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APPLICATION OF *MORINGA OLEIFERA* PLANT EXTRACTS FOR ENHANCING THE CONCENTRATION OF PHOTOSYNTHETIC PIGMENTS LEADING TO STABLE PHOTOSYNTHESIS UNDER HEAT STRESS IN MAIZE (*ZEA MAYS* L.)

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Abstract

The research experiment was conducted to know the photosynthetic parameters in maize plants exposed to high temperature after applying different methods and types of extracts of *Moringa oleifera* for mitigating heat stress. Two maize hybrid varieties namely SB-11 (heat tolerant) and ICI-984 (heat sensitive) raised under control (ambient temperature 25°C) and high temperature (35°C) conditions. To lessen the effect of thermal stress, these hybrid maize plants were exposed to exogenously application of aqueous leaf (fresh and shade dried) and flower extracts of *M. oleifera* using 3 methods such as seed priming, foliar spray and medium supplementation. The data regarding various photosynthetic pigment parameters like chlorophyll a, chlorophyll b, carotenoids and their ratios were recorded. The results depicted that foliar application of *M. oleifera* extracts was the most effective in increasing the amount of chlorophyll a, chlorophyll b and carotenoid contents and soil application of moringa extract was more favorable towards enhancing total chlorophyll pigments. *Moringa oleifera* dry leaves extract (MDLE) applied through foliar application and medium supplementation modes was the most effective in improving the photosynthetic pigments in heat tolerant hybrid under heat stress. Thus increased accumulation of chlorophyll a, chlorophyll b and reduced chlorophyll-to-carotenoids ratio shown by SB-11 (a heat tolerant maize hybrid) resulted in enhancing photosynthetic pigments leading directly to more photosynthesis and indirectly to the heat tolerance. This study would be helpful in alleviating the adverse effects of heat on photosynthetic machinery and thus increasing the yield of maize under heat stress.

Key words: Carotenoid; Chlorophyll; Foliar spray; Maize hybrids; *Moringa oleifera* extracts; Thermal stress.

Introduction

Moringa oleifera Lam. is mainly grown in the semi-arid, tropical, and subtropical areas and endemic of Pakistan, India, Bangladesh and Afghanistan (Fahey, 2005). *Moringa oleifera* widely known as miracle tree is being introduced as growth stimulant due to its specific attributes found in shoot. *Moringa oleifera* leaves have high concentrations 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 that was found to be beneficial for stressed plants (Anwar *et al.*, 2007; Amaglo *et al.*, 2010).

The occurrence of high temperature stress at critical developing phases especially at reproduction is considered as one of the primary limitations of plant adaptation to a fluctuating climate (Hall, 2001). The photosynthetic fluctuations are predominantly brought by high thermal stress resulting in shortening the life cycle of plant and its productivity (Barnabas *et al.,* 2008). The primary sites of thermal injury are chloroplast stroma and thylakoid membrane systems because of occurrence of photochemical regulation of carbon flux (Wise *et al.,* 2004). Such structural variations are accompanied by variations in energy allocation to the photosystems and leakage of ions from the cells of leaf exposed to heat (Wahid and Tariq, 2008). Reduced amount of photosynthetic pigments and enhanced chlorophyllase activity under heat stress usually lead to the reduced photosynthesis in plants (Sharkey and Zhang, 2010).

Maize (*Zea mays* L.) is the third most important cereal that is grown over a wider geographical and environmental range than that of any of the other cereals as a staple food in many countries (Frova *et al.,* 1999). Maize is a C_4 crop species from tropical origin, its productivity is severely reduced due to the prevailing climatic changes. Researchers all over the world are actively involved in understanding the responses of crop plants to heat stress condition, and finding ways and means of increasing the crop yields. In maize, heat stress decreases yield by negatively affecting the chlorophyll contents and net photosynthetic rate (Morales *et al.,* 2003). Augmented ambient temperature affects plant yield by detrimental impact on photosynthesis (Al-Khatib and Paulsen, 1990).

Yasmeen *et al.*, (2013) reported that *M. oleifera* contains significant amount of cytokinins, macronutrients, micronutrients and antioxidants in its leaves. It was revealed that extract derived from *M. oleifera* leaves enhanced crop production up to 20-35% (Foidle *et al.,* 2001). Ashraf & Foolad (2007) reported that the crop stress tolerance was increased against the abiotic stresses by the application of various compounds through seed priming or exogenous foliar spray. Such biologically active compounds can adjust plant responses to various stresses. To the best of our knowledge, this is the first report about the application of *M. oleifera* leaves extracts for the improvement of photosynthetic pigments in maize. Therefore, the present study was carried out to test the aqueous extracts of *M. oleifera* leaves and flowers exogenously applied as seed priming, foliar spray and root medium for improving photosynthetic pigments of maize plants exposed to heat stress.

Materials and Methods

Plant materials: This study was conducted at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. During this study, the effect of exogenous application of leaves and flowers extracts of *M. oleifera* was examined on two maize hybrids namely SB-11 (heat tolerant) and ICI-984 (heat sensitive) under ambient/control and heat stress conditions. The extracts were applied in three experiments i.e. seed priming, medium supplementation and foliar spray. Newly emerging leaves and flowers obtained from young fully grown trees of *M. oleifera* were selected and *M. oleifera* fresh leaf extracts (MFLE), *M. oleifera* dry leaf extracts (MDLE) and *M. oleifera* flower extracts (MFE) were prepared according to a method described by Price (2007). The seeds of SB-11 (heat tolerant) and ICI-984 (heat sensitive) of maize hybrids were used and sown in plastic pots containing 10 kg of washed river sand and half strength nutrient solution was applied on five day intervals (Yoshida *et al.,* 1976).

For seed priming experiment, the seeds of selected hybrids were soaked in 3, 5 and 5% dilution of the concentrated *M. oleifera* fresh leaf extracts (MFLE), *M. oleifera* dry leaf extracts (MDLE) and *M. oleifera* flower extracts (MFE), respectively. For medium supplementation experiment, 10 day old plants were applied with 3, 10 and 10% dilution of the concentrated MFLE, MDLE and MFE, respectively. For foliar spray, 10 day old plants were sprayed on the leaves with 3, 5 and 15% dilution of the concentrated MFLE, MDLE and MFE, respectively with a manual sprayer (added with Tween-80 as surfactant at the rate of 10%).

After treatment application, one set of 10 day old plants from each experiment was kept in the green net house (control treatment with ambient temperature about 25°C and relative humidity around 32%.), while the other set was kept in the plexiglass fitted canopies for high temperature stress (10ºC more than ambient temperature = 35°C and relative humidity 18%). The plants from all the experiments were grown for 10 days and the harvested samples of leaves were preserved at –80°C till analysis.

Data recording and statistical analysis method: The concentration of photosynthetic pigments such as chlorophyll (a, b and total) was determined following the method of Arnon (1949) whereas carotenoids were calculated following the method of Davies (1976). Fresh leaves (0.5 g) were grinded well and extracted in 20 ml of 80% acetone. The extract was vacuum filtered through Whatman No. 2 filter paper. The absorbance of the supernatant was measured at 645, 663 and 480 nm on spectrophotometer (Hitachi-220 Japan). The chlorophyll a, b, total chlorophyll and carotenoids were calculated by the following formulae:

$$\text{Chlorophyll a (mg/g fresh wt.)} = [12.7(\text{OD } 663) - 2.69(\text{OD } 645) \times V/1000 \times W$$
$$\text{Chlorophyll b (mg/g fresh wt.)} = [22.9(\text{OD } 645) - 4.68(\text{OD } 663) \times V/1000 \times W$$
$$\text{Total Chlorophyll (mg/g fresh wt.)} = [20.2(\text{OD } 645) + 8.02(\text{OD } 663) \times V/100 \times W$$
$$\text{Carotenoids (mg/g fresh wt.)} = [\text{OD } 480 + 0.114(\text{OD } 663) - 0.638(\text{OD } 645)/ 2500] \times 1000$$

where
V = Volume of the sample
W = Weight of the sample

Statistical analysis

The experiments were planned in completely randomized design with three replications. Analysis of variance (ANOVA) for all traits was performed using Statistix 8.1 computer package and means were subjected to Duncan's Multiple Range Test (DMRT) to determine the variation among various factors and their interaction separately (Steel *et al.,* 1996). Means of photosynthetic pigments under each treatment were presented through bar graphs. In all Figures, only those columns bars in graphs were labeled with alphabets where the interactive effect of hybrids × heat stress, treatments × extracts types were found significant (p<0.05).

Results

Chlorophyll a: For chlorophyll a contents, the data from the seed priming mode of extract application showed that all the priming treatments improved chlorophyll a in both hybrids. The amount of chlorophyll a was reduced by 10% in SB-11 and by 22% in ICI-984 under heat stress without extracts application. But the application of *M. oleifera* extracts was quite effective in increasing the chlorophyll a contents. Under control condition (no-stress), the chlorophyll a was improved by 15% in SB-11 and by 19% in ICI-984, while under heat stress; the increase was 14% in SB-11 and 17% in ICI-984. Among priming treatments, MFLE and MDLE were proved to be the most effective for chlorophyll a followed by MFE and water priming (Fig. 1a). Data regarding medium supplementation of extract showed that heat stress reduced the chlorophyll a contents in both the hybrids i.e. 10% in SB-11 and 22% in ICI-984. The application of all the extracts was effective in enhancing chlorophyll a contents under control (up to 14% in SB-11 and 11% in ICI-984) or heat stress (by 16% in SB-11 and 13% in ICI-984). Among various medium supplementation treatments, MDLE were the most effective for improving chlorophyll a contents followed by MFLE and MFE (Fig. 1b). As far as the foliar spray of the extracts is concerned, under heat stress (without extract application), the amount of chlorophyll a was substantially reduced in both hybrids (19% in SB-11 and 32% in ICI-984). However, the application of extracts was effective in improving the chlorophyll a contents under control (no-stress) condition by 14% in SB-11 and by 17% in ICI-984, while heat stress damage was reduced by 25% in SB-11 and 34% in ICI-984 (Fig. 1c).

Chlorophyll b: Under control condition (without extract treatment), there was 16 and 7% improvement in chlorophyll b contents in SB-11 an ICI-984, respectively. However, heat stress was quite damaging to this attribute showing a reduction of 11 and 18% in SB-11 and ICI-984, respectively. The application of all the extracts treatments improved the amount of chlorophyll b by 13% in SB-11 and by 16% in ICI-984. Among various priming treatments, MFLE and MDLE were confirmed to be the most effective for chlorophyll b contents (Fig. 2a). In case of medium supplementation of extract, the heat stress substantially reduced the amount of chlorophyll b i.e. 11 and 23% in SB-11 and ICI-984, respectively. The application of *M. oleifera* extracts improved chlorophyll b contents (7–10%) in both hybrids, while extracts application was highly effective under heat stress that improved chlorophyll b contents (18%) in SB-11 and 15% in ICI-984. Among various medium supplementation treatments, MDLE was found to be the most effective in improving heat tolerance (Fig. 2b). Data regarding foliar spray of extract suggested that both maize hybrids responded differently to the extracts and heat stress. Heat stress treatment without extracts spray decreased the chlorophyll b by 14% in SB-11 and by 28% in ICI-984. Under control condition, with extracts spray, there was nominal improvement in the chlorophyll b contents. However, the application of extracts was highly effective in enhancing chlorophyll b contents (28% and 30%, respectively in ICI-984 and SB-11) under heat stress. Among various extract application treatments, MDLE was highly significant followed by MFE and MFLE (Fig. 2c). Overall, foliar application mode was the most effective for chlorophyll b contents in both hybrids.

Total chlorophyll contents: During this study, the total chlorophyll contents were improved by 15 and 17% in SB-11 and ICI-984, respectively by applying *M. oleifera* extracts under control condition. But the heat stress was quite deterrent to the total chlorophyll contents in both hybrids although SB-11 exhibited a lesser reduction (13%) than that of ICI-984 which showed 16% loss of total chlorophyll contents. The application of all the extracts as foliar spray was quite effective in reducing the heat stress damage up to 14% in SB-11 and 17% in ICI-984. For this parameter, MLE was found to be the most effective followed by MFLE (Fig. 3a). Medium supplementation of extract improved the total chlorophyll contents under control (13 and 10% in SB-11 and ICI-984) and under heat stress (17 and 13% in SB-11 and ICI-984) conditions. Among various medium supplementation treatments, MDLE was the most effective followed by MFLE and MFE in improving heat tolerance (Fig. 3b). The foliar spray of extracts was quite effective in improving the total chlorophyll contents by 12 and 14% in SB-11 and ICI-984 under control condition and alleviating the heat stress damage in SB-11 by 26 and by 32% in ICI-984 (Fig. 3c). There were significant differences in the various factors but overall the soil application modes was the most effective followed by foliar spray in improving total chlorophyll contents of maize.

Chlorophyll a/b ratio: Seed pretreatment with extracts was effective in improving the total chlorophyll contents under control or heat stress conditions. The applied heat stress reduced the chlorophyll a/b ratio by 2% in SB-11, while it was increased by 4% in ICI-984. All the priming treatments slightly affected chlorophyll a/b ratio under normal condition (9.28% in SB-11 and 7% in ICI-984). During this experiment, MFE was found to be the most effective (Fig. 4a). As far as the medium supplementation of extracts is concerned, the application of all the extracts was effective in reducing chlorophyll a/b ratio under control by 4% in SB-11 and 12% in ICI-984 but enhanced under heat stress in SB-11 by 4% with MFE and in ICI-984 by 2% with MDLE. Under medium supplementation of extracts, MFE was quite effective in enhancing this ratio (Fig. 4b). The foliar spray of extracts indicated no clear trend of changes in the chlorophyll a/b ratio under control, while under heat stress; this ratio was reduced by 4-5% in both hybrids. Results showed that under control condition, the foliar spray of extracts generally decreased the chlorophyll a/b ratio (16%) in ICI-984. However, extracts supply under heat stress increased this ratio by 14% in SB-11 (Fig. 4c). Overall, medium supplementation and foliar spray with MDLE and MFE were the most effective for improving chlorophyll a/b ratio in maize.

Carotenoids: For the seed priming mode of extract application, results showed that both hybrids behaved differently for carotenoid contents. Heat stress damaged carotenoids contents almost equally in both hybrids (13–14%). Nevertheless, the extract application was quite helpful in increasing carotenoids contents in both hybrids (30% in SB-11 and 28% in ICI-984). Among various priming treatments, MFLE and MDLE were the most effective for carotenoid contents followed by MFE and water priming (Fig. 5a). As far as medium supplementation was concerned, heat stress without extracts substantially reduced the carotenoid contents in both hybrids although SB-11 incurred a lesser reduction (21%) than that of ICI-984 (34%) over respective controls. The application of all the extracts was effective in enhancing the carotenoid in both hybrids under control (up to 27%) and 41% under heat stress conditions (Fig. 5b). Foliar spray of the extracts proved effective in enhancing the carotenoid contents. Exposure to heat stress led to the reduction of leaf carotenoid contents by 20% in SB-11 and by 29% in ICI-984. The foliar spray of extracts was highly effective in improving the carotenoid contents under control condition in both hybrids (34% in SB-11 and 31% in ICI-984), while under heat stress this improvement was even greater (49% in SB-11 and 44% in ICI-984) (Fig. 5c). Overall, foliar application was the most effective followed by medium supplementation and priming in increasing carotenoid contents in both hybrids.

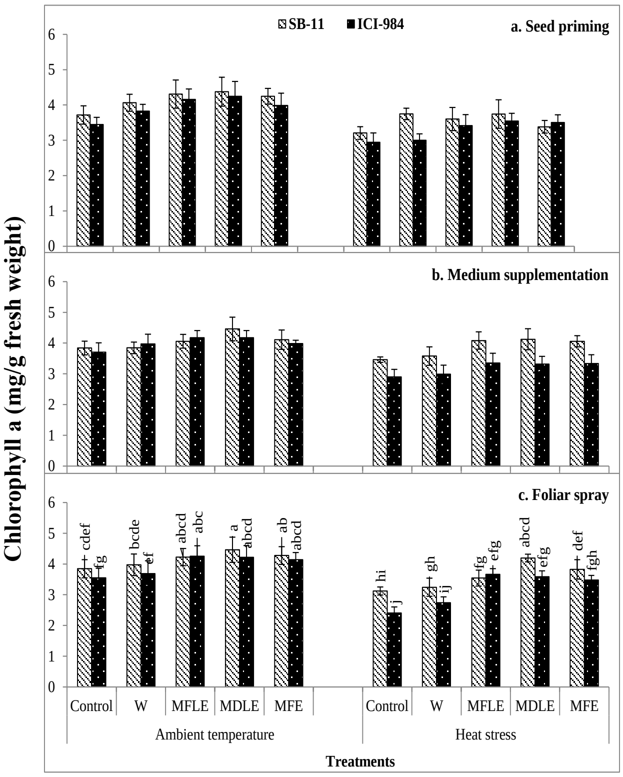

Fig. 1. Changes in chlorophyll-a of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes. Only significant means were labeled alphabetically.

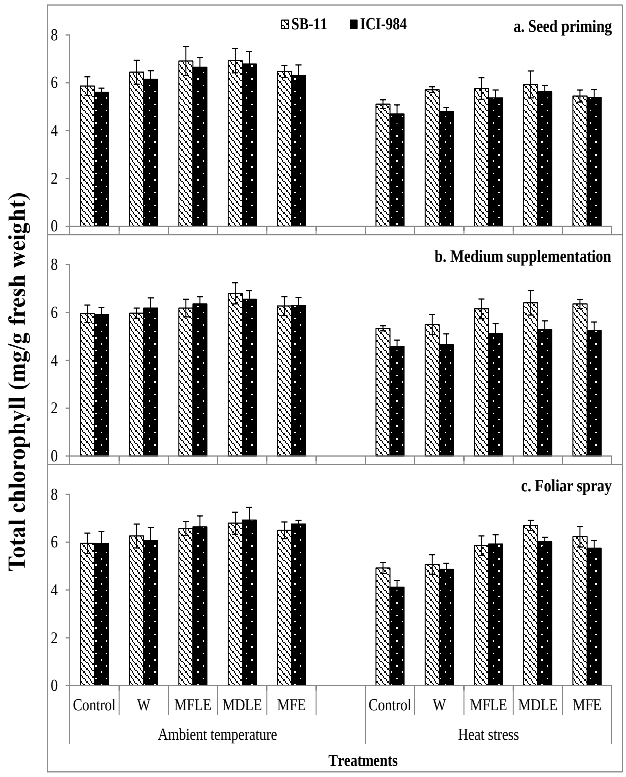

Fig. 3. Changes in total chlorophylls of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes. Only significant means were labeled alphabetically.

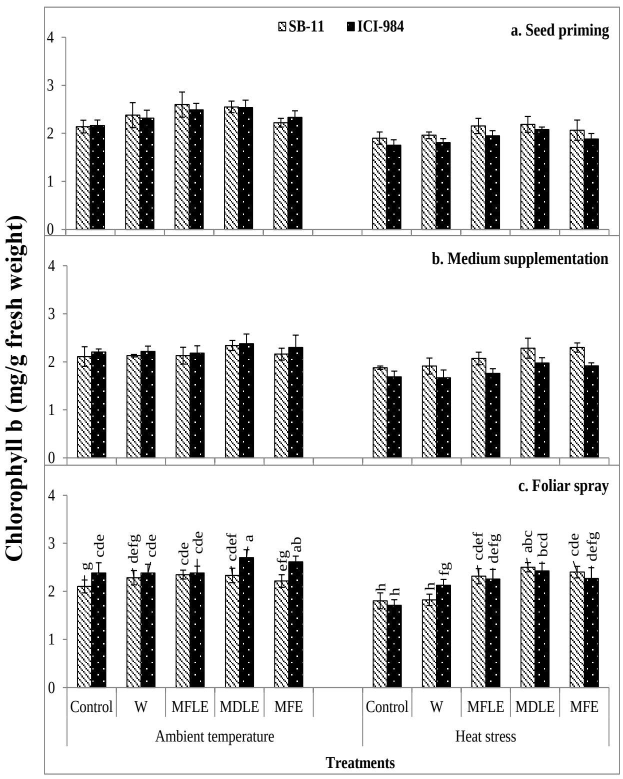

Fig. 2. Changes in chlorophyll-b of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes. Only significant means were labeled alphabetically.

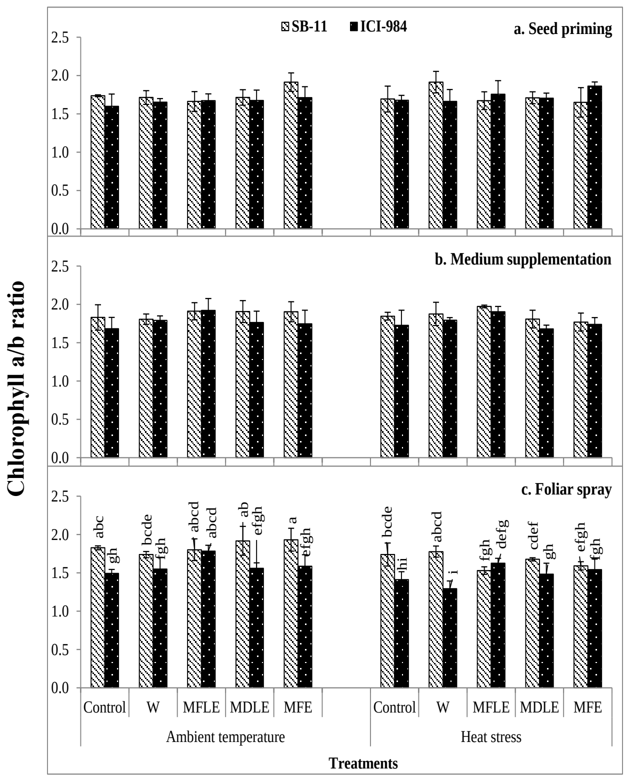

Fig. 4. Changes in chlorophyll a/b ratio of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes. Only significant means were labeled alphabetically.

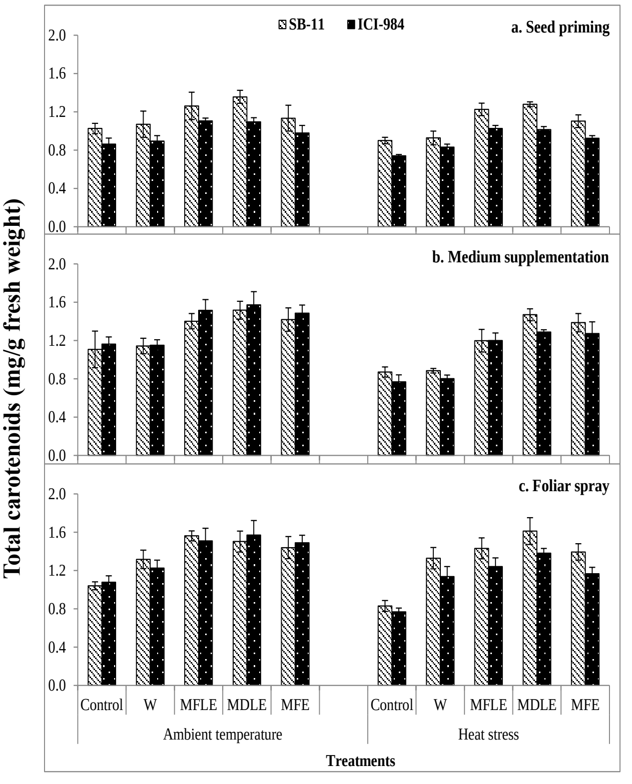

Fig. 5. Changes in carotenoids of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium and foliar spray application modes. Only significant means were labeled alphabetically.

Discussion

Photosynthesis is recognized as one of the most complex physiological processes in plants (Crafts-Brandner & Salvucci, 2002). Increased temperature significantly affects the photosynthetic capacity of plants chiefly in C_3 plants in comparison to C_4 plants (Yang *et al*., 2006). Photosynthesis is an important process of determining the ultimate plant productivity. Light harvesting during photosynthesis is important for the generation of reducing powers in the form of ATP and NADP mainly for the Calvin cycle and the maintenance of photosynthetic pigments is very important (Taiz *et al.*, 2015). High temperature has injurious impact on the cellular sites such as in stromal chloroplast (carbon metabolism) and photochemical thylakoid reactions (Marchand *et al*., 2005; Wang *et al*., 2009). Marutani *et al*., (2014) pointed out the changes occurring in chloroplasts that were structural change of thylakoids membrane, loss of grana stacking, and enlargement of grana under high temperature stress. The inhibition of activity of PS-11 and deterioration in thylakoid membranes had been correlated with the high temperature stresses (Morales *et al*., 2003). It was revealed in an earlier study that heatshock worsened quickly the number of photosynthetic pigments in plants (Marchand *et al*., 2005).

This study reveals that the heat stress reduced the photosynthetic pigment contents but the application of different parts of *M. oleifera* improved the photosynthetic pigment contents under control and heat stress conditions. The trend of accumulation of chlorophyll a, chlorophyll b and carotenoids was distinctly different in both hybrids, which led to changes in the chlorophyll a/b ratio and chlorophyll-to-carotenoids ratio. Reduced chlorophyll a/b ratio and chlorophyll to carotenoids ratio has been reported in previous research studies (Marchand *et al.*, 2005; Wahid, 2007). This is important in view of the fact that the carotenoids provide protection against oxidative damage caused by heat stress (Gill & Tuteja, 2010; Wahid *et al.*, 2014). It is however notable from the current results that MDLE and medium supplementation modes of extracts application were the most effective in improving the photosynthetic pigments contents in maize. It is reported that *M. oleifera* leaf extract is a rich source of growth promoting substances including cytokinins and vitamins (Yasmeen *et al.,* 2013). It has been reported that the water soluble compounds present in the aqueous extract of shade dried leaves improved the root structure and its proliferation, thereby improving leaf photosynthetic pigments by better root functioning under heat stress condition (Wahid, 2007).

Conclusion

It is important to point out that application dry leaf extract of *M. oleifera* as the medium supplement is a pragmatic approach for field use because of improving leaf photosynthetic pigments especially the carotenoids contents. The dry leaves can be used as mulch or soil amendment prior to sowing the maize crop. From the current findings, it can be conceived that an increased yield of 15 to 20% of maize may be achieved by using *M. oleifera* leaves as mulch under control or heat stress conditions without adding any additional inputs.

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