

## Effect of Thermopriming and Alpha-Tocopherol Spray in *Triticum aestivum* L. under Induced Drought Stress: A Future Perspective of Climate Change in the Region

(Kesan Penyebuan Terma dan Semburan Alfa-Tokoferol pada *Triticum aestivum* L. Teraruh Tekanan Kemarau: Suatu Perspektif Masa Depan terhadap Perubahan Iklim Serantau)

UBAID ULLAH SHAKIR<sup>1</sup>, SAMI ULLAH<sup>1</sup>, MUHAMMAD NAUMAN KHAN<sup>2,5</sup>, SAJJAD ALI<sup>2</sup>, USMAN ALI<sup>1</sup>, AKHTAR ZAMAN<sup>1</sup>, SARAH ABDUL RAZAK<sup>3,\*</sup> & FETHI AHMET OZDEMIR<sup>4</sup>

<sup>1</sup>Department of Botany, University of Peshawar, 25120, Pakistan

<sup>2</sup>Department of Botany, Bacha Khan University Charsadda, Pakistan

<sup>3</sup>Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Federal Territory, Malaysia

<sup>4</sup>Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, 12000 Bingol, Turkey

<sup>5</sup>Agriculture University Public School and College (Boys), The University of Agriculture, Peshawar, 25120, Pakistan

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### ABSTRACT

Because of global warming and decreased river flows, all of Pakistan's provinces, especially large parts of Sind and Baluchistan, have been experiencing water shortages for decades. Based on such climatic changes several management techniques have been recommended to cope through drought stress. This study is focused on the assumption that seed soaking of *Triticum aestivum* L. at low (4 °C) and high (80 °C) temperature (thermopriming) with exogenous spray of alpha-Tocopherol (150 mol/L) will increase seedling formation and crop production through drought stress of 5 and 10 days recommended to persuade resistivity in test species. This study also describes resistance mechanism of drought both in physiological and biochemical activities. Results concluded that chlorophyll a & b, carotenoids, sugar, protein and proline (µmg/g) contents were detected maximum in case of T1 (control) and T5 (5 days drought + 4 °C + α-Tocopherol) enhancing growth and osmolytes component in plant whereas; antioxidant enzymes bitterly respond under induced high drought stress and growth regulator at  $p \leq 0.05$ . The study showed the degree of resistance to various drought stressors best suited in agricultural country (Pakistan) signifying successful demonstration of priming method with the application of α-Tocopherol as growth regulator will help agricultural industries improve seed quality and germination rate.

Keywords: α-Tocopherol; antioxidant enzymes; drought stress; thermopriming; *Triticum aestivum* L.

### ABSTRAK

Disebabkan pemanasan global dan aliran sungai yang berkurangan, semua wilayah Pakistan, terutama sebahagian besar Sind dan Baluchistan telah mengalami kekurangan air selama beberapa dekad. Berdasarkan perubahan iklim tersebut, beberapa teknik pengurusan telah dicadangkan untuk mengatasi tekanan kemarau. Kajian ini memfokuskan pada anggapan bahawa rendaman benih *Triticum aestivum* L. pada suhu rendah (4 °C) dan tinggi (80 °C) (penyebuan terma) dengan semburan eksogen alfa-Tokoferol (150 mol/L) akan meningkatkan pembentukan anak benih dan pengeluaran tanaman melalui tekanan kekeringan selama 5 hari dan 10 hari yang disyorkan untuk mendapatkan daya tahan dalam spesies ujian. Kajian ini juga menjelaskan mekanisme tahan kekeringan dalam aktiviti fisiologi dan biokimia. Hasil kajian mendapati kandungan klorofil a & b, karotenoid, gula, protein dan prolin dikesan maksimum sekiranya T1 (kawalan) dan T5 (kekeringan 5 hari + 4 °C + α-Tokoferol) meningkatkan pertumbuhan dan komponen osmolit di dalam tumbuhan sedangkan enzim antioksidasi kurang bertindak balas di bawah tekanan kekeringan tinggi dan pengatur pertumbuhan pada  $p \leq 0.05$ . Kajian menunjukkan tahap ketahanan terhadap pelbagai tekanan kemarau yang paling sesuai di negara pertanian (Pakistan) yang menunjukkan kejayaan kaedah 'penyebuan' dengan penggunaan α-Tokoferol sebagai pengatur pertumbuhan akan membantu industri pertanian meningkatkan kualiti benih dan kadar percambahan.

Kata kunci: α-Tokoferol; enzim antioksidasi; penyebuan terma; tekanan kemarau; *Triticum aestivum* L.

## INTRODUCTION

The world's water supply is at a startling point and is on the verge of declining, which will worsen in the coming years as a consequence of global warming (Cook et al. 2007), while potential claim for promptly growing population stresses is likely to exacerbate the drought's responses (Somerville & Briscoe 2001). The response of plant to drought tolerance are influenced factors like time, duration, intensity and frequency of drought stress and poor soil-plant-environment interactions. Various morphological, physiological, and biochemical techniques have been recognized to the response of plant with respect to drought stress tolerance extending from dryness to dehydration respectively (Saint Pierre et al. 2012). Drought is a major concern in Pakistan, with one-quarter of the country's total agricultural land (4.9 million ha) classified as drought-prone, and the situation is deteriorating day by day. Water scarcity and poor rainfall are the two most important causes in the conversion of vast regions into deserts. Water and rivers have also reached a dead level, with river storage limited due to siltation, while Pakistan is sucking out ground water at an alarming pace, depleting 'Fossil' ground water at an alarming rate that could lead to catastrophe (Ali & Ashraf 2011).

Scarcity is a significant abiotic pressure that reduces plant development and productivity (Yuan et al. 2010). Drought intensity is variable because it is dependent on a variety of variables such as rainfall frequency and distribution, evaporative demands, and soil moisture storage capability. When plants are deprived of water, they go through a number of biochemical and physiological changes (Fujita et al. 2009). Generation of reactive oxygen species (ROS) lead to lipid peroxidation, protein degradation and nucleic acid damage (Jiang & Zhang 2002). Various genes that increase the tolerant level of plant to drought condition mostly involved in the synthesis of osmolytes like sugar and proline in response to different abiotic stresses.

Early development and stand formation technology will allow the crops to absorb more soil moisture, nutrient, and solar emission, increasing crop yield. A number of pre-sowing hydration therapies can help improve seed germination, with the goal of allowing water absorption and germination metabolism to progress to just short of radical (Bradford 1986) and is called seed priming. Priming is a practical technological method for improving quick and constant occurrence, high vigour, and higher crops of vegetables and flowering

plants, primarily (Bruggink et al. 1999). These benefits of priming are linked to a variety of different metabolic processes and physiological developments, including activating enzymes like POD, CAT, and SOD as well as adding osmo-protectants like soluble sugar, proline, and soluble protein, which are common stress-avoidance reactions (Farhad et al. 2011; Shehab et al. 2010). Enzymatic antioxidants can help to mitigate ROS-induced oxidative damage, while osmo-protectants can help to increase water absorption by enhancing water status (Farooq et al. 2009; Posmyk et al. 2009).

In short, in order to achieve reasonable crop yields as other countries, it is significant to mitigate the negative responses of scarcity stress. Pakistan is suffering from an extreme drought, with an arid state of 0.563 million km<sup>2</sup> out of a total area of 0.804 million km<sup>2</sup> with average rainfall of less than 60 cm (Anon 2012). To investigate the results of a short-term field experiment, the effects of  $\alpha$ -Tocopherol foliar spray on vegetative development, biological and biochemical attributes of wheat cultivar under induced drought stress. Such studies better suited in Pakistan because of an agricultural land where major farmLand have been affected by water scarcity and increase day by day due to global rise in temperature from 0.5-2 °C, hence a better and immediate adaptive way recommended by the present as well as related findings of other researchers to stabilize our growth rate.

## MATERIALS AND METHODS

### SITE DESCRIPTION AND EXPERIMENTAL DESIGN

The experiment was carried out in the green house of Department of Botany, University of Peshawar at 34° 1' 33.3012" N and 71° 33' 36.4860" E. Seeds of wheat (*Triticum aestivum* L.) collected from NARC (National institute of Agriculture and Research Centre) Islamabad, Pakistan were planted in earthenware pots (18 cm lower inside diameter, 18 cm upper inside diameter, 20 cm height and 2 cm of thickness) containing soil and sand in ratio 2:1. Pot were arranged in a complete randomize design (CRD) and were protected from rain and also from intense sunlight and warm wind. Three replicates were taken for each treatment. Prior to sowing, seeds were surface sterilized with a mixture of 10% chlorox and 95% ethanol. One third of the seeds was thermoprimered by keeping the seeds in distilled water at 4 and 80 °C. Pots were treated with 150 mol/L solution of  $\alpha$ -Tocopherol. Drought stress was induced when plants were 15 days old. Sampling was done after a month for

agronomic studies whereas, only foliar material was preserved at 4 °C in refrigerator for physiological and

biochemical analysis. Experimental design for this study is shown in Table 1.

TABLE 1. Experimental design for *Triticum aestivum* L. under induced drought stress

Treatments	Description
T1	Control
T2	5 days drought
T3	10 days drought
T4	5 days drought + 4 °C
T5	5 days drought + 4 °C + $\alpha$ -Tocopherol
T6	5 days drought + 80 °C
T7	5 days drought + 80 °C + $\alpha$ -Tocopherol
T8	10 days drought + 4 °C
T9	10 days drought + 4 °C
T10	10 days drought + 80 °C
T11	10 days drought + 80 °C + $\alpha$ -Tocopherol

ASSESSMENT AND MEASUREMENT OF AGRONOMIC CHARACTERS GERMINATION INDEX (GI) AND TOTAL BIOMASS (TBM)

Germination index and total biomass was determined using formula proposed by Kader (2005).

$$\text{Germination index} = (G1) - (10 \times n1) + (9 \times n2) + (8 + n3) + (1 \times n10) \quad (1)$$

ROOT SHOOT RATIO (RSR) AND WATER USE EFFICIENCY (WUE)

Chuyong and Acidri (2017) methods was followed for Root shoot ratio and water use efficiency determination.

$$\text{Root shoot ratio} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}} \quad (2)$$

$$\text{WUE} = \frac{\text{Total water use during experiment}}{\text{Total Biomass (g)}} \quad (3)$$

LEAF AREA RATIO (LAR) LEAF AREA INDEX (LAI)

Leaf area ratio and leaf area index was determined by formulas of Shah et al. (2017)

$$\text{LAR} = \frac{\text{Leaf Area}}{\text{Final Plant Dry weight}} \quad (4)$$

$$\text{LAI} = \frac{\text{Leaf Area (cm)}^2}{\text{Land Area (cm)}^2} \quad (5)$$

ABSOLUTE GROWTH RATE (AGR) AND RELATIVE WATER CONTENT (RWC)

Absolute growth rate and relative water content was determined by Ghule et al. (2013) method.

$$\text{AGR (Plant height)} = \frac{H_2 - H_1}{t_2 - t_1} \quad (6)$$

VIGOR INDEX (VI)

Bina and Bostani (2017) approach was used to study the vigor index.

$$\text{Relative water content} = \frac{Wf - Wd}{Ws - Wd} \quad (7)$$

$$\text{SVI} = \text{Mean seedling length} \times \% \text{ seed germination} \quad (8)$$

## MOISTURE CONTENT PERCENTAGE (%MC)

Moisture content percentage was determined by the following method of Ullah et al. (2016).

$$\%MC = \frac{\text{Fresh weight} - \text{Dry weight} \times 100}{\text{Fresh weight of sample}} \quad (9)$$

## TIMSON GERMINATION INDEX (TGI)

The number of seeds that grown on each day is indicated by this parameter. Al-Ansari and Ksiksi (2018) technique was used to calculate the Timson germinated index.

$$TGI = \frac{\sum G}{T} \quad (10)$$

## FINAL EMERGENCE PERCENTAGE (FEP)

This parameter was measured by the following formula as described by Babar et al. (2014).

$$FEP = \frac{\text{Final No of seedlings emerged} \times 100}{\text{Total No of seeds sown}} \quad (11)$$

## PHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS OF PLANTS

## DETERMINATION OF PLANT SOLUBLE PROTEIN CONTENT

Protein contents in leaves was estimated by the standard methods of Rostami and Ehsanpour (2009). Fresh leaves (0.2 g) was grounded in 1 mL of phosphate buffer pH 7.5 with a mortar and pestle and centrifuged for 10 min at 3000 rpm. In the test tube, 0.1 mL of supernatant from a given sample containing an undisclosed amount of protein was poured and volume of 1 mL of  $\text{Na}_2\text{CO}_3$ , NaOH, and Na-k tartrate was added. After ten min of shaking, 0.1 mL Folin phenol was applied. After 30 min of incubation, optical density was measured at 650 nm.

## DETERMINATION OF PHOTOSYNTHETIC PIGMENTS (CHLOROPHYLL A, B &amp; CAROTENOIDS)

Fresh leaf material (0.5 gm) was homogenized in 10 mL 80% acetone solution. Samples containing homogenized solution were kept in centrifuge machine and spun for 5 min. After centrifugation, the samples were kept in the dark overnight at 4 °C. On the following day, optical density of each sample was measured at 470, 645, and 663 nm for carotenoids and chlorophyll a & b quantification by following the protocol of Arnon (1949). The absorbance was recorded for each solution

and chlorophyll a and b contents are calculated using the following formula:

$$\text{Chlorophyll a (mg/mL)} = 12.7 A_{663} - 2.69 A_{645} \quad (12)$$

$$\text{Chlorophyll b (mg/mL)} = 22.9 A_{645} - 4.68 A_{663} \quad (13)$$

where as;

A<sub>645</sub> = absorbance at a wavelength of 645 nm

A<sub>663</sub> = absorbance at a wavelength of 663 nm (14)

$$\text{Total chlorophyll (mg/L)} = (20.2 \times A_{645}) + (8.02 \times B_{663})$$

## DETERMINATION OF LEAF PROLINE CONTENT

Proline content was quantified by the methods of Bates et al. (1973). Fresh leaves (0.5 gm) were grounded in 10 mL 3% aqueous sulphosalicylic acid and a homogenized mixture was prepared. The mixture was filtered, and 2 mL filtrate was taken. Similarly, 4 mL ninhydrin solution and 4 mL glacial acetic acid (20%) were mixed with 2 mL filtrate taken. The mixture was heated at 100 °C for 1 h and 4 mL toluene was added to it. OD readings were recorded at 520 nm.

## DETERMINATION OF PLANT SOLUBLE SUGAR CONTENT

Fresh plant materials (0.5 g) for sugar determination using the method of Marcińska et al. (2017) was homogenized with 10 mL of purified water and 0.1 mL of supernatant 1 mL treated with phenol at a concentration of 80% (w/v). After 4 h of incubation, the absorbance of each sample was measured at 420 nm. Optical density (OD) was noted at 490 nm.

## DETERMINATION OF PEROXIDASE ACTIVITY (POD)

Peroxidase (POD) activity was determined by following the methods of Maehly and Chance (1954). Leaf material (0.5 gm) was chopped in 2 mL morpholino ethane sulphonic acid (MES) and homogenized mixture was prepared. The samples were placed in centrifuge machine and spun for 15 min. After centrifugation, 0.1 mL supernatant was collected from each sample and 1.3 mL MES, 0.1 mL phenyl diamine and 1 mL hydrogen peroxide (30%) were added. OD was noted at 470 nm for 3 min via spectrophotometer.

## DETERMINATION OF SUPEROXIDE DISMUTASE ACTIVITY (SOD)

The content of superoxide dismutase (SOD) was

calculated using the method of Wang et al. (2014) with minor modifications. After centrifugation, the enzymatic extract will be treated with 0.1 mL of supernatant and 0.72 mL of methionine solution, NBT, EDTA solution, and riboflavin for 30 min in the dark and light. The OD was measured at 560 nm.

#### DETERMINATION OF CATALASE ACTIVITY (CAT)

Tybursk et al. (2009) method was followed for the determination of Catalase (CAT) with some modification. 1 mL of reaction mixture include 0.4 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.2 mL of enzyme extract and 0.4 mL of 30% H<sub>2</sub>O<sub>2</sub>. The decline in OD at 240 nm represented the disintegration of H<sub>2</sub>O<sub>2</sub> for 3 min.

#### STATISTICAL ANALYSIS

Whole experiment was designed in triplicates and subjected to Duncan Multiple Range Test (DMRT). Results are expressed as mean  $\pm$  standard deviation, whereas significance different at  $P \leq 0.05$  and means comparison were done using one-way ANOVA using SPSS Statistic-25 software.

#### RESULTS

Results given in Table 2 showed the shoot, root, soil, and leaf moisture content in *Triticum aestivum* L. plants treated with different concentration of  $\alpha$ -Tocopherol under drought stress. SHMC content showed higher moisture content in T1 compared with drought stress plants and  $\alpha$ -Tocopherol treated plants. However, the SHMC in 5 and 10 days drought stressed wheat plant treated with  $\alpha$ -Tocopherol showed higher moisture content compared with untreated drought stressed plants. It is noticeably that root moisture content and soil moisture content found at highest level in treatment T1 (5 days drought) at  $p > 0.5$  whereas no significant difference were observed in 5 and 10 days drought stressed plant treated with or without  $\alpha$ -Tocopherol. Furthermore, leaf area index was higher in control T1 treatments, while drought stress effect the LAI and reduced leaf area index and no significant difference were observed in LAI between stressed plants and treated with  $\alpha$ -Tocopherol. LAI effect on the moisture content of leaf and similar pattern were observed in leaf moisture content as in LAI. Higher leaf moisture content was observed in T1 compared with other treatments.

Table 3 shows the effect of thermopriming and  $\alpha$ -Tocopherol spray on different agronomic characteristic

of *Triticum aestivum* L. under drought stress. Results declared in Table 3 confirmed that the maximum germination index, timson germination index and final emergence percentage reported in T1 (control). Whereas the minimum germination index, timson germination index and final emergence percentage in T10 (10 days drought+80 °C). Moreover, and the final percent emergence efficiency results showed higher FEP in T1 control followed by T2 and T9; on the other hand, least FEP were observed in T6 and T10. Seed vigor index results showed higher SVI in control T1 plants however drought stress effect the SVI of wheat seedling by decreasing SVI percentage and lowest SVI were observed in T3 (10 days drought). Our results also demonstrated that the maximum water use efficiency reported and relative water content in T1 (control) while least WUE and RWC were observed in T10. Furthermore, total biomass results were also investigated and TBM showed higher content in T1 followed by T5 (5 days drought+4 °C+  $\alpha$ -Tocopherol) while lowest total biomass were investigated in T8 (10 days drought). Absolute growth rate reported maximum in T1 (5 days drought) while minimum in T3 (10 days drought).

#### EFFECT OF THERMOPRIMING AND $\alpha$ -TOCOPHEROL SPRAY UNDER DROUGHT STRESS ON PLANT PHYSIOLOGY OF *Triticum aestivum* L.

Different physiological, biochemical, and antioxidant activities of *Triticum* were investigated under drought stress with different thermopriming and  $\alpha$ -Tocopherol spray. Results in Figure 1 represented chlorophyll a and b content that are significantly affect by drought stress. Chlorophyll a result showed highest chlorophyll a value at  $p < 0.5$  in T1 (Control) followed by T5 (5 days drought + 4 °C+  $\alpha$ -Tocopherol) and T4 (treatment) compared with 5 days drought stress (T2), the treated plants showed significant difference and increase in chlorophyll content were observed, however least difference were observed between the treatment at 5 days drought stress treated plants. Similarly, maximum chlorophyll b content was detected in T1 (Control) while a significantly reduce in chlorophyll b content were observed in T3 and T10 (10 days drought+ 80 °C). Compared with 10 days drought stress (T3) there seem a significant difference in 5 and 10 days  $\alpha$ -Tocopherol treated plants. At T10 the chlorophyll b content were severely affected that might be due to drought stress combine with high temperature (80 °C). Figure 2 resulted that highest chlorophyll a/b ratio at  $p < 0.5$  were detected in T10 (10 days drought+ 80 °C), T11 (10

days drought+80 °C+ $\alpha$ -Tocopherol), while a significant reduced level of chlorophyll ratio was investigated in T1 (Control) and T4 (5 days drought+ 4 °C) at  $p < 0.5$ . Furthermore, total chlorophyll a and b were investigated

and as show in Figure 3 that maximum total chlorophyll value at  $p > 0.5$  was reported in T1 (Control) and T5 (5 days drought + 4 °C+ Tocopherol) followed by T4 (treatment).

TABLE 2. Effect of thermopriming and  $\alpha$ -Tocopherol spray under induced drought stress of *Triticum aestivum* L.

Treatments (T1-T11)	SHMC (%)	RMC (%)	SMC (%)	LMC (%)	RSR (%)	LAI (%)	LAR (%)
T1	83±4 <sup>a</sup>	97±0.4 <sup>a</sup>	18±1.5 <sup>a</sup>	90±4 <sup>a</sup>	1.3±0.4 <sup>a</sup>	42±2 <sup>a</sup>	0.04±0.01 <sup>a</sup>
T2	77±7 <sup>a</sup>	96±0.7 <sup>a</sup>	17±1.5 <sup>ab</sup>	87±5 <sup>ab</sup>	1.2±0.4 <sup>ab</sup>	27±5 <sup>ab</sup>	0.09±0.03 <sup>a</sup>
T3	68±1 <sup>ab</sup>	96±1.8 <sup>a</sup>	17±1.0 <sup>abc</sup>	81±11 <sup>ab</sup>	2.1±0.2 <sup>ab</sup>	32±1 <sup>abc</sup>	0.11±0.07 <sup>ab</sup>
T4	58±8 <sup>ab</sup>	95±0.3 <sup>ab</sup>	16±1.0 <sup>abc</sup>	85±1 <sup>abc</sup>	1.7±0.2 <sup>ab</sup>	34±9 <sup>abc</sup>	0.11±0.02 <sup>abc</sup>
T5	68±3 <sup>abc</sup>	95±0.3 <sup>ab</sup>	16±1.0 <sup>abc</sup>	88±2 <sup>abc</sup>	1.1±1.1 <sup>ab</sup>	21±1 <sup>bc</sup>	0.02±0.02 <sup>bc</sup>
T6	24±9 <sup>bcd</sup>	96±0.6 <sup>ab</sup>	16±1.0 <sup>abc</sup>	78±4 <sup>abcd</sup>	1.1±0.5 <sup>b</sup>	15±2 <sup>bc</sup>	0.07±0.02 <sup>c</sup>
T7	43±9 <sup>bcd</sup>	95±1.1 <sup>ab</sup>	17±1.0 <sup>bc</sup>	74±1 <sup>bcd</sup>	1.1±0.6 <sup>b</sup>	14±2 <sup>bc</sup>	0.02±0.02 <sup>c</sup>
T8	37±9 <sup>bcd</sup>	96±1.1 <sup>ab</sup>	17±1.1 <sup>c</sup>	63±6 <sup>cde</sup>	1.2±0.2 <sup>b</sup>	20±7 <sup>bc</sup>	0.04±0.01 <sup>c</sup>
T9	44±9 <sup>cd</sup>	96±0.8 <sup>ab</sup>	16±0.5 <sup>c</sup>	73±10 <sup>cde</sup>	1.2±0.3 <sup>b</sup>	22±2 <sup>bc</sup>	0.04±0.03 <sup>c</sup>
T10	35±7 <sup>cd</sup>	95±0.5 <sup>ab</sup>	18±1.0 <sup>c</sup>	65±13 <sup>de</sup>	1.1±0.1 <sup>b</sup>	18±3 <sup>c</sup>	0.03±0.03 <sup>c</sup>
T11	42±5 <sup>c</sup>	95±0.9 <sup>ab</sup>	16±1.0 <sup>c</sup>	78±6 <sup>c</sup>	1.1±0.1 <sup>b</sup>	17±5 <sup>c</sup>	0.03±0.01 <sup>c</sup>

Moisture content of shoot (SHMC), root (RMC), soil (SMC) and leaf (LMC) along with root shoot ratio (RSR), leaf area index (LAI) and leaf area ratio (LAR). Each data point is the mean of triplicated data with  $\pm$  SE. Different letters in columns indicate significant differences at  $P < 0.05$  based on DMRT

TABLE 3. Effect of thermopriming and  $\alpha$ -Tocopherol spray under drought stress on agronomic characteristics of *Triticum aestivum* L.

Treatment (T1-T11)	GI (%)	SVI (%)	TGI (%)	FEP (%)	RWC (%)	WUE (%)	TBM (%)	AGR (%)
T1	205±1 <sup>a</sup>	2057±12 <sup>a</sup>	5.8±0.3 <sup>a</sup>	82±2 <sup>a</sup>	97±2 <sup>a</sup>	29±7 <sup>a</sup>	588±80 <sup>a</sup>	0.26±0 <sup>a</sup>
T2	176±3 <sup>b</sup>	1786±14 <sup>ab</sup>	4.7±0.1 <sup>b</sup>	68±4 <sup>ab</sup>	96±1 <sup>a</sup>	20±5 <sup>b</sup>	253±76 <sup>ab</sup>	0.20±0 <sup>ab</sup>
T3	187±5 <sup>b</sup>	1240±25 <sup>c</sup>	5.0±0.1 <sup>b</sup>	65±2 <sup>bc</sup>	96±2 <sup>a</sup>	16±1 <sup>bc</sup>	239±18 <sup>ab</sup>	0.15±0 <sup>ab</sup>
T4	172±6 <sup>b</sup>	1768±30 <sup>bcd</sup>	4.5±0.1 <sup>b</sup>	64±0 <sup>bc</sup>	97±1 <sup>a</sup>	12±2 <sup>bcd</sup>	248±32 <sup>ab</sup>	0.16±0 <sup>ab</sup>
T5	184±1 <sup>b</sup>	2094±79 <sup>bcd</sup>	4.8±0.5 <sup>b</sup>	61±1 <sup>bc</sup>	97±1 <sup>a</sup>	20±9 <sup>bcd</sup>	581±32 <sup>a</sup>	0.18±0 <sup>ab</sup>
T6	178±8 <sup>b</sup>	1574±18 <sup>bcd</sup>	4.6±0.3 <sup>b</sup>	58±6 <sup>bc</sup>	95±1 <sup>a</sup>	12±6 <sup>bcd</sup>	464±19 <sup>ab</sup>	0.17±0 <sup>ab</sup>
T7	176±2 <sup>b</sup>	1666±95 <sup>cd</sup>	4.6±0.1 <sup>b</sup>	62±2 <sup>bc</sup>	90±2 <sup>b</sup>	12±5 <sup>cd</sup>	436±15 <sup>ab</sup>	0.17±0 <sup>ab</sup>
T8	176±4 <sup>b</sup>	1507±22 <sup>d</sup>	4.7±0.1 <sup>b</sup>	65±2 <sup>bc</sup>	91±1 <sup>b</sup>	11±2 <sup>c</sup>	373±90 <sup>b</sup>	0.16±0 <sup>ab</sup>
T9	181±1 <sup>b</sup>	1738±21 <sup>d</sup>	5.0±0.6 <sup>b</sup>	72±1 <sup>bc</sup>	92±1 <sup>b</sup>	10±1 <sup>c</sup>	375±72 <sup>b</sup>	0.17±0 <sup>b</sup>
T10	171±1 <sup>b</sup>	1689±44 <sup>d</sup>	4.4±0.8 <sup>b</sup>	58±1 <sup>c</sup>	91±2 <sup>b</sup>	9.1±0 <sup>c</sup>	415±28 <sup>b</sup>	0.18±0 <sup>b</sup>
T11	175±5 <sup>b</sup>	1819±22 <sup>d</sup>	4.5±0.2 <sup>b</sup>	64±2 <sup>c</sup>	92±1 <sup>b</sup>	9.6±0 <sup>c</sup>	437±26 <sup>b</sup>	0.19±0 <sup>b</sup>

Germination index (GI), seed vigor index (SVI), Timson germination index (TGI), final emergence percentage (FEP), relative water content (RWC), water use efficiency (WUE), total biomass (TBM) and absolute growth rate (AGR). Each data point is the mean of triplicated data with  $\pm$  SE. Different letters in columns indicate significant differences at  $P < 0.05$  based on DMRT

Total chlorophyll a and b were significantly reduced at 5 and 10 days of drought stress compared with control T1 plants. In addition, total carotenoid content was also investigated that showed no significant difference between control plants, drought stresses plants (5 and 10 days) and drought stress Tocopherol treated plants. Due to decrease in chlorophyll content under drought stress, we have investigated total sugar content of wheat plants to check drought stress effect and Tocopherol. Figure 4 concealed that maximum sugar value at  $p < 0.5$  was reported in T1 (Control), T5 and T7. Drought stress significantly decrease total sugar content compared with 5 day (T2) and 10 days (T3), however, compared with T2 and T3; Tocopherol treated plants showed significant difference in wheat plants. Among 5 days drought stress Tocopherol treated plants, non-significant difference was observed (T4-T7), however, a significant difference in sugar content were observed among treatments in T8-T11 Tocopherol plants at 10 days drought stress. This mean that high drought stress causes a decrease in sugar but thermopriming and  $\alpha$ -Tocopherol spray enhance sugar level to maintain osmo protective mechanism in plant under severe condition.

Furthermore, different antioxidant activities were investigated to observe the effect of thermopriming and  $\alpha$ -Tocopherol spray on *Triticum aestivum* L. under

drought stress. Drought stress severely effect total protein content as shown in Figure 5. Higher protein value at  $p < 0.5$  was reported in T1 (Control) and T5 (5 days drought+4 °C+ $\alpha$ -Tocopherol), while a decrease in total protein content were observed in T2 and T3. though significant ( $p < 0.5$ ) high proline content has been reported in T1 (Control) while non-significant in T2 (5 days drought), T3 (10 days drought), T4 (5 days drought+ 4 °C), T6 (5 days drought+80 °C), T7 (5 days drought+80 °C), T8 (10 days drought+4 °C), and T11 (10 days drought +80 °C+ $\alpha$ -Tocopherol) beside with significantly reduced protein and proline content in T10 (10 days drought+80 °C) has been reported which notify the degradation of proteins into amino acid at high drought stress. There seems a significant difference in total protein content among treatment T4-T7 and T8-T11 and increased in total protein content were observed in  $\alpha$ -Tocopherol treated wheat plants under drought stress. Proline content showed difference fluctuation and a significant difference were observed in control T1 and drought stressed plant T2 and T3, while least difference was investigated among treatments. Further POD and SOD were investigated and that showed a significant increase in SOD and POD content in 10 days treated plants compared with control T1 plants. Overall, there seem a significant difference compared with control plants

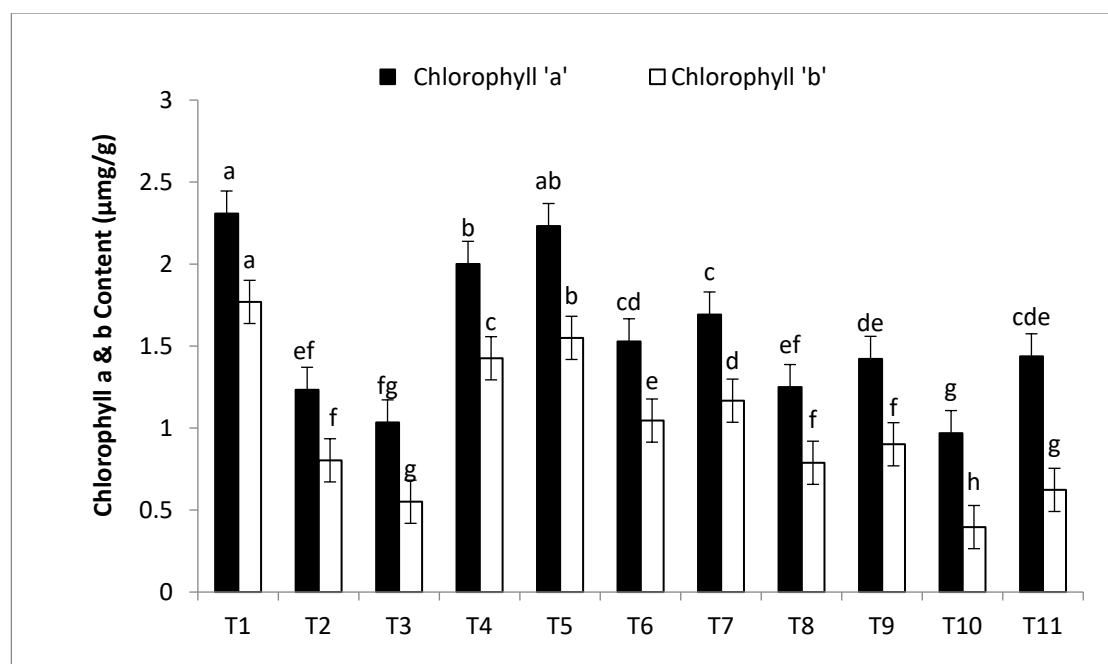


FIGURE 1. Effect of thermopriming and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on total chlorophyll a/b content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences indicate significant differences at  $P < 0.05$  based on DMRT

however least difference were observed with in 5 and 10 days treated plants (Figure 6).

Additionally, catalase content was quantified to observe the effect of thermopriming and  $\alpha$ -Tocopherol spray on *Triticum aestivum* L. under drought stress Figure 7. Catalase results showed that higher catalase content was detected in 10 days  $\alpha$ -Tocopherol treated plants

except T1. On the other hand, a significant decrease in catalase content were observed in T5-T7 compared with control and other treatments. Results clarified that stress condition initiate plant antioxidant mechanism with great synthesis of antioxidant enzymes for consumption of reactive oxygen species that damage plant cellular structures and function.

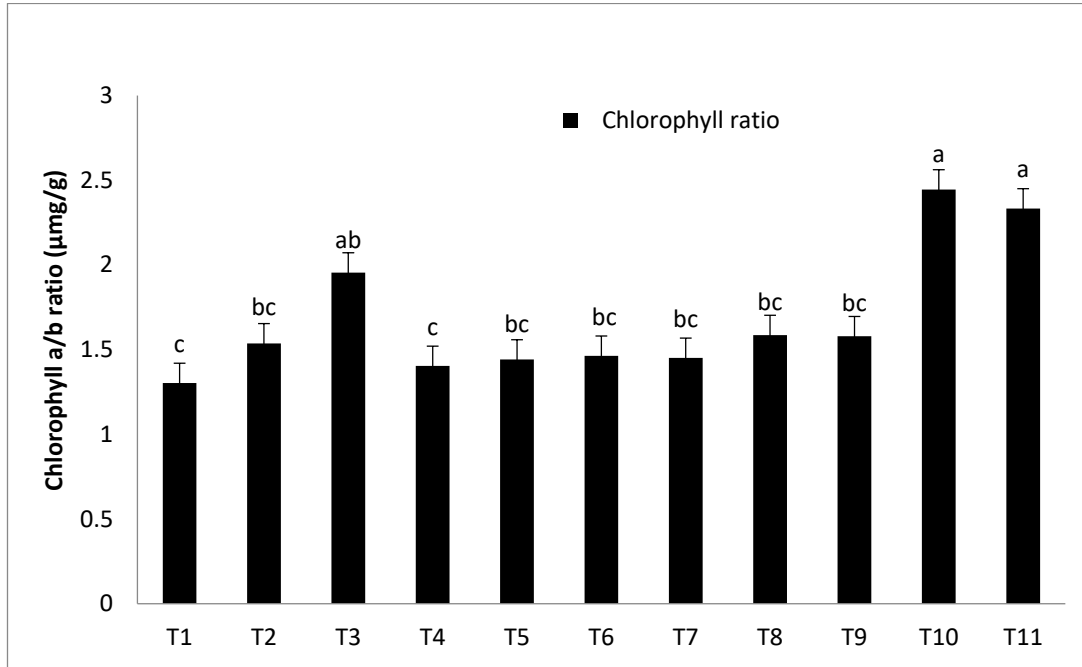


FIGURE 2. Effect of thermopriming and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on chlorophyll a & b ratio ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences indicate significant differences at  $P < 0.05$  based on DMRT

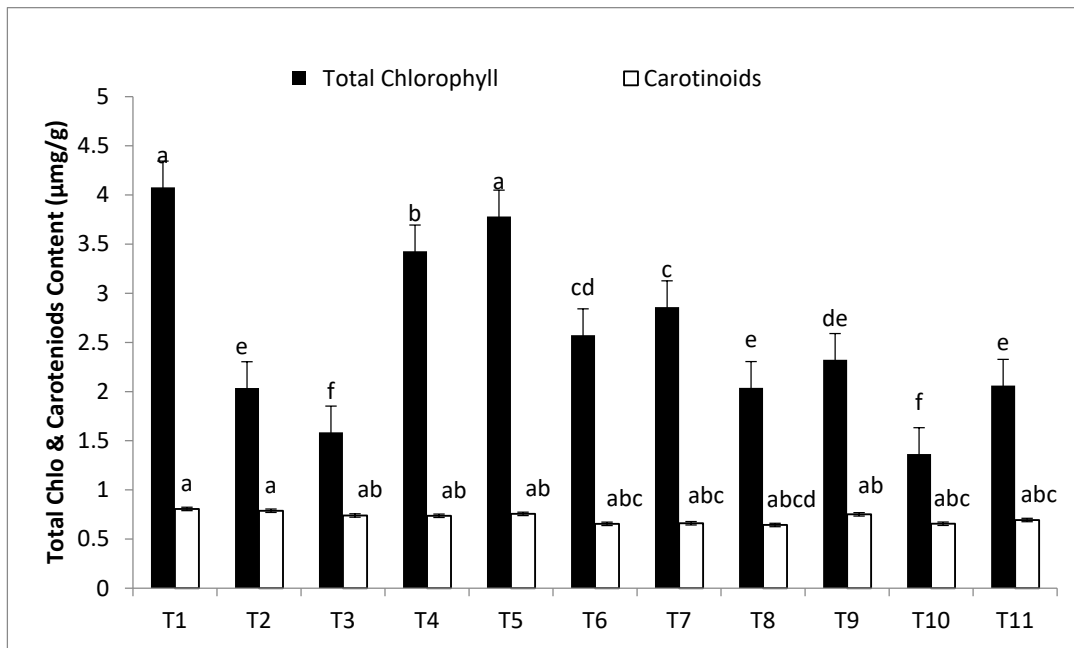


FIGURE 3. Effect of thermopriming and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on total chlorophyll and carotenoid content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences indicate significant differences at  $P < 0.05$  based on DMRT



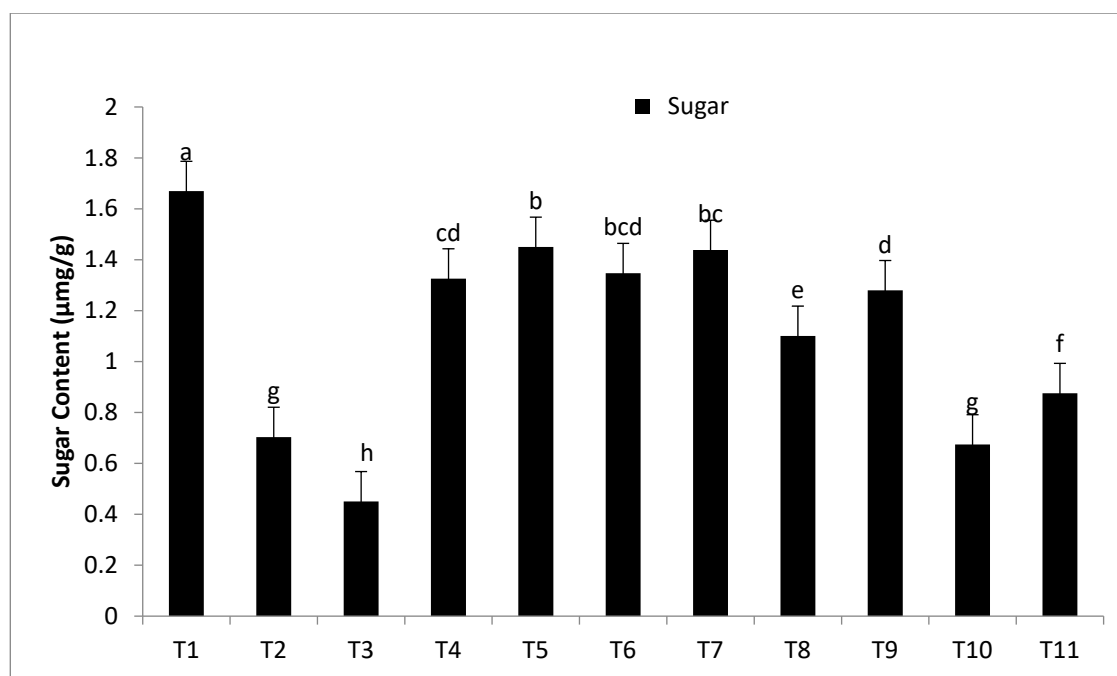


FIGURE 4. Effect of thermoprimering and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on sugar content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences at  $P < 0.05$  based on DMRT

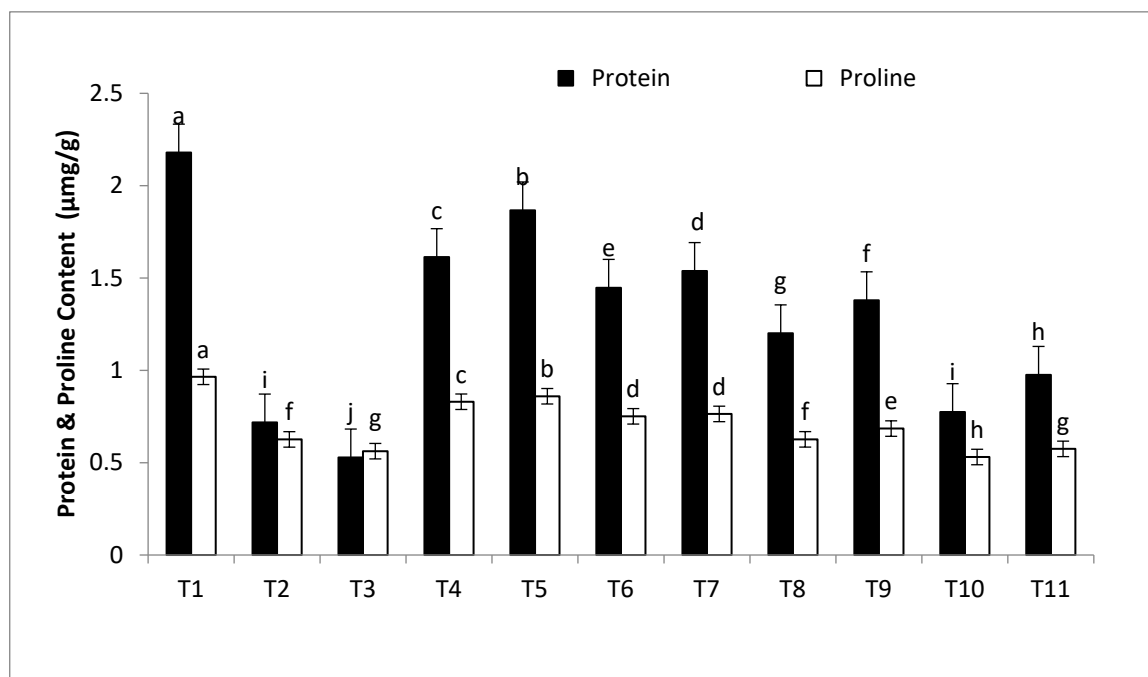


FIGURE 5. Effect of thermoprimering and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on protein and proline content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences at  $P < 0.05$  based on DMRT

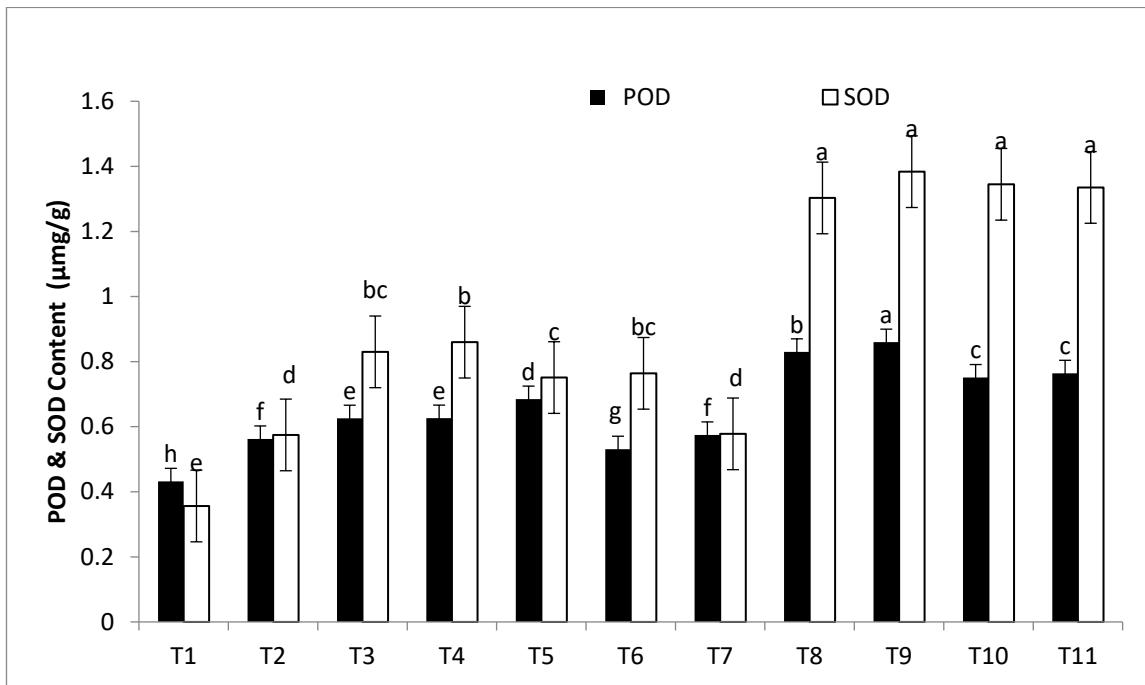


FIGURE 6. Effect of thermoprimering and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on POD and SOD content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences indicate significant differences at  $P < 0.05$  based on DMRT

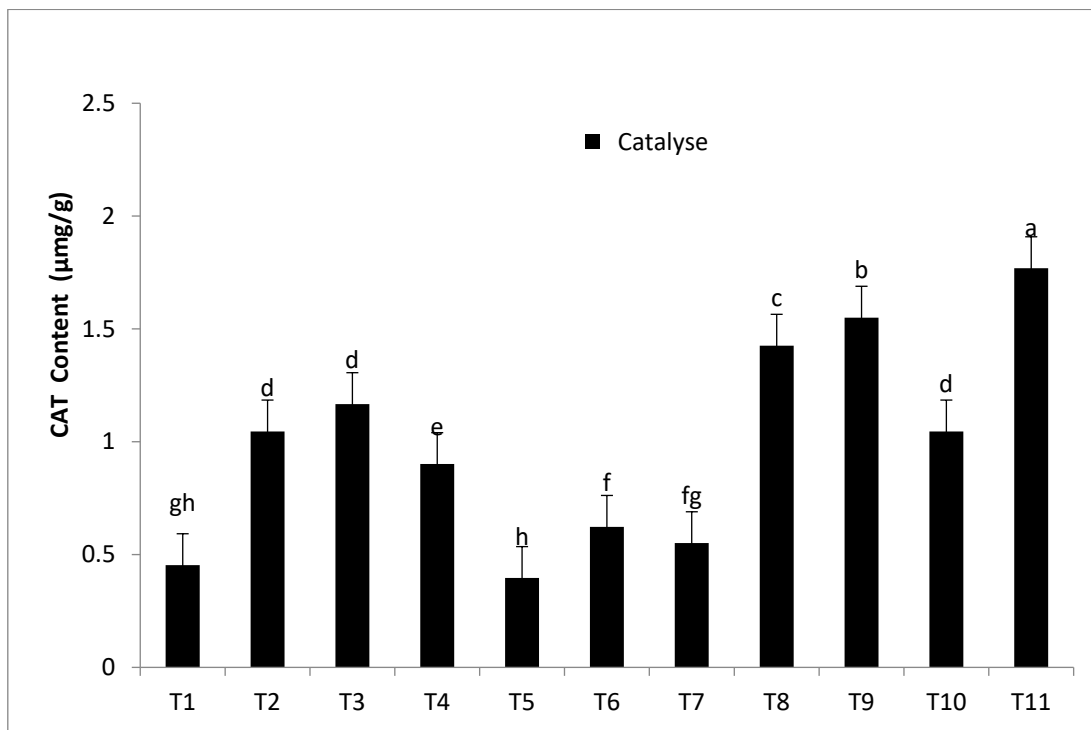


FIGURE 7. Effect of thermoprimering and  $\alpha$ -Tocopherol spray in wheat under induced drought stress on CAT content ( $\mu\text{mg/g}$ ). Each data point is the mean of triplicated data with  $\pm$  SE. Bar with different letters indicate significant differences indicate significant differences at  $P < 0.05$  based on DMRT

PRINCIPAL COMPONENT ANALYSIS OF THE BIOLOGICAL TRAITS

Analysis of Principal components results were based on 11 characters and recorded that PC1 account 66.841% of complete variance, which was significantly correlated

with all growth parameters including chlorophyll a & b, chlorophyll a/b ratio, CAT, TCC, SOD, CC, SPC, SSC, and TPC associated with growth parameters antioxidants. Similarly, PC2 described 13.840% of variance correlated with POD, hence mainly associated with antioxidant enzyme (Table 4 & Figure 8).

TABLE 4. Eigen values, variation explained cumulative variance, coefficient of determination of two principal components based on correlation matrix of biological components

Traits	Variance %			Component	
	Eigen values	Individual	Cumulative	PC1	PC2
Chl a ( $\mu\text{mg/g}$ )	7.353	66.849	066.849	0.908	0.221
Chl b ( $\mu\text{mg/g}$ )	1.523	13.841	080.690	0.981	0.125
TCC ( $\mu\text{mg/g}$ )	0.965	08.777	089.467	0.960	0.178
Chl a/b ratio ( $\mu\text{mg/g}$ )	0.673	06.116	095.583	0.677	0.006
CC ( $\mu\text{mg/g}$ )	0.240	02.179	097.761	0.253	-0.236
SSC( $\mu\text{mg/g}$ )	0.148	01.348	099.110	0.870	0.404
SPC ( $\mu\text{mg/g}$ )	0.048	00.438	099.548	0.914	0.366
TPC ( $\mu\text{mg/g}$ )	0.039	00.351	099.899	0.981	0.111
POD ( $\mu\text{mg/g}$ )	0.008	00.074	099.973	0.641	0.706
SOD ( $\mu\text{mg/g}$ )	0.003	00.027	100.000	0.733	0.649
CAT ( $\mu\text{mg/g}$ )	7.660	06.740	100.000	0.787	0.376

Chlorophyll a= Chl a, Chlorophyll b= Chl b, Total chlorophyll content= TCC, Chlorophyll a/b ratio= Chl a/b ratio, Carotenoid content= CC, Soluble sugar content =SSC, Soluble protein content= SPC, Total proline content= TPC, Peroxidase= POD, Superoxide dismutase= SOD, Catalase = CAT. Each data point is the mean of triplicated data with  $\pm$  SE. Different letters in columns indicate significant differences at  $P < 0.05$  based on DMRT

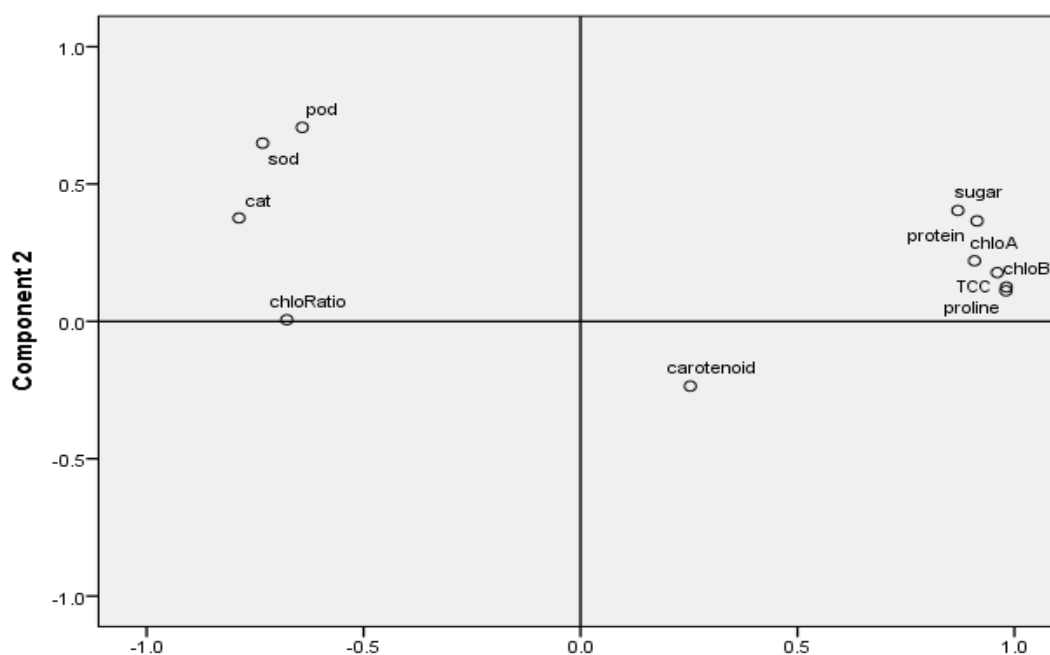


FIGURE 8. Loading plot of PC1 and PC2

REGRESSION AND CORRELATION ANALYSIS OF THE  
MEASURED TRAITS

Data about the analysis of correlation and regression (Tables 5-6) represented significant and positive relation between chl a of drought stress and chl a of primed seeds, while similar results were determined for chl a/b ratio, TPC, POD, and SOD. Correlation analysis showed

that estimated positive correlation between chlorophyll a & b, chl a/b ratio and TCC and negative with CC, SPC, and APOX. All correlations were significant at  $p = .01$  and  $0.05$ . Similarly, a positive correlation was observed between SSC and chl a/b ratio; TPC and chlorophyll b and chl a/b ratio; POD with chlorophyll a and TCC, SOD positively correlated with POD. APOX evaluated a positive correlation only with CC.

TABLE 5. Multicorrelation analysis of physiological and biochemical attributes

Traits ( $\mu\text{mg/g}$ )	Chl a ( $\mu\text{mg/g}$ )	Chl b ( $\mu\text{mg/g}$ )	TCC ( $\mu\text{mg/g}$ )	Chl a/b ratio ( $\mu\text{mg/g}$ )	CC ( $\mu\text{mg/g}$ )	SSC ( $\mu\text{mg/g}$ )	SPC ( $\mu\text{mg/g}$ )	TPC ( $\mu\text{mg/g}$ )	POD ( $\mu\text{mg/g}$ )	SOD ( $\mu\text{mg/g}$ )	CAT ( $\mu\text{mg/g}$ )
Chl a	1.0										
Chl b	0.93	1.0									
TCC	0.984	0.981	1.0								
Chl a/b ratio	0.404	0.694	0.554	1.0							
CC	0.24	0.26	0.254	0.143	1.0						
SSC	0.826	0.875	0.865	0.622	0.11	1.0					
SPC	0.895	0.925	0.926	0.575	0.119	0.964	1.0				
TPC	0.913	0.976	0.96	0.651	0.177	0.881	0.944	1.0			
POD	0.436	0.53	0.49	0.382	0.213	0.296	0.345	0.561	1.0		
SOD	0.525	0.638	0.59	0.522	0.265	0.037	0.425	0.649	0.922	1.0	
CAT	0.613	0.691	0.662	0.449	0.001	0.549	0.609	0.727	0.733	0.797	1.0

Chlorophyll a= Chl a, Chlorophyll b= Chl b, Total chlorophyll content= TCC, Chlorophyll a/b ratio= Chl a/b ratio, Carotenoid content= CC, Soluble sugar content =SSC, Soluble protein content= SPC, Total proline content= TPC, Peroxidase= POD, Superoxide dismutase= SOD, Catalase = CAT

TABLE 6. Regression and correlation analysis between physiological and biochemical attributes

Regression and correlation analysis between drought and wheat										
Chl a ( $\mu\text{mg/g}$ )	Chl b ( $\mu\text{mg/g}$ )	TCC ( $\mu\text{mg/g}$ )	Chl a/b ratio ( $\mu\text{mg/g}$ )	CC ( $\mu\text{mg/g}$ )	SSC ( $\mu\text{mg/g}$ )	SPC ( $\mu\text{mg/g}$ )	TPC ( $\mu\text{mg/g}$ )	POD ( $\mu\text{mg/g}$ )	SOD ( $\mu\text{mg/g}$ )	CAT ( $\mu\text{mg/g}$ )
0.766	0.847	0.817	0.614	0.348	0.74	0.756	0.865	0.853	0.887	0.817
0.586	0.717	0.667	0.377	0.12	0.547	0.571	0.748	0.727	0.786	0.667
Regression and correlation analysis between priming and wheat										
Chl a ( $\mu\text{mg/g}$ )	Chl b ( $\mu\text{mg/g}$ )	TCC ( $\mu\text{mg/g}$ )	Chl a/b ratio ( $\mu\text{mg/g}$ )	CC ( $\mu\text{mg/g}$ )	SSC ( $\mu\text{mg/g}$ )	SPC ( $\mu\text{mg/g}$ )	TPC ( $\mu\text{mg/g}$ )	POD ( $\mu\text{mg/g}$ )	SOD ( $\mu\text{mg/g}$ )	CAT ( $\mu\text{mg/g}$ )
0.756	0.897	0.897	0.614	0.348	0.79	0.756	0.885	0.803	0.807	0.897
0.586	0.717	0.897	0.377	0.12	0.547	0.571	0.748	0.727	0.786	0.667

Chlorophyll a= Chl a, Chlorophyll b= Chl b, Total chlorophyll content= TCC, Chlorophyll a/b ratio= Chl a/b ratio, Carotenoid content= CC, Soluble sugar content =SSC, Soluble protein content= SPC, Total proline content= TPC, Peroxidase= POD, Superoxide dismutase= SOD, Catalase = C

## DISCUSSION

Temperature, the most affective factor for plant response both morphologically and physiologically towards stress condition. Result concluded that thermoprimered seeds with 4 °C response significantly positive while 80 °C have evaluated negative effects on most of the agronomical characters. Table 2 parameters including root, shoot, leaf moisture content analysis confirmed that the maximum growth occur in control (T1) leaf area index (T6), leaf area ratio and root shoot ratio in T3. Similarly, germination index, timson germination index and final emergence percentage reported in T1 (control) whereas; the minimum in T11 (10 days drought+80 °C+Tocopherol) and percent emergence efficiency in T8 (10 days drought+ 4 °C). Table 3 demonstrated that the maximum water use efficiency reported in T1 (control), relative water content in T4 (5 days drought+4 °C), root shoot ratio in T3 (10 days drought) and total biomass in T5 (5 days drought+4 °C+ $\alpha$ -Tocopherol) whereas; the minimum water use efficiency reported in T11 (10 days drought+80 °C+ $\alpha$ -Tocopherol) relative water content in T7 (5 days drought+80 °C), root shoot ratio in T11 (10 days drought+80 °C+ $\alpha$ -Tocopherol) and total biomass in T3 (10 days drought). Similarly, the table portray the maximum absolute growth rate reported in T2 (5 days drought), leaf area index in T6 (5 days drought+80 °C), leaf area ratio. Jaleel et al. (2007) noticed plant under drought stress in combination to other factors changes in growth rate are of vital importance to drought tolerance in which the effect of drought on plant diminished growth due to loss of turgidity and incomplete mitosis. Saleem (2003) experimented on durum and bread wheat genotypes under induced drought stress in which the terminal drought caused a significant reduction in biomass, growth rate, spike yield, productivity, and grain yield at  $p < 0.001$ . Chen et al. (2012) proposed his research on the global impact of drought on net growth and productivity, which were examined during 1997-2009 through satellite his research was similar to that found by Zhao et al. (2007) from which he concluded a significant decline in productivity and growth attributes during and after the drought period which show no strong correlation between drought and net primary productivity.

Present study was based on the effect of induced drought stress on physiological and biochemical status of *Triticum aestivum* L. which is a global issue due to upcoming climate change and rise in temperature with its adverse effects on crop productivity and economic that cause an instability in economy of Pakistan being

developing agricultural region. Results concluded that thermo-primed seeds with 4 °C significantly positive and 80 °C have significantly negative effects on most of the physiological attributes. Maximum chlorophyll a & b value in T1 (Control) and T5 (5 days drought+4 °C+ $\alpha$ -Tocopherol), carotenoid content under treatment T1 (Control) followed by T5 (5 days drought+4 °C+ $\alpha$ -Tocopherol) beside with significantly reduced in given parameters under treatment T10 (10 days drought+80 °C). According to the findings of Jaleel et al. (2007), who studied chlorophyll composition in drought-stressed plants as well as other factors, changes in photosynthetic pigments are critical for drought tolerance, and the effect of drought on chlorophyll is controlled by carotenoids, which are specific for stress in plants. Our findings support the findings of Ramanjulu et al. (2000), who found that drought stress increases the level of carotenoid content in leaves when compared to plants grown in well water.

A significant decrease in the sugar and proline content reported under induced maximum drought stress and high temperature conditions in wheat with least significant increase under low drought stress and temperature reported that the specie recover from such low stress condition by synthesizing these osmolytes but long term exposure to drought stress (10 days) supposed to be caused by the future climate change will not only be controlled by the application of growth regulators but it also need biotechnological and breeding efforts. Similar results are explained by Hamada (2000) and Yadav et al. (2005) who evaluated increased sugar and proline accumulation in *Gossypium hirsutum* under that water stress. The breakdown of protein during drought stress, which deactivates the process of its production, was described as a little change in soluble protein concentration under treatment T3 (10 days drought). Sharma and Dubey (2005) found a significant decrease in the concentration of soluble protein in the roots and shoots of mild and high drought stressed seedlings, with the exception of the roots of Malviya-36 seedlings grown for 20 days, which showed no significant decline in soluble protein concentrations when compared to controls in both rice cultivars.

Oxidative stress caused by the degradation of biological components in the cell blocks both growth and development by reducing cell division, cell maturation, cell structure and therefore protection from such oxidative stress is of vital importance for germination of seed. Figure 6 showing that minimum POD and SOD values were reported in T1 (Control) beside with significantly

maximum POD content in T8 (10 days drought+4 °C) and SOD T9 (10 days drought+4 °C). The result showed that an increase in antioxidant enzymes participating in the scavenging of ROS under drought stress. Abedi and Pakniyat (2010) demonstrated that drought stress significantly increases the antioxidant enzymes of plant leaves particularly POD in comparison with the control condition. Drought stress increases leaf SOD content compared to well-watered plants while lowering root SOD enzymes under water deficit, according to Reddy et al. (2004). Antioxidant enzymes are required for the manufacture of different osmoprotectants in stressed plants.

#### CONCLUSION

Rise in temperature by global warming is an alarming dilemma that has been reduced most of our agricultural crops by reducing water quantity due to high evaporation, transpiration, and poor irrigation system. Present study evaluated that tocopherol spray along with thermoprimered treated seeds with chilling temperature (4 °C) has been significantly enhanced plant growth and development by activating both physiological and biochemical activities whereas; 80 °C cause reductions in most of agronomical and physiological attributes under treatment T10 (10 days drought+80 °C) and T11 (10 days drought+80°C+ $\alpha$ -Tocopherol). Conclusively, present species is tolerable to short term water deficit condition along with pre-sowing chilling temperature (4 °C) and growth regulator, such application of themoprimering at low temperature and foliar spray of growth regulator that is simple and cheap method will be helpful for our farmers and agriculturist to sustain our food scarcity during drought stress condition Pakistan.

#### ACKNOWLEDGEMENTS

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\*Corresponding author; email: sarahrazak@um.edu.my