MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Effect of Drought Stress on Seed Germination, Seedling Growth and Antioxidant Enzymatic Activity of *Moringa oleifera* Lam. Growing at Different Burial Depths

Abdullah Aldubise [1] **, *Abdulaziz A. Alqarawi*** [1] **, *Jahangir Ahmad Malik*** [1] **.**

[1] *Department of Plant Production, Faculty of Food & Agricultural Sciences, King Saud University, P.O. Box.2460, Riyadh 11451, Saudi Arabia*

**** Correspondence to:** yasran@mans.edu.eg; Tel. +201017229120*

Received:30/10/2022
Accepted:26/11/2022

AbstractDrought is the most significant environmental stress caused by changes in temperature, light intensity, rainfall levels, and moisture levels along the soil depth. Drought stress and seed burial depth have a negative influence on the morphological, physiological, and biochemical characteristics of plants, which change germination patterns. An experiment was conducted to ascertain the effects of drought stress at different burial depths on seed germination, seedling growth, and physiological parameters of *Moringa oleifera*. The activity of antioxidant enzymes was also evaluated to ascertain how plants might respond to enhanced reactive oxygen species (ROS) oxidation. For this reason, the *M. oleifera* seeds were treated with four levels of drought (100% filed capacity (FC), 75% FC, 50% FC, and 25% FC) and sown at three burial depths (2.5 cm, 5 cm, and 7.5 cm). The findings of this study showed that all germination indices and survival rates of *M. oleifera* seeds, except for 75% FC, were negatively impacted by drought and burial depth. The seeds at 75% FC displayed the highest germination parameters and survival rate. Similar effects were observed in the morphological traits and the photosynthetic pigment accumulation. However, phenol and proline contents showed an abrupt increase with the increase in drought stress at all the burial depths. The highest phenol and proline build-up was recorded in the seedlings growing at 2.5 cm burial depth and exposed to 25% FC stress. Moreover, the seedlings under drought stress at different burial depths showed increased ROS oxidative damage. The highest MDA and H_2O_2 contents were recorded in the seedlings exposed to the highest drought level and growing at a depth of 7.5 cm. However, the seedlings showed increased oxidative enzyme activity in response to the ROS-induced oxidative damage in the plants under drought stress. The highest of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) activities were recorded in the plants of 75% FC. These findings suggest that M. oleifera seeds can adapt to different environmental circumstances. The key explanations for its restoration under adverse environmental conditions must be the soil's field capacity and the depths at which M. oleifera seeds must be sown for successful germination and its emergence and establishment on the surface.

keywords: *Moringa oleifera*, Soil dry, burial depth, pigments, growth parameters, antioxidant enzyme activity

1. Introduction

Moringa oleifera Lam. is a fast-growing acclimatizing plant due to its multiple uses, medicinal values, and environmental importance [1,2]. This plant is extensively dispersed in Southeast Asia, Africa, Saudi Arabia, and South America and is endemic to northwest India [3-5]. Despite its ecological and economic importance, this valuable plant has been less considered in Saudi Arabia. This plant often grows in highland areas far from human access and is less noticeable due to anthropogenic activity in accessible regions. *M. oleifera* is used for multiple purposes, including food, fodder (pasture in silvopastoral systems),

fuel, and traditional and modern medicine [6]. *M. oleifera* can be grown under various environmental conditions [2] for different ecosystem services such as hedges, fences, soil erosion, soil improvement, and pollution control [7].

One major abiotic factor that restricts plant growth, development, and productivity is drought [8]. The most crucial phases of a plant's life cycle are seed germination and early seedling development [9], which are the most sensitive to environmental conditions [10]. The meagre unpredictable distribution of precipitation in the desert [11,12] restricts seedling emergence [13,14]. Water supply affects how soil moisture varies over time in the various soil layers [15]. At different sand depths, the moisture content of the soil varies [14,16]. According to [17], seed germination (SG) and seedling emergence (SE), both of which have an impact on a plant's ability to successfully establish itself, are significant phenological events in the life cycle of a plant [18]. In general, a variety of environmental conditions, such as water scarcity and burial depth, can have a significant impact on how seeds germinate and how quickly plants grow [19-21]. Poor soil aeration [22], low soil temperature variation [23], and/or darkness have been linked to the impacts of seed burying on seed germination [24]. The depth of seed burying at which germination takes place determines how well a plant establishes itself [25,26]. Due to a lack of seed reserves or decreased seed hydration at greater depths, some plant species' seeds may develop but fail to emerge with increasing burial depths [27].

To adapt to stressful situations, plants build up a variety of metabolites such as sugars, phenols, and proline [28]. However, this has a detrimental effect on seed germination and seedling growth [28]. The antioxidant system acts as the plants' defence mechanism to help it endure varied stress situations. Plant's antioxidant activity rises in direct proportion to how much moisture is present [29]. Numerous enzymatic antioxidants can help plants recover from oxidative damage. The enzymatic antioxidants superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and others can increase plant tolerance and lessen the effects of stressful situations [30,31]. Also, the sand burial of seeds can cause a reduction in basal respiration and enzyme activities and is depth-dependent [32].

In order to counteract the effect of dryness on seed germination and seedling growth at varied burial depths, precipitation, seed burial, and the buildup of antioxidant activity may all have an impact on how seedlings emerge in the desert habitat. Heavy winds have been seen transporting sand, burying the seed bank of desert plants and trees beneath the earth and depleting the region of seedlings. As reported earlier, *Moringa* plants can survive under moderate saline and stress conditions. However, no study has been conducted to study the combined effect of drought and seed burial depth on seed germination, seedling growth and the antioxidant activity of *M. oleifera* seeds. This study aims to investigate how *Moringa* seeds' germination and subsequent seedling growth can be affected when grown at different depths under different drought conditions. This study will help us to develop methods to establish Moringa plants in drought environments and to mitigate the negative effects of drought and seed burial on the germination of seeds and their subsequent seedling growth.

2. Materials and Methods

2.1. Collection and preparation of seeds

The *Moringa oleifera* Lam. seeds collected from Ahad AlMasariyah, Jizan, Saudi Arabia (16°41′57.3″N 42°54′17.7″E) were predated, deformed and discarded. The whole and healthy seeds were selected, scarified, and surface sterilized in 5% sodium hypochlorite for 3 minutes before the experiment. The viability of collected *M. oleifera* seeds was tested by setting the germination trail on petri dishes for ten days and calculated to be around 95%.

2.2. Soil and pot preparation

To imitate the habitat conditions, sand and soil were combined in a 1:1 ratio and placed in pots 14 cm in diameter, 13 cm in height, and 11 cm at the bottom, for the experiment. In each pot, five seeds were sowed.

2.3. Estimation of plant species

The experiment was carried out in containers under carefully controlled circumstances at the greenhouse at King Saud University in Riyadh's

Plant Production Department. Throughout the experiment, a constant 25/20°C (D/N) average temperature, a 14/10 h (L/D) light cycle, and a relative humidity of between 50 and 60% were all maintained. Four drought levels-control (100%), 75%, 50%, and 25% of the field capacity-were used as one component in a two-factorial pot experiment with a completely randomized design (CRD), and three seed burial depths-2.5 cm, 5 cm, and 7.5 cm-served as the second factor. All seed burial depths consisted of four replications. Therefore, each drought treatment had 12 pots, and a total of 48 pots were used, with five *M. oleifera* seeds sown in each pot. The seeds were sown at depths of 2.5 cm, 5 cm and 7 cm in plastic pots filled with soil mixture. To stop soil mixture from leaking out of pots' drainage outlets while allowing extra water to drain, strips of nylon mesh were placed over the openings at the bottom of the pot. Each pot was filled with soil mixture up to the bottom mark. The pots were then filled to the higher mark with more soil before seeds (n=5) were spread out over the soil surface

Germination measurements were obtained every day for the duration of the experiment. Only after the radicle broke through the surface of the earth was the seed regarded to have germinated. The germination counts were stopped, and the survival rate of the seedlings was calculated after five days of the last germinated seed i.e., the 20th day from the date of seed sowing. The germination parameters assessed were germination percent (GP), germination rate (GR), mean germination time (MGT), and seed vigour index (SVI). The following equations were used to calculate the values of GP, GR, MGT and SVI:

Germination % (GP) = (total no of seeds germinated)/(total no of seeds sown) x 100

Germination rate (GR)=∑Xi/Yi

where Yi is the day that corresponds to Xi and Xi is the number of seeds that have germinated

[33].

Mean germination time (MGT) = MGT = X1Y1 +X2Y2+…+XnYn / X1 +X2+…Xn

Where X is the number of seeds germinating per day, Y is the time in days that corresponds to X, and n is the number of days until the final count.

Seed vigor index (SVI) =(S.L.+R.L.) * G.P.

where S.L. = shoot length, R.L. = root length, and G.P. = germination percentage.

2.4. Measurement of growth parameters

The plants were harvested after one hundred and twenty days of growth and the morphological parameters that were calculated are as follows: Number of branches, number of leaves per plant, shoot length (SL) and root length (RL), shoot and root biomass, and leaf area. Following a 72-hour period of oven drying at 70°C, the shoot and root biomass was calculated.

2.5. Chlorophyll estimation

To estimate chlorophylls a and b, and total chlorophylls, 100 mg of fresh leaves were ground in acetone (80%) and centrifuged at 5000 rpm for 5 minutes using a Benchtop Centrifuge-5810R, Eppendorf, Hamburg, Germany [34,35]. After three hours of dark incubation, the samples were examined, and the absorbance at wavelengths 645 nm and 663 nm then determined by using a UV–VIS spectrophotometer (SHIMADZU, Kyoto, Japan, UV 1800). The data was estimated as µg/g FW.

2.6. Estimation of total phenolic content

The amount of total phenols was calculated using the Folin-Ciocalteu technique [36]. A 50 ml sample received 250 µl of undiluted Folin-Ciocalteu reagent. Following this, 750 µl of 20% (w/v) aqueous Na_2CO_3 was added, and the volume was then increased with distilled water to 5 ml. All of the reaction reagents, save the extract, were present in the controls. After two hours of incubation at 25°C, the absorbance was measured at 765 nm with a UV-VIS spectrophotometer (SHIMADZU, Kyoto, Japan, UV1800) and compared to a calibration curve for gallic acid. Total phenols were estimated as gallic acid equivalents (mg gallic acid/g extract). The amount of gallic acid equivalents (mg GAE/g DW) used to measure total phenols.

2.7. Estimation of proline content

According to the method of [37], the free proline content was extracted from 0.5 g of

plant samples in 3% (w/v) aqueous sulphosalycylic acid and quantified using glacial acetic acid and the ninhydrin reagent. At 520 nm, the absorbance of the toluene-containing chromophore in the liquid phase was measured in a UV/VIS spectrophotometer (SHIMADZU, Kyoto/Japan, UV18000). Using a calibration curve, the proline concentration was calculated and represented as (µg/g FW).

2.8. Estimation of Protein content

Using the dye-binding technique and bovine serum albumin as a reference, total proteins were calculated [38]. Absorbance at 595 nm were taken using a spectrophotometer.

2.9. Determination of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2)

Malondialdehyde (MDA) of *M. oleifera* plant growing under drought stress at three different burial depths was quantitatively estimated as the degree of lipid peroxidation in accordance with the methodology of Heath and Packer [39]. The Velikova *et al*. [40] recommended method was used to measure the hydrogen peroxide concentration of *M. oleifera*. After being homogenized in a cold mortar with 5 mL of 10% (w/v) TCA, 0.25 g of fresh material was centrifuged at 15,000 g (Benchtop Centrifuge-5810R, Eppendorf, Hamburg, Germany) for 15 min at 4°C. The concentration of H_2O_2 was then determined by recovering the supernatant. 1 mL of iodic potassium (1 M) and 0.5 mL of potassium phosphate buffer (10 mM, pH 7) were added to 0.5 mL of the supernatant. After an hour of incubation in the dark, the absorbance at 390 nm was measured. The content of H_2O_2 was quantified by using a standard curve prepared from the known H2O2 concentration.

2.10 Estimation of antioxidant enzyme activity

Following Beauchamp and Fridovich's approach from 1971, the activity of superoxide dismutase (SOD, EC: 1.15.1.1) was measured by determining its ability to stop the reduction of nitroblue tetrazolium chloride (NBT) at 560 nm. 50 mM sodium-phosphate buffer (pH 7.8), 1 mM NBT, l-methionine, enzyme extract, and 0.01 M EDTA with 0.2 mM riboflavin made up the reaction mixture. After the combination had been exposed to fluorescent lights for 15 minutes to initiate the photochemical reaction, it was placed in the dark, and the absorbance was measured. 50% inhibition of the photochemical reduction was used as the definition for one unit of enzyme activity. The SOD activity unit is (U/gFW). Peroxidase (POD, EC 1.11.1.7) activity was quantified via the [42]. 25 mM K-P buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2, and enzyme were all included in the reaction mixture. Activity was calculated as the rise in absorbance at 470 nm following one minute of guaiacol oxidation. Changes in absorbance at 340 nm brought by NADPH oxidation were used to gauge the activity of glutathione reductase (GR, EC:1.6.4.2) [43]. According to Noctor et al. [44], dehydroascorbate reductase (DHAR, EC:1.8.5.1) activity was assessed by combining the extracted enzyme with K-P buffer (50 mM) at pH 7.0, GSH (2.5 mM), and DHA (0.1 mM). The activity was expressed as U/mg protein after the absorbance at 265 nm was measured.

2.11. Statistical analysis

The data was analyzed using the software Statistix 8.1. A two-factor completely randomized design (CRD) factorial arrangement of the data was applied for the analysis of variance (ANOVA) (Gomez and Gomez, 2010). Significance of variance ($p< 0.05$) was found among treatments i.e., field capacity, burial depth and the interaction of field capacity and seed burial depth for most of the germination, morphological and antioxidant enzyme activity parameters. Upon showing significant effects, the least significant difference test (LSD, $p< 0.05$ probability level) was used to compute the mean difference between the treatments and their interaction [46].

3. Results

3.1. Effect of drought stress on germination parameters of *M. oleifera* growing at different seed burial depths

Both drought stress and burial depth showed a significant effect on seed germination indices (Table 1). Increasing drought stress decreased the germination percentage (GP), survival percentage, germination rate (GR), and seed vigor index (SVI) except at 75% field capacity, which showed increased germination indices (Figure 1 A, B, D& E). On the other hand, mean germination time (MGT) showed an

increase at the highest drought stress of 25% field capacity (Figure 1C). Similarly, the burial depths showed a varying effect on seed germination indices. The seeds sown at a 5 cm burial depth at 75% FC have the highest GP, followed by 2.5 cm depth in both control and 75% FC drought stress (Figure 1A). The survival rate showed a similar trend as that of GP, and the maximum survival (85 and 90%) was calculated in the seeds of the first two burial depths at 75% FC (Figure 1B). Although MGT was significantly affected by drought levels and burial depths, it was highest in seeds at burial depth 7.5 cm with the highest drought stress (25% FC) (Figure 1C). Moreover, burial depth had no direct effect on GR and SVI, but the increase in drought stress indicated a significant negative impact on both parameters (Figure 1D& E)

Table 1. Analysis of Variance for the germination and morphological parameters of *Moringa oleifera* grown under different drought stress at different seed burial depths.

SOV	Df	MS	F	P	MS	F	P
		Germination percentage (GP)			**Germination rate (GR)**		
Field capacity (FC)	3	4600	12.44	<0.0001***	2.083	11	<0.0001***
Depth(D)	2	908.33	2.46	0.101ns	0.812	4.29	0.022*
FC x D	6	241.67	0.5	0.6869ns	0.229	1.21	0.325ns
		Mean germination time (MGT)			**Survival percentage (SP)**		
Field capacity (FC)	3	19.8	6.97	0.0009***	9488.89	29.17	<0.0001***
Depth(D)	2	60.25	21.21	<0.0001***	758.33	2.33	0.112ns
FC x D	6	6.88	2.42	0.047*	147.22	0.45	0.837ns
		Seedling vigor index (SVI)			**Specific leaf area (SLA)**		
Field capacity (FC)	3	70 x 10^5	33.01	<0.0001***	2826.53	2.93	0.048*
Depth(D)	2	11 x 10^5	4.92	0.013*	779.6	0.81	0.454ns
FC x D	6	2 x 10^5	1.14	0.358ns	963.05	1	0.442ns
		Shoot length (SL)			**l**		
Field capacity (FC)		280.982	38.37	<0.0001***	77.694	44.27	<0.0001***
Depth(D)		27.978	3.82	0032*	4.127	2.35	0.110ns
FC x D		11.937	1.63	0.170ns	4.87	2.78	0.0269*
		Shoot biomass			**Root biomass**		
Field capacity (FC)		0.108	27.12	<0.0001***	1.093	80.42	<0.0001***
Depth(D)		0.004	1.06	0.357ns	0.08	4.29	0.022*
FC x D		0.005	1.48	0.214ns	0.018	1.32	0.274ns
		Chl_a			**Chl_b**		
Field capacity (FC)		145.34	162.4	<0.0001***	107.452	284.29	<0.0001***
Depth(D)		11.789	13.17	0.0001***	9.837	26.03	<0.0001***
FC x D		1.354	1.51	0.2048ns	1.706	4.51	0.001**
		Tot Chl			**Proline**		
Field capacity (FC)		96.995	284.87	<0.0001***	11.49	21.95	<0.0001***
Depth(D)		8.912	26.18	<0.0001***	2.43	4.65	0.0167*
FC x D		1.448	4.25	0.002**	0.77	1.48	0.2169ns

Significance (P < 0.001=***); (P < 0.01**); P < 0.05*); ns: non-significant (P > 0.05).

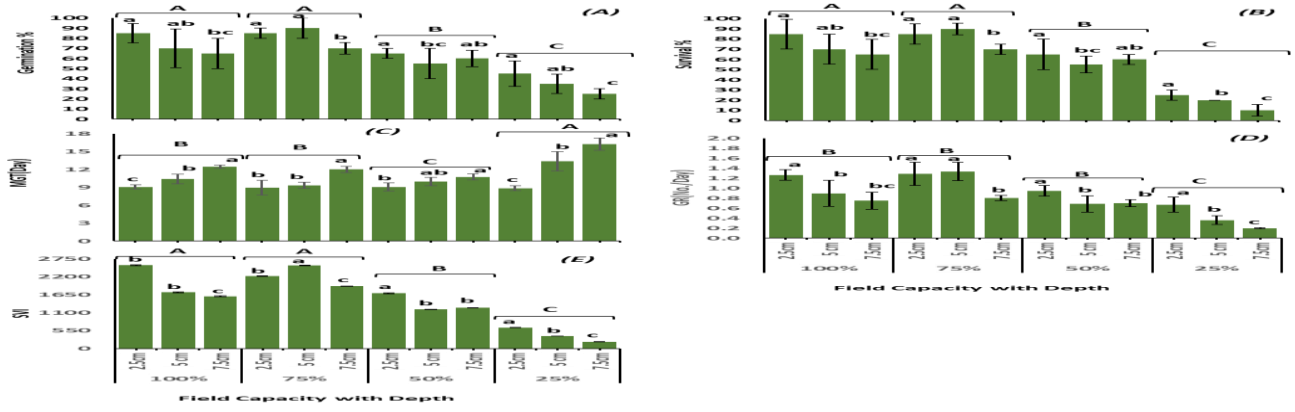

Fig 1. Effect of drought and seed burial depth on the germination parameters of *M. oleifera* seeds. (A) germination %; (B) survival rate; (C) mean germination time; (D) germination rate and (E) seed vigor index. The mean values are shown as colored bars, and the standard error is shown as black

lines on top. The importance of the drought stress and burial depth are shown, respectively, by capital and tiny letters indexed on the top of the bars ($p \leq 0.05$, LSD test).

Table 2. Analysis of Variance (Factorial) for the enzymatic activities of *Moringa oleifera* grown under different drought stress at different seed burial depths.

SOV	Df	MS	F	P value	MS	F	P value
		Total Phenol			**Total Protein**		
Field Capacity (FC)	3	887.17	295.72	<0.0001***	216846	33.77	<0.0001***
Depth(D)	2	163.79	11.6	0.0002***	15665	2.44	0.1028ns
FC x D	6	219.17	15.52	<0.0001***	2283	0.36	0.9105ns
		H_2O_2			Malondialdehyde **(MDA)**		
Field Capacity (FC)	3	166.55	230.97	<0.0001***	2708.62	272.23	<0.0001***
Depth(D)	2	10.61	14.71	<0.0001***	177.83	17.87	<0.0001***
FC x D	6	3.79	5.26	0.0007***	49.08	4.93	0.0011**
		Superoxide dismutase (**SOD**)			peroxidase **(POD)**		
Field Capacity (FC)	3	1021.6	97.81	<0.0001***	39.53	90.27	<0.0001***
Depth(D)	2	24.67	2.36	0.1100ns	0.85	1.93	0.1607ns
FC x D	6	53.67	5.14	0.0008***	4.95	11.3	<0.0001***
		glutathione reductase (**GR**)			dehydroascorbate reductase **(DHAR)**		
Field Capacity (FC)	3	3137	108.61	<0.0001***	1469.9	12.74	<0.0001***
Depth(D)	2	40.86	1.39	0.2631ns	58.59	0.51	0.6065ns
FC x D	6	62.33	2.12	0.0771*	117.01	1.01	0.4333ns
		monodehydroascorbate reductase (**MDHAR**)			**Proline**		
Field Capacity (FC)	3	5360.95	89.06	<0.0001***	0.39	15.45	<0.0001***
Depth(D)	2	31.32	0.52	0.5911ns	0.032	1.29	0.2886ns
FC x D	6	105.86	1.76	0.1384ns	0.163	6.46	0.0001***

Significance (P < 0.0001=***); (P < 0.001**); P < 0.05*); ns: non-significant (P > 0.05).

3.2. Effect of drought stress on growth parameters of *M. oleifera* growing at different seed burial depths

Table 1 indicates that the application of drought stress on *M. oleifera* plants grown at different burial depths had a significant effect on shoot length (SL), root length (RL), shoot biomass (SB), root biomass (RB), specific leaf area (SLA) and root shoot ratio (RSR) (p= <0.0001). Shoot length consistently declined with the increasing drought stress (Figure 2A).

SB and RB showed a continuous decline with an increase in drought stress with no direct impact on burial depth (Figure 2C & D). The SB of *M. oleifera* was highest at 100% FC for the plants sown at 2.5 cm depth, followed by the plants at 75% FC at 7.5 cm burial depth. RB followed the same trend, with the highest RB calculated in the plants with 100% FC. Furthermore, the drought stress and different burial depths had no significant effect on SLA (Table 1). However, the plants sown at 7.5 cm and exposed to the highest drought stress of 25% FC indicated a considerable increase in their SLA (Figure 1E). On the other hand, the RSR of *M. oleifera* plants showed a significant improvement at 5 cm burial depth in 100% and 50% FC but indicated a sudden decline at the highest drought level (Figure 2F).

The highest SL was recorded in the plants exposed to 100% FC growing at 2.5 cm burial depth, while the plants growing at 7.5 cm burial depth and applied with extreme drought stress (25%) showed a considerable reduction in their SL. Unlike SL, RL of *M. oleifera* grown at 100% and 75% field capacities showed a prominent improvement at a burial depth of 5 cm (Figure 2B). However, at the highest level of drought stress 50% and 25% FC, the RL showed a consistent decline.

3.3. Effect of drought stress on photosynthetic pigments, total phenol, proline and protein content of *M. oleifera* growing at different seed burial depths

The effect of the drought stress on the chlorophyll content of *M. oleifera* plants growing at different burial depths is shown in (Figure 3A-C). Drought, burial depth, and their interactions negatively impacted all the chlorophyll parameters (Table 1). The highest Chl_a, Chl_b, and total Chl were shown by the plants growing at 100% FC at a burial depth of 5 cm, which, however, showed a significant decline with the increase in drought stress at all the burial depths. The lowest production of these photosynthetic pigments was recorded in plants at 25% FC.

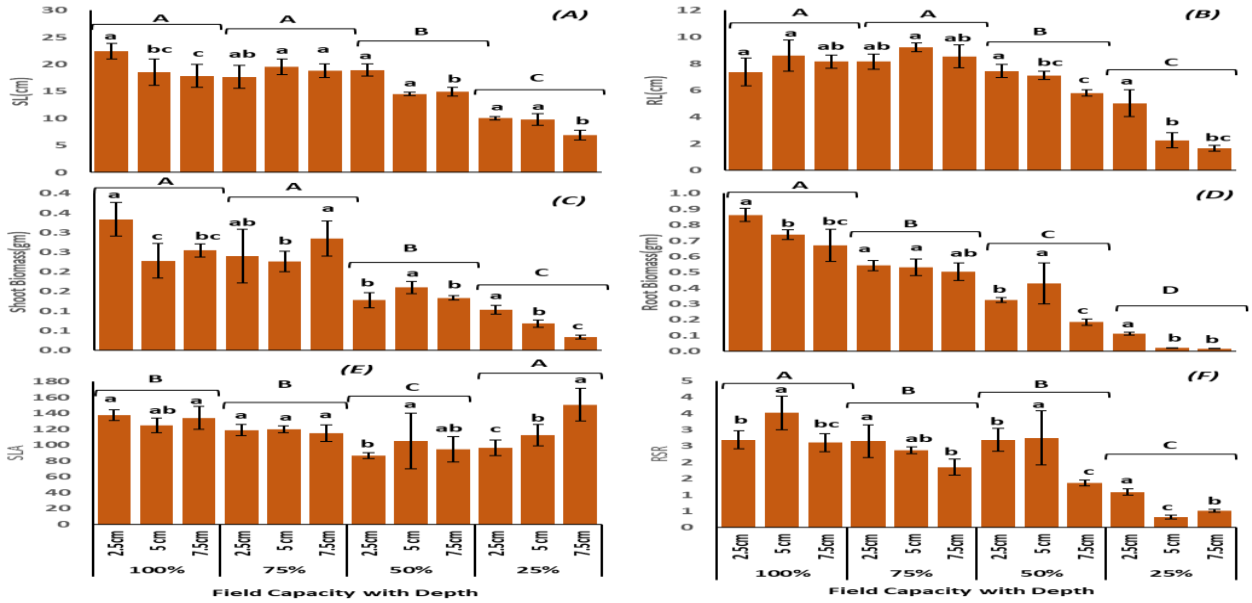

Fig 2. Effect of drought and seed burial depth on the growth parameters of *M. oleifera* (A) shoot length (SL); (B) Root length (RL); (C) shoot biomass (SB); (D) root biomass (RB); (E) specific leaf area (SLA) and (F) root shoot ratio (RSR). The mean values are shown as colored bars, and the standard error is shown as black lines on top. The importance of the drought stress and burial depth are shown, respectively, by capital and tiny letters indexed on the top of the bars ($p \leq 0.05$, LSD test).

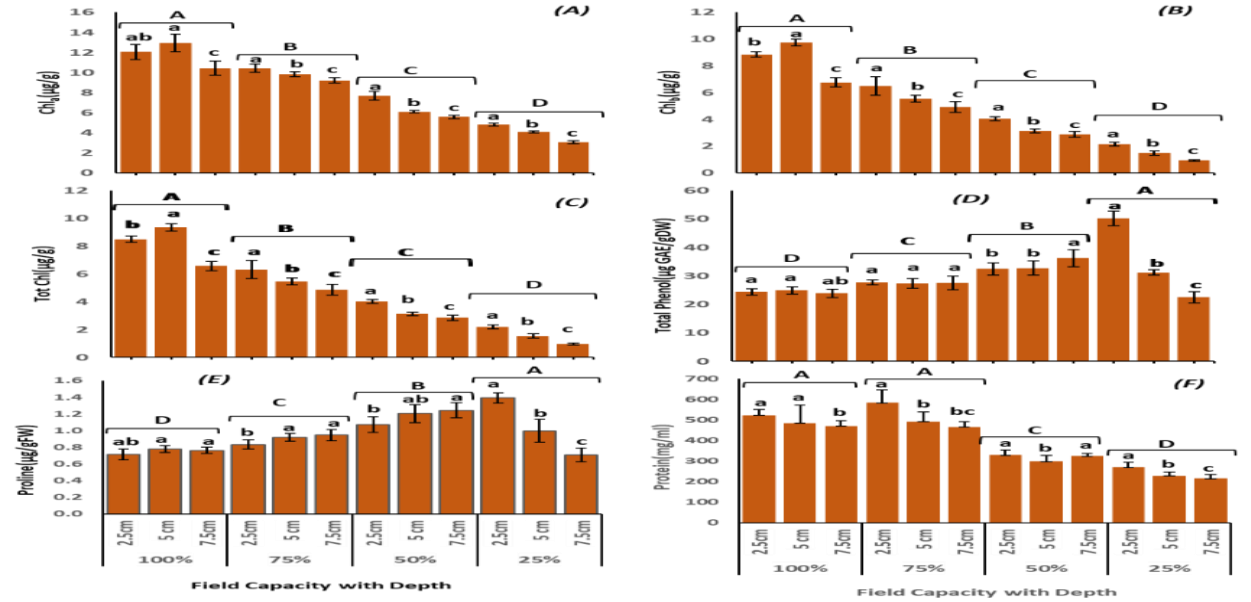

Fig 3. Effect of drought stress on (A) chlorophyll$_a$ (*Chl $_a$*); (B) chlorophyll$_b$ (*Chl $_b$*); total chlorophyll (Tot *chl*); (D) total phenol; (E) proline and (F) protein content of *M. oleifera* plants growing at different burial depths. The standard error (black lines) is displayed on top of the colored bars representing the mean values. The importance of the drought stress and burial depth are shown, respectively, by capital and tiny letters indexed on the top of the bars ($p \leq 0.05$, LSD test).

Contrary to photosynthetic pigments, the total phenol content indicated a significant increase in the plants growing at 25% and 50% FC (Figure 3E). Similarly, the production of proline significantly increased with the severity of drought stress (Figure 3F). The plants exposed to 25% FC showed the highest proline build-up at 2.5 cm depth, followed by the plants irrigated with 50% FC at 7.5 and 5 cm depth. However, the drought stress triggered the reduction of protein content except in the plants growing at 75% FC (Figure 3F).

3.4. Hydrogen peroxide H2O2 and MDA production in *M. oleifera* plants growing under drought stress at different burial depths

H_2O_2 levels were evaluated to determine the extent of drought-induced oxidative stress on *M. oleifera* growing at different seed burial depths (Figure 4A). The H_2O_2 in our results showed a significant increase in the plants exposed to extreme drought stress at different depths (Table 2). The highest increase in the H_2O_2 content was calculated in plants sown at 7.5 cm depth and applied with 25% FC irrigation. Similar results were depicted by the plants in the case of MDA production (Figure 4B). MDA production of *M. oleifera* under different levels of drought stress was evaluated to assess its membrane damage

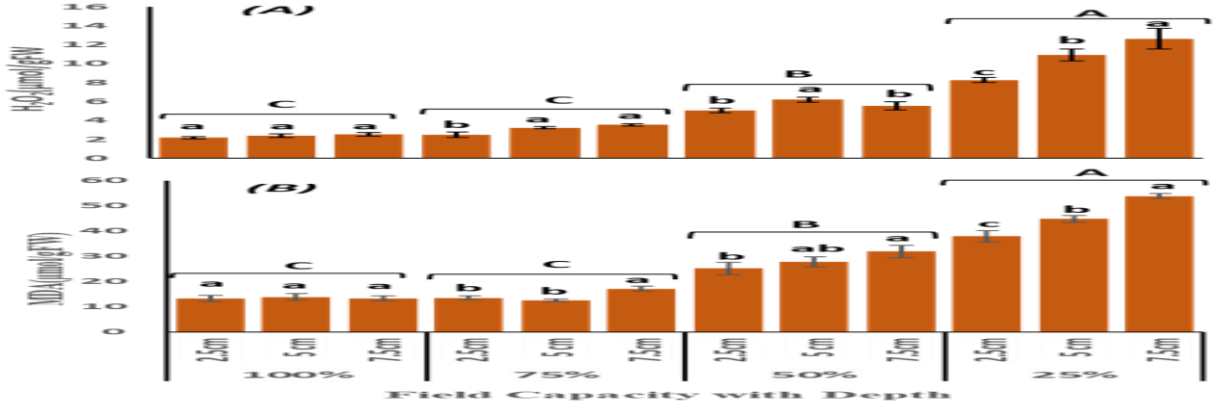

Fig 4. Effect of drought stress on (A) H_2O_2 and (B) MDA content of *M. oleifera* plants growing at different burial depths. The standard error (black lines) is displayed on top of the colored bars representing the mean values. The importance of the drought stress and burial depth are shown, respectively, by capital and tiny letters indexed on the top of the bars (p = 0.05, LSD test).

3.5. Effect of drought stress on antioxidant enzyme activity of *M. oleifera* growing at different seed burial depths

The enzyme activities of SOD, POD, GR, and DHAR were calculated to assess the antioxidant defense mechanism of *M. oleifera* in response to drought stress and seed burial depth. The antioxidant enzyme activity varied significantly in plants when irrigated with different levels of FC (Figure 5C-F). Plants at the FC level of 50% showed a significant increase in SOD, POD, GR, and DHAR activities at all burial depths. However, the enzyme activities abruptly declined at the highest drought stress of 25% FC.

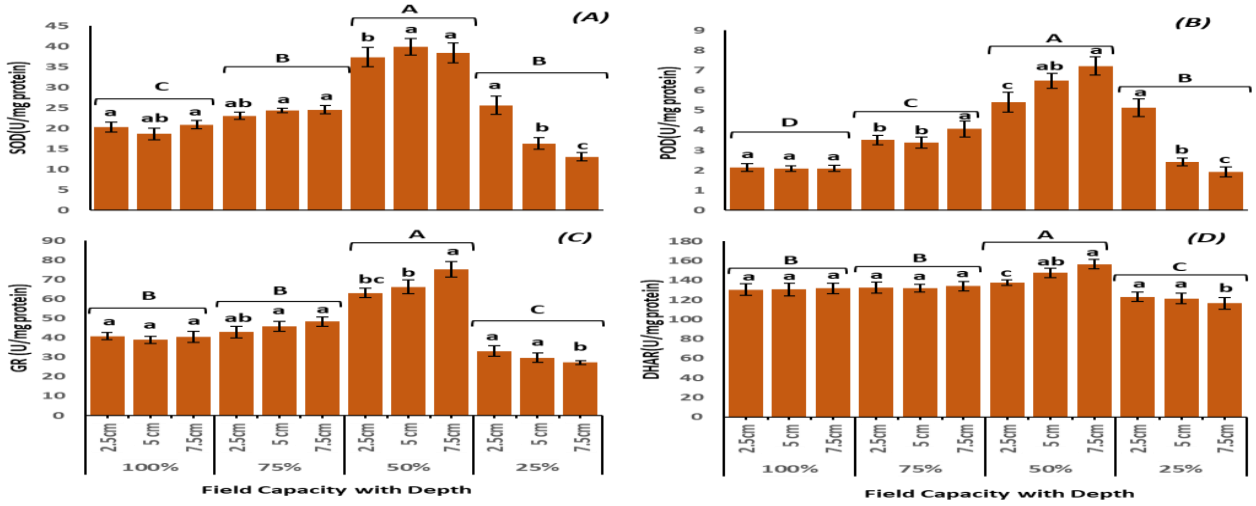

Fig 5. Effect of drought stress on (A) SOD; (B) POD (C) GR and (D) DHAR content of *M. oleifera* plants growing at different burial depths. The standard error (black lines) is displayed on top of the colored bars representing the mean values. The importance of the drought stress and burial depth are shown, respectively, by capital and tiny letters indexed on the top of the bars (p = 0.05, LSD test).

4. Discussion

The current study's findings suggest that numerous environmental conditions, including drought and seed burial depths, have an impact on the germination of *M. oleifera* seeds. By limiting exposure to air, maintaining high humidity, and protecting seeds from unusually low and high air temperatures, seed burying can either improve or inhibit germination and seedling establishment. Our study showed an inverse relationship between seed emergence, burial, and field capacity, as most of the seeds germinated significantly at a shallow 2.5 cm depth at both control and 75% of FC. The seeds of *M. oleifera* performed best at 75% FC with a 2.5 cm burial depth and at least at 25% FC with a 7.5 cm burial depth. Both the germination and seedling emergence declined with the increase in burial depth and decrease in field capacity for *M. oleifera*. A decrease in seedling emergence due to an increased burial depth has also been reported for various weeds [47]. The germination and seedling emergence variation could be explained by edaphic (water-holding capacity) and environmental factors [48]. Shallow burial can reduce the seed's exposure to air, maintaining a moist and protective environment against low and high air temperatures around seeds, thereby stimulating germination [49]. The deeply-buried seeds might not provide the necessary nutrients to enable the early shoot system to reach the soil's surface [50]. Also, seedling emergence might decrease at deep seed burials due to hypoxia and low rates of gaseous diffusion [51]. Fang et al. [52] also reported that the seedling emergence of *A. tauschii* was inversely related to burial depth, and seeds buried below 8 cm depth showed no emergence. The frequent sand movement could reduce *M. oleifera* emergence when seeds are deep buried under the soil, like the inhibition due to deep tillage of other community species such as *Al. japonicus* [53] and *Galium aparine* L. [54].

The effect of drought on the seedling growth of Moringa seedlings was observed using a range of response variables. There was a difference in the growth pattern at different drought stress levels and seed burial depths. Plant biomass, shoot length, and root length were highly responsive to changes in soil water content [55]. Our results agree with those of [56], who reported a significant reduction of growth traits in *Acacia tortilis*, *Salvadora persica*, and *Leptadenia pyrotechnica* nursery due to drought. Due to the lack of moisture content in the drought-treated soil, the whole seedlings of *M. oleifera* may become dehydrated when they are fully exposed to the outer environment. Our results indicate the survival rate of seedlings decreased with the increase in drought stress and burial depth of the seeds (Figure 1B). The root length determined the survival of the seedling at the time of survival and by the length of the desiccation period [57]. *M. oleifera* may be capable of morphological adjustments like reduction of shoot and root length in response to field capacity as a form of ecological adaptation or survival strategy.

The decline in chlorophyll content under abiotic stress is considered a common response [58, 59] and results in reduced photosynthesis and assimilation rates under water stress [60]. In this study, the chlorophyll synthesis of *M. oleifera* significantly decreased with the increase in drought stress and seed burial depth (Figure 3A-C). the reduction in chlorophyll content under drought stress is primarily due to chloroplast damage caused by an excess of reactive oxygen species (ROS) [61]. In this study, total phenolic content showed an increased trend from 75% to 25% FC, corresponding to their respective depth. These results agree with previous studies where drought response leads to reduced chlorophyll and phenolic content [62] and has been linked with protecting plant cells from ROS damage [61]. The activation of the antioxidant defense mechanism is crucial for decreasing ROS [63]. This significant accumulation of proline content resulted in decrease biomass content with increased drought stress. Accumulation of proline delays protein denaturation during severe drought stress [64].

The higher levels of SOD, POD, GR and DHA activities were observed under 25% drought stress at all burial depths. These results suggest that the plants have developed a complex defense mechanism of antioxidant enzymes to cope with the increased ROS production [65, 66]. Increased generation of H_2O_2 from damaged membranes can harm cellular organelles, protein structure, and

nucleic acid fragmentation while also compromising other physiological functions [67]. Such oxidative bursts may also cause an increase in MDA content which reflects the peroxidation of membrane lipids [67]. As a result, there was a significant increase in H_2O_2 and MDA under 25% FC at deep burial.

5. conclusion

In conclusion, our results indicate that *M. oleifera* seeds can adapt to various environmental conditions. The soil's field capacity and the burial depths of *M. oleifera* seeds to germinate and emerge on the soil surface must be the primary factors explaining its restoration in harsh environmental conditions. The results showed a reduction in germination parameters and morphological parameters and increased antioxidant enzyme activity of the *M. oleifera* seeds under different drought stress levels and seed burial depths. However, the study was the outcome of the controlled greenhouse conditions. Therefore, field experiments will be necessary before recommending planting seeds at specific depths with limited field capacity

6. References

1. El-Alfy, T.S., Ezzat, S.M., Hegazy, A.K., Amer, A.M. and Kamel, G.M. (2011) Isolation of biologically active constituents from Moringa peregrina (Forssk.) Fiori. (family: Moringaceae) growing in Egypt. Pharmacognosy Magazine, 7, 109.
2. Nouman, W., Siddiqui, M.T., Basra, S., Ahmed, M., Farooq, H., Zubair, M. and Gull, T. (2013) Biomass production and nutritional quality of Moringa oleifera as a field crop. *Turkish Journal of Agriculture Forestry*, **37**, 410-419.
3. Olson, M.E. and Fahey, J.W. (2011) Moringa oleifera: a multipurpose tree for the dry tropics. Revista Mexicana De Biodiversidad, **82**, 1071-1082.
4. Severino, L.S. and Auld, D.L. (2013) A framework for the study of the growth and development of castor plant. Industrial Crops Products, **46**, 25-38.
5. Hegazi, M. (2015) Influence of soil type, sowing date and diluted seawater irrigation on seed germination, vegetation and chemical constituents of Moringa oleifera, Lam. *Journal of Agricultural Science*, 7, 138.
6. Sharma V, Paliwal R, Pracheta, et al (2011). Phytochemical analysis and evaluation of antioxidant activities of hydroethanolic extracts of Moringa oleifera lam pods. *J Pharm Res***, 4**, 554-7.
7. Fuglie, L.J. (1999) The miracle tree: Moringa oleifera, natural nutrition for the tropics.
8. Ludlow, M.M. and Muchow, R.C.J.A.i.a. (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy, **43,** 107-153.
9. Ludewig, K., Zelle, B., Eckstein, R.L., Mosner, E., Otte, A. and Donath, T.W. (2014) Differential effects of reduced water potential on the germination of floodplain grassland species indicative of wet and dry habitats. Seed Science Research, **24**, 49-61.
10. Zhu, Y., Yang, X., Baskin, C.C., Baskin, J.M., Dong, M. and Huang, Z. (2014) Effects of amount and frequency of precipitation and sand burial on seed germination, seedling emergence and survival of the dune grass Leymus secalinus in semiarid China. *J Plant Soil*, **374**, 399-409.
11. Gutterman, Y. (1993) Seed germination in desert plants.,(Springer-Verlag: Berlin).
12. Gutterman, Y. (2002) Survival strategies of annual desert plants: Springer Science & Business Media.
13. Gillespie, I.G. and Loik, M.E. (2004) Pulse events in Great Basin Desert shrublands: physiological responses of Artemisia tridentata and Purshia tridentata seedlings to increased summer precipitation. *Journal of Arid Environments*, **59**, 41-57.
14. Tobe, K., Zhang, L. and Omasa, K. (2005) Seed germination and seedling emergence of three annuals growing on desert sand dunes in China. Annals of Botany, **95**, 649-659.
15. Chun-Wang, X. and Guang-Sheng, Z. (2001) Study on the water balance in three dominant plants with simulated precipitation change in Maowusu

sandland. *Journal of Integrative Plant Biology*, 43, 82.
16. Maun, M. (1998) Adaptations of plants to burial in coastal sand dunes. *Canadian Journal of Botany*, **76**, 713-738.
17. Bakhshandeh, E., Atashi, S., Hafez-Nia, M. and Pirdashti, H. (2013) Quantification of the response of germination rate to temperature in sesame (Sesamum indicum). Seed Science Technology, **41**, 473.
18. Forcella, F., Arnold, R.L.B., Sanchez, R. and Ghersa, C.M. (2000) Modeling seedling emergence. Field Crops Research, **67,** 123-139.
19. Honarmand, S.J., Nosratti, I., Nazari, K. and Heidari, H. (2016) Factors affecting the seed germination and seedling emergence of m uskweed (Myagrum perfoliatum). Weed Biology Management, **16**, 186-193.
20. Nosratti, I., Soltanabadi, S., Honarmand, S.J. and Chauhan, B.S. (2017) Environmental factors affect seed germination and seedling emergence of invasive Centaurea balsamita. Crop Pasture Science, **68**, 583-589.
21. Abdellaoui, R., Boughalleb, F., Zayoud, D., Neffati, M. and Bakhshandeh, E. (2019) Quantification of Retama raetam seed germination response to temperature and water potential using hydrothermal time concept. Environmental Experimental Botany, **157**, 211-216.
22. Zhu, Y.-J., Dong, M. and Huang, Z.-Y. (2005) Effects of sand burial and seed size on seed germination and seedling emergence of Psammochloa villosa. Chinese *Journal of Plant Ecology*, 29, 730.
23. Zhang, J. and Maun, M. (1990) Sand burial effects on seed germination, seedling emergence and establishment of Panicum virgatum. Ecography, **13**, 56-61.
24. Kazuo, I., Masayuki, K., Ryo, N., Yoshihiko, K. and Kunio, Y., 2005. Soluble carbohydrates and variation in vase-life of cut rose cultivars 'Delilah'and 'Sonia'. *The Journal of Horticultural Science and Biotechnology*, **80(3)**, pp.280-286.
25. Amini, V., Zaefarian, F. and Rezvani, M. (2015) Interspecific variations in seed germination and seedling emergence of three Setaria species. *Brazilian Journal of Botany*, **38**, 539-545.
26. Javaid, M.M., Florentine, S.K., Ali, H.H. and Chauhan, B.S. (2018) Environmental factors affecting the germination and emergence of white horehound (Marrubium vulgare L.): a weed of arid-zone areas. *The Rangeland Journal*, **40**, 47-54.
27. Dinelli, G., Marotti, I., Catizone, P., Bosi, S., Tanveer, A., Abbas, R. and Pavlovic, D. (2013) Germination ecology of Ambrosia artemisiifolia L. and Ambrosia trifida L. biotypes suspected of glyphosate resistance. Open Life Sciences, **8**, 286-296.
28. Tesfay, S., Modi, A. and Mohammed, F. (2016) The effect of temperature in moringa seed phytochemical compounds and carbohydrate mobilization. *South African Journal of Botany*, **102**, 190-196.
29. Chitiyo, S.T., Ncube, B., Ndhlala, A.R. and Tsvuura, Z. (2021) Biochemical responses of Moringa oleifera Lam. plants to graded moisture deficit. *South African Journal of Botany*, **138**, 41-49.
30. Khan, A. and Ashraf, M. (2008) Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat.
31. Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. Annual Review of Plant Biology, **59**, 651-681.
32. Liu, Y., Jia, R., Yang, H., Xing, Z., Shi, G. and Cui, Z. (2022) Effects of sand burial caused by ant nests on soil microbial biomass, basal respiration, and enzyme activity in/under biocrusts in vegetated areas of the Tennger Desert. Land Degradation Development, **33**, 1596-1607.
33. Malik, J.A., AlQarawi, A.A., AlZain, M.N., Dar, B.A., Habib, M.M. and Ibrahim, S.N.S. (2022a) Effect of Salinity and Temperature on the Seed Germination and Seedling Growth of Desert Forage Grass Lasiurus scindicus Henr. Sustainability, **14**, 8387.

34. Arnon, D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24, 1.
35. Malik, J.A., AlQarawi, A.A., Dar, B.A., Hashem, A., Alshahrani, T.S., AlZain, M.N., Habib, M.M., Javed, M.M. and Abd-Allah, E.F. (2022b) Arbuscular mycorrhizal fungi isolated from highly saline "sabkha habitat" soil alleviated the NaCl-induced stress and improved Lasiurus scindicus Henr. growth. Agriculture, 12, 337.
36. Ainsworth, E.A. and Gillespie, K.M. (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, **2** 875-877.
37. Bates, L.S., Waldren, R.P. and Teare, I. (1973) Rapid determination of free proline for water-stress studies. Plant and Soil, **39**, 205-207.
38. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72**, 248-254.
39. Heath, R.L. and Packer, L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, **125**, 189-198.
40. Velikova, V., Yordanov, I. and Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Science, **151**, 59-66.
41. Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, **44**, 276-287.
42. Hemeda, H. and Klein, B. (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *Journal of food Science*, **55**, 184-185.
43. Edwards, E.A., Rawsthorne, S. and Mullineaux, P.M. (1990) Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (Pisum sativum L.). Planta, **180**, 278-284.
44. Noctor, G., Mhamdi, A. and Foyer, C.H. (2016) Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. Plant, Cell and Environment **39**, 1140-1160.
45. Gomez, K.A. and Gomez, A.A. (2010) Statistical procedures for agricultural research.
46. Steel, R.C.D., Torrie, J.H., Deekey, D.A., (1997) Principles and Procedures of Statistics: A Bio metric Approach. New York, USA, pp. 400–428: McGraw Hill Book.
47. Benvenuti, S., Macchia, M. and Miele, S. (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. Weed Science, **49**, 528-535.
48. Benvenuti, S. and Macchia, M. (1995) Effect of hypoxia on buried weed seed germination. Weed Research, **35**, 343-351.
49. Baskin, C.C. and Baskin, J.M. (1998) Seeds: ecology, biogeography, and, evolution of dormancy and germination: Elsevier.
50. Wu, X., Li, J., Xu, H. and Dong, L. (2015) Factors affecting seed germination and seedling emergence of Asia minor bluegrass (Polypogon fugax). Weed Science, **63**, 440-447.
51. Benvenuti, S. (1995) Soil light penetration and dormancy of Jimsonweed (Datura stramonium) seeds. Weed Science**, 43**, 389-393.
52. Fang, F., Zhang, C., Wei, S., Huang, H. and Liu, W. (2012) Factors affecting Tausch's goatgrass (Aegilops tauschii Coss.) seed germination and seedling emergence. *Journal of Agricultural Science*, 4, 114.
53. Wang, H., Huang, Y., Zhang, L., Liu, W. and Wang, J. (2018) Japanese foxtail (Alopecurus japonicus) management in wheat in China: seed germination, seedling emergence, and response to herbicide treatments. Weed Technology, **32**, 211-220.
54. Wang, H., Zhang, B., Dong, L. and Lou, Y. (2016) Seed germination ecology of catchweed bedstraw (Galium aparine). Weed Science, **64**, 634-641.

55. Jibo, A. and Barker, M. (2019) Effects of water deficit on growth, biomass allocation and photosynthesis of A. Senegal seedlings from Nguru and Gujba provinces of Yobe state, north eastern Nigeria. ournal of Applied Sciences Environmental Management, **23**, 2221-2229.
56. Environmental Experimental Botany, 63, 224-231.
57. Elfeel, A. and Al-Namo, M.L.J.A.B.J.A. (2011) Effect of imposed drought on seedlings growth, water use efficiency and survival of three arid zone species (Acacia tortilis subsp raddiana, Salvadora persica and Leptadenia pyrotechnica*). Agriculture and Biology Journal of North America*, **2**, 493-498.
58. Gutterman, Y. and Gozlan, S. (1998) Amounts of winter or summer rain triggering germination and 'the point of no return'of seedling desiccation tolerance, of some Hordeum spontaneum local ecotypes in Israel. Plant Soil, **204**, 223-234.
59. Chaves, M.M., Maroco, J.P. and Pereira, J.S. (2003) Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology, **30**, 239-264.
60. Hussain, Z., Rasheed, F., Tanvir, M.A., Zafar, Z., Rafay, M., Mohsin, M., Pulkkinen, P. and Ruffner, C. (2021) Increased antioxidative enzyme activity mediates the phytoaccumulation potential of Pb in four agroforestry tree species: A case study under municipal and industrial wastewater irrigation. *International Journal of Phytoremediation*, **23**, 704-714.
61. Rahmati, M., Mirás-Avalos, J.M., Valsesia, P., Lescourret, F., Génard, M., Davarynejad, G.H., Bannayan, M., Azizi, M. and Vercambre, G. (2018) Disentangling the effects of water stress on carbon acquisition, vegetative growth, and fruit quality of peach trees by means of the QualiTree model. Frontiers in Plant Science, 9, 3.
62. Cotrozzi, L., Remorini, D., Pellegrini, E., Landi, M., Massai, R., Nali, C., Guidi, L. and Lorenzini, G. (2016) Variations in physiological and biochemical traits of oak seedlings grown under drought and ozone stress. Physiologia Plantarum, **157**, 69-84.
63. Zafar, Z., Rasheed, F., Abdullah, M., Salam, M.M.A. and Mohsin, M. (2019) Effects of water deficit on growth and physiology of Young Conocarpus erectus L. and Ficus benjamina L. saplings. *Bangladesh Journal of Botany*, **48**, 1215-1221.
64. Chen, Y., Hung, Y.-C., Chen, M., Lin, M. and Lin, H. (2019) Enhanced storability of blueberries by acidic electrolyzed oxidizing water application may be mediated by regulating ROS metabolism. Food Chemistry, **270**, 229-235.
65. Ain-Lhout, F., Zunzunegui, M., Barradas, D., Tirado, R., Clavijo, A. and Garcia Novo, F. (2001) Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. Plant soil, **230**, 175-183.
66. Laxa, M., Liebthal, M., Telman, W., Chibani, K. and Dietz, K.-J. (2019) The role of the plant antioxidant system in drought tolerance. Antioxidants, 8, 94.
67. Jia, H., Wang, L., Li, J., Sun, P., Lu, M. and Hu, J. (2020) Physiological and metabolic responses of *Salix* sinopurpurea and Salix suchowensis to drought stress. Trees, **34**, e563-577.
68. Lei, Y., Yin, C. and Li, C.J.P.P. (2006) Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of Populus przewalskii. Physiologia Plantarum, **127**, 182-191.