PHENOTYPIC AND MOLECULAR ANALYSIS FOR TERMINAL HEAT STRESS TOLERANCE IN WHEAT (*Triticum aestivum* L.)

By

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CERTIFICATE – I

This is to certify that this thesis entitled, "**Phenotypic and molecular analysis for terminal heat stress tolerance in wheat** ***(Triticum aestivum*** **L.)**", submitted for the degree of Doctor of Philosophy, in the subject of **Biotechnology and Molecular Biology** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Pritam Kumar** (2007BS8D) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that this thesis entitled, "**Phenotypic and molecular analysis for terminal heat stress tolerance in wheat *(Triticum aestivum* L.)**", submitted by **Pritam Kumar** (Admn. No. 2007BS8D), to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**, in the subject of **Biotechnology and Molecular Biology**, has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
AFLP	Amplified Fragment Length Polymorphism
AGE	Agarose gel electrophoresis
ASAP	Allele Specific Associated Primers
CAPS	Cleaved Amplification Polymorphic Sequence
cM	Centi Morgan
CTAB	Cetyl trimethyl ammonium bromide
CTD	Canopy temperature depression
Cv	Cultivar
DAF	DNA Amplification Fingerprinting
DAMD	Directed Amplification of Minisatellite Region DNA
DH	Double Haploid
DNA	Deoxy Ribonucleic Acid
dNTP	Deoxyribose nucleoside triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EST	Expressed Sequence Tag
GA	Genetic advancement
GCV	Genetic coefficient of variance
GFD	Grain filling duration
GFR	Grain filling rate
GPC	Grain Protein Content
GY	Grain yield
HSP	Heat shock protein
ISSR	Inter Simple Sequence Repeat Markers
ITMI	International Triticecae Mapping Initiative
KCl	Potassium Chloride
kDa	Kilo Dalton
M	Molar
MAS	Marker Assisted Selection
Mb	Megabase pair
mha	million hactare
$MgCl_2$	Megnesium Chloride
MT	Membrane thermostability
NaCl	Sodium Chloride
NIL	Near Isogenic Line
nM	nanomoles

NTSYS-PC	Numerical taxonomy and multivariate analysis system programme
PAGE	Poly-Acrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PCV	phenotypic coefficient of variance
pmoles	picomoles
PH	Plant height
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
RNA	Ribonucleic acid
Rnase	Ribonuclease
SCAR	Sequence Characterized Amplified Region
SDS	Sodium Dodecyl Sulphate
SSR	Simple Sequence Repeat
STMS	Sequence Tagged Microsatellite Site
STS	Sequence Tagged Site
Taq	*Thermus aquaticus*
TBE	Tris Boric EDTA
TE	Tris-EDTA
TGW	Thousand grain weight
Tris	Tris-hydroxymethyl amino methane
t/ha	tons per hactare
UPGMA	Unweighted pair group methods with arithmetic average
V/cm	Volts per centimeter

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CHAPTER-I

INTRODUCTION

Bread wheat (*Triticum aestivum* L. em Thell.) is an allohexaploid species with its extremely huge genome (2n = 6x = 42, approx. 16,000 Mbp). Wheat provides over 20% of calories, nearly 55% of the carbohydrate and protein in human nutrition, and is the staple food in more than 40 countries for over 35% of the world's population (Gupta *et al.,* 2009). It belongs to family *Poaceae*, the largest family within the monocotyledonous plants. It is the most widely cultivated food crop, from the southern regions of South America and Australia to the northern latitudes of Canada and China, and can grow over a wide range of elevations, climatic conditions and soil fertility (Fischer, 2011). Most wheat is grown for human food and about 10 percent is retained for seed and industry (for production of starch, pasta, malt, dextrose and gluten).

India is the second largest wheat producer in the world with production of 93.9 Mt from an area of 29.9 mha giving a productivity of 3.14 t/ha in 2011-12 (FAO, 2012). The major wheat producing countries are China, India, USA, the Russian Fedration and Australia. These five countries together contribute more than half of the global wheat production. In India, its production is highly concentrated in the northern belt of Uttar Pradesh, Punjab and Haryana, contributing 67 percent of total production. The population of India is increasing at a growth of 1.5 percent and the country would require about 109 million tons wheat by the year 2025 (DWR Report, 2007). Further, world demand for wheat by 2020 is estimated at 840 to 1000 million tons. Yield potential and yield gains are essential to meet this demand. The option for increasing wheat production by expanding area under cultivation has already been exploited to almost its maximum. Therefore, continuous efforts are required to develop high yielding wheat genotypes (Anonymous, 2011).

Wheat is one of the most broadly adapted cereals. It is cultivated in a large area in the subtropics under continual heat stress, defined as having mean daily temperature greater than 17.5 °C in the coolest month of the growing cycle (Fischer and Byerlee, 1991). Terminal heat stress largely refers to a rise in temperature at the time of grain filling duration (GFD). High temperature during grain filling is a major limitation to wheat production in many environments worldwide (Hays *et al.,* 2007; Paliwal *et al*., 2012). Heat stress is a problem in 40 % of temperate environments which covers more than 36 million ha (Reynolds *et al.,* 2001). A significant portion of the wheat grown in South Asia is considered to be affected by heat stress, of which the majority is present in India (Joshi *et al.,* 2007a). The most heat-stressed locations of South Asia are the Eastern Gangetic Plains, central and peninsular India and Bangladesh, whereas heat stress is considered moderate in northwestern parts of the Indian Gangetic Plains (Joshi *et al.,* 2007c; Singh *et al.,* 2007).

According to Hays *et al.* (2007), 32–35 °C is common in the Southern Great Plains of the US where roughly 30–40% of the country's wheat crop is grown and in the northern region of Israel where more than 70% of its wheat production is located. Furthermore, current estimates indicate that in India alone, more than 13.5 million ha of wheat growing area is heat stressed (Joshi *et al.,* 2007b). Both the proximity to the equator and the popular rice - wheat cropping system, which involve late sowing of wheat, are the major causes of exposure of wheat in India and other neighboring countries to high temperatures during grain filling (Rane *et al.,* 2007). The current trends in India indicate that the 'cool period' for wheat crop is shrinking, while the threat of terminal heat stress is increasing (Rane *et al.,* 2007; Joshi *et al.,* 2007b). Therefore, breeding for high-temperature tolerance in wheat is a major objective around the world. This need is expected to grow further in light of increased global warming (Lillemo *et al.,* 2005). Hence, it is important to incorporate late heat tolerance into wheat germplasm. Breeding for heat tolerance is still in its infancy stage and warrants more attention in future (Ortiz *et al.,* 2008; Ashraf, 2010).

High temperature stress adversely affects plant physiological processes; limiting plant growth and reducing grain yield (GY). At anthesis, high temperatures may result in pollen and anther sterility and restrict embryo development thereby reducing grain number. High temperature stress after anthesis affects the rate of grain filling, leading to reductions in GY (Al-Khatib and Paulsen,1984; Tashiro and Wardlaw, 1990). In order to adapt to high temperature stress, plants employ various physiological adaptive mechanisms such as earliness, high transpiration rate, cooler canopies, stay-green and reduced photosynthetic rates (Reynolds *et al.,* 1998). Although, significant variation for heat tolerance exists among wheat germplasm (Reynolds *et al.,* 1994; Joshi *et al.,* 2007b,c), no direct criteria are available for selection of heat tolerance. Phenotypic selection for heat tolerance has been performed using indirect selection for GFD (Yang *et al.,* 2002), thousand grain weight (TGW) and canopy temperature depression (CTD) (Reynolds *et al.,* 1994; Ayeneh *et al.,* 2002).

In recent years, molecular markers tightly linked to numerous traits of economic importance have been developed in many crops which allow indirect selection for desirable traits in early segregating generations at the seedling stage. This saves time, resources and energy that are needed not only for raising large segregating population for several generations, but also for estimating the parameter used for direct selection (Caetano and Gresshoff, 1997). Several types of molecular markers have been employed to evaluate genetic diversity and identification of QTLs in wheat, such as random amplified polymorphic DNA (RAPD) (Joshi and Nguyen, 1993), restriction fragment-length polymorphism (RFLP) (Kim and Ward, 2000), amplified fragment-length polymorphism (AFLP) (Barret and Kidwell, 1998, Burkhamer *et al.,* 1998), sequence tagged-site (STS) (Chen *et al.,* 1994), inter-simple sequence repeat (ISSR) (Devos and Galley, 1992; Nagaoka and Ogihara, 1997) and microsatellites (Roder *et al.,* 1998). Among various types of markers, use of microsatellite markers have been suggested as the most informative method as they are multiallelic, chromosome-specific and well distributed in the genome. Microsatellite markers have already been used in wheat for selecting specific genes (Peng *et al.,* 1999; Borner *et al.,* 2002), for identifying quantitative trait loci (QTLs) (Parker *et al.,* 1998) and for molecular marker assisted selection (Korzun *et al.,* 1998; Huang *et al.,* 2002). Heat tolerance trait is quantitative in nature and is controlled by a number of genes/quantitative trait loci (QTL) (Blum, 1988). Few QTLs for heat tolerance in wheat have been reported using different traits like grain filling duration, canopy temperature depression, yield (Yang *et al.,* 2002; Mason *et al.,* 2010; Pinto *et al.,* 2010; Barakat *et al*., 2012) and senescence related traits (Vijayalakshmi *et al.,* 2010).

During the past two decades, the use of marker-assisted selection (MAS) approaches has contributed greatly to a better understanding of the genetic basis of plant stress tolerance in crops, in particular tomato and maize (Liu *et al.,* 2006; Momcilovic and Ristic, 2007) and in some cases, has led to the development of plants with enhanced tolerance to abiotic stress (Lopes and Reynolds, 2010). Because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection, MAS is considered as an effective approach to improve stress tolerance. However, comparatively limited efforts have been made to identify genetic markers associated with heat tolerance in different plant species including wheat. Thus, there is an urgent need to understand the genetic factors affecting heat tolerance and to identify new diagnostic markers to be deployed in marker assisted breeding leading to improvement in grain yield of wheat under heat-stressed environments. Hence, keeping in view the importance of these aspects, the present study was planned with following objectives:

1 To raise mapping population using terminal heat stress tolerant and sensitive genotypes of wheat
2 To phenotype mapping populations for terminal heat stress tolerance
3 To identify QTLs linked to terminal heat stress tolerance in wheat

CHAPTER-II

REVIEW OF LITERATURE

Wheat is one of the most important staple food crops, which is cultivated throughout the world. It belongs to grass family *Poaceae* and genus *Triticum.* In this genus, four sets of chromosomes A, B, D and G are present (Miller, 1987). Cultivated species within *Triticum* are grouped on whether they have 14 (diploid), 28 (tetraploid) or 42 (hexaploid) chromosomes. However, most modern wheat varieties are hexaploid. Hexaploid wheat is of two types-the major group with genome AABBDD and second group is of a single hexaploid, *T. zhukovoskyi* with genome AABBGG. Main group (AABBDD) further has four subgroups-spelta, aestivum, compactum and sphaerococcum. The most important hexaploid wheat is *T. aestivum*.

T. aestivum is a major cereal crop, supplying nearly 55% of the carbohydrates consumed world-wide (Gupta *et al.*, 1999). High-temperature stress is one of the major constraints to wheat production worldwide (Rane *et al.*, 2007). In wheat, high temperature (>30°C) after anthesis can reduce the rate of grain-filling (Wardlaw and Moncur, 1995) while high temperatures imposed before anthesis can also decrease yield (Wardlaw *et al.*, 1989; Hunt *et al.*, 1991). Progress in developing cultivars that are tolerant to high temperature stress using conventional breeding approaches has been slow due to complexity of the trait and difficulty in phenotypic selection for heat tolerance. To overcome these problems and speed up the selection process, traditional breeding, along with molecular markers has been suggested as an effective strategy. The development of molecular linkage maps based on DNA markers in various crops allow dissection of genetic basis underlying complex traits such as drought, salinity and heat stress into individual components. In the present review, literature pertaining to effect of heat stress on various morpho- physiological traits and DNA markers for identification of QTLs for heat stress tolerance in wheat has been presented.

2.1. Heat stress

Heat stress is currently a documented major limitation to wheat productivity in the drier and hotter parts of the world (Fischer, 1986). Heat stress affects the metabolism and structure of plants, especially cell membrane and many basic physiological processes such as photosynthesis, respiration and water relations (Wahid *et al.,* 2007). Reduction of photosynthesis by high temperature stress appears to be a major factor that imposes source limitation to yield formation. Wardlaw and Wrigley (1994) reported that wheat yield decreases by 3 to 4% when temperature increases by 1°C above 15 °C under controlled conditions and kernel number declines by 12.5% by increasing temperature by 10 °C from

25/20°C to 35/20°C. Under high temperature, not only yield but also quality of wheat can decline (Fokar *et al*., 1998 ; Wardlaw *et al*., 2002). Yield reductions are routinely experienced by the wheat crop in the Southern Great Plains due to higher temperature during grain filling. Heat damage in these areas is manifested in reduction in kernel number, kernel weight, and grain filling duration (Hays *et al*., 2007). The GFR is the most important parameters associated with grain development, which determine the productivity of wheat genotypes (Yang and Zhang, 2006).

In general, the process of grain filling is regulated by both GFR and GFD (Wang *et al.,* 2009); however, their relative contribution remains debatable. Mashiringwani and Schweppenhauser (1992) reported that genotypic differences in grain yield of wheat were due to differences in GFR. It indicates that genetic differences in final grain weight were related to differences in GFR rather than GFD as GFD and GFR is also influenced by rise in temperature. However, GFR is generally accelerated to compensate for reduction in GFD. Under these conditions, reduction in kernel numbers might be attributed to sensitivity of pollen development to elevated temperatures. Under optimal conditions, pollen grains build up starch, storage protein and triacylglycerides that serve as the source of energy for pollen tube growth (Clement *et al*., 1994). The accumulation of starch in the pollen grains can be repressed under heat stress. Heat stress can also affect the activities of key enzymes involved in starch biosynthesis and related sucrose metabolism in wheat anthers (Dorion *et al*., 1996). Wardlaw (1974) suggested that heat stress can affect three major components of plant systems including source, sink, and transport pathways and that grain filling in wheat can be affected by the injury of these three components.

Under high temperature cellular injury occurs after a longer exposure and the plant efficiency can be severely affected. High temperature directly affects injuries such as protein denaturation and aggregation, and increased fluidity of membrane lipids (Dhanda and Munjal 2012). Other indirect or slower heat injuries involve inactivation of enzymes in chloroplast and mitochondria, protein degradation, inhibition of protein synthesis and loss of membrane integrity. Immediately after exposure to high temperature, stress related proteins are expressed as stress defense strategy of the cell. Expression of heat shock proteins (HSPs), protein with 10 to 200 kDa, is supposed to be involved in signal transduction during heat stress. Al- Whaibi (2011) indicated that HSPs protect cells from injury and facilitate recovery and survival after a return to normal growth conditions. Therefore, HSPs play an essential role in heat stress tolerance by acting as molecular chaperones (Lee and Vierling, 2000:Basha *et al*., 2004). Wahid *et al*. (2007) stated that the synthesis and accumulation of HSPs under heat stress can prevent the denaturation of other proteins.

Adaptability to heat stress can be caused by escape, avoidance or tolerance mechanisms (Blum, 1988). Plants can use these mechanisms to overcome damage due to heat stress. Leaf waxes and leaf rolling are considered mechanisms of avoidance. A waxy cuticle covers the aerial surfaces of the leaf in many plants (Chen *et al*., 2009). Heat stress causes the plants to lose more water through transpiration; therefore, the existence of epicuticular wax increases water use efficiency by decreasing cuticular transpiration and increasing the leaf boundary effects as well as decreasing leaf canopy temperature as a result of reflected solar radiation (Jefferson *et al*., 1989). Leaf rolling is an adaptation mechanism that can decrease leaf exposure to heat stress, by decreasing the number of stomata exposed and consequently transpiration. Rolled leaves are usually cooler than the straight leaves. As a result, genotypes that posses this mechanism are less affected by heat stress. Canopy temperature depression is highly suitable for selecting physiologically superior lines in warm, low relative humidity environments where high evaporative demands leads to leaf cooling of up to 10^0C below ambient temperature. This permits differences among genotypes to be detected relatively easily using infrared thermometry. Experiments done under natural field conditions have shown a close association between grain yield of wheat and CTD in hot environments (Reynolds *et al*., 1994; Fischer *et al*., 1998). Hatfield *et al*. (1984) indicated that the presence of awns in the spikes was not associated with heat tolerance. Other studies indicated the importance of awns in photosynthesis as well as of grain filling under heat stress in both wheat and barley (Ferguson *et al*., 1973; Johnson *et al*., 1974; Blum, 1986).

Although resistance to high temperature involves several complex tolerance and avoidance mechanisms, the membrane is thought to be a site of primary physiological injury by heat and measurement of solute leakage from tissue can be used to estimate damage to membranes, since membrane thermostability is reasonably heritable (Focker *et al.* 1998) and shows high genetic correlation with yield. Shanahan *et al.* (1990) suggested membrane thermostability test as useful screening procedure for selecting HT (heat tolerant) genotypes of spring wheat and explained relative injury was correlated with grain test weight than grain yield. Sikder *et al.* (1999) also suggested membrane thermostability test as suitable procedure for selecting heat tolerant and sensitive cultivars. Ibrahim and Quick (2001) assayed heat induced damage of plant membranes by membrane thermal stability on 12 days old F1 seedling of six wheat genotypes. Maternal sources effected (67%) may be important in hybrid seed and suggested heat tolerance based on membrane thermostability may be improved by using existing genetic variability available within the germplasm. Behl *et al.* (2010) conducted an experiment for the measurement of membrane thermo-stability in parents and their six F_1's at post-anthesis stage. Parents and F_1 showed significant variation for high temperature stress tolerance in late sown conditions. Genotype PBW 435 and the cross PBW 343 × PBW 435 exhibited less relative injury and greater thermotolerance possibly through maintaining cellular membrane integrity under high temperature stress. Variation in membrane thermostability of both field-acclimatized flag leaves and seedlings grown in controlled conditions was associated with heat tolerance in warm wheat-growing regions (Reynolds *et al.* 1994, Blum *et al*. 2001, Thiaw and Hall 2004).

Chlorophyll fluorescence, an indication of the fate of excitation energy in the photosynthetic apparatus, has been used indicator for heat stress tolerance. Maintaining high leaf chlorophyll content is also considered a desirable trait. It indicates a low degree of photo inhibition of the photosynthetic apparatus at high temperatures (Ristic *et al*., 2007). Studies have shown that the ability of plants to maintain leaf chlorophyll content under high temperatures stress is associated with grain yield and yield components (Ali *et al*., 2010; Yang *et al*., 2002).

2.2 Molecular markers:

The development of DNA marker technologies during the last two decades has revolutionized the genetic analysis of crop plants. Various molecular techniques have been used for construction of the physical and genetic linkage maps, assessing the genotypic profiles, genetic diversity and cultivar identification in many plants (Anand, 1999). On the basis of the principles and methods employed, molecular markers can be broadly classified in the following three groups according to Mohan *et al*., 1997; Gupta *et al*., 1999b; Gupta and Varshney, 2000):-

1. Hybridization based DNA markers such as restriction fragment length polymorphisms (RFLPs) and oligonucleotide fingerprinting.
2. PCR based DNA markers such as random amplified polymorphic DNAs (RAPDs), which can also be converted into sequence characterized amplified regions (SCARs), simple sequence repeats (SSRs) or microsatellites, sequence tagged sites (STS), amplified fragment length polymorphisms (AFLPs), inter-simple sequence repeat amplification (ISA), cleaved amplified polymorphic sequences (CAPS) and amplicon length polymorphisms (ALPs).
3. DNA chip and sequencing based DNA markers such as single nucleotide polymorphisms (SNPs).

The commonly used molecular markers for construction of the physical and genetic linkage maps are restriction fragment length polymorphisms (RFLPs) (Botstein *et al*., 1980), randomly amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), simple sequence repeats (SSRs) (Litt and Luty, 1989), amplified fragment length polymorphisms (AFLPs) (Vos *et al*., 1995), inter simple sequence repeat (ISSRs) (Zietkiewicz *et al*., 1994) etc. These molecular markers are especially useful for breeders to identify quantitative trait loci (QTL), where traits have a polygenic inheritance and variable heritability. In plants, using molecular markers, several genetic maps were initially prepared in tomato (Bernatzky and Tanksley, 1986). Subsequently, maps were constructed in different crops such as rice (McCouch *et al*., 1988, Kishimoto *et al*., 1989); maize (Burr *et al*., 1988; Beavis and Grant, 1991; Burr and

Burr, 1991), barley (Heun *et al*., 1991; Graner *et al*., 1991; Hinze *et al.* 1991) and wheat (Chao *et al*., 1989; Liu and Tsunewaki, 1991;; Devos *et al*., 1992; Devos and Gale, 1993; Röder *et al*., 1998). Wheat molecular genetic map first comprised RFLP markers (Chao *et.al.*, 1989, Devos *et.al.*, 1993) and over the time PCR based markers became the dominant marker type of genetic map construction in including RAPDs and microsatellites or SSRs (Roder *et.al.*, 1998, Pestova *et.al.*, 2000, Gupta *et.al.*, 2002)

The genomes of all eukaryotes contain a class of sequences, termed microsatellites (Litt and Luty 1989); or simple sequenced repeats (SSRs) (Tautz *et al.* 1986). Microsatellites with tandem repeats of a basic motif of <6 bp have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang *et al.* 1994).

SSR polymorphisms are based on the difference in the number of DNA repeats units at a given locus and provide a valuable source of genetic analysis. They detect a large number of alleles, level of polymorphism is high and follow Mendelian inheritance (Wu and Tanksley, 1993). The analysis of microsatellites is based on the polymerase chain reaction (PCR), which is much easier to perform than RFLP analysis and is highly amenable to automation.

Microsatellites show much higher level of polymorphism and informativeness in hexaploid bread wheat than other marker system (Plaschke *et al*, 1995; Roder *et al*, 1995). It has been studied that in wheat, (GA)n/(GT)n are found every 270kb of DNA, (AC)n at 292 kb and (AG)n at every 212 kb approximately (Ma *et al*, 1996). Roder *et al*. (1998) reported the random distribution of SSR loci over different chromosomes.The SSR markers have been used to develop linkage maps in hexaploid wheat by several workers (Table 2.1).

Linkage mapping of QTLs for thermotolerance in wheat

In general, the inheritance of traits can be classified into two types: qualitative and quantitative. Qualitative (or Mendelian) inheritance is observed when a trait or observable characteristic (such as color, shape, and height) is largely determined by only one or a few major loci or genes. Thus, phenotypes show discrete variation and provide information about the underlying genotypes. In contrast, quantitative (or complex) inheritance is determined by many factors or genes of varying effects. Thus, phenotypes show continuous variation, yield, some disease and insect resistance, abiotic stress tolerance, quality traits, and other traits of agronomic importance for example. Due to their importance, quantitative traits have been the subject of genetic studies for over a century (Fisher, 1918). Two common approaches are used to genetically dissect traits that show quantitative inheritance. One is quantitative trait loci (QTL) mapping using mapping populations segregating for the traits of interest. Another is association mapping or linkage disequilibrium mapping using a population of well chosen lines, accessions, or cultivars showing variation for the traits of interest.

QTL mapping in plants is usually conducted in a segregating experimental population derived from a cross between phenotypically different parental lines, such as F2, F_3, F_4, backcross (BC), recombinant inbred line (RIL), recombinant substitution line (RSL), doubled-haploid (DH) or near-isogenic line (NIL) populations (Kearsey and Farquhar, 1998). F2, F3 and F4 populations are frequently used for linkage map construction because they are easier to produce in a short time. RIL, RSL, and DH populations are immortal mapping populations and useful for QTL analysis because a large number of plants of each homozygous line allow replication (Varshney *et al.,* 2006). Of these, RILs produce robust genetic mapping information.

A suitable mapping population having a reasonable sample size and segregating for phenotypic traits is essential for the construction of a saturated genetic map and QTL analysis. It is critical to select parental lines with genetic diversity for the trait(s) of interest. This will enhance the possibility of identifying a large set of polymorphic markers covering the whole genome. In general, the larger the population size, the higher the mapping resolution and the more accurate and reliable the QTL detection (Liu, 1998; Mohan *et al.,* 1997). A study of the effects of population size on QTL mapping in barley reported that the number of QTL detected increased with increasing population size (Vales *et al.,* 2005). Even though major QTL can be identified in a small population, minor QTL are under detection. Thus, one advantage of a large population is the ability to detect minor QTL. However, population size is often governed by practical concerns as well as the cost of genotyping and phenotyping.

QTL mapping can be defined as the molecular marker-facilitated genetic dissection of the variation of complex phenotypes. The aim of QTL mapping is to identify chromosomal regions that affect the quantitative trait of interest, and to estimate the effect of QTL on the trait. In general, QTL analysis is performed with a well-developed genetic linkage map and reliable phenotypic data from a mapping population. The power of QTL mapping and the accuracy of QTL estimation mostly depend on the selection of two parents, type and size of the mapping population, marker density, genome coverage of the genetic linkage map, quality of phenotypic trait data, and statistical method used.

Recent mapping studies have been dominated by SSRs owing to their highly polymorphic nature, abundance, co-dominant inheritance and reproducibility (Roder *et al.,* 1995; Prasad *et al.,* 2000). Success in using information about QTLs to increase genetic gain depends greatly on the magnitude of QTL effects, precise estimation of QTL positions, stability of QTL effects across multiple environments, and whether QTLs are robust across relevant breeding germplasm. Prediction of QTL positions is enhanced by further fine mapping, which facilitates testing QTL effects and breeding values in additional populations. When the density of observed recombinations approaches the resolution of single genes, the causal genetic change for a QTL can be determined (Salvi and Tuberosa, 2005; Harjes *et al.*, 2008). Molecular isolation of QTLs permits the development of perfect or functional molecular markers at the potential resolution of the fundamental unit of inheritance, the nucleotide, and dramatically increases the specificity and precision by which genetic effects are estimated and manipulated in breeding programs.

Genetic linkage maps are powerful tools for many studies such as gene tagging, genome characterization, QTL analysis, evolutionary studies, and marker development for MAS (Chu *et al.,* 2010). Tanksley (1993) suggested that molecular markers could help breeders to track genetic loci without extensive, expensive, and time consuming field trials. This will not negate the need for field work but would rather reduce cost, increase breeding efficiency, and allow selection for heat-stress tolerance and other traits as well. These QTL were used by Ottaviano *et al.* (1991) to understand and delineate heat stress tolerance in cereals. Grain yield in cereals is generally controlled by a number of QTL and is highly affected by the environment, making it hard to make large gains in yield improvement (Kato *et al.,* 2000). Heat stress tolerance is a quantitatively inherited and normally distributed trait (Blum, 1988; Yang *et al.,* 2002). Therefore, determining the physiological mechanisms associated with heat tolerance and finding QTL associated with these mechanisms might be vital for heat tolerance in wheat breeding program. Most of the traits related with the yield and heat tolerance are controlled by several genes each with minor individual but significant effects when acting together. QTL mapping allows assessment of numbers, locations, magnitude of phenotypic effects, and patterns of gene action (Vinh and Paterson, 2005). However, several QTLs have been identified in wheat for heat tolerance during the reproductive phase. For instance, Yang *et al.* (2002b) analyzed F_2 and F_3 mapping population raised from a cross between Ventnor (heat tolerant) and Karl 92 (heat susceptible) cultivars of wheat using microsatellite (SSR) markers. Grain-filling duration (GFD) at 30/25 °C 16/8 h day/night was determined as a measure of heat tolerance. Out of 59 SSR primer pairs which were polymorphic, two markers viz., Xgwm 11 and Xgwm 293 were found to be located on the short arms of chromosomes 1B and 5A linked to grain filling duration by QTL analysis. In wheat, the quantitative trait loci (QTLs) analysis has, partly, been hindered by large genome size (Bennett *et al.,* 1982). The polyploid nature of the genome also makes molecular analysis complicated (Barnaba *et al.,* 2008) due to repetitions of DNA sequences.

Table 2.1: Molecular maps of wheat (*Triticum* spp.) using SSR markers

No.	Genome	Type of marker	No. of mapped loci	Mapping population	Reference
1	Bread wheat	RFLP, SSR	264, 2	Chinese Spring x Courtot DH population	Cadalen *et al.* (1997)
2	Bread wheat	STMS	279	'Opata85' x 'W7984'	Roder *et al.* (1998)
3	Bread wheat	SSR	53	'Chinese Spring' x 'Synthetic'	Stephenson *et al.* (1998)
4	Bread wheat	AFLP, SSR	620,42	'Garnet x 'Saunders' DH population	Penner *et al.* (1998)
5	Bread wheat	RFLP, Microsatellites	230	*T. aestivum* var 'Forno' x *T. spelta* 'Oberkulmer'	Messmer *et al.* (1999)
6	D-genome	STMS	65	'Opata85' x 'W7984'	Pestsova *et al.* (2000)
7	Durum wheat	RFLP, AFLP, SSR, Storage Proteins	306	'Jennah' x 'Khetifa'	Nachit *et al.* (2001)
8	Bread wheat	SSR	66	'Opata85' x 'W7984'	Gupta *et al.* 2002
9	Bread wheat	SSR	126	'Opata85' x 'W7984'	Nicot *et al.* (2004)
10	Bread wheat	SSR	48	'Opata85' x 'W7984'	Peng and Lapitan (2005)
11	Bread wheat	SSR	1406	'Opata85' x 'W7984'	Song *et al*. 2005
12	Bread wheat	EST -SSR	122	Svevo × Ciccio	Gadaleta *et al* (2009)

Kirigwi *et al*. (2007) found that QTL linked to grain yield, GFR, spike density, biomass production and drought susceptibility index (DSI) under drought stress conditions in a spring wheat population were on the proximal region of chromosome 4AL. Microsatellite locus Xwmc89 was associated with all significant QTLs covering a 7.7

centiMorgans (cM) region and generally explained the greatest proportion of phenotypic variation.

Mohammadi *et al*. (2008) characterized and mapped quantitative trait loci controlling heat tolerance in wheat, 144 recombinant inbred lines deriving from the cross of Kauz and MTRWA116 were assessed in a greenhouse and growth chamber at 35˚C. One hundred and sixty six SSR and 3 AFLP markers were used to construct a linkage map containing 18 linkage groups and covering 16 chromosomes of wheat. Using the composite interval mapping method, three QTLs were detected for heat tolerance and measured by the Fischer susceptibility index, on chromosomes 1B, 5B and 7B. The alleles of both parents contributed to heat tolerance. Kumar *et al.* (2010) identified three QTLs for stay green on the chromosomes 1AS, 3BS and 7DS using a recombinant inbred (RI) population developed by making crosses between the stay green parent 'Chirya 3' and non-stay green 'Sonalika'. Likewise,Vijayalakshmi *et al.* (2010) identified QTLs for senescence-related traits.

Pinto *et al.* (2010) identified a QTL on chromosome 4A-a for canopy temperature depression under heat stress. In addition to QTLs for senescence/stay-green traits, several have been mapped for wheat yield and its related traits under heat stress. For example, under heat stress a QTL at the same location as the QTL for canopy temperature accounted for 17% of yield variation (Pinto *et al*., 2010). Likewise, Mason *et al.* (2010) identified 15 and 12 QTLs associated with yield and its associated traits in 2005 and 2006, respectively. The results suggest that for heat tolerance main spike should be used for the identification of QTLs genomic regions Mason *et al.,* 2010). Barakat *et al.* (2011) by quantitative trait loci (QTL) analysis of the F_2 population of cross between Debra (heat-tolerant) and Yecora Rojo (heat-sensitive) in Saudi Arabia reported that three SSR markers; *Xgwm132, Xgwm577* and *Xgwm617* were linked to grain filling rate (GFR). The results showed that regression analysis for the relationship between the three markers (*Xgwm132*, *Xgwm577* and *Xgwm617*) and the phenotypes of F_2 plants were highly significant and the coefficients of determination (R^2) were 0.07, 0.25 and 0.03, respectively. This indicates that these three markers were associated with the grain filling rate as indicator for heat tolerant genes. The adjusted R^2 values suggested that the *Xgwm132*, *Xgwm577* and *Xgwm617* accounted for 7%, 25% and 3% of the total phenotypic variation of heat tolerance in the F_2 population, respectively.

Bulked segregant analyses (BSA) was used in conjunction with simple sequence repeats (SSR) analysis to find markers linked to genes of heat tolerance. Composite interval mapping was used for mapping quantitative trait loci (QTL). The results revealed that 12 SSR markers: *Wmc24, Wmc168, Wmc326, Xgwm30, Xgwm456, Wmc25*, *Wmc44*, *Wmc94*, *Wmc161*, *Wmc273*, *Wmc327* and *Xgwm566* were linked to GFR by QTLs analysis of the F_2 population (Barakat *et al.* 2012). Paliwal *et al.* (2012) recorded four different traits (1) heat susceptibility index (HSI) of thousand grain weight (HSITGW), (2) HSI of grain fill duration (HSIGFD), (3) HSI of grain yield (HSIYLD) and (4) canopy temperature depression (CTD) and these were used to determine heat tolerance. Days to maturity was also investigated. A linkage map comprising 160 simple sequence repeat markers was prepared covering the whole genome of wheat. Using composite interval mapping, significant genomic regions on 2B, 7B and 7D were found to be associated with heat tolerance.

CHAPTER - III

MATERIAL AND METHODS

The present investigation was conducted to study the phenotypic and molecular analysis of terminal heat stress tolerance in wheat *(Triticum aestivum* L. em Thell) in the Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University.

3.1 Materials

3.1.1 Plant material

Seeds of two thermotolerant (Hindi62 and DBW16) and two thermosensitive (WH711 and WH147) varieties of wheat were procured from Wheat Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar and used in the study.

The pedigree of the parental genotypes involved in crosses is given in Table 3.1

Table-3.1: Pedigree of Parental genotypes involved in two wheat crosses

Genotype	Pedigree
Hindi62	KENYA48F/L1/E144//N245.44-25
WH711	ALD'S'HUAC//HD2285/3/HFW17
DBW16	RAJ 3765/WR 484//HUW468
WH147	E.4870 -C-303/S339 PV18

3.1.2 Glassware and Plasticware

Glassware used was of the borosilicate quality and obtained from Borosil Glass Works Ltd., Mumbai or Corning Glass Company, USA. Disposable PCR tubes were procured from Tarsons Products Pvt. Ltd., India. Centrifuge tubes were obtained from Fisher Scientific International Co., USA.

3.1.3 Chemicals

Taq DNA polymerase, magnesium chloride and PCR buffer (10X) were obtained from Life Technologies Pvt. Ltd. India. dNTPs and standard molecular weight markers (100 bp DNA ladder) were obtained from Labmate Pvt Ltd. India. All other chemicals used in the present investigations were of Molecular biology grade and procured from Sigma Chemicals Co™. USA, Life Technologies Pvt. Ltd. India and SISCO Research Laboratories, India.

3.1.4 Primers

A total of 103 SSR primers distributed throughout the wheat genome, 25 RAPD and two known gene primers (*hsp101b* and *hsp16.9*) were used in the present investigation. Primers were got synthesized from Sigma Aldrich chemical Pvt. Ltd. A brief description of SSR and known gene primers used in the study is given in Table 3.2 and RAPD primer sequences are shown in Table 3.3.

3.2 Methods

3.2.1 Raising mapping populations

Two F_2 mapping populations comprising 90 plants each involving crosses between Hindi62 (heat tolerant) x WH711 (heat susceptible) and DBW16 (heat tolerant) x WH147 (heat susceptible) were developed. . In 2008-2009 winter season all the parental wheat genotypes were sown on 20th November 2008 and the crosses were made by hand. F_1 seeds were sown on 26th November 2009 to produce the F_2 mapping population. In the next season, these seeds were harvested and sown on 28th November 2010 to obtain F_2 plants for phenotypic and molecular analysis. F_2 mapping populations of both crosses viz. Hindi62 x WH711 and DBW16 x WH147 consisted of 90 plants each. F_3 seeds from individual F_2 plants were harvested and sown in two environments, normal sown (30th November 2011) and late sown (26th December 2011) to get F_3 mapping population.

3.2.3 Phenotyping mapping populations

For phenotyping, F_3 mapping populations obtained from two crosses viz. Hindi62 x WH711 and DBW16 x WH147 were sown in two environments, normal sown (30th November 2011) and late sown (26th December 2011). Heat stress was created by late sowing the crop. Data was recorded on individual plant basis in both the crosses in respect of characters viz. membrane thermostability index, chlorophyll stability index, grain yield, kernels/spike, thousand kernel weight (Test weight), days to heading, days to maturity, plant height, number of tillers/plant, biomass/plant and harvest index. These traits were recorded as follows:

Membrane thermostability index

For leaf membrane thermostability index, 2g of freshly harvested leaves were taken and immersed in a test tube containing 10 ml of distilled water at 35ºC for 4 h. EC of this solution was measured using EC meter (Digital conductivity meter NDC 732) and designated EC_1. The leaves were again shifted to 20 ml test tube containing 10 ml of distilled water and subjected to boiling temperature (100 ºC) for 30 min in water bath. After cooling the EC of the solution was again measured and designated as EC_2. Membrane thermostability index was calculated using the following equation:

Table 3.2: A brief description of SSR and known gene primers used in the present investigation

Sr. No	Primer name	Forward primer (5' - 3')	Reverse primer (5' -3')	Choromosom -al position	Annealing temperature (^{0}C)
1	Xgwm 95	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	2A	60
2	Xgwm 261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	2D	55
3	Xgwm 458	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	1D	60
4	Xgwm 111	TCTGTAGGCTCTCTCCGACTG	ACCTGATCAGATCCCACTCG	7D	55
5	Xgwm 617	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	5A	60
6	Xgwm 46	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	7B	60
7	Xgwm 186	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	5A	60
8	Xgwm 539	CTGCTCTAAGATTCATGCAACC	GAGGCTTGTGCCCTCTGTAG	2D	60
9	Xgwm 389	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	3B	60
10	Xgwm 455	ATTCGGTTCGCTAGCTACCA	ACGGAGAGCAACCTGCC	2D	55
11	Xgwm156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	6A	60
12	Xgwm 311	TCACGTGGAAGACGCTCC	CTACGTGCACCACCATTTTG	2A, 2B, 2D, 6B	60
13	Xgwm604	TATATAGTTCAATATGACCCG	ATCTTTTGAACCAAATGTG	5B	50
14	Xgwm 610	CTGCCTTCTCCATGGTTTGT	AATGGCCAAAGGTTATGAAGG	4A	60
15	Xgwm 428	CGAGGCAGCGAGGATTT	TTCTCCACTAGCCCCGC	7D	60
16	Xgwm382	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	2D	60
17	Xgwm 356	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	2A	55
18	Xgwm 388	CTACAATTCGAAGGAGAGGGG	CACCGCGTCAACTACTTAAGC	2B	60
19	Xgwm3	GCAGCGGCACTGGTACATTT	AATATCGCATCACTATCCCA	3D	55
20	Xgwm 18	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	1B	50
21	Xgwm 165	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	4A, 4B, 4D	60
22	Xgwm 102	TCTCCCATCCAACGCCTC	TGTTGGTGGCTTGACTATTG	2D	60
23	Xgwm 190	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	5D	60
24	Xgwm 526	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	2A	55
25	Xgwm 157	GTCGTCGCGGTAAGCTTG	GAGTGAACACACGAGGCTTG	2D	60
26	Xgwm 458	AATGGCAATTGGAAGACATAGC	TTCGCAATGTTGATTTGGC	1D	60
27	Xgwm 400	GTGCTGCCACCACTTGC	TGTAGGCACTGCTTGGGAG	7B	60
28	Xgwm 132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCCTTCCCC	6B, 6D	60
29	Xgwm193	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTTGGGG	6B	60

Sr. No	Primer name	Forward primer (5’ - 3’)	Reverse primer (5’ -3’)	Choromosom-al position	Annealing temperature (^{0}C)
30	Xgwm 33	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	1A	60
31	Xgwm 155	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	3A	60
32	Xgwm 630	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA	2B	60
33	Xgwm 513	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	4B	60
34	Xgwm 5	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	3A	50
35	Xgwm 296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	2D, 7D	55
36	WMC 48	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	4B	61
37	Wmc105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	4B	61
38	WMC 120	GGAGATGAGAAGGGGGTCAGA	CCAGGAGACCAGGTTGCAGAAG	1A	61
39	WMC 169	TACCCGAATCTGGAAAATCATT	TGGAAGCTTGCTAACTTTGGAG	3A	61
40	WMC 170	ACATCCACGTTTATGTTGTTGC	TTGGTTGCTCAACGTTTACTTC	2A	61
41	WMC 215	CATGCATGGTTGCAAGCAAAA	CATCCCGGTGCAAACTCTGAAA	3A, 5A, 5D	61
42	WMC 382	CATGAATGGAGGCACTGAAACA	CCTTCCGGTCGACGCAAC	2A, 2B	61
43	WMC 243	CGTCATTTCCTCAAACACACCT	ACCGGCAGATGTTGACAATAGT	2B	61
44	Xbarc188	CGTGAGATCATGTTATCAGGACAAG	GCGTTGAAAGGTGTTAGTGGGATGG	1B	58
45	Xbarc136	GCG AGC TCA CTG CAC ACT TAC CC	GCA ACG CAC CTT GAT AAT C	6B	52
46	BARC19	GCGACCCGAGTAGCCTGAA	GGTGGACCATTAGACGCTTACTTG	3A	62
47	BARC26	GCGCTGGGTAAAAAGTGAAATTC	TGCAAGTGGAGGGGGAGGCGAGAG	7D	53
48	BARC28	CTCCCCGGCTAGTGACCACA	GCGGCATCTTTCATTAACGAGCTAGT	5D	55
49	BARC45	CCCAGATGCAATGAAACCACAAT	GCGTAGAACTGAAGCGTAAAATTA	3A	52
50	BARC48	GCGAGCTGCAGAGGTCCATC	GCGTTAGTCTTCTTGGTCAATCAC	4D	55
51	BARC113	GCGCACAACAACGGACACTTAACAATT	GGGACTCATTTAGCTTCTACTCGCCATTA	6A	50
52	BARC133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	3B	50
53	BARC187	GTGGTATTTCAGGTGGAGTTGTTTTA	CGGAGGAGCAGTAAGGAAGG	1B	55
54	BARC236	GCG AAA ATT GTC ACC CTT ATC CAG TA	CGT TCG TAC ACA CCA TAG TCA CTT C	4A	50
55	BARC263	GGAAGCGCGTCAGCACTAGGCAAC	GGCTTCTAGGTGCTGCGGCTTTTGTC	1A	55
56	BARC275	GCG TTT GGT CAG AAT AGG GAA GAT	GCG TAT GTT CGT GTT AGT GTT GGT TAT GC	7A	50
57	BARC288	GGG TTT TGC TTG GTT GAC A	CGG GAC GAT TTT ATT TAG GAG T	4D	52
58	BARC297	GCG TAG GAG AGA TGC CCC AAA GGT T	GCG TGC GGA CTC GTG AAT CAT TAC A	2D	50
59	BARC344	GCG CGT CGA CAT GTA TTT CTT GAT	GCG TTT CAT CTG GTA TCT GGT GTA T	3B	52

Sr. No	Primer name	Forward primer (5’ - 3’)	Reverse primer (5’ -3’)	Choromosom -al position	Annealing temperature (^{0}C)
60	BARC350	GCA CCG CAC AAG ATT ACA	GCC CAA GGA GAG ATT ATT AGT T	4A	50
61	BARC359	GCG TCC GCC GAT GAA CTT CTG	GCG ACC CAA TGG AAT GTA TGT AAC	3A	58
62	BARC1005	CGC GTT TGC CTC TCT TGT GCT ATA C	CGC GAG ATA CCC GAA AGT TTT GAT	7A, 7B	53
63	CFA2104	CCTGGCAGAGAAAGTGAAGG	AGTCGCCGTTGTATAGTGCC	5A, 5D	60
64	CFA2262	ACAATGTGGAGATGGCACAA	TACCAGCTGCACTTCCATTG	2D, 3A	60
65	CFA2292	GGACCGTTTATCCGTAAGCA	GCCTATGCTGCTGATCCATT	1B	60
66	CFD233	GAATTTTTGGTGGCCTGTGT	ATCACTGCACCGACTTTTGG	2D	58
67	CFD239	CTCTCGTTCTCTCCAGGCTC	GAGAGGAGAGCTTGCCATTG	2D	61
68	WMC41	TCCCTCTTCCAAGCGCGGATAG	GGAGGAAGATCTCCCGGAGCAG	2D	59
69	WMC110	GCAGATGAGTTGAGTTGGATTG	GTACTTGGAAACTGTGTTTGGG	5A	60
70	WMC134	CCAAGCTGTCTGACTGCCATAG	AGTATAGACCTCTGGCTCACGG	1B	62
71	WMC149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG	2B, 2D, 5B	60
72	WMC232	GAGATTTGTTCATTTCATCTTCGCA	TATATTAAAGGTTAGAGGTAGTCAG	4A, 4B	57
73	WMC254	AGTAATCTGGTCCTCTCTTCTTCT	AGGTAATCTCCGAGTGCACTTCAT	1A, 4B	61
74	WMC296	GAATCTCATCTTCCCTTGCCAC	ATGGAGGGGTATAAAGACAGCG	2A	63
75	WMC349	ACACACACTCGATCGCAC	GCAGTTGATCATCAAAACACA	4B	60
76	WMC407	GGTAATTCTAGGCTGACATATGCTC	CATATTTCCAAATCCCCAACTC	2A	61
77	WMC413	CACTGGAAACATCTCTTCAACT	ACAGGAAAGGATGATGTTCTCT	4B	58
78	WMC416	AGCCCTTTCTACCGTGTTTCTT	TATGGTCGATGGACTGTCCCTA	1B, 6D	62
79	WMC398	AAGCGCACTTAACAGAAGAGGG	CGTGAGAGCGGTTCTTTG	6A	58
80	WMC475	AACACATTTTCTGTCTTTCGCC	TGTAGTTATGCCCAACCTTTCC	5A, 7B	62
81	WMC500.1	ATAGCATGTTGGAACAGAGCAC	CTTAGATGCAACTCTATGCGGT	6A	53
82	WMC601	ACAGAGGCATATGCAAAGGAGG	CTTGTCTCTTTATCGAGGGTGG	2D	61
83	WMC719	TTGTGGGAATCTACATCAGAAGG	AACAGCCACGCTCTATCTTCAGT	1B	61
84	WMC727	CATAATCAGGACAGCCGCAC	TAGTGGCCTGATGTATCTAGTTGG	5A	61
85	WMC758	TAGGGGAGGCGACGGAG	GTTGCTGGAGAGTGGATTGC	7B	61
86	WMC766	AGATGGAGGGGATATGTTGTCAC	TCGTCCCTGCTCATGCTG	1B	61
87	WMC819	GATTCGGTCGGTTGGCTAAG	GTTTGTGGTGGGTGGATTGC	2A	61
88	WMC827	ACGGTGACCTCAGTGCTCAC	ATGCTTGCCTCAGCAAAACC	3B	52
89	Xgwm219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	6B	60
90	Xgwm443	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	5A	55

Sr. No	Primer name	Forward primer (5’ - 3’)	Reverse primer (5’ -3’)	Choromosom -al position	Annealing temperature (^{0}C)
91	Xgwm515	AACACAATGGCAAATGCAGA	CCTTCCTAGTAAGTGTGCCTCA	5A	55
92	WMC642	ACGGCGAGAAGGTGCTC	CATGAAAGGCAAGTTCGTCA	1D	60
93	Xcfd223	CATGCATGGTTGCAAGCAAAA	CATCCCGGTGCAAACTCTGAAA	3D	58
94	Xgwm 261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	2D	55
95	Xgwm484	TGCTGCTACTTGTACAGAGGAC	CCGAATTGTCCGCCATAG	2D	61
96	WMC25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	2B	51
97	WMC44	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	1B	61
98	WMC94	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA	7D	51
99	WMC161	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	4A, 5D	61
100	WMC215	CATGCATGGTTGCAAGCAAAAG	CATCCCGGTGCAACATCTGAAA	2B	61
101	WMC273	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	7D	51
102	WMC327	TGCGGTACAGGCAAGGCT	TAGAACGCCCTCGTCGGA	5A	61
103	Xgwm566	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	3B	60

Known gene primers used in the present investigation

1	*Hsp101b*	TGGAGAGGAAGCGGATTC	CTGCTTCAGCTTCCGGAT		60
2	*Hsp16.9*	CAGCAATCAACACCACGATG	TGCCACTTGTCGTTCTTGTC		60

Table 3.3: A brief description of RAPD primers used in the present investigation

Sr. No.	Primer	Sequence 5'- 3'
1	RAPD-2	TTCGAGCCAG
2	RAPD-3	ACTCGCATCC
3	RAPD-4	GGCACTGAGG
4	RAPD-5	CCCCGATGGT
5	RAPD-6	CCACAGCAGT
6	RAPD-7	CCTGGGCTTC
7	RAPD-8	CCTGCGCTTA
8	RAPD-9	GGGCCGTTTA
9	RAPD-10	CCCGCCTTCC
10	RAPD-11	CCGGCCTTAA
11	RAPD-13	CCGGCCTTCC
12	RAPD-14	CTACCCGTGC
13	RAPD-15	TTCCCGGAGC
14	RAPD-16	GAGGTCCAGA
15	RAPD-17	GGGCCCGAGG
16	RAPD-18	GGGGGCTTGG
17	RAPD-20	GATGACCGCC
18	RAPD-45	CCCAGTCACT
19	RAPD-46	CCACGGGAAG
20	RAPD-47	CAGCACTGAC
21	RAPD-49	TCCCACGCAA
22	RAPD-58	GGCTTATGCC
23	RAPD-59	TGCGCCCTTC
24	RAPD-60	GTCCACACGG
25	RAPD-61	GTTGGTGGCT

$$\text{Membrane thermostability index} = 1\text{- \% injury} = 1- \frac{EC_1}{EC_1 + EC_2} \times 100$$

Chlorophyll stability index:

Chlorophyll stability index of the population under study was recorded by using chlorophyll fluorescence meter (Model OS-30p, manufactured by Opti-Sciences, Inc., Hudson, USA). Chlorophyll fluorescence meter works on continuous excitation fluorescence measurement principle. The fully expanded leaves were first acclimated to the dark for 2 min by fixing clips. The dark adapted samples were continuously irradiated for 1 s, provided by an array of three light emitting diodes in the sensor. The fluorescence signals were detected as F0, Fm and Fv/Fm after passing through the long pass filter.

Days to heading (DH):

Heading date for each plant was recorded using the Julian scale, in the field as the day when 50% of the heads had emerged from the flag leaf sheath. The date on which the first ear emerges from the flag leaf was recorded and numbers of days were calculated from date of sowing to date of ear emergence.

Days to maturity (DM)

Number of days from date of sowing to mature heads was counted.

Number of Kernels per spike (NKS)

The numbers of grains of three spikes/plant were counted and the mean number of grains per spike was calculated.

Number of tillers per plant (NTP)

The ear bearing tillers per plant were counted at the time of harvest.

1000-Kernel weight (Test weight)

A random sample of 1000-grains was taken from three plants/ and their weight was recorded in grams.

Plant height (PH)

Plant heights were evaluated by measuring the distance, in centimeters, from the soil surface to the estimated average height of two or three main tillers, excluding the awns from each cross.

Grain yield per plant (GY)

All the spikes of individual plant at maturity were threshed and grains were weighed in grams on an electrical balance.

Biomass/ Plant

Plants of both the crosses were harvested, dried at 70^0 C for 3 days and weighed by using digital weighing machine.

Harvest index

Harvest index of the population under study was calculated by using following formula

$$\frac{\text{100 X Seed dry mass (g)}}{\text{Plant dry mass (g)}}$$

Grain filling duration

Grain filling duration was calculated as period from anthesis to physiological maturity.

Grain filling rate

Grain filling rate was estimated as the ratio between single grain weight (SGW) per plant and grain filling duration.

3.2.4 Statistical analysis:

Statistical analysis was done using SPSS software package (SPSS, 1991). The data for different characters studied were analyzed following randomized block design model:

$Y_{ij} = m+a_i+b_j+e_{ij}$

Where,

Y_{ij} = An observation of the ith genotype in the jth block

m = general mean

a_i = ith genotype effect

b_j = jth block effect

e_{ij} = random error associated with ith genotype in jth block

The assumptions of the model are:

a) Observations are independent

b) The random errors (e_{ij}) are distributed normally and independently with mean zero and variance σ_e^2

c) The different effects in the model are additive

Analysis of variance tables were constructed for each of the studied traits.

Table 3.4: Analysis of variance

Source	Degree of freedom	Mean squares	Expectation of mean square
Replications	r – 1	MS_1	
Genotypes	g – 1	MS_2	$\sigma_e^2 + r\sigma_g^2$
Error	(r – 1) (g – 1)	MS_3	σ_e^2

Where,

r = Number of replications

g = Number of genotypes

MS_1, MS_2, MS_3 stand for mean squares due to block, genotype and error, respectively

σ_g^2 = Genotypic variance of the character

σ_e^2 = Error variance of the character

3.2.4.1 Computation of data recorded:

(i) **Mean:** The mean value of each character was worked out by dividing the total of corresponding number of observations under all the replications.

$$\overline{X} = \frac{\sum x_i}{n}$$

Where,

x_i = any observation in i^{th} genotype

n = number of observations.

(ii) **Median:** The median was computed by arranging the observations in ascending order of magnitude and the average of the two middle observations was recorded as median.

(iii) **Range:** Lowest and highest mean values for each character were recorded over the genotypes.

(iv) **Standard Error (S.E.):** It is the dispersion of family mean around the experimental or estimated population mean. Standard error of mean was calculated with the help of error mean squares from the analysis of variance.

$$SE(d) = \sqrt{\frac{2MS_e}{r}}$$

Where,

MS_e = Mean squares due to error

r = Number of replications

(v) Critical Differences (C.D.): Critical difference was calculated to compare means for all the characters. It was computed with the help of S.E. (d) and tabulated value of 't' at 5 per cent level of significance at error degree of freedom.

C.D. = S.E. (d) x t

(vi) Coefficient of variation (Experimental error): Coefficient of variation was estimated by the formula:

$$\mathrm{CV}(\%) = \frac{\sqrt{\sigma_e^2}}{\mathrm{Mean}} \times 100$$

Where, = σ_e^2 error variance

3.2.4.2 Genetic parameters:

The parameters were calculated as given here after:

(i) Genotypic variance:

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

(ii) Phenotypic variance:

$$\sigma_p^2 = \sigma_g^2 + \frac{MS_e}{r}$$

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

MS_g = Mean square due to genotype

MS_e = Mean square due to error/environment

r = Number of replications

3.2.4.3 Heritability (broad sense): It is the ratio of genotypic variance to phenotypic variance. Heritability in broad sense was calculated by using the formula:

$$h_B^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Where,

h^2 = Heritability in broad sense

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

3.2.4.4 Estimation of parameters of variation:

The genotypic, phenotypic and environmental coefficients of variations were estimated as below:

3.2.4.5 Genotypic coefficient of variation (GCV):

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

3.2.4.6 Phenotypic coefficient of variation (PCV):

$$\text{PCV} = \sqrt{\frac{\sigma_p^2}{\bar{x}}}\ \text{x}\ 100$$

3.2.4.7 Environmental coefficient of variation (ECV):

$$\text{ECV} = \frac{\frac{\sqrt{\sigma_e^2}}{r}}{\bar{x}}$$

3.2.4.8 Genetic advance (% of mean):

$$\text{G.A (\% of mean)} = \frac{K\frac{\sigma_g^2}{\sigma_p^2}}{\bar{x}}\ \text{x}\ 100$$

Where,

G.A = Genetic advance

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

K = Selection differential and at 5 per cent selection the value of K is 2.06

$\bar{x}$ = Grand Mean

3.3 Genomic DNA isolation

DNA isolation was done by CTAB method (Saghai-Maroof *et al.*1984).

3.3.1 Reagents

CTAB extraction buffer

Tris-HCl (pH 8.0)	0.2 M
EDTA (disodium, pH 8.0)	0.02 M
Sodium chloride	1.4 M

CTAB	2.0%
β-mercaptoethanol	2.0%
(Added just before use)	

TE buffer

Tris (pH 8.0)	10 mM
EDTA (disodium, pH 8.0)	1 mM

RNase solution:

RNase solution was prepared by dissolving 10 mg RNase (DNase free RNase-A, Sigma chemical Co. USA, No.R-5503) in 1 ml of (500 µl of 10 mM Tris and 500 µl of 15 mM NaCl) and then boiled at 100 ^{0}C for 15 minutes.

3.3.2 Procedure:

Leaf samples (about 5g) were taken from 2-3 week old seedlings and cut into small pieces and ground into fine powder using liquid nitrogen in a sterile pestle and mortar. The ground leaf tissue powder was transferred into a 50 ml polypropylene tube. To this, 15 ml of preheated (65ºC) CTAB buffer was added and the samples were thoroughly mixed by inverting the tubes several times. Incubation was done at 65ºC for 60-90 minutes with regular gentle mixing of samples. After incubation, samples were cooled to room temperature and 15 ml of Chloroform: Isoamyl alcohol (24:1) mixture was added. Contents were mixed well by inverting the tubes gently for 10-15 minutes. Tubes were then centrifuged at 10,000 rpm for 10 minutes and the upper aqueous layer was transferred into new sterilized 50 ml tubes using a micropipette. Equal volume of ice-cold isopropanol was subsequently added to precipitate the DNA and the tubes were kept undisturbed for 15 minutes. The DNA was then spooled out and put into 1.5 ml eppendorf centrifuge tubes. Centrifugation was done at 12,000 rpm for 5 min to pellet down the DNA. The supernatant was discarded and the DNA pellet was washed with 70% ethanol and the DNA pellet was dried overnight at room temperature.

DNA was subsequently dissolved in appropriate volume of T E buffer and samples were stored at -20°C till further use.

RNase treatment

RNA contamination was removed by adding 1 µl of 10 mg/ml RNase in the dissolved DNA samples. The samples were mixed gently and then incubated at 37ºC for 2-3 hrs. DNA was again extracted by adding equal volume of chloroform: isoamyl alcohol (24:1) mixture. Samples were mixed well and then centrifuged at 10,000 rpm for 10 minutes. Supernatant was transferred to sterilized eppendorf-tubes. DNA was precipitated by adding 1/10th volume of 5M sodium chloride and 2 volume of chilled absolute alcohol, followed by centrifugation to pellet down the DNA. Pellet was then washed with 70% alcohol, air-dried and finally dissolved in T.E. buffer and stored at -20°C till further use.

3.3.3 Qualitative and Quantitative Estimation of DNA

Quality and quantity of DNA was estimated by UV spectroscopy and agarose gel electrophoresis. For UV spectroscopy, an aliquot of DNA samples was suitably diluted and absorbance (A) was determined at 260 nm and 280 nm wavelength in spectrophotometer. Using the relationship of O.D. unit of 1.0 at 260 nm equivalent to 50 μg DNA per ml, the quantity of DNA was estimated from the following formula:

Concentration of DNA (μg/ml) = A_{260} x 50 x dilution factor

The DNA concentrations were also checked by visual assessment of band intensity in comparison with Lambda DNA of known concentration in 0.8% agarose gel.

Quality of DNA samples was checked both by UV-spectrophotometer and on agarose gel electrophoresis. Using spectrophotometer, the ratio of the absorbance at 260 nm and 280 nm was noted. Samples with a ratio of 1.8 to 2.0 were considered of good quality.

A_{260}/A_{280} = 1.8 (pure DNA)

Quality of DNA preparation was also tested by submerged horizontal agarose (0.8%) gel electrophoresis. Appearance of discrete band of high molecular weight near the wells was considered as indicator of good quality DNA.

3.3.4 PCR amplification

3.3.4.1 SSR Marker

For SSR primers, PCR reaction was carried out in 25μl of reaction mix containing 50 ng of template DNA, 200 μM of each dNTP, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM each forward and reverse primer and 1.2 U Taq DNA polymerase.

The following protocol was used for PCR amplification:

1.	Initial Denaturation	94°C for 4 min.	
2.	Denaturation	94°C for 1 min.	42 cycles
3.	Annealing*	50°C- 65°C for 1 min.	
4.	Extension	72°C for 2 min.	
5.	Final Extension	72°C for 14 min.	

*Annealing temperatures for various primers varied from 50°C- 65°C (Table 3.2).

Amplified products were stored at -20°C till further use.

3.3.4.2 RAPD Marker

The RAPD-PCR reactions were carried out in 20 µl of reaction mix containing 25 ng of template DNA, 200 µM of each dNTP, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 µM primer and 1.5 U Taq DNA polymerase.

The following protocol was used for PCR amplification:

1.	Initial Denaturation	94°C for 4 min.	
2.	Denaturation	94°C for 1 min.	
3.	Annealing	34°C for 1 min.	40 cycles
4.	Extension	72°C for 2 min.	
5.	Final Extension	72°C for 8 min.	

Amplified products were stored at -20°C till further use.

3.3.4.3 Known gene primers analysis

For amplification using known gene primers, a polymerase chain reaction (PCR) was performed in a 25 µl volume containing 100 ng of genomic DNA, 2.5 µl of 10X PCR buffer, 200 µM of each dNTP, 0.2 µM of each primer and 1.0 unit of *Taq* DNA polymerase.

The following protocol was used for PCR amplification:

1.	Initial Denaturation	94°C for 4 min.	
2.	Denaturation	94°C for 45 sec.	
3.	Annealing	60°C for 45 sec.	30 cycles
4.	Extension	72°C for 60 sec.	
5.	Final Extension	72°C for 5 min.	

Amplified products were stored at -20°C till further use.

3.3.5 Agarose Gel Electrophoresis

3. 3.5.1 Reagents

10X TBE buffer

Tris	108.0 g
Boric acid	55.0 g
0.5 M EDTA (pH 8.0)	40 ml
Final volume	1000 ml

6X Loading dye

Sucrose	4.0 g
Bromophenol blue	0.025 g
Xylene cyanol	0.025 g
Volume	10 ml

Loading dye solution was stored at 4ºC in the refrigerator.

3.3.5.2 Procedure

Amplified DNA fragments were resolved by submerged horizontal electrophoresis in 2.5 per cent (w/v) agarose gel (for SSR Markers), 1.5 per cent (w/v) agarose gel (for RAPD Markers) and 3 per cent (w/v) agrose gel (known gene primer) and visualized by staining with ethidium bromide. Agarose solution was prepared in 1X TBE and ethidium bromide (10 mg/ml) was added in the gel at a concentration of 3 µl/100 ml of gel and then mixed gently. It was poured in gel casting plate with appropriate comb with required well number and size. DNA samples were mixed with 0.20 volume of 6X loading dye solution and loaded in wells using micropipette. Electrophoresis was done using 1X TBE as running buffer at constant voltage (3 V/cm of gel). PCR amplification products were viewed under UV light and photographed using gel documentation system.

3.3.6 Allele Scoring

SSR, RAPD and known gene markers amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands for each wheat genotype. Only clear and unambiguous bands were scored. The size (in nucleotide base pairs) of amplified bands was determined based on its migration relative to standard molecular size marker.

3.3.7 Data Analysis

Based on presence/absence data, genetic similarity was calculated to estimate all pairwise differences in the amplification products for all genotypes. Based on this data, cluster analysis was done to estimate relationship among genotypes.

The data generated from the polymorphic fragments were analyzed by the equation given by Nei and Li (1979):-

$$\text{Similarity (F)} = \frac{2\,Mx}{My + Mz}$$

Dissimilarity = 1 – F

Where,

Mx = Number of shared fragments between genotypes y and z

My = Number of scored fragments of genotype y

Mz = Number of scored fragments of genotype z

The 0/1 matrix was used to calculate the similarity genetic distance using ‘SIMQUAL’ sub-programme of NTSYS-PC software (numerical taxonomy and multivariate analysis system programme) (Rohlf, 1993). Dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-PC. Principal component analysis (PCA) was done using the ‘CPCA’ sub-programme of NTSYS-PC software. Diagrams in both 2 and 3 dimensions were constructed.

3.3.8 QTL Mapping

QTL analysis for all markers was performed with the computer program WinQTLCart 2.5 (Wang *et al.,* 2005). Kosambi function (Kosambi, 1944) was used to convert the recombination frequency to genetic distances in centimorgans (cM). This analysis was carried out by evaluating the mapping populations as an F_2 using two-point analysis to identify linkage group at a LOD score of 3.0. Data was put in as 2, 0 and 1 to mark genotypes of parent A; parent B, and heterozygotes, respectively, for co-dominant marker.

CHAPTER–IV

RESULTS

DNA based molecular markers are important tools for crop improvement. The polymorphism detected by these markers can be used for: (i) Indirect selection of tagged loci affecting qualitative or quantitative traits, (ii) To identify and discriminate closely related cultivars, (iii) For pedigree analysis, (iv) To assess taxonomic and phylogenetic relationships, (v) Linkage mapping etc. Polymerase chain reaction (PCR) based markers like RAPD and SSR help us in the detection of polymorphisms at the molecular level from many individuals or pooled samples at a very fast rate. In present investigation was undertaken to identify QTLs linked to thermotolerance in wheat using F_2 populations derived from the crosses between Hindi62 xWH711 and DBW16 x WH147. The genomic DNA of parents and F_2 population was isolated and PCR amplification was done using RAPD and SSR primers. Both F_2 populations were advanced to raise F_3 populations and these were phenotyping under normal and late sown conditions.

The results obtained are being presented as under

4.1 Field evaluation of Hindi62 x WH711 F_2 population

The data on various phenotypic traits viz. membrane thermostability, chlorophyll stability index, grain yield, kernels/spike, thousand kernel weight (Test weight), days to heading, days to maturity, plant height, number of tillers/plant, biomass/plant and harvest index for Hindi62 × WH711 derived F_2 plants and parental wheat varieties given in Annexure I. A wide range of variation was observed for all the phenotypic traits in F_2 population and parental wheat genotypes (Table 4.1).

4.1.1 Membrane thermostability index

Membrane thermostability index of 90 F_2 plants of cross Hindi62 × WH711 varied between 46.7-63.96 (Hindi62–47.65, WH711– 64.54) (Annexure I). Out of 90 plants, 69 plants had membrane thermostability index below the mean value 50.93.

4.1.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_2 plants varied between 0.51-0.64 (Hindi62- 0.64 and WH711 – 0.52). Out of 90 plants, 42 plants had chlorophyll stability index below the mean value of 0.58.

4.1.3 Days to heading

Days to heading in 90 F_2 plants ranged from 104-117 (Hindi62-115 and WH711-108) (Annexure I). As many as 40 plants had days to heading above the mean value (110 days).

4.1.4 Plant height (cm)

Plant height of 90 F_2 plants varied between 96.3-136.6 cm (Hindi62-126.5 cm and WH711-98.2 cm) (Annexure I). Out of 90 plants, 30 plants had plant height below the mean value 116.7 cm.

4.1.5 Number of kernels per spike

Number of kernels per spike of F_2 population ranged from 16-57 (Hindi62-56.8 and WH711-38.6) (Annexure I). Of these, 63 plants had number of kernels per spike above the mean value 41.83.

4.1.6 Number of tillers per plant

Number of tillers per plant of 90 F_2 plants varied between 3-14 (Hindi62-15 and WH711-8) (Annexure I). Out of 90 F_2 plants 26 plants had number of tillers per plant below the mean value 10.

4.1.7 1000 kernel weight (Test weight)

Thousand kernel weight varied between 24.6-42.3 g (Hindi62-40.2 g and WH711-42.6 g) (Annexure I). Of these, 49 plants had kernel weight above the mean value of 33.2 g. Plant number 19 and 35 had 1000 kernel weight of 42.30 and 41.50 g respectively that is more than Hindi62.

4.1.8 Grain yield per plant

Grain yield per plant in F_2 population ranged from 4.82-21.1 g (Hindi62-20.2 g and WH711-12.5 g) (Annexure I). Of these 90 F_2 plants, 64 plants had grain yield above the mean value of 14.91 g.

4.1.9 Biomass per plant

Biomass per plant among 90 F_2 plants varied between 21.5 -83.12 g (Hindi62-88.5 g and WH711-42.73 g) (Annexure I). Out of 90 F_2 plants 66 plants had biomass per plant above the mean value of 60.3 g.

4.1.10 Days to maturity

Days to maturity in F_2 population ranged from 145-159 days (Hindi62-154 days and WH711-148 days). (Annexure I). Among 90 F_2 plants 24 plants had days to maturity above the mean value of 150 days.

4.1.11 Harvest index

Harvest index in F_2 population varied between 20.76-36.11 (Hindi62-17.72 and WH711- 20.28) (Annexure I). Out of 90 F_2 plants 39 plants had harvest index above the mean value of 24.77.

4.1.12 Grain filling duration

Grain filling duration in F_2 population varied between 33.3-48.3 (Hindi62-39 and WH711-40). Out of 90 F_2 plants 43 plants had grain filling duration above the mean value of 40.

4.1.13 Grain filling rate

Grain filling rate in F_2 population varied between 0.697-1.199 (Hindi62-1.03 and WH711-0.82). Out of 90 F_2 plants 48 plants had grain filling rate above the mean value of 0.83.

4.2 Estimates for genetic variability parameters for various traits

The genetic parameters were worked out separately for the characters. The components of variance, heritability, genetic advance, and genetic advance as % of mean for various traits have been presented in Table 4.2. The perusal of the table revealed that phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation. Tiller per plant (22.574) depicted highest GCV and biomass per plant highest PCV (25.971). Grain yield and biomass per plant had same GCV (22.55) estimates followed by kernel per spike (15.168, 15.955), test weight (13.820, 13.941), membrane thermostabilty index (10.229, 10.262), plant height (9.549, 9.609), chlorophyll index (6.519, 6.677), days to heading (3.166, 3.370), days to maturity (2.122, 2.283) and harvest index (1.837, 6.394).

Table 4.1: Phenotypic traits in parents and F_2 population from Hindi62 x WH711

Sr. No.	**Trait**	**Parents**		**F_2 Population**	
		Hindi62	**WH711**	**Mean**	**Range**
1.	Chlorophyll Index	0.648	0.523	0.583	0.51-0.64
2.	Membrane thermostability index	47.65	64.54	50.9352	46.7-63.96
3.	Plant height (cm)	126.5	98.2	116.7481	96.33-136.66
4.	Kernels per spike	56.8	38.6	41.8333	16-57
5.	Tillers per plant	15	8	10.5333	3-14
6.	Biomass/Plant (g)	88.5	42.7	60.3945	21.5 -83.1
7.	Grain Yield (g)	20.2	12.5	14.9114	4.82-21.1
8.	Test weight(g)	40.2	42.6	33.2385	24.6-42.3
9.	Days to heading	115	108	110.5	104-117
10.	Days to maturity	154	148	150.9185	145-159
11.	Harvest Index	17.72	20.28	24.7707	20.76-36.11
12.	Grain filling duration	39	40	40.39	33.3-48.3
13.	Grain filling rate	1.03	0.82	0.83	0.57-1.11

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading, days to maturity and harvest index and (<80%) tiller per plant, biomass per plant and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.953), membrane thermostability (0.994), plant height (0.988), kernel per spike(0.904) and test weight (0.983).

A further perusal of Table 4.2 regarding genetic advance indicated a good scope for improvement for almost all the traits studied as revealed by the genetic advance expressed as percentage of mean. The genetic advance in terms of per cent of mean at 1 per cent selection intensity has been indicated very high for tiller per plant (52.325), and high for kernel per spike (38.070), test weight

(36.168) and moderate for plant height (25.055), Membrane thermostability index (26.918 and low for days to heading (7.852), days to maturity (5.207) and Harvest index (1.393).

The extent of improvement to the tune of almost 40 per cent can be achieved by adopting 5 per cent selection intensity for characters viz., tillers per plant (40.829), biomass per plant (40.364) and grain yield (40.486). For the characters viz., kernel per spike (29.706), test weight (28.222), the scope of improvement is above 30 per cent just by applying selection intensity of 5 per cent. The scope of improvement to the extent of around 20 per cent is indicated for characters viz., plant height (19.55), membrane thermostability index (21.004).

Table4.2: Genetic parameters for phenotypic characters in F_2 generation of Hindi62 × WH711

Sr. No.	Characters	Genetic Parameters						
		GCV	PCV	Heritability	GA		Genetic advance as % of mean	
					5%	1%	5%	1%
1.	Chlorophyll Index	6.519	6.677	0.953	0.076	0.098	13.113	16.805
2.	Membrane thermostability index	10.229	10.262	0.994	10.698	13.711	21.004	26.918
3.	Plant height (cm)	9.549	9.609	0.988	22.824	29.251	19.550	25.055
4.	Kernel per spike	15.168	15.955	0.904	12.427	15.926	29.706	38.070
5.	Tiller per plant	22.574	25.711	0.771	4.301	5.512	40.829	52.325
6.	Biomass/Plant (g)	22.558	25.971	0.754	24.378	31.241	40.364	51.728
7.	Grain Yield (g)	22.558	25.823	0.761	6.037	7.737	40.486	51.885
8.	Test weight(g)	13.820	13.941	0.983	9.380	12.022	28.222	36.168
9.	Days to heading	3.166	3.370	0.883	6.770	8.676	6.127	7.852
10.	Days to maturity	2.122	2.283	0.864	6.132	7.859	4.063	5.207
11.	Harvest Index	1.837	6.394	0.830	0.269	0.345	1.087	1.393
12.	Grain filling duration	10.372	11.296	0.794	10.752	12.972	18.742	21.634
13.	Grain filling rate	9.846	11.462	0.852	10.684	13.563	17.356	19.854

4.3 Field evaluation of DBW16 x WH147 F_2 population

The data on various agronomic traits for DBW16 × WH147 derived F_2 plants and parental wheat varieties given in Annexure II. A wide range of variation was observed for all the agronomic traits in F_2 population and parental wheat genotypes (Table 4.3).

4.3.1 Membrane thermostability index

Membrane thermostability index of 90 DBW16 × WH147 F_2 plants varied between 43.9-64.3 (DBW16–51.35, WH147–62.53) (Annexure II). Out of 90 plants, 21 plants had membrane thermostability index above the mean value 51.29.

4.3.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_2 plants varied between 0.514-0.651 (DBW16- 0.62 and WH147 – 0.52). Out of 90 plants, 39 plants had chlorophyll stability index below the mean value of 0.587.

4.3.3 Days to heading

Days to heading in 90 F_2 plants ranged from 102-116 (DBW16-92 and WH147-96) (Annexure I). As many as 62 plants had days to heading above the mean value (110 days).

4.3.4 Plant height (cm)

Plant height of 90 F_2 plants varied between 89-141 cm (DBW16-98.6 cm and WH147-102.0 cm) (Annexure II). Out of 90 plants, 27 plants had plant height below the mean value 115.75 cm.

4.3.5 Number of Kernels per spike

Number of kernels per spike of F_2 population ranged from 22-52 (DBW16-46 and WH147- 33) (Annexure II). Of these, 64 plants had number of kernels per spike above the mean value 44.75.

4.3.6 Number of tillers per plant

Number of tillers per plant of 90 F_2 plants varied between 6-16 (DBW16-14 and WH147-10) (Annexure II). Out of 90 F_2 plants 54 plants had number of tillers per plant above the mean value 11.

4.3.7 1000 kernel weight (Test weight)

Thousand kernel weight varied between 22.4-42.2 g (DBW16-41.6 g and WH147-43.6 g) (Annexure II). Of these, 56 plants had kernel weight above the mean value of 33.7 g. Plant number 10, 26 and 67 had 1000 kernel weight of 42.33, 41.86 and 42.36 g respectively that is more than DBW16.

4.3.8 Grain yield per plant

Grain yield per plant in F_2 population ranged from 6.9-23.4 g (DBW16-16.2 g and WH147-10.1 g) (Annexure II). Of these 90 F_2 plants, 16 plants had grain yield above the mean value of 13.94 g.

4.3.9 Biomass per plant

Biomass per plant among 90 F_2 plants varied between 30.5-92.3g (DBW16-80.2 g and WH147-56.4 g) (Annexure II). Out of 90 F_2 plants 49 plants had biomass per plant above the mean value of 57.06 g.

4.3.10 Days to maturity

Days to maturity in F_2 population ranged from 142-158 days (DBW16-132 days and WH147-146 days). (Annexure II). Among 90 F_2 plants 51 plants had days to maturity above the mean value of 150 days.

4.3.11 Harvest index

Harvest index in F_2 population varied between 13.88-39.28 (DBW16-17.86 and WH147- 17.09) (Annexure II). Out of 90 F_2 plants 42 plants had harvest index above the mean value of 25.07.

4.3.12 Grain filling duration

Grain filling duration in F_2 population ranged from 34.3-43.3 (DBW16-40.3 and WH147- 40.0). Out of 90 F_2 plants 47 plants had grain filling duration above the mean value of 39.7.

4.3.13 Grain filling rate

Grain filling rate in F_2 population ranged from 0.59-1.11 (DBW16-1.03 and WH147- 0.84). Out of 90 F_2 plants 46 plants had grain filling rate above the mean value of 0.85.

Table 4.3: Phenotypic traits in parents and F_2 population from DBW16 x WH147

Sr. No.	Trait	Parents		F_2 Population	
		DBW16	WH147	Mean	Range
1.	Chlorophyll Index	0.62	0.52	0.5872	0.514-0.651
2.	Membrane thermostability index	51.3	62.5	51.2967	43.9-64.3
3.	Plant height (cm)	98.6	102	115.7519	89-141
4.	Kernels per spike	46.0	32.3	44.7593	22-52
5.	Tillers per plant	14	10	11.1519	6-16
6.	Biomass/Plant (g)	80.2	56.4	57.0667	30.5-92.3
7.	Grain Yield (g)	16.2	10.1	13.943	6.9-23.4
8.	Test weight(g)	41.6	43.6	33.7856	22.4-42.2
9.	Days to heading	92	96	110.6407	102-116
10.	Days to maturity	132	146	150.3	142-158
11.	Harvest Index	17.86	17.09	25.07	13.884-39.286
12.	Grain filling duration	40.3	40.0	39.7	34.3-43.3
13.	Grain filling rate	1.03	0.84	0.85	0.59-1.11

4.4 Estimates for genetic variability parameters for various traits

Phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation. Biomass per plant (24.389, 27.273) depicted highest GCV and

PCV estimates followed by grain yield (21.985, 25.322), kernel per spike (19.509, 19.963), test weight (15.220, 15.448), membrane thermostability (11.827, 11.848), plant height (9.455, 9.588), harvest index (8.045, 15.127), chlorophyll index (6.819, 6.842), days to heading (3.175, 3.444) and days to maturity (2.702, 2.917) (table 4.4).

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and days to maturity, biomass per plant and (<70%) tiller per plant, and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.993), membrane thermostability (0.996), plant height (0.973), kernel per spike(0.955) and test weight (0.971). A further perusal of Table 4.4 regarding genetic advance indicated a good scope for improvement for almost all the traits studied as revealed by the genetic advance expressed as percentage of mean. The genetic advance in terms of per cent of mean at 1 per cent selection intensity has been indicated very high for biomass per plant (57.577), grain yield (50.391), and high for kernel per spike (50.332), test weight (39.589) and moderate for plant height (24.617) and low for days to heading (7.729), days to maturity (6.609).

The extent of improvement to the tune of almost 40 per cent can be achieved by adopting 5 per cent selection intensity for characters viz., biomass per plant (44.928), grain yield (39.320) kernel per spike (39.274), tillers per plant (36.499). For the characters viz., test weight (30.892) is above 30 percent improvement. Membrane thermostability index (24.319) and plant height (19.208), the scope of improvement is above 20 per cent just by applying selection intensity of 5 per cent. The scope of improvement to the extent of around 10 per cent is indicated for characters viz., chlorophyll index (13.998) and harvest index (8.813).

Table4.4: Genetic parameters for phenotypic characters in F_2 generation of DBW16 x WH147

Sr. No.	Characters	Genetic Parameters						
		GCV	PCV	H^2	GA		Genetic advance as % of mean	
					5%	1%	5%	1%
1.	Chlorophyll Index	6.819	6.842	0.993	0.082	0.105	13.998	17.940
2.	Membrane thermostability index	11.827	11.848	0.996	12.475	15.987	24.319	31.167
3.	Plant height (cm)	9.455	9.588	0.973	22.234	28.494	19.208	24.617
4.	Kernel per spike	19.509	19.963	0.955	17.579	22.528	39.274	50.332
5.	Tiller per plant	20.225	23.087	0.767	4.070	5.216	36.499	46.775
6.	Biomass/Plant (g)	24.389	27.273	0.800	25.639	32.857	44.928	57.577
7.	Grain Yield (g)	21.985	25.322	0.754	5.482	7.026	39.320	50.391
8.	Test weight(g)	15.220	15.448	0.971	10.437	13.376	30.892	39.589
9.	Days to heading	3.175	3.444	0.850	6.673	8.552	6.031	7.729
10.	Days to maturity	2.702	2.917	0.858	7.748	9.930	5.155	6.607
11.	Harvest Index	8.045	15.127	0.283	2.209	2.831	8.813	11.294
12.	Grain filling duration	13.254	15.642	0.762	13.892	16.642	18.948	21.652
13.	Grain filling rate	11.872	14.248	0.846	14.622	17.346	18.152	22.734

4.5 Field evaluation of Hindi62 x WH711 F_3 population (normal sown)

The data on various phenotypic traits for Hindi62 × WH711 derived F_3 plants and parental wheat varieties given in Annexure III. A wide range of variation was observed for all the phenotypic traits in F_3 population and parental wheat genotypes (Table 4.5).

4.5.1 Membrane thermostability index

Membrane thermostability index of 90 Hindi62 × WH711 F_3 plants varied between 0.5145 - 0.6683 (Hindi62–46.6, WH711– 64.85) (Annexure III). Out of 90 plants, 22 plants had membrane thermostability index above the mean value 50.80.

4.5.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_3 plants varied between 0.51-0.66(Hindi62- 0.65 and WH711 – 0.52). Out of 90 plants, 45 plants had chlorophyll stability index below the mean value of 0.59.

4.5.3 Days to heading

Days to heading in 90 F_3 plants ranged from 103-112 (Hindi62-110 and WH711-105) (Annexure III). As many as 49 plants had days to heading above the mean value (107 days).

4.5.4 Plant height (cm)

Plant height of 90 F_3 plants varied between 95.3-128.6 cm (Hindi62-116.3 cm and WH711-100.6 cm) (Annexure III). Out of 90 plants, 54 plants had plant height above the mean value 112.3 cm.

4.5.5 Number of Kernels per spike

Number of kernels per spike of F_3 population ranged from 21.7-53.0 (Hindi62-52.9 and WH711-37.7) (Annexure III). Of these52 plants had number of kernels per spike above the mean value 40.3.

4.5.6 Number of tillers per plant

Number of tillers per plant of 90 F_3 plants varied between 6-15 (Hindi62-14 and WH711-10) (Annexure III). Out of 90 F_3 plants 7plants had number of tillers per plant above the mean value 14.0

4.5.7 1000 kernel weight (Test weight)

Thousand kerenel weight varied between 27.9-42.5 g (Hindi62-41.3 g and WH711-47.6 g) (Annexure III). Of these, 51 plants had kernel weight above the mean value of 34.8 g. Plant number 13, 16, 19and 35 had 1000 kernel weight of 42.0, 41.9, 42.5 and 41.90 g respectively that is more than Hindi62.

4.5.8 Grain yield per plant

Grain yield per plant in F_3 population ranged from 7.3-19.9 g (Hindi62-19.4 g and WH711-15.3 g) (Annexure III). Of these 90 F_2 plants, 54 plants had grain yield above the mean value of 15.3 g.

4.5.9 Biomass per plant

Biomass per plant among 90 F_3plants varied between 29.05-82.5 g (Hindi62-86.5 g and WH711-59.5 g) (Annexure III). Out of 90 F_3 plants 64 plants had biomass per plant above the mean value of 62.41 g.

4.5.10 Days to maturity

Days to maturity in F_2 population ranged from 142-153 days (Hindi62-149 days and WH711-146 days). (Annexure III). Among 90 F_3 plants 54plants had days to maturity above the mean value of 146 days.

4.5.11 Harvest index

Harvest index in F_3 population varied between 19.79-29.72 (Hindi62-22.43 and WH711- 25.70) (Annexure III). Out of 90 F_3 plants 40 plants had harvest index above the mean value of 24.80.

4.5.12 Grain filling duration

Grain filling duration in F_3 population varied between 33.6-44.3 (Hindi62-39 and WH711-41). Out of 90 F_3 plants 38 plants had grain filling duration above the mean value of 38.

4.5.13 grain filling rate

Grain filling rate in F_3 population varied between 0.697-1.199 (Hindi62-1.07 and WH711-0.90). Out of 90 F_3 plants 42 plants had grain filling rate above the mean value of 0.904.

Table 4.5: Phenotypic traits in parents and F_3 population from Hindi62 x WH711 under normal sown condition

Sr. No.	Trait	Parents		F_3 Population	
		Hindi62	WH711	Mean	Range
1.	Chlorophyll Index	0.6597	0.5238	0.5901	0.5145 - 0.6683
2.	Membrane thermostability index	46.65	64.85	50.80	45.67 – 63.67
3.	Plant height (cm)	116.3	100.6	112.3	95.3 – 128.6
4.	Kernel per spike	52.9	37.7	40.3	21.7 – 53.0
5.	Tiller per plant	14.7	10.7	14.09	6.7 – 15.3
6.	Biomass/Plant (g)	86.50	59.53	62.41	29.05 – 82.5
7.	Grain Yield (g)	19.4	15.3	15.3	7.3 – 19.9
8.	Test weight(g)	41.3	47.6	34.8	27.9 – 42.5
9.	Days to heading	110.7	105.3	107.8	103.0 – 112.0
10.	Days to maturity	149.3	146.7	146.6	142.0 – 153.0
11.	Harvest Index	22.43	25.70	24.80	19.79 – 29.72
12	Grain filling duration	39	41	38.8	33.6-44.3
13	Grain filling rate	1.07	0.90	0.904	0.697-1.199

4.6 Estimates for genetic variability parameters for various traits

Phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation. Biomass per plant (23.432, 26.930) depicted highest GCV and PCV estimates followed by grain yield (20.645, 25.342), tillers per plant (20.582-24.834), kernel per spike (17.432, 17.955)test weight (16.824, 16.928), membrane thermostability (10.754, 10.936), plant height (9.836, 9.954), chlorophyll index (6.264, 6.456), harvest index (4.898, 8.694), days to heading (4.178, 4.570) and days to maturity (2.763, 2.976) (table 4.6).

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading, days to maturity and harvest index, biomass per plant (<70%) tiller per plant, and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.986), membrane thermostability (0.982), plant height(0.998), kernel per spike(0.934) and test weight (0.976).

The extent of improvement to the tune of almost 40 per cent can be achieved by adopting 5 per cent selection intensity for characters viz., grain yield (42.984), biomass per plant (42.960), tillers per plant (42.648), kernel per spike (40.070).

Table 4.6 : Genetic parameters for phenotypic characters in F_3 generation of Hindi62 × WH711 under normal sown conditions

Sr. No.	**Characters**	**Genetic Parameters**						
		GCV	**PCV**	**Heritability**	**GA**		**Genetic advance as % of mean**	
					5%	**1%**	**5%**	**1%**
1.	Chlorophyll Index	6.264	6.456	0.986	0.068	0.128	13.243	17.228
2.	Membrane thermostability index	10.754	10.936	0.982	11.662	14.675	22.546	28.964
3.	Plant height (cm)	9.836	9.954	0.998	20.764	27.571	19.876	26.072
4.	Kernel per spike	17.432	17.955	0.934	13.787	15.386	32.724	40.070
5.	Tiller per plant	20.582	24.834	0.791	4.451	5.356	42.648	52.678
6.	Biomass/Plant (g)	23.432	26.930	0.782	23.571	30.321	42.960	51.240
7.	Grain Yield (g)	20.645	25.342	0.743	5.274	7.582	42.984	50.664
8.	Test weight(g)	16.824	16.928	0.976	10.126	11.486	30.460	37.346
9.	Days to heading	4.178	4.570	0.874	6.592	8.264	7.279	7.475
10.	Days to maturity	2.763	2.976	0.852	6.344	8.482	4.838	6.246
11.	Harvest Index	4.898	8.694	0.817	1.634	1.962	1.468	1.668
12.	Grain filling duration	17.46	18.245	0.734	12.962	13.964	21.538	27.864
13.	Grain filling rate	14.652	17.865	0.836	15.268	18.654	20.852	26.960

4.7 Field evaluation of Hindi62 x WH711 F_3 population (late sown)

The data on various agronomic traits for Hindi62 × WH711 derived F_3 plants and parental wheat varieties given in Annexure IV. A wide range of variation was observed for all the agronomic traits in F_3 population and parental wheat genotypes (Table 4.7).

4.7.1 Membrane thermostability index

Membrane thermostability index of 90 Hindi62 × WH711 F_3 plants varied between 46.7-66.8 (Hindi62–49.2, WH711– 62.30) (Annexure IV). Out of 90 plants, 22 plants had membrane thermostability index above the mean value 52.70.

4.7.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_3 plants varied between 0.502-0.672 (Hindi62- 0.64 and WH711 – 0.53). Out of 90 plants, 34 plants had chlorophyll stability index below the mean value of 0.59.

4.7.3 Days to heading

Days to heading in 90 F_3 plants ranged from 79-88 (Hindi62-81 and WH711-84) (Annexure IV). As many as 40 plants had days to heading above the mean value 83 days.

4.7.4 Plant height

Plant height of 90 F_3 plants varied between 78-126 cm (Hindi62-121.3 cm and WH711-92.6 cm). Of these, 24 plants had plant height below the mean value 11.4 cm.

4.7.5 Number of kernels per spike

Number of kernels per spike of F_3 population ranged from 24-52 (Hindi62-48.0 and WH711-32.3). Of these, 32 plants had number of kernels per spike below the mean value 39.40.

4.7.6 Number of tillers per plant

Number of tillers per plant of 90 F_3 plants varied between 4-15(Hindi61-13 and WH711-9). Out of 90 F_3 plants 25 plants had number of tillers per plant below the mean value 10.3.

4.7.7 1000 kernel weight (test weight)

Thousand kernel weight varied between 23.1-40.9 g (Hindi62- 38.5 g and WH711-40.4 g). Of these, 49 plants had kernel weight above the mean value of 31.7 g.

4.7.8 Grain yield per plant

Grain yield per plant in F_3 population ranged from 6.3-19.7 g (Hindi62-16.0 g and WH711-12.9 g). Out of these 90 F_3 plants, 57 plants had grain yield above the mean value of 13.05 g.

4.7.9 Biomass per plant

Biomass per plant among 90 F_3 plants varied between 20.8-84.6 g (Hindi62-61.8 g and WH711-45.7 g) (Annexure IV). Out of 90 F_3 plants 56 plants had biomass per plant above the mean value of 53.5 g.

4.7.10 Days to maturity

Days to maturity in F_3 population ranged from 112-122 days (Hindi62-113days and WH711-118 days). (Annexure IV). Among 90 F_3 plants 41 plants had days to maturity above the mean value of 116 days.

4.7.11 Harvest index

Harvest index in F_3 population varied between 20.05-31.28 (Hindi62-22.11 and WH711- 20.42) (Annexure IV). Out of 90 F_3 plants 38 plants had harvest index above the mean value of 24.82.

4.7.12 Grain filling duration

Grain filling duration in F_3 population ranged from 23.2-40.9 (Hindi62-34.3 and WH711-32.0). Out of 90 F_3 plants 32 plants had grain filling duration above the mean value of 33.9.

4.7.13 Grain filling rate

Grain filling rate in F_3 population ranged from 0.663-1.205 (Hindi62-1.122 and WH711-0.951).

Out of 90 F_3 plants 46 plants had grain filling rate above the mean value of 0.937.

4.8 Estimates for genetic variability parameters for various traits

The table 4.8 revealed that phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation. Biomass per plant (22.117, 27.665) depicted highest GCV and PCV estimates followed by grain yield (18.264, 23.344), kernel per spike (16.322, 17.115), test weight (14.640, 14.666), membrane thermostability (12.785, 12.819), plant height (9.989, 10.243), chlorophyll index (7.452, 7.484), harvest index (6.891, 9.245), days to heading (3.049, 3.221) and days to maturity (2.306, 2.484).

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and days to maturity and (<70%) tiller per plant, biomass per plant and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.953), membrane thermostability (0.994), plant height(0.988), kernel per spike(0.904) and test weight (0.983).

A further perusal of Table 4.8 regarding genetic advance indicated a good scope for improvement for almost all the traits studied as revealed by the genetic advance expressed as percentage of mean. The genetic advance in terms of per cent of mean at 1 per cent selection intensity has been indicated very high for biomass per plant (46.682) and high for grain yield (37.726), test weight (38.583) and moderate for plant height (25.717) and low for days to heading (7.619), days to maturity (5.649).

Table 4.7 Phenotypic traits in parents and F_3 population from Hindi62 x WH711 under late sown condition

Sr. No.	Trait	Parents		F_3 Population	
		Hindi62	WH711	Mean	Range
1.	Chlorophyll Index	0.643	0.534	0.592	0.502-0.672
2.	Membrane thermostability	49.2	62.3	52.7048	46.7-66.8
3.	Plant height (cm)	121.3	92.6	111.3667	78-126
4.	Kernel per spike	48.0	32.3	39.4037	24-52
5.	Tiller per plant	13.6	9.3	10.3185	4-15
6.	Biomass/Plant (g)	61.8	45.7	53.5215	20.8-84.6
7.	Grain Yield (g)	16.2	12.9	13.0522	6.3-19.7
8.	Test weight(g)	38.5	40.4	31.7522	23.1-40.9
9.	Days to heading	81	84	82.9926	79-88
10.	Days to maturity	113	118	116.9593	112-122
11.	Harvest Index	22.11	20.42	24.8257	20.05-31.28
12	Grain filling duration	34.3	32.0	33.9	23.2-40.9
13	Grain filling rate	1.122	0.951	0.937	0.663-1.205

The extent of improvement to the tune of almost 30 per cent can be achieved by adopting 5 per cent selection intensity for characters viz., tillers per plant (33.835), biomass per plant (36.426) and grain yield (29.438) kernel per spike (32.065) and test weight (30.107) . For the characters viz., membrane thermostability index (26.268) and plant height (20.067), the scope of improvement is above 20 per cent just by applying selection intensity of 5 per cent. The scope of improvement to the extent of around 10 per cent is indicated for characters viz., harvest index (10.580), chlorophyll index (15.284).

Table 4.8 Genetic parameters for phenotypic characters in F_3 generation of Hindi62 × WH711 under late sown conditions

Sr. No.	Characters	Genetic Parameters						
		GCV	PCV	Heritability	GA		Genetic advance as % of mean	
					5%	1%	5%	1%
1.	Chlorophyll Index	7.452	7.484	0.991	0.090	0.116	15.284	19.588
2.	Membrane thermostability index	12.785	12.819	0.995	13.845	17.742	26.268	33.664
3.	Plant height (cm)	9.989	10.243	0.951	22.348	28.640	20.067	25.717
4.	Kernel per spike	16.322	17.115	0.909	12.635	16.192	32.065	41.093
5.	Tiller per plant	20.835	26.429	0.621	3.491	4.474	33.835	43.361
6.	Biomass/Plant (g)	22.117	27.665	0.639	19.496	24.985	36.426	46.682
7.	Grain Yield (g)	18.264	23.344	0.612	3.842	4.924	29.438	37.726
8.	Test weight(g)	14.640	14.666	0.997	9.560	12.251	30.107	38.583
9.	Days to heading	3.049	3.221	0.896	4.934	6.323	5.945	7.619
10.	Days to maturity	2.306	2.484	0.861	5.155	6.607	4.408	5.649
11.	Harvest Index	6.891	9.245	0.556	2.627	3.366	10.580	13.559
12.	Grain filling duration	12.642	13.169	0.672	11.452	13.682	17.532	19.974
13.	Grain filling rate	11.844	13.652	0.762	13.854	14.978	16.422	18.836

4.9 Field evaluation of DBW16 x WH147 F_3 population (normal sown)

The data on various agronomic traits for DBW16 × WH147 derived F_3 plants and parental wheat varieties given in Annexure V. A wide range of variation was observed for all the agronomic traits in F_3 population and parental wheat genotypes (Table 4.9).

4.9.1 Membrane thermostability index

Membrane thermostability index of 90 DBW16 × WH147 F_3 plants varied between 44.63-64.70 (DBW16–52.43, WH147–59.53) (Annexure V). Out of 90 plants, 21 plants had membrane thermostability index above the mean value 51.34.

4.9.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_3 plants varied between 0.514-0.652 (DBW16- 0.63 and WH147– 0.54). Out of 90 plants, 41 plants had chlorophyll stability index below the mean value of 0.587.

4.9.3 Days to heading

Days to heading in 90 F_3 plants ranged from 102-111 (DBW16-89 and WH147-106) (Annexure V). As many as 44 plants had days to heading above the mean value (106 days).

4.9.4 Plant height (cm)

Plant height of 90 F_3 plants varied between 96.7-124.0 cm (DBW16-101.3 cm and WH147-108.0 cm) (Annexure V). Out of 90 plants, 4 plants had plant height above the mean value 120.91 cm.

4.9.5 Number of Kernels per spike

Number of kernels per spike of F_3 population ranged from 32-68 (DBW16-42 and WH147- 36) (Annexure V). Of these, 44 plants had number of kernels per spike above the mean value 46.39.

4.9.6 Number of tillers per plant

Number of tillers per plant of 90 F_3 plants varied between 6-13 (DBW16-12 and WH147-10) (Annexure V). Out of 90 F_3 plants 32 plants had number of tillers per plant above the mean value 11.9.

4.9.7 1000 kernel weight (Test weight)

Thousand kernel weight varied between 26.5-43.5 g (DBW16-40.7 g and WH147-47.0 g) (Annexure V). Of these, 56 plants had kernel weight above the mean value of 34.98 g. Plant number 7, 9, 10, 25, 47, 61, 62 and 68 had 1000 kernel weight of 40.7, 42.8, 43.0, 42.7, 40.6, 42.3, 43.5 and 42.7 g respectively that is more than DBW16.

4.9.8 Grain yield per plant

Grain yield per plant in F_3 population ranged from 9.9-21.3 g (DBW16-18.5 g and WH147-12.7 g) (Annexure V). Of these 90 F_3 plants, 40 plants had grain yield above the mean value of 14.84g.

4.9.9 Biomass per plant

Biomass per plant among 90 F_3 plants varied between 32.70-72.53g (DBW16-74.5 g and WH147-59.3 g) (Annexure V). Out of 90 F_3 plants 43 plants had biomass per plant above the mean value of 59.3 g.

4.9.10 Days to maturity

Days to maturity in F_3 population ranged from 141-151 days (DBW16-139 days and WH147-145 days). (Annexure V). Among 90 F_3 plants 46 plants had days to maturity above the mean value of 145.9 days.

4.9.11 Harvest index

Harvest index in F_3 population varied between 19.79-44.95 (DBW16-24.83 and WH147- 21.41) (Annexure V). Out of 90 F_3 plants 45 plants had harvest index above the mean value of 25.94.

4.9.12 Grain filling duration

Grain filling duration among 90 F_3 plants varied between 36.3-41.7 (DBW16-39 and WH147-40.3). Out of 90 F_3 plants 43 plants had grain filling duration above the mean value of 39.09.

4.9.13 Grain filling rate

Grain filling rate in F_3 population varied between 0.705-1.211 (DBW16-1.05 and WH147-0.89). Grain filling rate had mean value of 0.937.

Table 4.9 Range for various phenotypic traits in DBW16 x WH147 F_3 population (normal sown)

Sr. No.	Trait	Parents		F_3 Population	
		DBW16	WH47	Mean	Range
1.	Chlorophyll Index	0.638	0.547	0.587	o.514 – 0.652
2.	Membrane thermostability index	52.43	59.53	51.34	44.63 – 64.70
3.	Plant height (cm)	101.3	108.0	120.91	96.7 – 124.0
4.	Kernel per spike	42.0	36.3	46.39	32.0 – 68.0
5.	Tiller per plant	12.7	10.3	11.9	6.0 – 13.7
6.	Biomass/Plant (g)	74.50	59.33	59.33	32.70 – 72.53
7.	Grain Yield (g)	18.5	12.7	14.84	9.9 – 21.3
8.	Test weight(g)	40.7	47.0	34.98	26.5 – 43.5
9.	Days to heading	89.0	106.0	116.8	102.3 – 111.7
10.	Days to maturity	139.3	145.0	145.90	141.0 – 151.0
11.	Harvest Index	24.83	21.41	25.94	19.79 – 44.95
12	Grain filling duration	39	40.3	39.09	36.3-41.7
13	Grain filling rate	1.05	0.89	0.937	0.705-1.211

4.10 Estimates for genetic variability parameters for various traits

Phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation depicted highest GCV than PCV for biomass per plant (24.828, 28.566) estimates followed by grain yield (22.924, 26.642) and tillers per plant (22.530, 24.642).

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and (<70%) tiller per plant and biomass per plant. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.985), membrane thermostability (0.976), plant height (0.982), kernel per spike (0.938) and test weight (0.926). A further perusal of Table 4.10 regarding genetic advance indicated a good scope for improvement for almost all the traits studied as revealed by the genetic advance expressed as percentage of mean.

Table 4.10 Genetic parameters for phenotypic characters in F_3 generation of DBW16 × WH147 under normal sown

Sr. No.	Characters	Genetic Parameters						
		GCV	PCV	Heritability	GA		Genetic advance as % of mean	
					5%	1%	5%	1%
1.	Chlorophyll Index	6.578	6.970	0.985	0.093	0.164	14.632	18.924
2.	Membrane thermostability index	11.284	11.890	0.976	14.423	16.924	25.869	31.584
3.	Plant height (cm)	9.267	9.768	0.982	21.246	28.836	19.746	24.864
4.	Kernel per spike	20.378	21.457	0.938	16.268	21.472	41.354	50.994
5.	Tiller per plant	22.530	24.642	0.791	5.120	6.584	38.436	47.762
6.	Biomass/Plant (g)	24.828	28.566	0.842	25.442	32.762	46.168	56.543
7.	Grain Yield (g)	22.924	26.642	0.768	5.758	7.928	39.862	50.887
8.	Test weight(g)	17.340	17.688	0.926	10.644	14.746	32.467	39.728
9.	Days to heading	3.472	3.746	0.872	6.854	8.986	6.654	7.868
10.	Days to maturity	2.562	2.983	0.854	7.220	9.362	5.867	6.813
11.	Harvest Index	4.356	10.189	0.834	2.684	2.982	9.835	11.436
12.	Grain filling duration	12.587	13.982	0.758	10.468	12.534	16.863	18.274
13.	Grain filling rate	10.386	12.86	0.834	13.652	15.968	18.422	20.732

4.11Field evaluation of DBW16 x WH147 F_3 population (late sown)

The data on various agronomic traits for DBW16 × WH147 derived F_3 plants and parental wheat varieties given in Annexure VI. A wide range of variation was observed for all the agronomic traits in F_3 population and parental wheat genotypes (Table 4.11).

4.11.1 Membrane thermostability index

Membrane thermostability index of 90 DBW16 × WH147 F_3 plants varied between 46.2-65.4 (DBW16–48.4, WH147–63.0) (Annexure VI). Out of 90 plants, 21 plants had membrane thermostability index above the mean value 52.76.

4.11.2 Chlorophyll stability index

Chlorophyll stability index in 90 F_3 plants varied between 0.513-0.651 (DBW16- 0.636 and WH147– 0.549). Out of 90 plants, 26 plants had chlorophyll stability index below the mean value of 0.588.

4.11.3 Days to heading

Days to heading in 90 F_3 plants ranged from 78-88 (DBW16-81 and WH147-86) (Annexure VI). As many as 31 plants had days to heading above the mean value (83days).

4.11.4 Plant height (cm)

Plant height of 90 F_3 plants varied between 78-134 cm (DBW16-104.3 cm and WH147-108.0 cm) (Annexure VI). Out of 90 plants, 26 plants had plant height below the mean value 110.3 cm.

4.11.5 Number of Kernels per spike

Number of kernels per spike of F_3 population ranged from 22-56 (DBW16-46 and WH147- 31) (Annexure VI). Of these, 60 plants had number of kernels per spike above the mean value 43.42.

4.11.6 Number of tillers per plant

Number of tillers per plant of 90 F_3 plants varied between 4-18 (DBW16-13 and WH147-8) (Annexure VI). Out of 90 F_3 plants 34 plants had number of tillers per plant below the mean value 10.9.

4.11.7 1000 kernel weight (Test weight)

Thousand kernel weight varied between 20.2-39.6 g (DBW16-36.2g and WH147-39.3g) (Annexure VI). Of these, 57 plants had kernel weight above the mean value of 31.84 g. 12 plants had more 1000 kernel weight than DBW16. Plant number 9 and 10 had 1000 grain weight of 39.46 and 40.16 g respectively that is more than DBW16.

4.11.8 Grain yield per plant

Grain yield per plant in F_3 population ranged from 5.6-24.6 g (DBW16-14.7 g and WH147-9.03 g) (Annexure VI). Of these 90 F_3 plants, 50 plants had grain yield above the mean value of 14.06g.

4.11.9 Biomass per plant

Biomass per plant among 90 F_3 plants varied between 22.4-92.6g (DBW16-63.8 g and WH147-40.5 g) (Annexure VI). Out of 90 F_3 plants 56 plants had biomass per plant above the mean value of 56.14 g.

4.11.10 Days to maturity

Days to maturity in F_3 population ranged from 112-122 days (DBW16-113 days and WH147-120 days). (Annexure VI). Among 90 F_3 plants 44 plants had days to maturity above the mean value of 117 days.

4.11.11 Harvest index

Harvest index in F_3 population varied between 17.16-28.36 (DBW16-20.35 and WH147- 19.72) (Annexure VI). Out of 90 F_3 plants 27 plants had harvest index above the mean value of 25.21.

4.11.12 Grain filling duration

Grain filling duration in F_3 population varied between 29-45 (DBW16-34 and WH147-32). Out of 90 F_3 plants37 plants had grain filling duration above the mean value of 33.9.

4.11.13 grain filling rate

Grain filling rate in F_3 population ranged from 0.52-1.18 (DBW16-1.07 and WH147-0.92). Out of 90 F_3 plants 41 plants had grain filling rate above the mean value of 0.94.

Table 4.11 Range for various phenotypic traits in DBW16 x WH147 F_3 population (late sown)

Sr. No.	Trait	Parents		F_3 Population	
		DBW16	WH47	Mean	Range
1.	Chlorophyll Index	0.636	0.549	0.588	0.513-0.651
2.	Membrane thermostability index	48.4	63.0	52.7626	46.2-65.4
3.	Plant height (cm)	104.3	108.0	110.3593	78-134
4.	Kernel per spike	46	31	43.4259	22-56
5.	Tiller per plant	13	8	10.9407	4-18
6.	Biomass/Plant	63.8	40.5	56.1474	22.4-92.6
7.	Grain Yield (g)	14.7	9.0	14.0673	5.6-24.6
8.	Test weight(g)	36.2	39.3	31.847	20.2-39.6
9.	Days to heading	81	86	83.2148	78-88
10.	Days to maturity	113	120	117.1481	112-122
11.	Harvest Index	20.35	19.72	25.2153	17.16-28.36
12	Grain filling duration	34	32	33.9	29-45
13	Grain filling rate	1.07	0.92	0.94	0.52-1.18

4.12 Estimates for genetic variability parameters for various traits

Phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study indicating negligible influence of the environment on the characters studied in present investigation depicted highest GCV and PCV Grain yield (21.809, 28.239) estimates followed by biomass per plant (21.052, 25.298), kernel per spike (20.054, 20.467), test weight (16.121, 16.364), plant height (12.111, 12.369), membrane thermostability index (12.048, 12.073), harvest index (8.626, 15.780), chlorophyll index (6.743, 6.799), days to heading (2.728, 2.901) and days to maturity (2.087, 2.713) (table 4.12).

Heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and (<70%) tiller per plant and biomass per plant. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.984), membrane thermostability (0.996), plant height (0.959), kernel per spike (0.960) and test weight (0.970). A further perusal of Table 4.12 regarding genetic advance indicated a good scope for improvement for almost all the traits studied as revealed by the genetic advance expressed as percentage of mean. The genetic advance in terms of per cent of mean at 1 per cent selection intensity has been indicated very high for kernel per spike (51.872) and high for biomass per plant (46.250), tiller per plant (45.926), grain yield (44.464) and moderate for membrane thermostability index (31.744) and plant height (31.309) and low for days to heading (6.772), days to maturity (4.238).

4.13 Molecular marker analysis in Parents and F_2 Generation

A total twenty five RAPD and one hundred three simple sequence repeats (SSR) and two known gene primers were used to generate DNA profile of the four parental genotypes and 90 F_2 individual plants of each cross (Hindi62xWH711-Cross I) and (WH147xDBW16- Cross II) with a view to detect segregation pattern for terminal heat stress tolerance.

4.13.1 Qualitative and Quantitative Estimation of DNA

Genomic DNA was isolated from leaves of 2-3 weeks old 90 plants of each wheat cross-I & II using CTAB extraction method of Saghai-Maroof *et al.* (1984). DNA extraction procedure was standardized by using different concentrations of CTAB (1.5% to 2%) at different incubation times (1 h, 1.5 h and 2 h). Best DNA preparations were obtained with 0.2 M Tris buffer (pH 8.0), containing CTAB (1.8%), 1.4 M NaCl and β-mercaptoethanol (2%) with an incubation period of 90 min at 65ºC.

Quantity and quality of the DNA samples was assessed by determining absorbance at 260 nm and 280 nm using UV-spectrophotometer. DNA concentrations were also checked by visual assessment of band intensity in comparison with Lambda DNA of known concentration (50 ng/µl) using 0.8% agarose gel. A ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}) of various DNA samples ranged from 1.50 to 1.87 which indicated that DNA was free from contaminants like polysaccharides, proteins and RNA. The quality of DNA was also checked by agarose gel electrophoresis (0.8%). A single discrete band near the wells was observed in all genotypes showing that genomic DNA was intact, of high molecular weight and free from RNA contamination (Plate 1 a-c and Plate 2 a-c).

Table4.12: Genetic parameters for morphological characters in F_3 generation of DBW16 x WH147 (late sown)

Sr. No.	Characters	Genetic Parameters						
		GCV	PCV	H^2	GA		Genetic advance as % of mean	
					5%	1%	5%	1%
1.	Chlorophyll Index	6.743	6.799	0.984	0.081	0.104	13.777	17.655
2.	Membrane thermostability index	12.048	12.073	0.996	13.069	16.749	24.770	31.744
3.	Plant height (cm)	12.111	12.369	0.959	26.961	34.552	24.430	31.309
4.	Kernel per spike	20.054	20.467	0.960	17.577	22.526	40.476	51.872
5.	Tiller per plant	20.487	24.127	0.721	3.921	5.025	35.836	45.926
6.	Biomass/Plant	21.052	25.298	0.693	20.263	25.968	36.089	46.250
7.	Grain Yield (gm)	21.809	28.239	0.596	4.881	6.255	34.696	44.464
8.	Test weight(gm)	16.121	16.364	0.970	10.419	13.352	32.714	41.925
9.	Days to heading	2.728	2.901	0.884	4.397	5.635	5.284	6.772
10.	Days to maturity	2.087	2.713	0.592	3.874	4.965	3.307	4.238
11.	Harvest Index	8.626	15.780	0.299	2.449	3.139	9.713	12.448
12.	Grain filling duration	11.685	13.452	0.736	13.534	15.976	17.654	19.423
13.	Grain filling rate	10.523	12.658	0.832	14.642	17.242	18.643	20.286

4.13.2 RAPD analysis

4.13.2.1 Optimization of PCR amplification conditions

PCR amplification conditions viz. concentration of template DNA, primers, $MgCl_2$, Taq DNA polymerase and annealing temperature were also optimized for RAPD primers

. For this, varying concentrations of template DNA (15 ng, 25 ng, 50 ng and 100 ng), primer (0.25 μM, 0.5 μM and 1 μM), $MgCl_2$ (1.0 mM, 1.5 mM, 2.0 mM and 2.5 mM) and Taq DNA polymerase (0.8 U, 1 U and 1.5 U) were used in a reaction volume of 20 μl. Different annealing temperatures viz. 32°C, 34°C, 36°C and 38°C were used during PCR amplification for RAPD primers.

In general, inconsistent bands were obtained at annealing temperature of 32ºC. At 36°C and 38ºC annealing temperatures, very few DNA fragments were observed while at 34ºC annealing temperature more number of bands were observed than at 36°C or 38ºC, and consistent too. Hence 34ºC was kept as optimum annealing temperature for PCR amplification to study polymorphism. Concentration of $MgCl_2$ was varied from 1.0 to 2.5 mM and a concentration of 1.5 mM was found to be optimum for generating clear and reproducible bands. Varying concentrations i.e. 0.8 U, 1.0 U and 1.5 U of Taq DNA polymerase per reaction mix (20 μl) were also used.

In brief, reproducible and clear banding patterns were obtained in a reaction mixture of 20 μl containing 25 ng of template DNA, 200 μM of each dNTP, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 μM primer and 1.5 U Taq DNA polymerase. The annealing temperature of 34ºC was found to be optimum for generating clear and reproducible bands. Therefore, the thermocycler was programmed for an initial denaturation step of 4 min at 94ºC, followed by 40 cycles of denaturation (94ºC, 1 min), annealing (34ºC, 1 min) and extension (72ºC, 2 min) followed by a final extension of 72ºC for 8 min. and a hold temperature of 4ºC at the end that resulted in clear and reproducible bands.

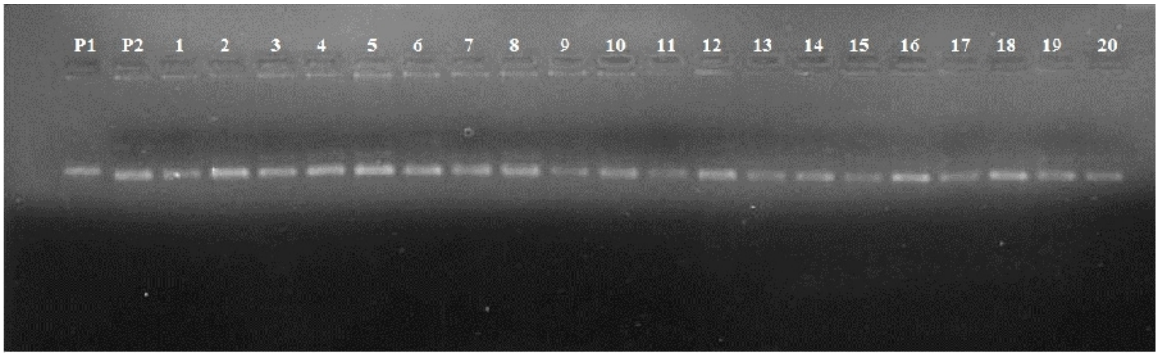
4.13.2.2 Polymorphism among parents and F_2 population of wheat using RAPD primers

Twenty-five RAPD primers were used for the present investigation, out of which 14 primers showed amplification and 11 primers showed no amplification in cross Hindi62xWH711. Using a total of 14 RAPD primers, 107 amplified bands were obtained of which 63 were polymorphic. The DNA amplification and polymorphism generated with 14 RAPD primers are presented in Table 4.13. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified bands varied between 2 (primer RAPD-61) and 13 (primer RAPD-9) with an average of 7 bands per primer. The polymorphism percentage ranged from as low as 16.7% (primer RAPD-6) to as high as 90.9% (primer RAPD-49). Average polymorphism across the population was found to be 58.87%. Overall size of PCR amplified products ranged between 280 bp and 4000 bp.

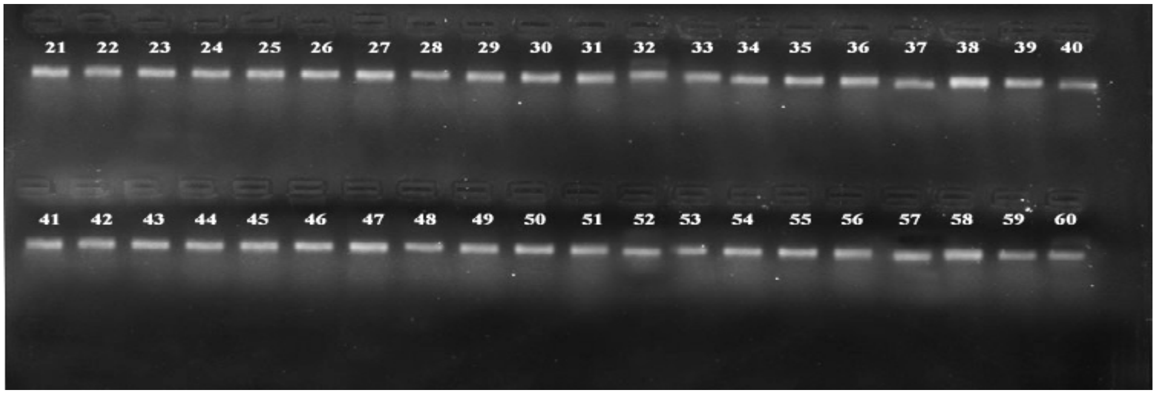
In cross DBW16 x WH147, 96 amplified bands were obtained of which 56 were polymorphic. The DNA amplification and polymorphism generated among the parents and F_2 population using RAPD primers are presented in Table 4.14. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified bands varied between 4 (primer RAPD-10) and 11 (primer RAPD-49) with an average of 6.85 bands per primer. The polymorphism percentage ranged from as low as 25% (primer RAPD-4) to as high as 83.7% (primer RAPD-3).

Table 4.13: DNA amplification profile and polymorphism generated in parents and F_2 population of cross (Hindi62 x WH711) wheat using 14 RAPD primers

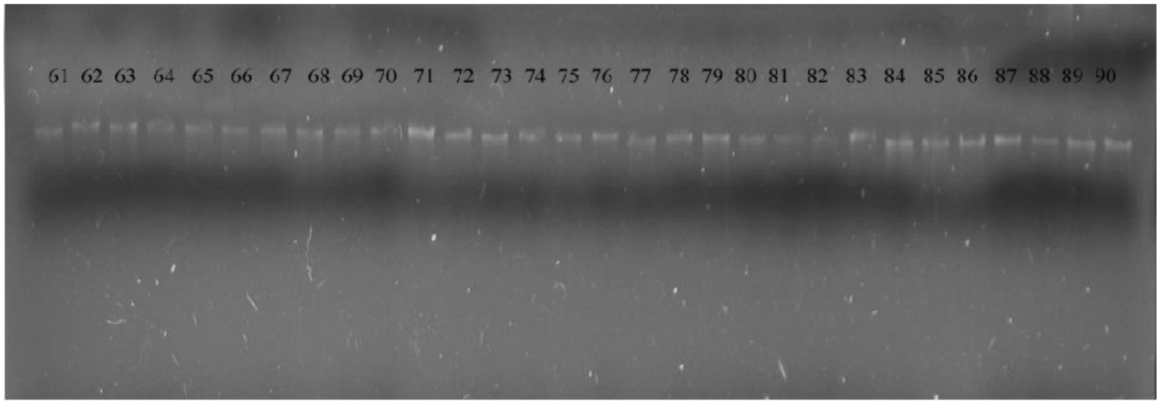
Sr. No.	Primer	Molecular Weight range (bp)	Total no. of bands	No. of Monomorphic bands	No. of Polymorphic bands	Percentage Polymorphism
1	RAPD-2	450-2100	7	2	5	66.7
2	RAPD-3	400-4000	7	1	6	85.7
3	RAPD-4	800-2500	4	3	1	25.0
4	RAPD-5	550-4000	7	3	4	57.1
5	RAPD-6	600-4000	6	5	1	16.7
6	RAPD-9	300-2400	13	9	4	30.8
7	RAPD-10	950-4000	4	2	2	50.0
8	RAPD-14	800-2000	7	2	5	71.4
9	RAPD-15	800-1800	8	2	6	75.0
10	RAPD-16	500-4000	9	4	5	55.6
11	RAPD-45	500-2600	5	3	2	40.0
12	RAPD-47	350-1600	8	2	6	75.0
13	RAPD-49	430-2500	11	1	10	90.9
14	RAPD-60	280-1500	9	3	6	66.7



(a)

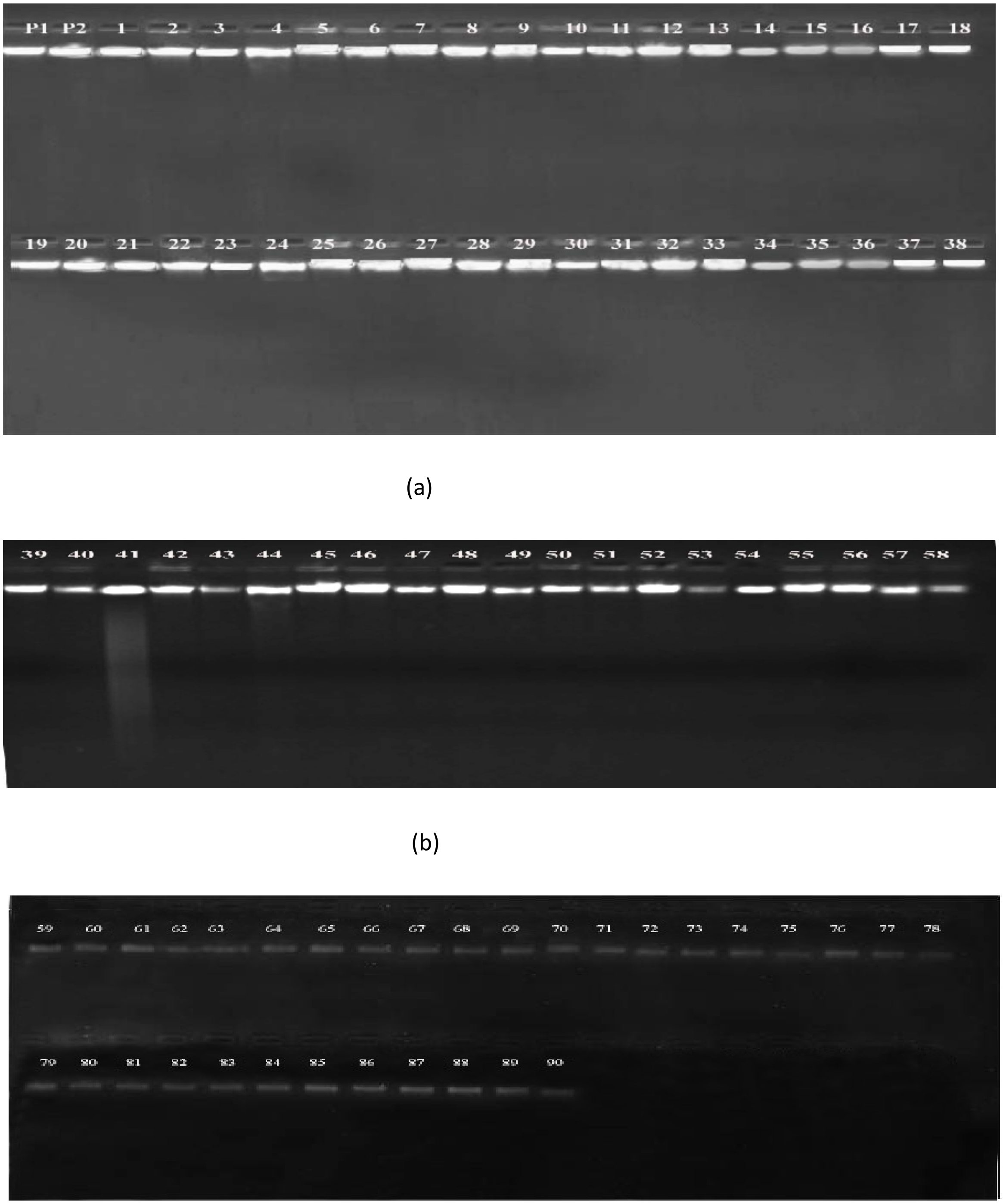


(b)



(c)

Plate 1: Agarose gel (0.8%) showing genomic DNA (a, b & c) from Hindi62 (P1), WH711 (P2) and F_2 plants (1-90) of Hindi62 x WH711

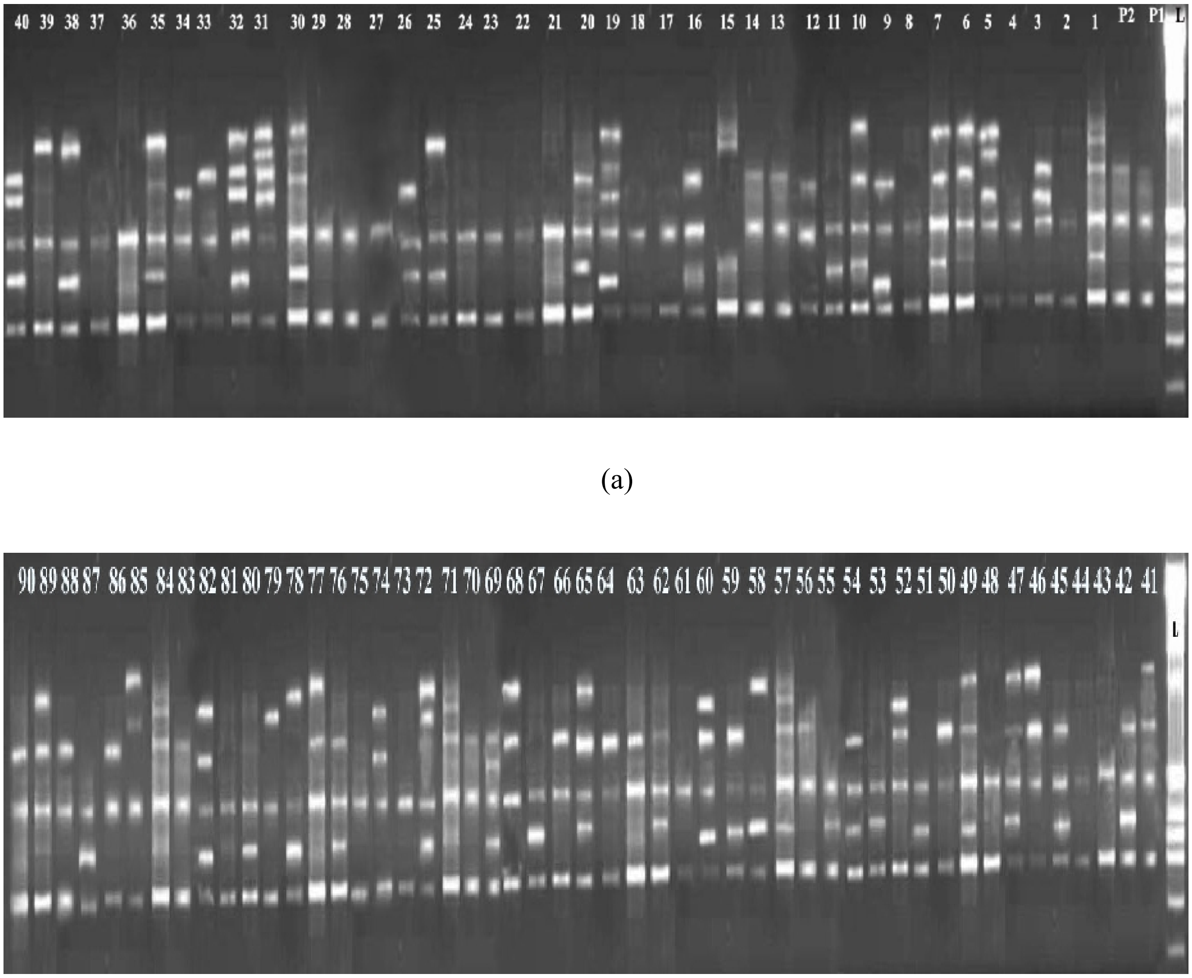


(a)

(b)

(c)

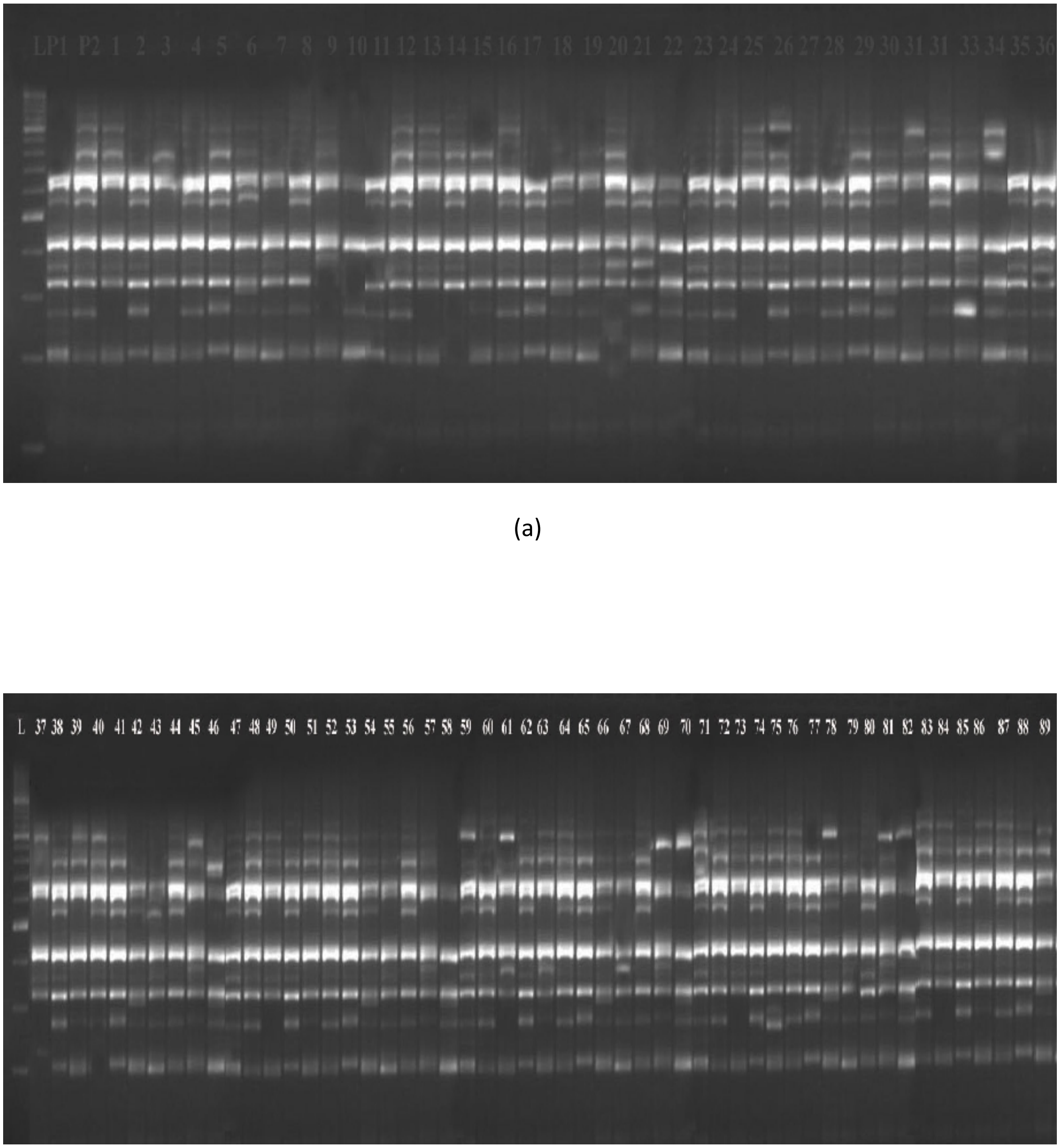
Plate 2: Agarose gel (0.8%) showing genomic DNA (a, b & c) from DBW16 (P1), WH147 (P2) and F_2 plants (1-90) of DBW16 x WH147



(a)

(b)

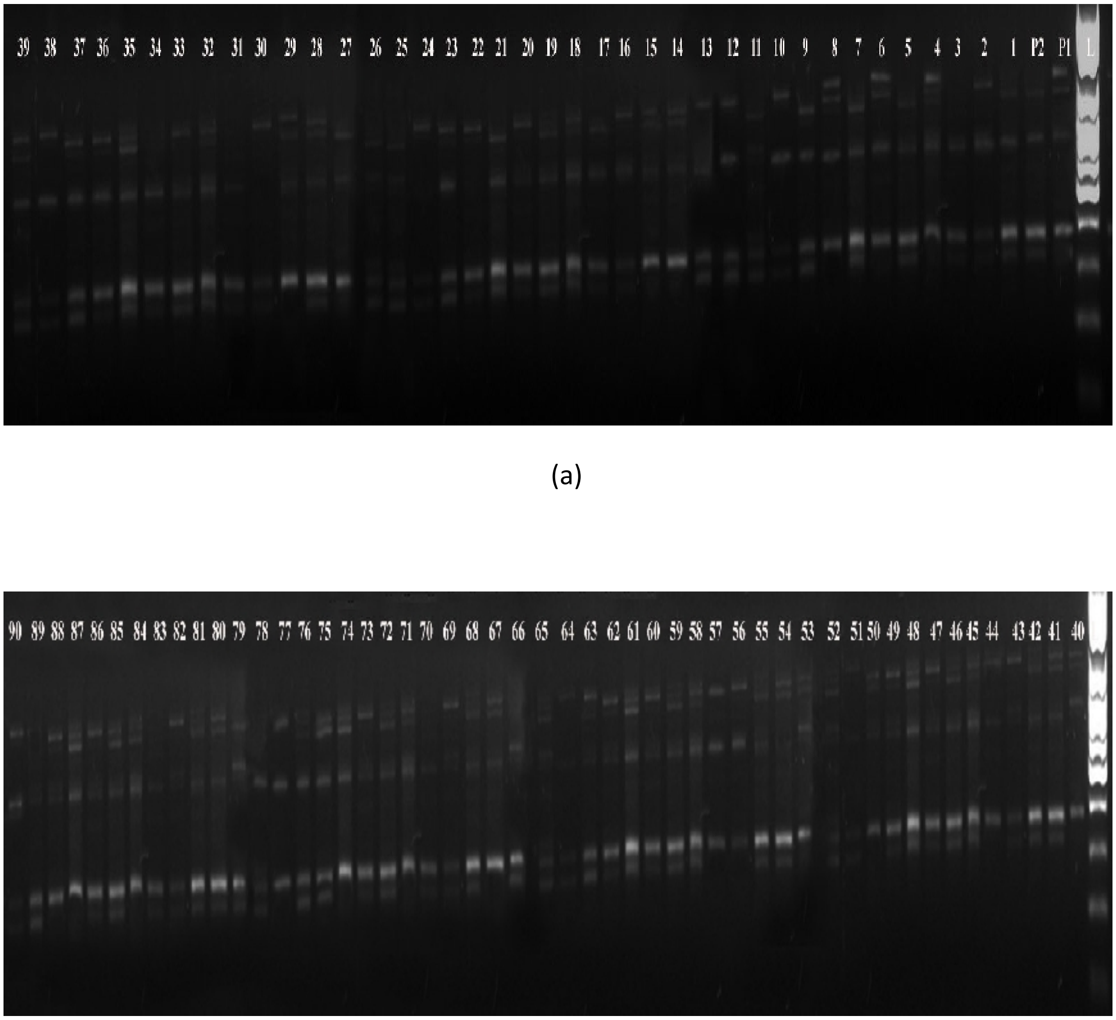
Plate 3: Agarose gel (1.5%) showing PCR amplification of genomic DNA from Hindi62 (P1) WH711 (P2) and F_2 plants (1-90) using RAPD-2 primer



(a)

(b)

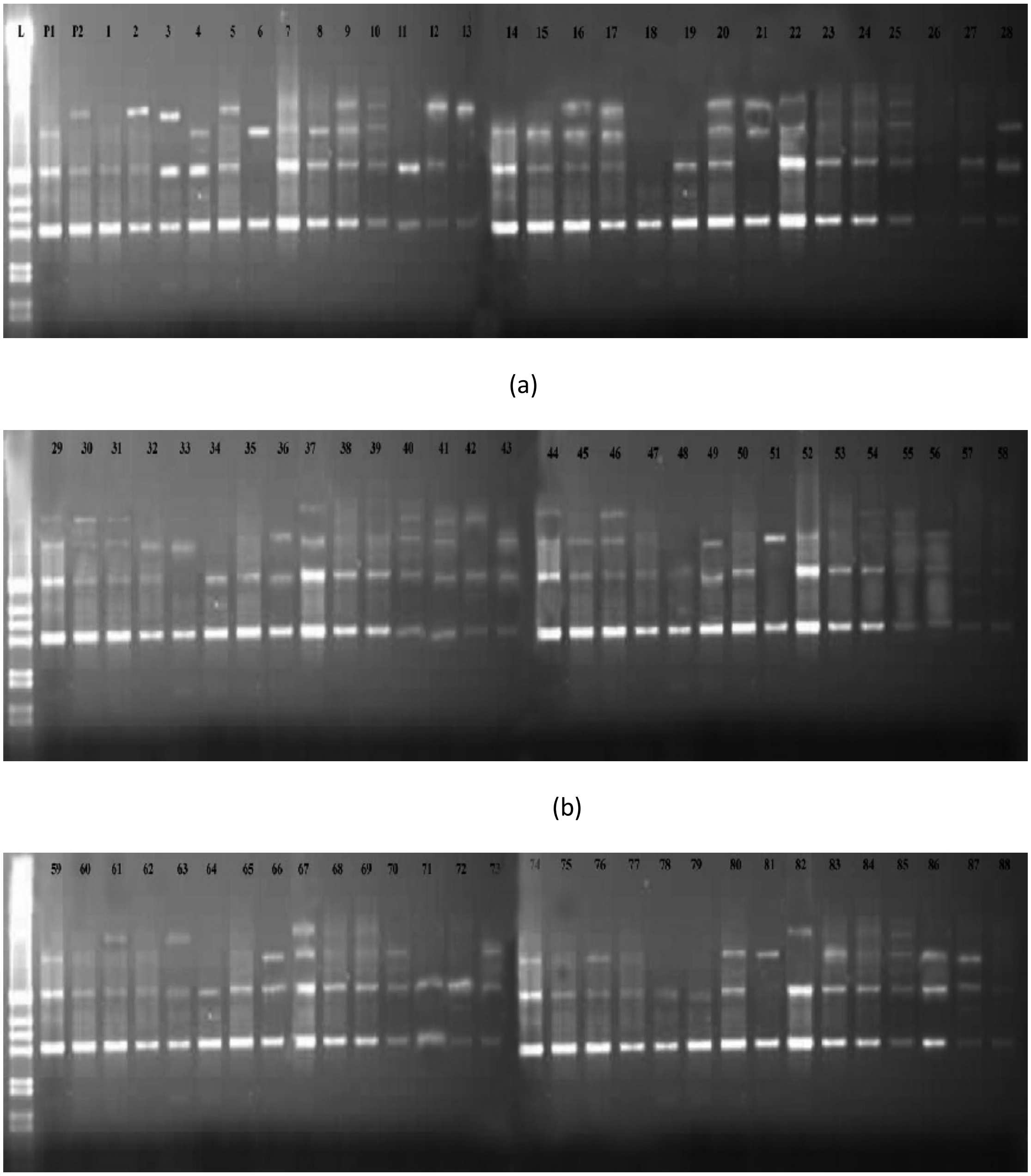
Plate 4: Agarose gel (1.5%) showing PCR amplification of genomic DNA from Hindi62 (P1), WH711 (P2) and F_2 plants (1-90) using RAPD-16 primer



(a)

(b)

Plate 5: Agarose gel (1.5%) showing PCR amplification of genomic DNA from DBW16 (P1), WH147 (P2) and F_2 plants (1-90) using RAPD-6 primer



(a)

(b)

(c)

Plate 6: Agarose gel (1.5%) showing PCR amplification of genomic DNA from DBW16 (P1), WH147 (P2) and F_2 plants (1-90) using RAPD-10 primer

4.13.2.3 Cluster Tree Analysis in Hindi62 x WH711 (Cross-I):

The hierarchical cluster analysis showed that the genotypes were mainly divided into two major clusters at a similarity coefficient of 0.67 (Fig. 4.1). Cluster–I was divided into two sub clusters at the similarity coefficient of 0.82.

Similarly, the cluster-II is divided into 2 sub clusters at a similarity coefficient of 0.72.These sub clusters are further divided into many sub clusters. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH711 (Figure 4.2, 4.3).

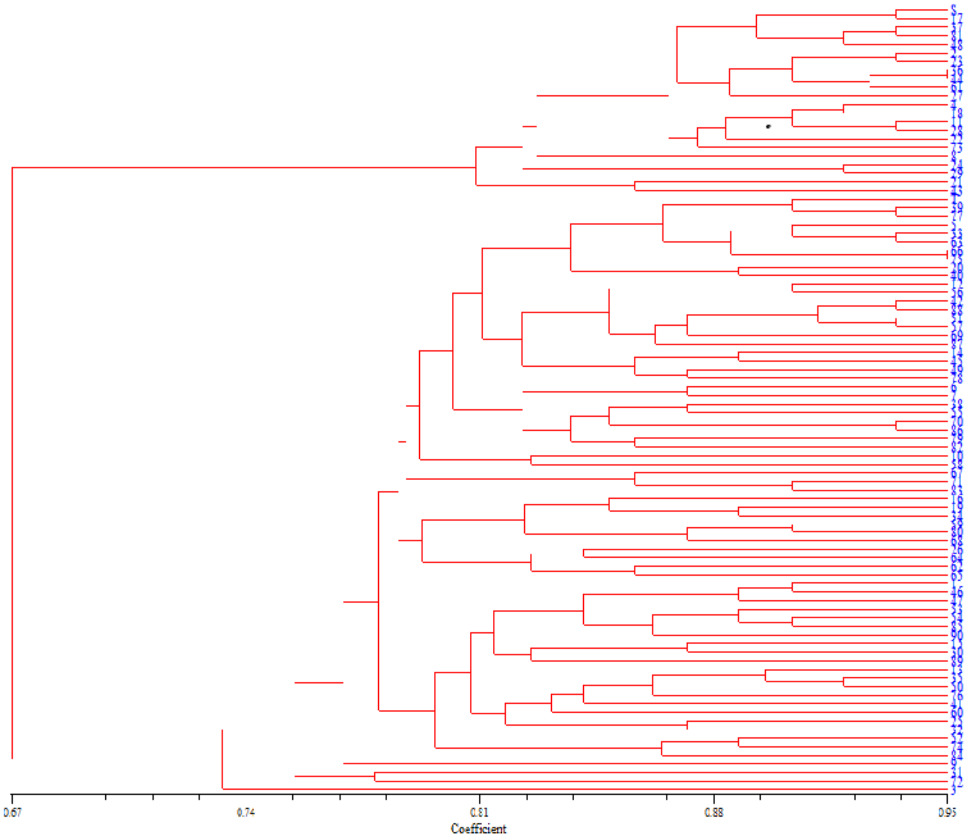


Fig.4.1: Dendrogram showing relationship among 90 individual plants of F_2 population of Hindi62 × WH711 along with both parents generated by UPGMA analysis based on single primers using polymorphic RAPD primer pairs

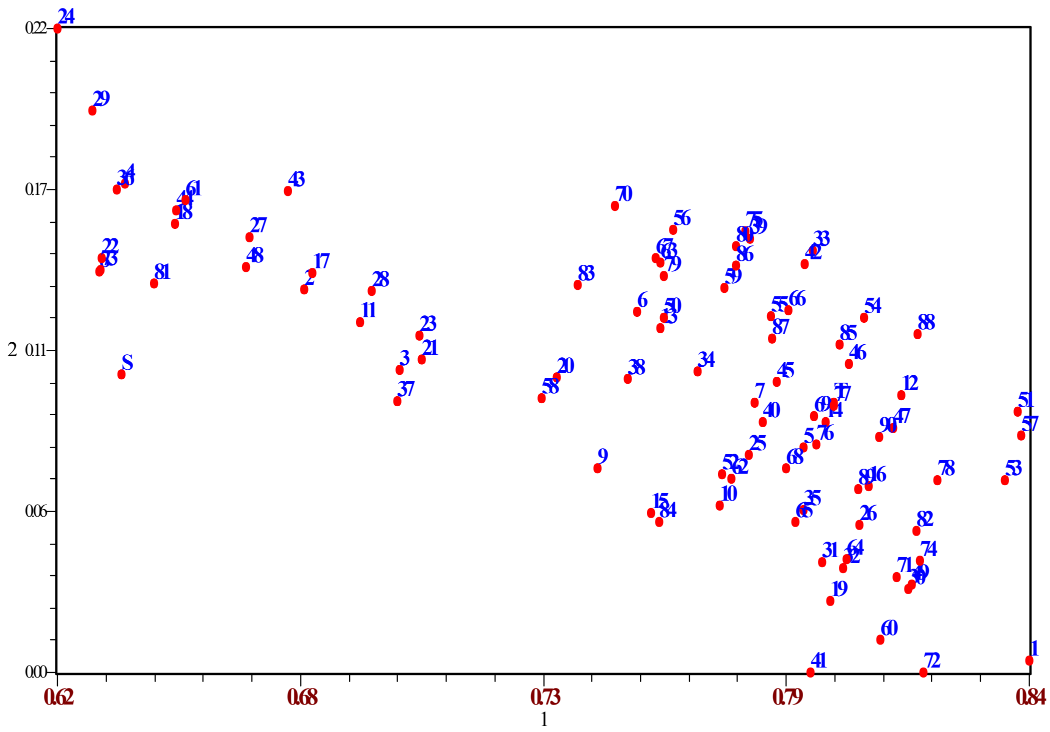


Figure 4.2: Two dimensional PCA scaling of Hindi62 x WH711 F_2 plants and parental wheat genotypes based on RAPD analysis.

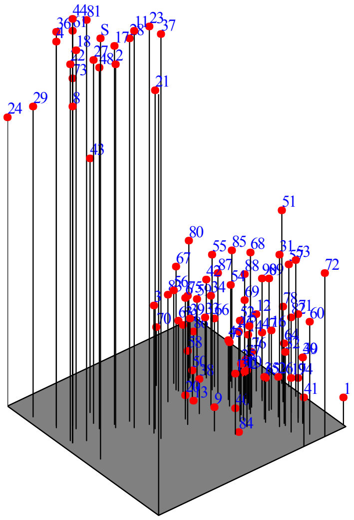


Figure 4.3: Three dimensional PCA scaling of Hindi61 x WH711 F_2 plants and parental wheat genotypes based on RAPD analysis.

4.13.2.4 Cluster Tree Analysis in DBW16 x WH147 (Cross-II):

The hierarchical cluster analysis showed that the genotypes were mainly divided into two major clusters at a similarity coefficient of 0.67 (Fig. 4.4). Cluster–I was divided into two sub clusters at the similarity coefficient of 0.78.

Similarly, the cluster-II is divided into 2 sub clusters at a similarity coefficient of 0.73.These sub clusters are further divided into many sub clusters. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards DBW16 (Figure 4.5, 4.6).

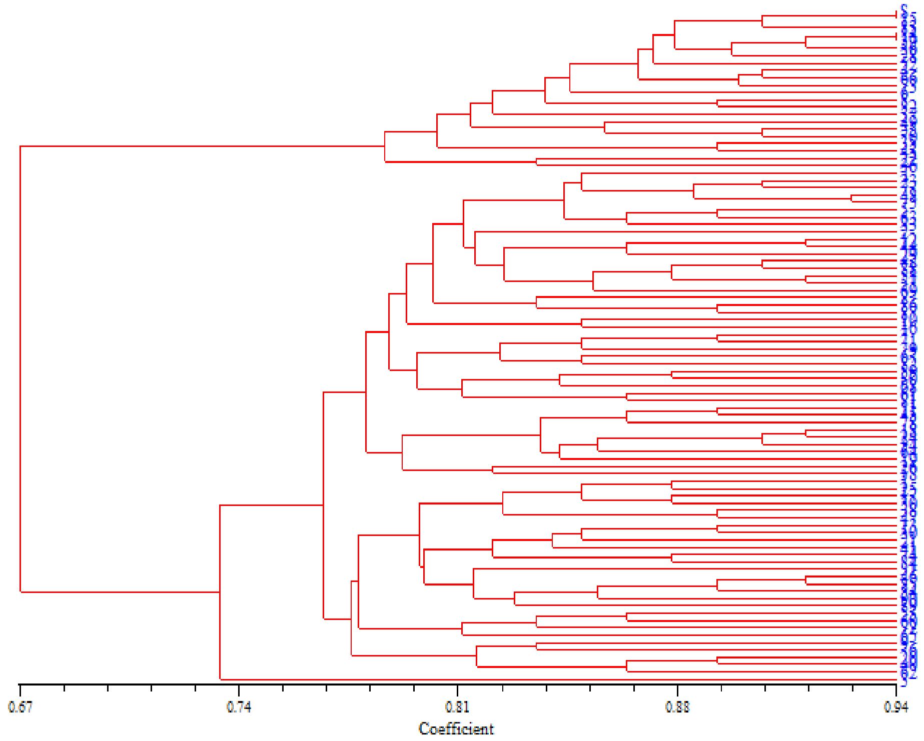


Fig.4.4: Dendrogram showing relationship among 90 individual plants of F_2 population of DBW16 x WH147 along with both parents generated by UPGMA analysis based on single primers using polymorphic RAPD primer pairs

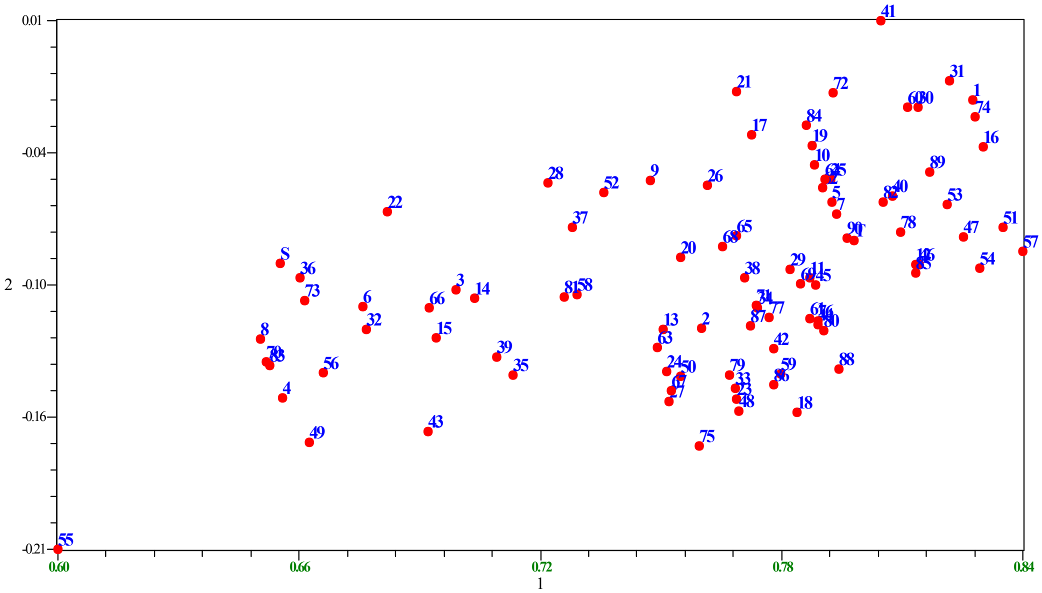


Figure 4.5: Two dimensional PCA scaling of DBW16 x WH147 F_2 plants and parental wheat genotypes based on RAPD analyais.

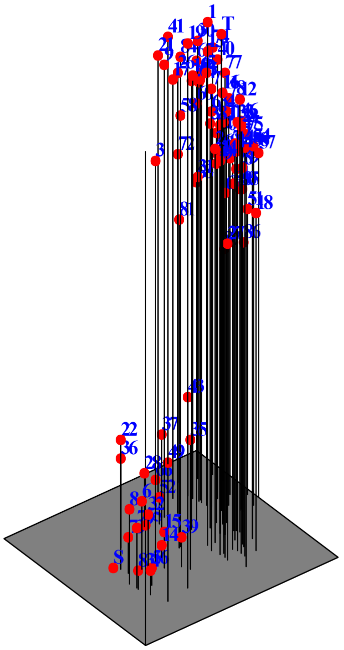


Figure 4.6: Three dimensional PCA scaling of DBW16 x WH147 F_2 plants and parental wheat genotypes based on RAPD analyais.

Table 4.14: DNA amplification profile and polymorphism generated in parents and F_2 population of cross (DBW16 x WH147) of wheat using RAPD primers

Sr. No.	Primer	Molecular Weight range (bp)	Total no. of bands	No. of Monomorphic bands	No. of Polymorphic bands	Percentage Polymorphism
1	RAPD-2	400-2000	5	2	3	60.0
2	RAPD-3	400-3500	6	1	5	83.7
3	RAPD-4	800-2500	4	3	1	25.0
4	RAPD-5	550-4000	6	3	3	50.0
5	RAPD-6	500-4000	6	4	2	33.7
6	RAPD-9	300-2000	9	5	4	44.6
7	RAPD-10	950-4000	4	2	2	50.0
8	RAPD-14	600-2000	6	2	4	64.4
9	RAPD-15	800-1800	8	2	6	75.0
10	RAPD-16	500-4000	7	4	3	57.1
11	RAPD-45	500-2600	5	3	2	40.0
12	RAPD-47	350-1600	6	3	3	50.0
13	RAPD-49	460-2500	8	5	3	62.5
14	RAPD-60	300-1500	7	3	4	42.8

4.13.3 SSR Analysis

4.13.3.1 Optimization of PCR Amplification Conditions for SSR

PCR amplification conditions viz. concentration of template DNA, primers, $MgCl_2$, Taq DNA polymerase and annealing temperature were optimized. Different temperature and time intervals during denaturation, annealing and elongation steps were also optimized which affect amplification, banding pattern and reproducibility. For this, varying concentrations of template DNA (25 ng, 50 ng and 75 ng), primer (0.2 μM, 0.3 μM and 0.4 μM), $MgCl_2$ (1.5 mM, 2.0 Mm and 2.5 mM), and Taq DNA polymerase (0.8U, 1U, 1.2U and 1.5U) were used in a reaction volume of 25 μl. Genomic DNA concentration varying from 25-75 ng was used for PCR amplification. Lower concentration of template DNA gave faint bands. Most satisfactory results of amplification were obtained with a concentration of 50 ng genomic DNA per reaction.

Among other parameters, the concentration of $MgCl_2$ is reported to be the most important factor for unambiguous and reproducible DNA amplification. Suboptimal concentrations of $MgCl_2$ often result in reduced amplicon products. In the present study concentration of $MgCl_2$ was tested from 1.5 to 3.0 mM and a concentration of 1.5 mM was found to be optimum for generating clear and reproducible bands. Varying concentrations i.e. 0.8U, 1.0U, 1.2U and 1.5U of Taq DNA polymerase per reaction mix (25 μl) were also used.

Reproducible and clear banding patterns were obtained in a reaction mixture of 25μl containing 50 ng of template DNA, 200 μM of each dNTP, 1X PCR buffer, 1.5mM $MgCl_2$, 0.2μM primer and 1.2U Taq DNA polymerase. Different annealing temperatures (55°C to 67°C) were used during PCR amplification for and SSR primers.

4.13.3.2 Polymorphism among F_2 population of wheat

One hundred and three SSR primers were used for the present investigation, out of which 67primers showed polymorphism between Hindi62 x WH711 parental genotypes. Using a total of 67 SSR primers, 180 amplified bands were obtained of which 131 were polymorphic. The DNA amplification and polymorphism generated among various plants of F2 population using SSR primers are presented in Table 4.15. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified alleles varied between 2 (primer Xgwm 190) and 6 (primer Xbarc 136) with an average of 3 alleles per primer. The polymorphism percentage ranged from as low as 20 (primer WMC 149) to as high as 100% (primer WMC 110). Average polymorphism across the population was found to be 77.09%. Overall size of PCR amplified products ranged between 90 bp and 1120 bp. Out of 103 SSR primers, 64 primers showed polymorphic bands/alleles between DBW16 and WH147 and were used to genotype 90 individual plants of F_2 population raised from DBW16 x WH147. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified alleles varied between 2 (primer Xgwm 169) and 5 (primer WMC 416) with an average of 2.48 alleles per primer.. Average polymorphism across the population was found to be 77.35%.

Table 4.15: DNA amplification profile and polymorphism generated in parents and F_2 population of wheat using 67 SSR primers in cross Hindi62 x WH711

Sr. No.	Primer	Number of alleles	Allele size (bp)
1	Xgwm 95	2	110, 140
2	Xgwm 261	2	165, 195
3	Xgwm 604	3	160, 180, 215
4	Xgwm 526	2	120, 170
5	Xgwm 111	2	120, 180
6	Xgwm 617	2	105, 195
7	Xgwm 46	2	150, 200
8	Xgwm 186	2	110, 185
9	Xgwm356	2	140, 165
10	Xgwm 389	2	100, 155
11	Xgwm 455	5	125, 160, 185, 240, 310
12	Xgwm 156	2	190, 225
13	Xgwm 610	2	185, 210
14	Xgwm 33	2	110, 350
15	Xgwm 513	3	95, 135, 185
16	Xgwm 18	2	100, 215
17	Xgwm 382	3	95, 150, 185

18	Xgwm 193	3	125, 140, 155
19	Xgwm 3	3	140, 150, 170
20	Xgwm 190	2	105, 125
22	Xbarc136	6	125, 225, 450, 700, 980, 1120
23	WMC 120	2	140, 165
24	WMC 169	2	115, 200
25	WMC 105	3	100, 170, 265
26	WMC 170	2	105, 140
27	Xbarc188	2	160, 215
28	BARC19	2	120, 145
29	BARC26	2	110, 210
30	BARC28	3	165, 200
31	BARC45	4	130, 200
32	BARC48	2	130, 240
33	BARC113	3	140, 190, 220
34	BARC133	5	130, 165, 190, 220, 250
35	BARC187	3	100, 140, 190
36	BARC236	2	110, 220
37	BARC263	2	130, 180
38	BARC275	2	110, 140
39	BARC288	2	95, 160
40	CFA2104	3	120, 220
41	CFA2262	3	90, 100, 110
42	CFA2292	2	120, 210
43	CFD239	2	105, 190
44	WMC 642	2	140, 175
45	WMC110	2	100, 200
46	WMC149	5	135, 160, 190,260, 280

47	WMC232	3	185, 210, 230
48	WMC254	5	105, 120, 150, 180, 200
49	WMC296	2	115, 210
50	WMC349	3	110, 140, 190
51	WMC398	2	100, 150
52	WMC413	4	100, 130, 180, 240
53	WMC416	5	90, 130, 165, 220, 320
54	WMC475	2	185, 220
55	WMC601	2	160, 200
56	WMC727	2	120, 320
57	WMC758	2	105, 145
58	XGWM219	5	180, 250
59	XGWM261	2	165, 200
60	XGWM443	4	150, 180, 200, 230
61	XGWM515	2	170, 210
62	XGWM642	3	165, 190, 225
63	XCFD223	3	130, 180, 240
64	Xgwm311	5	130, 150, 170, 180, 190
65	WMC25	2	110, 210
66	WMC215	2	130, 200
67	WMC273	3	120, 190

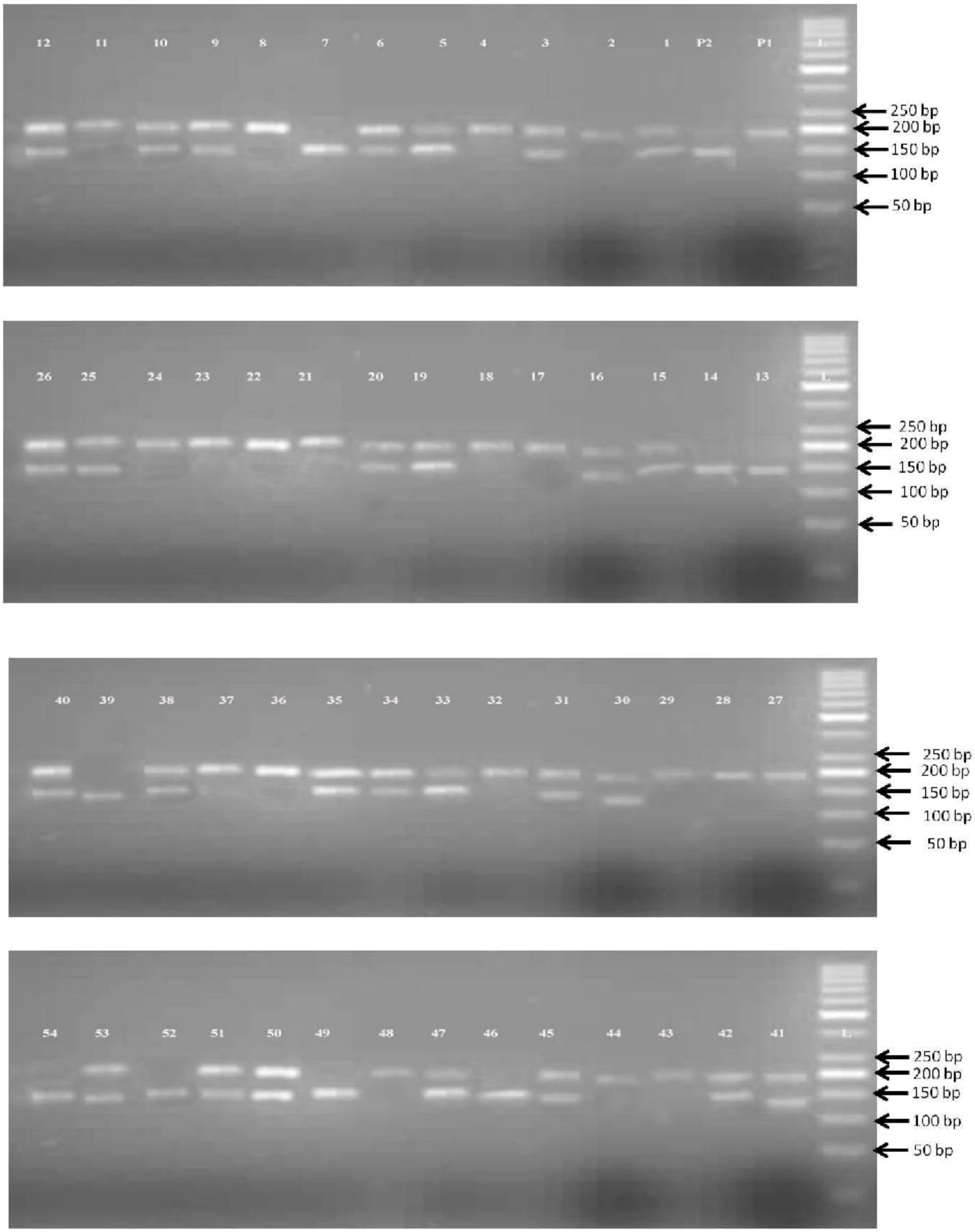


Plate-7(a): Agarose gel (2.5%) showing allelic polymorphism for primer Xgwm 356 among F_2 population of wheat cross-I (Hindi62 x WH711); Lane L-50 bp ladder Lane, P_1- Hindi 62 ; P_2- WH711

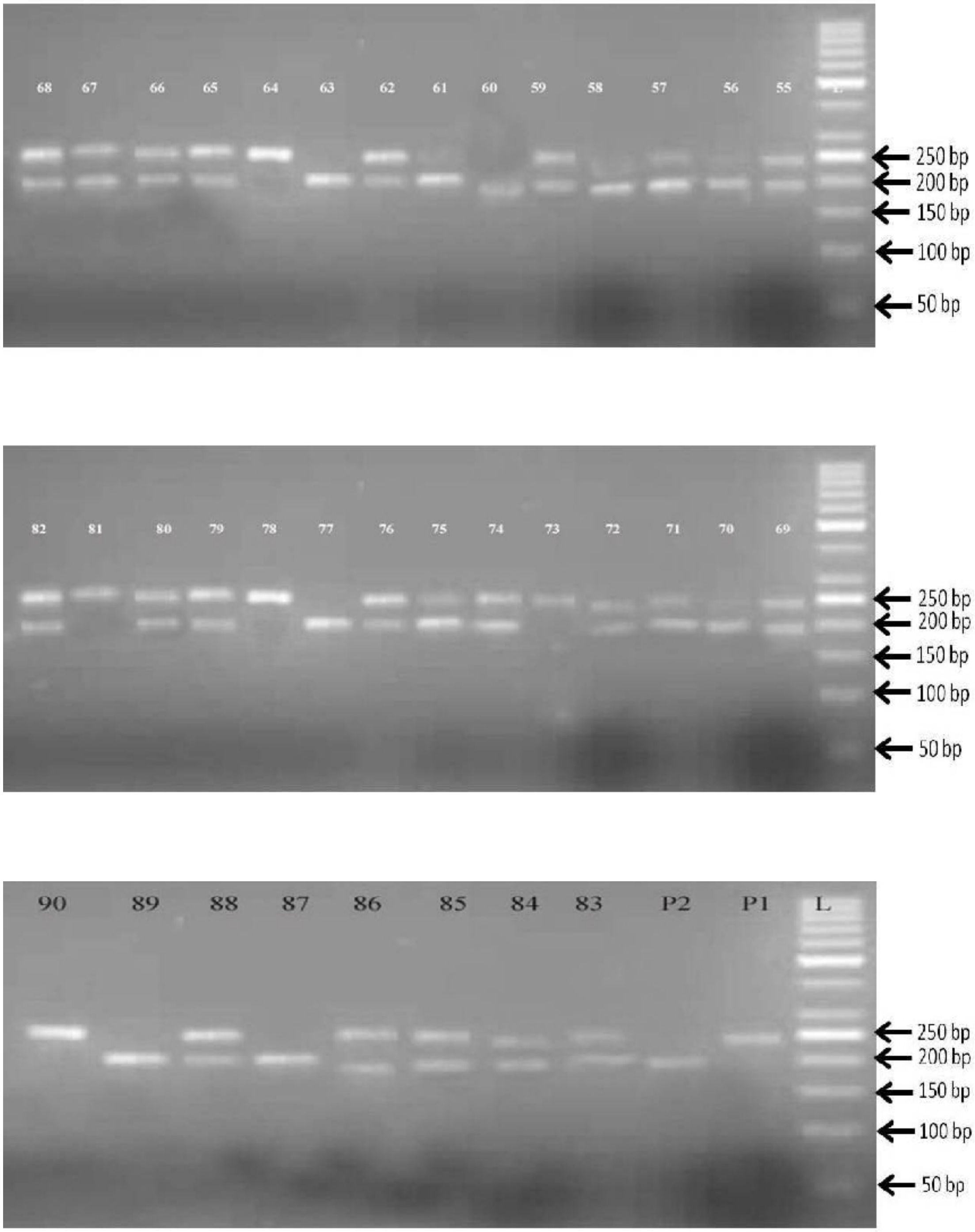
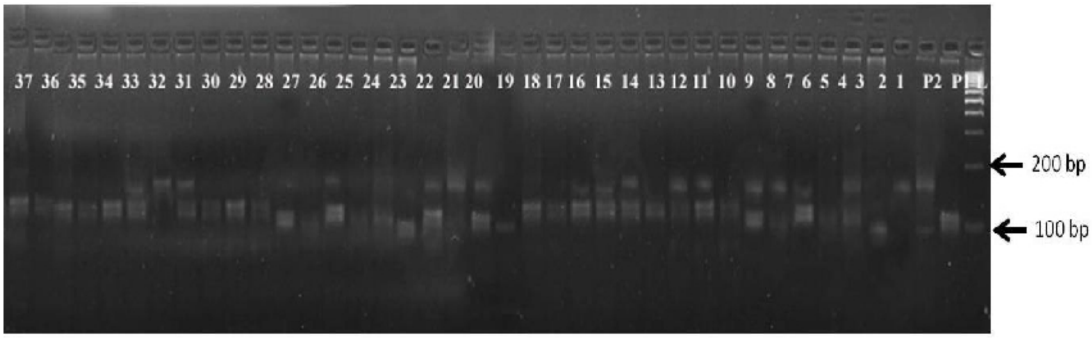
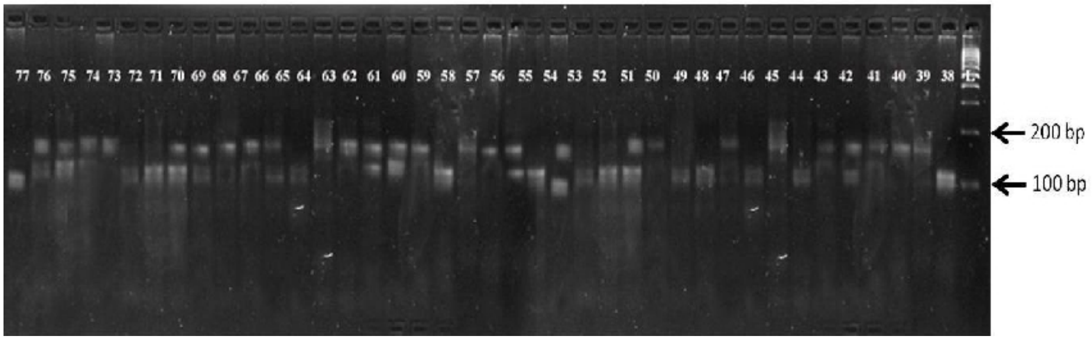


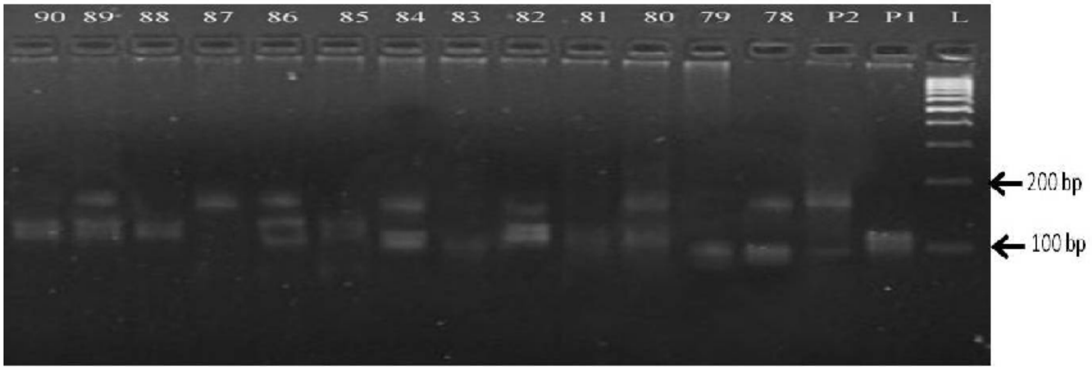
Plate-7(b): Agarose gel (2.5%) showing allelic polymorphism for primer Xgwm 356 among F_2 population of wheat cross-I (Hindi62 x WH711); Lane L-50 bp ladder Lane, P_1- Hindi 62 ; P_2- WH711



(a)



(b)



(c)

Plate-8: Agarose gel (2.5%) showing allelic polymorphism for primer WMC642 among F_2 population of wheat cross-I (Hindi62 x WH711); Lane L-100 bp ladder Lane, P_1- Hindi 62; P_2- WH711

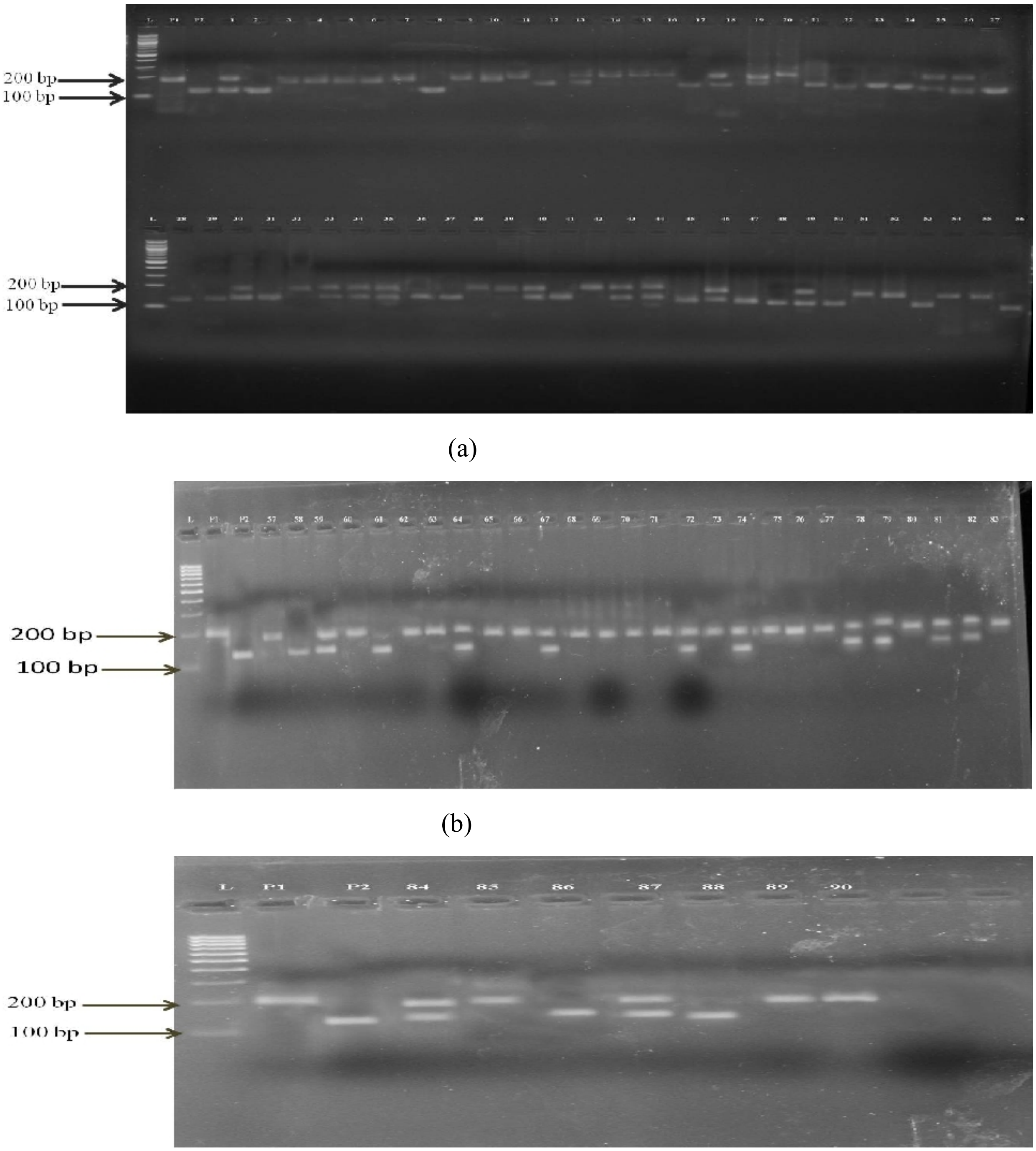


Plate-9: Agarose gel (2.5%) showing allelic polymorphism for primer Cfa2292 among F_2 population of wheat cross-I (Hindi62 x WH711); Lane L-100 bp ladder Lane, P_1- Hindi 62; P_2- WH711

4.13.3.3 Cluster Tree Analysis in Hindi62 x WH711 (Cross-I):

The hierarchical cluster analysis showed that the genotypes were mainly divided into two major clusters at a similarity coefficient of 0.53 (Fig. 4.7). Cluster–I was divided into two sub clusters at the similarity coefficient of 0.82.

Similarly, the cluster-II is divided into 2 sub clusters at a similarity coefficient of 0.62.These sub clusters are further divided into many sub clusters. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH711 (Figure 4.8, 4.9).

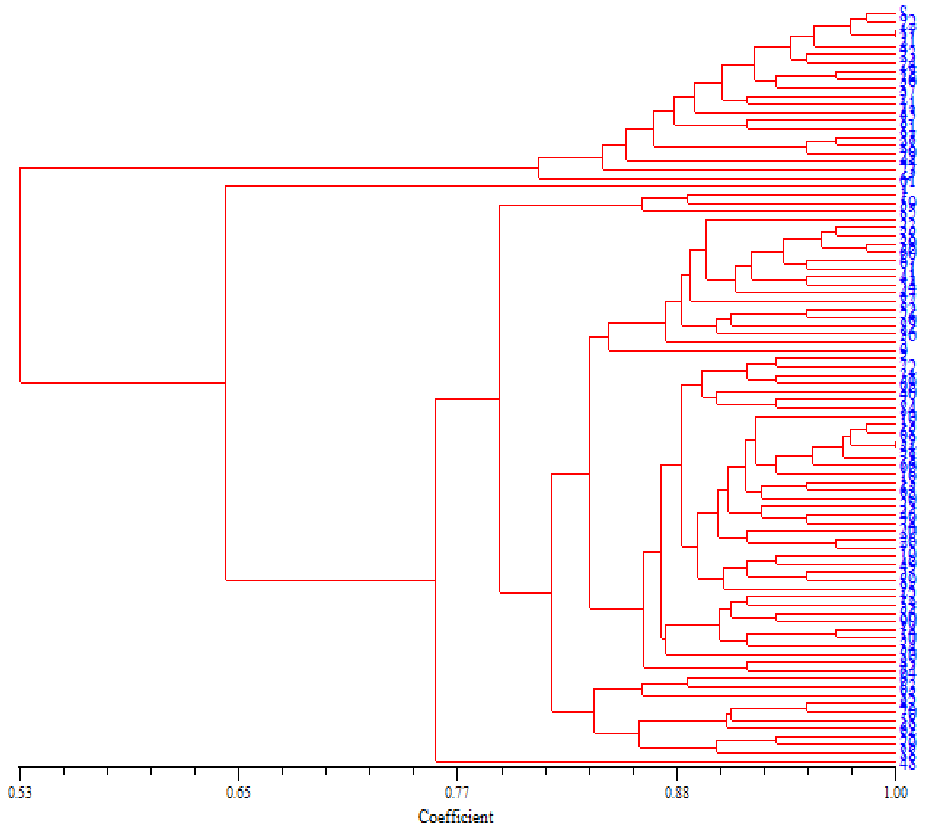


Fig.4.7: Dendrogram showing relationship among 90 individual plants of F_2 population of Hindi62 × WH711 along with both parents generated by UPGMA analysis based on single primers using polymorphic SSR primer pairs

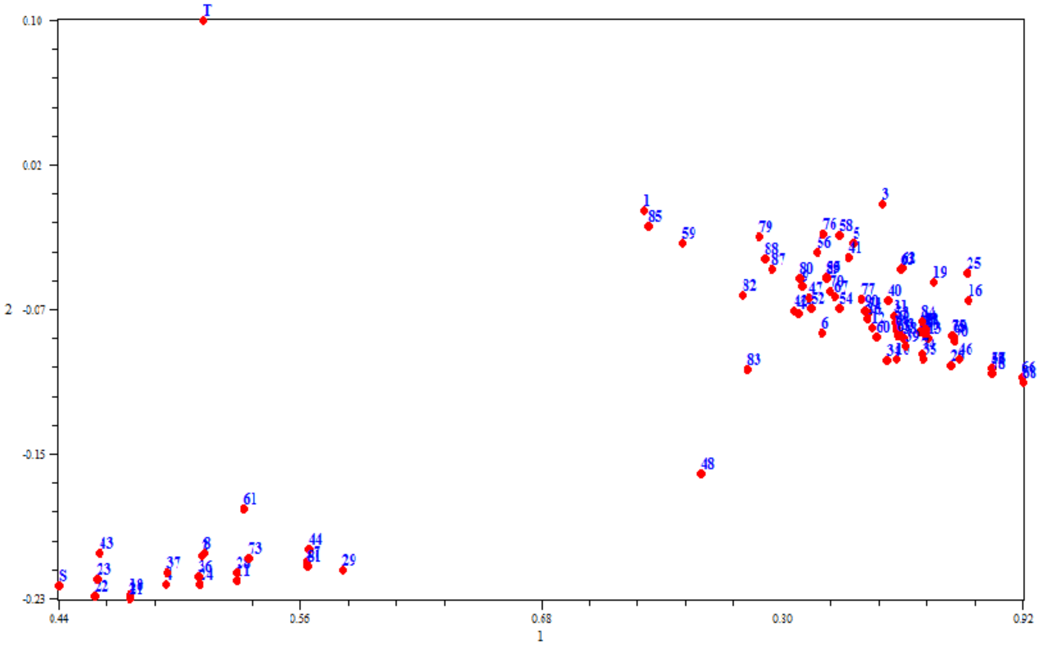


Figure 4.8: Two dimensional PCA scaling of Hindi61 x WH711 F_2 plants and parental wheat genotypes based on SSR analysis.

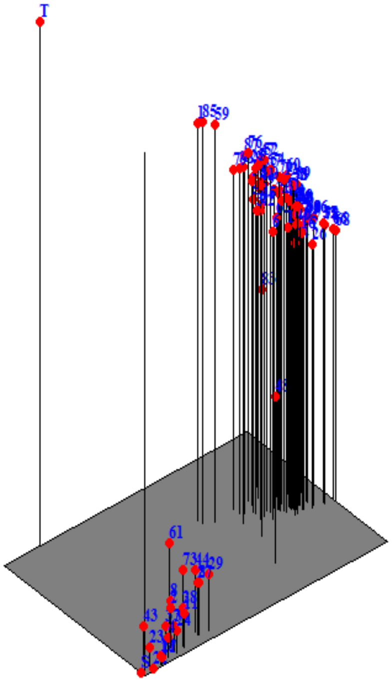


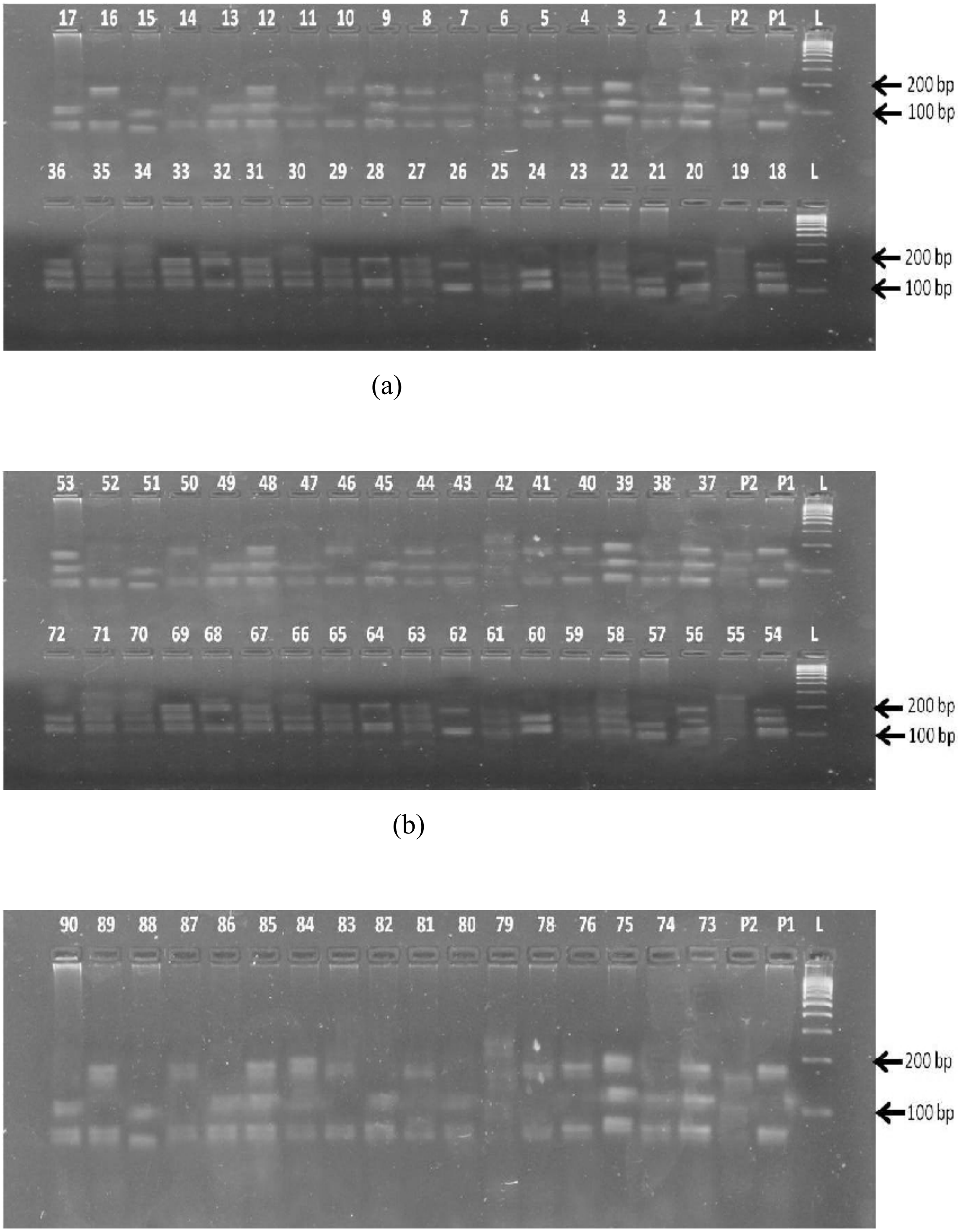
Figure 4.9: Three dimensional PCA scaling of Hindi61 x WH711 F_2 plants and parental wheat genotypes based on SSR analysis.

Table 4.16: DNA amplification profile and polymorphism generated in parents and F_2 population of wheat using 64 SSR primers in cross DBW16 x WH147

Sr. No.	Primer	Total No. of bands	Allele size (bp)
1	Xgwm 95	2	110, 160
2	Xgwm 261	2	165, 215
3	Xgwm 604	3	160, 185, 220
4	Xgwm 526	2	130, 190
5	Xgwm 111	2	140, 180
6	Xgwm 617	2	105, 190
7	Xgwm 46	2	135, 200
8	Xgwm 186	2	120, 185
9	Xgwm356	2	120, 180
10	Xgwm 389	2	100, 170
11	Xgwm 455	3	140, 220, 310
12	Xgwm 156	2	190, 240
13	Xgwm 610	2	160, 200
14	Xgwm 33	2	110, 250
15	Xgwm 513	3	90, 190
16	Xgwm 18	2	100, 205
17	Xgwm 382	2	95, 170
18	Xgwm 193	3	125, 160, 185
19	Xgwm 3	3	150, 165, 190

20	Xgwm 190	2	105, 140
21	Xbarc136	3	125, 230, 340
22	WMC 120	2	120, 195
23	WMC 169	2	110, 170
24	WMC 105	3	105, 135, 160
25	WMC 170	2	120, 170
26	Xbarc188	2	160, 200
27	BARC19	2	120, 150
28	BARC26	2	110, 190
29	BARC28	2	130, 200
30	BARC45	3	130, 165, 185
31	BARC48	2	130, 250
32	BARC113	3	140, 190, 220
33	BARC133	3	130, 180, 210
34	BARC187	3	60, 140, 180
35	BARC236	2	90, 160
36	BARC263	2	100, 140
37	BARC275	2	110, 160
38	BARC288	2	110, 165
39	CFA2104	3	120, 155, 180
40	CFA2262	3	90,120, 140
41	CFA2292	2	120, 190
42	CFD239	2	105, 160
43	WMC 642	2	130, 195
44	WMC110	2	100, 140

45	WMC149	3	110, 160, 180
46	WMC232	3	145, 170, 200
47	WMC254	3	100, 180, 240
48	WMC296	2	140, 210
49	WMC349	3	120, 160, 195
50	WMC398	2	100, 140
51	WMC413	4	100,120, 150, 160
52	WMC416	5	90, 125, 210, 290, 350
53	WMC475	2	185, 230
54	WMC601	2	140, 185
55	WMC727	2	120, 210
56	WMC758	2	130, 170
57	XGWM219	3	130, 190, 250
58	XGWM261	2	145, 200
59	XGWM443	4	135, 160, 210, 250
60	XGWM515	2	155, 230
61	XGWM642	3	160, 210, 245
62	XCFD223	3	150, 190, 260
63	Xgwm311	3	110, 140, 175
64	WMC25	2	130, 210

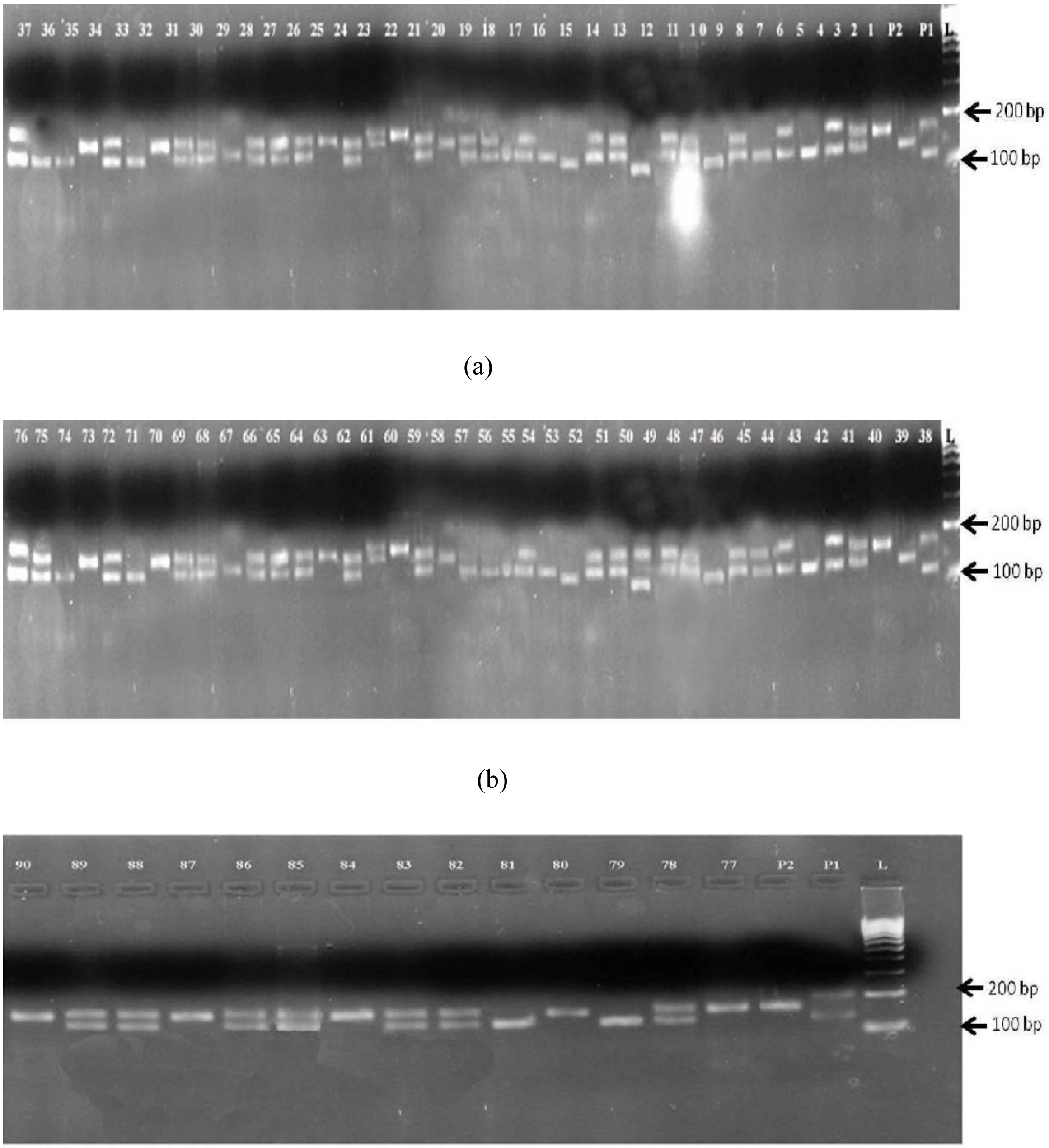


(a)

(b)

(c)

Plate-10: Agarose gel (2.5%) showing allelic polymorphism for primer BARC187 among F_2 population of wheat cross-II (DBW16 x WH147); Lane L-100 bp ladder Lane, P_1-DBW16; P_2- WH147

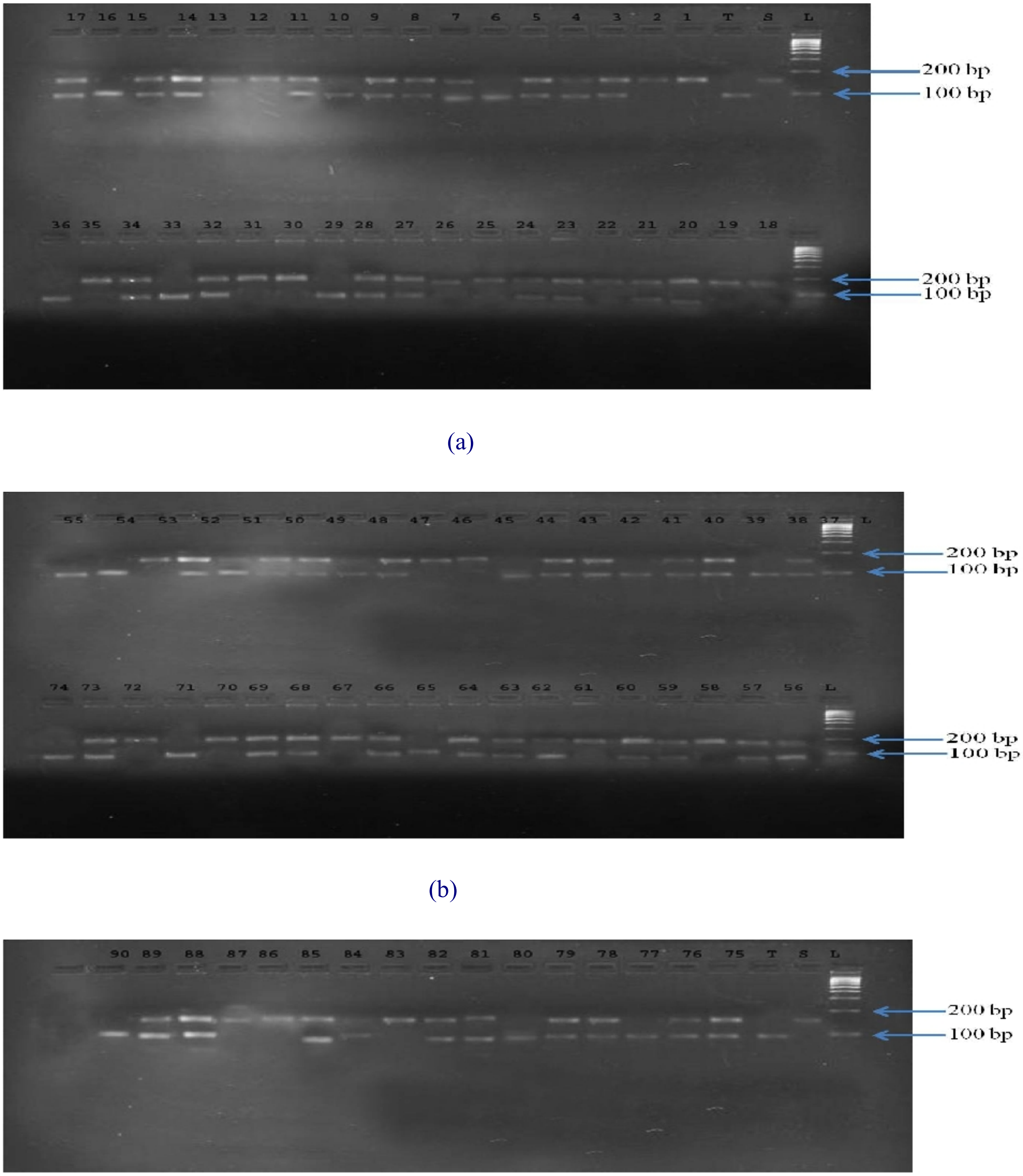


(a)

(b)

(c)

Plate11: Agarose gel (2.5%) showing allelic polymorphism for primer Xgwm356 among F_2 population of wheat cross-II (DBW16 x WH147); Lane L-100 bp ladder Lane, P_1-DBW16; P_2- WH147



(a)

(b)

(c)

Plate12: Agarose gel (2.5%) showing allelic polymorphism for primer Xgwm356 among F_2 population of wheat cross-II (DBW16 x WH147); Lane L-100 bp ladder Lane, P_1-WH147; P_2- DBW16

4.13.3.4 Cluster Tree Analysis in DBW16x WH147 (Cross-II):

The hierarchical cluster analysis showed that the genotypes were mainly divided into two major clusters at a similarity coefficient of 0.53 (Fig. 4.10). Cluster–I was divided into two sub clusters at the similarity coefficient of 0.82.

Similarly, the cluster-II is divided into 2 sub clusters at a similarity coefficient of 0.62.These sub clusters are further divided into many sub clusters. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH147 (Figure 4.11, 4.12).

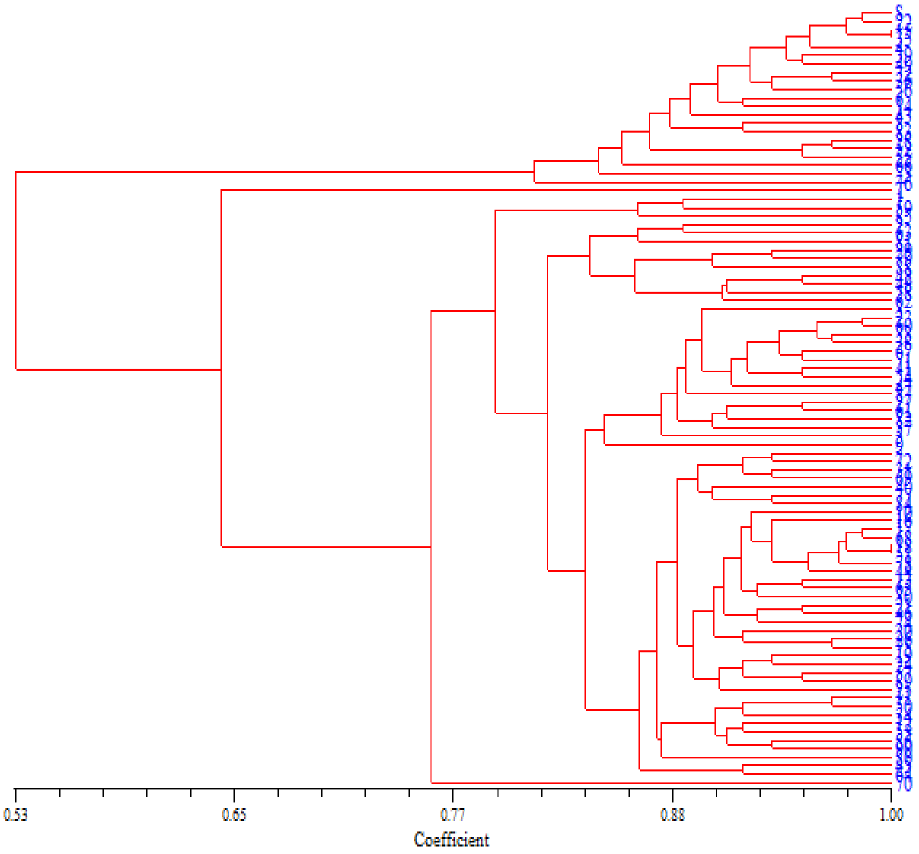


Fig.4.10: Dendrogram showing relationship among 90 individual plants of F_2 population of DBW16 x WH147 along with both parents generated by UPGMA analysis based on single primers using polymorphic SSR primer pairs

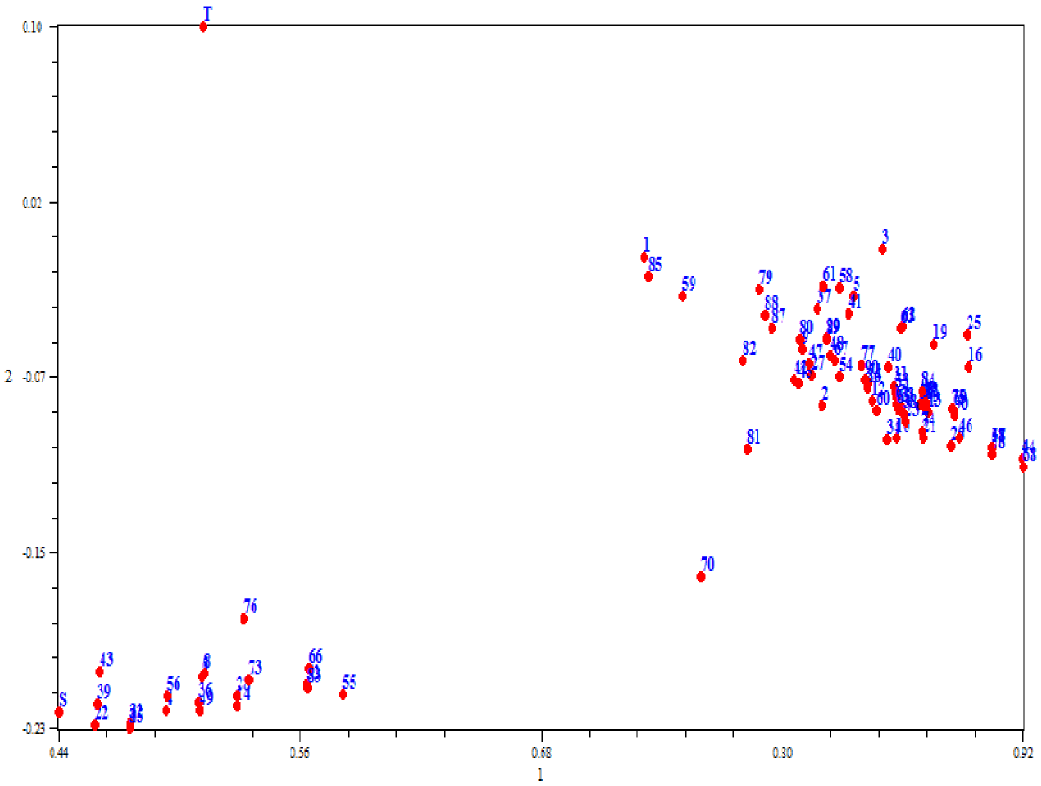


Figure 4.11: Two dimensional PCA scaling of DBW16 x WH147 F_2 plants and parental wheat genotypes based on SSR analysis.

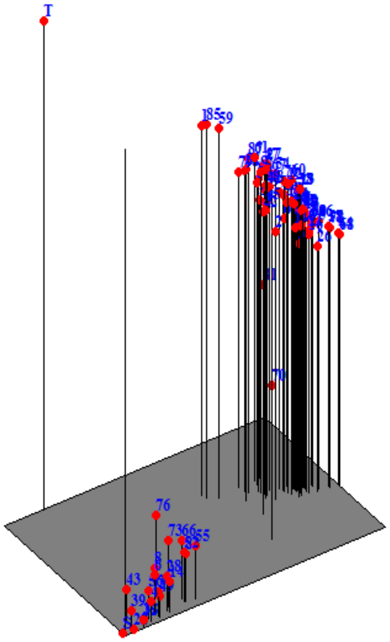


Figure 4.12: Three dimensional PCA scaling of DBW16 x WH147 F_2 plants and parental wheat genotypes based on SSR analysis.

4.13.4 Known gene primer analysis

4.13.4.1 Optimization of PCR Amplification Conditions for known gene primers

`For marker analysis, a polymerase chain reaction (PCR) was performed in a 25 µl volume containing 100 ng of genomic DNA, 2.5 µl of 10X PCR buffer, 200 µM of each dNTP, 0.2 µM of each primer and 1.0 unit of *Taq* DNA polymerase. The thermocycling program consisted of an initial denaturation at 94°C for 4 min, followed by 30 cycles of 45 sec at 94°C, 45 sec at 60°C, 60 sec at 72°C and a final cycle of 5 min at 72°C in S 1000 Thermal Cycler (Bio-Rad).

4.13.4.2 Polymorphism among parents and F_2 population of wheat using known gene primers

A band of 197 bp appeared on agarose gel (3.0%) upon electrophoresis of PCR amplified products of genomic DNA from thermosensitive wheat varieties, WH711 and WH147 as well as F_2 plants categorized as thermosensitive based on phenotypic data in both the crosses (Plate 13 & 14) by using *HSP16.9* known gene primer. This band was absent in thermotolerant parents viz. Hindi62 and DBW16 as well as thermotolerant F_2 plants. Similarly, using *HSP*101 gene primers, a band of 120 bp was observed in thermosensitive parents and F_2 genotypes of both crosses while it was absent in all thermotolerant F_2 genotypes and parents.

4.13.4 QTL Mapping

Multiple QTLs were detected for different traits. A total of 34 QTLs detected in both the crosses (Table- 17, 18). . In cross Hindi62 x WH711 three QTL were detected for chlorophyll index at map positions, 135.7 (1B), 184.6 (2A) and 36.4 (6B) by SSR marker WMC 416, Xbarc188, Xgwm526, WMC170, Xgwm219, WMC398 respectively. Six QTL were detected for membrane thermostability at map positions, 118.7 (1B), 58.2 (2A), 137.2 (2A), 176.7 (2A), 62.0 (5A) and 11.7 (6B) by SSR marker WMC416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219 and WMC398. For yield per plant 6 QTLs were detected at map position103.4 (1B), 51.6 (2A), 130.6 (2A), 178.9 (2A), 55.4 (5A), 22.6 (6B) by SSR markers WMC 416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219, WMC398. For grain filling duration one QTL was detected at chromosome no. 2A at a map position 171.9 and for grain filling rate 2 QTLs were detected on chromosome 2A and 6B at map position of 66.0 and 76.3 by SSR markers WMC642, Xgwm356, Xgwm219, WMC398 respectively. Similarly in cross DBW16 x WH147, 4 QTLs were detected for chlorophyll index on chromosome 1B (120.9), 2A (174.5), 6B (35.8) and 6B (88.5) respectively. Five QTLs were for membrane thermostability on chromosome 1A (47.6), 1B (120.6), 2A (100.4), 3A (109.5) and 6B (17.2). One QTL was identified for yield per plant and one for grain filling rate at chromosome 2A (180.7) and 4A (146.8) respectively. Five QTLs were identified for grain filling duration at a map position 113.5 (2A), 31.5 (2A), 88.5 (3A), 31.0 (5A) and 29.0(5B).

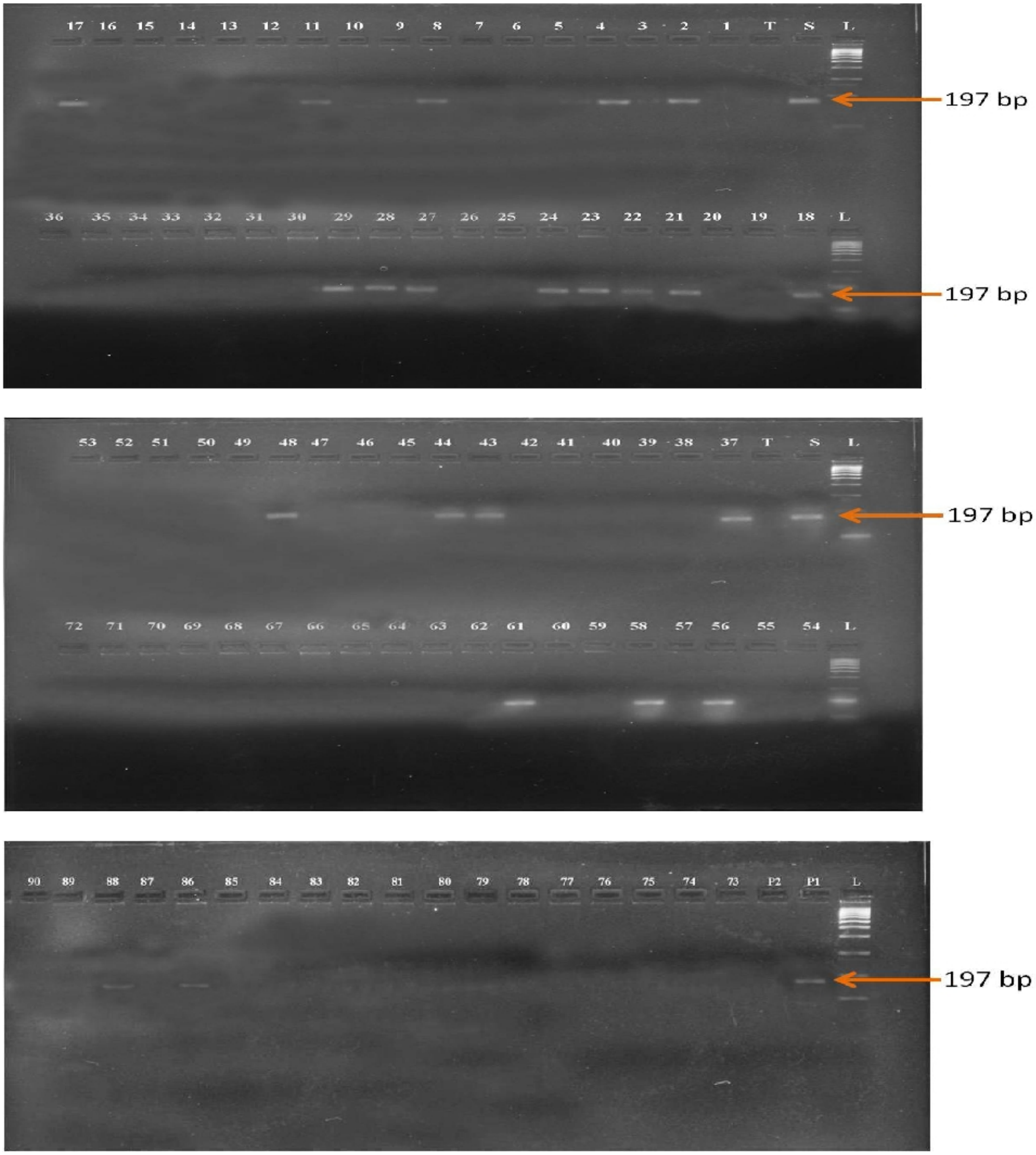


Plate13: Agarose gel (3.0%) showing allelic polymorphism for primer *HSP*16.9 among F_2 population of wheat cross-I (Hindi62 x WH711); Lane L-100 bp ladder Lane, P_1-WH711; P_2- Hindi62

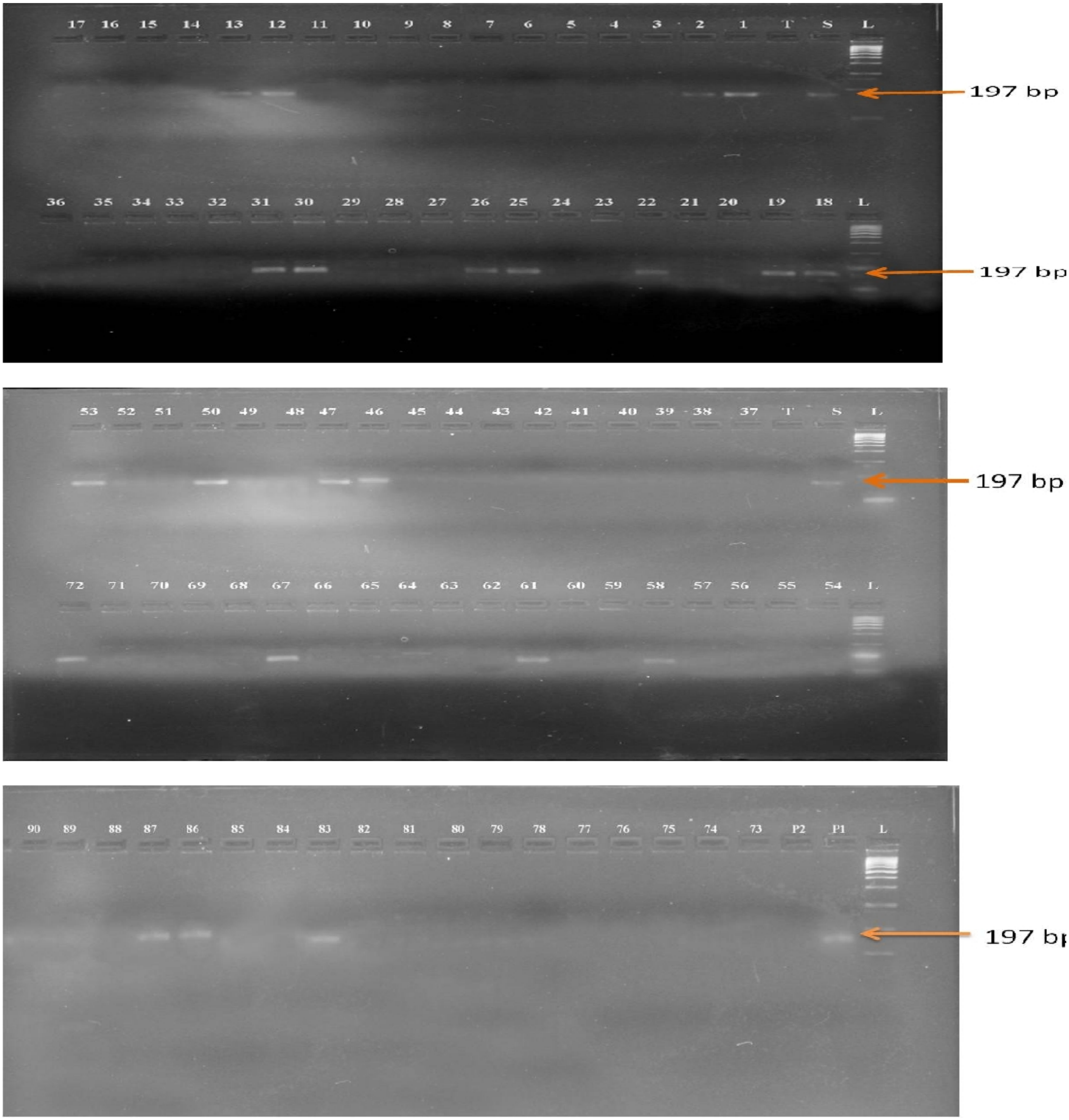


Plate14: Agarose gel (3.0%) showing allelic polymorphism for primer *HSP*16.9 among F_2 population of wheat cross-II (DBW16 x WH147); Lane L-100 bp ladder Lane, P_1-WH147; P_2- DBW16

Table-4.17: QTLs identified in cross Hindi62 x WH711

Trait	Chromosome	Flanking markers	Position of flanking markers	Position of QTL	LOD score	Additive effect	R^2	Phenotypic Variance (%)
Chlorophyll index	1B	WMC 416-Xbarc188	77.0-143.9	135.7	7.2	0.0293	0.041735	4.1
	2A	Xgwm526-WMC170	170.4-200.9	184.6	4.9	0.0444	0.044938	4.4
	6B	Xgwm219-WMC398	0.7-80.6	36.4	3.0	0.0118	0.075895	7.5
Membrane thermostability index	1B	WMC 416-Xbarc188	77.0-143.9	118.7	11.3	7.0364	0.04243	4.2
	2A	WMC407-WMC642	7.7-73.1	58.2	8.8	5.0437	0.03135	3.1
	2A	Xgwm356-Xgwm95	96.0-154.2	137.2	9.6	7.063	0.08012	8.0
	2A	Xgwm526-WMC170	170.4-200.9	176.7	10.8	4.2126	0.08578	8.5
	5A	Xgwm443-WMC475	18.6-83.7	62.0	8.9	5.3405	0.0737	7.3
	6B	Xgwm219-WMC398	0.7-80.6	11.7	8.2	3.0722	0.05889	5.8
Yield per plant	1B	WMC 416-Xbarc188	77.0-143.9	103.4	7.7	2.8383	0.077265	7.7
	2A	WMC407-WMC642	7.7-73.1	51.6	4.7	1.879	0.0224	2.24
	2A	Xgwm356-Xgwm95	96.0-154.2	130.6	5.4	2.2818	0.026281	2.62
	2A	Xgwm526-WMC170	170.4-200.9	178.9	7.3	2.963	0.079626	7.96
	5A	Xgwm443-WMC475	18.6-83.7	55.4	4.6	2.0857	0.0586	5.86
	6B	Xgwm219-WMC398	0.7-80.6	22.6	3.9	2.0975	0.038863	3.88
Grain filling duration	2A	Xgwm526-WMC170	170.4-200.9	171.9	4.6	1.0795	0.08247	8.2
Grain filling rate	2A	WMC642-Xgwm356	73.1-96.0	66.0	10.3	4.7789	0.482192	48.2
	6B	Xgwm219-WMC398	0.7-80.6	76.3	11.0	4.7249	0.417401	41.7

Table-4.18: QTLs identified in cross DBW16 x WH147

Trait	Chromosome	Flanking markers	Position of flanking markers	Position of QTL	LOD score	Additive effect	R^2	Phenotypic Variance (%)
Chlorophyll index	1B	WMC 416-Xbarc188	77.0-143.9	120.9	6.3	2.93	0.0417	4.1
	2A	Xgwm526-WMC170	170.4-200.9	174.5	3.1	4.84	0.044938	4.4
	6B	Xgwm219-WMC398	0.7-80.6	35.8	2.9	11.8	0.07495	7.4
	6B	Xgwm193-WMC105	88.2-93.8	88.5	2.6	6.59	0.09421	9.4
Membrane thermostability index	1A	WMC254-WMC120	31.0-67.2	47.6	8.9	14.8486	0.035566	3.55
	1B	WMC 416-Xbarc188	77.0-143.9	120.6	8.0	13.4406	0.03149	3.14
	2A	Xgwm356-Xgwm95	96.0-154.2	100.4	10.3	14.7827	0.02747	2.74
	3A	Barc19-WMC169	60.8-175.2	109.5	3.7	13.7034	0.04859	4.85
	6B	Xgwm219-WMC398	0.7-80.6	17.2	6.6	13.4124	0.07194	7.19
Yield per plant	2A	Xgwm526-WMC170	170.4-200.9	180.7	4.3	1.4071	0.0874	8.74
Grain filling duration	2A	Xgwm356-Xgwm95	96.0-154.2	113.5	5.2	1.018	0.03824	3.82
	2A	WMC407-WMC642	7.7-73.1	31.5	5.4	9.730	0.12683	12.6
	3A	BARC19-WMC169	60.8-175.2	88.5	5.8	11.470	0.15041	15.04
	5A	Xgwm443-WMC475	18.6-83.7	31.0	4.8	13.17	0.16872	16.87
	5B	Xgwm604-WMC149	0.9-30.5	29.0	2.9	13.47	0.24396	24.39
Grain filling rate	4A	Xgwm610-WMC232	50.5-181.9	146.8	3.3	10.96	0.3627	36.2

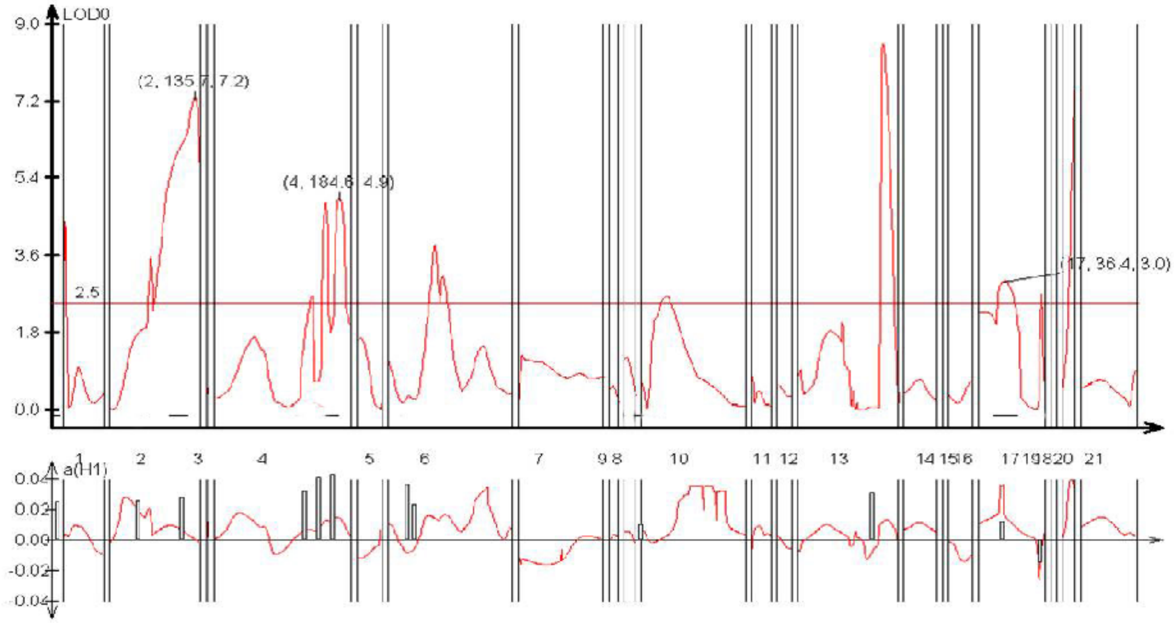


Fig. 4.13: Cartograph showing the mapping of QTLs combined for chlorophyll index in cross Hindi62xWH711

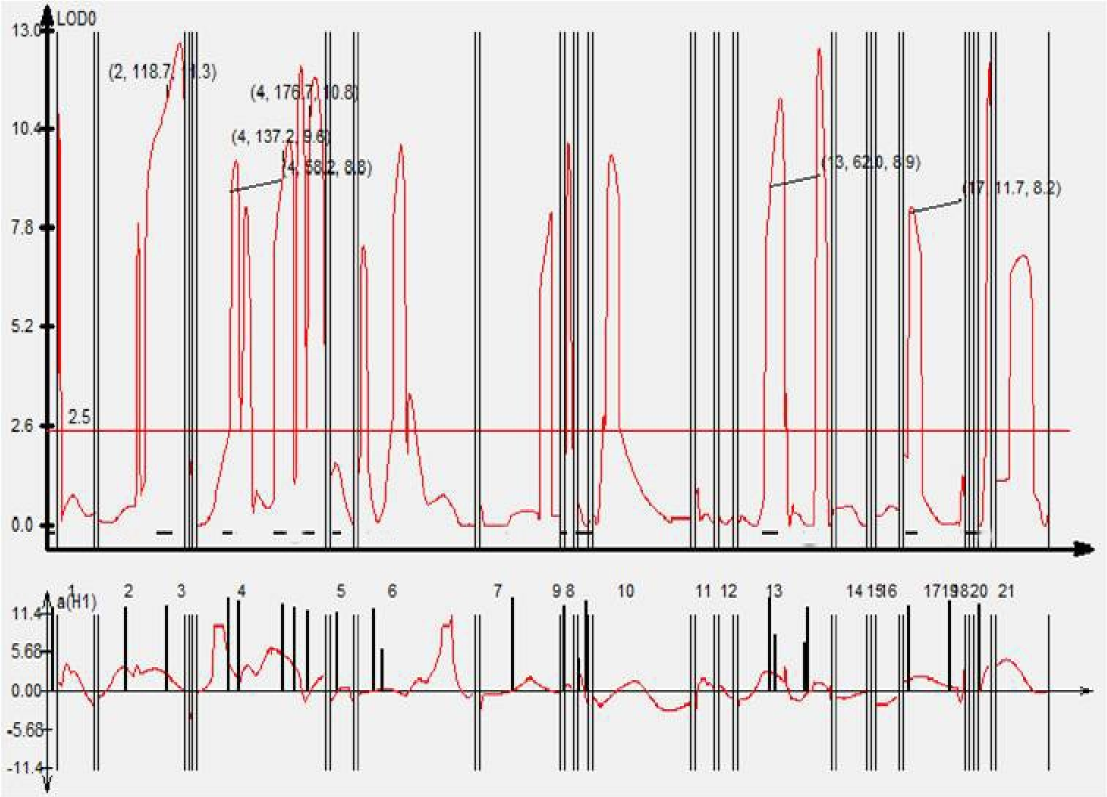


Fig. 4.14: Cartograph showing the mapping of QTLs combined for membrane thermostability index in cross Hindi62xWH711

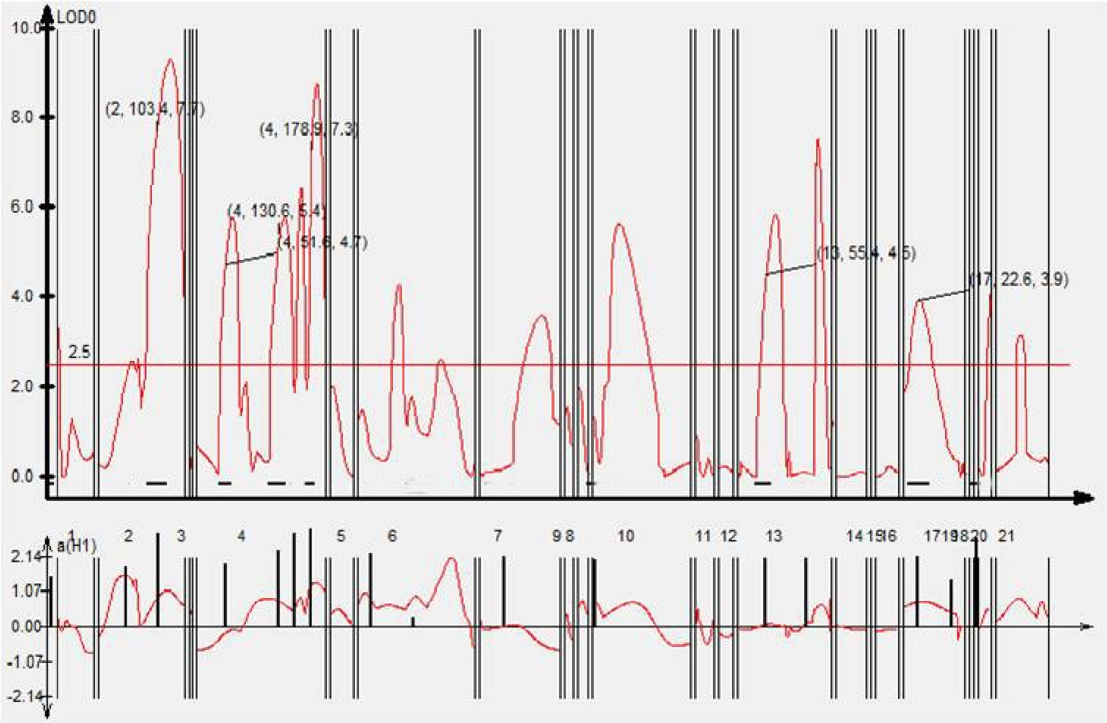


Fig. 4.15: Cartograph showing the mapping of QTLs combined for yield/plant in cross Hindi62xWH711

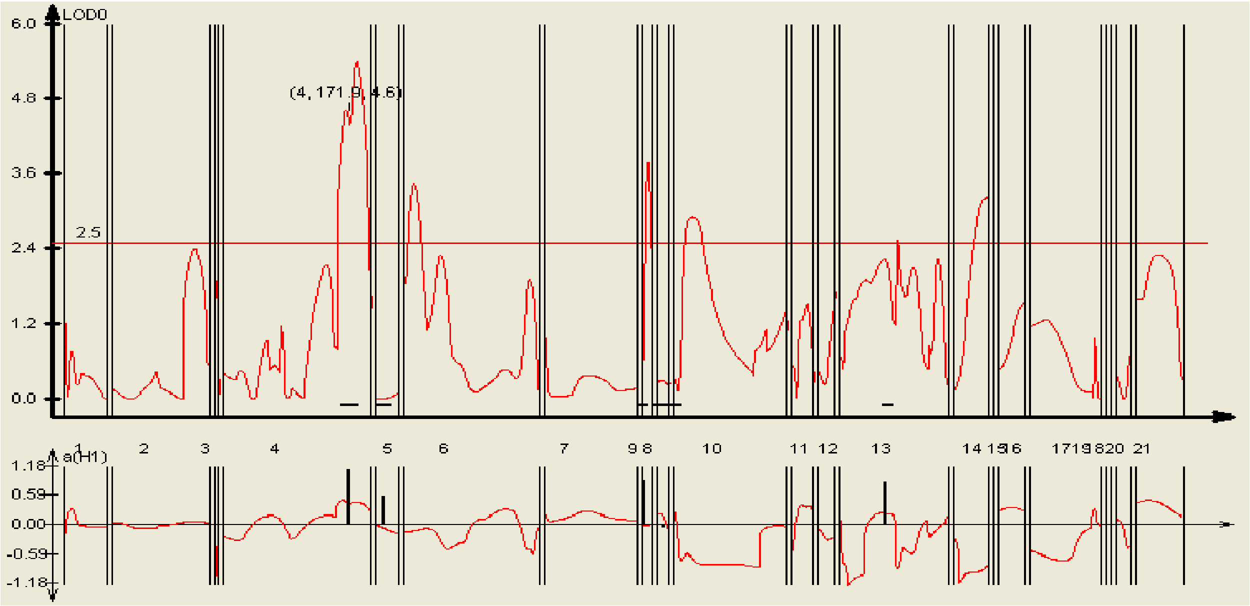


Fig. 4.16: Cartograph showing the mapping of QTLs combined for grain filling duration in cross Hindi62xWH711

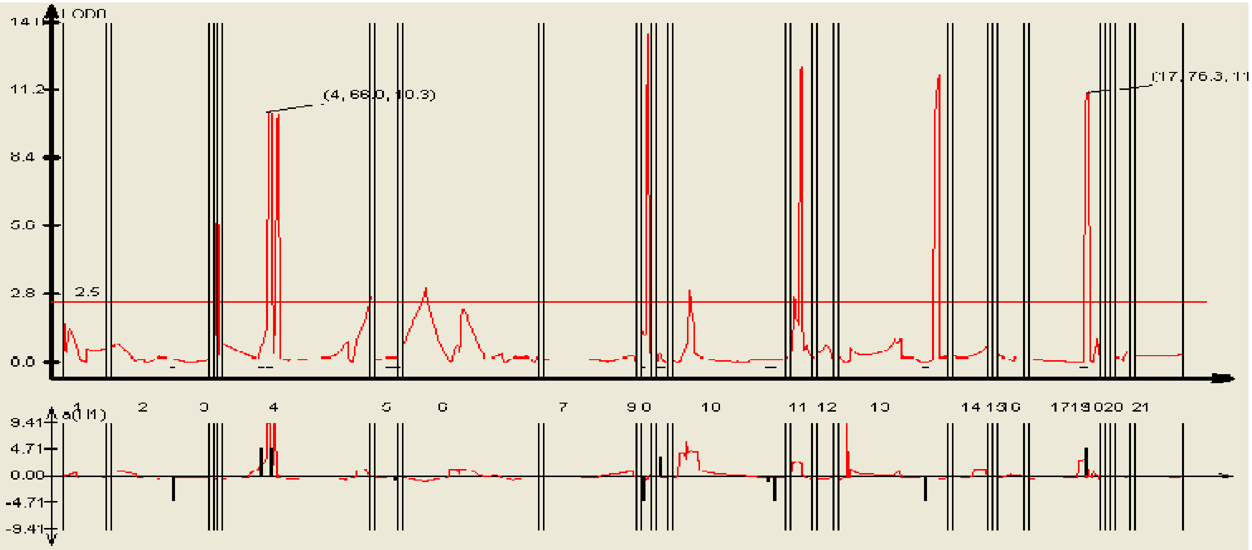


Fig. 4.17: **Cartograph showing the mapping of QTLs combined for grain filling rate in cross Hindi62xWH711**

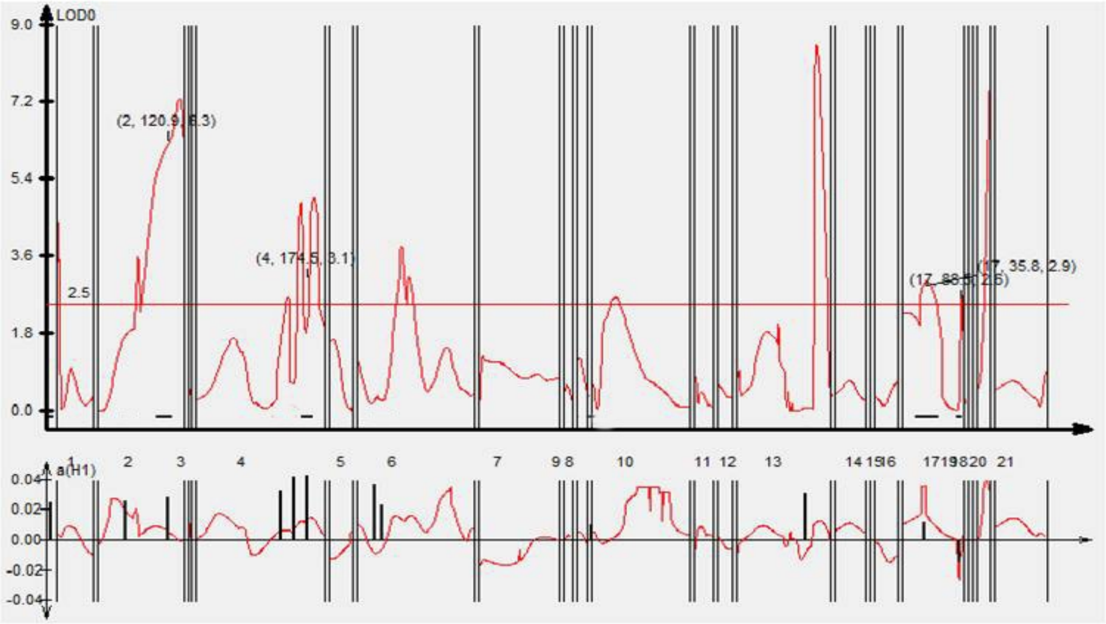


Fig. 4.18: **Cartograph showing the mapping of QTLs combined for chlorophyll stability in cross DBW16 x WH147**

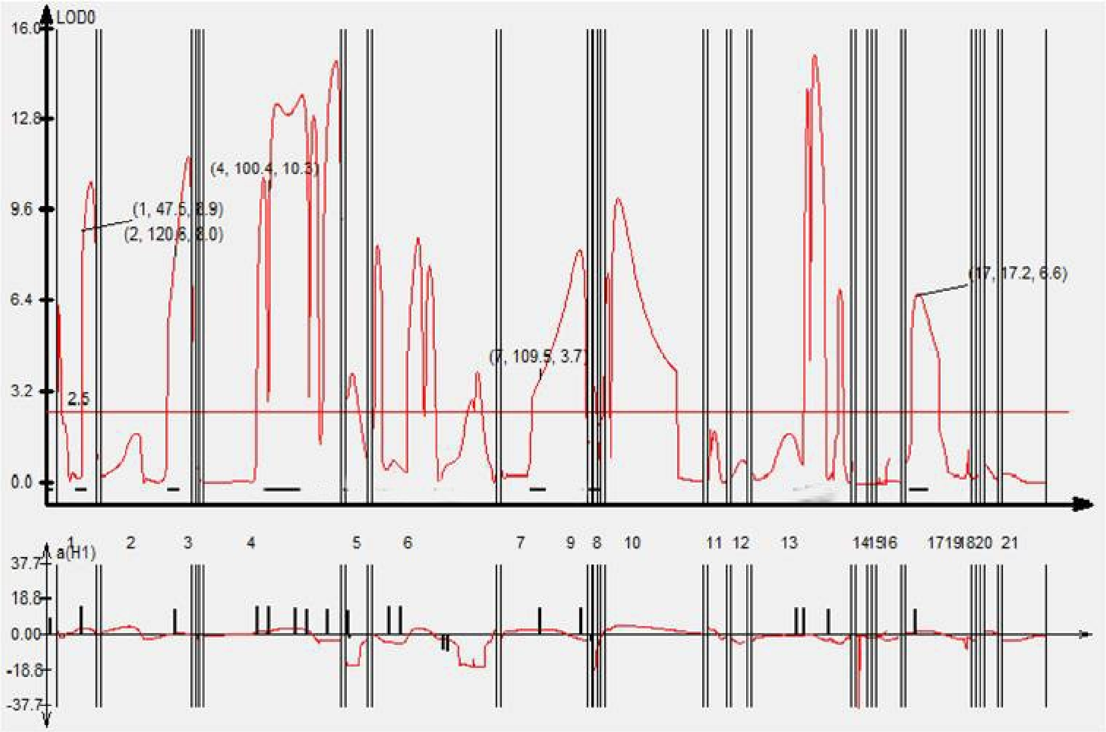


Fig. 4.19: **Cartograph showing the mapping of QTLs combined for membrane thermostability in cross DBW16 x WH147**

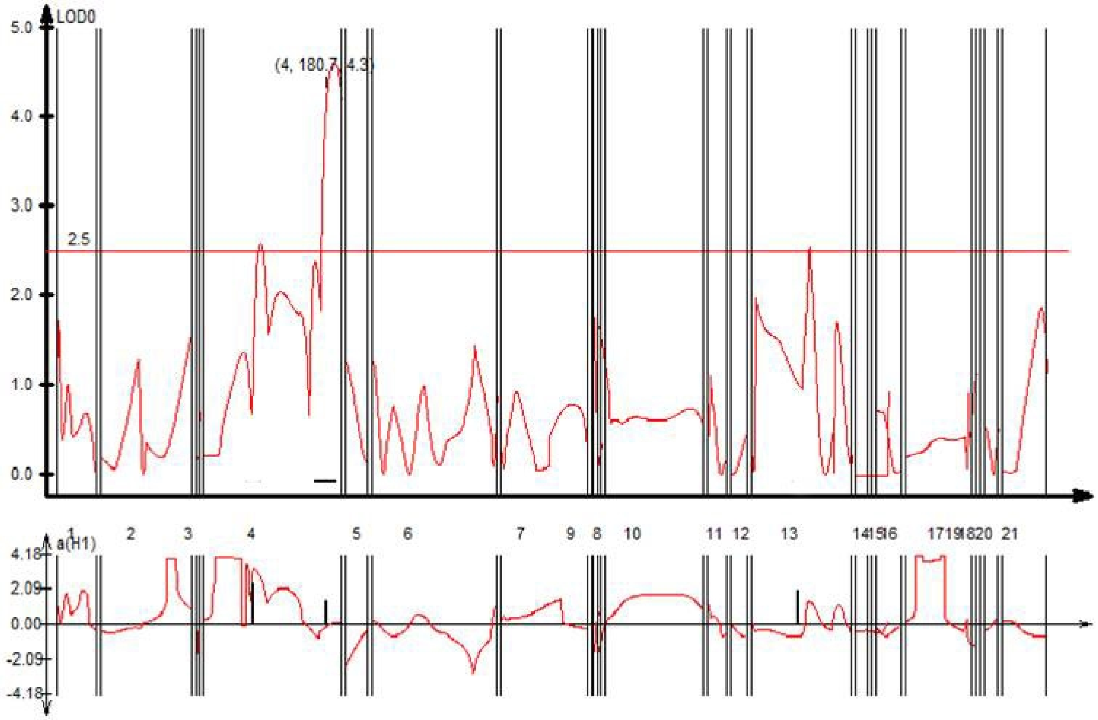


Fig. 4.20: **Cartograph showing the mapping of QTLs combined for yield/plant in cross DBW16 x WH147**

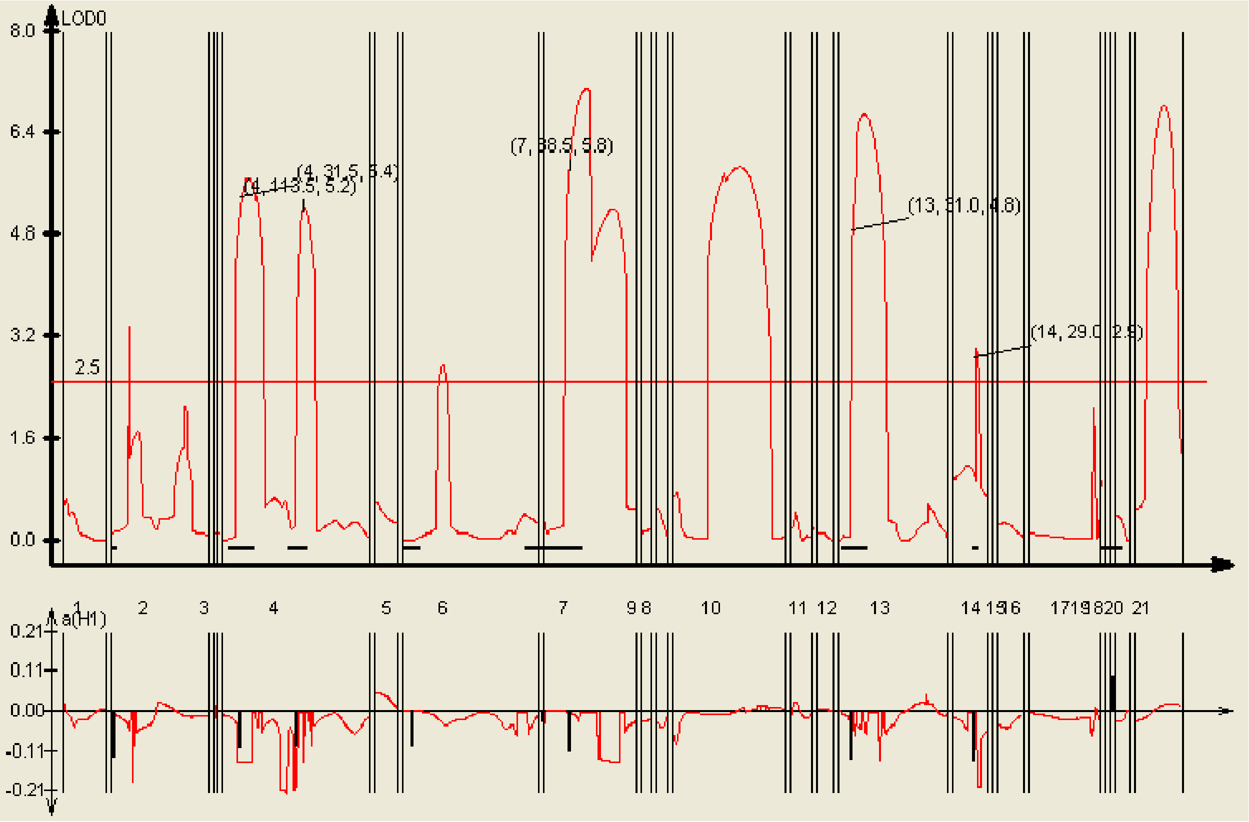


Fig. 4.21: Cartograph showing the mapping of QTLs combined for grain filling duration in cross DBW16 x WH147

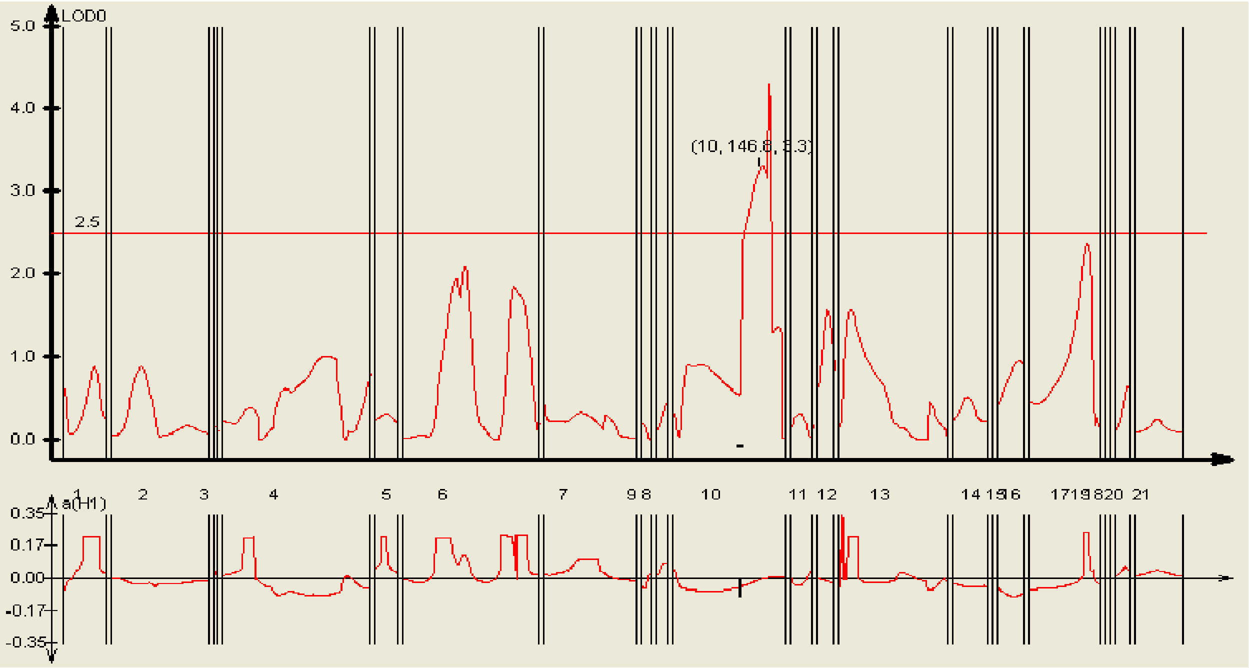


Fig. 4.22: Cartograph showing the mapping of QTLs combined for grain filling rate in cross DBW16 x WH147

Chapter – V

DISCUSSION

With the introduction of semi-dwarf, photoinsensitive, fertilizer responsive and the high yielding varieties of wheat, the wheat production in India has increased from 12 million tonnes in 1966 to 93.9 million tonnes in the 2011-12. Wheat exceeds every other grain crop in acreage and production and is, therefore, the most important cereal of the world. It is imperative to stabilize the wheat production while maintaining its quality by reducing the losses due to various biotic and abiotic stresses. Out of various abiotic stresses, terminal high temperature stress has reached at alarming stage due to global warming.

Yield loss due to heat stress is an agricultural problem in many areas in the world (Wahid *et al.,* 2007). High temperature stress during post-anthesis is a major cause of wheat yield reduction in many wheat-growing regions of the world. Heat stress during grain filling is commonly occurred in wheat. High temperatures, typically above 34°C, affect grain weight by reducing the duration of grain filling due to suppression of current photosynthesis (El-Khatib and Paulsen, 1984), and by direct inhibition of starch biosynthesis in the endosperm (Jenner, 1994). The grain filling rate (GFR) plays a significant role in the total grain yield of wheat (Beiquan and Kronstad, 1994). Direct selection for heat stress tolerance under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of trials. Often, no consistent high-temperature conditions can be guaranteed in field nurseries, as heat stress may or may not occur in the field. Furthermore, various stages of development in wheat are affected by heat stress. Consequently, the genetic dissection of the quantitative traits controlling the adaptive response of crops abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to molecular breeding programs aimed to improve the sustainability and stability of yield under adverse conditions (Collins *et al.,* 2008). Therefore, the use of molecular markers, QTLs and genomics based tools can play an important role in development of thermotolerant wheat genotypes. the present investigation was undertaken with the objective of phenotyping of population and identification of QLs associated with terminal heat tolerance.

Phenotyping of the population

Targeting genes for improvement of any trait in crop plants may require an appropriate population and right choice of parents. In the present investigations the mapping population was obtained from crosses between terminal heat stress tolerant and sensitive genotypes of wheat Hindi 62 x WH 711 (cross-1) and DBW 16 x WH 147 (cross-II) were made to raise F_1, F_2 & F_3 populations. Parent lines and above mapping populations of wheat were evaluated for terminal heat stress tolerance/ sensitivity and characterized morphologically for following traits *viz;* membrane thermostability index, chlorophyll stability index, grain yield, kernels/spike, thousand kernel weight (test weight), days to heading, days to maturity, grain filling duration, grain filling rate, plant height, number of tillers/plant, biomass/plant and harvest index.

A large variation for the chlorophyll index was observed 0.51 to 0.64 in F_2 (Hindi62 x WH711) population, whereas for membrane thermostability index and plant height the range was 46.7 to 63.96 and 96.33 cm, respectively. For kernel per spike, the range was found as 16 to 57, whereas for tiller per plant and biomass per plant range was 3 to 14 and 21.5 to 83.12, respectively. For grain yield/plant variation was recorded from 4.82 to 21.1 gm. The range varied for test weight, days to heading, days to maturity and harvest index as 24.6 to 42.3g, 104 to 117 days, 145 to 159 days and 20.76% to 36.11%, grain filling duration as 33.3-48.3 and grain filling rate as 0.697-1.199 respectively.

In F_3 population of above cross, the range for chlorophyll index was recorded from 0.50 to 0.67 whereas for membrane thermo stability index and plant height varied from 46.7 to 66.8 and 78 to 126 cm, respectively. For kernel per spike, the range was found as 24 to 52, whereas for tiller per plant and biomass per plant has been recorded as 4 to 15 and 20.8 to 84.6 gm, respectively. Grain yield/plant varied from 6.3 to 19.7 gm. Test weight, days to heading, days to maturity and harvest index ranges also showed large variation as 23.1 to 40.9 gm, 79 to 88 days, 112 to 122 days and 20.05% to 31.28%, grain filling duration as 33.6-44.3and grain filling rate as 0.697-1.199 respectively.

Similarly in DBW16 x WH147, F_2 population, chlorophyll index ranged from 0.51 to 0.65 whereas membrane thermostability index and plant height ranged between 43.9 to 64.3 and 89.0 to 141.0 cm, respectively. For kernel per spike, the range was found as 22 to 52, whereas for tiller per plant and biomass per plant it varied between 6 to 16 and 30.5 to 92.3, respectively. For grain yield, the range was recorded from 6.9 to 23.4 gm. The range for the physio-morphological traits such as test weight, days to heading, days to maturity and harvest index varied between 22.4 to 42.2 gm, 102 to 116 days, 142 to 158 days and 13.88 % to 39.28 %, grain filling duration as 34.3-43.3 and grain filling rate as 0.59-1.11 respectively. For F_3 population the range for the chlorophyll index has been recorded as 0.51 to 0.65 whereas for membrane thermostability index and plant height observed to be as 46.2 to 65.4 and 78 to 134 cm, respectively. For kernel per spike, the range was found as 22 to 56, whereas for tiller per plant and biomass per plant has been recorded as 4 to 18 and 22.4 to 92.6gm, respectively. For grain yield, the range was recorded as 5.6 to 24.6gm. The range for the characters viz., test weight, days to heading, days to maturity and harvest index has been obtained as 20.2 to 39.6gm, 78 to 88 days, 112 to 122 days and 17.16% to 28.36%, grain filling duration as 23.2-40.9 and grain filling rate as 0.663-1.205 respectively.

Heritability

In F_2 population of cross Hindi62 x WH711 broad sense heritability for chlorophyll index, membrane thermostability index, plant height, kernel per spike, test weight was high (>90%) and for days to heading, days to maturity and harvest index it was between 80-90% while for tiller per plant, biomass per plant and grain yield it was <80%. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.953), membrane thermostability (0.994), plant height (0.988), kernel per spike (0.904) and test weight (0.983). Similarly for F_3 population, heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and days to maturity and (<70%) tiller per plant, biomass per plant and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.953), membrane thermostability (0.994), plant height (0.988), kernel per spike (0.904) and test weight (0.983).

Heritability (broad sense) in cross DBW16 X WH147 for F_2 population estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and days to maturity, biomass per plant and (<70%) tiller per plant, and grain yield. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.993), membrane thermostability (0.996), plant height (0.973), kernel per spike (0.955) and test weight (0.971). Similarly for F_3 heritability (broad sense) estimates were high (>90%) for chlorophyll index, membrane thermostability, plant height, kernel per spike, test weight and (>80%) for days to heading and (<70%) tiller per plant and biomass per plant. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.984), membrane thermostability (0.996), plant height (0.959), kernel per spike (0.960) and test weight (0.970). Earlier Mehta *et al.* (1997) in wheat also observed high heritability implied that selection for most of the traits might be effective in this set of genotypes. The high heritability estimates coupled with high genetic advance as per cent of mean for grain yield per plant, number of effective tillers per plant, hundred grain weight, grain weight per spike, spike weight, spike length and number of grains per spike suggest that all these characters are governed by additive gene effect and as such are expected to exhibit improvement for such traits by direct selection to an extent of about more than 30 per cent over and above the respective average values for these traits. These observations are in agreement with the earlier reports of Ahmad *et al.* (1999); Dhanda and Munjal, 2009; Dhanda and Munjal, 2012 as observed in wheat.

Genetic diversity analysis based on RAPD markers

Among the several PCR-based techniques, random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland, 1990; Williams *et al.*, 1990) is a simple, rapid and efficient technique for identifying the degree of genetic similarity and variability among biological populations (Tingey and del Tufo, 1993; Gang and Weber, 1995).

Twenty-five RAPD primers were used for the present investigation, out of which 14 primers showed amplification and 11 primers showed no amplification. These primers perhaps did not find any complementary binding sequence on the genomic DNA of these wheat genotypes. Moreover, some primers might have some special requirement for amplification as suggested by Weeden *et al.* (1992) and Ahmad (1999).

A total of 107 alleles were obtained using 14 RAPD primers, out of which 44 were monomorphic and 63 were polymorphic. Average polymorphism across 4 wheat genotypes was 53.77 % which is quite consistent with that (64.3%) reported by Mukhtar *et al.* (2002) among 20 wheat genotypes. Cao *et al.* (2002) reported 45.3% polymorphic alleles in 29 wheat genotypes. However, in some earlier reports, high level of polymorphism has been reported. Frietas *et al.* (2000) reported 83% polymorphism among 14 genotypes of Brazilian wheat.

Size of amplified products ranged between 280 bp to 4000 bp. Teshale *et al.* (2003) obtained products ranging between 300 bp to 3000 bp and those obtained by Cao *et al.* (2002) ranged from 280 bp to 2800 bp.

Cluster analysis of parents and F_2 population of Hindi62 x WH711 the group analysis showed two major groups at a similarity coefficient of 0.67 (Fig. 4.1). Group–I was divided into two sub-groups at the similarity coefficient of 0.82.

Similarly, the group-II was divided into 2 sub-groups at a similarity coefficient of 0.72. These sub-groups are further sub divided into many minor groups. Two dimensional NTSYS-PC analysis showed that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH711 (Figure 4.2, 4.3).

Similarly in cross II (WH147xDBW16) the hierarchical group analysis showed that the genotypes were mainly divided into two major groups at a similarity coefficient of 0.67 (Fig. 4.4). Group–I was divided into two sub groups at the similarity coefficient of 0.78.

Similarly, the group-II is divided into 2 sub groups at a similarity coefficient of 0.73. These sub groups are further divided into many sub groups. Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F2 plants interspersed between the two parental lines with distribution of some plants towards DBW16 (Figure 4.5, 4.6). DNA of eleven wheat genotypes (five parental genotypes, three F1 crosses and three F2 populations) was amplified with eight different random primers. Eight RAPD primers detected 129 fragments and 91 of them (70.125%) were polymorphic. Eleven wheat genotypes were grouped into two clusters using dendrogram analysis. The results of similarity indices were compared with those of genetic variation for the parents combined into three crosses. The parental with the lowest similarity indices had the highest score for genetic variation in most of the morphological and agronomical traits.

Genetic diversity analysis based on SSR primers

Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability (Plaschke *et al.,* 1995; Fu *et al.,* 2005). In wheat, abundant wheat genomic SSR markers are now available and have been mapped (Roder *et al*., 1998), making them a useful resource for further studies. Microsatellite markers are useful and becoming popular for different applications in wheat breeding due to their high level of polymorphism and easy handling (Devos *et al*., 1995; Roder *et al.,* 1995; Bryan *et al*., 1997; Roy *et al.,* 1999; Lelley *et al*., 2000).

The present study addressed the utility of SSR markers in polymorphism study at the molecular level among 4 wheat genotypes and their F2 mapping population (Hindi62 x WH711 and DBW16 x WH147) wherein 103 SSR primers were used, which were earlier identified in the genomic regions of A, B, and D genomes of wheat. Out of 103 SSR primers 67 primers showed polymorphism between parental lines Hindi62 and WH711 while 64 primers were found to give polymorphism between DBW16 and WH147. Using 67 SSR primers, a total of 180 alleles were amplified which ranged from 1-6 alleles with an average of 2.72 alleles/primer. Similar results have been reported by Schuster et al. (2009) in a study on 36 wheat genotypes using 23 SSR markers. The total number of amplified allele varied between 2 and 5 with an average of 3.2 allele per locus, which is close to results of present investigation. Ahmed (2002) observed from two to eight alleles per locus, with a mean of 3.6, in 13 wheat genotypes of diverse origin analyzed with 43 SSR markers. Akkaya and Buyukunal-Bal (2004) obtained PIC values ranging from 0.36 to 0.87 with a mean of 0.68 when evaluating 19 SSR highly polymorphic loci in 11 wheat cultivars. They observed a mean of 3.26 alleles per locus within a range from two to five and for PIC values ranging from 0.20 to 0.79, a mean of 0.48. The microsatellite profiles, characterized with 23 pairs of polymorphic markers showed that the loci employed delineated all the 38 genotypes. Thus, these markers can be used to define the genetic profile of each genotype.

Salem *et al.* (2008) also reported 3.2allele per locus. Contrary to this, more number of alleles (5-13) have been reported by Manifesto *et al.* (2001), Parker *et al.* (2001) and Telku *et al.* (2006). Huang *et al*. (2002) investigated 998 wheat accessions of bread wheat obtained from Gatersleben Gene Bank and reported 18.1 alleles per locus.

The present study also showed that primer pair Xbarc 136 generated a maximum number of 6 alleles while primer pair Xgwm 157 produced a minimum number of alleles (1). This revealed significant differences in allelic diversity among various microsatellite loci. Many studies have also reported remarkable differences in allelic diversity among various microsatellite loci (Akagi *et al.,* 1997; McCouch *et al.,* 2001). The alleles revealed by markers showed a higher degree of polymorphism.

The polymorphism percentage ranged from as low as 20% to as high as 100%, giving an average percentage polymorphism of 72.77% in present investigation. Similarly Ijaz *et al.* (2009) reported high level of polymorphism ranging from 10.52% to 98.42%. Manifesto et al 2001 reported PIC values ranged from 0.40 to 0.84 with an average value of 0.72.

In present investigation, size of amplified DNA fragments using SSR primers varied from approx. 90bp to 1120bp. Abbas et al. (2008) obtained amplified DNA fragments that varied in size from 250bp to 1000bp and Manifesto et al. (2001) obtained amplified DNA fragments that varied in size from 115bp to 285bp. Based on data on presence/absence of bands, genetic similarity was calculated to estimate all pair wise differences in the amplification product for all genotypes. Based on this data, phylogenetic analysis was done to estimate relationship among genotypes.

The average linkage between wheat genotypes was used for constructing phylogenetic tree depicting the relationship among the 4 parental wheat genotypes and the selected F_2 populations of both crosses.

In cross I (Hindi62xWH711) the hierarchical group analysis showed that the genotypes were mainly divided into two major groups at a similarity coefficient of 0.53 (Fig. 4.7). Group–I was divided into two sub groups at the similarity coefficient of 0.82.

Similarly, the group-II was divided into 2 sub groups at a similarity coefficient of 0.62.These sub groups are further divided into many sub groups. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH711 (Figure 4.8, 4.9). Similarly in cross II (WH147xDBW16) The hierarchical group analysis showed that the genotypes were mainly divided into two major groups at a similarity coefficient of 0.53 (Fig. 4.10). Group–I was divided into two sub groups at the similarity coefficient of 0.82.

Similarly, the group-II is divided into 2 sub groups at a similarity coefficient of 0.62.These sub groups are further divided into many sub groups. . Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 90 F_2 plants interspersed between the two parental lines with distribution of some plants towards WH711 (Figure 4.11, 4.12).

Similar reports were recorded by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of wheat (Wang *et al.,* 2007; *Salem et al. (2008);* Ijaz and Khan *et al.* (2009. In the present study, information on genetic similarity among wheat genotypes obtained using SSr markers was similar to that obtained with RAPD primers indicating usefulness of RAPD primers in genetic diversity analysis.

Identification of QTLs for terminal heat stress tolerance in $F_{2:3}$ population

Heat tolerance is quantitative in nature and is controlled by a number of genes/QTLs (Blum 1998). QTLs for heat tolerance in wheat have been reported in earlier studies using different traits like GFD, CTD, TGW and other yield related traits (Yang *et al*. 2002, Manon *et al*. 2010, Pinto *et al*. 2010, Paliwal *et al*. 2011). In the present study QTL were identified associated with terminal heat stress traits in bread wheat F_2 populations. A total of 34 QTLs detected in both the crosses (Table- 17, 18). In cross Hindi62 x WH711, three QTL were detected for chlorophyll index at map positions, 135.7 (1B), 184.6 (2A) and 36.4 (6B) by SSR marker WMC 416, Xbarc188, Xgwm526, WMC170, Xgwm219, WMC398 and %phenotypic variance for these QTLs were 4.1, 4.4 and 7.5 respectively. Six QTL were detected for membrane thermostability index at map positions, 118.7 (1B), 58.2 (2A), 137.2 (2A), 176.7 (2A), 62.0 (5A) and 11.7 (6B) by SSR marker WMC416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219 and WMC398 with 4.2, 3.1, 8.0, 8.5, 7.3 and 5.8 % phenotypic variance respectively. For yield per plant, 6 QTLs were detected at map position103.4 (1B), 51.6 (2A), 130.6 (2A), 178.9 (2A), 55.4 (5A), 22.6 (6B) by SSR markers WMC 416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219, WMC398. For grain filling duration one QTL was detected at chromosome no. 2A at a map position 171.9 and for grain filling rate 2 QTLs were detected on chromosome 2A and 6B at map position of 66.0 and 76.3 by SSR markers WMC642, Xgwm356, Xgwm219, WMC398 respectively. Similarly in cross DBW16 x WH147, 4 QTLs were detected for chlorophyll index on chromosome 1B (120.9), 2A (174.5), 6B (35.8) and 6B (88.5) respectively. Five QTLs were for membrane thermostability index on chromosome 1A (47.6), 1B (120.6), 2A (100.4), 3A (109.5) and 6B (17.2). One QTL was identified for yield per plant and one for grain filling rate at chromosome 2A (180.7) and 4A (146.8) respectively. Five QTLs were identified for grain filling duration at a map position 113.5 (2A), 31.5 (2A), 88.5 (3A), 31.0 (5A) and 29.0(5B). Similar to our findings Ciuca and Petcu (2009) also reported QTL for membrane stability and injury index, located close to Xwmc9, Xwmc596, Xwmc603 and Xbarc108. The GFR is the most important parameters associated with grain development, which determine the productivity of wheat genotypes (Yang and Zhang, 2006). In general, the process of grain filling is regulated by both GFR and GFD (Wang et al., 2009); however, their relative contribution remains debatable. Mashiringwani and Schweppenhauser (1992) reported that genotypic differences in grain yield of wheat were due to differences in GFR. It indicates that genetic differences in final grain weight were related to differences in GFR rather than GFD as GFD and GFR is also influenced by rise in temperature. However, GFR is generally accelerated to compensate for reduction in GFD. The QTL for stem water-soluble carbohydrates at the flowering stage and accumulation efficiency of stem water-soluble carbohydrates was also detected (Yang et al. 2007) in the neighboring region of the QTL on chromosome 7BL. Pinto et al. (2010) reported important chromosomal regions of QTLs on chromosomes 1B, 2B, 3B, 4A, 4A, and 5A. Several co-localized QTL for yield and grain weight with agronomic and physiological traits have been reported in wheat under drought and heat stress and temperate irrigated conditions (Pinto et al. 2010; Mason et al. 2010). Paliwal et al., 2012 prepared a linkage map comprising 160 simple sequence repeat markers covering the whole genome of wheat. Using composite interval mapping, significant genomic regions on 2B, 7B and 7D were found to be associated with heat tolerance. These hot spot regions of QTL could carry the genetic information about relation between morpho-physiological traits under heat-stressed environments.

The presence of pleiotropic/co-localized QTLs and their importance has been reported in wheat for different traits (Kumar et al. 2010; Vijayalakshmi et al. 2010) including heat-stress-related traits (Mason et al. 2010, 2011; Pinto et al. 2010). According to Yang et al. (2007), the common QTL can be a result of: (a) two strongly linked genes affecting different traits; (b) a single gene that can produce a number of effects in related traits; (c) one gene governing two or more independent traits; and (d) two linked genes that affect the same traits. The co-location of QTL for different agronomic and physiological traits with QTL for yield suggests that it is possible to achieve genetic dissection of the crop performance under heat stress to facilitate a more efficient breeding approach (Pinto et al. 2010).

Molecular markers identified in this study are expected to benefit wheat breeders in selection of heat tolerant plants and development of improved wheat varieties and will also provide preliminary information for further fine mapping and cloning. In addition to several QTLs identified in the present study, primers designed for two known genes namely *HSP16.9* and

HSP101 were found to clearly differentiate between all heat tolerant and sensitive genotypes among parents as well as F_2 population of both the crosses. The primers based on *HSP16.9* gene were earlier reported to be associated with heat tolerance in wheat (Garg *et al*. 2012). In our study we could find a novel marker based on *HSP101* gene associated with thermotolerance in wheat. It is suggested that *HSP16.9* and *HSP101* gene primers used in the study are potential markers that can be used by breeders for improving tolerance to high temperature in wheat.

CHAPTER-VI

SUMMARYAND CONCLUSION

The present investigation was conducted to study the Phenotypic and molecular analysis of terminal heat stress tolerance in wheat. Crosses between terminal heat stress tolerant and sensitive genotypes of wheat Hindi 62 x WH 711 and DBW 16 x WH 147 were made to raise F_1, F_2 & F_3 populations. Data has been recorded on individual plant basis for all the two crosses in respect of characters viz. membrane stability index , chlorophyll stability index, grain yield, kernels/Spike, thousand kernel weight (Test weight), days to heading, days to maturity, plant height, number of tillers/plant, biomass/plant, harvest index, grain filling duration and grain filling rate.

The results are summarised as below:

Phenotyping of mapping populations:

- In the F_2 and F_3 populations of both crosses viz. Hindi62 x WH711 and DBW16 x WH147 under normal sown condition, the morphological characters showed significant variation and ranged for chlorophyll index (0.51-0.64), membrane thermostability (46.7-63.96), plant height (96.33-136.6 cm), kernel/spike (16-57), tiller/plant (3-14), biomass/plant (21.5-83.12), grain yield (4.82-21.80), test weight (24.6-42.3), days to heading (104-117), days to maturity (145-159), harvest index (20.76-36.11), grain filling duration (33.3-48.3) and grain filling rate (0.57-1.11).
- The phenotypic coefficient of variation (PCV) was higher than respective genotypic coefficient of variation (GCV) for all the traits under study. The estimates of heritability in broad sense were moderate to high for most of the characters indicating more than 0.9 as for chlorophyll index (0.953), membrane thermostability (0.994), plant height (0.988), kernel per spike (0.904), test weight (0.983), grain filling duration (0.794) and grain filling rate (0.852).
- The genetic advance in terms of per cent of mean at 1 per cent selection intensity has been indicated very high for tiller per plant (52.325), and high for kernel per spike (38.070), test weight (36.168) and moderate for plant height (25.055), Membrane thermostability index (26.918 and low for days to heading (7.852), days to maturity (5.207) and Harvest index (1.393).
- Similarly in F_3 population significant results were found and traits showed significant variation in both crosses.

- Under late sown conditions also F_3 population from both crosses showed significant variation in traits viz. membrane thermostability index (46.7-66.8), chlorophyll stability index (0.502-0.672), grain yield (6.3-19.7 g), kernels/spike (24-52), thousand kernel weight (23.1-40.9 g), days to heading (79-88), days to maturity (112-122), plant height (78-126 cm), number of tillers/plant (4-15), biomass/plant (20.8-84.6 g), harvest index (20.05-31.28), grain filling duration (23.2-40.9) and grain filling rate (0.663-1.205).

Molecular marker analysis:

- Out of 25 RAPD and 103 SSR primers, 14 RAPD and 67 SSR primers showed polymorphism among parental lines.
- RAPD primers resulted in amplification of 2-13 bands and average percent polymorphism of 58.87%.
- The total number of alleles using SSR primers varied between 2 (primer Xgwm 190) and 6 (primer Xbarc 136) with an average of 3 bands per primer. Average polymorphism across the population was found to be 77.07%.

QTL analysis:

- A total of 34 QTLs were detected in both the crosses (Table- 17, 18). . In cross-I (Hindi62 x WH711), three QTL were detected for chlorophyll index at map positions, 135.7 (1B), 184.6 (2A) and 36.4 (6B) by SSR marker WMC 416, Xbarc188, Xgwm526, WMC170, Xgwm219, WMC398 respectively. Similarly in cross-II (DBW16 x WH147), 4 QTLs were detected for chlorophyll index on chromosome 1B (120.9), 2A (174.5), 6B (35.8) and 6B (88.5) respectively
- In cross-I six QTL were detected for membrane thermostability index at map positions, 118.7 (1B), 58.2 (2A), 137.2 (2A), 176.7 (2A), 62.0 (5A) and 11.7 (6B) by SSR marker WMC416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219 and WMC398. In cross-II, five QTLs were detected for membrane thermostability on chromosome 1A (47.6), 1B (120.6), 2A (100.4), 3A (109.5) and 6B (17.2).
- For yield per plant 6 QTLs were detected at map position103.4 (1B), 51.6 (2A), 130.6 (2A), 178.9 (2A), 55.4 (5A), 22.6 (6B) by SSR markers WMC 416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219, WMC398 in

cross-I while in cross-II, one QTL was identified for yield per plant and one for grain filling rate at chromosome 2A (180.7) and 4A (146.8) respectively

- For grain filling duration, one QTL was detected at chromosome no. 2A at a map position 171.9 and for grain filling rate 2 QTLs were detected on chromosome 2A and 6B at map position of 66.0 and 76.3 by SSR markers WMC642, Xgwm356, Xgwm219, WMC398 respectively in cross-I while in cross-II, Five QTLs were identified for grain filling duration at a map position 113.5 (2A), 31.5 (2A), 88.5 (3A), 31.0 (5A) and 29.0(5B).

- *HSP*16.9 and *HSP* 101b primers showed clear distinction between thermotolerant and thermosensitive genotypes by amplifying a unique band of 197 bp and 120 bp in all thermosensitive genotypes which was absent in thermotolerant genotypes.

- In conclusion, several QTLs identified in the present study may prove useful for direct selection of thermotolerant genotypes in wheat breeding programmes. These will also prove useful in further fine mapping and cloning of genes for thermotolerance. A novel marker based on *HSP101* gene primers has been identified for the first time to be associated with thermotolerance in wheat. This marker along with *HSP16.9* gene based marker would prove useful in improving wheat for thermotolerance using marker assisted selection.

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ABSTRACT

Title of Thesis : **Phenotypic and molecular analysis for terminal heat stress tolerance in wheat (*Triticum aestivum* L.)**
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The present investigation was conducted to study the phenotypic and molecular analysis of terminal heat stress tolerance in wheat *(Triticum aestivum* L.) in the Department of Molecular Biology & Biotechnology, CCS Haryana Agricultural University, Hisar, India. Two F_2 mapping populations containing 90 plants each involving crosses between Hindi62 (heat tolerant) x WH711 (heat susceptible) and DBW16 (heat tolerant) x WH147 (heat susceptible) were developed. Seeds from individual F_2 plants were harvested and sown in two environments, normal sown (30th November 2011) and late sown (26th December 2011) to get F_3 mapping population. Heat stress was created by late sowing the crop. Data was recorded on individual plant basis for both the crosses in respect of characters viz. membrane thermostability index, chlorophyll stability index, grain yield, kernels/spike, thousand kernel weight (Test weight), days to heading, days to maturity, plant height, number of tillers/plant, biomass/plant, harvest index, grain filling rate and grain filling duration.103 SSR, 25 RAPD and 2 known gene primers were used in molecular marker analysis for terminal heat stress tolerance. For QTL identification data was analysed by WinQTL-Cartographer. In cross Hindi62 x WH711 three QTL were detected for chlorophyll index at map positions, 135.7 (1B), 184.6 (2A) and 36.4 (6B) by SSR marker WMC 416, Xbarc188, Xgwm526, WMC170, Xgwm219, WMC398respectively. Six QTL were detected for membrane thermostability index at map positions, 118.7 (1B), 58.2 (2A), 137.2 (2A), 176.7 (2A), 62.0 (5A) and 11.7 (6B) by SSR marker WMC416, Xbarc188, WMC407, WMC642, Xgwm356, Xgwm95, Xgwm526, WMC170, Xgwm443, WMC475, Xgwm219 and WMC398. For yield per plant 6 QTLs were detected at map position103.4 (1B), 51.6 (2A), 130.6 (2A), 178.9 (2A), 55.4 (5A), 22.6 (6B). For grain filling duration one QTL was detected at chromosome no. 2A at a map position 171.9 and for grain filling rate 2 QTLs were detected on chromosome 2A and 6B at map position of 66.0 and 76.3 respectively. Similarly in cross DBW16 x WH147, 4 QTLs were detected for chlorophyll index on chromosome 1B, 2A, 6B and 6B respectively. Five QTLs were for membrane thermostability index on chromosome 1A, 1B, 2A, 3A and 6B.One QTL was identified for yield per plant and one for grain filling rate at chromosome 2A and 4A respectively. Five QTLs were identified for grain filling duration at a map position 113.5 (2A), 31.5 (2A), 88.5 (3A), 31.0 (5A) and 29.0(5B). These QTLs may be used for further improvement of the traits they represent. *HSP*16.9 and *HSP* 101b primers showed clear distinction between thermotolerant and thermosensitive genotypes and are suggested as potential DNA markers for improving thermotolerance in wheat using marker-assisted selection.

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I, Pritam Kumar, Admn. No. 2007BS8D undertake that I give copy right to the CCS HAU, Hisar of my thesis entitled, "**Phenotypic and molecular analysis for terminal heat stress tolerance in wheat *(Triticum aestivum* L.)**".

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Annexure 1: Mean of different traits observed in F_2 population derived from cross of Hindi62×WH711

Plant No.	Chlorophyll index	Membrane thermostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yeild	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.6337	47.5000	119.0000	45.3333	12.3333	70.6467	16.4333	37.5333	109.3333	151.6667	23.1167	42.3334	0.89
2	0.5473	58.8333	105.0000	36.3333	5.0000	26.3867	7.5833	30.0000	106.0000	145.6667	29.9633	39.6667	0.76
3	0.5917	46.7000	137.6667	57.0000	11.6667	66.3800	17.0667	37.5667	116.6667	159.6667	25.7133	43	0.87
4	0.5313	60.8667	109.0000	35.6667	3.6667	21.5100	4.8233	27.9667	107.0000	145.3333	22.6767	38.3333	0.73
5	0.6423	48.3333	114.6667	47.3333	11.6667	66.8667	15.7400	37.8333	109.0000	153.6667	23.7400	44.6667	0.85
6	0.6213	48.4667	118.6667	49.0000	11.3333	65.2767	16.1033	38.2333	112.0000	154.3333	24.8733	42.3333	0.90
7	0.5910	47.8667	117.0000	45.0000	11.6667	66.9067	16.2067	38.2667	114.0000	152.0000	24.4633	38	1.01
8	0.5470	63.2667	102.3333	36.0000	6.3333	37.4967	8.7400	26.1333	106.3333	146.0000	23.2767	39.6667	0.66
9	0.6103	47.2667	137.6667	42.3333	12.6667	72.6933	17.5767	38.2667	109.3333	149.3333	24.2133	40	0.96
10	0.5750	48.4000	129.6667	45.0000	11.6667	67.4667	17.2800	39.3333	114.3333	150.6667	25.8467	36.3334	1.08
11	0.5243	59.3667	102.0000	35.0000	5.3333	31.0500	7.8033	26.9667	105.6667	146.0000	25.1533	40.3333	0.67
12	0.5780	48.4667	116.3333	45.0000	11.0000	63.8800	15.0733	37.8667	108.3333	156.0000	23.5433	47.6667	0.79
13	0.6310	47.7667	123.0000	47.3333	12.0000	70.7000	16.2567	38.3000	106.6667	149.0000	23.2367	42.3333	0.90
14	0.6390	48.4333	117.6667	47.6667	11.0000	64.8000	15.2200	39.3000	113.6667	149.0000	23.6800	35.3333	1.11
15	0.6123	47.7667	118.0000	46.3333	10.3333	60.6133	14.4800	39.6333	107.6667	151.0000	23.7267	43.3333	0.91

16	0.5760	48.5000	119.6667	42.0000	12.0000	70.6533	16.6100	41.4333	108.3333	149.3333	23.6433	41	1.01
17	0.5330	63.9667	104.0000	25.6667	6.3333	34.5367	8.8400	27.5333	109.3333	145.3333	25.4467	36	0.76
18	0.5217	61.8000	102.6667	16.6667	7.3333	42.7900	10.8067	28.6000	104.6667	146.0000	24.9533	41.3333	0.69
19	0.5960	47.5333	126.3333	43.0000	12.6667	72.5700	17.4000	42.3000	114.6667	154.0000	24.0167	39.3333	1.08
20	0.6357	48.3667	117.3333	43.3333	10.0000	57.8667	14.1567	34.3333	110.0000	150.0000	24.4267	40	0.86
21	0.5260	59.0667	100.0000	30.6667	7.6667	44.7900	10.8067	27.8333	105.3333	148.0000	24.1900	42.6667	0.65
22	0.5223	62.0000	101.3333	33.3333	6.6667	37.8300	9.2800	26.0667	105.3333	145.6667	24.4200	40.3334	0.65
23	0.5173	60.2667	102.0000	32.3333	7.0000	40.8200	9.9467	27.2667	105.6667	145.0000	24.2333	39.3333	0.69
24	0.5220	59.7000	114.3333	27.6667	7.3333	44.0500	10.3467	26.1667	105.3333	145.3333	24.0467	40	0.65
25	0.5720	48.3000	129.3333	41.3333	12.3333	72.7433	17.7167	35.4000	116.6667	150.6667	24.2667	34	1.04
26	0.5820	47.4000	132.6667	46.6667	13.3333	75.2500	18.7600	34.8000	113.0000	150.0000	24.9933	37	0.94
27	0.5270	58.7000	100.0000	32.6667	7.3333	42.5733	10.6000	24.7000	106.6667	145.0000	25.0800	38.3333	0.64
28	0.5203	57.9000	99.0000	27.3333	6.0000	35.2833	8.6200	26.6667	105.6667	150.0000	24.6467	44.3333	0.60
29	0.5223	59.2667	100.0000	33.6667	6.0000	34.7400	8.2600	25.9333	105.6667	150.3333	23.8267	44.6666	0.58
30	0.5830	47.6333	119.6667	40.0000	13.3333	78.0367	18.6733	39.3667	110.6667	151.0000	23.9900	40.3333	0.98
31	0.6170	48.4333	112.6667	44.0000	10.0000	57.5333	14.5333	33.2667	109.0000	149.6667	25.2700	40.6667	0.82
32	0.6340	47.7333	116.6667	41.0000	13.3333	76.5833	18.8800	35.6333	107.3333	150.0000	24.5133	42.6667	0.84
33	0.5773	48.3667	119.3333	42.0000	12.0000	69.6267	17.6800	34.7333	116.3333	150.3333	25.2967	34	1.02

34	0.6310	47.7333	113.6667	42.0000	12.3333	69.9133	17.5467	39.6667	114.0000	152.0000	25.1433	38	1.04
35	0.6407	47.6667	126.6667	44.3333	12.0000	69.0700	16.5167	41.5000	113.3333	154.0000	23.9133	40.6667	1.02
36	0.5200	61.1667	101.0000	33.3333	7.0000	40.5867	10.0133	25.7000	105.0000	149.3333	24.6267	44.3333	0.58
37	0.5233	59.3333	98.3333	32.3333	7.3333	41.8767	10.7733	24.8667	105.0000	149.0000	25.8367	44	0.57
38	0.5763	48.3667	109.3333	47.6667	13.0000	74.7800	18.5767	35.3333	111.6667	156.0000	24.9533	44.3333	0.80
39	0.5973	48.3000	117.6667	43.6667	11.3333	64.1467	16.1867	36.2667	109.3333	151.3333	25.1767	42	0.86
40	0.6130	49.2667	110.3333	46.0000	11.6667	66.5800	16.6600	31.7667	112.6667	153.3333	25.0400	40.6666	0.78
41	0.5970	47.7333	118.0000	45.0000	12.3333	70.4700	17.8400	34.8000	109.3333	151.0000	25.1067	41.6667	0.84
42	0.6290	47.3667	119.3333	42.0000	13.3333	78.5833	19.2133	34.6333	113.3333	150.0000	24.3467	36.6667	0.94
43	0.5253	60.4000	96.3333	34.3333	5.3333	30.4833	7.6267	26.1000	106.3333	145.3333	25.2633	39	0.67
44	0.5210	59.0000	103.6667	36.0000	5.0000	29.1500	7.2600	24.9000	105.6667	146.3333	25.0233	40.6666	0.61
45	0.5790	48.4667	118.3333	42.0000	13.3333	76.3833	19.2267	34.4667	110.0000	150.3333	25.0267	40.3333	0.85
46	0.5703	47.4667	114.0000	42.3333	11.6667	66.4000	16.3800	35.3000	109.3333	153.0000	24.7667	43.6667	0.81
47	0.5907	48.3333	107.3333	44.6667	11.3333	63.6800	16.6267	31.7667	109.3333	156.6667	26.0500	47.3334	0.67
48	0.5210	58.7667	98.0000	32.3333	6.6667	37.0300	9.4067	26.5333	105.6667	146.0000	25.3667	40.3333	0.66
49	0.5667	48.4000	124.0000	43.6667	11.6667	67.7800	16.4133	38.3333	111.0000	154.0000	24.2933	43	0.89
50	0.5780	47.7333	127.6667	41.3333	12.6667	77.0033	17.8933	31.5667	116.3333	150.3333	23.2433	34	0.93
51	0.6130	48.3333	116.6667	45.0000	11.3333	63.9133	16.3000	33.7333	113.6667	150.3333	25.5833	36.6666	0.92

52	0.6393	48.4000	119.3333	47.0000	11.3333	66.0800	16.5100	34.5667	115.3333	150.0000	24.9500	34.6667	1.00
53	0.6307	48.5000	117.0000	39.6667	11.3333	63.5700	15.9600	32.5000	109.0000	152.0000	25.0133	43	0.76
54	0.6140	48.4000	126.3333	43.6667	12.0000	68.6900	17.3933	34.9000	115.0000	153.3333	25.3700	38.3333	0.91
55	0.6107	47.6333	133.0000	44.3333	11.6667	67.2000	16.3000	36.4333	113.0000	150.0000	24.3867	37	0.98
56	0.6273	47.6667	121.3333	40.3333	12.6667	72.3033	17.7667	32.7000	109.0000	150.0000	24.5933	41	0.80
57	0.5980	48.4000	119.6667	48.6667	10.0000	57.1333	13.6500	31.7667	108.0000	153.3333	23.8967	45.3333	0.70
58	0.6140	48.5000	131.6667	46.6667	12.6667	71.8467	18.1467	34.6667	107.6667	156.0000	25.2733	48.3333	0.72
59	0.5707	47.6333	136.6667	45.3333	10.6667	60.6333	14.8267	32.8333	115.0000	153.0000	24.5067	38	0.86
60	0.5967	47.3667	117.6667	45.6667	12.0000	68.5033	17.0200	33.3333	113.0000	150.3333	24.8600	37.3333	0.89
61	0.5227	58.4000	98.3333	31.0000	7.0000	39.8300	10.0533	26.3667	105.6667	145.3333	25.2733	39.6666	0.66
62	0.5670	47.7333	117.0000	46.3333	11.3333	64.9000	15.9533	37.8333	108.0000	152.0000	24.6533	44	0.86
63	0.5737	47.5000	119.6667	44.0000	11.6667	66.1467	16.4200	36.3000	111.0000	150.0000	24.8100	39	0.93
64	0.5670	48.5667	119.6667	43.0000	11.0000	61.5033	15.6667	34.7000	110.3333	156.0000	25.3633	45.6667	0.76
65	0.6307	48.4000	117.3333	46.0000	12.3333	70.5133	17.6067	33.3000	113.6667	151.6667	24.9500	38	0.88
66	0.6107	48.5667	119.3333	42.0000	11.0000	61.8333	15.8867	31.8000	117.0000	150.3333	25.7633	33.3333	0.95
67	0.5893	48.4333	147.3333	47.0000	14.0000	79.5767	19.9333	33.0000	115.0000	149.6667	25.0233	34.6667	0.95
68	0.5670	51.3667	136.3333	51.3333	11.0000	62.7733	15.5933	31.8667	111.0000	151.3333	24.8533	40.3333	0.79
69	0.6167	48.5667	142.6667	45.6667	14.3333	83.1267	21.1000	36.4333	111.0000	152.3333	25.4533	41.3333	0.88

70	0.5920	48.5333	116.3333	45.3333	11.0000	63.3567	15.3200	38.0333	112.3333	153.6667	24.2167	41.3334	0.92
71	0.6380	47.5333	112.3333	43.0000	11.3333	62.3000	16.7533	25.5000	117.6667	156.6667	27.0500	39	0.65
72	0.5760	47.7333	109.3333	44.3333	11.3333	64.9467	15.7000	33.4667	110.3333	151.3333	24.2467	41	0.82
73	0.5380	59.8667	97.3333	33.3333	6.6667	37.7533	9.6267	24.6000	105.6667	145.6667	25.5700	40	0.62
74	0.5707	47.5000	118.3333	43.0000	11.3333	64.6567	15.0667	32.3333	110.6667	154.0000	23.5833	43.3333	0.75
75	0.5793	48.3000	119.3333	47.3333	11.6667	66.7033	16.7533	31.8000	114.0000	150.6667	25.3467	36.6667	0.87
76	0.5907	47.5000	123.6667	47.0000	12.0000	68.5133	17.2533	32.4333	110.0000	151.0000	25.2200	41	0.79
77	0.5820	48.3667	117.3333	49.0000	11.3333	64.9033	16.1800	32.8333	111.6667	152.0000	25.0367	40.3333	0.81
78	0.5893	48.8000	116.6667	44.6667	11.0000	63.3900	14.7667	34.5333	112.6667	156.0000	23.4133	43.3333	0.80
79	0.6380	47.5667	127.3333	41.6667	13.0000	73.2067	17.9133	39.8667	117.0000	154.3333	24.3500	37.3333	1.07
80	0.6127	48.4333	132.6667	43.6667	11.3333	64.1333	15.8867	32.4333	112.0000	152.0000	24.8733	40	0.81
81	0.5230	61.8667	97.0000	37.0000	6.6667	37.0633	9.4467	26.4333	106.3333	146.0000	25.7733	39.6667	0.67
82	0.6120	48.3667	119.3333	36.0000	12.0000	68.0900	15.1667	36.4000	110.6667	152.0000	22.3600	41.3333	0.88
83	0.6380	47.5667	121.0000	45.0000	12.0000	69.0000	16.8533	34.4667	111.6667	151.6667	24.5233	40	0.86
84	0.5707	48.5000	118.3333	48.0000	10.6667	61.7333	15.6667	37.3000	109.6667	153.3333	25.3900	43.6666	0.85
85	0.5750	47.7000	117.3333	49.0000	11.0000	64.1667	16.2000	36.4000	115.3333	156.3333	25.0200	41	0.89
86	0.5680	48.3333	118.6667	42.0000	11.6667	67.5467	16.7600	34.3000	114.3333	154.6667	24.6833	40.3334	0.85
87	0.5923	48.1667	127.3333	43.6667	11.3333	64.4533	16.4867	32.7333	116.3333	151.3333	25.9733	35	0.94

88	0.6310	48.5333	122.3333	48.6667	11.0000	63.5000	15.7933	31.3000	110.0000	150.6667	24.8767	40.6667	0.77
89	0.5880	48.3667	110.3333	46.3333	13.0000	70.3667	18.5800	32.1667	110.6667	156.3333	26.4633	45.6666	0.70
90	0.5690	48.5333	119.6667	47.6667	11.0000	60.7800	15.3000	34.3667	111.6667	153.0000	25.1600	41.3333	0.83
Hindi62	0.6486	47.6541	126.5982	56.8754	15.6822	88.5000	20.2000	40.2555	115.3333	154.3333	17.7265	39	1.03
WH711	0.5238	64.5482	98.2496	38.6824	8.6667	42.7333	12.5486	42.6881	108.6667	148.6667	20.2809	40	0.82
Mean	**0.5830**	**50.9352**	**116.7481**	**41.8333**	**10.5333**	**60.3945**	**14.9114**	**33.2385**	**110.5000**	**150.9185**	**24.7707**	**40.398**	**0.83**
C.V.	1.4418	0.8240	1.0661	4.9487	12.3071	12.8688	12.6216	1.8346	1.1545	0.8424	6.1242	0.9364	1.264
S.E.	0.0049	0.2423	0.7186	1.1952	0.7484	4.4872	1.0866	0.3521	0.7366	0.7340	0.8758	0.0654	0.926
C.D. 5%	0.0135	0.6763	2.0055	3.3356	2.0887	12.5227	3.0325	0.9826	2.0556	2.0485	-	1.874	1.248
C.D. 1%	0.0179	0.8923	2.6461	4.4011	2.7559	16.5228	4.0011	1.2964	2.7122	2.7029	-	2.875	1.982

Annexure II: Mean for different traits of parents and F_2 population of DBW16 X WH147

Plant No.	Chlorophyll index	Membrane thermostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yield	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.6340	48.4000	136.6667	45.6667	11.6667	60.0333	13.9333	35.6000	111.0000	152.6667	22.6797	41.7	0.85
2	0.6130	47.3000	129.3333	46.6667	12.0000	59.4667	14.9000	34.6333	112.6667	148.3333	24.8390	35.7	0.97
3	0.5943	48.3000	131.6667	47.0000	11.0000	62.4000	13.3000	36.8000	110.6667	154.0000	28.3953	43.3	0.85
4	0.5270	61.9000	101.6667	35.3333	7.3333	33.7333	8.8000	26.8000	104.3333	143.6667	22.5670	39.3	0.68
5	0.5873	47.5333	120.6667	50.00	11.3333	56.2000	13.4000	34.7000	112.0000	153.3333	29.2833	41.3	0.84
6	0.5213	60.4333	96.6667	31.0000	7.6667	33.5667	9.0667	26.9000	105.3333	145.3333	22.6353	40.0	0.67
7	0.5943	47.5333	122.0000	46.3333	12.3333	60.1333	15.7667	37.6000	115.0000	151.3333	32.8243	36.3	1.03
8	0.5227	63.1333	96.3333	38.3333	8.3333	33.6000	10.6667	25.8667	104.6667	142.6667	31.0613	38.0	0.68
9	0.5760	48.7000	112.6667	46.0000	13.0000	60.6000	14.5333	41.4000	112.0000	154.0000	24.4043	42.0	0.99
10	0.5837	48.3000	119.3333	41.0000	11.6667	57.9667	13.6667	42.3333	111.0000	151.6667	23.8957	40.7	1.04
11	0.5907	48.4333	112.6667	46.0000	10.6667	53.0000	13.0333	38.3000	116.0000	156.0000	25.9093	40.0	0.96
12	0.6407	48.4333	117.6667	46.6667	11.3333	55.7000	13.7667	37.9667	112.3333	151.6667	23.9553	39.3	0.97
13	0.6307	48.3333	123.3333	48.3333	10.6667	53.6333	13.1000	36.3333	111.3333	150.3333	28.2913	39.0	0.93
14	0.5213	60.8333	96.0000	35.0000	7.0000	35.6000	9.4000	24.9333	104.3333	143.3333	27.6177	39.0	0.64
15	0.5220	61.9667	120.0000	31.6667	7.6667	38.6000	9.8667	25.2667	105.0000	142.3333	21.8683	37.3	0.68

16	0.5720	47.2333	117.6667	45.6667	12.0000	62.8000	16.2000	34.8000	108.0000	153.3333	25.0463	45.3	0.77
17	0.5937	48.3333	120.3333	46.6667	11.0000	57.0333	13.6667	36.8333	112.6667	151.0000	25.9057	38.3	0.96
18	0.6137	47.7333	120.3333	46.3333	11.3333	57.1333	14.7333	34.6000	111.6667	152.0000	25.1770	40.3	0.86
19	0.6207	48.2333	123.6667	46.3333	10.3333	51.0667	12.7667	34.9000	113.0000	154.0000	26.5653	41.0	0.85
20	0.5923	48.6000	141.3333	48.3333	12.0000	45.4667	14.8333	36.9000	110.3333	150.3333	31.2397	40.0	0.92
21	0.5940	47.5000	126.3333	46.3333	17.6667	94.5000	20.4000	36.4000	113.6667	153.6667	22.8247	40.0	0.91
22	0.6210	47.3667	137.3333	46.3333	12.3333	76.2667	15.2333	31.6333	110.3333	151.0000	21.6250	40.7	0.78
23	0.5863	47.3667	124.3333	47.0000	10.6667	55.8667	13.3667	34.6333	115.0000	156.0000	23.3783	41.0	0.84
24	0.5877	48.3333	119.6667	43.6667	12.0000	66.6000	14.7667	33.6000	112.3333	152.3333	24.1090	40.0	0.84
25	0.6507	47.5333	117.3333	46.3333	11.6667	57.5667	14.3667	41.6000	111.3333	152.0000	24.7133	40.7	1.02
26	0.6193	47.6333	121.3333	48.3333	12.3333	72.7000	15.7667	41.8667	112.0000	150.0000	24.0880	38.0	1.10
27	0.5867	48.5333	127.6667	46.0000	11.6667	59.5667	14.6000	37.7000	111.0000	149.6667	27.7687	38.7	0.97
28	0.5223	59.9333	104.3333	30.3333	8.3333	40.3000	10.2667	25.5667	105.3333	142.3333	25.2167	37.0	0.69
29	0.5920	47.7667	123.6667	46.0000	13.3333	75.5667	17.1000	35.5667	112.0000	152.0000	24.0143	40.0	0.89
30	0.6170	48.3333	119.6667	48.0000	11.3333	56.8000	15.3333	34.7667	114.0000	155.0000	25.4597	41.0	0.85
31	0.6420	47.4333	123.0000	48.6667	12.6667	65.5667	17.3000	39.6667	112.6667	153.6667	28.7817	41.0	0.97
32	0.5667	62.5667	102.3333	32.6667	7.6667	34.1333	9.7667	26.1333	104.6667	142.6667	27.9883	38.0	0.69
33	0.6127	47.5333	121.6667	46.3333	12.3333	64.1667	16.1000	35.5000	112.0000	153.0000	24.5540	41.0	0.87

34	0.5950	47.3000	120.6667	54.3333	11.0000	65.8000	14.4667	34.7000	113.0000	154.0000	21.3120	41.0	0.85
35	0.5223	60.0000	104.6667	33.6667	7.3333	35.2000	10.1000	22.5667	105.6667	143.0000	26.5630	37.3	0.60
36	0.5230	61.9000	95.6667	32.6667	7.6667	37.4333	11.6667	26.1667	104.6667	144.3333	32.7767	39.7	0.66
37	0.5950	47.6667	118.3333	47.6667	11.0000	53.9333	13.8667	38.4667	110.6667	153.6667	26.1733	43.0	0.89
38	0.5777	48.1667	123.0000	46.3333	13.0000	72.4333	16.7667	34.7000	111.6667	151.0000	23.4480	39.3	0.88
39	0.5230	63.3333	96.0000	32.6667	8.3333	37.1333	10.4667	25.5667	106.3333	144.3333	27.5010	38.0	0.67
40	0.6190	48.4333	119.3333	45.0000	14.3333	77.6667	18.2667	33.6333	111.6667	150.3333	24.7817	38.7	0.87
41	0.6267	48.4667	120.6667	48.3333	11.3333	56.1000	14.2333	32.6667	112.3333	152.6667	25.9720	40.3	0.81
42	0.5607	47.5667	118.3333	46.6667	11.6667	57.3333	14.0333	34.6667	111.3333	152.3333	27.5957	41.0	0.85
43	0.5233	59.8000	100.6667	20.3333	8.3333	45.9000	10.7333	26.0000	104.3333	143.3333	23.5317	39.0	0.67
44	0.5867	48.3000	122.3333	48.3333	13.6667	77.1667	18.9667	34.7667	112.3333	150.0000	24.9897	37.7	0.92
45	0.6107	47.5667	117.0000	48.6667	12.6667	65.7667	17.2000	36.8000	113.3333	150.3333	24.4713	37.0	0.99
46	0.6380	48.5000	123.3333	45.0000	11.3333	57.9667	13.8667	37.7333	112.3333	154.6667	25.5540	42.3	0.89
47	0.6107	47.5667	122.0000	46.0000	10.6667	52.8000	13.2667	39.3667	111.6667	154.6667	26.1253	43.0	0.92
48	0.5860	48.3667	112.0000	42.3333	10.3333	50.2667	13.2000	36.5000	114.6667	155.6667	27.3660	41.0	0.89
49	0.5233	59.1000	94.6667	31.0000	7.3333	34.9000	9.2000	25.2333	104.6667	145.0000	26.9847	40.3	0.63
50	0.6360	48.4667	127.0000	48.0000	11.6667	59.1000	14.8333	32.3667	112.3333	153.6667	25.8540	41.3	0.78
51	0.5927	43.9667	122.3333	49.6667	12.0000	64.3333	14.8000	31.5667	111.0000	149.6667	24.0313	38.7	0.82

52	0.5240	64.2333	89.3333	30.3333	7.6667	35.6333	9.8333	26.6000	105.3333	144.0000	28.6003	38.7	0.69
53	0.6203	48.4000	121.0000	45.0000	12.3333	51.2333	16.1000	34.7000	112.0000	150.3333	27.8170	38.3	0.91
54	0.5950	48.3333	120.3333	46.3333	14.0000	72.7333	18.4333	31.7000	111.6667	151.0000	26.2020	39.3	0.81
55	0.5223	60.4000	98.3333	30.3333	6.6667	28.7000	9.1333	26.0667	106.3333	142.6667	32.6447	36.3	0.72
56	0.5213	63.3000	98.3333	33.6667	7.6667	42.6333	8.8333	27.1333	105.3333	143.6667	21.2400	38.3	0.71
57	0.5750	48.4667	121.6667	43.3333	14.3333	73.7667	20.6333	35.2333	115.3333	156.0000	26.0183	40.7	0.87
58	0.6207	47.3667	126.3333	47.6667	18.0000	100.6333	25.2667	32.7000	111.0000	152.3333	25.6203	41.3	0.79
59	0.6493	48.3000	116.6667	49.3333	11.6667	57.4667	15.2000	34.7000	112.6667	150.0000	27.2520	37.3	0.93
60	0.5767	47.5667	119.3333	46.0000	11.6667	56.7333	15.3000	37.7333	111.6667	150.6667	25.7217	39.0	0.97
61	0.6230	48.3667	119.6667	45.0000	14.3333	75.8000	18.7000	41.2333	114.3333	156.0000	26.7440	41.7	0.99
62	0.5907	49.4333	121.3333	47.0000	11.6667	59.4000	15.2667	41.4333	111.6667	154.0000	25.4157	42.3	0.98
63	0.5950	47.3667	112.3333	45.6667	11.0000	56.5000	13.2667	39.8000	111.0000	152.3333	21.2243	41.3	0.96
64	0.6120	48.3667	117.3333	49.6667	12.6667	69.5000	17.4667	38.6667	112.3333	148.6667	25.8293	36.3	1.06
65	0.5917	47.3000	123.0000	47.3333	13.6667	70.3000	17.9667	39.3667	112.0000	152.0000	26.6410	40.0	0.98
66	0.5277	63.0000	96.6667	32.0000	7.6667	40.3333	9.9667	26.1333	104.6667	144.6667	25.4067	40.0	0.65
67	0.6223	48.3667	119.0000	61.0000	13.6667	70.4667	17.3333	42.3667	115.3333	156.0000	24.3713	40.7	1.04
68	0.5857	47.5667	123.6667	47.3333	11.6667	61.6333	14.7333	41.7333	111.6667	149.3333	23.8693	37.7	1.11
69	0.5970	47.4000	114.6667	48.6667	10.6667	56.4333	13.5667	34.2333	113.6667	153.3333	23.6567	39.7	0.86

70	0.5203	59.2667	100.0000	32.0000	7.6667	37.4000	10.3333	26.6667	105.3333	145.3333	27.1643	40.0	0.67
71	0.5907	47.4667	126.3333	62.0000	12.6667	63.2000	16.5333	34.9667	112.3333	153.6667	24.0907	41.3	0.85
72	0.6350	47.6667	117.0000	61.6667	13.6667	79.0000	16.7000	33.7000	115.6667	155.3333	21.4377	39.7	0.85
73	0.5280	64.1000	98.3333	35.6667	7.0000	35.8000	7.8033	24.9667	105.0000	144.3333	21.9643	39.3	0.63
74	0.6290	47.5000	116.0000	74.0000	12.0000	62.8333	14.5667	36.8667	110.3333	150.3333	23.2787	40.0	0.92
75	0.5950	47.6000	123.6667	63.3333	11.0000	57.4333	12.4333	38.3667	110.0000	149.6667	22.0673	39.7	0.97
76	0.5230	61.2667	98.6667	32.6667	8.0000	36.9333	8.9333	26.6000	104.3333	142.6667	23.5973	38.3	0.69
77	0.5970	47.4333	117.0000	59.6667	12.3333	64.1000	14.0667	36.5667	111.3333	152.0000	22.9187	40.7	0.90
78	0.6207	47.5000	117.6667	47.6667	12.0000	60.0667	13.0000	35.7000	115.6667	155.6667	21.0807	40.0	0.89
79	0.6187	47.4667	120.6667	49.6667	11.6667	60.2667	13.4333	34.5667	112.0000	152.6667	22.2167	40.7	0.85
80	0.5950	47.7000	112.6667	45.3333	11.3333	57.5000	13.1000	36.4000	116.6667	151.0000	22.4413	34.3	1.06
81	0.6227	48.4667	121.3333	56.0000	13.3333	69.6667	14.8000	31.8333	111.6667	153.0000	21.2297	41.3	0.77
82	0.6390	48.5667	117.0000	48.6667	11.6667	60.7000	13.5000	31.3000	114.0000	154.0000	22.5763	40.0	0.78
83	0.5203	63.1333	99.0000	34.0000	8.0000	38.1667	8.7667	26.6667	103.6667	144.0000	23.1440	40.3	0.66
84	0.6427	48.4333	108.0000	48.3333	15.3333	78.0000	18.7667	36.7333	116.3333	151.3333	25.9923	35.0	1.05
85	0.6203	48.5333	116.3333	53.0000	11.6667	59.5000	14.0000	34.1667	114.3333	154.3333	23.7967	40.0	0.85
86	0.5197	61.2000	100.6667	32.0000	7.6667	35.8667	7.5333	24.0000	103.3333	144.0000	19.1930	40.7	0.59
87	0.5380	64.0333	96.6667	31.6667	8.6667	40.9333	9.2000	28.4667	105.0000	144.3333	22.8923	39.3	0.72

88	0.5947	48.5333	127.6667	46.0000	12.6667	68.0000	15.1667	37.7000	113.0000	151.0000	21.9177	38.0	0.99
89	0.6207	47.5000	121.0000	60.0000	15.0000	80.0667	18.0667	37.9000	112.0000	150.3333	23.6963	38.3	0.99
90	0.6280	47.8333	116.3333	48.3333	11.3333	62.1000	13.4667	33.4667	114.0000	153.6667	21.6143	39.7	0.84
DBW16	0.6219	51.3566	98.6667	46.0000	14.3333	80.2255	16.2000	41.6222	92.0000	132.3333	17.8662	40.3	1.03
WH147	0.5233	62.5333	102.0000	32.3333	10.6660	56.4000	10.1660	43.6667	96.0000	146.0000	17.0929	40.0	0.84
Mean	**0.5872**	**51.2967**	**115.7519**	**44.7593**	**11.1519**	**57.0667**	**13.9430**	**33.7856**	**110.6407**	**150.3000**	**25.0700**	**39.7**	**0.85**
C.V.	0.5665	0.7083	1.5881	4.2339	11.1334	12.2068	12.5647	2.6416	1.3336	1.1001	12.8106	1.764	1.283
S.E.	0.0019	0.2098	1.0613	1.0941	0.7168	4.0218	1.0115	0.5153	0.8519	0.9546	1.8542	0.846	0.742
C.D. 5%	0.0054	0.5855	2.9619	3.0534	2.0005	11.2240	2.8227	1.4380	2.3775	2.6642	5.1747	0.968	0.828
C.D. 1%	0.0071	0.7725	3.9080	4.0287	2.6395	14.8092	3.7244	1.8973	3.1369	3.5151	6.8276	1.368	1.286

Annexure III: Mean for different traits observed in F_3 population derived from cross Hindi62×WH711 under normal sown condition

Plant No.	Chlorophyll index	Membrane theromostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yield	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.6564	46.74	117.0	42.7	14.0	72.25	14.3	34.3	107.3	148.3	19.79	36	0.95
2	0.5625	59.35	103.6	38.3	8.7	32.46	9.3	31.0	105.0	142.7	28.65	32.7	0.95
3	0.5978	45.87	124.3	53.0	13.3	69.37	18.1	33.6	109.7	152.7	26.09	38	0.88
4	0.5431	61.26	107.3	32.7	6.7	29.05	7.3	31.0	105.3	142.0	25.13	31.7	0.98
5	0.6563	47.58	110.6	42.3	12.3	69.30	14.6	36.2	106.0	148.3	21.07	37.3	0.97
6	0.6683	48.98	119.0	45.0	10.6	68.88	17.3	34.1	108.3	146.0	25.12	32.7	1.04
7	0.5987	48.14	115.6	42.0	13.7	71.30	16.8	40.3	110.7	144.3	23.56	28.6	1.41
8	0.5634	63.67	104.3	38.0	8.9	42.50	10.2	28.3	104.0	144.0	24.00	35	0.81
9	0.6287	48.25	126.6	40.3	14.3	76.79	17.9	36.0	110.3	142.3	23.31	27	1.33
10	0.5785	48.95	120.0	41.0	15.3	64.30	18.5	39.9	111.0	144.3	28.77	28.3	1.41
11	0.5465	60.14	104.6	38.0	8.7	42.45	9.3	31.0	105.0	146.0	21.91	36	0.86
12	0.5976	48.85	114.3	40.0	13.3	66.50	13.9	36.1	107.3	148.0	20.90	35.7	1.01
13	0.6476	46.82	121.6	43.3	11.7	72.79	17.5	41.3	104.0	146.0	24.04	37	1.12
14	0.6415	48.58	115.3	41.7	13.0	66.90	18.1	42.0	110.7	147.0	27.06	31.3	1.34
15	0.6265	47.15	116.0	47.3	12.7	62.65	15.9	41.1	105.3	144.0	25.38	33.7	1.22

16	0.5894	49.20	117.3	39.0	14.3	71.50	17.3	41.9	106.0	146.3	24.20	35.3	1.19
17	0.5276	63.13	102.0	28.7	7.7	36.87	9.2	32.5	107.7	142.0	24.95	29.3	1.11
18	0.5376	61.34	100.3	21.7	9.3	44.50	11.3	31.6	105.0	140.3	25.39	30.3	1.04
19	0.5987	46.86	120.0	41.0	15.0	74.65	18.2	42.5	110.3	148.0	24.38	32.7	1.30
20	0.6426	48.53	114.3	39.3	12.3	55.30	16.3	36.1	110.0	144.0	29.48	29	1.24
21	0.5461	58.25	98.6	28.7	10.3	46.68	12.5	30.3	103.7	148.0	26.78	39.3	0.77
22	0.5145	61.75	100.3	29.3	8.3	40.62	11.0	29.2	103.0	142.7	27.08	34.7	0.84
23	0.5192	61.20	101.6	36.3	9.3	42.50	9.9	30.1	106.3	142.0	23.29	30.7	0.98
24	0.5312	58.40	112.0	31.7	10.7	46.75	10.9	28.2	104.7	147.3	23.32	37.6	0.75
25	0.5865	47.20	123.3	39.3	13.0	70.50	18.2	33.9	112.3	147.7	25.82	30.4	1.12
26	0.5965	46.80	124.0	42.7	15.3	72.65	19.5	35.3	110.0	144.0	26.84	29	1.22
27	0.5165	59.35	98.6	29.7	9.0	44.50	11.5	28.3	103.7	142.0	25.84	33.3	0.85
28	0.5256	56.80	98.3	31.3	7.3	38.25	10.8	29.1	106.3	144.0	28.24	32.7	0.89
29	0.5356	58.75	96.6	30.7	10.3	32.74	9.3	27.9	103.0	146.3	28.41	38.3	0.73
30	0.5965	46.30	113.3	43.0	13.7	74.30	19.7	39.9	108.3	145.0	26.51	31.7	1.26
31	0.6256	47.90	110.6	41.0	11.0	54.50	16.2	36.3	107.3	142.7	29.72	30.4	1.19
32	0.6462	46.85	112.3	37.0	12.7	72.75	19.9	38.6	105.0	146.0	27.35	36	1.07
33	0.5873	48.93	116.6	41.0	11.3	66.50	17.9	36.9	110.3	148.0	26.92	32.7	1.13

34	0.6412	46.84	110.6	38.0	13.0	72.30	16.5	40.2	111.0	146.0	22.82	30	1.34
35	0.6498	46.80	120.3	41.3	11.3	70.65	15.7	41.9	109.0	148.0	22.22	34	1.23
36	0.5198	62.85	99.6	36.3	8.3	44.50	10.7	29.7	103.7	146.3	24.04	37.6	0.79
37	0.5324	58.30	99.0	35.3	9.7	43.30	12.8	28.9	106.3	144.0	29.56	32.7	0.88
38	0.5856	46.87	108.3	43.7	11.3	76.54	19.2	35.7	111.0	148.0	25.08	32	1.12
39	0.6132	48.95	113.6	40.7	12.0	68.75	17.2	38.5	107.3	146.0	25.02	33.7	1.14
40	0.6254	48.20	106.3	42.0	13.3	72.30	18.7	33.9	109.7	147.0	25.86	32.3	1.05
41	0.5982	46.93	114.3	41.0	11.0	75.05	17.1	36.1	109.0	147.0	22.78	33	1.09
42	0.6186	48.75	116.8	39.0	13.0	82.50	19.5	34.9	110.3	148.0	23.64	32.7	1.07
43	0.5314	61.58	97.0	32.3	8.7	37.80	9.3	29.1	104.7	142.0	24.60	32.3	0.90
44	0.5198	57.26	100.3	38.0	7.3	34.30	8.9	28.2	106.3	144.0	25.95	32.7	0.86
45	0.5816	47.15	116.0	40.0	12.0	78.65	19.2	36.3	110.0	148.0	24.41	33	1.10
46	0.5814	45.85	110.6	44.3	13.3	68.30	16.9	35.9	107.0	153.0	24.74	41	0.88
47	0.5895	47.53	104.3	46.7	14.0	69.75	16.3	33.8	106.7	148.3	23.37	36.6	0.92
48	0.5318	59.45	96.6	34.3	9.3	40.30	9.9	29.5	104.0	144.0	24.57	35	0.84
49	0.5562	47.35	118.3	41.7	12.3	69.85	17.4	38.6	108.0	148.0	24.91	35	1.10
50	0.5798	45.67	113.0	44.3	11.7	81.00	18.9	33.2	110.3	147.3	23.33	32	1.04
51	0.6215	48.90	112.3	41.0	12.0	67.30	15.3	35.8	111.7	147.0	22.73	30.3	1.18

52	0.6463	48.75	117.6	45.0	13.7	68.05	15.9	35.1	107.0	148.0	23.37	36	0.98
53	0.6395	49.52	115.0	36.7	12.7	62.50	16.1	31.8	105.7	148.0	25.76	37.3	0.85
54	0.6276	48.65	116.3	39.7	10.7	72.05	17.9	36.5	110.3	148.0	24.84	32.7	1.12
55	0.6365	47.70	121.6	46.3	12.3	69.30	17.5	38.3	106.0	147.3	25.25	36.3	1.06
56	0.6176	48.95	113.0	38.3	13.0	74.05	18.3	35.2	105.3	148.0	24.71	37.7	0.93
57	0.5998	49.85	114.6	46.7	10.7	62.75	14.5	33.0	107.7	147.3	23.11	34.6	0.95
58	0.6268	46.50	122.3	43.7	13.3	76.85	19.1	36.7	105.3	148.3	24.85	38	0.97
59	0.5687	47.90	120.6	41.3	12.3	64.65	14.3	35.4	110.0	146.0	22.12	31	1.14
60	0.5894	46.84	114.3	42.7	13.7	71.50	17.5	36.3	109.3	148.0	24.48	33.7	1.08
61	0.5156	59.53	97.5	32.0	9.0	43.05	11.5	29.4	109.7	142.7	26.71	28	1.05
62	0.5865	46.98	113.3	44.3	13.0	67.90	16.9	39.8	105.0	147.3	24.89	37.3	1.07
63	0.5687	45.87	117.6	42.0	12.3	63.50	17.3	38.3	111.0	147.0	27.24	31	1.24
64	0.5518	48.95	114.6	45.0	13.0	65.50	15.0	34.9	106.7	148.3	22.90	36.6	0.95
65	0.6486	47.75	112.3	43.0	11.3	74.51	17.0	34.1	109.3	150.0	22.82	35.7	0.96
66	0.6268	48.95	116.0	39.0	10.0	65.05	15.3	33.2	111.3	147.3	23.52	31	1.07
67	0.5913	47.58	128.6	43.0	13.0	82.50	19.2	33.0	109.7	146.3	23.27	31.6	1.04
68	0.5546	51.84	120.3	48.3	10.3	66.77	14.1	35.7	106.0	148.0	21.12	37	0.96
69	0.6287	48.93	128.0	41.7	13.7	83.05	18.5	38.4	109.3	146.3	22.28	32	1.20

70	0.5875	47.85	112.6	43.3	12.3	68.75	15.3	38.9	107.3	148.0	22.25	35.7	1.09
71	0.6453	48.40	110.3	39.0	10.3	64.50	16.1	29.2	110.0	148.7	24.96	33.7	0.87
72	0.5682	46.52	104.6	41.3	13.3	68.95	15.0	36.3	107.3	150.0	21.75	37.7	0.96
73	0.5538	60.85	95.3	31.3	8.7	34.75	10.3	27.3	105.7	143.7	29.64	33	0.83
74	0.5910	48.74	113.3	45.0	123	67.70	14.5	37.0	109.3	148.3	21.42	34	1.09
75	0.5823	49.50	112.0	47.3	12.0	66.30	15.9	32.9	107.0	150.0	23.98	38	0.87
76	0.6054	46.95	118.6	44.0	11.7	70.51	17.0	32.5	105.3	147.3	24.11	37	0.88
77	0.5967	47.75	113.6	48.0	10.3	66.20	14.0	34.1	109.7	149.0	21.15	34.3	0.99
78	0.5913	49.70	110.3	41.7	12.0	66.50	15.1	34.8	110.0	147.7	22.71	32.7	1.06
79	0.6459	46.85	121.6	39.7	133	73.75	18.7	39.9	112.3	149.3	25.36	32	1.25
80	0.6267	48.93	120.3	44.7	12.3	68.13	15.0	36.2	110.7	150.0	22.02	34.3	1.06
81	0.5187	62.25	99.0	38.0	8.7	39.06	11.3	29.3	109.3	143.3	28.93	29	1.01
82	0.6230	47.80	115.0	33.0	11.0	65.09	17.0	38.1	110.0	148.7	26.12	33.7	1.13
83	0.6470	47.95	118.3	42.0	13.0	65.00	18.5	32.5	109.3	149.0	28.46	34.7	0.94
84	0.5692	47.85	114.3	51.0	9.7	64.73	16.0	37.9	105.7	147.3	24.72	36.6	1.04
85	0.5840	48.20	115.6	46.0	10.0	61.17	18.3	39.1	109.7	149.3	29.92	34.6	1.13
86	0.5714	47.95	113.3	39.0	12.7	67.05	17.1	36.3	110.0	150.7	25.50	35.7	1.02
87	0.5892	47.55	120.6	40.7	10.3	61.45	15.9	34.5	109.3	149.3	25.87	35	0.99

88	0.6296	48.95	119.3	46.7	11.0	64.50	15.1	33.9	110.0	148.0	23.41	33	1.03
89	0.5910	49.20	108.6	43.3	14.0	70.50	15.5	36.7	107.3	149.3	21.99	37	0.99
90	0.5584	48.85	115.3	48.7	10.0	62.12	16.1	37.1	106.3	148.0	25.92	36.7	1.01
Hindi62	0.6597	46.65	116.3	52.9	14.7	86.50	19.4	41.3	110.7	149.3	22.43	39.6	1.07
WH711	0.5238	64.85	100.6	37.7	10.7	59.53	15.3	47.6	105.3	146.7	25.70	41.4	0.90
Mean	**0.5901**	**50.80**	**112.3**	**40.3**	**14.09**	**62.41**	**15.3**	**34.8**	**107.8**	**146.6**	**24.80**	**33.87**	**1.04**
C.V.	1.326	0.9160	1.2750	4.4752	13.0764	11.9640	12.1668	2.2472	1.4673	0.6428	6.3576	1.584	1.278
S.E.	0.0065	0.3120	0.8670	1.4876	0.6842	5.2867	2.0437	0.4478	0.6854	0.8256	1.1442	0.0286	0.0164
C.D. 5%	0.0148	0.5977	2.1256	4.2686	2.4018	11.8672	2.9254	1.2465	2.3476	2.9758	-	0.818	0.752
C.D. 1%	0.0186	0.8612	2.2460	6.2548	2.1684	16.8674	3.2864	1.5784	2.9254	2.2970	-	0.964	0.914

Annexure 4: Mean for different traits of F_3 population of Hindi 62 X WH 711 under late sown condition

Plant No.	Chlorophyll index	Membrane thermostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yeild	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.6630	48.7000	117.0000	42.0000	13.0000	64.8667	14.4333	33.1000	84.3333	118.0000	22.2067	33.67	0.983
2	0.5077	63.1667	100.6667	32.0000	6.0000	29.3667	8.0000	23.2000	79.3333	114.3333	27.1300	35.00	0.663
3	0.6070	46.9333	118.0000	52.3333	11.6667	55.9667	13.4667	35.1667	82.6667	116.6667	24.0733	34.00	1.034
4	0.5210	60.8000	97.6667	33.6667	6.6667	32.7333	9.0000	25.2667	79.6667	112.6667	27.3767	33.00	0.766
5	0.6723	49.1667	116.3333	45.0000	12.3333	64.6333	15.0667	36.4667	83.6667	117.3333	23.3367	33.67	1.083
6	0.6333	47.7333	118.3333	44.3333	11.0000	57.1667	13.1000	36.8333	81.6667	115.6667	22.9067	34.00	1.083
7	0.6163	47.7333	119.3333	42.6667	12.0000	64.7000	14.2667	36.9000	81.0000	116.3333	22.5100	35.33	1.044
8	0.5290	65.2667	96.6667	33.3333	5.0000	27.2000	6.9000	24.7000	80.0000	112.6667	25.5433	32.67	0.756
9	0.6247	49.1333	126.3333	35.6667	12.0000	64.0000	14.8667	36.8000	82.3333	116.3333	23.2467	34.00	1.082
10	0.5990	49.1667	116.0000	43.6667	13.3333	70.8333	15.7000	37.9667	85.6667	119.6667	22.1233	34.00	1.117
11	0.5217	63.9000	92.0000	34.3333	5.0000	24.8000	6.9667	25.6000	78.6667	113.6667	27.9000	35.00	0.731
12	0.5937	47.1333	122.0000	46.3333	11.0000	56.3333	13.5000	36.5000	86.3333	119.3333	23.9067	33.00	1.106
13	0.6540	49.5333	121.6667	44.3333	11.6667	60.2667	14.9667	36.9667	84.0000	117.6667	24.8167	33.67	1.098
14	0.6453	49.9333	122.3333	44.0000	11.0000	58.3333	12.8667	37.7333	82.3333	116.3333	22.0467	34.00	1.110
15	0.6247	48.2333	117.3333	45.0000	9.6667	51.2667	12.7000	38.2667	81.3333	115.6667	24.7767	34.33	1.115

16	0.5937	49.3000	120.0000	40.6667	13.0000	63.2000	15.8667	39.5333	87.6667	121.3333	25.0900	33.67	1.174
17	0.5270	65.0000	92.3333	23.3333	5.6667	28.7667	8.2333	26.1667	80.0000	113.0000	28.7900	33.00	0.793
18	0.5293	62.3667	88.0000	16.3333	6.6667	33.4000	9.1667	27.4667	80.6667	112.6667	28.0067	32.00	0.858
19	0.6407	49.8000	121.6667	42.3333	11.3333	57.4667	14.3333	40.9667	86.3333	120.3333	24.9300	34.00	1.205
20	0.6523	47.2000	118.0000	40.6667	10.3333	53.8667	13.2667	32.9667	81.0000	116.3333	24.7467	35.33	0.933
21	0.5257	60.8000	95.3333	28.3333	6.3333	33.0667	8.8667	26.4667	81.0000	113.6667	26.8267	32.67	0.810
22	0.5077	62.7333	93.6667	32.6667	6.3333	32.4333	8.8000	24.7000	80.3333	114.3333	27.2300	34.00	0.726
23	0.5217	65.8000	85.0000	30.6667	6.3333	31.4333	8.7000	25.9333	80.3333	112.3333	27.9067	32.00	0.810
24	0.5183	64.8000	92.6667	25.6667	7.0000	35.8000	9.3667	24.9000	80.3333	114.3333	26.7333	34.00	0.732
25	0.5800	65.3667	118.6667	40.0000	12.0000	58.2333	14.8000	33.9000	85.3333	119.3333	25.6367	34.00	0.997
26	0.5850	49.6333	121.6667	45.6667	11.3333	55.4333	13.6667	33.4667	87.0000	121.3333	24.8000	34.33	0.975
27	0.5333	64.6667	89.3333	31.0000	7.0000	34.1000	9.4333	23.4000	82.0000	113.6667	27.9800	31.67	0.739
28	0.5337	66.8333	88.6667	25.6667	6.3333	32.8333	8.9667	25.3000	81.3333	114.3333	27.8233	33.00	0.767
29	0.5153	65.4000	86.3333	32.0000	6.6667	31.7000	8.9333	24.5667	80.3333	113.0000	28.3167	32.67	0.752
30	0.6107	49.2333	118.3333	35.3333	11.3333	57.4333	14.2000	37.8000	84.0000	118.6667	24.7167	34.67	1.090
31	0.6187	49.9000	116.0000	41.3333	8.3333	39.8667	11.0333	31.9000	81.0000	114.3333	27.7267	33.33	0.957
32	0.6243	50.8333	118.6667	37.3333	12.6667	63.7333	16.0000	34.2667	85.0000	118.6667	25.0500	33.67	1.018
33	0.6430	47.9000	116.3333	39.3333	10.6667	53.8000	14.1667	33.3667	80.6667	113.3333	26.3133	32.67	1.021

34	0.6100	50.9000	114.3333	37.6667	11.0000	52.3667	13.8667	38.3000	86.6667	120.6667	26.6833	34.00	1.126
35	0.6427	51.0333	120.0000	32.0000	11.0000	56.1333	13.6000	40.2667	83.0000	117.3333	24.2233	34.33	1.173
36	0.5260	65.4000	95.3333	29.6667	6.6667	40.3000	9.1333	24.3333	81.6667	114.0000	22.8933	32.33	0.753
37	0.5280	64.2667	88.3333	31.3333	7.6667	41.1667	9.4667	23.5000	79.6667	114.6667	23.2233	35.00	0.671
38	0.5863	50.9000	115.6667	45.3333	12.3333	60.4000	16.0000	34.0000	82.0000	115.3333	26.5467	33.33	1.020
39	0.5903	48.7000	117.3333	40.6667	10.0000	52.5333	12.9333	34.8333	85.6667	116.6667	24.6833	31.00	1.124
40	0.5760	49.9333	112.6667	42.6667	11.3333	58.2000	14.0333	30.4000	82.3333	118.6667	24.2300	36.33	0.837
41	0.6247	48.7000	114.0000	42.3333	12.0000	57.5333	15.2667	33.4000	82.0000	116.0000	26.6300	34.00	0.982
42	0.5940	49.5667	114.6667	41.3333	11.6667	57.8333	15.2667	33.2667	86.3333	120.6667	26.4100	34.33	0.969
43	0.5270	64.7333	94.6667	32.0000	5.6667	29.7333	8.1333	24.7667	78.6667	114.3333	27.4000	35.67	0.694
44	0.5167	65.0000	97.0000	31.6667	6.6667	36.9667	9.0333	23.5667	79.6667	114.6667	24.5500	35.00	0.673
45	0.5917	50.6333	115.3333	40.3333	13.6667	69.7667	17.2667	32.8667	86.3333	120.6667	24.7433	34.33	0.957
46	0.5923	49.9333	111.6667	41.3333	11.0000	61.7333	14.3333	33.8667	82.3333	117.3333	23.2767	35.00	0.968
47	0.5837	47.8333	106.3333	42.6667	12.0000	65.1000	15.6333	30.4333	86.3333	121.3333	24.1167	35.00	0.870
48	0.5117	65.2667	93.3333	30.0000	8.0000	42.7333	10.6000	25.2000	79.3333	113.6667	24.7967	34.33	0.734
49	0.5967	51.2000	113.6667	41.3333	11.3333	56.5667	14.0333	36.9667	81.3333	115.6667	25.0200	34.33	1.077
50	0.6183	51.0667	119.6667	39.0000	12.0000	61.1667	15.0667	30.2000	86.3333	120.6667	24.8533	34.33	0.880
51	0.5833	48.5667	112.6667	42.0000	11.3333	55.6333	13.9000	32.4000	81.6667	116.6667	25.0033	35.00	0.926

52	0.6150	49.4667	113.6667	44.0000	12.0000	63.1000	15.0667	33.2000	83.3333	117.6667	24.1433	34.33	0.967
53	0.6527	47.6000	113.6667	39.3333	11.6667	60.9333	13.6000	31.1667	83.0000	116.3333	22.4367	33.33	0.935
54	0.6407	48.7333	112.6667	41.0000	11.0000	58.6000	13.5333	33.5333	80.6667	115.3333	23.1200	34.67	0.967
55	0.6187	47.5333	123.3333	42.3333	11.6667	63.4667	14.5000	35.1333	85.3333	120.3333	23.1800	35.00	1.004
56	0.6247	48.2667	116.3333	39.3333	11.0000	56.8333	13.8333	31.3333	87.6667	121.3333	24.4633	33.67	0.931
57	0.6333	46.9333	115.3333	49.0000	10.6667	55.0667	12.3000	30.4000	79.3333	113.6667	22.2900	34.33	0.885
58	0.6127	48.2667	123.6667	46.0000	12.0000	70.4000	14.2000	33.3333	82.3333	117.3333	20.1800	35.00	0.952
59	0.6213	46.8667	121.6667	42.3333	10.0000	53.3333	12.1667	31.4667	82.3333	116.6667	22.7967	34.33	0.917
60	0.5867	48.4000	114.3333	45.0000	11.3333	60.2333	14.2667	31.9667	84.3333	118.3333	23.7800	34.00	0.940
61	0.5230	63.6000	91.3333	25.3333	7.0000	37.4667	9.4667	25.0000	79.3333	112.6667	25.3500	33.33	0.750
62	0.6180	46.4000	114.3333	44.6667	11.0000	52.5667	14.1667	36.4333	80.6667	116.0000	27.0800	35.33	1.031
63	0.6210	49.5000	116.0000	41.3333	11.6667	60.4333	14.9333	34.9333	83.6667	117.0000	24.6367	33.33	1.048
64	0.5933	48.5333	117.6667	42.6667	11.6667	60.2000	15.2000	33.3667	84.0000	118.0000	25.2500	34.00	0.981
65	0.5940	49.8333	115.6667	43.6667	11.6667	64.2000	14.7333	31.9667	83.3333	117.6667	22.9533	34.33	0.931
66	0.6347	46.5667	112.3333	42.0000	12.6667	68.5333	16.4000	30.4333	82.3333	116.3333	23.9300	34.00	0.895
67	0.6247	49.5000	125.6667	45.3333	9.3333	46.5000	11.8667	31.6667	81.3333	116.0000	25.7400	34.67	0.913
68	0.6157	61.2000	120.0000	47.6667	10.0000	49.3667	12.8333	30.5000	85.3333	118.3333	26.4200	33.00	0.924
69	0.6260	47.3000	125.3333	44.3333	10.6667	53.7667	14.0667	34.9000	85.0000	118.3333	26.2867	33.33	1.047

70	0.6257	49.3333	115.6667	44.0000	13.0000	70.0667	16.5333	36.6667	82.6667	116.6667	23.6267	34.00	1.078
71	0.6073	49.2000	110.3333	41.3333	9.0000	48.5667	12.3667	24.1333	83.3333	117.3333	25.4633	34.00	0.710
72	0.6427	48.0667	117.3333	40.3333	8.6667	45.7000	11.6333	32.4000	88.3333	122.6667	26.4633	34.33	0.944
73	0.5217	62.7667	81.0000	32.0000	5.3333	26.1667	7.4000	23.2667	79.6667	112.3333	28.4667	32.67	0.712
74	0.5883	47.5333	111.0000	41.0000	10.6667	57.8333	13.7333	30.9667	81.3333	115.3333	23.8533	34.00	0.911
75	0.5843	49.5333	114.0000	44.6667	10.0000	50.3333	12.5667	30.4333	86.3333	120.6667	24.9800	34.33	0.886
76	0.5837	48.5333	119.0000	45.3333	11.6667	61.7667	14.3667	31.1333	87.3333	121.6667	23.1933	34.33	0.907
77	0.6050	49.3333	114.6667	45.0000	12.3333	68.7667	15.6667	31.5000	82.3333	116.3333	22.8100	34.00	0.926
78	0.5940	47.9333	111.6667	43.6667	12.6667	73.8000	15.0667	33.3333	83.6667	117.3333	20.3333	33.67	0.990
79	0.5887	49.5333	121.3333	39.0000	11.3333	52.7667	13.9667	38.5333	81.3333	116.6667	26.4333	35.33	1.091
80	0.6417	49.6333	120.3333	42.0000	12.0000	68.3333	15.1000	31.1667	86.3333	120.6667	22.2633	34.33	0.908
81	0.5243	61.1667	120.0000	28.6667	9.3333	43.0000	11.7333	25.0667	80.6667	113.3333	27.3133	32.67	0.767
82	0.5867	47.7000	114.0000	33.6667	12.6667	66.3000	15.7000	35.1333	86.6667	120.0000	23.8033	33.33	1.054
83	0.6153	49.5333	118.0000	41.6667	12.0000	61.9667	14.8000	33.0333	82.3333	117.3333	23.9267	35.00	0.944
84	0.6537	47.7333	113.6667	44.6667	10.6667	56.2667	12.9000	35.9667	86.3333	120.6667	22.8100	34.33	1.048
85	0.5917	49.4667	118.0000	45.3333	11.6667	59.8333	14.8000	35.2000	87.6667	121.6667	25.0833	34.00	1.035
86	0.5860	48.7667	114.0000	39.6667	13.3333	73.4000	16.2667	32.9000	82.6667	118.3333	22.2333	35.67	0.922
87	0.5723	48.2000	119.3333	42.6667	13.6667	78.1000	16.4667	31.3667	84.3333	119.0000	21.0933	34.67	0.905

88	0.6033	49.5333	115.3333	46.0000	12.6667	66.3333	15.5000	30.0000	88.3333	122.6667	23.4800	34.33	0.874
89	0.6440	48.2000	111.6667	44.6667	11.0000	57.4000	14.4000	30.8000	81.3333	113.3333	25.1433	32.00	0.963
90	0.5923	49.5000	110.6667	45.3333	12.0000	60.3333	15.4333	32.8667	84.3333	118.6667	25.6967	34.33	0.957
Hindi62	0.6430	49.2000	121.3333	48.0000	13.6667	61.8000	16.2667	38.5333	81.3333	113.6667	22.1144	34.33	1.122
WH711	0.5343	62.3000	92.6667	32.3333	9.3333	45.7000	12.9000	40.4333	84.3333	118.3333	20.4222	32.00	0.951
Mean	**0.5920**	**52.7048**	**111.3667**	**39.4037**	**10.3185**	**53.5215**	**13.0522**	**31.7522**	**82.9926**	**116.9593**	**24.8257**	**33.95**	**0.937**
C.V.	0.6946	0.9287	2.2664	5.1499	16.2610	16.6177	14.5379	0.8661	1.0379	0.9257	6.1629	0.832	0.972
S.E.	0.0024	0.2826	1.4572	1.1716	0.9687	5.1350	1.0955	0.1588	0.4973	0.6251	0.8833	0.396	0.586
C.D. 5%	0.0066	0.7886	4.0667	3.2696	2.7035	14.3306	3.0574	0.4431	1.3880	1.7444	2.4652	0.924	0.798
C.D. 1%	0.0087	1.0405	5.3658	4.3140	3.5671	18.9081	4.0340	0.5846	1.8313	2.3016	3.2526	1.0764	1.358

Annexure V: Mean of different traits observed in F_3 population derived from cross DBW16×WH147 under normal sown condition

Plant No.	Chlorophyll index	Membrane theromostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yield	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.634	46.90	124.7	43.7	10.3	64.00	14.1	38.7	106.0	146.0	22.03	37	1.05
2	0.625	49.50	120.0	40.3	11.7	61.53	12.3	32.5	102.7	144.3	19.99	38.6	0.84
3	0.586	46.73	121.7	47.0	10.0	66.40	14.7	38.6	110.0	151.0	22.14	38	1.02
4	0.542	62.70	107.7	35.3	9.3	41.33	10.4	29.8	106.3	143.7	25.16	34.4	0.87
5	0.612	49.63	112.0	50.0	12.0	52.20	12.7	36.5	105.0	148.3	24.33	40.3	0.91
6	0.532	61.70	101.3	42.0	10.3	38.50	10.3	28.5	107.3	145.3	26.75	35	0.81
7	0.573	48.53	117.0	49.0	11.7	64.17	16.2	40.7	105.0	144.3	25.25	36.3	1.12
8	0.512	61.43	99.3	48.3	9.7	38.40	11.8	31.7	107.7	142.7	30.73	32	0.99
9	0.564	46.30	106.7	47.0	12.0	65.20	12.9	42.8	104.0	144.0	19.79	37	1.16
10	0.592	46.53	112.3	44.0	10.7	59.30	14.3	43.0	106.0	141.7	24.11	32.7	1.31
11	0.610	46.30	108.3	49.0	11.3	58.00	12.5	36.3	106.0	145.0	21.55	36	1.01
12	0.632	51.70	115.0	51.7	12.0	59.30	14.7	35.0	109.3	148.7	24.79	36.4	0.96
13	0.644	46.63	116.3	53.0	12.3	55.70	12.5	36.0	104.3	143.3	22.44	36	1.00
14	0.516	60.70	99.0	41.0	9.0	38.60	10.9	31.9	106.3	144.0	28.24	34.7	0.92
15	0.520	61.00	114.0	42.3	10.7	41.37	11.5	30.5	105.0	142.0	27.80	34	0.90

16	0.565	49.63	109.3	46.0	13.0	64.80	17.3	32.7	106.0	146.7	26.70	37.7	0.87
17	0.586	48.60	116.3	48.7	12.0	62.47	14.7	37.3	102.7	141.0	23.53	35.3	1.06
18	0.605	49.73	111.7	44.3	13.3	59.63	13.4	36.3	110.7	148.0	22.47	34.3	1.06
19	0.630	48.60	113.0	49.3	11.7	56.80	13.7	32.7	107.0	145.0	24.12	35	0.93
20	0.578	49.40	121.3	50.7	10.0	48.33	15.3	38.0	110.3	150.3	31.66	37	1.03
21	0.583	45.23	119.7	44.0	13.7	72.40	18.7	35.8	109.3	147.7	25.83	35.4	1.01
22	0.634	46.10	117.3	52.7	12.0	68.57	16.8	34.7	108.3	147.0	24.50	35.7	0.97
23	0.575	47.73	114.3	51.0	10.0	58.70	14.3	36.7	110.7	151.0	24.36	37.3	0.98
24	0.589	46.33	109.0	48.3	13.0	61.20	13.9	34.3	108.7	150.3	22.71	38.6	0.89
25	0.638	48.20	107.0	49.0	10.3	55.70	15.3	42.7	104.3	143.0	27.47	35.7	1.20
26	0.624	45.53	111.7	51.3	11.0	68.27	16.7	40.3	105.0	146.0	24.46	38	1.06
27	0.567	48.73	117.0	48.0	13.0	61.50	13.4	38.0	107.0	147.0	21.79	37	1.03
28	0.546	61.43	109.3	42.3	10.3	44.30	12.5	29.6	102.3	142.3	28.22	37	0.80
29	0.576	48.30	111.7	48.0	12.3	62.73	16.6	34.7	108.0	147.0	26.46	36	0.96
30	0.607	48.70	110.0	54.0	10.0	59.43	17.9	32.8	109.7	148.0	30.12	35.3	0.93
31	0.638	46.83	113.0	53.3	10.7	62.70	15.3	40.3	106.3	145.7	24.40	36.4	1.11
32	0.548	61.50	106.7	42.0	9.3	42.37	11.7	29.5	102.7	142.7	27.61	37	0.80
33	0.623	45.70	111.3	51.3	13.3	60.40	17.7	34.7	107.3	148.0	29.30	37.7	0.92

34	0.587	46.30	110.0	48.3	9.0	62.23	15.3	35.5	110.0	150.0	24.59	37	0.96
35	0.514	62.00	104.0	41.0	8.7	45.87	12.2	26.5	105.0	143.0	26.60	35	0.76
36	0.532	61.20	99.3	38.7	10.3	43.73	13.7	29.7	107.7	144.0	31.33	33.3	0.89
37	0.587	48.60	108.0	45.0	13.0	56.90	12.8	36.9	106.7	147.3	22.50	37.6	0.98
38	0.562	46.53	113.7	49.3	12.0	64.80	15.9	32.0	109.0	151.0	24.54	39	0.82
39	0.510	64.70	106.0	39.3	10.3	46.10	12.3	29.3	106.0	144.7	26.68	35.7	0.82
40	0.626	49.57	109.0	48.0	13.7	68.37	19.5	34.7	104.0	143.3	28.52	36.3	0.96
41	0.612	47.60	116.7	46.7	13.0	52.43	15.6	35.3	104.3	143.7	29.75	36.4	0.97
42	0.553	46.57	112.0	48.0	9.7	59.20	13.7	33.0	107.7	146.3	23.14	35.6	0.93
43	0.518	60.80	105.7	32.0	8.3	48.90	11.2	29.0	104.0	143.3	22.90	36.3	0.80
44	0.574	49.73	116.0	52.3	12.7	68.10	18.7	36.4	109.3	148.0	27.46	35.7	1.02
45	0.622	46.30	113.7	46.7	13.0	63.70	16.4	34.8	105.7	144.3	25.75	35.6	0.98
46	0.640	48.70	113.0	48.0	10.3	54.47	14.2	38.5	107.3	146.7	26.07	36.4	1.06
47	0.622	46.27	116.0	49.0	11.0	54.73	15.7	40.6	104.7	143.7	28.69	36	1.13
48	0.578	49.30	115.0	48.3	10.3	53.30	14.2	38.5	109.3	148.7	26.64	36.4	1.06
49	0.543	60.17	98.7	38.0	9.0	42.40	11.7	29.7	104.0	145.0	27.59	38	0.78
50	0.647	48.70	116.0	46.0	12.7	62.73	15.6	34.3	106.3	144.0	24.87	34.7	0.99
51	0.585	44.63	112.7	51.0	11.0	66.93	13.7	35.7	104.7	143.0	20.47	35.3	1.01

52	0.518	62.73	99.0	37.3	9.3	42.23	12.6	28.7	105.0	144.0	29.84	36	0.80
53	0.615	47.57	110.0	48.0	11.3	50.40	17.3	36.9	107.3	146.3	34.33	36	1.03
54	0.586	47.73	110.7	49.3	13.0	68.47	19.6	32.3	104.7	143.0	28.63	35.3	0.92
55	0.570	61.57	97.0	37.0	9.3	32.70	14.7	28.9	106.0	142.7	44.95	33.7	0.86
56	0.529	64.37	99.7	38.7	6.0	47.33	12.3	30.5	104.3	143.7	25.99	36.4	0.84
57	0.569	49.20	111.3	46.3	13.0	68.43	19.6	36.7	107.0	146.0	28.64	36	1.02
58	0.631	46.57	116.3	49.3	14.3	72.53	21.3	33.5	105.3	142.3	29.37	34	0.99
59	0.652	49.10	111.7	51.3	12.0	59.20	16.6	32.3	106.7	145.0	28.04	35.3	0.92
60	0.582	46.57	109.0	48.0	10.3	55.50	13.9	36.7	105.7	144.7	25.05	36	1.02
61	0.635	49.43	114.7	46.0	9.3	71.63	19.3	42.3	110.3	151.0	26.94	37.7	1.12
62	0.586	50.23	111.7	49.0	10.0	61.40	14.6	43.5	109.7	148.0	23.78	35.3	1.23
63	0.589	46.43	107.3	43.3	12.0	54.53	16.7	38.7	108.0	148.3	30.63	37.3	1.04
64	0.621	48.70	114.3	46.3	9.7	67.93	18.3	36.5	105.0	144.7	26.94	36.7	0.99
65	0.588	46.00	111.0	45.3	12.0	72.40	19.5	40.3	108.0	147.0	26.93	36	1.12
66	0.534	63.30	97.3	39.0	8.3	44.63	12.2	29.7	104.0	143.7	27.34	36.7	0.81
67	0.618	48.70	114.0	62.0	11.3	66.80	18.3	40.4	110.3	146.0	27.40	32.7	1.24
68	0.597	47.20	113.3	49.0	9.0	63.50	16.7	42.7	108.7	149.0	26.30	37.3	1.14
69	0.584	46.87	112.7	46.7	10.3	54.20	12.5	33.5	107.0	146.3	23.06	36.3	0.92

70	0.524	60.37	110.0	36.0	8.3	42.63	11.9	28.7	105.0	145.3	27.91	37.3	0.77
71	0.580	48.23	116.0	58.0	13.0	65.23	17.5	37.0	108.3	147.7	26.83	36.4	1.02
72	0.642	46.67	112.0	59.3	12.7	68.90	17.2	35.7	109.7	148.3	24.96	35.6	1.00
73	0.534	64.07	98.7	39.7	9.0	43.73	11.3	28.0	105.0	144.3	25.84	36.3	0.77
74	0.618	46.70	106.0	68.0	13.0	67.20	15.8	34.7	106.3	145.3	23.51	36	0.96
75	0.575	48.53	113.0	61.0	10.0	54.40	14.3	37.3	108.0	149.0	26.29	38	0.98
76	0.548	61.00	96.7	38.7	7.0	41.63	10.9	28.9	104.0	142.7	26.18	35.7	0.81
77	0.588	46.30	112.0	61.3	11.0	67.90	15.3	37.5	110.3	147.0	22.53	33.7	1.11
78	0.626	48.57	107.3	44.7	13.0	59.23	14.7	33.8	110.7	144.7	24.82	31	1.09
79	0.618	48.70	110.7	47.7	11.3	62.83	12.6	35.7	108.0	146.7	20.05	35.7	1.00
80	0.574	46.50	104.7	43.7	12.7	61.40	14.4	36.6	111.7	151.0	23.45	36.3	1.01
81	0.612	48.97	111.0	52.0	10.3	66.83	15.2	32.9	108.0	151.0	22.74	40	0.82
82	0.641	48.57	112.0	43.3	9.3	58.93	15.8	33.5	109.0	151.0	26.81	39	0.86
83	0.536	62.93	109.0	37.0	10.0	46.70	10.6	30.3	103.0	142.0	22.70	36	0.84
84	0.639	49.67	105.0	46.3	12.3	72.23	20.3	39.5	110.3	148.7	28.10	35.4	1.12
85	0.627	47.50	106.3	42.0	10.3	61.40	16.0	33.0	108.0	146.3	26.06	35.3	0.93
86	0.538	60.43	102.0	37.0	9.3	41.70	9.9	28.0	103.0	142.0	23.74	36	0.78
87	0.542	62.70	99.7	34.3	10.7	43.20	11.5	31.5	104.0	144.3	26.62	37.3	0.84

88	0.583	49.63	121.3	41.0	10.3	65.00	17.3	36.9	109.0	147.0	26.62	35	1.05
89	0.632	47.97	111.0	50.0	13.0	71.30	20.4	38.3	107.0	145.7	28.61	35.7	1.07
90	0.628	45.60	113.7	44.0	9.0	60.53	14.3	35.0	110.0	148.7	23.62	35.7	0.98
DBW16	0.638	52.43	101.3	42.0	12.7	74.50	18.5	40.7	89.0	139.3	24.83	39.3	1.05
WH147	0.547	59.53	108.0	36.3	10.3	59.33	12.7	47.0	106.0	145.0	21.41	40.3	0.89
Mean	**0.587**	**51.34**	**120.91**	**46.39**	**11.9**	**59.33**	**14.840**	**34.98**	**116.8**	**145.90**	**25.94**	**36.09**	**0.97**
C.V.	0.534	0.7256	1.6238	4.4553	11.4475	12.7589	12.9546	2.8564	2.1548	1.3665	12.6744	0.632	0.864
S.E.	0.0022	0.2168	1.2653	1.1843	0.8341	4.3586	1.6453	0.7468	1.6328	1.7698	1.6884	0.0078	0.0066
C.D. 5%	0.0052	0.6382	3.4856	3.2754	2.2576	12.3520	3.6232	1.6542	3.1886	2.9472	6.1352	0.0092	0.0086
C.D. 1%	0.0073	0.7586	4.3268	4.2654	2.9724	16.8548	3.9845	2.4856	3.4659	3.8529	6.9132	0.0198	0.0174

Annexure VI: Mean for different traits of F_3 population of DBW16 X WH147 under late sown condition

Plant No.	Chlorophyll index	Membrane theromostability index	Plant height	Kernel/spike	Tiller/plant	Biomass/plant	Grain yield	Test weight	Days to heading	Days to maturity	Harvest index	Grain filling duration	Grain filling rate
1	0.6403	46.3000	122.6667	42.3333	11.0000	53.7333	13.4667	33.7000	82.6667	116.3333	25.0733	33.67	1.001
2	0.6147	48.2000	123.3333	44.6667	12.0000	59.8333	14.6333	32.6667	86.6667	121.3333	24.5167	34.67	0.942
3	0.6080	48.6333	121.0000	46.6667	11.3333	57.6000	13.9000	34.7000	84.6667	118.6667	24.2167	34.00	1.021
4	0.5207	62.9000	100.3333	35.0000	7.3333	37.8333	9.8000	24.9000	80.3333	114.6667	25.9433	34.33	0.725
5	0.5917	48.8667	118.0000	48.0000	11.0000	56.6667	13.7000	32.9667	83.0000	118.3333	24.1667	35.33	0.933
6	0.5157	61.8667	97.6667	30.0000	7.0000	34.0667	8.6667	24.9333	80.6667	113.6667	25.5100	33.00	0.756
7	0.5950	48.5333	119.0000	43.3333	12.3333	61.0333	15.9000	35.6333	84.3333	118.3333	26.0567	34.00	1.048
8	0.5190	63.8333	76.3333	34.0000	8.3333	37.4000	10.5667	23.9333	79.3333	113.6667	28.8200	34.33	0.697
9	0.5863	49.2000	118.6667	44.0000	12.6667	64.5667	15.8333	39.4667	81.3333	115.3333	24.5500	34.00	1.161
10	0.5800	49.1333	120.6667	40.6667	12.0000	63.0000	14.5667	40.1667	86.6667	120.6667	23.1567	34.00	1.181
11	0.5910	48.5000	120.6667	44.3333	12.3333	63.2000	15.3000	36.3333	87.0000	121.0000	24.3167	34.00	1.069
12	0.6500	49.1000	118.0000	45.6667	11.0000	53.5000	13.5333	36.0667	83.0000	116.6667	25.2633	33.67	1.071
13	0.6347	49.5333	117.3333	47.0000	11.0000	58.1333	13.6667	34.4333	84.6667	117.6667	23.5333	33.00	1.043
14	0.5427	62.2333	88.3333	32.0000	6.6667	35.2333	8.2000	22.8333	81.3333	113.3333	23.3200	32.00	0.714
15	0.5200	64.1333	67.6667	30.3333	7.0000	38.0000	8.5667	23.3000	81.0000	113.6667	22.5500	32.67	0.713
16	0.5957	50.2000	112.0000	44.0000	12.0000	60.8000	15.2000	32.9000	85.6667	119.6667	25.0233	34.00	0.968
17	0.5917	48.5333	117.0000	46.3333	10.3333	54.7667	12.6333	34.9000	82.3333	116.3333	23.0733	34.00	1.026
18	0.6153	48.3333	117.3333	46.6667	12.6667	66.5333	14.9667	32.6333	86.6667	120.6667	22.5067	34.00	0.960

19	0.6240	49.6667	117.6667	45.6667	11.0000	57.9333	13.3333	33.1667	84.3333	119.0000	23.0700	34.67	0.957
20	0.5967	50.1000	134.3333	48.6667	11.0000	54.2333	13.8667	34.9667	84.0000	118.0000	25.5733	34.00	1.028
21	0.5940	48.5667	123.3333	44.0000	15.3333	81.4333	20.3000	34.4667	86.0000	120.0000	24.8600	34.00	1.014
22	0.6260	49.5333	133.0000	45.3333	16.0000	81.1667	21.3667	29.7667	85.3333	119.3333	26.2800	34.00	0.875
23	0.5623	48.8333	120.0000	45.0000	16.3333	74.0333	20.8333	32.7333	87.3333	121.3333	28.2900	34.00	0.963
24	0.5900	47.5333	117.3333	45.0000	12.6667	62.5333	16.3333	31.8333	81.6667	116.3333	26.1233	34.67	0.918
25	0.6460	49.2333	117.3333	45.3333	13.6667	70.3000	17.0667	39.6667	81.3333	115.6667	24.2733	34.33	1.155
26	0.6207	50.5333	120.0000	48.0000	12.0000	60.9667	14.7333	39.9333	83.6667	117.3333	24.1500	33.67	1.186
27	0.5603	49.5000	122.6667	44.6667	12.0000	61.4667	14.4667	35.7667	86.6667	120.6667	23.6367	34.00	1.052
28	0.5480	63.6000	90.0000	29.6667	7.3333	36.5000	15.9000	23.6667	78.6667	113.6667	44.7000	35.00	0.676
29	0.5933	48.2333	117.3333	46.3333	13.0000	68.0333	14.1000	33.5000	84.3333	118.6667	20.7833	34.33	0.976
30	0.6160	49.5000	119.6667	47.0000	11.0000	57.3000	14.1000	32.8333	86.3333	121.0000	24.6033	34.67	0.947
31	0.6460	50.6333	120.3333	46.6667	10.6667	55.2333	13.8000	37.7667	81.6667	116.3333	24.9267	34.67	1.089
32	0.5270	63.7000	90.6667	31.3333	8.0000	40.4333	9.7333	24.1667	83.6667	112.6667	24.0367	29.00	0.833
33	0.6153	49.2000	113.6667	46.0000	11.0000	57.0000	14.0333	33.5333	82.3333	116.3333	24.6033	34.00	0.986
34	0.5923	48.9667	115.3333	51.0000	13.0000	69.3333	16.2333	32.7667	84.0000	118.0000	23.5500	34.00	0.964
35	0.5233	63.9000	91.0000	32.0000	6.0000	32.1333	7.8900	20.6333	80.0000	113.6667	24.7100	33.67	0.613
36	0.5190	62.8000	90.0000	29.3333	7.3333	38.3000	8.8000	24.2333	80.3333	121.6667	23.0533	41.33	0.586
37	0.5940	49.8667	112.3333	46.3333	13.6667	70.8000	17.6000	36.5667	82.3333	115.6667	24.9000	33.33	1.097
38	0.5733	50.2000	117.6667	46.0000	11.6667	47.5667	14.7000	32.8000	86.6667	120.6667	33.8867	34.00	0.965

39	0.5260	63.4000	87.6667	31.0000	7.3333	35.2000	9.1000	23.6667	78.6667	124.3333	25.8233	45.67	0.518
40	0.6220	49.4667	115.3333	42.3333	11.3333	56.3667	13.9333	31.8000	82.3333	116.3333	24.8633	34.00	0.935
41	0.6257	49.5667	115.0000	48.0000	10.3333	51.0000	13.3667	30.7333	81.6667	115.6667	26.2167	34.00	0.904
42	0.6130	48.4667	115.0000	45.6667	11.3333	58.3000	14.3000	32.7333	85.6667	119.6667	24.4300	34.00	0.963
43	0.5260	62.8333	89.3333	22.3333	8.3333	44.2333	10.9000	24.0333	81.3333	114.3333	24.6500	33.00	0.728
44	0.5700	48.5000	121.0000	47.0000	12.0000	63.6333	15.6667	32.8667	82.0000	116.0000	24.7333	34.00	0.967
45	0.6163	48.8333	113.6667	47.3333	11.0000	54.1000	14.1000	34.7667	83.3333	117.3333	26.0233	34.00	1.023
46	0.6387	50.2000	120.0000	44.6667	12.0000	58.9667	15.2333	35.8333	82.6667	116.3333	25.9167	33.67	1.064
47	0.6143	50.8667	120.0000	45.6667	10.6667	56.8000	13.3333	37.2667	84.3333	118.3333	23.4600	34.00	1.096
48	0.5870	48.7000	112.6667	41.0000	10.6667	55.3000	13.5000	34.7667	87.6667	121.6667	24.3400	34.00	1.023
49	0.5213	62.2333	86.6667	28.3333	7.0000	37.3000	8.9000	23.3000	80.3333	112.0000	23.7933	31.67	0.736
50	0.6313	46.8667	117.6667	46.3333	13.0000	68.8000	16.3000	30.4667	83.0000	117.3333	23.7067	34.33	0.887
51	0.5863	48.5667	115.3333	49.6667	12.3333	65.7667	15.4667	29.6333	86.0000	116.6667	23.6133	30.67	0.966
52	0.5240	64.8333	85.6667	29.6667	6.6667	34.5667	8.4333	24.7000	80.6667	113.6667	24.6067	33.00	0.748
53	0.6243	48.8667	117.3333	45.0000	14.6667	77.4667	18.9333	32.5667	84.0000	118.0000	24.3333	34.00	0.958
54	0.6013	49.5333	114.3333	45.3333	10.6667	53.6667	13.7000	29.7667	84.6667	118.6667	25.4867	34.00	0.875
55	0.5350	63.0000	94.6667	28.6667	7.3333	37.1000	10.2333	24.1000	78.6667	114.3333	27.7700	35.67	0.676
56	0.5263	65.0333	92.6667	30.3333	7.0000	38.8000	9.3000	25.2667	81.6667	112.3333	23.7667	30.67	0.824
57	0.6150	50.5333	118.0000	43.6667	13.3333	69.0667	17.6333	33.2667	82.3333	116.0000	25.5233	33.67	0.988
58	0.6257	49.5333	122.3333	44.6667	14.3333	75.7333	19.3000	30.7333	83.3333	117.3333	25.5367	34.00	0.904

59	0.6363	50.8667	110.6667	48.3333	11.6667	62.2000	15.0000	32.7333	81.6667	115.6667	24.1167	34.00	0.963
60	0.5803	48.2333	114.3333	46.0000	11.0000	54.0333	14.1000	35.7667	82.0000	116.0000	26.0833	34.00	1.052
61	0.6303	48.7000	119.6667	44.3333	12.3333	67.2667	16.2333	39.2667	84.6667	118.6667	24.1167	34.00	1.155
62	0.5860	48.7000	118.3333	44.6667	11.3333	59.9333	14.8333	39.4667	83.6667	117.0000	24.7533	33.33	1.184
63	0.5937	49.7667	109.3333	42.3333	10.3333	55.8333	12.7333	37.9333	81.6667	115.6667	22.7900	34.00	1.116
64	0.6147	50.3667	116.0000	49.6667	12.3333	64.5333	15.9333	36.7667	81.3333	115.6667	24.7033	34.33	1.071
65	0.6210	48.5000	112.3333	44.6667	12.3333	62.9667	15.3667	37.4667	82.6667	116.6667	24.6133	34.00	1.102
66	0.5290	65.3333	90.3333	30.0000	7.6667	37.0000	9.6000	24.2000	81.3333	112.3333	26.2633	31.00	0.781
67	0.6247	49.5000	115.6667	60.3333	14.3333	72.9667	18.1000	40.3000	86.6667	120.6667	24.8067	34.00	1.185
68	0.5930	48.5667	118.3333	47.3333	10.3333	51.1333	12.9000	39.7667	88.3333	122.0000	25.2200	33.67	1.181
69	0.5940	50.1667	112.3333	46.0000	11.0000	57.2000	14.7333	32.3000	82.6667	116.3333	25.7833	33.67	0.959
70	0.5173	64.7333	93.3333	28.0000	7.6667	38.1000	9.5333	24.7333	80.0000	113.6667	25.0467	33.67	0.735
71	0.5847	49.5667	120.0000	60.6667	12.3333	63.2000	15.8667	32.9667	82.6667	116.0000	25.1233	33.33	0.989
72	0.6317	50.5000	114.0000	61.0000	13.0000	66.0333	16.8667	31.7667	85.6667	120.0000	25.5567	34.33	0.925
73	0.5243	63.0333	88.3333	32.0000	7.3333	35.3333	9.9333	23.1333	80.6667	114.3333	28.2233	33.67	0.687
74	0.6150	49.5333	115.0000	68.0000	11.3333	56.2667	14.2667	34.9333	83.6667	117.3333	25.3267	33.67	1.038
75	0.5970	48.5667	119.6667	61.6667	11.6667	63.4000	15.1000	36.2667	84.6667	117.6667	23.8267	33.00	1.099
76	0.5350	65.3333	91.3333	32.0000	8.0000	40.5667	9.9000	24.6333	81.0000	113.6667	24.4700	32.67	0.754
77	0.5883	50.4000	114.3333	56.6667	12.0000	63.8667	15.3000	34.6667	85.3333	119.3333	24.1500	34.00	1.020
78	0.6233	48.2333	109.3333	45.3333	10.6667	52.9667	13.3667	33.7333	82.6667	116.6667	25.1567	34.00	0.992

79	0.6227	51.3333	118.6667	48.3333	11.3333	59.5667	14.7333	32.6000	83.6667	117.6667	24.7400	34.00	0.959
80	0.6120	49.2333	109.6667	46.3333	10.6667	56.4667	14.4000	34.5000	83.6667	117.6667	25.5033	34.00	1.015
81	0.6247	48.4333	117.3333	52.6667	12.0000	65.9667	15.4333	29.8667	84.6667	117.0000	23.4933	32.33	0.924
82	0.6387	47.8000	115.6667	47.3333	10.6667	54.6667	13.5333	29.3667	81.6667	119.6667	24.7967	38.00	0.773
83	0.5350	63.7333	82.0000	35.6667	7.6667	38.7000	9.0333	24.7000	80.6667	112.3333	23.3733	31.67	0.780
84	0.6013	50.4333	108.0000	47.3333	15.0000	79.0000	19.2000	34.8000	86.6667	120.3333	24.3133	33.67	1.034
85	0.5950	48.8333	115.3333	51.0000	11.6667	61.2000	15.0000	32.0667	84.6667	118.3333	24.4900	33.67	0.952
86	0.5230	64.6333	88.3333	31.6667	7.6667	37.6333	9.2333	22.0667	79.3333	113.3333	24.7300	34.00	0.649
87	0.5267	65.5000	87.3333	29.0000	7.6667	38.9333	9.9333	26.5667	80.3333	112.6667	25.6867	32.33	0.822
88	0.5930	49.5333	122.3333	46.3333	14.0000	77.1000	27.8000	35.8333	86.6667	120.6667	37.0200	34.00	1.054
89	0.6073	49.1000	118.0000	61.6667	13.0000	67.3000	17.3000	36.0000	84.6667	118.6667	25.7467	34.00	1.059
90	0.6147	49.5000	113.0000	47.0000	12.6667	67.1667	16.8667	31.5667	83.3333	117.3333	25.1567	34.00	0.928
DBW16	0.6362	48.4333	104.3333	46.3333	13.0000	63.8667	14.7333	36.2667	81.6667	113.6667	20.3548	34.00	1.067
WH147	0.5493	63.0000	108.0000	31.6667	8.0000	40.5667	9.0333	39.3667	86.6667	120.6667	19.7206	32.00	0.918
Mean	**0.5880**	**52.7626**	**110.3593**	**43.4259**	**10.9407**	**56.1474**	**14.0673**	**31.8470**	**83.2148**	**117.1481**	**25.2153**	**33.91**	0.941
C.V.	0.8720	0.7666	2.5107	4.0941	12.7431	14.0284	17.9396	2.8134	0.9865	1.7334	13.2135	0.938	0.752
S.E.	0.0030	0.2335	1.5997	1.0265	0.8049	4.5476	1.4570	0.5173	0.4739	1.1724	1.9236	0.462	0.532
C.D. 5%	0.0083	0.6518	4.4644	2.8646	2.2464	12.6912	4.0662	1.4437	1.3227	3.2718	5.3684	1.264	1.468
C.D. 1%	0.0109	0.8599	5.8904	3.7797	2.9640	16.7450	5.3650	1.9048	1.7452	4.3170	7.0832	1.842	2.142