Bulgarian Journal of Agricultural Science, 32 (No 5) 2026, 1109–1124

DOI: https://doi.org/10.61308/PRPM3877

Seed origin effects on growth, physiological and biochemical traits of *Moringa oleifera* seedlings grown under drought and salinity conditions

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Abstract

Hajaji, A. N., Dguimi, H. M., Hamouda, R. A. E. & Youssef, A. (2026). Seed origin effects on growth, physiological and biochemical traits of *Moringa oleifera* seedlings grown under drought and salinity conditions. *Bulg. J. Agric. Sci., 32*(5), 1109–1124

The versatile plant *Moringa oleifera* has spread beyond its native range and is now widely cultivated across diverse geographic regions. Global climate change requires careful seed selection before planting to ensure adaptation to the target environment. In the present study, *M. oleifera* seeds were collected from two geographical origins (Bouhedma Park, Tunisia, and Jeddah, Saudi Arabia) and cultivated in the National Institute of Research in Rural Engineering, Waters and Forests area (INRGREF, Tunisia), under salinity, drought, and combined drought–salinity treatments. Growth parameters, photosynthetic pigments, proteins, total carbohydrate, phenolic compounds, Malondialdehyde and hydrogen peroxide (H_2O_2) contents, antioxidant enzyme activities such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase were determined. The data showed that six-month-old Saudi plants reached a height of 60 cm and a stem diameter of 10 mm. Tunisian plants of the same age reached a height of 55.8 cm and a stem diameter of 11.34 mm. Protein and total carbohydrate contents were higher in seeds from Saudi *Moringa* plants. Saudi *Moringa* plants also had higher leaf protein contents than Tunisian plants. Antioxidant enzyme activities and total phenolic compound contents increased in both Saudi and Tunisian plants under drought and high salinity. Consequently, current results showed that although some morphological and biochemical differences were observed between plants derived from Saudi and Tunisian seeds, *Moringa* trees showed substantial tolerance to drought and salinity. The tolerance of *Moringa* to severe environmental conditions may contribute to its potential for sustainable cultivation in stress-prone environments.

Keywords: abiotic stress; *Moringa oleifera*; seed origin; antioxidants; phytochemicals

Introduction

The United Nations Sustainable Development Goals and the African Orphan Crops Consortium have supported initiatives focusing on several selected crops in Africa, including *Moringa*. These initiatives aim to address the growing problem of hidden hunger (food deficient in minerals and vitamins), and boost the strong and nutritious system for the acutely malnourished African population (Alavilli et al., 2022; Hendre et al., 2019; Keatinge et al., 2017). Native to

the Indian subcontinent, *Moringa oleifera* L. is a fast-growing, drought-resistant tree that has been cultivated and/or occurs naturally in semi-arid climates worldwide (Alavilli et al., 2022; Farahat and Refaat, 2021; Harshika and Abdul Ajees, 2024). Thirteen species of *M. oleifera* are the most widely cultivated under tropical and subtropical climates (Olson and Fahey, 2011), naturalized in semi-arid climates (Alavilli et al., 2022), grown in different soil types (Fakayode et al., 2019) and at different altitudes (Palada and Chang, 2003). *Moringa* can grow without frequent irrigation and fertilization, although adequate water and nutrient availability can enhance its growth (Labbassi et al., 2024).

Moringa trees are cultivated all over the world for their edible parts, which are extraordinarily rich in nutrients and minerals, as well as for their many aspects and relevance in medicinal applications and food production (Islam et al., 2021; Trigo et al., 2021). *M. oleifera* leaf polysaccharides may be a viable prebiotic with health-promoting properties, that regulate the microbial flora of the intestinal tract, and anti-obesity effects (Seleiman et al., 2021; Wang et al., 2019). *Moringa* has potential uses as a multipurpose plant and a vital nutrient, known to improve health in animals and humans such as high phenolic content and higher concentration of the vitamins (Alqurashi and Aldossary, 2021; Bancessi et al., 2020). *Moringa* has various phytochemicals such as antioxidants, omega-6 fatty acids, and omega-3 (Alam et al., 2020). *Moringa* seeds are a good source of oil, which contains important compounds such as unsaturated fatty acids (Salama et al., 2020). *Moringa* is used in the pharmaceutical, food, cosmetic, animal-feed, and other sectors (Němec et al., 2020).

Abiotic stresses such as drought and salinity limit crop growth and productivity, because of their negative effects on biochemical and physiological processes in cells (Azeem et al., 2023; Ma et al., 2022). Dramatic effects were reported on photosynthetic efficiency, production of reactive oxygen species (ROS), antioxidant defense, proteins, lipids, and total phenolic contents (Taha et al., 2021). The influence of drought stress depends on several factors, such as the length and intensity of the stress, the stage of the plant‘s growth, and the plant‘s genetic tolerance to the stress (Rivas et al., 2013).

Like many other plants, *Moringa* is susceptible to various abiotic stresses, but it has considerable potential for stress tolerance (Swarup et al., 2020; Zanella et al., 2019). To decrease stress-induced damage such as salinity and drought, plants have diverse pathways, like increasing the levels of both non-enzymatic and enzymatic antioxidants (Hajaji and Ammarim 2021; Hajaji et al., 2024; Nazari et al., 2021; Osman and El-Naggar, 2022). Precisely, the most adaptive responses of *M. oleifera* to stress conditions are the accumulation of osmoprotectants, such as proline and soluble sugars, an accumulation of total polyphenol compounds and stimulation of radical scavenging activity (Bekka et al., 2022).

Currently, global climate change is contributing to the occurrence of simultaneous stresses such as drought and salinity.

This study aimed to evaluate the effects of drought and salinity applied separately or in combination in *M. oleifera* seedlings. *M. oleifera* seeds derived from two geographical areas (Saudi Arabia, Red Sea, and Tunisia, Mediterranean Sea). The study of the morphological and physiological *M. oleifera* changes under drought and salinity could help identify the most suitable seed origin for stress-affected environments.

Materials and Methods

Material origin and experimental site description

Seeds used in this study were collected in 2020 from *Moringa oleifera* trees growing in two distinct agro-climatic regions. The first batch of seeds was from Tunisia (National Park Bouhedma, 814 m relative to Mediterranean Sea T°), whereas the second one was from Saudi Arabia (Jeddah, characterized by 12 m relative to Red Sea T°). Experiments were conducted in the National Institute of Research in Rural Engineering, Waters and Forests (INRGREF) of Tunis area (Tunisia). The first part of the work was conducted under Tunisian climate conditions for eight months (May–December 2021). Changes in temperature and rainfall are presented in Table 1 (Deputy Direction of Climatology Climate Products Department/Tunisian National Institute of Metrology https://www.meteo.tn). The second part of our study was conducted in a greenhouse under controlled irrigation conditions.

Table 1. Minimum, maximum temperature and rainfall during height months (2021)

	Monthly	Min Temp	Max Temp
	Rainfall amount (mm)	(°C)	(°C)
May	30	15	26
June	12.5	18.4	31.66
July	4	21.25	33.33
August	0.31	24.41	37.27
September	4.8	23.18	35
October	51.66	17	25.1
November	20	12.61	21.25
December	55	9.1	17.16

Source: Authors' own elaboration

Experimental design and treatments

Disinfected and soaked *Moringa* seeds from the two geographical origins were used to establish two experimental groups. The first group of seeds was sown in pots placed in the INRGREF area to follow the growth response from May 2021 to December 2021. Irrigation was carried out every 10 days to prevent water deficiency. A second group of germinated seeds were transferred to a pot (greenhouse) containing a mixture of sand and vermiculite (1/3). During the first two weeks, culture was conducted under controlled conditions using Hoagland solution for soaking. After that, young seedlings were divided into six sub-groups to grow under different stress conditions. Two field capacities (25%, 12.5%), and two salinity doses (7.5, 10g/L NaCl) were applied to create drought and salt stress conditions. Besides, another group of germinated seeds was cultivated under combined stresses of drought (25% FC), and salinity (7.5g/L). Plants were harvested after four weeks.

Morphological parameters

Morphological changes of leaves (fresh and dry weight, water content, and number) and stem (height and diameter) were measured during seven months (June-December) in *M. oleifera* grown from Tunisian (T) and Saudi (S) seeds. In leaves, studied parameters are water content of different foliar stages, Leaf length, Leaf width, Leaf area, Leaf Perimeter and Leaf water content. In roots, parameters are Root length, Root diameter, Root volume and number of Ramified roots.

Relative growth rate (RGR: mg. $g^{-1}.d^{-1}$), (W, shoot weight) was determined following equation:

$$\mathbf{RGR\ mg.\ g^{-1}.d^{-1} = (lnw_4 - lnw_0)/(t_4 - t_0)}$$

The relative growth rate of the principal stem in height (RGRh: cm. $cm^{-1}\ d^{-1}$) (h – stem height) was calculated according to the following formula:

$$\mathbf{RGRh,\ cm.\ cm^{-1}.d^{-1} = (lnhf - lnh_i/tf - t_i),}$$

where *t* is the time at two-time intervals, *ti* at the beginning and *tf* – at the end of the experiment.

The different leaf parameters were measured using a planimeter (model CI-202, USA).

Determination of photosynthetic pigments content and fluorescence measurements extraction of photosynthetic pigments from fresh leaves was done with acetone solution (80%). The contents of Chla, Chl a+b, and carotenoid were determined by spectrophotometer (PG instrument, T60 UV-V /United Kingdom) (DI et al., 1956). The measurement of the maximum quantum efficiency of PSII photochemistry (Fv/Fm) was done using a modulated chlorophyll fluorometer OS1p.

Determination of nitrogen and crude proteins contents

Nitrogen content was determined by Kjeldahl method (AOAC, 1994), to determine the nitrogen content of the samples. The following formula was used:

$$N\ \% = [(V \times N \times 0.014)/m] \times 100.$$

(V: volume of hydrochloric acid used in the titration. N: normality of hydrochloric acid and m: mass of the sample in grams).

Crude protein content was determined using the following formula:

$$\text{Crude protein \%} = \%\ N \times 6.25.\ (N: \text{Nitrogen}).$$

Determination of malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) contents

Determination of MDA content, indicating lipid peroxidation level of cellular membrane, was done according to Heath and Packer (1968). The method was determined by measuring thiobarbituric acid reacting substances (TBARS). About 0.5 g of leaf samples were homogenized in trichloro-acetic acid (TCA) (0.1%). After centrifugation (15,000 × *g* for 5 min), 2 ml of the supernatant was mixed with 4 ml of thiobarbituric acid (TBA) (0.5%). The mixture was heated at 95°C for 30 min. The absorbance was measured by Spectrophotometer (PG instrument, T60 UV-V / United Kingdom), at 532 nm and 600 nm (non-specific value at 600 nm).

The content of H_2O_2 in fresh leaves was determined based on measurement of titaniumhydroperoxide complex content according to Patterson et al. (1984). Firstly, samples (0.5 g) were homogenized in acetone solution. After centrifugation (5 min at 10,000 g and 4°C), 0.75 ml of supernatant was dissolved in 0.75 ml of potassium phosphate buffer (10 mM, pH 7.0) and 1.5 ml of potassium iodide (1M). Colorimetric measure (PG instrument, T60 UV-V /United Kingdom) of absorbance was performed at wavelength 508 nm against a blank, and using H_2O_2 as a standard.

Total phenolic compounds, total flavonoids, and condensed tannins contents quantification

Secondary metabolites were extracted (maceration) from leaf DW by ethanol solution (70%).

Total phenolic compounds (TPC)

Total phenolic compounds (TPC) in dry leaf samples were quantified using the method of Li et al., (2005). This method is based on the use of Folin Ciocalteu and Na_2CO_3

as reagents. Gallic acid is the standard used to determine TPC content. Optic absorbance was determined at 760 nm using UV/Vis spectrophotometer (PG instrument, T60 UV-V /United Kingdom).

Total flavonoids

According to Dehpour et al. (2009), aluminium chloride was used as a reagent. Using a UV/Vis spectrophotometer (PG instrument, T60 UV-V /United Kingdom), absorbance was measured at 430 nm. Flavonoid content was quantified using quercetin as the standard.

Condensed tannins

The vanillin method in an acid medium described by Ba et al. (2010), was adopted to determine condensed tannin content. Absorbance was measured at 500 nm by a UV spectrophotometer (PG instrument, T60 UV-V /United Kingdom). Catechol was used as standard.

Proline and soluble sugars contents

The quantification of free proline in leaves was conducted using the method of Bates et al. (1973). Proline was extracted from dry leaf samples with a sulfosalicylic acid solution. Ninhydrin was supplied as a reagent. By spectrophotometer (PG instrument, T60 UV-V /United Kingdom), the absorbance was read at 520 nm using toluene as blank. Total soluble sugars were measured according to the method of Sunderwirth et al. (1964). Soluble sugars were extracted with ethanol solution (80%) at 70°C. Anthrone used as a reagent was added to extract sugar from dried leaves. Absorbance was measured at 640 nm.

Antioxidant enzymatic extraction and assays

Enzyme extractions were carried out at 4°C. The fresh plant tissues (0.5g) were powdered in liquid nitrogen and extracted at a ratio of 1:3 (w/v) in potassium phosphate buffer (0.1mM, pH 7.5) containing EDTA (0.5mM).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was estimated according to the method of Beyer and Fridovich (1987). It was assayed as the inhibition of the reduction of nitro-blue tetrazolium chloride (NBT). 3 mL of reaction mixture solutions (0.1 ml of sodium carbonate (1.5 M), 0.2 mL of methionine (200 mM), 0.1 mL of Nitro-blue tetrazolium (2.25 mM), 0.1 mL of EDTA (3mM), 1.5 mL of potassium phosphate buffer (pH 7.8)) was added to 1 mL distillate water and 0.5 mL of enzyme extract. Enzyme activity started after the supply of 0.1ml of riboflavin solution (60 µM), by placing the reaction mixture under a fluorescent lamp (15 W). The reaction was stopped by covering the light source. One unit of SOD corresponds to the amount of enzyme reducing 50% of NBT. Absorbance was determined at 560 nm.

Catalase (CAT)

Catalase activity (CAT) was assayed according to Cakmak (1993). Catalase activity was determined based on the residual H_2O_2 concentration using a titanium reagent. To 3 mL of reaction solution containing a mixture of 1mL H_2O_2 (6 mM) and 1.9 mL of phosphate buffer (0.1M, pH 7.0), the supply of 0.1mL enzyme extract initiated enzyme reaction. After 5 min the addition of 4 mL of titanium reagent caused stop of activity. A similar mixture of solutions without extract was prepared to serve as control tube. Absorbance was taken at 415 nM by spectrophotometer.

Ascorbate peroxidase (APX)

Ascorbate peroxidase activity (APX) was measured by monitoring the rate of ascorbate oxidation according to Nakano and Asada (1981). About 3 mL of reaction mixture of phosphate potassium buffer (200mM, pH 7), 0.05ml Ascorbate (5 mM), 0.05 mL (H_2O_2), was added to 50 µL of enzyme extract. The reaction was initiated by adding the enzyme extract. A blank tube was prepared similarly, but using boiled extract enzyme for each leaf sample. Recording the decrease in absorbance was realized at 290 nM using a spectrophotometer. The enzyme activity was calculated using the molar extinction coefficient (absorbance of one molar solution) of $E = 2.8/\ mM^{-1}\ cm^{-1}$. One unit of APX enzyme was the amount necessary to decompose 1.0 µmol of NADPH /minute at 25°C.

Glutathione reductase (GR)

Glutathione reductase activity (GR) was determined according to the method of Rao et al. (1996). The method was based on record of absorbance increase in presence of oxidized glutathione and 5.5-dithiobis-2-nitrbenzoic acid. A total volume (2.9 mL) of mixture of 1 ml potassium phosphate buffer (0.1 M, pH 7.5), 0.1 ml EDTA (0.1 mM), 0.5 ml DTNB (3 mM),0.1 mL NADPH (2 mM) was added with 0.1 mL enzyme extract and distilled water. Start of reaction was induced by the supply of 0.1 mL of oxidized glutathione (2mM). Absorbance at 340 nM was recorded by spectrophotometer at 25°C during 5 min.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and Tukey's HSD test (SPSS Statistics 27), to determine significant differences among treatment means

at a probability level of 5%, and also applied to two-way ANOVA, to determine the combination of seeds origin and stress conditions factors, with studied variables: leaf fresh weight, stem fresh weight, roots fresh weight, stem length, stem diameter, and root length. Pearson's correlation coefficient measures linear correlation between two data sets. Correlation analyses were used to correlate between fresh weights and dry weight, water content, leaves number, stem height, and stem diameter.

Results

Morphological parameters during seven months

Morphological traits of *M. oleifera* derived from Tunisian (T) and Saudi (S) regions were measured during seven months (June – December). The summarized results in Table 1 demonstrate that there was a significant difference in the growth parameters according to the seed's origin.

During the first four months, the Saudi-*M. oleifera*, showed better growth (leaf FW and DW, and plant height) than Tunisia-*M. oleifera*. However, the leaf water content and stem diameter were highest in Tunisia-*M. oleifera*. The plant height increased in Saudi-*M. oleifera* till October. Since October, leaf FW and DW and stem diameter increased more in Tunisia-*M. oleifera*, while the plant height in Saudi-*M. oleifera* was higher in all months compared to Tunisian-*M. oleifera*. In November, a significant decrease in leaf number of Tunisian seedlings, and a total falling leaves of Saudi seedlings were recorded (Table 2). October saw an increase in rainfall to 51.66 mm, which then declined in November. These factors may have had an impact on *M. oleifera*'s vegetative parameters during these months. The average minimum and maximum temperature during this time were 12.61 and 21.25°C, respectively. Therefore, we suggested that temperature is the principal factor affecting Tunisian and Saudi -*Moringa* trees.

Figure 1 illustrated the relative growth rate (RGR) of shoot and in height (RGRh) of Tunisian and Saudi *M. oleifera*. Results showed that RGR of Saudi-*M. oleifera* was higher than Tunisia-*Moringa* during the first three months (summer). After this period, the RGR started to slow down in several plants. But, compared to Tunisian *M. oleifera*, RGR in Saudi one was broken in November (Fig. 1A). In addition, the RGRh was enhanced during the first three months with a higher level in Tunisia-*M oleifera* (Fig. 1B). Regardless seed origin, RGRh was declined in December. Saudi originated plants began to grow more rapidly in the first grown season and begin dormancy (the leaves dropped) earlier in November and December, while, Tunisian originated plants could grow better under low temperatures (autumn). These patterns of seedling growth responses seemed to be related to the environmental conditions noted favourable for the different *M. oleifera* seed origin growth (Table 2).

Figure 2 (A, B, C, D) illustrates leaf parameter growth of different foliar stages of seven months old *Moringa* plants. The results indicated that the leaf foliar stage of Tunisian *Moringa* was higher than Saudi plants through six months. Also, results showed the complete disappearance of the seventh and eighth foliar stages in Saudi-*M. oleifera* plants. Results also showed, that leaf width, leaf area and leaf perimeter were higher in Tunisian plants (Fig. 2B, 2C and 2D). During the three first months, leaf water content was higher in Tunisian plants (Fig. 2E).

In contrast, in seven months old plants, the longest and largest diameter roots were found in Tunisian seed origin *Moringa* (Fig. 2F, 2G). Meanwhile, Saudi seed-origin plants present the largest root volume and ramified roots and the

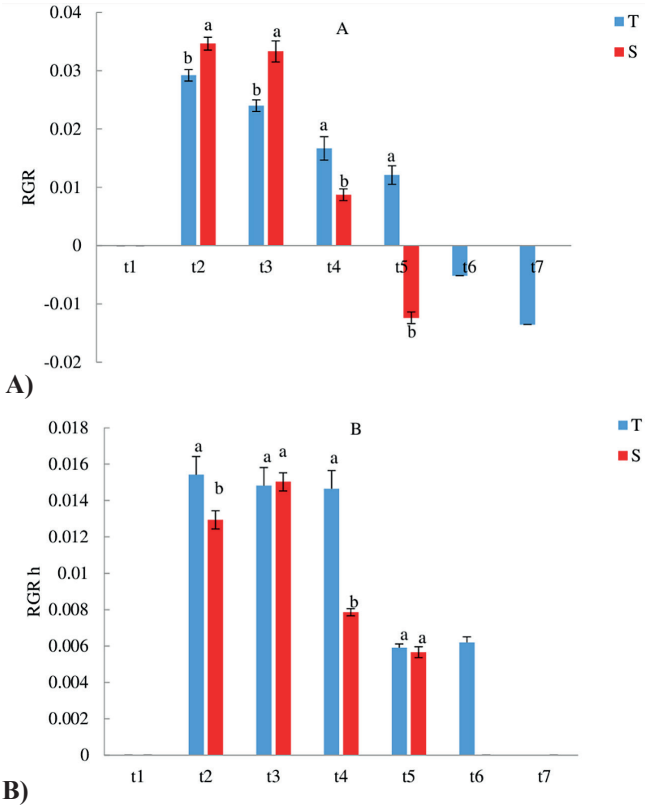


Fig. 1. Relative growth rate of shoot and in height (A and B) changes of Moringa *oleifera* of Tunisian (T) and Saudi (S) seeds origins during seven months of growth

Means showing different letters are statistically different ($p < 0.05$) from each other. Data was computed from five replications consisting of three plants in each replication.

Source: Authors' own elaboration

Table 2. Morphological parameters changes in *M. oleifera* issued from Tunisian (T) and Saudi (S) seeds during seven months

Origin	Date	Leaves			Stem		
		Fresh weight (g)	Dry weight (g)	Water content (%)	Number of leaves	Height (cm)	Diameter (cm)
T	June	0.2±0.01^{g}	0.01±0.00^{g}	14.748±1.05^{f}	3±0.01^{e}	10.093±1^{f}	6.353±0.52^{f}
	July	0.75±0.03^{f}	0.02±0.00^{f}	31.33±2.07^{c}	6±0.02^{c}	16.03±1.0^{e}	8.303±0.04^{e}
	August	1.51±0.01^{e}	0.07±0.00^{e}	19.13±1.024^{e}	8±0.1^{b}	25±2.07^{d}	9.05±0.14^{d}
	September	2.49±0.014^{c}	0.1±0.01^{b}	22.27±1.035^{d}	9±0.01^{a}	38.8±2.05^{c}	11.173±1.0^{c}
	October	3.58±0.018^{a}	0.75±0.03^{a}	46.773±0.24^{ab}	8±0.01^{b}	46.33±3^{b}	11.253±1^{b}
	November	3.068±0.02^{b}	0.051±0.00^{d}	59.157±3.0 ^{a}	4±0.01d	55.8±4.01^{a}	11.343±1^{a}
	December	2.04±0.01^{d}	0.045±0.00^{e}	44.344±1.25^{b}	2±0.02f	55.8±3.16^{a}	11.343±1.0^{a}
S	June	0.31±0.02^{e}	0.03±0.002^{e}	9.417±0.61^{d}	3±0.01^{d}	17.08±1.2^{e}	4.33±0.31^{e}
	July	0.89±0.04^{d}	0.078±0.00^{d}	10.43±0.05^{e}	6±0.01^{b}	25.18±1.5^{d}	5.297±0.34^{d}
	August	2.43±0.02^{b}	0.124±0.01^{c}	18.623±0.97^{a}	9±0.21^{a}	40±2.01^{c}	7.39±0.5^{c}
	September	3.16±0.02^{a}	0.25±0.015^{a}	11.295±1.01^{b}	9±0.32^{a}	50.63±2.1^{b}	9.8±0.32^{b}
	October	2.1 ^{c}	0.36±0.041^{b}	5.05±0.1^{e}	6±0.04^{c}	60±4.87^{a}	9.963±0.52^{a}
	November	0 f	0 f	0 f	0 e	60±4.87^{a}	9.965±0.52^{a}
	December	0 f	0 f	0 f	0 e	60±2.6^{a}	10±0.98^{a}

The means showing different letters are statistically different ($p < 0.05$). Data are computed from five replications consisting of three plants in each replication.
Source: Authors' own elaboration

(Fig. 2H, 2I). The characteristic of ramification seemed to be related to the purpose of occupation of a bigger volume of rhizospheres to enhance water acquisition. In current study, plants presented different ways of root establishment and significant difference in RGRh under the same environmental conditions, as explained by the *Moringa* plant's morphology and growth profiles.

Correlation analysis

Pearson correlation analysis was used to investigate the association between plant morphological parameters during seven months of cultivation *M. oleifera* derived from Tunisian, and Saudi seeds (Table 3). A positive correlation was observed between FW and DW, water content (r = 0.650*), water content (r = 0.720), stem height (r = 0.854*), stem diameter (r = 0.909**), while results revealed a weak positive correlation with leaves number (r = 0.331).

It is noticed a strong correlation between stem height and stem diameter (r = 0.938**). In Saudi plants, a strong positive correlation was obtained between FW, DW (r = 0.809*), and leaves number (0.927**), and between stem height and stem diameter (r = 0.987**).

Primary metabolites and pigment contents

Figure 3 demonstrated that the two plants contained vital amounts of carbohydrates and proteins (Fig. 3A). In Saudi originated plants, the Chla, Chlb and carotenoid levels were higher than that in Tunisian plants (Fig 3B). Figure 3C revealed a higher PSII efficiency in Saudi originated plants. The PSII efficiency enhancement might be attributed to the underground development of roots to absorb and translocate water to shoots.

Effect of salinity and drought stress on morphological parameters

Table 4 showed morphological changes in six-week-old *M. oleifera* grown from Tunisian (T) and Saudi (S) seeds, cultivated under drought (25 and 12.5% FC), salinity (0, 7.5, and 10g/l NaCl), simultaneously salinity (7.5 g/l NaCl) and drought (25% FC) conditions. Morphological parameters of *M. oleifera* derived from Tunisian (T) and Saudi (S) seed showed significant changes under different treatments. After four weeks, the leaf FW was higher in Saudi-*M. oleifera* compared to Tunisian-*M. oleifera* in control conditions. Leaf, stem and root FW decreased in both Saudi and Tunisian plants under drought, salinity and especially under the co-treatment salinity-drought. Stem length and stem diameter decreased progressively under salinity and drought. Root length increased under low salinity and low drought in both Tunisian and Saudi plants. Saudi-*M. oleifera* was less affected by drought and salinity than Tunisian-*M. oleifera*. Results showed that *Moringa* derived from Saudi seeds presented a higher potential of resistance than plants grown from Tunisian seeds. However, under co-stress drought and salinity, there was no significant difference between their abilities to tolerate the abiotic stresses.

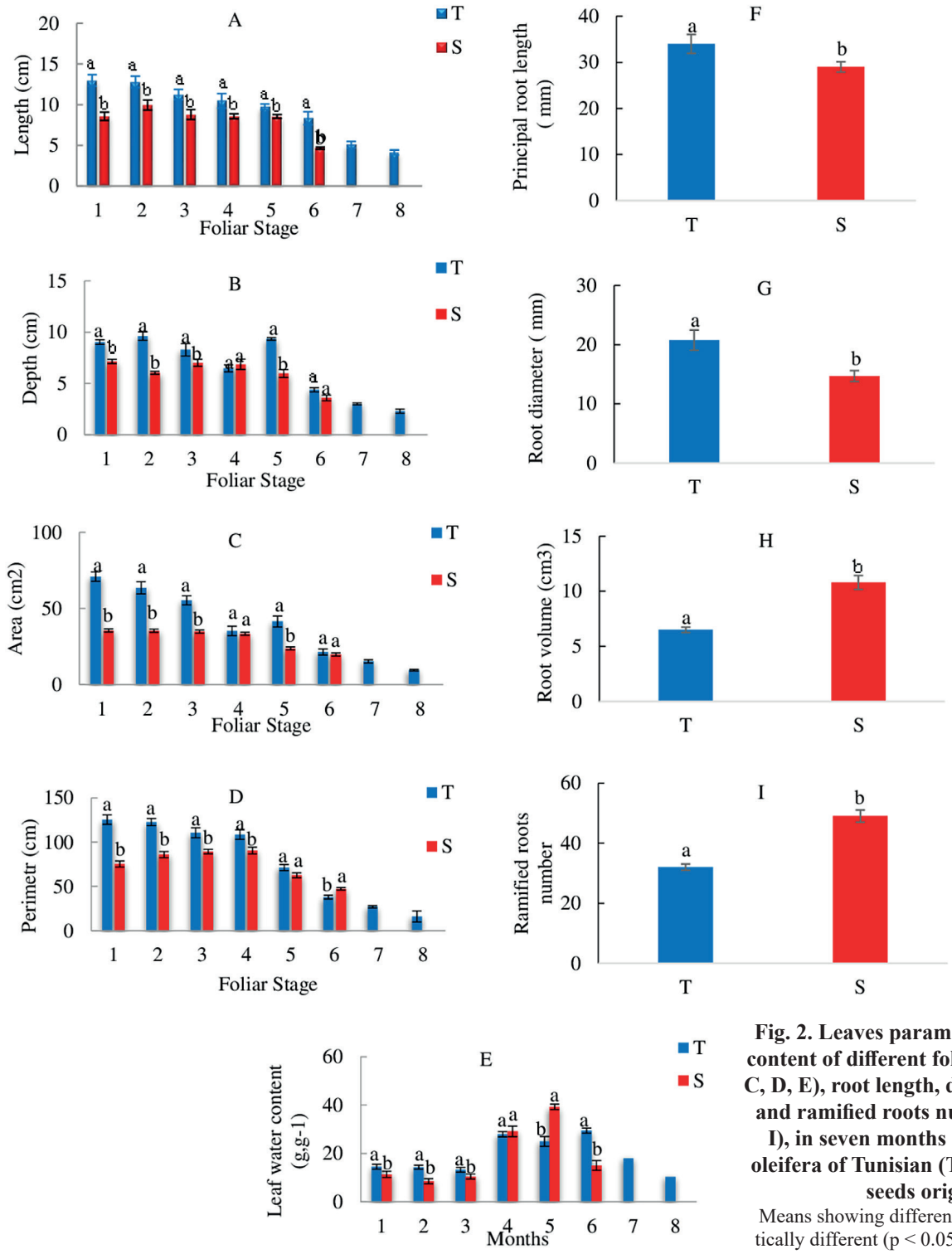


Fig. 2. Leaves parameters and water content of different foliar stages (A, B, C, D, E), root length, diameter, volume and ramified roots number (F, G, H, I), in seven months aged Moringa oleifera of Tunisian (T) and Saudi (S) seeds origins

Means showing different letters are statistically different ($p < 0.05$) from each other. Data was computed from five replications consisting of three plants in each replication

Source: Authors' own elaboration

Table 3. Pearson's correlation coefficient of morphological changes during seven months aged – *M. oleifera* derived from grown Tunisian (T) and Saudian (S) seeds

Seeds origin		Fresh weight (g)	Dry weight (g)	Water content (%)	Leaves number	Stem height (cm)	Stem diameter (cm)
T	Fresh weight (g)	1					
	Dry weight (g)	0.650	1				
	Water content (%)	0.720r	0.337	1			
	Leaves Number	0.331	0.438	-0.29	1		
	Stem height (cm)	0.854*	0.306	0.820*	-0.114	1	
	Stem diameter (cm)	0.909**	0.388	0.708	0.204	0.938**	1
S	Fresh weight (g)	1					
	Dry weight (g)	0.809*	1				
	Water content (%)	0.663	0.264	1			
	Leaves number	0.927**	0.672	0.862*	1		
	Stem Height (cm)	0.099	0.276	-0.570	-0.238	1	
	Stem diameter (cm)	0.203	0.332	-0.506	-0.147	0.987**	1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
Source: Authors' own elaboration

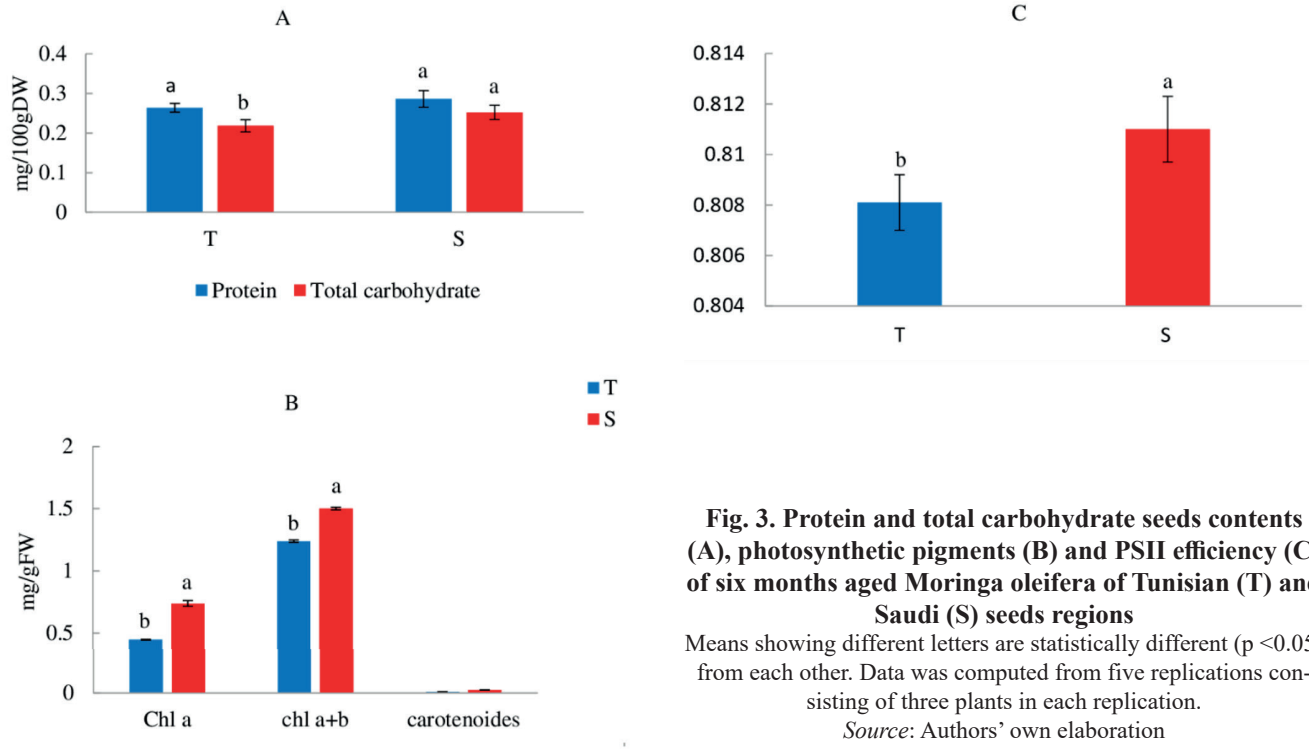


Fig. 3. Protein and total carbohydrate seeds contents (A), photosynthetic pigments (B) and PSII efficiency (C) of six months aged Moringa oleifera of Tunisian (T) and Saudi (S) seeds regions

Means showing different letters are statistically different ($p < 0.05$) from each other. Data was computed from five replications consisting of three plants in each replication.
Source: Authors' own elaboration

Effect of salinity and drought stress on secondary metabolites

MDA is a principal indicator that can prove that oxidative stress damage takes place in cells as a consequence of abiotic stress conditions. Data in Table 5 indicated that nevertheless applied stress, *Moringa* leaf contents of MDA were higher in Tunisian-*Moringa* compared to Saudi plants, especially, when stresses are co-applied, the lipid peroxidation of cell membranes had same level in leaves of both Tunisian and Saudi-*Moringa*. In contrast, hydrogen peroxide accumulated to a greater extent in *Moringa* leaves independently of the constraint type (drought, salt, drought-salinity).

Table 4. Morphological changes in six-week- old *M. oleifera* issued from Tunisian (T) and Saudi (S) seeds origins, cultivated under drought (25 and 12.5% FC), salinity (0, 7.5 and 10g/l NaCl), simultaneously salinity (7.5g/l NaCl) and drought (25% FC) conditions

Seeds origin	Treatments	Leaf fresh weight (gm)	Stem fresh weight (gm)	Roots fresh weight (gm)	Stem length (cm)	Stem diameter (cm)	Root length (cm)
T	Control	0.21±0.04^{e}	1.09±0.0^{f}	0.48±0.0^{cd}	15.36±0.2^{g}	5.43±0.1^{f}	3.4±0.20^{g}
	Drought 25% FC	0.129±0.0^{c}	0.59±0.0^{cde}	0.33±0.0^{bc}	11.03±0.1^{f}	3.2±0.09^{bc}	3.72±0.0^{h}
	Drought 12.5% FC	0.022±0.0^{a}	0.22±0.0^{a}	0.23±0.0^{ab}	8.1±0.041^{d}	1.41±0.0^{a}	1.47±0.1^{c}
	Salinity 7,5g/l NaCl	0.131±0.0^{c}	0.51±0.0^{cd}	0.26±0.0^{ab}	6.78±0.22^{c}	2.98±0.0^{bc}	3.26±0.2^{g}
	Salinity 10g/l NaCl	0.026±0.0^{a}	0.22±0.0^{a}	0.12±0.0^{a}	3.57±0.11^{a}	1.07±0.0^{a}	0.98±0.0^{a}
	Salinity (7.5g/L) +drought (25% FC)	0.021±0.0^{a}	0.19±0.0^{a}	0.09±0.0^{a}	9.25±0.20^{e}	2.73±0.1^{b}	1.33±0.1^{bc}
S	Control	0.30±0.0^{f}	1.48±0.0^{g}	0.53±0.0^{d}	15.8±0.0^{g}	5.39±0.1^{f}	2.79±0.0^{e}
	Drought 25% FC	0.19±0.1^{d}	0.74±0.0^{e}	0.44±0.0^{cd}	10.7±0.1^{f}	4.01±0.0^{e}	3.06±0.0^{f}
	Drought 12.5% FC	0.05±0.0^{b}	0.27±0.0^{ab}	0.24±0.0^{ab}	5.01±0.0^{b}	1.37±0.0^{a}	1.33±0.1^{bc}
	Salinity 7.5g/L NaCl	0.19±0.0^{d}	0.69±0.0^{de}	0.49±0.1^{cd}	9.33±0.1^{e}	3.89±0.0^{de}	2.82±0.0^{e}
	Salinity 10g/L NaCl	0.08±0.0^{c}	0.44±0.0^{bc}	0.2±0.0^{ab}	6.5±0.18^{c}	2,97±0.0^{bc}	1.28±0.1^{b}
	Salinity (7.5g/L) +drought (25% FC)	0.03±0.0^{ab}	0.55±0.00^{cd}	0.18±0.02^{ab}	11.43±0.28^{f}	3.37±0.16^{cd}	1.97±0.29^{d}
	Sig	0.000	0.000	0.000	0.000	0.000	0.000

Means showing different letters are statistically different ($p < 0.05$) from each other (in each column).
Data are computed from five replications consisting of three plants in each replication.
Source: Authors' own elaboration

Table 5. Effect of drought (25 and 12.5% FC), salinity (0, 7.5 and 10g/l NaCl), co-treatment salinity (7.5g/l NaCl) and drought (25%FC) on *M. oleifera* derived from Tunisian (T) and Saudi (S) seeds origins, that cultivated for four weeks on MDA, H_2O_2, total phenolic, flavonoids, condensed tannins, proline and soluble sugar of leaves

Seeds origin	Treatment	MDA nmol.g^{-1} (DW)	H_2O_2 nmol.g^{-1} (FW)	Total Phenolic eqAG g^{-1} (DW)	Flavonoids mg Qu g^{-1} (DW)	Condensed tanins mg eq catch g^{-1} (DW)	Proline µmol.g^{-1} (DW)	Soluble sugar µmol.g^{-1} (DW)
T	Control	9.12±0.8^{a}	1.33±0.1^{a}	0.31±0.02^{a}	0.20±0.01^{a}	0.19±0.01^{a}	3.2±0.02^{a}	2.77±0.12^{a}
	Drought 25% FC	60.25±3.25^{b}	2.57±0.11^{bc}	1.85±0.01^{cd}	0.43±0.015^{bc}	0.40±0.03^{bc}	7.55±0.031^{def}	5.03±0.4^{bc}
	Drought 12.5% FC	105.67±9.25^{d}	3.88±0.2^{d}	1.08±0.09^{b}	0.42±0.02^{bc}	0.39±0.03^{bc}	7.12±0.014^{cd}	4.44±0.22^{b}
	Salinity 7.5g/l NaCl	108.23±6.78^{d}	2.9±0.17^{c}	2.40±0.14^{c}	1.661±0.03^{g}	0.57±0.02^{e}	8.53±0.045^{fgh}	6.26±0.41^{de}
	Salinity 10g/L NaCl	147.1±6.52^{e}	4.02±0.25^{d}	2.011±.0.1^{d}	1.45±0.04^{f}	0.50±0.001^{de}	8.02±0.07^{defg}	6.1±0.31^{de}
	Salinity (7.5g/L)+ drought (25% FC)	168.02±8^{f}	3.85±0.27^{d}	1.427±0.07^{bc}	0.97±0.004^{d}	0.36±0.02^{b}	6.32±0.35^{c}	4.95±0.18^{bc}
S	Control	8.75±0.71^{a}	1.11±0.09^{a}	0.50±0.2^{a}	0.28±0.01^{ab}	0.233±0.02a	4.79±0.034^{b}	2.96±0.14^{a}
	Drought 25% FC	58.74±2.18^{b}	2.14±0.145^{b}	2.23±0.018^{d}	0.50±0.014^{c}	0.46±0.025^{cd}	8.83±0.02^{ghi}	6.84±0.32$_{ef}$
	Drought 12.5% FC	90.94±3.51^{c}	3.08±0.18^{c}	1.96±0.084^{d}	0.5±0.01^{c}	0.42±0.02b^{cd}	8.2±0.018^{efgh}	6.02±0.065^{de}
	Salinity 7.5g/l NaCl	100±3.4^{cd}	2.11±0.11^{b}	3.74±0.22^{f}	1.99±0.04^{h}	0.74±0.04^{fd}	9.68±0.036^{i}	7.84±0.56^{f}
	Salinity 10g/L NaCl	139.08±5.26^{e}	2.056±0.17^{b}	3.601±0.22^{f}	1.75±0.1^{g}	0.71±0.03^{f}	9.22±0.061^{hi}	7±0.01^{ef}
	Salinity (7.5g/L) +drought (25% FC)	167.8±9.01^{f}	3.86±0.13^{c}	1.74±0.01^{cd}	1.25±0.01e	0.44±0.01^{bcd}	7.48±0.12^{de}	5.66±0.26^{cd}
	Sig	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Means showing different letters are statistically different ($p < 0.05$) from each other (in each column). Data are computed from five replications consisting of three plants in each replication.
Source: Authors' own elaboration

To tolerate environmental stresses, stressed plants accumulated more total polyphenols, flavonoids, and condensed tannins. The results in Table 5 showed that stress increased secondary metabolite levels such as flavonoids and tannins. Osmo-protectant accumulation such as soluble proteins, soluble sugars, and amino acids, like proline, is another important physiological process adopted by plants to avoid stress. Results indicated that, regardless of seed origin, leaf proline,

and soluble sugar contents were increased under drought and salinity applied separately or simultaneously (Table 5). When the two stresses were co-applied, the osmolyte accumulation was reduced compared to severe salinity and water-deficient levels.

Effect of salinity and drought stress in nitrogen and crude protein contents

Figure 4 (A and B) showed that the most important decrease in crude protein content in *Moringa* from both Saudi and Tunisian seeds origins was detected when co-stresses were applied, followed by water deficit (12.5% FC) and salinity (10 g/L NaCl). Conversely, the changes in these parameters are more pronounced in Tunisian *Moringa* leaves.

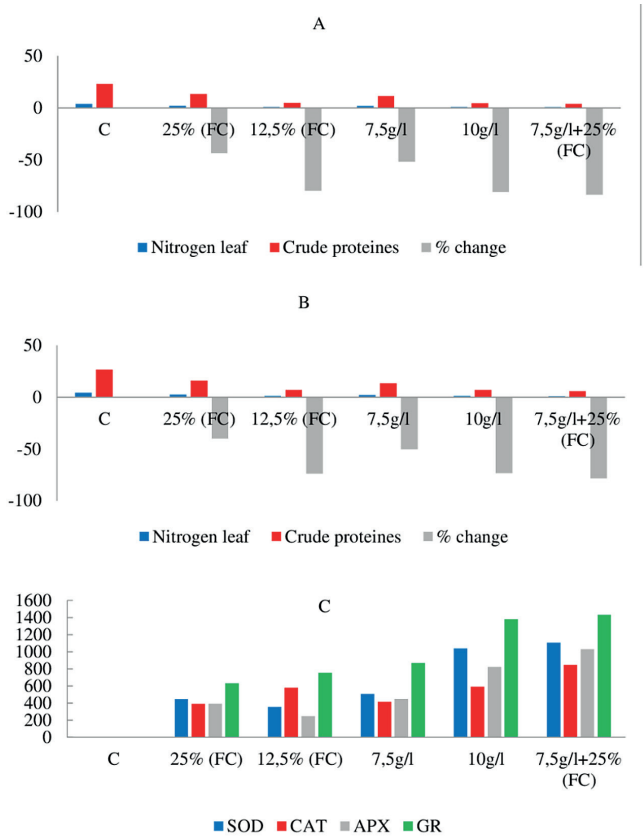


Fig. 4. Nitrogen leaf and crude proteins contents and SOD, CAT, APX and GR percent changes in six months aged *Moringa oleifera leaves of* Tunisian (A and C) and Saudi (B and D) seeds regions

Data was computed from five replications consisting of three plants in each replication

Source: Authors' own elaboration

Effect of salinity and drought stress in SOD, CAT, APX and GR

The activity of antioxidant enzymes such as superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), and Glutathione reductase (GR) is generally up-regulated in stressed plants. The change in antioxidant enzyme activity is one of the different ways adopted, to overcome stress conditions. Figure 4 (C and D) showed that all studied antioxidant enzymes were activated under stress. However, the highest increase in antioxidant enzyme activities in both Tunisian and Saudi-*Moringa* was observed with simultaneous treatment with 25% drought and 7.5% NaCl. The co-treatment drought-salinity resulted in accentuated oxidative stress levels. *Moringa* showed stimulation of antioxidant enzyme activities, which support plants to tolerate oxidative damage. Under salinity, the increase of antioxidant activation in *Moringa* plants help sustain nutritional quality despite stress. They mitigate environmental stresses. In fact, the decrease in photosynthetic efficiency induced overproduction of reactive oxygen species (ROS), which could generate lipid peroxidation. Under salinity, malondialdehyde (MDA) level increased, which is reflected as a marker of lipid peroxidation in plants. Plants detoxify ROS through the activity of ROS-scavenging enzymes such as CAT and APX. The SOD had higher activity in Saudi plants under drought (12.5% FC), salinity (10 g/L) and co-treatment drought-salt. Meanwhile, catalase activity was higher in Tunisian origin plant. GR had activity no significant differences due to plant origin.

Discussion

Morphological traits of *M. oleifera*, derived from Tunisian and Saudi regions, were measured during seven months (June – December). Results in Table 2 showed that there was significant difference in the growth parameters according to the seed's origin. At the first four months, the Saudi-*M. oleifera*, showed better growth (leaf FW and DW, and plant height) than Tunisian-*M. oleifera*. In October and November, an important change in rainfall and in the average minimum and maximum temperature was recorded. These factors had an impact on *M. oleifera*'s vegetative parameters during these months. In November, a significant decrease in leaf number of Tunisian seedlings, and a total falling leaves of Saudi seedlings were recorded. The rainfall and the temporal distribution of the precipitation pulses affected growth, duration of the flowering and fruiting phenophases in *M. oleifera* (João et al., 2022). The current results suggest that temperature is an important factor affecting Tunisian and Saudi *Moringa* trees. Amongst all climatic factors that af-

fect plant growth, temperature is one of the most important factors governing tree growth, physiology and productivity (Mashamaite et al., 2021). This result was previously discussed by Muhl et al. (2011), who showed that *M. oleifera* seedlings' growth increased exponentially with temperature. This study demonstrated a correlation between higher growth potential and a high temperature regime of 20–30°C.

According to Alshoaibi (2021), in Saudi Arabia, *M. oleifera* prefers a temperature regime of 32–35°C in order to grow larger. In contrast, the same author has confirmed that 25°C is the ideal temperature for the highest *M. oleifera* growth.

Current results also showed that RGR of Saudi-*M. oleifera* was higher than Tunisia-*Moringa* during the first three months (summer) (Fig. 1). The RGR and RGRh were declined in December in both Saudi and Tunisian plants (Fig. 1A and 1B). Saudi originated plants began to grow more rapidly in the first grown season and begin dormancy (the leaves dropped) earlier in November and December. In contrast, plants grown from Tunisian seeds could grow better under low temperatures (autumn). These patterns of seedling growth responses seemed to be related to the environmental conditions noted favourable for the different *M. oleifera* seed origin growth (Table 1). In current study, plants presented different ways of root establishment and significant difference in RGRh face to same environmental conditions, as explained by the *Moringa* plant's morphology and growth profiles. Current results confirmed the alternative hypothesis (Ha) that *Moringa oleifera* seed sources differed considerably in stem diameter and height (Santoso and Parwata, 2020). These results remained debated by several studies (Olaborode et al., 2022; Santoso and Parwata, 2020). Differences were also recorded in photosynthetic pigment level between Saudi and Tunisian plants (Fig. 3B). Results revealed a higher PSII efficiency in Saudi originated plants (Fig. 3C). The PSII efficiency enhancement might be attributed to the underground development of roots, to absorb and translocate water to shoots (Liao et al., 2022).

Morphological parameters of *M. oleifera* derived from Tunisian and Saudi seeds showed significant changes under salinity and drought stresses. Leaf, stem and root FW decreased in both Saudi and Tunisian plants under drought salinity, and especially under the co-treatment salinity-drought. Stem length and stem diameter decreased progressively under salinity and drought. Saudi-*M. oleifera* was less affected by drought and salinity than Tunisian-*M. oleifera*. Results showed that *Moringa* derived from Saudi seeds presented a higher potential of resistance to drought and salinity than plants grown from Tunisian seeds. Under co-stress drought – salinity, there was no significant difference between their abilities to tolerate this applied stress. Water limitation restricted water transport to the shoots and indirectly altered the photosynthetic machinery and metabolism, resulting in growth inhibition (Hajaji and Gouia, 2019; Li et al., 2020). Compared to drought, the stronger negative effects of salinity on *Moringa* are because of both salt-induced dehydration and osmotic stress. Indeed, water deficit can disrupt cellular structure by reducing cell turgor, thereby reducing leaf biomass (Miranda et al., 2021). Under abiotic stress causing changes in morphological traits and growth inhibition, plants adopted several strategies to tolerate stress, such as production of stress hormones, osmotic adjustment, and activation of stress-related genes (Hasan et al. 2020; Liu et al., 2018). Under stress, the leaf and stem growth parameter reduction is compensated by root expansion, density, and elongation. It presented one of the strategies developed by *Moringa* to alleviate the reduction of water availability (Farooq et al., 2009; Hsiao and Xu, 2000; Nouman et al., 2014, 2018). Compared to water deficit and salinity stress separately applied, their combination presented more accentuated effects. Such a note was mentioned by Chaves et al. (2009) and Cohen et al. (2021).

The co-treatment salinity- drought induced rapid gene expression alteration (Chaves et al. 2009). In this regard, there were very close responses to the combined stress of *Moringa* derived from the two seeds' origins. Therefore, it is extremely important to research the principal indicators that can prove that oxidative stress damage takes place in cells as a consequence of abiotic stress presence. Data in Table 5 indicated that under different studied stresses, MDA leaf contents were higher in Tunisian-*Moringa* compared to Saudi plants. Under co-treatment drought-salinity, MDA level was quite similar in Tunisian and Saudi-*Moringa* leaves. Hydrogen peroxide accumulated to a greater extent in *Moringa* leaves independently of the constraint type (drought, salt). According to previous studies (Miranda et al., 2021), it is largely demonstrated that, Malondialdehyde and hydrogen peroxide accumulated under water deficiency or salinity (Adnan et al., 2020; Goche et al., 2020). The results denoted that oxidative stress damage as lipid peroxidation in *Moringa* is more pronounced in *Moringa* treated by co-applied stress (drought-salinity) followed by salinity stress (10 and 7.5 g NaCl) and deficit water (12.5 and 25% FC). In agreement with Nouman et al. (2018) and Alhakmani et al. (2013), results also showed that *Moringa*'s sensitivity to environmental stresses could be explained in relation to the geographical area origin of seeds.

Thus, to investigate *Moringa*'s ability to tolerate environmental stresses, several adaptive processes were discussed in previous studies (Álvarez-Román et al., 2020; Batool et al. 2020; Daryanto et al. 2017). One of the physiological pro-

cesses is the accumulation of total polyphenols, flavonoids, and condensed tannins in stressed plants. The results in Table 5 showed that stress increased secondary metabolite levels such as flavonoids and tannins.

Osmo-protectant accumulation such as soluble proteins, soluble sugars, and amino acids, like proline, is another important physiological process adopted by plants to avoid stress. Results indicated that, regardless of seed origin, leaf proline, and soluble sugar contents were increased under drought and salinity applied separately or simultaneously (Table 5). Under co-treatment drought-salinity, the osmolyte accumulation was reduced compared to severe salinity and water-deficient levels. The crucial role of osmolyte production is the maintenance of homeostasis for the metabolism process, resulting in the improvement of the *Moringa*'s capacity to tolerate current stresses. In addition to maintaining cellular redox homeostasis and scavenging free radicals, proline and soluble sugars accumulate under water deficit or salinity stress (Dobra et al., 2010; Omari Alzahrani, 2021; Osakabe et al., 2020).

Under co-stresses, an important decrease in crude protein content in both Saudi and Tunisian plants was recorded. Conversely, the changes in these parameters are more pronounced in Tunisian *Moringa* leaves. It is largely suggested that differences in leaves' protein contents were in reality depending on species (Balfany et al., 2023; Görgüç et al., 2020; Nynäs et al., 2021). In this study, significant changes shown in *Moringa* are related to the type and level of applied stress. While proteins are presented in the majority of chloroplasts (Rubisco) to activate photosynthesis, and are used in carbohydrate synthesis, this may be due to the decline of protein level under stress conditions (Hasan et al., 2018; Raven, 2013).

Changes in antioxidant enzyme activity represent one of the mechanisms by which plants cope with stress conditions. (Ghasemlou et al., 2019). Current results showed that all studied antioxidant enzymes were activated under stress. The most important increase in antioxidant enzyme activities in both Tunisian and Saudi-*Moringa* was observed with co-treatment with drought – salinity (Fig. 4C and 4D). *Moringa* showed stimulation of antioxidant enzyme activities, which support plants to tolerate oxidative damage (Nouman et al., 2018). Under salinity, the increase of antioxidant activation in *Moringa* plants, help sustain nutritional quality despite stress; they mitigate environmental stresses (Nouman et al., 2014). In fact, the decrease in photosynthetic efficiency induced overproduction of reactive oxygen species (ROS), which could generate lipid peroxidation (Xi et al. 2021).

Under salinity, leaf MDA level increased (Table 5). Lipid peroxidation had adverse effects on cell growth processes (Kh et al., 2010). Plants detoxify ROS by the motivation of ROS scavenging enzymes such as CAT and APX. The antioxidant protection systems have vigorous roles in handover plant tolerance to environmental stresses (Bernstein et al., 2010; Mansour and Salama, 2019; Wang et al., 2016). The SOD had higher activity in Saudi plants under drought (12.5% FC), salinity (10 g/L) and co-treatment drought-salt. Catalase activity was higher in Tunisian origin plant. GR activity had no significant differences between Tunisian and Saudi plants.

Results showed that *Moringa* is an exceptional tree that could adapt and grow in various climates. Current results showed some differences in morphological and growth parameters between Tunisian and Saudi plants. Tunisian originated plants could grow better under low temperatures. Results showed that *Moringa* derived from Saudi seeds presented a higher potential of resistance to drought and salinity than plants grown from Tunisian seeds. Whatever the origin of the seeds, *Moringa* is always able to tolerate drought and salinity applied separately or simultaneously. *Moringa* tolerance efficiency was linked to morphological, physiological and biochemical processes highly advanced under environmental stresses.

Conclusion

Recent climatic changes are serious threats that affect the sustainable production of plants and, as a result, food security. As a consequence, there is a need to examine the potential of seeds derived from different geographical sources before planting them in various areas and climates. The current study showed that *Moringa oleifera* from Saudi (Jeddah, which lies along the Red Sea) selected seeds could adapt to Tunisian climatic conditions. While, under low daily minimum temperature average, Saudi-*Moringa* showed an earlier senescence compared to Tunisian one. On the other hand, to cope with water scarcity and salinity factors, *M. oleifera* employs various tolerance mechanisms such as morphological, physiological as well as biochemical responses. Results showed that resistance potential to drought and salinity seems to be different according to the origin of grown seeds. Compared to drought (25 and 12.5% FC) that hampers *Moringa* plant growth, salinity stress had more pronounced adverse effects (7.5 and 10 gNaCl/l), because of its more pronounced adverse effects on *Moringa* derived from both seeds' origins. The co-treatment of drought (25% FC) and salinity (7.5 gNaCl/l) induced more the oxidative stress compared to the separate application of drought and salinity. To tolerate salt and drought- induced oxidative stress, *Moringa* leaf tissues accumulated higher levels of polyphe-

nols, osmoprotectants, and antioxidants (SOD, CAT, APX, and GR). Results showed that regardless of the geographical seed sources, *Moringa* presented the potential to cope with drought and salinity, caused by global climate change, and maintain its biochemical functions. It is also important to note that *Moringa* derived from Saudi seeds presented a higher potential of resistance to drought and salinity than plants grown from Tunisian seeds.

Conflict of interest

No conflict of interest.

References

Adnan, M., Fahad, S., Zamin, M., Shah, S., Mian, I. A., Danish, S. & Datta, R. (2020). Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. *Plants, 9*(7), 900. https://doi.org/10.3390/plants9070900

Alam, M. W., Pandey, P., Khan, F., Souayeh, B. & Farhan, M. (2020). Study to investigate the potential of combined extract of leaves and seeds of *Moringa oleifera* in groundwater purification. *International Journal of Environmental Research and Public Health, 17*(20), 7468. https://doi.org/10.3390/ijerph17207468

Alavilli, H., Poli, Y., Verma, K. S., Kumar, V., Gupta, S., Chaudhary, V. & Jain, A. (2022). Miracle Tree *Moringa oleifera*: Status of the genetic diversity, breeding, *in vitro* propagation, and a cogent source of commercial functional food and non-food products. *Plants, 11*(22), 3132. https://doi.org/10.3390/plants11223132

Alhakmani, F., Kumar, S. & Khan, S. A. (2013). Estimation of total phenolic content, invitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pacific Journal of Tropical Biomedicine, 3*(8), 623–627. https://doi.org/10.1016/S2221-1691(13)60126-4

Alqurashi, R. M. & Aldossary, H. M. (2021). In vitro antioxidant and antimicrobial activity of *Moringa oleifera* leaf as a natural food preservative in chicken burgers. *Emirates Journal of Food and Agriculture, 33*(6), 450–457. https://doi.org/10.9755/ejfa.2021.v33.i6.2711

Alshoaibi, A. (2021). Seed germination, seedling growth and photosynthetic responses to temperature in the tropical tree *Moringa oleifera* and its relative desert, *Moringa peregrina*. *Egyptian Journal of Botany, 61*(2), 541–551. https://doi.org/10.21608/ejbo.2021.63271.1631

Álvarez-Román, R., Silva-Flores, P. G., Galindo-Rodríguez, S. A., Huerta-Heredia, A. A., Vilegas, W. & Paniagua-Vega, D. (2020). Moisturizing and antioxidant evaluation of *Moringa oleifera* leaf extract in topical formulations by biophysical techniques. *South African Journal of Botany, 129*, 404–411. https://doi.org/10.1016/j.sajb.2019.10.011

Association of Official Analytical Chemists (AOAC). (1994). Official methods of analysis (12th ed.). Washington, DC.

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H. & Rahimi, M. (2023). Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. *Scientific Reports, 13*(1), 2895–2908. https://doi.org/10.1038/s41598-023-29954-6

Ba, K., Tine, E., Destain, J., Cissé, N. & Thonart, P. (2010). Comparative study of phenolic compounds, antioxidant capacity of different Senegalese sorghum varieties, and amylolytic enzymes of their malt. *Biotechnology, Agronomy, Society and Environnement*, *14*(1), 131–139.

Balfany, C., Gutierrez, J., Moncada, M. & Komarnytsky, S. (2023). Current status and nutritional value of green leaf protein. *Nutrients, 15*(6), 1327. https://doi.org/10.3390/nu15061327

Bancessi, A., Bancessi, Q., Baldé, A. & Catarino, L. (2020). Present and potential uses of *Moringa oleifera* as a multipurpose plant in Guinea-Bissau. *South African Journal of Botany, 129*, 206–208. https://doi.org/10.3390/plants10020259

Bates, L. S., Waldren, R. A. & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil, 39*(1), 205–207.

Batool, S., Khan, S. & Basra, S. M. A. (2020). Foliar application of *Moringa* leaf extract improves the growth of *Moringa* seedlings in winter. *South African Journal of Botany, 129*, 347–353. https://doi.org/10.1016/j.sajb.2019.08.040

Bekka, S., Tayeb-Hammani, K., Boucekkine, I., Aissiou, M. E. A. & Djazouli, Z. E. (2022). Adaptation strategies of *Moringa oleifera* under drought and salinity stresses. *Ukrainian Journal of Ecology, 12*(4), 8–16. https://cyberleninka.ru/article/n/adaptation-strategies-ofmoringa-oleifera-under-drought-and-salinity-stresses/viewer

Bernstein, N., Shoresh, M., Xu, Y. & Huang, B. (2010). Involvement of the plant antioxidative response in the differential growth sensitivity to salinity of leaves vs roots during cell development. *Free Radical Biology and Medicine, 49*(7), 1161–1171. https://doi.org/10.1016/j.freeradbiomed.2010.06.032

Beyer, W. F., Jr. & Fridovich, I. (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry, 161*(2), 559–566. https://doi.org/10.1016/0003-2697(87)90489-1

Cakmak, I., Strbac, D. & Marschner, H. (1993). Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *Journal of Experimental Botany, 44*(1), 127–132. https://doi.org/10.1093/jxb/44.1.127

Chaves, M. M., Flexas, J. & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany, 103*(4), 551–560. https://doi.org/10.1093/aob/mcn125

Cohen, I., Zandalinas, S. I., Huck, C., Fritschi, F. B. & Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum, 171*(1), 66–76. https://doi.org/10.1111/ppl.13203

Daryanto, S., Wang, L. & Jacinthe, P. A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agricultural Water Management, 179*, 18–33. https://doi.org/10.1016/j.agwat.2016.04.022

Dehpour, A. A., Ebrahimzadeh, M. A., Fazel, N. S. & Mohammad, N. S. (2009). Antioxidant activity of the methanol extract

of *Ferula assafoetida* and its essential oil composition. *Grasas y Aceites, 60*(4), 405–412. https://doi.org/10.3989/gya.010109

DI, A., MB, A. & FR, W. (1956). Photosynthesis by isolated chloroplasts. IV. General concept and comparison of three photochemical reactions. *Biochimica et Biophysica Acta, 20*(3), 449–461. https://doi.org/10.1016/0006-3002(56)90339-0

Dobra, J., Motyka, V., Dobrev, P., Malbeck, J., Prasil, I. T., Haisel, D. & Vankova, R. (2010). Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline content. *Journal of Plant Physiology, 167*(16), 1360–1370. https://doi.org/10.1016/j.jplph.2010.05.013

Fakayode, O. A., Akpan, D. E. & Ojoawo, O. O. (2019). Size characterization of *Moringa* (*Moringa oleifera*) seeds and optimization of the dehulling process. *Journal of Food Process Engineering, 42*(6), e13182. https://doi.org/10.1111/jfpe.13182

Farahat, E. A. & Refaat, A. M. (2021). Predicting the impacts of climate change on the distribution of *Moringa peregrina* (Forssk.) Fiori A conservation approach. *Journal of Mountain Science, 18*(5), 1235–1245. https://doi.org/10.1007/s11629-020-6560-y

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development,*92 185–92212. https://doi.org/10.1051/agro:2008021

Ghasemlou, F., Amiri, H., Karamian, R. & Mirzaie-asl, A. (2019). Alleviation of the effects of drought stress on Verbascum nudicuale by methyl jasmonate and titanium dioxide nanoparticles. *Iranian Journal of Plant Physiology, 9*(4), 2911–2920. https://doi.org/10.30495/ijpp.2019.668857

Goche, T., Shargie, N. G., Cummins, I., Brown, A. P., Chivasa, S. & Ngara, R. (2020). Comparative physiological and root proteome analyses of two sorghum varieties responding to water limitation. *Scientific Reports, 10*(1), 11835. https://doi.org/10.1038/s41598-02068735-3

Görgüç, A., Özer, P. & Yılmaz, F. M. (2020). Simultaneous effect of vacuum and ultrasound assisted enzymatic extraction on the recovery of plant protein and bioactive compounds from sesame bran. *Journal of Food Composition and Analysis, 87*, 103424. https://doi.org/10.1016/j.jfca.2020.103424

Hajaji, A. N. & Ammari, Y. (2021). PSII photosystem protection by soil amendment with biochar and adequate nitrogen fertilizer availability attenuate cadmium inducedphotoinhibition and phytotoxicity in Solanum lycopersicum. *Journal of Plant Science and Crop Protection, 4*(1), 1–10

Hajaji, A. N. & Gouia, H. (2019). ROS scavenging and nitrogen fertilizer roles in alleviation of Cd-induced oxidative stress in Arabidopsis thaliana. *Russian Journal of Plant Physiology, 66*(3), 495–502. https://doi.org/10.1134/S1021443719030063

Hajaji, A. N., Heikal, Y. M., Hamouda, R. A., Abassi, M. & Ammari, Y. (2024). Multivariate investigation of *Moringa oleifera* morpho-physiological and biochemical traits under various water regimes. *BMC Plant Biology, 24*(1), 505.

Harshika, M. & Abdul Ajees, A. S. (2024). Environmental, industrial, and health benefits of *Moringa oleifera*. *Phytochemistry Rev*iews, *23*, 1497–1556. https://doi.org/10.1007/s11101-024-09927-x

Hasan, M. M., Alharby, H. F., Hajar, A. S., Hakeem, K. R., Alzahrani, Y. & Hajar, A. S. (2018). Effects of magnetized water on phenolic compounds, lipid peroxidation and antioxidant activity of *Moringa* species under drought stress. *Journal of Animal & Plant Sciences, 28*(3), 803–810.

Hasan, M. M., Alharby, H. F., Uddin, M. N., Ali, M. A., Anwar, Y., Fang, X. W. & Hajar, A. S. (2020). Magnetized water confers drought stress tolerance in *Moringa* biotype via modulation of growth, gas exchange, lipid peroxidation and antioxidant activity. *Polish Journal of Environmental Studies, 29*(1), 1–10. https://doi.org/10.15244/pjoes/110347

Heath, R. L. & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics, 125,* 189–198.

Hendre, P. S., Muthemba, S., Kariba, R., Muchugi, A., Fu, Y., Chang, Y. & Jamnadass, R. (2019). African Orphan Crops Consortium (AOCC): Status of developing genomic resources for African orphan crops. *Planta, 250*, 989–1003. https://doi.org/10.1007/s00425-019-03156-9

Hsiao, T. C. & Xu, L. K. (2000). Sensitivity of growth of roots versus leaves to water stress: Biophysical analysis and relation to water transport. *Journal of Experimental Botany, 51*(350), 1595–1616. https://doi.org/10.1093/jexbot/51.350.1595

Islam, Z., Islam, S. M., Hossen, F., Mahtab-ul-Islam, K., Hasan, M. R. & Karim, R. (2021). *Moringa oleifera* is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*, *2021*(1), 1–8. https://doi.org/10.1155/2021/6627265

João, L. A. M. J., Luan, D. F. A. M., João, C. A.N., Keven, W. S. G. S., Larice, B. F, S., Vilma, M. F., Reinaldo, A. P., Jaqueline, F. O. C., Ivanildo, C. S., Priscila, C. S. & Lívia**, F. G. C.** (2022). Influence of precipitation on reproductive phenophases in *Moringa oleifera* Lam. *Australian Journal of Crop Science, 16*(06), 833–837. doi: 10.21475/ajcs.22.16.06.p3629

Keatinge, J. D. H., Ebert, A. W., Hughes, J. A., Yang, R. Y. & Curaba, J. (2017). Seeking to attain the UN‘s Sustainable Development Goal 2 worldwide: The important role of *Moringa oleifera*. *Acta Horticulturae, 1158*, 1–10. https://doi.org/10.17660/ActaHortic. 2017.1158.1

Kh, K., Mohseni, R. & Saboora, A. (2010). Biochemical changes of Rosmarinus officinalis under salt stress. *Journal of Stress Physiology & Biochemistry, 6*(3), 114–122.

Labbassi, S., Abdelghani, T., Abdelaziz, M., Naima, C., Maryem, T., Chaima, A., Khadija, B., Driss, H. & Naima, A. A. (2024). Morphological and genetic diversity of *Moringa oleifera* grown in Morocco under a semi-arid climate. *Genetic Resources and Crop Evolution, 72*(2), 1699–1715. 10.1007/s10722-024-02047-7

Li, J. W., Ding, S. D. & Ding, X. L. (2005). Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. *Process Biochemistry, 40*(11), 3607–3613. https://doi.org/10.1016/j.procbio.2005.03.005

Li, P., Zhu, Y., Song, X. & Song, F. (2020). Negative effects of long-term moderate salinity and short-term drought stress on the photosynthetic performance of Hybrid Pennisetum. *Plant Physiology and Biochemistry, 155*, 93–104. https://doi.org/10.1016/j.plaphy.2020.06.033

Liao, Z., Zeng, H., Fan, J., Zhenlin, L. Chen, Z., Fucang, Z.,

Haidong, W., Minghui, C., Jinjin, G., Zhijun, L. & Peng, W. (2022). Effects of plant density, nitrogen rate and supplemental irrigation on photosynthesis, root growth, seed yield and water-nitrogen use efficiency of soybean under ridge-furrow plastic mulching. *Agricultural Water Management, 268,* 107688. https://doi.org/10.1016/j.agwat.2022.107688

Liu, X., Pan, Y., Zhu, X., Yang, T., Bai, J. & Sun, Z. (2018). Drought evolution and its impact on the crop yield in the North China Plain. *Journal of Hydrology, 564*, 984–996. https://doi.org/10.1016/j.jhydrol.2018.07.077

Ma, K. K., Greis, M., Lu, J., Nolden, A. A., McClements, D. J. & Kinchla, A. J. (2022). Functional performance of plant proteins. *Foods, 11*(4), 594. https://doi.org/10.3390/foods11040594

Mansour, M. M. F. & Salama, K. H. A. (2019). Cellular mechanisms of plant salt tolerance. In Microorganisms in saline environments: *Strategies and functions*, 169–210. *Springer*. https://doi.org/10.1007/978-3-030-18975-4

Mashamaite, C.V., Pieterse, P.J., Mothapo, P.N. & Phiri, E.E. (2021). *Moringa oleifera* in South Africa: A review on its production, growing conditions and consumption as a food source. *South African Journal of Science, 117*(3/4), 1–7. https://doi.org/10.17159/

Miranda, M. T., Da Silva, S. F., Silveira, N. M., Pereira, L., Machado, E. C. & Ribeiro, R. V. (2021). Root osmotic adjustment and stomatal control of leaf gas exchange are dependent on citrus rootstocks under water deficit. *Journal of Plant Growth Regulation, 40*, 11–19. https://doi.org/10.1007/s00344-020-10069-5

Muhl, Q. E., Du Toit, E. S. & Robbertse, P. J. (2011). Temperature effect on seed germination and seedling growth of *Moringa oleifera* Lam. *Seed Science and Technology, 39*(1), 208–213. https://doi.org/10.15258/sst.2011.39.1.19

Nakano, Y. & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology, 22*(5), 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nazari, Z., Honarvar, M. & Dianat, M. (2021). Evaluation of the effects of extraction method, duration and harvesting time on qualitative and quantitative features of Medicago sativa. *Journal of Food Measurement and Characterization, 15*, 4868–4875. https://doi.org/10.1007/s11694-021-01056-9

Němec, P., Ungrová, M., Alem, S., Novák, J. & Habrová, H. (2020). Biomass production of a young plantation of *Moringa stenopetala* (Baker f.) Cufod. and *Moringa oleifera* Lam. in southern Ethiopia. *South African Journal of Botany, 129*, 463–470. https://doi.org/10.1016/j.sajb.2020.01.018

Nouman, W., Basra, S. M. A., Yasmeen, A., Gull, T., Hussain, S. B., Zubair, M. & Gul, R. (2014). Seed priming improves the emergence potential, growth and antioxidant system of *Moringa oleifera* under saline conditions. *Plant Growth Regulation, 73*, 267–278. https://doi.org/10.1007/s10725-014-9887-y

Nouman, W., Olson, M. E., Gull, T., Zubair, M., Basra, S. M. A., Qureshi, M. K. & Shaheen, M. (2018). Drought affects size, nutritional quality, antioxidant activities and phenolic acids pattern of *Moringa oleifera* Lam. *Journal of Applied Botany & Food Quality, 91*, 79–87.

Nynäs, A. L., Newson, W. R. & Johansson, E. (2021). Protein fractionation of green leaves as an underutilized food source—Protein yield and the effect of process parameters. *Foods, 10*(11), 2533. https://doi.org/10.3390/foods10112533

Olaborode, O. S., Gardner, C. S. & Onokpise, O. U. (2022). Growth performance of selected *Moringa oleifera* seed origins in North Florida. *Journal of Plant Science and Physiology, 155*, 93–104. https://doi.org/10.29328/journal.jpsp.1001066

Olson, M. E. & Fahey, J. W. (2011). *Moringa oleifera*: A Multipurpose Tree for the Dry Tropics. *Revista Mexicana de Biodiversidad,* 82(4), 1071–1082. https://doi.org/10.22201/ib.20078706e.2011.4.678

Omari Alzahrani, F. (2021). Metabolic engineering of osmoprotectants to elucidate the mechanism(s) of salt stress tolerance in crop plants. *Planta, 253*(1), 1–17. https://doi.org/10.1007/s00425-020-03550-8

Osakabe, K., Wada, N., Miyaji, T., Murakami, E., Marui, K., Ueta, R. & Osakabe, Y. (2020). Genome editing in plants using CRISPR type I-D nuclease. *Communications Biology, 3*(1), 648. https://doi.org/10.1038/s42003-020-01366-6

Osman, A. R. & El-Naggar, H. M. (2022). Enhancing salinity stress tolerance and phenylalanine ammonia lyase gene activity using osmolytes in *Moringa* seedling production. *Annals of Agricultural Sciences, 67*(1), 127–135. https://doi.org/10.1016/j.aoas.2022.06.002

Palada, M. C. & Chang, L. C. (2003). Suggested cultural practices for *Moringa*. AVRDC International Cooperators' Guide. AVRDC Publication No. 03-545.

Patterson, B. D., MacRae, E. A. & Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochemistry, 139*(2), 487–492. https://doi.org/10.1016/0003-2697(84)90039-3

Rao, M. V., Paliyath, G. & Ormrod, D. P. (1996). Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of Arabidopsis thaliana. *Plant Physiology, 110*(1), 125–136. https://doi.org/10.1104/pp.110.1.125

Raven, J. A. (2013). Rubisco: Still the most abundant protein of Earth? *New Phytologist, 198*(1), 1–3.

Rivas, R., Oliveira, M. T. & Santos, M. G. (2013). Three cycles of water deficit from seed to young plants of *Moringa* oleifera woody species improves stress tolerance. *Plant Physiology and Biochemistry, 63*, 200–208. https://doi.org/10.1016/j.plaphy.2012.11.026

Salama, A., Hasanin, M. & Hesemann, P. (2020). Synthesis and antimicrobial properties of new chitosan derivatives containing guanidinium groups. *Carbohydrate Polymers, 241*, 116363. https://doi.org/10.1016/j.carbpol.2020.116363

Santoso, B. B. & Parwata, I. A. (2020). The Growth of *Moringa* Seedling Originated from Various Sizes of Stem Cutting. *International Conference Earth Science & Energy, Earth and Environmental Science, 519,* 012010. doi:10.1088/1755-1315/519/1/012010

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y. & Battaglia, M. L. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants, 10*(2), 259. https://doi.org/10.3390/plants10020259

Sunderwirth, S. G., Olson, G. G. & Johnson, G. (1964). Paper

chromatography-anthrone determination of sugars. *Journal of Chromatography A, 16*, 176–180. https://doi.org/10.1016/S0021-9673(01)82452-3

Swarup, S., Cargill, E. J., Crosby, K., Flagel, L., Kniskern, J. & Glenn, K. C. (2020). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science, 61*(2), 839–852. https://doi.org/10.1002/csc2.20377

Taha, S. R., Seleiman, M. F., Alhammad, B. A., Alkahtani, J., Alwahibi, M. S. & Mahdi, A. H. (2021). Activated yeast extract enhances growth, anatomical structure, and productivity of *Lupinus termis* L. plants under actual salinity conditions. *Agronomy, 11*(1), 74. https://doi.org/10.3390/agronomy11010074

Trigo, C., Castello, M. L., Ortola, M. D., Garcia-Mares, F. J. & Soriano, M. D. (2021). *Moringa oleifera*: An unknown crop in developed countries with great potential for industry and adapted to climate change. *Foods, 10*(1), 31. https://doi.org/10.3390/foods10010031

Wang, F., Bao, Y. F., Si, J. J., Duan, Y., Weng, Z. B. & Shen, X. C. (2019). The beneficial effects of a polysaccharide from *Moringa oleifera* leaf on gut microecology in mice. *Journal of Medicinal Food, 22*(9), 907–918. https://doi.org/10.1089/jmf.2018.4382

Wang, L., Wang, X. & Yin, S. (2016). Effects of salinity change on two superoxide dismutases (SODs) in juvenile marbled eel *Anguilla marmorata*. *Peer J., 4*, e2149. https://doi.org/10.7717/peerj.2149

Xi, Y., Kong, F. & Chi, Z. (2021). ROS Induce β-Carotene Biosynthesis Caused by Changes of Photosynthesis Efficiency and Energy Metabolism in *Dunaliella salina* Under Stress Conditions. *Frontiers in Bioengineering and Biotechnology*, 8, 613768. https://doi.org/10.3389/fbioe.2020.613768

Zanella, L., Gismondi, A., Di Marco, G., Braglia, R., Scuderi, F., Redi, E. L. & Canini, A. (2019). Induction of antioxidant metabolites in *Moringa oleifera* callus by abiotic stresses. *Journal of Natural Products, 82*(9), 2379–2386. https://doi.org/10.1021/acs.jnatprod.8b00801

Received: **April, 01, 2025**; *Approved*: **June, 06, 2025**; *Published*: **October, 2026**