Phenotypical, physiological and molecular assessment of drought tolerance of five Egyptian teosinte genotypes

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ABSTRACT
Prolonged drought presents a serious challenge to the agricultural sector. The main concern of this work was to assess the phenotypical, physiological, and molecular responses of five teosinte (Zea mays L. Schrad L) genotypes (Ba, Gm1, Gm2, Gm3 and Gm4). In a two-season (2020 and 2021) field experiment, fifteen-day-old teosinte plants were subjected to well-watered (15-day watering frequency) as a control, moderate drought (25-day watering frequency), and extreme drought (35-day watering frequency) treatments. Drought negatively affected growth, yield, chlorophyll, and POD activity of all genotypes, but promoted soluble sugars and proteins, osmoregulatory molecules (glycinebetaine, amino acids, and proline), non-enzymatic antioxidants (phenols, flavonoids, and alkaloids), and SOD activity. Furthermore, long-term water stress upregulated MOCOS, Rad17, NCED1, CAT1, and PSC5 genes expression, with Gm3 and Gm4 being the most drought-tolerant genotypes. These findings could be employed in breeding programs to develop tolerant genotypes to address the challenges posed by climate changes like drought.

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1. Introduction

Drought is a prevalent environmental issue that has a detrimental impact on plant development and performance, particularly in arid and semi-arid parts of the world. Drought leads to hyperosmotic stress and inhibits plant growth through interfering with a variety of biochemical and physiological processes, including membrane permeability, photosynthetic activity, redox homeostasis, secondary metabolism, pigmentation, turgor pressure, and relative water content (Chiappero et al. 2019). The reduced water uptake limits the plant’s ability to obtain essential nutrients and inhibits photosynthetic activity, resulting in reduced growth and development, as evidenced by smaller leaves, shorter stems, and poorer root growth (Talbi et al. 2020). Plants typically close their stomata as the main response to water scarcity, resulting in reduced intracellular CO₂ concentration and photosynthetic activity. This leads to slowing down the Calvin cycle, causing NADPH accumulation and decreased regeneration of electron acceptors (NADP⁺, NAD⁺ and FAD), promoting flux of electrons from the electron transport chain to oxygen and activating ROS (reactive oxygen species) excessive production (Robinson and Bunce 2000). Increased ROS production is detrimental because it debilitates cellular structures and inhibits metabolic pathways while also oxidizing biomolecules like lipids, proteins, and DNA, bringing on cell death (Noctor et al. 2014).

Several drought-tolerance strategies have been hypothesized based on biochemical and physiological responses. Many plants have evolved mechanisms that allow them to endure varying levels of water deficiency through altering the major metabolic processes, including CO₂ reduction, glycolysis, as well as C and N metabolism (Abid et al. 2016). Plant physiological responses help to sustain cellular metabolic activity, reduce water loss, and protect subcellular structures. Under drought conditions, osmotic adjustment is mediated by the substantial biosynthesis and accumulation of compatible osmolytes such as carbohydrates, proteins, polyamines, betaines, organic acids, and amino acids. Osmotic adjustment, a well-drought-responsive mechanism, attests to a decrease in osmotic potential in response to cumulative osmolyte accumulation to sustain cellular turgidity for the continuation of plant metabolic activity and growth (Zeng et al. 2015). Carbohydrates are important in osmotic adjustment, whereas amino acids and organic acids are integral to maintaining water potential gradients from soil to plants (Rahman et al. 2017). One of the first responses to a water shortage is stomatal closure. This immediate response is controlled by a complex network of signaling cascades. In this situation, abscisic acid operates as a master regulator of stomatal closure to avoid dehydration through transpiration (Ayyaz et al. 2021).

Drought stress is associated with a higher concentration of ROS, which are detrimental to plant growth. To overcome the harmful consequences of these ROS, plants enhance the transcription of antioxidant enzyme genes such as POD, CAT, SOD, and APX (Li et al. 2020). Furthermore, numerous recent investigations have shown the transcription of other drought-tolerance genes linked to osmoregulation and photosynthesis, as well as a variety of transcription factors (Wei et al. 2016; Jacob et al. 2017; Maghsoudi et al. 2018). Drought has also been demonstrated to cause the accumulation of some other solutes such as polyphenol and α-tocopherol, which protect plants from oxidative damage and enable them to sustain the high turgor pressure required for stomata apertures and gas exchange (White...
et al. 2000). Additionally, non-enzymatic antioxidants including leaf anthocyanins, carotenoids, phenolics, flavonoids, and ascorbic acid, also play a role in preventing ROS formation (Sircel et al. 2007). In addition, some secondary metabolites such as glucosinolates, indole alkaloids, non-protein amino acids, terpenes, cyanogenic glucosides, and phytoalexins have been reported to protect plants experiencing water stress in several plant species (Klein-wächter and Selmar 2015). Cui et al. (2019) ascribed the higher levels of secondary metabolites in drought-stressed plants to an adaptive mechanism to guarantee the metabolic balance under such circumstances.

Teosinte (Zea mexicana Schrad L.) is a maize-related green fodder commonly known as buffalo grass. Phenotypically, teosinte resembles maize, but they differ in cob morphology, tillering, and branching characteristics. It is well adapted to humid tropical and subtropical regions (Rana et al. 2018). Rapid growth, high yield, and resistance to biotic and abiotic challenges characterize teosinte as fodder. The value as fodder is comparable to maize, with the distinction of having a high carbohydrate content. Teosinte is a wonderful multi-cut fodder that produces a high output of nutritive green spectacular fodder in 65–70 days while requiring fewer inputs than maize (Mohan et al. 2017). Because of its versatility and biomass production competence, it can be grown in any rigorous fodder production system. Teosinte is an appropriate fodder crop with excellent adaptation to extreme environments such as acidic and flooded places, which many other fodder species cannot (Devkota et al. 2015).

Drought-tolerant genotype identification has been recognized as an economically sustainable and environmentally superior strategy for increasing forage production in water-stressed areas. Drought tolerance must therefore be assessed using phenomenology, physiology, biochemistry, and molecular behavior at various growth stages. Assessment of drought tolerance of different plant genotypes based on their physiological response to drought stress may be a powerful strategy for screening out well-developed alternative genotypes, but it requires a complete understanding of the yield determinant mechanisms (Jall and Ansari 2020). Furthermore, assessing the physiological changes occurring under drought stress may lead to the genetic enhancement of drought-tolerant genotypes. In this regard, teosinte may be one of the promising summer animal feed that may be cultivated with limited water supplies. The main target of this work was to explore the variability in drought response of five teosinte genotypes recently grown in Egypt under three distinct irrigation systems based on their phenological, biochemical, and molecular traits. We also planned to determine the most tolerant genotype that may be employed as parents of water-stress-tolerant hybrids in the newly reclaimed areas.

### 2. Materials and methods

#### 2.1. Plant material, experimental treatments and design

The Field Crops Research Institute, Agricultural Research Center, Giza, Egypt, gratefully provided seeds of five teosinte (Zea mexicana Schrad L.) genotypes. The supplied genotypes were collectively named Baladi (check genotype), Gemiza 1, Gemiza 2, Gemiza 3, and Gemiza 4. The experiment was conducted at El-Gemiza Experimental Research Station, El-Santa, El-Gharbia, Egypt (30° 79′ N, 31° 12′ E) during two consecutive summer seasons (2020 and 2021). The physio-chemical examinations of the soil used in this study are summarized in Table 1. A randomized complete block design in a split-plot arrangement with a plot area of 16 m² (4 x 4 m) was replicated three times in each season. Before sowing, teosinte seeds were washed with tap water, surface-sterilized for 8 min with 3% (v/v) Chlorox, then thoroughly rinsed five times with deionized water. Three teosinte seeds were planted in each hole, with holes 35 cm apart and row 60 cm apart on the 1st of May over the two seasons. Seeds were irrigated with the prescribed field capacity (265 L/m²) and seedlings were trimmed to one per hole 10 days following planting, after complete seedling establishment. The plots of each genotype were split into three plots and drought treatments were started from the second irrigation. Until the harvest, water was supplied to the first plot every 15 days, the second plot every 25 days, and the third plot every 35 days. The FAO Irrigation and Drainage for Computing Crop Water Requirements standards were used to calculate the average quantity of water utilized in each irrigation (2418.27 m³/ha) (Allen et al. 1998).

All other agricultural practices were done on time following the guidelines of the Egyptian Ministry of Agriculture and Land Reclamation. The supplementary material Table S1 provides further information on overall water requirements during the growing season as well as weather considerations.

### Table 1. The physio-chemical examinations of the soil used in this study.

| Parameters                      | Value | Parameters                      | Value |
|---------------------------------|-------|---------------------------------|-------|
| Particle size distribution (%)   |       | Total macronutrients (%)        |       |
| Coarse sand                     | 5.23  | N                               | 0.144 |
| Fine sand                       | 18.46 | P                               | 0.032 |
| Silt                            | 37.24 | K                               | 0.356 |
| Clay                            | 39.07 | Available macronutrients (mg. kg⁻¹) |       |
| Textures                        |       | Organic C (%)                   | 1.45  |
| Clay loam                       |       | C/N ratio                       | 10.07 |
| pH                              | 7.77  | P                               | 10.63 |
| EC (dSm⁻¹)                      | 1.67  | K                               | 315.72|
| Soluble ions (meq⁻¹)            |       | Extractable micronutrients (ppm)|       |
| Ca²⁺                            | 6.13  | C/N ratio                       | 10.07 |
| Mg²⁺                            | 5.32  | Extractable micronutrients (ppm)|       |
| Na⁺                             | 7.46  | Fe                              | 3.83  |
| K⁺                              | 0.23  | Mn                              | 3.15  |
| CO₃⁻                             | 0.00  | Zn                              | 4.46  |
| HCO₃⁻                           | 3.62  | Cu                              | 1.53  |
| Cl⁻                             | 8.13  |                                 |       |
| SO₄²⁻                            | 7.39  |                                 |       |

2.2. Assessment of growth traits

At the age of fifty days, ten plants from each plot were randomly collected and separated into shoots and roots. The harvested plants were measured for shoot height (cm.plant⁻¹), leaf area (cm²:leaf⁻¹), and shoot biomass (g.plant⁻¹). For shoot biomass calculation shoot samples were dried in an air-forced oven for seven days at 65 °C.

2.3. Biochemical analyses

#### 2.3.1. Measurement of total chlorophyll and soluble sugars and proteins

A portable chlorophyll meter (SPAD502 plus, Konica Minolta Inc., Japan) was used to measure total chlorophyll concentration in the fifth completely exposed leaf per plant from
the middle of the leaves. Soluble sugars were extracted from the finely powdered teosinte leaves using borate buffer. Using glucose as a standard sugar, the phenol-sulfuric acid method of Dubois was employed to determine total soluble sugars (TSS) as mg.g\(^{-1}\) DW (Dubois et al. 1965). Soluble proteins were extracted from the fresh teosinte leaves using potassium phosphate buffer (50 mM, pH 7). Coomassie brilliant blue reagent and a standard graph generated by bovine serum albumin as a standard protein were used to evaluate total soluble proteins (TSP) as mg.g\(^{-1}\) DW (Bradford 1976).

2.3.2. Enzymatic antioxidants
Teosinte fresh leaves were ground in liquid nitrogen before being extracted in a 50 mM potassium phosphate buffer, ethylenediaminetetraacetic acid (EDTA), and polyvinylpyrrolidone (PVP) solution. The mixture was centrifuged for 15 min at 5488 g in a cooling centrifuge and the supernatant was used in assaying three antioxidant enzymes as µM FW.min\(^{-1}\). Catalase (CAT; EC 1.11.1.6) activity was determined in the enzyme extract at 240 nm using phosphate buffer and H\(_2\)O\(_2\) (Kato and Shimizu 1987). The enzyme extract was mixed with phosphate buffer, guaiacol, and H\(_2\)O\(_2\), and the absorbance at 460 nm was monitored to determine peroxidase (POD; EC 1.11.1.7) activity according to Kato and Shimizu (1987). The activity of superoxide dismutase (SOD; EC 1.15.1.1) was evaluated by monitoring the absorbance at 560 nm following the photochemical reduction of nitroblue tetrazolium to formazan in the presence of methionine, as reported by Beyer and Fridovich (1987). Enzymatic activities were assessed as µM.g\(^{-1}\)FW.min\(^{-1}\) with the aid of the specific extinction coefficients of each enzyme (40.0, 26.6, and 21.1 m\(^{-1}\)cm\(^{-1}\) for CAT, POD, and SOD, respectively).

2.3.3. Osmomodulatory compounds determination
Dry powdered teosinte leaf extract were prepared using deionized water for 24 h with agitation, then centrifuged for 15 min at 2800 g for the determination of glycinebetaine (GB). The extract was combined with 2N HCl (1:1 v/v) and incubated at 4 °C for 24 h before centrifugation at 4032 g for 25 min. After dissolving the pellets in 1,2-dichloroethane, the organic fraction was completely evaporated. The total alka
doid content (TFC) was determined using the aluminum chloride colorimetric technique (Lesjak et al. 2014). The total flavonoids content (TFC) was determined using the modified Folin–Ciocalteu technique was used to determine the total phenolic content (TPC) according to (Velioglu et al. 1998). Leaf extracts were mixed with a two-fold diluted Folin-Ciocalteu’s reagent and a 7.5% (w/v) Na\(_2\)CO\(_3\) solution, then incubated in dark for 30 min before measuring the absorbance at 765 nm. TPC was calculated as mg.g\(^{-1}\) DW using a gallic acid standard curve. Total flavonoids content (TFC) was determined using the aluminum chloride colorimetric technique (Lesjak et al. 2014). The measuring mixture was composed of teosinte extract, methanol, 5% (w/v) AlCl\(_3\), and 0.5 M potassium acetate. After 30 min of shaking, the absorbance at 415 nm was measured. TFC was represented as mg.g\(^{-1}\) DW, with the standard curve constructed using quercetin as a standard flavonoid. Total alkaloid content (TAC) in teosinte ethanolic extracts was evaluated gravimetrically according to (Resmi et al. 2015). The extract was acidified using HCl, centrifugated, and treated with chloroform four times to extract the acidic solution. Ammonia solution was added drop-wise until pH reached 9.0 then treated again with chloroform. Water was used to wash the organic layer, which was subsequently passed through anhydrous sodium sulfate. The residue was dried at 60 °C after the organic fraction was completely evaporated. The total alka
doid weight was then calculated and represented as mg.g\(^{-1}\) DW.

2.4. Quantitative RT–PCR analysis
Total RNA was isolated from teosinte leaves using TRIZOL reagent (Invitrogen, USA), and cDNA was synthesized using a reverse transcription reagent kit according to the manufacturer’s instructions. The qRT–PCR was performed in triplicate using the SYBR Green PCR Master Mix (Fermentas, USA). In each reaction, a 25 µl reaction mixture comprising primer pairs of drought resistance genes (MOCOS, Rad17, P5CS, NCED1 or CAT1) or the reference gene (β-actin) (Table 2) were used, and data were retrieved during the extension phase. The drought-responsive genes employed in this investigation were designated based on our previous study on maize (Saad-Allah et al. 2022), which is a close relative to teosinte. The Rotor-Gene 6000 Real-Time PCR cycler was used to perform the qRT–PCR reactions (QIAGEN, ABI System, USA).

The amplification technique comprised a 10-min denaturat
tion period at 95 °C, accompanied by 40 cycles for 5 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. To eliminate the inclusion of non-specific products, melting curves were generated. The gene expression data were investigated using the 2\(^{-ΔΔCt}\) approach (Livak and Schmittgen 2001).

Table 2. Primer sequences of the candidate drought-responsive genes for qRT–PCR analysis.

| Gene name | Gene ID | Sequence (5′–3′) |
|-----------|---------|-----------------|
| ZmMOCOS   | 542228  | F′′GTATGGATCGAAAGTGGTTGGAC′′ R′′AAGTCGAACCTGGAACATC′′ |
| ZmRad17   | 542621  | F′′CACTAGATACGTCGTTGATG′′ R′′AGCTAATAGCTTCCCAATC′′ |
| ZmNCED1   | 10362760 | F′′TACCCTGATTTACAAACGGA′′ R′′GATGTTTGACCCGATGCT′′ |
| ZmCAT1    | 542369  | F′′CTTACGATGGTCGTTGGAAGG′′ R′′GTGCGCGGATTTGGTCGT′′ |
| ZmP5CS    | 778431  | F′′ACGCTGCTGCTGAAAAGTGCTGAG′′ R′′GAAGGCTGCTGAAAAGTGCTGAG′′ |
| Zmβ-actin | 100282267 | F′′ACTATCGTGGCCTGGGAT′′ R′′GTCCGTCTGCTGAAAAGTGCTGAG′′ |

F = Forward primer; R = Reverse primer.
2.5. Assessment of yield parameters

At harvest time, each plot’s plants were harvested separately. The number of tillers per plant was counted, and the tillers were taken from the plant and allowed to dry in the open air. The kernels of each teosinte genotype were removed from the tillers individually in each plot, and the weight of 1000 kernels (g), as well as kernels yield (kg ha⁻¹), were assessed.

2.6. Statistical analysis

The experiment was set up as a split-plot treatment with a completely randomized design. Drought was the main plot parameter (three levels: control, mild, and severe), and teosinte genotypes were the sub-plot parameter (Ba, Gm1, Gm2, Gm3, and Gm4). To examine the impacts of drought frequencies on teosinte genotypes, a two-way analysis of variance (ANOVA) at \( P \leq 0.05 \) was used for all the measured parameters. The means of water-stressed and control teosinte genotypes in the molecular analysis were separated using a one-way analysis of variance (One-way ANOVA) at the \( P \leq 0.05 \) level. Averaging the results of two separate studies with six replicates (3 each) yielded the mean and the standard deviation (SD). All statistical analyses were performed using the CoStat 6.311 software.

3. Results

3.1. Genotypic response of teosinte growth parameters to the watering frequency

Figure 1 demonstrates how five teosinte genotypes, Baladi (Ba), Gemiza 1 (Gm1), Gemiza 2 (Gm2), Gemiza 3 (Gm3), and Gemiza 4 (Gm4), responded to three watering intervals (15, 25 and 35 days) in terms of growth performance. Under typical irrigation interval (15 days), the four Gemiza genotypes outperformed the control genotype (Ba) in terms of shoot length, leaf area, and shoot biomass, with the superiority of Gm3 and Gm4 over other genotypes in these traits. All of the assessed growth attributes were significantly reduced when the watering interval frequency was increased, with the 35-day frequency being the most severe for all teosinte genotypes.

When compared to the typical watering frequency, the genotypes Ba, Gm1, Gm2, Gm3, and Gm4 exhibited shoot height reductions of 92.7, 49.0, 44.6, 55.3, and 53.7%, respectively. When compared to the typical watering frequency, the abovementioned genotypes showed decreases in leaf area of 48.7, 43.0, 45.7, 33.6, and 33.8%, respectively. Likewise, when compared to the typical watering frequency, shoot biomass was reduced by 38.5, 43.8, 48.8, 36.5, and 38.7% for the foregoing genotypes as a consequence of the longer watering interval. As a result, among the genotypes evaluated, Gm3 and Gm4 were the most drought-tolerant in terms of growth characteristics.

3.2. Genotypic response of teosinte chlorophyll, sugars, and proteins to the watering frequency

The amount of chlorophyll (SPAD), total soluble sugars (TSS), and total soluble protein (TSP) in the examined teosinte genotypes were significantly affected by the frequency of irrigation intervals (Figure 2). Gm3 and Gm4 genotypes were shown to possess the highest chlorophyll, TSS, and TSP levels under the normal irrigation frequency, but the check genotype (Ba) had the lowest chlorophyll and TSP, and the genotype Gm2 had the lowest TSS. Drought severity had a detrimental impact on chlorophyll content in teosinte genotype leaves, as it reduced as drought severity increased. The longest irrigation frequency period resulted in 47.3, 47.7, 39.7, 47.2 and 49.7% decreases in chlorophyll content of Ba, Gm1, Gm2, Gm3, and Gm4, respectively, as compared to the control irrigation interval (15 days). Total soluble sugars and proteins, on the other hand, revealed an increasing trend in teosinte leaves in response to water stress treatments. The largest accumulation of soluble sugars was achieved with a 25-day irrigation interval. The soluble sugars of Ba, Gm1, Gm2, Gm3, and Gm4 genotypes increased by 35.3, 50.3, 60.9, 24.6, and 41.6%, respectively, following this treatment when compared to the control one. However, the highest soluble protein yield, compared to the control watering interval, was determined after teosinte genotypes were exposed to severe water stress (35 days), resulting in increases of 38.3, 43.6, 41.5, 44.8, and 44.8% in the soluble proteins of Ba, Gm1, Gm2, Gm3, and Gm4 genotypes, respectively.

3.3. Genotypic response of teosinte CAT, POD, and SOD activities to the watering frequency

The activities of CAT, POD and SOD in the studied teosinte genotypes were significantly affected by the applied water stress treatments (Figure 3). Under control watering treatment, the check genotype (Ba) exhibited the lowest CAT activity, whereas the Gm4 genotype had the lowest POD and SOD activities. However, the highest activity was identified in the Gm3 genotype for CAT, but in the Gm1 genotype for POD and SOD. In all genotypes, increasing the watering interval had a negligible impact on CAT activity, compared to the corresponding control. However, as the watering interval was extended, POD activity significantly decreased in all genotypes investigated. On the other hand, in comparison to the control treatment (15 days watering interval) of all genotypes, SOD demonstrated a larger rising trend in activity after 35 days watering frequency. In the genotypes Ba, Gm1, Gm2, Gm3, and Gm4, SOD activity was increased by 74.0, 37.5, 50.4, 99.6, and 149.1%, respectively, compared to the control treatment. As a result of these findings, the Gm3 genotype has the most robust enzymatic antioxidant system for drought tolerance, with SOD serving as a key determinant of drought tolerance across all genotypes.

3.4. Genotypic response of teosinte osmoregulatory molecules to the watering frequency

Watering frequency demonstrated a significant effect on osmoregulatory molecules; glycinebetaine (GB), free amino acids (FAA) and proline (Pro), within the different teosinte genotypes (Figure 4). The amounts of GB, FAA, and Pro attained their highest levels in Gm3 and Gm4 genotypes at the classical irrigation frequency (15 days), whereas the Gm1 genotype had the lowest GB content and the check genotype (Ba) had the lowest FAA and Pro contents. Extending watering frequency significantly intensified the accumulation of osmoregulatory compounds in all studied teosinte genotypes. The 35-day watering distance prompted 32.1, 35.4, 20.4, and 19.3% increases in GB content of Ba, Gm1, Gm2,
and Gm3 teosinte genotypes, respectively, as compared with the control watering frequency; however, the genotype Gm4 was marginally influenced by watering severity (0.7% increase). Severe water stress also led to 57.5, 60.0, 52.4, 47.9, and 65.0% increases in FAA accumulation in Ba, Gm1, Gm2, Gm3 and Gm4 teosinte genotypes respectively,

Figure 1. The impact of different watering frequencies on the growth characteristics (shoot height, leaf area and shoot biomass) of five teosinte genotypes. Columns represents the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.
when compared to that of the control treatment. The frequency of watering interval has a direct relationship with the concentration of free Pro among the studied teosinte genotypes. Pro content had increased by 96.6, 65.6, 47.8, 38.5, and 45.9% in Ba, Gm1, Gm2, Gm3, and Gm4 genotypes, respectively, at severe watering stress treatment.

Figure 2. The impact of different watering frequencies on the physiological attributes (chlorophyll, soluble sugars and proteins) of five teosinte genotypes. Columns represent the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.
Figure 3. The impact of different watering frequencies on the enzymatic antioxidant activities (CAT, POD and SOD) of five teosinte genotypes. Columns represent the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey's post hoc HSD test.
Figure 4. The impact of different watering frequencies on the osmomodulatory compounds (glycinebetaine, amino acids and proline) of five teosinte genotypes. Columns represents the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.
Consequently, these results revealed that teosinte might withstand prolonged water deficits by synthesizing auxiliary osmoregulators.

3.5. Genotypic response of teosinte secondary metabolites to the watering frequency

In all of the teosinte genotypes investigated, longer watering intervals increased the level of secondary metabolites such as total phenolic compounds (TPC), total flavonoids content (TFC), and total alkaloids content (TAC) (Figure 5). The genotype Gm3 showed the highest TPC and TFC (24.2 and 4.81 mg·g⁻¹ DW, respectively) under the 15-day watering frequency (control) treatment, but the genotype Gm4 exhibited the highest TAC (0.68 mg·g⁻¹ DW). In general, all teosinte genotypes with the longest watering frequency (35 days) accumulated excessive secondary metabolites in their leaves. This treatment had resulted in 71.1, 60.6, 63.4, 53.7 and 62.3% increases in TPC, 109.7, 131.8, 121.3, 145.8 and 140.9% increases in TFC, and 72.7, 87.1, 113.5, 54.9 and 16.2% increases in TAC of Ba, Ba1, Ba2, Ba3 and Ba4 genotypes, respectively. Accordingly, the rise in TFC caused by this watering frequency interval outweighed the increases in TPC and TAC in the studied teosinte genotypes.

3.6. Genotypic response of teosinte yield parameters to the watering frequency

Water stress exposure, as measured by watering frequencies, exhibited substantial implications on the yield components of the investigated teosinte genotypes (Figure 6). In comparison to the other genotypes at the control irrigation frequency (15 days), the number of tillers/plant, the weight of 1000 kernels (g), and kernels yield (kg·ha⁻¹) were highest in the Gm4 genotype, followed by the Gm3 genotype, whereas the check genotype (Ba) displayed the lowest yield performance characteristics. Yield components reduction was related to watering spacing time, therefore a 35-day irrigation frequency resulted in a considerable reduction in teosinte productivity. The genotypes Ba, Gm1, Gm2, Gm3, and Gm4 experienced 35.3, 23.5, 26.4, 22.7, and 24.0% reductions in tiller number following the 35-day irrigation interval, respectively, as compared to the control irrigation cycle. Meanwhile, for the aforementioned genotypes, the reduction in 1000 kernel weight as a result of the harshest irrigation sequence was 45.9, 41.2, 36.6, 32.5, and 32.4%, respectively. Kernel production (kg·ha⁻¹), as an important measure of water stress tolerance, was significantly influenced by the length of the watering cycle. In comparison to the control watering interval, the longest irrigation regimen led to 21.1, 17.7, 19.7, 15.4, and 16.4% reductions in kernel yield for Ba, Gm1, Gm2, Gm3, and Gm4 genotypes, respectively. As a result, Gm3 and Gm4 genotypes were identified to be the most drought-tolerant genotypes in this study, with better yield characteristics than other genotypes.

3.7. qRT–PCR analysis of drought-responsive genes

The gene expression of key water stress-response genes, and thus the phenology and physiology, in the examined teosinte genotypes, was substantially impacted by the extended exposure to severe water stress (35 days watering interval). In teosinte, water stress had a significant impact on the expression patterns of specific genes related to water stress resistance, like molybdenum cofactor sulfurase (MOCOS), Δ1-pyrroline-5-carboxylate synthase 1 (P5CS), checkpoint clamp loader component (Rad17), 9-cis-epoxycarotenoid dioxygenase (NCED1) and cationic amino acid transporter 1 (CAT1) (Figure 7). Based on the expression patterns of the studied genotypes, both genotypes Gm3 and Gm4 demonstrated superior drought tolerance than other genotypes. The prolonged watering interval promoted MOCOS expression by 2.1 and 1.7 folds in Gm4 and Gm3 teosinte genotypes, respectively, while the check genotype (Ba) exhibited the lowest level of expression (0.9 fold) at the same watering timescale. In the meantime, the checkpoint clamp loader component gene showed expression of 1.3 and 1.2 folds (maximum expression) in Gm4 and Gm3 teosinte genotypes, respectively, but was only 0.8 fold in the check genotype (minimum expression). Likewise, the 9-cis-epoxycarotenoid dioxygenase gene was overexpressed by 1.7 and 1.6 folds in Gm4 and Gm3 teosinte genotypes, respectively, as compared to the check genotype (0.5 fold) because of the extended watering interval. Besides, the cationic amino acid transporter 1 drought resistance gene was found to be expressed by 1.0 and 0.8 folds in the Gm3 and Gm4 teosinte genotypes, respectively, compared to the least expressed genotype (Gm1), which was only expressed by a 0.5 fold, but the check genotype (Ba) showed a 0.7 fold in the expression of this gene. Interestingly, Δ1-pyrroline-5-carboxylate synthase 1 showed the highest expression among the studied genotypes within all teosinte genotypes. The expression level of this gene was 4.0 folds in the Gm4 genotype, and 3.3 folds in Gm2 and Gm3 genotypes, but it was 1.9 folds in the check genotypes. Consequently, the five teosinte genotypes revealed different responses to water stress. When compared to the Ba genotype, which demonstrated the lowest drought tolerance characteristics on the molecular scale, Gm4 and Gm3 genotypes were found to be the most drought-tolerant.

4. Discussion

The phenotypical, physiological and molecular responses of the five investigated teosinte genotypes were substantially affected by the water deficiency generated by extended irrigation frequency. These teosinte genotypes, on the other hand, displayed varied responses to water stress. In this investigation, we determined that genotypes Gm3 and Gm4 were relatively drought-tolerant, whereas genotypes Gm1 and Gm2 possessed drought vulnerability, while the check genotype seemed to have substantial susceptibility to deficit water stress. This is consistent with the finding that under deficit watering, genotypes Gm3 and Gm4 had the highest vegetative performance as assessed by shoot length, leaf area, and shoot biomass, whereas other genotypes were less drought-tolerant. Roots are unable to obtain adequate quantities of macro and micronutrients from the soil when the water supply is insufficient, resulting in metabolic disruptions and detrimental implications on growing plants. Underwater stress circumstances, disruptions in both cell division and cell expansion due to turgor loss and diminished photosynthesis and energy availability can explain the drop in growth attributes (Talbi et al. 2020). Nevertheless, prolonged drought has been reported to hamper several plant cellular activities including photosynthetic activity, gene expression, nitrogen assimilation, stomatal conductance, turgor balance, redox
homeostasis, hormonal balance, enzymatic activities, and membrane functioning, resulting in an overall decreased growth rate (Zia et al. 2021). As a result, differences in growth performance among the genotypes tested could be employed as a selection criterion in teosinte breeding programs, particularly in water-limited areas.

Figure 5. The impact of different watering frequencies on the secondary metabolites (phenols, flavonoids and alkaloids) of five teosinte genotypes. Columns represent the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.
Chlorophyll concentration in plant leaves is a major indicator of the plant’s responsiveness to water deficit as well as the ability of different genotypes of the same species to respond to water stress, allowing for the differentiation of susceptible and tolerant genotypes. Water stress reduced the chlorophyll content of the leaves in all of the teosinte genotypes. Column represents the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.

Figure 6. The impact of different watering frequencies on the yield attributes (number of tillers, thousand kernels weight and total kernels yield) of five teosinte genotypes. Columns represent the average of two independent experiments with 3 replicates each ± SD. Bars with different letters are significantly different at $P \leq 0.05$ according to Tukey’s post hoc HSD test.
genotypes studied, with the most pronounced reductions in the check (Ba) and Gm1 genotypes, particularly when the irrigation periods were distanced by 35 days. The decrease in chlorophyll concentration observed here after water stress is similar to the findings reported on Calamagrostis angustifolia (Wu et al. 2022) and Zea mays (Van Nguyen et al. 2022). Drought-tolerant genotypes exhibited more chlorophyll content than drought-sensitive genotypes, according to Khayatnezhad et al. (2011), because drought-tolerant cultivars exhibited higher amounts of superoxide dismutase enzymes. Water stress causes the overproduction of ROS, which provokes lipid peroxidation and eventually chlorophyll degeneration, resulting in chlorosis of the plant leaves (Khodabin et al. 2020). Higher ethylene production via increased expression of stress-inducing ethylene, which promotes lipid peroxidation and eventually chlorophyll degradation, resulting in chlorosis of the plant leaves (Khodabin et al. 2020). Higher ethylene production via increased expression of stress-inducing ethylene, which promotes lipid peroxidation and impairs membrane integrity, could be linked to the decrease in chlorophyll concentration. Ethylene comes into direct contact with the chloroplast due to lipid degradation and activates the chlorophyllase gene, leading to substantial chlorophyll degradation (Matile et al. 1997).

Total soluble sugars and proteins, in contrast to chlorophyll content, increased dramatically in teosinte leaves following prolonged watering intervals, particularly in Gm3 and Gm4 genotypes. These findings were previously reported in maize (Saad-Allah et al. 2022) and oak (Xiong et al. 2022). Plants accumulate large amounts of low molecular-weight compatible solutes, such as soluble sugars, proteins, and amino acids to adjust the osmotic potential of the plant tissues and improve water absorption during drought stress (Zhang et al. 2010). Soluble sugars function as a substrate for many biosynthetic pathways, signal transduction pathways, sugar sensing, metabolic regulation, ROS scavenging, membranes stabilization and energy generation. It protects plants against water stress via replacing water with hydroxyl groups, which keeps the hydrophilic association with proteins and membranes unbroken, or by vitrification (formation of biological glass in the cytoplasm) (Aslam et al. 2015). Increased proteolysis of starch and other structural sugars under drought conditions as well as the modification in photosynthetic and sugar metabolism rates might explain the increase in soluble sugars concentration (Kenawy et al. 2020). Drought stress also prevents sugars from being exported, causing sugar accumulation in the leaf and inactivation of photosynthetic genes (Krapp et al. 1993). Moreover, drought-induced increase in soluble proteins concentration was linked to the regulation of many physiological processes like photosynthesis, redox homeostasis, carbon metabolism, protein folding and transport, protein breakdown and nitrogen metabolism (Bowne et al. 2012). Drought stress alters gene expression by upregulating ABA-regulated genes that encode proteins related to late embryogenesis abundant (LEA) proteins. The LEA proteins prevent other proteins from aggregating because they are hydrophilic (Goyal et al. 2005). Furthermore, Kottapalli et al. (2009) stated that the higher ATP availability to accommodate increased stress-related energy utilization may alleviate water stress in drought-tolerant genotypes by stimulating protein synthesis. According to Ling et al. (2016), LEA proteins in addition to osmotines (stress-responsive proteins) protect cells from dehydration. As a result, the genotypes Gm3 and Gm4 have the capacity to overcome drought through enhancing cellular sugar and protein metabolism.

When teosinte was exposed to water stress, CAT activity relatively showed constant activity, nevertheless, POD activity decreased gradually as drought intensity rose, however, CAT activity increased significantly, with the maximum activity in all genotypes at a 35-day watering interval, particularly in the Gm3 genotype. Catalase (CAT) is a major enzyme in the elimination of oxidative stress in peroxisomes by dissipating H$_2$O$_2$ into water and oxygen. A simultaneous elevation in catalase activity has been described as part of the triggering of the detoxification enzymes in plants in response to diverse detrimental growth conditions (Leung 2018). Drought-induced increase in CAT activity was attributed to either the stimulation of its biosynthesis or a change in its subunits association (Kamarudin et al. 2018). Recently, Zhao et al. (2020) proposed that the activation of CAT under drought stress is due to the activation of some transcriptional factors that bind directly to the promoter region of the CAT expressing gene, regulating its expression. Some plant species have been reported to have stable CAT
activity during water stress like *Ctenanthe setosa* (Kadioglu et al. 2011). Accordingly, Contour-Ansel et al. (2006) stated that the influence of water stress on enzyme activity is conflicting, as it depends on the plant tolerance capacity and the severity of water stress. Peroxidase (POD) is an enzyme that utilizes an electron donor to reduce H$_2$O$_2$ generated by superoxide dismutase (SOD), resulting in water and an oxidized product of the hydrogen donor. In plant hormone signaling pathways, peroxidase is a stress-responsive enzyme. These pathways, which are controlled by ABA, methyl jasmonate and ROS signaling, are important in biotic and abiotic stress signaling crosstalk (Fujita et al. 2006). According to Dossa et al. (2017), the expression of many peroxidase-encoding genes was associated with increased POD activity under drought stress conditions. In accordance with our results, the decreased POD activity in plants under water stress has been reported in other studies (de Campos et al. 2011; Khan and Komatsu 2016). Other investigations, however, have found that water stress has no effect on POD activity (Bano et al. 2012; Ghabadi et al. 2013).

The initial step in ROS scavenging mechanisms is catalyzed by superoxide dismutase (SOD), which catalyzes the dismutation of two molecules of superoxide anion (O$_2^-$) into oxygen and H$_2$O$_2$. Drought stress increased SOD activity in all genotypes, but it was significantly higher in extended irrigation periods, especially in Gm3 and Gm4 genotypes, suggesting that SOD plays a role in drought tolerance by detoxifying O$_2^-$ and triggering the dismutation process, which transforms O$_2^-$ into H$_2$O$_2$. These findings support previous research that showed an increase in SOD activity as drought stress increased (Hella et al. 2018; Singh et al. 2020; Han et al. 2022). Based on the activities of CAT, POD, and SOD in teosinte genotypes under drought stress conditions, Gm3 genotype may be categorized as drought-tolerant genotypes when compared to other genotypes.

In response to water stress, all of the teosinte genotypes studied acquired significant levels of the osmolytes glycine-betaine (GB), free amino acids (FAA), and proline (Pro), with the amounts varying according to the watering frequency and genotype. Similar results were obtained in teak (Husen 2010), canola (Shafiq et al. 2014), and maize (Saad-Allah et al. 2022). Osmolytes offer an ideal environment for a range of physiological processes while also shielding plants from oxidative damage (Hayat et al. 2012). GB protects proteins from inactivation and conformational changes by sustaining normal cellular physiological functions in plants during environmental stresses, allowing for abiotic stress tolerance in a wide range of plant species, as well as increased growth and productivity (Jain et al. 2021). It efficaciously protects enzymatic and complex protein structures, acts as a shield for photosynthetic machinery components, such as Rubisco and the O$_2$-evolving photosystem II (Shahbaz et al. 2011), maintains the integrity of biological membranes at and diminishes ROS effects (Chen and Murata 2011). Although GB does not directly alleviate ROS effects, it does cause the activation/stabilization of ROS-scavenging enzymes and/or the suppression of ROS production (Jain et al. 2021). Proline is an imperative amino acid that is biosynthesized in plant cells in response to different stressors, including drought stress, in order to manipulate numerous physiological processes and alleviate ROS effects (Abdelaal et al. 2020). Free amino acids (FAA), in addition to functioning as osmoprotectants, contribute in mitigating drought-induced nitrogen bioavailability through reassimilation and promoting protein homeostasis (Živanović et al. 2020). Several investigations looked into drought-related FAA accumulation via increased proteolysis to adjust osmotic potential or detoxify ammonia (Pires et al. 2016; You et al. 2019). The increment in FAA pool could be ascribed to the inhibition of amino acids integration to protein synthetic pathway under water stress conditions. Under water stress scenarios, the suppression of amino acid incorporation into the protein synthetic pathway might account for the increase in FAA pool. Furthermore, according to Abid et al. (2018), FAA accumulation is caused by the rise in the precursor concentration rather than increased activity of amino acids biosynthesizing enzymes. Proline is a stress-responsive amino acid that is biosynthesized in plant cells in response to a wide range of stressors, including drought, and performs a number of actions that assist in relieving stress. Proline, according to Szabados and Savouré (2010), is important for regulating mitochondrial activities, cell death, and activation-specific gene expression, which assist plants to recover from stress. In line with our findings, Zahid et al. (2021) and Singh et al. (2021) reported a significant increase in proline content with water stress, as well as differences in proline content among genotypes, asserting that proline stimulates the stabilization of cellular membranes, scavenging of ROS, and buffering of cellular redox homeostasis under drought conditions. One of the reasons that teosinte genotypes can withstand severe drought stress might be because of the increases in osmoprotectants accumulation, particularly Gm3 and Gm4 genotypes.

Total phenolic content (TPC), total flavonoid content (TFC), and total alkaloids content (TAC) were all shown to be affected by the level of water stress as well as the studied genotype in this investigation. The concentration of these secondary metabolites in teosinte leaves peaked at the longest watering frequency, particularly in the Gm3 and Gm4 genotypes. In this context, Gao et al. (2020) stated that when photosynthesis is hampered by water stress, plants increase their phenolic and flavonoid content, and the more they increase, the better plants physiologically and biochemically adapt to drought. As phenols and flavonoids share the same biosynthetic pathway through the phenylpropanoid pathway, the increased content of these compounds has been attributed to the increased activity of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) under water stress (Oh et al. 2009). As a result, the accumulation of phenols and flavonoids may provide a particular adaptive response of plants to drought stress as they might have a critical role in quenching of ROS, shifting the metabolic pathway to aromatic amino acids biosynthesis, and sustaining cellular turgidity (Saad-Allah et al. 2022). Furthermore, (Ayaz et al. 2000) suggested that the increase in phenolic compounds might be coupled with the lignification of cell walls as a drought-adaptive strategy. As a result, phenolics and flavonoids safeguard the photosynthetic machinery, protect cell membranes, stabilize protein structures and stimulate plant growth under water stress conditions (Araújo et al. 2015). The antioxidative characteristics of flavonoids have been attributed to their hydroxyl groups, double bonds, and predisposition to glycosylation and methylation (Hodaei et al. 2018). Alkaloids, which are nitrogen-containing organic molecules with a heterocyclic ring structure that are biosynthesized via the shikimate route, have seemed to be
active metabolites in plants. In several plant species, alkaloids have been supposed to have a role in drought tolerance (Christiansen et al. 1997; Szabó et al. 2003; Jaleel et al. 2007). Genes governing the shikimic acid pathway and alkaloids biosynthesis have been reported to increase in response to diverse stress conditions (Karlic et al. 2015). Under stress conditions, alkaloids serve as nitrogen reservoirs, allowing plants to withstand stressful conditions. In addition, because the structure of many alkaloids is analogous to known growth hormones, they could function as growth regulators. Furthermore, some alkaloidal species act as antioxidants, contributing to the detoxification of ROS generated from different stressors (Matsura et al. 2014). According to the findings of secondary metabolite measurements in teosinte genotypes, Gm3 and Gm4 showed to have the highest TPC, TFC, and TAC among the genotypes investigated, indicating that they have the potential to withstand the long watering frequencies.

The findings of this study revealed that water stress had a significant influence on teosinte yield indices such as the number of tillers plant$^{-1}$, thousand kernels weight, and kernel yield hectare$^{-1}$, with responses differing across the genotypes investigated. The check genotype (Ba) was the most vulnerable to water stress, but the Gm3 and Gm4 genotypes instead indicated that they could somehow withstand it. According to Frahm et al. (2004), the loss in yield criterion due to water stress varies according to the timing and magnitude of stress imposed, as well as the genotype employed. Drought-induced oxidative damage at the cellular level, which is a principal cause of yield losses, and therefore, yield reduction, might explain the variation in drought tolerance among genotypes, therefore understanding how to keep yield balanced under drought is necessary. Reduced photosynthetic capacity and photosynthetic area of leaves, as well as inhibition of many enzyme activities, have been associated with lower productivity under water stress (Kazem et al. 2021). Furthermore, as established by Farooq et al. (2008), water stress reduces yield by disrupting variety of biochemical and physiological processes, such as respiration, photosimulation, stomatal conductance, nutrient availability, translocation in xylem and phloem, nutrient assimilation, and sugars metabolism. According to our results, Gm3 and Gm4 genotypes sustained higher yield attributes compared to the other genotypes. This might be explained by the fact that under drought stress, those two genotypes maintained higher photosynthetic pigments, antioxidant potential, and osmoregulatory molecules. Herein, both genotypes can be used in breeding programs to improve the growth performance of teosinte in water-stressed regions.

Water stress induces physiological, biochemical, and hormonal variations, which are associated with specific transcriptome responses. Drought signals are recognized, identified by membrane sensors, and then transmitted by various signal transduction pathways, resulting in drought-responsive gene expression (Kaur and Asthir 2017). Some studies suggest that drought-inducible genes, including transcriptional cascades, are amenable to complicated modulation (Todaka et al. 2015). Drought-responsive genes encode variety of proteins involved in signaling pathways, functional proteins that safeguard biological membranes, osmoregulatory proteins like osmotin, aquaporins, and sugar transporters, as well as antioxidant enzymes (Nakashima et al. 2014). Due to extensive water stress, the genes for molybdenum cofactor sulfurase (MOCOS), Δ1-pyrroline-5-carboxylate synthase 1 (P5CS), checkpoint clamp loader component (Rad17), 9-cis-epoxycarotenoid dioxygenase (NCED1) and the antioxidant defense gene for catalase (CAT1) were all upregulated in all teosinte genotypes, particularly in Gm3 and Gm4 genotypes. These results are consistent with those of Lu et al. (2013) and Saad-Allah et al. (2022) in maize.

MOCOS is a gene that encodes for molybdenum cofactor sulfurase, which is the main modulator in abscisic acid (ABA) biosynthesis in response to many stress treatments like salinity and drought (Yue et al. 2011). The increased expression of MOCOS accompanied by increased biosynthesis of ABA was reported to alleviate drought stress by increasing water-retaining ability, enhancing membrane stability, boosting accumulation of proline, and promoting root system growth and development (Zhong et al. 2010; Yue et al. 2011; Roychoudhury and Chakraborty 2021). Rad17 is a DNA-binding protein that helps to repair double-strand breaks caused by stress (Fukumoto et al. 2021). RAD17 contributes to the repair of DNA forks that have collapsed, as well as the prevention of late origin firing-presumably to protect forks from interacting with strand breaks again (Kemp et al. 2010). The increase of RAD17 transcription in drought-stressed teosinte plants showed that this protein potentially plays a pivotal role in DNA damage repair. Another gene that promotes drought-induced ABA biosynthesis is NCED1 (Wang et al. 2020). NCED1 overexpression has been identified to promote plant drought tolerance by boosting ABA accumulation (Xian et al. 2014). Furthermore, according to Bao et al. (2016), the overexpression of NCED1 could indeed improve ascorbic acid levels in water-stressed plants, thereby boosting their drought tolerance. Also, Changan et al. (2018) showed that the increased ABA accumulation, membrane integrity, and relative water content following water stress in rice plants were related to the overexpression of NCED1. Likewise, NCED1 expression has been reported to be interrelated to sugar metabolism and ABA accumulation in drought-stressed plants to improve drought tolerance (Zhang et al. 2014).

CAT1 is a catalase-encoding gene that has been proposed as a candidate gene for evaluating drought tolerance among different plant genotypes. The increased CAT1 gene expression in G3 and Gm4 genotypes corresponded to higher catalase activity in both genotypes as a result of the prolonged watering interval. The increased CAT1 expression in this study following water stress imposition, conferring drought tolerance, has been reported in many studies (Eftekhari et al. 2017; Iwuala et al. 2019; Amoah and See 2021). Consequently, Apel and Hirt (2004) confirmed that CAT1 was involved in the elimination of drought stress-induced H$_2$O$_2$. Another osmolyte regulating gene, P5CS is a proline synthesis-related gene that is activated by an ABA-dependent signaling pathway on exposure to drought conditions in response to ROS accumulation (La et al. 2019). The promotion of proline accumulation in teosinte leaves due to extended watering intervals in this study could be explained by the induced expression of the P5CS gene. Szabados and Savouré (2010) stated that water stress stimulates P5CS, which limits ROS synthesis and regulates both ROS generation and ion scavenging in the chloroplast (Szabados and Savouré 2010). In the light of the preceding results, the
high drought tolerance of Gm3 and Gm4 genotypes could be linked to enhanced expression of specific genes encoding drought-tolerance biomolecules that allow them to endure extended watering frequency.

5. Conclusion

In terms of growth performance, physiological attributes, enzymatic and non-enzymatic ROS detoxifying pathways, yield characteristics, and drought-response gene expression involved in teosinte tolerance, the current study concluded that drought stress exhibited differential responses in the five genotypes studied. Drought-tolerant genotypes Gm3 and Gm4 were found to have higher adaptability to water deficits by boosting the buildup of substantial quantities of low molecular-weight osmoprotectants, antioxidant molecules, as well as high SOD activity, especially at the extended watering frequency intervals. Following extended water stress, all genotypes displayed overexpression of the studied water-stress-responsive genes (MOCOS, Rad17, NCED1, CAT1, and P5CS), with the Gm3 and Gm4 genotypes surpassing other genotypes. Overall, our findings showed that Gm3 and Gm4 genotypes, which outperformed the check and Gm1 and Gm2 genotypes during all watering periods, can be classified as drought-tolerant teosinte genotypes that can be incorporated into areas where continuous periods of drought are expected, and that they can be used in drought-tolerance and genetic improvement protocols.

In the future, further biochemical and molecular research should be performed to determine the appropriate mechanisms of drought tolerance in Gm3 and Gm4 genotypes, supporting their installation in poor-water areas.

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No potential conflict of interest was reported by the author(s).

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