

## Evaluation of water deficit tolerance in maize genotypes using biochemical, physio-morphological changes and yield traits as multivariate cluster analysis

Piyanan PIPATSITEE, Rujira TISARUM, Thapanee SAMPHUMPHUANG, Sumaid KONGPUGDEE, Kanyarat TAOTA, Apisit EIUMNOH, Suriyan CHA-UM\*

National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Paholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand; [piyanan.pip@biotec.or.th](mailto:piyanan.pip@biotec.or.th); [rujira.tis@biotec.or.th](mailto:rujira.tis@biotec.or.th); [thapanee@biotec.or.th](mailto:thapanee@biotec.or.th); [sumaidkong@gmail.com](mailto:sumaidkong@gmail.com); [kanyaratao67@gmail.com](mailto:kanyaratao67@gmail.com); [apisiteiumnoh@gmail.com](mailto:apisiteiumnoh@gmail.com); [suriyanc7@gmail.com](mailto:suriyanc7@gmail.com) (\*corresponding author)

### Abstract

Drought is an abiotic stress that inhibits plant growth and development and, therefore, declines crop productivity, as seen in maize plant. The aim of this investigation was to identify the candidate maize varieties that can be grown under water limited conditions using physio-morphological and yield attributes. Eight genotypes of maize including 'Suwan4452' (drought tolerant) as a positive check, 'CP301', 'CP-DK888', 'DK7979', 'DK9901', 'Pac339', 'S7328', and 'Suwan5' were selected as test plants. Physiological, biochemical and morphological characteristics at seedling (24 day after sowing; DAS) and reproductive (80 DAS) developmental stages of plants under 20-day water withholding (WD), and yield traits at harvesting period were analysed. Leaf temperature in each genotype increased with the degree of water deficit stress, leading to leaf chlorosis, and reduction in maximum quantum yield of PSII ( $F_v/F_m$ ), photon yield of PSII ( $\Phi_{PSII}$ ), net photosynthetic rate ( $P_n$ ), overall growth and yield.  $P_n$  and stomatal conductance ( $g_s$ ) in drought tolerant genotype, 'Suwan4452', were decreased by 19.1% and 18.6%, respectively, whereas these in drought sensitive, 'Pac339', were significantly declined by 53.9% and 61.8%, respectively. Physio-morphological parameters, growth performance and yield-related traits of maize genotypes grown under water deficit conditions and well-watered conditions were subjected to Ward's cluster method for identification of water deficit tolerant cultivars. Maintaining photosynthetic abilities, osmotic adjustment and CWSI in drought tolerant genotypes of maize were evidently demonstrated to keep overall growth performance and yield attributes. Based on multivariate cluster analysis and PCA (principal component analysis), 'Suwan4452', 'CP-DK888' and 'S7328' were categorized as drought tolerant genotypes whereas 'Suwan5', 'Pac339', 'DK7979', 'CP301' and 'DK9901' were identified as drought susceptible cultivars. Hybrid maize cvs. 'CP-DK888' and 'S7328' may further be suggested to be grown in the rainfed area without irrigation.

**Keywords:** crop water stress index; normalized difference vegetation index; photosynthetic abilities; seedling stage; reproductive stage; yield traits

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## Introduction

Limited precipitation because of global climate change leads to reduction in soil water availability and changes in vegetation patterns (Tietjen *et al.*, 2017). Drought stress results in the loss of 12 million ha of agricultural land (0.4% in the globe or 20 million ton of grain loss) per year (Azadi *et al.*, 2018; Hall and Leng, 2019) and 0.9% of gross primary productivity per year (Du *et al.*, 2018). In agricultural sector, 75% of the total food production is generated from 30% of the irrigated land (accounting for > 80% of total water consumption), and it is expected that ~2,300 km<sup>3</sup> of fresh water will be used for irrigation by the year 2050 (Dalezios *et al.*, 2018). Annual loss of 0.75 billion USD at global level (year 1990-2004) and 6.2 billion EURO in Europe (year 2001-2006) has been estimated to be caused due to drought stress in the past 30 years (Gerber and Mirzabaev, 2017). In United States of America, crop loss is increased by 3 folds from 9 billion in 1988 to 27.6 billion in 2012 (Elliott *et al.*, 2018; He *et al.*, 2019). Cost of the water used for irrigation is another issue for the agronomists. For example, maize crop requires water consumption (during late vegetative growth stage) greater than 0.21 USD m<sup>3</sup> whereas its grain prices are only 0.19 USD kg<sup>-1</sup>. In this case, water cost is a key criterion for the cultivation of the maize crop. Water prices for irrigation ranged between 0.23-0.29 USD per m<sup>3</sup> (e.g., 0.15 USD per m<sup>3</sup> in Australia and 0.61 USD per m<sup>3</sup> in Israel), depending on crop species, availability of water resources and year of crop cultivation (Manning *et al.*, 2018).

Drought severity, risk assessment, agricultural loss and food insecurity in major crops such as maize, wheat, rice, millet, sorghum and soybean can be estimated using several indices, including Standardized Precipitation-Evapotranspiration Index (SPEI) (Chen *et al.*, 2016a); Standardized Precipitation Index (SPI) (Leng and Hall, 2019; Spinoni *et al.*, 2019), Drought Severity Index (DSI) (Zhang *et al.*, 2019a,b,c), Synthesized Drought Index (SDI) (West *et al.*, 2019), agricultural drought monitoring indices (Liu *et al.*, 2016), Normalized Difference Vegetation Index (NDVI) (Nanzad *et al.*, 2019) and Crop Water Stress Index (CWSI) (Ihuoma and Madramootoo, 2017). SPEI has been reported as an effective indicator of drought-induced yield loss (Standardized Yield Residuals Series; SYRS) for five major cereal crops (Chen *et al.*, 2016a). Physiological and morphological adaptations under drought stress maintain growth and yield of the crop (Tardieu *et al.*, 2018). Maize is amongst the top five C<sub>4</sub> cereal crop species, used as food, feed and biofuel products in USA (384.7 MT), China (231.8 MT), Brazil (64.1 MT), Argentina (39.8 MT) and Ukraine (28.1 MT) (Wang *et al.*, 2018). Approximately 39.3% of yield reduction in maize has been reported when soil water capacity is less than 40% (Sun *et al.*, 2017; Comas *et al.*, 2019). In USA, yield of maize was significantly declined if the water availability is 70% of the water required for the crop (Trout and DeJonge, 2017). Physiological changes in maize grown under drought conditions have been studied in relation to tolerance abilities, degree of severity of drought, plant developmental stages and their interactions (Casari *et al.*, 2019; Li *et al.*, 2019a; Pires *et al.*, 2020). Multiparameter indices such as Infra-red thermal (leaf/canopy temperature), Red-Green-Blue Imagery (RGB), and GreenSeeker (NDVI), with novel technologies have been used to screen the drought tolerant candidate maize varieties (Casari *et al.*, 2019; Gao *et al.*, 2019). NDVI (GreenSeeker™) has been validated to identify the healthy plants (green color of leaves indicating high amount of chlorophyll content in relation to SPAD) using camera or non-destructive methods. Moreover, CWSI is an alternative index, indicating temperature shift between control and stressed plants using IR Thermal camera to play as abiotic stress indices (leaf temperature or canopy temperature). Both parameters are particularly important for biological parameters of plants in response to drought conditions, especially in maize crop (Zhang *et al.*, 2019a; Zhang *et al.*, 2019b). Possible hypotheses for yield reduction in maize genotypes under drought stress include: i) plant developmental stages (maturation; R<sub>4</sub>-R<sub>6</sub> > late vegetative growth stage; V8-VT) (Mi *et al.*, 2018; Zhang *et al.*, 2019d), ii) genetic drought tolerance background (waxy corn > normal corn; Hao *et al.*, 2019; Zhao *et al.*, 2019), iii) water use efficiency of each genotype (Hao *et al.*, 2015a), iv) ovary abortion (especially in ovary apical zone) in the silky stage (Oury *et al.*, 2016a, b), v) ABA-repressed starch biosynthesis (Yang *et al.*, 2019), vi) abortion of kernel (Marwein *et al.*, 2017), vii) degree of severity of water deficit stress (Hao *et al.*, 2016; Cai *et al.*, 2017; Greaves and Wang, 2017), and viii) parental hybrid vigor for drought tolerant abilities of diallel

crosses (Makumbi *et al.*, 2018). Yield attributes play a major role in cluster ranking of drought tolerant candidates in different populations of maize crop using several evaluation methods such as Distance-based clustering analysis (Su *et al.*, 2019), Principal component analysis (Chen *et al.*, 2016b) and Ward's minimum variance method (Makumbi *et al.*, 2018; Hao *et al.*, 2015b, 2016, 2019; Zhao *et al.*, 2019). Moreover, PCA has been well established to confirm the morphological, physiological responses and biochemical changes of maize genotypes in responses to drought and re-watered conditions (Chen *et al.*, 2016b). In Thailand, maize is one of the most carbohydrate cereal crops to supply for food, animal feed and fuel (bioethanol). An adoption of hybrid maize varieties has been successfully reported (>90% plantation area) as modern technology to overcome the conventional methods (Poolsawas and Napisintuwong, 2019). However, the basic information of drought tolerant abilities in both hybrid and inbred maize varieties is still lacking as well as the elite variety with drought tolerant strategies is missing. Eight maize varieties, including 'Suwan5' (inbred variety), 'Suwan4452' (positive check; drought tolerant), 'CP-DK888', 'Pac339', 'DK9901', 'DK7979', 'CP301' and 'S7328', were selected as test plants. In Thailand, eight genotypes of maize in both inbred and hybrid varieties, belonging to private companies are dominated with high yield (>5788 kg ha<sup>-1</sup>), which cultivated >95% of whole area (Napisintuwong, 2020). We hypothesized that more adapted strategies in terms of physio-morphological, biochemical traits (at seedling and reproductive stages) and yield attributes (at harvesting stage) of drought tolerant maize genotypes than those in drought susceptible, playing a key role as multivariate indices for drought tolerant screening. In the present study, we aim to evaluate candidate drought tolerant maize varieties under water deficit rainfed trials using physio-morphological changes and yield traits.

## Materials and Methods

### *Plant materials and field experiment conditions*

Seeds of eight genotypes of maize (*Zea mays* L.), including an inbred variety 'Suwan5' and 7 hybrid varieties (Table S1), were cultivated in the farmer field at Phetchabun Province, Northern region of Thailand (Latitude 15° 44' 58.6" N, Longitude 101° 00' 37.4" E). Two cultivars, 'Suwan5' and 'Suwan4452' (drought tolerant positive check) of maize genotypes were provided by Suwan Farm Research Station, National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand. In addition, six hybrid maize genotypes i.e., 'CP-DK888', 'Pac339', 'DK9901', 'DK7979', 'CP301' and 'S7328' were purchased from the market. The isohyperthermic Ultic Paleustalfs soil (0-20 cm depth) was clayey in nature (sand 12.25%, silt 31.33% and clay 54.54%) with a pH of 7.65, electrical conductivity (EC<sub>e</sub>) of 0.95 dS m<sup>-1</sup>, 1.96% organic matter (OM), 7.78 mg kg<sup>-1</sup> available phosphorus (P) and 51.01 mg kg<sup>-1</sup> of exchangeable potassium (K) (Table S2). In year 2019, the experimental plots were established as 8 × 2 factorials in completely randomized design (CRD) under two water regimes: well-watered (WW) and water deficit (WD) with three replicates each (*n* = 3) in an area of 26.88 m<sup>2</sup> (4.8 m × 5.6 m). The plant and row spacing were set at 0.2 and 0.7 m, respectively. Fertilizers were applied twice, before cultivation and 30 days after sowing (DAS), using site-specific nutrient management (SSNM) with 62.5 kg ha<sup>-1</sup> of 46-0-0 fertilizer, 31.25 kg ha<sup>-1</sup> of 18-46-0 fertilizer, and 31.25 kg ha<sup>-1</sup> of 0-0-60 fertilizer for N, P and K, respectively. Furrow irrigation was applied to well-watered plots (WW, 30 ± 2% soil water content) at ten days interval to maintain the soil water content. On the other hand, water deficit plots (WD; 12 ± 2% soil water content) were maintained without water for twenty days before seedling (24 DAS) and reproductive stages (80 DAS) appeared. Weather station was installed at the experimental site to collect the online weather data, including rainfall (mm), air temperature (°C), relative humidity (%), and soil moisture content (%) (Figure S1). Digital images from UAV (unmanned aerial vehicle) with RGB camera were collected for both seedling (24 DAS) and reproductive (80 DAS) stages (Figure S2). UAV (DJI Phantom 4 Advanced) installed with multispectral sensors (RedEdge-M by Micasense) was used. Digital camera installed on the UAV captured images with three spectral bands of red, green, and blue, with image size ratio of 5472 × 3078 pixels and resolution of 20 megapixels. Multispectral sensors have five-narrow spectral bands such as Red, Green,

Blue, Near-Infrared, and Red Edge. The UAV images of both sensors were measured at vegetative and reproductive stages. UAV flight plan was fixed at an altitude of ~90 m aboveground with 80% front and side image overlap. The images were acquired under clear sky minimum possible cloud cover between 10 am to 2 pm. The ground sampling distance (GSD) of digital and multispectral images was fixed at 2.39 and 6.25 cm per pixel, respectively. The UAV images were georeferenced and ortho-mosaicked using Pix4D software. The digital imageries were analysed in the region of interest (ROI) according to the ratio of red (R), green (G), and blue (B) colour components using ArcGIS program.

#### *Normalized difference vegetation index (NDVI)*

Normalized difference vegetation index (NDVI) was calculated from red and near infra-red (NIR) regions of the spectrum using Trimble GreenSeeker™ (Trimble Inc., Sunnyvale, California, USA) handheld optical sensor (Govaerts and Verhulst, 2010). The measurement was taken from the maize canopy at the seedling (24 DAS) and reproductive stage (80 DAS) with three biological replicates per treatment ( $n = 3$ ).

#### *Leaf temperature and crop water stress index (CWSI)*

Thermal infra-red images were captured between 11 a.m. to 2 p.m. at 1.5 m from the maize canopy using an infra-red thermal imaging camera of FLIR E series (Model E50, FLIR Systems, Inc., Boston, USA), with long-wave of 7.5-13  $\mu\text{m}$ , at resolution of  $240 \times 180$  pixels with an emissivity set at 0.95 for the surfaces of natural vegetation. Artificial dry and wet references were used in the ambient temperature for 10 min and placed in the scene of thermal images. A black paper was used as the artificial dry reference, to absorb the radiation and, therefore, act as a non-transpiration leaf. Artificial wet was obtained by using a small absorbent cotton box filled with water to represent a fully transpiring leaf. Three biological replicates were used per treatment ( $n = 3$ ) at the seedling (24 DAS) and reproductive stages (80 DAS) (Figure S3). Temperature of leaf, dry reference and wet reference were analysed using FLIR Tool 5.1 software. CWSI was calculated by the equation provided by Idso *et al.* (1981).

$$CWSI = \frac{T_{Leaf} - T_{wet}}{T_{dry} - T_{wet}}$$

where  $T_{Leaf}$  is the leaf temperature ( $^{\circ}\text{C}$ ),  $T_{dry}$  is the dry reference temperature ( $^{\circ}\text{C}$ ) for fully closed stomata and  $T_{wet}$  is the wet reference temperature ( $^{\circ}\text{C}$ ) for fully transpiring leaf. CWSI index  $>0.4$  was represented as stress indicator when plants exposed to water deficit condition.

#### *Physiological characteristics*

Leaf greenness in the second fully expanded leaf of maize genotypes was estimated using Digital Chlorophyll Meter (Model SPAD-502Plus, Konica Minolta Inc., Osaka, Japan) following the method of Dwyer *et al.* (1991). In brief, the SPAD data in four points of different region of second fully expanded leaf blade were collected and averaged in each treatment.

Chlorophyll fluorescence emission was measured from the adaxial surface of second fully expanded leaf of maize using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode (Loggini *et al.*, 1999). A leaf kept in dark for 30 min was initially exposed to the modulated measuring beam of far-red light (LED source) with typical peak at a wavelength of 735 nm. Basal fluorescence ( $F_0$ ) and maximum ( $F_m$ ) fluorescence yields were measured under weak modulated red light ( $<85 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with 1.6 s pulses of saturating light ( $>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPF) and calculated using FMS software for Windows. The variable fluorescence yield ( $F_v$ ) was calculated using the equation:  $F_v = F_m - F_0$ . The ratio of variable to maximum fluorescence ( $F_v/F_m$ ) was calculated as the maximum quantum

yield of PSII photochemistry. The photon yield of PSII ( $\Phi_{\text{PSII}}$ ) in light was calculated as:  $\Phi_{\text{PSII}} = (F_m' - F) / F_m'$  after 45 s of illumination, when steady state was achieved (Maxwell and Johnson, 2000).

Net photosynthetic rate ( $P_n$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $E$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $g_s$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) in the second fully-expanded leaf of maize genotypes were measured using a Portable Photosynthesis System with an Infra-red Gas Analyzer (Model LI 6400, LI-COR Inc., Lincoln, Nebraska, USA) at 10.00 – 12.00 am. All parameters were measured by continuously monitoring the content of the air entering and exiting the IRGA headspace chamber, according to Cha-um *et al.* (2006). The air-flow rate of IRGA chamber was fixed at  $500 \mu\text{mol s}^{-1}$  and chamber temperature were set at  $28^\circ\text{C}$ . The light intensity was adjusted to  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD by 6400-02B red-blue LED light source. Leaf area was set at  $3 \times 2$  or  $6 \text{ cm}^2$  according to the size of IRGA microchamber.

#### *Morphological and yield-related traits*

Shoot height (cm) and total chlorophyll were assessed at the seedling (24 DAS) and reproductive stage (80 DAS). At the harvesting stage, yield traits (Figure S4), including number of ears per plant, ear weight (g), husk weight (g), cob weight (g), hundred grain weight (g), grain weight ( $\text{g ear}^{-1}$ ), total grain weight ( $\text{kg ha}^{-1}$ ) and above ground biomass ( $\text{kg ha}^{-1}$ ) were measured.

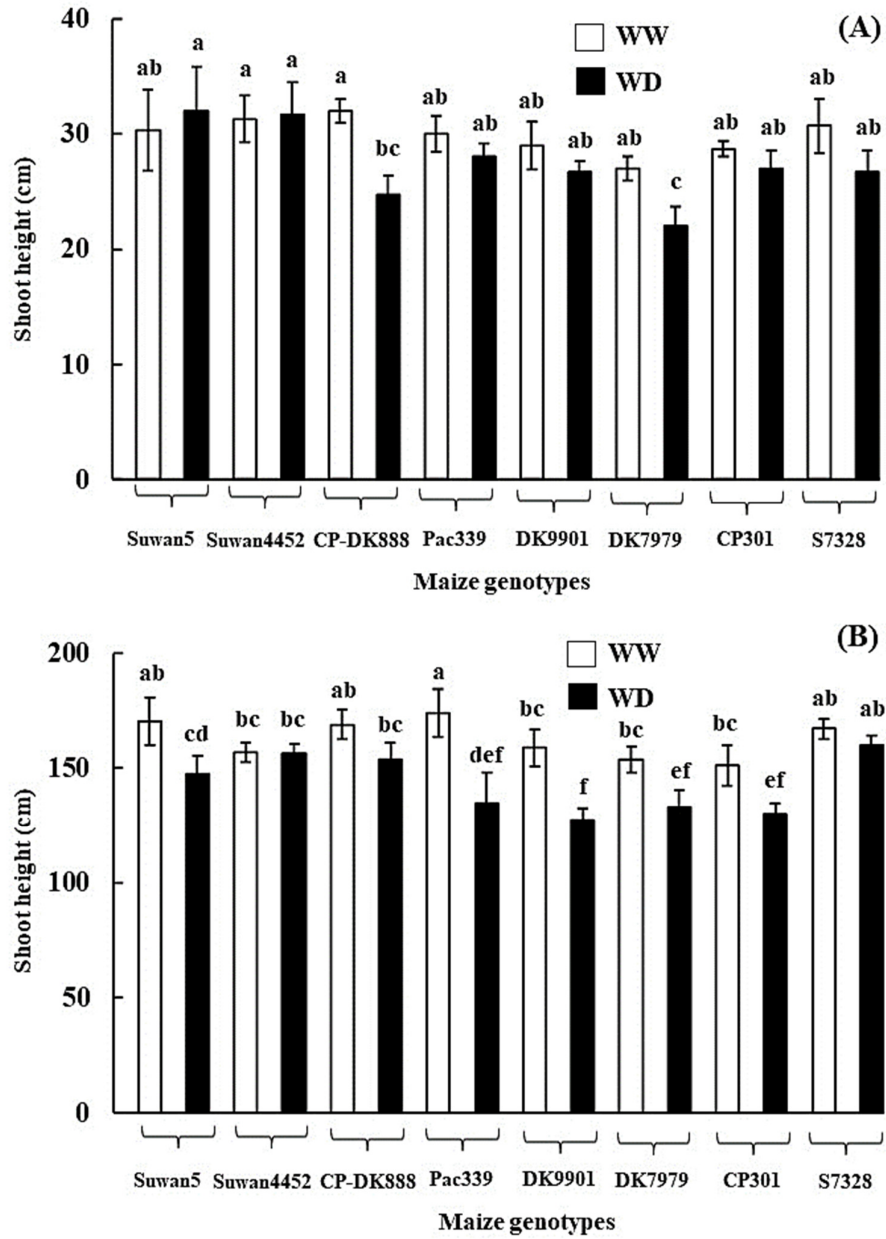
#### *Statistical analysis and cluster analysis*

The experiment was arranged as  $8 \times 2$  factorials in Completely Randomized Design (CRD) with three replicates ( $n = 3$ ) in well watering (WW) and water deficit conditions (WD). Analysis of variance (ANOVA) was calculated, then the mean values obtained from sixteen treatments were compared using Duncan's Multiple Range test (DMRT) and analysed by SPSS software (version 11.5 for Window®). In addition, water deficit tolerance indices were calculated by dividing the values of physio-morphological parameters and yield traits in water deficit stress by the control as per the following equation (Cha-um *et al.*, 2014). The cluster ranking groups were obtained based on Ward's cluster analysis using the water deficit tolerance indices of physio-morphological parameters and yield traits (Chen *et al.*, 2016b). In addition, drought tolerance indices (DTI) of morphological, physiological changes and yield-related traits were subjected to principal component analysis biplot for similarity assay according to Liu *et al.* (2015).

## **Results**

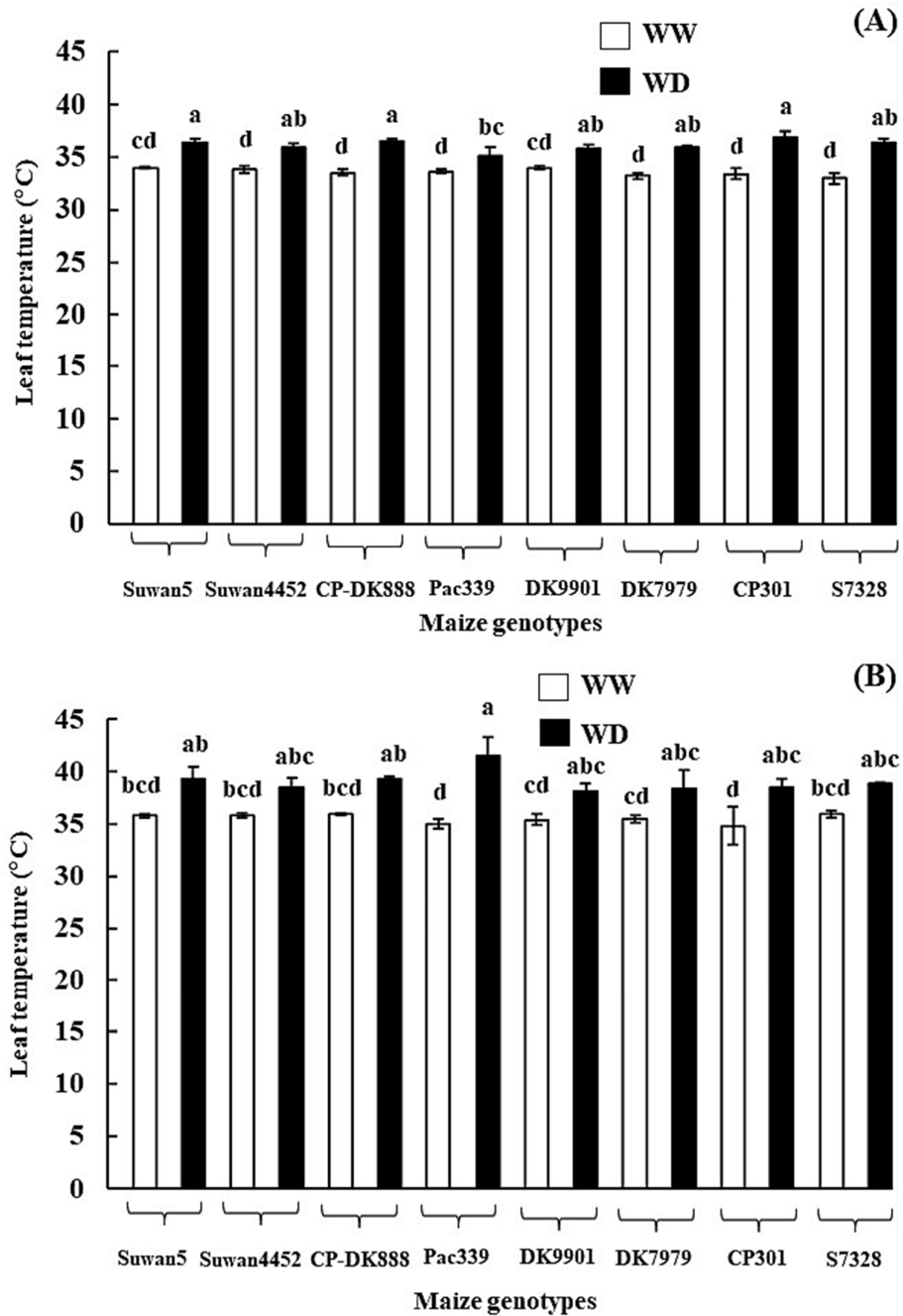
#### *Seedling stage (24 DAS 5 leaf stage)*

Shoot height of eight maize genotypes was measured to be 28-32 cm in well-watered plants. Under water deficit, shoot height in cv. 'CP-DK888' and 'DK7979' was significantly declined by 22.8% and 18.6%, respectively, whereas it was retained in other genotypes (Figure 1A). Leaf temperature in all the genotypes under water deficit condition was significantly increased by 1.05-1.11 folds over well-watered plants (Figure 2A), thereby leading to enhanced crop water stress index (CWSI) by 1.42-1.86 folds (Table 1). Normalized difference vegetation index (NDVI) in cvs. 'DK9901', 'CP301' and 'S7328', grown under water deficit conditions were decreased by 18.7%, 23.3% and 19.4% over the control, respectively, whereas NDVI in other genotypes was maintained (Table 1).



**Figure 1.** Shoot height of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD) for 24 days after sowing (seedling stage; A) and 80 days (reproductive stage; B) after sowing (DAS)

Data are presented as mean  $\pm$  SE ( $n = 3$ ). Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).



**Figure 2.** Leaf temperature, using Infra-red Thermal Camera, of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD) for 24 days (seedling stage; A) and 80 days after sowing (reproductive stage; B) after sowing (DAS). Data are presented as mean  $\pm$ SE ( $n = 3$ ). Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).

Leaf greenness (SPAD) in maize leaves under water deficit stress was stabilized in most of the genotypes, except in cv. 'Pac339' (degradation by 10.2% over the control) and 'DK7979' (degradation by 7.6% over the control) (Figure 3A). Maximum quantum yield of PSII ( $F_v/F_m$ ) in water deficit stressed plants was unchanged, whereas photon yield of PSII ( $\Phi_{PSII}$ ) in cvs. 'Pac339', 'DK7979' and 'S7328' were significantly diminished by

9.0%, 9.5% and 10.2% over the control, respectively (Table 1). Net photosynthetic rate ( $P_n$ ) in maize cvs. ‘Suwan5’, ‘Pac339’, ‘DK7979’, ‘CP301’ and ‘S7328’ grown under water deficit was significantly reduced by 13.2%, 22.5%, 8.8%, 24.8% and 13.3% over the control, respectively (Table 1). Transpiration rate ( $E$ ) in cvs. ‘Pac339’, ‘DK7979’, and ‘CP301’ was also declined by 35.1%, 11.3% and 22.8%, respectively, when exposed to water deficit condition (Table 1). In addition, stomatal conductance ( $g_s$ ) in maize genotypes at seedling stage was very sensitive to water deficit stress, and it was sharply declined by 19.4% (‘Suwan5’), 10.9% (‘Suwan4452’), 15.6% (‘CP-DK888’), 30% (‘Pac339’), 19.5% (‘DK9901’), 36.8% (‘CP301’) and 23.6% (‘S7328’), except in cv. DK7979 where it was retained (Table 1). Leaf osmotic potential ( $\Psi_s$ ) in water deficit stressed maize was decreased, especially in seedling stage (by 8.95% - 28.69% over well-watered) (Table 1), whereas it was maintained at reproductive stage (by 1.00 – 14.69% over control) (Table 2). This parameter was used as a good indicator for water availability in the leaf tissues, especially in water deficit stressed plants.

**Table 1.** Normalized Difference Vegetation Index (NDVI), Crop Water Stress Index (CWSI), maximum quantum yield of PSII ( $F_v/F_m$ ), quantum efficiency of PSII ( $\Phi_{PSII}$ ), net photosynthetic rate ( $P_n$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $E$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and leaf osmotic potential ( $\Psi_s$ ; MPa) in maize genotypes grown under different water regimes for 24 days after sowing (DAS) or seedling stage. Data are presented as mean  $\pm$ SE ( $n = 3$ ).

Varieties	Water regime	NDVI	CWSI	$F_v/F_m$	$\Phi_{PSII}$	$P_n$	$E$	$g_s$	$\Psi_s$
‘Suwan5’	WW	0.56 $\pm$ 0.0ab	0.25 $\pm$ 0.03cde	0.82 $\pm$ 0.01a	0.69 $\pm$ 0.00ab	26.34 $\pm$ 3.9b	4.76 $\pm$ 0.8ab	0.17 $\pm$ 0.0b	-2.57 $\pm$ 0.11a
	WD	0.48 $\pm$ 0.01b	0.40 $\pm$ 0.04ab	0.80 $\pm$ 0.00ab	0.64 $\pm$ 0.01bc	22.86 $\pm$ 2.56c	4.08 $\pm$ 0.55b	0.13 $\pm$ 0.02c	-2.80 $\pm$ 0.11ab
		(-15.2%)	(+1.6-fold)	(-2.6%)	(-6.8%)	(-13.2%)	(-14.3%)	(-19.4%)	(-8.95%)
‘Suwan4452’	WW	0.53 $\pm$ 0.04b	0.26 $\pm$ 0.02cde	0.79 $\pm$ 0.01ab	0.65 $\pm$ 0.00bc	28.66 $\pm$ 1.98ab	5.47 $\pm$ 0.50a	0.20 $\pm$ 0.03a	-2.37 $\pm$ 0.13a
	WD	0.53 $\pm$ 0.03b	0.37 $\pm$ 0.01abc	0.77 $\pm$ 0.01b	0.61 $\pm$ 0.02c	25.31 $\pm$ 1.55b	5.02 $\pm$ 0.54ab	0.17 $\pm$ 0.04b	-3.05 $\pm$ 0.08ab
		(-0.6%)	(+1.42-fold)	(-2.6%)	(-7.3%)	(-11.7%)	(-8.2%)	(-10.9%)	(-28.69%)
‘CP-DK888’	WW	0.55 $\pm$ 0.02ab	0.23 $\pm$ 0.02de	0.80 $\pm$ 0.00ab	0.70 $\pm$ 0.01ab	27.21 $\pm$ 3.57ab	5.29 $\pm$ 0.76a	0.20 $\pm$ 0.06a	-2.56 $\pm$ 0.14a
	WD	0.55 $\pm$ 0.05ab	0.41 $\pm$ 0.12ab	0.78 $\pm$ 0.01ab	0.66 $\pm$ 0.04b	25.65 $\pm$ 3.74b	4.90 $\pm$ 1.02ab	0.17 $\pm$ 0.04b	-3.16 $\pm$ 0.20b
		(0)	(+1.78-fold)	(-3.3%)	(-5.5%)	(-5.7%)	(-7.5%)	(-15.6%)	(-23.44%)
‘Pac339’	WW	0.58 $\pm$ 0.05a	0.21 $\pm$ 0.03de	0.81 $\pm$ 0.00a	0.73 $\pm$ 0.02a	29.41 $\pm$ 4.80a	5.78 $\pm$ 1.49a	0.20 $\pm$ 0.05a	-2.68 $\pm$ 0.02a
	WD	0.59 $\pm$ 0.02a	0.39 $\pm$ 0.05ab	0.79 $\pm$ 0.01ab	0.66 $\pm$ 0.05b	22.80 $\pm$ 5.51c	3.75 $\pm$ 0.90c	0.14 $\pm$ 0.05c	-3.16 $\pm$ 0.13b
		(0%)	(+1.86-fold)	(-1.6%)	(-9.0%)	(-22.5%)	(-35.1%)	(-30.0%)	(-23.44%)
‘DK9901’	WW	0.59 $\pm$ 0.00a	0.18 $\pm$ 0.04e	0.79 $\pm$ 0.01ab	0.70 $\pm$ 0.01ab	30.06 $\pm$ 2.66a	5.40 $\pm$ 0.34a	0.21 $\pm$ 0.03a	-2.56 $\pm$ 0.12a
	WD	0.48 $\pm$ 0.02b	0.33 $\pm$ 0.03abcd	0.78 $\pm$ 0.01ab	0.65 $\pm$ 0.04bc	28.49 $\pm$ 2.22ab	5.19 $\pm$ 0.34a	0.17 $\pm$ 0.01b	-3.13 $\pm$ 0.19b
		(-18.7%)	(+1.83-fold)	(-1.5%)	(-7.5%)	(-5.2%)	(-3.9%)	(-19.5%)	(-22.27%)
‘DK7979’	WW	0.51 $\pm$ 0.03b	0.19 $\pm$ 0.00e	0.80 $\pm$ 0.01ab	0.70 $\pm$ 0.01ab	29.35 $\pm$ 1.96a	5.47 $\pm$ 0.43a	0.18 $\pm$ 0.05b	-2.71 $\pm$ 0.10a
	WD	0.43 $\pm$ 0.06b	0.29 $\pm$ 0.01bcde	0.79 $\pm$ 0.01ab	0.63 $\pm$ 0.04c	26.77 $\pm$ 4.28b	4.85 $\pm$ 0.74ab	0.18 $\pm$ 0.02b	-3.28 $\pm$ 0 .16b
		(-17.1%)	(+1.53-fold)	(-1.7%)	(-9.5%)	(-8.8%)	(-11.33%)	(0%)	(-21.03%)
‘CP301’	WW	0.58 $\pm$ 0.03a	0.21 $\pm$ 0.01de	0.80 $\pm$ 0.01ab	0.70 $\pm$ 0.01ab	27.09 $\pm$ 4.22ab	4.83 $\pm$ 0.65ab	0.18 $\pm$ 0.05b	-2.77 $\pm$ 0.05a
	WD	0.45 $\pm$ 0.04b	0.38 $\pm$ 0.04abc	0.79 $\pm$ 0.01ab	0.63 $\pm$ 0.04c	20.37 $\pm$ 1.73c	3.73 $\pm$ 0.50c	0.11 $\pm$ 0.01c	-3.36 $\pm$ 0.20b
		(-23.3%)	(+1.81-fold)	(-1.1%)	(-10.2%)	(-24.8%)	(-22.8%)	(-36.8%)	(-21.30%)
‘S7328’	WW	0.62 $\pm$ 0.01a	0.26 $\pm$ 0.01cde	0.79 $\pm$ 0.01ab	0.69 $\pm$ 0.01ab	30.26 $\pm$ 2.81a	5.83 $\pm$ 0.38a	0.22 $\pm$ 0.05a	-2.60 $\pm$ 0.03a
	WD	0.50 $\pm$ 0.01b	0.44 $\pm$ 0.03a	0.80 $\pm$ 0.00ab	0.68 $\pm$ 0.01b	26.24 $\pm$ 0.77b	4.89 $\pm$ 0.12ab	0.17 $\pm$ 0.01b	-2.99 $\pm$ 0.12ab
		(-19.4%)	(+1.69-fold)	(0%)	(-1.4%)	(-13.3%)	(-16.1%)	(-23.6%)	(-15.00%)

Significant level									
Varieties	*	ns	ns	*	ns	ns	ns	*	
Water	**	**	*	**	*	*	*	**	
Var × Water	ns	ns	ns	ns	ns	ns	ns	**	

<sup>ns</sup>, <sup>\*</sup>, and <sup>\*\*</sup> represent non-significant difference, and significant difference at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

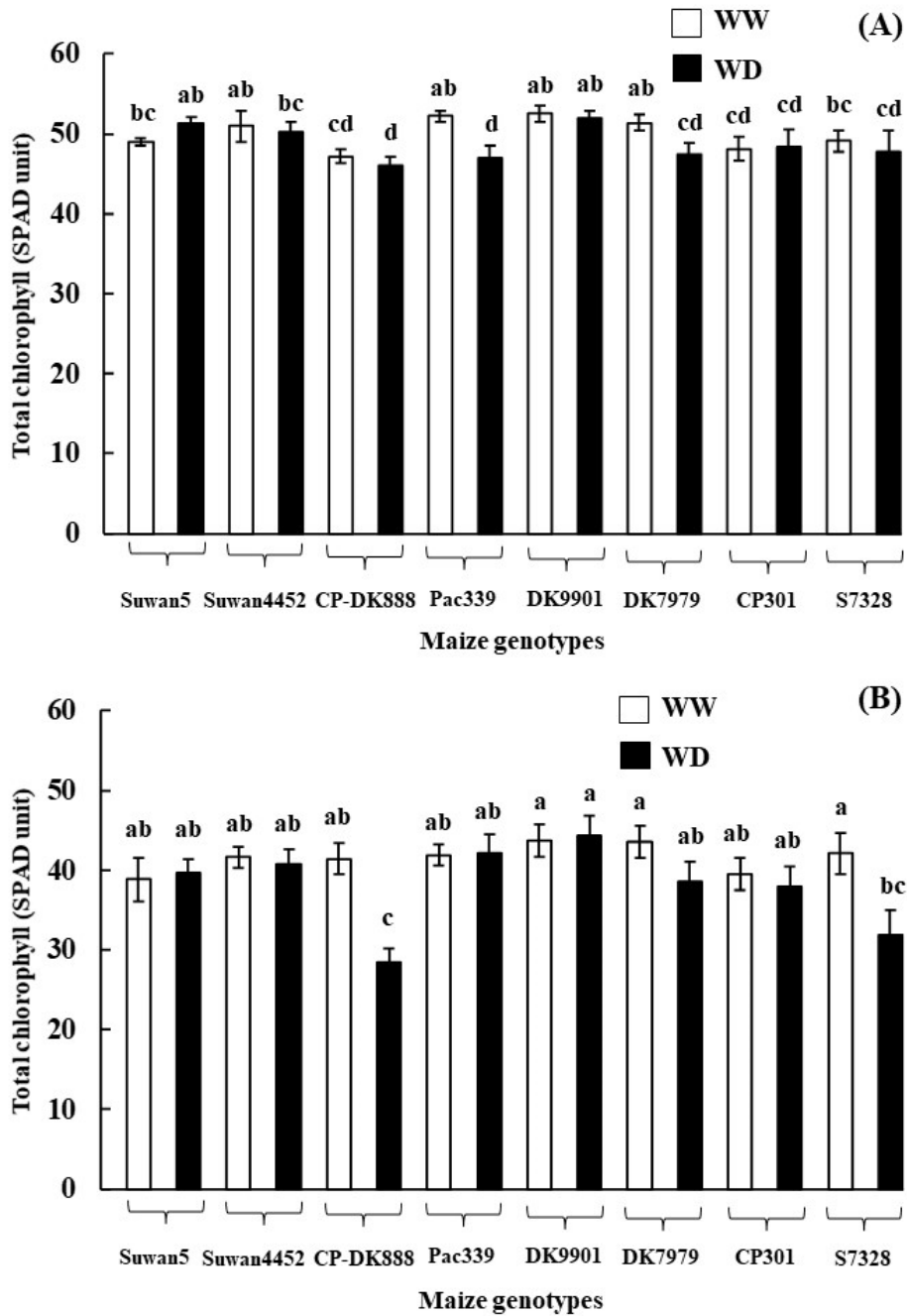
Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).

**Table 2.** Normalized Difference Vegetation Index (NDVI), Crop Water Stress Index (CWSI), maximum quantum yield of PSII ( $F_v/F_m$ ), quantum efficiency of PSII ( $\Phi_{PSII}$ ), net photosynthetic rate ( $P_n$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $E$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ;  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and leaf osmotic potential ( $\Psi_s$ ; MPa) in maize genotypes grown under different water regimes for 80 days after sowing (DAS) or reproductive stage. Data are presented as mean  $\pm$  SE ( $n = 3$ ).

Varieties	Water regime	NDVI	CWSI	$F_v/F_m$	$\Phi_{PSII}$	$P_n$	$E$	$g_s$	$\Psi_s$
'Suwan5'	WW	0.55± 0.03ab	0.32± 0.10bc	0.78± 0.04b	0.64± 0.01a	15.71± 4.11ab	3.31± 0.72b	0.10± 0.04b	-3.46± 0.13ab
	WD	0.43± 0.04c	0.62± 0.18a	0.81± 0.01a	0.64± 0.03a	11.75± 4.41b	2.23± 0.69c	0.05± 0.02c	-3.59± 0.23ab
		(-22.0%)	(+1.94-fold)	(0%)	(0%)	(-25.2%)	(-32.6%)	(-48.1%)	(-3.76%)
'Suwan44 52'	WW	0.57± 0.05a	0.24± 0.08c	0.82± 0.01a	0.63± 0.02a	16.69± 1.51ab	3.36± 0.29b	0.10± 0.02b	-3.51± 0.08ab
	WD	0.47± 0.01bc	0.32± 0.12bc	0.78± 0.02b	0.59± 0.02ab	13.50± 5.67b	3.13± 1.51b	0.08± 0.05bc	-3.55± 0.04ab
		(-17.5%)	(+1.33-fold)	(-4.2%)	(-6.0%)	(-19.1%)	(-7.1%)	(-18.6%)	(-4.31%)
'CP-DK888'	WW	0.56± 0.05ab	0.29± 0.06c	0.80± 0.02a	0.57± 0.06ab	18.97± 2.65a	3.82± 0.94b	0.12± 0.04ab	-3.79± 0.17bc
	WD	0.42± 0.02c	0.49± 0.07ab	0.74± 0.07b	0.60± 0.03a	11.75± 1.96b	2.59± 0.42c	0.06± 0.01c	-3.84± 0.13bc
		(-25.0%)	(+1.69-fold)	(-7.1%)	(0%)	(-38.1%)	(-32.0%)	(-53.3%)	(-1.32%)
'Pac339'	WW	0.58± 0.01a	0.36± 0.12b	0.82± 0.01a	0.63± 0.01a	17.91± 3.01ab	3.62± 0.69b	0.12± 0.03ab	-3.80± 0.15bc
	WD	0.49± 0.07bc	0.46± 0.10ab	0.80± 0.02a	0.59± 0.05ab	8.25± 3.24c	1.77± 0.62c	0.05± 0.02c	-4.16± 0.20c
		(-14.8%)	(+1.28-fold)	(-2.3%)	(-5.9%)	(-53.9%)	(-51.1%)	(-61.8%)	(-9.48%)
'DK9901'	WW	0.60± 0.05a	0.32± 0.10bc	0.82± 0.02a	0.63± 0.04a	20.54± 2.34a	4.67± 0.70a	0.17± 0.06a	-3.20± 0.05a
	WD	0.51± 0.05b	0.42± 0.13ab	0.82± 0.02a	0.61± 0.03a	14.17± 4.45b	2.58± 0.84c	0.07± 0.03bc	-3.43± 0.15ab
		(-14.2%)	(+1.31-fold)	(0%)	(-3.1%)	(-31.0%)	(-44.7%)	(-55.8%)	(-7.19%)
'DK7979'	WW	0.60± 0.07a	0.20± 0.03c	0.84± 0.01a	0.65± 0.03a	20.63± 2.93a	4.42± 0.66a	0.15± 0.04a	-3.22± 0.04a
	WD	0.50± 0.06bc	0.46± 0.17ab	0.82± 0.02a	0.57± 0.02b	16.42± 3.83ab	2.85± 0.65c	0.08± 0.03bc	-3.25± 0.07a
		(-17.3%)	(+2.30-fold)	(-2.6%)	(-11.7%)	(-20.4%)	(-35.4%)	(-45.2%)	(-1.00%)
'CP301'	WW	0.53± 0.00b	0.26± 0.03c	0.82± 0.01a	0.59± 0.03ab	19.59± 3.91a	4.40± 1.21a	0.18± 0.08a	-3.19± 0.05a
	WD	0.52± 0.05b	0.38± 0.05b	0.82± 0.01a	0.60± 0.05a	13.67± 3.15b	2.73± 0.43c	0.08± 0.02bc	-3.40± 0.10ab
		(-2.7%)	(+1.46-fold)	(0%)	(0%)	(-30.2%)	(-38.0%)	(-58.6%)	(-6.58%)
'S7328'	WW	0.55± 0.03ab	0.26± 0.05c	0.80± 0.02a	0.63± 0.04a	17.16± 2.87ab	3.50± 0.47b	0.12± 0.04ab	-3.54± 0.08ab
	WD	0.43± 0.05c	0.50± 0.08ab	0.83± 0.02a	0.59± 0.02ab	9.79± 4.07c	2.03± 0.87c	0.06± 0.03c	-4.06± 0.05c
		(-22.6%)	(+1.92-fold)	(0%)	(-6.0%)	(-42.9%)	(-42.0%)	(-51.8%)	(-14.69%)
Significant level									
Varieties	ns	ns	ns	ns	ns	ns	ns	*	
Water	**	**	*	*	**	**	**	**	
Var × Water	ns	ns		ns	ns	ns	**	**	

<sup>ns</sup>, <sup>\*</sup>, and <sup>\*\*</sup> represent non-significant difference, and significant difference at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).



**Figure 3.** Leaf greenness (SPAD) in the leaf tissues of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD) for 24 days after sowing (seedling stage; A) and 80 days (reproductive stage; B) after sowing (DAS). Data are presented as mean  $\pm$ SE ( $n = 3$ ). Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).

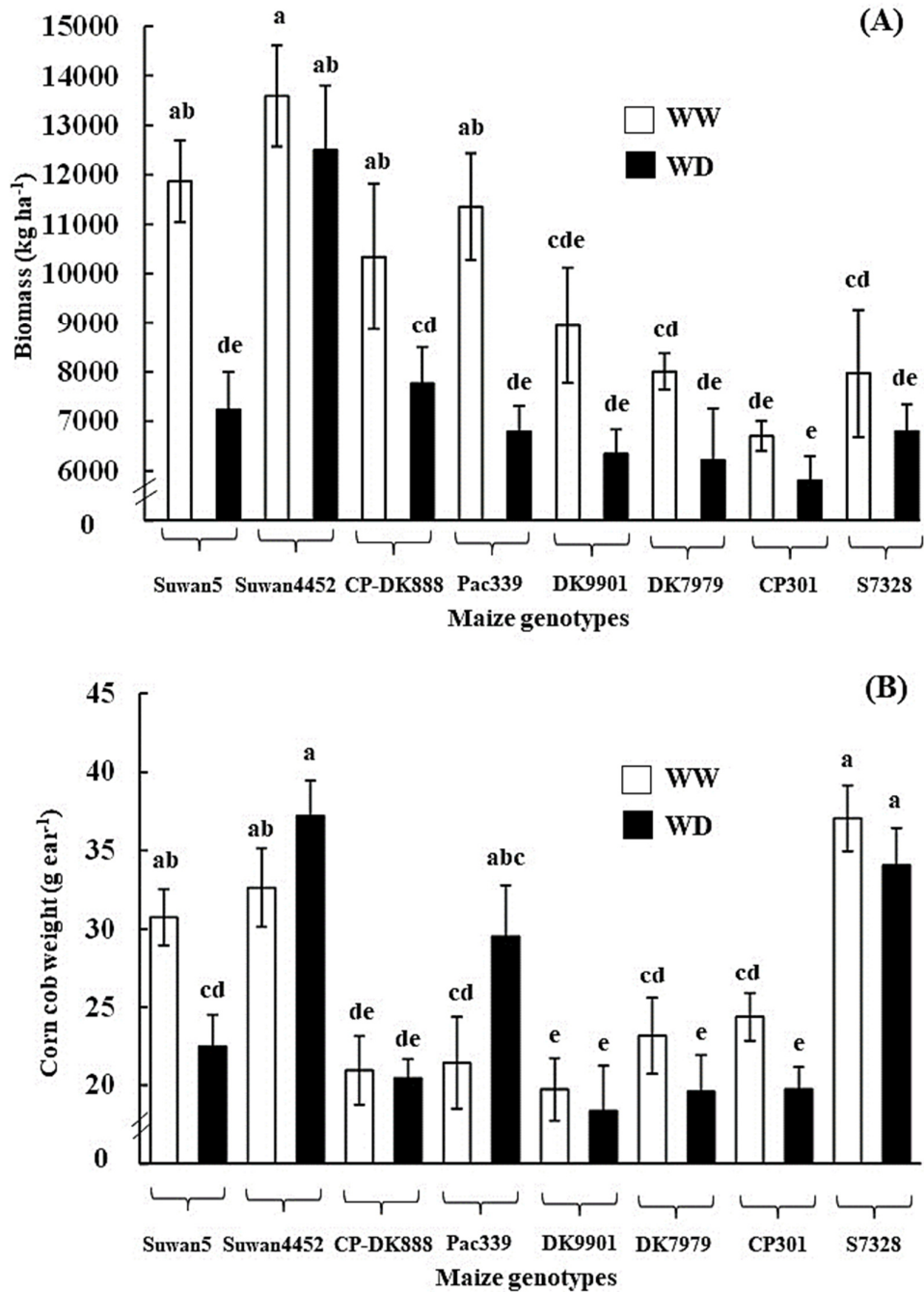
*Reproductive stage (80 DAS)*

Shoot height of maize cvs. 'Suwan4452', 'CP-DK888', and 'S7328' during flowering period under water deficit stress was retained, whereas it was sharply declined by 13.5%, 22.8%, 19.8%, 13.7%, and 13.9% in cvs. 'Suwan5', 'Pac339', 'DK9901', 'DK7979', and 'CP301', respectively, when compared to well-watered condition (Figure 1B). Leaf temperature of maize cvs. 'Pac339' and 'CP301' were increased by 1.19 and 1.10 folds, respectively, over the control, whereas it was unchanged in other genotypes (Figure 2B). NDVI in water deficit stressed leaves of maize genotypes was significantly declined (> 14.2% reduction over the control), except in cv. 'CP301' (Table 2). CWSI in maize plants was promoted by water deficit conditions (> 1.46 folds over the control) which act as a stress indicator, while it was retained in cvs. 'Suwan4452', 'Pac339' and 'DK9901' (Table 1).

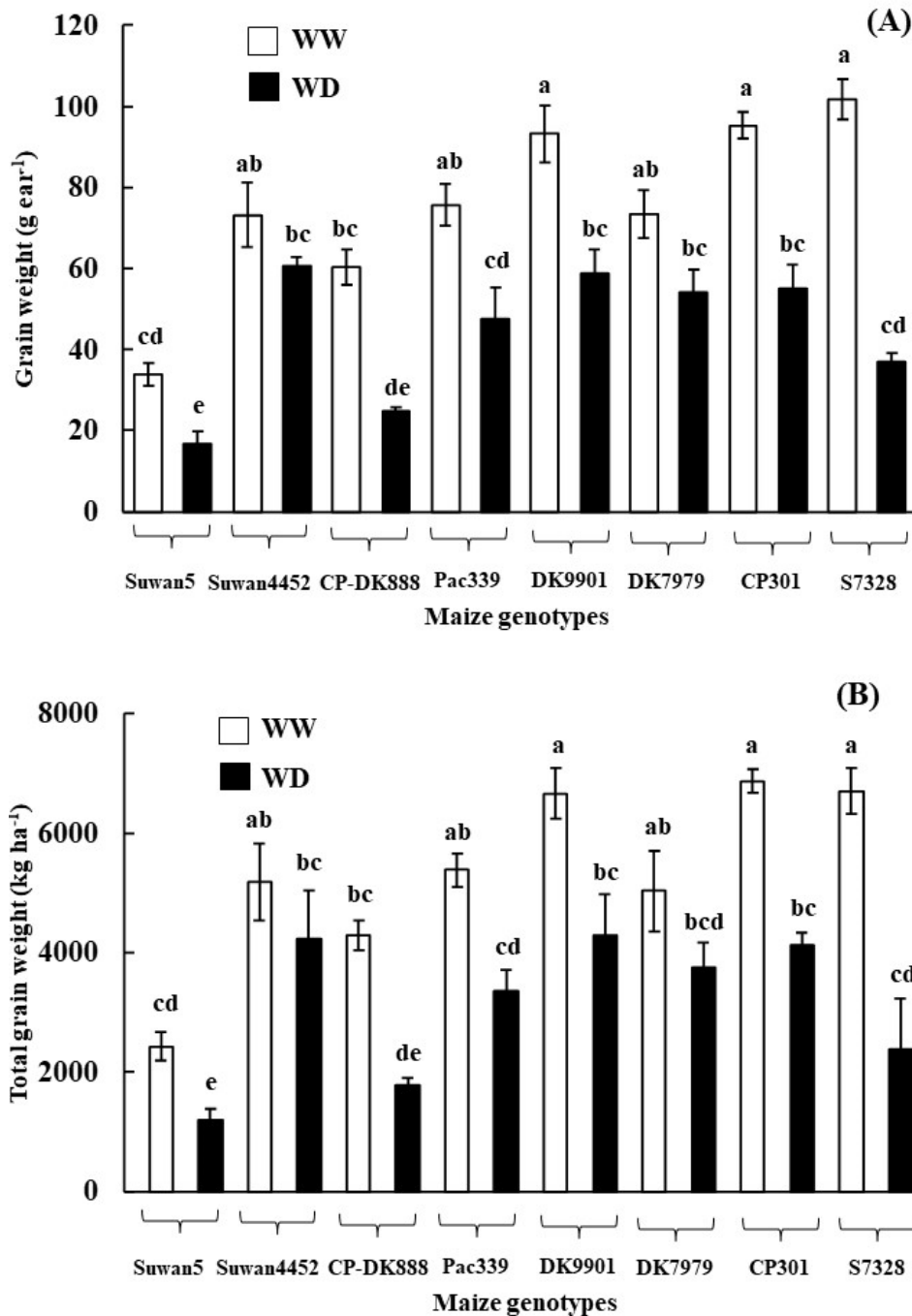
Interestingly, leaf greenness in maize cvs. 'CP-DK888' and 'S7328' under water deficit conditions was degraded by 31.3% and 24.1% over the control, respectively, whereas it was unchanged in other genotypes (Figure 3B).  $F_v/F_m$  in cvs. 'Suwan4452' and 'CP-DK888' under drought situation was diminished by 4.2% and 7.1% over the control, respectively. Moreover,  $\Phi_{PSII}$  in cv. 'DK7979' under drought stress was declined by 11.7% (Table 2).  $P_n$  in maize plant was a very sensitive parameter to water deficit stress as indicated by a sharp decline in cvs. 'CP-DK888' (38.1%), 'Pac339' (53.9%), 'DK9901' (31.0%), 'CP304' (30.2%) and 'S7328' (42.9%) (Table 2). Moreover, E and g in cv. Suwan4452 under water deficit conditions were stabilized whereas they were sharply dropped in other genotypes (Table 2).

*Yield attributes and cluster analysis*

At the harvesting stage, plant biomass in cvs. 'Suwan5', 'CP-DK888', and 'Pac339' of water deficit stressed plants was significantly dropped by 39.0%, 24.9% and 40.2% over the control, respectively (Figure 4A). Number of ears per plant in cv. Suwan5 was significantly declined by 30%, whereas it was retained in other genotypes (Table 3). Interestingly, ear weight in cvs. 'Suwan4452', 'Pac339', and 'DK7979' under water deficit condition was retained, whereas it was sharply declined (>27.5% reduction over the control) in other genotypes (Table 3). Husk weight in cv. 'Suwan5' was very sensitive to water deficit stress, leading to a reduction of 22.9% over the control. In addition, hundred grain weight in cv. 'S7328' was also susceptible to drought situation, leading to the reduction of 20.1% (Table 3). Corn cob weight in cvs. 'Suwan5', 'DK7979' and 'CP301' under drought stress was declined by 27.0%, 15.4% and 19.0%, respectively, when compared with well-watered plants (Figure 4B). Grain weight per ear (Figure 5A) and total grain weight (Figure 5B) in cvs. 'Suwan4452' and 'DK7979' under water deficit stress were maintained, whereas those were significantly decreased (>35% reduction over well-watered) in other genotypes.



**Figure 4.** Biomass (A) and corn cob weight (B) of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD) until harvest process  
Data are presented as mean  $\pm$ SE ( $n = 3$ ). Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT)



**Figure 5.** Grain weight per ear (A) and total grain weight (B) of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD) until harvest process. Data are presented as mean  $\pm$ SE ( $n = 3$ ). Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan's Multiple Range test (DMRT).

Ten indices including shoot height, leaf temperature, chlorophyll content, NDVI, CWSI,  $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $P_n$ ,  $E$ , and  $g_s$ , in 24-day-old seedlings of each maize genotype were subjected to Ward's cluster analysis. 'DK7979' and 'CP-DK888' maize genotypes were categorized as drought tolerant in the same group with 'Suwan4452' as drought tolerant positive check. In addition, 'Suwan 5', 'DK9901', and 'S7328' were identified as moderately

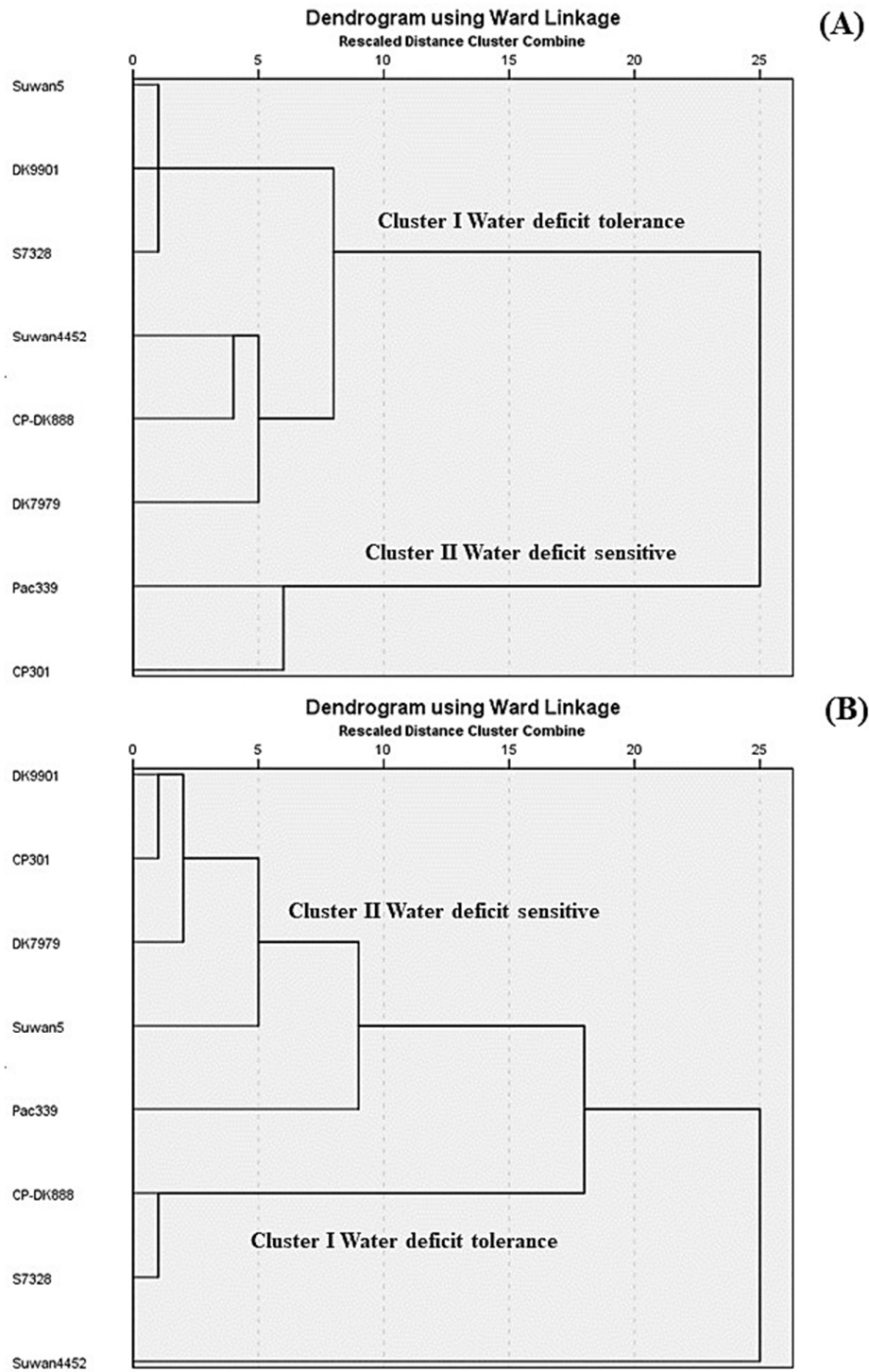
drought tolerant genotypes at the seedling stage. In contrast, ‘Pac339’ and ‘CP301’ genotypes were identified as drought susceptible ones (Figure 6A). According to flowering and yield harvesting stages, ‘Suwan4452’, ‘S7328’, and ‘CP-DK888’ were classified as drought tolerant genotypes, whereas ‘DK9901’, ‘CP301’, ‘DK7979’, ‘Suwan5’, and ‘Pac339’ were identified as drought sensitive (Figure 6B). Distribution of several drought tolerance indices of morphological, physiological changes and yield-related traits in both seedling (Figure 7A) and reproductive developmental stages (Figure 7B) using PCA was demonstrated. Moreover, the similarity of individual genotype of maize in responses to water deficit conditions at seedling and reproductive stages was evidently validated (Figure 7).

**Table 3.** Number of ears per plant, ear weight, husk weight and hundred grain weight of maize genotypes grown under different water regimes for 80 days after sowing (DAS) or reproductive stage. Data are presented as mean  $\pm$  SE ( $n = 3$ ).

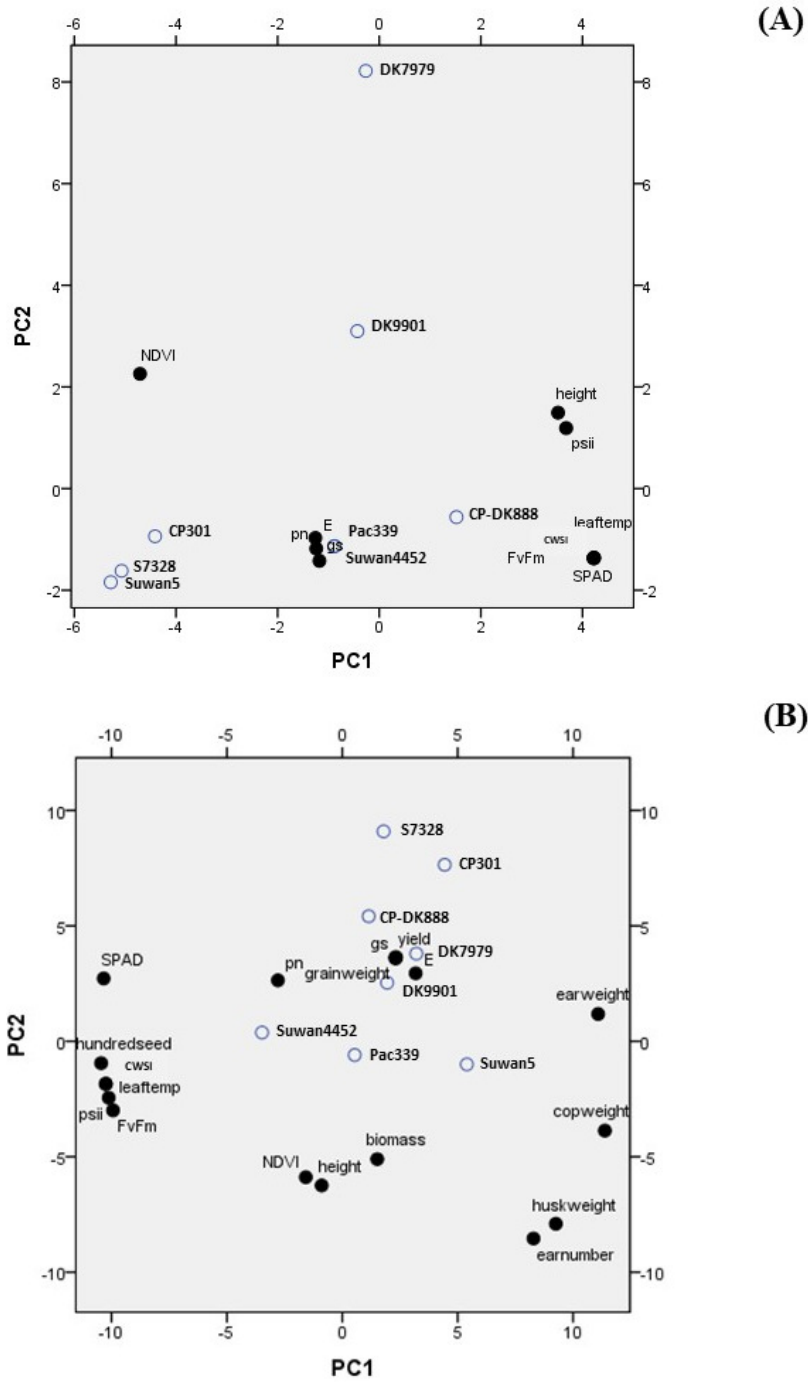
Varieties	Water regime	Number of ears per plant	Ear weight (g)	Husk weight (g)	Hundred grain weight (g)
‘Suwan5’	WW	1.00 $\pm$ 0.00a	106.00 $\pm$ 7.08cd	41.37 $\pm$ 3.34ab	22.41 $\pm$ 1.77bcd
	WD	0.70 $\pm$ 0.15b	71.22 $\pm$ 3.87e	31.91 $\pm$ 2.25c	23.28 $\pm$ 0.05bcd
		(-30.0%)	(-32.8%)	(-22.9%)	(0%)
‘Suwan4452’	WW	0.93 $\pm$ 0.03a	146.36 $\pm$ 21.51ab	40.49 $\pm$ 7.59ab	23.44 $\pm$ 0.40bcd
	WD	1.00 $\pm$ 0.00a	147.38 $\pm$ 9.84ab	49.68 $\pm$ 4.30a	23.33 $\pm$ 1.14bcd
		(0%)	(0%)	(0%)	(-0.5%)
‘CP-DK888’	WW	0.97 $\pm$ 0.03a	104.38 $\pm$ 7.87cd	23.11 $\pm$ 1.69cde	24.91 $\pm$ 0.36b
	WD	0.97 $\pm$ 0.03a	75.61 $\pm$ 3.56e	30.24 $\pm$ 4.82cd	24.61 $\pm$ 0.40b
		(0%)	(-27.6%)	(0%)	(-1.2%)
‘Pac339’	WW	1.00 $\pm$ 0.00a	122.57 $\pm$ 13.16bcd	25.44 $\pm$ 5.55cde	29.32 $\pm$ 0.36a
	WD	0.90 $\pm$ 0.00a	116.39 $\pm$ 19.43bcd	39.41 $\pm$ 4.24ab	27.89 $\pm$ 0.93a
		(-10.0%)	(-5.0%)	(0%)	(-4.9%)
‘DK9901’	WW	1.00 $\pm$ 0.00a	127.27 $\pm$ 8.95bc	14.33 $\pm$ 1.10e	21.14 $\pm$ 0.82cde
	WD	1.00 $\pm$ 0.00a	91.82 $\pm$ 18.20de	14.86 $\pm$ 4.30e	21.52 $\pm$ 1.69cde
		(0%)	(-27.8%)	(0%)	(0%)
‘DK7979’	WW	1.00 $\pm$ 0.00a	117.07 $\pm$ 12.63bcd	20.50 $\pm$ 1.67cde	20.85 $\pm$ 0.92de
	WD	1.00 $\pm$ 0.00a	92.78 $\pm$ 11.80de	19.13 $\pm$ 2.68de	20.92 $\pm$ 0.42de
		(0.0%)	(-20.7%)	(-6.7%)	(0%)
‘CP301’	WW	1.00 $\pm$ 0.00a	138.28 $\pm$ 5.47abc	18.60 $\pm$ 1.31de	24.17 $\pm$ 1.16bcd
	WD	1.00 $\pm$ 0.00a	92.43 $\pm$ 11.82de	17.71 $\pm$ 3.87de	24.39 $\pm$ 0.56bc
		(0%)	(-33.2%)	(-4.8%)	(0%)
‘S7328’	WW	0.97 $\pm$ 0.03a	163.95 $\pm$ 9.97a	25.18 $\pm$ 3.59cde	22.99 $\pm$ 1.01bcd
	WD	1.00 $\pm$ 0.00a	96.55 $\pm$ 12.81de	25.42 $\pm$ 1.63cde	18.37 $\pm$ 1.79e
		(0%)	(-41.1%)	(0%)	(-20.1%)
<b>Significant level</b>					
<b>Varieties</b>		*	**	**	**
<b>Water</b>		ns	**	*	*
<b>Var <math>\times</math> Water</b>		ns	**	ns	ns

<sup>ns</sup>, \*, and \*\* represent non-significant difference, and significant difference at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

Different letters in each column represent significant difference at  $p \leq 0.05$  according to Duncan’s Multiple Range test (DMRT).



**Figure 6.** Ward's cluster analysis using physio-morphological changes at seedling stage (A), and physio-morphological changes and yield traits at reproductive stage (B) of maize genotypes grown under different water regimes; well-watered (WW) and water deficit (WD)



**Figure 7.** Principal component analysis biplot of morphological and physiological traits of 8 maize genotypes grown under water deficit at seedling (A; 10 parameters) and reproductive developmental stages (B; 18 parameters including yield-related traits)

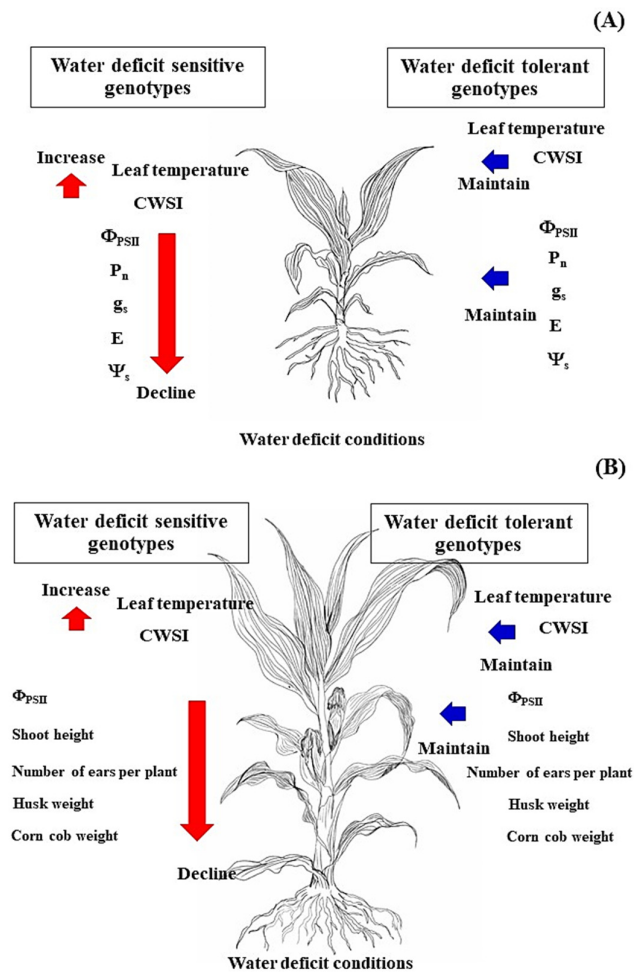
## Discussion

At the seedling stage, shoot height of maize seedlings was sensitive to water shortage, especially in ‘CP-DK888’ and ‘DK7979’. It corroborated a previous study reporting inhibition in shoot height at 4 leaf stage in drought susceptible maize genotypes, FLD01, 13, 16, 18, 29 and 31 (Adhikari *et al.*, 2019). Likewise, in summer maize (cv. ‘Nongda-108’), plant height was significantly declined, when plants were subjected to 35% field capacity (FC) (Ge *et al.*, 2012). In our study, leaf temperature in maize plants exposed to water deficit stress was increased, resulting in enhanced CWSI ( $>0.4$ ), whereas leaf greenness (SPAD) in cv. ‘Pac339’ under water deficit conditions was declined, leading to chlorophyll degradation and reduction in NDVI. Increasing leaf and canopy temperature in water-deficit stressed maize directly regulates CWSI (stress indicator depending on different growth stages (Han *et al.*, 2018), chlorophyll degradation and NDVI reduction, especially in drought susceptible genotypes (Han *et al.*, 2016). Chlorophyll degradation in leaf senescence under drought conditions of higher plants has been well reported, therefore delay leaf senescence in drought tolerant genotypes has evidently observed (Rolando *et al.*, 2015; Monteoliva *et al.*, 2021). Consequently,  $\Phi_{PSII}$ ,  $P_n$ ,  $g_s$  and E in maize genotypes, ‘Pac339’ and ‘CP301’, under water deficit condition were significantly dropped. Total chlorophyll and  $P_n$  in drought stressed maize plants were identified as sensitive parameters, which rapidly changed when subjected to drought conditions (Voronin *et al.*, 2019) and with the degree of drought stress (Ge *et al.*, 2012). Photosynthetic abilities,  $F_v/F_m$ ,  $P_n$  and  $g_s$  in drought sensitive maize were decreased, when compared with those of drought tolerant maize (Zhang *et al.*, 2015). In addition, leaf greenness,  $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $P_n$ ,  $g_s$  and E were higher in drought tolerant maize (Saglam *et al.*, 2014; Chen *et al.*, 2016b). Photosynthetic abilities, especially stomatal function have been reported as sensitive parameters to decline when plants exposed to drought situation (Chaves *et al.*, 2009; Drake *et al.*, 2017). Moreover, reduction  $\Psi_s$  in the leaf tissues of maize under drought was a good indicator to identify the plant response to water availability and played a role as index for drought tolerance in maize genotypes (Li *et al.*, 2019a).

At reproductive stage, overall growth performance in cvs. ‘Suwan4452’, ‘CP-DK888’ and ‘S7328’ under water deficit condition was retained, indicating as drought tolerant indices. In a previous report, morphological traits and biomass in drought-tolerant maize varieties were maintained, identifying these as drought tolerant genotypes (Shao *et al.*, 2016). In contrast, growth characters in maize crop grown under drought stress were significantly declined, identifying as drought susceptible (Ziyomo and Bernardo, 2013). Growth inhibition in maize depends on precipitation, especially in rainfed regions (Greaves and Wang, 2017; Li *et al.*, 2019b). Leaf temperature at flowering stage of maize cvs. ‘Pac339’ and ‘CP301’ was significantly increased, leading to an increased CWSI. In addition, total chlorophyll degradation in the drought stressed leaves was observed in cv. ‘CP-DK888’, leading to reduced NDVI. In general, chlorophyll content and NDVI are played an important role as the drought tolerant indices observed in maize genotypes (Maheswari *et al.*, 2016; Effendi *et al.*, 2019). Similarly, CWSI (increase leaf temperature or canopy temperature) was used to identify drought tolerant cultivar of maize crop (Nielsen and Schneekloth, 2018). Increase leaf temperature and CWSI index in drought stressed plants have been well established as rapid, simple, non-destructive method and low cost using Infrared thermal camera (Pipatsitee *et al.*, 2018; 2021). Physiological parameters, i.e.,  $F_v/F_m$ ,  $P_n$ ,  $g_s$  and E, in drought-stressed maize leaves were sharply declined, especially in drought susceptible genotypes such as Suwan5 inbred cultivar. Interestingly, the integrated physiological data (stomatal and non-stomatal functions) in relation to a degree of drought stress in higher plants have been reported, leading to identify a drought tolerant candidate (Gao *et al.*, 2021). Yield traits, including number of ears, ear weight, husk weight, 100-grain weight, corn cob weight, grain weight and total grain weight, are the major criteria to determine the drought tolerance in maize genotypes (El-Sabagh *et al.*, 2018; Su *et al.*, 2019). In the present study, ear weight, grain weight per ear and total grain weight in maize cv. CP301 were sensitive parameters to water deficit stress, leading to declined crop productivity.

For cluster analysis, ten parameters were subjected to classify the drought tolerant group including ‘Suwan4425’ (positive check), ‘CP-DK888’ and ‘DK7979’. Multivariate cluster analysis has been conducted to

categorize the drought tolerant genotypes of maize crop. For example, seedling morphological traits of maize genotypes were used to classify drought tolerant genotypes (Wattoo *et al.*, 2018). Overall growth traits (20 parameters) of 45-day-old seedlings of hybrid maize population were used as criteria for the identification of drought tolerant cultivars (Akinwale *et al.*, 2018). Also, plant growth attributes in maize population (Adhikari *et al.*, 2019) and single cross hybrid lines (Adewale *et al.*, 2018) were selected to classify the candidate drought tolerant maize cultivars at seedling stage. Therefore, the reproductive and maturation stages with yield attributes are required to evaluate the stability of grain productivity when exposed to drought stress (El-Sabagh *et al.*, 2018; Su *et al.*, 2019). Based on this validation, ‘S7328’ and ‘CP-DK888’ were classified as drought tolerant genotypes in the same group as cv. Suwan4452 (positive check). In the previous reports, STI (stress tolerance index), Harm (Harmonic mean) and GMP (Geometric mean productivity) were subjected to Ward’s cluster analysis to identify H6, SC400, H28, H11, H12 and SC250 as drought tolerant maize genotypes (Golbashy *et al.*, 2010). KSC720, KSC710GT and KSC700 were categorized as drought tolerant using STI, stress yield ( $Y_s$ ) and yield potential ( $Y_p$ ) indices in Ward’s method (Naghavi *et al.*, 2013). Moreover, 43 candidate genotypes of maize were identified as drought tolerant based on 20 morphological traits and grain yield using Ward’s cluster analysis (Hao *et al.*, 2011). Alternatively, maize genotypes, ‘BC504’, ‘BC652’, ‘BC404’, ‘KSC302’, ‘KSC320’ and ‘KSC647’ were identified as drought tolerant based on STI, GMP, and Harm using Fernandez’s cluster analysis (Jafari *et al.*, 2012).



**Figure 8.** A summary of evident physio-morphological traits of eight maize genotypes at seedling stages (A) and reproductive developmental stage as well as yield attributes (B) in responses to water deficit stress, playing as key indices for water-deficit tolerant classification

## Conclusions

Physio-morphological indices of maize at the seedling stage, including leaf temperature, CWSI,  $\Phi_{PSII}$ ,  $P_n$ ,  $g_s$ ,  $E$  and  $\Psi_s$ , were identified as potential parameters to classify the drought tolerant genotypes (Figure 8A). According to traits studied at reproductive stage (i.e., leaf temperature, CWSI,  $\Phi_{PSII}$  and shoot height) and yield attributes (i.e., number of ears per plant, husk weight, and corn cob weight) (Figure 8B), 'Suwan4452', 'CP-DK888' and 'S7328' were categorized as drought tolerant genotypes, whereas 'Suwan5', 'Pac339', 'DK7979', 'CP301' and 'DK9901' were identified as drought susceptible ones. Suwan4452 was established as drought tolerant in both seedling and reproductive stages with several physio-morphological and yield adapted traits. Based on the study, hybrid maize cvs. 'Suwan4452', 'CP-DK888' and 'S7328' may be suggested to be grown in the rainfed area without irrigation.

## Authors' Contributions

SC-U and AE conceived of the presented idea. PP, and RT participated in the design, performed experiments and analysis; TS, SK, KT encouraged to investigate yield attributes, morphological and biochemical analysis. All authors participated in interpretation of the data. PP and SC-U wrote the paper and part. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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