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Investigating the effects of drought stress on photosynthetic electron transport chain of two basil (*Ocimum basilicum* L.) cultivars by measuring 'Chlorophyll-a' fluorescence

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Abstract

Drought stress is one of the most important abiotic stresses that affects plant growth and development. In recent years, due to low precipitation and drought conditions, studying the effects of low water stress on plants performance have gained significance. Basil (*Ocimum basilicum* L.) belongs to Lamiaceae family which has many medicinal properties and is also being used as fresh edible vegetable. Two basil cultivars (green and purple) were grown in pots and were subjected to two water regimes of 100% (control) and 25% (drought stress) field capacity respectively. Handy PEA instrument was used to measure leaves chlorophyll fluorescence. Results showed that under drought stress the photosynthetic electron transport efficiency of green and purple basil cultivars were reduced by 57.73 and 47.11% respectively, as compared with their controls. Investigating the chlorophyll-a fluorescence also revealed that the activities of water photolysis complex, electron transport to Q_A acceptor and also the electron transport from the mid electron transport chain to photo system I were reduced under drought stress. With respect to electron transport efficiency in the middle of electron transport chain from b_6f complex to photo system I, purple basil performed better than green basil and as a result had higher tolerance to drought stress.

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Introduction

Plants are faced with multiple environmental stresses during their growth and development. Each of these stresses depending on plants sensitivity and growth stage, can cause various morphological, physiological, biochemical and molecular changes in plants and thus resulting in growth reduction and productivity (Imam and Zavarehi, 2005). Drought stress is one of the most important environmental stresses that limits plants growth (Cheong *et al.,* 2003) and considering the low rates of precipitation in recent years and high water consumption in agricultural sector in Iran, it has received considerable attention in recent years. Water shortage reduces approximately 25% of agricultural products globally and annually incurs great damages to the crop yields both directly and indirectly (Delkhosh *et al.,* 2006). Studying the effects of drought stress on plants physiological processes will be effective both in selecting varieties resistant to drought and also in efficient water supply and consumption. Water is a scarce resource in Iran that is affected by annual precipitation.

Plants growth and photosynthesis are affected by environmental conditions such as drought stress. For plants the ability to survive and to continue to do photosynthesis and grow under environmental stresses depends on their genetic potentials which are revealed by physiological and molecular responses (Oukaroum *et al.,* 2007). One of the most obvious responses of the plants to environmental stresses is caused by a drop in photosynthesis caused by impairment in the electron transport chain (Jafarinia and Shariati, 2012). Drought stress affects photosynthesis through both stomatal closure and thus preventing carbon dioxide to reach carbon fixation site in chloroplasts and by reducing cellular water potentials. The water shortage affects both roots and stems growth and may reduce the leaf area (Korkmaz *et al.,* 2007). During the last two decades, manyre searchers have identified a direct correlation between the components of photosynthesis especially of photosystem II, and changes in chlorophyll "a" fluorescence (Strasser *et al.,* 2004; Mehta *et al.,* 2010; Lazar, 2009). Therefore, photosystem II plays an important role in photosynthetic responses to environmental factors in the plants and the technique used to investigate chlorophyll fluorescence as a fast, sensitive and non-destructive method in the studies of plants ecophysiology has attracted much attention in recent years (Baker and Rosenqvist, 2004). Wang *et al.*, (2010) studied the effects of drought stress on accumulation of compatible osmolytes in wheat plant and compared control and treated plants in terms of their fluorescence parameters. Based on their results, plants which had accumulated more glycine betaine, both their photosynthetic water dissociation complex and their photosynthetic electron transport chain had higher efficiencies. Kocheva *et al.*, (2004) showed that short-term water stress had no effect on barley seedlings photosystem II efficiency, but was reduced under long-term water stress. In another study conducted on wheat seedlings under drought stress by Lu and Zhang (1999) it was shown that drought stress does not affect early photosynthetic photochemical reactions. But the excitation capacities of the photosystem II reaction centers were reduced. They also showed that while the rates of CO_2 fixation were reduced due to stomatal closure, the rates of electron transport in photosynthetic electron transport chain were also reduced. Drought stress also reduced the activity of photosynthetic water dissociation complex. Mark *et al.*, (2011) in a study on both normal and transgenic rice seedlings showed that using chlorophyll "a" fluorescence parameters, the better performance of transgenic rice seedlings under drought stress can be demonstrated. They also showed that the photosynthetic electron transport chain in transgenic rice seedlings had a better efficiency than in the normal seedlings. In another study by Oukaroum *et al.*, (2007) by measuring the parameters related to chlorophyll a fluorescence they showed that photosynthetic parameters such as quantum yield of primary reactions of photosynthesis, the amount of light harvesting antenna pigments and the rate of electron transport in the electron transport chain were reduced with the increase in the duration of water shortage.

Basil (*Ocimum basilicum* L.) is an annual plant belonging to the Lamicaceae family. This plant due to having antioxidants, vitamins A and C can prevent cellular damage (Simon*et al*., 1991). Basil has several medicinal properties such as sedative, heart tonic, mouth and gastrointestinal tract disinfectant and lactation. Concentrated basil brew and chewing its leaves are used to treat mouth fungal infection. Also smelling and chewing its leaves can be used as pain reliever (Javanmardi *et al*., 2002). The goal of this study is to examine the responses of two basil cultivars, green and purple, to drought stress by evaluating their chlorophyll-a fluorescence.

Materials and methods

Plant material

Seeds of two basil (*Ocimum basilicum*L) cultivars, green and purple, were purchased from Pakan Bazr Company in Isfahan, Iran. Seeds were germinated in 40 pots filled with one Kg soil each. The soils field capacity was determined. Half of the pots were kept at 100% Ten seeds were sown in each pot field capacity as control and the other half were kept at 25% field capacity as drought stress.

Plant growth conditions

Ten seeds were sown in each pot. After germination, three uniform seedlings in each pot were kept and the rest were discarded. When seedlings were at 4 leaves stage drought stress was imposed by keeping half of the pots at 25% field capacity. Four weeks after imposing drought stress when drought symptoms started to appear, the rates of leaves chlorophyll "a" fluorescence were determined.

Chlorophyll a fluorescence measurement

To investigate the effects of drought stress on chlorophyll "a" fluorescence in basil seedlings, the information obtained from OJIP wave emitted after exposing dark adapted leaves to light was used. Handy PEA (Hansatech, UK) was used to measure leaves fluorescence. To do this, the newest expanded leaves were chosen and attached to special clips which could provide a dark space on chosen leaves through special windows. This was done to make sure of providing and adapting the leaves surfaces to dark conditions. After that using Handy PEA, leaves were exposed to light and the preliminary information about the chlorophyll "a" fluorescence was recorded by the instrument. The recorded data were transferred to computer and using a method called OJIP-test, the preliminary fluorescence information were converted to biophysical parameters and analyzed by Biolyzer HP4 and PEA plus softwares. Excel 2003 was used to plot the graphs.

Results and discussion

The curves showing the rates of chlorophyll "a" fluorescence at different time intervals from 0.01 to 1000 milliseconds after light exposure for control and drought treated green and purple basil seedlings are shown in Fig. 1. Results shown by these curves indicate that between control and drought treated seedlings, from the stand point of chlorophyll "a" fluorescence, there are differences in some stages. It is also shown that the trends in chlorophyll "a" fluorescence changes for green and purple basil leaves are different at some points.

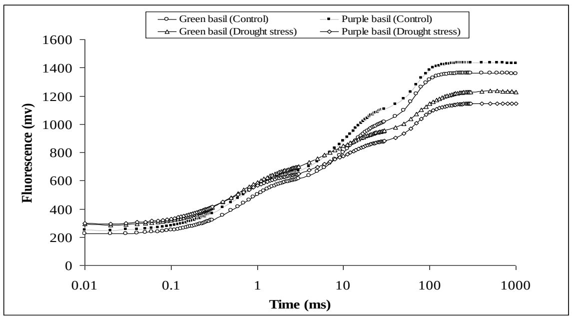

Fig. 1. Chlorophyll-a fluorescence kinetics of green and purple basil in control and in drought treated samples.

Results obtained from OJIP test showed that the parameters related to photosynthetic efficiency system from the beginning of photosystem II till the end of photosystem I (PI_{total}) have the required sensitivities to be used to show the differences between the two basil cultivars as to their responses to drought stress (Fig. 2). As the results are shown in

Fig. 2, for the green basil the PI $_{total}$ has changed from 7.92 in control to 3.35 for plants under drought stress indicating a reduction equivalent to 57.73% with respect to control. Also in purple basil the average photosynthetic efficiency index for control is 5.36 which has reduced to 2.83 under drought stress, showing a reduction equivalent to 47.11%. Results shown in Fig. 2 indicate that although drought stress has reduced the photosynthetic electron transport efficiency in the two basil cultivars, the purple basil has a higher photosynthetic electron transport efficiency under drought stress as compared to green basil.

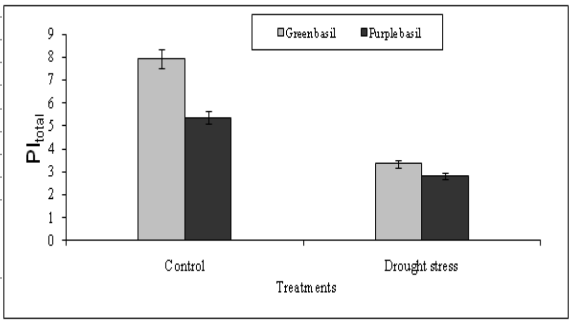

Fig. 2. Comparison of the total photosynthetic efficiency index of photosystem II from the beginning till the end of photosystem I (PI_{total}) in green and purple basil in control and under drought stress.

The results of photosynthetic efficiency index from the beginning of photosystem II till the end of photosystem I (PI $_{total}$) although as a suitable indices showed the differences in photosynthetic electron transport in these two basil cultivars under drought stress, it does not show in which segment of photosynthetic electron transport chain the purple basil has performed better which has resulted in suffering less under drought stress. In order to show the sites in photosynthetic electro transport chain in which the green and purple basils act differently in response to drought stress, Figs. 3 to 6 show step by step the differences in chlorophyll "a" fluorescence kinetics. In Fig. 3, changes in chlorophyll "a" fluorescence relative to control at time intervals between 0.01 to 1 millisecond after light exposure in both green and purple basils are shown. Our study indicates that under drought stress in chlorophyll "a" fluorescence kinetic curve, there is also a stage called K which shows the rate of chlorophyll "a" fluorescence 300 microseconds after light exposure depicted as F_k.. As shown in Fig. 3, 300 microseconds after light exposure, the relative fluorescence in green and purple basils has increased with respect to control. The increase in relative fluorescence at this stage indicates the presence of a difficulty in water dissociation complex next to photosystem II. The reason for this could be due to the separation of Mn ion from water dissociation complex preventing electron transfer from this complex to the next electro acceptor (Y_z) due to drought stress. Also, oxidative damages to the proteins making this complex could be the reason for the reduction in water dissociating complex under drought stress (Ait *et al.,* 2006). As shown in Fig. 3, although both the green and purple basils have lower water dissociating complex activity (higher relative chlorophyll "a" fluorescence) under water stress, the difference in relative chlorophyll "a" fluorescence between the two basil cultivars is not high enough at this stage. Results shown in this Fig. indicate that this section of photosynthetic electron transport chain is not the site to respond to water stress in green and purple basils and the two plants exhibit almost the same damage to their photosynthetic water dissociating complexes under water stress.

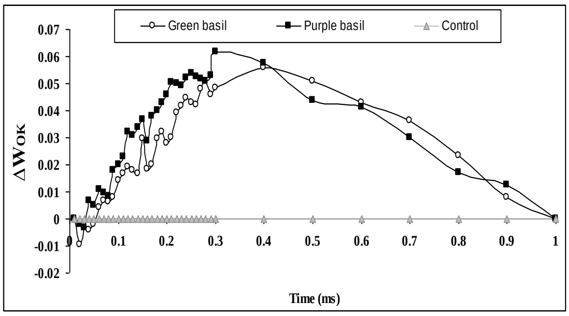

Fig. 3. The rates of chlorophyll "a" fluorescence changes in green and purple basils under drought treatment as compared to control plants at time intervals between 0.01 and 1 milli second after light exposure. dry treatment compared to the control

treatment in the intermediate moments from 01.0 to 1 millisecond after exposure.

Fig. 4 shows the rates of relative changes of chlorophyll “a” fluorescence in green and purple basils as compared to control from the beginning till 2 milliseconds after light exposure. This Fig. illustrates the trend in relative chlorophyll “a” fluorescence changes in green and purple basil plants at the O to J intervals under drought stress as compared to control. In fact, the OJ stage is a reflection of electron transport to initial electron acceptor or Q_A and along the electron transfer pathway, represents the stages before the electrons reach the primary electron acceptor, Q_A. The reason for the increase in chlorophyll “a” fluorescence at this stage could be due to the decrease in the amount of primary electron acceptor (Q_A) which takes place under drought stress. Thach and coworkers (2007) who studied the effects of drought stress on chlorophyll “a” fluorescence in *Graptophyllum* plants and Mehta and coworkers (2010) who investigated the effects of salt stress on wheat plants have shown the reduction in photosynthetic primary electron acceptor under stress conditions. Another reason for this reduction could be due to difficulty in electron transfer from chlorophylls in the reaction centers i.e. from P680 to Q_A as a result of reduction in active reaction centers and also increase in quiescent centers under stress conditions (Strasser *et al.*, 2004). Studies have shown that under stress conditions some the reaction centers lose their active states and will be converted to heat or quiescent centers (Strasser *et al.,* 2004; Mehta *et al.,* 2010; Lazar, 2009). These inactive centers will not be able to reduce Q_A and can play a role in increasing the fluorescence rates at this stage. Also, as shown in Fig. 3 there has been problem in the efficiency of water dissociating complex before electrons reaching Q_A which could be due to increase in fluorescence at this stage. However, results shown in Fig. 4 similar to the ones in Fig. 3, indicate that the differences between green and purple basils plants are not very high at this stage. Although the two plants under drought stress show an increase in the rates of chlorophyll “a” fluorescence as compared to control plants, there is no difference in their rates of fluorescence. Therefore, this stage of the curve cannot be the site of differences in the two plants behavior in response to drought stress.

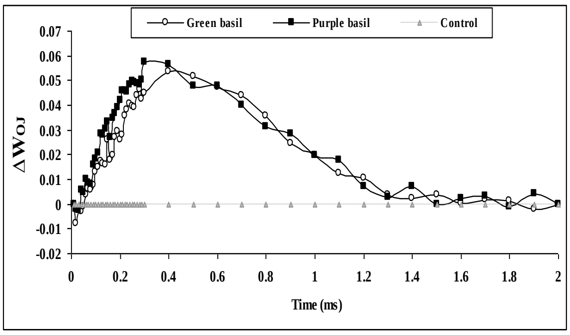

Fig. 4. The relative chlorophyll “a” fluorescence kinetic curves at O to J intervals in green and purple basil seedlings under drought stress as compared to control plants.

Fig. 5 demonstrates the amount of changes of the relative fluorescence Changes in the rates of chlorophyll “a” fluorescence in the intermediate stage between J to I for green and purple basil seedlings under drought stress as compared to control 2 to 20 milli seconds after light exposure are shown in Fig. 5. The increase in fluorescence at I stage could be due to inhibition in electron transfer from Q_A and Q_B toward the middle of electron transport chain which will result in an increase in relative fluorescence at this stage. Studies have shown that the height of the curve at J to I stage is related to the second electron acceptor i.e. Q_B (Xia and Zou, 2004 and Chen and Cheng, 2010). The increase in fluorescence at this stage shows that probably the electron carriers and the intermediate electron acceptors in the photosynthetic electron transport chain have been reduced by drought stress. Also the pattern of changes in chlorophyll “a” fluorescence curve shows that possibly the rates of electron transport from Q_A to Q_B and from $Q_B$to electron transport chain after Q_B in the photosystem II have decreased which can be one of the causes of the relative increase in fluorescence at this stage. This curve shows that under drought stress, the relative fluorescence of chlorophyll "a" in green basil seedlings at J to I stage is higher than purple basil. The difference in relative chlorophyll "a" fluorescence at this stage between the two basil plants is higher than the previous ones. The higher rates of relative chlorophyll "a" fluorescence in green basil at this electron transport segment, indicate the higher sensitivity of this plant to drought stress than the purple basil.

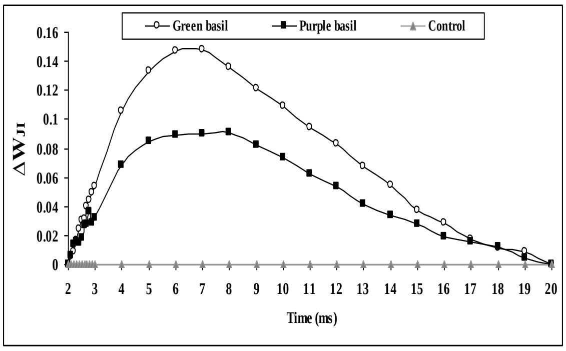

Fig. 5. The rates of changes in relative chlorophyll "a" fluorescence in green and purple basil under drought stress as compared to control at the intermediate stage from J to I of chlorophyll "a" fluorescence kinetic curve.

The rates of relative chlorophyll "a" fluorescence of green and purple basil seedlings under drought stress as compared to control plants at electron transport chain from I to P are shown in Fig. 6. This stage which is the terminal stage of OJIP, shows the rate of relative fluorescence of chlorophyll "a" from 20 milli seconds to the end of light exposure. The increase in the relative rates of chlorophyll "a" fluorescence at this time can be due to the reduced efficiency of electron transfer chain from the mid-acceptors in electron transport chain to the last electron acceptor at the photosystem I side. Another reason for the increase in the relative chlorophyll "a" fluorescence at this stage can be the decrease in the number of electron acceptors under drought stress. In fact, the reduction in the rate of electron transfer toward the photosystem I can boost the relative chlorophyll "a" fluorescence at this stage. In this Fig. 6, the differences in chlorophyll "a" fluorescence between green and purple basil are very obvious. As shown in Fig. 6, the relative chlorophyll "a" fluorescence is more in the green basil. The highest difference in the rate of chlorophyll "a" fluorescence between green and purple basil seedlings is observed at this stage. Therefore, the reason for the difference in these two plant responses to drought stress is probably due to a better performance of purple basil at this stage of photosynthetic electron transport chain and the higher sensitivity of green basil to electron transport from the middle acceptors in the electron transport chain i.e. cytochromes b 6, f and plastocyanine towards photosystem I.

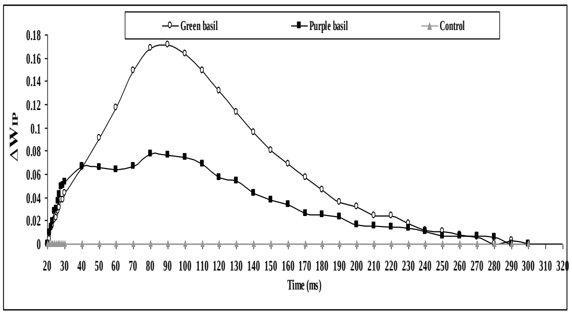

Fig. 6. Changes in relative chlorophyll "a" fluorescence in green and purple bails relative to control at intermediate stages from I to P in chlorophyll "a" fluorescence kinetic.

Conclusion

In general, the results of this study showed that the highest difference in photosynthetic electron transport activity in green and purple basil seedlings in response to drought stress has occurred at photosynthetic electron transport stages from J to P. In other words, at electron transport stage after Q_b till electrons reaching photosystem I of the photosynthetic electron chain the green basil is more sensitive to drought stress conditions and the reason for the better performance of purple basil is its higher performance at this stage. Also the results shown in Figs. 5 and 6 indicate that the highest difference in chlorophyll "a" fluorescence has been observed in approximately 90 milliseconds after light exposure between the two plants indicating the differences between the two plants in their cytochrom b_6f

complex performance in transferring electrons via plastocyanin to photosystem I.

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