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Moringa oleifera Lam. Seed Extract Enhances Tolerance to Drought Stress by Regulating Photosynthesis and Antioxidant Defense Mechanism in *Lessertia frutescens* L.

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Abstract. **Drought is a predominant environmental stress that limits plant growth and yield. Biostimulants including moringa (*Moringa oleifera* Lam.) seed extract (MSE) can alleviate adverse plant responses triggered by drought stress. Nonetheless, there is limited information regarding the functions of MSE in promoting drought tolerance in plants. Consequently, the current study investigated the effect of MSE on the enhancement of drought tolerance in cancer bush (*Lessertia frutescens* L.) plants under drought stress. The 6% MSE foliar spray was applied to cancer bush plants subject to standard [80% of soil water-holding capacity (SWHC)] and drought stress (60% of SWHC) in a terracotta pot experiment that was conducted twice sequentially in a tunnel. Plants that were not treated with MSE were used as control. The application of MSE effectively alleviated the adverse effect of drought stress on cancer bush by improving plant growth and yield characteristics, photosynthesis attributes, soluble protein, and free proline contents. The MSE mitigated lipid oxidation (malondialdehyde) of drought affected plants and enhanced the antioxidant enzyme activities. These results demonstrated that MSE application effectively alleviated drought stress in cancer bush plants. Therefore, MSE is an economical and eco-friendly biostimulant for enhancing plant performances under drought stress.**

Abiotic stresses including drought, salinity, and extreme low or high temperatures significantly limit agricultural productivity by reducing crop quality and yield by more than 50% (Campobenedetto et al. 2021; Francesca et al. 2021; Tinte et al. 2022). Recently, droughts have led in an economic loss of ~30 billion US dollars globally (Gupta et al. 2020; Repke et al. 2022). Drought stress can affect crop production by triggering changes in the plant's physiological, biochemical, and molecular processes (Goyal et al. 2023; Tinte et al. 2022). Furthermore, drought adversely affects photosynthesis by damaging chloroplasts, restricting the electron transfer, and generating cellular oxidative stress (Banerjee and Roychoudhury 2019; Petropoulos et al. 2020). Ultimately, this leads to crop yield loss, as well as plant death or failure (Ahmad et al. 2021; El Boukhari et al. 2023).

Drought promotes overproduction of reactive oxygen species (ROS) primarily superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) in cell organelles (Pinciroli et al. 2018; Tinte et al. 2022), leading to the initiation of oxidative stress through the peroxidation of cellular membranes (Desoky et al. 2021a) and the deterioration of cell structures including proteins, nucleic acids, membrane lipids, and photosynthetic pigments and activating programmed cell death (Desoky et al. 2021b). Plants adapt to drought through enzymatic and nonenzymatic antioxidant mechanisms that involve morphological, physiological, and biochemical changes that eliminate ROS-induced oxidative stress (Babaei et al. 2021; Goyal et al. 2023).

During drought conditions, plants can accumulate osmoprotective molecules including proline and other amino acids, soluble sugars, and essential minerals, which function to decrease cellular osmotic potential, thus contributing to preservation of water absorption and cell turgor (Annunziata et al. 2017; Antonucci et al. 2021). Another mechanism of plants to cope with drought comprises reduction of water loss through regulation of stomatal closure and production of small leaves, enhancing water uptake by promoting the root system growth and accumulating osmoprotective substances (Francesca et al. 2021; Amiri Forotaghe et al. 2022). Furthermore, recovering from stress has also been regarded as a drought resistance response strategy for the plant, allowing vital metabolic functions such as transpiration and photosynthesis to resume (Desoky et al. 2021a, 2021b; Goyal et al. 2023).

In addition to adaptation to drought stress, activation of the antioxidant capacity is a vital mechanism to scavenge excessive ROS and reduce ROS-induced damages that involves antioxidant enzymes and nonenzymatic antioxidants including phenolic compounds, carotenoids, ascorbic acid, and glutathione (Amir et al. 2021; Amiri Forotaghe et al. 2022). Synthetic fertilizers, which are expensive, toxic, and cause environmental pollution, have been effectively used worldwide to improve plant productivity and help plants cope with the negative effect of drought (Bibi and Rahman 2023; Piyasena and Hettiarachchi 2023). Although chemical fertilizers are an effective input to get higher crop productivity under water deficiency conditions, excessive application of synthetic fertilizers is associated with soil compaction and acidification, heavy metals pollution, and changes in soil microbiome (Duddigan et al. 2023; Piyasena and Hettiarachchi 2023). The application of synthetic fertilizers over the long term alters the bacterial composition of soil and decreases soil pH and microbial metabolic activity, leading to the decrease of beneficial bacteria (Fincheira et al. 2021; Piyasena and Hettiarachchi 2023). In addition, the overuse of inorganic fertilizers causes emissions and water contamination and reduces crop yields over time (Seleiman et al. 2020; Duddigan et al. 2023). Therefore, the modern agricultural sector needs to explore more environmentally friendly techniques that promotes agricultural sustainability and plant yield.

The environmentally friendly alternative to enhance crop yield during drought stress is the application of plant growth regulators and stress alleviators such as biostimulants (Goñi et al. 2018; Tinte et al. 2022). Biostimulants are involved in various processes including membrane stability, stomatal conductance, osmoprotection, ROS scavenging, nutrient and water use efficiency, and phytohormonal signalling (Ali et al. 2021; García-Sánchez et al. 2022) and activate stress responsive genes by the signal transduction and transcriptional regulations (Monteiro et al. 2022; Arif et al. 2023). Therefore, the application of biostimulants can alleviate the adverse effect of drought stress and improve plant growth and productivity (Arif et al. 2023; Gupta et al. 2023).

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Among natural biostimulants, moringa (*Moringa oleifera* Lam.) leaf extract (MLE) is an important biostimulator due to the presence of essential mineral nutrients, vitamins, antioxidants, and phytohormones that function to facilitate plant growth and development (Arif et al. 2023; Zulfiqar et al. 2020). The presence of nutrients such as calcium and potassium in MLE may improve plant growth and development through enzyme activation, osmoregulation, and photosynthesis (Rana et al. 2019; Zulfiqar et al. 2020). Antioxidants and phytohormones in MLE effectively decrease environmental stresses in plants including salinity, drought, and heat stress by promoting the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX), which aid in scavenging ROS and enhancing plant growth and yield attributes (Arif et al. 2023; Latif and Mohamed 2016; Zulfiqar et al. 2020). Previous studies have showed that MLE (2% to 6%) improved the growth and productivity of different plants such as squash (*Cucurbita pepo* L.) (Abd El-Mageed et al. 2017), pepper (*Capsicum annuum* L.) (Hala and Nabila 2017), maize (*Zea mays* L.) (Maswada et al. 2018), and strawberry (*Fragaria ×ananassa* Duch.) (Ismail and Ganzour 2021) subjected to environmental stress such as drought and salinity. These findings demonstrate that plant-derived biostimulants such as MLE may be an effective technique to enhance plant drought tolerance. Although all plant parts of moringa such as roots, leaves, flowers, fruit, pods, and seeds have high nutrient value due to the presence of essential phytochemicals and phytohormones (Gopalakrishnan et al. 2016; Ismail and Ganzour 2021), limited previous studies mainly investigated the biostimulant potential of moringa leaves; thus, little is known about the effect of moringa seeds on abiotic stress tolerance and growth of medicinal plants. Therefore, there is a need for future studies to investigate biostimulant potential of other plant parts of moringa on the growth and productivity of medicinal plants subjected to abiotic stress conditions.

Cancer bush [*Lessertia frutescens* L. (syn. *Sutherlandia frutescens* L.)] is an important medicinal plant native to South Africa (Govender et al. 2023). The leaves of cancer bush comprise a diversity of bioactive compounds including pinitol, triterpenoid saponins, flavonoids, and amino acids (Avula et al. 2010; Govender et al. 2023), to which several pharmacological activities such as analgesic, anti-inflammatory, hypoglycemic, and anticonvulsant activities have been ascribed (Avula et al. 2010; Nsibanyoni et al. 2023). For over 200 years, cancer bush has been widely used in traditional African medicine to treat various diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome, diabetes, cancer, tuberculosis, osteochondrosis, influenza, viral hepatitis, asthma and chronic bronchitis, rheumatoid arthritis, and hot flashes and irritability in menopause (Hlongwane et al. 2023; Nosov et al. 2023). Commercial plantations of cancer bush were established in the late 1990s in South Africa, particularly at Sanniesh in the North West Province (Hlongwane et al. 2023; Van Wyk 2011). The first branded products were tablets made from powdered leaves of a selected chemotype that became known as the SU1 type (Govender et al. 2023; Hlongwane et al. 2023); thus, cancer bush is a valuable medicinal plant in both modern and traditional medicine. However, the growth of cancer bush is mostly limited by drought stress, which extensively inhibits plant growth and yield, resulting to economic loses. Although the role of MLE biostimulant in enhancing the growth and yield of plants have been documented, little is known regarding the effect of moringa seed extracts (MSEs) on drought stress tolerance and productivity of medicinal plants. Also, little is known about the effect of plant-derived biostimulants on antioxidant defense mechanisms of medicinal plants. The MSE will have a positive effect on plant performance due to its antioxidants and phytohormones components; thus, this study investigated the effect of MSE on drought stress tolerance and productivity of cancer bush plants.

Materials and Methods

Plant growth conditions. This experiment was conducted under conditions similar to our previous studies (Buthelezi et al. 2022, 2023, 2024). It was carried out twice sequentially in the tunnel at Sefako Makgatho Health Sciences University, South Africa (25°37′8″S, 28°1′22″E), during the winter to summer seasons of 2021. Plants were grown in the tunnel that was 4 m high, 8 m long, and 4 m wide covered with a green colored net with 40% shading [ChromatiNet™; Carports and Pergola Builders (Pty) Ltd., Pretoria, South Africa]. During the experiment, the average temperature and relative humidity in the tunnel were 21.08 °C and 43.01%, correspondingly.

About 150 seedlings of cancer bush were purchased from the Plantland Akasia Garden Centre Nursery in Pretoria, South Africa (25°39′41.2″S, 28°04′34.5″E). The seedlings were then transported and kept in the tunnel at Sefako Makgatho Health Sciences University, where they were watered three times a day with an average of 200 mL of tap water per plant for a week. Afterward, healthy seedlings of about 30 cm long were transplanted into plastic terracotta pots (40 cm in diameter and 50 cm depth), containing 5 kg of Culterra potting soil per pot.

The Culterra potting soil used in the current study was obtained from Builders Express, Pretoria, South Africa. This product is composed of raw organic material such as general 2:3:2 (22), lawn 8:1:5 (25), limestone ammonium nitrate (28%), vita flora 5:1:5 (33) slow release nitrogen, and vital flora 3:1:5 (26) slow release nitrogen per 30 kg of soil (pH 7.7 and EC 2.01 $dS \cdot m^{-1}$) (Buthelezi et al. 2023). The soil also had total porosity of 48.90% and infiltration rate of 30.54 $mm \cdot h^{-1}$ and consisted of ammonium sulfate (21%), available potassium (120.32 $mg \cdot kg^{-1}$), available phosphorus (15.32 $mg \cdot kg^{-1}$), and total nitrogen (1.98 $mg \cdot kg^{-1}$).

Irrigation water applied. Soil water-holding capacity (SWHC) was evaluated according to Taha et al. (2020) using Eq. [1] as follows:

$$SWHC\ (\%) = total\ porosity\ (\%) - air\ space\ (\%) \quad [1]$$

Two irrigation treatments were used during the experiment after transplanting: 80% (160 mL of irrigation water), which was calculated as the ideal irrigation water for optimum growth of cancer bush plants, and 60% (120 mL of irrigation water) of SWHC. All pots were well irrigated with 200 mL of tap water before transplanting. The ideal irrigation treatment (80% of SWHC) was used as a control, whereas 60% of SWHC was regarded as a drought stress treatment. In our preliminary study (data not shown), water deficiency in 40% of SWHC significantly reduced growth and yield of cancer bush even when treated with MSE. The drought stress levels were applied to cancer bush plants three times a day. Culterra potting soil moisture content per terracotta pots was observed daily (HH2 moisture meter, version 4.0; Delta-T Devices Ltd., Cambridge, UK) and maintained through water application where applicable.

Moringa seed extract preparation and treatments. Moringa seeds were harvested in bulk from commercial orchards of Afrinest Moringa Farm in Tzaneen, Limpopo, South Africa (23°49′15.3″S, 30°10′08.7″E). The harvested seeds were then ground into fine powder using a blender (Mellerware Fusion 1L 300 W; Makro, Pretoria, South Africa) and extracted according to El-Sappah et al. (2023). Moringa seed powder (100 g) was mixed with 1 L of 80% aqueous ethanol and vortexed (Oxford LP Benchmate digital vortex mixer, VM-D; Selectech Laboratory Equipment Supplier, Pretoria, South Africa) for 1 min. Afterward, the mixture was kept at ambient conditions overnight. Then, the solution was centrifuged (laboratory centrifuge, TD4C; Labtex Co., Ltd., Bangladesh, South Asia) at 8000 g_n for 20 min and filtered twice using Whatman® no. 1 filter paper and then with nonabsorbent cotton. Consequently, the supernatant was mixed with distilled water (v/v) to attain the desired extract at 6% MSE to use as a foliar spray. In our preliminary study (data not shown), 6% and 8% MSE successfully enhanced plant growth and development of cancer bush with no and little significant differences compared with 2 and 4% MSE. Freshly prepared extract was then used within 24 h or kept in the refrigerator at −20 °C for further use. About 200 to 500 mL of 6% MSE foliar spray, depending on the plant growth stage, was applied to a single cancer bush plant/pot/irrigation level per week. Untreated plants, which were considered to be control, were sprayed with tap water at the same time of MSE treatment.

Experimental design and plant management. The two experiments were each conducted on 50 individual plants using a randomized complete block design. Plant pots were set up with an intrarow pot of 40 cm and

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interrow spacing of 50 cm. The treatments of this experiment were irrigation with 80% of SWHC + foliar spray with distilled water (control), irrigation with 80% of SWHC + foliar spray with MSE application, irrigation with 60% of SWHC + foliar spray with distilled water (control), and irrigation with 60% of SWHC + foliar spray with MSE. Overall, each treatment had 25 pot plants, making a total of 100 pots plants of the entire experiment. The MSE foliar pray was applied to cancer bush plants at 7 d intervals during the 10 weeks of the experiment.

Measurements of plant growth, ornamental, and root morphology characteristics. At harvest (10 weeks after transplanting), the growing medium (Culterra potting soil) was carefully washed from the roots, and the plants were divided into stem, leaves, flowers, and roots. Five plants per treatment were used to determine shoots and roots fresh weight using a precision balance [ME3002T; Labotec (Pty) Ltd., Johannesburg, South Africa]. Afterward, these were oven dried (DON-H; Bioevopeak, Co., Ltd., Shandong, China) at 70 °C until a constant weight was attained (dry weight). The number of inflorescences was counted. Fruit yield attributes such as fruit number, individual fruit mass, and overall fruit yield per plant were also recorded. Root scanning was performed following a method of Ertani et al. (2018), with minor adjustments. Fresh root systems were gently washed with tap water after harvest, placed on a transparent tray, and scanned before the sampling process with a root scanner (Epson Expression 10000XL 1.0 system; Regent Instruments Inc., Québec, Canada) to measure total root length, average diameter, volume, tips, forks, crossings, projected area, and surface area. The roots of three plants were scanned per replicate and treatment.

Photosynthetic parameters quantification. Leaf gas exchanges based on the CO_2 net assimilation rate (A), stomatal conductance (Gs), transpiratory rate (E), and intercellular CO_2 concentration (Ci) were performed on fully expanded leaves (2 to 6 weeks after transplanting) using an infrared gas analyzer (portable model LI-6400xt; Thermo Fisher Scientific Inc., Johannesburg, South Africa). Measurements were taken between 9:00 and 11:30 AM with constant photosynthetically active radiation (1000 μmol photons $m^{-2}{\cdot}s^{-1}$), atmospheric CO_2 concentration, room temperature, and humidity (Agliassa et al. 2021; Kałużewicz et al. 2017). These data were used to calculate water use efficiency (WUE = A/E) and carboxylation efficiency (CE = A/Ci) (Agliassaet al. 2021).

Determination of soluble protein. The total soluble protein concentration was determined according to Contartese et al. (2015). Briefly, 1 g of fresh leaf sample was extracted in 5 mL of 50 mM Tris-HCl buffer [pH 7.4; containing 0.2 M NaCl, 20 mM $MgSO_4$, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM β-mercaptoethanol, 0.5 mM phenylmethylsulfonic fluoride, 10 mM leupeptin, and 10% (v/v) glycerol]. The mixture was allowed to stand on ice for 10 min and then centrifuged at 20,000 g_n for 15 min. The supernatant was filtered through Miracloth® and then used for a Bradford (1976) assay, with bovine serum albumin as a standard. Bradford dye reagent was prepared by mixing the dye concentrate with distilled water 1:4. Afterward, 1 mL of sample extract was added to 0.1 mL of water and 2 mL of Bradford reagent in a test tube, mixed, and incubated at ambient temperature for 3 min. The absorbance was read at 595 nm using a spectrophotometer (UV-2700; Shimadzu, Kyoto, Japan).

Determination of free proline. Free proline was assessed according to Agliassa et al. (2021) and Hamedeh et al. (2022). Ground leaf tissue (0.05 g) was homogenized in 0.5 mL of ethanol (v/v) and incubated overnight at 4 °C in the dark. The homogenates were centrifuged [FC5706 230 V, United Scientific (Pty) Ltd., Johannesburg, South Africa] at 20,000 g_n for 15 min at 4 °C. Samples of 100 μL of the extract were added to 200 μL of acidic ninhydrin solution, and the mixture was incubated at 100 °C for 1 h. After cooling at ambient temperature, the absorbance was measured at 520 nm using a spectrophotometer (UV-2700). After preparing a calibration curve standard curve with L-proline (Sigma-Aldrich, Johannesburg, South Africa), free proline content is expressed as $mg{\cdot}g^{-1}$ dry weight (Contartese et al. 2015).

Determination of lipid peroxidation. Lipid peroxidation was measured by the quantification of malondialdehyde (MDA) in leaf tissues. The MDA concentration was analyzed by the reaction with thiobarbituric acid (Jacomassi et al. 2022). Briefly, the MDA was extracted with 10% trichloroacetic acid, and then the extract was mixed with 0.6% thiobarbituric acid and centrifuged at 15,000 g_n at 4 °C for 25 min. Afterward, 0.5 mL aliquots of the supernatants were added to 1.5 mL of 0.5% thiobarbituric acid solution in 20% trichloroacetic acid and incubated at 90 °C in a shacking water bath (Monochrome LCD; Thermo Fisher Scientific, Johannesburg, South Africa) for 30 min, and the reaction was stopped in an ice bath (17763; Sigma-Aldrich) for 15 min. Thereafter, the samples were centrifuged at 10,000 g_n for 5 min, and the absorbance was read at 532 and 600 nm. The MDA concentration was measured by subtracting the nonspecific absorption 600 nm from the absorption at 532 nm. The value for nonspecific absorption at 600 nm was subtracted using an absorbance and absorptivity coefficient of 155 $mM^{-1}{\cdot}cm^{-1}$. The results are expressed in MDA $nmol{\cdot}g^{-1}$ fresh weight.

Determination of antioxidant enzyme activities. To measure the activities of antioxidant enzymes, the total proteins were extracted using a method of Rahimi et al. (2022), with some alterations. Briefly, 0.3 g samples of leaf tissue were macerated in liquid nitrogen, homogenized with 0.1 M potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 1 mM phenylmethylsulfonic fluoride, and 1% (w/v) polyvinylpyrrolidone for extraction of the enzymes SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POX (EC 1.11.1), and APX (EC 1.11.1.11). The mixture was centrifuged for 20 min at 12,000 g_n at 4 °C, and the supernatant was used as crude enzyme extract.

Superoxide dismutase activity. SOD (EC 1.15.1.1) activity was evaluated using the method outlined by Repke et al. (2022) and Rahimi et al. (2022) with slight modifications. Briefly, SOD activity was determined in a reaction medium consisting of 50 mM sodium phosphate buffer (pH 7.8) containing 13 mM methionine, 75 μM *p*-nitro blue tetrazolium, 0.1 mM EDTA, and 2 μM riboflavin together with the enzyme extract. The absorbance was detected at 560 nm (UV-2700) after 15 min of light exposure (4000 lx). One unit of total SOD activity was calculated as the amount of protein per milligram causing 50% inhibition of *p*-nitro blue tetrazolium reduction. Enzymatic activity results are expressed as $U{\cdot}mg^{-1}$ proteins.

Catalase activity. CAT (EC 1.11.1.6) activity was determined according to the decomposition of H_2O_2 using the method of Rahimi et al. (2022), with some alterations. Briefly, an aliquot of 50 μL of crude extract was added to 950 μL of reaction medium containing 50 mM sodium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2. The absorbance was obtained at 240 nm using a spectrophotometer (UV-2700) after 1 min. CAT activity was calculated as μmol of decomposed H_2O_2 per minute. Enzymatic activity results are expressed as nmol H_2O_2 $min^{-1}{\cdot}mg^{-1}$ proteins.

Peroxidase activity. POX (EC 1.11.1) activity was measured in aliquots of crude extract added to the reaction medium consisting of 25 mM potassium phosphate buffer (pH 6.8), 20 mM pyrogallol, and 20 mM H_2O_2. The production of purpurogallin was measured at 420 nm using a spectrophotometer (UV-2700). Enzyme activity was calculated using the absorbance and molar extinction coefficient of 2.47 $mM^{-1}{\cdot}cm^{-1}$ (Akensous et al. 2022; Panda et al. 2003) and is expressed as μmol of purpurogaline $min^{-1}{\cdot}mg^{-1}$ protein.

Ascorbate peroxidase activity. APX (EC 1.11.1.11) activity was determined according to Akensous et al. (2022), with slight modifications. An aliquot of 100 μL of crude extract was added to 900 μL of reaction medium, consisting of 0.05 M sodium phosphate buffer (pH 7.0), 0.8 mM ascorbic acid, and 1.0 mM H_2O_2. Enzymatic activity was measured at 290 nm using a spectrophotometer (UV-2700), considering the molar extinction coefficient of 2.8 $Mm^{-1}{\cdot}cm^{-1}$. The results are expressed as μmol of ascorbic acid $min^{-1}{\cdot}mg^{-1}$ protein.

Statistical analysis. The collected data were subjected to analysis of variance using GenStat® statistical software (18.1 edition; VSN International, Hemel Hempstead, UK). Fischer's least significant differences were calculated and used to separate means at 5% significance level.

Results

Plant growth and yield characteristics. The foliar application of MSE under ideal

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irrigation (80% of SWHC) significantly ($P < 0.05$) improved all measured growth and yield parameters of cancer bush plants compared with the corresponding control (Fig. 1). Drought stress (60% of SWHC) significantly ($P < 0.05$) decreased shoot fresh weight, shoot dry weight, number of inflorescences per plant, and number of fruit per plant compared with normal irrigation (80% of SWHC). Individual fruit mass per plant and overall yield per plant of plants under both 80 and 60% of SWHC were not statistically significant. The application of MSE effectively ($P < 0.05$) increased shoot fresh weight, shoot dry weight, and number of inflorescences per plant of drought-stressed plants compared with the corresponding controls. Although not statistically significant, the number of fruit per plant, individual fruit mass per plant, and overall yield per plant of drought-stressed plants treated with MSE were improved compared with respective controls. As shown in Fig. 1, MSE significantly ($P < 0.05$) increased shoot dry and fresh weight, root fresh and dry weight, number of inflorescences per plant, number of fruit per plant, individual fruit mass per plant, and overall yield per plant of drought-stressed plants by 50.04%, 60.75%, 30.29%, 74.22%, 36.22%, 23.44%, 36.98%, and 19.70%, respectively, in comparison with corresponding controls. In addition, Fig. 2 shows that roots parameters such as total length, volume, tips, crossings, forks, and fresh and dry weight of drought-stressed plants treated with MSE were increased by 49.45%, 35.81%, 43.19%, 41.47%, 72.76%, 30.29%, and 82.03%, respectively compared with corresponding controls. Although not statistically significant, the root diameters of drought-stressed plants treated with MSE were increased by 37.27% compared with control. The positive effect of MSE treatment was more evident under drought stress than its application under normal irrigation and yielded minor to no significant differences between most evaluated traits of ideal-irrigated plants and those of drought-stressed plants treated with MSE.

Photosynthetic parameters. Well-watered (80% of SWHC) cancer bush plants treated with MSE had effectively ($P < 0.05$) improved Gs, E, and CE in comparison with control (Fig. 3). Well-irrigated (80% of SWHC) cancer bush plants treated with MSE had lower Ci than control. Although not statistically significant, Fig. 2A and 2F shows that plants treated with 80% SWHC + MSE treatment had improved A (16.91 μmol CO_2 $m^{-2}{\cdot}s^{-1}$) and WUE (5.12 mmol CO_2 H_2O^{-1}) compared with corresponding controls (80% SWHC) (15.81 μmol CO_2 $m^{-2}{\cdot}s^{-1}$ and 4.25 mmol CO_2 H_2O^{-1}, respectively). Nevertheless, drought stress (60% of SWHC) treatment significantly decreased A, Gs, E, CE, and WUE and increased Ci compared with well-irrigated plants (80% of SWHC). However, MSE treatment significantly ($P < 0.05$) enhanced A, Gs, E, CE, and WUE and decreased Ci of drought-stressed plants compared with the corresponding controls. The A, Gs, E, CE, and WUE were increased by 56.81%, 92.31%, 31.96%, 99.59%, and 84.62%, respectively, and Ci was decreased by 30.02% compared with corresponding controls. Overall, the application of MSE was effective in improving photosynthetic parameters including A, Gs, E, CE, and WUE and decreasing Ci of cancer bush plants, which was demonstrated more under drought stress than under normal irrigation. In addition, there were no significant differences between all assessed parameters of well-irrigated plants and those of drought-stressed plants treated with MSE.

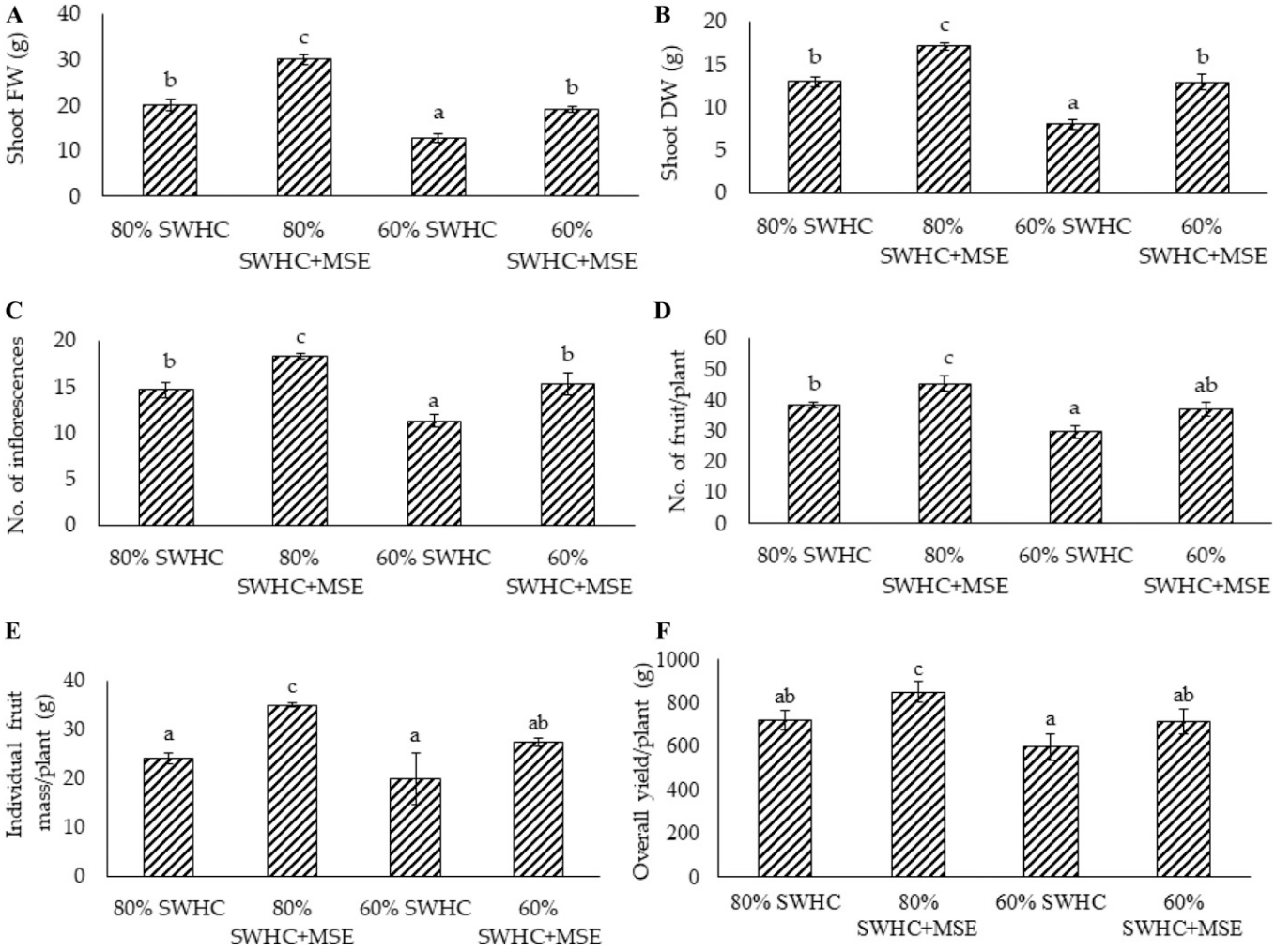

Fig. 1. Effect of foliar moringa seed extract (MSE) application on morphological growth and yield attributes of cancer bush plants under drought stress. (**A**) Shoot fresh weight (FW). (**B**) Shoot dry weight (DW). (**C**) Number of inflorescences per plant. (**F**) Number of fruit per plant. (**E**) Individual fruit mass per plant. (**F**) Overall yield per plant. Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC = soil water-holding capacity.

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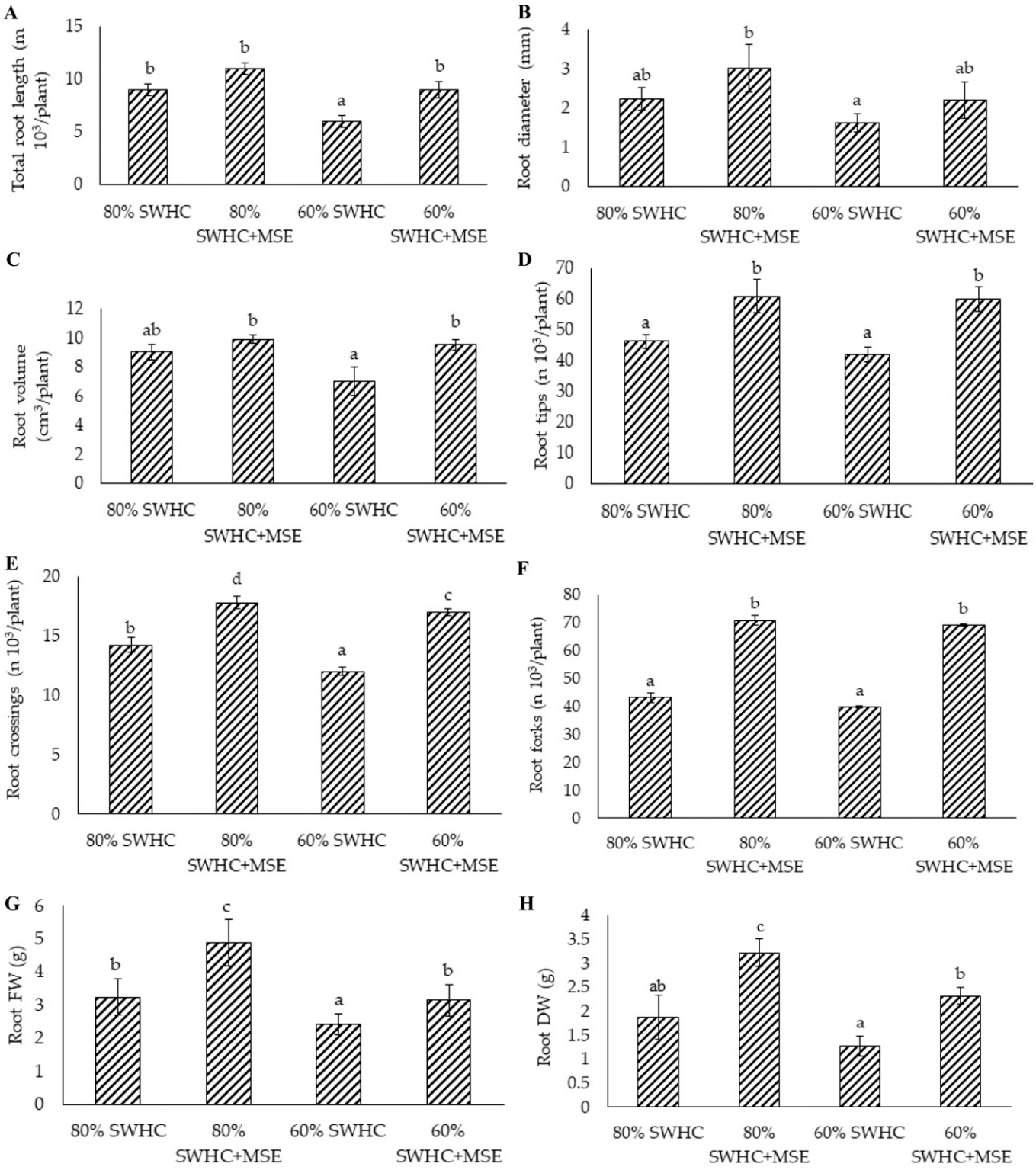

Fig. 2. Effect of foliar moringa seed extract (MSE) application on roots attributes of cancer bush plants under drought stress. (**A**) Total root length. (**B**) Root diameter. (**C**) Root volume. (**D**) Root tips. (**E**) Root crossings. (**F**) Root forks. (**G**) Root fresh weight (FW). (**H**) Root dry weight (DW). Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC = soil water-holding capacity.

Soluble protein and free proline concentration. Although not statistically significant, MSE induced the accumulation of soluble protein content, which was increased by 7.45% and significantly ($P < 0.05$) increased free proline content by 49.38% in well-watered (80% SWHC) cancer bush plants compared with corresponding controls (Fig. 4). Subjecting plants to drought stress (60% of SWHC) significantly reduced the above-mentioned parameters in comparison with well-irrigated (80% of SWHC) plants. However, MSE effectively ($P < 0.05$) increased the soluble protein (31.88%) and free proline (66.67%) of drought-stressed plants in comparison with corresponding controls. The effectiveness of MSE was more apparent under drought stress than under ideal irrigation. This is further supported by no significant differences between the contents of soluble protein and free proline of well-irrigated plants and that of drought-stressed plants treated with MSE.

Lipid peroxidation. As shown in Fig. 5, the application of MSE under ideal irrigation (80% of SWHC) significantly ($P < 0.05$) reduced MDA concentration of cancer bush plants compared with control. The MDA

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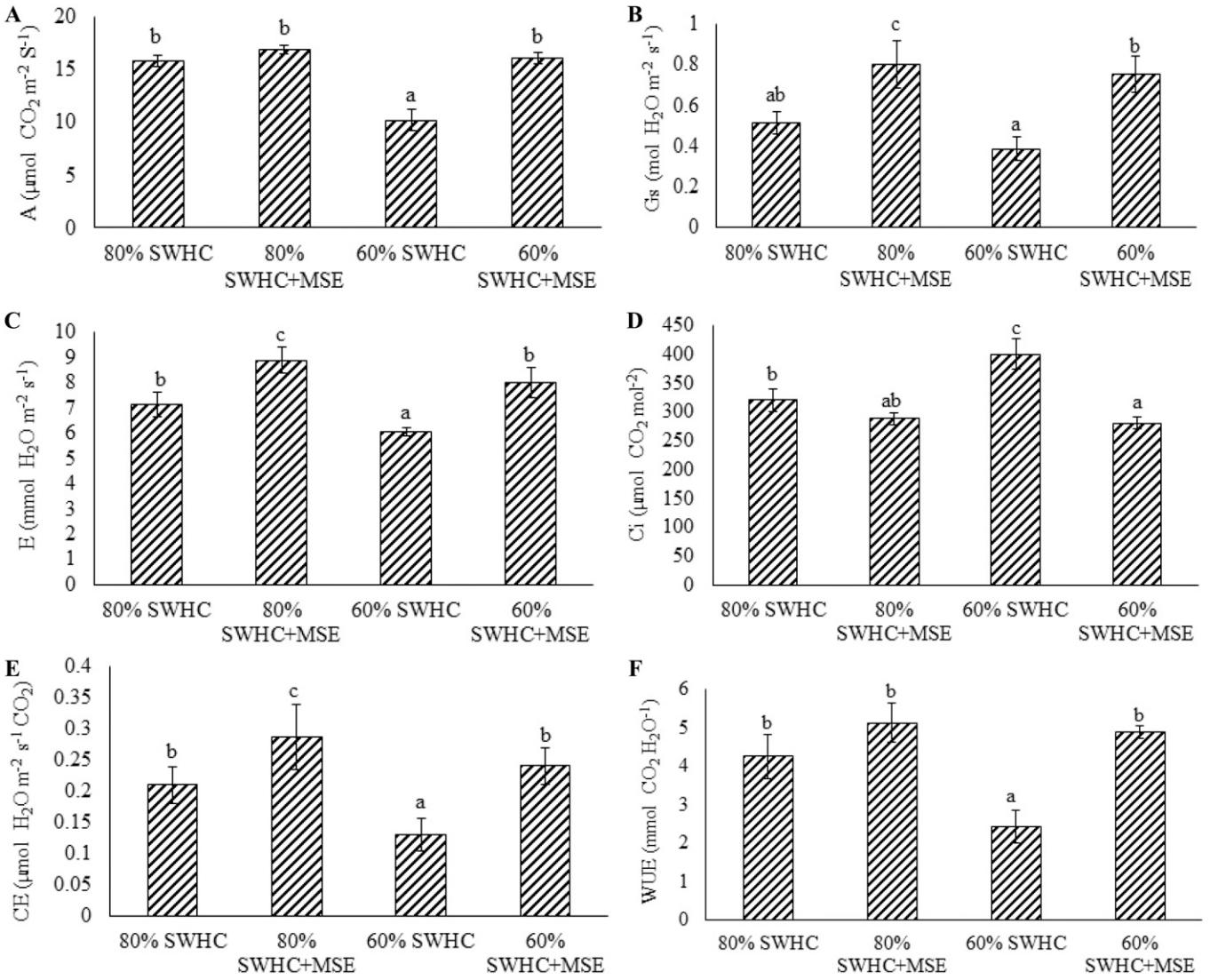

Fig. 3. Effect of foliar moringa seed extract (MSE) application on photosynthetic parameters of cancer bush plants under drought stress. (**A**) CO_2 net assimilation rate (A). (**B**) Stomatal conductance (*Gs*). (**C**) transpiratory rate (*E*). (**D**) Intercellular CO_2 concentration (Ci). (**E**) Carboxylation efficiency (CE). (**F**) Water use efficiency (WUE). Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC = soil water-holding capacity.

content was reduced by 48.75% compared with control. In addition, MSE treatment under drought stress (60% SWHC) further reduced the MDA content of cancer bush plants by 62.28% compared with control. Overall, plants that were well-irrigated (80% of SWHC), as well as those who were drought stressed (60% of SWHC), had high MDA content, whereas MSE significantly ($P < 0.05$) reduced MDA content of plants that were well irrigated and those that were drought stressed. The positive effect of MSE foliar application was more noticeable under drought stress than under ideal irrigation, with no or slight significant differences between well-irrigated plants and drought-stressed plants treated with MSE.

Antioxidants enzyme activities. Although not statistically significant, an enhancement of SOD and significantly ($P < 0.05$) higher activities of CAT, POX, and APX were observed in well-irrigated (80% of SWHC) MSE-treated plants, which were increased by 14.53%, 36.79%, 95.68%, and 29.58% in comparison with respective controls (Fig. 6). However, drought stress (60% of SWHC) significantly ($P < 0.05$) reduced all the above-mentioned antioxidant enzyme activities compared with well-irrigated (80% of SWHC) plants. The foliar application of MSE had a positive effect on SOD, CAT, POX, and APX activities of drought-stressed plants, which were significantly ($P < 0.05$) increased by 16.54%, 21.33%, 73.13%, and 65.85%, respectively, compared with respective controls. The positive effect of MSE treatment was evident under drought stress than under ideal irrigation, with no or minor statistical differences between well-irrigated plants and drought-stressed plants treated with MSE.

Discussion

Drought is one of the main abiotic factors reducing plant production and yield worldwide (Desoky et al. 2021a, 2021b). In the present study, both 80% of SWHC + MSE and 60% of SWHC + MSE treatments effectively ($P < 0.05$) alleviated cancer bush growth inhibition caused by drought stress through enhancing growth and yield traits such as shoot and root fresh and dry weight, number of inflorescences, number of fruit, individual fruit mass per plant, and overall yield per plant (Fig. 1) and root characteristics attributes including root length, diameter, volume, tips, crossings, and forks (Fig. 2) compared with corresponding controls. These positive effects of MSE on plants tolerant to drought stress could be due to the presence of phytohormones, which are important endogenic modulators that enhance numerous physiological processes that promote plant growth and development and enhanced plant resistance against drought stress (Arif et al. 2023; Zulfiqar et al. 2020). Our former studies showed that MSE is rich in phytohormones such as cytokinins, gibberellins, and indol acetic acid (0.94, 0.85, and 0.72 $\mu g \cdot g^{-1}$, respectively) and the activity of antioxidants

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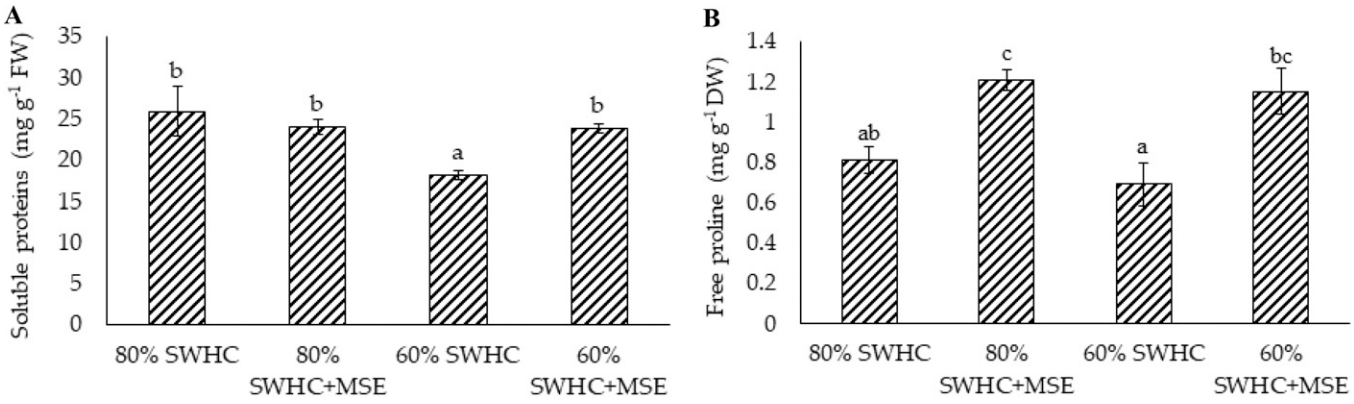

Fig. 4. Effect of foliar moringa seed extract (MSE) application on soluble proteins and free proline of cancer bush plants under drought stress. Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC, soil water-holding capacity.

such as 2,2′-diphenyl-1-picrylhydrazyl (70.28%) (Buthelezi et al. 2022, 2023), which might have played a role in enhancing the growth and phytochemical compositions of cancer bush plants under drought and heat stress. Similarly, Abd El-Mageed et al. (2017) and Hanafy (2017) reported that 3% and 30% MLE effectively improved the growth and yield of squash and soybean (*Glycine max* L.) plants grown under drought stress, respectively.

Photosynthesis is one of the processes that is negatively affected by drought stress (Tinte et al. 2022), leading to reduced growth and yield of plants (Gupta et al. 2023). In our study, drought stress negatively affected photosynthetic parameters, whereas MSE application effectively ($P < 0.05$) alleviated drought stress effect and improved photosynthesis of plants (Fig. 3). Under drought stress, MSE increased the CO_2 net assimilation rate compared with nontreated plants (Fig. 3A), demonstrating the improved photosynthetic capacity after MSE treatment. This could be attributed to the maintenance of partially open stomata (Zulfiqar et al. 2020), which favors CO_2 diffusion when the light is available for photosynthesis (Kaiser et al. 2020; Lawson and Matthews 2020). Under drought stress, the reduced photosynthesis is predominantly prompted by stomatal closure, which constraints CO_2 acquisition during the Calvin cycle (Claassens et al. 2020). In this study, the evident reduction of stomatal conductance in deficit irrigation-stressed plants compared with MSE-treated plants under drought stress (Fig. 3B) further confirmed the vital roles of CO_2 diffusion in enhancing plant photosynthesis (Lawson and Matthews 2020). Moreover, the increase of stomatal conductance in MSE-treated plants under drought stress resulted in keeping stomata open, which allowed the continuous addition of CO_2 in the Calvin cycle, promoting a higher flow of Ca^{2+} and K^+ at stomatal level, crucial ions to protect ionic and osmotic stress, and the enhancement of water rate and photosynthesis through photosynthetic efficiency (Van Oosten et al. 2017). Also, Li et al. (2021) stated that the higher stomatal conductance and improved photosynthesis are dependent on the low leaf temperature and faster water loss, which are also associated with the larger stomatal apertures under drought stress conditions. Ultimately, higher stomatal conductance and photosynthesis would lead to improved plant growth and yield with high nutrition contents (Hamedeh et al. 2022; Kaiser et al. 2020). This is further supported by the results of this study, which showed that the application of MSE increased stomatal conductance, photosynthesis (Fig. 3A and 3B), morphological growth and yield attributes (Fig. 1), and root characteristics (Fig. 2) of drought-stressed plants compared with corresponding controls.

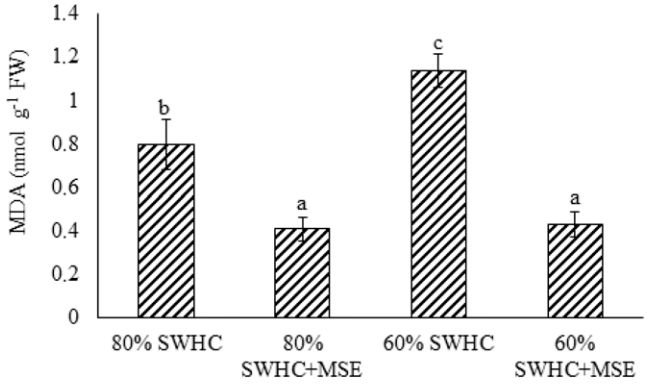

Fig. 5. Effect of foliar moringa seed extract (MSE) application on malondialdehyde (MDA) of cancer bush plants under drought stress. Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC = soil water-holding capacity.

Higher Ci and lower CE values were observed in drought-stressed plants, whereas the application of MSE on drought-stressed plants significantly ($P < 0.05$) reduced Ci and increased CE (Fig. 3D and 3E). Zhang et al. (2016) stated that lower CE values linked with higher Ci reflect biochemical alterations in the photosynthesis machinery of plants and lower activity of the ribulose bisphosphate carboxylase/oxygenase (Rubisco) enzyme. Hence, the reduced Ci and increased CE in drought-stressed plants treated with MSE could be associated with the Rubisco enzyme catalyzing CO_2 fixation during photosynthesis, thus promoting plant growth (Kurepa and Smalle 2019). This is further supported by the results of the current study, which shows that foliar application of MSE was effective in alleviating the negative effect of drought stress on the photosystem by improving the stomatal control and gas exchange (Fig. 3A–E) of drought-stressed plants compared with respective controls.

Drought stress significantly reduced E and WUE (Fig. 3C and 3F) in cancer bush plants, which could be because during drought stress, stomatal closure results in reduced leaf conductance, photosynthesis, and transpiration (Agliassa et al. 2021). However, the application of MSE effectively enhanced the E and WUE (Fig. 3C and 3F) of drought-stressed plants by improving stomatal conductance (Fig. 3B). Also, due to the sensitive response of leaf conductance to decreased leaf water potential, the more conservative use of water leads to higher WUE in drought-stressed plants, which may possibly be a mechanism for enhancing resource utilization (Mashilo et al. 2017; Wang et al. 2020). Furthermore, these observations may be due to the modified levels of phytohormones including gibberellins and abscisic acid in guard cells, which enhance stomatal conductance to improve CO_2 diffusion and WUE as observed in Fig. 3B and 3F under drought stress (Buthelezi et al. 2023). Our results are in agreement with those of Maswada et al. (2018), who demonstrated that MLE (1:30, w/v) improved stomatal condense and water use efficiency of maize under drought stress.

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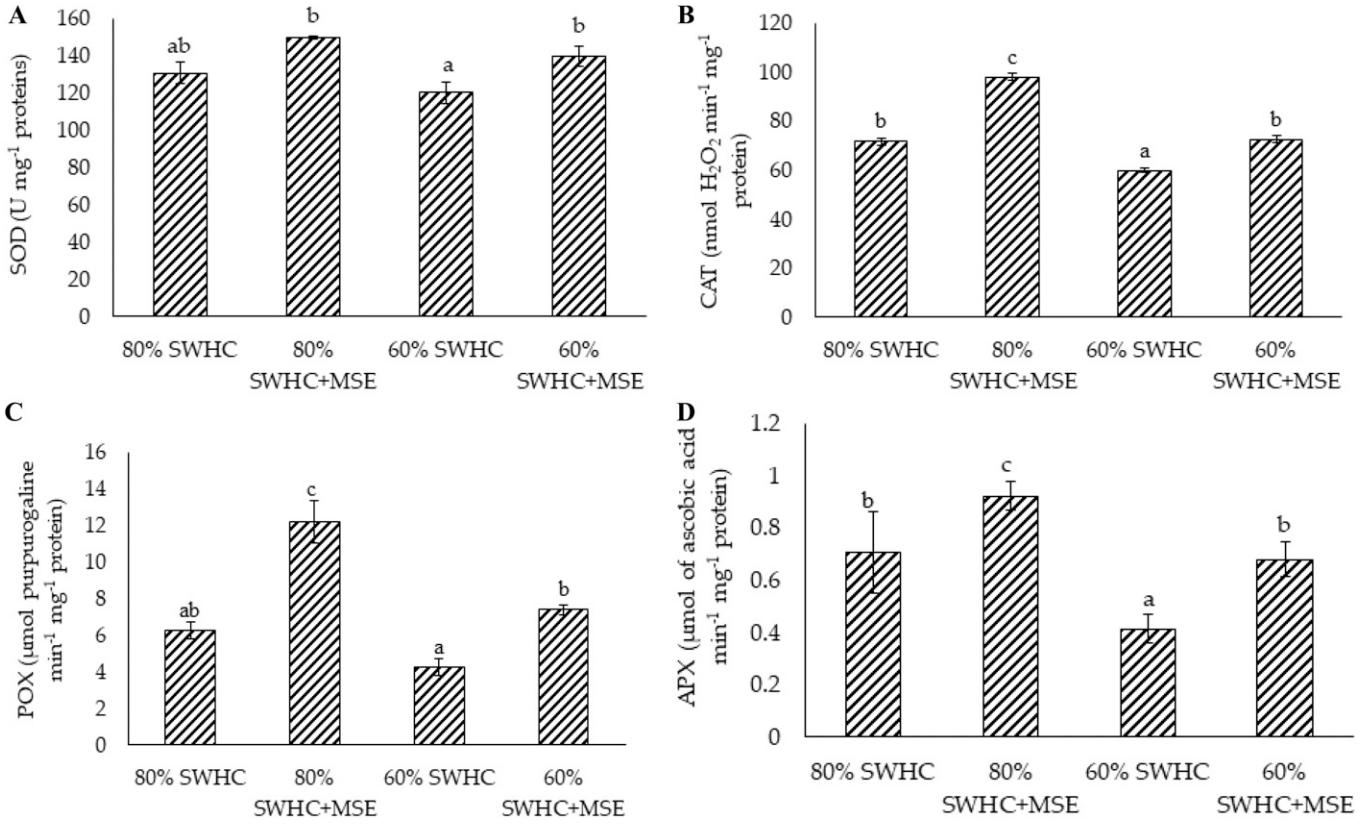

Fig. 6. Effect of foliar moringa seed extract (MSE) application on antioxidants enzyme activities of cancer bush plants under drought stress. (**A**) Superoxide dismutase (SOD). (**B**) Catalase (CAT). (**C**) Peroxidase (POX). (**D**) Ascorbate peroxidase (APX). Vertical bars represent standard error of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). SWHC = soil water-holding capacity.

Figure 4 shows that drought-stressed plants had significantly ($P < 0.05$) reduced soluble protein and free proline contents, whereas the application of MSE effectively increased the contents of soluble protein and free proline, which might have enhanced plant tolerance to drought stress (Goyal et al. 2023; Mahmood et al. 2017). In addition to functioning as an effective osmolyte, proline preserves turgidity under stress (Wang et al. 2020), improves the activities of antioxidant enzymes, and facilitates the partial opening of the stomata, enabling photosynthesis to continue under drought stress conditions (AlKahtani et al. 2021; Meena et al. 2019). This is also supported by the results of this study, which shows that the improved free proline contents in drought-stressed plants treated with 80% SWHC + MSE and 60% SWHC + MSE (Fig. 4B) could be due to the effectiveness of MSE application to enhance stomatal conductance (Fig. 3B), net photosynthetic CO_2 assimilation, and intercellular CO_2 concentration (Fig. 3A and 3D) in drought-stressed plants compared with respective controls. Moreover, proteins play a central role in reducing plant environmental stress, including serving as the enzymes in metabolism pathway, the regulators and components of transcription and translation machinery, and the components for plasma membrane, cell cytoskeleton, and intracellular compartments, thus improving plant performance under abiotic stress (Rasheed et al. 2020). This is further supported by the results of the current study, which shows the effectiveness of MSE to improve chemical compositions such as the soluble protein and free proline concentrations (Fig. 4) of drought-stressed plants, which might have provided osmoprotection and enhanced the growth and yield attributes (Figs. 1 and 2) of drought-stressed plants compared with untreated plants.

The MDA content is a commonly used parameter as a measure of lipid peroxidation in plant tissue that increases under oxidative stress (Meena et al. 2019). Drought-stressed plants (60% of SWHC) had higher content of MDA compared well-irrigated plants (80% of SWHC) (Fig. 5), indicating severe ROS production and oxidative damage (Seymen 2021). Thus, drought-stressed plants had reduced morphological growth and yield characteristics compared with well-irrigated plants (Fig. 1). However, the application of MSE significantly mitigated the oxidative damage in especially drought-stressed plants by favoring the activity of antioxidant enzymes, hence inhibiting accumulation of ROS (Goyal et al. 2023). The improvement of photosynthetic rates (Fig. 3) by the application of MSE also contributed to the decrease of cell damage by consuming free electrons that would form free radicals (Meena et al. 2019) and increased plant growth and yield characteristics (Figs. 1 and 2) in drought-stressed plants compared with corresponding controls. Moreover, oxidative damage is closely associated with the antioxidant defense mechanism, and plants with high stress tolerance exhibit higher activities of antioxidant enzymes and improved plant growth (Gupta et al. 2023; Rasheed et al. 2020). The results of this study showed that drought-stressed plants treated with MSE had high drought tolerance, which is further supported by improved plant growth attributes (Figs. 1 and 2) as a result of enhanced photosynthetic traits (Fig. 3) in comparison with untreated plants.

The enzymatic antioxidants play a crucial role in detoxifying ROS (Afzal et al. 2020; Denaxa et al. 2020). In the present study, the application of MSE treatment effectively ($P < 0.05$) increased the activities of SOD, CAT, POX, and APX in cancer bush plants under drought stress (Fig. 6). This indicates that the application of MSE was successful in reducing oxidative damage by promoting the activity of antioxidant enzymes (Fig. 6); chemical compositions such as free proline and soluble protein (Fig. 4); photosynthetic attributes (Fig. 3); and growth characteristics (Figs. 1 and 2) of drought-stressed plants in comparison with corresponding controls. SOD acts in the first step of defense against the accumulation of ROS through O_2^- dismutation in O_2 and H_2O_2, thus improving plant performance even under abiotic stress conditions (Desoky et al. 2021b; Hanafy 2017). Other antioxidant enzymes such as CAT, POX, and APX eliminate particularly H_2O_2 molecules or help plants to cope with excess H_2O_2 during environmental stress conditions (Gupta et al. 2023; Khanet al. 2022). This is further supported by the results of this

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study, which shows that MSE effectively reduced lipid peroxidation (Fig. 5) in drought-stressed plants compared with corresponding controls. The findings of this study correlated with those of Ferdiansyah et al. (2024) and Ibrahim et al. (2023), who demonstrated that 15% and 3.0% MLE significantly enhanced the activities of SOD, CAT, POX, and APX in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) plants subjected to drought stress.

Conclusions

The present study demonstrated that drought stress significantly decreased growth and yield of cancer bush plants, whereas the effectiveness of MSE treatment was more evident in drought-stressed plants. MSE significantly improved the growth, yield, and photosynthetic characteristics, soluble proteins, free proline, and antioxidant enzymes and reduced lipid peroxidation of drought-stressed plants. This study contributes to the present scientific background on the mode of action of especially plant-derived biostimulants and provides a way forward for future studies to further explore diverse sources of biostimulants and its application on plants under various environmental stress conditions. It is recommended that MSE may be used as a cost-effective and environmentally friendly alternative for enhancing plant performance under drought stress. Future studies should explore various sources of plant-derived biostimulants and their role in improving plant productivity under different environmental stress conditions, irrigation levels including 100% SWHC, and natural soil types.

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