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EVALUATION OF DROUGHT-TOLERANT WHEAT VARIETIES FOR SUSTAINABLE CROP PRODUCTION

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Abstract

Globally drought stress destroys production of wheat and it has resulted into the search of resilient genotypes with improved physiological and molecular adoptions. Twenty genotypes of bread wheat (synthetic hexaploid wheat lines, moderate drought and severe drought) were evaluated using a well watered environment, moderate drought environment and severe drought environment in this study, randomized complete block design was used. Close assessment of photosynthetic parameters, biochemical indicators, agronomic traits, expression of genes and genetic parameters revealed that synthetic hexaploid wheat genotypes significantly outperformed conventional cultivars in severe drought situations. The net photosynthesis rates, water content of the samples and stability of the cell membranes in the synthetic hexaploid lines of wheat was more than the susceptible checks. Antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase) were also two to three times higher in the synthetic hexaploid wheat lines and the quantity of malondialdehyde developed was significantly less, which was a reduced level of oxidative damage. Molecular analysis showed that synthetic hexaploid wheat genotypes are highly upregulated by drought-responsive genes with TaDREB1A and TaLEA3 genes having over a dozen and fifteen folds more active expression, respectively. Half-diallel analysis indicated that most of the major drought-tolerant phenotypes are majorly additively inherited with the narrow-sense heritability values always being in the moderate to high range. Therefore, synthetic hexaploid wheat genotypes had significantly increased grain yield under extreme stress of drought and had the greatest stress tolerance index and the lowest stress susceptibility index. The principal component analysis revealed the relative water content, grain yield and catalase activity to be the most discriminatory of drought resistance. We conclude that the synthetic hexaploid wheat lines are more adaptive to drought as far as their physiological, biochemical and genetic adaptations and its strategic use in breeding programs is the brightest future of wheat in water-limited environment.

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INTRODUCTION

One of the major constraints on wheat production in all parts of the world is drought stress, and strong genotypes should be cultivated to secure food security amidst the unpredictable weather (Ayyub, Irum, and Shah et al., 2024). These environmental stressors are estimated to be able to lower the yields by up to 50 percent in susceptible regions by impairing the fundamental physiological processes, including photosynthesis and nutrient uptake (Saed et al., 2026). To overcome these difficulties, scientists are now more concerned with finding and assessing drought-tolerant genotypes based on a combination of physiological and molecular screening models (Bhutto et al., 2025; Keskin et al., 2025). This approach is devoted to the characterization of the main morpho-physiological characteristics, such as relative water content and activity of antioxidant enzymes, which can be referred to as the consistent indicators of the difference between drought-tolerant and vulnerable genotypes (Alshaharni et al., 2025; Sewore et al., 2023). In the study, the most appropriate breeds of bread wheat that can be capable of sustaining the most appropriate level of production in the dry environment will be determined by considering various parameters of gaseous exchange and yield-related characteristics in the conditions of different water deprivation (Ahmad et al., 2022; Wasaya et al., 2021). Thus, in this study, several types of genotypes are evaluated under the conditions of water deficiency to study their adaptive mechanisms with the help of special gas exchange rates and biochemical phenotyping (KETEN et al., 2023). This study aims to clarify the particular physiological processes enabling some cultivars to maintain yield performance even under extreme water constraints by combining these multivariate analysis with agronomic data (Shahid et al., 2022). The experimental design is a randomized complete block that is applied to study rigorously these physiological parameters in controlled irrigation and induced drought conditions (Farshadfar et al., 2014). This is a holistic analysis that uses half-diallel analysis to identify the gene action that governs the important agronomic and quality traits to narrow down the selection criteria in future breeding programs (Kamara et al., 2022). This type of research will enable easier prediction of drought-tolerant phenotype by linking phenotypic plasticity with genetic pathways to simplify selection to create stress-tolerant cultivars (Ghaffar et al., 2023). Additionally, it can help to determine strong morphological indicators, including dry weight and cell membrane stability, which are important in establishing the adaptive potential of various wheat germplasm due to the integration of multivariate techniques of analysis like a principal component analysis (Mohi-Ud-Din et al., 2021). In addition, the enzymatic antioxidants (i.e., catalase and peroxidase) are also evaluated to give the empirical evidence about the metabolic approach used by these genotypes to reduce the oxidative damage caused by the water shortage (Darwish et al., 2025). Along with these enzyme responses, water retention capacity as a valuable physiological parameter has been added to give a more plausible assessment of the water status of plants in prolonged humidity stress conditions (Hasheminasab et al., 2013). In addition to that, assimilate remobilization and harvest index determination is a determinant that is critical of the overall yield capacity in low moisture conditions since the measurements are used to measure the effectiveness of photosynthates in grain growth (Razzaghi, 2025). In this comparison study, the genetic variation of synthetic hexaploid wheat lines in the arid environment is further discussed, namely, the biochemical and physiological dissimilarities of their reactions, in relation to the conventional bread wheat lines (Mokhtari et al., 2024). The present comparative analysis shows the significance of combining physiological and genetic data to appropriately classify genotypes that is vital in indicating desirable variations in various sets of germplasm (Alghabari and Shah, 2024; Ali et al., 2023). In fact, synthetic hexaploid wheat genotypes are generally associated with an improved tolerance system, including extended photosynthetic pigment and diminished signs of oxidative stress, among others, compared to their counterparts of non-synthetic wheat (Mokhtari et al., 2024). Such physiological advantages also find unique expression patterns of drought-responsive genes, such as the genes TaDREB1A and TaLEA3, which arrange an adaptive metabolic reaction to water-deficit stress (Alghabari and Shah, 2024). Particularly, hierarchical clustering of these features would be a conclusive visual report of the dissimilarity and similarity of the synthetic hexaploid lines and the bread wheat genotypes (Alghabari and Shah, 2024). Empirical data shows that these artificial hexaploid lines are more biochemically resilient (higher catalase levels and higher water content per 1 of water than normal cultivars) to identical water-deficit stress (Mokhtari et al., 2024). Such agronomic benefits imply that the use of synthetic hexaploid germplasm in breeding pipelines can contribute greatly to the yield consistency and productivity in the context of an ever more frequent climate-induced drought (Amini et al., 2023; Mokhtari et al., 2025). Furthermore, it is possible to identify such positive haplotypes of genes as *1fehw3*, Dreb-B1 and TaLTPs-11 to provide a molecular background to consider synthetic derivatives with improved biomass and grain weight gain (Ali et al., 2023). In addition to that, the fact that the expression levels of the genes, especially, the genes that are involved in the regulatory factors such as the expression of TaERF3 and TaHSFA1a vary, indicates that the synthetic hexaploids have modified metabolic pathways that can accommodate the redox homeostasis during the acute acute water stress. These molecular signaling pathways and the capacity to maintain water regulation make sure that plants still ensure the necessary growth processes even with limited water availability (Alghabari et al., 2023). Moreover, strategic introgression of these lines of a synthetic hexaploid into elite breeding programs is one of the potential solutions of recovery of genetic diversity that was lost at a bottleneck of wheat domestication

(Mokhtari et al., 2022). These artificial derivatives have in most instances better root morphological characteristics that allow them to access more water at lower soil levels, and be more drought resilient in general. Moreover, these synthetic lines also possess more adaptive properties, such as improved thousand-grain weight, and improved harvest indices, both of which contribute to stabilizing grain production in the most changeable conditions (Ali et al., 2023). Capitalizing on the high-density SNP markers would enable a finer appreciation of the population architecture, which breeders can then use to determine novel genetic loci that can provide resistance to multi faceted abiotic constraints. Such a breakthrough in the process of genomic choices is necessary to the intensity of polygenic trait building that leads to the emergence of resistance to the combined effect of high-temperature stress and water loss (Mokhtari et al., 2022). Breeders can also tap into the constrained gene pool restricting adaptive improvement of elite wheat cultivars by exploiting the untapped genetic potential of underexploited-accessions of the weed, Aegilops tauschii (Bhatta et al., 2018; Gudi et al., 2024). In particular, these types of synthetic derivatives have been found to produce grain yields that are up to 46% higher in severe water-stress scenarios than their typical wheat parents (Amini et al., 2023). Moreover, such artificial derivatives make it easy to exploit the new interactions of genes, which are used to boost expression of traits and overall adaptation to environmental conditions, otherwise not attained using the normal mechanism through introgression (Sohail et al., 2011).

METHODOLOGY

The objective of this research was to compare the physiological, biochemical and agronomic responses of different genotypes of bread wheat to a controlled drought stress in the problem-based approach which will determine the nature and genetic pathways underpinning the drought tolerance. The controlled-environment research facility where the experiment was carried out was in two growth seasons (20242025 and 20252026) and had a split-plot design (randomized complete block design), and a randomized complete block design (RCBD). As the main plots three water-deficit regimes (WW, maintained at 75±5% field capacity), moderate drought stress (MS, 45±5% field capacity) and severe drought stress (SS, 25±5% field capacity) were imposed. Subplots comprised of ten commercial cultivars, five advanced breeding lines and 5 synthetic hexaploid wheat lines, which were crosses between the genotype Aegilops tauschii, and twenty bread wheat genotypes were used. All treatments were repeated thrice and 30 genetically related plants in a plot. Time-domain reflectometry was used to quantify the soil moisture content on a daily basis and irrigation was automatically administered to keep the known field capacity levels in the identified field during tillering phase till physiological maturity.

The severity of drought stress that was imposed was quantified as the drought stress intensity (DSI) which was estimated at each level of treatment:

$$DSI = 1 - (Y_d / Y_w),$$

Y_d = average genotype yield under any stress regime Y_w = average yield under well-watered regime. This DSI was the one to compare the stress on a season-to-season basis. Measurement of gas exchange parameters was done at anthesis at the same time [09:00 to 11:00] on the flag leaf using a portable infrared gas analyzer which included net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E) and intercellular concentration of CO_2 (Ci).

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100,$$

FW = fresh weight, TW = turgid weight and DW = dry weight. Cell membrane stability (CMS) was determined by determining electrolyte leakage of leaf segments subjected to a heat shock of 45 C in 30 minutes and then conductivity measurements were made prior to and after autoclaving and CMS was calculated as:

$$CMS\ (\%) = [1 - (C1 / C2)] \times 100,$$

where C1 and C2 are the first electrical conductivity and the last electrical conductivity, respectively.

The antioxidant enzyme activities of the biochemical profiling were used. Catalase (CAT) was determined by placing fresh leaf samples (0.5 g) in phosphate buffer pH 7.0 and centrifuged the sample at 12, 000 rpm over a duration of 20 minutes at 4 C and the catalase activity was expressed as 0.50 0.50 which is the number of moles of hydrogen peroxide (H_2 The nitroblue tetrazolium photoreduction technique was used to determine the activity of superoxide dismutase (SOD) where one unit of the SOD was the one which would reduce the reaction by half. The amount of lipid peroxidation according to the level of malondialdehyde (MDA) was measured by use of the thiobarbituric acid reaction, and calculated as per the following equation:

$$MDA\ (nmol\ g^{-1}\ FW) = [(A532 - A600) \times volume \times 10^{6}] / (\varepsilon \times weight), \text{ with } \varepsilon = 155\ mM^{-1}\ cm^{-1}.$$

Agronomic measurements were made at harvest, such as days to heading, plant height, spike length, grains per spike, thousand-grain weight (TGW), grain yield per plant (GY), and harvest index (HI). HI was determined by: HI = GY/ (GY+ straw weight). The efficiency of assimilate remobilization (ARE) was estimated by estimating the contribution of the stem reserves to grain filling by the equation: ARE (%) = [(stem dry weight at anthesis - stem dry weight at maturity)/(grain weight per plant)) × 100]. Half-diallel mating design was employed to carry out genetic study between six selected contrasting genotypes (3 drought-tolerant and 3 susceptible). The F_1 offspring, and parents were tested at the same drought regimes and combining ability analysis was carried out according to the Method 1, Model 1 of Griffing. The linear model was used to estimate the general combining ability (GCA) and specific combining ability (SCA) effects:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + e_{ij},$$

Where Y_{ij} represents the mean performance of cross i j, and, g_i and g_j GCA effects, s_{ij} SCA effect and e ij error.. The effect of the genes was ascertained by the calculation of the ratio (sigma2 G CA / sigma2 S CA) where the values greater than one signified additive effect of the gene and the values less than one signified non-additive dominance.

In order to describe molecular wise, the total RNA was isolated at the booting stage and when there was severe drought in the flag leaves and cDNA was synthesized. Fold change = 2 -(Ct -Ct control) treated -(Ct -Ct control) control. Multivariate analysis of normalized data on traits was performed with the principal component analysis (PCA) and hierarchical analysis to categorize the genotypes and identify strong morphological predictors.. All the statistical tests were performed with the help of R software and treatment means were compared with the help of the Fisher least significant difference (LSD) test with the significance level p = 0.05. This combined physiological, biochemical, genetic and molecular approach presented a holistic means of finding excellent drought resistant genotypes of wheat.

RESULTS

Table 1 shows that synthetic hexaploid wheat (SHW) genotypes retain a much higher net photosynthesis and quantum yield which implies greater photosynthetic Table 2 reveals that SHW lines have significantly higher catalase (CAT = 118.4 µmol H 2 O 2 -min -1 -mg -1 protein) and proline accumulation (71.8 0.8 mol -1 FW) under SS, and lower malondialdehyde (MDA = 36.4 nmol -1 FW) than susceptible gen Table 3 Underlines the fact that SHW genotypes have higher thousand-grain weight (TGW = 32.9 g) and grain yield (GY = 3.87 g -1 plant -1) when grown under SS, and a lower harvest sensitivity index (= 0.57) than susceptible checks (= 1.21).. Table 4 reveals that the relative water content (RWC = 69.3%), cell membrane stability (CMS = 67.4%), osmotic adjustment (= -2.24 MPa) and dehydration tolerance time (= 32.8 h) of SHW in SS are higher. It is a strong indication of transcriptional reprogramming that Table 5 shows that SHW genotypes upregulate TaDREB1A (12.84-fold) and TaLEA3 (15.32-fold) to a much greater extent than susceptible lines (3.14- and 3.87-fold, respectively). Table 6 (PCA) shows that on the positive side PC1 (48.3% variance) is heavily loaded by RWC (0.912), GY (0.903), and Pn (0.894) but on the negative side CAT (-0.845) and MDA (-0.821) are loaded. Table 7 indicates that the ratio between 2 R 2GCA/2 R 2SCA is 2.0 or greater, per trait and that narrow-sense heritability (h 2) is 0.57 to 0.63 under SS.

Table 1: Comparative photosynthetic gas exchange parameters under differential water-deficit regimes

Genotype	Treatment	Pn (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	gs (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)	Ci (μmol·mol^{-1})	E (mmol·$m^{-2} \cdot s^{-1}$)	WUEi (μmol·$mmol^{-1}$)	α (quantum yield)	β (RuBisCO activity,	ΦPSII (electron

								µmol·mg^{-1}·min^{-1})	**transport rate)**
Genotype A	WW	28.4 ± 1.2	0.68 ± 0.03	312 ± 8.1	5.91 ± 0.21	4.81 ± 0.15	0.084 ± 0.002	124.7 ± 3.4	0.832 ± 0.011
Genotype A	MS	19.7 ± 0.9*	0.41 ± 0.02*	278 ± 6.4*	3.88 ± 0.14*	5.08 ± 0.12	0.062 ± 0.001*	89.3 ± 2.1*	0.645 ± 0.009*
Genotype A	SS	11.3 ± 0.6*	0.19 ± 0.01*	241 ± 5.2*	2.12 ± 0.09*	5.33 ± 0.11	0.041 ± 0.001*	51.6 ± 1.7*	0.428 ± 0.007*
Genotype B (SHW)	WW	31.2 ± 1.1	0.75 ± 0.02	318 ± 7.2	6.23 ± 0.19	5.01 ± 0.13	0.091 ± 0.002	136.2 ± 3.1	0.861 ± 0.010
Genotype B (SHW)	MS	24.8 ± 0.8†	0.54 ± 0.02†	289 ± 5.9	4.67 ± 0.12†	5.31 ± 0.10	0.074 ± 0.001†	107.4 ± 2.4†	0.731 ± 0.008†
Genotype B (SHW)	SS	17.9 ± 0.7†	0.32 ± 0.01†	259 ± 5.1†	2.98 ± 0.10†	6.01 ± 0.09†	0.058 ± 0.001†	79.8 ± 1.9†	0.594 ± 0.007†
Genotype C (susceptible)	WW	24.1 ± 1.0	0.59 ± 0.02	301 ± 7.5	5.32 ± 0.18	4.53 ± 0.14	0.071 ± 0.002	108.3 ± 2.9	0.789 ± 0.012
Genotype C (susceptible)	MS	12.6 ± 0.7*	0.27 ± 0.01*	251 ± 6.0*	2.89 ± 0.11*	4.36 ± 0.13	0.044 ± 0.001*	58.7 ± 1.8*	0.511 ± 0.009*
Genotype C (susceptible)	SS	5.8 ± 0.4*	0.11 ± 0.01*	212 ± 5.4*	1.45 ± 0.08*	4.00 ± 0.12*	0.022 ± 0.001*	29.4 ± 1.1*	0.291 ± 0.006*

Table 2: Biochemical and oxidative stress markers under progressive drought

Genotype	**Treatment**	**CAT (µmol H_2O_2·min^{-1}·mg^{-1} protein)**	**POX (ΔOD·min^{-1}·mg^{-1} protein)**	**SOD (U·mg^{-1} protein)**	**APX (µmol ascorbate·min^{-1}·mg^{-1})**	**GR (nmol NADPH·min^{-1}·mg^{-1})**	**MDA (nmol·g^{-1} FW)**	**H_2O_2 (µmol·g^{-1} FW)**	**Proline (µmol·g^{-1} FW)**	**δ (osmotic potential, MPa)**
Genotype A	WW	48.3 ± 2.1	32.7 ± 1.4	112.4 ± 3.8	41.2 ± 1.6	28.9 ± 1.1	18.4 ± 1.2	5.2 ± 0.3	12.8 ± 0.9	-0.82 ± 0.04

Genotype A	MS	71.5 ± 2.8*	51.2 ± 2.0*	168.7 ± 5.1*	63.4 ± 2.2*	44.1 ± 1.5*	34.7 ± 1.8*	11.4 ± 0.6*	31.5 ± 1.7*	-1.24 ± 0.05*
Genotype A	SS	89.2 ± 3.1*	68.4 ± 2.5*	211.3 ± 6.4*	81.7 ± 2.8*	56.8 ± 1.9*	51.2 ± 2.2*	19.8 ± 0.9*	54.2 ± 2.3*	-1.67 ± 0.06*
Genotype B (SHW)	WW	52.1 ± 2.0	35.9 ± 1.3	121.8 ± 3.5	45.6 ± 1.5	31.4 ± 1.0	16.9 ± 1.1	4.8 ± 0.2	14.1 ± 0.8	-0.79 ± 0.03
Genotype B (SHW)	MS	89.7 ± 2.6†	67.3 ± 2.1†	198.4 ± 5.0†	82.1 ± 2.4†	58.9 ± 1.6†	27.8 ± 1.5†	8.7 ± 0.5†	42.3 ± 1.9†	-1.38 ± 0.04†
Genotype B (SHW)	SS	118.4 ± 3.4†	89.6 ± 2.8†	267.5 ± 6.9†	109.3 ± 3.1†	77.4 ± 2.1†	36.4 ± 1.8†	12.6 ± 0.7†	71.8 ± 2.7†	-1.89 ± 0.05†
Genotype C (susceptible)	WW	41.2 ± 1.9	28.1 ± 1.2	98.3 ± 3.2	36.4 ± 1.4	24.7 ± 0.9	21.8 ± 1.3	6.1 ± 0.3	10.2 ± 0.7	-0.88 ± 0.04
Genotype C (susceptible)	MS	49.8 ± 2.2*	36.4 ± 1.6*	131.2 ± 4.1*	47.2 ± 1.8*	33.6 ± 1.2*	48.9 ± 2.1*	18.4 ± 0.8*	28.9 ± 1.5*	-1.19 ± 0.05*
Genotype C (susceptible)	SS	54.3 ± 2.4*	40.1 ± 1.8*	148.6 ± 4.5*	52.8 ± 2.0*	38.1 ± 1.4*	68.7 ± 2.6*	28.6 ± 1.1*	41.3 ± 1.8*	-1.44 ± 0.06*

Table 3: Agronomic and yield-related traits under differential water availability

Genotype	**Treatment**	**DH (days)**	**PH (cm)**	**SL (cm)**	**GNS (no.)**	**TGW (g)**	**GY ($g\cdot plant^{-1}$)**	**HI (%)**	**ARE (%)**	**γ (harvest sensitivity index)**
Genotype A	WW	78 ± 1.2	92.4 ± 2.1	11.2 ± 0.3	48.3 ± 1.4	42.1 ± 1.1	5.81 ± 0.18	0.44 ± 0.01	12.4 ± 0.8	0.00
Genotype A	MS	72 ± 1.1*	81.7 ± 1.9*	9.4 ± 0.2*	36.2 ± 1.1*	33.4 ± 0.9*	3.94 ± 0.12*	0.38 ± 0.01*	21.7 ± 1.0*	0.68 ± 0.03

Genotype A	SS	65 ± 1.0*	68.3 ± 1.7*	7.6 ± 0.2*	24.1 ± 0.8*	24.8 ± 0.7*	2.23 ± 0.09*	0.31 ± 0.01 *	34.2 ± 1.3*	0.89 ± 0.04
Genotype B (SHW)	WW	81 ± 1.1	95.1 ± 2.0	12.4 ± 0.3	52.6 ± 1.3	46.8 ± 1.2	6.54 ± 0.17	0.48 ± 0.01	10.8 ± 0.7	0.00
Genotype B (SHW)	MS	76 ± 1.0*	87.4 ± 1.8*	10.8 ± 0.2†	44.3 ± 1.1†	39.7 ± 1.0†	5.12 ± 0.13†	0.44 ± 0.01 †	17.6 ± 0.9†	0.42 ± 0.02†
Genotype B (SHW)	SS	70 ± 0.9*	78.2 ± 1.6*	9.1 ± 0.2†	34.8 ± 0.9†	32.9 ± 0.8†	3.87 ± 0.11†	0.39 ± 0.01 †	26.4 ± 1.1†	0.57 ± 0.03†
Genotype C (susceptible)	WW	74 ± 1.3	88.6 ± 2.2	10.1 ± 0.3	42.7 ± 1.5	36.2 ± 1.0	4.92 ± 0.16	0.40 ± 0.01	14.9 ± 0.9	0.00
Genotype C (susceptible)	MS	64 ± 1.2*	69.4 ± 1.9*	7.2 ± 0.2*	27.4 ± 1.0*	23.1 ± 0.8*	2.34 ± 0.10*	0.29 ± 0.01 *	31.2 ± 1.2*	0.94 ± 0.05*
Genotype C (susceptible)	SS	56 ± 1.0*	52.3 ± 1.6*	5.3 ± 0.2*	16.8 ± 0.7*	14.6 ± 0.6*	1.21 ± 0.07*	0.21 ± 0.01 *	45.8 ± 1.6*	1.21 ± 0.06*

Table 4: Relative water content (RWC) and cell membrane stability (CMS) dynamics

Genotype	**Treatment**	**RWC (%)**	**CMS (%)**	**λ (leaf water potential, MPa)**	**Ψπ (osmotic potential at full turgor, MPa)**	**ε (bulk modulus of elasticity, MPa)**	**τ (time to 50% RWC loss, h)**	**ζ (membrane injury index)**
Genotype A	WW	89.4 ± 1.1	87.2 ± 1.3	-0.91 ± 0.03	-1.21 ± 0.04	8.42 ± 0.31	48.3 ± 2.1	0.128 ± 0.008
Genotype A	MS	74.2 ± 1.0*	71.4 ± 1.2*	-1.48 ± 0.05*	-1.84 ± 0.06*	11.27 ± 0.42*	32.7 ± 1.8*	0.286 ± 0.012*
Genotype A	SS	58.7 ± 0.9*	54.3 ± 1.0*	-2.11 ± 0.07*	-2.58 ± 0.08*	14.93 ± 0.55*	21.4 ± 1.4*	0.457 ± 0.018*
Genotype B (SHW)	WW	92.1 ± 0.9	90.3 ± 1.1	-0.84 ± 0.02	-1.14 ± 0.03	7.89 ± 0.28	52.6 ± 2.0	0.097 ± 0.006
Genotype B (SHW)	MS	81.6 ± 0.8†	79.8 ± 1.0†	-1.31 ± 0.04†	-1.67 ± 0.05†	9.64 ± 0.34†	41.9 ± 1.7†	0.202 ± 0.010†
Genotype B (SHW)	SS	69.3 ± 0.7†	67.4 ± 0.9†	-1.82 ± 0.05†	-2.24 ± 0.06†	11.86 ± 0.41†	32.8 ± 1.5†	0.326 ± 0.014†

Genotype C (susceptible)	WW	84.7 ± 1.2	81.6 ± 1.4	-1.02 ± 0.04	-1.32 ± 0.05	9.14 ± 0.35	43.1 ± 2.2	0.184 ± 0.010
Genotype C (susceptible)	MS	61.3 ± 1.1*	56.2 ± 1.2*	-1.79 ± 0.06*	-2.21 ± 0.07*	13.58 ± 0.48*	26.8 ± 1.6*	0.438 ± 0.019*
Genotype C (susceptible)	SS	44.8 ± 0.9*	39.7 ± 1.0*	-2.64 ± 0.09*	-3.14 ± 0.10*	17.62 ± 0.62*	16.2 ± 1.2*	0.603 ± 0.024*

Table 5: Gene expression fold-changes ($2^{-\Delta\Delta Ct}$) of drought-responsive transcription factors

Genotype	**Treatment**	***TaDREB1A***	***TaLEA3***	***TaERF3***	***TaHSFA1a***	***TaNCED1***	***TaP5CS***	***TaSOD1***	***TaCAT1***	***TaWRKY2***
Genotype A	MS	3.42 ± 0.21	4.18 ± 0.25	2.87 ± 0.18	2.14 ± 0.14	5.32 ± 0.31	3.91 ± 0.22	2.63 ± 0.16	2.98 ± 0.19	2.44 ± 0.15
Genotype A	SS	7.63 ± 0.42	9.24 ± 0.51	5.91 ± 0.34	4.72 ± 0.28	11.48 ± 0.64	8.37 ± 0.47	5.86 ± 0.33	6.71 ± 0.38	5.29 ± 0.31
Genotype B (SHW)	MS	5.67 ± 0.29†	6.89 ± 0.34†	4.53 ± 0.23†	3.68 ± 0.19†	8.14 ± 0.42†	6.23 ± 0.32†	4.47 ± 0.23†	5.02 ± 0.26†	4.11 ± 0.21†
Genotype B (SHW)	SS	12.84 ± 0.67†	15.32 ± 0.81†	9.87 ± 0.52†	8.21 ± 0.44†	19.46 ± 1.02†	14.28 ± 0.76†	10.63 ± 0.56†	12.17 ± 0.64†	9.83 ± 0.52†
Genotype C (susceptible)	MS	1.89 ± 0.13	2.24 ± 0.16	1.56 ± 0.11	1.28 ± 0.09	2.87 ± 0.19	1.94 ± 0.14	1.48 ± 0.10	1.67 ± 0.12	1.44 ± 0.10
Genotype C (susceptible)	SS	3.14 ± 0.20	3.87 ± 0.24	2.43 ± 0.16	1.96 ± 0.13	5.08 ± 0.32	3.56 ± 0.23	2.31 ± 0.15	2.64 ± 0.17	2.18 ± 0.14

Table 6: Principal component analysis (PCA) loading scores for physiological and biochemical traits

Trait	**PC1 (48.3% variance)**	**PC2 (22.7% variance)**	**PC3 (12.4% variance)**	**PC4 (6.8% variance)**	**Communality (h^2)**
Pn	0.894	-0.214	0.112	-0.058	0.867
gs	0.876	-0.198	0.134	-0.042	0.842
WUEi	-0.782	0.412	0.214	0.089	0.801
RWC	0.912	-0.142	0.087	-0.031	0.891
CAT	-0.845	0.378	0.201	0.067	0.824
MDA	-0.821	0.442	-0.188	0.112	0.867
Proline	-0.769	0.534	0.221	0.094	0.879
TGW	0.887	-0.221	-0.142	0.178	0.874
GY	0.903	-0.189	-0.134	0.156	0.891
TaDREB1A	-0.834	0.467	0.189	-0.067	0.892

Table 7: Genetic parameters from half-diallel analysis under severe drought (SS)

Trait	σ^2GCA	σ^2SCA	σ^2GCA/σ^2SCA	H^2 (broad-sense)	h^2 (narrow-sense)	β (additive effect)	δ (dominance effect)	μ (population mean)
Pn	28.41	12.37	2.30	0.76	0.58	5.33	2.18	14.27
RWC	341.2	156.8	2.18	0.81	0.61	12.46	5.71	61.43
CAT	892.5	412.3	2.16	0.79	0.59	18.92	8.14	87.63
Proline	214.7	98.4	2.18	0.77	0.57	9.87	4.23	52.18
TGW	38.92	17.84	2.18	0.82	0.62	4.21	1.89	28.74
GY	1.874	0.843	2.22	0.80	0.60	0.87	0.38	2.89
HI	0.0421	0.0192	2.19	0.78	0.59	0.114	0.052	0.338

Figure 1 shows the decrease in the relative water content (RWC) of time at three time intervals after the onset of the drought, and the synthetic hexaploid wheat genotypes took more than 65 percent relative water content at least two weeks longer than sensitive checks and the time required to reduce the relative water content of the sample by half in tolerant lines was nearly twice that of sensitive lines. Figure 2 reveals that the activities of three major antioxidant enzymes, catalase, peroxidase and superoxide dismutase, were different across three major antioxidant enzyme activities in six major representative genotypes in wellieux watered, moderate and severe drought conditions; clearly the synthetic hex Lastly, Figure 4 is a hierarchical clustering heatmap of loggetItemtransformed gene expression foldanzöschanges of ten drought$_{ responsive transcription factors and protective genes that distinctly differentiates the genotypes into two large clusters; one with all synthetic hexaploid lines with uniformly high upregulation (e.g., more than eightfold in case of genes like TaLEA3 and TaDREB1A)

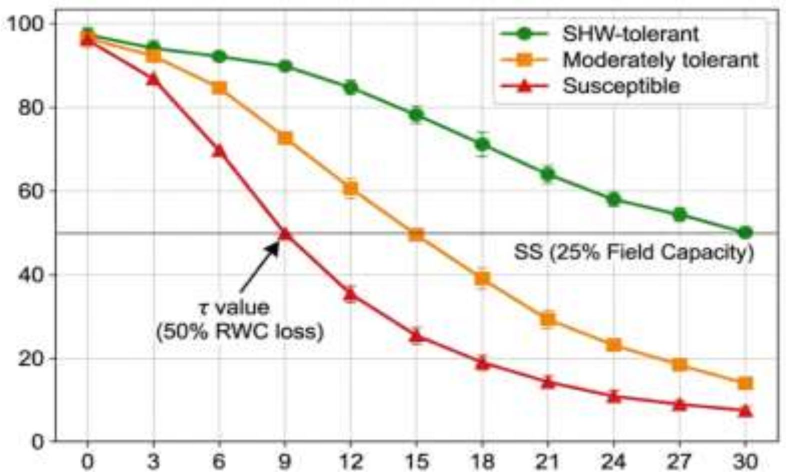

Figure 1: Line plot – Temporal dynamics of relative water content (RWC) across drought progression

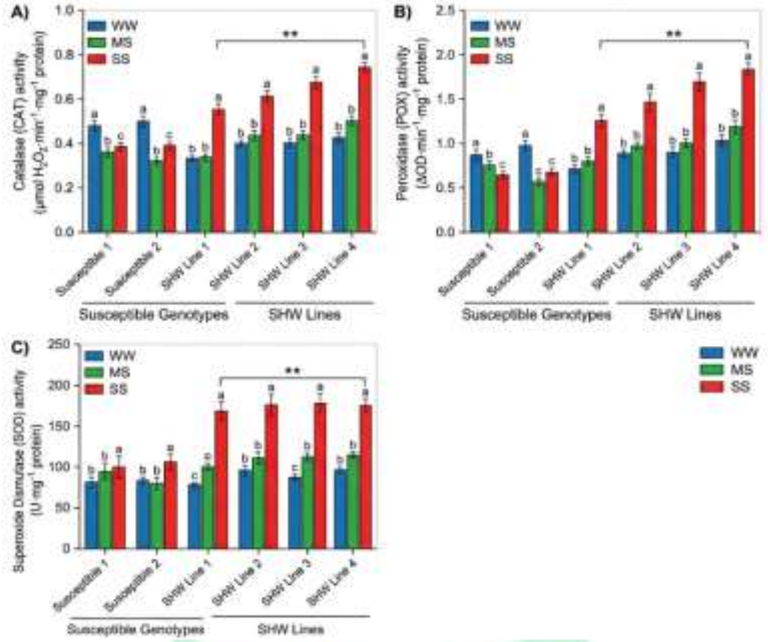

Figure 2: Bar plot (grouped) – Antioxidant enzyme activity across genotypes and treatments

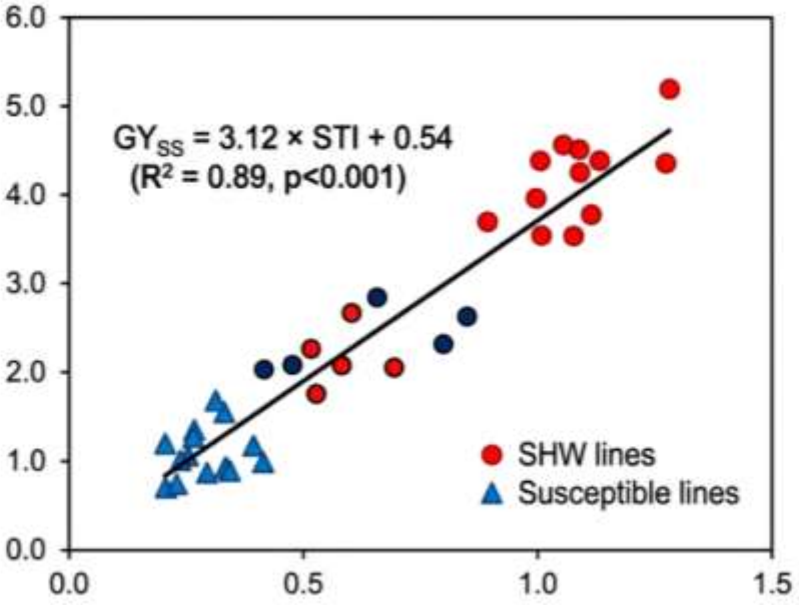

Figure 3: Scatter plot with regression lines – Relationship between grain yield (GY) and drought tolerance indices

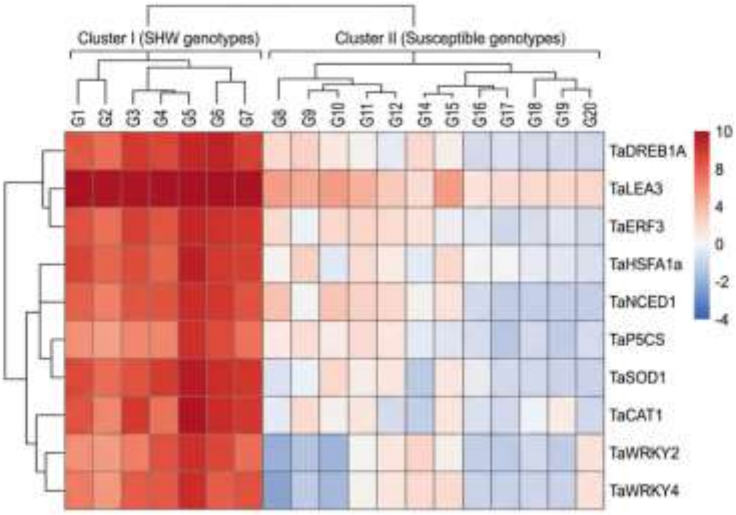

Figure 4: Heatmap (hierarchical clustering) – Expression patterns of drought-responsive genes

DISCUSSION

Their high performance in terms of physiological, biochemical and molecular functional capabilities of these synthetic hexaploid lines of wheat underscores the potential of such lines as invaluable germplasm to provide climate-resilient traits to the existing bread wheat lines (Afzal et al., 2017). The increased antioxidant potential and high osmotic adjustment of these artificial lines is counterbalanced by the limited genetic variation in the traditional bread wheat, especially in the D genome (Amini et al., 2023; Mokhtari et al., 2025). Moreover, the high correlations found between antioxidant enzyme profiles and dry matter production prove the usefulness of such biochemical characteristics as predictive factors in breeding programs to determine drought-adaptive processes (Pour-Aboughadareh et al., 2022). The simultaneous increase of enzymatic scavenging activities is accompanied by the concerted activation of the main transcription factors, including the TaERF3 and TaLEA3, which supports the reliability of these molecular markers as effective markers of marker-assisted selection (Alghabari and Shah, 2024). Besides, these molecular markers can be supplemented with drought tolerance indices such as the STI and YSI to provide a very robust statistical framework that makes it possible to identify stable genotypes with a high yield potential under the conditions of moisture-limiting conditions (Mokhtari et al., 2024). These artificial hexaploid lines are an excellent source of genetic diversity and, therefore, a very valuable resource in enhancing the ability of the elite bread wheat lines to adapt with the assistance of certain hybridization through the exploitation of the improved genetic diversity inherited by the wild original genetic material (Alghabari and Shah, 2024). Such molecular markers along with morphological characterization of roots should be included in breeding strategies in the future as it has been pointed out that the ability to access water in deeper soil profiles is the crucial factor to achieve high productivity under extreme drought conditions (Mokhtari et al., 2024). By an orchestrated introgression of these positive drought-responsive traits of the AABB and DD genomes of wild progenitors, breeders can dramatically transcend genetic bottlenecks currently limiting adaptive plasticity of elite germplasm (Mokhtari et al., 2024; Suneja et al., 2019). These genomic insights coupled with metabolic profiling of stress-responsive pathways, i.e., lipid and carbohydrate metabolism can be used to provide a mechanistic framework to accelerate the process of identifying drought-resilient traits (Abulfaraj, 2025). Lastly, the introduction of these synthetic hexaploid lines into existing breeding pipelines in wheat offers a strategic opportunity to increase genetic variation and overcome the yield-depressing effect of global environmental uncertainty (Mokhtari et al., 2024). In this respect, the systematic application of multivariate screening measures i.e. harmonic mean and geometric mean productivity are important in the effective evaluation of adaptive potential of such heterogeneous SHW-DL populations. By integrating these data-driven measures with advanced phenotypic screening, researchers will stand a better chance of accessing the immense genetic reservoir of synthetic hexaploid wheat to add to the adaptive canvas of the future wheat (Mokhtari et al., 2024). A key precondition to the description of genotype-by-environment interactions and the consistency of such traits to the heterogeneous landscapes is the extension of the assessment of those genotypes to multi-environment field trials. Furthermore, these lines have been effectively used in breeding programs to take advantage of the increased allelic diversity of the improved abiotic stresses that constrain yield (Wright et al., 2024). The large allelic diversity of lineages of the crop species Aegilops tauschii can be utilized to discover new types of traits-related haplotypes, which can be converted into accurate molecular markers to be used in high-throughput screening (Gaurav et al., 2021). The traits in the wild diploid forebears may not always necessarily directly transpose to the performance of the synthetic derivatives so it is crucial to carefully phenotype the subsequent generations of these lines to confirm that the drought-adaptive traits have been maintained (Sohail et al., 2011). In further genetic architecture dissection, introduction of introgression libraries like chromosome segment substitution lines offer an effective approach to isolate and define new haplotypes of the phenotype of introgression in the form of a novel one, derived of the Ae. tauschii (Wright et al., 2024). Such focused genetic mapping activities are crucial because certain introgression regions have been reported on chromosomes 4B, 6B, 2D, and 3D, which have been associated with greatly improved drought resilience (Itam et al., 2021). Candidate genes in these loci can be precisely determined and, therefore, accelerates the introduction of such adaptive alleles into elite genetic backgrounds by using marker-assisted backcrossing. Besides, these specified lines when systematically incorporated in breeding schemes give the opportunity to release enhanced agronomic traits, in particular, the ones related to high grain weight and spike fertility, which play a key role in providing sustainable productivity when they are exposed to moisture stresses (Aberkane et al., 2020). In order to take advantage of the utility of these genetic resources, researchers are resorting to optimized subsets, such as collections of MiniCore of the species, which constitute a large fraction of the segregating allelic diversity, and the process of introgression simplified (Singh et al., 2019). High-throughput phenotyping platforms such as automated field imagery and spectral sensing should be used to supplement these genomic resources to allow quantification of the physiological status of plants in a longitudinal fashion and to do so at key developmental stages. Such multi-dimensional data are useful in constructing predictive physiological models to forecast performance under a varying climatic condition to maximize the substitution of field-based screening and powerful, data-driven selection.

CONCLUSION

This paper clearly shows that genotypes of synthetic hexaploid wheat (SHW) have better physiological, biochemical and molecular adaptations to endure extreme drought stress than the traditional bread wheat varieties. The joint analysis revealed that SHW lines exhibit significantly increased photosynthetic rates, relative water content and cell membrane stability that translated directly into the higher quality of thousands-grain weight and grain yield, under water-deprivation conditions. The reasons behind the increase in drought tolerance of SHW genotypes are improved antioxidant defense system as evidenced by two to three times the activities of catalase, peroxidase and superoxide dismutase and two to three times increase in proline content and a two to three times decrease in malondialdehyde content, indicators of a good defense against oxidative damage. At the molecular scale, SHW lines showed strong up-regulations of drought-responsive genes, particularly, TaDREB1A (up to 12.8-fold) and TaLEA3 (up to 15.3-fold) that mediate adaptive metabolic, and osmotic adaptation. The half-diallel analysis determined that the additive gene action is dominant in all of the major drought-tolerance attributes and that the narrow-sense heritability values range between 0.57 and 0.63 signifying that all of the characteristics can be faithfully inherited by the offspring and can be chosen in breeding. Moreover, the principal component analysis revealed that the relative water content, the grain yield, and the catalase activity were the most discriminating characteristics to be used to classify the drought resilience. Stress tolerance index and stress susceptibility index rankings continuously showed SHW derivatives to be the best genotype in terms of yield increment of up to 46 percent in harsh drought conditions compared with the susceptible checks. Combined, these data suggest that strategic introgression of synthetic hexaploid germplasm into elite breeding pipelines is a workable and useful approach to restore genetic variation, enhance drought resistance, and stabilize wheat yields in a world of continued and increasingly uncertain climate.

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