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Drought tolerance mechanisms in contrasting wheat genotypes: coordination of ABA signaling, antioxidant defense, and stress-responsive genes

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Research Article

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Abstract

Background

Drought stress is a major constraint on wheat productivity, particularly under climate change scenarios characterized by increasing drought frequency and severity. Drought tolerance involves the coordinated regulation of hormonal signaling, osmotic adjustment, antioxidant defense, and stress-responsive gene expression.

Methods

This study evaluated physiological, biochemical and molecular responses of drought-tolerant and drought-sensitive wheat genotypes at the seedling stage. Drought stress was applied by withholding irrigation for five days. Abscisic acid (ABA) accumulation, osmolyte content, antioxidant enzyme activities, and the expression of thirteen drought-responsive genes were analyzed.

Results

Drought stress significantly increased ABA accumulation in the sensitive genotype BT, whereas tolerant genotype Utique maintained stable ABA levels. Tolerant genotypes Utique and D117 accumulated higher proline and soluble sugar contents, indicating improved osmotic adjustment. They also showed enhanced antioxidant defense, particularly higher superoxide dismutase (SOD) and catalase (CAT) activities. Gene expression analysis revealed contrasting responses: sensitive genotypes exhibited stronger induction of ABA biosynthesis genes (NCED and AAO), stress-related transcription factors (WRKY44 and NAC48), and repression of the photosynthesis-related gene LHCb1. In contrast, tolerant genotypes showed higher expression of genes associated with osmolyte biosynthesis (P5CS and BADH), ion homeostasis (NHX1 and AVP1), and water transport (PIP2;1).

Conclusions

The identified physiological and molecular traits highlight key mechanisms underlying drought tolerance and provide potential biomarkers for the selection of drought-resilient wheat genotypes in breeding programs targeting water-limited environments.

Introduction

The combined impacts of climate change and rapid population growth are exacerbating global food security challenges [1]. Among the major constraints to sustainable agricultural production, water scarcity has emerged as one of the most critical, particularly in North African countries, where only 22.7% of cultivated land is currently irrigated [2].

Durum wheat (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.) are among the four most extensively cultivated cereal crops worldwide, contributing substantially to both global grain production and human caloric intake [3]. However, wheat is highly sensitive to water deficit, and drought stress frequently causes significant yield losses [4]. In marginal rainfed environments, water limitation can reduce wheat productivity by approximately 35% compared with irrigated systems, posing a serious threat to future food security [1, 4, 5]. Drought-induced reductions in wheat growth and yield are primarily associated with osmotic stress, ion toxicity, oxidative damage and nutrient imbalance, which collectively disrupt key physiological processes [5].

Developing drought-tolerant wheat cultivars remains a major challenge due to the complex and multigenic nature of drought adaptation [4, 6]. Drought tolerance is governed by highly coordinated physiological, biochemical, and molecular mechanisms involving interconnected signaling pathways and gene regulatory networks [7]. Notably, among 146 stress-responsive genes identified in wheat seedlings under abiotic and biotic stress conditions, more than half have been associated with drought tolerance functions [8, 9].

A central component of the drought response is abscisic acid (ABA), which serves as a key phytohormonal signal regulating stomatal closure, osmotic adjustment and stress-responsive gene expression [4, 10, 11]. Drought stress also promotes the excessive accumulation of reactive oxygen species (ROS), necessitating the activation of efficient antioxidant defences systems. Enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), and glutathione reductase (GR) play essential roles in scavenging ROS and minimizing oxidative damage [12, 13]. Concurrently, compatible osmolytes, including proline and soluble sugars, accumulate to maintain cellular osmotic balance, stabilize proteins and membranes and preserve metabolic activity under stress conditions [13].

These hormonal, antioxidant and osmotic responses are closely integrated with extensive transcriptional reprogramming mediated by transcription factors (TFs), particularly members of the NAC and WRKY families, which regulate downstream stress-responsive genes [14–17]. Activation of these TFs induces the expression of genes involved in ABA biosynthesis and signaling (*NCED*, *AAO*), photosynthetic performance (*Rubisco*, *LHCb1*), antioxidant defense (*CAT*, *MnSOD*), osmolyte biosynthesis (*P5CS*, *BADH*), and water and ion transport systems, including aquaporins (*PIP2;1*), vacuolar H^+-pyrophosphatase (*AVP*), and Na^+/H^+ antiporters (*NHX*). Together, these mechanisms contribute to maintaining cellular homeostasis and enhancing drought tolerance [14–16, 18].

In the present study, four wheat genotypes exhibiting contrasting responses to drought stress were evaluated under controlled environmental conditions to investigate their physiological and molecular adaptation mechanisms at the seedling stage. Drought stress was imposed on 10-day-old seedlings by withholding irrigation for five days. Specifically, the objectives were to: (i) quantify leaf ABA accumulation under drought conditions; (ii) evaluate the activities of key antioxidant enzymes (SOD, CAT, POD, and GR) as indicators of ROS-scavenging capacity; (iii) determine the accumulation of compatible osmolytes, namely proline and soluble sugars and (iv) analyze the expression patterns of thirteen drought-

responsive genes. By integrating physiological, biochemical, and molecular analyses, this study provides a comprehensive understanding of drought tolerance mechanisms in wheat, identifies potential biomarkers for genotype selection and contributes to the development of breeding strategies aimed at enhancing early-stage drought resilience under water-limited environments.

Materials and Methods

Plant material and Experimental Design

Four wheat genotypes contrasting in drought tolerance were used in this study: the durum wheat genotypes D117 (drought-tolerant) and AG3 (drought-sensitive), and the bread wheat genotypes Utique (tolerant) and BT (sensitive). Seeds were dehulled, surface-sterilized, and germinated on 0.8% water–agar medium in darkness at 21°C following Bianco and Defez [19]. Five-day-old uniform seedlings were transplanted into pots containing a sand–perlite mixture (3:1, v/v) and grown under controlled conditions (16 h photoperiod, 19–23°C, 75% relative humidity). Plants were irrigated daily and supplied weekly with a nitrogen-free nutrient solution. Drought stress was imposed on 10-day-old seedlings by withholding irrigation for five days. Leaf samples from control and drought-stressed plants were collected at the end of the treatment for subsequent analyses.

Quantification of Abscisic Acid (ABA)

Leaf ABA content was determined in the wheat genotypes Utique and BT using a competitive enzyme-linked immunosorbent assay (ELISA) kit. Fresh leaf tissue (250 mg) was homogenized in extraction buffer, incubated overnight in the dark, and centrifuged (10,000 × g, 10 min, 4°C). ABA concentrations were then quantified according to the manufacturer's protocol using ABA antigen-coated microplates. In this competitive assay, endogenous ABA in the extracts competed with immobilized ABA for binding to specific antibodies. Following incubation and washing, bound antibodies were detected using an HRP-conjugated secondary antibody. Color development was measured at 450 nm and ABA concentration was calculated from a standard curve. Absorbance values were inversely proportional to ABA content in the samples.

Proline and soluble sugar contents

Proline content was determined using the colorimetric method described by Bates et al [20]. Absorbance was measured with a Biochrom Libra S22 spectrophotometer and proline concentrations were quantified using an L-proline standard curve.

Total soluble sugar content was quantified following the method of McCready et al [21]. Soluble carbohydrates were estimated colorimetrically using glucose as the reference standard, and

concentrations were calculated from a calibration curve prepared with a 0.1 g L^{-1} glucose solution. Results were expressed on a fresh weight basis.

Antioxidant Enzyme Activities

Total soluble proteins were extracted from wheat leaves and quantified according to Bianco and Defez [19]. Briefly, 500 mg of fresh leaf tissue was homogenized in Tris–HCl buffer and centrifuged at 13,000 × g for 30 min at 4°C. Protein concentration in the resulting supernatant was determined using the Bradford assay with bovine serum albumin (BSA) as the standard [22].

The activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and glutathione reductase (GR), were evaluated by kinetic spectrophotometric assays. SOD (EC 1.15.1.1) activity was measured based on the inhibition of nitroblue tetrazolium (NBT) photoreduction in the presence of riboflavin, following Rubio et al [23]. CAT (EC 1.11.1.6) activity was determined by monitoring the decomposition of H_2O_2 and expressed as μmol H_2O_2 consumed min^{-1} [24]. POX (EC 1.11.1.7) activity was assayed by measuring guaiacol oxidation in the presence of hydrogen peroxide [24]. GR (EC 1.6.4.2) activity was determined according to Tejera, Campos [25] by monitoring the decrease in NADPH absorbance associated with the reduction of oxidized glutathione (GSSG) over 180 s.

RNA Extraction and qRT-PCR Analysis

Total RNA was extracted from 100 mg of frozen leaf tissue using TRIzol™ reagent (Sigma-Aldrich) according to the manufacturer's instructions. After phase separation with chloroform and centrifugation (12,000 × g, 15 min, 4°C), RNA was precipitated with isopropanol, resuspended in RNase-free water, and treated with the TURBO DNA-free™ kit (Invitrogen) to remove residual genomic DNA. RNA integrity was verified by agarose gel electrophoresis, while concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific).

First-strand cDNA was synthesized from 2 μg of total RNA using the SuperScript™ III Reverse Transcriptase kit (Invitrogen). Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry on an Agilent AriaMx Real-Time PCR System (Agilent Technologies). Amplification conditions consisted of an initial denaturation at 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. Primer sequences for the thirteen target genes are listed in Table 1 [15, 26–32].

Table 1
Primer sequences used for the amplification of the studied genes

Functional category	Gene	Primer sequences	
Signaling	*NCED*	F 5'- CCTGCTGCCTCTTCTGCT -3'	[26]
		R 5'- ACCAAGTGCTCTTCCGTCTC – 3'	
	AAO	F 5'- TTGGCGTTGTGATTGCTG -3'	[26]
		R 5'- GCTCAAGGTTCTCGGTGCT – 3'	
Transcription factors	*NAC48*	F 5'- CCAAGGGCGACAAGACCAA – 3'	[15]
		R 5'- CGAGGCGCCTTTCTTGTTG – 3'	
	WRKY44	F 5'- CCAACGGCGGTGATAACTACAT-3'	[27]
		R 5'- GCTACTGGATGCTGCCTTCTG-3'	
Photosynthesis	*RUBISCO*	F 5'- GTGGTAGAGCGTGTTATGAGTGTC – 3'	[28]
		R 5'- CGGCACAAAAGACAAAACGGT – 3'	
	LHCb1	F 5'- GTAGTCGACCCACTCTACCC -3'	[28]
		R 5'- ATAGAAAACATGGCGAGGCG -3'	
ROS scavenging	*CAT*	F 5'- TGATACCCAAAGGCACCG – 3'	[29]
		R 5'- GCAGCCAGATAGAACACG -3'	
	MnSOD	F 5'- TTTTCCTCTAGCTGCTCCCC -3'	[29]
		R 5'- CGGAAAGGCACATGAAACCA -3'	
Osmotic adjustment	*P5CS*	F 5'- GGAGACAAGTCCCGTGTTGGTAG – 3'	[29]
		R 5'- GCAGCAACAGCCATTTCACGGAC – 3'	
	BADH	F 5'- GGCTGGGTTCAGTCATCAGT -3'	[30]
		R 5'- CCAAATTTGCATTGATGTGC – 3'	
Aquaporin & ion channels	*AVP1*	F 5'- GACCGGTCTTGCCATTGATG – 3'	[31]
		R 5'- CTGAGCCAATTGCGAATCCC – 3'	
	NHX1	F 5'- GCCTGGTTCACCCATAGAGA -3'	[31]
		R 5'- CACCGAAAGAATCCCAAGAG – 3'	
	PIP2:1	F 5'- GGCCGGACTGAAGTGTAGAT-3'	[32]
		R 5'- ACAGGACAAAGGTGTGGGAT-3'	

Relative gene expression levels were calculated using the $2^{-\Delta\Delta}$Ct method [33], with *tubulin* serving as the reference gene for normalization. Expression data were analyzed using AriaMx software (v1.6) and are presented as fold changes in drought-stressed plants relative to well-watered controls.

Statistical Analysis

Statistical analyses were conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was performed to evaluate differences among treatments. Data are presented as mean ± standard deviation (SD) based on three biological replicates, each with at least three technical replicates. When significant effects were detected, means were compared using Tukey's post hoc test at a 5% probability level ($P \leq 0.05$), and different letters indicate statistically significant differences among groups.

Levels of statistical significance are indicated in tables as ns (not significant), * ($P \leq 0.05$), and ** ($P \leq 0.01$). In addition, principal component analysis (PCA) was carried out using OriginPro 2023 (OriginLab) to explore multivariate patterns and identify key physiological and transcriptional traits associated with genotype-specific drought responses in wheat.

Results

ABA accumulation under drought stress

Leaf ABA content showed strong genotype-dependent variation under water deficit (Fig. 1A). The sensitive genotype BT exhibited a significant increase (98%) in ABA compared with control plants. In contrast, the tolerant genotype Utique showed no significant change in ABA levels under the same conditions.

Osmolyte accumulation

Drought stress significantly increased proline and soluble sugar contents in all genotypes, with marked differences in magnitude (Table 2). The highest increases were observed in D117 (proline: 72.5%; sugars: 106.5%), followed by Utique (proline: 34.9%; sugars: 61.2%), whereas BT and AG3 showed weaker responses. Overall, osmolyte accumulation varied significantly among genotypes.

Table 2
Effect of drought stress on proline and soluble sugar (SS) accumulation in leaves of the four studied wheat genotypes

Genotype	Treatment	Proline content (mg g^{-1} DW)	Total soluble sugar content (mg g^{-1} DW)
D117	Control	15.28 ± 0.50 ^{d}	43.72 ± 1.05 ^{de}
	Drought	26.37 ± 1.56 ^{bc}	90.27 ± 4.85 ^{b}
AG3	Control	21.30 ± 1.30 ^{c}	33.59 ± 3.43 ^{e}
	Drought	25.31 ± 2.20 ^{bc}	45.02 ± 1.04 ^{de}
Utique	Control	27.05 ± 1.49 ^{b}	76.60 ± 6.49 ^{c}
	Drought	36.48 ± 1.82 ^{a}	123.46 ± 1.59 ^{a}
BT	Control	28.86 ± 1.02 ^{b}	55.83 ± 1.40 ^{d}
	Drought	41.32 ± 1.59 ^{a}	68.83 ± 4.30 ^{c}
Treatment effect		41.755**	90.930**

Each value represents mean ± SD (n = 3). Different letters within the same column indicate significant differences between treatments (P ≤ 0.05) according to Tukey's test. Treatment effect test for both proline and SS was represented with F values. **Significant at $P \leq 0.01$

Antioxidant enzyme activities

Drought induced differential regulation of antioxidant enzymes (Fig. 1B-E). SOD activity increased significantly in D117 (2.5-fold) and Utique (1.5-fold), whereas no significant change was observed in AG3 and BT (Fig. 1B). CAT activity was strongly induced in D117 (+ 58%) but remained stable in other genotypes (Fig. 1C). POX activity increased in AG3 and Utique (Fig. 1D) while GR activity was strongly induced in BT (7-fold) with moderate increases in AG3 and Utique and no change in D117 (Fig. 1E).

Correlation analysis of physiological traits under drought

Correlation analysis revealed significant relationships between physiological traits (Table 3). ABA correlated positively with GR (r = 0.850**) and proline (r = 0.642*). Proline showed strong correlations with SOD, CAT, and GR. SOD was significantly correlated with CAT and POX, indicating coordinated antioxidant regulation under drought.

Table 3
Pearson's correlation coefficients among different physiological traits in leaves of studied wheat genotypes evaluated under drought stress conditions at seedling stage

ABA	ABA	Proline	SS	SOD	CAT	POX	GR
	1						
Proline	0,642*	1					
SS	-0,308	0,585**	1				
SOD	0,427	0,843**	0,476*	1			
CAT	0,145	0,786**	0,773**	0,667**	1		
POX	-0,404	-0,382	-0,046	0,658**	-0,356	1	
GR	0,850**	0,795**	0,207	0,583**	0,500*	-0,359	1
*Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$							

Gene expression responses

Drought stress induced significant transcriptional changes in all genotypes (Fig. 2A-D). ABA biosynthesis genes *NCED* (Fig. 2A) and *AAO* (Fig. 2B) were strongly upregulated in sensitive genotypes (AG3, BT) but weakly induced in tolerant genotypes (D117 and Utique).

Transcription factors showed differential regulation: *WRKY44* (Fig. 2C) was induced in all genotypes, whereas *NAC48* (Fig. 2D) showed strong induction in sensitive genotypes only.

Photosynthesis-related genes were also affected. *LHCb1* (Fig. 3A) was downregulated in all genotypes, with complete repression in sensitive ones. In contrast, *RUBISCO* (Fig. 3B) transcripts increased in all genotypes.

Antioxidant genes *MnSOD* (Fig. 3C) and *CAT* (Fig. 3D) were upregulated under drought, but stronger functional consistency between transcript and enzyme activity was observed in tolerant genotypes.

Osmotic adjustment genes *BADH* (Fig. 4A) and *P5CS* (Fig. 4B), showed higher induction in tolerant genotypes at biochemical level, while transcript accumulation was more pronounced in sensitive genotypes.

Ion transport and water channel genes *NHX1* (Fig. 4C), *AVP1* (Fig. 4D) and *PIP2;1* (Fig. 4E), were strongly induced in tolerant genotypes, with limited or weak expression in sensitive ones.

Multivariate analysis

PCA (Fig. 5) clearly separated tolerant and sensitive genotypes. PC1 (54.03% variance) discriminated genotypes based on stress-response strategies. Tolerant genotypes were associated with *PIP2;1*, *AVP1*,

NHX1 and moderate ABA signaling, while sensitive genotypes clustered with strong ABA biosynthesis (*NCED*, *AAO*) and transcription factor activation (*NAC48*).

Discussion

Drought tolerance in wheat results from the integration of hormonal regulation, osmotic adjustment, antioxidant defences and transcriptional reprogramming [4, 7, 11, 13, 26]. In this study, clear physiological and molecular differences were observed between tolerant (D117, Utique) and sensitive (AG3, BT) genotypes.

ABA plays a central role in drought signaling, particularly in stomatal regulation [26]. The strong ABA accumulation observed in BT (Fig. 1A) suggests rapid activation of water-conserving mechanisms, consistent with an isohydric strategy. In contrast, the absence of ABA induction in Utique indicates a more stable hormonal regulation that may allow sustained gas exchange under water deficit. These results support the view that drought tolerance is not associated with higher ABA accumulation, but rather with controlled ABA responsiveness [10, 34].

Osmotic adjustment was more efficient in tolerant genotypes, as reflected by higher accumulation of proline and soluble sugars (Table 2). These metabolites contribute to cellular osmotic balance and stress protection [5, 35]. The strong correlation between both compounds (Table 3) suggests coordinated regulation [35]. Limited osmolyte accumulation in AG3 indicates reduced capacity to maintain cellular hydration under drought stress [4, 34].

Drought-induced oxidative stress was evident in all genotypes, but antioxidant responses differed significantly (Fig. 1B-E). Tolerant genotypes showed higher SOD and CAT activities, indicating efficient primary ROS detoxification. In contrast, sensitive genotypes relied more on POX and GR, which may represent secondary or compensatory mechanisms. These results suggest that SOD–CAT activity is more strongly associated with drought tolerance than POX–GR activation [36].

At the molecular level, strong induction of ABA biosynthesis genes *NCED* and *AAO* in sensitive genotypes (Fig. 2A-B) suggests that excessive ABA production is linked to stress sensitivity rather than tolerance [11]. This may reflect stronger stress perception rather than adaptive regulation [37].

Transcription factors *WRKY44* and *NAC48* were broadly induced, but *NAC48* showed stronger activation in sensitive genotypes (Fig. 2C-D), supporting its role in ABA-dependent stress signaling and stomatal regulation [15]. In contrast, *WRKY44* appears to be involved in general stress responses rather than genotype-specific tolerance [14, 17, 38–39].

Photosynthetic adjustment was evident through repression of *LHCb1* in all genotypes (Fig. 3A), indicating reduced light-harvesting capacity under drought [40]. However, maintenance of *RUBISCO* expression (Fig. 3B) suggests a compensatory mechanism to preserve carbon fixation potential [41].

Antioxidant gene expression generally reflected enzyme activity more consistently in tolerant genotypes, indicating better coordination between transcriptional and biochemical levels (Fig. 3C-D). In sensitive genotypes, discrepancies suggest post-transcriptional limitations in ROS detoxification [12].

Genes involved in osmotic adjustment (*BADH*, *P5CS*) and ion homeostasis (*NHX1*, *AVP1*) were more functionally effective in tolerant genotypes (Fig. 4A-D), supporting better cellular water and ion balance [12, 35, 42–44]. Similarly, strong induction of *PIP2;1* in tolerant genotypes (Fig. 4E) suggests improved water transport capacity [45].

PCA (Fig. 5) confirmed that drought tolerance is associated with coordinated regulation of water transport, ion homeostasis, and moderate ABA signaling, whereas sensitivity is characterized by strong ABA biosynthesis and stress signaling activation.

Conclusion

The contrasting responses observed among wheat genotypes highlight the complexity of the physiological, biochemical, and molecular mechanisms underlying drought tolerance. In particular, the lack of ABA induction under water deficit in the tolerant genotype suggests a more flexible regulation of stomatal behavior, allowing the maintenance of gas exchange and sustained physiological activity under stress conditions.

Drought-tolerant genotypes also exhibited more efficient osmotic adjustment, as reflected by higher accumulation of proline and soluble sugars, together with a stronger activation of antioxidant defenses. In this context, superoxide dismutase (SOD) and catalase (CAT) emerged as key components of the reactive oxygen species (ROS) detoxification system, contributing to redox homeostasis and improved drought resilience.

At the molecular level, transcriptional profiling identified several drought-responsive genes, including *NCED*, *AAO*, *NAC48*, *PIP2;1*, *AVP1*, and *LHCb1*, which displayed clear genotype-dependent expression patterns under water deficit. These genes represent promising molecular markers for distinguishing drought-tolerant and drought-sensitive wheat genotypes at the seedling stage.

Overall, the integration of physiological, biochemical, and gene expression data provides a coherent framework for understanding drought adaptation in wheat and highlights potential targets for breeding programs aimed at improving productivity under water-limited conditions.

Abbreviations

ABA: abscisic acid; CAT: catalase; SOD: superoxide dismutase; POD: peroxidises; GR: glutathione reductase, ROS: reactive oxygen species; TFs; transcription factors, NBT: nitroblue tetrazolium.

Declarations

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Ethical approval This article does not contain any studies conducted on human or animal subjects.

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Consent for participation Not applicable.

Competing interests The authors declare no competing interests.

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Figures

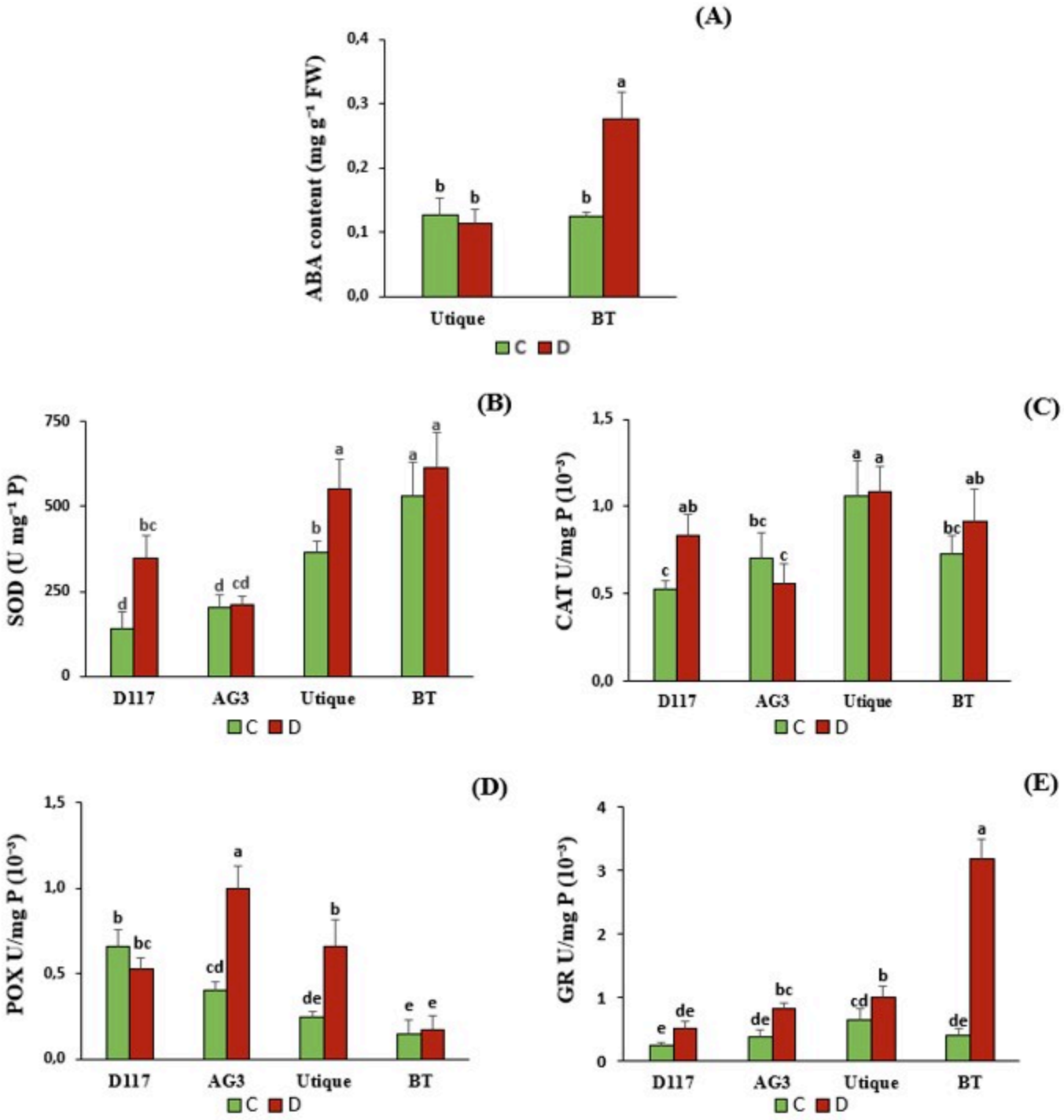

Figure 1

Effect of drought stress under (C) control and (D) drought conditions on (A) ABA content and the activities of antioxidant enzymes: (B) superoxide dismutase (SOD), (C) catalase (CAT), (D) peroxidase (POX), and (E) glutathione reductase (GR) in wheat leaves at the seedling stage.

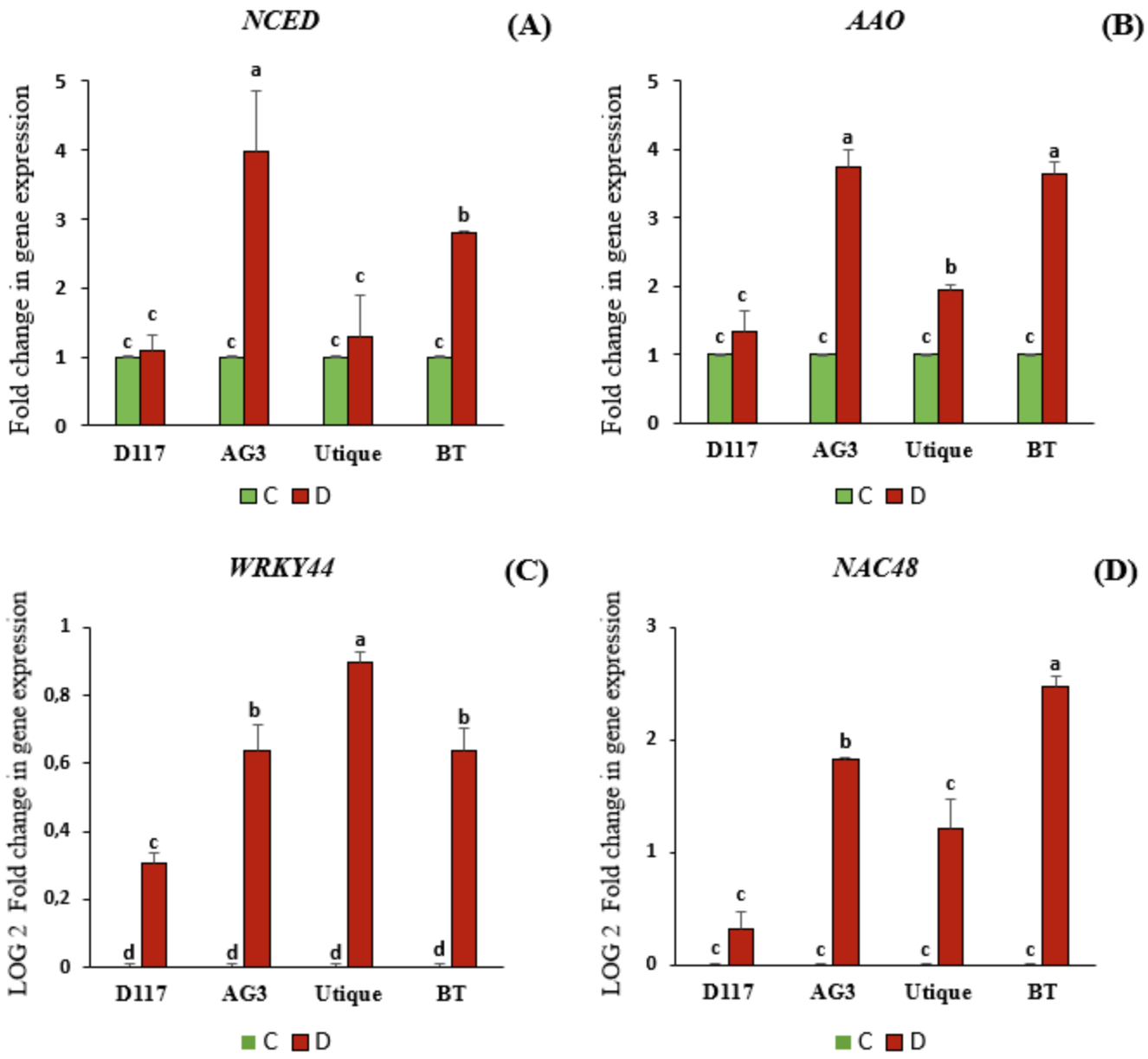

Figure 2

Effect of drought stress under (C) control and (D) drought conditions on the expression levels of drought-signaling genes NCED (A) and AAO (B) and transcription factors WRKY44 (C) and NAC48 (D), as determined by qPCR analysis in durum wheat genotypes (D117 and AG3) and bread wheat genotypes (Utique and BT) subjected to water deficit at the seedling stage.

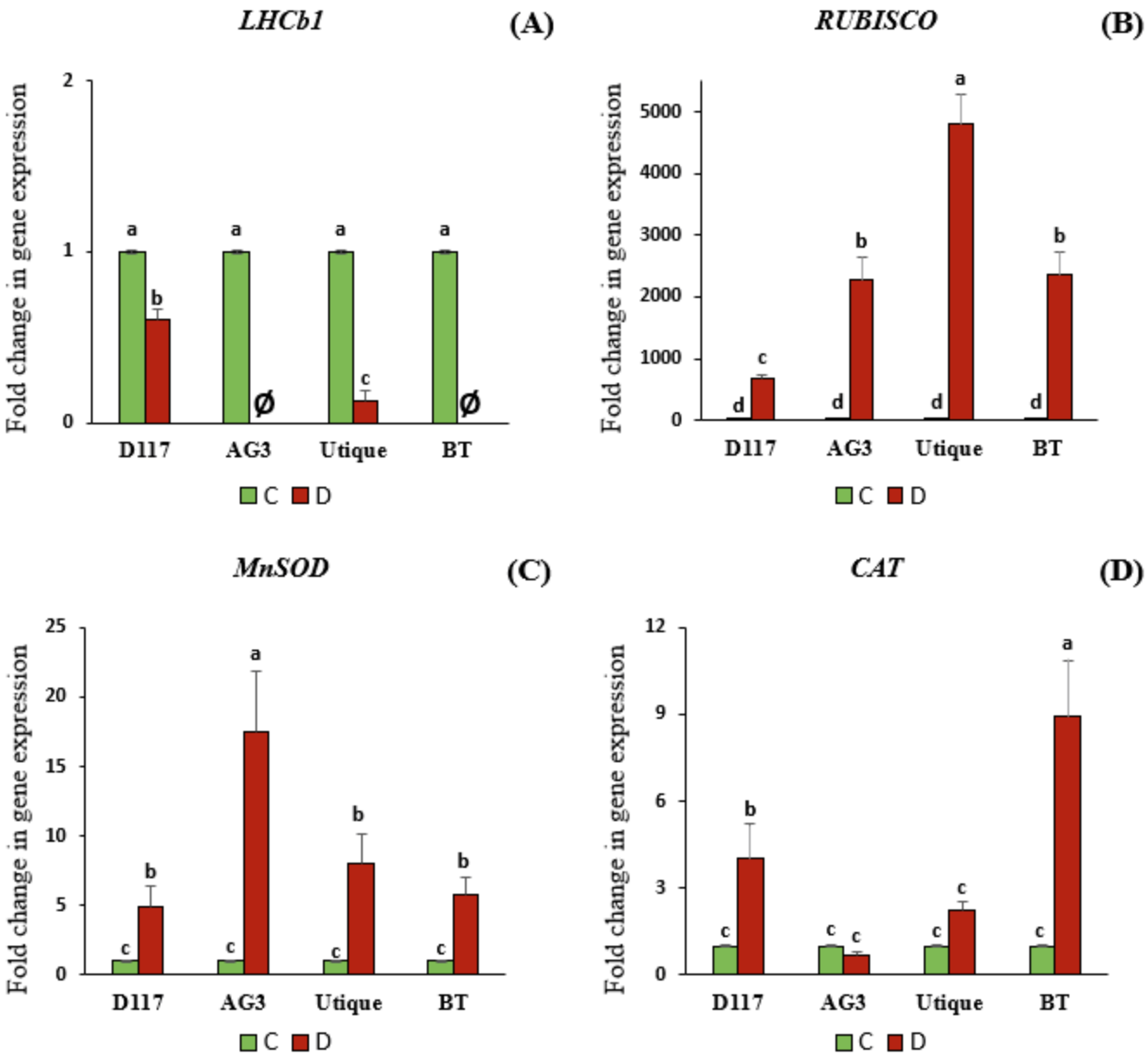

Figure 3

Effect of drought stress under (C) control and (D) drought conditions on the expression levels of LHCb1 (A), RUBISCO (B) in relation to photosynthetic performance and MnSOD (C) and CAT (D) genes associated with antioxidant defense responses, as determined by qPCR analysis, in durum wheat genotypes (D117 and AG3) and bread wheat genotypes (Utique and BT) subjected to drought stress at the seedling stage.

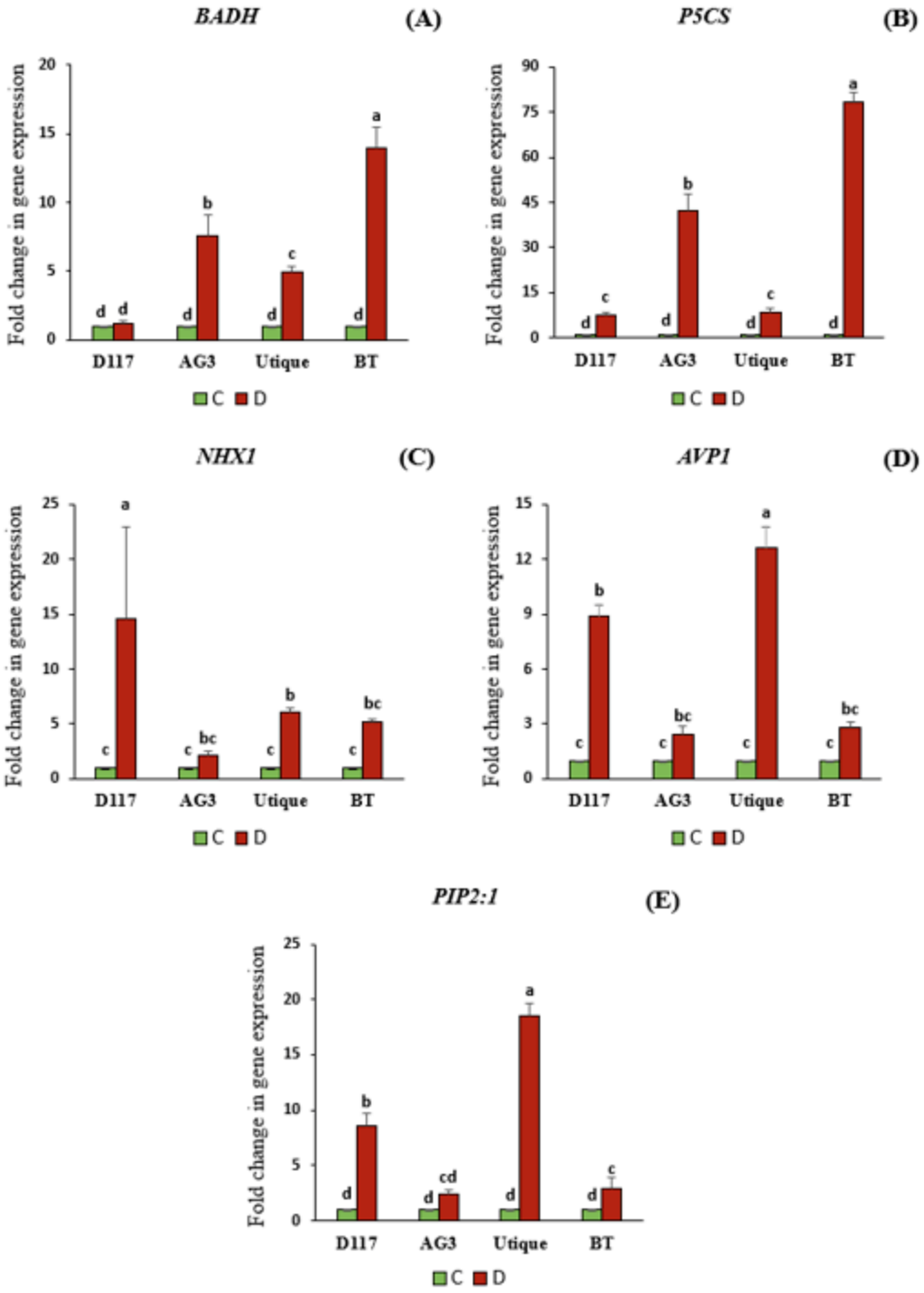

Figure 4

Effect of drought stress under (C) control and (D) drought conditions on the expression levels of BADH (A) and P5CS (B) genes involved in osmolyte biosynthesis and NHX1 (C), AVP1 (D) and PIP2;1 (E) genes associated with ion homeostasis and water transport, as determined by qPCR analysis, in durum wheat genotypes (D117 and AG3) and bread wheat genotypes (Utique and BT) subjected to drought stress at the seedling stage.

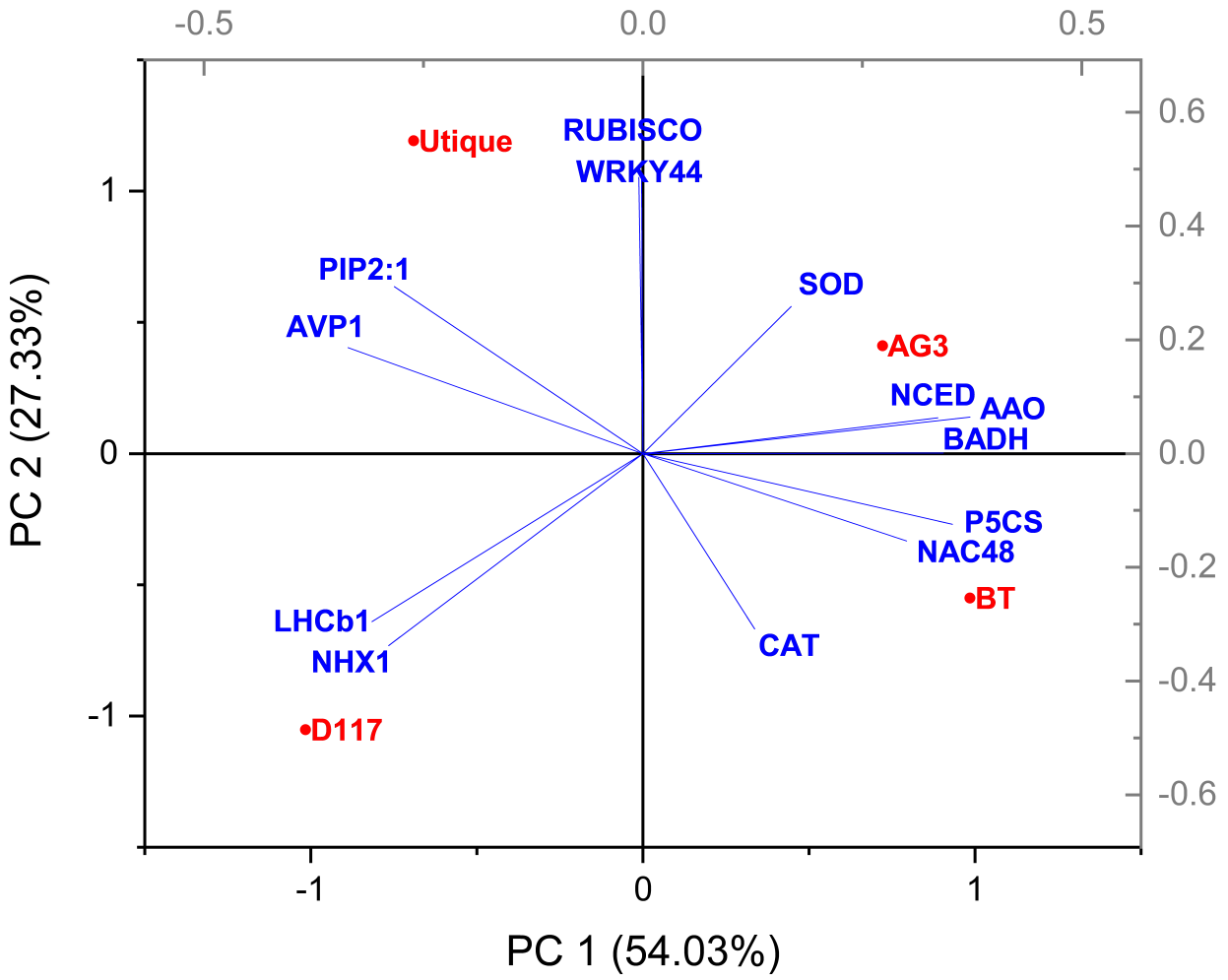

Figure 5

Biplot of principal component analysis (PCA) based on the expression profiles of thirteen drought-responsive genes in four wheat genotypes