



**Full Length Article**

## Improving Drought Resistance in Wheat (*Triticum aestivum*) by Exogenous Application of Growth Enhancers

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### Abstract

Water stress due to its severity badly effects productivity in cereals, while the foliar application of growth-enhancing substances may be helpful in reducing the drought effects. This study evaluated the efficacy of foliar applied growth enhancers i.e. *Moringa oleifera* leaf extract (MLE), benzyl amino purine (BAP, 50 mg L<sup>-1</sup>) and K (SOP, 2%) against water spray as control in wheat cv. Sehar-2006. Three irrigation levels were maintained at 100, 75 and 50% field capacity designated as well watered, moderate and severe drought stressed. Increasing drought stress significantly reduced plant growth and yield. The foliar applied MLE and BAP produced maximum leaf area, Chl *a* and *b* under well watered conditions and minimum in control under severe stress. Total leaf soluble proteins were also higher in well watered and drought stressed plants applied with BAP. Foliar applied MLE increased POD, catalase, ascorbic acid and leaf K<sup>+</sup> contents under moderate drought, while total phenolic contents under severe drought condition. BAP application exhibited enhanced SOD levels under severe drought. Highest grain yield per plant was also recorded for foliar applied MLE under well watered, moderate and severe water stress than other growth enhancers. Nonetheless, foliar applied growth enhancers particularly moringa leaf extracts ameliorated the adverse effects of drought by enhancing antioxidant levels and plant secondary metabolites. © 2013 Friends Science Publishers

**Keywords:** Antioxidants; Ascorbate; BAP; Chlorophyll; MLE; Phenolic contents; SOP

### Introduction

Drought is becoming a serious threat to crop production worldwide resulting in 67 and 82% reduction in K uptake under mild and severe water stress conditions (Baque *et al.*, 2006), which ultimately leads to reduction in the number of grains per spike and grain weight in wheat (Li *et al.*, 2000), and deteriorated quality proteins in mungbean (Garg *et al.*, 2004). The decrease in soil water potential causes alteration in minerals uptake by plant roots and reduction in leaf expansion under drought or salinity stress conditions (Kaminek *et al.*, 1997; Pospíšilová *et al.*, 2000). Similar to many other stresses, drought also causes oxidative stress with the generation of reactive oxygen species (ROS), which affect the photosynthetic activities of leaf causing overall growth retardation in plants (Farooq *et al.*, 2009). Antioxidants system of plants, different hormones like cytokinins and minerals like calcium, potassium and magnesium and vitamin-A help to scavenge these species to minimize the damage due to ROS (Banowitz, 1998).

The chlorophyll contents, which are severely affected

under drought conditions (Tas and Tas, 2007), can be significantly improved by exogenous application of plant growth regulators under water stress conditions (Zhang *et al.*, 2004). Moreover, water deficiency in soil rapidly lowers down the cytokinin level in plants which might be due to reduced cytokinin biosynthesis (Pospíšilová *et al.*, 2000). The plant growth regulators possessing cytokinin in good amount, showed protective effects under water deficit and prevented reduction in chlorophyll and protein contents. Lesser quantities of cytokinin, observed in xylem sap under drought stress (Dodd, 2003), also emphasized the need for exogenous application of cytokinin.

Recently, *Moringa oleifera* leaf extract (MLE) is being introduced as a natural plant growth enhancer, which not only improves the plant growth and biomass production but also induces tolerance under stress conditions (Yasmeen *et al.*, 2012a, b). Afzal *et al.* (2012) used MLE as priming tool for overcoming chilling injury to maize seedlings and found that it effectively improved the germination rate and percentage of hybrid maize seeds at low temperature. Moreover, reports show that foliar application of MLE

suppressed the drought effects on maize plants by improving the leaf area, plant height and biomass, and chlorophyll contents (Ali *et al.*, 2011). MLE is rich in zeatin, calcium, potassium, magnesium and other growth promoting substances, which make it a natural plant growth enhancer for plants (Yasmeen *et al.*, 2012a, b, 2013; Nouman *et al.*, 2012a, c). An increase in yield of late-sown wheat was also recorded when the plants were subjected to foliar application of MLE (Yasmeen *et al.*, 2012a). Drought is also a serious threat to wheat production by adversely affecting the vital physiological and biochemical processes, leading to reduced growth and final crop yield (Ahmadi and Baker, 2001). As MLE has been found to improve crop performance under low and high temperature and salinity stresses and proved as natural source of plant growth enhancer (Yasmeen *et al.*, 2013), but very little information is available highlighting the foliar applied moringa leaf extracts on wheat growth, yield and physiological mechanisms under drought stress. Nouman *et al.* (2012d) reported an increase in mineral contents of different rangeland grasses when the seed were subjected to MLE priming. Being a rich source of cytokinin, potassium and antioxidants, it was hypothesized that MLE might induce tolerance in wheat plants under drought conditions. So, the present study was carried out aiming at improving the wheat growth and yield under drought stress conditions.

## Materials and Methods

### General Experiment Details

The experiment was conducted during winter season 2009-2010 under net house conditions at Department of crop physiology, University of Agriculture, Faisalabad, Pakistan. Seeds of wheat cv. Sehar-2006 obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan was sown. The earthen pots each filled with 10 kg of a mixture of soil, sand and compost (1:1:1) were sown with wheat seeds (10 each). Fertilizer including urea, single super phosphate and potassium sulphate was added in pots @120-100-62.5 kg NPK ha<sup>-1</sup> respectively. Whole phosphorous and potash along with half nitrogen was mixed in soil prior to sowing. Remaining half nitrogen was applied after sowing with irrigation up to tillering. The experiment was conducted in completely randomized design (CRD) with two factor factorial arrangement using three replications. After completion of emergence, four plants were retained in each pot for collecting data regarding growth and biochemical parameters.

### Imposition of Drought Stress

Drought stress based on soil water holding capacity was imposed after uniform stand establishment. Three irrigation levels were maintained at 100, 75 and 50% field capacity designated as well watered, moderate and severe drought stressed respectively.

### Preparation of MLE

MLE was prepared according to procedure described by Nouman *et al.* (2012a) with modifications described by (Yasmeen *et al.*, 2012a). Young frozen leaves of *M. oleifera*, collected from experimental nursery area of Department of Forestry, Range management and Wildlife, University of Agriculture Faisalabad, Pakistan were ground with a pinch of water (1 L 10 kg<sup>-1</sup> moringa leaves). The extract was purified and centrifuged at 8,000 rpm for 15 min. and diluted 30 times by adding distilled water.

### Application of Growth Enhancers

One week after drought imposition and maintenance, growth enhancers i.e. MLE (Yasmeen *et al.*, 2012a), BAP (benzyl amino purine) (Amin *et al.*, 2007) 50 mg L<sup>-1</sup> and K (SOP, 2%) (Ismail, 2005) against water spray as control were applied at tillering, booting, jointing and heading growth stages of wheat using 25 mL/plant (Yasmeen *et al.*, 2012a).

### Growth and Plant Analysis

Leaf area per plant (cm<sup>2</sup>) was measured by using leaf area meter (CI-203, CID Inc., USA) one week after foliar applications. At maturity plants were harvested and threshed manually to record grain yield per plant.

### Biochemical Analysis

Leaves were randomly selected from each treatment one week after foliar applications of growth enhancers. Chlorophyll contents (*a* and *b*) were determined after extraction in 80% acetone following the method of Nagata and Yamashta (1992) by using UV-spectrophotometer (UV-4000, O.R.I. Germany). Total soluble proteins were quantified by following the protocol devised by Bradford (1976). For determination of enzymatic antioxidants, leaf samples were extracted in 50 mM phosphate buffer (pH 7.8). The extract was centrifuged at 15,000 rpm @ 4°C and the supernatant was used for further assay of catalase (CAT), peroxidase (POD) (Chance and Maehly, 1955) and super oxide dismutase (SOD) activities (Giannopolitis and Ries, 1977). Total phenolic contents (TPC) were determined by using the method described by Singleton and Rossi (1965) revised by Waterhouse (2001). Ascorbate contents were determined following the protocol of Ainsworth and Gillespie (2007).

### Mineral Contents

Wheat leaves were oven dried at 60°C till a constant weight was achieved. Dried wheat samples were then ground to pass through 2 mm sieve and digested in concentrated nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) at 2:1 ratio by following the method adapted by (Rashid, 1986). Leaf potassium (K<sup>+</sup>) and chloride (Cl<sup>-</sup>)

contents were determined by following the protocol of USDA Laboratory Staff (1954).

### Statistical Analysis

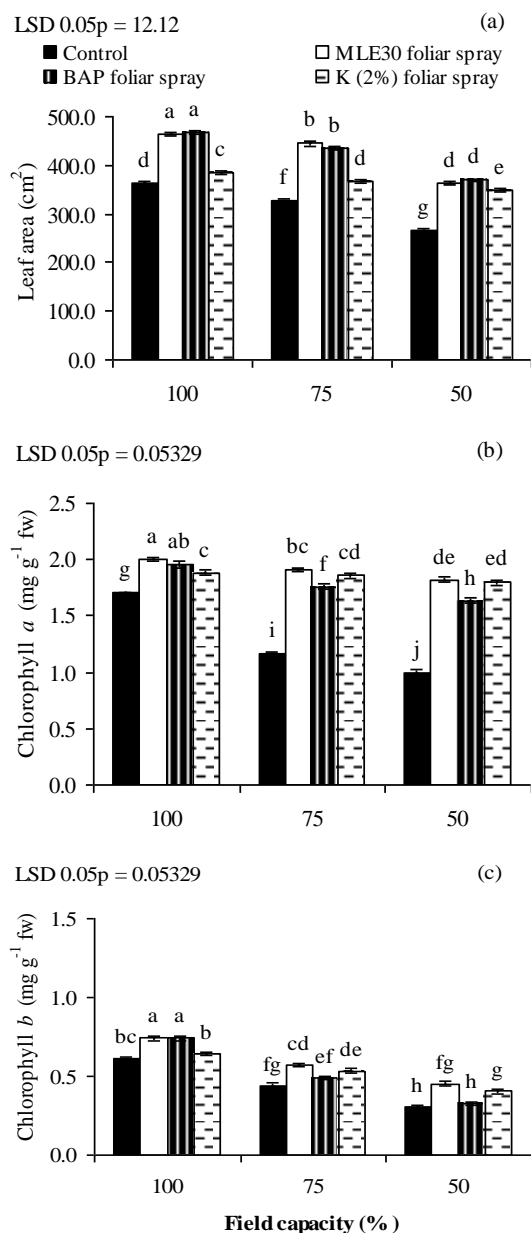
The data was computed and analyzed by using the MSTAT-C Program (MSTAT Development Team, 1989). LSD test ( $P < 0.05$ ) was calculated through Duncan's New Multiple Range test to compare differences among mean values (Steel *et al.*, 1996).

### Results

Increasing drought stress levels significantly reduced plant growth and yield, but exogenous applications of growth enhancers improved antioxidants activities. Comparison of means indicate that leaf area was gradually decreased with increasing drought stress (Fig. 1a). The minimum leaf area was obtained under extreme drought conditions. The interaction of drought and foliar application on leaf area was found significant. Highest leaf area was recorded for foliar applied MLE and BAP than control under well watered, moderate and severe drought stress. A minimum leaf area was recorded under severe drought stress. The increasing water stress decreased leaf chlorophyll *a* contents (Fig. 1b). A maximum chlorophyll *a* was observed under well watered conditions by exogenous application of MLE and BAP while in moderate and severe drought, foliar applied K and MLE exhibited higher leaf chlorophyll *a*. A similar trend was followed by all the treatments in case of chlorophyll *b* under each water stress treatment. Minimum chlorophyll *a* and *b* contents were noted under extreme water stress. Increase in drought stress decreased the total soluble proteins in wheat leaves (Fig. 2a). Maximum leaf protein content was evident at moderate stress followed by severe with minimum value under well watered conditions. Foliar spray with BAP showed the highest leaf protein contents under well watered and drought stress. Nevertheless, the exogenous application of growth enhancers performed better than control for leaf total soluble protein.

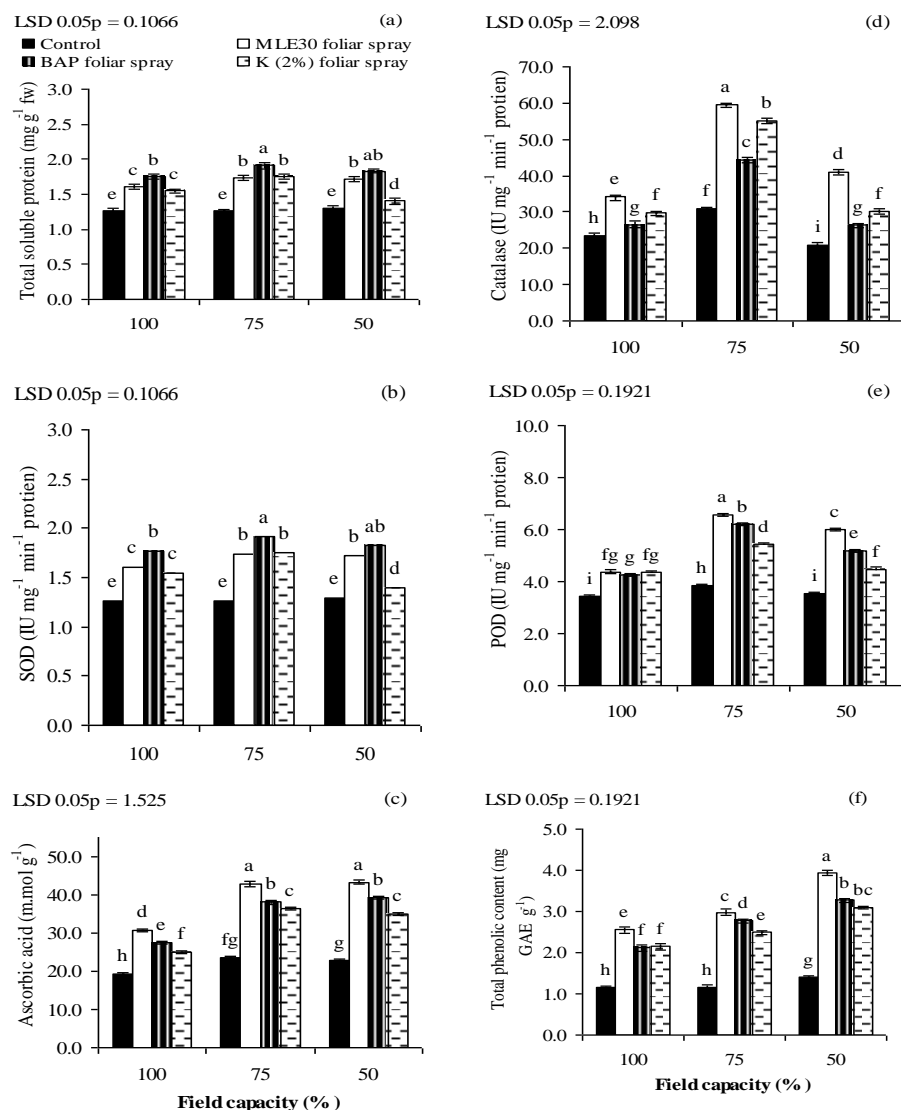
A rise in drought stress also amplified the antioxidants status of wheat leaves (Fig. 2b, d, e). The enzymatic and nonenzymatic antioxidants contents produced by exogenous application of growth enhancers were better than control under each water level. The maximum SOD was recorded for foliar applied BAP under severe drought. Increased POD and CAT activities were found under medium drought stress by foliar applied MLE.

Decreasing water level increased ascorbic acid contents for all treatments (Fig. 2c) and the highest value of ascorbic acid was obtained under moderate drought by exogenous application of MLE. However, ascorbic acid remained unaffected under severe drought and produced similar contents under moderate and severe drought for foliar applied MLE. The TPC contents were found in comparatively lesser quantities as compared to ascorbic



**Fig. 1:** Effect of exogenous application of different plant growth enhancers on leaf area (a), chlorophyll *a* (b) and chlorophyll *b* (c) of wheat cv. Sehar-2006 under drought conditions

acid (Fig. 2f). Maximum total phenolic contents were produced under severe water stress with MLE spray. Foliar spray of K<sup>+</sup> and BAP showed similar TPC contents under well watered and severe drought conditions. Minimum TPC were recorded for control under well watered conditions. MLE application accumulated largest leaf K<sup>+</sup> ions under moderate drought stress (Fig. 3a). Under severe drought, foliar applied MLE produced K<sup>+</sup> ion in similar quantity as under well watered conditions. The least K<sup>+</sup> was observed in control under severe drought stress.



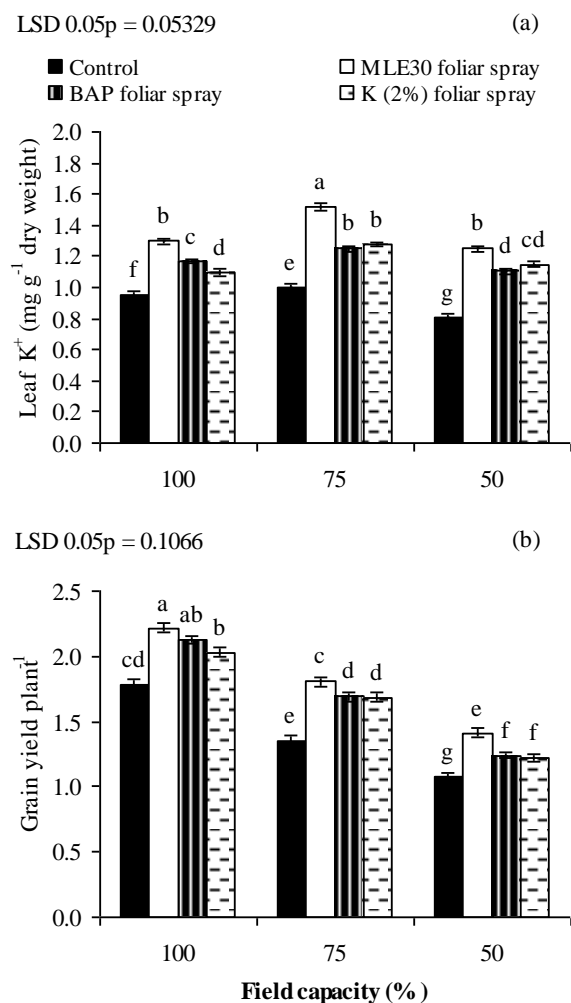
**Fig. 2:** Effect of exogenous application of different plant growth enhancer on total soluble protein (a), super oxide dismutase (b), ascorbic acid (c), catalase (d), peroxidase (e) and total phenolic contents (f) of wheat cv. Sehar-2006 crop under drought conditions

A highest grain yield per plant was observed under well watered conditions by MLE foliar spray (Fig. 3b). Water stress decreased grain yield but exogenous application of growth enhancers ameliorated the negative effects of water stress significantly than water spray only. However, MLE application produced more grain yield under moderate and severe water stress than BAP, K<sup>+</sup> including control.

## Discussion

Increasing water stress has direct impact on crop growth and yield reduction and similar observations for reduction in growth and grain yield of wheat were found in present study. Reduction in plant growth under restricted water

availability might have been due to reduction in photosynthetic area and photosynthetic capacity of leaves (Chaves *et al.*, 2011). Chlorophyll and protein contents affect the photosynthetic capacity of plants and considered as important adaptation traits under drought (Chernyad'ev and Monakhova, 2003). The chlorophyll contents should be maintained under water deficit to support photosynthetic capacity of plants. In the present study, chlorophyll *a* and *b* contents were drastically reduced due to drought effect, whilst MLE and K (2%) foliar application improved these contents in wheat leaves. It has been reported that the foliar application of plant growth regulators like BAP and ascorbic acid improved the chlorophyll contents in wheat leaves (Hanaa *et al.*, 2008). Moreover, foliar application of potassium compounds can also minimized the growth



**Fig. 3:** Effect of exogenous application of different plant growth enhancer on leaf K<sup>+</sup> contents (a) and grain yield plant<sup>-1</sup> (b) of wheat cv. Sehar-2006 under drought conditions

retarding effects under stress conditions (Ahmad and Jabeen, 2005). Yasmeen *et al.* (2013) also found increase in wheat chlorophyll contents when MLE was applied under saline conditions. The foliar application of algal extract, MLE and BAP may also stimulate earlier cytokinin formation thus preventing premature leaf senescence resulting in more leaf area with higher photosynthetic pigments (Hanaa *et al.*, 2008; Rehman and Basra, 2010; Ali *et al.*, 2011).

In the present study, reduced yield due to water stress was not only correlated with leaf area but also with chlorophyll *a* and *b* (Fig. 1). Exogenous application caused increase in yield under normal or water stress conditions, except for K<sup>+</sup> as foliar spray. However, foliar application of MLE caused maximum increase in grain yield of wheat under control or water stress conditions. Zhang *et al.* (2004) found that chlorophyll contents were

significantly improved by exogenous application of growth regulators under water deficit conditions. The phyto regulators exhibiting cytokinin activity showed protective effects in water deficit and prevent reduction in chlorophyll and protein contents. According to Schachtman and Goodger (2008) plant growth regulators such as cytokinins, activated under water stress conditions initiate defensive responses in plants to protect important processes in plants from water stress injury. In the presence of cytokinin like activity compounds e.g., Kartolin less reduction were observed in protein contents (Chernyad'ev and Monakhova, 2003). The positive effect of BAP on protein contents was also pronounced under drought stress. Besides adaptation role, hormones also regulate yield potential, by controlling floret survival and grain filling capacity in cereals (Young *et al.*, 1997). Less reduction in growth and yield observed in present study by foliar spray of MLE and BAP in drought stressed wheat plants could be attributed to hormonal influence especially rich zeatin contents of moringa leaves. Moringa leaves are a rich source of  $\beta$ -carotene, protein, vitamin C, calcium and potassium and act as good source of natural antioxidants such as ascorbic acid, flavonoid, phenolics and carotenoids (Dillard and German, 2000; Siddhuraju and Becker, 2003). The exogenous applications of MLE improve the antioxidant status and yield of wheat under drought stress. Yasmeen *et al.* (2013) reported increase in potassium contents of wheat leaves when MLE was exogenously applied to induce tolerance in wheat crop under saline conditions. Moreover, the increase in antioxidant enzymes (SOD, POD and CAT) was also reported in the same study. Moringa leaves have been reported as source of strong antioxidant system (Anwar *et al.*, 2007; Nouman *et al.*, 2012c). It has been reported that activation of self defense system by exogenous application of MLE is also associated with higher mineral contents present in moringa leaves making it excellent plant growth enhancer (Yasmeen *et al.*, 2013). Various studies have confirmed its growth promoting behavior exercised on different vegetable, cereal crops, range grasses and nuts (Nouman *et al.*, 2012a, b).

In conclusion, MLE proved to be a good natural source as plant growth enhancer, which can induce resistance in wheat crop against drought. Improved enzymatic and non-enzymatic antioxidant activities and increase in wheat mineral contents was also recorded during this study. Being a natural growth enhancer, MLE can be promoted as a good alternate of BAP which has been reported as drought mitigating effect.

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