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Exogenously Supplied Salicylic Acid and Trehalose Protect Growth Vigor, Chlorophylls and Thylakoid Membranes of Wheat Flag Leaf from Drought-Induced Damage

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ABSTRACT

In plants, it is well established that chloroplast is one of the main organelles to break down during leaves senescing by drought. The effects of exogenous salicylic acid and/or trehalose on flag leaf growth, total chlorophylls, lipid peroxidation and thylakoid membranes in flag leaves were investigated in drought-stressed wheat cultivars; Gemmieza-7 (drought sensitive cultivar) and Sahel-1 (drought tolerant cultivar) plants. Water stress induced a significant reduction in growth vigor of flag leaf, especially its biomass, the degree of succulence as well as leaf area and specific leaf area, total chlorophylls, lipid peroxidation and chloroplasts number. On the other hand, drought resulted in a marked increase in oleosomes volume in mesophyll cells of flag leaves in both wheat cultivars when compared with those of the well-watered plants. Gemmieza-7 appeared to be the most affected cultivars. Application of SA and/or Tre appeared to act in a reverse manner by inducing an additional increase in the values of abovementioned criteria. Furthermore, electron microscopic examination of mesophyll cells of wheat flag leaf revealed that water stress negatively affected the chloroplasts and oleosomes ultrastructure in the two cultivars during grain-filling. The chloroplasts showed to be more or less spherical with irregular shape after drought stress treatment. Moreover, disorganized membrane system with swollen thylakoids was identified. These changes in membrane structure are mainly due to the rapid oxidative damage evaluated as malondialdehyde. Moreover, many plastoglobuli were found in the chloroplasts of droughted plants than those recognized in control plants. This effect was more conspicuous with the sensitive one. The exogenous application of SA and/or Tre had a positive effect on the ultrastructure characteristics of mesophyll cells of wheat flag leaves.

Keywords: Wheat; Drought; Oleosomes; Chloroplasts ultrastructure

Abbreviations: SA: Salicylic Acid; Tre: Trehalose; TAGs: Triacylglycerols

INTRODUCTION

Flag leaf plays an important role in wheat plant life as it exports assimilates to spike and developing grains [1]. Reduction in the rate of leaf surface expansion, followed by a cessation of expansion is the general pattern of plant response to stress [2].

Permanent membrane integrity and function under a given level of dehydration has been used as a measure of drought tolerance [3]. Loss of water, degradation of photosynthetic pigment and lipid peroxidation are also significantly stimulated due to drought stress [4].

Leaf ultrastructural characteristics have been widely used to study plant responses to environmental changes [1,5]. Drought is well-known to cause gradual leaf senescence which is characterized by specific leaf ultrastructural changes. Certain changes in cell ultrastructure, such as thylakoid swelling, starch grains depletion and plastoglobuli

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accumulation, occur during leaf senescence in drought-stressed plants [6].

So far, most studies on these cultivars have been concerned with their morphological characteristics but few ultrastructural studies have addressed their tolerance to drought stress. Therefore, in the light of the above-mentioned survey and keeping in view the importance of wheat and water stress, it was of particular interest to assess up to what extent salicylic acid and/or trehalose could ameliorate the deleterious effects of drought stress on both wheat plants. Furthermore, the main goal of this study is to find out the positive effect of grain priming with salicylic acid and spraying the wheat plants with trehalose on increasing the drought tolerance of sensitive cultivar Gemmieza-7.

MATERIALS AND METHODS

Plant material and growth conditions

Two wheat genotype, Gemmieza-7 (drought sensitive cultivar) and Sahel-1 (drought tolerant cultivar) were selected. The sterilized grains from each cultivar were divided into two sets (≈ 500 g per set for each cultivar). Grains of the 1st set were soaked in distilled water to serve as a control, while those of the 2nd were soaked in salicylic acid (3 mM) for about 6 h. Grains were raised in plastic pots (20 cm in diameter) filled with 5.5 kg soil (clay/sand 2/1, v/v), where grains were sown (20 November 2011 and 2012) in each pot. The pots were then kept in a greenhouse at research area of Botany Department, Faculty of Science. The plants were subjected to natural day/night conditions (minimum/maximum air temperature and relative humidity were 15/25°C and 35/45%, respectively) at mid-day during the experimental period. The plants were irrigated to field capacity with tap water.

On the day 65 after planting (at the beginning of heading) the pots of the 1st set was allocated to four groups (20 pots per each group) as follows: control (cont.), water stress (WS), trehalose control, trehalose+water stress (trehalose+WS). The 2nd set group was allocated to four groups as follows: salicylic acid control (SA), salicylic acid+water stress (SA+WS), control trehalose+salicylic acid (SA+trehalose) and salicylic acid+trehalose+water stress (SA+trehalose+WS). For trehalose (1.5 mM) treatment, the plants were sprayed by trehalose 48 h before starting the stress period and weekly during the stress period.

Water deficit was applied by withholding water at the reproductive stage for 30 days within two periods: on the day 65 from planting (heading stage) and at the day 80 from planting (anthesis stage). Each droughted pot received 500 ml water at the end of 1st stress period. At the end of stress periods, rewatering to the field capacity was carried out. The draughted plants were irrigated to the field capacity during the stress period and all plants were left to grow until grain maturation under normal irrigation with tap water. At heading, the plants received 36 kg N ha^{-1} as urea and 25 kg P ha^{-1} as super-phosphate. Physiological observations were taken on fully expanded flag leaves where ten and three samples from flag leaves were taken from each treatment for morphological and biochemical analyses during grain-filling (21 days post-anthesis), respectively.

Agronomic traits were calculated using the following formula:

Leaf area = Length × Breadth × 0.75 [7],

Specific leaf area = Leaf area / Dry mass [8],

Degree of succulence = Water amount / Leaf area [9],

Degree of sclerophylly = Dry mass / Leaf area [10]

Measuring the plant photosynthetic pigment (chl a+chl b) was according to the method of Arnon [11]. Estimation of lipid peroxidation was assayed spectrophotometrically using thiobarbituric acid-malondialdehyde assay (TBA-MDA) [12].

Ultrastructural Studies Using Transmission Electron Microscope

The leaf tissues were processed for transmission electron microscopy TEM according to the method of Woods and and Gay [13]. The plant tissues were cut into 1 mm^2 pieces and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 24 h at room temperature, and then post-fixed in 1% osmium tetroxide in the same buffer. Leaf tissues were dehydrated in a graded ethanol series and infiltrated with Araldite. Sections showed silver-grey interference colours cut by Reichert ultramicrotome were collected on copper grids, stained with uranyl acetate and lead citrate [14]. Sections were examined and photographed by using Jeol 1010 TEM.

Counting of Chloroplasts Number

The number of chloroplasts in flag leaf mesophyll tissue of wheat plants was counted for each treatment in 1.0 µm thick sections cut parallel to the epidermis from the Araldite-embedded material using an ordinary light microscope. A computerized method was followed to count chloroplasts in semi-thin sections. The image of 10x magnification power of computerized light microscope (equivalent to 40x using an ordinary light microscope) was opened with "PAINT" program. The "Line" tool was then used to make standardization with the aid of the magnification bar already present in the image. This step aims at converting the measurement unit from pixels to microns, where it was found that 40 µ (length of the magnification bar) is equivalent to 57 pixels. Accordingly, the "Rectangle" tool was then used to draw a square of 71 × 71 pixels; since this area would be equivalent to 50 × 50=2500 $µ^2$. Such squares were recommended to be drawn in a random fashion on the semi-thin sections where the chloroplasts were counted in a

replicate of ten squares all of the same areas. Finally, the mean number of chloroplasts was calculated for each treatment.

MEASUREMENT OF OLEOSOMES VOLUME IN SEMI-THIN SECTIONS

A new technique developed using the image analysis for measuring the volume of oleosomes (in semi-thin sections from flag leaf mesophyll tissues was estimated to a leaf or cell unit volume for each treatment) was performed.

STATISTICAL ANALYSIS

The data were subjected to one-way analysis of variance (ANOVA), and different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/CoStat software, Version 6.311.

RESULTS

Changes in growth vigor of flag leaf

Perusal of the data shown in **Figures 1 and 2** cleared that, in comparison with control values, water stress resulted in general significant reduction ($p \leq 0.05$) in growth vigor of flag leaf, especially its biomass (fresh and dry masses), degree of succulence as well as leaf area and specific leaf area (non-significant increase in case of sensitive cultivar) of both wheat cultivars during grain-filling. On the other hand, water stress induced a clear increase ($p \leq 0.05$) in the degree of leaf sclerophylly of tolerant cultivar and a non-significant increase in case of the sensitive cultivar. Application of SA and/or Tre resulted in the enhancement of flag leaf growth (i.e., biomass, area and specific area as well as the degree of succulence) in well watered and water stressed wheat plants. Also, the application of these chemicals to the water-stressed plants caused a noticeable decrease ($p \leq 0.05$) in the degree of flag leaf sclerophylly intolerant cultivar and a non-significant decrease in case of sensitive cultivar beyond that of either control or stressed plants. Generally, SA and Tre treatment appeared to be the most effective treatment in counteracting the negative effects of water stress on all flag leaf growth criteria.

Changes in pigment content

In relation to wheat cultivar, the flag leaves of the control tolerant plants had higher pigment (chl a chl b and chl (a+b) content than the sensitive one **(Figure 3)**. On the other hand, SA and/or Tre treatments enhanced pigment production in stressed or unstressed wheat plants. In general, the interaction of SA and Tre appeared to be the most effective treatment in enhancing chlorophyll production in flag leaf of stressed and unstressed wheat plants.

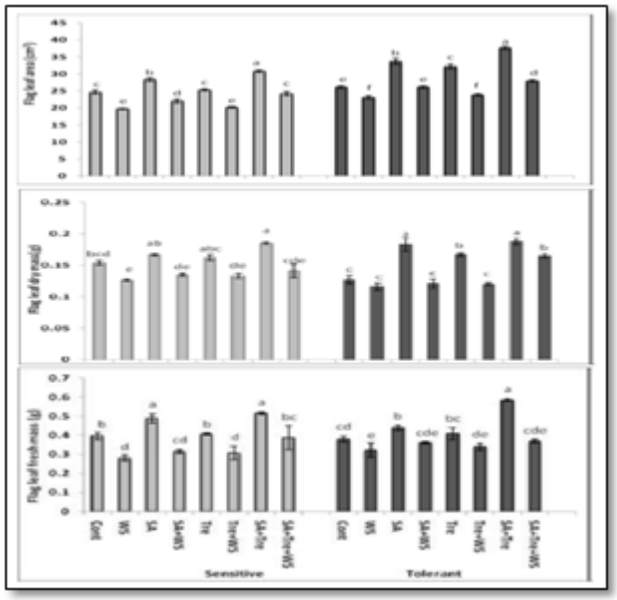

Figure 1. Effect of salicylic acid, trehalose and their interaction on growth vigor of flag leaf (flag leaf fresh and dry masses (g) as well as flag leaf area (cm^2)) of droughted wheat cultivars during grain-filling.
Vertical bars represent standard error of the mean (n=10).
Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/CoStat software, Version 6.311

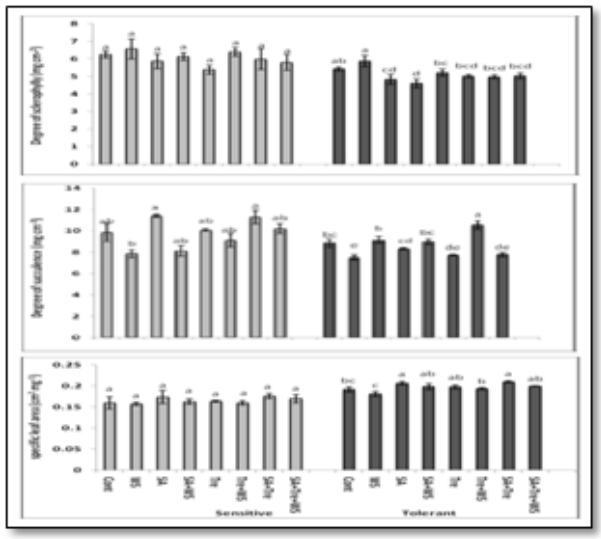

Figure 2. Effect of salicylic acid, trehalose and their interaction on growth vigor of flag leaf (specific leaf area (cm^2 mg^{-1}), degree of succulence (mg cm^{-2}) and degree of sclerophylly (mg cm^{-2})) of droughted wheat cultivars during grain filling.
Vertical bars represent standard error of the mean (n=10).
Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/CoStat software, Version 6.311

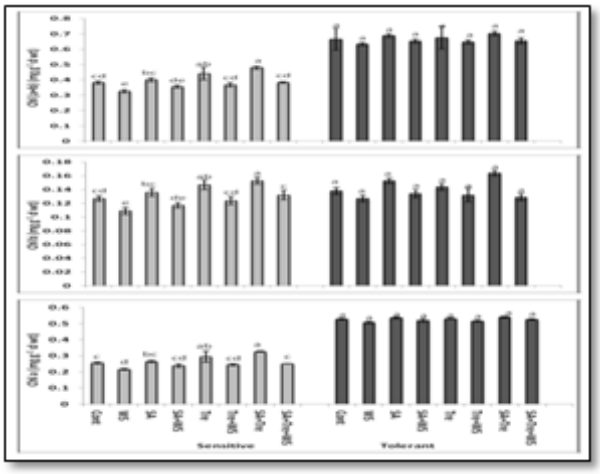

Figure 3. Effect of salicylic acid, trehalose and their interaction on pigment content (Chl a, Chl b and Chl (a+b) (mg g-1 d wt)) of droughted wheat cultivars during grain-filling.
Vertical bars represent standard error of the mean (n=3).
Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311

Changes in lipid peroxidation

As compared to the control values, the results in **Figure 4** reflected that water stress induced a marked increase ($p \leq 0.05$) in the values of lipid peroxidation of tolerant wheat cultivar and a non-significant increase in the sensitive cultivar. Application of SA and/or Tre caused a significant decrease ($p \leq 0.05$) in lipid peroxidation of tolerant wheat cultivar and a non-significant decrease in sensitive cultivar as compared with water-stressed plants. The interaction of SA and Tre was the most effective in decreasing the values of lipid peroxidation in flag leaf of stressed wheat plants.

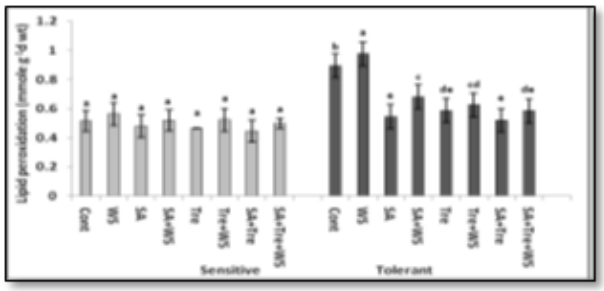

Figure 4. Effect of salicylic acid, trehalose and their interaction on lipid peroxidation as malondialdehyde (MDA) content (mmol g^{-1} d wt) in flag leaf of droughted wheat cultivars during grain-filling.
Vertical bars represent standard error of the mean (n=3).
Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/CoStat software, Version 6.311

Changes in chloroplasts number in mesophyll cells of wheat flag leaf

The pattern of results in **Figure 5** showed that in relation to control values, water stress caused a noticeable decrease ($p \leq 0.05$) in chloroplasts number in mesophyll cells of flag leaf in both cultivars during grain-filling when compared with those of the control plants. The most pronounced effect was recorded for the sensitive cultivar. On the other hand, application of SA and/or Tre caused the additional increase ($p \leq 0.05$) in these values. The most pronounced effect was recorded for the interaction of SA and Tre treatment.

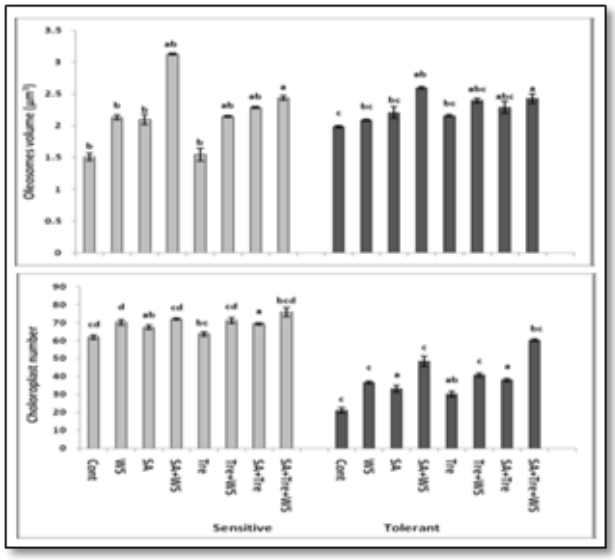

Figure 5. Effect of salicylic acid, trehalose and their interaction on chloroplasts number and oleosomes volume (μm^3) in flag leaf of droughted wheat cultivars during grain-filling.
Vertical bars represent standard error of the mean (n=10).
Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/CoStat software, Version 6.311

Changes in oleosomes volume in mesophyll cells of wheat flag leaf

Data in **Figure 5** cleared that, as compared to the control values, water stress led to the obvious increase ($p \leq 0.05$) in oleosomes volume in mesophyll cells of flag leaves in both wheat cultivars during grain filling. In general, comparing both cultivars, under stress conditions, larger oleosomes volume was observed in drought-sensitive cultivar than drought tolerant one. In comparison with control plants or with water stressed ones, application of SA and/or Tre resulted in an obvious increase in oleosomes volume. Generally, the magnitude of increases was more pronounced with the interaction of SA and Tre treatment.

Changes in the ultrastructure of chloroplasts and oleosomes of flag leaf of wheat cultivars sensitive wheat cultivar

Transmission electron microscopic examination of well-watered wheat flag leaf revealed that the shape of chloroplast from mesophyll cells of control plants was comparatively regular (tended to be oval or elliptical to somewhat) when compared with that of droughted wheat plants. Chloroplast contained starch grains and plastoglobuli. More specifically, mitochondrion was also observed. Grain presoaking in SA caused the chloroplast of well-watered wheat plants to be more regular. Chloroplast contained starch grains and a continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed. More specifically, chloroplasts also contained plastoglobuli. Conspicuous spherical globules, the oleosomes, appeared to be free in the vacuole of mesophyll cells. These inclusions had a sharply-defined osmiophilic interface and apparently lack a limiting membrane.

Regarding the ultrastructure of the flag leaf of well-watered wheat plants treated with Tre, the chloroplasts were normal and ellipsoidal in shape. Chloroplast was closely associated with the cell wall and the membrane system of grana and intergranal lamellae was somewhat organized with defined chloroplast envelope. Starch grains had approximately as the same size as those of control plants, but more in its number. Conspicuous spherical oleosomes appeared to be free in the vacuole of mesophyll cells. Application of an interaction of SA and Tre to well-watered plants resulted in regular or oval chloroplasts containing starch grains. A continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed. Furthermore, the chloroplast contained plastoglobuli and appeared to be dividing. More mitochondria were also observed.

For the flag leaf of droughted wheat plants, it was apparent that the chloroplasts began to be less or more spherical with an irregular shape. Moreover, the disorganized membrane system was identified with swollen thylakoids in these chloroplasts. The starch grains were disappeared. More conspicuous spherical oleosomes were appeared to be free in the vacuole of mesophyll cells and increased in number. Stressed cells also displayed extensive, but thin peripheral cytoplasmic regions devoid of chloroplasts. The chloroplasts of water-stressed cells appeared "bulbous" and discrete. Plastoglobuli were increased in number and become bigger. More mitochondria were also observed. Grain presoaking in SA caused a little change in the ultrastructure of chloroplasts of water-stressed wheat plants. Chloroplasts kept their ellipsoidal shape as the control. They contained starch grains and plastoglobuli. A continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed. The membrane system was slightly affected. Conspicuous spherical oleosomes had a sharply-defined osmiophilic interface and apparently lack a limiting membrane. More mitochondria were also observed.

Regarding the ultrastructure of the flag leaf of stressed wheat plants treated with Tre, the chloroplast shape was, like in SA treatment, more regular than the stressed ones. Chloroplasts were closely associated with the cell wall and the membrane system was slightly affected. They contained plastoglobuli which are increased in number and become bigger. Oleosomes were more spherical in shape and their interface was getting thicker and appeared more osmiophilic. More mitochondria were also identified. Application of an interaction of SA and Tre to water-stressed plants resulted in very slight changes in chloroplasts. They kept their ellipsoidal shape as the control. They were still closely associated with the cell wall with few exceptions and showed the organized membrane system. Chloroplasts contained bigger plastoglobuli.

Tolerant wheat cultivar: Regarding control tolerant cultivar, the chloroplasts were normal and ellipsoidal in shape. Although the membrane system of grana and intergranal lamellae was not clear enough, they also contained bigger plastoglobuli and starch grains. A continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed. Conspicuous oleosomes appeared to be free in the vacuole of mesophyll cells and contained electron-dense material. These inclusions had a sharply-defined osmiophilic interface and apparently lacked a limiting membrane. Grain presoaking in SA caused the chloroplast of well-watered wheat plants to be more regular. Although the membrane system of grana and intergranal lamellae was not clear enough, chloroplasts contained plastoglobuli and starch grains. Mitochondria were also identified. Conspicuous oleosomes appeared to be free in the vacuole of mesophyll cells and contained electron-dense material. These inclusions had a sharply-defined osmiophilic interface and apparently lacked a limiting membrane.

Regarding the ultrastructure of the flag leaf of well-watered wheat plants treated with Tre, the chloroplasts were normal and ellipsoidal in shape. They were closely associated with the cell wall. Oleosomes looked smaller with the defined osmophilic interface. Chloroplasts contained many plastoglobuli and starch grains. More mitochondria were also identified. More specifically, some projections originated from the chloroplasts in the form of tails were also observed. Application of an interaction of SA and Tre to well-watered plants resulted in the chloroplasts were normal and ellipsoidal in their shape. They were closely associated with the cell wall and they also contained few plastoglobuli and many starch grains. Conspicuous oleosomes appeared to be spherical in shape and free in the vacuole of mesophyll cells. Many mitochondria were also identified. The striking feature was the presence of small spherical bodies originated from the chloroplasts.

For the flag leaf of droughted wheat plants, it was apparent that the chloroplasts began to be less or more spherical with an irregular shape. Moreover, disorganized membrane system was identified with swollen thylakoids in these chloroplasts. Although the membrane system of grana and intergranal lamellae was not clear enough, the chloroplast moved away from the cell wall and many starch grains and plastoglobuli were present. Conspicuous oleosomes appeared to be free in the vacuole of mesophyll cells and contained electron-dense material. Many mitochondria were also observed. A cytoplasmic inclusion attached to the chloroplast was rarely found. Grain presoaking in SA caused a little change in the ultrastructure of chloroplasts of water-stressed wheat plants. Chloroplasts kept their ellipsoidal shape and contained many plastoglobuli. A continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed. The membrane system was slightly affected. Conspicuous oleosomes appeared to be free in the vacuole of mesophyll cells and had a sharply-defined osmiophilic interface and apparently lacked a limiting membrane.

Regarding the ultrastructure of the flag leaf of stressed wheat plants treated with Tre, the chloroplast shape was, like in SA treatment, more regular than the stressed ones. They kept their ellipsoidal shape as the control. They were still closely associated with the cell wall with few exceptions and showed the organized membrane system. They also contained many starch grains and few plastoglobuli. Small spherical oleosomes apparently lack a limiting membrane was found. Oleosomes appeared to be more ellipsoidal in shape and no changes in their ultrastructure were pronounced. Many mitochondria were also observed. Application of an interaction of SA and Tre to water-stressed plants resulted in very slight changes in chloroplasts. They kept their ellipsoidal shape as the control. Chloroplast contained starch grains and a continuous "end-to-end" distribution of chloroplasts around the cell periphery was observed and they were still closely associated with the cell wall. They also contained many and bigger plastoglobuli. Conspicuous spherical oleosomes had a sharply-defined osmiophilic interface and apparently lacks a limiting membrane was observed.

DISCUSSION

The ill effect of drought on flag leaf growth may be attributed to photosynthetic pigment decline. Moreover, the observed reduction in leaf biomass might be due to a combination of slower growth and development as a result of osmotic stress [15]. Stressing the studied wheat plants by drought caused a marked reduction in flag leaf area and specific leaf area. Netondo et al. [16] explained the decrease in leaf area under stress to early leaf senescence and death, reduced growth rate or delayed emergence. The reduction in flag leaf area reflects that wheat plants try to cope with the water stress. Furthermore, the cumulative degree of leaf succulence also decreased under stress conditions. These results were in accord with those obtained by Welch and Rieseberg [17]. On contrary to the trend recorded for the degree of leaf succulence, the cumulative degree of leaf sclerophylly was found to increase under stress conditions. Thus, the studied wheat plants appeared to induce adaptive feature to increase its tolerance to stress conditions by increasing the degree of its leaf sclerophylly.

Data clearly showed that drought-induced a drastic reduction in Chl a+b of the sensitive wheat cultivar and a non-significant decrease in drought tolerant cultivar wheat plants. In accordance with these results, Aldesuquy et al. [18] reported that when the leaves of wheat plants started to senesce, there was a gradual decline in chlorophylls [18]. Moreover, Netondo et al. [16] explained that the decline in leaf chlorophylls induced by water stress may occur through the decrease of its synthesis and/or the increase of its degradation.

Salicylic acid and/or Tre treatments enhanced chl a chl b, and Chl a+b, production in stressed or unstressed wheat plants. In general, the interaction of SA and Tre appeared to be the most effective treatment in enhancing the pigment production in flag leaf of stressed and unstressed wheat plants. Similar results were observed by ShiraniBidabadi et al. [19]. The manipulating effect of SA and Tre may be due to the fact that SA increases leaf longevity of droughted plants by keeping their chlorophylls content, therefore delay their senescence. In relation to these results, Chandra and Bhatt [20] showed that the increasing or decreasing effect of SA on chlorophyll content of cowpea. Moreover, Alam et al. [21] reported that interaction of Tre with drought improved chl (a+b) contents in Brassica species.

Drought stress caused a significant increase in lipid peroxidation. This increase lipid peroxidation my result from malondialdehyde (MDA) increased as those obtained by Aldesuquy and Ghanem [22] and Fazeli et al. [23]. On contrary, Salicylic acid and/or Tre treatments mitigated the ill effect of drought on lipid membrane degradation by reducing malondialdehyde (MDA).

Oleosomes, globules rich in neutral lipids, were present in flag leaf of untreated and treated wheat plants where there was a noticeable increase in oleosomes volume in control and droughted plants in both cultivars. Drought led to massive increase in oleosomes volume in mesophyll cells of flag leaf of both cultivars during grain-filling. The pattern of increase was higher in Gemmieza-7 than Sahel-1. This increase in oleosomes accompanied drought stress could be attributed to the fact that water stress often induces premature senescence and subsequently decreasing the storing fats through increases its hydrolysis by lipase into fatty acids and glycerol.

In comparison with control plants or with water stressed ones, application of SA and/or Tre resulted in marked increase in oleosomes volume. Generally, the magnitude of

increases was more pronounced with the interaction of SA and Tre treatment. In connection, oleosomes may, therefore, be multipurpose: (a) short-term energy reserves to release energy for plant growth and metabolism, (b) to provide the energy needed in the formation of grains, (c) adaptation to water-stress and (d) supply fatty acids for membrane synthesis during growth.

It is evident from the current studies that a number of chloroplasts were adversely influenced by drought in both wheat cultivars with more reduction in chloroplasts number in mesophyll cells of flag leaf in both cultivars when compared with those of the control plants. The most pronounced effect was recorded for the sensitive cultivar. On the other hand, application of SA and/or Tre caused an additional increase in these values. The most pronounced effect was recorded for the interaction of SA and Tre treatment. This observation was in accordance with the results obtained by Smethurst et al. [24]. This reduction may be a strategy of protection and/or acclimation. Moreover, this reduction may be a consequence of the reduction in the leaf area of wheat plants under salt stress.

Transmission electron microscopic examination of wheat flag leaf revealed that the shape of chloroplasts in well-watered plants was comparatively regular with a well-organized membrane system. On the other hand, the chloroplasts in the leaves of droughted plants began to be less or more spherical with an irregular shape. Water-stressed cells also displayed extensive but thin peripheral cytoplasmic regions devoid of chloroplasts. While a continuous distribution of chloroplasts around the cell's periphery was observed in control plants, the chloroplasts of droughted ones appeared discrete. Clear matching with those obtained by Stoyanova et al. [25] who studied leaf ultrastructure in maize plants grown under water stress. Furthermore, similar observations under different stress forms were also reported by Sam et al. [26] and Smethurst et al. [24].

With the lower availability of CO2 to chloroplasts, ROS build up and in-turn can increase lipid peroxidation of the chloroplast, inducing damage to thylakoid membranes and pigment breakdown resulting in the reduction of photosynthesis [25].

Stressing wheat plants with drought resulted in the appearance of plastoglobuli within the chloroplasts. During senescence, the number of plastoglobules increased as the thylakoids break down. Plastoglobule numbers also increased in plants subjected to environmental conditions [27,28].

The exogenous application of SA and/or Tre retained the ultrastructure characteristics of mesophyll cells of wheat flag leaves. In this connection, some researchers stated that the damage caused by heat was alleviated to some degree by trehalose treatment, with the more gently irregular grana lamellae and more lightly damaged chloroplast envelope. They further added that Tre increases the photosynthetic ability of thylakoids, which reflects a protective role of trehalose for the thylakoid membrane. Moreover, Kang et al. [29] reported that cell ultrastructure of SA-pretreated banana seedlings showed good health than those of control seedlings.

Hormones involved directly in the control of chloroplast biogenesis and function and affect chloroplast ultrastructure, pigment production and finally the rate of carbon assimilation [30].

CONCLUSION

Last but not least, together with these obtained results found in the available literature, we, therefore, conclude that exogenous SA, Tre or their interaction was an effective way to improve short-term drought tolerance. Although a number of reports indicated the protective role of SA and/or Tre under abiotic stress conditions, to date the research conducted on the ultrastructural roles of SA and/or Tre under stressful conditions is scarce. Therefore, further studies are required to elucidate the molecular mechanism and signalling pathways underlying the role of SA and/or Tre in the stress tolerance of plants. Complete elucidation of the ultrastructural roles of SA and/or Tre with its detailed protective mechanisms would be helpful for developing stress tolerance in plants.

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