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Astudy on antioxidant enzyme activities and gene expression in different barley genotypes under drought stress

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Abstract.barley is an ecologically important crop with wide adaptability in varied climatic conditions.The current study was conducted with the objective of testing the performance of different barley genotypes (Jau-87, Sanober-96, Frontiers-87 and Soorab-96) at enzymatic (superoxide dismutase, peroxidase and catalase) and genetic levels (HvDREB3, HvWRKY42, HvHsfB2c and HvERF4) under drought stress. Atri-replicate pot experiment was conducted underglasshouse conditions using a randomized complete block design (RCBD). The data collected for antioxidant enzymes were subjected to statistical analysis using Statistix8.1 program, while gene expression analysis was done using RT-PCR and expression was normalized using HvActin1-expressing gene. All genotypes recorded an increase in the activities of antioxidant enzymes due to drought stress. Correspondingly, all genotypes showed over-expression of all drought-associated genes under drought treatment compared to the control condition. Furthermore, all genotypes depicted complete parallelism in the expression of drought-associated genes and increased activity of antioxidant enzymes due to the involvement of these genes in regulating the metabolic pathways involved in the production of these antioxidant enzymes. Overall, the current study proved the complementary performance of all four genotypes in perspective of antioxidant and genetic responses; hence they can further serve as a valuable source for elucidating the dynamics of drought stress tolerance.

Keywords:Gene Expression; Antioxidant; Enzymes; Drought Stress

INTRODUCTION:

Barley is an important cereal crop with a wide range of adaptability in varying environmental conditions. This plant is ecologically important, with a comparatively high tolerance to drought as compared to other cereals (Sallam et al., 2019). Barley is considered a unique genomic model for elucidating the dynamics of abiotic stress due to its diverse genome and physio-biochemical properties (Kaur et al., 2021). Drought is amongst the strongest environmental constraints, including a series of physiological, morphological and biochemical changes in plants (Sallam et al., 2019). In fact, drought stress imposes directly or indirectly various biochemical changes at the cellular level. It creates homeostatic disequilibrium by triggering the production of various reactive oxygen species (ROS), resulting in redox ionic stress within the cellular system (Shah et al., 2017). To counteract the effect of oxidative stress, plants are equipped with a built-in

antioxidant system (Shah et al., 2022). Hence, they trigger the production of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase that have tendency to scavenge the ROS (Kaur et al., 2021).

On the other hand, to unravel the mystery of drought tolerance, it is crucialto elucidate the expression patterns of genes for characterizing a genotype as tolerant or susceptible. In this context,dehydration-responsive element binding (DREB) proteins are responsible for the functional modulation of some genes providing tolerance against drought stress in barley via osmotic homeostasis and adjustments (Yang et al., 2020). In the same way, heat shock factors (Hsf) play a pivotal role in providing tolerance against drought stress due to the production of heat shock proteins (Reddy et al., 2014). On the other hand, WRKY gene family provides tolerance to drought stress byenhancing the activities of antioxidant enzymes involved in ROS scavenging, as reported in model plants Arabidopsis and rice (Niu et al., 2012; Nan et al., 2020). Furthermore, ethylene responsive factor (ERF) isa diverse family of transcripts involved in the control of different pathways regulating the expression of different drought-related genes, hence elucidation of its expression,making itimportant for estimating the stress tolerance tendency of a genotype (Najafi et al., 2018). Therefore, evaluatingthe expression pattern of ERF genes in contrasting genotypes is highly important to tag genotypes as tolerant or susceptible (Ding et al., 2021). In general varying genetic expression in barley genotypes under the condition of drought stress is an indication of their different genetic makeup and responsiveness to stress. In this regard present study aimed to genetically characterize drought-tolerant barley genotypes suitable to cultivate in the environment where drought stress encounters the crop at any stage during the life cycle.

1. MATERIAL AND METHODS:

In current study four different drought tolerant barley cultivars Jau-87, Sanober-96, Frontiers-87 and Soorab-96, collected from National Agricultural Research Station, Islamabad, Pakistan were subjected to antioxidant enzymes and gene expression analysis using pot experiment. The tri-replicate experiment was conducted in the green house of Department of Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia using RCBD arrangement.

2.1 Crop Husbandry:

In the pot experiment, 8 seeds were sown in each pot with a size of 3L. The crop cultural practices like weeding and hoeing were provided as per requirement during the growth cycle. For molecular diagnostics, plant samples were collected by treating them with two types of environments, i.e., control and drought. Thinning was done when the plant attained the seedling stage and 4 plants were retained within each pot. Under normal conditions, the control group of the plant was placed open, while the drought group was kept under shelter. The control group was irrigated according to requirement, while the drought group was subjected to drought stress at the pre-anthesis stage by stopping irrigation for 16 days. After the completion of drought regimes, plants were watered normally.

2.2 Antioxidant Enzymes:

The activities of enzymes peroxidase, superoxide dismutase and catalase were measured using the respective kits according to the manufacturer’s instructions. For this, 2g frozenleaf samples of barley cultivars were homogenized using 2 mL of 0.1M Tris-HCl buffer and centrifuged at 20000 g for 15 minutes by optimizing temperature at 50C. Superoxide dismutase assay (Sigma-Aldrich,

Unites States), Peroxidase assay (Cell Biolabs Inc, United States) and Plant catalase assay kits (Sigma-Aldrich, United States) were used according to the manufacturer's instructions for the quantification of the activities these enzymes. The data for antioxidant enzyme activities were recorded on a weekly basis after the application of stress.

2.3 Gene Expression Analysis:

For expression studies of drought-related genes (HvDREB3, HvWRKY42, HvHsfB2c and HvERF4), RNA wasextracted according to the procedure opted by Li et al. (2019) using RNeasy kit (Qiagen, United States). For this purpose, plant samples were randomly selected. Moreover, cDNA was constructed following the method used by Ahmed et al. (2022). Besides, qRT-PCR analysis was performed, and gene expression was normalized with the help of the HvActin1-expressing gene. The primers used in the expression study are mentioned in Table 1.

Table 1. List of primers used in gene expression analysis

Primer	Sequence
HvDREB3	CAGAACCACTGGCTCCACCTC (F)
	ACGCTGCGGCAAAAGACGTCG (R)
HvWRKY42	GCCGGGCTTCGCTCTTCTC (F)
	CAGGGAGAAGTGGGCAAAT (R)
HvHsfB2c	GGCATTCCAGGCCGGCAGAT (F)
	CCAGGCATTCCCAGGTTCTC (R)
HvERF4	CCTCACCCGCGGCTGGCCCC (F)
	CCCCGCGGTGTCGTAGGCGC (R)
HvActin1	GCCGTGCTTTCCCTCTATG (F)
	GCTTCTCCTTGATGTCCCTTA (R)

Statistical analysis

For data collection, five plants from each treatment were randomly selected and their data were averaged. The recorded data of biochemical parameters were subjected to statistical analysis using the computer-based program, Statistix8.1.

2. RESULTS

3.1 Activity of Antioxidant Enzymes

All barley cultivars depicted statistically significant differences ($p \leq 0.05$) in the activities of superoxide dismutase, catalase and peroxidase due to drought stress as compared to the control (Table 2). In addition, activities of antioxidant enzymes were triggered significantly due to drought compared to the control treatment (Table 2). Among genotypes, the maximum enzymatic activity was recorded in Soorab-96, followed by Sanober-96, Frontiers-87 and jau-87

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

3.2 Gene Expression Analysis:

3.2.1 Expression profiling of HvDREB3:

Relative expression of gene HvDREB3 illustrated a significant increase in all barley genotypes under drought compared to control treatment (Figure 1). Among genotypes, Soorab-96 revealed maximum expression,

Table 2. Antioxidant enzyme activitiesin different barley cultivarsunder drought conditions.

	Catalase (Enzyme unit)			**Superoxide dismutase** (Enzyme unit)			**Peroxidase Activity**(Enzyme unit)		
Genotypes	Control	Drought	Difference	Control	Drought	Difference	Control	Drought	Difference
Jau-87	6.25.±0.26	8.57±0.21	2.16 ±0.04	22±0.60	30±0.51	8±0.10	0.35±0.02	0.55±0.01	0.20±0.02
Frontiers-87	6.55±0.27	9.10±0.23	2.45±0.03	21±0.55	29±0.50	8±0.15	0.25±0.02	0.50±0.02	0.25±0.01
Soorab-96	7.10±0.24	10.10±0.21	3.15±0.05	20±0.44	31±0.40	11±0.12	0.30±0.01	0.55±0.03	0.25±0.03
Sanober-96	6.10±0.22	10.25±0.24	4.20±0.06	23±0.43	30±0.41	7±0.10	0.34±0.02	0.60±0.05	0.26±0.02
LSD	0.20	0.32	0.12	1.21	1.00	0.51	0.04	0.03	0.03

followed by Sanober-96, Jau-87 and Frontiers-87. As a whole, all the expression of HvDREB3 was comparatively different among all barley genotypes under drought stress conditions.

3.2.2 Expression profiling of HvWRKY42:

The gene HvWRKY42 showed significant ($p \leq 0.05$) up-regulation in all barley genotypes due to drought stress as compared to the control condition (Figure 2). Relative expression analysis of HvWRKY42 recorded the maximum increase in Sanober-96 followed by Soorab-96, Frontiers-87 and Jau-87. As a whole, the expression of HvWRKY42 manifested a significant ($p \leq 0.05$) difference in all genotypes under drought stress.

3.2.3 Expression profiling of HvHsfB2c:

HvHsfB2c showed significantly ($p \leq 0.05$) high expression in all barley cultivars under

drought stress as compared to the control (Figure 3). Among wheat genotypes, Frontiers-87, followed by Jau-87, Sanober-96 and Soorab-96 revealed the maximum increase in the relative expression of *HvHsfB2c*. Overall all genotypes depicted a statistically distinct ($p \leq 0.05$) increase in the expression of the barley *HvHsfB2c* gene due to the application of drought stress.

3.2.4 Expression profiling ofHvERF4:

Relative expression of gene HvERF4 illustrated significant ($p \leq 0.05$) variation under both control and drought conditions in all barley cultivars (Figure 1). However, its relative expression was comparatively high in Sanober-96, followed by Soorab-96, Frontiers-87 and Jau-87. Overall, the expression of TaERF3 was significantly ($p \leq 0.05$) variable among all cultivars under the condition of drought stress.

Figure 1. Expression analysis of barley HvDREB3 gene in different barley genotypes under drought and control conditions.

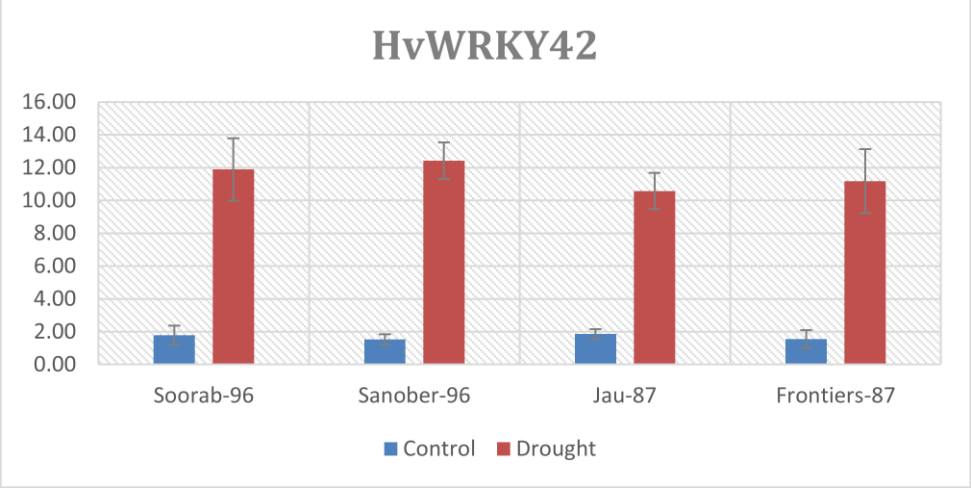
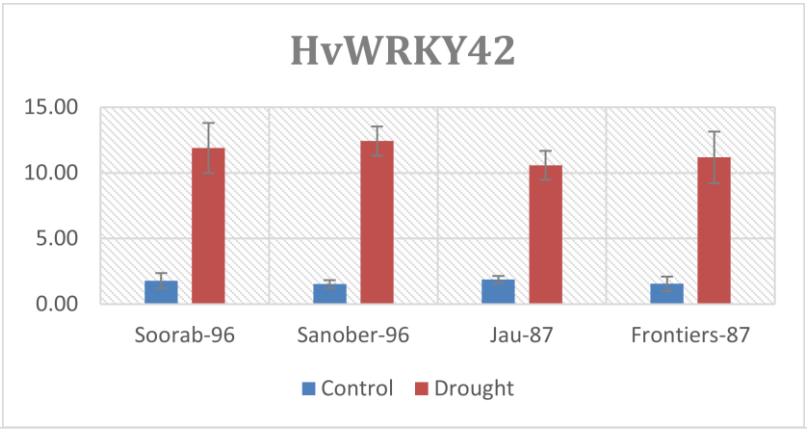


Figure 2. Expression analysis of barley HvWRKY42 gene in different barley genotypes under drought and control conditions.



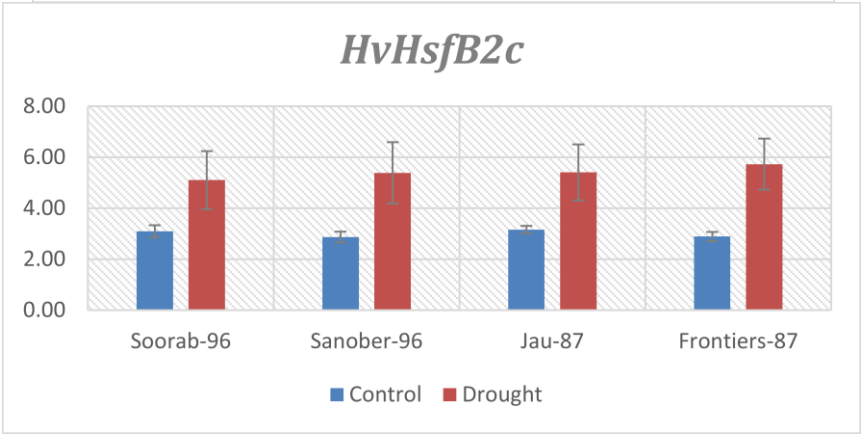


Figure 3. Expression analysis of barley HvHsfB2c gene in different barley genotypes under drought and control conditions.

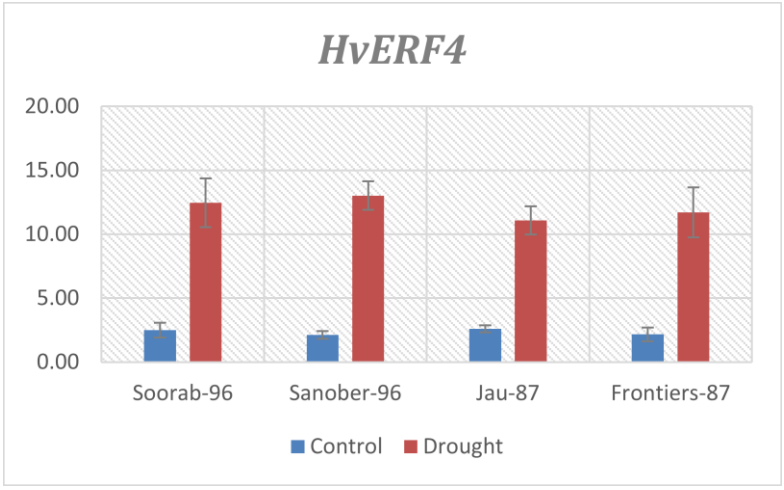


Figure 4. Expression analysis of barley HvERF4 gene in different barley genotypes under drought and control conditions

3. DISCUSSION

The current study intended to elucidate the effect of drought stress on the activities of antioxidant enzymes and the expression of drought-associated genes in different barley cultivars. Drought stress elicits various oxidative processes within the plant cell system due to the generation of reactive oxygen species (ROS) that interfere with redox homeostatic activities (Shah et al., 2017). To counteract the ROSdamage , the plant respond via different regulatory mechanisms (Shah et al., 2022). The tolerant barley genotypes depict enhancement in the activities of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase that potentially hinder the production of ROS through different scavenging processes (You and Chen, 2015). Probably due to the aforementioned reasons, current study recorded a dynamic increase in the activities of peroxidase, superoxide dismutase and catalase in all barley genotypes due to drought stress (Table 2).

Furthermore, our findings were consistent with the findings of Alghabari et al. (2021) and Shah et al. (2022), who recorded a dynamic increase in the activities of superoxide dismutase, peroxidase and catalase in wheat genotypes due to heat and drought stress respectively. Apart from this, plants elicitvarious homeostatic regulatory mechanisms to counter the effect of stress. Therefore,the expression of associated genes variesconsistently. All drought-associated genes, such as HvDREB3, HvWRKY42, HvHsfB2c and HvERF4, recorded significantly high expression in all barley genotypes due to drought stress (Figure 1-4). For instance, Yang et al. (2020) found that over-expression of HvDREB3 in barley under drought stress mediates various biochemical processes that protect the plant cellular system from oxidative damage caused by drought stress. In parallel with these findings, the current study recorded an increase in the expression of HvDREB3 in all barley genotypes under drought stress (Figure 1).

Besides, high expression of HvWRKY42 gene during drought stress regulates the antioxidant defense mechanism by boosting the activities of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase involved in the scavenging of ROS as reported by Wang et al. (2015) and Javadi et al. (2021). The present study authenticated these findings by recording the increase in the expression of HvWRKY42 gene in all barley cultivars under drought stress (Figure 2). Correspondingly, Reddy et al. (2014) concluded that over-expression of HvHsfB2c regulates the production of heat shock proteins that serves as osmoprotectants and saves plants from the negative impacts of drought stress. This was the probable reason whyall barley cultivars depicted up-regulation of HvHsfB2c due to heat stress (Figure 3). Similarly, Ding et al. (2021) noticed that up-regulation of HvERF4 under drought stress initiates the generation of some transcriptions factors regulating the ethylene-dependent signaling pathways that trigger the expression of various genes conferring drought tolerance in barley. Correspondingly,the current study confirmed these findings by reporting a significant increase in the expression of HvERF4 gene in all barley genotypes under drought stress (Figure 4).

Furthermore, all genotypes depicted complete parallelism in the expression of drought associated genes and increased activity of antioxidant enzymes that may be due to the involvement of these genes in regulating the metabolic pathways involved in the production of these antioxidant enzymes as reviewed by Shah et al. (2017).

4. CONCLUSION

Varying genetic expression in barley genotypes under drought stress indicatestheir different genetic makeup and responsiveness to stresson barley growth and stress regulation, whichhelped to mitigate adverse effects of drought on plant physiological and agronomic attributes, thus; efficient water management resulted in higher grain yield. Overall, the current study proved the complementary performance of all four genotypes in perspective on antioxidant and genetic responses, and therefore, they can further serve as a valuable source for elucidating the dynamics of drought stress tolerance.

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دراسة لنشاط الإنزيمات المضادة للتأكسد والتعبير الجيني لطرز جينية مختلفة من الشعير تحت إجهاد الجفاف

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مستخلص. يعتبر الشعير محصولًا مهمًا بيئيًا، حيث يتمتع بقدرة كبيرة على التكيف في الظروف المناخية المتنوعة. ولقد أجريت الدراسة الحالية بهدف اختبار أداء الطرز الوراثية المختلفة للشعير(Jau-87 و Sanober-96 و Frontiers-87وSoorab-96)على مستوى نشاطإنزيمات التأكسد (سوبر أكسيد ديسموتاز، بيروكسيدازوكاتلاز) والمستوى الجيني (HvDREB3 ، HvWRKY42 ، HvHsfB2cوHvERF4)تحت ظروفإجهاد الجفاف. أجريت تجربة أصص تحت ظروف الصوبة الزاجيةباستخدام ثلاث مكررات موزعة بنظام تصميم القطاعات كاملة العشوائية (RCBD) ، كما تم إخضاع البيانات التي تم جمعها من نشاط الإنزيمات المضادةللتأكسدللتحليل الإحصائي باستخدام برنامجStatistix8.1 ، بينما تم إجراء تحليل التعبير الجيني باستخدام RT-PCR وتم تطبيع التعبير باستخدام الجين الذي يعبر عنHvActin1. وقد أظهرت النتائج أن جميع الطرز الوراثية سجلت زيادة في نشاط الإنزيمات المضادة للأكسدة بسبب إجهاد الجفاف. في المقابل، أظهرت جميع الأنماط الجينية زيادة مرتفعةفي التعبير عن جميع الجينات المرتبطة بالجفاف تحت ظروف معاملة الجفاف مقارنةً بظروف معاملة الشاهد. علاوة على ذلك،أظهرتجميع الأنماط الجينية توازيًاكاملاً في التعبير عن الجينات المرتبطة بالجفاف وزيادة نشاط إنزيمات مضادات الأكسدة وذلك بسبب تداخلهذه الجينات في تنظيم المسارات الأيضية المشاركة في إنتاج تلك الإنزيمات. بشكل عام، أثبتت الدراسة الحالية الأداء التكميلي لجميع الأنماط الجينية الأربعة من منظور مضادات الأكسدة والاستجابات الجينية؛ ومن ثم يمكن أن تعدأيضًا مصدرًا قيمًا لتوضيح ديناميكيات تحمل الإجهاد الناتج عن الجفاف.

الكلمات الدالة: التعبير الجيني، مضاداتالأكسدة،إنزيمات، إجهاد الجفاف.