Physiological and Biochemical Responses of Turmeric (*Curcuma longa* L.) Under Drought Stress

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ABSTRACT

Unfavorable environmental conditions, particularly abiotic stresses such as drought, can hinder the growth and development of turmeric, leading to significant production losses. In this study, we investigated physiological and biochemical changes in a turmeric variety after introducing artificial drought stress by withholding water for 21 days. Net photosynthetic rate, chlorophyll content, moisture content, catalase activity, and rhizome curcuminoid content were measured in stressed vs. unstressed control plants. Drought stress reduced photosynthetic rates and decreased chlorophyll content by 27.9%, but increased the curcuminoids and catalase activity in senescing plants by 90.2% and 55.1 %, respectively, compared to control. The curcuminoid levels increased in general at the end of the 21-day drought-stress period. Curcumin content of drought stressed rhizomes increased by 50% over the control treatment but was not statistically significant. The total curcuminoid content increased by 106% over the control. Understanding the molecular and physiological responses of turmeric plants under drought stress will assist researchers in developing drought-tolerant cultivars of turmeric by incorporating the stress-tolerant traits into plant breeding programs. The information from our research can also assist agronomists in developing turmeric cultivation management practices by optimizing growth conditions or altering external inputs like irrigation or the addition of fertilizer.

Abbreviations. ANOVA: analysis of variance, DMSO: Dimethyl sulfoxide, ECSt: total electrochromic shift, Fv′/Fm′: The maximal quantum efficiency of PSII, LEF: Linear electron flow, NADP: Nicotinamide adenine dinucleotide phosphate, NPQt: Total non-photochemical quenching, Phi2: Quantum yield of PSII, PhiNO: Fraction of incoming light that is lost via non-regulated processes, PhiNPQ: Fraction of light dedicated to non-photochemical quenching, Pn: Net

photosynthetic rate, ROS: Reactive oxygen species, RMC: Relative moisture content, WUE: Water use efficiency.

INTRODUCTION

According to Ayurveda (ancient Indian herbal medical practice), turmeric (*Curcuma longa)* from the Zingiberaceae family has a long history of therapeutic uses (Govindarajan 1980).Turmeric contains a variety of phytochemicals, primarily curcuminoids, volatile oils, sugars, proteins, and resins (Tayyem et al., 2006). Curcuminoids, a type of secondary metabolites produced in the turmeric rhizomes belonging to the class of lipophilic diketones (Pandey et al., 2020) are the major bioactive components attributed to several medicinal properties (Sharifi-Rad et al., 2020) including anti-inflammatory (Panahi et al., 2014), antioxidant (Masuda et al., 2001), antimicrobial (Negi et al., 1999), anticancer (Giordano and Tommonaro, 2019; Zoi et al., 2021) and antitumor (Astinfeshan et al., 2019). Because of these attributes, turmeric has gained popularity in Western countries over the past decade or two (Priyadarsini, 2014). Turmeric rhizomes contain three major curcuminoids including: curcumin [**1**, (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, typically 60–70% of a crude extract], demethoxycurcumin (**3**, 20–27%), and bisdemethoxycurcumin (**4**, 10–15%), along with numerous and less abundant secondary metabolites (Shafabakhsh et al., 2019; (Ashrafizadeh et al., 2020). Besides its medicinal uses, turmeric has been used for improving flavor, aroma, and food preservation (Govindarajan, 1980). Turmeric has been used in cosmetics for decades because of its bright golden-yellow color due to curcuminoids. The same compounds are known for the popular yellow-colored Indian curries (Nair, 2020).

Plants often face unfavorable environmental conditions due to natural calamities and global climate changes. Environmental stress-related effects on plants can primarily decrease photosynthesis, chlorophyll content, moisture content, stomatal closure, and carbohydrate metabolism (Adam and Murthy, 2014; Siddique et al., 2016; Hajihashemi et al., 2018; Morales et al., 2020; Sherin et al., 2022). Drought is one of the critical abiotic stresses that cause most crop losses (Santhi et al., 2016). Drought stress can change a plant at morphological, anatomical, physiological, biochemical, and molecular levels (Seleiman et al., 2021). Drought stress reduces the photosynthetic efficiency by damaging the thylakoid membrane and reducing the chlorophyll content (Song et al., 2021). Chloroplasts are highly sensitive to drought stresses (Hussain et al., 2018; Song et al., 2021). The chloroplasts generate reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen during severe abiotic stress (Song et al., 2021), including drought stress (Hussain et al., 2018). Under drought stress, water use efficiency (WUE) and root conductivity decrease due to the inhibition of the metabolic mechanism of photosynthesis (Song et al., 2021). Soil water stress in arid and semi-arid regions severely limits plant growth and productivity (Mahajan and Tuteja, 2005). Secondary metabolites are involved in protective functions in response to both biotic and abiotic stress conditions. Bryant et al. hypothesized that defensive secondary compounds accumulated at the expense of biomass production among drought-tolerant plants (Bryant et al., 1983).

This research aimed to investigate the effects of drought stress on the physiological and biochemical changes of turmeric variety, CL11. We studied net photosynthetic rate, chlorophyll content, moisture content, catalase activity, and rhizome curcuminoid contents in drought-stressed vs. unstressed control plants. Our work lays the foundation for the development and cultivation of drought-resilient turmeric plants to meet the increasing demand for this crop.

MATERIALS AND METHODS

Plant materials and growth conditions. Freshly harvested rhizomes of turmeric variety CL11 from a previous year at the Alabama A&M University (AAMU) were used in this study. The CL11 rhizomes were shipped to California State University Northridge (CSUN) in 2022 for further use in drought stress studies. The rhizomes were planted in

six pots containing Vigoro Perlite soil-less potting mix at one rhizome per pot in the greenhouse at CSUN till they sprouted. The pots with sprouted plants were moved to a growth chamber with a temperature of 24-26°C and a light and dark cycle of 16 h and 8 h. All plants were grown with full irrigation when needed to maintain moisture-stress-free conditions for two months. The pots were then divided into two groups of three pots each. One set of three pots was designated as a control that received full watering every two days to avoid drought stress. The second set of three pots was subjected to drought stress by withholding watering completely for 21 days to mimic drought stress (The decision to impose a 21-day drought stress was based on results of a 'kill curve' experiment; data not shown). Fresh leaves and rhizomes were harvested from these two sets of treatments. Fresh leaves and rhizomes were harvested from these two sets of groups: control (C1, C2, and C3) or drought-stressed plants (S1, S2, and S3). All physiological measurements, such as net photosynthetic rate (Pn), chlorophyll content, relative water content (RWC), and catalase activity, were measured using the fresh leaves.

Photosynthetic rate. The leaf net photosynthetic rates were measured using a handheld instrument, MultiSpeQ (PhotosynQ, USA). The data on photosynthetic rates were saved in an online database platform called PhotosynQ platform (http://photosynq.org). The platform provided web-based tools to visualize and analyze the data (Kuhlgert et al., 2016). The measurements were taken at three different points in each biological replicate (three controlled plants and three stressed plants). The measurements taken by MultispeQ were NPQt (total non-photochemical quenching), Phi2 (quantum yield of PSII), PhiNO (fraction of light lost via nonregulated photosynthesis inhibitor processes, in other words, the lights which neither dissipated nor used for photosynthesis), PhiNPQ (fraction of light dedicated to non-photochemical quenching), qL (fraction of PSII open centers), ECSt (total electrochromic shift), Fv′/Fm′ (maximal quantum efficiency of PSII), leaf temperature, leaf thickness, and LEF (linear electron flow) (Table 3).

Chlorophyll content. For chlorophyll content analysis, only two stressed biological replicates (S1 & S3) were used as the Sample 2 (S2) plant did not have enough leaves.

Chlorophyll was estimated according to Hiscox and Israelstam (1979). Fresh 0.5 g of leaves were cut into small pieces and set in test tubes with 5 mL of DMSO, warmed in a water bath for 2.5 h at 50°C. The absorbance of each yellow or green supernatant was assessed at 645 nm and 663 nm with Dimethyl sulfoxide (DMSO) alone on a NanoDrop UV visible spectrophotometer (Thermo Scientific, USA). Complete chlorophyll content was determined by the following equation (Arnon, 1949):

Chlorophyll a (mg/ ml) = $(12.7\times Abs_{663\ nm}) - (2.69\times Abs_{645\ nm})$

Chlorophyll b (mg/ ml) = $(22.9\times Abs_{645\ nm}) - (4.68\times Abs_{663\ nm})$

Total chlorophyll (mg/ ml) = chlorophyll a + chlorophyll b

Relative moisture content. Relative moisture content (RMC) was assessed by following a published method (Barrs and Weatherley, 1965 used the term 'Relative Water Content'). Three leaves (technical replicates) from each biological replicate (three control plants and two drought-stressed plants) were cut into small pieces. Ten symmetrical pieces from each sample were weighed, and fresh weight was recorded. Then, the leaves were submerged in distilled water for four hours and weighed again (submerged weight). After weighing, they were dried for 14 hours in an incubator set at 43°C to 45°C, and their dry weight was recorded.

RMC was determined with the following formula:

RMC = (Fresh weight - Dry weight)/(Submerged weight - Dry weight)

Statistical analysis. The effect of water shortage on RMC was analyzed through one-factor ANOVA using R Studio 4.2.0 (https://www.rstudio.com/). The statistical analysis is shown in Supplementary Figures 1, 2, 3, and 4.

Catalase activity. Fresh leaves from the three control plants and two drought-stressed plants (4.0 g

of leaves from each biological replicate) were ground in 8 mL of distilled water. Then, 2 mL of the aqueous solution was pipetted into a 50 mL graduated cylinder, and 2 mL of 3% H_2O_2 was added. The catalase activity was calculated by the volume of effervescence (Euler and Josephson, 1927).

Curcumin content. At the end of the drought-stress experiment at CSUN, the fresh rhizomes were shipped to AAMU for further processing to determine curcuminoids content. The rhizomes of control and drought stressed treatments were weighed, cut into pieces, and dried in a forced air dryer set at 50ºC. The dried rhizomes were weighed and powdered. The dry powdered samples were analyzed at the Natural Products Utilization Research Unit, United States Department of Agriculture, Agricultural Research Service, MS, for determining curcuminoids content using HPLC/DAD-MS.

Extraction of Curcuminoids. A 50 mg turmeric sample was weighed. The powdered sample was sonicated in 3 mL of methanol for 30 min (room temperature) in a centrifuge tube, followed by centrifugation for 15 min at 3300 rpm. The supernatant was transferred to a 10 mL volumetric flask. The procedure was repeated three times, and respective supernatants were combined. The final volume was adjusted to 10.0 mL with methanol and mixed thoroughly. Then, the solution was transferred to a scintillation vial, labeled, and sealed. Meanwhile, an adequate volume (ca. 2 mL) was passed through a 0.2 µm nylon membrane filter. The first 1.0 mL was discarded, and the remaining volume was collected in a labeled LC sample vial. Each sample solution was injected in triplicate.

UHPLC/DAD-MS Analysis. The analysis was performed on a 1290 Infinity series UHPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector, binary pump, vacuum degasser, autosampler, and thermostated column compartment. The separation was achieved on an Agilent Poroshell EC-C_{18} (2.1 × 150 mm, 2.7 µm) column maintained at 30ºC throughout the analysis. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1 % formic acid (B). The gradient elution was as follows: 0 min 40% B, 0-8 min 80% B, 8-9 min 100 % B. A 3-min wash followed each run with 100 % B and an equilibration period of 6 min with 40% B. The eluent was performed at a flow rate of 0.2 mL/min with injection volume set at 2 µL. The DAD wavelength was set at 240 nm for αR-turmerone and 425 nm for bisdemethoxycurcumin, demethoxycurcumin, and curcumin.

The mass spectrometer was an Agilent 6120 quadrupole. The APCI source was set in a negative mode for bisdemethoxycurcumin, demethoxycurcumin, and curcumin with fragmentor voltage at 100 V and a positive mode for αR-turmerone with fragmentor voltage at 120 V. The MS parameters were as follows: capillary voltage 4.0 kV; gas temperature: 325°C; gas flow 12 L/min; nebulizer pressure 35 psi; vaporizer temperature: 225°C; mass range (m/z): 100 to 500.

RESULTS AND DISCUSSION

Under drought stress, plants undergo physiological changes to adapt to the shortage of water as well as nutrients (Ben-Jabeur et al., 2021). To detect the influences of drought stress, we measured the net photosynthetic rate, sugar and chlorophyll contents, catalase activity, and leaf relative water content.

Net photosynthetic rate (Pn). Plants under drought stress reduce linear electron flow (LEF) (Ben-Jabeur et al., 2021) to protect Photosystem II (PSII) and Photosystem I (PSI) (Huang et al., 2012). In the LEF of oxygenic photosynthesis, electrons from the oxygen-evolving complex are transferred to NADP+, reducing it to NADPH (Huang et al., 2012). There are several mechanisms to protect PSII: activation of non-photochemical quenching (NPQ), activation of antioxidants to scavenge ROS species, development of cyclic electron flow, alternative electron flow, and photorespiration (Bashir et al., 2021; Muhammad et al., 2021). ROS are produced as a result of over-reduction of reaction centers due to excess light, which is not adequately dissipated (Loggini et al., 1999; Ben-Jabeur et al., 2021). In our studies, the analysis of variance (ANOVA) suggested that drought stress has a significant effect

on some traits of plants, such as total non-photochemical quenching (NPQ), quantum yield of PSII (Phi2), fraction of light lost via nonregulated photosynthesis inhibitor processes (PhiNO), fraction of light dedicated to non-photochemical quenching (PhiNPQ), Electrochromic Shift (ECSt), Maximal Quantum Efficiency of PSII (Fv′/Fm′), Leaf temperature, and Linear Electron Flow (LEF) (Table 1 and 2). Drought stress increased the PhiNPQ and NPQ while decreasing Phi2, PhiNO, ECSt, Fv′/Fm′, and LEF (Figure 1 and Supplementary Figure 1).

Drought stress decreased Phi2 (Fig. 1F), PhiNO (Fig. 1D), ECSt (Fig. 1A), Fv′/Fm′(Fig. 1H), and LEF (Fig. 1B). Phi2 parameter measures captured light energy by photosystem II, which is used for ATP and NADPH production. The decrease in Electrochromic Shift (ECSt provides an indication of ATP synthase capacity) is mainly because of the reduction of intercellular carbon dioxide concentration due to stomatal closure and chlorophyll degradation under drought stress, which leads to lower production of ATP and NADPH, which reduces the LEF (Ben-Jabeur et al., 2021). The limitation of CO_2 can lead to a decrease in the PSII redox site and its operating efficiency. This could be a reason for the reduction of the quantum yield of PSII (Phi2) and Fv′/Fm′ (The maximal quantum efficiency of PSII (Ben-Jabeur et al., 2021).

Drought stress increased the PhiNPQ and NPQ. Increased NPQ can dissipate excess light energy to minimize the effects of photodamage and decrease ROS generation(Zhao et al., 2017), thereby protecting the photosystems.

So, in conclusion, Phi2 under drought stress is associated with increased PhiNPQ and NPQ and decreased PhiNO. We think a decrease in PhiNO may have a protective function as suggested by Ben-Jabeur et al., 2021. A decrease in PhiNO could be productive as less energy is dissipated as heat through a non-regulatory process, and more energy is utilized to sustain photosynthesis or unproductive as more energy in the photosystem can cause photoinhibition and photodamage. Hence, more research is needed to understand the role of lower PhiNO during drought stress. We propose a possible physiological response in the drought-stressed plants. We speculate drought induced stomatal closure led to intracellular decrease in CO_2, which assisted in dissipating excess light energy to protect the photosynthetic apparatus from photodamage. We speculate, a decrease in LEF, PhiNO, ECSt, Fv'/Fm', and Phi2 may have protective function of the photosystems (Fig. 2).

Chlorophyll content. Drought stress imposes a degradation of chlorophyll a, chlorophyll b, and total chlorophyll in plants, which can be considered one crucial trait of oxidative stress and degradation of chlorophyll (Anjum et al., 2011). The data in our study showed a significant reduction of chlorophyll a, chlorophyll b, and total chlorophyll (Figure 3; Supplementary Fig. 2, 3, and 4, which is similar to an earlier finding (Mafakheri et al., 2010), who reported a decrease in chlorophyll a, chlorophyll b, and total chlorophyll in three chickpea cultivars following drought stress. Chlorophyll, one of the essential contents of chloroplast, has a direct relationship with the photosynthesis ability of plants (Anjum et al., 2011). The lowered chlorophyll content in drought-stressed plants (Fig. 3) can cause the degradation of the photosynthetic rate (Fig. 1 B, D, F, H). The degradation of chlorophyll content can also be due to leaf senescence, which ultimately leads to lipid peroxidation in the chloroplast (Dhindsa et al., 1981).

Relative moisture content (RMC). RMC in drought-stressed plants is a tolerance indicator (Maghsoudi et al., 2016). Higher RMC in drought stress has been reported in wheat cultivars, indicating stress tolerance of wheat against drought stress (Maghsoudi et al., 2016). The difference in relative moisture content can be due to two reasons: either the ability of the plant to uptake water from soil or stomatal conductance in response to the shortage of water (Keyvan, 2010). In this study, RMC was decreased under drought stress (Fig. 4). However, when testing the data by ANOVA, the difference was not significant, indicating turmeric plants did not show any effect on moisture content under drought stress (Supplementary Figure 4).

Catalase activity. Catalase is an antioxidant enzyme that reduces oxidative stress by breaking

down H_2O_2 into O_2 and H_2O. These free radicals (O_2, H_2O_2, O H^- and O_2^-) are proven to have the ability to initiate lipid peroxidation in senescing leaves (Fong et al., 1973). A higher catalase activity is an indicator of detoxification of free radicals. In our case, we noticed a decline in catalase activity in drought-stressed plants at the end of the 21-day stress period. Lipid peroxidation is an indicator of cell membrane degradation under harsh environments. The lipid peroxidation lowers catalase activity and indicates the senescence of the stressed plant cells (Dhindsa et al., 1981). In our study, drought-stressed plants showed a decline in catalase activity (Fig. 5). Therefore, we assumed that the turmeric plants may reach senescence within 21 days of the stress and hence showed reduced catalase activity. We can conclude that prolonged drought stress could reduce catalase activity in turmeric cells, thereby losing the cells' abilities to fight against free radicals. It will be informative to perform a catalase activity assay within a few days of initiation of drought stress before the plants reach senescence.

Curcumin content. In this study, the mean curcuminoids and α*R*-turmerone content varied between control unstressed and stressed treatments (Fig. 6). Among the three curcuminoids assessed, two curcuminoids, bisdemethoxycurcumin, demethoxycurcumin, and α*R*-turmerone increased significantly by 160, 131, and 471%, respectively, in the drought stressed compared to the unstressed control rhizomes (Fig. 6). Abiotic stress can have similar impacts on the accumulation of secondary metabolites (Raven et al., 2005). For example, salinity stress induced an increase in curcumin content in *Curcuma longa* L (Mostajeran et al., 2014). In our observation, the greatest increase was in α*R*-turmerone, which spiked from 1.67 mg/g in control rhizomes to 9.57mg/g in drought-stressed rhizomes. Both bisdemethoxycurcumin and demethoxycurcumin increased significantly compared to the control. However, curcumin content of drought stressed rhizomes increased by 50% over the control treatment but was not statistically significant. The total curcuminoid content increased by 106% over the control. Previous research reported a decrease in the curcumin content of two turmeric varieties grown in India(Tripathi et al., 2015). In their study, water was not totally withheld for a prolonged period, as was the case in our study. Perhaps prolonged drought stress reduced key physiological traits and 'switched on' defense mechanisms, resulting in higher production of secondary metabolites, including curcuminoids, in our study.

A wide variation in yield and chemical composition of turmeric was observed across genotypes and agroclimatic conditions (Prasath et al., 2018). This variation in phyto-constituents (curcuminoids, oleoresin, and essential oil) of turmeric can likely be attributed to differences in climate and soil conditions that occur in different agro-climatic zones. Other reports indicate that the curcumin percentage differs remarkably when a high curcumin cultivar is cultivated in different places, thus affecting its commercial potential (Anandaraj et al., 2014; Sandeep et al., 2016). Kim et al. (2011) reported that secondary metabolite production differed in various environmental situations, including growth conditions, stress, or seasonal changes. It was also reported that specific phytochemicals were either genus or species-specific and found that these chemical components were affected by environmental factors such as temperature, precipitation, soil conditions, and seasonal (Raven et al., 2005). Environmental stressors impede plant growth, attributed to altered metabolic pathways, favoring the synthesis of secondary metabolites, notably defense-related compounds (Foyer and Harbinson, 2020). Osmotic stress induces the production of various reactive oxygen species, prompting the activation of cellular antioxidants like phenolic compounds (curcumin), terpenoids, tocopherol, ascorbate, and glutathione to mitigate cellular damage through scavenging mechanisms in response to osmotic stress (Foyer et al., 1997).

CONCLUSIONS

From our studies, we have concluded that turmeric plants are highly sensitive to drought. When subjected to drought stress, these plants exhibited adaptive physiological changes to withstand the

adverse conditions, including the minimization of photodamage. We observed an increase in the accumulation of secondary metabolites, such as curcumin, after 21 days of drought stress, likely as a response mechanism to water scarcity. However, it is essential to repeat this experiment with plants of various ages and under different environmental conditions to ensure the reliability of the data. Following a 21-day period of drought stress, we observed a decrease in the photosynthetic rate, degradation of chlorophyll content, and a decline in catalase activity. These data could be used to select phenotypic traits to develop drought-tolerant turmeric plants. By the end of the 21-day drought stress period, the turmeric plants showed signs of senescence and visible injury due to water deficiency. This information will be valuable in developing climate-resilient turmeric plants, including drought-tolerant turmeric plants with higher curcumin content.

Table 1: Analysis of Variance (ANOVA) of photosynthetic traits between the controlled and drought-stressed plant groups.

ANOVA[1]	Traits				
	ECSt	Fv´/Fm´	Leaf Temperature	Leaf Thickness	LEF
F value	6.839**	525.1	5.09	0	23.375
P-value	0.018*	1.163e-13 ***	0.383*	0.996	0.0001***

[1]one way ANOVA analysis of 3 controlled plants and 3 drought-stressed plants of different parameters collected using MultispeQ: total electrochromic shift (ECSt), maximal quantum efficiency of PSII (Fv′/Fm′), Leaf temperature, Leaf thickness, linear electron flow (LEF), ECSt, Fv´/Fm´, and LEF are statistically significant differences between control and drought-stressed groups. Leaf Temperature and Leaf Thickness do not show significant differences between control and drought-stressed groups.
*Symbols indicate the statistical significance

Table 2: Analysis of Variance (ANOVA) of photosynthetic traits between the controlled and drought-stressed plant groups.

ANOVA[1]	Traits				
	NPQt	Phi2	PhiNO	PhiNPQ	qL
F value	246.21	362.93	368.25	426.77	1.9744
P-value	3.882e-11 ***	2.023e-12 ***	1.809e-12 ***	5.804e-13 ***	0.1791

[1]one way ANOVA analysis of 3 controlled plants and 3 drought-stressed plants of different parameters collected using MultispeQ: total non-photochemical quenching (NPQt) , quantum yield of PSII (Phi2), fraction of light lost via nonregulated photosynthesis inhibitor processes (PhiNO), fraction of light dedicated to non-photochemical quenching (PhiNPQ), fraction of PSII open centres (qL).
*Symbols indicate the statistical significance

- **NPQt**, **Phi2**, **PhiNO**, and **PhiNPQ** show highly significant differences between control and drought-stressed groups (***).
- **qL** does not show statistically significant differences between control and drought-stressed groups, as its P-value is above the threshold of 0.05.

Table 3: Different terminologies of photosynthesis rate and their functions

Terminology	Definition/Function
Electrochromic Shift (ECSt)	ECSt refers to changes in the absorption spectra of photosynthetic pigments following the absorption of photons from sunlight. ECSt can be directly correlated with the production of ATP.
Maximal Quantum Efficiency of PSII (Fv′/Fm′)	This is a ratio of variable fluorescence (Fv′) to maximum fluorescence (Fm′), which determines the maximal quantum efficiency of PSII
Linear Electron Flow (LEF)	LEF refers to the flow of electrons in a light-dependent reaction of photosynthesis, ultimately leading to the synthesis of ATP and NADPH.
Quantum Yield of PSII (Phi2)	Phi2 is the amount of sunlight utilized by plants for photosynthesis. Phi2 parameter measures captured light energy by photosystem II.
PhiNO (Non-Regulatory Energy Dissipation)	PhiNO is the fraction of light energy that is neither utilized in photosynthesis and photochemistry nor dissipated as heat as non-photochemical photo quenching (NPQ).
Fraction of light dedicated to non-photochemical quenching (PhiNPQ)	PhiNPQ is the portion of the excess light that is not used dissipated as heat.
Quantum yield of photosynthesis (qL)	qL is directly proportional to the efficiency of photosynthesis and photosynthetic organisms in converting light energy to chemical energy.

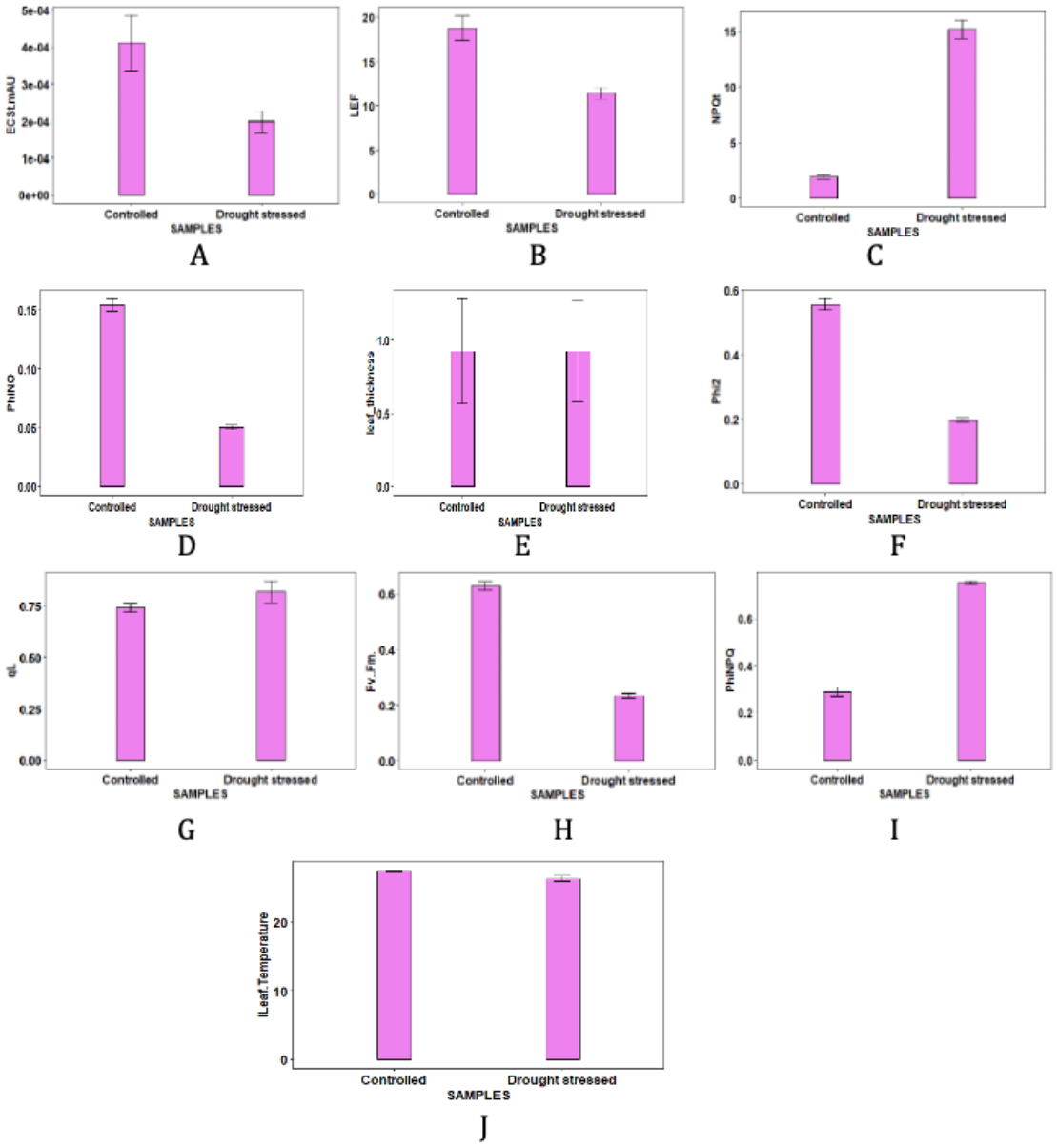

Figure 1. Photosynthetic parameters, leaf temperature and thickness in control and drought stressed turmeric (*Curcuma longa*) greenhouse-grown plants. This figure shows the data, which are the means calculated from three technological replicates from each biological replicates of turmeric plants under drought stress. Drought stress increased the NPQ (Fig. 1C), qL (Fig. 1G), and PhiNPQ (Fig 1I) and while decreasing ECSt (Fig.1A), PhiNO (Fig.1 D), Phi2 (Fig. 1F), Fv′/Fm′(Fig.1H). No changes were observed in leaf thickness (Fig. 1E) and leaf temperature (Fig. 1J).

Abbreviations used: total non-photochemical quenching (NPQt) , quantum yield of PSII (Phi2), fraction of light lost via nonregulated photosynthesis inhibitor processes (PhiNO), fraction of light dedicated to non-photochemical quenching (PhiNPQ), fraction of PSII open centers (qL), total electrochromic shift (ECSt), maximal quantum efficiency of PSII (Fv′/Fm′), Leaf temperature, Leaf thickness, linear electron flow(LEF).

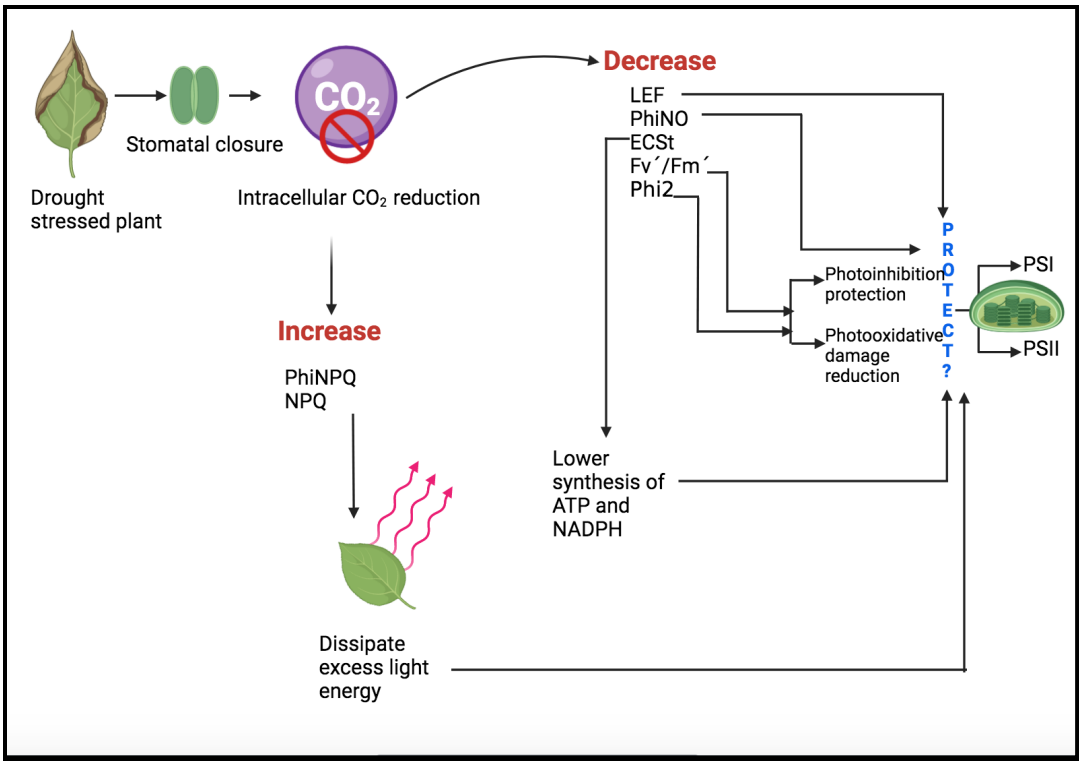

Figure 2. A schematic diagram representing possible physiological responses of drought stress in turmeric plants to protect photosystems. We speculate drought induced stomatal closure led to intracellular decrease in CO_2, which assisted in dissipating excess light energy to protect the photosynthetic apparatus from photodamage. We speculate, a decrease in LEF, PhiNO, ECSt, Fv'/Fm', and Phi2 may have protective function of the photosystems. Abbreviations used: total non-photochemical quenching (NPQt) , quantum yield of PSII (Phi2), fraction of light lost via nonregulated photosynthesis inhibitor processes (PhiNO), fraction of light dedicated to non-photochemical quenching (PhiNPQ), fraction of PSII open centers (qL), total electrochromic shift (ECSt), maximal quantum efficiency of PSII (Fv′/Fm′), Leaf temperature, Leaf thickness, linear electron flow(LEF). The figure was generated from www.biorender.com.

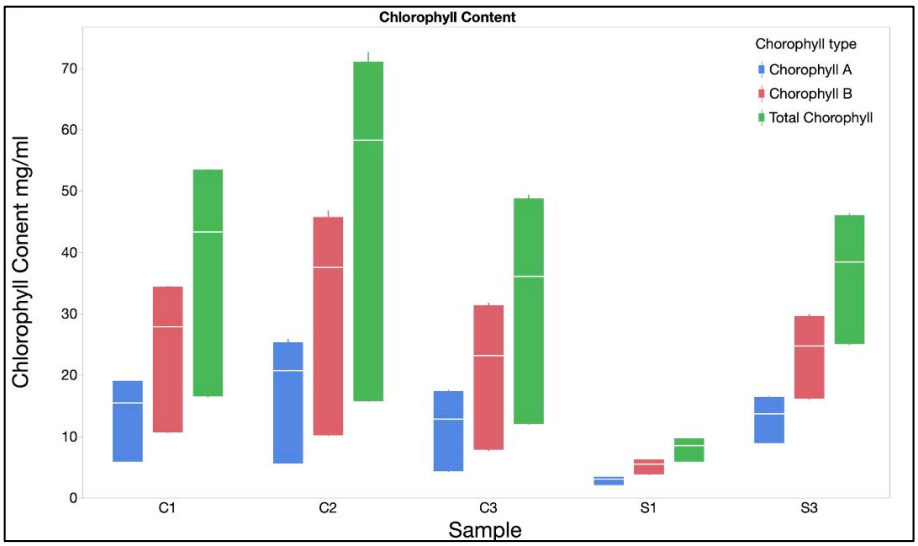

Figure 3. Total chlorophyll content in control (C1-C3) and drought-stressed (S1, S2) (*Curcuma longa*) greenhouse grown plants. Each bar represents 3 technical replicates within each biological sample. The black lines on top indicate the standard error, and the white line inside each bar marks the average of the three replicates.

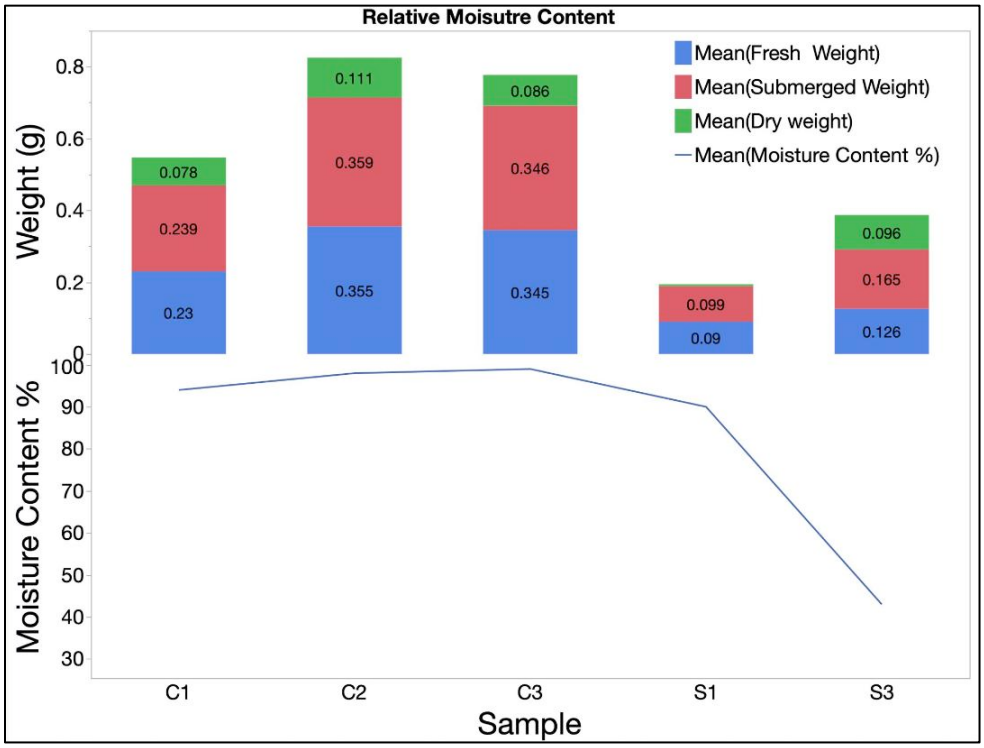

Figure 4. Relative moisture content in control (C1-C3) and drought-stressed (S1, S2) (*Curcuma longa*) greenhouse grown plants. The bar diagram figure at the top shows each sample's average fresh, submerged, and dry weight of leaves in g. The line diagram figure shows the percentage of relative moisture content (%)

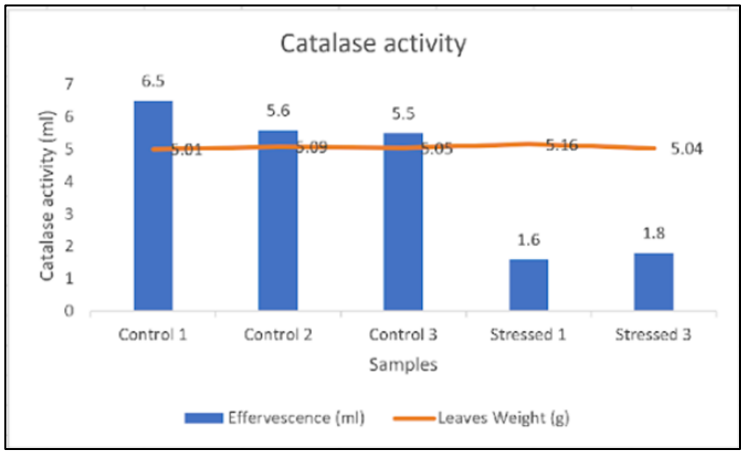

Figure 5. Catalase activity in control (C1-C3) and drought-stressed (S1, S2) (*Curcuma longa*) greenhouse grown plants. The catalase activity was much higher in control samples compared to drought-stressed samples. The leaf weight (line graph) shows no difference, showing that drought stress didn't affect the leaf weight, although it affected the catalase activity.

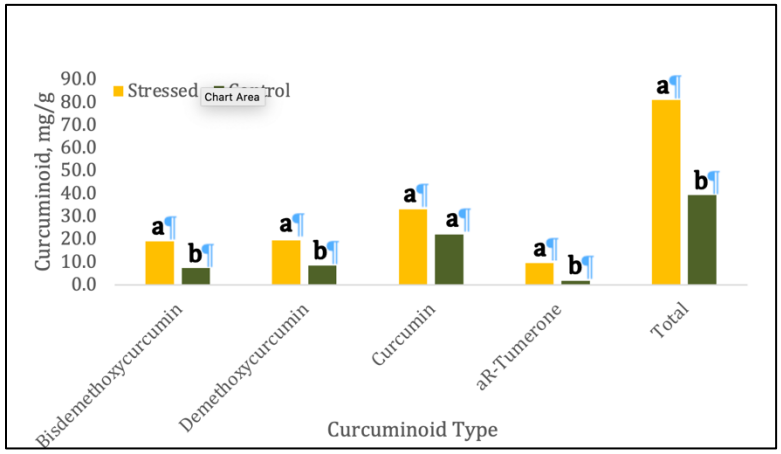

Figure 6. Increase of curcuminoid content of drought-stressed compared to control turmeric (*Curcuma longa*) greenhouse grown plants. Overall, curcumin contents were found to be upregulated following drought stress.

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