ASSESSMENT OF STRESS-TOLERANCE ATTRIBUTES IN WHEAT USING GENE – SPECIFIC MOLECULAR MARKERS

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*This is to certify that thesis entitled, "**ASSESSMENT OF STRESS-TOLERANCE ATTRIBUTES IN WHEAT USING GENE – SPECIFIC MOLECULAR MARKERS**" 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 **BIOTECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **RONA MAHMUD, Registration No. 13-05790** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: 02 December, 2015
Dhaka, Bangladesh

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DEDICATED

TO

MY BELOVED PARENTS

CHAPTER 1
INTRODUCTION

Wheat (*Triticum aestivum* L.) ranks first in respect of total cereals production in the world and it belongs to the grass family Gramineae (Poaceae). Although many people in the world eat rice as their principle cereal, wheat is very widely grown in Europe, West Asia and North Africa and eaten by millions of people. In Bangladesh, the second important cereal crop is wheat after rice. (Hossain and Teixeira da Silva, 2012). Around 50% of world cereal production is covered by wheat. Wheat supplies 72% of the calories and proteins in the average diet (Heyne, 1987). According to BBS estimate the national average yield was 3.04 t ha^{-1} (BBS, 2013-14), while 2.78 t ha^{-1} and 3.01 were also recorded during 2011-12 and 2012-13, respectively. The area has also increased to 0.453 million ha in 2013-14 from 0.358 and 0.417 million ha in 2011-12 and 2012-13, respectively. The total production also increased significantly to 1.375 million ton in 2013-14, which was 0.995 million ton in 2011-2012. In last few years the weather condition was also more or less favourable for wheat growth and development. Besides carbohydrates and proteins wheat contains several vitamins like vitamin B6, niacin, thiamin, riboflavin, pentothenic acid, vitamin K, E and Fe, P, Ca etc.

Wheat in Bangladesh is grown during the dry winter season. During the growing period the wheat crop faces various stress conditions both abiotic and biotic. A plant's first line of defense against abiotic stress is in its roots. If the soil holding the plant is healthy and biologically diverse, the plant will have a higher chance of surviving stressful conditions. (Brussaard *et al*., 2007).

In abiotic stress conditions (drought, heat, salinity, flood, cyclone, storm etc), plants undergo a variety of changes from physiological adaptation to plants undergo gene expression (Yamaguchi and Blumwald, 2005). Late sown crops are more prone to these stresses and hence there is a need for high yielding stress tolerant varieties. Although stress related agronomic traits are easier to be

characterized and can be directly selected based on their performance in the field, use of functional or gene-specific markers derived from polymorphic sites within the genes or linked to a particular character are more efficient and can directly identify the desired traits. Some of these markers for stress tolerance attributes that could be used for enhancing the selection efficiency in a wheat breeding programme are associated with rye translocation, dwarfing genes and heat shock proteins.

Rye translocations have been extensively used in wheat breeding, especially those involving the short arm of rye chromosome 1R (1RS), because they provide resistance to insects, diseases and reported improvements in yield potential and water-use efficiency (Singh, 1998). Translocation sources of different genetic and geographic origins have been used in breeding programs all over the world (Rabinovich, 1998). Triticale has long been used as a bridge to facilitate rye gene introgressions into wheat (Sebesta *et al*., 1995, Sethi, 1989). The genomic advantages of the wheat-rye translocations as well as their peliotropic detrimental effects depend on

- The wheat genetic background
- Wheat class and environmental conditions
- The sources of the transferred rye chromatin and its position in the wheat genome. (Kumlay *et al* ., 2003)

The 1BL/1RS wheat-rye chromosome arm translocation has been used worldwide by plant breeders for its positive effects on yield and above ground biomass.

Dwarf varieties of wheat carry a gene called reduced height gene (*Rht*). To increase lodging tolerance and for their high yield potential, semi dwarf varieties were mostly used for wheat improvements programmes and combinations of different alleles of this gene also contributed in protecting dwarf wheat varieties from abiotic stress conditions. Dwarfing genes are present in majority of the wheat varieties grown worldwide (Fick and Qualset, 1973). The Japanese variety Norin 10 (CT12699) and its derivatives, the

Olesen dwarf (CT14497) and the variety Tom Thumb (CT13563) from Tibet was three sources of reduced height in wheat. Dwarf genes can be classified as GA insensitive genes contains Rht1 and Rht2, derived from the cultivar 'Norin 10' have contributed significantly to a worldwide increase in potential grain yield.(Gale and Youssefian, 1985; Slafer *et al*., 1994; Calderini *et al*., 1995). These GA-insensitive dwarfing genes are probably present in around 90% of the world semi-dwarf wheat crop and were responsible for the worldwide green revolution in wheat cultivation (Borlaug 1968., Worland *et al*., 1990)

Dwarfing genes *Rht-B1* and *Rht-D1* were first transferred to US cultivars and then to CIMMYT lines and varieties which were later sent to many other countries for adaptation. Recently, the homoeologous genes *Rht-B1b* and *Rht-D1b* were isolated from wheat (Peng *et al*., 1999). The semi-dwarfing genes *Rht*-B1b (*Rht1)* and *Rht-D1b (Rht2)* widely present in commercial cultivars reduce plant height, increase harvest index, improve lodging resistance, and consequently increase grain yield. However, it has been reported that the highest grain yield was obtained in an irrigated location. Lines carrying both dwarfing alleles rendered lower grain yields in every environment. For stressed environments the best choice seemed to be related more with the right plant height than just the combination of alleles. The best results were observed with shorter lines within the tall class, without any dwarfing allele, or taller lines within the semi-dwarf class carrying *Rht-B1b+Rht-D1a.*

Drought is a major abiotic stress that adversely affects wheat production and quality in many regions of the world, the loss of which is equivalent to the total loss for other natural disasters, with increasing global climate change making the situation more serious (Kirigwi *et al*., 2007). Terminal or post-anthesis heat stress also frequently limits production of wheat in many regions. (Boote *et al*., 1994). Most studies found that cytoplasmic effects and nuclear-cytoplasmic interactions were involved in heat tolerance, but conclusion about other genetic characteristics varied. Heat shock proteins (HSPs) function as

molecular chaperons which are responsible for protein folding, assembly, translocation and degradation in many cellular process, stabilization of proteins and membranes and assist in protein refolding under stress conditions including high temperatures (Wang *et al*., 2003). Different types of HSP are synthesized in different tissues in response to duration and kinds of stress conditions (Zivy *et al*., 1987).

Scientists all around the world are currently conducting research for increasing stress tolerance in wheat varieties to increase production of wheat under unfavourable growing conditions by using gene specific molecular markers. Development of such varieties can help reducing stress related yield loss and increase wheat productions. To the best of our knowledge, no such study has yet been undertaken in Bangladesh. Hence, the aim of the present study was to characterize 24 wheat genotypes (including BARI released varieties) using gene specific molecular markers by PCR technology. Their phenotypic traits were also recorded from field grown plants for comparison.

Objectives

1. Molecular characterization of rye chromosome translocations in different wheat genotypes.
2. Characterization of dwarfing genes in wheat using PCR based markers.
3. To evaluate the presence and consequence of a HSP in different wheat varieties.

CHAPTER 2
RIVEIW OF LITERATURE

2.1. Rye Translocation

Wheat (*Triticum aestivum* L.) is the first before most produced food among the whole cereal crop. Recently the yield potential of wheat grain has been increased by acquiring traits for the biotic and abiotic tolerance (Villareal, *et al.*, 2006).

According to various studies, the characterization of rye translocation, dwarfing genes and heat shock protein for stress tolerance (rye translocation, dwarfing genes and heat shock protein) variety are reviewed below:

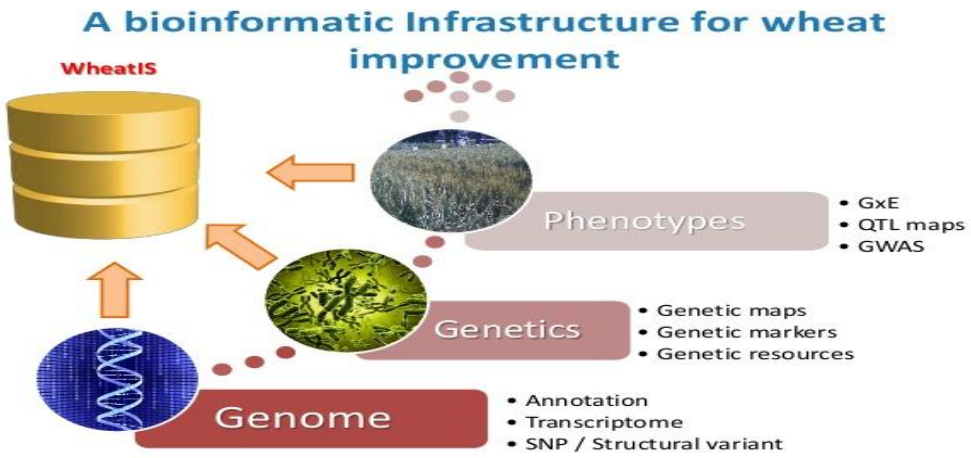

Figure 1. Different ways for wheat improvement. (Sources: Internet)

Niu *et al*. (2012) showed that WRKY-type transcription factors are involved in multiple aspects of plant growth, development and stress response. WRKY genes have been found to be responsive to abiotic stresses; however, the abiotic stress tolerance is largely unknown especially in crops. Forty-three putative TaWRKY genes were identified and two multiple stress-induced genes, TaWRKY2 and TaWRKY19, were further characterized. The two TaWRKY proteins may regulate the downstream genes through direct binding to the gene promoter or via indirect mechanism. Manipulation of TaWRKY2 and

TaWRKY19 in wheat or other crops could improve their performance under various abiotic stress conditions.

Fleury *et al*. (2010) reported that quantitative trait loci (QTL) and marker-assisted selection can improve the performance in the difficult environmental abiotic stress, such as high temperatures, high irradiance, and nutrient toxicities or deficiencies.

Habash *et al.* (2010) stated that the coupling of new genomic tools, technologies, and resources with application of genomic technologies will introduce new target traits for consideration in wheat breeding for resistance to drought. He has also shown that many traits related to the plant's response and adaptation to drought are complex and multigenic, and quantitative genetics coupled with genomic technologies have the potential to dissect complex genetic traits and to identify regulatory loci, genes and networks.

Nevo and Chen (2010) reported that advanced backcross of QTL analysis, the introgression libraries based on wild wheat and wild barley act as donors, and positional cloning of natural QTLs will play prevailing roles in elucidating the molecular control of drought and salt tolerance.

Witcombe *et al*. (2008) showed that marker-assisted selection for component traits of drought in rice and pearl millet and salinity tolerance in wheat has produced some positive results and the pyramiding of stable quantitative trait loci controlling component traits may provide a solution.

Kim *et al*. (2004) reported the effects of centric translocations of chromosome in various rye (*Secale cereale* L.) sources on agronomic performance of wheat grown in humid south-eastern conditions in North America. Various 1R substitution, 1RS translocation, and 1RL translocation lines in ‘Pavon 76’ were evaluated for agronomic performance. The 1RS translocation line was most favorable for agronomic performance. When compared with those of substitution, 1RL translocation, and controls. The 1RS significantly increased

grain yield. Thus, selection of 1RS source is important in producing constantly higher grain yield in 1RS translocation lines. Genetic recombination among different 1RS may also be used to create more genetic variation.

Nagy *et al*. (2003) stated that homologous recombination was expected between the short arm of the 1R chromosomes of the rye genotypes and the 1RS arm of the 1BL.1RS wheat/rye translocation. Seven sequence-specific PCR-based markers: Xiag95, RMS13, Bmac0213, GPI, Xpsr960, 5Sand SCM9, and ω-secalin proteins were used to detect recombination events in the BC_1F_2 generation. Segregation analysis demonstrated that barley SSR marker (Bmac0213) locus was present on the 1RS chromosome arm of 834 plants tested in four different BC_1F_2 populations, 246 individuals were found to carry recombined1BL.1RS translocation

Wojciechowski (2002) showed that the analysis of both pSc10C and pSc20H fragments were related to retro transposons, ubiquitously distributed in plant genomes. The FISH patterns showed that the two markers could be useful to select or track all wheat rye translocation lines derived from the whole arms of rye chromosomes, as well as to characterize the positions of the translocation breakpoints generated in the proximal and distal regions of rye arms.

Rabinovich *et al*. (1998) classified numerous wheat on the basis of geographic origins and genetic background of possessing the 1BL/1RS wheat-rye translocation, 1B(R) substitution and 1AL/1RS translocation. Wheat-rye translocations can determine high productivity, adaptive possibilities, and disease and insect resistance in wheat.

Friebe *et al.* (1995) showed that the wild relatives of common wheat, *Triticum aestivum*, and related species are an important source of disease and pest resistance and several useful traits have been transferred from these species to wheat. C-banding and *in situ* hybridization analyses are powerful cytological techniques allowing the detection of alien chromatin in wheat. Review summarized the available data on wheat-alien transfers conferring resistance to

diseases and pests. Ten of the 57 spontaneous and induced wheat-alien translocations were identified as whole arm translocations with the breakpoints within the centromeric regions. This presented information should be useful for further directed chromosome engineering aimed at producing superior germplasm.

Gregory (1995) stated that the homoeology of 2R chromosomes and the high level of compensation provided by the translocation it is probably associated with agronomic deficiencies that will prevent its exploitation in agriculture.

Lapitan *et al*. (1991) showed that the usefulness of tissue culture as a method of introgressing alien genes into wheat tissue may be a useful tool in alien gene introgression and manipulation of heterochromatin in triticale improvement and the karyotypes of amphidiploids were analyzed by C-banding to determine chromosome structural changes that occurred during tissue culture.

Rogowsky *et al*. (1991) reported that the translocation of the short arm of rye chromosome 1 (1RS) onto homeologous wheat chromosomes confers disease resistance and increased yield on wheat. This translocation was also associated with dough quality defects. Wheat-rye recombinants were used as a mapping tool to assign two RFLP markers to specific regions on chromosome arms 1DS and 1RS of wheat and rye.

Heslop Harrison *et al*. (1990) carried out an experiment using in situ hybridization technique by labeled biotin to show the sizes and translocation points of the rye chromosome segments in five wheat varieties which carry a translocation between wheat chromosome IB and the short arm of rye chromosome IR (1B/1R) These techniques using genomic probes are useful for detecting, characterizing and following alien chromosomes or chromosome segments through breeding programmes.

Chai *et al*. (1990) concluded that translocation has frequently resulted in unsatisfactory grain processing quality. Plants which were homozygous for the

1BL. 1RS could clearly be distinguished from the heterozygous ones. This co-dominant marker was successfully applied to genotype a segregating F_2 population and a local cultivar collection.

Zeller (1973) showed that several derivatives of derivatives of the variety Zorba have 20 wheat-chromosome pairs and an interchanged pair involving segments of wheat-chromosome 1B and rye-chromosome 1R (V), Chromosome 1R in Zorba carries linked genes for resistance to *Puccinia graminis*, *P. recondita* and *P. striiformis*, and it seems likely that the wheat varieties examined with alien variation transferred from rye possess these resistance genes.

2.2. Dwarfing genes

Ellis *et al.* (2007) concluted that microsatellite marker *Gwm261* has been used extensive to screen large numbers of diverse international germplasm to increase the population derived from a cross between Sunco *(Rht-B1b, Xgwm261*) and Tasman *(Rht-D1b, Xgwm261),* there were significant height differences associated with the segregation of *Rht-B1b* and *Rht-D1b*, but no height differences between *Xgwm261*genotypes, the presence of *Xgwm261* is only indicative of Rht8 in wheat cultivars that have inherited this allele from Akakomugi or a Strampelli wheat ancestor.

Butler *et al.* (2005) studied the effects of the *Rht-B1b* and *Rht-D1b* dwarfing alleles in a recombinant inbred line (RIL) spring wheat population under a range of soil moisture conditions. Most traits occurred across a relatively wide range of plant heights; with the best performing lines either shorter lines in the tall class or taller lines in the semi dwarf classes.

Borojevic and Borojevic (2005) showed that wheat is the main crop and often a strategic crop in many European countries. From a historical perspective, he described the transfer of ‘‘reduced height genes’’ (Rht genes) from Japanese wheat varieties to wheat varieties in Europe and their influence on the increase

of the total wheat production in the last century. Historic pathways of *Rht* genes were influenced directly or indirectly by wheat breeders exchanging seed samples and by some governments importing large quantities of wheat during historically critical periods for their countries.

Verma *et al*. (2005) stated that lodging is a major constraint to increasing yield in many crops, but is of particular importance in the small-grained cereals. This study investigated the genetic control of lodging and component traits in wheat through the detection of underlying quantitative trait loci (QTL). The analysis was based on the identification of genomic regions which affect various traits related to lodging resistance in a population of 96 doubled haploid lines of the cross 'Milan' x 'Catbird', mapped using 126 microsatellite markers. Although major genes related to plant height (*Rht* genes) were responsible for increasing lodging resistance in this cross, several other traits independent of plant height were shown to be important such as root and shoot traits, and various components of plant yield. Yield components such as grain number and weight were shown to be an indicator of plant susceptibility to lodging. QTL for lodging and associated traits were found on chromosomes 1B, 1D, 2B, 2D, 4B, 4D, 6D and 7D. QTL for yield and associated traits were identified on chromosomes 1B, 1D, 2A, 2B, 2D, 4D and 6A.

Bai *et al.* (2004) showed that wheat (*Triticum aestivum* L.) cultivars with greater coleoptile elongation are preferred in low-precipitation dryland regions and in early-planted management systems of the Great Plains, but the presence of GA3 (gibberellin)-insensitive dwarfing genes tends to restrict coleoptile elongation. The agronomic value of *Rht8* and the discovery of its diagnostic microsatellite marker, Xgwm 261, have accelerated breeders' interest in *Rht8* as an alternative dwarfing gene. The objectives were to determine allelic distributions at the marker locus in contemporary samples of hard winter and soft red winter wheat relative to samples of Chinese accessions from a *Rht8*-rich geographic region, and to compare coleoptile elongation in the presence or absence of *Rht8* determined by the Xgwm 261 marker. The 165-bp (primarily

hard winter wheats) and the 174-bp (primarily soft red winter wheats) alleles of *Xgwm 261* were most frequent. About 8% of all U.S. accessions carried the 192-bp allele diagnostic for *Rht8*, compared with 64% of the Chinese accessions. Coleoptile length varied among accessions from 4.4 to 11.4 cm. Frequency distributions for 192- and non-192-bp genotypes showed no advantage of the 192-bp allele to coleoptile elongation. None of the 192-bp genotypes from the Great Plains showed greater coleoptile length than 'TAM 107', a hard red winter cultivar without *Rht8* often chosen over contemporary cultivars for its greater emergence capacity with deeper seed placement. Since coleoptile elongation may be controlled by several quantitative trait loci, identifying only the presence of 192-bp allele of *Xgwm 261* may be misleading if the primary motivation for its deployment is to increase coleoptile length in a semi dwarf plant type.

Hedden (2003) showed that the spectacular increases in wheat and rice yields during the 'Green Revolution' was enabled by the introduction of dwarfing traits into the plants. Identification of the genes responsible for these traits shows that they interfere with the action or production of the gibberellin (GA) plant hormones. It was also known that the wheat *Rht* genes encode growth repressors that are normally suppressed by GA, and recent work shows that the rice *sd1* gene encodes a defective enzyme in the GA-biosynthetic pathway.

Ahmad and Sorrells (2002) showed that a wheat microsatellite locus, *Xgwm 261*, whose 192-bp allele closely linked to the dwarfing gene *Rht8*, on chromosome 2D, was used to screen 71 wheat cultivars from 13 countries to assess the variation at this locus. Screening of this wheat collection showed that a 165-bp allele and a 174-bp allele were the most frequent. None of the New Zealand cultivars possessed a 192-bp allele specific to *Rht8*, while only one cultivar from the US produced this important allele. The frequency of a 192-bp allele among these wheat cultivars was 5.63%. The highest allele frequency was observed for a 174-bp fragment (52.11%) followed by a 165-bp fragment (26.76%). The only durum wheat 'Cham 1', did not show any amplification due

to the absence of D genome. Four new novel alleles, 180-bp, 198-bp, 200-bp and 204-bp present in the US and New Zealand wheat cultivars are reported.

According to Wojciechowski *et al.* (2002) no significant differences in root length were found between semi-dwarfing lines and the control lines in any experiment, nor was there a significant difference between the root lengths of the two cultivars grown in the field. Total root length of the dwarf lines (Rht-B1c, Rht-D1c, and Rht12) was significantly different from that of the control although the effect was dependent on the experimental methodology; in gel chambers root length of dwarfing lines was increased by 40% while in both soil media it was decreased (by 24–33%).

Ellis *et al*., (2002) validated two PCR based gene specific perfect markers by testing 19 wheat varieties of known reduced height (Rht) genotype that indicated *Rht-B1b* and *Rht-D1b* dwarfs, double-mutant varieties and *Rht-B1a* and *Rht-D1a* tall alleles. Mapping analysis expected homoeologous regions of chromosomes 4B and 4D showed highly significant associations with height of both markers together accounting for 67% of the phenotypic variance in height. This marker-assisted selection improved wheat breeding programs.

According to Worland *et al.* (2001) , microsatellite marker WMS261 developed at I.P.K., Gatersleben, Germany, co-segregates with the semi-dwarfing gene *Rht8*. Screens of over 800 wheat varieties from 20 countries show 90% carry WMS-261 alleles with 165, 174 or 192 base pairs (bp). The 192-bp allele diagnostic of *Rht8* occurs in most screened Southern European varieties. Anallele with 165 bp occurs in the majority of CIMMYT Mexican varieties andin most varieties bred in countries that utilize CIMMYT germplasm. Agronomic studies of single chromosome recombinant line mapping populations segregating for WMS 261 alleles with 165, 174 or 192 bp, showed that compared to the 174-bp allele the 192-bp allele reduces height by around 8 cm and the 165-bp allele increases height by around 3cm. The results indicate the importance and widespread utilization of *Rht8* as a dwarfing gene in

southern Europe and suggest an adaptative significance to the height promoting 165-bp allele in CIMMYT material.

Korzun *et al*. (1998) reported that among over 100 international varieties of wheat it also demonstrated that a limited number of varieties carried novel WMS 261 variants of over 200 bp in plant height with the WMS 261-192-bp allele compared to the WMS 261-174-bp allele in the set of recombinant lines comparing 2D chromosomes of 'Mara' and 'Cappelle- Desprez'. A height reduction of around 3 cm was detected between the WMS 261-174-bp allele and the WMS 261-165-bp allele in the recombinant lines comparing 2D chromosomes of 'Cappelle-Desprez' and 'Ciano 67'.

Worland *et al.* (1998) used dwarfing gene *Rht8* to screen over 100 wheat varieties to determine the worldwide spread of *Rht8* in the very successful semi-dwarf varieties bred by CIMMYT, Mexico. The microsatellite analysis showed that CIMMYT wheat varieties lack *Rht8* and carry a WMS 261 allelic variant of 165 bp that has been associated with promoting height.

2.3. Heat-Shock Protein

Garg, *et al*. (2012) analyzed DNA fragments covering partial sequence of susceptible genotypes of wheat using HSP as a target gene. One SNP was found between these genotypes and the analysis of amino acid showed that that the base transaction is susceptible to heat stress. Single markers analysis explained 29.89% and 24.14% phenotypic variation for grain weight per spike and thousand grain weights respectively.

Mason *et al.* (2010) showed that heat stress adversely affects wheat production in many regions of the world and is particularly detrimental during reproductive development and grain filling. The objective of this study was to identify quantitative trait loci (QTL) associated with heat susceptibility index (HSI) of yield components in response to a short term heat shock during early grain filling in wheat. The results validated the use of the main spike for

detection of QTL for heat tolerance and identify genomic regions associated with improved heat tolerance that can be targeted for future studies.

Elizabeth *et al*. (2003) isolated the WHSP16.8 and WHSP16.9 cDNAs by screening a lambda gt11 expression library with antibodies to HMGc (a chromosomal protein of wheat) which is dependent on heat shock and have molecular weights (estimated by sodium dodecyl sulfate electrophoresis) consistent with the molecular weights of the proteins deduced from the sequences of the cDNAs.

Wang *et al*. (2003) proved that molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress related genes. The stress-response mechanisms and their biotechnological application, regulatory control, metabolic engineering, ion transportation, antioxidant and detoxification can be examined though heat-shock proteins.

Clarke and Critchley (2002) showed that that all chloroplast heat-shock proteins were synthesized on cytoplasmic ribosomes, translocated into the chloroplast, and located in the stroma. The synthesis of this protein and the formation of the heat-shock protein complex were dependent on functional cytoplasmic ribosomes heat-shock protein complex in wheat chloroplasts is a homogeneous octamer of 32-kD subunits.

Waters (1995) reported that over 20 different small heat-shock proteins are found in higher plants. The phylogenetic relationships of the small heat-shock proteins, estimated using parsimony and distance methods, reveal that gene duplication, sequence divergence and gene conversion have all played a role in the evolution of the small heat-shock proteins.

Zoran *et al*. (1992) studied the synthesis of heat shock proteins (HSPs) in the leaves of a drought- and heat-resistant (line ZPBL 1304), and a drought- and heat-sensitive (line ZPL 389) line of wheat. Heated plants of the drought- and heat-resistant line ZPBL 1304 synthesized a band of HSP(s) of approximately

45 kilo Daltons which was not found in heated plants of the drought and heat sensitive line ZPL 389.

Helm and Abernethy (1990) studied the relationship of pre-existing HSP mRNAs and the heat shock response during early imbibitions. Heat shocks (42°C, 90 minutes) were administered following 1.5, 16, and 24 hours at 25°C imbibitions. The behavior of the HSP 70 group of proteins during early imbibition was examined by RNA gel blot analysis. The mRNAs for the HSP 70 group were detectable at moderate levels in the quiescent embryo.

Xiaolin *at el.* (1990) characterized proteome alterations of maize *vp5* seeds and to identify ABA-dependent proteins during seed maturation, LEA proteins and HSPs which has displayed differential accumulations in *vp5* embryos: six out of eight identified LEA proteins decreased while nine HSPs increased in abundance on wheat varieties.

Krishnan *et al*. (1989) showed that the determination whether genotypic differences in acquired thermal tolerance were associated with changes in the pattern of heat shock protein synthesis, the HSP exhibiting apparent molecular weights of 16, 17, 22, 26, 33, and 42 Kilo Daltons.

CHAPTER 3
MATERIALS AND METHODS

The materials and methods used for conducting the experiments under the present study include the following aspects:

3.1. Location of the study

The experiment was conducted in the "Biotechnology Laboratory" of Bangladesh Agricultural Research Institute (BARI), Gazipur during the period of January to June, 2014.

3.2. Wheat genotypes used in the study:

Twenty four wheat genotypes from different origins were used in the study of which most were BARI released varieties and the rest were from foreign origin (Table 1).

Table 1. Name and origin of the wheat genotypes used in the experiments

Serial No.	Genotype	Origin	Serial No.	Genotype	Origin
1	Kheri	Bangladesh	13	BARI-Gom-19 (Sourav)	Bangladesh
2	Kalyansona	India	14	BARI-Gom-20 (Gaurav)	Bangladesh
3	Sonora-64	CIMMYT, Mexico	15	BARI-Gom-21 (Shatabdi)	Bangladesh
4	Sonalika	India	16	BARI-Gom-22 (Sufi)	Bangladesh
5	Pavon-76	CIMMYT, Mexico	17	BARI-Gom23 (Bijoy)	Bangladesh
6	Balaka	Bangladesh	18	BARI-Gom-24 (Prodip)	Bangladesh
7	Ananda	Bangladesh	19	BARI Gom-25	Bangladesh
8	Kanchan	Bangladesh	20	BARI Gom-26	Bangladesh
9	Akbar	Bangladesh	21	BARI Gom-27	Bangladesh
10	Barkat	Bangladesh	22	BARI Gom-28	Bangladesh
11	Aghrani	Bangladesh	23	Westonia-5907	CSIRO, Australia
12	Protiva	Bangladesh	24	Westonia-5924	CSIRO, Australia

Figure 2. Fresh leaves were collected from two week old seedlings for DNA extraction.

Twenty four (24) wheat genotypes were grown for DNA extraction and molecular studies. There were seventeen Bangladeshi (number 6-22), two Australian (number 23-24), two Indian (number 2, 4), two CIMMYT Mexican (number 2, 4) wheat genotypes. All wheat lines were grown in plastic pots in the green house of Biotechnology laboratory.

3.3. Reagents for DNA extraction and PCR amplification

1. Hot Start PCR mixture
2. *Taq* Polymerase
3. *Taq* buffer
4. Red dye
5. dNTPs
6. DNA Extraction buffer
7. Elusion buffer
8. Wash buffer
9. Ethidium bromide
10. 50% Tris EDTA
11. Water
12. Agarose
13. $MgSO_4$

14. Glacial acetic acid
15. Isopropanol
16. Absolute ethanol
17. Double distilled water
18. Wash buffer
19. NaOH
20. $MgCl_2$

3.4. Procedure of DNA extraction:

Genomic DNA was isolated by following the protocol of Genomic DNA Mini Kit (Plant, Version: 04-24-13)

Step 1 (Tissue dissociation):

For DNA extraction 150mg of fresh leaf tissue was collected and grinded by mortar with 400µl GP1 buffer and transferred them into a 1.5 ml micro-centrifuge tube.

Step 2 (Lysis):

After grinding with GP1 the sample was transferred into a 5µl RNase A containing 1.5 ml microcentrifuge tube and mixed by vortex.

- Incubated at 60°C for 20 minute, during incubation the tube was inverted on every 5 minute.
- Then, pre heated the required elution buffer (200 µl per sample) to 60° C (step 5)
- And added 100 µl of GP2 buffer and mixed by vortex then incubated on ice for 5 minutes.
- Then it was centrifuged at 16,000× g for 5 minute.
- After centrifugation, placed filter column in a 2 ml collection tube then transferred the mixture to the filter column.

- Then it was again centrifuged for 5 minute at 1,000× g then discarded the filter column.

The supernatant was carefully transferred from the 2ml collection tube to a new 1.5 ml microcentrifuge tube.

Step 3 (DNA Binding)

- Added a 1.5 volume of GP3 buffer (isopropanol added) for lysate then vortex immediately for 5 seconds. E .g . Added 750 of GP3 buffer to 500 µl of lysate.

- Then placed a GD Column in a 2ml collection tube.
- After that transferred the dried GD Column to a clean 1.5ml 700 µl of mixture to the GD column.
- It was centrifuged at 14-16000× g for 2 minutes.
- Then discarded the flow through and placed the GD Column back in the 2ml collection tube.
- Also added the remaining mixture to the GD column then it was centrifuged at 14-16000 × g for 2 minutes.
- Discarded the flow through and placed the GD column back in the 2 ml collection Tube.
- After that added 400 µl of W1 buffer to the GD column then centrifuge at 14-16000 × g for 30 seconds.
- Then discarded the flow through and placed the GD column back in the 2 ml collection Tube.

Step 4 (Wash)

- Added 600 µl of wash buffer (ethanol added) to the GD column.
- Then it was centrifuged at 14-16000 × g for 30 seconds.
- After centrifugation, discarded the flow through then placed the GD column back in the 2 ml collection tube.
- Again centrifuged GD column for 3 minutes at 14-16000 × g to dry the column matrix.

Step 5 (DNA elution)

- The sample was transferred the dry GD column to a cleaned 1.5 ml microcentrifuge tube.
- Then added 100µl of pre-heated elution buffer or TE to the centre of the column matrix.
- It was kept stand for 3-5 minutes to ensure the elution buffer or TE is completely absorbed.
- Finally it was centrifuged at 14-16000 × g for 30 seconds for eluting the purified DNA.

3.5 PCR primers

Table 2. List of PCR primers used in the study

SL No.	Trait	Locus	Primer/Marker Name	Primer sequence (5'-3')	Expected fragment size (bp)	Reference
1	Rye Translocation	5s-Rrna-R1	RIS	F:TAATTTCTGCTTGCTCCATGC R:ACTGGGGTGCACTGGATTAG	110	Koebner *et al.* 1994
2	Reduction in plant height	*Rht-B1a*	BF-WR1	F:GGTAGGGAGGCGAGAGGCGAG R:CATCCCCATGGCCATCTCGAGCTG	237	Ellis *et al*. 2002
		Rht-B1b	BF-MR1	F:CCAGATACACAACTGCTGGC R:TGATCTTGAGGTTCTCGTCG	237	Ellis *et al.* 2002
		Rht-D1a	DF2-WR2	F:GGCAAGCAAAAGCTTCGCG R: GGCCATCTCGAGCTGCAC	264	Ellis *et al.* 2002
		Rht-D1b	DF-MR2	F:CGCGCAATTATTGGCCAGAGATAG R:CCCCATGGCCATCTCGAGCTGCTA	254	Ellis *et al*. 2002
3	Heat Shock Protein	*HSP 16.9*	AF-R1	AF:CAGCAATCAACACCACGATG R1:TGCCACTTGTCGTTCTTGTC	*307*	Ellis *et al* 2002

3.5.1. Primer dilution

Primer is a short oligonucleotide sequence of 10-20 nucleotides used as template for DNA amplification.

To make stock solution of 100 pM primer was diluted in ddH_2O, and desired concentration (5 pM) was prepared by using the following standard equation:

$S_1V_1 = S_2V_2$

Where 'S_1' is stock concentration of Primer (ng/µL); 'V_1' is the volume of primer stock required; 'S_2' is desired concentration (ng/µL l); and 'V_2' is the total volume of the diluted primer to be made (µL).

3.6. Preparation of Stock Solution used for Gel Electrophoresis

For performing the gel electrophoresis, the following stock solutions and other solutions were prepared:

3.6.1. 50 x TAE Buffer (pH 8.3) (1 liter)

242.0 gm Trizma Base (MW=121.14) was dissolved into 700 ml of sterile de-ionizing distilled water. Then 57 ml glacial acetic acid was added to the solution. Finally 100 ml, 0.5 EDTA (pH 8.0) was added in it. They were mixed well. The pH of the solution was adjusted by mixing concentrated HCL at pH 8.3. The final volume of the solution was adjusted to 1000ml.

4.6.2. 6X Loading Dye

This is required to load samples in gel electrophoresis for future visualization.

Preparation of stock solution:

1. 10ml of a 2% bromophenol blue stock solution.
2. 10 ml of a 2% xylene cyanol stock solution.
3. 50% glycerol solution.

The stock solutions were mixed to prepare the final 6X concentration loading dye.

3.6.3. Ethidium Bromide solutions

For 1 mL solution, 10 mg Ethidium Bromide was added to 1mL of sterile de-ionized distilled water. It was then mixed by hand shaking .The solution was then transferred 10 ml dark bottle and stored at room temperature. Stock solution of 10 mg/mL can also be purchased directly from companies.

3.7. Agarose Gel Electrophoresis

1. The standard method used to separate, identify and purify the DNA fragments through electrophoresis.
2. 1.0 gm of agarose was heated to melt into 100 ml of TAE buffer; ethidium bromide was added as 0.5 μg/mL final concentration and poured into gel tray fixed with appropriate combs**.**
3. After the gel was solidified it was placed into gel running kit containing 1 x TAE buffer.
4. Digested plant DNA solutions were loaded with 6X gel loading dye and electrophoresis was performed.
5. For marker analysis 2-log Ladder was used.

3.7.1. Documentation of the DNA sample

i. After electrophoresis, the gel was taken out carefully from the electrophoresis chamber and placed in Gel documentation system (Alpha Innotech, SCOR-14 SOM, USA) for checking the DNA bands .

ii. The DNA was observed and photographed using Gel Documentation system.

3.7.2. Measurement of OD in the spectrophotometer:

DNA was quantified using a spectrophotometer. Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity.

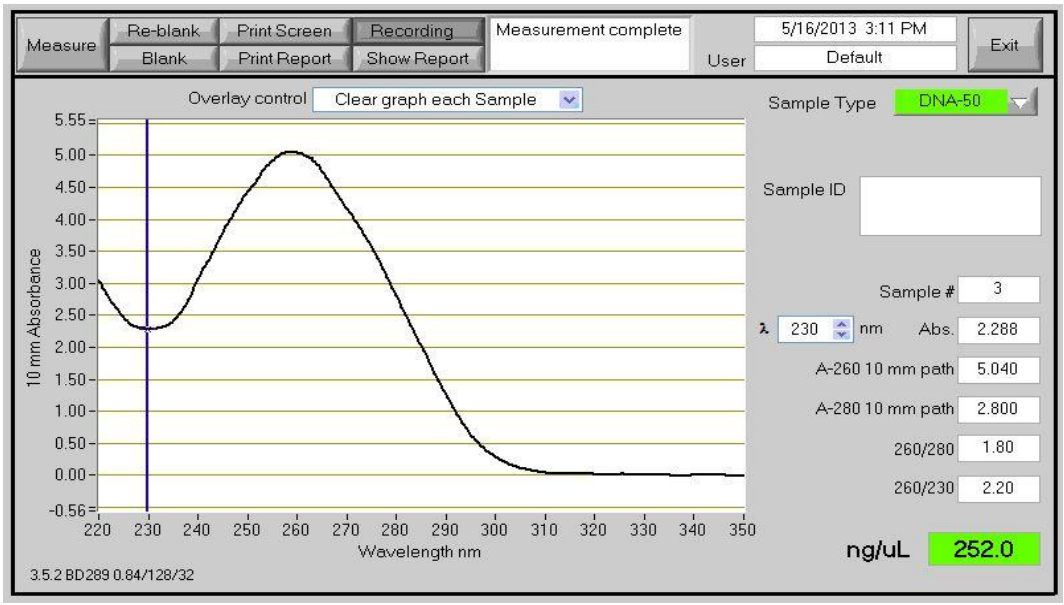

Figure 3: Measuring DNA concentration of wheat samples by Nanodrop spectrophotometer.

For the most consistent results, it is best to begin any measurement session with a blanking cycle. This will assure the user that the instrument is working properly and that the pedestal is clean.

Follow the steps below to perform a blanking cycle:

1. Loaded a blank sample (the buffer, solvent, or carrier liquid used with your samples) onto the lower measurement pedestal and lower the sampling arm into the ‘down’ position.

2. Clicked on the ‘Blank’ (F3) button.

3. When the measurement was completed, wiped the blanking buffer from both pedestals using a laboratory wipe.

4. Analyzed an aliquot of the blanking solution as though it were a sample. This is done using the 'Measure' button (F1). The result should be a spectrum with a relatively flat baseline. The blank was wiped from both measurement pedestals.

3.7.3 Calculation of the concentration of DNA:

Before PCR, DNA concentration was determined according to the following formula:

DNA concentration = A260 × Dilution factor × Conversion factor

$$= A260 \times \frac{\text{Volume of distilled water (µl)}}{\text{Amount of the DNA sample (µl)}} \times 50$$

=ng/ µl

= µg/ml [Since 1 µg = 10^{-3} ng i.e µg/ml=ng/ µl],

3.8 PCR amplification

PCR conditions were as follows (20µl total volume):1 X Hotstar Buffer, 1 X Hotstar Q solution, 100ng of template DNA ,4 nmol of dNTPs, 10 pmol each of the forward and reverse primers, 1 unit of Hotstar Taq polymerase (Quagen, Hilden Germany). The PCR products were separated by electrophoresis in 1.5% agarose gel at 100V for 1h and visualized over ultraviolet light in a gel documentation system. The PCR cycles for different primers are given below:

R1S	3 minutes at 94°C 30 seconds at 94°C 45 seconds at 60°C (35 cycles) 30 seconds at 72°C 7 minutes at 72°C Hold at 10°C forever
BF-WR1 BF-MR1 DF-MR2	5 minutes at 95°C 30 seconds at 94°C 30 seconds at 60°C (40 cycles) 30 seconds at 72°C 5 minutes at 72°C Hold at 10°C forever
DF2-WR2	3 minutes at 94°C 30 seconds at 94°C 30 seconds at 45°C (35 cycles) 30 seconds at 72°C 7 minutes at 72°C Hold at 10°C forever
HSP 16.9	3 minutes at 94°C 30 seconds at 94°C 30 seconds at 60°C (30 cycles) 7 minutes at 72°C Hold at 10°C forever

3.9. Phenotypic observation

Following observations were recorded for yield related traits of all genotypes. The investigated traits were given below:

3.9.1. Plant height (cm)

Plant height of each of ten randomly selected plants was measured from base of plant to the tip of spike excluding awns of the main shoot. Average plant height for each genotype was estimated.

3.9.2. Number of tillers per plant

Numbers of tillers of each genotype of selected plants were counted.

3.9.3. Number of productive tillers per plant

Numbers of tillers of each genotype of selected plants were counted at maturity in each plant and average was computed.

3.9.4. Number of grains per plant

The grains from ten randomly selected plants were counted after harvesting.

3.9.5. 1000-grain weight (g)

Weight of 1000 grains was measured with electronic balance.

3.9.6. Grain yield per plant (g)

All spikes of individual selected plants were threshed manually and weighed using electric balance (SCIENCETEC, 510D, USA). Average grain yield per plant was estimated for each genotype.

3.10. Statistical analysis

The means for all collected phenotypic data were calculated and the analyses of variance for all characters were performed. The collected data were analyzed by IRRISTAT Software (Version 4.0) and ‘R’ program.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Characterization of samples

Twenty four wheat genotypes (Table 1) were characterized for the presence of rye translocations, dwarfing genes and a heat shock protein by using gene specific molecular markers. A field observation trial was also conducted for recording different yield contributing characters of the varieties. The results of the experiments are presented below.

4.2 DNA extraction

Table 3. Concentration of extracted DNA from 24 wheat genotypes

Serial No.	Name of the genotype	Concentration of DNA (ng/µL)	Serial No.	Name of the genotype	Concentration of DNA(ng/µL)
1	Kheri	243.5	13	BARI Gom-19 (Sourav)	220.9
2	Kalyansona	306.0	14	BARI Gom-20 (Gaurav)	273.4
3	Sonora-64	436.0	15	BARI Gom-21 (Shatabdi)	199.2
4	Sonalika	387.1	16	BARI Gom-22 (Sufi)	267.7
5	Pavon-76	300.0	17	BARI Gom-23 (Bijoy)	366.5
6	Balaka	264.8	18	BARI Gom-24 (Prodip)	297.4
7	Ananda	148.7	19	BARI Gom-25	313.3
8	Kanchan	223.9	20	BARI Gom-26	414.3
9	Akbar	297.5	21	BARI Gom-27	263.0
10	Barkat	398.4	22	BARI Gom-28	175.7
11	Aghrani	247.5	23	Westonia-5907	258.2
12	Protiva	218.3	24	Westonia-5924	168.0

Good quality DNA was extracted from 14 day old seedlings of 24 wheat genotypes. DNA concentration obtained from 100mg leaf samples varied from 148.7 ng/µL (Ananda) to 436.0 ng/µL (Sonora 64) (Table 3). Concentration of all the samples were adjusted to 50 ng/µL prior to PCR analyses using specific primer combinations.

And then all genomic DNA were screened under gel documentation after PCR analysis. (Fig 4)

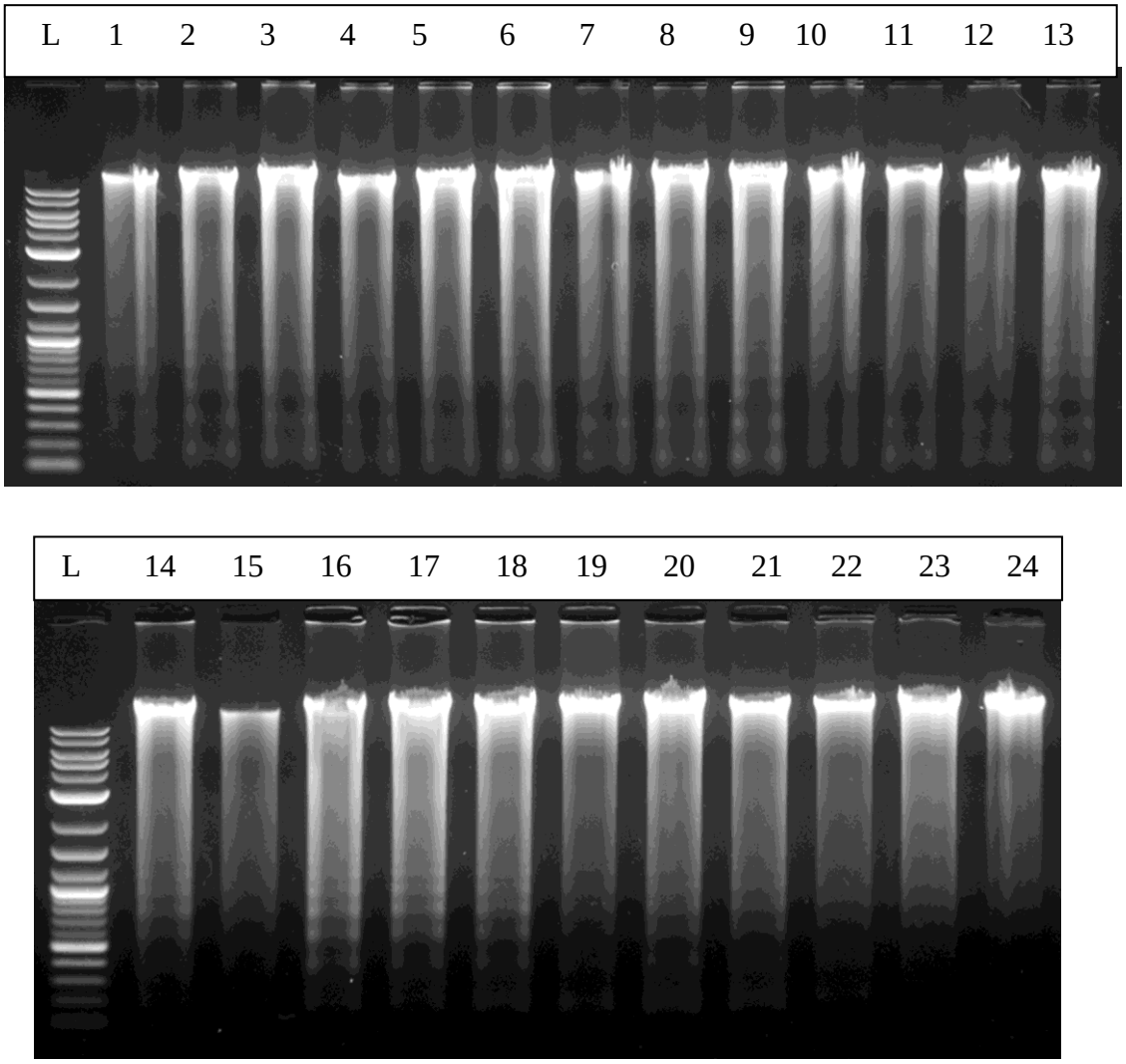

Figure 4. Gel Electrophoresis of 24 wheat genomic DNA.

4.3. Characterization of rye translocations

Wheat (*Triticum aestivum* L.) 1BL.1RS translocation which containes 1RS (short arm of chromosome 1) fragment from rye (*Secale cereal* L.) is the most widespread alien chromatin in wheat breeding programmes used in all over the world (Rabinovich, 1998; Grey-bosch, 2001). Increased genetic diversity in wheat breeding is desirable for dealing with present and future challenges caused by various stress condition pertaining to the onset of climate change.

Rye has already proved to be a good donor of genes for improving important traits and diversity in wheat breeding (Zeller and Hsam 1983). Many wheat cultivars carrying wheat-rye translocations, rye chromosome 1R (1RS), have proved successful worldwide. (Lukaszewski 1990; Rabinovich 1998; Schneider and Molnár-Láng 2009). They have also been reported to improve yield potential, stress tolerance, and adaptation in bread wheat (Friebe *et al*. 1990, 1995; Carver and Rayburn 1994; McKendry *et al.* 1996; Kim *et al.* 2003).

The short arm of this translocation carries resistance genes against leaf rust (Lr26), stem rust (Sr31), stripe rust (Yr9) and Powdery mildew (Pm8) (McIntosh, 1988). Furthermore, several studies indicated that this 1RS is able to increase yield and improve adaptation in specific genetic backgrounds of wheat (Carver and Rayburn, 1995; Moreno-Sevilla *et al*., Villareal et *al*., 1998) However, as most 1BL.1RS translocation wheats in convensional production can be traced back to a single genotypes (Schegel and Korzun, 1997), very little allelic variation is available in the rye arm of this translocation (Marais *et al.*, 1994; Hsam and Zeller, 1997).

PCR analysis of the selected 24 wheat genotypes were carried out using gene specific molecular markers to identify a single product of 110 bp (all 1BL.1RS translocation lines) from all templates containing rye DNA but not from templates lacking it (Driscoll and Sears, 1971).

Specific primer pairs for 1RS rye chromosome fragment amplified PCR products of approximately 110 bp from 16 genotypes indicating the presence of

translocation in these lines (Figure 5). No amplification product was obtained from rest of the 8 genotypes which include some of the best varieties like Shatabdi (No. 15) and BARI Gom-26 (No. 20). This indicates that under favourable growing conditions varieties can perform well without having rye translocations. However, under stress conditions, varieties with the rye translocation might show better adaptation.

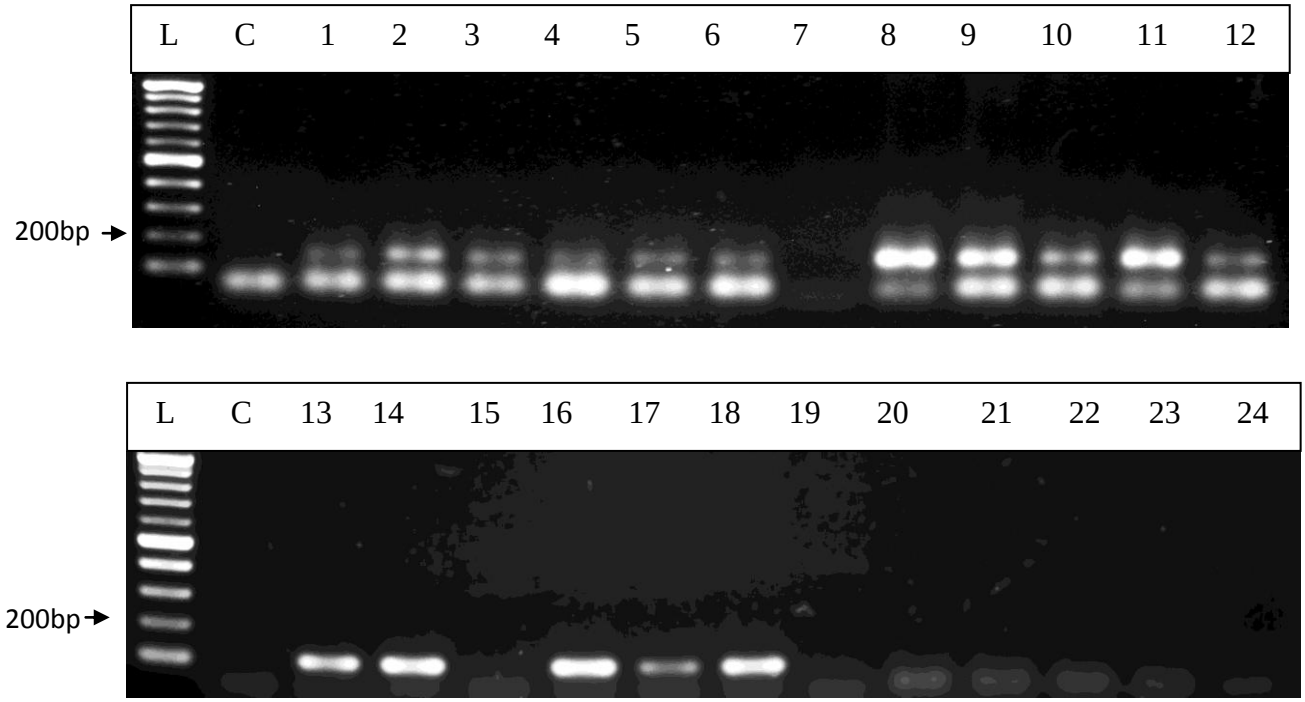

Figure 5. Amplification profiles of 24 wheat genotypes having wheat–rye translocation (1BL.1RS.L). C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom- 26, 21. BARI Gom- 27, 22. BARI Gom- 28, 23. Westonia- 5907 and 24. Westonia- 5924.

4.4. Presence of dwarfing genes

Twenty four wheat genotypes were screened by PCR analysis using the following primer sets, BF-WR1, BF-MR1, DF-MR2 and DF2-WR2. The expected product sizes were 237 bp for BF-MR1 and BF-WR1, 254 bp for DF-MR2, and 264 bp for DF2-WR2.

4.4.1 Screening for *Rht-B1b* (Dwarf)

An expected amplification product of approximately 237 bp for the *Rht-B1b* gene was amplified from six genotypes (Nos. 2, 5, 9, 11, 13, and 15). No amplification was observed from rest of the genotypes (Fig. 6) indicating the absence of this dwarfing allele in these genotypes.

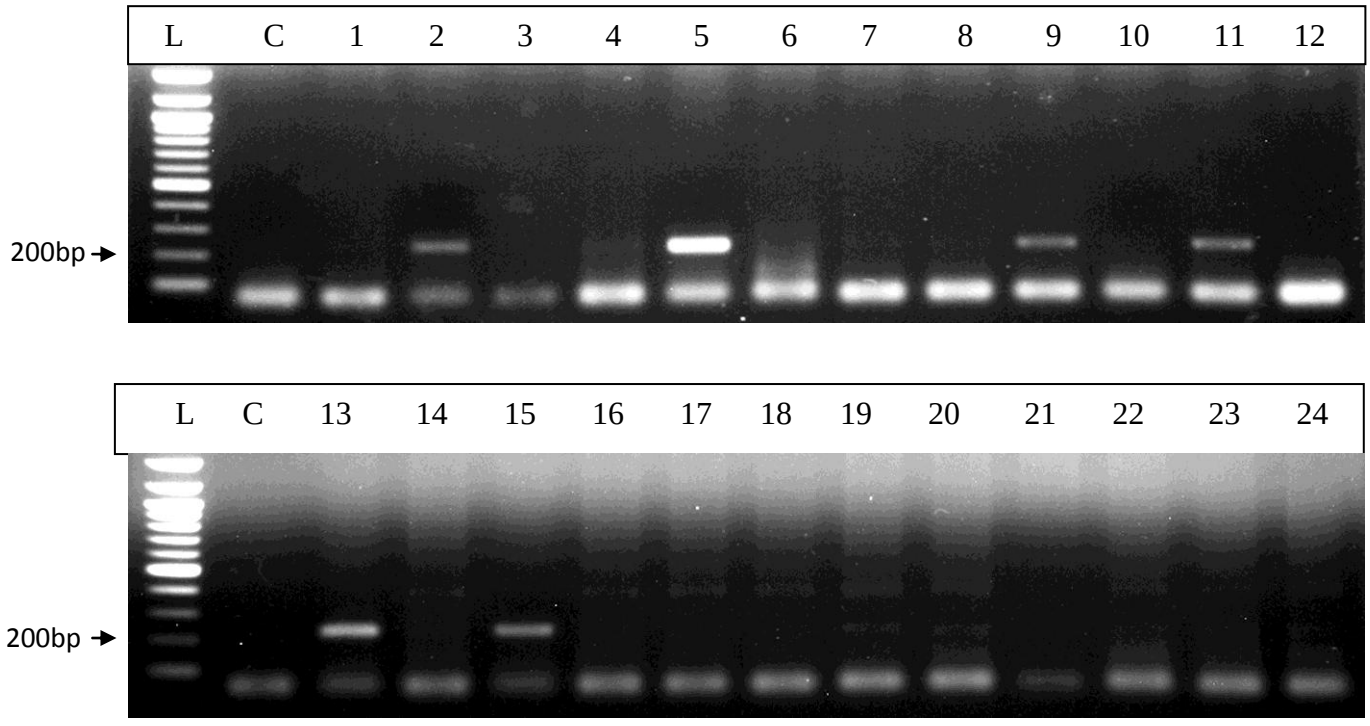

Figure 6. Amplification profiles of *Rht-B1b*. PCR primer pair of 24 wheat varieties. C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom- 26, 21. BARI Gom- 27, 22. BARI Gom- 28, 23. Westonia- 5907 and 24. Westonia- 5924.

4.4.2. Screening for *Rht-B1a* (Wild type)

Rht-B1a gene specific products were amplified from eighteen lines from the twenty four wheat genotype studied. The expected amplification products size was 237 bp. No amplified products were produced from rest of the samples (Fig. 7). The results obtained were exactly reverse of that obtained from the *RhtB1b* specific primer combination. This provides an accurate assessment of the presence of the dwarfing allele in this locus.

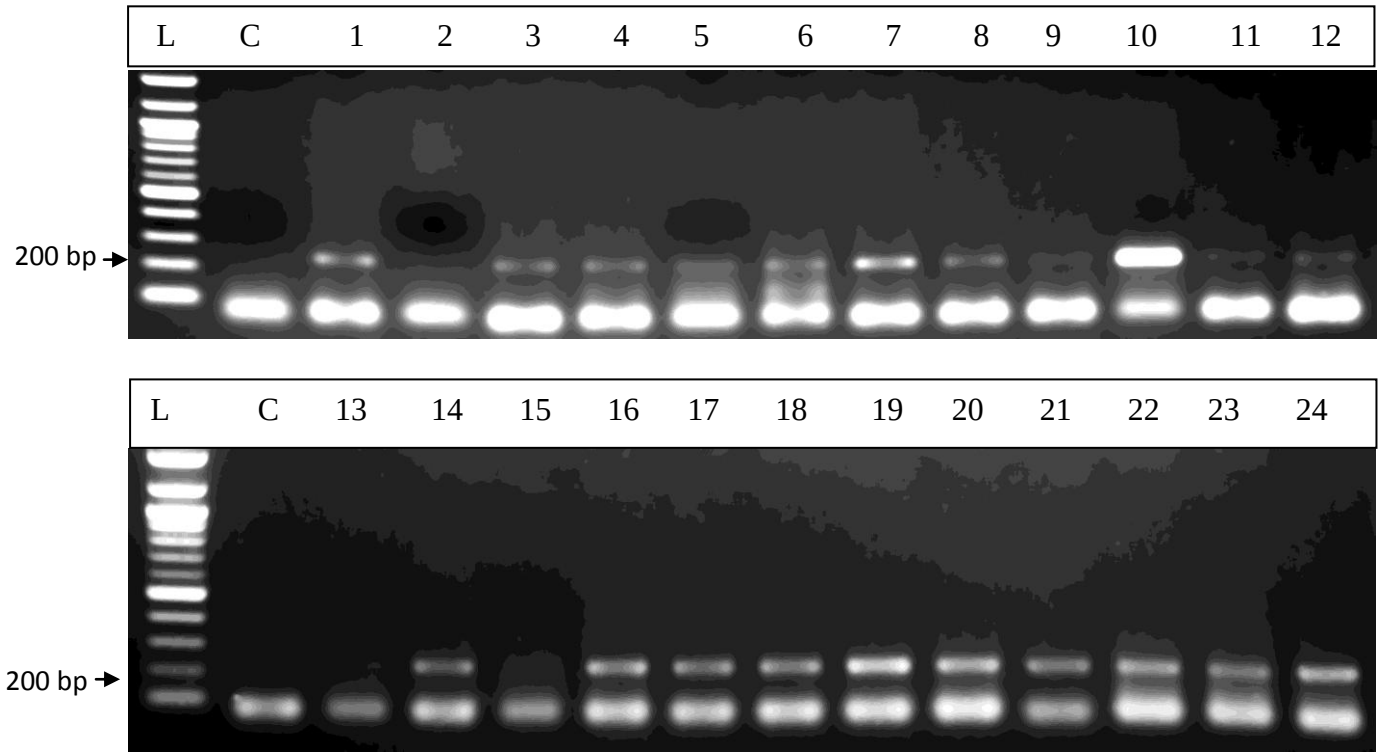

Figure 7. Amplification profiles of *Rht-B1a*. PCR primer analyses of 24 wheat varieties. C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom- 26, 21. BARI Gom- 27, 22. BARI Gom- 28, 23. Westonia-5907 and 24. Westonia- 5924.

4.4.3 Screening for *Rht-D1b* (Dwarf)

Rht- D1b gene specific products were amplified from nineteen wheat genotypes out of twenty four. The expected amplification products size was 254 bp. The amplified products were produced in wheat lines 2-10, 12, 14, and 17-24. No amplification was obtained from genotypes 1, 11, 13, 14 and 15 (Fig. 8). Specific amplified PCR product shows the presence of *Rht-D1b* dwarfing allele in those respective genotypes the rest of the genotypes contain the wild type allele.

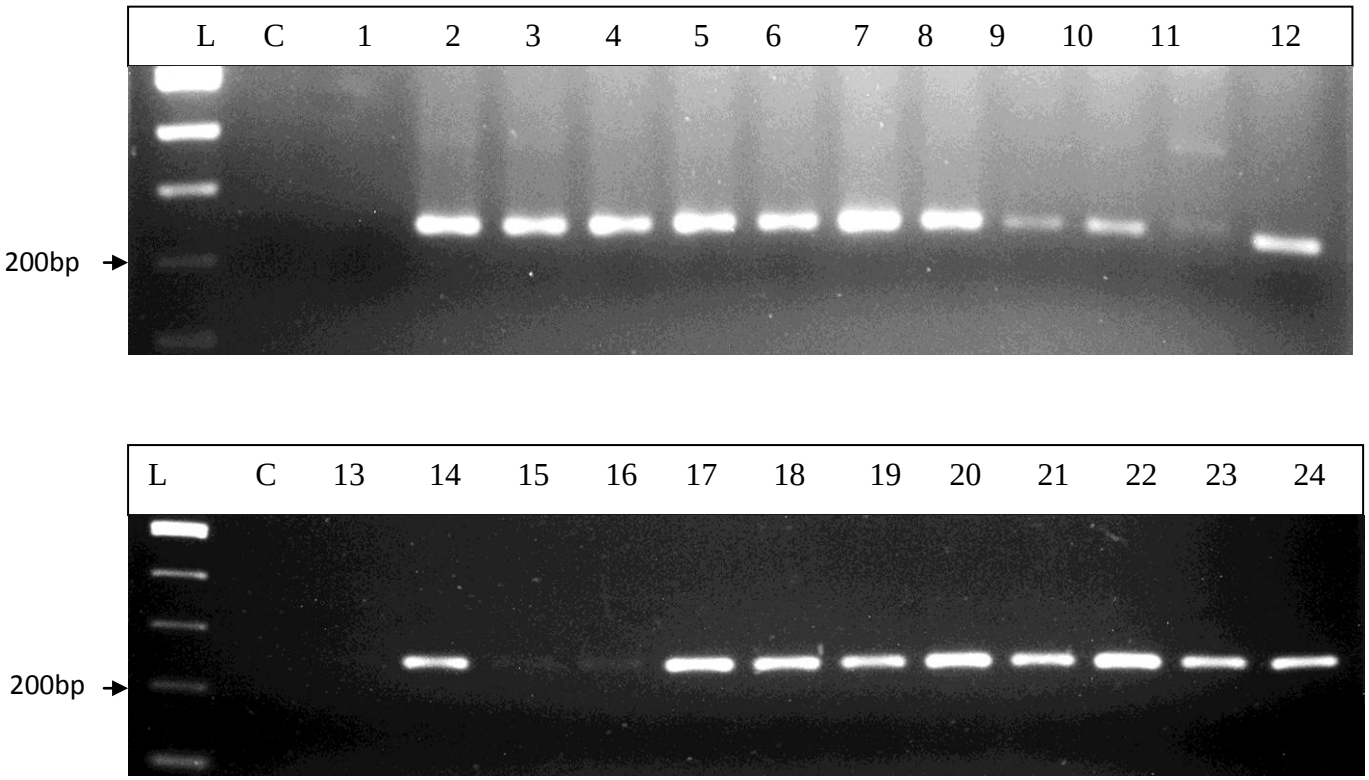

Figure 8. Amplification profiles of *Rht-D1b*. PCR primer pair analyses of 24 wheat varieties. C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom- 26, 21. BARI Gom- 27, 22. BARI Gom- 28, 23. Westonia- 5907 and 24. Westonia- 5924.

4.4.4 *Rht-D1*a (Wild type)

The expected amplification products size was 264 bp for the *Rht- D1a* allele. Specific products were amplified only from five genotypes (nos. 1, 11, 13, 15 and 16) indicating the presence of the wild type allele in these genotypes (Fig.9). No product was amplified from rest opf the genotypes. This result confirms the previous findings obtained with the *Rht-D1b* specific primers

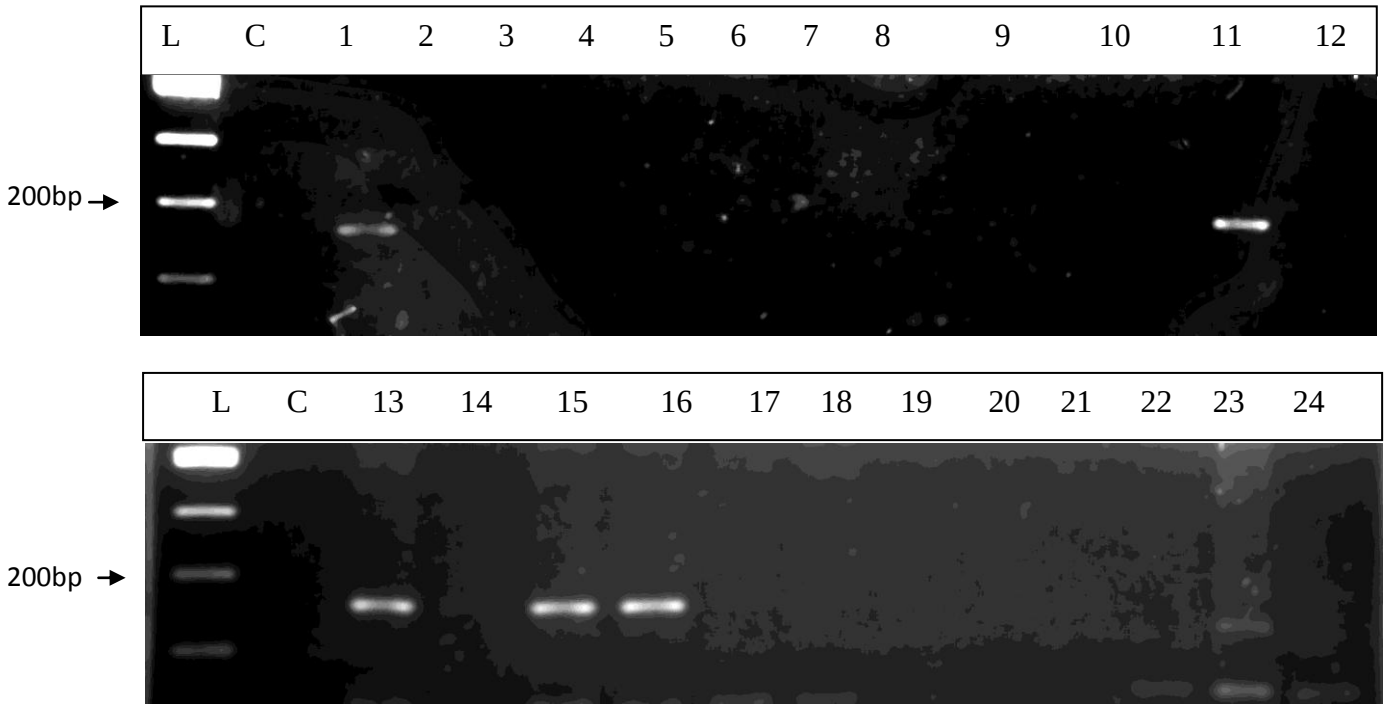

Figure 9. Amplification profiles of *Rht-D1a*. PCR primer pair analyses of 24 wheat varieties. C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom- 26, 21. BARI Gom- 27, 22. BARI Gom-28, 23. Westonia- 5907 and 24. Westonia- 5924

4.4.5 Summary of *Rht* dwarfing alleles

A range of wheat varieties with unknown *Rht* genotypes were selected and DNA samples from these lines were analysed by PCR using *Rht* gene specific molecular markers. Four sets of primers were used for the detection of dwarfing genes containing either *Rht-B1b* or *Rht -D1b* (Table 4) allele. Out of the 24 the combination of genotypes studied, 19 genotypes with either *Rht-B1b* or *Rht -D1b* allele showed semi-dwarf phenotype having heights between 78.94 (Westonia 5924) and 94.87 (Kanchan) cm (Fig. 9). Butler *et al*. (2005) evaluated the agronomic performance of *Rht-B1* and *Rht-D1* genes and reported that the highest grain yield was obtained in an irrigated location with the semi-dwarf combination *Rht-B1b* + *Rht-D1a*. Three of the semi-dwarf genotypes under the present study, 11 (Aghrani), 13 (Sourov) and 15 (Shatabdi) showed similar genotype (Table 5) and are expected to give better yield under irrigated conditions. Sixteen other semi-dwarf genotypes showed the presence of the combination Rht-*B1a* + *Rht-D1b* (Table 5) and some of those genotypes (e.g. Prodip and BARI Gom 26) are considered as some of the best varieties in Bangladesh. Two of the genotypes 1 (Kheri) and 16 (Sufi) had wild type alleles in both the loci and were expected to produce tall phenotypes, however, only 'Kheri' showed a tall phenotype and 'Sufi' produced a semi-dwarf phenotype which was probably influenced by some other genetic or environmental factors. Two genotypes showed the presence of double dwarf alleles (*Rht-B1b* + *Rht-D1b*) although they showed semi-dwarf nature of growth (Fig. 10). Butler *et al*. (2005) also showed that wheat lines carrying both dwarfing alleles rendered lower grain yields in every environment. For stressed environments the best choice seemed to be related more with the right plant height than the combination of alleles, the best results were observed with shorter lines within the tall class, without any dwarfing allele (like Sufi), or taller lines within the semi dwarf class carrying *Rht-B1b* + *Rht-D1a* allele combination as mentioned above.

Table 4. Summery table of dwarfing gene

Genotypes	*Rht* B1a (Wild type)	*Rht* B1b (Dwarf)	*Rht* D1a (Wild type)	*Rht* D1b (Dwarf)
	BF-WR1	BF-MR1	DF2-WR2	DF-MR2
Kheri	+	-	+	-
Kalyansona	-	+	-	+
Sonora- 64	+	-	-	+
Sonalika	+	-	-	+
Pavon- 76	-	+	-	+
Balaka	+	-	-	+
Ananda	+	-	-	+
Kanchan	+	-	-	+
Akbar	-	+	-	+
Barkat	+	-	-	+
Aghrani	-	+	+	-
Protiva	+	-	-	+
BARI-Gom-19 (Sourav)	-	+	+	-
BARI-Gom-20 (Gaurav)	+	-	-	+
BARI-Gom-21 (Shatabdi)	-	+	+	-
BARI-Gom-22 (Sufi)	+	-	+	-
BARI-Gom23 (Bijoy)	+	-	-	+
BARI-Gom-24 (Prodip)	+	-	-	+
BARI Gom25	+	-	-	+
BARI Gom26	+	-	-	+
BARI Gom27	+	-	-	+
BARI Gom28	+	-	-	+
Westonia 5907	+	-	-	+
Westonia 5924	+	-	-	+

*(+) Presence of PCR band and (-) Absence of PCR band.

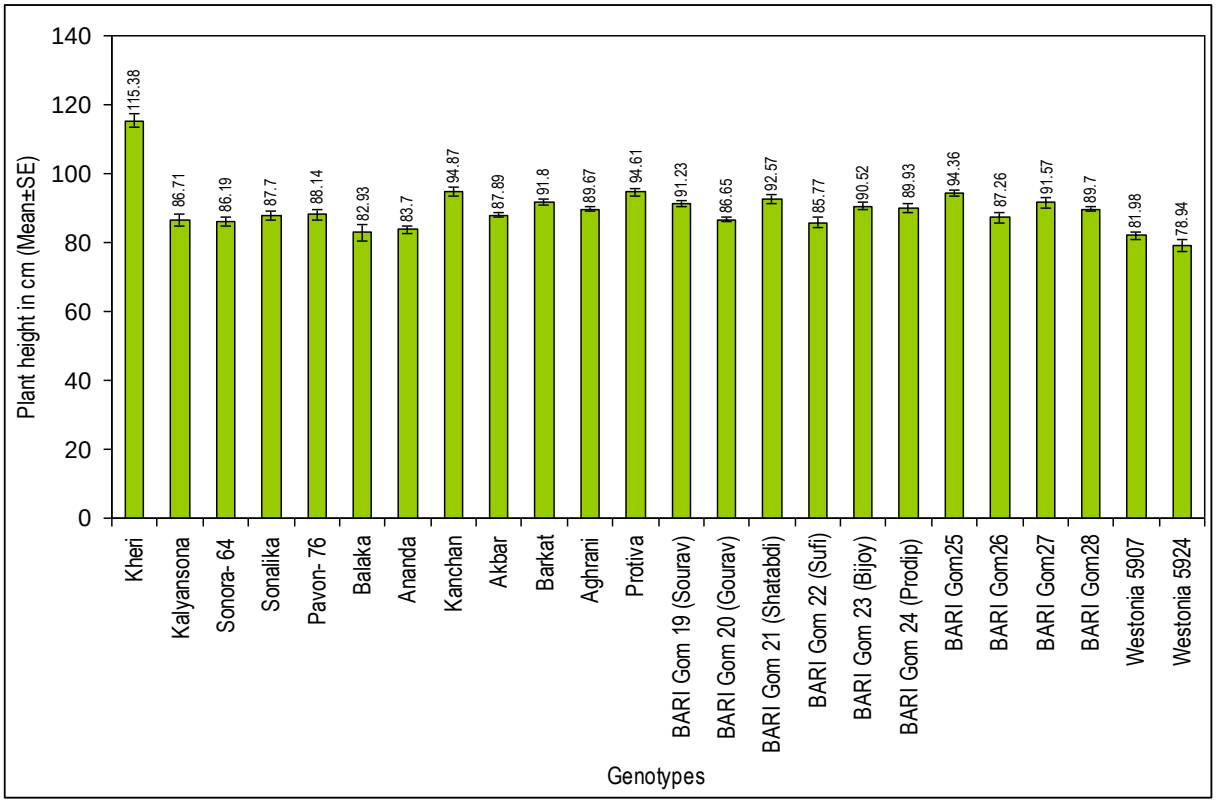

Figure 10. Distribution of wheat genotypes under study plant height in 24.

Table 5. Combination of *Rht* dwarfing alleles present in the wheat genotypes under study

Primers	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
Rht-B1b (Dwarf) BF-MR1	-	+	-	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
Rht-D1b (Dwarf) DF MR2	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+
Types	T	DD	SD	SD	DD	SD	SD	SD	DD	SD	SD	SD	SD	SD	SD	T	SD	SD	SD	SD	SD	SD	SD	SD

G = Genotype; T=Tall; SD=Semi Dwarf; DD= Double Dwarf

Taller wheat genotypes were not strong enough to support the heavy grain of the high yielding genotypes and fell over due to a process known as lodging with consequently large yield losses. But the semi dwarf plants possessed short strong stalks and did not lodge for this reasons the introduction of dwarfing genes into cereal crops was crucial to the green revolution. Genotypes carrying both dwarfing allele rendered lower grain yields in every environment (Butler *et al*., 2005). For stressed environment the semi-dwarf allele combinations seems to be the right choice for highest yields.

4.5 Heat shock protein (HSP 16.9) SNP markers

Expression patterns in heat shock protein genes have been linked with adaptation to thermal environments across a range of organisms (Hoffmann and Willi, 2008). One SNP was found between these genotypes and the analysis of amino acid sequence showed that the base transition (A/G) positioned at 31 amino acid resulted in missense mutation from aspartic acid to aspargine residue (Fig. 10). This validated SNP of 16.9 kDa HSP may serve as an informative molecular marker that can be used to improve thermo tolerance in wheat (Garg *et al.,* 2012). In another report, seven proteins were specifically expressed in heat tolerant wheat cultivar 'Fang' but not in heat susceptible cultivar 'Wyuna' after heat shock treatment. Characterization of seven proteins by tandem mass spectrometry revealed five different isoforms of 16.9 kDa HSP (Skylas *et al*., 2002). Previously rice (*Oryza sativa*) class I low-molecular mass (LMM) HSP, Oshsp16.9, has been shown to be able to confer thermo tolerance in *Escherichia coli*. The deletion amino acid residues in N-terminal domain of Oshsp16.9 led to the loss of chaperone activities (Yeh *et al.,* 2002).

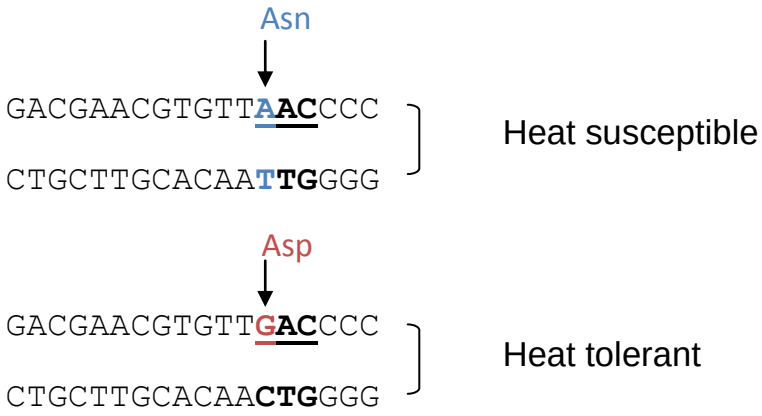

Figure 11. SNP marker in HSP 16.9 linked with terminal heat stress in wheat.

In the present study, allele specific primers based on the SNP were used to screen the wheat genotypes for their tolerance to heat stress. Thirteen of the 24 genotypes, which resulted into no PCR product (Fig. 12), were considered tolerant for heat stress (Table 6). This HSP derived SNP marker associated with terminal heat stress in wheat can be used by the breeders for improving tolerance to high temperatures in wheat breeding programmes.

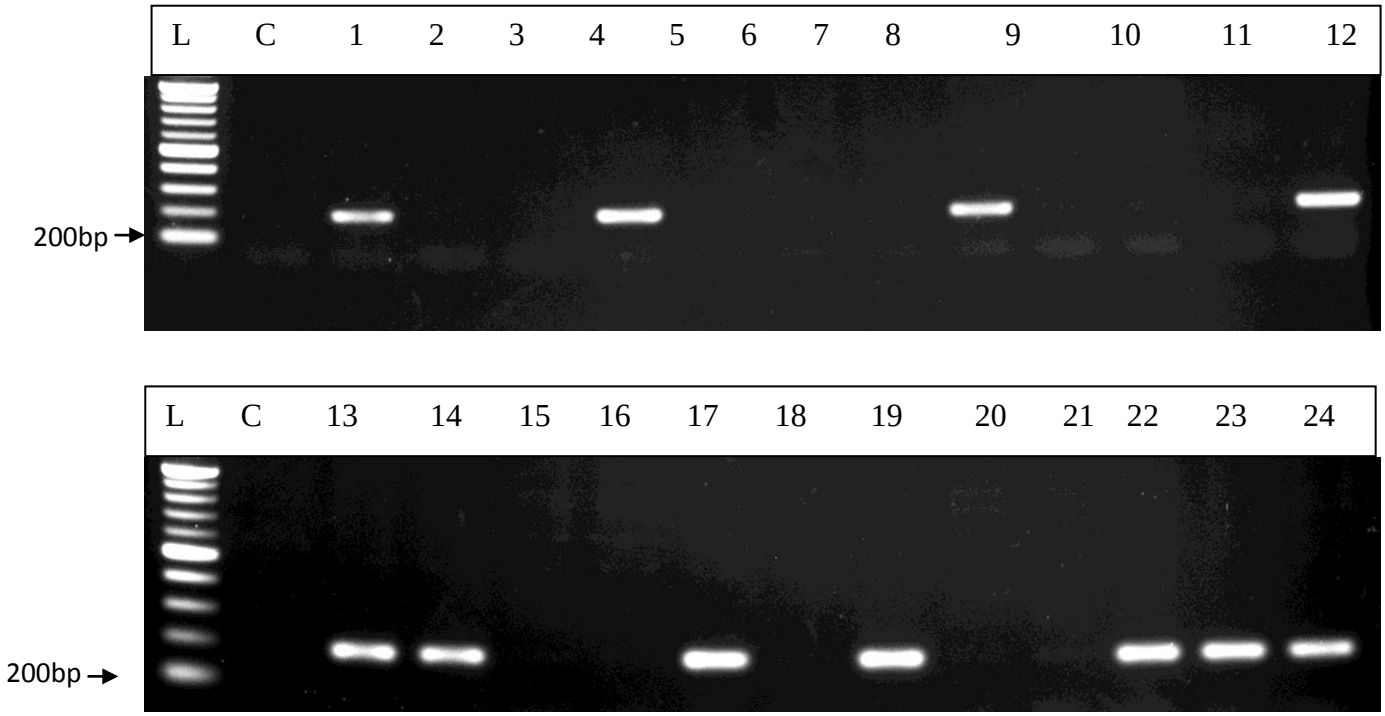

Figure 12. PCR primer pair AF-R1 analyses of 24 wheat varieties . C. negative (water) control, L. DNA ladder, 1. Kheri, 2. Kalyansona, 3. Sonora-64, 4. Sonalika, 5. Pavon-76, 6. Balaka, 7. Ananda, 8. Kanchan, 9. Akbar, 10. Barkat, 11. Aghrani, 12. Protiva, 13. BARI Gom-19 (Sourav), 14. BARI Gom-20 (Gaurav), 15. BARI Gom- 21 (Shatabdi), 16. BARI Gom- 22 (Sufi), 17. BARI Gom- 23 (Bijoy), 18. BARI Gom- 24 (Prodip), 19. BARI Gom- 25, 20. BARI Gom-26, 21. BARI Gom- 27, 22. BARI Gom- 28, 23. Westonia- 5907 and 24. Westonia- 5924.

Table 6. Heat tolerance of 24 wheat genotypes identified through SNP validation

Serial No	Genotypes	PCR	SNP	Heat Tolerance	Serial No	Genotypes	PCR	SNP	Heat Tolerance
1	Kheri	+	A	S	13	BARI-Gom-19 (Sourav)	+	A	S
2	Kalyansona	-	G	T	14	BARI-Gom-20 (Gaurav)	+	A	S
3	Sonora- 64	-	G	T	15	BARI-Gom-21 (Shatabdi)	-	G	T
4	Sonalika	+	A	S	16	BARI-Gom-22 (Sufi)	-	G	T
5	Pavon- 76	-	G	T	17	BARI-Gom23 (Bijoy)	+	A	S
6	Balaka	-	G	T	18	BARI-Gom-24 (Prodip)	-	G	T
7	Ananda	-	G	T	19	BARI Gom25	+	A	S
8	Kanchan	+	A	S	20	BARI Gom26	-	G	T
9	Akbar	-	G	T	21	BARI Gom27	-	G	T
10	Barkat	-	G	T	22	BARI Gom28	+	A	S
11	Aghrani	-	G	T	23	Westonia 5907	+	A	S
12	Protiva	-	A	S	24	Westonia 5924	+	A	S

*(+) Presence of PCR band and (-) Absence of PCR band; S – Heat susceptible, T – Heat tolerant

4.6. Observation of yield and yield components

An observation trial for recording the phenotypic characters of the wheat genotypes under study was set in the field. Data were recorded as mentioned in the material and methods section and presented in Table 7. ‘Kanchan’ showed the highest number (10.6) of effective tillers/plant where as BARI Gom-26 showed the minimum effective tillers (4.0), although they were similar in their yield capacity. ‘Akbar’ was the highest for non- effective tillers and ‘Prodip’ was the lowest. ‘Ananda’ showed the maximum number of grain per plant (379.1) where ‘BARI Gom-24’ showed minimum number of grain per plant

(134.1). ‘BARI Gom-24’ showed highest1000 grain weight (40.02 g) where ‘Pavon-76’ showed lowest 1000 grain weight (20.4 g). ‘Kanchan’ showed the highest yield per plant (10.92 g) where ‘BARI Gom-21’ showed lowest yield per plant (5.31 g). Some of the best varieties showed lower than expected performance in this trial probably due to cultural conditions or small sample size used for data recording.

Table 7. Yield and its components of the 24 wheat genotypes used in the present study.

Genotypes	Mean					
	Plant Height (cm)	No. of Total Tillers/ plant	No. of Productive Tillers/plant	Grain Number/plant	1000 Grain Weight (gm)	Yield /Plant (gm)
Kheri	115.38	11.6	10.1	324.2	22.51	7.29
Kalyansona	86.71	6.7	5.9	233.1	25.92	6.04
Sonora- 64	86.19	7.0	6.5	230.0	26.91	6.19
Sonalika	87.70	7.8	7.3	259.3	31.01	8.04
Pavon- 76	88.14	8.6	8.1	314.4	20.40	6.42
Balaka	82.93	8.5	7.7	270.4	27.06	7.314
Ananda	83.70	9.1	8.5	379.1	26.32	9.98
Kanchan	94.87	11.6	10.6	335.7	32.54	10.92
Akbar	87.89	11.6	10.4	298.3	33.82	10.09
Barkat	91.80	10.4	10	349.1	22.67	7.91
Aghrani	89.67	7.2	5.9	237	26.50	6.28
Protiva	94.61	8.0	7.3	217	33.52	7.27
BARI-Gom-19 (Sourav)	91.23	5.2	5.1	230.8	26.11	6.03
BARI-Gom-20 (Gaurav)	86.65	8.4	8.1	314.6	31.47	9.901
BARI-Gom-21 (Shatabdi)	92.57	5.1	4.8	167.8	31.66	5.312
BARI-Gom-22 (Sufi)	85.77	5.9	5.7	181.3	29.83	5.409
BARI-Gom23 (Bijoy)	90.52	5.5	5.3	154.3	34.47	5.319
BARI-Gom-24 (Prodip)	89.93	4.1	3.8	134.1	40.02	5.393
BARI Gom25	94.36	4.2	4.1	153.3	38.55	6.01
BARI Gom26	87.26	4.1	4.0	166	32.02	5.317
BARI Gom27	91.57	5.9	5.7	224.8	35.60	8.002
BARI Gom28	89.70	8.3	8.1	282.5	31.20	8.81
Westonia 5907	81.98	8.7	8.5	288.3	23.61	6.91
Westonia 5924	78.94	7.5	6.6	272.6	22.87	6.23
Mean	89.59	7.54	7.18	250.75	29. 40	7.18
LSD	7.07	4.28	4.06	12.43	4.12	0.40
CV (%)	4.63	33.30	33.64	2.91	8.23	3.19

CHAPTER 5
SUMMARY AND CONCLUSION

5.1 Summary

Wheat is one of the most important cereal crop in Bangladesh. Large area of the country where wheat is grown is vulnerable to various biotic stresses, like diseases or insects and abiotic stresses, such as heat, drought or salinity. Breeding varieties with increased tolerance to these stresses is pre-requisite for ensuring a sustainable yield. Knowledge gained about stress tolerance attributes in wheat would lead to major improvements in its production. The aim of the experiments presented here was to investigate some of the factors related to abiotic stress tolerance in wheat genotypes available in Bangladesh. The experiments were conducted in the "Biotechnology Laboratory" of Bangladesh Agricultural Research Institute (BARI), Gazipur during the period from January to June, 2014. Twenty four wheat genotypes (were characterized for the presence of rye translocations, dwarfing genes and a heat shock protein by using gene specific molecular markers. A field observation trial was also conducted for recording different yield contributing characters of the varieties.

It has been reported previously that rye translocation (1BL.1RS) in wheats provide resistance to insects, diseases and reported improvements in yield potential and water-use efficiency. Among the varieties studied, 16 were found to have this trans location from rye. However, the effects on yield and drought tolerance have been reported to be highly dependent upon genetic background and environmental conditions.

Then characterization for the presence of two dwarfing alleles (*Rht*-B1b, *Rht*-D1b) and two wild type alleles (*Rht*-B1a, *Rht*-D1a) with four pairs of gene specific primers revealed the combination of semi-dwarfing genotype in 19 genotypes. Other combinations include 2 double dwarf and 2 wild types. Semi-dwarf genotypes are considered to be the best for their high yielding character.

A heat-shock protein based marker was evaluated and 13 genotypes were found to carry the SNP mutation imparting terminal heat tolerance in wheat. This validated SNP of 16.9 kDa HSP may serve as an informative molecular marker that can be used to improve thermo tolerance in wheat.

5.2 Conclusions

Presence of rye chromatin, dwarfing alleles and heat shock protein combination can be considered for developed stress tolerance supportive genotype in wheat. On the basis of these PCR-based markers it may be concluded that Sonora-64, Balaka, Barkat, Aghrani and BARI Gom-24 (Prodip) are very good for stress tolerance and can be used for breeding stress tolerant wheat genotypes in Bangladesh. However, further studies are necessary for validating these genotypic characters with phenotypes observed in the field

5.3 Recommendations:

1. The best varities in terms of combined stress tolerance attributes were Sonora 64, Balaka, Barkat, Agrani and Prodip (BARI Gom 24).

2. PCR-based markers could be used for transferring desired stress tolerane traits alongside conventional breeding techniques.

3. Multiple stress-tolerance traits can be combined through marker-assisted pyramiding into future wheat varieties in Bangladesh.

REFERENCES

Ahmad, M and Sorrells, M. E. (2002). Distribution of microsatellite alleles linked to *Rht8* dwarfing gene in wheat. *Euphytica*. **123**: 235-240.

Bai, G., Das, M. K., Carver, B. F., Xu. X. and Krenzer, E. G. (2004). Covariation for microsatellite marker alleles associated with Rht8 and coleoptile length in winter wheat. *Crop Sci* . **44**: 1187-1194.

Bangladesh Bureau of Statistics (2014). Year book of agricultural statistics of Bangladesh. Estimates of wheat 2013-2014.

Boote, K. J., Bennet, I. M.,. Sinclair, T. R., and Paulsen, G. M. (1994). High temperature responses of crop plants. In: Physiology and Determination of Crop Yield. pp. 365–389.

Borlaug, N. E. (1968). Wheat breeding and its impact on world food supply. In: Proc. III Inter. Wheat Gen. Symp. *Aust. Acad. Sci.* pp. 1–36.

Borojevic, K. and Borojevic, K. (2005) The transfer and history of 'reduced height genes' (*Rht*) in wheat from Japan to Europe. *J. Hered.* **96**: 455-459.

Brussaard, L., Peter, C., Ruiter, D. and Brown, G. G. (2007). Soil biodiversity for agricultural sustainability. *Agric Ecosyst Environ.* **121**: 233–244.

Butler, J. D., Byrne, P. F., Mohammadi, V., Chapman, P. L. and Haley, S. D. (2005). Agronomic performance of *Rht* alleles in a spring wheat population across a range of moisture levels. *Crop Sci* . **45**: 939-947.

Calderini, D. F., Dreccer, M. F. and Slafer, G. A. (1995). Genetic improvement in wheat yield and associated traits. Re-examination of previous results and latest trends. *Plant Breed.* **114**: 108-112.

Carver, B.F. and Rayburn, A. L. (1994). Comparisons of related wheat stocks possessing 1B or 1RS·1BL chromosomes: Agronomic performance. *Crop Sci*. **34**: 1505–1510.

Chai, J. F., Zhou, R. H., Jia, J. Z. and Liu, X. (1990). Development and application of a new co dominant PCR marker for detecting 1BL-1RS wheat–rye chromosome translocations. *Plant Breed.* pp. 385–392.

Clarke, A. K. and Critchley, C. (2002). The Identification of a Heat-Shock Protein Complex in Chloroplasts of Barley Leaves. Proceedings of the fourth international wheat genetics symposium. *Alien genetic material*. **10**(5): 349-357.

Driscoll, C. J. and Sears, E. R. (1971). Individual additions of the chromosomes of "Imperial" rye to wheat. *J. Agron*. p. 6.

Elizabeth, F., McElwain and Spiker, S. (1992). Molecular and Physiological Analysis of a Heat- Shock Response in Wheat. *Plant Physiol*. **99**(4): 1455-1460.

Ellis, M. H., Bonnet, D. G. and Rebetzke, G. J. (2007). A 192bp allele at the *Xgwm261* locus is not always associated with the *Rht8* dwarfing gene in wheat (*Triticum aestivum* L.). *Euphytica*. **157**: 209-214.

Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzke, G. J. and Richards, R. A. (2002). 'Perfect' markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. *Theor. Appl. Genet.* **105**: 1038-1042.

Fick, G. N. and Qualset, C. O. (1973). Genes for dwarfness in wheat, *T. aestivum* L. *Genetics.* **75**: 531-539.

Fleury, D., Jefferies, S., Kuchel, H. and Langridge, P. (2010). Genetic and genomic tools to improve drought tolerance in wheat. *J Exp Bot.* **61**(12): 3211-22.

Friebe, B., Hatchett, J. H., Sears, R.G. and Gill B.S. (1990). Transfer of Hessian fly resistance from “Chaupon” rye to hexaploid wheat via a 2BS/2Rl wheat-rye chromosome translocation. *Theor. Appl. Genet.* **79**: 385–389.

Gale MD, Youssefian S, Russell GE (1985). Dwarfing genes in wheat. Progress in plant breeding, Butterworths and co London,United Kingdom. pp. 1–35. *Plant Physiol.* 2011 Dec; **157**(4): 1820–1831

Garg, G., Sareen, S., Dalal, S., Tiwary, R and singh, R. (2012). Heat shock protein based SNP marker for terminal heat stress in wheat (*Triticum aestivum* L.). *Aust. J. Crop Sci*. **6**(11): 1516-1521.

Graybosch, R. A. (2001). Uneasy unions: Quality effects of rye chromatin transfers to wheat. *J. Cer. Sci*. **33**: 3-16.

Gregory, P. J. (1995). Cytogenetical studies in wheat XVI. Chromosome location of a new gene for resistance to leaf rust in a Japanese wheat-rye translocation line. *J. Bot*. **91**(6): 1125-1128.

Habash, D. Z. Kehel, Z. and Nachit, M. (2010). Genomic approaches for designing durum wheat ready for climate change with a focus on drought. *J Exp Bot*. **61**(4): 1249.

Hedden, P (2003). The genes of the Green Revolution, Trends in Genetics. **19**: 5-9.

Helm, K. W. and Abernethy, R. H. (1990). Heat Shock Proteins and Their mRNAs in Dry and Early Imbibing Embryos of Wheat. *Plant Physiol.* **93**(4): 1626-1633.

Heslop-Harrison, J. S., Leitch, A. R., Schwarzacher, T and Anamthawat-Jónsson, K (1990). Detection and characterization of 1B/1R translocations in hexaploid wheat. Karyo biology Group. JI Centre for *Plant Sci. Res*. pp. 470-475.

Heyne, E. G. (1987). Wheat and Wheat improvement .2nd edition. Madison, Wisconsin, USA. pp 32-40.

Hoffmann, A. and Willi, Y. (2008). Detecting genetic responses to environmental changes. *Nature Rev Genet*. **9**:421-432.

Hossain, A. and Teixeira da Silva, J.A. (2012). Control of woodsorrel (Oxalis europaea Jord.) in wheat (*Triticum aestivum* L.) field by herbicides. *Wheat Info. Ser*. **114**: 9-16.

Hsam, S.L.K. and Zeller, F.J. (1997). Evidence of allelism between genes Pm8 and Pm17 and 17 and chromosomal location of powdery mildew and leaf rust resistance genes in the common wheat cultivar 'Amigo'. *Plant Breed*. **116**: 119-122.

Kim, W., Johnson, J. W., Baenziger, P. S., Lukaszewski, A. J. and. Gaines, C. S. (2004). Agronomic Effect of Wheat-Rye Translocation Carrying Rye Chromatin (1R) From Different Sources. *Crop Sci.* **44**:1254–1258.

Kim, W., Johnson, J. W., Graybosch, R. A. and Gaines, C. S. (2003). The effect of T1DL.1RS wheat-rye chromosomal translocation on agronomic performance and end-use quality of soft wheat. *Cer Res Commun.* **31**: 301–308.

Kirigwi F. M., Ginkel M. V., Brown-Guedira, G., Gill, B. S., Paulsen, G. M. and Fritz, A. K. (2007). Markers associated with a QTL for grain yield in wheat under drought. *Mol Breed.* **20**: 401–413.

Korzun, V., Roder, M. S., Ganal, M. W., Worland, A. J. and Law, C. N. (1998). Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). *Theor Appl Genet*. **96**: 1104-1109.

Krishnan, M., Nguyen, H.T. and Burke, J. J. (1989). Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol.* **90**:140–145.

Kumar, S., Sehgal, S. K., Kumar, U., Prasad, P. V. V and Joshi, A. K. and Gill, B. S. (2012). Genomic characterization of drought related traits in spring wheat. *Euphytica.* **186**: 265–276.

Kumlay, A.M., Baenziger, P.S., Gill K.S., Shelton D.R., Graybosch R.A., Lukaszewski A.J. and Wesenberg D.M. (2003). Understanding the effect of rye chromatin in bread wheat. *Crop Sci*. **43**:1643–1651.

Lapitan, N. L., Sears R. G. and Gill, B. S. (1984). Translocations and other karyotypic structural changes in wheat x rye hybrids regenerated from tissue culture. *Theor Appl Genet*. **68**(6): 547-54.

Lukaszewski, A. J. (1990). Frequency of 1RS·1AL and 1RS·1BL translocations in United States wheats. *Crop Sci*. **30**: 1151–1153.

Marais, G. F., Horn., M. and Toit, F. D. (1994). Intergenetic transfer (ryeto wheat) of a gene (s) for Russian wheat aphid resistance. *Plant Breed*. **113**: 265-271.

Mason, R. E., Mondal, S., B., paceco, A., Jampala, B., Ibrahim A. M. H. and Hays, D. B. (2010). QTL associated with heat sucsceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. *Euphytica.* **174**:423-436.

McKendry, A. L., Tague, D. N. and Miskin, K. E. (1996). Effects of T1BL·1RS on agronomic performance of soft red winter wheat. *Crop Sci.* **36**: 844–847.

Moreno- Sevilla, B., Baenziger, P. S., Peterson, C. J., Graybosch, R. A. and McVey, D.V. (1995). The 1BL/1RS translocation: Agronomic performance of F3-derived lines from a winter wheat cross. *Crop Sci.* **35**: 1051-1055.

Nagy, E. D., Eder, C., Molnár-Láng, M. and Lelley T. (2003). Genetic mapping of sequence-specific PCR-based markers on the short arm of the 1BL.1RS wheat-rye translocation. *Euphytica*. **132**(3):243-249.

Nevo, E. and Chen, G.(2010). Drought and salt tolerances in wild relatives for wheat and barley improvement. *Plant Cell Environ*. **33**(4):670-85.

Niu, C. F., Wei, W., Zhou, Q. Y., Tian, A. G., Hao, Y. J., Zhang, W. K., Ma, B., Lin, Q., Zhang, Z. B., Zhang, J. S and Chen, S. Y (2012). Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stresses tolerance in transgenic Arabidopsis plants. *Plant Cell Environ.* **35**(6): 1156-70.

Peng, J., Richards, D. E., Hartley, N.M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F., Sudhakar, D., Christou, P., Snape, J. W., Gale, M. D. and Harberd, N. P. (1999). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*. **400**:256–261.

Rabinovich, S. V. (1998). Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L. *Euphytica.* **100**(1-3): 323–340.

Rogowsky, P. M., Guidet, F. L. Y., Langridge, P., Shepherd, K. W and Koebner, R. M. D. (1991). Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. *Theor Appl Genet.* **82**(5): 537-544.

Salem, K., El-Zanaty A. M. and Esmail R. M. (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. *World J. Agric. Sci*., **4**(5): 538-544.

Schlegel, R. and Korzun. (1997). About the origin of 1RS.1BL wheat – rye chromosome translocations from Germany. *Plant Breed*. **116**: 537-540.

Schneider, A. and Molnár-Láng, M. (2009). Detection of the 1rs chromosome arm in Martonvásár wheat genotypes containing 1BL.1RS or 1AL.1RS translocations using SSR and STS markers. *Acta Agron. Hung.* **57**: 409–416.

Sebesta E.E., Wood E.A. jr., Porter D.R., Webster J.A., Smith E.L. (1995). Registration of Amigo wheat germplasm resistant to greenbug. *Crop Sci*. **35**: 293.

Sethi, G. S. (1989). Towards the introgression of rye genes into wheat. In: Mujeeb-Kazi A., Sitch L.A. (eds): Review of Advances in Plant Biotechnology, 1985–88.In: 2nd Int. Symposium Genetic Manipulations in Crops. *CIMMYT, IRRI*. pp. 145–156.

Singh, R. P., Huerta-Espino, J., Rajaram, S. and Crossa, J. (1998). Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. *Crop Sci.* **38**: 27–33.

Skylas, D. J., Cordwell, S. J., Hains, P. G., Larsen, M. R., Basseal, D. J., Walah, D. J., Blumonthal, C., Rathmell, W., Copeland, L. and Wrigley, C. W. (2002). Heat shock of wheat during grain filling: protein associated with heat tolerance. *J carel sci.* **35**:175-188.

Slafer, G. A., Satorre, E. H. and Andrade, F. H. (1994). Increases in grain yield in bread wheat from breeding and associated physiological changes .In: G.A.Slafer (Ed.), Genetic Improvement of field crops. pp. 1-68.

Verma V, Worland A. J, Sayers E. J, Fish L, Caligari P. D. S and Snape J. W. (2005). Identification and characterization of quantitative trait loci related to lodging resistance and associated traits in bread wheat. *Plant Breed.* **124**:234-241.

Villareal, R. L. Rajaram, S, Mujeeb-Kazi, A., Del Toro, E. (2006). The Effect of Chromosome 1B/1R Translocation on the Yield Potential of Certain Spring Wheats (*Triticum aestivum* L.). *Plant Breed.* **106**(1): 77-81.

Villareal, R. L., O. Banuelos, A., Mujeeb-kazi, S. and Rajaram, S. (1998). Agronomic performance of chromosomes 1B and T1BL.1RS, near-isolines in the spring bread wheat Seri M82. *Euphytica*. **103**: 195-202.

Wang, W. Vinocur, B. and Altman, A. (2003). Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*. **218**:1-4.

Waters, E. R. (1995). The Molecular Evolution of the Small Heat-Shock Proteins in Plants. *J. Genet.* **141**(2): 785–795.

Weng J, Nguyen H. T. (1992). Differences in the heat-shock response between thermo tolerant and thermo susceptible cultivars of hexaploid wheat. *Theor Appl Genet.* **84**:941-964.

Witcombe, J. R., Hollington, P. A., Howarth, C. J., Reader, S. and Steele, K. A. (2008). Breeding for abiotic stresses for sustainable agriculture. *Phil. Trans. R. Soc. B.* **363**(1492): 703-716.

Wojciechowski, T., Gooding, M. J., Ramsay, L. and Gregory, P. J. (2009). The effects of dwarfing genes on seedling root growth of wheat. *J Exp Bot.* **60**(9): 2565–2573.

Wojciechowski,T. (2002). Identification and chromosomal organization of two rye genome-specific RAPD products useful as introgression markers in wheat. *Genome.* pp. 157-164.

Worland, A. J., Korzun, V., Röder, M. S. and Ganal, M. W. (1998). Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the Rht8 locus of wheat

as revealed by microsatellite screening, *Theor Appl Genet*. **96**:1110-1120.

Worland, A. J., Sayers, E. J. and Korzun, V. (2001). Allelic variation at the dwarfing gene Rht8 locus and its significance in international breeding programmes. *Euphytica*. **119**:157-161.

Xiaolin, W., Gong, F., Yang, L., Hu, X., Tai, F. and Wang, W. (1990). Proteomic analysis reveals differential accumulation of small heat shock proteins and late embryogenesis abundant proteins between ABA-deficient mutant *vp5* seeds and wild-type *Vp5* seeds in maize. *Plant sci.* **5**: 801.

Yamaguchi, T. and Blumwald, E. (2005). Developing salt-tolerant crop plants: challenges and opportunities. *Tren. Plant Sci.* **10**: 615–620.

Yeh, C. H., Chen Y. M., Lin, C. Y. (2002). Functional regions of rice heat shock protein, Oshsp16.9, required for conferring thermotolerance in *Escherichia coli*. *Plant Physiol*. **128**:661-668.

Zeller, F. J. (1973). 1B/1R wheat-rye chromosome substitutions and translocations. Proceeding of the fourth International Wheat Genetics symposium genetic material. pp. 209-221.

Zhou F, Zhang, Z, Gregersen P. L, Mikkelsen J. D, de Neergaard E, Collinge D. B, Thordal-Christensen H. (1998). Molecular Characterization of the Oxalate Oxidase Involved in the Response of Barley to the Powdery Mildew Fungus. *Plant Physiol*. **117**: 33-41.

Zivy, M. (1987). Genetic variability for heat shock proteins in common wheat. *Theor Appl Genet*. **74**:209- 213.

Zoran, R., David J., Gifford, J. and Cass, D. D. (1992). Heat Shock Proteins in Two Lines of Zea mays L. That Differ in Drought and Heat Resistance. *Plant Physiol*. **99**(4): 1455–1460.