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Effect of Drought Stress on Germination, Enzymes and Different Metabolites in *Moringa oleifera*

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A B S T R A C T

The content of phenolic compounds in *Moringa* leaves (Fig. L) were increased with increasing the concentration of PEG. The amino acid content in *Moringa* leaves increased at 5% PEG then decreased gradually at 15, 25, 35 and 45% PEG. Drought stress by PEG has an adverse effect on plumule length, which was reduced steadily by increasing PEG concentration. The lowest of plumule length (3.6 cm) was recorded at 15% PEG which decreased by 60.4% of the control treatment. The germination percentage of *M. oleifera* seeds increased continuously as PEG concentration was increased up to 15%. The concentration of 15% PEG recorded the maximum of 100 % seed germination which increased by 12.5% over control. The results of RNA content in leaves of *M.oleifera* are represented in Fig. Q. Statistically, the content of RNA in the leaves of *Moringa* varied significantly ($P< 0.01$) in response to different concentrations of PEG.

Introduction

Moringa oleifera is a member of the Moringaceae family of shrubs and trees, which is thought to have originated in Agra and Oudh in India's northwest area, south of the Himalayan Mountains. This plant is native to northern India, Pakistan, Bangladesh, Afghanistan in the sub-Himalayan region, Pakistan, and Nepal's northern foothills (Leone *et al.*, 2015; Sodhi *et al.*, 2019).

All parts of the plant (leaves, fruits, immature pods, and flowers) are utilized for traditional food and human use; however, the leaves are the most widely used (Dhakad *et al.*, 2019). Moringa leaves are utilized as a medication because they are reported to contain several phytochemicals, making them useful for both prevention and treatment (Udikala *et al.*, 2017).

M. oleifera is extremely suited for human consumption because it is high in vitamins, minerals, protein, amino acids (methionine, tryptophan, lysine, and cysteine) and various phenolics (Anwar *et al.*, 2007; Rani and Arumugam, 2017). Polyphenols, flavonoids, glucosinolates, alkaloids, terpenoids, glycosides, ascorbic acid, phenolic acids and carotenoids are all found in the plant. It has the highest concentration of vitamins A, B, C, D, E, and K. K, Mg, Ca, Mn, Zn, Cu, and Fe are among the minerals found in this tree (Thurber and Fahey, 2009).

Drought is one of the most serious environmental stress affecting agricultural production around the world, and it can lead to significant yield decreases (Porudad and Beg, 2003; Mohammadkhani and Heidari, 2008). It is also one of the most significant abiotic limitations on crop plant development and productivity around the world (Meeta *et al.,* 2013).

Melibiose, mannitol and polyethylene glycol (PEG) are three popular osmoticum compounds, while PEG is the better at controlling the potential of water that cannot be absorbed by the plant (Chazen and Neumann, 1994; Verslues *et al.,* 2006). Other low molecular weight osmotic solutions, on the other hand, could be toxic to plants since they are rapidly absorbed (Hamza, 2012). The advantage of utilizing polyethylene glycol over other osmotic solutions is that PEG cannot enter plant cells due to its large molecular weight (6000-8000), hence water is withdrawn from the cell and cell wall without altering or harming the cell structure (Chazen and Neumann, 1994; Van den Berg and Zeng, 2006).

Drought and salt stresses inhibit seeds germination (He *et al.,* 2011; Li *et al.,* 2011; Majeed *et al.,* 2011). Because of the economic and environmental importance of drought, there is serious concern about the effects of climate change on future drought frequency, duration, and severity in many parts of the world (Davatgar *et al.,* 2009). Drought has a significant impact on seedling development as well as germination rates (Kalefetoğlu *et al.,* 2009). This type of stress is one of the most significant environmental factors affecting agricultural production around the world, and it can lead to significant decrease in the yield (Mohammadkhani and Heidari, 2008). According to Jones (1992), there are many mechanisms by which plants can maintain their physiological activity when they are exposed to water stress, including the accumulation of compatible solutes in plant cells, such as amino acids and carbohydrates (Ourcut and Nilsson, 2000). Compatible solutes are low molecular weight compounds that interfering with cellular biochemical reactions that occur naturally during osmotic stress.

These compounds may have a significant role in protecting enzymes and membrane structure, as well as eliminating reactive oxygen species (ROS), in addition to their primary function in osmoregulation (Ourcut and Nilsson, 2000).

Materials and Methods

Seed germination

Seeds of *M. oleifera* were provided from The National Research Centre Community, Dokki, Cairo, Egypt. The uniform seeds were sterilized for 1 min with regular shaking using 4% sodium hypochlorite (NaOCl) solution. The seeds were washed several times with sterilized distilled water. Various concentrations of polyethylene glycol 6000 (0, 10, 15, 20, 25, and 30 %) were prepared by dissolving in water. Among two sterilized wetted filter paper, five replicates of 12 cm diameter petri dishes with six seeds in each plate were prepared for germination.

Appropriate volume of PEG solution (4ml) was applied to each petri dish every two days. The seeds were incubated in an incubator at 25°C for 14 days in the dark. Petri plates were periodically checked, and appropriate solutions were applied to compensate for evaporation. The appearance of radicles was used as a germination criterion. Every day, the number of germinated seeds was counted, and seed quality parameters were recorded and calculated using the equation:

Germination percentage

Germination percentage [%]= (Number of germinated seeds/Total number of seeds)*100

Vigor Index

The value vigor index was calculated according to Abdul-Baki and Anderson(1973) and based on the formula:

(VI) = [Total seedling length (cm) × germination percentage (%)]

Germination Energy

Germination energy (%) was measured on the 9th day of the experiment. It's the percentage of seeds that germinated within 9 days after the beginning of the experiment compared to the total number of seeds tested (Ruan *et al.,* 2002).

Water content

To evaluate the water content, the seedlings were dried in an electric oven at 80 °C for 17 h, and the following formula was used to determine it (Evans, 1972).

Percentage of water content (%) = (Fresh weight – Dry weight)/ Fresh weight * 100

Pot experiment

Seeds were germinated in plastic pots containing sand: clay (1:1) soil (10 kg) and conducted in green house of Faculty of Science, Menoufia University, Egypt. The seeds were cultivated under normal day light conditions at a temperature of 25-35 ^{o} C. Before cultivation, seeds were sterilized for 2 min with a dilute solution of sodium hypochlorite, then washed three times with distilled water and soaked for 5 minutes in water.

Six seeds were allowed to germinate in each pot and then thinned to four seeds after germination. Seeds were cultivated in early morning and were completely irrigated with tap water. After seed germination (7 days after cultivation), seeds were irrigated with different concentrations of PEG (0, 5, 15, 25, 35, and 45%). Each treatment was replicated four times. Appropriate volume(250 ml) was applied to each pot and irrigated every two days. The fresh leaves were collected after 35 days for assay of enzymes activities.

Determination of total flavonoids

To estimate the flavonoids in *M. olifera*, Chang *et al.*, (2002) modified the aluminium chloride colorimetric method. The dried leaf sample of *M. olifera* (10 mg) was extracted with 80% ethanol before being combined with a specific volume of solution (0.5 ml), 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. The reaction mixture's absorbance at 415 nm was measured using a spectrophotometer after 30 minutes of incubation at room temperature (Metertek SP-850).

In the blank, 10% aluminium chloride was replaced with the equivalent volume of distilled water. The calibration curve was established using quercetin, and the amount of flavonoids in the extracts were measured in mg/g dry weight of the plant leaves was determined by reacting the flavonoids with aluminium chloride.

Estimation of total phenolic compounds

Using the Jindal and Singh (1975) method, the quantitative total phenolic content of *M. olifera* leaves was determined. A known amount (10 mg) of dried plant material leaves was extracted three times using 95% ethanol, the clear supernatants were collected and finished to a defined volume (25 ml) by 95% ethanol. Then, 1ml of this extract was combined with 1ml of the folin reagent and 1ml of Na2CO3 (20% w/v), and the mixture was then increased in volume by distilled water to a known level. Following this, exactly 30 minutes later, the absorbance of the produced colour was measured spectrophotometrically (Metertek SP-850) at 650 nm. To determine the amount of total phenolic compounds (mg/g d.wt.) in the plant leaves, a standard curve was made using various pyrogallol concentrations as in the previous method.

Determination of RNA content

The Sadasivam and Manickam method was used to determine the amount of RNA. Fresh weight leaves (0.2 g) were ground in 5 ml of ethanol, the resulting homogenate was filtered, and the filtrate was diluted with ethanol to a final volume of 10 ml.

The mixture was then heated at 1000 rpm with 10 ml of 1N perchloric acid added, and the supernatant

was diluted to 10 ml. One mL of the RNA extract was diluted to four mL with distilled water, combined with four mL of the orcinol reagent, and heated in a boiling water bath for twenty minutes. The mixture was then allowed to cool to room temperature, and the produced color's absorbance at 660 nm was measured spectrophotometrically.

Estimation of total soluble proteins content

The total soluble proteins content was estimated quantitatively in the borate buffer extract using the method described by Bradford, (1976). 0.1 ml of borate buffer extract was used, and 3 ml of the Commassi brilliant blue reagent (100 mg of Commassi brilliant blue G250 in 50 ml of 95% ethanol + 100 ml of 85% phosphoric acid + 1 L of distilled water) was added. After two minutes, the absorbance was measured spectrophotometrically at 595 nm. The protein content was determined as mg/g d.wt, using a calibration curve created by the protein Bovine Serum Albumin.

Estimation of amino acids content

According to Lee and Takahashi, ninhydrin tests were used to measure the amount of amino acids using glycine as a reference (1966). 10 ml of 95% ethanol were used to extract 0.1 gm of finely powdered leaves. The determination of amino acids was done using the clear supernatant. In a mixture of 1.9 ml of ninhydrin-citrate buffer-glycerol, 0.1 ml of plant extract or standard solution (glycine), and 0.5 ml of 1% ninhydrin solution in 0.5 M of citrate buffer (PH 5.5), 1.2 ml of 55% (v/v) glycerol solution, respectively, were added. The mixture was then mixed and boiled for 12 minutes in a water bath. It was then cooled in a water bath. After thoroughly shaking the tubes, the sample mixture's absorbance was determined at 570 nm. Using a glycine-prepared calibration curve, the amount of amino acids was estimated as mg/g dry weight.

Estimation of rosmarinic acid

Lopez-Arnoldos *et al.,* (1995) and Komali and Kalidas (1998) reported the method of rosmarinic acid extraction and detection. Fresh leaf tissue samples from 200 mg of *M. olifera* were homogenised in a porcelain mortar with 10 ml of 50% methanol, then left in a water bath at 55 °C for two hours before being centrifuged for ten minutes at 3500 rpm.

The rosmarinic acid content (mol-1 cm -1) was calculated after diluting one ml of the extract with nine ml of 50% methanol. The absorbance of the colour that resulted was measured at 333 nm using a spectrophotometer (Metertek SP-850) from the following equation: A= εbc Where: (A) is the absorbance at 333 nm, (C) is the concentration of rosmarinic acid, (ε) is the extinction coefficient (ε=19000 L mol-1 cm -1) and (b) is the width of cuvette (b = 1cm). Rosmarinic acid molecular weight was multiplied to obtain the content per mol, which was calculated as mg/g F.wt.

Results and Discussion

Germination percentage

The germination percentage of *M. oleifera* seeds (Fig. A) increased continuously as PEG concentration was increased up to 15%. The concentration of 15% PEG recorded the maximum of 100 % seed germination which increased by 12.5% over control. The lowest germination percentage (61.1%) was exhibited at 20% PEG which decreased by 31.3% of control treatment. However, the two concentrations 25% and 30% PEG inhibited completely seed germination percentage.

Radical length

Under control treatment, the radical length (Fig. B) did not surpass 7.1 cm. At 5% PEG, the maximum radical length (11.7 cm) was observed with 64.8 % over the control. However, lowest radical length (3.5 cm) was 50.7 % of the control at 20 % PEG. Moreover, the two concentrations 25 and 30% of PEG inhibited radical growth completely.

Plumule length

The results reported in Fig. C displayed the effect of

drought induced by PEG on plumule length of *M. oleifera*. Drought stress by PEG has an adverse effect on plumule length, which was reduced steadily by increasing PEG concentration.

The lowest of plumule length (3.6 cm) was recorded at 15% PEG which decreased by 60.4% of the control treatment. On the other hand, the concentrations 20, 25 and 30% PEG inhibited the plumule length.

Plumule/radical (length)(cm)

The results in Fig. Dshows the effect of drought induced by PEG on the ratio of plumule/radical (length) of *M. oleifera*. The ratio of plumule/radical (length) was decreased with increasing the concentration of PEG.

The lowest ratio plumule/radical (length) was 0.7 cm was found at15% PEG that decreased by 44.8% of the control. The concentrations 20, 25 and 30% PEG inhibited the ratio of plumule/radical (length).

Seedling length

The effect of drought induced by PEG on seedling length of *M. oleifera* is shown in Fig. E. The highest seedling length (20.2 cm) was observed at 5% PEG which increased by 22.5% over the control. On the other hand, the lowest seedling length was 3.5 cmat 20% PEG which decreased by 78.8% of the control.

Seedling fresh weight

The effect of drought induced by PEG on seedling fresh weight of *M. oleifera* is shown in Fig. F. The highest fresh weight (448.2 mg) was recorded at the control. However, the lowest fresh weight (28.3 mg) was found at a treatment with 20% PEG which decreased by 93.7% compared to control treatment. The concentrations 25 and 30% PEG abolished the fresh weight of seedlings.

Seedling dry weight

The dry weight of *M. oleifera* seedlings (Fig. G) was affected by drought stress which is induced by PEG. By increasing the drought stress, the seedling dry weight decreased continually. The concentration 20 % PEG resulted in the lowest dry weight (4.4 mg) of seedlings, which decreased by 90.8% of the control.

Moisture content (water content)

The effect of drought stress on moisture content of *M. oleifera* is shown in Fig. H. The moisture content of *M. oleifera* decreased under different concentrations of PEG. The lowest moisture content of seedling was 84.3 % at 20% PEG which decreased by 5.6% of the control.

Vigor index

Fig. I provides the data of the impact of the drought stress on the vigor index of *M. oleifera*. The highest of vigor index (1913.9) was observed at 5% PEG which increased by 30.5% of the control. At 20 % PEG, the vigor index declined to 213.9 with 85.4% of the control.

Germination energy

The effect of drought on germination energy of *M. oleifera* is shown in Fig. J. The highest of germination energy (100%) was at 15% PEG with increase of 20% control. While the lowest germination energy was 61.1% at 20% PEG which decreased by 26.6% of the control.

Biochemical analysis

Total flavonoids content

Statistically, the content of flavonoids in the leaves of *Moringa* (Fig. K) varied significantly ($P< 0.01$) in response to different concentrations of PEG. As PEG concentration was increased, the accumulation flavonoids in *Moringa* leaves significantly increased as well. At 45 % PEG, the accumulation of flavonoids increased by 380 % of the control treatment.

Phenolic compounds

The content of phenolic compounds in *Moringa*

leaves (Fig. L) were increased with increasing the concentration of PEG. The highest accumulation of phenolic compounds was recorded at 45% PEG, which increased by 63% over the control treatment.

Rosmarinic acid content

The results of rosmarinic acid content in leaves of *M. oleifera* were represented in (Fig. M). Statistically, the content of rosmarinic acid in the leaves of *Moringa* varied significantly ($P< 0.01$) in response to different concentrations of PEG.

As PEG concentration was increased, the accumulation rosmarinic acid in *Moringa* leaves significantly increased as well. The highest mean of rosmarinic acid content is at the concentration of 45% PEG. Rosmarinic acid content at these maxima was 0.067 mg/g fresh weight.

Antioxidant scavenging activity using ABTS

The antioxidant scavenging activity in *Moringa* leaves (Fig. N) was increased with increasing the concentration of PEG. The assay of antioxidant scavenging activity in the *Moringa* leaves varied significantly in response to different concentrations of PEG. The concentration of 45% induced the antioxidant scavenging activity to the maximum level of 95.2%.

Protein content

The result of protein content in leaves of *M. oleifera* were represented in Fig. O. Statistically, protein content in the leaves of *Moringa* varied significantly in response to different concentrations of PEG. The accumulation of protein in *Moringa* leaves exhibited little increase at low concentration of PEG (5%), while it decreased with increasing the concentration of PEG. The highest protein content (2.62 mg/g dry weight) was recorded at 5% PEG.

Amino acid content

Amino acids content in the leaves of *Moringa* (Fig. P) varied significantly in response to different concentrations of PEG. The amino acid content in *Moringa* leaves increased at 5% PEG then decreased gradually at 15, 25, 35 and 45% PEG, respectively but still lower than the control value. The highest amount of amino acids content was 4.53 mg/g dry weight at 5% of PEG.

RNA content

The results of RNA content in leaves of *M. oleifera* are represented in Fig. Q. Statistically, the content of RNA in the leaves of *Moringa* varied significantly ($P< 0.01$) in response to different concentrations of PEG. The RNA content in *Moringa* leaves was decreased with increasing the concentration of PEG.

The lowest content of RNA was recorded at35% and 45% PEG respectively. RNA content at these minima was 2.7 and 2.3 mg/g fresh weight, respectively.

Scientific research aims to cultivate and study a variety of plants, especially if one of them has a medical application. *M. oleifera* is an important medicinal plant that is used to cure a variety of diseases and disorders.

Drought stress is now recognized as the most detrimental environmental stress in the world, affecting a significant portion of agricultural land (Avramova *et al.*, 2015; Huang *et al.*, 2015; Langridge and Reynolds, 2015)

PEG induces water stress (Abbas *et al.*, 2014). PEG is a biologically inert chemical that is widely used to stimulate drought stress during early germination and seedling growth stages for studying the effects of water stress in various plant species (Kauser *et al.*, 2006; Khodarahmpour, 2011 and Shamim *et al.*, 2014).

The study was designed to examine the effect of drought stress induced by PEG on growth and metabolic profile of *M. oleifera*. However, seeds of *M. oleifera* were subjected to different concentrations of PEG to investigate the adverse effects of drought stress on vegetative growth and phytochemical constituents of *M. oleifera*.

The effect of drought stress on germination percentage of *M. oleifera* seeds was highly significant. Low concentrations of PEG increased germination percentage. This could be related to the ability of seed to imbibe water required for germination under the osmotic conditions provided by the PEG solution. High concentrations of PEG decrease germination percentage. Our findings agree with the findings of Hegarty, (1997); Turk *et al.,* (2004) and Basha *et al.,* (2015). The existence of several metabolic disorders causes a decrease in seed germination under stress conditions.

The drop in germination percentage may be due to decrease in water absorption into the seeds during imbibitions and seed turgescence phases (Hadas, 1977). Too high PEG concentrations caused the osmotic potential value around the seeds to become increasingly negative, making water difficult to be absorbed by the seeds.

The solution of low osmotic potential may hinder the imbibition process (Nurmauli and Nurmiaty, 2010). Kavar *et al.,* (2008) observed that the main responses to drought stress in plants are root growth, density, proliferation, and size, because roots absorb water from the soil. The effect of drought stress on root length of *M. oleifera* was highly significant. Root length increased under normal stress conditions, but reduced under severe stress conditions, stressing the importance of vigorous root growth during the seedling stage, which would result in better root structure at maturity (Nejad, 2011).

With increasing PEG concentration, root length decreased, and the decline was more noticeable at high concentration. Significant reduction in root parameters with increasing PEG concentration has been reported for tomato by Basha *et al.,* (2015), lentil (Muscolo *et al.,* 2014), maize (Soltani *et al.,* 2006; Dar *et al.,* 2018), chickpea (Kalefetoğlu Macarand Ekmekci, 2009) and wheat (Almansouri *et al.,* 2001; Jajarmi *et al.,* 2009), Shoot length decreased with increasing concentration of PEG.

This agrees with the report which stated that drought stress reduced shoot growth (Sankar *et al.,* 2007; Celikkol Akcay *et al.,* 2010; Kavas *et al.,* 2013; Toosi *et al.,* 2014 and Basha *et al.,* 2015). This could be attributed to a decrease in cell elongation caused by a decrease in turgor induced by a water shortage (Van den Berg and Zeng, 2006).

Drought stress caused by PEG reduced the fresh and dry weights of shoots, roots, and seedlings in this experiment. This agrees with the results of Akitha Devi and Giridhar (2015) which found that drought stress decreased the fresh and dry weight of the roots and shoots in Indian soybean varieties. However, the decrease in dry weight caused by stress could be linked to changes in carbon and nitrogen allocation and partitioning (Jaleel, 2009).

Tahir and Mehid, (2001) found that water stress reduced biomass in practically all genotypes of sunflower (*Helianthus annuus*). Several studies have shown that increasing drought stress can reduce the dry weights (Hamada, 1996; Abdalla and El-Khoshiban, 2007; Jongrungklang *et al.,* 2008 and Omidi, 2010).

The relative water content (RWC) of a plant is a measure of its water status, indicating metabolic activity in tissues and serving as the most important index of dehydration tolerance (Sinclair and Ludlow, 1986). Our results show a decrease in moisture content with increasing concentration of PEG. The reduction in RWC may be due to abscisic acid hormone production in roots, its accumulation in stomatal guard cells, and stomata closing. According to Schonfeld *et al.,* (1988) drought stress decreased the leaf's ability to hold water and its relative water content (RWC) because the plant's roots were not able to absorb as much water. In progressive mild stress, a relative greater RWC was seen than in severe stress, showing that plants can maintain their water content under mild stress, whereas this ability is lost under severe stress treatment. There are many studies which report that PEG-induced water stress decreased RWC in many plants such as in rice leaves (Hsu and Kao, 2003), barley (Yuan *et al.,* 2005), tomato (Zgalla *et al.,* 2005) and pigeonpea plants (Kumar *et al.,* 2011).

Plate.1 (A) Germination percentage (%) of *M. oleifera* under drought stress induced by PEG at seedling stage. (B) Radical length of *M. oleifera* under drought stress induced by PEG at seedling stage. (C) Plumulelength of *M. oleifera* under drought stress induced by PEG at seedling stage.(D) Ratio of plumule / radical (length) of *M. oleifera* under drought stress induced by PEG at seedling stage. (E) Seedling length of *M. oleifera* under drought stress induced by PEG at seedling stage. (F) Seedling fresh weight of *M. oleifera* under drought stress induced by PEG at seedling stage.

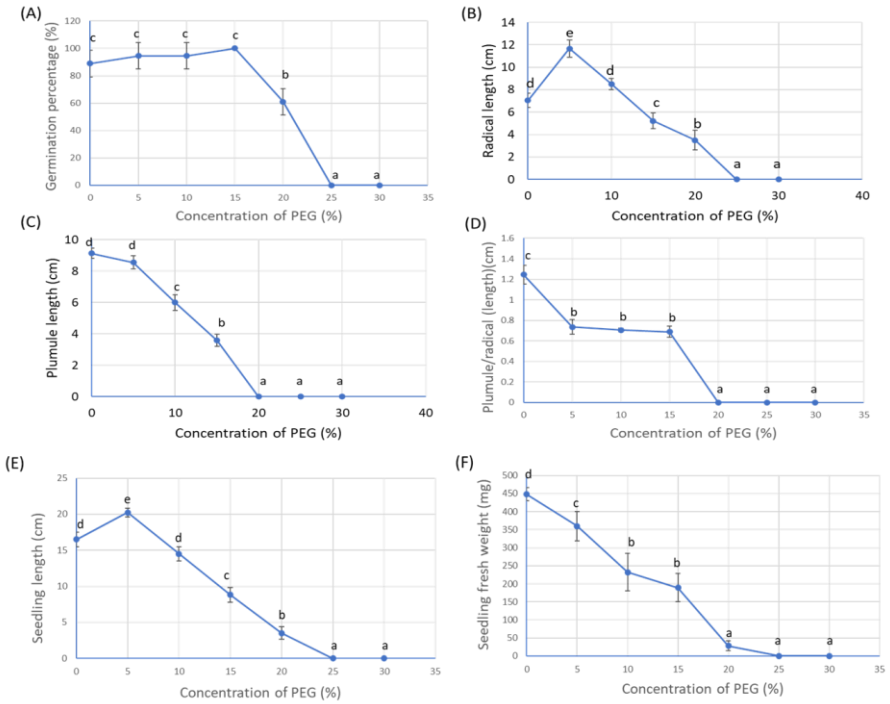


Plate.2 (G) Seedling dry weight of *M. oleifera* under drought stress induced by PEG at seedling stage. (H) Moisture content of*M. oleifera* under drought stress induced by PEG at seedling stage.(I) Vigor index of *M. oleifera* under drought stress induced by PEG at seedling stage. (J) Germination energy of *M. oleifera* under drought stress induced by PEG at seedling stage.

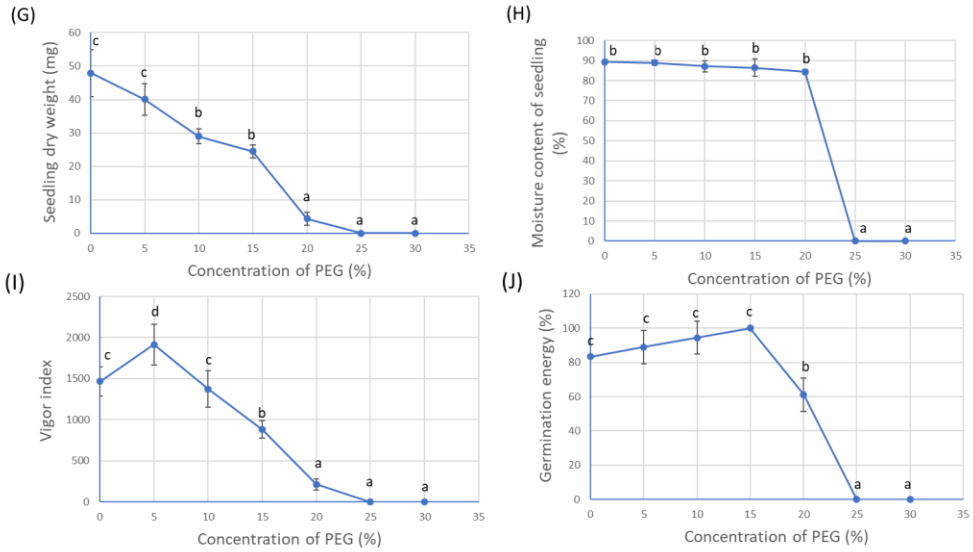
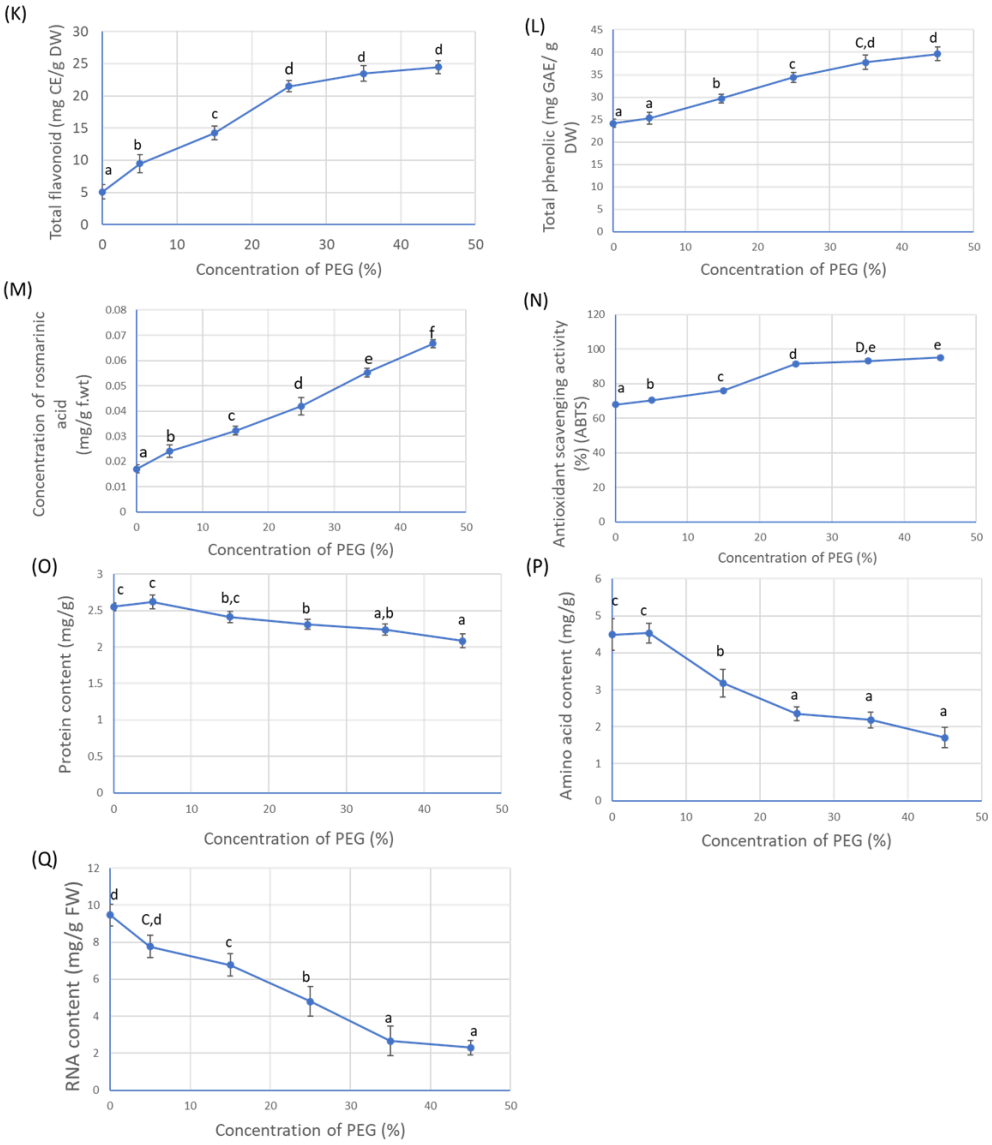


Plate.3 (K) Total flavonoid content of *M. oleifera* under drought stress induced by PEG. (L) Total phenolic content of *M. oleifera* under drought stress induced by PEG. (M) Rosmarinic acid content of *M. oleifera* under drought stress induced by PEG. (N) Antioxidant scavenging activity using ABTS of *M. oleifera* under drought stress induced by PEG. (O) Protein content of *M. oleifera* under drought stress induced by PEG. (P) Amino acid content of *M. oleifera* under drought stress induced by PEG. (Q) RNA content of *M. oleifera* under drought stress induced by PEG.



The total phenolic and flavonoid content of *M. oleifera* seedling increased with rising PEG concentrations. These results indicate that drought stress had a substantial impact on the total phenolic and flavonoids content of *M. oleifera*. Our results agree with *Halimeh et al.*, (2013) who reported phenolic and flavonoid content was increased in roots and shoots during drought condition in *Dracocephalum moldavica* L. Talbi *et al.*, (2020) also found that drought stress increased flavonoid accumulation and antioxidant capability in the saharan plant *Oudeneya africana*, improving plant adaptation to abiotic stress. Gao *et al.*, (2020) also reported that drought stress enhanced the accumulation of secondary metabolites including flavonoids in two Adonis species, which lowered H_2O_2 content and boosted drought resistance. It has been found that varied stress conditions raised phenolic concentration and flavonoids synthesis (Takahama and Oniki, 2000; Ruiz *et al.*, 2003).

The relationship between drought stress induced by PEG and the protein content of *M. oleifera* is highly significant. Under drought stress, the total soluble protein content *M. oleifera* leaves first increased and then decreased. The initial rise in total soluble proteins during drought stress appears to be related to the expression of new stress proteins, whereas the subsequent decline appears to be owing to a significant reduction in photosynthesis. Since there were insufficient materials for protein synthesis and a decrease in photosynthesis during drought stress (Havaux *et al.,* 1987).

One of the first signs of stress is a shift in the content of free amino acids in plants (Rai, 2002). The connection between drought stress induced by PEG and the amino acid concentration of *M. oleifera* is extremely important. The amino acid content of *M. oleifera* slightly increased then reduced as PEG concentrations increased. In general, it has been stated that under drought stress, amino acid accumulation rises in plants such as sunflower and sorghum due to osmotic adjustment to mitigate negative consequences (Morgan, 1984; Yadav *et al.*, 2005; Manivannan *et al.,* 2007). This report agrees with our results and the increase in amino acid accumulation in plants under drought stress may be due to the hydrolysis of protein. The increase of free amino acids in response to water stress reflects a dynamic adjustment in plant nitrogen metabolism (Stewart and Hanson, 1980; Hanson *et al.,* 1979).

Currently, stress tolerance has been studied using ABTS and DPPH to measure free radicals in plant samples (Cui *et al.,* 2010). The antioxidant scavenging activity of *M. oleifera* using ABTS or using DPPH improved with increasing PEG concentration. This study showed that the effect of drought stress on antioxidant scavenging activity of *M. oleifera* using ABTS and DPPH was significant. The considerable increase in both ABTS and DPPH radical scavenging capacity appeared to be related to the degree of plant seedling stress tolerance (Weidner *et al.,* 2009; Dominguez-Perles *et al.,* 2011). Our findings were consistent with those obtained from the screening of drought-tolerant sugarcane genotypes using biochemical markers against PEG (Abbas *et al.,* 2014).

The results show that the connection between RNA content of *Moringa oleifera* and the drought stress induced by PEG is highly significant. The RNA content of *Moringa oleifera* decreased with increasing concentrations of PEG. Our results agree with the reported results of He *et al.,* (1999) that there is a reduced RNA synthesis with increased water stress. During water stress, chloroplast RNAase was upregulated, causing RNA degradation. Additionally, it has also been shown that under conditions of water stress, ribosomes and polyribosomes are reduced. As a result, mRNAs get congested with ribosomes to shield them from degradation, which disrupts ribosome function (Scott *et al.,* 1979; Mason *et al.,* 1988).

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