Journal of Animal & Plant Sciences, 34(3): 2024, Page: 637-651
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.3.0750

EVALUATION OF BREAD WHEAT AND SYNTHETIC HEXAPLOID GENOTYPES UNDER DROUGHT CONDITION BASED ON PHYSIOLOGICAL, BIOCHEMICAL AND GENETIC INDICES

F. Alghabari[1*] and Z. H. Shah[1,2]

[1]Department of Arid Land Agriculture, King Abdulaziz University, Jaddah, Saudi Arabia.
[2]Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

[*]Corresponding author's email: fahadalghabari@hotmail.com

ABSTRACT

The present study intended to assess the physiochemical and genetic responses of ten different wheat (*Triticum aestivum* L.) genotypes including bread wheat and synthetic hexaploids (SHs) under drought and control conditions. The experiment was performed in two factorial arrangements with genotypes as one and treatments as other factor. The drought treatment was applied at pre-anthesis stage by withholding water for 10-15 days and continued till the appearance of the symptoms of drought. The data for biochemical contents, physiological traits and gene expression was conducted in randomly selected wheat samples at the onset of drought symptoms. The enzymatic activity, proline and glycine betaine showed significant increase in wheat genotypes due to drought treatment, with maximum increase in SHs lines followed by bread wheat genotypes Galaxy-2013, Aas-2011 and AARI-2011. Conversely, all SHs recorded less reduction in physiological traits as compared to bread wheat genotypes due to application of drought stress. Besides correlation, PCA and heatmap analysis proved significantly high impact of drought on the correlation and expression of physiological and biochemical traits as compared to control treatment. Furthermore, in all wheat genotypes, the expression of drought associated genes *TaDREB1A*, *TaGROS-A*, *TaERF3, TaCPK34, TaLEA3, TaHSFA1a, TaWRKY44, TaZFP34* and *TaEXPA2*changed complementary to the corresponding physiological and biochemical traits. The most significant change in expression of these genes with respect to corresponding physiological and biochemical traits under drought stress was recorded in SHs, Galaxy-2013, Aas-2011 and AARI-2011.The drought tolerant genotypes screened on the basis of physiochemical and genetic indices can further be used as an effective germplasm while devising a breeding program for drought tolerance adaptability optimization.

Keywords: Gene Expression; Antioxidant; Synthetic Hexaploids; Statistix8.1; Drought

INTRODUCTION

Wheat is an eminent crop whose yield is drastically reduced due to different constraints like drought, heat and soil salinity. About 70% area of wheat is under rain fed conditions where drought encounters in any way during growing season (Mahmood *et al.,* 2022). Drought causes a substantial loss in wheat yield upto 27.5% particularly at terminal stage (Zhang *et al.,* 2018).. In this perspective the choice of drought tolerant germplasm with tendency to counter stress is very important. Drought stress reveals its potential impact at cellular level by altering various homeostatic mechanisms due to production of reactive oxygen species (ROS) and inhibiting the antioxidant system (Alghabari *et al.,* 2021). Besides, abiotic stress such as drought impairs physiological processes such as stomatal conductivity (Gs), transpiration (Tr), chlorophyll activity and cell membrane stability (Alghabari *et al.,* 2021). Moreover, moisture stress perturbs photosynthetic activities by reducing chlorophyll and by damaging enzymes involved in chlorophyll synthesis (dos-Santos *et al.,* 2022). The drought stress creates the osmotic stress that disturbs plant water balance; therefore, plants produce various osmo-protectants like proline and glycine betaine (Wang *et al.,* 2010). In perspective, no one can deny the significance of proline owe to its significant role in nullifying the hazardous impacts of drought through initiating the antioxidant defense system involved in the scavenging of ROS (Pour-Aboughadareh *et al.,* 2019). Likewise glycine betaine is an important osmolyte having tendency to sustain plant growth and ensure plant survival due to its vital role in counteracting metabolic dysfunctions imposed by drought stress (Annunziata *et al.,* 2019; Ahmad *et al.,* 2022). Wheat is a potential system to unknot the mysteries of drought tolerance by elucidating the expression pattern of various genes involved in drought associated signaling pathways(Shah *et al.,* 2017; Alghabari *et al.,* 2021). For instance, over-expression of auxin regulated genes involved in organ size (*ARGOS*) increases drought tolerance in wheat and maize via regulation of ethylene dependent signaling pathways (Ahmed *et al.,* 2022). Dehydration responsive element binding (*DREB*) proteins are involved in functional regulation of some candidate genes imparting drought stress tolerance wheat by regulating osmotic homeostasis through various regulatory pathways(Niu*et al.,* 2012). On the other hand genes associated with late embryogenesis abundant (*LEA*) proteins are highly stress responsive that activate in wheat under water deficit conditions and trigger various processes for imparting tolerance against stress (Sivamani *et al.,* 2000; Yu *et al.,* 2019). Likewise, heat shock factors (Hsf) exhibit central role in providing tolerance to drought and heat stress due to their role as osmo-protectant in osmotic homeostasis (Shah *et al.,* 2022). Drought stress initiate oxidative stress within plant cellular system through the production of reactive oxygen species (ROS), whereas genes of *WRKY* family enhance tolerance to drought stress through regulation of antioxidant defense system as reported in model plants *Arabidopsis* and rice(Niu *et al.,* 2012; Nan*et al.,* 2020. However, limited research has been conducted in wheat in this perspective due to large genome size. Besides, genes from expansin (*EXPA*) family such as *TaEXPA2* has tendency to adjust the architect of plant cell wall and imparts tolerance when over expressed and vice versa(Yang *et al.,* 2020). Calcium dependent kinases (*CPK or CDPK*) play important role in drought tolerance by mediating calcium dependent pathways under drought stress and have tendency to transmit calcium signals directly that regulate variety of cellular responses (Li *et al.,* 2020). Zinc finger proteins (*ZFPs*) play crucial role in drought tolerance as they regulate the expression of multiple genes involved in ROS scavenging (Zang *et al.,* 2016). In this context the elucidation of expression pattern of *TaZFP13D* is important to validate tolerance against drought inducible stress (Bouard and Houde*,* 2022). In addition ethylene responsive factor (*ERF*) is one of the diverse families of transcription factors involved in regulation of various molecular networks controlling the expression of various drought and heat stress responsive genes (Najafi *et al.,* 2018). Therefore evaluation of expression pattern of *ERF* genes in contrasting wheat genotypes is highly important to tag genotypes as tolerant or susceptible (Magar*et al.,* 2022).. Although some studies from recent past have been conducted on wheat to elucidate the impacts of drought stress on physiological, biochemical and genetic traits of wheat (Alghabari *et al.,* 2021; Shah *et al.,* 2022) however present study intended to unify the role of drought associated genes with their respective physiological and biochemical traits. Unification of physiological, biochemical and genetic traits will provide in depth knowledge of drought tolerant mechanisms in wheat. Besides, to our knowledge this will be the first study to comparatively evaluate the drought responses of diverse synthetic hexaploid (SH) wheat and bread wheat cultivars.

MATERIALS AND METHODS

In present study drought tolerant bread wheat and synthetic hexaploid (SH) wheat genotypes (Table 1) collected from National Agricultural Research Center (NARC) Islamabad, Pakistan were evaluated for physiological, biochemical and genetic studies in a pot experiment. The SH wheat genotypes were selected due to their enriched diversity and high resistance against abiotic stresses (Li *et al.,* 2018), while the elite bread wheat cultivars were selected based upon their suitability to rain fed areas (Din *et al.,* 2020). The experiment was conducted in three replicates at research area of Department of Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia using two factorial arrangements in randomized complete block design (RCBD), with genotypes as one and treatments as other factor.

Table 1. List of drought tolerant wheat genotypes used for genes expression analysis.

Genotypes	Pedigree
NARC-2009	INQALAB 91*2/TUKURU
Chakwal-86	FORLANI/ACC//ANA or Fln/ACS//ANA
AARI-2011	SH-88/90A-204//MH97
Iqbal-2000	BURGUS/SORT 12–13//KAL/BB/3/PAK 81
Aas-2011	PRL/PASTOR//2236
Galaxy-2013	Pb96/Watan/MH-97
SH1	TC870344/GU1//TEMPORALERA M 87/AGR/3/WBLL1
SH2	GPO8 KAZAKSTAN 6 WM98-99/4/KAUZ//ALTAR 84/AOS/3/ KAUZ/5/ /KAUZ//ALTAR 84/ AOS/3/KAUZ
SH3	CROC_1/AE.SQUAROSSA (205)//BORL95/3/KENNEDY
SH4	PAM94/3/ALTAR 84/AEGILOPS SQUAROSA(TAUS)//OPATA/PASTOR

Crop Growth and Application of Treatments: For experiment, the 2L pots were filled with soil containing clay and compost in 4:1 along with 10 g of N, K, P (10:5:5) fertilizer. Each pot was sown with five seeds. For each treatment five pots were used, while each treatment was replicated three times. The weeding and hoeing were performed as per requirement during crop growing period. Plants were exposed to drought and control conditions to obtain the samples for biochemical, physiological and genetic analysis. Thinning was performed at seedling stage and each pot was left with three plants. During normal condition, the pots set exposed to control condition were placed in open environment and the pots set exposed to drought were placed under shelter. The drought stress was applied during pre-anthesis stage. Plants of control set were watered till optimum level as per requirement. Furthermore, the drought stress was applied by withdrawing irrigation for 10-15 days until the appearance of drought symptoms such as wilting or conditional leaf rolling. Afterward drought stress was withheld and plants were watered normally. The experiment was conducted in glasshouse and the experimental conditions were optimized at 60% humidity, 25/15±2 ^{0}C day/night temperature with a photo period of 10 hour. The experiment was started at the mid of November,2022, and kept continued till the end of March, 2023. The 70 to 80 days plants were exposed to stress, and the data were taken on the onset of stress symptoms.

Measurement of Biochemical Contents

Activity of Antioxidant Enzymes: The enzymatic activities were recorded according to the method opted by Shah *et al.* (2022). For this objective1g of frozen leaf of wheat genotypes was crushed in 1 mL ice-cold Tris-HCl buffer with concentration 0.1M. Subsequently the mixture was optimized at 4^{0}C and subjected to centrifugation for 15 mins at 20000. Furthermore, supernatant was separated to measure the enzymatic activities. SOD-kit (Sigma-Aldrich, USA) was used to determine the activity of superoxide dismutase (SOD) in accordance to provided instructions of manufacturer. Besides, flourimetric POD-kit (Cell Biolabs Inc, USA) was used to assess the activity of peroxidase (POD) following the given manufacturer's method. Furthermore, plant catalase-kit (Sigma-Aldrich, USA) was used to record the activity of catalase by following the protocol provided by manufacturer. The data for antioxidant enzymes was collected on weekly basis since after inception of stress.

Proline and Glycine Betaine: UV-Vis spectrophotometer **(**DeNovix, USA**)** was used to estimate proline content on the basis of its reactivity with ninhydrin. Furthermore, glycine betaine (GB) was quantified with the HPLC(Shimadzu Corp, Japan) in accordance to the method described by *Ma et al.,* (2007).

Physiological Analysis: Among physiological traits the total chlorophyll content was recorded with the help of SPAD-502 (Spectrum Technologies, UK) apparatus while stomatal conductivity (Gs), rate of photosynthesis (Pn) and rate of transpiration (Tr) were measured from flag leaf using IRGA-apparatus (ADC Bioscientific, UK). On the other hand cell membrane stability percentage (CMSP) was calculated by following formula CMSP= [(1-(A1/A2))/(1-(B1/B2)) } x 100, whereas A1=Conductivity of wheat stress treated samples before autoclaving, A2= Conductivity of wheat stress treated samples after autoclaving; B1=Conductivity of wheat control samples before autoclaving, B2= Conductivity of wheat control samples after autoclaving.

Gene Expression Analysis: For expression analysis of drought associated genes such as *TaHSFA1a,TaDREB1A, TaZFP13D,TaGROS-A, TaERF3, TaCPK34, TaLEA3,TaWRKY44, and TaEXPA2*, RNA was extracted following the protocol of Li *et al.,* (2019) from randomly selected samples of wheat genotypes using QiagenRNeasy kit (Qiagen, German Town, USA). Furthermore, Ahmed *et al.,* (2022) procedure was opted for the synthesis of cDNA by using 2 µg of RNA according to the instructions of manufacturer. Furthermore, qRT-PCR analysis was performed and normalization of genes expression was done by using TaActin1-expressing gene. The list of primers along with their forward and reverse primers is given in Table 2.

Table 2. List of primers used in gene expression analysis.

Primer	Sequence
TaDREB1A	CGGAACCACTCCCTCCATCTC (F)CGGTTGCCCCATTAGACGTCA (R)
TaLEA3	AGTACACCAAGGAGTCCGC (F) CATTGTCACCACCCATGCC (R)
TaARGOS-A	GATCATCTTCCACCACCATCTC (F) GCACCTACATGGGTGTTCTT (R)
TaWRKY44	GCCCCCCTTCGCTCTTCTC (F) CAGCACACCAGAAATGGGCTAAT (R)
TaHSFA1a	AGCATTCCAGGATTCCCAGAT (F) CCAGGCATTGCGAAATTCTC (R)
TaEXPA2	TCAGGTTCACCATCAACGGG(F) TCTCACACACCACTCCATGC (R)
TaERF3	CTTCTCCTGCGGCTGGCTCC (F) CTCGGCGGTGTCGTAGGTGC (R)
TaCPK34	GCCAAGGACCTCGTTAGAAAGA (F) TCTTGAACATCTCCTTGAGCCC (R)
TaZFP13D	AGTTAATGACGAATAGGTTCGC (F)CGGGCCAGCCACCGCCACCACC (R)

Statistical Analysis: Statistix ver. 8.1 (McGraw-Hill, 2008) was used for analysis of variance (ANOVA) at a 5 % probability level. Moreover, correlation, principal component analysis (PCA) and heatmap analysis were conducted using RStudio version 1.1.456 (RStudio Team, 2020). The R packages "factoextra" and "FactoMineR" were used for PCA. The heatmap cluster analysis was performed by R packages "pheatmap" and complex Heatmap, while the Pearson's correlation was performed using R packages "GGally" and "ggplot2".

RESULTS

Biochemical Contents: All genotypes illustrated statistically significant ($p \leq 0.05$) variation in the catalytic activity of antioxidant enzymes (SOD, CAT and POD) and in the concentrations of proline and glycine betaine (GB) under both control and drought conditions (Table 3). Activities of antioxidant enzymes depicted more significant variation owe to drought as compared to control treatment (Table 3). Besides, the SH wheat lines showed more dramatic increase in the activities of antioxidant enzymes (SOD, POD and CAT) as compared to bread wheat cultivars due to drought stress (Table 3). On the other hand, among bread wheat cultivars Galaxy-2013 and Aas-2011 recorded significant rise in the activities of SOD, POD and CAT under drought stress as compared to other bread wheat cultivars (Table 3). All wheat genotypes manifested significant difference in proline and glycine betaine (GB) contents under drought as compared to control conditions (Table 3). Overall as compared to bread wheat cultivars, the synthetic hexaploid (SH) wheat lines revealed significantly high level and high difference in the concentrations of antioxidant enzymes, proline and glycine betaine (GB) both at drought and control conditions (Table 3).

Physiological Traits: The physiological attributes such as total chlorophyll (chl), photosynthesis (Pn), transpiration (Tr), stomatal conductance (Gs) and cell membrane stability percentage (CMSP) showed significant difference ($p \leq 0.05$) in all wheat genotypes under both control and drought conditions (Table 4).The drought stress made significant reduction in physiological traits in all wheat genotypes as compared to control conditions (Table 4). Besides, among synthetic hexaploid (SH) wheat lines the physiological traits depicted least variation traits under control and drought treatments as compared to bread wheat cultivars (Table 4). Moreover, among bread wheat cultivars Galaxy-2013 and Aas-2011 recorded less difference between physiological traits at control and drought conditions as compared to other bread wheat cultivars (Table 4). As a whole as compared to bread wheat cultivars, the synthetic hexaploid (SH) wheat lines revealed significantly high level and less difference in physiological activities at control and drought conditions (Table 4).

Correlation and Principal Component Analysis: The correlation analysis of physiological and biochemical traits illustrated varied extent of paired association among them. Overall the traits did not show significant association under control conditions; however they depicted more significant and strong paired association under drought condition (Figure 1). Among traits, photosynthesis (Pn) showed statistically significant correlation with stomatal conductance (Gs) and total chlorophyll content. Besides, all antioxidant enzymes including SOD, POD and POX showed significantly high paired association in positive direction among them and with glycine betaine (GB) and proline content. On the other hand cell membrane stability percentage (CMSP) demonstrated positive paired association with the SOD, POD,POX, Pn, Gs and total chlorophyll content. Conversely the transpiration rate (Tr) depicted significant association in negative direction with Pn and Gs as shown in Figure 3.

The PCA biplot revealed the differential impacts on the extent of expression and association of physiological and biochemical traits under control and drought condition both in bread wheat and synthetic hexaploid (SH) wheat cultivars (Figure 2). The varying orientation of eclipses in biplot under drought and control conditions confirmed the variation in association and expression of traits owe to changing environmental conditions. On the other hand PCA biplot for wheat genotypes under drought conditions showed the vectors representing physiological and biochemical traits in more proximity as compared to control conditions (Figure 3). Furthermore, under control conditions the bread wheat cultivars (Inqilab-2009, AARI-2011, Chakwal-86, Iqbal-200, Aas-2011, Galaxy-2013) responded differently as compared to synthetic hexaploid (SH) wheat genotypes in context of traits association as shown by their disperse distribution in PCA biplot (Figure 3). However, under saline conditions bread wheat genotypes were more closely spaced in one quadrant of PCA biplot, while synthetic hexaploid genotypes were more closely spaced in other quadrant of PCA biplot (Figure 3). This was a confirmation of distinct genetic makeup of bread wheat and synthetic hexaploid (SH) wheat genotypes.

Table 3. Effect of drought stress on antioxidant enzymes activities, proline and glycine betaine in different wheat genotypes.

Genotypes	Catalase Activity (Enzyme unit)		SOD Activity (Enzyme unit)		POD Activity (Enzyme unit)		Proline (μg g^{-1}FW)		Glycine betaine (μg g^{-1}FW)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
NARC-2009	6.3±0.25^{cf}	8.5±0.21^{f}	0.40±0.01^{gj}	0.60±0.02^{fgi}	23±0.61^{ac}d	31±0.53^{gj}	20±0.21^{bcefj}	28±0.12^{j}	60±0.41^{i}	146±0.42^{h}
Chakwal-86	6.7±0.28^{ce}	9.0±0.23^{e}	0.30±0.03^{ci}	0.55±0.03^{h}	22±0.56^{a}	30±0.51^{i}	20±0.13^{bcef}	25±0.13^{hi}	63±0.53^{c}	155±0.43d^{g}
AARI-2011	7.0±0.23^{a}	10.0±0.21d	0.35±0.02^{gh}	0.60±0.02^{fg}	21±0.45^{a}	32±0.43^{h}	21±0.22^{bce}	25±0.12^{h}	65±0.52^{ah}	160±0.52^{ef}
Inqlab-2000	6.0±0.24d	10.0±0.24d	0.37±0.03^{fg}	0.63±0.04^{f}	24±0.47^{ac}	31±0.45^{g}	22±0.33^{abc}d	29±0.14^{fg}	64±0.63^{g}	163±0.54^{ce}
Aas-2011	6.5±0.23^{c}	12.0±0.21^{c}	0.33±0.02^{cef}	0.75±0.03^{e}	23±0.66^{a}	34±0.61^{f}	23±0.32^{a}	30±0.13^{f}	63±0.52^{cf}	155±0.53d
Galaxy-2013	7.5±0.26^{b}	13.0±0.25^{b}	0.30±0.02^{ce}	0.70±0.03d	22±0.53^{a}	35±0.63^{e}	21±0.22^{bc}	28±0.13^{e}	58±0.52^{e}	160±0.43^{c}
SH1	7.0±0.22^{a}	15.0±0.23^{a}	0.25±0.03d	0.80±0.02^{b}	21±0.53^{a}	37±0.43d	22±0.23^{ab}	36±0.12^{bc}d	65±0.53^{a}	170±0.52^{b}
SH2	7.5±0.24^{b}	15.5±0.22^{a}	0.30±0.02^{c}	0.85±0.04^{c}	23±0.52^{a}	39±0.31^{c}	23±0.12^{a}	35±0.14^{c}	63±0.52^{c}	180±0.54^{a}
SH3	7.0±0.23^{a}	16.0±0.22^{b}	0.21±0.01^{ab}	0.80±0.03^{b}	20±0.50^{b}	38±0.34^{b}	24±0.11^{a}	37±0.13^{ab}	66±0.51^{b}	180±0.53^{a}
SH4	7.0±0.22^{a}	15.0±0.20^{a}	0.25±0,03^{a}	0.90±0.05^{a}	22±0.48^{a}	40±0.42^{a}	23±0.13^{a}	38±0.15^{a}	65±0.53^{a}	177±0.55^{a}
LSD	0.25	0.35	0.05	0.04	1.25	1	1.15	1.14	1.00	0.04

*Means indicated vary significantly at $p \leq 0.05$ during tri-replicate experiment due to effect of treatments.

Table 4. The effect of control and drought conditions on physiological traits of different bread wheat and synthetic hexaploid wheat genotypes.

Treatments	Photosynthesis Rate (μmm^{-2}S^{-1})		Stomatal Conductance (mmm^{-2}S^{-1})		Transpiration Rate (mmm^{-2}S^{-1})		Total Chlorophyll (g kg^{-1})		CMSP	
Genotypes	**Control**	**Drought**	**control**	**Drought**	**Control**	**Drought**	**control**	**Drought**	**Control**	**Drought**
NARC-2009	31±0.35^{a}	21±0.21^{d}	825±2.3^{fg}	635±2.5^{ij}	13±0.10^{d}	6±0.10 ^{f}	1.45±0.01^{j}	0.60±0.01^{ef}	77±0.25^{fg}	44±0.21^{g}
Chakwal-86	32±0.41^{e}	24±0.21^{f}	830±2.4^{f}	638±2.4^{i}	14±0.11^{a}	7±0.10 ^{e}	1.50±0.02^{i}	0.62±0.02^{e}	76±0.23^{f}	46±0.22^{ef}
AARI-2011	31±0.31^{a}	22±0.20^{c}	866±2.5^{e}	656±2.3^{h}	15±0.12^{c}	8±0.11^{d}	1.56±0.01^{h}	0.68±0.01^{d}	78±0.24^{e}	47±0.25^{e}
Inqilab-2000	32±0.31^{e}	23±0.21^{e}	850±2.5^{d}	655±2.2^{g}	14±0.11^{b}	8±0.11^{d}	1.66±0.03^{g}	0.69±0.02^{d}	80±0.21^{acd}	46±0.24^{ef}
Aas-2011	31±0.32^{a}	21±0.22^{d}	840±2.1^{c}	648±2.3^{f}	14±0.11^{b}	8±0.10^{d}	1.45±0.02^{f}	0.54±0.03^{c}	81±0.26^{ac}	46±0.22^{ef}
Galaxy-	29±0.31^{d}	22±0.23^{c}	855±2.6^{a}	673±2.3^{e}	14±0.10^{b}	9±0.11^{c}	1.53±0.03^{cde}	0.57±0.01^{c}	82±0.27^{a}	47±0.24^{e}
2013	30±0.32^{c}	26±0.20^{a}	860±2.3^{a}	686±2.5^{d}	14±0.10^{b}	9±0.10^{c}	1.69±0.02^{cd}	0.85±0.02^{b}	83±0.25^{ac}	55±0.22^{cd}
SH1	31±0.33^{a}	27±0.21^{b}	858±2.1^{a}	682±2.5^{c}	15±0.11^{a}	10±0.11^{b}	1.73±0.02^{abc}	0.89±0.01^{b}	82±0.24^{a}	56±0.23^{bc}
SH2	33±0.31^{b}	27±0.23^{b}	845±2.3^{b}	675±2.4^{b}	15±0.12^{a}	10±0.10^{b}	1.74±0.01^{ab}	0.89±0.01^{b}	81±0.23^{b}	57±0.21^{b}
SH3	31±0.31^{a}	26±0.20^{a}	858±2.5 ^{a}	698±2.5^{a}	15±0.11^{a}	11±0.10^{a}	1.76±0.03^{a}	0.95±0.02^{a}	82±0.22^{a}	58±0.22^{a}
SH4										
LSD value at 0.05	1.00	0.85	5.46	3.70	0.95	0.73	0.05	0.06	1.60	1.12

*Means indicated vary significantly at $p \leq 0.05$ during tri-replicate experiment due to effect of treatments.

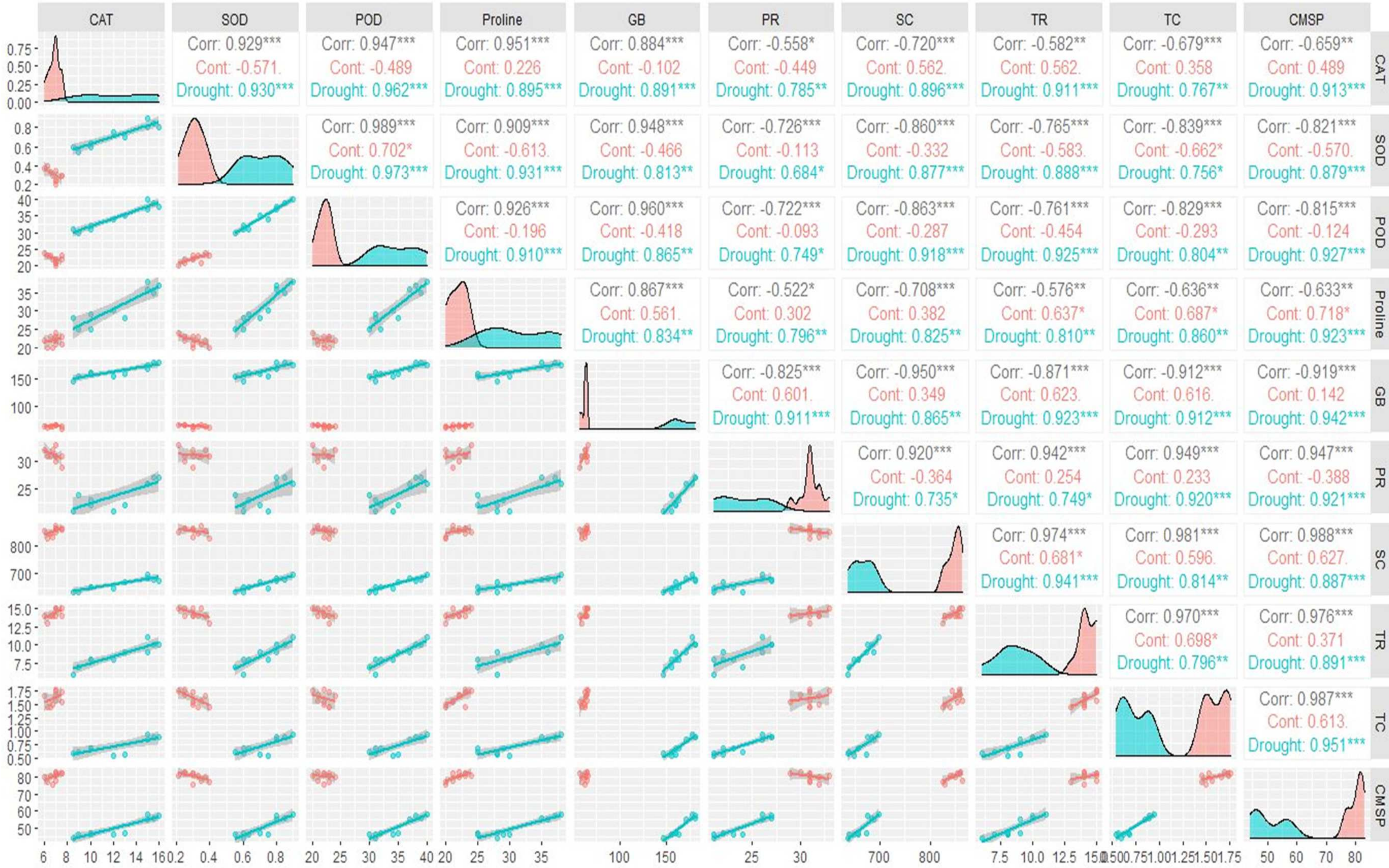

Figure 1. Correlogram indicating the extent of paired association of physiological and biochemical traits in bread wheat and synthetic hexaploid wheat genotypes under control and drought treatments. (CAT); Catalase, (SOD); Sodium dismutase, (POD); Peroxidase, (PC); Proline Content, (GB); Glycine Betaine, (PR); Photosynthesis Rate, (SC); Stomatal Conductance, (TR); Transpiration Rate, (TC); Total Chlorophyll, Cell Membrane Stability Percentage (CMSP).

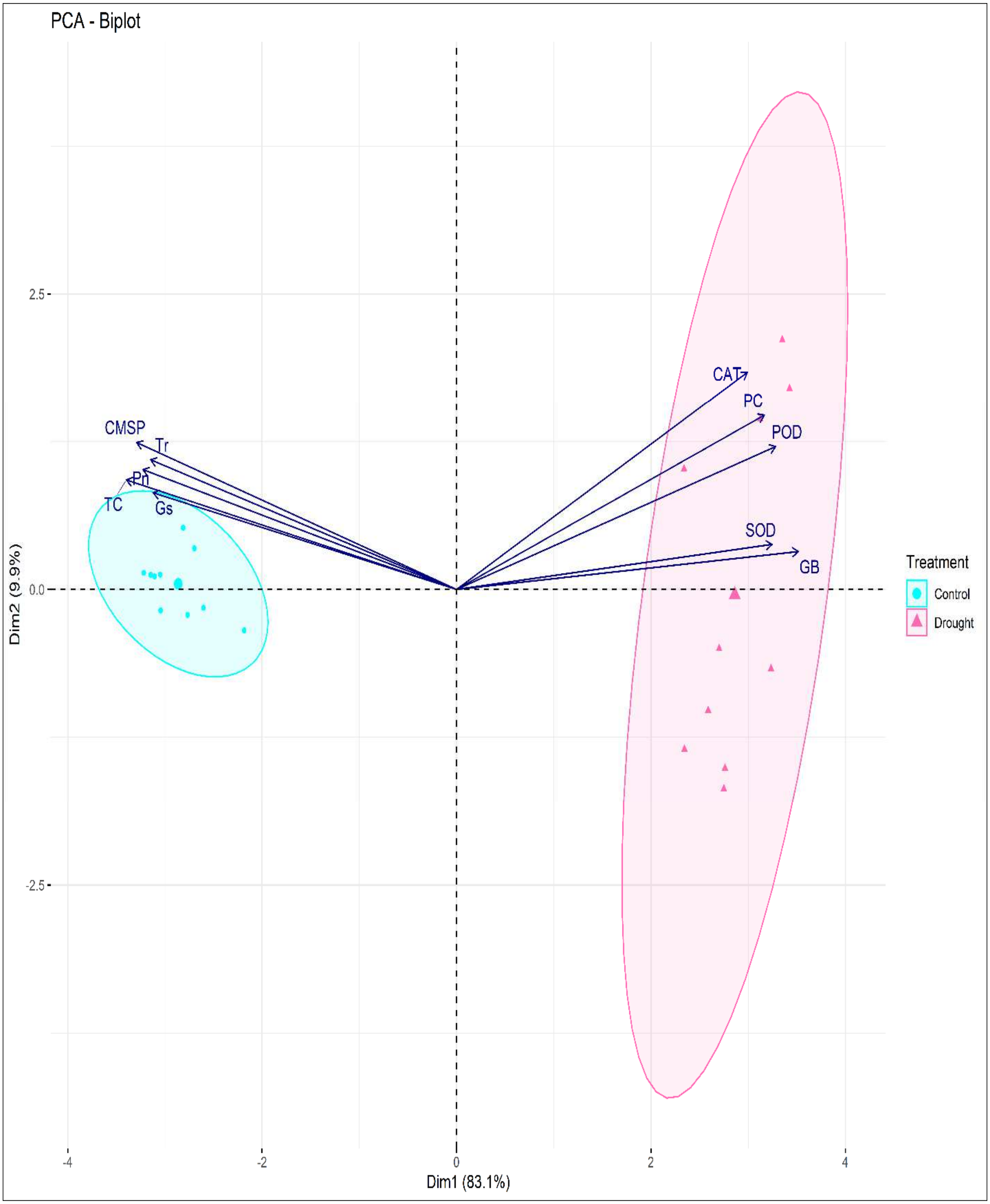

Figure 2.PCA biplot ellipses orientation illustrating the differential impacts of control and drought treatments on the extent of association of traits. (CAT); Catalase, (SOD); Sodium dismutase, (POD); Peroxidase, (PC); Proline Content, (GB); Glycine Betaine, (PR); Photosynthesis Rate, (SC); Stomatal Conductance, (TR); Transpiration Rate, (TC); Total Chlorophyll, Cell Membrane Stability Percentage(CMSP).

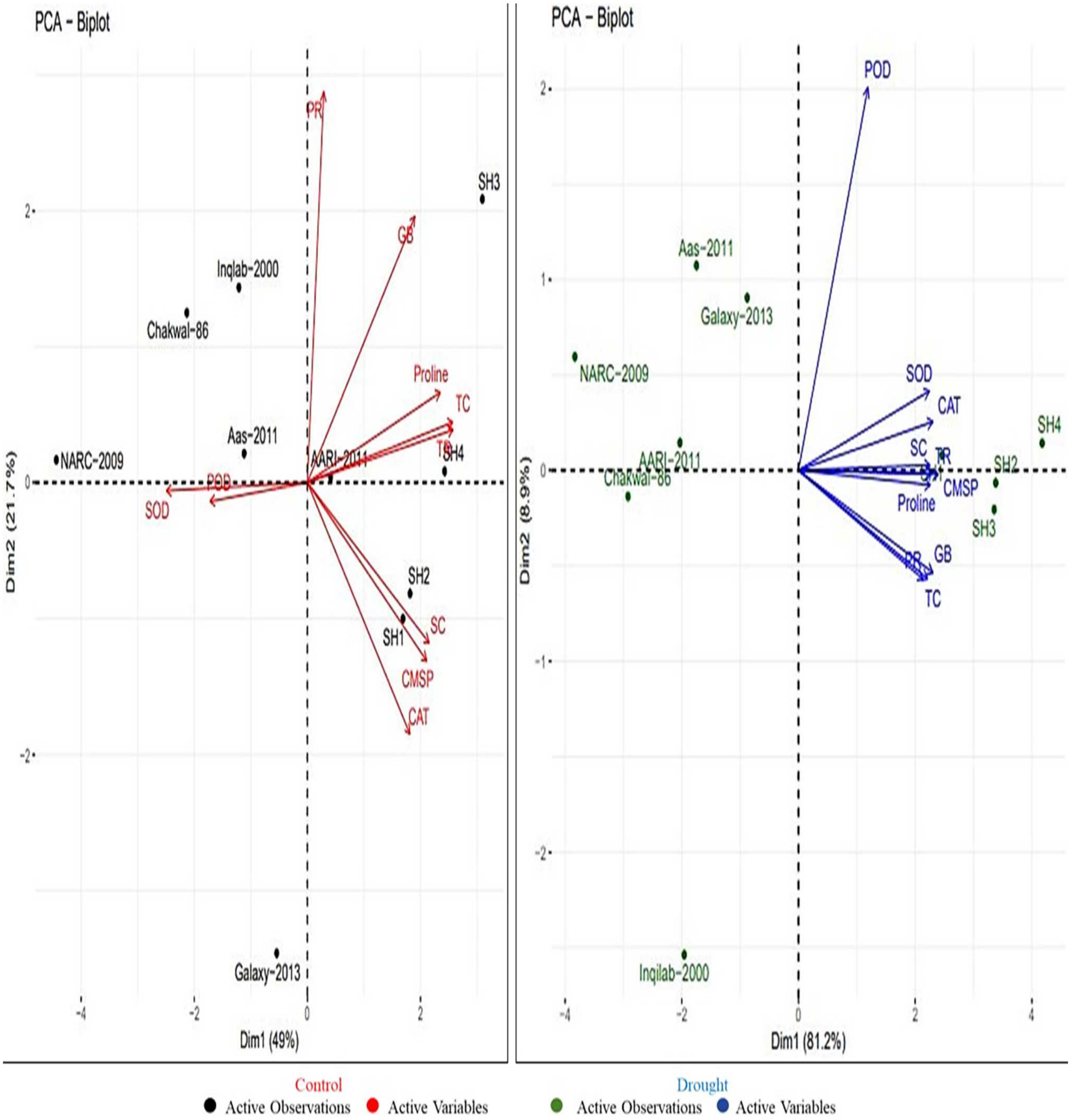

Figure 3.PCA biplot categorizing genotypes into different quadrants based on the trait's association under control and drought stress condition. (CAT); Catalase, (SOD); Sodium dismutase, (POD); Peroxidase, (PC); Proline Content, (GB); Glycine Betaine, (PR); Photosynthesis Rate, (SC); Stomatal Conductance, (TR); Transpiration Rate, (TC); Total Chlorophyll, Cell Membrane Stability Percentage(CMSP).

Heatmap and Hierarchical Cluster Analysis: Moreover, heatmap cluster analysis further confirmed the varying expression and extent of association of traits in wheat genotypes under saline conditions as compared to control conditions (Figure 4). Hierarchical cluster analysis further categorized genotypes into four sub clusters based upon their similarity and dissimilarity of responses (Figure 5). Synthetic hexaploid (SH) wheat genotypes depicted analogous physiological and biochemical response, therefore they occupied same cluster (Figure 5). Correspondingly Aas-2011 and Galaxy-2013 formulated second, Chakwal-86 and AARI-2011 formulated third, while Inqilab-2009 formulated cluster based upon similarity and dissimilarity of responses (Figure 5).

Gene Expression and Regulation: Relative expression of gene *TaERF3* showed significant ($p \leq 0.05$) variation under both control and drought conditions in all wheat genotypes (Figure 6). However, its relative expression was comparatively high in set of SH wheat lines followed by bread wheat cultivars Galaxy-2013 and Aas-2011. Overall, the expression of *TaERF3* was significantly ($p \leq 0.05$) variable among all genotypes due to drought application.

The *TaGROS-A* significantly ($p \leq 0.05$) up-regulated in all genotypes under drought treatment as compared to control, with maximum expression in Aas-2011 followed by Galaxy-2013, AARI-2011, Inqilab-2000 and SH wheat genotypes (Figure 6). Overall the expression of *TaGROS-A* varied considerably in all wheat genotypes during treatment of drought stress.

TaLEA-3 exhibited dramatic increase in expression under drought stress as compared to control treatment in all wheat genotypes (Figure 6). The maximum increase in *TaLEA-3* expression was recorded in SH wheat genotypes followed by Galaxy-2013, AARI-2011 and Aas-2011 while minimum increase was recorded in NARC-2009.In general, the increase in relative of *TaLEA-3* varied significantly ($p \leq 0.05$) in all wheat genotypes due to water deficit condition.

*TaHSFA1a*recorded significantly ($p \leq 0.05$) high expression in all genotypes under water deficit condition (Figure 6). Among wheat genotypes, Aas-2011 followed by Galaxy-2013, AARI-2011, Inqilab-2000 and SHs revealed the maximum increase while Chakwal-86 revealed minimum increase in the relative expression of *TaHSFA1a.*As a whole all genotypes depicted statistically distinct ($p \leq 0.05$) increase in *TaHSFA1a* expression due to application of drought stress.

Correspondingly *TaWRKY44* revealed significant ($p \leq 0.05$) upregulation in all wheat genotypes due to drought treatment as compared to normal water application (Figure 6). Relative expression of *TaWRKY44* showed maximum increase in Inqlab-2000 followed by Galaxy-2013, AARI-2011, Aas-2011 and set of SH wheat genotypes. However, genotypes Chakwal-86 and NARC-2009 recorded relatively low increase in expression. As a whole increase in *TaWRKY44* expression manifested significant ($p \leq 0.05$) variations in all genotypes due to application of drought stress.

The relative expression *TaZFP13D* varied significantly ($p \leq 0.05$) in all wheat genotypes owe to drought stress (Figure 6). Among genotypes SHs depicted maximum increase in relative expression followed by Aas-2011, Galaxy-2013 and AARI-2011. Besides, NARC-2009 demonstrated minimum increase in relative expression of *TaZFP34.* Overall the expression of *TaZFP13D*depicted statistically distinct ($p \leq 0.05$) increase in all genotypes due drought stress.

The relative expression of *TaEXPA2* varied significantly ($p \leq 0.05$) in all bread wheat while non-significantly in SHs genotypes due drought stress application(Figure 6). Among bread wheat genotypes Chakwal-86 followed by NARC-2009, Aas-2011, Galaxy-2013 and Inqilab-2000 recorded maximum increase in *TaEXPA2* expression due to drought stress. However bread wheat cultivars recorded significant ($p \leq 0.05$) difference in *TaEXPA2* expression as compared to SHs under drought stress.

All genotypes showed significant ($p \leq 0.05$) variation in the expression of *TaCPK34* under drought treatment (Figure 6). *TaCPK34* depicted maximum expression in SHs followed by Aas-2011, Galaxy-2013, AARI-2011, Inqilab-2000 and Chakwal-86, while minimum expression in NARC-2009. Overall, among genotypes *TaCPK34* expression varied in statistically distinct ($p \leq 0.05$) way due to drought stress.

Drought associated gene *TaDREB1A* illustrated significant ($p \leq 0.05$) variation in expression due to drought implementation (Figure 6). In general this gene depicted maximum expression in bread wheat cultivars as compared to SH wheat genotypes. Besides, all wheat genotypes manifested significant ($p \leq 0.05$) difference in the expression of *TaDREB1A* under drought stress.

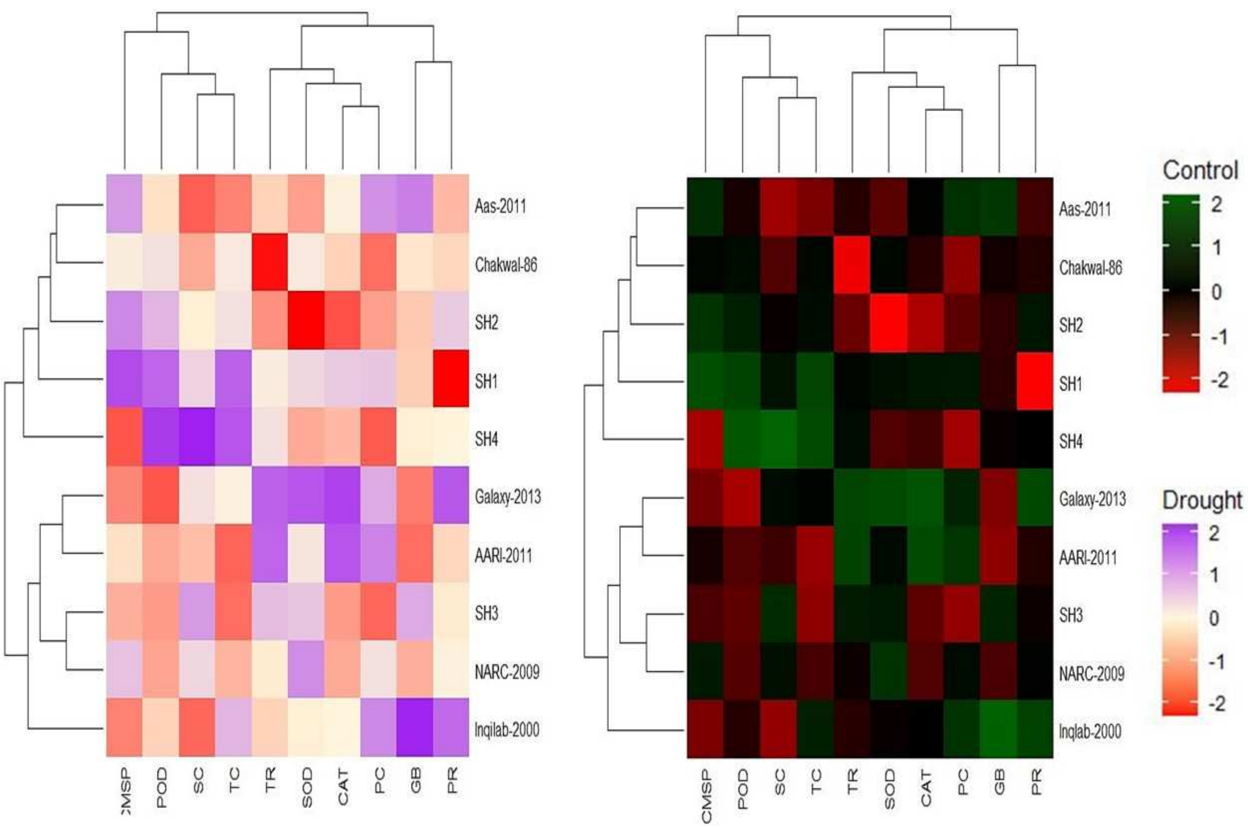

Figure 4.Heatmap analysis among genotypes and traits for the expression of physiological and biochemical traits under control and salinity stress treatment. (CAT); Catalase, (SOD); Sodium dismutase, (POD); Peroxidase, (PC); Proline Content, (GB); Glycine Betaine, (PR); Photosynthesis Rate, (SC); Stomatal Conductance, (TR); Transpiration Rate, (TC); Total Chlorophyll, Cell Membrane Stability Percentage(CMSP).

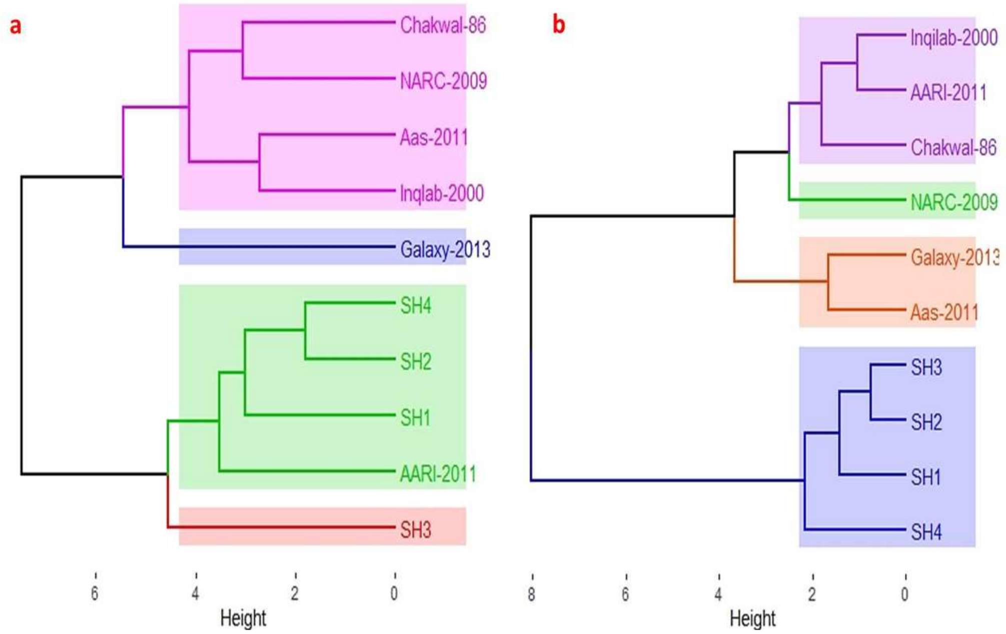

Figure 5.Hierarchical clustering of bread wheat and synthetic hexaploid wheat genotypes based of similarity and dissimilarity of physiological and biochemical traits under control (a) and drought (b) conditions.

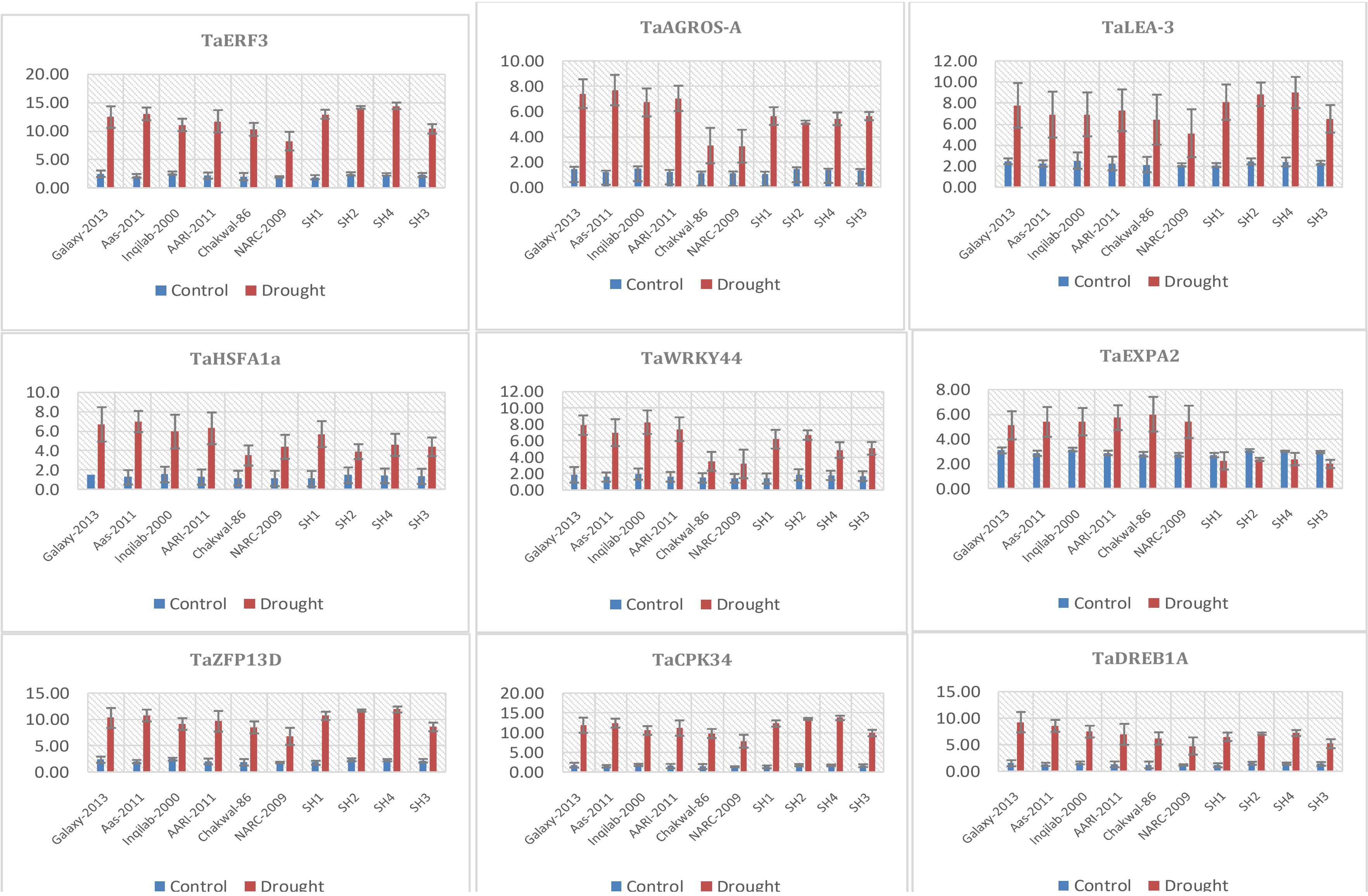

Figure 6. Relative gene expression of drought associated genes under control and drought conditions in bread wheat and synthetic hexaploid wheat genotypes.

DISCUSSION

The present study aimed to elucidate the impacts of drought stress on the physiological, enzymatic, biochemical and genetic traits of elite drought tolerant bread wheat cultivars and synthetic hexaploid (SH) wheat genotypes. Drought stress elicits various oxidative processes within plant cell system due to production of free radicals in the form of reactive oxygen species (ROS) that interfere with redox homeostatic activities (Shah *et al.,* 2017). To nullify the deleterious impacts of ROS plant are naturally provided with some tolerant mechanisms, hence they respond through regulations of various mechanisms (Shah *et al.,* 2022).The tolerant wheat genotypes initiates ROS scavenging mechanism via increasing the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase(POD) and catalase(CAT) to counter the production of ROS (You and Chan*,* 2015). This was confirmed by Shah *et al.,* (2022) who recorded an increase in the activities of SOD, POD and CAT in wheat genotypes exposed to drought stress. Correspondingly present research noticed significant increase in the activities of POD, CAT and SOD in all wheat genotypes due to drought stress (Table 3). The differential activities of antioxidant enzymes in wheat genotypes corresponds to their differential genetic architect and genes regulation mechanisms (Li*et al.,* 2018). In this perspective SH wheat lines manifested comparatively high enzymatic activities as compared to bread wheat cultivars as they are enshrined with various genetic networks conferring drought tolerance.

Abiotic stresses interfere with plants physiological processes leading towards the interruption plant whole metabolic system (Shah *et al*., *2017*). For example, drought stress has the tendency to destroy the components of plant photosystem and the enzymes involved in chlorophyll biosynthesis, therefore drought causes significant decline in chlorophyll (chl) and photosynthesis (Pn) as explicated by Fahad *et al.,* (2017). Furthermore, drought enhances the generation of ROS that disrupts membrane integrity owe to lipid-peroxidation and protein degradation that damages membrane structural assembly and resulting in high electrolyte leakage (Qamer *et al.,* 2021). Apart from this, abiotic stresses perturb plant water relations that disturb the exchange processes such as stomatal conductance (Gs) and transpiration (Tr) (Yang *et al.,* 2021). Correspondingly, present study recorded significant reduction in chlorophyll, Pn, Gs, Tr and CMSP (Table 3, 4). Our findings were further authenticated by Qaseem *et al.,* (2019), Algahabari *et al.,* (2021), Shah *et al.,* (2022) and who recorded significant reduction in chlorophyll, Pn, Gs, Tr and CMSP due to different versions of abiotic stresses. Moreover, plants naturally have tendency to retain their osmotic balance through production of various osmo-protectants such as proline and glycine betaine (GB) that facilitate the plant to tolerate the negative effects of abiotic stresses (Wang *et al.,* 2010; Qaseem *et al.,* 2019). Corresponding to these findings current study noticed a dynamic rise in proline and glycine betaine (GB) content of all wheat genotypes due to drought stress (Table 3). Like other living entities plants have tendency to retain the equilibrium of physiochemical processes. The physiological and biochemical events of plants are deeply interconnected (Annunziata *et al.,* 2019). Despite having plasticity and in build tolerant mechanisms the interconnection of physiological and biochemical processes perturbs under the conditions of drought stress. Abiotic stresses such as drought perturbs the physio-chemical equilibrium operating in plants (Niu *et al.,* 2012). In this perspective the response of plants varies depending upon the nature of their genotype (Shah *et al.,* 2022). Correspondingly current study recorded variation in the association and expression of traits with nature of stress and type of genotypes (Figure 1-4). Accordingly current study reported strong paired association among physiological and biochemical traits.The GB is an important quaternary ammonium compound in plant that occurs abundantly in plant exposed to dehydration (Krasensky and Jonak, 2012). GB is abundantly present in chloroplast where it plays essential role in protection and adjustment of thylakoid membrane, thereby maintaining the vital physiological contents and processes such as chl and Pn (Ali *et al.,* 2020). Similarly, the amino acid proline accumulates in plants in large amount under abiotic stresses. In addition to its role as an osmo-protectant, proline plays important role in the stabilization of sub-cellular structures, enhancement of membrane integrity, ROS scavenging and redox-ionic homeostasis (Hayat *et al*., 2012). Various plant species naturally accumulate proline and GB as major osmo-protectant during abiotic stress. Therefore, plants with high GB and proline during dehydration stress exhibit higher Chl, Pn and Gs with increased activities of antioxidant enzymes to harness the overproduction of ROS (Ashraf and Majid, 2007). This was the most probable reason for high drought tolerance of SHs and bread wheat genotypes Galaxy-2013, Aas-2011 and AARI-2011. Besides, the drought tolerance of plant is highly associated with expressions of genes that are somehow associated with the production of proteins involved in ROS scavenging and other homeostatic modulations (Shah *et al.,*2022). Besides, drought tolerance is a complicated phenomenon which is not easy to understand. Various genes along with signaling pathways are involved in enhancing the drought tolerance potential of plants (Shah *et al.,* 2017). All drought associated genes such as *TaDREB1A*, *TaGROS-A*, *TaERF3, TaCPK34, TaLEA3, TaHSFA1a, TaWRKY44, TaZFP34 and TaEXPA2* depicted significantly high relative expression under drought treatment. For instance upregulation of *TaERF3*under drought stress enhances the production of some transcriptions factors mediating the ethylene dependent signaling pathways that provoke the expression of different genes conferring drought tolerance in wheat (Magar *et al.,* 2022). Correspondingly current study confirmed these findings by reporting significant increase in expression of *TaERF3* in all wheat genotypes under drought stress (Figure 6). Besides, high expression of genes *TaWRKY44*, *TaZFP13D, TaGROS1A* and *TaCPK34*during drought stress regulate the antioxidant defense mechanism by boosting the catalytic kinetics of enzymes SOD, CAT and POD involved in scavenging of ROS as reported by Wang *et al.,* (2015), Bouard and Houde (2022), Ahmed *et al.,* (2022) and Li *et al.,* (2020) respectively. In consistent with these studies present study found significant rise in the expression of these genes (Figure 6) along with increase in the activities of SOD, CAT and POD (Table 3). Besides, transgenic wheat lines with over-expression of *TaLEA3* depict enhanced drought tolerance under water deficient conditions as concluded by Yu *et al.,* (2019). In the same way, Yang *et al.,* (2020) noticedthe high expression of the *TaEXPA2* gene in transgenic wheat genotypes that enhanced the drought enduring tendency of wheat genotypes through various biochemical and morphological modulations. Liu *et al.,* (2018) found the over-expression of *TaDREB1* in wheat due to osmotic stress induced by drought. Furthermore, over-expression *TaHSFA1* mediates the production of heat shock protein that protects plants from the hazardous impacts of various abiotic stresses as reported by Shah *et al.,* (2022). Corresponding with these findings current study recorded dramatic increase in the expression of *TaLEA3, TaEXPA2, TaDREB1A* and*TaHSFA1a* due to drought stress in drought tolerant genotypes under study (Figure 6). Apart from this the relative expression of these genes manifested significant variation in all wheat genotypes under same intensity of drought stress. This was an indicator of their different genetic architect inherited from their pedigree. Interestingly, the genetic and biochemical behavior of SH wheat genotypes was different from bread wheat genotypes in complementary way as confirmed by hierarchical clustering (Figure 5).This can be attributed to enriched genetic diversity that they inherited from their closed wild progenitors that modulate the expression pattern of aforementioned genes unanimously as reviewed by Li *et al.* (2018). Furthermore, among bread wheat genotypes Galaxy-2013, Aas-2011 and AARI-2011 depicted high expression of drought associated genes in addition to increased activity of antioxidant enzymes due to drought stress, which is an indicator of their high counter tendency against drought stress as reviewed by Shah *et al.*(2017). Overall, current study proved bread wheat cultivars Galaxy-2013, Aas-2011 and AARI-2011 along with SH wheat genotypes can be an effective stock of drought tolerant genes for rendering drought tolerance to other elite bread wheat cultivars through appropriate breeding program.

Acknowledgements: This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G: 185-155-1442. The author, therefore, acknowledge with thanks DSR for technical and financial support.

Authors Contribution: FA came with idea and executed the experiment while ZHS performed analysis, wrote and reviewed the manuscript.

Conflict of Interests: Authors declare no conflict of interests.

REFERENCES

Ahmad, A., Z. Aslam, T. Javed, S. Hussain, A. Raza, R. Shabbir, F. Mora-Poblete, T. Saeed, F. Zulfiqar, M.M. Ali, M. Nawaz, M. Rafiq, H.S. Osman, M. Albaqami, M.A.A. Ahmed and M. Tauseef (2022). Screening of Wheat (*Triticum aestivum* L.) Genotypes for Drought Tolerance through Agronomic and Physiological Response. Agronomy 12(2) 287. doi: 10.3390/agronomy12020287

Ahmed, K.,G. Shabbir, M. Ahmed, S. Noor, A.M.U. Din, M. Qamar and N. Rehman (2022). Expression profiling of TaARGOS homoeologous drought responsive genes in bread wheat. Sci. Rep.12(1): 3595. doi:10.1038/s41598-022-07637-y

Alghabari, F.,Z.H. Shah, A.A. Elfeel and J.H. Alyami (2021). Biochemical and Physiological Responses of Thermostable Wheat Genotypes for Agronomic Yield under Heat Stress during Reproductive Stages. Agronom*y*11(10):*20*80. doi: 10.3390/agronomy11102080

Ali S., Z. Abbas, M.F. Seleiman, M. Rizwan, I.S. Yava, B.A. Alhammad, A. Shami, M. Hasanuzzaman and D. Kalderis .(2020). Glycine Betaine Accumulation, Significance and Interests for Heavy Metal Tolerance in Plants. Plants 9(7):896. doi: 10.3390/plants9070896.

Annunziata, M.G.,L.F. Ciarmiello, P. Woodrow, E. Dell'Aversana and P. Carillo (2019). Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Front. Plant Sci.7: 10, 230. doi: 10.3389/fpls.2019.00230

Ashraf, M. F. M. R. and R. Majid. (2007). "Roles of glycine betaine and proline in improving plant abiotic stress resistance." Environ. Exp. Bot. 59(2): 206-216. doi:10.1016/j.envexpbot.2005.12.006

Bouard, W. and M.Houde (2022). The C2H2 Zinc Finger Protein TaZFP13D increases drought stress tolerance in wheat. Plant Stress 100119. doi: 10.1016/j.stress.2022.100119

Din, A., M. Ahmad, F,M. Watto, S. Ahmed, I. Ali and M. K.N. Shah. (2020). Drought tolerance screening in thirty common wheat (Triticum aestivum L.) genotypes. Sarhad J. Agric, *36*:168-177. doi: 10.17582/journal.sja/2020/36.1.168.177

dos-Santos, T.B.,A.F. Ribas, S.G.H. de Souza, I.G.F. Budzinski and D.S. Domingues (2022). Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. Stresses *2*: 113-135. doi:10.3390/stresses2010009

Fahad, S.,A. Bajwa, U. Nazir, S.A. Anjum, A. Farooq, A. Zohaib, S. Sadia, W. Nasim, S. Adkins, S. Saud *et al* (2017). Crop production under drought and heat stress: plant responses and management options. Front. Plant Sci.*8*: 1147. doi: 10.3389/fpls.2017.01147

Hayat S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel and A. Ahmad. (2012). Role of proline under changing environments: a review. Plant Signal. Behav. 7(11):1456-66. doi: 10.4161/psb.21949.Krasensky, J. and C. Jonak. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 63(4):1593-608. doi: 10.1093/jxb/err460.

Li, A.,D. Liu,W. Yang, M. Kishii and L. Mao (2018). Synthetic hexaploid wheat: yesterday, today, and tomorrow. Engineering4(4): 552-558. doi:10.1016/j.eng.2018.07.001

Li, S.,R. Wang, H. Jin and Y. Ding, C. Cai (2019). Molecular characterization and expression profile analysis of heat shock transcription factors in Mungbean. Front. Genet.*9*: 736. doi:10.3389/fgene.2018.00736

Li, G.Z., H. Li, M.J. Xu, P.F. Wang, X.H. Xiao and G.Z. Kang (2020). Functional characterization and regulatory mechanism of wheat CPK34 kinase in response to drought stress. BMC Genomics21(1): 1-14.doi: 10.1186/s12864-020-06985-1

Liu, M., Z. Wang, H.M. Xiao and Y. Yang (2018). Characterization of TaDREB1 in wheat genotypes with different seed germination under osmotic stress. Hereditas155: 26.doi: 10.1186/s41065-018-0064-6

Ma, Z., A. Shayeganpour, D.R. Brocks, A. Lavasanifar and J. Samuel (2007). High-performance liquid chromatography analysis of curcumin in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. Biomed. Chrom. 21(5): 546-552.doi: 10.1002/bmc.795.

Magar, M. Maya, H. Liu and G. Yan (2022). Genome-wide analysis of AP2/ERF superfamily genes in contrasting wheat genotypes reveals heat stress-related candidate genes. Front. Plant Sci. 13: 853086. doi: 10.3389/fpls.2022.853086

Mahmood, N.,M. Arshad, H. Kächele, A. Ullah and K. Müller(2022). Economic efficiency of rainfed wheat farmers under changing climate: evidence from Pakistan. Environ. Sci. Poll. Res. 27(27): 34453-34467. doi: 10.1007/s11356-020-09673-5

Najafi, S.,K. Sorkheh and F. Nasernakhaei (2018). Characterization of the APETALA2/ethylene-responsive factor (AP2/ERF) transcription factor family in sunflower. Sci. Rep.8:11576. doi:10.3390/plants9040455

Nan, H.,W. Li, Y.L. Lin and L.Z. Gao (2020). Genome-wide analysis of WRKY genes and their response to salt stress in the wild progenitor of Asian cultivated rice, Oryza rufipogon. Front. Genet.11: 359. doi:10.3389/fgene.2019.00363

Niu, C.F., W. Wei, Q.Y. Zhou, A. Tian, Y.J. Hao, W.K. Zhang, B. Ma, Q. Lin, Z.B. Zhang, J.S. Zhang *et al* (2012). Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant Cell Environ.35: 1156–1170. doi:10.1111/j.1365-3040.2012.02480.x

Pour-Aboughadareh, A.,M. Omidi, M.R. Naghavi, A. Etminan, A.A. Mehrabi, P. Poczai and H. Bayat (2019). Effect of water deficit stress on seedling biomass and physio-chemical characteristics in different species of wheat possessing the D Genome. Agronomy 9: 522. doi:10.3390/agronomy9090522

Qamer, Z., M.T. Chaudhary, X. Du, L. Hinze and M.T. Azhar (2021). Review of oxidative stress and antioxidative defense mechanisms in Gossypium hirsutum L. in response to extreme abiotic conditions. J. Cotton Res.4(1): 1-9. doi:10.1186/s42397-021-00086-4

Qaseem, M.F., R. Qureshi and H. Shaheen (2019). Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. Sci Rep. 9: 6955.doi:10.1038/s41598-019-43477-z

Shah, Z.H., H.M. Rehman, T. Akhtar, I. Daur, M.A. Nawaz, M.Q. Ahmad, I.A. Rana, R.M. Atif, S.H. Yang and G. Chung (2017). redox and ionic homeostasis regulations against oxidative, salinity and drought stress in wheat (a systems biology approach). Front Genet. 8: 141. doi: 10.3389/fgene.2017.00141

Shah, S.M.D.M.,G. Shabbir, S.I. Malik, N.I. Raja, Z.H. Shah, M. Rauf, Y.A. Zahrani, F. Alghabari, H. Alsamadany, K. Shahzad and S.H. Yang (2022). Delineation of physiological, agronomic and genetic responses of different wheat genotypes under drought condition. Agronomy *12*: 1056. doi:10.3390/agronomy12051056

Sivamani, E.,A. Bahieldin, J.M. Wraith, T. Al-Niemi, W.E. Dyer, T.H.D. Ho and R. Qu (2000). Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. Plant Sci.155: 1–9. doi:10.1016/S0168-9452(99)00247-2

Wang, G.P., X.Y. Zhang, F. Li, Y. Luo and W. Wang (2010). Overaccumulation of glycine betaine enhances tolerance to drought and heat stress in wheat leaves in the protection of photosynthesis. Photosynthetica48(1): 117-26. doi:10.1007/s11099-010-0016-5

Wang, X.,J. Zeng, Y. Li, X. Rong, J. Sun, T. Sun, M. Li, L. Wang, Y. Feng, R. Chai *et al* (2015). Expression of TaWRKY44, a wheat WRKY gene, in transgenic tobacco confers multiple abiotic stress tolerances. Front. Plant Sci.*6*: 615. doi: 10.3389/fpls.2015.00615

Yang, J.,G. Zhang, J. An, Q. Li, Y. Chen, X. Zhao, J. Wu, Y. Wang, Q. Hao, Wang, *et al* (2020). Expansin gene TaEXPA2 positively regulates drought tolerance in transgenic wheat (*Triticum aestivum* L.). Plant Sci. 298: 110596. doi:10.1016/j.plantsci.2020.110596

Yang, X.,M. Lu, Y. Wang, Y. Wang, Z. Liu and S. Chen (2021). Response Mechanism of Plants to Drought Stress. Horticulturae *7*: 50. doi:10.3390/horticulturae7030050

You, J. and Z. Chan (2015). ROS Regulation During Abiotic Stress Responses in Crop Plants. Front. Plant Sci. 8(6):1092. doi:10.3389/fpls.2015.01092

Yu, Z., X. Wang, Y. Tian, D. Zhang and L. Zhang (2019). The functional analysis of a wheat group 3 late embryogenesis abundant protein in *Escherichia coli* and *Arabidopsis* under abiotic stresses. Plant Signal., Behav. 14(11):1667207.doi: 10.1080/15592324.2019.1667207

Zang, D.,H. Li, H. Xu, W. Zhang, Y. Zhang, X. Shi andY. Wang (2016). An arabidopsis zinc finger protein increases abiotic stress tolerance by regulating sodium and potassium homeostasis, reactive oxygen species scavenging and osmotic potential., Front. Plant Sci.7: 1272.doi:10.3389/fpls.2016.01272

Zhang, J., S. Zhang, M. Cheng, H. Jiang, X. Zhang, C. Peng, X. Lu,M. Zhang and J. Jin. (2018). effect of drought on agronomic traits of rice and wheat: A Meta-Analysis. Int. J. Environ. Res. Public Health *15*, 839. doi:10.3390/ijerph15050839.