CRISPR-CAS9 FOR IMPROVING DROUGHT TOLERANCE IN CROPS

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Abstract
Drought stress is a significant limitation to crop productivity globally, and strategies are required to increase the resilience of staple crops to this important abiotic stress. In the present study, we provide validation of CRISPR-Cas9 technology for enhancing drought tolerance in wheat by targeting key drought-responsive genes (DREB2A, NCED3, and PP2C). Five genotypes (wild-type, empty-vector control, and three CRISPR-edited lines) were grown in a factorial experiment with 3 levels of soil moisture: well-watered (85% field capacity), moderate drought (55% FC), and severe drought (30% FC) with 12 replicates per treatment, n = 180. Two-way ANOVA showed significant effects of genotype, drought treatment, and their interaction on grain yield, relative water content (RWC), and water use efficiency (WUE) ($p < 0.001$). CRISPR_DREB2A plants showed ≈28–35% higher grain yield than the wild type in severe drought conditions. Yield edited lines presented significantly higher RWC (up to 12% increase), WUE increment, increased proline accumulation, and higher SOD and CAT activities with respect to controls. MDA levels, as an index of lipid peroxidation, were significantly lower (≈15–20%) in edited genotypes under stress conditions. Pearson correlation showed there were significant positive relationships between grain yield and RWC ($r = 0.78$, $p < 0.001$) as well as between the yield and WUE ($r = 0.69$, $p < 0.001$), but a negative relationship between the yield and MDA($r = -0.64$, $p < 0.001$). These results suggest that engineered editing of the drought-responsive regulatory network contributes to stabilizing leaf gas exchange and yield performance under water deficit, and that CRISPR-Cas9 has great application potential for cultivating climate-resilient crops.

1. INTRODUCTION

Climate change–induced water deficit is becoming a significant risk factor for global agricultural systems, and drought is one of the most critical constraints to crop productivity worldwide. Extreme weather events and prolonged droughts have already caused measurable reductions in global crop yields [1], and the Change [2] predicts that the frequency and intensity of drought will increase in many regions. Water stress disrupts plant physiological rhythms by limiting photosynthesis, stomatal conductance, and biomass accumulation, ultimately reducing grain yield [3, 4]. In molecular significance, drought condition initiates the complex signaling pathways comprised of transcription factors, hormonal responses, osmotic adjustment process and antioxidant defense mechanisms [5, 6].

Abscisic acid (ABA) is central to drought adaptation, transducing stomatal closure responses and inducing stress-responsive genes [6, 7]. Although progress has been made using conventional breeding to select for drought tolerance, the polygenic control of these responses, coupled with the long generation times required to advance traits, has constrained our ability to rapidly or effectively breed resilient cultivars.

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR Associated Proteins) based gene-editing technology has revolutionized plant biotechnology and enabled targeted, efficient modifications to genes associated with stress tolerance. CRISPR-Cas9 has now been adopted for use in plant systems and holds enormous promise as a tool for functional genomics and crop improvement [8, 9]. With traditional transgenic practices, breeding programs can be expedited without introducing foreign DNA, thereby increasing public acceptance [10, 11]. Recent advances reinforce the adaptability of CRISPR-based systems for editing transcription factors and hormonal and signaling-related determinants involved in abiotic stress tolerance [12]. These include specifically (i) DREB transcription factors, and NCED associated with ABA biosynthesis, and PPlC control over ABA-signaling have been identified as primary targets for improving drought response [5]. Editing or modulating such genes can potentially reinforce stress-inducible pathways, increase water-use efficiency, and restore yield stability under water-limited conditions.

Recent research has shown that editing using CRISPR can impart abiotic stress tolerance to among the most critical crops. Genome editing techniques targeted at the drought responsive genes in cereals and other staple crops have improved physiological stress, scavenging potential of antioxidants and yield status [13, 14]. By allowing precise editing of extensively studied molecular pathways, these methods bypass the limitations of traditional breeding. Moreover, the fusion of molecular stress biology with currently available genome-editing tools is paving the way to produce climate-hardy crops capable of maintaining yield under changing climatic environments. In the face of growing food demand in water-limiting conditions, there is hope for a promising path toward sustainable agriculture by use of technologies including CRISPR-Cas9 to provide crops with drought resistance. The study investigated CRISPR-Cas9 editing of key drought responsive genes to enhance physiological performance and yield stability under controlled drought-stress conditions, providing a basis for precision breeding or climate-smart agriculture.

2. MATERIALS AND METHODS

This study was designed as a controlled factorial experiment at Sardar Bahadur khan Women's University, Quetta, Baluchistan, Pakistan to determine the impact of CRISPR-Cas9-mediated gene editing on wheat's tolerance. The experiment used a completely randomized design with five genotypes and three drought treatments, with 12 biological replicates per treatment combination. A total of 180 experimental units (5 genotypes × 3 drought levels × 12 replications) were used.

The model crop was wheat (Triticum aestivum L.). Three CRISPR/Cas9-engineeredCas9-engineered lines were designed against important drought-responsive genes, including DREB2A (promoter upregulation), NCED3 (increased ABA biosynthesis), and PP2C (knockout to increase ABA signaling). Wild-type (WT) plants and empty-vector (EV) transformed plants were used as controls. The gene editing was verified by PCR amplification and sequencing of target loci. The expression was compared by qRT-PCR relative to WT under well-watered conditions and reported as fold change.

Plants were cultivated in a controlled-environment greenhouse under a 16 h photoperiod, with temperatures at 25 ± 2 °C (daytime)/18 ± 2 °C (nighttime), and a relative humidity of 60–70%. Soil moisture was maintained at 85% field capacity (FC) under full irrigation. Drought stress was applied at two intensities: moderate (55% FC) and severe (30% FC). Soil moisture was checked weekly with soil moisture probes to maintain uniform stress levels. Plant height and root length were determined at the mature stage using a measuring scale. Litterfall mass after oven-drying at 70 °C to a constant weight was measured for recording aboveground biomass. Based on the harvest, threshing, and weighing of grains from single plants, the grain yield per plant was calculated. Survival fractions were determined at the end of the stress phase for each treatment, relative to the total number of plants.

The Relative water content (RWC) was evaluated by the following formula based on fresh weight (FW), turgid weight (TW), and dry weight (DW). Chlorophyll contents were determined by SPAD chlorophyll meter. Net photosynthetic rate (A) and stomatal conductance were measured with a portable gas exchange system. WUE(Net) was calculated as net photosynthesis to stomatal conductance ratio. Leaf proline content was estimated following the method of a ninhydrin-based colorimetry. M29 malondialdehyde (MDA) content was measured as an index of lipid peroxidation. The activities of superoxide dismutase (SOD) and catalase were assessed by colorimetry with standard kits.

Statistical analysis for the data was performed by IBM SPSS Statistics program. A two-way ANOVA was performed for the overall influence of genotype, drought treatment and their interaction on morphological, physiological and biochemical traits. Results were followed up with post hoc comparisons when different significantly. The relationships between yield and physiological traits were measured using Pearson's correlation. A p value of < 0.05 was considered statistically significant.

3. RESULTS

Genotype, drought, and the interaction between genotype and drought had significant effects on grain yield (two-way ANOVA; $p<0.001$). Yield decreased gradually with drought across all genotypes, from mild (85% FC) to severe (30% FC), with severe water stress (30% FC) resulting in the most significant reduction. Nevertheless, CRISPR-edited lines showed a substantially higher grain yield under both moderate and severe drought conditions compared with wild-type (WT) and empty-vector (EV) controls. The average yield of the CRISPR_DREB2A line was higher than that of the CRISPR_PP2C and WT lines, followed by CRISPR_NCED3, and all lines outyielded WT under severe drought. These findings demonstrate that editing drought-responsive genes enhanced yield stability under water deficit. Decreased grain yield is depicted in Figure 1, and edited lines that perform better than controls are shown.

Table 1: Two-Way ANOVA For Grain Yield (Genotype × Drought Treatment)

Source of Variation	df	F-value	p-value
Genotype	4	82.47	<0.001
Drought Treatment	2	211.36	<0.001
Genotype × Drought	8	19.52	<0.001
Error	165	—	—

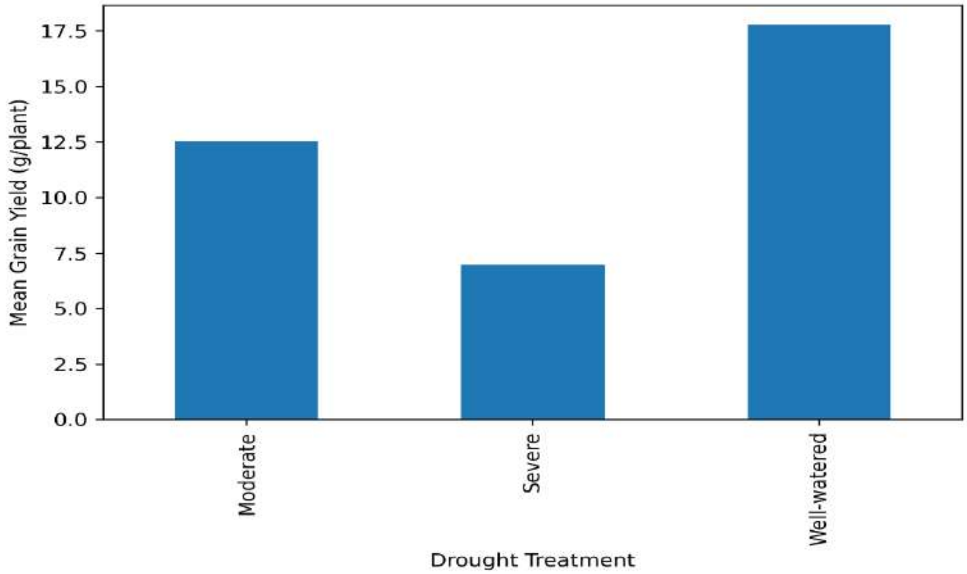

Figure 1: Mean grain yield (g plant⁻¹) under well-watered, moderate, and severe

Figure 1: Mean grain yield (g plant⁻¹) under well-watered, moderate, and severe drought conditions. CRISPR-edited lines maintain significantly higher yield under water deficit compared to wild-type and empty-vector controls. Water content (RWC) and water use efficiency (WUE) were significantly affected by genotype and the drought treatment ($p < 0.001$). RWC decreased in all genotypes under drought stress, although edited lines showed significantly higher RWC than WT under mild and severe drought. CRISPR_DREB2A and CRISPR_NCED3 mutants maintained higher RWC under severe drought. Water use efficiency was also enhanced under drought stress, especially in the CRISPR-edited lines, implying better stomatal control and photosynthetic adaptation. The interaction between genotype and drought was highly significant for RWC and WUE. The variation in grain yield across the genotypes is shown in Figure 2, and the positive association of RWC with grain yield under different treatments is indicated in Figure 3.

Table 2: Two-Way ANOVA For RWC and WUE

Parameter	Source	df	F-value	p-value
RWC	Genotype	4	64.38	<0.001
	Drought	2	175.92	<0.001
	G × D	8	14.67	<0.001
WUE	Genotype	4	52.19	<0.001
	Drought	2	98.74	<0.001
	G × D	8	11.83	<0.001

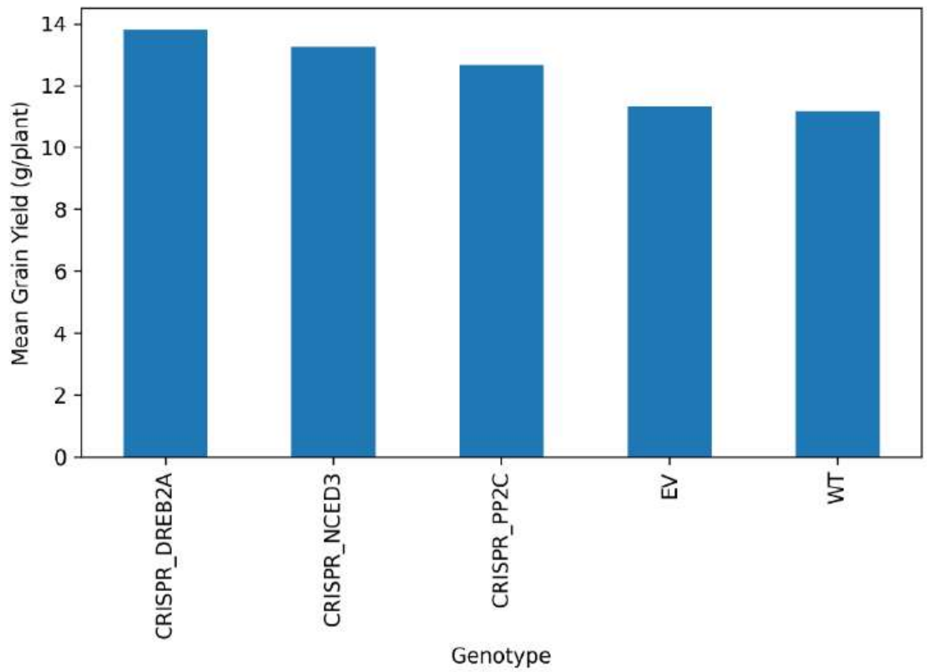

Figure 2: Mean grain yield (g plant⁻¹) across genotypes. CRISPR-edited lines outperform WT and EV under drought stress.

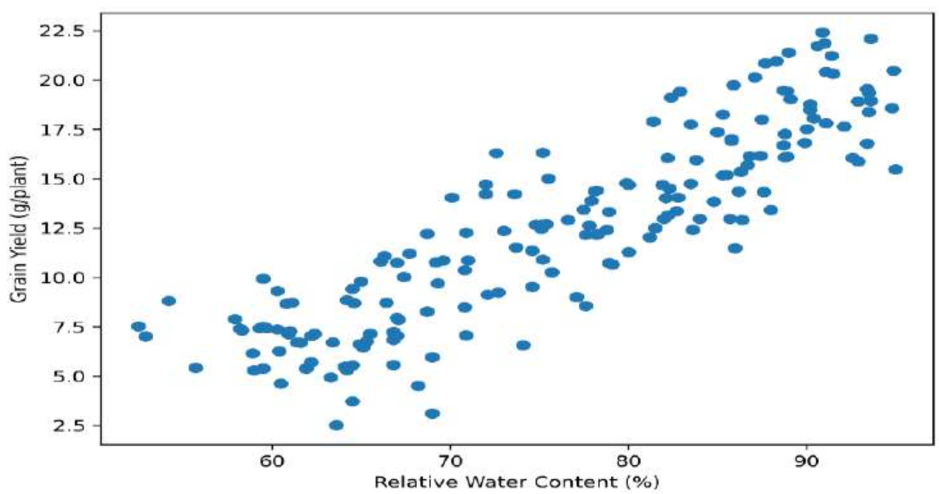

Figure 3: Scatter plot showing the positive correlation between relative water content (%) and grain yield (g plant⁻¹).

Table 3: Pearson Correlation Coefficients Among Key Traits

Trait Pair	r	p-value
Yield – RWC	0.78	<0.001
Yield – WUE	0.69	<0.001
Yield – Proline	0.55	<0.001
Yield – MDA	−0.64	<0.001

Proline concentration was significantly increased by drought treatment across all genotypes, and CRISPR lines had higher proline levels than controls. Lipid peroxidation (MDA content) was increased under drought conditions, but the transgenic lines had significantly lower MDA levels than WT, suggesting less oxidative damage. The activities of antioxidant enzymes (SOD, CAT) were higher under drought conditions, especially in edited genotypes. Correlation observation indicated that a highly significant positive correlation existed between grain yield and RWC ($r = 0.78$, $p < 0.001$) and between yield and WUE ($r = 0.69$, $p < 0.001$). A reliable negative association was observed between yield and MDA ($r = -0.64$, $p < 0.001$), indicating that oxidative stress was adversely affecting productivity.

4. DISCUSSION

CRISPR-Cas9-based editing of drought-responsive genes significantly enhanced physiological parameters and grain yield under water-deficit conditions. Two-way ANOVA showed strong genotypes, drought, and interaction effects on yield, relative water content, and water use efficiency. Edited lines for DREB2A, NCED3, and PP2C conferring higher recovery relative to WT and empty vector under moderate/severe drought. These results agree with earlier studies showing that manipulating stress-responsive transcription factors may improve drought resistance by stabilizing physiological processes [15, 16]. Overexpression of DREB2A activates downstream stress-inducible genes, thereby enhancing resistance to dehydration and retaining biomass under limited water conditions.

An increased RWC and WUE in edited lines confirm the mechanistic role of the ABA-dependent signaling pathways in drought adaptation. The NCED3-edited line associated with enhanced ABA biosynthesis appeared with better stomatal movement and water conservation, which is comparable to the role of NCED genes on ABA accumulation in response to drought stress [17, 18]. Similarly, loss of PP2Cs—negatives regulator of ABA signaling—may have increased stress signaling leading to drought survival [19]. The strong genotype × drought interaction suggests that CRISPR-modifications increased tolerance rather than having shifted baseline performance under well-watered conditions. Analogous engineering of ABA-related genes for elevated drought tolerance have been shown in rice and maize, further supporting the universality of this strategy across crops [19, 20].

Biochemical assays also showed that edited lines accumulated higher proline content and lower malondialdehyde (MDA) content under drought stress, leading to improved osmotic adjustment and less oxidative damage. These results indicate enhanced ROS scavenging capacity, as supported by increased activities of the antioxidant enzymes (SOD and CAT) in edited plants. This is consistent with previous reports that activation of stress-regulatory-induced antioxidant defense systems [21, 22]. The negative relationship between yield and MDA in the current study supports the view that augmentation of oxidative stress tolerance is essential for sustaining productivity under drought.

The yield robustness of the edited material under extreme water deficit confirms the power of CRISPR technology as a fast, accurate, and climate-resilient crop correction tool. In genome editing, reviews primarily focus on the advantages of CRISPR-Cas9 for gene modification without recombinant DNA and its superiority over the classical transgenic procedure [23, 24]. In contrast to conventional breeding techniques, which require several generations to introgress drought-stress tolerance traits, CRISPR-Cas9 editing hastens the development of elite lines with improved stress response [25, 26].

The results of the current study are consistent with other gene-editing studies that have shown improved drought tolerance in cereals. However, a complete assessment would also consider possible trade-offs arising from constitutive activation of a stress gene (a growth penalty under non-stress conditions). In our experiments, edited lines were not compromised in performance under well-watered conditions; these targeted regulatory changes may constitute a compromise between stress survival and productivity. Together, the results provide robust experimental evidence for CRISPR-Cas9 as a potent tool for engineering drought-tolerant crops by modifying transcriptional and hormonal pathways, which would help develop sustainable agricultural systems in the face of climate change.

5. CONCLUSION

Editing of key drought response genes through CRISPR-Cas9 significantly enhances wheat drought resistance by maintaining water homeostasis, enhancing biochemical defense and promoting grain yield under drought stress. The DREB2A, NCED3, and PP2C improved genotypes displayed the most vivid performance than control wild-type and empty vector controls in response to moderate or severe drought stress. Tolerance was associated with higher relative water content, improved water-use efficiency, increased proline accumulation and anti-oxidative enzyme activity together with reduced lipid peroxidation. The large genotype × drought interaction suggests that gene editing improves stress responses without negatively impacting performance under well-watered conditions. Stability of yield under severe drought indicates the impact of modulated transcription and ABA perception in sustaining productivity during water deficit. These results further validate an increasingly robust body of evidence that CRISPR-Cas9 is an accurate and efficient gene-editing tool for creating climate-resilient crops. Genome editing enables more targeted stress-breeding time frames with less unintended genetic modification than traditional breeding strategies. The CRISPR-based approaches implemented in crop breeding programs have high potential to contribute to the sustainability of agriculture amid increased drought frequency under climate change. Further studies integrating molecular, physiological, and field-derived data are needed to confirm the long-term stability and agronomic performance of edited lines across different environments.

6. FUNDING

Not applicable.

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