SCREENING OF WHEAT (*Triticum aestivum* L.) GENOTYPES FOR DROUGHT TOLERANCE USING COMBINATION OF MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL TRAITS

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This is to certify that thesis entitled, "SCREENING OF WHEAT (Triticum aestivum L.) GENOTYPES FOR DROUGHT TOLERANCE USING COMBINATION OF MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL TRAITS" submitted to the Faculty of AGRICULTURE, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN GENETICS AND PLANT BREEDING**, embodies the result of a piece of bona fide research work carried out by ATIKUR RAHMAN, Registration No.12-05032 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated : June, 2018
Place : Dhaka, Bangladesh

(Prof. Dr. Mohammad Saiful Islam)
Supervisor

Dedication

It is dedicated to My Beloved Parents

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ABSTRACT

The experiment was conducted during the period from November 2017 to April 2018 in rabi season in the experimental field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka to unravel the role of morpho-physiological and biochemical characters in spring wheat under drought stress. Twenty diverse genotypes including 16 lines from the Wheat Research Center (BARI)'s heat and drought nurseries, and four local checks were evaluated under field conditions. Mean performance, genetic parameters, Pearson's correlation coefficient, and principal component biplot analysis were calculated. The longest plant (84.20 cm) was recorded in genotype Shatabdi, while the shortest plant (63.96 cm) was found in wheat genotype BARI GOM-30. The highest grain yield per plant (5.57 g) was recorded in Prodip, while the lowest grain yield per plant (4.15 g) was observed in the wheat genotype SAWYT-312. Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the yield contributing traits. In correlation study, significant negative association was recorded for grain yield per plant of wheat genotypes with days to 50% of heading (-0.397), while the non-significant negative association for number of grains/spike (-0.183), chlorophyll content (-0.097), dry matter content (-0.003) and root length (-0.058). On the other hand, significant positive association was recorded for grain yield per plant with plant height (0.688), number of spike/m^2 (0.269), number of spikelet's/spike (0.630), peduncle length (0.640) and weight of 1000 grains (0.201), while non-significant positive association was observed with leaf area index (0.007). The positive correlation observed between grain yield and proline content under-drought stress conditions provides evidence that proline accumulation might ultimately be considered as a tool for effective selection of drought tolerant genotypes. The study selected 8 genotypes with high grain yields under drought stressed conditions and favorable adaptive traits useful for breeding. In consideration of yield contributing characters and yield Prodip performed better under drought condition followed by DTWYT-22, SAWYT-326, SAWYT-331, Shatabdi, BARI Gom-28, and BARI Gom-30. The proline content hight found in SAWYT-312 (220.2), and also found in SAWYT-303 (194.74), SAWYT-345(178.53) and lowest in SAWYT-324(46.16).

Key words: wheat, drought, physiology, biochemical

SOME COMMONLY USED ABBREVIATIONS

Full word		Abbreviation
Academy	:	*Acad.*
Advanced	:	*Adv.*
Agricultural	:	*Agril.*
Agriculture	:	*Agric.*
Agro ecological zone	:	AEZ
Agronomy	:	*Agron.*
Analysis of variance	:	ANOVA
And others (at elli)	:	*et al.*
Annals	:	*Ann.*
Applied	:	*App.*
As follows	:	*viz.*
At the rate of	:	@
Bangladesh Agricultural Research Institute	:	BARI
Bangladesh Bureau of Statistics	:	BBS
Botany	:	*Bot.*
Breeding	:	*Breed.*
Bulletin	:	Bull.
Centimeter	:	cm
Coefficient of variation	:	CV
Congress	:	*Congr.*
Degree Celsius	:	^{o}C
Degree of freedom	:	df
Drought Tolerant Wheat Yield Trail	:	DTWYT
Ecology	:	*Ecol.*
Environmental coefficient of variation	:	ECV
Environmental variance	:	δ^2_e
Genetic advance	:	GA
Genetics	:	*Genet.*
Genotypic coefficient of variation	:	GCV
Genotypic variance	:	δ^2_g
Gram	:	g
Gross domestic product	:	GDP
Hectare	:	ha
Heritability in broad sense	:	h^2_b
Hydrogen ion concentration	:	pH

Full word		Abbreviation
International Maize and Wheat Improvement Centre	:	CIMMYT
Journal	:	*J.*
Least significant difference	:	LSD
Mean sum of square	:	MSS
Meter	:	m
Molecular	:	*Mol*
Muriate of Potash	:	MP
Nature/Natural	:	*Nat.*
Number	:	No.
Percent	:	%
Phenotypic coefficient of variation	:	PCV
Phenotypic variance	:	δ^2_{ph}
Plant	:	*Pl.*
Principal component analysis	:	PCA
Proceedings	:	Proc.
Randomized Complete Block Design	:	RCBD
Research	:	*Res.*
Semi-Arid Wheat Yield Trial		SAWYT
Science/Scientific	:	*Sci.*
Sher-e-Bangla Agricultural University	:	SAU
Society	:	*Soc.*
Square meter	:	m^2
Standard deviation	:	SD
Standard Error	:	SE
Symposium	:	Symp.
Technical	:	Tech.
That is	:	*i.e.*
Theory	:	*Theor.*
Ton	:	*t*
Triple Super Phosphate	:	TSP
Tropical	:	Trop.
Wheat Research Centre	:	*WRC*

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CHAPTER I

INTRODUCTION

The physiological responses of plants to a deficit of water include leaf wilting, a reduction in leaf area, leaf abscission, and the stimulation of root growth by directing nutrients to the underground parts of the plants. Plants are more susceptible to drought during flowering and seed development (the reproductive stages), as plant's resources are deviated to support root growth. Crop plants undergo several environmental stresses, which lead to significant reduction in production (Farooq *et al.,* 2011). Among these environmental stresses drought is considered one of the most devastating factor, which cause significant reduction in crop productivity (Noorka and Heslop-Harrison, 2014). It disrupts normal growth, impairs water relations and reduces water use efficiency in plants (Aroca, 2012).

Wheat (*Triticum aestivum* L.) is one of the most important winter crops and is temperature sensitive and the second most important grain crop after rice. Wheat is among the major staple crops, with about 720 million tons being produced globally. However, its production is projected to decrease across the continent due to recurring droughts that are associated with climate change (Knox *et al.,* 2012). Thus, the wheat yields need to be increased in order to meet the food demands of growing populations (Ray *et al.,* 2013). Therefore, breeding drought tolerant wheat genotypes with relevant agronomic and adaptive traits is key to enhance productivity and food security among wheat growing communities. Phenotyping remains a key criterion for screening breeding materials based on drought adaptive and constitutive morpho-physiological characteristics including yield and its components (Monneveux *et al.,* 2012; Passioura, 2012). It was found that the expression patterns of an abiotic stress-inducible dehydration responsive element binding protein-2 (DREB2) and Bax inhibitor-1 gene in tomato (Islam and Wang, 2009; 2012) and zinc finger protein gene in rice (Islam and Wang, 2008) which is responsible for drought stresses.

Yield of wheat are comparatively low in Bangladesh and this low yield however is not an indication of low yielding potentiality of this crop, but may be attributed to a number of reasons. Among different factors, seeds of high yielding varieties and water

availability or drought stress are the major reasons of yield reduction. Drought is the most common factor that limits the productivity of wheat crops in Bangladesh. Fischer (1999) showed that under drought, yield reduction in spring wheat is on average of 60% of productivity. So efforts to identify drought tolerance characters among the existing varieties/lines to incorporate the tolerance character into the newly developed varieties are an important aspect to increase the yield of wheat in the climatic condition of Bangladesh.

Screening of genotypes on the basis of seedling traits is easy, less expensive and less laborious. Similarly, seedling traits exhibit moderate to high variability with additive gene action across environment (Rauf *et al.,* 2008), thus have a benefit of efficient selection at early stage. Vigorous seedling is extremely important in determining the yield of crop in short period of time (Noorka and Khaliq, 2007). Under rainfed conditions of arid and semi-arid regions, low moisture is limiting factor during germination (Misra, 1990; Misra *et al.,* 2002). A variety with water stress tolerance has more impregnable rooting abilities to encourage the absorption of soil moisture and diminishes the effects of water shortfall on growth (Zhong and Wang, 2012). Root, the foremost part of wheat plant, is attacked first by water stress. Long roots ensure availability of moisture from then dept. of soil and guarantee the adaptation in drought stress conditions. Seedling growth is also reported to be affected by limited water supply but effect is different for different cultivars (Noorka, 2014). Environmental variability greatly affects the performance of genotypes. Previous studies indicated that proline is among key biochemicals that accumulate in significant proportions in plants that are exposed to various kinds of stress, including dehydration (Hong-Boa *et al.,* 2006; Khamssi, 2014).

Biochemical analysis including mannitol, glycine betaine, trehalose and proline contents, have long been proposed to be useful as a complementary strategy for selection of drought tolerant genotypes in plant breeding (Mwadzingeni *et al.,* 2016). Proline, which is an α-amino acid, has been associated with several osmoprotection roles, including; osmotic adjustment (Marek *et al.,* 2009; Zadehbagheri et al., 2014) and gene signaling to activate anti-oxidizing enzymes that scavenge reactive oxygen species (ROS) (De Carvalho *et al.,* 2013). Information on the correlation between proline

accumulations at critical growth stages of wheat with drought stressed yield and other agronomic traits is limited. Most previous studies quantified proline at the seedling stages without considering the ultimate grain yield. Exploration of proline content under severe stress in a pool of diverse genotypes at critical growth stages and description of its correlation with the yield and its component traits will provide useful information for rapid germplasm screening when breeding for drought tolerance. However, some of the of the physiological and biochemical changes related to drought tolerance in wheat genotypes are to be identified for developing drought tolerant varieties.

There is therefore a need to intensively screen a large pool of wheat breeding lines for drought tolerance using yield, yield related traits and proline analyses. So in the context of the above mentioned situation and in respect of wheat cultivation in Bangladesh, the present piece of work was undertaken with 20 wheat genotypes for fulfilling the following objectives:-

i.   To find out drought tolerant wheat genotype to use in breeding cope with drought stress in future climate change situation.

ii.   To determine the genotypic variation for drought tolerance among diverse wheat genotypes based on morpho-physiogenic traits and proline analysis.

iii.   To study the morpho-physiological and biochemical under drought stress situation.

CHAPTER II

REVIEW OF LITERATURE

Comprehensive studies on wheat breeding were performed in many countries for its improvement. In Bangladesh, a fair amount of research was also being carried out by various agricultural universities and research institutes. An effort has been made here to review the findings of the studies relevant to present investigation.

2.1 Influence of water availability or drought stress:

Qadir *et al.,* (2018) Reported that the current study is an effort to identify the best wheat genotype, which can tolerate drought inarid environmental conditions of Lasbela district, Balochistan. For this purpose, five genotypes of wheat, *viz.,* Amber, Mehran, Khirman, Imdad-05 and Sehar-2006 were selected. These five wheat genotypes seeds were sown in winter of 2015-16 at Lasbela University campus for 45 days. The biomass, leaf size, leaf weight and moisture constituents of leaves were collected when these varieties started to show the signs of drought stress in the form of wilting. The results showed that the Sehar-2006 is a promising genotype when grown in Lasbela's local environmental condition compared with other genotypes. On the other hand, Mehran is one most vulnerable genotype when grown in this region. Three genotypes (Imdad-05, Khirman and Amber) showed mix response in relation to water stresses. The research highlighted that Sehar-2006 is the best species to be cultivated by the farmers and it can provide good vigor and high production for future development.

Ahmed *et al.,* (2019) reported that diminishing water resources as a result of excessive use of water for irrigation and climate change posture a severe global threat to food security. Herein, an experiment was conducted to determine the selection criteria for drought-tolerant bread wheat genotypes at the seedling stage using morphological and photosynthetic pigmentation-related traits. A panel of 105 wheat landraces, historical Pakistani varieties, and advance breeding lines were evaluated under normal and drought stress using factorial completely randomized design. The root length, fresh weight, dry weight, cell membrane thermo-stability, and chlorophyll *b* were positively

correlated among themselves under both normal and stress conditions. Hence, selection of any one of these traits enhances the performance of other traits. The shoot length was non-significant and negatively associated with all other studied characters except relative water content. The results suggested that selection for shoot length could not improve genetic gain for drought tolerance. Out of 10 principal components (PCs), the first three PCs were showed significant genetic variation under both conditions. The first three PCs showed 74.6% and 76% cumulative genetic variation under normal and drought conditions, respectively. Based on PCA, 10 drought-tolerant and five drought-susceptible genotypes were identified. Overall results suggested that selection for root length, fresh weight, dry weight, cell membrane thermo-stability, and chlorophyll *b* at the seedling stage would improve genetic gain for drought tolerance. The outperforming genotypes under drought stress conditions can be useful in future wheat breeding programs, and early selection for the traits recommended in this study will be effective for developing high-yielding and drought-tolerant wheat varieties.

Khakwani *et al.* (2011) investigated that plants of six wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04, and Dera-98) were grown under three water regimes i.e. 100% Field Capacity (FC), 35% FC and 25% FC. Results of this experiment showed highly significant difference among wheat varieties in all the studied traits and water stress conditions decreased them significantly. The superior variety Hashim-8 which indicated higher relative water content (RWC), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI) whereas stress susceptibility index (SSI) and tolerance (TOL) was observed at its lowest. These traits are recognized as beneficial drought tolerance indicators for selecting a stress tolerant variety. Similarly, total grain yield per plant, biological yield per plant and harvest index was also higher in the same wheat variety, which put it as a good candidate for selection criteria in wheat breeding program for drought resistance.

Iqbal *,* (2019) reported that wheat is most important crop and mostly grows in rainfed areas. In cereal crops, wheat having highest protein content. In the abiotic stresses, mostly drought effects wheat productivity and at growth stages. According to climate change, frequency of drought increases in arid and semi-arid region because of water

shortage. Drought effects all growth stages of wheat and more critical at flowering and grain filling stage. Losses of wheat productivity depend on the severity and duration of drought because of reducing in photosynthesis, stomata closure, metabolic activity decrease, oxidative stress increase and result in poor grain formation ultimately yield loss. Easy method to get yield from drought areas are to develop drought tolerance genotypes according to marks. Heritable variation required for the improvement, but heritability is low because of the genotypic and environmental interaction. Different genotypes of wheat behave different in drought. A comprehensive study helps us understanding of some important markers. Breeders can select well adaptive drought genotypes on the base of morphological markers (avoid leaf senescence, flag leaf, root system, grain development, stay green character, cuticular wax and stomata conductance.), physiological markers (abscisic acid (ABA), proline, chlorophyll content, jasmonic acid (JA) and cell stability). Several genes which are doing job for drought stress tolerance and change the enzymes and proteins like, late embryogenesis abundant, rubisco, responsive to abscisic acid, glutathione-S-transferase, carbohydrates, helicase, and proline during drought stress. Drought stress alters some gene Review Article Iqbal; AJBGE, 2(1): 1-13, 2019; Article no.AJBGE.46253 2 expression and cannot work properly due to the influence of environmental factors. Researchers used biotechnological tools to identify the specific genes for drought tolerances. These markers help us to identified drought tolerance genotypes for breeding program. This review paper covers morphological, and physiological for the development of drought tolerance genotypes.

2.2 Influence of variety on wheat:

Babgohari *et al.,* (2017) investigated that understanding the interrelationships between agronomic and water status characters helps development of drought tolerant cultivars. In the present study, 34 wheat genotypes were used to investigate joint variability of water status characters and yield related traits under normal irrigation regimes and drought stress in 2014-2015 growing season. The results indicated that selection of genotypes based on loadings in factors number 1, 3 and 4 would be of beneficial in terms of increasing grain yield related traits under drought stress conditions. In canonical correlation analysis (CCA), the first (U1, V1) and second (U2, V2) pairs

canonical variables (CV) explained 75% and 67% of the total joint variability of agronomic and physiological traits under drought stress conditions, respectively. The first (V1) physiological CV that had positive correlation with water saturated deficit (WSD, $r = 0.63$) and excised leaf water loss (ELWL, $r = 0.35$) was more correlated with grain yield and harvest index under drought stress. The second (V2) physiological CV which was more influenced by variations in initial water content (IWC, $r = 0.6$) and leaf water content (LWC, $r = 0.65$) was associated with spike length and spikelet per spike variations. Significant between-groups mean squares advocated that classification of genotypes into four groups was the best possible branching under both conditions. Therefore, making crosses between genotypes of cluster numbers 1 (with high grain yield and its components) and 3 (having shortest height) can be used for the production of extreme or novel phenotypes for grain yield and dwarfness in the progenitors in further breeding programs for drought tolerance.

Haque *et al.,* (2010) screening of wheat genotypes for drought tolerance was done at the research field of Agronomy Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during November, 2009 February 2010. Thirty (30) wheat genotypes collected from Wheat Research Centre of BARI, were evaluated against drought at vegetative stage (stress was imposed from CRI stage to before anthesis by withholding irrigation) with control (no drought). Exposure of plants to drought led to noticeable reduction in yield and yield contributing characters such as plant height (1-13 %), number of spikes per plant (10-48 %), TDM (16-45 %), number of seeds per spike (7-43 %), 100- seed weight (49-69%) and seed yield (15-65 %). Under drought stress condition, BCN, BAW- 923(C7), KAN (C9) and BAW- 1138 produced higher seed yield than other genotypes, which gave above 80% seed yield compared to control. These genotypes also showed higher values of all other yield-contributing characters under drought stress. Based on the yield of genotypes under control (YP) and drought stress (YS) conditions, three quantitative drought tolerance indices including relative yield (RY), stress susceptibility index (SSI) and stress tolerance index (STI) used to evaluate drought responses of these genotypes. According to stress tolerance index, Shatabdi, BCN, KAN (C9), BAW- 923/4, BAW- 923/BAW- 824, Garuda and Oasis (RC5 Jo) showed higher values (STI >0.8) though Shatabdi,

Oasis (RC5 Jo) and Garuda were discarded from the selection because they produced very lower yield in stress condition and STI was able to identify cultivars producing high yield in both conditions. The genotypes BCN, BAW- 923 (C7), KAN (C9) and BAW -1138 showed higher values in relative yield (RY >80%) and lower values in stress susceptibility index (SSI) and stress tolerance index (STI) used to evaluate drought responses of these genotypes. According to stress tolerance index, Shatabdi, BCN, KAN (C9), BAW- 923/4, BAW- 923/BAW- 824, Garuda and Oasis (RC5 Jo) showed higher values (STI >0.8) though Shatabdi, Oasis (RC5 Jo) and Garuda were discarded from the selection because they produced very lower yield in stress condition and STI was able to identify cultivars producing high yield in both conditions. The genotypes BCN, BAW- 923 (C7), KAN (C9) and BAW -1138 showed higher values in relative yield (RY >80%) and lower values in stress susceptibility index (SSI<0.06) On the basis of STI, SSI and RY, the genotypes BCN, BAW 923- (C7), KAN (C9), BAW -923/4, BAW- 923/BAW- 824 and BAW1138 were selected as drought tolerant at vegetative stage.

2.3 Genetic variability for yield characters and yield of wheat:

Chachar *et al.,* (2016) denoted that climate change is emerging phenomena and causing frequent drought which lead to scaricity of water, which ultimately nagetively affecting wheat (*Triticum aestivum* L.) yield all around the world. The aim of this study was to explore the potential drought tolerant wheat genotypes for candidate genes exploration. This study was conducted during the year 2014-2015 at Plant Physiology Division, Nuclear Institute of Agriculture (NIA) Tandojam. The six wheat genotypes (cv. MT-1/13, MT-2/13, MT-3/13, MT-4/13 Chakwal-86 and Khirman) were investigated for their response at germination and seedling stage under different water stress treatments (0, -0.5, -0.75 and -1.0 MPa) in controlled conditions. The results of experiments with reference to genotypes revealed that genotype Chakwal-86 shows maximum seed germination (82.58 %), while the genotype Khirman shows maximum shoot length (7.23 cm), root length (15.1 cm), shoot fresh wt. (5.85 g 10-1shoots), root fresh wt. (3.45 g 10-1roots), shoot dry wt. (1.33 g 10-1shoots), root dry wt. (0.69 g 10-1roots). Among the genotypes tested Khirman and MT-4/13 are the tolerant genotypes had the

potential to perform better under drought conditions, whereas MT-4/13 and Chakwal-86 were moderate tolerant under water stress conditions. Moreover, the genotypes i.e. MT-1/13 and MT-2/13 are the sensitive genotypes under drought environment. It is concluded from present in-vitro studies that osmotic stress significantly reduced the seed germination shoot/root length fresh and dry weight in all six wheat genotypes. The maximum reduction was found at higher osmotic stress induced by PEG-6000 (-1.0 MPa) significantly.

Jatoi *et al.,* (2011) reported that twelve wheat cultivars of diverse characters and origin were studied at Sindh Agriculture University, Tandojam, Pakistan. The experiment was laid-out in factorial design with two treatments (non-stress and stress at anthesis) and three replications during crop season 2007-08. The analysis of variance revealed significant differences between treatments and among the cultivars. The treatment × cultivar interactions were also significant for all the characters except grain yield per plant. Significant interactions indicated that cultivars performed differentially over the stress conditions, yet consistently for grain yield. The cultivars TD-1, SKD-1 and Sarsabz showed minimum reduction in physiological as well as yield characters in stress at anthesis, however low to high reductions were observed in stress conditions as compared to non-stress. Correlations among morphological, physiological and morpho-physiological traits such as plant height, grains per spike, seed index, grain yield per plant, harvest index, relative water content, stomatal conductance, leaf area and spike fertility were generally reliable indicators for screening drought tolerant wheat cultivars and potentially with higher yields. It is further observed that improvement in any of these traits will lead to increased grain yield under water stress conditions.

Variation, heritability and genetic advance for days to maturity were usually studied for developing early maturing varieties. Nessa *et al.,* (1994) reported low genotypic and phenotypic coefficients of variation for days to maturity in wheat. The difference between them was very small indicating at less influence of environment on this trait. They observed high heritability and moderate genetic advance for this trait. Sharma and Kaul (1986) carried out an experiment with wheat and observed high genotypic and phenotypic coefficients of variation for this trait. High heritability and moderate genetic

advance for this character was also reported. Tripathi *et al.,* (1973) conducted an experiment with 16 varieties of wheat and reported moderate genotypic and phenotypic coefficients of variation. But they also observed low genotypic and phenotypic coefficient of variation along with high heritability with moderate genetic advance for this character

2.4 Relationship between yield contributing characters of wheat:

Ahmad *et al.* (2013) investigated that drought significantly reduces yield of many crop plants including wheat in the world. Identification of wheat genotypes that can thrive on limited water is vital to boost the wheat production of rainfed areas. Forty wheat genotypes were screened 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. Data were recorded on various seedling parameters like germination percentage, germination rate index, root length, shoot length, coleoptile length and seedling vigor. 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.

Meena *et al*., (2015) showed that moisture stress is a major constraint in productivity across the wheat growing zones of India. Climate change and uneven rainfall further aggravate the situation under moisture stress environments. Wheat genotypes capable of giving increased yield under a broad range of optimal and sub-optimal water availability are considered desirable. This study was undertaken to evaluate various selection indices of moisture stress and their applicability in identifying drought tolerant wheat genotypes which can adapt to various moisture stressed environmentsin different

wheat growing zones of India i.e., North Western Plain Zone, North Eastern Plain Zone, Central Zone and Peninsular Zone. A set of wheat genotypes were tested under moisture stress condition of different irrigation regimes. Irrigation treatments were arranged as main plots and varieties as sub plots. Fifteen wheat varieties representing major wheat growing zones of India were tested for water stress tolerance during two consecutive years. It was found that yield under irrigated conditions (Ypi), yield under stress conditions (Ysi) and lower stress tolerance index (STI), were marked indices for stress tolerance. Significantly positive correlation of Ypi and Ysi with STI, mean productivity (MP), geometric mean productivity (GMP) were obtained during both the years of the study. The indices of STI, MP and GMP could be used as the desirable indices for screening drought tolerant varieties. On the basis of findings of these indices wheat varieties NI-5439, WH-1021 and HD-2733 were found having higher stress tolerance and with better yield potential under both normal and restricted irrigation conditions of India.

Moradi *et al.,* (2015) reported that drought is one of the major factors limiting crop production in arid and semi-arid regions. In order to identify drought tolerant bread wheat genotypes using agro-morphological traits, physiological criteria and new integrated selection index, 20 bread wheat genotypes were studied in a randomized complete block design (RCBD) with three replications under irrigated and rainfed conditions. The results of ANOVA in the rainfed condition showed significant differences for all the characters investigated except for Chlorophyll a, b (Chl a, Chl b) and relative chlorophyll content (RCC), indicating the presence of genetic variation and possibility of selection for drought tolerant genotypes under drought condition. Dunkan's multiple rang test revealed that the genotype (18), (15) and (3) had higher grain's yield while genotypes (10) and (11) exhibited lower value for these trait under rainfed condition. In our study, genotypes (10), (4) and (11) displayed the lowest and genotypes (18), (3) and (19) the highest values for integrated selection index (ISI). Principal component analysis (PCA) showed that the integrated selection index (ISI) was correlated with relative water loss (RWL), Chlorophyll a (Chl a), Total chlorophyll (TChl) and grain yield under irrigation and rainfed conditions. The Results indicating that these screening techniques can be useful for selecting drought tolerant genotypes.

In consideration to all indices, genotypes (18), (3) and (6) showed the best mean rank and low rank and rank sum in water deficit stress condition, hence they were identified as the most drought tolerant genotypes which is almost in agreement with the results of our new index (ISI), while genotypes (10), (4) and (11) as the most sensitive. Therefore, this genotype recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in bread wheat.

Soleimani *et al.,* (2014) investigated that cultivation of drought adapted genotypes is the best approach to avoid yield loss under water deficit condition. In order to screen for drought tolerance 82 Iranian wheat genotypes were evaluated by recording relative water content (RWC), days to maturity, leaf rolling and leaf silvering under stress condition in a field trial. In next experiment ten genotypes (six tolerant and four sensitive) selected from field experiment were assessed for drought adaptability measuring RWC, osmotic adjustment (OA), catalase (CAT) and peroxidase (POD) activity and stomatal characteristics. The genotypes were grown under normal and stress condition with three replications. There was high variability among genotypes in response to drought. Azadi and Ohadi showed significant enhancement in CAT activity while in POD activity maximum increase was recorded for Homa. Kohdasht (0.59MPa) and Arta (0.15MPa) had the highest and lowest OA, respectively. Higher OA ability indicates the potential for accumulating osmolytes in cells to absorb water more easily under stress condition. Stomatal closure in response to drought was more significant in Kohdasht and Arta while in Homa almost no change in stomatal closure was detected. Significant negative correlation (r = -0.65, p <0.05 was obtained between RWC and stomatal opening implying the dominance of stomatal conductance control for water retention in genotypes with higher RWC. The activity of POD in Homa, a drought sensitive genotype, was significantly high under stress condition. Presumably the scavenging of H_2O_2 may be the last defense barrier of this genotype against drought. Kohdasht had the highest ability of water retention under stress condition mostly due to its excellent OA and stomatal closure. These physiological characters can be transferred to high yield genotypes to improve drought adaptability.

Safarian *et al.,* (2013) investigated that wheat (*Triticum aestivum* L.) is one of the most widely cultivated crops in rainfed areas of Iran, where drought is the main limiting factor on yield. The object of this study was the identification of drought-tolerant genotypes in bread wheat. Forty bread wheat genotypes were tested in separate experiments under drought stress and normal conditions in two years (2009–2010 and 2010–2011). Nine drought-tolerance/susceptibility indices including stress susceptibility index (SSI), mean productivity (MP), tolerance (TOL), stress tolerance index (STI), geometric mean productivity (GMP), yield index (YI), yield stability index (YSI), linear regression coefficient (β) and drought response index (DRI) were determined. Simultanously applied factor analysis used two factors instead of nine indices in this study. Mahdavi was recognized as the most drought-tolerant genotype in both years based on factor analysis. In this study an equation was developed for estimating the Stress Tolerance Score (STS). The results of the equation were identical to those of factor analysis in both years. The equation was much easier to use than factor analysis and is suggested as a screening tool for the identification of drought-tolerant genotypes. In this study, Mahdavi was the most drought-tolerant genotype also corresponding to this equation.

Bowne *et al*., (2012) reported that drought has serious effects on the physiology of cereal crops. At the cellular and specifically the metabolite level, many individual compounds are increased to provide osmo protective functions, prevent the dissociation of enzymes, and to decrease the number of reactive oxygen species present in the cell. We have used a targeted GC-MS approach to identify compounds that differ in three different cultivars of bread wheat characterized by different levels of tolerance to drought under drought stress (Kukri, intolerant; Excalibur and RAC875, tolerant). Levels of amino acids, most notably proline, tryptophan, and the branched chain amino acids leucine, isoleucine, and valine were increased under drought stress in all cultivars. In the two tolerant cultivars, a small decrease in a large number of organic acids was also evident. Excalibur, a cultivar genotypically related to Kukri, showed a pattern of response that was more similar to Kukri under well-watered conditions. Under drought stress, Excalibur and RAC875 had a similar response; however, Excalibur did not have the same magnitude of response as RAC875. Here, the results are discussed in the

context of previous work in physiological and proteomic analyses of these cultivars under drought stress.

Inheritance of grain yield in bread wheat is a complex one. Grain yield in cereals is determined by some yield components. Grafious (1964) suggested that these yield components had expressed their genetic and environmental effects through grain yield. Nessa *et al.,* (1994) reported high genotypic and phenotypic coefficients of variation for grain yield/plant. High heritability and high genetic advance for grain yield/plant in bread wheat was also noticed. Sharma and Kaul (1986) reported high genotypic and phenotypic coefficients of variation with high heritability and high genetic advance. Pathak and Nema (1985) also observed high genotypic and phenotypic coefficients of variation with high heritability and high genetic advance for grain yield in bread wheat. Tripathi *et al.,* (1973) observed high genotypic and phenotypic coefficients of variation in bread wheat for grain yield/plant. High heritability and moderate genetic advance was also noticed for this trait. High genetic advance for grain yield as observed in these studies was probably the root cause of high coefficient of variation and high heritability for grain yield in wheat.

Bogale *et al.*, (2011) investigated that the experiment was conducted to assess the differential morpho-physiological response to stimulated water deficit and to determine the relationship between some of these morphological and physiological traits and yield components of eighteen durum wheat genotypes grown in pots under lath house condition. Water deficit significantly affected gas exchange and chlorophyll fluorescence parameters. It reduced the net photosynthesis rate (Pn), transpiration rate (E) and stomatal conductance (gs) measured both at anthesis and grain-filling stages. Similarly, the value of initial fluorescence (Fo) was increased while variable fluorescence (Fv), maximum fluorescence (Fm) and optimum quantum yield fluorescence (Fv/Fm) were decreased under water deficit. RWC of the leaves was decreased by 36.7% while SLA increased by 12.6% due to moisture stress relative to the well-watered control. No significant correlations were found between chlorophyll fluorescence parameters and grain yield under water deficit condition. Similarly, no significant correlations were found between leaf gas exchange parameters and grain

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yield. On the other hand, peduncle length and excursion were positively correlated with grain yield while negatively correlated with drought susceptibility index under water deficit condition. Leaf posture and rolling had also a profound effect on grain yield and other attributes. Erect leaved genotypes had more grain yield, HI, kernel numbers per spikelet and grain-filling rate but had lower kernel weight than droopy leaved. Similarly, genotypes exhibited strong leaf rolling under water deficit condition had more grain yield, kernel numbers per spike and water use efficiency. The genetic variability found for leaf posture, leaf rolling, peduncle length and excursion among the Ethiopian durum wheat genotypes suggests the opportunity for selection superior and adapted genotype in water-limited environments. These can be achieved by integrating these morphological traits as indirect selection in conjunction with other yield components.

Rampino *et al.,* (2006) denoted that water deficit is a severe environmental stress and the major constraint on plant productivity with   an evident effect on plant growth. The aim of this work was to study *Triticum* and *Aegilops* seedlings differing in their response to drought stress at the physiological and chemical levels. The identification of resistant and sensitive genotypes was firstly based on the relative water content (RWC) measurement. Further characterization of genotypes contrasting in their response to water stress was performed at the physiological level by determination of RWC, water loss rate (WLR) and free proline content after different hours of dehydration.

Several scientists had observed positive direct effect on grain yield/plant via yield component characters in bread wheat. Das (1972) studied path analysis and reported that the highest direct effect was obtained for number of spikes/plant on grain yield. Shamsuddin (1987) studied path analysis and observed that spikes/plant, grains/spike and 1000-grain weight had direct effects on yield/plant. Shamsuddin and Ali (1989) studied genotypic and phenotypic correlation and path analysis in spring wheat and reported that grains/spike displayed considerable amount of direct effects on grain yield followed by spike length and 1000-grain weight. Paroda and Joshi (1970) observed that grains/spike and 1000-grain weight had positive direct effects on yield. Das and Mondal (1984) observed that number of grains/spike had a moderate direct effect on grain yield.

They identified that number of grains/spike was one of the major component of yield in bread wheat. Shelembi and Wright (1991) reported that number of grains/spike had direct and strong effect on grain yield.

Many researchers had also observed negative direct effect on grain yield/plant via plant height (Barma*et et al.,* 1991), vegetative period (Rahman *et al.* 1983) and grain filling period (Razzaque*et et al.,* 1981) in bread wheat.

Bhular *et al.,* (1985) suggested from path analysis that 1000-grain weight was one of the most important yield components in durum wheat. Amin *et al.,* (1990) observed that 1000-grain weight contributed maximum positive and direct effect to grain yield. Khan *et al.* (1994) found that 1000-grain weight exhibited positive association and high direct effect on grain yield and suggested hybridization program should include genotypes with greater number of grains/spike, high grain weight and high grain yield to obtain further improvement grain yield in bread wheat.

Several statistical methods were usually used for discriminating among the genotypes *viz.*Mahalanobis' generalized distance (Mahalanobis, 1936), the algorithm method of Williams and Lambert (1960), Cooper's statistical classification with quadratic forms (Cooper, 1963) and Principal component analysis. The principal component analysis (PCA) resulted in the reduction of enormous variables to three independent linear combination principal component variables.

Balasch *et al.,* (1984) reported that in classifying a number of tomato varieties/lines, different multivariate techniques were used and Principal Component Analysis (PCA), as a simple multivariate technique, was compared with factorial analysis and Mahalanobis' D^2 distance. It was marked that three methods gave similar results. But factorial discriminate and Mahalanobis' D^2 distance methods required collecting data plant by plant, while the PCA method required taking data by plots.

Principal Component Analysis was performed in soybean (Chowdhury, 1994) and in pea (Mian *et al.,* 1991) in order to assess genetic diversity among the germplasms of these crops.

Joshi and Kohli (2003) assessed the nature and magnitude of genetic divergence using non-hierarchical Euclidean cluster analysis in 73 tomatoes for different quantitative and qualitative traits

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted to find out the role of morpho-physiological and biochemical character in spring wheat under drought stress. Materials used and methodology followed for conducting the research along with data recording and analyzing procedure were described briefly as follows.

3.1. Description of the experimental site

3.1.1. Experimental period

The experiment was conducted during the period from November 2017 to April 2018 in rabi season.

3.1.2 Site description

The present piece of research work was conducted in the experimental area of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the site is $23^070^/$N latitude and $90^035^/$E longitude with an elevation of 8.2 meter from sea level.

3.1.3 Climatic condition

The geographical location of the experimental site was under the subtropical climate and its climatic conditions is characterized by three distinct seasons, namely winter season from the month of November to February and the pre-monsoon period or hot season from the month of March to April and monsoon period from the month of May to October (Edris *et al.,* 1979). Details of the meteorological data of air temperature, relative humidity, rainfall and sunshine hour during the period of the experiment was collected from the Weather Station of Bangladesh, Sher-e-Bangla Nagar, Dhaka and details has been presented in Appendix II.

3.1.4 Geographical location and soil characteristics

Geographical location of the experimental site was described as 23°74' N latitude and 90° 35' E longitude at an altitude of 8.6 meter above the sea level. The soil belonged to "The Modhupur Tract", AEZ-28(FAO,2000). Top soil was silty clay in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. Soil pH was 5.6 and had organic carbon 0.45%. The experimental area was flat having available

irrigation and drainage system and above flood level. The selected plot was medium high land. The details have been presented in Appendix III.

3.2 Experimental details

3.2.1 Planting materials

In this experiment 20 wheat genotypes (Table 1) were used as experimental materials which were produced in the 2017-2018 cropping season, and the purity and germination percentage were leveled as 98% and 95%, respectively. These genotypes were collected from Wheat Research Centre (WRC) of Bangladesh Agricultural Research Institute (BARI), Rajshahi centre, Rajshahi.

Table 1. Name of wheat genotypes used in the present study

SL.	Genotypes	SL.	Genotypes
01.	Shatabdi (Check)	11.	SAWYT-347
02.	Prodip (Check)	12.	DTWYT-02
03.	BARI GOM-28 (Check)	13.	DTWYT-03
04.	BARI GOM-30 (Check)	14.	DTWYT-16
05.	SAWYT-303	15.	DTWYT-22
06.	SAWYT-312	16.	SAWYT-326
07.	SAWYT-313	17.	SAWYT-327
08.	SAWYT-317	18.	SAWYT-331
09.	SAWYT-323	19.	SAWYT-344
10.	SAWYT-324	20.	SAWYT-345

SAWYT: Semi-Arid Wheat Yield Trial, DTWYT: Drought Tolerant Wheat Yield Trial

3.2.2 Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The total area of the experimental plot was 535.5 m^2 with length 76.5 m and width 7.0 m. The total area was divided into three equal blocks. Each block was divided into 20 plots where 20 wheat genotypes were allotted at random. There were 60 unit plots altogether in the experiment. The size of the each plot was 2.0 m × 1.0 m. The distance maintained between two blocks and two plots were 1.0 m and 0.5 m, respectively.

3.3 Growing of crops

3.3.1 Land preparation and fertilization

The land was prepared by ploughing with power tiller followed by harrowing and laddering. All the stubbles and weeds were removed from the field. In the following day Cowdung was applied 126 kg. According to recommended fertilizer doses TSP, MP and Gypsum was applied 3 kg, ½ kg and 1 kg respectively. 1 kg Urea was also applied during final land preparation and rest 2 kg was split applied at tillering and panicle initiation stage.

3.3.2 Sowing of seeds

Furrows were made for sowing the wheat seeds when the land was in proper joe condition and seeds were sown at 18 November, 2017 (Plate 1). Seeds were sown continuous with maintaining 20 cm line to line distance and plant to plant 5 cm. After sowing, seeds were covered with soil and slightly pressed by hand.

Plate1: Seed sowing in the field

3.3.3 Application of fertilizers and manure

The fertilizers N, P, K and S in the form of Urea, TSP, MP and Gypsum, respectively were applied. Cowdung was applied @ 10 t ha^{-1} during 15 days before seeds sowing in the field. The entire amount of TSP, MP and Gypsum, 2/3rd of Urea were applied during the final preparation of land. Rest of urea was top dressed after first irrigation (BARI, 2011). The dose and method of application of fertilizer are presented below in Table 2.

Table 2. Doses and method of application of fertilizers in wheat field

Fertilizers	Dose (per ha)	Application (%)	
		Basal	1st installment
Urea	220 kg	66.66	33.33
TSP	180 kg	100	--
MP	50 kg	100	--
Gypsum	120 kg	100	--
Cowdung	10 ton	100	--

Source: BARI, 2011, Krishi Projukti Hatboi, Joydebpur, Gazipur.

3.3.4 After care

After the germination of seeds, various intercultural operations such as weeding, top dressing of fertilizer and plant protection measures were accomplished for better growth and development of the wheat seedlings as per the recommendation of BARI. Plate 2 and 3 showing field inspection at different stages in the field.

Plate 2: Field inspection at seedling stage

Plate 3: Field inspection at floweing stage

3.3.4.1 Drainage and irrigation

Drainage was made for suitable irrigation channel. At crown initiation stage, irrigation was a must. Otherwise, yield would be drastically low. So, after 18 days, 1st irrigation was given. 2nd irrigation was given after 58 days at panicle initiation stage and 3rd irrigation was given after 75 days at grain filling period.

3.3.4.2 Thinning and weeding

Weak and densely grown plants were discarded. Various weeds like bathua, nunia, bonnomasur etc. was weeded out through raker and nirani.

Plate 4: Field inspection with supervisor

3.3.4.3 Plant protection

75 g of Autostin fungicide was sprayed with 40 L water due to fungal attack. The attack was not severe and was mitigated efficiently. Plate 4 and Plate 5 showing field inspection at nearly maturarity stage.

3.3.4.4Application of drought stress

At crown root initiation stage, irrigation was a must. Otherwise, yield would be drastically low. Therefore, after 20 days, 1st irrigation was given and 2nd irrigation was given after 48 days at panicle initiation stage. After that no supplementary irrigation was provided to create drought stress. No rainfall was recorded in the experimental field area (Weather station, Agargoan, 2017-18).

3.4 Harvesting, threshing and cleaning

The crop was harvested manually depending upon the maturity and bundled separately, properly tagged and brought to threshing floor. Enough care was taken during threshing and cleaning of wheat grain. Fresh weight of grain was recorded plot wise from 1 m^2 area. The grains were dried, cleaned and weighed for individual plot. The weight was adjusted to a moisture content of 14%. Yields of wheat grain was recorded and converted into per plant.

3.5 Data collection

3.5.1 Days to 50% heading

Days to 50% heading was recorded by calculating the number of days from sowing to 50% heading by keen observation of the experimental plots.

3.5.2 Plant height

The height of plant was recorded in centimeter during at harvest. Data were recorded as the average of 10 plants selected at random from the inner rows of each plot that were tagged earlier. The height was measured from the ground level to the tip of the plant by a meter scale.

3.5.3 Number of productive tillers

Number of productive tillers was counted from each of the sample plants and was averaged over per plant.

3.5.4 Spike length (cm)

The length of spike length was measured as the average of 10 plants selected at random from the inner rows of each plot. The length was measured from the base to tip of the spike.

3.5.5 Number of spikelets/spike

The total number of spikelets/spike was counted as the number of spikelets from 10 randomly selected spikes from each plot and average value was recorded.

3.5.6 Empty spikelet/spike

The number of empty spikelets/spike was counted as the number of empty spikelets per spike from 10 randomly selected spikes from each plot.

3.5.7 Number of grains/spike

The total number of grains/spike was counted by adding the number of filled and unfilled grains from 10 randomly selected spikes from each plot and average value was recorded.

3.5.8 Days to 50% maturity

Days to starting of maturity was recorded by calculating the number of days from sowing to starting of maturity as spikes become brown color by keen observation of the experimental plot (Plate 5).

Plate 5. Investigating maturity of grains for harvesting in the field

3.5.9 Weight of 1000 grains

One thousand grains were counted randomly from the total cleaned harvested grain of each individual plot and then weighed in grams and recorded.

3.5.10 Chlorophyll SPAD value content

Chlorophyll content of 10 selected leaves was determined from plant samples by using an automatic machine immediately after removal of leaves from plants to avoid rolling and shrinkage (Plate 6).

Plate 6: Determination of cholorophyll (SPAD-502 value) contents in the field

3.5.11 Determination of proline content: Proline analysis was carried out in the following manner. Samples of the second top leaves from the flag leaf was harvested from the stressed plots of the pot experiments. The leaf samples temporarily stored at ultra-low temperature (−20°C) then freeze dried. The dry leaf tissue was ground and 0.1 g samples homogenized in 10 mls of 3% aqueous sulfosalicylic acid. Proline extractions were done following the acid-ninhydrin method according to Bates *et al.,* (1973). This followed by UV-visible spectrophotometer analysis of the absorbance of the proline

extract in toluene at a wavelength of 520 nm, using a model UV-1800 spectrophotometer, Shimadzu Corporation, Kyoto, Japan. The proline concentration was calculated using the following formula:

Proline content(μg per gram of dry leaf tissue)=[(μgproline/ml)×mltoluene)/115.5μg/μ mole]/[(gsample)/5].

3.5.12 Grain yield per plant

Grains obtained from m^{-2} from each unit plot were sun-dried and weighed carefully. The dry weight of grains of central 1 m^2 area used to record grain yield m^{-2} and converted this into per plant yield.

3.6 Statistical analysis: The data obtained for different characters were statistically analyzed to observe the morpho-physiological character in spring wheat under drought stress. The mean values of all the characters were calculated and analysis of variance and variability were performed. Physiological, chlorophyll and proline data were analyzed. Combined analysis of variance was performed following a test of homogeneity of variances. To describe the magnitude of the relationships among agronomic traits, chlorophyll and proline content, Pearson's correlation coefficients (*r*) was calculated separately for the stress and control treatments using the SPSS. Principle component analysis (PCA) based on the correlation matrix were performed using SPSS to identify influential traits for selection. PCA biplots were plotted separately for the stressed using GenStat to show the relationships among studied genotypes based on recorded traits.

3.7 Measures of genetic variability

According to formula given by Johnson *et al.,* (1955).

Genotypic Variance (σ_g^2),

$$\sigma_g^2 = \frac{GMS-EMS}{r}$$

Where,
GMS = Genotypic mean sum of square
EMS = Error mean sum of square
r = No. of replication

Phenotypic Variance (σ_{ph}^2),

$$\sigma_{ph}^2 = \sigma_g^2 + \text{EMS}$$

Where,

σ_g^2 = Genotypic Variance
EMS   = Error mean sum of square

Environmental Variance (σ_e^2),

$$\sigma_e^2 = \sigma_{ph}^2 - \sigma_g^2 - \sigma_{ge}^2$$

Where,

σ_e^2 = Environmental Variance
σ_{ph}^2 = Phenotypic variance
σ_g^2 = Genotypic Variance
σ_{ge}^2 = Interaction between genotype and environment

Genotypic Coefficient of Variation,

$$\text{GCV} = \frac{\sqrt{\sigma_g^2}}{\overline{x}} \times 100$$

Where,

σ_g^2 = Genotypic Variance
$\overline{x}$ = Population mean

Phenotypic Coefficient of Variation,

$$\text{PCV} = \frac{\sqrt{\sigma_{ph}^2}}{\overline{x}} \times 100$$

Where,

σ_{ph}^2 = Phenotypic variance
$\overline{x}$ = Population mean

Environmental Coefficient of Variation,

$$\text{ECV} = \frac{\sqrt{\sigma_e^2}}{\overline{x}} \times 100$$

Where,

σ_e^2 = Environmental Variance
$\overline{x}$ = Population mean

Heritability in broad sense,

$$h_b^2 = \frac{\sigma_g^2}{\sigma_{ph}^2} \times 100$$

Where,

σ_g^2 = Genotypic Variance
σ_{ph}^2 = Phenotypic variance

Genetic advance,

$$\text{GA} = h_b^2 \cdot \text{K} \cdot \sigma_{ph}$$

Where,
h_b^2 = heritability in broad sense
K   = Selection differential, value is   2.06 at 5% selection intensity
σ_{ph} = Phenotypic standard deviation

Genetic advance in percent of mean,

$$\text{GA (\%)} = \frac{GA}{\bar{x}} \times 100$$

Where,

GA = Genetic advance
$\bar{x}$ = Population mean

3.8. Multivariate analysis

Genetic divergence among genotypes was assessed by different multivariate analysis. Rao (1952) suggested that the quantification of genetic diversity through biometrical procedure had made it possible to choose genetically diverse parents for hybridization program.

3.9. Principal Component Analysis (PCA)

PCA could be computed by Correlation matrix and genotype scores obtained from the first components (accounted for maximum variance) and succeeding components with latent roots greater than unity. Linear combination of a set of variate could be found from it. It was being used to investigate the interrelationships among several characters with yield and could be done from the sum of squares and product matrix for the character. Contribution of different morphological characters towards divergence was discussed from the latent vectors of the first two principal components.

3.10 Cluster Analysis (CA)

Cluster analysis was used to divide the genotypes of a data set into some number of mutually exclusive groups. Clustering was done using non-hierarchical classification. In GENSTAT, the algorithm was used to search for optimal values of chosen criterion proceeds as follows. Starting from some initial classification of the genotypes into required number of groups, the algorithm repeatedly transferred genotypes from one group to another so long as such transfer improved the value of the criterion. When no further transfer could be found to improve the criterion, the algorithm had switched to a second stage, which examined the effect of swooping on two genotypes of different classes and so on.

3.11 Calculation of D^2 values

The Mahalanobls's distance (D^2) values were calculated from transformed uncorrelated means of characters according to Rao (1952) and Singh and Chaudhary (1985).

$$\mathbf{D^2} = \sum_1^x (Y_i^j - Y_j^k)^2 \quad (j \neq k)$$

Where,

Y = Uncorrelated variable (i=1,2….to x)

x = No. of characters

3.4.6 Computation of cluster distances

$$\textbf{Intra cluster distance} = \frac{\sum D_i^2}{n}$$

Where,

$\sum D_i^2$ = Sum of distances between all possible combinations (n) of genotypes included in a cluster

n   = All possible combinations

And

$$\textbf{Inter cluster distance} = \frac{\sum D_{ij}^2}{n_i \times n_j}$$

Where,

$\sum D_{ij}^2$ = Sum of distances between all possible combinations (n) of the   populations in cluster i and j

n_i = No. of population in cluster i

n_j = No. of population in cluster j

3.12 Selection of genotypes for future breeding purpose

According to Singh and Chaudhary (1985), following points should be considered while selecting genotypes for hybridization program.

➢ Choice of cluster from which genotype(s) would be selected for use as parent(s)
➢ Selection of particular genotype(s) from the selected cluster(s)
➢ Relative contribution of the characters to the total divergence
➢ Other important traits from the genotype performance

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted to To determine the genotypic variation for drought tolerance among diverse wheat genotypes based on morpho-physiogenic traits and proline analysis. Mean performance, variability, correlation matrix and principal component biplot analysis were done on different yield contributing characters and yield of wheat genotypes were estimated. The experimental results have been presented under the following heads:

4.1 Evaluation of mean performance of different yield contributing characters and yield of wheat under drought stress

Mean performance and analysis of variance was estimated and presented in Table 3 and 4. Turkey's test revealed highly significant variation among 20 wheat genotypes in terms of all the studied characters. Significantly high level of variation for different yield contributing characters and yield revealed the indicative possibilities of improving the genetic yield potential of wheat genotypes under drought stress.

4.1.1 Days to 50% heading

Statistically significant variation was recorded for different wheat genotypes on days to 50% heading under the present trial (Table 3). Data revealed that the average days to 50% heading was around 63.00 days with a range from 53.33 to 68.00 days and more than 50% genotypes have required more than that average day for staring 50% of heading. The highest days to 50% starting of heading (68.00) was observed in the genotype of DTWYT-16 which was statistically similar (67.67) with the wheat genotypes of SAWYT-312, while the lowest days (53.33) from BARI Gom-30 (Table 4). Qasim *et al.,* (2008) reported that days to heading varied for different cultivars of wheat. Mohsen *et al.,* (2013) reported that the effect of cultivars was significant for days to starting of heading in wheat. Sulewska (2004) also reported that day to starting of heading in wheat varied from genotype to genotype.

4.1.1.1 Plant height

Different wheat genotypes showed statistically significant variation in terms of plant height (Table 3). The average plant height was around 76.32 cm with a range from 63.96 cm to 82.20 cm but for plant height most of the genotypes within 70-80 cm in height. The longest plant (82.20 cm) was recorded in genotype Shatabdi which was statistically similar (78.93 cm, 81.49 cm, 81.80 cm, 76.53 cm, 79.11 cm, 77.66 cm,80.38 cm,79.19 cm,79.45 cm and 80.15 cm) with Prodip, SAWYT-303, SAWYT-312,SAWYT-317, SAWYT-324, SAWYT-326, SAWYT-327, SAWYT-344, SAWYT-345, SAWYT-347, and SAWYT-326 again the shortest plant (63.96 cm) was found in wheat genotype BARI GOM-30 (Table 4). Gupta *et al.,* (2001) reported that plant height decreased to a greater extent when water stress was imposed at the anthesis stage while imposition of water stress at booting stage caused a greater reduction in plant height. Islam *et al.,* (1993) reported that plant height significantly affected by variety. Litvinrnko *et al.,* (1997) reported that plant height itself governed by genetically.

4.1.1.2 Number of productive tillers

Statistically significant variation was observed for different wheat genotypes in terms of number of productive tillers (Table 3). The average number of productive tillers was 2.89 with a range from 2.33 to 3.55. The maximum number of productive tillers (3.53) was found in SAWYT-324 which was statistically similar with BARI Gom-28, BARI Gom-30, SAWYT-313 and DTWYT-16, whereas the minimum number of productive tillers (2.33) was observed in the wheat genotype DTWYT-03 which was statistically similar (3.00) with SAWYT-345 (Table 4).

4.1.1.3 Spike length (cm)

Spike length (cm) varied significantly for different wheat genotypes (Table 3). The average spike length was 11.36 cm with a range from 9.11 cm to 14.09 cm. The longest spike length (14.09 cm) was recorded in BARI GOM- 30. On the other hand, the shortest spike length (9.11 cm) was recorded in the wheat genotype BARI Gom-28 which was statistically similar (9.5 and 9.73 cm) with SAWYT- 317 and DTWYT-16 (Table 4).

4.1.1.4 Number of spikelet's/spike

Statistically significant difference was observed for different wheat genotypes in terms of number of spikelet's/spike (Table 3). The average number of spikelet's/spike was around 16.71 with a range from 13.67 to 19.40. The maximum number of spikelet's/spikes (19.40) was found in SAWYT-345, while the minimum number (13.67) was observed in the wheat genotype SAWYT- 317 (Table 4).

4.1.1.5 Empty spikelet/ spike

Number of empty spikelet/ spike showed statistically significant variation for different wheat genotypes under the present trial (Table 3). The average number of empty spikelet/ spike was around 1.47 with a range from 0.37 to 3.07. The maximum number of empty spikelet/ spike (3.07) was attained in BARI Gom-28 whereas the minimum number empty spikelet/ spike (0.37) were observed in the wheat genotype SAWYT- 347. Zarea and Ghodsi (2004) reported that number of spike/m^2 decreased with increasing irrigation intervals. When a 20 and 30-day irrigation interval were applied, number of spike/m^2 were higher in cultivars C-75-14 and C-75-9 (Table 4).

Table 3. Analysis of variance (ANOVA) for yield contributing characters and yield of spring wheat under drought condition

Characters	Mean sum of square		
	Replication (r-1) = 2	Genotype (g-1) = 19	Error (r-1)(g-1) = 38
Days to 50% heading	8.116	59.385**	9.590
Plant height(cm)	1.480	74.616**	12.980
Number of productive tillers	0.864	3.600*	0.192
Spike length(cm)	0.340	4.177**	1.818
Number of spikelet /spike	0.002	9.276**	2.970
Empty spikelet/spike	0.891	1.672**	0.757
Number of grains /plant	17.428	77.855**	18.951
Days to physical maturity	22.216	16.044**	4.181
Thousand seeds weight	41.12	56.26**	7.72
Chlorophyll content	58.763	161.065**	2.926
Proline content	144.803	6848.573**	168.396
Grain yield per plant	0.28	0.58*	0.09

**: Significant at 0.01 level of probability;   *: Significant at 0.05 level of probability

Table 4. Mean performance of yield contributing characters and yield of spring wheat under drought condition

Genotypes	Days to 50% heading	Plant height (cm)	Number of productive tillers	Spike length (cm)	Number of spikelet's /spike	Empty spikelet/ spike	Number of grains /plant	Days to physical maturity	Thousand seeds weight	Chlorophyll content	Proline content	Grain yield per plant
Shatabdi	59.00a-d	82.20a	2.47	12.90ab	18.73ab	0.40	42.07a	103.67a-c	50.03ab	31.99g-i	84.70f-j	5.50ab
Prodip	59.33a-d	78.93a	2.50	12.34ab	18.37ab	1.73	35.47a-d	103.67a-c	48.79a-c	50.99a	105.42e-h	5.57a
BARI GOM-28	53.33d	65.27bc	3.17	9.11b	13.88b	3.07	22.39d	107.00a-c	46.10a-e	40.41b-d	63.95ij	5.45ab
BARI GOM-30	55.67cd	63.96c	3.33	14.09a	14.53ab	2.83	26.67cd	107.00a-c	42.80a-e	53.26a	82.57h-j	5.24a-c
SAWYT-303	67.67a	81.49a	3.13	11.20ab	16.12ab	1.07	33.13a-d	107.00a-c	46.07a-e	31.04h-j	194.74ab	4.80a-e
SAWYT-312	65.67ab	81.80a	2.70	11.53ab	17.54ab	1.13	34.50a-d	108.00a-c	39.73de	43.95b	220.20a	4.15e
SAWYT-313	62.33a-d	74.78a-c	3.47	10.72ab	15.67ab	0.83	32.23a-d	109.00a-c	39.47de	38.71b-e	104.93e-h	4.23de
SAWYT-317	66.33ab	76.53a	2.70	9.58b	13.67b	1.23	30.07a-d	110.00a	48.57a-c	37.66c-f	163.08b-d	4.79a-e
SAWYT-323	67.00ab	71.84a-c	2.73	11.56ab	14.60ab	1.80	29.80a-d	109.33ab	49.60ab	30.28ij	72.49h-j	4.77a-e
SAWYT-324	65.00a-c	79.11a	3.53	11.07ab	16.20ab	1.67	33.53a-d	108.67a-c	40.73c-e	39.92b-d	46.16i	4.42c-e
SAWYT-326	66.33ab	77.66a	2.73	11.33ab	15.73ab	1.53	28.17b-d	109.00a-c	38.93e	25.95jk	144.99c-e	4.35c-e
SAWYT-327	65.00a-c	80.38a	2.87	11.51ab	18.33ab	0.93	34.97a-d	109.00a-c	41.47b-e	37.10c-g	83.77g-j	4.66a-e
SAWYT-331	66.33ab	74.49a-c	2.73	12.41ab	18.23ab	2.00	33.43a-d	108.33a-c	47.77a-d	23.27k	94.07f-i	4.95a-e
SAWYT-344	68.00a	79.19a	3.27	11.30ab	17.33ab	1.67	35.80a-d	106.67a-c	39.10e	36.31d-h	142.79c-e	4.23de
SAWYT-345	63.67a-c	79.45a	3.00	12.59ab	19.40a	1.07	41.60ab	107.33a-c	48.50a-c	40.88b-d	178.53bc	5.13a-d
SAWYT-347	60.00a-d	80.15a	2.73	11.89ab	18.68ab	0.37	37.73a-c	106.00a-c	50.40a	39.84b-d	123.26d-g	5.36ab
DTWYT-02	59.00a-d	76.35ab	2.87	10.63ab	17.20ab	2.40	30.13a-d	105.00a-c	50.23a	33.00f-i	165.18bc	4.89a-e
DTWYT-03	57.67b-d	73.37a-c	2.33	11.17ab	16.91ab	1.60	33.87a-d	104.00a-c	49.43ab	33.80e-i	124.50d-f	5.01a-e
DTWYT-16	57.67b-d	75.46ab	3.13	9.73b	18.20ab	0.40	41.47ab	103.00bc	48.50a-c	41.83bc	97.81f-i	4.98a-e
DTWYT-22	58.67a-d	73.98a-c	2.47	10.49ab	14.93ab	1.73	28.60a-d	102.67c	41.87a-e	36.29d-h	65.97h-j	4.60b-e
Mean	62.18	76.32	2.89	11.36	16.71	1.47	33.28	106.72	45.40	37.32	117.96	4.85
Range	53.33-68.00	63.96-82.20	2.33-3.55	9.11-14.09	13.67-19.40	0.37-3.07	22.39-42.07	102.67-110.00	38.93-50.40	23.27-53.26	46.16-220.20	4.15-5.57
CV(%)	4.98	4.72	15.15	11.87	10.31	59.08	13.08	1.92	6.12	4.58	11.00	6.15
HSD (5%)	9.612	11.182	1.362	4.185	5.349	2.701	13.511	6.347	3.45	5.309	40.277	0.92

Means with the same letter are not significantly different.   HSD = Turkey's Honest Significant Difference (HSD) Test

4.1.1.6 Number of grains/plant

Different wheat genotypes showed statistically significant differences in terms of number of grains/plant (Table 3). The average number of grains/plant was around 33.28 with a range from 22.39 to 42.07. The maximum number of grains/plant (42.07) was recorded in Shatapdi and the minimum number (22.39) in wheat genotype BARI Gom-28 (Table 4). Good quality wheat variety for producing maximum yield through the highest yield contributing characters that plays an important and major role for wheat production. Islam *et al.,* (1997) reported that grain/spike was significantly affected by variety.

4.1.1.7 Days to 50% of maturity

Days to 50% of maturity varied significantly due to different wheat genotypes (Table 3). Data revealed that the average days to starting of maturity was around 106.72 days with a range from 102.67 to 110.0 days and in an average 50% genotypes had required less than that average day required for staring of heading. The highest days to starting of maturity (110.00) was found in genotype SAWYT-317 whereas the lowest days (102.67) were attained in the wheat genotypes DTWYT-22 (Table 4).

4.1.1.8 Weight of 1000 grains

Weight of 1000 grains showed statistically significant variation for different wheat genotypes under the present trial (Table 3). The average weight of 1000 grains was 45.40 g with a range from 38.93 g to 50.40 g. The highest weight of 1000 grains (50.40 g) was found in SAWYT-347 which was statistically similar (50.23 g) with DTWYT-02. On the other hand, the lowest weight of 1000 grains (38.93 g) was attained in the wheat genotype SAWYT-326 which was statistically similar (39.10 g) with SAWYT-344 (Table 4). *Malik et al.,* (2010) reported that that the yield contributing parameters were significantly higher when crop was irrigated with five irrigations, while 1000-grains weights were not affected significantly. Islam *et al.*, (1993) reported that 1000-grain weight was significantly affected by variety.

4.1.1.9 Chlorophyll content

Statistically significant variation was recorded for different wheat genotypes in terms of chlorophyll content (Table 3). Data revealed that the average chlorophyll was around

37.32 with a range from 23.27 to 53.26. The highest chlorophyll content (53.26) was attained in BARI Gom-30 which was statistically similar (50.99) with wheat variety Prodip, whereas the lowest chlorophyll content (23.27) was found in the wheat genotype DTWYT-331 (Figure 1). Zarea and Ghodsi (2004) reported also reported similar findings earlier.

4.1.1.10 Proline content

Proline content varied significantly for different wheat genotypes under the present trial (Table 3). The average proline content was 117.96 with a range from 46.16 to 220.20. The highest proline content (220.20) was observed in SAWYT-312 while the lowest proline content (46.16) was found in the wheat genotype SAWYT-324 (Figure 2).

4.1.1.11 Grain yield per plant

Different wheat genotypes showed statistically significant differences in terms of grain yield per plant (Table-3). The average grain yield per plant was 4.85 g with a range from 4.15 g to 5.57 g. The highest grain yield per plant (5.45 g) was recorded in Prodip, while the lowest grain yield per plant (4.15 g) was observed in the wheat genotype SAWYT-312 (Table 4). Razi-us-Shams (1996) observed that the effect of irrigation treatments on yield and yield contributing characters were statistically significant. Irrigation increased the grain yields.
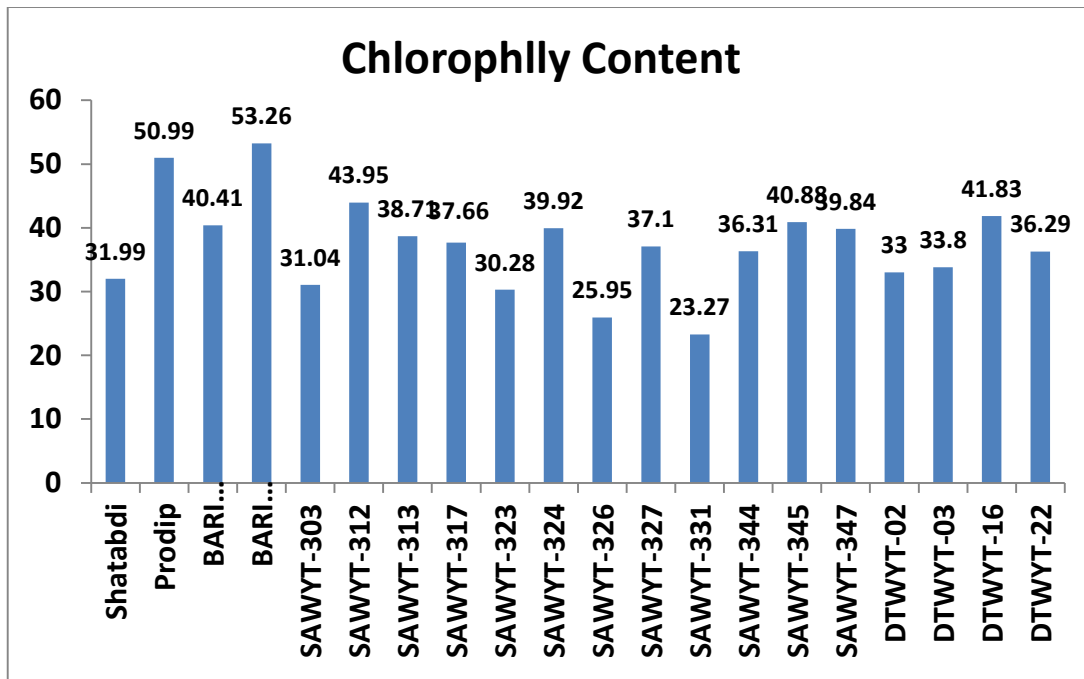
Figure 1: Chlorophyll content for different wheat genotypes
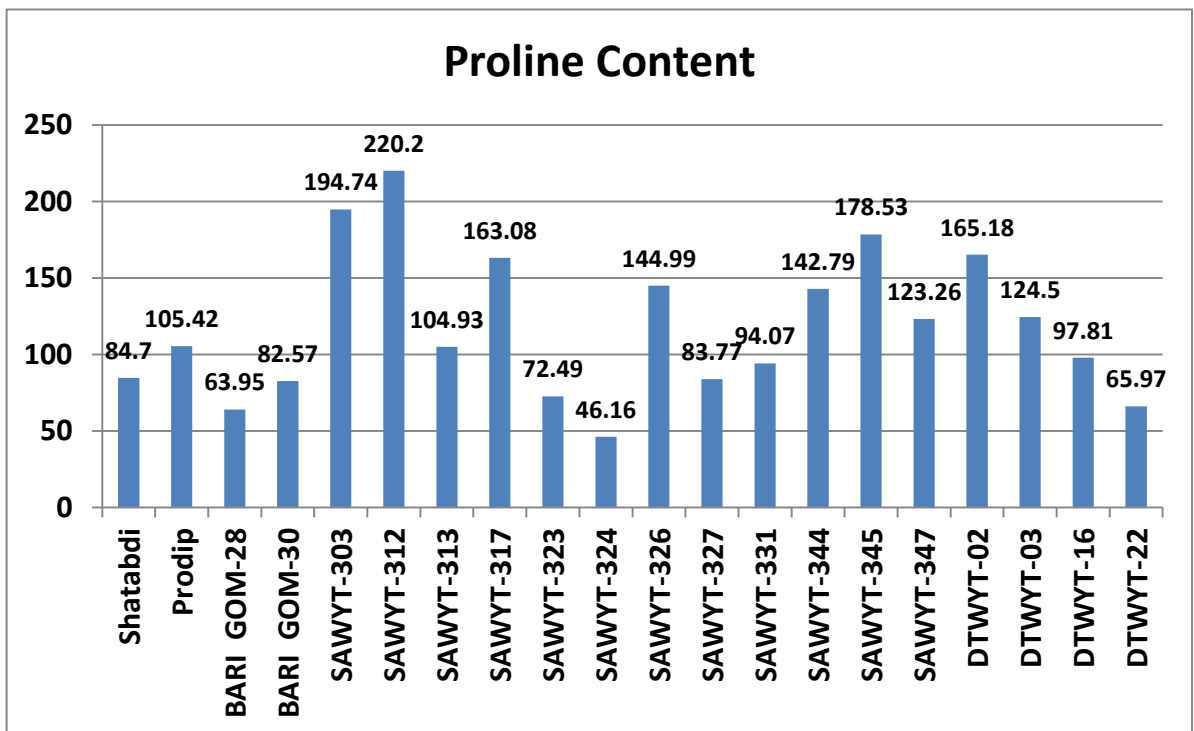
Figure 2: Proline content for different wheat genotypes

4.2 Variability study for 12 traits of wheat

Genotypic and phenotypic variance, heritability, genetic advance and genetic advance in percentage of mean was estimated for twelve traits in 20 collected genotypes of wheat and presented in Table 5.

4.2.1 Days to starting 50% of heading

Days to starting of 50% heading refers to phenotypic variation (26.19) was higher than the genotypic variance (16.60) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (8.23%) and genotypic (6.55%) co-efficient of variation, environmental variance (9.59) (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment. High heritability (63.38%) in days to starting of 50% heading attached with moderate genetic advance (6.68) and moderate genetic advance in percentage of mean (10.74). The high heritability along with moderate genetic advance in percentage of mean of days to 50% starting of heading indicated the possible scope for improvement through selection of the character. Sharma and Garg (2002) found high heritability coupled with high genetic advance for number of days to heading.

4.2.2 Plant height

In terms of plant height, phenotypic variation (33.53) was higher than the genotypic variance (20.55) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (7.59%) and genotypic (5.94%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of plant height. High heritability (61.28%) in plant height attached with moderate genetic advance (7.31) and moderate genetic advance in percentage of mean (9.58). The high heritability along with moderate genetic advance in percentage of mean of plant height indicated the possible scope for improvement through selection of the character and breeder may expect reasonable benefit in next generation in consideration of this trait. Kumar and Shukla (2002) observed high heritability coupled with high genetic advance for plant height. Wang *et al.*, (2003) observed very high broad sense and narrow sense heritability for plant height

4.2.3 Number of productive tillers

Phenotypic variation (0.25) was higher than the genotypic variance (0.06) for number of productive tillers content indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (17.23%) and genotypic (8.18%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of number of productive tillers. Moderate heritability (22.53%) in number of productive tillers attached with low genetic advance (0.23) and low genetic advance in percentage of mean (8). Moderate high estimate of heritability and low genetic advance were registered for days to flowering of male suggested that this character was predominantly controlled by environment with complex gene interaction.

4.2.4 Spike length

Spike length in refers to phenotypic variation (2.60) was higher than the genotypic variance (0.79) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (14.21%) and genotypic (7.81%) co-efficient of variation (Table 5). That mean the very close to phenotypic and genotypic variance which indicated that environment had played a little role with little genetic variation among the genotypes of this trait i.e. environmental influence was minimum. High heritability (30.20%) in spike length attached with low genetic advance (1.0) and low genetic advance in percentage of mean (8.84). The high heritability estimate coupled with low expected genetic advance for this trait indicated the less importance of both additive and non-additive genetic effects for the controlling the character

4.2.5 Number of spikelet's/spike

Phenotypic variation (5.07) was higher than the genotypic variance (2.10) in consideration of number of spikelet's/spike, that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (13.48%) and genotypic (8.67%) co-efficient of variation (Table 5). The difference between phenotypic and genotypic variation was high indicated great influence of the environment for the expression of this character. Therefore, the breeder

must have to simultaneous consideration of genetic work predicted environment for improving the trait. High heritability (41.44%) in number of spikelet's/spike attached with low genetic advance (1.92) and moderate genetic advance in percentage of mean (11.50). The high heritability along with low genetic advance in percentage of mean of number of spikelet's/spike indicated that this trait possessed high variation. Sarkar *et al*., (2001) observed high broad sense heritability for spikelet's per spike. Pramad Kumar and Mishra (2004) found high heritability with high genetic advance in percentage of mean for spikelets per spike of wheat.

Table 5.Genetic parameters of different yield contributing characters and yield of spring wheat under drought condition

Parameters	Phenotypic variance (σ²p)	Genotypic variance (σ²g)	Environmen- tal variance (σ²e)	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Environmen- tal coefficient of variation (%)	Heritability	Genetic advance (%)	Genetic advance (% mean)
Days to 50% heading	26.19	16.60	9.59	8.23	6.55	4.98	63.38	6.68	10.74
Plant height(cm)	33.53	20.55	12.98	7.59	5.94	4.72	61.28	7.31	9.58
Number of productive tillers	0.25	0.06	0.19	17.23	8.18	15.17	22.53	0.23	8.00
Spike length(cm)	2.60	0.79	1.82	14.21	7.81	11.87	30.20	1.00	8.84
Number of spikelet /spike	5.07	2.10	2.97	13.48	8.67	10.31	41.44	1.92	11.50
Empty spikelet/spike	1.06	0.31	0.76	69.97	37.49	59.08	28.71	0.61	41.38
Number of grains /plant	38.59	19.63	18.95	18.66	13.31	13.08	50.89	6.51	19.57
Days to physical maturity	8.14	3.95	4.18	2.67	1.86	1.92	48.60	2.86	2.68
Thousand seeds weight	23.90	16.18	7.72	10.77	8.86	6.12	67.70	6.82	15.02
Chlorophyll content	55.64	52.71	2.93	19.98	19.45	4.58	94.74	14.56	39.00
Proline content	2395.12	2226.73	168.40	41.49	40.01	11.00	92.97	93.73	79.46
Grain yield per plant	0.25	0.16	0.09	10.34	8.31	6.15	64.60	0.67	13.75

4.2.6 Number of empty spikelet's/ spikes

Number of empty spikelet's/ spikes refers that phenotypic variation (1.06) was higher than the genotypic variance (0.31) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (69.97%) and genotypic (37.49%) co-efficient of variation (Table 5). That mean the very close to phenotypic and genotypic variance which indicated that environment had played a little role with little genetic variation among the genotypes of this trait i.e. environmental influence was minimum. Moderate heritability (28.71%) in number of empty spikelet's/ spikes attached with low genetic advance (0.61) and high genetic advance in percentage of mean (41.38). The moderate heritability along with high genetic advance in percentage of mean of number of empty spikelet's/ spikes this trait possessed high variation.

4.2.7 Number of grains/plant

Number of grains/plant refers that phenotypic variation (38.59) was higher than the genotypic variance (19.63) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (18.66%) and genotypic (13.31%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of number of grains/spike. High heritability (50.89%) in number of grains/ plant attached with low genetic advance (6.51) and moderate genetic advance in percentage of mean (19.57). The high heritability along with moderate genetic advance in percentage of mean of number of grains/ plant indicated the possible scope for improvement through selection of the character and breeder may expect reasonable benefit in next generation in consideration of this trait. Sharma and Garg (2002) found high heritability coupled with high genetic advance for number of grains per plant.

4.2.8 Days to physical maturity

Phenotypic variation (8.14) was higher than the genotypic variance (3.95) in terms of days to physical maturity, that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (2.67%) and genotypic (1.86%) co-efficient of variation (Table 5). The difference between

phenotypic and genotypic variation was high indicated great influence of the environment for the expression of this character. Therefore, the breeder must have to simultaneous consideration of genetic work predicted environment for improving the trait. Moderate heritability (48.60%) in days to physical maturity attached with low genetic advance (2.86) and low genetic advance in percentage of mean (2.68). The moderate heritability along with low genetic advance in percentage of mean of days to physical maturity indicated the less possible scope for improvement through selection of the character.

4.2.9 Weight of 1000 grains

Weight of 1000 grains in consideration of phenotypic variation (23.90) was higher than the genotypic variance (16.18) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (10.77%) and genotypic (8.86%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of weight of 1000 grains. High heritability (67.70%) in weight of 1000 grains attached with high genetic advance (6.82) and high genetic advance in percentage of mean (15.02). The high heritability along with low genetic advance in percentage of mean of weight of 1000 grains indicated the possible scope for improvement through selection of the character and breeder may expect reasonable benefit in next generation in consideration of this trait. Kumar and Shukla (2002) observed high heritability coupled with high genetic advance for 1000-kernel weight.

4.2.10 Chlorophyll content

In terms of chlorophyll content, phenotypic variation (55.64) was higher than the genotypic variance (52.71) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (19.98%) and genotypic (19.45%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of chlorophyll content. High heritability (94.74%) in chlorophyll content attached with moderate genetic advance (14.56) and moderate genetic advance in percentage of mean (39.00). High estimate of heritability and low genetic advance were

found for chlorophyll content suggested that this character was not predominantly controlled by environment with complex gene interaction.

4.2.11 Proline Content

Proline content in consideration of phenotypic variation (2395.12) was higher than the genotypic variance (2226.73) indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (41.49%) and genotypic (40.01%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of proline content. High heritability (92.97%) in proline content attached with high genetic advance (93.73) and high genetic advance in percentage of mean (79.46). The high heritability along with high genetic advance in percentage of mean of proline content indicated the possible scope for improvement through selection of the character and breeder may expect reasonable benefit in next generation in consideration of this trait.

4.2.12 Grain yield per plant

For grain yield per plant in context of phenotypic variation (0.25) was higher than the genotypic variance (0.16) that indicating that high environmental influence on this characters which was supported by narrow difference between phenotypic (10.34%) and genotypic (8.31%) co-efficient of variation (Table 5). The moderate difference for this parameter was also suggested a considerable influence of environment for the expression of grain yield per plant. High heritability (64.60%) in grain yield per plant attached with low genetic advance (0.67) and high genetic advance in percentage of mean (13.75). The high heritability estimate coupled with moderate expected genetic advance for this trait indicated the importance of both additive and non-additive genetic effects for the controlling the character. The heritability estimates provides the basis for selection on the phenotypic performance. Gupta and Verma (2000) observed high heritability and genetic advance for grain yield per plant. Sharma and Garg (2002) found high heritability coupled with high genetic advance for grain yield per plant.

4.3 Correlation Matrix

To measure the mutual relationship among yield and yield contributing characters of wheat genotypes correlation matrix analysis was done and also to determine the

component characters on which selection could be based for improvement in yield of 20 genotypes of wheat in Table 6.

4.3.1 Days to 50% heading

Significant positive association was recorded for days to 50% heading of wheat genotypes with plant height (0.331) and days to maturity (0.451), and proline content (0.361) while the non-significant positive association for number of productive tillers (0.138), spike length (0.109), spikelet's/ spike (0.056), grain yield per plant (0.62) and proline contents (0.361). On the other hand, significant negative association was recorded for chlorophyll content (-0.388) and grain yield per plant (-0.397) and non-significant negative association was observed with empty spike per plant (-0.084) and thousand seed weight (-0.201) (Table 6). The results revealed that increase of days to 50% heading decreases most of yield contributing characters and yield in wheat. Patel and Jam (2002) found that kernel yield had a positive and highly significant correlation with days to heading.

4.3.2 Plant height

Data revealed a significant positive association was recorded for plant height of wheat genotypes with spikelet's /spike (0.508), grain per plant (0.565), proline content(0.415), plant hight(0.331). On the other hand, significant negative association was recorded for plant height with empty spike per plant (-0.466) and non-significant negative association was observed with number of productive tillers (-0.115), spike length (-0.004), days to physical maturity (-0.122), thousand seed weight (-0.0), chlorophyll content (-0.176) and grain yield per plant (-0.166) (Table 6). The results revealed that plant height increase considerably with highest yield and yield contributing characters. This suggested that plant height for different genotypes were more potential to allocate their photosynthesis towards highest yield. Kumar et al. (2002) reported that grain yield per plant had direct positive correlation with plant height.

4.3.3 Number of productive tillers

Significant positive association was recorded for days to 50% heading of wheat genotypes with days to maturity (0.276) while the non-significant positive association for spike length (0.041), empty spikelet's/ plant (0.0.37), chlorophyll contents (0.104), days to 5o% heading (.138). On the other hand, significant negative association was

recorded for thousand seed weight (-0.349) and non-significant negative association was observed with spikelet per spike (-0.015), grain per plant (-0.018). proline content (-0.107) and yield per plant (-0.226),plant height(-0.115). (Table 6).

4.3.4 Spike length

Data revealed a significant positive association was recorded for Spike length of wheat genotypes with spikelet's /spike (0.309). On the other hand, non-significant negative association was observed with thousand seed weight (-0.028), proline content (-0.023) plant height(-0.004) and non-significant positive association was observed with empty spikelet's per plant (0.062).grain per plant (0.208), days to physical maturity (0.112), chlorophyll content(0.131) and grain yield per plant (0.087),productive tiller(0.041),days to 50% heading. (Table 6).

4.3.5 Number of spikelet's/spike

Significant positive association was recorded for number of spikelet's/spike of wheat genotypes with grain per plant (0.785), spike length (0.309), plant height (0.508), while the significant negative association was found for empty spike per plant (-0.314). The non-significant positive association for number of thousand seed weight (0.186), chlorophyll content (0.003), proline content (0.158), grain per plant (0.147), days to 50% heading(0.056). On the other hand, non-significant negative association was recorded for productive tiller (-0.015), days to physical maturity (-0.215) (Table 6). Lad et al. (2003) observed that the grain yield exhibited highly significant and positive correlation with spikelets per spike.

4.3.6 Number of empty spikes per plant

Number of empty spikes per plant of wheat genotypes showed significant negative association with grain per plant (-0.662), plant height (-0.466), number of spikelet,s/spike (-0.314), whereas the non-significant negative association for days to physical maturity (-0.022), days to 50% heading (-0.084), thousand seed weight (-0.124), proline content (-0.122). On the other hand, significant positive association was recorded for spike length (0.062), productive tiller (0.037), chlorophyll content (0.105) and grain yield per plant (0.090) (Table 6). Kumar et al. (2002) reported that grain yield per plant had direct positive correlation with number of spikes per plant and 1000-grain weight in some advanced wheat lines.

4.3.7 Number of grains/plant

Number of grains/spike of wheat genotypes showed significant positive association with thousand seed weight (0.279), spikelet's /spike(0.785), plant height(0.565) whereas the non-significant negative association for days to physical maturity (-0.200), productive tiller(-0.018). On the other hand, non-significant positive association was observed with spike length (0.208), days to 50% heading (0.062), chlorophyll content (0.052), proline content (0.144), and grain yield per plant (0.150) (Table 6). Dokuyucu (2002) studied correlation coefficients, which showed that grain yield, was positive and significantly related,with grains per spike .

Table 6. Correlation matrix of different yield contributing characters and yield of wheat under drought condition Pearson's correlation coefficients (r) describing association of twelve traits of 20 wheat genotypes evaluated under drought stress

	PH	NT	SL	SPS	ESP	GPP	DM	TSW	CC	PC	GYP
DH	0.331**	0.138	0.109	0.056	-0.084	0.062	0.451**	-0.201	-0.388**	0361**	-0.397**
PH		-0.115	-0.004	0.508**	-0.466**	0.565**	-0.122	-0.000	-0.176	0.415**	-0.166
NT			0.041	-0.015	0.037	-0.018	0.276*	-0.349**	0.104	-0.107	-0.226
SL				0.309**	0.062	0.208	0.112	-0.028	0.131	-0.023	0.087
SPS					-0.314**	0.785**	-0.215	0.186	0.003	0.158	0.147
ESP						-0.662**	-0.022	-0.124	0.105	-0.122	0.090
GPP							-0.200	0.279*	0.052	0.144	0.150
DM								-0.193	-0.183	0.039	-0.344**
TSW									-0.042	0.005	0.707**
CC										-0.047	0.244*
PC											-0.190

DTH, days to 50% heading; PH, plant height; TN, number of productive tillers; SL, spike length; SPS, number of spikelet's per spike; ESP, empty spikelet's per plant; DM, days to maturity; TSW, thousand seed weight; CC, chlorophyll content; PC, proline content; GYP, grain yield per plant. **: Significant at 0.01 level of probability;   *: Significant at 0.05 level of probability

4.3.8 Days to physical maturity

Data revealed a significant positive association was recorded for days to physical maturity of wheat genotypes with, days to 50% heading(0.451),productive tillers(0.276), while the non-significant positive association for spike length(0.112), proline content (0.039). On the other hand, significant negative association was recorded for grain yield per plant (-0.344) and non-significant negative association was observed with plant height (-0.122), spikelet,s/spike(-0.215), thousand seed weight (-0.193), chlorophyll content (-0.183),empty spikelet,s/plant(-0.022).grain/plant(-0.200). (Table 6).

4.3.9 Weight of 1000 grains

Weight of 1000 grains of wheat genotypes showed significant positive association with grain yield per plant (0.707), grain/plant(0.279), whereas the non-significant positive association for spikelet's per spike (0.186), proline content (0.005). On the other hand, significant negative association was recorded for weight of 1000 grains with Number of productive tiller(-0.349), whereas non-significant negative association was observed with plant height (-0.000),chlorophyll content(0-.042),days to 50% heading(-0.201),Spike length(-0.028),empty spikelet`s per plant (-0.124), days to physical maturity (-0.193), (Table 6). Sarkar et al. (2001) found highly positive correlation of 1000-grain weight with grain yield. Kumar et al. (2002) reported that grain yield per plant had direct positive correlation with 1000-grain weight in some advanced wheat lines.

4.3.10 Chlorophyll content

Statistically significant negative association was recorded for chlorophyll content of wheat genotypes with days to 50% heading(-.388),significant positive association for grain yield per plant(0.244), while the non-significant positive association for spikelets per spike (0.003), number of productive tiller (0.104),Spike length(0.131),Empty spike per plant(0.105),grain per plant(0.052) . On the other hand, non-significant negative association was recorded for number of plant height (-0.176),days of maturity (-0.183),thousand seed weight(-0.042), proline content(0.047). (Table 6).

4.3.11 Proline content

Statistically significant positive association was recorded for root number of wheat genotypes with days to 50% heading (0.361), Plant hight 0.415), again non-significant positive association of number of spikelet`s per spike(0.158),grain per plant (0.144),days of maturity(0.039),thousand seed weight(0.005), the non-significant negative association for chlorophyll content (-0.047)), empty spikelet`s per plant(-0.122),Spike length (-0.023),Number of productive tiller(-0.107), grain yield per plant(-0.190). (Table 6).

4.3.12 Grain yield per plant

Significant negative association was recorded for grain yield per plant of wheat genotypes with days to 50% of heading (-0.397), days to physical maturity (-0.344) while the non-significant negative association for plant height (-0.166), number of productive tillers (-0.226), proline content (-0.190). On the other hand, significant positive association was recorded for grain yield per plant with thousand seed weight (0.707), chlorophyll content (0.244), while non-significant positive association was observed with spike length (0.087), spikelet`s per spike (0.147), empty spikelet per plant (0.090),number of grain per plant (0.150) ((Table 6). Payal et al. (2007) observed Positive direct effects of biological yield per plant, number of grains per ear, tillers per plant, 1000 kernel weight, days to heading and days to maturity on grain yield.

4.4 Principal Component Analysis (PCA)

The rotated component matrix (Table 5) shows the proportion of total variance explained by different principal components and their correlations with variable traits. From the stress treatment, three principal components were important, contributing 0.6863% of the total variation observed. The first two principal components were the most influential with a cumulative contribution to the total variation of 0.5646 %. Variables DH, PH, SL, SPS, GPP, TSW, PC and GYP had high positive loading into the first principle component while SL, SPS, ESP, GPP, TSW, and GYP had high positive loading into the second principal component. These were followed by SL, SPS, ESP, TSW, PC and GYP which had high positive loading into the third principal components respectively.

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Table 7: Rotated component matrix of ten phenotypic traits and chlorophyll and proline Content of 20 wheat genotypes evaluated under drought stressed

Variables	PC1	PC2	PC3
DH	0.1746	-0.4725	0.1202
PH	0.4543	-0.1917	0.0062
NT	-0.1897	-0.2055	-0.5192
SL	0.1513	0.1138	-0.3394
SPS	0.4474	0.1164	-0.1880
ESP	-0.4250	0.0827	0.0535
GPP	0.4774	0.0778	-0.2053
DM	-0.1271	-0.4159	-0.0059
TSW	0.1455	0.3834	0.3699
CC	-0.0880	0.2035	-0.6076
PC	0.2231	-0.2038	0.1097
GYP	0.0280	0.5054	0.0496
Explained variance (eigenvalue)	3.6376	3.1371	1.4610
Proportion of total variance (%)	0.3031	0.2614	0.1217
Cumulative variance (%)	0.3031	0.5646	0.6863

DTH, days to 50% heading; PH, plant height; TN, number of productive tillers; SL, spike length; SPS, number of spikelet's per spike; ESP, empty spikelet's per plant; DM, days to maturity; TSW, thousand seed weight;   CC, chlorophyll content; PC, proline content; GYP, grain yield per plant.

4.5 Principal Component Biplot Analysis

The relationships between the different variables and genotypes with respective principal components are further illustrated by the principal component biplots in Figures 3, 4 and 5 for the stressed conditions respectively. Doding et al. (2012) reported that Smaller angles

between dimension vectors in the same direction indicated high correlation of the variable traits in terms of discriminating genotypes. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. Under stress, most of the genotypes were scattered in the positive side of the first principal component, with genotypes such as SAWYT-303, SAWYT-326, SAWYT-331, and SAWYT-344 excelling in yield which was contributed mostly by high spikelet`s per spike, grain per plant, spike length and thousand seed weight, as well as optimum values for other yield components (Figure 3). In the relationship between PC2 and PC3, the genotypes were also more concentrated on the positive side of the 2nd principal component with genotype Shatabdi and BARI GOM-28 being more inclined in the direction of GYP and TSW (Figure 4). Under combination of PC1 and PC3 most of the genotypes were scattered in the positive side of the principal component, with genotypes such as SAWYT-326, SAWYT-303, DTWYT-03, and SAWYT-331 excelling in yield which was contributed mostly by high PH, PC,GYP, and TSW as well as optimum values for other yield components (Figure 5)

Figure 3. Biplot graph showing PC1 and PC2. DTH, days to 50% heading; PH, plant height; TN, number of productive tillers; SL, spike length; SPS, number of spikelet`s per spike; ESP, empty spikelet`s per plant; DM, days to maturity; TSW, thousand seed weight; CC, chlorophyll content; PC, proline content; GYP, grain yield per plant
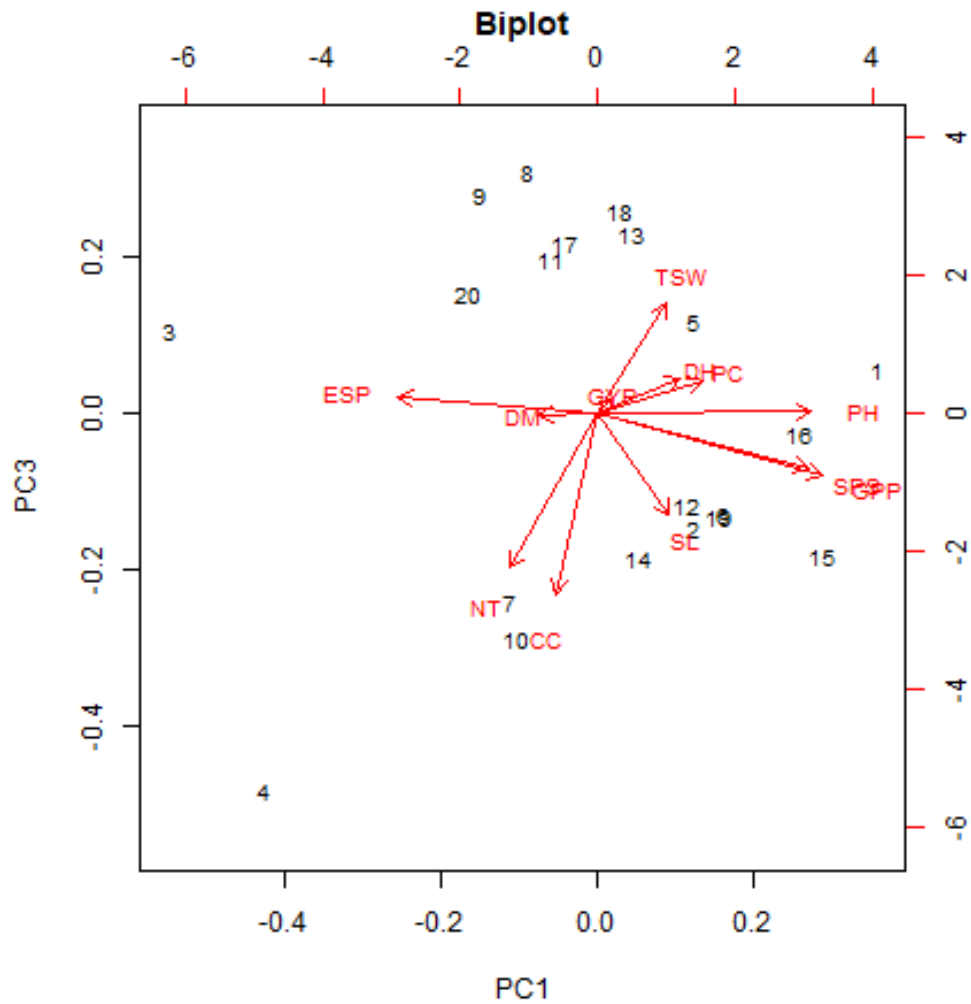
Figure 4. Biplot graph showing PC1 and PC3. DTH, days to 50% heading; PH, plant height;TN, number of productive tillers; SL, spike length; SPS, number of spikelet`s per spike; ESP, empty spikelet`s per plant; DM, days to maturity; TSW, thousand seed weight; CC, cholorophyll content; PC, proline content; GYP, grain yield per plant
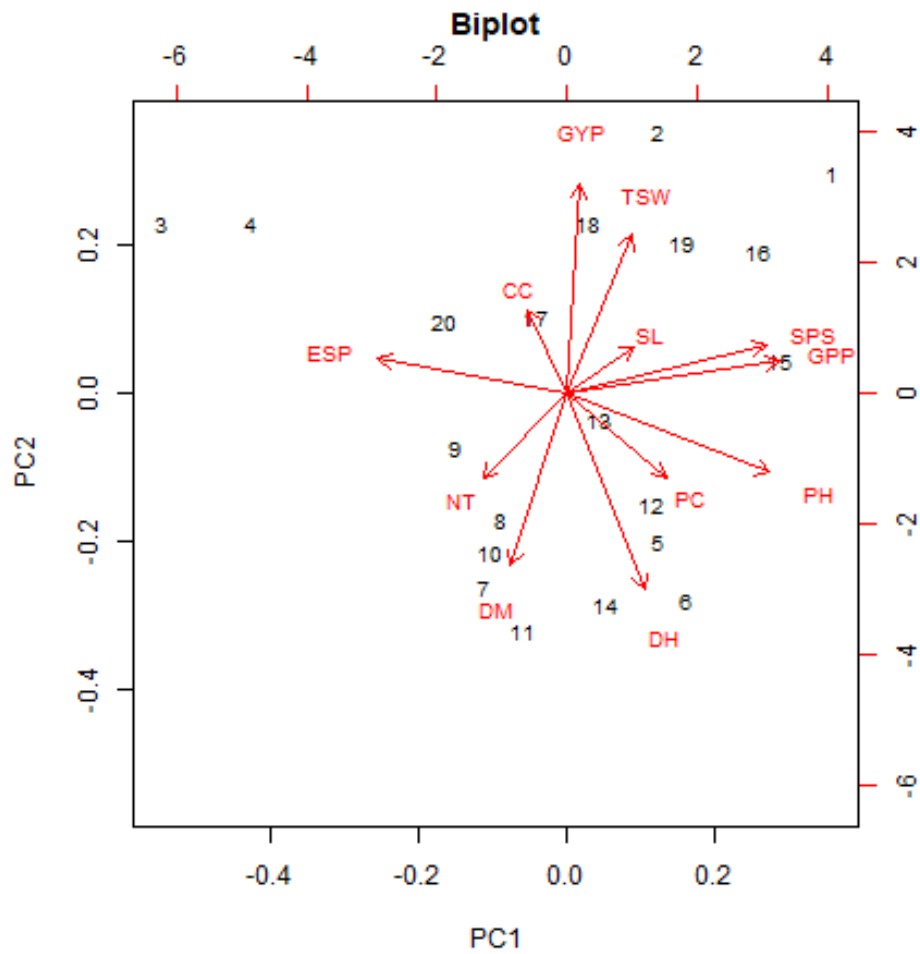
Figure 5. Biplot graph showing PC2 and PC3. DTH, days to 50% heading; PH, plant height;TN, number of productive tillers; SL, spike length; SPS, number of spikelet`s per spike; ESP, empty spikelet`s per plant; DM, days to maturity; TSW, thousand seed weight; CC, chlorophyll content; PC, proline content; GYP, grain yield per plant
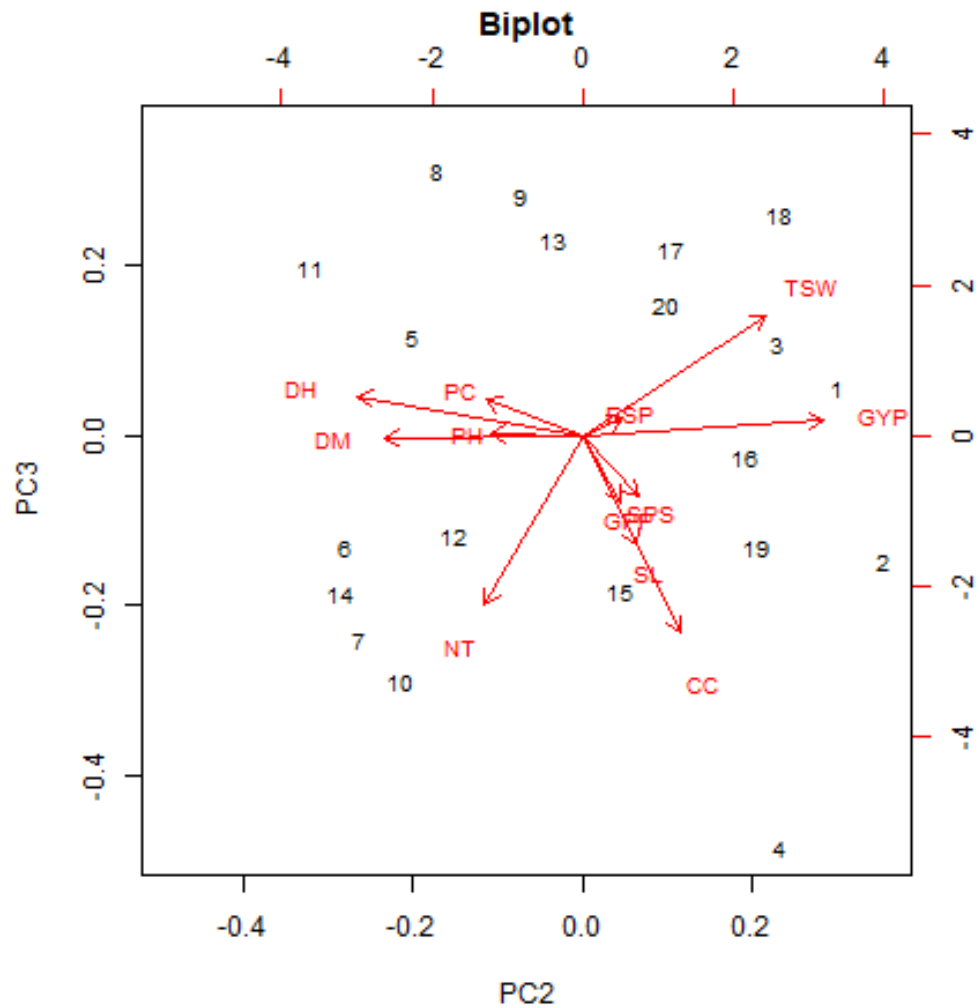
CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted during the period from November 2017 to April 2018 in rabi season in the experimental area of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka to find out the role of morpho-physiological character in spring wheat under drought stress. In this experiment 20 wheat genotypes were used as experimental materials. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. During the experimental period no irrigation was provided for creating drought environment. Mean performance, variability, correlation matrix, was done on different yield contributing characters and yield of wheat genotypes. The highest days to 50% starting of heading (68.00) was observed in the genotype of DTWYT-16 which was statistically similar (67.67) with the wheat genotypes of SAWYT-312, while the lowest days (53.33) from BARI Gom-30. The longest plant (84.20 cm) was recorded in genotype Shatabdi the shortest plant (63.96 cm) was found in wheat genotype BARI GOM-30. The maximum number of productive tillers (3.53) was found in SAWYT-324, whereas the minimum number of productive tillers (2.33) was observed in the wheat genotype SAWYT-331. The longest spike length (14.09 cm) was recorded in BARI GOM-30. On the other hand, the shortest spike length (9.11 cm) was recorded in the wheat genotype BARI Gom-28. The maximum number of spikelet's/spikes (19.40) was found in DTWYT-22, while the lowest number (13.67) was observed in the wheat genotype SAWYT- 317. The highest number of empty spikelet/ spike (3.07) was attained in BARI Gom-28 whereas the minimum number empty spikelet/ spike (0.37) were observed in the wheat genotype SAWYT-326. The maximum number of grains/plant (42.07) was recorded in Shatapdi and the minimum number (22.39) in wheat genotype BARI Gom-28. The highest days to starting of maturity (110.00) was found in genotype SAWYT-317 whereas the lowest days (102.67) were attained in the wheat genotypes SAWYT-324. The highest weight of 1000 grains (50.40 g) was found in SAWYT-326. On the other hand, the lowest weight of 1000 grains (38.93 g) was attained in the wheat genotype SAWYT-347. The

highest chlorophyll content (53.26) was attained in BARI Gom-30, whereas the lowest chlorophyll content (23.27) was found in the wheat genotype DTWYT-03. The highest proline content (220.20) was observed in SAWYT-312 while the lowest proline content (46.16) was found in the wheat genotype SAWYT-324. The highest grain yield per plant (5.57 g) was recorded in Prodip, while the lowest grain yield per plant (4.15 g) was observed in the wheat genotype SAWYT-312.

In correlation study, Significant negative association was recorded for grain yield per plant of wheat genotypes with days to 50% of heading (-0.397), days to physical maturity (-0.344) while the non-significant negative association for plant height (-0.166), number of productive tillers (-0.226), proline content (-0.190). On the other hand, significant positive association was recorded for grain yield per plant with thousand seed weight (0.707) and chlorophyll content (0.244) while non-significant positive association was observed with spike length (0.087), spikelet`s per spike (0.147), empty spike per plant (0.090), and grain per plant (0.150)

In consideration of yield contributing characters and yield Prodip performed better under drought condition followed by DTWYT-22, SAWYT-326, SAWYT-331, Shatabdi, BARI Gom-28, BARI Gom-30. Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the yield contributing traits indicating that high environmental influence on the studied characters. Correlation analysis revealed that the characters thousand seed weight and chlorophyll content had highly positive correlation with yield per plant. Under stress, most of the genotypes were scattered in the positive side of the first principal component, with genotypes such as SAWYT-303, SAWYT-326, SAWYT-331, and SAWYT-344 excelling in yield which was contributed mostly by high spikelet`s per spike, grain per plant, spike length and thousand seed weight, as well as optimum values for other yield components.

The positive correlation of grain yield and proline content found under-drought stress conditions provides already evidence that proline accumulation might ultimately be

considered as a tool for effective selection. Further studies are required to quantify proline content of diverse genotypes at different stress levels to explore the rate of proline accumulation in different genotypes during time of stress exposure and yield potential of genotypes.

RECOMMENDATION

●» Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the yield contributing traits indicating that high environmental influence on the studied characters.

●» Under stress, most of the genotypes were scattered in the positive side of the first principal component, with genotypes such as SAWYT-303, SAWYT-326, SAWYT-331, and SAWYT-344 excelling in yield which was contributed mostly by high spikelet's per spike, grain per plant, spike length and thousand seed weight, as well as optimum values for other yield components. The proline content hight found in SAWYT-312 (220.2), and nearable found in SAWYT-303 (194.74),SAWYT-345(178.53), and lowest in SAWYT-324(46.16).

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APPENDICES

Appendix I. Map showing the experimental site under the study
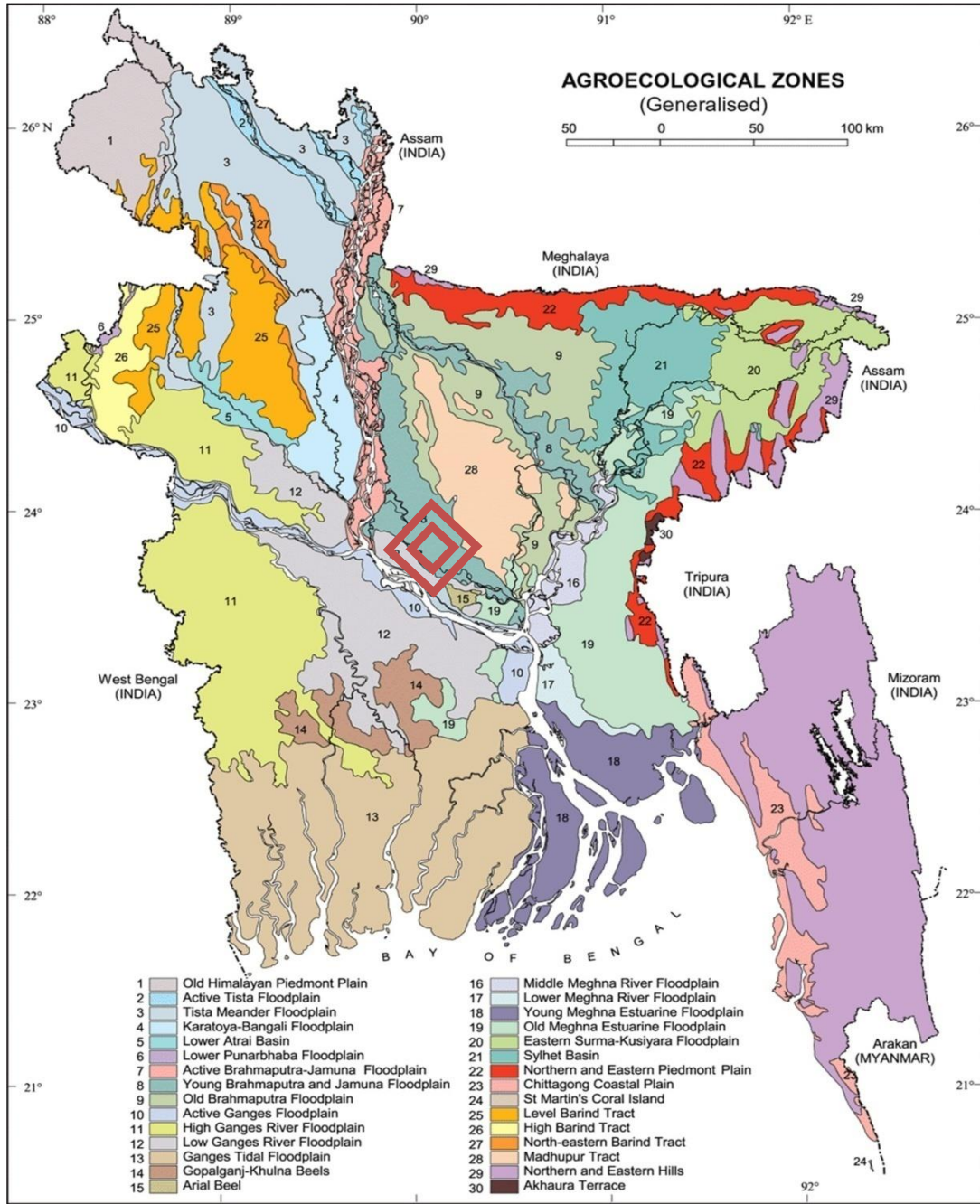
The experimental site under study

Appendix II. Monthly average of air temperature, relative humidity and total rainfall of the experimental site during the period from November, 2017 to April, 2018

Month	Air temperature (ºC)*		Relative humidity (%)*	Rainfall (mm)
	Maximum	**Minimum**		
November, 2017	26.82	16.05	77	00
December, 2017	21.4	13.5	75	00
January, 2018	25.5	12.7	67	00
February, 2018	28.1	16.2	65	00
March, 2018	31.4	19.4	53	12

*Monthly average

* Source: Bangladesh Meteorological Department (Climate and weather Division), Agargaon, Dhaka.

Appendix III. Soil properties of experimental field analyzed by Soil Resource Development   Institute (SRDI), Khamarbari, Farmgate, Dhaka.

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Research Field, SAU, Dhaka
AEZ	Madhupur Tract (AEZ 28)
General Soil Type	Shallow red brown terrace soil
Land Type	High land
Soil Series	Tejgaon
Topography	Fairly leveled

B. Physical and chemical properties of the soil before experiment

Characteristics	Values
% Sand	28
% Silt	40
% Clay	32
Texture	Loamy
pH	5.6
Organic matter (%)	0.82
Total N (%)	0.05
Available P (ppm)	20.01
Exchangeable K (me/100g Soil)	0.11
Available S (ppm)	43

Source: Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka