

## Article

# Morpho-Physiological and Biochemical Responses of Maize Hybrids under Recurrent Water Stress at Early Vegetative Stage

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**Abstract:** Rapid climate change may put future food security under threat, which emphasizes the significance of assessing the morpho-physiological and biochemical traits associated with maize tolerance against recurrent water stress at the early vegetative stage. Three maize varieties (V1, SUWAN2301; V2, SUWAN4452; and V3, S7328) and three water levels (I<sub>1</sub>, daily watering as the control; I<sub>2</sub>, watering every two days as the short stress; and I<sub>3</sub>, watering every four days as the prolonged stress) were employed in a factorial design pot experiment. During the experiment, I<sub>1</sub>'s soil moisture content (SMC) was maintained at almost 100% of its field capacity (FC), whereas I<sub>2</sub> and I<sub>3</sub>'s volumetric SMC dropped to an average of 22.10% and 11.57%, respectively, following a stress phase. Fourteen distinct characteristics of maize were investigated at 5, 9, and 13 days after watering treatment initiation (DAWTI). The findings revealed that water levels significantly influenced all the tested traits ( $p < 0.05$ ), except for a few traits at 5 or 9 DAWTI only, whereas the maize variety significantly influenced most of the studied attributes ( $p < 0.05$ ). Except for proline content in leaf (PrL) and root (PrR); total soluble sugar in leaf (TSSL) and root (TSSR); and root length to shoot length ratio (RL:SL), the value of all analyzed characters was higher under I<sub>1</sub> compared to I<sub>2</sub> and I<sub>3</sub>. However, during the first recovery period (RP), the recovery rate (RR) of stem perimeter (SP), root length (RL), root dry weight (RDW), leaf water potential (LWP), leaf greenness (LG), and TSSL were higher in I<sub>2</sub>, whereas leaf area (LA) and RL:SL was higher in I<sub>3</sub>. However, in the second RP, the RR of plant height (PH), SP, RL, LWP, LG, and TSSL were higher in I<sub>3</sub>, whereas LA, RDW, RL:SL, PrL, PrR, and TSSR were higher in I<sub>2</sub> compared to each other. Under I<sub>3</sub>, the RR of biochemical traits, i.e., PrL (29.45%) and TSSR (20.23%), were higher in the first RP, and PrR (20.74%) and TSSL (15.22%) were higher in the second RP. However, the variety V1 could recover more after a re-watering, and, in the second RP, it performed better in the case of LA (120.14%), PH (18.41%), SP (19.94%), RL (17.74%), Shoot dry weight (SDW) (56.82%), RDW (11.97%), LG (0.05%), PrR (42.55%), TSSL (18.54%), and TSSR (22.87%) than other varieties. The maize varieties performed differently under I<sub>1</sub> and I<sub>3</sub> according to the principal component analysis and stress tolerance index. The variety V1 exhibited superior performance under both water levels. The biplot analysis highlighted the importance of traits, such as PrL, RL, TSSL, TSSR, PrR, and RL:SL, in water-stressed conditions. However, re-watering following a water stress period triggered the recovery rates in most traits, particularly after the second four-day stress period, and variety V1 performed better as well. Nonetheless, more research on a genomic and molecular level is required to gain a deeper understanding of the precise processes of drought tolerance in maize, particularly under recurring water stress circumstances.

**Keywords:** maize; water deficit; proline; total soluble sugar; absolute growth rate; ggbiplot



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## 1. Introduction

Adversely rapid climate change is already having a significant impact on agriculture, and the frequency and severity of drought is expected to increase in the future. This is because rising temperatures are leading to more evaporation and less precipitation [1,2]. If the current trends continue, it is predicted that 30% of the world's water supply will be depleted, and the number of drought-affected regions will more than double by 2050 [3,4]. This poses a major threat to food security [5], and, consequently, affects major sources of income and employment for millions of people around the world.

Maize is an important economic crop, cultivated in both temperate and tropical regions [6,7] including Thailand [8]. Thailand produced 5,500,000 tons of grain in 2022–2023 from 1,250,000 hectares of maize cultivation, which is about 5% more than the average for the previous five years. However, this amount fell short of the nation's needs [9]. In many tropical countries, maize is highly vulnerable to drought stress, and millions of impoverished maize consumers may be at risk of hunger and food insecurity unless growers develop climate-resilient cultivars that increase yields [10]. The severity of the drought, the duration of exposure, and the growth stage all have an impact on maize yield loss [11]. Drought stress cannot be predicted because it might occur at any stage of the plant's life cycle [11], though the emphasis is often given more on the flowering stage than the seedling stage. Drought during this stage is also just as significant as drought during the flowering stage [12]. At the early growth stage, drought stress may affect seed vigor, imbibition, germination potential, germination rate, plumule and radicle development, and root and shoot growth. By screening maize cultivars when they are still in the seedling stage, it is possible to identify inbred lines that are tolerant to drought and can thrive during later growth stages [13].

It is well known that drought is one of the environmental factors that could influence the growth, development, and production of many crops, including maize [14]. When faced with certain environmental conditions, such as drought, some plants can adapt to the environment [15–18]. Maize undergoes various development stages, from germination to harvest maturity, which include seedling establishment, vegetative growth and development, and reproductive growth. These stages are highly susceptible to the detrimental effects of drought stress [19].

Drought conditions lead to a decrease in cell division and cell expansion. This, in turn, results in a reduction in leaf area and vegetative growth, as well as a decrease in cell size due to lower water potential. Leaf elongation is determined by turgor pressure, light interception, and flux assimilation [19–21].

The aboveground parts of the plant, including the leaves and stem, are affected by drought. Leaves may become curled and folded, leading to a decrease in leaf area, light interception, and photosynthetic activity. This can increase the leaf-to-stem ratio, indicating a higher level of growth retardation in the stem compared to the leaves [21–23]. Meanwhile, the underground part of the plant relies heavily on the roots as primary detectors or sensors of drought stress. Drought stress significantly impacts the structural traits of roots, such as length, volume, density, and number, which can disrupt the overall growth of the plant. Additionally, roots are responsible for functional traits like spatial and temporal water uptake. For example, mild stress can cause an increase in root length in maize, while severe stress can lead to a decrease in root length [24,25]. The reproductive and vegetative stages are two stages of maize growth. The vegetative phase starts with VE-emergence and lasts until VT-tassel emergence, which occurs after the V1-one leaf collar visible, V2-two leaf collar visible, V3-three leaf collar visible, V4-four leaf collar visible, and V5-V6-five to six leaf collars visible [26]. The occurrence of visible wilting for four consecutive days during the vegetative growth stages of maize, specifically from V1 to V5, will significantly diminish maize production from 5 to 10% [26].

Plants have many mechanisms they can use in response to water stress and resistance. Accumulation of compatible solutes is an important mechanism. Many studies have indicated that the accumulation of compatible solutes, such as proline and soluble sugars,

enhances plant resistance to various stresses like drought [27–30], high temperature, and high salinity. The primary function is to prevent water loss in order to maintain cell turgor and to maintain the gradient of water uptake into the cell. In addition, they contribute to the protection of enzymes and the integrity of membrane structures. These solutes exhibit high hydrophilic properties, allowing them to potentially substitute water molecules around nucleic acids, proteins, and membranes during water shortages [31–39].

Re-watering after a water stress period may have a substantial effect on recovering plant growth and development. Research on drought [40] has demonstrated that plants adapt to repeated stress episodes. Water deficit stress can inhibit leaf growth, and, when plants are rewatered, the leaves expand fully again [41]. Rewatering drought-treated plants resulted in the elimination of leaf wilting, but, during the water recovery period, the mean values of the metrics related to photosynthesis remained lower than those of the control plants. It is probable that either the recovery period was too short or the photosystem's damage from drought stress was too great. These findings suggest that there might be a strong correlation between the physiological water content and the water content of seedling leaves [41]. When plants experience environmental stressors, a variety of biological processes are triggered [42–44], but those connected to photosynthesis are the most susceptible to water shortage and recovery [45–47].

Rapid seedling tests are feasible for elucidating the genetic response of root growth to low water potential. Traditional breeding programs have helped to generate genotypes that are high-yielding and drought-tolerant [48–50]. It is assumed that the ability to osmotically adjust and protect cellular components from stress will be a critical factor influencing tolerance to episodic drought during the vegetative phase. It is also worth investigating the response of maize plants regarding the differential accumulation of compatible solutes (proline and soluble sugar) under water deficit at the early vegetative growth stage considering rapid recovery at pre-drought levels upon rewatering. Therefore, the present study was conducted to investigate the morpho-physiological and biochemical responses of maize varieties at the early vegetative stage under water stress conditions.

## 2. Materials and Methods

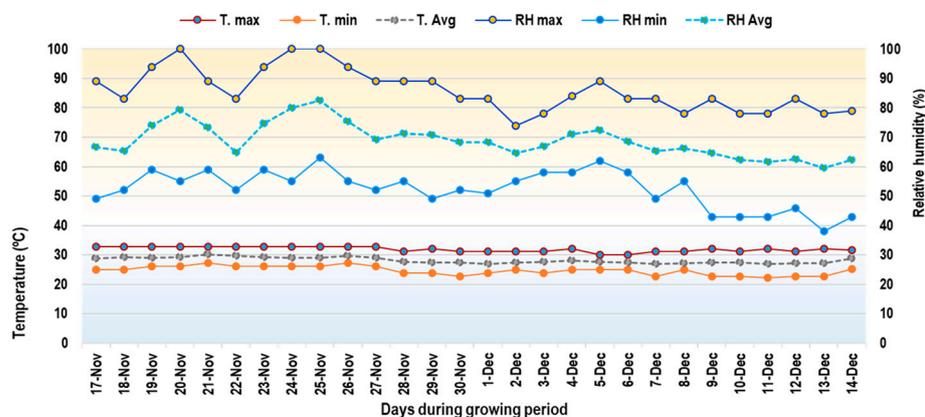
### 2.1. Plant Materials, Treatments and Growth Conditions

The experiments were conducted in a greenhouse under the Faculty of Agriculture, Kasetsart University, Thailand. Factorial Randomized Block Design was used in this study with two factors and four replicates. The first factor was three single-cross maize varieties (V): V1: SUWAN2301 (from National Maize and Sorghum Research Center-NCSRC, Thailand); V2: SUWAN4452 (from NCSRC, Thailand); and V3: S7328 (from Syngenta Company, Thailand). The second factor was water deficit condition, with 3 levels of irrigation (I): I<sub>1</sub>: watering every day; I<sub>2</sub>: watering at 2-day intervals; and I<sub>3</sub>: watering at 4-day intervals. Three maize varieties were selected assuming their drought tolerance levels (V1 > V2 > V3) according to Kanavittaya [51] and Molla et al. [52].

Seeds were disinfected using a 2% sodium hypochlorite (NaOCl) solution and then imbibed in distilled water for 24 h. Next, the incubated seeds were planted in air-dried soil-filled 5 L plastic pots at a depth of 2 cm. Finally, three pots with three plants each were kept for each treatment. For inadvertent usage, three additional pots for each treatment under the same management were kept next to the main experimental plot. Pot soil was prepared with a 1:1 ratio of Chia Tai soil and Field soil (collected from 0 to 30 cm depth of maize growing area, Nakhon Ratchasima, Thailand). The soil properties of Chia Tai soil were loam textured with porosity 50–60%, nitrogen 1.5%, phosphorous 1.0%, potassium 0.8%, organic matter 4%, C/N ratio 11, pH 6.5–7.5, EC 1–2.5 mS cm<sup>-1</sup>, and bulk density 3.5–4.5 g cm<sup>-3</sup>. The properties of field soil were nitrogen 0.09%, phosphorous 72 mg kg<sup>-1</sup>, potassium 140 mg kg<sup>-1</sup>, calcium 2400 mg kg<sup>-1</sup>, magnesium 210 mg kg<sup>-1</sup>, and organic matter 2.28%, and its texture was silty clay with sand 9.6%, silt 40.2%, and clay 50.2%. The soil mixture was allowed to dry for about two weeks, being regularly turned over the entire time. The gravimetric approach was used to determine the soil's moisture content (0.16%)

once it was sufficiently dry [53]. To determine how much air-dried soil (4.0064 kg) would be required to make 4 kg of oven-dried soil, the soil’s moisture content was used. Each pot was filled with soil that had dried in the air and weighed the same as 4 kg of oven-dried soil. Three identical transparent 2 L containers were filled with some of the air-dried potting soil to evaluate how much water would be required to bring the soil to 100% field capacity (FC). After that, water was evenly applied to the soil’s surface until it was completely saturated. The containers were immediately wrapped in plastic and left in a cool, shaded area for three days. This was performed in order to enable the extra water to entirely drain through the pot’s bottom hole and to allow the soil to totally saturate and reach 100% FC. A 100 g sample of moist soil was taken from each container after 3 days at a depth of 5–10 cm below the soil surface. The gravimetric method was then used to calculate the moisture content of the wet soil [54]. The potting soil’s moisture content at 100% FC (44.12%) was calculated from the mean of the three replicates. In the pot experiment, air-dried soil was used to fill the pots; hence, the amount of water needed to make the oven-dried equivalent mass 100% FC had to account for the moisture that was already present in the air-dried soil (44.12%–0.16% = 43.96%). To raise a 1 g mass of oven-dried soil to 100% FC, however, 0.4396 g/g of water is required, which is represented by the value of 43.96%. Thus, it was determined that 1758.4 g of water would be required to obtain 4 kg (4000 g) of oven-dried soil to 100% FC. It is simpler to predict the mass of pots following water additions if the water needed to raise the soil to FC is reported as a mass. The mass of water that was to be added was calculated by weighing the pots, but the water was actually added in volume form. In order to account for the density of water (1 g/cm<sup>3</sup>), the amount of water added was always adjusted to its volume equivalent (mass density). By calculating the volume of water needed for 4 kg of oven-dried soil to reach 100% FC, a volume of 1758.4 cm<sup>3</sup> was calculated. Each pot’s mass, soil mass, and plant mass were taken into account when determining how much water needed to be added [55]. The plant’s changing mass over time was also taken into account.

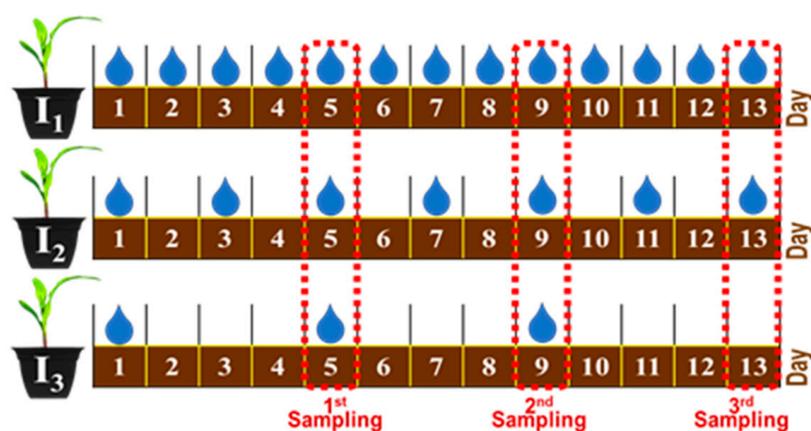
The pot experiment was conducted from 17 November to 14 December 2019. Sufficient soil moisture was maintained in all pots before starting the watering treatment levels for smooth germination and initial plant growth. Treatment-wise, deficit watering was started after the V<sub>2</sub> stage (two leaves with visible collar [26]) of the maize plant, i.e., 16 days after planting. Watering was performed at 8:00 am each day, with sufficient water to reach the soil FC. Well-watered pots were watered regularly to maintain 100% FC. During the experimentation, the volumetric soil moisture content was monitored every 12 h (7:00 am and 7:00 pm) using the WET-2 sensor (Delta-T Devices, Cambridge, England). The maximum, minimum, and average temperature and relative humidity of the tropical environment during the growing period are shown in Figure 1.



**Figure 1.** Temperature, °C (T. max, maximum temperature; T. min, minimum temperature; and T. avg, average temperature) and relative humidity, % (RH max, maximum relative humidity; RH min, minimum relative humidity; and RH avg, average relative humidity) during the whole growing period.

## 2.2. Sampling and Measurements

Data were collected on leaf water potential using pressure bomb, leaf area, plant height, stem perimeter, root length, shoot dry weight, root dry weight, leaf greenness, root length to shoot length ratio, absolute growth rate, proline, and total soluble sugar (TSS) content in leaf and root. They were measured at the end of every interval of day 2 (of  $I_2$ ) and day 4 (of  $I_3$ ) for 3 consecutive destructive sampling dates matching with the  $I_3$  sampling dates, i.e., 5, 9, and 13 d, just before rewatering (Figure 2).



**Figure 2.** Schematic view of irrigation and destructive plant sampling schedule after watering treatment initiated in  $I_1$  = watering every day;  $I_2$  = 2 days of watering interval; and  $I_3$  = 4 days of watering interval.

### 2.2.1. Leaf Water Potential

Treatment-wise, the fully expanded topmost three leaves in each replicate were used at 8:00 am to measure leaf water potential (LWP) with a Scholander pressure chamber (Soil Moisture Equipment Corp., Model 3005, Santa Barbara, CA, USA).

### 2.2.2. Proline Content Analysis

Proline content was measured by following the Bates method [56,57]. To prepare the sample, a fresh gram of the sample was crushed using a mortar and pestle. The crushed sample was then mixed with 5 mL of 3% sulfosalicylic acid to achieve homogenization. The resulting mixture was subjected to centrifugation at  $6000 \times g$  rpm for 15 min. From the resulting mixture, 1 mL of the supernatant was collected and combined with 1 mL of ninhydrin and 1 mL of acetic acid. The mixture was then heated in a water bath for an hour and subsequently incubated on ice for 5 min. Furthermore, 2 mL of the solution was extracted by vigorously shaking it with 2 mL of toluene using a vortex. The upper phase was taken, and absorbance was measured with a spectrophotometer (BioDrop Duo +- Micro-volume Spectrophotometer) at 520 nm. To determine the proline content of the sample, a standard curve was made using pure proline. The content of proline was expressed in unit of  $\mu\text{mol}$  per gram fresh weight ( $\mu\text{mol g}^{-1}$  FW).

### 2.2.3. Total Soluble Sugar (TSS) Content Analysis

Total soluble sugar content was measured by using the anthrone method [58]. A total of 0.5 g of the fresh sample was crushed in a mortar, and 5 mL of 80% ethanol was added. The mixture was filtered through filter paper (Wathman No. 1). A total of 12.5 mL of 80% ethanol was added to the solution. A total of 1 mL of the solution was extracted and mixed with 1 mL of 0.2 anthrone. The mixture was then heated at  $100^\circ\text{C}$  for 10 min. To terminate the reaction, the mixture was subsequently incubated on ice for 5 min. The determination of the total soluble sugar content was performed using a spectrophotometer (BioDrop Duo +- Micro-volume Spectrophotometer) at a wavelength of 620 nm. The calculation of the total soluble sugar content was accomplished by generating a standard

curve using a standard glucose solution. The final results were expressed in milligrams per gram of fresh weight ( $\text{mg g}^{-1}$  FW).

#### 2.2.4. Calculations and Other Data

(i) Absolute growth rate (AGR) was calculated using [59] the following formula:

$$\text{AGR (g day}^{-1}\text{)} = (W2 - W1)/(t2 - t1) \quad (1)$$

where  $t1$  and  $t2$  are the 1st and 2nd harvesting times, and  $W1$  and  $W2$  are dry weight at time  $t1$  and  $t2$ , respectively.

(ii) Leaf area (LA) was determined according to the method described by Radford [60].

$$\text{LA} = K (L \times W) \quad (2)$$

where  $LA$  = leaf area ( $\text{cm}^2$ );  $K$  = constant (0.75);  $L$  = leaf length (cm); and  $W$  = maximum leaf width (cm).

(iii) Stress tolerance index (STI) was calculated using [61] the following formula:

$$\text{STI} = (Y_{si} \times Y_{pi})/(Y_{p2}) \quad (3)$$

where  $Y_{si}$  and  $Y_{pi}$  are biomass yield under stress and non-stress conditions, respectively, for variety "i".  $Y_p$  is mean biomass yield under non-stress condition.

(iv) Recovery rate (RR) was calculated using the following formula:

$$\text{RR} = \frac{(\text{2nd observation} - \text{1st observation}) \times 100}{\text{1st observation}}$$

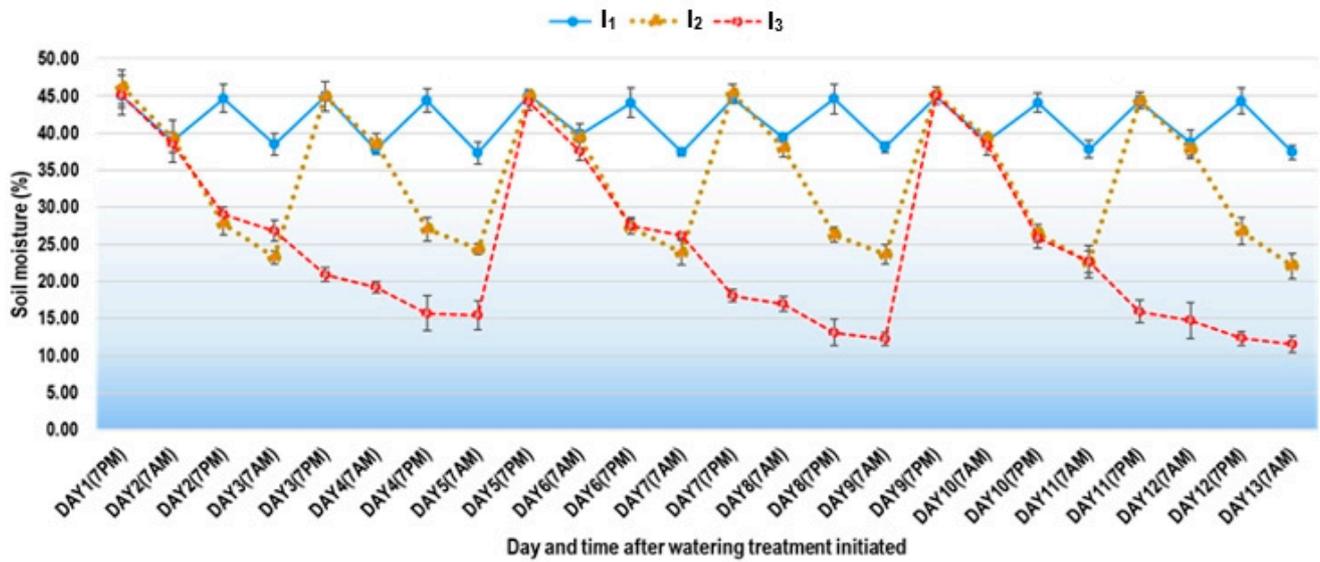
#### 2.3. Statistical Analysis

Different data were subjected to analysis of variance (ANOVA) for a factorial Randomized Complete Block (RCB) design design. The mean values were compared with the Fisher's Protected Least Significant Difference (LSD) procedure at  $p = 0.05$ . Statistical software R [62], version 4.2.3, using ggplot2 package, CropStat 7.2, and RStudio statistical packages were employed for analysis. The two R packages FactoMineR and factoextra were used to generate a principal component analysis (PCA) biplot. The R package corrplot was used to analyze the correlation matrix. In order to better reflect the impact of water stress on crop growth, this PCA only uses two treatments of well-watered and prolonged water deficit for analyzing.

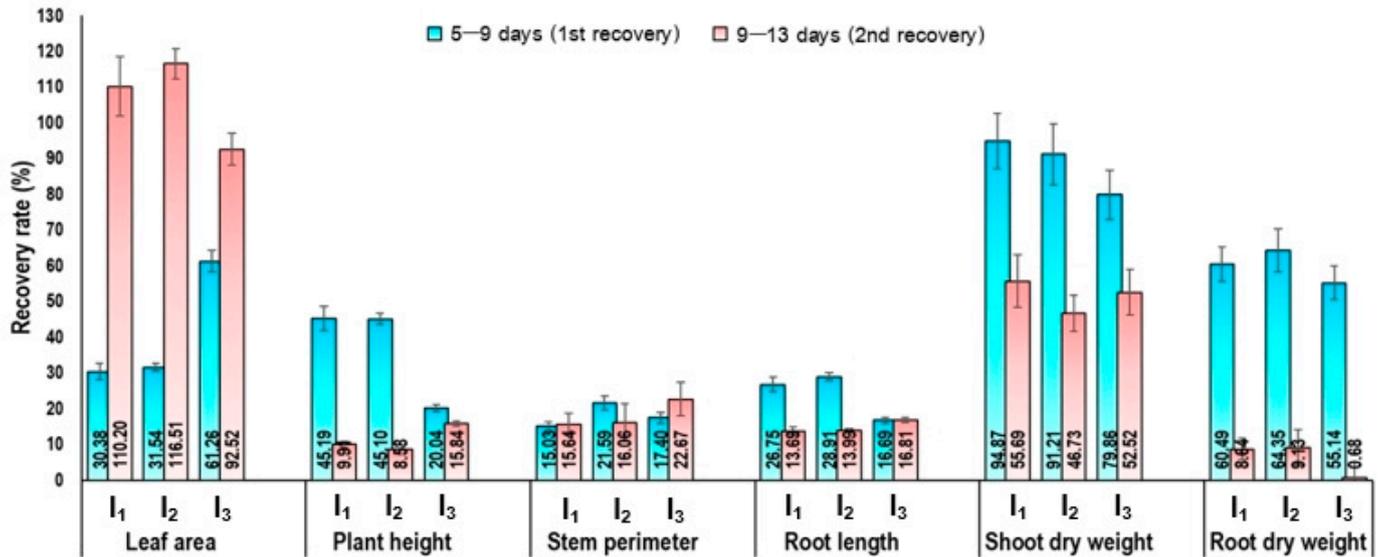
### 3. Results

The results of ANOVA presented in Tables 1–3 for morphological, physiological, and biochemical traits, respectively, revealed the individual effect of each irrigation level and variety, as well as their interactions, on the 14 investigated traits. In the case of morphological traits, the effect of irrigation level was significant for all traits except RL at 9 and 13 DAWTI, whereas the effect of variety was significant for all traits with the exception of some stages, i.e., PH at 5 DAWTI, SP at 9 and 13 DAWTI, RL at 9 DAWTI, SDW at 5 and 9 DAWTI, and RDW at 5 DAWTI. All traits were unaffected by the interaction between irrigation level and variety. In the case of physiological traits, all traits were significantly affected by irrigation level except leaf greenness at 5 and 9 DAWTI, whereas, except for leaf greenness, all traits were significantly affected by variety. The effect of the interaction between irrigation level and variety was significant for LWP at 5 and 13 DAWTI but non-significant for other traits. All biochemical traits were significantly affected by irrigation level, whereas the effect of variety was significant for all traits except PrL at 13 DAWTI, PrR at 9 DAWTI, and TSSL at 5 and 9 DAWTI. The interaction effect was significant for all traits except for PrR at 9 and 13 DAWTI and TSSL at 9 DAWTI. The volumetric soil moisture dynamics during the water stress period (after watering treatment

started) are presented in Figure 3. The recovery rates of maize, as influenced by irrigation level and variety, were distinct and are presented in Figures 4–9.



**Figure 3.** Changes in soil moisture content (%) after 16 days of planting when watering treatment was initiated in I<sub>1</sub> = watering every day; I<sub>2</sub> = 2 days of watering interval and I<sub>3</sub> = 4 days of watering interval. Vertical bars are twice the standard deviation.



**Figure 4.** Recovery rate of different morphological traits of maize as affected by irrigation level at 5–9 days (1st recovery) and at 9–13 days (2nd recovery). I<sub>1</sub>, 1 day watering interval; I<sub>2</sub>, 2 days watering interval; I<sub>3</sub>, 4 days watering interval. Vertical bars are twice the standard deviation.

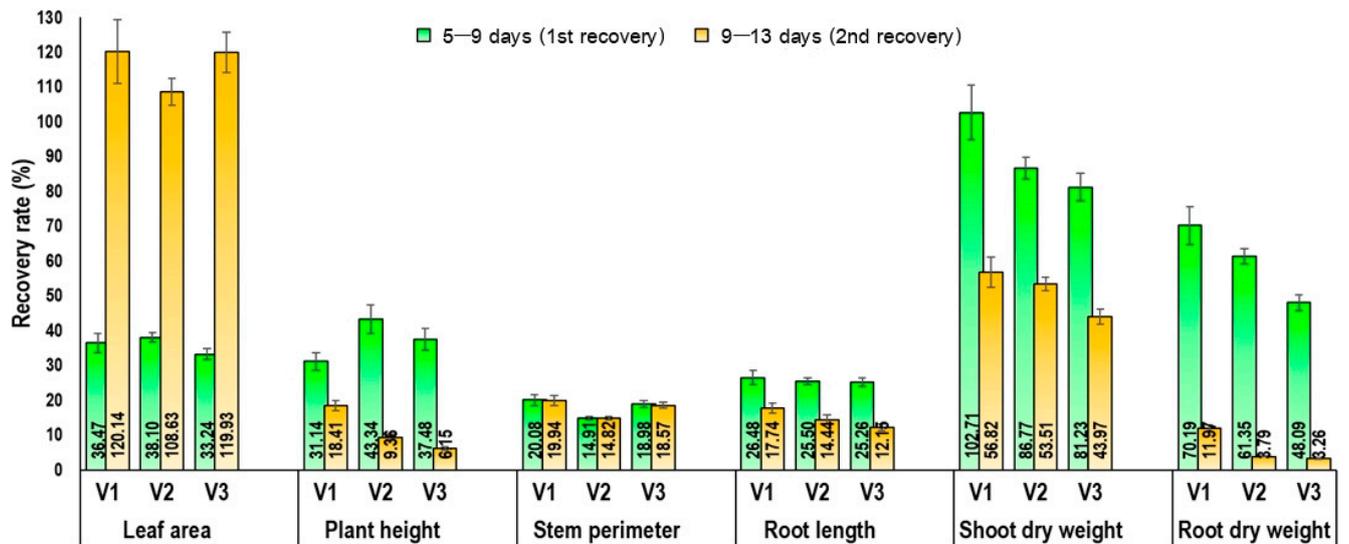


Figure 5. Recovery rate of different morphological traits of maize as affected by variety at 5–9 days (1st recovery) and at 9–13 days (2nd recovery). V1, SUWAN2301; V2, SUWAN4452; V3, S7328. Vertical bars are twice the standard deviation.

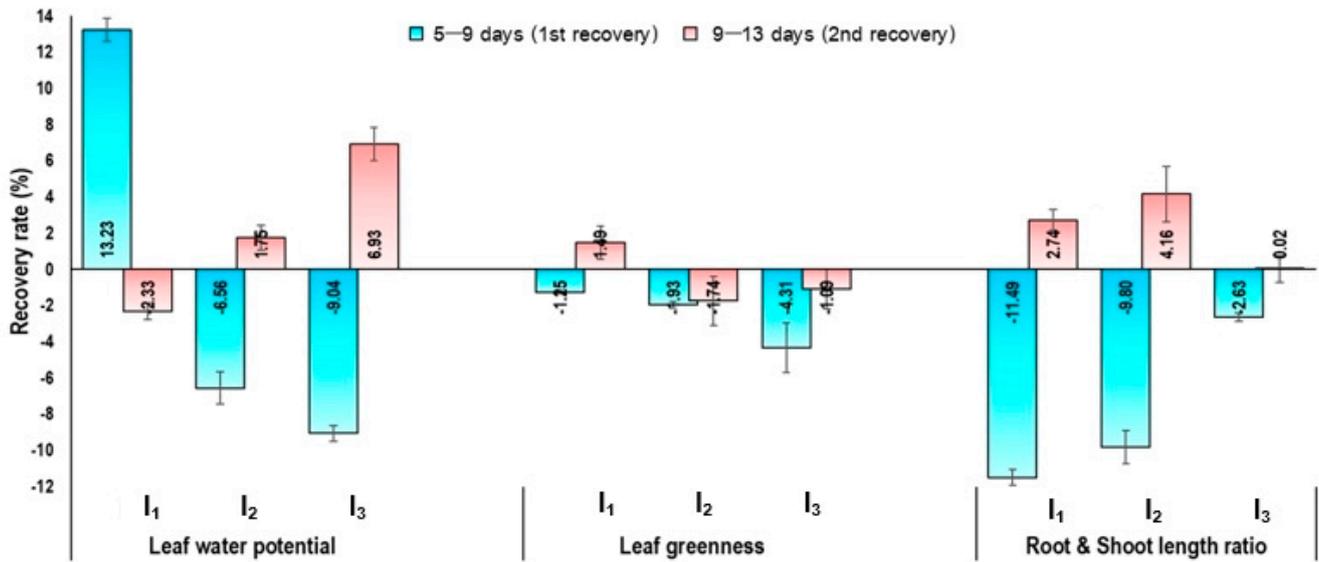


Figure 6. Recovery rate of different physiological traits of maize as affected by irrigation level at 5–9 days (1st recovery) and at 9–13 days (2nd recovery). I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals. Vertical bars are twice the standard deviation.

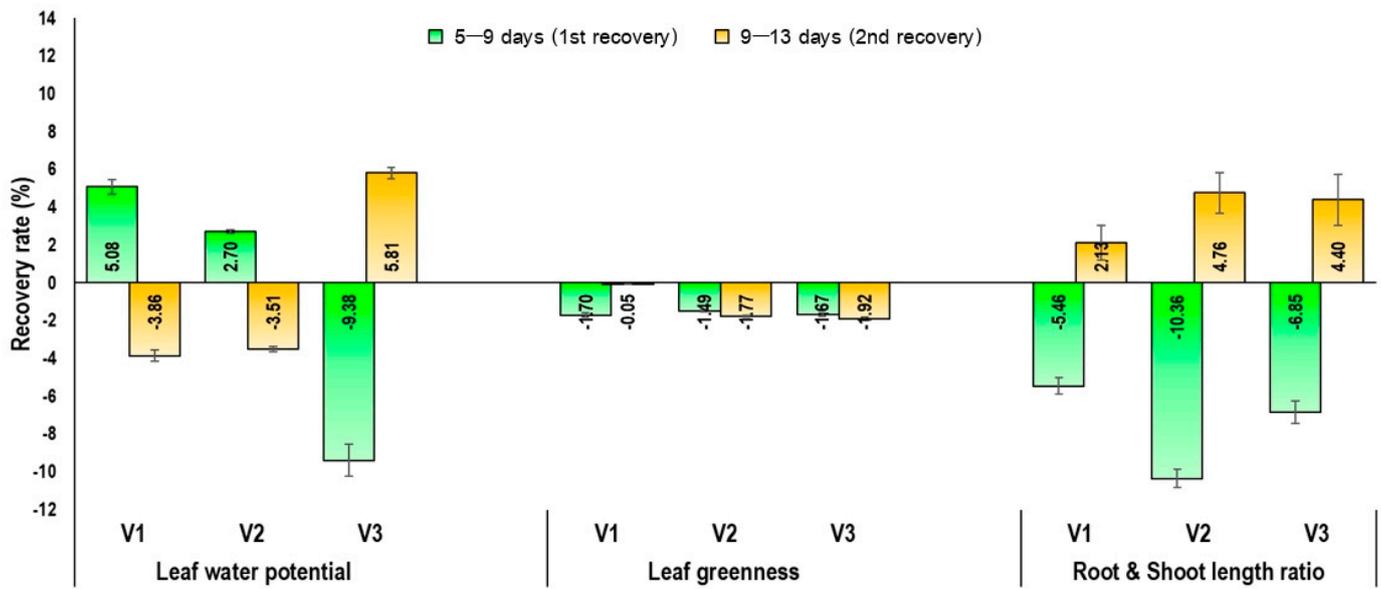


Figure 7. Recovery rate of different physiological traits of maize as affected by variety at 5–9 days (1st recovery) and at 9–13 days (2nd recovery). V1, SUWAN2301; V2, SUWAN4452; V3, S7328. Vertical bars are twice the standard deviation.

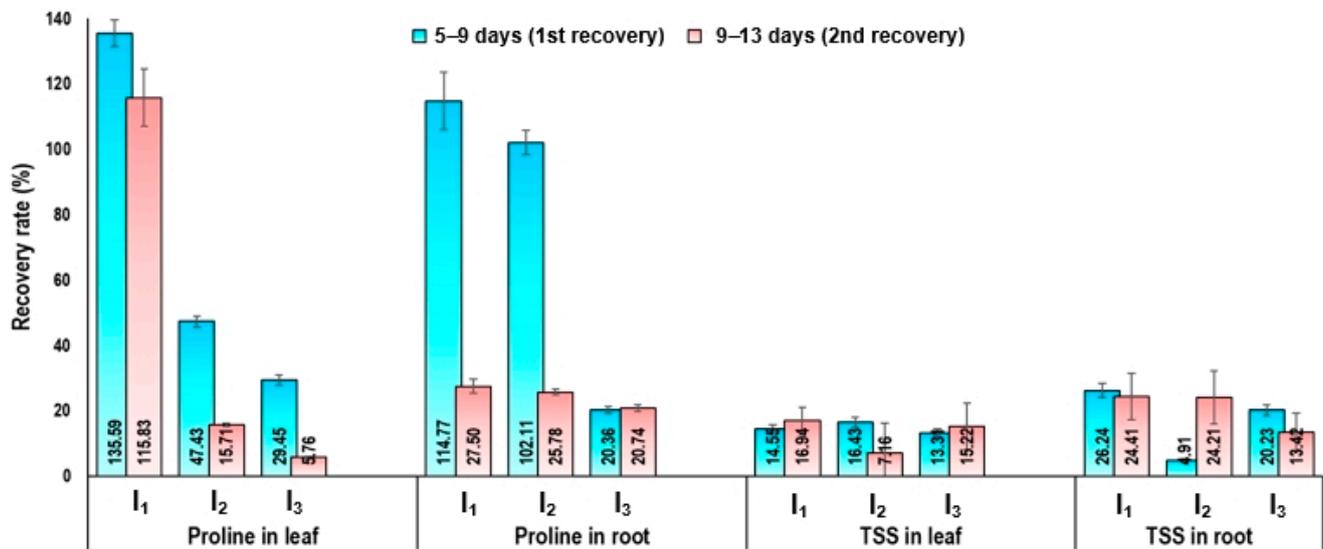


Figure 8. Recovery rate of different biochemical traits of maize as affected by irrigation level at 5–9 days (1st recovery) and at 9–13 days (second recovery). I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals. Vertical bars are twice the standard deviation.

**Table 1.** Analysis of variance of the effects of irrigation level, variety, and their interactions on different morphological parameters of maize.

Source of Variance	df	Mean Sum Square																	
		Leaf Area per Plant (cm <sup>2</sup> )			Plant Height (cm)			Stem Perimeter (cm)			Root Length (cm)			Shoot Dry Weight (g Plant <sup>-1</sup> )			Root Dry Weight (g Plant <sup>-1</sup> )		
		5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI
Replication	3	14.67	66.51	502.6	11.313	11.010	43.739	0.699	0.50	2.09	11.08	17.45	2.48	0.873	0.445	7.225	0.267	0.092	0.319
Irrigation level (I)	2	2018.28 **	1652.54 **	10,690.4 **	70.440 **	480.935 **	445.625 **	11.785 **	14.60 **	13.60 **	33.16 **	6.20 NS	18.93 NS	13.818 **	63.254 **	151.392 **	1.729 **	5.431 **	9.216 **
Variety (V)	2	316.65 **	952.90 **	1621.9 **	16.361 NS	114.104 **	70.392 *	2.206 **	1.77 NS	1.66 NS	20.51 *	17.91 NS	51.89 *	0.761 NS	2.875 NS	16.913 **	0.246 NS	2.901 **	5.619 **
I × V	4	67.77 NS	73.82 NS	216.5 NS	1.000 NS	18.191 NS	13.816 NS	0.250 NS	0.81 NS	0.12 NS	0.98 NS	5.49 NS	1.73 NS	0.596 NS	0.131 NS	1.894 NS	0.023 NS	0.194 NS	0.088 NS
Error	24	40.24	54.02	218.5	8.661	20.207	18.296	0.384	0.71	0.81	4.08	8.04	13.67	0.255	1.126	2.141	0.187	0.296	0.241

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; NS = non-significant; DAWTI = days after watering treatment initiated; df = degree of freedom.

**Table 2.** Analysis of variance of the effects of irrigation level, variety, and their interactions on different physiological parameters of maize.

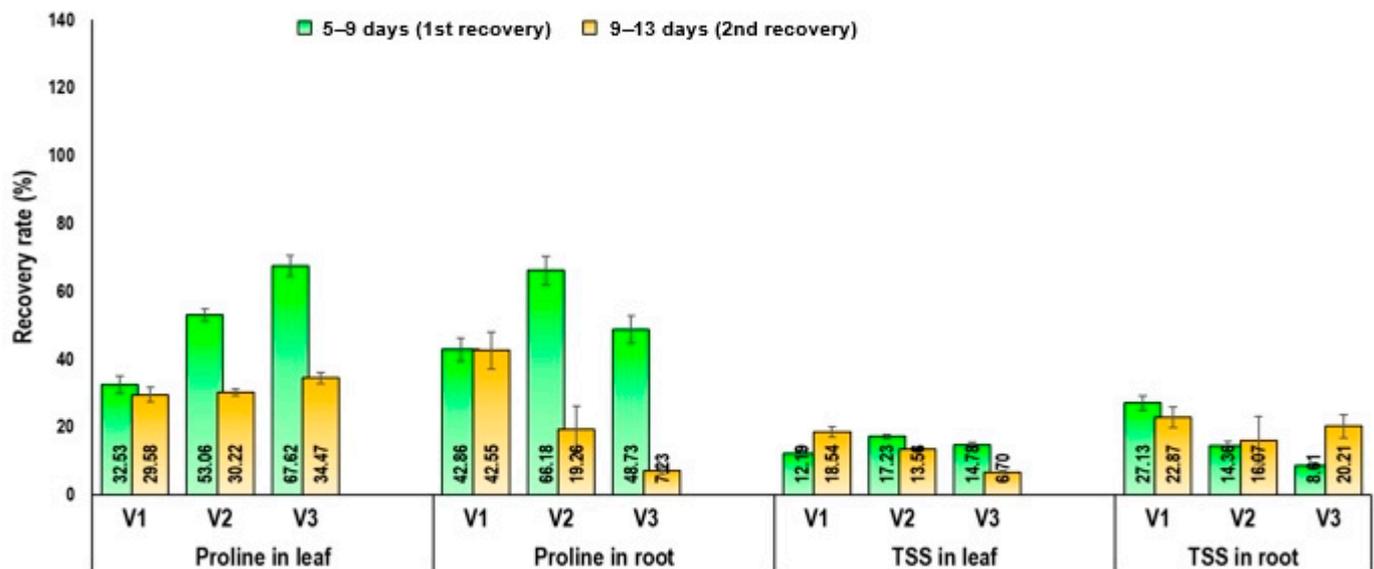
Source of Variance	df	Mean Sum Square									
		LWP (MPa)			LG (SPAD Value)			RL:SL			AGR (g day <sup>-1</sup> )
		5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5–13 DAWTI
Replication	3	0.018	0.003	0.026	76.913	11.077	82.713	0.001	0.010	0.014	0.049
Irrigation level (I)	2	2.803 **	2.033 **	2.427 **	43.621 NS	73.961 NS	106.266 **	0.274 **	0.396 **	0.345 **	1.679 **
Variety (V)	2	0.163 **	0.152 **	0.111 **	6.417 NS	5.544 NS	12.387 NS	0.078 **	0.100 **	0.042 *	0.495 **
I × V	4	0.022 *	0.023 NS	0.039 **	2.722 NS	4.269 NS	3.365 NS	0.001 NS	0.000 NS	0.002 NS	0.014 NS
Error	24	0.007	0.010	0.008	17.224	27.472	20.414	0.006	0.016	0.010	0.018

\* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; ns = nonsignificant; dawti = days after watering treatment initiated; df = degree of freedom; lwp = leaf water potential; lg = leaf greenness; rl:sl = root length to shoot length ratio; agr = absolute growth rate.

**Table 3.** Analysis of variance of the effects of irrigation level, variety, and their interactions on different biochemical parameters of maize.

Source of Variance	df	Mean Sum Square											
		PrL ( $\mu\text{mol g}^{-1}$ FW)			PrR ( $\mu\text{mol g}^{-1}$ FW)			TSSL ( $\text{mg g}^{-1}$ FW)			TSSR ( $\text{mg g}^{-1}$ FW)		
		5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI
Replication	3	0.016	0.007	0.045	0.009	0.006	0.016	0.027	0.021	0.026	0.007	0.003	0.009
Irrigation level (I)	2	1.393 **	1.390 **	0.526 **	2.157 **	1.647 **	1.527 **	0.170 **	0.202 **	0.282 **	0.084 **	0.134 **	0.105 **
Variety (V)	2	0.143 **	0.063 **	0.073 NS	0.055 **	0.025 NS	0.194 **	0.017 NS	0.012 NS	0.081 **	0.009 **	0.044 **	0.068 **
I $\times$ V	4	0.048 **	0.049 **	0.073 *	0.027 **	0.026 NS	0.011 NS	0.020 *	0.006 NS	0.024 *	0.014 **	0.010 *	0.016 *
Error	24	0.004	0.009	0.026	0.003	0.013	0.026	0.005	0.016	0.009	0.001	0.003	0.005

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; NS = nonsignificant; FW = fresh weight; DAWTI = days after watering treatment initiated; df = degree of freedom; PrL = Proline in leaf; PrR = Proline in root; TSSL = total soluble sugar in leaf; TSSR = total soluble sugar in root.



**Figure 9.** Recovery rate of different biochemical traits of maize as affected by variety at 5–9 days (1st recovery) and at 9–13 days (second recovery). V1, SUWAN2301; V2, SUWAN4452; V3, S7328. Vertical bars are twice the standard deviation.

### 3.1. Soil Moisture Content

Soil moisture content (SMC) was monitored using the WET-2 sensor under different irrigation level during the 13 days of different water stress periods started after 16 days of planting to see the dynamics of soil water. The pot soil moisture was chronologically decreased after watering to next rewatering. In the  $I_1$  treatment, watering was performed every morning, and the pot soil moisture content of this treatment was maintained at an average of 37–46% throughout the experiment. In the  $I_2$  and  $I_3$  treatments, watering was performed at 2-day and 4-day intervals, respectively. The results showed that the soil moisture declined prominently. The soil moisture content of  $I_2$  and  $I_3$  decreased to an average of 22.10% and 11.57%, respectively, at the end of each stress cycle (Figure 3).

### 3.2. Morphological Parameters of Maize

#### 3.2.1. Leaf Area

Leaf area was measured to understand how water stress and variety affect the plant's growth and development. In response to irrigation level and variety, the LA was differently affected (Tables 1, 4 and 5). Under water deficit level, at 13 DAWTI, the highest LA was measured to be 156.24 cm<sup>2</sup> in  $I_1$ , whereas the lowest LA was measured in the  $I_3$  treatment (98.01 cm<sup>2</sup>) (Table 4). The LAs at all sampling dates were relatively higher in V1 and relatively lower in V3 compared to other varieties (Table 3). The variety V1 could produce maximum LA under all water deficit levels at all sampling dates except at 5 DAWTI in well-watered condition. Furthermore, V3 produced the minimum LA at all levels. Under the short water deficit level ( $I_2$ ), the variety V1 lost only 7.00, 7.22, and 13.78%, whereas V2 and V3 lost 14.31, 17.44, and 15.82% and 23.49, 17.96, and 2.79%, at 5, 9, and 13 DAWTI, respectively. Under the prolonged water deficit level ( $I_3$ ), the variety V1 lost only 30.75, 23.14, and 37.70%, whereas the V2 and V3 lost 53.79, 41.12, and 41.02% and 49.46, 30.03, and 42.13%, at 5, 9, and 13 DAWTI, respectively. Therefore, it was found that V1 lost relatively less LA at all levels.

**Table 4.** Main effect of irrigation level on different morphological characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

Source of Variation	Leaf Area per Plant (cm <sup>2</sup> )			Plant Height (cm)			Stem Perimeter (cm)			Root Length (cm)			Shoot Dry Weight (g Plant <sup>-1</sup> )			Root Dry Weight (g Plant <sup>-1</sup> )		
	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI
I <sub>1</sub>	57.01 a	74.33 a	156.24 a	26.95 a	39.12 a	43.00 a	6.77 a	7.79 a	9.01 a	20.21 b	25.62	29.12	4.81 a	9.37 a	14.58 a	2.89 a	4.64 a	5.04 a
I <sub>2</sub>	48.63 b	63.97 b	138.50 b	23.83 b	34.58 b	37.55 b	5.81 b	7.07 b	8.20 b	20.41 b	26.31	29.99	4.40 a	8.41 b	12.35 b	2.52 b	4.14 b	4.52 b
I <sub>3</sub>	31.57 c	50.91 c	98.01 c	22.17 b	26.62 c	30.83 c	4.79 c	5.62 c	6.90 c	23.18 a	27.05	31.60	2.78 b	5.00 c	7.63 c	2.13 c	3.31 c	3.33 c
F test (I)	**	**	**	**	**	**	**	**	**	**	NS	NS	**	**	**	**	**	**
LSD <sub>0.05</sub>	5.34	6.19	12.46	2.48	3.79	3.60	0.52	0.71	0.76	1.70	2.39	3.12	0.43	0.89	1.23	0.36	0.46	0.41

\*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test.

**Table 5.** Main effect of variety on different morphological characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

Source of Variation	Leaf Area per Plant (cm <sup>2</sup> )			Plant Height (cm)			Stem Perimeter (cm)			Root Length (cm)			Shoot Dry Weight (g Plant <sup>-1</sup> )			Root Dry Weight (g Plant <sup>-1</sup> )		
	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI
V1	47.05 a	64.21 a	141.35 a	23.13	30.33 b	35.92 b	5.74 ab	6.89 ab	8.26	21.51 a	27.21	32.03 a	3.81 b	7.73 ab	12.12 a	2.60	4.43 a	4.96 a
V2	46.17 a	63.76 a	133.02 a	25.46	36.50 a	39.92 a	6.24 a	7.18 a	8.24	21.39 a	26.84	30.72 ab	4.28 a	8.00 a	12.28 a	2.59	4.18 a	4.34 b
V3	40.40 b	53.83 b	118.39 b	24.36	33.49 ab	35.55 b	5.39 b	6.41 b	7.61	19.90 b	24.93	27.96 b	3.89 ab	7.05 b	10.15 b	2.35	3.48 b	3.59 c
F test (V)	**	**	**	NS	**	*	**	NS	NS	*	NS	*	NS	NS	**	NS	**	**
LSD <sub>0.05</sub>	5.34	6.19	12.46	2.48	3.79	3.60	0.52	0.71	0.76	1.40	2.39	3.12	0.43	0.89	1.23	0.36	0.46	0.41

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test.

### 3.2.2. Plant Height

Plant height (PH), as an important growth parameter, was measured at 5, 9, and 13 DAWTI, and we found that it was significantly affected by water deficit level (I) and variety (V), while the interaction between water deficit level and varieties ( $I \times V$ ) was non-significant (Tables 1, 4 and 5). Plant height was measured from the base of the ground to where half of the leaves were fully unfolded. The main impact of water deficit levels was observed at 13 DAWTI. The highest plant height of 43.00 cm was recorded for the daily watering ( $I_1$ ) treatment, followed by 37.55 cm and 30.83 cm for the 2-day and 4-day watering intervals ( $I_2$  and  $I_3$ ), respectively. As the water deficit level increased, there was a reduction in plant height (Table 4). V2 showed, significantly, the highest plant height (25.46, 36.50, and 39.92 cm at 5, 9 and 13 DAWTI, respectively) followed by V3 and V1, which were not significantly different from each other (Table 5). It was found that V2 could produce maximum PH under all water deficit levels at all sampling dates, while V1 produced the minimum PH at all levels. The variety V1 lost plant height 8.51, 2.26, and 9.32%, whereas V2 and V3 lost 11.50, 15.91, and 18.75% and 14.39, 14.63, and 8.83%, at 5, 9, and 13 DAWTI, respectively, at the short water deficit level ( $I_2$ ). Under the prolonged water deficit level ( $I_3$ ), the variety V1 lost only 15.47, 24.06, and 22.98%, whereas V2 and V3 lost 18.09, 35.23, and 31.77% and 19.38, 34.95, and 29.45%, at 5, 9, and 13 DAWTI, respectively.

### 3.2.3. Stem Perimeter

The strength and plant water availability of maize plants may vary depending on stem thickness. Stem perimeter (SP) was mostly affected by water deficit level, whereas the varieties (except 5 DAWTI) and their interactions were insignificant at all DAWTI (Tables 1, 4 and 5). The SP was measured from the base of the plant at ground level. The main impact of water deficit levels was visible at 13 DAWTI. The highest SP of 9.01 cm was recorded for the daily watering ( $I_1$ ) treatment, followed by 8.20 cm and 6.90 cm for the 2- and 4-day watering intervals ( $I_2$  and  $I_3$ ), respectively. As the water deficit level increased, there was a reduction in stem perimeter (SP) (Table 4). V2 showed, significantly, the highest SP, followed by V1 and V3 at 5 and 9 DAWTI, but it was little bit higher in V1 at 13 DAWTI (Table 5).

### 3.2.4. Root Length

The root system is responsible for absorbing water and nutrients from the soil, and it is, therefore, essential for plant survival under water stress. Root length was significantly different due to water deficit (at 5 DAWTI) and variety (at 5 and 13 DAWTI) (Tables 1, 4 and 5). The root length was non-significantly influenced by interaction between water deficit levels and varieties ( $I \times V$ ) at all three sampling dates. The root length was higher under  $I_3$  in all cases compared to  $I_1$  and  $I_2$ . Maximum root length was measured in V1 (22.51, 27.21, and 32.03 cm at 5, 9, and 13 DAWTI, respectively). The minimum values were recorded from V3 at all dates (Table 5).

### 3.2.5. Shoot Dry Weight

Shoot dry weight (SDW) was measured to understand the plant's productivity under different treatments, and we found that water deficit levels significantly affected the SDW at 5, 9, and 13 DAWTI, whereas variety significantly influenced it during later stage, at 13 DAWTI (Tables 1, 4 and 5). The interaction between water deficit levels and varieties was non-significant at all dates. The main effect of water deficit levels showed in the maximum SDW obtained from  $I_1$  (4.81, 9.37, and 14.58 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) and minimum SDW from  $I_3$  (2.78, 5.00, and 7.63 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively). The SDW was higher in V2 (4.28, 8.00, and 12.28 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) and the minimum was seen in V3 (3.89, 7.05, and 10.15 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) (Table 5).

### 3.2.6. Root Dry Weight

As an important character of dry matter partitioning under water stress, root dry weight (RDW) was measured. The water levels significantly influenced the RDW at 5, 9, and 13 DAWTI, where variety significantly influenced them at 9 and 13 DAWTI (Tables 1, 4 and 5). The interaction between water deficit levels and variety was insignificant at all dates. The RDW under the main effect of the water deficit levels was at its maximum in  $I_1$  (2.89, 4.64, and 5.04 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) and at its minimum from  $I_3$  (2.13, 3.31, and 3.33 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively). The RDW was higher in V1 (2.60, 4.43, and 4.96 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) and lower in V3 (2.35, 3.48, and 3.59 g plant<sup>-1</sup> at 5, 9, and 13 DAWTI, respectively) (Table 5). Under the prolonged water deficit level ( $I_3$ ), the variety V1 lost its RDW only 20.90, 18.57, and 26.28%, whereas V2 and V3 lost 27.21, 35.26, and 37.07% and 30.91, 32.68, and 40.00%, at 5, 9, and 13 DAWTI, respectively.

### 3.2.7. Recovery Rate of Morphological Traits

To sustain itself in a water stress-prone environment, the recovery capacity of a plant after a stress period is worth characterizing. To assess the recovery rate (RR) of different traits of maize as influenced by irrigation level and variety, observations were considered for a period after re-watering. After a stressful period, the plants were re-watered once and left again for a certain period without watering. After a re-watering, the plants recovered, as well as faced the next water stress. After the first stress period under prolonged stress ( $I_3$ ), the plants were re-watered first at 5 DAWTI, at 9 DAWTI, and, finally, at 13 DAWTI. The second stress period was considered to be the first recovery period (5–9 days), and the third stress period was the second recovery period (9–13 days). But, in the case of short water stress ( $I_2$ ), the plant was stressed every 2 days and re-watered as well. In the well-watered group, ( $I_1$ ) the plants received water daily (no stress). To coincide with the prolonged stress period, two common recovery periods were considered. Figure 4 reveals that the RR for morphological traits was affected in diverse ways by irrigation levels. The RR of LA and SP was higher in the second recovery period (RP) than in the first RP under all irrigation levels, where the RR of PH, RL, SDW, and RDW was greater in the first RP than in the second RP, except for RL under  $I_3$  (Figure 4). However, the RR of LA, PH, SP, RL, and RDW were higher due to short stress compared to the control at all RPs, and the RR of PH, SP, and RL was greater in the second RP, due to prolonged stress, compared to the control. Figure 5 showed that different varieties had various recovery capabilities for morphological traits. The RR of LA (120.14%), PH (18.41%), SP (19.64%), RL (17.74%), SDW (56.82%), and RDW (11.97%) showed a maximum value in V1 in the second RP compared to the other varieties (Figure 5). The RR of LA (38.10%) and PH (43.34%) attained a maximum value in V2 in the first RP. The RR of V3 was poor in almost all cases, under both RPs.

## 3.3. Physiological Parameters of Maize

### 3.3.1. Leaf Water Potential

Under water stress conditions, a plant's water status may limit its growth and development, and, for this reason, the leaf water potential (LWP) was measured. We found that it was significantly influenced by water deficit levels (I), variety (V), and the interaction between water deficit levels and varieties ( $I \times V$ ) at 5, 9, and 13 DAWTI ( $p < 0.05$ ), except 9 DAWTI (Tables 2, 6 and 7). The maximum leaf water potential was observed in the well-watered condition. It decreased with the increase in the water stress period. The lowest one was found in the 4-day watering interval period at all sampling dates. Under short and prolonged water stress levels, the maximum LWP was  $-0.71$ ,  $-0.67$ , and  $-0.70$  MPa and  $-1.03$ ,  $-0.89$ , and  $-0.98$  MPa, respectively, in V1, whereas the lowest was  $-0.93$ ,  $-0.83$ , and  $-0.87$  MPa and  $-1.41$ ,  $-1.27$ , and  $-1.38$  MPa, respectively, in V3. There is a main effect of water deficit levels. The leaf water potential was the highest in the well-watered group ( $I_1$ ) ( $-0.25$ ,  $-0.29$ , and  $-0.28$  MPa at 5, 9, and 13 DAWTI, respectively) and the lowest was in the prolonged water stress level group ( $I_3$ ) ( $-1.22$ ,  $-1.11$ , and  $-1.18$  MPa at

5, 9, and 13 DAWTI, respectively) (Table 6). The variety V1 showed the maximum LWP at all sampling dates (−0.66, −0.59, and −0.66 MPa at 5, 9, and 13 DAWTI, respectively).

**Table 6.** Main effect of irrigation on different physiological characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

Source of Variation	LWP (MPa)			LG (SPAD Value)			RL:SL			AGR (g day <sup>−1</sup> )
	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5–13 DAWTI
I <sub>1</sub>	−0.25 a	−0.29 a	−0.28 a	42.01 a	41.48 a	42.10 a	0.76 c	0.67 b	0.69 c	1.491 a
I <sub>2</sub>	−0.81 b	−0.76 b	−0.77 b	40.35 ab	39.57 ab	38.89 b	0.86 b	0.77 b	0.81 b	1.243 b
I <sub>3</sub>	−1.22 c	−1.11 c	−1.18 c	38.21 b	36.56 b	36.16 b	1.05 a	1.02 a	1.02 a	0.756 c
F test (I)	**	**	**	NS	NS	**	**	**	**	**
LSD <sub>0.05</sub>	0.07	0.09	0.08	3.50	4.42	3.01	0.07	0.11	0.08	0.11

\*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test. LWP = Leaf water potential; LG = Leaf greenness; RL:SL = Root length to shoot length ratio; AGR = Absolute growth rate.

**Table 7.** Main effect of variety on different physiological characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

Source of Variation	LWP (MPa)			LG (SPAD Value)			RL:SL			AGR (g day <sup>−1</sup> )
	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5 DAWTI	9 DAWTI	13 DAWTI	5–13 DAWTI
V1	−0.66 a	−0.69 a	−0.66 a	40.67	39.98	39.96	0.98 a	0.93 a	0.91 a	1.333 a
V2	−0.74 b	−0.76 b	−0.73 a	40.55	39.95	39.24	0.85 b	0.76 b	0.80 b	1.218 b
V3	−0.89 c	−0.80 b	−0.85 b	39.35	38.69	37.95	0.84 b	0.78 b	0.81 b	0.938 c
F test (V)	**	**	**	NS	NS	NS	**	**	*	**
LSD <sub>0.05</sub>	0.07	0.06	0.08	3.50	4.42	3.81	0.07	0.11	0.08	0.11

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test. LWP = Leaf water potential; LG = Leaf greenness; RL:SL = Root length to shoot length ratio; AGR = Absolute growth rate.

### 3.3.2. Leaf Greenness (SPAD)

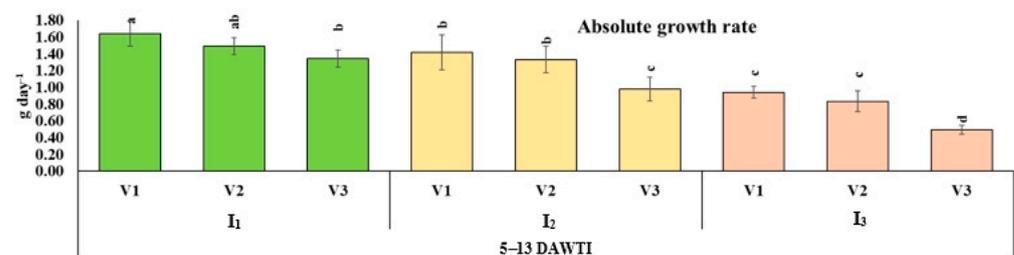
Leaf greenness depends on chlorophyll content, which is the green pigment in leaves and is responsible for photosynthesis. Leaf greenness, measured by SPAD chlorophyll meter, was significantly influenced by water deficit levels at 13 DAWTI (Tables 2, 6 and 7). At 13 DAWTI, it was found that the SPAD value was at its maximum in well-watered conditions (42.10), and the lowest, in prolonged water stress level group, was 36.16. In general, the SPAD value decreased with the increase in the water stress period. The highest SPAD was found to be 43.82 when watering every day (I<sub>1</sub>) (Table 2). It can be concluded that variety has no significant effect on the SPAD value at 5, 9, and 13 DAWTI (Table 7).

### 3.3.3. Root Length to Shoot Length Ratio

Dry matter partitioning is very important under water stress, and it was found that the root length to shoot length ratio (RL:SL) was significantly affected by water deficit levels (I) and variety (V). The interaction between water deficit levels and varieties (I × V) was insignificant at 5, 9, and 13 DAWTI (Tables 2, 6 and 7). Under the water deficit levels, at 13 DAWTI, the highest RL:SL was measured to be 1.02 in I<sub>3</sub>, whereas the lowest RL:SL was measured in I<sub>1</sub> treatment (0.69) (Table 6). The RL:SL at all sampling dates was relatively higher in V1 and lower in V2 (Table 7). It was found that V1 could produce a maximum RL:SL under all water deficit levels at all sampling dates, and V2 produced the minimum RL:SL at the prolonged water stress level. Under the short water deficit level (I<sub>2</sub>), the RL:SL in variety V1 increased 15.10, 14.33, and 18.10%, whereas V2 and V3 increased 11.94, 17.52, and 23.12% and 11.69, 13.98, and 9.45%, at 5, 9, and 13 DAWTI, respectively. Under the prolonged water deficit level (I<sub>3</sub>), the variety V1 increased 35.21, 48.09, and 43.23%, whereas V2 and V3 increased 37.70, 58.32, and 55.48% and 43.53, 52.16, and 47.66%, at 5, 9, and 13 DAWTI, respectively.

### 3.3.4. Absolute Growth Rate

A smooth growth rate under water stress conditions is a desirable characteristic for a variety. In the period from 5 to 13 DAWTI, the water deficit levels (I) and varieties (V) had a significant impact on the absolute growth rate (AGR), whereas the interaction between these two factors ( $I \times V$ ) had no statistically significant effect at  $p < 0.05$  (Tables 2, 6 and 7). Under water deficit levels, the highest AGR was measured at 1.491 g day<sup>-1</sup> in I<sub>1</sub>, whereas the lowest AGR was measured in I<sub>3</sub> treatment (0.756 g day<sup>-1</sup>) (Table 6). The maximum AGR was observed in V1 (11.33 g day<sup>-1</sup>), followed by V2 (1.218 g day<sup>-1</sup>) and V3 (0.938 g day<sup>-1</sup>). As shown in Figure 10, it was found that V1 could maintain maximum AGR (1.42 and 0.94 g day<sup>-1</sup> under I<sub>2</sub> and I<sub>3</sub> water deficit levels), and V3 performed poorly in AGR under both short and prolonged water stress levels.



**Figure 10.** Absolute growth rate (AGR) of three maize varieties (V1, SUWAN2301; V2, SUWAN4452; V3, S7328) under three irrigation levels (I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals) at 5, 9, and 13 days after watering treatment initiated (DAWTI). Vertical bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.

### 3.3.5. Recovery Rate of Physiological Traits

The RR of different physiological traits of maize was also measured in the same way as morphological traits were. Figure 6 shows that the RR for physiological traits was affected in diverse ways by irrigation levels. The RR of LWP was at its maximum in I<sub>3</sub> (6.93%), followed by I<sub>2</sub> (1.75%), and, in the control, it was negative (−2.33%) in the second RP (Figure 6). The RR of leaf greenness was negative in both water stress levels at all RP, and it is more negative in the first RP, whereas it could recover a little bit in the second RP. The RR of RL:SL was negative in the first RP under all irrigation levels, whereas it was relatively less negative in I<sub>3</sub> (−2.63%) and at its maximum in I<sub>1</sub> (−11.49%). The RR of RL:SL was increased in the second RP, and it was at its maximum in I<sub>2</sub> (4.16%). Figure 8 shows that the RR of physiological traits varied due to different varieties. The RR of LWP was mostly negative in all varieties in the second RP, except V3, which is also negative in the first RP (Figure 7). The RR of leaf greenness, in all varieties, was negative in all of the RPs, but V1 mostly recovered in the second RP. The RR of RL:SL was decreased in the first RP, but it was drastically increased in the second RP in all varieties.

## 3.4. Biochemical Parameters of Maize

### 3.4.1. Proline Content in Leaf and Root

Under water stress, plants can produce proline to protect the cells from damage caused by water stress through stabilizing proteins and membranes. Under water stress conditions, dry matter partitioning is relatively more evident in the roots than in the shoots in a tolerant maize variety, and, for this reason, the proline content was measured from both the leaves and the roots. The water level was significantly affected the proline content in both leaf (PrL) and root (PrR) at 5, 9, and 13 DAWTI (Table 8). The proline content was also significantly influenced by variety at 4 and 9 DAWTI (for leaf), as well as at 5 and 13 DAWTI (for root). The proline content was also influenced by the water deficit levels and the interaction between water deficit level and variety ( $I \times V$ ) at all sampling dates, as well as in the case of leaf and root at 5 DAWTI (Table 3). Water stress enhanced the

proline content in both leaf and root. It was more prominent under the prolonged water stress level at all sampling dates (Table 8). The maximum proline content in both leaf and root was observed in I<sub>3</sub> at 13 DAWTI. These were 1.19 and 1.35  $\mu\text{mol g}^{-1}$  FW, respectively. The variety V1 produced a relatively higher amount of proline in both leaf and root at 5, 9, and 13 DAWTI (0.63, 0.83, and 1.07  $\mu\text{mol g}^{-1}$  FW and 0.60, 0.85, and 1.09  $\mu\text{mol g}^{-1}$  FW, respectively). The capacity for producing proline in both leaf and root was relatively poor in V3 (Table 9). Figures 11 and 12 show that the maximum proline contents of leaf and root were in V1 at all water deficit levels, and poor performance was observed in V3, except at 5 DAWTI. Under the short water deficit level (I<sub>2</sub>), V1 could produce 14.29 and 42.86%; 14.89 and 0.71%; and 14.10 and 11.54% higher leaf proline compared to V2 and V3 at 5, 9, and 13 DAWTI, respectively. But under the prolonged water deficit level (I<sub>3</sub>), V1 could produce 27.48 and 36.94%; 10.77 and 28.85%; and 12.41 and 26.28% higher leaf proline compared to V2 and V3 at 5, 9, and 13 DAWTI, respectively. In comparison to V2 and V3 at 5, 9, and 13 DAWTI, under the short water deficiency level (I<sub>2</sub>), V1 could produce 23.68 and 26.32%; 27.27 and 23.38%; and 14.04 and 14.61% more root proline, respectively. Conversely, under the prolonged water deficit level (I<sub>3</sub>), V1 could produce 26.07 and 13.68%; 2.76 and 8.66%; and 13.83 and 21.67% higher root proline compared to V2 and V3 at 5, 9, and 13 DAWTI, respectively.

**Table 8.** Main effect of irrigation on different biochemical characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

Source of Variation	PrL ( $\mu\text{mol g}^{-1}$ FW)			PrR ( $\mu\text{mol g}^{-1}$ FW)			TSSL ( $\text{mg g}^{-1}$ FW)			TSSR ( $\text{mg g}^{-1}$ FW)		
	5	9	13	5	9	13	5	9	13	5	9	13
	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI
I <sub>1</sub>	0.20 c	0.46 c	1.00 b	0.25 c	0.53 c	0.68 b	0.59 c	0.67 b	0.79 b	0.33 c	0.42 b	0.52 b
I <sub>2</sub>	0.45 b	0.67 b	0.77 c	0.32 b	0.64 b	0.81 b	0.66 b	0.77 b	0.83 b	0.39 b	0.41 b	0.51 b
I <sub>3</sub>	0.87 a	1.13 a	1.19 a	1.02 a	1.22 a	1.35 a	0.82 a	0.93 a	1.07 a	0.50 a	0.60 a	0.68 a
F test (I)	**	**	**	**	**	**	**	**	**	**	**	**
LSD <sub>0.05</sub>	0.06	0.08	0.14	0.05	0.10	0.14	0.06	0.11	0.08	0.03	0.05	0.06

\*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test. PrL = Proline in leaf; PrR = Proline in root; TSSL = total soluble sugar in leaf; TSSR = total soluble sugar in root.

**Table 9.** Main effect of variety on different biochemical characteristics of maize at 3 watering levels, sampling at 5, 9, and 13 days after watering treatment initiated (DAWTI).

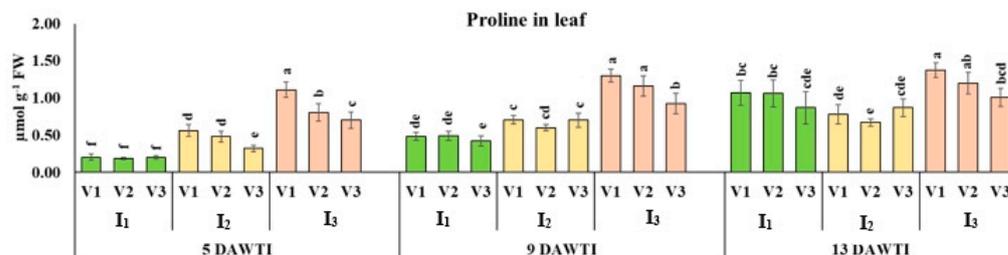
Source of Variation	PrL ( $\mu\text{mol g}^{-1}$ FW)			PrR ( $\mu\text{mol g}^{-1}$ FW)			TSSL ( $\text{mg g}^{-1}$ FW)			TSSR ( $\text{mg g}^{-1}$ FW)		
	5	9	13	5	9	13	5	9	13	5	9	13
	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI	DAWTI
V1	0.63 a	0.83 a	1.07 a	0.60 a	0.85	1.09 a	0.74 a	0.83	0.98 a	0.41 a	0.52 a	0.64 a
V2	0.49 b	0.75 ab	0.98 ab	0.46 c	0.76	0.91 b	0.68 ab	0.79	0.90 a	0.43 a	0.49 a	0.57 b
V3	0.41 c	0.68 b	0.92 b	0.53 b	0.78	0.84 b	0.66 b	0.76	0.81 b	0.38 b	0.41 b	0.49 c
F test (V)	**	**	NS	**	NS	**	NS	NS	**	**	**	**
LSD <sub>0.05</sub>	0.06	0.08	0.14	0.05	0.10	0.14	0.06	0.11	0.08	0.03	0.05	0.06

\*\* significant at  $p < 0.01$ ; NS = nonsignificant; DAWTI = days after watering treatment initiated. Means within a column with the same or no letters are not significant at  $p < 0.05$  based on LSD test. PrL = Proline in leaf; PrR = Proline in root; TSSL = total soluble sugar in leaf; TSSR = total soluble sugar in root.

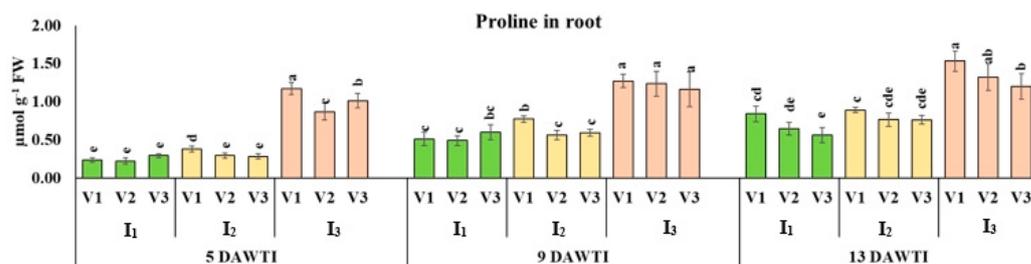
### 3.4.2. Total Soluble Sugar (TSS) Content in Leaf and Root

Total soluble sugar (TSS) is an osmotic solute and may vary in roots and shoots in different varieties under water stress. The TSS content in leaf (TSSL) and root (TSSR) were significantly ( $p < 0.05$ ) influenced by water deficit levels (I), variety (V), and the interaction between water deficit levels and varieties (I  $\times$  V) at all DAWTI, except TSS in leaf under varietal effect at 5 and 9 DAWTI (Tables 3, 8 and 9). The TSS levels of leaf and root were increased in water-stressed plants. It was greater in the prolonged water deficit treatment (I<sub>3</sub>) at all sampling dates. The TSS level was relatively higher in leaf than root in all varieties. Maximum TSS in leaf and root was produced by V1, followed by V2 and V3 (Table 9). The maximum TSS level in both leaf and root was observed in V1 under I<sub>3</sub> level at 13 DAWTI

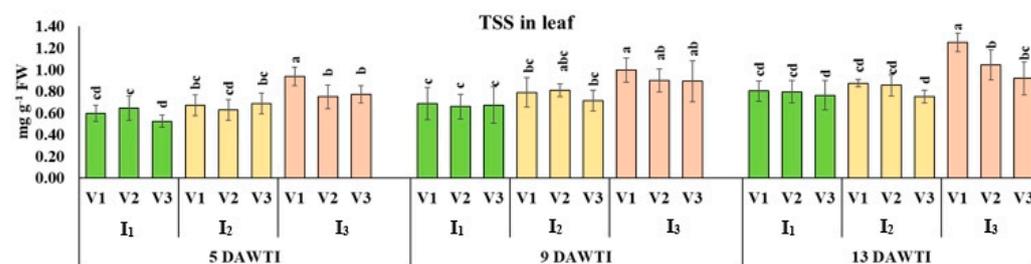
(1.25 and 0.82 mg g<sup>-1</sup> FW, respectively). The variety V1 produced relatively higher TSS in both leaf and root at 5, 9, and 13 DAWTI under I<sub>3</sub> level (0.94, 1.00, and 1.25 mg g<sup>-1</sup> FW and 0.55, 0.69, and 0.82 mg g<sup>-1</sup> FW, respectively). The capacity for producing TSS in both leaf and root was relatively poor in V3 (Table 9). Figures 13 and 14 show that the maximum TSS levels in leaf and root were in V1 at water stress, whereas poor performance was observed in V3, except at 5 DAWTI of root TSS.



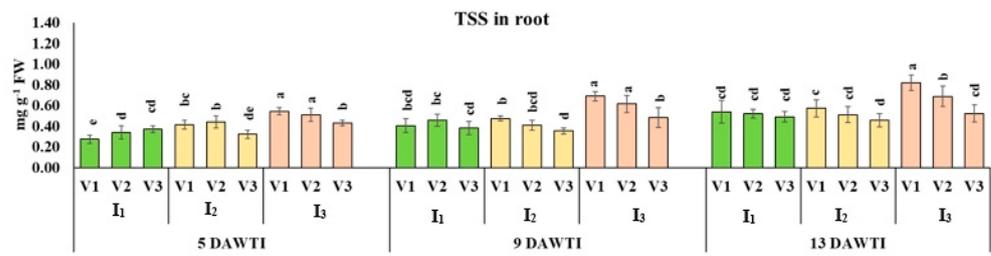
**Figure 11.** Proline in leaf (PrL) of three maize varieties (V1, SUWAN2301; V2, SUWAN4452; V3, S7328) under three irrigation levels (I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals) at 5, 9, and 13 days after watering treatment initiated (DAWTI). Vertical bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.



**Figure 12.** Proline in root (PrR) of three maize varieties (V1, SUWAN2301; V2, SUWAN4452; V3, S7328) under three irrigation levels (I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals) at 5, 9, and 13 days after watering treatment initiated (DAWTI). Vertical bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.



**Figure 13.** TSS in leaf (TSSL) of three maize varieties (V1, SUWAN2301; V2, SUWAN4452; V3, S7328) under three irrigation levels (I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals) at 5, 9, and 13 days after watering treatment initiated (DAWTI). Vertical bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.



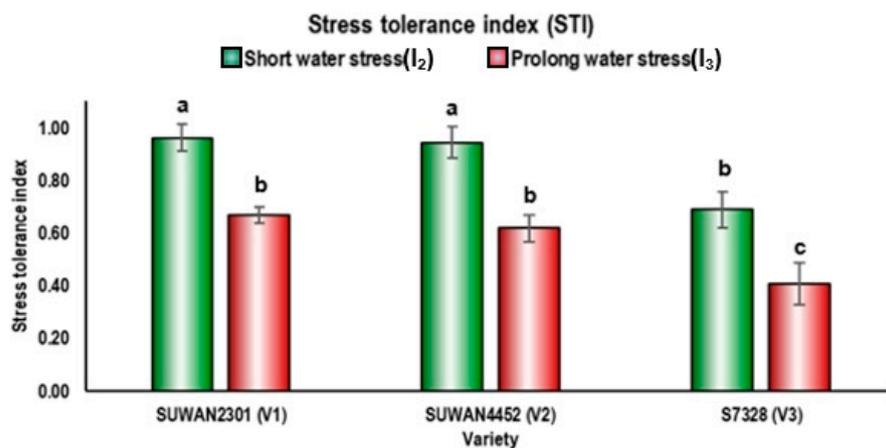
**Figure 14.** TSS in root (TSSR) of three maize varieties (V1, SUWAN2301; V2, SUWAN4452; V3, S7328) under three irrigation levels (I<sub>1</sub>, 1-day watering intervals; I<sub>2</sub>, 2-day watering intervals; I<sub>3</sub>, 4-day watering intervals) at 5, 9, and 13 days after watering treatment initiated (DAWTI). Vertical bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.

### 3.4.3. Recovery Rate of Biochemical Traits

The RR of different biochemical traits of maize was also measured in the same way as the same as morphological traits. Figure 8 shows that the RR for biochemical traits was affected in diverse ways by irrigation levels. Due to water stress, the RR of PrL was increased more in the first RP than in the second RP. The RR for PrL was calculated to be higher in I<sub>1</sub>, but the actual PrL amount was much lower in this plant compared to I<sub>2</sub> and I<sub>3</sub> (Table 6). Table 6 also showed that, after the first water stress, before re-watering, the PrL increased dramatically in I<sub>2</sub> and I<sub>3</sub>, and that is why, in the later stage, the increment of RR was relatively low. Almost the same trend was also found in the case of PrR, TSSL, and TSSR. Figure 9 shows that the RR of PrR (42.55%), TSSL (18.54%), and TSSR (22.87%) was higher in V1 in the second RP as compared to the other varieties. The RR of PrL and PrR was higher in V1 and V2, respectively, in the first RP.

### 3.5. Stress Tolerance Index

The stress tolerance index (STI) was calculated for the different varieties based on the total dry biomass under both the short water stress and the prolonged water stress levels as compared to the well-watered condition. From Figure 15, it can be seen that the stress tolerance index differed due to water levels and variety. The maximum STI value was calculated in the short water stress level in all varieties, compared to prolonged stress, whereas the variety V1 gave the maximum STI value under the short water stress condition. However, the STI value decreased under prolonged water stress, with V1 still having the highest value (0.67), while V3 displayed the lowest STI value (0.40) under both the short and prolonged water stress levels.

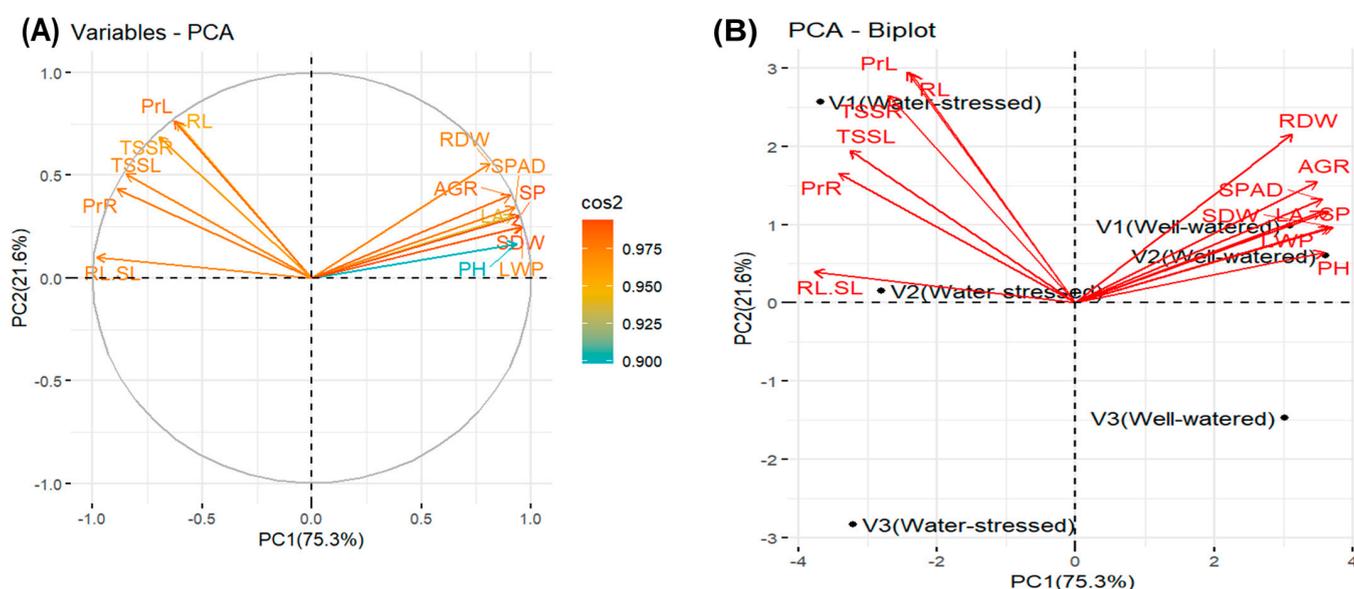


**Figure 15.** Stress tolerance index based on total dry biomass of 3 maize varieties under short water stress (I<sub>2</sub>, 2-day watering intervals) and prolonged water stress (I<sub>3</sub>, 4-day watering intervals). Vertical

bars are twice the standard deviation. Means in the figure with the same or no letters are not significant at  $p < 0.05$  based on the least significant difference test.

### 3.6. Principal Component Analysis

Principal component analysis (PCA) was performed on a dataset consisting of three maize varieties and 14 different variables to reduce the dimensions of the data and identify potential correlations among the measured characteristics in this study (Figure 16). Under both prolonged water stress and well-watered conditions, the association exists among different factors and varieties; together, their respective major components are displayed in biplot form (Figure 16A,B). The biplot results proved that there was a clear division between the well-watered group and water stress treatment (Figure 16B). The first two PCs accounted for the highest proportion of variance (96.9%), which was produced with PCA-biplot PC1 (75.3%) and PC2 (21.6%). The results of the biplot showed that traits such as PrL, RL, TSSL, TSSR, PrR, and RL:SL clustered together in the leftmost region of the biplot, scattered around the varieties under water-stressed conditions.



**Figure 16.** (A) Principal component analysis (PCA) for variables of 3 maize varieties based on the variance in 14 physio-morphological and biochemical variables when grown under well-watered and prolonged water-stressed conditions. Arrows indicate the strength of the variable's influence on the first two principal components (PCs). The longer and dark red arrows indicate a higher contribution, and the shorter and darker blue arrows indicate the lower contribution of the variables. (B) PCA-Biplot of 3 maize varieties and 14 variables under well-watered and water-stressed conditions. PH, plant height (cm); LWP, leaf water potential (MPa); SPAD, leaf greenness; LA, leaf area (cm<sup>2</sup>); RL, root length (cm); PrL, proline in leaf ( $\mu\text{mol g}^{-1}$  FW); PrR, proline in root ( $\mu\text{mol g}^{-1}$  FW); TSSL, total soluble sugar in leaves ( $\text{mg g}^{-1}$  FW); TSSR, total soluble sugar in roots ( $\text{mg g}^{-1}$  FW); RL:SL, ratio of root length and shoot length; RDW, root dry weight ( $\text{g plant}^{-1}$ ); SDW, shoot dry weight ( $\text{g plant}^{-1}$ ); AGR, absolute growth rate ( $\text{g day}^{-1}$ ); SP, stem perimeter (cm); V1, SUWAN2301; V2, SUWAN4452; and V3, S7328.

Furthermore, other variables, including RDW, AGR, SPAD, LA, SP, LWP, SDW, and PH, grouped on the right side of the biplot on the variety under well-watered conditions, whereas PrL and RDW were approximately positioned at the center of the biplot, as shown, demonstrating relatively similar performance under both water conditions. In terms of contribution of variables to the two PCs biplot, the variable RL:SL showed the highest contribution to PC1 (9.15%), followed by SDW (8.81%), LWP (8.64%), SP (8.57%), PH (8.26%), SPAD (8.14%), LA (8.05%), AGR (7.79%), PrR (7.51%), TSSL (6.81%), and RDW

(6.27%). The contribution of variables to PC2 was mainly explained by PrL (19.43%), RL (19.06), TSSR (15.65), RDW (10.37), and TSSL (8.50%).

#### 4. Discussion

Different morpho-physiological and biochemical processes in plants are impacted by drought, much like by other environmental conditions. In the present study, we conducted a comparative morphological, physiological, and biochemical analysis under water stress and water recovery conditions.

##### *Morphological responses of maize under water stress*

The responses of plants to water stress vary significantly depending on the intensity and duration of the stress, as well as on the plant species and its stage of growth [63]. Water deficit significantly influenced the growth and development of the aboveground components, including plant height, leaf area, and leaf greenness, during the early vegetative phase of this study. Additionally, the growth and development of the underground components, specifically root length, were significantly influenced by both the levels of water deficit and the varieties tested. Among the varieties, SUWAN2301 (V1) exhibited the highest root length. It has been demonstrated that, in situations of water deprivation, decreased light harvesting through decreased leaf area results in decreased cell division and dry matter accumulation [46,47].

In the early vegetative stage of maize, water stress lowered leaf area considerably [64]. In the present study, the interaction of irrigation and variety for leaf area was non-significant and might be due to the ways in which different varieties respond to water stress differently. A variety that loses comparatively less leaf area than others may be more tolerant overall. Therefore, it was found that V1 lost relatively less LA at all levels. Having relatively more leaf area under stress conditions might help a variety to produce more photosynthate through proper photosynthesis. Our findings are consistent with those of previous studies [46,65].

The plant heights for the different varieties were mostly determined by varietal character. However, the reduction rate of PH was different under water stress, and these results are supported by several researchers [66–70]. The seedlings' growth was slowed down by the drought stress. Although the seedlings' growth rates increased once again during the recovery stage, their height was noticeably less than that of the controls. Efeoglu et al. [71] also supported these results. In the early vegetative stage, water stress dramatically reduced maize's plant height [64]. The interaction of irrigation and variety for PH was non-significant due to different tolerance capacities of different varieties against water stress. Hence, V1 could resist drought relatively better in the case of PH at all levels of water stress. The plant height of V2 is genetically higher than others. Under water stress, a tolerant variety can uptake more water and photosynthesis and can continue relatively better than a susceptible variety. Thus, the plant height of tolerant varieties are relatively taller than in susceptible varieties. This can also be clearly seen in the ggbiplot, where it is found that V1 is more tolerant than V2.

Varietal characteristics may influence the stem perimeters of different varieties, which may be reduced under water stress [68,69]. Though the plant height of V1 was relatively lower than V2, the SP was higher in V1 and might be influenced by the total dry matter production and water reservation.

Water stress significantly decreased the SDW of maize [67], especially in the early vegetative stage. Though the plant height was lower in V1 than V3, the SDW was higher in V1 than V3. Plants may allocate resources differently under water stress conditions. While the taller maize variety may prioritize stem growth, it may allocate fewer resources to root development and biomass production. This could limit the plant's ability to absorb water and nutrients, impacting overall biomass accumulation.

Water stress significantly decreased the root dry weight (RDW) of maize in the early vegetative stage [67]. The variety that lost relatively less RDW could be more tolerant under water stress through uplifting more water from soil. The variety V1 lost relatively less RDW

at all levels. According to a previous work, short water stress periods during the rapid vegetative growth stage caused a loss of 28–32% of final dry matter in the plants [64]. A relatively greater RDW under stress conditions might help a variety to produce more total biomass by maintaining proper growth through uplifting more water and nutrients from the soil than other varieties. After a short water stress, the assimilate production is hampered, and the plant tries to uptake more water, but, by this time, it receives irrigation; therefore it does not deepen its root system. Rather, it produces more lateral roots. After prolonged water stress, the assimilate production is hampered, and root biomass is hampered. The plant tries to uptake more water from the deeper layers, and has enough time to deepen its root system, even though it has less root biomass.

The ability of plants to recover from drought has gained relevance recently, especially in the case of crops [27,72–75]. Water stress significantly decreased the maximum morphological traits of maize in the present study. But, after a water stress period, when re-watering was performed, it was found that recovery was more prominent in stressed plants than in the control plants, and these findings are corroborated by Zhang et al. [41]. This tendency might make a variety more tolerant against water stress. The recovery trend was relatively higher in the first RP, and it was a little bit lower in the second RP. This might be due to a slight decrease in the oxidative damage protectant under recurrent stress conditions [41].

#### *Physiological responses of maize under water stress*

According to several studies [76–84], drought results in decreased growth, decreased water content, and decreased chlorophyll pigment content. The results revealed that leaf water potential decreased with the increment of the water stress period. However, the LWP was reduced more in variety V3 in all water stress conditions at 5, 9, and 13 DAWTI, followed by V2 and V1. While the soil moisture values were decreased, the plant leaf water potential also decreased after exposure to water deficit conditions [26]. According to Reddy et al. [85], under well-watered conditions, LWP did not differ between tolerant and susceptible genotypes, but, under stressed conditions, tolerant genotypes exhibited a lower reduction in LWP than the greater reduction of susceptible genotypes, which is in agreement with this study. Additionally, Souza et al. [78] observed an LWP decline in drought-stressed maize. However, in the present study, the leaf water potential recovered dramatically after rewatering in the second recovery period, which is also corroborated by several other scientists [71,86–93]. A higher water status may encourage greater stomatal conductance, which results in CO<sub>2</sub> flux and transpiration-based leaf cooling. Under repeated drought, maize performance is associated with the induction of mechanisms related to leaf water status maintenance [94–96].

The leaf greenness was significantly reduced due to water stress, with a decrease of 7.62%, observed during a short water stress period, and a decrease of 14.11%, observed during a prolonged water stress period. Sah et al. [67] emphasized the stay-green traits as a drought tolerant characteristic. In maize plants, severe and prolonged water stress experienced during the seedling stage can result in damage to the photosynthetic membrane structure. This damage leads to a decrease in chlorophyll content, ultimately affecting plant growth negatively compared to plants that were not subjected to water stress during that stage. But, interestingly, in the present study, the leaf greenness regained a big portion after rewatering in the second recovery period, and these results are corroborated by several other researchers [86,90–93]. Water stress significantly increased the RL:SL of maize in the early vegetative stage. The interaction of irrigation and variety for leaf area was insignificant, and it might be due to the different ways in which different varieties respond to water stress. The variety in which RL:SL increases more might be relatively more tolerant than others. It was found that V1 increased relatively more in terms of RL:SL at all levels. The relatively larger RL:SL under stress conditions might help this variety to tolerate the adverse situation. In general, initially and the under well-watered condition, a maize variety may perform as per initial establishment ratio or varietal characters. After stress, a

plant may allocate more dry matter to its roots than to its shoots; Therefore, RL:SL increases. Tolerant varieties can allocate more DM to its roots; therefore, its RL:SL is relatively higher.

Water stress significantly decreased the AGR of all varieties, which generally agreed with previous studies [97–99]. However, the variety V1 could maintain relatively better AGR under all level of water stress, and it might be influenced by more uplifting of water through longer roots than others. The larger leaf area in V1 under water stress conditions might be also help to produce more assimilate, which, subsequently, enhances the AGR in V1.

The recovery of physiological traits after the water stress period indicates the acceptable performance of a plant [95,100]. Furthermore, Kränzlein et al. [23] found, in a pot experiment, that preserving the relative water content of the leaves is a necessary quality for reaching larger stress recovery potentials during repeated water deficit stress. V1 could recover more compared to other varieties, and it maintained more leaf water and leaf greenness [41], which might enhance the ability of this variety to withstand water stress. The relatively higher water content in V1 under water stress conditions might help this variety to distribute assimilate properly, and, as a result, it was found that it could increase leaf greenness more than other varieties.

#### *Biochemical responses of maize under water stress*

According to the references [57,64,100–102], maize roots and leaves have higher proline and total soluble sugar contents, which leads to high leaf water potential. When plants are under water stress, the osmotic pressure of the plant cell regulates a number of processes through the accumulation of non-toxic solutes inside the cell because the cell's water potential drops, which raises the concentration of dissolved material and keeps the cell turgid [18]. In the present experiment, proline content, total soluble sugar content, root length, and root length to shoot length ratio were increased in response to water deficit levels. These findings corroborate the argument made by [58] that drought-stricken plants may exhibit an increase in sugar content in their leaves. Moreover, one of the mechanisms plants use to withstand drought stress relies on regulating the osmotic potential of the cell, especially if drought stress increases gradually from mild to severe [19]. Water stress significantly increased the proline content in both the leaves and roots of maize in the early vegetative stage. The proline content increased more under the prolonged water stress level. Plants modify the osmotic potential in their leaves during drought by accumulating solutes [103], particularly proline [104]. The variety V1 produced relatively more proline at all levels. More proline under stress conditions might help a variety to protect itself from oxidative damage [105] under stress conditions.

Water stress significantly increased TSS levels in both the leaves and roots of maize in the early vegetative stage. The TSS level was increased more under the prolonged water stress level. In the case of the prolonged water deficit level, V1 could produce higher leaf TSS compared to V2 and V3. On the other hand, in the prolonged water deficit level, V1 could produce higher root TSS compared to V2 and V3. The differences in the responses to drought stress among the maize cultivars show that each cultivar has a different ability to synthesize proline and total soluble sugar with an increase in drought stress treatment [64]. Chen et al. [86] found no significant relationship between soluble sugar and osmotic potential with drought resistance or recovery. On the other hand, a number of studies have revealed a favorable relationship between osmoregulation and photosynthesis [29,30].

Though the RRs for PrL, PrR, TSSL, and TSSR were relatively lower after the second (first recovery) and third (second recovery) water stress in both I<sub>2</sub> and I<sub>3</sub> compared to I<sub>1</sub>, the actual amounts of those traits were much lower in the I<sub>1</sub> plants as compared to in I<sub>2</sub> and I<sub>3</sub>. After the first water stress, and before re-watering, the PrL, PrR, TSSL, and TSSR increased dramatically in I<sub>2</sub> and I<sub>3</sub>, and that is why, in the later stage, the increment of RR seemed relatively low. A higher recovery tendency in PrR, TSSL, and TSSR was observed in the V1 variety, which makes it more tolerant against water stress, maybe through protecting against oxidative damage [106] caused by the stress and helping to produce more biomass in this variety. This result is also supported by Maywald et al. [106],

who reported that repeated water deprivation treatments caused an osmotic adjustment in all of the plants' leaves.

Principal component analysis (PCA) was used to identify the most vital selection parameters for drought tolerance in maize varieties. PCA-biplot is a multivariate analytic technique which combines traits and objects in two dimensions or more while minimizing overlapping variations to make it easier to identify important characteristics for selection [39–41]. The results showed that the variables PrL, RL, TSSR, RL:SL, RDW, TSSL, SDW, LWP, SP, PH, SPAD, LA, and AGR were the most important traits characterizing variation among maize varieties. The PCA biplot showed that PrL, TSSL, RL:SL, PrR, TSSR, and RL are clustered together in the biplot, closely scattering around the varieties under water stress conditions. This indicates that these six traits are very important in selecting the best characters under water stress conditions. The morphological, physiological, and biochemical characteristics should be taken into account, as they may be utilized in water stress resistance selection and the identification of cultivars that are resistant to water stress. This finding is supported by numerous studies [107–111]. These identified traits can be used to develop breeding programs aimed at increasing the water stress tolerance of maize.

## 5. Conclusions

In this study, we tested 14 traits in three maize varieties across three distinct water levels. Overall, the water deficit levels and varieties had considerable effects on most of the studied characteristics, but the variation due to varieties had significant effects on all the analyzed traits. The approach in this study clearly showed that morphological, physiological, and biochemical variables varied significantly when individual maize varieties were examined under three different water regimes. Proline content, TSS level, and root length showed higher values under water stress conditions, as opposed to other remaining traits. Re-watering following a water stress period triggered the recovery rates in most traits, which inferred that recurrent water stress at a tolerable level can improve the tolerance of maize plants by enhancing recovery capacity. In this study, discriminant analyses, such as PCA, supported the used method as a clear differential approach, showing that proline content in roots and leaves, TSS in leaves and roots, and root length influenced the performance of maize varieties under stress conditions, while the other remaining traits were the most discriminative under normal conditions. Additionally, variety SUWAN2301 was the most water-stress-tolerant variety, as evaluated for all tested traits using the water stress tolerance index. S7328 was the least water stress tolerant variety. As a result, when the water supply is scarce, farmers in the tropics can use this study's findings to maintain or increase their crop output while using less water, helping to mitigate the effects of global climate change and sustaining crop productivity. The findings of this study may motivate plant breeders to investigate the genetic potential of present maize varieties.

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