

EXOGENOUS APPLICATION OF GROWTH PROMOTERS CAN IMPROVE THE CHICKPEA PRODUCTIVITY UNDER TERMINAL HEAT STRESS CONDITIONS BY MODULATING THE ANTIOXIDANT ENZYME SYSTEM

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Heat stress causes irrecoverable damage to plant growth, thus adversely affecting the crop productivity. This study was planned to minimize the heat stress induced-losses to chickpea plants with the exogenous application of growth promoters i.e. moringa leaf extract (MLE), jasmonic acid 100 μ M and proline 10uM including distilled water as a control. Two chickpea cultivars (Thal-2006 and Noor-2013) cultivated under optimum sowing time (25th October) and late sowing (25th November) to expose heat stress at the reproductive phase. Results indicated that heat stress severely reduced the chickpea growth and productivity. It was observed that exogenously applied MLE and proline improved productivity even under heat stress conditions as compared to control. However, exogenous application of MLE had a greater influence on the studied parameters than proline, although the insignificant differences were documented in some of the traits. Exogenous application of MLE produced significantly taller plants with improved 100-grains weight, economic yield, biological yield and harvest index. However, foliar spray of proline produced significantly higher enzymatic antioxidants activities than MLE that modulated the adverse impacts of heat stress on yield contributing attributes. Our results suggest that exogenous application of plant growth promoters particularly moringa leaf extract and proline modulated the heat stress induced losses to chickpea plants by improving their antioxidant defense mechanism and enhanced the productivity.

Keywords: Antioxidant, temperature stress, chickpea, proline, moringa leaf extract.

INTRODUCTION

Chickpea is an important grain legume crop and essential constituent of crop rotations in the world. However, high temperature often restricted the growth and productivity of chickpea. Optimum temperature at different growth stages adjusting the crop growth and scheduling of blooming thus affecting the economic yield (Teixeira *et al.*, 2013). High temperature may restrict crop development and productivity at various phenological stages. Environment changes increased the average temperature around 0.74 °C per 100 years (IPCC, 2007). However, during the last 50 years, the linear heating tendency has been approximately twofold the rate of the preceding 100 years. Forecasts to the expiration of the twenty first century appraise to increase in worldwide average temperature between 1.8 and 4 °C, relying on greenhouse emissions and variations in precipitation patterns (IPCC, 2007). These variations in temperature will definitely influence crop growth and reduce productivity up to 30% (ICRISAT, 2009).

Global rise in temperature, variable rainfall and moisture deficit are the alarming indications can have erratic production patterns all over the world. Unfavorable influences of abiotic stresses on chickpea plants can be minimized with the exogenous application of bio-stimulants and synthetic growth promoters that brings significant

variations in plant physiology and biochemistry (Verma *et al.*, 2016). Among various growth promoters, proline, jasmonic acid and moringa leaf extract (MLE) possess a definite role and are studied as one of the fundamental management techniques under adverse environmental conditions.

Foliar spray of proline is getting an important position in modern agronomic investigation to survive under abiotic stress circumstances (Sadak and Ahmed, 2016). As it is a proteinogenic amino acid, extremely important for numerous fundamental biological developments inside the plant tissues. At optimal level, it has several roles such as osmoprotectant, the balance of redox status, storage of nitrogen, conservation the configuration of cytosolic pH and protein, reduced the lipid membrane peroxidation and acts as an antioxidant in adverse environmental conditions (Hayat *et al.*, 2012). It also plays a substantial role in standardizing uptake of nutrients under abiotic stress conditions (Sadak and Ahmed, 2016).

Similarly, Jasmonic acid (JA) is an endogenous regulator derived from the metabolism of membrane fatty acids, which has received considerable attention and plays an important role in regulating stress responses, plant growth and development (Shan and Liang, 2010). In response to many stress factors, jasmonic acid is involved in the signal transduction pathway. The primary function of JA in high temperature conditions is to prevent heat stress and the rapid

flow of water into the cell to perform cell turgor. In addition to the osmoregulation function, compatible solvents protect enzymes, knock down free oxygen radicals and preserve membrane structures and integrity. JA regulates anthocyanin production, senescence and bolus settings, which are responsible for the regulation of many physiological and metabolic processes in different plants, such as vegetation growth, stamens and trichome development (Yosefi *et al.*, 2018). In addition, JA activates the plant defense mechanism in response to pathogen invasion, insect-driven injuries, and abiotic stresses (Pauwels and Gosens, 2011). Foliar spray of JA stable cell viability in stressed plants determined by electrolyte leakage analysis (Clarke *et al.*, 2009).

Exogenous application of these plant growth promoters is the most effective approach involved in promoting plant growth and development by minimizing or alleviating the adverse effects of heat stress on the plant growth and metabolism in different crops (Sadak and Dawood, 2014), but this application is expensive. Therefore, there is continuous need to explore the safe and inexpensive natural plant growth promoters (Yasmeen *et al.*, 2018). *Moringa oleifera* L. is one of such alternative, being investigated to determine its impact on the plants development and productivity in heat stress circumstances. It is enormous cradle of mineral nutrients, zeatin, riboflavin, phenols, vitamin A and C, antioxidant and certain osmoprotectant that making it a prospective biostimulant (Yasmeen *et al.*, 2013). Moringa leaf extract (MLE) had considerably been consumed to enhance the stress tolerance in numerous crop plants by causing modulations in plant biochemistry mandatory for stress tolerance (Ismail *et al.*, 2016). Its foliar application could be used to improve the development, productivity and the quality attributes of cotton (Arif *et al.*, 2019b). Exogenous application of MLE has encouraging impacts on the plant development and improves the process of photosynthesis, regulate internal hormonal contents and anti-oxidative resistance activities in heat stress circumstances (Yasmeen *et al.*, 2014). Moreover, zeatin as supposed to be improved the antioxidant system and protects the plant cells from adverse influences of reactive oxygen species that ultimately strengthen the seedling growth and encouraging the plant development in heat stress circumstances (Rehman *et al.*, 2015).

Although a lot of work has been done on different crops to mitigate the adverse effects of heat stress by exogenous application of above studied growth promoters. However, only a limited studies showing the potential of proline, jasmonic acid and moringa leaf extract to promote the growth and productivity of chickpea under heat stress conditions are available. Therefore, we hypothesized that the foliar application of selected growth promoters contributes towards improving the physiological and antioxidant attributes and enhance the chickpea productivity under heat stress conditions. Keeping in view the above-mentioned discussion, the present two years study was planned to investigate the

influence of heat stress and role of natural and synthetic growth promoters in mitigating its adverse effects on chickpea.

MATERIALS AND METHODS

Experimental detail: A field study was carried out at Land Reclamation Research Station, 7/3-L Ahmad Pur Sial District Jhang (27.2038 °N, 77.5011 °E) during two consecutive winter seasons 2017-18 and 2018-19. Experimental treatments included two chickpea cultivars (Thal 2006 and Noor 2013), two sowing dates (25th October and 25th November) and three foliar spray of growth promoters (moringa leaf extract (MLE30) (Arif *et al.*, 2019a), jasmonic acid 100 µM (Ahmad and Murali, 2015) and proline 10uM (Kaur *et al.*, 2011) including distilled water as a control. The experiment was designed in completely randomized blocks with factorial arrangements and had three replications.

Preparation of moringa leaf extract (MLE): Moringa leaves are potential source of zeatin that enhances the antioxidant properties of many enzymes and protects the cells from injuries. Young leaves of *Moringa oleifera* plants were harvested and washed several times with distilled water then stored in freezer at -5 °C for 12 hours. Moringa 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 different methodologies, moringa leaf extract (MLE) was analyzed for chemical composition. Eighteen chemical constituents were identified in the moringa leaf extract; they are total soluble protein (1.40 mg g⁻¹), super oxide dismutase (191.86 IU min⁻¹ mg protein⁻¹), peroxidase (21.99 mmol min⁻¹ mg protein⁻¹), catalase (7.09 mmol min⁻¹ mg protein⁻¹), total phenolic contents (8.19 mg g⁻¹), ascorbic acid (0.36 mmol g⁻¹), gibberellins (0.74 mg g⁻¹), zeatin (0.96 mg g⁻¹), nitrogen (1.93%), phosphorus (0.18%), Potassium (2.19%), Calcium (2.43%), Magnesium (0.012%), Zinc (38.33 mg kg⁻¹), Copper (3.50 mg kg⁻¹), iron (544 mg kg⁻¹), manganese (49.67 mg kg⁻¹) and boron (21.33 mg kg⁻¹).

Crop Husbandry: A fine seedbed was prepared by two dry cultivations and soaking irrigation and at optimum moisture experimental soil was cultivated twice each followed by planking. Chickpea was sown by hand drill using 60 kg ha⁻¹ seed rate. To get optimum plant populations, chickpea plants were thinned out by maintaining one plant per hill at 15 days after sowing. Crop was once irrigated at flowering stage to demand of crop to save from moisture stress. Recommended dose of Urea @ 20 kg N ha⁻¹ and DAP @ 22 kg P ha⁻¹ was applied at the time of seedbed preparation. Intercultural operation was carried out at 50 days after sowing immediately after hand weeding. All other practices were kept identical for all the experimental units.

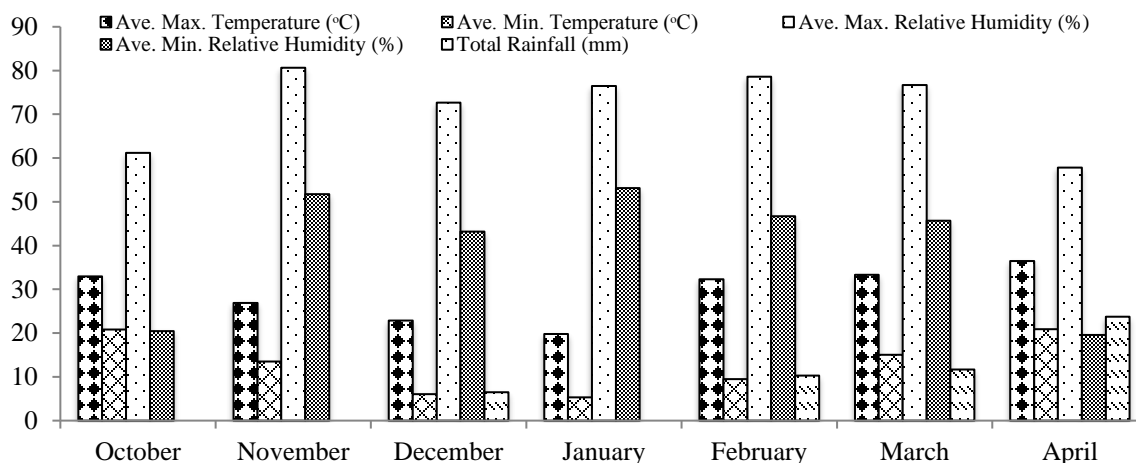


Figure 1a: Meteorological data for chickpea growth period during 2017-18

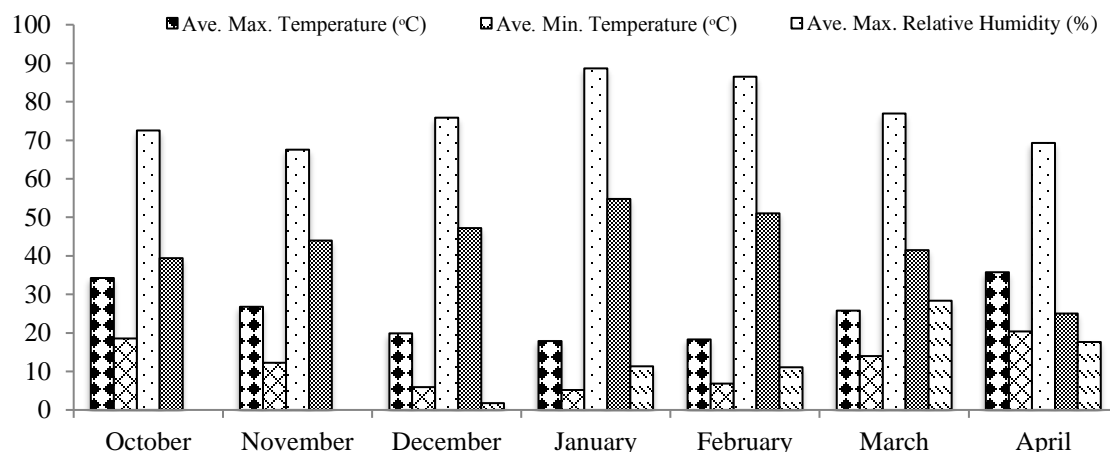


Figure 1b: Meteorological data for chickpea growth period during 2018-19

The weather statistics from chickpea sowing to final harvesting was collected from observatory of Land Reclamation Research Station, 7/3-L Ahmad Pur Sial District Jhang during the both growing seasons (Figure 1a & b). Data on total rainfall, mean maximum and minimum relative humidity and average maximum and minimum temperature was documented on circadian and averaged on respective month.

Data collection: After 30 days of sowing, 10 randomly selected plants were tagged from each experimental unit to record the final plant height, number of pods per plant and 100 grains weight. Ten days after every exogenous application of growth promoters, ten leaves sample were taken from each experimental unit to observe the antioxidant enzyme activities. All the leaves samples freeze, dried and then 0.5 g powder taken from freeze-dried leaves sampled obtained previously selected tagged plants were homogenized with 50mM Na₂HPO₄-NaH₂PO₄ buffer containing 0.2 mM EDTA and 2% insoluble polyvinyl pyrrolidone in a chilled pestle and mortar. The slurry was centrifuged at 12,000×g for

20 min and the supernatant was used for enzyme activities assay. Standard protocols were adopted to measure peroxidase (POD), catalase (CAT) (Chance and Maehly, 1955) and superoxide dismutase (SOD) (Giannopolitis and Reis, 1997). The mature crop was harvested on the 9th and 15th April 2018 and 2019, respectively and threshed manually to determine grain yield and biological yield.

Statistical analysis: Data collected during the growth period was statistically analyzed by adopting the Fisher's Analysis of Variance techniques and used 5% probability level to test least significant differences among various treatment means (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Plant height (cm): Plant height is an imperative morphological attribute for its role in light interception. Exogenous application of growth promoters under heat stress conditions significantly improved the plant height of chickpea cultivars during both growing years (Table 1). Exogenous application of MLE on black gram cultivar Thal 2006 cultivated at optimal sowing time (25th October) produced

Table 1. Effect of growth promoters on plant height (cm), pods per plant and 100-grain weight (g) of chickpea.

Treatments	Plant height (cm)		No. of pods per plant		100 grain weight (g)	
	2018	2019	2018	2019	2018	2019
V ₁ H ₁ F ₁	57.05ad	60.69ad	53.89ae	57.22ad	222.30be	228.97ad
V ₁ H ₁ F ₂	65.08a	67.67a	59.65ab	64.25a	239.20a	243.04a
V ₁ H ₁ F ₃	60.57ac	63.16ac	56.77ad	60.77ac	234.40ac	237.74ab
V ₁ H ₁ F ₄	62.06ab	65.27ab	61.38a	64.71a	237.66ab	241.12a
V ₁ H ₂ F ₁	49.28de	52.28cd	45.20fg	48.77df	202.16fg	207.50fg
V ₁ H ₂ F ₂	57.21ad	58.01ad	49.02dg	53.55bf	223.14ae	219.54cf
V ₁ H ₂ F ₃	54.07be	56.34ad	47.72eg	50.85df	215.21dg	212.87ef
V ₁ H ₂ F ₄	55.41ae	57.62ad	52.11bf	55.44be	219.86ce	214.86df
V ₂ H ₁ F ₁	56.26ae	58.62ad	50.06cg	51.06df	213.12dg	216.45cf
V ₂ H ₁ F ₂	62.24ab	65.35ab	55.89ae	60.22ac	235.82ac	234.15ab
V ₂ H ₁ F ₃	58.30ad	59.35ad	55.53ae	57.19ad	228.08ad	225.75be
V ₂ H ₁ F ₄	58.42ad	64.03ac	57.88ac	62.08ab	233.96ac	230.54ac
V ₂ H ₂ F ₁	45.70e	49.16d	42.23g	45.56f	199.76g	197.43g
V ₂ H ₂ F ₂	53.99be	57.54ad	48.59dg	51.14df	216.36df	213.02ef
V ₂ H ₂ F ₃	51.44ce	52.87cd	49.75cg	48.41ef	209.03eg	205.89fg
V ₂ H ₂ F ₄	52.59be	54.08bd	51.19cf	53.19cf	213.90dg	209.10fg
LSD $p=0.05$	10.573	12.162	8.3611	8.7137	16.247	14.179

Whereas V₁ = Thal 2006, V₂ =, Noor 2013 H₁ = 25th October, H₂ = 25th November, F₁ = distilled water, F₂ = MLE30, F₃ = Jasmonic acid and F₄ = Proline

considerably taller plants (Table 1). While minimum plant height was observed with the foliar spray of distilled water on Noor 2013 plants cultivated on 25th November. This reduction in plant height might be due to negative impact of heat stress, which showed that deviations from optimal temperature result in disruption of membrane, metabolic modifications and production of oxidative stress (Liu *et al.*, 2011). While, application of growth promoters has an effective role in mitigating the oxidative stress injury on the biologically more important mesophyll tissue. MLE being rich source of macro as well as micronutrients, vitamins, antioxidant and growth promoting hormones mitigates the adverse affects of heat stress that ensure stimulated the plant growth (Arif *et al.*, 2019b).

Number of pods per plant: Pods per plant propensity are one of the most important yield contributing traits of chickpea. Exogenously applied growth promoters significantly improved the pods per plant even under heat stress conditions (Table 1). Exogenous application of proline on black gram cultivar Thal 2006 cultivated at optimum sowing time (25th October) produced significantly higher number of pods per plant against the lowest was observed with the foliar spray of distilled water on Noor 2013 plants cultivated on 25th November (Table 1). It might be due to the fact that heat stress during flowering stage can cause failure of either pollination or pollen conceptions or even both, resulting in fewer numbers of pods developments (Boote *et al.*, 2005). On the other hand, foliar spray of proline on plants cultivated under heat stressed conditions significantly improved the pods per plant might be due to the protection of green leaf area duration during pods filling stage and reallocation of soluble

carbohydrates throughout pods filling phase (Husain *et al.*, 2018).

100 grains weight (g): The weight of single grain exhibited the amount of its development that is the fundamental yield determinant and plays a dynamic role in productivity. Exogenous applied growth promoters significantly improved the 100 grains weight even under heat stress conditions (Table 1). Exogenous application of MLE on black gram cultivar Thal 2006 cultivated at optimal sowing time (25th October) produced significantly greater 100 grains weight against the lowest was observed with the foliar spray of distilled water on Noor 2013 plants cultivated on 25th November (Table 1). Heat stress might disturb the pollination (Boote *et al.*, 2005) and successive fertilization leading to reduce fruit set (Snider *et al.*, 2010). However, foliar applied MLE proved to be potentially effective in alleviation of the adverse effects of heat stress. Higher grain weight with the foliar spray of MLE possibly attributed to stabilized membrane integrity and enhanced antioxidant activity enabled the maize plant to maintain photosynthesis due to higher chlorophyll contents at maturity even under heat stress (Rashid *et al.*, 2018).

Biological yield (Kg ha⁻¹): Exogenous application of growth promoters significantly improved the biological yield per unit area even under heat stress conditions (Table 2). Exogenous application of MLE on black gram cultivar Thal 2006 cultivated at optimum sowing time (25th October) produced significantly higher biological yield, which was statistically similar to foliar spray of proline on same cultivar cultivated

Table 2. Effect of growth promoters on biological yield (kg ha⁻¹), grain yield (kg ha⁻¹) and harvest index (%) of chickpea.

Treatments	Biological yield (Kg ha ⁻¹)		Grain yield (Kg ha ⁻¹)		Harvest index (%)	
	2018	2019	2018	2019	2018	2019
V ₁ H ₁ F ₁	5834.7ae	6201.3ac	1753.7ad	1828.4bd	30.06ad	29.54ac
V ₁ H ₁ F ₂	6720.9a	7120.9a	2119.7a	2231.8a	31.58a	31.34a
V ₁ H ₁ F ₃	6295.0ac	6728.4ab	1903.0ac	2024.9ac	30.31ac	30.13ac
V ₁ H ₁ F ₄	6586.7a	6886.7a	2069.1a	2115.6ab	31.40ab	30.72ac
V ₁ H ₂ F ₁	4390.4fg	4723.8d	1248.8ef	1391.4ef	28.48de	29.49ac
V ₁ H ₂ F ₂	5008.3eg	5541.6bd	1527.7be	1704.0ce	30.49ac	30.78ab
V ₁ H ₂ F ₃	4969.0eg	5369.0cd	1461.3df	1590.7df	29.42ce	29.71ac
V ₁ H ₂ F ₄	5368.1bf	5568.1bd	1618.7be	1708.5ce	30.07ad	30.68ac
V ₂ H ₁ F ₁	5107.4cg	5440.7cd	1518.7ce	1605.9df	29.53ce	29.40bc
V ₂ H ₁ F ₂	6328.4ab	6728.4ab	1919.6ab	2028.0ac	30.29ad	30.14ac
V ₂ H ₁ F ₃	5969.7ae	6136.3ac	1802.8ad	1832.1bd	30.13ad	29.89ac
V ₂ H ₁ F ₄	6247.0ad	6680.4ab	1916.1ab	2015.2ac	30.62ac	30.15ac
V ₂ H ₂ F ₁	4007.1g	4407.1d	1123.2f	1273.2f	28.06e	28.89c
V ₂ H ₂ F ₂	5057.0dg	5290.4cd	1537.1be	1628.1df	30.30ac	31.04ab
V ₂ H ₂ F ₃	4943.0eg	4843.0d	1462.2df	1459.7df	29.67be	30.07ac
V ₂ H ₂ F ₄	5128.1bg	5428.1cd	1569.1be	1666.7ce	30.58ac	30.73ac
LSD 0.05 _p =	1210.7	1194.5	394.40	374.00	1.8133	1.8820

Whereas V₁ = Thal 2006, V₂ =, Noor 2013 H₁ = 25th October, H₂ = 25th November, F₁ = distilled water, F₂ = MLE30, F₃ = Jasmonic acid and F₄ = Proline.

on 25th October. While minimum biological yield was observed with the foliar spray of distilled water on Noor 2013 plants cultivated on 25th November (Table 2). Exogenous application of MLE and proline plays a fundamental responsibility in providing tolerance in heat stress conditions. MLE being rich source of minerals, ascorbate and phenolics affect plant hormone and consequently increase plant growth even under heat stress conditions. This metabolite improves the pods filling rate and duration that contributes to higher biological yield (Yasmeen *et al.*, 2012). Similarly, MLE has also been reported to keep secure in heat stress conditions (Khan *et al.*, 2013).

Grain yield (Kg ha⁻¹): Exogenously applied growth promoters significantly improved the grain yield per unit area even under adverse environmental conditions (Table 2). Exogenous application of MLE on black gram cultivar Thal 2006 cultivated at optimum sowing time (25th October) produced significantly higher grain yield against the lowest was observed with the foliar application of distilled water on Noor 2013 plant cultivated on 25th November (Table 2). This reduction in grain yield might be due to adverse influence of heat stress, which showed that beyond optimum temperature negatively affects the various morphological and physiological attributes (Iqbal *et al.*, 2020). However, foliar spray of growth promoters proved to be potentially effective in alleviation of the adverse influence of heat stress. Foliar spray of MLE produced significantly higher grain yield might be due to its role as growth enhancer because its enriched with protein contents that essential for the formation of the protoplasm, vitamin C, essential nutrients such as potassium,

calcium and magnesium that use as suitable source of natural antioxidant combinations that make it superb plant growth promoter (Arif *et al.*, 2019b).

Harvest index (%): Statistically analyzed data exhibited that exogenously applied of growth promoters significantly improved the harvest index percentage (Table 2). Exogenous application of MLE on black gram cultivar Thal 2006 cultivated at optimal sowing time (25th October) produced significantly higher harvest index percentage against the lowest was perceived with the foliar application of distilled water on Noor 2013 plants cultivated on 25th November (Table 2). Lower harvest index might be due to problem in the reallocation of photo-assimilates from source to mature grain, which ensued in small and shriveled grains (Hall, 2004). Exogenously applied MLE enhanced the grain yield and its constituents that ultimately enhanced the harvest index (Yasmeen *et al.*, 2012).

Catalase (IU min⁻¹mg⁻¹ protein): Catalase (CAT) enzyme activities are generally measured as a sign of the plant tolerance level against the stress condition. Exogenously applied of growth promoters significantly improved the catalase production (Table 3). Exogenous application of proline on black gram cultivar Thal 2006 cultivated at late sowing time (25th November) produced significantly higher catalase as compared to other treatments. Whereas, minimum catalase was observed with the application of distilled water on Noor 2013 cultivated on 25th October (Table 3). Exogenous application of growth promoters especially proline significantly improved the production of catalase both under optimum and heat stress conditions. In addition to the

Table 3. Effect of growth promoters on enzymatic antioxidant activities of chickpea.

Treatments	Catalase (IU min ⁻¹ mg ⁻¹ protein)		Superoxide dismutase (IU min ⁻¹ mg protein ⁻¹)		Peroxidase (IU min ⁻¹ mg ⁻¹ protein)	
	2018	2019	2018	2019	2018	2019
V ₁ H ₁ F ₁	31.33f	30.36e	55.95f	59.28ef	6.87fg	6.70f
V ₁ H ₁ F ₂	34.02f	32.35e	63.23f	66.23ef	8.19fg	7.96f
V ₁ H ₁ F ₃	31.78f	31.12e	58.30f	62.30ef	7.12fg	7.25f
V ₁ H ₁ F ₄	34.76ef	33.10e	66.16f	68.83e	8.49f	8.02f
V ₁ H ₂ F ₁	57.98bc	59.65bd	131.79de	138.12d	13.21de	12.55de
V ₁ H ₂ F ₂	65.35ab	67.68ab	171.20ab	164.54ab	18.94a	16.88ab
V ₁ H ₂ F ₃	62.04ab	63.71ac	163.16ac	155.49bc	16.60b	15.27bc
V ₁ H ₂ F ₄	68.21a	71.55a	179.41a	170.75a	19.22a	17.65a
V ₂ H ₁ F ₁	28.57f	30.24e	51.30f	54.03f	6.39g	6.49f
V ₂ H ₁ F ₂	32.18f	31.91e	62.20f	63.87ef	8.01fg	7.75f
V ₂ H ₁ F ₃	30.78f	30.25e	61.10f	59.76ef	7.78fg	7.44f
V ₂ H ₁ F ₄	32.27f	33.01e	64.27f	65.60ef	8.14fg	7.81f
V ₂ H ₂ F ₁	43.60de	50.27d	122.21e	132.54d	11.59e	11.26e
V ₂ H ₂ F ₂	47.49d	55.52cd	148.36cd	155.70bc	14.07cd	13.57cd
V ₂ H ₂ F ₃	44.97d	51.63d	142.87ce	146.20cd	13.37de	13.11de
V ₂ H ₂ F ₄	49.16cd	56.16cd	157.07bc	160.40ac	15.84bc	14.18cd
LSD 0.05 _p =	9.3239	10.156	21.317	14.694	1.9976	2.0767

Whereas V₁ = Thal 2006, V₂ =, Noor 2013 H₁ = 25th October, H₂ = 25th November, F₁ = distilled water, F₂ = MLE30, F₃ = Jasmonic acid and F₄ = Proline.

role of osmo-protection, proline protects the enzymes, protein structure, cell organelles and membranes by checking lipid peroxidation and facilitates the energy supply for plant growth, and effective quencher of reactive oxygen species (ROS) formed through life cycle of plant and enhanced the activity of antioxidant enzymes, therefore proline increases resistance to unfavorable climatic conditions (Kaushal *et al.*, 2011).

Superoxide dismutase (IU min⁻¹mg⁻¹ protein): Exogenous application of growth promoters significantly improved the superoxide dismutase (SOD) production (Table 3). Exogenous application of proline on black gram cultivar Thal 2006 cultivated at late sowing time (25th November) produced significantly higher superoxide dismutase as compared to other treatments. Whereas, minimum superoxide dismutase was observed with the application of distilled water on Noor 2013 cultivated on 25th October (Table 3). Higher superoxide dismutase activity with the exogenous application of proline indicates that the antioxidant defense system would play an imperative role in the heat stress tolerance of chickpea cultivar (Osman, 2015).

Peroxidase (IU min⁻¹mg⁻¹ protein): Exogenously applied growth promoters significantly improved the peroxidase synthesis (Table 3). Exogenous application of proline on black gram cultivar Thal 2006 cultivated at late sowing time (25th November) produced significantly higher peroxidase as compared to other treatments. Whereas, minimum peroxidase was observed with the application of distilled water on Noor 2013 cultivated on 25th October (Table 3). Lower peroxidase activity is observed in control plots sensitivity to heat stress.

Exogenous application of growth promoters especially proline significantly improved the production of peroxidase both under normal and heat stress conditions. Higher peroxidase activity showed tolerance to environmental stress and play an imperative role in reducing damages in stress situations (Osman, 2015). The enhancement of antioxidant enzymes might be efficient in enhancing the growth and productivity of various crops under abiotic stress circumstances (Azevedo *et al.*, 2012).

Conclusion: Heat stress drastically reduced the chickpea productivity. This decline in productivity was the consequence of reduced plant height, number of pods per plant and grain weight that was correlated with damaged morpho-physiological characteristics of chickpea plants. Under such conditions, exogenous application of plant growth promoters particularly moringa leaf extract and proline modulated the heat stress induced losses to chickpea plants. These growth promoters also enhance the antioxidant enzymes (SOD, POD and CAT) activities of chickpea plants and improved the productivity even under heat stress conditions.

REFERENCES

- Ahmad, M.A. and P.V. Murali. 2015. Exogenous jasmonic acid alleviates adverse effects of drought stress in *Allium cepa* L. Int. J. Geol. Agric. Environ. Sci. 3:1-9.
- Arif, M., N. Hussain and A. Yasmeen. 2019a. Influence of bio-stimulant and potassium sources on the productivity of cotton. The J. Anim. Plant Sci. 29:1643-1653.
- Arif, M., S.H.S. Kareem, N.S. Ahmad, N. Hussain, A. Yasmeen, A. Anwar, S. Naz, J. Iqbal, G.A. Shah and M.

- Ansar. 2019b. Exogenously applied bio- stimulant and synthetic fertilizers to improve the growth, yield and fiber quality of cotton. *Sustainability*. 11:1-14.
- Azevedo, R.A., P.L. Gratão, C.C. Monteiro and R.F. Carvalho. 2012. What is new in the research on cadmium-induced stress in plants? *Food Energy Secur.* 1:133-140.
- Boote, K.J., L.H. Allen, P.V.V. Prasad, J.T. Baker, R.W. Gesch, A.M. Snyder, D. Pan and J.M.G. Thomas. 2005. Elevated temperature and CO₂ impacts on pollination, reproductive growth, and yield of several globally important crops. *J. Agric. Meteorol.* 60:469-474.
- Chance, B. and A.C. Maehly. 1955. Assay of catalase and peroxidase. *Meth. Enzymol.* 2:764-775.
- Clarke, S.M., S.M. Cristescu, O. Miersch, F.J. Harren, C. Wasternack and L.A. Mur. 2009. Jasmonates act with salicylic acid to confer basal thermotolerance in *Arabidopsis thaliana*. *New Phytol.* 182: 175-187.
- Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol.* 59:309-314.
- Hall, A.E. 2004. Breeding for adaptation to drought and heat in cowpea. *Eur. J. Agron.* 21:447-454.
- Hayat S., M. Irfan, A.S. Wani, M.N. Alyemeni and A. Ahmad. 2012. Salicylic acids local, systemic or inter systemic regulators?. *Plant Signal Behav.* 7:93-102.
- Husain, A.J., A.G. Muhmood and A.H. Alwan. 2018. Interactive effect of GA₃ and proline on nutrients status and growth parameters of Pea (*Pisum sativum* L.). *Indian J. Ecol.* 45:201-204.
- ICRISAT. 2009. Climate change in the semiarid tropics. Available at: www.icrisat.org/rds/Climate_Change_SAT_flyer.pdf.
- Iqbal, H., C. Yaning, H.U. Rehman, M. Waqas, Z. Ahmed, S.T. Raza and M. Shareef. 2020. Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors. *Chil. J. Agric. Res.* 80:30-40.
- IPCC. 2007. Fourth assessment report: synthesis. http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf.
- Ismail, H., J.D. Maksimovic, L. Maksimovic, B.D. Shabala, Y. Živanovic and S.E. Tian. 2016. Jacobsen and S. Shabala. 2016. Rutin, a flavonoid with antioxidant activity, improves plant salinity tolerance by regulating K⁺ retention and Na⁺ exclusion from leaf mesophyll in quinoa and broad beans. *Funct. Plant Biol.* 43:75-86.
- Kaur, G., S. Kumar, P. Thakur, J.A. Malik, K. Bhandhari, K. Sharma and H. Nayyar. 2011. Involvement of proline in response of chickpea (*Cicer arietinum* L.) to chilling stress at reproductive stage. *Sci. Hortic.* 128:174-181.
- Kaushal, N., K. Gupta, K. Bhandhari, S. Kumar, P. Thakur and H. Nayyar. 2011. Proline induces heat tolerance in chickpea (*Cicer arietinum* L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. *Physiol. Mol. Biol. Plants.* 17:203-213.
- Khan, M.I.R., N. Iqbal, A. Masood, T.S. PER and N.A. Khan. 2013: Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal Behav.* 8e26374.
- Liu, H.C., H.T. Liao and Y.Y. Charng. 2011. The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in *Arabidopsis*. *Plant Cell Environ.* 34:738-751.
- Osman, H.S. 2015. Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. *Ann. Agric. Sci.* 60:389-402.
- Pauwels, L. and A. Goossens. 2011. The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *Plant Cell.* 23:3089-3100.
- Rashid, N., S.M.A. Basra, M. Shahbaz, S. Iqbal and M.B. Hafeez. 2018. Foliar applied moringa leaf extract induces terminal heat tolerance in quinoa. *Int. J. Agric. Biol.* 20:157-164.
- Rehman, H.U., M. Kamran, S.M.A. Basra, I. Afzal and M. Farooq. 2015. Influence of seed priming on the performance and water productivity of direct seeded rice in alternate wetting and drying. *Rice Sci.* 22:189-196.
- Sadak, M.S. and M.G. Dawood. 2014. Role of ascorbic acid and α tocopherol in alleviating salinity stress on flax plant (*Linum usitatissimum* L.). *J. Stress Physiol. Biochem.* 10:93-111.
- Sadak, M.S. and M.R.M. Ahmed. 2016. Physiological role of cyanobacteria and glycinebetaine on wheat plant grown under salinity stress. *Inter. J. Pharm. Tech. Res.* 9:78-92.
- Shan, C. and Z. Liang. 2010. Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. *Plant Sci.* 178:130-139.
- Snider, J.L., D.M. Oosterhuis and E.M. Kawakami. 2010. Genotypic differences in thermotolerance are dependent upon pre-stress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*. *Physiol. Plant.* 138:268-277.
- Steel, R.G.D., J.H. Torrie and D.A. Deekey. 1997. Principles and procedures of Statistics. A Biometrical Approach. 3rd ED. Mc Graw Hill Book. Int. Co. New York. PP: 400-428.
- Teixeira, E.I., G. Fischer, H. van Velthuizen, C. Walter and F. Ewert. 2013: Global hot-spots of heat stress on agricultural crops due to climate change. *Agric. For. Meteorol.* 170:206-215.
- Verma, V., P. Ravindran and P.P. Kumar. 2016. Plant hormone-mediated regulation of stress responses. *BMC Plant Biol.* 16:1-10.

- Yasmeen, A., M. Arif, N. Hussain, S. Naz and A. Anwar. 2018. Economic analyses of sole and combined foliar application of moringa leaf extract (MLE) and k in growth and yield improvement of cotton. *Int. J. Agric. Biol.* 20:857-863.
- Yasmeen, A., S.M.A. Basra and A. Wahid. 2012. Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. Leaf extract. *Chil. J. Agric. Res.* 72:92–97.
- Yasmeen, A., S.M.A. Basra, M. Farooq, H.U. Rehman and N. Hussain. 2013. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regul.* 69:225-233.
- Yasmeen, A., W. Nouman, S.M.A. Basra, A. Wahid, H.U. Rehman, N. Hussain and I. Afzal. 2014. Morphological and physiological response of tomato (*Solanum lycopersicum* L.) to natural and synthetic cytokinin sources: a comparative study. *Acta Physiol Plant.* 36:3147-3155.
- Yosefi, M., S. Sharafzadeh, F. Bazrafshan, M. Zare and A. Amiri. 2018. Application of jasmonic acid can mitigate water deficit stress in cotton through yield-related physiological properties. *Acta Agrobot.* 71:1741.

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