



Full Length Article

Foliar Spray of Moringa Leaf Extract, Sorgaab, Hydrogen Peroxide and Ascorbic Acid Improve Leaf Physiological and Seed Quality Traits of Quinoa (*Chenopodium quinoa*) under Terminal Heat Stress

Nabila Rashid¹, Abdul Wahid^{1*}, Shahzad M.A. Basra² and Muhammad Arfan¹

¹Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan

²Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan

*For correspondence: drawahid@uaf.edu.pk; drawahid2001@yahoo.com

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a newly emerging crop of the world, but is sensitive to episodes of heat stress especially at terminal growth stage. Therefore, strategies need to be adopted for improving terminal heat stress tolerance in quinoa. In this study, an elite quinoa genotype (UAF Q7) was grown to examine the effect of terminal heat stress in terms of physiological attributes and seed yield and quality attributes and their possible improvement with the foliar spray of water and optimized levels of moringa fresh leaf extract (MLE, 3%), Sorgaab (3%), hydrogen peroxide (H₂O₂, 100 μM) and ascorbic acid (AsA, 500 μM). Analysis of leaves in close proximity to the inflorescence revealed that heat stress reduced the chlorophyll (Chl) a, b and carotenoids. Heat stress also inhibited the net rate of photosynthesis (*P_n*), transpiration rate (*E*), and stomatal conductance (*g_s*) as a function of closure of stomata and increased the sub-stomatal CO₂ concentration (*C_i*) as a function of reduced ability of mesophyll parenchyma to assimilate CO₂ present intercellular. The foliar spray of MLE, Sorgaab, H₂O₂ and AsA was promising in averting (up to 55–70%) the terminal heat stress induced changes on the photosynthetic pigments and gas exchange attributes. A prominent increase in the concentration of leaf H₂O₂ and malondialdehyde (MDA) due to heat stress was markedly declined with the foliar spray of the abovementioned foliar spray agents. The activity of antioxidants including catalase, peroxidase and dismutase was improved with all foliar spray treatments but was the highest with MLE and Sorgaab. Heat stress induced deterioration in the seed yield and seed nutritional quality was substantially improved with all the foliar spray treatments but more distinctly with MLA and Sorgaab and lesser with water. In crux, terminal heat stress induced reductions in physiological, seed yield and seed nutritional quality of quinoa can be successfully recuperated with foliar spray especially of MLE and Sorgaab. © 2020 Friends Science Publishers

Keywords: Photosynthesis; Oxidative damage; MLE; Seed quality; Heat stress; Quinoa

Introduction

High temperature is one of the main growth limiting factors during leaf formation and grain filling because it declines the metabolism and photosynthetic partitioning (Qaseem *et al.* 2019). High ambient temperature is most detrimental to plants when it occurs at pollination and grain filling stages; known as terminal heat stress, which disturbs metabolic activities (Farooq *et al.* 2011a). Gaseous exchange attributes i.e., stomatal conductance and photosynthetic processes are badly influenced due to enhanced production of reactive oxygen species (ROS) in various cellular organelles (Wahid *et al.* 2007; Ruehr *et al.* 2019), which lead to “oxidative burst” (Petrov and Van Breusegem 2012). Furthermore, it also disturbs the pollen tube formation, which, consequently, causes the death of pollen grains (Hinojosa *et al.* 2019).

Quinoa is abiotic stress tolerant pseudocereal crop,

belonging to family Amaranthaceae and native to Andean Region (Ruiz *et al.* 2014). Due to superior nutritional profile, it is cultivated all over the world. Quinoa seeds are gluten-free and have all essential amino acids with good quality protein (Mota *et al.* 2016). Quinoa grain is rich in minerals, vitamins, antioxidants, dietary fiber as well as Omega-3 and -6 fatty acids and also an excellent source of phenolic acid (vanillic acid, ferulic acid) and flavonoids such as quercetin and kaempferol (Tang *et al.* 2015).

Globally, interest in quinoa cultivation has been increasing because it can survive in harsh environmental conditions like salinity (Iqbal *et al.* 2018), drought (Alandia *et al.* 2016), low temperature (González *et al.* 2015). However, studies showed that it is generally less heat resilient plant; especially terminal heat stress severely lower the photosynthetic pigments, antioxidant activity and economic yield of quinoa (Rashid *et al.* 2018). High

temperature stress causes more damage at reproductive phase of plants than the vegetative stages (Prasad and Djanaguiraman 2014). For example, many plant species have been observed to be more affected by heat stress at anthesis stage, which decreases pollen growth and its viability (Xu *et al.* 2017; Djanaguiraman *et al.* 2018; Hinojosa *et al.* 2019). Lesjak and Calderini (2017) reported that at flowering stage, heat stress decreased quinoa yield by 23 to 31%.

Exogenous use of plant growth promoters, antioxidants, mineral elements, organic and inorganic substances have a central role in improving plant growth and development as well as in mitigating abiotic stress effects, and, as consequence of which, economical yield is improved (Wahid *et al.* 2007). Foliar use of plant growth promoters play vital role in plants to mitigate abiotic stresses by changing plant phenomena (Rashid *et al.* 2018). In addition to chemicals, various organic sources, for instance humic acids, seaweed extracts, protein hydrolysates, amino acids and plant extracts, also promote plant productivity (Nardi *et al.* 2016).

Among various plant extracts, it has been observed that 3% aqueous extract each of moringa (MLE) and that of sorghum (Sorgaab) is very effective for plant growth promotion (Yasmeen *et al.* 2012; Mahboob *et al.* 2018). MLE is used as plant growth promoter because it improves seed germination rate, plant development and yield by 25–30% (Phiri and Mbewe 2010). Quinoa leaves are rich in antioxidants such as vitamin B and vitamin E (tocopherol), which make it resilient to abiotic stresses (Lowell and Fuglie 1999). MLE is rich in zeatin and vitamins (Batool *et al.* 2011), which stimulates crops growth, development and improve grain yield not only under normal conditions but also under stressful conditions (Yasmeen *et al.* 2012). Sorgaab is widely used to promote crop growth (Alsaadawi and Dayan 2009). Farooq *et al.* (2011b) noted that external use of Sorgaab on crop increased biological membrane stability, morpho-physiological attributes and yield, because it is rich in ferulic and coumaric acids and promotes the plant growth (Sene *et al.* 2001; Maqbool and Sadiq, 2017). Similarly, Jahangeer (2011) noted that foliar application of 3% Sorgaab enhanced the crop yield by 22–42%. It not only increases the crop growth and yield but also mitigates the adversities of abiotic stresses by scavenging ROS and delaying leaf senescence (Cheema *et al.* 2012).

There are many synthetic plant growth enhancers such as ascorbic acid (AsA), which have low molecular weight and play vital role to mitigate environmental stress effects. AsA is widely distributed antioxidant in plants, and has major role in scavenging ROS, produced during environmental stresses (Sharma *et al.* 2012). Afzal *et al.* (2006) studied that under abiotic stress conditions, pre-sowing treatment of seeds with ascorbic acid improved germination percentage, seedling growth, antioxidative defense and rate of photosynthesis in wheat (*Triticum aestivum* L.). AsA also mediates the biosynthesis of tocopherol, which protect crops from various abiotic

stresses (Conklin and Barth 2004). Hydrogen peroxide (H₂O₂), though an oxidant, is an important signaling molecule at low concentration, and helps regulate defense mechanism in plants (Kumar *et al.* 2010). Maize (*Zea mays* L.) seed pretreatment was found to be quite befitting for improving heat tolerance in maize at early growth stages (Wahid *et al.* 2008). Foliar spray of different synthetic compounds increased the plant resistance against environmental stresses especially in cereals (Kumar *et al.* 2010; Ahmad *et al.* 2013).

It is evident from the above that exogenous application of various agents is an important strategy for improving plant growth and physiological phenomena. Like many other plants, quinoa is also found to be susceptible to heat stress but studies lack on its physiological and yield responses under terminal heat stress. It is predicted that foliar spray treatment may improve the quinoa photosynthetic, antioxidative response and seed quality of subjected to terminal heat stress. The objective of this two years study was to find the relative effectiveness of plant extracts (MLE and Sorgaab), H₂O₂ and AsA on the pigment composition, gas exchange, antioxidant response of leaves proximal to inflorescence, and to examine the changes in seed yield and quality attributes of quinoa grown under terminal heat stress.

Materials and Methods

Experimental details and treatments application

Pot experiments were conducted in the wire house of Old Botanical Garden, Department of Botany, University of Agriculture, Faisalabad-Pakistan during two successive seasons (2016–17 and 2017–18) to study the mitigation effect of MLE (3%), Sorgaab (3%), H₂O₂ (100 µM) and AsA (500 µM) in quinoa (*Chenopodium quinoa* Willd.) under terminal heat stress. Quinoa genotype UAF-Q7, obtained from Alternate Crops Lab, Department of Agronomy, University of Agriculture Faisalabad, was used in these experiments. Ten seeds were sown in each pot containing 10 kg loamy soil, which were thinned to two plants after one week of germination. The design of the experiment was Completely Randomized (CRD) with three replicates.

Preparation of MLE and sorgaab

Fresh leaves were collected from fully grown moringa trees located at research farm of Department of Agronomy, University of Agriculture, Faisalabad. Moringa leaf extract was prepared according to the methodology described by Price (2000). Before extraction, healthy and disease free leaves were rinsed with distal water and kept in freezer overnight. Extraction was done mechanically. The extract was filtered using Whatman filter paper and further diluted with water to make 3% solution. For preparing Sorgaab, sorghum leaves were collected, chopped into pieces and dried under shade. The chopped material was soaked for 24

h in distilled water in 1:10 (w/v) ratio (Bhatti *et al.* 2000). Soaked material was filtered, and the filtrate was diluted to make 3% concentration.

Terminal heat stress and foliar spray treatments

For heat stress, at anthesis stage (68 days old plants after sowing), pots were divided into two groups; one group was shifted to open door plexi-glass fitted canopies, with a light transmission index of about 0.8, for high temperature stress, and other group was kept in the wire house just outside the canopies. Temperature was 7–10°C higher inside the canopy than ambient condition during daytime. The plants were supplied with water as and when needed to keep the soil moisture 50–60% and 500 mL of Hoagland nutrient solution after 20 days interval. Weekly ambient and canopy minimum and maximum temperature data were recorded (Fig. 1).

Foliar spray of pre-optimized levels of MLE (3%), Sorgaab (3%), H₂O₂ (100 µM) and AsA (500 µM) was done two times at anthesis and grain filling stages with a hand pump. Tween-20 at 2% concentration was used as surfactant in all the spray solutions. In both the pot groups, one set of plants was unsprayed and the other was sprayed with distilled water (controls).

Leaf physiological and biochemical analysis

All these determinations were made in triplicate ten days after second foliar spray. A fully expanded leaf subtending the inflorescence was selected for taking the physiological measurements. Leaf gas exchange parameters of intact leaf including net rate of photosynthesis (P_n), transpiration rate (E), stomatal conductance (g_s) and substomatal CO₂ concentration (C_i) were measured using broad leaf chamber of Infra-Red Gas Analyzer (IRGA; LiCor Model Li-6400, Analytical Development Co. Ltd., Hoddesdon, England) under clear sunny days. The set of conditions for these determinations were air flow 327 mM/m/s, atmospheric pressure 99 kPa, photosynthetically active radiations on leaf surface 345 µmol/m²/s and CO₂ concentration 408 ppm while ambient temperature was 32°C.

For the estimation of pigment composition, 0.5 g of the selected leaf was immediately extracted in 80% acetone in a mini blender, filtered and volume made up to 20 mL. Absorbance of the extract was taken at 645 and 663 nm for the determination of chlorophylls (Chl) a and b with the method of Arnon (1949) and at 480 nm for carotenoids (Davies 1976).

The leaf H₂O₂ content was measured by using the method of Velikova *et al.* (2000). The MDA was estimated by following the protocol of Heath and Packer (1968). To get the leaf extract, 0.5 g fresh leaves were grinded in 10 mL phosphate buffer of pH = 7.8. The leaf extract was centrifuged at 15000 rpm for 20 min. The supernatant of enzyme extract was stored in Eppendorff tubes at -20°C and

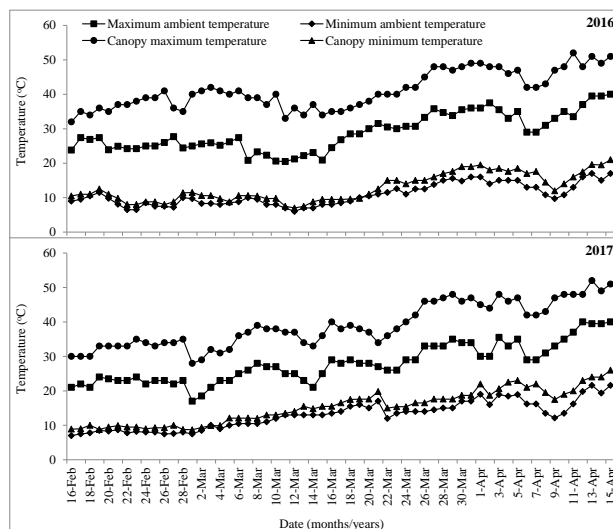


Fig. 1: Minimum and maximum temperature recorded during pot experiments over two years (2016 and 2017) inside and outside the open door glass fitted canopy. One set of potted plants were shifted into the canopy when the plants reached anthesis stage (68 days after sowing)

further used to determine the amount of soluble proteins and activities of antioxidants. The activity of SOD was measured by using the protocol of Giannopolitis and Ries (1977). Catalase activity was measured with the method of Beers *et al.* (1952). The POD activity was measured by using the procedure of Chance and Maehly (1955).

Seed yield components and seed quality determination

At maturity, panicle length was taken of intact plant. After removal, the panicle was measured for dry weight. The seeds were removed and seed yield per plant was recorded, while 1000 seeds weight was taken. Total aboveground dry matter (AGDM) was taken after collecting and drying the shoot mass. The harvest index (HI) was calculated as: $HI (\%) = (\text{seed yield}/\text{AGDM}) \times 100$.

To determine nutritional quality, the seeds were dried in an oven at 60°C for four days. The seeds were digested in a mixture of HNO₃ and HClO₄ (3:1) for 2 h by gradually increasing the temperature of heating block to 250°C. After clearing, the samples were filtered and volume up to 25 mL. This extract was used to measure the K and Ca using flame photometer (Sherwood Model 410, UK), while phosphate-P, Mg, Zn and Fe with the protocols given by Yoshida *et al.* (1976). For seed sulfate-S, the method of Tendon (1993) was used. For nitrate-N, the H₂SO₄ and H₂O₂ digested seed samples were measured with the method of Kowalenko and Lowe (1973).

Statistical analysis

Data were collected and analyzed statistically by two-way

analysis of variance in CRD-factorial arrangement. Data regarding biochemical, mineral elements and yield components and seed quality attributes were analyzed using statistical software “Statistix8.1”. Comparison of individual treatment means was done by using least significant difference (LSD) test at 5% probability level.

Results

Leaf photosynthetic pigments and gas exchange attributes

Results of present study showed that heat stress significantly ($P < 0.001$) lowered photosynthetic pigments (Chl. a, b and Car) contents but foliar spray of various plant growth promoters alleviated the adverse effects of heat stress. All the spray treatments enhanced Chl a and b and Car content under control condition (57%), but such an increase was relatively lesser under heat stress. Foliar applications improved these attributes by 8 to 22% under control condition, while 14 to 107% improvements were observed under heat stress in both the years (Fig. 2).

Data showed that high temperature lowered P_n , E and g_s during both the years while C_i decreased during 2016–17 but increased during 2017–18 under heat stress. Foliar spray of H_2O_2 , Sorgaab, MLE and AsA improved the net P_n , E and g_s to varying extents under control and heat stress. Likewise, C_i decline with the use of H_2O_2 , Sorgaab, MLE and AsA under control and heat stress conditions. Overall, it was noted that foliar treatments increased all gas exchange attributes except C_i under control and heat stress conditions (Fig. 3).

Oxidative stress and antioxidants activity

High temperature increased the internal level of hydrogen peroxide, the data recorded in first year (2016) experiment (Fig. 4). Heat stress significantly increased the MDA level in quinoa leaves in both year studies. Overall, order of MDA level reduction by foliar spray treatments was: MLE > Sorgaab > AsA > H_2O_2 > H_2O . Quinoa plants showed more SOD activity under high temperature than ambient conditions in the years 2016–17 and 2017–18 studies. Highest activity was observed by the use of Sorgaab both under stressed and normal condition (Fig. 4).

As regards antioxidative response, data revealed that heat stress reduced the CAT activity by 19% at first year, whereas by 21% at second year of experimentation. All the spray treatments led to improved CAT activity irrespective of the stress treatments. Highest CAT activity was observed under heat stress and less was under ambient condition. A similar, trend was also observed for POD activity (Fig. 5).

Seed yield and seed nutrients parameters

Heat stress reduced the panicle length by 34 and 50% during

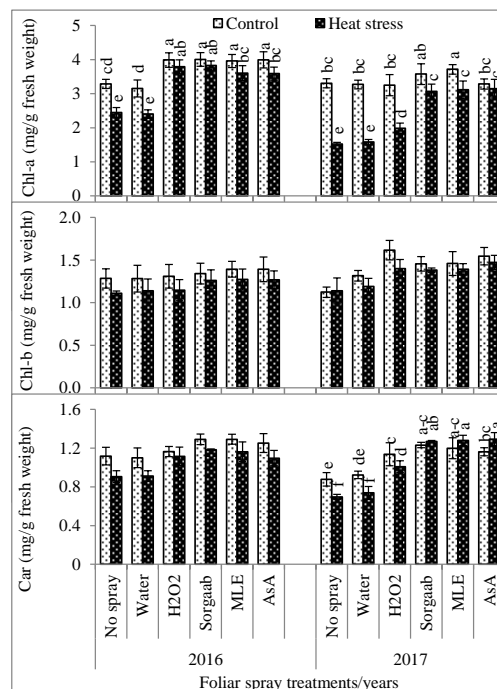


Fig. 2: Photosynthetic content of quinoa leaves under control and heat stress conditions. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

the years 2016 and 2017, respectively as compared to control. However, all foliar spray treatments effectively improved panicle length compared to unsprayed plants. Overall, MLE and Sorgaab displayed highest panicle length in both years of study. Significant reduction in panicle weight and 1000-grain weight under heat stress was observed as compared to ambient temperature (Fig. 6). However foliar spray of H_2O_2 , AsA, Sorgaab and MLE significantly improved panicle weight and 1000-grain weight under stressed and normal condition. Overall, Sorgaab and MLE showed maximum improvement in these attributes in both years of experiments. Heat stress was quite damaging to the seed yield per plant while foliar spray treatments under control or heat stress improved this attribute markedly. A maximum increase in seed yield was 111 and 100% under control condition but was 76 and 126% under heat stress with MLE in both the years. The AGDM yield although was reduced under heat stress condition but marginally. However, there was significant difference among the treatments in this attribute during the year 2016–17 but no such difference was recorded during the year 2017–18. For HI there were significant differences among the foliar spray treatments in both the years. The HI increased with the foliar spray of all the treatments while highest increase of 70 and 40% was noted with MLE under control and heat stress in 2016–17 and respective increase of 56 and 55% was observed in 2017 (Fig. 6).

Heat stress without foliar spray reduced the quinoa seed nutrient contents while foliar spray was effective in

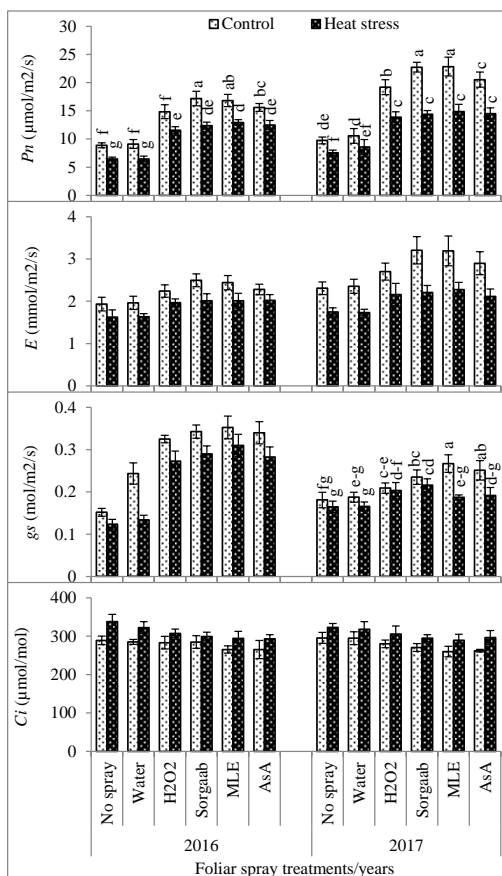


Fig. 3: Gas exchange attributes of quinoa leaves under control and heat stress conditions. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

enhancing them (Fig. 7). As compared to unsprayed control, seed nitrate-N content was improved by 55% in sprayed seeds and by 71% in heat stressed plants in 2016 while by 45 and 96%, respectively in 2017. Compared with unsprayed control, seed phosphate-P was increased by 31% (MLE) under control, and by 42% (Sorgaab) under heat stress in 2016 but by 18 and 49% with Sorgaab in 2017. Heat stress declined the seed K contents while foliar spray treatments improved K contents both under control and heat stress conditions. A maximum improvement in seed K during 2016 was noted with MLE up to 24% under control and 5% under heat stress, while in 2017, this increase was 38% (Sorgaab) under control condition while by 23% (MLE) under heat stress in 2016. Data indicated that in 2016, the seed Ca contents were improved the most with Sorgaab (63%) under control and with MLE and H₂O₂ (19%) under heat stress, while in 2017 such improvement under control condition was noted with AsA (68%) and under heat stress with Sorgaab (82%). Heat stress induced reduction in seed Mg contents was improved with all the foliar spray treatment but the most with Sorgaab under control (38%) and heat stress (18%) in 2016 but with MLE under control (64%) and heat stress (22%) in 2017.

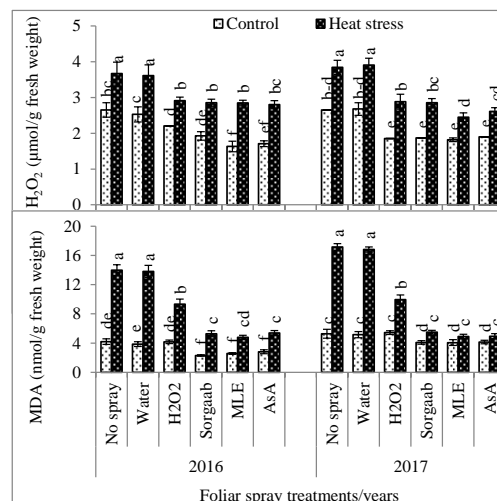


Fig. 4: Oxidative damage attributes of quinoa leaves under control and heat stress. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

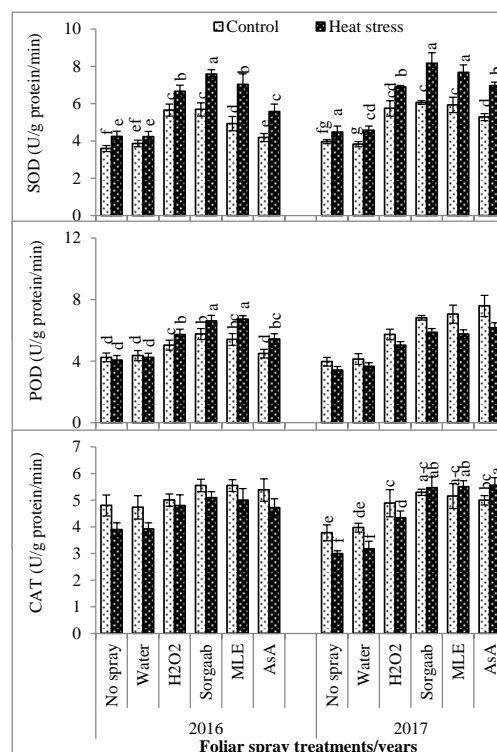


Fig. 5: Antioxidant defense of quinoa leaves under control and heat stress conditions. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

Likewise, heat stress also reduced the seed sulfate-S content significantly but foliar spray reduced the heat stress effect and improved this nutrient by 65 and 103% with MLE under control and heat stress, respectively in 2016 while by 58 and 76% with MLE under both conditions in 2017 (Fig. 7).

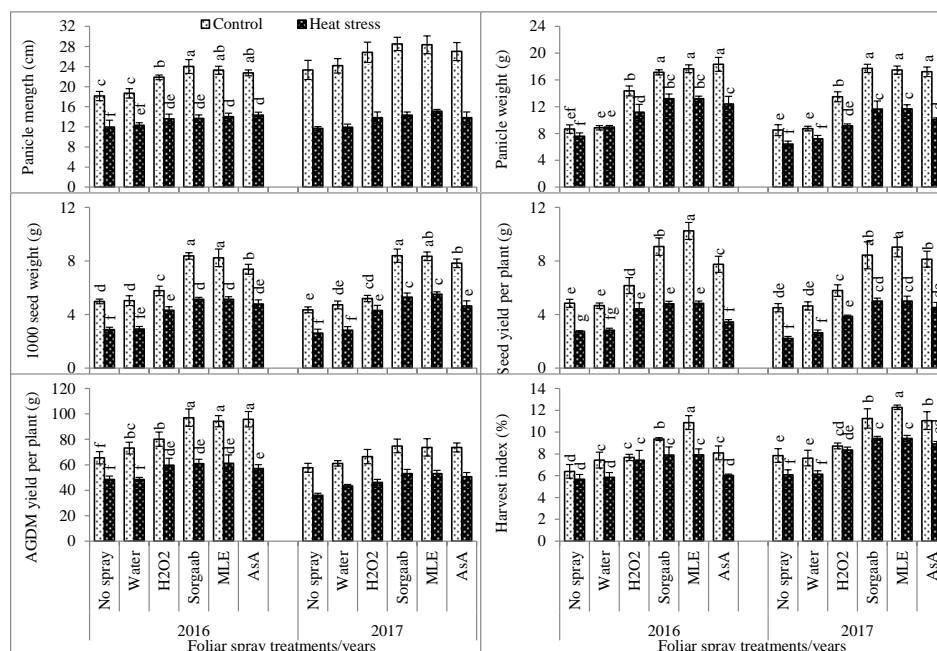


Fig. 6: Yield and yield components of quinoa plants under control and heat stress conditions. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

Discussion

In this research, it has been noted that high temperature declined the physiological attributes of quinoa leaves in both experimental years. Photosynthetic pigments are of two type i.e., primary (Chl *a*, *b* and total) and secondary (Car). Both function to harness light; the reason why the maintenance of these pigments is very important (Taiz *et al.* 2015). The PSII is more thermo-labile because high temperature damages PSII by making it more susceptible to ROS (Takahashi and Murata 2005). Moreover, heat stress causes the excessive biosynthesis of ethylene, which is involved in chlorophyll breakdown and ultimately promotes senescence, while stay green character is necessary to tolerate high ambient temperature episodes (Farooq *et al.* 2011a, b). Results of current experiments revealed that heat stress reduced the photosynthetic pigments Chl *a*, *b*, total Chl and Car in both the years (Fig. 2). Exogenous spray of water, H₂O₂, Sorgaab, MLE and AsA treatments improved all photosynthetic pigments under control and heat stressed conditions. A greater increase was noted under control condition than under heat stress. The changes in leaf chlorophyll content may be due to reduced biosynthesis or increased degradation of chlorophyll under heat stress (Hussain *et al.* 2019).

Productivity of crops is dependent on the amount of CO₂ fixation and assimilates formation by the leaves. Photosynthetic process is quite sensitive to high temperature and is greatly reduced due to disruption in chloroplast structure and decrease in stomatal conductance due to loss in guard cell. In this study, the gas exchange properties of quinoa leaves were studied in terms of changes in *Pn*, *E*, *gs* and *Ci*. Present study revealed that glass-canopy had great

influence and reduced the gas exchange attributes of quinoa plants, which may be due to a decrease in photosynthesis (Fig. 3). Previous experiments on wheat revealed that *Pn* was lowered with increased ambient temperature because of decline in RUBISCO activity (Feng *et al.* 2014). Conversely, Yang *et al.* (2016) reported that high temperature significantly increased *Pn* and *gs* in quinoa. Nonetheless, treatment of plant with different plant growth regulators alleviated the lethal effects of abiotic stresses and ensured the optimum rate of gas exchange attributes. It has been documented that exogenous application of H₂O₂ enhanced the *Pn* in melon (*Cucumis melo* L.) leaves (Ozaki *et al.* 2009) and soybean (*Glycine max* L.) plant (Ishibashi *et al.* 2011).

Primary effect of high temperature on cereals is oxidative damage. It has frequently been observed that thermal stress associated with the accumulation of ROS including H₂O₂, superoxide radical, hydroxyl ion and singlet oxygen, which cause cellular injury (Hussain *et al.* 2019). In plants, ROS are continuously formed in cellular organelles as a byproduct in different metabolic pathways (Heyno *et al.* 2011) and under stressed condition which caused macromolecules denaturation (Hussain *et al.* 2019). ROS cause the lipid peroxidation, which lead to the membrane leakage and loss of membrane integrity and its function (Xu *et al.* 2006). In the present study, there was an increased production of H₂O₂ under glasshouse condition of both experimental years (Fig. 4). When the ROS level exceeds beyond the limits, the lipid peroxidation and formation of MDA commences, while antioxidants level decreases under harsh environment, as reported in many crops like maize (Hussain *et al.* 2019), soybean (Guler and Pehlivan, 2016)

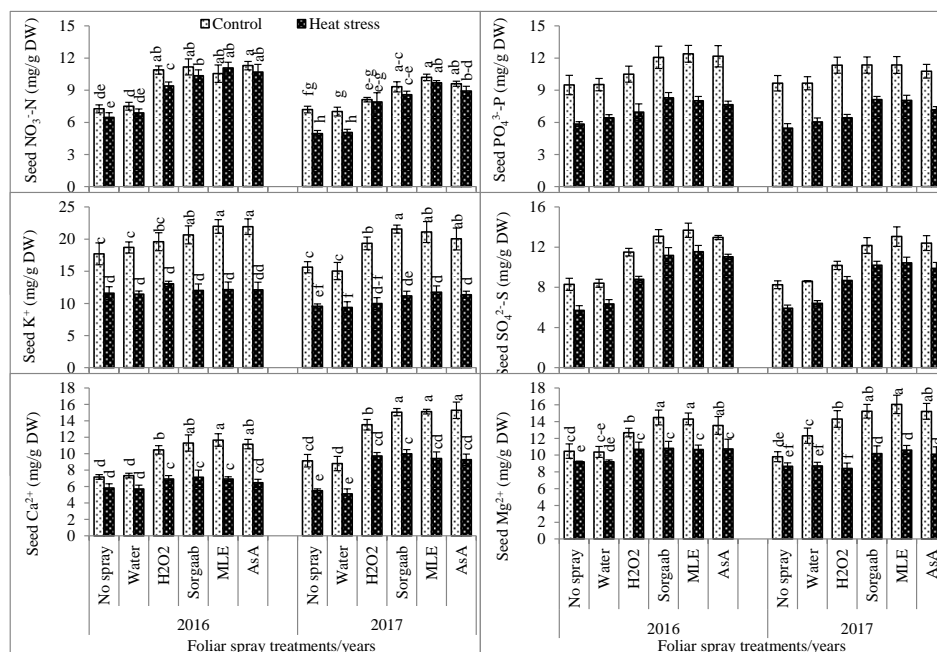


Fig. 7: Nutrient contents of quinoa seed under control and heat stress conditions. The plants were foliar sprayed with selected levels of various growth promoters in the years 2016 and 2017

and quinoa (Iqbal *et al.* 2018). However, foliar spray of MLE and Sorgaab was quite effective in reducing the production of H_2O_2 and MDA, which was due to possible antioxidative potential of both of these plant extracts in enhancing the quinoa tolerance to oxidative damage (Qaseem *et al.* 2019).

Antioxidants activity can be enhanced by the application of various organic and inorganic growth promoters. For instance, foliar application of H_2O_2 at low concentration decreased the MDA content and enhanced SOD, POD and CAT antioxidants level in different crops under normal and abiotic stress conditions (Guler and Pehlivan 2016; Iqbal *et al.* 2018), which convert ROS into water and other non-toxic molecules (Wahid *et al.* 2008). AsA also act as antioxidant as well as substrate peroxidase during chloroplast electron transport chain to scavenge deleterious ROS in the cells (Foyer and Noctor, 2009). Thus the foliar application of AsA increases the growth under heat stress by inducing tolerance against oxidative damage (Batoool *et al.* 2016). In the present research exogenous application of different plant growth promoters (H_2O_2 , Sorgaab, MLE and AsA) mitigated the high temperature stress in quinoa by enhancing antioxidants (SOD, POD, CAT) activity (Fig. 5). Kovinich *et al.* (2015) found that various types of anthocyanins are synthesized in Arabidopsis leaves under different environmental stresses, which enhance plant tolerance by scavenging ROS and improve the biological membrane properties.

Heat stress causes the reduction in cereals economic yield and yield related attributes, because it changes the phenological development of crops by reducing the time to grain fill and metabolism and mobilization of reserves. As a

results small sized and low quantity grains is produced (Nahar *et al.* 2010; Qaseem *et al.* 2019). A rise in environmental temperature can also prolong grain filling time with least vegetative growth. Moreover, it interferes with assimilates partitioning resulting in smaller size, low quality and altered protein profile of grains (Akter and Islam 2017). In addition, terminal heat stress caused spikelets sterility, and pollen abortion as well as poor pollen tube growth on stigma in wheat crop (Qaseem *et al.* 2019). However, adverse abiotic stress effect on seed yield and quality can be diminished by foliar spray of growth promoters (Yasmeen *et al.*, 2013b). In the present study data were recorded for panicle length, panicle weight and 1000 seed weight and HI (Fig. 6). It has been reported that yield and yield related parameters of quinoa were significantly reduced under high temperature stress compared to ambient group of plants, thereby showing its high susceptibility to heat stress. Nonetheless, the foliar application of different plants growth promoters increased the yield and yield related attributes under ambient and glass canopy conditions in both years 2016 and 2017. Foliar application of the selected treatments improved the quinoa yield and yield related parameters, but exogenous use of Sorgaab and MLE produced greatest improvement almost in all yield regarding attributes of both year study. Increase in the yield and its attributes was possibly because the aqueous plant extracts are excellent sources of minerals, antioxidants and secondary metabolites, which help the plant to withstand harsh conditions by improving source and sink activity and water uptake pathway (Yasmeen *et al.*, 2013b). Resultantly improved 1000 seed weight and HI were accomplished with

the foliar spray in both the years, although relatively better in 2016 due to more favoring meteorological conditions.

Moreover, in the present study quinoa seed nutrient contents including nitrate-N, phosphate-P, K, Ca, Mg and sulfate-S were significantly improved with the foliar spray of the selected growth enhancers (Fig. 7). The results showed that nutritional level of quinoa grains are badly affected by under heat stress, while exogenous application of Sorgaab, MLE, AsA and low level H₂O₂ enhanced these attributes to great extent, thus improving the quality of quinoa seed for consumption. The increase in these attributes may be due to better absorption of nutrients via roots and thus efficiently available towards seed filling by partitioning of assimilates from proximal leaves of the panicles (Taiz *et al.* 2015). A greater effectiveness of Sorgaab and MLE in enhancing the seed nutrient contents can be attributed to the presence of phenolic and terpenoid compounds in the aqueous extract (Shah *et al.* 2016), which when used in appropriately diluted concentration can improve the plant growth under heat and other stress effects (Maqbool and Sadiq, 2017). Likewise, MLE, though has more of the cytokinins and vitamins, is important in enhancing the economic yield (Basra and Lovatt 2016), while AsA is a vitamin and has important metabolic role in plants under stress (Chen *et al.* 2017). This indicated that all these growth enhancers by virtue of their own specific effects at least partially rescued the quinoa plants from heat damage and enabled to display better seed yield.

It is important to note that at low concentration H₂O₂ act as signaling molecule and trigger for various antioxidants activation thus prevents the plants from oxidative damage. Maswada and Abd El-Rahman (2014) stated that foliar application at low level H₂O₂ mitigates abiotic stress and enhances crop biomass, mineral absorption and photo assimilates. Fresh MLE is a rich source of minerals, antioxidants, secondary metabolites and cytokinins (Batool *et al.* 2016). External use of MLE protects the crops from damaging environmental effects as well as improved quinoa plant physiological attributes under control and abiotic stresses (Yasmeen *et al.* 2012). Sorgaab is an excellent source of allelochemicals mainly phenolics, which act as antioxidants and promote growth under adverse condition (Cheema *et al.* 2012; Maqbool and Sadiq 2017).

Conclusion

The damaging effects of heat stress to the physiological characteristics of quinoa were partially nullified with foliar spray of AsA, H₂O₂, Sorgaab and MLE during both the years at terminal growth stage. MLE and Sorgaab were more promising, which may be related to the action of growth-promoters and stress-alleviating compounds in both these extracts. The foliar-spray treatments possibly mediate the resource allocation during seed filling resulting in improved seed yield and nutritional quality of quinoa grain under

terminal heat stress. The benefit of foliar applications was greater during the year 2017 in reducing terminal drought effect when the temperature was relatively more subversive.

References

- Afzal I, SMA Basra, A Hameed, M Farooq (2006). Physiological enhancements for alleviation of salt stress in wheat. *Pak J Bot* 38: 1649–1659
- Ahmad I, SMA Basra, I Afzal, M Farooq, A Wahid (2013). Growth improvement in spring maize through exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide. *Intl J Agric Biol* 15:95–100
- Akter N, MR Islam (2017). Heat stress effects and management in wheat. A review. *Agron Sustain Dev* 37:37
- Alsaadawi IS, FE Dayan (2009). Potentials and prospects of sorghum allelopathy in agroecosystems. *Allelopath J* 24:255–270
- Arnon DI (1949). Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15
- Basra SMA, C Lovatt (2016). Exogenous applications of *Moringa oleifera* leaf extract and cytokinins improve plant growth, yield and fruit quality of cherry tomato (*Solanum lycopersicum*). *HortTechnology* 26:327–337
- Batool A, A Wahid, M Farooq (2016). Evaluation of aqueous extracts of moringa leaf and flower applied through medium supplementation for reducing heat stress induced oxidative damage in maize. *Intl J Agric Biol* 18:757–764
- Beers RF, and I.W. Sizer, 1952. Determination of glutathione disulfide in biological samples. *Meth. Enzymol.*, 113: 548–555
- Bhatti MQL, ZA Cheema, T Mahmood (2000). Efficacy of Sorgaab as natural weed inhibitor in raya. *Pak J Biol Sci* 3:1128–1130
- Chance B, AC Maehly (1955). Assay of catalase and peroxidase. *Meth Enzymol* 2:764–775
- Cheema ZA, M Farooq, A Khaliq (2012). Application of allelopathy in crop production: Success story from Pakistan. In: *Allelopathy: Current Trends and Future Applications*, pp:113–143. Cheema ZA, M Farooq, A Wahid (eds.). Springer, Heidelberg, Germany
- Chen K, M Zhang, H Zhu, M Huang, Q Zhu, D Tang, X Han, J Li, J Sun, J Fu (2017). Ascorbic acid alleviates damage from heat stress in the photosystem II of tall fescue in both the photochemical and thermal phases. *Front Plant Sci* 8; Article 1373
- Conklin PL, C Barth (2004). Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens and the onset of senescence. *Plant Cell Environ* 27:959–971
- Davies BH (1976). Carotenoids. In: *Chemistry and Biochemistry of Plant Pigments*, 2nd edn. Goodwin TW (ed.). Vol 2, pp:38–165. Academic Press, London, UK
- Djanaguiraman M, R Perumal, IA Ciampitti, SK Gupta, PVV Prasad (2018). Quantifying pearl millet response to high temperature stress: Thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. *Plant Cell Environ* 41:993–1007
- Farooq M, H Bramley, JA Palta, KHM Siddique (2011a). Heat stress in wheat during reproductive and grain-filling phases. *Crit Rev Plant Sci* 30:491–507
- Farooq M, K Jabran, ZA Cheema, A Wahid, KHM Siddique (2011b). The role of allelopathy in agricultural pest management. *Pest Manage Sci* 67:493–506
- Feng B, P Liu, G Li, ST Dong, FH Wang, LA Kong, JW Zhang (2014). Effect of heat stress on the photosynthetic characteristics in flag leaves at the grain-filling stage of different heat-resistant winter wheat varieties. *J Agron Crop Sci* 200:143–155
- Foyer CH, G Noctor (2009). Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxidant. Redox Signal* 11:861–905
- Alandia G, SE Jacobsen, NC Kyvsgaard, B Condori, F Liu (2016). Nitrogen sustains seed yield of quinoa under intermediate drought. *J Agron Crop Sci* 202:281–291
- Giannopolitis CN, SK Ries (1977). Superoxide dismutase I. Occurrence in higher plants. *Plant Physiol* 59:309–314

- González JA, S Eisa, S Hussin, FE Prado (2015). Quinoa: An incan crop to face global changes in agriculture. In: *Quinoa: Improvement and Sustainable Production*, pp:1–18. Murphy KS, J Matanguihan (eds.). Wiley-Blackwell, Hoboken, New Jersey, USA
- Guler NS, N Pehlivan (2016). Exogenous low-dose hydrogen peroxide enhances drought tolerance of soybean (*Glycine max* L.) through inducing antioxidant system. *Acta Biol Hung* 67:169–183
- Heath RL, L Packer (1968). Photoperoxidation in isolated chloroplasts. *Arch Biochem Biophys* 125:189–198
- Heyno E, V Mary, P Schopfer, A Krieger-Liszskay (2011). Oxygen activation at the plasma membrane: Relation between superoxide and hydroxyl radical production by isolated membranes. *Planta* 234:35–45
- Hinojosa L, JB Matanguihan, KM Murphy (2019). Effect of high temperature on pollen morphology, plant growth and seed yield in quinoa (*Chenopodium quinoa* Willd.). *J Agron Crop Sci* 205:33–45
- Hussain HA, S Men, S Hussain, Y Chen, S Ali, S Zhang, K Zhang, Y Li, Q Xu, C Liao, L Wang (2019). Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci Rep* 9; Article 3890
- Iqbal H, C Yaning, M Waqas, M Shareef, ST Raza (2018). Differential response of quinoa genotypes to drought and foliage-applied H₂O₂ in relation to oxidative damage, osmotic adjustment and antioxidant capacity. *Ecotoxicol Environ Saf* 164:344–354
- Ishibashi Y, H Yamaguchi, T Yuasa, M Iwaya-Inoue, S Arima, SH Zheng (2011). Hydrogen peroxide spraying alleviates drought stress in soybean plants. *J Plant Physiol* 168:1562–1567
- Kowalenko CG, LE Lowe (1973). Determination of nitrates in soil extracts. *Soil Sci Soc Amer Proc* 37:660–668
- Kovinich N, G Kayanja, A Chanoca, MS Otegui, E Grotewold (2015). Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. *Plant Signal Behav* 10; Article 7
- Kumar M, G Sirhindi, R Bhardwaj, S Kumar, G Jain (2010). Effect of exogenous H₂O₂ on antioxidant enzymes of *Brassica juncea* L. seedlings in relation to 24-epibrassinolide under chilling stress. *Ind J Biochem Biophys* 47:378–382
- Lesjak J, DF Calderini (2017). Increased night temperature negatively affects grain yield, biomass and grain number in Chilean quinoa. *Front Plant Sci* 8; Article 352
- Lowell J, NY Fuglie (1999). *The Miracle Tree: Moringa Oleifera, Natural Nutrition for Tropics*. Church World Service, Dakar, Senegal
- Maqbool N, R Sadiq (2017). Allelochemicals as growth stimulators for drought stressed maize. *Amer J Plant Sci* 8:985–997
- Maswada HF, LA Abd El-Rahman (2014). Inducing salinity tolerance in wheat plants by hydrogen peroxide and lithovit a nano-caco3 fertilizer. *J Agric Res Kafu El-Sheikh Univ* 40:693–716
- Mota C, M Santos, R Mauro (2016). Protein content and amino acids profile of pseudocereals. *Food Chem* 193:55–61
- Nahar K, KU Ahmed, M Fujita (2010). Phenological variation and its relation with yield in several wheat (*Triticum aestivum* L.) cultivars under normal and late sowing mediated heat stress condition. *Nat Sci Biol* 2:51–56
- Mahboob W, MA Khan, MU Shirazi, S Faisal, Asma (2018). Seed priming induced high temperature tolerance in wheat by regulating germination metabolism and physio-biochemical properties. *Intl J Agric Biol* 20:2140–2148
- Nardi S, D Pizzeghello, M Schiavon, A Ertani (2016). Plant biostimulants: Physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Sci Agric* 73:18–23
- Ozaki K, A Uchida, T Takabe, F Shinagawa, Y Tanaka, T Takabe, T Hayashi, T Hattori, AK Rai, T Takabe (2009). Enrichment of sugar content in melon fruits by hydrogen peroxide treatment. *J Plant Physiol* 166:569–578
- Petrov VD, F Van Breusegem (2012). Hydrogen peroxide-a central hub for information flow in plant cells. *AoB Plants* 2012; Article ls014
- Phiri, C. and D.N. Mbewe, 2010. Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes. *Intl J Agric Biol* 12:315–315
- Prasad PVV, M Djanaguiraman (2014). Response of floret fertility and individual grain weight of wheat to high temperature stress: Sensitive stages and thresholds for temperature and duration. *Funct Plant Biol* 41:1261–1269
- Price ML (2000). *The Moringa Tree, ECHO Development Note*. North Fort Myers, Florida, USA
- Qaseem MF, R Qureshi, H Shaheen (2019). Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. *Sci Rep* 9; Article 6955
- 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. *Intl J Agric Biol* 20:157–164
- Ruehr N, R Grote, S Mayr, A Arneith (2019). Beyond the extreme: Recovery of carbon and water relations in woody plants following heat and drought stress. *Tree Physiol* 39:1285–1299
- Ruiz K, SB Biondi, R Oses, IS Acuna-Rodriguez, F Antognoni, EA Martinez-Mosqueira, A Coulibaly, A Canahua-Murillo, M Pinto, A Zurita-Silva, D Bazile, SE Jacobsen, MA Molina-Montenegro (2014). Quinoa biodiversity and sustainability for food security under climate change: A review. *Agron Sustain Dev* 34:349–359
- Sene M, T Dore, F Pellissier (2001). Effect of phenolic acids in soil under and between rows of a prior sorghum crop on germination, emergence and seedling growth of peanut. *J Chem Ecol* 26:624–637
- Shah SH, EA Khan, H Shah, N Ahmad, J Khan, GU Sadozai (2016). Allelopathic sorghum water extract helps to improve yield of sunflower (*Helianthus annuus* L.). *Pak J Bot* 48:1197–1202
- Sharma P, AB Jha, RS Dubey, M Pessarakli (2012). Reactive Oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot* 2012; Article 217037
- Taiz L, E Zeiger, MI Moller, A Murphy (2015). *Plant Physiology and Development*, 6th edn. Sinauer Associates, Sunderland, Massachusetts, USA
- Takahashi S, N Murata (2005). Interruption of the Calvin cycle inhibits the repair of photosystem II from photodamage. *Biochim Biophys Acta* 1708:352–361
- Tang Y, X Li, B Zhang, PX Chen, R Liu, R Tsao (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. *Food Chem* 166:380–388
- Tendon HLS (1993). *Methods of Analysis of Soil, Plants, Water and Fertilizers*. Fertilization Development and Consultation Organisation, New Delhi, India
- Velikova V, I Yordanov, A Edreva (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci* 151:59–66
- Wahid A, S Gelani, M Ashraf, MR Foolad (2007). Heat tolerance in plants: An overview. *Environ Exp Bot* 61:199–223
- Wahid A, S Sehar, M Perveen, S Gelani, SMA Basra, M Farooq (2008). Seed pretreatment with hydrogen peroxide improves heat tolerance in maize at germination and seedling growth stages. *Seed Sci Technol* 36:633–645
- Xu J, N Driedonks, MJ Rutten, WH Vriezen, GJ de Boer, I Rieu (2017). Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (*Solanum lycopersicum*). *Mol Breed* 58:37–58
- Xu S, J Li, X Zhang, H Wei, L Cui (2006). Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. *Environ Exp Bot* 56:274–285
- Yang A, SS Akhtar, M Amjad, S Iqbal, SE Jacobsen (2016). Growth and physiological responses of quinoa to drought and temperature stress. *J Agron Crop Sci* 202:445–453
- Yasmeen A, SMA Basra, A Wahid, W Nouman, H Rehman (2013b). Exploring the potential of *Moringa oleifera* leaf extract (MLE) as a seed priming agent in improving wheat performance. *Turk J Bot* 37: 512–520
- 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.*, 72: 92–97
- Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*, International Rice Research Institute (IRRI), Los Banos, The Philippines