A STUDY OF QUANTITATIVE TRAITS GOVERNING WHEAT GRAIN QUALITY BY MOLECULAR APPROACHES

A thesis submitted to the
University of Pune

For the degree of

DOCTOR OF PHILOSOPHY

In

BIOCHEMISTRY

By
RAMYA PRASHANT

Research Guide
Dr. VIDYA S. GUPTA

Plant Molecular Biology Group
Division of Biochemical Sciences
National Chemical Laboratory
Pune 411008 (INDIA)

April 2011

INTER-INSTITUTIONAL COLLABORATIVE RESEARCH EFFORT

Research work embodied in this thesis
was carried out at

NATIONAL CHEMICAL LABORATORY, PUNE (INDIA)

In collaboration with

PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA (INDIA)

AND

AGHARKAR RESEARCH INSTITUTE, PUNE (INDIA)

Certificate

Certified that the work in the Ph.D. thesis entitled ***'A study of quantitative traits governing wheat grain quality by molecular approaches'*** submitted by ***Mrs. Ramya Prashant*** was carried out by the candidate under my supervision. The material obtained from other sources has been duly acknowledged in the thesis.

Date:
Pune

Dr. Vidya S. Gupta
(Research Guide)

DECLARATION

I hereby declare that the thesis entitled **'A study of quantitative traits governing wheat grain quality by molecular approaches'** submitted for Ph.D. degree to the University of Pune has not been submitted by me for a degree at any other university.

Date: Ramya Prashant

National Chemical Laboratory

Pune

To

Prashant, my husband

Jyotsna and Tejas, my little ones

And

The Plant Molecular Biology group

Contents

Acknowledgements

I wish to express my sincere gratitude to my Research supervisor Dr. Vidya Gupta, National Chemical laboratory (NCL), Pune for accepting me as a student and for her continued academic guidance. Her enthusiasm for science, openness to ideas, teaching methods and generosity were the sources of inspiration. As my mentor, she always ensured my academic and emotional well-being. Her special instinct to sense my apprehensions and gentle nudge towards the right directions made sure that my work progressed continuously. She helped me see the bigger picture always, which kept me going in challenging times. She encouraged my research interests, gave complete freedom to plan my work, make mistakes, and learn from them. The sessions of daily discussion and corrections that I had the privilege of spending with her in the past six months during the writing of this thesis were a great learning experience about patience, meticulousness and the proper use of the language of science. I feel truly blessed to have had the good-fortune of being associated with her.

I thank Dr. Meena Lagu, NCL for her enthusiasm in knowing my progress on a daily basis. She gladly took sincere efforts in facilitating experiments and rejoiced at my smallest of achievements. Lavish in praise, she celebrated each well-written paragraph, a good gel and completed field trips. Her encouragement towards meticulous record keeping and making day-to-day task lists helped me a lot. Her ability to spread cheer will always be fondly remembered.

My express my heartfelt gratitude to Dr. Narendra Kadoo, NCL for his constant help and encouragement. Naren set realistic targets keeping in mind my strengths and weaknesses and made sure in that I achieved them. He built my confidence in using analytical softwares and statistical tools and helped me improve my writing skills. I am sincerely thankful for his valuable guidance and generous support during the crucial stages of thesis writing, without which it could not have had the present form.

Prof. HS Dhaliwal and Dr. Parveen Chhuneja, Punjab Agricultural University, Ludhiana are gratefully acknowledged for developing the population required for this study, conducting field trials at Ludhiana and providing the harvests for phenotyping at NCL. I thank Dr. VS Rao, former Director, Agharkar Research Institute (ARI) for providing field facilities at the Agricultural Research Station, Hol farm, Pune.

I express my grateful thanks to Dr. MD Bhagwat, Mr. Chavan and Dr. Honrao, ARI for their valuable advice during the planning and conducting of field trials at Hol. Their enthusiasm and energy during harvesting of wheat were truly inspiring. I gratefully acknowledge the timely help from Dr. Shubhada Tamhankar, Dr. Manoj Oak, Dr. Ravi Patil and Mr. Surve, ARI during the field trials. I also thank all the field personnel who helped during the field trials at Hol, many of whom became good friends over the years.

Thanks are due to Dr. Mohini Sainani and Dr. Ashok Giri, NCL for encouragements during my PhD tenure. I express my sincere thanks to Dr. Bhushan Dholakia for being a

true support right from the time he introduced me to molecular markers, linkage maps and QTLs to the time I put my thesis together. In my initial days, he took time to introduce me to the work of wheat research laboratories around the world, which helped me to keep myself updated about the current wheat research. I also express my gratitude to Dr. Manje Gowda and Dr. Rebecca Zwart for their advice during data analysis. I express my heartfelt thanks for the sincere support rendered by Aditi, Krishna, Laxmi, Charu and Prajakta who closely worked with me during the molecular and field work. I gratefully thank Reema, Rasika, Gayatri, Sheetal and Mrs. Indira Mohandasan for their crucial help during thesis writing.

I acknowledge the Council of Scientific and Industrial Research, Government of India for the research fellowship that enabled me to pursue PhD at NCL. I express my sincere thanks to Dr. Sivaram and Dr. Sourav Pal, the former and present Directors of NCL, for providing the generous research facilities at this institute.

The members of the Plant Molecular Biology created a great working atmosphere. The moments of cheer that they brought in brightened each day. They not only created a healthy competitive world, but were also a great support system. The 'wheat team' enthusiastically came forward to help during sowing, tagging and harvesting. Shashi, Neeta, Rashmi, Pankaj, Rajendra, Elan, Richa, Prashant Hosmani and Nikhil participated in lively discussions and offered timely help. Even the 'non-wheat' members like Abhijit, Prabhash, Hemangi, Manasi and Pradeep volunteered and helped me whenever required during harvesting. The cheerful camaraderie that prevailed during the field trips, made the hectic harvesting sessions in the peak summers, a whole lot of fun. My days at PMB were made unforgettable with the company of lab-mates like Radhika, Gauri Apte, Trupti, Sofia, Varsha, Sheetal, Sagar, Ram, Rohini, Arun, Sadhana, Suvarna who were my peers. The younger members like Sandip, Vitthal, Yashwant, Yojana, Neha, Medha, Priyanka, Gouri, Rahul, Amey, Rakesh…. and many others, brought in fresh perspectives when they joined PMB. Ajit, PMB's blithe spirit, and Jagtap Kaka's affectionate insistence to have the afternoon tea first and then continue working will always be a happy memory.

I feel overwhelmed when I think of all the good wishes and help that came my way during this work and the times that lead to it. I remember gratefully, all the encouragement and loving guidance my teachers at Mysore and at the National Centre for Biological Sciences, Bangalore showered on me, which helped me reach the present stage.

Ashwini, my dear friend was a true source of inspiration with her endless source of energy and enthusiasm. She eased many a trying time I was in with practical help and soothing words. The memories of her generosity and compassion will always be treasured. The blessings and good wishes of my friends in Pune like the Jhaveri, Venkataraman, Jamma and Parekh families; Suruchi and Yogesh, Shylesh, Chandra, Sarita and Kiran, Sowmya and JB, Pratibha, Swaroopa and Megha are fondly remembered.

I gratefully acknowledge Mrs. Meena Ghadge, Mrs. Shraddha Mokashi and Mrs. Meena Petkar and the Amber and Sparkles day care teams, without whose sincere help, I could not have managed.

I am truly fortunate to have a loving and supportive family, which was a great asset during my PhD tenure. Prashant, my husband, was a pillar of strength; he supported me unconditionally in everything I did, unwavering, every single day, urging me to do my very best. His belief in my abilities and whole-hearted support in all possible big and small ways carried me through. I am thrilled to see the immense pride I see in his eyes when this work nears completion. Nothing would have been possible without him by my side.

Dear lovely daughter Jyotsna and little son Tejas, your patience and love during the time your Amma worked for and wrote her 'big black book' were endless. My precious little ones, thank you.

With deepest gratitude, I remember the efforts of my grandmother (Ajji), my parents (Amma and Appa) and my sister Ranju. Ajji's resilience and positive attitude were always an inspiration, while Ranju, in her own quiet ways showed me the virtues of discipline. Amma and Appa created an academic environment, encouraged wide reading, gave me the freedom to pursue the path of my choice and facilitated in multitude ways for me to enjoy it.

My sincere thanks are due to my parents-in-law for their timely help. Discussions with my father-in-law (Anna) about statistical issues will always be warmly remembered. The methodical approach of my mother in law (mummy) towards housework helped me learn to manage my time in a better way.

I express my heartfelt gratitude to all the people involved during the conception and progress of this research project, some of whom might have been left out due to oversight, but whose inputs have helped me reach this stage.

Above all, I thank The Almighty for the association with wonderful individuals, the opportunities for work and the strength to carry out at least some of them.

Ramya

Thesis abstract and organization

Wheat is one of the most important food crops, cultivated worldwide and consumed in various forms. India is the second largest wheat producing country in the world after China. Wheat is the raw material for the manufacture of diverse food products such as bread, traditional unleavened breads, pasta, noodles, cakes and biscuits. Because of its importance in national food security and global trade, wheat plays a major role in the financial welfare of India. India is experiencing a slow but steady increase in demands for ready to use food products especially in the urban areas. The wheat processing and bakery industry is dependent on flour milling industry, which in turn requires high quality wheat to meet consumer specifications. Wheat kernel morphometric traits influence milling yield and flour quality and kernel appearance can have impacts on its market price. Hence, improving kernel characters is an important breeding objective. Information on chromosomal regions controlling kernel characters and identification of markers closely linked to them can help accelerate breeding for better kernel characters. A systematic study was carried out to genetically dissect five kernel morphometric traits, namely, thousand kernel weight (TKW), kernel length (KL), kernel width (KW), kernel length-width ratio (LWR) and factor form density (FFD). A number of genomic regions influencing these traits and significant genetic interactions were identified. Additionally, the important role of genotype×environment interactions in the expression of kernel morphometric traits was demonstrated. The details of these studies are reported in the present thesis.

This thesis has been organized in the following manner-

LIST OF ABBREVIATIONS

°C	Degree Celsius
A	Additive (effect)
A + AE	Additive + Additive × environmental effect
AFLP	Amplified fragment length polymorphism
AM	Association mapping
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
APS	Ammonium persulfate
Avg	Average
BAC	Bacterial artificial chromosome
bp, kb, Mb	Base pairs, kilo base pair, mega base pair
cDNA	Complementary DNA
Chr	chromosome
CIM	Composite interval mapping
cM	centiMorgan
CS	Chinese Spring
CTAB	Cetyltrimethylammonium Bromide
CV	Coefficient of variation
DArT	Diversity arrays technology
df	Degrees of freedom
DH	Doubled haploid
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
E	Environment main-effect
Env	Environment
EDTA	Ethylenediaminetetraacetic acid
Eqtl	Epistatic QTL
EST	Expressed sequence tags
et al.	*et alia* (and others)
F	Variance ratio/ *F* statistic
FFD	Factor form density
G	Genotype main-effect
g	Relative centrifugal force
g, mg, ng, kg	Gram, milligram, microgram, kilogram
G×E	Genotype×environment
GPC	Grain protein content
ha	hectare
HCl	Hydrochloric acid
HMW	High molecular weight
i.e.	That is
IPCA	Interaction principal component axi/es
ISSR	Inter simple sequence repeat
kcal	Kilocalories
KL	Kernel length
KW	Kernel width
L, ml, µl	Liter, milliliter, microliter
LOD	Log of the odd (Base 10 logarithm of the likelihood ratio)

Cont…

Lud	Ludhiana
LWR	Kernel length-width ratio
m, cm, mm, nm	Meter, centimeter, millimeter, nanometer
M, mM, μM	Molar, millimolar, micromolar
mA	milliamperes
MAS	Marker assisted selection
$MgCl_2$	Magnesium chloride
min	minute
Mqtl	Main-effect QTL
MSS	Mean sum of squares
Mt-CIM	Multi trait-composite interval mapping
ncl	National Chemical Laboratory
NIL	Near isogenic line
No./no.	number
ns	Non-significant
NWPZ	North-Western Plains Zone
PCR	Polymerase chain reaction
Pun	Pune
PZ	Peninsular Zone
QQ	QTL×QTL
QTL	Quantitative trait locus
R^2	Coefficient of determination
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RNA	Ribonucleic acid
rpm	Revolutions per minute
RS	Rye Selection 111
SAMPL	Selective amplification of microsatellite polymorphic loci
SD	Standard deviation
SIM	Simple interval mapping
SKCS	Single kernel characterization system
SMA	Single marker analysis
SNP	Single nucleotide polymorphism
SRAP	Sequence related amplified polymorphism
SS	Sum of squares
SSR	Simple sequence repeat
STS	Sequence tagged site
TEMED	Tetramethylethylenediamine
TKW	Thousand kernel weight
TRAP	Target region amplification polymorphism
Tris	Tris hydroxymethyl amino methane
U	Units (enzymatic)
V	Volt
v/v	Volume/volume
W	Watt
w/v	Weight/volume

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION
AND
REVIEW OF LITERATURE

Introduction

1.1 Wheat: A global food crop

Wheat is one of the most important food crops of the world that feeds nearly half of the world's population. Owing to its rich genetic diversity and ability to adapt to a wide range of environments, amenability to both traditional and mechanical agricultural practices, endurance to long duration storage and most important of all, the versatility in being processed into diverse end-products, wheat has long established itself as one of the major food source of the world (Dixon *et al*., 2009; Shewry, 2009). Apart from being the food for the growing human population, wheat is a raw material for producing carbohydrate and protein-based products like starch, dextrose, alcohol, bio-fuel and gluten. Inferior quality or surplus wheat, milling by-products and straw are used as livestock feeds. The chief contributor to the global wheat produce is the hexaploid bread wheat (*Triticum aestivum* L.), which is consumed in hundreds of forms all over the world with processing at the household or industrial levels. The tetraploid durum wheat (*Triticum durum* Desf.) is cultivated on a smaller scale compared to bread wheat and is used for making pasta products. In addition to these, small-scale production of other wheat species such as spelt (*Triticum spelta* L.) and emmer (*Triticum dicoccum* Schrank, Schulb) is practiced in parts of the world for their use in local culinary practices or as health food (Cornell and Hoveling, 1998; Bonjean and Angus, 2001). Wheat is the chief source of vegetable protein in human food, especially in underprivileged regions where adequate consumption of proteins from pulses and animal sources is difficult due to their higher cost (Shewry, 2009). The energy yield of wheat is approximately 320 kcal per 100 g contributed mainly by carbohydrates (60-70%) and proteins (10-15%) (USDA, 2010). Because of its widespread use, the world dietary energy consumption sourced from wheat (534 kcal/person/day) is at par with that of world's largest cultivated crop rice (536 kcal/person/day; FAOSTAT, 2009).

1.2 Wheat: A vital part of food trade in India and the world

The annual world production of wheat was estimated to be nearly 680 million tonnes during the years 2009 and 2010 (FAOSTAT, 2011; IGC, 2011).

Approximately 120 million tonnes of wheat amounting to roughly 50 billion dollars (FAOSTAT, 2011, http://faostat.fao.org/site/342/default.aspx) were involved in total imports in the world. India is the second largest producer of wheat with nearly 80 million tonnes of annual output (**Figure 1.1**) grown in about 28 million hectares with an yield of approximately 2.9 metric tonnes/ha (Directorate of Economics and Statistics, Government of India, http://dacnet.nic.in/eands/). India contributes approximately 12% to global wheat production and has maintained its position in world wheat production scenario consistently in the past nine years (Directorate of Wheat Research, Karnal, India, http://www.dwr.in/). In India, *T. aestivum*, *T. durum* and *T. dicoccum* are grown commercially with *T. aestivum* occupying 95% of the area under wheat cultivation followed by durum (4%) and emmer or dicoccum wheats (1%) (Gupta R *et al*., 2002). Wheat is grown in India in winter (Rabi season) from October to February/March under rain-fed conditions and from November to March/April under irrigated conditions. During the 2009-10 season, the wheat production in India was estimated to be 80.8 million tonnes and the advance estimate for the year 2010-11 is at 81.47 million tonnes (http://dacnet.nic.in/eands/), which has promised self-sufficiency and also revived the expectations of India becoming an exporter of wheat (Statement from the Union Agriculture Minister, Government of India, Reuters, February 23, 2011, New Delhi).

1.3 Wheat: A prominent system in plant genome research

The taxonomical position of wheat is as follows – Kingdom-Plantae, Subkingdom-Tracheobionta, Superdivision-Spermatophyta, Division-Magnoliophyta, Class-Liliopsida, Subclass- Commelinidae, Order- Cyperales, Family- Poaceae and Genus- *Triticum* (http://www.gramne.org/). Cultivated wheat species and their wild relatives display complex genome organization with diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) chromosome compositions. *Triticum aestivum* is an allohexaploid with three distinct but genetically related (homeologous) genomes A, B and D (Feldman *et al*., 1995). The genome size of bread wheat is among the largest in crop plants with approximately 16,000 Mb (Arumuganathan and Earle, 1991). In addition to this, wheat harbors >80% of repetitive DNA in the genome sequence (Smith and Flavell, 1975) and relatively smaller gene-rich regions (Erayman, *et al*., 2004).

Because of such intriguing genetic architecture, wheat is one of the most extensively studied crop species that has revealed interesting information on genome organization related to genome rearrangements (Bennetzen, 2007; Devos, 2010), polyploidy and genome evolution (Levy and Feldman, 2004; Adams and Wendel, 2005; Feldman and Levy, 2009) and transposable elements (Sabot *et al*., 2004; Cantu *et al*., 2010). Wheat is also a model system for the study of crop domestication. Many studies have thrown light on the genome rearrangements during domestication of wheat (Balter, 2007; Dubcovsky and Dvorak, 2007; Sang, 2009) that lead to changes in characters like spike morphology and threshing properties, heading date and kernel size and shape that enhanced its suitability for agricultural practices (Peng *et al*., 2003; Purugganan and Fuller, 2009; Gegas *et al*., 2010).

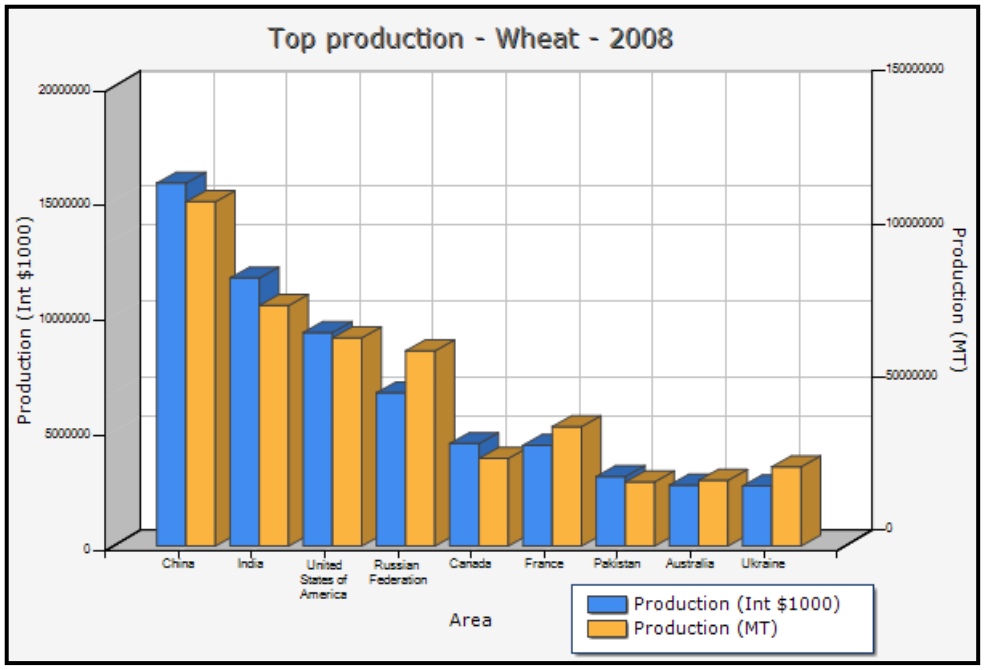


Figure 1.1: Position of India in global wheat production (FAOSTAT, 2011)

1.4 Wheat kernel: The fruit that feeds the world

Wheat kernel is a caryopsis, the characteristic fruit type borne by the members of the grass family *Poaceae.* A fertilized egg cell in the ovary develops into a single seed in which, the fruit wall (pericarp) and the seed coat are united (**Figure 1.2**),

and hence cannot be separated from each other. The kernel has the *germ* or *embryo* at one end, and a bundle of hairs, which is referred to as the *beard* or *brush* at the other end. The outer layers of the kernel constitute the *bran* and enclose the nutrient-rich *endosperm,* which is the source of wheat flour, the raw material for its multitude end products. Up to 80% of the total proteins in the wheat kernels belong to a class of prolamin storage proteins classified as gluten proteins that confer wheat its bread making properties (Shewry, 2009; Belderok, 2010a). Wheat flour milling involves the separation of the bran from the endosperm and reduction of endosperm to the desired particle size by a gradual milling and sieving process (Belderok, 2010b). Wheat milling has evolved from the ancient grinding processes to the modern automated industrial roller mills that demand grains with specific quality characteristics to ensure maximum flour yield (**Figure 1.3**, Cornell and Hoveling, 1998). There are several factors that affect milling yield including kernel hardness (Cane *et al*., 2004; Nadolska-Orczyk *et al*., 2009), the amount of germ, thickness of bran, depth of the crease along with kernel size and shape (Marshall *et al*., 1984; Marshall *et al*., 1986).

1.5 Kernel size and shape influence flour milling yield and market price in wheat

Wheat genotypes show extensive variation for kernel size and shape (**Figure 1.4**). Various studies have examined the influence of kernel size and shape on flour milling yield in wheat. Using wheat sieved into small, medium and large kernels, Shuey (1960) reported an attempt to predict the flour milling yield by calculating a potential flour yield multiplication factor. This factor was found to be progressively lower for smaller kernels. Marshall *et al.* (1986) stratified commercial wheat cultivar samples based on kernel size and reported positive correlation between kernel size and milling yield. Test weight or hectoliter weight is a commercial grading factor that denotes the weight for a given volume of a wheat sample. A high positive correlation between test weight and milling yield was reported earlier (Barmore, *et al*., 1965; Marshall *et al*., 1986). Test weight showed significant correlations with kernel shape factors (Troccoli and di Fonzo,

1999; Bergman *et al*., 2000; Sun *et al*., 2009) possibly because these parameters determine the way the individual grains pack.

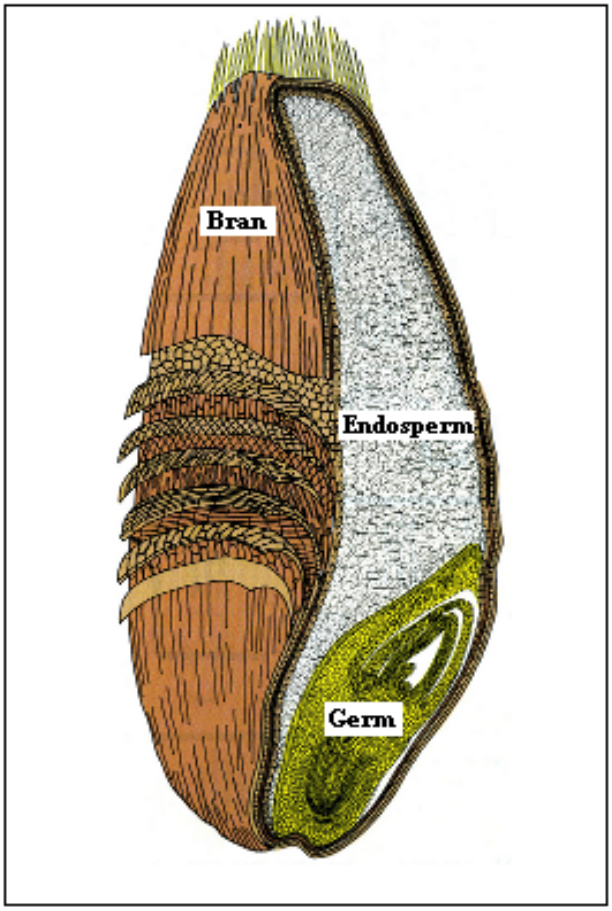


Figure 1.2: Longitudinal section of a wheat kernel depicting its three major parts (http://wbc.agr.mt.gov/consumers/diagram_kernel.html)

Since non-invasive methods are very useful during breeding programmes as the sample under testing will be left intact for sowing in future, the use of image analysis of whole grain samples has been evaluated to predict milling yield in wheat. Grain area, length, width and volume in association with test weight could explain up to 66% of variation in milling yields, whereas, test weight could explain for only 17% of the variation (Berman *et al*., 1996). Image analysis being relatively low in labor requirement and cost, can be a suitable milling yield prediction method for a breeding programme if standardized methodically.

Figure 1.3: Transformation of wheat flour milling from small-scale grinding to large-scale roller milling process

Thousand kernel weight (TKW), a measure of the mass of the wheat kernel is used by wheat breeders and flour millers as a complement to test weight to gauge potential flour extraction. Wheat with a higher TKW can be expected to have a greater potential flour extraction (Wheat and Flour Testing Methods, 2008). Breseghello and Sorrells (2006b) showed positive correlation of kernel length, weight and area with milling score; a parameter derived from flour yield, endosperm separation index and friability. In durum wheat, Matsuo and Dexter (1980) showed that TKW had significant positive correlation with semolina yield and Novaro *et al.* (2001) demonstrated that kernel volume, together with TKW or test weight, were the best predictors of semolina yield.

For faster analysis of large samples of wheat on a commercial scale, single kernel characterization system (SKCS) is an appropriate method as it can simultaneously measure physical features of wheat such as kernel hardness, kernel diameter and kernel weight (Wheat and Flour Testing Methods, 2008). In addition to this, using such devices will result in faster measurement of kernel features

during early selection and quality testing of breeding material in wheat improvement programmes (Ohm *et al*., 1998). Furthermore, recording wheat non-grade data like kernel size and shape by rapid methods based on SKCS were recommended for prediction of milling yield and end-product quality at the grain elevators prior to milling (Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources http://www.fapc.okstate.edu/files/factsheets/fapc129.pdf).

In the flour milling process, wheat is tempered and passed through a series of roller mills to separate the endosperm from the outer bran. When there is a wide variation in kernel size, small kernels escape grinding while they pass through the roller mills or are partially broken in the initial breaking process and consequently require additional processing. This additional processing requires more milling time and energy costs. Furthermore, inefficient milling may decrease the quality of the flour derived by bran contamination that results in increased ash or mineral content in the flour. Ash can affect flour color, imparting a darker color to finished products (Wheat and Flour Testing Methods, 2008). Kernel size uniformity is a potentially important physical quality attribute that can enhance processing efficiency, quality control and milling yield (Yoon *et al*., 2002; Tsilo *et al*., 2010). Yoon *et al.* (2002) recommended industrial-level grain sorting to achieve increased kernel size uniformity. In view of their importance in milling yield determination, mathematical modeling of the wheat grain morphology, calculation of features like bran proportion inside the crease have also been attempted to predict milling yield (Marshall *et al*., 1984; Mabille *et al*., 2004).

1.6 Importance of kernel size in aspects other than milling yield

Wheat kernel morphometric traits have also been implicated in the quality of seedling growth, in planning planting and harvesting, verification of wheat varieties and classes, determination of market price, end-use quality and agronomic yield.

1.6.1 Early plant growth and agricultural practices

Botwright *et al.* (2002) reported increased embryo size and early seedling vigor associated with the large seeded wheat genotypes, which translated to greater

Figure 1.4: Variation in kernel size and shape among wheat genotypes

biomass production at anthesis and maturity and increased grain yield. Bredemeier *et al.* (2001) reported better seedling growth and tillering in plants grown from larger kernels and Chastain *et al.* (1995) reported similar observations, which resulted in 4.2% greater grain yield compared to that from plants originated from small seeds in an environment dependent manner.

Kernel length and kernel width are some of the physical features taken into consideration while designing sowing drills and harvest machines (Öğüt *et al.*, 1992). TKW has been recommended to account for seed size variations when calculating seeding rates, calibrating seed drills and estimating combine losses (Agri-facts, 2007).

1.6.2 Market price and end use quality

For practical application of grain morphometric traits in commercial scenario, image processing was used by Zayas *et al.* (1985) and Myers and Edsall (1989) to

identify wheat varieties and by Neuman *et al.* (1987) and Zayas *et al.* (1986) to discriminate between wheat commercial classes. Appearance of a wheat sample can influence its market price with bold and lustrous grains claiming consumer preference both at household and industrial levels. During varietal development in India, breeding material is evaluated for Grain appearance score taking into consideration grain size, shape, soundness, color and luster (Gupta R *et al*., 2008).

Baker *et al.* (1999) showed that wheat segregated based on dough factor, a milling and baking quality parameter that indicates the amount of flour-water dough that can be produced from a given unit of wheat also showed differences in flour yield, kernel size and kernel shape. In the studies of Morgan *et al.* (2000) positive relationships between kernel weight and water absorption during baking were reported.

1.6.3 Kernel weight is a wheat yield component

Kernel weight measured in terms of TKW is an agronomic yield component. Because of the ease in measuring kernel weight and positive correlation with grain yield, recommendations were made to use it to predict wheat agronomic yield (Alexander *et al*., 1984; Baril, 1992). Wiersma *et al.* (2001) used a recurrent selection method of intermating and repeated selection in an initiative to select for high yielding wheat on the basis of kernel weight and estimate its resultant functional gains. As a result, there were significant increases in kernel weight, length, width and area and about 5% higher flour extraction at the end selection cycles, while, both bran and shorts contents decreased. These results in terms of assuming that the outer layers of the kernels did not increase in thickness during selection in proportion to the increase in the volume of the endosperm possibly resulted in higher flour extraction compared to the proportion of bran and shorts extraction (Wiersma *et al*., 2001). However, yield did not change across cycles due to compensation among yield components.

1.7 Kernel size from the perspective of cereal domestication

The study of kernel size of cultivated and ancient wheats has revealed interesting information on food crop domestication. Increase in grain size is one of the main

components of the domestication in cereals. The transition from the diploid wild einkorn (*Triticum monococcum* ssp. *aegilopoides*; A_mA_m) and tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*; BBAA) to the domesticated forms (*T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *dicoccum*, respectively) was associated with a trend toward larger grain size as indicated by archaeobotanical evidence from the region around the Fertile Crescent (Feldman, 2001; Fuller, 2007). Grain size is often used as an indication of human intervention in plant reproduction (Brown *et al*., 2009). In their recent report, Gegas *et al.* (2010) discussed on the basis of their observations on a wide range wheat species that kernel shape does not appear to be a major component of the wheat domestication syndrome, but has been a relatively recent breeding target dictated by the market and industry requirements in contrast. This is unlike to rice (*Oryza sativa*), where the domestication process involved strong selection for both grain size and shape (Kovach *et al*., 2007).

1.8 Need for improving kernel morphometric characters in breeding programmes

During the past four decades, wheat breeders made a significant contribution to the increase in global food production through the use of higher yielding, water- and fertilizer responsive, and disease resistant varieties supported by strengthened input delivery systems, tailored management practices and improved marketing. With the global population projected to increase steadily to around 9 billion, the demand for wheat is expected to increase to more than 900 million tonnes in 2050 (Dixon *et al*., 2009). Increased attention to wheat quality is being given due to significant changes in wheat consumption patterns and end use in developing countries. By urbanization, consumer trends have shifted towards processed wheat products and have given rise to enhanced role of industrial wheat processing in the developing world. It will be important to understand and address the implications for wheat quality due to the shift from home preparation to mechanized processing. Achieving improvements at the producer level for the supply of wheat with desired quality characteristics for the processing industry requires concerted efforts at several levels. These include documentation of quality standards

correlated with specific end uses, systems to provide quality assurance, segregation of grain based on qualities upon delivery along with farmer education and outreach (Meng *et al*., 2009). Above all this, identification and breeding for varieties possessing specific qualities can result in higher incentives for the grower. In view of the importance of kernel morphometric traits and kernel size and shape uniformity in influencing flour milling yield and quality, breeding for their improvement is an important objective for enhancing wheat quality. Information on the genomic regions influencing such traits can result in achieving breeding targets with better efficiency (Moose and Mumm, 2008). Furthermore, in the current climate change situation, abiotic stress can influence wheat quality, with higher temperatures causing shorter grain-filling period resulting in reduced grain size (Hodson and White, 2009). In view of this, the knowledge of genetic basis of kernel morphometric traits is essential for accelerated wheat breeding with better kernel characters.

1.9 Understanding the genetic basis of wheat kernel morphometric traits: A literature survey

Kernel size and shape have been studied in terms of various representative traits in previous studies (**Table 1.1**). For example, Campbell *et al.* (1999) used TKW, kernel length (KL), kernel width (KW), kernel area and certain measures derived from the above parameters like shape factor and density factor as representatives of kernel size and shape. Various methods have been used by earlier studies to dissect the genetic basis of kernel morphometric traits, a summary of which is given below.

1.9.1 Chromosome substitution and aneuploid analysis

Previous studies such as Kuspira and Unrau (1957) and Halloran (1976) used chromosome substitution lines in wheat to identify the chromosomes involved in the control of wheat kernel size. In the later years, aneuploid techniques that exploited the advantage of the allopolyploid nature of wheat were the methods of choice for estimation of the number and chromosomal location of segregating genetic factors influencing kernel characters (Bannier, 1979; Khrabrova and Maistrenko, 1980; Chojecki *et al*., 1983; Zheng *et al*., 1993; Sharma *et al*., 1995;

Giura and Saulescu, 1996; Varshney *et al*., 2000). These studies indicated the complexity in the control of kernel size traits with loci distributed on a number of chromosomes. Among these studies, Giura and Saulescu (1996) reported the identification of chromosomes involved in the control of both kernel size and shape, with chromosomes 4A and 6D influencing kernel weight, 1B, 2B, 3A, 4A and 4B controlling KL, 1A and 1B for KW and 6A and 6D for kernel factor form density (FFD).

1.9.2 Molecular marker-based quantitative trait locus (QTL) mapping approaches

The complex inheritance and chromosomal distribution of kernel size and shape genetic components indicated that these traits are controlled by multiple loci (QTLs). With the development and standardization of molecular marker methods (Gupta *et al*., 1999) and QTL mapping approaches (Collard *et al*., 2005), identification of specific chromosomal regions associated with kernel characters and their effects on phenotypic variance became possible. A summary of previous QTL mapping studies on kernel size and shape is given in **Table 1.1**. The study of various kernel characters in the same genetic background in these studies enabled the analysis of their inter-relationships and identification of common genomic regions with effects on multiple traits. In addition, QTL analysis of TKW has been reported in many individual reports for wheat kernel weight and also in the studies on yield and related traits (**Table 1.2**). It may be noted that considering the reports listed in **Table 1.1** and **1.2**, all the 21 chromosomes of hexaploid wheat were noted in association with kernel weight. The involvement of different subsets of chromosomes in each study indicates the influence of the environment and the genetic background on the expression of kernel traits, because of which the identification of the associated genes becomes more challenging.

1.9.3 Association mapping

Association mapping identifies QTLs by examining the marker-trait associations that can be attributed to the strength of linkage disequilibria between markers and functional polymorphisms across a set of diverse germplasm (Zhu *et al*., 2008). Because of the constraints such as the limited number of recombination events that occur during the construction of mapping populations and the cost for

propagating and evaluating a large number of lines, QTL mapping based on linkage analysis in plants typically localizes QTLs to 10 to 20 cM intervals (Doerge, 2002; Holland, 2007). On the other hand, association mapping that exploits historical and evolutionary recombination events at the population level offers increased mapping resolution, reduced research time and greater allele number (Breseghello and Sorrells, 2006a; Yu and Buckler, 2006). The use of association mapping to identify loci for kernel morphometric traits was reported by Breseghello and Sorrells (2006b). In this study, the markers on chromosome 2D were associated with KL and KW, 5A with KL, KW, kernel area and kernel weight and 5B with KL, KW and kernel weight. Similarly, Roy *et al.* (2006), Yao *et al.* (2009), Neumann *et al.* (2011) and Reif *et al.* (2011) reported TKW-associated markers in bread wheat by association mapping (**Table 1.2**).

1.9.4 Genes for kernel size identified in wheat

A vital part of the genetic dissection of complex traits is the identification of single Mendelian genes underlying the QTLs and their characterization. Both map-based cloning and candidate gene approaches have been used in wheat for the identification of genes underlying QTLs of interest (Uauy *et al*., 2006; Simons *et al*., 2006; Cloutier *et al*., 2007; Su *et al*., 2011). In bread wheat, fine mapping of a region on chromosome 7D controlling grain weight was reported by Röder *et al.* (2008), which might eventually lead to map-based cloning of the associated gene. Based on the candidate gene approach using information on a gene associated with control of rice grain shape and size, its ortholog in wheat *TaGW2* was cloned (Su *et al*., 2011). Association analysis of modern varieties using information on two haplotypes of *TaGW2* suggested that its effect in improving grain weight was mainly associated with increased grain width, with little effect on grain length and grain thickness. In addition to this, assessment of transcript levels implied that the effect of *TaGW2* on grain size was due to the level of gene expression. Recently, a triplicated set of *Triticum aestivum* Sucrose synthase 2 (*Tasus2*) genes was cloned and assigned to the chromosomes 2A, 2B and 2D (Jiang *et al*., 2011). Sucrose synthase is involved in the conversion of sucrose to starch in endosperm. Three single nucleotide polymorphisms (SNPs) detected for the *TaSus2-2B* locus formed two haplotypes, which were significantly associated with TKW. More recently, a

hexaploid wheat cell wall invertase gene *TaCwi-A1* on chromosome 2A was cloned and association between its allelic variations and TKW was reported (Ma *et al*., 2011).

1.10 Studies on seed size and shape in other plants

Investigation of the genetic basis of seed morphometric traits is an important area of research in other cereals, legumes and model plants. In barley, oat and rye, QTL analyses have been performed to detect multiple QTLs affecting these traits (Groh *et al*., 2001; Marquez-Cedillo *et al*., 2001; Ayoub *et al*., 2002; Wricke, 2002; De Koeyer *et al*., 2004; Falke, *et al*., 2009; Szűcs *et al*., 2009). In rice, several studies have focused on kernel appearance, which has important implications on its market price (Fitzgerald *et al*., 2009). QTLs associated with each aspect of grain size and shape have been identified in diverse rice populations over the past 15 years (http://www.gramene.org/). Recently, genes underlying three seed size, shape and weight QTLs have been cloned and characterized; namely, a grain-length QTL *GS3* on chromosome 3 (Fan *et al*., 2006), a seed width QTL *SW5* on chromosome 5 (Shomura *et al*., 2008) and a grain weight QTL *GW2* on chromosome 2 (Song *et al*., 2007) of rice. In all these cases, cultivars carrying the recessive allele(s) have longer, wider and/or heavier seeds than those carrying the wild type (WT) alleles. The authors concluded that the genes, directly or indirectly, affected cell division, and recessive alleles at *SW5* and *GW2* were associated with larger numbers of cells in the hull (maternal) tissue (Fan *et al*., 2006; Song *et al*., 2007; Shomura *et al*., 2008; Fitzgerald *et al*., 2009). A fourth gene, *GIF1* on rice chromosome 4, cloned from a population of induced mutants, is a cell-wall invertase gene that is not associated with cell division, but rather influences grain weight by affecting the rate of grain-filling (Wang *et al*., 2008). In legumes like chickpea, soybean and common bean, several QTLs controlling seed size and shape parameters have been mapped (Hossain *et al*., 2010; Pérez-Vega *et al*., 2010; Xu *et al*., 2011). In the model plant *Arabidopsis*, a number of genes involved in seed size control have been cloned and characterized (Garcia *et al*., 2003; Jofuku *et al*., 2005; Luo *et al*., 2005; Schruff *et al*., 2006; Adamski *et al*., 2009; Zhou *et al*., 2009; Wang *et al*., 2010).

Table 1.1: Summary of previous reports of QTL analysis of various kernel morphometric traits in the same mapping population

Reference	Traits	Mapping Population (Type and size)	No. of field trial locations (Year-location combinations)	Molecular Marker type (QTL analysis method)	QTL chromosomal location
Campbell *et al.* (1999)	TKW, KL, KW, KA, PP, SF, DF	NY6432-18×Clark's Cream (RIL, 78)	4 (6)	RFLP (ANOVA/Multiple regression)	1A, 1B, 2A, 2B, 2D, 3B, 3D, 4A, 5A, 7A
Dholakia *et al.* (2003)	KWt, KL, KW, FFD	WL711×PH132 (RIL, 106)	1 (1)	RAPD/ ISSR/SSR (SMA)	2B, 2D, 6B, 5B, 7B
Ammiraju *et al.* (2004)	KWt, KL, KW, FFD	Rye Selection 111 × Chinese Spring (RIL, 113)	1 (1)	RAPD/ISSR/SSR (SMA)	1A, 1D, 2D, 4B, 5A, 5B, 6A, 6B
Breseghello and Sorrells (2007)	Kwt, KL, KW, HAP, Sph, VP, PA, SA, Sur, KV	W7984×Opata 85 (RIL, 115)	2 (2)	RFLP/SSR/ gene-specific probes (SMA/CIM)	1B, 2A, 2B, 2D, 4A, 4B, 5B, 5D, 7B
		AC Reed × Grandin (DH, 101)	2 (2)	RFLP/AFLP/SSR (SMA/CIM)	1A, 1B, 2B, 2D, 3A, 4B, 6A, 7B
Sun *et al.* (2009)	TKW, KL, KW	Chuan 35050×Shannong (RIL, 131)	3 (4)	ISSR/SRAP/ TRAP/SSR/ EST-SSR (Mixed-model-based QTL mapping)	1A, 1B, 2A, 2B, 4A, 4B, 5D, 6A
Gegas *et al.* (2010)	TKW, KL, KW, KA, LWR, FFD	Avalon × Cadenza (DH, 202)	1 (2)	SSR/DArT (CIM)	1D, 2B, 2D, 3A, 3B, 4B, 4D, 5A, 6A, 7D
		Beaver × Soissons (DH, 65)	1 (2)		1A, 2D, 4B, 3A

Table 1.1 cont…

Reference	Traits	Mapping Population (Type and size)	No. of field trial locations (Year-location combinations)	Molecular Marker type (QTL analysis method)	QTL chromosomal location
		Shamrock × Shango (DH, 76)	1 (2)		2D, 3D, 5A, 6B, 7A, 7B
		Spark × Realto (DH, 112)	1 (1)		1A, 1B, 5A, 4D
		Savannah × Rialto (DH, 98)	1 (1)		2A, 2B, 3A, 6A
		Malacca × Charger (DH, 100)	1 (1)		1A, 2A, 3A, 5A, 6A, 7A
Tsilo *et al.* (2010)	Kwt, KD, TKW, Proportions of large, medium and small kernels	MN98550×MN99394 (RIL, 139)	3 (3)	SSR/DArT (CIM)	1B, 2A, 5B, 6B, 7A
Sun *et al.* (2010)	Kwt, kernel diameter	Ning7840×Clark (RIL, 132)	3(7)	SSR (CIM)	2A, 3A, 5A, 6A, 7A
Manickavelu *et al.* (2011)	KL, KW, KH	Chinese Spring × KT19-1 (*T. spelta*) (RIL, 147)	1 (2)	SSR (CIM)	2B, 3A, 4A, 4B, 4D, 5A

DF	Density factor (TKW/mean KA)	KP	Kernel perimeter	SA	Section area
FFD	Factor form density (KWt/KL*KW)	KV	Kernel volume (derived from SA and KL)	SF	Shape factor (4π(area)/perimeter^2)
HAP	Horizontal axes proportion (100*KW/KL)	KW	Kernel width	Sph	Sphericity (100*SA/PA)
KA	Kernel area	KWt	Kernel weight	Sur	Surface (derived from VP and KL)
KH	Kernel height	LWR	Length width ratio (KL/KW)	TKW	Thousand kernel weight
KL	Kernel length	PA	Projection area	VP	Vertical perimeter

Table 1.2: Chromosomal locations of kernel weight QTLs in hexaploid wheat reported in previous studies. Kernel weight was measured in these studies as thousand kernel weight (TKW), unless otherwise mentioned.

Reference	Chromosomes	Molecular marker type	Method
Araki *et al.* (1999) (50-grain weight)	4A	RFLP	SMA/SIM/CIM
Shah *et al.* (1999)	3A	RFLP	Single factor ANOVA/ multiple regression
Kato *et al.* (2000) (50-grain weight)	5A	RFLP	SIM/CIM
Varshney *et al.* (2000)	1A	SSR	SMA
Ammiraju *et al.* (2001)	1D, 2D, 6B	ISSR	SMA
Börner *et al.* (2002)	1B, 2D, 3A, 3B, 5A, 6A, 6B, 7B, 7D	RFLP	SIM
Campbell *et al.* (2003)	3A	SSR	SMA/CIM
Huang *et al.* (2003)	2D, 4D, 5B, 7A, 7B, 7D	SSR	SMA/SIM
Groos *et al.* (2003)	1D, 2B, 2D, 3A, 5B, 6A, 6D, 7A, 7D	RFLP/SSR/AFLP	Single factor ANOVA/multiple regression
Huang *et al.* (2004)	1B, 1D, 2A, 2D, 3A, 3B, 3D, 4B, 6A, 7A, 7D	SSR	SMA/SIM
Quarrie *et al.* (2005)	1B, 1D, 2A, 2B, 3A, 3D, 4B, 5A, 5B, 5D, 6B, 6D, 7B	RFLP/SSR/AFLP	SMA /CIM
McCartney *et al.* (2005)	2A, 3D, 3D, 4A, 4B, 4D, 6D,	SSR/SNP	CIM
Dilbirligi *et al.* (2006)	3A	RFLP/SSR	SMA/CIM
Huang *et al.* (2006)	2B, 2D, 3B, 4B, 4D, 6A	SSR/ STS-PCR	CIM
Kumar *et al.* (2006)	1A, 2B, 7A	SSR/AFLP/SAMPL	SMA/CIM
Quarrie *et al.* (2006)	7A	RFLP/SSR/AFLP	SMA
Roy *et al.* (2006)	-	AFLP	AM
Habash *et al.* (2007)	2B, 4A, 4B, 5A, 6A, 6B, 6D, 7B, 7D	RFLP/SSR/AFLP	CIM
Kuchel *et al.* (2007a)	6A, 7D	SSR	CIM

Table 1.2 cont...

Reference	Chromosomes	Molecular marker type	Method
Li *et al.* (2007)	1D, 3B, 5D, 6A, 7D	ISSR/SSR/ EST-SSR/TRAP/ SRAP/ HMW-glutenin subunit loci	Mixed model based interval mapping
Elangovan (2007)	1A, 1B, 1D, 2B, 4B, 5A, 5B, 5D, 6A, 6B, 7D	SSR/ HMW-glutenin subunit loci	CIM
Cuthbert *et al.* (2008)	2D, 3B, 5A, 7A	SSR	CIM
Mir *et al.* (2008)	1A, 1B, 1D, 2B, 4B, 5A, 6A, 7A, 7D, several epistatic QTLs	SSR/ AFLP/SAMPL	Inclusive CIM/ two-locus analysis
Röder *et al.* (2008)	7D	SSR	SMA (Fine mapping)
Hai *et al.* (2008)	2B, 7B	SSR	CIM
Liao *et al.* (2008)	1A, 3D, 4B	SSR	CIM
Wang *et al.* (2009)	1A, 1B, 2A, 2D, 3B, 4A, 6D, 7D	SSR/EST	CIM
Yao *et al.* (2009)	2A	SSR/EST-SSR	AM
Fontaine *et al.* (2009)	1B, 2D, 4B, 5D, 6A	RFLP/SSR	CIM
Su *et al.* (2009)	2A, 2D, 3A, 4A, 5B, 6A, 6B, 7A	SSR/AFLP/ EST-SSR/EST	CIM
McIntyre *et al.* (2010) (grain weight in mg)	1B, 1D, 2B, 4D, 5D, 6B	SSR/AFLP/DArT	CIM
Pinto *et al.* (2010)	1A, 1B, 1D, 2A, 2B, 3B, 4A, 4B, 4D, 5A, 5B, 6A, 6D, 7A	SSR/AFLP/DArT	CIM
Neumann *et al.* (2011) Grain weight per spike/TKW	1A, 1B, 1D, 2B, 2D, 5A, 6B, 7A, 7B	DArT	AM
Reif *et al.* (2011)	2D, 4A, 4D, 5B, 7D	SSR	AM

1.11 Tools for estimating the number and location of QTLs

Linkage-based QTL mapping techniques have been the most frequently used methods for identifying genomic regions controlling economically important traits in crop plants (Bernardo, 2008; Asins *et al*., 2010). In this method, genetic maps of molecular markers are used to identify chromosomal regions harboring genes

or QTLs associated with specific traits by statistical procedures testing marker-trait associations (Paterson, 1997; Lynch and Walsh, 1998; Kearsey, 1998; Hackett, 2002). Considering the large genome size of hexaploid wheat (16,000 Mb), identification of closely linked markers by such QTL analysis procedures is the convenient first step on the road to marker assisted selection (MAS) and map-based cloning of genes controlling complex traits. The closely linked and gene-based markers can be utilized for rapid and easier selection of desired traits through MAS and the isolated genes can be used for the generation of transgenic varieties (Varshney *et al*., 2004). The key steps in the process of identification of marker-trait associations involve the choice of molecular marker system and mapping population, genotyping and construction of a linkage map, phenotypic evaluations and QTL analysis and finally, validation (Collard *et al*., 2005). The basic features of these stages are described below.

1.11.1 Molecular marker systems and mapping populations for QTL analyses

Genetic markers are specific locations on a chromosome, which serve as landmarks for genome analysis. Molecular markers that reveal polymorphisms at the DNA level based on hybridization or polymerase chain reaction (PCR) have proved immensely useful in identifying genes as well as in crop improvement (Semagn *et al*., 2006a; Jones *et al*., 2009; Gupta *et al*., 2010). Restriction fragment length polymorphism (RFLP) and PCR based markers like random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers were largely employed in wheat QTL analysis in the last decade of the 20th century (Gupta *et al*., 1999). The development of simple sequence repeat (SSR) markers for wheat in the later part of 1990s that yielded higher levels of polymorphism and reliability, resulted in a surge of QTL mapping studies in wheat (Ganal and Röder, 2007). SSRs or microsatellites are a common feature of most eukaryotic genomes and consist of tandem repeats of di-, tri- or tetra-nucleotide motifs. They exhibit high variability in the number of repeats due to frequent gain or loss of repeat units caused by strand slippage and mispairing during DNA replication. Polymorphism can be assessed by PCR-based assay using primers complementary to unique sequences flanking the microsatellite repeat region. Differences in the number of tandem repeats are readily assayed by

measuring the molecular weight of the resulting PCR fragments with gel- or gel-free detection techniques.

SSR markers have several advantages such as being co-dominant, abundant in the wheat genome, reproducible, locus-specific and amenable to fluorescence-based automation and multiplexing (Gupta and Varshney, 2000). The primer information about newly characterized SSR markers distributed among research groups is all that is required for sharing these markers for further use. The GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml) harbors a large repository of SSR primer sequences that are publicly available. Many SSR- based consensus maps are also available that can serve as reference for marker order and for getting information on nearby markers during map saturation (Paillard *et al*., 2003; Somers *et al*., 2004; Komugi website http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp). In addition to these advantages, SSR markers can be used as anchor markers during further saturation of linkage maps with random markers like AFLP (Quarrie *et al*., 2005; Kumar *et al*., 2006). Physical locations of many SSRs in the wheat genome are known (Qi *et al*., 2003; Sourdille *et al*., 2004), which might be useful for approximating the position of the QTLs of interest and for mapping closer markers for the associated traits. More recently, large numbers of SSR markers developed from the expressed region of wheat genome [Expressed sequence tag (EST)-SSRs] have been characterized and made available in the public domain (Yu *et al*., 2004; La Rota *et al*., 2005; Xue *et al*., 2008). In addition to these, an array-based high-throughput marker system–diversity arrays technology (DArT) has been used in many QTL mapping studies in wheat (Akbari *et al*., 2006; Gegas *et al*., 2010; Tsilo *et al*., 2010). The development of sequence-based markers like single nucleotide polymorphisms in wheat that promises an abundant supply of easily scorable markers is gaining momentum in the recent years (Ganal and Röder, 2007).

The task of constructing a molecular marker linkage map begins with recording the segregation of markers in defined populations. Various types of mapping populations derived from crosses involving diverse parents have been used earlier for gene tagging and QTL mapping in wheat (Gupta and Varshney,

2004). The examples are, F_2 or backcross populations derived from F_1 plants through selfing or backcrossing to one of the parental lines, respectively; recombinant inbred lines (RILs) derived by single seed descent for five or more generations and doubled haploids (DHs) derived from F_1 plants through anther or ovule culture or distant hybridization. Though simpler to construct in terms of time and resources, F_2 and backcross populations are transient; since they exhibit segregation of markers and traits upon selfing (Young, 2001). The RILs that tend towards homozygosity with each successive generation derived by selfing and DHs that are derived by chromosome doubling after the meiosis occurred in F_1 plants have a 'fixed' genetic make up and hence can be permanently maintained and evaluated in repeated year-location experiments that can reveal genotype × environment effect on the traits. In addition, several plants representing each genotype can be evaluated for traits of interest and in multiple replicates, which can improve the accuracy of QTL analysis. RILs have an additional advantage of being the product of several meioses, so that each RIL contains a different combination of linkage blocks from the original parents. However, generation of RILs is time-consuming and certain genomic regions have a tendency to stay heterozygous even after repeated selfing. Additionally, dominance effects at QTLs cannot be estimated, which would be possible with F_2 populations (Burr and Burr, 1991; Knapp and Bridges, 1990; Austin and Lee, 1996).

Doubled haploids have been used in many studies (Huang *et al*., 2006; Breseghello and Sorrells, 2007; Gegas *et al*., 2010) and, because of their shorter development time, have an advantage over RILs. However, the cost and difficulty in developing DH populations have proved prohibitive in the widespread use of this population type for mapping. Some of the studies have reported an alternative in the advanced backcross QTL (AB-QTL) analysis that integrates QTL analysis with germplasm development in crosses between adapted and wild germplasm (Tanksley and Nelson, 1996). It relies on the partial isolation of wild QTLs by several backcrosses to an adapted parent to eliminate much of the wild genetic background. QTLs identified in this advanced material can then be isolated in near-isogenic lines (QTL-NILs) by further backcrossing and selfing. This approach can provide a means to tap the genetic diversity in exotic germplasm to

isolate beneficial genes and also to conduct trials at multiple environments to evaluate the heritability of the identified QTLs. Using the AB-QTL method, many QTLs have been identified and transferred from wild/donor germplasm to elite breeding lines in wheat (Huang *et al*., 2003 and 2004; Narasimhamoorthy, *et al*., 2006; Röder *et al*., 2008).

1.11.2 Development of linkage maps

A linkage map indicates the position and relative genetic distance between markers along chromosomes. Genes or markers that are close together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart (Collard *et al*., 2005). Genetic distances between DNA markers are based on frequencies of crossing over and are represented on the genetic map in centiMorgans (cM). For linkage mapping, the segregation of markers in a mapping population is recorded and linkage between two loci is estimated based on the probability *r* of each crossover occurring between them at a single meiosis. This is done by tabulating the distinct genotype combinations observed at these loci and then estimating a value for *r* that agrees with the observed frequencies of these combinations (Nelson, 2005). The estimated *r* for each pair of markers is converted into genetic distance using specific map functions (Wu *et al*., 2007). The loci are then grouped into linkage groups that represent candidate chromosome segments.

Further, loci within these groups are arranged at relative positions most compatible with their linkages. Loci are assigned to linkage groups using the odds ratios (logarithm of odds–LOD value or LOD score), which refers to the ratio of the probability that two loci are linked with a given recombination value over a probability that the two are not linked. Several researchers have used LOD value 3.0 as the minimum threshold value in order to decide whether or not loci were linked (Semagn *et al*., 2006b). A LOD value of 3.0 between two markers indicates that linkage is 1000 times more likely than no linkage (Stam, 1993). The use of computer programs is inevitable in linkage mapping since several hundreds of markers are often used in a single population for map construction. The most widely used mapping software is MAPMAKER (Lander *et al*., 1987). The information from several mapping studies in a species can be integrated to

generate a consensus map. Both individual linkage maps and consensus maps can be constructed using the software JoinMap (Stam, 1993; Van Oijen, 2006). Many SSR-based high-density maps and consensus maps are available in wheat (GrainGenes http://wheat.pw.usda.gov/ggpages/map_shortlist.html and Komugi http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp databases).

1.11.3 QTL mapping

QTL analysis is based on the fact that if there is close proximity (linkage) between a marker and a QTL influencing the trait of interest, the marker locus and the QTL will not segregate independently and hence, differences in those marker genotypes will be associated with differences in trait phenotypes (Kearsey, 1998). QTL mapping involves testing for such associations between the quantitative trait and the marker alleles segregating in the population. The detection of marker–QTL linkage is based on a statistical test of a null hypothesis (H_0) that there is no QTL and hence no linkage exists between the marker and the QTL against an alternative hypothesis (H_1) (Narain, 2010). The key stages in QTL mapping are detection of marker-trait associations, determining the putative position of the QTL in the linkage map and estimation of the effect of allelic substitution at the particular locus. The three common approaches used for linkage-based QTL mapping are single marker analysis (SMA), simple interval mapping (SIM) and composite interval mapping (CIM) (Kearsey and Pooni, 1996; Hackett, 2002; Nelson, 2005; Wu *et al*., 2007).

1.11.3.1 Single marker association

It is the simplest method for detecting QTLs that does not necessitate the use of a linkage map. The mapping population is partitioned into different genotypic groups based on the marker genotype. Tests are performed to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993). A significant difference between phenotypic means of the groups indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait. The statistical methods used for SMA include *t*-tests, analysis of variance (ANOVA) and linear regression. Linear regression is most commonly used because the coefficient of determination (R^2)

from the marker explains the phenotypic variation arising from the QTL linked to the marker. Softwares like QGENE (Nelson, 1997) or MapManager QTX (Manly *et al*., 2001) are commonly used for SMA. As an extension of this method, multiple regression methods may be used (Cordell *et al*., 1998; Whittaker, *et al*., 1996), in which the genotypes at several markers are simultaneously used to predict the trait outcome. However, with SMA, if a QTL is located farther from the test marker, it might not be detected as a result of recombination between the marker and the QTL. Furthermore, missing data will influence the computation and since one locus is tested at a time, a large number of markers are required to get a reasonable picture of the QTLs involved (Collard *et al*., 2005).

1.11.3.2 Simple interval mapping

Simple interval mapping (Lander and Botstein, 1989) offers better estimates of the genetic location and phenotypic effect of QTLs compared to SMA. The whole chromosome is divided into short intervals using markers and each interval is treated separately for QTL detection and estimation of its effect. The maximum likelihood method leading to LOD score statistics is used for this purpose (Narain, 2010). The LOD score values obtained for each interval are plotted against the linkage group positions to give a likelihood map. The maximum value of the LOD scores provides a possible position of the QTL for the given genomic region. Softwares like QGENE (Nelson, 1997), MAPMAKER/QTL (Lincoln *et al*., 1993) and QTL Cartographer (Basten *et al*., 1994, 2001) offer SIM procedures. Although SIM has advantages over SMA in identifying specific marker intervals with putative QTLs, it ignores the fact that most quantitative traits are influenced by numerous QTLs, the proximity of which may influence the estimations of positions and effects of the QTLs in the test region (Collard *et al*., 2005).

1.11.3.3 Composite interval mapping

By using additional markers as cofactors during interval mapping, CIM attempts to eliminate the influences of genetic background on QTL detection. Zeng (1993, 1994) and Jansen and Stam (1994) independently demonstrated the use of the CIM method, which constructs test statistics by combining interval mapping using two flanking markers and multiple regression analysis on other markers. With this

method, some of the interference from QTLs outside the interval being tested is removed (Wu *et al*., 2007). Similar to SIM, a profile of the likely sites for a QTL based on LOD scores is obtained in CIM. The determination of threshold LOD for declaring the presence of a significant QTL is commonly performed by permutation tests (Churchill and Doerge, 1994). In this test, the phenotypic values of the population are shuffled, while the marker genotypic values are held constant and QTL analysis is performed. This process is repeated usually for 1000 times or more and significance levels can then be determined based on the level of false-positive marker-trait associations (Collard *et al*., 2005). The commonly used softwares for CIM include QTL Cartographer (Basten, 2001), MapManager QTX (Manly *et al*., 2001) and MapQTL (van Ooijen, 2004).

1.11.3.4 Exploring epistatic and pleiotropic QTLs

The genetic architecture of complex traits is not just one-dimensional with QTLs involved in additive gene action, but also involves complex epistatic interactions among many QTLs (Wu *et al*., 2007). Analysis of QTL×QTL interactions has been attempted based on two-way analysis of variance and multiple regression models with terms for pair-wise interactions between markers (Chase *et al*., 1997; Pressoir *et al*., 1998). Multiple interval mapping (MIM) method available in the QTL Cartographer software can also incorporate epistasis in the QTL model (Kao *et al*., 1999). An alternative approach based on a mixed linear model to map epistatically interacting QTLs was proposed by Wang *et al.* (1999) and Yang *et al.* (2007) that can be utilized with the help of QTLMapper and QTLNetwork softwares, respectively.

Studies involving multiple traits segregating in the same population might detect distinct QTLs mapped in the same chromosomal locations. A simultaneous analysis of quantitative trait data for multiple traits from a cross is necessary to detect possible pleiotropic actions in such chromosomal regions. Methods for analyzing several traits simultaneously using single markers were developed by Ronin *et al.* (1995) and with interval mapping by Korol *et al.* (1995) and Jiang and Zeng (1995). The software MultiQTL (http://www.multiqtl.com/) and program *JZmapQTL* of QTL Cartographer offer multiple trait analyses based on

the interval mapping methods. Hackett (2002) provides an overview of additional information on methods for epistatic and multiple trait QTL analysis.

The inheritance of quantitative traits in hexaploid wheat is highly intricate because of the large number of QTLs with a wide range of effects often distributed across the three genomes. In addition, low levels of marker polymorphism in wheat, especially in the D genome poses difficulties in constructing linkage maps with increased genome coverage and density in single mapping populations (Dubcovsky and Dvorak, 2007; Chao *et al*., 2009). However, the development of extensive genetic and genomic resources for wheat and related cereals in the recent years is rapidly transforming the analysis of complex traits in wheat. A general scheme of the use of these resources and the technologies has been represented in **Figure 1.5** and described in the following sections.

1.12 Recent advances in genetic and genomic tools for high-resolution QTL mapping and gene discovery in wheat

1.12.1 Genetic and germplasm resources

As a result of more than hundred years of continuous research, large assets of wheat genetic resources in the form of germplasm collections, genetic stocks and various mapping populations have been assembled. Because of the presence of homeologous chromosomes, wheat can tolerate the loss of segments, arms or whole chromosomes. Exploiting this phenomenon, aneuploid stocks in wheat were developed that included monosomic, nulli-tetrasomic and ditelosomic stocks, which helped in localizing different traits to specific chromosomal arms (Sears, 1954, 1966). The development of a set of more than 400 deletion stocks facilitated the localization of molecular markers to physical locations on the chromosomes (Endo and Gill, 1996; Sourdille *et al*., 2004; Goyal *et al*., 2005; http://www.k-state.edu/wgrc/Germplasm/Stocks/aneuploid.html). The GrainGenes database provides information about the collections of useful plant material at various institutions in the form of aneuploids, mutants, alien substitutions, additions, translocations, deficiencies, synthetic hexaploids and mapping populations (http://wheat.pw.usda.gov/GG2/).

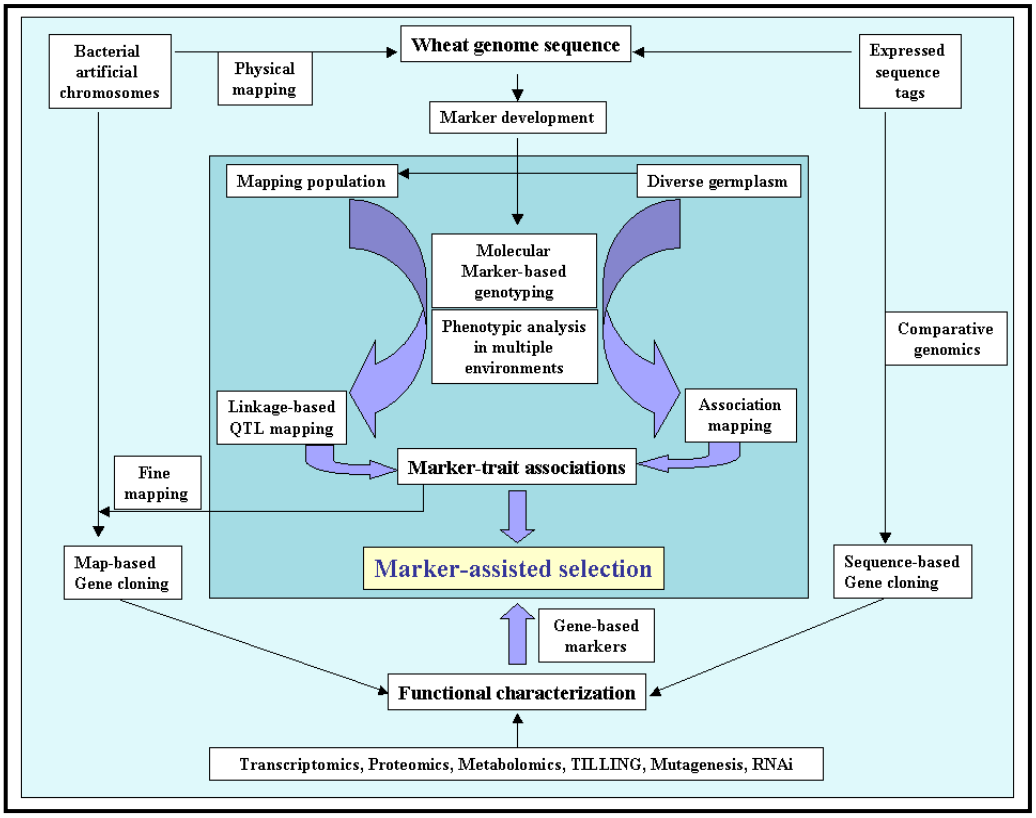


Figure 1.5: A general scheme of wheat resources and techniques to develop better varieties

Abundant wheat germplasm is the source of variability that creates opportunities and provides support for both identification of useful genes and breeding programmes. A core collection is a subset of a larger germplasm collection that contains, with a minimum of repetitiveness, the maximum possible genetic diversity of the species in question (Gouesnard *et al*., 2001). A worldwide core collection arrayed in 384-well plates is available for bread wheat (Balfourier *et al*., 2007). In addition to this, specific core collections with germplasm from important wheat producing regions are also being made available (Hao *et al*., 2008). As a tool to aid the identification of initial germplasm to screen in breeding programmes, a database called the Focused Identification of Germplasm Strategy (FIGS Bread Wheat Landrace Database - http://www.figstraitmine.com/) has been assembled that houses a virtual collection of nearly 16,000 bread wheat landraces held by three gene banks (Australian Winter Cereals Collection, Australia; ICARDA Genetic Resources Unit, Syria and the Vavilov Research Institute of

Plant Industry in Russia). All accessions have been geo-referenced. This website allows the user to efficiently interrogate the data associated with this collection and provides the capacity to identify custom subsets of accessions with single and multiple trait(s) that may be of importance to breeding programmes.

1.12.2 Genomic resources

The rapid advances in molecular biology in terms of techniques and their automation, next-generation sequencing technologies (Bräutigam and Gowik, 2010), development of statistical and bioinformatics tools has resulted in the fast-expanding field of wheat genomics (Walsh, 2009; Mochida and Shinozaki, 2010). Physical mapping, which is the ordering of genetic components on chromosome maps in terms of physical distances, is crucial for high quality sequence assembly during genome sequencing. The availability of deletion stocks (Endo and Gill, 1996), bacterial artificial chromosome (BAC) libraries (reviewed by Gupta P *et al*., 2008), techniques like fluorescence *in situ* hybridization (FISH) and chromosome flow sorting (Kato *et al*., 2005; Doležel *et al*., 2007) and radiation hybrid mapping (Paux *et al*., 2008) in wheat have facilitated intensive efforts in physical mapping of wheat chromosomes (Moolhuijzen *et al*., 2007).

A large number of marker types are being developed in view of covering the entire wheat genome for the construction of high-resolution genetic maps, discovery of marker-trait associations in biallelic/association mapping experiments, mapping and cloning genes for economically important traits, and for implementation of efficient breeding methods and rapid development of superior varieties. Among these, mapped SSRs are still a preferred marker type with more than 2,500 primer sequences available in public domain (GrainGenes database-http://wheat.pw.usda.gov/). Large-scale EST sequencing and mapping projects (Lazo *et al*., 2004; Qi *et al*., 2004) have lead to the development of EST-SSRs, sequence tagged sites (STSs) and transcript/functional maps to enrich marker availability for genome mapping (Eujayl *et al*., 2002; Nicot *et al*., 2004; Yu *et al*., 2004; La Rota *et al*., 2005; Varshney *et al*., 2004; Xue *et al*., 2008; http://wheat.pw.usda.gov/ITMI/2002/EST-SSR/). The use of EST-SSRs in QTL mapping may help relating genes of known function to phenotypic variation (Eujayl *et al*., 2002) and associated QTLs. Furthermore, deletion bin location

information on approximately 770 SSRs (Sourdille *et al*., 2004) and many DArT markers (http://www.cerealsdb.uk.net/dart/index.htm) are also available.

More recently, large-scale development of SNP markers has attracted the attention of wheat researchers worldwide (Akhunov *et al*., 2009; Bérard *et al*., 2009; Chao *et al*., 2009; Edwards *et al*., 2009). SNP markers are based on the variation of a specific nucleotide at a given sequence position between individuals. Predominantly, such variation occurs as biallelic alternative bases or insertion/deletions of individual or small numbers of nucleotides. They are the smallest unit of genetic variation and being the basis of most genetic variation between individuals, they occur in virtually unlimited numbers. SNPs in coding sequences may create changes in the amino acid sequence within a protein (if they are not silent) and might have an effect on protein function and eventually on the traits associated with the expression of such genes (Johnson *et al*., 2001; Ganal and Roder, 2007). Moreover, SNP markers are amenable for cost-efficient, high-throughput and multiplex-ready analysis techniques (http://wheatgenomics.plantpath.ksu.edu/IWSWG/Genotyping).

SNP markers are usually identified through the comparative sequencing of individual lines or varieties or the bioinformatics analysis of EST data generated from multiple lines (Rafalski, 2002a and 2002b). Based on EST information, large numbers of potentially genome-specific primers have been generated and tested for SNP identification by the wheat HapMap project involving multiple research groups (http://wheat.pw.usda.gov/SNP). As of May 2006, this database contains 17,174 primers, 1,102 wheat polymorphic loci, and 2,224 polymorphic sequence tagged sites in diploid ancestors of polyploid wheat (http://wheat.pw.usda.gov/SNP/new/index.shtml). The International wheat SNP working group (IWSWG) aims at rapid advances in SNP identification utilizing the next generation sequencing platforms and panels of diverse genotypes for SNP discovery based on sequencing cDNA libraries, physically mapped ESTs and targeted or whole genome re-sequencing (http://wheatgenomics.plantpath.ksu.edu/IWSWG/snp_projects). The results of this large-scale project are being amassed, for instance, Matthew Hayden (Australia) reports the identification of nearly 42,000 SNPs by targeted re-

sequencing efforts of 30 wheat genotypes (IWSWG, March 2011). SNP markers are already in use for identifying marker-trait associations (Zhang *et al*., 2008) and diversity analysis (Chao *et al*., 2009).

Single feature polymorphism (SFP) is a new microarray-based type of marker that is detected by hybridization of DNA or complementary RNA (cRNA) to oligonucleotide probes. In wheat, a high-density map of 877 SFPs with SSR anchors was developed based on Affymetrix arrays (Bernardo *et al*., 2009). Another marker system called insertion site-based polymorphism (ISBP) has been developed in hexaploid wheat using BAC-end sequences (Paux *et al*., 2006) that exploited information on the sequence flanking a transposable element to design one primer in the transposable element and the other in the flanking DNA sequence. Since transposable elements display unique insertion sites that are highly polymorphic, the resulting amplicon represents a putative locus/genome-specific molecular marker. The utility of ISBP markers in mapping (Paux *et al*., 2008) and suitability for MAS (Paux *et al*., 2010) has been demonstrated.

Similarly, various sequence-based marker systems are being developed in wheat that include the conserved orthologous set (COS) markers (Quraishi *et al*., 2009). Based on rice–wheat orthologs that can be useful in cross-genome map-based cloning of candidate genes underpinning quantitative traits, 695 COS markers were developed, which were assigned to 1,535 wheat loci. Similarly, Ishikawa *et al.* (2007) developed PCR-based landmark unique gene (PLUG) markers based on the orthologous gene conservation between rice and wheat, and on the intron polymorphisms among the three orthologous genes derived from the A, B and D genomes of wheat. Primer information on 960 PLUG markers, of which chromosomal location for 531 markers based on nullisomic- tetrasomic analysis and deletion bin locations of 154 markers are known (Ishikawa *et al*., 2009). PLUG markers can help distinguish BAC clones originating from A, B or D genomes that can be particularly useful in map-based cloning of QTLs.

Vast functional genomics resources that can be used for the analysis of gene function are available for wheat. The number of wheat EST sequences that are accessible in the public domain is 1,071,361 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html, dbEST release, 1

March, 2011). In addition, cDNA libraries prepared from various wheat developmental stages and stress conditions and their sequenced clones (Wilson *et al*., 2004; http://www.cerealsdb.uk.net/; Mochida *et al*., 2009; http://trifldb.psc.riken.jp/index.pl) as well as targeting induced local lesions in genomes (TILLING) methods and resources (Slade and Knauf, 2005) are present to explore gene function. Microarray-based gene expression analysis using platforms such as Affymetrix wheat gene chip (http://www.affymetrix.com) with >55,000 gene representatives and a ~10,000 feature unigene set arrayed on to glass slides based on sequences from 26,000 ESTs from 35 cDNA libraries relating to different stages of grain development and various stress treatments (http://www.cerealsdb.uk.net/) have been used for gene expression analysis (Wilson *et al*., 2005; Jordan *et al*., 2007; Wan *et al*., 2008). The quantitative expression of individual genes in developing seeds of a DH mapping population used for QTL mapping yielded >500 expression QTLs (eQTLs) across the wheat genome. The eQTL positions when aligned with existing phenotypic trait data identified genomic regions and candidate genes that control seed quality traits (Jordan *et al*., 2007). Furthermore, transgenic and RNA interference (RNAi), proteomics and metabolomics methods in wheat are standardized that can help in functional characterization of identified genes controlling traits of interest (Jones, 2005; Fu *et al*., 2007; Mochida and Shinozaki, 2010).

Genome information from related cereals can be utilized in an alternative approach of comparative genomics to generate markers for map saturation and identify and clone genes based on conserved DNA sequences in wheat (Salse and Feuillet, 2007; http://www.gramene.org/). Rice, barley and maize genome sequence have been used to obtain markers for high-resolution mapping, candidate gene identification and homology based cloning in wheat (Bagge *et al*., 2007; He *et al*., 2008; Su *et al*., 2011). The available genome sequences for rice, *Brachypodium*, sorghum and maize and the continued efforts in barley and wheat genome sequencing (http://www.ncbi.nlm.nih.gov/genomeprj?term=plant) promise to reveal information with comparative genomics on important genes that might help in breeding better crop varieties.

1.12.3 The ongoing efforts to sequence the hexaploid wheat genome

Genome sequencing can reveal the basic nucleotide sequence information that can help in understanding the molecular basis of phenotypic variation, which in turn can improve the exploitation of genetic diversity and accelerate development of new crop varieties. The mammoth task of sequencing the large and repetitive sequence-rich hexaploid wheat genome is underway. A wheat genome sequencing project by John Innes Centre, Liverpool University and Bristol University, United Kingdom, sequenced the wheat variety Chinese Spring using Roche 454 technology and released sequence raw data that represents 5X genome coverage. At this level of coverage, it is expected to have at least one read for > 95% of the genome. The draft assembly of the gene-rich regions of the genome and the raw sequence reads is available in the public domain (http://www.cerealsdb.uk.net/search_reads.htm).

A global effort in the form of The International Wheat Genome Sequence Consortium (IWGSC – http://www.wheatgenome.org/) is also in progress to sequence the wheat genome. In one of the initial stages, a bacterial artificial chromosome (BAC) - based integrated physical map of 995 Mb of the largest chromosome 3B was constructed. For this, a chromosome-specific BAC library was used to assemble 82% of the chromosome into 1036 contigs that were anchored with 1443 molecular markers, providing a major resource for genetic and genomic studies. This established a template for the route to construct physical maps and eventually sequence and assemble the information for all the 21 chromosomes in a meticulous effort to decode the wheat genome.

As of March 2010, the physical mapping status of 3AS was also reported as finished (Bread wheat-Physical mapping status-March 2010 - http://www.wheatgenome.org/General-Documents). Currently, sequencing of megabase level contigs of physically mapped chromosomes are underway (Feuillet and Eversole, 2007; Choulet *et al*., 2010). Recently, India has become a part of this genome project with the physical mapping of chromosome 2A being performed at the Punjab Agricultural University (PAU), Ludhiana. The genome sequence of wheat eventually will be a rich source of sequence-based molecular markers especially in regions of interest associated with specific traits. It can also

accelerate gene discovery, provide extensive information for efforts like analysis of structure-function relationships of proteins, study of gene distribution, genome rearrangements by duplications, translocations and transposable elements, comparative genomics and application of information near selected markers for prebreeding efforts to minimize linkage drag during MAS (Varshney *et al*., 2009a; Edwards and Batley, 2010).

1.13 Marker assisted selection in wheat

The development of molecular marker systems for crop plants and identification of genetic linkage or association between markers and traits and the use of such information for selection and fixation of superior plant phenotypes in the development of improved cultivars gave rise to a new set of tools in plant breeding –marker assisted selection (MAS) (Semagn *et al*., 2006d; Collard and MacKill, 2008). If there is a strong association between marker and the QTL (or gene) of interest controlling the desired phenotype, then selection (directly) can be focused on the markers in order to (indirectly) increase the frequencies of linked QTL alleles of interest. Compared to classical (phenotypic) selection, MAS should prove to be i) easier when traits are difficult to record; ii) faster when phenotyping involves long-duration testing, can permit selection at an early stage of development and in off-season nurseries for the desired trait; iii) cheaper if the cost of genotyping is lower than the cost of phenotyping as in case of traits like abiotic or biotic stress and finally iv) more efficient unless parameters like genetic background or genotype×environment interactions influence marker-trait associations (Hospital, 2009). For the use of a particular marker or marker type in MAS, i) its linkage with the target locus should be reliable; ii) quantity and quality of DNA required for the assay should not be high; iii) the technical procedure for marker assay should be easy and amenable to high throughput and multiplexing; iv) be highly polymorphic in breeding material to discriminate between different genotypes and finally v) its use in screening breeding material should be cost-effective (Collard and MacKill, 2008). Markers are used at various levels in breeding procedures (**Figure 1.6**). These include evaluation of breeding material for purity, genetic diversity or parental selection; marker assisted

backcrossing procedures involving foreground and background selection, QTL or gene pyramiding, early generation selection and strategic combinations of phenotypic selection and MAS (Collard and MacKill, 2008; Hospital, 2009; Varshney *et al*., 2009b).

Varietal development in wheat is being benefited by MAS in recent years. Many research organizations and private companies in several countries and collaborative efforts such as MASWheat and HarvestPlus (http://maswheat.ucdavis.edu/index.htm; http://www.harvestplus.org/) and Consultative Group on International Agricultural Research (CGIAR) are involved in MAS for favorable traits in wheat. A large number of disease resistance traits such as various rusts, *Fusarium* head blight, barley yellow dwarf virus, nematodes, Hessian fly and Russian wheat aphid and quality traits like grain protein content, tolerance to pre-harvest sprouting, bread making quality and gluten strength have been targeted for wheat improvement through MAS (reviewed by Gupta *et al*., 2010). Different procedures involved in MAS along with examples of specific traits selected through them are illustrated in **Figure 1.6**. Various wheat varieties have been developed and released or many existing varieties have been improved using marker-assisted introgression of desired gene(s) (Gupta *et al*., 2010). For instance, BIOINTA 2004, a hard red winter wheat, was selected and released for its excellent grain yield potential, resistance to leaf rust (caused by *Puccinia triticina* Eriks.) conferred mainly by the *Lr47* gene selected by MAS, and its good bread-making quality (Bainotti *et al*., 2009).

A compilation of various efforts in MAS in wheat and also in other crops is available at the website of Generation Challenge Programme (GCP) molecular marker toolkit (http://s2.generationcp.org/gcp-tmm/web/index.php/). Compared to other marker systems, SSR markers have been used more often in a majority of the MAS efforts reported (Gupta *et al*., 2010; GCP molecular marker toolkit - http://s2.generationcp.org/gcp-tmm/web/index.php/). Many traits such as tolerance to drought, heat, salinity, water-logging and metal toxicity that might help enhancing wheat production and quality parameters like enhanced protein content, micronutrients, and end-product quality will be the targets of MAS in the coming years.

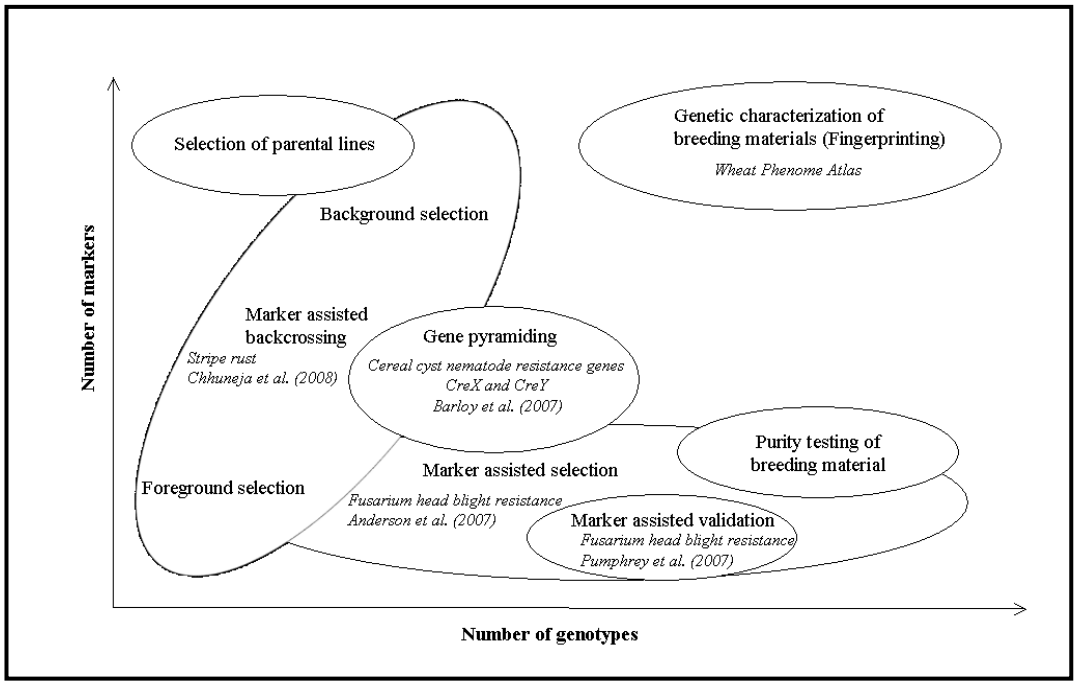


Figure 1.6: Marker-based selection procedures. The requirements of numbers of markers and genotypes for various applications are illustrated. Specific examples from wheat are also shown. [Modified from Bagge and Lübberstedt (2008)].

Presently, some of the obstacles in MAS include availability of a large supply of reliable markers, effects of genetic background and genotype×environment interactions. The wheat genome sequencing project and various large-scale QTL mapping efforts will ensure enhanced availability of markers for future MAS projects (Edwards and Bately, 2010). Furthermore, a massive resource of phenotype and marker information in wheat is being assembled for the establishment of a Wheat Phenome Atlas (http://apps.cimmyt.org/english/docs/brochure/whtPhenomeAtlas.pdf). This will include 17 million phenotypic data points for 80 traits from 10,000 international field trials collected during 40 years. These data will be compiled for approximately 13,000 wheat lines with known pedigree data. Genotyping of these lines will be achieved using 26 million DArT marker data points and will be integrated with the phenotypic data. The Wheat Phenome Atlas will be useful for molecular wheat breeding by facilitating identification of genetic markers for a range of agronomic traits. Previously, it was assumed that most markers associated with QTLs from preliminary mapping studies could be directly useful

in MAS. However, in recent years, three aspects have been accepted to avoid the failure of MAS by the influence of the genetic background – i) QTL confirmation and verification of its position in multiple populations; ii) QTL validation by surveying the favorable allele in germplasm collections with variation for the phenotype and iii) evaluation of epistatic interactions of the selected loci (Bernardo, 2008; Collard and MacKill, 2008; Varshney *et al*., 2009b). In many cases, the effects of QTLs involved in MAS might become inconsistent because of genotype×environment interaction (Kang, 2002). The extent of such interactions is often unknown because the QTL mapping studies have been limited to only a few years or locations. Therefore, to develop broadly adaptable varieties, breeding strategies based on QTL evaluation in many environments is necessary to realize the potential of MAS (Liu *et al*., 2004; Collard and MacKill, 2008).

Thus, considering the potential of the molecular marker technology for wheat improvement, the collective efforts of researchers committed to plant molecular biology, genetics and crop improvement can further expand their contribution through molecular breeding and help meet the global needs for sustainable increases in the productivity of high quality wheat.

Genesis of my thesis

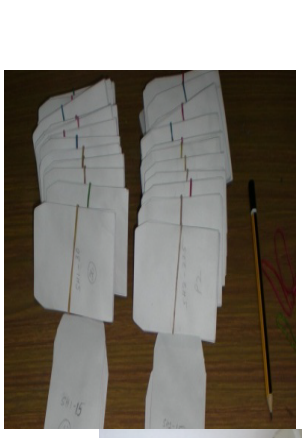
Wheat is the backbone of food security not only in India but also for billions of people around the world. It is one of the main sources of energy in the Indian diet and has been under cultivation in the Indian subcontinent from pre-historic times. India is self-sufficient in wheat production, but further increase in output can help India take part in the global export market. For food processing industries in both domestic and global scenario, wheat with desired quality attributes and high milling yield is desired. In view of improving the quality of Indian wheat, research work on mapping of wheat quality traits was initiated at Plant Molecular Biology group of National Chemical Laboratory, Pune, India in collaboration with various other wheat breeding research organizations. Since it was my aspiration to carry out research in plant molecular biology and crop biotechnology, which would eventually be useful to Indian farmers, I decided to join this program for my PhD work. I focused my work on wheat kernel size and shape traits, which are

associated with its market price, milling yield and quality with an aim to map genomic regions controlling them. Targeted selection using molecular markers closely linked to kernel morphometric trait QTLs during plant breeding can help develop wheat with improved kernel size and shape. In view of this, genetic dissection of wheat kernel size and shape was performed with focus on five quantitative traits, namely, thousand kernel weight (TKW), kernel length (KL), kernel width (KW), kernel length-width ratio (LWR) and factor form density (FFD). These traits were studied using a hexaploid wheat mapping population in diverse locations for multiple years to analyze genetic and environmental influence on them. The objectives of my research project were as follows -

Objectives

- Construction of a molecular marker linkage map of hexaploid wheat using the recombinant inbred line (RIL) population from the cross Rye Selection 111 (RS) × Chinese Spring (CS)
- Phenotypic evaluation of the parents RS, CS and the RIL population for multiple years at Ludhiana (Punjab) and Pune (Maharashtra), that represent two diverse agro-climatic and wheat growing zones in India
- Analysis of phenotypic correlations among the traits and evaluation of sources of variation
- QTL analysis of kernel characters employing the linkage map and phenotypic data.

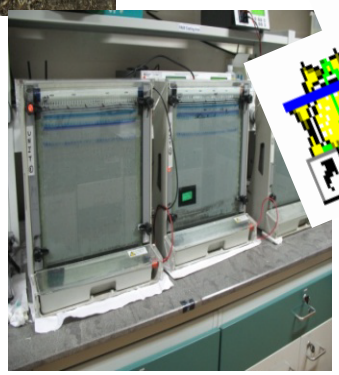
MATERIALS AND METHODS











Materials and Methods

2.1 Phenotypic evaluation

2.1.1 Plant material and mapping population development

The recombinant inbred line (RIL) mapping population used in this study was derived from a cross between the hexaploid wheat genotypes Rye Selection 111 (RS) × Chinese Spring (CS). RS has larger sized kernels than CS (**Figure 1.4**). The cross and subsequent advancement of F_2 generation plants by single seed descent method to the F_6 generation were performed at Punjab Agricultural University (PAU), Ludhiana, Punjab, India. The mapping population thus derived consisted of 185 RILs and was used for phenotypic evaluation and molecular marker analysis. The parents and the population were grown in randomized block design with two replications in 1 m rows planted with 10 seeds each and separated by gaps of 23 cm. Fertilizer was applied in a ratio of 120 kg Nitrogen: 60 kg Phosphorus: 40 kg Potassium per ha in field experiments. The experiments were conducted during the wheat-growing Rabi season (November to April) under irrigated conditions using standard agricultural practices.

2.1.2 Phenotypic evaluation of the parents and RIL population

Phenotypic evaluation of RS, CS and RILs was performed at Ludhiana, Punjab and Pune, Maharashtra that represent two diverse agro-climatic and wheat growing zones in India. According to the Planning Commission, Government of India [http://planningcommission.gov.in/sectors/agricul.html], the country is broadly divided into fifteen regions based on agro-climatic features, particularly soil type, variation in climate and rainfall and water resources availability. Considering this, Ludhiana is located in the Trans-Gangetic Plains region and Pune in the Western Plateau and Hills region (**Table 2.1**, **Figures 2.1** and **2.2**). Similarly, the wheat growing regions of India are divided into six zones based on agro-climatic conditions, disease spectrum and soil status (Mishra *et al*., 2007). When the wheat-growing zones are taken into account, Ludhiana is located in the North-Western Plains zone and Pune in the Peninsular zone (**Figure 2.1**). For the RS×CS population, field trials were conducted at PAU, Ludhiana and at the agricultural research station of Agharkar Research Institute (ARI) at Hol, Pune. At

Ludhiana, field trials were conducted during the years 1999-2000, 2002-03, 2004-05 and 2006-07 and at Pune during 2004-05, 2005-06, 2006-07 and 2008-09. The harvests from parental lines and RILs were brought to NCL, Pune for kernel trait evaluation and molecular analysis. Phenotypic evaluation of RILs was performed starting with F_6 generation. Thousand kernel weight (TKW) was recorded for all the year-location trials, while for kernel length (KL), kernel width (KW), length-width ratio (LWR) and factor form density (FFD), phenotypic analysis was performed for five year-location combinations (2002-03 and 2004-05 at Ludhiana and 2004-05, 2005-06 and 2006-07 at Pune).

For RS, CS and RILs in both the replications, weight of 250 grains was recorded using an electronic balance (Contech, India) and multiplied by 4 to obtain TKW values. KL and KW were recorded using Vernier calipers for twenty random kernels one at a time from each RIL and parents and the average value was used for further analysis. Using TKW, KL and KW, kernel LWR that represents the overall kernel shape and FFD that describes the differences in grain density and the deviation of a shape from a cylindrical form were calculated. LWR was calculated as KL / KW (Redoña and Mackill, 1998; Gegas *et al*., 2010). FFD was given by TKW / (KL * KW) as described by Giura and Saulescu (1996). For all the traits, average values of the two replications were used for statistical analyses and QTL mapping. Each year-location combination was considered as one environment and each environment was designated by its trait, year and location information. For example, TKW2003Lud indicated TKW assessed for the harvest of the season 2002-03 at Ludhiana.

2.2 Molecular analysis

2.2.1 Chemicals, enzymes and oligonucleotides

Molecular biologicals such as DNA molecular weight markers, λ-bacteriophage DNA and Taq DNA polymerase were obtained from Bangalore Genei (India). MetaPhor® agarose was from Cambrex Bioproducts (USA). Some of the essential chemicals used in DNA extraction, PCR and PAGE such as Cetyltrimethylammonium bromide (CTAB), formamide, bind and repel silanes were procured from Sigma (USA) and Fluka (Germany). Other chemicals used in electrophoresis like agarose, acrylamide, N, N'-methylene bisacrylamide, etc.

Table 2.1: Geographical location and important features of Ludhiana and Pune

Feature	Ludhiana	Pune
Location	Plains of North-West	Deccan Plateau
Agro-climatic region	Trans-Gangetic Plains	Western Plateau and Hills
Wheat growing zone	North-Western Plains Zone	Peninsular Zone
Latitude	30º 52' N	18º 32' N
Longitude	75º 56' E	73º 51' E
Altitude (m)	255	559
Soil type	Sandy, clayey loam	Light to medium black

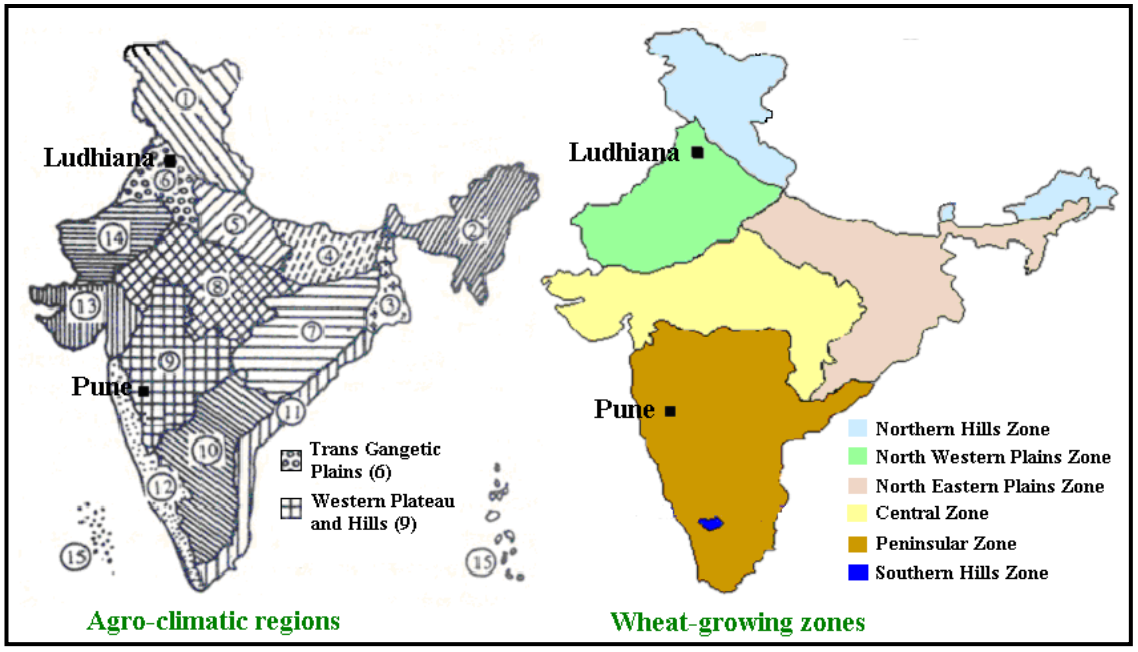


Figure 2.1: Geographical locations of Ludhiana and Pune in India (http://planningcommission.gov.in; http://www.dwr.in/)

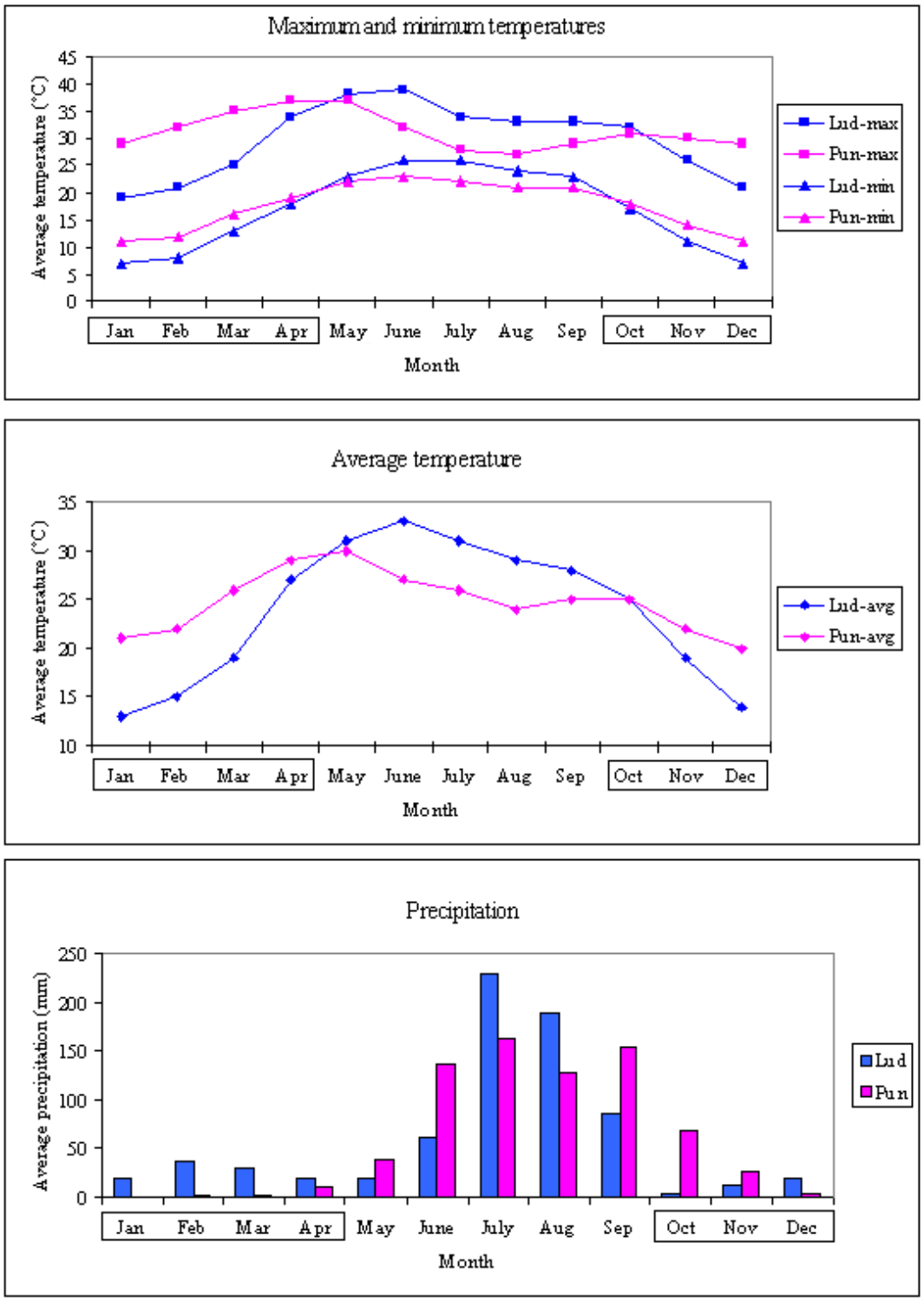


Figure 2.2: Temperature and rainfall variation at Ludhiana and Pune. The months from October to April fall in the wheat-growing Rabi season in India. [Data obtained from http://www.weather.com]

were obtained from Sisco Research Laboratories (SRL, India). Commonly used chemicals required for laboratory reagents like inorganic acids and salts, Tris base, etc. were of high quality (analytical grade) and supplied by Qualigens (India) and SRL. SSR oligonucleotide primers were custom synthesized from Sigma Genosys (India). Aliquots of *psp* primers were obtained from John Innes center, UK.

2.2.2 Genomic DNA extraction

Leaf tissue from fifteen-day old seedlings of RS, CS and RILs of F_9 generation grown in glass house conditions was harvested, instantly frozen in liquid nitrogen and stored at -80°C till further use. Reagents were prepared by CTAB method of Stein *et al*. (2001). The frozen leaf tissue (1 g) was ground to a fine powder in liquid nitrogen using mortar and pestle and transferred to centrifuge tubes. It was mixed with 10 ml of 1× CTAB buffer (details in Section 2.2.8) and the resulting emulsion was incubated at 65°C for 1 hour in a water bath. After incubation, the emulsion was allowed to cool down to room temperature and an equal volume of Chloroform-Isoamyl alcohol (24:1) mixture was added. After thorough mixing of the contents, the tubes were centrifuged at 10,000 rpm (~12,000 *g*, Sorvall RC 5B centrifuge with SS34 rotor) for 10 min at room temperature. The aqueous layer formed after centrifugation was transferred to fresh tubes. Further, 1 µl RNase A (1,000 U/ml) per 100 µl of the aqueous layer was added and incubated at 37°C for 15 min. For DNA precipitation, an equal volume of ice-cold isopropanol was added and mixed gently. The precipitated DNA was either spooled out or the pellets were recovered by centrifugation at 10,000 rpm for 5 min at 4°C. The DNA spool or the pellet was washed with 1 ml of DNA wash solution-1 (Section 2.2.8) by incubation for 5 min at room temperature followed by centrifugation at 10,000 rpm for 1 min at 4°C. The supernatant was removed and washing was repeated with DNA wash solution-2 (Section 2.2.8). The DNA pellets were air dried and dissolved in Tris-EDTA (TE) buffer (Sambrook and Russell, 2001) and stored at –20°C until further use.

2.2.3 DNA quantification

Undiluted genomic DNA was loaded on 0.8% agarose gel in 0.5× Tris-acetate EDTA (TAE) (Sambrook and Russell, 2001) containing ethidium bromide (EtBr) to check for quality. It was diluted suitably (1/100 or 1/200) and 3-5 µl of each was loaded on 0.8% agarose gel containing EtBr in 0.5× TAE along with known concentrations (50, 100, 200 and 300 ng) of λ bacteriophage DNA. The DNA concentration of the sample was estimated by comparison of the fluorescence intensity of the sample with those of λ bacteriophage DNA. Purity and concentration of the extracted DNA for each sample was further checked using a spectrophotometer at 230-300 nm. The DNA quality was determined by calculating the ratio A260/A280 nm and it was ensured that the ratio ranged between 1.7 and 2.0.

2.2.4 SSR analysis

Microsatellite polymorphism was surveyed using parental DNA with 826 primer pairs. They were mainly public domain SSR primers with the nomenclature *gwm*, *gdm*, *barc*, *cfa*, *cfd*, *ksum*, *psp*, *DuPw* and *wmc* (Röder *et al*., 1998; Stephenson *et al*., 1998; Pestosova *et al*., 2000; Eujayl *et al*., 2002; Guyomarc'h *et al*., 2002; Song *et al*., 2002; Sourdille *et al*., 2003; Yu *et al*., 2004 and GrainGenes database website http://wheat.pw.usda.gov/ggpages/SSR/WMC/). In addition to these, twenty-six primer pairs obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), and TraitGenetics, Gatersleben, Germany were also used. PCR was performed in 15 µl reaction volume with 1× buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.6U Taq DNA polymerase, 0.5 µM each of forward and reverse primers and 40-50 ng genomic DNA. The reaction conditions were as follows – initial denaturation of 94°C for 5 min, 35 cycles of 94ºC for 1 min, annealing temperature (dependent on the primer pair) for 1 min, extention at 72ºC for 2 min and final extention for 5 min at 72ºC.

2.2.5 Resolution of PCR products

For the assessment of parental polymorphism, the PCR products were initially resolved separately using agarose gel electrophoresis with 3% MetaPhor® agarose (Cambrex Bioproducts, USA) and with 6% denaturing polyacrylamide gel

electrophoresis. Polymorphic primers with RS and CS SSR alleles showing unambiguous difference in molecular weight were separated from the primers that were monomorphic or yielded multiple bands. Further, polymorphic primers that revealed SSR alleles with band size difference large enough to be differentiated with the resolution of MetaPhor® agarose were distinguished from those that could only be revealed by PAGE. The annealing temperature during PCR and the duration of electrophoresis were optimized for each primer pair to ensure minimum number of bands and distinct separation of bands, respectively.

2.2.6 Agarose gel electrophoresis

Agarose gels with superior resolution were prepared by suspending 3% (w/v) of MetaPhor® agarose in 0.5× Tris-borate EDTA (TBE) buffer (Sambrook and Russell, 2001). The mixture was boiled in microwave oven till a homogeneous suspension was formed. It was cooled to 40-50ºC and 1 µl of EtBr (10 mg/ml) per 100 ml of gel was added and mixed gently. The gel was cast on trays fitted with 30 well combs and allowed to solidify at room temperature. The samples were prepared by mixing 15 µl amplified PCR products with 4 µl Bromophenol blue dye solution (Sambrook and Russell, 2001) and loaded on the gel immersed in 0.5× TBE buffer along with *PhiX174*/*Hae*III molecular weight marker. Electrophoresis was carried at 150 V, 70 mA for 1.5-3 hours. Visualization of the amplified products and gel documentation were done under UV light using ImageMaster VDS gel documentation system (Amersham Pharmacia Biotech, USA).

2.2.7 Polyacrylamide gel electrophoresis

The PCR products of polymorphic SSR primers that could not be resolved on Metaphor® agarose gels were separated on polyacrylamide gels using the sequencing gel units from Life Technologies (USA). The gel assembly consisted of a bind plate (42×33cm) to which the polyacrylamide gel of 0.4 mm thickness adhered and a repel plate (40×33cm) that helped in separating the bound gel from the assembly. Both the plates were washed with detergent, rinsed with water and dried thoroughly. Traces of water were removed by wiping with absorbent paper dipped in ethanol. A 3% solution of 3-(Trimethoxysilyl) propylmethacrylate (bind

silane) in chloroform was applied to the bind plate to encourage the binding of polyacrylamide. The repel plate was treated with 10% solution of dichlorodimethyl silane (repel silane) in chloroform to allow complete release of the gel from this plate. The two plates were assembled using spacers, metal clamps and a rubber gasket to create a mould for casting the gel. The gel solution was prepared by mixing 65 ml of the urea-acrylamide mix (Section 2.2.8) with 1 ml of freshly prepared 10% APS and 25 µl of TEMED. The gel solution was poured into the assembled mould using a pointed beaker. Shark teeth combs were inserted in reverse orientation to create a uniform gel-front and secured with metal clamps. The gel was allowed to polymerize for 60 min before subjecting to electrophoresis.

The gel plate assembly was separated from the rubber gasket, the inverted shark-teeth combs were removed carefully and the unit was placed and secured in the gel-running assembly. The sequencing gel was prerun at 60 W (approximately 40 mA; 1500 V) using 0.5× TBE for 30 min. The gel front was cleaned to remove urea and polyacrylamide gel debris and the combs were inserted back such that closely spaced wells formed when the shark-teeth pierced the gel-front. The PCR products were mixed with 6 µl of PAGE gel loading dye (Section 2.2.8) and denatured for 5 min at 94°C using a dry heating block and immediately cooled on ice. Six microlitres of each sample was loaded in the wells along with 20 bp Ladder as DNA molecular weight marker. Electrophoresis was performed at 60 W for 1.5-2.5 hours as optimized for each polymorphic primer.

The glass plate assembly was removed from the electrophoresis assembly and cooled to room temperature. The bind and repel plates were separated and the bind plate with the adhered gel was subjected to silver staining as described by Sanguinetti *et al*. (1994). The gel was immersed for 10 min in a solution of fixer (Section 2.2.8) and transferred to the silver stain solution (Section 2.2.8). After incubating for 20 min, the gel was placed in a tray containing a solution of developer (Section 2.2.8) and agitated gently to reveal silver-impregnated dark bands. The gel plates were drained and treated with stop solution (Section 2.2.8) to end the staining process. The gel was thoroughly washed with water and air-

dried. The results were documented either by a digital computer scanner or by photography using a digital camera.

2.2.8 Composition of reagents used

Genomic DNA extraction

1× CTAB DNA extraction buffer	*DNA Wash solution-1*	*DNA Wash solution-2*
2% (w/v) CTAB 200 mM Tris-HCl, pH 8.0 20 mM EDTA, pH 8.0 1.4 M Sodium chloride 1% (w/v) polyvinylpyrrolidone-K30 1% (v/v) β-mercaptoethanol	76% Ethanol 200 mM Sodium acetate	76% Ethanol 10 mM Ammonium acetate

Polyacrylamide gel electrophoresis

40% Acrylamide gel mix	*Urea-Acrylamide mix*	*PAGE loading dye*
29:1 ratio of Acrylamide and N, N'-Methylene-bisacrylamide	6% Acrylamide gel mix 0.5 × TBE 7.5 M urea	0.1% (w/v) Xylene cyanol 0.1% (w/v) Bromophenol blue 90% (v/v) Formamide 2% (v/v) 0.5 M EDTA, pH 8.0

Visualization of PCR products on PAGE gels

Fixer	*Silver stain*	*Developer*	*Stop solution*
10% Ethanol 0.5 % Acetic acid	0.2% Silver nitrate	1.5% Sodium hydroxide 0.25% Formaldehyde	10% Acetic acid

2.3 Construction of linkage map

2.3.1 Scoring of marker data

The SSR allele sizes in RS and CS were recorded and the band pattern in the RILs was compared with that of the parents. The genotype of the RILs with allele size comparable to RS was scored as 'a' and that of CS as 'b'. Some of the RILs that indicated both the parental alleles were scored as 'h'. RILs showing ambiguous patterns that were neither similar to RS nor CS were scored as missing data. Segregation of the SSR marker alleles in the RIL population was assessed using the $\chi 2$ test for goodness-of-fit to the expected 1:1 ratio for 'a' and 'b' alleles by the software JoinMap v. 4.0 (Van Ooijen, 2006).

2.3.2 Linkage analysis and map construction

Linkage analysis was performed using JoinMap v. 4.0 on the basis of a regression mapping procedure with a weighted least-squares method that sequentially adds markers into the map (Stam, 1993). The markers were initially grouped with LOD thresholds from 3.0 to 5.0 and after inspecting the grouping results; the final grouping was done using a minimum conservative LOD threshold of 4.0. During grouping, marker linkages with recombination frequency <0.45 were used. The "jump in goodness-of-fit" threshold for locus removal was set to 5.0; the "ripple" command was used each time after adding a locus to a linkage group and three "mapping rounds" were performed for each linkage group. The Kosambi mapping function (Kosambi, 1944) was used to calculate map distances. Wherever possible, some of the unlinked markers and smaller linkage groups were shifted to other groups based on published map information using the 'Move Selected Loci' function from the 'Grouping' menu. In some cases, the 'fixed order' function of JoinMap was used to produce the map of each linkage group on the basis of the marker order information in previously published SSR maps. MapChart ver 2.0 (Voorrips, 2002) was used for graphical representation of the linkage map.

2.4 Statistical analysis

The phenotypic data were subjected to statistical analysis using Microsoft Excel 2000 and IRRISTAT v. 5.0 (International Rice Research Institute, Manila,

Philippines). Trait statistics and Pearson correlation coefficients were assessed using Microsoft Excel 2000 while, Analysis of variance (ANOVA) and Additive main effects and multiplicative interaction (AMMI) procedures were performed using IRRISTAT.

2.4.1 Correlation among the traits

The Pearson correlation coefficient '*r*' between pairs of traits was calculated using data from the years in which all the five kernel traits were recorded and also for the average trait value across those environments for each trait. The statistical significance of Pearson correlation coefficients was calculated using an online calculator available at http://www.danielsoper.com/statcalc/calc44.aspx.

Spearman rank correlation coefficients 'ρ' and their levels of significance were calculated between year-location combinations for each kernel character. An online calculator (Wessa, 2009) was used for this purpose available at the website http://www.wessa.net/rwasp_spearman.wasp/.

2.4.2 AMMI and biplot analysis

The phenotypic data from all the environments for each trait were subjected to the AMMI procedure (Gauch, 1988; Gauch and Zobel, 1989; Zobel *et al*., 1988) through the "Cross site analysis" module of IRRISTAT. The AMMI model integrates the analysis of variance (ANOVA) for the additive effects with the principal component analysis (PCA) for the multiplicative effects. In this method, the additive main effects of the genotype (G) and environment (E) are first accounted for by ANOVA and further, PCA is applied to the interaction component to extract a new set of co-ordinate axes that summarize the genotype × environment (G×E) interaction patterns. The AMMI model as shown below, indicates the number of significant interaction principal component axes (IPCA) that can represent a large proportion of the variance, which is unexplained by the ANOVA. The contributions of G and E main effects and G×E effects to total variance and the contribution of individual IPCA to G×E effects were calculated using the total sum of squares and individual sums of squares of G, E, G×E and significant IPCA.

The patterns in G×E interaction component were further subjected to Biplot analysis (Gabriel, 1971; Yan and Tinker, 2006) using IRRISTAT. Biplots, a type of exploratory graph in the form of a two-variable scatter plot, were used to graphically represent the extent of participation of both RILs and environments in G×E interaction. The first two significant IPCA axes derived for both RILs and environments were used for constructing biplots in which, the RILs were displayed as points while environments were displayed as vectors.

AMMI model: $Y_{ij} = \mu + g_i + e_j + {}^{h}\sum_{n=1} \lambda_n \alpha_{ni} \gamma_{nj} + R_{ij}$

Y_{ij}: value of the i^{th} genotype in the j^{th} environment

μ: grand mean

g_i: mean of the i^{th} genotype minus grand mean

e_j: mean of the j^{th} environment minus the grand mean

λ_n: singular value for the principal component analysis axis n

α_{ni}: principal component score for PCA axis n of the i^{th} genotype

γ_{nj}: principal component score for PCA axis n of the j^{th} environment

R_{ij}: residual

h: number of IPCA retained in the AMMI model

2.4.3 Heritability analysis

Heritability was estimated using the components of ANOVA by the formula $h^2 = 1-[M_2/M_1]$ where M_1 and M_2 are the mean squares of genotype and genotype × environment, respectively (Huang *et al*., 2006).

2.5 QTL mapping

QTL analysis was carried out using two softwares, namely, QTL Cartographer v. 2.5 (Wang S *et al*., 2007) and QTLNetwork v. 2.0 (Yang *et al*., 2007). QTL Cartographer performs composite interval mapping (Zeng, 1994) based on a linear model without taking the interactions among QTLs and QTLs with the environment into consideration. Whereas QTLNetwork, which is based on a mixed-linear model, can detect QTLs with significant individual effects, QTL×QTL and QTL×environment interactions.

2.5.1 Composite interval mapping

The positions and effects of QTLs were detected by composite interval mapping using the data from all the environments. Also, the average data across year-location combinations were considered as an added environment for each trait. Model 6 of the CIM was implemented for scanning intervals of 2 cM for putative QTLs. In this model, CIM fits parameters for a target QTL in one interval by simultaneously fitting partial regression coefficients for background markers to account for variance caused by non-target QTLs. For Model 6, five markers were set as the number of cofactors to control the effects of genetic background and a window size of 10 cM was used for forward stepwise regression and backward elimination of markers. QTLs with LOD value ≥ threshold LOD, as determined by 1000 permutation tests at $P \leq 0.05$ (Churchill and Doerge, 1994; Doerge and Churchill, 1996) were declared as significant and those with LOD ≥ 2.0 but less than the threshold LOD were considered as putative QTLs. The proportion of observed phenotypic variance explained by a QTL was estimated as the coefficient of determination (R^2). Following the common practice in QTL mapping studies (Sourdille *et al*., 2003; Huang *et al*., 2006), the signs of the additive effects of the QTLs were used to identify the parental origin of the favourable alleles, i.e., positive and negative signs indicated that the better parent and poor parent contributed towards higher trait values for all traits evaluated, respectively.

The QTLs for a trait with identical, overlapping or adjacent marker intervals in a linkage group were treated as the same and given a common name

according to the Catalog of gene symbols for wheat (McIntosh *et al*., 2003). The QTLs identified using CIM for various traits were grouped based on the linkage group positions and QTL maps were drawn using MapChart ver. 2.0 (Voorrips, 2002). If the support intervals of QTLs for different traits overlapped in a chromosomal region, those QTLs were considered to represent a QTL cluster. Such chromosomal regions may suggest the presence of either cosegregating QTLs for distinct traits or pleiotropic regions influencing multiple traits. The test of pleiotropy versus close linkage based on the statistical method of Jiang and Zeng (1995) was conducted by Multi-trait composite interval mapping (Mt-CIM) using QTL Cartographer.

2.5.2 Detection of QTL interactions

QTL analysis was conducted using QTLNetwork v. 2.0 with phenotypic data from all year-location combinations for each kernel trait in the framework of mixed linear model (Wang *et al*., 1999; Yang *et al*., 2007, 2008). It attempts to model the genetic architecture of a complex trait by proposing a full-QTL model and integrating the effects of main-effect QTLs that have significant individual effects on the trait, epistatic interactions, QTL × Environment interactions and environmental influence on the epistatically interacting QTLs into one mapping system. In addition to this, it graphically represents the genetic architecture and interactions. In this method, marker interval analysis is first performed to select candidate marker intervals that might be linked with QTL(s). These selected marker intervals are subsequently used as cofactors in a one-dimensional (1D) genome scan for putative QTLs (**Figure 2.3a**). Next, a two-dimensional (2D) genome scan is performed to search for epistasis based on the previously detected QTLs and candidate marker interval interactions (**Figure 2.3b**). During the analyses, *F*-statistic is computed and the critical *F*-value is estimated with the help of a permutation test (Doerge and Churchill, 1996) to control genome-wide false positive rate. In this study, during 1D genome scans, a walk speed of 1 cM was employed, which is the space between two adjacent putative QTL test points along the genome. The testing window size was 10 cM to define a window adjacent to the testing interval in each direction where no marker will be selected as cofactor during genome scans. Similarly, a window of 10 cM was set as filtration window to distinguish whether two adjacent test statistic peaks represent

two distinct QTLs or not. Critical *F* value was calculated by 1000-permutation test with $P \leq 0.05$ as experimental-wise significance level for candidate interval selection, putative QTL detection and determination of QTL effects.

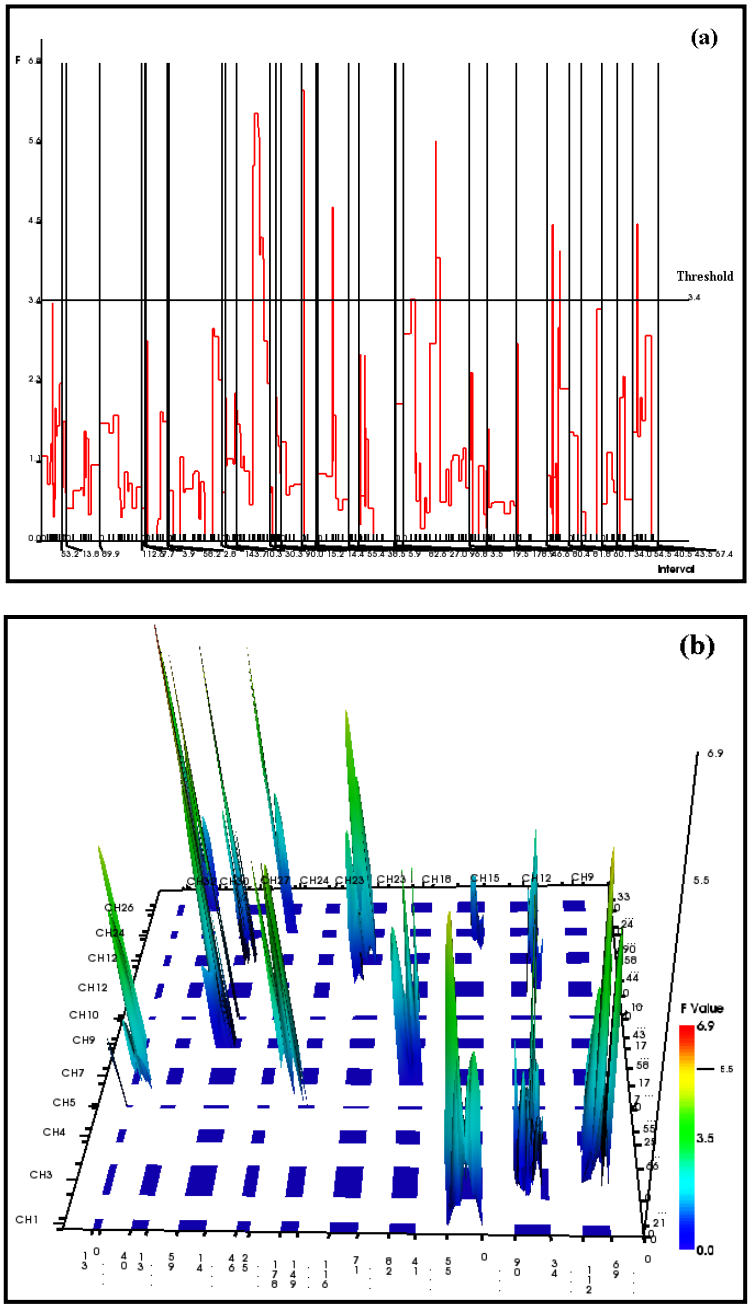
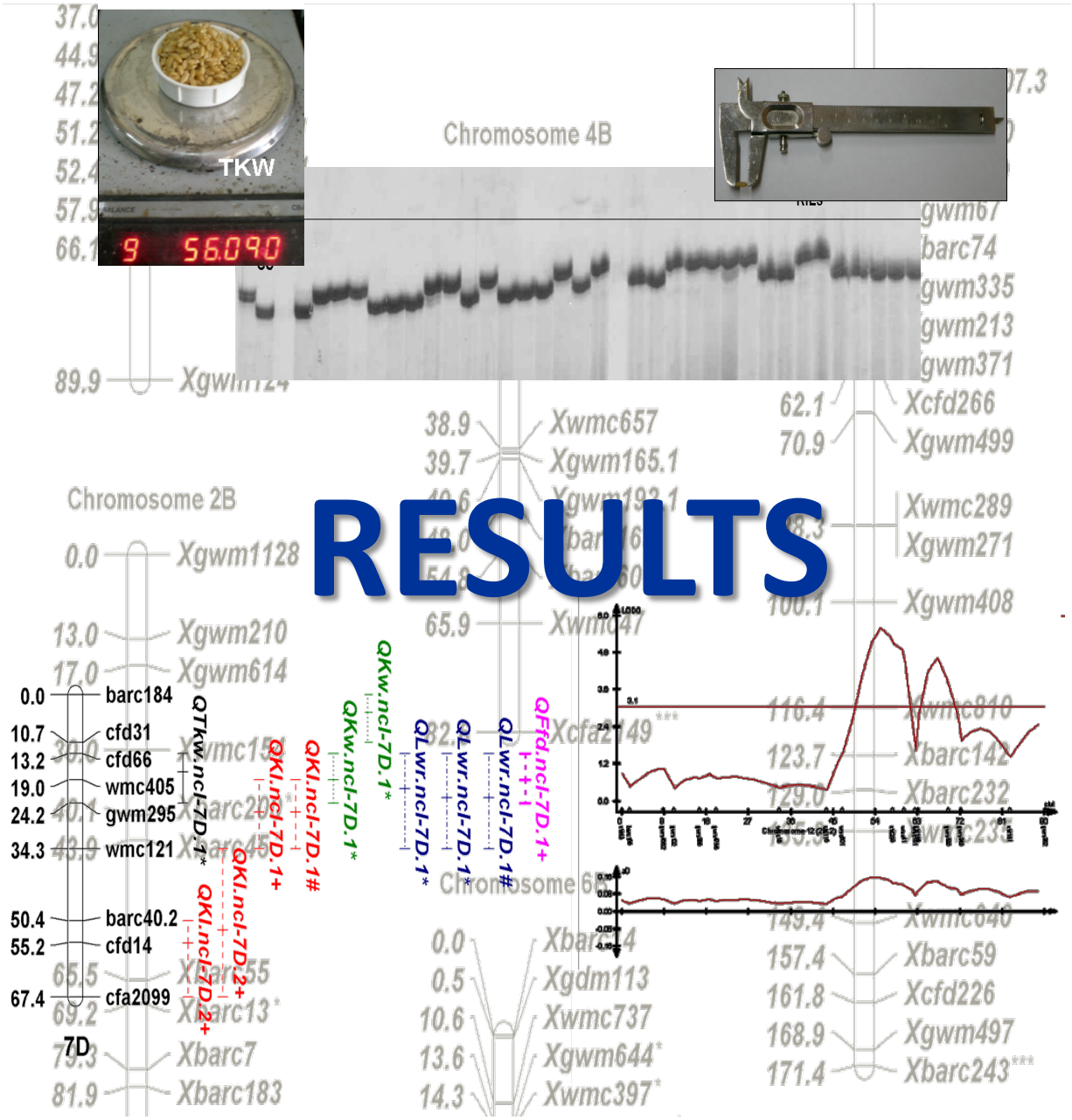


Figure 2.3: An example of *F*-statistic profiles generated by a 1D genome scan (a) and a 2D genome scan (b) using QTLNetwork software to detect QTLs and epistatic effects, respectively.


TKW
Chromosome 4B
Chromosome 2B
RESULTS
0.0 barc184
10.7 cfd31
13.2 cfd66
19.0 wmc405
24.2 gwm295
34.3 wmc121
50.4 barc40.2
55.2 cfd14
67.4 cfa2099
7D
QTkw.ncl-7D.1*
QKl.ncl-7D.1+
QKl.ncl-7D.1#
QKw.ncl-7D.1*
QKl.ncl-7D.2+
QLwr.ncl-7D.1*
QLwr.ncl-7D.1#
QFfd.ncl-7D.1+
Xgwm1128
Xgwm210
Xgwm614
Xbarc7
Xbarc183
Xwmc657
Xgwm165.1
Xcfa2149
Xgdm113
Xwmc737
Xgwm644
Xwmc397
Xcfd266
Xgwm499
Xwmc289
Xgwm271
Xgwm408
Xbarc59
Xcfd226
Xgwm497
Xbarc243
gwm67
barc74
gwm335
gwm213
gwm371

Results

The Rye Selection 111 (RS) × Chinese Spring (CS) mapping population comprising 185 recombinant inbred lines (RILs) was utilized for genetic analysis of kernel morphometric traits. Phenotypic analysis, simple sequence repeat (SSR) marker analysis, linkage map construction and QTL mapping for kernel quality traits were performed as described in Chapter 2. The results are summarized in the following sections.

3.1 Phenotypic evaluation

The parents RS, CS and the RIL population derived from their cross were analyzed for kernel size and shape for the harvests of Ludhiana and Pune for multiple years as detailed in Chapter 2. The parents, RS and CS showed marked difference for all the five kernel traits recorded, namely TKW, KL, KW, LWR and FFD **(Table 3.1).** RS exhibited higher trait values than CS for TKW, KL, KW and FFD in all the environments. However, for LWR, the environments LWR2005Lud and LWR2007Pun indicated lower trait value for RS than CS. In other three environments, RS displayed higher LWR than CS.

The mean, phenotypic range, standard deviation and coefficient of variation for the RILs are shown in **Table 3.1**. In both Ludhiana and Pune, the RIL population displayed a wide phenotypic range for all the five kernel traits. The values of coefficient of variation (CV) were comparable for the same traits in different environments. Frequency distributions of traits indicated continuous variation in all the year-location combinations (**Figure 3.1**).

When transgressive segregants were noted for kernel traits, RILs with TKW lower than that of the poor parent CS were observed in all the environments except TKW2009Pun. Transgressive segregants with TKW higher than that of the better parent RS were seen only in Pune location. For KL, KL2003Lud and KL2007Pun indicated some RILs with trait value lower than CS, whereas, only KL2006Pun showed transgressive segregants with higher KL than RS. The environments KW2005Lud, KW2005Pun and KW2006Pun displayed transgressive segregants exceeding the KW of RS, while in all the environments,

transgressive segregants with lower KW than CS were observed. For LWR and FFD, all the year-location combinations indicated transgressive segregants that were superior to RS and inferior to CS.

Table 3.1: Phenotypic performance of RS, CS and RILs in Ludhiana and Pune for kernel characters

Trait	Environment	RS	CS	RILs			
				Mean	*SD*	*Range*	*CV (%)*
TKW (g)	TKW2000Lud	54.6	19.6	31.15	7.39	15.4-52.1	23.7
	TKW2003Lud	52.2	20.5	29.32	7.08	11.5-46.0	24.1
	TKW2005Lud	50.3	17.6	24.57	5.44	10.0-40.3	22.1
	TKW2007Lud	54.4	22.2	28.99	7.22	10.8-44.2	24.9
	TKW2005Pun	52.8	16.1	31.61	7.40	15.5-55.5	23.4
	TKW2006Pun	51.5	14.5	25.65	6.41	13.3-52.3	24.9
	TKW2007Pun	51.0	16.6	27.03	5.56	13.4-52.4	20.5
	TKW2009Pun	53.6	17.2	30.13	6.84	17.6-59.6	22.7
KL (mm)	KL2003Lud	7.66	5.31	5.96	0.39	5.14-7.14	6.5
	KL2005Lud	6.70	5.10	5.92	0.30	5.10-6.60	5.1
	KL2005Pun	7.61	5.20	6.38	0.34	5.40-7.50	5.3
	KL2006Pun	7.53	5.35	6.22	0.38	5.40-7.65	6.1
	KL2007Pun	7.52	5.37	6.12	0.33	5.27-7.41	5.4
KW (mm)	KW2003Lud	3.64	2.97	2.95	0.32	2.10-3.61	10.8
	KW2005Lud	3.30	2.20	2.82	0.34	2.00-3.50	12.1
	KW2005Pun	3.36	2.38	3.01	0.29	2.20-3.60	9.6
	KW2006Pun	3.19	2.35	2.65	0.29	2.00-3.35	10.9
	KW2007Pun	3.20	2.17	2.56	0.22	2.07-3.15	8.6
LWR	LWR2003Lud	2.10	1.79	2.04	0.22	1.59-2.99	10.9
	LWR2005Lud	2.03	2.32	2.13	0.27	1.63-3.10	12.7
	LWR2005Pun	2.26	2.18	2.14	0.20	1.78-2.98	9.2
	LWR2006Pun	2.36	2.28	2.36	0.25	1.86-3.33	10.5
	LWR2007Pun	2.35	2.47	2.41	0.20	1.77-2.98	8.3
FFD (g/mm^2)	FFD2003Lud	1.87	1.30	1.64	0.23	0.98-2.45	13.9
	FFD2005Lud	2.27	1.57	1.48	0.29	0.71-2.86	19.8
	FFD2005Pun	2.06	1.30	1.63	0.26	0.72-2.34	15.9
	FFD2006Pun	2.14	1.15	1.54	0.22	0.82-2.35	14.6
	FFD2007Pun	2.12	1.42	1.73	0.29	0.91-2.57	16.8

The environment is designated by its trait, year and location information.

SD: standard deviation, Range represents the RIL minimum and maximum trait values and CV was calculated as RIL SD/mean expressed as percentage.

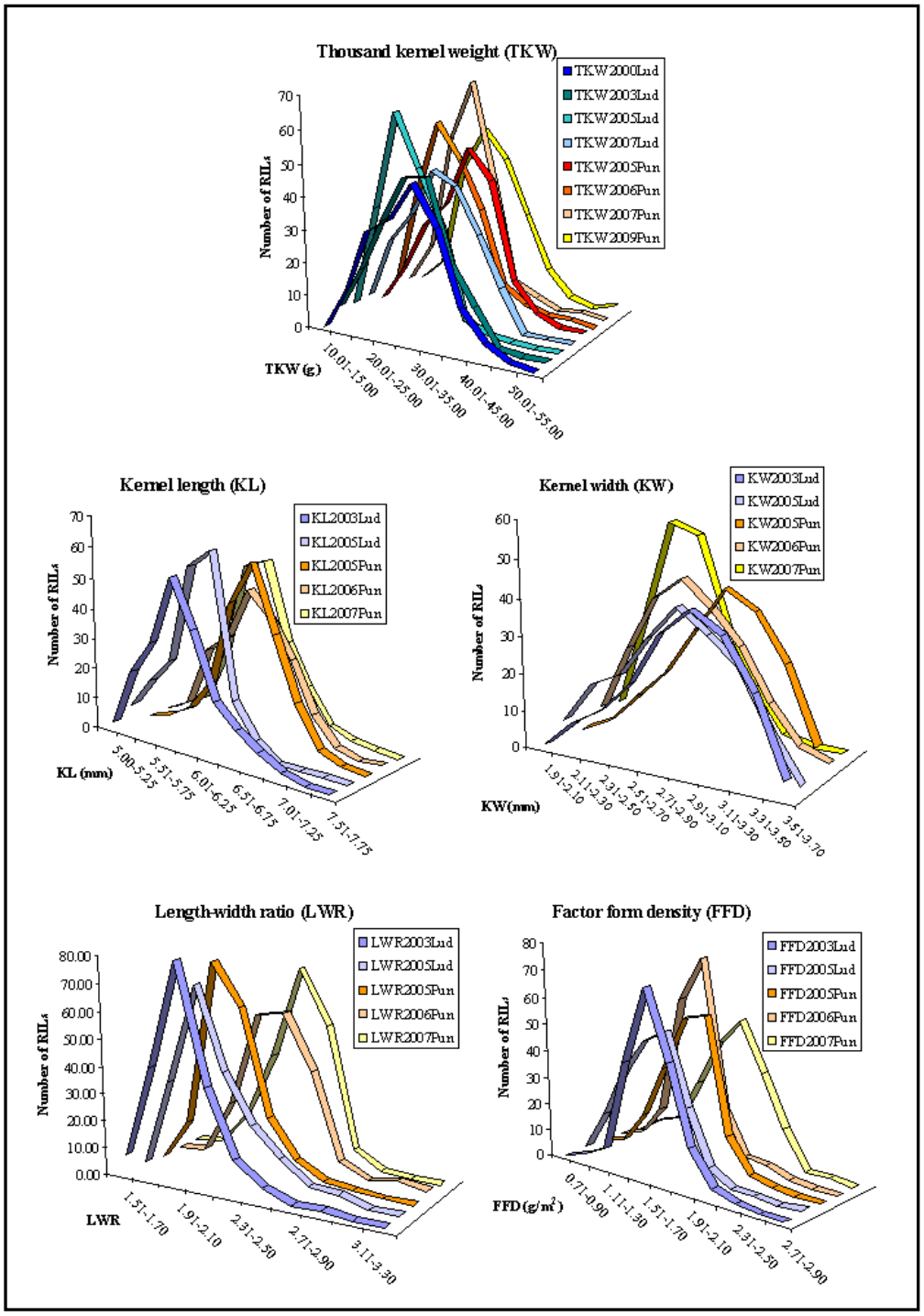


Figure 3.1: Frequency distributions of kernel characters

3.2 Correlation analysis

3.2.1 Correlation among kernel characters

Pearson correlation coefficients were calculated between pairs of kernel traits recorded from the same environment (**Table 3.2**). TKW showed positive and highly significant correlation with KL, KW and FFD in all the environments and with average trait values across environments. Among these, correlation between TKW and KW at Ludhiana (year 2003) and TKW and FFD with average data across environments indicated the highest correlation coefficient (r = 0.87, P<0.001). Negative and significant correlations were observed between TKW and LWR. Correlation between KL and KW were positive and significant in all the environments. However, KL showed positive and significant correlation with LWR only in some of the environments. Positive correlation with FFD was displayed by KL in four environments, while in Ludhiana (year 2005), it indicated negative correlation (r = -0.23, P<0.01). Kernel width exhibited highly significant negative correlation with LWR in all the environments with the highest being in Ludhiana (year 2005, r = -0.90, P<0.001). Correlations of KW with FFD were positive and significant in four out of six environments tested. Negative and significant correlations in all the environments were recorded between LWR and FFD except in Ludhiana (year 2005) where it was non significant and Pune (year 2006) in which the correlation coefficient suggested positive and moderately significant relationship.

3.2.2 Correlation among year-location environments for kernel characters

Spearman rank correlations between environments for kernel traits were positive and significant in most of the environments indicating the consistency in the performance of the RILs across environments (**Table 3.3**). Notably, for TKW and KL, all the pairs of environments tested indicated correlations that were highly significant (P<0.001). Comparatively, in case of KW, LWR and FFD, correlations between some of the year-location combinations were not significant or were significant only at P<0.05 or P<0.01 that indicated environmental influence on these traits.

3.3 AMMI analysis

Additive main-effect and multiplicative interaction (AMMI) analysis was employed to initially partition the total variance for each kernel trait by ANOVA into variance due to genotype (G) and environment (E) main-effects and G×E interaction effects. AMMI analysis further extracted interaction principal component axes (IPCA) to derive patterns in G×E that could significantly represent the G×E interaction component (**Table 3.4**). The number of significant IPCA derived for the kernel traits varied from two to four. The individual sum of squares of G and E main-effects, G×E interaction effects and the IPCA were divided by total sum of squares and expressed as percentage to calculate their contributions to the total variance (Tarakanovas and Ruzgas, 2006).

Table 3.2: Pearson correlation coefficients '*r*' between kernel characters

Traits	2003 Ludhiana	2005 Ludhiana	2005 Pune	2006 Pune	2007 Pune	Average
TKW-KL	0.56***	0.21**	0.54***	0.65***	0.50***	0.52***
TKW-KW	0.87***	0.56***	0.70***	0.77***	0.41***	0.79***
TKW-LWR	-0.59***	-0.46***	-0.49***	-0.43***	ns	-0.48***
TKW-FFD	0.85***	0.73***	0.86***	0.81***	0.81***	0.87***
KL-KW	0.43***	0.28***	0.46***	0.44***	0.33***	0.37***
KL-LWR	ns	ns	ns	0.15*	0.30***	0.27***
KL-FFD	0.19**	-0.23**	0.16*	0.32***	ns	0.22**
KW-LWR	-0.83***	-0.90***	-0.84***	-0.82***	-0.79***	-0.79***
KW-FFD	0.59***	ns	0.32***	0.34***	ns	0.54***
LWR-FFD	-0.50***	ns	-0.28***	-0.17*	0.15*	-0.40***

ns- non significant ***P<0.001 **P<0.01 *P<0.05

Average – Average value across five year-location combinations for each character

Table 3.3: Spearman rank correlation coefficients 'ρ' across environments for kernel characters

Trait	Environment	2003Lud	2005Lud	2007Lud	2005Pun	2006Pun	2007Pun	2009Pun
TKW	2000 Lud	0.60***	0.35***	0.53***	0.46***	0.51***	0.30***	0.49***
	2003 Lud	-	0.51***	0.59***	0.59***	0.59***	0.51***	0.60***
	2005 Lud	-	-	0.49***	0.31***	0.42***	0.47***	0.51***
	2007 Lud	-	-	-	0.50***	0.57***	0.44***	0.60***
	2005 Pun	-	-	-	-	0.66***	0.52***	0.63***
	2006 Pun	-	-	-	-	-	0.58***	0.76***
	2007 Pun	-	-	-	-	-	-	0.53***
KL	2003 Lud	-	0.43***	-	0.48***	0.54***	0.45***	-
	2005 Lud	-	-	-	0.56***	0.53***	0.41***	-
	2005 Pun	-	-	-	-	0.68***	0.61***	-
	2006 Pun	-	-	-	-	-	0.67***	-
KW	2003 Lud	-	0.42***	-	0.43***	0.53***	ns	-
	2005 Lud	-	-	-	0.31***	0.42***	0.15*	-
	2005 Pun	-	-	-	-	0.53***	0.25***	-
	2006 Pun	-	-	-	-	-	0.19**	-
LWR	2003 Lud	-	0.42***	-	0.43***	0.46***	0.16*	-
	2005 Lud	-	-	-	0.30***	0.37***	0.15*	-
	2005 Pun	-	-	-	-	0.55***	0.29***	-
	2006 Pun	-	-	-	-	-	0.16*	-
FFD	2003 Lud	-	0.26***	-	0.30***	0.36***	0.34***	-
	2005 Lud	-	-	-	ns	0.20**	0.25***	-
	2005 Pun	-	-	-	-	0.44***	0.38***	-
	2006 Pun	-	-	-	-	-	0.47***	-

ns- non-significant ***P<0.001 **P<0.01 *P<0.05

The contributions of G, E, G×E effects and the IPCA to total variance and the contributions of IPCA for G×E effects for kernel traits are shown in **Table 3.5** and **3.6**, respectively**.** The IPCA1 and IPCA2 scores for the RILs and environments were used for constructing AMMI2 biplots to graphically represent the G×E interaction patterns (**Figure 3.2**). A summary of AMMI and biplot analysis for the kernel traits is given below.

3.3.1 Thousand kernel weight

The AMMI analysis of variance of TKW tested in eight environments showed that approximately 52% of the total sum of squares (TSS) was attributed to genotype main-effect, 37% to genotype × environment interactions and only 11% to environment effect. Four significant IPCA explained up to 73% of the genotype × environment effect. The first two IPCA used for the AMMI2 biplot explained 43.9% of the G×E sum of squares. The vectors of the environments A (TKW2000Lud), C (TKW2005Lud) and E (TKW2005Pun) were longer than the rest that could be indicative of the greater involvement of those environments in G×E interactions. The RIL vectors represented by points clustered close to the origin, and depicted the stability in the performance of RILs across environments.

3.3.2 Kernel length

The contribution of genotype main-effects (53%) to variation in kernel length in the RS×CS population was higher compared to that of environmental main-effects (19%) or G×E effects (28%). Two significant IPCA explained up to 64% of G×E interactions. The RILs clustered close to the origin of the biplot and the environments A (KL2003Lud) and B (KL2005Lud) showed increased involvement in the G×E interactions.

3.3.3 Kernel width

The contributions from genotype main-effects (36%) and G×E interactions (39%) were similar in case of KW and the environmental main-effects contributed a substantial effect of 25%. Three significant IPCA were derived and explained up to 85% of G×E interactions. The AMMI2 biplot represented nearly 65% of G×E variation. The dense clustering of RILs near the origin of the biplot indicated their

stability, while three environments, A (KW2003Lud), B (KW2005Lud) and E (KW2007Pun) suggested greater involvement in G×E interactions.

3.3.4 Length-width ratio

Genotype (36%), environment (28%) and G×E (35%) contributions to total variance of LWR were comparable to each other. Approximately 87% of G×E interaction was explained by three significant IPCA. The AMMI2 biplots represented up to 67% of G×E interaction indicating that the first two IPCA were sufficient to represent a large proportion of variation due to G×E. The RILs indicated greater phenotypic stability by clustering near the biplot origin, while the environments B (LWR2005Lud), D (LWR2006Pun) and E (LWR2007Pun) had long vectors that showed increased participation in G×E interactions.

3.3.5 Factor form density

FFD indicated higher contribution from G×E interactions (49%) compared to genotype (41%). When compared with the other kernel traits, environment main-effects (10%) contributed minimum to the total variance. Three significant IPCA explained approximately 84% of G×E interaction effects. The AMMI2 biplot could explain up to 64% of G×E interaction. Four out of five Environment vectors representing FFD2003Lud, FFD2005Lud, FFD2005Pun and FFD2007Pun were considerably long depicting the extent of participation of these year-location combinations in the G×E interactions. RILs 26, 56 and 111 were slightly distant from the dense cluster of RILs located near the origin and reflected the greater influence of the environment on their phenotypic performance.

3.4 Heritability of the kernel characters

Heritability, which suggests the proportion of the phenotypic variation in the population that can be assigned to variation in genotype, was calculated for kernel traits using the method described in Chapter 2. The traits indicated moderate (FFD, h^2=0.688) to high (TKW, h^2=0.879) heritability (**Table 3.7**).

Table 3.4: Sources of variation and pattern of G×E interactions for kernel traits revealed by AMMI analysis

TKW

Source	df	SS	MSS	*F*
G	184	38606.50	209.82	9.78***
E	7	8493.81	1213.40	56.57***
G×E	1288	27625.70	21.45	-
IPCA 1	190	6541.62	34.43	1.79***
IPCA 2	188	5576.77	29.66	1.74***
IPCA 3	186	4201.95	22.59	1.45***
IPCA 4	184	3726.24	20.25	1.44***
G×E Residual	540	7579.09		
Total	**1479**	**74726.00**		

KL

Source	df	SS	MSS	*F*
G	184	73.83	0.40	7.43***
E	4	26.67	6.67	123.48***
G×E	736	39.74	0.05	-
IPCA 1	187	14.48	0.08	1.68***
IPCA 2	185	11.07	0.06	1.53***
IPCA 3	183	7.54	0.04	ns
IPCA 4	181	6.66	0.04	ns
Total	**924**	**140.25**		

KW

Source	df	SS	MSS	*F*
G	184	38.40	0.21	3.60***
E	4	27.03	6.76	116.62***
G×E	736	42.64	0.06	-
IPCA 1	187	14.74	0.08	1.55***
IPCA 2	185	12.93	0.07	1.70***
IPCA 3	183	8.50	0.05	1.30*
IPCA 4	181	6.47	0.04	ns
Total	**924**	**108.07**		

LWR

Source	df	SS	MSS	*F*
G	184	24.42	0.13	4.10***
E	4	19.06	4.77	147.16***
G×E	736	23.83	0.03	-
IPCA 1	187	9.01	0.05	1.79***
IPCA 2	185	6.93	0.04	1.77***
IPCA 3	183	4.78	0.03	1.52**
IPCA 4	181	3.11	0.02	ns
Total	**924**	**67.31**		

FFD

Source	df	SS	MSS	*F*
G	184	28.60	0.16	3.39***
E	4	7.01	1.75	38.17***
G×E	736	33.77	0.05	-
IPCA 1	187	13.22	0.07	1.89***
IPCA 2	185	8.25	0.04	1.32*
IPCA 3	183	6.95	0.04	1.28*
IPCA 4	181	5.36	0.03	ns
Total	**924**	**69.38**		

ns- non-significant *P<0.05, **P<0.01 and ***P<0.001

df-degrees of freedom, SS-Sum of squares, MSS-Mean sum of squares

Table 3.5: Contributions (%) of sources of variation to kernel characters

Trait	G	E	G×E
TKW	51.66	11.37	36.97
KL	52.64	19.02	28.34
KW	35.53	25.01	39.46
LWR	36.27	28.32	35.41
FFD	41.22	10.10	48.68

Table 3.6: Contributions (%) of IPCA to total variance and G×E interaction

	TKW		KL		KW		LWR		FFD	
	SS_{Total}	$SS_{G\times E}$	SS_{Total}	$SS_{G\times E}$	SS_{Total}	$SS_{G\times E}$	SS_{Total}	$SS_{G\times E}$	SS_{Total}	$SS_{G\times E}$
IPCA1	8.75	23.68	10.32	36.44	13.64	34.57	13.39	37.81	19.05	39.15
IPCA2	7.46	20.19	7.89	27.86	11.96	30.32	10.30	29.08	11.89	24.43
IPCA3	5.62	15.21	ns	ns	7.87	19.93	7.10	20.06	10.02	20.58
IPCA4	4.99	13.49	ns	ns	ns	ns	ns	ns	ns	ns
Total	**26.82**	**72.57**	**18.21**	**64.30**	**33.47**	**84.82**	**30.79**	**86.95**	**40.96**	**84.16**

ns: non-significant

Table 3.7: Heritability estimates of kernel characters

Trait	Heritability
TKW	0.879
KL	0.865
KW	0.796
LWR	0.769
FFD	0.688

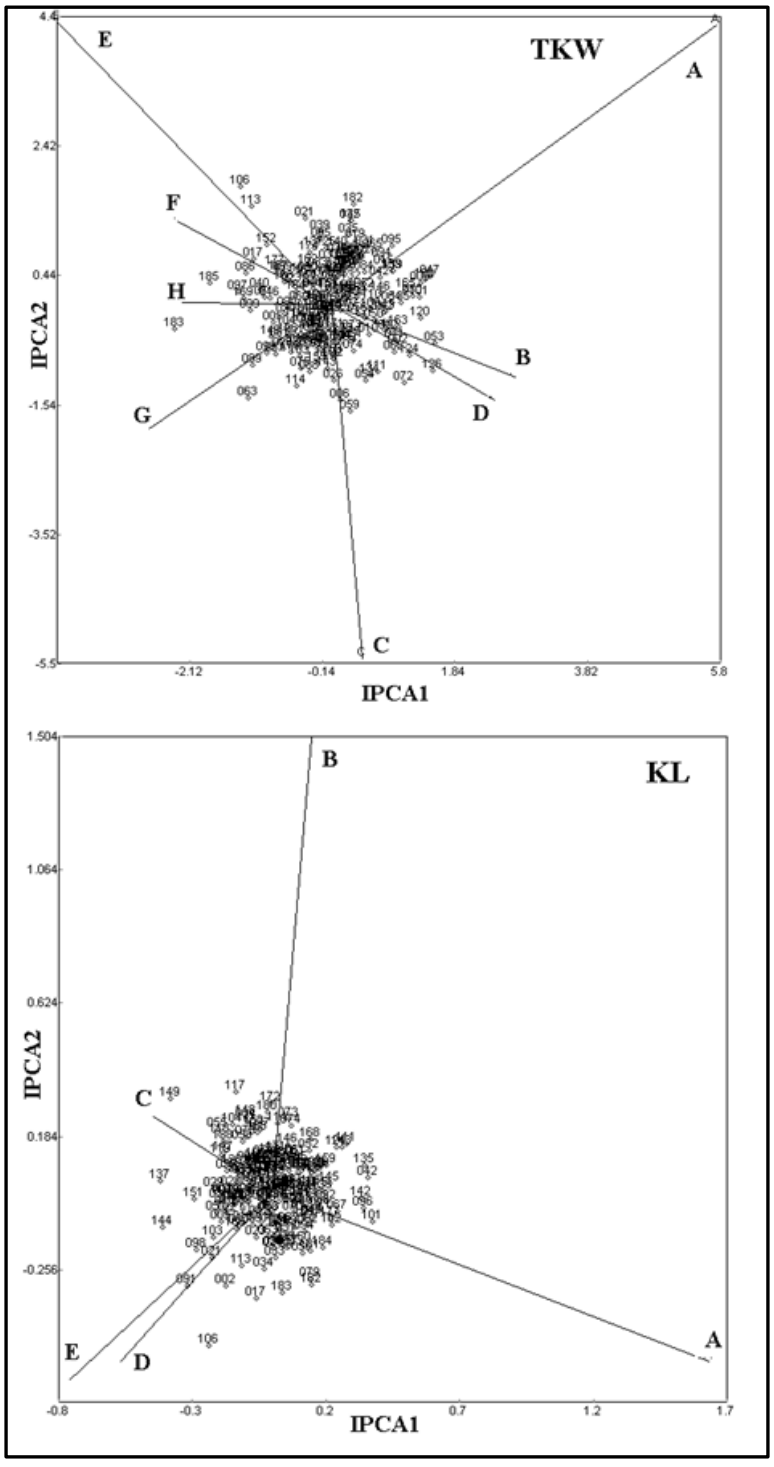


Figure 3.2: AMMI2 biplots for kernel characters.

TKW: A-TKW2000Lud, B-TKW2003Lud, C-TKW2005Lud, D-TKW2007Lud, E-TKW2005Pun, F-TKW2006Pun, G-TKW2007Pun and H-TKW2009Pun. Other traits: A-2003 (Ludhiana); B-2005 (Ludhiana); C-2005 (Pune); D-2006 (Pune) and E-2007 (Pune)

Figure 3.2 Continued

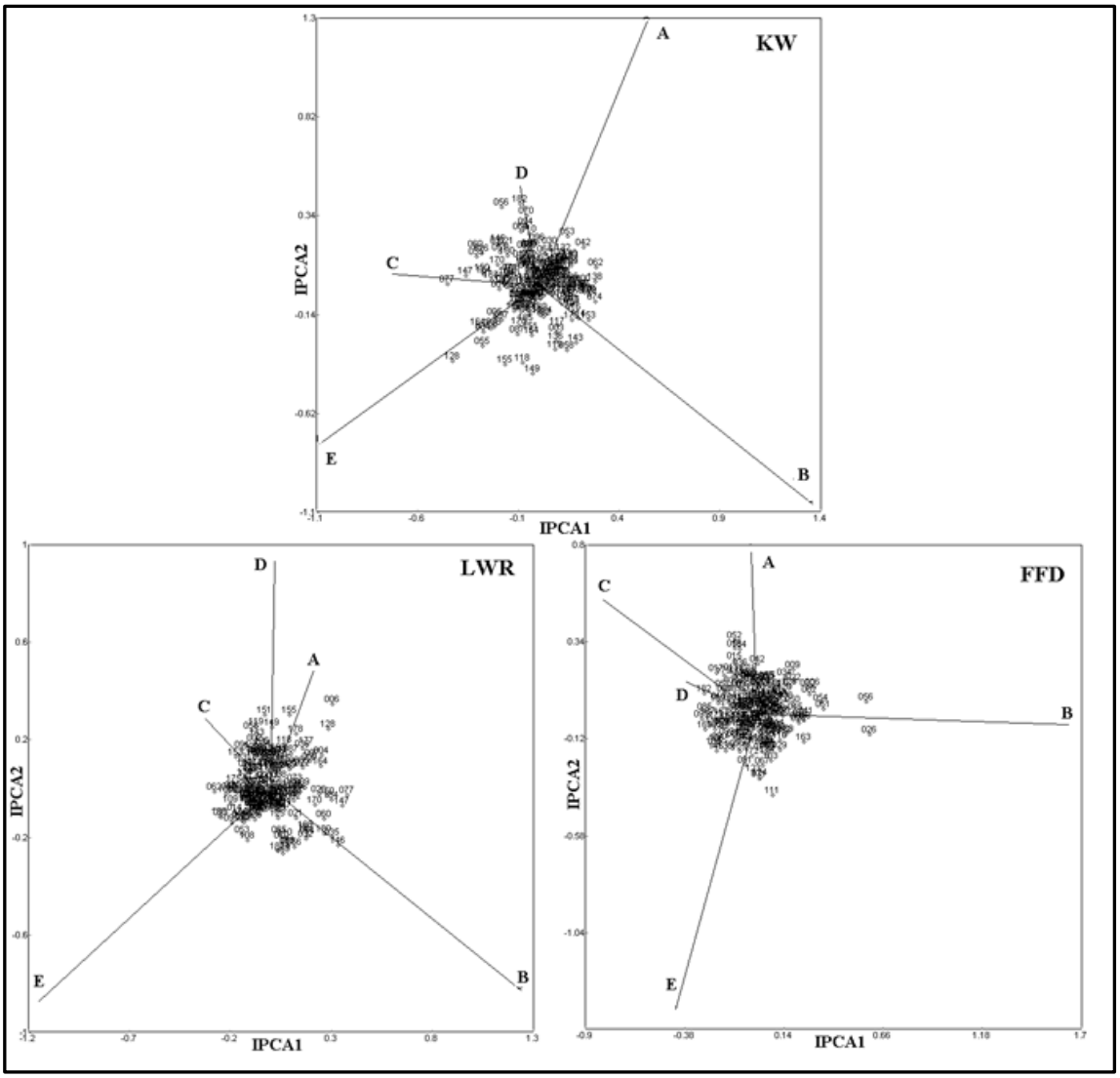


Figure 3.2: AMMI2 biplots for kernel characters.

3.5 Construction of linkage map of RS×CS population

Parental polymorphism was evaluated with previously mapped SSRs selected from each chromosome (Röder *et al*., 1998; Stephenson *et al*., 1998; Pestosova *et al*., 2000; Song *et al*., 2002; Eujayl *et al*., 2002; Guyomarc'h, *et al*., 2002; Sourdille *et al*., 2003; Yu, *et al*., 2004 and GrainGenes website http://wheat.pw.usda.gov/ggpages/SSR/). The primers that showed polymorphism in parents were used for genotyping the RIL population. The marker segregation scores of the RILs were used for linkage mapping using JoinMap 4 software (Van Ooijen, 2006).

3.5.1 Parental polymorphism

A total of 826 SSR primer pairs were used for the assessment of parental polymorphism (**Table 3.8**). The number of polymorphic bands that were reproducible and segregated in the RILs ranged from 1-4. In total, 245 primers were polymorphic (29.66%) and yielded 267 bands that segregated in the RIL population.

The list of primers that yielded multiple polymorphic bands and their location in the RS×CS population determined by linkage mapping is shown in **Table 3.9**. Among the primer pairs that amplified more than one locus, eight amplified loci on homeologous chromosomes, five amplified on other chromosomes as well, while two could not be compared.

3.5.2 RIL population genotyping

The 245 polymorphic primer pairs were resolved using MetaPhor® agarose or polyacrylamide gel electrophoresis (PAGE) based on the molecular weight difference between the SSR alleles. A representative gel picture of RIL genotyping using MetaPhor® agarose is shown for the marker *Xpsp3000* in **Figure 3.3(a)**. PAGE gel pictures for the marker *Xcfa2250* showing a single polymorphic band and for the primer pair of *cfd59* with three polymorphic bands segregating in the RILs are indicated in **Figures 3.3(b)** and **3.3(c)**, respectively. The segregation of 267 polymorphic bands was scored in the 185 RILs to record the genotypic data. Deviation from 1:1 parental allele segregation ratio was observed in 43 markers (16.1%). Among these, 24 markers indicated deviation at the significance level of 5% and the rest at 1% or higher.

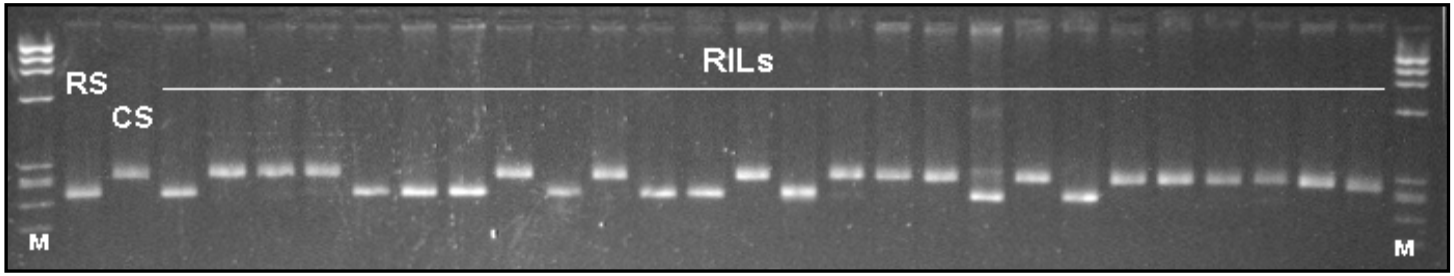
Table 3.8: SSR primer polymorphism detected by initial parental survey

Chromosome	Primers surveyed	Polymorphic	Chromosome	Primers surveyed	Polymorphic
1A	68	24	**5A**	62	15
1B	14	6	**5B**	77	24
1D	58	14	**5D**	97	20
2A	33	10	**6A**	14	6
2B	77	19	**6B**	43	16
2D	66	22	**6D**	16	9
3A	15	5	**7A**	26	5
3B	20	5	**7B**	16	6
3D	20	5	**7D**	23	8
4A	18	7			
4B	51	14			
4D	12	5			

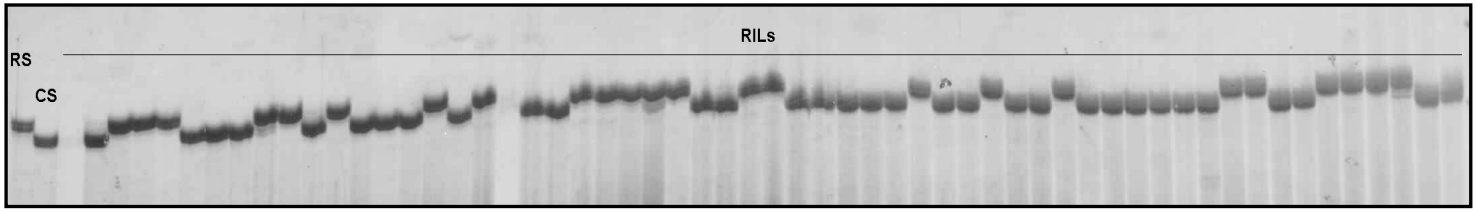
Table 3.9: Map locations of loci derived from SSR primers that yielded more than one polymorphic and segregating bands

No.	SSR	Loci	Chr	No.	SSR	Loci	Chr
1	*barc40*	*Xbarc40.1*	1B	8	*gwm165*	*Xgwm165.1*	4B
		Xbarc40.2	7D			*Xgwm165.2*	4D
2	*barc146*	*Xbarc146.1*	6A			*Xgwm165.3*	4A
		Xbarc146.2	6B	9	*gwm192*	*Xgwm192.1*	4B
3	*barc240*	*Xbarc240.1*	1D			*Xgwm192.2*	4A
		Xbarc240.2	1A	10	*gwm273*	*Xgwm273.1*	1B
		Xbarc240.3	-			*Xgwm273.2*	6B
		Xbarc240.4	-	11	*gwm1307*	*Xgwm1307.1*	5D
4	*cfa2185*	*Xcfa2185.1*	5A			*Xgwm1307.2*	5A
		Xcfa2185.2	5D			*Xgwm1307.3*	5B
5	*cfd19*	*Xcfd19.1*	6D	12	*gdm33*	*Xgdm33.1*	-
		Xcfd19.2	1D			*Xgdm33.2*	-
6	*cfd48*	*Xcfd48.1*	1D	13	*wmc150*	*Xwmc150.1*	5A
		Xcfd48.2	1B			*Xwmc150.2*	5D
		Xcfd48.3	-			*Xwmc150.3*	4A
7	*cfd59*	*Xcfd59.1*	1D	14	*wmc269*	*Xwmc269.1*	1B
		Xcfd59.2	1B			*Xwmc269.2*	7B
		Xcfd59.3	1A	15	*wmc505*	*Xwmc505.1*	3A
						Xwmc505.2	-

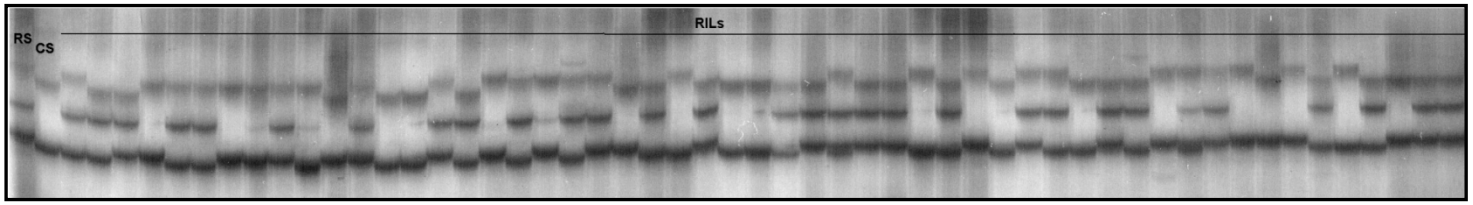
Loci excluded from linkage mapping are indicated by '-'



a: SSR alleles for the marker *Xpsp3000-1B* resolved using 3% MetaPhor® agarose



b: SSR alleles for the marker X*cfa2250-5A* resolved using 6% denaturing PAGE



c: Multiple loci derived from the SSR primer pair of *cfd59*

Figure 3.3: Resolution of PCR products in RS and CS and their segregation pattern in a subset of RILs

3.5.3 Construction of linkage map

During linkage analysis, five RILs were excluded as they had missing data as high as 40%, while four marker bands were excluded with as high as 30% missing data. They were eventually not included in QTL analysis as well. In the initial rounds of mapping, three marker loci, namely, *Xcfd48.3*, *Xbarc240.3* and *Xbarc240.4* mapped very close (<0.1 cM) to other loci derived from the same primer pairs. Hence they were removed during the subsequent analyses. The 43 markers, which deviated from the 1:1 segregation ratio were initially excluded from linkage analysis. After constructing the framework map of markers that conformed to the segregation ratio, the skewed markers were introduced to linkage groups using the "Move selected loci" function of JoinMap.

Out of the 260 markers used for linkage analysis, 251 linked to generate 32 linkage groups at LOD ≥ 4 and nine markers remained unlinked. Linkage groups were allocated to chromosomes when the groups had two or more SSR loci that had been assigned to a particular wheat chromosome in previously published maps (Röder *et al*., 1998; Somers *et al*., 2004; and the *Triticum aestivum* maps in the GrainGenes database). All the 21 chromosomes of hexaploid wheat were represented in the resultant map and the number of linkage groups matching to each chromosome ranged from 1 to 3 (**Table 3.10**). Eleven chromosomes were represented by a single linkage group for each; chromosome 2A was represented by 3 linkage groups and 1A, 1D, 2B, 2D, 4A, 5A, 5B, 5D and 6D by two linkage groups each. Thus, the B genome had nine groups followed by D genome and A genomes with 11 and 12 groups, respectively. The number of markers per chromosome ranged from 5 (3A, 3B, 3D and 7A) to 25 (5B). Chromosome 5B had the maximum map length of 190.91 cM while 3B represented by five markers showed a map length of 14.44 cM. The framework map categorized based on A, B and D genomes is depicted in **Figure 3.4 (a-c)**. The map length of A genome in the RS×CS SSR map was 414.04 cM, while that of B and D genome was 635.34 cM and 605.91 cM, respectively. The mean interval between loci was the smallest for A genome (5.6 cM), followed by B genome (6.98 cM) and 7.05 cM for D genome. The total map length was 1655.28 cM and the mean interval between loci was 6.59 cM. Large gaps within the

linkage groups were minimum with the largest of gaps observed on chromosomes 6A, 5A and 5D spanning 44 cM, 31.86 cM and 26.61 cM, respectively.

The overall arrangement of the markers was similar to the published microsatellite maps developed by Röder *et al*. (1998), wheat consensus map by Somers *et al*. (2004) and the more recent high density SSR map of Nanda2419× Wangshuibai (Xue *et al*., 2008). In seven cases, there were minor variations in the order of markers, such as inversion of order of two markers or two markers belonging to two different published maps placed in the same linkage group. In these cases, "fixed order" command of JoinMap software was applied, which accepted the command and markers were placed in an order as given in the fixed order. Out of the 43 markers that deviated from the 1:1 segregation ratio, 36 mapped to different chromosomes and seven remained unlinked. Among the mapped distorted markers, 19 skewed towards CS and 17 towards RS. The skewed markers were located on the chromosomes 1A, 1D, 2B, 2D, 3D, 4A, 4B, 5B, 6A, 6B, 6D, 7A and 7D. Interestingly, large clusters of skewed markers were detected on chromosomes 2B (6 distorted out of 20 markers) and 7D (7 distorted out of 9 markers). Among other chromosomes, 6B showed 4 distorted markers and 1D, 4A and 6A had three distorted markers each.

3.6 QTL analysis

QTL analysis of kernel characters in the RS×CS population was performed using various methods. Composite interval mapping was performed using QTL Cartographer v. 2.5 to detect the number and effects of QTLs controlling kernel characters. Multi-trait composite interval mapping was performed to identify genomic regions associated with more than one kernel trait. QTL analysis based on mixed linear model was employed to identify main-effect QTLs with significant individual effects on the traits, QTL × QTL interactions and the interaction of QTLs with the environment.

3.6.1 Composite interval mapping of kernel characters

Thousand kernel weight recorded in eight environments and the average across environments was used for CIM. For the other kernel traits, phenotypic data recorded in five year-location combinations and the average data were used.

Table 3.10: Distribution of mapped markers on 21 wheat chromosomes in the RS×CS map

Subgenome	Chromosome	Linkage groups	Markers	Length (cM)
A	1A	2	17 + 5	53.16 + 13.82
	2A	3	2 + 8 + 2	3.86 + 58.24 +2.79
	3A	1	5	15.16
	4A	2	6 + 2	38.50 + 5.94
	5A	2	14 + 2	96.78 + 3.54
	6A	1	6	81.76
	7A	1	5	40.50
	Subtotal	12	74	414.04
B	1B	1	10	89.87
	2B	2	18 + 2	143.69 + 10.26
	3B	1	5	14.44
	4B	1	9	82.56
	5B	2	2 + 23	19.54 + 171.37
	6B	1	16	60.12
	7B	1	6	43.50
	Subtotal	9	91	635.34
D	1D	2	13 + 2	112.64 + 7.70
	2D	2	6 + 17	30.26 + 90.04
	3D	1	5	55.39
	4D	1	6	27.04
	5D	2	11 + 8	46.59 + 80.39
	6D	2	4 + 5	33.96 + 54.46
	7D	1	9	67.44
	Subtotal	11	86	605.91
Total		**32**	**251**	**1655.28**

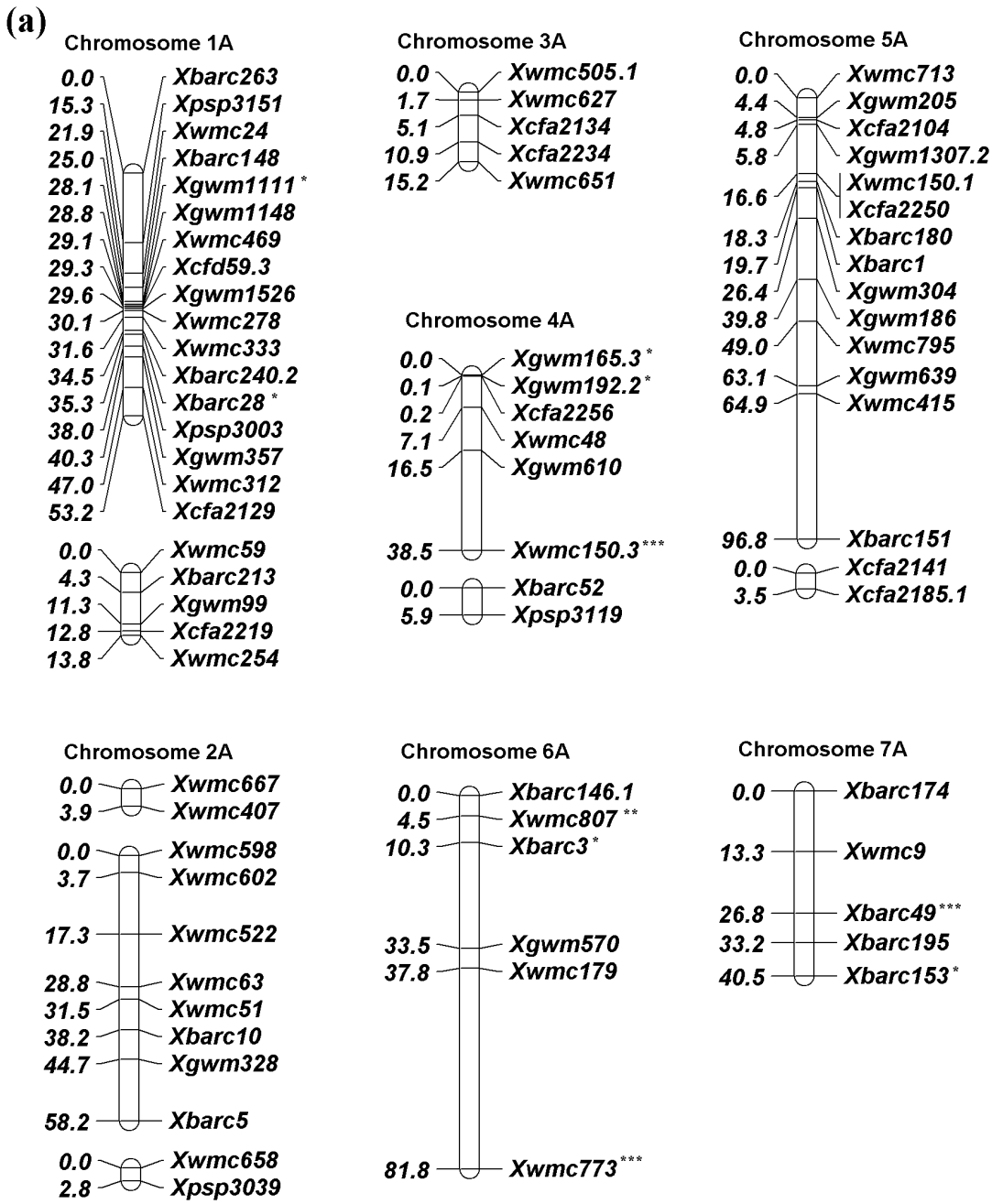


Figure 3.4: Linkage map representing the 21 chromosomes of hexaploid wheat. (a) Linkage groups representing wheat A genome. Marker names are on right and distance in cM on the left. Markers deviating from the 1:1 segregation ratio are marked * P<0.05, **P<0.01 and ***P<0.001

(b)

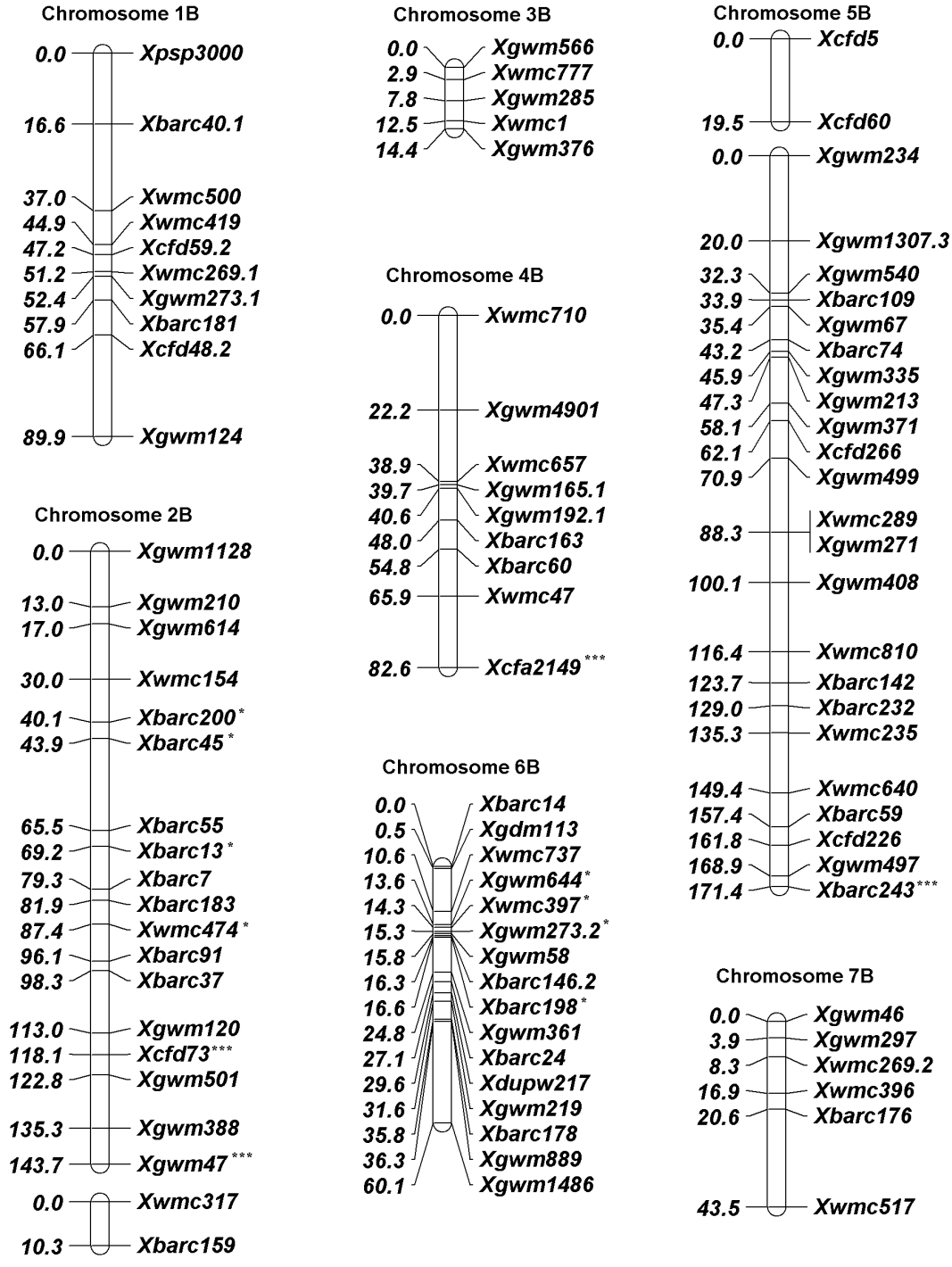


Figure 3.4(b): Linkage groups representing wheat B genome

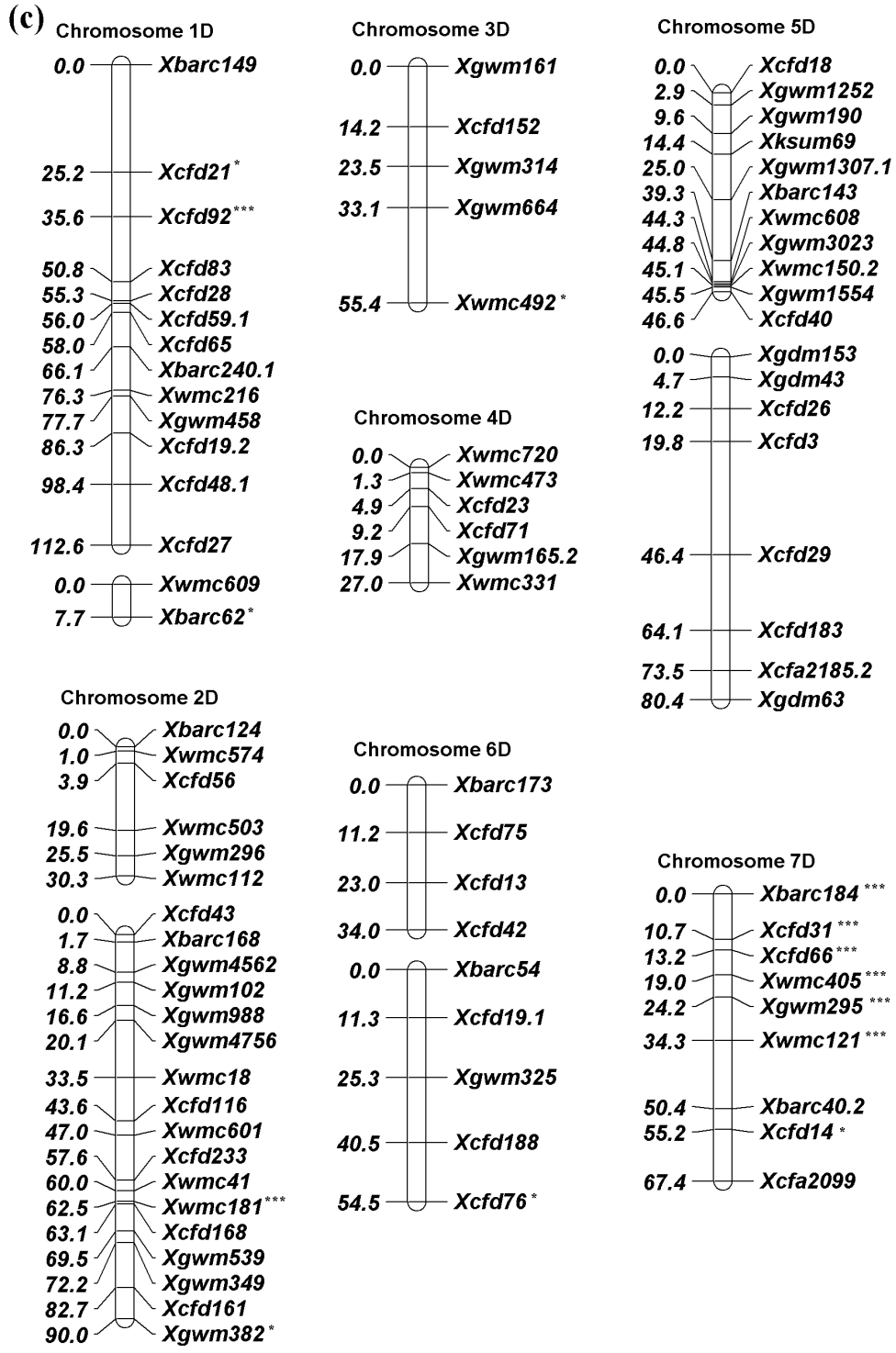


Figure 3.4(c): Linkage groups representing wheat D genome

Considering all the five kernel traits, 104 QTL peaks with LOD ≥ 2.0 were detected, which were located on 16 of the 21 wheat chromosomes. Among the QTL peaks observed, 46 (44.23%) indicated LOD scores ≥ threshold LOD computed by 1000-permutation tests (Churchill and Doerge, 1994; Doerge and Churchill, 1996), 21 (20.19%) with LOD scores between 2.5 and threshold LOD and 37 (35.58%) showed LOD scores between 2.0-2.5. Ludhiana and Pune showed 42 and 43 QTL peaks, respectively. The average data across environments for the kernel traits together exhibited 19 peaks. For each trait, some of the QTL peaks detected for Ludhiana displayed overlap with those observed for Pune and the remaining were location-specific (**Table 3.11**). The 104 peaks detected by CIM represented 59 QTLs when QTL peaks with adjacent, overlapping or the same marker intervals were given the same name according to McIntosh *et al*. (2003). Among these 59 QTLs, 11 (18.64%) QTLs were detected in three or more environments, 16 (27.12%) in two environments and 32 (54.24%) in single environments (**Table 3.12**). Furthermore, the better parent RS contributed 36 QTLs (61.02%) and the poor parent CS contributed 23 QTLs (38.98%) as suggested by the additive effects of the QTLs **(Table 3.13).** Interestingly, among the 36 QTLs contributed by RS, eight (22%) were detected in both Ludhiana and Pune, while only 3 out of 23 (13%) CS-derived QTLs were detected in both the locations. Among the RS-derived QTLs, 50% were detected only in Pune; while for CS-derived QTLs, nearly 57% were detected only in Ludhiana. The D genome harbored the maximum number of 26 QTLs (44.07%), followed by the B genome with 19 QTLs (32.2%) and the A genome with 14 QTLs (23.73%). The relative distribution of QTLs in the genome for each kernel character is shown in **Table 3.14** and **Figure 3.5**.Among the seven homeologous groups, maximum number of QTLs was displayed on Group 2 with 23 (38.98%) QTLs. Group 1 and Group 3 chromosomes indicated the least number of QTLs with 3 QTLs each (5.08%). The summary of QTLs detected for the kernel traits with their positions, LOD scores, additive effects and contribution to phenotypic variation (R^2) are shown in **Tables 3.15-3.19** and their map locations are depicted in the **Figure 3.6**. The details of the QTL analysis for each kernel trait are given below.

3.6.1.1 Thousand kernel weight

For TKW, 16 QTLs were identified which were distributed on nine chromosomes with contributions ranging from 4.1 to 14.23% (**Table 3.15**). Of these, 10 were contributed by the superior parent RS and six by CS. All the homeologous groups except Group 4 indicated TKW QTLs at least in one environment. The chromosomes of Group 2 harbored the highest with nine QTLs, while others were uniformly distributed among the rest of the homeologous groups. The A, B and D genomes showed approximately equal number of TKW QTLs. Three QTLs, namely, *QTkw.ncl-1A.1*, *QTkw.ncl-2B.2 and QTkw.ncl-5B.1* were detected in three or more environments. Ten QTLs were indicated in a single environment, while three QTLs on the chromosomes 2A, 2B and 3D, respectively, were detected in two year-location combinations.

3.6.1.2 Kernel Length

Eight kernel length QTLs distributed on six chromosomes with contributions ranging from 4.28 to 17.14% were identified in the RS×CS population (**Table 3.16**). RS contributed six QTLs, while two QTLs derived favorable alleles from CS. A and D genomes contributed similarly with four and three QTLs each, while the B genome contributed only one QTL, located on chromosome 2B. The homeologous groups 1 and 3 did not show QTLs for KL, while the remaining groups indicated similar number of QTLs. The QTLs *QKl.ncl-2B.1*, *QKl.ncl-2D.1*, *QKl.ncl-4A.1 and QKl.ncl-6A.1* were detected in three or more environments. Three QTLs, namely, *QKl.ncl-5A.1*, *QKl.ncl-7D.1* and *QKl.ncl-7D.2* were detected in two environments and interestingly, only one QTL *QKl.ncl-6A.2* was identified in a single environment.

3.6.1.3 Kernel width

The chromosomes 2B, 2D, 5B, 7A and 7D together displayed eight QTLs or KW, with contributions ranging from 3.67 to 11.26% **(Table 3.17).** Contribution of RS and CS to the number of QTLs was comparable, with three and five QTLs, respectively. *QKw.ncl-5B.1* was detected at Ludhiana in two years, at Pune in year 2007 and with average KW across environments. Among the rest of the QTLs, five QTLs located on the chromosomes 2B, 2D (*QKw.ncl-2D.1* and *QKw.ncl-*

2D.2), 7A and 7D were detected in two environments each, while *QKw.ncl-2B.2* and *QKw.ncl-5B.2* were detected only in one year-location combination.

3.6.1.4 Length-width ratio

Twelve QTLs for LWR located almost in equal numbers on the A (3 QTLs), B (4 QTLs) and D (5 QTLs) genomes were detected with contributions ranging from 5.05 to 19.08% (**Table 3.18)**. All the homeologous groups except Group1 indicated at least one QTL for LWR. The contribution of superior QTL allele was mainly from the better parent RS with nine out of 12 QTLs displaying positive additive effect. The QTLs *QLwr.ncl-5B.1* and *QLwr.ncl-7D*.1 were detected in four and three environments, respectively. Nine QTLs were identified in single environments, while *QLwr.ncl-5D.1* was documented in LWR2005Pun and LWR average.

3.6.1.5 Factor form density

In all, 15 QTLs were detected for FFD with maximum presence (8 QTLs) in the D genome, followed by B genome (5 QTLs) and lastly, the A genome (2 QTLs). All the homeologous groups indicated at least one QTL for FFD (**Table 3.19**). RS and CS contributed eight and seven QTLs, respectively, which underscored the importance of superior allele input from the poor parent. The contribution to phenotypic variance ranged from 4.15 to 11.91%. Only one QTL, namely, *QFfd.ncl-2B.2* was detected in three environments. Among the rest, *QFfd.ncl-2D.1*, *QFfd.ncl-2D.2*, *QFfd.ncl-4D.1* and *QFfd.ncl-5B.1* were observed in two environments each and the rest in single year-location combinations.

3.6.1.6 Chromosomes with QTLs for multiple traits

Most of the chromosomes (12 out of 16) harbored QTLs for more than one trait **(Figure 3.5).** Chromosomes 2B, 2D and 7D showed QTLs for TKW, KL, KW, LWR and FFD. Chromosome 5B harbored QTLs for TKW, KW, LWR and FFD, while, QTLs for three of the kernel traits were detected on 4A, 6B and 6D. Chromosomes 1A, 3D, 5D, 6A and 7A showed QTLs for two kernel traits each. QTLs for only one trait each were identified on chromosomes 2A, 3B, 4D and 5A.

Table 3.11: Number of QTL peaks detected at Ludhiana and Pune by Composite interval mapping

Trait	Number of QTL peaks					
	Total	Ludhiana	Pune	Overlapping	Location-specific	Trait average
TKW	27	15	09	10 (6L + 4P)	14 (9L + 5P)	03
KL	22	03	14	09 (3L + 6P)	08 (0L + 8P)	05
KW	16	07	05	05 (3L + 2P)	07 (4L+ 3P)	04
LWR	18	08	07	03 (2L + 1P)	12 (6L + 6P)	03
FFD	21	09	08	02 (1L + 1P)	15 (8L + 7P)	04
Total	104	42	43	29(15L + 14P)	56 (27L + 29P)	19

Overlapping: QTL peaks for Ludhiana and Pune that shared the same or overlapping marker intervals, thus representing the same QTL. L-Ludhiana and P-Pune. In some cases, one peak for Ludhiana showed overlap with one or more peaks for Pune and vice versa.

Trait average: Eight environments for TKW and five each for the other traits

Table 3.12: Proportion of kernel trait QTLs detected in one or more environments

Trait	No. and proportion of QTLs		
	1 Env (%)	2 Env (%)	≥ 3 Env* (%)
TKW (16)	10 (62.5)	3 (18.8)	3 (18.8)
KL (8)	01 (12.5)	3 (37.5)	4 (50.0)
KW (8)	02 (25.0)	5 (62.5)	1 (12.5)
LWR (12)	09 (75.0)	1 (08.3)	2 (16.7)
FFD (15)	10 (66.7)	4 (26.7)	1 (06.6)
Total (59)	32 (54.2)	16 (27.1)	11 (18.6)

Env: Environment; % percentage of total QTLs; * Stable QTL

Table 3.13: Contribution of QTLs from the bold-grained parent RS and small-grained parent CS

Trait	Total QTLs	Source of favorable alleles									
		RS					CS				
		Com	Lud	Pun	Avg	Total	Com	Lud	Pun	Avg	Total
TKW	16	4	2	3	1	**10**	-	5	1	-	**6**
KL	08	3	-	3	-	**6**	-	-	2	-	**2**
KW	08	1	-	2	-	**3**	1	3	1	-	**5**
LWR	12	-	4	5	-	**9**	1	1	1	-	**3**
FFD	15	-	3	5	-	**8**	1	4	2	-	**7**
Total	59	8	9	18	1	**36**	3	13	7	-	**23**
% contribution		22.2	25.0	50.0	2.8	100	13.0	56.5	30.4	0.0	100
Contribution of QTLs (%)						**61.02**					**38.98**

Com: Common - QTLs detected both in Ludhiana and Pune; Lud: Ludhiana;

Pun: Pune; Avg: Average across environments

Table 3.14: Distribution of QTLs in the RS×CS population

Chromosomes	Number of QTLs						
	TKW	KL	KW	LWR	FFD	Total	Contribution (%)
A genome	4	4	1	3	2	14	23.73
B genome	5	1	4	4	5	19	32.20
D genome	7	3	3	5	8	26	44.07
Group 1	2	-	-	-	1	3	5.08
Group 2	9	2	4	3	5	23	38.98
Group 3	1	-	-	1	1	3	5.08
Group 4	-	1	-	1	3	5	8.47
Group 5	1	1	2	2	2	8	13.56
Group 6	2	2	-	3	2	9	15.25
Group 7	1	2	2	2	1	8	13.56

Contribution: To the total number of QTLs

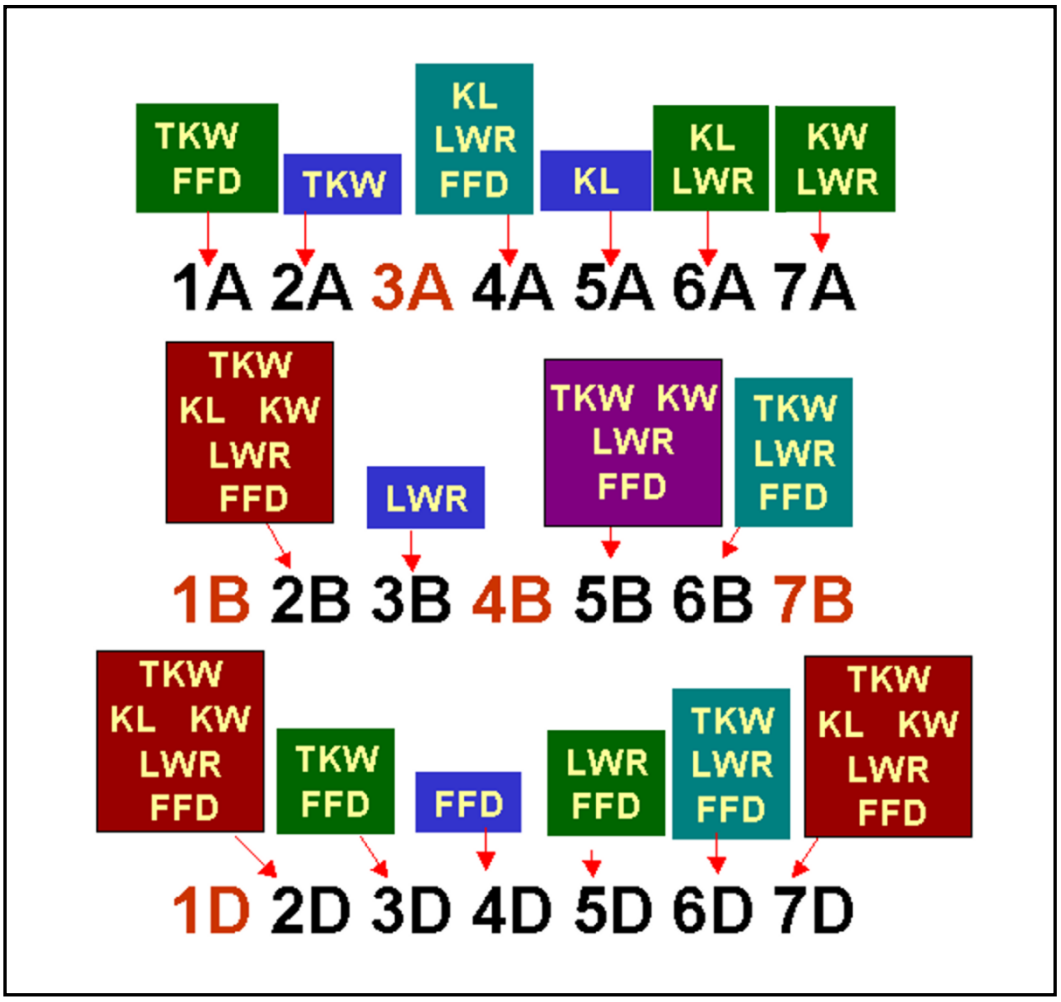


Figure 3.5: Distribution of kernel character QTLs in the RS×CS population detected by composite interval mapping. Chromosomes in red color did not show QTL for any of the kernel traits. Colored boxes display the traits for which the respective chromosomes harbored QTLs. Where QTLs for all the five kernel traits were detected, brown boxes are shown. Similarly, purple box represents four traits followed by ocean green, green and dark blue with QTLs for three, two and one trait, respectively.

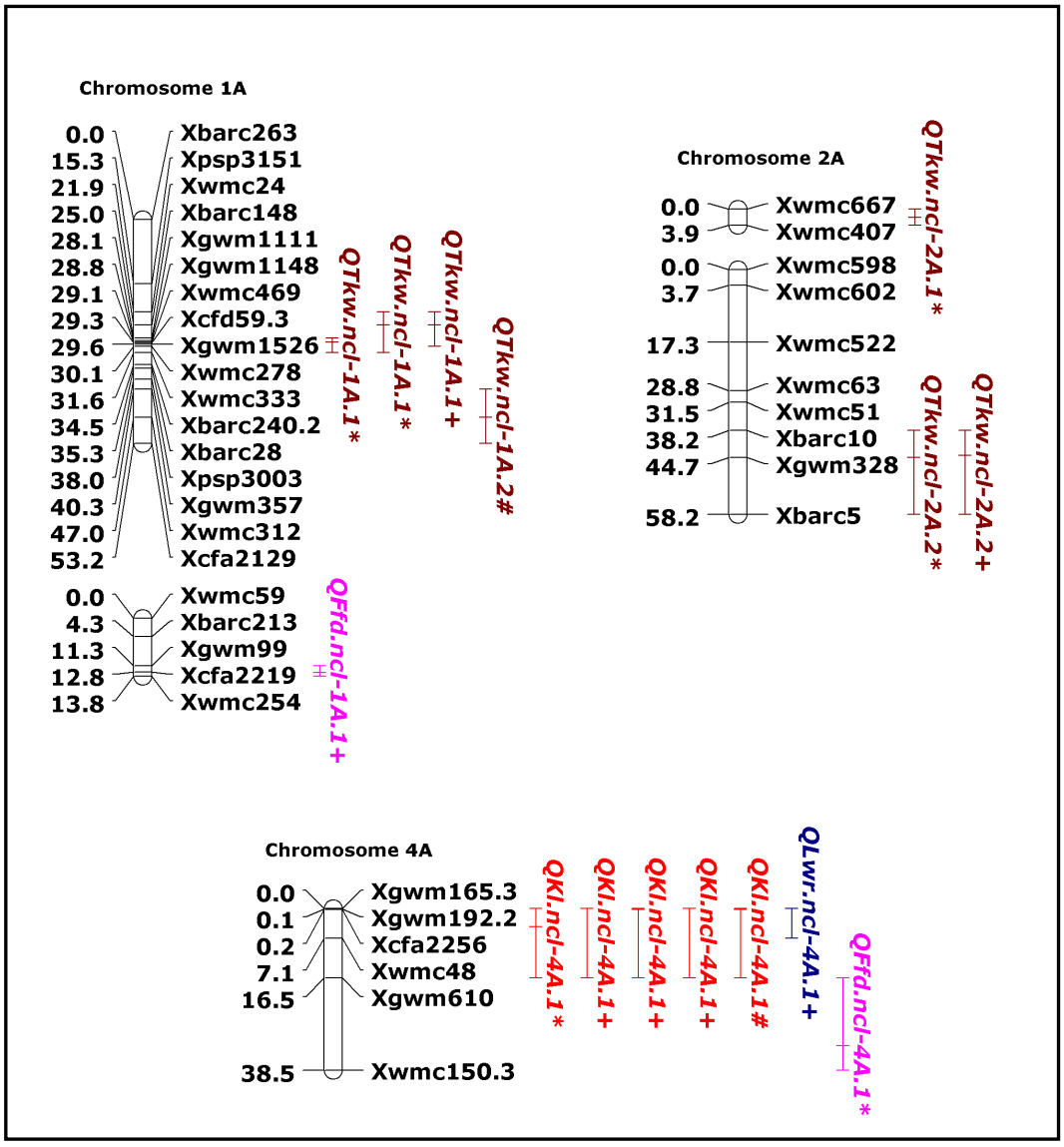


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population. QTL support intervals as vertical bars are shown on the right side of linkage groups. The horizontal mark on the QTL bars indicates the closest marker. Each trait is given a separate color – **Brown-TKW**, **Red-KL**, **Green-KW**, **Blue-LWR** and **Pink-FFD**. *QTL detected for Ludhiana location, + Pune and # Average across environments.

Figure 3.6: Continued

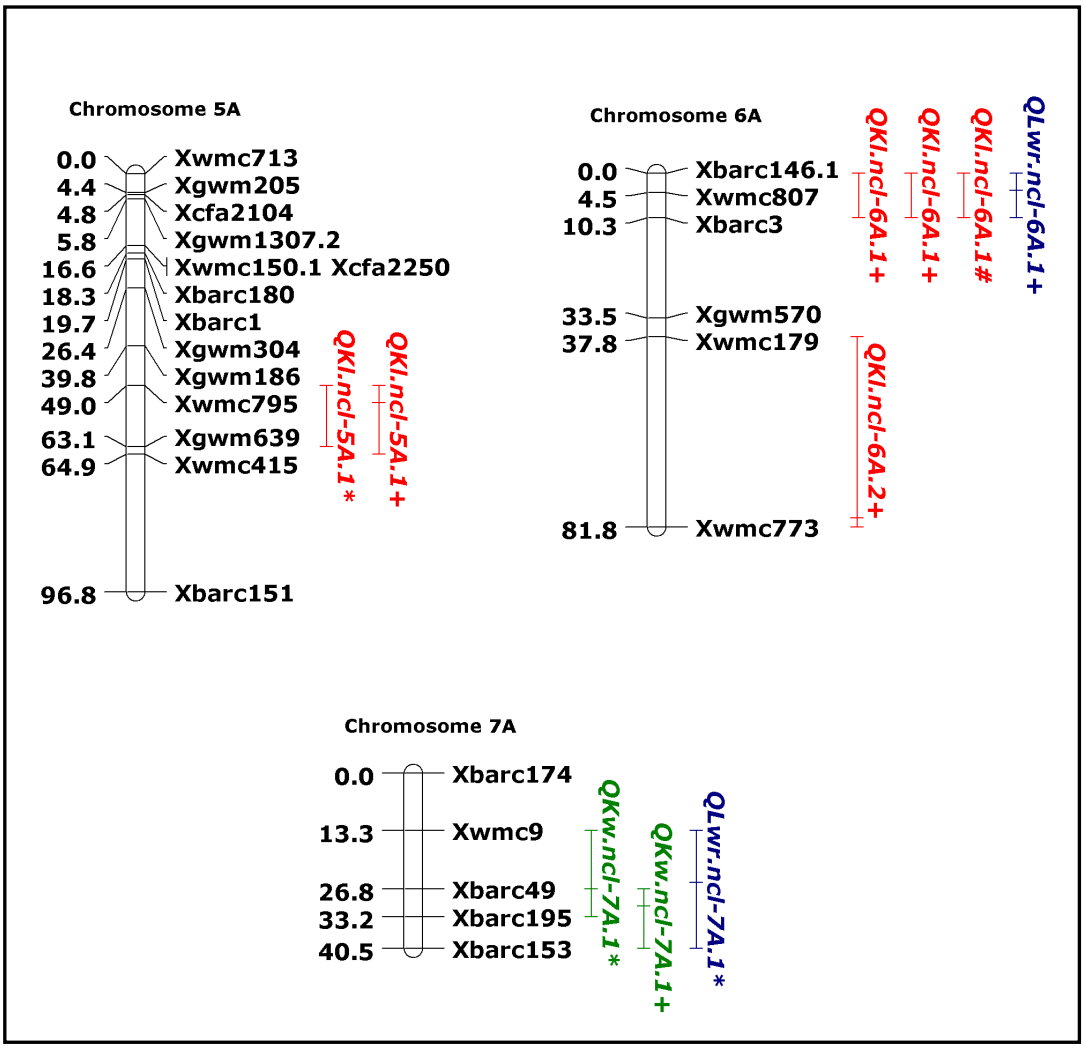


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population

Figure 3.6: Continued

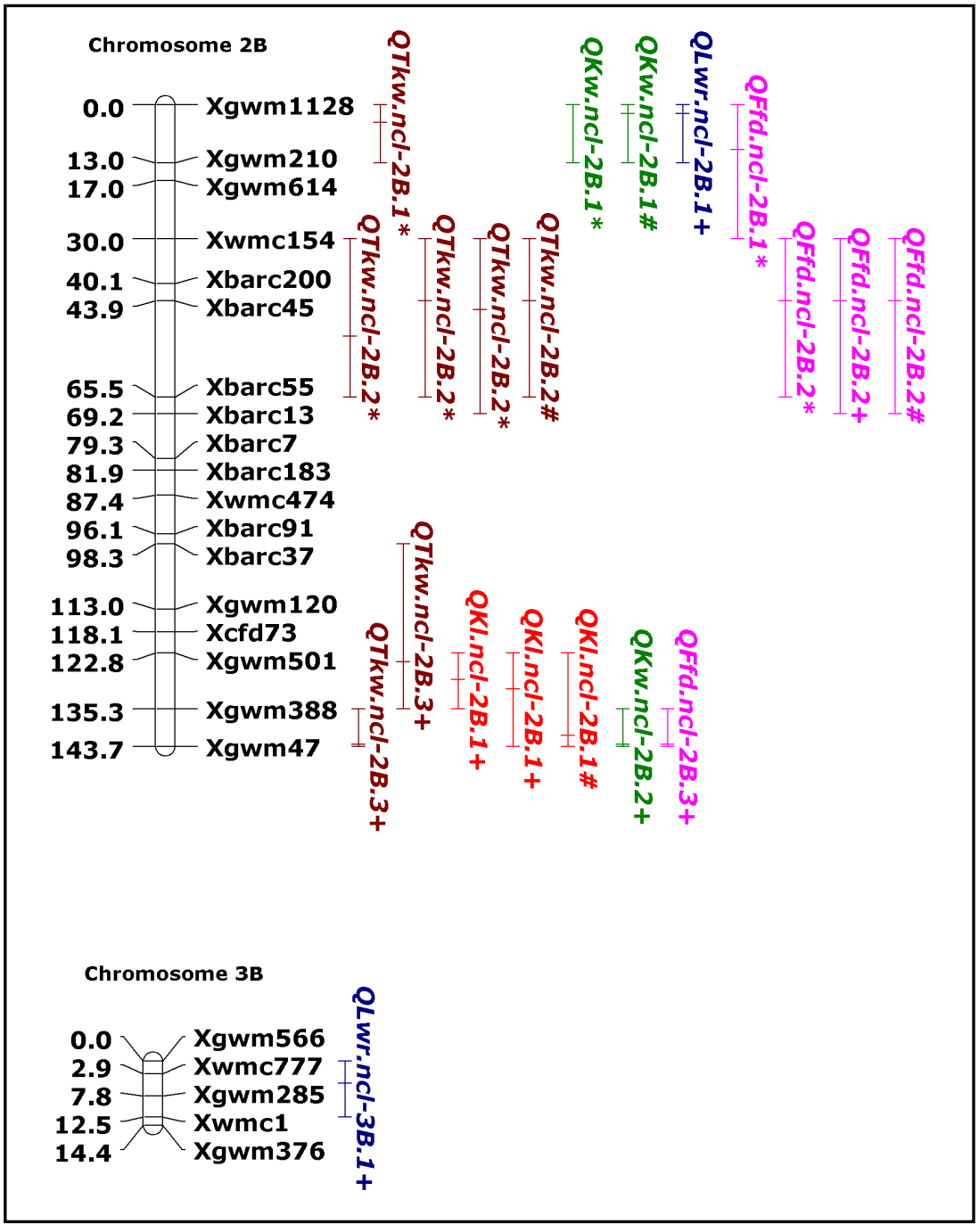


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population

Figure 3.6: Continued

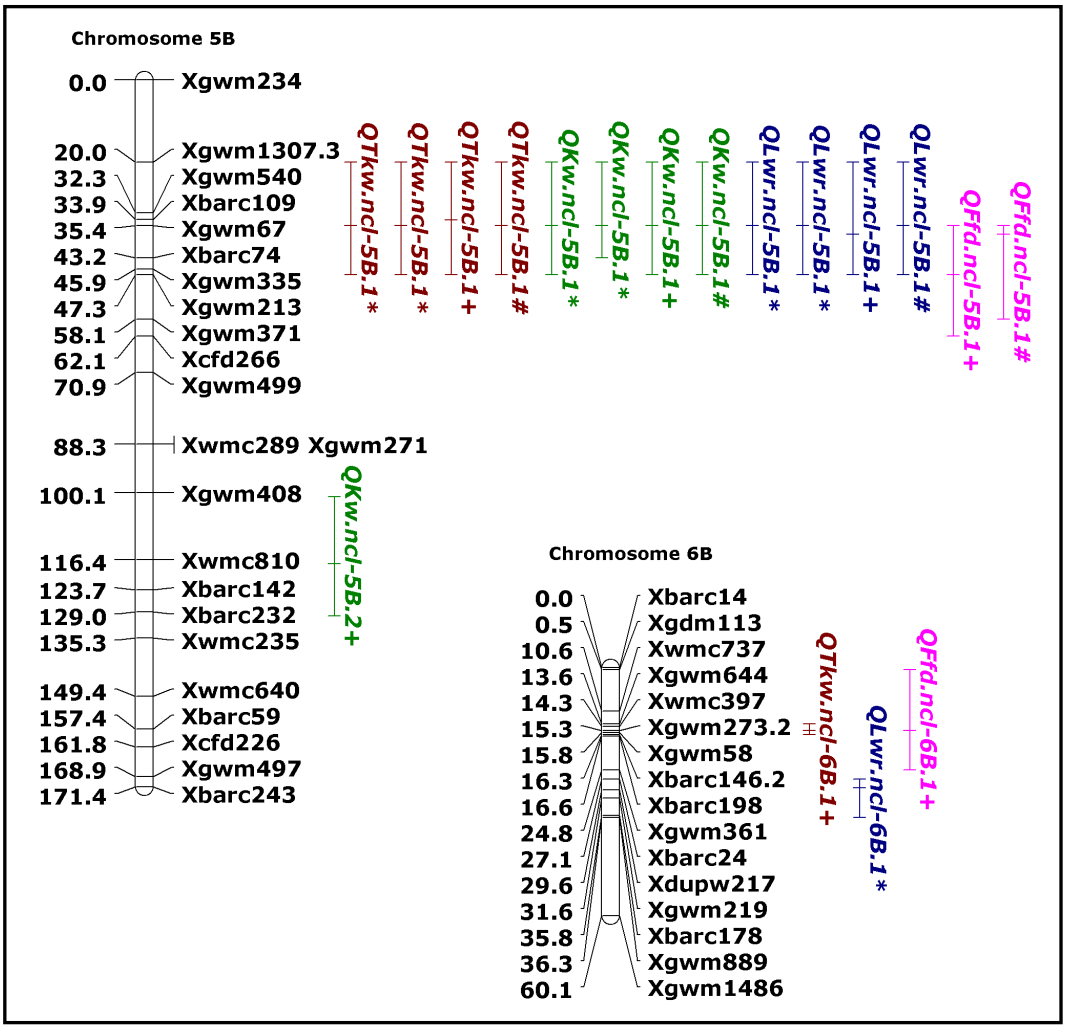


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population

Figure 3.6: Continued

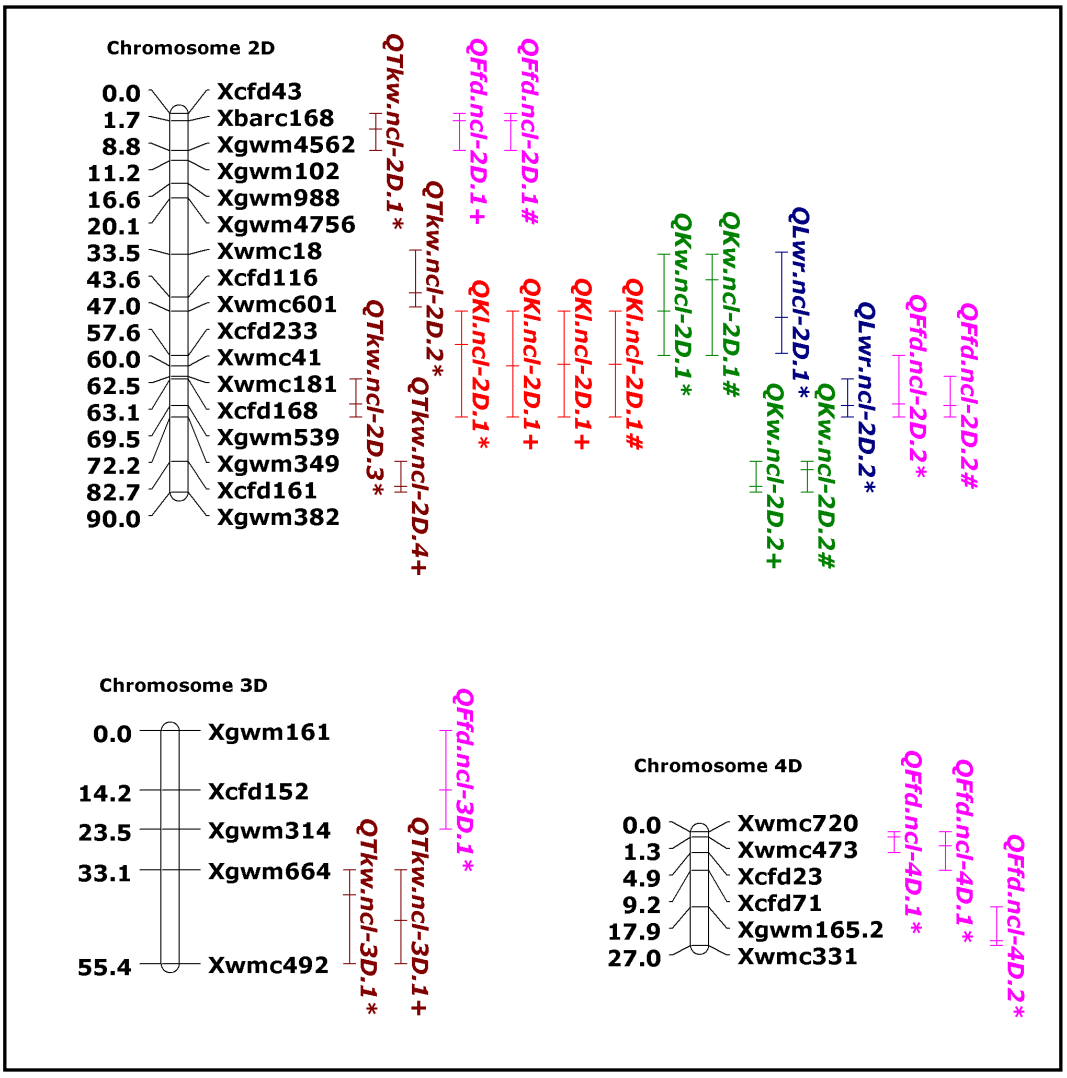


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population

Figure 3.6: Continued

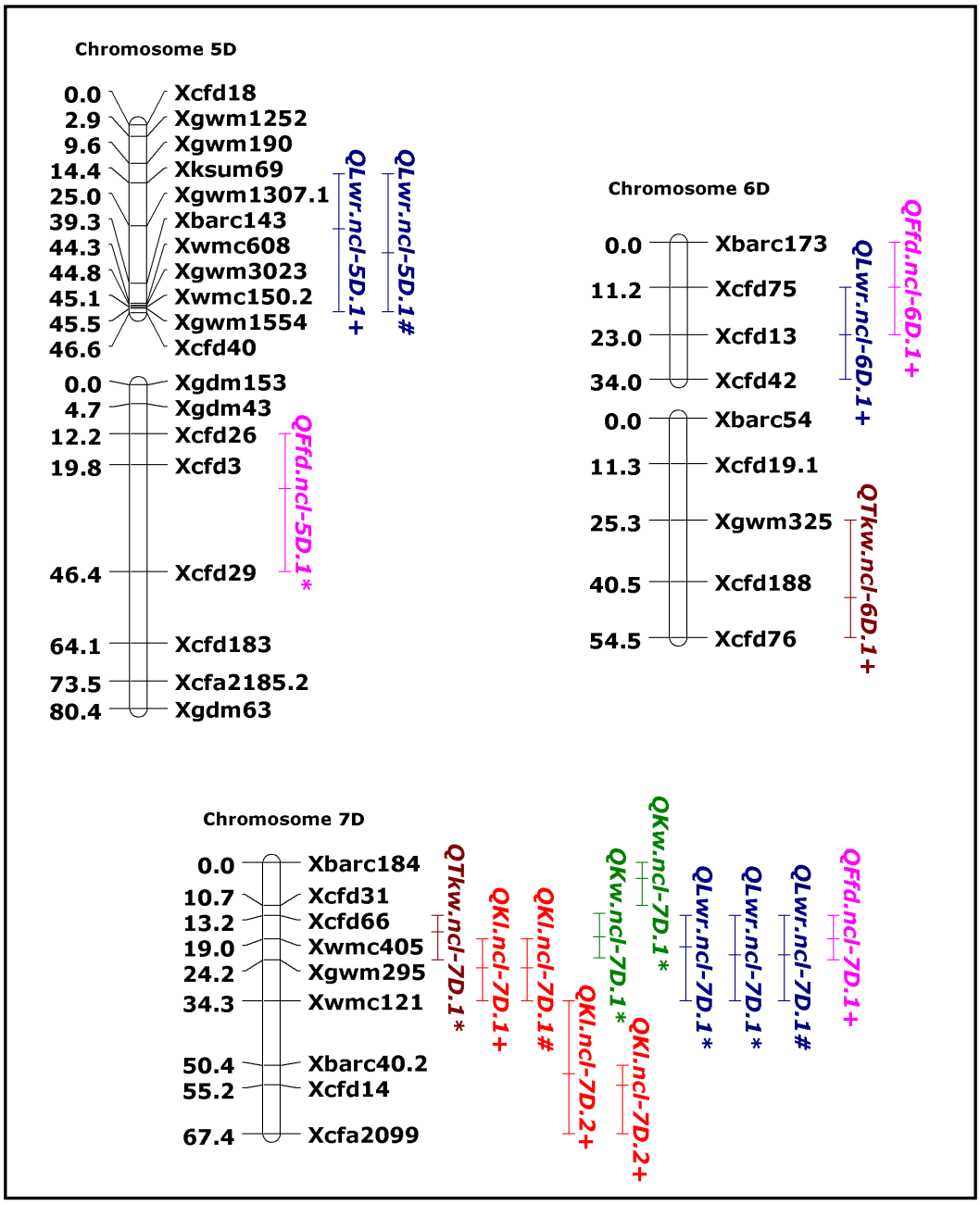


Figure 3.6: Distribution of kernel trait QTLs on the linkage map of the RS×CS population

Table 3.15: Composite interval mapping of Thousand kernel weight

QTL	Chr	Environment	Flanking markers		Closest marker	Position (cM)	LOD	Additive Effect	$R^2 \times 100$
			Left	Right					
QTkw.ncl-1A.1	1A	TKW2000Lud	*Xgwm1111*	*Xwmc333*	*Xwmc469*	29.13	2.56	1.78	5.63
QTkw.ncl-1A.1	1A	TKW2007Lud	*Xwmc24*	*Xwmc333*	*Xbarc148*	24.97	3.06	1.75	5.79
QTkw.ncl-1A.1	1A	TKW2006Pun	*Xwmc24*	*Xwmc278*	*Xbarc148*	24.97	2.25	1.43	4.89
QTkw.ncl-1A.2	1A	TKW average	*Xgwm357*	*Xcfa2129*	*Xwmc312*	47.01	2.12	1.05	4.16
QTkw.ncl-2A.1	2A	TKW2000Lud	*Xwmc667*	*Xwmc807*	*Xwmc667*	2.01	2.59	1.89	6.23
QTkw.ncl-2A.2	2A	TKW2005Lud	*Xbarc10*	*Xbarc5*	*Xgwm328*	44.68	2.55	1.29	5.38
QTkw.ncl-2A.2	2A	TKW2009Pun	*Xbarc10*	*Xbarc5*	*Xbarc10*	44.21	2.02	1.58	5.17
QTkw.ncl-2B.1	2B	TKW2003Lud	*Xgwm1128*	*Xgwm210*	*Xgwm1128*	4.01	3.49	-2.30	10.21
QTkw.ncl-2B.2	2B	TKW2000Lud	*Xwmc154*	*Xbarc55*	*Xbarc45*	51.92	3.99	-2.83	14.05
QTkw.ncl-2B.2	2B	TKW2005Lud	*Xwmc154*	*Xbarc55*	*Xbarc45*	43.92	4.86	-1.84	10.50
QTkw.ncl-2B.2	2B	TKW2007Lud	*Xwmc154*	*Xbarc13*	*Xbarc45*	45.92	5.71	-2.71	13.54
QTkw.ncl-2B.2	2B	TKW average	*Xwmc154*	*Xbarc55*	*Xbarc45*	43.92	4.83	-1.67	10.14
QTkw.ncl-2B.3	2B	TKW2005Pun	*Xgwm388*	*Xgwm47*	*Xgwm388*	143.32	5.99	-3.14	14.23
QTkw.ncl-2B.3	2B	TKW2009Pun	*Xbarc37*	*Xgwm388*	*Xgwm501*	124.78	2.61	-1.84	7.05
QTkw.ncl-2D.1	2D	TKW2005Lud	*Xcfd43*	*Xgwm4562*	*Xbarc168*	3.74	2.24	1.35	5.97
QTkw.ncl-2D.2	2D	TKW2003Lud	*Xwmc18*	*Xwmc601*	*Xcfd116*	43.56	2.43	-1.71	5.58
QTkw.ncl-2D.3	2D	TKW2005Lud	*Xcfd168*	*Xgwm349*	*Xcfd168*	69.09	3.54	-1.55	7.60
QTkw.ncl-2D.4	2D	TKW2005Pun	*Xcfd161*	*Xgwm382*	*Xcfd161*	88.67	2.46	1.73	5.41
QTkw.ncl-3D.1	3D	TKW2007Lud	*Xgwm314*	*Xwmc492*	*Xgwm664*	39.14	2.38	1.87	6.62
QTkw.ncl-3D.1	3D	TKW2009Pun	*Xgwm314*	*Xwmc492*	*Xgwm664*	45.14	2.23	2.04	8.75
QTkw.ncl-5B.1	5B	TKW2003Lud	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	3.18	1.96	7.42
QTkw.ncl-5B.1	5B	TKW2005Lud	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	2.76	1.31	5.57
QTkw.ncl-5B.1	5B	TKW2007Pun	*Xgwm1307.3*	*Xgwm213*	*Xbarc109*	33.96	3.43	1.52	7.31
QTkw.ncl-5B.1	5B	TKW average	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	2.72	1.20	5.45
QTkw.ncl-6B.1	6B	TKW2005Pun	*Xgwm644*	*Xbarc146.2*	*Xgwm273.2*	15.26	2.90	1.75	5.55
QTkw.ncl-6D.1	6D	TKW2005Pun	*Xgwm325*	*Xcfd76*	*Xcfd188*	44.47	2.08	1.62	4.89
QTkw.ncl-7D.1	7D	TKW2003Lud	*Xcfd66*	*Xgwm295*	*Xcfd66*	17.16	3.34	-2.45	10.16

Table 3.16: Composite interval mapping of Kernel length

QTL	Chr	Environment	Flanking markers		Closest marker	Position (cM)	LOD	Additive Effect	$R^2 \times 100$
			Left	Right					
QKl.ncl-2B.1	2B	KL2005Pun	*Xgwm501*	*Xgwm388*	*Xgwm501*	128.78	2.25	-0.09	6.37
QKl.ncl-2B.1	2B	KL2007Pun	*Xgwm501*	*Xgwm47*	*Xgwm501*	130.78	2.39	-0.10	7.69
QKl.ncl-2B.1	2B	KL average	*Xgwm501*	*Xgwm47*	*Xgwm388*	141.32	2.21	-0.08	6.64
QKl.ncl-2D.1	2D	KL2003Lud	*Xwmc601*	*Xgwm349*	*Xwmc601*	55.01	5.61	0.16	17.14
QKl.ncl-2D.1	2D	KL2005Pun	*Xwmc601*	*Xgwm349*	*Xwmc41*	60.04	5.24	0.11	9.71
QKl.ncl-2D.1	2D	KL2006Pun	*Xwmc601*	*Xgwm349*	*Xcfd233*	59.64	4.51	0.12	8.85
QKl.ncl-2D.1	2D	KL average	*Xwmc601*	*Xgwm349*	*Xcfd233*	59.64	6.33	0.10	12.61
QKl.ncl-4A.1	4A	KL2005Lud	*Xgwm165.3*	*Xgwm610*	*Xcfa2256*	4.25	3.82	0.09	9.52
QKl.ncl-4A.1	4A	KL2005Pun	*Xgwm165.3*	*Xgwm610*	*Xgwm165.3*	0.01	3.53	0.09	6.35
QKl.ncl-4A.1	4A	KL2006Pun	*Xgwm165.3*	*Xgwm610*	*Xcfa2256*	0.25	6.35	0.13	12.22
QKl.ncl-4A.1	4A	KL2007Pun	*Xgwm165.3*	*Xgwm610*	*Xgwm165.3*	0.01	2.48	0.08	5.39
QKl.ncl-4A.1	4A	KL average	*Xgwm165.3*	*Xgwm610*	*Xcfa2256*	0.25	5.82	0.09	10.90
QKl.ncl-5A.1	5A	KL2003Lud	*Xgwm186*	*Xgwm639*	*Xwmc795*	49.04	2.08	0.09	4.84
QKl.ncl-5A.1	5A	KL2007Pun	*Xgwm186*	*Xwmc415*	*Xwmc795*	53.04	2.16	0.08	6.02
QKl.ncl-6A.1	6A	KL2005Pun	*Xbarc146.1*	*Xbarc3*	*Xbarc146.1*	0.01	3.25	0.08	5.89
QKl.ncl-6A.1	6A	KL2006Pun	*Xbarc146.1*	*Xbac3*	*Xbarc146.1*	0.01	2.33	0.08	4.28
QKl.ncl-6A.1	6A	KL average	*Xbarc146.1*	*Xbarc3*	*Xbarc146.1*	0.01	3.41	0.07	6.20
QKl.ncl-6A.2	6A	KL2007Pun	*Xwmc179*	*Xwmc773*	*Xwmc179*	79.77	2.06	-0.09	6.06
QKl.ncl-7D.1	7D	KL2006Pun	*Xwmc405*	*Xwmc121*	*Xgwm295*	26.24	3.64	0.13	9.22
QKl.ncl-7D.1	7D	KL average	*Xwmc405*	*Xwmc121*	*Xgwm295*	26.24	2.90	0.08	7.40
QKl.ncl-7D.2	7D	KL2007Pun	*Xwmc121*	*Xcfa2099*	*Xbarc40.2*	52.46	2.23	0.08	5.44
QKl.ncl-7D.2	7D	KL2005Pun	*Xbarc40.2*	*Xcfa2099*	*Xcfd14*	55.25	2.89	0.08	5.29

Table 3.17: Composite interval mapping of Kernel width

QTL	Chr	Environment	Flanking markers		Closest marker	Position (cM)	LOD	Additive Effect	$R^2 \times 100$
			Left	Right					
QKw.ncl-2B.1	2B	KW2003Lud	*Xgwm1128*	*Xgwm210*	*Xgwm1128*	0.01	2.03	-0.06	3.67
QKw.ncl-2B.1	2B	KW average	*Xgwm1128*	*Xgwm210*	*Xgwm1128*	2.01	3.28	-0.06	8.11
QKw.ncl-2B.2	2B	KW2006Pun	*Xgwm388*	*Xgwm47*	*Xgwm388*	143.32	2.66	-0.09	6.65
QKw.ncl-2D.1	2D	KW2003Lud	*Xwmc18*	*Xcfd233*	*Xwmc601*	47.01	3.34	-0.08	5.90
QKw.ncl-2D.1	2D	KW average	*Xwmc18*	*Xcfd233*	*Xwmc18*	39.56	3.28	-0.06	8.96
QKw.ncl-2D.2	2D	KW2005Pun	*Xcfd161*	*Xgwm382*	*Xcfd161*	88.67	2.16	0.07	5.41
QKw.ncl-2D.2	2D	KW average	*Xgwm349*	*Xgwm382*	*Xcfd161*	84.67	2.04	0.05	5.12
QKw.ncl-5B.1	5B	KW2003Lud	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	5.43	0.10	9.88
QKw.ncl-5B.1	5B	KW2005Lud	*Xgwm1307.3*	*Xbarc74*	*Xgwm67*	35.44	2.04	0.08	4.44
QKw.ncl-5B.1	5B	KW2007Pun	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	2.28	0.05	5.07
QKw.ncl-5B.1	5B	KW average	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	3.94	0.06	8.31
QKw.ncl-5B.2	5B	KW2006Pun	*Xgwm408*	*Xbarc232*	*Xwmc810*	116.40	2.93	0.07	6.18
QKw.ncl-7A.1	7A	KW2003Lud	*Xwmc9*	*Xbarc195*	*Xbarc49*	26.76	2.85	-0.08	5.09
QKw.ncl-7A.1	7A	KW2006Pun	*Xbarc49*	*Xbarc153*	*Xbarc49*	30.76	2.26	-0.07	5.73
QKw.ncl-7D.1	7D	KW2003Lud	*Xcfd66*	*Xgwm295*	*Xwmc405*	18.97	6.11	-0.12	11.26
QKw.ncl-7D.1	7D	KW2005Lud	*Xbarc184*	*Xcfd31*	*Xbarc184*	4.01	3.01	-0.12	9.82

Table 3.18: Composite interval mapping of Kernel length-width ratio

QTL	Chr	Environment	Flanking markers		Closest marker	Position (cM)	LOD	Additive Effect	$R^2 \times 100$
			Left	Right					
QLwr.ncl-2B.1	2B	LWR2006Pun	*Xgwm1128*	*Xgwm210*	*Xgwm1128*	2.01	3.43	0.07	8.26
QLwr.ncl-2D.1	2D	LWR2003Lud	*Xwmc18*	*Xcfd233*	*Xwmc601*	49.01	8.57	0.10	18.09
QLwr.ncl-2D.2	2D	LWR2005Lud	*Xcfd168*	*Xgwm349*	*Xgwm539*	69.47	2.99	0.07	6.67
QLwr.ncl-3B.1	3B	LWR2005Pun	*Xgwm566*	*Xwmc1*	*Xwmc777*	4.89	2.24	0.05	5.44
QLwr.ncl-4A.1	4A	LWR2007Pun	*Xgwm165.3*	*Xwmc48*	*Xgwm165.3*	0.01	2.57	0.05	5.41
QLwr.ncl-5B.1	5B	LWR2003Lud	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	5.77	-0.07	9.63
QLwr.ncl-5B.1	5B	LWR2005Lud	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	3.35	-0.07	7.05
QLwr.ncl-5B.1	5B	LWR2007Pun	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	37.44	2.07	-0.05	5.05
QLwr.ncl-5B.1	5B	LWR average	*Xgwm1307.3*	*Xgwm213*	*Xgwm67*	35.44	4.93	-0.05	10.67
QLwr.ncl-5D.1	5D	LWR2005Pun	*Xcfd26*	*Xcfd29*	*Xcfd26*	25.82	2.95	-0.06	10.31
QLwr.ncl-5D.1	5D	LWR average	*Xcfd26*	*Xcfd29*	*Xcfd3*	31.82	2.22	-0.05	7.72
QLwr.ncl-6A.1	6A	LWR2006Pun	*Xbarc146.1*	*Xbarc3*	*Xbarc146.1*	4.01	2.39	0.06	5.11
QLwr.ncl-6B.1	6B	LWR2003Lud	*Xbarc24*	*Xgwm889*	*Xbarc24*	29.13	2.09	-0.05	4.29
QLwr.ncl-6D.1	6D	LWR2005Pun	*Xcfd75*	*Xcfd42*	*Xcfd13*	23.00	2.59	0.05	6.00
QLwr.ncl-7A.1	7A	LWR2003Lud	*Xwmc9*	*Xbarc153*	*Xwmc9*	25.34	3.40	0.06	6.41
QLwr.ncl-7D.1	7D	LWR2003Lud	*Xcfd66*	*Xwmc121*	*Xwmc405*	20.97	9.48	0.11	19.08
QLwr.ncl-7D.1	7D	LWR2005Lud	*Xcfd66*	*Xwmc121*	*Xwmc405*	22.97	3.12	0.09	8.21
QLwr.ncl-7D.1	7D	LWR average	*Xcfd66*	*Xwmc121*	*Xwmc405*	22.97	4.26	0.06	9.42

Table 3.19: Composite interval mapping of Kernel factor form density

QTL	Chr	Environment	Flanking markers		Closest marker	Position (cM)	LOD	Additive effect	R^2×100
			Left	Right					
QFfd.ncl-1A.1	1A	FFD2007Pun	*Xgwm99*	*Xwmc254*	*Xcfa2219*	12.85	2.16	0.06	4.40
QFfd.ncl-2B.1	2B	FFD2003Lud	*Xgwm1128*	*Xgwm154*	*Xgwm1128*	10.01	2.12	-0.05	5.53
QFfd.ncl-2B.2	2B	FFD2005Lud	*Xwmc154*	*Xbarc55*	*Xbarc45*	43.92	2.93	-0.08	6.61
QFfd.ncl-2B.2	2B	FFD2007Pun	*Xwmc154*	*Xbarc13*	*Xbarc45*	43.92	3.97	-0.09	8.54
QFfd.ncl-2B.2	2B	FFD average	*Xwmc154*	*Xbarc13*	*Xbarc45*	43.92	5.58	-0.06	11.91
QFfd.ncl-2B.3	2B	FFD2005Pun	*Xgwm388*	*Xgwm47*	*Xgwm388*	143.32	5.15	-0.10	11.41
QFfd.ncl-2D.1	2D	FFD2005Pun	*Xcfd43*	*Xgwm4562*	*Xbarc168*	1.74	3.48	0.07	6.75
QFfd.ncl-2D.1	2D	FFD average	*Xcfd43*	*Xgwm4562*	*Xbarc168*	1.74	2.65	0.04	5.33
QFfd.ncl-2D.2	2D	FFD2005Lud	*Xcfd233*	*Xgwm349*	*Xcfd168*	69.09	2.14	-0.07	4.77
QFfd.ncl-2D.2	2D	FFD average	*Xwmc181*	*Xgwm349*	*Xgwm539*	69.47	2.47	-0.04	4.91
QFfd.ncl-3D.1	3D	FFD2003Lud	*Xgwm161*	*Xgwm314*	*Xcfd152*	14.19	2.03	0.05	4.15
QFfd.ncl-4A.1	4A	FFD2003Lud	*Xgwm610*	*Xwmc150.3*	*Xgwm610*	32.55	2.14	-0.06	7.14
QFfd.ncl-4D.1	4D	FFD2003Lud	*Xwmc720*	*Xcfd23*	*Xwmc473*	1.29	2.49	-0.05	5.16
QFfd.ncl-4D.1	4D	FFD2005Lud	*Xwmc720*	*Xcfd71*	*Xwmc473*	3.29	2.99	-0.09	7.51
QFfd.ncl-4D.2	4D	FFD2005Lud	*Xgwm165.2*	*Xwmc331*	*Xwmc165.2*	25.92	3.12	0.09	7.50
QFfd.ncl-5B.1	5B	FFD2007Pun	*Xgwm67*	*Xcfd266*	*Xgwm213*	47.32	2.61	0.07	5.27
QFfd.ncl-5B.1	5B	FFD average	*Xgwm67*	*Xgwm371*	*Xgwm67*	37.44	3.07	0.05	7.03
QFfd.ncl-5D.1	5D	FFD2003Lud	*Xcfd26*	*Xcfd29*	*Xcfd3*	25.82	2.65	0.07	9.02
QFfd.ncl-6B.1	6B	FFD2005Pun	*Xgdm113*	*Xgwm361*	*Xgwm273.2*	15.26	4.04	0.07	7.88
QFfd.ncl-6D.1	6D	FFD2006Pun	*Xbarc173*	*Xcfd13*	*Xcfd75*	11.21	2.17	0.05	5.17
QFfd.ncl-7D.1	7D	FFD2007Pun	*Xcfd66*	*Xgwm295*	*Xwmc405*	18.97	2.77	-0.08	5.80

3.6.1.7 QTL clusters for kernel characters

When the chromosomal regions harboring QTLs for various kernel characters were examined, chromosomes 2D and 7D showed overlap in the regions *Xwmc18-Xgwm539* and *Xbarc184-Xwmc121*, respectively, for all the five kernel characters, whereas a 13 cM region on 2B between the markers *Xgwm1128-Xgwm210* and a 26 cM region between *Xgwm1307.3-Xgwm213* on chromosome 5B (**Figure 3.7**) supported QTLs for TKW, KW, LWR and FFD. In addition to this, a common genomic region *Xgwm501-Xgwm47* with QTLs for TKW, KL, KW and FFD was detected on chromosome 2B (**Figure 3.6**). Many chromosomes indicated common QTL regions for combinations of two kernel characters. TKW and FFD QTLs showed overlap on 2B, 2D and 6B, while common KL and LWR QTLs regions were detected on 4A and 6A. LWR indicated a coincident QTL on 6D with FFD and with KW on 7A. However, apart from this, there were few intervals, which contributed only to individual traits (**Figure 3.6**). Chromosomes 1A, 2A, 3D and 6D showed QTLs for TKW, which did not share the support intervals with any other trait. Such differential QTLs for KL were detected on 5A, 6A and 7D. For KW, *QKw.ncl-5B.2* did not share its support interval with any other trait QTL. Though LWR and FFD were derived from the trait values of TKW, KL and KW, some of their QTLs showed positions independent of the positions of TKW, KL and KW. Such differential QTLs for LWR were displayed on the chromosomes 3B, 5D and 6B; and for FFD on 1A, 3D, 4A, 4D and 5D in the RS×CS population.

3.6.2 QTL analysis of kernel characters based on mixed linear model

A mixed-linear model-based QTL mapping methodology using the software QTLNetwork v. 2.0 (Yang and Zhu, 2007) was used to detect main-effect QTLs with significant additive effects, QTL×QTL interactions and interactions of QTLs with environment. For TKW, eight year-location combinations and for the other kernel traits, five year-location combinations were involved in QTL analysis. In all, 19 main-effect QTLs located on the chromosomes 2B, 2D, 4A, 5B, 6A and 7D were detected, of which five indicated Q×E interactions. QTL×QTL interactions were detected for all the five kernel traits, which were largely between QTLs that

did not show significant individual effects. Some of the chromosomes indicated main-effect and epistatic QTLs

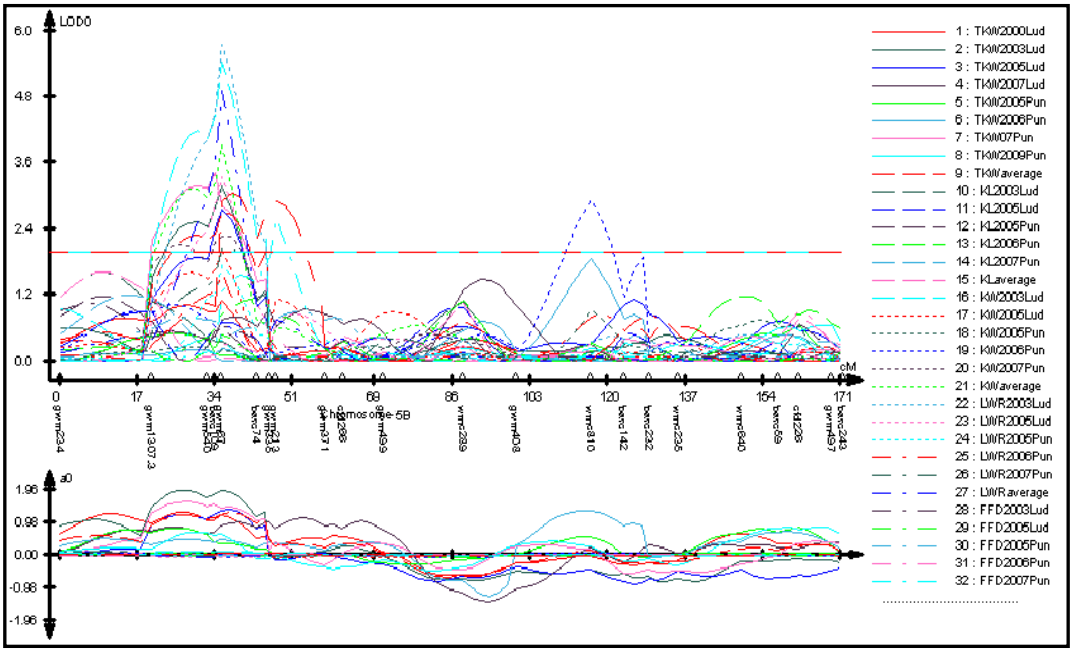


Figure 3.7: A QTL cluster for kernel characters on chromosome 5B

for more than one trait, with their support intervals indicating varying degrees of overlap. The short-arm of chromosome 2B was noteworthy, which harbored main-effect QTLs for TKW, KW, LWR and FFD and epistatic QTLs for TKW, KL and KW. The relative distribution of main-effect and epistatic QTLs on the 21 wheat chromosomes is shown in **Figure 3.8**. The details of this analysis for each kernel trait are given below. The map locations of QTLs detected by QTLNetwork as well as their relative positions with the loci detected by CIM analysis were analyzed for each of the five kernel traits and are also described here.

3.6.2.1 Thousand kernel weight

For TKW, chromosomes 2B and 5B together indicated three main-effect QTLs (**Table 3.20, Figure 3.9**). The main-effect QTL on 2BL indicated environmental interactions at Ludhiana (year 2005) and Pune (year 2005). CS contributed the alleles that increased TKW to the 2B QTLs, whereas, the 5B main-effect QTL derived the superior alleles from RS. TKW indicated the highest number of seven epistatic interactions of which, the main-effect QTL on 2B in the marker interval

Xbarc45-Xbarc55 showed interaction with minor QTLs on 1D and 2D (**Table 3.21, Figure 3.9**). When the relative distribution of QTLs detected by QTLNetwork and QTL Cartographer was considered (**Figure 3.10**), the main-effect QTLs on 2B and 5B showed overlap with the TKW stable QTLs *QTkw.ncl-2B.2* and *QTkw.ncl-5B.1* detected by CIM, respectively. Whereas, only two epistatic QTLs located on 2B and another on 6D indicated partial overlap with the support intervals of QTLs detected by CIM in the respective regions.

3.6.2.2 Kernel length

Kernel length indicated six main-effect QTLs, the highest among the kernel characters (**Table 3.22, Figure 3.11**). Of these, a QTL located in the marker interval *Xbarc40.2-Xcfd14* on 7D showed interaction with environment at Ludhiana (year 2000). The poor parent CS contributed a main- effect QTL on 2B, while the others were derived from RS. Two QTL×QTL interactions were observed, both between minor QTLs located on 2B and 6B (**Table 3.23, Figure 3.11**). Influence of the environment on an epistatic interaction between minor QTLs in the marker intervals *Xwmc317-Xbarc159* (2B) and *Xbarc178-Xgwm88* (6B) was observed at Ludhiana (year 2000). The main-effect QTLs for KL showed co-location with QTLs detected in multiple environments for KL by CIM analysis (**Figure 3.12**), whereas, there was no correspondence between the support intervals of any of the epistatic QTLs and QTLs detected by CIM.

3.6.2.3 Kernel width

Three main-effect QTLs were detected for KW located on chromosomes 2B, 5B and 7D, respectively (**Table 3.24, Figure 3.13**). The QTL harbored by the interval *Xwmc405-Xgwm295* on chromosome 7D showed QTL × Environment interactions in two environments. The 5B main-effect QTL had the origin of its superior alleles in RS, while the same for the 2B and 7D QTLs was from the inferior parent CS. In addition to these QTLs, a single pair of epistatically interacting QTLs located on chromosomes 1D and 2B was also identified (**Table 3.25, Figure 3.13**). Similar to KL, some of the QTLs detected by CIM in multiple environments showed overlap with the main-effect QTLs detected for KW in the

respective regions, while the epistatic QTLs were located in chromosomal regions that were distinct from any of the QTL regions detected by CIM (**Figure 3.14**).

3.6.2.4 Length-width ratio

Out of the five main-effect QTLs detected for LWR, two indicated Q×E interactions (**Table 3.26, Figure 3.15**). The QTL on chromosome 2B indicated environmental interaction at Pune (year 2006) and the other on 7D at Ludhiana (year 2000), while both of them interacted with the environment at Pune (year 2007). Alleles that increased LWR were derived from the inferior parent CS for the main-effect QTL on 5B, whereas, for the other four QTLs, RS contributed the superior alleles. Epistatic QTLs were also identified for LWR, with two pairs between chromosomes 2A and 5B and between 4A and 7D, respectively (**Table 3.27, Figure 3.15**). Two of the five main-effect QTLs detected for LWR coincided with stable LWR QTLs identified by CIM in the respective chromosomal regions while, none of the epistatic QTLs shared support intervals with QTLs identified by CIM for the same trait (**Figure 3.16**).

3.6.2.5 Factor form density

Compared to the other kernel traits, the number of main-effect QTLs detected was lower, with two QTLs, one each on 2B and 2D (**Table 3.28, Figure 3.17**). The 2B main-effect QTL originated from CS, while the superior alleles for the QTL on 2D were derived from RS. Two epistatic interactions, between minor QTLs on chromosomes 1B and 5B, and between 1D and 7B were identified that did not show environmental influences (**Table 3.29, Figure 3.17**). The two main-effect QTLs identified for FFD showed overlap with QTLs detected by CIM in multiple environments (**Figure 3.18**). In contrast, the genomic regions harboring interacting loci for FFD were separate from any of the QTLs for the same trait detected by CIM.

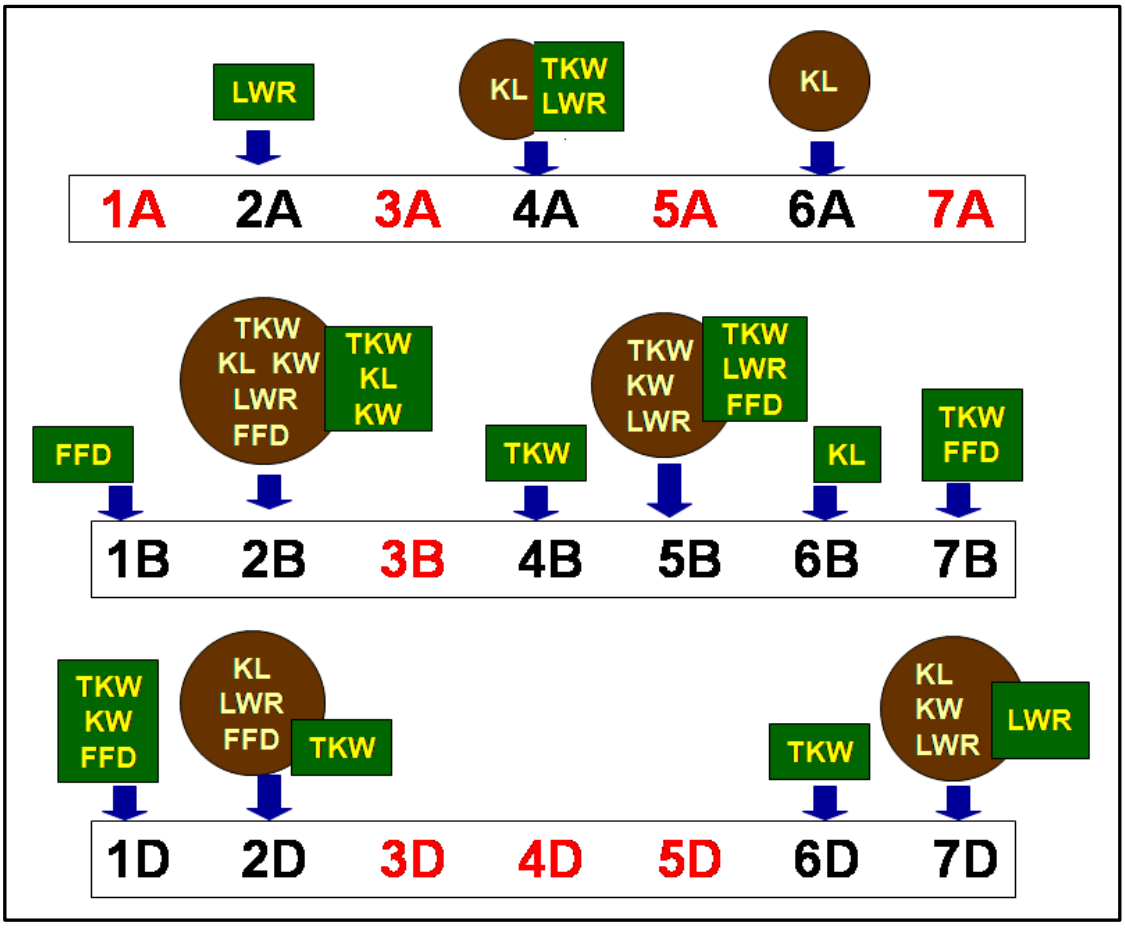


Figure 3.8: Chromosomal distribution of main-effect and epistatic QTLs detected by QTLNetwork. Trait names in circular shapes denote the location of main-effect QTLs and those in rectangles indicate epistatic QTLs for the same. Chromosomes on which kernel character QTLs were not detected are shown in red.

Table 3.20: Main-effect QTLs for Thousand kernel weight and their interaction with the environment

Interval	Chr	Position	Range	A	Environmental effect
Xbarc45-Xbarc55	2B	48.9	42.1-53.9	-1.90	-
Xgwm388-Xgwm47	2B	143.3	139.3-143.3	-1.06	0.827(AE3), -1.027(AE5)
Xgwm67-Xbarc74	5B	35.4	23.0-38.4	1.71	-

A: The additive effect of the main-effect QTL; a positive value indicates superior allele contribution from RS and negative, from CS. E3 Ludhiana (year 2005) E5 Pune (year 2005)

Table 3.21: Epistatic QTLs for Thousand kernel weight

Intervals with epistatic QTLs	Chr	Position	Range	AA
Xbarc240.1-Xwmc216	1D	71.1	67.1-76.1	0.997
Xbarc45-Xbarc55	2B	48.9	42.1-53.9	
Xwmc609-Xbarc62	1D	7.0	5.0-7.0	-1.075
Xwmc47-Xcfa2149	4B	81.9	78.9-81.9	
Xbarc45-Xbarc55	2B	48.9	42.1-53.9	-1.229
Xbarc124-Xwmc574	2D	0.0	0.0-1.0	
Xbarc55-Xbarc13	2B	65.5	60.9-68.5	-0.697
Xbarc176-Xwmc517	7B	42.6	36.6-42.6	
Xbarc37-Xgwm120	2B	99.3	97.1-104.3	1.422
Xcfd266-Xgwm499	5B	69.1	66.1-73.9	
Xgwm610-Xwmc150.3	4A	37.5	34.5-37.5	-1.376
Xcfd188-Xcfd76	6D	46.5	41.5-50.5	
Xgwm4901-Xwmc657	4B	22.2	14.0-25.2	1.042
Xcfd13-Xcfd42	6D	33.0	31.0-33.0	

AA: Epistatic effects; a positive value indicates that the parental two-locus genotypes have a positive effect and that the recombinants have a negative effect

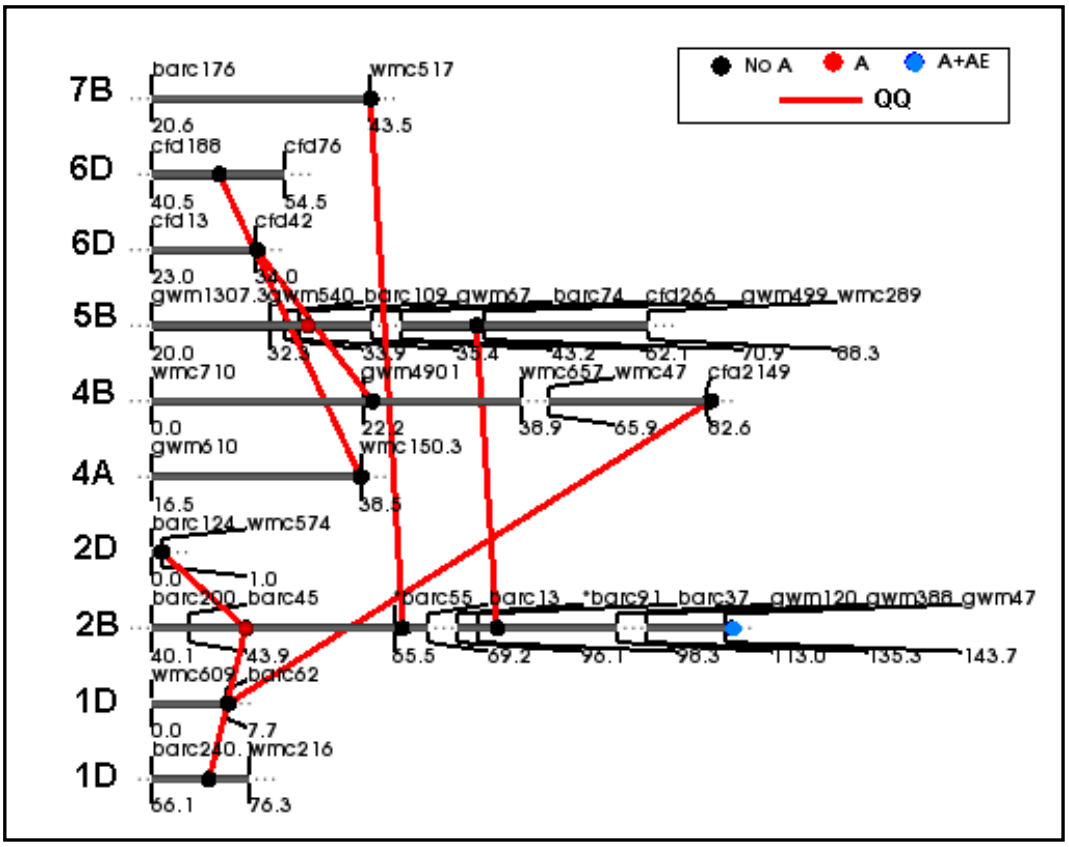


Figure 3.9: Main-effect and epistatic QTLs for Thousand kernel weight

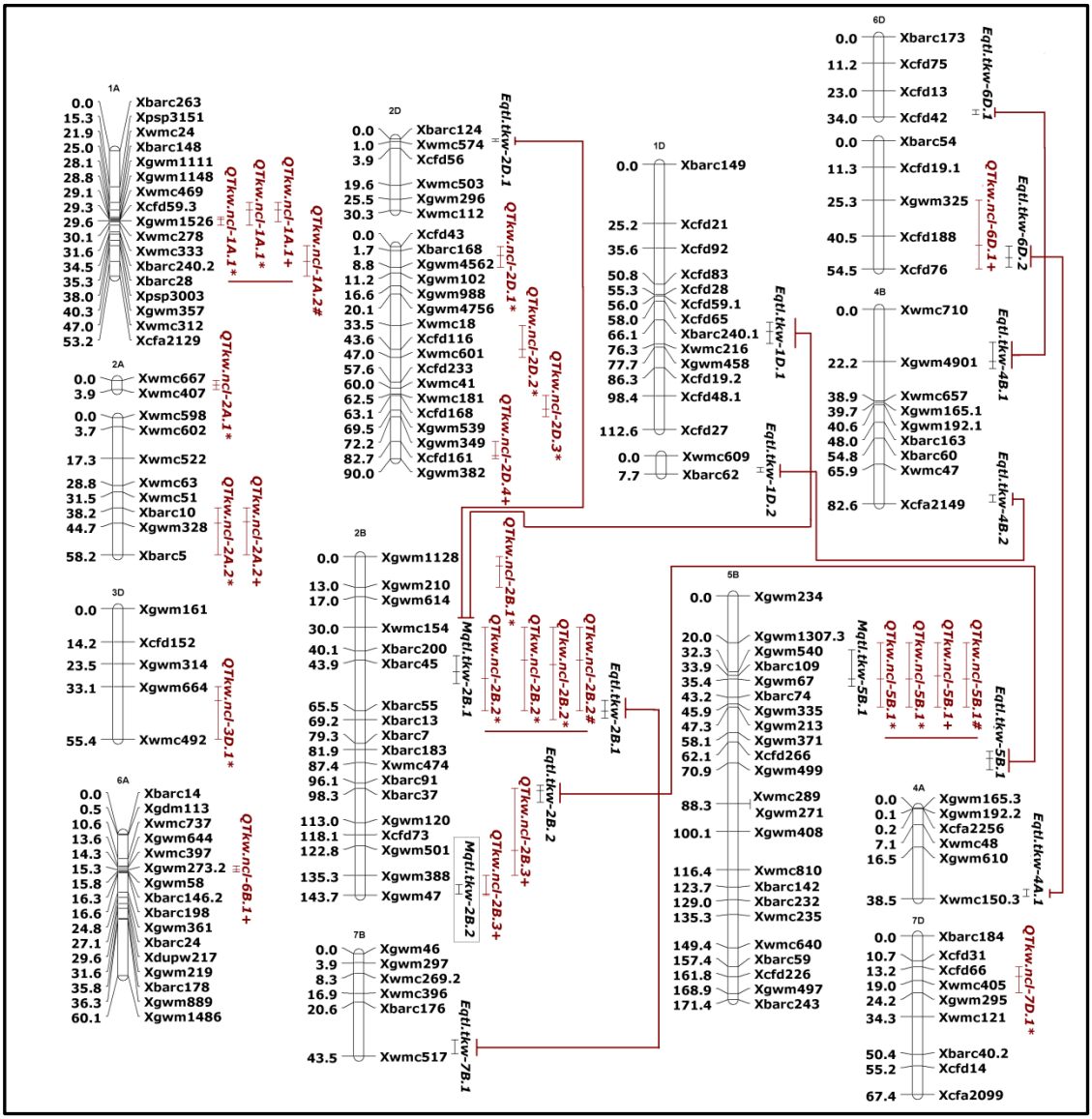


Figure 3.10: Overview of QTL positions detected by QTL Cartographer and QTLNetwork for Thousand kernel weight. QTLs identified by CIM are in color and named according to McIntosh *et al*. (2003). Horizontal colored bars indicate stable QTLs detected by CIM. Main-effect QTLs are indicated as *Mqtl.trait-chromosome.QTL no*. Main-effect QTLs influenced by environment are highlighted by boxes. Epistatic QTLs are shown as *Eqtl.trait- chromosome.QTL no*. and their interactions are represented by colored lines.

Table 3.22: Main-effect QTLs for Kernel length and their interaction with the environment

Interval	Chr	Position	Range	A	Environmental effect
Xgwm388-Xgwm47	2B	142.3	139.3-143.3	-0.04	-
Xcfd233-Xwmc41	2D	58.6	56.0-61.0	0.09	-
Xgwm165.3-Xgwm192.2	4A	0.0	0.0-3.2	0.09	-
Xbarc146.1-Xwmc807	6A	2.0	0.0-4.5	0.07	-
Xwmc405-Xgwm295	7D	24.0	21.0-24.2	0.05	-
Xbarc40.2-Xcfd14	7D	54.4	51.4-55.2	0.05	-0.0368(AE1)

E1:KL2003Lud

Table 3.23: Epistatic QTLs for Kernel length

Intervals with epistatic QTLs	Chr	Position	Range	AA	Environmental effect
Xbarc200-Xbarc45	2B	41.1	38.0-43.1	0.0625	-
Xbarc178-Xgwm889	6B	35.8	33.6-36.3		
Xwmc317-Xbarc159	2B	10.0	5.0-10.0	-0.0546	-0.048 (AAE1)
Xbarc178-Xgwm889	6B	35.8	33.6-36.3		

E1:KL2003Lud

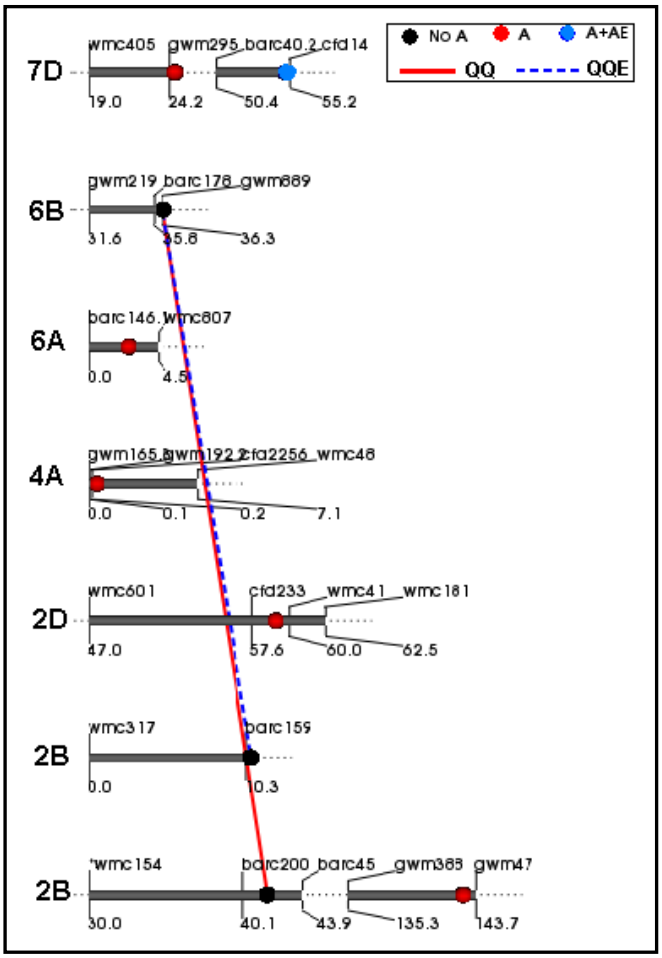


Figure 3.11: Main-effect and epistatic QTLs for Kernel length

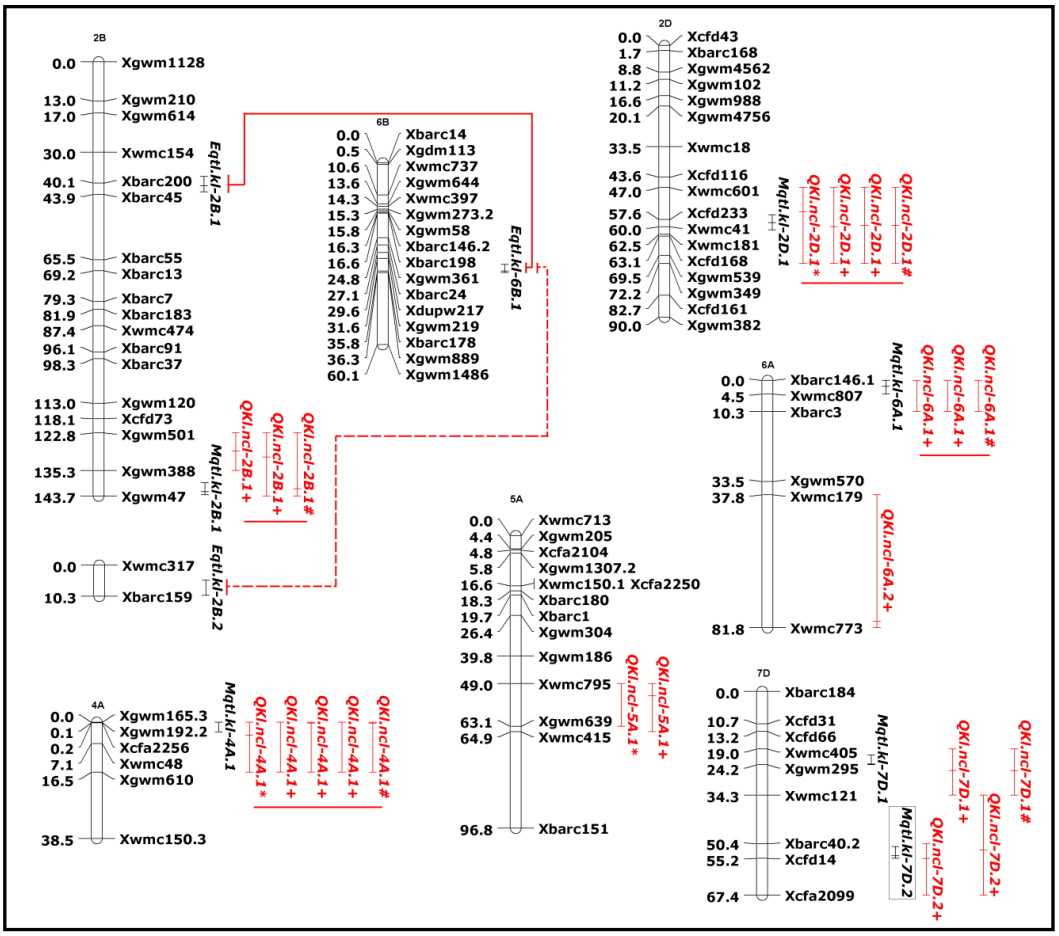


Figure 3.12: Overview of QTL positions detected by QTL Cartographer and QTLNetwork for Kernel length. QTLs identified by CIM are in color and named according to McIntosh *et al*. (2003). Horizontal colored bars indicate stable QTLs detected by CIM. Main-effect QTLs are indicated as *Mqtl.trait-chromosome.QTL no*. Main-effect QTLs influenced by environment are highlighted by boxes. Epistatic QTLs are shown as *Eqtl.trait- chromosome.QTL no*. and their interactions are represented by colored lines.

Table 3.24: Main-effect QTLs for Kernel width and their interaction with the environment

Interval	Chr	Position	Range	A	Environmental effect
Xgwm1128-Xgwm210	2B	1.0	0.0-7.0	-0.04	-
Xgwm67-Xbarc74	5B	35.4	33.9-38.4	0.04	-
Xwmc405-Xgwm295	7D	19.0	17.2-21.0	-0.02	-0.0695 (AE1), 0.0507 (AE5)

E1: KW2003Lud, E5: KW2007Pun

Table 3.25: Epistatic QTLs for Kernel width

Intervals with epistatic QTLs	Chr	Position	Range	AA
Xcfd19.2-Xcfd48.1	1D	94.3	90.3-98.3	0.1012
Xwmc154-Xbarc200	2B	34.0	27.0-39.0	

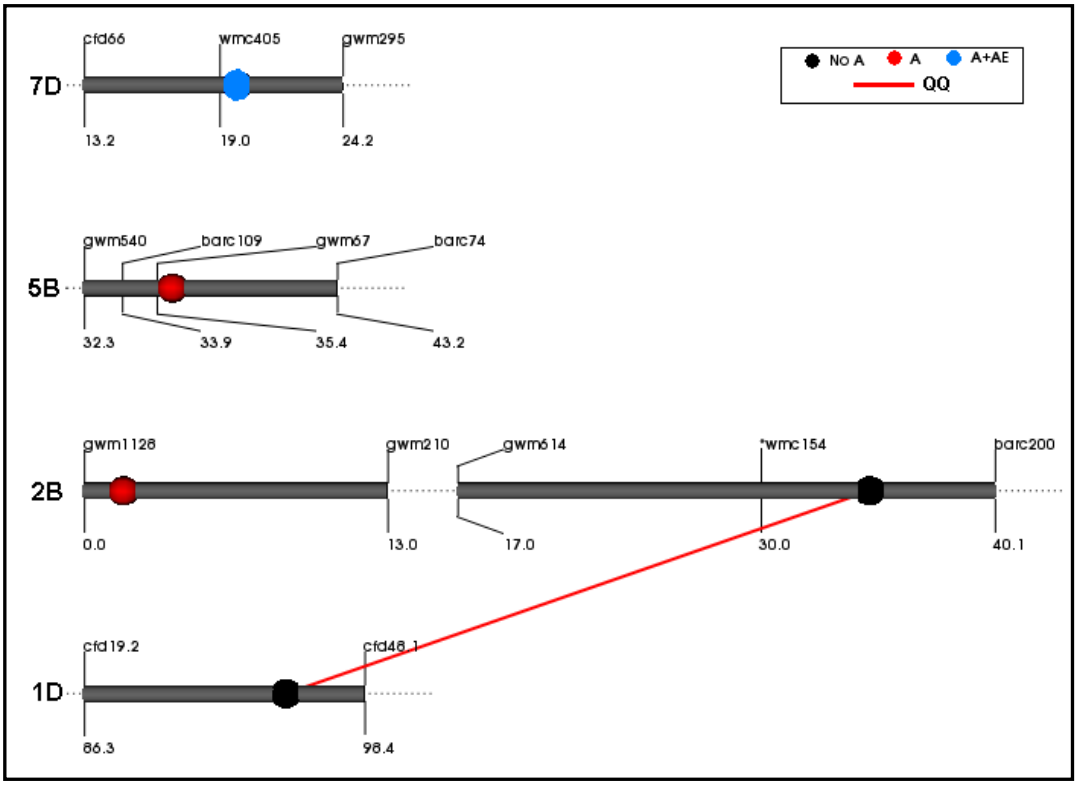


Figure 3.13: Main-effect and epistatic QTLs for Kernel width

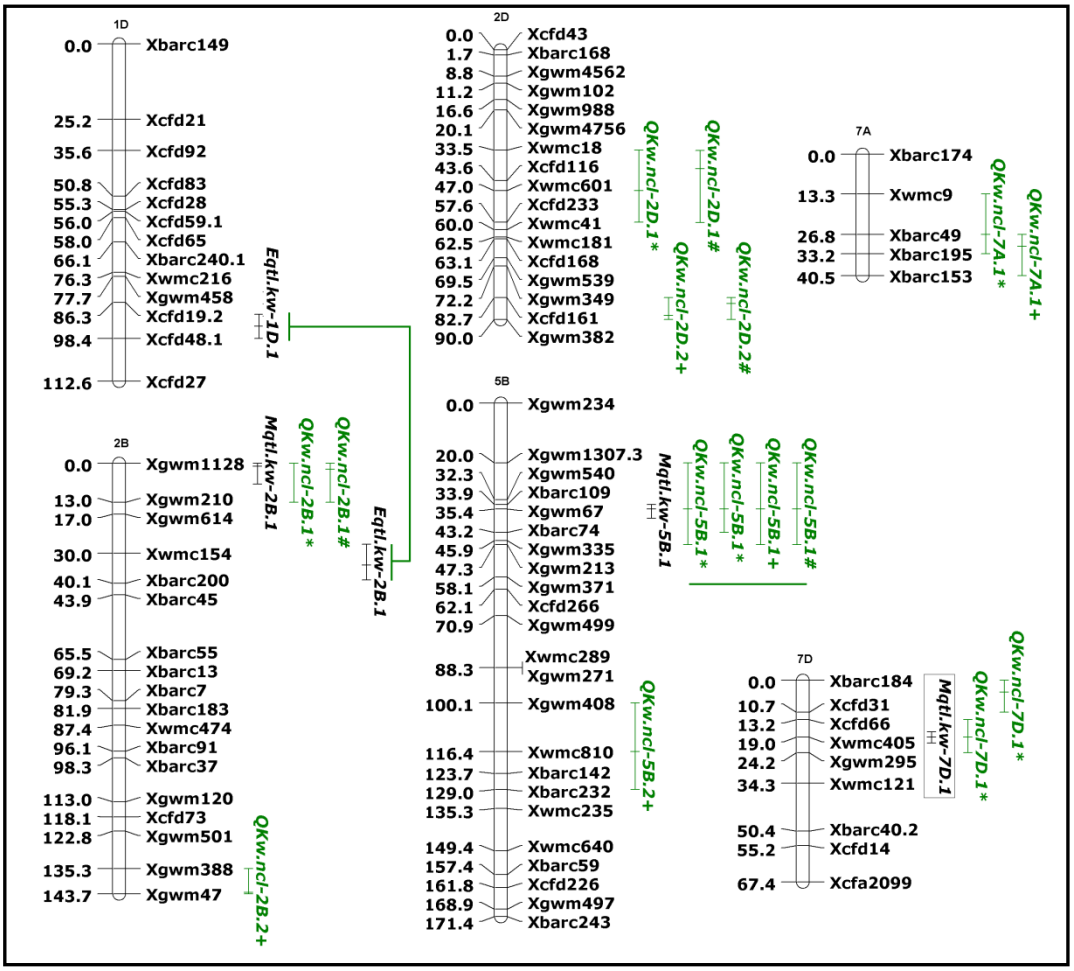


Figure 3.14: Overview of QTL positions detected by QTL Cartographer and QTLNetwork for Kernel width. QTLs identified by CIM are in color and named according to McIntosh *et al*. (2003). Horizontal colored bars indicate stable QTLs detected by CIM. Main-effect QTLs are indicated as *Mqtl.trait-chromosome.QTL no*. Main-effect QTLs influenced by environment are highlighted by boxes. Epistatic QTLs are shown as *Eqtl.trait- chromosome.QTL no*. and their interactions are represented by colored lines.

Table 3.26: Main-effect QTLs for kernel length-width ratio and their interaction with the environment

Interval	Chr	Position	Range	A	Environmental effect
Xgwm1128-Xgwm210	2B	0.0	0.0-4.0	0.04	0.0257 (AE4), -0.0275 (AE5)
Xgwm539-Xgwm349	2D	69.5	66.1-70.5	0.04	-
Xcfa2256-Xwmc48	4A	4.2	0.0-9.1	0.05	-
Xgwm67-Xbarc74	5B	35.4	33.9-37.4	-0.06	-
Xwmc405-Xgwm295	7D	20.0	17.2-23.0	0.06	0.043 (AE1), -0.0475 (AE5)

E1: LWR2003Lud, E4: LWR2006Pun, E5: LWR2007Pun

Table 3.27: Epistatic QTLs for kernel length-width ratio

Intervals with epistatic QTLs	Chr	Position	Range	AA
Xbarc10-Xgwm328	2A	42.2	39.2-47.7	-0.0408
Xgwm408-Xwmc810	5B	115.1	107.1-116.1	
Xgwm610-Xwmc150.3	4A	25.5	17.5-33.5	0.0777
Xwmc121-Xbarc40.2	7D	45.3	39.3-53.4	

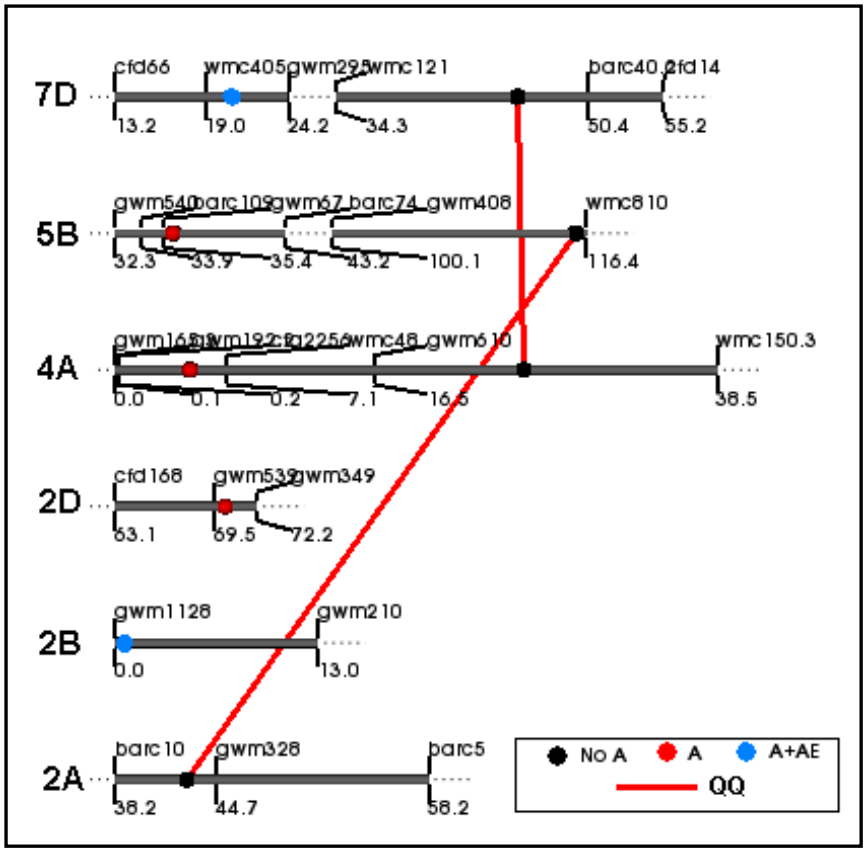


Figure 3.15: Main-effect and epistatic QTLs for Length-width ratio

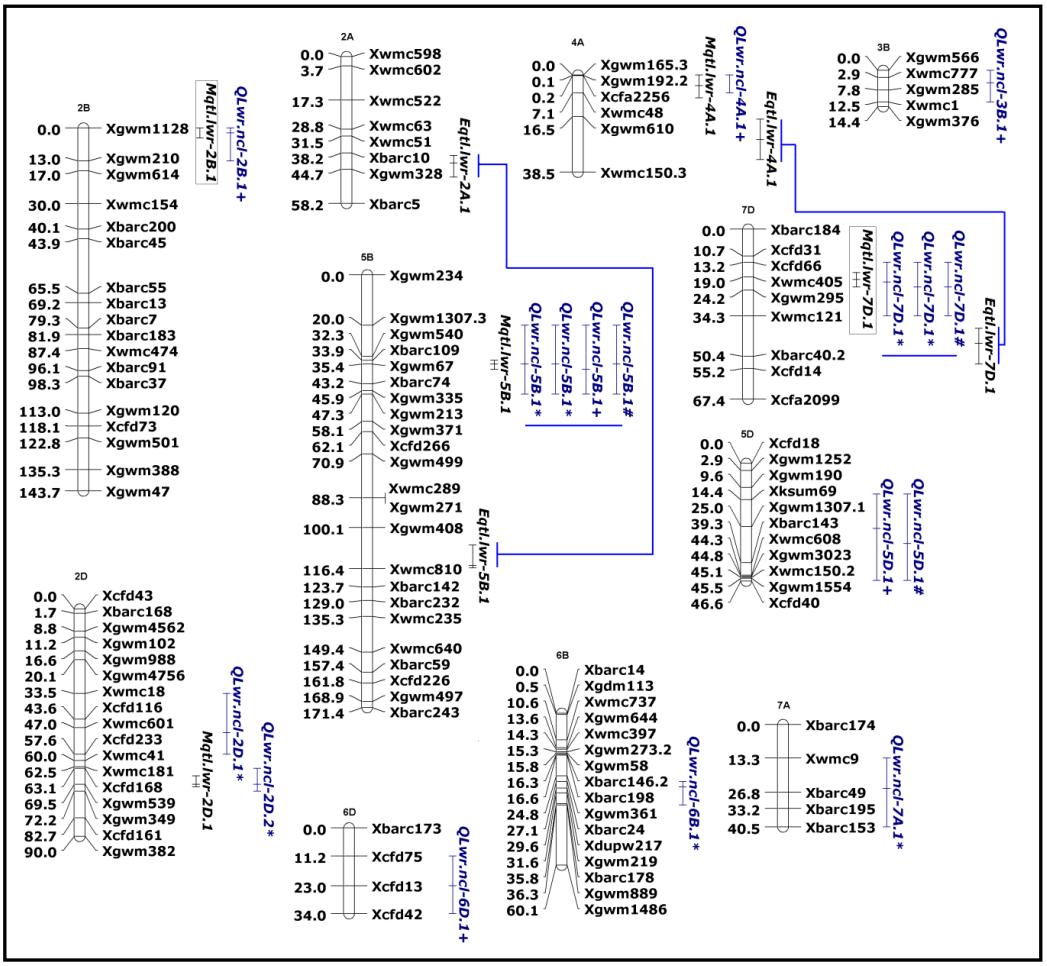


Figure 3.16: Overview of QTL positions detected by QTL Cartographer and QTLNetwork for kernel length-width ratio. QTLs identified by CIM are in color and named according to McIntosh *et al*. (2003). Horizontal colored bars indicate stable QTLs detected by CIM. Main-effect QTLs are indicated as *Mqtl.trait-chromosome.QTL no*. Main-effect QTLs influenced by environment are highlighted by boxes. Epistatic QTLs are shown as *Eqtl.trait- chromosome.QTL no*. and their interactions are represented by colored lines.

Table 3.28: Main-effect QTLs for Factor form density

Interval	Chr	Position	Range	A
Xbarc45-Xbarc55	2B	43.9	42.1-47.9	-0.06
Xbarc168-Xgwm4562	2D	1.7	0.0-5.7	0.03

Table 3.29: Epistatic QTLs for Factor form density

Intervals with epistatic QTLs	Chr	Position	Range	AA
Xcfd59.2-Xwmc269.1	1B	50.2	47.2-54.4	0.0382
Xgwm271-Xgwm408	5B	89.3	82.9-96.3	
Xcfd21-Xcfd92	1D	26.2	15.0-32.2	-0.0466
Xbarc176-Xwmc517	7B	20.6	17.9-32.6	

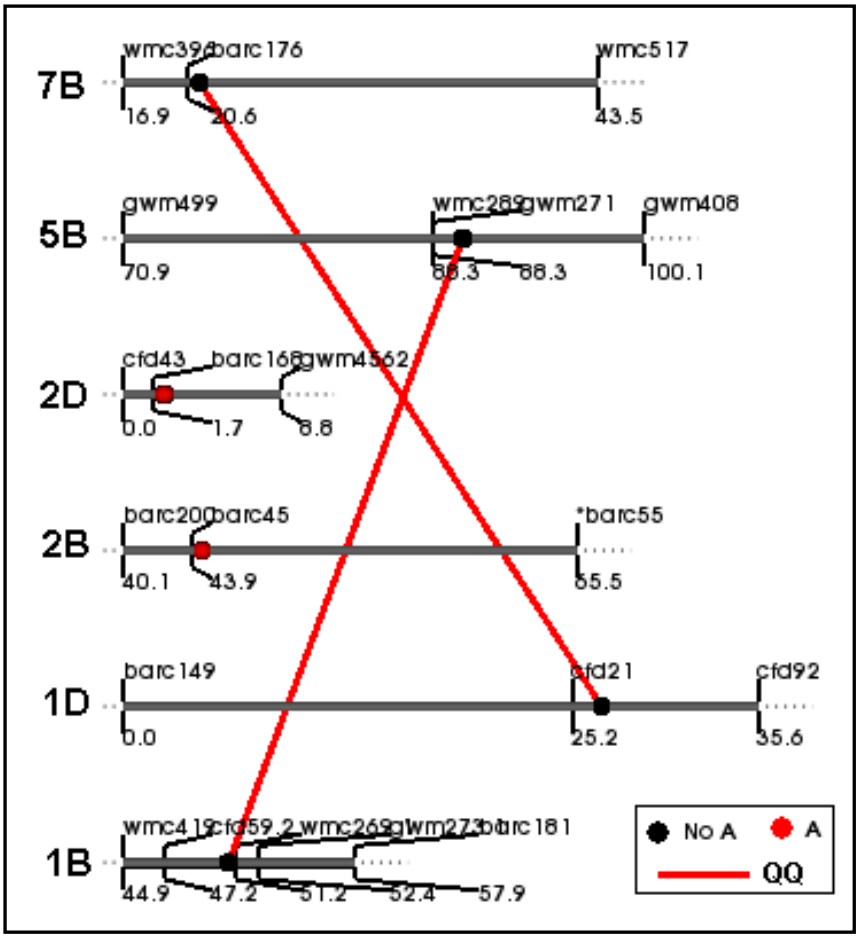


Figure 3.17: Main-effect and epistatic QTLs for Factor form density

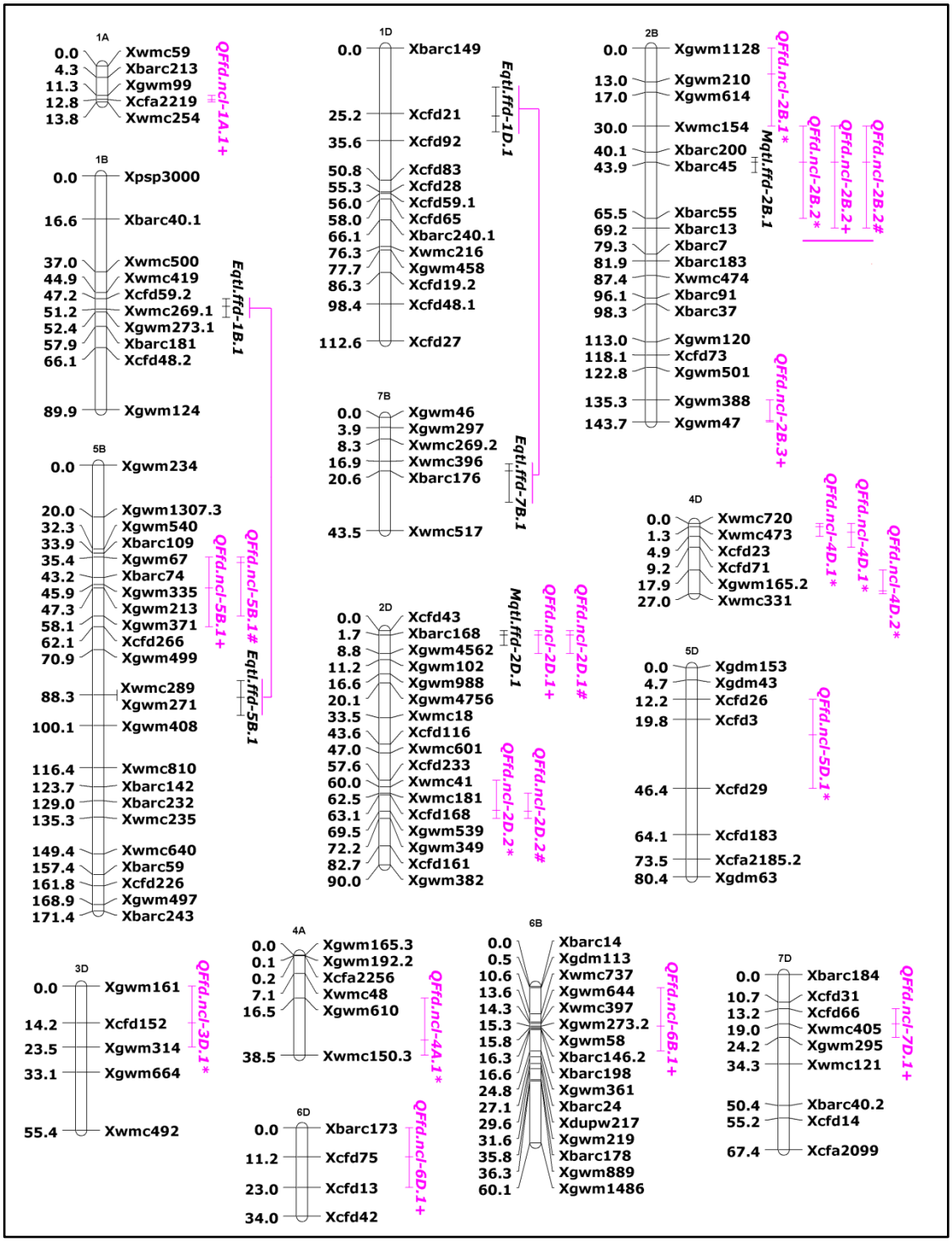


Figure 3.18: Overview of QTL positions detected by QTL Cartographer and QTLNetwork for Factor form density. QTLs identified by CIM are in color and named according to McIntosh *et al*. (2003). Horizontal colored bars indicate stable QTLs detected by CIM.Main-effect QTLs are indicated as *Mqtl.trait-chromosome.QTL no*. Main-effect QTLs influenced by environment are highlighted by boxes. Epistatic QTLs are shown as *Eqtl.trait- chromosome.QTL no*. and their interactions are represented by colored lines.

3.6.3 Multi-trait composite interval mapping (Mt-CIM) of kernel traits

Composite interval mapping (CIM) showed QTL clusters for kernel characters in the RS×CS population suggesting pleiotropism or close linkage in the underlying loci. In addition to this, QTLNetwork indicated chromosomal regions with overlap in support intervals of main-effect or epistatic QTLs for different kernel traits. Mt-CIM was therefore performed using QTL Cartographer with trait data recorded for all the five kernel characters in the same environment and the average data across such environments.

Pleiotropic QTLs were detected for various trait combinations on chromosomes 2B, 2D, 4A, 5B and 7D (**Table 3.30**, **Figure 3.19**). Chromosome 2B indicated a putative pleiotropic QTL in the region *Xgwm1128-Xgwm614* with effect on TKW, KW, LWR and FFD for Ludhiana (year 2003). Similarly, for TKW, KW and LWR, both 5B and 7D suggested the presence of QTLs with multiple effects (Ludhiana, year 2003). Three chromosomes, namely, 2B, 2D and 4A showed pleiotropic QTLs for combinations of two traits (**Table 3.30**). Among these, a pleiotropic region for KW and LWR (Pune, year 2006) on chromosome 2B between markers *Xgwm1128-Xgwm210* displayed overlap with the pleiotropic QTL region for TKW, KW, LWR and FFD for Ludhiana (year 2003). Similarly, overlap in the pleiotropic region for TKW and FFD in the year 2005 at Ludhiana and for average data was observed on chromosome 2B in the region between the markers *Xbarc200 and Xbarc55*. On chromosome 5B, common chromosomal regions supported pleiotropic QTLs for TKW, KW and LWR (Ludhiana, year 2003 and Average data) between the markers *Xgwm1307.3 and Xgwm213*.

Thus, in the RS×CS population, 59 QTLs were identified for five kernel traits, TKW, KL, KW, LWR and FFD from two agro-climatic zones of wheat growing in India, namely, Ludhiana and Pune. The QTLs identified in this study could throw light on the loci with significant individual effects on each kernel trait, epistatically interacting genomic regions in the control of kernel traits and the influence of G×E interactions and presence of putative pleiotropic loci in their determination. The detection of genomic regions with pleiotropic effects on various trait combinations within a location, within a specific year as well as with different year-location combinations suggested interdependence of these morphometric traits in wheat. These have been discussed with reference to available literature in the next chapter.

Table 3.30: Putative pleiotropic regions for kernel characters. Multi-trait composite interval mapping was performed for year-location combinations in which all the five kernel traits were recorded

Environments	Chr	Marker interval
TKW2003Lud, KW2003Lud, LWR2003Lud and FFD2003Lud	2B	*Xgwm1128-Xgwm614*
TKW2003Lud, KW2003Lud and LWR2003Lud	5B	*Xgwm540-Xgwm213*
TKW2003Lud, KW2003Lud and LWR2003Lud	7D	*Xcfd66-Xgwm295*
KW2003Lud and LWR2003Lud	2D	*Xcfd116-Xcfd233*
TKW2005Lud and FFD2005Lud	2B	*Xbarc200-Xbarc55*
KW2005Lud and LWR2005Lud	2B	*Xbarc45-Xbarc13*
KW2005Pun and FFD2005Pun	2B	*Xgwm388-Xgwm47*
TKW2005Pun and FFD2005Pun	2D	*Xcfd43-Xgwm4562*
KL2005Pun and KW2005Pun	2D	*Xgwm349-Xcfd161*
KW2006Pun and LWR2006Pun	2B	*Xgwm1128-Xgwm210*
TKW average and FFD average	2B	*Xbarc45-Xbarc55*
KL average and LWR average	4A	*Xcfa2256-Xwmc48*
TKW average, KW average and LWR average	5B	*Xgwm1307.3-Xgwm213*

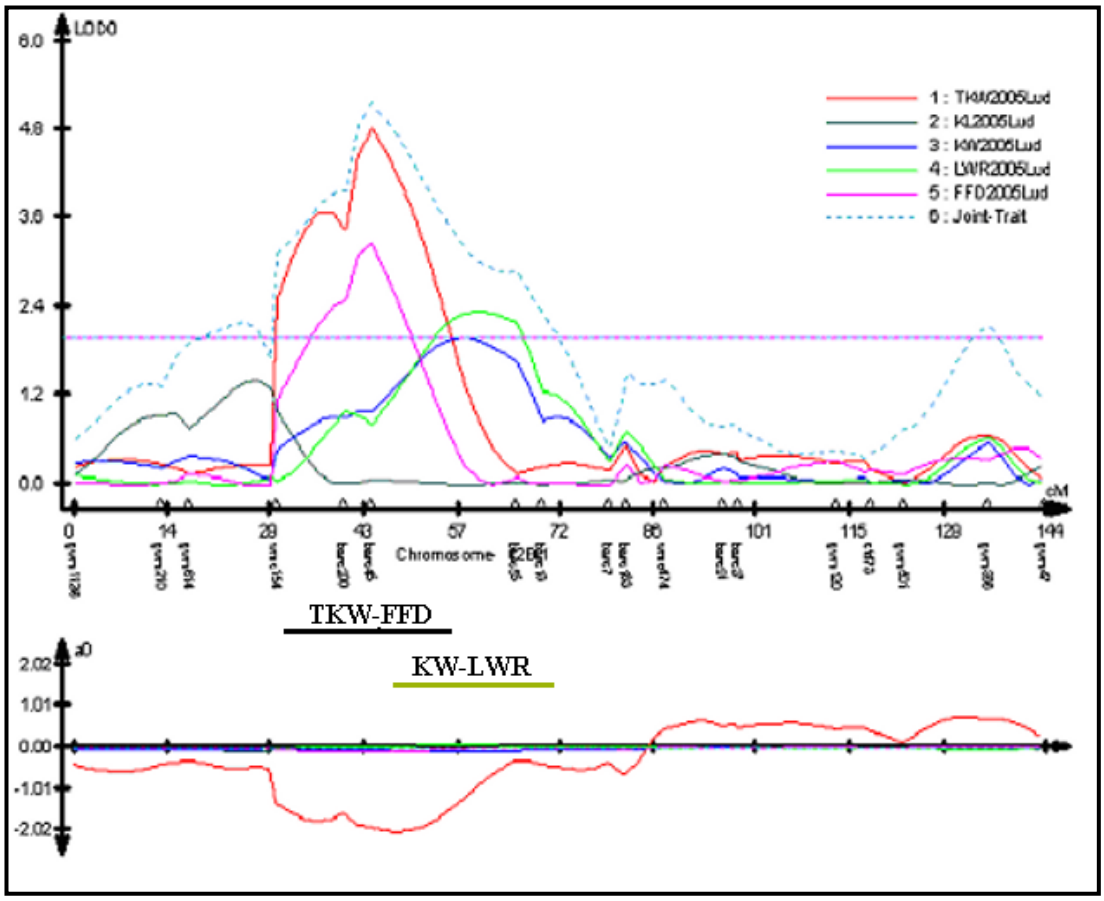
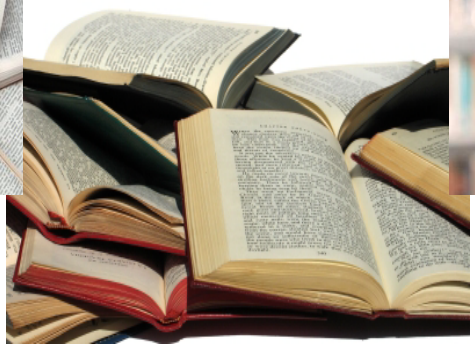
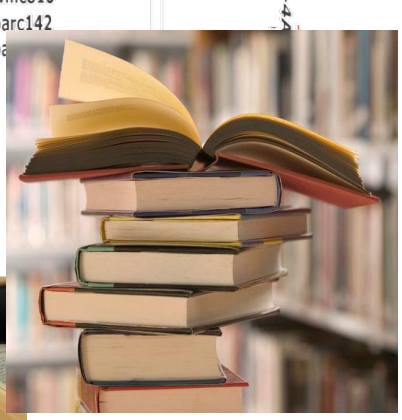


Figure 3.19: Putative pleiotropic regions for TKW and FFD; and KW and LWR, respectively on chromosome 2B


DISCUSSION
0.0 Xwmc598
3.7 Xwmc602
17.3 Xwmc522
28.8 Xwmc63
31.5 Xwmc51
38.2 Xbarc10
44.7 Xgwm328
58.2 Xbarc5
3D
0.0 Xgwm161
14.2 Xcfd152
23.5 Xgwm314
33.1 Xgwm664
55.4 Xwmc492
6A
0.0 Xbarc14
0.5 Xgdm113
10.6 Xwmc737
13.6 Xgwm644
14.3 Xwmc397
15.3 Xgwm273.2
15.8 Xgwm58
2B
0.0 Xgwm1128
13.0 Xgwm210
17.0 Xgwm614
65.5 Xbarc55
69.2 Xbarc13
79.3 Xbarc7
81.9 Xbarc183
87.4 Xwmc474
96.1 Xbarc91
98.3 Xbarc37
113.0 Xgwm120
118.1 Xcfd73
122.8 Xgwm501
135.3 Xgwm388
Xgwm47
63.1 Xcfd168
69.5 Xgwm539
72.2 Xgwm349
82.7 Xcfd161
90.0 Xgwm382
112.6 Xcfd27
0.0 Xwmc609
7.7 Xbarc62
5B
0.0 Xgwm234
35.4 Xgwm67
43.2 Xbarc74
45.9 Xgwm335
47.3 Xgwm213
58.1 Xgwm371
62.1 Xcfd266
70.9 Xgwm499
88.3 Xwmc289
Xgwm271
100.1 Xgwm408
116.4 Xwmc810
123.7 Xbarc142
129.0
40.6 Xgwm192.1
48.0 Xbarc163
54.8 Xbarc60
65.9 Xwmc47
82.6 Xcfa2149
4A
0.0 Xgwm165.3
0.1 Xgwm192.2
0.2 Xcfa2256
7.1 Xwmc48
16.5 Xgwm610
QTkw.ncl-2A.2*
QTkw.ncl-2A.2+
QTkw.ncl-3D.1*
QTkw.ncl-2D.4+
QTkw.ncl-2B.2*
QTkw.ncl-2B.2#
QTkw.ncl-2B.3+
QTkw.ncl-5B.1*
QTkw.ncl-5B.1+
QTkw.ncl-5B.1#
Eqtl.tkw-1D.2
Eqtl.tkw-4B.2
Eqtl.tkw-2B.1
Eqtl.tkw-2B.2
Eqtl.tkw-5B.1
Mqtl.tkw-5B.1
Mqtl.tkw-2B.1





Discussion

The past two decades witnessed the rapid and wide-ranging development of molecular marker-based techniques for identification of genomic regions controlling various traits of agricultural importance (Gupta and Xu, 2008; Jones *et al*., 2009; Varshney *et al*., 2009b). The knowledgebase built using these techniques is being employed to improve the quality and yield of various crop plants (Fitzgerald *et al*., 2009; Hospital, 2009; Generation Challenge Programme molecular marker toolkit - http://s2.generationcp.org/gcp-tmm/web/index.php). Wheat, being one of the most important food crops in the world, is in constant need for improvement in agronomic yield and quality to meet the requirements of the growing population and processing industry. Many better varieties of wheat have already been developed and many are in pipeline in the publically funded marker assisted selection (MAS) projects, which can be made available to farmers (Gupta *et al*., 2010).

Kernel morphometric traits play a significant role in the determination of wheat market price by their influence on the visual appeal of the produce and also on the flour-milling yield. With the large-scale milling industries creating demand for wheat with better kernel characters, these traits have gained increased importance in the recent years and have directed studies to identify their genetic determinants (Dholakia *et al*., 2003; Sun *et al*., 2009; Gegas *et al*., 2010; Tsilo *et al*., 2010). In the current study, genetic analysis of kernel morphometric traits was performed using an RIL mapping population derived from the cross Rye Selection 111 (RS) × Chinese Spring (CS) and quantitative trait loci (QTL) were identified on 16 of the 21 hexaploid wheat chromosomes. The inter-relationships among the kernel traits as well as interactions between genetic components and environment were analyzed by performing phenotypic evaluations of the same population in multiple years and locations. The results are discussed in this chapter in the context of the available literature and their potential utility in wheat improvement.

4.1 Measurement of kernel morphometric traits

Diverse tools have been employed to record kernel morphometric traits in wheat. Schuler *et al*. (1995) and Sun *et al*. (2009) reported the aligning of samples of 20-25 kernels length-wise along a ruler to measure average kernel length (KL), and then breadth-wise to measure average kernel width (KW). Several researchers used photography and digital image analysis with various softwares to record multiple kernel shape traits (Berman *et al*., 1996; Campbell *et al*., 1999; Bergman *et al*., 2000; Breseghello and Sorrells, 2007). A method to record kernel morphometric traits using the advantages of fixed resolution and image acquisition distances of a digital scanner along with an image-processing software was described by Shouche *et al*. (2001). Gegas *et al*. (2010) and Tsilo *et al*. (2010) employed digital grain analyzer and single kernel characterization system (SKCS), respectively to simultaneously obtain various kernel trait measurements. For the phenotypic analysis of the RS×CS population in this study, Vernier calipers was employed as described previously (Dholakia *et al*., 2003; Aluko *et al*., 2004; Ammiraju *et al*., 2004), since this method provided tangible measurements of kernel shape determinants although it was a laborious and time-consuming method.

4.2 Phenotypic evaluation in multiple years and locations

Phenotypic evaluation of quantitative traits in diverse locations for multiple years can lead to the identification of QTLs that are unique to a location or stable across the locations in a specific year or in multiple years (Groos *et al*., 2003; Hittalmani *et al*., 2003; Quarrie *et al*., 2005). Both these kinds of QTLs are important in development of varieties by MAS. The varieties suitable to a particular location can be developed keeping in mind the biotic and abiotic stresses in a region; while, varieties suitable for multiple locations might be of increased commercial importance (Asins, 2002; Kang, 2002). Furthermore, detection of a particular QTL region in multiple years points at its stability, which might be a crucial parameter for it to become a potential target for MAS (Bernardo, 2008). In view of this, phenotypic analyses of kernel morphometric traits in the RS×CS population were performed at Ludhiana and Pune in India for eight year-location

combinations for thousand kernel weight (TKW) and in five year-location combinations for kernel length (KL), kernel width (KW), kernel length-width ratio (LWR) and factor form density (FFD). Continuous variation was observed for all the five traits at both the locations suggesting the presence of multiple QTLs with complex gene action, while variation in their frequency distribution profiles across years indicated various degrees of interactions between the genes and the environments (**Table 3.1, Figure 3.1**). The RIL population showed both, negative and positive transgressive segregants, suggesting the possibility of contribution of some of the favorable alleles for kernel traits from the poor-parent CS and unfavorable alleles at some loci from the bold-grained parent RS (**Table 3.1**). Similar observations on trait variation for various kernel characters were observed in other QTL mapping studies (Campbell *et al*., 1999; Dholakia *et al*., 2003; Sun *et al*., 2009; Gegas *et al*., 2010) as well.

Furthermore, KW transgressive segregants with trait values higher than RS were observed for more years in Pune than in Ludhiana and for TKW and KL, such transgressive segregants were observed only at Pune location. For LWR and FFD, however, both the locations revealed transgressive segregation in all the years. These observations point to better expression of kernel quality traits at Pune than at Ludhiana. Similarly, in the case of grain protein content (GPC) evaluated at Ludhiana and Pune for the WL711×PH132 RIL population (Dholakia *et al*., 2001), higher GPC was recorded at Pune compared to Ludhiana. Additionally, many bread-making quality traits exhibited higher trait values at Pune than at Ludhiana in an RIL population evaluated for two consecutive years at both the locations (Elangovan, 2007; Elangovan *et al*., 2008). Hence, it may be suggested that Pune, representing the Peninsular zone, might elicit better phenotypic responses for some of the quality traits compared to the North-western plains zone represented by Ludhiana.

4.3 Significant correlations between kernel morphometric traits

The correlation coefficients that represented the proportion of common variation and the relationship between kernel traits were calculated within the same year-location combinations and among the average data cross environments in which

all the five traits were recorded (**Table 3.2**). Significant positive correlation that was highly consistent across environments was observed for TKW–KL, TKW–KW and TKW–FFD in the RS×CS population. Similarly, Dholakia *et al*. (2003) and Gegas *et al*. (2010) reported significant correlations between TKW, KL, KW and FFD. Bergman *et al*. (2000) and Sun *et al*. (2009) also reported positive and significant correlations between TKW-KL and TKW-KW. Tsilo *et al*. (2010) observed correlations as high as 0.90 and 0.92 ($P<0.001$) for TKW with single kernel diameter determined based on SKCS and percentage of large kernels, respectively. In contrast, the percentage of medium and small kernels showed strong negative correlation with TKW in the same population. These observations collectively suggest that the overall increase in kernel dimensions might result in higher kernel weight and highlight the importance of selection for better kernel shape in wheat. Interestingly, TKW indicated negative correlation with LWR in the present study. When viewed with the observation of correlation coefficients being higher between TKW-KW compared to those between TKW-KL, the negative correlation of TKW with LWR suggested that KW was more important in increasing TKW than KL. Dholakia *et al*. (2003) and Sun *et al*. (2009) also reported higher correlation coefficients between TKW-KW compared to TKW-KL. Furthermore, correlations between the KL and KW in the RS×CS population were positive similar to the reports of Dholakia *et al*. (2003), Sun *et al*. (2009) and Gegas *et al*. (2010). Significant correlations among the kernel traits suggested the possibility of some of these QTLs being co-segregating or pleiotropic.

4.4 Kernel traits are controlled mainly by G and G×E components

High heritability for TKW, KL, KW and LWR and moderate levels for FFD were observed in the RS×CS population (**Table 3.7**) indicating significant genotype (G) contributions to kernel trait expression. Similar high estimates of heritability were also shown for TKW in other studies (Campbell *et al*., 1999; Huang *et al*., 2006; Kumar *et al*., 2006; Cuthbert *et al*., 2008; Wang *et al*., 2009; Gegas *et al*., 2010 and Tsilo *et al*., 2010). For kernel shape traits, high (Campbell *et al*., 1999; Gegas *et al*., 2010) to moderate (Sun *et al*., 2009; Gegas *et al*., 2010) levels of heritability were reported. Significant positive rank correlations between most of

the year-location combinations for kernel traits in the RS×CS population (**Table 3.3**) supported the central role of genotypic contributions to trait expression.

An attempt to partition the variation in kernel traits was made with the help of AMMI analysis by estimating the relative contributions of genotype (G), environment (E) and G×E effects (Gauch, 1992). A similar approach was used in earlier studies in crop plants for evaluating sources of variation for agronomic and grain quality traits in mapping populations (Groos *et al*., 2003; Hittalmani *et al*., 2003; Cho *et al*., 2007 and Elangovan *et al*., 2008). In the present study, by AMMI analysis, KL and TKW showed the highest contributions from G, followed by FFD, LWR and KW (**Table 3.5).** Only in case of FFD and KW, the G×E contribution was higher than the G effect, while the E effect was minimum in case of all the five traits. Genotype main-effects based on ANOVA were also found to be a significant source of variation in wheat kernel traits such as kernel weight, length, width, and area as suggested by Campbell *et al*. (1999) and Breseghello and Sorrells (2007).

Though the contributions of G effects were vital for kernel traits in the RS×CS population, substantial G×E component indicated by AMMI analysis suggested the influence of environment on the expression of underlying genetic components. Further partitioning of G×E component into multiple significant interaction principal component axes (IPCA) by the AMMI model could summarize the patterns in G×E effects (Crossa, 1990). For the kernel traits in the RS×CS population, 2-4 significant IPCA axes could be derived (**Tables 3.4** and **3.6**), which explained up to 87% (for LWR) of G×E variation and indicated that a large proportion of noise-rich residual deduced by ANOVA was actually due to G×E interactions (Gauch, 1988 and 2006, Gauch and Zobel, 1988, Nachit *et al*., 1992). Groos *et al*. (2003) and Elangovan (2007) reported two and three significant multiplicative terms deriving patterns in G×E interaction for TKW, respectively. However, AMMI analysis has so far not been reported in previous studies for the other four kernel morphometric traits.

The IPCA scores of a genotype in the AMMI analysis are indicative of its adaptability over environments and the graphical representation of the analysis of

the interaction component in the form of biplots (Kroonenberg, 1995; Yan and Tinker, 2006) can reveal useful information about the participation of genotypes and environments in G×E interactions (Kang, 2002; Tarakanovas and Ruzgas, 2006). Examination of the IPCA scores of the RILs in the present study showed that for TKW, 89.86% of them had absolute scores less than 1. Further, none of the RILs indicated IPCA scores higher than 1 in case of the other four traits and the maximum values of their absolute IPCA scores ranged from 0.38 (LWR) to 0.59 (FFD). The proximity of the IPCA scores of the RILs to zero and their dense clustering near the origin in biplots (**Figure 3.2**) indicated their high degree of stability and general adaptation to diverse environments across multiple years for kernel traits. The lengths of the Environment vectors indicated the enhanced participation of Ludhiana location in G×E interactions in all the traits except LWR (**Figure 3.2**). Among the years at Ludhiana, year 2005 indicated increased effect on G×E interaction for all the traits. In case of Pune, year 2005 indicated higher participation in G×E interactions for TKW and year 2007, for KW, LWR and FFD.

G×E interactions pose major challenges for breeders and have significant implications on plant breeding initiatives (Kang, 2002). An important challenge is selecting genotypes that perform well across many sites in the face of drastically changing genotype rankings across sites. Developing separate populations for each site will not only be laborious but also expensive (McKeand *et al*., 1990). The favorable performance of a genotype in a particular environment may be overlooked if its average performance across environments is poor. Conversely, selecting a variety that, on average, performed well but did poorly when grown in a particular environment may result in loss of years of selection for a desired phenotype (Denis and Gower, 1996). Hence evaluation of G×E interaction component is recommended during varietal development by many researchers (Pinnschmidt and Hovmøller, 2002; Nurminiemi *et al*., 2002; Williams *et al*., 2008; Hristov *et al*., 2010). Analyzing the G×E effects in the expression of kernel traits in the present study suggested the possibility of detection of genomic regions associated with kernel traits specifically in individual locations as well as in specific year-location combinations. However, high contribution of G effects

coupled with high heritability, significant rank correlations among environments and RIL clustering near biplot origins suggested the possibility of identification of stable QTLs across environments.

4.5 Size of the mapping population used in the present study

In this study, phenotypic evaluation was performed using all of the 185 RILs of the RS×CS population; while linkage mapping and QTL analysis were conducted using 180 RILs as described in Chapter 3. Except for the Avalon × Cadenza population with 202 doubled haploid (DH) lines used by Gegas *et al*. (2010), the size of the RS×CS mapping population is higher than those used in many previous studies on kernel characters that ranged from 65 to 147 RILs or DHs (Campbell *et al*., 1999; Dholakia *et al*., 2003; Ammiraju *et al*., 2004; Breseghello and Sorrells, 2007; Sun *et al*., 2009; Gegas *et al*., 2010; Tsilo *et al*., 2010, Sun *et al*., 2010; Manickavelu *et al*., 2011). The use of RILs in the present study facilitated their phenotypic evaluation simultaneously in two locations for multiple years and enabled the evaluation of G×E interactions. With the availability of phenotypic data for multiple year-location combinations for each trait, the consistency of QTL regions could also be assessed.

4.6 Features of linkage map of the RS×CS population

The linkage map of the RS×CS population was constructed using 260 polymorphic SSR markers. The use of SSR markers was considerably advantageous over other maker systems as described in Section 1.11. The level of polymorphism observed between RS and CS in this study was 29.66%. Parental genotypes of various mapping populations derived from intraspecific crosses have shown SSR polymorphism as low as 21% (Marza *et al*., 2006) to as high as 43% (McCartney *et al*., 2005) in bread wheat. Multiple polymorphic bands that segregated in the RILs of the RS×CS population were observed for 6% of the polymorphic primers (**Table 3.9**). Such SSR primer pairs yielding multiple bands with location in homeologous or non-homeologous positions have also been reported earlier in wheat (Röder *et al*., 1998; Gupta P *et al*., 2002; Elouafi and Nachit, 2004; McCartney *et al*., 2005). The presence of such multiple loci for

SSRs has been attributed to the polyploid nature of wheat genome and occurrence of duplications between homeologous or non-homoeologous chromosomes and translocations (Gupta P *et al*., 2002).

The framework map constructed for the RS×CS population consisted of 32 linkage groups representing all the 21 chromosomes of hexaploid wheat with a total of 251 SSRs (**Figure 3.4**). The presence of more than one linkage group matching different regions of a chromosome was probably due to the low levels of polymorphism observed in the RS×CS population for some of the chromosomal segments. In some chromosomes such as 1A, 1D, 2A and 6D, the marker density in the consensus map developed by Somers *et al*. (2004) and the high-density intervarietal map of Xue *et al*. (2008) was also found to be less in the regions that separated two linkage groups belonging to the same chromosome in the present RS×CS map. Similar linkage maps with more than 21 linkage groups representing the hexaploid wheat genome have been routinely used for QTL mapping (Groos *et al*., 2003; Elouafi and Nachit, 2004; Breseghello *et al*., 2005; McCartney *et al*., 2005; Huang *et al*., 2006; Li *et al*., 2007; Landjeva *et al*., 2010). Even though the number of markers used for linkage group construction in the RS×CS population was significantly high, the total map length was 1655.28 cM, which could mainly be due to high marker density in some of the chromosomes (1A, 2D, 5A, 5B, 5D and 6B) and lesser coverage of Group-3 and -7 chromosomes and the distal chromosomal regions (**Table 3.10**, **Figure 3.4**). The average marker interval of 6.59 cM and absence of large gaps in the linkage groups made the RS×CS map highly suitable for QTL analysis.

In the linkage map, the chromosomes 2B, 6B and 7D displayed large concentration of markers showing segregation distortion (**Figure 3.4**). In addition to these, chromosomes 1D, 4A and 6A showed two adjacent markers each with deviation from the 1:1 segregation ratio. Recently, Xue *et al*. (2008) showed segregation distortion regions (SDRs) in 10 of the 21 wheat chromosomes in the Nanda2419×Wangshiubai mapping population. Both RS×CS and the Nanda2419×Wangshuibai maps showed SDRs on chromosomes 2B and 6B. In many earlier reports, chromosomes 2B (Campbell *et al*., 1999; Messmer *et al*., 1999; Sourdille *et al*., 2003; Paillard *et al*., 2003; Breseghello *et al*., 2005;

Suenaga *et al*., 2005) and 6B (Campbell *et al*., 1999; Sourdille *et al*., 2003; Suenaga *et al*., 2005; Liu *et al*., 2005; Semagn *et al*., 2006c) exhibited SDRs. In the case of chromosome 7D, a region associated with a segregation distortion locus was mapped using an *Aegilops tauschii* F_2 population (Faris *et al*., 1998). Similar to the RS×CS population, Quarrie *et al*. (2005) showed SDRs on chromosomes 4A and 6A, while Semagn *et al*. (2006c) presented regions with high segregation distortion on chromosomes 1D and 4A. In addition, Paillard *et al*. (2003) indicated SDR on 1D and Suenaga *et al*. (2005) on chromosome 6A. Linkage map regions with high segregation distortion have been observed both in RILs (Messmer *et al*., 1999; Xue *et al*., 2008) and DH mapping populations (Cadalen *et al*., 1997; Quarrie *et al*., 2005; Suenaga *et al*., 2005).

Segregation distortion has been proposed to be due to factors such as chromosomal rearrangements (Tanksley, 1984), alleles inducing gametic or zygotic selection (Nakagarha, 1986), reproductive differences between the two parents (Foolad *et al*., 1995), lethal genes (Blanco *et al*., 1998), sterility induced by the distant genetic parental background or selective survival of RILs caused by single seed descent method (Nachit *et al*., 2001) as discussed by Elouafi and Nachit (2004) or loci affecting viability in anther culture in doubled haploid populations (Manninen, 2000). However, inclusion of distorted loci in the linkage map did not affect the marker order in the RS×CS map. In addition to this, the marker order was comparable to the previously published maps, which resulted in the use of the RS×CS map for QTL analysis without eliminating the skewed markers. Similar use of linkage maps with skewed markers for QTL analysis has also been reported in previous studies (Zanetti *et al*., 2001; Groos *et al*., 2003; Sourdille *et al*., 2003; Breseghello *et al*., 2005; Suenaga *et al*., 2005).

4.7 Kernel morphometric traits are determined by a large number of QTLs

The continuous trait distributions and broad phenotypic range of the RILs suggested the effects of many loci for the control of kernel traits in the present population. QTL analysis for kernel morphometric traits was performed using two softwares, QTL Cartographer 2.5 and QTLNetwork 2.0. QTL Cartographer

detected 59 QTLs by composite interval mapping (CIM), which showed varying individual effects. The number of QTLs identified for individual kernel traits ranged from eight (KL and KW) to sixteen (TKW). These QTLs were distributed on various chromosomes with the chromosomes of Group 2 and those of the D genome indicating the highest number of QTLs. For each kernel trait, at least one QTL peak was detected in each of the year-location combinations using CIM (**Tables 3.15-3.19**). Furthermore, QTLNetwork identified a number of main-effect QTLs and epistatic interactions controlling the kernel traits. Similar to these results, many QTLs for each trait such as TKW, KL, KW, LWR, FFD and kernel diameter were detected in previous studies (Sun *et al*., 2009; Gegas *et al*., 2010; Tsilo *et al*., 2010). A discussion on the QTLs detected in the RS×CS population is presented in the following sections.

4.8 QTLs detected for kernel traits at Ludhiana and Pune

In the present study, based on CIM analysis, the total number of QTL peaks detected for Ludhiana and Pune were similar (**Table 3.11**). Considering trait wise, KW, LWR and FFD exhibited a similar number of QTL peaks for both these locations. However, higher number of QTL peaks was detected for TKW at Ludhiana and for KL at Pune. Furthermore, when the QTLs detected in multiple environments were considered, some QTLs were detected at both the locations, some were location-specific and the others were identified in one of the locations as well as with the average data for the respective trait (**Table 4.1**). For all the five kernel traits, QTLs that were observed in both the locations as well as location-specific QTLs detected in multiple years were observed. As discussed in earlier studies, such location-specific QTLs highlight the role of G×E interactions in the expression of complex traits (Jansen *et al*., 1995; Veldboom and Lee, 1996a and b). However, it needs to be noted that QTLs that are detected across locations in the present study might still have significant G×E effects as they have contributed to the respective traits in specific years (**Tables 3.15-3.19**).

Table 4.1: Kernel trait QTLs detected across locations, at a single location and QTLs showing overlap with those of average data for the respective trait

Trait	Overlap among peaks for each QTL detected in multiple environments		
	Across locations	**Within location**	**Only with average data**
TKW	*QTkw.ncl-1A.1*	*QTkw.ncl-2B.2**	-
	QTkw.ncl-2A.2	*QTkw.ncl-2B.3*	
	QTkw.ncl-3D.1		
	*QTkw.ncl-5B.1**		
KL	*QKl.ncl-2D.1**	*QKl.ncl-2B.1**	
	*QKl.ncl-4A.1**	*QKl.ncl-6A.1**	*QKl.ncl-7D.1*
	QKl.ncl-5A.1	*QKl.ncl-7D.2*	
KW	*QKw.ncl-5B.1**	*QKw.ncl-7D.1*	*QKw.ncl-2B.1*
	QKw.ncl-7A.1		*QKw.ncl-2D.1*
			QKw.ncl-2D.2
LWR	*QLwr.ncl-5B.1**	*QLwr.ncl-7D.1*	*QLwr.ncl-5D.1*
FFD	*QFfd.ncl-2B.2**	*QFfd.ncl-4D.1*	*QFfd.ncl-2D.1*
			QFfd.ncl-2D.2
			QFfd.ncl-5B.1

* Overlap with average data QTL for the trait also observed

4.9 Stable QTLs for kernel characters detected by CIM

Multiple year-location analysis of the five kernel morphometric traits displayed QTLs that were detected in more than one environment, ranging from three for LWR to seven for KL (**Tables 3.15-3.19**). More prominently, at least one QTL for

each of the five traits was detected in three or more environments (**Table 3.12**). Their validation using closely linked markers in different mapping populations and diverse wheat genotypes might yield valuable tools for marker-assisted selection procedures. Such stable QTLs can also be considered as appropriate candidates for fine mapping and targeted analysis of the genomic regions harboring them (Collard and Mackill, 2008). The stable QTLs detected for each kernel trait and their positions with reference to previous reports are discussed below.

4.9.1 Thousand kernel weight

For TKW, chromosomes 1A, 2B and 5B harbored stable QTLs in the RS×CS population (**Table 3.15**; **Figure 3.6**). *QTkw.ncl-1A.1* was detected in two years at Ludhiana and for Pune (year 2006). Kumar *et al*. (2006) also reported a QTL for TKW associated with this chromosomal region in the same population with field trials conducted in three locations in North India. In earlier studies, Campbell *et al*. (1999) and Wang *et al*. (2009) also reported kernel weight QTLs on 1A by single-marker analysis (SMA) using RFLP markers and by CIM using SSR markers, respectively. Chromosome 2B harbored a QTL detected in three years at Ludhiana and for TKW Average. The closest marker for this QTL in all these environments was *Xbarc45*. A QTL on the chromosome arm 2BS in the same population was detected for TKW by Kumar *et al*. (2006), but in a region more distal on the chromosome arm. The reason for this difference could possibly be the smaller population size of 100 RILs and clustered AFLP markers mapped among the anchored SSRs in the QTL region by Kumar *et al*. (2006). Some other researchers (Zanetti *et al*., 2001; Groos *et al*., 2003; Huang *et al*., 2006; Breseghello and Sorrells, 2007; Hai *et al*., 2008 and Gegas *et al*., 2010) also reported TKW QTLs on 2B. A hexaploid wheat sucrose synthase 2 gene (*TaSus2-2B*) was mapped between the markers *Xbarc102.2* and *Xbarc91* on 2BS (Jiang *et al*., 2011), which was associated with kernel weight and width. The position of *Xbarc91* is close to the position of *QTkw.ncl-2B.2* in the RS×CS map. Hence, it would be interesting to see if the *TaSus2-2B* gene indeed underlies *QTkw.ncl-2B.2*. The third stable QTL (*QTkw.ncl-5B.1*) for TKW in the present population

was located on chromosome 5B and was detected both at Ludhiana and Pune as well as with TKW average data. *Xgwm67* was the closest marker in three out of four environments for this QTL. Groos *et al*. (2003) and Su *et al*. (2009) also reported a TKW QTL on 5B close to the position of *QTkw.ncl-5B.1*. Huang *et al*. (2003) reported a TKW QTL on 5B with *Xgwm544* as the closest marker. The positions of *Xgwm544* and *Xgwm67* are apart by approximately 5 cM in the wheat consensus map of Somers *et al*. (2004). In addition, Breseghello and Sorrells (2006) and Reif *et al*. (2011) also reported loci controlling TKW on chromosome 5B by association mapping.

4.9.2 Kernel length

Kernel length indicated the highest number of stable QTLs (four) in the present study, with chromosomes 2B, 2D, 4A and 6A harboring one stable QTL each (**Table 3.16, Figure 3.6**). Among these, *QKl.ncl-2B.1* was detected in two years at Pune (years 2005 and 2007) and for KL Average. Campbell *et al*. (1999) and Dholakia *et al*. (2003) also reported KL-associated markers on 2BL by SMA. In the studies of Sun *et al*. (2009), a KL QTL on 2BL close to *Xgwm120* was reported, which is close to the markers associated with *QKl.ncl-2B.1*. Similarly, the chromosomal region harboring *QKl.ncl-2B.1* was also found to be associated with KL in the Malacca × Charger population (Gegas *et al*., 2010). *QKl.ncl-2D.1* was detected in four environments, which were associated with both Ludhiana and Pune as well as with KL Average. Campbell *et al*. (1999) and Dholakia *et al*. (2003) reported an RFLP marker and an SSR marker *Xgwm261*, respectively associated with KL on 2DL. Breseghello and Sorrells (2006) detected a KL-associated 2D marker *Xgwm539*, which is close to the location of *QKl.ncl-2D.1* in the RS×CS map as well as in the wheat consensus map (Somers *et al*., 2004). A KL QTL close to the chromosomal region associated with *QKl.ncl-2D.1* was also reported in the Beaver × Soissons population (Gegas *et al*., 2010) and in a *T. aestivum* × *T. spelta* RIL population reported by Manickavelu *et al*. (2011). The KL stable QTL *QKl.ncl-4A.1* was detected in five environments – Ludhiana (year 2005), Pune (years 2005, 2006 and 2007) as well as in KL Average in the present study. Though Sun *et al*. (2009) reported a QTL for KL in two environments on

4A, it was situated at distal end of 4AL, while *QKl.ncl-4A.1* was located on 4AS close to the centromere. Chromosome 4A was also associated with KL in monosomic studies (Giura and Saulescu, 1996) and the region associated with *QKl.ncl-4A.1* has been reported by Manickavelu *et al*. (2011). Similarly, *QKl.ncl-6A.1* was detected on 6A in two environments at Pune (years 2005 and 2006) and for KL Average. In all these environments, the closest marker for the QTL was *Xbarc146.1*. Ammiraju *et al*. (2004) reported the association of the marker *Xgwm334* located on 6AS with KL using the RS×CS population by SMA. In the Avalon × Cadenza population, a KL QTL was detected on 6A, but on the distal long arm of the chromosome. A kernel size and shape-associated gene *TaGW2* was mapped near the centromere of chromosome 6A, which is close to the position of *QKl.ncl-6A.1* in the RS×CS population (Su *et al*., 2011). However, *TaGW2* is involved in grain development, mainly affecting KW and TKW, and not by significantly influencing KL.

4.9.3 Kernel width

Chromosome 5B showed *QKw.ncl-5B.1*, a stable QTL for KW detected in four environments namely, Ludhiana (years 2003 and 2005), KW2007Pune and KW Average (**Table 3.17, Figure 3.6**). *Xgwm67* was the closest marker for this QTL in all the environments. In previous studies, Breseghello and Sorrells (2006) reported KW loci by association mapping on 5B. Tsilo *et al*. (2010) reported a QTL on 5B for kernel diameter, which could probably be analogous to KW. Other single marker analysis or interval mapping studies of KW have so far not reported QTL for KW on 5B.

4.9.4 Kernel length-width ratio

Chromosomes 5B and 7D each indicated a stable QTL for LWR in the RS×CS population (**Table 3.18, Figure 3.6**). *QLwr.ncl-5B.1* was observed in Ludhiana (years 2003 and 2005) and at Pune (year 2007) and for LWR Average. Other studies have so far not reported 5B in association with LWR. *QLwr.ncl-7D.1* was detected for two years at Ludhiana (years 2003 and 2005) and for LWR Average. For all these environments, *Xwmc405* was the closest marker for this stable QTL. The region on 7DS associated with *QLwr.ncl-7D.1* was also found to harbor a

LWR QTL in the Avalon × Cadenza population in two environments (Gegas *et al*., 2010).

4.9.5 Factor form density

In the RS×CS population, a stable QTL for FFD, namely, *QFfd.ncl-2B.2* was observed on chromosome 2B in three environments, one each at Ludhiana and Pune and for FFD Average (**Table 3.19**, **Figure 3.6**). In all these environments, *Xbarc45* was the closest marker. In earlier studies, chromosome 2B was not associated with FFD. However, a similar feature Density factor computed by kernel weight / area was associated with 2BS (Campbell *et al*., 1999).

4.10 Main-effect QTLs for kernel characters

QTLNetwork enabled the identification of main-effect QTLs for all of the five kernel traits studied in the RS×CS population. The main-effect QTLs indicated genomic regions associated with kernel traits with significant individual effects. Interestingly, the location of most of the main-effect QTLs corresponded with the support intervals of either QTLs detected in multiple environments, or with QTLs having higher contributions for the respective kernel characters detected by QTL Cartographer (**Tables 3.15-3.19, Figures 3.10, 3.12, 3.14, 3.16, 3.18**). For example, two of the main-effect QTLs for TKW detected on chromosomes 2B and 5B, coincided with the locations of the stable QTLs *QTkw.ncl-2B.2* and *QTkw.ncl-5B.1*, respectively. The third main-effect QTL located on distal 2BL co-located with *QTKW.ncl-2B.3* that was detected in two environments with contributions of 14.23% and 7.05%, respectively (**Table 3.15**). Similarly, coincidences between QTLs detected by CIM and main-effect QTLs were also reported by Kumar *et al*. (2007).

4.11 Contribution of favorable alleles from RS and CS based on CIM analysis

The presence of transgressive segregants in the RS×CS population suggested that favorable allele contribution to kernel trait QTLs could be from RS, the superior parent as well as from CS, the poor parent. Though RS contributed majority of

kernel trait QTLs (61.02%), a significant proportion (38.98%) had their origin from CS. For TKW, KL and LWR, the contribution of QTLs from RS was higher than that from CS; while for KW and FFD, interestingly, both RS and CS contributed similar number of QTLs (**Table 3.13**). In case of stable QTLs for all the five kernel traits together, seven out of 11 had favorable allele contribution from RS, while four had their source of superior alleles in CS (**Table 4.2**). Since the stable QTLs on 2B were derived from CS, it raised the question whether their detection was due to the segregation distortion towards CS alleles observed on this chromosome as described in Chapter 3 (Section 3.5). However, chromosome 2B was associated in earlier studies with TKW (Zanetti *et al*., 2001; Groos *et al*., 2003; Huang *et al*., 2006; Kumar *et al*., 2006; Hai *et al*., 2008; Gegas *et al*., 2010 Jiang *et al*., 2011) and KL (Gegas *et al*., 2010), which ruled out this possibility. Similarly, in case of chromosome 7D, a SDR with markers skewed towards CS harbored QTLs for all the five kernel traits in the RS×CS population. However, QTLs for TKW, KW and FFD showed favorable allele contribution from CS, while KL and LWR indicated the same from RS. This ruled out the possibility of interference of skewed markers on QTL detection of these traits and further suggested greater interdependence of TKW, KW and FFD compared to their relationship with KL and LWR.

When the favorable allele contribution was analyzed in case of the QTL cluster on 5B, both TKW- and KW- stable QTLs displayed superior allele contribution from RS, while for *QLwr.ncl-5B.1*, the QTL showed contribution from CS in spite of high degree of correspondence in their support intervals (**Tables 3.15, 3.17-3.18**). TKW and KW displayed highly significant positive correlations in all environments, while the same for LWR with TKW and KW were decidedly negative (**Table 3.2**). The opposite effects of RS and CS alleles in the region of *QLwr.ncl-5B.1* on TKW and KW compared to LWR supported the results of the correlation analysis and indicated greater influence of the horizontal axis (KW) than the vertical axis (KL) on variation in TKW in this RIL population. Likewise, Su *et al*. (2011) reported a gene *TaGW2* that contributed to kernel size by influencing KW and TKW, but not KL. Its rice ortholog *OsGW2* (Song *et al*., 2007), contributed to kernel weight by primarily influencing KW, while kernel

thickness and length had minor contributions in increasing kernel weight. On the other hand, in case of rice *GS3*, the increase in grain weight was mainly due to increase in grain length and not due to grain width and thickness (Fan *et al*., 2006; Mao *et al*., 2010).

When the expression of the number of QTLs derived from RS and CS in different environments was investigated, an interesting pattern was noted. A large proportion of RS-derived QTLs were preferentially expressed in Pune while similar tendency towards Ludhiana location was observed for CS-derived QTLs (**Table 3.13**). This may suggest the differential patterns of G×E interactions of the QTLs with positive allele contribution from the two parental genotypes. Furthermore, when QTLs that were expressed in both the locations were compared, RS contributed more QTLs in both Ludhiana and Pune compared to CS, which might suggest the need for the availability of increased information on particular QTLs before they are chosen for molecular breeding approaches.

Table 4.2: Source of favorable alleles from parental genotypes for stable QTLs for kernel characters

Source of favorable alleles for stable QTLs	
RS	**CS**
QTkw.ncl-1A.1	*QTkw.ncl-2B.2*
QTkw.ncl-5B.1	*QKl.ncl-2B.1*
QKl.ncl-2D.1	*QLwr.ncl-5B.1*
QKl.ncl-4A.1	*QFfd.ncl-2B.2*
QKl.ncl-6A.1	
QKw.ncl-5B.1	
QLwr.ncl-7D.1	

4.12 Differential and co-locating QTLs for kernel characters

In the RS×CS population, CIM analysis showed some of the chromosomal regions with QTLs for individual kernel characters (differential QTLs), while others with co-locating QTLs for more than one kernel trait (**Figure 3.6**). Interestingly, apart from QTLs for TKW, KW and KL, there were differential QTLs observed for LWR and FFD as well. Gegas *et al*. (2010) also reported some independent LWR or FFD QTLs in Avalon × Cadenza, Savannah × Rialto and Shango × Shamrock DH populations. This observation may suggest that only TKW, KL and KW might not determine the overall kernel morphology and traits like LWR and FFD might also add to the information on its genetic determination. Among the differential QTLs for kernel traits in the RS×CS population, noteworthy were *QTkw.ncl-1A.1*, *QTkw.ncl-2A.2*, *QTkw.ncl-3D.1*, *QKl.ncl-5A.1*, *QKl.ncl-7D.2*, *QLwr.ncl-5D.1* and *QFfd.ncl-4D.1*, which were observed in more than one environment, while *QLwr.ncl-5D.1* and *QFfd.ncl-5D.1* showed contributions of approximately 10 and 9% respectively, which were among the highest detected for these traits (**Tables 3.15, 3.16. 3.18, 3.19**).

Among the co-locating QTLs, noteworthy were the regions on 2D and 7D, which showed overlapping QTLs for all the five kernel characters studied and regions on 2B and 5B with overlapping QTLs for four of the kernel characters analyzed in the RS×CS population (**Figure 3.6**). QTLNetwork also showed co-locating main-effect QTLs for TKW, KW and LWR on 5B, for KL, KW and LWR on 7D, for TKW and KL; TKW and FFD and KW and LWR in different regions of 2B and for KL and LWR on 4A (Figures 3.10, 3.12, 3.14, 3.16, 3.18). Interestingly, epistatic QTLs on 2B for KL and KW also displayed co-location. In previous studies, Sun *et al*. (2009), Gegas *et al*. (2010) and Tsilo *et al*. (2010) showed common QTL regions detected on a number of chromosomes for multiple traits. Since such co-locating QTLs for different traits detected in the RS×CS population and in other previous studies displayed large support intervals, they might harbor discrete but co-segregating QTLs controlling separate traits. It could also be possible that in some cases the influence of a genomic region on more than one trait might reflect that the inheritance of underlying gene(s) could be pleiotropic i.e. linked functionally through common mechanistic bases.

4.13 Pleiotropic QTLs for kernel characters

QTL Cartographer v. 2.5 offers multi-trait CIM (Mt-CIM) based on the statistical methods developed by Jiang and Zeng (1995). Mt-CIM suggested the presence of pleiotropic loci for kernel traits on five chromosomes, namely, 2B, 2D, 4A, 5B and 7D (**Table 3.30**) in the present RS×CS population. Among these, chromosome 2B harbored pleiotropic loci for TKW, KW, LWR and FFD. Pleiotropic regions were also observed for different combinations of two traits, except for KL-TKW and KL-FFD. When the support intervals for KL QTLs detected by CIM were observed in comparison with those for TKW and FFD, only partial overlap between them was noted (**Figure 3.6**). This might suggest that loci controlling KL might be distinct from those controlling TKW and FFD. In previous studies, even though Tsilo *et al*. (2010) and Sun *et al*. (2009) reported QTL clusters for kernel size and shape, statistical analyses for pleiotropy were not performed in both the cases. In the present study, although indications of loci with pleiotropic effects were pointed out by Mt-CIM, fine mapping and further identification and characterization of the underlying gene(s) is needed to obtain stronger evidence of their pleiotropic role in controlling kernel characters (Xie *et al*., 2008).

4.14 Epistatic QTLs for kernel characters

Epistasis refers to the phenotypic effects of interactions among alleles at multiple loci and makes a substantial contribution to variation in complex traits (Philips, 1998; Carlborg and Haley, 2004). Several recent genetic analyses using molecular markers in various plant species have clearly shown that, in addition to single locus QTLs, epistatic interactions play an important role on the determination of quantitative traits (Xing *et al*., 2002; Kulwal *et al*., 2004; Wang B *et al*., 2007; Lin *et al*., 2008; Gutierrez-Gonzalez *et al*., 2010). However, CIM methods used for dissecting QTL effects in commonly used softwares like QTL Cartographer usually assume the absence of epistasis among QTLs (Zeng 1993, 1994). This assumption was made largely for simplification of the statistical models, but its violation might result in biased estimates of the positions and effects of QTLs (Wang *et al*., 1999). In the present study, QTLNetwork v. 2.0 was employed,

which can systematically map the QTLs involved in digenic epistasis as well as QTL × Environment interactions based on the mixed-model approaches (Yang *et al*., 2007). By this analysis, several epistatic interactions were detected for kernel morphometric traits in the RS×CS population (**Figures 3.9-3.18**)**.** In case of kernel characters, though Sun *et al*. (2009) employed QTLNetwork, the role of epistatic QTLs and the distinction between epistatic and main-effect QTLs were not reported in their studies. Hence, with reference to the literature surveyed during the current analysis, the role of epistatic interactions in the control of wheat kernel shape traits is being reported for the first time, while the same for TKW has been reported in earlier studies (Mir *et al*., 2008). Most of the epistatic interactions detected in the present study on the RS×CS population were between QTLs that did not display significant individual effects. Indeed, most of them, did not share marker intervals with single-locus QTLs detected by QTL Cartographer. Only in case of TKW, partial overlap was observed between three of the epistatic QTLs and QTLs detected by CIM (chromosomes 2B and 6D). Interestingly, as an exception, one main-effect QTL on 2B for TKW whose location was coincident with one of the stable TKW QTLs detected by CIM was found to be involved in two epistatic interactions. Similar involvement of main-effect QTLs in epistatic interactions was observed by Gutierrez-Gonzalez *et al*. (2010) in soybean. This highlights the importance of evaluating the QTLs with a potential to be MAS targets for their involvement in epistatic interactions.

4.15 Role of G×E effects as suggested by QTL studies

The performance of individual QTLs across environments can be tracked by QTL analysis using data collected from phenotypic trials in multiple environments. The importance of QTL × Environment interactions in the expression of quantitative traits has been discussed in many studies (Hittalmani *et al*., 2003; Talukder *et al*., 2005; Kuchel *et al*., 2007b; Snape *et al*., 2007). In the RS×CS population, CIM detected 32 out of 59 QTLs in only one environment (**Table 3.12**), while QTLNetwork detected the influence of environment on both, main-effect QTLs (five out of 19) and epistatic interactions (one out of 14) (**Figures 3.6 and 3.9-3.15**) reflecting the significant role for G×E interaction in kernel trait expression.

It was interesting to note that even though FFD displayed higher contributions from G×E interactions compared to the other traits, none of the main-effect QTLs or the two epistatic interactions identified suggested QTL × Environment interactions (**Figure 3.17**). However, the presence of some additional unidentified QTLs in the present population might be involved in environmental interactions and might have resulted in the increased G×E contribution for FFD in AMMI analysis. Influence of the environment on main-effect and QTL×QTL interactions was reported in studies on different crop species, for various traits such as yield traits in rice (Xing *et al*., 2002), leaf and sheath blight in maize (Lin *et al*., 2008), grain protein content (Kulwal *et al*., 2004) and flour color in wheat (Zhang *et al*., 2009) and isoflavone accumulation in soybean (Gutierrez-Gonzalez, 2010) that emphasized the importance of their analysis in genetic dissection of complex traits.

4.16 Complexity in the determination of kernel characters in bread wheat

The study of kernel characters in the RS×CS population revealed that their expression was due to the role of a number of QTLs with varied effects, QTL×QTL interactions, G×E interaction effects and to a certain extent, environmental main-effects. In addition to these, QTL clusters and putative pleiotropic QTLs added more dimensions in the genetic control of these traits. Furthermore, increasing the genomic coverage of the RS×CS framework map using more SSRs, EST-SSRs and high-throughput markers like DArT might lead to the identification of more QTLs and epistatic interactions. The regions of stable QTLs in the present RS×CS map warrant further attention. Their fine mapping and possible short listing of candidate genes in the region might lead to the discovery of underlying gene(s) that could shed light on the genetic basis of kernel characters in wheat.

4.17 Potential utility of kernel character QTLs revealed in the RS×CS population for MAS

Since kernel traits require the maturity and harvest of each generation during selection, MAS might prove beneficial for their breeding. In addition to this, knowledge of markers linked to QTLs for kernel traits might be useful in elite background recovery during marker-assisted backcross breeding. In the RS×CS population, several stable QTLs were observed that could be of potential use in diverse marker-based selection schemes. However, QTLs such as *QTkw.ncl-2B.2* that was involved in epistatic interactions and *QLwr.ncl-7D.1* that showed the influence of environment might pose difficulties during selection. The TKW stable QTL on chromosome 5B, *QTkw.ncl-5B.1* might be useful as a potential target since it was not involved in either G×E or epistatic interactions. Selection for the superior allele contributed by RS at this locus might increase the kernel weight and width and result in bolder grains but with lower LWR as suggested by the additive effects (**Tables 3.15, 3.17, 3.18**). The validation of stable kernel character QTLs identified in the present study in different mapping populations and diverse germplasm might provide useful markers for development of better quality wheat.

SUMMARY AND FUTURE DIRECTIONS

Summary and future directions

Wheat kernel characters related to its size and shape influence the milling yield and harvests with bold grains ensure better returns for the farmer. Five kernel morphometric traits, namely, thousand kernel weight (TKW), kernel length (KL), kernel width (KW), kernel length-width ratio (LWR) and factor form density (FFD) were analyzed in a population of hexaploid wheat. A detailed analysis of phenotypic variation in these traits was performed using multiple year-location data recorded from two diverse wheat-growing regions in India. Based on quantitative trait locus (QTL) analysis using a framework linkage map and phenotypic data, a number of chromosomal regions associated with kernel morphometric traits were detected. A summary of the results is presented below.

Important outcomes

- *Phenotypic evaluations and analysis of trait variance*: Field trials of the recombinant inbred line (RIL) population of the hexaploid wheat cross Rye Selection 111 (RS) × Chinese Spring (CS) for many year-location combinations suggested the increased participation of Genotype main-effects and genotype×environment interactions in trait variation compared to Environment main-effects. The traits showed significant phenotypic correlations among each other that suggested underlying genetic interrelationships.

- *Framework map of RS×CS population*: The simple sequence repeat (SSR) marker framework map constructed for the present population consisted of 251 markers in 32 linkage groups that represented all the 21 bread wheat chromosomes. This linkage map with total length of 1655.28 cM and average interval of 6.59 cM was used for QTL analysis.

- *QTL analysis of kernel morphometric traits*: For the five kernel traits together, composite interval mapping (CIM) revealed 59 QTLs distributed

on 16 chromosomes. The number of QTLs for each trait ranged from 8 (for KL and KW) to 16 (for TKW). QTL clusters and putative pleiotropic regions were detected for various trait combinations. Mixed model-based QTL mapping identified 19 main-effect QTLs with individual effects and many epistatic interactions for the five kernel traits under study.

- *Identification of stable QTLs*: Stable QTLs that were detected in multiple year-location combinations were identified for all the five kernel morphometric traits by CIM. TKW showed stable QTLs on chromosomes 1A, 2B and 5B and KL on 2B, 2D, 4A and 6A. A single stable QTL each for KW and FFD was detected on 5B and 2B, respectively. Similarly, LWR stable QTLs were present on the chromosomes 5B and 7D. A majority of the regions associated with these stable QTLs were also detected as main-effect QTLs for respective traits by mixed model based QTL mapping.

- *QTL × Environment interactions for kernel traits*: The importance of QTL × Environment interactions in kernel trait determination was revealed in the present study. This was exemplified by the detection of some QTLs exclusively in Ludhiana or Pune locations and others in both the locations for all the kernel traits with CIM analysis. A number of main-effect QTLs and a single epistatic interaction were involved in QTL × Environment interactions as shown by mixed model based QTL mapping approach.

Future directions

The information on the kernel morphometric trait determination revealed by the present analysis can be used for further studies and also for wheat improvement. Some of the efforts that can be taken in this regard are suggested –

- Increase in genome coverage of RS×CS linkage map with high throughput diversity arrays technology (DArT) markers along with markers from the genic regions of wheat genome might reveal more QTLs associated with kernel traits.
- Marker information from stable QTL regions is of potential use for selection during breeding. Confirmation of such QTL positions in other populations and validation of closely linked markers with diverse genotypes might contribute suitable markers for selection of better kernel characters in wheat.
- Fine mapping of chromosomal regions harboring stable QTLs and pleiotropic QTLs may provide useful information on underlying loci. The new marker systems and the sequencing projects might provide valuable resources for this task. Presently, a locus for TKW – *QTkw.ncl-1A.1* is being focused for fine mapping. For this, four crosses between selected RILs of RS×CS population with high TKW (RIL105 and RIL108) and low TKW (RIL28 and RIL91) have been initiated.

BIBLIOGRAPHY

गोधूमाः क्षलिता शुभ्राः शोषिता रविरश्मिभिः।७५
घरट्टैश्चूर्णिताःश्लक्ष्णाश्चालन्या वितुषीकृताः।
गोधूमचूर्णकं किञ्चित्घृत्त विमिश्रितम।७६
लवणेन च संयुक्तं क्षीर नीरेणपिण्डितम्।
सुमहत्यां काष्ठपात्र्यां करास्फालैर्विमर्दयेत्।७७
मर्दितं चिक्कणीभूतं गोलकान् परिकल्पयेत्।
स्नेहाभ्यक्तैः करतलैः शालिचूर्णैर्विरूक्षितान्।७८
प्रसारयेत गोलकांस्तान् करसञ्चारवर्तनैः।
विस्तृतामण्डकाःश्लक्षाः सितपद्मसमप्रभाः।७९

Wheat was washed, dried in the sun, ground, and cleaned in a sieve. The flour was mixed with clarified butter and salt and made into balls. The balls were turned into cakes with the palms of hands and were cooked in a pot-sherd. They were baked on live charcoals before eating. Sometimes a wooden roller and a piece of stone were used to change the balls into circular cakes before baking.

Someshwara (1100 AD) Manasollas III, 1375-79

..and a process similar to the one given in this 900 year-old Sanskrit treatise is still used in millions of households to prepare their daily bread

Bibliography

Adams K, Wendel J (2005) Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* 8: 135–141

Adamski N, Anastaslou E, Eriksson S, O'Neill C, Lenhard M (2009) Local maternal control of seed size by KLUH/CYP78A5-dependent growth signaling. *Proceedings of the National Academy of Sciences USA* 106: 20115-20120

Agri-facts: Practical information for Alberta's agriculture industry. Agdex 100/ 22-1 (2007) www.agric.gov.ab.ca

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden M, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics*, 113(8): 1409–1420

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. *Theoretical and Applied Genetics* 119: 507–517

Alexander W, Smith E, Dhanasobhan C (1984) A comparison of yield and yield component selection in winter wheat. *Euphytica* 33: 953–961

Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard J (2004) QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*. *Theoretical and Applied Genetics* 109: 630–639

Ammiraju J, Dholakia B, Jawdekar G, Santra D, Röder M, Singh H, Lagu M, Dhaliwal H, Rao V, Ranjekar P, Gupta V (2004) Identification of chromosomal regions governing grain size and shape in wheat (*Triticum aestivum* L.). *Journal of Genetics and Breeding* 58: 91-100

Ammiraju J, Dholakia B, Santra D, Singh H, Lagu M, Tamhankar S, Dhaliwal H, Rao V, Gupta V, Ranjekar P (2001) Identification of inter simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theoretical and Applied Genetics* 102: 726–732

Anderson J, Chao S, Liu S (2007) Molecular breeding using a major QTL for *Fusarium* head blight resistance in wheat. *Crop Science* 47: 112-119

Araki E, Miura H, Sawada S (1999) Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. *Theoretical and Applied Genetics* 98: 977–984

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* 9: 208-218

Asins M (2002) Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding* 121: 281-291

Asins M, Bernet G, Villalta I, Carbonell E (2010) QTL analysis in plant breeding. In *Molecular techniques in crop improvement.* SM Jain and DS Brar (eds.) Springer Science and Business Media BV. p3-22

Austin D, Lee M (1996) Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. *Theoretical and Applied Genetics* 92: 817–826

Ayoub M, Symons S, Edney M, Mather D (2002) QTLs affecting kernel size and shape in a two-rowed by six-rowed barley cross. *Theoretical and Applied Genetics* 105: 237–247

Bagge M, Lübberstedt T (2008) Functional markers in wheat: technical and economic aspects. *Molecular Breeding* 22: 319–328

Bagge M, Xia X, Lübberstedt T (2007) Functional markers in wheat. *Current Opinion in Plant Biology* 10: 211–216

Bainotti C, Fraschina J, Salines J, Nisi J, Dubcovsky J, Lewis S, Bullrich L, Vanzetti L, Cuniberti M, Campos P, Formica M, Masiero B, Alberione E, Helguera M (2009) Registration of 'BIOINTA 2004' Wheat. *Journal of Plant Registrations* 3: 165–169

Baker S, Herrman T, Loughin T (1999) Segregating hard red winter wheat into dough factor groups using single kernel measurements and whole grain protein analysis. *Cereal chemistry* 76: 884-889

Balfourier F, Roussel V, Strelchenko P, Exbrayat-Vinson F, Sourdille P, Boutet G, Koenig J, Ravel C, Mitrofanova O, Beckert M, Charmet G (2007) A worldwide bread wheat core collection arrayed in a 384-well plate. *Theoretical and Applied Genetics* 114: 1265–1275

Balter M (2007) Seeking Agriculture's Ancient Roots. *Science* 316: 1830-1835

Bannier E (1979) Monosomic analysis of the inheritance of 1000 grain weight in the winter wheat varieties, Karkaz and Orlando. *Arch Zuchtungsforsch* 9: 209-214

Baril C (1992) Factor regression for interpreting genotype-environment interaction in bread-wheat trials. *Theoretical and Applied Genetics*. 83: 1022–1026

Barloy D, Lemoine J, Abelard P, Tanguy A, Rivoal R, Jahier J (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. *Molecular Breeding* 20: 31-40

Barmore M, Bequette P (1965) Weight per bushel and flour yield of Pacific Northwest white wheat. *Cereal Science Today* 10: 72-77

Basten C, Weir B, Zeng Z (1994) Zmap-a QTL Cartographer. In: J.S.G.C. Smith, B.J. Benkel,W.F. Chesnais, J.P. Gibson, BW Kennedy & EB Burnside (eds.), Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software, Guelph, Ontario, Canada.

Basten C, Weir B, Zeng Z (2001) QTL Cartographer. Department of Statistics, North Carolina State University, Raleigh, NC.

Belderok B (2010a) The wheat grain. *Plant Foods for Human Nutrition* 55: 15-20

Belderok B (2010b) Milling of wheat. *Plant Foods for Human Nutrition* 55: 21-29

Bennetzen J (2007) Patterns in grass genome evolution. *Current Opinion in Plant Biology* 10: 176–181

Bérard A, Le Paslier M, Dardevet M, Exbrayat-Vinson F, Bonnin I, Cenci A, Haudry A, Brunel D, Ravel C (2009) High-throughput single nucleotide polymorphism genotyping in wheat (*Triticum* spp.) *Plant Biotechnology Journal* 7: 364–374

Bergman C, Gualberto D, Campbell K, Sorrells M, Finney P (2000) Kernel morphology variation in a population derived from a soft by hard wheat cross and associations with end-use quality traits. *Journal of Food Quality* 23: 391–407

Berman M, Bason M, Ellison R, Peden G, Wrigley C (1996) Image analysis of the whole grains to screen for flour-milling yield in wheat breeding. *Cereal chemistry* 73: 323-327

Bernardo A, Bradbury P, Ma H, Hu S, Bowden R, Buckler E, Bai G (2009) Discovery and mapping of single feature polymorphisms in wheat using Affymetrix arrays. *BMC Genomics* 10: 251

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science* 48: 1649–1664

Blanco A, Bellomo M, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta M, Porceddu E, Sciancalepore A, Simeone R, Tanzarella O (1998) A genetic linkage map of durum wheat. *Theoretical and Applied Genetics* 97: 721–728

Bonjean A, Angus W (eds.) (2001) The world wheat book: A history of wheat breeding. Lavoisier Publishing. France

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder M, Weber W (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 105: 921–936

Botwright T, Condon A, Rebetzke G, Richards R (2002) Field evaluation of early vigour for genetic improvement of grain yield in wheat. *Australian Journal of Agricultural Research* 53(10): 1137-1145

Bräutigam A, Gowik U (2010) What can next generation sequencing do for you? Next generation sequencing as a valuable tool in plant research. *Plant Biology* 12(6): 831–841

Bredemeier C, Claudio MM, Büttenbender D (2001) Effect of seed size on initial plant growth and grain yield of wheat. *Pesquisa Agropecuária Brasileira* 36: 1061-1068

Breseghello F, Finney P, Gaines C, Andrews L, Tanaka J, Penner G, Sorrells M (2005) Genetic loci related to kernel quality differences between a soft and a hard wheat cultivar. *Crop Science* 45: 1685–1695

Breseghello F, Sorrells M (2006a) Association analysis as a strategy for improvement of quantitative traits in plants. *Crop Science* 46: 1323–1330

Breseghello F, Sorrells M (2006b) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics* 172: 1165–1177

Breseghello F, Sorrells M (2007) QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. *Field Crops Research* 101: 172–179

Brown T, Jones M, Powell W, Allaby R (2009) The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology and Evolution* 24: 103–109

Burr B, Burr F (1991) Recombinant inbred lines for molecular mapping in maize: Theoretical and practical considerations. *Trends in Genetics* 7: 55–60

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell., and comparison with a map from a wide cross. *Theoretical and Applied Genetics* 94: 367–377

Campbell B, Baenziger P, Gill K, Eskridge K, Budak H, Erayman M, Dweikat I, Yen Y (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. *Crop Science* 43: 1493–1505

Campbell K, Bergman C, Gualberto D, Anderson J, Giroux M, Hareland G, Fulcher R, Sorrells M, Finney P (1999) Quantitative trait loci associated with kernel traits in a soft×hard wheat cross. *Crop Science* 39: 1184–1195

Cane K, Spackman M, Eagles H (2004) Puroindoline genes and the effects on grain quality traits in southern Australian wheat cultivars. *Australian Journal of Agricultural Research* 53: 89-95

Cantu D, Vanzetti L, Sumner A, Dubcovsky M, Matvienko M, Distelfeld A, Michelmore R, Dubcovsky J (2010) Small RNAs, DNA methylation and transposable elements in wheat. *BMC Genomics* 11: 408

Carlborg O, Haley C (2004) Epistasis: too often neglected in complex trait studies? *Nature reviews* 5: 618-625

Chao S, Zhang W, Akhunov E, Sherman J, Ma Y, Luo M, Dubcovsky J (2009) Analysis of gene-derived SNP marker polymorphism in US wheat (*Triticum aestivum* L.) cultivars. *Molecular Breeding* 23: 23–33

Chase K, Adler F, Lark K (1997) Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. *Theoretical and Applied Genetics* 94: 724-730

Chastain T, Ward K, Wysocki D (1995) Stand establishment responses of soft white winter wheat to seedbed residue and seed size. *Crop Science* 35: 213-218

Chhuneja P, Kaur S, Garg T, Ghai M, Kaur S, Prashar M, Bains N, Goel R, Keller B, Dhaliwal H, Singh K (2008) Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. *Theoretical and Applied Genetics* 116: 313–324

Cho Y, Kang H, Lee J, Lee Y, Lim S, Gauch H, Eun M, McCouch S (2007) Identification of quantitative trait loci in rice for yield, yield components, and agronomic traits across years and locations. *Crop Science* 47: 2403–2417

Chojecki A, Gale M, Bayliss M (1983) Reciprocal monosomic analysis of grain size in wheat. In: Sakamoto S (ed) *Proceedings of the sixth International Wheat Genetics Symosium* Maruzen, Koyoto, Japan p1061-1071

Choulet F, Wicker T, Rustenholz C, Paux E, Salse J, Leroy P, Schlub S, Le Paslier M, Magdelenat G, Gonthier C, Couloux A, Budak H, Breen J, Pumphrey M, Liu S, Kong X, Jia J, Gut M, Brunel D, Anderson JA, Gill B, Appels R, Keller B, Feuillet C (2010) Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces. *The Plant Cell* 22: 1686-1701

Churchill G, Doerge R (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963–971

Cloutier S, McCallum B, Loutre C, Banks T, Wicker T, Feuillet C, Keller B, Jordan M (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large *psr567* gene family. *Plant Molecular Biology* 65: 93-106

Collard B, Jahufer M, Brouwer J, Pang E (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* 142: 169–196

Collard B, Mackill D (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B* 363: 557–572

Cordell H, Todd J, Lathrop G (1998) Mapping multiple linked quantitative trait loci in non-obese diabetic mice using a stepwise regression strategy. *Genetics Research* 71: 51–64

Cornell H, Hoveling A (1998) Wheat Chemistry and Utilization. Technomic publishing company, Inc, United States of America

Crossa J (1990) Statistical analysis of multi-location trials. *Advances in Agronomy* 44: 55–85

Cuthbert J, Somers D, Brûlé-Babel A, Brown P, Crow G (2008) Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 117: 595-608

De Koeyer D, Tinker N, Wight C, Deyl J, Burrows V, O'Donoughue L, Lybaert A, Molnar S, Armstrong K, Fedak G, Wesenberg D, Rossnagel B, McElroy A (2004) A molecular linkage map with associated QTLs from a hulless × covered spring oat population. *Theoretical and Applied Genetics* 108: 1285–1298

Denis J, Gower J (1996) Asymptotic confidence regions for biadditive models: interpreting genotype–environment interactions. *Applied Statistics* 45: 479–493

Devos K (2010) Grass genome organization and evolution. *Current Opinion in Plant Biology* 13: 139–145

Dholakia B, Ammiraju J, Santra D, Singh H, Katti M, Lagu M, Tamhankar S, Rao V, Gupta V, Dhaliwal H, Ranjekar P (2001**)** Molecular marker analysis of protein content using PCR-based markers in wheat. *Biochemical Genetics* 39: 325-338

Dholakia B, Ammiraju J, Singh H, Lagu M, Röder M, Rao V, Dhaliwal H, Ranjekar P, Gupta V (2003) Molecular marker analysis of kernel size and shape in bread wheat. *Plant Breeding* 122: 392-395

Dilbirligi M, Erayman M, Campbell B, Randhawa H, Baenziger P, Dweikat I, Gill K (2006) High-density mapping and comparative analysis of agronomically important traits on wheat chromosome 3A. *Genomics* 88: 74–87

Dixon J, Braun H, and Crouch J (2009) Overview: Transitioning Wheat Research to Serve the Future Needs of the Developing World. In *Wheat Facts and Futures 2009*. Dixon, J., H.-J. Braun, P. Kosina, and J. Crouch (eds.). Mexico, D.F.: CIMMYT p1-25

Doerge R (2002) Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics* 3: 43–52

Doerge R, Churchill G (1996) Permutation tests for multiple loci affecting a quantitative character. *Genetics* 142: 285–294

Doležel J, Kubálaková M, Paux E, Bartoš J, Feuillet C (2007) Chromosome-based genomics in the cereals. *Chromosome Research* 15: 51-66

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploidy wheat under domestication. *Science* 316: 1862–1866

Edwards D, Batley J (2010) Plant genome sequencing: applications for crop improvement *Plant Biotechnology Journal* 8: 2-9

Edwards K, Reid A, Coghill J, Berry S, Barker G (2009) Multiplex single nucleotide polymorphism (SNP)-based genotyping in allohexaploid wheat using padlock probes. *Plant Biotechnology Journal* 7: 375–390

Elangovan M (2007) Molecular dissection of breadmaking quality in wheat (*Triticum aestivum* L.) PhD Thesis, University of Pune, Pune, India

Elangovan M, Rai R, Dholakia B, Lagu M, Tiwari R, Gupta R, Rao V, Roder M, Gupta V (2008) Molecular genetic mapping of quantitative trait loci associated with loaf volume in hexaploid wheat (*Triticum aestivum*). *Journal of Cereal Science* 47: 587–598

Elouafi I, Nachit M (2004) A genetic linkage map of the Durum × *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. *Theoretical and Applied Genetics* 108: 401–413

Endo T, Gill B **(**1996) The deletion stocks of common wheat. *Journal of Heredity* 87: 295-307

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger P, Gill K (2004) Demarcating the gene-rich regions of the wheat genome. *Nucleic Acids Research* 32(12): 3546–3565

Eujayl I, Sorrells M, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theoretical and Applied Genetics* 104: 399–407

Falke K, Wilde P, Wortmann H, Geiger, Miedaner T (2009) Identification of genomic regions carrying QTL for agronomic and quality traits in rye (*Secale cereale*) introgression libraries. *Plant Breeding* 128: 615-623

Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theoretical and Applied Genetics* 112: 1164–1171

FAOSTAT (2009) Table D9, FAO Statistical Yearbook 2009 Statistics Division. Food and Agricultural Organization (FAO)

FAOSTAT (2011) Statistics division, Food and Agricultural Organization FAO, Rome. http://www.fao.org/ag

Faris J, Laddomada B, Gill B (1998) Molecular mapping of segregation distortion loci in *Aegilops tauschii*. *Genetics* 149: 319–327

Feldman M (2001) Origin of cultivated wheat. In The World Wheat Book: A History of Wheat Breeding, A.P. Bonjean (ed.) Andover, UK: Intercept p3–56

Feldman M, Levy A (2009) Genome evolution in allopolyploid wheat- a revolutionary reprogramming followed by gradual changes. *Journal of Genetics and Genomics* 36: 511-518

Feldman M, Lupton F, Miller T (1995) Wheats. In: J Smartt, N Simmonds, (eds.) *Evolution of crop plants*, Second edition London: Longman Scientific and Technical Press, p184–192

Feuillet C, Eversole K (2007) Physical mapping of the wheat genome: A coordinated effort to lay the foundation for genome sequencing and develop tools for breeders. *Israel Journal of Plant Sciences* 55: 307–313

Fitzgerald M, McCouch S, Hall R (2009) Not just a grain of rice: the quest for quality. *Trends in Plant Science* 14: 133-139

Fontaine J, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat *Theoretical and Applied Genetics* 119: 645–662

Foolad M, Arulsekar S, Becerra V, Bliss F (1995) A genetic map of Prunus based on an interspecific cross between peach and almond. *Theoretical and Applied Genetics* 91: 262–269

Fu D, Uauy C, Blechl A, Dubcovsky J (2007) RNA interference for wheat functional gene analysis. *Transgenic Research* 16: 689–701

Fuller D (2007) Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the Old World. *Annals of Botany (London)* 100: 903–924

Gabriel K (1971) The biplot graphic display of matrices with application to principal component analysis. *Biometrika* 58: 453–467

Ganal M, Röder M (2007) Microsatellite and SNP markers in wheat breeding *R.K. Varshney and R. Tuberosa (eds.), Genomics Assisted Crop Improvement: Vol. 2: Genomics Applications in Crops*. p1–24

Garcia D, Saingery V, Chambrier P, Mayer U, Jurgens G, Berger F (2003) *Arabidopsis haiku* mutants reveal new controls of seed size by endosperm. *Plant Physiology* 131: 1661-1670

Gauch H (1988) Model selection and validation for yield trials with interaction. *Biometrics* 44: 705–715

Gauch H (1992) Statistical Analysis of Regional Yield Trials: AMMI Analysis of Factorial Designs. Elsevier, Amsterdam, The Netherlands, 278

Gauch H (2006) Statistical analysis of yield trials by AMMI and GGE. *Crop Science* 46: 1488-1500

Gauch H, Zobel R (1988) Predictive and postdictive success of statistical analyses of yield trials. *Theoretical and Applied Genetics* 76: 1–10

Gauch H, Zobel R (1989) Accuracy and selection success in yield trial analyses. *Theoretical and Applied Genetics* 77: 473-481

Gegas V, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan J, Snape J (2010) A genetic framework for grain size and shape variation in wheat. *The Plant Cell* 22: 1046–1056

Giura A, Saulescu N (1996) Chromosomal location of genes controlling grain size in a large grained selection of wheat (*Triticum aestivum* L). *Euphytica* 89: 77–80

Gouesnard B, Bataillon TM, Decoux G, Rozale C, Schoen DJ, David JL (2001) MSTRAT: An Algorithm for Building Germ Plasm Core Collections by Maximizing Allelic or Phenotypic Richness. *Journal of Heredity* 92(1): 93-94

Goyal A, Bandopadhyay R, Sourdille P, Endo TR, Balyan HS, Gupta PK (2005) Physical molecular maps of wheat chromosomes. *Functional & Integrative Genomics* 5(4): 260–263

Groh S, Kianian S, Phillips R, Rines H, Stuthman D, Wesenberg D, Fulcher R (2001) Analysis of factors influencing milling yield and their association to other traits by QTL analysis in two hexaploid oat populations. *Theoretical and Applied Genetics* 103: 9–18

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics* 106: 1032–1040

Gupta P and Xu Y (2008) Genomics of Major Crops and Model Plant Species. In *International Journal of Plant Genomics*. Hindawi Publishing Corporation. doi:10.1155/2008/171928

Gupta P, Balyan H, Edwards K, Isaac P, Korzun V, Röder M, Gautier M, Joudrier P, Schlatter A, Dubcovsky J, De la Pena R, Khairallah M, Penner G, Hayden M, Sharp P, Keller B, Wang R, Hardouin J, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and Applied Genetics* 105: 413–422

Gupta P, Langridge P, Mir R (2010) Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding* 26: 145-161

Gupta P, Mir R, Mohan A, Kumar J (2008) Wheat Genomics: Present Status and Future Prospects. *International Journal of Plant Genomics*. Hindawi Publishing Corporation Article ID 896451 doi:10.1155/2008/896451

Gupta P, Varshney R (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163–185

Gupta P, Varshney R, Sharma P, Ramesh B (1999) Molecular markers and their application in wheat breeding. *Plant Breeding* 118: 369-390

Gupta R, Mohan D, Sewa Ram, Mishra B (eds.), DWR progress report (2008), Volume 4. Wheat quality, All India coordinated wheat and barley improvement project. Directorate of wheat research, Karnal, India

Gupta R, Sewa Ram, Chauhan DS (2002) Quality of Indian wheat, Directorate of wheat research, Karnal – 132001, India, Research bulletin No. 14: 92

Gutierrez-Gonzalez J, Wu X, Gillman J, Lee J, Zhong R, Yu O, Shannon G, Ellersieck M, Nguyen H, Sleper D (2010) Intricate environment-modulated genetic networks control isoflavone accumulation in soybean seeds. *BMC Plant Biology* 10:105

Guyomarc'h H, Sourdille P, Charmet G, Edwards K, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. *Theoretical and Applied Genetics* 104: 1164–1172

Habash D, Bernard S, Schondelmaier J, Weyen J, Quarrie S (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. *Theoretical and Applied Genetics* 114: 403-419

Hackett C (2002) Statistical methods for QTL mapping in cereals. *Plant Molecular Biology* 48: 585-599

Hai L, Guo H, Wagner C, Xiao S, Friedt W (2008) Genomic regions for yield and yield parameters in Chinese winter wheat (*Triticum aestivum* L.) genotypes tested under varying environments correspond to QTL in widely different wheat materials. *Plant Science* 175: 226–232

Halloran G (1976) Genetic analysis of hexaploid wheat, *Triticum aestivum* using intervarietal chromosome substitution lines- protein content and grain weight. *Euphytica* 25: 65-71

Hao C, Dong Y, Wang L, You G, Zhang H, Ge H, Jia J, Zhang X (2008) Genetic diversity and construction of core collection in Chinese wheat genetic resources *Chinese Science Bulletin* 53: 1518-1526

He X, Zhang Y, He Z, Wu Y, Xiao Y, Ma C, Xia X (2008) Characterization of *phytoene synthase 1 gene (Psy1)* located on common wheat chromosome 7A and development of a functional marker. *Theoretical and Applied Genetics* 116: 213–221ping in wheat

Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar H, Zhuang J, Zheng K, Liu G, Wang G, Sidhu J, Srivantaneeyakul S, Singh V, Bagali P, Prasanna H, McLaren G, Khush G (2003) Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. *Theoretical and Applied Genetics* 107: 679–690

Hodson D, White J (2009) Climate Change: What Future for Wheat? In *Wheat Facts and Futures 2009*. Dixon, J., H.-J. Braun, P. Kosina, and J. Crouch (eds.). Mexico, D.F.: CIMMYT 52-61

Holland J (2007) Genetic architecture of complex traits in plants. *Current Opinion in Plant Biology* 10: 156–161

Hospital F (2009) Challenges for effective marker-assisted selection in plants. *Genetica* 136: 303–310

Hossain S, Ford R, McNeil D, Pittock C, Panozzo J (2010) Inheritance of seed size in chickpea (*Cicer arietinum* L.) and identification of QTL based on 100-seed weight and seed size index. *Australian Journal of Crop Science* 4(2): 126-135

Hristov N, Mladenov N, Djuric V, Kondic-Spika A, Marjanovic-Jeromela A, Simic D (2010) Genotype × environment interactions in wheat quality breeding programs in southeast Europe. *Euphytica* 174: 315-324

Huang X, Cloutier S, Lycar L, Radovanovic N, Humphreys D, Noll J, Somers D, Brown P (2006) Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 113: 753–766

Huang X, Cöster H, Ganal M, Röder M (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 106: 1379–1389

Huang X, Kempf H, Ganal M, Röder M (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and synthetic wheat (*Triticum aestivum L.*). *Theoretical and Applied Genetics* 109: 933-943

IGC 2011, Grain market report, International grains Council GMR no. 408, 24 Feb 2011

Ishikawa G, Nakamura T, Ashida T, Saito M, Nasuda S, Endo T, Wu J, Matsumoto T (2009) Localization of anchor loci representing five hundred annotated rice genes to wheat chromosomes using PLUG markers. *Theoretical and Applied Genetics* 118: 499–514

Ishikawa G, Yonemaru J, Saito M, Nakamura T (2007) PCR-based landmark unique gene (PLUG) markers effectively assign homoeologous wheat genes to A, B and D genomes. *BMC Genomics* 8:135

Jansen R, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136: 1447–1455

Jansen R, Van Ooijen J, Stam P, Lister C, Dean C (1995) Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. *Theoretical and Applied Genetics* 91: 33-37

Jiang C, Zeng Z (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* 140: 1111–1127

Jiang Q, Hou J, Hao C, Wang L, Ge H, Dong Y, Zhang X (2011) The wheat (*T. aestivum*) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. *Functional and Integrative Genomics* 11: 49–61

Jofuku K, Omidyar P, Gee Z, Okamuro J (2005) Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. *Proceedings of the National Academy of Sciences USA* 102: 3117-3122

Johnson G, Esposito L, Barratt B, Smith A, Heward J (2001) Haplotype tagging for the identification of common disease genes. *Nature Genetics* 29: 233–237

Jones H (2005) Wheat transformation: current technology and applications to grain development and composition. *Journal of Cereal Science* 41: 137-147

Jones N, Ougham H, Thomas H, Pašakinskienë I (2009) Markers and mapping revisited: finding your gene. *New Phytologist* 183: 935–966

Jordan M, Somers D, Banks T (2007) Identifying regions of the wheat genome controlling seed development by mapping expression quantitative trait loci. *Plant Biotechnology Journal* 5: 442–453

Kang M (2002) Genotype–Environment Interaction: Progress and Prospects In: *Quantitative Genetics, Genomics and Plant Breeding* (ed. M.S. Kang) CAB *International, USA*

Kao C, Zeng Z, Teasdale R (1999) Multiple interval mapping for quantitative trait loci. *Genetics* 152: 1203–1216

Kato A, Vega J, Han F, Lamb J, Birchler J (2005) Advances in plant chromosome identification and cytogenetic techniques. *Current Opinion in Plant Biology* 8: 148-154

Kato K, Miura, H, Sawada S (2000) Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. *Theoretical and Applied Genetics* 101: 1114–1121

Kearsey M (1998) The principles of QTL analysis (a minimal mathematics approach). *Journal of Experimental Botany* 49: 1619–1623

Kearsey M, Pooni H (1996) The genetical analysis of quantitative trait. Chapman and Hall, London

Khrabrova M, Maistrenko O (1980) Monosomic genetic analysis of caryopsis protein content and weight in bread wheat Diamant 2. *Genetika* 16: 1425-1434

Knapp S, Bridges W (1990) Using molecular markers to estimate quantitative trait locus parameters: power and genetic variances for unreplicated and replicated progeny. *Genetics* 126: 769–777

Korol A, Ronin Y, Kirzhner V (1995) Interval mapping of quantitative trait loci employing correlated trait complexes. *Genetics* 140: 1137-1147

Kosambi D (1944) The estimation of map distances from recombination values. *Annals of Eugenics* 12: 172-175

Kovach M, Sweeney M, McCouch S (2007) New insights into the history of rice domestication. *Trends in Genetics* 23: 578–587

Kroonenberg P (1995) Introduction to biplots for G×E tables. Department of Mathematics, Research Report 51. University of Queensland, Australia. http://three-mode.leidenuniv.nl/document/biplot.pdf

Kuchel H, Williams K, Langridge P, Eagles H, Jefferies S (2007a) Genetic dissection of grain yield in bread wheat. I. QTL analysis. *Theoretical and Applied Genetics.* 115: 1029–1041

Kuchel H, Williams K, Langridge P, Eagles H, Jefferies S (2007b) Genetic dissection of grain yield in bread wheat. II. QTL-by-environment interaction. *Theoretical and Applied Genetics.* 115: 1015–1027

Kulwal P, Singh R, Balyan H, Gupta P (2004) Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. *Functional and Integrative Genomics* 4: 94–101

Kumar N, Kulwal P, Balyan H, Gupta P (2007) QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. *Molecular Breeding* 19: 163–177

Kumar N, Kulwal P, Gaur A, Tyagi A, Khurana J, Khurana P, Balyan H, Gupta P (2006) QTL analysis for grain weight in common wheat. *Euphytica* 151: 135–144

Kuspira J, Unrau J (1957) Genetic analysis of certain characters in common wheat using whole chromosome substitution lines. *Canadian Journal of Plant Science* 37: 300-326

La Rota M, Kantety R, Yu J, Sorrells M (2005) Nonrandom distribution and frequencies of genomic and EST-derived microsatellite markers in rice, wheat, and barley. *BMC Genomics* 6: 23

Lander E, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121: 185–199

Lander E, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174–181

Landjeva S, Lohwasser U, Börner A (2010) Genetic mapping within the wheat D genome reveals QTL for germination, seed vigor, and longevity, and early seedling growth. Euphytica 171: 129-143

Lazo G, Chao S, Hummel D, Edwards H, Crossman C, Lui N, Matthews D, Carollo V, Hane D, You F, Butler G, Miller R, Close T, Peng J, Lapitan N, Gustafson J, Qi L, Echalier B, Gill B, Dilbirligi M, Randhawa H, Gill K, Greene R, Sorrells M, Akhunov E, Dvorak J, Linkiewicz A, Dubcovsky J, Hossain K, Kalavacharla V, Kianian S, Mahmoud A, Miftahudin M, Xi F, Conley E, Anderson J, Pathan M, Nguyen H, McGuire P, Qualset C, Anderson O (2004) Development of an expressed sequence tag (EST) resource for wheat (*Triticum aestivum* L.): EST generation, unigene analysis, probe selection and bioinformatics for a 16,000-locus bin-delineated map. *Genetics* 168: 585-593

Levy A, Feldman M (2004) Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biological Journal of the Linnean Society* 82: 607-613

Li S, Jia J, Wei X, Zhang X, Li L, Chen H, Fan Y, Sun H, Zhao X, Lei T, Xu Y, Jiang F, Wang H, Li L (2007) An intervarietal genetic map and QTL analysis for yield traits in wheat. *Molecular Breeding* 20: 167–178

Liao X, Wang J, Zhou R, Li R, Jia J (2008) Mining favorable alleles of QTLs conferring 1000-grain weight from synthetic wheat. *Acta Agronomica Sinica* 34: 1877–1884

Lin H, Tan D, Zhang Z, Lan H, Gao S, Rong T, Pan G (2008) Analysis of digenic epistatic and QTL × environment interactions for resistance to banded leaf and sheath blight in maize (*Zea mays*) *International Journal Agriculture and Biology* 10: 605–611

Lincoln S, Daly M, Lander E (1993) Mapping genes controlling quantitative traits using MAPMAKER/QTL. Version 1.1. Whitehead Institute for Biomedical Research Technical Report, 2nd edition.

Liu P, Zhu J, Lu Y (2004) Marker-assisted selection in segregating generations of self-fertilizing crops, *Theoretical and Applied Genetics* 109:370–376

Liu Z, Anderson J, Hu J, Friesen T, Rasmussen J, Faris J (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. *Theoretical and Applied Genetics* 111: 782–794

Luo M, Dennis ES, Berger F, Peacock W, Chaudhury A (2005) *MINISEED3 (MINI3)*, a WRKY family gene, and *HAIKU2 (IKU2)*, a *leucine-rich repeat (LRR) KINASE* gene are regulators of seed size in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* 102: 17531-17536

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA

Ma D, Yan J, He Z, Wu L, Xia X (2011) Characterization of a cell wall invertase gene *TaCwi-A1* on common wheat chromosome 2A and development of functional markers. *Molecular Breeding* DOI 10.1007/s11032-010-9524-z

Mabille F, Mueangdee N, Abecassis J (2004) Modelling the morphology of wheat grain and applications. International workshop. Modelling quality traits and their genetic variability for wheat. 18-21 July 2004, Clermont-Ferrand, France. p 44

Manickavelu A, Kawaura K, Imamura H, Mori M, Ogihara Y (2011) Molecular mapping of quantitative trait loci for domestication traits and β-glucan content in a wheat recombinant inbred line population. *Euphytica* 177: 179–190

Manly K, Cudmore R, Meer J (2001) MapManager QTX, cross-platform software for genetic mapping. *Mammalian Genome* 12: 930–932

Manninen OM (2000) Associations between anther culture response and molecular markers on chromosome 2H, 3H and 4H of barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics 100: 57-62*

Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q (2010) Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *Proceedings of the National Academy of Sciences USA* 107: 19579-19584

Marquez-Cedillo L, Hayes P, Kleinhofs A, Legge W, Rossnagel B, Sato K, Ullrich S, Wesenberg D (2001) QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. *Theoretical and Applied Genetics* 103: 625–637

Marshall D, Ellison F, Mares D (1984) Effects of grain shape and size on milling yields in wheat. I. Theoretical analysis based on simple geometric models. *Australian Journal of Agricultural Research* 35: 619–630

Marshall D, Mares D, Moss H, Ellison F (1986) Effects of grain shape and size on milling yields in wheat. II. Experimental studies. *Australian Journal of Agricultural Research* 37: 331-342

Marza F, Bai G, Carver B, Zhou W (2006) Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. *Theoretical and Applied Genetics* 112: 688–698

Matsuo R, Dexter J (1980) Relationship between some durum wheat physical characteristics and semolina milling properties. *Canadian Journal of Plant Science* 60: 49-53

McCartney C, Somers D, Humphreys D, Lukow O, Ames N, Noll J, Cloutier S, McCallum B (2005) Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 × 'AC Domain'. *Genome* 48: 870–883

McIntosh R, Yamazaki Y, Devos K, Dubcovsky J, Rogers W, Appels R (2003) Catalog of Gene Symbols for Wheat. Tenth International Wheat Genetics Symposium Paestum, Italy

McIntyre C, Mathews K, Rattey A, Chapman S, Drenth J, Ghaderi M, Reynolds M, Shorter R (2010) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. *Theoretical and Applied Genetics* 120: 527–541

McKeand S, Li B, Hatcher A, Weir R (1990) Stability parameter estimates for stem volume for loblolly pine families growing in different regions in the southeastern United States. *Forest Science* 36: 10–17

Meng E, Loyns A, Peña R (2009) Wheat Quality in the Developing World: Trends and Opportunities In *Wheat Facts and Futures 2009*. J Dixon, H Braun, P Kosina, and J Crouch (eds.). Mexico, D.F.: CIMMYT 26-41

Messmer M, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of a wheat × spelt cross. *Theoretical and Applied Genetics* 98: 1163–1170

Mir R, Kumar N, Prasad M, Girdharwal N, Kumar J, Balyan H, and Gupta P (2008) Single-locus and two locus QTL analysis to detect main-effect and epistatic QTL for grain weight in bread wheat. *In:* Proc 11th Internat Wheat Genet Symp (Appels E, Eastwood R, Lagudah W, Langridge P, Mackay M, McIntyre L, and Sharp P, eds). Sydney University Press, Australia. pp. 1-3

Mishra B, Chatrath R, Mohan D, Saharan M, Tyagi B (2007) DWR Perspective Plan: Vision 2025. Directorate of Wheat Research, Karnal-132001, Haryana (India)

Mochida K, Shinozaki K (2010) Genomics and bioinformatics resources for crop improvement. *Plant Cell Physiology* 51: 497-523

Mochida K, Yoshida T, Sakurai T, Ogihara Y, Shinozaki K (2009) TriFLDB: A database of clustered full-length coding sequences from *Triticeae* with applications to comparative grass genomics. *Plant Physiology* 150: 1135–1146

Moolhuijzen P, Dunn D, Bellgard M, Carter M, Jia J, Kong X, Gill B, Feuillet C, Breen J, Appels R (2007) Wheat genome structure and function: genome sequence data and the International Wheat Genome Sequencing Consortium. *Australian Journal of Agricultural Research* 58: 470–475

Moose S, Mumm R (2008) Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. *Plant Physiology* 147: 969–977

Morgan B, Dexter J, Preston K (2000) Relationship of kernel size to flour water absorption for Canada Western Red Spring Wheat. *Cereal chemistry* 77: 286-292

Myers D, Edsall K (1989) The application of image processing techniques to the identification of Australian wheat varieties. *Plant Varieties and Seeds 2*: 109-116

Nachit M, Elouafi I, Pagnotta M, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut J, Tanzarella O, Porceddu E, Sorrells M (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L var durum). *Theoretical and Applied Genetics* 102: 177-186

Nachit M, Nachit G, Ketata H, Gauch H, Zobel R (1992) Use of AMMI and linear regression models to analyze genotype-environment interaction in durum wheat. *Theoretical and Applied Genetics* 83: 597-601

Nadolska-Orczyk A, Gasparis S, Orczyk W (2009) The determinants of grain texture in cereals. *Journal of Applied Genetics* 50: 185-197

Nakagarha M (1986) Geographic distribution of gametophyte genes in wide crosses of rice cultivars. Rice genetics. Proc Int Rice Genet Symp IRRI, Manila, The Philippines, 73–82

Narain P (2010) Quantitative genetics: past and present. *Molecular Breeding* 26: 135–143

Narasimhamoorthy B, Gill B, Fritz A, Nelson J, Brown-Guedira G (2006) Advanced backcross QTL analysis of a hard winter wheat × synthetic wheat population. *Theoretical and Applied Genetics* 112: 787–796

Nelson J (1997) QGene-software for marker-based genomic analysis and breeding. *Molecular Breeding* 3: 239-245

Nelson J (2005) Methods and software for genetic mapping. In:The handbook of plant genome mapping. Genetic and physical mapping. K Meksem and G Kahl (eds.) Wiley-VCH Verlag GmbH and Co. KgaA, Weinheim.

Neuman M, Sapirstein H, Shwedyk E, Bushuk W (1987) Discrimination of wheat class and variety by digital image analysis of whole grain samples. *Journal of Cereal Science* 6: 125-132

Neumann K, Kobiljski B, Denčić S, Varshney R, Börner A (2011) Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). *Molecular Breeding* 27: 37–58

Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P (2004) Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theoretical and Applied Genetics* 109: 800–805

Novaro P, Colucci F, Venora G, D'egidio M (2001) Image analysis of whole grains: A noninvasive method to predict semolina yield in durum wheat. *Cereal chemistry* 78: 217-221

Nurminiemi M, Madsen S, Rognli O, Bjornstad A, Ortiz R (2002) Analysis of the genotype × environment interaction of spring barley tested in the Nordic region of Europe: relationships among stability statistics for grain yield. *Euphytica* 127: 123–132

Öğüt H, Aydin C, Mengeç H (1992) Determination of some physical and mechanical properties of different wheat varieties. In: *Proceedings of the national Symposium on Mechanization in Agriculture*, Samsun, Turkey, 247-253

Ohm J, Chung O, Deyoe C (1998) Single kernel characteristics of hard winter wheats in relation to milling and baking quality. *Cereal Chemistry* 75: 156-161

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.) *Theoretical and Applied Genetics* 107: 1235–1242

Paterson A (1997) QTL analyses: Power, precision, and accuracy. In *Molecular Dissection of Complex Traits* (ed.) Boca Raton, FL, CRC Press, 1997, 145–162

Paux E, Faure S, Choulet F, Roger D, Gauthier V, Martinant J, Sourdille P, Balfourier F, Le Paslier M, Chauveau A, Cakir M, Gandon B, Feuillet C (2010) Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. *Plant Biotechnology Journal* 8: 196–210

Paux E, Roger D, Badaeva E, Gay G, Bernard M, Sourdille P, Feuillet C (2006) Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B. *The Plant Journal* 48: 463–474

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A physical map of the 1Gb bread wheat chromosome 3B. *Science* 322: 101–104

Peng J, Ronin Y, Fahima T, Roder M, Li Y, Nevo E, Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. *Proceedings of the National Academy of Sciences USA* 100: 2489–2494

Pérez-Vega E, Pañeda A, Rodríguez-Suárez C, Campa A, Giraldez R, Ferreira JJ (2010) Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population

of common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 120: 1367–1380

Pestsova E, Ganal M, Roder M (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43: 689–697

Philips P (1998) The language of gene interaction. *Genetics* 149: 1167-1171

Pinnschmidt H, Hovmøller M (2002) Genotype × environment interactions in the expression of net blotch resistance in spring and winter barley varieties. *Euphytica* 125: 227-243

Pinto R, Reynolds M, Mathews K, McIntyre C, Olivares-Villegas J, Chapman S (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. *Theoretical and Applied Genetics* 121: 1001–1021

Pressoir G, Albar L, Ahmadi N, Rimbault I, Lorieux M, Fargette D, Ghesquière A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. II. Evidence of a complementary epistasis between two QTLs. *Theoretical and Applied Genetics* 97: 1155-1161

Pumphrey M, Bernardo R, Anderson J (2007) Validating the *Fhb1* QTL for *Fusarium* head blight resistance in near-isogenic wheat lines developed from breeding populations. *Crop Science* 47: 200–206

Purugganan M, Fuller D (2009) The nature of selection during plant domestication. *Nature* 457: 843–848

Qi L, Echalier B, Friebe B, Gill B (2003) Molecular characterization of a set of wheat deletion stocks for use in chromosome bin mapping of ESTs. *Functional and Integrative Genomics* 3: 39–55

Qi L, Echalier E, Chao S, Lazo G, Butler G, Anderson O, Akhunov E, Dvorak J, Linkiewicz A, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis C, Greene R, Kantety R, La Rota C, Munkvold J, Sorrells S, Sorrells M, Dilbirligi M, Sidhu D, Erayman M, Randhawa H, Sandhu D, Bondareva S, Gill K, Mahmoud A, Ma X, Miftahudin, Gustafson J, Conley E, Nduati V, Gonzalez-Hernandez J, Anderson J, Peng J, Lapitan N, Hossain K, Kalavacharla V, Kianian S, Pathan M, Zhang D, Nguyen H, Choi D, Fenton R, Close T, McGuire P, Qualset C, Gill B (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics* 168: 701–712

Quarrie S, Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes J, Leverington M, Ceoloni C, Dodig D (2006) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. *Journal of Experimental Botany* 57: 2627–2637

Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash D, Farmer P, Saker L, Clarkson D, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M, Hollington P, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring×SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theoretical and Applied Genetics* 110: 865–880

Quraishi U, Abrouk M, Bolot S, Pont C, Throude M, Guilhot N, Confolent C, Bortolini F, Praud S, Murigneux A, Charmet G, Salse J (2009) Genomics in cereals: from genome-wide conserved orthologous set (COS) sequences to candidate genes for trait dissection. *Functional and Integrative Genomics* 9: 473–484

Rafalski J (2002a) Application of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology* 54: 357–374

Rafalski J (2002b) Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science* 162: 329–333

Redoña E, Mackill D (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. *Theoretical and Applied Genetics* 96: 957-963

Reif J, Gowda M, Maurer H, Longin C, Korzun V, Ebmeyer E, Bothe R, Pietsch C, Würschum T (2011) Association mapping for quality traits in soft winter wheat. *Theoretical and Applied Genetics* 122: 961–970

Röder M, Huang X, Börner A (2008) Fine mapping of the region on wheat chromosome 7D controlling grain weight. *Functional and Integrative Genomics* 8: 79–86

Röder M, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal M (1998) A microsatellite map of wheat. *Genetics* 149: 2007–2023

Ronin Y, Kirzhner V, Korol A (1995) Linkage between loci of quantitative traits and marker loci: multi-trait analysis with a single marker. *Theoretical and Applied Genetics* 90: 776-786

Roy J, Bandopadhyay R, Rustgi S, Balyan H, Gupta P (2006) Association analysis of agronomically important traits using SSR, SAMPL and AFLP markers in bread wheat. *Current Science* 90(5): 683-689

Sabot F, Simon D, Bernard M (2004) Plant transposable elements, with an emphasis on grass species. *Euphytica* 139: 227–247

Salse J, Feuillet C (2007) Comparative genomics of cereals In *Genomics-Assisted Crop Improvement: Vol. 1: Genomics Approaches and Platforms*, *R.K. Varshney and R. Tuberosa (eds.)* 177–205

Sambrook, J and Russell D (2001) *Molecular Cloning, A Laboratory Manual* Cold Spring Harbor Laboratory, New York

Sang T (2009) Genes and mutations underlying domestication transitions in grasses. *Plant Physiology* 149: 63–70

Sanguinetti C, Dias Neto E, Simpson A (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 915-919

Schruff M, Spielman M, Tiwari S, Adams S, Fenby N, Scott R (2006) The *AUXIN RESPONSE FACTOR 2* gene of *Arabidopsis* links auxin signaling, cell division, and the size of seeds and other organs. *Development* 133: 251-261

Schuler S, Bacon R, Finney P, Gbur E (1995) Relationship of test weight and kernel properties to milling and baking quality in soft red winter wheat. *Crop Science* 35: 949–953

Sears E **(**1954**)** The aneuploids of common wheat. *Research Bulletin, University of Missouri Agriculture Experiment Station* 572: 1-59

Sears E **(**1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: C*hromosome Manipulations and Plant Genetics,* Eds. Riley R and Lewis KR pp. 29-44, Oliver & Boyd, Edinburgh.

Semagn K, Å Bjørnstad, Ndjiondjop M (2006a) An overview of molecular marker methods for plants. *African Journal of Biotechnology* 25: 2540-2568

Semagn K, Å Bjørnstad, Ndjiondjop M (2006b) Principles, requirements and prospects of genetic mapping in plants. *African Journal of Biotechnology* 25: 2569-2587

Semagn K, Bjørnstad A, Skinnes H, Marøy A, Tarkegne Y,William M (2006c) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. *Genome* 49: 545–555

Semagn K, Å Bjørnstad, Ndjiondjop M (2006d) Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnology* 5: 2588-2603

Shah M, Gill K, Baenziger P, Yen Y, Kaeppler S, Ariyarathne H (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. *Crop Science* 39: 1728–1732

Sharma R, Joshi B, Tomar S (1995) Aneuploid analysis of a recombinant of wheat and rye for yield and yield contributing characters. *Indian Journal of Genetics and Plant Breeding* 55: 416-423

Shewry P (2009) Wheat. *Journal of Experimental Botany* 60(6): 1537–1553

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. *Nature Genetics* 40: 1023 – 1028

Shouche S, Rastogi R, Bhagwat S, Sainis J (2001) Shape analysis of grains of Indian wheat varieties. *Computers and Electronics in Agriculture* 33: 55–76

Shuey W (1960) A wheat sizing technique for predicting flour milling yield. *Cereal Foods World* 5: 71-75

Simons K, Fellers J, Trick H, Zhang Z, Tai Y, Gill B, Faris J (2006) Molecular characterization of the major wheat domestication gene *Q*. *Genetics* 172: 547-555

Slade A, Knauf V (2005) TILLING moves beyond functional genomics into crop improvement. *Transgenic Research* 14: 109–115

Smith D, Flavell R (1975) Characterization of wheat genome by renaturation kinetics. *Chromosoma* 50: 223-242

Snape J, Foulkes J, Simmonds J, Leverington M, Fish L, Wang Y, Ciavarrella M (2007) Dissecting gene × environmental effects on wheat yields via QTL and physiological analysis. *Euphytica* 154: 401–408

Somers D, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 109: 1105–1114

Song Q, Fickus E, Cregan P (2002) Characterization of trinucleotide SSR motifs in wheat. *Theoretical and Applied Genetics* 104: 286–293

Song X, Huang W, Shi M, Zhu M, Lin H (2007) A QTL for rice grain width and weight encodes a previously unknown RING type E3 ubiquitin ligase. *Nat Genet* 39: 623–630

Sourdille P, Cadalen T, Guyomarc'h H, Snape J, Perretant M, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. *Theoretical and Applied Genetics* 106: 530–538

Sourdille P, Singh S, Cadalen T, Brown-Guedira G, Gay G, Qi L, Gill B, Duour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Functional and Integrative Genomics* 4: 12–25

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *The Plant Journal* 3: 739-744

Stein N, Herren G, Keller B (2001) A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. *Plant Breeding* 120: 354-356

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. *Theoretical and Applied Genetics* 97: 946-949

Su J, Zheng Q, Li H, Li B, Jing R, Tong Y, Li Z (2009) Detection of QTLs for phosphorus use efficiency in relation to agronomic performance of wheat grown under phosphorus sufficient and limited conditions. *Plant Science* 176: 824–836

Su Z, Hao C, Wang L, Dong Y, Zhang X (2011) Identification and development of a functional marker of TaGW2 associated with grain weight in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 122: 211–223

Suenaga K, Khairallah M, William H, Hoisington D (2005) A new intervarietal linkage map and its application for quantitative trait locus analysis of ''gigas'' features in bread wheat. *Genome* 48: 65–75

Sun X, Marza F, Ma H, Carver B, Bai G (2010) Mapping quantitative trait loci for quality factors in an inter-class cross of US and Chinese wheat. *Theoretical and Applied Genetics* 120: 1041–1051

Sun X, Wu K, Zhao Y, Kong F, G Han, Jiang H, Huang X, Li R, Wang H, Li S (2009) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. *Euphytica* 165: 615–624

Szűcs P, Blake V, Bhat P, Chao S, Close T, Cuesta-Marcos A, Muehlbauer G, Ramsay L, Waugh R, Hayes P (2009) An integrated resource for barley linkage map and malting quality QTL alignment. *The Plant Genome* 2: 134–140

Talukder Z, McDonald A, Price A (2005) Loci controlling partial resistance to rice blast do not show marked QTL × environment interaction when plant nitrogen status alters disease severity. *New Phytologist* 168: 455-464

Tanksley S (1984) Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, *Capsicum annuum*. *Chromosoma* 89: 352-360

Tanksley S (1993) Mapping polygenes. *Annual Review of Genetics* 27: 205-233

Tanksley S, Nelson J (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics* 92: 191–203

Tarakanovas P, Ruzgas V (2006) Additive main effect and multiplicative interaction analysis of grain yield of wheat varieties. Lithuania Agronomy Research 4: 91-98

Troccoli A, Di Fonzo N (1999) Relationship between kernel size features and test weight in *Triticum durum*. *Cereal chemistry* 76: 45-49

Tsilo T, Hareland G, Simsek S, Chao S, Anderson J (2010) Genome mapping of kernel characteristics in hard red spring wheat breeding lines. *Theoretical and Applied Genetics* 121: 717–730

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314: 1298-1301

USDA National Nutrient Database for Standard Reference, Release 23 (2010). Nutrient Data Laboratory, U.S. Department of Agriculture, Agricultural Research Service. http://www.ars.usda.gov/ba/bhnrc/ndl

Van Ooijen J (2004) MapQTL® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, The Netherlands

Van Ooijen J (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, The Netherlands

Varshney R, Hoisington D, Naik S, Graner A (2009b) Molecular plant breeding: methodology and achievements. DJ Somerset al. (eds.) methods in Molecular biology, plant genomics, 513: 283-304 Humana Press, Springer Science and Business media LLC

Varshney R, Korzun V, Börner A (2004) Molecular maps in cereals: methodology and progress. In: Cereal genomics PK Gupta, RK Varshney Kluwer academic Publishers. Printed in China p35-82

Varshney R, Nayak S, May G, Jackson S (2009a) Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology* 27(9): 522-530

Varshney R, Prasad M, Roy J, Kumar N, Singh H, Dhaliwal H, Balyan H, Gupta P (2000) Identification of eight chromosomes and a microsatellite marker on 1AS associated with QTLs for grain weight in bread wheat. *Theoretical and Applied Genetics* 100: 1290–1294

Veldboom L, Lee M (1996a) Genetic mapping of quantitative trait loci in maize in stress and non-stress environments: I. Grain yield and yield components. *Crop Science* 36: 1310-1319

Veldboom L, Lee M (1996b) Genetic mapping of quantitative trait loci in maize in stress and non-stress environments: II. Plant height and flowering. *Crop Science* 36: 1320-1327

Voorrips R (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity* 93: 77-78

Walsh B (2009) Quantitative genetics, version 3.0: where have we gone since 1987 and where are we headed? *Genetica* 136: 213–223

Wan Y, Poole R, Huttly A, Toscano-Underwood C, Feeney K, Welham S, Gooding M, Mills C, Edwards K, Shewry P, Mitchell R (2008) Transcriptome analysis of grain development in hexaploid wheat. *BMC Genomics* 9: 121

Wang A, Garcia D, Zhang H, Feng K, Chaudhury A, Berger F, Peacock W, Dennis E, Luo M (2010) The VQ motif protein IKU1 regulates endosperm growth and seed size in *Arabidopsis*. *The Plant Journal* 63: 670-679

Wang B, Wu Y, Guo W, Zhu X, Huang N, Zhang T (2007) QTL analysis and epistasis effects dissection of fiber qualities in an elite cotton hybrid grown in second generation. *Crop science* 47: 1384-1392

Wang D, Zhu J, Li Z, Paterson A (1999) Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. *Theoretical and Applied Genetics* 99: 1255–1264

Wang R, Hai L, Zhang X, You G, Yan C, Xiao S (2009) QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai × Yu8679. *Theoretical and Applied Genetics* 118: 313-325

Wang S, Basten C, Zeng Z (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Wang, E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhan L, He W, Lu b, Lin H, Ma H, Zhang G, He Z (2008) Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nature Genetics* 40: 1270-1274

Wessa P (2009) Spearman ran Correlation (v1.0.0) in Free Statistics Software (v1.1.23-r3), Office for Research Development and Education, http://www.wessa.net/rwasp_spearman.wasp/

Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality, Version 2, Wheat Marketing Center, Kansas State University, September 2008

Whittaker J, Thompson R, Visscher P (1996) On the mapping of QTL by regression of phenotype on marker-type. *Heredity* 77: 23–32

Wiersma J, Busch R, Fulcher G, Hareland G (2001) Recurrent selection for kernel weight in spring wheat. *Crop Science* 41: 999-1005

Williams R, O'Brien L, Eagles A, Solah A, Jayasena V (2008) The influences of genotype, environment and genotype×environment interaction on wheat quality. *Australian Journal of Agricultural Research* 59: 95–111

Wilson I, Barker G, Beswick R, Shepherd S, Lu C, Coghill J, Edwards D, Owen P, Lyons R, Parker J, Lenton J, Holdsworth M, Shewry P, Edwards K (2004) A transcriptomics resource for wheat functional genomics. *Plant Biotechnology Journal* 2: 495–506

Wilson I, Barker G, Lu C, Coghill J, Beswick R, Lenton J, Edwards K (2005) Alteration of the embryo transcriptome of hexaploid winter wheat (*Triticum aestivum* cv. Mercia) during maturation and germination. *Functional and Integrative Genomics* 5: 144–154

Wricke G (2002) Two major genes for kernel weight in rye. *Plant Breeding* 121: 26-28

Wu R, Casella G, Ma C (2007) Statistical genetics of quantitative traits: linkage, maps, and QTL. Springer Science and Business Media, LLC New York

Xie X, Jin F, Song M, Suh J, Hwang H, Kim Y, McCouch S, Ahn S (2008) Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* × *O. rufipogon* cross. *Theoretical and Applied Genetics* 116: 613–622

Xing Y, Tan Y, Hua J, Sun X, Xu C, Zhang Q (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theoretical and Applied Genetics* 105: 248–257

Xu Y, Li H, Li G, Wang X, Cheng L, Zhang Y (2011) Mapping quantitative trait loci for seed size traits in soybean (*Glycine max* L. Merr.). *Theoretical and Applied Genetics* 122: 581–594

Xue S, Zhang Z, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhu H, Wu J, Xu H, Zhao D, Tian D, Zhang C, Ma Z (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. *Theoretical and Applied Genetics* 117: 181–189

Yan W, Tinker N (2006) Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science* 86: 623–645

Yang J, Hu C, Hu H, Yu R, Xia Z, Ye X (2008) QTL Network: Mapping and visualizing genetic architecture of complex traits in experimental populations. *Bioinformatics* 24: 721-723

Yang J, Zhu J, Williams R (2007) Mapping the genetic architecture of complex traits in experimental populations. *Bioinformatics* 23: 1527-1536

Yao J, Wang L, Liu L, Zhao C, Zheng Y (2009) Association mapping of agronomic traits on chromosome 2A of wheat. *Genetica* 137: 67–75

Yoon B, Brorsen B, Lyford C (2002) Value of increasing kernel uniformity. *Journal of agricultural and resource economics* 27: 481-494

Young N (2001) Constructing a plant genetic linkage map with DNA markers. In: Philips RL and Vasil IK (eds.) DNA-based markers in plants, Kluwer Academic Publishers, The Netherlands, 31-48

Yu J, Buckler E (2006) Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology* 17: 155–160

Yu J, Dake T, Singh S, Benscher D, Li W, Gill B, Sorrells M (2004) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome* 47: 805–818

Zanetti S, Winzeler M, Feuillet C, Keller B, Messmer M (2001) Genetic analysis of bread-making quality in wheat and spelt. *Plant Breeding* 120: 13-19

Zayas I, Lai F, Pomeranz Y (1986) Discrimination between wheat classes using image analysis. *Cereal chemistry* 63: 52-56

Zayas I, Pomeranz Y, Lai F (1985) Discrimination between Arthur and Arkan wheat by image analysis. *Cereal Chemistry* 62: 478-480

Zeng Z (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proceedings of the National Academy of Sciences of the United States of America* 90: 10972–10976

Zeng Z (1994) Precision mapping of quantitative trait loci. *Genetics* 136: 1457–1468

Zhang K, Chen G, Zhao L, Liu B, Xu X, Tian J (2009) Molecular genetic analysis of flour color using a doubled haploid population in bread wheat (*Triticum aestivum* L.). *Euphytica* 165: 471-484

Zhang W, Chao S, Manthey F, Chicaiza O, Brevis J, Echenique V, Dubcovsky J (2008) QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. *Theoretical and Applied Genetics* 117: 1361–1377

Zheng Y, Yan J, Yang J (1993) Localization of genes for grain weight in common wheat. *Acta Agronomica Sinica* 19: 304-308

Zhou Y, Zhang X, Kang X, Zhao X, Zhang X, Ni M (2009) SHORT HYPOCOTYL UNDER BLUE1 associates with *MINISEED3* and *HAIKU2* promoters in vivo to regulate Arabidopsis seed development. *Plant Cell* 21: 106-117

Zhu C, Gore M, Buckler E, Yu J (2008) Status and Prospects of Association Mapping in Plants. *The Plant Genome* 1: 5-20

Zobel R, Wright M, Gauch H (1988) Statistical analysis of a yield trial. *Agronomy Journal* 80: 388–393

RAMYA PRASHANT

Plant Molecular Biology Group,
Biochemical Sciences Division,
National Chemical Laboratory,
Pashan, Pune, India 411008

Email: dixitrp@rediffmail.com
Phone: +91-20-25902247
Fax: +91-20-25902648
Mobile: +91-9689528866

CAREER PROFILE

Pursuing PhD at National Chemical Laboratory, Pune, India, under the guidance of Dr. Vidya Gupta, Head, Biochemical Sciences Division, National Chemical Laboratory, Pune, India from May 2004.

RESEARCH INTERESTS

Molecular dissection of complex traits, development of molecular markers and linkage maps, genetic improvement of crops like small millets, orphan legumes, dicoccum wheat, carbon and nitrogen partitioning during seed development.

RESEARCH EXPERIENCE

- Carried out PhD research on quantitative traits governing wheat grain quality by molecular approaches. The project involved field trials and molecular marker analysis to identify QTLs controlling kernel characters in hexaploid wheat.
- Junior Research Fellow (Council of Scientific and Industrial Research, India) from June 2002 to June 2004 under the supervision of Dr. Vidya Gupta, Plant Molecular Biology Group, Biochemical Sciences Division, National Chemical Laboratory, Pune, India. I was involved in molecular marker analysis of wheat grain protein content using random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers.
- Junior Research Fellow in the research group of Dr. Satyajit Mayor, National Centre for Biological Sciences (NCBS), Bangalore, India from August 1999 to September 2000. My work involved expression of various lipid-tagged green fluorescent protein molecules in Chinese hamster ovary (CHO) fibroblasts and study of their subcellular expression pattern by fluorescence microscopy.
- MSc dissertation on “A study on the effect of Indian saw-scaled viper (*Echis carinatus*) venom on blood coagulation” under the guidance of Dr. Kemparaju, Professor, Department of Studies in Biochemistry, University of Mysore, Mysore, India. The project involved preliminary studies on temporal patterns in blood coagulation in the presence of *Echis carinatus* venom and the influence of various protease inhibitors on its procoagulant activity.
- Summer Research Fellow in 1996 and 1997 in the laboratory of Prof. K. VijayRaghavan at National Centre for Biological sciences (NCBS), Bangalore,

India. The project involved characterization of a putative regulatory region of a gene encoding a transcription factor required for nervous system and muscle development in the fruit fly *Drosophila melanogaster*.

TECHNICAL SKILLS

- Development of linkage maps and in-depth analysis of quantitative trait loci (QTLs) using appropriate softwares
- Collection and statistical analysis of phenotypic data from field experiments
- DNA extraction, restriction digestion, cloning, PCR, agarose and polyacrylamide gel electrophoresis, molecular marker analysis using markers like RAPD, ISSR, SSR, etc.
- Basic fluorescence microscopy, animal cell culture and transient transfection, handling *Drosophila* and germline transformation by microinjection

EDUCATION

MSc (*1999*) in **Biochemistry**, **University of Mysore**, Mysore, India. Score 76%

BSc (*1997*) in **Biochemistry, Botany** and **Microbiology**, **University of Mysore**, Mysore, India. Score 87%

HONORS/SCHOLARSHIPS

- Qualified for Research Fellowship by Council of Scientific and Industrial Research (CSIR), Government of India in years 1999 (June and December), 2000 and 2001 through Nation-wide exams
- 91.6 percentile in Graduate Aptitude Test in Engineering (GATE) 1999 in Life Sciences conducted at all-India level for assessment of Master's degree graduates in Life Sciences
- Selected for All India Summer Research Fellowship in 1996 and 1997 sponsored by Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, India and Rajiv Gandhi Foundation, New Delhi, India to work as a trainee at National Centre for Biological Sciences (NCBS), Bangalore, India
- Named as Rajiv Gandhi Science Talent Research Scholar both in 1996 and 1997 by JNCASR and Rajiv Gandhi Foundation based on the performance as a summer research fellow at NCBS
- National Merit Scholarship sponsored by Government of India in 1997, 1998 and 1999
- University topper in BSc (1997) with gold medals for highest scores in Biochemistry, Botany and Microbiology

PUBLICATIONS

Ramya P, Chaubal A, Kulkarni K, Gupta L, Kadoo N, Dhaliwal HS, Chhuneja P, Lagu M and Gupta V (2010) Molecular characterization of kernel characters in bread wheat. *Journal of Applied Genetics* **51**(4): 421-429.

CONFERENCE ABSTRACTS

- 'Identification of stable QTLs and analysis of QTL×QTL and genotype × environment interactions for wheat kernel size and shape', poster presented at the 3rd International group Meeting "Wheat productivity enhancement under changing climate". February 9-11, 2011, University of Agricultural Sciences, Dharwad, Karnataka, India
- 'Identification of genomic regions controlling kernel characters in bread wheat by molecular markers' presented at 'Plant genomics and biotechnology: Challenges and opportunities'- International Conference at Indira Gandhi Agricultural University, Raipur, India, Oct 26-28, 2005

REFERENCES

Dr. Vidya Gupta
Scientist 'G' and Head,
Biochemical Sciences Division,
National Chemical Laboratory,
Pashan, Pune, India 411008
Phone: +91-20-25902237
Fax: +91-20-25902648
Email: vs.gupta@ncl.res.in

Dr. Narendra Kadoo
Scientist,
Biochemical Sciences Division,
National Chemical Laboratory,
Pashan, Pune, India 411008
Phone: +91-20-25902724
Fax: +91-20-25902648
Email: ny.kadoo@ncl.res.in

Dr. VS Rao
Faculty and Registrar,
Indian Institute of Science Education and Research, Pune
Formerly, Director, Agharkar Research Institute,
Pune, India 411008
Phone: +91-20-25908001
Email: srao48@gmail.com

PERSONAL

Date of Birth: February 7, 1977 **Sex**: Female **Nationality**: Indian

“Wheat Fields”
by Van Gogh