

Effect of *Moringa oleifera* Lam. leaf aqueous extract on growth attributes and cell wall bound phenolics accumulation in maize (*Zea mays* L.) under drought stress

Kiran Pervez, Faizan Ullah*, Sultan Mehmood, Adnan Khattak

Dept. of Botany University of Science and Technology, Bannu, KP, Pakistan

*Corresponding author: drfaizanwazir@gmail.com

Abstract

Drought stress is one of the major abiotic stresses affecting maize yield and quality. Scientists are struggling for the production of natural growth stimulants to improve drought stress tolerance of crop plants. *Moringa oleifera* (*M. oleifera*) is a good source of natural antioxidants and natural phenolics and its leaf extracts has been used as a bioregulator for improving growth of various crop plants. Present study evaluated the effect of different concentrations (such as 25, 12.5, 6.25, 3.13 and 1.56 %) of *Moringa* aqueous leaf extract (MALE) on growth and cell wall bound phenolics of maize cv. Azam under drought stress (soil humidity 10%). Exogenous application of MALE significantly improved maize leaf soluble proteins, leaf relative water content (LRWC), shoot and root fresh weight and dry weight, root area, root length and root width under drought stress. Drought stress caused accumulation of cell wall bound phenolics which were decreased by foliar application of MALE. It was inferred that MALE could be a potential bioregulator for improving growth of maize under drought stress.

Keywords: Drought stress tolerance; leaf relative water content; leaf soluble proteins; phenolics; root area.

1. Introduction

Climate change is associated with altered patterns of rainfall, elevated levels of CO₂ and temperature, which are adversely affecting agriculture of the world (Aydinalp & Cresser, 2008). The consequences of climate change are floods, drought, desertification, salinity, rising temperature and weather extremes (Nelson *et al.*, 2009; Saifuddin *et al.*, 2016). Due to lack of proper resources, developing countries will be at risk of climate change in near future (IPCC 2007). On the other hand population of world is increasing rapidly with increasing demand for food. Therefore, to feed the ever increasing world population, there is need to overcome the problems related to climate change harmful to agriculture. Drought stress, salt stress and high temperature stress are the common non-living factors adversely influencing the growth and productivity of plants. Among these various abiotic stresses, drought stress is very important because it affects almost every aspect of plant life (Walthall *et al.*, 2012). Decline in water supply to plant alter its cell membrane structure, disturb metabolic activity, leads to low relative water content, low mineral uptake and chlorophyll content (Pospíšilová *et al.*, 2000; Egilla *et al.*, 2005; Tas & Tas, 2007; Ullah *et al.*, 2012). Previous studies have shown that root growth, canopy height and leaf area index were declined in maize by water stress (Hirich *et al.*, 2012).

Adverse effects of drought stress on crop plants can be minimized by the exogenous application of synthetic growth regulators, antioxidants, organic and inorganic chemicals and nutrients (Farooq *et al.*, 2009). However, continuous use of commercially available plant hormones and synthetic

compounds as osmo-protectants are usually not cost effective and environment friendly (Pizzale *et al.*, 2002). It is due to the eco-toxicological effects of these synthetic compounds that presently consumers are asking for natural products, which have resulted in increased application of natural antioxidants in agriculture (Kaur & Kapoor, 2001). Bioregulators are substances that manipulate growth, development and composition of plants and function by interaction with the endogenous phytohormone groups (Bibi *et al.*, 2016). Their actions include growth promotion or retardation, flower induction, hastening maturity or senescence, enlarged biomass production etc. Plant based phytochemicals provide a promising source for new growth regulating compounds (Narwal, 2004). Plant phenolics compounds are known for their function as antioxidants due to their free radical-scavenging capabilities (Fauconneau *et al.*, 1997; Jain *et al.*, 2013; Hassan *et al.*, 2015; Temizel *et al.*, 2015). The same bioactive compounds present in plant extracts may function either as growth stimulator or inhibitor depending upon the concentration in which it is applied (Popa *et al.*, 2008; Popa *et al.*, 2002). *Moringa oleifera* Lam. is a fast growing small tree (512- m tall). It has potential to cope with prolonged drought stress conditions (Martin, 2000). The foliar application of the leaf extracts of the *M. oleifera* were reported stimulatory on growth of soybean, sorghum, tea, melon, chilli and tomato (Fuglie, 2000; Yasmeen *et al.*, 2014). *M. oleifera* leaf is a good source of natural phenolics with higher antioxidant activity (Pakade *et al.*, 2013). Moreover, its extracts also contain zeatin (natural derivative of cytokinin), vitamins and mineral elements such as K, Ca and Fe (Siddhuraju & Becker, 2003).

Maize (*Zea mays* L.) is an important cereal crop feeding a large portion of the world population. It also provides food for poultry, fodder for livestock and raw material for the industry (Edmeades, 2013). Previous studies have shown that growth and grain yield of maize is severely affected by drought stress. Early stage of seedling growth and establishment is very sensitive to drought stress (Ali *et al.*, 2011). Thus cessation of elongation and expansion of cell stops growth of seedling (Anjum *et al.*, 2003). Therefore, in current climate scenario the protection of maize crop from adverse effects of drought stress using natural growth regulators is very important.

The present investigation was aimed to determine effects of shade dried *Moringa oleifera* aqueous leaf extract (MALE) on performance of maize plants under drought stress. The research work was based on hypothesis that foliar spray of *M. oleifera* extract can improve the content of endogenous phenolics of maize plants associated with enhanced tolerance to drought stress.

2. Materials and methods

2.1 Plant material

The fully expended and healthy leaves of *Moringa oleifera* were collected from wild in the district Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The collection from field did not involve any endangered plant species.

2.2 Preparation of aqueous extracts

The leaves were shade dried and ground finely using an electric grinder. Fifty grams of the plant material was soaked in 200 ml of distilled water. The mixture was kept at 25°C for 48 hours and filtered using Whatman No. 1 filter paper. The extract obtained was designated as 25 % extract which was further diluted to make 12.5%, 6.25%, 3.125% and 1.56% extracts.

2.3 Total phenolics determination of *M. oleifera* extracts

Folin Ciocalteu reagent was used for determination of total phenolics content with slight modifications (Singleton *et al.*, 1965; Chun *et al.*, 2003). The 1 ml of the aqueous extract was mixed with a freshly prepared 0.5 ml of Folin-Ciocalteu reagent. The solution obtained was kept at room temperature for 5 min prior to addition of 7.5% sodium carbonate solution. The volume of mixture was raised to 8 ml using autoclaved distilled water and kept for 2h. Measurements of the absorbance were made at 765nm by using a spectrophotometer (Hitachi's U-510 Tokyo Japan). Standard curve was generated by using various concentrations of gallic acid and the measurements were compared to the

plotted standard curve. The content of total phenolics was articulated as mg gallic acid equivalents / ml extract.

2.4 Bioassay

Seeds of maize var. Azam were obtained from Agriculture Research Station Sara-e-Naurang, Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The seeds were surface sterilized with 0.2% solution of mercuric chloride for 23- minutes. After sterilization seeds were thoroughly washed with autoclaved distilled water and dried on a filter paper. The seeds were grown into plastic pots (8x12cm²) filled with a mixture of autoclaved clay and sand in ratio of 1:1. Five seeds were grown per pot. The pots were arranged in complete randomized design (CRD) and placed in a glass house. After establishment of seedlings for 21 days, plants were supplied with a foliar spray of *M. oleifera* aqueous extracts (till the formation of drip) and subjected to 10 days drought stress. Another foliar spray of the MALE was made six days after imposition of drought stress. For all the drought treatments soil moisture content was 10 %. For control treatments, soil humidity was maintained about 25%. Soil moisture content was determined on dry weight basis (Bano *et al.*, 2012).

The treatments were: Control plants having normal water supply, plants sprayed with 25 % MALE, plants sprayed with 12.5 % MALE, plants sprayed with 6.25 % MALE, plants sprayed with 3.125 % MALE, plants sprayed with 1.56 % MALE, plants sprayed with 25 % MALE and subjected to drought stress, plants sprayed with 12.5 % MALE and subjected to drought stress, plants sprayed with 6.5% MALE and subjected to drought stress, plants sprayed with 3.125% MALE and subjected to drought stress, plants sprayed with 1.56% MALE and subjected to drought stress.

After 10 days of drought stress, plants were harvested and observed for various physiological and morphological attributes.

2.5. Determination of relative water content of maize leaves
Determination of leaf relative water content (LRWC) was based on the method of Gaoz (2000).

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

FW represents sample fresh weight; TW represents sample turgid weight; DW represents sample dry weight.

2.6. Extraction and determination of cell wall bound phenolics

The method of Campbell & Ellis (1992) was used for the extraction of ester bound phenols incorporated in the cell wall. A total of 0.5 g leaf tissue was extracted in 80% methanol. The suspension thus obtained was subjected to centrifugation (12,000 g) for 20 min. The supernatant obtained was discarded and an alcohol insoluble residue containing

plant cell wall material was used for the extraction of cell wall bound phenolics. The 10 mg cell wall material was suspended in 1 mL NaOH (0.5 M) for 1 h at 96°C. These mild saponification conditions resulted in the release of cell wall-esterified hydroxycinnamic acid derivatives. The supernatant obtained was acidified (pH 2) with HCl and centrifuged at 12,000 g for 15 min and extracted with anhydrous diethyl ether (1 ml). The extract thus obtained in diethyl ether was reduced to dryness in a speedvac. The precipitate obtained was suspended in 0.25 ml of aqueous methanol (50%) and centrifuged (12000 g). Folin-Ciocalteu reagent was used for determination of wall-esterified phenolic acids content in the solution (Latif *et al.*, 2016).

2.6.1. Folin-Ciocalteu assay

The method for determination of phenolics is based on the reduction of the phospho-molybdene/phospho-tungstate occurring in the Folin-Ciocalteu reagent (Swain & Hillis, 1959). The supernatant (50 µl) was diluted to 0.25 ml by using 50% methanol. The mixture thus prepared was added to 0.25ml of freshly prepared diluted Folin-Ciocalteu reagent (50% v/v). After incubation for 3 min, 0.5 ml of the saturated aqueous sodium carbonate was added to the mixture and was kept in the dark at 25°C in a water bath for 60 min to complete the reaction. The absorbance absorption was measured at 765nm using a spectrophotometer (Hitachi, U-510 Tokyo Japan). The measurements were compared with a standard curve prepared by using various concentrations (0 to 32 µg) of gallic acid solution. The concentration of cell wall bound phenolic compounds was expressed as µg gallic acid equivalents / g fresh weight.

2.7. Determination of soluble proteins content of maize plants

The method of Lowery *et al.* (1951) was used for extraction and determination of leaf total soluble proteins content. Bovine serum albumen (BSA) was used as standard.

2.8. Determination of morphological traits of maize

After harvest, measurement of shoot fresh weight and shoot dry weight, root fresh weight and root dry weight was made by using an electronic balance. Root length, root width, root edges and root area was determined using Root Law Software (Washington State Research Foundation, USA).

2.9. Statistical analyses

The data were treated by analysis of variance (one way ANOVA). The mean values were compared by least significant differences (LSD) test (Steel & Torrie, 1984).

3. Results and discussion

A number of allelochemicals are present in cells and tissues of plants (Ashrafi *et al.*, 2008). Recent researches have shown that phenols were the most effective substances on germination, seedling growth and cell division (Khan *et al.*, 2011; Ullah *et al.*, 2014). Moringa oleifera aqueous leaf extract (MALE) showed the presence of phenolic compounds (Figure 1). Maximum phenolic compounds (24.32 mg gallic acid eq./ml extract) were recorded in 25% extract followed by 12.5 % extract (17.65 mg gallic acid eq./ml extract), 6.25% extract (12.52 mg gallic acid eq./ml extract), 3.13% extract (9.19 mg gallic acid eq./ml extract) and 1.56% extract (7.15 mg gallic acid eq./ml extract). Phenolic compounds have significant allelopathic potential for application in agriculture as herbicides, fungicides and insecticides (Santana *et al.*, 2009).

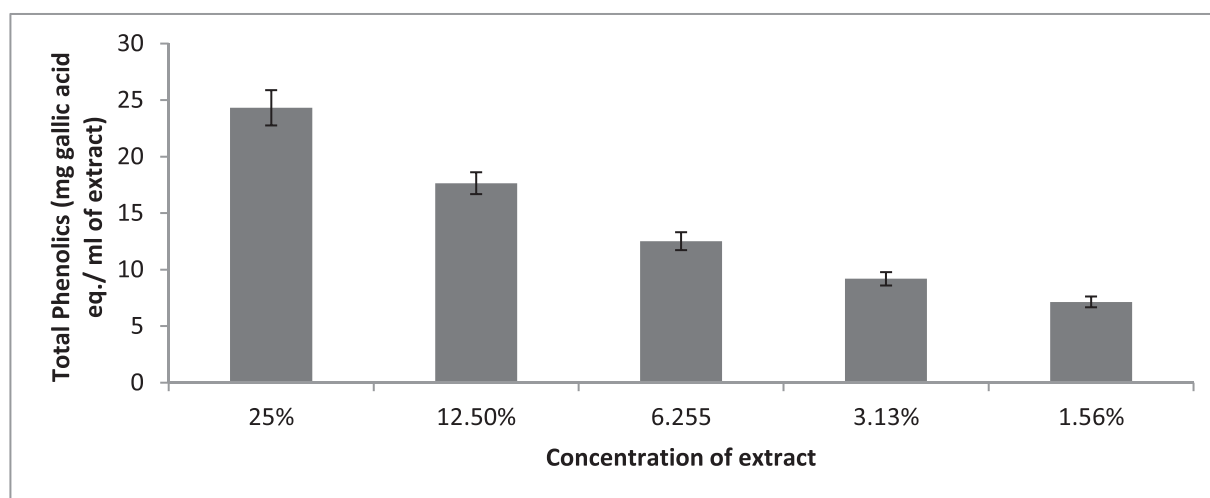


Fig. 1. Total Phenolics content of MALE

Under unstressed condition foliar application of MALE at 25% significantly increased shoot weight as compared to control (Table 1). Under drought stress shoot weight was significantly reduced. The reduction in shoot weight caused by drought stress was ameliorated by foliar application of MALE at 25%, 12.5%, 6.25% and 3.125%.

Root fresh and dry weights were significantly affected by MALE at $p > 0.05$ (Table 1). Under unstressed condition MALE at 25%, 12.5% and 6.25% significantly increased root weight as compared to untreated control. Drought stress significantly reduced root weight. Adverse effects of drought stress on root weight were minimized by MALE at all the concentrations. However, stimulatory effects MALE were higher at 25% and 12.5%.

The reduction in shoot weight under drought stress is an adaptation of maize plants to drought stress conditions (Ali *et al.*, 2011). The beneficial effects of MALE on maize growth can be attributed to the enhanced meristematic activity in growth zones as was also reported for sea weed extracts (Wu & Lin, 2000). The amelioration of adverse effects of drought stress on growth attributes of maize by MALE might be due to its phenolics composition and zeatin content, a natural derivative of cytokinin (Siddhuraju & Becker, 2003; Yasmeen *et al.*, 2013; Hura *et al.*, 2013). The promotive effects of cytokinin on plant growth might be due to their stimulatory effects on cell division and differentiation (Werner *et al.*,

2001). These results are in confirmatory to Ali *et al.* (2011), who have reported mitigation of drought stress in maize plants by foliar application of frozen moringa leaf extract.

Drought stress significantly reduced root width. However foliar application of MALE significantly ameliorated adverse effects of drought stress on root width. Under unstressed condition MALE has no significant effect on root width (Table 1).

Under unstressed conditions MALE did not affect root area. Drought stress caused significant reduction in root area (Table 1). The reduction in root area as a result of drought stress was significantly minimized by foliar application of MALE. The beneficial effect of MALE at 25% was significantly more pronounced. Optimizing root architecture can overcome yield limitations in crop plants imposed by water or nutrient deficiencies. Plants having better root system can withstand not only environmental stresses but also increase its efficiency to survive under nutrient deficiency conditions (Coque & Gallais, 2006). During present investigation, maize plants receiving foliar spray of MALE exhibited better root system, which helped them to tolerate drought stress in a better way that might contribute to better crop yield. The Moringa leaf extract has been reported as an effective bio-regulator to improve growth and yield of crops such as *Phaseolus vulgaris* and maize (Mvumi *et al.*, 2013; Ali *et al.*, 2011).

Table 1. Effect of *Moringa oleifera* aqueous leaf extract (MALE) on morphological growth attributes of maize under drought stress

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root area (cm ²)	Root width (cm ²)
Control	1.6±0.019 ^{bc}	0.32±0.04 ^{bc}	1.95±0.03 ^c	0.40±0.06 ^e	6.00±0.17 ^{ab}	0.051±0.003 ^a
Drought stress	0.5±0.013 ^g	0.09±0.03 ^g	0.75±0.07 ⁱ	0.12±0.02 ⁱ	0.87±0.12 ^f	0.008±0.000 ^b
25% MALE	2.1 ±0.17 ^a	0.4±0.13 ^a	2.56±0.04 ^a	0.89±0.04 ^{ab}	6.42 ±0.13 ^a	0.042±0.001 ^a
12.5% MALE	1.5±0.14 ^{bc}	0.31±0.01 ^{bc}	2.6±0.12 ^a	0.91±0.09 ^a	5.99 ±0.29 ^{ab}	0.052±0.005 ^a
6.25% MALE	1.71±0.11 ^b	0.35±0.05 ^b	2.45±0.14 ^b	0.85±0.04 ^b	6.30±0.16 ^a	0.041±0.006 ^a
3.125% MALE	1.52±0.09 ^{bc}	0.29±0.07 ^{bc}	1.91±0.05 ^{cd}	0.72±0.09 ^c	6.15±0.29 ^{ab}	0.052±0.003 ^a
1.56% MALE	1.47 ±0.13 ^{bc}	0.27±0.04 ^{bc}	1.83±0.09 ^e	0.61±0.03 ^d	6.00±0.23 ^{ab}	0.051±0.007 ^a
25% MALE + drought stress	1.61 ±0.21 ^c	0.26±0.09 ^c	1.89±0.10 ^d	0.42±0.08 ^e	5.49±0.18 ^b	0.048±0.002 ^a
12.5% MALE+ drought stress	1.3 ±0.07 ^d	0.23±0.02 ^d	1.79±0.12 ^e	0.38±0.01 ^f	4.61 ±0.09 ^c	0.051±0.001 ^a
6.25% MALE+ drought stress	1.2 ±0.04 ^{de}	0.21±0.05 ^{de}	1.69±0.15 ^f	0.36±0.05 ^{fg}	3.27 ±0.18 ^d	0.049±0.001 ^a
3.125% MALE+ drought stress	1.0 ±0.06 ^{ef}	0.18 ±0.02 ^{ef}	1.59±0.09 ^g	0.30±0.07 ^{gh}	2.00±0.15 ^e	0.052±0.000 ^a
1.56% MALE+ drought stress	0.87 ±0.03 ^e	0.14±0.01 ^{fg}	1.48±0.13 ^h	0.29±0.02 ^h	1.81±0.14 ^e	0.048±0.007 ^a
LSD	0.026	0.052	0.054	0.039	0.682	0.017

Means sharing common English letters are statistically similar

Leaf relative water content (LRWC) is a key physiological parameter which can decide the tolerance potential of plants to induced drought stress (Sanchez-Blanco *et al.*, 2002). Drought stress significantly reduced leaf relative water content (LRWC) of maize plants. Foliar application of MALE significantly ameliorated adverse effects of drought stress on LRWC. The beneficial effects of MALE on LRWC under drought stress were higher at 25% and 12.5% concentration (Table 2). The inhibitory effects of drought stress on LRWC have been reported previously (Valentovic *et al.*, 2006; Bano *et al.*, 2012). The beneficial effect of MALE on LRWC may be attributed to its phytochemical composition (Yasmeen *et al.*, 2014). A phenolic compound salicylic acid was reported to improve LRWC of barley plants under drought stress (Habibi, 2012). The observed higher LRWC in MALE treatments under drought stress may be attributed to the increase in the content of phenolics in the cell wall and, therefore, have increased the hydrophobic character of the cell wall. Consequently, it is possible to suppose that such an unfriendly for water apoplast environment will limit water transport from the metabolically active inside of the cell, capillary transport of water in the apoplast and finally the cuticular transpiration (Hura *et al.*, 2013).

Leaf soluble proteins content was significantly affected by exogenously applied MALE as compared to control (Table 2). Higher soluble proteins content was recorded for 25% MALE followed by 12.5% MALE. Drought stress

significantly reduced leaf soluble proteins content. Foliar application of MALE at 25% and 12.5% minimized the adverse effects of drought stress on leaf soluble proteins content. Under drought stress MALE at 25% and 12.5% showed 19% and 17% increase in leaf soluble proteins content respectively as compared to drought treatment. The accumulation of soluble proteins in plants under drought stress, serve as a means of osmotic adjustment (Ma & Turner, 2006). The marked increases in leaf soluble proteins content due to foliar application of MALE under drought stress may reveal the role of MALE in the induction of stress proteins. The MALE was a rich source of natural phenolics. A study conducted by Ullah *et al.* (2012) showed that exogenous application of a phenolic compound, salicylic acid, prevented adverse effects of drought stress on leaf soluble proteins content of canola.

Under unstressed conditions the foliar application of MALE at all the concentrations did not significantly affect the endogenous level of cell wall bound phenolic compounds in maize leaves (Table 2). Drought stress caused the accumulation of cell wall bound phenolic compounds. Leaf soluble phenolic contents were not affected for foliar applied MALE at 25% and 12.5% under drought stress. However, the treatments having drought stress and applied with 6.5 %, 3.125 % and 1.625% MALE exhibited significantly higher cell wall bound phenolics as compared to untreated control.

Table 2. Effect of *Moringa oleifera* aqueous leaf extract (MALE) on physiological and biochemical growth attributes of maize under drought stress

Treatment	Leaf relative water content (%)	Total soluble proteins content (mg/g f.w)	Cell wall bound phenolics (μg gallic acid eq./ g f.w)
Control	85.33 \pm 1.89 ^{ab}	79.20 \pm 6.23 ^d	24.68 \pm 1.33 ^d
Drought stress	51.67 \pm 1.21 ^h	63.00 \pm 4.19 ^e	48.63 \pm 2.45 ^a
25 % MALE	86.33 \pm 1.09 ^a	98.90 \pm 7.48 ^a	22.62 \pm 1.00 ^d
12.5 % MALE	82.33 \pm 1.05 ^{abc}	86.02 \pm 5.39 ^b	23.66 \pm 1.09 ^d
6.25 % MALE	82.66 \pm 1.00 ^{abc}	83.67 \pm 7.09 ^c	24.00 \pm 0.99 ^d
3.125 % MALE	81.33 \pm 0.97 ^{bcd}	81.13 \pm 4.34 ^d	23.00 \pm 1.09 ^d
1.56 % MALE	81.67 \pm 0.67 ^{bc}	81.34 \pm 4.92 ^d	24.00 \pm 1.08 ^d
25 % MALE + drought stress	79.00 \pm 0.59 ^{cd}	79.16 \pm 5.14 ^{de}	25.70 \pm 1.01 ^d
12.5 % MALE+ drought stress	77.33 \pm 1.05 ^{de}	78.16 \pm 6.18 ^e	25.66 \pm 1.12 ^d
6.25 % MALE+ drought stress	74.67 \pm 1.03 ^{ef}	77.12 \pm 7.23 ^{ef}	34.00 \pm 1.32 ^c
3.125 % MALE+ drought stress	72.33 \pm 1.03 ^f	76.00 \pm 4.09 ^f	33.33 \pm 1.12 ^c
1.56 % MALE+ drought stress	67.00 \pm 0.56 ^g	70.00 \pm 3.98 ^g	40.65 \pm 1.75 ^b
LSD	4.305	2.300	3.193

Means sharing common English letters are statistically similar

The enhanced content of cell wall bound phenolics under drought stress may be due to the fact that phenolic compounds function as natural antioxidants and exhibit free radical-scavenging capabilities (Fauconneau *et al.*, 1997; Azhar *et al.*, 2011). Previous studies have shown that drought stress increased the content of phenolic compounds (rosmarinic acid, ursolic acid and oleanolic acid) of *Prunella vulgaris* L plants (Chen *et al.*, 2011). An enhancement of some flavonoids and phenolic compounds was recorded in some cultivars of cherry tomato under moderate water stress (Sánchez-Rodríguez *et al.*, 2011). Increase in the content of cell wall bound phenolics under drought stress is a trustworthy indicator of drought stress tolerance in plants (Hura *et al.*, 2013). During present studies, drought stress increased the content of cell wall bound phenolics. However, the foliar spray of MALE reversed the effect of drought stress on cell wall bound phenolics accumulation. The MALE was a good source of natural phenolics which prevented maize plants from adverse effects of drought stress.

4. Conclusion

Moringa leaf was a good source of natural phenolics and nutrients. Drought stress caused accumulation of cell wall bound phenolics. The foliar spray of MALE inhibited the accumulation of cell wall bound phenolics under drought stress. Foliar application of MALE to maize plants induced the accumulation of soluble proteins, which ameliorated the adverse effects of drought stress on growth attributes of maize plants. We therefore recommend the foliar application of MALE at optimized concentration (12.5%) to improve the performance of maize plants under drought stress.

References

- Ali, Z., Basra, S.M.A., Munir, H., Mahmood, A. & Yousaf, S. (2011). Mitigation of drought stress in maize by natural and synthetic growth promoters. *Journal of Agriculture Forestry and Social Science*, **7**:56-62.
- Anjum, F., Yaseen, M., Rasul, E., Wahid, A. & Anjum, S. (2003). Water stress in barley (*Hordeum vulgare* L.). I. Effect on morphological characters. *Pakistan Journal of Agriculture Science*, **40**:43-44.
- Ashrafi, Z.Y., Sadeghi, S., Mashhadi, H.R. & Hassan, M.A. (2008). Allelopathic effects of Sunflower (*Helianthus annuus*) on germination and growth of wild barley (*Hordeum spontaneum*). *Journal of Agriculture Technology*, **4**:219-229.
- Aydinalp, C. & Cresser, M.S. (2008). The effect of global climate change on agriculture. *American- Eurassian Journal of Agriculture and Environmental Science*, **3**:672-676.
- Azhar, N., Hussain, B., Ashraf, Y.M. & Abbasi, K.Y. (2011). Water stress mediated changes in growth, physiology and secondary metabolites of Desi Ajwain (*Trachyspermum amni* L.). *Pakistan Journal of Botany*, **43**:15-19.
- Bano, A., Ullah, F. & Nosheen, A. (2012). Role of abscisic acid and drought stress on the activities of antioxidant enzymes in wheat. *Plant Soil Environment*, **58**:181-185.
- Bibi, A., Ullah, F., Mehmood, S., Bibi, K. & Khan, S.U. *et al.*, (2016). *Moringa oleifera* Lam. Leaf extract as bioregulator for improving growth of maize under mercuric chloride stress. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*, **66**:469-475.
- Campbell, M.M. & Ellis BE. (1992). Fungal elicitor-mediated responses in pine cell cultures: cell wall-bound phenolics. *Phytochemistry*, **31**:737-742.
- Chen, Y., Guo, Q., Liu, L., Liao, L. & Zhu, Z. (2011). Influence of fertilization and drought stress on the growth and production of secondary metabolites in *Prunella vulgaris* L. *Journal of Medicinal Plants Research*, **5**:1749-1755.
- Chun, O.K, Kim, D.O. & Lee, C.Y. (2003). Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal of Agriculture and Food Chemistry*, **51**:8067-8072.
- Coque, M. & Gallais, A. (2006). Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. *Theoretical and Applied Genetics*, **112**:1205-1220.
- Edmeades, G.O. (2013). Progress in achieving and delivering drought tolerance in maize – an update. Ithaca, NY: ISAAA
- Egilla, J.N., Davies, F.T. & Boutton, T.W. (2005). Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosasinensis* at three potassium concentrations. *Photosynthetica*, **43**:135-140.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. & Basra, S.M.A. (2009). Plant drought stress Effects, mechanisms and management. *Agronomy for Sustainable Development*, **28**:185-212.
- Fauconneau, B., Waffo-Teguo, P., Huguet, F., Barrier, L. & Decendit, A. *et al.*, (1997). Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using in vitro tests. *Life Science*, **61**:2103-2110.

- Fuglie, L.J. (2000).** New uses of Moringa studied in Nicaragua. ECHO Development Notes, No.68, June, 2000.
- Gaoz, F. (2000).** Experimental technology in plant physiology. World Books Publishing Company, China.
- Habibi, G. (2012).** Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress. *Acta Biol Szeged*, **56**:57-63.
- Hassan, M.A., Fuertes, M.M., Sanchez, F.J.R., Vicente, O. & Boscaiu, M. (2015).** Effects of Salt and Water Stress on Plant Growth and on Accumulation of Osmolytes and Antioxidant Compounds in Cherry Tomato. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **43**:1-11.
- Hirich, A., Rami, A., Laajaj, K., Choukr-Allah, R. & Jacobsen, S.E. et al., (2012).** Sweet corn water productivity under several deficit irrigation regimes applied during vegetative growth stage using treated wastewater as water irrigation source. *World Academy of Science and Engineering Technology*, 61.
- Hura, T., Hura, K.A., Ostrowska, M., Grzesiak, K. & Dziurka H. (2013).** The cell wall-bound phenolics as a biochemical indicator of soil drought resistance in winter triticale. *Plant Soil Environment*, **59**:189-195.
- Jain, P., Jain, S., Pareek, A. & Sharma, S. (2013).** A comprehensive study on the natural plant phenols: perception to current scenario. *Bulletin of Pharmacological Research*, **3**:90-106.
- Kaur, C. & Kapoor, H.C. (2001).** Review. Antioxidants in fruits and vegetables the millennium's health. *International Journal of Food Sciences and Technology*, **36**:703-725.
- Khan, M.A., Qureshi, R.A., Ullah, F. & Gilani, S.A. (2011).** Phytotoxic effects of selected medicinal plants collected from Margalla Hills, Islamabad Pakistan. *Journal of Medicinal Plants Research*, **5**:4671-4675.
- Latif, F., Ullah, F., Mehmood, S., Khattak, A. & Khan, A.U. et al., (2016).** Effects of salicylic acid on growth and accumulation of phenolics in *Zea mays* L. under drought stress. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*, **66**:325-332.
- Lowry, O.H., Rosebrough, N.H., Farr, A.L. & Randall, J.R. (1951).** Protein measurement with folin phenol reagent. *Biochemistry Journal*, **193**:265-275.
- Ma, Q. & Turner, D.W. (2006).** Osmotic adjustment segregates with and is positively related to seed yield in F3 lines of crosses between *Brassica napus* and *B. juncea* subjected to water deficit. *Australian Journal of Experimental Agriculture*, **46**:1621-1627.
- Martin, L.P. (2000).** The Moringa Tree: Revised in 2000 by Kristin Davis, pp. 1-14.
- Muzaffar, S., Barket, A. & Niyaz, A.W. (2012).** effect of catechol, gallic acid and pyrogallol on the germination, seedling growth and the level of endogenous phenolics in cucumber (*Cucumis sativus* L.). *International Journal of Life Science and Biotechnol Pharma Res*, **1**:50-55.
- Mvumi C., Tagwira, F. & Chiteka A.Z. (2013).** Effect of moringa extract on growth and yield of maize and common beans. *Greener Journal of Agriculture Science*, **3**:55-62.
- Narwal, S.S. (2004).** Allelopathy in crop production. Scientific Publishers, Jodhpur, India. Pp: 326-332.
- Nelson, G.C., Rosegrant, M.W., Koo, J., Robertson, R. & Sulser, T. et al., (2009).** Climate change: Impact on agriculture and costs of adaptation. Washington, DC, IFPRI. IPCC. 2007b. General guidelines on the use of scenario data for climate impact and adaptation assessment. Nairobi.
- Pakade, V., Cukrowska, E. & Chimuka, L. (2013).** Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa. *South African Journal Science*, **109** (3/4):1-5.
- Pizzale, L., Bortolomeazzi, R., Vichi, S. & Conte, L.S. (2002).** Antioxidant activity of sage and oregano extracts related to their phenolic compound content. *Journal of Science Food and Agriculture*, **82**:1645-1651.
- Popa, V.I., Dumitru, M., Volf, I. & Anghel, N. (2008).** Lignin and polyphenols as allelochemicals. *Industrial Crops Production*, **27**:144-149.
- Popa, V.I., Agache, C., Belega, C. & Popa, M. (2002).** Polyphenols from spruce bark as plant growth regulator. *Crop Research*, **24**:398-406.
- Pospíšilová, J., Synovial, H. & Rulcova, J. (2000).** Cytokinin and water stress. *Biology Plantarum*, **43**:321-328.
- Saifuddin, M., Osman, N., Idris, R.M. & Halim, A. (2016).** The effect of Pre-aluminium treatment on morphology and physiology of potential acidic slope plants. *Kuwait Journal of Science*, **43**:199-220.
- Sanchez-Blanco, M.J., Rodriguez, P., Morales, M.A., Ortuño, M.F. & Torrecillas, A. (2002).** Comparative growth and water relations of *Citrus albidus* and *Citrus monspeliensis* plants during water deficit conditions and recovery. *Plant Science*, **162**:107-113.

- Sánchez-Rodríguez, E., Moreno, D.A., Ferreres, F., Rubio-Wilhelmi, M.M. & Ruiz, J.M. (2011).** Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. *Phytochemistry*, **72**:723-729.
- Santana, C.M., Ferrera, Z.S., Padrón, M.E. & Rodríguez, J.J. (2009).** Methodologies for the extraction of phenolic compounds from environmental samples: New Approaches. *Molecule*, **14**:298-320.
- Siddhuraju, P. & Becker, K. (2003).** Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam). *Journal of Agriculture and Food Chemistry*, **15**:2144-2155.
- Singleton, V., Joseph, A. & Rossi, Jr. (1965).** Colorimetry of total phenolics with phosphor molybdic cphosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**:144-158.
- Steel, R.G.D. & Torrie, J.H. (1984).** Principles and procedures of statistics (2nd Ed.). M.C Graw Hill Book Co; Singhapore pp. 172- 177.
- Swain, T. & Hillis, W. E. (1959).** Phenolic constituents of *Prunus domestica* quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, **10**:63-68.
- Tas, S. & Tas, B. (2007).** Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkey. *World Journal of Agriculture Science*, **3**:178-183.
- Temizel, K.E. (2015).** Estimation of the phenolics content of St. John's wort (*Hypericum perforatum* L.) grown under different water and salt levels based on reflectance spectroscopy *Kuwait Journal of Science*, **42**:210-222.
- Ullah, F., Ullah, A., Wazir, S.M. & Shinwari, Z. K. (2014).** Phytotoxic effects of safflower yellow exposure on seed germination and early seedling growth of canola (*Brassica napus* L). *Pakistan Journal of Botany*, **46**:1741-1746.
- Ullah, F., Bano, A. & Nosheen, A. (2012).** Effects of plant growth regulators on growth and oil quality of canola (*Brassica napus* L.) under drought stress. *Pakistan Journal of Botany*, **44**:1873-1880.
- Valentovic, P., Luxova, M., Kolarovic, L. & Gasparikova, O. (2006).** Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environment*, **4**:186-191.
- Walthall, C.L., Hatfield, J., Backlund, P., Lengnick, L. & Marshal, E. et al., (2012).** Climate Change and Agriculture in the United States: Effects and Adaptation." USDA Technical Bulletin 1935. Washington, DC. 186 pp.
- Werner, T., Motyka, V., Strnad, M. & Schmulling, T. (2001).** Regulation of plant growth by cytokinin. *Proceedings of National Academy of Sciences USA* **98**: 10487-10492.
- Wu, Y.T. & Lin, C.H. (2000).** Analysis of cytokinin activity in commercial aqueous seaweed extract. *Garten Bauwissen Schaft*, **65**:170-173.
- Yasmeen, A., Nouman, W., Basra, S.M.A., Wahid, A., Rehman, H.U. et al., (2014).** Morphological and physiological response of tomato (*Solanum lycopersicum* L.) to natural and synthetic cytokinin sources: a comparative study. *Acta Physiologia Plantarum*, **36**:3147-3155.
- Yasmeen, A., Basra, S.M.A., Farooq, M., Rehman, H.U., Hussain, N. & Athar, H.R. (2013).** Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regulation*, **69**:225-233.

Submitted : 11/12/2015

Revised : 27/10/2016

Accepted : 30/10/2016

تأثير الاستخراج المائي من أوراق نبات مورينجا أوليفيرا *Moringaoleifera Lam.* على سمات النمو وتراكم الفينولات المرتبطة بجدار الخلية في نبات الذرة (*Zea mays L.*) تحت ضغط الجفاف

كيران برفيز، فايزان أولاء*، سلطان محمود، عدنان خاتاك

قسم علم النبات - جامعة العلوم والتكنولوجيا، بانو، كي بي، باكستان

*drfaizanwazir@gmail.com

خلاصة

يشكل إجهاد الجفاف أحد أهم الضغوط غير الحيوية التي تؤثر على محصول الذرة وجودته. ويجتهد العلماء لإنتاج محرضات للنمو الطبيعي في النباتات لتحسين قوة تحمل إجهاد الجفاف في المحاصيل. يُعتبر نبات مورينجا أوليفيرا (*M. oleifera*) مصدراً جيداً للمواد المضادة للأوكسدة الطبيعية والفينولات الطبيعية، وقد استخدمت مستخلصات أوراقها كمحفزات لنمو النبات لتحسين نمو العديد من المحاصيل. وقد قيمت الدراسة الحالية تأثير التركيزات المختلفة من الخلاصة المائية في أوراق نبات المورينجا (MALE) (مثل 12.5 و 25 و 6.25 و 3.13 و 1.56%) على نمو النبات والفينولات المرتبطة بجدار الخلية في الذرة (*cv. Azam*) تحت إجهاد الجفاف (ونسبة رطوبة التربة 10%). وعمل الاستعمال الخارجي للخلاصة المائية في أوراق نبات المورينجا (MALE) على تحسين البروتينات القابلة للذوبان في أوراق الذرة، والمحتوى النسبي للماء في الورقة (LRWC)، والوزن الرطب والوزن الجاف للأفرخ والجذور، ومساحة الجذور، وطول وعرض الجذر تحت إجهاد الجفاف. وقد تسبب إجهاد الجفاف في تراكم الفينولات المرتبطة بجدار الخلية والتي تم تخفيضها عن طريق استخدام الخلاصة المائية في أوراق نبات المورينجا. وتم استنتاج أن الخلاصة المائية في أوراق نبات المورينجا من الممكن أن تكون محفزاً محتملاً لنمو النبات لتحسين نمو الذرة تحت إجهاد الجفاف.