agronomy

MDPI

Article

Drought Tolerance Evaluation and Verification of Fifty Pakchoi (*Brassica rapa* ssp. *chinensis*) Varieties under Water Deficit Condition

Jingrui Li [1,2,3], Kumail Abbas [1], Weihong Wang [1], Binbin Gong [1,2,3], Lin Wang [1], Shenglin Hou [4], Hui Xia [3,5], Xiaolei Wu [1,2,3], Lida Chen [1] and Hongbo Gao [1,2,3,*]

1 College of Horticulture, Hebei Agricultural University, Baoding 071000, China
2 Key Laboratory of North China Water-Saving Irrigation Engineering, Baoding 071000, China
3 Collaborative Innovation Center of Vegetable Industry in Hebei, Baoding 071000, China
4 Hebei Academy of Agriculture and Forestry, Shijiazhuang 050050, China
5 College of Urban and Rural Construction, Hebei Agricultural University, Baoding 071000, China
* Correspondence: hongbogao@hebau.edu.cn

Abstract: Reduced available water resources have become the main limiting factor for the production of leafy vegetable, such as pakchoi, which can be effectively addressed by growing water-efficient varieties. Therefore, it is particularly important for evaluation and verification of drought tolerant varieties. In this study, 50 different varieties of pakchoi were detected for their morphological and physiological indicators under both normal-irrigated and water-deficiency environments. Based on systematic analysis of these evaluation indicators using different evaluation methods, the significant indicators were identified and the prediction model was established followed by validation of different drought tolerant of pakchoi varieties. The results showed that considerable differences were observed in all the indicators detected under conditions of water deprivation compared with normal irrigation. Frequency distribution revealed that the indictors sensitivity with significant changes to water-deficient conditions was ordered as follows: aboveground fresh and dry weight, followed by belowground fresh and dry weight, root volume, root length, root activity, and soluble sugar. Correlation analysis showed that each indicator was significantly or extremely significantly associated with other indices, indicating that there is a certain degree of association between the indices. Principal component analysis (PCA) turned 16 indicators into four independent components, with a cumulative contribution ratio of 80.147%. According to the results of drought tolerance comprehensive evaluation value (D-value) analysis and cluster analysis, 50 varieties were ranked in relation to drought tolerance and classified into five categories, among them Jinhui, Qingguan, Dongyue, Xiazhijiao, and Hanszifei, which were classified as highly drought-tolerant cultivars. Moreover, the stepwise regression model was established and demonstrated that soluble sugar, average root diameter, belowground fresh weight, root volume, and leaf number were selected as key indicators which can be used for screening and identifying drought-pakchoi varieties. Furthermore, the tolerance capacity of pakchoi varieties was further validated using one representative variety selected from five groups and treated with water-deficit stress. It was demonstrated that the established model was verified consistent with drought tolerance of pakchoi varieties, and tolerance capacity was closely related to increasing epidermal stomatal density, maintaining high photosynthesis, and increasing antioxidant enzyme activity to reduce damage (ROS). The results proposed the key drought tolerance indicators and evaluation methods, which provide the basis for the screening of leaf-vegetable varieties with different drought-tolerances, as well the identified varieties could be used for effective water-saving production.

Keywords: pakchoi; water deficit; drought tolerance; indicators screening; tolerance verification; systematic evaluation; physiological response

Citation: Li, J.; Abbas, K.; Wang, W.; Gong, B.; Wang, L.; Hou, S.; Xia, H.; Wu, X.; Chen, L.; Gao, H. Drought Tolerance Evaluation and Verification of Fifty Pakchoi (*Brassica rapa* ssp. *chinensis*) Varieties under Water Deficit Condition. *Agronomy* **2023**, *13*, 2087. https://doi.org/10.3390/agronomy13082087

Academic Editor: Michael Fuller

Received: 2 July 2023
Revised: 2 August 2023
Accepted: 4 August 2023
Published: 8 August 2023

1. Introduction

Pakchoi (*Brassica rapa* L. ssp. *Chinensis*), a fresh leafy vegetable with high leaf-water content and a shallow root system, is widely cultivated and consumed around the world, especially in China, accounting for 30–40% [1,2]. However, a shortage of available water resources has become the main limiting factor for the production [3,4]. To meet the requirement of such a huge population, water saving is a significant approach to enhance leaf-vegetable yield especially such as pakchoi, which can be effectively addressed by growing water-efficient varieties. Therefore, it is great important for screening a high level of drought tolerance in pakchoi varieties and verification of drought tolerance for its stable production [5]. This can become an effective measure to save manpower in the agricultural sector and increase water use efficiency [6].

At present, many countries and regions have made effective efforts in selecting drought-tolerant crop varieties in agricultural production [7,8]. Among them, in areas with frequent high-temperature risks and water shortages, drought-tolerant varieties have shown a prominent role in overcoming water consumption to achieve the goal of water conservation [9]. Many researches have shown that planting varieties with high drought tolerance potential had shown significant water-saving efficiency and increases yield [10,11]. Presently, the screening of drought-tolerant varieties mainly focus on agronomic and field crops, such as wheat [12–14], barley [14], maize [15], cotton [16], rice [17], millet [18], and potato [19]; however, there is no key report on the comprehensive evaluation and verification based on indicators that screen pakchoi drought-tolerant varieties for morphological, physiological, and biochemical indicator changes.

Over the last two decades, several indicators and indicators features have been proposed to identify the most drought-tolerant varieties [20]. Generally, drought stress indicators based on yield loss under drought circumstances have been utilized to identify drought-tolerant varieties [7]. Drought susceptibility is also typically measured by comparing its yield reduction under both normal and water deficient environments [11]. However, due to multiple aspects of crop genotype tolerance and susceptibility, the tolerance of plants to drought is also a comprehensive performance of multiple factors [21–23] and a single indicator cannot fully or accurately evaluate drought tolerance as it is too one-sided and unrepresentative. Until now, no single indicator can adequately represent plant drought tolerance [24]. So, the identification of plant drought tolerance needs to be a comprehensive evaluation of its morphological, physiological, and biochemical indicators to achieve the purpose of selecting accurate drought-tolerant varieties.

Furthermore, the majority of evaluation indicators for assessing drought tolerance have been primarily focused on crops, which may not be applicable for screening pakchoi varieties due to variations in growth morphology, harvested parts, and water requirements.

In the stages of plant growth and development, it is crucial to identify drought tolerance indicators markers for genotype screening process [25–28]. Previous studies have discovered various indicators for tolerant crop varieties under drought. These indicators markers include leaf water potential, net photosynthesis, water use efficiency, maximum quantum efficiency of PSII, proline, betaine content, soluble sugars, chlorophyll, malondialdehyde (MDA), antioxidant enzyme activity, leaf area, and yield output [20,29–33]. Nonetheless, the abundance and complexity of these indicators have been acknowledged. Studies have demonstrated the significance of identifying key indicators and streamlining evaluation methods in order to effectively select drought-tolerant varieties [14,15]. To address this, a novel and advanced indicator called D-value was proposed. This indicator aims to evaluate and screen varieties under both normal and drought conditions, providing a more comprehensive and effective approach [24]. The utilization principal component analysis (PCA) enables simplification of extensive and intricate datasets by transformation the original multiple indicators into a reduced set of representative indicators [34]. Multiple linear regression enables the creation of predictive models that utilize morphology and physiological indicators to elucidate the level of water-deficit tolerance [35]. Therefore, it is of utmost importance to identify a simple set of indicators for evaluating drought stress or effective modelling using a combination of different evaluation methods that will apply for the rapid identification drought stress tolerance of elite pakchoi germplasm or cultivars.

The limited adaptability of modern varieties primarily results in reduced yield as a consequence of water deficit, posing a significant threat to crop drought tolerance [25]. However, plants possess various mechanisms, such as osmotic adjustments or osmoregulation through the accumulation of proline, sugars and other substances, which allow them to thrive best and attain significant output yield [36]. Additional mechanisms involve in mitigating oxidative damage (ROS) through heightened antioxidant activity system (SOD, POD, CAT) and protect the plants from oxidative stress [37]. Furthermore, enhanced water use efficiency through the regulation of the stomatal activity leads to an increase in CO_2 concentration, elevated chlorophyll levels, and ultimately, ameliorate photosynthesis [16,38,39]. Moreover, root growth also plays a crucial role in maintaining water and nutrient supply when subjected to water deficit.

However, there is a lack of comprehensive studies analyzing the morphological, physiological, and biochemical characteristics using a multivariate approach to identify marker indicators associated with drought tolerance in pakchoi varieties. Therefore, it is crucial to identify simple and effective indicators for evaluating drought stress, enabling quick evaluation and validation of elite germplasm or varieties tolerant to drought stress. In this study, 50 different pakchoi varieties were cultivated under two irrigation conditions: normal irrigation (with a soil water content of 70–80% of field capacity) and water shortage (with a soil water content of 30–40% of field capacity). By determining the morphological and physiological indicators, the drought tolerance of pakchoi varieties was assessed and classified through systematic analysis using techniques such as PCA, D-value calculation, and cluster analysis. Additionally, a prediction model was developed using stepwise regression to validate the drought tolerance capacity and mechanisms of different pakchoi varieties. These findings provide a basis for screening drought tolerance indicators and offer valuable insights into the tolerance and susceptibility of leafy vegetable varieties to drought.

2. Materials and Methods

2.1. Experimental Plant Material

In this study, 50 varieties of pakchoi were selected, including loose leaf, girdling different type, green and red leaf different leaf color, green and white different petiole color, narrow and hypertrophy different petiole type, and so on (Table 1).

2.2. Experimental Design

2.2.1. Screening of Pakchoi for Drought Tolerance and Development of Drought Tolerance Indicators

All of the experimental samples were planted in the greenhouse of Dingxing County. The test materials were divided into normal (70–80% of field capacity) and water deficit treatments (30–40% of field capacity). The ZL6 data collector was used to monitor the soil moisture content, and the water deficit treatment duration was 15 days. Measurement of plant height during harvest period. Measurement of stem diameter (SD), leaf number (LN), root length (RL), average root diameter (ARD), root surface area (RSA), root volume (RV), aboveground fresh weight (AFW), belowground fresh weight (BFW), aboveground dry weight (ADW), belowground dry weight (BDW), root activity (RA), leaf relative water content (RWC), SPAD, relative electrolytic leakage (REL), soluble sugar (SS), and soluble protein (SP) during harvest period.

2.2.2. Response of Pakchoi to Water Deficit with Varying Drought Tolerance Levels

Based on the ranking of the D-value of the comprehensive evaluation value of drought tolerance in the preliminary test and the classification grade of cluster analysis, one type of Pakchoi with varying drought tolerance grades was chosen at random as the test material. In this experiment, 70% to 80% of the maximum field water capacity was designated as the control treatment, whereas 30% to 40% of the minimum field water capacity was designated as the water deficit treatment. Measurement of key indicators that identified indicators in regression models, and epidermal stomatal, photosynthesis, antioxidant enzyme activity, reactive oxygen species and MDA content during harvest period.

Table 1. Names and numbers of 50 pakchoi varieties.

Number	Name	Feature	Number	Name	Feature
1	Feicuiyihao	Petiole emerald green	26	Naiyouheiyw	Petiole thickness
2	Zijinkuaicai	Purple leaves	27	Aijiao	Petiole short
3	Lexialiangpin	Petiole narrow	28	815 Kuaicai	Leaf thickness
4	Shuoyuannaibaicai	Petiole white	29	537 Xiaobaicai	Girdling
5	Meihuiqinggengbaicai	Petiole hypertrophy	30	Sucuibai	Petiole white
6	Huangjinkuaicai	Yellow-green leaves	31	Heimeigui	Dark-green leaves
7	Qingshuangjimaocai	Petiole green-white	32	Lvsongxiaobaicai	Petiole slender
8	Vuguanheiyebaicai	Petiole strong green	33	Qingguan	Leaf nearly circular
9	Jinhui	Leaf thickness	34	Heiniu	Petiole white
10	Dongyue	Light-green leaves	35	Dongchunsansan	Petiole green
11	Feicuierhao	Petiole hypertrophy	36	Lifei	Petiole white
12	Xiazhijiao	Petiole hypertrophy	37	Guoxiayihao	Petiole hypertrophy
13	Dongxiu	Petiole light green	38	Zhongyannaibaicai	Petiole white
14	Zijinyoucai	Purple leaves	39	Jingguanerhao	Petiole green
15	Hansizifei	Dark-purple leaves	40	Heiyouyihao	Petiole slender
16	Zijiaren	Purple leaves	41	Heixinwu	Petiole short
17	Shanghaiqing	Light-green leaves	42	Heiyoubaicai	Dark-green leaves
18	Jinzuanziyi	Light-purple leaves	43	Huangxinwu	Petiole short
19	Kuishanheiyetianbaicai	Dark-green leaves	44	Ruiguanqingbang	Petiole slender
20	Baigengsijixiaobaicai	Petiole slender	45	Ziyuyoucai	Purple leaves
21	Teaibaixue	Petiole short	46	Yuexiaqinggengcai	Petiole green
22	Naiyouxiaobaicai	Petiole long	47	Xinguanhua	Petiole green
23	Beijingheidatou	Leaf nearly circular	48	Qingbangyoucai	Leaf-blade thin
24	Kangresijitianbaicai	Tender green leaves	49	Jingpinwuyueman	Petiole green
25	Xinsijisuzhouqing	Girdling	50	Woyouganju	Petiole short

2.3. Determination of Indicators and Methods

2.3.1. Determination of Growth Indices

An electronic balance (Ohouse/CP 114) was used to choose uniformly growing plants, count the number of leaves, and then the fresh weight and dried weight of the upper part of the plant and the lower part of the plant was measured, respectively. Dry weight was determined by sterilization in a 105 °C oven for 30 min and then baking to a consistent weight at 70 °C. The root system was scanned with the MRS-9600TFU2L and then analyzed with LA-S root analysis software, which measured the root system's overall dimensions, including root length, surface area, volume, and average diameter. The root system was thoroughly cleaned and laid out on the scanner panel before scanning could begin.

2.3.2. Determination of RWC

Take functional leaves from the same part of each plant, measure the fresh weight of the leaves first, then completely immerse the leaves in distilled water to make them absorb water to a saturated state. After taking them out, dry the leaves and weigh them to obtain the saturated weight of the plant leaves. Place the plant leaves in an oven and bake them for 105 °C for 30 min to before being dried at 85 °C until they reach a consistent weight. The formula for calculating the relative water content (RWC) of leaves is: relative water content of leaves = (fresh weight of leaves − dry weight of leaves)/(saturated weight of leaves − dry weight of leaves) × 100% by method proposed by Meher et al., 2018 [40].

2.3.3. SPAD and REL Assay

SPAD was measured with a SPAD502 chlorophyll meter. SPAD measurements at the 2/3 position on the third fully expanded leaves from the top of the plant.

The REL was measured by the immersion method. The leaves were cut into strips, placed in a test tube, and 15 mL of pure water was added. The strips were removed after pumping for 15 min with a vacuum pump and allowed to stand for 20 min, and the conductivity was measured at room temperature (20–25 °C). Then, the sample was placed in a 100 °C boiling water bath for 15 min, removed and cooled for 10 min, and its conductivity was measured after boiling.

2.3.4. SS and SP Content Assay

Weigh 0.5 g of the sample, add 4 mL of distilled water and grind it to a homogenate. Extract it in boiling water for 30 min. Take 0.1 mL of the supernatant and add it to a 10 mL centrifuge tube. Dilute to 10 mL with pure water. Adding 1 mL of pure water, 1 mL of sample extraction solution, slowly add 5 mL of anthrone sulfate to the wall in cold water, mix well in cold water, then they are placed together in an 80 °C water bath for 10 min to develop color. And measure the OD-value at 630 nm. The SS content were calculated based on the standard curve.

Take 0.5 g of sample, add 2 mL of distilled water, and grind it into a homogenate. Transfer it to a test tube, let it sit for 30 min, and then centrifuge. Suck 0.2 mL of supernatant, add 0.8 mL of distilled water and 5 mL of coomassie brilliant blue solution, fully mix them, and then colorimetric at 595 nm to determine the absorbance. The SP content were calculated based on the standard curve.

2.3.5. Measurement of Stomatal Characteristics of the Leaves

Nail polish-imprinted slices were observed with CellSens image analysis software (3.17.0.16686) and imaged under an Olympus BX51 fluorescence microscope (Olympus Soft Imaging Solutions GmbH). Three slices were prepared for each variety. The stomatal length, stomatal width, stomatal aperture, and stomatal density in each field were measured by ImageJ image processing software. After observations were made and counting was performed, the average value of the six fields was calculated [41].

2.3.6. Chlorophyll Content and chl a, chl b Calculations

The photosynthetic pigment was determined by ethanol acetone extraction colorimetry. Use a punch to drill a hole on one side of the main vein at the same node and sample 0.2 g. Soak the leaves in a 1:1 mixture of anhydrous ethanol and acetone (10 mL, V), after the leaves turn white the absorbance values at 663 nm and 645 nm were measured, and chlorophyll a and chlorophyll b were calculated.

2.3.7. Measurement of Photosynthetic Parameters

The LI-6400XT (Lincoln, NE, USA), a portable photosynthetic instrument, was used to take a single reading from the leaves of five different types of pakchoi on a sunny morning. For each treatment, three plants were chosen for their steady development, strong growth, and lack of disease and insect pests. For each plant, three usable leaves were chosen at random, and within each leaf, three alternative orientations were chosen to determine its photosynthetic qualities. The net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration are the measurable indicators (Ci).

2.3.8. Enzymatic Activity, MDA Content and O_2^- Production Rate Assay

Weigh 0.5 g of pakchoi leaves, add 50 mmol/L, pH 7.8 PBS buffer solution, grind in ice bath, then centrifugate at 4 °C at 12,000× g rpm for 10 min, take out the supernatant to determine SOD activity, POD activity and CAT activity, according to a previously described method [42,43]. MDA content was determined by the thiobarbiturate method. A total of

0.5 g of leaves was used to determine the O_2^- production rate according to a previously published method for determination [44].

2.4. Data Processing and Statistical Analysis

The indicator drought-tolerance coefficient (DC) was calculated according to Formula (1), where CKi and Ti represent the indicator measureD-values of normal irrigation and water deficit treatment, respectively.

$$\mathrm{DC} = \frac{\mathrm{Ti}}{\mathrm{CKi}} \tag{1}$$

For the DC value of each indicator, correlation, frequency analysis and principal component analysis were performed. The factor weight coefficient (ωi), membership functional value [μ(xi)] and drought comprehensive evaluation value (D) were calculated according to Equations (2)–(4).

Where Pi denotes the contribution rate of the ith comprehensive indicator and xi max and minimum represent the maximum and minimum values of the ith comprehensive indicator, respectively.

$$\omega\mathrm{i} = \mathrm{Pi} \div \sum\nolimits_{\mathrm{i}}^{\mathrm{n}} \mathrm{Pi} \tag{2}$$

$$\mu(\mathrm{xi}) = \frac{\mathrm{xi} - \mathrm{xi,min}}{\mathrm{xi,max} - \mathrm{xi,min}} \text{ or } \mu(\mathrm{xi}) = 1 - \frac{\mathrm{xi} - \mathrm{xi,min}}{\mathrm{xi,max} - \mathrm{xi,min}} \tag{3}$$

$$\mathrm{D} = \sum\nolimits_{\mathrm{i}=1}^{\mathrm{n}} \left[\mu(\mathrm{xi}) \times \left(\mathrm{Pi} \div \sum\nolimits_{\mathrm{i}}^{\mathrm{n}} \mathrm{Pi}\right)\right] \tag{4}$$

The comparison series is the DC value of each indicator, and the reference series is the D-value for gray correlation analysis. Finally, cluster analysis was carried out for the D-value of pakchoi, and the drought tolerance grade was divided. Finally, taking d as the dependent variable, the DC values of each indicator are analyzed by stepwise regression, and the regression equation is calculated.

2.5. Data Analysis

Microsoft Excel 2010 was used for data processing. PCA, stepwise regression analysis, correlation and significance were evaluated by using IBM SPSS Statistics 22nd edition software (Armonk, NY, USA). OriginPro 2022 (Origin Lab Corp, Northampton, MA, USA) was used for drawing.

3. Results

3.1. Screening of Drought-Tolerant Varieties and Identification of Drought-Tolerant Indicators of Pakchoi under Water Deficit Stress Treatment

3.1.1. Analysis of the MeasureD-Value of the Variety Indicator of Pakchoi

Water deficit treatment has a significant effect on the measureD-values of 16 indicators across 50 kinds of pakchoi (Table 2). Varieties of pakchoi selected for this study showed a wide range of diversity in terms of material type, with coefficients of variation ranging from 0.05 to 0.62. Additionally, the selected indicators were sensitive to water deficit, and exhibited a positive treatment effect. Furthermore, the correlation coefficient of the selected Chinese cabbage varieties under water deficit treatment and normal water supply treatment ranged from 0.07 to 0.95, indicating that the sensitivity of each indicator to water deficit varies, making it challenging to directly consider the drought tolerance of each pakchoi variety by directly using the measureD-values of each indicator.

Table 2. Difference analysis of 16 indicators of pakchoi under normal irrigation and water deficit irrigation.

Indicators	Treatment	Average Value	Coefficient of Variation	Standard Error	T-Value	*p*-Value	Correlation Coefficient
SD	NI	9.79	0.19	0.26	9.99	0.00	0.63
	WDI	7.16	0.33				
LN	NI	18.01	0.17	0.28	11.15	0.00	0.82
	WDI	14.90	0.23				
RL	NI	66.11	0.17	1.39	15.78	0.00	0.63
	WDI	44.17	0.25				
ARD	NI	0.75	0.14	0.01	14.41	0.00	0.42
	WDI	0.55	0.12				
RSA	NI	18.98	0.27	0.48	12.54	0.00	0.75
	WDI	13.00	0.30				
RV	NI	1.40	0.40	0.05	11.22	0.00	0.75
	WDI	0.79	0.40				
AFW	NI	188.64	0.50	7.48	8.42	0.00	0.85
	WDI	125.68	0.48				
BFW	NI	3.45	0.51	0.16	6.76	0.00	0.75
	WDI	2.34	0.51				
ADW	NI	8.88	0.57	0.27	10.47	0.00	0.95
	WDI	6.10	0.62				
BDW	NI	0.31	0.45	0.01	9.99	0.00	0.86
	WDI	0.21	0.53				
RA	NI	4.33	0.31	0.17	8.49	0.00	0.49
	WDI	2.88	0.34				
RWC	NI	94.39	0.05	1.82	10.46	0.00	0.21
	WDI	75.36	0.17				
SPAD	NI	45.23	0.17	0.73	5.14	0.00	0.52
	WDI	41.45	0.22				
REL	NI	26.92	0.26	2.01	−6.94	0.00	0.39
	WDI	40.87	0.38				
SS	NI	31.38	0.41	2.28	2.76	0.01	0.07
	WDI	25.07	0.36				
SP	NI	1.19	0.49	0.09	−2.30	0.03	0.09
	WDI	1.40	0.35				

NI: normal irrigation; WDI: water deficit irrigation; SD: stem diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein.

3.1.2. Evaluation of Drought Tolerance Indicator Coefficients of Pakchoi

Different indicators for pakchoi changed by varying degrees after being subjected to drought treatment compared to the normal treatment (Table 3). Although all the variations of a given indicator have a variation coefficient of between 0.13–0.79 for their DC values, the drought tolerance reflected by these values varies among varieties, showing that the sensitivity of these indicators to water shortage varies as well. Even within the same time interval, DC values for different indicators have very distinct distributions. Stem diameter, leaf number, root surface area, root average diameter, relative electrolyte leakage, relative water content, SPAD, and soluble protein distribution frequency are all greater than or equal to 80% from $0.6 \leq$ DC, demonstrating that they are insensitive to water deficiency. Furthermore, the aboveground fresh weight, underground fresh weight, aboveground dry weight, belowground dry weight, root volume, root length, root activity, and soluble sugar distribution frequency were 68.0%, 74.0%, 78.0%, 68.0%, 50.0%, 76.0%, 72.0%, and 72.0%, respectively. The aboveground fresh weight, aboveground dry weight, belowground dry weight, root activity and soluble sugar indicators were more sensitive to water deficit than the other indicators.

Table 3. DC of indicators in 50 pakchoi varieties.

Varieties	SD	LN	RL	ARD	RSA	RV	AFW	BFW	ADW	BDW	RA	RWC	SPAD	REL	SS	SP
1	0.64	0.75	0.32	0.88	0.64	0.61	0.51	0.61	0.47	0.60	0.72	0.86	0.82	0.65	0.67	1.13
2	0.69	0.74	0.36	0.91	0.42	0.33	0.47	0.57	0.57	0.54	0.67	0.87	0.79	0.68	0.57	0.98
3	0.99	0.88	0.77	0.86	0.82	0.62	0.58	0.88	0.71	0.88	0.87	0.79	0.76	0.91	1.68	2.10
4	0.60	0.80	0.51	0.78	0.63	0.62	0.47	0.65	0.64	0.67	0.79	0.83	0.95	0.68	0.75	1.26
5	0.39	0.89	0.57	0.81	0.53	0.49	0.79	0.27	0.78	0.38	0.52	0.78	0.81	0.50	0.47	0.81
6	0.56	0.62	0.50	0.77	0.45	0.44	0.64	0.47	0.67	0.47	0.32	0.63	0.79	0.52	0.36	0.68
7	0.34	0.68	0.57	0.76	0.74	0.79	0.47	0.46	0.50	0.45	0.37	0.72	0.75	0.35	0.43	0.77
8	0.83	0.90	0.67	0.76	0.52	0.34	0.59	0.79	0.71	0.82	0.78	0.92	0.87	0.75	0.79	1.27
9	0.98	1.00	0.87	0.61	0.88	0.72	0.95	0.97	0.85	0.97	0.95	0.96	1.08	0.97	3.63	2.84
10	0.94	0.98	0.85	0.74	0.84	0.60	0.88	0.92	0.79	0.93	1.02	0.94	1.20	0.93	3.55	2.55
11	0.88	1.00	0.86	0.80	0.76	0.77	0.84	0.84	0.68	0.86	0.87	0.84	1.06	0.90	1.40	1.95
12	0.87	0.99	0.95	0.97	0.90	0.60	0.72	0.84	0.93	0.90	0.74	0.82	0.94	0.88	2.30	2.55
13	0.85	0.95	0.61	0.73	0.78	0.54	0.88	0.80	0.91	0.86	0.76	0.93	1.10	0.87	1.21	1.76
14	0.79	0.75	0.48	0.88	0.73	0.65	0.50	0.62	0.89	0.52	0.76	0.87	1.10	0.80	0.92	1.40
15	0.84	0.92	0.68	0.87	0.75	0.79	0.80	0.87	0.77	0.86	0.85	0.89	1.03	0.84	2.11	2.32
16	0.87	0.69	0.84	0.82	0.75	0.54	0.80	0.85	0.71	0.74	0.91	0.88	0.99	0.81	0.94	1.44
17	0.81	0.95	0.75	0.68	0.76	0.65	0.80	0.74	0.85	0.70	0.88	0.82	0.93	0.86	1.09	1.62
18	0.80	0.96	0.65	0.84	0.69	0.68	0.61	0.79	0.67	0.85	0.84	0.86	0.85	0.78	1.23	1.83
19	0.84	0.80	0.50	0.77	0.67	0.64	0.72	0.89	0.62	0.84	0.71	0.83	0.97	0.80	0.98	1.54
20	0.82	0.89	0.74	0.78	0.86	0.66	0.97	0.79	0.76	0.77	0.83	0.92	0.98	0.76	1.13	1.70
21	0.24	0.94	0.81	0.88	0.92	0.78	0.58	0.28	0.85	0.26	0.46	0.56	1.00	0.39	0.50	0.93
22	0.73	0.80	0.62	0.65	0.62	0.58	0.57	0.74	0.74	0.68	0.74	0.83	0.76	0.79	0.74	1.21
23	0.42	0.69	0.69	0.83	0.51	0.42	0.69	0.48	0.75	0.41	0.41	0.55	0.87	0.33	0.33	0.56
24	0.67	0.76	0.57	0.89	0.64	0.65	0.63	0.62	0.46	0.69	0.55	0.63	0.69	0.70	0.62	1.10
25	0.61	0.84	0.70	0.76	0.76	0.89	0.53	0.48	0.44	0.47	0.73	0.79	0.80	0.67	0.69	1.15
26	0.75	0.78	0.61	0.68	0.83	0.52	0.64	0.78	0.58	0.67	0.67	0.80	1.02	0.61	0.65	1.12
27	0.68	0.88	0.63	0.78	0.71	0.68	0.73	0.65	0.71	0.67	0.96	0.74	0.87	0.73	0.90	1.39
28	0.94	0.78	0.62	0.74	0.62	0.56	0.84	0.91	0.77	0.86	0.86	0.97	0.78	0.82	1.05	1.56
29	0.99	0.86	0.71	0.78	0.80	0.65	0.86	0.97	0.76	0.94	0.78	0.97	0.92	0.92	1.52	2.03
30	0.37	0.79	0.69	0.77	0.80	0.56	0.44	0.36	0.68	0.33	0.49	0.40	0.67	0.39	0.38	0.69
31	0.39	0.66	0.71	0.76	0.66	0.47	0.38	0.29	0.59	0.30	0.41	0.49	0.88	0.27	0.97	0.42
32	0.76	0.89	0.68	0.83	0.61	0.61	0.55	0.69	0.70	0.67	0.60	0.74	0.87	0.61	0.73	1.15
33	0.96	0.96	0.77	0.70	0.90	0.84	0.83	0.98	0.71	0.88	0.83	0.98	0.95	0.88	3.59	2.67
34	0.77	0.66	0.41	0.64	0.58	0.50	0.74	0.72	0.68	0.64	0.86	0.79	0.82	0.65	0.60	1.01
35	0.46	0.69	0.81	0.72	0.73	0.82	0.49	0.52	0.61	0.48	0.26	0.67	0.87	0.36	0.39	0.73
36	0.71	0.76	0.61	0.72	0.82	0.56	0.46	0.75	0.43	0.57	0.71	0.81	0.99	0.62	0.62	1.09
37	0.57	0.98	0.72	0.78	0.66	0.48	0.60	0.54	0.82	0.46	0.61	0.64	0.99	0.77	0.60	1.08
38	0.74	0.85	0.55	0.71	0.77	0.51	0.69	0.63	0.61	0.56	0.82	0.73	0.93	0.86	0.75	1.21
39	0.85	0.95	0.72	0.60	0.71	0.44	0.94	0.88	0.61	0.70	0.76	0.95	1.01	0.86	0.80	1.31
40	0.76	0.73	0.62	0.68	0.62	0.42	0.70	0.65	0.71	0.73	0.55	0.79	1.16	0.75	0.58	0.99
41	0.60	0.84	0.85	0.65	0.61	0.45	0.63	0.75	0.69	0.63	0.36	0.84	0.93	0.58	0.49	0.91
42	0.98	0.75	0.61	0.61	0.68	0.49	0.65	0.86	0.44	0.87	0.86	0.96	0.77	0.83	0.83	1.36
43	0.73	0.85	0.81	0.55	0.47	0.34	0.85	0.66	0.77	0.61	0.45	0.85	0.77	0.78	0.48	0.84
44	0.92	0.80	0.77	0.62	0.51	0.61	0.75	0.87	0.72	0.85	0.83	0.96	0.89	0.77	0.86	1.35
45	0.49	0.62	0.84	0.69	0.73	0.62	0.52	0.45	0.65	0.48	0.27	0.52	1.02	0.30	0.30	0.55
46	0.87	0.88	0.62	0.77	0.45	0.28	0.88	0.87	0.81	0.83	0.89	0.90	0.87	0.73	0.83	1.31
47	0.70	0.65	0.59	0.58	0.60	0.48	0.65	0.49	0.49	0.63	0.66	0.76	0.90	0.74	0.48	0.87
48	0.87	0.88	0.69	0.68	0.57	0.36	0.98	0.84	0.88	0.76	0.89	0.92	0.96	0.64	0.76	1.27
49	0.73	0.77	0.76	0.59	0.71	0.47	0.63	0.62	0.81	0.58	0.51	0.65	1.07	0.87	0.56	0.96
50	0.86	0.76	0.66	0.55	0.77	0.62	0.82	0.76	0.52	0.67	0.70	0.83	0.92	0.80	0.63	1.10
AV	0.73	0.82	0.67	0.74	0.69	0.57	0.68	0.69	0.69	0.67	0.69	0.80	0.91	0.71	0.99	1.33
CV	0.26	0.13	0.20	0.13	0.18	0.24	0.23	0.27	0.19	0.27	0.28	0.17	0.13	0.26	0.79	0.43

SD: stream diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein.

3.1.3. Correlation Analysis for Various Indicators

The correlation analysis revealed that the indicators were significantly or highly significantly related to one another (Figure 1). SP was significantly correlated with other indicators except for ARD and was significantly correlated with RL; similarly, SS showed a significant association with other indicators except for ARD and was significantly correlated with ADW. Moreover, there were no correlations found among ARD with other indicators except with AFW.

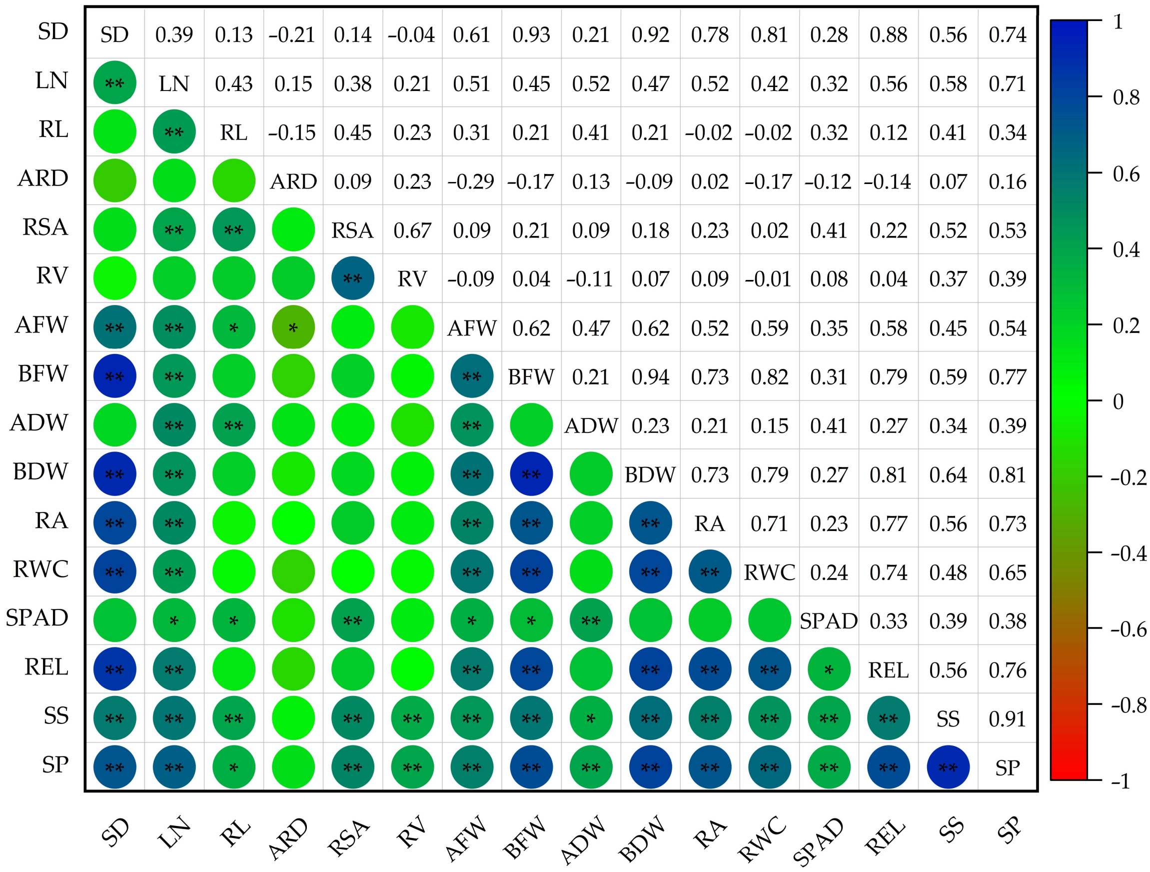

Figure 1. Correlation of drought tolerance coefficient of each indicator of leaf lettuce varieties. SD: stream diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein. * and ** indicate significant correlations at the $p < 0.05$ and $p < 0.01$ levels, respectively.

3.1.4. PCA for Indicators in Pakchoi Varieties

Through PCA, the cumulative contribution rate of the first four factors in the characteristic value of each factor is 80.147%, and the eigen root is >1.198 (Table 4). Therefore, the first four factors were extracted, and the original 16 indicators were replaced by four new independent comprehensive indicators (PCA1, PCA2, PCA3, and PCA4). PCA1 showed higher load capacity on SD, LN, AFW, BFW, BDW, RA, RWC, SPAD, REL, SS, and SP. In addition, PCA2 represented higher loads on RL, RSA, and RV. For PCA3, ADW exhibited higher load capacity, and for PCA4, ARD constituted the highest variability.

Table 4. Eigenvectors and contribution rates of all indicators in pakchoi varieties by PCA.

Indicators		PCA1	PCA2	PCA3	PCA4
SD		0.886	−0.356	0.093	−0.089
LN		0.690	0.333	−0.156	0.303
RL		0.342	0.545	−0.484	−0.259
ARD		−0.08	0.379	0.396	0.758
RSA		0.391	0.742	0.191	−0.315
RV		0.184	0.684	0.500	−0.238
AFW		0.719	−0.175	−0.408	−0.024
BFW		0.897	−0.258	0.083	−0.111
ADW		0.413	0.247	−0.626	0.483
BDW		0.904	−0.231	0.116	−0.032
RA		0.812	−0.197	0.251	0.135
RWC		0.796	−0.400	0.124	−0.013
SPAD		0.455	0.292	−0.400	−0.158
REL		0.872	−0.216	0.076	0.005
SS		0.788	0.352	0.094	0.004
SP		0.928	0.251	0.179	0.103
	Characteristics root	7.625	2.425	1.575	1.198
	Contribution rate (%)	47.659	15.159	9.843	7.487
	Cumulative contribution rate (%)	47.659	62.817	72.66	80.147
	Factor weights	0.595	0.189	0.123	0.093

Note: SD: stream diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein.

3.1.5. Comparative Study of Pakchoi Drought Tolerance

According to the results of PCA, the comprehensive score of each D-value of the 50 varieties was calculated according to the proportion of the variance contribution rate of each principal component to the cumulative variance contribution rate of the extracted principal components. Furthermore, the D-values of the 50 varieties ranged from 0.108 to 0.710, with a mean of 0.313 and a coefficient of variance of 0.476, showing that the D-values were more representative. The 50 varieties were sorted based on the size of the D-value, with the extremely drought-tolerant types being Jinhui, Dongyue, and Qingguan, and the highly sensitive varieties being Huangjinkuaicai, Sucuibai, Heidatou, Ziyuyoucai, and Heimeigui. The other varieties fell somewhere in the middle (Table 5).

3.1.6. Drought Tolerance Levels Dissected Using Cluster Analysis

Cluster analysis resulted in the classification of the 50 varieties of pakchoi into five categories based on the D-value at $\lambda = 0.2$ (Figure 2). The first category included particularly drought-tolerant cultivars such as Jinhui, Qingguan, Dongyue, Xiazhijiao, and Hanszifei. The second category contained 12 varieties with moderate drought tolerance; the third category contained 15 drought-tolerant varieties; the fourth category contained 12 sensitive varieties; and the fifth category contained 6 highly sensitive varieties.

3.1.7. The Classification of Complete Evaluation Indicators of Pakchoi Varieties under Drought Conditions

The values of the SD, LN, RV, AFW, BFW, BDW, RA, RWC, REL, SS, and SP membership functions and the above three drought tolerance evaluation indicators (D-value) all increased with increasing drought tolerance grade. Note the grading statistics of drought tolerance evaluation indicators of 50 varieties (Table 6).

Table 5. D-value of drought tolerance evaluation of pakchoi varieties.

Varieties	Membership Function Value				D-Value	Sorting
	μ1	μ2	μ3	μ4		
1	0.152	0.183	0.848	0.696	0.294	20
2	0.121	0.000	0.609	0.982	0.238	35
3	0.393	0.542	0.870	0.732	0.512	6
4	0.189	0.282	0.652	0.643	0.306	18
5	0.070	0.282	0.163	0.929	0.202	38
6	0.035	0.107	0.250	0.625	0.130	49
7	0.029	0.405	0.587	0.268	0.191	41
8	0.244	0.099	0.424	0.768	0.288	23
9	0.637	0.992	0.772	0.518	0.710	1
10	0.598	0.939	0.739	0.643	0.684	3
11	0.389	0.573	0.620	0.536	0.466	7
12	0.475	0.885	0.663	1.000	0.625	4
13	0.357	0.397	0.391	0.732	0.404	10
14	0.232	0.397	0.565	0.857	0.362	13
15	0.445	0.710	0.815	0.804	0.574	5
16	0.293	0.305	0.478	0.482	0.336	17
17	0.316	0.435	0.435	0.607	0.380	12
18	0.322	0.435	0.783	0.768	0.441	9
19	0.283	0.260	0.685	0.518	0.350	16
20	0.334	0.458	0.500	0.554	0.398	11
21	0.059	0.779	0.196	0.661	0.268	30
22	0.209	0.198	0.522	0.536	0.276	27
23	0.018	0.252	0.065	0.696	0.132	48
24	0.143	0.260	0.707	0.554	0.273	28
25	0.154	0.458	0.761	0.268	0.296	19
26	0.195	0.267	0.467	0.250	0.247	34
27	0.244	0.374	0.587	0.679	0.351	15
28	0.322	0.160	0.587	0.661	0.355	14
29	0.414	0.435	0.685	0.571	0.466	8
30	0.006	0.466	0.337	0.536	0.183	42
31	0.000	0.473	0.261	0.393	0.158	45
32	0.184	0.328	0.478	0.679	0.294	21
33	0.588	1.000	1.000	0.464	0.705	2
34	0.176	0.023	0.500	0.518	0.219	37
35	0.043	0.473	0.359	0.107	0.169	44
36	0.166	0.275	0.641	0.196	0.248	33
37	0.156	0.389	0.196	0.786	0.264	32
38	0.211	0.260	0.522	0.500	0.285	25
39	0.287	0.160	0.304	0.304	0.267	31
40	0.180	0.160	0.207	0.393	0.200	39
41	0.139	0.237	0.130	0.304	0.172	43
42	0.273	0.038	0.739	0.214	0.280	26
43	0.158	0.038	0.000	0.429	0.141	47
44	0.285	0.137	0.446	0.393	0.287	24
45	0.012	0.427	0.109	0.071	0.108	50
46	0.275	0.015	0.293	0.929	0.289	22
47	0.127	0.076	0.413	0.179	0.157	46
48	0.275	0.099	0.163	0.732	0.270	29
49	0.166	0.282	0.109	0.286	0.192	40
50	0.219	0.176	0.467	0.000	0.221	36

μ1, μ2, μ3, and μ4 represent the subordinate function values of the five factors. D-value: drought tolerance comprehensive evaluation value.

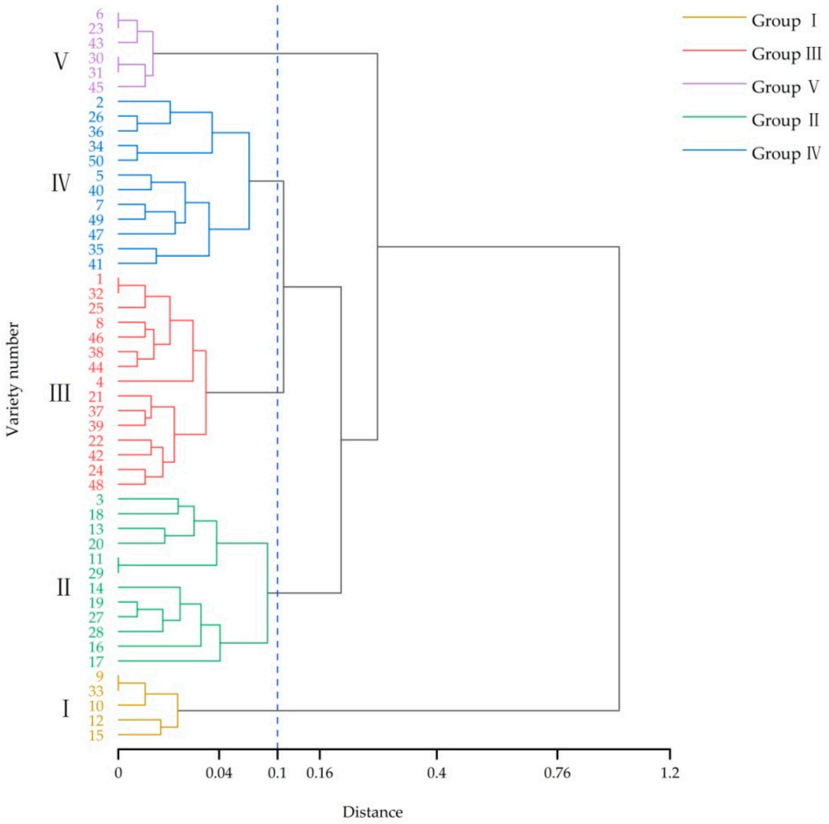

Figure 2. Cluster diagram of drought tolerance in pakchoi based on the D-value. Group I, Group II, Group III, Group IV, and Group V represent different drought tolerance levels.

Table 6. Classification of comprehensive evaluation of indicators for pakchoi varieties under water deficit conditions.

Indicators	**Membership Function**				
	I	**II**	**III**	**IV**	**V**
SD	0.920	0.855	0.727	0.646	0.493
LN	0.971	0.865	0.851	0.746	0.706
RL	0.823	0.681	0.638	0.618	0.707
ARD	0.778	0.788	0.747	0.689	0.726
RSA	0.856	0.746	0.646	0.663	0.603
RV	0.711	0.636	0.551	0.537	0.474
AFW	0.835	0.760	0.660	0.623	0.587
BFW	0.917	0.811	0.690	0.612	0.454
ADW	0.812	0.754	0.660	0.614	0.684
BDW	0.908	0.790	0.659	0.582	0.433
RA	0.880	0.836	0.734	0.571	0.390
RWC	0.919	0.867	0.813	0.776	0.573
SPAD	1.040	0.943	0.878	0.919	0.835
REL	0.901	0.830	0.714	0.626	0.432
SS	3.033	1.172	0.727	0.541	0.470
SP	2.584	1.694	1.206	0.945	0.623
D-value	0.660	0.402	0.282	0.207	0.138

I, II, III, IV, and V represent different drought tolerance levels. SD: stem diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein.

3.1.8. PCA of Individual Indicators

According to the PCA results of 12 individual indicators of pakchoi, the cumulative contribution rate of the total variance of the four principal components is greater than 80%. Pakchoi SD, AFW, BFW, BDW, and other indicators are the main factors of PCA1; LN, RL, ADW, and SPAD are the main factors of PCA2; RSA and RV are the main factors of PCA3; and ARD are the main factors of PCA4 (Table 7). To avoid collinearity of indicators, select indicators with higher contribution rates from each principal component, including, SS, ARD, BFW, ADW, RV, LN, and construct a regression model.

Table 7. Component matrix after principal component rotation of 16 indicators.

Indexes	PCA1	PCA2	PCA3	PCA4
SD	0.948			
LN	0.452	0.598		
RL		0.700		
ARD				0.921
RSA			0.873	
RV			0.883	
AFW	0.611	0.525		
BFW	0.916			
ADW		0.857		
BDW	0.921			
RA	0.860			
RWC	0.894			
SPAD		0.589		
REL	0.878			
SS	0.584		0.494	
SP	0.770		0.446	

SD: stem diameter; LN: leaf number; RL: root length; ARD: average root diameter; RSA: root surface area; RV: root volume; AFW: aboveground fresh weight; BFW: belowground fresh weight; ADW: aboveground dry weight; BDW: belowground dry weight; RA: root activity; RWC: leaf relative water content; SPAD: soil and plant analyzer development; REL: relative electrolytic leakage; SS: soluble sugar; SP: soluble protein.

3.1.9. Screening of Drought Tolerance Indicators by Stepwise Regression Analysis

Stepwise regression analysis on the DC and D-values of the selected SS, ARD, BFW, ADW, RV, LN, and the coefficient of determination (R^2) was 0.976; the F test value was highly significant, indicating that the regression equation was optimal, the predictions were accurate, and the model was a good fit for the given data (Table 8). In the evaluation of pakchoi drought tolerance, the drought tolerance of pakchoi varieties can be identified by measuring the indicators closely related to the D-value, such as soluble sugar, average root diameter, belowground fresh weight, root volume, and leaf number, thus simplifying the identification work.

Table 8. Drought tolerance model prediction in pakchoi varieties by stepwise regression analysis.

Multiple Regressive Equations	Coefficient of Determination R^2	F-Value	*p*-Value
$y = -0.427 + SS \times 0.120 + ARD \times 0.298 + BFW \times 0.203 + RV \times 0.147 + LN \times 0.214$	0.976	393.394	<0.01

SS: soluble sugar; ARD: average root diameter; BFW: belowground fresh weight; RV: root volume; LN: leaf number.

3.2. Verification of the Drought Regression Equation and Assessment System for Pakchoi Water-Deficit Tolerance

3.2.1. Regression Equation Verification

One pakchoi variety was randomly selected from each of the five drought tolerance classes and used to validate the regression equation: I-Jinhui (I-JH), II-Dongxin (II-DX), III-Suoyuannaibaicai (III-SY), IV-Xinganhua (IV-XGH), and V-Ziyuyoucai (V-ZY). After measuring BFW, ARD, RV, RA, SS, and SP, the y value of five was 0.128–0.450, consistent with drought tolerance of different varieties. The validation results were consistent with the ranking order using D-values, indicating the drought tolerance is accuracy (Table 9).

Table 9. Verification of regression equation for non-heading Chinese cabbage varieties.

Varieties	BFW	ARD	RV	RA	SS	SP	y Value	Storing	D-Value
I-JH	0.817	0.618	0.726	0.994	1.869	1.808	0.450	1	0.710
II-DX	0.815	0.733	0.599	0.818	1.653	1.642	0.407	2	0.404
III-SY	0.681	0.786	0.644	0.721	1.420	1.214	0.331	3	0.306
IV-XGH	0.602	0.635	0.486	0.560	0.881	0.622	0.158	4	0.157
V-ZY	0.545	0.695	0.617	0.498	0.390	0.511	0.128	5	0.108

BFW: belowground fresh weight; ARD: average root diameter; RV: root volume; RA: root activity; SS: soluble sugar; SP: soluble protein.

3.2.2. The Effect of Water Deficit on Pakchoi Stomatal Morphology

Stomatal densities increased both in the upper and the lower epidermis of five verities, under water deficit (Figure 3). In addition, the stomatal density of varieties that are sensitive to drought was higher than that of varieties that are drought-tolerant. Upper epidermis stomatal density of I-JH and V-ZY increased 88.92% and 37.5%, respectively; lower epidermis stomatal density of I-JH and V-ZY increased 68.0% and 4.3%, respectively. Stomatal length of upper and lower epidermis stomatal in I-JH, II-DX, III-SY, and IV-XGH significantly increased and V-ZY significantly decreased. Stomatal width of I-JH and V-ZY decreased 46.2% and 19.0% in upper epidermis, and 30.1% and 12.2% in lower epidermis. Stomatal aperture of upper epidermis and lower epidermis in I-JH, II-DX, III-SY, and IV-XGH significantly decreased (Table 10).

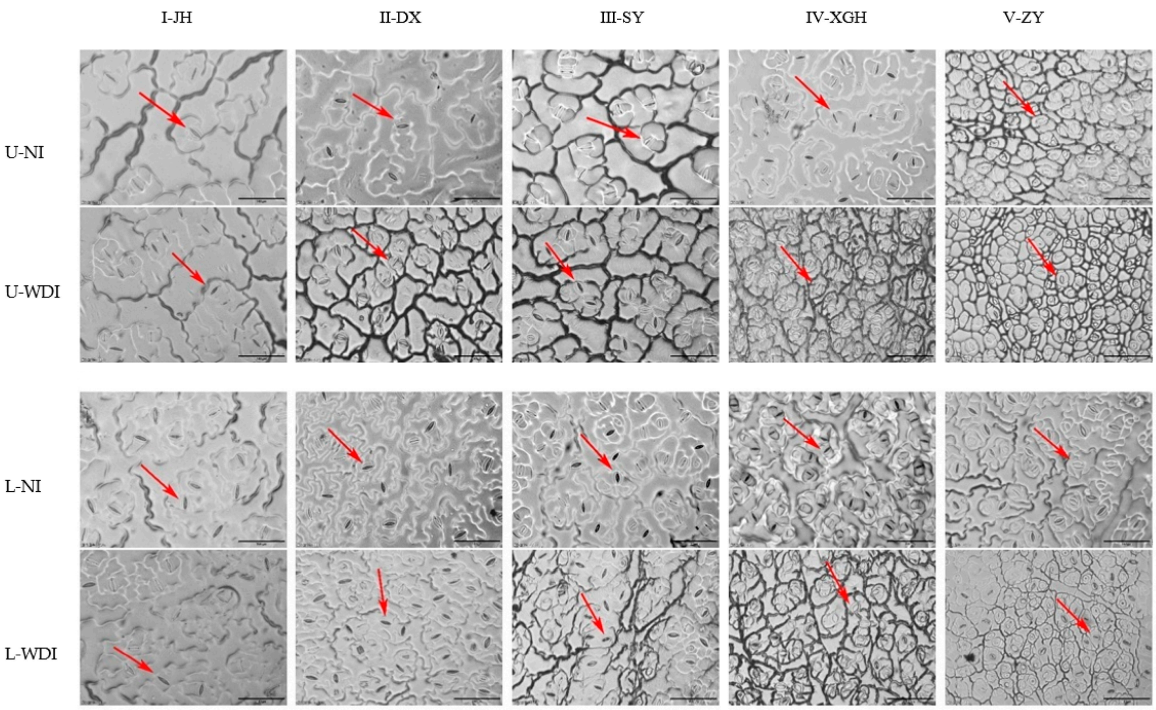

Figure 3. Differences in stomatal indicators in the upper and lower epidermis of five cultivars under water deficit conditions. S-NI: normal irrigation treatment of upper epidermis; S-WDI: irrigation treatment of water deficit in upper epidermis; X-NI: normal irrigation treatment of lower epidermis; X-WDI: irrigation treatment of water deficit in lower epidermis. The direction indicated by a red arrow is stomatal. The scale bar represent 100 μm.

Table 10. Effect of water deficit treatment on stomatal morphology of pakchoi.

Varieties	Treatments	Stomatal Density (No·mm^{-2})		Stomatal Length (μm)		Stomatal Width (μm)		Stomatal Aperture (μm)	
		Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis	Upper Epidermis	Lower Epidermis
I-JH	NI	12.00 ± 0.57 g	16.67 ± 0.33 h	35.93 ± 0.51 a	26.34 ± 0.15 d	25.52 ± 0.24 a	22.15 ± 0.11 b	7.77 ± 0.11 bc	7.49 ± 0.19 c
	WDI	22.67 ± 0.33 e	28.01 ± 0.57 f	24.45 ± 0.32 d	20.05 ± 0.31 f	17.46 ± 0.25 d	17.03 ± 0.10 f	5.42 ± 0.21 e	4.76 ± 0.12 f
II-DX	NI	10.67 ± 0.32 g	25.00 ± 0.55 g	34.17 ± 0.21 a	33.55 ± 0.64 a	22.41 ± 0.81 b	22.49 ± 0.12 b	7.82 ± 0.13 b	8.41 ± 0.21 b
	WDI	19.66 ± 0.31 f	36.02 ± 0.98 de	24.63 ± 0.51 d	28.49 ± 0.23 c	18.60 ± 0.24 c	17.09 ± 0.21 f	5.88 ± 0.22 e	6.03 ± 0.22 d
III-SY	NI	14.00 ± 0.58 g	28.04 ± 0.51 f	24.94 ± 0.25 d	24.66 ± 0.12 e	18.34 ± 0.31 cd	18.35 ± 0.20 e	7.96 ± 0.14 ab	11.29 ± 0.37 a
	WDI	22.00 ± 0.51 e	38.67 ± 0.33 d	19.35 ± 0.54 f	21.91 ± 0.11 f	16.48 ± 0.54 e	14.72 ± 0.14 g	7.20 ± 0.10 d	8.58 ± 0.25 b
IV-XGH	NI	24.33 ± 0.33 d	32.00 ± 0.58 e	24.25 ± 0.47 e	30.33 ± 0.22 b	18.97 ± 0.22 c	20.59 ± 0.22 d	7.98 ± 0.21 ab	7.26 ± 0.21 c
	WDI	36.00 ± 0.54 c	41.00 ± 0.51 c	20.26 ± 0.26 f	27.31 ± 0.12 c	18.37 ± 0.11 cd	18.24 ± 0.18 e	7.31 ± 0.11 c	5.52 ± 0.22 e
V-ZY	NI	40.00 ± 0.44 b	47.00 ± 0.57 b	25.35 ± 0.48 c	25.31 ± 0.13 d	17.15 ± 0.14 d	23.63 ± 0.11 a	8.55 ± 0.13 a	7.83 ± 0.11 bc
	WDI	45.00 ± 0.53 a	49.00 ± 0.55 a	27.76 ± 0.43 b	24.18 ± 0.27 e	16.96 ± 0.15 e	21.06 ± 0.12 c	8.32 ± 0.29 a	7.24 ± 0.33 c

NI: normal irrigation; WDI: water deficit irrigation. Different lowercase letters indicates significant differences among various varieties and treatments at $p < 0.05$ level.

3.2.3. Effect of Water Deficit on the Chlorophyll Content of Pakchoi

Five drought-tolerant varieties of pakchoi showed the same pattern of change in terms of the leaf chlorophyll content after being subjected to the water deficit treatment (Table 11). The levels of chlorophyll a, chlorophyll b, and total chlorophyll all decreased, but the decrease in chlorophyll b was more significant than the decrease in chlorophyll a. This led to an upward trend in the ratio of chlorophyll a/b. Chlorophyll a decreased by 3.8%, 4.6%, 7.7%, 17.9%, and 19.0% with the reduction in drought tolerance level; chlorophyll b decreased by 5.1%, 6.5%, 12.6%, 28.6%, and 50.9%; and the total chlorophyll content decreased by 4.2%, 5.1%, 9.0%, 21.5%, and 30.1%. Furthermore, the following table shows that the chlorophyll a content of the five varieties decreased, whereas the chlorophyll a/b ratio showed the significant increase of 1.4%, 1.7%, 5.5%, 13.9%, and 25.7%.

Table 11. Effect of water deficit treatment on the chlorophyll content of pakchoi.

Varieties	Treatments	Chlorophyll a (mg. g^{-1})	Chlorophyll b (mg. g^{-1})	Chlorophyll Content (mg. g^{-1})	Chlorophyll a/b
I-JH	NI	1.14 ± 0.22 ab	0.44 ± 0.08 ab	1.58 ± 0.31 ab	2.62 ± 0.07 b
	WDI	1.10 ± 0.14 abc	0.41 ± 0.06 ab	1.51 ± 0.20 ab	2.65 ± 0.02 a
II-DX	NI	1.03 ± 0.13 abc	0.37 ± 0.05 ab	1.40 ± 0.18 ab	2.80 ± 0.14 ab
	WDI	0.99 ± 0.04 abc	0.35 ± 0.01 ab	1.34 ± 0.05 ab	2.85 ± 0.02 ab
III-SY	NI	0.82 ± 0.15 bc	0.30 ± 0.05 b	1.12 ± 0.20 b	2.69 ± 0.06 ab
	WDI	0.75 ± 0.12 c	0.27 ± 0.03 b	1.02 ± 0.15 b	2.83 ± 0.15 ab
IV-XGH	NI	1.05 ± 0.04 abc	0.37 ± 0.02 ab	1.42 ± 0.06 ab	2.85 ± 0.06 ab
	WDI	0.85 ± 0.17 bc	0.26 ± 0.06 b	1.11 ± 0.23 b	3.25 ± 0.13 a
V-ZY	NI	1.25 ± 0.42 b	0.72 ± 0.63 a	1.97 ± 1.05 a	2.30 ± 0.97 b
	WDI	1.02 ± 0.08 abc	0.35 ± 0.02 ab	1.37 ± 0.10 ab	2.89 ± 0.04 ab

NI: normal irrigation; WDI: water deficit irrigation. Different lowercase letters indicates significant differences among various varieties and treatments at $p < 0.05$ level.

3.2.4. Differences in Photosynthetic Characteristics of Pakchoi Varieties under Water Deficit Stress

The photosynthetic characteristics of the selected pakchoi varieties showed significant differences when grown under normal irrigation and water deficit conditions (Table 12). I-JH decreased 9.2% in the net photosynthetic rate (Pn), while ZY decreased 18.3%. Both of these results were in comparison to the normal irrigation treatment. I-JH and V-ZY decreased in stomatal conductance (Gs) by 32.0% and 15.5%, respectively, when compared with the normal irrigation treatment. In addition, the transpiration rate (Tr), which was consistent with the change in stomatal conductance, was significantly lower in the drought-tolerant varieties compared to the sensitive varieties, and the I-JH decreased 44.5% under the condition of water deficit, while the V-ZY decreased 18.3%. I-JH showed a decrease in intercellular CO_2 concentration (Ci) that was 35.8% higher than that of any other group.

Table 12. Difference of photosynthetic characteristics of different pakchoi.

Varieties	Treatments	Pn	Gs	Ci	Tr
I-JH	NI	8.24 ± 0.01 b	23.33 ± 0.33 a	135.71 ± 2.82 a	4.44 ± 0.10 a
	WDI	7.69 ± 0.11 c	21.33 ± 0.33 b	118.23 ± 5.32 b	3.63 ± 0.08 b
II-DX	NI	8.74 ± 0.06 a	14.67 ± 0.33 c	66.21 ± 1.14 d	3.61 ± 0.26 b
	WDI	7.04 ± 0.02 d	12.00 ± 0.12 d	55.96 ± 2.12 e	2.84 ± 0.48 c
III-SY	NI	6.06 ± 0.08 e	14.33 ± 0.32 c	73.68 ± 1.86 c	2.31 ± 0.06 cd
	WDI	4.84 ± 0.05 g	10.67 ± 0.67 c	44.65 ± 2.02 f	1.57 ± 0.06 e
IV-XGH	NI	7.09 ± 0.10 d	14.67 ± 0.33 c	48.12 ± 2.42 f	3.76 ± 0.27 ab
	WDI	5.33 ± 0.18 f	10.00 ± 0.58 e	27.55 ± 0.25 g	2.26 ± 0.14 cd
V-ZY	NI	7.62 ± 0.22 c	12.33 ± 0.33 d	59.64 ± 2.05 de	3.72 ± 0.15 ab
	WDI	5.41 ± 0.10 f	8.33 ± 0.33 f	27.26 ± 0.43 g	2.00 ± 0.39 e

NI: normal irrigation; WDI: water deficit irrigation; Pn: net photosynthetic rate; Gs: stomatal conductance; Ci: intercellular carbon dioxide concentration; Tr: transpiration rate. Different lowercase letters indicates significant differences among various varieties and treatments at $p < 0.05$ level.

3.2.5. Effect of Water Deficit on Oxidative Damage and Enzymatic Activity in Pakchoi Varieties

Under water deficit treatment, SOD, POD, CAT activity, MDA, and the rate of O_2^- production rate in five different pakchoi varieties increased. Compared to normal irrigation, SOD, POD, and CAT activities in I-JH increased by 63.2%, 71.3%, and 79.1% under the water deficit treatment compared to the normal irrigation treatment, while in V-ZY increased by 12.8%, 17.3%, and 17.2%, respectively (Figure 4A–C). The MDA content increased by 21.3%, 40.5%, 58.6%, 76.7%, and 84.3% in five varieties (Figure 4D). O_2^- generation rate increased by 13.6%, 41.2%, 56.5%, 64.0%, and 76.5%, respectively, in five varieties (Figure 4E). Both the MDA content and the O_2^- generation rate increased in response to a water deficit, although the increase in drought-tolerant types was much smaller than in sensitive types.

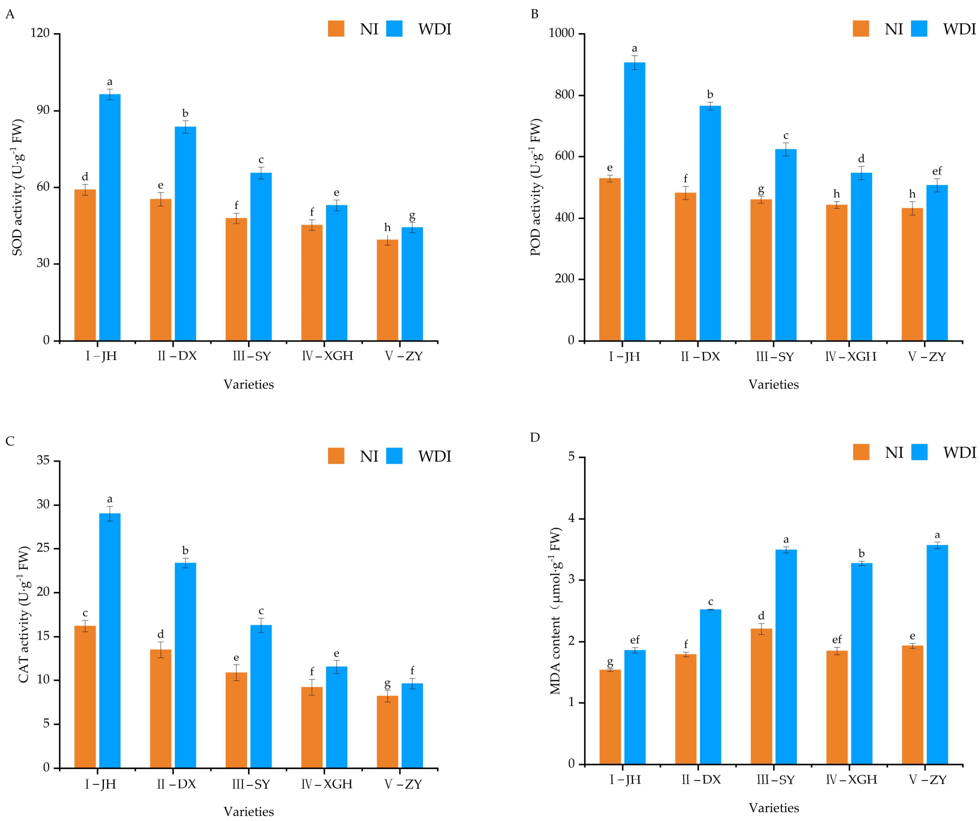

Figure 4. *Cont.*

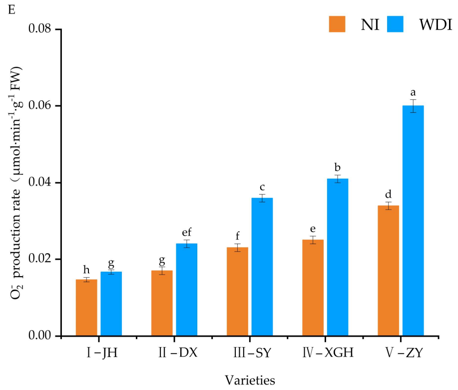

Figure 4. SOD of pakchoi with different drought tolerance levels under normal irrigation and water deficit treatments (**A**). POD of pakchoi with different drought-tolerance levels under normal irrigation and water deficit treatments (**B**). CAT of pakchoi with different drought-tolerance levels under normal irrigation and water deficit treatments (**C**). MDA of pakchoi with different drought-tolerance levels under normal irrigation and water deficit treatments (**D**). Superoxide anion production rate of pakchoi with different drought-tolerance under normal irrigation and water deficit treatments (**E**). NI: normal irrigation; WDI: water deficit irrigation. Vertical bars represent standard errors. Different lowercase letters indicates significant differences among various varieties and treatments at $p < 0.05$ level.

4. Discussion

Drought stress exerts a profound impact on the morphology of plants, diminishing their performance across multiple dimensions, including physiological characteristics, morphology, and yield. The presence of water stress adversely affects various aspects of plant growth and development, resulting in reduced overall plant performance [45]. Numerous researchers have undertaken drought-tolerance assessments by focusing on individual or limited aspects such as morphology, photosynthesis, and physiology. This approach stems from the understanding that yield is influenced by the intricate processes of growth and development, and plant growth serves as a reliable measure of drought adaptability. As a result, to comprehensively evaluate drought tolerance, it is imperative to incorporate indicators related to both yield and morphological characteristics into the assessment framework [46,47]. The evaluation of drought-tolerance in cotton materials has predominantly been conducted using morphological and yield indicators in most studies [48,49]. Furthermore, these indicators, which focus on morphological and yield aspects, provide only a partial understanding of crop drought-tolerance, disregarding the crucial roles of photosynthesis, physiology, and biochemistry. Osmotic adjustment, including parameters like MDA and proline content, is recognized as a significant component of drought-tolerance and should be considered in comprehensive assessments [50–52]. Hence, when conducting evaluations and verifications of drought-tolerance, it becomes essential to integrate multiple indicators encompassing morphology, physiology, biochemical changes, and representative indices. This comprehensive approach ensures a more holistic understanding of drought-tolerance screening in plants.

Our study involved the cultivation of 50 pakchoi varieties in the field, where we assessed 16 indicator variables closely associated with drought tolerance, encompassing morphological, physiological, and biochemical indicators. From the seedling stage to harvesting, the variance analysis of the drought tolerance coefficient revealed notable differences among the pakchoi varieties grown under different water treatments, particularly under drought stress (Table 3). These findings indicate that the selected pakchoi varieties exhibit sufficient genetic diversity to serve as representative samples for the region. Notably, drought stress exerted a significant influence on all the studied indicators ($p < 0.05$), as evidenced by decreasing drought tolerance coefficients (<1) and increasing drought tolerance coefficients (>1) (Table 2). Additionally, the coefficient of variation (CV) values for most indicators were higher under water deficit conditions compared to normal treatment. This observation suggests that the pakchoi cultivars chosen for this study demonstrate ample diversity, manifesting noticeable effects of drought stress and yielding representative results.

To mitigate inherent variations among cultivars, the evaluation of different pakchoi varieties under drought stress was conducted using relative values. However, drought tolerance is a complex indicator influenced by multiple factors, and assessing it solely based on individual or single-type indicators can lead to errors. Currently, there is no single indicator that can provide a complete and accurate evaluation of drought-tolerance. Therefore, it is crucial to identify more comprehensive indicators and employ suitable evaluation methods for plant assessments. Furthermore, many indicators exhibit correlations with each other, resulting in overlapping responses as indicators of crop stress tolerance (Figure 1). Hence, utilizing multivariate analysis methods becomes essential in evaluating and screening comprehensive indicators associated with drought-tolerance. PCA can effectively reduce multiple variables to underlying factors, thus addressing missing data issues and facilitating efficient grouping of drought-tolerant varieties [53,54]. By PCA, we were able to transform 16 individual indicators of pakchoi varieties under drought stress into four distinct independent comprehensive indices. Notably, the cumulative contribution rate of the first four independent comprehensive indicators exceeded 80%, suggesting that a significant portion of the data pertaining to the 16 indicators was effectively encompassed by these comprehensive indicators (Table 4). The drought tolerance membership function value serves as a multivariate indicator that combines the drought tolerance coefficients of various indicators, providing a comprehensive representation of the overall performance of plants under drought stress. This indicator effectively captures and reflects the collective response of plants to drought conditions. Utilizing the principal component scores, the membership function values were computed, and subsequently, the D-value was determined by incorporating the respective weights. This facilitated the ranking of pakchoi varieties based on their drought tolerance, with higher D-values indicating superior drought tolerance. In previous studies, the waterlogging tolerance of 12 onion cultivars and the salt tolerance of wheat and maize varieties were classified into two groups based on their respective characteristics. This classification was determined by evaluating the Euclidean distances between the cultivars or varieties, providing insights into their relative tolerance levels [55–57]. Similarly, the drought tolerance of cotton cultivars was classified based on the membership function and D-value. This classification method enabled the categorization of cotton cultivars into distinct groups according to their respective levels of drought tolerance [58]. In this study, pakchoi varieties differed significantly in various morphological, physiological, and biochemical characteristics, indicating that there was sufficient genetic diversity among the selected pakchoi varieties. We used PCA to convert the 16 drought-tolerance indicators of pakchoi varieties into 4 independent composite indicators. D-values of different pakchoi varieties were obtained by the membership function. Furthermore, the use of PCA in conjunction with the membership function and cluster analysis makes assessing stress-tolerance in crops more reliable and practical. Hierarchical clustering analysis classified 50 pakchoi varieties into five categories based on the D-value (Figure 2): highly drought-tolerant, moderately drought-tolerant, drought-tolerant, highly drought-sensitive, and drought-sensitive. Step wise regression analysis revealed that among the 16 indicators studied, 6 drought-tolerance indicators (BFW, ARD, RV, RA, SS, and SP) exhibited significant effects on the drought tolerance of pakchoi. These identified indicators can serve as primary indicators for evaluating and screening drought-tolerant pakchoi varieties in future studies. Additionally, we developed a robust regression model for assessing the drought tolerance of pakchoi, represented by the equation: $y = -0.427 + SS \times 0.120 + ARD \times 0.298 + BFW \times 0.203 + RV \times 0.147 + LN \times 0.214$ ($R^2 = 0.976$, p-value < 0.01) (Table 9). This model provides a reliable means for evaluating the drought-tolerance of pakchoi, incorporating multiple indicators as predictors. For the validation of the regression model, varieties were randomly selected from each group based on their D-values. The high contribution of each of the six indicators, as well as the high D-value, indicates that these varieties possess a high level of drought tolerance and ranking is as follows: I-JH, II-DX, III-SY, IV-XGH, and V-ZY (Table 10). The results of the grey relational analysis provided additional confirmation of the accuracy of the regression analysis, further enhancing the scientific reliability and credibility of the identified indicators in assessing drought tolerance.

Comprehensive evaluation methods were highly effective to screen drought-tolerant varieties. Many morphological and physiological are included in the process of plants responding to drought-tolerance. It is also very important to further verify the classification of plants by using comprehensive evaluation methods by different indicators. Among many drought-tolerant indicators, stomatal behavior, patterning, and morphology are important factors that contribute to water use efficiency. Stomata exhibit a diverse range of shapes, sizes, and numbers across different plant species. Variation in size and density of stomata may arise due to genetic factors and/or growth under different environmental conditions. With the rise of drought stress, the stomata closed down and their density in the upper and lower epidermis also decreased to prevent evapotranspiration [59,60]. The drought-tolerant wheat cultivar 'Changhan 58' showed lower stomatal density under well-watered and water-stressed conditions, and the drought-sensitive cultivar 'Xinong 9871' had a non-significantly larger decrease under water-stressed conditions [61]. In this current study, we explored potential modifications in stomatal indicators among five groups of drought-tolerant varieties in order to enhance their drought tolerance. These modifications include reducing stomatal density, length, width, and stomatal aperture in both the upper and lower epidermis of the leaf under water deficit conditions (Figure 3, Table 11). We observed that the reduction in stomatal density and opening of stomatal aperture was more pronounced in group I-JH compared to group V-ZY, indicating that I-JH exhibits higher levels of drought-tolerance. Drought stress experienced during the vegetative stage led to a significant reduction in the levels of chlorophyll a, chlorophyll b, and total chlorophyll content. However, the limited water supply throughout the vegetative phase had only a mild impact on these chlorophyll contents. Importantly, the ratio of chlorophyll a to chlorophyll b remained unaffected, suggesting that chlorophyll b is not more susceptible to drought stress compared to chlorophyll a (Table 12). These findings are consistent with a study by Nyachiro et al. [61] which observed a notable decrease in chlorophyll a and b levels due to water scarcity in six cultivars of Triticum aestivum [62]. Similar changes in chlorophyll levels, either decreased or unchanged, have been reported in other species under drought conditions, although the severity and duration of drought may influence the outcome [63]. The decrease in total chlorophyll content during drought stress indicates a reduced capacity for capturing light energy. To prevent the generation of reactive oxygen species, which are primarily produced due to excessive energy absorption in the photosynthetic apparatus, the degradation of absorbing pigments may be employed as a protective mechanism [64].

When exposed to drought stress, all five varieties exhibited a reduction in transpiration rate and stomatal conductance (Table 12). This decrease can be attributed to the initial response of plants to drought, wherein stomata close to limit the exchange of gases between the leaf interior and the atmosphere. The I-JH showed a substantial reduction in stomatal conductance of 32.0% compared to the other drought sensitive varieties. This reveled that I-JH, when faced drought, instantly closed stomata to overcome drought stress and increase the survival efficiency. Moreover, in response to drought, there was an observed increase in the Ci concentration. Numerous experiments have consistently demonstrated a correlation between a decrease in Gs and a concurrent decrease in Pn [65,66]. According to the findings of Chaves and Oliveira, the impact of Gs on Pn is primarily significant under severe drought-stress conditions. The reduction in Pn observed in plants experiencing drought stress can be attributed to both stomatal factors, such as stomatal closure, as well as non-stomatal factors, including impairments in metabolic processes [67]. Currently, there is a consensus among most researchers that under mild and moderate drought stress, the primary cause of decreased photosynthesis is stomatal closure, which leads to a deficit of CO_2 in the chloroplasts [68]. Likewise, the impact of drought stress on Tr exhibited a striking resemblance to its effect on Pn. The provision of ample water supply resulted in a significant increase in stomatal conductance, net photosynthesis, and transpiration [69]. The increase in Ci in drought-tolerant variety, I-JH indicates the predominance of non-stomatal limitations (mesophyll tolerance) to photosynthesis. Based on the observations, it can be inferred that during drought stress conditions, photosynthesis is primarily restricted by factors related to stomatal regulation [70].

ROS play a significant role in the metabolic pathways of plant drought tolerance. When plants experience drought stress, they often generate elevated levels of ROS, including increased production rates of O_2^- and higher H_2O_2 content. To counteract the detrimental effects of ROS, plants synthesize antioxidants that help scavenge these reactive molecules. At the onset of drought stress, H_2O_2 production intensifies, leading to the synthesis of CAT, which helps neutralize H_2O_2 and mitigate its negative impact on cells. SOD catalyzes the conversion of superoxide into molecular oxygen and H_2O_2, which is subsequently converted into water and oxygen within the cytosol and chloroplasts, safeguarding cells against the toxic effects of ROS [71]. The results of Alscher et al. revealed that SOD and POD counter act against the ROS by enhancing their activity under drought conditions. This helps alleviate the harmful effects of ROS and enhances the defense system by increasing antioxidant enzyme activity [72]. In our study, SOD, POD, and CAT activities in I-JH significantly increased more when exposed to drought as compared to others varieties in the remaining four groups. It is due to the tolerance of pakchoi varieties in each group to drought stress. Therefore, antioxidant enzymes are commonly utilized as physiological indicators to identify plant stress tolerance. In response to water deficit, both the content of MDA and the generation rate of O_2^- increased. However, the magnitude of increase observed in drought-tolerant types was considerably smaller compared to sensitive types. Comparative analysis of various drought-tolerant soybean varieties revealed that the drought-tolerant varieties exhibited higher antioxidant enzyme activity of SOD, CAT, and POD compared to the sensitive varieties under different durations and intensities of drought treatment [48,73].

Hence, when assessing and analyzing water deficit tolerance, it is essential to consider comprehensive set of indicators that includes, morphology, physiology, and biochemical parameters. This will increase the efficiency of identifying and screening of pakchoi reduced the phenotyping cost and time. However, the molecular mechanism of drought-tolerance in pakchoi and the breeding of targeted high drought- tolerant varieties need further research.

5. Conclusions

This study evaluated 16 drought-related indices, including morphology, physiology, and biochemical indicators of 50 pakchoi varieties grown in normal-irrigated and water-deficient conditions. PCA, membership function value analysis, multiple regression analysis, D-value, and cluster analysis were performed to effectively evaluate the drought-tolerance of the pakchoi varieties. A total of six indicators associated with pakchoi drought-tolerance, such as BFW, ARD, RV, RA, SS, and SP, were evaluated, and a digital model for pakchoi drought tolerance evaluation was established. Furthermore, five drought-tolerant pakchoi varieties were chosen from each group by cluster ranking based on the D-value: I-JH, II-DX, III-SY, IV-XGH, and V-ZY. In the meantime, to validate the tolerance level of each variety by stomatal conductance, chlorophyll, ROS, antioxidant enzymes will aid in the rapid evaluation of drought tolerance. The findings presented here will facilitate the rapid evaluation of drought tolerance and enable the screening of drought-tolerant pakchoi materials more effectively.

Author Contributions: Experiments and editing of the manuscript, J.L.; Writing of the manuscript, K.A.; Methods and experiments, W.W.; Data analysis, L.W.; Supervision of the experimental design, B.G.; Supervision of the experimental design, H.X.; Collection of the literature, L.C.; Editing of the manuscript, S.H. and X.W.; Experimental design, H.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Research and Develop Program of Hebei (21326903D), the Hebei Facility Vegetables Innovation Team of Modern Agro-industry Technology (HBCT2023100211, HBCT2023100215).

Data Availability Statement: All data generated in the study were included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gao, H.; Yue, X.; Li, Z.; Yuan, S.; Shi, W.; Yan, Z.; Xu, D.; Zhong, F.; Chang, H.; Jiang, A. The molecular regulation of folate accumulation in pakchoi by low intensity white light-emitting diode (led) illumination. *Sci. Hortic.* **2023**, *314*, 111937. [CrossRef]
2. Suruban, C.; Kader, M.A.; Solaiman, Z.M. Influence of various composted organic amendments and their rates of application on nitrogen mineralization and soil productivity using chinese cabbage (*Brassica rapa.* L. var. Chinensis) as an indicator crop. *Agriculture* **2022**, *12*, 201. [CrossRef]
3. Wang, J.; Li, C.; Li, L.; Reynolds, M.; Mao, X.; Jing, R. Exploitation of drought tolerance-related genes for crop improvement. *Int. J. Mol. Sci.* **2021**, *22*, 10265. [CrossRef] [PubMed]
4. Chen, L.; Shen, Y.; Yang, W.; Pan, Q.; Li, C.; Sun, Q.; Zeng, Q.; Li, B.; Zhang, L. Comparative metabolic study of two contrasting chinese cabbage genotypes under mild and severe drought stress. *Int. J. Mol. Sci.* **2022**, *23*, 5947. [CrossRef]
5. Park, B.J.; Liu, Z.; Kanno, A.; Kameya, T. Genetic improvement of Chinese cabbage for salt and drought tolerance by constitutive expression of a *B. napus* LEA gene. *Plant Sci.* **2005**, *169*, 553–558. [CrossRef]
6. Scardigno, A. New solutions to reduce water and energy consumption in crop production: A water–energy–food nexus perspective. *Curr. Opin. Environ. Sci. Health* **2020**, *13*, 11–15. [CrossRef]
7. Sun, F.; Chen, Q.; Chen, Q.; Jiang, M.; Qu, Y. Yield-based drought tolerance index evaluates the drought tolerance of cotton germplasm lines in the interaction of genotype-by-environment. *PeerJ* **2023**, *11*, e14367. [CrossRef] [PubMed]
8. Hou, D.; Wei, Y.; Liu, K.; Tan, J.; Bi, Q.; Liu, G.; Yu, X.; Bi, J.; Luo, L. The response of grain yield and quality of water-saving and drought-resistant rice to irrigation regimes. *Agriculture* **2023**, *13*, 302. [CrossRef]
9. Singh, H.K.; Solankey, S.S.; Ray, P.K.; Singh, P.; Shamim, M.; Singh, R.N.; Kumar, A. Impact of climate change on underexploited vegetable crops production and mitigation strategies. *Adv. Res. Veg. Prod. Under Chang. Clim.* **2023**, *2*, 149–166.
10. Zhang, G.; Zhang, Y.; Zhao, D.; Liu, S.; Wen, X.; Han, J.; Liao, Y. Quantifying the impacts of agricultural management practices on the water use efficiency for sustainable production in the loess plateau region: A meta-analysis. *Field Crops Res.* **2023**, *29*, 108787. [CrossRef]
11. Saed-Moucheshi, A.; Pessarakli, M.; Heidari, B. Comparing relationships among yield and its related traits in mycorrhizal and nonmycorrhizal inoculated wheat cultivars under different water regimes using multivariate statistics. *Int. J. Agron.* **2013**, *2013*, 682781. [CrossRef]
12. Guellim, A.; Hirel, B.; Chabrerie, O.; Catterou, M.; Tetu, T.; Dubois, F.; Ahmed, H.B.; Kichey, T. Screening for durum wheat (*Triticum durum* desf.) cultivar resistance to drought stress using an integrated physiological approach. *J. Crop Sci. Biotechnol.* **2020**, *23*, 355–365. [CrossRef]
13. Abdolshahi, R.; Nazari, M.; Safarian, A.; Sadathossini, T.; Salarpour, M.; Amiri, H. Integrated selection criteria for drought tolerance in wheat (*Triticum aestivum* L.) breeding programs using discriminant analysis. *Field Crops Res.* **2015**, *174*, 20–29. [CrossRef]
14. Nevo, E.; Chen, G. Drought and salt tolerances in wild relatives for wheat and barley improvement. *Plant Cell Environ.* **2010**, *33*, 670–685. [CrossRef]
15. Masood, M.; Ahsan, M.; Sadaqat, H.; Awan, F. Screening of maize (*Zea mays* L.) inbred lines under water deficit conditions. *Biol. Clin. Sci. Res. J.* **2020**, *2020*, 7. [CrossRef]
16. Ahmad, A.; Ilyas, M.Z.; Aslam, Z.; Roman, M.; Ali, A.; Naeem, S.; Nazar, M.; Rehman, S. Physiological screening of cotton (*Gossypium hirsutum* L.) genotypes against drought tolerance. *Pure Appl. Biol.* **2020**, *9*, 140–147. [CrossRef]
17. Ndikuryayo, C.; Ndayiragije, A.; Kilasi, N.L.; Kusolwa, P. Identification of drought tolerant rice (*Oryza sativa* L.) genotypes with asian and african backgrounds. *Plants* **2023**, *12*, 922. [CrossRef] [PubMed]
18. Choudhary, M.; Tripathi, M.; Gupta, N.; Tiwari, S.; Tripathi, N.; Parihar, P.; Pandya, R. Screening of pearl millet (*Pennisetum glaucum* [L.] R. Br.) germplasm lines against drought tolerance based on biochemical traits. *Curr. J. Appl. Sci. Technol.* **2021**, *40*, 1–12. [CrossRef]
19. Romero, A.P.; Alarcón, A.; Valbuena, R.I.; Galeano, C.H. Physiological assessment of water stress in potato using spectral information. *Front. Plant Sci.* **2017**, *8*, 1608. [CrossRef]
20. Cai, K.; Chen, X.; Han, Z.; Wu, X.; Zhang, S.; Li, Q.; Nazir, M.M.; Zhang, G.; Zeng, F. Screening of worldwide barley collection for drought tolerance: The assessment of various physiological measures as the selection criteria. *Front. Plant Sci.* **2020**, *11*, 1159. [CrossRef]
21. Lonbani, M.; Arzani, A. Morpho-physiological traits associated with terminal drought stress tolerance in triticale and wheat. *Agron. Res.* **2011**, *9*, 315–329.
22. Mwadzingeni, L.; Shimelis, H.; Tesfay, S.; Tsilo, T.J. Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Front. Plant Sci.* **2016**, *7*, 1276. [CrossRef]

23. Munns, R.; James, R.A.; Sirault, X.R.; Furbank, R.T.; Jones, H.G. New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. *J. Exp. Bot.* **2010**, *61*, 3499–3507. [CrossRef]
24. Xu, Y.P.; Pan, Y.D.; Liu, Q.D.; Yao, Y.H.; Jia, Y.C.; Ren, C.; Huo, K.C.; Chen, W.Q.; Zhao, F.; Bao, Q.J. Drought resistance identification and drought resistance indexes screening of barley resources at mature period. *Acta Agron. Sin.* **2020**, *46*, 448–461.
25. Brito, G.G.; Sofiatti, V.; Lima, M.M.; Carvalho, L.P.; Silva Filho, J.L. Physiological traits for drought phenotyping in cotton. *Acta Sci. Agron.* **2011**, *33*, 117–125. [CrossRef]
26. Levi, A.; Ovnat, L.; Paterson, A.H.; Saranga, Y. Photosynthesis of cotton near-isogenic lines introgressed with qtls for productivity and drought related traits. *Plant Sci.* **2009**, *177*, 88–96. [CrossRef]
27. Santos, I.C.; Almeida, A.A.; Anhert, D.; Conceição, A.S.; Pirovani, C.P.; Pires, J.L.; Valle, R.R.; Baligar, V.C. Molecular, physiological and biochemical responses of *Theobroma cacao* L. Genotypes to soil water deficit. *PLoS ONE* **2014**, *9*, e115746. [CrossRef] [PubMed]
28. Bo, W.; Fu, B.; Qin, G.; Xing, G.; Wang, Y. Evaluation of drought resistance in *iris germanica* L. Based on subordination function and principal component analysis. *Emir. J. Food Agric.* **2017**, *29*, 770–778. [CrossRef]
29. Feng, F.; Song, M.; Chen, Q.; Yao, Z.; Li, Y.; Liu, Y.; Wang, X.; Qu, Y. Analysis and comprehensive evaluation on principal component of relative indices of drought resistance at the seedling stage of cotton. *J. Xinjiang Agric. Univ.* **2011**, *34*, 211–217.
30. Chen, Y.; Shi, Y.; Luo, J.; Wang, D.; Hou, Y.; Li, Z.; Zhang, B. Screening of drought tolerant agronomic trait indices of colored cotton varieties (lines) in gansu province. *Acta Agron. Sin.* **2012**, *38*, 1680–1687. [CrossRef]
31. Patil, B.; Babu, A.; Pawar, K. Assessment of genotypic variability for growth, biophysical parameters, yield and yield-attributing characters under drought stress in cotton. In *Presented at Prospects in Bioscience: Addressing the Issues*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 103–110.
32. Shi, Y.; Chen, Y.; Luo, J.; Pei, H.; Zhang, Y.; Nan, H. Identification and evaluation of drought tolerant indices of colored cotton. *Crops* **2013**, *29*, 62–67.
33. Zou, J.; Hu, W.; Li, Y.X.; He, J.Q.; Zhu, H.H.; Zhou, Z.G. Screening of drought resistance indices and evaluation of drought resistance in cotton (*Gossypium hirsutum* L.). *J. Integr. Agric.* **2020**, *19*, 495–508. [CrossRef]
34. Donde, R.; Kumar, J.; Gouda, G.; Gupta, M.K.; Mukherjee, M.; Baksh, S.Y.; Mahadani, P.; Sahoo, K.K.; Behera, L.; Dash, S.K. Assessment of genetic diversity of drought tolerant and susceptible rice genotypes using microsatellite markers. *Rice Sci.* **2019**, *26*, 239–247. [CrossRef]
35. Quevedo, Y.M.; Moreno, L.P.; Barragan, E. Predictive models of drought tolerance indices based on physiological, morphological and biochemical markers for the selection of cotton (*Gossypium hirsutum* L.) varieties. *J. Integr. Agric.* **2022**, *21*, 1310–1320. [CrossRef]
36. Kaur, G.; Asthir, B. Molecular responses to drought stress in plants. *Biol. Plant.* **2017**, *61*, 201–209. [CrossRef]
37. Yang, X.; Lu, M.; Wang, Y.; Wang, Y.; Liu, Z.; Chen, S. Response mechanism of plants to drought stress. *Horticulturae* **2021**, *7*, 50. [CrossRef]
38. Parida, A.K.; Dagaonkar, V.S.; Phalak, M.S.; Umalkar, G.; Aurangabadkar, L.P. Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. *Plant Biotechnol. Rep.* **2007**, *1*, 37–48. [CrossRef]
39. Niu, J.; Zhang, S.; Liu, S.; Ma, H.; Chen, J.; Shen, Q.; Ge, C.; Zhang, X.; Pang, C.; Zhao, X. The compensation effects of physiology and yield in cotton after drought stress. *J. Plant Physiol.* **2018**, *224*, 30–48. [CrossRef]
40. Meher Shivakrishna, P.; Ashokreddy, K.; Rao, D.M. Effect of peg-6000 imposed drought stress on rna content, relative water content (rwc), and chlorophyll content in peanut leaves and roots. *Saudi J. Biol. Sci.* **2018**, *25*, 285. [CrossRef]
41. Bian, Z.; Zhang, X.; Wang, Y.; Lu, C. Improving drought tolerance by altering the photosynthetic rate and stomatal aperture via green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions. *Environ. Exp. Bot.* **2019**, *167*, 103844. [CrossRef]
42. Giannopolitis, C.N.; Ries, S.K. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* **1977**, *59*, 309–314. [CrossRef]
43. Dhindsa, R.S.; Plumb-Dhindsa, P.; Thorpe, T.A. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [CrossRef]
44. Elstner, E.F.; Heupel, A. Inhibition of nitrite formation from hydroxylammoniumchloride: A simple assay for superoxide dismutase. *Anal. Biochem.* **1976**, *70*, 616–620. [CrossRef]
45. Claeys, H.; Inzé, D. The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiol.* **2013**, *162*, 1768–1779. [CrossRef] [PubMed]
46. Kapanigowda, M.H.; Payne, W.A.; Rooney, W.L.; Mullet, J.E. Transpiration ratio in sorghum [*Sorghum bicolor* (L.) Moench] for increased water-use efficiency and drought tolerance. *J. Arid Land Stud.* **2012**, *22*, 175–178.
47. Dolferus, R. To grow or not to grow: A stressful decision for plants. *Plant Sci.* **2014**, *229*, 247–261. [CrossRef] [PubMed]
48. Sun, F.; Chen, Q.; Chen, Q.; Jiang, M.; Gao, W.; Qu, Y. Screening of key drought tolerance indices for cotton at the flowering and boll setting stage using the dimension reduction method. *Front. Plant Sci.* **2021**, *12*, 619926. [CrossRef]
49. Li, Z.W.; Chen, Y.L.; Luo, J.J.; Shi, Y.T.; Chen, Z.X. Screening and evaluation for drought resistance of cotton varieties. *Agric. Res. Arid. Areas* **2017**, *35*, 240–247.
50. Chen, D.; Wang, S.; Cao, B.; Cao, D.; Leng, G.; Li, H.; Yin, L.; Shan, L.; Deng, X. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Front. Plant Sci.* **2016**, *6*, 1241. [CrossRef]

51. Fang, Y.; Xiong, L. General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell Mol. Life Sci.* **2015**, *72*, 673–689. [CrossRef]
52. Wei, L.; Zhang, D.; Xiang, F.; Zhang, Z. Differentially expressed mirnas potentially involved in the regulation of defense mechanism to drought stress in maize seedlings. *Int. J. Plant Sci.* **2009**, *170*, 979–989. [CrossRef]
53. WU, X.L.; Bao, W.K. Statistical analysis of leaf water use efficiency and physiology traits of winter wheat under drought condition. *J. Integr. Agric.* **2012**, *11*, 82–89. [CrossRef]
54. Maheswari, M.; Tekula, V.L.; Yellisetty, V.; Sarkar, B.; Yadav, S.K.; Singh, J.; Kumar, A.; Amirineni, S.; Narayana, J.; Maddi, V. Functional mechanisms of drought tolerance in maize through phenotyping and genotyping under well watered and water stressed conditions. *Eur. J. Agron.* **2016**, *79*, 43–57. [CrossRef]
55. Huqe, M.A.S.; Haque, M.S.; Sagar, A.; Uddin, M.N.; Hossain, M.A.; Hossain, A.; Rahman, M.M.; Wang, X.; Al-Ashkar, I.; Ueda, A. Characterization of maize hybrids (*Zea mays* L.) for detecting salt tolerance based on morpho-physiological characteristicsion accumulation and genetic variability at early vegetative stage. *Plants* **2021**, *10*, 2549. [CrossRef]
56. Gedam, P.A.; Shirsat, D.V.; Arunachalam, T.; Ghosh, S.; Gawande, S.J.; Mahajan, V.; Gupta, A.J.; Singh, M. Screening of onion (*Allium cepa* L.) genotypes for waterlogging tolerance. *Front. Plant Sci.* **2022**, *12*, 727262. [CrossRef]
57. Uzair, M.; Ali, M.; Fiaz, S.; Attia, K.; Khan, N.; Al-Doss, A.A.; Khan, M.R.; Ali, Z. The characterization of wheat genotypes for salinity tolerance using morpho-physiological indices under hydroponic conditions. *Saudi J. Biol. Sci.* **2022**, *29*, 103299. [CrossRef] [PubMed]
58. Zahid, Z.; Khan, M.K.R.; Hameed, A.; Akhtar, M.; Ditta, A.; Hassan, H.M.; Farid, G. Dissection of drought tolerance in upland cotton through morpho-physiological and biochemical traits at seedling stage. *Front. Plant Sci.* **2021**, *12*, 627107. [CrossRef]
59. Liu, X.J.; Yin, B.Z.; Hu, Z.H.; Bao, X.Y.; Wang, Y.D.; Zhen, W.C. Physiological response of flag leaf and yield formation of winter wheat under different spring restrictive irrigation regimes in the Haihe plain, China. *J. Integr. Agric.* **2021**, *20*, 2343–2359. [CrossRef]
60. Golldack, D.; Li, C.; Mohan, H.; Probst, N. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Front. Plant Sci.* **2014**, *5*, 151. [CrossRef]
61. Nyachiro, J.; Briggs, K.; Hoddinott, J.; Johnson-Flanagan, A. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. *Cereal Res. Commun.* **2001**, *29*, 135–142. [CrossRef]
62. Mafakheri, A.; Siosemardeh, A.; Bahramnejad, B.; Struik, P.; Sohrabi, Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop Sci.* **2010**, *4*, 580–585.
63. Kyparissis, A.; Petropoulou, Y.; Manetas, Y. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., *labiatae*) under mediterranean field conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. *J. Exp. Bot.* **1995**, *46*, 1825–1831. [CrossRef]
64. Herbinger, K.; Tausz, M.; Wonisch, A.; Soja, G.; Sorger, A.; Grill, D. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiol. Bioch.* **2002**, *40*, 691–696. [CrossRef]
65. Conductance, L. Diurnal variations in leaf conductance and gas exchange in natural environments jd tenhunen, rw pearcy, and ol lange. *Stomatal Funct.* **1987**, *1*, 323–351.
66. Bornkamm, R. The physiology of plants under stress. *Agric. Ecosyst. Environ.* **1989**, *26*, 157. [CrossRef]
67. Chaves, M.M.; Oliveira, M.M. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. *J. Exp. Bot.* **2004**, *55*, 2365–2384. [CrossRef]
68. Konieczna, W.; Warchoł, M.; Mierek-Adamska, A.; Skrzypek, E.; Waligórski, P.; Piernik, A.; Dąbrowska, G.B. Changes in physio-biochemical parameters and expression of metallothioneins in avena sativa l. In response to drought. *Sci. Rep.* **2023**, *13*, 2486. [CrossRef]
69. de Souza, C.R.; MarocoJ, P.; dos Santos, T.P.; Rodrigues, M.L.; Lopes, C.; Pereira, J.S.; Chaves, M.M. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agric. Ecosyst. Environ.* **2005**, *106*, 261–274. [CrossRef]
70. Basu, P.; Ali, M.; Chaturvedi, S. Adaptation of photosynthetic components of chickpea to water stress. In Proceedings of the 4th International Crop Science Congress, Brisbane, Australia, 26 September 2004.
71. Sarker, U.; Oba, S. Drought stress effects on growth, ros markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in amaranthus tricolor. *Appl. Biochem. Biotech.* **2018**, *186*, 999–1016. [CrossRef]
72. Alscher, R.G.; Erturk, N.; Heath, L.S. Role of superoxide dismutases (sods) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [CrossRef]
73. Zhou, Q.; Li, Y.; Wang, X.; Yan, C.; Ma, C.; Liu, J.; Dong, S. Effects of different drought degrees on physiological characteristics and endogenous hormones of soybean. *Plants* **2022**, *11*, 2282. [CrossRef] [PubMed]