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Drought affects size, nutritional quality, antioxidant activities and phenolic acids pattern of *Moringa oleifera* LAM.

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Summary

To observe variation in growth performance, antioxidant activities, and nutritional quality of *Moringa oleifera* we exogenously applied benzyl amino purine (BAP), ascorbic acid, and moringa leaf extract (MLE) to moringa plants at three field capacity levels, 100, 75, and 40% in a completely randomized design with three replications. We observed a decrease in growth, chlorophyll *a* and *b*, total phenolic contents, antioxidant activities, crude protein, and mineral contents of moringa leaves at 100 and 40% field capacity in comparison with 75% field capacity. BAP best improved growth performance of moringa plants, improving shoot length, root length, number of leaves and photosynthetic pigments, followed by MLE at 75% field capacity, while moringa plants showed reduced growth at 40% field capacity which was increased by BAP and MLE foliar application. Maximum contents of gallic acid, *p*-coumaric acid and sinapic acid were found in moringa leaves when the plants were sprayed with ascorbic acid while *p*-hydroxybenzoic acid and caffeic acid were maximally increased under 75% field capacity when the plants were subjected to BAP followed by MLE. The lowest and highest crude protein, calcium, potassium, magnesium, and phosphorous contents were recorded under 40 and 75% field capacity, with MLE improving these contents under both conditions. It can safely be concluded that moringa plants showed retarded growth under 100 and 40% field capacity, and that the effects of deficit in nutritional quality were mitigated by applying BAP and MLE. Among these two plant growth regulators, MLE can be preferred being a natural source.

Keywords: Ascorbic acid; benzyl amino purine; RP-HPLC; *Moringa* leaf extract

Introduction

Moringa oleifera LAM., Moringaceae, is grown in contexts as diverse as an agricultural crop, for living fences, and as livestock fodder for its multiple attractive attributes. Moringa has multiple compounds with strong antioxidant potential and is a rich source of crude protein, minerals, and polyphenolics (NOUMAN et al., 2016; OLSON et al., 2016). Its various parts are used in folk practices against an array of ailments (e.g. ANWAR et al., 2007). Plant scientists mostly focus on moringa leaves because they not only are a source of potentially useful compounds but leaf extracts are used as growth regulators which have been tested on various crops and rangeland grasses (NOUMAN et al., 2012a, b, 2014a; YASMEEN et al., 2013). Moringa is tolerating typical seasonal conditions of a dry tropical and subtropical environment.

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Abiotic stress affects plant metabolism, resulting in impaired plant growth resulting in reduced crop yield and economic loss (MAHAJAN and TUTEJA, 2005). The nutritional and medicinal importance of moringa demands improvement in its production, especially under stress conditions, given that a large percentage of world agriculture is facing stress. Moringa is a promising crop under stressful conditions given evidence that it can provide acceptable yields despite marginal conditions. For example, in one study, moringa growth behavior, nutritional quality, and antioxidant system tolerated salinity up to 8 dS m^{-1} with only slight reductions in biomass production and nutritional quality (NOUMAN et al., 2012a). Moreover, there is evidence that moringa tolerance to saline conditions can be enhanced by the application of plant growth regulators as seed priming agents (NOUMAN et al., 2014a). In addition to salinity, drought is another abiotic stress that causes significant decrease in yield (MAHAJAN and TUTEJA, 2005). In addition to yield decrease, drought also results in changes in nutritional quality of plants and bioactive compounds such as phenolic acids (FAROOQ et al., 2009). With increasing climate change, drought events are becoming more erratic. Therefore, we need crops that can deal well with drought, rather than requiring abundant irrigation. Moringa is a suitable option which is known to be drought-tolerant.

Moringa is well known for its nutritional quality and antioxidant compounds (ANWAR et al., 2007). These contents vary with different cultural practices, changing climatic conditions, soil nutrients availability, and soil types (NOUMAN et al., 2013, 2014b; OLSON et al., 2016). Drought stress is no exception, with water availability to plants strongly influencing nutrient uptake and the production of plant biomass and its nutritional quality and phenolic acid composition. For example, saline conditions, which comprise a form of drought stress that is an osmotic factor of salinity, can cause a significant decline in nutritional quality of moringa leaves (NOUMAN et al., 2014a). Moreover, variation in phenolic acids under abiotic stress conditions has been reported in different plants such as *Tithonia diversifolia*, *Solanum lycopersicum* and *Triticum aestivum* (ALI and GHADA 2014; CHAKHCHAR et al., 2016; SAMPAIO et al., 2016), but there is no study available illustrating the variation in these contents in moringa leaves under drought stress. Such data would be useful because they would help identify currently low-yield agricultural lands that could be made much more productive if planted with moringa, provided that a means could be identified of obtaining acceptable yields of phenolic acids on these lands. Phenolic acids not only help mitigating environmental stress; phenolic acid-rich plants are also used as therapeutic herbal medicines, with moringa (leaves, flowers, roots, green pods, and seeds) being used in traditional medicine (ANWAR et al., 2007; PAVARINI et al., 2012; MIRANDA et al., 2015). So, it is imperative to study variation in nutritional attributes and phenolic acid composition under varying abiotic stress conditions.

Plants mitigate drought stress through bioactive compounds such as phenolic acids, flavonoids, isothiocyanates, etc., whose quantities can be enhanced by addition of plant growth regulators. Different plant growth regulators have been used to mitigate drought stress in different plants, especially agricultural crops (FAROOQ et al., 2009), but regulators have not been tested for moringa under water deficit. Enhanced abiotic tolerance in moringa plants under saline conditions has been reported for moringa plants with the assistance of plant growth regulators (NOUMAN et al., 2014a). So, it can be hypothesized that moringa growth, nutritional quality, antioxidant activities, and composition of phenolic acids can also be affected under drought. Because moringa is used as a food and fodder crop and medicinal plant in different regions including drought prone areas, the variation in its nutritional attributes, antioxidant potential, and phenolic acids requires study. Here, we investigated variation in growth behavior, nutritional quality, enzymatic antioxidant activities, and the composition of phenolic acids under varying levels of drought stress.

Materials and methods

Seed material

To test the growth performance, nutritional quality, antioxidant activities and composition of phenolic acids of *Moringa oleifera* leaves under drought conditions, moringa plants were grown in the greenhouse of the Department of Forestry and Range Management, Bahauddin Zakariya University, Multan, Pakistan. We collected seeds from locally grown moringa plant on the campus of Bahauddin Zakariya University, Multan, Pakistan in May 2013.

Experimental layout

The experiment was laid out in a two factor (drought stress and plant growth regulators) factorial completely randomized design with three replications under greenhouse conditions (Temp: 34±3 ºC, 14 h day light, 26±3 ºC, 10 h night with 28±4% average humidity). Moringa seeds were sown in earthen pots filled with 3 kg of soil (clay loam), sand, and green manure (1:1:1). We planted five seeds in each pot, keeping three plants. When plants reached an age of two weeks, three field capacity levels (100, 75, and 40%) were induced and maintained for two months till the end of the experiment. Field capacity i.e., 100% was considered as half of the soil saturation capacity. Exogenous foliar application of benzylaminopurine (BAP, 50 mg L^{-1}), ascorbic acid (50 mg L^{-1}), and moringa leaf extract (MLE, 3.3%) were carried out, in addition to a control (no spray) and water spray to investigate the efficacy of these plant growth regulators to mitigate drought stress. Water spray was used as a positive control.

Plant vigour evaluation

The growth performance of moringa plants was noted on the day of harvest. For this, shoot and root lengths, number of leaves and roots were noted. The roots emerging from the main tap root were counted as number of roots.

Chlorophyll and total phenolic contents

Chlorophyll *a* and *b* contents were quantified following NAGATA and YAMASHTA (1992), while total phenolic contents were quantified following SINGLETON and ROSSI (1965) as revised by WATERHOUSE (2001).

Antioxidant enzyme assay

To determine the variation in the activities of these enzymatic antioxidants, 1 g of fresh moringa leaves was ground in 10 mL of 50 mM cooled phosphate buffer of pH 7.8, filtered through Whatman No. 1 filter papers and centrifuged at 15000 rpm for twenty minutes at 4 ºC. The supernatant was removed with a micropipette and stored in opaque Eppendorf tubes. The supernatant was used to determine SOD activity at 560 nm with a UV spectrophotometer (UV-4000, O.R.I. Germany) following GIANNOPPOLOTIS and RIES (1977). Assay ingredients (1 mL of 1.3 µM Riboflavin, 500 µL of 13 mM Methionine, 500 µL of 75 nM EDTA and 950 µL of 50 mM phosphate buffer of pH 7.8) were pipetted into a quartz cuvette followed by 50 µl of enzyme extract, and 1 ml of 50 µM NBT. After introducing the NBT, the cuvette was immediately placed under a fluorescent lamp (30 W) for 5 minutes until blue formazane was produced by NBT photo-reduction. In addition, one cuvette was placed in a dark chamber containing the same assay ingredients except the enzyme extract for the same duration. After 5 minutes, the absorbance was recorded at 560 nm. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition at the rate of NBT reduction at 560 nm in comparison with tubes having no enzyme extract.

To determine CAT activity, 2 mL phosphate buffer of pH 7.0 and 900 µL of 5.9 mM H_2O_2 were combined in a quartz cuvette placed in a UV spectrophotometer (UV-4000, O.R.I. Germany). After placing in the spectrophotometer, 100 µL enzyme extract was introduced into the cuvette and recording of absorbance at 240 nm was immediately started. The absorbance was noted every 20 seconds for 5 minutes or until constant reading. CAT activity was measured as units (µmol of H_2O_2 decomposed per minute) per mg of protein (CHANCE and MAEHLY, 1955).

POD activity was also determined using the protocol of CHANCE and MAEHLY (1955) with minor modifications. 2 mL of 50 mM phosphate buffer of pH 7.0, 400 µL of 20 mM guaiacol and 500 µL of 40 mM H_2O_2 were introduced into a cuvette and placed in UV spectrophotometer. Then enzyme extract was added to the cuvette while in the spectrophotometer. The recording of absorbance reading at 470 nm was immediately started and was noted after every 20 seconds for 5 minutes or until constant reading. POD one unit activity was defined as the change of 0.01 absorbance unit per minute per mg of protein.

Crude protein and mineral analyses

Moringa leaf samples were dried at 60 ºC for 70 hours until constant weight and ground to pass a 2 mm sieve. The samples were digested by using 10 mL of nitric acid (HNO_3) and 5 mL of perchloric acid ($HCLO_4$) (AOAC, 2003). Potassium (K) contents were measured using a flame photometer (Jenway PEP-7, Jenway, UK). Calcium (Ca), magnesium (Mg), and phosphorous (P) contents were measured using an atomic absorption spectrophotometer (Model: Z-8200, Hitachi, Japan). Crude protein (CP) contents were determined according to AOAC (2003) guidelines. For this, dried and grinded moringa leaves (5 g) were digested in sulfuric acid (H_2SO_4) with a mixture of K_2SO_4: $CuSO_4$: $FeSO_4$ (10: 05: 01) with micro Kjeldhal's apparatus for nitrogen digestion, distillation and quantification.

Separation and quantification of phenolic acids by reverse phase high performance liquid chromatography (RP-HPLC)

Phenolic acids are bioactive compounds which indicate plant response to drought stress. To study the variation in the selected phenolic acids (*p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid, gallic acid and sinapic acid) of moringa leaves under drought by RP-HPLC, methanolic extracts were prepared. These phenolic acids were selected due to their reported nutraceutical activity.

Extraction / Hydrolysis

Hydrolysis of sample extracts followed NUUTILA et al. (2002) with slight modification. For this, 1000 mg of crude extract was mixed in 10 mL of 50% aqueous methanol (1.2 M HCl) in a refluxing flask at 80 °C for 2 h. After refluxing, the extract was allowed to cool to

room temperature and the volume adjusted to 10 mL with methanol and filtered through 45 µm (Millipore) before injecting into an RP-HPLC column.

Chromatographic system and conditions used for phenolic acids analysis

Phenolic acids analysis was performed on an Agilent 1200-series HPLC system (Agilent Technology Germany) equipped with a gradient model LC (G1312B) binary pump system, auto sample injection (G1367B), degasser (G1379), a UV-VIS detector (G513C), and column oven (G1316B). Separation of individual phenolic acids was done on a hypersil Cold C18 column (250×4.6mm, 5 µm particle size) (Thermo Fisher Scientific Inc., Massachusetts, United States). A non-linear gradient system consisting of solvent **A** (acetonitrile: methanol 70:30) and solvent B (water with 0.5% glacial acetic acid) at a flow rate of 1mL/min was used for elution. The gradient programming used for the separation of phenolic acids included: 10-15% **A** from 0 to 5 min; 15 to 20% **A** from 5 to 18 min; 20-40% **A** from 18-40 min and maintained at 40% **A** from 40-45 min; 40-10% **A** from 45 to 50 min and kept at 10% **A** from 50-55 min). Detection of the targeted phenolic acids was made at 280 nm. The analytes were identified by matching the retention time and spiking samples with pure standards and quantification was based on an external standard calibration method. The selected phenolic acids were identified at different retention times (Fig. 1). Gallic acid was identified at 3.96 min (retention time) while *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid and sinapic acid were identified at 11.97, 12.44, 19.04 and 22.13 min (retention time), respectively.

Statistical analysis

The replicated data were pooled and a CRD two factor factorial design was used for analysis of variance (ANOVA) using *Statistix 8.1*. A significance level of $p<0.05$ was used for determining differences among the interaction of field capacity levels and foliar application of plant growth regulators. The differences among the obtained mean values of above mentioned parameters were compared and computed by using Tukey's HSD test considering 5% probability level (Steel et al., 1997).

Results

Plant vigour evaluation

Exogenously applied PGRs significantly improved seedling vigour of moringa plants. BAP and MLE were the treatments that most improved moringa shoot length at 75% field capacity (24.16 and 22.00 cm, respectively) while at 40% field capacity, MLE was most effective. (Tab. 1). In general, moringa plants grow best in areas of low water availability. Perhaps as a result, the plants grew less in height under 100% field capacity. Moringa plants that were not subjected to foliar application (control) had varied root length, with the maximum being recorded at 75% field capacity followed by 100 and 40% levels. The results showed that moringa can endure 75% field capacity with no reduction in root length, but under 40% field capacity level, a significant decrease was observed. A similar trend was observed in moringa leaf score, BAP maximally increased leaves of moringa plants at 75% field capacity followed by MLE (35.11 and 29.56, on average respectively) while no significant improvement was recorded at 40% field capacity. Moreover, maximum roots were counted in moringa plants grown at 40% field capacity when the plants were exogenously applied with ascorbic acid (Tab. 1).

Chlorophyll and total phenolic contents

It was noted that BAP and MLE performed best in improving chlorophyll *a* and *b* contents. Moringa leaves showed variation in expressing chlorophyll *a* contents under different field capacity levels which were further improved when subjected to foliar application of plant growth regulators. Maximum chlorophyll *a* contents were observed when moringa plants were sprayed with BAP and MLE at 75% field capacity level (28.87 and 26.73 µg g^{-1}, respectively) followed by ascorbic acid (23.47 µg g^{-1}) at the same field capacity. A similar trend was observed in chlorophyll *b* contents (Tab. 2). Moreover, maximum total phenolic contents were recorded when moringa plants were sprayed with BAP or MLE at 75% field capacity level (651.67 and 639.08 µg g^{-1}, respectively). At 40% field capacity level, MLE performed well in improving total phenolic contents (621.09 µg g^{-1}) which were 57% more than untreated plants at 40% field capacity (Tab. 2).

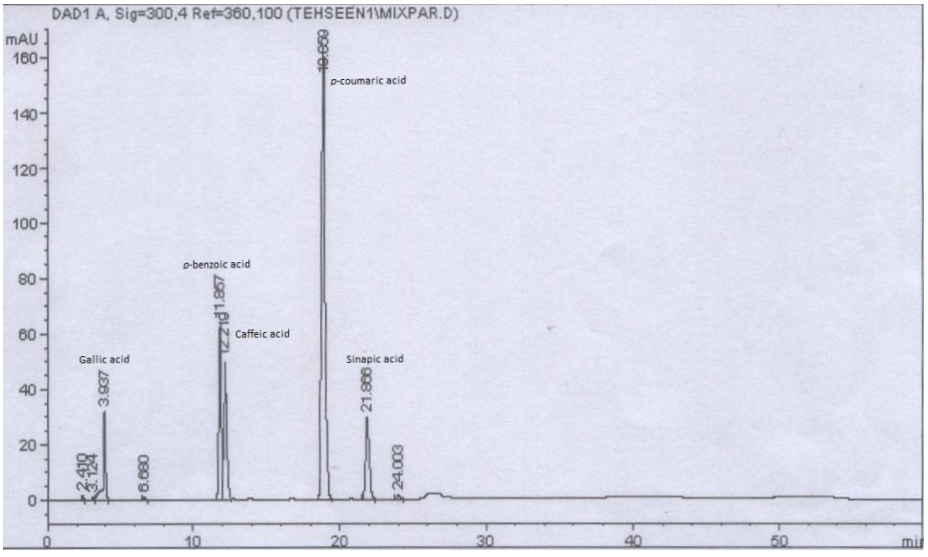

Fig. 1: A typical RP-HPLC chromatogram of mixture of phenolics acid standards. Peak identification: 1: gallic acid (RT 3.96), 2: *p*-hydroxyl benzoic acid (RT 11.97), 3: caffeic acid (RT 12.44), 4: *p*-coumeric acid (RT 19.04), 4: sinapic acid (RT 22.13)

Tab. 1: Effect of foliar application of plant growth regulators on shoot length (cm), root length (cm), number of leaves and roots of moringa seedlings under different field capacity levels.

Treatments	Shoot Length (cm)			Root Length (cm)		
	100%	75%	40%	100%	75%	40%
Control	11.24±0.60 e	15.28±0.83 b-e	12.33±0.65 de	5.06±0.47 de	6.21±0.47 b-e	4.62±0.18 e
Water	11.61±1.23 de	15.40±0.93 b-e	12.88±1.25 de	5.49±0.77 c-e	6.21±0.18 b-e	4.77±0.07 e
BAP	17.36±0.30 a-e	24.16±0.60 a	17.99±1.83 a-e	8.23±0.81 a-c	10.11±0.94 a	6.21±0.47 b-e
Ascorbic acid	16.26±1.76 b-e	18.29±1.82 a-e	14.28±2.05 c-e	6.93±0.61 b-e	7.37±0.31 a-e	5.63±0.53 c-e
MLE	18.58±2.57 a-d	22.00±1.21 ab	20.19±0.58 a-c	7.66±0.71 a-d	8.52±1.51 ab	6.64±0.35 b-e
	Leaves			Roots		
	100%	75%	40%	100%	75%	40%
Control	16.44±1.16 d-f	25.44±1.16 a-d	13.44±1.19 gh	9.44±0.83 f	13.22±1.21 d-f	21.56±1.77 a-e
Water	19.33±3.06 c-f	27.67±1.47 a-c	13.78±0.54 ef	14.11±1.80 c-f	20.11±0.54 a-e	25.11±2.65 ab
BAP	28.78±4.53 a-c	35.11±1.19 a	16.56±1.83 d-f	11.44±0.14 ef	17.78±2.52 b-f	23.89±4.91 a-d
Ascorbic acid	21.22±4.01 b-e	26.56±3.83 a-d	11.22±0.98 ef	17.78±0.59 b-f	23.33±0.63 a-d	29.44±2.14 a
MLE	24.00±0.85 b-d	29.56±3.25 ab	9.67±1.03 f	12.56±2.81 ef	18.00±0.24 b-f	24.33±5.89 a-c

Means not showing same letters differ significantly at 5% probability level, Critical value for comparison for Shoot Length: 7.16, Critical value for comparison for Root Length: 2.80, Critical value for comparison for Leaves: 10.15, Critical value for comparison for Roots: 10.67

Tab. 2: Effect of foliar application of plant growth regulators on chlorophyll *a*, chlorophyll *b* and total phenolic contents of moringa leaves under different field capacity levels.

Treatments	Chlorophyll *a* (µg g^{-1})			Chlorophyll *b* (µg g^{-1})		
	100%	75%	40%	100%	75%	40%
Control	18.67±0.47 d-f	20.03±0.35 c-e	16.37±0.51 ef	5.17±0.53 fef	6.67±1.54 d-f	3.43±0.75 f
Water	18.97±0.32 d-f	20.83±0.46 cd	17.20±0.25 d-f	5.23±0.50 ef	8.33±0.39 b-e	4.77±0.23 ef
BAP	20.13±0.71 c-e	28.87±0.29 a	17.97±2.01 d-f	7.30±0.32 c-e	16.70±0.49 a	7.33±1.42 c-e
Ascorbic acid	19.17±0.22 de	23.47±0.29 bc	15.13±2.42 f	5.90±0.81 ef	10.67±0.59 bc	6.30±0.90 ef
MLE	19.00±0.19 d-f	26.73±0.53 ab	21.10±0.89 cd	6.23±0.36 ef	11.20±1.14 b	10.03±1.33 b-d
	Total Phenolic Contents (µg g^{-1})					
	100%		75%		40%	
Control	363.53±16.35 c		431.67±14.26 bc		394.64±13.82 bc	
Water	370.19±28.12 bc		455.38±19.69 c-e		418.34±14.28 bc	
BAP	567.97±18.07 ab		651.67±36.53 a		557.6±21.78 a-c	
Ascorbic acid	462.79±15.74 b-e		562.04±20.55 a-c		490.93±14.73 a-c	
MLE	495.38±19.31 a-c		639.08±13.36 a		621.09±23.10 a	

Means not showing same letters differ significantly at 5% probability level, Critical value for comparison for Chlorophyll *a*: 3.91, Critical value for comparison for Chlorophyll *b*: 3.65, Critical value for comparison for Total Phenolic Contents: 198.68

Enzymatic antioxidant activities

The antioxidant enzymes SOD, POD, and CAT were significantly affected by varying field capacity and foliar application of plant growth regulators. An increase in SOD activity was observed in moringa plants with decrease in field capacity while the plants subjected to foliar application of plant growth regulators improved SOD activities in moringa plants. Maximum SOD activity was observed in BAP applied moringa plants (418.55 unit mg^{-1} protein) at 75% field capacity, with lower levels (390.91 unit mg^{-1} protein) under application of ascorbic acid (Fig. 2). Moreover, maximum POD activities (1260.93 units mg^{-1} protein) were recorded at 40% field capacity when moringa plants were exogenously applied with ascorbic acid followed by MLE (1067.55 units mg^{-1} protein) (Fig. 3). Catalase activity showed varying trends with decreasing field capacity. Maximum CAT activity was observed at 75% field capacity, followed by 100 and 40% field capacity levels. At 75% field capacity, BAP and MLE maximally improved CAT activity followed by ascorbic acid (125.16, 112.60 and 97.60 unit mg^{-1} protein, respectively). The lowest CAT activity was recorded at the lowest field capacity in untreated moringa plants (Fig. 4).

Crude protein and mineral analyses

Moringa plants were also analyzed for their nutritional quality. Maximum crude protein (CP) contents were recorded at 75% field

capacity when the plants were subjected to MLE foliar application (29.77 mg kg^{-1}). BAP foliar application was found effective at 75 and 100% field capacities (28.68 and 26.62 mg kg^{-1}, respectively) (Fig. 5). Maximum calcium contents were exhibited by BAP and MLE at 75% field capacity level followed by ascorbic acid at same level (3951.87, 3917.6 and 3322.76 mg kg^{-1}, respectively) while the lowest calcium contents were found in moringa plants grown at 40% field capacity (Fig. 6). Maximum potassium contents were observed at 75% field capacity with foliar application of MLE > BAP > ascorbic acid (3122.47, 2203.76 and 2014.29 mg kg^{-1}). At 100% field capacity, maximum potassium contents were found in moringa plants sprayed with MLE > ascorbic acid > BAP (Fig. 7). MLE and ascorbic acid foliar applications were equally effective in improving magnesium contents of moringa leaves at 75 and 100 field capacity levels while a drastic reduction in these contents was recorded at 40% field capacity (Fig. 8). In the case of phosphorous, BAP was the best foliar application for improving phosphorous contents followed by MLE at 75% field capacity (2787.48 and 2707.79 mg kg^{-1}, respectively). These both treatments were also found at 100% field capacity (Fig. 9).

Separation and quantification of phenolic acids by RP-HPLC

As per HPLC analysis, five phenolic acids (gallic acid, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid and sinapic acid) were

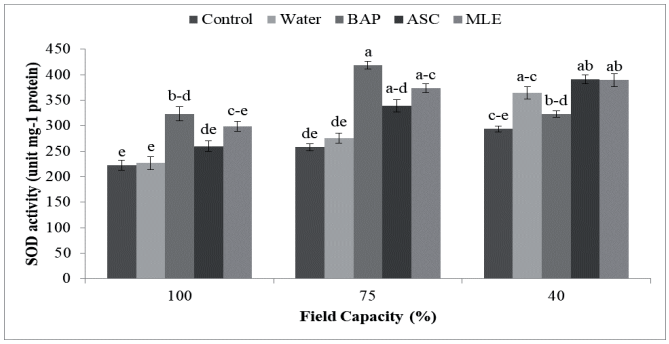

Fig. 2: Effect of foliar application of plant growth regulators on superoxide dismutase activity in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 88.42

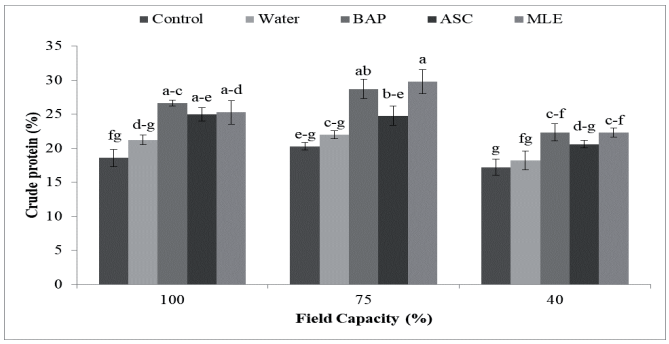

Fig. 5: Effect of foliar application of plant growth regulators on crude protein (%) in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 4.88

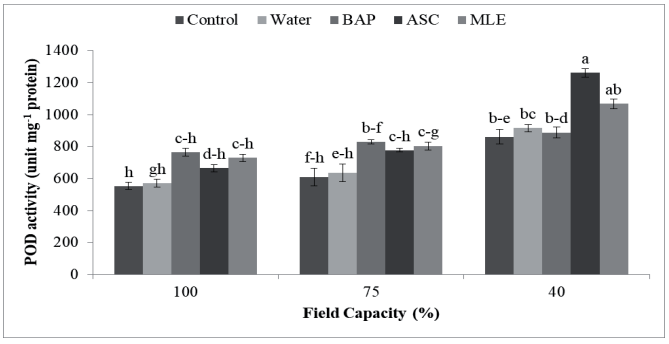

Fig. 3: Effect of foliar application of plant growth regulators on peroxidase activity in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 247.91

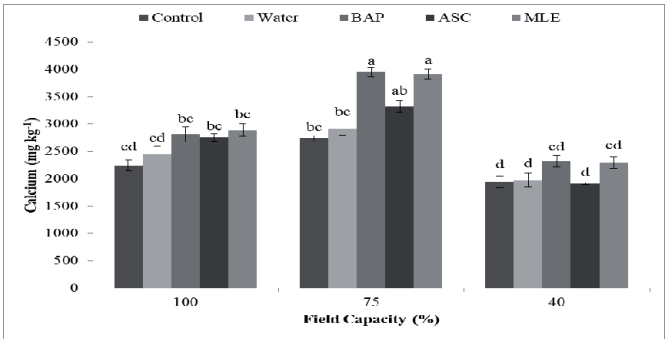

Fig. 6: Effect of foliar application of plant growth regulators on calcium content (mg kg^{-1}) in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 703.19

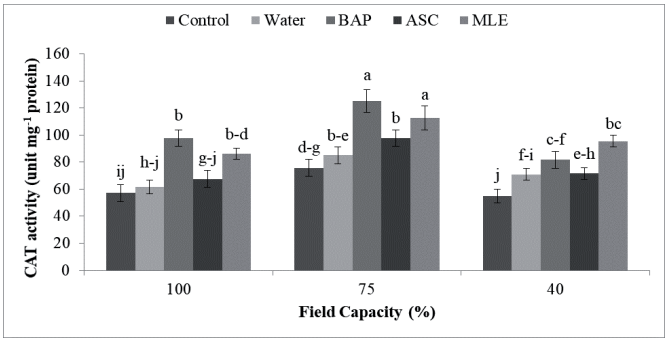

Fig. 4: Effect of foliar application of plant growth regulators on catalase activity in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 25.49

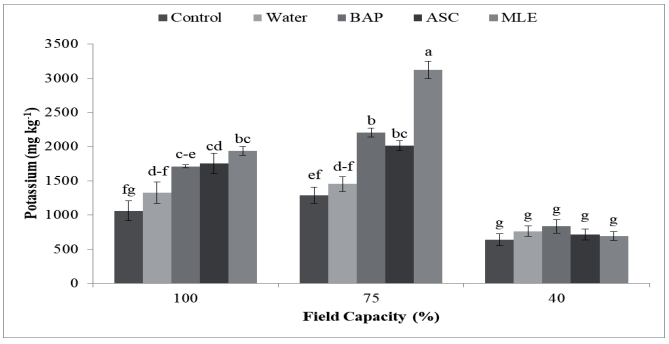

Fig. 7: Effect of foliar application of plant growth regulators on potassium content (mg kg^{-1}) in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 439.29

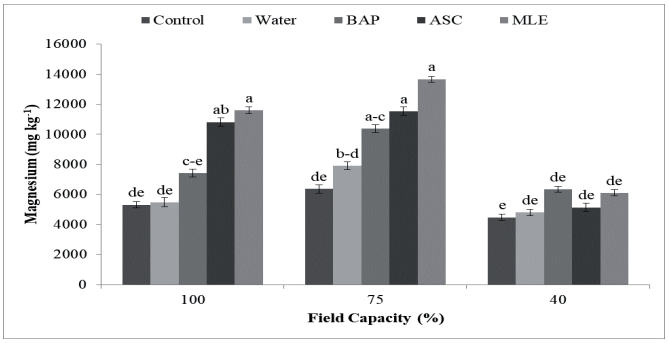

Fig. 8: Effect of foliar application of plant growth regulators on magnesium content (mg kg^{-1}) in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 3382.0

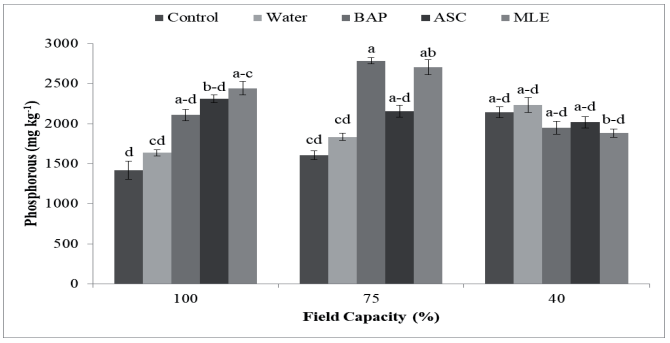

Fig. 9: Effect of foliar application of plant growth regulators on phosphorous content (mg kg^{-1}) in moringa leaves under different field capacity levels: Means not showing same letters differ significantly at 5% probability level, Critical value for comparison: 848.75

identified and quantified in the moringa leaves. A significant variation in these selected phenolic acids was observed in moringa leaves with decrease in field capacity and with application of foliar plant growth regulators. BAP mostly increased *p*-hydroxybenzoic and caffeic acid content (25.62 and 26.48 µg g^{-1}, respectively), whereas higher amount of gallic, *p*-coumaric and sinapic acids was recorded when moringa plants were sprayed with ascorbic acid (20.93, 19.06 and 12.35 µg g^{-1}, respectively). As a conclusion, increase in *p*-hydroxybenzoic acid and caffeic acid can be associated with BAP exogenous application while maximum content of gallic acid, *p*-coumaric acid and sinapic acid were associated with foliar application of ascorbic acid. MLE application ranked 3rd in improving or maintaining selected phenolics (Tab. 3). As a result of correlation matrices, *p*-hydroxybenzoic acid, gallic acid and sinapic acid contents were found as positively correlated with shoot length while gallic acid showed a positive correlation with root length. Moreover, *p*-coumaric acid was positively correlated with number of roots, and activities of enzymatic antioxidants (Tab. 4).

Discussion

Study of the stress physiology of plants examines factors such as salinity, heat, cold, and drought, which affect agricultural yield, nutritional quality, antioxidant systems, and phenolic compounds. In the present study, we found that growth of moringa plants was affected at 100, 75 and 40% field capacity which was mitigated by applying plant growth regulators, especially in the case of root growth and expansion. Prolific and extended root growth is a plastic response to water deficit allowing plants to uptake water and nutrients from deep soil (Nguyen et al., 1997; Kavar et al., 2007). Root extension and proliferation under drought stress has been noted in moringa seedlings, as well as in crops as diverse as tea, onion, and cotton (Farooq et al., 2009; Nouman et al., 2014a). Increase in number of roots at 75 and 40% field capacity might involve lower osmotic pressure under stress conditions which trigger root elongation in search of water and nutrients (Hsiao and Xu, 2000). Similar reactions of moringa plants have been previously reported under saline conditions (Nouman et al., 2012a, 2014a).

Decline in number of leaves and increase in number of roots under 40% field capacity likely represents an adaptive plastic allocation pattern that reduces transpiration loss through leaves with a compensating water absorption surface area by proliferated roots (Jefferis and Rudmik, 1991; Houle et al., 2001). In the present study, foliar application of synthetic and natural plant growth regulators improved moringa tolerance to drought conditions. Among these, BAP and MLE were effective in inducing drought tolerance. BAP and MLE have been previously reported as effective plant growth regulators to improve plant survival and vigour under stress conditions (Ali et al., 2011; Nouman et al., 2012b, 2014a) whereas this was the first time that they were tested to investigate the response of moringa plants under water deficit with the application of plant growth regulators, especially moringa leaf extract. Moreover, Rivas et al. (2012) and Arauja et al. (2016) reported that moringa plants can withstand under water stress conditions while the variation in its chlorophyll contents, nutritional quality and phenolic acid contents were studied in the present investigation.

The growth behavior of plants can be linked to chlorophyll content and plant survival under stress conditions might be attributed to the existence of total phenolic contents which serve as antioxidants. Decrease in chlorophyll content under water deficit is a common phenomenon that can be compensated by using plant growth regulators (Bijanzadeh and Emam, 2010; Din et al., 2011). Drought tolerant plants have higher chlorophyll content, mitigating drought stress, with chlorophyll content being improved by exogenous application of plant growth regulators (Farooq et al., 2009). In the present investigation, a similar improvement in chlorophyll content was observed under stress conditions when plants were sprayed with MLE and BAP Increase in chlorophyll content by the application of plant growth regulators has been previously reported in moringa plants under saline conditions (Nouman et al., 2014a). It can be concluded here that moringa plants show higher chlorophyll content under low field capacities and these can be further improved with the exogenous application of MLE and BAP.

In addition to chlorophyll content, antioxidant enzymes are another factor that affects plant tolerance in stressful conditions. Plants produce reactive oxygen species, which affect plant vigour and results in a lower nutritional quality. Enzymatic antioxidants enable plants to resist such oxidative damage. Higher antioxidant activities have been reported in drought tolerant plants in comparison to intolerant ones (Lum et al., 2014). In the present study, a similar trend was observed. With increasing water deficit, SOD, POD, and CAT activity increased, further increased by plant growth regulators especially BAP, ascorbic acid and MLE. Previously, an increase in antioxidant activity in moringa plants under saline conditions was reported (Nouman et al., 2014a). Because antioxidants help buffer environmental challenges, they help to maintain nutritional quality despite stress.

Nutrient uptake, antioxidant compounds i.e., phenolic acids and water availability are intimately related, and this relationship might shift under stress conditions. Plant growth regulators can assist plants in mitigating drought stress enabling regulation of phenolic acids (Garg, 2003). The present investigation showed that moringa plants can even perform better at 75% field capacity than at 100%

Tab. 3: Effect of foliar application of plant growth regulators on selected phenolic acids (µg g^{-1}) of moringa leaves under different field capacity levels.

Treatments	*p*-hydroxybenzoic acid			Caffeic acid		
	100%	75%	40%	100%	75%	40%
Control	12.75±0.48 b	16.11±0.97 ab	14.76±0.62 ab	8.83±0.33 d	14.70 cd	13.72±0.42 cd
Water	15.48±0.67 ab	16.89±0.66 ab	16.06±0.88 ab	10.80±0.34 cd	16.84±0.59 cd	16.66±0.65 a-d
BAP	21.81±1.01 ab	29.74±1.23 a	25.32±0.97 ab	21.34±0.88 a-d	29.93±0.93 a	28.17±0.92 ab
Ascorbic acid	23.65±1.24 ab	24.97±0.98 ab	21.70±1.28 ab	20.04±0.76 a-d	24.05±1.24 a-d	22.60±1.36 a-c
MLE	13.54±0.57 ab	28.01±1.01 ab	23.48±1.19 ab	16.31±1.03 b-d	21.46±0.79 a-d	16.20±0.74 b-d
	Gallic acid			*p*-coumaric acid		
	100%	75%	40%	100%	75%	40%
Control	8.78±0.29 c	10.36±0.37 c	9.19±0.35 bc	6.37±0.21 f	7.13±0.29 f	8.20±0.28 d-f
Water	8.89±0.21 c	12.60±0.40 c	10.07±0.40 bc	6.27±0.28 f	7.33±0.31 ef	8.63±0.32 c-f
BAP	11.94±0.39 bc	17.58±0.57 a-c	14.29±0.56 a-c	12.08±0.39 c-e	12.87±0.59 b-d	13.52±0.48 bc
Ascorbic acid	20.18±0.64 ab	23.76±1.11 a	18.86±0.60 a	17.76±0.55 ab	19.24±0.51 a	20.18±0.79 a
MLE	16.42±0.62 a-c	14.71±0.45 bc	14.71±0.49 a-c	7.47±0.28 ef	9.35±0.31 c-f	10.28±0.39 c-f
	Sinapic acid					
	100%		75%		40%	
Control	ND		5.83±0.21 c-e		4.05±0.16 ef	
Water	ND		5.80±0.20 c-e		4.92±0.19 d-f	
BAP	7.16±0.29 b-e		9.68±0.37 b-d		9.98±0.36 bc	
Ascorbic acid	11.03±0.34 ab		15.60±0.46 a		10.43±0.43 bc	
MLE	7.03±0.27 b-e		9.26±0.41 b-d		7.93±0.29 b-e	

Means not showing same letters differ significantly at 5% probability level, Critical value for comparison for *p*-hydroxybenzoic acid: 16.69, Critical value for comparison for caffeic acid: 13.17, Critical value for comparison for gallic acid: 8.74, Critical value for comparison for *p*-coumaric acid: 4.90, Critical value for comparison for sinapic acid: 5.04.

Tab. 4: Correlation among growth parameters (shoot length, number of leaves, root length, number of roots), enzymatic antioxidant activities (superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)) and the selected phenolic acid contents in moringa leaves.

Phenolic acids	Shoot length	Leaves	Root length	Roots	SOD	POD	CAT
p-Hydroxybenzoic acid	0.92**	0.44 ^{NS}	0.49 ^{NS}	0.45 ^{NS}	0.73*	0.20 ^{NS}	0.88*
Caffeic acid	0.83*	0.26 ^{NS}	0.31 ^{NS}	0.62 ^{NS}	0.85*	0.38 ^{NS}	0.78*
Gallic acid	0.99**	0.79*	0.82*	0.01 ^{NS}	0.35 ^{NS}	0.25 ^{NS}	0.99*
p-Coumaric acid	0.17 ^{NS}	0.52 ^{NS}	0.47 ^{NS}	0.99**	0.97**	0.93**	0.09 ^{NS}
Sinapic acid	0.88*	0.35 ^{NS}	0.40 ^{NS}	0.54 ^{NS}	0.79*	0.29 ^{NS}	0.84*

field capacity with the application of plant growth regulators. We observed a significant increase in moringa leaf mineral and crude protein contents. Based on findings regarding phenolic acid composition, it can be predicted that phytochemicals of moringa leaves vary with changes in water availability and application of plant growth regulators. Under stress conditions, changes in phenolic acids have been previously reported in different plants like *Solanum lycopersicum* and *Salvia officinalis* (Dixon and Paiva, 1995; Bettaieb et al., 2011; Ali and Ghada, 2014). The present investigation supports the finding that with moringa leaves exhibited lower phenolic acids at 100% field capacity while an increase was recorded with decrease in field capacity. Moringa leaves exhibited maximum phenolic acid contents when grown at 75% field capacity while the least quantities were recorded at 100% field capacity. The results of the present study also suggest that plants exhibit varying amount of phenolic acids under different field capacity which could improve antioxidant system to mitigate stress conditions. Moreover, the change in phenolic acids can be correlated with growth behavior of *M. oleifera*. In addition, a positive correlation was found among the selected phenolic acids and SOD, POD and CAT activities. Similarly, shoot length, number of leaves and root length were positively correlated with gallic acid (0.99, 0.79 and 0.82, respectively). These findings support the notion that phenolic acids protect plant tissues against oxidative damage inhibiting decrease in nutritional quality of moringa leaves under abiotic stress (Ali et al., 2014). Caffeic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid and are considered as potential antioxidants due to their polyhydroxy nature (Natella et al., 1999; Sgherri et al., 2004). By studying correlation among these phenolic acids, growth parameters and enzymatic antioxidant activities, *p*-hydroxybenzoic acid and caffeic acid were positively correlated with SOD and CAT activities while *p*-coumaric acid was significantly correlated with SOD and POD activities. As activities of enzymatic antioxidants and composition of phenolic acids increased under unfavorable growing conditions of moringa, it can be conclu-

ded that moringa expedites its antioxidant activities improving the phenolic acids to mitigate unfavorable conditions.

Keeping in view the findings of the present study, moringa plants can grow better under low water availability areas where other crops are difficult to cultivate. It can be suggested to farmers that it is preferable to cultivate moringa as fodder for their livestock under water deficit areas rather to leave the land as fallow. By these means, the farmers cannot only bring their abandoned land under cultivation but they can also have fodder for their livestock being rich in essential nutrients and further can be used as nutraceutical as phenolic rich source.

Conclusion

It can safely be concluded here that moringa can be cultivated as a fodder crop in areas facing water shortage and even the tolerance can be improved by the exogenous application of BAP and MLE. Among these both, MLE is more suitable for farmers as it is economical, easily available and environment friendly being directly extracted from moringa leaves while BAP is synthetic plant growth regulator which is relatively expensive than MLE.

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