MORPHO-PHYSIOLOGICAL ANALYSIS OF DROUGHT AND HEAT TOLERANCE IN WHEAT

A

Thesis

By

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Student No. 1305033

MASTER OF SCIENCE (MS)
IN
CROP PHYSIOLOGY AND ECOLOGY

DEPARTMENT OF CROP PHYSIOLOGY AND ECOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY DINAJPUR

JUNE 2015

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DEDICATED

TO

MY LATE FATHER

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July 2015 *The author*

ABSTRACT

The present investigation was done to study the eco-physiological basis of heat and drought tolerance of wheat. It was conducted in a split plot design with three replications. The treatment factors were A. Main plot treatment: Four growing conditions viz. i. Well water growing condition (Giving three irrigations), ii. Water limited drought condition (Giving one irrigation at 20 DAS for seedling establishment). iii. Normal growing condition (sowing novmber 30, 2014) iv. Late growing heat stress growing (sowing December 30, 2014), and B. Sub plot treatment: Two wheat varieties: BARI gom-28 and Kanchan. From the results it was found that the days required to different phenological stages were significantly varied between two wheat varieties at different growing conditions. The requirements of days were decreased for both the varieties to attaining different growth stages at stressed (heat and drought either combined or single) condition compared to stress free (normal sowing with well watered) condition. To attain crown root initiation, tillering, heading, anthesis, physiological maturity and harvesting maturity stages, the maximum number of days required for BARI Gom-28, where as the minimum number of days required for Kanchan at both in stressed and stress free condition. At stressed condition both the varieties showed higher canopy temperature depression (CTD) compared to stressed free condition. Under highly stressed (late sowing heat stress with drought) conditions BARI Gom-28 showed the highest canopy temperature depression (6.1 ^{0}C), where as variety Kanchan had the lowest CTD (4.5 ^{0}C) at stress free condition. The chlorophyll content was decreased by both the wheat varieties at stressed (both heat and drought) condition compared to stress free conditions. At stress free condition, BARI Gom-28 had the maximum flag leaf chlorophyll content (2.8 mg/g fresh weight) and the lowest chlorophyll content was found in Kanchan at late sowing with drought condition (1.4 mg/g fresh weight). The proline content of all wheat varieties were increased at all the stressed conditions compared to stress free condition. BARI Gom-28 gave the significantly highest amount of proline (2.30 µmole/g fresh weight) at late sowing heat stress with drought, where as variety Kanchan produced the lowest proline content (1.65 µmole/g fresh weight) at normal sowing well watered condition. The stressed conditions reduced the plant height, spike length, spike number, spike plant^{-1}, floret sipkelet^{-1}, grains sipkelet^{-1} and grains sipke^{-1} of both the varieties compared to stress free conditions. Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher value of these characters compared to Kanchan. The floret sterility of both wheat

varieties were increased in different magnitude at all the stressed conditions compared to stress free condition. BARI Gom-28 gave the significantly lowest floret sterility (20.0%) at stress free condition. Under highly stressed condition (late sowing heat stress with drought) variety Kanchan showed the highest floret sterility (33.33%) compared to BARI Gom-28. It was observed that the stressed conditions reduced the grain yield of both wheat varieties compared to stress free conditions. Under stress free condition BARI Gom-28 attained significantly higher grain yield (4.2 t ha^{-1}) compared to Kanchan (3.6 t ha^{-1}). At highly stressed condition BARI Gom-28 also had the higher grain yield (2.6 t ha^{-1}) than the Kanchan (2.0 t ha^{-1}). However, considering all stress combination BARI Gom-28 attained the highest grain yield (4.2 t ha^{-1}) at well watered normal sowing condition whereas, variety Kanchan showed the lowest plant yield (2.0 t ha^{-1}) at heat stress drought condition. BARI Gom-28 showed the significantly highest biological yield (9.5 t ha^{-1}) at stress free condition compared to other growing conditions. Whereas, Kanchan produced the lowest biological yield (5.5 t ha^{-1}) at highly stressed condition .BARI Gom-28 attained the highest harvest index compared to Kanchan at all the growing conditions. Due to lower stress susceptibility BARI Gom-28 was regarded as stress (both heat and drought) tolerant (SSI<1) and Kanchan was considered as stress sensitive due to higher SSI value (SSI>1)

ABBREVIATION AND ACRONYMS

%	Percentage
@	At the rate of
AEZ	Agro Ecological Zone
BARC	Bangladesh Agricultural Research Council
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BRRI	Bangladesh Rice Research Institute
cm	Centimeter
CV%	Co-efficient of Variance
DAE	Department of Agricultural Extension
DAT	Days after transplanting
DSI	Drought susceptible index
DMRT	Duncan's Multiple Range Test
et al.	And others
FAO	Food and Agriculture Organization
g	Gram
HSTU	Hajee Mohammad Danesh Science and Technology University
HYV	High Yielding Varieties
IAA	Indole acetic acid

IRRI	International Rice Research Institute
J.	Journal
K	Potassium
kg	Kilogram
m^{-2}	Per square meter
mg	Milligram
MoP	Muriate of potash
N	Nitrogen
^oC	Degree Celsius
P	Phosphorous
P_2O_5	Phosphate
pH	Potential of H^+ concentration
ppm	Parts per million
S	Sulphur
t ha^{-1}	Ton (s) per hectare
TSP	Triple super phosphate
UNDP	United Nations Development Programme
viz.	Such as

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wheat varieties

Chapter I

INTRODUCTION

Among the environmental stresses drought and heat stresses are seemed to be most adverse factors for wheat productivity (Boyer 1982). Wheat (*Triticum aestivum* L.) is a thermosensitive cool season crop. But globally, about seven million hectares of wheat is affected by heat stress through the life cycle and about 40% crop faces terminal heat stress (Ruwali and Bhaswar 1998). On the other hand, wheat is produced under diverse environmental conditions ranging from well irrigated to water stress situations and wheat yield are reduced 50-90% of their irrigated potential by drought on at least 60 Mha in developing world (Skomand *et al*. 2001).

Drought induces decrease in leaf water content and increased stomatal closure, it decreases the supply of CO_2 to mesophyll tissue and the rate of photosynthesis is decreased (Araus *et al.* 2002). Supra-optimal temperature during grain filling in the field associated with acceleration of phasic development (Shpilar and Blum 1991), accelerated senescence (Renolds *et al.* 1998), reduction of photosynthesis (Al-Khatib and Paulsen 1999). The net effect of heat stress at the reproductive stage is to lower the kernel weight due to reduced grain filling period, grain filling rate or combined effect of both (Tashiro and Wardlaw 1989). These stresses at reproductive stage are more harmful to plant process than any other growth stage. At anthesis markedly it reduces photosynthesis, reproductive development and finally grain yield (Araus *et al.* 2002)..

Bangladesh furnishes a good example of drought and heat stresses where wheat is grown in under hot and humid climate and in a short winter. Wheat is grown in a short winter season (usually December to March) which is dry as well as inadequate soil moisture. The national average of wheat yield is about 2.13 t/ha in Bangladesh (BBS 2010) which about 50% lower than the potential yield of some released varieties. The yield gap between the potential and the national average is associated with many limiting factors of which high temperature and drought stresses are the vital factors. About 40% of the total wheat area in the country is irrigated and rest of the area is cultivated under rainfed condition (BBS 2010). Therefore, about 60% of area is planted lately due to following previous rice crop (Badruddin *et al.* 1994). Due to late planting the crop frequently encounters high temperature stress during the reproductive stage of growth causing significant yield reduction. This problem will be further increased due to climate change. Due to raise in world temperature soil losses its moisture holding capacity as a result drought effect is accelerated. Thus the best option for yield improvement and yield stability of wheat drought condition is to develop drought tolerant wheat varieties.

A physiological approach would be the most attractive way to develop new varieties rapidly but it involves a deeper understanding of the yield determining process. The use of physiological tolerance along with morphological traits has been shown to be applicable and their relationship with stress tolerance indices are considered strong enough to be exploited as a selection tool in the breeding of stress tolerance cultivars. With considering above facts the proposed research will be undertaken to find out the eco-physiological basis of drought and heat tolerance of wheat which would be the guideline to identify /screening drought and heat tolerance wheat genotypes in a breeding program.

The yield is a complex process. It is a function of interactions among the factors responsible for wheat growth and depends on the environmental factors for expression of yield potential. In Bangladesh wheat improvement program for water and heat stress environment has taken separately did not take account the growth and physiological sensitivity in relation to drought and heat tolerance. But Heat and drought stress are simultaneously present on wheat crop in Bangladesh. There is very little information about the combined effect of drought and heat stress on wheat physiology especially on photosynthesis and yield potential of wheat genotypes. So, the challenge is to introduce both heat and drought tolerance characters into same genotype. The selection of drought and heat tolerant genotypes by screening advance lines through a standard procedure and to evaluate their performance through physiological and yield attributes at water limited drought condition in Bangladesh environments would be an important step to develop heat tolerant and drought variety(s) and also achieving high yield potential of wheat under drought and heat stress conditions.

Therefore, the present investigation was done with the following objectives:

i. To Screen the wheat genotypes for different morpho-physiological traits in relation to drought and heat tolerance.

iii. To find out the relationship of yield with these traits.

iv. To evaluate the performance of wheat genotypes on the basis of selected traits at field condition and identify the relative drought and heat tolerant genotypes.

Chapter II

REVIEW OF LITERATURE

Water and heat stress on wheat has a profound effect on growth and yield of the crop. In this regards many researches throughout the world have several observations on the effect of water deficit stress on wheat. But there is very little information on it. However, some of the information on the morpho-physiological characters affected by water stress is given below.

Improvement of drought and heat tolerance in crop plants requires identification of relevant resistance mechanisms and development of a suitable methodology for their measurement in large breeding populations. Drought and heat tolerance are manifested by the relative ability to the plant tissue to sustain a smaller reduction in physiological or metabolic activity as its water potentials decreases (Blum and Ebercon 1981).

Bewley (1979) reported the critical role of cell membrane stability under conditions of moisture stress as a major component of drought tolerance. The rate of injury of cell membranes by drought may be estimated through measure of electrolyte leakage from cells. The rate of injury of cell membranes is commonly used as a measure of tolerance to additional plant stresses.

Arabinda and Dolgodvorov (1994) studied the different stages of growth of two newly developed high yielding spring wheat varieties, namely Moskovskaya 35 and Minovoskya spring. They divided the growth cycle into three major developmental phases viz., vegetative, panicle development and grain filling and ripening phase. They observed that the vegetative phase started with the emergence of seedlings upto the point of panicle initiation and needed 36, 36, and 39 days after sowing in Moskovskaya 35 and 37, 36, and 38 for Minovoskya spring in 1983, 1984 and 1985 respectively. The panicle developmental phase included the heading and flowering stages and required 56, 60, 67 days after sowing for Moskovskaya 35 and 58, 60 and 68 days after sowing for Minovoskya spring in the first, second and third year year respectively. The third growth phase began with the fertilization of florets in the panicle and continued upto maturity of the plant and required 107, 108 and 112 days after sowing for Moskovskaya 35 and 108, 110 and 113 for Minovoskya spring in 1983, 1984 and 1985 respectively.

Sikder (2009) examined phenology of heat tolerant and heat sensitive wheat cultivar under normal and late sowing heat stress condition. He observed that the number of days for attaining different phenological (root initiation, tillering, booting, heading, anthesis, grain filling and maturity) stages were higher for normal sowing condition compared to late sowing condition. He also reported that the heat tolerant cultivars required higher number of days for attaining different phenological stages than the heat sensitive cultivars in both the sowing times.

Renolds *et al. (*1994) reported that genetic variability existed for canopy temperature depression (CTD) among the wheat germplasms under heat stress condition while CTD correlated significantly with yield for all the stages of development. Canopy temperature depression was correlated better with yield between 1200 and 1600 hours than it was in the morning or late afternoon

Renolds *et at.* (1998) mentioned that potential to keep canopy cool was one of the important traits of high temperature tolerant wheat genotypes. This was reflected by canopy temperature depression (CTD), which was expressed as difference between the ambient temperature and canopy temperature. This trait showed high genetic correlation with yield and high values of proportion of direct response to selection indicated that the trait is heritable and therefore amenable to early generation selection. Further, this trait could be measured almost instantaneously by using special designed infrared thermometers on score of plants in a small breeding plot.

Rahman et al. (2000) to study the effect of irrigation and nitrogen fertilization on the leaf photosynthesis (LPn), Crop growth rate (CGR), leaf area index (LAI) and dry matter (DM) production of wheat and reported that both irrigation and N application created a significant impact on LPn, LAI, CGR and DM production of wheat. Irrigation scheduling which included irrigation at grain filling stage (80 DAS) coupled with N application up to 120 kg/ ha significantly increased LPn, LAI, CGR and DM production.

Blum *et al*. (2001) concluded that cell membrane thermostability is a fair index of genetic variation for heat tolerance and has a reasonable relationship to plant performance under heat stress environment and is, therefore, considered as a possible selection criterion for heat tolerance.

Abdorrahmani et al. (2005) found that drought stress reduced dry matter production, crop growth rate and relative growth rate.

Blum *et al.* (1994) observed leaf senescence of two wheat cultivars, (V5 and V2183) under optimum and heat stress conditions. Under heat stress, leaf senescence was accelerated and V5 lost more chlorophyll than V2183. Therefore, in terms of leaf chlorophyll content, V5 inherently tended to senescence faster than V2183, and this tendency was amplified by heat stress. They concluded that V5 more heat susceptible than V2183 in terms of leaf longevity, *in vivo* chlorophyll stability and grain abortion under heat stress.

Singh and Patel (1996) subjected two wheat cv WH-283 and WH-331 in a pot experiment to water stress by withholding water until wilting occured at tillering, flowering or grain filling stages and observed that leaf water potential, osmotic potential, relative water content and photosynthesis decreased in both the cultivars and at all the growth stages respiration rate and accumulation of proline increased with water stress. The effect was more pronounced in WH-283 than the WH-331. Water stress reduced grain yield at flowering more than water stress at tillering or grain filling stage.

Proline accumulation capacity in plant is closely related with plant-antidrought, specially under soil water deficits. Many reports from wheat and other crops have proved this (Wang and Li 2000, Wang et al. 2003 and Errabii et al. 2006)

Vendruscolo et al. (2007) found that proline is involved in tolerance mechanism against oxidative stress and this is the main strategy of plants to avoid detrimental effects of water deficit stress.

Maiti Et. Al (2000) reported that proline accumulation is a stress tolerance mechanism of resistant plants against various abiotic stresses such as drought, high temperature and salinity.

Maralian Et.Al (2010) conducted a field study to evaluate the effect of water stress on proline accumulation rate and wheat grain yield. A bred wheat line (N84-12) was evaluated by contrasting irrigation regimes i.e., well watered and water deficit stress before tillering stage (T1) and after heading stage (T2). To impose water deficit stress plant was not irrigate before T1 and T2. The analysis of variance showed that water deficit stress significantly affected proline accumulation rate and harvest index in $P \leq 0.01$. The highest proline accumulation rate was observed under T2 condition and grain yield was decreased by water deficit stress compared to well watered condition.

Ashraf et al. (1994) evaluate four wheat genotypes (two tolerant: Chakwal-86 and DS-4, two susceptible: DS-17 and Pavon) to drought induced by PEG-6000 solutions. Water stress reduced chlorophyll (a, b, and total) contents and reduction was more pronounced in drought susceptible genotypes. Total phenol, peroxidase activity and cholophyll a/b ratio increased under drought conditions.

Hasan *et al.* (2007) conducted an experiment to evaluate the perfomance of three heat tolerant (Aghrani, Kanchan and CB 30) and one heat sensitive (Sonora) wheat cultivars under normal and post-anthesis heat stress condition in relation to proline content in flag leaf and grain at 8 DAA. They concluded that relative to normal growing condition the sharp increase in flag leaf proline along with larger decrease in kernel proline under heat stress condition clearly indicated the sensitivity of the heat sensitive cultivar (Sonora) to post-anthesis heat stress.

Islam *el al,* (1993) studied the performance of the existing (Sonalika) and newly released wheat varieties (Ananda, Kanchan, Barkat, Akbar and Aghrani) seeded from 1 November to 15 January at 15 days interval. Grain yield, number of spike/m^2, number grain per spike and 1000 grain weight were significantly affected by sowing date and variety. The highest grain yield was obtained with variety Kanchan when sown on 15 November which was identical to Akbar and Barkat. Aghrani performed better than all other varieties when sown in December and January. Sonalika variety also showed lower yield than other varieties when seeding done in December and January. Different yield components of these six varieties at maturity reacted differently to late seeded conditions. Delay in sowing caused significant reduction in grain weight due to higher temperature at grain filling stage.

Podsiado (1999) grew four spring wheat cultivars on sandy soil with or without irrigation. Irrigation generally increased the chlorophyll and carotinoid contents but decreased nitrate reductase activity and increased acid and alkaline phosphate activities of flag leaf.

Blum *et al.* (1994) reported that grain filling of wheat is seriously impaired by heat stress due to reducing in current leaf and ear photosynthesis at high temperatures. An alternative source of carbon for grain filling is stored stem reserves. They evaluated the hypothesis that the mobilization of stored stem reserves into growing grain is an important source of carbon for supporting grain filling under heat stress through two experiments with two spring wheat cultivars (V_5 and V_{2183}) of very similar phenology and plant stature, which had previously been found to differ in grain shrivelling under drought and heat stress conditions in the field. Variety V_5 was more heat susceptible than V_{2183}. The rates of stem dry weight (DM) loss was monitored under optimum (control) and high (stress) temperatures in glass house (Expt 1) and growth chamber (Expt 2). In Expt 1, irrespective of the environment, stem DM reserves may have accounted for about half of grain weight per ear in V_5, while it was less than quarter in V_{2183}. The contribution of stem reserves to grain mass was consistently higher in V_5 than in V_{2183} under both stress and non-stress conditions. In Expt 2, stem weight loss in the controls could account for 62.3% and 40.2% of grain weight per ear, in V_5 and V_{2183} respectively. However, under heat stress these values increased to greater than 100%.

Rahman (1997) conducted an experiment with 8 wheat cultivars under irrigated and rainfed condition. He reported that moisture retention capacity (MRC) and relative leaf water content (RLWC) were higher in irrigated plants than the rainfed plants. He found variation in MRC and RLWCamong the cultivars in different magnitude.

Prakash and Ramchandran (2000) indicated leaf water potential as a tool to measure the water stress condition was reduced in moisture deficit brinjal plant.

Canopy temperature (CT) has been expected to be a useful physiological parameter to screen wheat genotypes for tolerance of water stress and yield potential but it is strongly influenced by environmental conditions (Blum et al. 1989).

Inagaki and Nachit (2007) conducted a study on wheat under water deficit stress. Infrared thermograph was used to monitor the changes in the leaf temperature. CT of 11 wheat genotypes were also comparedunder moderate water stress in the field. They suggested that leaf and canopy temperature was a reliable indicator of thermal changes in wheat foliage under water deficit.

Sikder *et al.* (2001) evaluated yield performance of ten wheat varieties in relation to heat tolerance by seeding them optimum seeding time and late seeding time. Out of ten varieties four varieties were comparatively heat tolerant, three were moderately tolerant and three were heat sensitive. The heat tolerant and moderately tolerant varieties showed better performance in yield and yield components at optimum and late sowing time than those of heat sensitive ones. They also reported that heat tolerant and moderately tolerant varieties showed higher relative performance in grain number per ear, 1000 grain weight and main shoot grain weight compared to sensitive varieties.

Siddique Et.Al. (1999) conducted an experiment on drought effects on four wheat cultivars (Kanchan, Sonalika, Kalyansona and C306). These cultivars were grown in pots and subjected to four levels of water stress at vegetative or anthesis stage or both. Exposure of plant to drought led to noticeable decreases in leaf water potential and relative water content with a concurrent increase in leaf temperature. The higher leaf water potential and relative water content as well as lower leaf temperature was associated with a higher photosynthesis rate. Drought stress plant displayed a higher CT than well watered plant in both vegetative growth and anthesis growth stages. Successive stresses at both developmental stages raised the canopy temperature much higher than in plants stressed once.

Water stress adversely affects the plant establishment, and thereafter growth and development. Cell enlargement, gas exchange and assimilates partitioning are hindered by drought stress. Under extreme condition it may severely disturbs several metabolic processes which may results in diminished photosynthesis, check cell enlargement, cell division and finally cell death (Kramer 1983).

Siddique et al. (1999) found reduce spikelet per spike, grain per spike and grain yield at drought stress. They reported that the reduction of spikelet per spike was more severe at vegetative drought than at anthesis drought of wheat plant. They indicated that ecological condition at vegetative stage played an important role in determining the number of spikelet per spike.

Karim Et. Al. (2000) stated that water stress reduced grain yield by reducing productive tillers per plant, fertile spikelet per plant, number of grains per plant and individual grain weight. They also mentioned that water stress at reproductive stage is more harmful to plant processes than that of any other growth stages. This is because of water stress at anthesis markedly reduces photosynthesis, reproductive development and finally grain yield.

Sikder Et Al. (2011) tested four wheat varieties (Prodip, Sufi, Bijoy and Pavon-76) under well watered and non-irrigated water stress condition to evaluate their morphological and yield performance in relation to water stress. They found that plant height, ear bearing tiller number, grain number per spike, seed size and grain yield were significantly affected by combined effect of growing conditions (water level) and varieties. Under water stress condition all the varieties showed reduced value of those parameters at different magnitude compared to well water condition. Varieties Prodip, Sufi and Bijoy had showed relatively better performance and lower reduction under non-irrigated water stress condition in those characters compared to Pavon-76.

Chapter III

MATERIALS AND METHODS

The experiment was conducted at research field and laboratory of the Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of July 2014 to June 2015. Details of the methodology of the experiment followed during the research period are presented in this chapter.

3.1 Experimental site and soil

The location of the experimental site was at 25º38´ N latitude and 88º41´ longitude and at the elevation of 34.4 m above the sea level. The experimental site was medium high land and belonging to the Agro-ecological Zone-1 (AEZ-1) named Old Himalayan Piedmont Plain (FAO and UNDP 1988). The soil characteristic was given at appendix I and II.

3.2 Weather in experimental period

The field experiment was performed during November 2013 to April 2014. The weather information was shown in appendix III.

3.3 Experiment

The experiment was carried out at the research farm of Crop Physiology and Ecology Department, HSTU, Dinajpur. It was laid out in a split plot design with three replications. The unit plot size will be 3m x 2m having a plot to plot and block to block distance of 0.75m and 1.0 m, respectively. For crop cultivation the management practice recommended by WRC followed.

The treatment factors A and B were

A. Main plot treatment: Four growing conditions

i. Well water growing condition (Giving three irrigations) and

ii. Water limited drought condition (Giving one irrigation at 20 DAS for seedling establishment).

iii. Normal growing condition (sowing novmber 30, 2014)

iv. Late growing heat stress growing (sowing December 30, 2014)

B. Sub plot treatment: Two wheat varieties: BARI gom-28 and Kanchan.

3.4 Phenology

The following phenological stages were recorded in days when 50% plants of each plot reached a definite stage as the representative of that stage. Duration of anthesis and grain filling (days) were also recorded.

i. Crown root initiation – When 50% plants of the plot produced crown root.
ii. Tillering- When 50% plants of the plot produced maximum tiller.
iii. Heading - When 50% plants of the plot emerged spike.
iv. Anthesis - When 50% plants of the plot gave flowering.
v. Physiological maturity - When 50% plants of the plot produced matured soft grain.
vi. Maturity. - When 50% plants of the plot produced hard grain and ready for harvesting.

3.6 Physiological parameters

3.6.1 Canopy temperature depression (CTD) ^{0}C

A hand held infra-red thermometer (Model: Crop TRAK Item no. 2955L – Spectrum Technologies, Inc.) was used to measure this trait i.e. the difference between ambient air temperature and canopy temperature in degree centigrade. The CTD was recorded at 5 days after anthesis during noon period under bright sunlight and less wind.

3.6.2 Chlorophyll Estimation

Chlorophyll was estimated on fresh weight basis extracting with 80% acetone by using spectrophotometer (Model: SPECTRO UV-VIS RS, Labomed Inc, USA) according to Witham *et al.* (1986) using the following formulae:

mg chlorophyll a/g tissue = [12.7(D663 – 2.69(D645)] x [v/(1000 x w)]

mg chlorophyll b/g tissue = [22.9(D645 – 4.68(D663)] x [v/(1000 x w)]

mg chlorophyll (a+b)/g tissue = [20.2(D645 – 8.02(D663)] x [v/(1000 x w)]

3.6.3 Estimation of proline

Proline contents of the flag leaf at 8 DAA in all the wheat cultivars grown in two different growing conditions were estimated. At 8 DAA, the flag leaves from each replication of each cultivar were collected and immediately kept in the ice-bag and were brought to Crop Physiology and Ecology Laboratory of HSTU for proline estimation. One gram fresh weight of the flag leaf was taken for proline estimation. Subsequently proline was estimated as Troll and Lindsley (1955).

At first ninhydrin reagent was prepared in such a way so that it was utilized for proline estimation within two hours of preparation. For preparing ninhydrin reagent, addition of 30 ml glacial acetic acid and 30 ml 6M orthophosphoric acid were mixed with 1.25 g of ninhydrin. It was subsequently heated and stirred gently to dissolve but the temperature was not allowed to exceed 70^0C. Proline standards were prepared for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 ppm with distilled water.

The fresh samples were crushed in mortar and pestle and homogenized the material in 10 ml 3% sulphosalicylic acid until no large segments of plant material remained. Homogenate was filtered through Whatman No. 2 filter paper and washed with 3% sulphosalicylic acid and the volume was set to 25 ml. Two ml of the filtrate and each standard proline solutions were then reacted with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid in a pyrex test tube and boiled for one hour at 100^0C in water bath covering the tube with aluminium foil to prevent excess evaporation. Subsequently, it was cooled in ice bath and 4 ml of toluene was added to each tube using a dispensor. Each tube was then shaken vigorously for 15 to 20 seconds in an electrical shaker and allowed the layer to separate for 30 minutes. The absorbance of layer was measured through spectrophotometer at 520 nm with pure toluene as a blank. Proline content was expressed on a fresh weight basis from the standard curve, using standard L-proline according to the method developed by to Troll and Lindsley (1955).

3.7 Yield and yield attributes at harvest

3.7.1 Plant height

Plant height was measured from the base of the plant to the tip of the spike. Five plant samples from each plot were taken and means were calculated.

3.7.2 Spike length

Spike length was measured from the base of the spike to the tip of the spike excluding the own. Five spikes from each plot were taken and means were calculated.

3.7.3 Spikes per plant

Spikes of five plant samples from each plot were taken and means were calculated.

3.7.4 Florets per spike and spikelet

Floret number per spike and spikelet was counted from five spikes of each plot and means were calculated.

3.7.5 Grain per spike and spikelet

Grain number per spike and spikelet was counted from five spikes of each plot and means were calculated.

3.7.6 Floret sterility

Floret sterility was measured on the basis of following equation-

Floret sterility (%) = (1- Number of kernel per spike/Number of floret per spike) $\times 100$

3.7.7 1000 seeds weight

1000 cleaned sun dried grains were counted from the seed stock obtained from each treatment combination and weighted using electronic balance.

3.7.8 Grain weight and biological weight

Grain and straw yield per plot was calculated first then converted to ton per hectare. Biological yield also measured.

3.7.9 Harvest index

Harvest index was calculated by dividing economic yield by biological yield of plant in each pot by multiplying with 100 and expressed in percentage.

$$\text{Harvest index (\%)} = \frac{Economic\ yield}{Biological\ yield} \times 100$$

3.8.0Stress (Drought and heat) susceptibility index (SSI)

Stress susceptibility index (SSI) was calculated for grain yield as described by Fischer and Maurer (1976).

$SSI = (1- Y/Y_p) / (1- X/X_p)$

Where, Y = Variable of a cultivar in a stress environment

Y_p = Variable of a cultivar in a stress-free environment

X = Mean of Y of all the cultivars

X_p = Mean of Y_p of all the cultivars.

(S < 1.0, stress tolerant and S > 1.0, stress susceptible)

3.8 .1Statistical analysis

The data was analyzed by partitioning the total variance with the help of computer by using MSTAT program. The treatment means will be compared using Duncun's Multiple Range Test (DMRT).

CHAPTER IV

RESULTS AND DISCUSSSION

The results of the present investigation have been presented under the following heads with several tables and figures.

4.1 Phenological stages

The effect of growing conditions and wheat varieties on various phenological stages is presented in Table 1. From the results it was found that the combined effect of four growing conditions and two wheat genotypes on phenological stages was significant except crown root initiation stage (CRI). It was observed that the crown root initiation stage required lower number of days for both the varieties with all the growing conditions compared to the other successive growth stages like tillering, heading, anthesis, physiological maturity and harvesting maturity. In CRI stage, the maximum number of days (20) required for BARI Gom-28 at normal sowing with well watered condition, which was followed by Kanchan at normal sowing with well watered condition and BARI Gom-28 at normal sowing with drought condition. Where as, the minimum number of days (16) required for Kanchan at late sowing heat stress with drought condition. The requirement of days were decreased for both the varieties to attaining the CRI stage at stressed (heat and drought) condition compared to stress free (normal sowing with well watered) condition.

Table 1: Days required for attaining different phenological stages of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Crown root initiation stage	Tillering stage	Heading stage
Water Level	Sowing time				
Well watered	Normal Sowing	BARI Gom 28	20 a	28 a	64 a
		Kanchan	19 a	25 ab	62 a
	Late Sowing (Heat stress)	BARI Gom 28	19 a	24 bc	61 ab
		Kanchan	18 a	22 bc	57 bc
	Normal	BARI Gom	19 a	23 bc	60 ab

Drought	Sowing	28			
		Kanchan	18 a	22 bc	57 bc
	Late Sowing (Heat stress)	BARI Gom 28	17 a	21 bc	54 cd
		Kanchan	16 a	20 c	51 d
CV (%)			12.25	8.91	4.26

Growing conditions		Wheat Varieties	Anthesis stage	Physiological Maturity Stage	Harvest Maturity Stage
Water Level	Sowing time				
Well watered	Normal Sowing	BARI Gom 28	70 a	106 a	116 a
		Kanchan	67 ab	102 b	112 b
	Late Sowing (Heat stress)	BARI Gom 28	65 b	100 bc	110 bc
		Kanchan	61 cd	90 d	105 d
Drought	Normal Sowing	BARI Gom 28	64 bc	100 bc	109 c
		Kanchan	60 d	97 c	102 e
	Late Sowing (Heat stress)	BARI Gom 28	59 d	93 d	100 e
		Kanchan	55 e	86 e	95 f
CV (%)			3.63	2.20	

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

In case of tillering stage, BARI Gom-28 at normal sowing with well watered condition required the maximum number of days (28) followed by Kanchan at normal sowing with well watered condition and BARI Gom-28 at normal sowing with well drought condition, where as the minimum number of days (20) required for Kanchan at late sowing heat stress with drought condition.

At normal sowing with well watered condition, BARI Gom-28 required the highest number of days (64) to attaining heading stage followed by Kanchan at normal sowing with well watered condition and BARI Gom-28 at normal sowing with well drought condition, where as the lowest number of days (51) required for Kanchan at late sowing heat stress with drought condition. The requirement of days were decreased for both the varieties to attaining the heading stage at stressed (heat and drought) condition compared to stress free (normal sowing with well watered) condition.

In case of anthesis stage, BARI Gom-28 at normal sowing with well watered condition required the maximum number of days (70) which was followed by Kanchan at normal sowing with well watered condition (67 days) and BARI Gom-28 at normal sowing with drought condition (65 days), where as the minimum number of days (55) required for Kanchan at late sowing heat stress with drought condition.

At well watered with normal sowing condition, BARI Gom-28 required the maximum number of days (106) to attaining physiological maturity stage followed by Kanchan at normal sowing with well watered condition and BARI Gom-28 at normal sowing with drought condition, where as the lowest number of days (86) required for Kanchan at late sowing heat stress with drought condition. The requirements of days were decreased for both the varieties to attaining the physiological stage at stressed (heat and drought) condition compared to stress free (normal sowing with well watered) condition.

Finally, BARI Gom-28 required the highest number of days (116) to attaining harvest maturity stage at normal sowing with well watered condition, followed by Kanchan at normal sowing with well watered condition (112 days) and BARI Gom-28 at normal sowing with drought condition (110 days), where as the lowest number of days (91) required for Kanchan at late sowing heat stress with drought condition.

In the present study the requirements of days were decreased for both the varieties to attaining different growth stages at stressed (heat and drought) condition compared to stress free (normal sowing with well watered) condition. Similar results were found by Islam (2014), and Sikder (2009). They found that drought and heat stress conditions reduced the number of days form attaining different growth stages in wheat. In the present study late sowing and drought caused unfavourable condition for growth resulting in reduced number of days for attaining different phenological stages.

4.2 Physiological parameters

4.2.1 Canopy temperature depression (^{0}C)

Canopy temperature depression (CTD) at 5 days after anthesis of two wheat varieties under different growing conditions is presented in Table 2. Results showed that the combined effect of growing conditions and wheat varieties on canopy temperature depression was significant. At stressed (heat and drought) both the varieties showed higher CTD (^{0}C) compared to stressed free (normal sowing and well watered) conditions. Under highly stressed (late sowing heat stress with drought) condition BARI Gom-28 showed the highest Canopy temperature depression (6.1 ^{0}C), where as variety Kanchan had the lowest CTD (4.5 ^{0}C) at normal sowing with well watered condition.

Table 2: Canopy Temperature Depression (CTD) of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	CTD(^{0}C) (Mean±SE)	Range
Water Level	Sowing time			
Well watered	Normal Sowing	BARI Gom 28	5.1(±0.63) ab	4.0 - 6.2
		Kanchan	4.5(±0.09) b	4.2 - 4.8
	Late Sowing (Heat stress)	BARI Gom 28	5.5(±0.40) ab	4.8 - 6.2
		Kanchan	4.8(±0.41) ab	4.1 - 5.5
Drought	Normal Sowing	BARI Gom 28	5.4(±0.46) ab	4.6 - 6.2
		Kanchan	4.8(±0.52) ab	3.9 -5.7
	Late Sowing (Heat stress)	BARI Gom 28	6.1(±0.51) a	5.2 – 7.0
		Kanchan	5.2(±0.35) ab	4.6 – 5.8
CV (%)			12.0	

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level

These performances in CTD of two wheat varieties under four growing conditions were reflected to their yield performance. Similar results were reported by Sikder and Paul (2010) and

Renolds et al. (1998) that potential to keep canopy cool is one of the important traits of high temperature tolerant wheat genotypes. This is reflected by canopy temperature depression which is expressed as difference between the ambient temperature and canopy temperature.

4.2.2Chlorophyll content of flag leaf

The combined effect of growing conditions and wheat varieties on chlorophyll content of flag leaf during anthesis stage was significant which is presented in Table 3. The chlorophyll content was decreased by both the wheat varieties at stressed (both heat and drought) condition compared to stressed free (well watered and normal sowing) conditions. At well watered with normal sowing condition, BARI Gom-28 showed the maximum flag leaf chlorophyll content (2.8 mg/g fresh weight) followed by Kanchan (2.3 mg/g fresh weight) at well watered with normal sowing condition. Therefore, the lowest chlorophyll content was found in Kanchan at late sowing with drought condition (1.4 mg/g fresh weight).

Under highly stressed (late sowing heat stress with drought) conditions BARI Gom-28 showed the higher flag leaf chlorophyll content 1.5 mg/g compared to Kanchan at the same stressed condition.

Table 3: Flag leaf chlorophyll and proline content of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Chlorophyll content (mg/g fresh weight)	Proline content (µmole/g fresh weight)
Water Level	Sowing time			
Well watered	Normal Sowing	BARI Gom 28	2.8 a	1.71 c
		Kanchan	2.3 ab	1.65 c
	Late Sowing (Heat stress)	BARI Gom 28	2.0 bc	2.00 b
		Kanchan	2.0 bc	1.70 c
Drought	Normal Sowing	BARI Gom 28	1.8 bc	2.10 b
		Kanchan	1.6 c	1.75 c
	Late Sowing (Heat stress)	BARI Gom 28	1.5 c	2.30 a
		Kanchan	1.4 c	1.80c
CV (%)			11.68	4.47

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level

Bojovic and Stojanovic (2005) and Sikder *et al*. (2011) also found the similar pattern of chlorophyll content in wheat under stressed conditions, which is an agreement of the present study.

4.3.2 Proline content of leaf

The combined effect of growing conditions and wheat varieties on proline content of flag leaf during anthesis stage was significant which is presented in Table 3. The proline content of both wheat varieties were increased at all the stressed conditions compared to stress free condition. From the results it was found that BARI Gom-28 gave the significantly highest amount of proline (2.30 µmole/g fresh weight) at late sowing heat stress with drought condition followed by BARI Gom-28 (2.10 µmole/g fresh weight) under normal sowing with drought condition. Where as, variety Kanchan produced the lowest proline content (1.65 µmole/g fresh weight) at normal sowing well watered condition which was statistically similar to BARI Gom 28 (1.71 µmole/g fresh weight) at same growing condition.

In the present study it was observed that stressed condition (heat and drought) increased the proline content in flag leaf of wheat varieties compared to stress free condition. Similar results also reported by some researcher that proline accumulation is higher in stressed condition than the stress free condition and stress tolerant wheat genotypes had higher leaf proline content than the stress sensitive genotypes (Sikder 2010) Wang and Li 2000

4.3 Yield and yield attributes

4.3.1 Plant height

The effect of growing conditions and wheat varieties on plant height is presented in Table 4. From the results it was found that the interaction effect of four growing conditions and two wheat varieties was significant. It was observed that the stressed conditions (heat and drought either combined or single) reduced the plant height of both the varieties compared to stress free conditions.

Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher plant height (105 cm) compared to Kanchan (100 cm). At highly stressed condition (late sowing heat stress with drought) variety BARI Gom-28 also had the higher plant height (97 cm) than the Kanchan (95 cm). Therefore, considering all stress combination variety BARI Gom-28 attained the highest plant height (105 cm) at well watered normal sowing condition where as, variety Kanchan showed the lowest plant height (95 cm) at heat stress drought condition.

Table 4: Plant height, spike length and spike number plant^{-1} of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Plant height (cm)	Spike length (cm)	Spike plant^{-1} (no.)
Water Level	Sowing time				
Well watered	Normal Sowing	BARI Gom 28	105 a	11.5 a	7.5 a
		Kanchan	100 bcd	9.0 b	6.0 bc
	Late Sowing (Heat stress)	BARI Gom 28	103 ab	8.9 b	6.5 ab
		Kanchan	98 cde	7.5 b	5.5 bc
Drought	Normal Sowing	BARI Gom 28	101 bc	8.8 b	6.6 ab
		Kanchan	96 e	7.6 b	5.7 bc
	Late Sowing (Heat stress)	BARI Gom 28	97 de	7.5 b	5.0 c
		Kanchan	95 e	6.9 c	4.9 c
CV (%)			1.64	13.73	11.28

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

In the present investigation, stressed conditions (heat and drought either combined or single) created unfavourable conditions for wheat growth causing reduced plant height. Reduced plant height of wheat crop under stressed condition (heat and drought) also reported by Sikder (2010), Siddique *et al*. (1999) and Sikder *el al.* (2011)

4.3.2 Spike length

The interaction effect of growing conditions and wheat varieties on spike length was significant which is presented in Table 4. From the results it was found that BARI Gom-28 showed the significantly highest spike length (11.5 cm) at stress free condition (normal sowing with well watered condition) compared to other treatments combinations. Where as, Kanchan produced the lowest spike length (6.9 cm) at highly stressed condition (late sowing drought). At stressed condition (heat and drought combined or single) both the varieties showed reduced spike length at different magnitude compared to their spike length under stress free condition. Similar results also found Islam (2014) and Sikder et al. (2011) in wheat under water stress and Sikder (2010) under heat stress condition. Spike length was reduced when it was grown in, high temperature and high temperature resulted in smaller organs (Bagga and Rawson 1977).

4.3.3 Spike plant^{-1}

The combined effect of growing conditions and wheat varieties on spike plant^{-1} was significant which is presented in Table 4. From the results it was found that the stressed condition (heat and drought combined or single) showed reduced spike number per plant of both wheat varieties e.g. BARI Gom-28 and Kanchan compared to stress free condition. At stress free condition BARI Gom-28 attained the highest spike plant^{-1} (7.5) compared to Kanchan (6.0) at same growing condition.

Under highly stressed (late sowing drought condition) again the variety BARI Gom-28 attained the highest spike plant^{-1} (5.0), where as variety Kanchan had the lowest spike plant^{-1} (4.9). In other stress combinations BARI Gom-28 showed better performance in spike number per plant. Results from other studies showed that spike number per plant was markedly influenced by heat /and drought stress in wheat (Handy *et al.,* 2003; Sikder, 2010 and Sikder *et al*., 2011)

4.3.4 Floret sipkelet^{-1}

The effect of growing conditions and wheat varieties on floret sipkelet^{-1} is presented in Table 5. From the results it was found that the interaction effect of four growing conditions and two wheat varieties was significant. It was also observed that the stressed conditions (heat and drought either combined or single) reduced the floret sipkelet^{-1} of both varieties compared to stress free conditions. Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher floret sipkelet^{-1} (5.0) compared to Kanchan (4.0). At highly stressed condition (late sowing heat stress with drought) variety BARI Gom-28 also had the higher floret sipkelet^{-1} (3.0) than the Kanchan (3.0). Therefore, considering all stress combination variety BARI Gom-28 attained the highest floret sipkelet^{-1} (5.0) at well watered normal sowing condition where as, variety Kanchan showed the lowest plant height (3.0) at heat stress drought condition.

In the present study, stressed conditions (heat and drought either combined or single) causing reduced floret sipkelet^{-1}. Reduced floret sipkelet^{-1} of wheat crop under stressed condition (heat /and drought) also reported by Sikder (2010), Siddique *et al*. (1999) and Islam(2014)

Table 5: Floret sipkelet^{-1}, grain spikelet^{-1} and floret spike^{-1} of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Floret spikelet^{-1} (no.)	Grain spikelet^{-1} (no.)	Floret spike^{-1} (no.)
Water Level	Sowing time				
Well watere	Normal Sowing	BARI Gom 28	5.0 a	4.5 a	90.5 a
		Kanchan	4.0 bc	3.5 b	78.0 c

d	Late Sowing (Heat stress)	BARI Gom 28	4.5 ab	3.6 b	80.0 b
		Kanchan	3.8 bcd	3.2 c	71.0 e
Drought	Normal Sowing	BARI Gom 28	4.6 ab	3.7 b	81.0 b
		Kanchan	3.7 bcd	3.0 cd	70.0 e
	Late Sowing (Heat stress)	BARI Gom 28	3.5 cd	3.9 d	75.0 d
		Kanchan	3.0 d	2.5 e	68.0 f
CV (%)			12.00	3.38	1.03

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

4.3.5 Grains sipkelet^{-1}

The combined effect of growing conditions and wheat varieties on grains sipkelet^{-1} was significant which is presented in Table 5. From the results it was found that BARI Gom-28 showed the significantly highest grains sipkelet^{-1} (4.5) at stress free condition (normal sowing with well watered condition) compared to all other growing conditions. Where as, Kanchan produced the lowest grains sipkelet^{-1} (2.5) at highly stressed condition (late sowing heat stress with drought). At stressed condition (heat and drought combined or single) both the varieties showed reduced grains sipkelet^{-1} at different magnitude compared to their grains sipkelet^{-1} under stress free condition. Similar results also found Islam (2014) and Sikder *et* *al.* (2011) and Sikder (2010) in wheat under heat and drought stress condition.

4.3.6 Floret sipke^{-1}

The interaction effect of growing conditions and wheat varieties on floret sipke^{-1} was significant which is presented in Table 5. From the results it was found that the stressed condition (heat and drought combined or single) showed reduced floret sipke^{-1} of both wheat varieties e.g. BARI Gom-28 and Kanchan compared to stress free condition. At stress free condition (normal sowing with well watered) BARI Gom-28 attained the highest floret sipke^{-1} (90.5) compared to Kanchan (78.0) at the same growing condition. Under highly stressed (late sowing drought condition) again the variety BARI Gom-28 attained the highest floret sipke^{-1} (75.0) where as variety Kanchan had the lowest floret sipke^{-1} (68.0). In other stress conditions BARI Gom-28 also showed better performance in spike floret sipke^{-1}. Results from other studies showed that floret sipke^{-1} was markedly influenced by heat and drought stress in wheat (Islam (2014) and Sikder *et al.*, 2011). Significantly reduction in floret number per spike in wheat under heat stress was also reported by Shpilar and Blum (1991) and Zeng *et al*. (1985).

4.3.7 Grains sipke^{-1}

The effect of growing conditions and wheat varieties on grains sipke^{-1} is presented in Table 6. From the results it was found that the combined effect of four growing conditions and two wheat varieties was significant. It was observed that the stressed conditions (heat and drought either combined or single) reduced the grains sipke^{-1} of both the varieties compared to stress free conditions.

Table 6: Grain spike^{-1} (no.), Floret sterility (%) and 1000 seed weight (g) of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Grain spike^{-1} (no.)	Floret sterility (%)	1000 seed weight (g)
Water Level	Sowing time				
Well watered	Normal Sowing	BARI Gom 28	65.0 a	20.00 e	55.0 a
		Kanchan	60.0 b	22.53 d	53.0 a
	Late Sowing (Heat stress)	BARI Gom 28	59.0 b	23.08 d	45.0 b
		Kanchan	55.0 b	26.25 c	44.0 bc
Drought	Normal Sowing	BARI Gom 28	59.5 b	24.54 d	46.0 b
		Kanchan	56.0 b	28.18 b	43.0 bc
	Late Sowing (Heat stress)	BARI Gom 28	50.0 c	29.41 b	42.0 bc
		Kanchan	48.0 c	33.33 a	40.0 c
CV (%)			4.88	3.03	4.86

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level

Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher grains sipke^{-1} (65.0) compared to Kanchan (60.0). At highly stressed condition (late sowing heat stress with drought) variety BARI Gom-28 also had the higher grains sipke^{-1} (50.0) than the Kanchan (48.0). Therefore, considering all stress combination variety BARI Gom-28 attained the highest floret sipkelet^{-1} (65.0) at well watered normal sowing condition where as, variety Kanchan showed the lowest grains sipke^{-1} (48.0) at heat stress drought condition.

In the present study, stressed conditions (heat and drought either combined or single) causing reduced grains sipke^{-1}. Reduced grains sipke^{-1} of wheat crop under stressed condition (heat /and drought) also reported by Sikder (2010), Siddique *et al*. (1999) and Islam (2014)

4.3.8 Floret sterility

The interaction effect of growing conditions and wheat varieties on floret was significant which is presented in Table 6. The floret sterility of both wheat varieties were increased in different magnitude at all the stressed conditions compared to stress free condition. From the results it was found that BARI Gom-28 gave the significantly lowest floret sterility (20.0) at stress free condition (normal sowing with well watered condition) followed by Kanchan (22.53) under the same growing condition. Under highly stressed condition (late sowing heat stress with drought) variety Kanchan showed the highest floret sterility (33.33) compared to others.

In the present study it was observed that stressed condition (heat and drought) increased the floret sterility of wheat varieties compared to stress free condition. Similar results also reported by Nazrul, 2014 that floret sterility is higher in water stress condition than the well watered condition.. Results from other studies indicated that high temperature resulted in higher floret sterility (Rawson, 1986). Late planting wheat experienced high temperature at the reproductive stage causing abortion of florets. Number of grains that developed in an ear is dependent on the number of florets and effective fertilization of them after anthesis (Evans and Wardlaw, 1976). On the other hand, reduction of grain number per ear is associated with high temperature during anthesis stage (Saini *et al.,* 1983; Zeng *et al*., 1985). Murty *et al*. (1979) stated that unfavourable environmental condition, particularly temperature attributed reduction in grain per ear and grain growth. In the most of the cases with increasing temperature, there was progressive decrease in number of grains that confirmed the grain abortion. At the grain filling stage, the increased temperature induced sterility of pollen tube that causes grain abortion in ear. Higher temperature prevailed at delayed sowing was responsible for higher grain abortion (Saini *et al*. 1983).

4.3.9 Thousand seed weight

The combined effect of growing conditions and wheat varieties on 1000 seed weight was significant which is presented in Table 6. From the results it was found that the stressed condition (heat and drought combined or single) showed reduced 1000 seed weight of both wheat varieties e.g. BARI Gom-28 and Kanchan compared to stress free condition. At stress free condition BARI Gom-28 attained the highest 1000 seed weight (55 g) which was at par with Kanchan (53) at the same growing condition.

Under highly stressed (late sowing with drought condition) again the variety BARI Gom-28 attained the highest 1000 seed weight

(42.0) where as variety Kanchan had the lowest spike plant^{-1} (40.0). In other stress combinations BARI Gom-28 showed better performance in 1000 seed weight .

Results from other studies it was found that Tashiro and Wardlaw (1989) reported that the effect of heat stress in the reproductive stage was lower the grain weight. Late sowing condition caused reduced seed size was also reported by Hu and Rajaram (1993). Al-Khatib and Paulsen (1990) concluded that high relative grain weight is a potential selection criterion for high temperature tolerant genotypes. Thousand seed weight was markedly reduced by drought stress in wheat (Sikder *et al*., 2011)

4.3.10 Grain yield ((t ha^{-1})

The effect of growing conditions and wheat varieties on grain yield is presented in Table 7. From the results it was found that the interaction effect of four growing conditions and two wheat varieties was significant. It was observed that the stressed conditions (heat and drought either combined or single) reduced the grain yield of both wheat varieties compared to stress free conditions.

Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher grain yield (4.2 t ha^{-1}) compared to Kanchan (3.6 t ha^{-1}). At highly stressed condition (late sowing heat stress with drought) BARI Gom-28 also had the higher grain yield (2.6 t ha^{-1}) than the Kanchan (2.0 t ha^{-1}). However, considering all stress combination variety BARI Gom-28 attained

the highest yield (4.2 t ha^{-1}) at well watered normal sowing condition whereas, variety Kanchan showed the lowest plant yield (2.0 t ha^{-1}) at heat stress drought condition.

Table 7: Grain yield, Biological yield and Harvest Index of two wheat varieties as influenced by growing conditions

Growing conditions		Wheat Varieties	Grain yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index (%)
Water Level	Sowing time				
Well watered	Normal Sowing	BARI Gom 28	4.2 a	9.5 a	44.21a
		Kanchan	3.6 b	8.0 abc	43.8 ab
	Late Sowing (Heat stress)	BARI Gom 28	3.5 b	8.5 ab	43.4 ab
		Kanchan	2.9 c	7.6 bcd	38.2 e
Drought	Normal Sowing	BARI Gom 28	3.5 b	8.6 ab	40.7 cd
		Kanchan	2.8 c	7.0 bcd	40.0 d

	Late Sowing (Heat stress)	BARI Gom 28	2.6 c	6.6 cd	39.39 e
		Kanchan	2.0 d	5.5 d	36.4 f
CV (%)			6.85	11.98	2.20

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

In the present study, yield components were significantly affected by growing conditions. Results from other studies showed that late sowing causes lower grain yield of wheat genotypes compared to optimum sowing (Sikder, 2010; Islam *et al*. 1993 and Bhatta *et al.* 1994). Bhatta et al. (1994) reported that some genotypes of wheat showed stability in grain yield between optimum and late planting. Numerous researchers also found reduced grain yield at high temperature stress conditions compared to optimum temperature Al-Khatib and Paulsen, 1990, and Hu and Rajaram 1993). Al-Khatib and Paulsen (1990) concluded that high relative grain yield which is the result of stable and / or long, duration of photosynthetic activity at heat stress condition is a selection criteria for heat tolerance of wheat genotypes.

Baser *et al*. (2004) found that 40% grain yield reduced due to water stress drought condition. Sikder *et al*. (2011) also found that water stress significantly inhibited the grain yield compared to well watered condition.

4.3.12 Biological yield (t ha^{-1})

The interaction effect of growing conditions and wheat varieties on biological yield was significant which is presented in Table 7. From the results it was found that BARI Gom-28 showed the significantly highest biological yield (9.5 t ha^{-1}) at stress free condition (normal sowing with well watered condition) compared to other treatments combinations. Where as, Kanchan produced the lowest biological yield (5.5 t ha^{-1}) at highly stressed condition (late sowing drought condition).

At stressed condition (heat and drought either combined or single) both the varieties showed reduced biological yield at different magnitude compared to their biological yield under stress free condition. Similar results also found in other studies on wheat that greater bilogical yield under well watered condition compared to water stress condition (Saren and Janna, 2001; Islam, 2014 and Sikder *et al*., 2011). Bhatta *et al*. (1994) and Sikder (2010) also reported reduced biological yield under high temperature. Hu and

Rajaram (1994) concluded that biomass could be considered as potential selection criteria for grain yield under high temperature.

4.3.13 Harvest index (%)

As a useful index of assessing the phytomass converted into useful economic yield, the harvest index was significantly influenced by the combined effect of growing conditions and wheat varieties and it is presented in Table 7. From the results it was found that the stressed condition (heat and drought combined or single) showed reduced harvest index of both wheat varieties e.g. BARI Gom-28 and Kanchan compared to stress free condition. At stress free condition BARI Gom-28 attained the highest harvest index (44.21) compared to Kanchan (43.4) at same growing condition.

Under highly stressed (late sowing drought) condition again the variety BARI Gom-28 attained the highest harvest index (39.39) where as variety Kanchan had the lowest harvest index (36.4). In other stress combinations BARI Gom-28 showed better performance in harvest index per plant. In the present study lower harvest index at stressed condition was due to lower grain yield and biological yield compared to stress free condition. Similar result was found Bhatta *et al*. (1994), Sikder *et al.* (2011), Islam (2014) and Sikder (2010) in wheat.

4.3.11 Stress susceptibility index (SSI)

Stress susceptibility index (SSI) based on grain yield of two wheat varieties is shown in Figure 1. From the results of stress susceptible index (SSI) based on grain yield it was found that BARI Gom-28 had lower stress susceptibility (SSI = 0.92), whereas Kanchan was more stress susceptible (SSI = 1.11) . So, variety BARI Gom-28 is regarded as stress (both heat and drought) tolerant and Kanchan is stress sensitive.

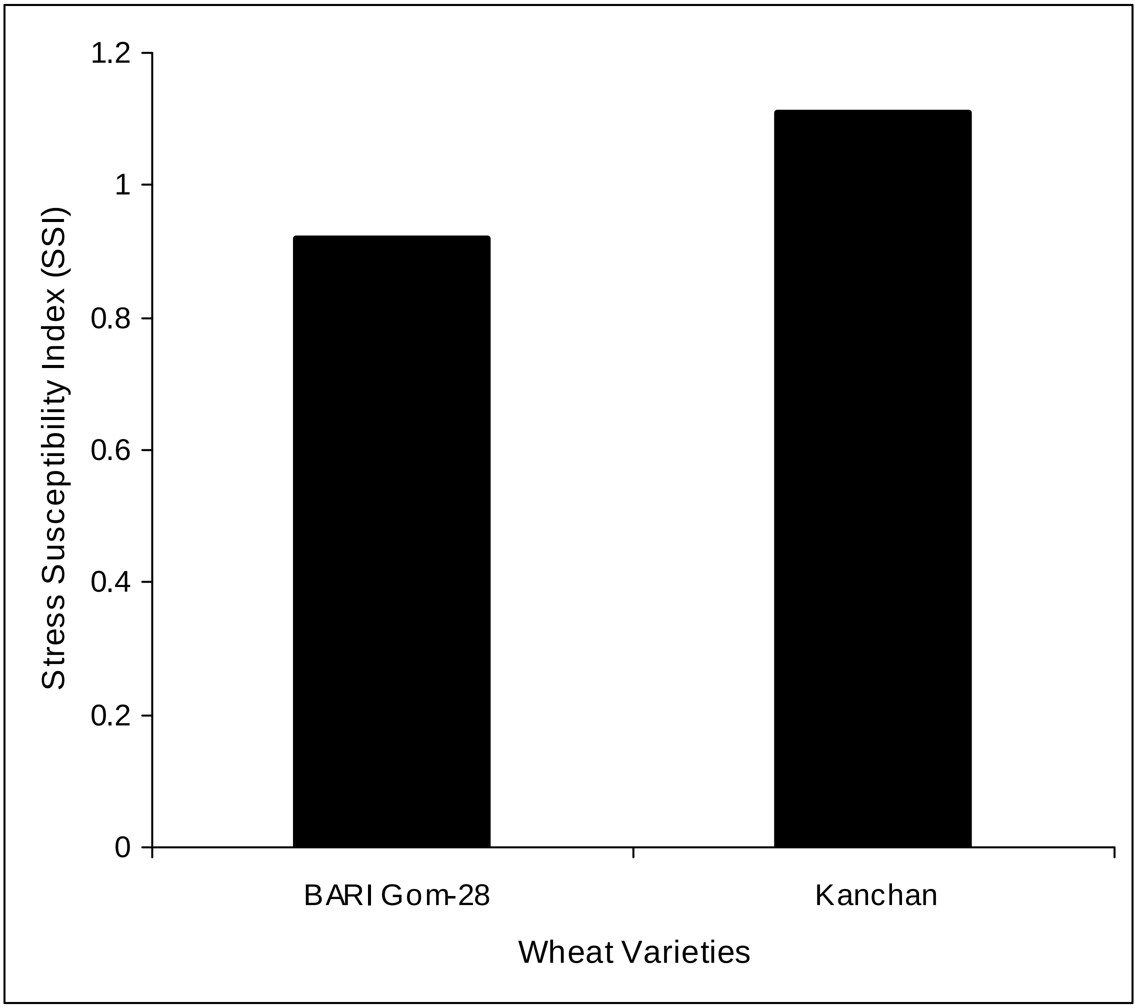

Figure 1: Stress susceptibility index (SSI) of two wheat varieties

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation was done to study the eco-physiological basis of heat and drought tolerance of wheat. It was conducted at research field and laboratory of the Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of July 2014 to June 2015. It was laid out in a split plot design with three replications. The unit plot size will be 3m x 2m having a plot to plot and block to block distance of 0.75m and 1.0 m, respectively. The treatment factors were A. Main plot treatment: Four growing conditions viz. i. Well water growing condition (Giving three irrigations), ii. Water limited drought condition (Giving one irrigation at 20 DAS for seedling establishment). iii. Normal growing condition (sowing novmber 30, 2014) iv. Late growing heat stress growing (sowing December 30, 2014), and B. Sub plot treatment: Two wheat varieties: BARI gom-28 and Kanchan.

From the results it was found that the days required to different phenological stages were significantly varied between two wheat varieties at different growing conditions. The requirements of days were decreased for both the varieties to attaining different growth stages at stressed (heat and drought) condition compared to stress free (normal sowing with well watered) condition.

The crown root initiation (CRI) stage required lower number of days for both the varieties with all growing conditions compared to the other successive growth stages like tillering, heading, anthesis, physiological maturity and harvesting maturity. To attain CRI stage, the maximum number of days (20) required for BARI Gom-28 at normal sowing with well watered condition, where as, the minimum number of days (16) required for Kanchan at late sowing heat stress with drought condition.

In tillering stage, BARI Gom-28 at normal sowing with well watered condition required the maximum number of days (28) to attaining it followed by Kanchan at normal sowing with well watered condition, where as the minimum number of days (20) required for Kanchan at late sowing heat stress with drought condition.

At normal sowing with well watered condition, BARI Gom-28 required the highest number of days (64) to attaining heading stage, where as the lowest number of days (51) required for Kanchan at late sowing heat stress with drought condition. The requirement of days were decreased for both the varieties to attaining the heading stage at stressed (heat and drought) condition compared to normal sowing with well watered condition.

In anthesis stage, BARI Gom-28 at normal sowing with well watered condition required the maximum number of days (70) to attaining it, where as the minimum number of days (55) required for Kanchan at late sowing heat stress with drought condition.

At well watered with normal sowing condition, BARI Gom-28 required the maximum number of days (106) to attaining physiological maturity stage followed by Kanchan at normal sowing with well watered condition, where as the lowest number of days (86) required for Kanchan at late sowing heat stress with drought condition. Therefore, BARI Gom-28 required the highest number of days (116) to attaining harvest maturity stage at normal sowing with well watered condition, where as the lowest number of days (91) required for Kanchan at late sowing heat stress with drought condition.

At stressed (heat and drought) both the varieties showed higher Canopy temperature depression (CTD) (^{0}C) compared to stressed free (normal sowing and well watered) conditions. Under highly stressed (late sowing heat stress with drought) conditions BARI Gom-28 showed the highest Canopy temperature depression (6.1 ^{0}C), where as variety Kanchan at normal sowing with well watered condition had the lowest CTD (4.5 ^{0}C).

The chlorophyll content was decreased by both the wheat varieties at stressed (both heat and drought) condition compared to stress free (well watered and normal sowing) conditions. At well watered with normal sowing condition, BARI Gom-28 required the maximum flag leaf chlorophyll content (2.8 mg/g fresh weight) and the lowest chlorophyll content was found in Kanchan at late sowing with drought condition (1.4 mg/g fresh weight).

The proline content of all wheat varieties were increased at all the stressed conditions compared to stress free condition. BARI Gom-28 gave the significantly highest amount of proline (2.30 µmole/g fresh weight) at late sowing heat stress with drought, where as variety Kanchan produced the lowest proline content (1.65 µmole/g fresh weight) at normal sowing well watered condition

The stressed conditions (heat and drought either combined or single) reduced the plant height of both the varieties compared to stress free conditions. Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher plant height (105 cm) compared to Kanchan (100 cm). At highly stressed condition late sowing heat stress with drought) variety BARI Gom-28 also had the higher plant height (97 cm) than the Kanchan (95 cm). In spike length BARI Gom-28 showed the significantly highest spike length (11.5 cm) at stress free condition compared to other growing conditions. Where as, Kanchan produced the lowest spike length (6.9 cm) at highly stressed condition (late sowing drought). At stressed condition (heat and drought combined or single) both the varieties showed reduced spike length at different magnitude compared to their spike length under stress free condition.

The stressed condition (heat and drought combined or single) showed reduced spike number per plant of both wheat varieties compared to stress free growing condition. Under highly stressed (late sowing drought condition) again the variety BARI Gom-28 attained the highest spike plant^{-1} (5.0) where as variety Kanchan had the lowest spike plant^{-1} (4.9). It was also observed that the stressed conditions (heat and drought either combined or single) reduced the floret sipkelet^{-1} of both the varieties compared to stress free conditions. Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher floret sipkelet^{-1} (5.0) compared to Kanchan (4.0).

From the results it was found that BARI Gom-28 showed the significantly highest grains sipkelet^{-1} (4.5) at stress free condition (normal sowing with well watered condition) compared to all other growing conditions. Where as, Kanchan produced the lowest grains sipkelet^{-1} (2.5) at highly stressed condition (late sowing heat stress with drought). The stressed condition (heat and drought combined or single) showed reduced floret sipke^{-1} of both wheat varieties compared to stress free condition. At stress free condition (normal sowing with well watered) BARI Gom-28 attained the highest floret sipke^{-1} (90.5) compared to Kanchan (78.0).

It was observed that the stressed conditions (heat and drought) reduced the grains sipke^{-1} of both the varieties compared to stress free conditions. Under stress free condition (normal sowing with well watered condition) BARI Gom-28 attained significantly higher grains sipke^{-1} (65.0) compared to Kanchan (60.0). The floret sterility of both wheat varieties were increased in different magnitude at all the stressed conditions compared to stress free condition. BARI Gom-28 gave the significantly lowest floret sterility (20.0) at stress free condition (normal sowing with well watered condition) followed by Kanchan (22.53). Under highly stressed condition (late sowing heat stress with drought) variety Kanchan showed the highest floret sterility (33.33) compared to others.

It was observed that the stressed conditions (heat and drought either combined or single) reduced the grain yield of both wheat varieties compared to stress free conditions. Under stress free condition BARI Gom-28 attained significantly higher grain yield (4.2 t ha^{-1}) compared to Kanchan (3.6 t ha^{-1}). At highly stressed condition variety BARI Gom-28 also had the higher grain yield (2.6 t ha^{-1}) than the Kanchan (2.0 t ha^{-1}). However, considering all stress combination variety BARI Gom-28 attained the highest grain yield (4.2 t ha^{-1}) at well watered normal sowing condition where as, variety Kanchan showed the lowest plant height (2.0 t ha^{-1}) at heat stress drought condition. BARI Gom-28 showed the significantly highest biological yield (9.5 t ha^{-1}) at stress free condition compared to other growing conditions. Where as, Kanchan produced the lowest biological yield (5.5 t ha^{-1}) at highly stressed condition.

It was found that the stressed condition (heat and drought combined or single) showed reduced harvest index of both wheat varieties compared to stress free condition. At stress free condition BARI Gom-28 attained the highest harvest index (44.21) compared to Kanchan (43.4) at same growing condition. Under highly stressed (late sowing with drought) condition again the variety BARI Gom-28 attained the highest harvest index (39.39) where as variety Kanchan had the lowest harvest index (36.4).

From the stress susceptible index (SSI) based on grain yield it was found that BARI Gom-28 had lower stress susceptibility (SSI = 0.92), whereas Kanchan was more stress susceptible (SSI = 1.11). So, variety BARI Gom-28 is regarded as stress (both heat and drought) tolerant and Kanchan is stress sensitive.

Finally, from the overall results it might be concluded that in addition to less stress susceptibility, longer growth phase, higher chlorophyll content, smaller changes in proline level, higher ability to maintain a cooler canopy environment and also higher grain yield and important yield components at stress condition may be used as indicators of stress tolerance (heat and drought) in wheat. Based on these characters variety BARI Gom-28 was found to be the stress tolerant. The present results also lead to understand that BARI Gom-28 may be used as heat and drought tolerant variety and a more stress tolerant variety can be developed through combining of these characters as far as possible.

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APPENDICES

Appendix I: Morphological and Physical characteristics of soil

Morphology	Characteristics
Location	Crop Physiology and Ecology research field, HSTU, Dinajpur
AEZ	Old Himalayan Piedmont Plain (AEZ-1)
General Soil type	Non-Calcareous Brown Floodplain Soil
Parent material	Piedmont alluvium

Soil series	Ranishankail
Drainage	Moderately well drained
Flood level	Above flood level
Topography	High land
Physical Characteristics	Value
sand (2-0.02mm)	60.0
silt (0.02-0.002mm)	27.0
clay (< 0.002mm)	13.0
Textural class	Sandy loam

Source: The Morphological and physical characteristics of soil samples were done by SRDI, Dinajpur, Bangladesh

Appendix II: Chemical characteristics of initial soil samples

Characteristics	Content	Interpretation
pH	5.40-5.50	Moderately acidic
Organic carbon (%)	0.69	Low
Organic matter (%)	1.19	Low
Bulk density (g cm-3)	1.30-1.57	Medium
CEC (meq/100g soil)	5.60	Low
Total N (%)	0.07	Very low
Available P (ppm)	16.75	Medium
Exchangeable K (meq/100g soil)	0.17	Medium low

Source: The chemical analysis of initial soil samples were done in SRDI, Dinajpur, Bangladesh

Appendix III: Weather data for growing season of wheat, 2014-15

Month	Relative humidity (%)	Temperature		Total rainfall (mm)
		Minimum (°C)	Maximum (°C)	
November/13	89	14.5	28.0	0.0
December/13	82	10.9	25.8	0.0
January/14	85	10.5	22.0	0.0
February/14	87	11.5	25.1	30.0
March/14	86	16.6	30.7	1.0
April/14	85	18.8	29.5	21.0

Source: Wheat Research Centre, Dinajpur