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GROWTH PROMOTERS MODULATE THE ANTIOXIDANT SYSTEM TO MITIGATE WATER STRESS IN QUINOA

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Abstract

The abrupt climatic changes leading to reduce water availability create serious threat for successful cultivation of profitable nontraditional quinoa crop. Therefore, a field investigation was planned to reduce the drought-induced losses to quinoa with the use of growth promoters i.e., moringa (*Moringa oleifera* L.) leaf extract (MLE30) and proline applied through seed and/or plant foliage individually or in combination including untreated control. Quinoa plants exposed to water stress (only pre-soaking irrigation + rainfall) at vegetative and reproductive stage. Results showed that water stress adversely affected the growth and yield contributing attributes that ultimately reduced the quinoa productivity. It was observed that application of growth promoters improved the growth and productivity even under water stress conditions. However, MLE30 seed priming + foliar spray exerted more impact on studied attributes than proline. Application of MLE30 improved the growth and yield contributing parameters and produced significantly higher chlorophyll contents, proline content, ascorbic acid, superoxide dismutase, peroxidase and catalase that modulate the adverse effects of water stress leading to higher quinoa seed yield under water stress conditions. These consequences suggest that application of MLE30 enhanced water stress tolerance in quinoa plants by improving the antioxidant system.

Key words: Moringa leaf extract, Proline, Quinoa, Priming, Foliar spray, Water stress.

Introduction

Quinoa (*Chenopodium quinoa* Willd) is pseudo cereal crop non-traditional food crop in Pakistan, which may substitute a share of food gap throughout the world. Its seed is reported to contain a well-balanced and significant amount of the nine essential amino acids required to fulfill our daily protein requirement (Pereira *et al*., 2019). Quinoa has high proportion of dietary fiber that can easily be absorbed by body systems and makes it a perfect nutrition to detoxify the body, abolishing poisons and surplus products (Repo-Carrasco *et al*., 2003). Leaves and seeds of quinoa comprise of α-and β-tocopherol and carotenoids that works as cell guards and provides an imperative foundation of antioxidants (Bhargava *et al*., 2006). Moreover, its seeds used in food manufacturing due to its cooking, extrusion and starch features and it also have characteristics beverages quality (Aluwi *et al*., 2017; Zannini *et al*., 2018). Generally, in cereals while particularly in quinoa have the capability to absorb liquid and stay longer in the gastro-intestinal conditions. Hence, quinoa is convenient for the preparations of easy digestible food and suggested as a beneficial staple food industry (Ogungbenle, 2003).

Climate change in recent decades not only increases the air temperature but also interrupt the amount and distribution of precipitation possibly leading to periodic water deficit cycles in the coming years (Wang *et al*., 2014). Water stress is one of the major threats to crop plants thus disrupting all levels of crop plants from morpho-physiological, molecular and biochemical features (Pulvento *et al*., 2010; Muscolo *et al*., 2015). Although quinoa is known as water stress tolerant crop, proficient of cultivating and producing seed yield in the semi-arid environments, however, severe water stress adversely affected the crop growth and productivity (Tuisima and Fernández, 2015; Choukr-Allah *et al*., 2016). Water stress during reproductive stage reduced the seed setting time and accelerates the remobilization of carbon reserves to grain (Rashid *et al*., 2018).

Scattered precipitation and water deficit are the threatening signs may exhibit the sporadic production patterns throughout the world. Antagonistic influences of water stress on crop plants can be minimized with the application of growth promoters (Farooq *et al*., 2009). Among various growth promoters, foliar spray of proline is getting substantial consideration in current farming to survive under water stress conditions (Sadak, 2016). Proline is a proteinogenic amino acid accumulates in cytoplasm of plants and can play an essential role in improving plant defense mechanism (Szabados and Savoure, 2010). As it is considered to assist in oxidative phosphorylation in mitochondria, encourages the production of ATP, enhanced the photosynthetic pigments content, ameliorated stomatal conductance and CO_2 assimilation and improve the activities of numerous antioxidants that protects cell membranes from oxidative stress and facilitated growth (Ben Ahmed *et al*., 2010; Molla *et al*., 2014). Moreover, leaf application of proline on water stressed plants improved the endogenous level of proline (Hasanuzzaman *et al*., 2014) that resulted in boosting the photosynthetic system and soluble protein (Shahid *et al*., 2014). In addition to these, proline also accelerates the transduction of signals, acts as an alternative of nitrogen and carbon, and supports protein complexes and DNA, therefore executing a numeral imperative function in plants in water stress situations (Szabados & Savouré, 2010).

In addition to proline, foliar application of moringa (*Moringa oleifera* L.) leaf extract (MLE 30), as natural growth promoter may be feasible substitute supplement to artificial chemical sources used to enhance the tolerance under water stress conditions in numerous plant species (Hussain *et al*., 2020; Khan *et al*., 2021). In fact, it is immense source of essential mineral nutrients, vitamins,

growth hormones, antioxidants and osmoprotectants that making it a potential growth promoter (Yasmeen *et al*., 2018; Rashid *et al*., 2021). Foliar spray of MLE30 has encouraging impacts on the plant growth, leaf area, rate of photosynthesis, adjustment in source – sink relationship, hormonal content and improved the anti-oxidative activities and fortified the plant defense system and improved the secondary metabolites levels under environmental stress conditions (Batool *et al*., 2016). All these features result in stronger plant growth and improving the crop performance in water stress environments (Rashid *et al*., 2021). However, the responses of MLE 30 application to alleviate the impact of water stress in quinoa at critical growth stages have not been well established yet. Therefore, the present study was conducted to explore the role of MLE as an organically natural plant growth enhancer in comparison with proline for improving the productivity of quinoa under various irrigation water-regimes at the critical growth stages through modulating the antioxidant system.

Material and Methods

A field experiment was planned to investigate mitigation of the water stress in quinoa under influence of growth promoters at Research farm area of Bahauddin Zakariya University, Multan, Pakistan during winter 2018-19 and 2019-20. The investigations were carried out in a Randomized Complete Block with factorial arrangement having 3 repeats. Experimental treatments were designed in eight combinations for application of growth promoters i.e., MLE30 seed priming, proline seed priming, MLE30 foliar spray, proline foliar spray, MLE30 seed priming + MLE30 foliar spray, MLE30 seed priming + proline foliar spray, proline seed priming + proline foliar spray, proline seed priming + MLE30 foliar spray) including control. Irrigation was skipped to impose water stress at vegetative and reproductive stage (excluding pre-soaking irrigation + seasonal rainfall) including control plots (received only seasonal rainfall). Foliar treatments were applied twice on 16th January and 15th February and 13th January and 11th February during 2018-19 and 2019-20, respectively by using a hand sprayer.

Prior to sowing, composite soil samples were collected from experimental area at the depth of 0-30 cm and assessed for physico-chemical features. The soil texture of experimental area containing sand 28 and 29%, silt 52 and 49%, clay 19 and 23%, having pH 8.0 and 7.8, EC 1.8 and 2.0 dS m^{-1}, organic matter 0.54 and 0.59%, total nitrogen 0.079 and 0.084%, available phosphorus 8 and 11 mg Kg^{-1} and available potassium 183 and 186 mg Kg^{-1} in 2018-19 and 2019-20, respectively.

Preparation and analysis of moringa leaf extract: Leaves of moringa trees are potential source of zeatin that improves the antioxidant properties of numerous enzymes which protects the cells from injuries. Young leaves and tender twigs of fully grown *Moringa oleifera* plants were harvested and washed several times with distilled water then stored in freezer at -5˚C for 12 hours. Frozen leaves were crushed in a juicer machine for extraction according to the procedure explained by Yasmeen *et al*., (2018). The extract was filtered twice by using Whatman No.1 filter paper and then centrifuged at 8000 g for 20 minutes and diluted 30 times with distilled water. Using distinctive procedures, moringa leaf extract (MLE) was analyzed for chemical composition. 18 chemical constituents were observed in the moringa leaf extract; they are super oxide dismutase (191.86 IU min^{-1}mg^{-1} protein), peroxidase (21.99 IU min^{-1}mg^{-1} protein), catalase (7.09 IU min^{-1}mg^{-1} protein), zeatin (0.96 mg g^{-1}), gibberellins (0.74 mg g^{-1}), total soluble protein (1.40 mg g^{-1}), total phenolic contents (8.19 mg g^{-1}), ascorbic acid (0.36 m mole g^{-1}), nitrogen (1.93%), phosphorus (0.18%), Potassium (2.19%), Calcium (2.43%), Magnesium (0.012%), Zinc (38.33 mg k g^{-1}), Copper (3.50 mg k g^{-1}), iron (544 mg k g^{-1}), manganese (49.67 mg k g^{-1}) and boron (21.33 mg k g^{-1}).

The weather data from the sowing of quinoa to the harvesting period was collected from meteorological observatory of CCRI, Multan during 2018-19 and 2019-20 (Fig. 1a & b). Data regarding total precipitation, average relative humidity and mean maximum temperature and mean sunshine hours was documented on daily basis and averaged on the particular month.

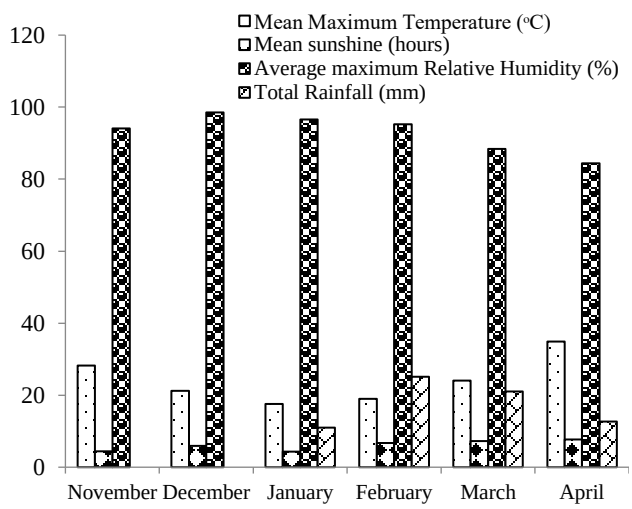

Fig. 1a. Metrological data for quinoa growth period during 2018-19.

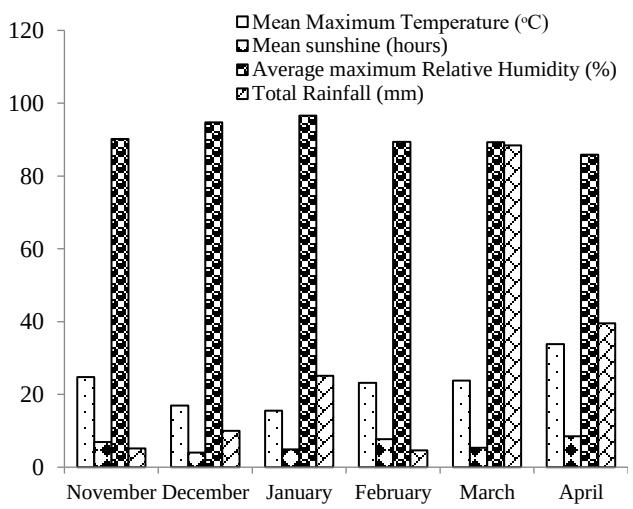

Fig. 1b. Metrological data for quinoa growth period during 2019-20.

Crop husbandry: Irrigation (rauni) was applied to selected experimental plots 6 days before the fine seedbed preparation. At optimum level of soil moisture, seedbed was prepared by cultivating the field thrice each followed by planking to preserve moisture appropriate for germination during both growing seasons. Seeds of quinoa cultivar UAF-Q-7 obtained from University of Agriculture Faisalabad were sown by using hand drill at the depth of 2-3 cm using @ 10Kg ha^{-1} seed rate and keeping 25 cm row to row distance and 10 cm plant to plant distance on 4th and 1st November 2018-19 and 2019-20, respectively. Irrigation was applied according to the treatments. At the time of sowing suggested dose of fertilizers was applied @ 40-50-50 N-P-K Kg ha^{-1}. Remaining quantity of nitrogenous fertilizer @ 40 Kg per hectare was applied at flowering stage. Weeds were manually removed after three weeks of sowing. No insect pest damage was observed on quinoa crop during both growing seasons. All other agronomic practices were maintained similar for all treatments during the growth period. Crop was harvested on physiological maturity on 23rd and 19th of April during 2018-19 and 2019-20, respectively.

Data collection

Twenty randomly tagged plants from each experimental unit were selected 30 days after sowing to record plant height, number of branched panicles per plant, biological yield and seed yield. Leaf area of twenty randomly tagged quinoa plants was calculated with help of leaf area meter at 45, 60 and 75 days after emergence. Leaf area index was computed by using succeeding formula suggested by Sestak *et al*., (1971).

$$LAI = \frac{\text{Leaf area per plant}}{\text{Land area covered}}$$

Crop growth rate represents the productivity of dry weight per unit area for specific time span and it was recorded as illustrated by Hunt (1978).

$$CGR\ (g\ m^{-2}\ day^{-1}) = \frac{W_2 - W_1}{t_2 - t_1}$$

whereas W_1 and W_2 are the total dry weight at time t_1 and t_2

To determine chlorophyll-concentrations, leaf sample of 0.5 g was grounded in ten-milliliter acetone (80%) in pestle and mortar. This grounded sample was moved to falcon tubes. Then falcon tubes comprising grounded sample were kept in centrifuge for ten minutes at 15000 rpm. Developed supernatant was taken in quartz-cuvette that was placed in spectrophotometer (UV4000) to observe absorbance of supernatant at 663 and 645 nm wavelengths against 80% acetone as blank. Chlorophyll *a* and *b* concentrations were calculated by using following formula illustrated by (Arnon, 1949).

Chlorophyll "*a*" (mg g^{-1}) = [(0.0127×A663–0.00269×A645)×100]/0.5
Chlorophyll "*b*" (mg g^{-1}) = [(0.0229×A645–0.00468×A663)×100]/0.5

After seventy days of emergence, well-developed fresh leaves were separated from five selected quinoa plants of each experimental unit during early in the morning. These leaves were enveloped in aluminium foil and then packed in plastic zipper-bags and put in icebox and later kept in freezer till analysis. Data regarding enzymatic antioxidants were recorded within three days. Leaf sample of 0.1g was grounded in one-milliliter phosphate buffer solution (50 mM; pH 7.8) in ice-cold pestle and mortar. Grounded sample was moved to ice-cold Eppendorf tubes. Then Eppendorf tubes comprising grounded sample were kept instantly in centrifuge for twenty minutes at 15000 rpm at -4°C and the supernatant was received to consume in analyses to record the enzymatic antioxidant activities such as SOD (Giannopolitis & Ries, 1977), POD and CAT (Chance & Maehly, 1955) by recording absorbance at 560, 470 and 240, respectively. Moreover, leaf ascorbate contents were recorded at 525 nm following the procedure explained by Yin *et al*., (2008).

Free proline intensities were estimated by adopting the rapid colourimetric method described by Bates *et al*., (1973). Dry leaf samples of 0.5g were extracted by grinding in ten milliliter 3% (v/v) sulpho salicylic acid and then centrifuged at 10,000×g for ten minutes. 02 ml of the supernatant was kept in a test tube, to which two milliliter of a freshly prepared acid ninhydrin solution was added. The test tubes were incubated in a water bath to 90°C for thirty minutes and the reaction was dismissed in an ice bath. Every reaction mixture was extracted with five-milliliter toluene and vortex-mixed for fifteen seconds. The test tubes were permitted to keep in the dark for at least twenty minutes at ambient temperature, to permit separation of the toluene and aqueous stages. Each toluene stage was then sensibly collected into a sterile test tube and its absorbance was read at 520 nm. The free proline content in every sample was estimated from a typical curve prepared by adopting analytical grade proline.

Statistical analysis

All the collected data was statistically analyzed by adopting appropriate computer-based software MSTAT C. The Duncan's Multiple Range test (DMR) was applied for comparison the variations among treatment means at 5% level of probability (Steel *et al*., 1997).

Results

Statistical analysis of the data showed that application of growth promoters on quinoa plants cultivated under water deficit regimes at different growth stages significantly affected the growth parameters during 2018-19 and 2019-20. All the priming and foliar treatments increased growth attributes as compared to control. Combined application of MLE30 through seed and foliage on quinoa plants cultivated under water stress at reproductive stage produced maximum leaf area index, crop growth rate and plant height against the minimum which was noticed from control plants (Figs. 2a & b, 3a & b and Table 1).

Table 1. Effect of growth promoters on plant height, number of branched panicles per plant and biological yield of quinoa plants.

Water stress	Growth promoters application	Plant height (cm)		Branched panicle plant^{-1}		Biological yield (Kg ha^{-1})	
		2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Control	Control	81.43i	88.09d	9.23m	9.70m	5530.7i	5707.3f
	MLE30 seed Priming	85.98fi	94.08cd	12.02jk	11.36ik	5723.0fi	6056.3bf
	Proline seed Priming	84.37gi	92.37cd	10.41lm	10.74kl	5632.0hi	5898.7df
	MLE3o foliar spray	88.18ci	98.27ad	13.51di	13.31df	5886.7di	6253.3af
	Proline foliar spray	86.35fi	95.32 bd	12.79hj	13.14eg	5790.0ei	6090.0bf
	MLE30 seed priming + MLE30 foliar spray	89.50bh	101.50ad	14.30af	14.04be	6036.3bh	6359.7af
	MLE30 seed priming + proline foliar spray	87.60di	98.26ad	12.86gj	13.30df	5956.0ci	6232.7af
	Proline seed priming + proline foliar spray	89.14ch	96.80ad	13.70di	13.40df	5966.7ci	6206.7af
	Proline seed priming + MLE3o foliar spray	88.12di	100.45ad	13.77ch	14.11be	5905.3ci	6238.7af
Water stress at vegetative growth stage	Control	82.90hi	91.24cd	10.08lm	10.02lm	5626.0hi	5759.3ef
	MLE30 seed Priming	87.06ei	96.72ad	11.87jk	12.03hj	5895.3ci	6128.7af
	Proline seed Priming	85.06gi	94.92 cd	10.96kl	11.63hk	5685.7gi	6019.0cf
	MLE3o foliar spray	90.03ah	104.07ad	13.13ej	13.61ce	6107.3ag	6440.7ae
	Proline foliar spray	87.70di	101.70ad	12.96fj	13.30df	5843.0ei	6209.7af
	MLE30 seed priming + MLE30 foliar spray	91.80ag	107.33ac	15.28ab	14.99ab	6349.7ac	6683.0ac
	MLE30 seed priming + proline foliar spray	88.66ci	102.01ad	14.22bg	14.72ab	6159.7af	6493.0ad
	Proline seed priming + proline foliar spray	87.76di	102.51ad	14.41ae	14.54ac	6201.0ae	6501.0ad
	Proline seed priming + MLE3o foliar spray	90.92ag	109.85ac	14.79ad	14.96ab	6222.0ae	6555.3ad
Water stress at reproductive growth stage	Control	87.18ei	95.81ad	12.32ik	11.15jk	5854.7ei	6021.3cf
	MLE30 seed Priming	91.17ag	106.83ac	13.14ej	12.48fh	6187.7ae	6384.3af
	Proline seed Priming	89.84ah	103.17ad	12.44hj	12.21gi	6101.7ag	6235.0af
	MLE3o foliar spray	95.22ad	108.56ac	14.64ad	14.31ad	6345.7ac	6679.0ac
	Proline foliar spray	93.62af	106.95ac	14.30af	14.32ad	6314.7ad	6548.0ad
	MLE30 seed priming + MLE30 foliar spray	97.22a	113.88ab	15.65a	15.32a	6477.0ab	6810.3a
	MLE30 seed priming + proline foliar spray	94.58ae	105.27ad	14.62ad	14.96ab	6522.3a	6755.7ab
	Proline seed priming + proline foliar spray	95.81ac	104.85ad	15.17ac	14.83ab	6466.3ab	6633.0ac
	Proline seed priming + MLE3o foliar spray	96.88ab	114.25a	15.62 ab	15.29a	6496.7a	6763.3ab
LSD 0.05*p*=		7.6742	18.736	1.4209	1.0297	457.93	720.28
Standard error		3.8244	9.3372	0.7081	0.5131	228.21	358.95

Table 2. Effect of growth promoters on seed yield and chlorophyll contents of quinoa plants.

Water stress	Growth promoters application	Seed yield (Kg ha^{-1})		Chlorophyll *a* (mg g^{-1})		Chlorophyll *b* (mg g^{-1})	
		2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Control	Control	1269.7o	1306.3j	1.98k	2.04j	0.64l	0.70j
	MLE30 seed Priming	1382.3ln	1522.3fi	2.13gj	2.15fj	0.79k	0.82hj
	Proline seed Priming	1298.7no	1445.3hj	2.05ik	2.11hj	0.79k	0.77ij
	MLE3o foliar spray	1433.7jm	1627.0cg	2.19ei	2.21dj	0.84jk	0.87gj
	Proline foliar spray	1354.0mo	1537.3fh	2.12hk	2.18ej	0.81k	0.84hj
	MLE30 seed priming + MLE30 foliar spray	1517.3fj	1657.3bg	2.25dh	2.29dh	1.02fh	0.96eh
	MLE30 seed priming + proline foliar spray	1434.7jm	1601.3dh	2.18fi	2.23di	0.88ik	0.90gi
	Proline seed priming + proline foliar spray	1445.7im	1579.0eh	2.18fi	2.27dj	0.90hk	0.91gi
	Proline seed priming + MLE3o foliar spray	1477.0hk	1648.7bg	2.26dh	2.28dh	1.02fh	0.98eh
Water stress at vegetative growth stage	Control	1299.7no	1366.3ij	2.01jk	2.04ij	0.80k	0.79ij
	MLE30 seed Priming	1443.7jm	1543.7eh	2.22dh	2.26dh	0.98gi	0.94fi
	Proline seed Priming	1391.7kn	1505.0gi	2.18fi	2.25dh	0.94gj	0.92fi
	MLE3o foliar spray	1557.7dh	1671.0bg	2.23dh	2.30ch	1.05eg	1.03dg
	Proline foliar spray	1484.0gk	1567.3eh	2.20dh	2.26dh	0.99gi	0.97eh
	MLE30 seed priming + MLE30 foliar spray	1646.3ad	1813.0ab	2.32cf	2.33af	1.24bd	1.14be
	MLE30 seed priming + proline foliar spray	1581.3cf	1648.0bg	2.19ei	2.25dh	1.14df	1.10cf
	Proline seed priming + proline foliar spray	1539.7ei	1639.7cg	2.25dh	2.26dh	1.18cd	1.14be
	Proline seed priming + MLE3o foliar spray	1564.0dh	1707.3ae	2.27dg	2.34af	1.23bd	1.17ad
Water stress at reproductive growth stage	Control	1457.0il	1457.0hj	2.18fi	2.14gj	0.96gj	0.93fi
	MLE30 seed Priming	1606.0bf	1639.3cg	2.35cd	2.32bg	1.22bd	1.13be
	Proline seed Priming	1523.3ej	1590.0dh	2.29cf	2.27dh	1.16de	1.13be
	MLE3o foliar spray	1686.0ab	1752.7ad	2.43bc	2.40ad	1.29ac	1.22ac
	Proline foliar spray	1573.0dg	1689.7bf	2.34ce	2.35ae	1.24bd	1.18ad
	MLE30 seed priming + MLE30 foliar spray	1709.7a	1859.7a	2.59a	2.52a	1.38a	1.33a
	MLE30 seed priming + proline foliar spray	1668.3ac	1758.3ad	2.55ab	2.49ac	1.31ab	1.29ab
	Proline seed priming + proline foliar spray	1614.3be	1707.7ae	2.54ab	2.49ab	1.32ab	1.28ab
	Proline seed priming + MLE3o foliar spray	1688.0ab	1778.0ac	2.55ab	2.51ab	1.34ab	1.29ab
LSD 0.05*p*=		94.629	169.13	0.1905	0.1478	0.1237	0.1748
Standard error		47.158	84.283	0.0736	0.0949	0.0617	0.0871

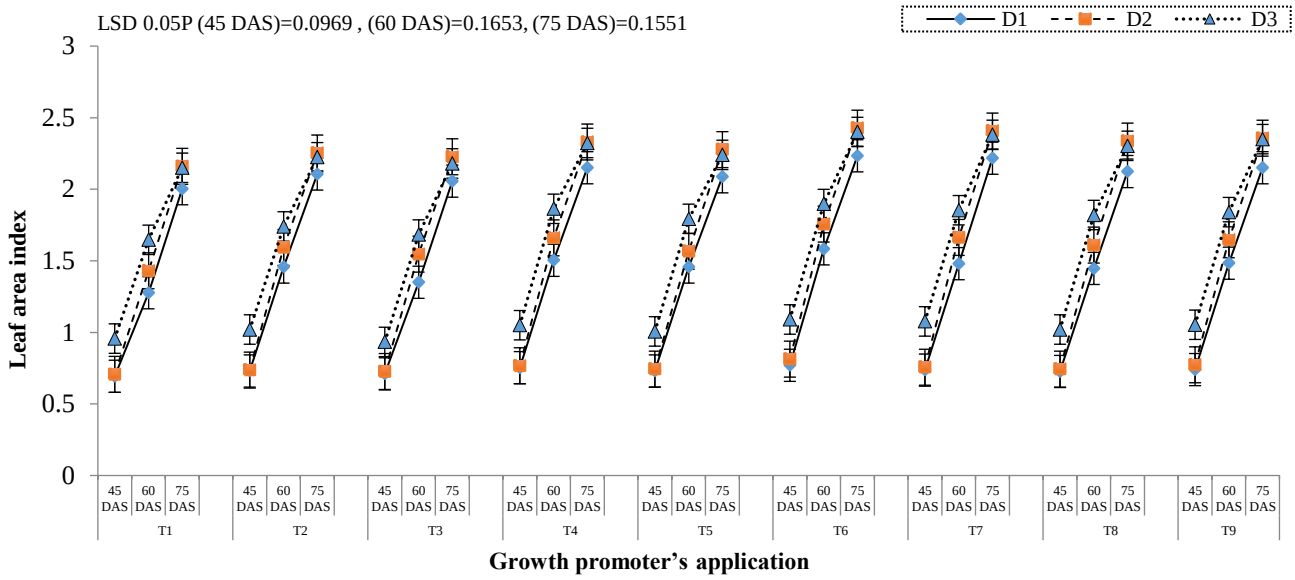

Fig. 2a. Effect of growth promoters on leaf area index of quinoa plants during 2018-19.

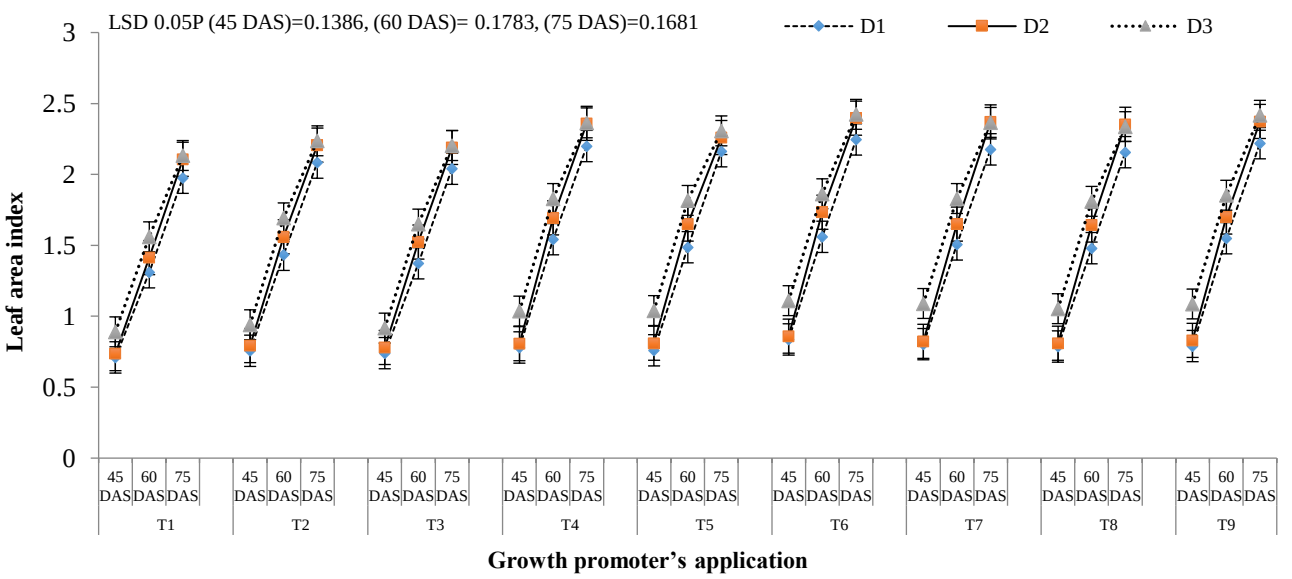

Fig. 2b. Effect of growth promoters on leaf area index of quinoa plants during 2019-20.

whereas T_1= Control, T_2 = MLE30 seed Priming, T_3 = Proline seed Priming, T_4 = MLE30 foliar spray, T_5 = Proline foliar spray, T_6 = MLE30 seed priming + MLE30 foliar spray, T_7 = MLE30 seed priming + Proline spray, T_8 = Proline priming + Proline spray and T_9 = Proline priming + MLE30 spray, D_1= Control, D_2 = Water deficit at vegetative stage, D_3 = Water deficit at reproductive stage.

The analysis of variance of the data indicated that application of growth promoters on quinoa plants cultivated under water stress at distinctive stages of growth significantly affected the yield contributing attributes during both growing seasons (Tables 1 & 2). Seed priming + foliar spray of MLE30 on quinoa plants cultivated under water stress conditions at reproductive growth stage exhibited maximum number of branched panicles per plant and seed yield per unit area. While minimum number of branched panicles per plant and seed yield was observed from control plots during 2018-19 and 2019-20. Whereas MLE30 seed priming + proline spray and seed priming + foliar spray of MLE30 on quinoa plants cultivated under water deficit conditions at reproductive growth stage produced maximum biological yield as compared to other treatments during 2018-19 and 2019-20, respectively. Whereas minimum biological yield was observed from control plots.

Interaction between water deficit at various growth stages and growth promoter's application showed significant effect on chlorophyll *a* and *b* during 2018-19 and 2019-20 (Tables 2 & 3). Seed priming + foliar spray of MLE30 on quinoa plants cultivated under water deficit conditions at reproductive growth stage produced maximum chlorophyll *a* and *b* against the minimum was documented from control plots.

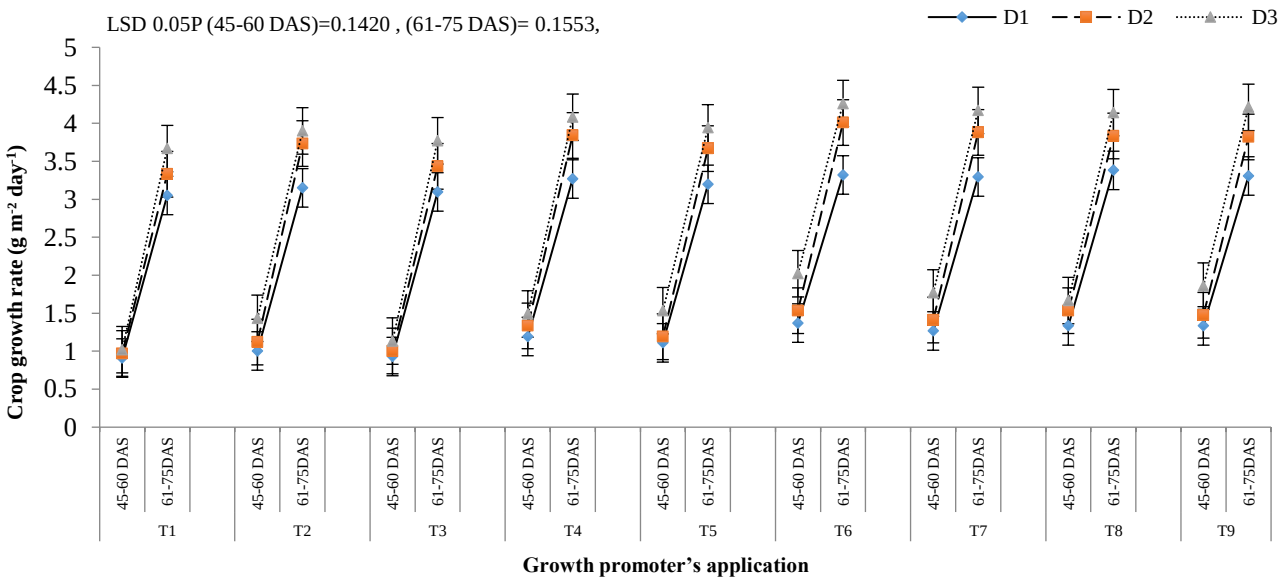

Fig. 3a. Effect of growth promoters on crop growth rate (g m^{-2} day^{-1}) of quinoa plants during 2018-19.

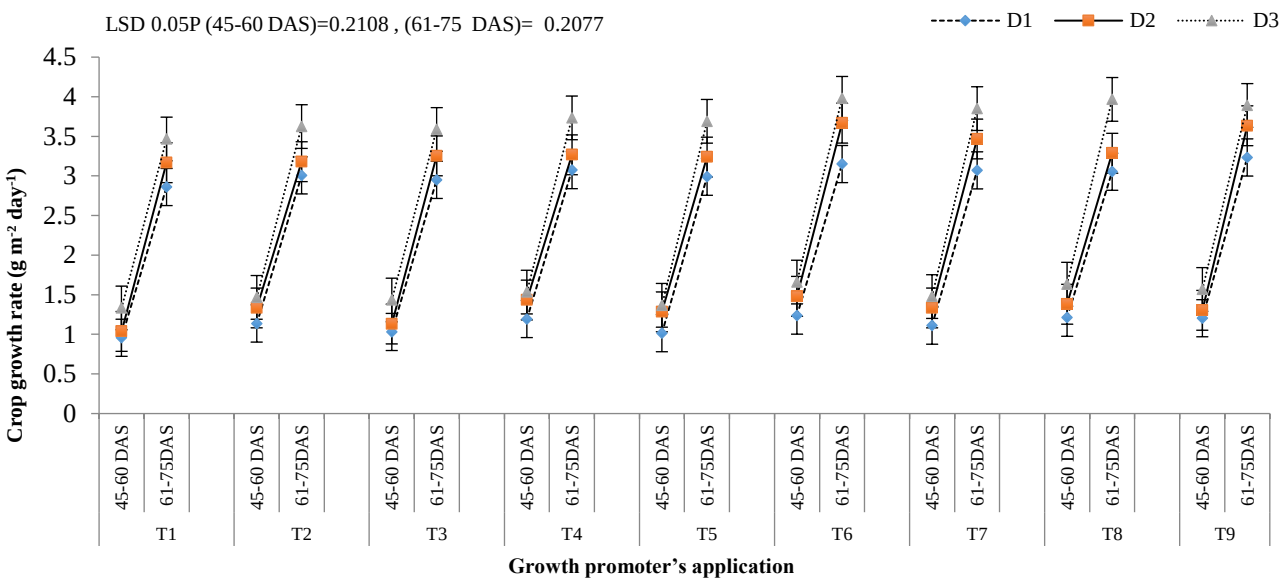

Fig. 3b. Effect of growth promoters on crop growth rate (g m^{-2} day^{-1}) of quinoa plants during 2019-20.

whereas T_1= Control, T_2 = MLE30 seed Priming, T_3 = Proline seed Priming, T_4 = MLE30 foliar spray, T_5 = Proline foliar spray, T_6 = MLE30 seed priming + MLE30 foliar spray, T_7 = MLE30 seed priming + Proline spray, T_8 = Proline priming + Proline spray and T_9 = Proline priming + MLE30 spray, D_1= Control, D_2 = Water deficit at vegetative stage, D_3 = Water deficit at reproductive stage.

The analysis of variance of the data indicated that application of growth promoters on quinoa plants cultivated under water stress at distinctive phases of growth significantly affected the proline contents during both growing seasons (Table 3). Combined exogenously applied proline through seed and foliage produced maximum proline contents from un-irrigated plots. Minimum proline contents were observed from quinoa plants cultivated under water stress at reproductive stage.

Interaction between water deficit at various growth stages and growth promoters' application showed significant effect on ascorbic acid during both years (Table 3). Seed priming + foliar spray of MLE30 and proline seed priming + MLE30 foliar spray on quinoa plants cultivated under water deficit conditions at vegetative growth stage produced maximum ascorbic acid during 2018-19 and 2019-20, respectively. While minimum ascorbic acid was recorded from control plots.

The analysis of variance of the data indicated that application of growth promoters on quinoa plants cultivated under water stress at distinctive growth stages of significantly affected the superoxide dismutase, peroxidase and catalase contents during both growing seasons (Table 4). Combined application of MLE30 through seed and foliage produced maximum superoxide dismutase, peroxidase and catalase contents from un-irrigated plots. Minimum superoxide dismutase, peroxidase and catalase contents were observed from quinoa plants cultivated under water stress at reproductive stage.

Table 3. Effect of growth promoters on proline and ascorbic acid of quinoa plants.

Water stress	Growth promoters application	Proline content (µg g^{-1})		Ascorbic acid (m.mol g^{-1})	
		2018-19	2019-20	2018-19	2019-20
Control	Control	5.52gh	5.1867ik	8.57n	8.433p
	MLE30 seed Priming	6.16bf	5.76fi	10.76lm	10.390o
	Proline seed Priming	5.99cg	5.88dh	10.27m	10.207o
	MLE3o foliar spray	6.65ab	6.28bf	12.19jk	11.853lm
	Proline foliar spray	6.56ac	6.3933af	11.27kl	11.38mn
	MLE30 seed priming + MLE 30 foliar spray	6.61ac	6.6167ac	13.29fh	12.890ik
	MLE30 seed priming + proline foliar spray	6.86a	6.7267ab	12.44hj	12.573jl
	Proline seed priming + proline foliar spray	7.02a	6.96a	12.23ij	12.30km
	Proline seed priming + MLE3o foliar spray	6.66ab	6.5333ac	13.17gi	12.840ik
Water stress at vegetative growth stage	Control	5.19hj	5.2533hj	12.17jk	10.823no
	MLE30 seed Priming	5.90dg	6.0167cg	14.39de	13.553hi
	Proline seed Priming	5.67fh	5.86eh	14.10dg	13.433hj
	MLE3o foliar spray	6.40ae	6.34af	17.29ab	14.287eh
	Proline foliar spray	6.19bf	6.36af	16.86bc	14.527dg
	MLE30 seed priming + MLE 30 foliar spray	6.41ae	6.5233ac	18.13a	14.797dg
	MLE30 seed priming + proline foliar spray	6.49ad	6.5100ad	16.89bc	14.823cg
	Proline seed priming + proline foliar spray	6.59ac	6.5567ac	16.74bc	15.070bf
	Proline seed priming + MLE3o foliar spray	6.45ae	6.4767ae	18.02a	15.353bd
Water stress at reproductive growth stage	Control	4.28k	4.6167k	12.31ij	10.647no
	MLE30 seed Priming	4.69jk	5.0400jk	14.23df	13.897gh
	Proline seed Priming	4.58jk	4.8933jk	13.83eg	14.160fh
	MLE3o foliar spray	5.09hj	5.2233ik	15.01d	14.880cf
	Proline foliar spray	4.84ik	5.1400ik	15.03d	15.120bf
	MLE30 seed priming + MLE 30 foliar spray	5.56fh	5.39gj	16.18c	15.850b
	MLE30 seed priming + proline foliar spray	5.84eg	5.3300hj	15.04d	15.777bc
	Proline seed priming + proline foliar spray	5.73fh	5.50gj	14.88d	15.210be
	Proline seed priming + MLE3o foliar spray	5.43gi	5.2600hj	16.14c	16.883a
LSD 0.05 *p*=		0.6425	0.6348	0.9525	0.9621
Standard error		0.3202	0.3163	0.4747	0.4795

Table 4. Effect of growth promoters on antioxidant activities of quinoa plants.

Water stress	Growth promoters application	Superoxide dismutase (IU $min^{-1}mg^{-1}$ protein)		Peroxidase (IU $min^{-1}mg^{-1}$ protein)		Catalase (IU $min^{-1}mg^{-1}$ protein)	
		2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Control	Control	8.53dg	8.68cd	5.27gl	5.60fi	7.58ch	7.65ch
	MLE30 seed Priming	8.76ce	8.73cd	5.45fk	5.78eh	7.72bg	7.79cf
	Proline seed Priming	8.71ce	8.77c	5.61ei	5.74eh	7.72bg	7.78cf
	MLE3o foliar spray	8.34di	8.74cd	5.42fk	5.71eh	7.68bg	7.74cg
	Proline foliar spray	9.01cd	8.85c	6.15be	6.49bd	7.96be	8.01be
	MLE30 seed priming + MLE30 foliar spray	10.82a	10.54a	7.35a	7.69a	8.64a	8.74a
	MLE30 seed priming + proline foliar spray	9.26bc	9.59b	6.64bc	6.82bc	8.09ac	8.21ad
	Proline seed priming + proline foliar spray	9.75b	9.69b	6.83ab	6.98b	8.32ab	8.48ab
	Proline seed priming + MLE3o foliar spray	8.67cf	8.80c	6.03cf	6.28ce	7.87bf	7.80bf
Water stress at vegetative growth stage	Control	7.68in	7.81fj	4.74lm	5.04im	6.74il	6.91ik
	MLE30 seed Priming	8.26ej	7.93ei	5.02hm	5.35gk	7.14gi	7.09gj
	Proline seed Priming	8.17ek	7.83fi	4.97im	5.30hl	7.17gi	7.13fj
	MLE3o foliar spray	7.71im	7.83fi	4.83jm	5.10im	6.93hk	7.02hk
	Proline foliar spray	8.24ej	8.02eh	5.51ej	5.57fi	7.19gi	7.36ei
	MLE30 seed priming + MLE30 foliar spray	9.80b	9.90ab	6.30bd	6.67bc	8.05ad	8.22ac
	MLE30 seed priming + proline foliar spray	7.82gm	8.10dg	5.69dh	5.79eh	7.29fi	7.40ei
	Proline seed priming + proline foliar spray	8.45dh	8.21cf	5.98cf	5.93dg	7.42dh	7.53di
	Proline seed priming + MLE3o foliar spray	7.99fk	7.92ei	5.29gl	5.46gj	6.98hj	7.13fj
Water stress at reproductive growth stage	Control	6.98n	7.18j	4.54m	4.71m	5.62o	5.75m
	MLE30 seed Priming	7.52kn	7.42hj	4.80km	4.90jm	5.92mo	5.89m
	Proline seed Priming	7.51kn	7.41hj	4.94im	4.87jm	6.10lo	5.90m
	MLE3o foliar spray	7.26ln	7.29ij	4.61lm	4.76lm	5.80no	5.86m
	Proline foliar spray	7.61jn	7.53gj	4.96im	5.02im	6.19lo	6.06lm
	MLE30 seed priming + MLE30 foliar spray	8.82ce	8.49ce	5.84dg	6.05df	7.32ei	7.65ch
	MLE30 seed priming + proline foliar spray	7.79hm	7.73fj	5.08hm	5.21hm	6.33kn	6.40km
	Proline seed priming + proline foliar spray	7.88gl	7.81fj	5.16gm	5.31hl	6.47jm	6.61jl
	Proline seed priming + MLE3o foliar spray	7.15mn	7.31ij	4.62lm	4.84km	5.85mo	5.95lm
LSD 0.05*p*=		0.7155	0.6459	0.6795	0.5913	0.6559	0.6840
Standard error		0.3566	0.3219	0.3386	0.2947	0.3268	0.3409

Discussion

Water stress is one of the strategic menaces to plants, as it interrupts the plant–water relations from cellular, molecular and organs to the entire plants (Muscolo *et al*., 2015). Results of the current investigation showed that water stress adversely affected the crop growth parameters including leaf area index, crop growth rate and final plant height of quinoa plants during both growing seasons. Growth of crop plants is the consequence of intricate interactions between cell division and expansion and their distinction, such as inherited, morpho-physiological and environmental conditions (Anjum *et al*., 2011). Moisture stress adversely affected the cell division and elongation because of lower turgor pressure (Taiz and Zeiger, 2006; Sehgal *et al*., 2018). However, seed priming and foliar spray of MLE30 improved the quinoa plants growth parameters even under severe water stress conditions. Higher leaf area index might be due to the combine impact of growth promoting hormones and availability of essential minerals present in moringa leaf extract (Khan *et al*., 2020). High crop growth rate and plant height could be due to improved leaf area indices which is a symbol of greater photosynthesis rate and carbohydrate production that improved crop growth performance (Khan *et al*., 2020). Furthermore, existence of essential nutrients and growth encouraging hormone i.e., zeatin in moringa leaf extract that improved the growth with increase the cell division rate under stress conditions (Aregheore, 2002).

Severity of water stress has a straight effect on crop productivity and adversely affected the number of branched panicles per plant, biological and seed yield. This decline in quinoa yield under water stress condition may be related to reduce chlorophyll contents and photosynthetic capability of leaves (Chaves *et al*., 2011). However, application of growth promoters improved the yield and yield contributing parameters even under severe water stress conditions. Seed priming and foliar spray of MLE30 produced maximum number of branched per panicle, biological yield and grain yield. MLE30 being natural and rich source of essential plant mineral nutrients and hormones plays a fundamental role in the improvement of quinoa yield (Rashid *et al*., 2018). Previously Yasmeen *et al*., (2012) observed that application of MLE30 increase the wheat productivity under adverse environmental circumstances due to a "stay green phenomenon," that enhanced the grain-filling period. In fact, cytokinin is present in MLE that might be intricate in the stay green character (Khan *et al*., 2017).

Chlorophyll contents in the leaves of quinoa plants were considerably reduced under water deficit conditions. This decline in chlorophyll contents under moisture deficit situations could be due to the inefficiency of the thylakoid membrane, with higher deprivation as compared to the production of chlorophyll contents through the synthesis of proteolytic enzymes, diminishing the process of photosynthesis and obstructing the accumulation of ions (Jaleel *et al*., 2008; Mafakheri *et al*., 2009). However, exogenously applied MLE30 significantly improved the leaves chlorophyll contents in quinoa plants. It might be due to the fact that leaves of moringa comprise of considerable amounts of definite pigments with strong antioxidant characteristics including chlorophyll and carotenoids (Owusu, 2008). Furthermore, moringa leaves have numerous macronutrients such as magnesium, which is an integral part of chlorophyll that improved the concentration of chlorophyll in crop plants (Yameogo *et al*., 2011).

In order to withstand water stress conditions, plants use their enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, peroxidase and ascorbic acid that help in scavenging the ROS in plants (Ahmad & Umar, 2011). Seed priming and exogenous application of MLE30 in water stress circumstances significantly boosted the synthesis of antioxidants to scavenge the raised level of reactive oxygen species. Earlier it was observed that exogenously applied MLE30 promptly reduced the oxidative damage and improved antioxidant system in soybean (Hanafy, 2017). Similarly, Zaki & Rady (2015) noticed that use of moringa leaf extract for seed priming or exogenous application increased the antioxidants activities in bean crop plants. In fact, higher antioxidant activities could be related to the induction of antioxidant responses, which guard the crop plants from oxidative injury (Foyer & Noctor, 2005).

Conclusion

Water stress adversely affected the growth and yield contributing attributes that ultimately reduced the quinoa productivity. However, application of MLE30 improved the growth and yield contributing parameters and produced significantly higher chlorophyll contents, proline content, ascorbic acid, superoxide dismutase, peroxidase and catalase that modulate the adverse effects of water stress leading to higher quinoa seed yield under water stress conditions. These consequences suggest that application of MLE30 enhanced water stress tolerance in quinoa plants by improving the antioxidant system.

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