March 2023, the Meteorological Bureau of Qianxinan Prefecture in Guizhou Province reported a prolonged period of moderate drought weather in Anlong County, lasting for more than half a year [14]. This extended drought had a significant impact on plant growth in the region. Consequently, there is a critical need to identify and select new drought-tolerant plant strains capable of adapting to severe drought conditions prevalent in arid areas.

In mesophyll cells, vacuoles and cytoplasm occupy the vast majority of the intracellular space, and their water absorption mode is mainly osmotic water absorption. The phospholipid bilayer is the fundamental scaffold that forms the cell membrane. Under electron microscopy, it can be divided into three layers, with an electron dense band (hydrophilic part) about 2.5 nm thick on each side of the membrane, and a transparent band (hydrophobic part) 2.5 nm thick sandwiched in the middle. Therefore, a cell (organelle) can be seen as a concentric spherical capacitor, but this type of capacitor becomes a complex capacitor that combines the functions of an inductor and a resistor due to the peripheral and intrinsic proteins on the membrane. Therefore, the electrophysiological characteristics of crop leaf cells are closely related to the material and energy metabolism of crop leaves. Researchers have invented a comprehensive method that can quickly and quantitatively detect the material and energy metabolism of different crops, screen high-yield and stress-resistant crop varieties, and obtain comparable results. Additionally, biophysical indicators can be used to characterize the adaptation characteristics of different crops to droughts and low nutrition, greatly improving the efficiency of crop-variety selection and reducing costs. This provides technical support for intelligent breeding and is an important component of smart agriculture [15,16]. The electrophysiological parameters of plants represent the fastest physiological response to environmental stimuli, providing valuable insights into plant growth under stress. In this study, various strains of *D. officinale* cultivated in the wild forests of Anlong County were utilized. The electrophysiological parameters of *D. officinale* leaves in a drought environment were measured, including the intracellular water metabolism, nutrient transport, metabolic capacity, chlorophyll fluorescence, and chlorophyll content. These measurements facilitated an analysis of physiological variations among different *D. officinale* strains subjected to drought stress. In addition, the correlation between photosynthetic physiological and electrophysiological signals of different strains of *D. officinale* under drought stress was explored with the mechanism of the effect of electrophysiological parameters on plant growth under drought stress. This study holds significant importance in monitoring the drought-resistance mechanism of *D. officinale*, promoting the evaluation and regulation of field growth environments and enhancing the breeding of drought- and low-nutrition-tolerant strains. Additionally, it provides theoretical support for understanding the physiological mechanism of *D. officinale* under drought stress and the breeding of drought-tolerant strains of *D. officinale*. However, the environmental selection for this experiment is relatively exclusive and has limitations in practical conditions. Further research is needed in other natural environments.

2. Materials and Methods

2.1. Plant Growth and Treatment

The experimental site is located in Zhegui Village, Pojiao Township, Anlong County, Guizhou Province, China, and belongs to the karst ecological areas. The geographical coordinates are approximately 24°59′26″ N and 105°25′12″ E, and the altitude is 950 to 1110 m. The different strains (LH1, GH1, and GH2) of *D. officinale* selected from the wild resources of *D. officinale* in Anlong County were cultivated in the wild here for more than 5 years. Three *D. officinale* strains experienced a medium-to-high drought in the area for more than half a year, with all strains being planted at the same time and slope direction and from the same tree species. A fully unfolded leaf was taken from the fourth leaf position of each plant.

2.2. Measurement of Real-Time Electrophysiological Parameters of Plant Leaves under Different Clamping Forces

Using an LCR tester (6300, GWinstek, Suzhou, China), and referring to Wu [17], the physiological capacitance, physiological resistance, and physiological impedance of the *D. officinale* leaves were measured. The electrophysiological parameter was used for determination. The test voltage and frequency of the tester were 1.5 V and 3.0 kHz [18,19], respectively. The mature leaves of *D. officinale* with the same leaf position were selected as the detection material. Fresh leaves were extracted and soaked in pure water for 30 min, then the water on the surface of the leaves was removed for determination, and three plants of each strain of *D. officinale* were tested (n = 3). The time periods of all tests were from 10:00 to 12:00, and the measured temperature (T) was 20.0 ± 2.0 °C. The real-time X_C and X_L of the plant leaves under different clamping forces were calculated according to Equations (1) and (2).

$$X_C = \frac{1}{2\pi fc} \tag{1}$$

$$\frac{1}{-X_L} = \frac{1}{Z} - \frac{1}{R} - \frac{1}{Xc} \tag{2}$$

where X_C is the capacitive reactance, f is the frequency, C is the capacitance, X_L is the inductive reactance, Z is the impedance, R is the resistance, and π is pi with a value of 3.1416 [20].

2.3. Fitting Determination of Inherent Electrophysiological Parameters of Plant Leaves

Temperature (K) in Table 1 shows the temperature. C_{i1} and C_{o1} are the electrolyte concentrations of resistance to plant leaves inside and outside the cell membrane, respectively. Using C_{T1} to express the total electrolyte concentration inside and outside the membrane in response to plant leaf resistance, we can obtain the following formula: $C_{T1} = C_{i1} + C_{o1}$. f_{o1} is the proportional coefficient between the electrolyte concentration C_{i1} in response to the resistance of plant leaves in the cell membrane and the resistance. n_R (mol) is the electrolyte transfer number in response to the resistance of plant leaves, and F_0 is the Faraday constant (96,485 C/mol). P is the pressure on the plant leaf cells, a is the electromotive force conversion energy coefficient, V is the volume of the plant leaf cells, and F (N) is the holding force.

Table 1. Intrinsic electrophysiological parameters of *D. officinale* leaves.

Parameter	Fitting Equation
Resistance (R)	$R = \frac{f_{o1}}{C_{T1}} + \frac{f_{o1}}{C_{T1}} e^{\frac{n_R F_0 E^0}{R_0 T}} e^{(-\frac{d n_R F_0}{a R_0 T} F)}; y_1 = \frac{f_{o1}}{C_{T1}};$ $k_1 = \frac{f_{o1}}{C_{T1}} e^{\frac{n_R F_0 E^0}{R_0 T}}; b_1 = \frac{d n_R F_0}{a R_0 T}; R = y_1 + k_1 e^{-b_1 F}$
Impedance (Z)	$Z = y_2 + k_2 e^{-b_2 F}$
Capacitive reactance (X_C)	$X_C = y_3 + k_3 e^{-b_3 F}$
Inductive reactance (X_L)	$X_L = y_4 + k_4 e^{-b_4 F}$
Capacitance (C)	$C = y_0 + K_0 F$
Effective thickness (d)	$d = \frac{U^2 K_0}{2}$
Intracellular water-holding capacity (IWHC)	$IWHC = \sqrt{C^3}$
Intracellular water-use efficiency (IWUE)	$IWUE = \frac{d}{IWHC}$
Intracellular water-holding time (IWHT)	$IWHT = C \times Z$
Water/nutrient transfer rate (STR)	$STR = \frac{IWHC}{IWHT}$
Relative flux of nutrient unit (UNF)	$UNF = \frac{R}{X_C} + \frac{R}{X_L}$
Nutrient transport rate (NTR)	$NTR = \frac{\sqrt{c^3}}{c \times z}$
Nutrient transport capacity (NTC)	$NTC = UNF \times NTR$
Nutrient active flow (UAF)	$UAF = \frac{X_C}{X_L}$
Nutrient active transshipment capacity (NAC)	$NAC = UAF \times NTR$
Nutrient active transport capacity (NAT)	$NAT = \frac{R}{X_L}$
Nutrient passive transport capacity (NPT)	$NPT = \frac{R}{X_C}$
Nutrition utilization efficiency (NUE)	$NUE = \frac{100}{NAT+NPT}$
Resistance to low nutrition (RLN)	$RLN = 100 \times \frac{NAT}{NAT+NPT}$
Metabolic flux (MF)	$MF = \frac{1}{R \times Z \times X_C \times X_L}$
Metabolic rate (MR)	$MR = STR \times NAC$
Metabolic activity (MA)	$MA = \sqrt[6]{MF \times MR}$

2.4. Determination of Chlorophyll Fluorescence Parameters

The PAM-2500 chlorophyll fluorimeter (WALZ company, Bad Waldsee, Germany) was used to determine chlorophyll fluorescence parameters. After the leaves were placed in the dark for 20 min, the initial fluorescence intensity (F_0), maximum fluorescence intensity (F_m), efficiency of the maximum light energy conversion of PSII (F_v/F_m), actual photochemical quantum yield of PSII (YII), non-photochemical quenching coefficient (qN), photochemical quenching coefficient (qP), and electron transfer rate (ETR) were measured (n = 3).

2.5. Determination of Chlorophyll Content

About 0.5~1 g of fresh leaves was weighed, cut up, and ground into a mortar. Five milliliters of pure acetone was added with a little $CaCO_3$ (Jinshan company, Chengdu, China) and quartz sand (Jinshan company) for the homogeneous grinding of leaves. Five milliliters of 80% acetone (Jinshan company) was also added into a mortar and ground until the tissue was whitened and placed for 3~5 min in the dark. The homogenate was filtered into a 25 mL volumetric bottle with a layer of dry filter paper. The pigment around the mortar and filter paper was filtered into the volumetric flask with 80% acetone. The volume was then adjusted to the scale with 80% acetone after all the filter paper and residue had been whitened. The filtered extract of 1 mL was diluted with 4 mL of 80% acetone and then transferred to a colorimetric dish for determination. With 80% acetone as the control, the absorbance values of OD663, OD645, and OD652 were measured by an ultraviolet spectrophotometer (UV-1800PC, Aoyi instruments, Shanghai, China). The contents of chlorophyll a, chlorophyll b, and the total chlorophyll in the extract were calculated according to the formula, and then the content per gram fresh weight (or dry weight) was calculated (n = 3) [21].

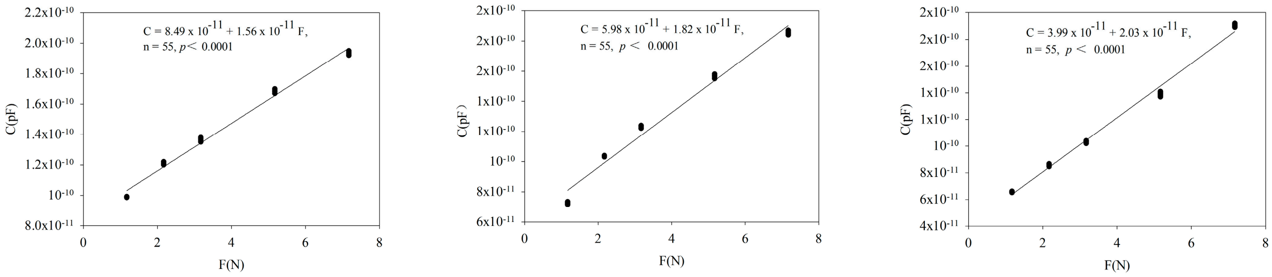
2.6. Statistical Analysis

The SigmaPlot data 12.0 statistical software was used for a fitting analysis, and the SPSS 20.0 data statistical software was used for significance and correlation analyses. The data are shown as means ± SE. Graphs were prepared using Origin 2018. A one-way analysis of variance (ANOVA) and the least significant difference test (LSD) were used for significance testing ($p < 0.05$). An online drawing tool was used for plotting (the website is https://hiplot.com.cn/home/index.html, accessed on 20 June 2024).

3. Results

3.1. Verification of Fitting Equation between Leaf Electrophysiological Parameters and Clamping Force

The fitting equation parameters of the electrophysiological parameters and holding force of leaves of different strains of *D. officinale* are shown in Table 1. Among these, the equations of R, Z, X_C, and X_L of different strains of leaves were constructed with the clamping force, and the correlation coefficients (R_2) of the fitting equations were 0.9941–0.9985, 0.9931–0.9985, 0.9933–0.9989, and 0.9912–0.9985, respectively. Additionally, the p-values of all parameters of the fitting equation were less than 0.0001. Figures 1 and 2 randomly show the fitting curve and correlation coefficient between the parameters of C, R, Z, X_C, X_L and the holding force, respectively. It was observed that there was a significant correlation between the clamping force and the electrophysiological parameters of *D. officinale* leaves. This confirmed the correctness of the formula and supported the real existence of the internal mechanism relationship between the parameters. Additionally, it provided a fast, efficient, accurate, and real-time technology for monitoring the physiological state of plant leaves, which held significant practical significance.



(**a**) (**b**) (**c**)

Figure 1. Fitting of leaf capacitance to clamping force for different strains of *D. officinale*: LH1 (**a**), GH1 (**b**), and GH2 (**c**).

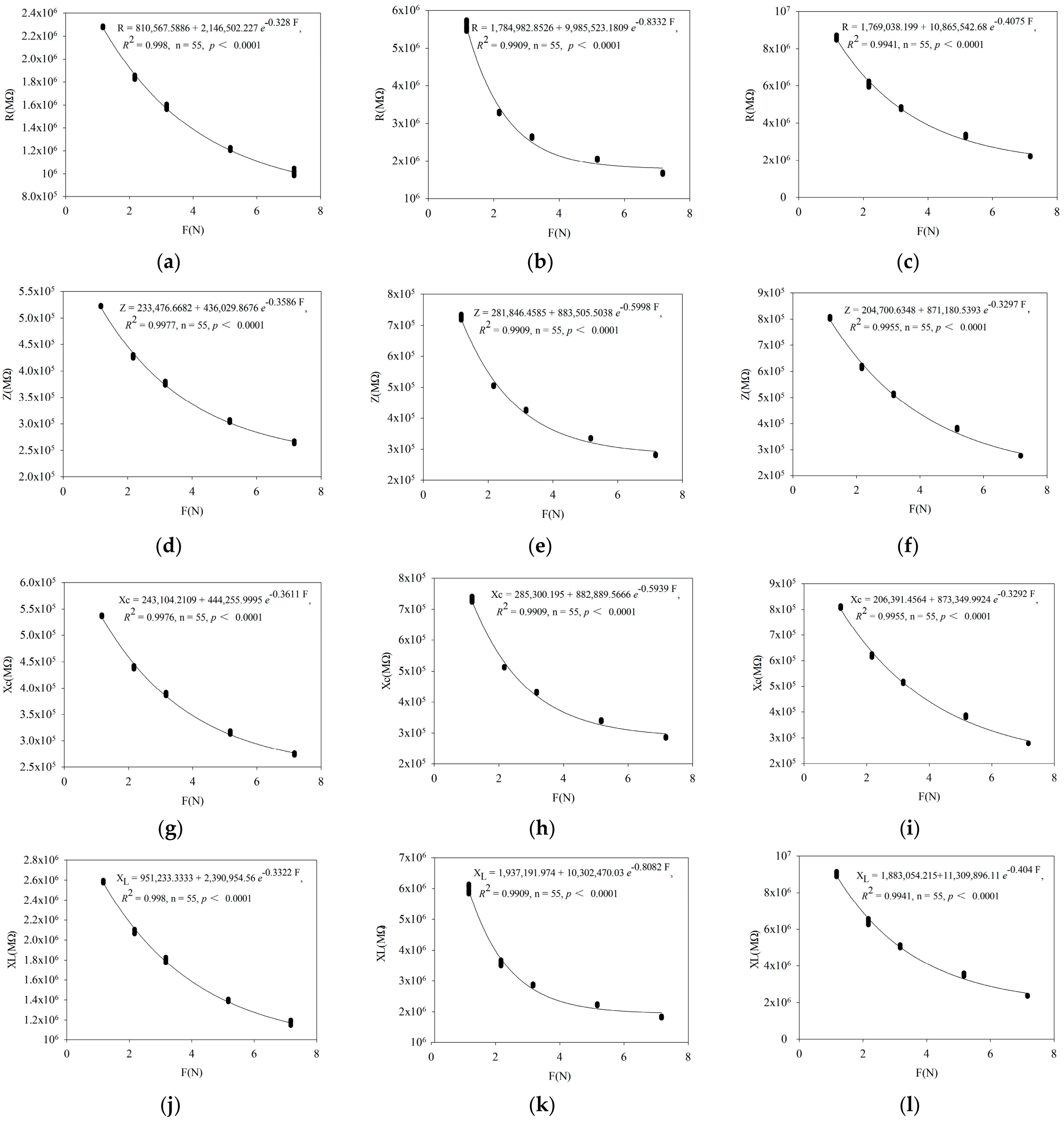


Figure 2. Fitting of leaf resistance, impedance and capacitive and inductive resistance to clamping force for different strains of *D. officinale*: LH1 (**a**,**d**,**g**,**j**); GH1 (**b**,**e**,**h**,**k**); and GH2 (**c**,**f**,**i**,**l**).

3.2. Differences in Electrophysiological Information, Intracellular Water Utilization, Nutrient Transport, and Metabolic Ability of Leaves of Different Strains

According to the parameters of the fitting equation, the electrophysiological parameters of leaves of different strains of *D. officinale* were calculated. From Table 2, the inherent C of LH1 leaves was significantly ($p < 0.05$) higher than that of GH1 and GH2, namely, by 59.87% and 76.31%, respectively. The inherent R and inherent X_L of LH1 leaves were extremely significantly ($p < 0.01$) reduced by 76.62% and 75.26% compared with GH2, and the inherent Z and inherent X_C of LH1 leaves were significantly ($p < 0.05$) reduced by 43.44% and 42.6% compared with GH2. In other words, the electrophysiological parameters of leaves of the LH1 strain were lower than those of the GH1 and GH2 strains, except for the inherent C. The results show that under the same stress, the damage of leaf cells of the

LH1 strain was less than that of the GH1 and GH2 strains, and the ion permeability of the LH1 strain was less affected.

Table 2. Electrophysiological parameters of leaves.

Strains	**Capacitance (C, pF)**	**Resistance (R, MΩ)**	**Impedance (Z, MΩ)**	**Capacitive Reactance (X_C, MΩ)**	**Inductive Reactance (X_L, MΩ)**
LH1	78.35 ± 16.95 aA	3.89 ± 0.82 cB	0.69 ± 0.15 bA	0.70 ± 0.15 bA	4.28 ± 0.81 cB
GH1	49.01 ± 7.83 bA	9.96 ± 2.33 bAB	1.09 ± 0.16 aA	1.10 ± 0.16 aA	10.49 ± 2.23 bAB
GH2	44.44 ± 7.942 bA	16.64 ± 3.63 aA	1.22 ± 0.24 aA	1.22 ± 0.24 aA	17.30 ± 3.67 aA

Note: Values show the mean ± SD, n = 3. Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

The IWHC and IWUE of the LH1 strain are significantly ($p < 0.05$) higher than those of strains GH1 and GH2 in Table 3. Among these, the IWHC of LH1 was 103.24% and 134.95% higher than that of GH1 and GH2, respectively, and the IWUE of LH1 was 105.50% and 138.30% higher than that of GH1 and GH2, respectively. The STR of LH1 was significantly ($p < 0.05$) lower than that of GH1 and GH2, namely, by 53.33% and 44.0%, respectively. There was no significant ($p > 0.05$) difference in IWHT among the three strains. The results show that under the same intensity of drought stress, the damage incidence of leaf cells of the LH1 strain was less than that of GH1 and GH2. After rewatering the leaves, the LH1 strain could recover to a higher cell water-holding capacity to ensure the transport of intracellular water and nutrients. Under low leaf moisture conditions, the two GH strains satisfied the water demands of the leaf and the basic function of the leaf by improving the retention time of water and the utilization efficiency of water and nutrients.

Table 3. Intracellular water utilization parameters of leaves.

Strains	**Effective Thickness (d, m)**	**Intracellular Water-Holding Capacity (IWHC)**	**Intracellular Water-Use Efficiency (IWUE)**	**Relative Intracellular Water-Holding Time (IWHT)**	**Water/Nutrient Transfer Rate (STR)**
LH1	17.92 ± 2.07 aA	701.65 ± 225.99 aA	0.028 ± 0.01 bA	52.11 ± 0.50 bA	13.44 ± 4.22 aA
GH1	18.67 ± 2.36 aA	345.23 ± 83.98 bA	0.06 ± 0.02 aA	52.73 ± 0.35 abA	6.54 ± 1.56 bA
GH2	15.08 ± 6.97 aA	298.64 ± 77.25 bA	0.05 ± 0.02 aA	52.94 ± 0.07 aA	5.64 ± 1.46 bA

Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

As displayed in Table 4, the nutrient transport parameters were calculated for different strains of *D. officinale* based on the fitting equation parameters. LH1 leaves exhibited a significantly higher active NAC compared to GH1 and GH2, with increases of 212.59% and 438.24%, respectively ($p < 0.01$). Moreover, the LH1 strain demonstrated a significantly higher NUE, RLN, and UAF compared to GH2, with enhancements of 125.53%, 121.18%, and 132.03%, respectively ($p < 0.05$).

However, the LH1 leaves showed a significantly lower NAT and NPT compared to GH2, with reductions of 5.21% and 58.19%, respectively ($p < 0.05$). These findings indicate that the LH1 leaves were less affected by the same intensity of drought stress compared to those of GH2. Upon rewatering, the LH1 strain exhibited a greater recovery in NUE and UAF, highlighting its stronger RLN and NAT compared to GH2. Conversely, GH2 demonstrated the ability to meet the basic nutrient needs of leaves by enhancing NPT.

According to Table 5, the MF, MR, and MA of the LH1 leaves were significantly ($p < 0.01$) higher than those of the GH1 and GH2 leaves, namely, they were 1557.57%, 554.43%, and 116.51% higher than those of GH1 and 5007.21%, 1162.17%, and 196.38% higher than those of GH2. The results reveal that under the same intensity of drought stress, the metabolic ability of the LH1 leaves was less affected by stress, and LH1 showed a stronger MF and MA than GH1 and GH2 after rewatering the leaves. This further demonstrates that evaluating plant metabolic ability based on electrophysiology has good feasibility, as evidenced by the metabolic parameters measured in this study.

Table 4. Nutritional operating parameters of leaves.

Strains	Nutrient Passive Transport Capacity (NPT)	Nutrient Active Transport Capacity of (NAT)	Nutrition Utilization Efficiency (NUE)	Resistance to Low Nutrition (RLN)	Active Flow of Nutrient Unit (UAF)	Nutrient Active Transshipment Capacity (NAC)
LH1	5.79 ± 2.061 bA	0.91 ± 0.03 bA	15.81 ± 4.30 aA	14.28 ± 3.51 aA	167,811.39 ± 46,945.73 aA	2,155,671.32 ± 500,151.86 aA
GH1	9.29 ± 2.96 abA	0.95 ± 0.02 abA	10.44 ± 3.49 abA	9.82 ± 3.01 abA	109,715.16 ± 37,627.76 abA	689,626.06 ± 143,716.37 bB
GH2	13.85 ± 3.73 aA	0.96 ± 0.01 aA	7.01 ± 1.55 bA	6.73 ± 1.43 bA	72,323.58 ± 16,253.62 bA	400,501.28 ± 115,524.01 bB

Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

Table 5. Metabolic capacity parameters of leaves.

Strains	Metabolic Flux (MF)	Metabolic Rate (MR)	Metabolic Activity (MA)
LH1	147,451.78 ± 74,782.09 aA	29,373,575.45 ± 11,514,173.85 aA	125.34 ± 20.46 aA
GH1	8895.65 ± 3415.29bB	4,488,409.55 ± 1,238,046.06 bB	57.89 ± 6.54 bB
GH2	2887.13 ± 2009.22bB	2,327,233.86 ± 1,102,590.90 bB	42.29 ± 8.32 bB

Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

3.3. Differences in Leaf Growth of Different Strains

In Table 6, it can be seen that the leaf area, leaf circumference, and leaf width of the LH1 leaves were significantly ($p < 0.05$) higher than those of GH2, increasing by 30.65%, 8.35%, and 21.67%, respectively. However, there was no significant ($p > 0.05$) difference in leaf length among the three strains. The results show that under the same intensity of drought stress, GH2 reduced leaf growth, decreased water and nutrient consumption, and ensured the basic needs of plant growth to resist the water and nutrient shortages caused by stress. These findings further confirm the good drought resistance of LH1.

Table 6. Growth index parameters of leaves.

Strains	Leaf Area (LA, mm^2)	Leaf Circumference (LC, mm)	Leaf Length (LL, mm)	Leaf Width (LW, mm)
LH1	638.98 ± 14.02 aA	127.10 ± 3.43 aA	51.28 ± 1.37 aA	16.90 ± 0.46 aA
GH1	580.37 ± 47.80 abA	122.80 ± 4.49 abA	51.28 ± 1.96 aA	15.76 ± 0.60 abA
GH2	489.07 ± 73.54 bA	117.31 ± 3.29 bA	49.06 ± 1.14 aA	13.89 ± 2.16 bA

Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

3.4. Differences in Chlorophyll Fluorescence in Leaves of Different Strains

The related parameters of chlorophyll fluorescence of *D. officinale* leaves were determined under natural drought stress, and the results are presented in Figure 3. Under stress, the F_v/F_m and ETR of the PSII system of the LH1 strain were significantly ($p < 0.05$) higher than those of the GH1 and GH2 strains, namely, 5.52% and 10.80% higher than GH1 and 7.13% and 7.37% higher than GH2, respectively. The photochemical quenching coefficient (qP) of LH1 was higher than that of GH1 and GH2, namely, 13.94% and 16.86% higher than that of GH1 and GH2, respectively. The qN of LH1 was 15.98% and 16.86 lower than that of GH1 and GH2, respectively. The qP reflects the opening degree of the PSII reaction center to some extent. Non-photochemical quenching is a way to protect the photosynthetic apparatus from damage through the heat dissipation of excess light energy [22]. The results reveal that under long-term drought stress, the PSII photochemical efficiency of GH1 and GH2 was negatively affected, the quantum efficiency of electron transport was inhibited, and the damage to the photosynthetic response of their plants was greater than that of LH1.

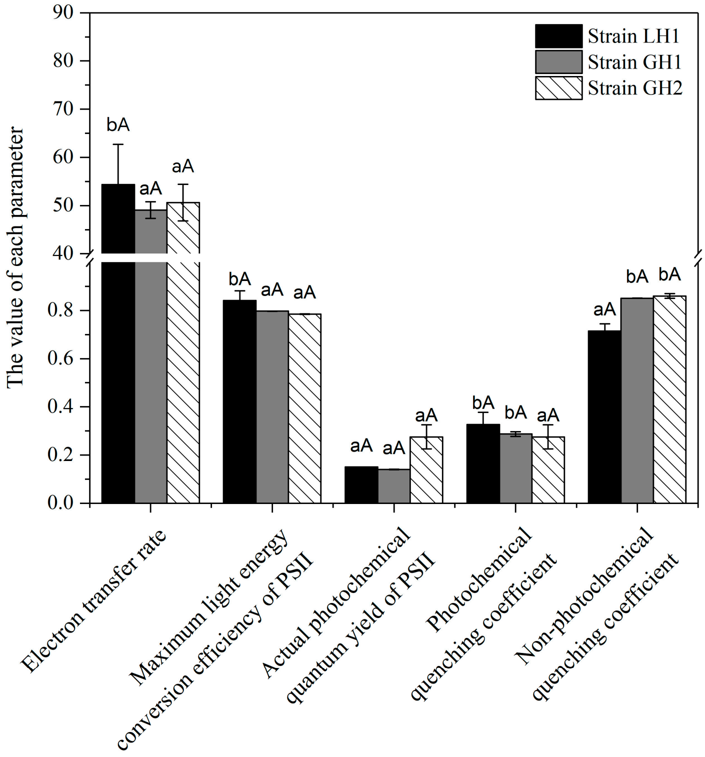


Figure 3. Leaf fluorescence kinetic parameters of different strains of *D. officinale*. Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

3.5. Differences in Chlorophyll Content in Leaves of Different Strains

The results reveal significant differences in chlorophyll content among the LH1, GH1, and GH2 strains (Figure 4). The chlorophyll content in LH1 was the highest, with chlorophyll a, chlorophyll b, and the total chlorophyll contents at 2.87, 2.28, and 5.15 mg, respectively. Compared to GH2, chlorophyll a, chlorophyll b, and the total chlorophyll contents increased by 30.07%, 53.05%, and 39.33% compared to those of GH1, respectively. These increases were 192.78%, 201.07%, and 196.39% higher than GH1. The results show that long-term drought stress would accelerate the decomposition rate of chlorophyll in the leaves of *D. officinale* and then affect the photosynthesis of the plant. The photosynthesis of plant leaves decreases, and the synthesis of organic matter in the body decreases, which in turn affects the normal growth of plants.

3.6. Correlation Analysis between Electrophysiological Parameters and Intracellular Water Utilization, Nutrient Transport, and Metabolic Parameters in Leaves of Different Strains

The electrophysiological parameters of *D. officinale* leaves were significantly correlated with the efficiency of light energy conversion, chlorophyll content, and growth index (Figure 5). Among the growth indexes of *D. officinale*, there was a significant positive correlation between LC and RLN ($p < 0.01$), UAF ($p < 0.01$), NUE ($p < 0.01$), chlorophyll a/b ($p < 0.05$), CT ($p < 0.05$), and NAC ($p < 0.05$). There was a significant negative correlation between LC and NPT ($p < 0.01$), NAT ($p < 0.01$), IWHT ($p < 0.01$), R ($p < 0.05$), and X_L ($p < 0.05$). Similarly, the results for LA, LW, and LC were similar to those for leaf circumference. The electrophysiological indicators closely related to the growth of *D.*

officinale are RLN, UAF, NUE, and NAC. The higher the value, the more vigorous the growth of *D. officinale*, which can better utilize low nutrient conditions for normal growth and has a higher nutrient flow, utilization rate, and transport capacity. The higher the values of electrophysiological indicators such as NPT, NAT, IWHT, R, and X_L, the poorer the growth of *D. officinale* and the weaker the water absorption capacity of plant cells. In order to resist this kind of damage, *D. officinale* accumulates a large amount of soluble substances, increases the cell water-holding time, ensures the normal supply of water under drought stress, and improves the transportation capacity of nutrients to ensure the timely supply of nutrients.

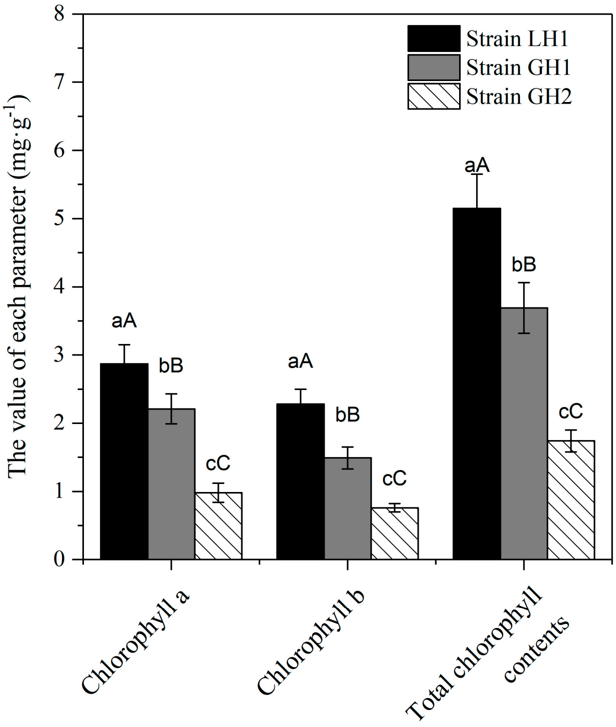


Figure 4. Chlorophyll content in leaves of different strains of *D. officinale*. Small letters indicate significant differences at the 5% level ($p < 0.05$), and capital letters indicate significant differences at the 1% level ($p < 0.01$).

In the leaf chlorophyll fluorescence index, the FV/FM of D. officinale was significantly correlated with NAC ($p < 0.01$), MA ($p < 0.01$), MF ($p < 0.01$) and MR ($p < 0.01$), STR ($p < 0.05$), and IWHC ($p < 0.05$), respectively. However, it has a significant negative correlation with NPT ($p < 0.01$), R ($p < 0.01$), XL ($p < 0.01$), NAT ($p < 0.05$), and IWHT ($p < 0.05$). In addition, the ETR was significantly positively correlated with NAC ($p < 0.01$), MA ($p < 0.01$), MF ($p < 0.01$), MR ($p < 0.01$), FV/FM ($p < 0.05$), STR ($p < 0.05$), and IWHC ($p < 0.05$), respectively. However, it was negatively correlated with IWUE ($p < 0.05$). In the chlorophyll content index of leaves, there was a significant positive correlation between CT and FV/FM ($p < 0.01$), NAC ($p < 0.01$), MA ($p < 0.01$), MF ($p < 0.05$), MR ($p < 0.05$), STR ($p < 0.05$), and IWHC ($p < 0.05$). Conversely, it is negatively correlated with NPT ($p < 0.01$), R ($p < 0.01$), X_L ($p < 0.01$), NAT ($p < 0.05$), Z ($p < 0.05$), and X_C ($p < 0.05$). The electrophysiological indicators positively correlated with the chlorophyll function of *D. officinale* are NAC, MA, MF, MR, etc. The higher the value, the higher the chlorophyll content, photosynthetic efficiency, and metabolic and nutrient transport capacity of *D. officinale*. On the other hand, the higher the value of electrophysiological indicators such as NPT, R, and X_L, the more severe the damage to the chlorophyll content of *D. officinale*, hindering the metabolic and nutrient transport capacity and resulting in slow growth.

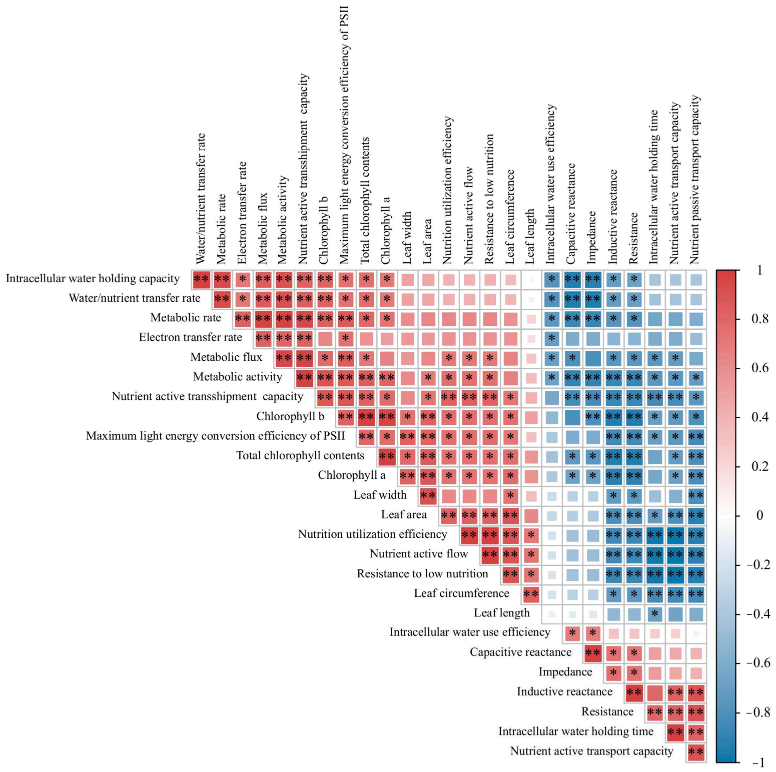


Figure 5. A corrplot of electrophysiological, intracellular water utilization, nutrient transport, and metabolic parameters in *D. officinale* leaves. The asterisk denotes statistically significant differences * $p < 0.05$, ** $p < 0.01$.

4. Discussion

4.1. Inherent Electrophysiological Information of Leaves of Different Strains of D. officinale

Generally, the real-time electrophysiological information inherent in plants cannot be detected. However, this study established a fitting equation between the electrophysiological parameters of C, R, Z, X_C, X_L, and the clamping force. It calculated the values of C, R, Z, X_C, and X_L in the leaves when the clamping force was 0, representing the inherent values of C, R, Z, X_C, and X_L of plant leaves [13]. This method overcomes the problems existing in traditional measurement methods, such as a lack of representativeness and comparability, poor stability and repeatability, great influence by the natural environment, and operational complexity. When the plant growth and metabolism activity are exuberant, the various ion-storage capacities (charge) in the cell can be understood as a generalized charging phenomenon.

The stronger the plant grows and the more charged it is, the greater the C and the smaller the R, Z, X_C, and X_L. The results show that the C of the LH1 leaves was significantly higher than that of GH2, but the R and X_L of the GH2 leaves were significantly higher than those of LH1, Z, and X_C and significantly higher than those of the LH1 leaves. The leaf area, leaf circumference, leaf length, and leaf width of GH2 were significantly lower than those of LH1, which indicated that GH2 had a higher R, Z, X_C, and X_L and a lower C than LH1, which further indicated that *D. officinale* of the LH1 strain grew more vigorously under drought stress and had stronger drought resistance.

4.2. Differences in Intracellular Water and Nutrient Metabolism in Leaves of Different Strains of D. officinale Based on Electrophysiological Information

The water balance inside plants is crucial for their growth. Leaf water potential can reflect the water status of plants and the degree of influence of the soil–vegetation–atmosphere continuous system on the water content in plants [23]. It can also reflect the ability of plants to transport nutrients and maintain plant growth and development [24].

In addition, a lower leaf moisture content and water potential indicate a soil water deficit, and plants can adapt to water-deficient environments by reducing water demands [25]. In this study, based on the C, R, Z, X_C, and X_L of *D. officinale* leaves, the cellular nutrient transport volume, nutrient unit active transport capacity, nutrient transport rate, nutrient transport capacity, and active nutrient transport capacity of *D. officinale* leaves were defined for the first time. The results accurately reflect the nutrient transport strategies of different strains of *D. officinale* and can monitor the nutrient transport status of *D. officinale* in real time. Compared with GH2, LH1 exhibited more vigorous life activity, while leaves with a low water content adapted to growth by improving the intracellular water-use efficiency and intracellular water-holding time [26]. These results demonstrate that based on electrophysiology, the cell water-holding capacity, intracellular water-use efficiency, cell water-holding time, and intracellular water-transfer rate of plants can be used to evaluate plant water use. These results are consistent with previously conducted work, wherein the nutrient flux per unit area (UNF), nutrient transfer rate (NTR), and nutrient transport capacity (NTC) in plants based on R, Xc, X_L, Z, and C were defined to reflect nutrient transport characteristics [27].

4.3. Effects of Drought Stress on Chlorophyll Fluorescence Parameters and Chlorophyll Content of Different Strains of D. officinale

Chlorophyll is the most important pigment in photosynthesis. Severe drought stress can slow down chlorophyll synthesis and cause its disintegration. Chlorophyll fluorescence serves as a simple and convenient method to explore the mechanism of photosynthesis and its response to external environmental factors. This method involves the absorption of light energy photochemical reactions and utilizes fluorescence imaging as a probe [28]. Previous studies have indicated that as drought stress increases, the water potential of plant leaves decreases. The limiting factors of photosynthesis shift from stomatal factors to non-stomatal factors. These factors include the decrease in phosphoenolpyruvate carboxylase (PEP) and RuBisCo activity, the weakening of the Hill reaction in the chloroplast, and limitations in RuBP regeneration, among others [29]. In this study, the F_v/F_m, qP, and ETR indexes of the GH1 and GH2 strains were significantly lower than those of the LH1 strains. This indicated that under long-term drought conditions, the leaf photosynthesis of the GH1 and GH2 strains was more inhibited than that of the LH1 strains, and the PSII reaction center was more affected. The GH1 and GH2 strains convert excess light energy into heat-dissipated energy, and the light system is mostly distributed in the direction of non-photochemical quenching to protect plants [30]. Chlorophyll is crucial for plant photosynthesis, aiding in the collection and transfer of light energy absorption, which are essential for growth. The color of plant leaves, primarily determined by chlorophyll as the main pigment, serves as an indicator of plant health and nutritional status, correlating with the degree of plant stress. Under water-scarcity conditions, the more stable the chlorophyll content, the stronger the drought resistance of crops, which will not change with changes in the ecological environment [31]. Some researchers believe that drought stress will lead to changes in the chlorophyll content and its precursor substances, leading to a reduced chlorophyll content, while others believe that chlorophyll in drought-resistant plants exhibits an increased chlorophyll content [32]. In this study, under the same drought-stress intensity, the LH1 strain exhibited higher chlorophyll a, chlorophyll b, and total chlorophyll contents in leaves compared to the GH1 and GH2 strains, indicating higher drought resistance in the LH1 strain.

4.4. Correlation among Electrophysiological Index, Growth Index, and Chlorophyll Index of D. officinale Leaves

The parameters defined based on electrophysiological information, such as NUE, RLN, UAF, NAT, NPT, can well characterize the intracellular water utilization, nutrient transport, and metabolic capacity of *D. officinale* under different degrees of stress [33,34]. Among these, RLN, UAF, NUE, NAC, NPT, NAT, IWHT, R, and X_L can accurately evaluate the growth of *D. officinale* leaves. The chlorophyll fluorescence index of *D. officinale* leaves can be accurately evaluated by the NAC, MA, MF, MR, STR, IWHC, NPT, R, X_L, and IWHT of plant leaves. NAC, MA, MF, MR, STR, IWHC, NPT, R, X_L, NAT, Z, and X_C can accurately evaluate the chlorophyll content of *D. officinale* leaves. Plants accumulate a large amount of organic osmoregulatory substances such as proline and soluble sugars when subjected to drought stress. In order to cope with adverse environments, plants reduce their osmotic potential to facilitate water absorption and improve their drought resistance. As osmoregulatory substances, proline, soluble sugars, and soluble proteins can play a role in regulating the osmotic potential within plants, enhancing their ability to regulate water. This leads to changes in the intracellular osmotic potential, electrophysiological information, and physiological changes [27]. From the research results, it can be seen that electrophysiological information can be a new method for monitoring small changes in plants, digitizing physiological indicators such as chlorophyll, the photosynthetic capacity, water use, and nutrient transport [35,36]. Our result supports a previous study which reported that the leaves of Broussonetia papyrifera plants grown in agricultural soil had higher C, d, IWHC, WTR, water-content values and lower Z and Xc values than those grown in moderately rocky desertified soil. The C, d, IWHC, WTR, and water-content values of the leaves of herbaceous plants were higher than those of woody plants [26].

5. Conclusions

For karst areas with seasonal water scarcity, selecting and cultivating drought-resistant crop varieties rationally is beneficial for regional plant recovery and economic growth. This study clarified the theoretical relationship between the clamping force and C, R, Z, X_C, and X_L parameters of *D. officinale* leaves. The parameters, defined based on electrophysiological information, can well characterize the differences in intracellular water utilization, nutrient transport, and metabolic capacity among different *D. officinale* strains. Through a correlation analysis, a close relationship between the corresponding electrophysiological indexes and leaf growth, chlorophyll fluorescence, and chlorophyll content was found. Among these R, X_L, NAC, NPT, NAT, MA, MF, MR, STR, IWHC, IWHT and other factors can accurately evaluate the chlorophyll content and function of *D. officinale* leaves. R, X_L, NAC, NPT, and NAT are closely related to the growth status and chlorophyll function of leaves of *D. officinale*. This relationship can be used as an important electrophysiological index for evaluating the drought resistance of *D. officinale*. The results show that the LH1 strain of *D. officinale* demonstrated the physiological characteristics of a high capacitance, low resistance, impedance, capacitance, and inductance, which further reflected its better growth index, higher chlorophyll content, and stronger efficiency of light energy use. The results indicate that the real-time online leaf electrophysiological information measured by the method used in this study can characterize the energy and material metabolism of crops, greatly improving the efficiency of crop-variety selection and reducing costs. The results also confirm that the strain LH1 exhibited less leaf damage, adequate water/nutrient supply, exuberant life activities, and excellent drought resistance. The LH1 strain demonstrates an ability to adapt to the drought environment of karst woodlands under severe drought stress. Therefore, the monitoring method based on electrophysiological information provides a new technical means for screening resistant varieties of *D. officinale* through the imitation of wild cultivation. These conclusions can be used to obtain real-time information on the transmission of water and nutrients within plant cells and provide theoretical support for studying the adaptation mechanisms of crops to adverse environments.

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