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Effects of Various Levels of Water Stress on Morpho-Physiological Traits and Spectral Reflectance of Maize at Seedling Growth Stage

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Abstract: Water stress (drought and waterlogging) is one highly important factor affecting food security in China. Investigating the effects of soil moisture stress on the morphological and physiological characteristics of maize seedlings is crucial for ensuring food production. The use of spectral monitoring to observe crop phenotypic traits and assess crop health has become a focal point in field crop research. However, studies exploring the contribution of crop phenotypic and physiological data to the Normalized Difference Vegetation Index (NDVI) are still limited. In this study, a 35-day pot experiment was conducted with seven soil moisture gradients: 50%, 60%, 70%, 80% (control group, CK), 90%, 100%, and 110% treatment. In order to investigate the effects of soil moisture stress on seedling phenotypes, antioxidant enzyme activities, and NDVI, an ASD FieldSpec 4 Hi-Res NG portable spectrometer was used to collect spectral data from maize (*Zea mays* L. B73) leaves. The contributions of maize phenotypic and physiological traits to NDVI were also examined. The results indicated that (1) the 50% and 110% treatments significantly affected maize seedling phenotypes compared to the CK group; (2) the activities of superoxide dismutase (SOD) and peroxidase (POD) in the leaves increased under water stress, while the activities of glutathione peroxidase (GSH-PX) and ascorbate peroxidase (APX) decreased; (3) soil moisture stress (drought and waterlogging) reduced photosynthetic pigments, chlorophyll content (SPAD), and NDVI, with inhibitory effects intensifying as the stress level increased; (4) Redundancy analysis showed that antioxidant enzymes explained 69.87% of the variation in seedling height, leaf area, and NDVI. Soil moisture stress, chlorophyll, and SPAD explained 58.14% of the variation in these parameters. The results demonstrated that maize seedlings were highly sensitive to soil moisture changes, and the SPAD value contributed significantly to NDVI ($p < 0.01$). This study provides valuable insights for future research in precision agriculture management

Keywords: maize seedlings; soil moisture stress; NDVI; antioxidant enzymes

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1. Introduction

Maize, as a crucial crop globally utilized for food, feed, and fuel, holds significant economic importance [1]. However, in recent years, the frequency of drought and waterlogging events caused by climate change has severely threatened global maize growth. Previous studies have identified soil water content (SWC) as the direct cause of both drought and waterlogging [2,3]. When soil moisture is insufficient, maize experiences drought stress; when soil moisture is excessive, maize faces waterlogging stress. The nature of these changes invariably impacts the normal growth of maize. For instance, both excessive and insufficient soil moisture (waterlogging or drought) lead to a reduction in maize yield and quality [4,5]. They can also cause changes in phenotypic physiology and leaf spectral characteristics [6].

Soil moisture can affect the growth and development of crops, with the underlying mechanisms possibly varying in several aspects. For example, water influences the vitality of crop roots, photosynthesis, respiration, and the synthesis and decomposition of organic matter [7], ultimately affecting crop yield. Drought during the seedling stage of maize promotes vertical root growth but reduces root absorption capacity, hindering the growth of the above-ground parts' biomass [8]. In addition, drought stress significantly reduces the photosynthetic capacity of maize. The photosynthetic rate is significantly limited by stomatal factors during early or moderate drought stress [9]. In the case of insufficient CO_2 supply, drought stress significantly reduces the transport of photosynthetic electrons and photochemical efficiency [10]. As the duration of stress extends or the intensity of stress increases, the biological membranes and enzymes involved in biological processes are damaged, and non-stomatal factors begin to play a dominant role in limiting photosynthesis. On the other hand, waterlogging stress plays a dominant role during the seedling growth stage. Waterlogging not only leads to soil hypoxia but also reduces root vitality and affects the absorption of nutrient elements. Therefore, it restricts root growth and development and subsequently impacts the growth of above-ground organs. For instance, leaf stomata close, reducing the entry of carbon dioxide, lowering the net photosynthetic rate, and decreasing leaf area. As the severity of drought and waterlogging increases, crops develop complex internal regulatory networks at phenotypic, metabolic, and molecular levels to cope with or adapt to water stress. Various antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA), and proline (Pro) [11–13], are involved in these complex regulatory networks. It has been reported that these antioxidant enzymes play critical roles in responding to drought and waterlogging stress. Both drought and waterlogging can lead to the accumulation of reactive oxygen species (ROS) and MDA in plants. This accumulation triggers the activation of antioxidant enzymes (such as SOD and POD) to mitigate and eliminate the ROS accumulation caused by drought and waterlogging [14].

Hyperspectral imaging, with its greater number of wavelengths and higher resolution, is capable of capturing subtle variations in surface features. In recent years, it has been widely used for monitoring crop traits [15] and managing water and fertilizer [16,17]. Previous studies have extensively utilized hyperspectral data to investigate crop traits, such as predicting canopy water content in rice [18] and identifying crops, like soybean, corn, and others, based on hyperspectral images [19]. However, there is a paucity of research exploring the impact of maize phenotypic and physiological characteristics on spectral properties. Previous research has demonstrated that soil moisture not only affects crop phenotypes and physiology but also causes changes in leaf spectral absorption and reflection [20,21]. Soil moisture affects leaf structure and water status, with characteristic spectral bands mainly located in the near-infrared and visible light range. The spectral responses in these bands can be used to calculate the Normalized Difference Vegetation Index (NDVI). Hyperspectral data, with its fine spectral resolution, is particularly adept at capturing subtle spectral variations [22]. The portable ground spectrometer ASD is characterized by mobility, timeliness and high resolution, which enables fast, real-time, accurate and non-destructive acquisition of crop leaf spectral information [23]. Therefore, in this experiment we chose to use ASD to collect leaf spectral information. Research on the exploration of spectral response patterns of crops under drought and waterlogging stress, the selection of characteristic bands, and the development of quantitative models requires further study to establish a more comprehensive understanding of this field.

The seedling stage is particularly relevant to the entire growth period and has a significant impact on the later growth and yield of maize. Water stress during the seedling stage can also lead to a sharp reduction in the total biomass of maize. It is, thus, of great importance to analyze the impact of soil moisture on maize seedlings for molecular breeding of maize. This study aims to fill the current knowledge gap regarding the impact of soil moisture on maize production. In addition, NDVI is sensitive to changes in vegetation, and NDVI values at different growth stages can reveal the response of maize to varying conditions, such as moisture availability and light intensity, which can subsequently inform irrigation management practices. Exploring the spectral differences of water stress is important for accurately identifying crop stress types. Therefore, we selected the seedling stage and utilized precise water conditions to investigate the effects of soil moisture on the phenotype, physiological characteristics, and Normalized Difference Vegetation Index (NDVI) of maize seedlings, as well as the contributions of phenotype and physiological characteristics to NDVI. Specifically, the objectives of this study are: (1) to determine the phenotypic and physiological responses of maize Inbred Line B73 seedlings to soil moisture; (2) to determine the impact of soil moisture on the leaf NDVI of maize B73 seedlings; and (3) to investigate the contributions of the phenotypic and physiological characteristics of B73 seedlings to NDVI.

2. Materials and Methods

2.1. Experiment Design

The experiment was conducted in a greenhouse at the Xinxiang Comprehensive Experimental Station of the Chinese Academy of Agricultural Sciences (35°18′ N, 113°54′ E), from August to October 2023. The tested maize variety was the "inbred line B73", developed by the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences. At first, uncontaminated soil (silty clay loam with a dry soil bulk density of 1.47 g/cm^3 and a field capacity of 27%) was dried and thoroughly mixed, and then sieved to remove stones, grassroots, and other impurities (Table 1). Then, 21 pots (15 cm in diameter) were prepared and labelled according to the water stress stage and gradient. Each pot was filled with 1.5 kg of the above-mixed soil without any fertilizer application (as the soil's inherent fertility is sufficient for seedling growth during the observation period). After that, six seeds were sown evenly in each pot and, when the first leaf unfolded, two healthy seedlings were retained per pot, with the others removed. From days 1 to 21, a uniform irrigation amount (75% of field capacity) was applied. Starting from day 22, irrigation levels were differentiated based on field capacity, with seven irrigation levels set at 50 ± 5%, 60 ± 5%, 70 ± 5%, 80 ± 5%, 90 ± 5%, 100 ± 5%, and 110 ± 5%. The 80 ± 5% treatment served as the control group (CK) (Table 2). In total, there were seven treatments, with three replicates each, amounting to 21 pots arranged randomly. Watering was regulated daily at the same time for 21 pots using the "weighing method" for 14 days.

Table 1. The physical and chemical properties of soil.

Soil Texture	Soil pH	Dry Soil Bulk Density $g \cdot cm^{-3}$	Soil Field Capacity	Soil Organic Matter $g \cdot kg^{-1}$	Total Nitrogen $g \cdot kg^{-1}$	Total Phosphorus $g \cdot kg^{-1}$
silty clay loam	8.80	1.47	27.00%	18.85	0.73	0.94

Note: Soil pH was determined in 1:5, soil to CO_2-free water suspension by pH meter (120 P-02A, Thermo Fisher Scientific, Waltham, MA, USA) [24]; dry soil bulk density was measured by ring knife method [25]; soil field capacity was measured by infiltration method [25]; total nitrogen was determined by microcalorimetric method [26]; total phosphorus was determined by perchloric acid–sulfuric acid method [26].

Table 2. Experimental Design for Soil Moisture in Young Maize Seedlings.

Soil Water Content	Lower Limit of Soil Moisture Control	Upper Limit of Soil Moisture Control
CK	75	85
50%	45	55
60%	55	65
70%	65	75
90%	85	95
100%	95	105

Note: The values in the table are the lower and upper limit index of soil moisture control, which is the percentage of soil water in field capacity.

2.2. Measurement of Morphological and Physiological Indicators of Maize Leaves

On the 7th and 14th days after differentiated irrigation, three plants with representative growth conditions were randomly selected from each treatment for phenotypic analysis. The rolled leaf phenotype was observed, and plant height (measured from the soil to the tip of the highest leaf using a soft tape measure, straightening the leaf if it is curved), leaf length, and maximum width were measured using a ruler. The leaf area per plant was then calculated [27]:

$$LAI = \sum(L \times W \times 0.75) \tag{1}$$

where LAI represents leaf area (cm²), L is the maximum leaf length (cm), and W is the maximum leaf width (cm). The coefficient of 0.75 is an empirical factor.

At noon on the 7th and 14th days of experimental treatment, the chlorophyll values of the selected three maize plants were measured using a SPAD-502 PLUS portable chlorophyll meter (Konica Minolta Holdings, Inc., Japan) under ample sunlight. Measurements were taken on the second fully expanded leaf from the top of each selected seedling. Each leaf was measured three times at different positions, and the chlorophyll content of each leaf was based on the average of the three readings. On the 14th day of experimental treatment, leaf samples were collected and chlorophyll was extracted by the anhydrous ethanol method and chlorophyll a ($Chl\ a$), chlorophyll b ($Chl\ b$) and total chlorophyll (Chl) were calculated by the following equations [28]:

$$Chl\ a = (12.7D_{663\text{nm}} - 2.69D_{645\text{nm}}) \times \frac{V}{1000 \times m} \tag{2}$$

$$Chl\ b = (22.9D_{645\text{nm}} - 4.68D_{663\text{nm}}) \times \frac{V}{1000 \times m} \tag{3}$$

$$Chl = (20.21D_{645\text{nm}} + 8.02D_{663\text{nm}}) \times \frac{V}{1000 \times m} \tag{4}$$

where $D_{663\text{nm}}$ and $D_{645\text{nm}}$ represent the absorbance at 663 and 645 nm, respectively; V is the volume of the solution to be measured (mL); and m is the fresh mass of the leaf (g).

On the 14th day of experimental treatment, leaf samples (the second fully expanded leaf) were collected from each group. The yellow or grey tips of the collected leaves were removed, and the samples were immediately frozen in liquid nitrogen and then stored in an ultra-low temperature freezer at −80 °C for subsequent physiological analysis. The activities of MDA (TBA method), SOD (hydroxylamine method), POD (hydrogen peroxide method), Pro [29], glutathione peroxidase (GSH-PX) [30], and ascorbate peroxidase (APX) [31] in the samples were measured using assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) (http://www.njjcbio.com, accessed on 15 October 2023, ultraviolet-visible spectrophotometry) to investigate the effects of soil moisture on the antioxidant enzymes of maize seedlings.

2.3. Acquisition of Corn Leaf Spectra

On the 14th day of the experimental treatments at noon, an ASD FieldSpec4 spectrometer (Analytica Spectra Devices Inc., Boulder, CO, USA) was used to measure the reflectance spectra of individual maize leaves. The spectrometer covers a range of 350 to 2500 nm with a spectral resolution of 1 nm. Due to the absence of blade clamps, to minimize potential interference from pots and soil, the leaves were placed on a blackboard, exposing only the leaves. The probe was positioned perpendicular to the leaf surface at a distance of 1.5 cm. Calibration was performed using a white reference board before data collection. Spectra were collected from the second fully expanded leaf of each plant, with two measurements taken from each section. Post-measurement, the hyperspectral images for each treatment were preprocessed, including splicing, correction, and averaging, to obtain the leaf spectra under different stress conditions. The wavelength range from 400 nm to 1100 nm was selected to calculate the Normalized Difference Vegetation Index (*NDVI*):

$$NDVI = (NIR - R)/(NIR + R) \tag{5}$$

where *NIR* (770 nm–900 nm) represents the reflectance in the near-infrared band, and *R* (630 nm–690 nm) represents the reflectance in the red band.

2.4. Statistical Analysis

The mean and standard deviation for each measured index (height, leaf area, SPAD, Chl a, Chl b, Chl, SOD, POD, MDA, Pro, GSH-PX, APX, NDVI) were calculated using Excel 2019 (Microsoft Corp., Redmond, WA, USA). Based on the results of normality tests, one-way ANOVA was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to analyze the differences in plant height, leaf area, SPAD, Chl a, Chl b, Chl, SOD, POD, MDA, Pro, GSH-PX, APX, and NDVI under different soil moisture treatments. Differences between treatments were determined by Duncan's least significant difference (LSD) test at a probability level of $p < 0.05$. Paired *t*-tests for plant height, leaf area, and SPAD values on Day 7 and Day 14 were conducted using GraphPad prism9.3.0 (La Jolla, CA, USA). In the Random Forest [32], morphological and physiological data (plant height, leaf area, SPAD, Chl a, Chl b, Chl, SOD, POD, MDA, Pro, GSH-PX, APX) were standardized and entered to determine their contribution to NDVI, and variable significance analyses were carried out using Random Forest and rfPermute packages in R 4.3.3. The contribution of each predictor variable to the modelling performance was ranked by calculating two different metrics: %IncMSE (percentage increase in mean squared error) and IncNodePurity. Generally, a larger %IncMSE indicates a higher importance of the predictor variable (Shen et al., 2016) [33]. Redundancy analysis (RDA) was performed using Canoco5 to explain the influence of physiological traits on phenotypic characteristics.

3. Results

3.1. Phenotypic Response of Corn during the Seedling Stage

As we can see, water stress significantly reduced plant height (Figure 1a). Water stress treatments with 50%, 60%, and 110% showed more pronounced effects ($p < 0.05$). Compared to the control group, plant height decreased by 25.85%, 20.41%, and 20.75% under 50%, 60%, and 110% water stress, respectively. Plant height decreased by 16.33%, 9.86%, and 16.33% ($p < 0.05$) under 70%, 90%, and 100% water stress, respectively.

Water stress also significantly reduced the leaf area index (LAI) (Figure 1b). Compared to the control group (80%), LAI decreased by 34.72%, 30.04%, 23.09%, 22.58%, and 31.88% ($p < 0.05$) under 50%, 60%, 70%, 100%, and 110% water stress, respectively. The 90% water stress treatment had a less pronounced effect on LAI, with a reduction of 18.11% ($p < 0.05$). As the severity of water stress increased, the decline in LAI became more significant.

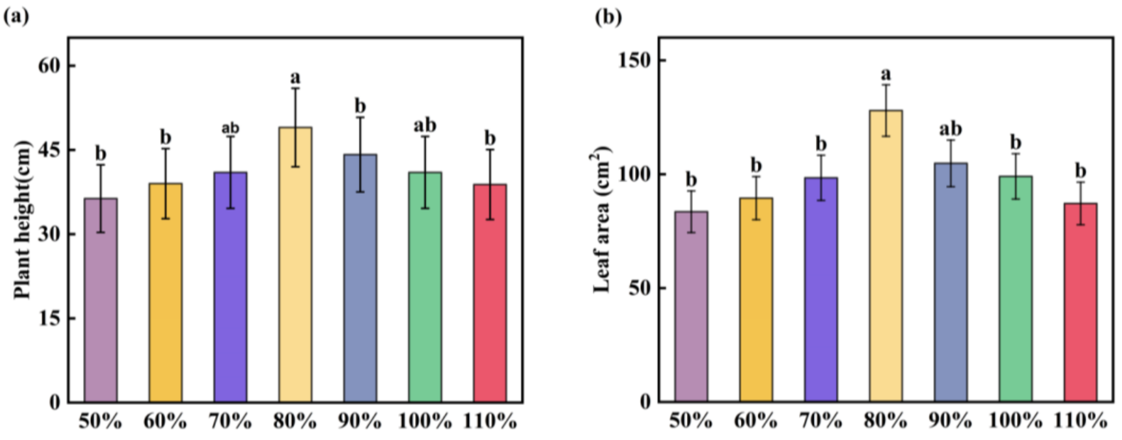
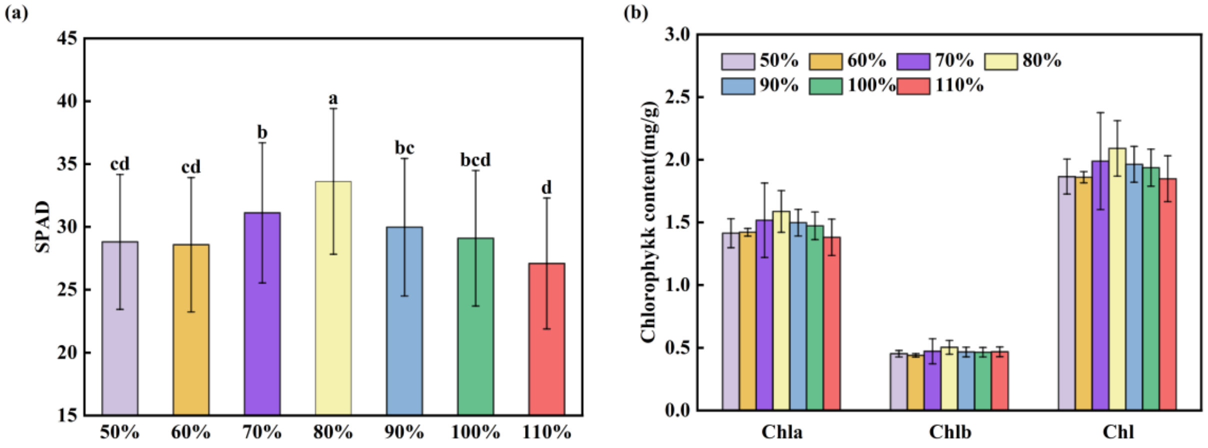


Figure 1. Effect of water stress on plant height and leaf area of maize seedlings on day 14. (**a**) Differences in plant height. (**b**) Differences in leaf area. (Note: different lowercase letters indicate significant at 0.05 level).

3.2. Effects of Soil Moisture on the Physiological Traits of Maize Seedling Leaves

We can see that water stress significantly reduced SPAD values (Figure 2a). Compared to the control group (80%), the 110% stress treatment reduced SPAD values by 19.42%. The 50%, 60%, and 100% stress treatments reduced SPAD values by 14.32%, 14.98%, and 13.46%, respectively. The 70% and 90% stress treatments both reduced SPAD values by 10.84%. Chlorophyll a, chlorophyll b, and total chlorophyll levels decreased, but the differences were not significant ($p \geq 0.05$) (Figure 2b).



Note: Different lowercase letters indicated significant at 0.05 level. (b) does not have letters marked because Chl a, Chl b, and total Chl are not significant at the $P<0.05$ level.)

Figure 2. Effect of water stress on chlorophyll content and SPAD values of maize seedlings on day 14. (**a**) Differences in chlorophyll content (**b**) Differences in chlorophyll a, chlorophyll b and total chlorophyll content.

However, water stress significantly increased the activities of SOD, POD, and MDA in maize seedlings (Figure 3). Compared to the control group (80%), the 50% and 110% stress treatments resulted in the highest increases in activity, followed by the 60% and 70% stress treatments, and then the 90% and 100% stress treatments. Specifically, SOD activity increased by 18.62%, 9.39%, 12.84%, 6.83%, 8.05%, and 16.48% under 50%, 60%, 70%, 90%, 100%, and 110% water treatments, respectively, compared to the control group (Figure 3b). POD activity increased by 43.81%, 37.90%, 19.28%, 7.33%, 12.26%, and 43.74% under the same respective stress treatments compared to the control group (Figure 3c). MDA activity increased by 45.65%, 11.22%, 20.75%, 15.23%, 35.61%, and 48.87% under the same respective stress treatments compared to the control group (Figure 3a).

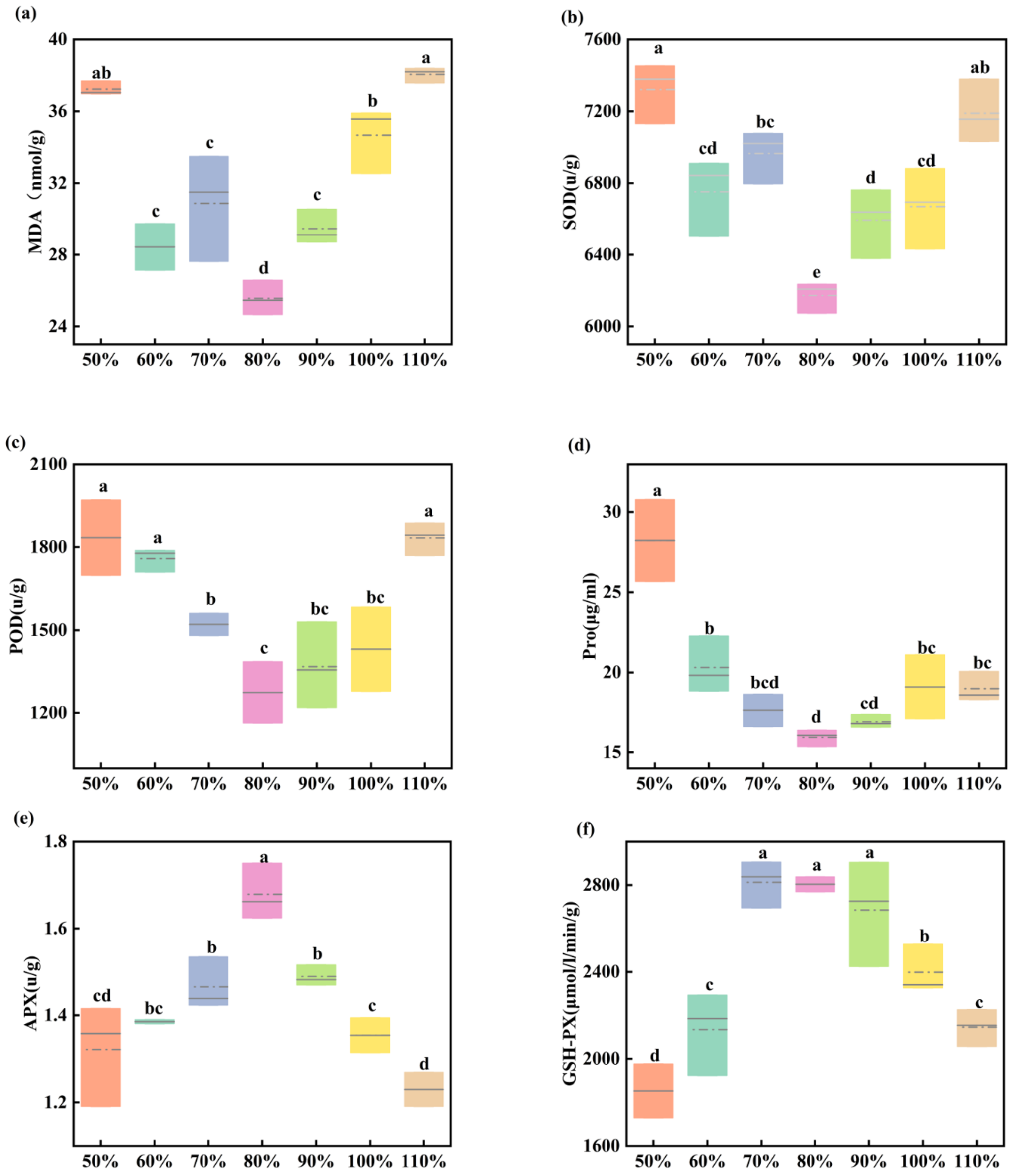


Figure 3. Effect of water stress on antioxidant enzymes in day 14 maize seedlings. (**a**) Malondialdehyde (MDA) content. (**b**) Superoxide dismutase (SOD) content. (**c**) Peroxidase (POD) content. (**d**) Proline (Pro) content. (**e**) Ascorbate peroxidase (APX) content. (**f**) Glutathione peroxidase (GSH-PX) content. The horizontal line inside the box in a box plot indicates the median of the data. Different letters indicate significance at the $p < 0.05$ level.

Moreover, water stress significantly increased the Pro content in maize seedlings (Figure 3d). The results showed that the 50% stress treatment significantly increased Pro content by 77.26%, compared to the control group. The 60%, 100%, and 110% stress treatments increased Pro content by 27.58%, 19.91%, and 19.27%, respectively, while the 70% and 90% stress treatments did not cause a significant increase in Pro content.

Water stress significantly reduced the activity of GSH-PX in maize seedlings (Figure 3f). Compared to the control group, the 50%, 60%, 100%, and 110% stress treatments decreased GSH-PX activity by 33.93%, 23.89%, 14.47%, and 23.46%, respectively. However, the 70% and 90% stress treatments did not cause a significant decrease in GSH-PX activity. Water stress also significantly reduced the activity of APX in maize seedlings (Figure 3e). Compared to the control group, the 50%, 60%, 70%, 90%, 100%, and 110% stress treatments decreased APX activity by 21.28%, 17.47%, 12.71%, 11.28%, 19.34%, and 26.73%, respectively.

3.3. Influence of Soil Moisture on Maize Leaf Spectra

Figure 4a shows the original leaf spectra (OR) under water stress, where 400 nm–700 nm is the red and blue region of the visible band where chlorophyll absorbs light energy resulting in low reflectance of the plant in this band, and 700 nm–1000 nm is the near-infrared (NIR) band, where there is a significant increase in the reflectance of the plant, creating a distinct red edge. From the statistics of NDVI across the seven moisture gradients, it is evident that the NDVI value was highest in the control group (80%) while it was lowest under 50% stress. Compared to the control group, the NDVI values decreased by 17.13%, 12.15%, and 5.54% under 50%, 60%, and 70% stress, respectively. The NDVI values decreased by 8.48%, 14.61%, and 9.15% under 90%, 100%, and 110% stress, respectively. The reduction in NDVI value was more significant under 100% stress compared to 110% stress (Figure 4b).

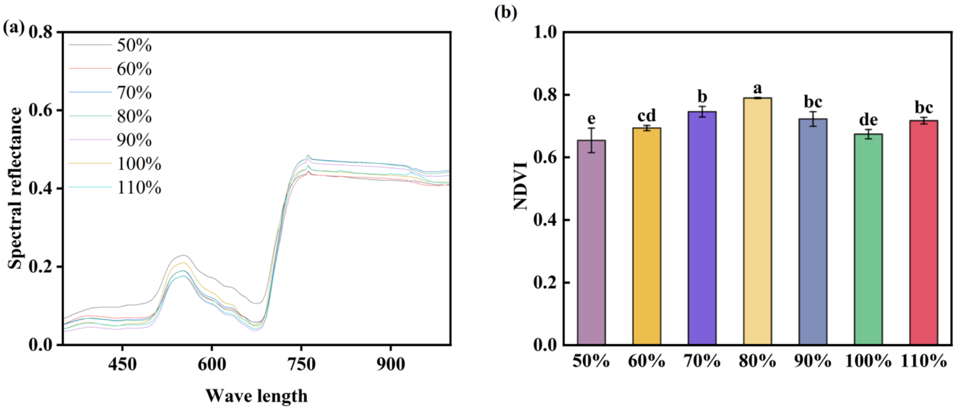


Figure 4. Effects of water stress on leaf spectra of maize seedlings on day 14. (**a**) Raw spectra of leaves (OR). (**b**) Differences in NDVI values of maize under different water stresses. Different letters indicate significance at the $p < 0.05$ level.

3.4. t-Test of Maize Phenotypic Characteristics

To evaluate the effects of soil moisture on plant height, leaf area index (LAI), and SPAD values, paired sample *t*-tests were conducted for Day 7 and Day 14 (Figure 5). The paired sample *t*-test results showed a significant difference in plant height between Day 7 (mean = 39.56, sd = 3.84) and Day 14 (mean = 41.33, sd = 4.45) under soil moisture treatment, t (20) = 3.23, $p < 0.01$ (Figure 5a). A paired sample *t*-test was also conducted for the leaf area index on Day 7 and Day 14. The results indicated that there was a significant difference in LAI between Day 7 (mean = 74.28, sd = 15.31) and Day 14 (mean = 98.63, sd = 18.3) under soil moisture treatment, t (20) = 8.74, $p < 0.001$ (Figure 5b). For SPAD values, the paired sample *t*-test showed a significant difference between Day 7 (mean = 28.34, sd = 2.67) and Day 14 (mean = 29.75, sd = 2.55) under soil moisture treatment, t (20) = 3.194, $p < 0.01$ (Figure 5c).

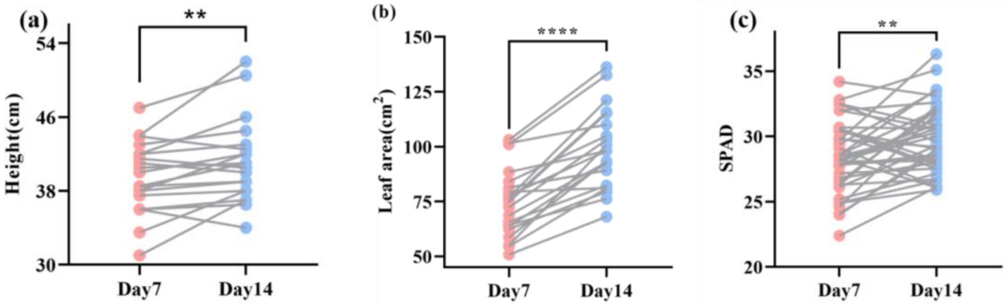


Figure 5. Paired *t*-tests for phenotypic characterization of maize seedlings under 7 and 14 days of water stress. (**a**) Paired *t*-test for plant height, (**b**) leaf area index paired *t*-test, and (**c**) SPAD values paired *t*-test. Significance levels for paired *t*-tests are shown below: **, $p < 0.01$; ****, $p < 0.0001$.

3.5. Contribution of Maize Phenotypic and Physiological Traits to NDVI

Using Random Forest, morphological and physiological data were standardized and entered to determine their relative importance to NDVI (Figure 6a). The results showed that SWC (soil water content), height, SPAD and Chl a were the four variables that contributed the most to NDVI, with 15.45%, 13.93%, 11.63% and 2.54%, respectively. Among them, the contribution of SPAD value to NDVI was very significant ($p < 0.01$).

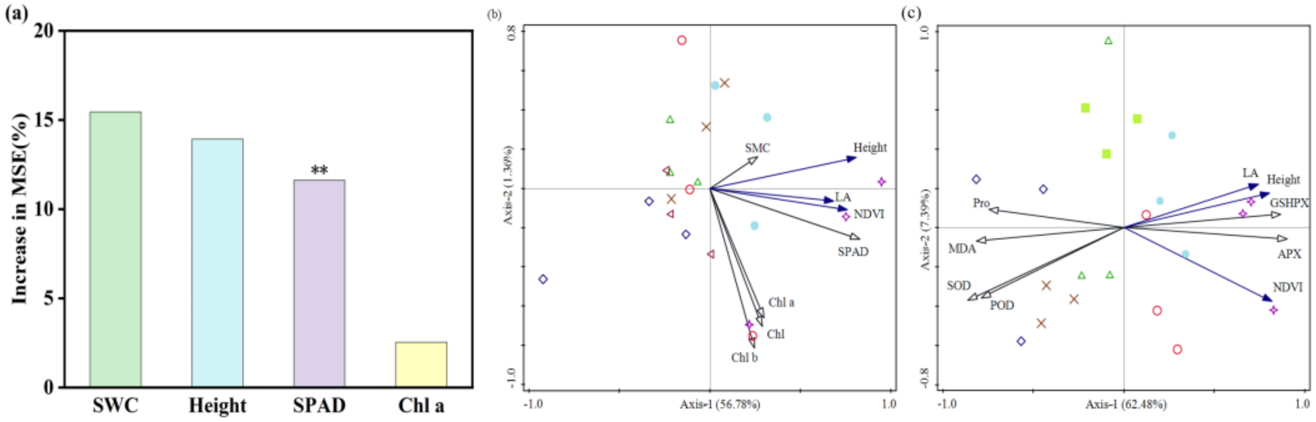


Figure 6. Random Forest and Redundancy Analysis. (**a**) Importance ranking of NDVI predictors using the Random Forest model ($p < 0.01$). (**b**) Importance of antioxidant enzymes to phenotypic traits and NDVI (percentage increase in mean squared error, %IncMSE). (**c**) Importance of chlorophyll, soil moisture content, and SPAD to phenotypic traits and NDVI. (Note: ** indicates significance at $p < 0.01$. Different shapes represent different plants).

Figure 6b shows the redundancy analysis (RDA) results for the correlations among SWC, SPAD, Chl a, Chl b, Chl, and morphological traits (height, LAI, NDVI). The correlations of SWC, SPAD, Chl a, Chl b, Chl, and morphological traits with the first and second axes were 0.8494 and 0.4315, respectively. All these variables explained 58.14% of the variation in morphological traits. SPAD and SWC individually explained 39.2% ($p < 0.01$) and 14.6% ($p < 0.01$) of the variation in morphological traits, respectively. Furthermore, SPAD and SWC were significantly positively correlated with height, LAI, and NDVI.

Figure 6c shows the redundancy analysis (RDA) results for the correlations between antioxidant enzymes (MDA, SOD, POD, APX, GSH-PX) and morphological traits (height, LAI, NDVI). The correlations of antioxidant enzymes and morphological traits with the first and second axes were 0.8910 and 0.6858, respectively. All antioxidant enzymes explained 69.87% of the variation in morphological traits. APX and Pro individually explained 51% ($p < 0.01$) and 10.3% ($p < 0.05$) of the variation in morphological traits, respectively. Furthermore, APX and GSH-PX were significantly positively correlated with height, LAI, and NDVI.

4. Discussion

4.1. The Response of Maize Seedling Phenotypic Traits to Soil Moisture

When soil moisture is insufficient to meet the water demands of crops, drought stress occurs. Drought stress may result in stomatal closure, inhibition of photosynthesis [34], reduction in chlorophyll content, alteration of crop metabolism, and decreased accumulation of dry matter, subsequently affecting crop yield and quality. Our experimental phenotypic results indicated that the degree of leaf curling intensified as soil moisture decreased, and in some cases, there was cell death at the leaf tips of seedlings. Compared to the control group (CK), the length and width of leaves were significantly shorter under 50% stress. Maize seedlings exhibited smaller growth under 50% and 60% stress conditions, which may be attributed to reduced photosynthetic rates and decreased SPAD values. Our findings are consistent with previous research, suggesting that photosynthesis and cellular metabolism are suppressed under drought conditions [35], ultimately inhibiting seedling growth.

When soil moisture exceeds the plant's water requirements for an extended period, waterlogging stress occurs. This results in the exclusion of air from the soil, causing inadequate oxygen supply to plant roots and restricting plant growth. Additionally, research by Vandoorne et al. [36] showed that, under waterlogging stress, some of the stomata on the leaves were closed, gas exchange parameters were reduced, and carbon dioxide was unable to enter through the stomata while, at the same time, intercellular carbon dioxide was constantly being absorbed, resulting in an insufficient supply of carbon dioxide to the cells, a reduction in the photosynthetic rate, a decrease in the chlorophyll content, and a reduction in the leaf area. Our experimental results indicated that 100% and 110% stress significantly reduce plant height.

Many biological processes activated in plants under environmental stress are particularly sensitive to drought and waterlogging. Processes related to photosynthesis are among the most commonly affected [37,38]. Chlorophyll b is responsible for absorbing and transferring light energy, while chlorophyll a converts light energy into chemical energy. The decrease in chlorophyll a, chlorophyll b, and total chlorophyll levels is attributed to the increase in O^{2-} and H_2O_2. Therefore, photosynthetic parameters have been widely used to assess the drought tolerance of plants. Previous studies have shown that waterlogging stress disrupts the membrane lipid structure of chloroplasts and mitochondria and reduces chlorophyll content [39]. Subsequently, the decrease in photosynthetic enzyme activity and the reduction in photosynthetic rate inhibit plant growth and biomass accumulation, ultimately leading to a decrease in crop yield. Our experimental results indicated that treatments with 100% and 110% stress resulted in a decrease in SPAD values by 13.46% and 19.42%, respectively. This indicates that waterlogging significantly reduces SPAD values, especially with increasing severity of waterlogging. Our findings are consistent with the results of Ren et al. [40] regarding summer maize, where waterlogging stress affected SPAD values and these decreased. In our study, we used both the SPAD meter and the ethanol extraction method to measure chlorophyll content, because each has distinct advantages. The SPAD meter's measurement is based on the absorption of red and near-infrared light, which allows it to quickly respond to changes in leaf surface and cellular characteristics, such as those caused by water stress that affect leaf transparency and optical properties. This aligns with the calculation method of NDVI, resulting in a high correlation between the two in the random forest analysis. On the other hand, Chl a, Chl b, and total Chl provide accurate absolute chlorophyll content data, which is valuable for understanding the physiological state of chlorophyll, but they do not directly reflect changes in light absorption and reflection. As a result, Chl a, Chl b, and total Chl did not show significant contributions to NDVI in the Random Forest analysis. By using both methods, they can complement each other, offering a more comprehensive understanding of chlorophyll content and a deeper insight into environmental changes.

4.2. Impact of Soil Moisture on the Antioxidant System of Maize

An important aspect of how crops respond to abiotic stress is the regulation of the antioxidant system. Normally, the levels of reactive oxygen species (ROS) within crops are relatively low. Prolonged drought and waterlogging stress, however, can lead to the generation of substantial amounts of ROS (such as OH^-, O^{2-}, and H_2O_2) within the plants. This increase in ROS results in lipid peroxidation and disruption of membrane homeostasis [41]. Ultimately, this leads to the accumulation of malondialdehyde (MDA) [12,42], and, to a certain extent, damages cell membranes in turn. Malondialdehyde (MDA) is an indicator of lipid peroxidation and has been shown to respond to oxidative stress. As such, MDA content is often used as a measure of plant tolerance to abiotic stress [43]. In this study, the 50%, 60%, 100%, and 110% stress treatment groups significantly increased MDA content, and the MDA levels tended to rise with the increasing severity of stress. The increase in ROS and malondialdehyde (MDA) leads to an elevation in antioxidant enzyme activities [12], which helps to eliminate or reduce ROS, preventing oxidative damage and mitigating lipid peroxidation in crops. For instance, the activities of superoxide dismutase (SOD) and peroxidase (POD) will increase to scavenge free radicals and protect plants from ROS-induced damage [11]. SOD catalyzes the conversion of O^{2-} into H_2O_2, while POD converts H_2O_2, into O_2 and H_2O [44], thereby detoxifying the effects induced by ROS [45]. Many studies [11,46] also confirmed that drought and waterlogging stress can increase SOD and POD activities due to the upregulation of antioxidant genes. Our results showed that the 50%, 60%, 100%, and 110% stress treatment groups significantly increased SOD and POD activities compared to the control group (CK). Moreover, as the severity of stress increased, the activities of SOD and POD also showed an upward trend. Proline (Pro) is an important osmolyte in plant tissues [47]. When plants are subjected to drought and waterlogging stress, Pro accumulates to maintain cell water potential balance. Additionally, Pro also acts as an antioxidant, protecting the cells from free radical damage and maintaining the cellular environment, thus enhancing the plant's resilience [48]. Most studies indicate that proline content increases under waterlogging stress [49]. Our experimental results indicated that the 50% stress treatment significantly increased Pro content compared to the control group, while 100% and 110% stress treatments increased Pro content a bit. However, the 90% stress treatment did not significantly affect Pro content. The synergistic action of GSH-PX, APX, and other antioxidants can reduce the production of H_2O_2, and MDA. Water stress significantly decreased the activities of GSH-PX and APX enzymes.

4.3. Effects of Soil Moisture on Leaf NDVI of Maize Seedlings

The spectral characteristics of plant leaves may also change under drought and waterlogging stress conditions [50]. We processed the raw spectra (OR) of leaves under water stress using View Spec Pro software (ViewSpec pro version 5.6). The results showed that the NDVI exhibited a similar trend of change as the severity of drought and waterlogging stress increased. This could be attributed to the loss of water in maize leaves under drought stress, leading to cell loss of elasticity, reduced photosynthesis, and increased pigment content. Consequently, the reflectance of the leaves in the near-infrared spectrum increased while the reflectance in the visible spectrum decreased, causing the NDVI values to decrease correspondingly with the intensification of drought stress.

Conversely, when maize seedlings are subjected to waterlogging stress, the overly saturated soil deprives the maize roots of adequate oxygen supply and causes root damage. This impairs photosynthesis, resulting in leaf chlorosis and a decrease in the reflectance in the near-infrared spectrum. As a consequence, the NDVI values decrease correspondingly with the intensification of waterlogging stress. Overall, the SPAD and NDVI values were significantly reduced under 50% and 110% soil moisture treatments compared to the control treatment group. These results indicate that NDVI can effectively reflect the physiological status of maize seedlings under different soil moisture conditions, aiding in the monitoring and management of water stress in agricultural systems. We can better understand the impact of environmental stress on plant physiology by utilizing spectral characteristics, and then improve strategies to maintain crop health and productivity in adverse conditions.

4.4. Contribution of Maize Seedling Phenotypic and Physiological Characteristics to NDVI

In this study, we applied a Random Forest model to evaluate the impact of maize morphological and physiological data on the NDVI of maize leaves. Through the model's variable importance assessment, we found that SWC (Soil Water Content), height, SPAD values, and Chl a (Chlorophyll a) were the variables contributing the most to NDVI prediction. The high contribution of SWC emphasized the critical role of water in maize growth. Existing research also indicated that an appropriate water supply not only affects the growth rate and biomass accumulation of maize but is also directly related to the photosynthetic capacity of the leaves [51,52]. Changes in soil moisture can significantly influence the spectral reflectance characteristics of the leaves, thereby altering NDVI values. This is consistent with our findings. Height is a direct indicator of maize growth, and its importance in NDVI prediction reflects the close relationship between maize growth status and leaf spectral characteristics [53]. An increase in height is usually accompanied by an increase in NDVI, which may be due to the fact that the taller the plant, the better it grows, the larger the plant leaf area becomes, and the more chlorophyll it contains, which in turn leads to a correlation between plant height and NDVI. The high contribution of SPAD values and Chl a further corroborates the central role of chlorophyll in photosynthesis and vegetation indices. Specifically, the SPAD value ($p < 0.01$) significantly influences NDVI, as both SPAD values and Chl a content directly affect leaf light absorption properties and spectral reflectance characteristics. This is consistent with our analysis of the experimental results, enriching our understanding of the factors influencing NDVI.

The RDA results in Figure 6b showed that SWC and SPAD values are located in the positive region, indicating a strong positive correlation with height, LAI, and NDVI. Similarly, Chl a, Chl b, and total chlorophyll (Chl) also showed a strong positive correlation with LAI and NDVI. This is consistent with the previous analysis with the Random Forest model, confirming that these variables significantly contribute to NDVI prediction. SWC is located in the positive region of both the first axis (Axis-1) and the second axis (Axis-2) in the diagram, reflecting the significant impact of soil moisture on maize growth and photosynthetic pigments. This effect is particularly pronounced in drought conditions, where water availability significantly influences NDVI. Related studies also highlight that height is a crucial indicator of maize growth status, with taller maize plants typically exhibiting better photosynthetic capacity and biomass accumulation [54]. Chl a, Chl b, total chlorophyll (Chl), and SPAD content directly affect photosynthetic efficiency and spectral reflectance characteristics, thereby influencing NDVI values. Although the contributions of Chl b and total chlorophyll are slightly lower than that of Chl a, their roles as accessory pigments in photosynthesis are also significant and cannot be overlooked [55]. These characteristics are reflected in NDVI. In Figure 6c, the role of the antioxidant enzyme system in plant responses to environmental stress is further validated. MDA, SOD, POD, Pro, APX, and GSH-PX are important antioxidant enzymes in plants. They effectively scavenge reactive oxygen species, protecting plant cells from oxidative damage [12]. The figure showed that APX and GSH-PX are positively correlated with height, LA, and NDVI, whereas SOD and POD are negatively correlated with these variables. This suggests that height, LA, and NDVI may decrease under higher oxidative stress. Pro and MDA exhibit a negative correlation with NDVI along the first axis, further validating the protective role of Pro in stress response [56] and the indicative role of MDA in oxidative stress [57]. These findings are consistent with our experimental results. Overall, this study utilizes the Random Forest model and RDA to analyze the contribution of maize phenotypic and physiological data to NDVI. These results provide a scientific basis for further optimizing maize production management and precision agriculture. However, the water treatment time set in this experiment was 14 d, the growth of maize seedlings was observed, and it was impossible to understand the process of change in the growth physiology of maize with the increase in water treatment time, as well as during the whole growth period, so it is necessary to conduct further research on the basis of this experiment, i.e., to set different water treatment times and to study the changes in the physiology and spectra of maize under different water treatment conditions as well as in different growth periods.

5. Conclusions

Phenotypic, physiological and NDVI differences in maize seedlings under stress and the contribution of phenotypic and physiological characteristics to NDVI were investigated by a soil moisture stress test in maize seedlings. The following conclusions were obtained:

(1) Phenotypic analysis indicates that both drought and waterlogging stress reduce photosynthesis in maize seedlings, resulting in decreased chlorophyll content (SPAD values). As drought and waterlogging stress intensify, the inhibitory effects also increase, leading to smaller and yellowing seedling leaves. The 50% and 110% treat-

ments have the most significant impact on maize phenotypes, while the 70% and 90% treatments do not produce significant effects on maize phenotypes.

(2) Physiological reflections indicate that both drought and waterlogging stress affect the antioxidant enzyme system in maize (reducing GSH-PX and APX enzyme activity, while increasing SOD, POD, and Pro enzyme activity). However, APX and GSH-PX exhibit patterns opposite to those of SOD and POD. Therefore, SOD, POD, APX, and GSH-PX may participate in regulating maize growth under water stress. NDVI values also decrease, consistent with changes in SPAD values and photosynthetic rates. This correlates with the phenotype of relatively small and yellowing leaves under water stress treatments.

(3) The results from the Random Forest model and RDA validate the contribution of maize phenotypic and physiological characteristics to NDVI, with SPAD making a significantly meaningful contribution to NDVI.

In summary, this study provides a basis for further investigation into maize seedling responses to abiotic stress and offers valuable insights for exploring precision agricultural management.

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