PHYSIOLOGICAL EVALUATION OF WHEAT GENOTYPES UNDER WATER DEFICIT STRESS

A THESIS

BY

MD. SOHEL RANA

Student No. 1405045

Session: 2014-2015

Thesis Semester: January-June, 2015

MASTER OF SCIENCE

IN

CROP PHYSIOLOGY AND ECOLOGY

DEPARTMENT OF CROP PHYSIOLOGY AND ECOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITYDINAJPUR

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JUNE, 2015

DEDICATED
TO
MY BELOVED PARENTS

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June 2015 The Author

ABSTRACT

To evaluate wheat genotypes under PEG induced water deficit stress during germination and early seedling growth twenty wheat genotypes were tested in three levels of water potential (Tap water, -2 bars and -4 bar) at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur during September 2014 to October 2014. Speed of germination as expressed by rate of germination, co-efficient of germination and germination vigor index of all wheat genotypes were delayed with the increment of water stress induced by PEG. Shoot length and root length and seedling dry weight of 10 days old seedlings were found to be affected due to the increment of water stress. But the magnitude of reduction of these parameters with the increment of water stress were not similar to all wheat genotypes. Among the twenty wheat genotypes BARI Gom 25, E 28 and BAW 1170 showing higher Stress Tolerance Index (STI) and BAW 1140 showing the lowest Stress Tolerance Index (STI) based on seedling dry weight both at moderate and higher water deficit stress were selected as tolerant and sensitive genotypes. These four wheat genotypes grown under well water and water deficit stress condition at the research field of Crop Physiology and Ecology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of November 2014 to April 2015 to evaluate the drought sensitivity of wheat genotypes.Water deficit stress tolerant wheat genotypes showed greater stability of flag leaf chlorophyll, greater ability to retain water in leaf, higher level of proline in flag leaf and kernel, higher level of soluble sugar in flag leaf and greater ability to keep the canopy cooler compared to sensitive genotype under water deficit condition. Greater spike dry matter accumulation at peak, longer grain growth duration, better yield components such as spikes $m^{-2}$and grains $spike^{-1}$ under water deficit stress contributed to better tolerance of BARI Gom 25, E 28 and BAW 1170. The order of tolerance based on grain yield was BAW 1170 > BARI Gom 25 > E 28 > BAW 1140 and the order of tolerance based on above ground biological yield was BAW 1170 > E 28 > BARI Gom 25 > BAW 1140.

CONTENTS

TITLE **Page no.**

LIST OF TABLES

Table no. **Title of the Table** **Page no.**

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

Wheat, next to rice is the staple food of the people Bangladesh grown over an area of 0.43 million hectare with an annual production of about 1.303 million metric tons with an average of 3.03 t ha^{-1} (Anonymous 2014). The consumption of wheat is increasing with increasing food diversity in our country. The average yield of this crop in Bangladesh is however low compared to other wheat growing countries because wheat is mainly grown under non-irrigated conditions during the dry winter (November to April) in Bangladesh. Although the vast storage of soil moisture resulted from monsoon rain supports the plant growth favorably at the early stages of growth, the plant suffers from water stress at the reproductive stage when the residual soil moisture depletes (Karim *et al*. 2000).

Water stress adversely affects plant establishment and thereafter growth and development (Harris *et al*. 2002). Cell enlargement and assimilates partitioning is hindered by the stress. Under extreme condition, it may severely disturb several metabolic processes, which may result in diminished photosynthesis, checked cell enlargement and division and finally cell death (Kramer 1983). Water stress causes a declines in relative water content (RWC), chlorophyll and carotenoid content, membrane stability and nitrate reductase activity and increases accumulation of abscisic acid (ABA), proline (Chandrasekar *et al*. 2000), soluble sugar (Munns and Weir 1981, Kameli and Loesel 1993) and leaf and canopy temperature (Siddique *et al*. 2000). Water deficit reduces the interception of solar radiation in the canopy due to early senescence as well as rolling up the leaves (Muller 2001). Water stress at reproductive stage is more harmful to plant process than any other growth stage.

However, this problem will be further increased due to climate change. Due to raise in world temperature soil losses it´s moisture holding capacity as a result drought effect is accelerated. Thus the best option for yield improvement and yield stability of wheat under soil moisture deficit condition is to screen or develop drought tolerant wheat varieties and to ameliorate the adverse effect of water deficit stress through agronomic strategies**.**

Polyethylene glycol (PEG) widely used to induce water stress is a non-ionic water soluble polymer which is not expected to penetrate into the plant tissue rapidly (Kawasaki 1983). Selection for drought tolerance at early stage of seedlings is most frequently practiced using poly ethylene glycol (PEG 6000) in the medium (Rauf *et al.*, 2006). Earlier studies focusing on identification of the drought tolerant wheat genotypes using different concentrations of PEG 6000 have showed significant differences for different seedling traits (Rauf *et al.* 2006, Singh *et al.* 2008). The seedling traits when pooled together could discriminate between drought tolerant and susceptible genotypes (Noorka and Khaliq 2007).

A physiological approach would be the most attractive way to develop new varieties rapidly (Turner and Nicolas 1987), but it involves a deeper understanding of the yield determining process (Blum 1983). The use of physiological tolerance along with agronomic traits has been shown to be applicable and their relationship with water stress tolerance indices are considered strong enough to be exploited as a selection tool in the breeding of water stress tolerance cultivars (Allakhverdiev *et al*. 2000). Little studies have been done in Bangladesh on these which may act as supporting aspect for increasing wheat production in Bangladesh. Therefore the present study was carried out to expand wheat cultivation and to sustain wheat yield under drought prone area with the following objectives-

1. To evaluate wheat genotypes under PEG induced water deficit stress during germination and early seedling growth.
2. To evaluate the sensitivity of physiological traits of wheat resulting reduced grain yield under water deficit stress.

CHAPTER II

REVIEW OF LITERATURE

Wheat (*Triticum spp*) is one of the important cereal crops which received much attention of the various research workers throughout the world. Growth and development of wheat is depending on many environmental factors. Water deficit stress on wheat has a profound effect on growth and yield of the crop. In this regards many researchers throughout the world have made several observations on the effect of water deficit stress on wheat. But there is a little information on it is available in Bangladesh. However here is some of the information on the morpho- physiological characters affected by water deficit stress. In wheat along with other related crops have been reviewed below-

2.1. Effect of polyethylene Glycol (PEG) induced water deficit stress on germination and seedling growth of wheat

Selection for drought tolerance at early stage of seedlings is most frequently practiced using poly ethylene glycol (PEG 6000) in the medium (Rauf *et al.* 2006). PEG 6000 molecules are inert, non ionic and virtually impermeable chains and have frequently been used to induce water stress without causing any significant physiological damage to crop plants (Carpita *et al.* 1979). PEG can be used to modify the osmotic potential of nutrient solution culture and can induce plant water deficit in a relatively controlled manner, appropriate to experimental protocols (Lagerwerff *et al.*1961). Earlier studies focusing on identification of the drought tolerant wheat genotypes using different concentrations of PEG 6000 have showed significant differences for different seedling traits (Rauf *et al.*2006, Singh *et al.*

2008). The seedling traits when pooled together could discriminate between drought tolerant and susceptible genotypes (Noorka and Khaliq 2007).

Datta *et al*. (2011) tested five selected wheat varieties viz., K 9107, HD 2954, RAJ 4125, HUW 234, NW 2036 in artificial drought stress created by preparing solution of PEG-6000 having osmotic potentials of -1.0 bars during winter season of January to April, 2008. In order to assess the relative capability of drought tolerance various morpho-physiological parameters (water uptake by seeds, relative water content, fresh weight, dry weight, germination percentage, root length, shoot length, Tolerance index, % difference from growth, pH of seed leachate) were studied. Among the wheat varieties RAJ 4125 variety was found to be mostly drought tolerant among the other experimental varieties having significant higher germination percentage, drought tolerance index value.

With a view to understand the parameters which can be used as a quick criteria for drought tolerance, Almaghrabi (2012) evaluated eight wheat (*Triticumaestivum*L.) cultivars, four local cultivars (Madini, Kaseemi, Yamanei and Tabokei) and four introduced cultivars (Sakha 93, Giza 168, Seds 12 and Masr 1) to drought stress induced by polyethylene Glycol (PEG) 6000 at different concentration 0.0, 60, 120, 180, 240 and 300 g/l PEG during germination and seedling growth stage of plant development. Five germination parameters: final germination percentage, mean daily germination, germination index, mean germination time and coefficient of velocity of germination and eight seedling growth parameters; shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, seedling length and root number were measured under experiment conditions. Mean comparison showed that the highest value for most of parameters was recorded for Sakha 93 and Madini cultivars followed by Yamanei, Kaseemi and Tabokei. Results of variance analysis made clear that different osmotic potential had significant effect on all parameters except root dry weight. In contrast, using all germination and seedling growth parameters, except root number, under study can used as a selectable parameters to discrimination between tolerance and sensitive cultivars under drought stress in breeding programs and laboratory experiment would appear to be suitable for screening under drought stress.

An experiment was carried out by Khakwani *et al.* (2011) under laboratory conditions where seeds of six wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were raised in Petri dishes and were either treated with distilled water (control) or 15% polyethylene glycol (PEG) 6000 solution. Variety Hashim-8 gave maximum germination percentage (93.33%) whereas maximum coleoptile (1.78cm) and shoot length (5.77cm) was observed in variety DN-73 which was statistically at par with variety Hashim-8. Similarly, root length (3.63cm), fresh shoot (0.15g) and root weight (0.12g) was maximum in variety Dera-98 which was statistically at par with variety Hashim-8.

Raza *et al.* (2012) tested eight wheat (*Triticum aestivum* L.) genotypes namely (Pasban-90, Inqalab-91, Auqab-2000, AS-2002, Sahar-2006, Shafaq-2006, Lasani-2008, and FSD-2008) for screening in laboratory against water deficit stress. Three techniques were used for the screening. These techniques are (I) seed germination at -0.6 MPa external water potential (PEG-6000), (2) seedling growth after 14 days at -0.6 MPa (PEG-6000), and (3) plant water relations of seven-week-old pot-grown plants. Significantly highest germination stress tolerance index, root length stress tolerance index and the water content was recorded in Lasani-2008 and lowest was recorded in Auqab-2000. Dry matter percentage was recorded significantly highest in pasban-90 while the lowest dry matter percentage was observed in auqab-2000. FSD-2008 gained maximum plant height and was recorded minimum in Inqilab-91. Relative saturation deficit was significantly highest in Auqab-2000 while the significantly lowest relative saturation deficit was recorded in case of Lasani- 2008.

Khayantnezhad *et al.* (2010) evaluated ten wheat cultivars in six levels of drought treatments (0, -3, -6, -9, -12 and -15 bar) induced by PEG. They found significant decrease in all traits studied in stress level. Ardabil cultivar had the longest root with 72.6 mm and Seimareh had the longest length of plumule. The highest germination percentage belonged to Ardabil 4057 (78%) and the lowest germination percentage belonged to Marahheh(49%). Ardabil 4057 showed the highest velocity of germination and Ahar showed the lowest velocity of germination. Traits in tolerant cultivars did not show a significant decline up to -3 bar. Considering all germination indices, Ardabil 4057 was the most tolerant and Ahar 11010 was the most sensitive cultivar.

Homayoun *et al.* (2011) evaluated five wheat genotypes in five levels of drought treatments (distilled water, -2, -4, -6 and -8 bar) induced by PEG. They found significant decrease in percentage of germination, germination rate, length of radical and plumule and radical and plumule dry weight in stress level. On the basis of the results, Seimareh genotype was found to be the most resistant cultivar in stress condition that they have the highest level of all traits in their study.

In order to investigate the synthetic wheat genotype reactions to drought stress in germination stage Jahanbin *et al*. (2012) conducted an experiment consisting of 12 genotypes from synthetic wheat and four levels of poly ethylene glycol density (PEG) with osmotic potentials of 0, -4/0, -8/0, and -2/1 MPa. In this study, germination indices including: germination rate (GR), germination index (GI), mean of emergence time (MET), final germination percentage (FGP), and germination rate index (GRI) were calculated. The analysis of variance for germination indices showed that there was a significant difference between different levels of osmotic potentials and genotypes for all the investigated characteristics. The mutual effects of variance in the amount have been observed significant between the genotype and the levels of poly ethylene glycol (drought) for germination index, final germination percentage, germination rate index, length of radicle, length of plumule. Based on Fernandez index (STI), according to their germination criteria, S2 genotype was the most resistant type and S9 was the most sensitive one.

Seven wheat cultivars (Azar, Omid, Dorom, Tabaci, Keraceharvand, Arvand and Gv 3-20) were evaluated in six levels of drought treatments (distilled water,-3,-6,-9,-12,-15 bar) by Jajarmi (2009). Results indicated significant differences among cultivars and drought stress levels. In all traits, a significant decrease was observed with increase in stress level. It seems that the length of stem among the other traits has more sensitivity to drought stress. Drought stress reduced the radical length at more than -6 bars. Keraceharvand variety had the longest length of root with 63.58 mm. Azar had the longest length of plumule. The highest germination percentage belonged to Gv 3-20 (78%). The lowest germination percentage belonged to Arvand with (36%). The percentage of germination and the velocity of germination lessened when drought stress exceeded more than -12bars. Gv 3- 20, tabaci had the highest velocity of germination. The highest coefficient of velocity of germination belonged to Tabaci. Traits in tolerant cultivars did not show a significant decline up to -3 bars. Considering all germination indices, the most tolerant cultivar was Gv-3-20 and Arvand was the susceptible cultivar.

Two durum wheat (*Triticum durum* Desf.) cultivars were tested by Sayar *et al.* (2010) for salt and drought tolerance at germination, seedling emergence and early seedling growth in NaCl and PEG-8000 solutions of different osmotic potentials (–0.2, –0.4, –0.6 and –0.8 MPa). Daily and final germination and emergence percentage, as well as germination and seedling emergence rate, seedling growth, fresh and dry weight were recorded under controlled conditions. Results showed that germination and emergence rates were delayed by both solutions in both cultivars, but Omrabia showed higher germination and emergence rates than BD290273 in NaCl while BD290273 was less affected by NaCl and PEG solutions at the emergence stage. Sodium chloride had a lesser effect on both cultivars in terms of germination rate, emergence rate, final germination and emergence percentage than did PEG-8000. This conclusively proves that the adverse effect of PEG-8000 on germination, emergence and early seedling growth was due to the osmotic effect rather than to the specific ion. Seedling growth was reduced by both stresses. However, NaCl usually caused less damage than PEG to durum wheat seedlings, suggesting that NaCl and PEG acted through different mechanisms.

Forty wheat genotypes were screened by Ahmad *et al.* (2013) for drought tolerance using 0, 7.5, 15 and 22.5% Polyethylene Ethylene Glycol 6000 solutions at PMAS

Arid Agriculture University, Rawalpindi, Pakistan during 2009-10. The seedling traits showed a decreasing trend in response to increased concentrations of PEG 6000. Wheat genotype Lyalpur-73 was found the best for germination percentage (87.5). The genotypes C-591 had maximum germination rate index (2.4). Wheat genotypes Pasban 90 and WC-18 possessed maximum root length (9.9) and seedling vigor (7.4) respectively. The genotype Auqab-2000 showed maximum shoot length (8.3). Wheat genotypes Pak-81 along with CB 335 had maximum coleoptile length (1.9). Germination percentage and germination rate index showed positive correlation with all other traits. Root length showed positive association with shoot length and coleoptile length. While shoot length had positive correlation with coleoptile length and seedling vigour.

2.2. Effect of water deficit stress on physiological traits of wheat

Stress exposed plants immediately lower down relative water content of their leaves, the decrease in leaf water potential and osmotic potential is also reported (Grover *et al.* 2004, Jing and Huang 2002). Singh *et al.* (1990) observed significant differences in water potential among wheat genotypes under water stress. While Sinclair and Ludlow (1985) proposed that leaf relative water content (RWC) was a better indicator of water status than water potential.

Photosynthesis, which is the basic process influencing crop productivity, is inhibited by water stress (Chaves and Oliveira 2004). The major effect of water deficiency is the closure of stomata that leads to reduced transpiration rate and a lowered internal concentration of CO_2, which in turn inhibits the Calvin-cycle even at moderate water deficits (Horton *et al.*1996). Galle *et al*. (2002) characterized some drought tolerant wheat cultivars exposing them to low water potential by growth response, stomatal conductance of leaves, by changes in water relations and ion accumulation of tissues and by fluorescence induction parameters. They found two strategies of acclimation to drought stress: plants using the first strategy save tissue water content by a fast decrease of stomatal conductance, maintain pressure potential and photosynthetic activity of leaves. In the second group the closure of stomata occurs later resulting in an intensive loss of water and a fast decrease of water potential in the leaves and tissues restore their turgor after a relatively long acclimation phase.

Osmoregulation is one of the mechanisms that plants use to combat the detrimental effects of water loss by synthesizing compatible solutes, typically certain polyols, sugars, amino acids, betaines and related compounds (Bohnert *et al*. 1996, Ramanjulu and Bartels 2002). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been known to be usually higher in stress tolerant than in stress-sensitive plants (Ashraf and Foolad 2007). Soluble sugars have been shown to increase in the leaves of wheat under water stress (Munns and Weir 1981, Kameli and Loesel 1993). They are also considered to play an important role in osmotic adjustment which is widely regarded as adaptive response to water stress conditions (Turner and Jones 1980, Kameli and Loesel 1993).

The water stress induced decrease in membrane stability indicates the extent of lipid peroxidation caused by active oxygen species (Baisak *et al*. 1994, Menconi *et al*. 1995). Lower lipid peroxidation and higher membrane stability (lower ion leaching) have also been reported in tolerant genotypes of wheat (Kraus *et al*. 1995). Sairam and Saxena (2000) found decrease in the membrane stability index of leaves with increasing age as well as under water stress.

Nine commercial wheat cultivars of Pakistan viz; Inqulab-91, AS-2002, Sehar-2006, Shafaq-2006, Bhakkar-2002, Auqab-2000, GA-2002, Chakwal-50 and Fareed-06 were evaluated for their physiological performance and drought resistance index under different levels of soil moisture. Germination of these cultivars was tested nder PEG induced osmotic stress of -2, -4, -6, and -8 bars. Highest germination percentage was observed for Fareed-06, Chakwal-50, GA-2002 and AS-2002 at -8 bars osmotic stress. To study physiological responses and drought resistance index, plants were grown in clay pots and water equivalent to 75%, 50% and 25% field capacity was applied. Decrease in soil moisture significantly affected relative water content, leaf succulence, chlorophyll content, cell membrane stability index, number of grains/spike and 100 grain weight. Significant variation was found among cultivars. Based on physiological responses and drought resistance index performance of Chakwal-50, GA-2002 and Bhakkar-2002 were found relatively better under limited moisture conditions (Razzaq *et al*. 2013).

2.3. Effect of water deficit stress on yield attributes and yield of wheat

Large differences in kernels per spikelet and kernel weight indicated that these two variables were responsible for yield adjustments to stress during spikelet and kernel development phase (Duggan and Fowler 2006). Baser *et al*. (2004) studied the effect of water stress on the yield and yield components of winter wheat and found a decrease of about 40% in yield under water stressed conditions as compared to control. Weight of grains/spike was reported by many researchers as the most closely linked variable related to grain yield per unit area and was often used in selecting high yielding wheat strains (Kumbhar *et al.* 1983). And 1000-grain weight had also been shown as the main yield component accounting for 20% of variation in wheat grain yield (Collaku 1989). Less decrease in wheat yield under water stress is usually observed in water stress tolerant cultivars as compared to sensitive ones (Gaspar *et al.* 2005, Khakwani *et al.* 2011).

CHAPTER III

MATERIALS AND METHODS

The present investigation was conducted in two experiments. Details of the methodology of the study followed during the research period are presented in this chapter.

Experiment -1: Evaluation of wheat genotypes under PEG induced water deficit stress during germination and early seedling growth

3.1.1. Location and duration

The experiment was conducted at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur during September 2014 to October 2014.

3.1.2. Experimental design and treatments

The experiment was carried out in two factors completely randomized design with three replications.

The treatment factors A and B were -

1. Factor A: Three levels of water potential-
   i. Control (Tap water)
   ii. Moderate stress (-2 bars) induced PEG
   iii. Higher stress (-4 bars) induced by PEG.
2. Factor B: Twenty wheat genotypes including advance lines and popular varieties.

3.1.3. Collection of seed

Twenty wheat genotypes (Table 1) including six varieties and fourteen advanced lines collected from Wheat Research Centre (WRC) of BARI, Dinajpur were used for the present study.

Table 1. List of wheat genotypes used for the present study

Sl. No.	Genotypes	Remarks
1.	Shatabdi	Variety
2.	Sourab	Variety
3.	BARI Gom 25	Variety
4.	BARI Gom 26	Variety
5.	BARI Gom 27	Variety
6.	BARI Gom 28	Variety
7.	E 30	Advanced line
8.	E 23	Advanced line
9.	E 2	Advanced line
10.	E 38	Advanced line
11.	E 24	Advanced line
12.	E 34	Advanced line
13.	E 28	Advanced line
14.	BAW1138	Advanced line
15.	BAW1170	Advanced line
16.	BAW1157	Advanced line
17.	BAW1157	Advanced line
18.	BAW1163	Advanced line
19.	BAW1140	Advanced line
20.	BAW1151	Advanced line

3.1.4. Placement of seed for germination

Seeds of twenty wheat genotypes collected from Wheat Research Centre (WRC) of BARI, Dinajpur, Bangladesh were surface sterilized by dipping the seeds in 1% mercuric chloride solution for 2 minutes and rinsed thoroughly with sterilized water. Three levels of water potential (Tap water, -2 bars and -4 bar) was developed dissolving calculating amount of PEG in tap water. Calculation was done using the following formula-

$$OP=(-1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C + (2.67 \times 10^{-4}) \times CxT + (8.39 \times l0^{-7}) \times C^2T$$

where,

C=PEG concentration; T=Tempearture.

as described by (Michel 1983). Thirty seeds of each genotype were placed for germination in 11cm petridish on sand bed irrigated with respective treatments. The petridishes were irrigated daily with required amount of respective solution. Each treatment was replicated thrice. Seedlings were allowed to grow up to 7 days after placement of germination.

3.1.5. Data recorded on germination characters

Germination was counted at 24-hour interval and continued upto 7th day (168 hrs). A seed was considered germinated as plumule and radicle came out and were >2 mm long.

The rate of germination was calculated following Krishnasamy and Seshu (1990)

$$\text{Rate of germination (\%)} = \frac{\text{No. of seeds germinated at 48 h}}{\text{No. of seeds germinated at 168 h}} \times 100$$

Co-efficient of germination and vigor index were calculated using the following formulae (Copeland 1976).

$$\text{Co-efficient of germination} = \frac{100\,(A_1 + A_2 + \cdots + A_n)}{A_1 T_1 + A_2 T_2 + \cdots A_n T_n}$$

$$\text{Vigor index} = \frac{A_1}{T_1} + \frac{A_2}{T_2} + \cdots + \frac{A_n}{T_n}$$

Where,

A = Number of seeds germinated

T = Time (days) corresponding to A

n = No of days to final count

3.1.6. Data recorded on shoot length, root length and seedling dry weight

At 7th day after placement for germination, all other seedlings were removed from each petridish keeping 10 healthy seedlings and allowed to grow upto 10 days by adding required amount of respective solution daily. At 10 days after placement for germination, five seedlings from each petridish were sampled. Shoot and root length of individual seedling were recorded manually with scale. Then the seedlings were dried separately at 70^0C for 72 h in an electric oven (Model- E28# 03-54639, Binder, Germany) and weight were recorded with an electrical balance (Model- AND EK-300 i). The mean length (cm) and dry weight were calculated for each treatment combination.

3.1.7. Stress tolerance index (STI)

Stress tolerance index was calculated as Goudarzi and Pakniyat (2008) using the following formula-

$$\text{Stress tolerance index} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

3.1.8. Statistical analysis

The data were analyzed by partitioning the total variance with the help of computer using MSTAT program. The treatment means were compared using Duncan's Multiple Range Test. Correlation analysis was also done.

Experiment -2: Physiological evaluation of wheat genotypes for water deficit stress tolerance

3.2.1. Location and duration

The experiment was set up at the research farm of Crop Physiology and Ecology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur during November 2014 to April 2015. The experimental site is situated under the Dinajpur Sadar Upazila and located at 25º39′ N latitude and 88º41′ E longitude with an elevation of 37.58 meter above the sea level.

3.2.2. Soil and climate

The experimental field was a medium high land belonging to the non-calcarious dark gray floodplain soil under the agro-echological zone of Old Himalayan Piedmont Plain (AEZ-1). The soil is sandy loam under the Order Inceptisol. The experimental site is situated in the sub-tropical region characterized by heavy rainfall during the months from May to September and scantly rainfall in the rest of the year. Details of the soil characteristics of the experimental site is presented in Appendix I and details of the meteorological data in respect to temperature, rainfall and relative humidity during the growing period of the experimental site are presented in the Appendix no. II.

3.2.3. Experimental design and layout

The experiment was conducted in a split plot design with three replications. The unit plot size was 2.5 m x 2 m. The distance between plots was 0.5 m and block to block distance was 1.0 m. The two growing conditions were placed in the main plots as main plot treatments whereas four wheat genotypes were placed randomly in the sub plots as sub plot treatments.

3.2.4. Experimental treatments

The treatments were-

A. Main plot: Two water regimes
    1. Well water: Three irrigations were applied at crown root initiation (CRI), anthesis and grain filling stages.
    2. Water deficit stress: No irrigation was applied and the crop was protected from rainfall by rainout shelter.

B. Sub-plot: Four wheat genotypes including three tolerant and one susceptible selected based on STI for seedling dry weight from experiment-1
    1. BARI Gom 25
    2. E 28
    3. BAW 1170
    4. BAW 1140

3.2.5. Land preparation

The land was ploughed by a power tiller and was leveled by harrowing and laddering carefully. The weeds and stubble were removed and main plots, sub-plots and blocks were prepared.

3.2.6. Fertilizer application

A fertilizer dose of 140-35-75-18-2-0.5 kg ha^{-1} N, P, K, S, Zn and B was applied in the form of Urea, Triple Supper phosphate (TSP), Murate of potash (MP), Gypsum and Boric acid, respectively. After land preparation, full dose of P, K, S, Zn, B and two third of N were incorporated thoroughly into the soil as basal dose. The remaining amount of N was applied at 25 after seedlings emergence.

3.2.7. Sowing of seed

Seeds of four wheat genotypes (BARI Gom 25, E 28, BAW 1170 and BAW 1140) were sown on 30 November 2014 in rows of 20 cm apart, at the rate of 120 kg ha^{-1}. Slight irrigation was given for uniform germination after sowing.

3.2.8. Irrigation

Half of plots (well watered) were irrigated three times (at 25, 55 and 75 days after sowing) but the remaining plots (water stressed) were not irrigated throughout the growing period to maintain water deficit stress condition and the crop was protected from rainfall by rainout shelter.

3.2.9. Intercultural operations

After sowing, care was taken against birds up to 15 days. The crop was kept weed free and for controlling diseases, Tilt 250 EC was sprayed for avoiding Stripe Rust when necessary.

3.2.10. Data collection

3.2.10.1. Spike dry matter accumulation

At anthesis 80 main shoots were tagged from each plot. Four tagged main shoot spike were harvested at every 4^{th} day beginning from anthesis to quantify spike dry matter accumulation pattern. Collection of main shoot spike in all genotypes was continued up to 40 days after anthesis (DAA) for both well watered and water deficit stress condition. The harvested spikes were kept in oven at 70 ^{o}C for 72 hours. After oven drying, spikes were weighted with an analytical balance (AND Electronic Balance Model EK 300 i).

3.2.10.2. Estimation of proline

Proline content of flag leaf and kernel at 16 days after anthesis in all wheat genotypes grown in two different growing conditions were estimated. Flag leaves and spike from each replication of each genotype were collected and immediately kept in the ice-bag and brought to Crop Physiology and Ecology Laboratory of HSTU for proline estimation. The kernels from spike were separated and 0.5g of fresh weight of both flag leaf and kernels were taken for proline estimation. Subsequently proline was estimated as Bates (1973).

At first Ninhydrin reagent was prepared in such a way so that it was utilized for proline estimation within two hours of preparation. To prepare Ninhydrin reagent, addition of 30 ml glacial acetic acid and 20 ml 6M orthophosphoric acid was 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°C. Proline standards were prepared for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ ml with distilled water.0.5g of fresh sample was crushed in mortar and pastte 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. 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, boiled for one hour at 100°C 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 (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 520 nm with pure toluene as a blank. The proline content was determined from a standard curve and calculated on a freash weight basis as follows:

µmoles proline / g of fresh plant material =

{(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample/5)

3.2.10.3. Estimation of soluble sugar

The soluble sugar content of flag leaf collected at 16 days after anthesis in case of all wheat genotypes in both growing conditions were determined as Yoshida *et al.* (1976).

The dried flag leaf samples were ground finely in mortar and pestle. 50 mg of finely ground sample was placed into a 15 ml centrifuge tube and 10ml of 80% ethanol was added. The tubes were covered with aluminium foil and were kept in a water bath at 80-85^{0}C for 30 minutes. Then the samples were centrifuged and decanted into a 50 ml beaker. This extraction was repeated three times. The alcohol extract was evaporated on a water bath at 80-85^{0}C until most of the alcohol was removed (e.g. the volume was reduced to about 3 ml). The volume was made up to 25 ml with distilled water.

5 ml of sugar extract was transferred to a 100 ml volumetric flask and made up to volume with distilled water. 5 ml of this diluted sugar extract was taken into a pyrex test tube and then these tubes containing the glucose standards (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/5ml) were put into an ice bath. To each tube 10ml

of anthrone reagent (2g of anthrone was dissolved in one liter of concentrated sulphuric acid and was stored in a refrigerator. A fresh solution was prepared every 2 days) was added slowly, allowing the reagent to run down the side of the test tube. The solution was stirred slowly with a glass rod. The tubes were put into boiling water bath for exactly 7.5 minutes and were cooled immediately in ice. Then the absorbance was measured at 630 nm and sugar content as mg/g dry weight was estimated using standard curve.

3.2.10.4. Estimation of chlorophyll

Chlorophyll content of the flag leaf at 8 and 24 days after anthesis was estimated according to Witham *et al.* (1986). 0.25g of leaf was taken from middle position of the flag leaf in a brown bottle containing 25ml of 80% aqueous acetone. The bottles were kept in dark for 48 hours. The optical density of this green colored solution was determined against 80% acetone as blank using spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 645 and 663 nm. Total chlorophyll of fresh leaf was determined using following formula-

Total chlorophyll (mg g^{-1} FW) = $[20.2(D_{645}) + 8.02(D_{663})] \times [V/(1000 \times W)]$

Where,

V = Volume of 80% aqueous acetone (ml)

W = Weight of fresh leaf (g)

D_{645} = Absorbance at 645nm wavelength

D_{663} = Absorbance at 663nm wavelength

3.2.10.5. Relative leaf water content

Relative flag leaf water content was determined at 16 days after anthesis. Five flag leaves for each treatment combination collecting from the field was sealed immediately in plastic bags and quickly transferred to the laboratory. Fresh weight was taken within 2 hours after excision. Turgid weight was obtained after soaking leaves in distilled water in test tubes for about 16 to 18 hours at room temperature. Dry weight was obtained after oven drying the leaf samples for 72 h at 70^{o}C. Relative leaf water content was calculated from the equation of Schonfeld *et al* . (1988).

$$RLWC\ (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.2.10.6. Canopy temperature Depression (CTD)

The difference between ambient air temperature and canopy temperature in degree centigrade is known as Canopy temperature Depression (CTD). The hand held infra-red thermometer (Model: Crop TRAC item no. 2955L-Spectrum Tecnologies, Inc.) was used to measure this trait. The CTD was recorded at 8 days and 24 days after anthesis during noon period under bright sunlight and less wind.

3.2.10.7. Plant height

Plant height was measured from the base of the plant to the tip of the spike excluding the awn. Ten plants sample from each treatment were taken and means were calculated.

3.2.10.8. Spike character

Ten spikes from the main shoot were collected randomly per plot to collect data on spike characters. Spike length (excluding awn) was measured manually using scale and number of grains spike^{-1} was counted manually.

3.2.10.9. Number of spikes m^{-2}, grain yield m^{-2} and straw yield m^{-2}

The samples were collected from an area of 2m × 1m from the center of each plots by cutting the plant at ground level. Then spikes were counted and collected in a cloth bag (2´ × 1.5´). The samples were dried in sun, threshed and cleaned manually and weight of grain and straw were taken after drying in the sun.

3.2.10.10. Thousand grain weight

From each plot thousand grains were taken randomly from dried sample and weighed.

3.2.10.11. Grain and above ground biological yield

Grain and above ground biological yield were expressed in t/ha, grain yield also adjusted to 12% moisture.

3.2.10.12. Soil moisture content

The soil samples are collected from desired depths from several places, in air tight containers. The samples are weighed and then they are dried in an oven at 105°C for about 24 hours or till constant weight. The samples are then taken out and weighed again and the loss in weight is the amount of water soil.

Soil moisture content (per cent by weight):

$$= \frac{\text{Weight of moist sample} - \text{weight of oven dry sample}}{\text{Weight of oven dry sample}} \times 100$$

3.2.10.13. Stress susceptibility index

Stress susceptibility index (SSI) was calculated for grain yield as described by Fisher and Maurer (1978).

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

Where,

Y = Grain yield of genotype in a stress environment

Y_p = Grain yield of genotype in a stress-free environment

X = Mean Y of all genotypes

X_p = Mean Y_p of all genotypes

(Higher SSI indicates greater susceptibility)

3.2.10.14. Statistical analysis

The data were analyzed by partitioning the total variance with the help of computer by using MSTAT program. The treatment means were compared using Duncun's Multiple Range Test (DMRT) at $P \leq 5\%$ level. Correlation analysis was also done.

CAHPTER IV

RESULTS AND DISCUSSION

The results of the present study have been presented in several tables and figures. Adequate discussion and possible interpretations whenever suitable have been provided in this chapter.

Experiment -1: Evaluation of wheat genotypes under PEG induced water deficit stress during germination and early seedling growth

4.1.1. Germination characters

Rate of germination: Rate of germination which indicates the speed of germination was significantly influenced by the interaction effect of water potential levels and wheat genotypes during germination (Table 2). Rate of germination was higher at control (with a range from 82.66 in BAW 1170 to 98.66 in Shatabdi and BARI Gom 27 and a mean of 93.28), moderate at moderate stress (with a range from 77.00 in BAW 1140 to 95.66 in E 34 and a mean of 88.36), and lower at higher water deficit stress (with a range from 63.33 in BAW 1140 to 91.33 in E 34 and a mean of 81.44). The results also showed that the speed of germination was reduced with the increment of water stress but the degree of reduction in rate of germination was not similar for all wheat genotypes at moderate and higher water deficit stress compared to control. At moderate water deficit stress, wheat genotypes- BARI Gom 25, BARI Gom 26, BARI Gom 28, E 30, E 2, E 24, E 34, BAW 1138, BAW 1170 and BAW 1151 showed less than 5% reduction and wheat genotypes- Shatabdi, Sourav, BARI Gom

Table 2. Rate of germination of different wheat genotypes as influenced by PEG induced water deficit stress

Wheat genotypes	Rate of germination (mean ± SE)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (-2 bar)	Higher stress (- 4 bar)		
Shatabdi	98.66± 1.70	93.00± 1.09	87.33± 2.18	-5.74	-11.48
Sourav	90.00± 1.01	83.33± 1.49	82.66± 1.47	-7.41	-8.16
BARI Gom 25	97.33± 3.05	94.66± 0.62	88.66± 4.98	-2.74	-8.91
BARI Gom 26	97.33± 2.40	94.33± 1.41	88.00± 1.56	-3.08	-9.59
BARI Gom 27	98.66± 2.00	89.33± 2.33	86.66± 4.00	-9.46	-12.16
BARI Gom 28	93.66± 1.33	91.66± 6.67	78.66±2.60	-2.14	-16.02
E 30	94.33± 0.54	93.00± 0.77	89.66± 0.83	-1.41	-4.95
E 23	94.00± 1.14	86.66±2.60	83.33± 1.20	-7.81	-11.35
E 2	91.00± 0.83	89.66± 0.54	85.00± 0.77	-1.47	-6.59
E 38	90.66± 0.77	85.28± 0.11	71.88± 0.83	-5.93	-20.71
E 24	94.66± 1.14	92.66± 1.67	85.00± 1.14	-2.11	-10.20
E 34	97.00± 0.77	95.66± 0.54	91.33± 0.77	-1.38	-5.85
E 28	97.33± 0.83	88.00± 0.11	77.33± 1.20	-9.59	-20.55
BAW 1138	92.66±2.60	90.66± 1.14	83.33±2.60	-2.16	-10.07
BAW 1171	97.33± 0.83	89.33± 0.77	87.33± 0.54	-8.22	-10.27
BAW 1170	82.66± 1.67	80.66±2.60	70.00± 0.11	-2.42	-15.32
BAW 1157	91.33± 1.20	83.00± 0.54	80.66± 1.14	-9.12	-11.68
BAW 1163	87.33± 0.11	81.33± 0.83	73.66± 1.20	-6.87	-15.65
BAW 1140	90.33± 1.20	77.00± 1.14	63.33±2.60	-14.76	-29.89
BAW 1151	89.33± 0.83	88.00± 0.54	75.00± 1.67	-1.49	-16.04
Mean	93.28	88.36	81.44		
Significant level	**				
CV (%)	2.08				

27, E 23, E 38, E 28, BAW 1171, BAW 1157, BAW 1163 and BAW 1140 showed more than 5% reduction in rate of germination compared to control.

At higher water deficit stress, wheat genotypes- Sourav, BARI Gom 25, BARI Gom 26, E 30, E 2 and E 34 showed less 10% reduction and wheat genotypes- Satabdi, BARI Gom 27, BARI Gom 28, E 23, E 38, E 24, E 28, BAW 1138, BAW 1171, BAW 1170, BAW 1157, BAW 1163, BAW 1140 and BAW 1151 showed more than 10% reduction in rate of germination compared to control.

Co-efficient of germination: Co-efficient of germination which indicates the speed of germination was significantly influenced by the interaction effect of water potential levels and wheat genotypes during germination (Table 3). Co-efficient of germination was found higher at control (with a range from 21.61 in BAW 1163 to 22.98 in E 24 and a mean of 22.31), moderate at moderate stress (ranging from 21.47 in BAW 1170 to 22.04 in BARI Gom 26, BARI Gom 28, E 23 and E 34 with a mean of 21.76), and lower at higher water deficit stress (with a range from 21.01 in BAW 1170 and BAW 1140 to 21.99 in E 30 and a mean of 21.59). The results on co-efficient of germination showed that the speed of germination was reduced with the increment of water deficit stress but the degree of reduction in co-efficient of germination was not similar for all wheat genotypes at moderate and higher water deficit stress compared to control. At moderate water deficit stress, wheat genotypes- BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 23, E 34, E 28, BAW 1138, BAW 1170 and BAW 1151 showed less than 2% reduction and wheat genotypes-Satabdi , Sourav, E 30, E 2, E 38, E 24, BAW 1171, BAW 1157, BAW 1163 and BAW 1140 showed more than 2%

Table 3. Co-efficient of germination of different wheat genotypes as influenced by PEG induced water deficit stress

Wheat genotypes	Co-efficient of germination (mean ± SE)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (-2 bar)	Higher stress (- 4 bar)		
Shatabdi	22.85± 0.26	21.91± 0.12	21.97± 0.18	-4.11	-3.85
Sourav	22.48± 0.27	21.75± 0.25	21.64± 0.21	-3.25	-3.74
BARI Gom 25	22.16± 0.25	21.93± 0.18	21.31± 0.18	-1.04	-3.84
BARI Gom 26	22.39± 0.06	22.04± 0.26	21.54± 0.25	-1.56	-3.80
BARI Gom 27	22.03± 0.18	21.95± 0.29	21.17± 0.19	-0.36	-3.90
BARI Gom 28	22.15± 0.25	22.04± 0.12	21.75± 0.06	-0.50	-1.81
E 30	22.23± 0.26	21.72± 0.31	21.99± 0.53	-2.29	-1.08
E 23	22.23± 0.22	22.04± 0.12	21.84± 0.26	-0.86	-1.75
E 2	22.55± 0.06	21.72± 0.53	21.77± 0.12	-3.68	-3.46
E 38	22.93± 0.18	21.95± 0.29	21.77± 0.18	-4.27	-5.06
E 24	22.98± 0.15	21.98± 0.12	22.09± 0.18	-4.35	-3.87
E 34	22.31± 0.25	22.04± 0.25	21.98± 1.43	-1.21	-1.48
E 28	22.19± 0.12	21.89± 0.53	21.22± 0.06	-1.35	-4.37
BAW1138	22.42± 0.53	22.00± 0.18	21.56± 0.31	-1.87	-3.84
BAW1171	22.21± 1.43	21.49± 0.18	21.83± 1.43	-3.24	-1.71
BAW1170	21.89± 0.25	21.47± 0.12	21.01± 0.53	-1.92	-4.02
BAW1157	22.12± 0.18	21.64± 0.31	21.71± 0.18	-2.17	-1.85
BAW1163	21.61± 0.29	20.50± 0.12	21.27± 1.43	-5.14	-1.57
BAW1140	22.63± 0.25	21.50± 0.12	21.01± 0.19	-4.99	-7.16
BAW1151	21.88± 1.43	21.70± 0.25	21.43± 0.25	-0.82	-2.06
Mean	22.31	21.76	21.59		
Significant level	**				
CV (%)	1.41				

reduction in rate of germination compared to control. At higher water deficit stress, wheat genotypes- BARI Gom 28, E 30, E 23, E 34, BAW 1171, BAW 1157 and BAW 1163 showed less than 2% reduction and wheat genotypes- Satabdi, Sourav, BARI Gom 25, BARI Gom 26, BARI Gom 27, E 2, E 38, E 24, E 28, BAW 1138, BAW 1170, BAW 1140 and BAW 1151 showed more than 2% reduction in co-efficient of germination compared to control.

Germination vigor index: Different levels of water potential interacted significantly wheat genotypes to influence germination vigor index which also expresses the speed of germination (Table 4). Germination vigor index was found higher at control (ranging from 34.50 in BAW 1163 to 43.62 in BAW 1171 with a mean of 39.61), moderate at moderate stress (ranging from 29.00 in BAW 1163 to 40.86 in E 30 with a mean of 37.09), and lower at higher water deficit stress (with a range from 26.29 in BAW 1163 to 37.96 in BARI Gom 27 and a mean of 34.65). The results on germination vigor index showed that the speed of germination was reduced with the increment of water deficit stress but the degree of reduction was not similar for all wheat genotypes at moderate and higher water deficit stress compared to control.At moderate water deficit stress, wheat genotypes- BARI Gom 25, BARI Gom 27, E 30, E 2, E 24 and BAW 1151 showed less than 5% reduction and wheat genotypes- Satabdi , Sourav, BARI Gom 26, BARI Gom 28, E 23, E 38, E 34, E 28, BAW 1138, BAW 1171, BAW 1170, BAW 1157, BAW 1163 and BAW 1140 showed more than 5% reduction in germination vigor index compared to control.At higher water deficit stress, wheat genotypes- Sourav, BARI Gom 25, BARI Gom 27, E 24, E 34 and BAW 1151 showed less than 10% reduction and wheat genotypes- Satabdi,

Table 4. Germination vigor index of different wheat genotypes as influenced by PEG induced water deficit stress

Wheat genotypes	Germination vigor index (mean ± SE)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (-2 bar)	Higher stress (-4 bar)		
Shatabdi	43.32± 1.83	37.49± 1.28	36.88± 0.87	-13.46	-14.87
Sourav	36.47± 0.48	34.17± 2.17	33.97± 0.48	-6.31	-6.86
BARI Gom 25	38.98± 0.91	38.02± 1.00	37.73± 0.91	-2.46	-3.21
BARI Gom 26	41.48± 0.87	37.57± 0.34	37.22± 0.34	-9.43	-10.27
BARI Gom 27	38.94± 1.00	38.47± 1.28	37.96± 0.18	-1.21	-2.52
BARI Gom 28	38.93± 1.25	36.87± 0.91	34.03± 0.34	-5.29	-12.59
E 30	42.76± 2.17	40.86± 0.72	36.01± 0.87	-4.44	-15.79
E 23	39.62± 0.34	37.43± 1.25	34.94± 0.76	-5.53	-11.81
E 2	41.59± 0.18	40.51± 0.76	36.71± 0.18	-2.60	-11.73
E 38	38.01± 0.18	33.77± 0.18	32.76± 0.48	-11.15	-13.81
E 24	37.99± 1.00	36.78± 1.00	36.38± 0.34	-3.19	-4.24
E 34	39.69± 0.48	37.28± 0.87	35.78± 0.18	-6.07	-9.85
E 28	40.04± 2.11	37.47± 1.28	34.93± 0.34	-6.42	-12.76
BAW1138	40.45± 0.48	36.89± 0.76	35.91± 1.00	-8.80	-11.22
BAW1171	43.62± 1.25	40.27± 1.00	33.71± 2.11	-7.68	-22.72
BAW1170	40.81± 0.72	38.72± 0.34	35.90± 1.25	-5.12	-12.03
BAW1157	40.04± 0.72	36.55± 1.00	33.89± 0.18	-8.72	-15.36
BAW1163	34.50± 1.00	29.00± 2.11	26.29± 0.76	-15.94	-23.80
BAW1140	40.34± 0.76	33.23± 1.25	27.75± 2.11	-17.63	-31.21
BAW1151	38.06± 0.72	37.02± 1.00	34.31± 1.28	-2.73	-9.85
Mean	39.61	37.09	34.65		
Significant level	**				
CV (%)	1.65				

BARI Gom 26, BARI Gom 28, E 30, E 23, E 2, E 38, E 28, BAW 1138, BAW 1171, BAW 1170, BAW 1157, BAW 1163 and BAW 1140 showed more than 10% reduction in vigor index compared to control.

As water is one of the primary requirement in seed germination (Evenari 1981) the water stress developed by PEG reduced germination ability of seed greatly. Slower germination of wheat under water deficit stress was found in present study. It may be due to lower surface contact of water with seed (Abdul- Baki and Anderson 1970) which restricts the water availability to the seeds (Uniyal and Nautiyal, 1998; Soltani et al., 2002). Water deficit stress may also lead to degradation and inactivation of the essential hydrolytic and other group of enzymes required for germination (Wilson 1971). Differential degree of sensitivity in speed of germination to different water potentials was also found among the wheat genotypes in the present study. It may be due to variability in genetically tolerance to water stress the wheat genotypes. Nayyar *et al*. (1995), Noorka and Khaliq (2007), Khayantnezhad *et al.* (2010) and Singh *et al.* (2008) also found differential sensitivity in germination velocity among different wheat genotypes in their studies.

4.1.2. Seedling growth

Shoot length: Shoot length of 10 days old seedling was significantly influenced by influenced by the interaction effect of water potential levels and wheat genotypes (Table 5). The shoot length was found to be higher at control at control (with a range from 15.43 cm in BAW 1151 to 23.78 cm in Sourav and a mean of 20.81 cm), moderate at moderate stress (ranging from 10.56 cm in BAW 1151 to 18.54 cm in BARI Gom 27 with a mean of 16.00 cm) and lower at higher water deficit stress

Table 5. Shoot length (mean ± SE) of different wheat genotypes at 10 days after placement for germination as influenced by PEG induced water deficit stress

Wheat genotypes	Shoot length (cm)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (-2 bar)	Higher stress (-4 bar)		
Shatabdi	23.18± 0.37	17.41± 0.15	12.88± 0.39	-24.89	-44.43
Sourav	23.78± 0.43	15.43± 0.29	10.72± 0.37	-35.11	-54.92
BARI Gom 25	20.73± 0.15	17.74± 0.15	14.72± 0.08	-14.42	-28.99
BARI Gom 26	21.71± 0.37	15.85± 0.32	10.72± 0.13	-26.99	-50.62
BARI Gom 27	23.63± 0.32	18.54± 0.28	8.92± 0.37	-21.54	-62.25
BARI Gom 28	17.43± 0.39	13.53± 0.08	8.25± 0.43	-22.37	-52.67
E 30	22.26± 0.37	17.83± 0.29	10.41± 0.15	-19.90	-53.23
E 23	19.78± 0.37	15.42± 0.13	10.33± 0.39	-22.04	-47.77
E 2	17.43± 0.43	15.85± 0.18	9.86± 0.29	-9.06	-43.43
E 38	23.00± 0.15	17.88± 0.32	11.97± 0.37	-22.26	-47.95
E 24	20.75± 0.39	16.36± 0.08	11.82± 0.43	-21.15	-43.03
E 34	19.18± 0.43	15.84± 0.18	11.98± 0.15	-17.41	-37.53
E 28	22.61± 0.37	18.06± 0.13	15.54± 0.43	-20.12	-31.27
BAW1138	23.70± 0.08	17.24± 0.18	10.46± 0.15	-27.25	-55.86
BAW1171	18.93± 0.15	15.42± 0.37	9.88± 0.39	-18.54	-47.80
BAW1170	20.96± 0.37	17.62± 0.43	15.47± 0.29	-15.94	-26.93
BAW1157	19.96± 0.29	15.74± 0.28	9.88± 0.32	-21.14	-50.50
BAW1163	21.53± 0.39	15.33± 0.15	10.65± 0.37	-28.79	-50.53
BAW1140	20.25± 0.37	12.25± 0.29	7.32± 0.37	-39.51	-63.85
BAW1151	15.43± 0.43	10.56± 0.39	10.37± 0.15	-31.56	-32.79
Mean	20.81	16.00	11.11		
Significant level	**				
CV (%)	2.96				

(with a range from 7.32 cm in BAW 1140 to 15.54 cm in BAW 1138 and a mean of 11.11 cm). The shoot length was found to be reduced with the increment of water deficit stress but the degree of reduction was not similar for all wheat genotypes. At moderate water deficit stress, wheat genotypes- BARI Gom 25, E 30, E 2, E 34, BAW 1171and BAW 1170 showed less than 20% reduction and wheat genotypes- Satabdi , Sourav, E 30, E 2, E 38, E 24, BAW 1171, BAW 1157, BAW 1163 and BAW 1140 showed more than 20% reduction in shoot length compared to control.

At higher water deficit stress, wheat genotypes- Satabdi, BARI Gom 25, E 23, E 34, E 2, E 38, E 24, E 28, BAW 1171, BAW 1170 and BAW 1151 showed less than 50% reduction and wheat genotypes- Sourav, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 30, BAW 1138, BAW 1157, BAW 1163 and BAW 1140 showed more than 50% reduction in shoot length compared to control.

Root length: Root length of 10 days old seedling was significantly influenced by influenced by the interaction effect of water potential levels and wheat genotypes (Table 6). The root length was found to be higher at control at control (with a range from 9.94 cm in BAW 1163 to 13.88 cm in BARI Gom 27 and a mean of 12.00 cm), moderate at moderate stress (ranging from 8.98 cm in BAW 1163 to 12.50 cm in BAW 1171 with a mean of 10.98 cm) and lower at higher water deficit stress (with a range from 7.42 cm in BAW 1140 to 11.43 cm in E 34 and a mean of 9.46 cm). The root length was found to be reduced with the increment of water deficit stress but the degree of reduction was not similar for all wheat genotypes. At moderate water deficit stress, wheat genotypes- Satabdi, Sourav, BARI Gom 25, BARI Gom 28, E 30, E 23, E 2, E 38, E 24, E 34, BAW 1138, BAW 1171, BAW 1170 and BAW 1163.

Table 6. Root length (mean ± SE) of different wheat genotypes at 10 days after placement for germination as influenced by PEG induced water deficit stress

Wheat genotypes	Root length (cm)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (- 2 bar)	Higher Stress (- 4 bar)		
Shatabdi	10.72± 0.18	9.93± 0.17	8.86± 0.31	-7.37	-17.35
Sourav	11.74± 0.20	10.80± 0.08	8.44± 0.28	-8.01	-28.11
BARI Gom 25	11.94± 0.09	11.50± 0.31	10.46± 0.18	-3.69	-12.40
BARI Gom 26	12.44± 0.17	10.72± 0.20	9.16± 0.17	-13.83	-26.37
BARI Gom 27	13.88± 0.28	12.10± 0.18	10.8± 0.40	-12.82	-22.19
BARI Gom 28	12.10± 0.18	11.15± 0.31	8.38± 0.08	-7.85	-30.74
E 30	10.90± 0.20	10.00± 0.40	9.82± 0.20	-8.26	-9.91
E 23	11.96± 0.17	11.58± 0.13	11.34± 0.28	-3.18	-5.18
E 2	12.95± 0.18	12.47± 0.31	8.54± 0.17	-3.71	-34.05
E 38	12.51± 0.18	12.28± 0.40	11.47± 0.13	-1.84	-8.31
E 24	11.09± 0.40	10.26± 0.18	9.80± 0.40	-7.48	-11.63
E 34	12.73± 0.28	12.31± 0.18	11.43± 0.28	-3.30	-10.21
E 28	11.61± 0.31	10.22± 0.13	9.76± 0.18	-11.97	-15.93
BAW1138	12.12± 0.17	11.12± 0.20	8.45± 0.28	-8.25	-30.28
BAW1171	13.34± 0.40	12.50± 0.08	10.52± 0.17	-5.92	-21.14
BAW1170	12.48± 0.28	11.34± 0.09	10.24± 0.18	-9.14	-17.95
BAW1157	13.67± 0.08	12.27± 0.31	8.45± 0.13	-10.24	-38.19
BAW1163	9.94± 0.20	8.98± 0.18	8.00± 0.18	-9.66	-19.52
BAW1140	11.08± 0.17	9.00± 0.28	7.42± 0.20	-18.77	-33.03
BAW1151	10.86± 0.18	9.10± 0.13	7.88± 0.40	-16.21	-27.44
Mean	12.00	10.98	9.46		
Significant level	**				
CV (%)	4.02				

showed less than 10% reduction and wheat genotypes- BARI Gom 26, BARI Gom 27, E 28, BAW 1157, BAW 1140 and BAW 1151 showed more than 10% reduction in root length compared to control.At higher water deficit stress, wheat genotypes- Satabdi, BARI Gom 25, E 30, E 23, E 38, E 24, E 34, E 28, BAW 1170 and BAW 1163 showed less than 20% reduction and wheat genotypes- Sourav, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 2, BAW 1138, BAW 1171, BAW 1157, BAW 1140 and BAW 1151showed more than 20% reduction in root length compared to control.

Seedling dry weight: Seedling dry weight of 10 days old seedling was significantly influenced by influenced by the interaction effect of water potential levels and wheat genotypes (Table 7). The seedling dry weight was found to be higher at control at control (with a range from 83.66 mg in BARI Gom 28 to 150.66 mg in Satabdi and a mean of 115.64 mg), moderate at moderate stress (ranging from 74.66 mg in BARI Gom 28 to 116.33 mg in Satabdi with a mean of 92.30 mg) and lower at higher water deficit stress stress (ranging from 67.33 mg in E 24 to 100.00 mg in BARI Gom 25 with a mean of 82.33 mg). The seedling dry weight was found to be reduced with the increment of water deficit stress but the degree of reduction was not similar for all wheat genotypes. At moderate water deficit stress, wheat genotypes- BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 38, E 24, E 34, E 28, BAW 1171, BAW 1170 and BAW 1163 showed less than 20% reduction and wheat genotypes- Satabdi, Sourav, E 30, E 23, E 2, BAW 1138, BAW 1157, BAW 1140 and BAW 1151showed more than 20% reduction in seedling dry weight compared to control.

At higher water deficit stress, wheat genotypes- BARI Gom 25, BARI Gom 27, BARI Gom 28, E 30, E 34, E 28, BAW 1138, BAW 1171, BAW 1170 and BAW

Table 7. Seedling dry weight (mean ± SE) of different wheat genotypes at 10 days after placement for germination as influenced by PEG induced water deficit stress

Wheat genotypes	Seedling dry weight (mg)			% change at moderate stress over control	% change at higher stress over control
	Control	Moderate stress (-2 bar)	Higher stress (-4 bar)		
Shatabdi	150.66± 0.61	116.33± 0.52	95.33± 0.83	-22.79	-36.73
Sourav	125.00± 1.40	83.33± 0.35	83.33± 0.76	-33.34	-33.34
BARI Gom 25	122.33± 0.93	110.30± 0.46	100.00± 0.11	-9.83	-18.25
BARI Gom 26	131.66± 0.52	113.66± 0.61	89.66± 1.40	-13.67	-31.90
BARI Gom 27	111.00± 0.11	96.00± 0.46	83.66± 0.93	-13.51	-24.63
BARI Gom 28	83.66± 0.61	74.66± 1.40	68.33± 0.76	-10.76	-18.32
E 30	115.33± 0.83	86.33± 0.58	86.33± 0.35	-25.15	-25.15
E 23	109.00± 1.53	75.00± 0.61	68.00± 0.52	-31.19	-37.61
E 2	105.00± 0.93	77.66± 1.40	73.00± 0.76	-26.04	-30.48
E 38	118.66± 0.58	95.00± 1.40	78.66± 0.61	-19.94	-33.71
E 24	102.33± 1.53	84.33± 0.93	67.33± 0.83	-17.59	-34.20
E 34	92.66± 1.40	90.66± 0.31	68.66± 0.33	- 2.16	-25.90
E 28	97.00± 0.76	88.33± 0.52	86.00± 0.35	-8.94	-11.34
BAW1138	127.33± 0.61	95.33± 0.83	96.00± 1.53	-25.13	-24.61
BAW1171	116.33± 0.33	96.66± 0.93	92.66± 0.58	-16.91	-20.35
BAW1170	94.00± 1.53	85.33± 0.35	84.66± 1.40	-9.22	-9.94
BAW1157	116.00± 0.52	89.33± 1.40	82.00± 0.46	-22.99	-29.31
BAW1163	124.33± 1.40	105.00± 0.33	78.66± 0.11	-15.55	-36.73
BAW1140	136.00± 0.46	83.00± 0.58	80.66± 0.52	-38.97	-40.69
BAW1151	134.66± 0.52	99.66± 1.53	83.66± 0.35	-25.99	-37.87
Mean	115.65	92.30	82.33		
Significant level	**				
CV (%)	2.64				

1157 showed less than 30% reduction and wheat genotypes- Satabdi, Sourav, BARI Gom 26, E 23, E 2, E 38, E 24, BAW 1163, BAW 1140 and BAW 1151 showed more than 30% reduction in seedling dry weight compared to control.

Water deficit stress developed by PEG reduced the shoot length, root length and seedling dry weight of wheat genotypes in the present study. Similar results were found by Datta *et al*. (2011), Almaghrabi (2012), Khakwani *et al.* (2011), Raza *et al.* (2012) and Jajarmi (2009) in their studies. Because increasing the level of water stress not only inhibit the germination characteristics but the extension growth of the seedlings was also observed (Pratap and Sharma 2010). Water stress also reduced the seed reserve utilization (Soltani *et al*. 2006 and Harb 2013) solubilization of sugars (Harb 2013) during germination which contributed to lower seedling dry weight of wheat. Different wheat genotypes showed differential degree in reduction in shoot length, root length and seedling dry weight in the present study. This may be due to differential genetic sensitivity of wheat genotypes to water deficit stress. Differential sensitivity of wheat genotypes to water deficit stress in relation to shoot length, root length and seedling dry weight was also found by Datta *et al*. (2011), Almaghrabi (2012), Khakwani *et al.* (2011), Raza *et al.* (2012) and Jajarmi (2009) in their studies.

4.1.3. Relationship among STI based on germination characteristics and early seedling growth

Table 8 shows the relationship among the STI (Stress Tolerance Index) based on different germination characteristics and seedling growth parameters at moderate water deficit stress (- 2 bar). Significant positive correlation was found between the STI based on rate of germination and germination vigor index (0.516**), rate of

Table 8. Relationship among the STI (Stress Tolerance Index) based on rate of germination, co-efficient of germination, germination vigor index, shoot length, root length and seedling dry weight at moderate water deficit stress

	Rate of germination	Co-efficient of germination	Germination vigor index	Shoot length	Root length	Seedling dry weight
Rate of germination						
Co-efficient of germination	0.284^{NS}					
Germination vigor index	0.516**	0.656**				
Shoot length	0.456*	0.275^{NS}	0.572**			
Root length	0.393*	-0.021^{NS}	0.255^{NS}	0.645**		
Seedling dry weight	0.377^{NS}	0.403*	0.299^{NS}	0.565**	0.241^{NS}	

Table 9. Relationship among the STI (Stress Tolerance Index) based on rate of germination, co-efficient of germination, germination vigor index, shoot length, root length and seedling dry weight at higher water deficit stress

	Rate of germination	Co-efficient of germination	Germination vigor index	Shoot length	Root length	Seedling dry weight
Rate of germination						
Co-efficient of germination	0.605**					
Germination vigor index	0.539**	0.091^{NS}				
Shoot length	0.115^{NS}	0.125^{NS}	0.324^{NS}			
Root length	0.162^{NS}	0.150^{NS}	0.229^{NS}	0.365^{NS}		
Seedling dry weight	0.122^{NS}	0.097^{NS}	0.249^{NS}	0.448*	0.091^{NS}	

germination and shoot length (0.456*), rate of germination and root length (0.393*), co-efficient of germination and germination vigor index (0.656**), co-efficient of germination and seedling dry weight (0.403*), germination vigor index and shoot length (0.572**), shoot length and root length (0.645**) and shoot length and seedling dry weight (0.565**).

The relationship among the STI (Stress Tolerance Index) based on different germination characteristics and seedling growth parameters at higher water deficit stress (- 4 bar) is presented in Table 9. Significant positive correlation was found between the STI based on rate of germination and co-efficient of germination (0.605**), rate of germination and germination vigor index (0.539**) and shoot length and seedling dry weight (0.448*).

4.1.4. Stress tolerance index based on seedling dry weight

Stress tolerance index (STI) of twenty wheat genotypes based on seedling dry weight (10 days old) of at moderate water deficit stress is presented in Figure 1. These STI values indicated a wide difference in water deficit stress tolerance among the wheat genotypes. Wheat genotypes- BARI Gom 25, E 34, E 28 and BAW 1170 showed more than 0.90 STI, the wheat genotypes- Sourav, E 23 and BAW 1140 provided less than 0.70 STI and the other wheat genotypes (Satabdi, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 30, E 2, E 38, E 24, BAW 1138, BAW 1171, BAW 1157, BAW 1163 and BAW 1151) showed STI in between 0.70 to 0.90.

Figure 2 shows the stress tolerance index (STI) of twenty wheat genotypes based on seedling dry weight (10 days old) of at higher water deficit stress. These STI values also indicated a wide difference in water deficit stress tolerance among the wheat

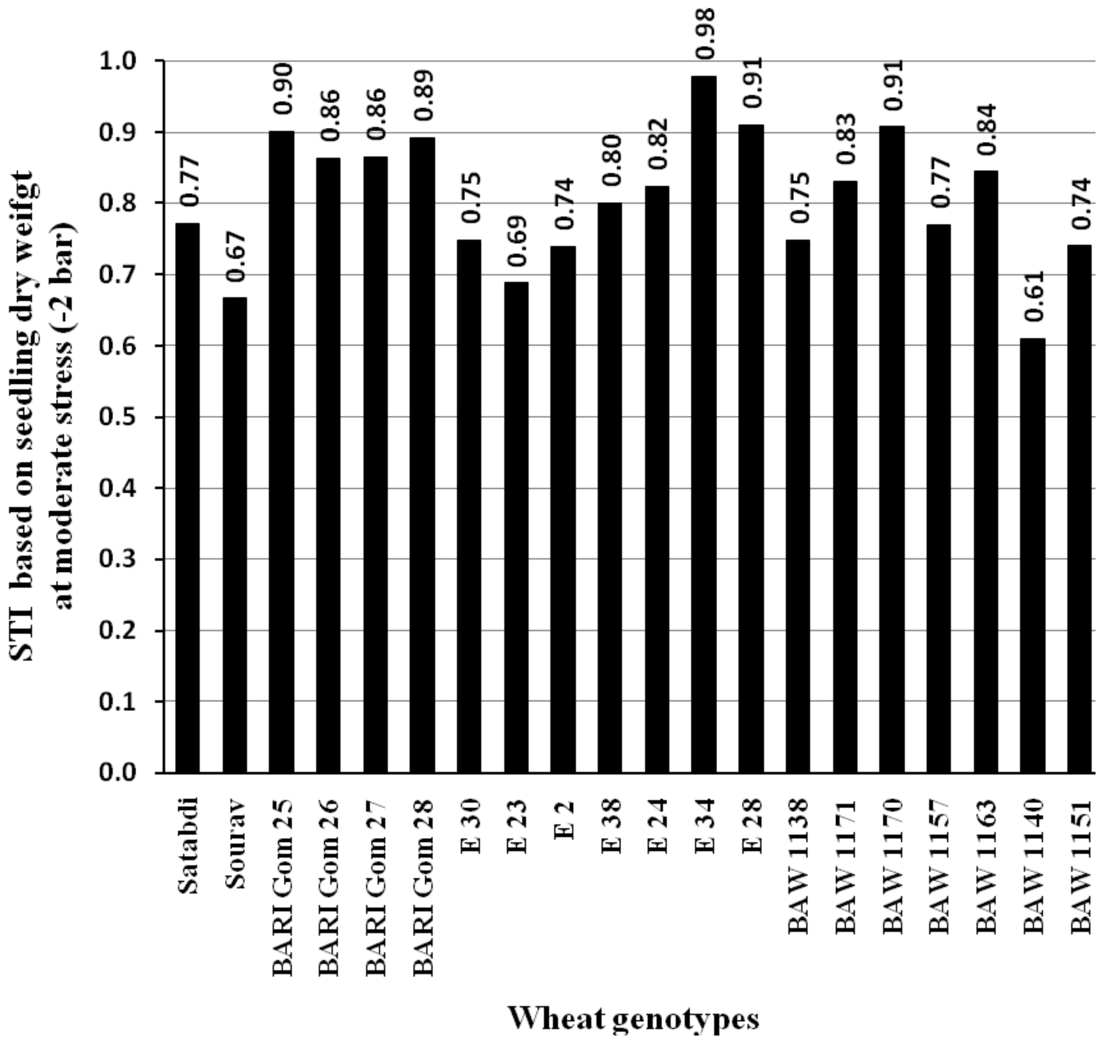

Fig. 1. Stress tolerance index (STI) based on seedling dry weight of different wheat genotypes at moderate water deficit stress (- 2 bar).

genotypes. Wheat genotypes- BARI Gom 25, BARI Gom 28, E 28 and BAW 1170 showed more than 0.80 STI, the wheat genotypes- Satabdi, Sourav, BARI Gom 26, E 23, E 38, E 24, BAW 1163, BAW 1140 and BAW 1151 provided less than 0.70 STI and the other wheat genotypes (BARI Gom 27, E 30, E 2, E 34, BAW 1138, BAW 1171 and BAW 1157) showed STI in between 0.70 to 0.80. Among the wheat genotypes tested in the study- BARI Gom 25, E 28 and BAW 1170 showing higher STI and wheat genotype- BAW 1140 showing lower STI both at moderate and higher water deficit stress were selected for the field experiment.

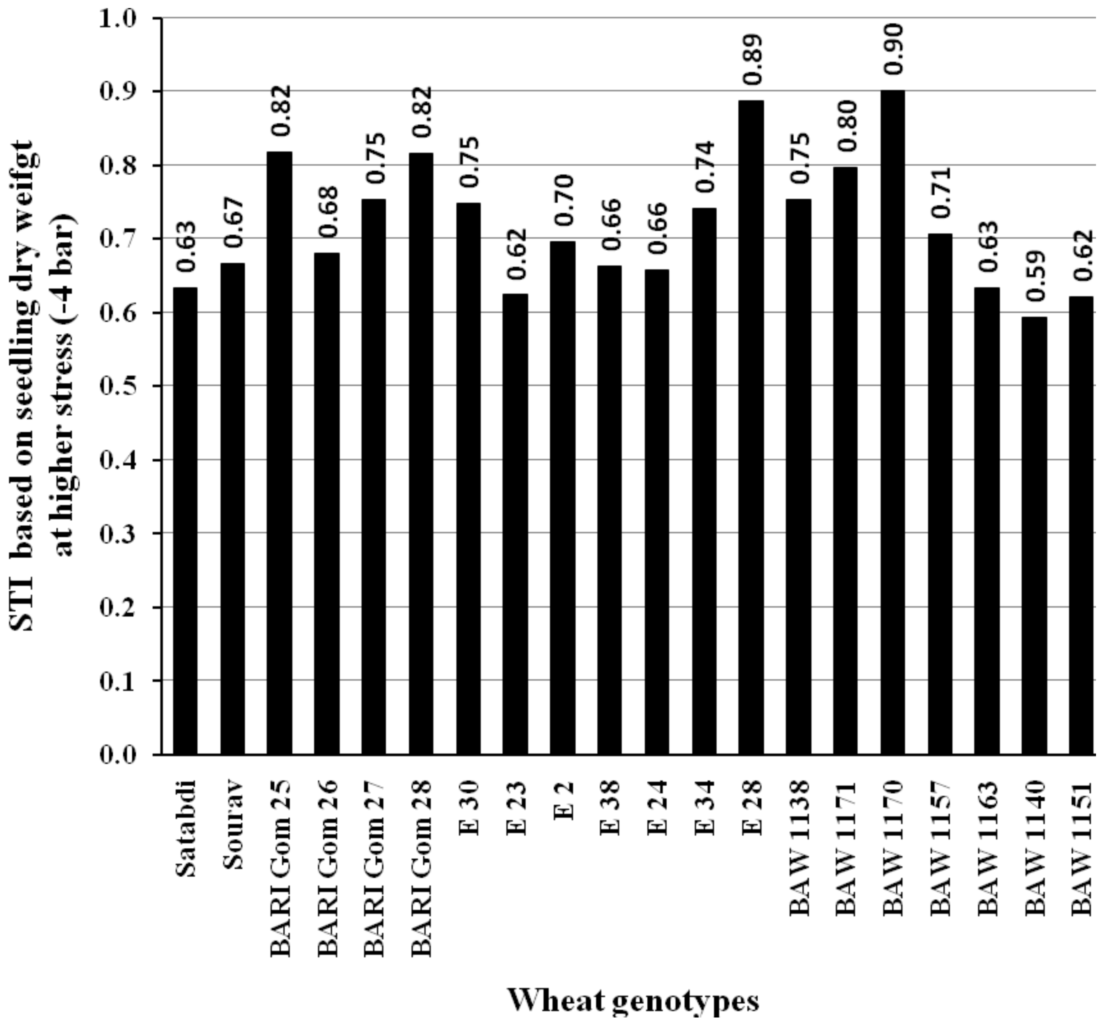

Fig. 2. Stress tolerance index (STI) based on seedling dry weight of different wheat genotypes at higher water deficit stress (- 4 bar).

Experiment -2: Physiological evaluation of wheat genotypes for water deficit stress tolerance

4.2.1. Soil moisture content

Soil moisture content at 0-15cm depth of well watered and water stressed plots at different days after sowing is presented in Figure 3. Figure 3 shows that well watered plot maintained higher soil moisture (7.40%, 9.27% and 8.89%) than that of water stressed plot (4.36%, 3.87% and 3.30%) at 90, 100 and 110 days after sowing, respectively. This figure also shows that soil moisture of water stressed plot was reduced gradually with the advancement of time after sowing.

4.2.2. Phenological parameters

Wheat genotypes and water regimes interacted significantly to influence the number of days required to attain anthesis and physiological maturity (Table 10). Under water stress condition, all the wheat genotypes required shorter time (68-76 days) to attain anthesis compared to well water condition (71-79 days). BAW 1170 required maximum days (79 days) to attain anthesis under well water condition but anthesis occurred 3 days earlier in this genotype under water stress condition. E 28 required minimum days (71 days) to attain anthesis under well water condition but anthesis occurred 3 days earlier in this genotype under water stress condition. In BARI Gom 25 and BAW 1140 anthesis was 1 and 2 days earlier, respectively under water stress condition compared to well water condition.

Under water stress condition, all the wheat genotypes required shorter time (102 - 109 days) to attain physiological maturity compared to well water condition (105-110 days). BAW 1170 and BAW 1140 required maximum days (110 days) to attain

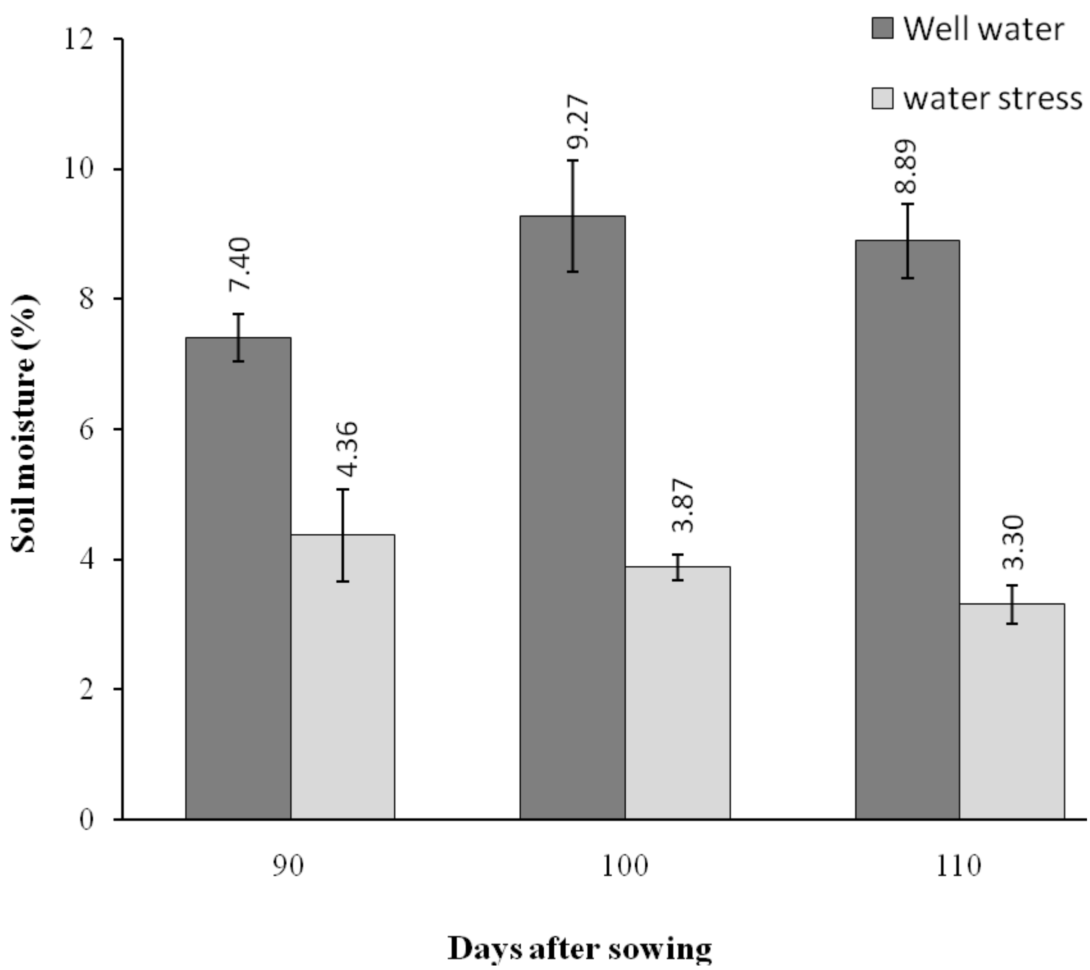

Fig. 3. Soil moisture content (0-15 cm depth) at different days after sowing as influenced by water regimes.

Table 10. Effect of water regimes on days required to attain anthesis and physiological maturity of different wheat genotypes

Wheat genotypes	Water regimes	Days to anthesis		Days to physiological maturity	
		Number of days	Early in days over control	Number of days	Early in days over control
BARI Gom 25	Well watered	74 c	-	105 b	-
	Water stress	73 d	1	104 b	1
E 28	Well watered	71 e	-	105 b	-
	Water stress	68 f	3	102 c	3
BAW 1170	Well watered	79 a	-	110 a	-
	Water stress	76 b	3	109 a	1
BAW 1140	Well watered	72 d	-	110 a	-
	Water stress	70 e	2	104 b	6
Level of significance		*		**	
CV (%)		0.48		1.04	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at P ≤ 5%

physiological maturity under well water condition whereas BAW 1170 attained at physiological maturity 1 days earlier and BAW 1140 attained at physiological mature stage 6 days earlier under water stress condition. BARI Gom 25 and E 28 required minimum days (105 days) to attain physiological maturity under well water condition whereas BARI Gom 25 attained at physiological maturity 1 day earlier and E 28 attained at physiological mature stage 3 days earlier under water stress condition.

The time required for the phoenological development of crops is one of the most important factors for yield adaptation in any environment (Motzo and Giunta 2007). In principle, the length of growing period and phenological development of crops can affect the yield either by consuming more resources or by decreasing the environmental tensions or by reducing the length of the periods (Attarbashi *et al*. 2002). In the present study, water deficit stress accelerated anthesis and physiological maturity stage. Similar results were also reported by various researchers that water deficit stress reduced the number of days to anthesis (Kiliç and Yagbasanlar 2010, Bayoumi *et al*. 2008, Sial *et al*. 2009, Khakwani *et al.* 2012, Kiliç *et al*. 1999) and days to maturity (Kiliç and Yagbasanlar 2010, Saleem *et al*. 2007).

4.2.3. Flag leaf chlorophyll content

Flag leaf chlorophyll content at 8 days after anthesis (DAA) was significantly influenced by the interaction effect of wheat genotypes and water regimes (Table 11). Under water stress condition, flag leaf chlorophyll was increased by 8.12% in BARI Gom 25, 7.65% in BAW 1170 and 5.37% in BAW 1140 whereas it was same

Table 11. Effect of water deficit stress on flag leaf chlorophyll content of different wheat genotypes

Wheat genotypes	Water regimes	Flag leaf chlorophyll content at 8 DAA		Flag leaf chlorophyll content at 24 DAA	
		mg g^{-1} FW	% change over control	mg g^{-1} FW	% change over control
BARI Gom 25	Well watered	1.60 c	-	1.44 a	-
	Water stress	1.73 bc	+8.12	1.24 a	-13.88
E 28	Well watered	1.84 b	-	1.28 a	-
	Water stress	1.84 b	0.0	0.99 b	-22.65
BAW 1170	Well watered	1.96 ab	-	1.44 a	-
	Water stress	2.11 a	+7.65	1.39 a	-3.47
BAW 1140	Well watered	1.86 b	-	1.24 a	-
	Water stress	1.96 ab	+5.37	0.88 b	-29.03
Level of significance		**		**	
CV (%)		6.69		8.15	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

both at well water and water stress condition in E 28. Flag leaf chlorophyll content at 24 days after anthesis (DAA) was also influenced significantly by the combined effect of wheat genotypes and water regimes (Table 10). Due to water stress condition, all the wheat genotypes showed lower flag leaf chlrophyll content (0.88-1.39 mg chlorophyll g^{-1}FW) compared to well water condition (1.24-1.44 mg chlorophyll g^{-1}FW) except E 28 **.** But the degree of reduction was not same for all wheat genotypes. Under water stress condition, flag leaf chlorophyll was reduced by 13.88% in BARI Gom 25, 22.65% in E 28, 3.47% in BAW 1170 and 29.03 % in BAW 1140.

Chlorophyll content has been known as an index for evaluation of source, therefore decrease in leaf chlorophyll content can be considered as a non-stomatal limiting factor in the drought stress conditions (Herzog, 1986). The decrease in chlorophyll content under drought stress has been considered as a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Keyvan, 2010). In the present study, flag leaf chlorophyll content at 8 days after anthesis remained same or increased under water stress condition, it might be due to the support of the vast storage of soil moisture resulted from monsoon (Karim *et al*. 2000). Deora *et al.* (2001) also reported that chlorophyll content in wheat increased in stressed leaves as compared to normal leaves. Flag leaf chlorophyll content at 24 days after anthesis was reduced due to water stress, as the plant suffers from severe water stress at this stage when the residual soil moisture depletes. The sensitive genotype BAW 1140 was found to affect more than the tolerant genotypes (BARI Gom 25, E 28 and BAW 1170) in chlorophyll degradation in the present study. Ashraf *el al*. (1994) and Farshadfar *et al*. (2014) working with what genotypes also reported that water stress reduced chlorophyll content and the reduction was more pronounced in drought susceptible genotypes.

4.2.4. Relative water content of flag leaf

Relative leaf water content of flag leaf at 16 days after anthesis was significantly influenced by the interaction effect of wheat genotypes and water levels (Table 12). At well watered condition, the highest flag leaf water content was found in BAW 1140 (83.84%) which was followed by BAW 1170 (80.96%) and BARI Gom 25(80.72%) while E 28 showed the lowest relative leaf water content (80.42%).

Under water stress condition, all the wheat genotypes showed lower relative leaf water content compared to well water condition except BARI Gom 25 in which the relative leaf water content was even increased by 2.15%. Among the other three genotypes BAW 1140 affected more (3.57%) than E 28 (1.24%) and BAW 1170 (3.24%) in reducing leaf water content.

Relative water content (RWC) in leaves is an important character, which is directly related to soil water content (Sarker *et al*. 1999). Relative water content has been reported as an important indicator of water stress in leaves (Merah, 2001). Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance (Anjum *et al*. 2011). A decrease in the relative water content in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006), Singh and Patel (1996) and Siddique *et. al.* (1999). Farshadfar *et al.* (2012), Hasheminasab *et al*. (2012) and Farshadfar *et al*. (2014) also reported relative water content as a useful character for selecting drought tolerant wheat genotypes.

Table 12. Effect of water deficit stress on relative water content of flag leaf of different wheat genotypes at 16 days after anthesis

Wheat genotypes	Water regimes	Relative water content of flag leaf	
		%	% change over control
BARI Gom 25	Well watered	80.72 b	-
	Water stress	82.87 a	+2.15
E 28	Well watered	80.42 b	-
	Water stress	79.42 c	- 1.24
BAW 1170	Well watered	80.96 b	-
	Water stress	78.33 c	-3.24
BAW 1140	Well watered	83.84 a	-
	Water stress	80.84 b	-3.57
Level of significance		**	
CV (%)		1.03	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

4.2.5. Flag leaf and kernel proline content

Wheat genotypes and water regimes interacted significantly to influence flag leaf and kernel proline content at 16 days after anthesis (Table 13). Under well condition, BARI Gom 25 contained the highest amount of proline in flag leaf (11.98 µmole g^{-1} FW) which was followed by E 28 (10.28 µmole g^{-1} FW) and BAW 1170 (9.95 µmole g^{-1} FW) while BAW 1140 contained the lowest amount of proline in flag leaf (9.76 µmole g^{-1} FW).

Under water stress condition, flag leaf proline was increased by 1 to 5.35% in three tolerant genotypes (BARI Gom 25, E 28 and BAW 1170), whereas the flag leaf proline was reduced by 0.57 % in sensitive genotype (BAW 1140) because of the variety does not have the ability to accumulate proline under water stress condition compared to well water condition.

Under well condition, BAW 1140 and BAW 1170 contained the highest amount of proline in kernel leaf (21.38 µmole g^{-1} FW) which was followed by BARI Gom 25 (20.73 µmole g^{-1} FW), whereas E 28 contained the lowest amount of proline in kernel (17.21 µmole g^{-1} FW) at 16 days after anthesis. Under water stress condition, kernel proline was increased by 1.39% in BARI Gom 25 and 1.10% in E 28. BAW 1170 and BAW 1140 failed to accumulate enough quantity of proline in kernel to tolerate water deficit stress. The kernel proline was reduced by 2.99% in BAW 1170 and 5.68% in sensitive genotype (BAW 1140) compared to well water condition.

Plants accumulate proline in large quantities in response to environmental stresses (Ashraf, 1994; Ali *et al*. 1999; Rhodes *et al*. 1999; Ozturk and Demir, 2002; Hsu *et al*. 2003 and Kavi Kishore *et al*. 2005). Accumulation of proline under stress in

Table 13. Effect of water deficit stress on flag leaf and kernel proline content of different wheat genotypes at 16 days after anthesis

Wheat genotypes	Water regimes	Flag leaf proline content		Kernel proline content	
		µmole g^{-1} FW	% change over control	µmole g^{-1} FW	% change over control
BARI Gom 25	Well watered	11.98 b	-	20.73 b	-
	Water stress	12.62 a	+5.34	21.02 b	+1.39
E 28	Well watered	10.28 d	-	17.21 c	-
	Water stress	10.83 c	+5.35	17.40 c	+1.10
BAW 1170	Well watered	9.95 d	-	21.38 a	-
	Water stress	10.05 d	+1.00	18.39 c	-2.99
BAW 1140	Well watered	9.76 d	-	21.38 a	-
	Water stress	9.19 e	-0.57	15.70 d	-5.68
Level of significance		*		**	
CV (%)		2.87		3.37	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at P ≤ 5%

many plant species has been correlated with stress tolerance, and its concentration has been known to be usually higher in stress tolerant than in stress sensitive plants (Ashraf and Foolad 2007). High levels of proline enable a plant to maintain low water potential. The accumulation of compatible osmolites lowering water potential involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (Kumar *et al*. 2003). Sankar *et al*. (2007) reported that high proline accumulation in plants could provide energy for growth and survival, and thereby help the plant to tolerate stress. It is well documented that accumulated proline plays a role as a compatible solute in plants, regulating and reducing water loss from the cell under water deficit conditions and osmotically stressful conditions (Bayoumi *et al.* 2008). Proline may also play a role as an enzyme stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanein 2004).

4.2.6. Flag leaf soluble sugar content

Soluble sugar content in flag leaf at 16 days after anthesis was influenced significantly by the combined effect of wheat genotypes and water regimes (Table 14). Under well watered condition, E 28 contained the highest amount of soluble sugar in flag leaf (61.02 mg g^{-1} DW) which was followed by BAW 1170 (54.04 mg g^{-1} DW) and BAW 1140 (51.13 mg g^{-1} DW) whereas BARI Gom 25 contained the lowest amount of soluble sugar in flag leaf (48.40 mg g^{-1} DW). Under water stress condition, flag leaf soluble sugar was increased by 2.96 to 25.42% in three tolerant genotypes (BARI Gom 25, E 28 and BAW 1170), whereas the flag leaf soluble sugar

Table 14. Effect of water deficit stress on leaf soluble sugar content of different wheat genotypes at 16 days after anthesis

Wheat genotypes	Water regimes	Leaf soluble sugar content	
		mg g^{-1} DW	% change over control
BARI Gom 25	Well watered	48.40 cd	-
	Water stress	60.70 b	+25.42
E 28	Well watered	61.02 b	-
	Water stress	69.24 a	+13.45
BAW 1170	Well watered	54.04 bd	-
	Water stress	55.64 bc	+2.96
BAW 1140	Well watered	51.13 cd	-
	Water stress	46.40 d	- 9.63
Level of significance		*	
CV (%)		7.53	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

was reduced by 9.63% in sensitive genotype (BAW 1140) compared to well water condition. Sugars produced during photosynthesis play a significant role in plant growth and development under abiotic stresses by regulating carbohydrate metabolism. It is also considered to play an important role in osmotic adjustment which is widely regarded as adaptive response to water stress conditions (Turner and Jones 1980, Kameli and Loesel 1993, 1995).

Soluble sugar content has been shown to increase in wheat leaves under water scarcity have been also reported by Chorfi and Taibi (2011), Nazarli and Faraji (2011), Kameli and Losel (1995). Prado *et al*. (2000) and Siddique *et al.* (2000) reported that adaptation to water stress has been attributed to the stress induced increase in sugar levels.

4.2.7. Canopy temperature depression (CTD)

Canopy temperature depression at 8 days after anthesis was not influenced significantly by the interaction effect of wheat genotypes and water regimes but at 24 days after anthesis it was influenced significantly by the interaction effect of wheat genotypes and water regimes (Table 15). At 24 days after anthesis, all the wheat genotypes maintained higher CTD (4.16-5.62°C) under well water condition compared to that (3.18-3.60°C) under water stress condition. CTD was reduced by 30.22% in BARI Gom 25, 32.91% in E 28, 15.86% in BAW 1170 and 35.94% in BAW 1140.

The results of the present study indicated that the tolerant genotypes maintained comparatively a cooler canopy under water deficit stress compared to the sensitive

Table 15. Canopy temperature depression at 8DAA and 24 DAA of different wheat genotypes as influenced by water levels

Wheat genotypes	Water levels	Canopy temperature depression at 8DAA		Canopy temperature depression at 24 DAA	
		°C	% change over normal	°C	% change over normal
BARI Gom 25	Well watered	8.05	-	4.93 b	-
	Water stress	7.50	-6.83	3.44 d	-30.22
E 28	Well watered	8.46	-	4.74 b	-
	Water stress	7.60	-10.16	3.18 d	-32.91
BAW 1170	Well watered	8.86	-	4.16 c	-
	Water stress	7.48	-15.57	3.50 d	-15.86
BAW 1140	Well watered	7.62	-	5.62 a	-
	Water stress	7.49	-1.71	3.60 cd	-35.94
Level of significance		NS		**	
CV (%)		5.17		4.45	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

genotype. Buttar *et al*. (2005), Olivares-Villegas *et al.* 2007 and Lopes and Reynolds (2010) also reported higher canopy temperature in stressed wheat compared to non-stressed wheat. Relatively lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status by various plant adaptive traits (Blum et al. 1989). Low canopy temperature under drought is associated with drought tolerance in wheat. Canopy temperature measurements had been widely used to study genotypic response to drought (Mason and Singh 2014). Blum (1989) evaluated the wheat genotypes for stable performance under various environmental conditions on the basis of canopy temperature.

4.2.8. Spike dry matter accumulation

Both under well water and water deficit stress condition, a typical sigmoid pattern of dry matter accumulation in spike were found in all wheat genotypes (Figure 4) and (Appendix III). Under well water condition, the spike dry weight was observed to be increased up to 2.64 g at 36 DAA in BARI Gom 25, 2.47 g at 40 DAA in E 28, 2.45 g at 40 DAA in BAW 1170 and 2.68 g at 40 DAA in BAW 1140 and decline there after slowly. Under water deficit stress condition, maximum dry matter accumulation in spike was reduced in all wheat genotypes except in E 28 and days required to attain maximum dry weight were reduced in all genotypes except BAW 1170.The reduction in maximum spike dry weight were lower in tolerant wheat genotypes (7.58% in BARI Gom 25, 0.0% in E 28 and 10.61% in BAW1170) compared to that in sensitive genotype (19.03% in BAW 1140). Number of days required to attain maximum dry weight were reduced by o to 4 days in tolerant genotypes (BARI Gom

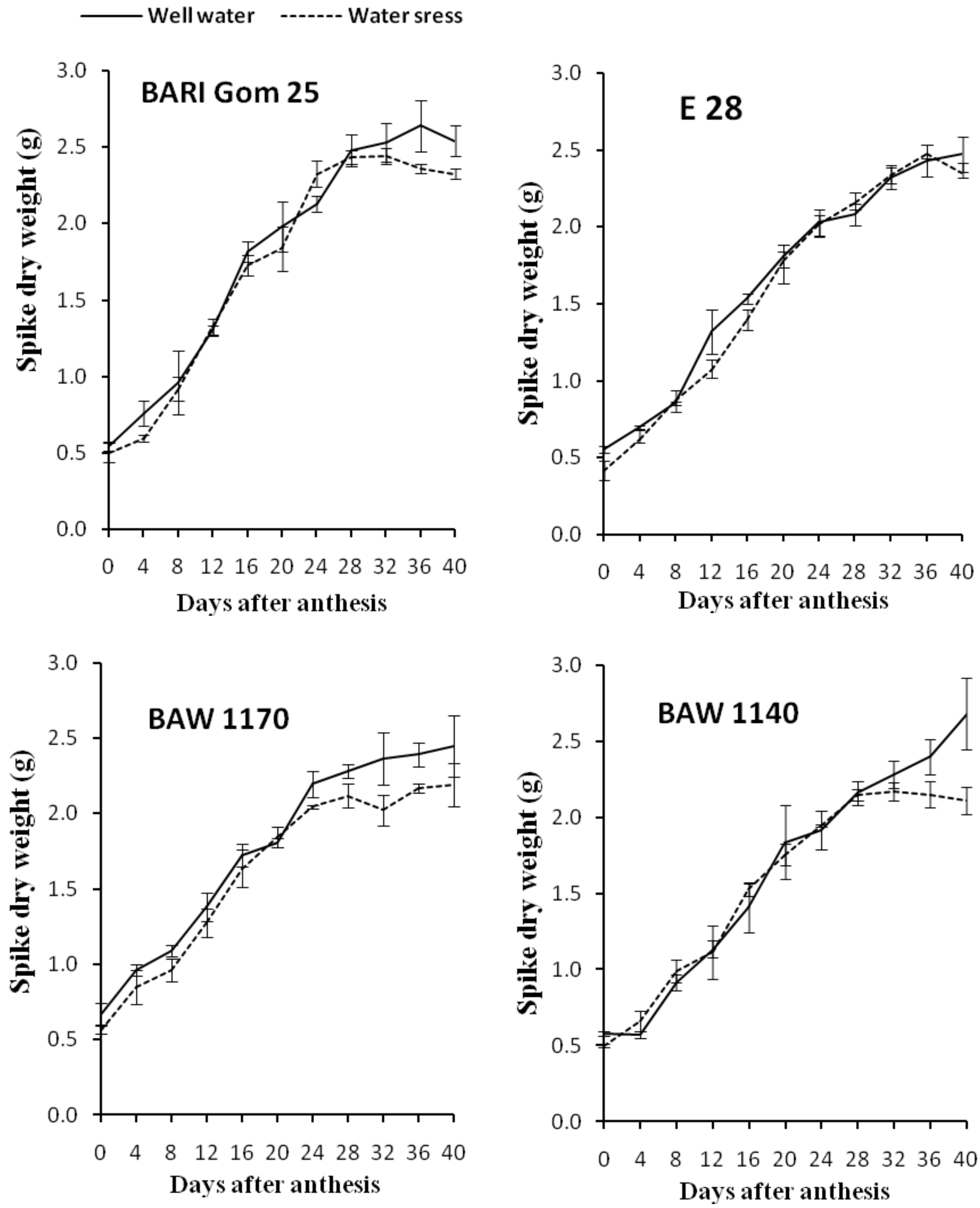

Fig. 4. Spike dry matter accumulation in different wheat genotypes at different days after anthesis as influenced by water regimes.

25, E 28 and BAW1170) but it was 8 days in sensitive wheat genotype (BAW 1140). The declining tendency in dry matter after attaining the highest level could be due to respiratory loss of spike. Thus it indicated the importance of harvesting the crop and quickly drying the wheat grains when it attain at physiological maturity. Similar pattern of spike dry matter accumulation in wheat was also found by Hasan *et al*. (2007) under heat stress condition and Chowdhury (2012) under water stress condition.

4.2.9. Plant height

Height of plant was not significantly influenced by the interaction effect of water regimes and wheat genotypes but all the wheat genotypes became shorter under water stress condition (91.34 cm to 95.74 cm) compared to that (94.18 cm to 99.67 cm) under well water condition (Table 16).

In water deficit stress, plant suffers from physical drought and consequently the reduction in plant height was observed. Baque (2003) reported that water stress significantly reduced plant height. Reduced plant height under water stress also reported by Monayari *et al.* (1984), Siddique *et al.* (1999), and Jamal *et al*.(1995) for wheat.

4.2.10. Spike length

The interaction effect of water regimes and wheat genotypes on spike length was found significant (Table 16). At well water condition BARI Gom 25 produced the longest spike (12.96 cm) which was followed by BAW 1170 (11.13 cm) and E 28 (11.01 cm). BAW 1140 produced significantly the shortest spike (9.83 cm). Due to

Table 16. Effect of water deficit stress on plant height and spike length of different wheat genotypes at harvest

Wheat genotypes	Water regimes	Plant height		Spike length	
		cm	%change over control	cm	% change over control
BARI Gom 25	Well watered	98.17	-	12.96 a	-
	Water stress	91.79	-6.49	10.82 b	-16.51
E 28	Well watered	94.18	-	11.01 b	-
	Water stress	91.34	-3.01	10.72 b	- 2.70
BAW 1170	Well watered	99.67	-	11.13 b	-
	Water stress	98.55	-1.12	10.55 bc	- 5.49
BAW 1140	Well watered	96.96	-	9.83 cd	-
	Water stress	95.74	-1.25	9.20 d	- 6.84
Level of significance		NS		**	
CV (%)		3.35		3.92	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

water deficit stress spike length was reduced in all wheat genotypes. But the magnitude of reduction was not same for all wheat genotypes. The reduction was 16.51% in BARI Gom 25, 2.70% in E 28 and 5.49% in BAW1170 and 6.84% in BAW 1140.

Baque (2003) reported that ear length significantly reduced due to water deficit stress. The reduction of ear length was well distinct under severe water stress condition. Hafiz (2007) reported that spike length increased with increasing irrigation frequency in Barley. Similar results were reported by Rahman (1997) and Naser (1996).

4.2.11. Number of spikes m^{-2}

The combined effect of water regimes and wheat genotypes on number of spikes per m^2 was significant (Table 17). Under well water condition, the highest number of spikes per m^2 was observed in BAW 1140 (345) which was followed by E 28 (312) and BAW 1170 (287) whereas the lowest number of spikes per m^2 was observed in BARI Gom 25 (263).

Due to the effect of water deficit stress number of spikes per m^2 was affected vigorously in all wheat genotypes. But the degree of reduction was different in different genotypes. Under water deficit stress condition more reduction in number of spikes per m^2 was observed in sensitive genotype (17.01% in BAW 1140) compared to three tolerant genotypes (15.99% in BARI Gom 25, 11.95% in E 28 and 6.96% in BAW 1170).

Afzal *et al.* (2006) and Mushtaq *et al*. (2011) found that number of spikes per m^2 was increased with the increase of irrigation frequency. Passioura (2007) also found 37% reduction in spike m^{-2} when moisture stress was imposed during spike emergence

Table 17. Effect of water deficit stress on yield attributes of different wheat genotypes

Wheat genotypes	Water regimes	Spikes m^{-2}		Grains $spike^{-1}$		1000 grain weight	
		Number	% change over control	Number	% change over control	g	% change over control
BARI Gom 25	Well watered	263 d	-	44.33 ab	-	50.36	-
	Water stress	221 e	-15.99	41.33 c	-6.76	52.14	+3.53
E 28	Well watered	312 b	-	46.67 a	-	38.46	-
	Water stress	275 cd	-11.95	46.00 a	-1.41	40.07	+4.18
BAW 1170	Well watered	287 c	-	38.67 d	-	41.91	-
	Water stress	267 cd	-6.96	37.67 d	-2.65	44.34	+5.79
BAW 1140	Well watered	345 a	-	43.33 bc	-	38.98	-
	Water stress	268 cd	-17.01	37.33 d	-13.84	41.95	+7.61
Level of significance		**		**		NS	
CV (%)		3.84		3.16		4.91	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

and anthesis stage. These results are in line with the findings of Rashid *et al*. (2003) and Rafiq *et al*. (2005).

4.2.12. Number of grains spike^{-1}

Number of grains spike^{-1} was significantly influenced by the combined effect of water regimes and wheat genotypes (Table 17). Under well water condition, the highest number of grains spike^{-1} was found in E 28 (46.67) which was followed by BARI Gom 25 (44.33) and BAW 1140 (43.33) whereas the lowest number of grains spike^{-1} was observed in BAW 1170 (38.67).

Due to the effect of water deficit stress number of grains spike^{-1} was reduced in all wheat genotypes. But the degree of reduction was different in different genotypes. Under water deficit stress condition more reduction in number of grains spike^{-1} was observed in sensitive genotype (13.84% in BAW 1140) compared to three tolerant genotypes (6.76% in BARI Gom 25, 1.41% in E 28 and 2.65% in BAW 1170).

Water deficit stress limits seed numbers by either influencing the amount of dry matter produced by the time of flowering or by directly influencing pollen or ovule function, which leads to decreased seed set (Prasad *et al.* 2008). Similar response of different wheat genotypes to different moisture levels was found by Motzo *et al.* (1996), Briggs *et al*. (1999) and Shahram *et al*. (2003).

4.2.13. Thousand grain weight

The interaction effect of water regimes and wheat genotypes on 1000-grain weight was not found to be significant in the present study (Table 17). In general, all wheat genotypes provided greater grain size under water stress condition (40.07 to 52.14 g 1000-grain^{-1}) compared to well water condition (38.46 to 50.36 g 1000-grain^{-1}).

Due to water deficit stress condition 1000-grain weight was increased in all genotypes in the present study. The increment was may be due to the reduction in grain number per spike which favors the larger grain size.

4.2.14. Grain yield

The combined effect of water regimes and wheat genotypes on grain yield was found to be significant in the present study (Table 18). Under well water condition, the highest grain yield was recorded in BAW 1140 (4.22 t ha^{-1}) which was followed by E 28 (3.89 t ha^{-1}) and BARI Gom 25 (3.63 t ha^{-1}) whereas the lowest grain yield was observed in BAW 1170 (3.17 t ha^{-1}).

Due to water deficit stress grain yield was reduced in all wheat genotypes. But the degree of reduction was different in different genotypes. Under water deficit stress condition more reduction in grain yield was observed in sensitive genotype (29.49%% in BAW 1140) compared to three tolerant genotypes (16.25% in BARI Gom 25, 16.45% in E 28 and 15.77% in BAW 1170).

Reduced number of spike per m^2 and grains per spike were the major responsible factors for reducing the grain yield under water deficit stress condition in the present experiment. Results from other studies also showed that water stress environment reduced grain yield in wheat compared to control (Baser *et al*. 2004, Gupta *et al*. 2001 and Dencic *et al*. 2000). Less decrease in wheat yield under water stress is usually observed in water stress tolerant cultivars as compared to sensitive ones (Gaspar *et al.* 2005, Khakwani *et al.* 2011).

Table 18. Effect of water deficit stress on grain and straw yield of different wheat genotypes

Wheat genotypes	Water regimes	Grain yield		Biological yield	
		t ha^{-1}	% change over control	t ha^{-1}	% change over control
BARI Gom 25	Well watered	3.63 b	-	8.59 b	-
	Water stress	3.04 c	-16.25	6.04 c	-29.69
E 28	Well watered	3.89 b	-	8.18 b	-
	Water stress	3.25 c	-16.45	6.19 c	-24.33
BAW 1170	Well watered	3.17 c	-	8.27 b	-
	Water stress	2.67 d	-15.77	6.31 c	-23.70
BAW 1140	Well watered	4.22 a	-	9.33 a	-
	Water stress	3.06 c	-27.49	6.36 c	-31.83
Level of significance		**		*	
CV (%)		5.54		3.64	

In a column, values followed by similar letter(s) did not differ significantly by DMRT at $P \leq 5\%$

4.2.15. Above ground biological yield

Above ground biological yield was influenced significantly by the combined effect of water regimes and wheat genotypes (Table 18). Under well water condition, the highest above ground biological yield was found in BAW 1140 (9.33 t ha^{-1}) which was followed by BARI Gom 25 (8.59 t ha^{-1}) and BAW 1170 (8.27t ha^{-1}) whereas the lowest above ground biological yield was observed in E 28 (8.18 t ha^{-1}).

Due to water deficit stress above ground biological yield was reduced in all wheat genotypes. But the degree of reduction was different in different genotypes. Under water deficit stress condition more reduction in above ground biological yield was observed in sensitive genotype (31.83% in BAW 1140) compared to three tolerant genotypes (29.69% in BARI Gom 25, 24.33% in E 28 and 23.70% in BAW 1170). The results of the present experiment are also in line with the findings of Wang *et al*. (2004) who reported that that skipping irrigation at different growth stages significantly reduced the biological yield in different wheat genotypes. Water stress experienced by a wheat crop during growth stages is known to have cumulative effects expressed as a reduction in total biomass as compared to well watered conditions (Mirbahar *et al*. 2009). Almeselmani *et al*. (2011) observed that biomass yield decreased beyond 70% due to drought in wheat.

4.2.16. Correlation analysis between relative performances of various parameters

Correlation analysis between relative performances of various parameters studied in this investigation is presented in Table 19. Relative performance of grain yield maintained a significant positive correlation with the relative performance of flag leaf chlorophyll content at 24 DAA (0.766**), relative flag leaf water content at 16

Table 19. Relationship among the relative performances of physiological traits, grain yield and straw yield of different wheat genotypes

	Relative flag leaf chlorophyll content at 24 DAA	Relative flag leaf relative water content at 16 DAA	Relative flag leaf proline content at 16 DAA	Relative kernel proline content at 16 DAA	Relative flag leaf soluble sugar content at 16 DAA	Relative canopy temperature depression at 24 DAA	Relative grain yield	Relative above ground biological yield
Relative flag leaf chlorophyll content at 24 DAA								
Relative flag leaf relative water content at 16 DAA	0.229 NS							
Relative flag leaf proline content at 16 DAA	0.455*	0.814**						
Relative kernel proline content at 16 DAA	0.300NS	0.863**	0.984**					
Relative flag leaf soluble sugar content at 16 DAA	0.345*	0.980**	0.912**	0.937**				
Relative canopy temperature depression at 24 DAA	0.446*	-0.052 NS	0.539**	0.440*	0.147 NS			
Relative grain yield	0.766**	0.584**	0.904**	0.813**	0.730**	0.704**		
Relative above ground biological yield	0.665**	-0.090 NS	0.482**	0.344*	0.111 NS	0.956**	0.748**	

DAA (0.584**), flag leaf proline content at 16 DAA (0.904**), kernel proline content at 16 DAA (0.813**), flag leaf soluble sugar content at 16 DAA (0.730**), canopy temperature depression at 24 DAA (0.704**) and above ground biological yield (0.748**).

4.2.17. Water stress susceptible index (SSI)

Figure 5 shows stress susceptibility index of different wheat genotypes based on grain yield. BAW 1170 showed the lowest water stress susceptibility index (0.81) this was followed by BARI Gom 25 (0.84) and E 28 (0.85). BAW 1140 showed the highest water stress susceptibility index (SSI) value (1.42) (Figure 5). These stress susceptibility index values indicated that BAW 1170 was less susceptible genotype and BAW 1140 was the most susceptible genotype.

Figure 6 shows stress susceptibility index of different wheat genotypes based on above ground biological yield. BAW 1170 showed the lowest water stress susceptibility index (0.86) this was followed by E 28 (0.88) and BARI Gom 25 (1.08). BAW 1140 showed the highest water stress susceptibility index (SSI) value (1.16). These stress susceptibility index values also indicated that BAW 1170 was less susceptible genotype and BAW 1140 was the most susceptible genotype.

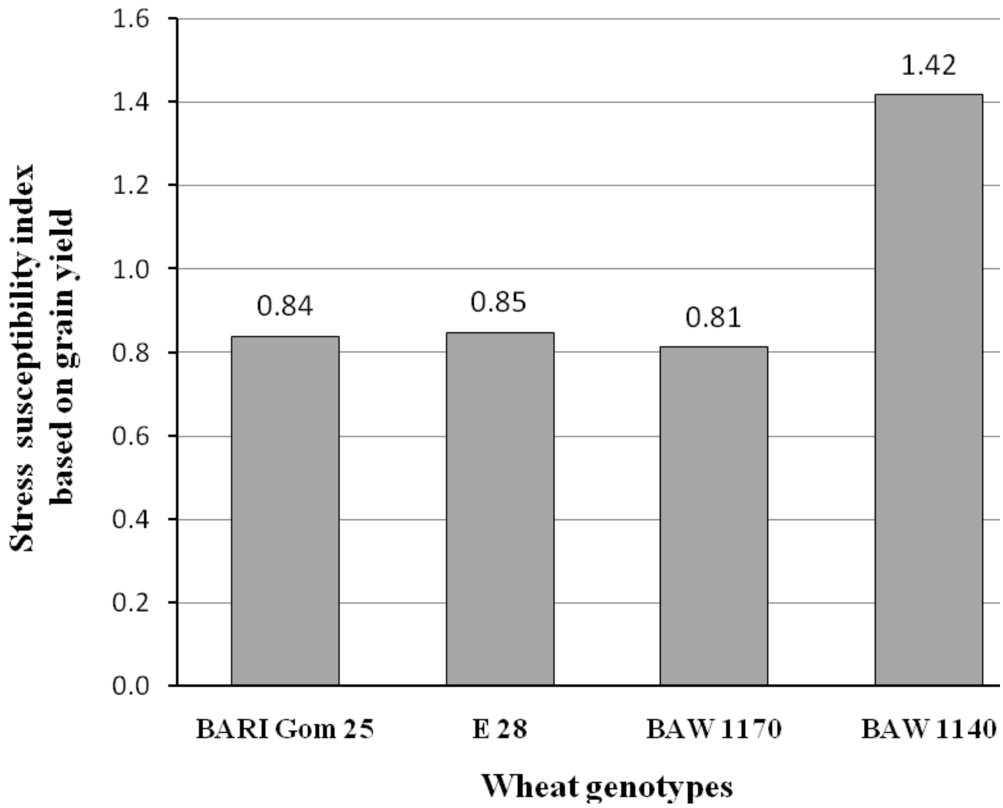

Fig. 5. Stress susceptibility index of different wheat genotypes based on grain yield.

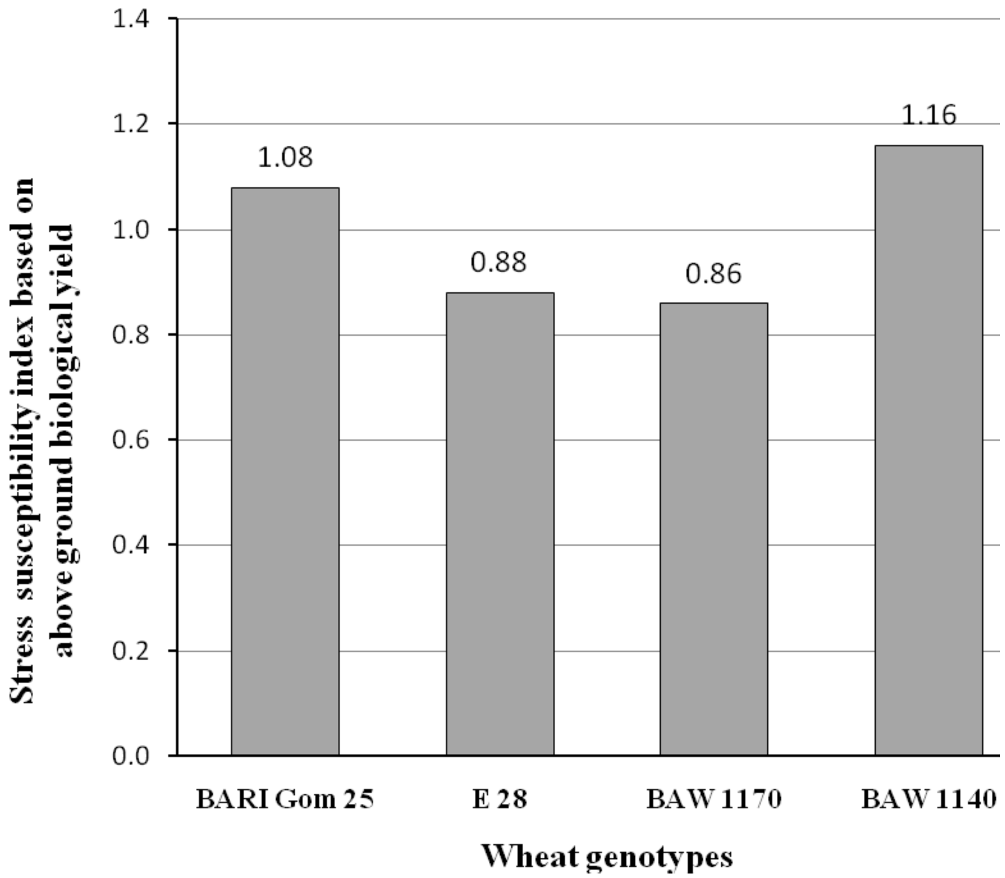

Fig. 6. Stress susceptibility index of different wheat genotypes based on above ground biological yield.

CHAPTER V

SUMMARY AND CONCLUSION

Selection of wheat genotypes tolerant to drought may be a feasible and economical approach for utilizing drought affected soil. To evaluate wheat genotypes under PEG induced water deficit stress during germination and early seedling growth twenty wheat genotypes were tested at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur September 2014 to October 2014. The seeds of twenty wheat genotypes were placed for germination in petridish (11 cm diameter) on sand bed irrigated with solution of three levels of water potential (Tap water, -2 bars and -4 bar) developed dissolving calculating amount of PEG in tap water.

Speed of germination as expressed by rate of germination, co-efficient of germination and germination vigor index of all wheat genotypes was delayed with the increment of water stress induced by PEG. Shoot length and root length and seedling dry weight of 10 days old seedlings were found to be affected due to the increment of water stress. But the magnitude of reduction of these parameters with the increment of water stress was not similar for all wheat genotypes.

Stress tolerance index (STI) based on seedling dry weight (10 days old) indicated a wide difference in stress tolerance among the wheat genotypes. At moderate water deficit stress, BARI Gom 25, E 34, E 28 and BAW 1170 showed more than 0.90 STI, the wheat genotypes- Sourav, E 23 and BAW 1140 provided less than 0.70 STI and the other wheat genotypes (Satabdi, BARI Gom 26, BARI Gom 27, BARI Gom 28, E 30, E 2, E 38, E 24, BAW 1138, BAW 1171, BAW 1157, BAW 1163 and

BAW 1151) showed STI in between 0.70 to 0.90. At higher water deficit stress, BARI Gom 25, BARI Gom 28, E 28 and BAW 1170 showed more than 0.80 STI, the wheat genotypes- Shatabdi, Sourav, BARI Gom 26, E 23, E 38, E 24, BAW 1163, BAW 1140 and BAW 1151 provided less than 0.70 STI and the other wheat genotypes (BARI Gom 27, E 30, E 2, E 34, BAW 1138, BAW 1171 and BAW 1157) showed STI in between 0.70 to 0.80.

Among the wheat genotypes tested in the study- BARI Gom 25, E 28 and BAW 1170 showing higher STI and wheat genotype- BAW 1140 showing lower STI both at moderate and higher water deficit stress were grown at the research field of Crop Physiology and Ecology department, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of November 2014 to April 2015 to evaluate the sensitivity of physiological traits of wheat resulting reduced grain yield under water deficit stress.

The experiment was laid out in a Split plot design with three replications. The main pot treatment consisted of two water regimes (Well water condition and Water deficit stress) and four wheat genotypes (BARI Gom 25, E 28, BAW 1170 and BAW 1140). The unit plot size was 2.5m × 2.0m. The distance between blocks was 1.0m and between plots was 0.50m. Seeds were sown on 30 November 2014 in rows of 20 cm apart, at the rate of 120 kg ha^{-1}. Observations were made on soil moisture content at different days after sowing, days required to anthesis and physiological maturity, flag leaf chlorophyll content, proline content in flag leaf and kernel, relative leaf water content, flag leaf soluble sugar content and canopy temperature depression.

Observation were also made on spike dry matter accumulation, plant height, spike length, yield attributes, grain yield and above ground biological yield.

Soil moisture of water stressed plot was found to be reduced gradually with the advancement of time after sowing. Under water stress condition, all the wheat genotypes required shorter time to attain anthesis and physiological maturity compared to well water condition. But the sensitive genotype (BAW 1140) attained at physiological maturity 6 days earlier whereas the tolerant genotypes (BARI Gom 25, E 28 and BAW 1170) attained at this stage 1 to 3 days earlier under water stress condition.

Flag leaf chlorophyll content both at 8 and 24 days after anthesis (DAA) was significantly influenced by the interaction effect of wheat genotypes and water regimes. At 8 days after anthesis, flag leaf chlorophyll content was found to be increased in three tolerant genotypes but remained same in sensitive BAW 1140 due to water deficit stress. At 24 days after anthesis, more reduction in flag leaf chlorophyll (29.03 %) was found in sensitive BAW 1140 compared to that in three tolerant genotypes (3.47 to 22.65%).

Due to water stress, relative leaf water content was increased by 2.15% BARI Gom 25 but it was reduced by 3.57% in BAW 1140, 1.24% in E 28 and 3.24% in BAW 1170 compared to well water condition.

Wheat genotypes and water regimes interacted significantly to influence flag leaf and kernel proline content at 16 days after anthesis. Under water stress condition, flag leaf proline was increased by 1 to 5.35% in three tolerant genotypes (BARI Gom 25, E 28 and BAW 1170) whereas the flag leaf proline was reduced by 5.84%

in sensitive genotype (BAW 1140) compared to well water condition. Kernel proline was increased by 1.39% in BARI Gom 25 and 1.10% in E 28 but it was reduced by 13.98% in BAW 1170 and 26.65% in sensitive genotype (BAW 1140) due water deficit stress.

Under water stress condition, flag leaf soluble sugar was increased by 2.96 to 25.42% in three tolerant genotypes (BARI Gom 25, E 28 and BAW 1170) whereas the flag leaf soluble sugar was reduced by 9.63% in sensitive genotype (BAW 1140) compared to well water condition.

Canopy temperature depression at 8 days after anthesis was not influenced significantly by the interaction effect of wheat genotypes and water regimes but at 24 days after anthesis it was influenced significantly by the interaction effect of wheat genotypes and water regimes. At 24 days after anthesis, the tolerant genotypes maintained comparatively a cooler canopy under water deficit stress compared to the sensitive genotype.

More reduction in maximum spike dry weight (19.03%) and grain growth duration (8 days) was found in sensitive BAW 1140 compared to the tolerant wheat genotypes (up to 10.61% in maximum spike dry weight and up to 4 days in grain growth duration) due water deficit stress.

The combined effect of water regimes and wheat genotypes on grain yield was found to be significant in the present study. Due to water deficit stress grain yield was reduced in all wheat genotypes but the degree of reduction was more in sensitive genotype (29.49%% in BAW 1140) compared to three tolerant genotypes (16.25% in BARI Gom 25, 16.45% in E 28 and 15.77% in BAW 1170). Reduction in number of spike per m^2 and grains per spike were found as the major responsible factors for reducing the grain yield under water deficit stress condition.

Due to water deficit stress more reduction in above ground biological yield was observed in sensitive genotype (31.83% in BAW 1140) compared to three tolerant genotypes (29.69% in BARI Gom 25, 24.33% in E 28 and 23.70% in BAW 1170).

Relative performance of grain yield maintained a significant positive correlation with the relative performance of flag leaf chlorophyll content at 24 DAA (0.766**), relative flag leaf water content at 16 DAA (0.584**), flag leaf proline content at 16 DAA (0.904**), kernel proline content at 16 DAA (0.813**), flag leaf soluble sugar content at 16 DAA (0.730**), canopy temperature depression at 24 DAA (0.704**) and above ground biological yield (0.748**).

The order of stress susceptibility index based on grain yield was found as BAW 1170 (0.81) < BARI Gom 25 (0.84) < E 28 (0.85) < BAW 1140 (1.42) and the order of stress susceptibility index based on above ground biological yield was found as BAW 1170 (0.86) < E 28 (0.88) < BARI Gom 25 (1.08) < BAW 1140 (1.16).

From the overall results it may be concluded that-

- Speed of germination as expressed by rate of germination, co-efficient of germination and germination vigor index and seedling growth was reduced with the increment of water deficit stress induced by PEG.
- Tolerance based on seedling dry weight may be used as effective selection criterion for screening wheat genotypes for water deficit stress tolerance.

- Water deficit stress tolerant wheat genotypes showed greater stability of flag leaf chlorophyll, greater ability to retain water in leaf, higher level of proline in flag leaf and kernel, higher level of soluble sugar in flag leaf and greater ability to keep the canopy cooler compared to sensitive genotype under water deficit condition.

- Greater spike dry matter accumulation at peak, longer grain growth duration, better yield components such as spikes m^{-2} and grains $spike^{-1}$ under water deficit stress contributed to better tolerance of BARI Gom 25, E 28 and BAW 1170.

- The order of tolerance based on grain yield was BAW 1170 > BARI Gom 25 > E 28 > BAW 1140 and the order of tolerance based on above ground biological yield was BAW 1170 > E 28 > BARI Gom 25 > BAW 1140.

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APPENDICES

Appendix I. Characteristics of soil of the experimental site

General characters	Description	
Location	Crop Physiology and Ecology, 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**	
Bulk density (g cm-3)	0.86-1.07	
Particle size (%)		
Sand (2-0.02mm)	60.0	
Silt (0.02-0.002mm)	27.0	
Clay (< 0.002mm)	13.0	
Textural class	Sandy loam	
Chemical characteristics	**Content**	**Interpretation**
pH	5.40-5.50	Moderately acidic
Organic carbon (%)	0.69	Low
Organic matter (%)	1.19	Low
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: Analysis of initial soil samples was done in SRDI, Dinajpur, Bangladesh

Appendix II: Weather data for growing season of wheat, 2014-2015

Month	Relative humidity (%)	Temperature		Total rainfall (mm)
		Minimum (^oC)	Maximum (^oC)	
November	81	15.9	29.2	0.0
December	85	11.9	24	0.0
January	86	10.5	22	24
February	80	13.4	26.9	0.0
March	74	16.9	30.7	25.5
April	78.4	20.1	31	66

Source: Wheat Research Centre, Dinajpur

Appendix III: Spike dry weight of different wheat genotypes at different days after anthesis as influenced by water deficit stress

Genotypes	Water levels	Spike dry weight of different wheat genotypes at different days after anthesis (g $spike^{-1}$)										
		0	4	8	12	16	20	24	28	32	36	40
BARI Gom 25	Well watered	0.54	0.75	0.96	1.30	1.81	1.98	2.12	2.48	2.53	2.64	2.54
	Water stress	0.50	0.59	0.92	1.32	1.72	1.83	2.32	2.43	2.43	2.35	2.32
E 28	Well watered	0.55	0.69	0.85	1.31	1.53	1.80	2.02	2.08	2.31	2.43	2.47
	Water stress	0.41	0.61	0.87	1.07	1.39	1.77	2.01	2.15	2.33	2.47	2.36
BAW 1170	Well watered	0.67	0.96	1.09	1.38	1.72	1.80	2.19	2.28	2.36	2.39	2.45
	Water stress	0.56	0.84	0.96	1.27	1.63	1.84	2.04	2.11	2.02	2.16	2.18
BAW 1140	Well watered	0.57	0.57	0.92	1.13	1.40	1.83	1.91	2.16	2.28	2.40	2.68
	Water stress	0.49	0.66	0.99	1.11	1.52	1.75	1.49	2.14	2.17	2.14	2.11