INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0089/2018/20–1–157–164
DOI: 10.17957/IJAB/15.0469
http://www.fspublishers.org

Full Length Article

Foliar Applied Moringa Leaf Extract Induces Terminal Heat Tolerance in Quinoa

Nabila Rashid[1*], Shahzad M.A. Basra[2], Muhammad Shahbaz[1], Shahid Iqbal[3] and Muhammad Bilal Hafeez[2]
[1]*Department of Botany, University of Agriculture Faisalabad, 38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture Faisalabad, 38040, Pakistan*
[3]*Department of Agronomy, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan*
[*]For correspondence: rasheed.nabila@yahoo.com

Abstract

Quinoa is a great value crop due to superior nutritional profile and abiotic stress tolerance however, its performance decreases under high temperature that can be improved by foliar application of moringa leaf extract (MLE) rich in antioxidants, growth promoters and minerals. This pot study focused to induce terminal heat tolerance in quinoa by foliar application of MLE. Foliar application of fresh MLE (MLEF), one month stored MLE (MLE1), two months stored MLE (MLE2), three months stored MLE (MLE3) and distilled water (control) were applied three times at fortnightly interval after start of high temperature treatment (76 days after sowing) on quinoa grown under ambient and heat stress conditions (±10–15ºC of ambient). Quinoa growth drastically declined in heat stress conditions however, MLEF foliar application mitigated adverse effects of heat stress as indicated by more photosynthetic rate and intrinsic water use efficiency due to improved leaf chlorophyll and antioxidants found at flowering and grain filling stages. The efficacy of MLE declined with storage period. Nine percent (9%) increase in seed yield per plant under normal and 36% increase in heat stress conditions, with respective to their controls harvested from MLEF treated plants. Interestingly seed quality including protein and minerals contents did not affected by heat stress in this study. © 2018 Friends Science Publishers

Keywords: *Chenopodium quinoa*; Abiotic stress; PGPR

Introduction

Quinoa (*Chenopodium quinoa* Willd) is a pseudocereal which produces highly nutritious grains (Repo-Carrasco *et al*., 2003; Ruiz *et al*., 2014) and occupies major cultivated area of Latin America (Jacobsen *et al*., 2005). Cultivation of this superior quality seed crop has got attention all over the world in order to enhance future food security both due its superior nutritional profile and its abiotic stress tolerance. Quinoa tolerates drought (Sun *et al*., 2014) and salinity (Riccardi *et al*., 2014) depending on genotypes. Its seed is gluten free, rich in minerals (Ca, K, Fe, Zn and Mn), vitamins (A, B2 and E), contains all essential amino acids and health supportive poly-unsaturated fatty acids like Omega-3 and -6 (Repo-Carrasco *et al*., 2003).

Basic production technology of quinoa has been developed for its successful introduction and cultivation in Pakistan (Basra *et al*., 2014). Out of 160 accessions only four survived or acclimated during Rabi season (October to April) of the country that might be due to high temperature at reproductive stages because quinoa has been originated from cold regions (Bazile *et al*., 2015). As for wheat, quinoa also experiences abrupt high temperature at reproductive stages in Pakistan conditions (Basra *et al*., personal communication). The rise in temperature during early spring is reason of terminal heat stress (Mahmood *et al*., 2010) causing early maturity and poor grain development. This terminal heat stress is further increasing day by day (Mitra and Bhatia, 2008) due to global warming.

Abrupt rise in temperature is more detrimental when occurs at pollination and grain filling developmental stages and termed as terminal heat stress (Hays *et al*., 2007; Farooq *et al*., 2011). Heat stress disturbs gaseous exchange relations i.e., photosynthesis and stomatal conductance (Wahid *et al*., 2007), induces metabolic modulations (Farooq *et al*., 2011) which promotes the excess generation of reactive oxygen species (ROS) in different cell organelles leading to "oxidative burst" (Wang *et al*., 2011), disturbs development of pollen tube and also causes pollen mortality (Saini *et al*., 1983), ethylene production also increases, thus ultimately leads to grain abortion and early crop maturity (Hays *et al*., 2007). Oxidative burst cause damages to chloroplast along with inhibition of chlorophyll biosynthesis (Farooq *et al*., 2011).

Exogenous application of; plant growth regulators (PGRs) had widely been used to enhance stress tolerance in many plant species by causing modulations in biochemistry and plant physiology required for stress tolerance (Ismail *et*

To cite this paper: 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

al., 2016; Bakhtavar *et al*., 2015). However, most of them are synthetic, costly and environmentally unsafe. Cost effective exogenous use of plant based extracts containing PGRs, hormones and antioxidants in sufficient amount had also been reported for the improvement of crop performance with higher economic returns (Bakhtavar *et al*., 2015). Among different plant sources explored to extract antioxidants and PGRs, juice extracted from moringa (*Moringa oleifera*) tree leaves had gained enormous fame having bio-stimulant potential (Bakhtavar *et al*., 2015).

Plant biostimulant, moringa leaf extract; MLE (3%) when used in seed soaking medium or applied as foliar spray positively improves growth of normal grown and abiotic stressed plants by altering plant metabolic processes (Semida and Rady, 2014). *Moringa oleifera* Lam is a multipurpose tree which belongs to Moringaceae family and is native of sub-continent i.e. Pakistan and India (Shahzad *et al*., 2013).

The MLE extracted from fresh moringa leaves contain, antioxidant, secondary metabolites and osmoprotectants in considerable amount (Rady *et al*., 2013). Moreover, MLE is a very rich source of main growth promoter zeatin: a natural derivative of cytokinin, minerals elements and vitamins, hence declared as a potential biostimulant for growth improvement (Rehman *et al*., 2015).

The chemical composition of MLE may vary with storage duration. Many secondary metabolites present in leaf extract are also temperature and pH sensitive and may be altered or denatured during storage duration ultimately reduces biological efficacy (Khan *et al*., 2017). In view of above reports present study was conducted with dual objectives to compare efficacy of fresh and stored moringa leaf extracts by supporting quinoa growth to alleviate heat stress based on stay green character, induction of antioxidant defense system, gas exchange relations, yield and produce quality.

It is also important to highlight, no previous report for use of bio-stimulant potential of fresh as well as stored MLE in quinoa for terminal heat stress alleviation.

Materials and Methods

All research activities were conducted at University of Agriculture Faisalabad Pakistan (31.41°N, 73.07°E and 184 m above sea level) with following details.

Extraction and Storage of MLE

Fresh moringa leaves were collected from already established moringa trees at research farm area of Department of Crop Physiology, University of Agriculture Faisalabad. Before extraction process, mature, disease free and healthy leaves were rinsed with water and kept in freezer overnight. Extraction was done with a locally assembled machine. The extract was sieved. Freshly extracted leaf extract was shifted in three separate autoclaved air tight opaque glass bottles having capacity of 1000 mL and placed in dark shelves at room temperature up to different storage durations (one, two and three months).

Pot Study

A pot experiment was executed in the wire-house conditions similar to quinoa growing season of the department of Botany, during 2014–2015 (November-April). Seeds of a well-adapted quinoa genotype UAF-Q-7 were obtained from Department of Agronomy. Initially, ten seeds were sown in each pot containing 20 kg loamy soil, which thinned to five plants in each pot after complete emergence. At the start of trial, basal dose of N-P-K were applied @ 0.5-0.45-0.38 N-P-K g per pot using urea (46% N), diammonium phosphate (18% N, 46% P_2O_5) and sulphate of potash (50% K_2O) as fertilizer sources.

Imposition of Terminal Heat Stress

After 76 days of sowing, half numbers of pots were shifted to open door plexi glass fitted canopies with a light transmission index of about 0.8. Inside and outside canopy temperature and relative humidity details during course of experimentation are presented in Fig. 1. Inside canopy temperature was 7–10°C more as compared to outside canopy. Plants were kept under ambient and high temperature regimes up to harvesting.

Moringa Leaf Extracts (MLE) Foliar Spray

Three percent solutions were prepared from freshly extracted moringa leaf extract (MLEF), one month stored moringa leaf extract (MLE1), two months stored moringa leaf extract (MLE2) and three month stored leaf extract (MLE3). Spray with distilled water as control and of diluted solutions were done three times at fortnightly interval after start of high temperature treatments with hand sprayer @ 50 mL per plant in the morning (9:00 am).

Measurements of Leaf Photosynthetic Rate (A), Stomatal Conductance (gs) and Water use Efficiency (WUE)

Net CO_2 assimilation rate (A) and stomatal conductance (gs) was measured by putting fully developed leaf (two leaves per plant and four plants per treatment) in chamber of a portable infrared gas analyzer (Analytical development company, Hoddeson, UK). Intrinsic water use efficiency was calculated by dividing A by gs. These measurements were made one week after first spray.

Leaf Biochemical Analysis

Fully expanded leaves from three tagged plants per pot were collected at flowering and grain filling stage. Leaf samples were collected early in the morning before 6:00 am, rapped in aluminum foils, shifted immediately in thermos bottles containing liquid nitrogen and transferred in plastic zipper bags, and stored at in biomedical freezer (-80°C) up to

further analysis. Following biochemical analysis was done within one week using spectrophotometer (UV 4000).

Leaf chlorophyll contents (*a, b* and total) were determined by grinding 0.5 g leaf tissue along with ten mL acetone (80%) using pestle and mortor. The resulting homogenized material was shifted in falcon tubes and centrifuged for ten min at 3000 rpm. Chlorophyll contents were quantified by taking absorbance of supernatant at 663 and 645 nm wavelengths.

0.1 g leaf tissue was grinded along with one mL (50 mM) phosphate buffers (pH 7.8) in pre chilled pestle and mortar, grinded material was centrifuged at 15000 rpm for 20 min at -4°C and supernatant was collected to use in assays to determine the activities of antioxidant enzymes, SOD (Giannopolitis and Ries, 1977), POD and CAT (Chance and Maehly, 1955) by recording absorbance at 560,470 and 240 respectively.

Non-enzyme antioxidants, total leaf phenolic contents (TPH) were estimated by taking absorbance at 765 nm wavelength using reference standards of gallic acid. Leaf ascorbate contents were measured at 525 nm following protocol describes by Yin *et al*. (2008) using ascorbic acid standards as reference.

Plant Growth and Yield

Harvesting was done when plants turned light brown, panicle and shoot lengths were recorded on centimeter scale. After that panicles were detached from stem and dried by placing under shade on paper sheets in seed store (25-30°C ambient temperature). After ten days seeds were threshed manually to calculate seed yield per plant and 100 seed weight. Shoots without panicles were dried in an oven at 65°C for one week to determine dry matter, later on total shoot dry mass (g) was calculated by sum of shoot dry matter and seed weight.

Seed Minerals Content

Seed mineral elements (K, Ca, Mg, Mn, Zn and Fe) were determined using an atomic absorption spectrophotometer (AAS; Shimadzu instruments, Inc., Spectra AA-220, Kyoto, Japan) after digesting in a di-acid mixture (3:1) of HNO_3:$HCLO_4$ at hot plate for two h following AOAC (1990) method No. 3.014-016.

Seed Protein Determination

Flour of seed samples was made by grinding in electric coffee seed grinder. Nitrogen content in flour samples was estimated by Kjeldahl's method. Nitrogen percentage was calculated by using Equation as briefed in AACC (2000) method No 46-10.

$$Nitrogen\ \% = \frac{Titer\ of\ 0.1\ N\ H2H_2SO_4\ used \times 0.0014 \times 250}{Weight\ of\ sample \times vol.\ of\ aliquot\ sample} \times 100$$

Seed protein percentage was calculated by multiplying % nitrogen with 5.7.

Statistical Analysis

Each treatment was tetra replicated and data were statistically analyzed by two-way ANOVA technique under completely randomized design (CRD) comprising split plot arrangements. Temperature treatments were set as main plot factor (A) and foliar spray treatments were considered as sub plot factor (B). Significance levels of treatments were computed using software "statistix" (ver8.1, Tallahasse, FL, USA).

Results

Plant Growth and Yield Responses

Shoot biomass, inflorescence length, seed yield and 100-grain weight significantly reduced under heat stress conditions as compared to ambient temperature treatments. However, foliar spray of moringa leaf extract (MLEF) significantly enhanced shoot biomass and seed yield/plant under both heat stress and ambient temperature conditions (Table 1).

Leaf Photosynthesis (A), Stomatal Conductance (gs) and Intrinsic Water use Efficiency

Values of A and gs were found significantly less in heat stressed plants as compared to normal grown plants. However, MLEF foliar application improved A and gs, both under heat stress and ambient temperature regimes. Furthermore, maximum intrinsic water use efficiency was found higher in MLEF treated plants grown under heat stressed conditions (Fig. 2).

Leaf Antioxidant Enzymes

Activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased markedly under heat stress plants (Fig. 3; Table 2) at flowering and grain filling stages. Foliar application of moringa leaf extracts significantly enhanced the activities of SOD, POD and CAT at both growth stages. Moreover, MLEF foliar application found better as compared to other treatments in improving the activities of SOD, POD and CAT in quinoa leaves.

Leaf Non-Enzyme Antioxidants

Leaf ascorbate contents were also improved by MLEF applications at flowering and grain filling stages under both ambient and high temperature regimes (Fig. 4; Table 2). Overall, ascorbate contents were found to be lower at grain filling stage as compared to flowering under ambient condition. Furthermore, leaf total phenolic content did not affect by temperature stress and foliar application sunder both ambient and temperature stress conditions.

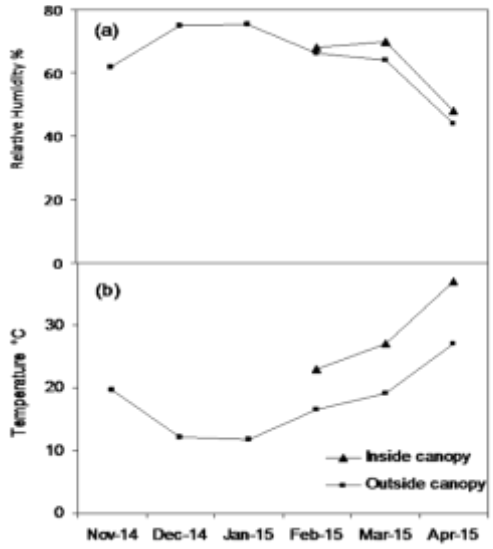

Fig. 1: Monthly mean temperature and mean relative humidity during experimental period i.e., inside and outside of plexi glass canopies

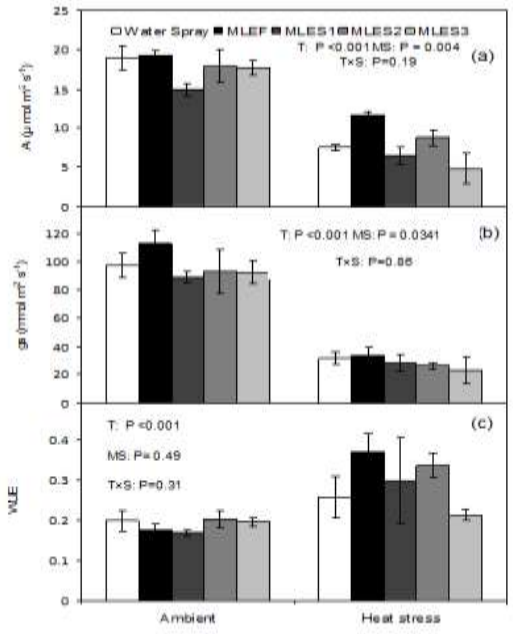

Fig. 2: Photosynthetic rate (A), stomatal conductance (gs) and interinsic water use effiecincy (WUE) of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2, month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes measured after one week of first spray

Leaf Chlorophyll Contents

Significant decrease in leaf chlorophyll (Chl *a*, Chl *b* and total chlorophyll contents) contents were observed under heat stress as compared to ambient temperature conditions with more apparent at grain filling stage (Fig. 5; Table 2). Foliar application of fresh moringa leaf extract (MLEF) caused increase in Chl *a* contents at flowering stage both under normal and heat stress conditions and at grain filling stage under heat stress. Overall, low leaf total chlorophyll contents were observed at grain filling stage.

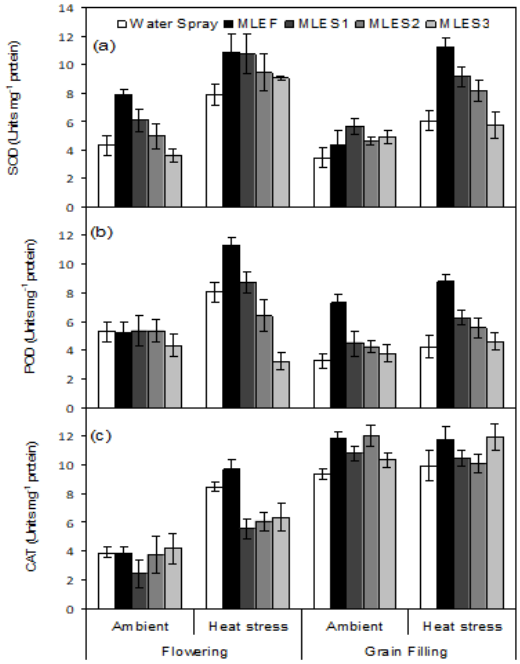

Fig. 3: Leaf antioxidant enzymes activity of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2, three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes measure during flowering and grain filling stage

Seed Protein and Minerals Content

No significant improvement for seed protein and mineral contents were found for all treatments except manganese (Mn) contents which was found higher in seeds harvested from plants grown under heat stress conditions and treated with different moringa foliar sprays (Tables 3 and 4; Fig. 6).

Discussion

Cultivation of high quality grain crops such as quinoa is one of the promising options to ensure food security but abrupt rise in temperature during early spring causing early maturity and poor grain development ultimately low yield of quinoa. Heat stress triggers physiological and biochemical modulations in plants, which enforce detrimental growth inhibition and ultimately low biomass production (Hays *et al*., 2007; Farooq *et al*., 2011).

Table 1: Growth and yield of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2 and three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes

Variables	Ambient					Heat stress					P- values		
	WS	MLEF	MLES1	MLES2	MLES3	WS	MLEF	MLES1	MLES2	MLES3	T	S	T×S
Plant height (cm)	66.8±2.1	91.9±3.2	86.6±6.3	85.5±4.5	70.8±2.4	80.0±2.9	85.2±9.1	90.4±6.1	85.5±12.3	72.2±10.5	0.591	0.036	0.703
Shoot biomass (g)	19.0±1.1	25.7±3.8	29.4±3.6	24.45±3.6	16.1±2.7	10.9±1.4	15.2±3.1	13.9±1.7	11.6±1.6	9.6±1.6	<0.001	0.012	0.461
Inflorescence length (cm)	14.9±1.2	14.0±0.6	15.5±0.5	15.0±1.0	13.5±1.2	10.0±0.3	11.7±0.7	12.7±0.6	10.3±0.8	10.9±1.9	<0.001	0.399	0.567
Seed Yield/Plant (g)	5.1±0.6	5.6±0.5	4.6±0.5	4.5±0.4	4.0±0.8	2.5±0.2	3.4±0.3	2±0.3	1.6±0.1	1.8±0.2	<0.001	0.0104	0.916
100 seed weight (g)	0.34±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0	<0.001	0.728	0.153

T and S indicate temperature and moringa spray treatments, T×S indicate their interaction (± SE)

Table 2: P values for biochemical attributes of quinoa influenced by MLE foliar spray under ambient and heat stress regimes at flowering and grain filling stage

	P values					
	Flowering			Grain filling		
Variables	T	MS	T×S	T	M	T×S
SOD	<0.001	0.004	0.696	<0.001	<0.001	0.004
POD	<0.001	<0.001	0.001	0.002	<0.001	0.949
CAT	<0.001	0.0144	0.126	0.988	0.058	0.159
Ascorbate	0.752	0.022	0.272	<0.001	0.005	0.003
Total leaf phenolics	0.822	0.221	0.112	<0.001	0.154	0.680
Chlorophyll *a (CHA)*	<0.001	0.052	0.120	0.147	0.023	0.023
Chlorophyll *b (CHB)*	<0.001	0.422	0.626	0.535	0.833	0.718
Total chlorophyll contents(TCH)	<0.001	0.099	0.244	0.280	0.224	0.018

T and S indicate temperature and moringa spray treatments while T×S indicate their interaction

Table 3: Seed mineral contents of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2 and three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes

Seed mineral contents (mg kg^{-1})	Ambient Temperature					Heat stress				
	Water Spray	MLEF	MLES1	MLES2	MLES3	Water Spray	MLEF	MLES1	MLES2	MLES3
Ca	715.6±162.6	837.5±197.2	550±113.6	550±113.6	500±102.0	380±69.4	442.5±117.1	562.5±62.5	562.5±62.5	500±102.0
Zn	33±2.5	37.2±3.9	36±5.4	27.7±4.6	34.3±4.5	29.21±1.7	32±4.5	31.6±4.0	34.3±3.7	26.6±4.5
Mn	33.1±1.1	31.0±1.0	29.3±0.4	30.6±1.2	30.6±1.5	15.3±1.8	29.4±0.5	29.7±1.0	29.9±0.8	23.5±2.5
Mg	2355.7±199.8	2516.6±282.8	2683.8±167.3	2343.8±161.0	1900.2±188.0	2687.7±21.1	2718±172.0	2524.9±113.9	1457±10.4	2079.5±37.2
Fe	105.2±1.4	116.1±4.5	93.8±5.7	94.1±9.1	94.8±4.5	97.5±7.6	89.1±5.8	90.9±2.3	95.1±9.3	95.1±9.3
Na	148.7±4.2	145±3.5	162.5±16.1	162.5±7.2	156.25±6.2	173.75±1.2	168.75±6.2	162.5±12.5	156.25±6.2	150±17.6
K	12250±853.9	12250±595.1	13250±520.4	13000±408.2	12875±657.4	12343.7±320.2	14375±515.3	14125±1852.6	14000±2791.3	14375±718.0

T and S indicate temperature and moringa spray treatments, T×S indicate their interaction (± SE)

Table 4: Statistical significance (P-values) for quinoa seed mineral contents as influenced by MLE foliar treatments under ambient and heat stress regimes

P-values	Ca	Zn	Mn	Mg	Fe	Na	K
Temperature treatments	0.067	0.271	<0.001	0.513	0.092	0.244	0.146
Moringa spray treatments	0.829	0.799	<0.001	<0.001	0.452	0.875	0.762
Interaction	0.218	0.470	<0.001	<0.001	0.219	0.275	0.934

MLE is found to rich in essential macro- and micronutrients (Mg, Ca, P, Fe, Mn, Zn and Cu) (Yasmeen *et al*., 2012). Antioxidants including, soluble phenolics, total carotenoids, ascorbic acid, catalase, superoxide dismutase, and peroxidases along with K^+, amino acid proline, total sugar as osmoprotectant had been found in very excess concentrations. Furthermore, it is also rich in growth promoters zeatin as cytokinin, indole-3-acetic acid (IAA) and gibberallins (GAs). With this extra enormous composition of MLE and its role as biostimulant (Yasmeen *et al*., 2012; Rady *et al*., 2013), was investigated in alleviation of heat stress in quinoa of present study.

In current study, terminal heat stress reduced growth, biomass and yield of quinoa compared to ambient temperature regimes. However, foliar applied moringa leaf extract (MLE) especially freshly extracted (MLEF) proved to be potentially effective in alleviation of the adverse effects of heat stress. Heat stress triggers metabolic changes especially excess ethylene production leading to early senescence and chlorophyll degradation. Thus, maintenance of stay green character is very important and considered as best indicator of thermo tolerance (Farooq *et al*., 2011). In

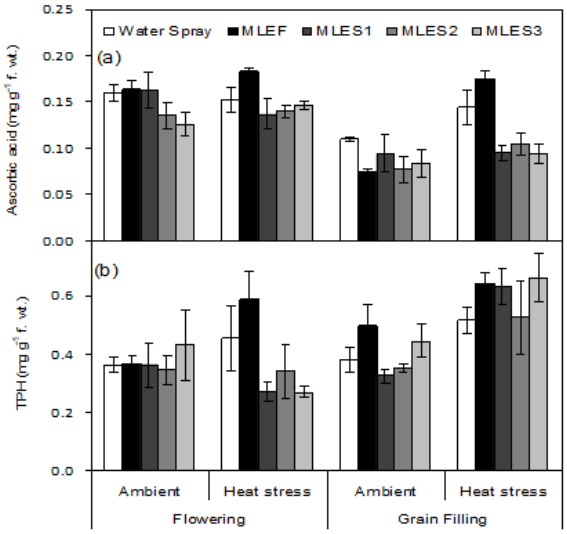

Fig. 4: Leaf non-enzymes antioxidant activity (Ascorbic acid and total Phenol:TPH) of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2, three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes measured during flowering and grain filling stage

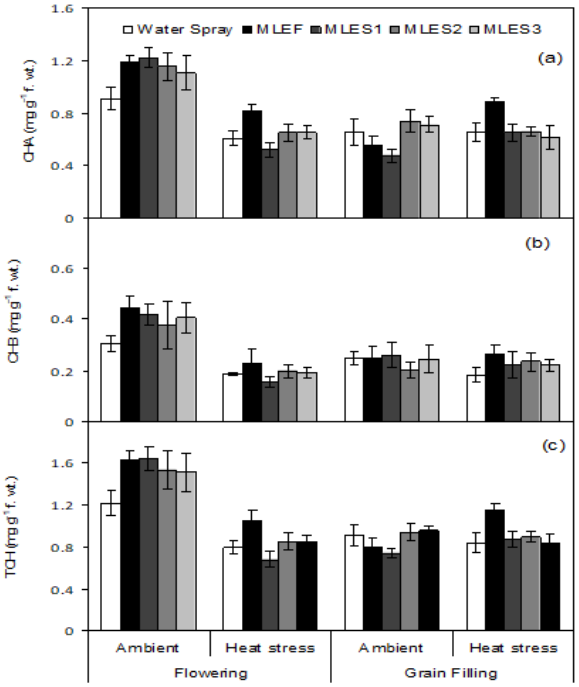

Fig. 5: Leaf cholorophyll contents (Chlorophyll *a*, Chlorophyll *b* and total Chlorophyll) of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2, three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes measured during flowering and grain filling stage

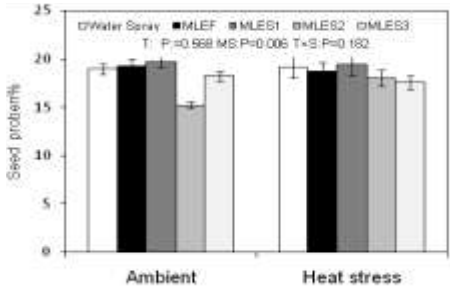

Fig. 6: Seed protien content of quinoa influenced by moringa leaf extracts spray (MLE) i.e., freshly extracted. MLEF, one month stored. MLE1, two month stored. MLE2, three month stored. MLE3 and water spray as control. WS under ambient and heat stress regimes measured after one week of first spray

this study leaf chlorophyll, *a* and total chlorophyll contents decreased at high temperature especially during grain filling stage, while MLE foliar application improved chlorophyll contents. This type of improvement was also reported by Yasmeen *et al*. (2013) in leaves of salt stressed wheat plants. It happens might be due to zeatin rich MLE application, which play role in stimulation of cytokinen biosynthesis perchance through activation of cytokinein dependent isopentenyl transferase (ipt) biosynthesis, increasing chlorophyll concentrations thus avoiding premature senescence of leaves that results in to more availability of photosynthetic active leaf area (Ali *et al*., 2011).

In present study, foliar spray of MLEF increased activities of enzymic antioxidants (SOD, POD and CAT) and non-enzymes (ascorbate and total leaf phenolics) both in heat stressed and normal grown plants. The increase in antioxidant activities in wheat plants has also been reported after spray of MLE by Yasmeen *et al.* (2013) under salt stress. Farooq *et al*. (2017) and Waqas *et al.* (2017) confirmed that spray of leaf extract on plant induces production of endogenous antioxidants. This induction of self-defense system might be associated with higher minerals (K^+ and Ca^{2+}) and growth promoters (zeatin and gibberellins) present in moringa leaves (Yasmeen *et al*., 2013; Semida and Rady, 2014) because minerals are activator and cofactor of many antioxidant enzymes. Furthermore, this increased activity of antioxidants was found more prominent in leaves during grain filling stage. Among different physiological process, photosynthesis is most sensitive to heat stress and its reduction affects growth and yield of plants (Wahid *et al*., 2007). This reduction occurs by two way (i) disruptions in chloroplast structure, which reduces chlorophyll contents (Xu *et al*., 2006) (ii) reduction in stomatal conductance occurs due to loss in turgor of guard cells as a result of rapid water loss by process "transpirational cooling" a necessary evil to alleviate heat stress effects. All these modulations leads to oxidative burst, which further induces inactivation of

chloroplast enzymes resulting in to reduced leaf photosynthetic rate and water use efficiency. Same response was observed in present study. Prominent reduction was found in these gaseous exchange attributes due to abrupt elevated temperature but MLEF foliar application was found supportive to improve these processes, which might be linked with improved "stay green" character along with enhanced leaf antioxidant activities. enhanced and maintained chlorophyll contents might be reason of stay green character, furthermore maintenance of chlorophyll contents could also be liked with improved antioxidant activities both enzymatic and non-enzymatic which have role to detoxify or scavenge toxic ROS which may otherwise be detrimental to macromolecules including chlorophylls (Sharma *et al*., 2012).

All these improvements in antioxidant defense system, chlorophyll formation and sufficient gas relations were harvested in the form of more shoot biomass and seed yield due to foliar application of MLEF on heat stressed and normal grown plants. Yasmeen *et al*. (2012) also reported that presence of growth-promoting substance in MLE foliar spray could extend seasonal leaf area duration, the grain-filling period and delays crop maturity that ultimately resulted into more yields (economic +biological) of wheat crop under late sown conditions.

Interestingly, seed quality attributes i.e. proteins and minerals were not affected by heat stress and comparable with values of same attributes found in seeds harvested from normal grown plants. This could be linked with inherent character of well adapted genotype of this region (Iqbal *et al*., 2017). Less improvement by stored MLE was might be due to denaturing of antioxidant enzymes, non-enzymes antioxidants vitamins and growth hormone zeatin. Many secondary metabolites present in leaf extract are also pH and heat sensitive and may be degraded during storage. For instance, presence of various conjugates of hormones present might decrease the biological activity of moringa leaf stored extracts. This would be also due to microbial and chemical decomposition (Khan *et al*., 2017).

Conclusion

Overall, heat stress found detrimental in terms of less growth and yield of quinoa and foliar application of moringa leaf extract was supportive to mitigate elevated temperature effects. Foliar application of fresh, moringa leaf extract (3%) at both flowering and grain filling stage was most effective in maintaining stay green character, induction of antioxidant defense system, improving gas relations which resulted in to improved yield performance of quinoa under ambient and heat stress conditions.

References

AACC, 2000. *Approved Methods of the American Association of Cereal Chemists*, 10th edition. American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA

Ali, Z., S.M.A. Basra, H. Munir, A. Mahmood and S. Yousaf, 2011. Mitigation of drought stress in maize by natural and synthetic growth promoters. *J. Agric. Soc. Sci*., 7: 56–62

AOAC, 1990. *Official Methods of Analysis*, 15th edition. Association of Official Analytical Chemists, Inc., Washington, USA

Bakhtavar, M.A., I. Afzal, S.M.A. Basra, A.U.H. Ahmad and M.A. Noor, 2015. Physiological Strategies to Improve the Performance of Spring Maize (*Zea mays* L.) Planted under Early and Optimum Sowing Conditions. *PloS One*, 10: e0124441

Chance, M. and A.C. Maehly. 1955. Assay of catalases and peroxidases. *Methods Enzymol.* 2:764.

Basra, S.M.A., S. Iqbal and I. Afzal, 2014. Evaluating the response of nitrogen application on growth, development and yield of quinoa genotypes. *Int. J. Agric. Biol*., 16: 886–892

Bazile, D., H.D. Bertero and C. Nieto, 2015. *State of the Art Report on Quinoa Around the World in 2013*. Rome: Food and Agriculture Organization of the United Nations (FAO) and CIRAD (Centre de cooperation internationale en recherche agronomi quepourle développement)

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique, 2011. Heat stress in wheat during reproductive and grain-filling phases. *Crit. Rev. Plant Sci*., 30: 1–17

Farooq, M., M. Rizwan, A. Nawaz, A. Rehman and R. Ahmad. 2017. Application of natural plant extracts improves the tolerance against combined terminal heat and drought stresses in bread wheat. *J. Agron. Crop Sci*., 203: 528–538

Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol.*, 59: 309–314.

Hays, D.B., J.H. Do, R.E. Mason, G. Morgan and S.A. Finlayson, 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. *Plant Sci*., 172: 1113–1123

Ismail, H., J.D. Maksimovic, V. Maksimovic, L. Shabala, B.D. Živanovic, Y. Tian, S.E. 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

Iqbal, S., S.M.A. Basra, I. Afzal and A. Wahid, 2017. Exploring Potential of Well Adapted Quinoa Lines for Salt Tolerance. *Int. J. Agric. Biol*., 19: 933–940

Jacobsen, S.E., C. Monteros, J.L. Christiansen, L.A. Bravo, L.J. Corcuera and A. Mujica, 2005. Plant responses of quinoa (*Chenopodium quinoa* Willd.) to frost at various phonological stages. *Eur. J. Agron*., 22: 131–139

Khan, S., S.M.A. Basra, I. Afzal and A. Wahid, 2017. Screening of moringa landraces for leaf extract as biostimulant in wheat. *Int. J. Agric. Biol*., 19: 999–1006

Mahmood, S., A. Wahid, F. Javed and S.M.A. Basra, 2010. Heat stress effects on forage quality characteristics of maize (*Zea mays*) cultivars. *Int. J. Agric. Biol*., 12: 701–706

Mitra, R. and C.R. Bhatia, 2008. Bioenergetic cost of heat tolerance in wheat crop. *Curr. Sci.*, 94: 1049–1053

Rady, M.M., C. Bhavya Varma and S.M. Howladar, 2013. Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Sci. Hortic.*, 162: 63–70

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

Repo-Carrasco, R., C. Espinoza and S.E. Jacobsen, 2003. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). *Food Rev. Int*., 19: 179–189

Riccardi, M., C. Pulvento, A. Lavini, R.D. Andria and S.E. Jacobsen, 2014. Growth and ionic content of quinoa under saline irrigation. *J. Agron. Crop Sci*., 200: 246–260

Ruiz, K., S.B. Biondi, R. Oses, I.S. Acuna-Rodriguez, F. Antognoni, E.A. Martinez-Mosqueira, A. Coulibaly, A. Canahua-Murillo, M. Pinto, A. Zurita-Silva, D. Bazile, S.E. Jacobsen and M.A. Molina-Montenegro, 2014. Quinoa biodiversity and sustainability for food security under climate change: A review. *Agron. Sustain. Dev.*, 34: 349–359

Saini, H.S., M. Sedgley and D. Aspinall, 1983. Effect of Heat stress during floral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum* L.). *Funct. Plant Biol.*, 10: 137–144

Semida, W.M. and M.M. Rady, 2014. Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and anti-oxidant capacity in *Phaseolus vulgaris* L. grown under NaCl stress. *J. Hortic. Sci. Biotech*., 89: 338–344

Shahzad, U., M.A. Khan, M.J. Jaskani, I.A. Khan and S.S. Korban, 2013. Genetic diversity and population structure of *Moringa oleifera*. *Conserv. Genet.*, 14: 1161–1172

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli, 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot*., 2012: 1–26.

Sun, Y., F. Liu, M. Bendevis, S. Shabala and S.E. Jacobsen, 2014. Sensitivity of two quinoa (*Chenopodium quinoa* Willd.) varieties to progressive drought stress. *J. Agron. Crop Sci*., 200: 12–23

Wahid, A., S. Gelani, M. Ashraf and M.R. Foolad, 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot*., 61: 199–223

Wang, X., J. Cai, D. Jiang, F. Liu, T. Dai and W. Cao, 2011. Pre-anthesis high-temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. *J. Plant Physiol*., 168: 585–593

Waqas, M., C. Yaning, H. Iqbal, M. Shareef, H. Rehman and Y. Yang, 2017. Paclobutrazol improves salt tolerance in quinoa: Beyond the stomatal and biochemical interventions. *J. Agron. Crop Sci*., 203: 315–322

Xu, S., J. Li, X. Zhang, H. Wei and L. Cui, 2006. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turf grass species under heat stress. *Environ. Exp. Bot*., 56: 274–285.

Yasmeen, A., S.M.A. Basra, M. Farooq, H. ur Rehman, N. Hussain and H.R. Athar, 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., S.M.A. Basra, R. Ahmad and A. Wahid, 2012. Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. *Chil. J. Agric. Res.*, 2: 92-97

Yin, H., Q. Chen and M. Yi, 2008. Effects of short term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. *Plant Growth Regul*., 54: 45–54.

(Received 25 January 2017; Accepted 07 September 2017)