Primming improves germination and seed reserve utilization, growth, antioxidant responses and membrane stability at early seedling stage of Saudi sorghum varieties under drought stress

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Abstract

Seeds of three sorghum (*Sorghum bicolor* (L.) Moench.) varieties from southwest Saudi Arabia were used to investigate the potential of osmopriming with polyethylene glycol (PEG 8000) to improve germination performance, seed reserve utilization and early seedling growth and drought stress tolerance. The primed (PS) and unprimed (UPS) seeds of the three sorghum varieties were germinated for 8 days under increasing PEG-induced osmotic stress. The treatments were arranged in a completely randomized design, in a factorial arrangement, with three sorghum cultivars (‘Zaydia’, ‘Shahbi’ and ‘Ahmar’) and four osmotic potentials (0.0; -0.4; -0.8 and -1.2 MPa) with four replicates of 50 seeds each. The results showed that drought stress affected seed germination and seedling emergence and establishment, but increased the activity of the antioxidant enzyme catalase (CAT). The strongest inhibition of germination and growth occurred at the highest PEG concentration and a significant difference was noticeable between the studied varieties. We confirmed also that seed osmopriming improved seed germination performance, seedling growth and enhanced the CAT activities while reduced malonyldialdehyde (MDA) accumulation and electrolyte leakage (EL) in the drought-stressed seedlings. Seed priming have enhanced also the α-amylase and total proteases activities in all varieties. The largest increase of these hydrolysing enzymes was shown in ‘Ahmar’. Furthermore, the PEG priming lead to improvement of the weight of utilized (mobilized) seed reserve (WUSR), seed reserve depletion percentage (SRDP), and total seedling dry weight (SLDW) of sorghum seedlings under water stress conditions. Still the highest values or all three parameters were found in the ‘Ahmar’ variety. Under increasing drought stress conditions, ‘Ahmar’ showed the highest yield stability index (YSI) and the least EL and MDA contents in comparison to the other two varieties during the seedling establishment stage. Therefore, the former variety can tolerate better a rigorous water stress condition. ‘Zaydia’ appears to be the most vulnerable to drought stress. Thus, the use of species or varieties with eminent seed metabolic quality is an advantageous trait in drought-prone regions.

Keywords: drought tolerance; early seedling growth; germination performance; PEG priming; seed reserve mobilization; *Sorghum bicolor*; varieties
Introduction

Drought stress is a serious agronomic problem in arid and semi-arid areas of the world and is one of the key factors reducing plant growth and productivity. Although sorghum \[Sorghum bicolor\] (L.) Moench. is commonly considered tolerant to abiotic stresses mainly drought and salinity as compared to other cereal crops but still it is sensitive at diverse growth stages to many stresses (Ejeta and Knoll, 2007; Teshome et al., 2018). The recognition of the genetic and the physiological mechanisms underlying drought tolerance in sorghum is vital for physiologists and plant breeders in order to boost productivity (Ejeta and Knoll, 2007; Tsag o et al., 2014). Still the traits related to drought tolerance of the sorghum varieties in Saudi Arabia are not yet identified while there is a variation within a lot of them. For these varieties, water shortage take place habitually during the early growing season in spring (Zhang et al., 2015). In addition, in these semi-arid areas, the rainfall fluctuation as a result of climate change frequently causes variations in soil water content which may harmfully affect the seedling establishment and plant growth and yield (Hamidi and Safarnejad, 2010). If prolonged over to a certain extent drought stress will inevitably generate reactive oxygen species (ROS) (Tounekti et al., 2012, Tounekti et al., 2018). These ROS react with cellular constituents, causing protein degradation, lipid peroxidation and DNA damage (Tounekti et al., 2018). Efficient scavenging of ROS produced under stressful conditions requires the action of several non-enzymatic and enzymatic antioxidants in cellular organelles (Tounekti et al., 2012; Hasanuzzaman et al., 2013; Zrig et al., 2019). Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and several other enzymes are well known enzymatic antioxidant components (Zrig et al., 2019). Plants can also deal with the soil moisture stress by the osmotic adjustment process (Tounekti et al., 2018).

In general, seed germination and seedling emergence and establishment are less tolerant to abiotic stress as compared to adult plants; thus, water deficit stress during these early stages may result in high mortality rates and low crop performance (Zhang et al., 2015). Seed priming is one of the efficient and effective techniques that can be used to reduce the undesirable effects of stress and to improve crop performance (Ghassemi-Golezani et al., 2008; Patade et al., 2011; Jisha et al., 2013; Zhang et al., 2015). Generally primed seeds demonstrate a faster and more harmonized germination and the emerged seedlings are more vigorous and tolerant to abiotic stresses than seedlings emerged from unprimed seeds (Bradford, 1986; Hur, 1991). Priming is a pre-sowing treatment that consists of soaking the seeds in an osmoticum of low water potential to induce a physiological state that enables seed to germinate more efficiently and later allow plant reacts more rapidly and more efficiently to a stress (Jisha et al., 2013; Rahimi 2013). Research demonstrated that seeds osmoprimed with polyethylene glycol (PEG) is effective to improve germination, emergence, and seedling establishment of several plants, especially under stress conditions (Zhang et al., 2015; Shihab and Hamza, 2019). For example, Shihab and Hamza (2019) reported that germination and seedling establishment of sorghum under drought, cold stress, and high salinity were improved following seed osmopriming with PEG. Similar improvements were also noted for rice (\[Oryza sativa\] L.) under drought after seed osmopriming with PEG (Li et al., 2011). Still, the physiological processes behind the efficient germination and the vigorous seedling formation after seed priming are ambiguous. Therefore, comprehensive research is reasonable to reveal such physiological mechanisms involved in drought stress tolerance of these sorghum varieties originated from Saudi Arabia after osmopriming with PEG. These varieties represent a highly valuable genetic pool because of their presumed water stress tolerance since they grow and provide good yields in an area often inflicted by severe droughts. The present study considered for the first time to our knowledge the ability of PEG osmopriming to induce seed germination performance, seed reserve mobilisation and early seedling growth and antioxidant defence of three sorghum varieties largely cultivated in the south-western Saudi Arabia. Still, the activities of some enzymes associated to seed reserve mobilization (α-amylase and total proteases) were also explored.
Materials and Methods

Plant material

Seeds of three sorghum [Sorghum bicolor (L.) Moench.] varieties were obtained from Seed collection of the Center for Environmental Research and Studies (CERS), Jazan University. These varieties are originated from the Southwest of Saudi Arabia where they have been grown for many centuries and are still widely used by farmers in their traditional agro-ecosystems. They are opening pollinated from which rural farmers retain seed for planting in the next season. These varieties varied in colour from white, red to brown. These varieties are locally known as ‘Zaydia’ (white variety), ‘Shahbi’ (brown variety) and ‘Ahmar’ (red variety).

Seed priming and germination tests

The sorghum seeds from three sorghum varieties (‘Zaydia’, ‘Shahbi’ and ‘Ahmar’) were surface-sterilized by using 6% (v/v) sodium hypochlorite for 5 min at room temperature and then rinsed with sterile distilled \( H_2O \). Sorghum seeds were primed in 20% (w/v) polyethylene glycol 8000 (PEG 8000) solution for 48 h at 18 °C in dark aseptic conditions. Seeds were then washed with distilled \( H_2O \) and dried at room temperature to their original weight. Unprimed seeds were used as a control. Afterward, primed (PS) and unprimed (UPS) seeds of the three sorghum varieties were germinated in growth chamber at 25 °C in Petri dishes containing filter paper properly moistened with increasing concentration of PEG 6000 which correspond to four osmotic potential (\( \Psi_s \)) levels: 0 (control); -0.4; -0.8 and -1.2 MPa. The equation of Michel and Kaufmann (1973) was used to determine the appropriate PEG-6000 concentrations for each osmotic potential value. A completely randomized design, in a factorial plan was used (three varieties x four osmotic potentials x four replicates). Germinated seeds were recorded every 12 h for 8 days.

Measurements of seed germination and seedling growth

The germination capacity (GC), germination rate index (GRI), mean germination time (MGT), and early growth of sorghum seedlings were calculated as follow:

Germination capacity (%): \( GC (%) = \frac{n_g}{n_0} \times 100 \), where \( n_g \) is the number of germinated seeds, and \( n_0 \) is the total number of seeds (50 seeds).

Germination rate index (seed day\(^{-1}\)): \( GRI = \Sigma \frac{n_i}{t_i} \), where \( n_i \) is the number of germinated seeds on a given day, and \( t_i \) is the time in days from the sowing day (Maguire, 1962).

Time to 50% germination \( (T'50) \) = \( \frac{\left(t_i + \left(\frac{n_f}{n_i} - n_t\right)\right)(t_i - t_f)}{n_i - n_j} \), where \( N \) is the final number of germination and \( n_i, nj \) are cumulative number of seeds germinated at times \( t_i \) and \( t_f \) when \( ni < N/2 < nj \).

Mean germination time (day): \( MGT = (\Sigma n_i t_i)/\Sigma n_i \), where \( n_i \) is the number of germinated seeds on a given day, and \( t_i \) is the time in days from the sowing day (Labouriau, 1983).

Seedling vigour index: \( SVI = SL \times \Sigma \frac{n_i}{t_i} \), where \( SL \) is the shoot length, \( n_i \) is the number of germinated seeds on a given day, and \( t_i \) is the time in days from the sowing day (Zhang et al., 2007).

The shoot and root length (mm) were measured at the 8\(^{th}\) day on 20 randomly selected seedlings. The dry matter of shoots and roots were recorded after oven drying at 65 °C for 72 h. The data of total dry matter production were recorded for each crop at each PEG treatment and used to calculate the drought tolerance indices as follow:

Yield stability index: \( YSI = \frac{Y_{stress}}{Y_{control}} \), where \( Y_{stress} \) and \( Y_{control} \) are the total dry matter yield (mg per seedling) under drought stress and non-stress conditions (control), respectively (Bouslama and Schapauge, 1984). The shoot and root lengths were measured at the 8\(^{th}\) day of sowing. The root: shoot ratio (RSR) was also calculated.
**Determination of seed reserve utilization**

For the evaluation of seed reserve utilization, four replicates of sorghum seeds (25 seeds per replicate) were weighed (W1), dried at 104 °C for 24 h and then reweighed (W2). Seed water content (WC) was calculated as \([W1-W2]/W2\).

According to the corresponding WC, the initial seed dry weight (ISDW; mg per seed) of each sorghum was calculated as \([W1 \times (1-WC)/25]\).

Primed and unprimed seeds of the three sorghum varieties were then germinated in Petri dishes with increasing PEG treatments. After eight days of germination, the shoots and roots of seedlings were separated from the seeds by hand. The seedlings dry weight (SLDW) and the remnant seed dry weight (RSDW; mg per seed) were obtained after oven drying at 104 °C for 24 h (Soltani et al., 2006). The weight of the utilised (mobilized) seed reserve (WUSR) was calculated as (ISDW - RSDW).

The seed reserve utilization efficiency (SRUE) was calculated as (SLDW / WUSR) (Soltani et al., 2006).

The seed reserve depletion percentage (SRDP) was calculated as WUSR/ ISDW.

**Determination of protease activity**

The protease (EC 3.4.2.21) was assayed according to the method described by Li et al. (2011) with modifications. 1 g of germinated seeds homogenized in a mortar in 10 mL of extraction medium (50 mM Tris-HCL pH 7.5, 5 mM β-mercaptoethanol). The homogenate was centrifuged at 20,000xg for 30 min at 4 °C, and the supernatant was used for protease activity measurements. 1 ml of enzyme extract and 1ml of substrate solution (0.6% casein in 0.1 M Tris HCL buffer, pH 8.0) were incubated for 10 min at 40 °C. The reaction was stopped by incubating at 90 °C for 5 min and by adding 2 mL of 10% (v/v) trichloracetic acid (TCA). Each test tube was allowed to stand for 15 min at room temperature. The mixture was centrifuged at 20,000 x g for 10 min. 500 μL of the supernatant was added to 1 mL of 1 M NaOH, and the absorbance was taken at 280 nm (UV-2600, Shimadzu, Suzhou, China). A control was used with the addition of TCA prior to sample incubation. A tyrosine standard calibration curve is constructed to determine the amount of tyrosine released after the proteolytic activity. A series of tyrosine standard solutions at different concentrations (5 - 50 μg/mL) were prepared from the 0.18mg/mL L-tyrosine stock solution with deionized water.

**α-amylase activity of the germinated seeds**

α-amylase activity was determined according to the method of Jones and Varner (1967). Frozen germinated seeds (after 4 days) were ground in 5 ml ice-cold buffer (0.05 mol L⁻¹ acetate buffer) then centrifuged (16,000 x g) for 15 min at 4 °C. The supernatant was taken for measurement. The substrate for α-amylase was 1% solution of soluble starch in 0.1 mol L⁻¹ acetate buffer. The reaction was started by the addition of 1.0 ml of starch substrate for one hour. The mixtures were put in a boiling water bath for 5 min then cooled to room temperature. The reaction was stopped by the addition of 1 ml of iodine reagent. To this reaction mixture, 5.0 ml of distilled water was added, mixed and measured the absorption at 620 nm. The α-amylase activity was calculated as the amount of starch hydrolysed per minute per mg of protein.

**Electrolyte leakage (EL) and malonyldialdehyde (MDA) contents in seedlings**

Six seedlings per treatment per variety were considered for both analyses. The EL(%) was determined as described by Ghoulam et al. (2002). Briefly, fresh seedlings (50 mg) were placed in tubes containing 10 mL of double-distilled water then incubated at 25 °C for 24 h in a rotary shaker and the initial electrical conductivity of the medium (EC₁) was measured using a conductivity meter. Then, samples were autoclaved at 121 °C for 20 min to release all electrolytes and cooled to 25 °C, after which the final electrical conductivity (EC₂) was measured. The EL (%) was calculated as follow: EL (%) = (EC₁/EC₂) x 100.
The MDA content was determined after 8 days of germination according to Hodges et al. (1999) which takes into account the possible influence of interfering compounds in the thiobarbituric acid (TBA)-reactive substances assay.

**Catalase enzyme activity in seedlings**

Six seedlings per treatment per variety were considered for CAT analyses. Fresh seedlings (100 mg) were grinded in liquid N<sub>2</sub> then extracted with 1 mL of cold K<sub>2</sub>HPO<sub>4</sub> buffer (50 mM, pH 7.5) containing 2% (w/v) PVP and EDTA (1 mM). After centrifugation for 10 min at 4 °C at 22,000 × g, the supernatants were used to measure protein content and CAT by spectrophotometer (Aebi, 1984). One milliliter of reaction mixture containing 50 mM K<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.0) and 250 µl of enzyme extract is initiated by adding 60 mM of H<sub>2</sub>O<sub>2</sub>. The CAT activities were measured at 240 nm for 3 min and expressed in enzyme units using an extinction coefficient of 39.3 mM<sup>-1</sup> cm<sup>-1</sup>. One unit of activity is equivalent to 1 mM of H<sub>2</sub>O<sub>2</sub> degraded per minute and is expressed as unit per gram of protein.

**Statistical analysis**

The three-way ANOVA analysis was performed using SAS software (SAS Institute Inc. 2004). Three replicates per variety and per treatment were used. Turkey’s test was used for the comparison of means.

**Results**

**Effect of seed priming and drought stress on germination**

The results of variance analysis (Table 1) showed significant effects (P < 0.05) for the PEG priming, varieties and osmotic potential levels, as well as for interactions, for the majority of the studied germination traits. The germination response of seeds from the tested varieties was negatively affected (P < 0.001) by the PEG-induced drought stress (Figure 1). For all germination traits, the variety of Ahmar was superior compared to others two varieties. The germination capacity (GC%) in primed (PS) and unprimed (UPS) seeds was notably reduced by increasing the medium water potential. Still, the values of GC% of the PS were significantly superior compared to the values of the UPS. At the highest water potential, the PS of ‘Ahmar’, ‘Shahbi’ and ‘Zaydia’ varieties reached a GC% of 84.12%, 79.07% and 74.72% respectively, while the UPS presented GC% values of 77.21%, 72.56% and 69.34% respectively under the similar conditions. For all varieties, the mean germination time (MGT) has been delayed significantly (P < 0.001) by increasing drought stress. MGT did not differ significantly between varieties (Table 1). The PS of ‘Ahmar’, ‘Shahbi’ and ‘Zaydia’ presented MGT values of 2.17±0.09, 2.36±0.14 and 2.44±0.12 respectively at the highest water potential level in comparison to 1.91±0.15, 1.99±0.15 and 2.19±0.20 for respectively the UPS (Figure 1). The seed priming considerably accelerated the T50 of the tested sorghum varieties under drought stress (P < 0.01, Table 1) with different pattern between them. The PS of ‘Ahmar’, ‘Shahbi’ and ‘Zaydia’ varieties accelerated their germination under severe drought stress of -1.2 MPa to reach 2.98±0.15, 3.08±0.10 and 3.26±0.12 days respectively. The values of 3.45±0.17, 3.47±0.12 and 3.72±0.13 days were respectively measured for the UPS of the same cultivars (Figure 1). The germination rate index (GRI) of sorghum seeds varied from 63.84 to 66.74 seed day<sup>-1</sup> and was considerably reduced with the increase of the drought stress (Figure 1). The seed priming significantly accelerated the GRI under increasing water potential with diverse behaviours between the varieties (P < 0.001).
Table 1. Results of variance analysis of water deficit, PEG priming, sorghum varieties and their interactions for GC, GRI, MGT, T50, SVI, SRD, SRUE, SLDW, WUSR, protease activity, α-amylase activity

| Dependent variables | df | GC | GRI | MGT | T50 | SVI | SRD | SRUE | SLDW | WUSR | Protease | α-amylase |
|---------------------|----|----|-----|-----|-----|-----|-----|-----|------|------|----------|-----------|
| Varieties (V)       | 2  | 7.49"" | 9.45"" | 0.81ns | 1.4/na | 12.92"" | 18.37"" | 11.91"" | 5.77"" | 3.35ns | 4.84"" | 5.80"" |
| Water deficit       | 3  | 43.41"" | 260.69"" | 8.14"" | 14.45"" | 556.63"" | 41.64"" | 88.67"" | 135.2"" | 9.31"" | 10.22"" | 17.18"" | 12.84"" |
| Priming (P)         | 1  | 9.26"" | 2.62 ns | 49.71"" | 52.98"" | 2.29ms | 2.84ms | 1.29ms | 9.31"" | 10.22"" | 17.18"" | 12.84"" |
| V x WD              | 6  | 0.32 ns | 8.53"" | 0.33 ms | 0.064 | 4.62"" | 1.55 ms | 15.94"" | 0.64ms | 0.92 ms | 5.17"" | 8.34"" |
| V x P               | 2  | 0.01ns | 0.01ms | 0.01ms | 0.028 | 0.001 | 0.04 m | 0.01ms | 0.01ms | 0.01ms | 0.01ns | 0.30ms |
| WD x P              | 3  | 0.04ns | 0.01ms | 0.11ms | 0.21ms | 0.34ms | 0.26ms | 0.035ns | 0.55ms | 0.40ms | 0.07ms | 0.03ns |
| V x WD x P          | 6  | 0.03ms | 0.01ms | 0.12ms | 0.18ms | 0.21ms | 0.13ms | 0.02ms | 0.43ms | 0.31ms | 0.02ns | 0.01ns |

GC: germination capacity. GRI: germination rate index. MGT: mean germination time. T50: Time to 50% germination. SVL: seedling vigor index. SRD: Seed reserve depletion percentage. SRUE: Seedling reserve utilization efficiency. SLDW: Seedling total dry weight. WUSR: Weight of utilized seed reserve.

Numbers represent F-values at 0.05 probability level, *, **, Significant at the 0.05, and 0.01, probability levels, respectively; ns: non-significant at 0.05 probability level.

Figure 1. Effect of PEG priming and drought stress induced by PEG6000 on germination capacity (GC), germination rate index (GRI), mean germination time (MGT) and Time to 50% germination (T50) of sorghum varieties. Data represent the mean (±SE) of at least four replicates.

Seed reserve mobilisation

Results of variance analyses showed significant effects for the PEG priming, varieties and water deficit levels, as well as for interactions, for most of the measured traits concerning the seed reserve mobilisation and seedling growth (Table 1). Still, osmopriming did not significantly affect the SRUE. It varied mostly between varieties, with the highest values was recorded for ‘Zaydia’ under control conditions. Under increasing drought stress, PS of all varieties had a greater SRDP, WUSR and SLDW than UPS (Figures 2 and 3). For all varieties,
the highest values of these parameters were recorded for the PS and under the lowest water potential (control treatment). Significant ($P < 0.001$) variability between the varieties was recorded in this regards (Table 1). The highest values of seed mobilisation parameters were found in ‘Ahmar’ compared to the other two varieties. When primed the seeds of ‘Ahmar’ showed the lowest decrease of SLDW (66.4%), SRUE (20.8%), WUSR (60.3%) and SRDP (59.8%) under the highest drought stress (-1.2 MPa). For the same conditions, ‘Zaydia’ showed the largest decrease of these parameters with 77.6%, 70.4%, 70.9 and 72.7% respectively (Figure 3).

Figure 2. Effect of PEG priming and drought stress induced by PEG6000 on seedling vigor index (SVI) and seedling total dry weight (SLDW) of sorghum varieties. Data represent the mean ($\pm$SE) of at least four replicates.

Changes in total proteases and α-amylase activities

The results of variance analysis (Table 1) showed significant effects for the PEG priming, varieties and osmotic potential levels, as well as for interactions, for total proteases and α-amylase activities. The total proteases and α-amylases in the seeds exhibited a significant decline in activity with increasing drought stress (Figure 4). For both PS and UPS, the lowest decreases of both hydrolysing enzymes were shown by Ahmar under the highest water potential (-1.2 MPa). When primed the seeds of ‘Zaydia’ showed the largest decrease of total proteases (56.6%) and α-amylases (67.8%) under the highest drought stress (-1.2 MPa). Seed priming was found to increase the α-amylase and total proteases activities to many folds in all sorghum varieties. Still the largest increase of these hydrolysing enzymes was shown in Ahmar followed by ‘Shahbi’ and finally the ‘Zaydia’.

Effect of seed priming and drought stress on early seedling growth

The results of variance analysis (Table 2) showed significant effects for the PEG priming, varieties and osmotic potential levels, as well as for interactions, for shoot and root lengths as well as for the root to shoot ratio (RSR). Our results showed that the shoot and root lengths of sorghum seedlings were significantly ($P < 0.001$) reduced with the increase of osmotic potential levels in all sorghum varieties (Table 2). For all traits considered, the variety of ‘Ahmar’ was superior compared to others two varieties. For all varieties, the maximum values for shoot length and root length was observed in control (0 MPa) and the minimum values were observed under severe drought stress (-1.2 MPa). The reductions of the shoot lengths were greater than the reductions observed for the root lengths under drought stress conditions, which obviously increased the RSR in the all varieties. ‘Ahmar’ showed the highest RSR compared the other two varieties at all levels of osmotic potential. Seeds priming improved significantly the shoot and root lengths, particularly in ‘Ahmar’ that reached 0.86±0.07 and 0.90±0.10 cm respectively for shoot and root lengths under water potential of -1.2 MPa (Table 2).
The YSI of sorghum seedlings ranged from 0.49 to 0.69, depending on the variety, and was considerably decreased with the increase of drought stress in the medium (Figure 5). ‘Ahmar’ variety had higher YSI compared to the other two varieties when subjected to an increasing osmotic potential. For -1.2 MPa water potential the decrease of the YSI was lower for ‘Ahmar’ (50.8%) when compared to ‘Zaydia’ (62.3%).

**Figure 3.** Effects of PEG priming on seed reserve depletion percentage (SRD), seed reserve utilization efficiency (SRUE), and weight of utilized seed reserve (WUSR) of three varieties of sorghum under different drought stress conditions (0, -0.4, -0.8 and -1.2 MPa). Data represent the mean (±SE) of at least four replicates.
Figure 4. Effects of PEG priming on the total protease activity and α-amylase activity in the seeds of three varieties of sorghum under different drought stress conditions (0, -0.4, -0.8 and -1.2 MPa). Data represent the mean (±SE) of at least four replicates.

Figure 5. Effects of PEG priming on Yield stability index (YSI), electrolytes leakage (EL), catalase (CAT) and malondialdehyde (MDA) contents in leaves of three varieties of sorghum under different drought stress conditions (0, -0.4, -0.8 and -1.2 MPa). Data represent the mean (±SE) of at least four replicates.
Table 2. Effects of PEG priming (PS) on some growth parameters of sorghum varieties as compared to the unprimed controls under (UPS) 0, -0.4, -0.8 and -1.2 MPa of PEG.

| Sorghum varieties | Osmotic potential (MPa) | Shoot length (cm) | Root length (cm) | Root to shoot ratio |
|-------------------|------------------------|-------------------|------------------|--------------------|
|                   | UPS                    | PS                | UPS              | PS                |
| 'Ahmar'           | Control                | 9.90±0.73         | 10.50±0.75       | 3.53±0.58         | 3.75±0.62         | 0.36±0.06 | 0.36±0.06 |
|                   | -0.4                   | 3.30±0.17         | 3.68±0.13        | 3.00±0.25         | 3.19±0.26         | 0.91±0.07 | 0.87±0.08 |
|                   | -0.8                   | 1.14±0.10         | 1.29±0.10        | 1.27±0.12         | 1.34±0.15         | 1.11±0.14 | 1.04±0.15 |
|                   | -1.2                   | 0.76±0.04         | 0.86±0.07        | 0.85±0.03         | 0.90±0.10         | 1.11±0.09 | 1.05±0.09 |
| 'Zaydia'          | Control                | 8.94±0.47         | 9.47±0.50        | 3.52±0.29         | 3.82±0.30         | 0.39±0.05 | 0.40±0.05 |
|                   | -0.4                   | 2.70±0.11         | 3.00±0.12        | 2.98±0.37         | 3.21±0.34         | 1.10±0.13 | 1.07±0.14 |
|                   | -0.8                   | 1.01±0.08         | 1.38±0.09        | 0.95±0.05         | 1.20±0.05         | 0.94±0.06 | 0.87±0.06 |
|                   | -1.2                   | 0.68±0.06         | 0.72±0.05        | 0.63±0.03         | 0.67±0.04         | 0.94±0.04 | 0.93±0.05 |
| 'Shahbi'          | Control                | 7.88±0.38         | 8.48±0.40        | 4.73±0.52         | 5.33±0.55         | 0.60±0.07 | 0.63±0.07 |
|                   | -0.4                   | 2.87±0.17         | 2.91±0.18        | 3.21±0.11         | 3.54±0.11         | 1.12±0.08 | 1.22±0.08 |
|                   | -0.8                   | 0.99±0.08         | 1.29±0.09        | 0.95±0.05         | 1.14±0.05         | 0.96±0.06 | 0.89±0.06 |
|                   | -1.2                   | 0.63±0.06         | 0.72±0.05        | 0.63±0.03         | 0.76±0.04         | 1.01±0.04 | 1.05±0.08 |

Means followed by standard errors and different letters are significantly different at P < 0.05. *, **, Significant at the 0.05, and 0.01, probability levels, respectively; ns: non-significant at 0.05 probability level.

Changes in membrane permeability and MDA contents in sorghum seedlings

The data showed that the independent variables including PEG priming, varieties and osmotic potential levels, and their interactions had significant effect on membrane permeability (EL) and leaf MDA contents (Table 3). Differences between varieties were not significant with regard to the EL. The Figure 5 indicated that EL of the seedlings of all varieties, either originated from UPS or PS, significantly increased with increasing drought stress. Results suggest that seed priming was effective in decreasing membrane permeability by reducing significantly EL of all sorghum seedlings (P < 0.001). The variety of ‘Ahmar’ was superior compared to others two varieties. The ‘Zaydia’ variety presented the highest EL values (78.8%) under the highest drought stress (-1.2 MPa) in the seedlings originated from UPS, while this harm was significantly less severe when originated from PS (72.1%).

MDA levels in plant leaves were determined to evaluate lipid peroxidation. Results showed that for all sorghum varieties, the MDA accumulation increase progressively with the increase of the osmotic potential of the medium and remarkably decreased when priming with PEG (Figure 5). Ahmar showed the least MDA increase (least lipid peroxidation) under the water stress, mainly in seedling originated from PS. The seedling of ‘Ahmar’, ‘Shahbi’ and ‘Zaydia’ varieties originated from the primed seeds accumulated 157.46 ±14.85, 193.18±12.00, and 196.64±7.98 nmol g⁻¹DW under -1.2 MPa treatments respectively.

Changes in catalase activities in sorghum seedlings

The data showed that the independent variables including PEG priming, varieties and osmotic potential levels, and their interactions had significant effect on CAT activities (Table 3). Results showed that for all sorghum varieties CAT activities were significantly improved by the rise of the medium osmotic potential either in seedlings originated from PS or UPS (Figure 5). Seedlings of both PS and UPS of ‘Zaydia’ variety showed a considerable CAT activity under drought stress level of -1.2 MPa in comparison to the other two varieties. Under the highest water potential these activities were 69.00±6.75, 71.73±5.27 and 82.34±6.02 IU min⁻¹ g⁻¹ FW for ‘Ahmar’, ‘Shahbi’ and ‘Zaydia’ respectively (Figure 5).
Table 3. Results of variance analysis of water deficit, PEG priming, sorghum varieties and their interactions for catalase (CAT) activity, malondialdehyde (MDA) contents and electrolytes leakage (EL).

| Dependent variables | df  | CAT     | MDA     | EL     |
|---------------------|-----|---------|---------|--------|
| Varieties (V)       | 2   | 9.19*   | 10.50*  | 2.74ns |
| Water deficit (WD)  | 3   | 32.99*  | 42.63*  | 79.02**|
| Priming (P)         | 2   | 7.25*   | 4.81*   | 16.64* |
| V x WD              | 6   | 0.67ns  | 0.61 ns | 7.17*  |
| V x P               | 4   | 5.01*   | 4.04*   | 0.007ns|
| WD x P              | 6   | 0.29ns  | 0.28ns  | 2.70 ns|
| V x WD x P          | 12  | 0.12ns  | 0.15ns  | 1.32 ns|

Numbers represent F-values at 0.05 probability level, *, ns: Significant and non-significant at 0.05 probability level.

Discussion

Seed priming is one of the methods that can be taken to counteract the harmful effects of abiotic stress (Chen and Arora, 2011; Patade et al., 2011; Jisha et al., 2013). The present study considered for the first time to our knowledge the ability of PEG osmopriming to induce seed germination performance and drought stress tolerance of three sorghum varieties largely cultivated in the south-western Saudi Arabia. Significant effects were shown for PEG priming, sorghum varieties and osmotic potential levels, as well as for their interactions with regards to the most of the traits considered (Table 1). For instance, seed osmopriming have invigorated all sorghum seeds ensuing a larger germination performance and early seedlings growth under increasing water deficit conditions (Figures 1 and 2). We confirmed that priming improved GC%, GRI, increased the germination speed (decreased MGT and T50) and SVI in all sorghum varieties. Still the positive effect of PEG priming on the sorghum tolerance to water deficit varied significantly among the tested varieties, which may be attributed to the seed reserve composition, the tegument characteristics, and the effectiveness of reserve mobilisation through the several enzymatic reactions (Nascimento and West, 1998, Bove et al., 2001). Ahmar variety showed motivating values in terms of GC, GRI, MGT and T50 even under water stress, mainly when osmoprimed. These results are in agreement with previous findings on some other sorghum varieties (Khan et al., 2014; Shihab and Hamza, 2019). The positive effect of osmopriming was also reported in other plant species as alfalfa, soybean, cumin, canola, sunflower and rice (Sun et al., 2010; Patade et al., 2011; Rouhi et al., 2011; Sadeghi et al., 2011; Ansari et al., 2012; Amooaghaie, 2013; Rahimi, 2013). Generally, priming was shown to ease the adherence of seed coat which may allow a simple emerging the radical (Nascimento and West, 1998). The effect of increasing osmotic potentials on the germination of sorghum seeds was previously reported and results showed that GRI and GC, as well as the amount of water absorbed by seeds, were noticeably lowered by increasing the osmotic potential of the medium (Oliveira and Gomes-Filho, 2009; Shihab and Hamza, 2019). Besides, with increasing water deficit, the starch and ATP production process were reduced causing a lower GRI and GC and later a lower SVI (Figure 2).

Seeds of diverse species and genotypes have different levels of food storage mainly starch, proteins and lipids (Nascimento and West, 1998; Bove et al., 2001). Such chemical composition of the seeds has great effects on germination performance and seedling growth rate (Finch-Savage and Bassel, 2016). Furthermore, germination may also dependent on the capacity of the seed to utilize (mobilize) these reserves in an appropriate way for better germination characteristics (Sikder et al., 2009). Our results showed that for all sorghum varieties and under increasing drought stress, PS had a greater WUSR, SRDP and SLDW than UPS (Figure 3). Still the highest values of all these seed reserve mobilisation parameters were found in Ahmar variety compared to the other two varieties. The reason for the superiority of Ahmar variety maybe returns to its superiority in the GC% (Figure 1). This indicates that the seeds produced seedlings have a vigour which should have the ability to make new materials efficiently and quickly to transfer it to the developing embryonic axis, resulting in increased accumulation of dry matter (Shihab and Hamza, 2019). Thus, the use of species or varieties with high seed
metabolic efficiency is an advantageous trait in drought-prone regions. Besides, the decline in WUSR and SRDP of seeds and seedling growth of all sorghum varieties under increasing water deficit was also previously reported for wheat, tomato, mountain rye and mung bean (De and Kar, 1995; Soltani et al., 2006; Ansari et al., 2012). Generally, the reduction of SRDP and WUSR by drought stress could be due to decrease in gibberellic acid and other hydrolytic enzymes in the germination process (McDonald, 1999). Starch digestion is primarily controlled by α-amylase; while the protein degradation is controlled by different proteases (Bishnoi et al., 1993). Our results showed that the total proteases and α-amylases in the seeds exhibited a significant decline in activity with increasing drought stress (Figure 4). Previous reports showed that under water stress α-amylase activity were affected in *Cicer arietinum* cotyledons and in *Medicago sativa* germinating seeds (Hamidi and Safarnejad, 2010; Khadraji et al., 2017). Furthermore, seed priming was found to enhance the α-amylase and total proteases activities in all sorghum varieties. The largest increase of these hydrolysing enzymes was shown in the Ahmar variety. This may suggest that priming have increased the process of proteolysis in the seeds. It was considered that the activities of proteases preserve the respiratory supplies of the growing roots and shoots (Maheshwari and Dubey, 2008). Moreover, the increased level of α-amylase activity seems to be liable for parallel increase in the level of non-reducing sugars in PS (Farooq et al., 2006). It appeared that seed priming either induces the de novo synthesis or promotes the activities of existing enzymes (Wang et al., 2009).

The YSI has been considered a good drought tolerance index for crops and varieties (Bouslama and Schapaugh, 1984). The YSI was measured in order to assess the stability of the sorghum seedlings under stressful conditions. Our results showed that the values of YSI varied between varieties, and was considerably decreased with the increase of drought stress in the medium (Figure 5). Ahmar variety had higher YSI compared to the other two varieties when subjected to an increasing osmotic potential. It appeared therefore that the ‘Ahmar’ variety can tolerate better a rigorous water stress conditions compared to the ‘Shahbi’ and ‘Zaydia’. Generally, drought tolerant plants have the ability to protect their cell membranes by boosting the synthesis of antioxidant enzymes and low antioxidant molecules (Foyer and Noctor, 2005; Tounekti et al., 2012). These antioxidants could neutralize the lethal effect of hydrogen peroxide (H$_2$O$_2$), superoxide (O$_2^-$) and hydroxyl (•OH) radicals in the tissues (Tounekti et al., 2012). Our results showed significant increases of CAT activities in all sorghum seedlings when subjected to an increasing osmotic potential. Still such CAT improvement varied between varieties with the highest increase was shown by ‘Zaydia’ (the more vulnerable to drought stress). The CAT enzyme plays a key role in the protection and repairing systems under drought stress and particularly when seeds were primed by PEG (Kibinza et al., 2011). In fact CAT enzyme accumulates in the cytosol at the same time with H$_2$O$_2$, localization during seed priming (Bray, 1995). To explore the effect of PEG osmopriming on membrane stability we measured MDA content and the degree of membrane permeability (EL) in these seedlings subjected to drought stress. MDA is generated when polyunsaturated fatty acids in the cellular membrane undergo oxidative damage (Tounekti et al., 2012). Our results showed that MDA contents were increased in all sorghum varieties when subjected to an increasing osmotic potential, while membrane stability was decreased (increase of EL) suggesting oxidative damage to the plant tissues (Figure 5). Under increasing drought stress conditions, ‘Ahmar’ variety showed the best membrane health status since it showed the least MDA accumulation and EL amid the three varieties. The low accumulation of MDA in the Ahmar variety (tolerant variety) could be explained by the breakdown of the ROS via increasing CAT activities (Figure 5) which ensure protection from oxidative damage (Sharma et al., 2012). Seed priming was shown to be useful in improving plant drought adaptation by enhancing considerably the cells membrane stability (Figure 5). Rouhi et al., (2012) showed that the germination performance and antioxidant enzyme activities, mainly CAT, in PEG primed seedlings of Berseem clover (*Trifolium alexandrinum* L.) were significantly improved compared to those in control group. However, the ameliorative effect of seeds osmopriming on stress tolerance of spinach plants may decrease in relatively mature seedlings (Chen and Arora, 2011). It appeared therefore that seed priming largely improved the germination performance and CAT activity in all sorghum seedlings, which have enhanced later the early seedling growth under critical drought stress. Growth and development processes in
plants are usually very vulnerable to water shortage (Tounekti et al., 2018). Our results confirmed that increasing the water deficit in the medium affects physiological function leading to growth retardation in plants (Table 2). In water stress conditions, seedling growth is affected due to the decline of water uptake by the plants and lower cell turgor pressure (Taiz et al., 2017). Different sorghum varieties responded differently to PEG-induced water stress in the medium. Seedlings that emerge fast have better chance to grow better by increasing their length and accumulate more dry matter than those germinate later. Our results showed that RSR was improved with the increase of PEG levels (Table 2) which suggesting that the fraction of dry matter allocated to shoots was lessened compared to the roots. Previous studies showed that the shoot of the wheat seedlings was more affected by the drought stress of the medium than other growth aspects (Boutraa et al., 2010). Furthermore, the difference in shoot length, root length and RSR in response to increasing osmotic potential (Table 2) confirmed variation in seedling utilization of the mechanisms including physiological, morphological, and molecular levels. ‘Ahmar’ variety had the highest plant height and was notably different to other two varieties. Generally, the highest seedling length is associated with higher GRI and less time of germination. Previous reports showed that severe water deficit resulted in considerably lower growth vigour of wheat seedlings (Shahi et al., 2015). Our results showed clearly that ‘Ahmar’ variety is more tolerant to drought stress during the seedling establishment stage. ‘Zaydia’ was found to be vulnerable to drought stress. The higher shoot growth of ‘Ahmar’ variety may be associated to the intrinsic features of the variety and size of seeds. The mass of one thousand seeds of ‘Ahmar’ is 37.06 g, while the weight of a thousand seeds of the ‘Shahbi’ variety is 35.48 g and ‘Zaydia’ is 33.25 g. Large sized seeds have a larger amount of reserves mainly carbohydrates to be translocated to the shoot growth when compared to the small-sized seeds (Shahi et al., 2015). Therefore, the use of high-quality seeds of drought tolerant varieties is essential for a successful crop production and food security particularly during the increasing uncertainty due to climate change.

Conclusions

It can conclude that the variance between cultivars to tolerate drought stress belonged to their intrinsic characteristics. ‘Ahmar’ variety can tolerate better a rigorous water stress conditions during the seedling establishment stage compared to the ‘Shahbi’ and ‘Zaydia’. However, regardless of the variety, seed priming improved seed germination performance and seed reserve mobilisation and later increased early seedling growth and antioxidant defence system of all sorghum varieties therefore resulting in increased stress tolerance. Furthermore, seed priming was found to enhance the α-amylase and total proteases activities in all sorghum varieties. The largest increase of these hydrolysing enzymes was shown in the ‘Ahmar’ variety. Osmotic priming appears to have boosted antioxidant defense systems resulting in increased stress tolerance. Therefore, the practice of seed priming should be encouraged in drought-prone locations where irrigations is not available.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.
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