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Research Article

Determination of Critical Level of Brassinosteroid (24-epibrassinoloid) for Heat-tolerance in Okra (*Abelmoschus esculentus* L.)

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Abstract: This study aimed at determining suitable dose of 24-epibrassinoloid (24-EBL) for improving morphological, physiological, and photochemical efficiency of PS II (Fv/Fm) characteristics in thermo-tolerant (*Sabaz pari* and *Green wonder*) and thermo-sensitive (MF-03 and Click-5769) genotypes of okra (*Abelmoschus esculentus* L.). The study was performed in a growth chamber at 40 °C by applying different levels of 24-EBL (i.e., 0 *µM* (control treatment), 0.25 *µM*, 0.50 *µM*, 0.75 *µM*, 1.00 *µM*, 1.25 *µM* and 1.50 *µM*). Foliar application of 24-EBL improved root and shoot growth, plant fresh and dry weight, leaf area, photosynthesis rate, chlorophyll content and photochemical efficiency of PS II in thermo-tolerant as well as of thermo-sensitive okra cultivars as compared to control plants. However, increase in the above stated characteristics was greater in thermo-tolerant okra genotypes as compared to thermos-sensitive genotypes, at all tested levels of 24-EBL. Greater reduction in electrolyte leakage in both thermo-sensitive and thermo-tolerant okra genotypes was observed with 1.50 *µM* 24-EBL. Considering increase in plant growth and biomass, leaf area, chlorophyll content, photosynthesis rate, photochemical efficiency of PS II and reduction in electrolyte leakage, both in thermos-tolerant and thermo-sensitive okra genotypes, it was concluded that 1.50 *µM* 24-EBL was the most suited level of brassinosteroid to improve the thermo-tolerance potential of okra genotypes.

Keywords: Okra, brassinosteroid, physiological, Fv/Fm, electrolyte leakage

1. INTRODUCTION

Plants are static in nature and they pass through different type of environmental (biotic and abiotic) stress during their growth life cycle [1]. These environmental stresses create bad effects on growth, development and production of plants. Nature has blessed the plants with different growth controlling hormones (gibberellins, auxins, cytokinins, abscisic acid, salicylic acid, ethylene, brassinosteroids, jasmonates, etc.), osmolytes (glycine betain, proline, free amino acids etc.) and antioxidants (peroxidase, super oxide dismutase, catalase, ascorbate peroxidase etc.) to mitigate the adverse effect environmental stresses [2-4]. The activation of these hormones may vary in plants based upon the nature and duration of environmental stress [5-6]. The hazardous effects of external environmental stress in plants can also be reduced through the foliar application of natural or synthetic plant growth hormones [7-10], osmolytes [11-13], antioxidants [14], etc.

Brassinosteroid can be extracted from flowers of brassica (*Brassica compestris*) plants and can be foliar applied to promote growth in plants under environments stress conditions [15-20]. Okra (*Abelmoschus esculentus* L.) is a famous crop of tropical and subtropical areas, which is classified in annual, often cross pollinated vegetable crops with

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11,480,000 hectares of cultivation and 7,896,300 tons of production around the globe [21-22]. Due to sudden environmental temperature fluctuations and increase in global temperature growth and production of crop plants has become adversely affected. Therefore, the present study was proposed to investigate the effect of foliar application of brassinosteroid on growth of thermo-tolerant and thermo-sensitive okra cultivars and to find a suitable level of brassinosteroid for better growth of okra which could be used in further study of reproductive study of okra plants under high temperature stress conditions.

2. MATERIALS AND METHODS

The present study was conducted in stress physiology lab, Department of Horticulture, University College of Agriculture, University of Sargodha, Punjab, Pakistan. Seeds of selected okra (*Abelmoschus esculentus* L. Moench) genotypes were collected from the Ayyub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Seeds were disinfected with 5% sodium hypochlorite solution for 15 min, followed by repeated washing with double distilled water and then these seeds were sown in plastic pots filled with peat moss (Sia Pindstrup Ltd., Talsi, Latvia). Three seeds per pot were sown in 4 inches plastic pots with perforated bottom. After seed's emergence, plant density per pot was adjusted to one by removing unhealthy and less vigorous seedling plants. Half strength Hoagland solution was drenched in pots as nutrition source. The nutrient solution was applied after every 10 d interval by adding 30 mLL^{-1} of distilled water and the plants were irrigated as per requirement by observing the moisture of rooting media. Growth chamber was adjusted to 25/23 °C day/night temperature with RH 75%, light intensity 550 µmol m^{-1} s^{-1} from fluorescent tubes and photoperiod 11.5 h. After the emergence of 5-6 true leaf temperature of growth chamber was raised to 40/28 °C while keeping all the other environmental conditions same as for 25/23 °C. Fifteen days after the induction of high temperature stress (40/28 °C) different levels of 24-epibrassinoloid i.e., 0 μM (control), 0.25 μM, 0.50 μM, 0.75 μM, 1 μM, 1.25 μM and 1.50 μM were foliar applied to identify the best suited level of 24-EBL for mitigation of adverse effects of heat stress and induction of thermos-tolerance in tested okra plants.

2.1 Growth Attributes

One week after foliar application of 24-EBL, plants from each replication were gently uprooted and gently washed with distilled water to remove growth media particles and then blotted with filter paper to remove surface water of leaves and shoots and then fresh weight of plants was measured through digital balance. The plants were oven dried (Memmert-110, Schawabach, Germany) at 72 °C for 48 h to measure dry weight per plants. Root length and shoot length was measured meter rod. The leaf area was calculated using leaf area meter (Ll-3100; LI-COR Inc., Lincoln, NE, USA).

Three young fully developed and healthy leaves per plant were selected and placed individually in the chamber of a portable Infrared Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, UK) for the measurement of net photosynthesis rate (Pn). Chlorophyll contents were determined by Chlorophyll content meter (Model CL-01, Hansatech Instruments, UK).

2.3 Photochemical Efficiency of PS II

Photochemical efficiency of PS II (Fv/Fm) was measured using a portable fluorometer (FMS2, Hansatech Ltd., Kings Lynn, Norfolk, England). Leaves were dark acclimated for 30 min and measurements of maximum yield of the photosystem II photochemical reactions (Fv/Fm) and quantum yield of the photosystem II electron transport (UPSII) were carried out according to the method of Yu et al. [23].

2.4 Electrolyte Leakage

For measurement of electrolyte leakage method of Shi et al. [24] was used with some modifications. The ten leaf discs (1cm in diameter) were placed in 50 ml autoclave vials, rinsed three times with 20 ml of distilled water to remove the electrolytes released during leaf disc excision. Vials were then filled with 20 ml of distilled water and were incubated at room temperature on shaker at 100 rpm for 24

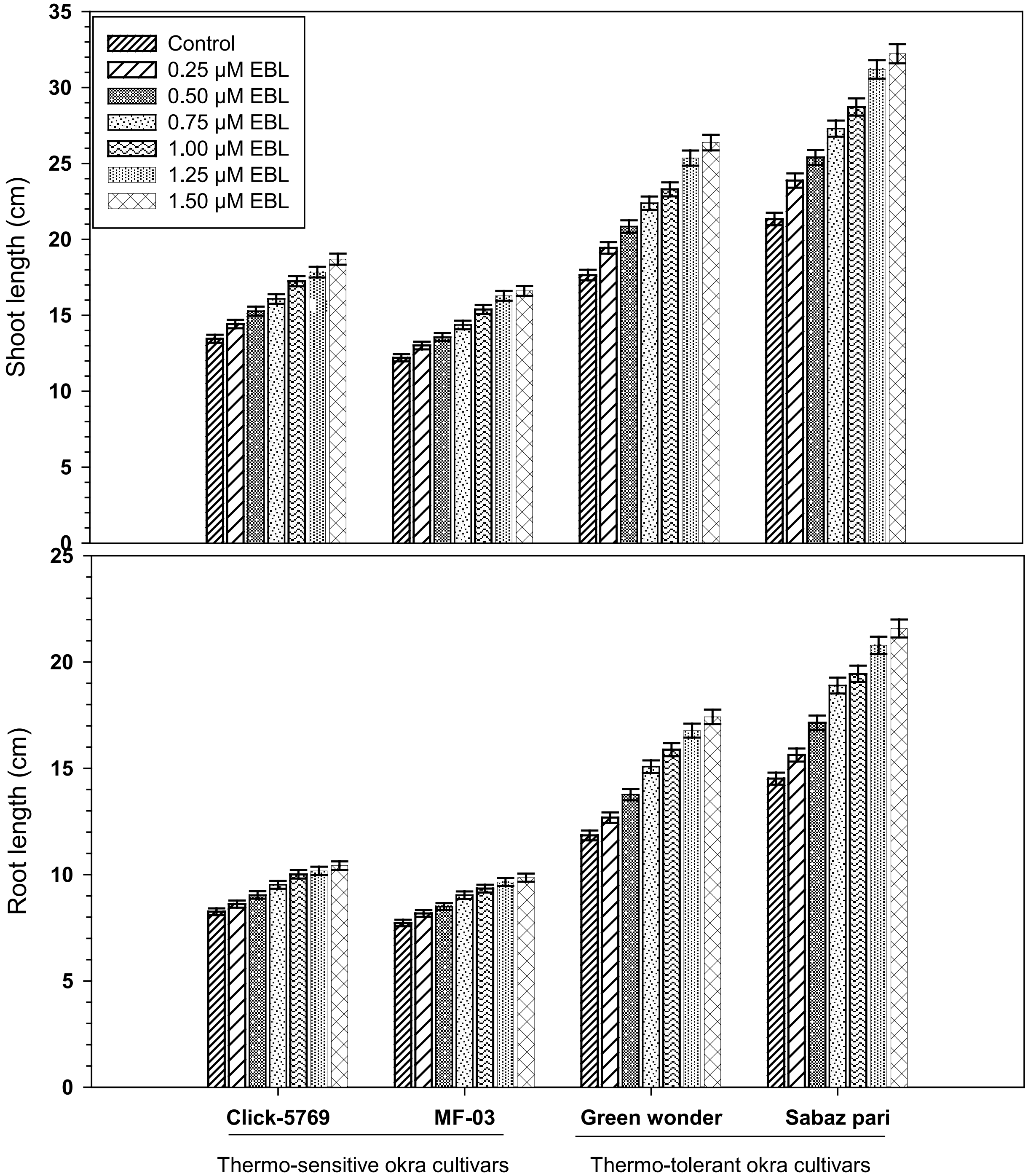

Fig. 1. Effect of 24-Epibrassinoloid application on root and shoot length of thermo-tolerant and thermo-sensitive okra genotypes.

hours. Electrical conductivity (EC_1) of the shaking solution was determined at the end of incubation period. After the measurement of EC_1, tubes were autoclaved at 120 ˚C for 15 mints and the electrical conductivity (EC_2) was measured followed by their cooling at room temperature. Electrolyte leakage was calculated as percentage of EC_1/EC_2.

2.5 Statistical Design and Analysis

The experiment was laid out under Completely Randomize Design (CRD) with five replications. There were five pots per replicate. The data were analysed by standard statistical procedures as described by Gomez and Gomez [25]. Tukey HSD test was used to evaluate the significance of

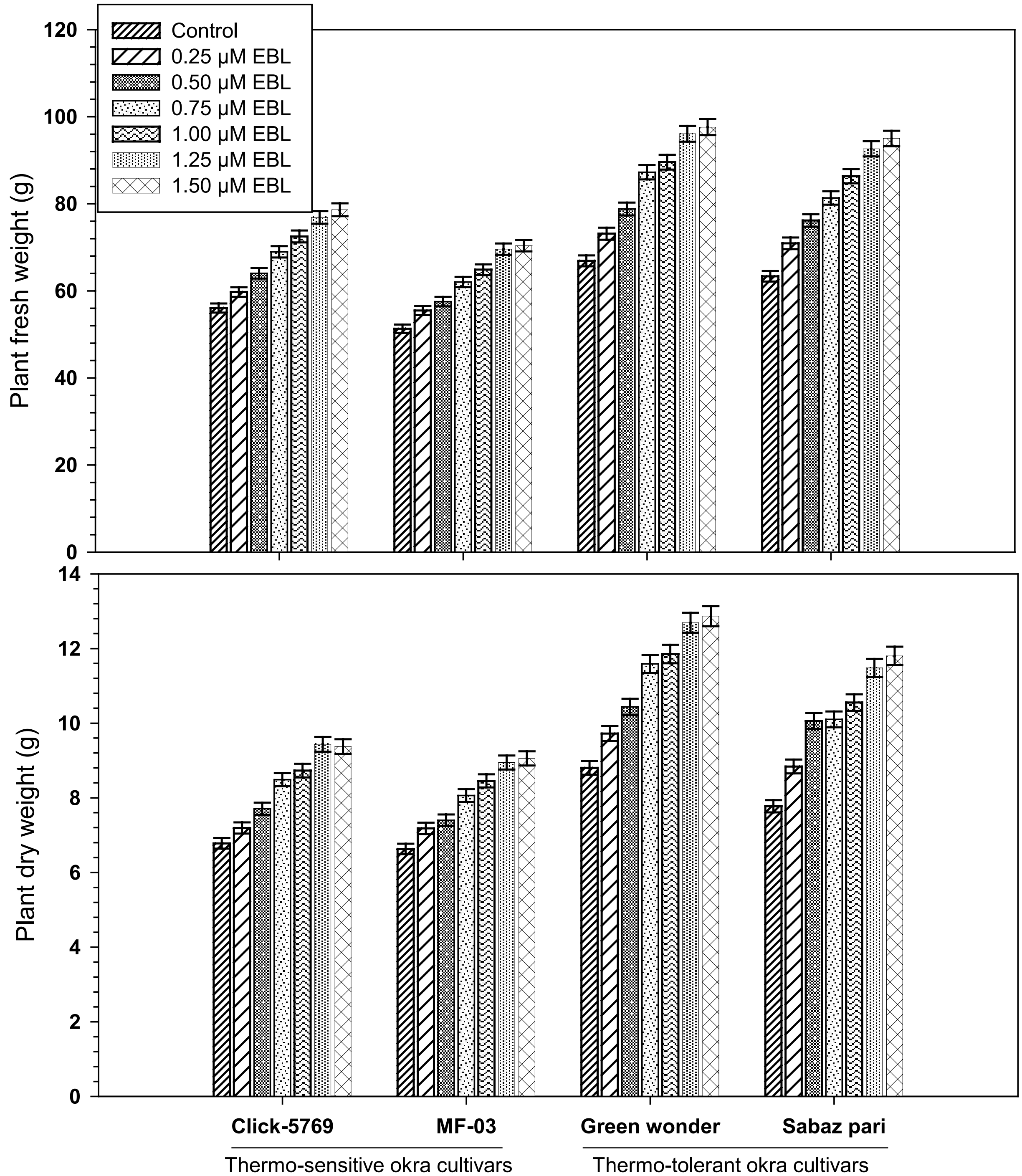

Fig. 2. Effect of 24-Epibrassinoloid application on fresh and dry weight of thermo-tolerant and thermo-sensitive okra genotypes.

differences between the treatments at $P < 0.05$ (n = 5). Data were analyzed using statistical package STATISTIX 8.1.

3. RESULTS

The data regarding thermo-tolerant and thermo-sensitive okra genotypes showed an increase in plants growth through the foliar application of 24-epibrassinoloid at 40 °C. The thermo-tolerant okra cultivar *Sabaz pari* showed greater increase in root and shoot length 48.67 % and 51.68 %, respectively. Thermo-sensitive okra genotype click-5769 and MF-03 showed least increase in root

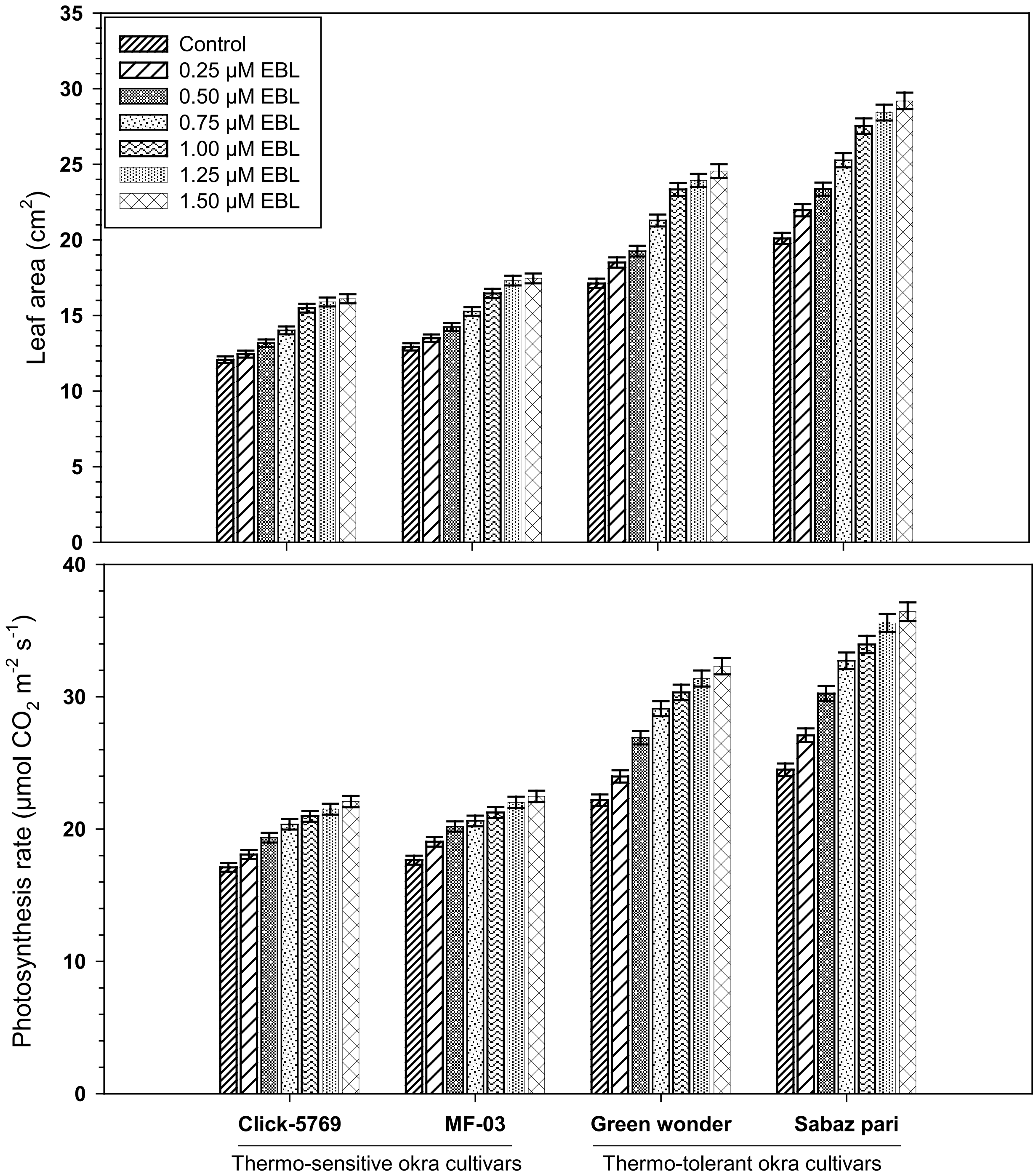

Fig. 3. Effect of 24-Epibrassinoloid application on leaf area and photosynthesis efficiency of thermo-tolerant and thermo-sensitive okra genotypes.

and shoot length 26.28% and 36.19% respectively wit foliar application of 1.5 *μM* 24-epibrassinoloid as compared to control (Fig. 1a, 1b). Foliar application of 24-epibrassinoloid enhanced the fresh and dry weigh of thermo-tolerant and thermo-sensitive okra plants but a greater increase in fresh and dry weigh 49.97% and 51.80% respectively was observed in thermo-tolerant okra genotypes *Sabaz pari*. The least increase in plant fresh weight 26.16 % was observed in thermo-sensitive genotype MF-03 while, plant dry weigh was observed lower 25.61 % in Click-5769 at 1.5 *μM* foliar application of 24-epibrassinoloid as compared to control (Fig. 2a, 2b).

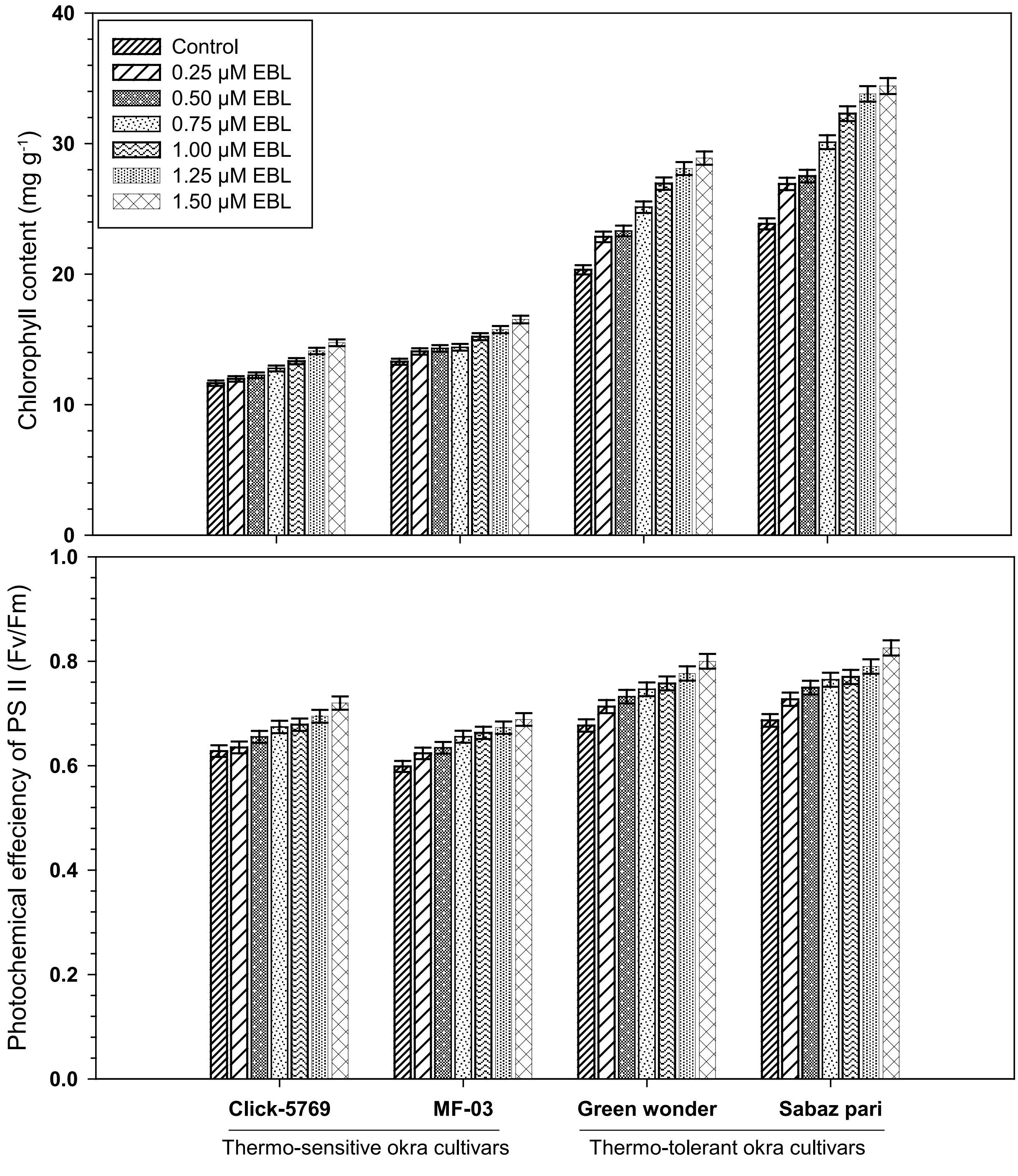

Fig. 4. Effect of 24-Epibrassinoloid application on chlorophyll content and photochemical efficiency of PS II (Fv/Fm) of thermo-tolerant and thermo-sensitive okra genotypes.

Leaf area and photosynthesis rate was observed higher in thermo-tolerant okra genotype *Sabaz pari* 43.41 % and 48.79 % respectively, whereas it was increased least 33.43 % and 27.31 % in thermo-sensitive okra genotypes click-5769 and MF-03 respectively, as compared to control plants (Fig. 3).

Chlorophyll contents and photochemical efficiency of PS II was increased in both thermo-tolerant and thermo-sensitive okra genotypes with increasing the concentration of foliar 24-epibrassinoloid. In comparison with control the greater increase in chlorophyll contents and photochemical efficiency of PS II 44.26 % and 40.18 % respectively was observed in thermo-tolerant

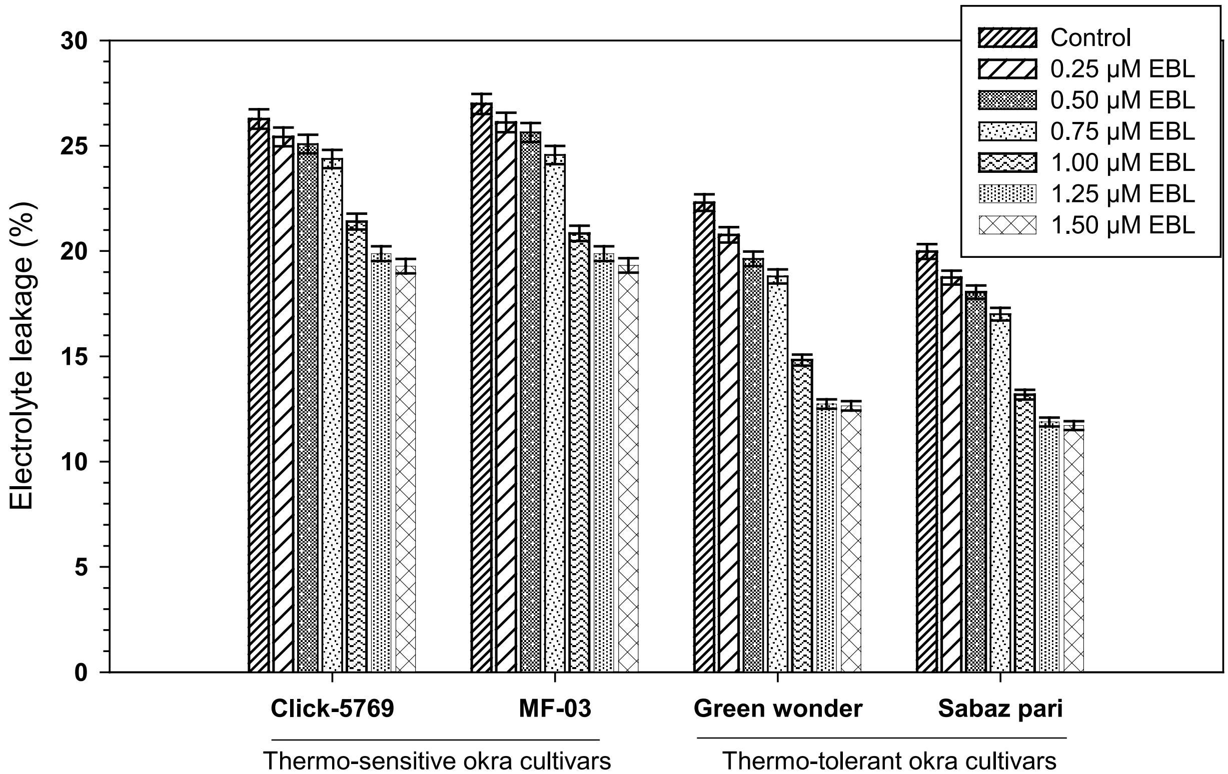

Fig. 5. Effect of 24-Epibrassinoloid application on electrolyte leakage of thermos-tolerant and thermos-sensitive okra genotypes.

okra genotype *Sabaz pari*. The least increase in chlorophyll contents and photochemical efficiency of PS II 24.33 % and 29.35 % respectively, was observed in thermo-sensitive MF-03 and click-5769 respectively (Fig. 4a, 4b).

In case of electrolyte leakage thermo-sensitive okra genotypes showed less reduction i.e. 26.60 % in electrolyte leakage. Thermo-tolerant green wonder showed higher reduction i.e. 43.28 % in electrolyte leakage in response to foliar application of 1.5 μM foliar application of 24-epibrassinoloid and compared with control (Fig. 5).

4. DISCUSSION

Plants growth regulators (PGR) can promote and inhibit the growth of plants depending upon the concentration of PGR used on plants. For example, auxin is a plant growth promoter at its lower concentration but it can be a weedicide when applied with a higher concentration on plants [26]. In our study, different levels of 24-epibrassinoloid (24-EBL) were tested to find a super optimal dose of 24-EBL for the growth of thermo-tolerant and thermo-sensitive okra genotypes screened from a preliminary experiment (data not shown). The results showed that under the varying concentrations of foliar applied 24-EBL plant growth was promoted in both thermo-tolerant and thermo-sensitive okra genotypes. The greater increase in growth parameters like root and shoot length, plant fresh and dry weigh and leaf area was observed in thermo-tolerant genotypes as compared to thermo-sensitive okra cultivars. This increase in okra plants growth was observed maximum under at 1.25 μM and 1.5 μM concentration of 24-EBL. The thermos-tolerant okra genotypes showed greater increase in plant growth due to their better adoptability under high temperature conditions as compared to thermo-sensitive okra genotypes, and high ability to absorb 24-EBL for improving their secondary metabolic activities which help them to grow better under high temperature conditions. El-Bassiony et al. [27] found that foliar application of 25 and 50 ppm of brassinosteroid increase the growth in snap bean plant under high temperature stress conditions. Similarly, Kumar et al. [28] reported that foliar application of 24-EBL promote growth and antioxidant enzyme activities in mustard (*Brassica juncea*) at seedling stage, and the foliar application of 24-EBL can increase plant's tolerance under heat-induced oxidative damage.

Thermal or high temperature stress affect the rate of photosynthesis rate and chlorophyll contents in plants that effect their growth and reduces productivity of plant [29-30] through the production of reactive oxygen species the cause oxidative damage in plants [31-32]. In response of stress, plants modify their morphological, physiological and metabolic activities through producing compatible solutes, maintaining cell turgor by osmotic adjustment, and regulating the antioxidant system to re-establish the cellular redox balance and homeostasis [33-34]. One of the change in plant physiological activities under high temperature stress conditions is that they reduce their photosynthesis activities to coop the adverse effects of thermal stress [35-36], similarly, Yang et al. [37] reported reduction in photosynthesis and chlorophyll contents occur in plants under high temperature conditions, whereas thermos-tolerant cultivars shows less reduction in photosynthesis rate, chlorophyll contents, and stomatal conductance under high temperature stress, and exhibit more plant growth than thermo-sensitive plant genotypes. In our study, reduction of photosynthesis rate was observed higher in thermo-sensitive okra cultivars as compared to thermo-tolerant cultivars due to their less ability to withstand under high temperature conditions. However, the foliar application of 24-EBL improved the photosynthesis rate and chlorophyll contents in both thermo-tolerant and thermo-sensitive okra genotypes, but thermo-tolerant okra cultivars responded more better than thermo-sensitive genotypes at all levels of 24-EBL application. Whereas, foliar application of 1.5 μM of 24-EBL was observed more effective than other levels of 24-EBL. Which may be due to greater production of antioxidant enzymes, osmolytes (not studies in this experiment) and suppressing the effect of ROS activity in okra plants.

Thermal stress causes cell membrane injury in plants due to lipid per oxidation and denaturation of proteins structure [38] that may cause reduction in plants growth, early wilting and death of plants under high temperature conditions [39]. The application of hormones and PGR can cause a reduction of adverse effect of stress in plants by modulating there metabolic activities under stressed conditions [40-41]. In our study, 1.5 μM foliar application of 24-EBL reduced the cell membrane injury more in thermo-tolerant okra genotypes as compared to thermo-sensitive genotype. Hasanuzzaman et al. [41] reported that exogenous application of plant growth promoting substances help to reduce the adverse effect of heat stress on plants through their growth promoting and antioxidant abilities. Similar results had also been reported by Kumar et al. [28], Miura et al. [42], Balal et al. [43] in chickpea, Arabidopsis and cucumber respectively. Wise et al. [44] and Yin et al. [45] reported that photochemical efficiency of PS II become reduced under plant's stress conditions, whereas, foliar application of PGR can improve the chlorophyll florescence in plants [46]. In our study, photochemical efficiency of PS II was observed more under the foliar application of 1.5 μM 24-EBL in thermo-tolerant okra genotypes as compared to thermo-sensitive genotypes under the same level of 24-EBL foliar application. On the basis of above information, it can be concluded that foliar application of 1.5 μM 24-EBL can promote the growth of okra plants by promoting growth, photosynthesis, chlorophyll contents, photochemical efficiency of PS II and reducing the electrolyte leakage under high temperature stress conditions.

5. CONCLUSIONS

Under high temperature conditions foliar spray of 24-EBL improved growth of both thermo-tolerant (cv. *Sabaz pari* and Green wonder) as well as thermo-sensitive (cv. MF-03 and Click-5769) okra genotypes. With foliar application of 1.5 μM 24-EBL increase in plant growth and physiological traits of thermo-tolerant genotypes was greater compared with thermo-sensitive genotypes. Therefore, under high temperature conditions, foliar sparys of 1.5 μM 24-EBL may be applied to enhance thermo-tolerance in okra crop.

6. ACKNOWLEDGEMENTS

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