Heat stress alters chlorophyll fluorescence, photosynthesis and antioxidative enzyme activities in wheat cultivars

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Heat stress becomes one of the most limiting factors to crop growth and yield. To investigate the impact of heat stress on physiological and biochemical responses in wheat cultivars, a pot experiment was conducted in the Department of Crop Botany, Bangladesh Agricultural University in Mymensingh, during November 2016 to March 2017. The experiment was laid out in a two factorial CRD design with three replications. The factors were (i) four wheat cultivars (BARI GOM-25, BARI GOM-26, BARI GOM-27 and BARI GOM-28) and (ii) heat stress (control and heat). The heat stress (38/25 °C day/night) was imposed to the wheat plants for three days at early grain filling stage in a climate chamber. The control plants were remained in the field at around 20−25 °C. Heat stress declined the leaf greenness (SPAD), the maximum photochemical efficiency of PSII ($F_{v}/F_{m}$), photosynthesis rate (A) and grain weight in all the cultivars in comparison to control. The leaf greenness (SPAD) and $F_{v}/F_{m}$ were declined with the progress of heat stress treatment in all four cultivars. In both cases, lowest reduction was observed in BARI GOM-28 whereas, the highest reduction in SPAD and $F_{v}/F_{m}$ were observed in BARI GOM-26 and BARI GOM-27, respectively. The percent reductions in photosynthesis and grain weight were significantly higher in BARI GOM-28 in comparison to other cultivars. Increased activities of catalase, peroxidase and ascorbate peroxidase enzymes were observed under heat stress in all four cultivars. Therefore, it may be concluded that the cultivar BARI GOM-28 showed more tolerance to heat stress than the other cultivars based on the measured physiological and biochemical traits.

**Keywords:** Heat stress, wheat, chlorophyll fluorescence, gas exchange, antioxidants

1 Introduction

An increase in global surface mean temperature between 1.8 and 5.8 °C is predicted by several climate models at the end of the 21st century (IPCC, 2007). In future climate scenarios, greater fluctuations in temperature and increased frequency of heat waves or hot days will more frequent (Pittock, 2003). In Bangladesh, the temperature is increased by 0.035 °C per year over the past two decades (Poulton and Rawson, 2011). Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world and next to rice in Bangladesh. Wheat is sensitive to high temperature at different phenological stages of development and it is reported that the reproductive phase is more detrimental to heat stress than the vegetative phase...
because of the direct impact of heat stress on grain number and dry weight (Wollenweber et al., 2003). To cope with the drastic effect of high temperature stress, plants alter their growth patterns and physiological processes (Duan et al., 2007).

Plants adapt to heat stress at different growth and developmental stages by changing a series of morphological, physiological and biochemical changes (Wahid et al., 2007). The grain filling period was reduced in wheat at a temperature of above 30 °C affecting final grain weight due to the down-regulation of photosynthesis and inhibition of starch synthesis in the endosperm (Zhao et al., 2008). A temperature range of 15−18 °C is required for the optimum kernel weight in wheat; heat stress reduces the grain filling duration and this reduction affected the accumulation of assimilates into the grain (Stone and Nicolas, 1996). Wheat plants often experience heat stress under late planting conditions in Bangladesh and therefore, research in relation to the development of heat tolerant cultivars should be emphasized. High temperature accelerates the life cycle in wheat which reduces the less accumulation of assimilates throughout the growth period and thus, reduces the whole plant biomass (Nahar et al., 2010). Plants exposed to high temperature usually exhibit reduced chlorophyll biosynthesis (Dutta et al., 2009). Photosynthesis is the most heat-sensitive physiological process and a partial or even complete reduction in the rate of photosynthesis due to heat stress can be determined (Fahad et al., 2017).

The photo-system II (PSII), carbon fixation by Rubisco and ATP-generating system are the primary targets of high temperature stress (Wahid et al., 2007). An increased photosynthetic photon flux density (PPFD) and high temperature in the leaf inhibits the PSII thermo-tolerance adjustment (Crafts-Brandner, 2002). Heat stress induces imbalanced flow of electrons to the PSII acceptor site which may result in serious damage to oxygen evolving complex (Ronde et al., 2004). The generation of reactive oxygen species (ROS) due to photo-inhibition and moderately high temperature restrict the repair of damaged PSII (Wahid et al., 2007). Plants maintain enzymatic and non-enzymatic antioxidative defense systems to scavenge the ROS or cope with the oxidative stress. The most effective mechanism of defense is usually enzymatic defense (Møller et al., 2007). Enzymes which play active role in the system are super oxide dismutase (SOD), peroxidase (POD), glutathion reductase (GR) and catalase (CAT) (Farooq et al., 2011). For the adaption of new crop varieties to the changing climatic conditions, understanding the mechanisms of high temperature tolerance to crop is needed which will help in developing heat tolerant wheat cultivars. Immediate attention has been given to develop heat tolerant wheat cultivars by combining different approaches. Physiological and biochemical responses of wheat to heat stress have been found effective against determining cultivars resistance or susceptibility. Plant photosynthesis is the key physiological process and it is directly related to the plant biomass production. The chlorophyll fluorescence and gas exchange measurements are the important early stress detectors and can provide valuable information in order to determine genotypic resistance to heat stress.

In this study, four wheat cultivars were used from wheat research institute, Bangladesh Agricultural Research Institute (BARI) of Bangladesh. All the cultivars were moderately heat tolerant but no information is available regarding the tolerance level/rank among the four cultivars. The present study was therefore, undertaken to investigate the effects of short term heat stress on photosystem II efficiency, photosynthesis and antioxidative enzyme activities in four wheat cultivars at grain filling stage and to evaluate their genetic potential of heat tolerance based on physio-chemical descriptors.

2 Materials and Methods

2.1 Plant materials and growth conditions

The experiment was conducted in the net house, climate chamber and Plant Physiology Laboratory in the Department of Crop Botany, Bangladesh Agricultural University in Mymensingh during November 2016 to March 2017. Seeds of four wheat cultivars viz. BARI GOM-25, BARI GOM-26, BARI GOM-27 and BARI GOM-28 were collected from Bangladesh Agricultural Research Institute (BARI), Dinajpur. The seeds of all four wheat cultivars were sown on 21 November 2016 in the pot (4 L) and kept in the net house until heat stress treatment. The pot soil was fertilized with urea, triple super phosphate (TSP), muriate of potash (MoP) and gypsum at the rate of 1 g, 0.5 g, 0.2 g and 0.3 g pot−1, respectively. The whole amount of TSP, MoP and gypsum and one-third of urea were applied 20 days after seed sowing (DAS). The rest amount of urea was applied in two equal splits at 40 and 60 DAS. Care was taken to protect the seeds and seedlings from birds up to 20 DAS. Various intercultural operations were done in order to ensure and maintain the normal growth of the crop. Weeding operation was done as per experimental treatments. The plants were irrigated two times in a week.

The experiment was laid out in a two factorial completely randomized design (CRD) with three replications. Two heat treatments were implied viz. control (20−25 °C in net house) and heat (38/25 °C day/night in a climate chamber). Total number of plastic pots required in the experiment was 24. Each pot containing a single plant was used as single replicate. The short term heat stress was imposed to the plants of all cultivars during the early grain filling
stage i.e. 80 DAS (Takahashi et al., 1993). Three plants of each cultivar from the net house were transferred to the climate chamber (Wisecube, Model WTH-E155, WITEG, Germany) maintaining 38/25 °C day/night temperature and kept inside the climate chamber for three days. The photosynthetic photon flux density (PPFD) inside the climate chamber was 200 µmol m$^{-2}$ s$^{-1}$. The relative humidity (RH) was set to 70/50% day/night. The plants were irrigated during the heat stress treatment to avoid water limiting conditions to the plants. The heat stress was implied for three days and then the plants were transferred back to the net house for further observations.

2.2 Data collection

The leaf greenness (SPAD) of the plants grown in control and heat stress conditions was measured on day 0, 1, 2 and 3 of heat stress treatment. A SPAD meter (SPAD-502, Konica Minolta, Japan) was used to measure the leaf greenness. The SPAD values were taken at three different parts of the flag leaf (base, middle and top) and mean value was used for data analysis. The maximum photochemical efficiency of photosystem II ($F_v/F_m$) was measured in the flag leaf of three plants of each cultivar of different treatments on day 0, 1, 2 and 3 of heat stress period. The chlorophyll fluorescence measurement ($F_v/F_m$) was done by a fluorometer (Pocket PEA, Hansatech, Norfolk, UK). The leaves were dark adapted for 30 min using the leaf clips and then the $F_v/F_m$ was measured using the Pocket PEA by applying a saturated PPFD of 3500 µmol m$^{-2}$ s$^{-1}$ (Haque et al., 2014).

The gas exchange parameters such as rate of photosynthesis ($A$), stomatal conductance ($g_s$) and transpiration rate ($E$) were measured by an LCI-SD photosynthetic system (ADC Bio Scientific Ltd, Hertfordshire, UK). The measurements were taken in the flag leaves on final day of heat treatment. The $A$, $g_s$ and $E$ were measured at a PPFD of 200 µmol m$^{-2}$ s$^{-1}$ at both heat stress (38 °C) and control (25 °C) plants. The ambient CO$_2$ level (400 ppm) was maintained during the measurements.

The leaf samples for antioxidative enzyme analysis were collected immediately after three days of heat stress and stored in a freezer (−28 °C) until analysis. The samples for the control plants were collected at the same time. Approximately 0.5 g of fresh leaf sample was taken for extraction and grinded with 3 mL KH$_2$PO$_4$ buffer using mortar and pestle. Then the extract was taken into a 1.5 mL Eppendorf tube and centrifuged for 10 min at 12000 rpm in a centrifuge. The samples were kept in ice box during the spectrophotometric analysis. The catalase (CAT) activity was determined according to Aebi (1984) and the ascorbate peroxidase (APX) was assayed using the method described by Nakano and Asada (1981). The guaiacol peroxidase (PX) activity was measured according to Mika (2003).

2.3 Data analysis

The statistical software ‘R’ (R Core Team, 2013) was used for ANOVA in order to evaluate the variations of parameters among the cultivars and between the treatments. The DMRT test was done by the Tukey’s post-hoc test of R program.

3 Results

3.1 Leaf greenness

Heat stress significantly affected the physiological processes, antioxidative enzyme activities and grain weight of four wheat cultivars (Table 1). The cultivars showed variability in response to heat stress based on different physio-chemical attributes (Table 1). The leaf greenness (SPAD) was significantly reduced in the plants of four wheat cultivars grown under heat stress treatment (Table 1; Fig. 1A).

Figure 1. The leaf greenness (SPAD values) and maximum photochemical efficiency of PSII ($F_v/F_m$) in four wheat cultivars at different days of heat stress treatment. The symbol D0 denotes the day before stress imposition (control) whereas the symbols D1, D2 and D3 denote day 1, 2 and 3 of stress period, respectively. Vertical bars are SEM (n = 3).
Table 1. Analysis of Variance (ANOVA) for various measured traits of wheat cultivars grown in different stress treatments

| Parameters                      | Sources of variation | % Reduction |
|---------------------------------|----------------------|-------------|
|                                 | Cultivars (C) | Stress treatments (S) | C × S |
| Leaf greenness (SPAD)           | *           | ***        | NS     | *     |
| \(F_v/F_m\)                     | NS         | ***        | NS     | NS    |
| Photosynthesis rate (\(A\))    | ***        | ***        | NS     | **    |
| Stomatal Conductance (\(g_s\)) | ***        | ***        | NS     | **    |
| Transpiration rate (\(E\))     | *          | ***        | NS     | ***   |
| 100 grain weight                | NS         | **         | NS     | ***   |
| Ascorbate peroxidase (APX)      | **         | ****       | *      | *     |
| Catalase (CAT)                  | ***        | *          | NS     | NS    |
| Peroxidase (PX)                 | ***        | ***        | ***    | **    |

\(F_v/F_m\) = Maximum photochemical efficiency of PSII; * = Significant at 5% level, ** = Significant at 1% level, *** = Significant at 0.1% level, and NS = Non-Significant

The leaf greenness was decreased with the increase of heat stress period in all four wheat cultivars (Fig. 1A). A significant variation in leaf greenness among the cultivars was observed (Table 1). The reduction in SPAD values was maximum at first day of heat stress treatment and the cultivar BARI GOM-28 showed the lowest reduction in SPAD values in comparison to the other cultivars (Fig. 1A and Fig. 2). Percent reduction in SPAD values due to heat stress as compared to control was higher in BARI GOM-26 and lower in BARI GOM-27 which is followed by BARI GOM-28 (Fig. 2).

3.2 Chlorophyll florescence

The maximum photochemical efficiency of PSII (\(F_v/F_m\)) was significantly declined by the stress treatments compared to control in four wheat cultivars (Table 1; Fig. 1B). Similar to SPAD values the \(F_v/F_m\) also declined with the progress of heat stress treatment (Fig. 1B). Although the variations in \(F_v/F_m\) among the wheat cultivars were not statistically significant (Table 1) but the maximum reduction in \(F_v/F_m\) was observed in BARI GOM-27 at day three of heat treatment (Fig. 1B). The cultivar BARI GOM-28 showed greater heat tolerance by showing lower percent reduction in \(F_v/F_m\) (Fig. 2).

3.3 Gas exchange parameters

The gas exchange parameters were significantly affected by heat stress in comparison to control in all four wheat cultivars (Table 1; Fig. 3). A significant variation among the cultivars in relation to gas exchange parameters was also observed (Table 1; Figs. 3 and 4). The highest percent reduction over control in photosynthesis rate (\(A\)) was found in BARI GOM-25 followed by BARI GOM-26 and BARI GOM-27 whereas the lowest reduction in \(A\) was observed in BARI GOM-28 (Fig. 3). The stomatal conductance (\(g_s\)) was reduced due to heat stress and varied significantly among the cultivars (Fig. 3B and Fig. 4). The highest and lowest percent reduction over control in \(g_s\) was observed in BARI GOM-25 and BARI GOM-28, respectively (Fig. 4). The transpiration rate (\(E\)) was also significantly declined under heat stress in all cultivars compared to control (Fig. 3). The highest percent reduction in \(E\) was found in BARI GOM-25 and lowest in BARI GOM-27 (Fig. 4).
Figure 3. The photosynthesis rate ($A$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance ($gs$, mol m$^{-2}$ s$^{-1}$) and transpiration rate ($E$, mol m$^{-2}$ s$^{-1}$) in four wheat cultivars grown in two stress treatments (control and heat). Vertical bars are SEM ($n = 3$).

3.4 Antioxidative enzyme activities

The antioxidative enzyme activities in all four wheat cultivars were significantly increased under heat stress in comparison to control (Table 1; Fig. 5). The wheat cultivars also showed variability in relation to antioxidative enzymes activities (Table 1 and Figs. 5 and 6). The maximum catalase (CAT) activity was observed in BARI GOM-28 whereas the CAT activities were lowest in BARI GOM-25 followed by BARI GOM-26. The ascorbate peroxidase (APX) and peroxidase (PX) activities were greatly increased in BARI GOM-27 and BARI GOM-28 under heat stress in comparison to the other two cultivars (Fig. 6). The cultivars BARI GOM-27 and BARI GOM-28 showed greater heat tolerance showing increased activities of APX and PX under heat stress condition (Fig. 6).

Figure 4. The percent reduction in photosynthesis rate ($A$), stomatal conductance ($gs$) and transpiration rate ($E$) under heat stress as compared to control in four wheat cultivars. Vertical bars are SEM ($n = 3$). Means with different letters within each parameter are significantly different at 5% level of significance.

3.5 Grain yield

The grain weight varied significantly between the treatments and among the cultivars (Table 1; Fig. 7). The 100 grain weight was decreased significantly in all four cultivars due to heat stress. The four cultivars varied significantly in terms of 100 grain weight under heat stress condition. The maximum grain weight was observed in BARI GOM-28 whereas, the lowest grain weight was observed in BARI GOM-26 due to heat stress (Fig. 7). The percent reduction in 100 grain weight was lowest in BARI GOM-28 whereas the highest reduction in grain weight was observed in BARI GOM-26 (Fig. 7).

4 Discussion

The present study was conducted to evaluate the performance of four wheat cultivars under short term heat stress with a view to select heat-tolerant wheat cultivar. Results revealed that all the measured parameters such as leaf greenness (SPAD), maximum photochemical efficiency of PSII ($F_v/F_m$), photosynthesis rate, antioxidant activities and grain weight were affected by heat stress. The reduction in leaf
greenness indicates the decline of chlorophyll content in the leaves.

The biosynthesis of chlorophyll in leaves usually affected by high temperature (Dutta et al., 2009). The leaf chlorophyll content in plants declines by high temperature either due to the reduced biosynthesis of chlorophyll or due to higher degradation of chlorophyll. Heat stress deactivated the various enzymes that are responsible for chlorophyll biosynthesis (Dutta et al., 2009). Karim et al. (2000) measured the thylakoid membrane stability, chlorophyll content and plant growth traits for the assessment of heat tolerance in wheat. They observed a drastic heat-induced damage in thylakoid membrane stability and chlorophyll loss in winter wheat. The heat-induced chlorophyll degradation is faster in developed leaves than the developing leaves (Karim et al., 1999). Although the cultivars did not vary significantly in relation to \( F_0 / F_m \) but the lowest reduction was observed in BARI GOM-28 in comparison to other cultivars. High temperature greatly affects the thermo-tolerance adjustment of the PSII and the activity of PSII can be partially or fully inhibited by the high temperature stress (Crafts-Brandner, 2002; Camejo et al., 2005). Sharkova (2001) reported that high temperature stress damaged the different components of PSII in wheat and barley. Haque et al. (2014) studied the heat tolerance and post-stress recovery in four wheat cultivars using chlorophyll fluorescence and they found a profound reduction of \( F_0 / F_m \) in wheat cultivars due to heat stress. Zhao et al. (2008) showed that the moderately elevated temperature (30–37.5 °C) had no effect but severely elevated temperature (higher than 37.5 °C) had significantly reduced the \( F_0 / F_m \) and photochemical quenching (\( q_p \)) in wheat. Heat stress significantly declined the rate of photosynthesis in all four wheat cultivars and the cultivars varied significantly in photosynthetic rate in response to heat stress. These results are in agreement with many earlier studies (Ashraf and Harris, 2013; Feng et al., 2013; Mathur et al., 2014).

Several studies showed that the rate of photosynthesis is a key physiological phenomena affected by heat stress and the reduction in leaf photosynthesis are mainly due to the reduction in leaf area, improper functioning of photosynthetic machinery and accelerated leaf senescence (Wahid et al., 2007; Farooq et al., 2011). Limited \( \text{CO}_2 \) availability due to the stomatal closure under heat stress allows the plant more susceptible to photo-damage (Lawlor and Cornic, 2002). The decreased rate of photosynthesis in this study could be also due to the disturbance of photosynthetic pigment production, declining activity of PSII and impairment of the regeneration capacity of RuBP. Mishra et al. (1993) reported significant reduction in photosynthetic apparatus of wheat due to high light and temperature stress. The chlorophyll fluorescence and photosynthetic rate could be used as fast and reliable tools for the screening of heat-tolerant wheat cultivars.

Heat stress significantly decreased the grain yield in all four wheat cultivars in this study. Earlier researches suggested that the heat stress in reproductive phases especially during post-anthesis stage are harmful for final grain weight and grain number (Wardlaw and Wrigley, 1994). These reduction in grain weight and number may be related with the problems during meiosis process and the growth of the ovaries. A number of studies have been suggested that the heat-

Figure 5. Catalase (CAT, \( \mu \text{mol min}^{-1} \text{ g}^{-1} \text{ FW} \)), ascorbate peroxidase (APX, \( \mu \text{mol min}^{-1} \text{ g}^{-1} \text{ FW} \)) and peroxidase (PX, \( \mu \text{mol min}^{-1} \text{ g}^{-1} \text{ FW} \)) activities in four wheat cultivars grown in two stress treatments (control and heat). Vertical bars are SEM (n = 3).

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Figure 6. The percent increase in catalase (CAT), peroxidase (PX) and ascorbate peroxidase (APX) activity under heat stress as compared to the respective control in four wheat cultivars. Vertical bars are SEM (n = 3). Means with different letters within each parameter are significantly different at 5% level of significance.

Figure 7. 100 Grain weight in four wheat cultivars grown in two stress treatments (control and heat). Line graph shows the percent reduction (Y axis) in 100 grain weight over control in four wheat cultivars. Vertical bars are SEM (n = 3). Cultivar means of percent reduction with different letters within each parameter are significantly different at 5% level of significance.

induced reduction in grain weight could be considered as a selection criterion of heat stress tolerance in wheat (Sharma et al., 2008; Sareen et al., 2012; Bennani et al., 2016). All the four wheat cultivars exhibited an increased activity of antioxidative enzymes indicating an enhanced production of reactive oxygen species (ROS) under heat stress. Plants exposed to heat stress primarily cause oxidative stress to plants by the formation of ROS which damages lipids and proteins causing disturb to proper cell functioning (Balla et al., 2007). Among the antioxidative defense systems in plants, the enzymatic defense against the ROS is most effective (Møller et al., 2007).

The anti-oxidative enzymes such as catalase (CAT), peroxidase (PX) and ascorbate peroxidase (APX) minimize the oxidative damage directly by scavenging the ROS produced by heat stress (Anjum et al., 2011). The synthesis of higher levels of antioxidative is a good strategy of plants under abiotic stress conditions to counter with the detrimental effects of ROS (Sharma and Dubey, 2005). In this study, the enhanced ROS production due to heat stress might be greatly scavenged by the increased activities of CAT and PX in BARI GOM-28 showing better tolerance to heat stress in comparison to other cultivars.

5 Conclusions

Heat stress at early grain filling stage greatly affected the chlorophyll content, maximum photochemical efficiency of PSII, rate of photosynthesis and grain weight in four wheat cultivars. The catalase and peroxidase enzyme activities were significantly increased in response to heat stress in all wheat cultivars. The study exhibited significant genotypic variation in heat tolerance and BARI GOM-28 showed better tolerance to heat stress than the other cultivars based on the measured physiological and biochemical traits.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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