Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2023i1.636
*Biol. Clin. Sci. Res. J., Volume, **2023**: 636*
Original Research Article

ESTIMATING THE PHOTOSYSTEM'S EFFICIENCY, ANTIOXIDANTS ACTIVITY AND PLANT WATER RELATIONS OF TOMATO UNDER WATER DEFICIT CONDITIONS

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(Received, 17th August 2023, Revised 28th December 2023, Published 30th December 2023)

Abstract *Due to physiological repercussions, the tomato crop has decreased productivity caused by drought throughout its entire life cycle. The tomato harvest has exceeded expectations due to a worldwide water resource limitation. The objective of subjecting the tomato cultivars Flanto and Sahel, to a self-imposed drought was to investigate the interrelationships among biochemical, physiological, enzymatic, and water-related factors. Five varying levels of moisture strongly impacted all plant attributes: 100%, 80%, 60%, 40%, and 20% when subjected to drought stress. The lack of water resulted in notable improvements in some metrics. Some examples of these traits include linear electron flow (LEF), photosynthetic efficiency (PSE), inward light dissipation ratio (qP), catalase activity (CAT), ascorbate peroxidase activity (APX), and leaf water potential (LEWP). Consider, for example, the intriguing discoveries concerning photosystem II, non-photochemical quenching, chlorophyll levels, leaf osmotic potential, and leaf turgor potential.*

Keywords: *Tomato; Photosystem II; Antioxidants; Plant water relations; Drought*

Introduction

Abiotic stresses, originating from the natural environment, impede plant growth and reduce agricultural productivity (Hussain et al., 2018). Open-field crops are very vulnerable to the effects of abiotic stressors at every stage of their growth. These non-living elements might hinder the growth and harvesting of crops, and they can also happen simultaneously (Wang *et al.*, 2016). There is a consensus that drought stress negatively impacts crop development and growth, posing a significant limitation to agricultural crop growth (Flexas *et al*., 2009; Latif *et al*., 2016; Robin *et al*., 2003). Plants cannot grow when they face water shortage caused by drought because it disrupts several biochemical and physiological processes (Farooq *et al*., 2008). Plants undertake several chemical and physiological changes, such as increased ethylene production, chlorophyll modifications, and photosynthetic machinery disruption, to prevent photosynthesis when water levels are low (Lata and Prasad, 2011). Plant's capacity to control its photosynthetic rate is one indicator of its resistance to drought. Drought stress has several detrimental impacts on plant cells, including decreased cell division and photosynthetic rates (Hussain et al., 2021). (Subrahmanyam et al., 2006) drought may cause significant harm to photosystem II when it disrupts cell integrity.

However, several contrary results have also been reported (Colom and Vazzana, 2003). But measurements of chlorophyll fluorescence are now being adopted as a measure to estimate the efficiency of photosynthetic apparatus (Massacci et al., 2008) under stressed environments, as it is a non-destructive method to estimate plants response to stress factors for measuring the most influenced entity i.e. the photosynthetic apparatus. Drought causes a significant decline in transpiration and leaf water potential, leading to a drop in plant nutrient uptake. (Fahad *et al*., 2017). The decline in plant productivity and quality is caused by a shortage of water, resulting in the production of reactive oxygen species, shrinking of cells, and reduced integrity of cell membranes, finally leading to leaf senescence (Tiwari et al., 2016). Previous research indicates that water constraint directly impacts plants, elevating reactive oxygen species (ROS) levels. Consequently, this leads to detrimental effects on DNA, lipids, proteins, and cell membranes. (Fahad *et al*., 2017). Plants undergoing metabolic alterations due to drought potentially results in reduced crop yields.

[Citation Ghaffar, R., Shaheen, M.R., Hussain, R., Anjum, S., Saeed, M.A, Shabbir, A., Amjad, M.U. (2023). Estimating the photosystem's efficiency, antioxidants activity and plant water relations of tomato under water deficit conditions. *Biol. Clin. Sci. Res. J.*, **2023***: 636*. doi: https://doi.org/10.54112/bcsrj.v2023i1.636]

This, in turn, substantially influences global food security and the economy (López-Serrano *et al*., 2019). According to (Tiwari et al., 2016), when free radicals build up due to drought stress, it alters protein structure and function, lipid peroxidation, and cell death. Due to climate change, droughts are expected to become more frequent and severe.

Agricultural systems are now encountering challenges in response to various climate changes. The diminished irrigation water supply adversely impacts the productivity of almost all types of field crops, particularly vegetables. Tomatoes are the predominant vegetable crop grown worldwide, farmed in various settings such as open fields, greenhouses, and net houses. It is a significant crop used as a standard crop for plant research (Zhou *et al*., 2015). Like other vegetable crops, tomato plants may suffer harm from high temperatures and lack of moisture. The degree to which these forces affect tomato genotypes varies (Sánchez-Rodríguez *et al*., 2010). The study by (Shao et al., 2005) emphasizes the need to investigate tomato crops' response to water deficiencies in combination with antioxidant defence mechanisms. This study examines the photochemical effectiveness of PS-II and the crop's sensitivity to key stressors seen in other crops. The present study aimed to expound the tomato's photosystem deterioration, changes in water relations, altered biochemical attributes and antioxidant activity response under drought conditions.

Materials and Methods

The research experiment was performed at the Department of Horticultural Sciences under the Faculty of Agriculture and Environmental Sciences at Islamia University of Bahawalpur in Punjab, Pakistan. The 9-liter containers were sand-filled for growth and Hoagland's nutrient solution. The seeds of two tomato types (Sahel and Flanto) were planted in them. The experimental design consisted of five treatments and four replications of a randomized block design. The treatments were designed by calculating the sand's water saturation percentage, i.e. 100%, 80%, 60%, 40%, and 20%. To calculate the saturation percentage, we filled the 09-liter plastic pot with sand, saturated it with water, weighed it, and then put it in the oven for 72 hours. After the sand had dried completely, we measured the difference in weight to determine how much water would be needed to achieve 100% saturation. The water needed for the remaining procedures was determined by calculating the water needed for 100% saturation. The seedlings were subjected to drought stress four weeks after germination by keeping the sand moisture level at the percentage determined for each treatment. After four weeks of therapy, the following characteristics were noted.

Physiological Attributes

The following variables were measured using a portable photosynthesis system (MultispeQ V2.0, manufactured by PHOTOSYNQ INC., USA): Photosystem-II (quantum yield of photosystem-II), non-photochemical quenching (NPQ), photochemical quenching coefficient (qP), PhiNO (ratio of incoming light that is lost via non-regulated processes), Fv/Fm (maximum photochemical efficiency of PS-II), with LEF standing for linear electron flux. We employed three leaves from each plant in each replication to determine the average Photosystem-II parameters. Twelve leaves were used for each treatment.

Measurement of enzymatic attributes

Super oxide dismutase (SOD) (U mg-1 protein)

One of the criteria utilized to assess the activity of SOD was its capacity to impede the photoreduction of nitroblue tetrazolium (NBT), following the methodologies delineated by Giannopolitis and Ries (1977). The three-millimolar reaction solution consisted of the following components: fifty millimolar NBT, 1.5 millimolar riboflavin, thirteen millimolar methionine, 75 millimolar EDTA, fifty millimolar phosphate buffer (pH 7.8), and twenty to fifty millimliters of enzyme extract. The reaction solution within the cylinders was exposed to 15 fluorescent lamps operating at a light intensity of 78 mmol m-2 s-1 for 15 minutes. The absorbance of the irradiation solution was determined by utilizing spectrophotometer readings acquired at 560 nm (Hitachi-650, Japan). An enzyme concentration is considered to have one unit of SOD activity if it reduces the photosensitivity of NBT by half.

Peroxidase and Catalase

Catalase (CAT) and peroxidase (POD) activity levels were evaluated utilizing the revised method established by Chance and Maehly in 1955. The CAT reaction solution comprised 50 mM phosphate buffer at a pH of 7.0, 5.9 mM H2 O2, and 0.1 mL of enzyme extract, weighing 3 mL. At intervals of twenty seconds, the reaction mixture's absorbance fluctuations were recorded at a wavelength of 240 nanometers. The measured CAT activity parameter was the absorbance change rate of 0.01 units per minute. The reaction solution to determine the presence of POD contained 0.1 mL of enzyme extract, 20 mM guaiacol, and 40 mM H2 O2 in a volume of 3 mL. The phosphate buffer had a pH of 5.0. At a specific wavelength of 470 nm, the variations in the absorbance of the reaction solution were measured over twenty seconds. The one-unit POD activity was specified as a 0.01-unit-per-minute increase in absorbance. The enzymatic activity of the protein concentration was determined.

Ascorbate peroxidase (APX)

The enzyme that facilitates the reduction of hydrogen peroxide with ascorbate as the reducing agent is referred to by its name. The ascorbate

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peroxidase (APX) was isolated at a pH of 7 using a phosphate buffer solution. APX's enzymatic activity was assessed using the protocol developed by Nakano and Asada in 1981. Enzyme extract, sodium phosphate buffer (50 mmol L-1, pH = 7), EDTA (0.2 mmol L-1), ascorbic acid (0.5 mmol L-1), and BSA (50 milligrammes) constituted the reaction mixture. A 0.1 mmol L-1 hydrogen peroxide (H2O2) solution was introduced into the reaction mixture to initiate the reaction. The absorbance measurement was performed at a designated wavelength of 290 nanometers precisely two minutes after the initiation of the procedure. To ascertain the enzyme's activity, the absorbance change was divided by 2.8 mmol-1 cm-1, which is the molar extinction coefficient of ascorbate. The enzyme activity is assessed by calculating the rate of H2O2 degradation per minute per milligramme of protein, assuming that 1.0 moles of ascorbate are required to counteract 1.0 moles of H_2O_2. Three samples were used for each treatment to measure enzymatic attributes. Each sample was a composite of four replicates.

Plant Water Relations

Leaf water potential (-Ψw) (-MPa)

Twelve leaves were used for each treatment, with three mature leaves chosen from each replication. To determine the leaf water potential (Ψw), a fully expanded leaf was chopped with a razor and after that inserted in the gasket of a pressure chamber instrument (Model-615, manufactured by PMS Instrument Company, USA). The data was computed between 10:00 and noon.

Leaf osmotic potential (Ψs) (-MPa)

After being utilized in the pressure chamber apparatus to find leaf Ψs, the same leaf was transferred to a plastic bag and stored in a freezer at a low temperature (-20°C) for one week. Following a thirty-minute defrosting period at room temperature, cell sap was collected using a disposable syringe from the frozen leaf material. With the assistance of a plastic syringe, 10 µL of the sap that had been harvested was put on an Osmometer (Wescor, Model-5500), and the reading was recorded.

Leaf turgor potential (Ψp)

We calculated the turgor potential (Ψp), which is the discrepancy between Ψw and Ψs, using the equation shown below: $(\Psi p) = \Psi w - \Psi s$

Biochemical Attributes

Chlorophyll contents (SPAD value)

A chlorophyll metre (CCM-200plus Bio-Scientific USA) was used to measure the amounts of chlorophyll in the leaves for this objective. The SPAD value and average were calculated using three leaves per plant in each replication. Each treatment made use of a total of twelve leaves.

Statistical Analysis

Statistical analysis was performed on the collected data using Fisher's analysis of variance and HSD (Tukey Test) to ascertain the treatments' significance. To estimate the phenotypic correlation coefficient and conduct a statistical analysis, the program Statistix 8.1 was used.

Results

Drought stress significantly affected most tomato varieties' physiological attributes, antioxidant activity, and plant water relations (Sahel and Flanto). Drought stress significantly changed linear electron flow. The highest linear electron flow (164.72±31.27) was recorded in Flanto at 60% moisture level, whereas the lowest (56.33±27.08) was recorded in Sahel at 100% moisture level. An evident impact of drought stress was detected on PhiNO. Sahel gave a maximum (0.315±0.010) value of PhiNO at a 20% moisture level, while a minimum (0.143±0.012) value was recorded in Flanto at a 60% moisture level (Table 1).

Table 1. Effect of drought stress on Photosystem-II (PS-II), Non-photo chemical quenching (NPQ), linear electron flow (LEF) and PhiNO (Ratio of incoming light via non-regulated process)

Treatment (Sand Water Content)	Photosystem II		Non-photo chemical quenching		Linear electron flow		PhiNO	
	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto
100%	0.45ab	0.20cd	1.10c	2.53bc	56.3d	133.5ab	0.29ab	0.16ef
80%	0.48a	0.19d	1.02c	5.05a	83.2bcd	153.7a	0.26cc	0.15f
60%	0.32bc	0.22cd	1.71c	3.87ab	121.9abc	164.7a	0.28b	0.14f
40%	0.39ab	0.42ab	1.51c	2.08bc	81.7bcd	92.9bcd	0.27bc	0.18f
20%	0.45ab	0.25cd	0.82c	5.56a	70.8cd	154.2a	0.32a	0.21d
LSD	0.135		1.99		52.7		0.02	

Drought stress significantly affects Fv/Fm. Sahel gave a maximum (0.694±0.025) Fv/Fm value at 100% moisture condition, and Flanto gave a minimum (0.433±0.021) value at 20% moisture level. The highest (0.728±0.016) qP was observed in Flanto at 100% level, whereas the lowest (0.519±0.031) was noted in Sahel at 20% moisture level. Both varieties of tomato also revealed significant results for chlorophyll content (Table 2).

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Table 2. Effect of drought stress on Fv/Fm (Maximum Photochemical Efficiency of PSII), qP (Photochemical quenching coefficient) and chlorophyll content

Treatment (Sand Water Content)	Fv/Fm		qP		Chlorophyll content	
	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto
100%	0.69a	0.61bc	0.59a	0.73a	46.9abc	49.3abc
80%	0.65ab	0.48d	0.53a	0.60b	36.2c	50.4abc
60%	0.59c	0.45d	0.53a	0.58b	44.9abc	57.8a
40%	0.62bc	0.46d	0.55a	0.57b	52.0ab	41.4bc
20%	0.59c	0.43d	0.52a	0.55b	53.2ab	49.2abc
LSD	0.052		0.08		14.36	

For SOD, POD, CAT, and APX, Flanto possessed maximum superoxide dismutase (24.51±0.58 U mg-1 protein) at 60% moisture level, while the lowest was in Sahel (11.39±2.34 U mg-1 protein) at 20% level. The highest peroxidase activity was noted in Flanto (2.06±0.08 U mg-1 protein) at 60% moisture level, and the lowest was in Sahel (1.15±0.03 U mg-1 protein) at 20% level. Flanto showed maximum catalase activity (0.153±0.022 U mg-1 protein) at 60% moisture level, while the lowest was recorded in Sahel (0.068±0.010 U mg-1 protein) at 80% moisture level. The data showed maximum ascorbate peroxidase activity (16.19±0.95 U mg-1 protein) in Flanto at 20% moisture level, and the lowest (7.36±1.22 U mg-1 protein) was recorded in the Sahel at the same moisture level (Table 3).

Table 3. Effect of drought stress on superoxide dismutase (U mg^{-1} protein), peroxidase (U mg^{-1} protein), catalase (U mg^{-1} protein), and ascorbate peroxidase (µmol H_2O_2 g^{-1} FW)

Treatment (Sand Water Content)	SOD		POD		CAT		APX	
	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto
100%	15.2a	18.4ab	1.7a	1.7a	0.09cd	0.12b	7.9bc	11.7b
80%	14.8a	18.7ab	1.3b	1.3b	0.06d	0.12b	10.6bc	12.8ab
60%	17.6a	24.5a	1.9a	2.1a	0.10bc	0.15a	11.8ab	13.7ab
40%	12.2a	19.1ab	1.3b	1.3b	0.07cd	0.08b	14.7a	13.5ab
20%	11.4a	16.1b	1.2b	0.9c	0.07cd	0.07cd	7.4c	16.2a
LSD	7.22		0.35		0.029		4.04	

It was observed that Flanto has the lowest LWP of (0.236±0.042 -MPa) at 20% moisture level, whereas the highest LWP (0.409±0.055 -MPa) was observed in the Sahel at 60% moisture level. Similarly, the results recorded for LWP, LOP, and LTP were significant (Table 4).

Table 4. Effect of drought stress on leaf water potential (-MPa), leaf osmotic potential (-MPa) and Leaf Turgor Potential (MPa)

Treatment (Sand Water Content)	LWP		LOP		LTP	
	Sahel	Flanto	Sahel	Flanto	Sahel	Flanto
100%	0.34bc	0.34a	0.54ab	0.44b	0.20abc	0.21abc
80%	0.39ab	0.32a	0.45ab	0.62a	0.16bcd	0.30a
60%	0.41a	0.32a	0.43b	0.56a	0.10d	0.24ab
40%	0.38abc	0.30ab	0.51ab	0.57ab	0.13cd	0.27a
20%	0.31c	0.24b	0.54ab	0.48ab	0.23abc	0.25ab
LSD	0.07		0.173		0.99	

The study found a direct relationship between the maximal photochemical efficiency of photosystem II (Fv/Fm) and the proportion of incoming light that is not used for regulated activities (PhiNO) in Photosystem II. Conversely, a negative association observed between non-photochemical quenching, leaf electron flow, photochemical quenching, chlorophyll concentration, superoxide dismutase, catalase, and ascorbate peroxidase. Concerning photochemical efficiency, there was a direct relationship between non-photochemical quenching and leaf electron flow, superoxide dismutase, ascorbate peroxidase, and leaf turgor potential. In contrast, it exhibited an inverse relationship with PhiNO, Fv/Fm, and leaf water potential. The flow of electrons through the leaf showed a strong negative correlation with PhiNO (the fraction of incoming light lost due to unregulated processes) and Fv/Fm (the maximum photochemical efficiency of PS-II). In contrast to the earlier statement, superoxide dismutase, catalase, and ascorbate peroxidase exhibited notable positive correlations. The

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photochemical quenching coefficient, superoxide dismutase, catalase, ascorbate peroxidase, and leaf turgor potential exhibited significant negative correlations. Significant positive associations were seen with Fv/Fm (Maximum Photochemical Efficiency of PS-II). A positive correlation was identified between leaf water potential (LWP) and the maximum photochemical efficiency of PS-II (Fv/Fm). Conversely, a negative association was discovered with superoxide dismutase, ascorbate peroxidase, and lipid transfer protein (LTP). A strong positive relationship was found between catalase and the photochemical quenching coefficient. An evident positive association was discovered in the interactions of superoxide dismutase, peroxidase, and catalase. A strong positive association was found between peroxidase, catalase, and leaf water potential (LWP). A clear inverse relationship was seen between LWP and LTP. Table 5 shows a strong positive association between the osmotic potential of leaves and LTP.

Table 5: Correlation among various attributes studied

Traits	NPQ	LEF	PhiNO	FvFm	qP	CC	SOD	POD	CAT	APX	LWP	LOP	LTP
PS-II	-.689**	-.595**	.686**	.511**	-.423*	-.386**	-.490**	-.233	-.611**	-.421*	.278	-.161	-.285
NPQ		.646**	-.597**	-.700**	.201	.101	.377*	-.153	.300	.479**	-.431*	.170	.499**
LEF			-.623**	-.592**	.218	.216	.505**	.084	.450*	.512**	-.242	-.175	.160
PhiNO				.664**	-.536**	-.153	-.685**	-.198	-.622**	-.500**	.327	-.222	-.489**
FvFm					.043	-.139	-.483**	.164	-.254	-.552**	.632**	-.208	-.463**
qP						.151	.249	.308	.377*	.054	-.031	.047	.314
CC							.042	.188	.285	.133	-.195	.154	.131
SOD								.486**	.722**	.282	-.150	.087	.293
POD									.669**	-.212	.435*	.009	-.194
CAT										.102	-.008	.278	.249
APX											-.279	-.079	.134
LWP												-.239	-.505**
LOP													.623**

Where: PS-II= Photosystem 2, NPQ= Non photochemical quenching, LEF= linear electron flow, PhiNO=Ratio of incoming light, Fv/Fm= Maximum Photochemical Efficiency of PSII, qP= Photochemical quenching coefficient, CC= Chlorophyll contents, SOD= superoxide dismutase, CAT= Catalase, APX= ascorbate peroxidase, LWP= leaf water potential, LOP= leaf osmotic potential, LTP= leaf turgor potential

Discussion

Photosystem-II (PS-II) measurements indicate the proportion of light absorbed and used in photo-chemical processes. The linear electron flow (LEF), a dependable indicator of net photosynthesis, arises from this source. The well-established truth is that PS-II's actual quantum yield maximizes the real yield of the active reaction centre as it absorbs incoming light energy (Schreiber, 2004). Under drought and other stresses, PS-II is thought to be the most sensitive component (Baker, 2008) and gives a clear picture to estimate the magnitude of loss owing to the prevailing stress. Abiotic stress reduces photochemical efficiency and electron transport activity, which may be explained by disturbances in the photosynthetic system (Fatima et al., 2023; Guidi et al., 2019; Rasheed and Malik, 2022). To evaluate how water stress affects tomato plants, researchers used the maximum quantum efficiency of PS-II (Fv/Fm), which is both a leading indicator of a plant's stress response (Abbas et al., 2021; ALI, 2022; Hussain et al., 2021) and the most important factor in determining a plant's photosynthetic status (Čaňová et al., 2012). Contrary to previous studies, drought stress significantly reduces both genotypes' photochemical efficiency (Fv/Fm). Previous studies on LEF (linear electron flow) have shown that photoinhibition occurs because drought stress increases the NPQ (estimate of the non-photochemical quenching) (Guidi et al., 2019; Haider et al., 2023; Ullah et al., 2023).

Plants undergo oxidative stress when they generate reactive oxygen species (ROS), such as hydroperoxide, hydroxyl radicals, superoxide radicals, and similar compounds (Panda et al., 2003). Increased concentrations of reactive oxygen species (ROS) impede the activity of enzymes, lipids, nucleic acids, and damaged proteins, activating the programmed cell death pathway and eventually leading to cell death (Sharma et al., 2012). The

plants' DNA may experience substantial harm when exposed to adverse environments (Tuteja et al., 2009). Plants use enzymatic and non-enzymatic antioxidants as a protective measure to defend against oxidative damage and maintain a consistent amount of reactive oxygen species (Ozgur et al., 2013). Drought stress enhances the activity of the catalase (CAT) enzyme in tomato genotypes. In line with the prior research conducted by (Sánchez-Rodríguez *et al*., 2010), the present study demonstrated increased CAT activity in both genotypes due to water stress. Studies have shown that genotypes with greater water stress resistance have elevated enzyme activity levels. Furthermore, the catalase activity is a dependable indicator of a plant's ability to withstand stress (Nazir *et al.*, 2017). (de Azevedo Neto et al., 2006) discovered that the levels of ascorbate peroxidase (APX) were elevated in both susceptible and resistant varieties of maize when cultivated under challenging conditions. A further inquiry demonstrated that the efficacy of APX in wheat plants was enhanced under conditions of drought-induced stress (Nikolaeva et al., 2010). Our analysis also found a similar increase in APX activity in response to drought stress for CAT. Drought stress led to increased superoxide dismutase (SOD) activity in many maize cultivars throughout their experiment (Moussa and Abdel-Aziz, 2008). Drought stress circumstances led to an augmentation in the activity of the SOD enzyme in thin-leaf lupins (Yu and Rengel, 1999). The drought stress has a considerable impact on the activity of superoxide dismutase (SOD) in tomato plants. The findings of this research support the findings of the previous experiment, showing that drought-induced stress led to increased superoxide dismutase (SOD) activity in both genotypes (Noctor et al., 2000). An efficient method to assess the water status of a plant is by examining its leaf water potential (Noctor *et al.*, 2000). The findings support previous research indicating drought stress significantly decreases leaf water potential (Calcagno et al., 2011; Siddique et al., 2000).

Conclusions

In conclusion, our study on the effects of drought stress on tomato crop productivity reveals a significant decrease in important plant characteristics under different moisture conditions, indicating the physiological consequences experienced by the crop throughout its growth stages. Our study aims to enhance our comprehension of the interaction between biochemical, physiological, enzymatic, and water-related aspects in two well-known tomato cultivars, Flanto and Sahel, due to the worldwide limitation on water resources resulting in subpar tomato harvests. The experiment was performed to find the effect of drought stress on these cultivars at five different moisture levels (100%, 80%, 60%, 40%, and 20%) revealing a range of responses in all plant parameters being studied. The varied degrees of drought stress significantly affected linear electron flow (LEF), photosynthetic efficiency (PSE), inward light dissipation ratio (qP), catalase activity (CAT), ascorbate peroxidase activity (APX), and leaf water potential (LEWP). Significant enhancements in specific characteristics associated with photosystem-II, non-photochemical quenching, chlorophyll levels, leaf osmotic potential, and leaf turgor potential were noted in response to water constraint. These findings not only enhance our understanding of the complex connections between moisture levels and plant physiology, but also provide possible opportunities for improving drought resilience in tomato crops. In Future more cultivars with different experiment techniques should be used to reduce the negative impact of water shortage on tomato farming.

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[Citation Ghaffar, R., Shaheen, M.R., Hussain, R., Anjum, S., Saeed, M.A, Shabbir, A., Amjad, M.U. (2023). Estimating the photosystem's efficiency, antioxidants activity and plant water relations of tomato under water deficit conditions. *Biol. Clin. Sci. Res. J.*, **2023***: 636*. doi: https://doi.org/10.54112/bcsrj.v2023i1.636]

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Ethical approval was given from Ethical Review committee of department.

Consent for publication

The consent form was approved from Ethical Review committee of department.

Funding

Not applicable

Conflict of Interest

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.

Acknowledgement

Not Applicable

Author Contribution

GR conducted research and wrote up initial draft of manuscript. MRS, RH, AS, and MAS provided resources. RR and MUA made final editing in the manuscript. All authors approved final version of manuscript.