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Drought-induced expression of *PvDERB1F* and *PvDREB5A* with promoted antioxidant activities possibly enhanced drought stress tolerance in Common bean (*Phaseolus vulgaris* L.)

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Drought-induced expression of *PvDERB1F* and *PvDREB5A* with promoted antioxidant activities possibly enhanced drought stress tolerance in Common bean (*Phaseolus vulgaris* L.)

Abstract: The common bean (*Phaseolus vulgaris* L.) is an important source of protein, fiber, vitamins, and minerals, making it essential for food programs in Botswana. Prioritizing its integration into diversified farming is crucial for achieving social, environmental, and economic benefits. Previous studies primarily focused on performance under rainfed, while the effect of drought stress remains unclear. The study aimed at evaluating the effect of drought stress on four (4) genotypes: DAB541, DAB515, CAL96, and GK011, while Tepary serves as the control. The study identifies CAL96 and DAB541 genotypes as the most promising genotypes for drought tolerance as demonstrated by their increased biomass production. The increase in biomass production may be due to the overexpression of the *Phaseolus vulgaris* dehydration-responsive element binding (*PvDREB*) genes, namely *PvDREB1F* and *PvDREB5A*. Higher proline and lower malondialdehyde (MDA) levels correlated with increased catalase (CAT) and ascorbate peroxidase (APX), which are linked to hydrogen peroxide (H_2O_2) scavenging activity. Conversely, GK011 and DAB514 exhibited decreased dry biomass, downregulated *PvDREB1F*, *PvDREB5A*, and *PvDREB6B*, along with greater levels of MDA and H_2O_2 and a steady activity of APX and CAT. This suggested an enhanced membrane lipid peroxidation and a loss of membrane integrity.

Key words: *Phaseolus vulgaris*, drought stress, *DREB* genes, lipid peroxidation, antioxidants, ROS scavenging

S sušo vzpodbujenim izražanjem genov *PvDERB1F* in PvDREB5A morda lahko s povečano antioksidacijsko aktivnostjo povečamo toleranco navadnega fižola (*Phaseolus vulgaris* L.) na sušni stres

Izvleček: Navadni fižol (*Phaseolus vulgaris* L.) je pomemben vir beljakovin, vlaknin, vitaminov in mineralov, zaradi česar je bistven v programih prehane v Botswani. Njegovo prednostno vključevanje pri povečavanju raznolikosti kmetijske pridelave je bistveno za doseganje socialnih, okoljskih in ekonomskih ciljev. Predhodne raziskave so se prvenstveno usmerjale na njegovo uspevanje v razmerah namakanja z dežjem med tem, ko je ostajal učinek sušnega stresa nepojasnjen. V tej raziskavi so bili ovrenoteni učinki sušnega stresa na štiri genotipe in sicer DAB541, DAB515, CAL96 in GK011, pri čemer je 'Tepary' služil kot kontrola. V raziskavi sta bila genotipa CAL96 in DAB541 prepoznana kot najbolj obetajoča glede tolerance na sušo, kar se je pokazalo v njuni povečani izgradnji biomase. Povečana tvorba biomase bi lahko bila zaradi močno povečanega izražanja genov v fižolu, odzivnih na dehidracijo (*PvDREB*), kot sta gena *PvDREB1F* in *PvDREB5A*. Večja vsebnost prolina in manjša vsebnost malondialdehida (MDA) sta soupadali v povečanjem aktivnosti katalaze (CAT) in askorbat peroksidaze (APX), kar je povezano z odstranjevanje vodikovega peroksida (H_2O_2). Nasprotno sta genotipa GK011 in DAB514 pokazala zmanjšanje suhe biomase, zmanjšano izražanje *PvDREB1F*, *PvDREB5A*, in *PvDREB6B* genov s hkratnim povečanjem vsebnosti MDA in H_2O_2 in enakomerno aktivnostjo APX in CAT. To nakazuje povečano peroksidacijo membranskih lipidov in izgubo delovanja membran.

Ključne besede: *Phaseolus vulgaris*, sušni stres, DREB geni, peroksidacija lipidov, antioksidati, ROS odstranjevanje

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1 INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important grain legume that represents a valuable source of protein in the diet (Broughton et al., 2003). *P. vulgaris* has been hailed as one of the priority crops for integration into diversified agricultural systems in Botswana (Ministry of Agriculture, 2023). This policy has been motivated by common bean being considered the most important food legume crop in the human diet, mainly for its rich protein, carbohydrates, vitamins, dietary fibre and minerals (Beebe et al., 2013; Mangole et al., 2022). It was further stated that the common bean is consumed in hospitals and school feeding schemes, and it is also offered as part of the government's supplementary feeding scheme for underage children at clinics nationwide (Mangole et al., 2022). Although it is important, production has been reported to be low, resulting in a high import bill. In that respect, various common bean genotypes from neighbou countries have been introduced.

Previous research on the introduced genotypes has been focused on planting date, nutritional value and yield stability under rainfed conditions (Moatshe-Mashiqa et al., 2021; Molosiwa et al., 2019). In addition, drought stress is a factor of economic importance in common bean production in Botswana. Southern Africa Drought Resilience Initiative (SADRI) (2021) indicated that the 2018/19 drought season resulted in two-thirds of crop failure. Botswana is a southern African country with an arid to semi-arid climate, resulting in desert-like conditions and very variable rainfall patterns across about a third of the country. This is caused by drastic and rapid changes in the global climate that can occur during the common bean's life cycle, such as initial seedling establishment, vegetative growth, flowering and/or grain filling are exacerbated (Rao et al., 2013). This, therefore, calls for screening of the introduced genotypes for drought stress tolerance.

The response to drought stress is mediated by subtle changes in gene expression that lead to changes in the composition of the plant transcriptome, proteome and metabolome and ultimately the phenotype (Ansari et al., 2017, 2018; Deeba et al., 2012; Lin et al., 2016). The adaptive strategies used by drought-tolerant plants are of major importance for the selecting and adopting crops with improved performance under erratic water deficit conditions, such as Botswana's. Pholo-Tait et al. (2022), reported the role of *Phaseolus vulgaris ALLANTONAISE (PvALN)* in drought stress in common bean genotypes. Meanwhile, transcriptional factors, especially the dehydration-responsive element binding (DREB) factor family members comprise critical targets for selection of crops that confer tolerance to abiotic stress. *DREBs* regulates gene expression through a mechanism that involves recognizing the dehydration responsive element (DRE), which consists of the conserved motif A/GCCGAC (Sakuma et al., 2002). Amongst the six subgroups (A-1- A-6) of DREB genes, the expression of *DREB2 (A-2), DREB5 (A-5; RAP2.1)* and *DREB6* genes (A-6; *RAP2.4*) showed to be highly induced under drought stress in *Arabidopsis* (ChunJuan & JinYuan, 2010; Nakashima, Ito, & Yamaguchi-Shinozaki, 2009). In another study, overexpression of *GmDREB1* increased the drought resistance of transgenic soybeans while increasing yield (Chen et al., 2022). Similarly, transgenic tobacco lines expressing the transcription factor gene of the DREB 5-A subgroup of *Ricinus communis* L. *(RcDREB1)* showed improved growth, drought tolerance and higher pollen viability (do Rego et al., 2021). Subsequent research showed that under drought stress conditions, the expression level of *DREB1* rose more in wheat genotypes that were drought tolerant than in those that were drought sensitive (Rustamova et al., 2021). Previous studies reported that drought stress tolerance in the common bean is due to the overexpression of *Phaseolus vulgaris* DREB genes such as *PvDREB1F, PvDREB5A* and *PvDREB6B* (Konzen et al., 2019). Therefore, these DREB genes offer the possibility of serving as a basis for screening different genotypes of common beans for drought tolerance.

Interestingly, the differential expression of DREB genes has been shown to also alter the functional expression of nonenzymatic antioxidant defense (Ghaffari et al., 2019; Moloi & van der Merwe, 2021; Wei et al., 2016). The overexpression of DREB genes has been reported to induce a decrease in malondialdehyde (MDA) content, and such an inverse correlation plays a vital role in drought stress tolerance. MDA is one of the final products of polyunsaturated fatty acid peroxidation in cells. It is widely used as a reliable marker for determining the degree of membrane damage in tissues under stress and the ability of plants to tolerate drought stress (Blokhina et al., 2003; Morales & Munné-Bosch, 2019; Vendruscolo et al., 2007). The production of reactive oxygen species (ROS) under drought stress correlated positively with an increase in MDA content, resulting in increased permeability of the plasma membrane and extravasation of the content of cells. This inevitably impairs the production of biomolecules, such as lipids, proteins, and nucleic acids (Kong et al., 2016; Ripullone et al., 2021). On the other hand, lower concentrations of MDA have been reported to be associated with lower production of ROS. In soybean, tomato and wheat (Amoah & Seo, 2021; Raja et al., 2020; Saruhan Guler & Pehlivan, 2016), low levels of MDA suggested lower production of ROS and ultimately a reduction in membrane damage under drought stress. The overexpression of DREB genes suppressed the

production of MDA, resulting in an improved reactive oxidative species (ROS) scavenging capability. This was demonstrated in transgenic *Arabidopsis*, where overexpression of *AtDREB1A* resulted in a lower MDA content (Morales & Munné-Bosch, 2019). Similarly, it was observed that the regulation of antioxidant mechanisms by *Arabidopsis thaliana DREB1A* (*AtDREB1A)* was associated with a reduction in MDA levels in peanut (*Arachis hypogaea* L.) under water deficiency (Bhalani et al., 2019). In that respect, the production of MDA has been used as a robust reliable marker for determining the degree of injury to a drought-stressed plant (Alché, 2019; Kong et al., 2016; Morales & Munné-Bosch, 2019).

In addition to MDA, osmolytes such as proline play an important role in plants under drought stress. Proline protects plants through cellular osmotic adjustment, ROS detoxification, protection of membrane integrity, and enzyme/protein stabilization (Ghaffari et al., 2019). Drought stress significantly enhanced the accumulation of proline in the leaves of canola at the flower initiation stage and pod filling stage under drought stress. This response justified that proline accumulation under drought stress is an adaptive response that enhances survival and tissue water status (Morsi et al., 2023). This response is ascribed to enhanced osmotic modifications to acclimatize to recompense for plant survival and, accordingly, assist in tolerating drought stress (Morsi et al., 2023). Conversely, a negative correlation between proline levels and plant growth has been reported in common beans. Higher levels of proline induced by stress inhibited growth in highly drought-sensitive cultivars and as such proline has been proposed as a suitable large-scale screening biochemical marker for common bean under water deficit and salt stress (Arteaga et al., 2020). Likewise, overexpression of the *DREB* gene has been shown to enhance proline accumulation (Nguyen et al., 2019). Transgenic *Arabidopsis* plants overexpressing *DREB1A* showed a positive correlation of increased accumulation of proline. Transgenic rice overexpressing *Oryza sativa DREB1A* (*OsDREB1A)* also accumulated proline in stressed and controlled conditions (Dubouzet et al., 2003). Similar results were also demonstrated in soybean, in which overexpression of the *Glycine max DREB6* and *DREB2 (GmDREB6; GmDREB2)* genes increased proline accumulation and tolerance to drought stress (Nguyen et al., 2019; Pham et al., 2020). Furthermore, peroxidase and catalases are among the versatile enzymatic hydrogen peroxide (H_2O_2) scavenging systems (Caverzan et al., 2012; Foyer & Shigeoka, 2011; Shigeoka, 2002), hence contributing to the regulation of redox homeostasis and signaling pathways (Sohag et al., 2020; Tyagi et al., 2021). Given that specific DREB genes induce tolerance to drought stress in *P. vulgaris* (Konzen et al., 2019), it is worth noting that genetic variability exists among genotypes. The study aimed to evaluate the physiological and molecular responses of introduced common bean genotypes for drought tolerance. The objectives were to evaluate both enzymatic and nonenzymatic antioxidant responses, as well as conducting transcriptional screening for drought stress tolerance using DREB genes.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS AND TREATMENTS

The study was conducted at Sebele Research Station (24° 34'25"S and 25° 58'0"E) under the growth cabinet growth conditions. The growth cabinet was set at a long-day photoperiod of 16 h light and 8 h dark with the temperature set at 30 °C (day) and 25 °C (night). The light intensity was set at 300 μE m^{-2} s^{-1} light, while the humidity was maintained at 60 %. The study was conducted on four (4) common bean varieties, namely, DAB541, DAB514, CAL96, and GK011 The choice of common genotypes was based on their performance in the previous studies, demonstrating their potential in terms of promising previous results on production and productivity stability under rainfed, as well as nitrogen fixation capability under water deficit conditions (Molosiwa et al., 2019; Pholo-Tait et al., 2022). Tepary bean, which is the commonly grown bean landrace in Botswana was used at the control. Seeds were directly sown on a mixture (1:2) of sterilized sandy soil and loose jiffy growth media. The experiment was subjected to a factorial complete randomized design with six (6). replications. The two treatments consisted of a maximum water holding capacity (control) and a drought stress treatment in which water was withhold for fourteen days (Nakashima et al., 2009).

2.2 DRY LEAF BIOMASS DETERMINATION

Six (6) replicates of leaf plant material were harvested at the end of the drought stress period. Samples were oven-dried at 65 °C) for three (3) consecutive days. Thereafter, the samples were weighed for total leaf dry biomass production.

2.3 PROLINE AND MALONDIALDEHYDE (MDA) CONTENTS

Leaf tissue material (0.1 g) was ground in liquid nitrogen and homogenized in 3 % (w/v) aqueous sulfo-

salicylic acid. The homogenate was centrifuged at 4000 × g for 10 minutes, while the resulting supernatant was added to a mixture of equal volumes of acid-ninhydrin and acetic acid. The mixture was incubated at 98 °C for 30 minutes and cooled to 25 °C, followed by the addition of toluene. The upper layer of the solution was aliquoted and used for proline determination. The proline content was quantified by spectrophotometer at an absorbance rate of 520 nm and calculated based on the standard curve using proline as a standard (Bates et al., 1973). Malondialdehyde (MDA) content was measured to determine the degree of membrane lipid peroxidation (Zhang and Huang, 2013). The total ground leaf samples (0.1 g) were homogenized in 0.1 % (w/v) trichloroacetic acid (TCA). The homogenate was centrifugated at 10,000 × g for 10 min. The supernatant was mixed with 20 % TCA consisting of 0.5 % thiobarbituric acid (TBA). The mixture was then heated at 95 °C for 15 min, followed by cooling on ice. Cooled samples were centrifuged at 4800 rpm for 10 min, after which the absorbance was measured at 450, 532 and 600 nm.

2.4 ACTIVITIES OF ANTIOXIDANT ENZYMES

Leaf tissues (250 mg) were homogenized in 0.1 % (w/v) TCA, centrifuged at 12 000 × g for 15 min before the addition of 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (KI) to the supernatant. The enzyme activity was read at an absorbance rate of 390 nm, while the H_2O_2 concentration was calculated using a standard curve (Velikova et al., 2000). Ascorbate peroxidase (APX) and catalase (CAT) were extracted from ground leaf tissue (0.2 g) in liquid nitrogen. The materials were homogenized in a mixture of 0.2 M sodium phosphate buffer (pH 7.8) and 0.1 mM EDTA. Consecutively, the homogenate was centrifuged at 15000 × g for 20 minutes at 4 °C followed by APX and CAT enzymatic activity assays. APX enzymatic activity was assessed in a reaction mixture consisting of an extract, 1 M potassium phosphate buffer (pH 7.8), 10 mM hydrogen peroxide, and 10 mM ascorbate. The reaction mixture without an extract was used as a blank. The reaction was initiated with the addition of H_2O_2 at 25 °C room temperature. The oxidation rate of ascorbate was determined by the decrease in absorbance at 290 nm for 3 min. CAT enzymatic activity (Aebi, 1974) was performed in a reaction mixture containing 0.01 M H_2O_2 and 0.05 M potassium phosphate buffer (pH 7.0). The CAT enzyme was added to initiate the reaction, while the decrease in absorbance at 240 nm during the initial 3 min was used to measure H_2O_2 activity.

2.5 REAL-TIME QUANTITATIVE ANALYSIS OF DREB GENES

CTAB protocol (Hu et al., 2002) was followed to isolate total RNA from three biological replicates of leaf tissue material (250 mg). RNA was purified using a Qiagen RNase-free DNase Kit (Cat #79254) and eluted in RNase-free water according to the manufacturer's instructions. RNA concentrations were checked using a NanoDrop ND-1000 UV–Vis Spectrophotometer (Thermo Fisher Scientific), while RNA integrity was validated by visualizing the RNA on a 1 % agarose gel. RNase-DNase free RNA was reverse transcribed to complementary DNA (cDNA) using an oligo (dT^{18}) primer and M-MLV (H-) reverse transcriptase (Promega, Anatech, South Africa) following the manufacturer's instructions. cDNA template was checked by RT-PCR using reference genes, namely, the *SKP1/ASK-INTERACTING PROTEIN 16 (PvSKIP16), ACTIN 11 (PvACT11),* and *TUBULIN BETA-8 (Pvβ-TUB8)* genes (Borges et al., 2012), which serve as internal control genes (Table 1).

Real-time PCR was performed to test for the relative expression of the drought stress-related marker genes *PvDREB1F, PvDREB5A* and *PvDREB6B* (Konzen et al., 2019) on four bean genotypes to evaluate their tolerance to drought stress (Table 1). RNA templates were replicated thrice (3) and diluted to a concentration of 10 ng μl^{-1}. The PCR experiment was conducted using a Luna Universal One-step RT-qPCR Kit (New England Biolabs, USA) consisting of 1X Luna Universal One-Step Reaction Mix (10 µl), 1 x Luna WarmStart® RT Enzyme Mix (1 µl), 0.4 µM of each primer (0.8 µl), 1 µg of diluted RNA and RNase-free water. Triple-replicate RT-qPCRs were performed in 96-well plates using a LineGene 9600 Bioer PCR machine (Hangzhou Bioer Technology) following SYBR Green/FAM detection. The RNA template was reverse transcribed at 55 °C for 60 s, followed by initial denaturation at 95°C for 60 s, 40 cycles of denaturation at 95°C for 10 s and extension at 60 °C for 35 s, and a melting step at 95 °C. PCR efficiency (E) was calculated using LinRegPCR (version 2014.5), while threshold (Ct) values were used to determine the relative expression level of a given gene using the $2^{-\Delta\Delta}$Ct method (Livak & Schmittgen, 2001; Schmittgen & Livak, 2008). The relative expression of genes common bean genotypes was then compared to that of Tepary bean as the control.

2.6 STATISTICAL DATA ANALYSIS

The data were subjected to analysis of variance

Table 1: Reference and target genes for the real-time PCR-based relative expression of genes.

Reference genes (Borges et al., 2012)				
NCBI ID	Gene	Forward primer (5'- 3')	Reverse Primer (5'- 3')	Amplicon size (bp)
62703083	*ACTIN-11 (PvACT11)*	TGCATACGTTGGTGATGAGG	AGCCTTGGGGTTAAGAGGAG	190
171656465	*TUBULIN BETA-8 (PvB-TUB8)*	AATGTGAAGTCCAGCGTGTG	CTTCCCCAGTGTACCAATGC	163
187434529	*SKP1/ASK-INTERACTING PROTEIN 16 (PvSKIP16)*	CACCAGGATGCAAAAGTGG	ATCCGCTTGTCCCTTGAAC	163
Biotic stress genes (Konzen et al., 2019)				
Phytozome accession ID	Gene	Forward primer (5'- 3')	Reverse Primer (5'- 3')	Amplicon size (bp)
Phvulv091025959m.g	*PvDREB1F*	TGCGTCGAGCAATTAGAGAA	TCCTGATGCGTCTGGTATTG	153
Phvulv091010162m.g	*PvDREB5A*	TTGGGTACTTTTCCCACTGC	GCCTTCCATGTCATCATCCT	177
Phvulv091016691m.g	*PvDREB6B*	AATTCTGCATCTCCCTCACG	GCTGGGCTTGATTTAGACGA	167

(ANOVA) using the statistical software SPSS (version 22 of Windows; SPSS). One-way analyses of variance followed by Tukey's HSD test comparison at $p \leq 0.05$ were performed to determine the relevant differences between the control and drought-stressed variants of the respective genotype.

3 RESULTS

3.1 DRY LEAF BIOMASS IN RESPONSE TO DROUGHT

Similarly, compared with that of the control plants, the growth rate of the drought-stressed plants in terms of dry biomass was not affected in the CAL96 or DAB541 genotype. Interestingly, drought stress significantly reduced dry biomass production in the GK011 and DAB514 genotypes. This response translated to significant dry biomass production inhibition of 0.68 g (28 %) and 0.94 g (33 %) in GK011 and DAB514, respectively (Table 2).

3.2 LIPID PEROXIDATION LEVELS AND PROLINE PRODUCTION

The lipid peroxidation in leaves as determined by the MDA content, varied significantly between stressed and control plants for the GK011 tepary bean and DAB514 common bean. The most significant drought-induced increase in the accumulation of MDA of 5.06 μmol g^{-1} fresh mass (117.3 %) was demonstrated in tepary bean, followed by 2.75 μmol g^{-1} fresh mass (42.04 %) in the DAB514 genotype. However, compared with those in the control plants, the MDA content in the stressed plants was not significantly greater for CAL96 and DAB541 (Table 2).

A significant increase in the biosynthesis of proline of 1.09 (23.6 %) and 1.70 (39.6 %) mg^{-1} fresh mass was induced in the CAL96 and DAB541 drought-treated plants, respectively, in comparison to their corresponding control plants. However, drought stress significantly inhibited the production of proline by 0.94 mg^{-1} fresh mass (22 %) in GK011 tepary beans (Table 2).

3.3 ANTIOXIDANT ENZYMATIC ACTIVITIES

The enzymatic activities varied between the stressed plants and the control plants in terms of H_2O_2 concentration were observed for all the genotypes except for the DAB514 genotype. Drought stress significantly promoted this enzyme activity by 5.43 μmol g^{-1} fresh mass (68.6 %) in GK011 tepary bean and 2.82 μmol g^{-1} fresh mass (47.1 %) in DAB514 common bean. The reverse was observed in CAL96, which exhibited a significant 2.5 μmol g^{-1} fresh mass (27 %) reduction in H_2O_2 enzymatic

Table 2. Dry biomass production and accumulation of metabolites in response to drought stress. Values are represented as the mean ± SEM (n = 6) of independent biological replications. Values followed by the same letter do not differ from each other by Tukey's test ($p \leq 0.05$).

Genotype	Dry biomass (g)		MDA content (mg^{-1} fresh mass)		Proline (mg^{-1} fresh mass)	
	Control	Drought stress	Control	Drought stress	Control	Drought stress
GK011	2.40 a	1.73 b	5.67 a	9.29 b	4.19 a	3.25 bc
CAL96	2.77 a	2.76 c	10.99 b	10.06 b	4.60 a	5.69 e
DAB514	2.86 a	1.92 b	6.95 ad	11.23 bc	3.82 b	3.48 bc
DAB541	3.67 d	3.60 d	9.67 bcd	5.35 a	4.29 b	5.98 e

activity in response to drought stress (Fig. 2A). While drought stress increased APX activity in DAB514 (3.37 µmol mg^{-1} FM protein min^{-1} 19,6 %), drought stress did not affect APX activity in the GK011 and CAL96 genotypes (Fig. 2B). Drought stress also induced variations in catalase activity between the stressed plants and the control plants. Although plants exposed to drought stress presented significant 2.71 µmol mg^{-1} FM protein min^{-1} (14.5 %) and 3.30 µmol mg-1 FM protein min^{-1} (15.4 %) increases in catalase activity in CAL96 and DAB541, respectively, such increases in enzyme activity in GK011 and DAB514 were not significant (Fig. 2C).

3.3 RELATIVE EXPRESSION OF DEHYDRATION-RESPONSIVE ELEMENT BINDING (DREB) GENES

The qPCR analysis was conducted on a highly intact RNA template that showed clear gel bands corresponding to 18S and 28S rRNA and the absence of a smear (Fig. 2A). The first qPCR experiment on the GK011 tepary bean and CAL96 common bean genotypes demonstrated an increase in the relative expression of the *PvDREB1F, PvDREB5A*, and *PvDREB6B* genes in GK011 tepary bean plants. Drought stress induced at least a 1-fold decrease in the relative expression of *PvDREB1F* and *PvDREB6B* as well as a 2-fold decrease in *PvDREB5A* in GK011 tepary bean plants. Similar results were observed for the CAL96 genotype, in which the relative expression of *PvDREB5B* and *PvDREB6B* were inhibited. However, there was a significant 1.2-fold increase in the expression of *DREB1F* in drought-stressed plants compared to that in the corresponding control plants. (Fig. 2B). A study between GK011 tepary bean and DAB514 revealed the distinct suppression of the differential expression of all three DREB genes in both genotypes. The highest average levels of 1.7-fold and 1.3-fold inhibition of *PvDREB5A* relative expression were revealed in the GK011 and DAB514 genotypes, respectively. Taken together, these findings indicated that drought stress induced significant and marked downregulation of the differential expression of *PvDREB5A* compared with that of the other two DREB genes in both the GK011 and DAB514 genotypes (Fig. 2C). A similar trend of a downregulated expression of *PvDREB1F, PvDREB5A,* and *PvDREB6B* in the which DREB gene were analyzed in GK011 and DAB541. Amongst the three genes, *PvDREB5A* was highly differentially expressed (2.9-fold). Drought stress downregulated the relative expression of PvDREB1F and *PvDREB6B* in the DAB541 genotype. In contrast, a marked increase in the expression of *PvDREB5A* (1.9-fold) was detected in DAB541 drought-stressed plants compared with control plants (Fig. 2D).

4 DISCUSSIONS

Climate-adaptive strategies such as the use of drought-tolerant plants in Botswana are highly important for the selection and introduction of crops with improved performance under fluctuating water deficit conditions. This study adopted the considerable effort that is devoted to the selection of crops using plant morphophysiological parameters coupled with molecular and biochemical selection approaches. Intriguingly, drought stress upregulated the differential expression of *PvDREB1F*, which might have contributed to the maintenance of dry biomass production in the CAL96 common bean genotypes. This finding was in agreement with that of a previous study on rice, which demonstrated the overexpression of *OsDREB1F* under salt, drought, and low-temperature tolerance (Wang et al., 2008). The induced overexpression of *PvDREB1F* was accompanied by an increase in proline and antioxidant enzymes in the present study. Interestingly, an increase in proline level positively correlated with the maintained levels of MDA, hence suggesting the prevention of lipid peroxidation. In addition, an increase in CAT activity suggested an enhanced CAT enzymatic antioxidative defense mechanism that plays a role in the detoxification of H_2O_2 thereby maintaining equilibrium (Apel & Hirt, 2004; Ghaffari

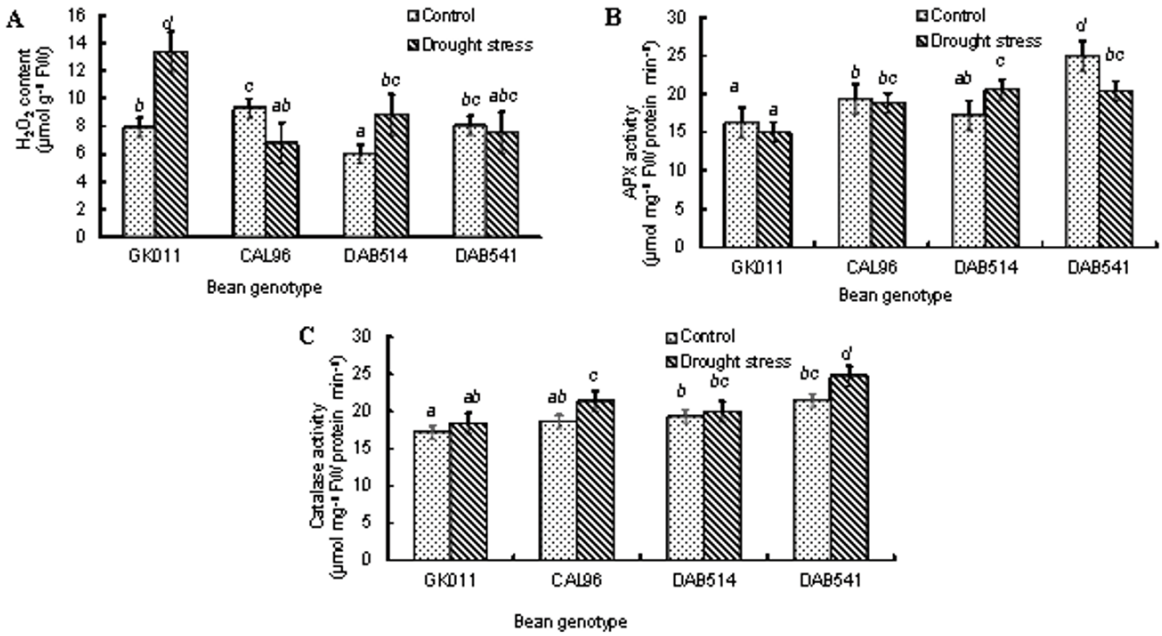

Figure 1: The effect of drought stress on the accumulation of H2O2 (A), APX activity (B) and CAT activity in common bean genotypes. Mean values represent ± SEM (n = 6) of independent biological replications. Different lower-case letters indicate significant ($p \leq 0.05$) differences between mean values according to Tukey's tests made separately for each genotype for the drought stresses against the control.

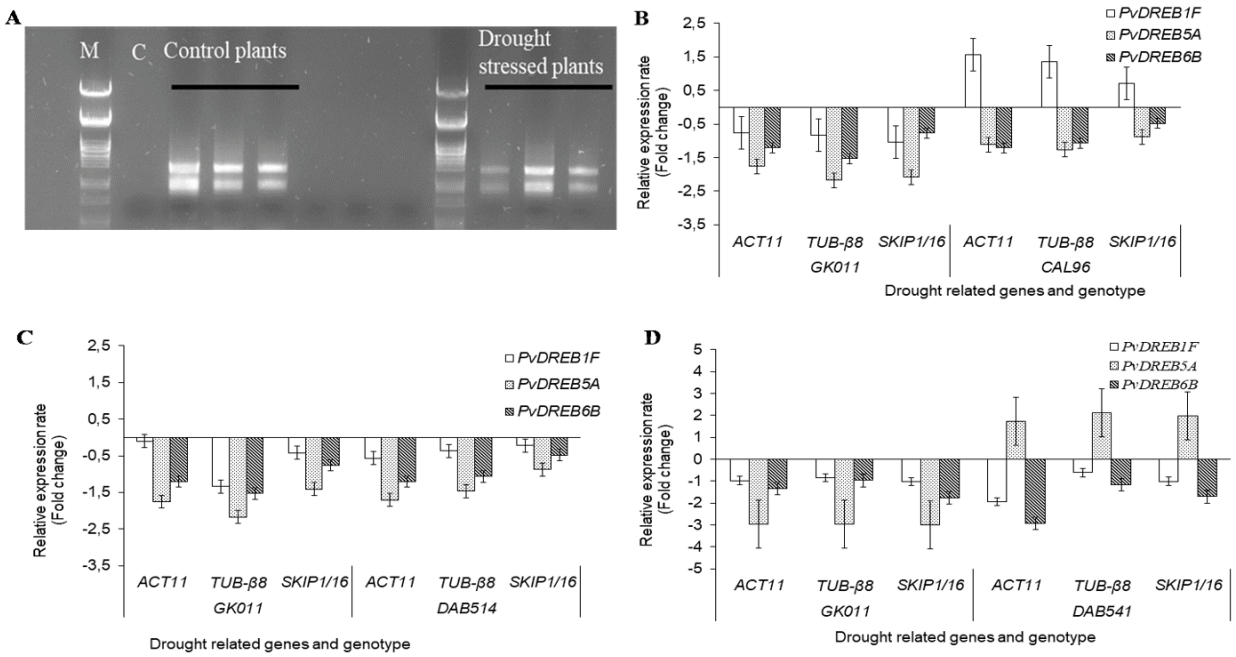

Figure 2: Relative expression of DREB genes run on high intact RNA template (A) for CAL96 (B), DAB514 (C) and DAB541 (D) common bean genotype in response to drought. Real-time PCR was conducted in the respective common bean genotypes against the GK011 tepary bean. Relative expression rates of DREB genes were normalized against three internal reference genes (PvACT11; PvTUB-β8; PvSKIP1/16). Bars represent the mean ± standard error of the mean (SE) of pooled three technical samples from three independent samples. Values sharing a common letter are not significantly different at $p < 0.05$.

et al., 2019; Gomes et al., 2022). The drought tolerance mechanism for CAL96 likely occurs through the expression of *PvDREB1F,* maintenance of ROS homeostasis and prevention of lipid peroxidation-related cell death. These are additional proposed drought stress tolerant mechanisms that also support the previous study that indicated that CAL96 could confer drought tolerance through the promotion of allantoin pathways (Pholo-Tait et al., 2022).

The expression of a DREB 5-A subgroup of transcription factor genes from castor bean in tobacco has been reported to be associated with drought tolerance (do Rego et al., 2021). Similarly, the overexpression of *PvDREB5A* in our study suggested the presence of transcriptionally related drought tolerance mechanisms that resulted in the maintenance of growth in terms of leaf dry biomass. Contrary to inhibited growth as a result of high levels of protein (Arteaga et al., 2020), the growth rate was not affected by an increase in proline in this study. In concert with the previous report (Porch et al., 2009), the increased proline levels might have acted as a component of signal transduction pathways that regulate the overexpression of *PvDREB5A*. In addition to its adaptive role in mediating osmotic adjustment and protecting subcellular structure, an increase in proline in the DAB541 genotype could have played a role in maintaining higher activities of CAT. The latter could have subsequently maintained a steady activity of CAT-enzyme scavenging of H_2O_2 reactive oxygen species (Chen et al., 2022; Molinari et al., 2007; Noctor et al., 2018). Furthermore, the overexpression of *PvDREB5A* correlated with a reduction in MDA in DAB541 and this is in line with the findings of a study on maize (Moussa & Abdel-Aziz, 2008). High levels of proline could have contributed to the reduction in MDA levels (Soares et al., 2019). This promoted an inverse correlation along with an increase in CAT activity, suggested a reduced lipid peroxidation and improved redox buffering as a result of effective scavenging of H_2O_2 (Dong et al., 2018).

On the contrary to CAL96 and DAB541 genotypes, drought stress-induced suppression of the differential expression of *PvDREB1F, PvDREB5A*, and *PvDREB6B* in GK011 and DAB514 genotypes. The downregulated differential expression of genes positively correlated with greater levels of proline, MDA, and H_2O_2 in the GK011 and DAB514 genotypes. Contrary to a previous study on common bean (Arteaga et al., 2020), decreased levels of proline resulted in an inhibited growth GK011 bean genotype. Rather, the inhibited growth rate might have been attributed to the promoted lipid peroxidation and eventual cell death due to the high accumulation of MDA and levels and the promoted H_2O_2 content (Ghaffari et al., 2019; Sivakumar et al., 2000; Soares et al., 2019). Previous reports indicated that an increase in MDA content resulted in cell membrane rupture, hence increasing membrane leakage in *P. vulgaris* L. (Zlatev et al., 2006), *Avena* species (Pandey et al., 2010) and wheat (Tatar & Gevrek, 2008). In that respect, the downregulated expression of DREB genes and increased levels of MDA and H_2O_2 suggested a promoted disequilibrium between H_2O_2-related ROS production and H_2O_2 scavenging activity (Yang et al., 2020) due to the stable cooperative activities of APX and CAT (Apel & Hirt, 2004; Gomes et al., 2022). Such disequilibrium could have resulted in an oxidative burst due to lipid peroxidation and protein denaturation (P. Sharma et al., 2012; Yang et al., 2020). This could have resulted in greater levels of oxidative damage possibly through enhanced membrane lipid peroxidation and loss of membrane integrity to withstand the cellular-level effects of water loss and ultimately caused cellular damage and death, hence inhibiting plant growth (Kong et al., 2016; Ripullone et al., 2021; V. Sharma et al., 2019).

An increase in MDA content, APX activity and greater levels of H_2O_2 was accompanied by a decrease in dry biomass in DAB514. As previously discussed above, increased levels of MDA suggested an increase in lipid peroxidation and subsequently promoted plant cell damage. Despite an increase in APX activity, the promoted production of H_2O_2 substantiated the need for APX for cooperative ROS enzymatic scavenging activity. This is in concert with previous studies which reported that increased APX activity in *Salvinia molesta* D. Mitch. and *Vallisneria natans* (Lour.) H. Hara resulted in H_2O_2 accumulation, lipid peroxidation, and subsequently decreased growth rates (Gomes et al., 2022).

5 CONCLUSIONS

The current study demonstrated that CAL96 and DAB541 plants are possibly tolerant to drought stress. The CAL96 common bean genotype could confer drought tolerance through the overexpression of *PvDREB1F* and enhance the scavenging mechanism that involves the active role of proline in maintaining lipid peroxidation and the cooperative scavenging of H_2O_2 by CAT and APX. In the DAB541 common bean genotype, drought stress tolerance is associated with the overexpression of *PvDREB5A* and increased levels of proline, which cooperatively play a major role in the suppression of lipid peroxidation through reduced levels of MDA, hence stabilizing the membrane. In addition, such drought tolerance could have been attributed to H_2O_2 enzymatic scavenging activity, in which increased CAT activity enhanced the maintenance of steady H_2O_2 levels. This finding therefore supported a previous study (Pholo-Tait et al., 2022) that the CAL96 and DAB541 genotypes serve

as promising drought-tolerant common bean genotypes. However, future reverse genetic approach studies that involve silencing *PvDREB1F* and *PvDREB5A* will unambiguously conclude their drought-induced tolerance role. This will further substantiate the importance of the inherent genotypic traits to serve as potential parent material in marker-assisted breeding to improve common bean varieties for drought tolerance and stress-induced ROS

6 CONFLICTS OF INTEREST

There is no conflict of interest regarding the manuscript.

7 DATA AVAILABILITY

Original data could be obtained upon reasonable requests from corresponding author.

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