MOLECULAR DISSECTION OF BREADMAKING QUALITY IN WHEAT (*Triticum aestivum* L.)

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CERTIFICATE

Certified that the work in the Ph.D. thesis entitled **'Molecular dissection of breadmaking quality in wheat (*Triticum aestivum* L.)'** submitted by **Mr. Elangovan Mani** was carried out by the candidate under my supervision. The material obtained from other sources has been duly acknowledged in the thesis.

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DECLARATION

I hereby declare that the thesis entitled **'MOLECULAR DISSECTION OF BREADMAKING QUALITY IN WHEAT (*Triticum aestivum* L.)'** submitted for Ph.D. degree to the **University of Pune** has not been submitted by me for a degree at any other university.

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CONTENTS

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LIST OF ABBREVIATIONS

°C	Degree centigrade
AACC	American association of cereal chemists
AFLP	Amplified fragment length polymorphism
APS	Ammonium per sulphate
AS-PCR	Allele specific PCR
BAC	Bacterial artificial chromosome
BMQ	Bread making quality
bn, mn	Billion, Million
bp	Base pair
cM	Centimorgan
cm	Centimeter
CTAB	Cyltrimethyl ammonium bromide
cv	Cultivar
DH	Doubled haploid
DNA	Deoxyribonucleic acid
dNTPs	Deoxy ribonucleotide tri phosphate
DT	Ditelosomic
DWR	Directorate of wheat research
EDTA	Ethylenediamine tetra acetate
EST	Expressed sequence tags
g/gm, mg, µg, ng	Gram, Milligram, Microgram, Nanogram
Gpc	Grain protein content
h	Hour
HCl	Hydrochloric acid
ha	Hectare
HMW	High molecular weight
Tw	Test weight or Hectolitre weight
IAA	Iso-amyl alcohol
ISSR	Inter simple sequence repeat
ITMI	International triticeae mapping initiative
K_2O	Potassium oxide
Kb, Mb	Kilobase pair, Megabase pair

KCl	**Potassium chloride**
kDa/kD	**Kilo Dalton**
kg	**Kilogram**
Kg/ha	**Kilogram per hectare**
KH	**Kernel hardness**
L, mL, µL	**Liter, Milliliter, Microliter**
LMW	**Low- molecular weight**
LOD	**Log of the odd (Base 10 logarithm of the likelihood ratio)**
M, mM, µM	**Molar, Millimolar, Micromolar**
MAS	**Marker assisted selection**
$MgCl_2$	**Magnesium chloride**
Mha	**Million hectare**
min	**Minute**
µM	**Micromolar**
mm	**Millimeter**
mM	**Millimolar**
mmole, µmole	**Millimole, Micromole**
mRNA	**Messenger RNA**
MT	**Metric tonnes**
Mt	**Million tonnes**
MW	**Molecular Weight**
N_2	**Nitrogen**
NaCl	**Sodium chloride**
NaOH	**Sodium hydroxide**
NIL	**Near isogenic line**
NIR	**Near infrared reflectance**
nM	**Nanomoles**
NT	**Nulli-tetrasomic**
PAGE	**Polyacrylamide gel electrophoresis**
PCR	**Polymerase chain reaction**
pmoles	**Picomoles**
PVP	**Polyvinyl pyrrolidone**
QTL	**Quantitative trait loci**
RAPD	**Random amplified polymorphic DNA**

RFLP	**Restriction fragment length polymorphism**
RIL	**Recombinant inbred line**
RNA	**Ribonucleic acid**
rpm	**Revolutions per minute**
RT-PCR	**Reverse transcriptase-polymerase chain reaction**
s	**Second**
SCAR	**Sequence characterized amplified region**
SDS	**Sodium dodecyl sulphate**
SSR	**Simple sequence repeat**
STMS	**Sequence tagged microsatellite site**
STS	**Sequence tagged site**
TAE	**Tris-acetate EDTA**
TEMED	**Tetramethylethylenediamine**
Tgw	**Thousand-grain weight**
Tm	**Melting temperature**
Tris	**Tris-hydroxymethyl amino methane**
var	**Variety**

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 1

Introduction

Wheat for Breadmaking

1.1 Wheat: A global perspective

Most of the wheat is grown for human food and is the staple food for 35% of the world's population. However, about 10% is retained for seed industry for production of starch, paste, malt, dextrose, gluten, alcohol, and other products of wheat. Inferior or surplus wheat and various milling by-products are used for livestock feeds. The composition of wheat grain, a major source of energy in the human diet, varies with the differences in climate and soil. On an average, the kernel contains 12% water, 70% carbohydrates, 12% protein, 2% fat, 1.8 % minerals, and 2.2% crude fibers. Approximately 100 grams of wheat contains nearly 330 calories, with vitamins such as thiamin, riboflavin, niacin, and small amounts of vitamin A. However, the milling processes remove the bran and germ, where these vitamins are found in the greatest abundance (CGIAR, 2005).

Wheat is a cereal grass of the *Gramineae (Poaceae)* family and of the genus Triticum. It is an edible grain, one of the oldest and the most important of the cereal crops. Modern wheat (*Triticum aestivum*) is the first grain to be domesticated and its cultivation started in the Middle East about 9-11,000 years ago. Now, wheat is not only the most important widely adapted crop to a range of environments, but also produced, traded and consumed worldwide (Oleson 1994). Though grown under a wide range of climates and soils, wheat is best adapted to temperate regions with annual rainfall between 30 and 90 cm. Winter and spring wheats are two major types of the crop in the temperate regions, with severity of the winter determining whether a winter or spring type is to be cultivated. Winter wheat is always sown in the fall; spring wheat is generally sown in the spring but can be sown in the fall where winters are mild. Therefore, today wheat is grown all over the world, with different varieties sown suitable to various climates. In Canada, the harsh winters require a fast growing grain, with wheat sown and matured in about 90 days, while in India different varieties are required to cope with the dry sun-baked lands of northern India. Wheat grown in dry climates is generally of hard type, having protein content of 11- 15% and strong gluten (elastic protein), while the wheat of humid areas is softer, with protein content of about 8-10% and weak gluten. Accordingly, under different climatic conditions, 15% of the world's arable land is devoted to wheat alone than to any other food crop (FAO, 2005). In the late 20th century about 230,000,000 hectares

were sown annually, with a total production of almost 600 mt. The world's largest producer is China, with an estimated annual yield of almost 97 mt followed by India with about 68.6 mt production (FAO, 2005). Other leading producers are; USA, France, Russia, Canada, Germany, Turkey, Kazakhstan, Ukraine and Pakistan (Fig. 1.1).

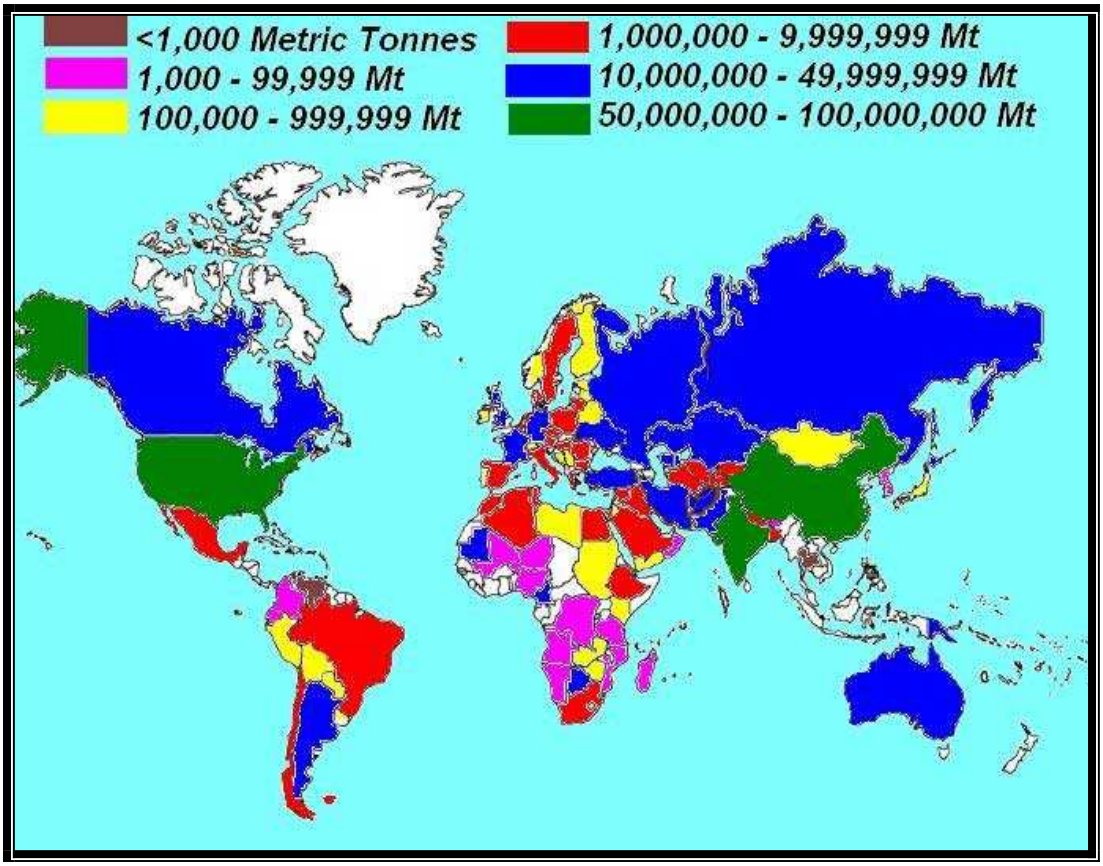


Fig.1.1 Distribution of wheat around the globe with wheat production statistics (FAOSTAT, 2005)

1.2 Wheat production in India: Some facts and concerns

Today, wheat is grown on nearly 26 Mha in India as a result of steadily increased crop area during the last 30 years. Indian wheat programme never looked back after 1966-67 and crossed 50 mt, during the green revolution period. India ranked high wheat output in 1983-84, when the country harvested 54 mt of wheat and it stood at 65.47 mt during 1994-95 (FAO, 2001). In the last 3 decades wheat production went up 4-5 times with an annual growth rate of 5.64%. The boost in increased production was a cumulative effect of research efforts, development of high yielding varieties, expansion of area, development of irrigation infrastructure, enhanced use of

fertilizers, plant protection measures and so on. Following this giant leap in production, annual wheat output stagnated. In 2005-06, India grew 68.6 mt while consumption stood at 75 mt leading to shortfall of 7 mt. This necessitated import of wheat from other countries. To makeup for the shortfall wheat worth of $1.29 bn was imported in 2006 and of $572 mn was imported in 2005. Prior to that, wheat worth of $414 mn and $556 mn was imported in 2004 and 2003, respectively (FAO, 2007).

This situation highlights two important points; the first one being the increased wheat demand. This is mainly because of the expansion of urban middle class population and alteration in their dietary preferences. Many traditional rice eaters have included one chapatti based meal and products such as biscuits, cakes and pizzas. The rise of diabetes as a major disease may further contribute to a shift away from rice to wheat in future (Herald Tribune, 2006). Secondly, climate change is among the top global risks facing the world in 2007 which is changing harvest patterns across the globe, and India is no exception. The above-average temperature has been recorded in 2007 by the meteorological department in major parts of Northern and Central India. It forced the wheat crop to start maturing faster in some places in Punjab, Haryana and Madhya Pradesh; the traditional and major wheat growing belt of India leading to reduced yields. In 2005-06, heat in February and March led to drop in yields and impacted India's wheat output. The same incident was repeated in the year 2006-07. The agriculturists and planning commission experts are of the opinion of subjecting our agricultural strategy to a thorough revamp. The eleventh five year plan advocated a second green revolution driven by Biotechnology in order to enhance crop production which may lead to India's second productivity leap in wheat (GOI, 2007).

1.3 Wheat: A model system for polyploid genetics

Wheat is one of the most extensively studied crop species, particularly in the area of cytogenetics. The pioneering cytogenetic work by Kihara, Sakamura, Sax, Sears, Riley (Riley and Chapman 1958; Riley 1965) showed that the species of the genus *Triticum* form a polyploid series, with a basic number of $x = 7$. Thus, there are the diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) species. Hexaploid wheat is a segmental allopolyploid containing three distinct but genetically related (homoeologous) genomes A, B and D from *Triticum urartu*, *Aegilops speltoides* and *Triticum tauschii*, respectively. An extensive catalogue of genetic and

cytogenetic stocks was developed in the years following the groundbreaking isolation of aneuploid lines by Sears (1954). This work led to the concept of chromosome engineering, which takes advantage of the effect of the *Ph* genes that restrict pairing and recombination to homologous chromosomes (Riley and Chapman 1958). Ease of chromosome manipulation makes wheat a model system for the study of polyploid cytogenetics and genomics. Though diploid, tetraploid and hexaploid genotypes are still available as germplasms, only hexaploid (Bread wheat) and tetraploid (Durum wheat) are grown world wide to meet economic purpose and for various end uses.

1.4 Wheat: Suitable for various end products

Wheat is the basic ingredient of many breakfast cereals, cookies, cakes and it is an important component of many processed items. The end use quality depends on the protein content of the wheat and it is listed in Table 1.1 (Edwards 1997). The uniqueness of wheat, among all cereals, resides in its flour, which upon mixing with water, produces cohesive dough that can retain the gases produced during fermentation thereby producing a leavened product (bread) of desirable attributes (Hoseney 1994). In western countries, leavened pan bread is the obvious wheat-based food, but now this form is common worldwide. Leavened bread is made from the hard type wheat, which produces flour best suited for breadmaking, while the softer type of wheat produces flour suitable for cakes, crackers, cookies, pastries and household flours. In India, wheat is mainly used to make rotis, parathas, chapattis and naans (unleavened flat bread). Among these, chapatti is the most popular traditional Indian homemade product and around 85% of the total wheat produced in the country is utilized for this purpose.

Durum wheat lacks D genome and cannot be used for leavened product; it is exclusively used for semolina (from the endosperm) for making pastas, or alimentary pastes. Pasta is another common form of wheat-based food and many forms of noodles are most evident in Asia, together with steamed breads. More common in the Middle East is the wide range of flat and pocket bread.

1.5 Wheat and bread making quality

Bread making quality (BMQ) of wheat is considered to be a complex trait influenced by interactions of many biochemical traits such as seed protein quality and content (Payne et al. 1987), starch quality and content (Gray and BeMiller, 2003) and oil

Table 1.1:- Wheat quality characteristics for diverse end-uses

Type	Grain hardness	Grain protein (%)	Gluten (dough) strength type
Leavened breads			
Pan-type, buns	Hard	>13	Strong-extensible
Hearth, French	Hard/Medium	11-14	Medium-extensible
Steamed	Hard/Soft	11-13	Medium/Weak
Unleavened (flat) breads			
Arabic	Hard/Medium	12-14	Medium-extensible
Chapati, tortilla	Medium	11-13	Medium-extensible
Crackers	Medium/Soft	11-13	Medium
Noodles			
Yellow alkaline	Medium	11-13	Medium/Strong
White	Medium/Soft	10-12	Medium
Cookies, cakes, pastries	Soft/Very soft	8-10	Weak/Weak-extensible

Source: Edwards (1997)

content (Helmerich and Koehler, 2005) supplemented by various physico-chemical traits such as moisture content, water retention capacity, vacuole formation, grain hardness and texture (Huang et al. 2006). The interaction between gluten proteins, along with water forms a gluten matrix, which traps the carbondioxide evolved during yeast fermentation on dough (Giannou et al. 2003). Lipids also improve the keeping quality, softness, moistness and contribute to bread texture. Both endogenous lipids and added fats are known to play an important role during breadmaking (Collar et al. 1999). Lipids embedded into the protein matrix are essential as they interact with proteins during dough mixing and contribute to the viscoelastic properties of the gluten network, required for expansion and gas retention during proofing (Demiralp et al. 2000). The expansion of gas vesicles depends on the perfect combination of elasticity and extensibility of the gluten. The water absorption and availability of the dough depends on the amount of pentosans such as arabinoxylans in the dough system, which is made available by arabinoxylanase (Courtin and Delcour, 2002).

1.5.1 Bread making: A classical way

Bread, the most popular wheat product, has been a staple food for the man kind throughout the recorded history (Knott 1987). It comes in wide array of shapes, sizes, color, textures and tastes. Considerable variation also exists in the ingredients used in making the bread. Flour, water, yeast and salt are the four most important ingredients of bread. Flour and water together produce the bread structure; yeast is responsible for leavening the bread through fermentation process and salt, for taste and to promote a better interaction between the proteins during dough development. Regardless of the ingredients used and the attributes desired, wheat flours are transformed into bread through a process consisting of three main steps, namely i) dough formation, ii) leavening of the latter during fermentation and iii) stabilization and drying of the structure during baking (Ammar 1997).

Dough formation: Dough is formed by mixing flour and water. Three goals are achieved by mixing which include, i) the production of a macroscopically homogenous system, ii) development of a three-dimensional protein network with gas retention capacity, and iii) the incorporation of air cells into the dough (Bloksma 1990a; Eliasson and Larsson, 1993a). Bernardin and Kasarda (1973) reported that upon contact with water, gluten protein fibrils are formed and they extend into

surrounding water, wherein gluten proteins are hydrated rather than dissolved (Eliasson and Larsson, 1993a). The friction that occurs during mixing between the flour particles and the mixer surfaces on one hand and between neighboring flour particles on the other hand, wears away the hydrated surfaces, thereby continuously exposing new, unhydrated surfaces (Faubion and Hoseney, 1990; Hoseney 1994). Before mixing, the dry gluten is referred to as a "glassy polymer" (Levine and Slade, 1990) and undergoes "glassy transition" upon hydration and mixing to yield an amorphous mass in which the component proteins are better able to interact and form dough (Hoseney et al. 1986). Dough is optimally mixed or developed when all the proteins and starch are hydrated (Hoseney 1998). The evolution from a discontinuous system (flour particles) to a continuous one (protein–starch matrix) can be explained at molecular level by cross-linking of protein molecules originating from different flour particles through thiol-disulfide interchanges among various thiol groups (-SH) occurring on the surface of the molecule and disulfide bonds (-S-S-) linking polypeptides within the same flour particle (Bloksma and Bushuk, 1988).

Another purpose of mixing is to incorporate air into the dough to produce gas nuclei that will subsequently expand during fermentation to form the gas cells. Since early 1940s, it has been known that yeast fermentation does not create new gas cells. Rather, these develop from air nuclei incorporated by occlusion into the continuous dough phase during mixing (Bloksma 1990b; Dobraszczyk and Morgenstern, 2003). At the end of mixing, the diameter of the gas nuclei ranges from 10 to 100 μm and their number is estimated to vary between 10^{11} to 10^{13} nuclei per m^3 of dough (Bloksma, 1981).

Leavening of dough during fermentation: Leavening of dough is required to obtain a porous bread product. It is achieved by the entrapment of carbon dioxide in the gluten-starch matrix. Yeast fermentation is one of the mechanisms that produces carbon dioxide in dough and is used in the making of most breads, both as a source of carbon dioxide and to produce a desirable texture, aroma and flavour in the finished product (Eliasson and Larsson, 1993b). Upon hydration, yeast cells are activated and metabolize the fermentable sugars to produce ethanol and carbon dioxide. After saturating the aqueous phase, the carbon dioxide diffuses in the gaseous state and the resulting pressure inside gas cells provides the driving force for their expansion. Gas retention is achieved by two consecutive mechanisms. First gas cells are embedded in

a continuous protein-starch matrix, which stretches under the excess pressure produced by the release of carbon dioxide. The ability of the matrix to be stretched into thin membranes is determined by the extensibility of the bulk dough phase (Bloksma and Bushuk, 1988; Bloksma 1990a). At this stage, response of the dough is determined largely by the extensibility of the bulk dough phase (Bloksma 1990a; Gan et al. 1995). Secondly, as the gas cells expand and tensile stress increases, the protein-starch matrix ruptures and the integrity of the gas cells as well as their gas retention capacity is ensured by a thin, lamellar liquid film lining the inner surface of the cell. This "liquid film" theory has been proposed by MacRitchie (1976) and later supported by scanning electron microscopic analysis (Gan et al. 1990). Also, the factors affecting the stability of the liquid film have been reviewed by Gan et al. (1995). Surface active compounds, presumably water soluble pentosans and polar liquids, are believed to play a role in stabilizing the liquid film once the protein-starch membrane ruptures. At this advanced stage of fermentation, the physical properties of wheat dough approach those of foam (Bloksma 1990a; Gan et al. 1995).

Baking: The primary function of baking is to stabilize and dry the whole dough structure to yield a product that can be consumed. Several phenomena take place during baking which transform foam-like dough with a discontinuous gas phase embedded in a continuous bulk dough phase into a sponge-like loaf with continuous gas phase and a dry, discontinuous solid phase. As the temperature inside the dough raises, the yeast cells become more active and produce more carbon dioxide. This additional expansion, referred to as "oven spring" stops when the temperature reaches approximately 60ºC, which is the point of thermal inactivation of the yeast (Kulp, 1988). Also, expansion is achieved when the liquid phase evaporates and gas cells expand as a result of increased temperature (Bloksma 1990b; Hoseney 1994). Oven spring is topped by the onset of starch gelatinization which occurs at approximately 65ºC (Hoseney 1994) and continues until the end of baking. Gelatinization is accompanied by the swelling of the starch granules due to absorption of free water present in the system. It has also been shown that water absorbed on the surface of the gluten protein is transferred to the starch granules, further contributing to the belief that the cause of the dramatic increase in dough viscosity observed at temperatures above 60ºC, which results in the increase of tensile stress on the thin gas cell walls causing the weakest cells to rupture (Bloksma 1990a; Eliassson and Larsson, 1993b;

Gan et al. 1995). The extent of increase in viscosity is related to the amount of starch in the flour and no increase in viscosity is observed if the starch is removed (Dreese et al. 1988). The gas–continuous structure of the bread crumb starts to form around 72ºC (Hoseney 1994). Ultimately, the dough looses its gas retention capacity and becomes sponge-like structure.

1.5.2 Methods to evaluate bread making process

In order to understand BMQ of wheat many direct and indirect methods are being exploited.

I) Indirect methods

The indirect methods to evaluate breadmaking quality in wheat include mixograph, farinograph, sedimentation volume and extensigraph (Bushuk 1985). Different regions of the world give different weightage to these parameters at early and later stage selection during variety development (Table 1.2).

i) SDS Sedimentation volume test (Sv): The sodium dodecyl sulfate (SDS) sedimentation volume test is an indirect parameter of BMQ and its suitability in early generation quality testing with affordability has become popular in predicting BMQ. Sedimentation volume (Sv) is quantitatively inherited and influenced by environmental factors (Carrillo et al. 1990; Silvela et al. 1993). The SDS-Sv test is a simple, small-scale method that gives a quick estimate of wheat gluten strength (Axford et al. 1979) and is based on the property of the endosperm storage proteins to swell and flocculate in a weak acid solution (lactic acid).

It involves measurement of the Sv of ground wheat dispersed in a solution of 2% (w/v) SDS and 0.05 M lactic acid. It is reported (Axford et al. 1978; 1979) that the SDS-Sv test is more accurate than the Pelshenke test or the Zeleny test (Axford et al. 1979) for prediction of Lv prepared either by mechanical development (Chorleywood breadmaking process, CBP) or traditional long fermentation.

The Sv is well correlated with BMQ (Axford et al. 1979) as well as with gluten strength (Dick and Quick, 1983). Blackman and Gill (1980) reported that the Sv correlated (r-0.68 and 0.73, respectively) with Lv and loaf score (CBP method) for 25 varieties and breeder lines of hard and soft winter wheats. The Sv gave a fair measure of the intrinsic differences in protein quality of the varieties.

Table 1.2:- Wheat quality testing adopted for genotype evaluation

Location	Early generation (F_2-F_5)	Advanced generation (F6 and higher)
United States	a) Protein b) Hardness c) SDS sedimentation d) Mixograph	a) Milling (extraction, ash) b) Flour protein c) Water absorption d) Farinograph (time, stability, etc.) e) Dough characteristics f) Loaf volume g) Bread characteristics
Europe	a) Protein b) Zeleny sedimentation c) SDS sedimentation d) High molecular weight glutenin subunits	a) Hagberg falling number b) Alveograph (W, P/L) c) Gluten elastic recovery d) Extensometer e) Loaf volume f) Baking score g) Machinability test
CIMMYT	a) Protein b) Hardness c) SDS sedimentation	a) Hagberg falling number b) Grain and flour protein c) High molecular weight glutenin subunits d) Flour SDS sedimentation e) Mixograph (time, tolerance) f) Alveograph (W, P/L) g) Loaf volume; Crumb structure
Asia	a) Protein b) Hardness c) SDS sedimentation d) High molecular weight glutenin subunits	a) Flour protein b) Loaf volume and crumb structure c) Baking score d) Grain and flour protein e) Mixograph (Time and tolerance)

Preston et al. (1982) evaluated over 30 varieties of Canadian hard spring wheat grown over three year period at different locations and concluded that SDS-Sv test had good potential to differentiate between bread wheat lines. Interestingly, the relationship between SDS-Sv and remix Lv varied significantly depending on the Grain protein content (Gpc). Samples with a protein content less than 13% showed high correlation (r=0.78) between SDS-Sv test and remix Lv, while wheat samples with protein content over 14% yielded insignificant correlations. Moonen et al (1982) also reported good correlations between SDS-Sv and Lv (r=0.82) using 60 European wheat cultivars.

ii) Mixograph: Mixograph is routinely used to test and evaluate the quality and functionality of wheat and flours related for cereal based food production. It is a rapid tool used to measure the baking quality. It is also a direct method to relate and understand BMQ, which is considered as a complex trait because of the influence of different parameters of wheat grain, e.g. Gpc, kernel hardness, moisture content, starch content and dough polymer structure. Thus mixograph, which considers above parameters, can help in the appropriate selection of good BMQ lines, which in turn, if studied separately is labour intensive and time consuming. Also its extensive use in breeding since 1933 when it was first introduced (Swanson and Working, 1933), the wider acceptance of its analysis and reduced sample size and fast interpretation have improved its utilization in breeding programs (Finney and Shogren, 1972; Finney 1989; Rath et al. 1990; Shogren 1990). Different data parameters received by mixogram have also been used to differentiate wheat flours into good and bad baking quality (Bruinsma et al. 1978; Buckley et al. 1990; Finney and Shogren, 1972; Neufeld and Walker, 1990; Slaughter et al. 1992; Wikstrom and Bohlin, 1996).

The main advantage of mixograph is the speed of the analysis, its relative simplicity and small amount of flour required for each test. Further simplification of the testing process was described by Sibbit and Harris (1944) and Bruinsma et al. (1978), who used ground wheat instead of flour. These properties make mixographic analysis particularly amenable to early quality evaluations of breeding lines. In fact, mixographs have been used extensively in breeding programs to provide general information on the mixing properties (dough development time and tolerance to mixing) of breeding lines and the ‘strength’ of their flours. However, because of the

limitations, evaluation should rely primarily on the general shape and pattern of the curves rather than on specific values measured (Shuey 1975).

Mixing properties can be translated into several numerical parameters: (Johnson et al. 1943; Kunertb and D'Appolonza, 1985; Shuey 1975) Peak time or time (in minutes or seconds) at which the curve reaches its maximum height, which corresponds to the optimum mixing time or time for optimum dough development (Hoseney 1985; Faubion and Hoseney, 1990). Peak height of the center of the curve from the baseline at the time of maximum height provides an indication of flour "strength". Tolerance to over mixing is assessed by several parameters including the height of the curve at a specific time after the peak and the angle between the ascending and descending portion of the curve. The area under the curve is a comprehensive parameter reflecting both flour "strength" and tolerance to over mixing.

Though mixograph peak time and mixing tolerance were two data parameters routinely used to explain the BMQ, with the availability of computer aided analysis of mixogram, many parameters can be analysed and selected as a function of dough properties (Buckley et al. 1990; Martinant et al. 1998; Neufeld and Walker, 1990; Wikstrom and Bohlin, 1996). Application of mixograph can be further enhanced by QTL mapping for mixograph traits which not only help in the identification of markers tightly linked with the loci but also to identify loci controlling various dough parameters. A significant progress has been already made in dissecting important agronomic traits like, kernel hardness (Ha locus) and grain protein content (Gpc locus) using phenotypic data generated with Near Infra Red spectroscopy (NIR), which has shown strong association with these traits. Similarly mapping of the mixograph traits, which is a measure of the functionality of dough will help in genetic dissection of the QTL associated with BMQ traits. Among many mixogram traits, few were considered for QTL analysis, which explained maximum dough characteristics [McCartney et al. 2006 (8 parameters), Huang et al. 2006 (8), Nelson et al. 2006 (37), Dobraszczyk and Schofield, 2002 (15), Campbell et al. 2001 (4), Ohm and Chung 1999 (10) and Martinant et al. 1998 (11)].

Recently, a direct-drive mixogram requiring only 2 g of flour has been described by Rath et al. (1990), extending the potential use of mixographic analysis to a single plant from segregating breeding populations. Gras and O'Brien (1992)

analyzed 2 g flour samples from F_2 plants and their F_3 progenies from three different crosses and reported a wide range in mixogram parameters with an acceptable error associated with their determination. Contrary to previous reports (Bakers and Campbell, 1971; Branlard et al. 1991) they obtained medium to high heritability estimates to peak time using offspring/parent regression analysis. However, they reported a low heritability estimate for peak height. Also, 2 g mixograph proved to be the most valuable for studying the functional properties of single purified polypeptides corresponding to the high molecular weight glutenin subunits (Bekes et al. 1994a and 1994b).

iii) Farinograph: The Brabender Farinograph (Brabender OHG, Germany) is the most universally used dough-testing instrument (Sheuy 1975). Mixing is provided by two sigma type blades rotating in opposite direction from each other, at different speeds (Kunertb and D'Appolonza, 1985). The resistance to mixing of water flour dough is transmitted from the blades to a dynamometer connected to a scale and lever system, which moves a tracing pen. The farinograph mixing bowl (50 or 300 g) is hollow allowing for water circulation, thereby ensuring operation at constant temperature. The kneading type mixing action of farinographs is gentler than harsher Pullfold-repull mixing pattern in mixographs (Kunertb and D'Appolonza, 1985; Spies 1990). The main advantage of farinograph over mixograph is the temperature control and its accuracy in determining the water absorption of dough. The main disadvantage of farinograph is the relatively large amount of flour needed to perform a test and difficulty for its use in early stages of breeding process.

iv) Extensigraph: The Brabender Extensigraph measures the force required to stretch a cylindrical piece of water-salt-flour dough prepared according to a AACC method 54-10, 2005. The dough is usually mixed to maximum consistency in a farinograph prior to being molded and allowed to rest at constant temperature for variable periods of time, depending on the protocol used (Bloksma and Bushuk, 1988). The stretching is performed by a hook moving downward at constant speed through the middle of the dough piece, transmitting the force to a dynamometer through a series of balances and levers. The resulting extensigram is a plot of force (in extensigraph units) versus time. As the speed of the hook is constant, time can be equated to extension (Bloksma and Bushuk, 1988). The commonly computed parameter with extensigraph is R_{max} or the

maximum resistance (flour strength) to stretching that corresponds to the maximum height of the curve (in Brabender units).

v) Alveograph: The Alveograph (Chopin, s.a – Tripette et Renault, Villeneuve la Garenne, France) was invented in 1920 by Marcel chopin. It measures the pressure required to blow a bubble in a sheeted piece of dough. The test piece is water –salt-flour dough which is mixed, sheeted, cut and allowed to rest according to a defined protocol (AACC method, 54-30, 2005). The alveograph usually tests dough pieces with fixed water content corresponding to 50 g of the flour weight on a 15% moisture basis, regardless of the absorption capacity of the flour tested (Faridi and Rasper, 1987). Different levels of starch damage can have markedly different hydration requirements, which in turn affects the alveographic measurement. The main advantage of the alveograph over the extensigraph is that the type of deformation generated. While the extensigraph stretches the dough in only one direction (uniaxial deformation) at a constant rate, the alveograph expand the dough in all directions (biaxial deformation) at a deformation rate that varies as the bubble grows. The mode of stretching in alveograph simulates the expansion occurring during the fermentation and oven rise (Hoseney 1994).

II) Direct methods:

i) Loaf volume: Loaf volume (Lv) is usually considered as one of the most important and direct measures of BMQ (Weegels et al. 1996). However, Lv estimation of bread demands large sample material, cost and labour. Visco-elastic property of wheat proteins is therefore studied to dissect the genetic effect of loci governing this trait (Ma et al. 2005). Diagnostic markers are available for BMQ influencing parameters like HMW, LMW, PinA, PinB, secalin and Gpc (Gale 2005), however, studies on Lv, a main direct estimate of BMQ are limited. Moreover, QTL studies on Lv have suggested that the HMW and LMW loci cannot be used as indirect measure of Lv (Rousset et al. 2001). Loci on chromosome 3A (Law et al. 2005) and chromosome 2A (Kuchel et al. 2006) were found to control Lv directly. Identifying such loci for Lv would help in implementation of an early progeny selection for BMQ using marker assisted breeding. Besides Lv, Sv and Gpc play an important role in determining the end use and marketability of wheat and have direct correlation with BMQ. As the QTLs governing above traits are influenced by environment, identification of markers

linked to these traits help in their genetic dissection as well as in marker assisted selection (MAS). Therefore, reliable assessment of BMQ parameters with molecular markers has received considerable attention in recent years. Such reports of QTLs for Lv, Sv, Gpc, Tw, Tgw and Mixograph parameters are tabulated in Table 1.3.

ii) Grain protein content (Gpc): Bread making process demands Gpc to be above 12.5% (Turner et al. 2004) and Gpc is often environmentally influenced and has low heritability (Simmonds 1995). More recently, QTL studies have been used for dissecting different loci governing Gpc to enable appropriate selection (Blanco et al. 1996; Börner et al. 2002; Groos et al. 2003; Joppa et al. 1997; Prasad et al. 1999, 2003; Perretant et al. 2000; Uauy et al. 2006; Zanetti et al. 2001). *Gpc-B1* gene mapped on chromosome arm 6BS (Joppa et al. 1997; Olmos et al. 2003) has shown promising increase in protein content in both tetraploid and hexaploid wheat (Mesfin et al. 1999; Chee et al. 2001) across different environments and it also affects Tgw (Uauy et al. 2006). Though Gp*c-B1* accounts for 66% of protein content variation (Joppa et al. 1997), there are many loci on different chromosomes controlling Gpc which need to be identified. The genetic components of Gpc have been extensively studied in bread wheat (Joppa et al. 1997; Prasad et al. 1999; Perretant et al. 2000; Zanetti et al. 2001). The greatest influence was detected by Joppa et al. (1997), who found a QTL explaining 66% of the phenotypic variation for Gpc located on chromosome 6B. Groos et al. (2003) identified 'stable' QTLs (i.e. detected in at least four of the six locations) for Gpc on chromosomes 2A, 3A, 4D and 7D, each explaining about 10% of the phenotypic variation. Further, the role of grain hardness related proteins Pin-a and Pin-b in affecting the BMQ were demonstrated by Igrejas et al. (2001) through the studies on dough strength and Lv.

iii) Thousand grain weight and Test weight: Grain yield is perhaps the most commonly studied but poorly understood trait related to agronomic performance of wheat. Few results are available for yield and generally, the studies have focused only on a single chromosome (Hyne and Snape, 1991; Araki et al. 1999; Shah et al. 1999). For yield, Groos et al. (2003) identified only one important QTL on chromosome 7D, explaining up to 15.7% of the phenotypic variation. Some studies have examined the influence of the *Rht* dwarfing genes (Hyne and Snape, 1991) on yield. More results are available on yield components, such as thousand-kernel weight (Tkw) or thousand grain weight (Tgw) and test weight (Tw) affecting the economic value of wheat

(Campbell et al. 1999; Prasad et al. 1999; Zanetti et al. 2001). Tgw is positively correlated with flour yield and it is the most stable component of yield trait (Varshney et al. 2000). It is influenced by many QTLs located on different chromosomes (Chojecki et al. 1983; Giura and Saulescu, 1996; Varshney et al. 2000). Also, Groos et al. (2003) identified three QTLs governing Tgw on chromosomes 2B, 5B and 7A for various environments. Tw measures relative plumpness of the grain and it is one of the important traits deciding the marketability of grain. It is a function of both kernel density and random kernel packing volume, often considered as an initial indicator of grain quality (Yamazaki and Briggle, 1969). Tgw and Tw also contribute to bread making quality through their influence on milling properties (Berman et al. 1996).

The shape and size of wheat grains is a primary determinant of the value of the grain (Campbell et al. 1999). It has an effect on many factors of the agronomy and on end use of the crop and therefore, is an important trait for genetic study. Wheat milling units obtain higher flour yields from larger grains (Wiersma et al. 2001, Giura and Saulescu, 1996) and therefore, place pressure on breeders to ensure new varieties that exhibit large grains. This has an effect on the specific weight of the grain sample, with large and full grains producing higher specific weights than small and shrivelled grains. The market price of grain samples throughout the world is partly determined by specific weight, which is influenced by seed size and shape of the grain. It has been suggested in a number of studies that increasing kernel size is an important factor in increasing crop yield. However, this may have some negative implications on the end use of the crop, with larger kernelled lines often showing decrease in protein content due to the extra grain size being composed mainly of starch rather than protein. It has also been shown that large seeds may give a better start for plants grown in the field, with greater seedling size and speed of growth coming from larger seeds (Bredemeier et al. 2001).

Yield and Gpc are both largely influenced by environmental conditions such as soil fertility, rainfall or temperature. Many authors (Bhullar and Jenner, 1985; Wardlaw and Wrigley, 1994; Daniel and Triboi, 2000) have shown that temperature and nitrogen nutrition influence both grain weight and Gpc. Therefore, descriptors of environmental conditions, such as climate or soil traits are candidate covariates for interpreting genotype x environment interactions (GEI) for yield and Gpc. An

Table 1.3:- BMQ trait QTLs reported for *Triticum aestivum*

Trait	QTLs / Loci/ Chromosome	Reference
Gpc		
	QGpc.ndsu-6Bb	Distelfeld et al. (2004)
	Gpc-B1	Uauy et al. (2005)
	QGpc.ccsu-2D.7	Kulwal et al. (2005)
	QPro.mgb-4B	Blanco et al. (2002)
	QPro.mgb-6A.1	Blanco et al. (2002)
	QGpc.crc-4D	Huang et al. (2006)
	QGpc.crc-7B	Huang et al. (2006)
	QGpc.ccsu-3D.1	Prasad et al. (2003)
	QGpc.ccsu-2B.1	Prasad et al. (2003)
	QGpc.ccsu-7A.1	Prasad et al. (2003)
	QGpc.crc-4A	McCartney et al. (2006)
	1B	Perretant et al. (2000); Turner et al. (2004)
	2B	Turner et al (2004); Groos et al. (2003)
	2D	Dholakia et al. (2001)
	3A, 5B, 7A	Groos et al. (2003)
	3B	Zanetti et al. (2001)
	6A	Perretant et al. (2000); Groos et al. (2003)
	6B	Mesfin et al (1999)
Sev		
	QSv.crc-1B	Huang et al.(2006)
	QSv.crc-2D	Huang et al.(2006)
	QSv.crc-5D	Huang et al.(2006)
	Qsev.mgb-1A	Blanco et al.(1998)
	Qsev.mgb-IB	Blanco et al.(1998)
	QSsd.crc-1B	McCartney et al. (2006)
	QSsd.crc-2A	McCartney et al. (2006)
	QSsd.crc-6A	McCartney et al. (2006)
	Glu-B1	Blanco et al. (1998); Zanetti et al. (2001)
	Glu-D1	Rousset et al. (2001); Martin et al. (2001)
	3A	Nelson et al (2006)
Tgw		
	QGwt.crc-3D	McCartney et al. (2003)
	QGwt.crc-4A	McCartney et al. (2003)
	QGw.ccsu-2B.1	Kumar et al. (2006)
	QGw.ccsu-7A.1	Kumar et al. (2006)
	QTgw.crc-2B	Huang et al.(2006)
	QTgw.crc-4B	Huang et al.(2006)
	QTgw.ipk-1B	Huang et al. (2004)
	QGw1.ccsu-1A	Kumar et al. (2006)
	QTgw.ipk-5A	Huang et al. (2004)
	1A	Varshney et al. 2000
	1B	Groos et al. (2003); Tahir et al. (2006); Huang et al. (2006)
	4B	Elouafi and Nachit, (2004)
	5A	Zanetti et al. (2001)
	6B	Elouafi and Nachit, (2004)
	7A	Dholakia et al. (2003); Ammiraju et al. (2001)
	7B	Elouafi and Nachit, (2004)

Table 1.3 contd.

Tw	*QTwt.crc-1B*	McCartney et al. (2003)
	2B	Campbell et al. (1999)
	6B	Elouafi and Nachit, (2004)
Grain weight per ear		
	QGwe.ipk-3B.1	Huang et al. (2004)
	QGwe.ipk-6A	Huang et al. (2004)
	QGwe.ipk-7D	Huang et al. (2004)
	QGwe.ocs-4A.1	
Lv	*QBlvl.crc-4D*	McCartney et al. (2006)
	1A, 1B, 3A,5B, 7A,7B	Groos et al. (2007)
	1A, 1B, 1D	Rousset et al. (2001)
	2A,	Kuchel et al. (2006)
	2B, 7B,7D	Campbell et al. (2001)
	3A	Law et al. (2005)
	3B	Nelson et al. (2006)
	3B	Mansur et al. (1990)
Mixograph		
Mixing stability	1A, 1B,	Nelson et al. (2006)
Mixing strength	3B	Nelson et al. (2006)
Midline Right Integral	1B	Nelson et al. (2006)
Dough Score	1B, 2B, 3A	Groos et al. (2007)
Mpv	*QMpkh.crc-4D*	McCartney et al. (2006)
	Glu-A1	Campbell et al. (2001)
	QPkh.crc-1B, QPkh.crc-1D, QPkh.crc-4D	Huang et al.(2006)
Mpt	*QMmdt.crc-1B, QMmdt.crc-4D*	McCartney et al. (2006)
	QMdt.crc-1B ,QMdt.crc-1D, QMdt.crc-3B	Huang et al.(2006)
	Glu-D1	Campbell et al. (2001)
	GluD3	Perretant et al. (2000)
	1D	Arbelbide and Bernardo (2006)
	5B	Zanetti et al. (2001)
Peak Band width	QMpbw.crc-2B, QMpbw.crc-4D	McCartney et al. (2006)
Mti	*QMteg.crc-1, QMteg.crc-2B,*	McCartney et al. (2006)
	QMteg.crc-4D, QMteg.crc-7D	McCartney et al. (2006)
	QTeg.crc-1B , QTeg.crc-5D	Huang et al. (2006)
Extensibility	5A	Ma et al. (2005)

accurate evaluation of these traits is made difficult by the importance of the GEI (Robert et al. 2001; Dholakia et al. 2003) and thus, determination of molecular markers linked to these traits would help plant breeders to develop cultivars that combine high yield with high Gpc. For many years, a negative relationship between these two traits was observed but little was known whether this association was due to a close genetic relationship or to opposite environmental effects on the two traits. Earlier studies of Groos et al. (2003) highlighted that no negative relationship could be established between yield and Gpc. By using factorial regression on GxE they concluded that some genetic regions were involved in the differential reaction of genotypes to specific climatic factors, such as mean temperature and the number of days with a maximum temperature above 25°C during grain filling. However, there are exceptional varieties that combine excellent yields with high levels of Gpc, and simultaneous increase in yield and Gpc have been reported in target populations (Cox et al. 1985). Efforts to improve Gpc without selecting for low yield can be accelerated by the identification of genes that affect Gpc and direct selection of the alleles with positive effects.

1.6 Gluten and breadmaking quality

Considerable studies have been accomplished in gluten and breadmaking quality (BMQ) during the last two decades. BMQ was first related to the expression of a single glutenin allele by the work of Payne et al. (1979, 1981). Their first report (Payne et al. 1979) illustrated a strong association (r=0.72) between the amount of the Glu-A1 encoded HMW glutenin subunits 1 and Sv in a segregating population of 60 F_2 plants from a cross between two British lines differing in their BMQ. A second study (Payne et al. 1981) based on the analysis of progenies from six crosses confirmed this association (qualitatively) and established an additional relationship between Sv and allelic composition at the Glu-D1 locus. In the latter case, lines expressing the allele corresponding to HMW subunits 5+10 had a markedly higher Sv than those expressing the alternative allele corresponding to subunits 2+12. Also, the results suggested that both the loci affected Sv in an additive fashion. These findings stimulated a great deal of research and were subsequently confirmed and extended by numerous studies involving different wheat collections from many countries as well as recombinant inbred lines and segregating populations from a number of crosses. Some of these investigations are discussed below.

Moonen et al. (1982) studied the relationship between the allelic composition at the HMW glutenin loci and Sv, Lv and gel protein content (SDS- insoluble protein) of flour samples from 60 European wheat cultivars as well as 36 F_6 progenies from a cross between cultivars Atlas 66 and Atys. They reported that the presence of allele 5+10 (versus 2+12) was associated with a significantly higher Sv, a greater Lv and proportion of a gel protein. However, HMW glutenin subunit 1 was not associated with better quality.

Payne et al. (1987) and Garcia-Olmedo et al. (1982) evaluated some of the alleles at the high molecular weight (HMW) glutenin loci (Glu-A1, Glu-B1 and Glu-D1) and scored their importance to wheat quality. Later, the combined effects of alleles at both the HMW and low molecular weight (LMW) glutenin loci on dough strength were investigated (Eagles et al. 2002; Gupta et al. 1989; Nieto-Taladriz et al. 1994). The HMW and LMW glutenins through disulphide bonds interact to make a gluten network capable of holding the gas evolved during fermentation of the dough (Shewry and Tatham, 1997).

Payne et al. (1987) summarized the relationship between BMQ and HMW subunits composition by ranking the alleles expressed at the Glu-1 loci according to their known or presumed contribution to BMQ and assigning them scores from 1 to 4. For the Glu-A1 alleles 1 and 2*, 3 points are assigned and the null allele 1 point. For the Glu-B1 locus alleles 17+18 and 7+8 are given 3 points, 7+9 are assigned 2 points and 7 or 6+8 are given 1 point. For Glu-D1, alleles 5+10 are assigned 4 points whereas alleles 2+12 or 3+12 are given 2 points. A comprehensive Glu-1 score (varying from 3 to 10) is then obtained by summing the scores for the individual loci.

A large number of many such factors were further implicated, for example, lipids (Pomeranz and Chung, 1978), pentosans (D'Appolonia et al. 1970), hydrolytic enzymes and LMW 'soluble' proteins within the albumin and globulin fractions (Pogna et al. 1991; Zawitowska et al. 1986) to affect BMQ in wheat. Some of these proteins are enzymes, involved in metabolic processes, while others are amylase and protease inhibitors playing protective roles in plants (Bietz 1988). Along with QTLs affecting grain protein content (Dholakia et al. 2001; Groos et al. 2003; Prasad et al. 2003; Turner et al. 2004), few studies reported additional genetic loci that influence dough rheology and baking quality (Groos et al. 2004; Law et al. 2005; Perretant et al. 2000).

1.7 Quality mapping using molecular markers

Most of the economically important traits of crop plants are controlled by QTLs and their expression is often influenced by the environment in which the plants are grown. The heritability of these traits is low and selection by conventional plant breeding method may delay the process or lead to failure in crop improvement. The estimation of the traits such as BMQ needs large sample size, technical facilities and labour. However, these estimates cannot be reliable if the heritability of the trait under study is governed by QTLs. Marker assisted selection (MAS) is widely adopted to transfer the QTLs and theoretically, it is more effective than phenotypic selection when correlation between the marker genotype scores and the phenotypic values is greater than the square root of heritability of the trait (Dudley 1993). Tagging of DNA markers with the phenotypic traits needs comprehensive study, which is discussed below.

1.7.1 Methodology of QTL mapping in plants using DNA markers

I) Microsatellite markers as a valuable marker system with special reference to wheat

Simple sequence length polymorphism (SSLP markers), also known as simple sequence repeats (SSRs), or microsatellites, consist of tandemly repeated di-, tri- or tetra-nucleotide motifs and are a common feature of most eukaryotic genomes. The number of repeats is highly variable because slipped strand mis-pairing causes frequent gain or loss of repeat units. With high level of allelic diversity, microsatellites are valuable as molecular markers, particularly for studies of closely related individuals. PCR-based markers are designed to amplify fragments that contain a microsatellite using primers complementary to unique sequences surrounding the repeat motif (Weber and May, 1989). Differences in the number of tandem repeats are readily assayed by measuring the molecular weight of the resulting PCR fragments. As the differences may be as small as two base pairs, the fragments are separated by electrophoresis on polyacrylamide gels or using capillary DNA sequencers that provide sufficient resolution. Without prior sequence knowledge, microsatellites can be discovered by screening libraries of clones. Clones containing the repeat motif must be sequenced to find unique sites for primer design flanking the repeats. Microsatellite marker development from pre-existing sequence is far more direct. Good reviews of microsatellite marker development include those of McCouch et al. (1997) and Zane et al. (2002). Microsatellites discovered in non-coding

sequence often have a higher rate of polymorphism than microsatellites discovered in genes. However, in some species such as spruce (*Picea* spp.) with highly repetitive genomes, SSR markers developed from gene sequences have fewer instances of null alleles, i.e. failure of PCR amplification (Rungus et al. 2004). Microsatellite markers have several advantages. They are co-dominant; the heterozygous state can be discerned from the homozygous state. The markers are easily automated using fluorescent primers on an automated sequencer and it is possible to multiplex (combine) several markers with non-overlapping size ranges on a single electrophoresis run. The results are highly reproducible, and markers are easily shared among researchers simply by distributing primer sequences. Although SSRs are abundant in most eukaryotic genomes, their genomic distribution may vary. Uneven distributions of microsatellites limit their usefulness in some species.

II) Mapping populations

The construction of a linkage map is a process that follows the segregation of molecular markers in a segregating population and placing them in linear order based on pair wise recombination frequencies. Thus, a mapping population with high number of polymorphisms over the total genome is highly desirable. Towards this end, various ways have been used to create mapping populations which are illustrated in Fig.1.2. Populations used for mapping are usually derived from F_1 hybrids between two lines (either homozygous or heterozygous) which shows allelic differences for selected probes. Specific populations can be further developed by various ways depending on the genetic features of the plant species being analysed. The commonly used populations are doubled haploid (DH) lines, recombinant inbred lines (RIL), F_1, F_2 and backcross (BC) populations.

Of these mapping populations, RILs offer unique advantages in QTL studies (Burr and Burr, 1991, Knapp and Bridges, 1990, Austin and Lee, 1996); i) a well-characterized RIL population can be permanently propagated and used indefinitely without further genotyping ii) a trait value for each genotype can be evaluated on several sister plants, which minimizes the environmental variation and improves the accuracy of QTL analysis iii) experiments can be replicated over years and environments using identical genotypes thus allowing the detection of QTL that cause GEI iv) maps constructed using RILs have a higher genetic resolution as compared to F_2 populations (Burr and Burr, 1991), as chromosome pairing and recombination during an extended period of inbreeding increases the probability of chromosome

recombination. Disadvantage of using RILs is that the development of RILs is time consuming and not readily accessible to self-incompatible species like cultivated potato. Furthermore, the dominant effects at QTL cannot be estimated as done in F_2 population. RILs have been used in maize, rice, arabidopsis, wheat and oat for various studies such as map construction (Cho et al. 1998), QTL mapping (Frova and Gorla, 1993; Austin and Lee, 1996; Lister et al. 1993; Blanco et al. (1998) and candidate gene analysis (Faris et al. 1999; Kianian et al. 1999). RIL populations are commonly used to dissect the QTLs associated with quality traits in wheat (Kuchel et al. 2006; Campbell et al. 1999).

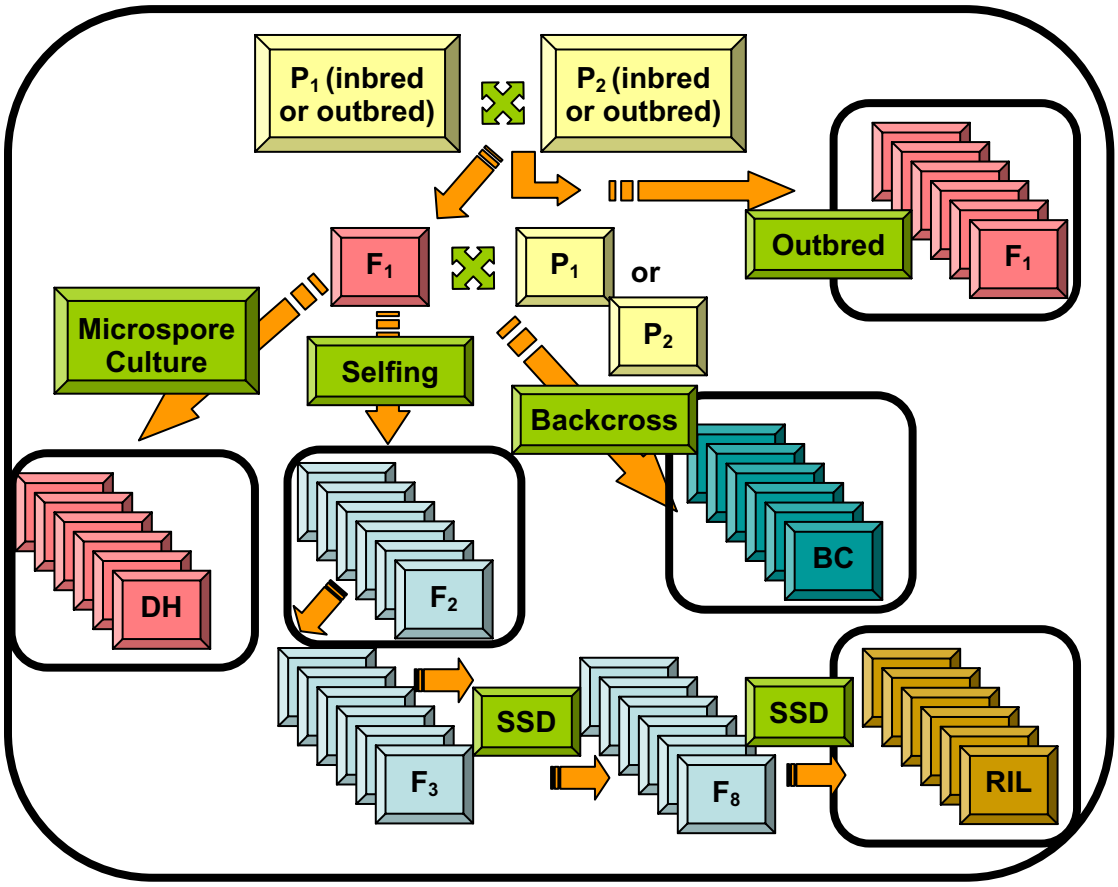


Fig. 1.2 Common strategies for the construction of mapping populations. A DH mapping population is generated from pollen of F_1 by anther or microspore culture followed by doubling of haploid. Recombinant inbred lines (RIL) are developed by successively selfing single F_2 plants by single-seed descent (SSD) method until it reaches homozygosity in the F_8 generation. A BC (Back cross) population is derived from crossing of an F_1 individual with one of the parents of F_1

III) QTL mapping approaches

Genetic maps act as the first step towards understanding the genetics of individual crop plants. Genetic maps based on molecular marker technologies are now available for all major cereal species, including wheat (Snape et al. 2006). At present, genetic maps are widely used to locate genes of interest so that the maps can be fully annotated with the locations of genes governing quality, agronomic performance, disease resistance, adaptability, or any other trait. This helps in direct manipulation of the desired trait by MAS. Much of the variation for important quality traits in wheat is quantitative in nature and controlled by many genes of small effect acting together, so called Quantitative trait loci. QTL analysis in crop species with complex genomes is an important tool, which allows the location of multiple loci such as those involved in quality differences (Snape et al. 2006). This analysis is complicated in wheat by the complexity of its polyploid genome, with the three genomes interacting in the regulation of one trait. In addition, low levels of polymorphism in molecular markers, especially in D genome of wheat, make it very difficult to construct complete genetic maps. To produce complete maps, researchers have in the past resorted to studying very wide crosses such as that of the ITMI population to increase the chances of finding polymorphic markers (Snape et al. 2005). However, to study quality traits, crosses need to be made between much more closely related varieties to ensure the results are relevant to the commercial market.

Single marker analysis: The simplest approach to identify QTLs, with data on an experimental cross is to perform analysis of variance (ANOVA) at each of the marker loci (Soller et al. 1976). At each genetic marker, the population is split into two groups, according to their genotypes at the marker and compares the two group phenotypes means by a t-test. It is accompanied with a LOD score, defined as the (base 10) log-likelihood ratio comparing the hypotheses (Broman 2002). Marker loci with large LOD scores are indicated to be linked to a QTL.

a) the phenotypes in the two groups are normally distributed with distance means but a common variance and

b) the phenotypes for all individuals follow a common normal distribution, independent of genotype.

The above approach has following weaknesses- i) if a QTL is not located at a marker, its effect will be attenuated as a result of recombination between the marker and the QTL ii) At each genetic marker, we must discard individuals whose genotypes are missing will be discarded iii) When the markers are widely spaced a QTL may be quite far from all markers, and so the power for QTL detection will decrease. iv) This approach considers only one locus at a time (Single QTL model); in the presence of several QTLs, this approach fails.

Interval mapping (IM): To overcome the disadvantage of single QTL model, multiple QTL model was proposed to give greater power for QTL detection, better separation of linked QTLs and to allow the examination of interactions between QTLs (though such interactions will not be considered here).

Lander and Botstein (1989) developed interval mapping, which overcomes the first three weaknesses of ANOVA at marker loci described above. This method, which continues to be the most popular approach for QTL mapping, makes use of a genetic map using linked markers and like ANOVA assumes the presence of single QTL. Each location in the genome is positioned, one at a time, as the location of the putative QTL.

Given the marker genotype data (and assuming no crossover interference), one can calculate the probability that an individual has genotype HH (or HL) at a putative QTL. These QTL probabilities depend only on the genotypes at the flanking markers. In interval mapping one assumes that given QTL genotypes, the phenotype follows a normal distribution with mean μ_H or μ_L according to whether the QTL genotype is HH or HL, respectively and a common standard deviation σ.

With the given genotypes at the markers flanking the QTL, the conditional QTL genotype probabilities, the marker genotype data, as mixing proportions, QTLs can be detected. For the QTL at each position in the genome (or in practice, at steps of 0.05 cM), three parameters are calculated μ_H, μ_L and σ and also a LOD score; the (base 10) log-likelihood ratio, by comparing the hypothesis that there is a single QTL at the given location with the hypothesis that there is no QTL anywhere in the genome. The LOD score, as a function of chromosome position, forms a profile log-likelihood. The genomic region which has large LOD score indicates the genomic interval which harbors the QTL. Churchill and Doerge, (1994) suggested permutation

test to generate genome wide threshold LOD, using the assumptions that there are no QTLs (ie. the phenotypes are simple normally distributed; independent of the marker data).

Advantages of interval mapping are two fold - i) it makes more complete use of the marker genotype data (making proper allowance for the missing data) and ii) it considers positions between markers as putative locations for a QTL effects. In case of dense genetic markers and relatively complete marker genotype data, interval mapping provides little advantage over ANOVA. The disadvantage being similar to ANOVA, which assumes single QTL model and it is not ideal in the presence of multiple especially linked QTLs.

Composite interval mapping (CIM): Methods that make use of multiple–QTL models can provide increased sensitivity, resolve linked QTLs and allow the examination of interactions between QTLs. The simplest multiple-QTL method is multiple regression, the obvious extension of ANOVA at the marker loci. Cowen (1989) appears to be the first to have recommended the use of multiple regressions in this context.

Jansen and Zeng independently developed a method which attempts to reduce the multi-dimensional search for identifying multiple QTLs to a one–dimensional search (Jansen 1993; Jansen and Stam, 1994; Zeng 1994). This is actually a hybrid of interval mapping and multiple regression on marker genotypes. One includes other markers (on the same chromosomes and on different chromosome) as repressors while performing interval mapping, in an effort to control for the effects of QTLs in other intervals, so that there will be greater power for QTL detection and also the effects of background QTLs will be precisely estimated. Zeng (1994) referred to this approach as composite interval mapping (CIM). Similar to interval mapping, LOD threshold is calculated by whole genome scanning.

Multiple interval mapping (MIM): MIM uses multiple marker intervals simultaneously to construct multiple putative QTL in the model for QTL mapping. Multiple–interval mapping is much like CIM, but the additional repressors are not required to reside at the marker loci. Therefore, when compared with the current methods such as IM and CIM, MIM tends to be more powerful and precise in detecting QTL (Kao et al. 1999). To detect QTL using the MIM model, model

selection procedures are considered because all possible subset selection is not feasible. There are at least three basic model selection techniques, forward, backward, and stepwise selections, for exploring the relationship between the independent and dependent variables (Draper and Smith, 1981; Kleinbaum et al. 1988; Miller 1990). As MIM uses multiple QTL, the computation burden is heavy when compared with the one-QTL model (CIM and IM). MIM has the potentiality to be more powerful and more precise in QTL mapping by directly conditioning putative QTL and incorporating possible epistasis in the model. Thus, more genetic variation can be controlled in the model. With the estimates of QTL parameters, other composite genetic parameters, such as the genetic variance components and heritabilities can also be estimated. Based on the MIM results, genotypic values of individuals can also be estimated to allow desired genotypes to be selected in MAS under various requirements (e.g. cost, efficiency, and trait correlations) (Kao et al. 1999).

1.7.2 Marker assisted selection (MAS) for crop improvement

Molecular markers are powerful tools that can be used for MAS and also as landmarks for map-based cloning of genes. Molecular markers associated with QTLs have been reported for many important traits. Once a linkage between a QTL and molecular marker is determined, the QTL can be transferred into genetic background by MAS.

Molecular markers are increasingly being used to tag genes or QTLs of agronomic importance, offering the possibility of their use in MAS for wheat breeding (Gupta et al. 1999; Jahoor et al. 2004). In addition to their use in MAS, molecular markers have been used to isolate genes via map-based cloning (Stein and Graner 2004). Some molecular markers detect homeoloci; the same sequence is present on all three members of a homoeologous group. Such homoeoloci have helped in the construction of comparative maps in different cereals, and which may demonstrate the presence of major translocations thought to have occurred during speciation.

The potential value of genetic markers, linkage groups and their association with agronomic traits has been known for more than 80 years. The usefulness of MAS was recognized as early as 1923 when Sax demonstrated in beans an association between seed size and seed coat pigmentation. The concept of selection based on

genotype rather than phenotype created strong interest among plant breeders (Tanksley et al. 1989; Paterson et al. 1995). The molecular-marker based (RFLP) map in plants was first demonstrated in tomato and consisted of 57 loci (Bernatzky and Tanksley, 1986). Since then, maps have been constructed for nearly all crop plants (Philips and Vasil, 2001), allowing in principle, the application of MAS in plant breeding as originally proposed by Sax (1923) and Thoday (1961). The rationale relies on the discovery of phenotype/genotype associations between genome regions (as assayed by molecular markers) and traits in segregating populations (such as F_2, RIL, DH, BC, etc.). These are derived by analysis of segregation of simply inherited traits and by QTL analysis for complex traits (Lee 1995). The identification of markers tightly linked to target genes/QTLs and their conversion, if necessary, to a PCR platform has made MAS feasible in some plant breeding programmes (Langridge and Chalmers, 1998). MAS can increase the efficiency and accuracy of selection, especially for traits that are difficult to phenotype or are recessive (Varshney et al. 2006a).

Markers selected for use in MAS should be reliable and easily shared among researchers. Co-dominant markers are preferred to avoid the need for progeny testing. Sometimes less desirable markers for MAS such as RAPDs, ISSRs and AFLPs are useful for finding markers linked to the desired allele. Once such a marker is found, it is possible to extract and sequence the corresponding band. This sequence can be used to develop co-dominant markers such as cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel, 1993) or to sequence characterized amplified regions (SCARs) (Paran and Michelmore, 1993). SCAR and CAPS markers are co-dominant and simplify the screening of large number of individuals. When a genetic map exists, markers can be positioned on the map and other linked markers can be substituted. The additional markers are useful for high resolution mapping to find markers more closely linked to the desired allele or ultimately for positional cloning of the underlying gene. Following are some of the major components of MAS aimed at enhancing the efficiency of plant breeding:

(1) Accelerating the selection of small number of traits that are difficult to follow due to complex inheritance or strong environmental influence.

(2) Selection for traits of substantial economic importance, in cases where the biological assays are unreliable and/or not cost-effective.

(3) Accumulating disease-resistance genes by gene pyramiding can be difficult without MAS. Once an effective resistance gene is present in a breeding line, it is difficult to select for additional resistance genes due to epistatic effects. Using molecular markers additional resistance genes can be accumulated into elite lines while maintaining pre-existing resistance genes.

1.7.3 Mapping and tagging of quality traits in wheat

The wheat genome is very large (16,000 Mb) compared to other cereal crops like Rice (390 Mb), Sorghum (1,000 Mb), Maize (2,500) and Barley (6,000 Mb) etc. Development of markers for quality traits in wheat has been very challenging because of its polyploid nature and presence of high proportion of repetitive DNA. At present 9,417 markers including RFLP, SSR, STS, EST-SSR, etc have been mapped on wheat. The RFLPs have been used as the preliminary system of markers and played role of the first generation markers to map the wheat genome by various workers like Chao et al. (1989), Liu et al. (1991), Anderson et al. (1992), Devos et al. (1992), Devos and Gale, (1992) and Boyko et al. (1999).

With further advancement of technology, new types of PCR based markers, namely RAPD, ISSR, STMS, SSR and AFLP occupied status of the second-generation markers and led to complete establishment of genetic linkage maps. Kojima et al. (1998) and Peng et al. (2000) have used RAPD markers for the construction of linkage maps while SSR markers were utilized by Cadalen et al. (1997), Roder et al. (1998), Stephenson et al. (1998), Penner et al. (1998), Messmer et al. (1999), Nachit et al. (2001) and Somers et al. (2004) for linkage group construction.

Efforts on wheat genome mapping using DNA markers have been initiated with the formation of the International Triticeae Mapping Initiative (ITMI). It was conceived in 1989, originally as a five-year effort to develop RFLP maps for crops of the Triticeae, mainly wheat and barley. The mapping effort was organised around the seven homoeologous chromosome groups of the Triticeae for which co-ordinators were appointed. Additional co-ordinated topics included related diploid genomes, genetics of abiotic stress resistance, and Triticeae informatics (database and RFLP probe repository). Some 130 scientists from most of the wheat growing countries are affiliated to ITMI including scientists from India.

Newer approaches, based on SNPs, EST-SSRs are perceived as third generation markers and expected to be used increasingly (Edwards and Mogg, 2001) as they enable development of markers within functionally relevant parts of the genome. The growth in the popularity of the EST repeats is evident through the Triticeae repeat database (TREP) (Fig. 1.3). In wheat, on an average one SNP per 540 bp was detected in coding regions (Somers et al. 2003). A number of SNP-based markers were identfied for γ-gliadins (Zhang et al. 2003), the waxy starch gene *Wx-D* (Yanagisawa et al. 2003), *Lr1* (Tyrka et al. 2004), a ferredoxin-dependent gene involved in nitrogen metabolism (Boisson et al. 2005), *Glu-D3* (Zhao et al. 2006) and a Karnal bunt resistance QTL (Brooks et al. 2006). To aid in rapid development of tools to harness SNPs, the Biotechnology and Biological Sciences Research Council (BBSRC) of UK initiated the cereal consortium 'Investigating gene function' (IGF) and developed a consortium to investigate cereal gene function. This consortium has identified and spotted 244,000 SNPs on array, with the help of Agilent Biosystems (UK) for high throughput SNP screening, which comprises SNP from different tissues of wheat plant (Barker 2007).

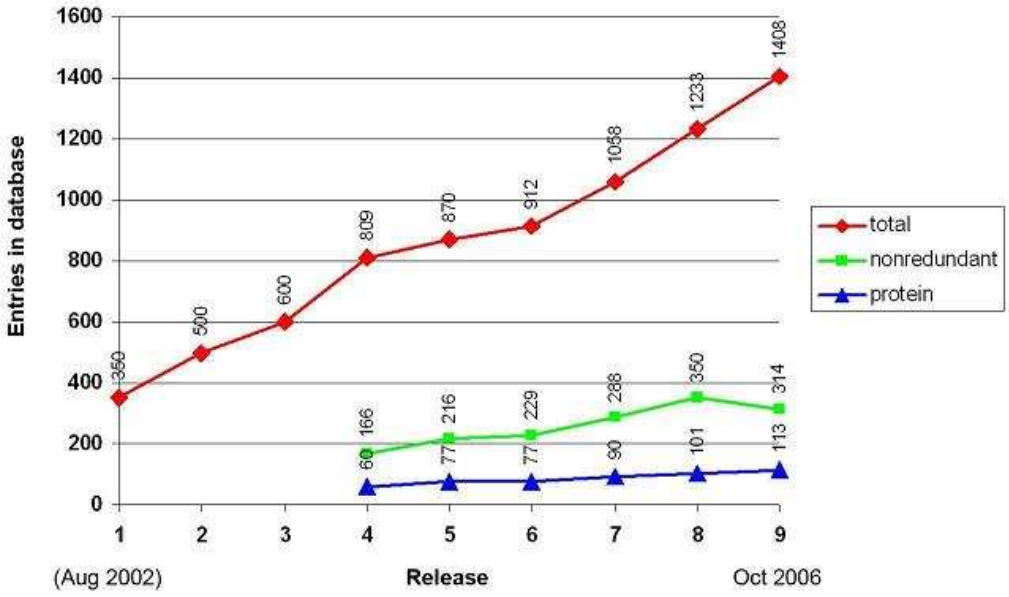


Fig. 1.3 Growth of the TREP database. The exponential growth of total sequences with decrease in "non redundant repeats" and stable growth of hypothetical protein is depicted (TREP, 2007)

I) SSR markers and linkage maps

Wheat microsatellite constortium (WMC), INRA Wheat SSR Club (Cfa and Cfd, Gpw), SSR markers from Betsville Agrciulture Research Centre (BARC) (ww.scabusa.org) and wheat microsatellite from IPK, Germany (gwm) played a criticial role in achieving the high density consensus map of wheat (Somers et al. 2004). Though 4,700 SSR markers were mapped in wheat using various mapping populations throughout the world (Gill et al. 2004), a single consensus map with a total of 1,235 microsatellite loci were mapped using ITMI and three other populations such as 'RL4452'/'AC Domain' (RD, 91 DH lines), 'Wuhan'/'Maringa' (WM, 93 DH lines), and 'Superb'/'BW278' (SB, 186 DH lines). This high density public microsatellite map of wheat covered total genome of 2,569 cM, giving an average interval distance of 2.2 cM per marker (Somers et al. 2004).

II) EST-SSR markers

Strategies were devised to sequence the gene space of the large and hexaploid wheat genome by International Genome Research on Wheat Consortium (IGRWC). In 2005, ETGI (Europrean Triticeae Genomics Initiative) was started to promote European Research Area (ERA) in the development of wheat, barley, and rye genomics. The ETGI is a platform for the coordination and representation of Triticeae genomics research at the European level and serves as a link to the ITMI. International wheat genome sequencing consortium (IWGSC) (http://www.wheatgenome.org) which was established in 2005, joined hands with INRA, France to coordinate the efforts in sequencing the complete genome of wheat.

In Japan, the National Bioresource Project (NBRP) was launched in 2002 to maintain and distribute seed stocks and DNA clones of "Wheat" as NBRP-WHEAT. It led to formation of an integrated database of wheat called KOMUGI. The outputs of these research consortia on wheat were periodically updated through web-based resources like NCBI, GenBank, TGIR wheat genome database, KOMUGI and Graingenes, for the benefits of wheat researchers around the globe. About 41,277 unigene EST are available in NCBI, 8,80,515 EST sequences in GenBank and 5,56,545 in KOMUGI wheat database (October, 2007). The EST sequences deposited in these databases were mined for SSR repeats using bioinformatic approaches and

16,000 EST SSRs were identified. Some of these EST SSR include 80 PK, 301 CFE, 48 CWEM reported in the Graingenes database (http://wheat.pw.usda.gov/).

1.7.4 MAS for wheat

In order to aid marker-assisted selection, Australian wheat breeding programme, has developed protocols for more than fifty molecular markers for resistance genes and quality traits. About 50,000 assays exclusively for more than a dozen loci, such as tolerance to high soil boron (*o1*), tolerance to late maturity amylase (*LMA*) (7BL), barley yellow dwarf virus resistance *(BYDV2*) (7DL), cereal cyst nematode resistance *Cre1* (2BL), *Cre8* (6BL), waxy or granule bound starch synthase (*Wx B1*) (4A), high molecular weight glutenin subunits (*Glu-D1*) (1DL), leaf rust resistances (*Lr46*) (1BL), (*Lr34*) (7DS), height or dwarfing genes (*Rht1*) (4BS), (*Rht2*) (4DS), (*Rht8*) (2DS), root lesion nematode resistance (*Rlnn1*) and yellow flour colour (7AL), stem rust resistances (*Sr2*) (3BS), (*Sr36*) (2B) and VPM (Ventricosa x Persicum x Marne), a source for eyespot resistance gene *Pch1* are being performed annually to implement molecular markers in wheat breeding (Varshney et al. 2006b). The different allele based markers for wheat quality traits are listed in Table 1.4.

Along with Australia, the United States Department of Agriculture (USDA) started "MAS wheat" project (http://maswheat.ucdavis.edu/) with a goal to use marker-assisted backcrossing to incorporate 27 different disease and pest resistance genes and 20 alleles with beneficial effects on breadmaking and pasta quality into 180 lines adapted to the primary US production regions. This resulted in more than 3,000 MAS backcrosses, 239 backcross derived lines and 45 released MAS-derived germplasms released in USA (Sorrells 2007).

In United Kingdom, equivalent assays for MAS have been incorporated in a number of breeding programmes, where they are used as guides to parental selection and/or in early generation selection. Prominent among these are markers for the genes *Rht-1* (responsible for the "Green Revolution" semi-dwarfism), *Pinb* (grain texture), *Pch1*, *Lr37/Yr17* (a gene complex conferring resistance to two of the most important leaf fungal pathogens) and the wheat/rye translocation 1B/1R (which is associated with high levels of yield). Currently, wheat breeding in northwest Europe is almost exclusively carried out by private companies, with some research underpinning by the

Table 1.4:- Gene - Allele based markers in MAS

Gene	Allele	Trait	References
HMW-Glutenin			
Dx, Dy	Various	Dough strength	Marchylo et al. (1989a,b), D'Ovidio et al. (1994), De Bustos et al. (2001)
Dx5	Glu-D1d	Dough strength	Smith et al. (1994), D'Ovidio and Anderson (1994), Ahmad (2000), Ma et al. (2005), Radovanovic and Cloutier (2003)
Ax, Ay	Various	Dough strength	Lafiandra et al. (1997)
Ax null	Glu-A1c	Dough strength	D'Ovidio et al. (1995), Lafiandra et al. (1997)
Ax	Various	Dough strength	De Bustos et al. (2000))
Dy10/Dy12	Glu-D1d/a	Dough strength	Ahmad (2000)
Bx7/Bx17	Glu-B1b	Dough strength	Ahmad (2000), Ma et al. (2003)
Ax2*B	-	Dough strength	Juhasz et al. (2001), D'Ovidio and Anderson (1994)
Ax1, Ax2*	Glu-A1a, b	Dough strength	Ma et al. (2003), Radovanovic and Cloutier (2003)
Bx7*,Bx7OE	B1al	Dough strength	Radovanovic and Cloutier (2003), Butow et al. (2003)
Bx	B1 al, b, i	Dough strength	Butow et al. (2004)
LMW-Glutenin			
	Glu-B1	Pasta quality	D'Ovidio (1994), D'Ovidio et al.(1994)
i-type LMWGS	Glu-A3 a–f	Dough strength and extensibility	Zhang et al. (2004)
	Glu-A3	Dough strength extensibility	
Puroindolines			
pinA	PinA-D1a,b	Grain hardness	Gautier et al. (1994)
pinB	PinB-D1a,b	Grain hardness	Giroux and Morris (1997)
pinB	PinB-D1c	Grain hardness	Limello and Morris (2000)
pinB	Various	Grain hardness	Morris (2002)
Granule bound starch synthase I			
GBSS1-4A	Wx-B1a	FSV	Briney et al. (1998), Gale et al. (2001)
GBSS1-7D	Wx-D1a	FSV	Shariflou et al. (2001)
GBSS1	Various	FSV	McLauchlan et al. (2001) and Nakamura et al. (2002)
GBSS-7D	Wx-D1e	FSV	Yanagisawa et al. (2003)
Wheat/rye translocations			
Rye chromatin	1B/1R	Dough properties	Francis et al. (1995), Koebner (1995)
u-secalin, LMWGS	Sec-2 (Rye 2RS)	Dough stickiness	de Froidmont (1998), Zhang et al. (2003), Andrews et al. (1996)
u-secalin		Dough stickiness	Skerritt et al. (1996)

Source: Gale (2005)

public sector. Though MAS has been widely employed by these companies, marker information remains confidential (Koebner and Summer, 2007).

1.7.5 Fine mapping / Map based cloning in wheat

Though many disease resistance and tolerance genes has been fine mapped, cloned and sequenced in wheat, recently Gpc-B1, a QTL locus responsible for high protein content, which functions as an early regulator of senescence has been sequenced and well characterized (Uauy et al. 2006) by map based cloning approach. Some of the resistance genes characterized by fine mapping and map based cloning revealed that they encode coiled coil (CC), nucleotide-binding site (NBS) - leucine rich repeats (LRR) and are listed below

Leaf rust resistance genes *Lr10* (1 CC- NBS- 14 LRR)	(Feuillet et al. 2003)
Lr21 (NBS- 13 LRR)	(Huang et al. 2003)
Powdery mildew resistance gene *Pm3b* (1 CC-NBS-28 LRR)	(Yahiaoui et al. 2004)
Adult plant resistance to fusarium head blight *Qfhs.ndsu on* 3BS	(Liu and Anderson, 2003)
Stem rust *Sr2*	(Kota et al. 2006)
Leaf rust *Lr34*	(Spielmeyer et al. 2003)
Major chromosome pairing loci *Ph1*	(Griffiths et al. 2006)
Wheat vernalization genes *vrn1, vrn2*	(Yan et al. 2003; 2004)
Wheat domestication gene Q	(Simons et al. 2006)
Semi-dwarf gene (*sd1*)	(Spielmeyer et al. 2002)

1.8 Advances in wheat genomics: RFLPs to RNAi

In a crop improvement program, the aim of QTL analysis is not only to genetically dissect the complex trait, but also transfer the QTLs, to develop a stable and high

performing cultivar. A breeder is, no doubt interested in enhancing the genotypic mean for any trait, but is also interested in its stability, which depends on GEI. These are considered to be important for quality traits in wheat (Peterson et al. 1992; Robert and Denis, 1996), because of which, an assessment of stability of the trait of interest would permit better genotype characterisation.

In wheat, several molecular-marker approaches have facilitated the identification of chromosomal regions associated with agronomically important traits like dwarfing and vernalization response (Korzun et al. 1997), leaf rust resistance (Feuillet et al. 1995, 1997; Naik et al. 1998), kernel hardness (Sourdille et al. 1997), cadmium uptake (Penner et al. 1995), pre-harvest sprouting tolerance (Roy et al. 1998), protein content (Prasad et al. 1999; Mesfin et al. 1999; Blanco et al. 1996), resistance to common bunt (Demeke et al. 1996), powdery mildew resistance (Qi et al. 1996), kernel traits (Campbell et al. 1999), and flour viscosity (Udall et al. 1999).

The recent advances in wheat research has enabled tremendous achievements (Sorrells 2007) such as

- Improved transformation protocols
- More and better molecular markers, large insert libraries
- New statistical methods
- Better methods of characterizing environments
- New and improved equipments for phenotyping various traits
- Virus induced gene-silencing protocols
- Microarrays for gene expression
- Improvement in DNA sequencing time frame and quality
- Introduction of transgenic commercial crops

A major challenge in the postgenome era of plant biology is to determine the functions of all the genes in a plant genome. A straightforward approach to this problem is to reduce or knock out expression of a gene to induce a mutant phenotype that is indicative of the gene function (Travella et al. 2006). In functional gene analysis and isolation of agronomically important genes, wheat is clearly lagging

behind compared to other major food crops such as Maize, Rice, and also species such as Tomato. This is mainly due to the lack of efficient tools to study gene function in polyploid species. The genomics of wheat is characterized by its polyploid nature. Hexaploid wheat has a large genome (16,000 Mb) that consists of three closely related homoeologous genomes (A, B, and D) and has a high content (80%) of repetitive DNA (Flavell et al. 1974). Each genome possesses large chromosomes with a total of 5 gigabases (Gb) of DNA (Bennett and Smith 1976). In contrast, rice harbors a small genome with a total of 450 Mb of DNA (Sasaki 2003).

Genes are found to be organized in gene islands or as single genes separated by large regions of nested repetitive elements (Feuillet and Keller, 2002). Due to the hexaploid nature of its genome, bread wheat has three (or a multiple of three) copies of most genes. It was found that many of these homoeologous genes are expressed (Mochida et al. 2003) and that there is a high degree of functional gene redundancy in hexaploid wheat. The differences in the sizes of the genomes between rice and wheat are largely due to the inclusion of repetitive sequences (Feuillet and Keller, 2002) Genome maps of cDNA markers show close synteny between the rice and wheat genomes (Sorrells et al. 2003; Peng et al. 2004; Conley et al. 2004; Linkiewicz et al. 2004; Singh et al. 2004; La Rota and Sorrells, 2004). This strongly suggests that the different characteristics of rice and wheat are due to distinct gene functions and/or gene expression patterns but not different gene constituents.

Functional genomic studies of wheat, in particular transcription analysis are important because the wheat genome is too large to be entirely sequenced in the near future. Recently, a large number of wheat expressed sequence tags (ESTs) have been accumulated from various developing tissues in the wheat life cycle (Ogihara et al. 2003; Lazo et al. 2004; Zhang et al. 2004). These ESTs were assigned into contigs corresponding to transcripts from three homoeologous genomes (Mochida et al. 2003). By grouping the three homoeologs, about 14,000 unique genes were identified. Because the total number of protein-coding genes is approximately 27,000 in Arabidopsis (Wortman et al. 2003) and less than 40,000 in rice (Bennetzen et al. 2004), it appeared that the 14,000 ESTs included approximately 40 to 50% of the total genes in wheat.

Rapid explosion of EST sequences and ability to study the expression of thousands of genes at one time using Microarray has made a dramatic improvement in

the field of functional genomics. The DNA microarrays have become indispensable for functional genomics because they can systematically investigate transcriptional profiles. A variety of DNA microarrays and chips have been developed and applied to analyse important organisms (Mockler and Ecker, 2005; Stoughton 2005). In plants, DNA microarrays and chips have been applied for studies of stress tolerance and growth. These are very powerful systems for the study of model systems such as Arabidopsis and rice because their complete genome sequences are available (The Arabidopsis Genome Initiative 2000; Sasaki et al. 2002; Yu et al. 2002) and a large number of full-length cDNAs have been collected (Seki et al. 2002a; Kikuchi et al. 2003). In addition, bioinformatics tools and public databases [e.g. The Arabidopsis Information Resource (TAIR), Rhee et al. 2003; and RicePipeline, Yazaki et al. 2004] have facilitated the analysis of the DNA microarray or chip data. Microarray analysis has allowed the investigation of gene expression related to important physiological and agronomic traits, including responses to hormones, abscisic acid and gibberellin (Seki et al. 2002b; Yazaki et al. 2003; Rabbani et al. 2003) and stress responses to high salinity (Kawasaki et al. 2001; Kreps et al. 2002; Seki et al. 2002b; Rabbani et al. 2003; Takahashi et al. 2004), drought (Kreps et al. 2002; Seki et al. 2002b; Rabbani et al. 2003; Oono et al. 2003), cold (Kreps et al. 2002; Seki et al. 2002b; Rabbani et al. 2003; Yamaguchi et al. 2004), high light (Rossel et al. 2002; Kimura et al. 2003), hyperosmolarity (Takahashi et al. 2004), oxidation (Takahashi et al. 2004) and iron deficiency (Thimm et al. 2001).

Though transcript profiling helps to understand the way genes get expressed, the confirmation requires development of mutant for a particular phenotype. Insertional mutagenesis is a useful tool for this type of study and is based on transposon/T-DNA insertions (Page and Grossniklaus, 2002). However, this approach is limited by the time required to saturate a genome by lethal knockouts, and is restricted to a few plant species. In addition, it is complicated by the problem of genetic redundancy caused by multigene families and polyploidy. In contrast to insertional mutagenesis, RNA interference (RNAi) is based on sequence-specific RNA degradation that follows the formation of double-stranded RNA (dsRNA) homologous in sequence to the targeted gene (Marx 2000; Carthew 2001; Baulcombe 2004).

RNAi allows silencing one, several, or all members of a multigene family or homoeologous gene copies in polyploids by targeting sequences that are unique or shared by several genes (Lawrence and Pikaard, 2003; Miki et al. 2005). dsRNA is detected by the host plant genome as aberrant and is cleaved by the action of Dicer-like enzymes (Hamilton and Baulcombe, 1999; Zamore et al. 2000; Djikeng et al. 2001; Tang et al. 2003) into two distinct classes of small interfering RNA (siRNA): long and short siRNAs (Hamilton et al. 2002; Tang et al. 2003). These two classes of small RNAs were proposed to have distinct RNA silencing functions - approximately 21-mers to direct post transcriptional signaling via mRNA degradation and the approximately 24-mers to trigger systemic silencing and the methylation of homologous DNA (Hamilton et al. 2002). Biochemical experiments in wheat germ extracts provided clear evidence that each class of siRNAs is generated by distinct Dicer-like enzymes (Tang et al. 2003). Through sequence complementarity, siRNA in association with an RNA-induced silencing complex directs the cleavage of endogenous RNA transcripts. siRNAs are also responsible for amplifying the silencing signal by priming endogenous RNA, which can be converted to dsRNA by the action of RNA-directed RNA polymerase (RdRP) encoded in the plant genome (Lipardi et al. 2001; Sijen et al. 2001). Tang et al. (2003) showed that wheat germ extracts contained an RdRP. Thus, a few trigger dsRNA molecules suffice to inactivate a continuously transcribed target mRNA for long period of time.

Apart from functional genomics, the proteomics of wheat requires detailed studies. The functionality and visco-elastic property of wheat dough is due to glutenin and gliadin and they form more than 85% of the extractable endosperm protein (Hurkman and Tanaka, 2004). Albumins and globulins of wheat endosperm have received relatively little attention because of their low abundance and perceived secondary role in flour quality (Vensel 2005). Development of suitable tools to detect these proteins will also help in better understanding of dough function.

1.9 Wonder wheat: A future path in wheat molecular research

Increasing the yield potential to feed the growing populations, addressing the malnutrition problem in rural areas and a nationwide effort to develop elite cultivars with pyramided desired genes could be the three priority areas of wheat research. The two best examples for nutrition enhancement in cereals are Quality Protein Maize (QPM) and Golden rice. The QPM was developed by transferring the opaque 2 zein

mutation into normal maize lines through backcross, it resulted in nearly double the lysine and tryptophan and a generally more balanced aminoacid content that greatly enhanced its nutritive value (Dreher et al. 2000). The Golden rice was developed by genetically engineering the rice, by construction of artificial biochemical pathway with two genes, with one from the *Erwinia ureidovora* and other from daffodils (Paine et al. 2005). Other examples of nutritionally enhanced crops in the pipeline include high beta carotene in oil seed rape, improving nutritive value of Andean potatoes by manipulating potato's own genes to block natural but bitter compounds called glycoalkoloids and ferritin rich lettuce. With the successful examples of QPM and Golden Rice, producing "wonder wheat" having enhanced nutritive value with balanced amino acids, adequate antioxidants and essential fatty acids could be one of the most important future paths in wheat molecular research.

1.10 Genesis of my thesis

In recent years, India has imported considerable amount of wheat to meet growing market demand for 'quality wheat' due to changed life-style of majority of Indian population. However, Indian wheat has been selected and improved for chapatti/roti making characters and is not highly suitable for making products such as bread, pasta, noodles and cookies. To meet the growing market for high BMQ wheat, it is essential to enhance the quality of Indian wheat. Wheat varieties producing good quality bread with smooth crust, uniform grain size and high loaf volume will be preferred not only within the country but also in the export market. In view, of improving the quality of Indian wheat, research work on tagging of wheat quality traits was initiated at Plant Molecular Biology group of National Chemical Laboratory, in collaboration with various other wheat breeding research organizations. Since it was my ambition to carry out research in crop biotechnology, which will be eventually useful to Indian farmers, I decided to join this programme for my Ph.D. work. I focused my work on mapping for bread making quality traits and made an attempt to identify molecular markers that are associated with them. The breadmaking quality is a complex trait and yield related traits are also important in the perspective of wheat growers. I channeled my research in such a way that bread-making quality is studied in every stage of bread making such as wheat grain, flour, dough and bread with the following specific objectives

1) Construction of wheat framework map using HI977 x HD2329 RIL population.

2) QTL analyses of yield traits Tgw, Tw segregating in the HI977xHD2329 population.
3) QTL analysis of BMQ related traits viz; Sv, Gpc and rheological parameters using mixograph for the population.
4) QTL analysis of Lv using HI977 x HD2329 cross.

Organization of my thesis

I have organized my thesis in the following order:

Chapter 1: Introduction - Wheat for bread making

Chapter 2: Materials and methods

Chapter 3: Results

Chapter 4: Discussion

Chapter 5: Thesis summary and future directions

Bibliography

Bio-data

Chapter 2

Materials and methods

2.1 Chemicals, enzymes and oligonucleotides

The *Taq*-DNA polymerase was procured from Bangalore Genei (India) and used with the 10 X buffer supplied, unless otherwise stated. Oligonucleotides were custom synthesized from Sigma Genosys (India) and Inter Simple Sequence Repeat (ISSR) primers were from University of British Columbia (Canada). Laboratory reagents were obtained from the following companies: Sigma Genosys (India), Qualigens (India), Bangalore Genei (India), GE Health care (USA), Promega (USA) and Cambrex Bioproducts (USA).

2.2 Plant material

For the molecular dissection of BMQ, 50 wheat genotypes from various wheat breeding centers in India were studied for various BMQ parameters such as Gpc, Sv, Lv, Tgw, Tw etc, as well as for their heritability at Directorate of Wheat Research (DWR), Karnal, India. Two cultivars HI977 and HD2329 were identified as stable and contrasting parents based on HMW glutenin, Sv, Lv and Gpc, which also showed high heritability (>0.7). The cultivar HI977 had good BMQ with Glu-A1 (2*), Glu-B1 (17 + 18) and Glu-D1 (5 +10) while HD2329 had poor BMQ with Glu-A1 (2*), Glu-B1 (7 + 9) and Glu-D1 (2 + 12). The pedigree for HI977 is [Gallo / AUST II 61.157 /2/ Ciano 67 / NO66 /3/ Yaqui50-Enano / 3*Kalyansona] and for HD2329 is [HD 2252 / UP 262].

Table 2.1:- BMQ and yield traits of the parents

Traits	HI977	HD2329
Protein (%)	13.5	11.2
Moisture (%)	11.3	11.1
Sedimentation vol. (cc)	66.0	42.0
Test wt. (Kg/L)	77.1	79.1
1000 grain wt. (g)	41.0	39.0
Glu-1D	$X_5 + Y_{10}$	$X_2 + Y_{12}$
Glu-1B	$X_{17} + Y_{18}$	$X_7 + Y_9$
Loaf vol. (cc)	545	484

The RIL population comprising 105 lines was developed at DWR, Karnal, India by single seed descent from F_2 generation onwards, bulked plant-wise at F_8 generation. The parents HI977 and HD2329 along with the RILs were grown at three different

agroclimatic regions (Karnal- North Western plain zone, Kota- Central zone and Pune- Peninsular zone) for two consecutive years (2003-04, 2004-05), in an augmented block design. The Fig. 2.1 represents the HI977 x HD2329 population grown at Hol, farm, Pune in the year 2004-05. 2.1).

The RILs were not replicated within a location (Hessler et al. 2002) and the design comprised an Augmented Randomized Complete Block (RCB) design having 8 blocks with 20 lines and 5 replicating checks, in each block. The lines were grown in 2 rows with 2 m x 0.23 m spacing in between the lines. The fertilizer was applied in a ratio of 120Kg N_2: 60Kg P: 40Kg K_2O in field experiments. The phenotypic data was collected in the successive years from all the RILs in a row to avoid biased selection. The RIL population was used to identify QTL for various yield and BMQ traits at various stages of bread making (Fig. 2.2) using different protocols as detailed below.

2.3 DNA extraction

2.3.1 CTAB DNA extraction method

Total genomic DNA of the parents as well as RILs of cross the HI977 x HD2329 were extracted from 15 day old seedlings grown in the glass house by modified Cetyltrimethylammonium bromide (CTAB) method of Rogers and Bendich (1988). The young leaf tissue (10 g) was ground to a fine powder in liquid nitrogen using mortar and pestle. To this, 2X CTAB buffer was added and the contents were mixed to form an emulsion, which was incubated at 60 °C for 20 min. After incubation, the emulsion was allowed to cool down to room temperature and then was equally distributed in SS34 centrifuge tubes. In each tube, an equal volume of Chloroform:IAA (24:1) mixture was added; the tubes were capped and gently swirled to mix the contents. The tubes were centrifuged at 10,000 rpm for 10 min at room temperature in a Sorvall RC-5B centrifuge (Du Pont, USA). The aqueous layer formed after centrifugation was recovered and distributed in fresh SS34 tubes. To this, an equal volume of CTAB precipitation buffer was added; the contents were thoroughly mixed and kept at room temperature for 15 min. Subsequently, the pellet of the precipitated DNA obtained by centrifuging the tubes at 10,000 rpm for 10 min at 15 °C was dried and dissolved in high salt TE buffer. The dissolved DNA was reprecipitated by adding two volumes of chilled ethanol and the precipitated DNA was either spooled out or

Fig. 2.1: HI977 x HD2329 population grown at HoI farm, Pune, India in the year 2004-05

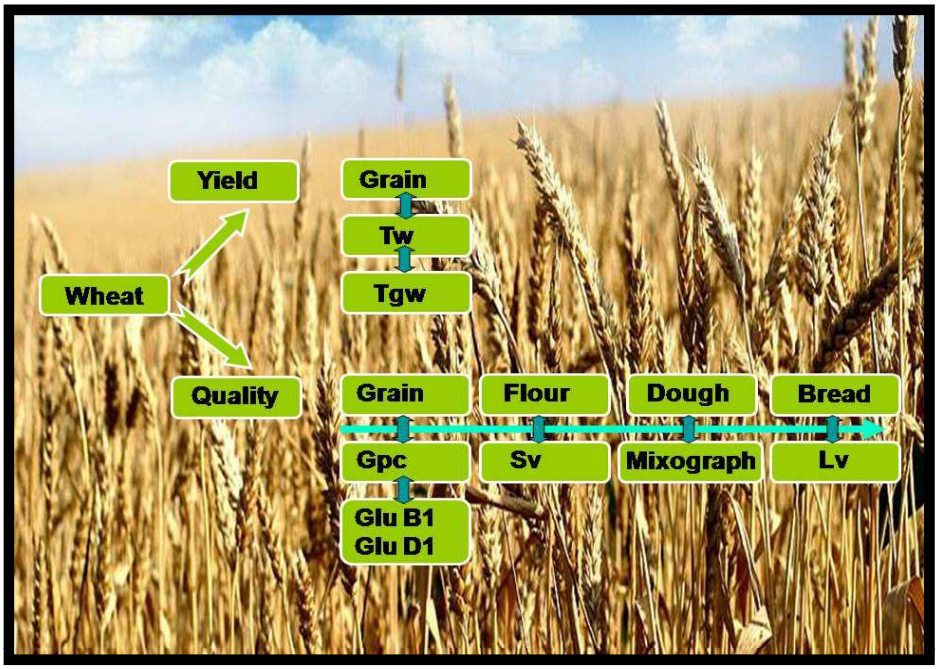


Fig. 2.2: The BMQ and yield traits recorded at different stages of wheat viz;–grain, flour, dough and bread analysed in the present study

pelleted by centrifugation at 10,000 rpm for 10 min at 4 °C. The DNA pellet was washed with 70% ethanol, dried and dissolved in an appropriate volume of TE buffer. To remove RNA from the samples, 100 mg/ml RNase-A (DNase free) was added and incubated at 37 °C for 1h. Subsequently, an equal volume of Chloroform:IAA (24:1) mixture was added, the contents were mixed and centrifuged at 10,000 rpm for 10 min at 15°C. Following centrifugation, the aqueous layer containing DNA was recovered and stored at –20 °C until further use.

2.3.2 Rapid DNA extraction

About 300 mg of two weeks old, fresh leaf tissue of wheat plants grown in the glasshouse was used for rapid DNA extraction as described by Stein et al. (2001). The plants were grown in a 24-well format tray and identified by labels. The leaves were harvested and immediately transferred into high density polythene bag by cutting them into smaller pieces using scissors. These bags were made by using household bag sealing machine with each compartment of size 10 cm x 5 cm spaces. After inserting leaves into the bags, 1.2 ml of cetyltrimethylammonium bromide (CTAB) extraction buffer was added and air was carefully removed from the bags before they were double sealed. The bags were placed on a piece of cardboard covered with a layer of PE (0.1 mm LD-PE transparent) attached to the bench and a second layer of PE foil was placed over the bags. The leaf material was completely homogenized using a hand homogenizer or pestle, followed by incubation for 1 h at 65 °C. The bags were separated from each other and the solution was transferred into a 2.2 ml microcentrifuge tube after folding each bag to a cone tip, which was cut and inserted into the tube. The solution was extracted with 800 µl cold choloroform / isoamylalcohol (24 : 1) by vertically rotating in hand for 15 min followed by centrifugation at 10,000 rpm for 15 min. The supernatants (800 µl) pipetted into 2.2 ml polypropylene tube (PP) and DNA was precipitated with chilled 0.7 volume of isopropanol (560 µl), added to each sample. The DNA was pelleted by centrifugation (20 min at 1850 rpm, at RT) and the supernatant was removed and the tubes were inverted upside down on a stack of paper towels. The DNA pellets were washed with 0.7 ml of DNA washing solution 1, followed by incubation for 5 min at RT before centrifugation (10 min at 1850 rpm). The supernatant was removed as described earlier and the washing was repeated with DNA washing solution 2. The DNA pellets were subsequently air dried for at least 10 min and finally resuspended in TE buffer.

To remove RNA from the samples, RNase A (DNase free, 10 mg/ml stock solution) equivalent to 0.3% of the total volume of sample was added to the dissolved DNA and incubated at 37 ºC for 1 h. Then it was stored at –20 ºC until further use.

2.4 DNA quantification

Extracted genomic DNA (1µl) was loaded on a 0.8% agarose gel in 0.5X TAE buffer containing ethidium bromide. The DNA concentration of the sample was estimated by visual comparison of the band with known dilutions of λ bacteriophage DNA(50 ng, 100 ng, 200 ng, 500 ng etc). Purity and concentration of the extracted DNA for each sample was also checked spectrophotometrically at 230nm, 260nm, 280nm and 320nm. 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. The A260/A230 ratio denoted the contamination of DNA with organic compounds, the DNA quality was best if the ratio was greater than 1.5. The absorption at A320 nm, was recorded to ensure that the DNA solution was without any turbidity.

2.5 PCR amplification using various DNA primers

2.5.1 ISSR analysis

A set of one hundred ISSR primers (UBC 801-900) were used for the analysis using both the parents. The primers, which gave clear and reproducible polymorphic patterns, were used for further analysis.

A 25 µl reaction consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 2.0 % formamide, 0.4 mM spermidine, 0.2 mM of primer, 1 unit of *Taq* DNA polymerase and 20 ng of genomic DNA. All ISSR-PCR amplifications were performed in PTC-200 thermocycler (MJ Research, USA). The thermal cycling protocol as described by Nagaoka and Ogihara (1997) was followed.

Initial denaturation:	94 ^{o}C for 5 min
45 cycles:	94 ^{o}C for 30 s
	50 ^{o}C / 60 ^{o}C for 45 s
	72 ^{o}C for 2 min
1 cycle:	72 ^{o}C for 5 min

2.5.2 SSR analysis

In all, 914 SSR primer pairs were used for the parental analysis. Out of these, 739 *wms* primer pairs (later designated as "*gwm*") were selected from all chromosomal regions of wheat, from the previously published microsatellite map of wheat by Roder et al. (1998), along with 17 *psp* primer pairs kindly provided by Dr. M. D. Gale, John Innes Research Center, UK. In addition to the above primers 27 *cfa*, 30 *cfd*, 56 *barc*, *45 wmc* were also used for the analysis. The PCR amplifications of *gwm* primers selected from wheat microsatellite linkage map were performed in 25 µl reaction volume following the protocol by Roder et al. (1998) with minor modifications in annealing temperature and reaction conditions in Perkin-Elmer (Norwalk, CT, USA) thermocyclers. The reaction mixture contained 250 nm of each primer (left and right), 0.2 mM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase and 100 ng of template DNA as advised by Roder et al. (1998) involving

Initial denaturation:	92 ^{o}C for 3 min
45 cycles:	92 ^{o}C for 1 min
	50 ^{o}C/ 55 ^{o}C /60 ^{o}C for 1min
	72 ^{o}C for 2 min
1 cycle:	72 ^{o}C for 10 min

The PCR amplifications using "*psp*" primers were done in 30µl reaction volume. Each reaction contained 100 ng of DNA, 10X reaction buffer, 2mM dNTPs, 2mM of primers and 1.5 units of *Taq* DNA polymerase and was performed according to the protocol described by Stephenson et al. (1998):

Initial denaturation:	94 ^{o}C for 5 min
30 cycles:	94 ^{o}C for 1 min
	-0.5 ^{o}C/s (RAMP)
	55 ^{o}C/ 58 ^{o}C /61 ^{o}C /63 ^{o}C or 65 ^{o}C
	+ 0.5 ^{o}C/s (RAMP)
	72 ^{o}C for 1 min
1 cycle:	72 ^{o}C for 5 min

The 25 µl PCR reaction mixture for *cfa, cfd, wmc, barc* primers contained 100 nM of each primer, 200 mM of each dNTP, 0.4 mM spermidine, 2% formamide, 1.5 mM $MgCl_2$, 0.8 units of *Taq* DNA polymerase and 25 ng of template DNA. The PCR thermal cycling programme was as follows.

Initial denaturation:	95 ^{o}C for 5 min
35 cycles:	94 ^{o}C for 1 min
	50 ^{o}C/ 55 ^{o}C/ 60 ^{o}C or 63 ^{o}C for 1 min (Based on GC content of individual microsatellite)
	72 ^{o}C for 2 min
1 cycle:	72 ^{o}C for 5 min

2.6 Resolution of PCR products using various methods

2.6.1 Agarose gel electrophoresis

The amplified products were resolved on 2% agarose gels in 0.5 X TAE buffers, visualized and further gel documented. About 4 g of agarose was dissolved in 0.5X TAE buffer by slow, circular motion. The mixture was boiled in microwave oven for 3 min and proper care was taken to avoid over boiling/ frothing of agarose. The agarose solution was cooled to 40-50 ^{o}C and poured on gel casting trays fitted with 24 well combs. About 4 µl of Bromophenol blue loading dye was added to 25 µl amplified PCR product and was loaded on the gel. The gel electrophoresis was carried at 100V, 50 mA for 45 min to 90 min and stained with 200 ml ethidium bromide staining solution with 5 µl of ethidium bromide stock (10 mg/ ml) for 10-15 min, with slow circular motion. Following staining, the gels were destained with plain ultrapure water gently for 5 min. The stained gels were visualized on gel documentation system (Amersham Pharmacia Biotech, USA) and digital images were stored in tiff format.

All SSR- amplified products except of *gwm* primers were resolved initially on 2.5% metaphor-agarose gels in 0.5 X TBE buffer, visualized and gel documented.

2.6.2 Polyacrylamide gel electrophoresis

The SSR primer products unable to resolve on Metaphor gels were resolved on 0.4 mm polyacrylamide using the sequencing gel unit from Life Technologies, USA. The Bind plate-larger glass plate (33.3 x 41.9 cm) was treated with 4 ml of γ-methacryloxypropyl-trimethoxysilane (PlusOne Bind Silane), in 1 ml of acidic ethanol (0.5% glacial acetic acid in 95% ethanol) to covalently attach the gel onto the glass plate. The plates were dried for 5 min and the excess silane was removed using a paper tissue moistened with 95% ethanol. The Repel plate- smaller glass plate (33.3 x 39.4 cm) was treated with 1 ml of a 2% solution of dimethyldichlorosilane in octamethyl cyclo-octasilane (PlusOne Repel-Silane ES, Amersham Pharmacia Biotech) for complete release of gel from this plate. The plates were dried for 5 min and excess silane was removed with a tissue moistened in distilled water. The gels solution was prepared by mixing 50 ml of the urea:acrylamide solution in TBE with 200 µl of freshly prepared 10% ammonium persulfate and 84 µL of TEMED. The gel solution poured into the assembled gel plates (0.4 mm thickness) using a pointed beaker. The gel was allowed to polymerize for 60 min.

Pre run: The sequencing gel was run at 60 W (42 mA; 1500 V) for 60 min or until the gel temperature reaches 55 °C in 1 X TBE. About 12.5 µL of denaturing buffer added to the 25 µL amplification reaction. The samples were denatured for 3 min at 94 °C in the thermocycler and immediately placed on ice. About 8 µl was loaded to the gel as quickly as possible and the electrophoresis was performed at 60 W for 80/100 min at 50-55 °C.

Silver staining: The gels bound to binding plates were removed from repel plates and fixed with fixer solution for 20 min. in gentle circular motion. The gel plates were drained and silver stained with staining solution for 30 min. After staining, the plates were drained free of staining solution and developed using developer solution for 5-10 min. After the appearance of sharp and dark bands, the gel was treated with stop solution to end the staining process. The gel was completely washed with ultrapure water and further dried for gel documentation.

2.6.3 Automated Laser Flourescence gel separation

All *wms* primers were resolved on Automated Laser Fluorescence (ALF) sequencer (Amersham Pharmacia Biotech, USA) using short gel cassettes (Fig. 2.3). Denaturing gels (0.35 mm thick) with 6% polyacrylamide were prepared using SequaGel XR

(Biozym, Germany). The gels were run in 1X TBE buffer [0.09 M Tris-borate (pH 8.3) and 2 mM EDTA] with 600 V, 50 mA, and 50 W with 2 mW laser power and a sampling interval of 0.84 s. The gels were reused four to five times. In each lane, fragments with known sizes were included as standards.

Fig. 2.3: The Automated Laser Fluorescence (ALF) Express

The fragment sizes were calculated using the fragment analyzer program 'Fragment Manager ver 1.2' (Pharmacia) by comparison with the internal size standards, loaded in the gels.

2.7 Extraction of HMW and LMW glutenins

HMW and LMW glutenins were extracted according to Singh et al. (1991). A single grain of wheat was crushed gently in one eppendorf tube (~ 20 mg flour). After crushing, 50% n-propanol was added. The mixture was incubated at 65 ºC for 30 min with vortexing twice intermittently and then spin at 10,000 rpm at RT for 1 min. The supernatant was discarded and the same step was repeated twice. After the final spin, supernatant, which contained gliadins, was discarded and the precipitate was subjected to drying. Glutenin extraction buffer was then added to the residue containing 1% freshly prepared dithiothreitol. The mixture was vortexed thoroughly and kept at 65 ºC for 30 min and then spun at 10,000 rpm for 5 min. Glutenin extraction buffer (pH - 8.0)

containing 1.4 % freshly mixed 4-vinylpyridine was added to each tube and incubated for 15 min for protein alkylation. The sample was then centrifuged at 10,000 rpm for 2 min. An aliquot of the supernatant was transferred to a new tube containing the dilution sample buffer. After brief vortexing, the tubes were kept at 65 ºC for 15 min and then centrifuged at 10,000 rpm for 2 min, followed by storing at –20 ºC.

2.7.1 Polyacrylamide gel electrophoresis (PAGE) of glutenin proteins

A 1.5 mm thick slab gel was casted by preparing 10% separating gel acrylamide (0.8% cross linker) and 3% stacking gel acrylamide as follows:

Separating gel- total volume	60.0 ml
2X separating gel buffer pH 8.9	30.0 ml
30% acrylamide	20.0 ml
Distilled water	10.0 ml
TEMED	200 µl
10% APS	120 µl

Stacking gel- total volume	9.0 ml
2X Stacking gel buffer pH 6.8	4.5 ml
Stock acrylamide (30%)	1.0 ml
Distilled water	3.5 ml
TEMED	20 µl
10% APS	60 µl

Preparation of discontinuous polyacrylamide gel: The glass plates were cleaned thoroughly and were sandwiched together to form a gel of 1 mm thickness. The separating gel solution was prepared by combining all reagents except APS and TEMED. The solution was degassed for 15 min under vacuum. APS and TEMED were added to the degassed separating gel solution and poured immediately into the gel assembly upto the mark. The gel solution was overlaid with distilled water to get a clear line gel formation. The gel was allowed to polymerize for about 45 min and then the overlayed distilled water was completely rinsed off. The stacking gel solution was prepared by combining all the reagents except APS and TEMED. The solution was

degassed for 15 min under vacuum. A suitable comb was then placed in the gel sandwich and tilted slightly to prevent air from being trapped. APS and TEMED were added to the stacking gel solution and poured into the gel sandwich. The comb was properly aligned and the gel was allowed to polymerize for about 30 min. The comb was removed gently and the wells were rinsed completely with distilled water and finally with running buffer.

The sample was electrophoresed at 80 mA for about 5 h (at least 45 min after the blue dye ran out). After electrophoresis, the gel was removed from the glass plate and placed in freshly prepared Coomassie Brilliant Blue R.250 staining solution for overnight with gentle agitation (Lawrence and Sheperd, 1980). The staining solution was drained out and the gel was rinsed with water three or four times for destaining. After destaining, the gel was scanned using HP6000 scanjet scanner and saved as a digital image.

2.8 Evaluation of phenotypic traits

The phenotypic data was collected in successive years from all the bulked RILs in a row to avoid biased selection. The grains harvested from each RIL were separately processed by hand threshing and removed from all contaminating materials including trash, chaff, broken and shriveled grains and sand grits. The data on Sv, Gpc, Tw and Tgw were recorded on the bulk yield of the lines.

2.8.1 Test weight (Tw)

Test weight is obtained by weighing one liter of grain using hectoliter weight-measuring funnel of the SINARFP Auto 6080 moisture analyzer (Tecator, Sweden) and expressed in kg per hectoliter.

2.8.2 Thousand grain weight (Tgw)

'Thousand grain weight' is the measure of weight of 1000 grains expressed in grams. About 1000 grains were measured using electronic counter and weighed in electronic weighing balance (Misra and Gupta, 1995).

2.8.3 Grain protein content (Gpc)

Gpc was determined using NIR with an Infrared analyzer 300 (Technicon, NY, USA) (Fig. 2.4) previously calibrated with Kjeldahl protein (N x 5.7) as described by "American Association of Cereal Chemists" (AACC 2000).

Fig. 2.4: The samples filled in the sample cups of Infrared analyzer 300 (Technicon, NY, USA)

The moisture content required for grain conditioning was determined simultaneously and protein content was calculated for standard 14% moisture content (Misra and Gupta, 1995).

2.8.4 Sedimentation volume (Sv)

Sedimentation volume was determined by sedimentation test, based on the fact that gluten protein absorbs water and swells considerably when treated with lactic acid in the presence of SDS (Misra and Gupta, 1995). Sv was measured using SDS sedimentation test to identify the gluten strength following the procedure of Dick and Donnelly (1980).

2.8.5 Mixograph

Mixing tests were conducted using a computerized 10 g Mixograph (National Mfg. Co., Lincoln, Nebraska, U.S.A.) (Fig. 2.5). The wheat grains were tempered to a moisture content of 14% milled, the whole grain flour was collected and kept overnight. The 10 gm of whole grain flour was added with 6.2 ml of distilled water, to determine the mixing characteristics of the flour at 62% absorption according to Pon et al. (1989) and mixed for 8 min with a mixing head speed of 88 rpm. The data point was recorded at 10 points per second (pps). The complete population (with 5 repeating checks), was analysed without repeat except for every 11^{th} sample to estimate the repeatability. The

mixogram data were analysed with the software Mixsmart v. 3.40 and 44 parameters were recorded, which those 9 parameters were considered for QTL analysis.

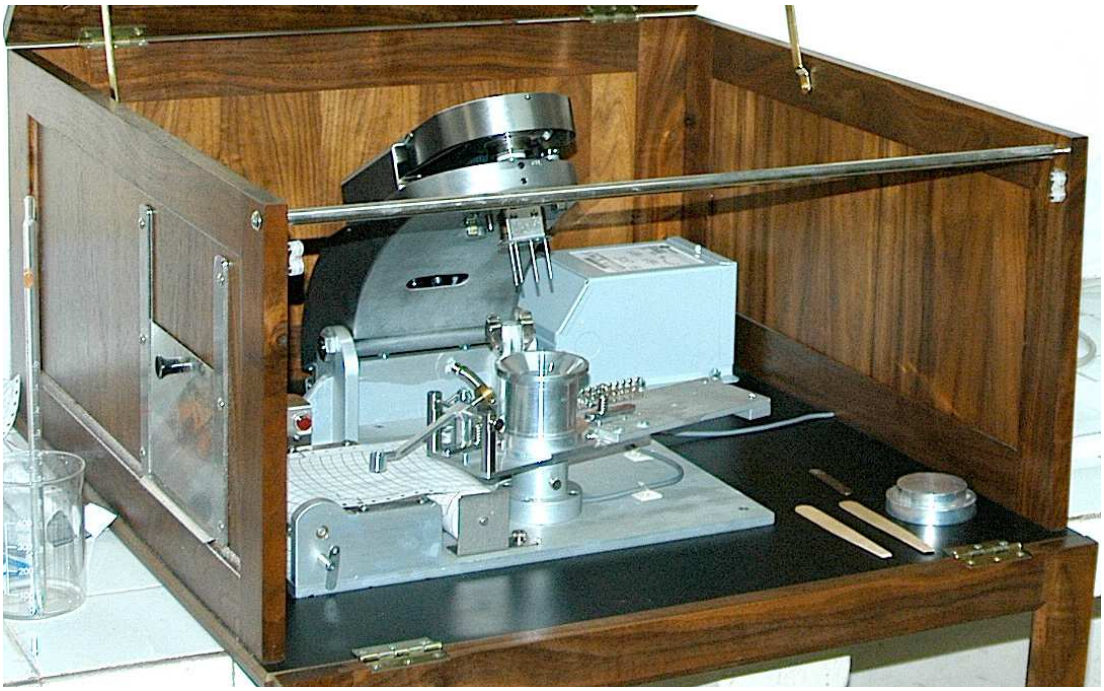


Fig. 2.5: The stationary mixing bowl of 10gm Mixograph Instrument (National Mfg. Co., Lincoln, Nebraska, U.S.A.)

The results from the Mixsmart® software were investigated by constructing a curve with two envelopes and one midline. Strong correlation between midline and top envelope parameters were observed, however best repeatability was observed with midline parameters (Martinant et al. 1998). Among the 44 mixograph traits, following 9 traits representing overall picture of the dough development, such as optimum dough development, break down and consistency were selected.

1) **Midline peak time** (Mpt): The time in minutes required for optimum dough development. The maximum resistance offered by the dough to mixing and the time taken to reach this point is influenced by the protein quality.
2) **Midline right value** (Mrv): The height of the peak at 1 min after MPT. At this point the dough is subjected to over mixing after optimum development of the dough.

   Midline curve tail value (Mtv)- It indicates the total breakdown or loss of strength during mixing (Martinant et al. 1998).
3) **Band width**: The width of the mixogram curve and the angle of descent indicate the tolerance of the dough to over mixing. Well defined curves with

wide bands and low angles of descent indicate strong tolerance to over mixing and superior protein quality.

Midline right width (Mrw)- Width of the peak at 1 min after Mpt. It exhibits dough tolerance during over mixing.

Midline curve tail width (Mtw)- Width of the peak at the end of the mixing period (8 Min.). It denotes the consistency of the dough at the end of the mixing (Ohm et al. 1998).

4) **Area/Integral**: The area under the mixogram curve from the start to any given point, denotes the energy used during the mixing process. It increases with grain hardness and the amount of glutenin present.

Midline right integral (Mri)**-** It is a total area under the mixogram midline curve from the starting point to one min after the peak time (Nelson et al. 2006*).*

Midline curve tail area (Mti)- It is an area under the curve till the end of the mixing process (Breseghello et al. 2005).

Envelope peak integral (Epi)- Total area under the mixogram envelope curve calculated from the starting point to envelope peak time.

5) **Weakening slope** (Ws): It indicates the rate of breakdown while mixing. It calculated as the difference of curve height at peak time and curve height at tail (Mtv) (Martinant et al. 1998**)**.

2.8.6 Loaf volume

The grain samples were tempered (AACC method 26-10, American Association of Cereal Chemists, 2000) and milled using a Brabender Senior Quadramat Mill (AACC method 26-21A) with a ~70% extraction rate. The bread making performance of the flour was determined using the straight dough (AACC method No. 10-10 B), with the remixing procedure of Irvine and McMullan (1960) with a minor modification. The bread formula for each loaf included 100 g flour (14% moisture), 60 ml water, 5 g sugar, 2 ml clarified butter, 2.0 g salt and 2.5 g yeast.

Fig. 2.6: The milling unit (mill), mixer, bread moulding pan, humidity chamber, baking oven and rapeseed displacement unit (RD unit)

The following baking schedule (Fig. 2.6) was adopted: mixing all ingredients except clarified butter for 1 min, fermentation for 1 h 40 min at 30 ± 1 ºC and 88% r.h., kneaded again with clarified butter for 40 s, degassing, proofing for 50 min. at 32 ± 1 ºC and 90% r.h., and baking for 12 min. at 220 ± 1 ºC using rotatory oven. Loaves were then placed on a wire grid for about 2 h and Lv was measured by the rapeseed displacement method (Dhingra and Jood, 2004).

2.9 Construction of frame work map

2.9.1 Scoring of marker data

The genotype of each sample in case of ISSR analysis was scored as presence or absence of amplified DNA locus. In case of SSR primers, scoring was done based on the size variation of the alleles in the parents. The RILs with a genotype of HI977 were given score 'A' and that of HD2329 were given 'B'. In case of HMW glutenin data, the Glu-D1 and Glu-B1 were scored separately, and given scores 'A' and 'B' depending upon the parental genotypes. Segregation ratio of each marker in the RIL population was tested for goodness of fit to 1:1 using Chi-square test.

2.9.2 Linkage group construction

The Linkage groups were constructed using the MAPMAKER software (Lander et al. 1987) and protocols for analysis were followed as described by Nelson (1995a). This software calculates the multipoint map distance estimates for a recombinant-inbred population and converts to estimates of gametic recombination *R* by inverse application of the mapping function (Kosambi 1944). These estimates are transformed via the function $r = 2R/(1 + 2R)$ (Haldane and Waddington, 1931) to estimates of the expected recombination r after selfing to homozygosity.

The recombination value of 0.4 and a LOD value of 3.0 were used as threshold level for the MAPMAKER program. If the recombination value of any two loci were more than 0.4, then they were declared as separate linkage groups. The choice of a stringent LOD threshold of 3.0 for ordering of markers suggests comparison with other genome maps (Nelson et al. 1995a).

The linkage groups were constructed by comparing the order of the markers in published maps and other resources [Roder et al. 1998; Roder (unpublished); Somers et al. 2004]. The linkage groups were built using a few unskewed markers and added further using TRY command in MAPMAKER. The stability of each group was tested using RIPPLE command and further fine tuned. The linkage maps and distance between loci were collected as MAPMAKER output and further utilized as input file for QTL mapping. The genetic map was finally drawn using the computer program Mapchart Ver. 2.1 (Voorips 2002).

2.10 Statistical analysis, QTL mapping and QTL clusters

2.10.1 The analysis of variance (ANOVA)

ANOVA for phenotypic data was performed using IRRISTAT ver. 5.0 (IRRI, Philippines) (Fig. 2.7). As the plant material was grown in Augmented Randomised Balanced Design, the single site module for balanced data was used to analyze the data for each trait. The output consisted of adjusted values for each treatment, based on the variation in checks, grown in each block. The adjusted means were calculated for each treatment by interpolation of block effects. Further Standard error for comparing any two treatments in any block was used as a standard for comparing the parents.

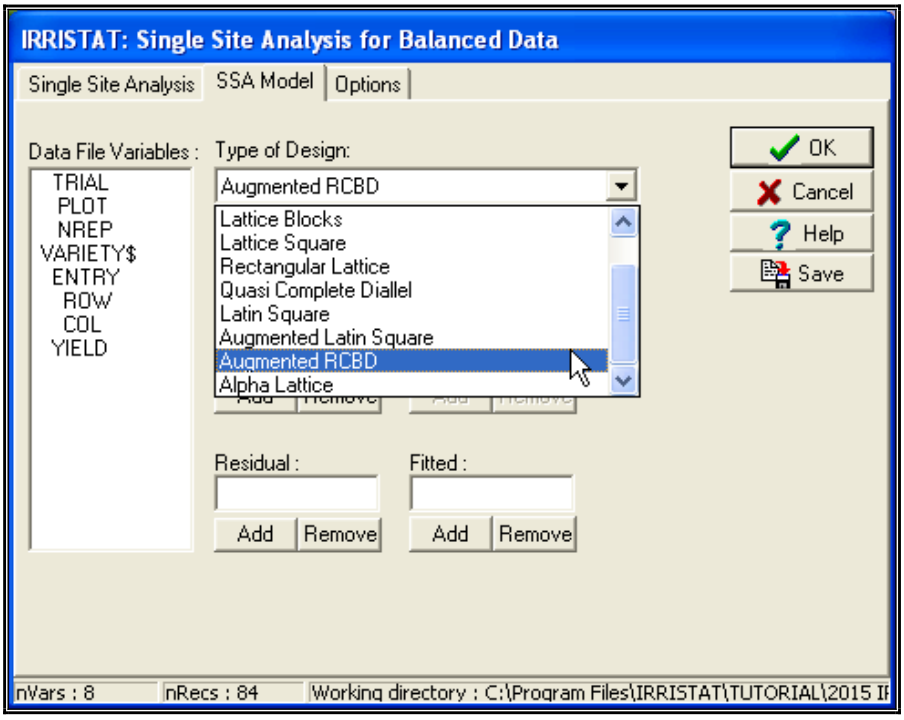


Fig. 2.7: The single site module of IRRISTAT software ver. 5.0

The GxE interaction (GEI) of RILs with the environments was deciphered by using AMMI (Additive Main effects and Multiplicative Interaction) model with IRRISTAT (IRRI, 2000) software through "Cross site analysis module". Two year's data at three sites was treated as six environments in the analysis. The sum of squares was first partitioned into genotype, environment, and GEI, then, the sum of squares for GEI term was further partitioned by principal components analysis using the AMMI model (Crossa et al.1990; Gauch 1992) using the formula

$$Yij = \mu + gi + ej + \sum_{k=1}^{n} \lambda_k \alpha_{jk} \gamma_{ik} + \mathrm{R}ij$$

where Yij is the value of the i^{th} genotype in the j^{th} environment, μ is the grand mean, gi is the mean of the i^{th} genotype minus grand mean, ej is the mean of the j^{th} environment minus the grand mean, λ_k is the singular value for the principal component analysis axis k, α_{ik} and γ_{jk} are the principal component scores for principal component analysis axis k of the i^{th} genotype and j^{th} environment, respectively, and Rij is the residual.

2.10.2 QTL mapping

The treatments were adjusted for the variation arose due to block effect and utilized for QTL mapping. The Win QTL cartographer ver. 2.5 (Basten et al. 1994; 2000) was used

for Composite interval mapping (Fig. 2.8). The threshold LOD scores for detection of QTLs were calculated based on 1000 permutations (Doerge and Churchill, 1996).

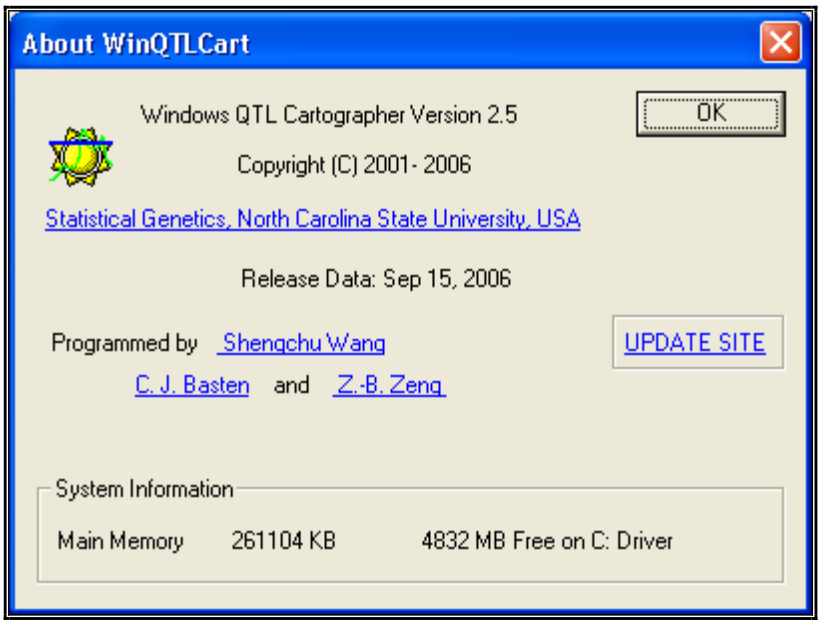


Fig. 2.8: The Windows QTL cartographer for CIM QTL mapping

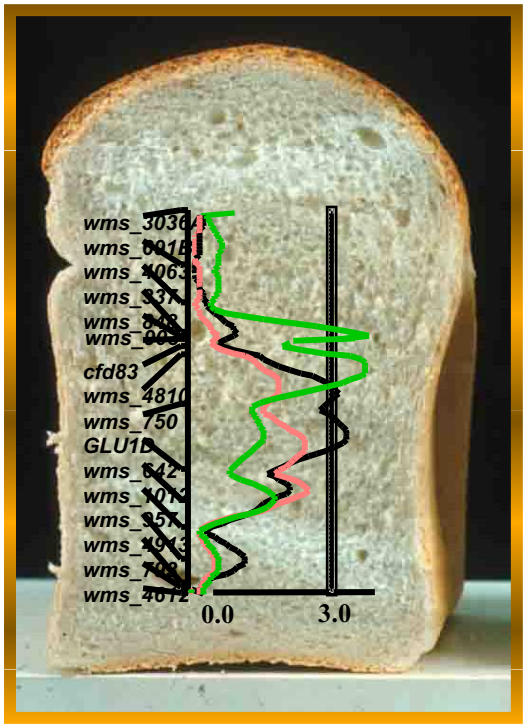
The Model 6 of the CIM was used with forward regression and backward elimination module of QTL Cartographer for scanning intervals of 2 cM between the markers and putative QTLs with a window size of 10 cM. Five markers were used as the background control for forward-backward stepwise regression. The position, genetic effects and percentage of phenotypic variation of the QTLs were estimated at the significant LOD peak in the region under consideration. But the flanking markers with LOD value above threshold were also considered and used for indicating the base of QTL peaks.

2.10.3 QTL clusters

The QTLs identified using CIM for various traits were grouped chromosome wise and QTL plots were drawn using QTL Mapchart (Voorrips 2002). The QTLs were designated as QX.ncl-Y, where X denotes the phenotypic trait abbreviation and Y represents the chromosome on which the quantitative trait locus was located. Each QTL was defined on one line, along the linkage group map with the extent of QTL intervals and peak of the QTL. Different styles and colours of lines were used to represent various QTLs of different traits.

Chapter 3

Results



The Recombinant Inbred Line (RIL) population of HI977 x HD2329, comprising 105 lines was grown for two consecutive years (2003-04, 2004-05), in an augmented block design at three agroclimatically different locations. The parental survey and linkage groups were constructed as per procedure discussed in the chapter 2 viz; Materials and methods. The QTL analysis was also performed for various yield and BMQ traits and the results are as follows

3.1 Construction of framework map

A total of 1014 PCR based primers comprising 914 SSR and 100 ISSR primers and 2 protein based markers (HMW-glutenin) were used for parental survey. The details of the primers used for population screening are illustrated in the Table 3.1. A representative gel picture for the HMW-glutenin SDS-PAGE gel is illustrated in the Fig. 3.1, while ALF chromatogram of SSR marker *Xgwm429* in the Fig. 3.2. The marker data as defined in the previous chapter were converted into allele scores and fed in input file of Mapmaker ver. 3.0.

3.1.1 Parental survey analysis

Among all the primers used for parental survey, the highest level of polymorphism (Table 3.1) was revealed by *barc* primers (42.86%), followed by *gwm* (40.46%) and *cfd* (16.67%). The least polymorphism was recorded with ISSR primers (3%). This shows the superiority of SSR primers over ISSR primers in revealing the genetic polymorphism. It was further observed that 20-30% polymorphism was observed with markers on chromosomes 3B, 5D, 6D and 7A; 30-40% polymorphism in chromosomes 3A, 3D, 4A and 7D; 40-50% polymorphism in 1A, 2D and 7B; 50-60% polymorphism in 2A, 2B, 4D and 5A; 60-70% polymorphism in chromosomes 1B, 1D, 6A and 6B. The highest polymorphism of 71% was revealed in 4B and 5B chromosomes.

3.1.2 Population survey

The genotypic data of HI977x HD2329 population was generated using 212 SSRs, three ISSRs and two HMW glutenin loci (Glu-B1 and Glu-D1), which revealed polymorphism between the parents. The PCR products of SSR primers were scored as A, B (codominant) or C, D (Null allele). ISSR PCR amplicons were scored as dominant markers and HMW glutenin bands were scored as codominant markers.

Table 3.1:- Primers used for parental analysis

Primer	Source	No. of primers	Polymorphism	% of polymorphism
ISSR	Univeristy of British Columbia, Canada	100	3	3.00
Barc	Betsville Agriculture Research Centre, USA	56	24	42.86
Psp	Gale, John Innes centre, UK	17	2	11.76
Wmc	Wheat Microsatellite Constoritum	45	2	4.44
Wms	Roder, IPK, Germany	739	299	40.46
Cfa	Sourdile, INRA, France	27	2	7.41
Cfd	Sourdile, INRA, France	30	5	16.67
Total		1014	337	33.23

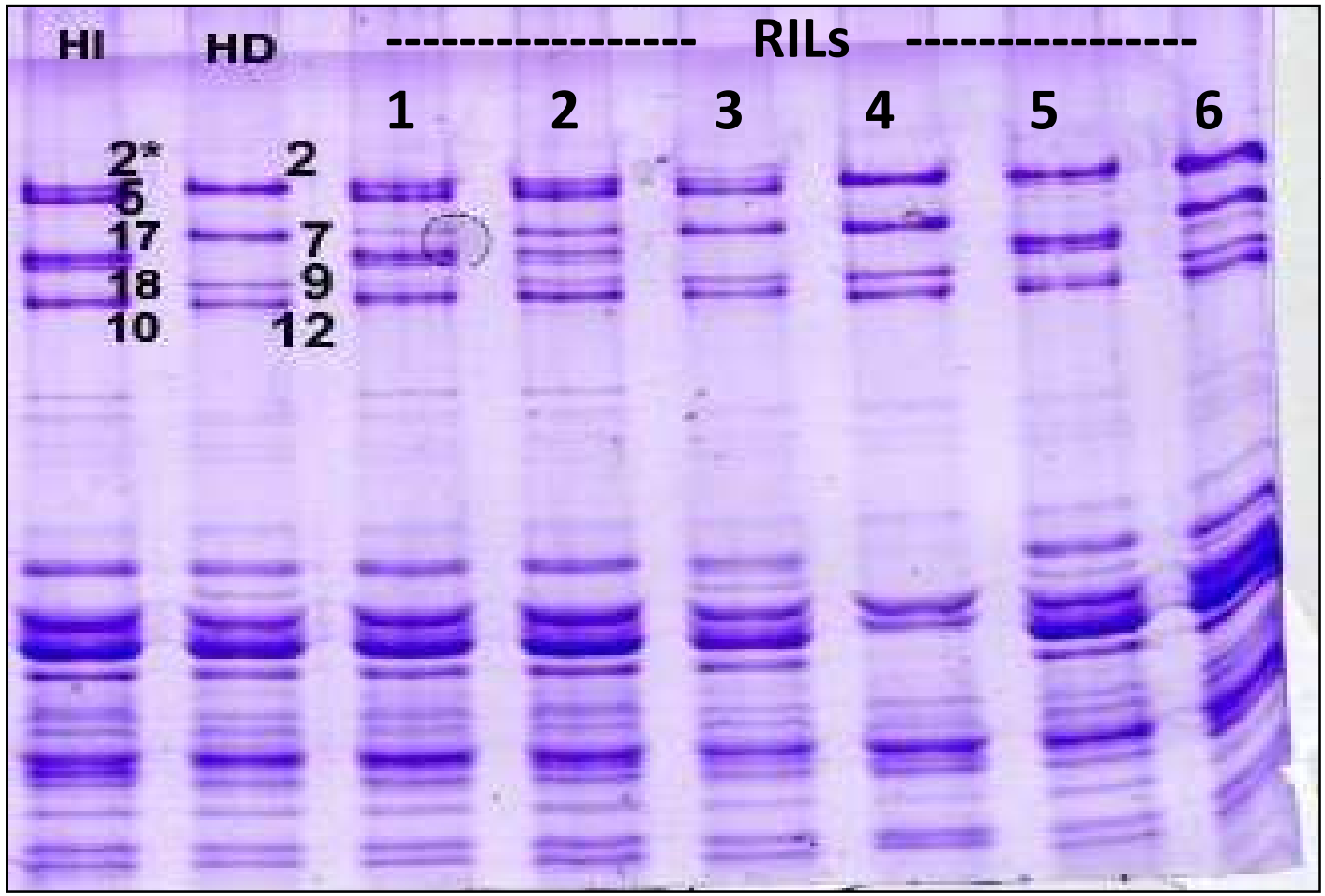


Fig.3.1: HMW glutenin pattern of HI977 and HD2329 along with a few representative RILs. The Glu-A1 loci of HI977 (2*),Glu-B1 subunits (17 +18) and Glu-D1 (5+10), also Glu-B1 of HD2329 (7+9) and Glu-D1(2+12) are displayed.

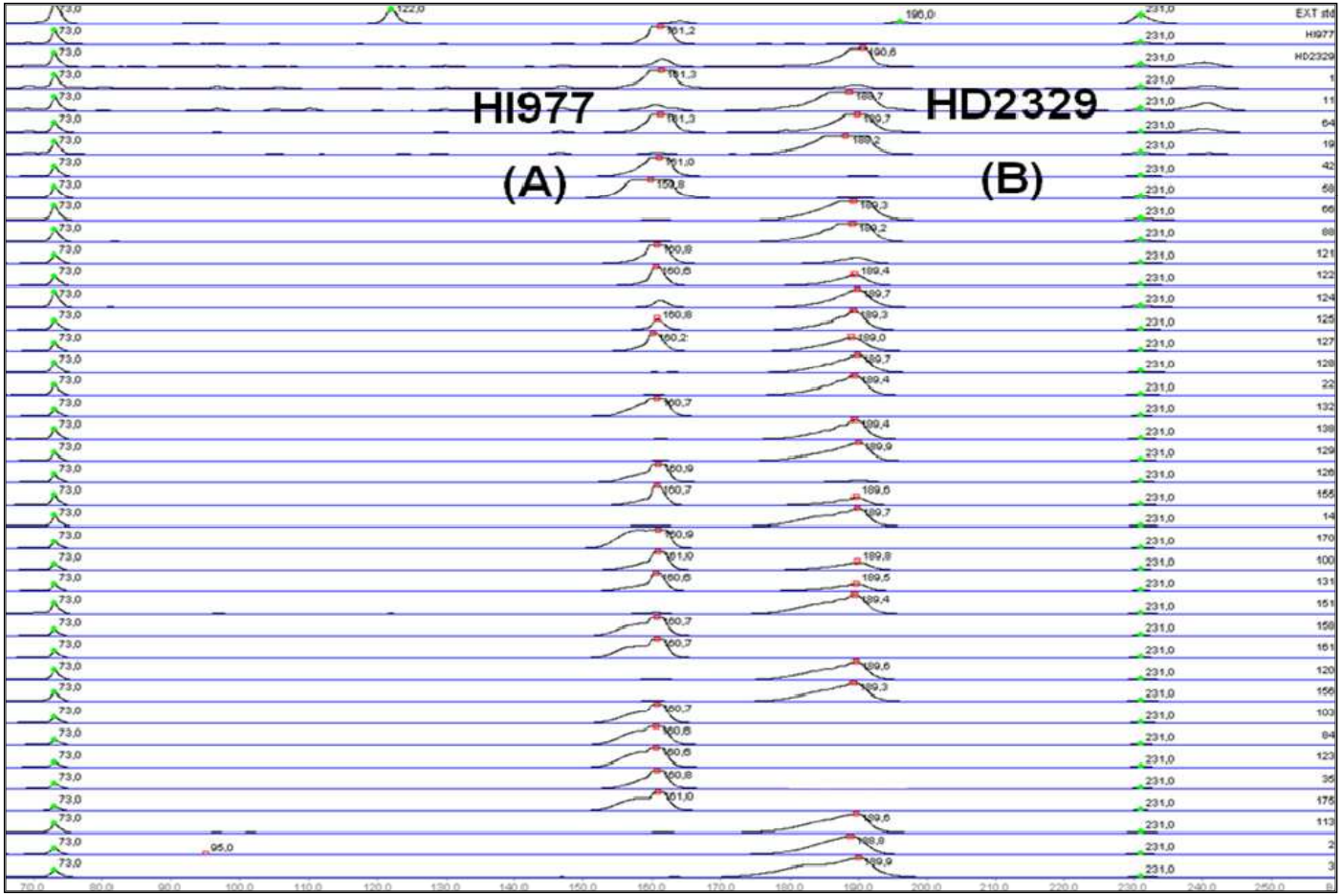


Fig. 3.2: Gel chromatogram of the SSR marker *Xgwm429*, with allele size of 160bp for A allele and 190bp for B allele. The first lane consists of external standards (73bp, 122bp, 196bp, 231bp), other lanes consist of internal standards (73bp and 231bp)

3.1.3 Linkage group construction

The polymorphic primers revealed by parental survey were selected for population screening based on position in established maps. Polymorphic markers were selected every 20 cM to cover the whole linkage group and population screening was attempted. Of the 217 markers used for population screening, 202 SSRs, with two HMW glutenin loci formed 19 linkage groups. Linkage groups were assigned to chromosomes when the groups had two or more SSR loci that had been assigned to a particular wheat chromosome in previously published maps (Roder et al. 1998; Somers et al. 2004). Two linkage groups for chromosome 4A and 7A could not be achieved, due to less polymorphic markers observed between the parental genotypes HI977 and HD2329 during parental survey covering the entire chromosomes. At a LOD score of 3, nine markers (4.1%) were unlinked and four markers mapped in the same loci, hence were not considered for the linkage analysis. The frame work map categorized based on A, B and D genome has been depicted in Fig 3.3, 3.4 and 3.5, respectively. The length of A genome of the map was 909 cM, while that of B and D genome was 1100.7 cM and 1152 cM, respectively.

The overall arrangement of the markers was same as the published microsatellite map of Roder et al. (1998) and wheat consensus map of Somers et al. (2004). The distribution of markers was quite even except for a few linkage groups viz., 1A (51.8 cM), 5A (50.4 cM) and 6D (51.2 cM) having a gap of more than 50 cM. The chromosomes 1B and 4D had maximum of 23 and minimum of 4 markers, respectively. The chromosome 2B had maximum linkage distance (300.3 cM), followed by 5D (297.0 cM), 1B (276.1 cM), 6B (252.2 cM) and 1A (248.9 cM) chromosomes. The total map length was 3161.8 cM and the mean interval between loci was 15 cM. The marker loci were subjected to χ^2 test at P <0.05% and identified that 41.17% (84) loci were skewed, compared to 58.83% (120 loci) fitting in the ratio of 1:1.

3.2 Phenotypic characteristics of the parents and the progeny population

The parents HI977, HD2329 and their RIL progeny were analysed for various phenotypic traits contributing to grain quality and the yield at three different locations, as detailed in the previous chapter. The parents, HI977 and HD2329 showed a

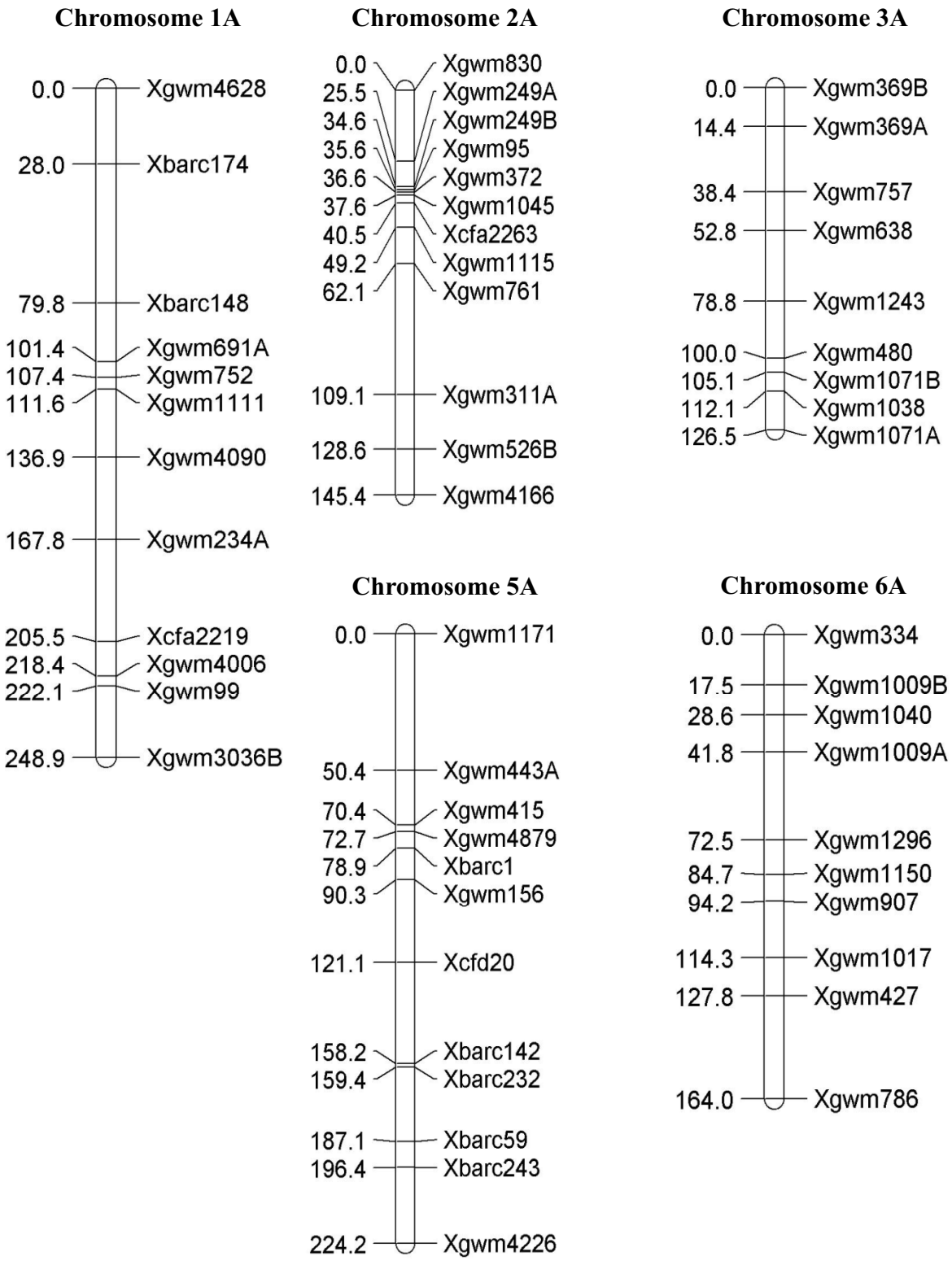


Fig. 3.3: Linkage groups of 'A' genome chromosomes of wheat

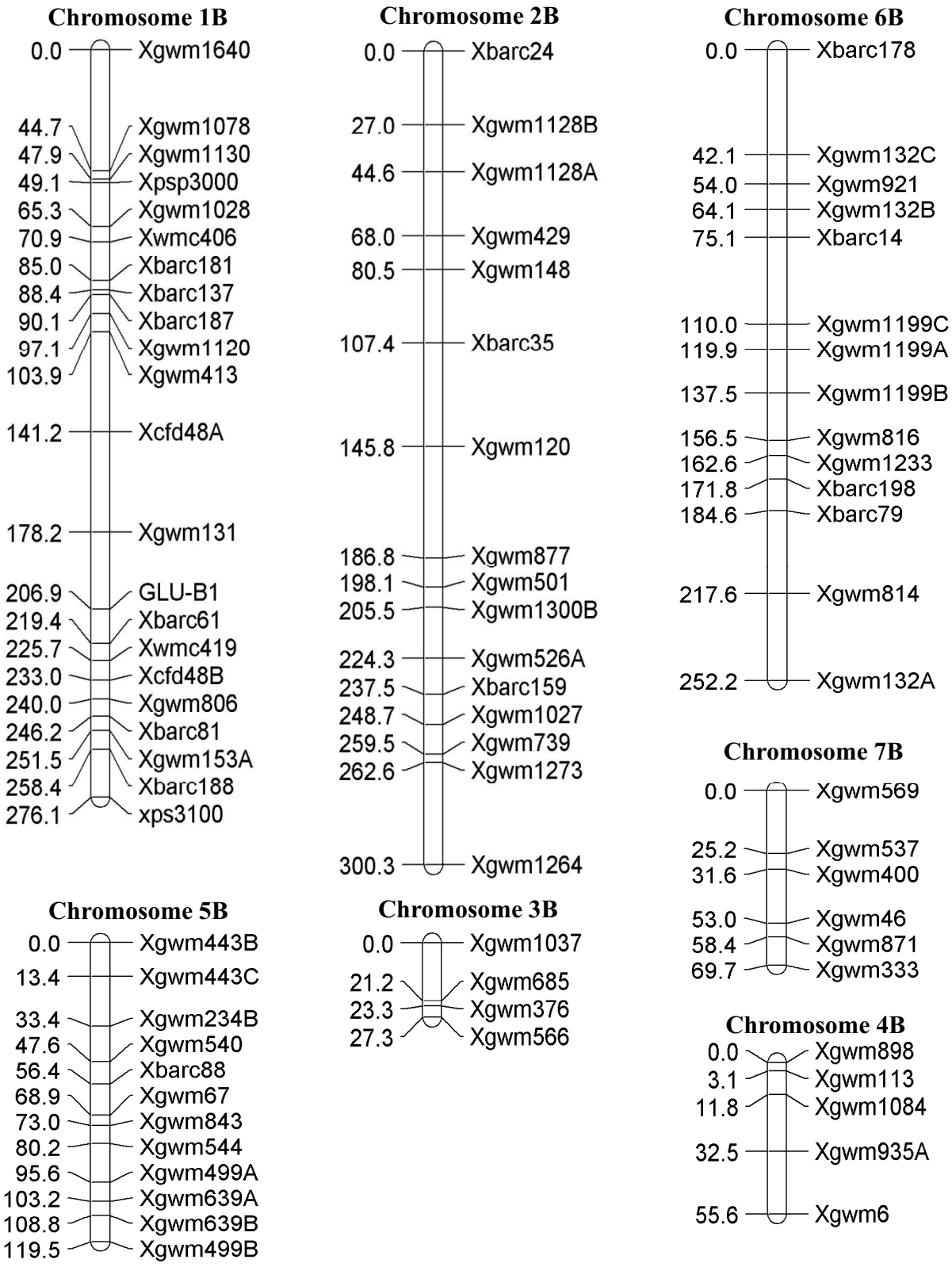


Fig. 3.4: Linkage groups of 'B' genome chromosomes of wheat

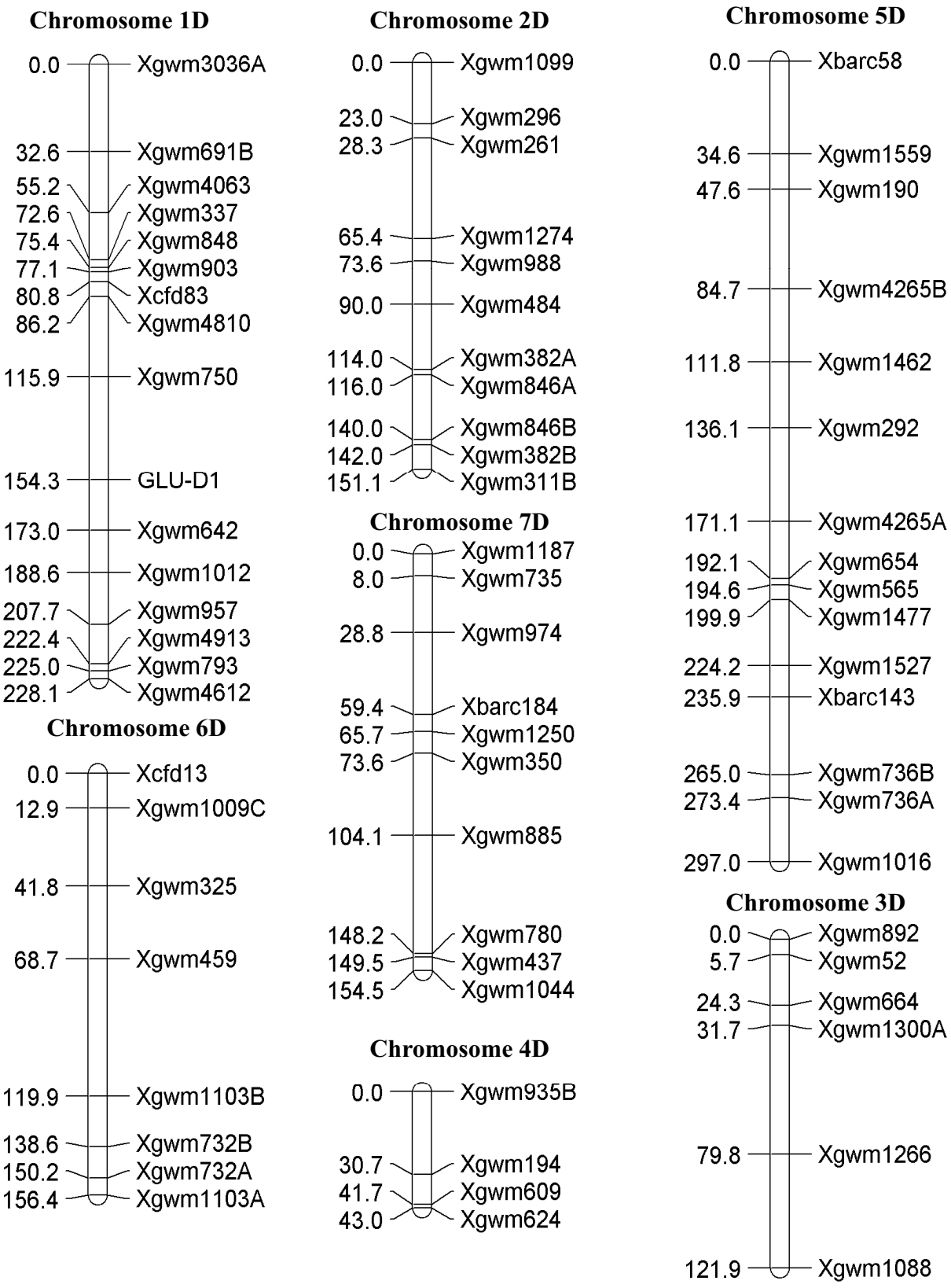


Fig. 3.5: Linkage groups of 'D' genome chromosomes of wheat

statistically significant difference for all the traits across different environments (Table 3.2 and 3.3).

The Table 3.2 depicts the location wise range of five traits, namely Tw, Tgw, Sv, Gpc and Lv in parents and in RILs. The phenotypic distribution for each trait in the population is shown graphically in Fig. 3.6 and 3.7. In the Fig. 3.6, Tw for Kota location (KotTw1 and KotTw2) and Sv for Pune location (PunSv1 and PunSv2) while in Fig. 3.7, the Gpc for Karnal location (KarGpc1 and KarGpc2), and Lv for Karnal (KarLv1, KarLv2) and Pune (PunLv1, PunLv2) locations showed a good overlapping of the data, recorded for both the years.

The parents HI977 and HD2329, showed a highly significant difference between them for Sv (51.82 to 54.19 ml, 38.76 to 39.63 ml) and Lv (573.13 to 594.69 cc., 496.88 to 533.13 cc.), respectively in each environment compared to other traits like Gpc, Tw and Tgw (Table 3.2). Although the difference in Gpc between the two parents, HI977 (11.68 to 13.60 %) and HD2329 (11.52 to 13.74 %) was rather small, significant difference was observed in the RIL population (9.35 to 14.91%). Similarly for Tgw and Tw, the difference between the parents was less while range in population was found to be wide (Table 3.2). Continuous phenotypic variation and transgressive segregation for all the five traits observed in the RIL population revealed the quantitative inheritance of these traits.

The mixogram of the parents HI977 and HD2329 has been represented in the Fig. 3.8. For mixograph traits, an average of all traits was calculated based on six data sets (2004-05, 2005-06) with three locations and are depicted in the Table 3.3. Based on the nature of curves (Fig. 3.8) the overall dough strength of HI977 is comparatively higher than HD2329. HI977, the better parent for BMQ recorded higher values than HD2329 for all the parameters except Weakening slope (Ws), which denotes the weakness of the dough, thus HI977 dough mixing ability is superior to HD2329. Trangressive segregants were identified for all mixograph traits with wide range, suggesting that both the parents carry both favourable and unfavourable alleles. The mixing time in HI977 was 3.06 min. compared to HD2329 (2.02 min.), while the transgressive segregants ranged from 0.87 min. to 6.98 min. The mixing peak time (Mpt) is also higher in HI977, while Midline curve tail values are similar in both the parents (Table 3.3). The mixograph traits when plotted locationwise (Fig. 3.9), showed

Table 3.2:- BMQ traits distribution in the cross HI977 x HD2329

Trait	Description	HI977	HD2329	S.E	Average	Range	
						Min	Max
KarLv	Karnal Loaf volume (cc.)	573.13	496.88	10.39	537.81	477.13	629.13
KotLv	Kota Loaf volume (cc.)	582.82	503.13	4.85	536.44	448.63	614.38
PunLv	Pune Loaf volume (cc.)	594.69	533.13	7.33	557.30	485.75	607.13
KarSv	Karnal Sedimentation volume (cc.)	52.51	39.63	2.33	46.23	35.93	61.13
KotSv	Kota Sedimentation volume (cc.)	54.19	38.76	1.86	46.20	32.03	62.23
PunSv	Pune Sedimentation volume (cc.)	51.82	39.19	1.11	46.26	34.96	61.48
KarTw	Karnal Test weight (kg)	73.13	72.73	1.33	72.50	60.31	81.83
KotTw	Kota Test weight (kg)	79.29	79.79	0.54	79.77	75.52	82.78
PunTw	Pune Test weight (kg)	82.51	83.36	0.43	83.01	80.13	85.20
KarTgw	Karnal Thousand grain weight (g)	31.62	32.24	0.92	31.52	20.87	41.32
KotTgw	Kota Thousand grain weight (g)	39.86	40.08	0.76	39.57	31.53	46.92
PunTgw	Pune Thousand grain weight (g)	41.38	43.12	1.39	40.96	32.45	47.85
KarGpc	Karnal Grain protein content (%)	12.95	13.02	0.27	12.87	11.05	14.78
KotGpc	Kota Grain protein content (%)	11.68	11.52	0.36	11.55	9.35	14.02
PunGpc	Pune Grain protein content (%)	13.60	13.74	0.22	13.64	11.94	14.91

S.E. represents the standard error for the parents ($P \leq 0.05$), average for each trait was calculated from overall population and range represents minimum and maximum of the RILs.

Table 3.3:- Mixograph parameters distribution in the population

Trait	Description	HI977	HD2329	Average	Range	
					Min	Max
EPI	Envelope peak integral (% Tq* Min.)	109.01	79.15	69.56	1.24	188.80
MPT	Mixing peak time (Min.)	3.06	2.02	2.96	0.87	6.98
MRI	Midline right integral (% Tq * Min.)	172.12	140.47	153.27	60.25	303.10
MRV	Midline right value (%)	51.98	46.88	42.86	31.39	60.32
MRW	Midline right width (% / Min.)	32.09	27.61	22.05	10.36	51.53
MTI	Midline curve tail integral (% * Min.)	377.95	360.75	309.69	224.50	424.10
MTV	Midline curve tail value (%)	43.54	43.89	25.66	4.12	51.18
MTW	Midline curve tail width (% / Min.)	20.76	16.00	25.71	1.49	48.69
WS	Weakening slope (%)	8.53	17.64	8.72	0.11	77.88

Average for each trait was calculated from overall population, grown in six environment. Range represents minimum and maximum of the RILs.

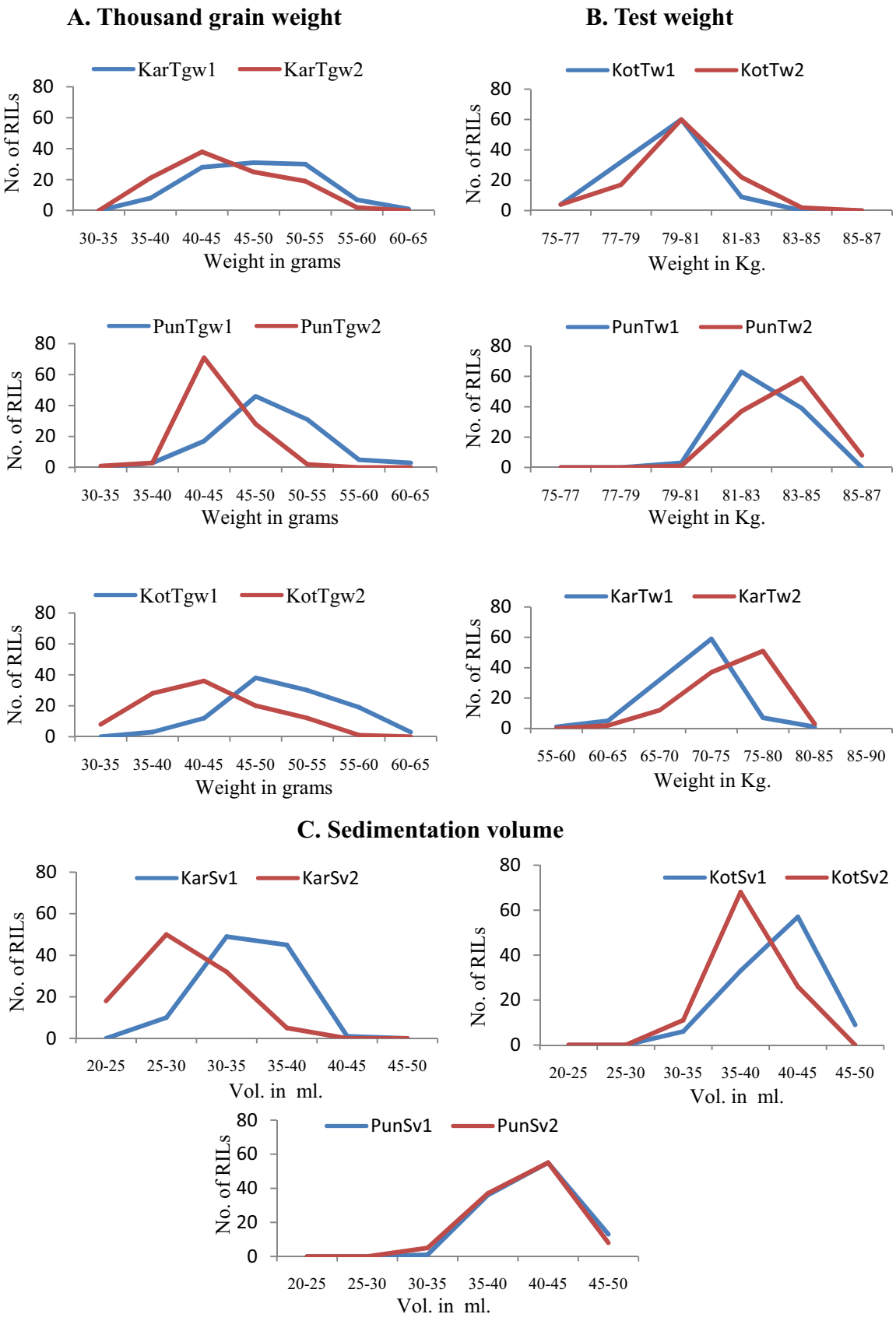


Fig. 3.6 : Frequency distribution of Tgw, Tw and Sv in the RIL population of HI977 x HD2329

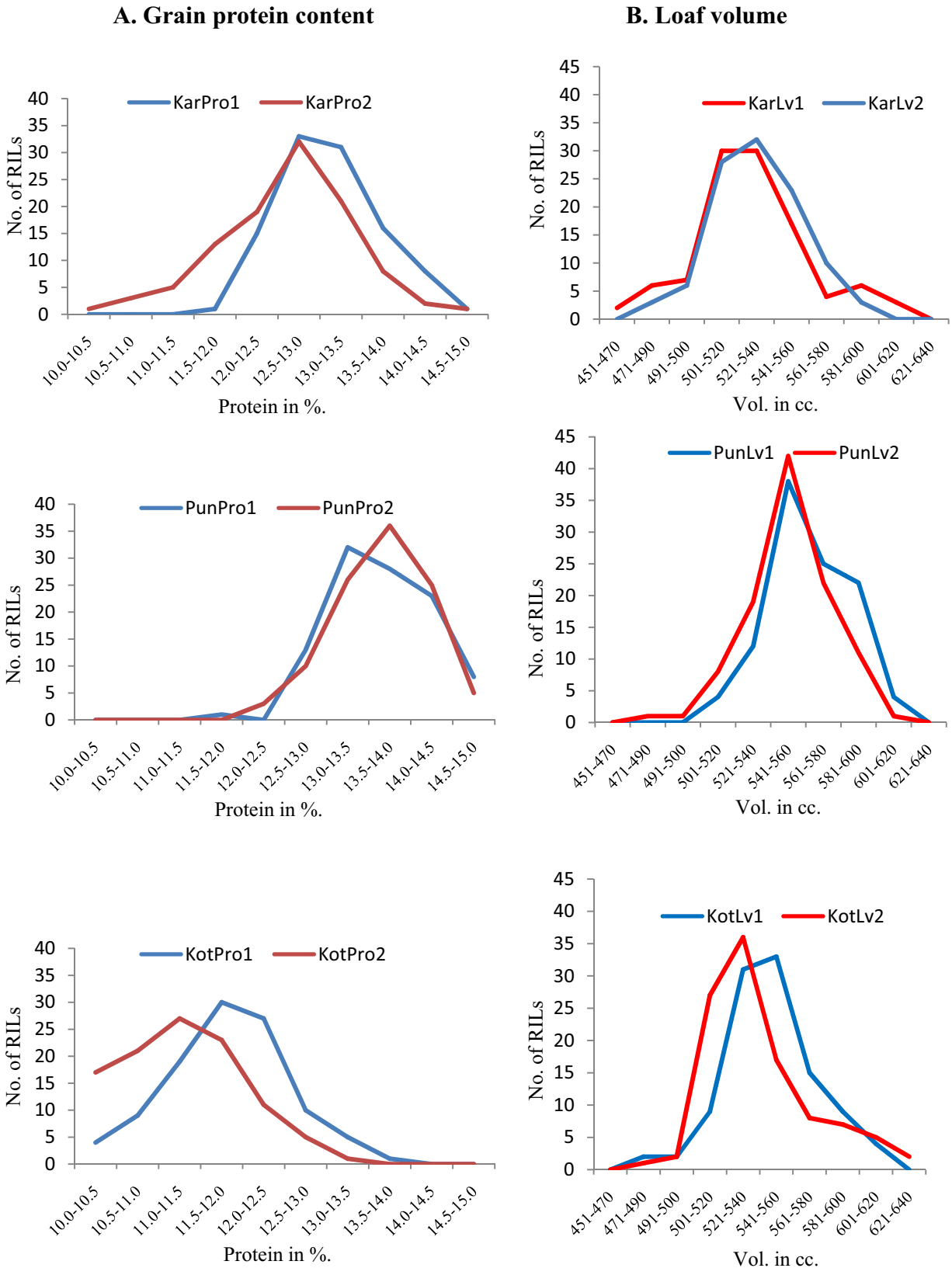
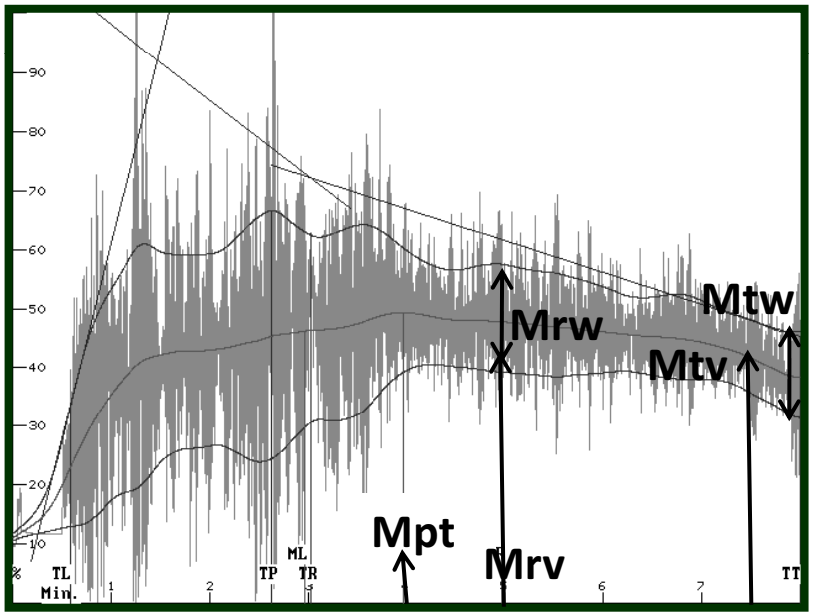
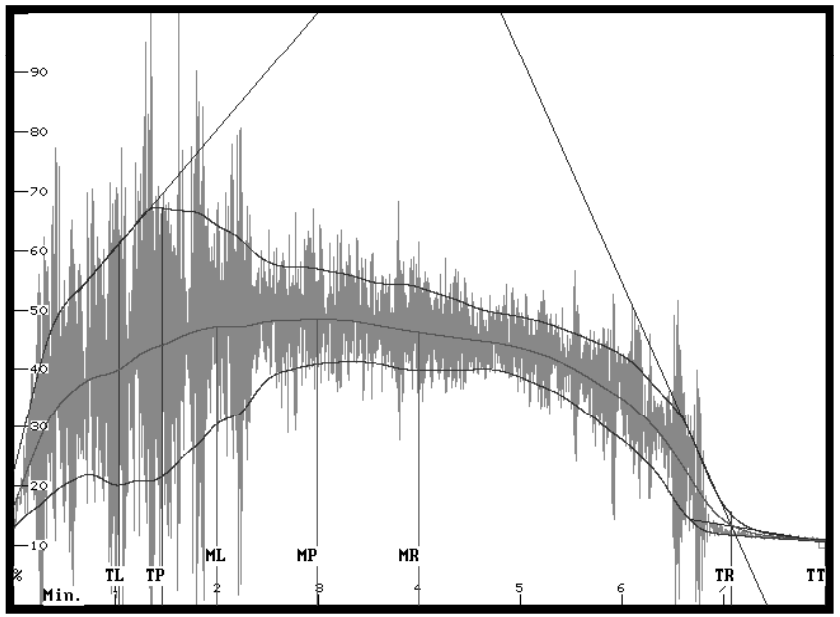


Fig. 3.7: Gpc and Lv frequency distribution in the HI977xHD2329 population



HI977 – Good bread making



HD2329 – Poor bread making

Fig. 3.8: Mixogram showing the strong (HI 977) and weak (HD2329) dough characteristics

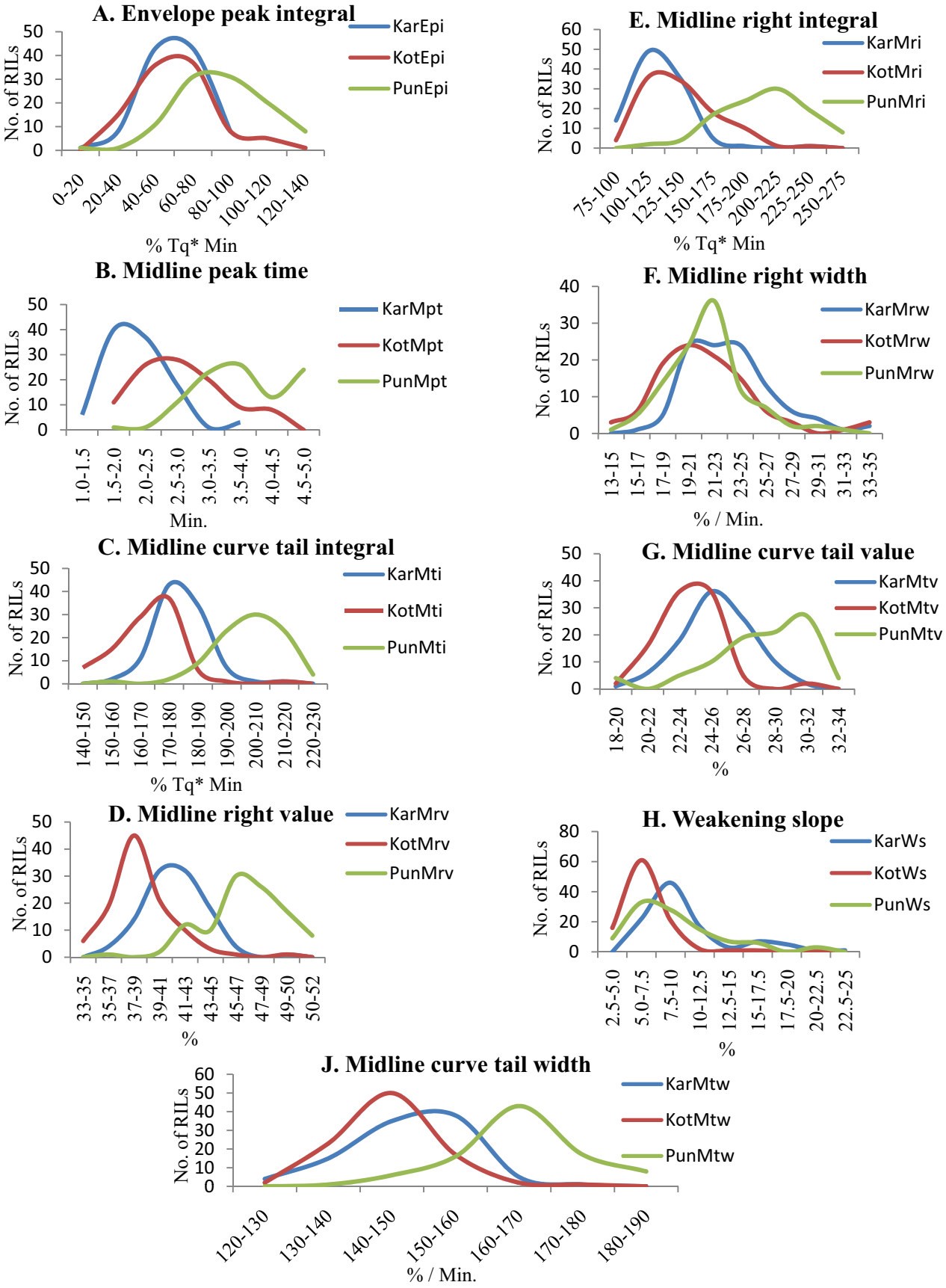


Fig. 3.9: Frequency distribution of mixograph parameters

a different pattern for all the traits emphasizing the role of environment in deciding the expression of these traits.

3.3 Correlation among yield and BMQ traits

3.3.1 Correlation among Tw, Tgw, Gpc and Sv

Rank correlation among the traits recorded at different locations and Pearson's correlation between traits within same location are presented in Table 3.4. Correlations were positive and significant between KarTgw and KotTgw for both the years. The correlation between PunTgw1 and KotTgw1 was significant, while the correlation was negative and insignificant between PunTgw2 and KotTgw2. Similar observation was made for the trait Sv. The correlation for Gpc and Tw was insignificant between all the locations recorded in 2003-04, while it was positive and significant between KarGpc2 and KotGpc2 (in the year 2004-05) and also for PunGpc1 and KotGpc2. In case of the trait Tw, Karnal with Kota and Kota with Pune recorded positive and significant correlation. Sv recorded at Karnal and Kota location, showed significant positive correlation among them and also displayed a positive correlation with PunTgw2. The study on correlation among the traits revealed that Tw and Gpc had positive and significant correlation in Karnal location, while it was insignificant in the other two locations. Positive and significant correlation was identified between Gpc and Sv, only in Kota location.

3.3.2 Correlation among Lv data recorded at different locations

Rank correlation for all the possible combinations of Lv is presented in Table 3.5. The correlation among the traits recorded at the same location for two years, different locations for each year and different locations for two years was significantly small in all the cases, except for KarLv1 Vs KotLv1 (0.862). Between the two years data, comparatively less correlation was observed in the second year (2004-05). KarLv2 did not show any correlation with KotLv2 and PunLv2.

3.3.3 Correlation among mixograph traits

The mixograms analyzed with Mixsmart ver. 3.4 software generated 44 mixograph parameters. To enhance the feasibility of analysis, the 44 traits from 6 data sets (3 locations x 2 years) were correlated together and cluster diagrams were constructed to identify closely related traits. Table 3.6 shows clusters of mixograph traits based on their correlations and the representative traits selected from each cluster for further

Table 3.4:- Correlation between BMQ and yield parameters

Trait	KarGpc1	KarGpc2	KotGpc1	KotGpc2	PunGpc1	PunGpc2	KarTw1	KarTw2	KotTw1	KotTw2	PunTw1	PunTw2
KarGpc2	-0.123											
KotGpc1	0.049	0.135										
KotGpc2	0.231	0.259**	0.018									
PunGpc1	0.163	0.089	0.183	0.365***								
PunGpc2	0.05	-0.141	-0.029	0.076	0.031							
KarTw1	0.431***	0.107	0.162	-0.197*	-0.099	0.001						
KarTw2	0.134	0.502***	0.098	-0.089	-0.086	0.167	0.054					
KotTw1	0.12	-0.051	-0.128	-0.087	-0.06	0.189	0.075	0.371***				
KotTw2	0.078	-0.007	0.333***	-0.037	0.157	0.158	0.012	0.237*	0.205*			
PunTw1	0.011	0.091	-0.121	0.047	-0.107	-0.154	0.018	-0.137	0.101	0.262**		
PunTw2	0.006	-0.081	0.023	-0.166	-0.178	0.084	0.113	0.136	0.357***	0.468***	0.312**	
KarTgw1	0.096	-0.071	0.053	0.036	0.094	-0.035	0.234*	0.062	0.133	-0.022	0.083	-0.01
KarTgw2	0.075	0.12	-0.027	0.088	0.099	0.216*	-0.024	0.138	-0.003	-0.076	-0.027	-0.047
KotTgw1	0.146	-0.08	-0.08	-0.038	0.101	-0.01	-0.024	-0.058	0.238*	-0.062	-0.016	-0.042
KotTgw2	0.145	0.116	0.15	0.019	0.213*	0.121	0.08	-0.058	-0.083	0.207*	-0.005	-0.105
PunTgw1	0.051	0.009	-0.223*	0.156	-0.127	0.066	0.106	-0.223*	-0.015	-0.252**	0.134	-0.032
PunTgw2	0.077	0.134	0.176	0.082	-0.025	-0.041	0.107	0.147	0.175	0.093	0.093	0.024
KarSev1	0.041	0.222**	0.194*	0.16	0.109	-0.243*	0.076	-0.068	-0.152	0.097	0.122	-0.067
KarSev2	0.281**	0.146	0.11	0.149	0.149	0.048	0.059	0.06	0.096	0	0.049	0.001
KotSev1	-0.013	0.116	0.323***	0.136	0.016	-0.182	0.089	0.108	-0.132	0.152	0.132	0.045
KotSev2	0.236*	0.092	0.325***	0.318***	0.286**	-0.049	-0.1	-0.151	-0.171	-0.163	0.005	-0.188
PunSev1	-0.254**	0.016	0.184	0.004	-0.014	-0.247*	0.019	0.122	-0.143	-0.085	0.113	-0.133
PunSev2	0.174	-0.096	0.044	-0.024	-0.014	0.314***	-0.067	0.089	0.083	0.184	-0.169	0.137

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

Table 3.4 (contd.)

Trait	KarTgw1	KarTgw2	KotTgw1	KotTgw2	PunTgw1	PunTgw2	KarSev1	KarSev2	kotSev1	KotSev2	PunSev1
KarGpc2											
KotGpc1											
KotGpc2											
PunGpc1											
PunGpc2											
KarTw1											
KarTw2											
KotTw1											
KotTw2											
PunTw1											
PunTw2											
KarTgw1											
KarTgw2	0.159										
KotTgw1	0.258**	0.186									
KotTgw2	0.022	0.334***	0.163								
PunTgw1	0.112	0.175	0.271**	0.129							
PunTgw2	0.236*	0.052	-0.291**	-0.191	0.043						
KarSev1	0.141	0.066	-0.102	0.08	-0.034	0.380***					
KarSev2	0.101	0.044	-0.065	0.048	0.092	0.430***	0.483***				
KotSev1	0.048	0.062	0.207*	-0.045	-0.071	0.354***	0.844***	0.411***			
KotSev2	-0.024	0.143	-0.028	0.076	0.230*	0.244*	0.253**	0.489***	0.161		
PunSev1	-0.052	0.09	-0.330***	-0.108	-0.146	0.167	0.167	0.11	0.301**	0.125	
PunSev2	-0.151	0.264**	0.106	0.455***	-0.054	-0.201*	-0.145	-0.016	-0.162	-0.147	-0.154

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

Table 3.5:- Spearman rank correlation between Lv recorded at different environments

Traits	KarLv1	KarLv2	KotLv1	KotLv2	PunLV1
KarLv2	0.585***				
KotLv1	0.862***	0.477***			
KotLv2	0.506***	-0.083^{ns}	0.513***		
PunLv1	0.559***	0.082^{ns}	0.544***	0.473***	
PunLv2	0.273**	0.135 ^{ns}	0.213*	0.241*	0.532***
ns- non significant	***P<0.001	**P<0.01	*P<0.05		

Table 3.6:- Clusters of mixograph traits and relationship among the cluster traits

Location	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Separate
	MRI	M10S	E10V	M10I	M10W	MTW	EPI	MPW	MLW	WS
	MPI	MTS	M10V	MTI	E10W	ETW	ELI	+	+	MLS
	MLI	E10S	MTV	MRV				EPW	E10I	ERV
	MRT	ETS	ETV	MPV				ELW	ETI	ERW
	MPT							+		MRW
	MLT							EPV		MRS
								ELV		ERS
										MLV
										ELS
										ERI
										ERT
										EPT
										ELT
Karnal 2005-06	Y	Y	Y	Y	Y	Y	Y	Y	Y	
Karnal 2004-05	Y	Partial	Partial	Y	Y	Y	Partial	N	Y	
Pune 2005-06	Y	Y	Y	Y	Y	Y	Partial	Partial	Partial	
Pune 2004-05	Y	Y	Y	Y	Y	Y	N	Partial	Partial	
Kota 2005-06	Y	Y	Y	Y	Y	Y	N	Y	Partial	
Kota 2004-05	Y	Partial	Partial	Y	Y	Y	N	Partial	Y	

M - Midline
E - Envelope
R - Right
L - Left
10 - Data at 7.5 min.
W - Width

V - Value
T - Curve Tail
E - Envelope
S - Slope
P - Peak
I - Integral

Y - All traits in the cluster had correlation (R) value > 0.8, among them
P - Atleast one of the traits in cluster has R value < 0.8, among them
N - None of the traits in the cluster has R value > 0.8

analysis. Thus, nine traits were selected and previous reports were also checked for the suitability of these nine traits and repeatability. Furthermore, correlation between Lv and 9 mixograph traits were performed and observed to be insignificant (at $P \leq 0.05$) for all the locations.

3.4 AMMI analysis

The ANOVA for Tw, Tgw, Gpc, Sv and Lv with AMMI model is presented in Table 3.7. Since Lv is the direct measure of BMQ, AMMI analysis was performed with this trait and not with the component traits of mixograph, which contributes to Lv. Contribution to the sum of squares due to Genotype, GxE interaction (GEI) and Environment were calculated as percentage of total sum of squares (Tarakanovos and Ruzgas, 2006). For all the traits AMMI model (Table 3.7) deciphered the GEI into 4 principal components, the first interaction principal component axes (IPCA 1) and the second component (IPCA II) score accounted for a large portion of the sum of squares with GEI for all traits.

The AMMI analysis of variance of Tw tested in six environments showed that 79.70% of the Total sum of squares (TSS) was attributable to environmental effect, only 4.5% to genotypic effect, and 15.8% to GEI effect (Table 3.7), while for Sv the environmental effect was 23.23% of the TSS, 28.73% to genotypic effects and 48.03% to GEI effect. Similarly, the Gpc showed that 62.38% of the TSS was due to environmental effects, only 9.11% due to genotypic effect and 28.51% to GEI effect. The highest contribution of TSS was realized for Tgw through environment (68.25%), the genotypic effects and GEI were 7.91 and 23.83%, respectively. The major components of TSS due to Lv were contributed by genotype (38%) and GEI effect (47 %).

3.5 Identification of QTLs associated with yield and BMQ related traits

Total 269 QTLs were identified for 14 traits with data recorded at 6 environments. The percentage of contribution of QTLs for HI977 (better parent) was 55 %, while HD2329 (poor parent) was 45 % (Table 3.8). This shows that the poor BMQ parent has also significantly contributed for most of the traits through QTLs.

The B genome had the maximum number of QTL mapped (115) followed by D and A (76) genome. The QTL distribution in homeologous chromosomes for group 1

Table 3.7:- Analysis of variance for Tgw, Tw, Sv, Gpc and Lv

	Tgw					Sv				
Source	df	S.S.	M.S.	F	explained (%)	df	S.S.	M.S.	F	explained (%)
Genotype (G)	104	1489.51	14.32		7.91	104	5554.49	53.41		28.73
Environment (E)	5	12850.2	2570.04		68.25	5	4491.09	898.22		23.23
GxE	520	4487.56	8.63		23.84	520	9285.46	17.86		48.03
IPCA 1	108	1483.38	13.74	1.884***	7.88	108	3034.29	28.10	1.852***	15.7
IPCA 2	106	1196.56	11.29	1.911***	6.36	106	2896.88	27.33	2.493***	14.99
IPCA 3	104	754.31	7.25	1.391*	4.01	104	1723.32	16.57	2.052***	8.91
IPCA 4	102	613.85	6.02	1.37	3.26	102	1263.76	12.39	3.374***	6.54
GXE residual	100	439.46				100	367.2			
TOTAL	629	18827.3				629	19331			
	Tw					Gpc				
G	104	736.67	7.08		4.5	104	72.52	0.7		9.11
E	5	13044.9	2608.99		79.7	5	496.65	99.33		62.38
GxE	520	2586.36	4.97		15.8	520	226.98	0.44		28.51
IPCA 1	108	1373.86	12.72	4.322***	8.39	108	67.87	0.63	1.627***	8.53
IPCA 2	106	933.7	8.81	9.668***	5.7	106	59.39	0.56	1.719***	7.46
IPCA 3	104	132.59	1.27	1.761***	0.81	104	38.87	0.37	1.24	4.88
IPCA 4	102	87.52	0.86	1.462*	0.53	102	30.56	0.30	0.99	3.84
GXE residual	100	58.69				100	30.29			
TOTAL	629	16368				629	796.15			
	Lv									
G	104	198394	1907.64		38					
E	5	75628	15125.6		15					
GxE	520	247452	475.86		47					
IPCA 1	108	114578	1060.91	3.290***	22					
IPCA 2	106	65326	616.27	2.792***	12.5					
IPCA 3	104	39949	384.12	2.811***	7.6					
IPCA 4	102	19530	191.46	2.373***	3.7					
GXE residual	100	8070			1.5					
TOTAL	629	521474								

The AMMI components were denoted as IPCA1, IPCA2, IPCA3 and IPCA4 ANOVA was calculated from the values of RILs across all six environments, significance of AMMI components were indicated with asterisk symbol (*)

***P<0.001 **P<0.01 *P<0.05

Table 3.8:- Contribution of QTLs by parents

Traits	Total QTLs	HI977	HD2329
Tw	26	10	16
Tgw	17	9	8
Gpc	11	7	4
Sv	14	5	9
Epi	18	15	3
Mpt	22	9	13
Mri	18	9	9
Mrv	16	10	6
Mrw	22	16	6
Mti	10	2	8
Mtv	18	2	16
Mtw	18	12	6
Ws	27	21	6
Lv	30	21	9
Total	267	148	119
contribution of QTLs (%)		55.43	44.57

through group 7 was 59 (22%), 42 (16%), 31 (12%), 9 (3%), 55 (20%), 63 (24%) and 8 (3%), respectively. The highest numbers of QTL were identified for the trait Lv (30), Ws (27). Tw (26) Mpt (22), Mrw (22), Mtw (18), Epi (18), Mri (18), Mtv (18), Tgw (17), Mrv (16), Sv (14) Gpc (11) and Mti (10). The position of all major QTL for respective traits above the threshold LOD 3.0 (incase of Lv LOD >2.0) score are depicted in Fig. 3.10, 3.11, 3.12 and 3.13.

3.5.1 QTLs for Tw

About 26 QTL were identified for Tw from 16 chromosomes (Table 3.9). Three common QTL (*QTw.ncl-5D.2, QTw.ncl-6B.2, QTw.ncl-6D.1)* were detected in two locations, Pune and Karnal. The contribution of the phenotypic variation ranged from 7.1% to 32.3%. HD2329 contributed for Tw through 16 QTL and HI977, for the remaining 10 QTL. Majority of QTL are on group 1 (8) followed by group 2 (5) and group 6 (4).

3.5.2 QTLs for Tgw

For Tgw, 17 QTL were identified on 11 chromosomes with majority mapped on group 1 (6) followed by group 5 (5) (Table 3.10). The chromosomes 2B, 5A and 5B each had 2 QTL controlling Tgw. *QTgw.ncl-5B.1* explained 27.69% of variation with HI977 allele, followed by *QTgw.ncl-1B.1* (27.12%) and *QTgw.ncl-2B.2* (26.52%) contributed by poor parent, suggesting the importance of alleles from HD2329. Two QTL (*QTgw.ncl-1B.3* and *QTgw.ncl-4B.1*) with positive additive effect contributed 15.1% and 14.6% respectively, towards phenotypic variation of Tgw.

3.5.3 QTLs for Sv

A total of 14 QTL were identified for Sv on 11 chromosomes, with majority mapped on group 1, group 5 and group 6 chromosomes (Table 3.11). Among these 14 QTL, 9 had negative additive effect, suggesting the contribution of alleles from inferior parent HD2329, especially for group 6 QTLs. Group 1 chromosome QTLs with positive and negative additive effect contributed 11.55% to 15.88% towards phenotypic variation, spanning through all the three locations.

3.5.4 QTLs for Gpc

In all, 11 QTL were detected on 7 chromosomes contributing 7.8% to 25.44% for Gpc (Table 3.12). Out of these, 7 were contributed by HI977 alleles and 4 by HD2329 alleles. The chromosome 1B had maximum QTL (4) followed by 6A (2). The highest

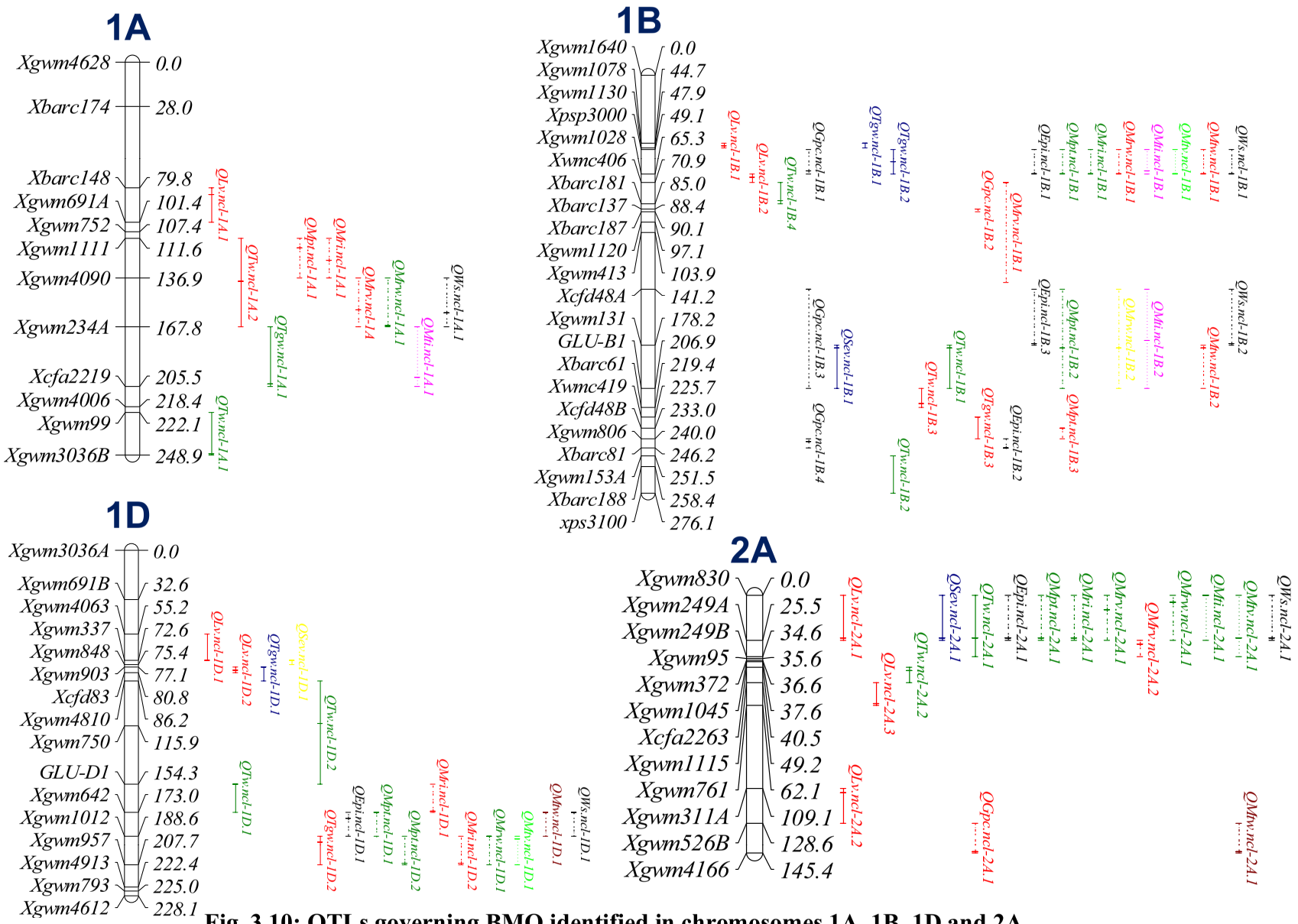


Fig. 3.10: QTLs governing BMQ identified in chromosomes 1A, 1B, 1D and 2A

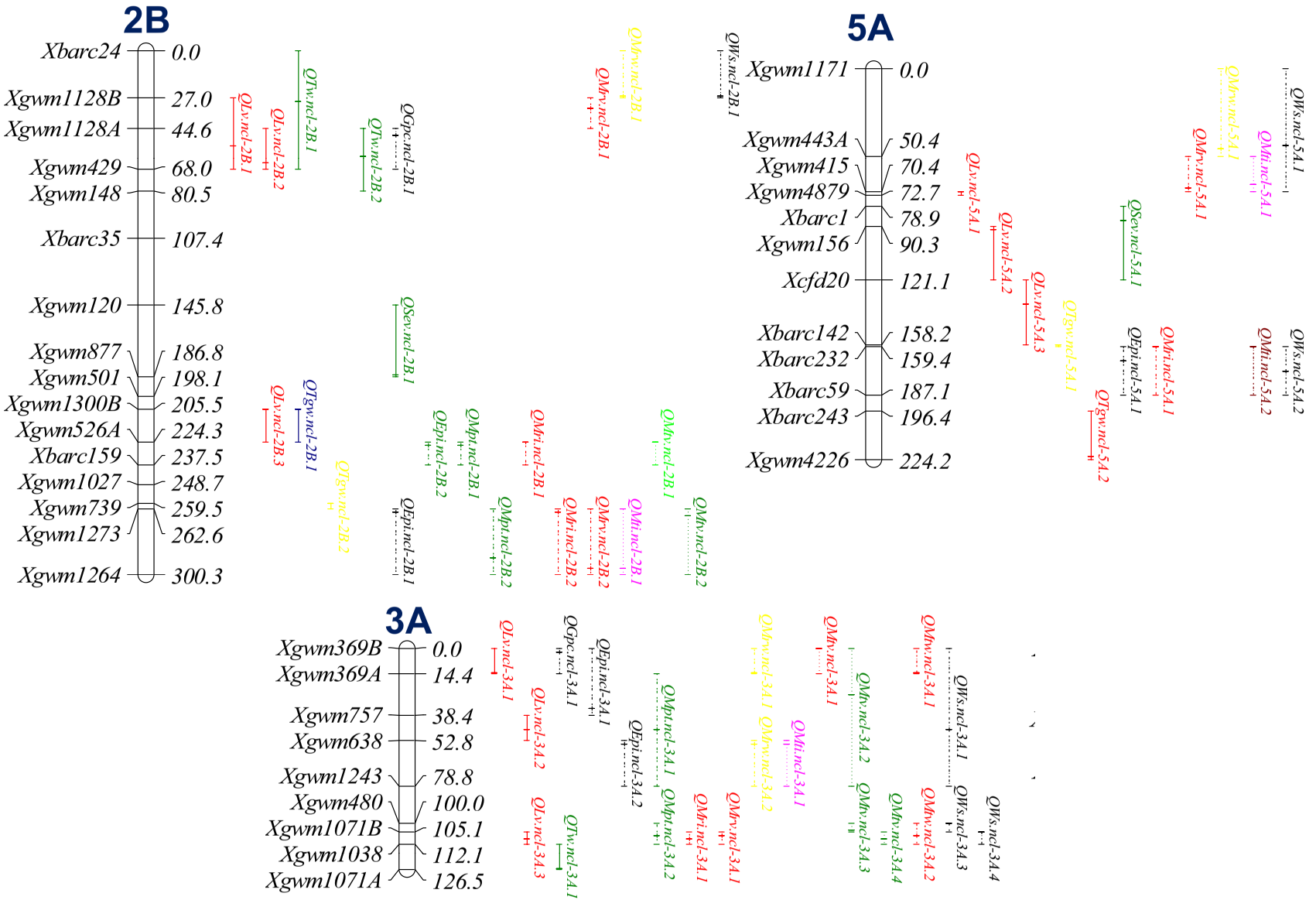


Fig. 3.11: QTLs governing BMQ identified on chromosomes 2B, 3A and 5B

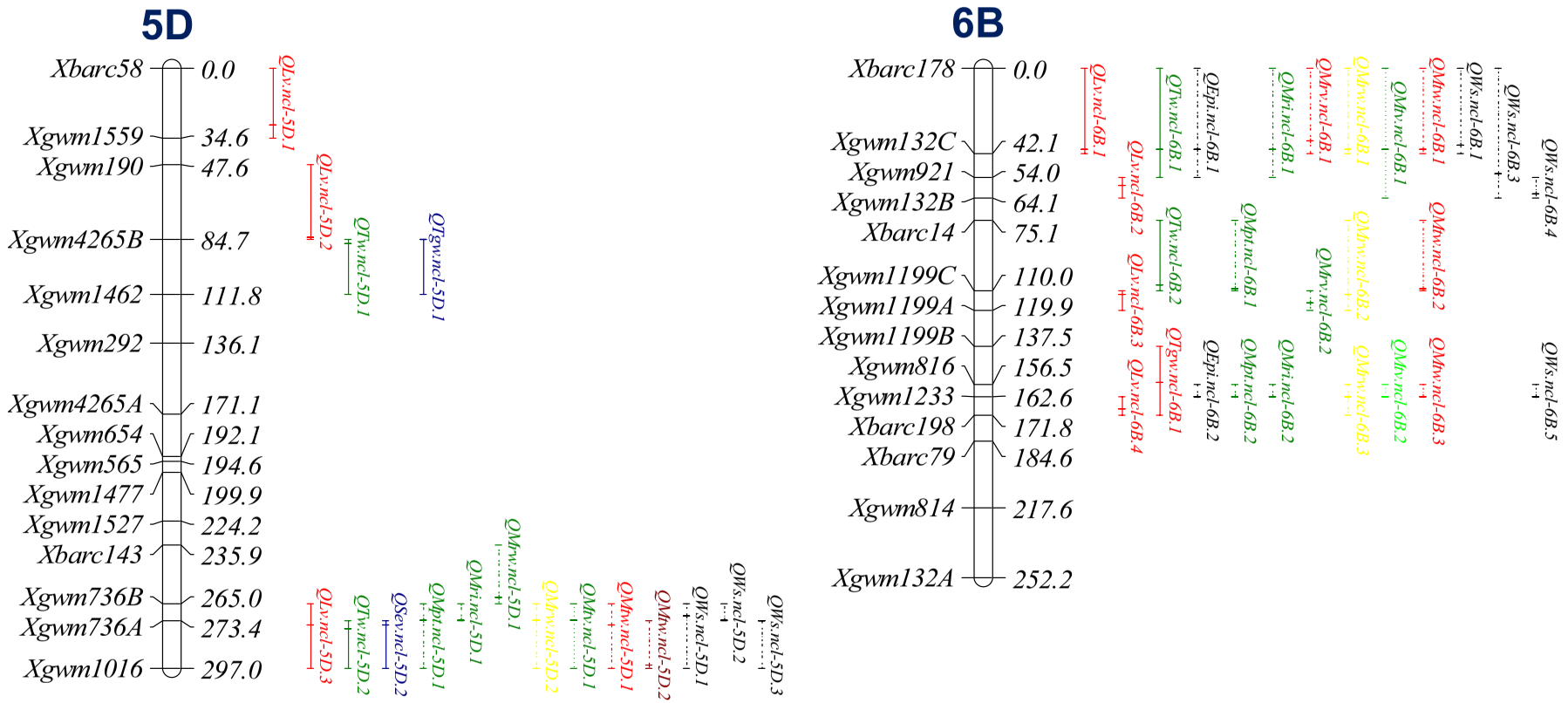


Fig. 3.12 : QTLs governing BMQ identified on chromosomes 5D and 6B

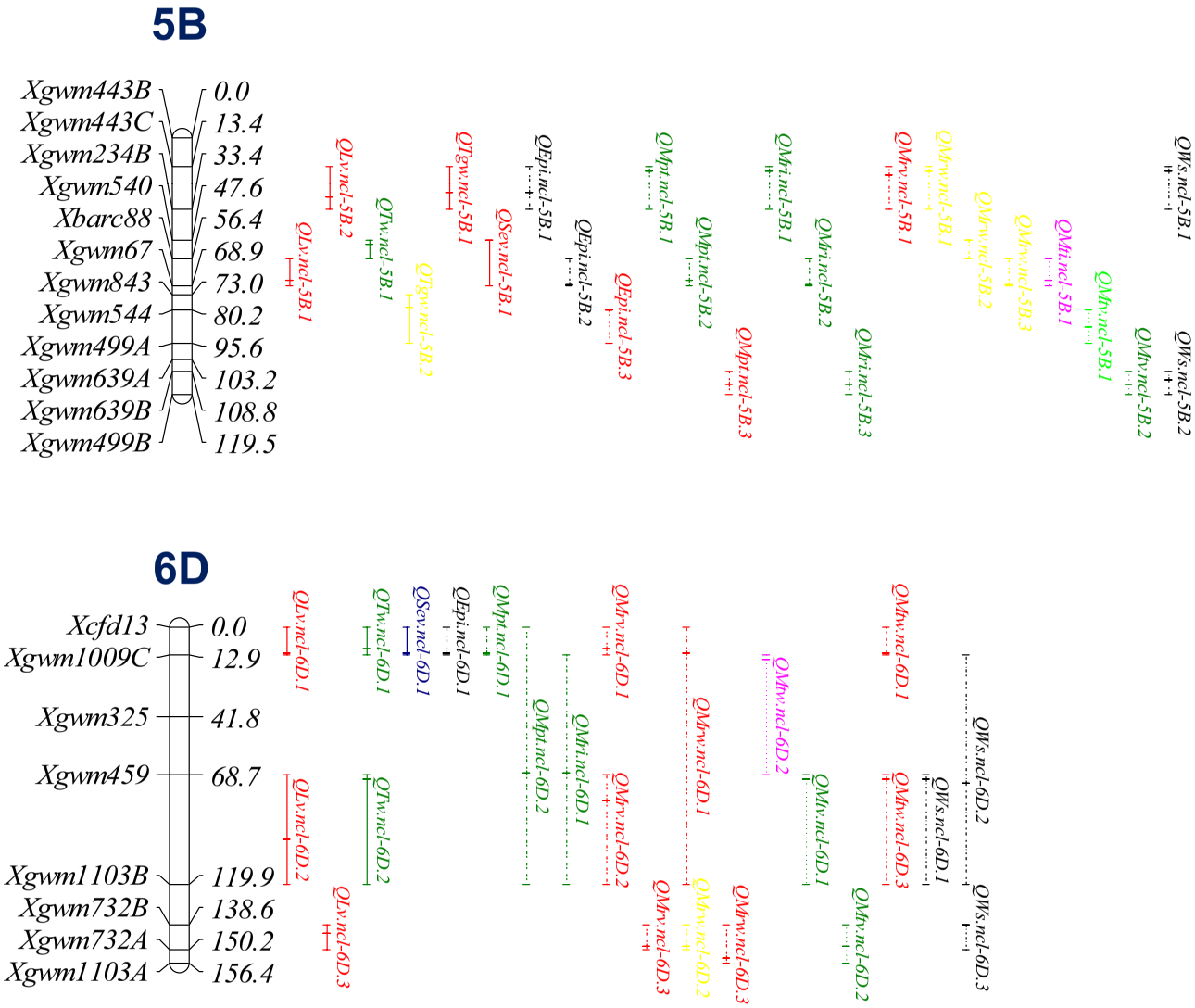


Fig. 3.13: QTLs governing BMQ identified on chromosomes 5B and 6D

Table 3.9:- Composite interval mapping for Tw

QTL	Chromosome	Trait	Marker		LOD	Position	Additive	R^2 x 100
			Right	Left				
QTw.ncl-1A.1	1A	KotTw2	Xgwm99	Xgwm3036B	3.95	248.11	-0.51	10
QTw.ncl-1A.2	1A	PunTw1	Xgwm1111	Xgwm234A	3.53	138.91	0.29	7.46
QTw.ncl-1B.1	1B	PunTw2	Xgwm131	Xglu1B	3.59	180.21	-5.3	15.88
QTw.ncl-1B.2	1B	PunTw1	Xgwm153A	Xpsp3100	5.19	251.51	-0.44	9.51
	1B	KotTw1	Xgwm153A	Xpsp3100	4.39	251.51	-0.5	11.19
QTw.ncl-1B.3	1B	PunTw1	Xglu1B	Xbarc61	3.55	216.91	0.41	9.03
QTw.ncl-1B.4	1B	KarTw2	Xwmc406	Xbarc181	3.52	82.91	1.46	12.23
QTw.ncl-1D.1	1D	KotTw2	Xglu1D	Xgwm642	3.08	154.31	0.44	8
QTw.ncl-1D.2	1D	PunTw1	Xgwm4810	Xglu1D	5.5	114.21	-0.37	11.12
QTw.ncl-2A.1	2A	PunTw2	Xgwm830	Xgwm249A	3.35	24.01	-5.12	15.4
QTw.ncl-2A.2	2A	KarTw2	Xcfa2263	Xgwm1115	3.15	42.51	-1.3	9.76
QTw.ncl-2B.1	2B	KarTw2	Xbarc24	Xgwm429	3.87	29.01	-4.07	32.3
QTw.ncl-2B.2	2B	KotTw2	Xgwm1128A	Xgwm148	3.25	60.61	-0.55	12.48
QTw.ncl-2D.1	2D	KotTw1	Xgwm968	Xgwm484	3.95	73.61	0.46	10.02
QTw.ncl-3A.1	3A	KotTw1	Xgwm1038	Xgwm1071A	4.91	126.11	-0.96	15.52
QTw.ncl-3B.1	3B	KarTw2	Xgwm1037	Xgwm376	3.27	23.21	1.33	8.9
QTw.ncl-3D.1	3D	PunTw1	Xgwm1266	Xgwm1088	5.24	79.81	0.33	9.06
QTw.ncl-4B.1	4B	PunTw2	Xgwm935A	Xgwm6	3.21	54.51	1.61	7.14
QTw.ncl-4D.1	4D	KotTw2	Xgwm194	Xgwm609	4.8	40.71	-0.54	13.27
QTw.ncl-5B.1	5B	KotTw1	Xgwm540	Xbarc88	5.9	49.61	0.75	18.68
QTw.ncl-5D.1	5D	KarTw1	Xgwm4265B	Xgwm1462	4.33	86.71	-1.59	15.47
QTw.ncl-5D.2	5D	PunTw2	Xgwm736A	Xgwm1016	3.17	275.41	-5.01	14.5
	5D	KarTw2	Xgwm736A	Xgwm1016	4.27	277.41	-3.65	27.38
QTw.ncl-6B.1	6B	KarTw2	Xbarc178	Xgwm921	3.29	40.01	-4.09	21.36
QTw.ncl-6B.2	6B	KarTw2	Xbarc14	Xgwm1199C	3.7	107.11	-3.92	23.13
	6B	PunTw2	Xbarc14	Xgwm1199C	3.03	107.11	-4.9	15.86
QTw.ncl-6D.1	6D	KarTw2	Xcfd13	Xgwm1009C	3.13	10.01	-3.51	25.54
	6D	PunTw2	Xcfd13	Xgwm1009C	3.9	12.01	-5.09	15.71
QTw.ncl-6D.2	6D	KarTw2	Xgwm459	Xgwm1103B	3.03	70.71	-4.07	21.21
QTw.ncl-7D.1	7D	PunTw1	Xgwm974	Xbarc184	4.81	28.81	0.33	8.88

Table 3.10:- Composite interval mapping for Tgw

QTL	Chromosome	Trait	Marker		LOD	Position	Additive	R^2 x 100
			Right	Left				
QTgw.ncl-1A.1	1A	PunTgw2	Xgwm234A	Xcfa2219	3.31	203.81	-1.03	7.48
QTgw.ncl-1B.1	1B	KotTgw1	Xgwm1078	Xgwm1130	5.23	44.71	-1.25	12.34
QTgw.ncl-1B.2	1B	KotTgw1	Xpsp3000	Xgwm1028	3.58	57.11	-1.87	27.12
QTgw.ncl-1B.3	1B	PunTgw2	Xwmc419	Xgwm806	7.07	225.71	1.4	15.17
QTgw.ncl-1D.1	1D	KotTgw1	Xgwm903	Xgwm4810	3.41	77.11	-1.05	7.85
QTgw.ncl-1D.2	1D	PunTgw2	Xgwm1012	Xgwm957	3.76	192.61	1.31	11.4
QTgw.ncl-2B.1	2B	KarTgw1	Xgwm1300B	Xgwm526A	3.05	205.51	1.06	8.35
QTgw.ncl-2B.2	2B	KarTgw2	Xgwm739	Xgwm1273	9.05	259.51	-2.42	26.52
QTgw.ncl-4B.1	4B	KotTgw2	Xgwm898	Xgwm113	5.75	2.01	1.16	14.64
QTgw.ncl-5A.1	5A	KarTgw2	Xbarc142	Xbarc232	3.09	158.21	1.32	7.53
QTgw.ncl-5A.2	5A	PunTgw2	Xbarc243	Xgwm4226	6.01	222.41	1.43	14.47
QTgw.ncl-5B.1	5B	PunTgw1	Xgwm443C	Xgwm234B	3.59	25.41	1.72	27.69
QTgw.ncl-5B.2	5B	KotTgw2	Xgwm843	Xgwm499A	3.67	79.01	-1.36	13.19
QTgw.ncl-5D.1	5D	KotTgw1	Xgwm4265B	Xgwm1462	3.46	84.71	-1.18	8.13
QTgw.ncl-6A.1	6A	KarTgw1	Xgwm1017	Xgwm427	3.71	126.31	-1.2	12.23
QTgw.ncl-6B.1	6B	PunTgw2	Xgwm1199B	Xbarc198	4.76	155.51	1.23	10.13
QTgw.ncl-7B.1	7B	KotTgw2	Xgwm400	Xgwm871	4.08	55.01	-0.94	9.75

Table 3.11:- Composite interval mapping for Sv

QTL	Chromosome	Trait	Marker		LOD	Position	Additive	R^2 x 100
			Right	Left				
QSev.ncl-1B.1	1B	KarSev2	Xgwm131	Xglu1B	3.59	180.21	-5.3	15.88
QSev.ncl-1B.2	1B	PunSev2	Xwmc419	Xgwm806	6.16	225.71	-1.21	14.88
QSev.ncl-1D.1	1D	KotSev1	Xgwm337	xgwm848	4.37	72.61	2.04	11.55
QSev.ncl-2A.1	2A	KarSev2	Xgwm830	Xgwm249A	3.35	24.01	-5.12	15.4
QSev.ncl-2B.1	2B	PunSev2	Xgwm120	Xgwm877	6.3	185.81	-1.12	16.24
QSev.ncl-3D.1	3D	KarSev1	Xgwm52	Xgwm664	4.15	7.71	2.35	14.14
QSev.ncl-4B.1	4B	PunSev2	Xgwm898	Xgwm1084	3.79	5.11	0.94	9.27
QSev.ncl-4B.2	4B	KarSev2	Xgwm935A	Xgwm6	3.21	54.51	1.61	7.14
QSev.ncl-5A.1	5A	PunSev2	Xbarc1	Xcfd20	4.12	86.91	-0.99	11.09
QSev.ncl-5B.1	5B	PunSev1	Xgwm540	Xgwm67	3.05	47.61	1.57	8.31
QSev.ncl-5D.2	5D	KarSev2	Xgwm736A	Xgwm1016	3.17	275.41	-5.01	14.5
QSev.ncl-6A.1	6A	PunSev2	Xgwm1009A	Xgwm1296	3.77	41.81	-1.68	8.47
QSev.ncl-6A.2	6A	KarSev2	Xgwm907	Xgwm1017	3.03	107.11	-4.9	15.86
QSev.ncl-6D.1	6D	KarSev2	Xcfd13	Xgwm1009C	3.9	12.01	-5.09	15.71

Table 3.12:- Composite interval mapping for Gpc

QTL	Chromosome	Trait	Marker		LOD	Position	Additive	R^2 x 100
			Right	Left				
QGpc.ncl-1B.1	1B	KarGpc1	Xpsp3000	Xgwm1028	3.12	63.11	0.45	20.73
QGpc.ncl-1B.2	1B	PunGpc1	Xbarc137	Xbarc187	3.64	88.41	0.23	11.04
QGpc.ncl-1B.3	1B	KarGpc1	Xcfd48A	Xglu1B	5.07	141.21	0.24	12.49
QGpc.ncl-1B.4	1B	KarGpc2	Xgwm806	Xbarc81	3.4	242.01	-0.28	9.97
QGpc.ncl-2A.1	2A	PunGpc2	Xgwm526B	Xgwm4166	3.25	144.61	0.18	7.88
QGpc.ncl-2B.1	2B	KotGpc2	Xgwm1128A	Xgwm429	6.68	48.61	-0.46	18.04
QGpc.ncl-3A.1	3A	KarGpc1	Xgwm369B	Xgwm369A	3.09	2.01	-0.39	25.44
QGpc.ncl-3D.1	3D	KarGpc2	Xgwm1266	Xgwm1088	3.53	79.81	0.26	8.55
QGpc.ncl-6A.1	6A	KotGpc2	Xgwm1296	Xgwm1150	8.08	84.51	0.47	18
QGpc.ncl-6A.2	6A	KarGpc2	Xgwm1150	Xgwm907	3.16	86.71	0.25	9.02
QGpc.ncl-7D.1	7D	PunGpc2	Xgwm735	Xbarc184	3.7	26.01	-0.22	11.14

Right and Left represent flanking markers to the corresponding QTL

[a]Nomenclature for QTLs in wheat: the Q for QTLs should be followed by a trait designator, a laboratory designator, a hyphen (-) and the symbol for the chromosome in which the QTL is located

[b]Positive value is associated with an increasing effect from HI 977alleles and negative value is associated with an increasing Kar- Karnal, Kot- Kota, Pun- Pune 1- 2003-04, 2-2004-05,

contribution (25.44 %) was from *QGpc.ncl-3A.1* with allele from HD2329, followed by *QGpc.ncl-1B.1* (20.73%) with allele from HI977.

3.5.5 QTLs for mixograph traits

The QTLs governing mixograph traits Epi, Mpt, Mri, Mrv, Mrw, Mti, Mtv, Mtw and Ws along with their additive effects and contribution are represented in Tables 3.13, 3.14, 3.15, 3.16, 3.17, 3.18, 3.19, 3.20 and 3.21, respectively. Among 169 QTLs identified for mixograph, 21 QTLs were detected on 6B, followed by 18 on 1B, 3A & 5B chromosomes, 17 on 6D, 13 on 2B and 10 on 2A & 5D chromosomes. The mixograph trait Ws had maximum QTLs (27) followed by Mpt (22), Mrw (22), Mtw (18), Epi (18), Mri (18), Mtv (18), Mrv (16) and Mti (10). The Mri had one QTL on Chromosome 1D (*QMri.ncl-1D.1*), which was consistent across three locations, followed by *QEpi.ncl-6D.1, QMpt.ncl-1D.2, QMpt.ncl-3A.1, QMpt.ncl-3A.2, QMrw.ncl-6B.2, QMti.ncl-5A.1, QMtw.ncl-1D.1, QMtw.ncl-5D.1, QMtw.ncl-6D.1, QMtw.ncl-6D.2, QWs.ncl-1B.2, QWs.ncl-2A.1, QWs.ncl-3A.1, QWs.ncl-6B.1, QWs.ncl-6B.2* and *QWs.ncl-6D.1* which were consistent across two locations.

3.5.6 QTLs for Lv

Of the total 30 QTLs, 15 were detected on chromosomes 1D, 2A, 2B, 3A, 5A, 5D, 6B and 6D explaining 5.93 to 22.5% phenotypic variation of Lv recorded in Karnal (Table 3.22). Similarly 9 QTLs were identified on chromosomes 1A, 1B, 2A, 2B, 5A, 5B, 5D and 7B that explained 6.5 to 36.71 % variance of Lv for Kota location. Also 9 unique QTLs were detected for Pune location on chromosomes 1B, 2B, 3A, 5A, 5D, 6B and 6D, explaining 5.8 to 44.6 % variance due to Lv. Contribution of the positive allele from poor parent was realized through chromosome 1A QTL *QLv.ncl-1A.1* for trait KotLv2 having negative additive effect. Two QTLs were detected on chromosome 1B, for the trait KotLv2 and PunLv2. Chromosome 1D had two QTLs for the trait KarLv1, which explained 10.8% and 7.3% genetic variance. The chromosome 2A, had 3 QTLs governing KarLv2 among which, two had positive additive effect and the third one had negative additive effect. Chromosome 2B had three QTLs each governing KarLv2, KotLv2 and PunLv2, with contribution 10.4%, 10.2% and 5.8%, respectively. Three QTLs were detected on chromosome 3A, two for KarLv2 and one for PunLv1, having 19.1%, 18.5% and 16.7% contribution, respectively. The traits KotLv2, KarLv1 and PunLv1, each had one QTL on chromosome 5A, while KotLv2 had two

Table 3.13:- Composite interval mapping for Envelope peak integral (Epi)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QEpi.ncl-1B.1	1B	KotEpi2	*Xpsp3000*	*Xgwm1028*	3.07	65.11	28.45	15.08
QEpi.ncl-1B.2	1B	KarEpi1	*Xgwm806*	*Xbarc81*	2.95	246.01	-5.74	7.78
QEpi.ncl-1B.3	1B	KotEpi2	*Xcfd48A*	*Xgwm131*	3.34	177.21	26.87	15.58
QEpi.ncl-1D.1	1D	KarEpi1	*Xgwm642*	*Xgwm1012*	5.94	177.01	11.28	24.33
QEpi.ncl-2A.1	2A	KotEpi2	*Xgwm830*	*Xgwm249A*	3.22	24.01	23.94	16.63
QEpi.ncl-2B.1	2B	KotEpi2	*Xgwm1273*	*Xgwm1264*	3.03	264.61	27.43	15.39
QEpi.ncl-2B.2	2B	PunEpi2	*Xgwm526A*	*Xbarc159*	3.42	226.31	-14.38	10.68
QEpi.ncl-3A.1	3A	KotEpi2	*Xgwm369B*	*Xgwm757*	3.05	34.41	26.91	15.53
QEpi.ncl-3A.2	3A	KotEpi2	*Xgwm638*	*Xgwm1243*	3.03	54.81	21.50	17.68
QEpi.ncl-3D.1	3D	PunEpi2	*Xgwm1300A*	*Xgwm1266*	4.42	35.71	-17.10	15.81
QEpi.ncl-4B.1	4B	KotEpi2	*Xgwm935A*	*Xgwm6*	5.76	32.51	10.75	16.64
QEpi.ncl-5A.1	5A	KarEpi2	*Xbarc232*	*Xbarc59*	3.33	167.41	7.92	15.34
QEpi.ncl-5B.1	5B	KotEpi2	*Xgwm443C*	*Xgwm234B*	4.11	25.41	11.52	25.49
QEpi.ncl-5B.2	5B	KotEpi2	*Xbarc88*	*Xgwm67*	3.75	68.41	26.41	15.82
QEpi.ncl-5B.3	5B	PunEpi2	*Xgwm544*	*Xgwm499A*	3.05	80.21	19.41	8.09
QEpi.ncl-6B.1	6B	KotEpi1	*Xbarc178*	*Xgwm921*	4.31	40.01	37.07	27.47
QEpi.ncl-6B.2	6B	KotEpi2	*Xgwm816*	*Xgwm1233*	3.17	162.51	28.21	15.16
QEpi.ncl-6D.1	6D	KarEpi2	*Xcfd13*	*Xgwm1009C*	3.07	12.01	22.36	20.75
	6D	KotEpi2	*Xcfd13*	*Xgwm459*	3.51	12.01	25.66	16.20

Table 3.14:- Composite interval mapping for Midline peak time (Mpt)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMpt.ncl-1A.1	1A	PunMpt2	*Xgwm1111*	*Xgwm4090*	5.17	117.61	0.52	20.55
QMpt.ncl-1B.1	1B	PunMpt1	*Xpsp3000*	*Xgwm1028*	4.24	65.11	-0.90	10.72
QMpt.ncl-1B.2	1B	PunMpt1	*Xcfd48A*	*GLU-B1*	4.05	180.21	-0.90	10.76
QMpt.ncl-1B.3	1B	PunMpt1	*Xcfd48B*	*Xgwm806*	3.46	233.01	0.23	5.86
QMpt.ncl-1D.1	1D	KarMpt1	*Xgwm642*	*Xgwm1012*	7.24	173.01	0.24	15.95
		PunMpt1	*GLU-D1*	*Xgwm1012*	9.47	175.01	0.42	23.68
QMpt.ncl-1D.2	1D	KarMpt2	*Xgwm1012*	*Xgwm957*	6.44	206.61	0.27	17.80
QMpt.ncl-2A.1	2A	PunMpt1	*Xgwm830*	*Xgwm249A*	4.06	24.01	-0.90	10.76
QMpt.ncl-2B.1	2B	KarMpt1	*Xgwm526A*	*Xbarc159*	4.18	226.31	0.18	9.84
QMpt.ncl-2B.2	2B	KotMpt1	*Xgwm1273*	*Xgwm1264*	3.82	290.61	0.74	26.21
QMpt.ncl-3A.1	3A	PunMpt1	*Xgwm369A*	*Xgwm1243*	4.21	46.41	-0.90	10.74
	3A	PunMpt2	*Xgwm638*	*Xgwm1243*	3.17	54.81	-1.08	13.98
QMpt.ncl-3A.2	3A	PunMpt1	*Xgwm1071B*	*Xgwm1038*	3.04	105.11	-1.05	8.03
	3A	KarMpt2	*Xgwm480*	*Xgwm1038*	4.32	107.11	0.40	23.56
QMpt.ncl-3D.1	3D	PunMpt2	*Xgwm1300A*	*Xgwm1266*	3.17	79.71	-0.30	5.89
QMpt.ncl-5B.1	5B	KotMpt1	*Xgwm443C*	*Xgwm234B*	3.12	15.41	0.75	21.47
QMpt.ncl-5B.2	5B	KotMpt1	*Xbarc88*	*Xgwm67*	3.46	66.41	0.74	22.19
QMpt.ncl-5B.3	5B	PunMpt1	*Xgwm639B*	*Xgwm499B*	4.04	114.81	-0.90	10.71
QMpt.ncl-5D.1	5D	PunMpt1	*Xgwm736B*	*Xgwm1016*	3.94	273.01	-0.90	10.75
QMpt.ncl-6A.1	6A	PunMpt2	*Xgwm334*	*Xgwm1009B*	3.65	14.01	-0.76	23.37
QMpt.ncl-6B.1	6B	PunMpt1	*Xbarc14*	*Xgwm1199C*	4.31	109.11	-0.90	10.75
QMpt.ncl-6B.2	6B	PunMpt1	*Xgwm816*	*Xgwm1233*	4.32	162.51	-0.90	10.73
QMpt.ncl-6D.1	6D	PunMpt2	*Xcfd13*	*Xgwm1009C*	3.82	12.01	-1.11	13.94
QMpt.ncl-6D.2	6D	PunMpt1	*Xcfd13*	*Xgwm1103B*	4.37	67.81	-0.90	10.75
QMpt.ncl-7D.1	7D	PunMpt1	*Xgwm735*	*Xgwm974*	4.00	26.01	0.27	9.74

Table 3.15:- Composite interval mapping for Midline right integral (Mri)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMri.ncl-1A.1	1A	KotMri2	*Xgwm1111*	*Xgwm4090*	4.27	125.61	17.53	23.75
QMri.ncl-1B.1	1B	PunMri1	*Xpsp3000*	*Xgwm1028*	3.38	65.11	-43.26	13.10
QMri.ncl-1D.1	1D	KarMri1	*GLU-D1*	*Xgwm1012*	4.80	168.31	10.33	13.61
	1D	KotMri1	*GLU-D1*	*Xgwm642*	5.17	172.31	13.69	12.68
	1D	PunMri1	*GLU-D1*	*Xgwm1012*	3.61	168.31	15.67	16.92
QMri.ncl-1D.2	1D	KarMri2	*Xgwm1012*	*Xgwm957*	6.79	206.61	10.15	17.18
QMri.ncl-2A.1	2A	PunMri1	*Xgwm830*	*Xgwm249A*	4.20	24.01	-39.38	16.09
QMri.ncl-2B.1	2B	KarMri1	*Xgwm526A*	*Xbarc159*	4.68	224.31	7.70	8.63
QMri.ncl-2B.2	2B	KarMri1	*Xgwm1273*	*Xgwm1264*	6.05	264.61	52.33	17.53
QMri.ncl-3A.1	3A	KotMri2	*Xgwm1071B*	*Xgwm1038*	4.44	109.11	21.27	29.96
QMri.ncl-4D.1	4D	PunMri1	*Xgwm194*	*Xgwm609*	4.87	38.71	15.27	15.93
QMri.ncl-5A.1	5A	KarMri2	*Xbarc232*	*Xbarc59*	4.61	159.41	7.95	10.28
QMri.ncl-5B.1	5B	PunMri1	*Xgwm443C*	*Xgwm234B*	3.35	15.41	-39.31	15.73
QMri.ncl-5B.2	5B	PunMri1	*Xbarc88*	*Xgwm67*	4.75	68.41	-39.62	16.05
QMri.ncl-5B.3	5B	PunMri1	*Xgwm639B*	*Xgwm499B*	3.44	114.81	-39.72	15.57
QMri.ncl-5D.1	5D	PunMri1	*Xgwm736B*	*Xgwm736A*	3.99	273.01	-38.69	16.13
QMri.ncl-6B.1	6B	PunMri1	*Xbarc178*	*Xgwm921*	3.82	40.01	-37.86	16.27
QMri.ncl-6B.2	6B	PunMri1	*Xgwm816*	*Xgwm1233*	3.85	162.51	-39.60	15.79
QMri.ncl-6D.1	6D	PunMri1	*Xgwm1009C*	*Xgwm1103B*	4.29	67.81	-39.08	16.08
QMri.ncl-7D.1	7D	KotMri2	*Xgwm350*	*Xgwm885*	3.13	73.61	12.23	9.42

Table 3.16:- Composite interval mapping for Midline right value (Mrv)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMrv.ncl-1B.1	1B	KotMrv1	*Xwmc406*	*Xbarc181*	3.45	70.91	1.06	13.33
QMrv.ncl-1A.2	1A	KotMrv2	*Xgwm4090*	*Xgwm234A*	3.22	156.91	-2.29	37.78
QMrv.ncl-2A.1	2A	PunMrv1	*Xgwm830*	*Xgwm249A*	4.46	8.01	-2.16	31.57
QMrv.ncl-2A.2	2A	KotMrv2	*Xgwm249A*	*Xgwm249B*	4.00	27.51	2.39	39.73
QMrv.ncl-2B.1	2B	KotMrv1	*Xgwm1128B*	*Xgwm1128A*	4.69	33.01	-2.20	39.96
QMrv.ncl-2B.2	2B	KotMrv1	*Xgwm1273*	*Xgwm1264*	4.15	296.61	-3.66	33.22
QMrv.ncl-3A.1	3A	KotMrv2	*Xgwm1071B*	*Xgwm1038*	3.14	107.11	2.59	27.14
QMrv.ncl-3D.1	3D	KarMrv1	*Xgwm1266*	*Xgwm1088*	3.80	79.81	1.05	9.38
QMrv.ncl-5A.1	5A	KotMrv2	*Xgwm443A*	*Xgwm415*	4.48	68.41	-1.69	17.65
QMrv.ncl-5B.1	5B	KotMrv2	*Xgwm443C*	*Xgwm234B*	3.89	17.41	2.82	30.45
QMrv.ncl-6A.1	6A	KotMrv2	*Xgwm334*	*Xgwm1040*	4.28	14.01	2.80	29.92
QMrv.ncl-6B.1	6B	KotMrv2	*Xbarc178*	*Xgwm132C*	3.35	36.01	2.73	30.53
QMrv.ncl-6B.2	6B	PunMrv1	*Xgwm1199C*	*Xgwm1199A*	3.84	116.01	-2.60	26.71
QMrv.ncl-6D.1	6D	KotMrv2	*Xcfd13*	*Xgwm1009C*	3.49	10.01	2.82	30.54
QMrv.ncl-6D.2	6D	KotMrv2	*Xgwm459*	*Xgwm1103B*	3.40	80.71	2.28	36.74
QMrv.ncl-6D.3	6D	KotMrv2	*Xgwm732B*	*Xgwm732A*	3.31	148.61	2.77	30.57

Table 3.17:- Composite interval mapping for Midline right width (Mrw)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMrw.ncl-1A.1	1A	PunMrw2	*Xgwm4090*	*Xgwm234A*	6.24	166.91	-7.20	26.92
QMrw.ncl-1B.1	1B	PunMrw2	*Xpsp3000*	*Xgwm1028*	5.65	65.11	8.78	29.13
QMrw.ncl-1B.2	1B	KarMrw1	*Xcfd48A*	*GLU-B1*	5.45	180.21	7.80	22.66
QMrw.ncl-1D.1	1D	PunMrw1	*Xgwm1012*	*Xgwm957*	3.08	188.61	-1.45	8.67
QMrw.ncl-2A.1	2A	PunMrw1	*Xgwm830*	*Xgwm249A*	4.80	4.01	-2.20	25.28
QMrw.ncl-2B.1	2B	KarMrw1	*Xbarc24*	*Xgwm1128B*	4.35	26.01	5.37	17.70
QMrw.ncl-2D.1	2D	KarMrw1	*Xgwm846A*	*Xgwm846B*	3.11	138.01	4.92	22.42
QMrw.ncl-3A.1	3A	KarMrw1	*Xgwm369B*	*Xgwm369A*	4.35	14.01	7.48	23.60
QMrw.ncl-3A.2	3A	KarMrw1	*Xgwm638*	*Xgwm1243*	3.00	54.81	5.32	21.63
QMrw.ncl-5A.1	5A	KarMrw1	*Xgwm1171*	*Xgwm443A*	3.51	46.01	-4.07	24.29
QMrw.ncl-5B.1	5B	KotMrw2	*Xgwm443C*	*Xgwm234B*	3.49	15.41	4.93	29.59
QMrw.ncl-5B.2	5B	KotMrw1	*Xgwm540*	*Xbarc88*	4.72	47.61	-2.63	11.70
QMrw.ncl-5B.3	5B	KotMrw2	*Xbarc88*	*Xgwm67*	3.46	68.41	5.76	25.87
QMrw.ncl-5D.1	5D	PunMrw2	*Xbarc143*	*Xgwm736B*	6.76	261.91	-6.00	32.56
QMrw.ncl-5D.2	5D	KotMrw2	*Xgwm736B*	*Xgwm1016*	3.62	273.01	5.39	30.22
QMrw.ncl-6A.1	6A	KotMrw2	*Xgwm334*	*Xgwm1040*	3.94	16.01	5.05	30.38
QMrw.ncl-6B.1	6B	KarMrw1	*Xbarc178*	*Xgwm921*	6.73	40.01	7.55	31.86
QMrw.ncl-6B.2	6B	KotMrw2	*Xbarc14*	*Xgwm1199C*	3.33	107.11	5.07	30.06
	6B	KarMrw1	*Xbarc14*	*Xgwm1199A*	3.45	112.01	4.63	20.08
QMrw.ncl-6B.3	6B	KotMrw1	*Xgwm816*	*Xbarc198*	4.24	162.51	8.08	19.40
QMrw.ncl-6D.1	6D	PunMrw2	*Xcfd13*	*Xgwm1103B*	7.11	12.01	8.78	29.13
QMrw.ncl-6D.2	6D	KotMrw1	*Xgwm732B*	*Xgwm732A*	5.15	148.61	7.56	24.84
QMrw.ncl-6D.3	6D	PunMrw2	*Xgwm732B*	*Xgwm1103A*	5.23	154.21	8.78	29.12

Table 3.18:- Composite interval mapping for Midline curve tail integral (Mti)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMti.ncl-1A.1	1A	KarMti1	*Xgwm234A*	*Xcfa2219*	5.71	199.81	-10.91	25.19
QMti.ncl-1B.1	1B	KotMti1	*Xpsp3000*	*Xgwm1028*	3.00	63.11	-19.49	19.87
QMti.ncl-1B.2	1B	KotMti1	*Xcfd48A*	*GLU-B1*	3.11	175.21	-21.20	19.59
QMti.ncl-2A.1	2A	PunMti1	*Xgwm830*	*Xgwm249A*	3.54	0.01	-9.69	10.71
QMti.ncl-2B.1	2B	KotMti1	*Xgwm1273*	*Xgwm1264*	4.58	296.61	-23.45	28.60
QMti.ncl-3A.1	3A	KotMti1	*Xgwm638*	*Xgwm1243*	3.17	54.81	-22.45	19.16
QMti.ncl-4D.1	4D	KotMti1	*Xgwm194*	*Xgwm609*	4.24	36.71	9.28	16.47
QMti.ncl-5A.1	5A	KarMti1	*Xgwm443A*	*Xgwm415*	3.02	66.41	-6.46	11.74
	5A	KotMti2	*Xgwm443A*	*Xgwm415*	6.72	68.41	-7.49	22.33
QMti.ncl-5A.2	5A	KarMti2	*Xbarc232*	*Xbarc59*	4.05	159.41	6.76	10.52
QMti.ncl-5B.1	5B	KotMti1	*Xbarc88*	*Xgwm67*	4.58	66.41	-18.19	23.48

Table 3.19:- Composite interval mapping for Midline curve tail value (Mtv)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMtv.ncl-1B.1	1B	KarMtv1	*Xpsp3000*	*Xgwm1028*	3.02	65.11	-5.19	13.66
QMtv.ncl-1D.1	1D	KotMtv2	*Xgwm1012*	*Xgwm957*	3.72	190.61	1.10	14.56
QMtv.ncl-2A.1	2A	PunMtv1	*Xgwm830*	*Xgwm249B*	6.55	24.01	-9.41	32.96
QMtv.ncl-2B.1	2B	KotMtv1	*Xgwm526A*	*Xbarc159*	4.66	224.31	-1.00	10.58
QMtv.ncl-2B.2	2B	PunMtv1	*Xgwm1273*	*Xgwm1264*	7.24	266.61	-9.36	32.99
QMtv.ncl-2D.1	2D	PunMtv1	*Xgwm846A*	*Xgwm846B*	4.92	138.01	-9.33	27.82
QMtv.ncl-3A.1	3A	PunMtv2	*Xgwm369B*	*Xgwm369A*	5.38	0.01	6.47	12.69
QMtv.ncl-3A.2	3A	PunMtv1	*Xgwm369B*	*Xgwm1243*	7.39	26.41	-9.34	33.00
QMtv.ncl-3A.3	3A	PunMtv1	*Xgwm480*	*Xgwm1071B*	4.31	104.01	-8.93	33.82
QMtv.ncl-3A.4	3A	PunMtv2	*Xgwm1071B*	*Xgwm1038*	7.06	109.11	-1.99	30.87
QMtv.ncl-5B.1	5B	KotMtv1	*Xgwm544*	*Xgwm499A*	3.78	88.21	-1.43	11.31
QMtv.ncl-5B.2	5B	PunMtv1	*Xgwm639B*	*Xgwm499B*	5.60	114.81	-9.42	32.97
QMtv.ncl-5D.1	5D	PunMtv1	*Xgwm736B*	*Xgwm1016*	7.47	273.01	-9.35	32.99
QMtv.ncl-6B.1	6B	PunMtv1	*Xbarc178*	*Xgwm132B*	7.15	40.01	-9.32	33.01
QMtv.ncl-6B.2	6B	KarMtv1	*Xgwm816*	*Xgwm1233*	3.03	162.51	-5.19	13.66
QMtv.ncl-6D.1	6D	PunMtv1	*Xgwm459*	*Xgwm1103B*	7.34	70.71	-9.34	32.99
QMtv.ncl-6D.2	6D	PunMtv1	*Xgwm732B*	*Xgwm1103A*	5.86	148.61	-8.98	33.63
QMtv.ncl-7D.1	7D	KotMtv1	*Xgwm974*	*Xbarc184*	3.38	58.81	-0.78	8.03

Table 3.20:- Composite interval mapping for Midline curve tail width (Mtw)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QMtw.ncl-1B.1	1B	PunMtw1	*Xpsp3000*	*Xgwm1028*	3.48	65.11	11.82	15.85
QMtw.ncl-1B.2	1B	PunMtw1	*Xgwm131*	*GLU-B1*	4.53	180.21	9.54	22.06
QMtw.ncl-1B.3	1B	KarMtw2	*Xcfd48A*	*Xgwm131*	3.48	175.21	-5.70	25.22
QMtw.ncl-1D.1	1D	KotMtw1	*Xgwm642*	*Xgwm1012*	4.60	173.01	2.06	12.71
	1D	KarMtw2	*Xgwm642*	*Xgwm1012*	3.62	173.01	2.45	9.31
QMtw.ncl-2A.1	2A	KarMtw1	*Xgwm526B*	*Xgwm4166*	4.67	144.61	-1.90	13.39
QMtw.ncl-3A.1	3A	PunMtw1	*Xgwm369B*	*Xgwm369A*	4.42	14.01	11.56	20.01
QMtw.ncl-3A.2	3A	PunMtw1	*Xgwm480*	*Xgwm1038*	6.06	107.11	7.08	30.66
QMtw.ncl-3D.1	3D	KarMtw2	*Xgwm1300A*	*Xgwm1266*	3.94	41.71	-2.34	15.91
QMtw.ncl-4D.1	4D	KotMtw1	*Xgwm194*	*Xgwm609*	4.83	40.71	1.96	13.29
QMtw.ncl-5D.1	5D	PunMtw1	*Xgwm736B*	*Xgwm1016*	4.56	275.41	11.63	19.97
	5D	KarMtw2	*Xgwm736B*	*Xgwm736A*	3.94	273.01	-5.64	22.78
QMtw.ncl-5D.2	5D	KarMtw1	*Xgwm736A*	*Xgwm1016*	5.62	295.41	2.28	18.13
QMtw.ncl-6A.1	6A	KotMtw1	*Xgwm1009B*	*Xgwm1040*	3.15	19.51	3.47	23.74
QMtw.ncl-6A.2	6A	KarMtw2	*Xgwm334*	*Xgwm1009B*	2.97	16.01	-5.57	22.53
QMtw.ncl-6B.1	6B	PunMtw1	*Xbarc178*	*Xgwm132C*	4.50	40.01	11.13	20.23
QMtw.ncl-6B.2	6B	PunMtw1	*Xbarc14*	*Xgwm1199C*	4.20	109.11	11.73	19.96
QMtw.ncl-6B.3	6B	PunMtw1	*Xgwm816*	*Xgwm1233*	3.57	162.51	11.82	15.84
QMtw.ncl-6D.1	6D	PunMtw2	*Xcfd13*	*Xgwm1009C*	3.41	6.01	-1.80	30.02
	6D	PunMtw1	*Xcfd13*	*Xgwm1009C*	5.39	12.01	11.39	24.41
QMtw.ncl-6D.2	6D	KarMtw2	*Xgwm1009C*	*Xgwm459*	3.13	14.91	-5.57	22.86
	6D	PunMtw1	*Xgwm459*	*Xgwm1103B*	4.31	70.71	11.10	20.18

Table 3.21:- Composite interval mapping for Weakening slope (Ws)

QTLs	Chromosome	Trait	Left marker	Right marker	LOD	Position	Additive	R^2x 100
QWs.ncl-1A.1	1A	KarWs2	*Xgwm4090*	*Xgwm234A*	5.23	158.91	-5.96	25.29
QWs.ncl-1B.1	1B	PunWs2	*Xpsp3000*	*Xgwm1028*	16.93	65.11	15.91	16.87
QWs.ncl-1B.2	1B	KarWs1	*Xcfd48A*	*GLU-B1*	10.89	177.21	6.02	24.99
		PunWs2	*Xcfd48A*	*Xgwm131*	16.69	177.21	15.91	16.87
QWs.ncl-1D.1	1D	KarWs2	*Xgwm642*	*Xgwm1012*	4.46	173.01	-2.24	9.57
QWs.ncl-2A.1	2A	KarWs1	*Xgwm830*	*Xgwm249A*	9.83	24.01	6.02	24.98
		PunWs1	*Xgwm830*	*Xgwm249B*	5.53	27.51	5.01	26.91
QWs.ncl-2B.1	2B	KarWs1	*Xbarc24*	*Xgwm1128B*	3.32	26.01	6.22	15.39
QWs.ncl-3A.1	3A	KarWs1	*Xgwm369B*	*Xgwm1243*	10.69	46.41	6.02	24.99
		KotWs1	*Xgwm638*	*Xgwm1243*	3.18	54.81	3.22	21.46
QWs.ncl-3A.3	3A	KarWs2	*Xgwm480*	*Xgwm1071B*	3.06	100.01	1.80	6.10
QWs.ncl-3A.4	3A	PunWs2	*Xgwm1071B*	*Xgwm1038*	16.31	105.11	15.91	16.87
QWs.ncl-5A.1	5A	PunWs1	*Xgwm1171*	*Xgwm415*	6.04	44.01	-5.58	25.16
QWs.ncl-5A.2	5A	PunWs1	*Xbarc232*	*Xbarc59*	3.15	173.41	-2.93	14.97
QWs.ncl-5B.1	5B	KarWs1	*Xgwm443C*	*Xgwm234B*	10.17	15.41	6.02	24.99
QWs.ncl-5B.2	5B	PunWs2	*Xgwm639B*	*Xgwm499B*	16.78	112.81	15.91	16.87
QWs.ncl-5D.1	5D	PunWs1	*Xgwm736B*	*Xgwm1016*	5.82	271.01	5.14	30.18
QWs.ncl-5D.2	5D	KarWs2	*Xgwm736B*	*Xgwm736A*	5.51	273.01	6.92	31.50
QWs.ncl-5D.3	5D	PunWs2	*Xgwm736A*	*Xgwm1016*	15.83	273.41	15.91	16.87
QWs.ncl-6A.1	6A	KarWs1	*Xgwm334*	*Xgwm1009B*	10.07	16.01	6.03	24.99
QWs.ncl-6A.2	6A	PunWs2	*Xgwm1009B*	*Xgwm1040*	16.12	17.51	15.91	16.87
QWs.ncl-6B.1	6B	PunWs1	*Xbarc178*	*Xgwm132C*	6.13	38.01	5.95	24.49
		KarWs1	*Xbarc178*	*Xgwm921*	10.53	48.11	6.02	24.99
QWs.ncl-6B.2	6B	PunWs2	*Xbarc178*	*Xgwm132B*	16.72	52.11	15.91	16.87
		PunWs1	*Xgwm921*	*Xgwm132B*	2.98	62.01	-4.53	26.41
QWs.ncl-6B.3	6B	KarWs1	*Xbarc14*	*Xgwm1199A*	10.80	109.11	6.02	24.99
QWs.ncl-6B.4	6B	PunWs2	*Xgwm816*	*Xgwm1233*	16.96	162.51	15.91	16.87
QWs.ncl-6B.5	6B	KarWs1	*Xgwm1233*	*Xbarc198*	8.96	162.61	6.03	24.99
QWs.ncl-6D.1	6D	KarWs1	*Xgwm459*	*Xgwm1103B*	10.13	70.71	6.02	24.98
	6D	PunWs1	*Xgwm1009C*	*Xgwm1103B*	5.97	72.71	5.87	24.61
QWs.ncl-6D.2	6D	PunWs2	*Xgwm732B*	*Xgwm732A*	10.40	138.61	-10.71	24.38
QWs.ncl-6D.3	6D	KarWs1	*Xgwm732A*	*Xgwm1103A*	9.84	154.21	6.03	24.99
QWs.ncl-7B.1	7B	KotWs1	*Xgwm400*	*Xgwm46*	3.54	31.61	-1.11	13.40

Right and Left represent flanking markers to the corresponding QTL

[a]Nomenclature for QTLs in wheat: the Q for QTLs should be followed by a trait designator, a laboratory designator, a hyphen (-) and the symbol for the chromosome in which the QTL is located

[b]Positive value is associated with an increasing effect from HI 977 alleles and negative value is associated with Kar- Karnal, Kot- Kota, Pun- Pune 1- 2003-04, 2-2004-05,

Table 3.22:- Composite interval mapping for Lv

QTL	Chromosome	Trait	Marker		LOD	Position	Additive	R^2x 100
			Right	Left				
QLv.ncl-1A.1	1A	KotLv2	*Xbarc148*	*Xgwm691A*	3.19	83.81	-12.31	13.5
QLv.ncl-1B.1	1B	PunLv2	*Xgwm1078*	*Xgwm1130*	2.14	46.71	6.24	6.07
QLv.ncl-1B.2	1B	KotLv2	*Xgwm1028*	*Xwmc406*	3.32	67.31	29.07	29.18
QLv.ncl-1D.1	1D	KarLv1	*Xgwm4063*	*Xgwm337*	4.4	72.61	9.02	10.82
QLv.ncl-1D.2	1D	KarLv1	*Xgwm903*	*Xcfd83*	2.81	79.11	7.54	7.33
QLv.ncl-2A.1	2A	KarLv2	*Xgwm830*	*Xgwm249A*	3	24.01	37.11	20.14
		KotLv2	*Xgwm830*	*Xgwm249A*	2.05	22.01	29.2	30.33
QLv.ncl-2A.2	2A	KarLv2	*Xgwm1045*	*Xcfa2263*	2.07	111.41	-11	9.89
QLv.ncl-2A.3	2A	KarLv2	*Xgwm1115*	*Xgwm761*	2.94	61.21	11.67	9.21
QLv.ncl-2B.1	2B	KotLv2	*Xgwm1128*	*Xgwm429*	2.24	54.61	-10.78	10.2
QLv.ncl-2B.2	2B	KarLv2	*Xgwm501*	*Xgwm1300B*	2.86	64.11	11.99	10.48
QLv.ncl-2B.3	2B	PunLv2	*Xgwm1300B*	*Xgwm526A*	2.3	205.51	6.17	5.85
QLv.ncl-3A.1	3A	KarLv2	*Xgwm369B*	*Xgwm369A*	2.49	14.01	38.24	19.14
QLv.ncl-3A.2	3A	KarLv2	*Xgwm757*	*Xgwm638*	2.34	46.41	39.2	18.49
QLv.ncl-3A.3	3A	PunLv1	*Xgwm1071B*	*Xgwm1038*	2.17	109.11	10.81	16.72
QLv.ncl-5A.1	5A	KotLv2	*Xgwm415*	*Xgwm4879*	2.68	70.41	10.08	6.5
QLv.ncl-5A.2	5A	KarLv1	*Xgwm156*	*Xcfd20*	2.17	92.31	6.24	5.93
QLv.ncl-5A.3	5A	PunLv1	*Xcfd20*	*Xbarc142*	2.13	135.11	10.71	17.85
QLv.ncl-5B.1	5B	KotLv2	*Xbarc88*	*Xgwm67*	6.22	66.41	31.36	36.71
QLv.ncl-5B.2	5B	KotLv2	*Xgwm443C*	*Xgwm234B*	2.05	27.41	-14.18	14.47
QLv.ncl-5D.1	5D	PunLv1	*Xbarc58*	*Xgwm1559*	2.31	28.01	-7.75	9.62
QLv.ncl-5D.2	5D	KarLv1	*Xgwm190*	*Xgwm4265B*	2.67	83.61	6.89	6.99
		KotLv1	*Xgwm190*	*Xgwm4265B*	2.01	77.61	9.04	9.59
QLv.ncl-5D.3	5D	KarLv2	*Xgwm736B*	*Xgwm1016*	2.95	275.41	34.94	22.49
QLv.ncl-6B.1	6B	KarLv2	*Xbarc178*	*Xgwm132C*	2.62	40.01	35.33	20.55
QLv.ncl-6B.2	6B	PunLv1	*Xgwm921*	*Xgwm132B*	2.77	58.01	17.02	44.11
QLv.ncl-6B.3	6B	KarLv2	*Xgwm1199C*	*Xgwm1199A*	3.67	112.01	29.6	22.33
QLv.ncl-6B.4	6B	KarLv1	*Xgwm1233*	*Xbarc198*	2.22	168.61	-10.68	17.93
QLv.ncl-6D.1	6D	KarLv2	*Xcfd13*	*Xgwm1009C*	3.47	12.01	37.21	20.22
		PunLv2	*Xcfd13*	*Xgwm1009C*	7.07	4.01	14.93	36.06
QLv.ncl-6D.2	6D	PunLv1	*Xgwm459*	*Xgwm1103B*	3.3	98.71	-16.51	44.2
QLv.ncl-6D.3	6D	PunLv1	*Xgwm732B*	*Xgwm732A*	3.11	142.61	-17.11	44.69
QLv.ncl-7D.1	7D	KotLv2	*Xgwm350*	*Xgwm885*	3.53	99.61	-14.51	15.03

Right and Left represent flanking markers to the corresponding QTL

[a]Nomenclature for QTLs in wheat: the Q for QTLs should be followed by a trait designator, a laboratory designator, a hyphen (-) and the symbol for the chromosome in which the QTL is located

[b]Positive value is associated with an increasing effect from HI 977 alleles and negative value is associated with an increasing effect from HD 2329 alleles

KarLv1- Karnal loaf volume 2003-04, KarLv2 -Karnal loaf volume 2004-05, KotLv1-Kota loaf volume 2003-04, KotLv2 Kota loaf volume 2004-05, PunLv1-Pune loaf volume 2003-04 and PunLv2-Pune loaf volume 2004-05

QTLs on chromosome 5B. Chromosome 5D had three QTLs for PunLv1, KarLv1, KotLv1 and KarLv2. The QTL (*QLv.ncl-5D.2*) governed both KarLv1 and KotLv1, with contribution of 6.99 and 9.59% respectively. Four QTLs were identified on chromosome 6B of which two were for KarLv2, one for PunLv1 with positive additive effect and one for KarLv1 with negative additive effect.

Three QTLs were identified on chromosome 6D, *QLv.ncl-6D.1* for KarLv2 and PunLv2 and two QTLs were detected on the same chromosome for PunLv1. A representative figure indicating 6D QTL using QTL cartographer software has been depicted in Fig. 3.14. A single QTL was observed on chromosome7D for KotLv2 explaining 15.03% genetic variance. The QTL *QLv.ncl-3A.1* on chromosome 3A appeared to be a putative common QTL for KarLv1 and KarLv2, as the LOD value for KarLv1 was below the threshold level (2.0) (data not shown). Similarly a putative common QTL for KotLv1 and KotLv2 was detected on chromosome 5D (*QLv.ncl-5D.2*) (data not shown). PunLv1 and PunLv2 had one putative common QTL on chromosome 6D (*QLv.ncl-6D.1*) (data not shown).

3.6 QTL clusters formed by QTLs affecting yield, dough rheology and BMQ traits

A total of 45 QTL clusters were detected on 14 chromosomes (Table 3.23) and the QTL positions in each chromosome were depicted in Fig. 31.0, 3.11, 3.12 and 3.13. About, 6 clusters were identified on chromosomes 1B followed by 5 (5B), 4 (1D, 2B, 3A, 6B, 6D), 3 clusters (1A, 5A, 5D) and 1 (2A, 3D, 4B, 4D, 6A). A cluster on chromosome 6D had a maximum of 12 QTLs, while 10 QTLs were identified in the cluster of 2A chromosome. Mixograph traits often colocated together, than with other traits and three traits with Ws, Mrv and Mrw clustered often with Lv trait. In chromosome 2A *QLv.ncl-2A.1* colocated with Sv, Epi, Mpt, Mri, Mrv, Mtw, Mti and Ws and in chromosome 6D *QLv.ncl-6D.1* with Tw, Sv, Epi, Mpt, Mrv and Mrw. Also, Tw QTLs cluster more often colocated with Sv than with others, while the QTL cluster of Tgw and Sv, was identified only on chromosome 1B.

Table 3.23:- QTL clusters governing BMQ traits

Chromosome	Marker		Traits
	Right	Left	
1D	*Xgwm903*	*Cfd83*	Lv, Tgw
	Glu-D1	*Xgwm642*	Tw, Mri
	Xgwm642	*Xgwm1012*	Epi, Mpt, Mtw, Ws
	Xgwm1012	*Xgwm957*	Tgw, Mpt, Mri, Mrw, Mtv
2A	*Xgwm830*	*Xgwm249A*	Lv, sv, Tw, Epi, Mpt, Mri, Mrv, Mrw, Mti, Mtv, Ws
	Xgwm526B	*Xgwm4166*	Gpc, Mtw
2B	*Xbarc24*	*Xgwm1128B*	Mrw, Tw
	Xgwm1128B	*Xgwm1128A*	Mrv, Tw
	Xgwm1128A	*Xgwm429*	Lv, Tw, Gpc
	Xgwm1300B	*Xgwm526A*	Lv, Tgw
	Xgwm526A	*Xbarc159*	Epi, Mpt, Mri, Mtv
	Xgwm739	*Xgwm1264*	Epi, Mpt, Mri, Mrv, Mti, Mtv
1A	*Xgwm1111*	*Xgwm4090*	Mri, Mpt
	Xgwm4090	*Xgwm234A*	Ws, Mrw, Mrv
	Xgwm234A	*Xcfa2219*	Tgw, Mti
1B	*Xgwm1130*	*Xpsp3000*	Lv, Tgw
	Xpsp3000	*Xgwm1028*	Gpc, Tgw, Epi, Mpt, Mri, Mrw, Mti, Mrv, Mtw, Ws
	Xcfd48A	*Xgwm131*	Epi, Ws
	Xcfd48A	*GLU-B1*	Gpc, Mpt, Mrw, Mti
	Xgwm131	*GLU-B1*	Sv, Tw, Mtw
	Xgwm806	*Xbarc81*	Gpc, Epi
3A	*Xgwm369B*	*Xgwm369A*	Lv, Gpc, Mrw, Mtv
	Xgwm638	*Xgwm1243*	Epi, Mrw, Mti, Ws, Mtv
	Xgwm480	*Xgwm1071B*	Mtv, Ws
	Xgwm1071B	*Xgwm1038*	Lv, Mri, Mrv, Mtv, Mtw, Ws
5A	*Xgwm1171*	*Xgwm443A*	Mrw, Lv
	Xgwm443A	*Xgwm4879*	Mrv, Mti
	Xgwm156	*xcfd20*	Lv, Sv
	Xbarc232	*Xbarc59*	Epi, Mti, Ws
5B	*Xgwm443C*	*Xgwm234B*	Lv, Tgw, Epi, Mpt, Mri, Mrv, Mrw
	Xgwm540	*Xbarc88*	Tw, Mrw
	Xbarc88	*Xgwm67*	Lv, Mpt, Mri, Mti
	Xgwm544	*Xgwm499A*	Tgw, Epi, Mtv
	Xgwm639B	*Xgwm499B*	Mpt, Mri, Mtv, Ws
5D	*Xgwm736B*	*Xgwm1016*	Lv, Mpt, Mrw, Mtv, Mtw, Ws
6D	*Xcfd13*	*Xgwm1009C*	Lv, Tw, Sv, Epi, Mpt, Mrv, Mtw
	Xgwm1009C	*Xgwm1103B*	Mpt, Mri, Mrw, Ws
	Xgwm459	*Xgwm1103B*	Lv, Tw, Mrv, Mtv, Mtw, Ws
	Xgwm732B	*Xgwm732A*	Lv, Mrv, Ws
	Xgwm732B	*Xgwm1103A*	Mrw, Mtv

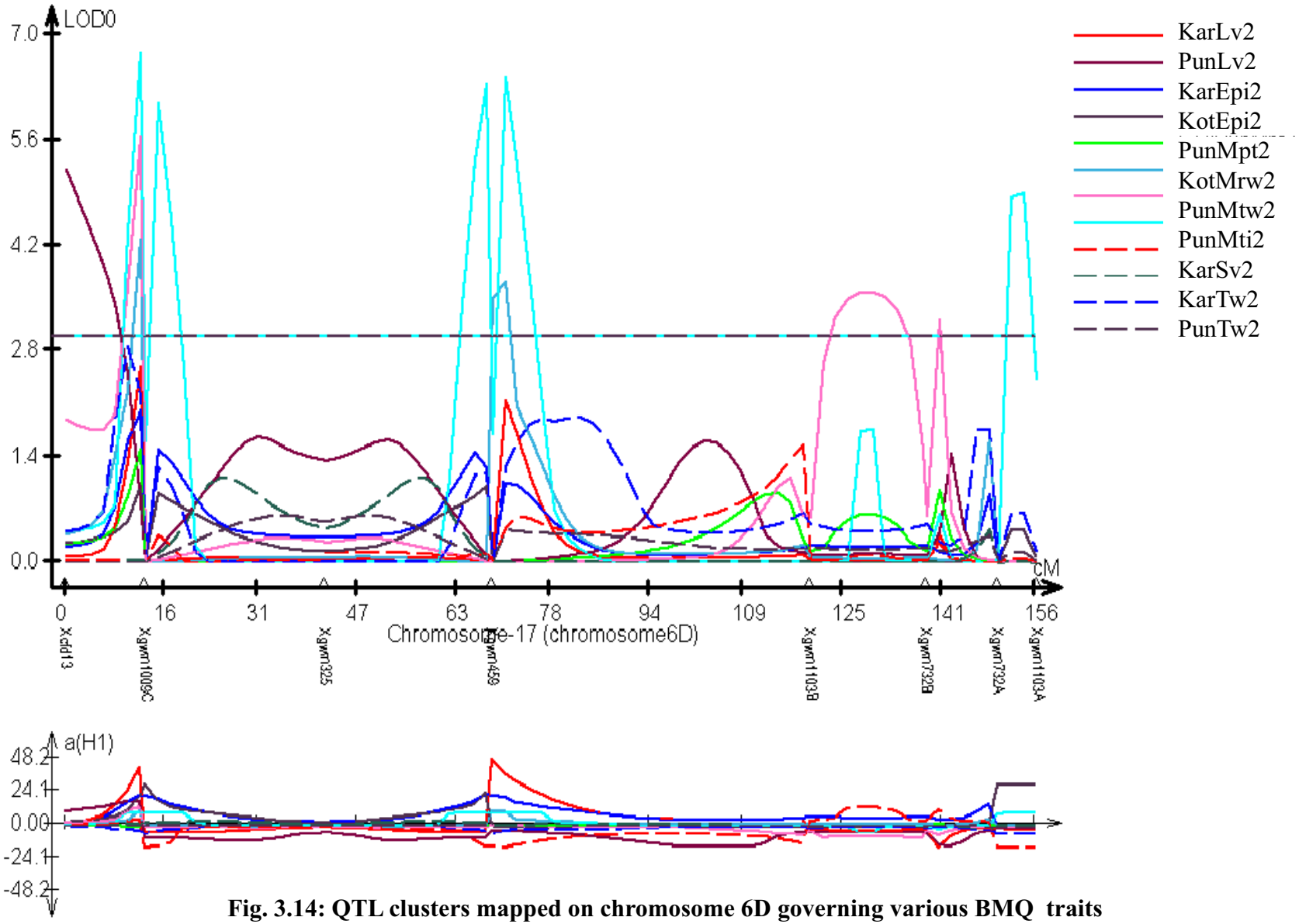
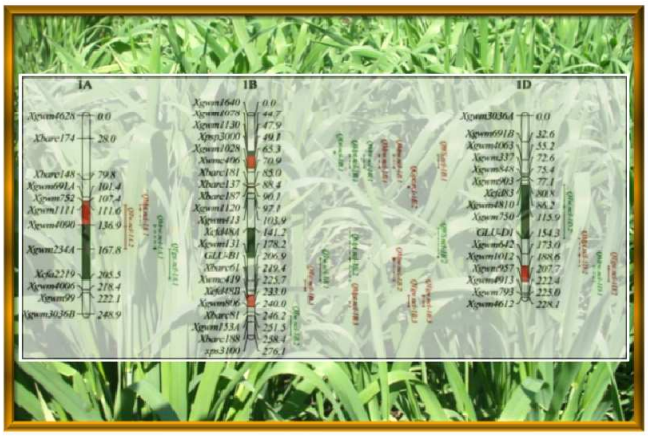


Fig. 3.14: QTL clusters mapped on chromosome 6D governing various BMQ traits

Chapter 4

Discussion



A part of the research work described in this chapter has been published as a full-length paper in Journal of Cereal Science and communicated to Plant Breeding

Many advances have been accomplished during the past decade, in the construction of molecular maps for crop plants using various molecular marker tools such as RFLP, RAPD, ISSR, SSR, AFLP and SNP (Subudhi and Nguyen, 2004). These maps play an important role in the genetic analysis of agronomic traits including QTL analysis, dissecting QTLs into individual components and map-based gene cloning. However, the availability of linkage maps using intervarietal cross with QTL positions of economic traits is limited in the polyploid species like wheat. Construction of linkage maps based on codominant SSR markers, helps in detecting good polymorphism, validation in other populations and easy comparison with existing maps as compared to maps with many dominant markers such as AFLP. Furthermore, intraspecific maps with codominant markers are usually considered to be suitable and preferred for MAS against desirable traits located on specific chromosomal region (Torado et al. 2006). We constructed a framework map using an intraspecific cross, in order to dissect BMQ and yield related traits in hexaploid wheat.

4.1 Features of the framework map

The present map reported in this work has been constructed including both SSR markers and HMW glutenin (protein marker), to create a functional map with reference to glutenin. Map construction using intraspecific populations in the present case was rather tedious because of lower rate of polymorphism compared to interspecific populations. Among the different sets of primers used for parental survey, SSR markers proved to be more polymorphic (Table 3.1) in this cross, which agrees with the fact that the SSR markers reveal polymorphism due to variation in the lengths of microsatellites at specific individual loci due to various mechanisms such as strand slippage, duplication/deletion of repeats etc. as compared to other markers (Gupta and Varshney 2000).

Recently, Groos et al. (2002), Sourdille et al. (2003) and Paillard et al. (2003) attempted to construct linkage maps with SSR and RFLP markers in intraspecific populations of common wheat, which spanned a total map length of 2,360, 3,685 and 3,086 cM, respectively. The map length reported in the present study (3,161.8 cM) was comparatively less than the previously published maps of distance (3,551 cM) reported by Nelson et al. (1995*a*, 1995*b*, 1995*c*), Van Deynze et al. (1995), and Marino et al. (1996), of map distance 4,110 cM reported by Chalmers et al. (2001) and of distance 3,685cM reported by Sourdille et al. (2003). This could be due to the

formation of only 19 linkage groups instead of 21 groups in the present map. However, even with 19 linkage groups, it was longer than the consensus map (2,569 cM) of Somers et al. (2004) and other maps (3,086 cM) of Paillard et al. (2003) and (2,360 cM) of Groos et al. (2002), which might be due to linkage gap and low marker density compared to the consensus map as well as the other maps detailed above.

In our present map skewed markers represented higher proportion (about 41.17 %) of the total markers. Framework maps with skewed markers have been constructed in wheat by Suenaga et al. (2005) and Nachit et al. (2001). Also, molecular markers representing skewed segregation have been reported earlier in several Triticeae species (Blanco et al. 1998; Heun et al. 1991; Liu and Tsunewaki, 1991). These distortions could be due to 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 the single-seed descent method (Nachit et al. 2001). It has been reported that the distorted loci may lead to spurious linkages and a reduced estimate of recombination value (Kammholz et al. 2001). Therefore, the unskewed markers were first used to construct linkage groups in the present case and then the skewed markers were introduced, there by eliminating the possibility of spurious linkage by distorted loci. Secondly, the order of the loci was checked using 'ripple' command and compared with those in the reported maps. The order was observed to be the same with a few exceptions even at a high LOD value of 3.0. The present map showed mean interval of 15 cM between the two loci and uniform distribution of markers indicating usefulness of the map for QTL analysis (Campbell et al.1999; He et al. 2001; Suenaga et al. 2005). In future, linkage map construction, with inclusion of markers tagging known functional genes like EST derived SSR, would be desirable and useful. It could take genetic mapping to a functional level, in exploration and tagging of agronomic traits with known genes.

4.2 Precise phenotyping at multiple locations during multiple years

Growing genotypes under well-adapted conditions with strong phenotypic expression can lead to over estimation of the genetic component and it could be avoided by including contrasting environments and seasons in which observations are made (Moralejo et al. 2004). In accordance, the experimental material consisting of RIL

population developed with the cross HI977 x HD2329 was grown in three different agroclimatic conditions in India for two consecutive years. Variation in agroclimatic conditions of these locations included variation in sowing and harvesting dates, rainfall, average temperature, temperature during grain filling, soil types etc. leading to phenotype estimations at environment representing all the wheat producing zones in India. Measurable characters contributing to BMQ were further considered for precise quantification of the phenotypic traits (Nelson et al. 2006), which is a basic requirement in any QTL analysis.

4.3 Normal distribution of BMQ and yield traits

The population means for Tw, Tgw, Sv, Gpc , Lv and mixograph traits in all the environments posed a normal distribution (Table 3.2), without skewing towards either of the parents, suggesting absence of epistatic effects between the QTLs (Blanco et al. 2006). The RIL population developed for the present study showed both positive and negative transgressive segregants, suggesting the possibility of finding positive BMQ alleles in the parents with poor BMQ (HD2329) while negative alleles in the parent with better BMQ (HI977). This was especially witnessed in the Mixograph parameters, where transgressive segregants in wide range were identified for Ws (0.11 to 77.88) and Epi (1.24 to 188.80) (Table 3.3). Such segregants for various BMQ traits were reported for Gpc (Dholakia et al. 2001; Nelson et al. 2006; Huang et al. 2006) , seed size and shape (Ammiraju et al. 2001) and mixograph traits (Huang et al. 2006).

4.4 Correlation between the traits

Correlations between the specific traits for two years at one location, for various locations per year and among the traits were analysed (Table 3.4 and 3.5). Significant and positive correlation between Tgw trait was recorded at Kota and Karnal locations, but the correlation was insignificant between PunTgw2 and KotTgw2; also the correlation was insignificant between datasets recorded at same location for different years. However, Kumar et al. (2006) reported a significant and positive correlation between Tgw recorded at 6 different locations. It is likely that, the locations in which our population was grown belong to three different agroclimatic zones and the variation in climatic conditions in subsequent years cannot be ruled out. Similar trend was observed in case of Gpc, however, positive and significant correlation was observed within Tw traits recorded at Kota and Pune. For Sv, significant correlation

was observed only in Karnal location, while it was insignificant among the other two locations. Low correlation among the Lv traits at the same location and lower rank correlation in the year 2003-04 as compared to 2004-05 suggested influence of environment on these traits. Similar influence of environment on genotypes was recently reported by Fufa et al. (2005) and Tarakanovos and Ruzgas (2006) in case of wheat yield.

4.5 GxE interactions contribute higher than environment alone for Lv

In order to understand the variation due to environment, AMMI analysis was performed to convert GxE into further components. Previous studies on predictive assessment revealed that AMMI with only two interaction principal component axes was the best predictive model (Zobel et al. 1988). The variation due to genotype was higher for Sv and Lv (28.73%, 38%), while it was less for Tgw, Tw and Gpc with 7.9%, 4.5% and 9.11%, respectively. Sv and Lv constituted a minimum variation of 23% and 15%, respectively due to the environment alone while it was greater than 60% for Tgw, Tw and Gpc. The variation due to environment for Tgw, Tw and Gpc, shows that the heritability for these traits is affected, when grown in different agroclimatic zones. Selection for these traits, by phenotype, will often lead to failure and therefore, selection using molecular markers is desired. In case of Lv, IPCA 1, which was the major component of GxE, explained only 22%, followed by AMMI component 2 (12%), indicating the poor fit of this model to help in selection of a single stable genotype for Lv. Similar situation with high environmental variation, compared to treatments (genotypes) was reported by Tarakanovos and Ruzgas (2006) in case of wheat varieties. In our study, significant effect of GxE to total variation (47%) was observed for Lv (Table 3.6), which was three times compared to variation due to environment (15 %). This GxE interaction was not due to rainfall (data not shown), as the experiments were carried out under well irrigated condition. The effect of rainfall was estimated by grouping the data set based on rainfall data and performing AMMI analysis (data not shown). It was identified that though the variation due to rainfall existed, still, it could not be considered as the only important factor contributing to GxE variation. Moreover, correlation studies revealed that, KarLv2 did not show any correlation with PunLv2 and KotLv2 and PunLv2 depicted low correlation with all the other locations for both the years. This further confirmed the role of environmental variation in the trait values. Therefore, pooled QTL analysis

was not performed on all the traits and the data sets were analysed separately. Such an analysis necessitates identification of QTLs across different environments and molecular markers for these QTLs can be exploited in breeding programme to develop region specific genotypes. Other studies have shown that molecular markers can be effectively deployed to aid in selection of traits influenced by high GxE (Koebner and Summers, 2002).

4.6 QTL mapping for various traits under study

In deciphering the BMQ QTLs, we used a population of 105 RILs from HI977 x HD2329 cross, which could be considered as a smaller population for QTL mapping of such complex traits. However, Price (2006) postulated that QTL positions identified using small populations were nearly same as that of large mapping population. The QTL anlaysis for important traits such as wheat frost tolerance (cbf3, 74 lines) (Vaguifalvi et al. 2003), wheat grain protein (Gpc, 74 lines) (Distelfeld et al. 2004) and barley photo period response (Ppd H1, 94 lines) (Turner et al. 2005) , using population less than 100 individuals, accurately predicted the underlying genes, governing these traits. However, it has been reported that the sampling affects the confidence interval and maximum LOD may not be found at true QTL position (Darvasi et al. 1993).The QTL identification carried out in the present study using 105 RILs can be considered predictive for further studies.

4.6.1 Tgw QTL

Tgw is preferred as a measure of grain quality (Kumar 2006) and it has favourable effect on milling quality and flour yield (Campbell et al. 1999; Schuler et al. 1995). Tgw is considered as one of the important yield components and selection for this trait directly increases the yield (Quarrie et al. 2005). Though its correlation with quality parameters is reported (Zanetti et al. 2001), selection for quality trait alone may not help in improving this trait.

Tgw depends on number of cells in the endosperm tissue and accumulation of starch, as starch constitutes major portion of dry matter in the grain weight. Starch accumulation depends on number and size of starch granules. The starch granule number though is genotype dependent, number of starch granules of different sizes categorized as A, B and C depend on the starch accumulation in grain development period. The number per cell and volume of these large starch granules are the major

determinants of endosperm cell weight and there exists separate genetic control of these parameters. It should therefore, be possible genetically to combine these attributes to achieve direct increase in mature grain weight (Chojecki et al. 1986). However, duration and rate of starch filling is largely determined by day/night temperature of the growing environment, suggesting a major role of environment in influencing Tgw. Also Yong et al (2004) and Zhang et al (2005) reported that location versus year interaction variance component was higher for Tgw, than its genetic variance confirming the effect of environment on expression of Tgw trait.

Tgw was controlled by at least a dozen chromosomes, with 17 QTLs identified in our study, covering all groups of chromosomes except group 3. Tgw QTLs each identified on chromosomes 1B, 2B and 5B (Table 3), *viz*. *QTgw.ncl-1B.2, QTgw.ncl-2B.2, QTgw.ncl-5B.1* explained maximum phenotypic variation (27.12 %, 26.52 %, and 27.69 % respectively). Tgw QTLs on 2B chromosome were identified as important (Tahir et al. 2006; Huang et al. 2006; Groos et al. 2003), since granule bound starch synthase genes were located on this chromosome (Vrinten and Nakamura 2000). Interestingly the two QTLs *QTgw.ncl-2B.2*, *QTgw.ncl-1B.2* had negative additive variance, implying the positive role of BMQ inferior parent (HD 2329) to Tgw. Zanetti et al. (2001) revealed absence of relationship between dough quality parameters and Tgw, however in our study, HD2329, the poor BMQ parent contributed for major Tgw QTLs.

Enzymes involved in starch synthesis such as sucrose transporter and starch synthase I have been mapped on group 4 and group 7, respectively. In support of this, our study also showed Tgw QTL one each on both, 4B and 7B chromosomes (Table 3.10). Similar QTL were reported by Elouafi and Nachit (2004) and Huang et al. (2006). Physiological studies with source sink relationship to Tgw could throw light in understanding the factors controlling Tgw and its direct correlation with yield in maize. The glutamate dehydrogenase (GDH) and Glutamine synthase pathway is the main route for ammonium assimilation in plants and three QTLs of GlutamateDH activity in the flag leaves were co-located with grain yield QTL (5H) in maize (Hirel et al. 2001). The comparative analysis in wheat-barley stresses the importance of group 5 chromosomes in wheat. The *QTgw.ncl-5A.2* in our study was in similar position reported by Zanetti et al (2001), while the other QTLs on chromosomes 5A and 5B for Tgw, were in comparable position reported by Zanetti et al (2001) and

Groos et al (2003). The *QTgw.ncl-6B.1*, with positive additive effect, explained 10.13% variation due to Tgw and was at comparable position to the Tgw QTL reported by Elouafi and Nachit (2004).

4.6.2 Tw QTLs

A total of 26 QTLs were identified for Tw of which four QTLs were consistent in at least two locations. The large number of QTLs detected for this trait, might be due to the fact that, Tw also affects Tgw, grain length and grain width. At Kota, two major QTLs (*QTw.ncl-3A.1* and *QTw.ncl-5B.1*) on chromosomes 3A and 4D and at Pune 2 major QTLs (*QTw.ncl-1B.1* and *QTw.ncl-2A.1*) on 1B and 2A were detected. At Karnal, seven major QTLs were detected from which three (*QTw.ncl-5D.2*, *QTw.ncl-6B.2* and *QTw.ncl-6D.1*) were also detected at Pune location. Although Tw often has positive correlation with yield and Tgw (Huang et al. 2006), in our study, correlation between Tgw and Tw were insignificant for most of the locations due to the QxE interaction. High environmental interactions for traits such as Tgw and Tw were also reported by Peterson et al (1992) and Zhang et al (2005), suggesting that independent and simultaneous improvement in both, could well be achieved by selection. The highest contribution to the phenotypic variation for Tw was from *QTw.ncl-2B.1*, which accounted for 32.3 % variation for Tw, followed by 5B, 6D and 6B chromosomes (Table 3.9). The QTL reported by Campbell et al (1999) on 2BS chromosome, also explained 31 % variation due to Tw, and was located in comparable location with *QTw.ncl-2B.2*. Further, the *QTw.ncl-6B.1* was contributed by HD2329 at comparable location to the QTL reported by Elouafi and Nachit (2004). The *QTw.ncl-6B.2*, *QTw.ncl-6D.1* and *QTw.ncl-6D.2* were new loci not reported earlier. Interestingly, it was observed that all the QTLs with contribution > 20 % were from the parent HD2329.

4.6.3 Sv QTLs

Considerable difference in Sv was observed between the parental lines compared to the other traits (Table 3.2). Fourteen QTLs for Sv were detected on chromosomes 1B, 1D, 2A, 2B, 3D, 4B, 5A, 5B, 5D, 6A and 6D in our study. Blanco et al (1998) reported a positive and significant relationship between *Glu-B1* locus and Sv. In our study two QTLs influencing Sv were identified on 1B chromosome. The *QSev.ncl-1B.1* colocating with *Glu-B1* locus, and the *QSev.ncl-1B.2* below the Glu-B1 locus, stressed the importance of *Glu-B1* loci on Sv. Interestingly, both these QTLs were

contributed through HD2329 alleles. Guillaumie et al (2004) identified an eQTL influencing the HMW quantity of Glu-B1x, Glu-D1x and Glu-D1y subunits, spanning a storage protein activator (SPA) and Glu-B1 gene within its confidence interval. Huang et al (2006) and Zanetti et al (2001) detected a QTL at Glu-B1 locus with considerable contribution to Sv. The *QSev.ncl-1B.1* near the Glu-B1 might be a comparable locus to the above reported other QTLs.

At Karnal location, seven QTLs (*QSev.ncl-1B.1*, *QSev.ncl-2A.1*, *QSev.ncl-3D.1, QSev.ncl-4B.2, QSev.ncl-5D.2, QSev.ncl-6A.2* and *QSev.ncl-6D.1*) were detected on chromosomes 1B, 2A, 3D, 4B, 5D, 6A and 6D, respectively. For Kota location, only 1 QTL (*QSev.ncl-1D.1*), but for Pune location six QTLs (*QSev.ncl-1B.2, QSev.ncl-2B.1, QSev.ncl-4B.1, QSev.ncl-5A1, QSev.ncl-5B.1* & *QSev.ncl-6A.1)* were observed. Rousset et al (2001) reported a Sv QTL on Glu-D1 locus, while Martin et al (2001) identified a positive and significant correlation of Glu-D1d (5 +10) with Sv compared to Glu-D1a (2+12), in 1B/1R translocated lines. However, Huang et al (2006) and Zanetti et al (2001) could not identify any QTL on Glu-D1 locus. Similarly in our study, though the parental lines carried Glu-D1d (HI977) and Glu-d1a (HD2329), we could not identify any QTL on Glu-D1 locus. This result supported the observation that group 7 and group 6 chromosomes, along with other chromosomes, regulate the expression of the HMW glutenin genes (Wanous et al. 2003). Two QTLs were detected on chromosomes 2A *(QSev.ncl-2A.1)* and 2B *(QSev.ncl-2B.1)*, with 15.4 and 16.24 % PVE, which are in similar position to those reported by Zanetti et al (2001) on chromosome 2A and 2B.

Three Sv QTLs each were detected on group 5 and group 6 chromosomes, with negative additive effects except the *QSev.ncl-5D.1,* which was contributed by HI977. The *QSev.ncl-5A.1* explained 11.09 % of PVE due to Sv and contributed by an allele from HD2329, which was comparable to Sv QTL earlier reported on 5A (Zanetti et al. 2001;Blanco et al.1998). The highest contributing QTL (15.86 %) for Sv was detected on 6A *(QSev.ncl-6A.2)* with negative additive effect, but its position was different from the QTL stated by Blanco et al.(1998). Among the 14 QTLs identified for Sv, nine QTLs were contributed by the poor parent (HD2329), suggesting the importance of allele from the inferior parent.

4.6.4 Gpc QTLs

QTL analysis for Gpc revealed 11 QTLs with phenotypic variation explained (PVE) ranging from 7.88 % to 25.44 % located on seven chromosomes namely 1B, 2A, 2B, 3A, 3D, 6A and 7D. This was in well accordance with the previous reports that Gpc was influenced by more than 12 chromosomes of diploid and hexaploid wheat (Blanco et al. 2002; Joppa et al. 1997; Mesfin et al. 1999, Huang et al. 2006, Börner et al. 2002; Groos et al. 2003; Prasad et al. 2003). Though the difference in protein content between the parents (0.2%) was less, transgressive segregants were observed for Gpc (Table 3.2). These transgressive segregants for high Gpc might be due to minor genes segregating in the population (Chee et al 2001) and the different Gpc controlling alleles in the parents, confirming the suitability of this population for Gpc QTL analysis.

At Karnal location, two major QTL (*QGpc.ncl-1B.1* and *QGpc.ncl-3A.1*) were identified on chromosome 1B and 3A, respectively. Similarly two major QTLs (*QGpc.ncl-2B.1* and *QGpc.ncl-6A.1*) were found on chromosome 2B and 6A at Kota location. The chromosome 1B had maximum number of QTL (4), with one QTL (*QGpc.ncl-1B.3*) near *Glu-B1* locus. Perretant et al. (2000) and Turner et al. (2004) have also reported a QTL on chromosome 1B, at a comparable location to the above QTL. Similarly, *QGpc.ncl-2A.1* controlling PunGpc2 had similar location reported by Groos et al. (2003). The *QGpc.ncl-2B.1* detected in Kota location was similar to the QTL reported by Turner et al (2004) on chromosome 2B. The *QGpc.ncl-3A.1* accounted for the highest PVE of 25.44 % and was close to the Gpc loci identified by Groos et al (2003) and homeologous to QTL reported by Zanetti et al (2001). The *QGpc.ncl-3D.1* identified in our study was on 3DL, while Prasad et al (2003) reported *QGpc.ccsu-3D.1* on 3DS in the population of WL711 x PH132 cross. The *QGpc.ncl-6A.1* on chromosomes 6A was in comparable location to the reported Gpc QTL (Perrentent et al. 2000 and Groos et al. 2003). The QTL (*QGpc.ncl-7D.1*) identified in Pune location and the position of this QTL was different from the 7D Gpc QTL reported by Prasad et al (2003) however, was near to the loci reported by Groos et al (2004).

In our study the maximum PVE explained by the QTL was 25.44 %, while Joppa et al (1997) identified a QTL on 6B (Gpc-B1) accounting for 66 % variation in diploid wheat. Such single major QTL was not identified in our study, as well as in

other reports by Prasad et al (2003) and Groos et al. (2004). Further, only a putative QTL was identified on 6B below the threshold LOD 3.0, which might be due to GxE interaction, leading to small effect of this 6B diploid Gpc locus in hexaploid wheat (Mesfin et al. 1999). In earlier study on cross WL711 x PH132 from our laboratory, major Gpc QTL was not detected on chromosome 6B (Dholakia et al. 2001) which suggested that different combination of loci governing GPC, might be present in hexaploid wheat. The QTL on 7D chromosome (*QGpc.ncl-7D.1*) was detected, with negative additive effect contributing to 11.14 % for PVE. Prasad et al (2003) reported a Gpc QTL on chromosome 7D, comparable to the location of the above QTL.

4.6.5 Mixograph QTLs

Dough development is the outcome of adding mechanical energy (strain) while mixing water with flour. It is a complex and dynamic process and is influenced by several flour components including proteins, starch, and lipids that vary among cultivars and even among batches of flour from the same cultivar (Blechl et al. 2007). As the dough development time increases, its threshold intensity of resistance is raised. The threshold intensity is related to the strain rate required to extend the largest glutenins (Singh and MacRitchie 2001).

The Mixograph trait 'Epi' is the total energy required for the dough to reach "dough development stage" or it is "resistance" in terms of energy offered by the dough till it reaches the stage of peak resistance. Don et al. (2003) suggested a link between glutenin particles and protein particles in wheat endosperm and the energy to peak was governed by initial glutenin particle size. The functional relationship between Gluten macro polymer (GMP) in dough prepared from wheat flour–water mixtures and input energy suggested that, as the input energy increased with time, then a decrease in the GMP was witnessed (Peighambardoust et al. 2006). These reports stressed the strong relationship between the energy input and dough development, accordingly two QTLs, *QEpi.ncl-1B.3* and *QEpi.ncl-1D.1* were identified near the Glu-B1 and Glu-D1 loci in our study (Fig. 3.10). The QTL (*QEpi.ncl-6D.1*) was the only QTL of Epi detected in two locations among the six data sets.

Mixing peak time (Mpt), is the time required for the dough to reach dough development stage and one of the most important trait studied using mixograph

(Nelson et al. 2006; Martinant et al. 1998). A good relationship between Mpt and Epi were reported in previous reports (Huang et al. 2006), in concurrence we identified positive correlation between Epi and Mpt recorded at all the three environments (data not shown). The colocating QTLs of Epi and Mpt on Chromosome 1B (*QEpi.ncl-1B.1* and *QMpt.ncl-1B.1*), and 1D (*QEpi.ncl-1D.1* and *QMpt.ncl-1D.1*) suggested the importance of dough functionality through gluten loci or LMW. Three QTLs, one on 1D (*QMpt.ncl-1D.1*) and two on 3A (*QMpt.ncl-3A.2*) were consistent for two locations, Karnal and Pune, while QMpt.ncl-3A.1 was consistent for both the years at Pune location. Payne et al. (1987) and Garcia olmedo (1982), revealed the importance of Glu-D1 in deciding the dough strength through HMW glutenin. The QTL (*QMdt.crc-1D*) reported by Huang et al. (2006), QTLs on 1D reported by Campbell et al. (2001) and Arbelbide and Bernardo (2006) (Table 1.1) were comparable to the *QMpt.ncl-1D.1*, identified in our study. Also this QTL explained a variation of 16 to 23.7 % due to Mpt and was contributed by the good BMQ parent HI977.

Midline right integral (Mri) is the status of energy consumption one minute after reaching the dough development stage. It denotes decrease in the resistance offered by the dough after complete gluten network establishment during dough development process. The rapid decrease in the resistance offered by the dough can be evaluated using slope values, but due to their poor repeatability (Martinant et al. 1998), they were not considered for the present analysis. The *QMri.ncl-1D.1* was detected in all the data sets of first year (2004-05), had a positive additive effect and explained variation of 12.6 to 16.9% due to Mri. It was near the locus Glu-D1, suggesting again the importance of HMW glutenin on dough rheology.

Midline curve tail integral (Mti), denotes the total energy imparted on the dough during the entire mixing process (Mc Cartney et al. 2006). The entire mixograph experiment was done by subjecting the dough for 8 min mixing duration. One consistent QTL (*QMti.ncl-5A.1*) on two locations (Table 3.17) was detected on chromosome 5A. The Mc Cartney et al. (2006) reported a QTL (*QMteg,crc-1B*), for the trait Mti, which was in comparable location to the *QMti.ncl-1B.1* identified in our study. The *QMti.ncl-5A.1* was contributed by HD2329 and explained 17 to 23% variation due to Mti. This QTL was of specific interest due to its association with 5A chromosome, which harbours hardness loci (Groos et al. 2004) and grain hardness affects the energy required for dough mixing (Martinant et al. 1998).

Midline curve tail value (Mtv), explains the loss of dough strength, which is subjected to overmixing and is calculated at the end of mixing process (Martinant et al. 1998). It is considered as one of the important parameters in predicting the 1B/1R translocation (Graybosch, 2001). Though HD2329 did not have 1B/1R translocation, the dough tolerance was less compared to HI977. Among the 18 QTLs detected for this trait 16 were contributed by HD2329, which implied the role of HD2329 in complementing the dough strength.

Midline curve tail width (Mtw) denotes the tolerance of the dough and the total breakdown of the dough due to over mixing (Ohm et al, 1999, Martinant et al. 1998). Experiments have confirmed that the degradation of GMP due to over mixing accounted for rapid breakdown of the dough (Lasztity 2002) and it depended on the initial concentration of the GMP during the dough development process (Don et al. 2003). Four QTLs, *QMtw.ncl-1D.1*, *QMtw.ncl-5D.1*, *QMtw.ncl-6D.1*, *QMtw.ncl-6D.2* identified on chromosomes 1D, 5D and 6D were found to be consistent under two locations. Presence of Mtw QTLs on chromosomes other than group 1, might indicate the role of LMW and Ha locus, other than HMW glutenin. This result supported the idea that dough properties are influenced by the aggregative protein polymers in agreement with Martinant et al. (1998) and Gupta et al. (1995). The *QMtw.ncl-6D.1* was consistent in Pune location and contributed by HD2329 in the first year (PunMtw1) and HI977 in the second year (PunMtw2).

Weakening slope (Ws) is the difference between the strength of the dough during the peak time and at the end of mixing time (Martinant et al. 1998). This dough strength is attributed by wheat glutenin proteins, which forms a continuous and viscoelastic network during dough mixing. The properties of the viscoelastic network appear to be governed by different glutenins. The different quaternary structures, which result from polymers (involving disulfide bridges) and aggregates (involving hydrogen bonding) of different size, influence the quantity of unextracted polymeric protein (UPP) and dough parameters (Aussenac et al. 2001). The dough strength reaches its peak during optimum dough development stage and further mixing leads to a continuous degradation in the structure of the protein matrix, due to an increase in the number of broken polymer network (Shewry et al. 2003). In all, four consistent QTLs were identified each on chromosomes 1B, 2A, 3A and 6D, while two were detected on 6B chromosome. On 1B chromosome *QWs.ncl-1B.2* was detected near

the Glu-B1 loci, two QTLs (*QWs.ncl-6B.1* and *Qws.ncl-6B.2*) were detected on 6B, while one (*QWs.ncl-6D.1*) was on 6D chromosome. The facts that Glu-B1 is one of the constituent of GMP (Weegels et al. 1996) and gliadins harbor on group 6 chromosomes (Branlard et al. 2001), show the importance of gliadin and HMW glutenin. The gliadins and the LMW glutenin subunits appear to act as a ‘‘solvent’’ which modifies the rheological properties of the dough either by interfering with the polymerization of the HMW glutenin subunits, or by altering the relative amounts of the different types of glutenin subunits available. However, no QTLs were detected near the Glu-D1 loci, and QTLs on chromosome 2A (*QWs.ncl-2A.1*) and on 3A (*QWs.ncl-3A.1*) further suggested that, there were loci other than HMW, in our study. The mechanical behavior of dough is strongly dependent on water content, during mixing process (Shewry et al. 2003) and constituents of dough like starch and pentosan influence the dough behavior through water absorption.

4.6.6 Lv QTLs

Lv is due to the ability of dough to hold the gas produced during fermentation within its evenly distributed discrete cells and maintain the firmness after baking (Simmonds, 1989). Law et al (2005) predicted a QTL on chromosome 3A for Lv, but did not arrive at a conclusive location of that QTL. Significant QTLs governing Lv (Table 3.22) were identified on chromosomes 2A, 5D and 6D, which explained 7 to 36 % phenotypic variance in our study. Three QTLs detected on chromosomes 2A (*QLv.ncl-2A.1*), 5D (*QLv.ncl-5D.2*) and 6D (*QLv.ncl-6D.1*) were found to be represented in more than one location. Especially *QLv.ncl-6D.1* detected in both KarLv2 and PunLv2, while the LOD value was less than 2 for KotLv2 and PunLv1. CIM analysis failed to identify a single major consistent QTL across all the locations, which could be due to the fact that most of the QTLs detected with higher LOD score in one environment were not detected in the other environments. This proves that in an individual environment, QTLs may often escape detection at the threshold value of LOD score due to Q×E interactions that lead to variable expression of QTLs (Kulwal et al. 2005). Though consistency of QTLs is affected due to larger GxE effect across environments, development of an ideotype for a specific environment could be well achieved through the environment specific QTLs (Dholakia et al. 2001; Asins 2002). In this study we have identified significant number of QTLs on group 6 chromosomes controlling Lv, of which the QTL *QLv.ncl-6D.1* was a prominent one (Fig. 3.14).

Effect of glutenin and gliadin loci on Lv: The HMW loci of chromosome 1B and 1D were also placed in the present framework map (Fig. 3.4 and 3.5). The relationship of Glu–B1 and Glu-D1 loci with BMQ in deciding the BMQ is well established (Payne et al. 1987; Garcia-Olmedo et al.1992). In our study the relationship between Lv and glutenin loci could not be achieved, and it supports similar results of Kuchel et al. (2006). However, Skerritt et al. (2003) showed a relationship between glutenin alleles and dough rheology but reported a lack of association between the glutenin alleles and BMQ. According to Rousset et al. (2001) BMQ was under complex control and the *Glu-1* loci were only a component of genetic control of these characters. Similarly, Hamer et al. (1992) questioned the accuracy of using HMW glutenin subunits to predict baking quality changes in the dough rheology traits. Our study was supported by recent other findings on Lv, revealing the importance of loci other than HMW in governing BMQ potential in wheat (Law et al. 2005; Kuchel et al. 2006).

The group 6 chromosomes were considered important for BMQ, due to their association with gliadins (the monomeric, hydrophobic proteins). The α, β and some γ gliadins are encoded by tightly clustered genes at a single locus on each of the short arm of group 6 chromosomes, namely *Gli-A2*, *Gli-B2* and *Gli-D2* (Branlard et al. 2001). A correlation between gliadin surface hydrophobicity and Lv was also reported (örnebro et al. 2003). Further Wanous et al. (2003) explained the influence of group 6 chromosomal arms on expression of HMW glutenin, through competition for aminoacid in protein synthesis. Recently Kawaura et al. (2005) suggested the importance of chromosome arms 6BS and 6DS having higher α and β gliadin gene expression compared to 6AS and showed variable expression at 10 and 20 days after anthesis in Chinese spring. Also expression of α/ß gliadin and LMW glutenin multigenes was shown to be independently regulated irrespective of their phylogenetic relationship in response to wheat seed maturation (Duan and Schuler, 2005; Kirch et al. 2005). We identified a consistent QTL (*QLv.ncl-6D.1*) for Lv on chromomse 6D, for KarLv2 and PunLv2, explaining 20% and 36% phenotypic variation. This QTL (Fig. 3.14) did not affect KotLv2, provided evidence for GxE interaction. In our study, we identified a QTL *QLv.ncl-2A.1* on short arm of chromosome 2A, while Kuchel et al (2006), identified a locus on the long arm of 2A chromosome for Lv. Zanetti et al. (2001) identified a strong QTL controlling

sedimentation volume on chromosome 2A. Probably the QTL *QLv.ncl-2A.1* could be a new locus governing Lv. Law et al. (2005) also mapped a QTL associated with Lv to chromosome 3AL and they proposed a single gene (*Lvl 1*) responsible for Lv. In our study we identified a putative QTL on chromosome 3AS contributing to Lv, which may be a different locus for Lv.

Implications of QxE on Lv: The QxE interaction in the present case might be due to difference in day temperature at different locations. It has been reported that the BMQ enhanced upto 30°C (Randall and Moss, 1990) and decreased at further temperature rise (above 35°C) in a genotype-dependent manner (Blumenthal et al. 1993; Stone and Nicolas, 1995). D'ovidio and Masci, (2004) have also reported that heat stress can affect the aggregation behaviour of LMW glutenins. Furthermore, heat stress of wheat genotypes carrying Glu-D1 locus leads to change in glutenin particle size, resulting in abnormal dough characteristics (Don et al. 2003). Recently, Ma et al. (2005) have deciphered the environmental effect of LMW and its interaction with HMW in deciding the dough quality.

4.7 Co-locating QTLs and QTL clusters

In wheat, associations of qualitatively inherited genes together represent gene-rich regions and they form the hot spots of recombination (Gill et al. 1996). QTL are usually spread over all the chromosomes, but clusters of QTL in certain chromosomal regions have been observed as well (Huang et al. 2006). QTLs affecting several traits are common (Hayes et al. 1997), may be due to pleiotropy or close linkage.Such QTL clusters were observed on chromosomes 1B, 1D, 2B, 3A, 5B, 6B and 6D. Like single genes, these QTLs for different traits were mapped in the same genomic regions forming clusters (Huang et al. 2006). Since most of the QTL clusters in this study were located in the centromere region of the chromosomes, clustering may be explained by the suppression of recombination in these regions (Tanksley et al. 1992). Several clusters of yield QTL were also identified previously in wheat, either controlling yield itself or a yield component (Groos et al. 2003; Quarrie et al. 2005).

Of the 45 QTL clusters identified on 14 chromosomes (Table 3.23), 1B had 6 clusters; this might be due to high polymorphism in the chromosome 1B and comparatively larger compared to other chromosomes (Fig. 3.10). Ten QTLs of the traits Gpc, Tgw, Epi, Mpt, Mri, Mtw, Mrw, Mti, Mtv and Ws clustered in the interval

between the markers *Xpsp3000* and *Xgwm1028*, stressing the importance of this region in controlling Gpc and Tgw along with Mixograph traits. The QTL cluster of Tgw and Sv, was identified only on chromosome 1B, detected in Pune location, with significant negative correlation. The correlation between Sv and Tw, was insignificant in all the locations, but in the clusters, they often colocated, compared to other traits. This might be due to absence of epistatis among these traits or low effects of these QTLs to traits. Also, the present population being small, the sampling effects might have affected the magnitude of confidence interval of Sv and Tw QTLs (Darvasi et al. 1993).

Also 11 QTLs in the cluster were identified on chromosome 2A between the markers Xgwm 830 and Xgwm249A. These QTLs colocated with Lv, Sv, Tw, Epi, Mpt, Mri, Mrv, Mrw, Mti, Mtv and Ws (Fig. 3.10). The QTLs of Lv and Ws were consistent at more than one location and all the QTLs in this interval had positive additive effect except Ws, which had negative additive effect. The colocation of mixograph traits along with Lv, stressed the role of these component traits in governing Lv. On Chromosome 5D, within the interval of *Xgwm736A* and *Xgwm1016* , ten QTLs comprising Lv, Tw, Sv, Mpt, Mrv, Mtw, Mrw and Ws were observed (Fig. 3.12). Among these QTLs, only Mtw (*QMtw.ncl-5D.1*) was the consistent one. On chromosome 6B, eight QTLs were identified in the interval of Xbarc178 - Xgwm132C including Lv (*QLv.ncl-6B.1*), Tw, Epi, Mri, Mrv, Mtv, Mtw and Ws (Fig. 3.12). Among these only Ws (*QWs.ncl-6B.1*) was observed to be consistent (Table 3.21).

The same type of cluster was observed on chromosome 6D within the interval of *Xcfd13-Xgwm1009C*, where in QTLs for 7 traits were detected, namely Lv, Tw, Sv, Epi, Mpt, Mrv and Mtw. Among these QTLs, *QEpi.ncl-6D.1*, *QMtw.ncl-6D.1*, *QWs.ncl-6D.1* and *QLv.ncl-6D.1* were consistent in more than one location (Table 3.12, 3.19, 3.20 and 3.21, respectively). The graphical picture of the chromosome 6D, along with the traits, Lv, Epi, Mpt, Mrw, Mtw, Sv and Tw was depicted in the Fig. 3.14. The QTLs *QTw.ncl-6D.1*, *QSv.ncl-6D.1* and *QMtw.ncl-6D.1*, detected in Karnal and Pune locations had negative additive effect, while it was positive additive effect for all the other QTLs. Such a combination helps in marker assisted selection using the markers flanking this interval. Saturating this region with more markers would

help in identifying close flanking markers for these QTL and pyramiding of these QTL clusters (Ashikari et al. 2005) will result in overall improvement of other traits.

In our study, the inconsistency of the BMQ QTLs is due to strong interaction of traits with environment (GxE), which may be due to QxQ and QxE. Multiple composite interval mapping helps to identify interacting QTLs (QxQ) of component traits with main trait. Studies on epistatic QTLs, on complex traits were widely performed to unravel the gene networks and their interaction (Kulwal, 2005). However, caution should be taken, while concluding the epistasis based on population size less than 200. Simulation studies of Yang et al. (2007), concluded that the false positive rate of epistasis is higher than 0.15 and the power of detecting epistasis is relatively low in RIL populations with individual number less than 200. Hence, further analysis on epistatic QTLs may not be relevant and appropriate on our HI977 x HD2329 population, due to its population size (105 lines). However, colocating QTLs for various traits would be useful in determining the breeding strategies.

4.8 Future strategy

The salient challenge of applied genetics and functional genomics is identification of the genes underlying a trait of interest so that they can be exploited in crop improvement programmes (Rensink and Buell 2005). Modern quantitative genetics is useful for investigating specific properties of individual genes contributing to quantitative traits, through QTL mapping (Paterson 1995), but classical quantitative genetics describes the aggregate behavior of suites of genes influencing a trait. Though functional genomics will help in identifying the candidate gene responsible for any QTL, epistatic interaction of genes due to genome plasticity makes it possible to produce various phenotypes from little genetic variation (Morgante and Salamini, 2003). The genome plasticity of wheat has helped it to compensate for diversity bottlenecks caused by domestication (Dubcovsky and Dvorak 2007). This implies development of ideal genotype with good plasticity towards BMQ is possible, provided a right combination of alleles responsible for BMQ are aggregated which could only be possible using molecular markers. Towards this endeavor, we have compiled all QTLs identified on Pune location for traits including Tw, Tgw, Gpc, Sv, Lv and mixograph traits and the QTL clusters were coloured based on their additive effect (Red- HI977 and Green-HD2329) (Fig. 3.15, 3.16, 3.17 and 3.18). Graphical presentation of combinations of QTLs / genes predicting better genotype is possible

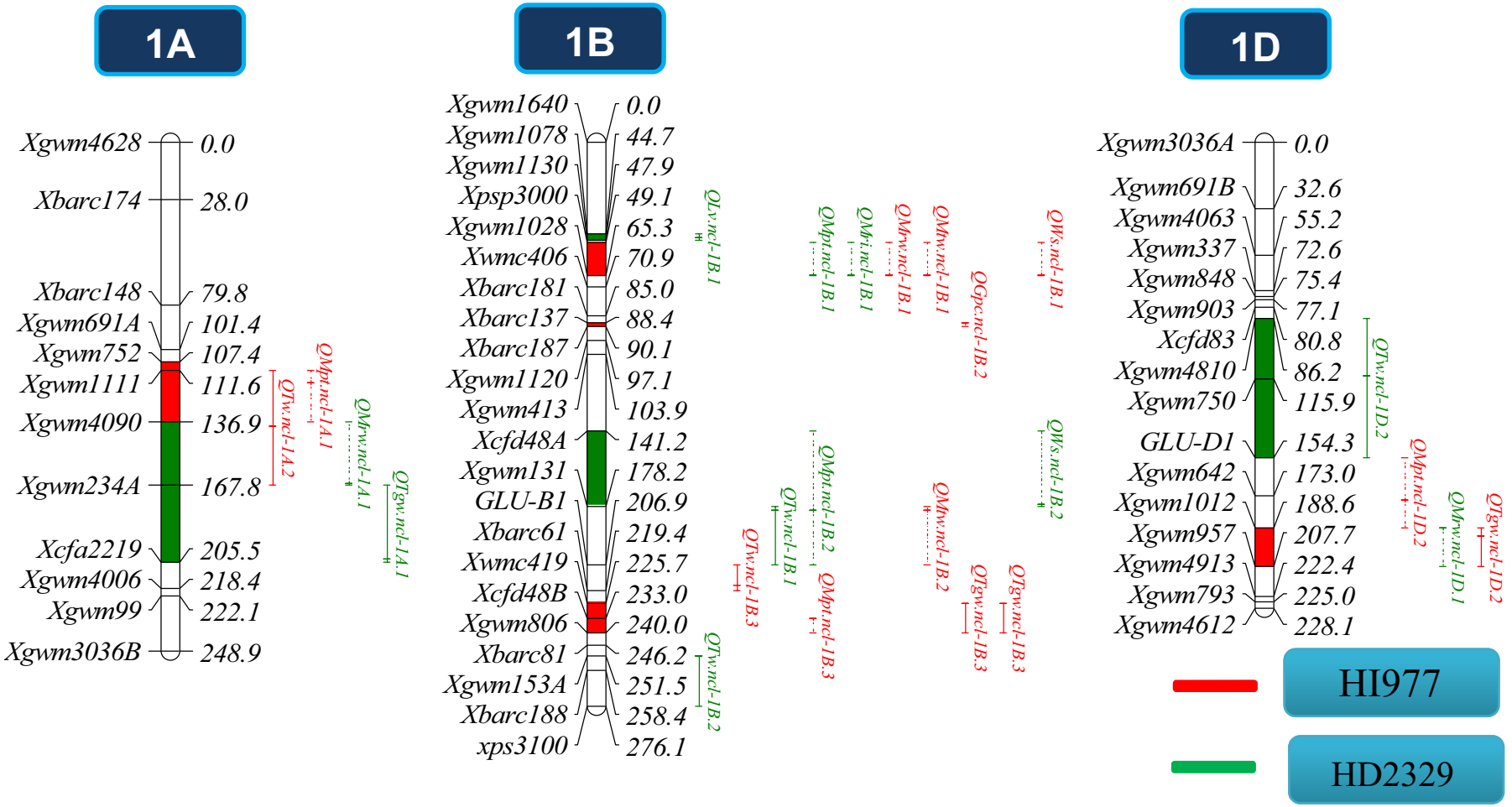


Fig. 4.1: BMQ QTLs identified on Pune location for chromosomes 1A, 1B and 1D

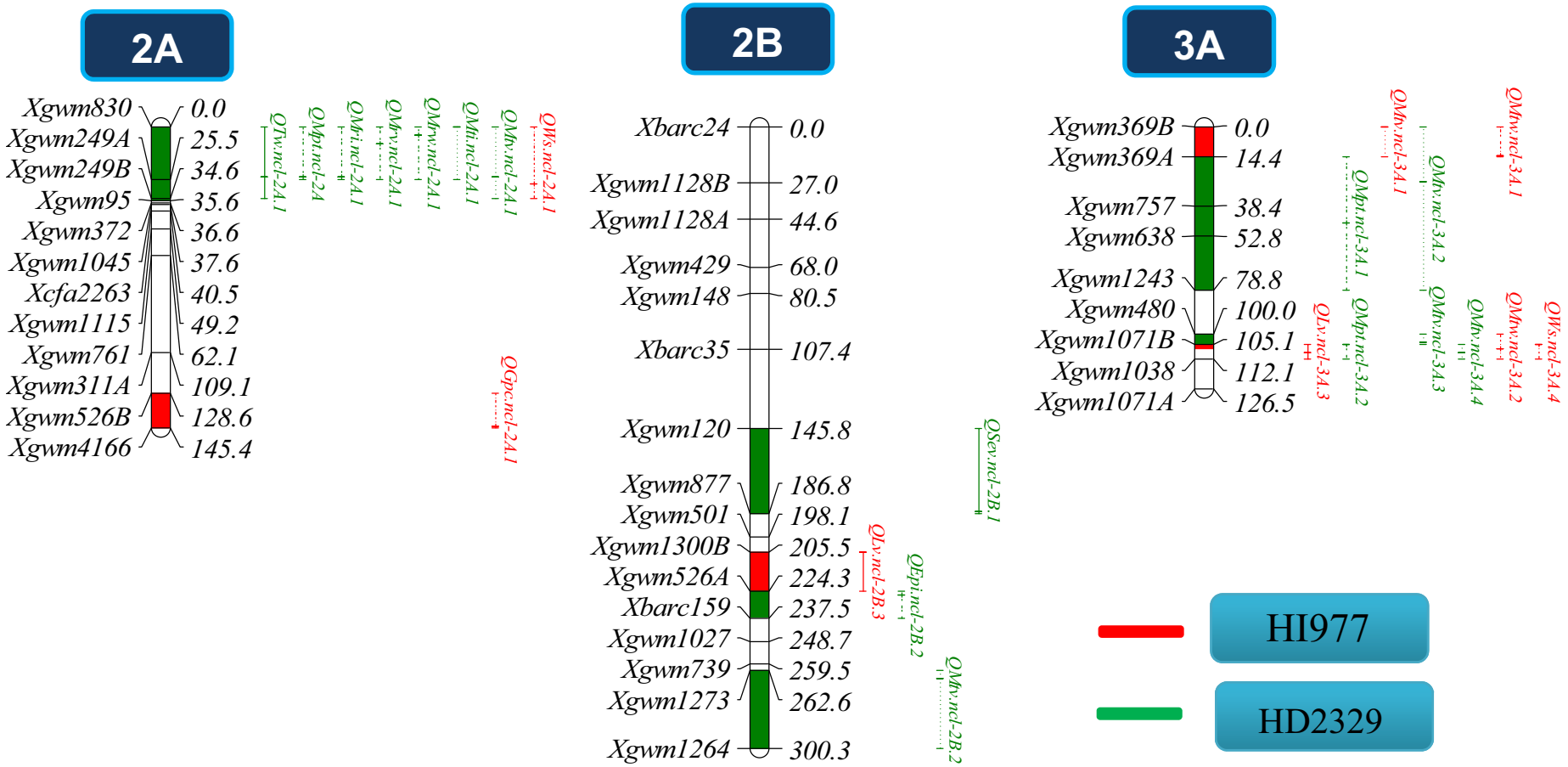


Fig. 4.2: BMQ QTLs identified on Pune location for chromosomes 2A, 2B and 3A

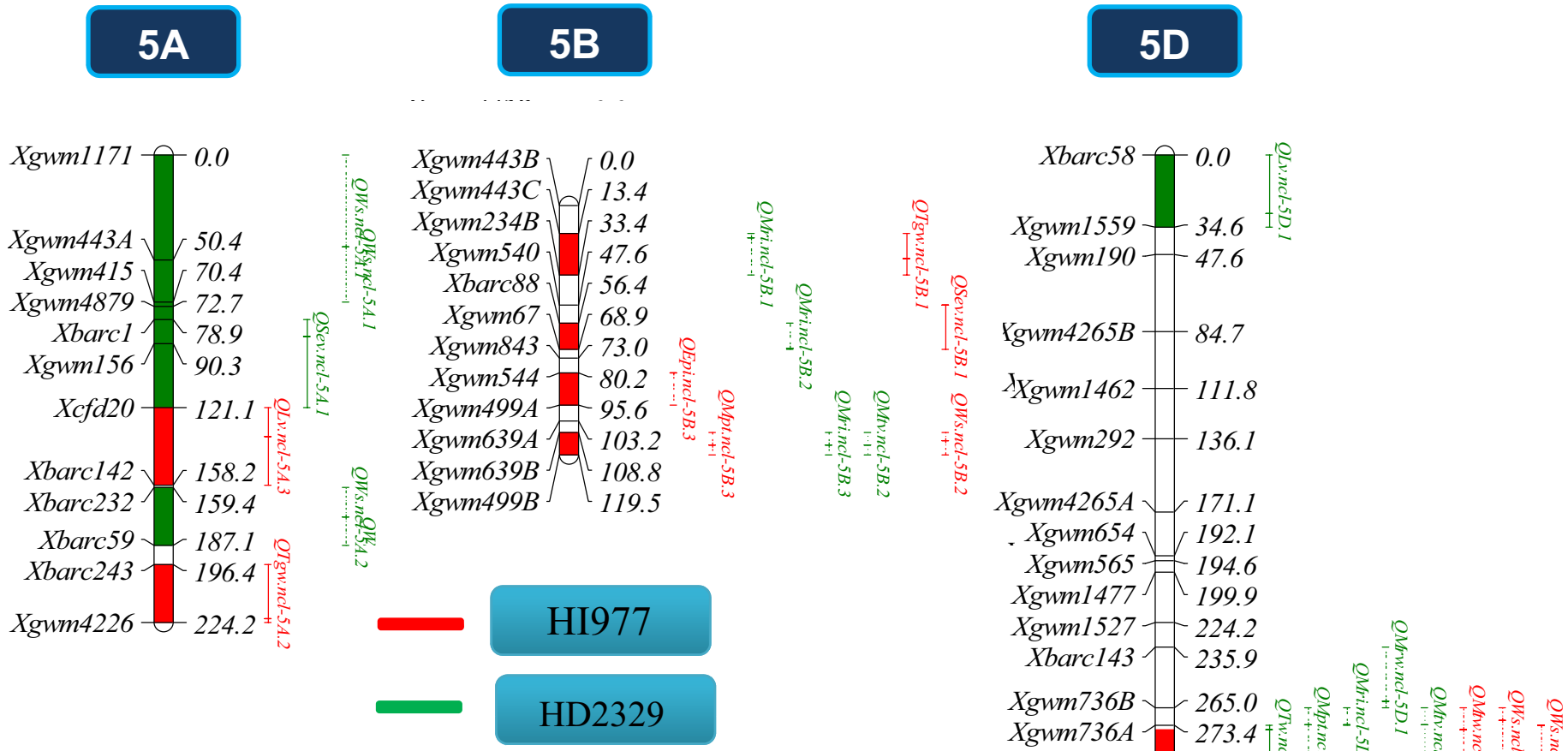


Fig. 4.3: BMQ QTLs identified on Pune location for chromosomes 5A, 5B and 5D

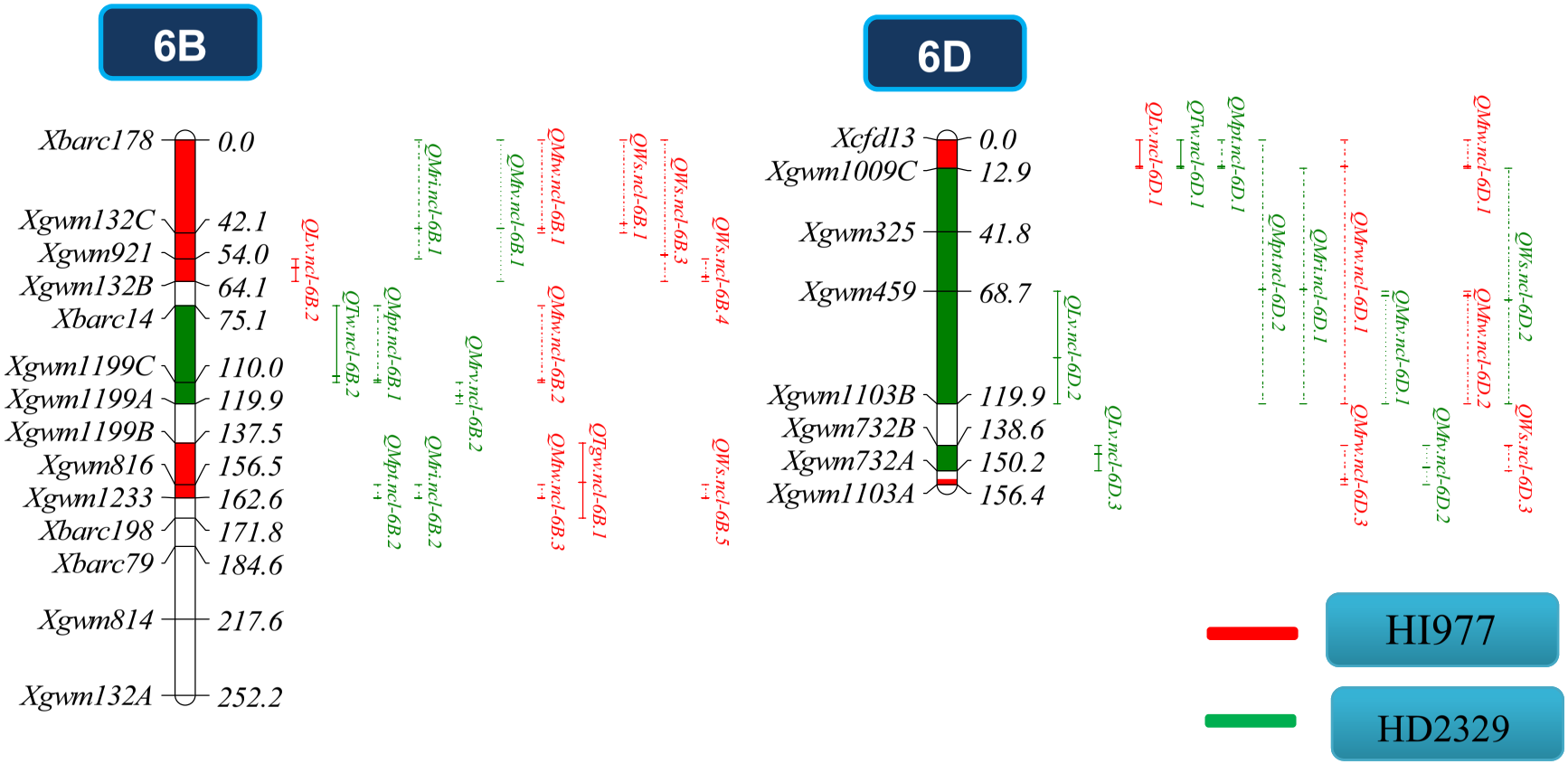


Fig. 4.4: BMQ QTLs identified on Pune location for chromosomes 6B and 6D

for any trait. This would give a clear idea to a wheat breeder for making appropriate crosses to generate superior genotypes.

In the present study, QTL mapping for BMQ has been the major objective, due to its higher QxE interactions. The inconsistency of QTLs, for many traits was due to such environmental interaction, affecting the expression of QTLs. Development of an ideotype specific for an environment can well be achieved with location specific QTLs. Also, buffering genotypes with many QTLs governing a typical trait, will benefit in wider adapatation and expression of these QTLs, as roughly 30 % of the QTL show GxE interaction. Futher, its percentage is expected to be large as the difference among the target environment becomes larger (Hemamalini et al. 2000). The molecular markers could be very useful in transferring the QTLs that have poor heritability and high environmental interaction. These QTLs may represent minor proteins in wheat grain that modify gluten functionality (e.g. by affecting the degree of cross-linking and thus size distribution of the polymer), or transcription factors that control glutenin expression level (Ma et al. 2005). Asins (2002) suggested identification of many QTLs regardless of their effect and environmental sensitivity, as it would help in genetic dissection of this complex trait, apart from the postulated role of QTL analysis in MAS. Our study stresses the importance of location specific combination of QTLs for better phenotype and also gives clues to the breeding community regarding combining QTLs that are best expressed in specific environment.

Chapter 5

Summary and future directions

Wheat is one of the most important widely adapted crop, consumed worldwide and cultivated for the last 11,000 years (Oleson, 1994). It is grown in 230 Mha worldwide and 600 mt of wheat is harvested every year. India is the second largest wheat producing country in the world after China. Most of the wheat produced in the world is traded as the main raw material for the manufacture of hundreds of diverse food products such as all kinds of bread, chapatti, roti, paratha, naan, pasta, macaroni, noodles, cakes, biscuits and cookies.

Why quality wheat required?

The rapid expansion of Indian urban middle class population and the change in food consumption demands wheat having good BMQ. Bread with high loaf volume, smooth crust and uniform smaller grain in the crumb is always preferred. Traditional varieties grown in India are best suited only for flat bread preparation (Roti) and hence attempts need to be made for improvement of Indian wheat for good BMQ.

Rationale in genetic analysis of BMQ

BMQ is considered to be a complex trait influenced by many QTLs and also by interaction of many biochemical traits such as protein content (Payne et al. 1987), starch quality and content (Gray and Bemiller 2003) and oil content (Helmerich and Koehler 2005), supplemented by various physico-chemical traits such as moisture content, water retention capacity, vacuole formation, grain hardness and texture (Huang et al. 2006). The QTLs governing BMQ show environmental interaction and selection by phenotype will yield poor results, because phenotypic expression depends on the environment in which it is grown. So the selection for this trait needs to carry out using genotype rather than phenotype. Development of DNA markers will be highly helpful in identifying the genotype, carrying BMQ alleles.

Important research findings

A systematic study was carried out to dissect the BMQ contributing traits from seed to loaf and a genetic proof of relationship between mixograph, BMQ traits and Lv was deciphered in this study.

Wheat framework map: Framework map of wheat from intraspecific cross HI977 x HD2329 was established with 19 linkage groups of the size 3,161.8 cM with 217 markers. About 202 SSRs, with two HMW glutenin loci were used for linkage group construction. The length of A genome of the map was 909 cM, while that of B and D

genome was 1,100.7 cM and 1,152 cM, respectively. The framework map had mean interval of 15 cM and uniform distribution of markers for most of the chromosome and it is useful for QTL mapping.

QTLs governing Tw, Tgw, Sv and Gpc: In all, 68 QTL mapped on 19 chromosomes were identified for these traits, among which 11 to 26 QTLs ranged for each trait. Our study confirmed the QTLs previously identified by researchers and also detected many new QTLs, for these BMQ traits.

QTLs governing mixograph: A total of 169 QTLs for these 9 traits were identified among which 77 were contributed by HD2329 and 92 by HI977. This study helped in splitting the dough rheology into further mixograph component traits and helped in establishing a relationship with Lv. Such comprehensive study of mixograph QTL mapping has been reported for the first time.

QTLs governing Lv: A total of 30 QTLs were identified for Lv on 12 chromosomes, which explained 5.85 to 44.69 % of phenotypic variation. It is for the first time, that I have identified 30 QTLs and shown that there are loci other than Glu-B1 and Glu-D1, which play major role in governing Lv.

Important inferences

- In my study, identification of QTLs governing the complex trait of BMQ has not only helped in genetic dissection of the trait, but could be further used by plant breeders to select the ideal genotype specific for a given environment.
- A significant role of chromosomes 1B, 2A, 3A, 6B and 6D in governing Lv and also influencing the other BMQ traits has been discovered.
- The QTL (*QLv.ncl-6D.1*) on 6D chromosome between the interval *Xcfd13* and *Xgwm1009C* is of special interest and saturation of this region with more SSR markers is underway.
- Development of single superior and stable genotype over a wide range of environments though desirable, is very difficult to achieve due to GxE interaction.
- Particularly, the ideal genotype fit for Pune location has been presented in this study and similar plans could be drawn for other locations.

Thus, my study stresses the importance of location specific combination of QTLs for better phenotype and also gives clues to the breeding community regarding combining QTLs that are best expressed in specific environment.

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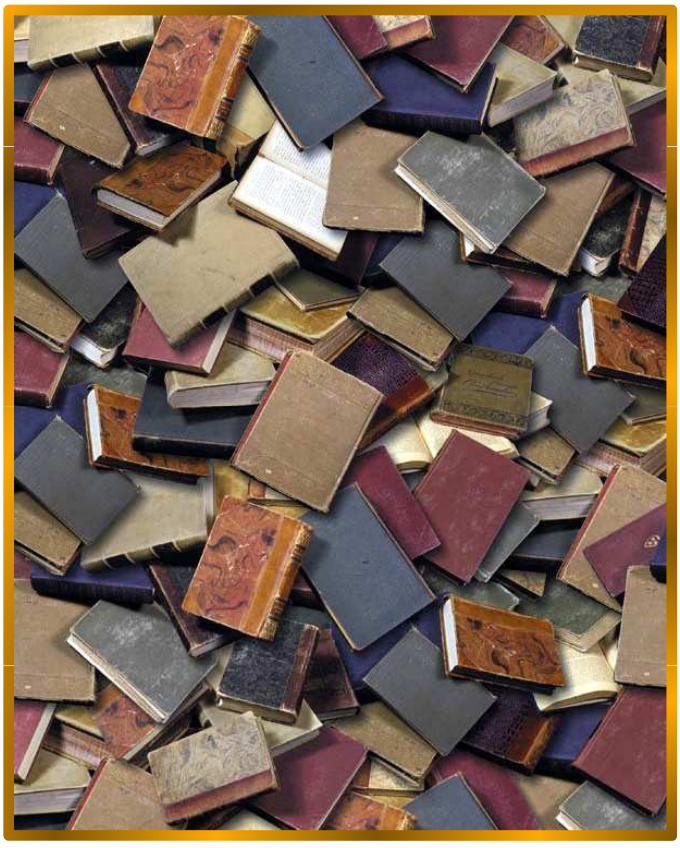

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Awards and Trainings

- Awarded **Junior Research Fellowship** and Lectureship through Nation wide exam conducted by **Council of Scientific and Industrial Research**, India.
- Short term training in Central Forensic Science Lab, Kolkata India on "**DNA markers for application in Forensic science**".
- Online Certificate course on "**Introduction to Bioinformatics**" conducted by **S-Star** organization and **National University of Singapore**.
- Awarded six months fellowship by **DAAD-IAESTE** (International Association for exchange of students for technical experience) and undergone research training in "**Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany**" (Sep 2005 – Feb 2006).

- Participated one-month Management course (Jan 2007 – Feb 2007) "**Technology Led Entrepreneurship Development Programme**", conducted by **Indian Institute of Management**, Bangalore, India and won prize in **Business plan competition**.

- As a team leader, presented a business plan on "**Visual marker for genetically modified fruits and vegetables**" and qualified in business plan competition organized by **Dept. of Biotechnology, India** and **UK Embassy**, **India** for **YES Biotech, UK** (September 14th 2007).

Work experience and Project work

Carried out Thesis entitled *"Heterosis and combining ability in sesame (Sesamum indicum. L.)* " as a part of M. Sc. (Agriculture) for a period of one year.

Experience in breeding self pollinated field crops like Wheat, Rice, Sesame, and Groundnut, cross pollinated crops like Cotton, Sunflower and Maize.

Wheat dough quality analysis through Mixograph, SDS-PAGE gels.

Most of the molecular biology techniques like PCR, Gene cloning, Plasmid Cloning, Protein expression and purification, Gel electrophoresis, RNA extraction, RP-HPLC, PAGE gels and genotyping.

Statistical software: IRRISTAT for ANOVA, AMMI and Genetic analysis packages: Mapmaker for Linkage analysis, QTL Cartographer & QTL Network for QTL mapping.

Publications

1) **Elangovan M,** Rai R, Dholakia BB, Lagu D, Tiwari R, Gupta RK, Rao VS, Röder MS, Gupta VS (2007) Molecular genetic mapping of quantitative trait loci associated with Loaf volume in hexaploid wheat (*Triticum aestivum*). Journal of Cereal science DOI 10.1016/j.jcs.2007.07.003 (In press)

2) **Elangovan M,** Rai R, Dholakia BB, Lagu D, Tiwari R, Gupta RK, Rao VS, Röder MS, Gupta VS (2007) Mapping quantitative trait loci associated with yield and quality traits in hexaploid wheat (*Triticum aestivum*). Plant Breeding (communicated)

3) **Elangovan M,** Rai R, Oak M, Dholakia BB, Tiwari R, Gupta RK, Rao VS, Röder MS, Gupta VS (2007) QTL mapping of rheological traits using Mixograph on hexaploid wheat (*Triticum aestivum*) (under preparation)

4) **Elangovan M,** Rai R, Gupta VS (2007) Molecular dissection of bread making quality in wheat (under preparation)

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Appendix – I

Composition of Solutions

SDS-sample buffer

2% SDS

0.05% Xylenecyanol

0.02% Bromophenol blue

0.08 M Tris-HCl, pH8.0

40% Glycerol

Staining solution

0.3% Silver nitrate

TAE (Tris/acetate/EDTA)

(electrophoresis buffer)

50X stock solution, pH 8.0

2 M Tris base

2 M Glacial acetic acid

50 mM $Na_2EDTA.2H_2O$

High salt TE buffer

(Rogers and Bendich 1988)

1 M NaCl

10 mM Tris-HCl, pH 8.0

1 mM EDTA

CTAB DNA extraction buffer

(Stein et al. 2001)

2% (w/v) CTAB

200 mM Tris-HCl, pH 8.0

20 mM EDTA, pH 8.0

1.4 M NaCl

1% (w/v) polyvinylpyrrolidone-K30

1% (v/v) β -mercaptoethanol

TBE (Tris/borate/EDTA)

(electrophoresis buffer)

10x stock solution, pH 8.0

890 mM Tris base

890 mM Boric acid

0.5 M EDTA, pH 8.0

CTAB DNA extraction buffer

(Rogers and Bendich 1988)

100 mM Tris-HCl, pH 8.0

20 mM EDTA

1.4 M NaCl

1% PVP and 2% β-mercaptoethanol

CTAB precipitation buffer

(Rogers and Bendich 1988)

500 mM Tris-HCl, pH 8.0

10 mM EDTA

Acidic ethanol

95% Ethanol

0.5% Glacial acetic acid

Fixer solution

10% Ethanol

0.5 % Acetic acid

Developer solution

3% NaOH

0.2% Formaldehyde

Acrylamide-Bis Acrylamide solution

29:1 of Acrylamide and

N, N'-methylene bisacrylamide

Ethidium bromide, 10 mg/ml

0.2 g ethidium bromide in 20 ml H_2O

Stored at 4°C in dark.

Denaturing gel mix

6% polyacrylamide solution

0.1 X TBE

7 M urea

Glutenin extraction buffer

50% propan-1-ol

0.08 M Tris-HCl, pH 8.0

Denaturing dye

10 mM NaOH

0.05% Bromophenol blue

20 mM EDTA in Formamide

DNA Washing solution I

76% Ethanol

200 mM Sodium acetate

DNA Washing solution II

76% Ethanol

10 mM Ammonium acetate

TE (Tris/EDTA) buffer

10 mM Tris-HCl, pH 8.0

1 mM EDTA, pH 8.0