



Full Length Article

Evaluation of Aqueous Extracts of Moringa Leaf and Flower Applied through Medium Supplementation for Reducing Heat Stress Induced Oxidative Damage in Maize

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Abstract

Heat stress induced oxidative damage is a major reason for reduced productivity in crop plants. This problem needs to be addressed by different ways and means but ecofriendly means, may be the application of aqueous extracts of different parts of plants. In this study, optimized concentration of 3% aqueous moringa fresh leaf extracts (MFLE), 10% aqueous moringa dry leaf extract (MDLE) and 15% moringa flower extract (MFE) were used for medium supplementation to find out effectiveness and possible mechanism involved in abolishing the heat-induced oxidative damage in selected heat tolerant and heat sensitive maize (*Zea mays* L.) hybrids. For the induction of heat stress, one set of ten day old plants was grown in plexiglass fitted canopies wherein the average temperature was 7–10°C higher than ambient while a control set was grown in the open net house. The plants were harvested after ten days of exposure to heat stress. Results revealed that heat stress induced the production of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA), while reduced the biosynthesis of ascorbic acid, niacin, riboflavin, soluble phenolics and anthocyanins both in the shoot and root. Although application of all the extracts was beneficial in improving heat tolerance in both the maize hybrids, MDLE was the most effective followed by MFLE and MFE. The changes were observed in terms of reduction in the H₂O₂ and MDA generation, vitamins and phenolics synthesis. In conclusion, MDLE supplemented via medium was the most effective for improving heat tolerance in maize hybrids, although improvisation was substantially greater in maize hybrid SB-11. The extracts had a positive effect on the accumulation of vitamins and antioxidants with the production of ROS and minimizing the membrane peroxidation. © 2016 Friends Science Publishers

Keywords: Leaf extract; ROS; Medium supplementation; Maize

Introduction

Environmental factors adversely affect the metabolism, growth and yield of plants (Monclus *et al.*, 2006; Arshad *et al.*, 2008). Salinity, water stress, flooding, pollutants, radiations and extremes of temperatures all restrict crop productivity (Lawlor, 2002; Suralta and Yamauchi, 2008). Of these, high ambient temperature is recognized as most damaging stresses and ever-changing components of the environment. A 0.2°C rise in temperature per decade will be responsible for 1.8–4.0°C rise in air temperature till 2100 (IPCC, 2007; Innes *et al.*, 2015).

Heat stress causes modifications in the plant metabolism with the generation of reactive oxygen species (ROS), which produce oxidative damage to the membranes (Hasanuzzaman *et al.*, 2013). Enhanced production of ROS (e.g., H₂O₂, OH⁻, O₂⁻ and O^{2•} etc.) auto-catalyze the peroxidation of lipids, which increases lipids membrane fluidity, thus losing self-control of cells upon moving water ions and organic solute across the plant membranes

(Wahid *et al.*, 2007; Koo *et al.*, 2015). Plants have natural ability to combat stress episodes to some extent by physical modification or by the production of specific compounds in response to stress signals that assist in metabolism, maintaining homeostasis and re-establish the cellular redox balance (Valliyodan and Nguyen, 2006; Janska *et al.*, 2010). Alterations in expression of genes for osmoprotectants accumulation, detoxifying enzymes, transporters and regulatory proteins directly control the defense mechanism (Semenov and Halford, 2009; Krasensky and Jonak, 2012; Koo *et al.*, 2015).

In addition to the induction of natural defense system, the heat stress tolerance in plants can be enhanced by exogenous application of various stress-relieving agents. Although a great progress has been achieved in improving heat tolerance using a variety of inorganic chemicals and plant growth regulators (Wahid *et al.*, 2008; Farooq *et al.*, 2009), the use of natural sources, especially the plant extracts is limited. In an increasing number of recently studies, it has been shown that the use of moringa fresh leaf

aqueous extract improved the plant growth and yield both under normal (Basra *et al.*, 2011) and sub-optimal conditions (Yasmeen *et al.*, 2013). However, the work has only been restricted to the use of fresh leaves, whilst the use of dry leaf or other part of the moringa plant has not been made.

Moringa (*Moringa oleifera* Lam.) is an important plant species with great medicinal and agricultural uses (Siddhuraju and Becker, 2003). Its leaves are a potential source of natural antioxidants (Santos *et al.*, 2012), synthesize high amounts of growth promoters, antioxidants, ascorbate, zeatin, phenolics, certain minerals i.e. Ca, Mg, K, Mn, B, Fe, P and Zn, and vitamins in a natural balanced composition (Nambiar *et al.*, 2005; Anwar *et al.*, 2007; Amaglo *et al.*, 2010; Ilyas *et al.*, 2015). Phytochemical constituents of moringa proved beneficial in improving stress tolerance in plants. Moringa leaf extract, when applied to salt stressed plants, promoted seed emergence, improved proteins synthesis, induced antioxidant activities and finally increased grains yield of wheat (Basra *et al.*, 2011). The presence of large amount of phenolic compounds, having an ability to combat ROS and high antioxidant activity, and polyphenols contents (Randhir *et al.*, 2004; Ilyas *et al.*, 2015) is important in stress tolerance. Moreover, antioxidants like glutathione and ascorbic acid are synthesized in the chloroplast in significant amounts, which can improve plant tolerance against oxidative stress (Foyer and Noctor, 2003).

The available studies have shown that moringa leaf extract has the potential to improve the plant growth and development. It is surmised that with the synthesis of antioxidants and vitamins in significant amounts, the moringa plant can be an excellent source for improving oxidative stress tolerance in plant species. The objective of this study was to determine the possible role of aqueous extracts of moringa fresh and dry leaves and floral parts via medium supplementation in the alleviation of heat stress induced oxidative damage in hybrid maize, a commercially important crop.

Materials and Methods

Young leaves of moringa were harvested from young full grown trees located at the experimental nursery area of Department of Forestry, Range Management and Wildlife, University of Agriculture, Faisalabad. The preparation of moringa fresh leaf extract was performed according to Price (2007). The extracts of leaves was done with a locally fabricated extraction machine. For moringa fresh leaf extract and moringa flower extract, the leaves and flowers, respectively were extracted immediately after they were excised. For moringa dry leaf extract, the leaves were dried in the shade for one week. The dry leaf extract was prepared by adding water to the dried leaves to make a slurry after extraction. After sieving through cheese cloth, all the extract was centrifuged for 15 min at 8000 × g. Dilutions of the

extract were prepared with distilled water for use as seed priming, medium supplementation and foliar spray.

To test the comparative responses of moringa extracts on heat tolerant (SB-11) and heat sensitive (ICI-984) maize hybrids, the seeds were sown in plastic pots containing sand. Ten day old seedlings of uniform size were supplemented with different treatments of moringa extracts. Hence, five treatments used were: no extract treatment, water treatment, 3% moringa fresh leaf extract, 10% moringa dry leaf extract and 10% moringa flower extract. Plants were harvested after ten days of extract supplementation and uprooted carefully, washed with tap water. Shoot and root dry weight was determined after putting in the paper bags and drying the respective tissues in an oven at 70°C continuously for seven days.

Extent of oxidative stress was assessed by measuring the tissue concentration of H₂O₂ and MDA with the methods of Velikova *et al.* (2000) and Heath and Packer (1968), respectively. Ascorbic acid was determined with the method of Mukherjee and Choudhuri (1983), while niacin and riboflavin concentrations were determined as described by Okwu and Josiah (2006). For total soluble phenolics determination, method described by Julkenen-Titto (1985) was used, while anthocyanins were determined according to the method of Stark and Wray (1989).

Design of the experiment was completely randomized with three replications. Analyses of variance (ANOVA) for all attributes were performed using COSTAT computer package and DMRT was applied to determine the differences among various factors and their interactions separately at individual growth stages (Steel *et al.*, 1996).

Results

Oxidative Damage

Hydrogen peroxide contents: Both the hybrids responded positively to medium supplemented extract under either condition, although SB-11 responded better than ICI-984. Under control condition, shoot H₂O₂ was reduced by ~37% in SB-11 and by 19% in ICI-984. Applied heat stress although increased the shoot H₂O₂ in both the hybrids, the application of extracts reduced the heat stress damage by 59% in SB-11 and by 39% in ICI-984 with MDLE. Although all the medium supplementation treatments reduced H₂O₂ this attribute in both the hybrids, moringa fresh and dry leaf extracts were more effective than the others (Fig. 1a). Medium supplemented extract reduced root H₂O₂ in SB-11 by ~21% with moringa dry leaf extract while by ~5% in ICI-984 with moringa flower extract. Applied extracts spray under heat stress reduced the root H₂O₂ in both the hybrids, being greater in SB-11 by 77% with moringa dry leaf extract, while by 55% in ICI-984 with moringa flower extract spray. Among the various medium supplementation and moringa flower extract were more effective followed by moringa fresh leaf extract (Fig. 1b).

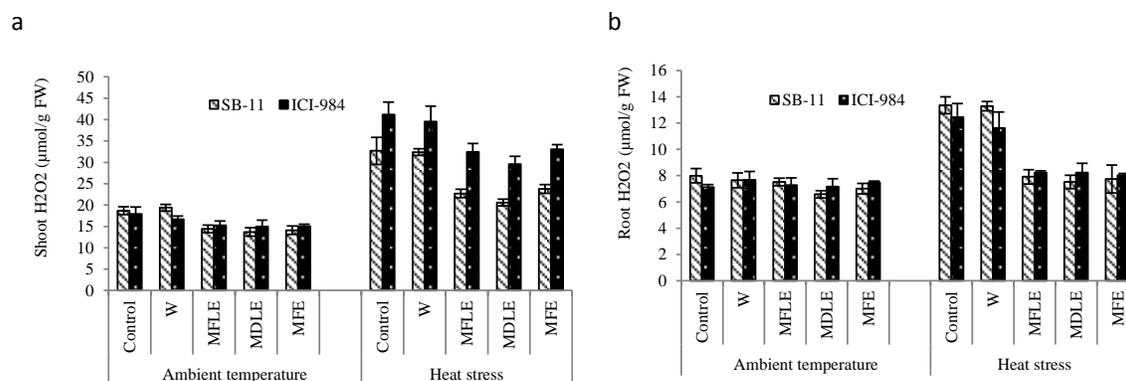


Fig. 1: Mean Performance of different moringa extracts regarding shoot (a) & root (b) hydrogen per oxide contents of maize hybrids under varying temperature regimes. In this and subsequent figures, W, water; MFLE, moringa fresh leaf extract, MDLE, moringa dry leaf extract, and MFE, moringa flower extract

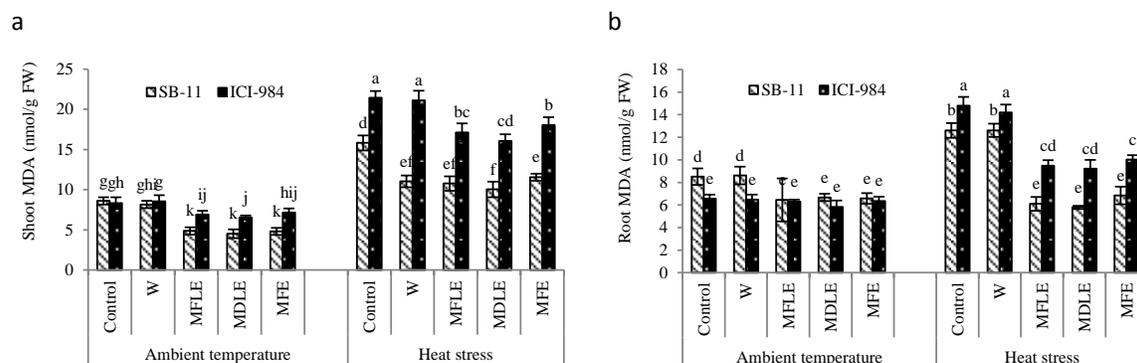


Fig. 2: Mean Performance of different moringa extracts regarding shoot (a) & root (b) malondialdehyde contents of maize hybrids under varying temperature regimes

Malondialdehyde (MDA) contents: The medium supplementation of extract showed that under control condition, shoot MDA reduced by 90% in SB-11 and by 28% in ICI-984 with moringa dry leaf extract. Heat stress although enhanced shoot MDA in both the hybrids, the medium supplementation of extracts reduced the heat stress induced MDA by 58% in SB-11 and by 33% in ICI-984 with moringa dry leaf extract. All the medium supplementation treatments although improved this attribute in both the hybrids, moringa dry leaf extract and moringa fresh leaf extract were more effective. Overall, moringa dry leaf extract and medium supplementation were more effective in changing shoot MDA in the hybrids (Fig. 2a). Medium supplementation of moringa extract reduced the root MDA in both the hybrids although the extent of reduction was much greater in SB-11 than in ICI-984 under either condition. Medium supplementation of extracts to control plants of SB-11 reduced root MDA by ~32% with moringa fresh leaf extract, while 12% with moringa dry leaf extract. However, the medium supplementation of moringa dry leaf extract reduced root MDA by 118% in SB-11 while ~61% in ICI-984. Although all the medium supplementation of extracts

improved this attribute in both the hybrids under either condition, moringa dry leaf extract was more effective than the other modes. Although significant differences in the extracts application through medium supplementation mode, yet moringa dry leaves extract was the most effective in declining root MDA (Fig. 2b).

Vitamins Contents

Ascorbate: With medium supplementation of extracts, under control conditions, the shoot ascorbate was improved by 29% in SB-11 and by 26% in ICI-984 with moringa dry leaf extract. Although reduced under heat stress in both hybrids, the shoot ascorbate was improved by 48% in SB-11 and by 33% with moringa dry leaf extract in ICI-984. Among the various medium supplementation treatments, moringa dry leaf extract was the most effective followed by moringa fresh leaf extract and moringa flower extract in both the hybrids under either conditions. With medium supplementation of extracts, both the hybrids responded positively, although SB-11 responded better than ICI-984 (Fig. 3a). Under control condition, SB-11 showed 36% while ICI-984 indicated 12% increase in root ascorbate with

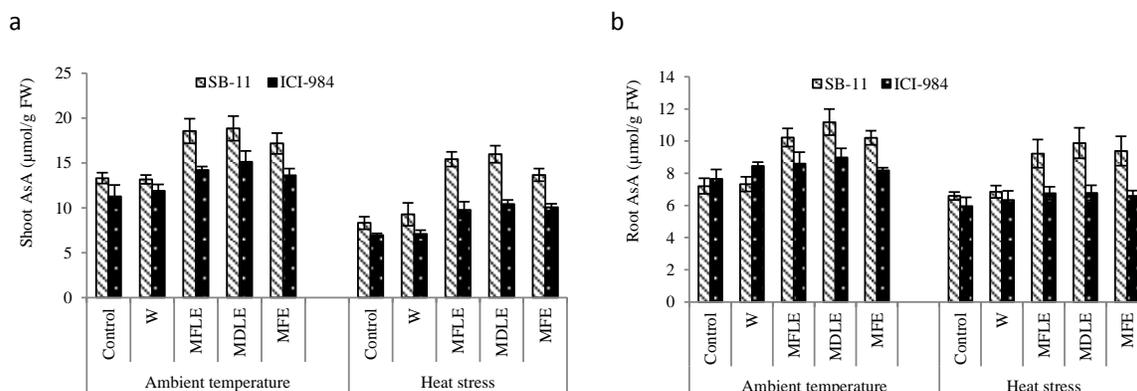


Fig. 3: Mean Performance of different moringa extracts regarding shoot (a) & root (b) ascorbic acid contents of maize hybrids under varying temperature regimes

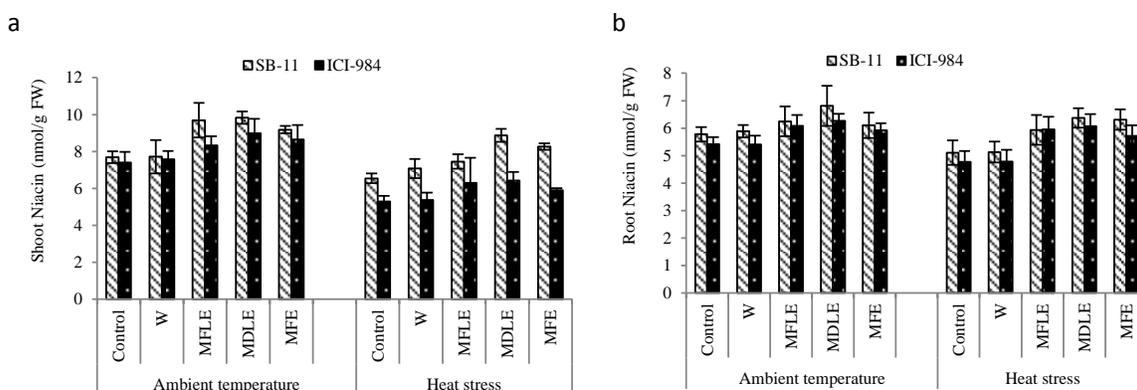


Fig. 4: Mean Performance of different moringa extracts regarding shoot (a) & root (b) niacin contents of maize hybrids under varying temperature regimes

moringa dry leaf extract. Heat stress treatment although reduced the root ascorbate in both the hybrids, the medium supplementation of moringa dry leaf extract improved the root ascorbate by 33% in SB-11 and by 12% in ICI-984. Although medium supplementation improved this attribute in both the hybrids, moringa dry leaf extract was the most effective followed by moringa flower extract in SB-11 and moringa fresh leaf extract in ICI-984 under either condition (Fig. 3b).

Niacin: Both the hybrids responded positively to the medium supplementation of extracts, since an increased shoot niacin both under control and heat stress conditions was noted, although SB-11 responded better than ICI-984. Under control condition, there was 22% increase in the shoot niacin in SB-11 and 18% in ICI-984 with moringa dry leaf extract. However, under heat stress SB-11 manifested 26% while ICI-984 indicated 18% increase in shoot niacin. All the medium supplementation treatments improved shoot niacin in both the hybrids, but moringa dry leaf extract was the most effective followed by moringa flower extract for SB-11 and moringa fresh leaf extract for ICI-984 (Fig. 4a). The medium supplementation of extracts to both the hybrids resulted in a greater improvement in root niacin in SB-11

under control and ICI-984 under heat stress. Under control condition SB-11 and ICI-984 indicated 15 and 14% improvement in this attribute, while under heat stress, SB-11 showed 20% while ICI-984 22% increase in niacin over controls. Although all the medium supplementations improved this attribute in both the hybrids, the effectiveness of moringa dry leaf extract was much greater followed by moringa fresh leaf extract and moringa flower extract (Fig. 4b). Overall, moringa dry leaves extract was the most effective in improving the root niacin contents in both the hybrids.

Riboflavin: The shoot riboflavin improved in both the hybrids with medium supplementation of extract under control or heat stress, although ICI-984 performed better than SB-11 under control condition while SB-11 responded better under heat stress. Under control condition, ~8 and 10% improvement in this parameter was noticed in SB-11 and ICI-984. However, under heat stress, 19% increase in SB-11 and 8% increase in ICI-984 was recorded in shoot riboflavin with moringa dry leaf extract in both hybrids. Among the medium supplementation treatments, moringa dry leaf extract followed by moringa fresh leaf extract and moringa flower extract were promising. Medium

supplemented moringa dry leaves extract was more effective in improving shoot riboflavin in both the hybrids under control or heat stress conditions (Fig. 5a). As regards root riboflavin, the medium supplementation of extracts improved the root riboflavin almost equally under control condition in both the hybrids while under heat stress SB-11 excelled the ICI-984. Under control condition, with moringa dry leaf extract, SB-11 showed ~15% while ICI-984 exhibited 16% improvement in root riboflavin. Heat stress although reduced this attribute in both the hybrids, extracts supply improved it by ~26% in SB-11 and by ~20% in ICI-984 under heat stress. Despite the fact that medium supplementation of extracts enhanced this attribute in both the hybrids, moringa dry leaves extract was the most effective followed by moringa fresh leaf extract in changing root riboflavin contents in the hybrids (Fig. 5b).

Phenolics Contents

The medium supplementation of extracts improved the shoot phenolics in both the hybrids under both the conditions, although ICI-984 was on a greater advantage than SB-11 did so under heat stress condition. Under control condition, there was an improvement of 26% in SB-11 with moringa dry leaf extract while 40% in ICI-984 with moringa fresh leaf extract spray in this attribute. Contrarily, under heat stress SB-11 indicated 51% while ICI-984 exhibited 38% improvement in this attribute with moringa dry leaf extract followed by moringa flower extract and moringa fresh leaf extract (Fig. 6a). Although all the exogenous application modes improved the shoot phenolics to a variable extent, the medium supplementation was more effective in improving this attribute especially under control condition. The medium supplementation of extracts improved the root phenolics greatly in both the hybrids under either condition. Under control condition, both SB-11 and ICI-984 showed 63% improvement in root phenolics with medium supplementation of moringa dry leaf extract. Although applied heat stress reduced root phenolics in both the hybrids, the medium supplementation of extracts effectively improved root phenolics in SB-11 (63%) than ICI-984 (56%) with moringa dry leaf extract. Among the medium supplementation of extracts, moringa dry leaf extract was the most effective followed by moringa flower extract and moringa fresh leaf extract (Fig. 6b). All the extracts although were helpful in improving root phenolics in both the hybrids, the differences were evident in the extracts types and prevailing temperature conditions. Overall, medium supplementation mode was effective in increasing the root phenolics of both the hybrids.

Anthocyanins Contents

With medium supplementation of moringa extracts, under control condition, maximally there was ~50 and 60%

improvement respectively in both the hybrids in shoot anthocyanins with moringa dry leaf extract. Although heat stress reduced the shoot anthocyanins substantially in control plants, it was improved by 68% in SB-11 with moringa dry leaf extract while 54% in ICI-984 with moringa fresh leaf extract. All the medium supplementation treatments improved this attribute in both the hybrids but moringa dry leaf extract was the most effective followed by moringa fresh leaf extract and moringa flower extract (Fig. 7a). Data regarding root anthocyanins showed that medium supplementation under either condition improved the root anthocyanins in both the hybrids, although SB-11 showed a greater improvement. Under control condition, the improvement in root anthocyanins was 71% in SB-11 with moringa fresh leaf extract and moringa dry leaf extract while 61% in ICI-984. Showing a little improvement in plants without medium supplementation in both the hybrids, heat stress further improved the root anthocyanins by 45% in SB-11 and 37% in ICI-984 with moringa dry leaf extract supplementation. Among the medium supplementation treatments, moringa dry leaf extract was the most effective followed by moringa fresh leaf extract (Fig. 7b). Not only there was significant difference in the types of extracts application, the Medium Supplementation with moringa dry leaves extract was most effective among the other extracts.

Discussion

Reactive oxygen species (ROS) are signaling molecules and therefore a physiological necessity for normal cellular metabolism (Bailey-Serres and Mittler, 2006). However, heat and other abiotic stresses cause its production (Asthir, 2015). Results of this experiment suggested that heat stress induced the oxidative damage (H_2O_2 and MDA production) on both the maize hybrids (Fig. 1-2), although the heat tolerant hybrid (SB-11) was significantly less affected than heat sensitive hybrid (ICI-984). H_2O_2 is a longer lived ROS than most other ROS, and is thus more damaging (Wahid *et al.*, 2014). MDA is membrane lipid peroxidation product and its accumulation has been linear with the generation of ROS including H_2O_2 (Ayala *et al.*, 2014). The plants have developed internal systems to undo the adverse effects of ROS and minimize the production of MDA. These strategies are enzymatic and non-enzymatic in nature, which include, among others, the vitamins and some secondary compounds (Kasote *et al.*, 2015). Despite the fact that heat stress induced the production of ROS, the application of aqueous moringa dry leaf extract, moringa fresh leaf extract and moringa flower extract quite effectively alleviated the production of both MDA and H_2O_2 , as evident from their contents in shoot and root, and enhanced the production of ascorbate, riboflavin and niacin in the shoot and root under control or heat stress in both the hybrids although performance of SB-11 was better than ICI-984 (Fig. 3-7).

It has been reported that moringa leaf is a rich source of cytokinins, antioxidants and vitamins

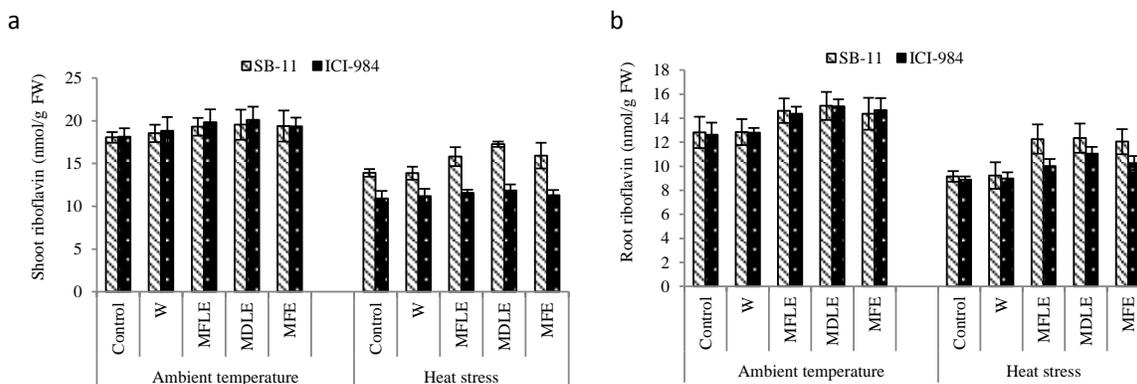


Fig. 5: Mean Performance of different moringa extracts regarding shoot (a) & root (b) riboflavin contents of maize hybrids under varying temperature regimes

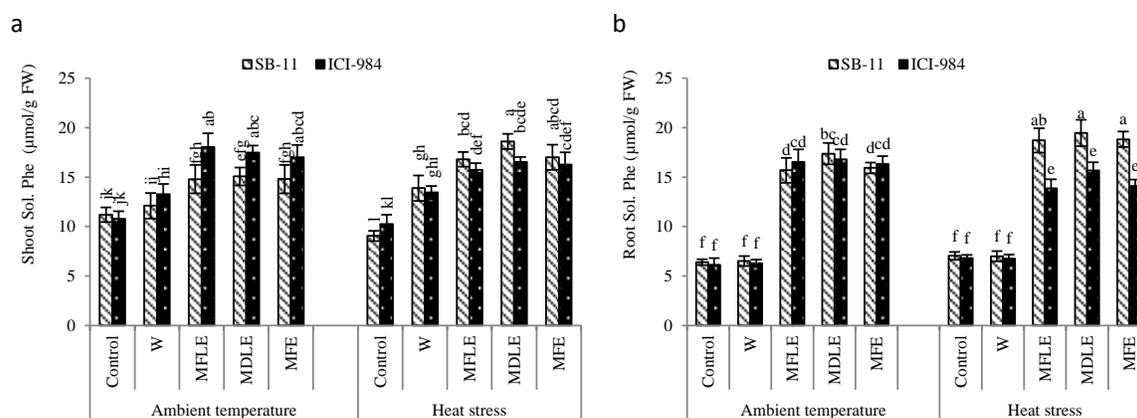


Fig. 6: Mean Performance of different moringa extracts regarding shoot (a) & root (b) soluble phenolic compounds contents of maize hybrids under varying temperature regimes

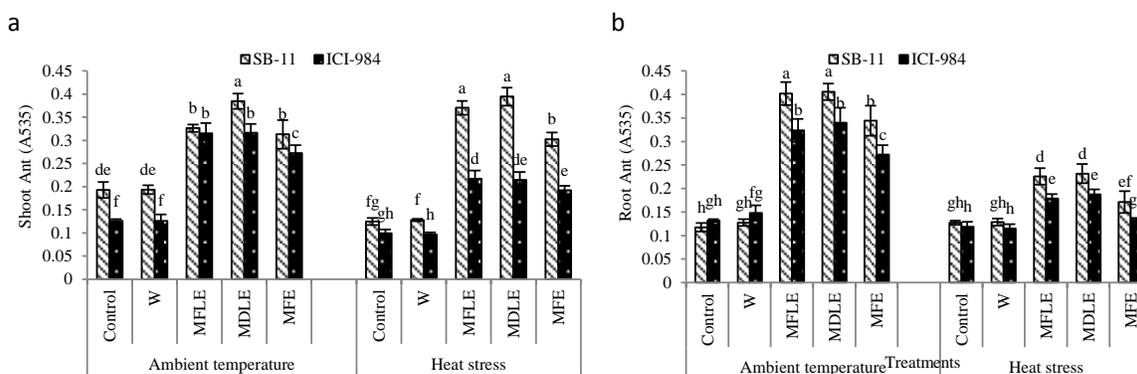


Fig. 7: Mean Performance of different moringa extracts regarding shoot (a) & root (b) ascorbic acid contents of maize hybrids under varying temperature regimes

(<http://itsmoringa.com/1/moringa-antioxidants>; Abdalla, 2013), while the phytochemical constituents of moringa flower is unknown, although flower petal, calyx, corolla etc. are a rich source of anthocyanins. Phenolic compounds, especially the anthocyanins, act as antioxidants under the

environmental stress conditions (Chalker-Scott, 1999; Wahid, 2007), which are reported to be present in moringa leaves (Siddhuraju and Becker, 2003; Manguro and Lemmen, 2007; Ilyas *et al.*, 2015). The alleviation of oxidative stress in terms of reduced production of H₂O₂

(a ROS) under heat stress seems to be highly related to the enhanced production of vitamins especially the ascorbate, soluble phenolics and anthocyanins, although their contents were lesser than control samples under heat stress in this research. However, differences in the effectiveness of aqueous extracts appear to be due to their phytochemical composition, which needs to be confirmed.

The application type and application mode of extracts are of great importance in view of the effectiveness and availability in any medium. Previously moringa dry leaves extract has been used as seed priming strategy most of the times under normal conditions for promoting plant growth (Yasmeen *et al.*, 2013, 2014; Basra *et al.*, 2015), while medium supplementation of moringa extracts has been rarely used. Medium supplementation of extracts was quite effective in the alleviation of heat stress effect. Amongst the moringa extracts, dry leaves extract was the most effective in alleviation of oxidative damage under heat stress. This indicated that moringa dry leaf extract contains significant amount of antioxidants, which appeared to effectively counteract either the production of ROS or their scavenging produced under heat stress (Asthir, 2015). Moringa fresh leaf and flower extracts also showed great promise in lessening the oxidative damage to both the shoot and root, although root was on a greater advantage.

It is of great interest to note that heat stress induced changes in H₂O₂, MDA, vitamins and phenolics were modulated in the both shoot and root tissue despite the fact that only shoot tissue was exposed to high temperature and root received normal supply of water and minerals. Root tissue is more sensitive to applied heat stress as compared to aerial parts (Wahid *et al.*, 2007; Hasanuzzaman *et al.*, 2013). The exogenous supply of moringa leaf and flower extracts alleviated the H₂O₂ and MDA production (Fig. 1-2) and improved the antioxidants synthesis in the root, although great differences were noticed in the extracts types and modes of their application (Fig. 3–7). From the comparative effectiveness of the application modes it was evident that medium supplementation was the most effective among all the application modes. It is plausible that changes in root functioning are due to some sort of shoot to root signaling.

Conclusion

Medium supplementation of moringa dry leaf extract proved to be better strategies to alleviate heat stress induced oxidative damage on maize hybrid varieties. It is likely that the medium supplementation had a direct positive effect on the maize roots, and the healthy and prolific root system due to its defined role in the provision of water and nutrients to the aerial parts.

Acknowledgement

Financial support to first author from Higher Education

Commission of Pakistan is highly acknowledged. The authors are very grateful to S.M.A. Basra for his useful advice in the successful planning and execution of this project. This paper is part of Ph.D. thesis of first author.

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(Received 28 December 2015; Accepted 24 March 2016)