INTROGRESSION AND MOLECULAR MAPPING OF HIGH Fe AND Zn CONTENT FROM *AEGILOPS* INTO WHEAT

A THESIS

Submitted in partial fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

in

BIOTECHNOLOGY

by

VIJAY KUMAR TIWARI

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **INTROGRESSION AND MOLECULAR MAPPING OF HIGH Fe AND Zn CONTENT FROM *AEGILOPS* INTO WHEAT** in fulfilment of the requirements for the award of the Degree of Doctor of Philosophy and submitted in the Department of Biotechnology of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from January 2005 to December 2008 under the supervision of Dr. H. S. Dhaliwal and Dr. G. S. Randhawa, Department of Biotechnology Indian Institute of Technology Roorkee, Roorkee.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

(VIJAY KUMAR TIWARI)

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

(G.S. RANDHAWA)
Supervisor

(H. S. DHALIWAL)
Supervisor

Date: **December 30, 2008**

The Ph.D. Viva-Voce Examination of **Mr. Vijay Kumar Tiwari**, Research Scholar, has been held on 8.05.2009

8.5.2009

Signature of Supervisors

Signature of External Examiner

ABSTRACT

Biofortification through genetic manipulations is the best approach for improving micronutrient content of the staple food crops for alleviating the micronutrient deficiency of Fe and Zn affecting more than 2 billion people worldwide. Identification of sources with high grain Fe and Zn content in wheat germplasm and understanding its genetics are the pre-requisites for their manipulation. *Triticum* and seven *Aegilops* species with non-progenitor S, U and M genomes along with 14 semi-dwarf bread and durum wheat cultivars, grown over two years at IIT Roorkee, were analyzed for iron and zinc content. The wheat and durum cultivars had very low content and limited variability for iron and zinc content. The *Aegilops* species showed up to 2-3 fold higher grain iron and zinc content than the cultivars. An Interspecific hybridization was made between a wheat line and an *Aegilops kotschyi* accession 3790 selected for high grain iron and zinc content. Wheat x *Ae. kotschyi* F_1 hybrid with low chromosome pairing was highly male and female sterile. This was extensively back crossed with wheat cultivars to get some seed set. Flag leaf analysis of sterile F_1 hybrid showed intermediate content of iron and zinc between their parents; suggesting superiority of *Aegilops kotschyi* for better uptake and translocation for the micronutrients. The BC_1F_1 and BC_2F_1 plants were allowed to self and plants with high grain iron and zinc content were selected among subsequent generations. Grain iron and zinc content of the selected derivatives showed 60% to 140% increased Fe and Zn content as compared to the recipient wheat cultivars. On the basis of morphological and cytological analysis 13 plants with high grain iron and zinc content were selected. Selected plants were finally subjected to cytological as well as molecular

characterization. Application of anchored wheat SSR markers indicated alien introgression of group 2 and group 7 chromosomes of *Ae. kotschyi* in the high grain iron and zinc containing derivatives suggesting that the genes controlling high grain micronutrient content in the *Ae. kotschyi* accession are on the group 2 and group 7 chromosomes.

Four different interspecific hybrids involving three accessions of *Aegilops longissima* Schweinf. & Muschl. with high grain iron and zinc content and three *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. cultivars with low micronutrient content were made for durum wheat biofortification and were investigated for chromosome pairing, fertility, putative amphiploidy and micronutrient content. The chromosome pairing in the 21 chromosome F_1 hybrids (ABS^l) varied from 0-6 rod bivalents and occasionally one trivalent. All the F_1 hybrids however, unexpectedly showed partial but variable fertility. The detailed meiotic investigation indicated the simultaneous occurrence of two types of aberrant meiotic divisions viz., first division restitution (FDR) and single division meiosis (SDM) leading to dyads and unreduced gamete formation and fertility. The F_2 seeds being putative amphiploids ($AABBS^lS^l$) had nearly the doubled chromosome number (42) of the F_1 hybrids, regular meiosis and fertility. The F_1 hybrids and putative amphiploids were intermediate between the two parents for different morphological traits. The putative amphiploids with bold seed size had higher grain ash and ash iron and ash zinc content as compared to durum wheat cultivars, suggesting that *Ae. longissima* also possesses better genetic system(s) for uptake and seed sequestration of iron and zinc which could be transferred to elite durum and bread wheat cultivars and exploited. The

amphiploids can be used to transfer useful variability and development of alien addition and substitution lines in wheat background.

Wild *Triticum* and *Aegilops* species including *Triticum boeoticum* (A^mA^m) have higher grain Fe and Zn content compared to the bread and durum wheat cultivars. A *Triticum boeoticum* accession pau5088 had relatively higher grain Fe and Zn content. A recombinant inbred line (RIL) population involving the accession with *T. monococcum* (pau14087) was evaluated for grain Fe and Zn content at two locations over two years. The grain Fe and Zn concentrations in the RIL population ranged from 13.1 to 70.9mg/kg and 16.6 to 69.0mg/kg, respectively. A linkage map with 179 molecular markers available for the RIL population was used for mapping QTL for accumulation of grain Fe and Zn. The QTL mapping led to the identification of two QTL for accumulation of grain Fe on chromosomes 2A and 7A and one QTL for accumulation of grain Zn on chromosome 7A. The QTL for accumulation of grain Fe were designated as *QFe.pau-2A* and *QFe.pau-7A* and these mapped in marker interval *Xwmc382-Xbarc124* and *Xqwm473-Xbarc29,* respectively, each explaining 12.6% and 11.7% of the total phenotypic variation. The QTL for accumulation of grain Zn, which mapped in the marker interval *Xcfd31-Xcfa2049*, designated as *QZn.pau-7A* and accounted 18.8% of the total phenotypic variation. The chromosomal locations of the QTLs have been validated from the introgression of group 2 and group 7 chromosomes of *Ae. kotschyi* in the high grain iron and zinc derivatives.

Seed samples of 63 landraces of wheat were collected from farmers' fields of hilly areas of Himalaya in Uttarakhand were analyzed for morphological trait, grain iron and zinc content, hardness index, HMW subunit and diversity for SSR markers . Genetic diversity

among 78 genotypes (cultivars and landraces of wheat) was studied using morphological traits, micro-satellite markers, micronutrient content, grain texture and SDS-PAGE of HMW-GS. The dendrograms based on molecular markers and morphological data clearly separated landraces of wheat from cultivars with few exceptions. The landraces had higher diversity for HMW-glutenin subunits coded by *Glu-B1*, with distinct subunit combinations 6 + 8, 7 + 9, 13 + 16, than within the wheat cultivars analyzed. Most of the landraces are clearly distinct from the indigenous and modern wheat cultivars released in India in the 20th century. Useful variation was found for high grain iron content and grain hardness. The landraces with resistance to yellow rust and powdery mildew, and higher grain iron content and distinct HMW-GS subunits can be exploited as such or used in appropriate breeding programs. Some of the landraces with very soft grains can be used for biscuit making. It will be desirable to conserve and protect the landraces as geographical indications of Uttarakhand.

Development of addition, substitution and translocation lines of *Ae. kotschyi* and *Ae. longissima* in wheat background will be of great help in wheat biofortification. The precise transfer of *Ae. kotschyi* and *Ae. longissima* grain Fe and Zn content and their marker assisted pyramiding in elite wheat cultivars can easily double the micronutrient content over the existing levels in wheat cultivars. Fine mapping and cloning of the putative QTL for high Fe and Zn content will lead to thorough understanding of the pathways for their uptake, translocation and sequestration in grains.

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30/12/2008

(Vijay Kumar Tiwari)

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ABBREVIATIONS USED

Abbreviation	Extended form
%	Percentage
AAS	Atomic Absorption Spectrometer
BC	Backcross
BC_1	First back cross generation
BC_2	Second back cross generation
bp	Base pairs
CGIAR	Consultative Group of International Agricultural Research
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
CTAB	Cetyl-trimethyl ammonium bromide
DArT	Diversity Array Technology
DMA	2-deoxymugineic acid
DMSO	Dimethyl sulphoxide
dNTPs	Deoxy Nucleotide Triphosphates
EDTA	Ethylenediaminetetraaceticacid
EMS	Ethane methyl sulphonate
epi-HDMA	epihydroxy-2hydroxy mugineic acid
epi-HMA	3-epi-hydroxymugineic acid
EST	Expressed sequence tag
F_1	First Filial Generation
FAO	Food and Agricultural organisation
Fig.	Figure
FISH	Fluoresence *in situ* hybridization

GISH	Genomic *in situ* hybridisation
HMW-GS	High Molecular Weight glutenin subunit
HPLC	High Performance Liquid Chromatography
ICPMS	Inductively Coupled Plasma Mass Spectrometer
IRT	Iron regulatory transporter protein
IZiNCG	International Zinc Nutrition Consultative Group
MA	Mugineic acid
mg/kg	Milligram per kilogram
MTP	Metal tolerance proteins
NRAMP	Natural Resistance Associated Macrophage Proteins
IRT	Iron regulatory protein
PCR	Polymerase Chain Reaction
PMCs	Pollen Mother Cells
ppm	Parts per million
QTL	Quantitative trait loci
RDA	Recommended dietary allowance
RAPD	Random amplified polymorhic DNA
RFLP	Restriction fragment length polymorphism
SDS-PAGE	Sodium dodecyl sulphate- Polyacrylamide gel electrophoresis
SEM-EDX	Scanning Electron Microscopy – Energy Dispersive X Ray analysis
SSRs	Simple Sequence Repeats
TAE	Tris Acetate
TE	Tris EDTA
TEMED	Tetramethylene diamine
VIT1	Vacuolar Iron Transporter

WHO	World Health Organisation
YS	Yellow Stripe
ZIP	Zinc regulated- Iron regulated transporter proteins
µg	Micrograms

Chapter I

Introduction

1. Introduction

The nutritional health and well being of humans are entirely dependent on plant foods either directly or indirectly (DellaPena, 1999). During the past 40-50 years, major emphasis of plant breeding programs has been to increase productivity to meet the calorific requirements of the world population. Despite a linear increase in food production over years, nearly half of the world population, though with adequate staple food intake, suffers from the deficiency of vitamin A and micronutrients like iron and zinc which has been termed as hidden hunger (Welch and Graham, 1999). Iron deficiency ranks among the most widespread nutrient deficiencies, estimated to affect over two billion people worldwide (Stoltzfus and Dreyfuss, 2001). In India alone, the prevalence of anemia ranges from 47% to 98% in different states (FAO, 1998). Mineral density in staple food crops such as rice and wheat in Asia, maize in Sub-Saharan Africa and Latin America is very low.

Availability of useful variability and understanding of its genetic architecture are prerequisites for an effective breeding programme for the wheat improvement. However there is little existing variability for micronutrients in the cultivated germplasm of cereals for their improvement. Wheat germplasm comprising traditional cultivars, landraces and related progenitor and non progenitor species harbor useful variability for numerous traits including micronutrient content. There are plenty of examples of alien chromosome /segments introgression in wheat. A number of genes for various traits including diseases resistance, biotic, abiotic stress tolerance, and pest resistance have been introgressed into wheat from related progenitor and non progenitor species (Kuraparthy *et al.,* 2007; Marais *et al.,* 2005; Friebe *et al.,* 1996) and commercially exploited. Sufficient

variability for the grain iron and zinc content is not available in the cultivated germplasm of wheat but wild relatives of wheat such as *Triticum boeoticum, Triticum monococcum, Triticum dicoccoides* and *Aegilops tauschii* show a wider range of grain micronutrient density (Cakmak *et al.*, 2000; Ortiz-Monasterio and Graham 2000).

Losses during processing and their reduced bioavailability due to the presence of antinutritional factors such as phytic acid, tannins, lignins, food fibers and polyphenols further aggravate the problem. Among various strategies such as biofortification, dietary diversification, fortification and supplementation; biofortification through genetic engineering and molecular breeding of plants for high mineral content in the grains is considered as the most feasible and cost effective approach.

Synthetic amphiploids have played an important role in genetics and germplasm enhancement of wheat. Amphiploids serve as immortal store house of genomic combinations between the alien and cultivated species. The utilization of synthetic amphiploids as a means of introducing important traits of related wild species into wheat cultivars has been well established. (Jiang *et al.*, 1994; Wojciechowska *et al.*, 2005). Meiotic restitution in interspecific hybrids leading to unreduced viable gamete formation has been responsible for evolution of many polyploid species of cultivated crop plants and amphiploids (Kellogg, 2003; Stebbins, 1971). *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. and *T. aestivum* L. are the two most important allopolyploids that evolved in nature by the phenomenon of meiotic restitution leading to chromosome doubling (Jauhar, 2007; Matsouka and Nasuda, 2004). The amphiploids have been extensively used for dissecting alien genomes through development of alien translocation, addition and substitution lines.

Realizing the importance of biofortification, several studies have been done for the evaluation of germplasm and advanced breeding lines for variability for grain Fe and Zn content (Cakmak *et al.*, 2000; Morgounov *et al.*, 2007). However, only a few studies are available on the genetics of accumulation of micronutrients in the grains of major cereals like wheat and rice (Shi *et al.*, 2008). So far, no major locus or QTLs have been mapped for grain Fe content in wheat. Understanding the genetic basis of accumulation of micronutrients in grains will provide the basis for devising the plant breeding strategies for improving grain micronutrient content by marker assisted selection.

Investigation for the evaluation of several non progenitor *Aegilops* species, wheat cultivars and landraces, for higher grain iron and zinc content and their utilization for wheat biofortification through molecular breeding and mapping of iron and zinc related QTL was taken with following broad objectives:

1. Analysis of the variability in grain iron and zinc content of some popular bread and durum wheat cultivars of northern India, and related wild *Triticum* and *Aegilops* species.
2. Wide hybridization and introgression of the useful variability from *Aegilops. kotschyi* into wheat cultivars.
3. Molecular and cytological characterization of introgressive derivatives with high grain iron and zinc content.
4. Development and characterization of *T. turgidum* ssp. *durum- Ae. longissima* amphiploids with high grain iron and zinc content.
5. Mapping of QTL for grain iron and zinc content in diploid wheat *T. monococcum* x *T. boeoticum* RIL population.

6. Collection and characterization of a set of landraces from Uttarakhand for iron and zinc content, hardness index, and HMW glutenin subunits.

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Chapter II

Review of Literature

2. Review of Literature

2.1 Origin of problem:

Micronutrient deficiency also called as hidden hunger, is one of the most widespread problem for human population affecting more than 3 billion people (FAO, 2002; Welch and Graham, 1999). Out of the 22 minerals required by human beings for normal growth, dietary deficiency of iron and zinc is the most common and wide spread. Global prevalence of iron and zinc deficiency is shown in Fig 2.1. Nearly 60-80 % of the world population suffers from iron and more than 30 % from zinc deficiency (White and Broadley, 2005). Iron deficiency is considered as the most serious micronutrient malnutrition problem especially in the developing countries of the world. South East Asia shows the highest occurrence of anemia in women, with over 50% of pregnant women affected (FAO/WHO, 2002). In India about 53% of children and 70-80% of adolescent girls and pregnant women suffer from iron deficiency (Vijayaraghavan, 2002). Iron Deficient Anemia (IDA), the most common consequence of severe iron deficiency occurs as a result of reduction in the oxygen carrying capacity of red blood cells. Iron deficiency symptoms are more prominently visible during pregnancy, menstrual loss, periods of growth and development or when iron is lost because of parasitic infections, such as hookworm. Iron Deficient Anemia (IDA) during pregnancy can result in serious consequences for both mother and baby. Anemic mothers have higher risk of mortality during child birth, and an increased incidence of low-birth weight babies.

Zinc deficiency is also widespread as iron deficiency. According to Caulfield and Black (2004), the global average prevalence of zinc deficiency is around 31%. Zinc is an essential micronutrient with several key roles in gene expression, cell development and replication (Hambrige, 2000). Stein *et al.* (2005) reported that zinc deficiency is responsible for stunted child growth during early childhood and the prevalence of stunting in South Asia and sub Saharan region is very high. It is estimated that 800,000 child deaths world wide are attributable to zinc deficiency annually. Zinc deficiency leads to impaired growth, immune dysfunction, increased morbidity and mortality, adverse pregnancy outcomes, and abnormal neuro-behavioural development. Zinc deficiency during pregnancy results in complications and even death. Even after birth it leads to improper development of child and reduced cognitive abilities. In adults it adversely affects the ability to work and longevity (Monasterio *et al.*, 2007). The severity of zinc deficiency has increased to the extent that zinc fortification has been jointly recommended by WHO and FAO (Allen *et al.*, 2003; WHO, 2004). Requirement of iron and zinc content by reference weight is presented in Fig. 2.2. Iron requirement of infants and children is around 8-10 times higher than that of adult men or women. Zinc requirement for an infant child is about 2-3 times higher than that of adult men and women (Lutter and Rivera, 2003).

2.2 Reasons for iron and zinc deficiency

2.2.1 Low concentration

A major portion of the world population depends upon cereals for their food requirements. Cereals are rich sources of carbohydrates and proteins but have inherently low concentrations of Zn and Fe in grain, particularly when grown on micronutrient

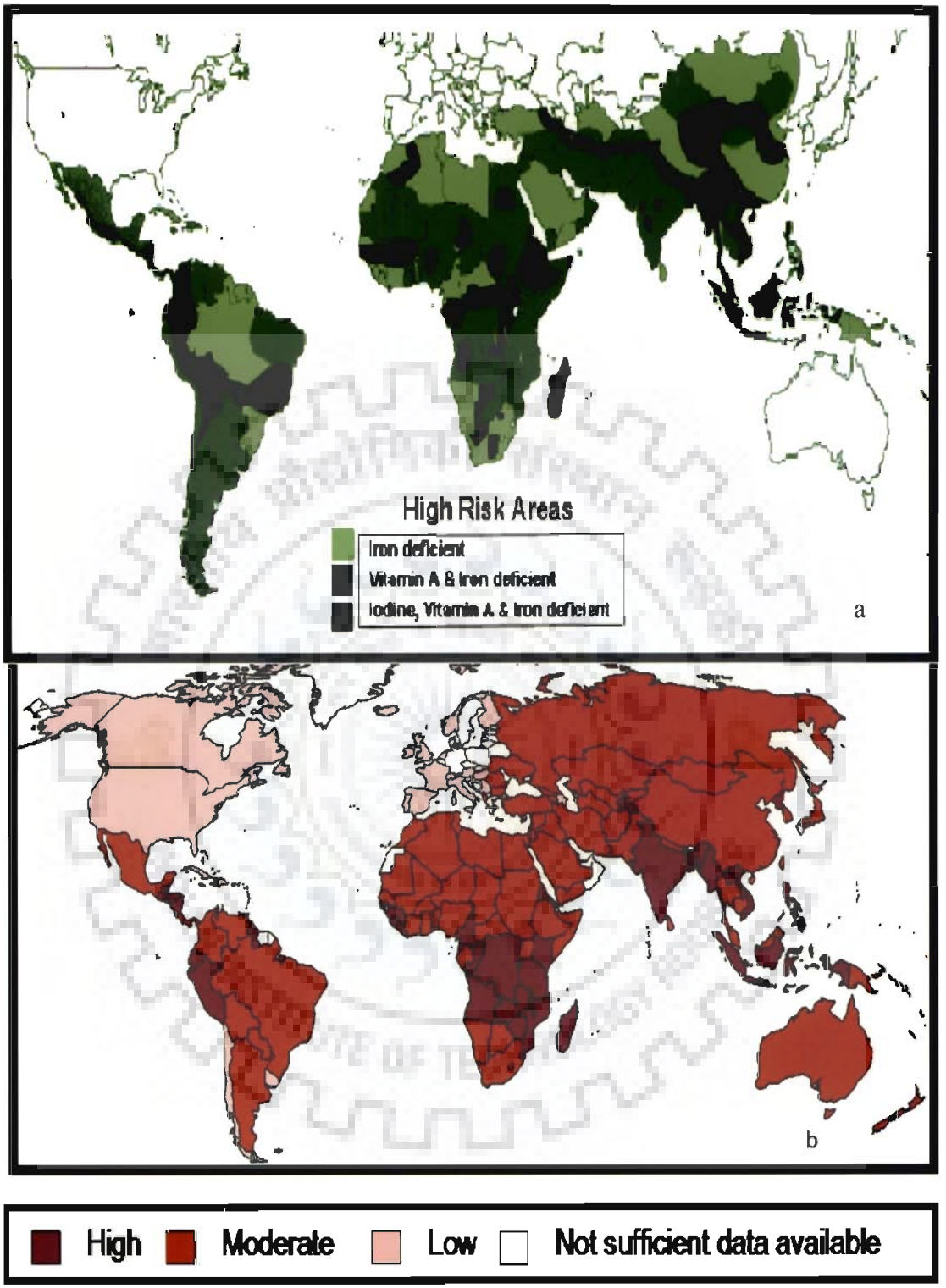

Fig. 2.1 Global prevalence of micronutrient deficiency (a) high risk areas for Iron, vitamin A and iodine deficiencies (b) high moderate and low areas under zinc deficiency. Source; WHO, 2000).

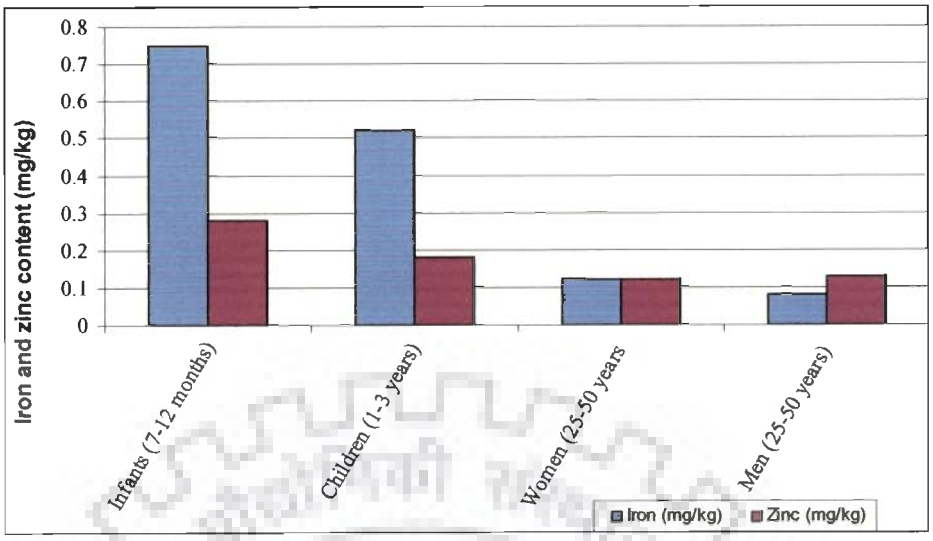

Fig.2.2 Estimated average requirement of iron and zinc by reference weight (Lutter and Rivera, 2003).

deficient soil. In wheat and rice, the most widely eaten food by the poor in the developing countries, only a small fraction of iron is transported to the grain from the senescing leaves. In contrast more than 70% of zinc is mobilized to the grains (Grusak and Dellapenna, 1999; Grusak *et al.,* 1999). In cereals, these micronutrients are primarily stored in husk, the aleurone layer and embryo which are lost during milling and polishing processes. Other important issues include intake of non-diversified diet, presence of anti-nutritional factors in grain (phytic acid, tannins etc) which chelates with metal cations (Fe^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+} etc) and make the availability difficult for human system. About 50 % of the global cereal growing regions are on soils having low levels of available soil Zn (Cakmak *et al.,* 2000). For a better Zn or Fe nutrition for human beings, cereal grains should contain around 40-60 mg/kg Zn or Fe but in

the present condition available amount is in the range of 10-30 mg/kg (Cakmak *et al.*, 2000).

2.2.2 Bioavailability of micronutrients

Another determinant of micronutrient utilization for human growth is their bioavailability, which is defined as the fraction of the ingested nutrient that is utilized for normal physiological function or storage. It depends on the different forms of these micronutrients present in body such as iron exist as ferric ions as thousand of atoms within the 24 subunits spherical cage of ferritin protein which is largely localized in leaves and in amyloplasts in seeds. Ferritin bound iron has relatively high bioavailability. Phytic acid, present in almost all of the cereals such as maize, wheat, rice, rye, puts a major hindrance in absorption of iron and zinc. Both iron and zinc are highly associated with phytic acid along with phosphate in highly non-available form. Other antinutrients in plant foods that reduce iron and zinc bioavailability are fibers, certain tannins and haemagglutinins. There are certain organic acids, heme-protein, some aminoacids, long chain fatty acids, β-carotene, that promotes Fe and Zn bioavailability (Graham *et al.*, 2001).

2.3 Strategies to address micronutrient malnutrition

Most sustainable and ideal solution of hidden hunger in developing countries is to increase the consumption of diversified diet including meat, fish, fruits, vegetables, legumes among the poorest. Some short term public health interventions (supplementation and fortification) can reduce the extent of micronutrient malnutrition. Supplementation is the best strategy for the acute cases of micronutrient malnutrition (Monasterio *et al.*, 2007). Approaches such as fortification of basic

preserved food with required elements (minerals) have contributed significantly (Lyddon, 2004). However since biofortified food do not reach to poor /large populations residing in remote areas, million of people do not benefit from above mentioned strategy.

2.4 Biofortification

Biofortification stands for increasing the bioavailability of micronutrient content of food crop through plant breeding (Welch and Graham, 2004). It can be defined as an example of the application of agricultural research and technologies to reach a public health objective. It holds the greatest potential for enhancing iron and zinc content in cereals (Welch and Graham, 2004). The ways of meeting this aim are screening for high iron and zinc containing varieties, and genetic engineering for plants with increased iron-zinc carriers like ferritin. These approaches are combined with modern plant breeding techniques to develop improved varieties (Welch, 2005). The biofortified cereals can reach the poor in rural areas, have low recurrent costs, are sustainable in the long term, and requiring investment only for genetic improvement. Breeding for specific nutritional qualities requires nutrient density traits in high yielding cultivars. Micronutrient-dense cereal varieties have the added benefit of improved resistance to disease and environmental stresses, and they are particularly efficient in trace mineral-deficient soils and arid regions (Monasterio *et al.*, 2007; Welch, 2005).Conventional and molecular breeding and genetic engineering techniques have been considered to be the most feasible and cost effective approaches for biofortification of cereals with high iron and zinc content (Lonnerdal, 2003; Bouis, 1999). Molecular mapping and gene tagging help to allow scientists to identify the specific plant gene /genetic material that control nutrient contents.

2.4.1 Conventional and molecular breeding techniques

2.4.1.1 Rice:

Iron content of rice is lowest in cereals, often containing only 5 or 6 mg of iron per kilogram after milling (Gregorio *et al.,* 2002). IRRI examined the variability of iron and zinc content in rice grain among 1,138 samples from various countries. The iron concentration in brown rice samples ranged from 6.3 to 24.4 ppm with a mean value of 12.2 ppm. For zinc, the range was 13.5 to 58.4 ppmwith a mean of 25.4 ppm. Few rice varieties Jalmagna, Zuchem, Xua Bue Nuo, Madhukar, IR64 and IR36, were found to have highest iron and zinc contents. Jalmagna, a traditional variety, grown in deepwater areas of eastern India, has twice the iron content of IR36 and IR64. Its zinc concentration was also 40% more than that of IR-36. A tendency for high iron and zinc was also found in the aromatic lines. This wealth of diversity can be utilized for developing biofortified varieties through plant breeding.

2.4.1.2 Maize:

Maize, a staple food crop in southern and eastern Africa is low in iron and zinc content (CIMMYT, 1998). It has been found by Bänziger and Long (2000), that genetic variability of zinc and iron is potentially available in white grained tropical maize germplasm. Promising genetic variability was also found in both improved maize germplasm and land races (Bänziger and Long, 2000). The range in Fe and Zn concentrations were 16.4 – 22.9 μg/g (mean 19.6 μg/g) and 14.7 – 24.0 μg/g (mean 19.8 μg/g), respectively.

2.4.1.3 Wheat:

Cultivated wheat genotypes have very low iron and zinc content in grains which are largely distributed in embryos and the peripheral tissue of bran (Welch and Graham, 1999). Under biofortification programme of CIMMYT, synthetic wheat has been made by crossing tetraploid emmer wheat with *Ae. taushii* accessions with high iron and zinc content (Calderini and Monasterio, 2003a, 2003 b; Monasterio and Graham, 2000). Wild relatives of *Aegilops* species have been reported to have iron and zinc content upto 3 fold higher than the cultivars (Rawat *et al.*, 2008; Chhuneja, 2006).

2.4.1.4 Rye:

Rye is a crop very efficient in nutrient use. A chromosome from rye (1R) was translocated to wheat as 1B/1R translocation. It was done to introduce a new source of leaf rust resistance. Two different sets of 1B/1R near-isogenics were studied for their contribution to mineral concentration in the grain. Both sets showed a significant increase in iron and zinc concentration between 6% and 12% in favour of the 1B/1R translocation as compared with the 1B/1B type, suggesting that some genes associated with high iron and zinc in the grain might be present in the 1R chromosome (Monasterio and Graham, 2000).

All the above examples in different crops clearly elucidate the rich variability in germplasm collections of various cereals for grain micronutrients which can be utilized for biofortification of cultivars effectively using molecular breeding.

2.4.2 Genetic Engineering Approaches

Biotechnology is playing a crucial role towards the goal of increasing micronutrient content in cereals. Lucca *et al.* (2001), generated some transgenic lines in rice variety, Taipei 309 with genes introduced for ferritin (pfe) metallothionein-like (rgMT) and phytase (*phy A*) genes into rice from different sources. One direct approach towards the same is to simply enhance the iron-content by increasing a protein ferritin which stores iron in animals, plants and bacteria. Ferritin protein consists of 24 polypeptide subunits and can sequester 4000 iron atoms in a non-reactive form as hydrous ferric oxide phosphate. The *ferritin* gene has been isolated and sequenced in plants, including soybean, french bean, pea and maize. Goto *et al.* (1999), reported improvement in the iron content of rice by transferring the entire coding sequence of the soybean ferritin gene into Japonica rice. These transgenic seeds stored up to three times more iron than the normal seeds. In the transformation experiments carried out by Lucca *et al.* (2001) ferritin gene was isolated from *Phaseolus vulgaris*. The ferritin transformants recorded an improved iron accumulation in the seeds with the iron content in mature transformed seeds between 11.53 + 0.16 μg/g and 22.07 + 0.70 μg/g. This 2-3 fold increase in iron content in the transgenic plants would be of nutritional importance. Daily consumption of 300 g of rice would yield about 6 mg of iron as compared to the present 3 mg. Transgenic wheat with *Aspergillus niger* phytaseA gene expressing high level of phytase has been developed for obtaining higher availability of iron and zinc (Lucca *et al.*, 2001; Pederson *et al.*, 2000).

However, success with transgenics has remained low because of the poor understanding of the complex mechanisms controlling mineral homoeostasis in plants

and little understanding of large genome crops such as wheat. Moreover germplasm collections have very rich variability for grain micronutrients which can be transferred to crops more easily with greater reliability and economy with one time expenditure.

2.5 Wheat biofortification through molecular breeding

Wheat is one of the most important staple food crops of the world, occupying 17% of crop acreage worldwide, feeding about 40% (nearly half) of the world population and providing 20% of total food calories and protein in human nutrition (Gupta *et al.*, 2008). Total cultivation area for wheat is 213 million hectares with an annual production of 619 million tonnes and an average yield of 2.9 tonnes /ha. Wheat shares 28% total cereal grain production after maize (31%). It has a global impact on world's economy and primary staple crop to feed ever growing human population (Feuillet *et al.*, 2007; http://faostat.fao.org/). Being staple food of one third of the world's population (FAO, 2004), it would be highly appropriate to biofortify wheat to overcome the menace of hidden hunger.

2.6 Evolution of wheat:

The A and D genome donors of hexaploid wheat *Triticum aestivum* (AABBDD) have been unequivocally identified as *Triticum urartu* Tumanian ex Gandilyan (AA) and *Ae. taushii* (DD) (Faris *et al.*, 2002; McFadden and Sears, 1946 ; Kihara, 1944) while *Ae. speltoides* Taush (SS) or a closely related species contributed the B genome (Faris *et al.*, 2002 and Riley *et al.*, 1958), however still some controversy surrounds the B genome donor (Jauhar, 2007; Dvoák, 1998; Wang *et al.*, 1997; Sarkar and Stebbins 1956,). The two diploid progenitors, *Triticum urartu* and *Aegilops speltoides,* hybridized in nature about half a million years ago and hybrid

resulted in to tetraploid wild emmer wheat (*T. turgidum* var. *dicoccoides* Körn), presumably in one step as a result of functioning of unreduced (2*n*) gametes in the BA hybrid (amphihaploid) (Step 1 in Fig. 2.3) (Huang *et al.*, 2002).

As the B-genome donor is believed to have acted as the female parent (Wang *et al.*, 1997), genome of the hybrid is indicated as BA. The corresponding (homoeologous) chromosomes of the closely related constituent genomes are hence capable of pairing with one another. *Ph1*, a chromosome pairing regulator, (*Pairing homoeologous* gene), arose as a mutation on the long arm of chromosome 5B at the time of origin of tetraploid emmer wheat. The *Ph1* gene acts as a suppressor for homoeologous chromosome pairing and ensures diploid-like meiosis and disomic inheritance in polyploid wheats (Jauhar, 2007; Jauhar and Joppa, 1996; Riley and Chapman, 1958). Second cycle of spontaneous hybridization took place some 7000 years ago (Step 2 in Fig. 2.3) resulting in the hexaploid wheat (2n = 6x = 42, genome BBAADD), from tetraploid wheat by acquiring a third genome, DD, from another *Aegilops tauschii* Coss. (2*n* = 2*x* = 14, DD genome) about 8000 years ago (Jauhar, 2007; Huang *et al.*, 2002; McFadden and Sears 1946).

2.7 Wheat gene pool and classification:

Wild *Triticum*, *Aegilops* and related species are very important sources of numerous genes of agronomic importance including resistance against biotic and abiotic stresses. The genus *Aegilops* comprises 11 diploids 10 tetraploids and 2 hexaploid species (Table 2.1). Out of these species various diploid and polyploid species were used to develop alien introgression lines (Schneider *et al.*, 2008). Detailed information of classification of *Triticum* and *Aegilops* genera can be archived by following link: **http://www.k-state.edu./wgrc/Taxonomy/taxaeg.html** .

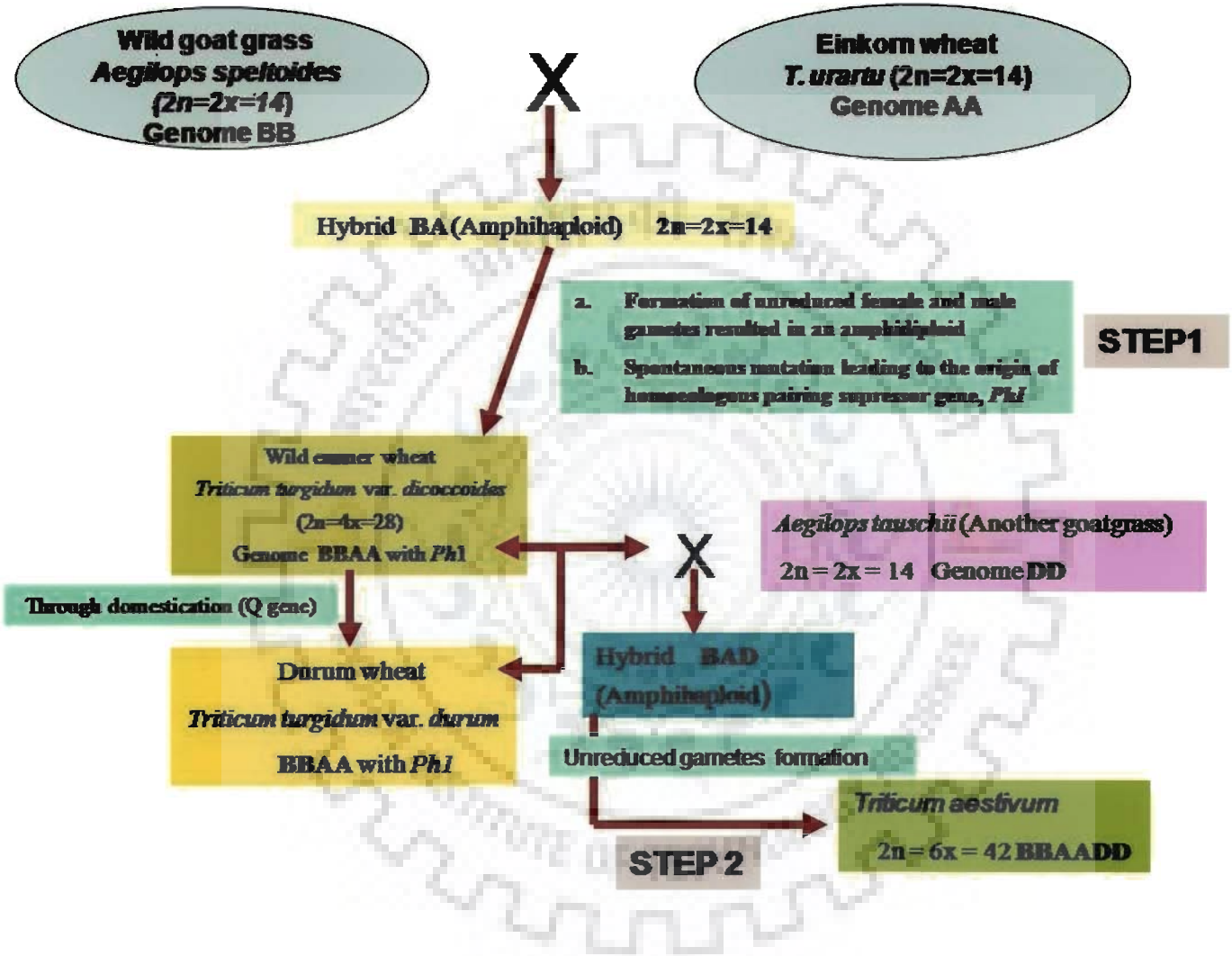

Fig. 2.3. Steps in wheat evolution from diploid *T. urartu* and *Aegilops speltoides* to tetraploid *T. durum / T. dicoccoides* and from *T. durum / T. dicoccoides* and *Ae. tauschii* to hexaploid *T. aestivum* under the effect of unreduced gamete formation and origin of homeologous pairing, suppressor gene (*PhI*).

Table 2.1 Genomic constitution of *Triticum* and *Aegilops* species

Species	**Genomic constitution**
Triticum aestivum L. (common or bread wheat)	ABD
Subspecies: *compactum* (Host)	
Triticum turgidum L. (pollard wheat)	AB
Subspecies: *carthlicum* (Nevski)	
Triticum zhukovskyi Menabde & Ericz.	A^tA^mG
Triticum timopheevii (Zhuk.) Zhuk. (cultivated form) Subspecies: *armeniacum* (Jakubz.) van Slageren (wild form)	A^tG
Triticum monococcum L. Subspecies: *aegilopoides* (Link) Thell.	A^m
Triticum urartu Tumanian ex Gandilyan	A
Aegilops speltoides Tausch	S
Aegilops longissima Schweinf. & Muschl.	S^l
Aegilops searsii Feldman & Kislev ex Hammer	S^s
Aegilops sharonensis Eig	S^{sh}
Aegilops bicornis (Forssk.) Jaub. & Spach	S^b
Aegilops tauschii Coss. var. *tauschii*, var. *Strangulata*	D
Aegilops uniaristata Vis.	N
Aegilops comosa Sm. in Sibth. & Sm. var. *heldreichii*	M
Aegilops caudate L.	C
Aegilops umbellulata Zhuk.	U
Aegilops mutica Boiss.	T
Aegilops cylindrica Host	D^cC^c
Aegilops ventricosa Tausch	D^VN^V
Aegilops crassa Boiss.	$D^{c1}M^c(D^{c1}X^C)$
var. *glumiaristata*	$D^{c1}D^{c2}M^c(D^{c1}D^{c2}X^c)$
Aegilops juvenalis (Thell.) Eig	DMU ($D^cX^cU^j$)

Aegilops vavilovii (Zhuk.) Chennav.	DMS ($D^cX^cS^v$)
Aegilops triuncialis L.	UC^t
Aegilops columnaris Zhuk.	UM (UX^{CO})
Aegilops neglecta Req. ex Bertol. (syn. *Ae. triaristata)*	UM (UX^n)
var. *recta* (Zhuk.) Hammer	UMN (UX^tN)
Aegilops geniculata Roth (syn. *Ae. Ovata)*	UM (UM^0)
Aegilops biuncialis Vis.	UM (UM^0)
Aegilops kotschyi Boiss.	US (US^1)
Aegilops peregrina (Hack. in J. Fraser) Maire & Weiller (syn. *Ae. variabilis)*	US (US^1)

(Source: FAO Document repository, 2002)

2.8 Distribution of genetic diversity: Gene pools

The tribe *Triticeae* comprises more than 500 species of 26 genera (**http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi**). In relation to the cultivated species, each of these can be considered to be a part of the primary, secondary or tertiary gene pool, depending on how closely related they are at the genomic level (Feuillet *et al.*, 2007). The primary gene pool of wheat includes the hexaploid landraces, cultivated tetraploids, wild *T. dicoccoides* and diploid donors of the A and D genomes to tetraploid and hexaploid wheats. Transfers of genes from these two genomes occur as a consequence of direct hybridization and recombination (homologous) with conventional breeding approaches. The secondary gene pool includes polyploid *Triticum* and *Aegilops* species, which share one genome with the three genomes of wheat. The diploid species of the Sitopsis section are included in this pool, and hybrid products within this gene pool demonstrate reduced chromosome

pairing. Gene transfers occur as a consequence of direct crosses, breeding protocols, homologous exchange between the related genome or through use of special manipulation strategies among the non-homologous genomes. Embryo rescue is a complementary aid for obtaining hybrids. Diploid and polyploid species with genomes that are non-homologous to wheat reside in the tertiary gene pool; hence, gene transfers require special techniques that assist homoeologous exchanges (Kazi and Rajaram, 2002).

2.9 Wheat Cytogenetics and Molecular biology

Data on the genomic structure of wheat cultivars has accumulated after almost a century of research, started with the pioneering genetic experiments of Nilsson Ehle (1909) and the cytological studies of Sakamura (1918) and Sax (1918). The method of nuclear genome analysis based on chromosome pairing behaviour in interspecific hybrids (Sax, 1922; Kihara, 1919) provided information on genome constitution, phylogeny and the evolution of *Triticum* and *Aegilops* species (Lilienfeld, 1951). In the 1930s, Sears (Sears and Sears, 1978; Sears, 1954) began studies with wheat aneuploids that ushered in the era of formal cytogenetic analysis and gene mapping of individual chromosomes and arms in wheat (McIntosh *et al.*, 1998). Modern staining techniques were used to analyse the substructures of cereals and a cytogenetic karyotype of wheat was developed (Gill*et al.*, 1991; Gill and Kimber, 1974a). Non-isotopic methods of mapping DNA sequences *in situ* (*in situ* hybridization) on chromosomes were used to construct a molecular karyotype of wheat (Rayburn and Gill, 1985; Jiang and Gill, 1994a). These early molecular cytogenetic methods of genome analysis have greatly facilitated cytogenetic analysis in wheat and related species, especially the analysis of alien transfers (Friebe *et al.*, 1991, 1996a). DNA restriction fragment length polymorphism (RFLP) analysis and linkage

mapping was applied to wheat in the late 1980s (Chao *et al.*, 1989; Kam-Morgan *et al.*, 1989). The genetic linkage maps of common wheat (Cadalen *et al.*, 1997; Marino *et al.*, 1996; Van Deynze *et al.*, 1995; Nelson *et al.*, 1995a, 1995b, 1995c; Devos and Gale, 1993; Liu and Tsunewaki, 1991), durum wheat (Blanco *et al.*, 1998) and two of the progenitor species have been developed (Dubcovsky *et al.*, 1996). The low level of polymorphism revealed by RFLP markers in wheat (Liu *et al.*, 1990; Chao *et al.*, *1989*) has often hampered the establishment of intervarietal genetic linkage maps. Development of linkage maps is a pre-requisite for the dissection of complex agronomic traits through QTL analysis and the use of these QTLs in plant breeding via marker-assisted selection. The first intervarietal map of bread wheat, based on RFLP markers, was published in 1997 (Cadalen *et al.*, 1997). Development and mapping of microsatellite markers in wheat (*Gupta et al.*, 2002; Guyomarc'h *et al.*, 2002; Röder *et al.*, *1998*;), as well as the transfer of microsatellites isolated from *Aegilops tauschii* to bread wheat (Guyomarc'h *et al.*, 2002; Pestsova *et al.*, 2000), enabled the development and improvement of intervarietal linkage maps in wheat.

The density of wheat genetic maps was improved with the development of microsatellite (SSR) markers leading to construction of SSR maps of wheat (Gupta *et al.*, 2002; Pestsova *et al.*, 2000; Röder *et al.*, 1998). Somers *et al.* (2004) added more SSR markers to these earlier maps and prepared a high-density SSR consensus map. The availability of a high-density consensus map and molecular markers (PCR based) has greatly improved the genetic characterization of hexaploid wheat (Somers *et al.*, 2004). An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* x *T. monococcum* RIL population was constructed by (Singh *et al.*, 2007).

Detailed information about the map can be achieved by following link: (http://wheat.pw.usda.gov/report?class=mapdataandname=T.%20boeoticum%20x%20monococcum)

2.10 Molecular physical maps of wheat:

2.10.1 Molecular marker-based physical maps

Molecular markers in bread wheat have also been used for the preparation of physical maps, which were then compared with the available genetic maps involving same markers. These maps allowed comparisons between genetic and physical distances to give information about variations in recombination frequencies and cryptic structural changes (if any) in different regions of individual chromosomes.

Several methods have been employed for the construction of physical maps. Physical maps of wheat chromosomes were already reported by different workers (Goyal *et al.*, 2005). Recently a very high resolution physical map of chromosome 3B (1GB) of wheat chromosome has been constructed by Paux *et al.* (2008)

2.10.2 Deletion Mapping:

In wheat, physical mapping of genes to individual chromosomes began with the development of aneuploids (Sears 1954), which led to mapping of genes to individual chromosomes. Later, deletion lines of wheat chromosomes developed by Endo and Gill (1996) were extensively used as a tool for physical mapping of molecular markers. Using these deletion stocks, genes for morphological characters were also mapped to physical segments of wheat chromosomes directly in case of unique and genome specific markers or indirectly in case of duplicate or triplicate loci through the use of intergenomic polymorphism between the A, B, and D sub-

genomes. In addition to physical mapping of genomic SSRs, ESTs and EST-SSRs were also subjected to physical mapping. A major project (funded by NSF, USA) on mapping of ESTs in wheat was successfully completed by a consortium of 13 laboratories in USA leading to physical mapping of ~16,000 EST loci (http://wheat.pw.usda.gov/NSF/progress_mapping; Qi *et al.*, 2004).

2.10.3 *In silico* physical mapping:

Nearly 16,000 wheat EST loci assigned to deletion bins constitute a useful source for *in silico* mapping. Markers with known sequences can be mapped to wheat chromosomes through sequence similarity with mapped EST loci available at GrainGene database (http://www.wheat.pw.usda.gov/GG2/blast.html). Applying this approach, mapped 157 SSR containing wheat unique sequences [out of 429 class I unigene derived microsatellites (UGMS) markers developed in wheat] to chromosome bins. These bin mapped UGMS markers provide valuable information for a targeted mapping of genes for useful traits, for comparative genomics and for sequencing of gene-rich regions of the wheat genome (Gupta *et al.*, 2008).

2.10.4 Radiation hybrid mapping:

Hybrid sterility and lack of recombination between wheat and alien chromosomes are the major barriers in alien gene transfer in wheat. Radiation hybrid mapping, a recent approach (do not rely on meiotic recombination) for wheat, is being successful in generating high resolution radiation hybrid maps. Kalavacharla *et al.* (2006) generated a radiation hybrid map of 1D chromosome of wheat with the resolution of about 200kb/break. Hossain *et al.* (2004) were able to locate an alien

scs^{ae} gene (Species Cytoplasm-Specific) in wheat by using radiation hybrid mapping approach.

2.10.5 Map based cloning:

The first genes cloned in wheat by map-based cloning included three resistance genes, against fungal diseases (*Lr21;* Huang *et al.,* 2003 and *Lr10*; Feuillet *et al.,* 2003) (*Pm3b*; Yahiaoui *et al.,* 2004). Map based cloning of a number of genes for various important traits including disease resistance, vernalization response, grain protein content, free threshing habit and tolerance to abiotic stresses have been recently completed (Gupta *et al.,* 2008).

2.11 Alien introgression for wheat improvement:

Aegilops germplasm has been utilized extensively for the wheat improvement and various addition, substitution, translocation lines for different chromosomes of *Aegilops* species have already been reported by many workers (Schineder *et al.*, 2007). Some examples of alien addition , substitution and translocation lines are given in Table 2.4. Already, there are spectacular examples of alien chromosome segments transferred to wheat, either by physical means (irradiation) or by genetic manipulation that have immensely improved the productivity of the wheat crop including *Lr 9* from *Aegilops umbellulata, Pm8, Sr31, Lr26, Yr9, Pm 17, Gb2* from *Secale sereale* and *Lr 19, Lr24, Sr24, Sr 26* from *Agropyron elongatum.*

Table 2.2. List of agronomically important genes cloned in wheat

Genes	Traits	References
Lr1	Leaf rust resistance	Ling *et al.* (2003); Cloutier *et al.* (2007)
Lr10	Leaf rust resistance	Feuillet *et al.* (2003)
Lr21	Leaf rust resistance	Huang *et al.* (2003a)
VRN1	Vernalization response	Yan *et al.* (2003a)
VRN2	Vernalization response	Yan *et al.* (2004
VRN3	Vernalization response	Yan *et al.* (2006)
Q	Free threshing character	Faris *et al.* (2003); Simons *et al.* (2006)
Pm3b	Powdery mildew resistance	Yahiaoui *et al.* (2004); Brunner *et al.* (2005)
GPC-B1	High grain protein content	Distelfeld *et al.* (2004); Uauy *et al.* 2006
Yr5	Resistance to stripe rust	Ling *et al.* (2005)
B	Boron tolerance	Schnurbusch *et al.* (2007)
Tsn1	Host-selective toxin Ptr ToxA	Lu *et al.* (2006)
Ph1	Chromosome pairing locus	Griffiths *et al.* (2006)
Sr2	Stem rust resistance	Kota *et al.* (2006)
Fr2	Frost resistance	http://www.agronomy.ucdavis.edu/Dubcovsky

Table 2.3. Addition, substitution and translocation lines of *T. aestivum- Aegilops* species

Plant material	***Aegilops* chromosomes**	**References**
Triticum–Aegilops addition lines		
Triticum aestivum–Ae. comosa addition line	2M	Riley *et al.* (1968)
Triticum aestivum–Ae. umbellulata addition lines	1U, 2U, 5U, 6U, 7U	Kimber (1967)
	complete set, except 3U; 4U mono	Friebe *et al.* (1995a)
Triticum aestivum–Ae. mutica (*Ambylopyrum muticum*) addition lines	A, C, E, F	Dover (1973)
Triticum aestivum-Ae. variabilis (Ae.peregrina) addition lines	A, C, E, G, H, J, M, N, O, P, Q	Driscoll (1974)
Triticum aestivum–T. peregrinum (*Ae. peregrina*) addition lines	Complete set	Friebe *et al.* (1996b)
Triticum aestivum–Ae. variabilis (*Ae. peregrina*) addition lines	Not identified	Spetsov *et al.* (1997)
Triticum aestivum–Ae. longissima addition lines	Complete set	Feldman (1975)
	Complete set	Friebe *et al.*, (1993)
	3S, 6S, 7S	Hart and Tuleen (1983)
	3S, G, B	Feldman, unpublished, see Shepherd and Islam (1988)
Triticum aestivum–Ae. ventricosa addition lines	C, G, H, 4U, 5U, 6U	Dosba *et al.* (1978)
	2M, 4M	Delibes *et al.* (1981)
	Not identified	Rivoal *et al.* (1986)
Triticum aestivum-Ae. sharonensis addition line	4S(4D) substitution; 2S, 3S, 5S, 7S[4S (4D)] addition	Miller *et al.* (1982), Miller (1983)
Triticum aestivum–Ae. bicornis addition lines	$3S^b$,$7S^b$	Riley and Chapman unpublished, see Shepherd and Islam (1988)
Triticum aestivum-Ae. searsii addition lines	Complete set	Pietro *et al.* (1988)
		Friebe *et al.* (1995b)
Triticum aestivum–Ae. markgrafii (*Ae. caudata*) addition lines	Complete set except chromosome A	Schubert and Blüthner (1995)
Triticum aestivum–Ae. geniculata addition lines	Complete set (6Umono)	Friebe *et al.* (1999)

Triticum aestivum–Aegilops speltoides addition lines	Complete set	Friebe *et al.* (2000)
Triticum aestivum-Ae. biuncialis addition lines	2M, 3M, 7M, 3U, 5U	Schneider *et al.* (2005)
Triticum–Aegilops substitution lines		
Triticum aestivum–Ae. umbellulata–Ae. sharonensis double substitution line	1U (1D) and 4S(4D)	Reader and Miller (1987)
Triticum aestivum–Ae. umbellulata substitution lines	1U, 2U, 5U, 7U (1, 2, 5, 7 A, B, D)	Riley *et al.* (1971), (1973)
Triticum aestivum–Ae. caudata substitution lines	5C (5A, 5D)	Muramatsu (1973)
Triticum aestivum–Ae. sharonensis substitution lines	4S(4A), 4S(4B), 4S(4D)	Miller (1983)
Triticum aestivum-Ae. longissima substitution line	A, C, D	Netzle and Zeller (1984)
Triticum aestivum–Ae. tauschii substitution lines	Complete set	Conner *et al.* (1988)
Triticum aestivum–Ae. longissima substitution lines	5S(5A), 5S(5B), 5S(5D)	Millet *et al.* (1988)
Triticum aestivum–Ae. searsii substitution lines	21 whole chomosome., 31 ditelo somic substituion	Friebe *et al.* (1995b)
Triticum aestivum–Ae. variabilis (*Ae. peregrina*) substitution lines	Not identified	Spetsov *et al.* (1997)
Triticum aestivum–Ae. ovata (*Ae. geniculata*) substitution lines	5M(5D)	Dhaliwal *et al.* (2002)
Triticum–Aegilops translocation lines		
Triticum aestivum–Aegilops translocation lines		Friebe *et al.* (1996 b)
Triticum aestivum–Ae. comosa translocation lines	6U/6BL, 6U/4A, 6U/2D, 6U/6BS	Sears (1956)
	2DL/2M, 2DS/2M	Riley *et al.* (1966), (*1968*)
Triticum aestivum–Ae. umbellulata translocation lines	1U/1B	Koebner and Shepherd (1987)
	6U/6B, 6U/4B, 6U/2D, 6U/7B	Friebe *et al.* (*1995a*)
Triticum aestivum–Ae. ovata (*Ae. geniculata*) translocation lines	5M/2AL, 5M/1BL, 5M/5BS	Dhaliwal *et al.* (*2002*)

(Source; Schineder *et al.*, 2008)

One of the best examples of introgression of chromatin from a relative into wheat is the 1BL/1RS chromosomal translocation (Feuillet *et al.*, 2007). The 1RS chromosome from rye carries several genes whose protein products increase grain yield by providing race-specific disease resistance to major rust diseases (including *Lr29/Yr26* leaf and yellow rust resistance genes), improved adaptation and stress tolerance, superior aerial biomass and higher kernel weight. The wild relatives of crop plants are sources of useful genes, but such genes when transferred to agricultural crops are often associated with deleterious traits. Because most of the recombination and the disease resistance genes are localized towards the end of wheat chromosomes, cryptic terminal alien segments, carrying rust resistance genes, were transferred from *Aegilops geniculata* (U^gM^g) and *Ae. triuncialis* (U^tC^t) into common wheat without usual linkage drag. A number of genes for resistance against various wheat diseases have been introgressed into wheat from related progenitor and non progenitor species (Kuraparthy *et al.*, 2007; Marais *et al.*, 2005; Friebe *et al.*, 1996) and commercially exploited. Some examples of alien gene transfer for disease resistance and quality improvement have been listed in Table 2.3.

2.12 Characterization of the alien introgressions

2.12.1 C-Banding:

C-banding is used to identify the alien chromosome with the target gene, the recipient wheat chromosome and the translocated chromosome. The C-banding involves staining of all constitutive heterochromatin through denaturation-reassociation of DNA, with the highly repetitive DNA reassociating faster and appearing as dark

bands (Gill and Kimber, 1974a). C-band is used to describe a pair of laterally adjacent stained dots, one belonging to each of the two chromatids comprising each metaphase chromosome. Thus, two adjacent bands consist of two pair of dots longitudinally juxtaposed. Anonymous (1972) defined a band as a 'part of a chromosome clearly distinguishable from adjacent parts by virtue of its lighter or darker staining ability'. Consequently all dark C-bands should be considered as landmark bands, which are diagnostic in the identification of individual chromosomes (Gill *et al.*, 1991).

The C-banding technique has been used to construct wheat karyotypes. Such karyotypes have been utilized to identify individual chromosomes (Gill and Kimber, 1974), to differentiate lines with polymorphic banding patterns, to study structural aberrations and other wheat cytogenetics research. To accomplish these studies in a standardized manner a karyotype and generalized nomenclature has been available illustrating chromosome bands, banding polymorphisms and various structural aberrations for *Triticum aestivum* L. cultivar 'Chinese Spring' (Gill *et al.*, 1991).

Table 2.4 List of alien genes transferred to wheat for resistance against various diseases.

Disease	Donor species	Resistance gene(s)
Leaf rust (*Puccinia recondita*)	*Ae. umbellulata*	*Lr9*
	Ae. speltoides	*Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51*
	Ae. tauschii	*Lr21*, *Lr22*, *Lr32*, *Lr39*, *Lr40*, *Lr41*, *Lr42*, *Lr43*
	Ae. ventricosa	*Lr37*
	Ae. geniculata	Several leaf rust resistance
	Ae. geniculata	*Lr57*
Stem rust (*Puccinia graminis*)	*Ae. speltoides*	*Sr32*, *Sr39*
	Ae. comosa	*Sr34*
	Ae. ventricosa	*Sr38*
	Ae. tauschii	*Sr33*
Stripe rust (*Puccinia stiiformis*)	*Ae. comosa*	*Yr8*
	Ae. tauschii	*Yr28*
	Ae. ventricosa	*Yr17*
	Ae. geniculata	*Yr40*
Powdery mildew (*Erysiphe graminis*)	*Ae. speltoides*	*Pm12*
	Ae. longissima	*Pm13*
	Ae. speltoides	*Pm32*
	Ae. tauschii	*Pm19*, *Pm35*
	Ae. geniculata	*Pm29*
Eyespot (*Tapesia yallundae*)	*Ae. ventricosa*	*Pch1*

(Source: Schineder *et al.*, 2008).

2.12.2 *In-situ* hybridization studies in wheat

The *in-situ* hybridization (ISH) involving radioactively labeled probes were initially used to localize repetitive DNA sequences, rRNA and alien DNA segments in wheat (Flavell and Smith 1974; Gerlach and Peacock 1980; Gerlach *et al.,* 1983). Later, fluorescence *in situ* hybridization (FISH), McFISH (multicolor FISH, simultaneous detection of more than one probe) and GISH (genome *in situ* hybridization, total genomic DNA as probe) were used in several studies. FISH with some repeated sequences as probes was used for identification of individual chromosomes (Zhang *et al.,* 2004b; Badaeva, 2002; Pederson and Langridge, 1997; Mukai *et al.,* 1993). FISH was also utilized to physically map rRNA multigene family (Mukai *et al.,* 1991), RFLP markers (Ma *et al.,* 2001; Zhang *et al.,* 2000b), and unique sequences (Li *et al.,* 2003; Turnbull *et al.,* 2003; Rahman *et al.,* 2001) and also for detecting and locating alien chromatin introgressed into wheat (Biagetti *et al.,* 1999; Schwarzacher *et al.,* 1992; Mukai and Gill 1991). A novel high-resolution FISH strategy, using super-stretched flow-sorted chromosomes was also used extended DNA fibre-FISH (Yamamoto and Mukai, 1998, 2005; Lavania *et al.,* 2003) to fine map DNA sequences (Fukui *et al.,* 2001; Valárik *et al.,* 2004) and to confirm integration of transgenes into the wheat genome (Jackson *et al.,* 2001). Recently, BACs were also utilized as probes for the so-called BAC-FISH which helped not only to discriminate between the three sub-genomes, but also in the identification of intergenomic translocations, molecular cytogenetic markers, and individual chromosomes (Zhang *et al.,* 2002). BAC-FISH also helped in localization of genes (BACs carrying genes) and in studying genome evolution and organization among wheat and its relatives (Papa *et al.,* 2000; Zhang *et al.,* 2004a, 2004b). Genomic in

situ hybridization (GISH), in which total genomic DNA is labelled and used as a probe, can be considered as a powerful technique widely applicable to the study of interspecific hybrids and their derivatives. GISH has been successfully applied in wheat cytogenetic studies, especially in mitotic chromosomes (Schwarzacher *et al.*, 1992). Genomic in situ hybridization is an important tool to identify alien chromosomes in wheat background and is of great importance in plant breeding programmes. Total genomic DNA can be labelled and used as a probe to identify alien introgression in wheat (Mukai and Gill, 1991). GISH studies identified the parental origin of each chromosome in hybrids of *Hordeurn chilense* Roem. and Schult and *H. vulgare* L. with *S. africanum* Stapf. and in the hybrid of *H. vulgate x H. bulbosum* L. (Schwarzacher *et al.*, 1992), as well as alien chromosomes and chromosome segments from *S. cereale* and *H. vulgare* in hexaploid wheat cultivars (Mukai and Gill, 1991) and triticale (Le and Armstrong, 1991).

2.13 Iron and zinc status of wheat

2.13.1 Iron and zinc status of wheat cultivars and related species:

Most of the *Triticum aestivum* L. and *T. turgidum* L. ssp. d*urum* (Desf.) cultivars have lower grain iron and zinc content than the related wild *Triticum* and *Aegilops* species (Rawat *et al.*, 2008; Chhuneja *et al.*, 2006; Cakmak *et al.*, 2000; Monasterio and Graham 2000). For the identification of useful variability for wheat biofortification major emphasis has been on the screening of progenitor species including diploid wheat, *T.monococcum* L., *T. turgidum* L. ssp. *dicoccoides* (Körn, ex Asch. and Graebn.), *T. turgidum* L. ssp. *dicoccon* (Schrank), *Ae. tauschii* L. etc. (White and Broadley 2005; Cakmak *et al.*, 2004; Monasterio and Graham 2000 and

Cakmak *et al.*, 2000). Scientists at CIMMYT, Mexico have used synthetic hexaploid wheat from crosses between *T. durum* and *Ae. tauschii* with high iron and zinc contents in breeding programmes and have developed wheat lines with higher level of these micronutrients which are being tested in farmers' fields in India, Pakistan and other countries (Calderini and Monasterio, 2003). However the level of enhancement of iron and zinc using wheat synthetics has not been very impressive because of the limited variability for iron and zinc in the progenitor wild parents. Therefore, screening of non-progenitor species for additional variability for micronutrients is very critical.

2.13.2 Germplasm screening for higher iron and zinc content:

Wild wheat and related species serve as an important source of new genetic variability for increasing micronutrient content in seeds (Cakmak *et al.*, 2000). The most feasible approach to biofortify wheat cultivars is to exploit genetic

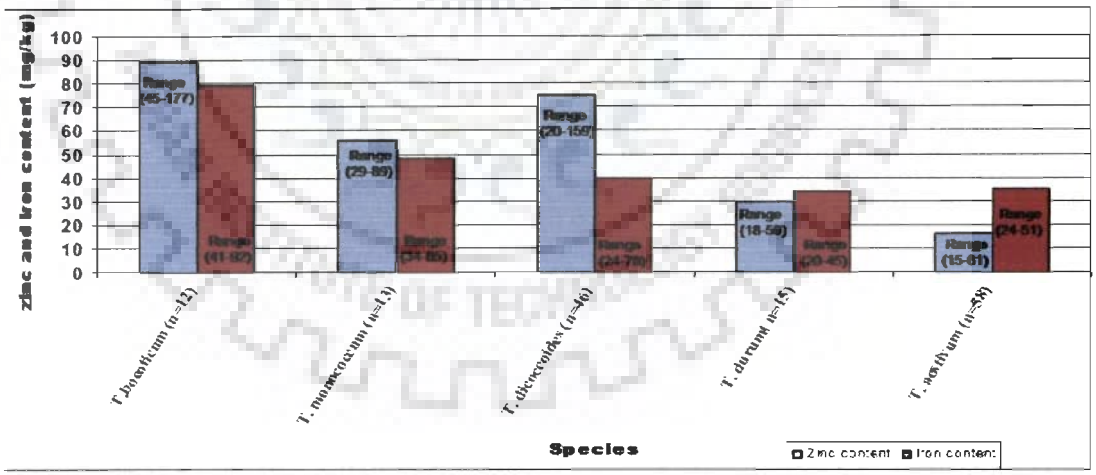

Fig 2.4. Concentration and content (total amount) of iron and zinc in seeds of different wild, primitive and modern wheat (Cakmak *et al.*, 2000).

variation in wild relatives for higher contents of these micro nutrients. Fig 2.6 shows concentrations and total Fe and Zn content in seeds of different wild primitive and modern wheat verities. The germplasm of *Triticum* and related species has been extensively evaluated for useful variability for higher grain and zinc. Wild *Aegilops* species is an excellent source of high grain iron and zinc content character (Rawat *et al.*, 2008; Chunneja *et al.*, 2006). Studies of Cakmak *et al.* (2000), in seeds of wild, primitive and modern wheats suggest that 25 accessions of wild diploid wheat (*T. boeoticum* and *T. urartu*) and 46 wild tetraploid (*T. dicoccoides*) wheat accessions presented substantial variation in seed concentration of iron and zinc. However the concentration of Fe and Zn in seeds of modern tetraploid (*T. durum*) and hexaploid (*T. aestivum*) cultivated wheats are much lower and less variable than the former. These examples suggest that wild wheats can be considered as a major source of genetic diversity for increasing zinc and iron density in seeds of modern wheat as high zinc concentrations in their seeds are genetically determined and not related to environmental conditions.

Development of amphiploids is an important step for successful gene introgression. Amphiploids serve as reliable store house of genomic interaction between the alien and cultivated species (Jiang *et al.*, 1994). *Aegilops* species are often used as sources of desirable agronomic characters that could be introduced into cultivated cereals. Simonenko *et al.* (1998) used *Aegilops*-rye amphiploids for introgression of genetic material to wheat. Synthetic amphiploids developed by crossing *Triticum durum* and *Ae. tauschii* at CIMMYT, Mexico (Mujeeb-Kazi, 1995) also showed wide variability for Fe and Zn concentrations in the grains (Calderini and

Ortiz-Monasterio, 2003). In addition to the above species, some of the S genome *Aegilops* species with higher grain Fe and Zn have also been identified (Rawat *et al.*, 2008; Chhuneja *et al.*, 2006). Realizing the importance of biofortification, several studies were undertaken for the evaluation of germplasm and advance breeding lines for variability of grain Fe and Zn content (Cakmak *et al.*, 2000; Monasterio and Graham, 2000; Chhuneja *et al.*, 2006; Morgounov *et al.*, 2007). However, only a few studies are available on the genetics of accumulation of micronutrients in the grains of major cereals like wheat and rice (Shi *et al.*, 2008; Stangoulis *et al.*, 2007). So far, no major locus or QTLs have been mapped for grain Fe content in wheat. Understanding the genetic basis of accumulation of micronutrients in grains will provide the basis for devising the plant breeding strategies for improving grain micronutrient content and for marker assisted selection.

Meiotic restitution in interspecific hybrids leading to unreduced viable gamete formation has been responsible for evolution of many polyploid species of cultivated crop plants and amphiploids (Kellogg, 2003; Islam and Shepherd, 1980; Harlan and de Wet, 1975; Stebbins, 1971). *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. and *T. aestivum* L. are two of the most important allopolyploids that evolved in nature by the phenomenon of meiotic restitution leading to chromosome doubling (Jauhar, 2007; Matsouka and Nasuda, 2004; Kihara and Lilienfeld, 1949). *T. turgidum ssp dicoccoides* (AABB) arose more than half a million years ago by the hybridization of the A genome donor *T. urartu* Tuman. (Dvörak *et al.*, 1993) with the B genome donor (most probably *Ae. speltoides*) Tausch (Wang *et al.*, 1997) as a result of unreduced gamete formation in these F_1 plants (Jauhar, 2003). In the sterile interspecific hybrids without any or with limited chromosome pairing during meiosis, the univalent

chromosomes do not undergo the usual equational division and instead come to metaphase-I plate resulting in a monad. During the second meiotic metaphase the sister chromatids of the univalents get equationally divided leading to only dyads with unreduced and viable gametes with the F_1 hybrid chromosome number. Such a mechanism of unreduced gamete formation is called first division restitution (FDR) (Jauhar, 2007; Xu and Joppa, 2000). In another mechanism of unreduced gamete formation, the sister chromatids of the univalents get equationally separated to two poles during the prolonged meiotic metaphase-I leading to dyads with unreduced F_1 chromosome number and viable gametes without the second meiotic metaphase. Such a mechanism is called as single division meiosis (SDM) (Matsouka and Nasuda, 2004; Maan and Sasakuma, 1977). Either FDR or SDM or both the mechanisms have been reported in some partially to highly fertile interspecific hybrids. In Triticeae several subspecies of *T. turgidum* have been reported to control FDR or SDM mediated development of fertile gametes in interspecific F_1 hybrids (Matsouka and Nasuda, 2004; Jauhar, 2003; Pignone, 1993; Fukuda and Sakamoto, 1992a, b; Xu and Dong, 1992; Islam and Shepherd, 1980; Vardi and Zohary, 1967; Kihara and Lilienfeld, 1949).

2.14 Iron and zinc in plants

2.14.1 Physiological bases for micronutrient accumulation in grains / seeds

The physiological basis for micronutrient efficiency in crop plants and the processes which control the accumulation of micronutrients in edible portions of seeds is not yet very clearly understood. There is a tightly controlled homeostatic mechanism that regulates uptake and distribution of micronutrient metal in plants. Metal uptake and distribution in plants, allowing adequate but nontoxic levels of these

nutrients to accumulate in plant tissues. The first important barrier to micronutrient uptake resides at the rhizosphere (Palmgren *et al.*, 2008; Krämer *et al.*, 2007). For the increased micronutrient metal uptake by roots, the levels of the available micronutrients in the root-soil interface must be increased to allow for more absorption by root cells. It could be achieved by stimulating certain root-cell processes that alter micronutrient solubility and movement to root surfaces, such as by stimulating the rate of root cell efflux of H, metal-chelating compounds and reductants, and by increasing root absorption surface area such as the number and extent of fine roots and root hairs. Second, root-cell plasma membrane absorption mechanisms (e.g., transporters and ion channels) must be sufficient and specific enough to allow for the accumulation of micronutrient metals once they enter the apoplasm of root cells from the rhizosphere. Fig. 2.5 shows the locations of transporters involved in iron and zinc uptake from the soil. Third, micronutrients, after being taken up by root cells, must be efficiently translocated to edible plant organs. Phloem sap loading, translocation and unloading rates within reproductive organs are important characteristics for the seeds and grains, which must be considered in increasing micronutrient metal accumulation in edible portions of seeds and grains (Welch, 1999). A large number of factors have their impact on the uptake of Iron and zinc in cereals, amount of these micronutrients depends mainly on their uptake by roots, translocation to the leaves and finally their mobilization to the grain. There are vast differences for iron and zinc uptake among different genotypes of cereal crops under different nutrient availability status. Cultivation of rice, wheat, maize and other cereals involves calcareous and saline sodic soil which contain iron in highly non- available form i.e. Fe $(OH)_3$ leads to lower iron uptake. Roots of various graminaceous plants secrete phytosiderophores, chelators of iron and zinc

which are then absorbed by plant as mugeneic acid-chelator complex (Takagi and Kamei, 1998; Cakmak *et al.,* 1994). Cereals such as barley and rye have genes on 4H and 5R chromosomes respectively for synthesis of mugeneic acid, hydroxymugeneic acid and epihydroxy mugeneic acid for more efficient chelation of iron and zinc uptake. Wheat could synthesize mugeneic acid only (Mori *et al.,* 1990; Mori *et al.,* 1989). Thus mugeneic acid in different forms act as enhancer for the uptake of iron and zinc in their deficiency.

2.14.2 Genes involved in iron and zinc transport

Iron is an essential element for the normal growth of human beings as well as it also plays a vital role in seed germination, plant metabolism, growth and crop productivity (Nozoye *et al.,* 2007; Garrido *et al.,* 2006).

In plants various genes are involved in iron and zinc homeostasis.In *Arabidopsis*, Nramp metal transporter family was found to be involved in Fe homeostasis (Lanquar *et al.,* 2005; Bereczky *et al.,* 2003; Kaiser *et al.,* 2003; Curie *et al.,* 2001; Thomine *et al.,* 2000). Under deficiency of iron and zinc, expression of other metal transporters such as IRT1 (iron regulatory transporter gene 1) and FRO2 (ferric reductase oxidase gene 2) which were involved in the uptake of ferrous ions was enhanced in *Arabidopsis.* The Zinc-regulated and Iron-regulated transporter Proteins (ZIP) have been isolated from both graminaceous (Ishimaru *et al.,* 2005; Ramesh *et al.,* 2003) and nongraminaceous plants (Moreau *et al.,* 2002; Lasat *et al.,* 2000; Grotz *et al.,* 1998). The iron deficiency induced genes (IDI-1, IDI-2), adenine phosphoribosyltransferase (APT) and formate dehydrogenase (FDH) genes which were involved in methionine cycle in barley, were also up regulated (Ashida *et al.,* 2003).

In barley, expression of H*v*NAS1, H*v*NAAT-A, H*v*NAAT-B, H*v*DMAS 1, H*v* IDS_2, H*v* IDS_3 is increased in iron deficient roots (Khurram *et al.*, 2006; Takahashi *et al.*, 1999; Okamura *et al.*, 1994; Nakanishi *et al.*, 1993), similarly in rice expression of OsNAS1, OsNAS2, OsNAS3, OsNAAT1 and OsDMAS1 was increased in roots and shoots under iron and zinc deficiency (Inoue *et al.*, 2008, 2003; Suzuki *et al.*, 2008). Enhanced expression of Yellow stripe1 gene in roots and shoots of *Zea mays* has been found under zinc deficiency (Schaaf. 2004; Curie *et al.*, 2001). All graminaceous plants release low molecular weight compounds, called mugeneic acid family phytosiderophores (MAs) for acquisition of Fe (III) or Zn (II) complexes from the rhizosphere (Takagi, 1976; Takagi *et al.*, 1984).

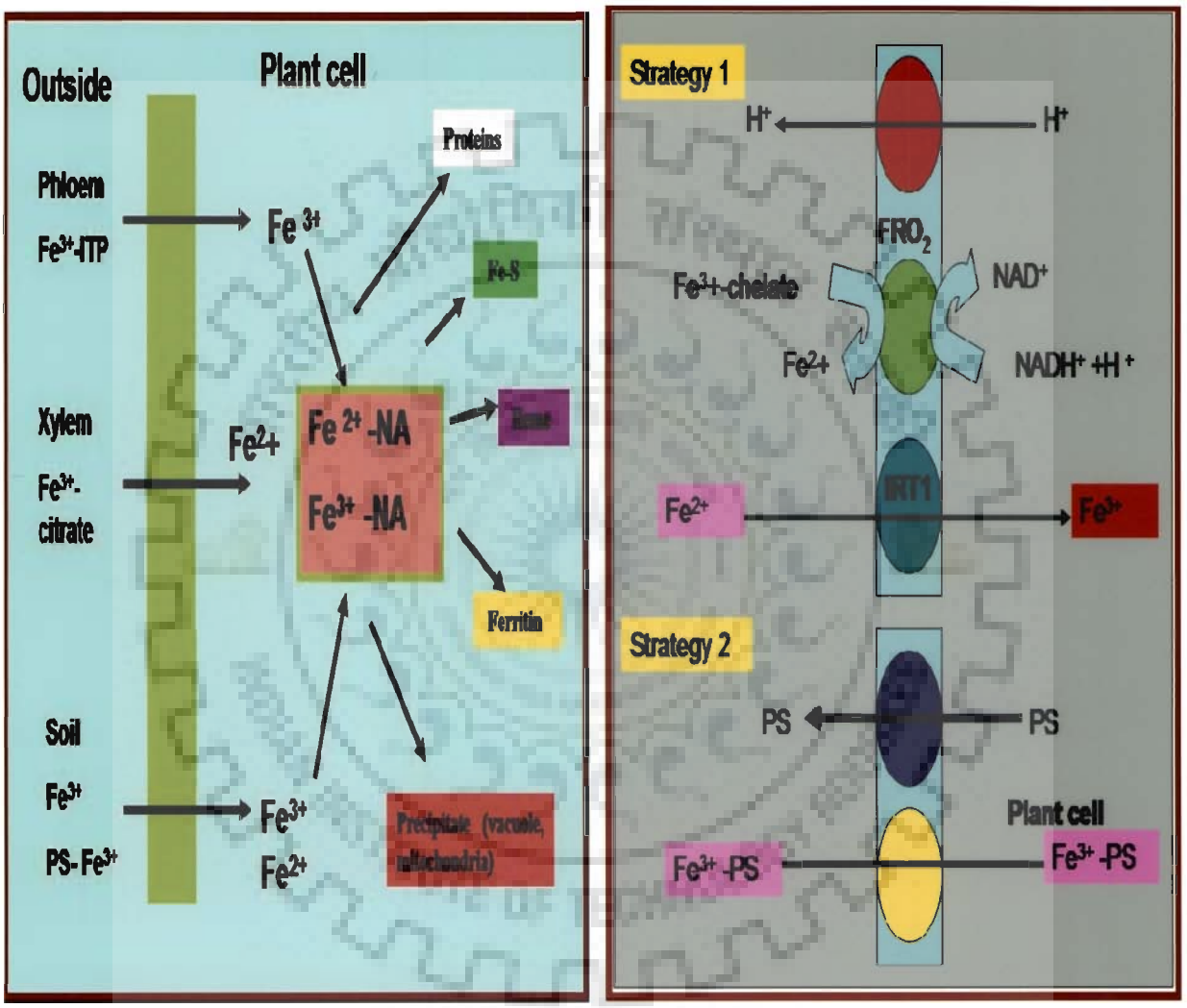

Figure 2.5 Schematic presentation of metal transporters for iron uptake from soil to cells

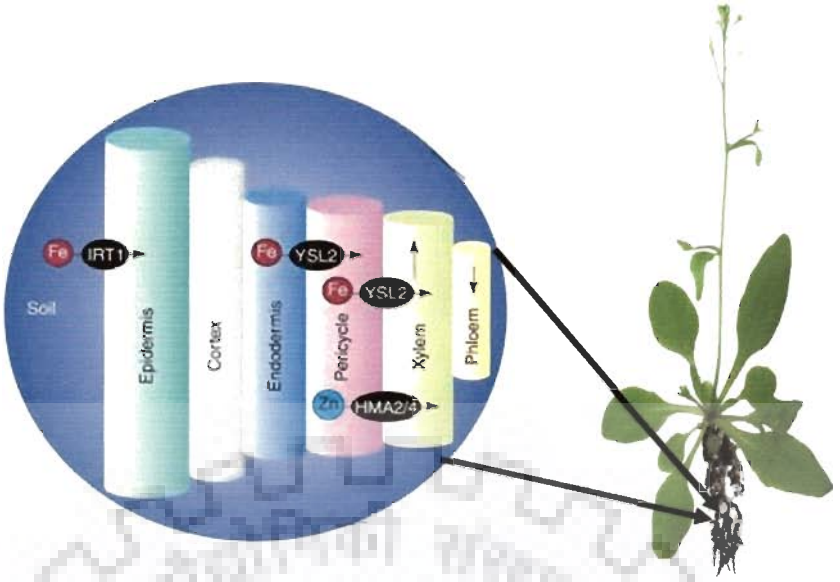

Fig.2.6. Location of transporters involved in iron uptake from roots to shoot

Enhanced expression of genes involved in 2'- deoxymugineic acid biosynthesis was observed under both iron and zinc deficiency (Higuchi *et al.,* 2001) and also these MAs have been found in the xylem and phloem of rice and barley plants indicating their role in long distance transport of micronutrients (Kawai *et al.,* 2001; Mori *et al.,*1991). Thus, with all these physiological changes graminaceous plants are able to sustain micronutrient deficiency. Uauy *et al.* (2006) provided new insight into homeostatic mechanisms of grain nutrient acquisition by establishing a direct link between senescence and distribution of nutrients. According to Uauy *et al.* (2006) wheat genome contains three *NAM* genes, and transcript analysis reveals a parallel increase in expression of all these genes in flag leaves at grain maturity. Abrogating expression of these *NAM* genes by RNA interference transgenesis results in delayed whole-plant senescence and more than 30% reduction of grain zinc, iron, and protein content, suggesting a quantitative contribution from each *NAM* gene.

Table 2.5. Metal transporters for iron and zinc uptake and translocation

Proteins	Tissue expression	Cellular localization	Inducing conditions	Proposed/known substrates
Metal efflux proteins				
P1B-ATPase (8)				
AtHMA2/HMA4	Vasculature of root and shoot, anther	PM		Zn, Cd
CDF (12)				
AtMTP1	Root, shoot, flower	VM		Zn
AhMTP1	Root	VM	+Zn	Zn
TgMTP1		PM		Zn
Metal uptake proteins				
YSL (8)				
ZmYS1	Root, shoot		−Fe	Fe^{3+}–PS, Fe^{3+}-, Fe-, Ni-, Cu–NA
AtYSL1	Silique, leaf (xylem parenchyma), flower		+Fe	Fe–NA
AtYSL2	Root (endoderm pericycle), shoot	PM	+Fe, downregulated by −Zn	
OsYSL2	Leaf (phloem), root, seed	PM	−Fe	Fe-, Mn–NA
NRAMP (6)				
AtNRAMP3/NRAMP4	Root, shoot, seed	VM		Fe
ZIP (16)				
OsZIP4	Root, shoot (phloem meristem)	PM	−Zn	Zn
MtZIP1	Root, leaf		−Zn	Zn
MtZIP3	Root, leaf		Downregulated by −Mn, −Fe	Fe
MtZIP4	Root, leaf		−Zn	Mn
MtZIP5	Leaf		−Zn, −Mn	Zn, Fe
MtZIP6	Root, leaf			Zn, Fe
MtZIP7	Leaf			Mn
TjZNT1				Ni, Cd, Mn, Zn

(Source: Colangelo and Guerinot, 2006)

This delay in senescence and decrease in grain nutrients is associated with increased residual nitrogen, zinc, and iron in the flag leaf, thereby demonstrating a role for *NAM* genes in nutrient redistribution to the developing grain during leaf senescence.

Chapter III

Materials and Methods

3. Materials and methods

3.1 Plant materials

3.1.1 Introgressive derivatives

The experimental materials comprising eighty accessions of nine related *Aegilops* and wild *Triticum* species of wheat from different geographical regions were obtained from the wheat germplasm collection maintained at the Punjab Agricultural University, Ludhiana, India. The related wild species, wheat and durum cultivars were grown at the experimental fields of the Indian Institute of Technology, Roorkee, Roorkee for two consecutive seasons of 2004-05 and 2005-06 as unreplicated single row of two meter length with plant to plant distance of 10 cm and row to row spacing of 30 cm with recommended fertilizers and irrigation as that of wheat. Grains, spikelets and spikes were harvested and threshed from cultivars and wild accessions at physiological maturity. Due to frequent shattering of spikes in various wild species, collection of mature spikelets and spikes had to be done repeatedly at different intervals over two-three weeks. Due to tough glumes and hard threshing in wild species the grains had to be taken out manually.

3.1.2 Interspecific crosses and backcross derivatives:

For transfer of useful variability for higher concentration of iron and zinc from selected wild donors, interspecific crosses were made using wheat and durum cultivars as the maternal parent. A bread wheat line Chinese Spring with Ph^1 transferred from *Ae. speltoides* obtained from Dr. B.S. Gill of Kansas State University, Kansas was used for making crosses for induced homoeologus pairing while some interspecific crosses were also made with wheat and durum cultivars

without Ph^l gene. In the consecutive season of 2005-06 the F_1 hybrids were backcrossed with elite cultivars. The BC_1 plants were either crossed with recurrent parent next year or allowed to self depending upon the fertility of the plants. The BC_2F_1 and BC_1F_2 seeds were analysed for their micronutrient content and the selected progenies were sown next year. Finally BC_2F_3 and BC_1F_4 seeds were put to rigorous chemical analysis to select a few derivatives with exceptionally higher micronutrient content than the control wheat cultivar WL711. These selected derivatives were characterized on the basis of morphology, cytology, HMW-glutenin subunit profiles, microsatellite markers and Genomic *in-situ* hybridization (GISH).

3.1.3 *T. monococcum /T. boeoticum* and their derived RIL population

The plant materials used for mapping of the QTL for grain Fe and Zn consisted of a set of 93 RILs derived from a cross *T. boeoticum* acc. pau5088/*T. monococcum* acc. pau14087 (hereafter referred to as *Tb5088* and *Tm14087*, respectively) through single seed descent method. Detailed information on these accessions and molecular linkage map generated using this population described by Singh *et al.* (2007) is available at GrainGenes.

(http://wheat.pw.usda.gov/report?class=mapdata&name=T.%20boeoticum%20x%20monococcum)

The parents and the RILs were planted as single 1.5m rows with row-row spacing of 50cm at two locations, Punjab Agricultural University (PAU), Ludhiana (30°52'N; 75°56'E) and Indian Institute of Technology (IIT), Roorkee, Roorkee (29°52'N; 77°53'E) for two years 2004-05 and 2005-06. These environments hereon are referred to as PAU2005, PAU2006, IIT2005 and IIT2006, respectively. Standard

agronomic practices were followed for raising the RIL population at both the locations. Seeds of individual lines were harvested manually and hand threshed to avoid any soil contamination. Weight of hundred seeds of parents and each RIL were recorded from the crop raised at PAU by weighing 100 grains on an electronic balance (Shimadzu.Aux220).

3.1.4 *T. turgidum* ssp *durum*/ *Aegilops longissima* amphiploids:

Plant materials received from the Punjab Agricultural University, Ludhiana, India consisted of three accessions of *Ae. longissima* viz., 28, 3770 and 3507 and two cultivars of *T. turgidum* ssp. *durum* namely PDW274 and PDW233. The materials were grown in two replications in the experimental fields of the Indian Institute of Technology Roorkee, Roorkee as single row of two meter length with plant to plant distance of 10 cm and row to row spacing of 30 cm with recommended fertilizers and irrigation as that of wheat (50:25:25 Kg/acre of N, P_2O_5, K, respectively). F_1 hybrids were produced using durum wheat cultivars as the female parents and *Ae. longissima* accessions as the male parents (Table 1). Selfed seeds obtained in all the partially fertile F_1 hybrids were grown in the next year to get fertile amphiploids.

3.1.5 Landraces

Seed samples of 63 landraces of wheat were collected from farmers' fields of hilly areas of himalayas in Uttarakhand state of India during April 2004, 2005 and were given Indian Institute of Technology Roorkee (IITR) accession numbers. The landraces were collected purely on the basis of gross plant morphology including plant height, awnness, seed color etc. and information from farmers cultivating them. Only those wheat plots with taller plants, fewer tillers per plant, smaller spikes and

seeds grown under rainfed and limited irrigation and fertilizer conditions for specific purpose from the village were considered as the landraces and sampled.

These landraces were grown along with 14 wheat cultivars, developed and released in northern India during the 20th century, at the Department of Biotechnology Research farm, Indian Institute of Technology Roorkee, Roorkee, India during winter season of 2007-2008 with a plant to plant distance of 10cm and row to row distance of 23cm.

3.2 Chemical analyses

3.2.1 Grain:

For chemical analysis whole grain samples from cultivated and wild accessions were washed with N/10 HCl (Merck), dried till constant weight and dried in hot air oven at 80°C for 4 hours. Grain samples (0.5g) were digested in a mixture of two parts of concentrated nitric acid (Merck) and one part perchloric acid (Merck) as per the standard procedure described by Zarcinas *et al.* (1987). Digestion was continued till white residue was obtained. Required volume was made after the completion of digestion process and digests were analyzed by Atomic Absorption Spectrophotometer (GBC- Avanta Garde M). A minimum of five replications of chemical analysis was made in each of cultivars and wild accessions. Grain iron and zinc status of selected donors and selected derivatives was also reconfirmed by Inductively Coupled Plasma Mass Spectrometer ICPMS (Perkin Elmer). All the standards used in this study were purchased from Merck, Germany.

3.2.2 Flag leaf:

Flag leaves from selected potential donors, recipient parents and their F_1 hybrids were collected at the pre-anthesis stage, washed thoroughly with N/10 HCl, dried at 80° C for 8 hrs in oven prior to digestion. Dried leaf samples were then digested as a minimum of five replications using diacid mixture of nitric acid and perchloric acid (Zarcinas *et al.*, 1987). Iron and zinc concentrations in the digests were analyzed by AAS.

3.2.3 Grain ash

Grains (1 g) were ashed in muffle furnace at 500° C for 10 hours. The ash was carefully collected and weighed. Further the ash samples were processed like grains for iron and zinc analyses.

3.3 Cytological Studies

For meiotic analysis spikes of interspecific F_1 plants were fixed in Cornoy's solution (6 ethanol: 3 chloroform: 1 acetic acid) for 24 hours and transferred to 70% ethanol. Anthers at various stages of meiotic division-I were squashed in 2% acetocarmine and the pollen mother cells (PMCs) were scored for chromosomal number and pairing in all the crosses. Photographs were taken with a digital camera (Canon PC1049, No. 6934108049). Pollen stainability was recorded by staining the pollen grains after squashing the anthers in Iodine-Potassium Iodide solution.

3.4 Scoring of morphological data

Morphological data such as plant height, ear shape, waxiness, rachis strength, grain colour, harvest index etc. were noted in the field at maturity except waxiness which was recorded at the time of grain setting when the plants were green.

3.5 Protein analysis

3.5.1 HMW Glutenin Subunit Extraction Reagents

Extraction buffer:

SDS (sodium dodecyl sulphate, Merck) 2.0g, ß- mercaptoethanol (HiMedia) 5ml

Total volume made upto 100ml with distilled water after setting pH to 6.8

Dye:

Bromophenol Blue (Merck) 0.5g, Glycerol 50ml (SRL), Distilled water 50ml

3.5.2 Extraction Procedure

Single seed was crushed and weighed (let its weight be X mg). Extraction buffer (13.2 x X) was added to it in an eppendorf tube and vortexed for 1.5 minutes and then incubated in water bath at 80°C for 18 minutes. 1.2 x X dye was added to it and then it was centrifuged at 4000rpm for 10 minutes. Supernatant was retained.

3.5.3 High Molecular Weight (HMW) glutenin SDS-PAGE Reagents

40% Acrylamide :

Acrylamide (SRL)100g, total volume was made upto 250ml with distilled water.

2% Bis-acrylamide :

Bisacrylamide (SRL) 2.0g, total volume was made upto 100ml with distilled water.

Stain :

Commassie Brilliant Blue R-250 (SRL) 2.0g, 100% Methanol 800ml (SRL), 100% Trichloro Acetic acid (SRL) 200ml. Total volume was made upto 2000ml with distilled water.

1.5M Running Gel Buffer:

Tris 18.17g, SDS 0.4g. Total volume was made upto 100ml with distilled water after setting pH to 8.8.

0.5M Stacking Gel Buffer (pH 6.8):

Tris 6.06g, SDS 0.4g. Total volume was made upto 100ml with distilled water after setting pH to 6.8.

10X Tank buffer (pH 8.3):

Tris 30.3g, Glycine 142.0g, SDS 10.0g. Total volume was made upto 1000ml with distilled water after adjusting pH to 8.3.

Ammonium per sulphate (APS, SRL) 0.06g in 600µl distilled water.

Tetramethylene diamine (TEMED)

Butan-2-ol (SRL)

Running gel (10%)

Acyrlamide	: 5.0ml
Bis-acrylamide	: 1.3ml
Running gel buffer	: 5.0ml
Distilled water	: 8.7ml
APS	: 250µl
TEMED	: 50µl

Stacking Gel (5%)

Acrylamide	: 0.55ml
Bisacrylamide	: 0.30ml
Stacking gel buffer	: 1.25ml
Distilled water	: 2.90ml
APS	: 55.55µl
TEMED	: 20µl

SDS-PAGE Procedure:

The 10% running gel was poured in the preset gel casting unit (Atto, Japan) and overlaid with butanol. It was allowed to polymerize for 40 minutes. Butanol was drained and thoroughly washed with distilled water. Then stacking gel was poured

and comb was inserted. It was left for 15minutes. Comb was removed and gel was washed by distilled water with gentle pressure. Chilled 1X tank buffer was poured in the assembly, gel was inserted into it and samples (10 µl) were loaded in the wells.

3.6. Isolation and purification of genomic DNA from leaf tissues

DNA was extracted from young leaves of the parents and selected BC_2F_2 and BC_1F_4 plants using CTAB method described by Murray and Thompson (1980).

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I.I.T. ROORKEE

3.6.1 DNA extraction reagents

DNA Extraction buffer:

200mM Tris-Cl (pH 8.0), 20mM ethylene diamine tetra-acetic acid (pH 8.0), 140mM NaCl, 2% CTAB (Cetyl-trimethyl ammonium bromide), 0.01% β mercaptoethanol

DNA isolation and purification reagents:

TE buffer (10 mM Tris-Cl (pH 8.0) 1mM EDTA (pH 8.0)), RNase solution (10mg/ml), Phenol: Chloroform: Isoamyl alcohol (25:24:1), isoproponal, absolute ethanol, 70% ethanol

3.6.2 DNA isolation procedure

About 5-7 g of young, healthy and disease free leaves from each plant were collected and kept in the plastic bags on ice. Leaves were frozen in liquid nitrogen and crushed to fine powder using autoclaved and pre-chilled mortar and pestle. The powder was transferred to 50 ml Oakridge tubes containing pre-warmed (65°C) DNA extraction buffer (15ml for approximately 3g of leaves). It was gently mixed and incubated in 65°C water bath for 1 hour, mixing briefly every 15 minutes. Equal

volumes of phenyl: chloroform: isoamyl alcohol (25:24:1) solution was added to the samples followed by gentle mixing for 15 minutes to ensure emulsification of phases. The samples were centrifuged at 10,000 rpm for 20 minutes at 25°C. Supernatants were transferred to the falcon tubes with the help of micropipettes. Equal volume of ice cold propan-2-ol was added and left overnight at 4°C for complete precipitation of DNA. DNA was spooled out using large bore pipette tips into the 1.5ml microcentrifuge tubes. It was centrifuged at 8000 rpm to get a pellet of DNA. Supernatant was discarded and pellet was washed with 400µl 70% ethanol. It was centrifuged at 8000rpm for 5 minutes. Ethanol was drained out, pellets were air dried and resuspended in 500µl TE buffer. Subsequently RNAse treatment at final concentration of 100µg/mL was done at 37°C for 1 hour. The DNA was re-extracted with fresh chloroform: isoamyl alcohol followed by reprecipitation with ethanol and pelleting by centrifugation (8000 rpm, 4°C). Pellet was collected, air dried (37°C) for few hours and dissolved in appropriate volume of 1X TE. For DNA quantification, spectrophotometric readings of the DNA samples were taken at wavelengths 260nm and 280nm. Ratio of OD260/OD280 was checked to be around 1.8 as a measure of DNA purity. At wavelength 260 nm, the concentrations of DNA (OD260x 50x dilution factor) were determined and subsequently samples were diluted to 50ng/µl concentration. Electrophoresis (Sambrook, 1989) was carried out finally for the qualitative and quantitative analysis in 0.8% agarose gel with 0.5µg/ml ethidium bromide (10mg/ml) in 1X TAE.

3.7 Application of microsatellite markers

Two hundred ninety six wheat microsatellite markers representing all the 21 chromosomes of wheat covering both chromosomal arms for checking initial parental

polymorphism between wheat cultivars and *Ae. kotschyi* accession 396 were selected from molecular maps of Räder *et al.* (1998), Pestsova *et al.* (2000) and Somers *et al.* (2004). PCR was carried out according to Räder et al. (1998) with some modifications. Two terminal transferable and polymorphic markers of each chromosome arm were applied in the finally selected derivatives to identify the introgressed chromosome. Finally the chromosome/ chromosome segment introgressed was characterized by applying additional polymorphic markers of homologous chromosomal introgressed into the selected derivatives.

3.7.1 Composition of reaction mix:

PCR Buffer (10X) - 2µl

dNTP mix (1mM each dATP, dCTP, dGTP and dTTP) - 4µl

Primer f (5mM) - 1µl

Primer r (5mM) - 1µl

Taq polymerase - 1 unit

$MgCl_2$ (25mM) - 1.2 µl

DNA (50ng/ µL) - 2µl

Total volume-20µl

3.7.2 PCR conditions:

The PCR was carried on Eppendorf Thermocycler with following conditions:

Initial denaturation at 94°C for 4 min; 35 cycles of - denaturation at 94°C for 1 min and annealing at 50-68°C depending upon the primer T_m for 1 min; extension at 72°C for 1 min; final extension at 72°C for 7 min

3.7.3 Resolution of the amplified SSR product:

A volume of 2µl of 10X gel loading dye (0.4% W/V Bromophenol Blue, 0.4% W/V Xylene Cyanol FF, 50% Glycerol) was added to the 20µl PCR product. The PCR products were loaded on 3% high resolution agarose (Amresco) having 0.5µg/ml ethidium bromide (10mg/ml) and prepared with 1X TAE buffer. The gels were visualized and photographed using BioRad gel documentation system.

3.7.4 Genomic *in situ* Hybridization:

Genomic in-situ hybridization was done in order to finally visualize the alien introgression in the selected derivatives using the method described by Dou *et al.*, 2006. Seeds were germinated at room temperature. Root tips were collected at a length of 0.5–2 cm, pretreated in ice-water for 24 h, and Waxed in 99% ethanol–glacial acid (3:1). Slides were prepared by squashing in 45% acetic acid. Genomic DNA of *Ae. longissima* and *Ae. umbelullata* were used as probes in GISH. Clones pAs1 (Rayburn and Gill, 1986 a,b) and a synthesized 30-base length $(AAG)_{10}$ repetitive oligomer were used as probes in FISH. pAs1 is regarded to be a D-genome-specific clone. The pattern of FISH with this clone permits identification of the D-genome chromosomes, though there are other weak hybridization signals on some B and A-genome chromosomes (Rayburn and Gill 1986a; Pedersen and Langridge 1997). The pattern of AAG-satellites together with pAs1 can identify the entire

chromosome complement of bread wheat by two-color FISH (Pedersen and Langridge 1997).

3.8 SKCS (single kernel characterization system)

The Single Kernel Characterization System (SKCS 4100) was used for measuring single kernel weight, width, moisture content, and hardness in wheat grain with greater speed than existing methods.

3.8.1 Procedure:

About 25 to 30 clean seeds were taken and loaded into the SKCS machine. The data for single kernel weight, width, moisture content, and hardness were then recorded for all the wheat cultivars and landraces.

3.8.2 Statistical Analysis

Pearson correlation coefficient was calculated for all the four environments to test the correspondence of Fe and Zn data in different environments as well as to study correlation, if any, between Fe and Zn accumulation in the grains of the RIL population. Pearson correlation coefficient was also estimated for Fe and Zn and the 100-grain weight recorded at PAU during 2005 and 2006. Students't-test was used to test the significance of the correlation coefficient.

3.9 QTL mapping

The grain Fe and Zn data for all the four environments individually and pooled data were used for detecting the QTLs governing grain Fe and Zn concentrations in this population. The positions and effects of QTLs were determined following composite interval mapping (CIM) using the software QTL Cartographer v. 2.5 (Wang *et al.*, 2007). The significant threshold LOD scores for detection of the QTLs

were calculated based on 1,000 permutations at P≤0.05 (Churchill and Doerge, 1994). Cartographer Z map QTL, Model 6 with a window size of 10 cM was used for the CIM. The number of markers for the background control was set to 5. Proportion of observed phenotypic variation explained (PVE) due to a particular QTL was estimated by the coefficient of determination (R^2) using maximum likelihood for CIM.

3.10 Genetic diversity analysis

Morphological data for qualitative traits was converted to binary form while quantitative data was kept as such. For calculating squared Euclidean distances by NTSYS-PC software was used. For the purpose of assessing genetic diversity on the basis of SSR markers and preparation of dendrogram, polymorphic microsatellite markers were scored in binary format giving unity score to presence of band and zero to the absence of band (Cao *et al.*, 1999, 2000; Prasad *et al.*, 2000). Genetic similarities were estimated from allelic binary format dataset using Dice similarity coefficient method. The software package, NTSYS pc version 2.02 was used for estimation of genetic similarities among the accessions (Rohlf, 1998). Allelic data from all the SSR loci were used to estimate similarity based on the number of shared amplified bands. Similarity between any two accessions was estimated using SIMQUAL module of NTSYS, which computes a variety of similarity coefficients (association coefficients) for qualitative (nominal) data. Similarity matrix was used to study the cluster analysis using the unweighed Pair Group Method With Arithmetic Average (UPGMA) algorithm from NTSYS-PC V.2.02 (Rolhf, 1992)

Chapter IV

Results

4. RESULTS

Results of this study are presented in 4 sections. Details of each section is given below

1. Development of introgressive derivatives of *Aegilops kotschyi* with high grain iron and zinc and their cytological and molecular characterization
2. Development of spontaneous *T. turgidum-Ae. longissima* amphiploids with high grain iron and zinc content
3. Molecular mapping for QTL for high grain iron and zinc content in diploid wheat RIL population
4. Collection and characterization of a set of landraces collected from high hilly regions of Himalaya of Uttarakhand state

4.1 Development of introgressive derivatives of A*egilops kotschyi* with high grain iron and zinc and their cytological and molecular characterization

4.1.1. Screening of germplasm

Mean grain iron and zinc content of fourteen wheat cultivars grown over two years is given in Fig. 4.1. All the durum and bread wheat cultivars showed lower content and limited variability for grain iron and zinc content. Grain iron ranged from 20 mg/kg to 25 mg/kg and zinc, varied from 15mg/kg to 20 mg/kg. In order to biofortify semidwarf wheat cultivars, accessions of ten different progenitor and non- progenitor *Triticum* and *Aegilops* species were screened for their grain iron and zinc content (Fig. 4.2). On an average *T. boeoticum*, *T. dicoccoides*, *T. arraraticum* had shown 63 percent increase in grain iron content over cultivars while for zinc this increase was nearly two times. Various non- progenitor wild *Aegilops* species *i.e. Ae. kotschyi* (US), *Ae. peregrina* (US), *Ae. ventricosa* (DN), *Ae. longissima* (S), *Ae. geniculata* (UM) and *Ae. cylindrica* (CD) showed nearly 2.5 to 3 fold higher grain iron and zinc content over cultivars. Variation for micronutrient content was also found within and among various *Aegilops* species. Maximum range of variability for iron content was found in *Ae. kotschyi* (44 mg/kg to 78 mg/kg) where as for zinc it varied from 25 mg/kg to 54 mg/kg. In general, all accessions of *Ae. kotschyii* had higher grain iron content than that of zinc. Only *T. dicocoides* showed higher concentration of grain zinc content (51.2) mg/kg over grain iron content (34.8 mg/kg). Based on highly reproducible results, *Ae. kotschyi* acc. no. 3790 with high iron (73 mg/kg) and zinc content (41 mg/kg) was selected as a donor for transfer of useful variability for high grain iron and zinc content to *T. aestivum.*

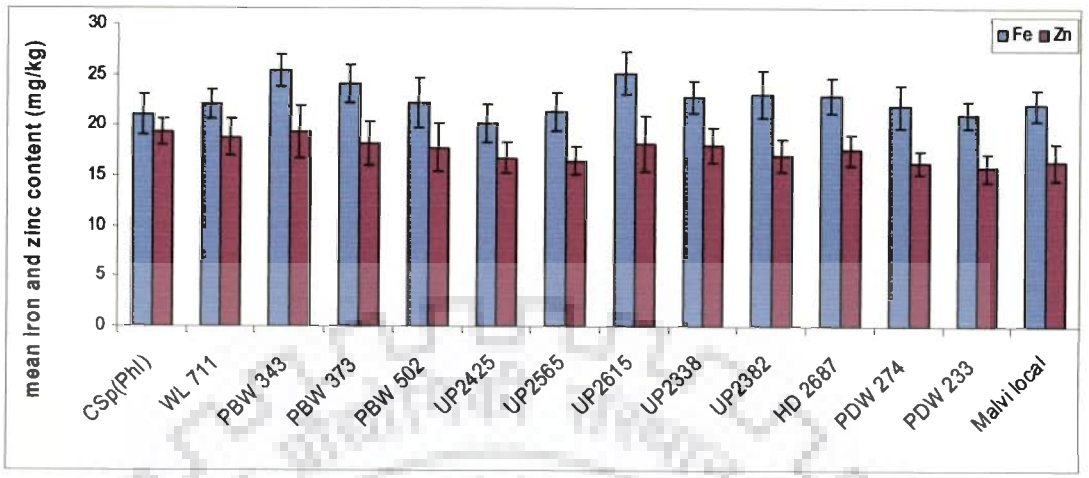


Fig. 4.1. Mean grain iron and zinc content of bread and drum wheat cultivars over 2 years. Error bars represent standard deviation for the trait.

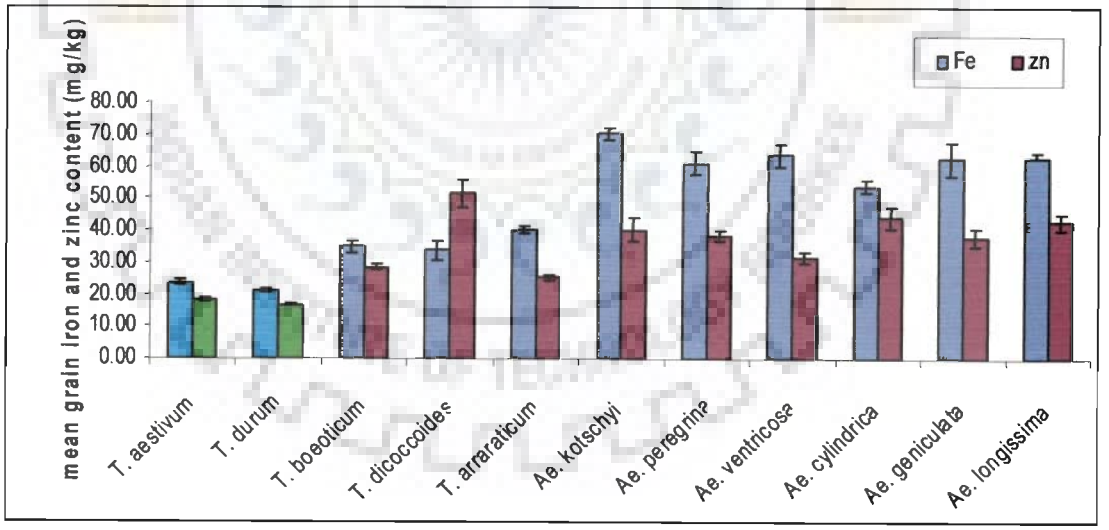


Fig 4.2. Grain iron and zinc content of bread and drum wheat cultivars, wild *Triticum* and *Aegilops* species over 2 years. Error bars represent standard Error of the means.

4.1.2. The F_1 hybrids

F_1 hybrids between *T. aestivum* Chinese Spring (*Ph*^{I}) as female parent and *Ae. kotschyi* 3790 as male parent were developed.

4.1.2.1. Cytology

Very little pairing was observed in the F_1 hybrids because of difference in their genomic constitution (Fig. 4.3). The F_1 hybrid was completely male sterile (pollen stainability, 21.5 %) and had no selfed seed set. The mean chromosome pairing at metaphase I in the F_1 hybrid was 29.40I +2.86 II+ 0.16 III. The sterile F_1 hybrid was extensively backcrossed with one or more wheat cultivars to recover the useful variability in fertile wheat background.

4.1.2.2 Flag leaf analysis

The leaf iron was found to be positively correlated with grain iron (r=+0.82) and leaf zinc was positively correlated with grain zinc content (r=+0.92) (Rawat *et al.*, 2008). Flag leaf analysis of sterile F_1 hybrid between Chinese Spring (*Ph*^{I})/*Ae. kotschyi* 3790 was done as it had no seed set. Iron and zinc content in flag leaves of F_1 hybrids revealed an increase of 50 % and 57% over those of the wheat parent, respectively (Fig. 4.4). These results suggest the presence of a superior genetic system of *Aegilops kotschyi* parent for the uptake and translocation of micronutrients from the soil to leaves as compared to the wheat parent.

4.1.3. BC_1F_1 plants

4.1.3.1. Morphology

Morphological data of the BC_1 plants, their chromosome numbers, and fertility status are presented in Table 4.1. BC_1F_1 plants showed morphological characteristics varying

from wheat to that of F_1 hybrids. Low percentage of seed set was observed in these backcrossed plants. Out of thirteen BC_1F_1 plants, only plants with nearly 42 chromosomes were found fertile. Most of the sterile plants had higher frequency of univalent's rather than bivalents. Extensive backcross was done with female parent in order to achieve some seed set in BC_1F_1 generation.

4.1.3. 2. Cytology of BC_1F_1 plants

Chromosome number varied from 39 in BC_1F_1 CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-1 to 56 in BC_1F_1 CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4 (Table 4.1).Univalent frequency ranged from 2 in CS (*Ph*^{l})/*Ae. kotschyi* /UP 2338-2 to 23 in CS (*Ph*^{l})/*Ae. kotschyi* 3790/ UP2338-1. Bivalent frequency ranged from eight (CS (*Ph*^{l})/*Ae. kotschyi* / UP2338-1) to twenty (CS (*Ph*^{l})/*Ae. kotschyi* /UP 2338-2). Presence of one to two trivalent was also observed in some backcrossing derivatives (Fig. 4.5). Increased pairing in the BC_1F_1 plants indicated their higher stability.

4.1.3.3. Micronutrient analysis of flag leaves of BC_1F_1 derivatives:

Leaves of BC_1 hybrids were analyzed in order to monitor the increase in their iron and zinc content as the little seed set was too precious to digest at this stage (Table 4.2.) Iron content of the backcross derivatives showed up to three fold higher iron content. Maximum increase in the iron content was observed in BC_1F_1 CS (*Ph*^{l})/ *Ae. kotschyi* 3790// PDW 274-1. Similarly increase in zinc content was observed in BC_1F_1CS (*Ph*^{l}) / *Ae. kotschyi* 3790// UP2338-1, CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-3 whereas in some derivatives reduction over wheat parent was also seen. Higher content of these

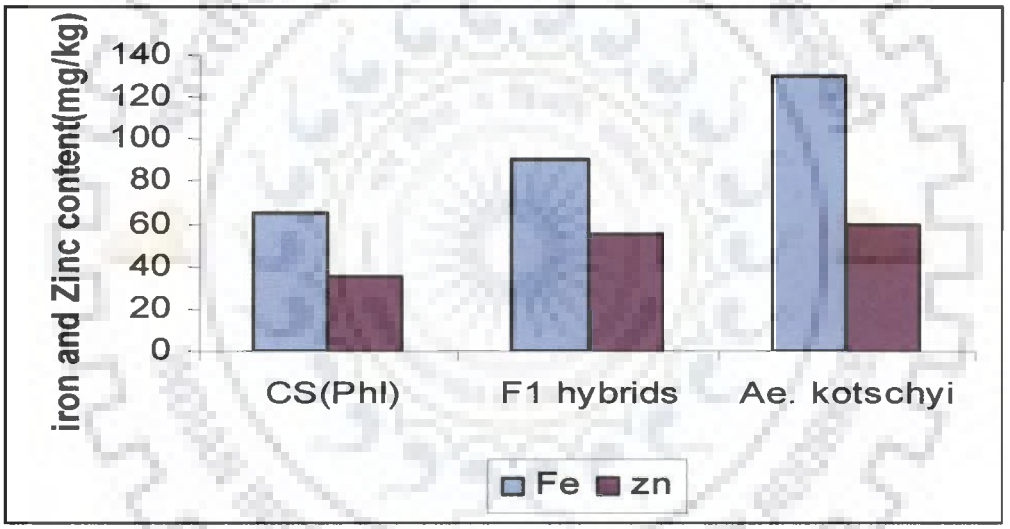


Fig. 4.4 Iron and zinc content in flag leaves of wheat parent, F_1 hybrid and *Ae. kotschyi* accc. 3790

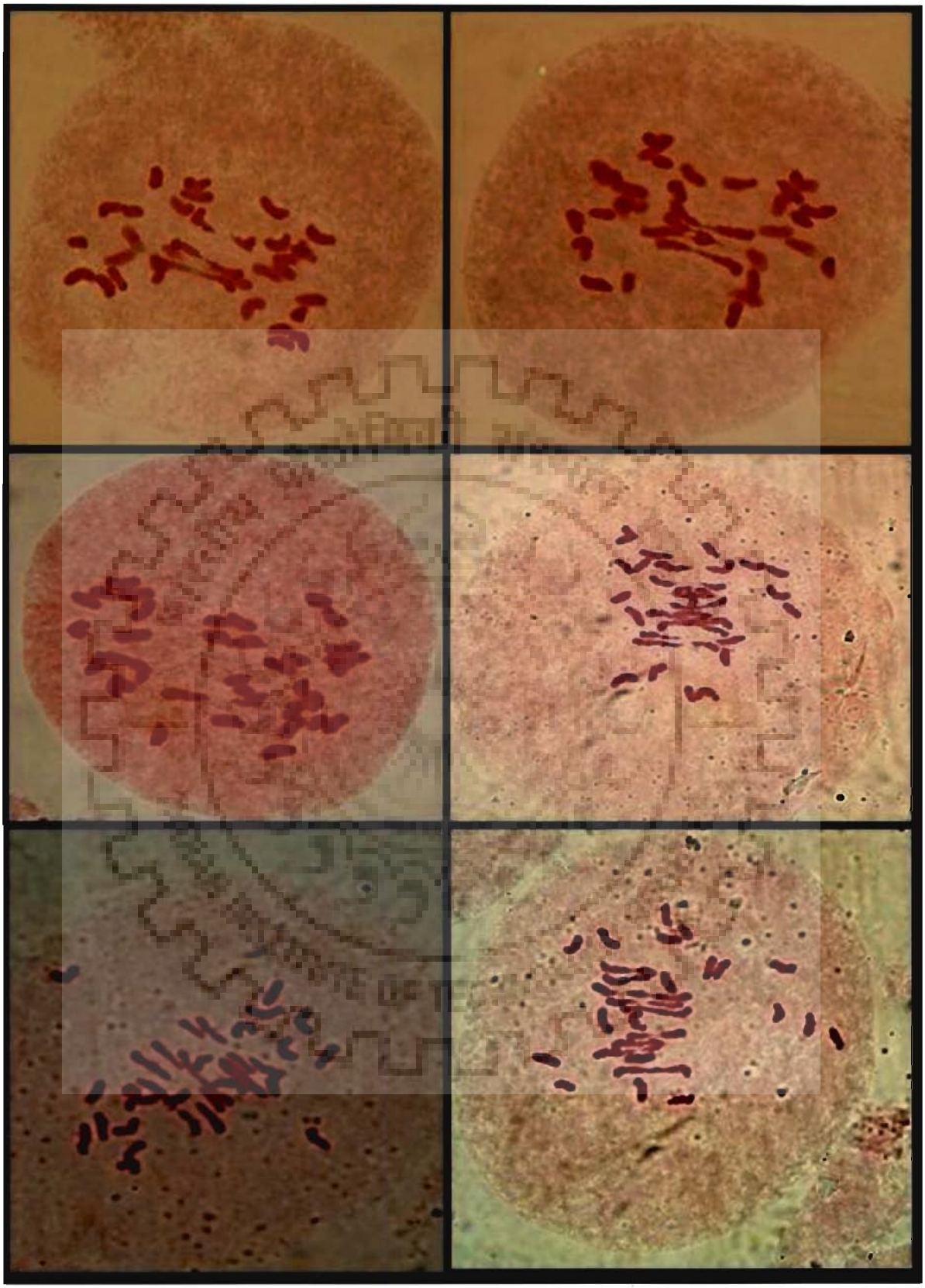


Fig 4.3. Meiotic chromosome pairing in the interspecific hybrids of Chinese Spring (*Ph*^{l}) X *Ae. kotschyi* 3790 (a) 3 II + 29I (b) 1 III +2 II+ 28 I (c) 2 II +31 I (d) 8II+19I (e) 1 III+ 7 II+18I (f) 2 III+ 5II+ 19I

Table 4.1 Morphology, chromosome number and fertility of the parents and BC_1F_1 plants

Pedigree	No. of Tillers	Height (cm)	Waxiness and awnness	Chr. No.	Pollen stainability	Female Fertility
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// WL711 -1	16	60	Awnless nonwaxy	46	8.6 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// WL711 -2	6	45	Awnless nonwaxy	41	7.3 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// WL711 -3	8	65	Awnless nonwaxy	41	82.4 %	Fertile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// WL711 -4	23	70	Awnless, nonwaxy	56	87.2 %	Fertile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// WL711 -5	20	55	Awnless, nonwaxy	43	20.5 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// UP2425-1	15	80	Awnless nonwaxy	36	9.2 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// UP2338-1	7	30	Awned waxy	39	16.2 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// UP2338-2	17	90	Awnless nonwaxy	42	86.2 %	Fertile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// UP2338-3	9	45	Awnless nonwaxy	40	8.7 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// HD 2687-1	26	45	Awned nonwaxy	41	15.6 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// HD 2687-2	12	55	Awnless nonwaxy	40	8.1 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// PDW 274-1	3	40	Awned nonwaxy	39	12.4 %	Sterile
CS (*Ph*^{I})/ *Ae. kotschyi* 3790// PDW 274-2	45	30	Awned nonwaxy	49	11.8 %	Sterile
WL 711	19.8	93	Awned waxy	42	88.4 %	Fertile
CS (*Ph*^{I})	18.3	100	Awnless nonwaxy	42	80.9 %	Fertile
Ae. kotschyi 3790	230.5	33	Awned nonwaxy	28	88.6%	Fertile

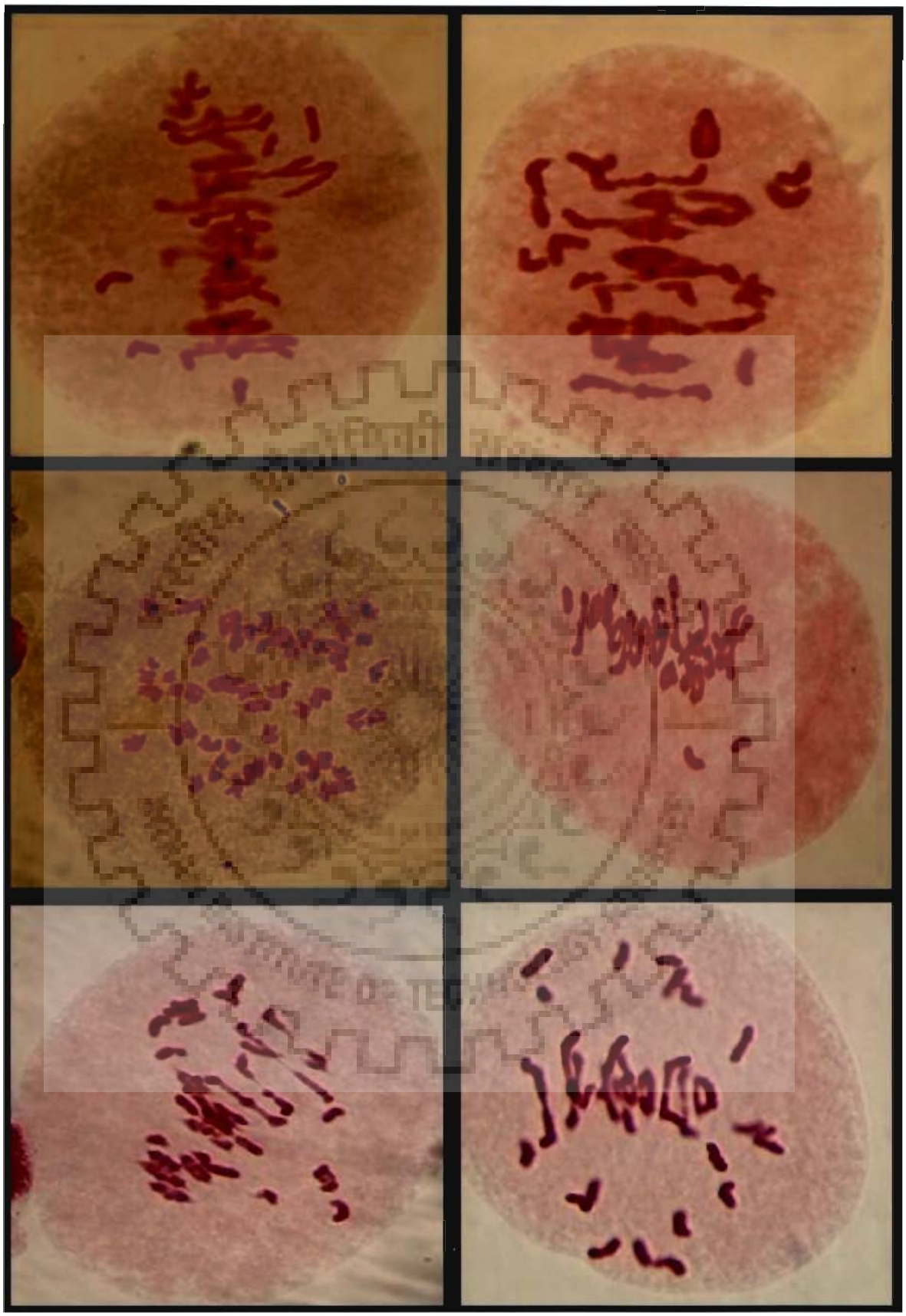


Fig. 4.5. Chromosome pairing at metaphase-I of PMCs of some selected BC_1 plants. (a) CS-3790/PDW274-2. (b) CS-3790/WL711-5. (c) CS 3790/WL711-4. (d) CS-3790/UP 2338-2.(e) CS-3790/ HD 2687-1. (f) CS 3790/ UP2338-1

micronutrients over control indicated the transfer of the required trait from *Aegilops* species. Some BC_1 plants were self fertile with good seed set. They were allowed to self whereas the others were extensively backcrossed with recurrent wheat parent to get fertile BC_2F_2 plants.

4.1.4. BC_2F_1, BC_1F_2 and BC_1F_3 Derivatives

4.1.4. 1. Morphology

Morphological characters of BC_2F_1 and BC_1F_2 plants are given in Table 4.3. Some seeds of the fertile BC_1F_1 CS (*Ph*^{l})/*Ae. kotschyi* 3790// UP2338-2, was sent to off season nursery at Keylong, H.P, India and thus we had one year advanced generation of this progeny *i.e.* BC_1F_3 117. Most of the backcrossed derivatives had nearly recovered background of the recurrent wheat parent. They had comparable tiller number, plant height, and head type with good seed set. Some of the derivatives had red grain color like *Aegilops* parent. Sterile or partially fertile plants with low seed sets were discarded.

Table 4.2 Iron and zinc content in flag leaves of some partially fertile and sterile BC_1 plants.

S.No.	BC_1 Plants	Iron (mg/kg)	Zinc (mg/kg)
1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-1	112.5	30.3
2	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2	127.9	29.4
3	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-3	156.9	35.1
4	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// HD2687-1	172.4	38.2
5	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// HD2687-2	162.2	24.3
6	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// PDW 274-1	191.0	24.9
7	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// PDW 274-2	184.3	30.8
8	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// PDW 274-3	178.4	29.2
9	*T. aestivum* lr. CS (*Ph*^{l})	66.7	35.0
10	*T. aestivum* cv. WL711	56.5	29.3
11	*Ae. kotschyi* acc. 3790	135.6	55.8

4.1.4. 2. Cytology

The chromosome number of the derivatives varied from 42 to 56 with reduced univalent and increased bivalent frequency (Table 4.1). Derivatives with high grain iron and zinc content and nearly 42 chromosomes with lower number of univalent were selected (Fig.4.6).

4.1.4. 3. Grain micronutrient analysis

Nearly 50 BC_2 and BC_1F_2 fertile derivatives were analyzed for their grain micronutrient content. Some of the backcross derivatives BC_2 58-4, BC_2 58-5, BC_2 58-9, BC_2 58-11, BC_2 66-1, $BC_1F_2$77-2, $BC_1F_2$77-23, $BC_1F_2$77-50 had up to three fold increase in both grain iron and zinc content while some such as BC_2 58- 14, $BC_2$73-1, $BC_1F_2$77-5 showed increase in only zinc content (Table 4.3). Some derivatives with low grain iron and zinc content were also present.

4.1.4.4. Grain ash micronutrient analysis

Grain ash and micronutrient analysis of some seeds of BC_2F_2 and BC_1F_3 were carried out and the ash analysis data is given in Table 4.4. Grain ash analysis was done for further confirmation of micronutrient content of the derivatives and the actual increase in micronutrient on grain ash weight basis. The seeds of *Ae. kotschyi* had higher ash percentage (2.21 %) than the wheat parents WL711 (1.58 %) and Chinese Spring (1.65 %). *Ae. kotschyi* 3790 had 39 % increase in ash content over wheat control indicating its higher inorganic content. Among the four derivatives, BC_1F_3 77-2-1-19, showed maximum increase in grain ash iron and zinc content. The results suggested that high grain ash iron and zinc content is not due to concentration effect but because of efficient uptake and translocation system of *Ae. kotschyi*. Intermediate grain ash iron and zinc content of derivatives clearly indicated introgression of gene(s) / QTL of genetically superior system of *Ae. kotschyi* into wheat cultivars.

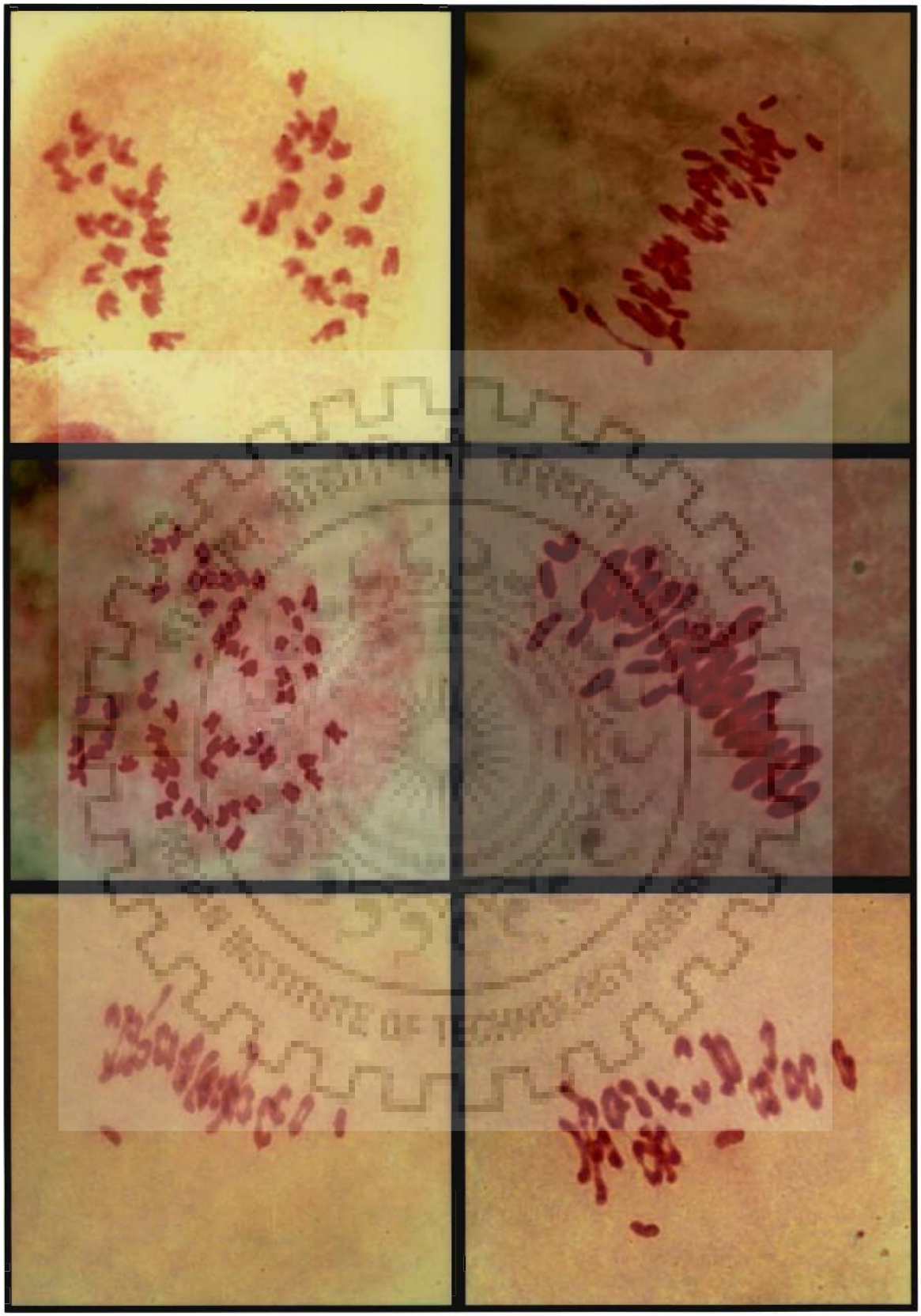


Fig. 4.6. Chromosome pairing at metaphase-I of PMCs of some selected BC_2F_1 and BC_1F_2 derivatives (a) $BC_1F_2$77-50-1, 21 II (b) BC_2 58-1, 20II+6 I (c) BC_1F_2, 77-38 56 (d) $BC_2$66-1, 20II+ 8I (e), $BC_1F_2$77-4, 18II+2I (f) BC_1F_2 77-2, 20II+ 2I

Table 4.3Morphology, Chromosome number and grain Fe and Zn content of some BC_2 and BC_1F_2 plants

I.D. No.	*Pedigree*	*No. of tillers*	*Plant Ht (cm)*	*Head type and waxiness*	*Chr. No.*	*Seed colour and shape*	*Grain iron (mg/kg)*	*Grain zinc (mg/kg)*
BC_2 56-1	CS (*Ph^l*)/ *Ae. kotschyi* 3790//WL711-3///WL711-1	33	75	Square, Non-waxy	41	Red, slighty shrivelled seeds	31.4	30.1
BC_2 58-1	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-1	40	93	Square, waxy	49	Amber, bold, round seeds	60.6	27.2
BC_2 58-4	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4	17	90	Square , waxy	56	Red, plump, slender seeds	47.5	28.5
BC_2 58-5	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5	21	115	Square , waxy	44	Red, bold, slender seeds	38.7	39.4
BC_2 58-8	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-8	20	125	Square, waxy	49	Red, slender, plump seeds	69.0	29.6
BC_2 58-9	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-9	36	90	Square, waxy	56	Red, shrivelled, slender	66.0	50.2
BC_2 58-11	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-11	20	92	Square, waxy	44	Red, slender, plump seeds	52.3	46.9
BC_2 58-14	CS (*Ph^l*)/ *Ae. kotschyi* 3790// WL711-4///WL711-14	30	105	Square, waxy	44	Amber, bold, slender	21.3	55.5
BC_2 63-1	CS (*Ph^l*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-1	18	105	Square, waxy	48	Amber, round seeds	26.8	26.2
BC_2 63-2	CS (*Ph^l*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-2	10	118	Square, waxy	44	Red, round, plump seeds	45.7	38.7
BC_2 63-3	CS (*Ph^l*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-3	16	130	Square, waxy	44	Amber, plump seeds	28.7	32.8
BC_2 66-1	CS (*Ph^l*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1	39	95	Square, waxy	42	Red, angular seeds, slender	60.1	49.5

I.D. No.	*Pedigree*	*No. of tillers*	*Plant Height (cms)*	*Awnness and waxiness*	*Chr. No.*	*Seed colour and shape*	*Grain iron (mg/kg)*	*Grain zinc (mg/kg)*
BC_2 66-2	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1	39	95	Square, waxy	42	Red, angular seeds, slender	43.2	39.5
$BC_2$73-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2425-2///WL711-1	25	85	Square, nonwaxy	44	Red, slender, plump seeds	29.6	42.1
$BC_1F_2$77-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-1	14	105	Square, nonwaxy	44	Red, round, small seeds	30.1	31.2
$BC_1F_2$77-2	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-2	57	125	Square, nonwaxy	49	Red, slightly, shrivelled seeds	59.3	43.2
$BC_1F_2$77-4	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-4	16	125	Square, nonwaxy	46	Red, bold, plump seeds	30.4	38.7
$BC_1F_2$77-5	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-5	12	132	Square, nonwaxy	44	Red, bold, plump seeds	26.3	42.1
$BC_1F_2$77-23	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-23	18	120	Square, nonwaxy	44	Amber, bold, slender seeds	49.3	37.9
$BC_1F_2$77-33	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-33	29	115	Square, nonwaxy	42	Red, bold, slender seeds	36.8	31.5
$BC_1F_2$77-36	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-36	31	100	Square, nonwaxy	42	Red, slighty shrivelled seeds	36.5	27.9
$BC_1F_2$77-38	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-38	4	105	Square, nonwaxy	46	Red, round, shrivelled seeds	51.4	29.4
$BC_1F_2$77-46	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-46	14	110	Square, nonwaxy	42	Red, round, bold seeds	36.5	27.9
$BC_1F_2$77-50	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-50	7	40	Square, nonwaxy	42	Red, plump medium sized seeds	43.7	37.4

Table 4.4 Grain ash and grain ash iron and zinc content in wheat cultivars, *Ae. kotschyi* 3790 and their BC_1F_2 and BC_2F_1 derivatives grain

.D. No.	Grain material	Ash %	Fe(µg/g) of ash	% Change in ash Fe over WL711	Zn (µg/g) of ash	% Change in ash Zn over WL711
Control	WL711	1.58	1,607	-	1,342	0
-	CS(*Ph*^{l})	1.65	1,702	5.9	1,181	-11.9
-	*Ae. kotschyi* 3790	2.21	2,828	75.9	2,215	65.1
BC_1F_3 77-2-1-8	BC_1 F_3CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-1-8	1.81	2,525	57.15	1,932	43.95
BC_2F_2 66-2-5	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1	1.94	2,472	53.82	2,107	57.00
BC_2F_2 63-2-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2///WL711-2	2.15	2,639	64.31	1,868	39.19
BC_1F_3 77-2-1-19	BC_1 F_3CS (*Ph*^{l})/ *Ae. kotschyi* 396// UP2338-2-1-19	2.02	2,755	71.42	2,248	67.55

4.1.5. BC_2F_2, BC_1F_3 and BC_1F_4 derivatives

Plants of selected progenies were sown in 10 rows at two locations. A large number of plants of each selected plant progenies were extensively screened for morphological, chemical, cytological and molecular characterizations (Table 4.5.).

4.1.5.1 Morphology

Morphological details of each plant of BC_2F_2, BC_1F_3 and BC_1F_4 families were recorded (Table 4.5) There was higher morphological variation among different families than within plant of same families. Various morphological characters associated with particular chromosome, such as (waxy or non waxy of group 2), head type (square or spelta group of 5), grain color (red or amber, of group 3) were taken into account for monitoring the introgressed segment from *Ae. kotschyi*. A few plants showed brittle rachis character which is associated with group 3 chromosomes and present in *Aegilops* species only. Plants with good background recovery and harvest index were selected for grain micronutrient analysis. Some of the plants with very good harvest index such as

instance BC_1F_3 77-46-45 (40) and $BC_1F_3$77-33-2 (44.6) were selected. Grain and plant morphology of few selected derivatives are given in Fig 4.8 and Fig 4.7.

4.1.5.2. **Cytology**

Chromosome number of the BC_2F_2, BC_1F_3 and BC_1F_4 derivatives varied from 40-43 with 1-2 univalent and with 19-21 bivalents. Some higher range of chromosome number was also observed in some derivatives (Table 4.6). Chromosome number in the derivatives 77-46-3 and 77-36-8 was 42 with 20-21 bivalents. Cytological details of some selected derivatives are given in Fig 4.9, 4.10, 4.11. Chromosome number (41-43) and pairing varied among the derivative plants. However most of the derivatives had nearly normal wheat chromosome configuration with bivalent frequency ranging from 18.76 to 20.70 and 0.60 to 4.45 univalents.

4.1.5.3 Grain iron and zinc content

Plant progenies were extensively screened on the basis of minimum linkage drag. Around 20-30 plants were selected from each plant family and extensively subjected to chemical analysis. Plants with good harvest index and background recovered were preferred. Minimum of six replications for grain samples per plants were prepared, digested and analyzed using AAS (Table. 4.8).The derivatives showed wide range of grain iron and zinc content. Plants with more than 60 % of increase in both grain iron and zinc content along with good harvest index were selected. Some of the derivatives such as BC_2F_2 58-11(bulk), $BC_2F_2$66-1-89, $BC_1F_4$77-36-6, $BC_1F_4$77-46-3 had nearly 100% increased grain iron and zinc content along with nearly 33% harvest index very close to that of wheat cultivars. Grain ash and ash iron and zinc content of finally selected derivatives were estimated for actual increase in the micronutrient contents over wheat cultivars.

Table 4.5 Morphological characteristics of some representative plants of BC_2F_2 and BC_1F_3 derivatives

ID. No.	Pedigree	No. of tillers	Height (cm)	Days to Flowering	Waxiness	Head type	Rachis	Grain color	No of seeds per spike	Harvest Index (%)
BC_2F_2 58-4-10	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-4-10	7	107	105	Waxy	Awend, square	NB	Red	18	40.3
BC_2F_2 58-4- 13	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-4-13	5	121	110	Waxy	Awned, clubed	NB	Red	26	23.5
BC_2F_2 58-4-17	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-4-17	3	108	102	Nonwaxy	Awned, square	NB	Red	12	11.1
BC_2F_2 58-4-39	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-4-39	9	85	108	Nonwaxy	Awned square	NB	Amber	21	31.7
BC_2F_2 58-4-116	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-4-116	14	100	112	Nonwaxy	Awned, square	NB	Red	22	16.3
BC_2F_2 58-5-12	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-12	15	120	105	Nonwaxy	Awned, square	NB	Red	23	35.0
BC_2F_2 58-5-13	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-13	6	132	110	Waxy	Awnless, square	NB	Red	21	16.0
BC_2F_2 58-5-17	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-17	7	125	115	Nonwaxy	Awned, square	NB	Red	23	12.7
BC_2F_2 58-5-19	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-19	13	115	106	Nonwaxy	Awned, square	NB	Amber	23	7.1
BC_2F_2 58-5-33	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-33	10	128	109	Nonwaxy	Awned, spelta	NB	Red	24	30.0
$BC_2F_2$58-11(x)	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-11	15	110	102	Nonwaxy	Awned, square	NB	Amber	23	32.0
BC_2F_2 63-2-13	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-13	15	120	103	Waxy	Awnless, square	NB	Red	23	32.8
BC_2F_2 63-2-16	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-16	12	110	101	Waxy	Awnless, spelta	NB	Red	21	21.4
BC_2F_2 63-2-20	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-20	11	122	104	Waxy	Awnless, square	NB	Red	20	23.7
BC_2F_2 66-1-6	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-6	16	65	102	Nonwaxy	Awned, clubbed	NB	Red	17	25.6
BC_2F_2 66-1-10	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-10	15	96	110	Waxy	Awned, spelta	NB	Amber	15	22.0
BC_2F_2 66-1-16	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-16	35	110	115	Waxy	Awned, spelta	NB	Red	18	23.9
BC_2F_2 66-1-30	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-30	13	95	110	Waxy	Awned, spelta	NB	Red	21	17.8
BC_2F_2 66-1-89	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-89	12	110	102	Waxy	Awned, spelta	NB	Red	22	34.6

BC_1F_377-14-8	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-14-8	17	85	110	Waxy	Awned, square	NB	Amber	17	9.0
BC_1F_377-23-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-23-1	20	110	95	Nonwaxy	Awnless, square	NB	Amber	20	33.0
BC_1F_377-23-7	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-23-7	22	100	100	Nonwaxy	Awnless, square	NB	Amber	18	19.3
BC_1F_377-33-2	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-33-2	24	115	105	Nonwaxy	Awned, square	NB	Amber	21	43.9
BC_1F_377-36-6	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-36-6	26	92	115	Nonwaxy	Awnless, square	NB	Red	18	35.0
BC_1F_377-36-8	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-36-8	20	115	100	Nonwaxy	Awnless, square	NB	Red	23	15.5
BC_1F_377-36-20	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-36-20	5	100	120	Nonwaxy	Awnless, square	NB	Red	17	15.5
BC_1F_377-46-3	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-46-3	10	130	108	Nonwaxy	Awnless, square	NB	Amber	22	32.0
BC_1F_377-46-6	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-46-6	14	136	105	Waxy	Awnless, square	NB	Red	18	28.0
BC_1F_377-46-15	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-46-15	8	67	120	Waxy	Awnless, square	NB	Amber	21	12.0
BC_1F_377-46-45	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-46-45	21	105	115	Nonwaxy	Awnless, square	NB	Red	24	44.6
BC_1F_377-50-8	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-50-8	29	132	115	Nonwaxy	Awnless, square	NB	Amber	26	32.6
BC_1F_377-50-9	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-50-9	41	160	106	Waxy	Awnless, square	NB	Red	21	15.4
BC_1F_377-50-15	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-50-15	43	150	108	Nonwaxy	Awnless, square	NB	Red	23	36.4
BC_1F_4117-18-8	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-18K-8R	8	85	115	Nonwaxy	Awned, square	NB	Red	16	7.3
BC_1F_4117-18-17	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-18K-17R	16	110	115	Nonwaxy	Awned, square	NB	Red	24	35.7
BC_1F_4 117-18-22	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-18K-22R	12	82	109	Nonwaxy	Awned, square	NB	Red	17	36.4
BC_1F_4 117-33-17	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-33K-17R	28	112	120	Waxy	Awned, square	NB	Amber	24	15.0
BC_1F_4 117-33-27	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-33K-27R	11	105	110	Waxy	Awned, square	NB	Amber	17	9.0
BC_1F_4 117-36-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2R-36K-1R	20	120	105	Nonwaxy	Awned, square	NB	Red	19	12.0

Table 4.6 cytological details of some selected BC_2F_2 and BC_1F_3 plants.

ID. No.	*2n*	*PMCs*	***Mean ± S.E. (Range) Univalent (I)***	***Mean ± S.E. (Range) Bivalent (II)***
BC_2F_2 58-5-12	42	25	2.56 ± 0.34	19.72 ± 0.39
BC_2F_2 58-5-33	43	25	2.20 ± 0.29	20.40 ± 0.35
$BC_2F_3$58-11(x)	42	25	1.70 ± 0.29	20.15 ± 0.37
BC_2F_3 63-2-13	41	25	2.60 ± 0.46	19.20 ± 0.51
BC_2F_3 66-1-89	41	25	1.16 ± 0.21	20.42 ± 0.34
$BC_1F_4$77-23-1	42	25	1.26 ± 0.15	20.37 ± 0.17
$BC_1F_4$77-33-2	41	25	1.72 ± 0.29	20.14 ± 0.35
$BC_1F_4$77-36-6	42	25	1.00 ± 0.37	20.50 ± 0.44
$BC_1F_4$77-46-3	42	25	0.60 ± 0.42	20.70 ± 0.51
$BC_1F_4$77-50-8	42	25	4.45 ± 0.29	18.76 ± 0.37
$BC_1F_4$77-50-15	41	25	2.20 ± 0.31	19.42 ± 0.54
$BC_1F_5$117-18-17	42	25	0.74 ± 0.18	20.63 ± 0.21
BC_1F_5 117-18-22	42	25	2.18 ± 0.19	19.91 ± 0.23

Table 4.7. Grain ash and ash iron and zinc content of some selected derivatives

I.D. No.	Grain material	Ash %	Fe (µg/g) of ash	% Change in ash Fe over WL711	Zn (µg/g) of ash	% Change in ash Zn over WL711
Control	WL711	1.6	1,708	-	1,442	-
-	CS(*Ph*^{l})	1.7	1,802	5.5	1,581	9.0
-	*Ae. kotschyi* 3790	2.5	3,259	90.8	2,552	76.4
BC_2F_3 58-5-12	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-12	1.8	2,674	56.5	1,958	35.5
BC_2F_3 58-5-33	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-5-33	1.8	2,781	62.8	2,453	70.1
$BC_2F_3$58-11(x)	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// WL711-4///WL711-11	1.9	2,951	72.7	2,386	65.5
BC_2F_3 63-2-13	CS (*Ph*^{l})/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-13	1.8	2,739	60.3	2,092	45.1
BC_2F_3 66-1-89	CS (*Ph*^{l})/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-89	2.0	3,126	83.0	2,443	69.0
$BC_1F_4$77-23-1	CS (*Ph*^{l})/ *Ae. kotschyi* 3790//UP2338-2-23-1	2.1	2,919	70.4	2,168	50.3

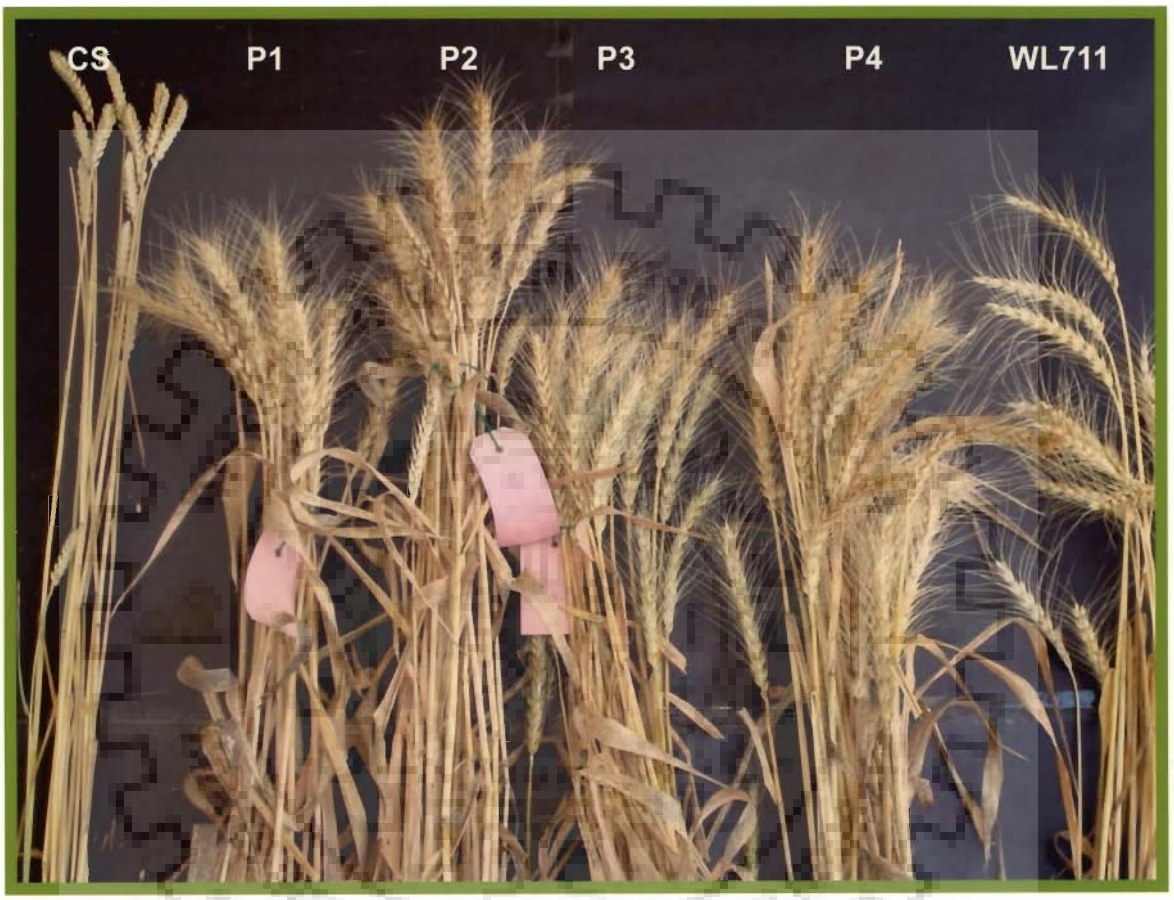


Fig 4.7 Morphology of selected derivatives 58-11

Fig 4.8 Grain morphology of selected derivatives with their parents

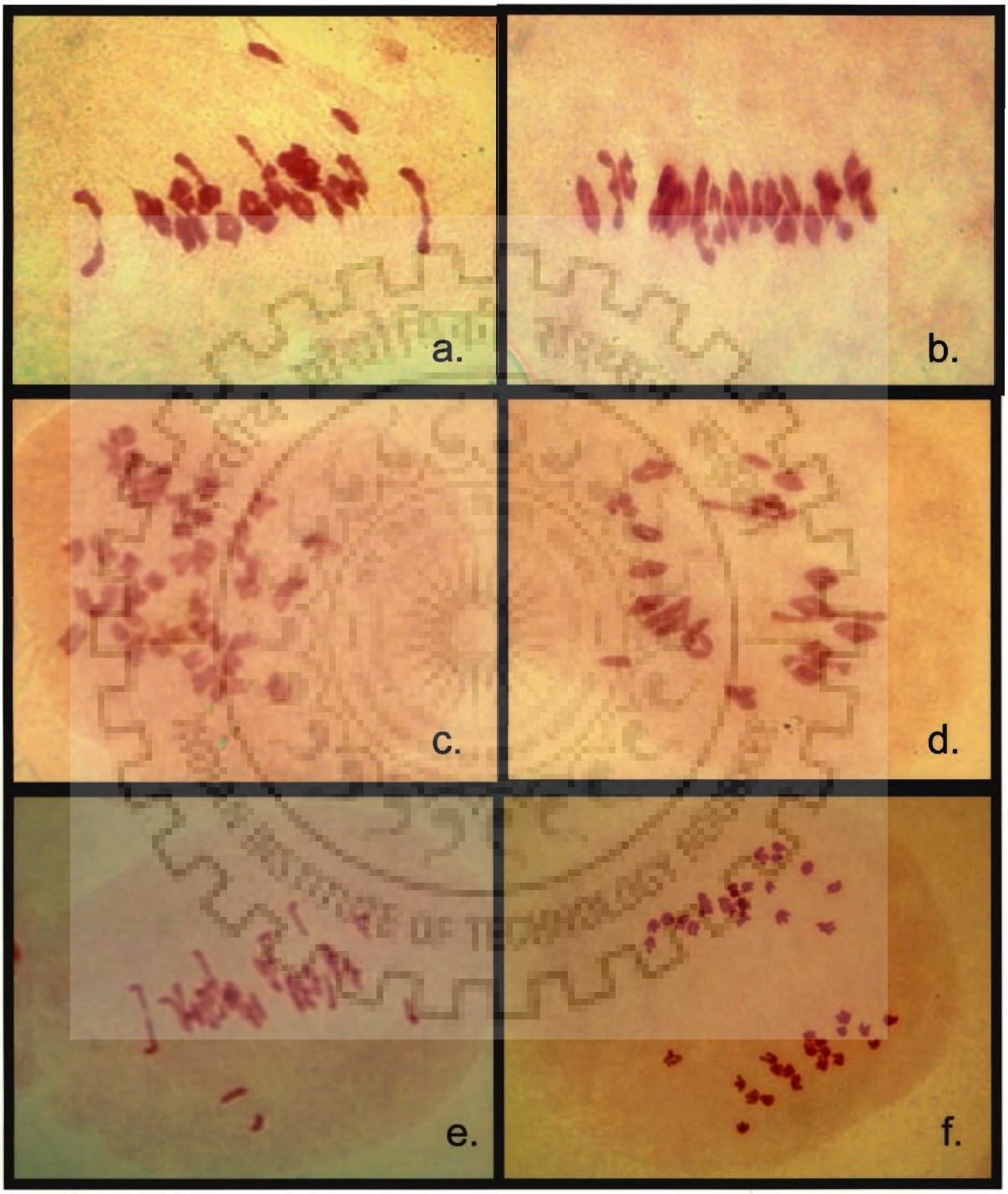


Figure 4.9 . Chromosome number and pairing of selected derivatives. (a) and (b); BC_1F_4 77-46-3 (42, 21 II), (c) and (d); BC_1F_4 77-36-8(40, 19II+2I), (e) and (f);BC_2F_3 66-1-89 (42,19II+1III+1I).

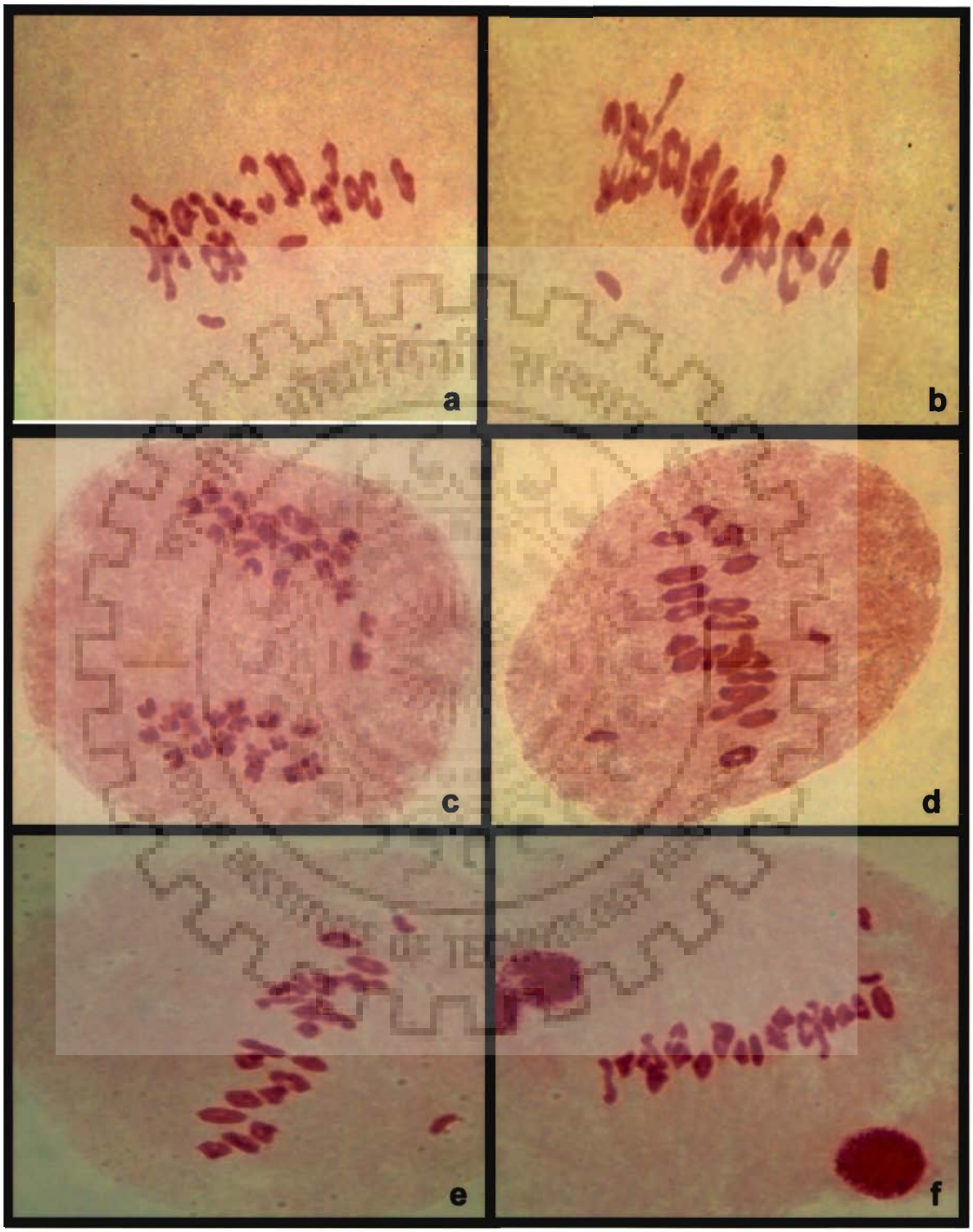


Figure 4.10 Chromosome number and pairing of selected derivatives. (a) and (b); BC_1F_4 77-50-15(42, 20II+2I), (c) and (d); BC_1F_4 77-50-8(41, 20II+1I), (e) and (f);BC_2F_3 63-2-13 (42, 20II+1I).

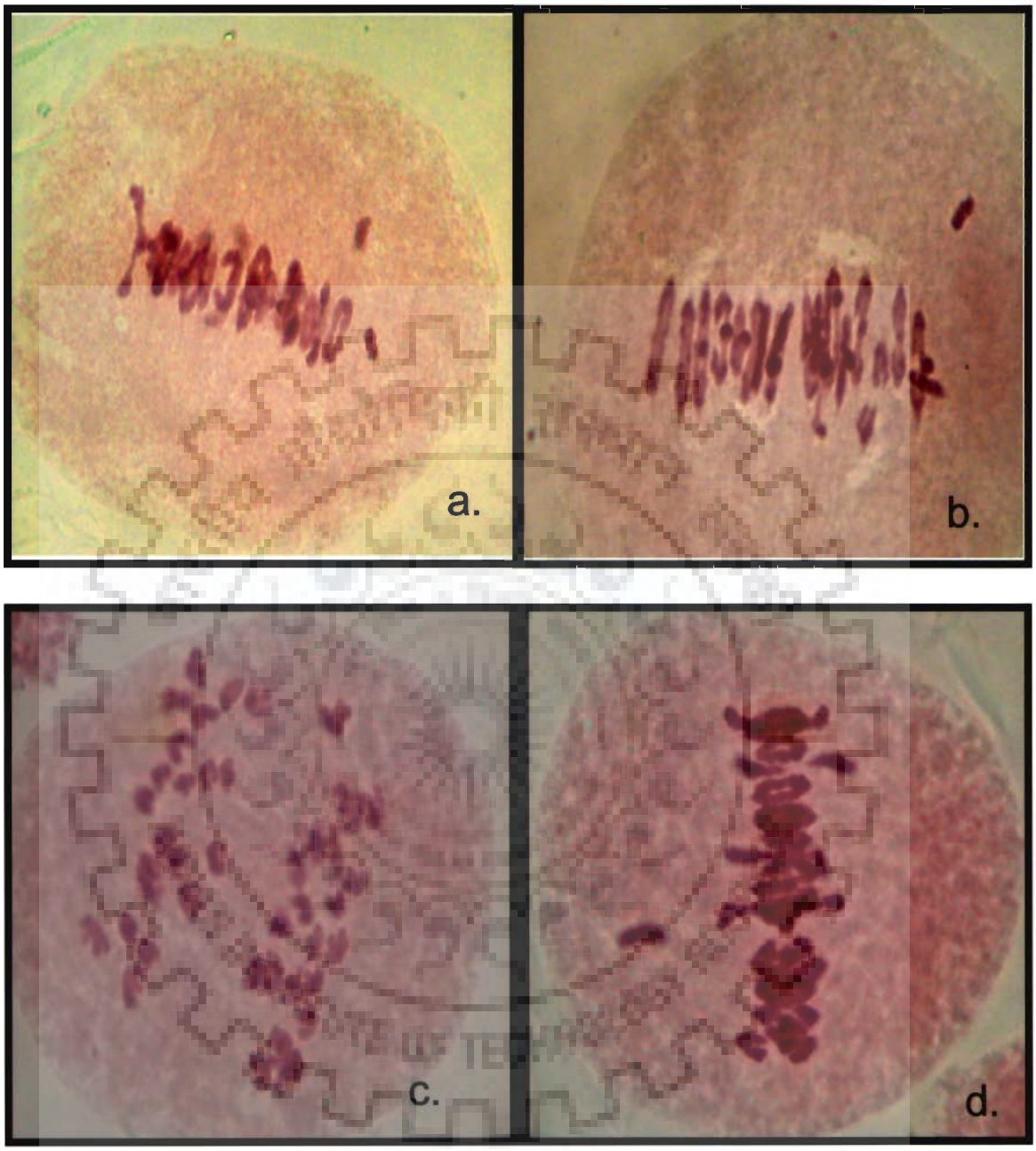


Figure 4.11 . Chromosome number and pairing in back cross derivative. (a) and (b); BC_1F_4 117-18- 22-(20II+ 2I), (c) and (d); $BC_1F_4$117-18-17 (21II)

Table 4.8 Grain iron zinc content in some BC_2F_3 and BC_1F_4 derivatives

ID. No.	Pedigree	Grain iron content (mg/kg)				% Change	Grain zinc content (mg/kg)				% Change
		I	II	III	Mean±SE	over WL711	I	II	III	Mean±SE	over WL711
Control	WL 711	22.0	20.4	25	22.4 ± 1.4	-	19	18.7	21.5	19.7 ± 1.7	-
BC_2F_3 58-4- 10	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4-10	15.9	17.2	14.3	15.8 ± 0.8	-29.3	25.0	21.3	23.5	23.2 ± 2.1	17.7
BC_2F_3 58-4- 13	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4-13	28.9	30.1	25.3	28.1 ± 1.4	28.4	31.9	32.4	35.3	33.1 ± 1.7	68.0
BC_2F_3 58-4- 17	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4-17	24.3	22.6	20.4	22.4 ± 1.1	8.2	29.6	30.1	31.9	30.5 ± 1.2	55.0
BC_2F_3 58-4- 39	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4-39	20.3	18.6	22.5	20.5 ± 1.1	-9.8	25.1	22.7	20.4	22.7 ± 2.4	15.4
BC_2F_3 58-4- 116	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-4-116	42.4	38.4	40.7	40.5 ± 1.2	88.4	25.1	22.0	28.6	25.2 ± 3.3	28.1
BC_2F_3 58-5-12	**CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5-12**	**37.6**	**38.6**	**33.4**	**36.5 ± 1.6**	**67.1**	**33.1**	**36.6**	**33.2**	**34.3± 1.5**	**70.1**
BC_2F_3 58-5-13	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5-13	27.0	20.1	22	23.0 ± 2.1	20.0	18.6	15.6	17.4	17.2 ± 1.5	-12.7
BC_2F_3 58-5-17	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5-17	20.3	21.8	19.4	20.5± 0.7	-9.8	32.3	30.8	28.9	30.7 ± 1.7	55.7
BC_2F_3 58-5-19	CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5-19	42.3	44.7	48.3	45.1± 1.7	88.0	45.2	42.5	44.1	43.9 ± 1.4	123.0
BC_2F_3 58-5-33	**CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-5-33**	**38.6**	**40.1**	**36.7**	**38.5 ± 1.0**	**71.6**	**33.4**	**38.2**	**35.1**	**35.6 ± 2.4**	**80.5**
$BC_2F_3$58-11(x)	**CS (*Ph^I*)/ *Ae. kotschyi* 3790// WL711-4///WL711-11**	**49.1**	**43.1**	**44.1**	**45.4 ± 1.9**	**118.2**	**43.2**	**38.9**	**40.3**	**40.8 ± 2.2**	**107.1**
BC_2F_3 63-2-13	**CS (*Ph^I*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-13**	**42.7**	**48.2**	**45.2**	**45.8 ± 1.6**	**89.8**	**36.7**	**38.4**	**39.2**	**38.1 ± 1.3**	**93.4**
BC_2F_3 63-2-16	CS (*Ph^I*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-16	25.3	21.5	22.1	23.0 ± 1.2	12.4	22.1	20.4	20.6	21.0 ± 0.9	6.8
BC_2F_3 63-2-20	CS (*Ph^I*)/ *Ae. kotschyi* 3790// UP2338-2///WL711-2-20	28.1	26.1	20.5	24.9 ± 2.3	24.9	19.0	20.0	21.0	20.0 ± 1.0	1.5
$BC_2F_3$66-1-6	CS (*Ph^I*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-6	33.9	30.6	31.4	32.0 ± 1.0	50.7	40.9	45.1	44.3	43.4 ± 2.2	120.5
BC_2F_3 66-1-10	CS (*Ph^I*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-10	26.1	22.4	19.4	22.6 ± 1.9	16.0	43.7	40.1	42.8	42.2 ± 1.9	114.2
$BC_2F_3$66-1-16	CS (*Ph^I*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-16	25.5	19.3	21	22.0 ± 1.9	13.6	42.3	44.1	40.3	42.2 ± 1.9	114.4
BC_2F_3 66-1-30	CS (*Ph^I*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-30	24.6	22.1	20.2	22.3 ± 1.3	9.3	39.3	35.1	30.1	34.8 ± 4.6	76.8
BC_2F_3 66-1-89	**CS (*Ph^I*)/*Ae. kotschyi*3790//PDW 274-2///PBW373-1-89**	**49.7**	**48.2**	**49.1**	**43.7 ± 1.8**	**117.7**	**49.8**	**50.2**	**52.1**	**45.5 ± 3.0**	**145.5**

ID. No.	Pedigree	Grain iron content (mg/kg)				% Change	Grain zinc content (mg/kg)				% Change
		I	II	III	Mean±SE	over WL711	I	II	III	Mean±SE	over WL711
$BC_1F_4$77-14-3	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-14-3	36.1	35	32.1	34.4 ± 2.1	60.4	34.1	30.1	28.6	30.9 ± 2.8	57.0
$BC_1F_4$77-14-8	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-14-8	20	22.1	25	22.4 ± 2.5	-11.1	26.0	28.1	27	27.0 ± 1.1	37.2
$BC_1F_4$77-23-1	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-23-1**	**40.9**	**42.1**	**44.7**	**42.6 ± 1.9**	**81.8**	**35.9**	**36**	**33.2**	**35.0 ± 1.6**	**77.8**
$BC_1F_4$77-23-7	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-23-7	26	28	27.2	27.1 ± 1.0	15.6	20.1	22	19.6	20.6 ± 1.3	4.3
$BC_1F_4$77-33-2	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-33-2**	**39.3**	**40.4**	**42.5**	**40.7 ± 1.6**	**74.7**	**37.2**	**33.5**	**38.9**	**36.5 ± 2.8**	**85.4**
$BC_1F_4$77-36-6	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-36-6**	**44.3**	**45.9**	**44.3**	**44.8 ± 0.9**	**96.9**	**44.8**	**45.6**	**43.3**	**44.6 ± 1.2**	**126.2**
$BC_1F_4$77-36-8	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-36-8	47.3	45.2	46.2	46.2 ± 1.1	110.2	40.6	46.1	45.3	44.0 ± 3.0	123.3
$BC_1F_4$77-36-20	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-36-20	20.4	23.2	25	22.9 ± 2.3	-9.3	19	20.5	21.3	20.3 ± 1.2	2.8
$BC_1F_4$77-46-3	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-46-3**	**47.6**	**45.1**	**42.5**	**45.1 ± 2.6**	**111.6**	**43.1**	**44.2**	**39.7**	**42.3 ± 2.3**	**114.8**
$BC_1F_4$77-46-6	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-46-6	35.7	32	33.1	33.6 ± 1.9	58.7	48.1	45.3	42.3	45.2 ± 2.9	129.6
$BC_1F_4$77-46-15	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-46-15	26.1	29.3	25.4	26.9 ±2.1	16.0	20	17.3	15.8	17.7 ± 2.1	-10.1
$BC_1F_4$77-46-45	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-46-45	42.9	35.6	37.3	38.6 ± 3.8	90.7	39.1	42.3	40.4	40.6 ± 1.6	106.0
$BC_1F_4$77-50-8	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-50-8**	**31.5**	**36.2**	**33.1**	**33.6 ± 2.4**	**40.0**	**45.2**	**41.6**	**42.8**	**43.2 ± 1.8**	**119.2**
$BC_1F_4$77-50-9	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-50-9	27.1	22.8	25.1	25.0 ± 2.2	20.4	28.6	25.1	29.1	27.6 ± 2.2	40.1
$BC_1F_4$77-50-15	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2-50-15**	**39.3**	**35.7**	**33.7**	**36.2 ± 2.8**	**74.7**	**50.1**	**49.3**	**47.2**	**48.9 ± 1.5**	**148.0**
$BC_1F_5$117-18-8	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-18K-8R	17.3	16.6	15.4	16.4± 1.0	-23.1	27.9	29.3	25.4	27.5 ± 2.0	39.7
$BC_1F_5$117-18-17	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-18K-17R**	**39.3**	**44**	**42.7**	**42.0 ± 2.4**	**74.7**	**43.6**	**48.6**	**45.4**	**45.9 ± 2.5**	**132.8**
BC_1F_5 117-18-22	**CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-18K-22R**	**41.4**	**32.8**	**39.4**	**37.9 ± 4.5**	**84.0**	**44.5**	**48.2**	**45.2**	**46.0 ± 2.0**	**133.3**
BC_1F_5 117-33-17	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-33K-17R	39.3	44.3	40.2	41.3 ± 2.7	74.7	36.2	33.5	37.1	35.6 ± 1.9	80.7
$BC_1F_5$117-33-27	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-33K-27R	29.1	23.5	25.6	26.1 ± 2.8	29.3	17.5	19.3	17.2	18.0 ± 1.1	-8.6
BC_1F_5 117-36-1	CS (*Ph^l*)/ *Ae. kotschyi* 3790//UP2338-2R-36K-1R	27.1	25.6	28.3	27.0 ± 1.4	20.4	20.5	23.5	22.5	22.2 ± 1.5	12.5

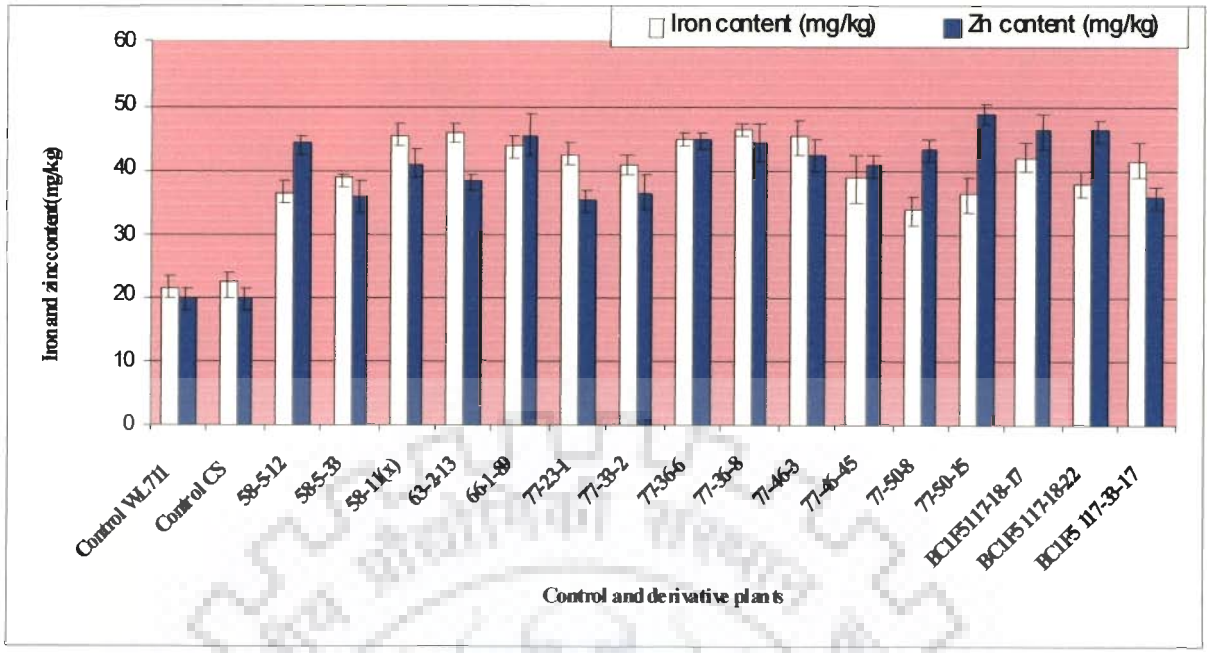


Fig. 4.12. Grain mineral content of selected BC_2 $F_3$and BC_1 F_4 derivatives analysed using ICPMS

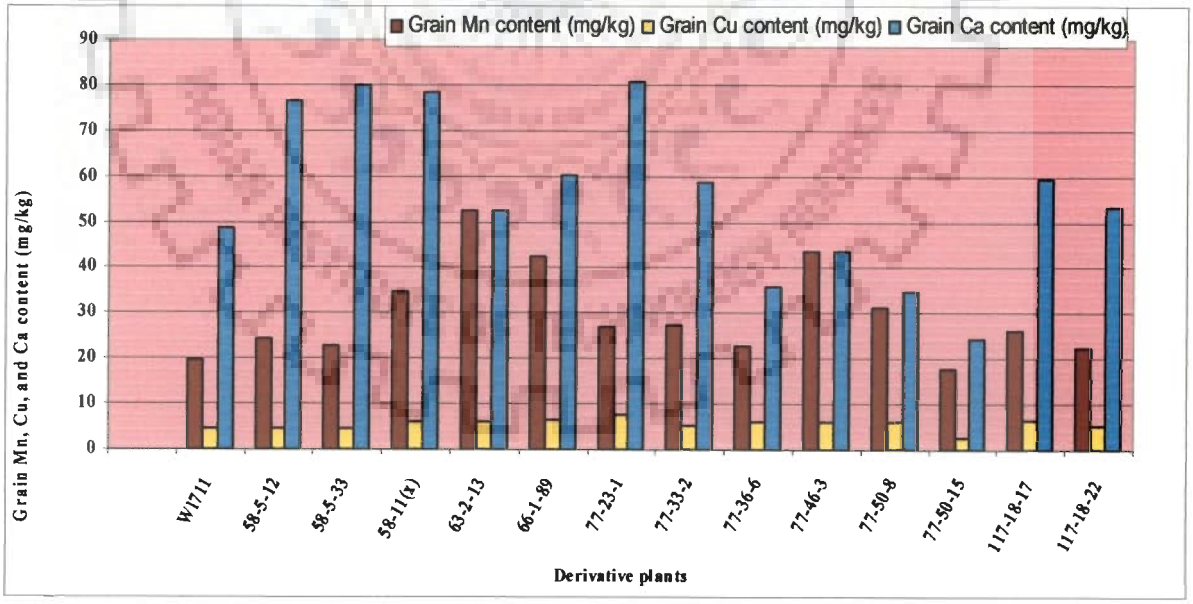


Fig. 4.13. Grain Mn, Cu, Ca content of selected BC_2 $F_3$and BC_1 F_4 derivatives analyzed using ICPMS

Some of the derivatives had upto 83% increase in iron and around 70% in zinc in the grain ash (Table. 4.7) Readings for aluminum were also recorded of the digested samples using ICPMS as aluminum is an indicator of contamination during handling or digestion. But Al was no detectable proving that the samples were free of any contamination and the readings of iron and zinc were reliable. Grain Fe and Zn content of selected plants analyzed by ICPMS are given in Fig 4.12. Grain Cu, Mn and Ca were also analysed using ICPMS (Fig 4.13). These derivatives showed upto 2 folds increase for Mn over wheat parent. Maximum percentage increase in Cu content (73.1) was found in $BC_1F_4$77-23-1 followed by Ca (66.5). Some derivatives such as BC_2 F_3 58-5-19 had 88 and 123 % increase in grain iron and zinc content respectively but had very low harvest index (7.1).

4.1.5.4 High molecular weight Glutenin subunit (HMW-GS) profile

The HMW glutenin subunit profile of some of the selected derivatives is given in Fig. 4.14. With the exception of 58-11(bulk), no other derivative showed HMW-glutenin subunit of *Ae. kotschyi* parent indicating no introgression of 1U/1S. This also suggests that group 1 chromosome of *Ae. kotschyi* 3790 either do not have gene(s) /QTLs for high grain iron and zinc content or have been preferentially eliminated. The 58-11 bulk had group 1U/1S introgressed in 4 seeds out of a total of 10 seeds, indicated that group 1 chromosome(s) of *Ae. kotschyi* is segregating.

Thirteen backcross derivatives were finally selected for characterization on the basis of cytology, high molecular weight glutenin subunit profile and molecular markers.

4.1.6 *In situ* hybridization:

In situ hybridization of selected derivatives was carried out to confirm alien introgression. Probes pAS1 (D genome specific) and AAG (B genome specific probe) were used. U genome chromsomes appeared green and S genome chromosomes were pink in color. On the basis of karyotype chromosome 7U of *Ae. umbellulata* was found introgressed in derivative 63-2-13 and 117-18-17 (Fig.4.15). Genomic *in situ* hybridization confirmed presence of one chromosome of *Ae. umbellualata* in derivative 63-2-13 and 77-50-15 and the same was also supported by molecular marker results. FISH and GISH studies confirmed introgression of an *Ae. longissima* chromosome in derivative 77-50-15.

4.1.7 Molecular markers analysis

To characterize the alien chromosomes introgressed in the derivatives, anchored wheat microsatellite markers were applied to the selected high grain iron and zinc content. Firstly distal markers were checked for introgression as most of the translocations have been reported to be in the telomeric ends of the chromosomes. A list of all the markers applied with their annealing temperatures has been provided in Appendix-I. Extensive polymorphic survey was carried using these SSR markers among cultivars and *Aegilops kotschyi, Ae. peregrina* and *Ae. longissima* species. Total number of applied markers, transferable markers and those found polymorphic for the parents are given in Table 4.9. Few transferable and polymorphic markers are shown in Fig 4.16 and 4.17. B genome markers had highest transferability (69.9 %) to *Ae. kotschyi*, whereas the A and D

genome markers had almost similar transferability (62.6 % and 60.9 % respectively) to *Ae. kotschyi*. 'A' genome markers had the highest transferability among the transferable markers. For each chromosome 4 distal (2 from both arms) distinctly polymorphic markers were applied on the selected derivative plants. On the basis of markers results it was found that either group 2 and 7 were present additionally in the selected derivatives (Table 4.9). Group of introgressed alien chromosomes were further confirmed by using additional distinct polymorphic markers of group 2 and 7. The details of the chromosomes identified are given in Table 4.7 and Fig 4.18, 4.19. Out of 3 genomes of group 2, anchored markers of wheat only 2D markers showed introgressed *Ae. kotschyi* 2U/2S chromosome introgression. Markers wmc539 and gdm148 indicated introgression of long arm of 2U/2S in the derivative plants 66-1-89,77-33-2, 77-36-6, 77-50-8, 77-50-15, 77-46-3, 117-18-17, 117-18-22 and 77-23-1. Short arm of group 2 alien chromosomes was present in plants On the basis of 2DS markers wmc25, barc11 and wmc601 it was found that in derivatives 58-11 (bulk), 66-1-89, 4, 5 and 13. This shows that Pl- 4, 5 and 13 had complete chromosome 2U/2S whereas other plants had introgressed chromosome segments/ arms. Anchored marker of 7DL wmc488 and wmc809 showed presence of 7U/7S in derivative plants 63-2-13, 58-5-33, 77-36-6, 77-50-8, 77-50-15, 117-18-17and 77-23-1 whereas anchored markers of 7DS of wheat cfd41 confirmed the introgression of alien group 7 chromosomes (7U/7S) was present in plants 58-5-33 and 66-1-89. However in derivative 58-5-12 markers didn't show introgression of any alien chromosome(s). This may be because of very small transfer conferring high Fe and Zn content. Backcross derivatives varied in the morphology, cytology and they had variable grain iron and zinc content.

Table 4.9. Transferability and polymorphism of anchored wheat microsatellite markers between wheat and *Aegilops* species

Homoeologous genome	Chromosome	Tested markers	Transferable markers	Polymorphic markers	% Transferable	% Polymorphic	Average genome wise transferability
A	1A	12	7	4	58.3	57.1	62.6
	2A	28	18	9	64.3	50.0	
	3A	20	14	6	70.0	42.9	
	4A	12	7	4	58.3	57.1	
	5A	12	7	4	58.3	57.1	
	6A	16	10	5	62.5	50.0	
	7A	24	16	6	66.7	37.5	
B	1B	12	8	5	66.7	62.5	69.9
	2B	27	20	9	74.1	45.0	
	3B	18	12	3	66.7	25.0	
	4B	14	10	4	71.4	40.0	
	5B	14	9	4	64.3	44.4	
	6B	15	11	4	73.3	36.4	
	7B	26	19	7	73.1	36.8	
D	1D	14	8	5	57.1	62.5	60.9
	2D	26	18	7	69.2	38.9	
	3D	15	9	5	60.0	55.6	
	4D	15	9	5	60.0	55.6	
	5D	14	8	4	57.1	50.0	
	6D	16	10	4	62.5	40.0	
	7D	25	15	6	60.0	40.0	
	Total	375	245	110	64.5	46.9	

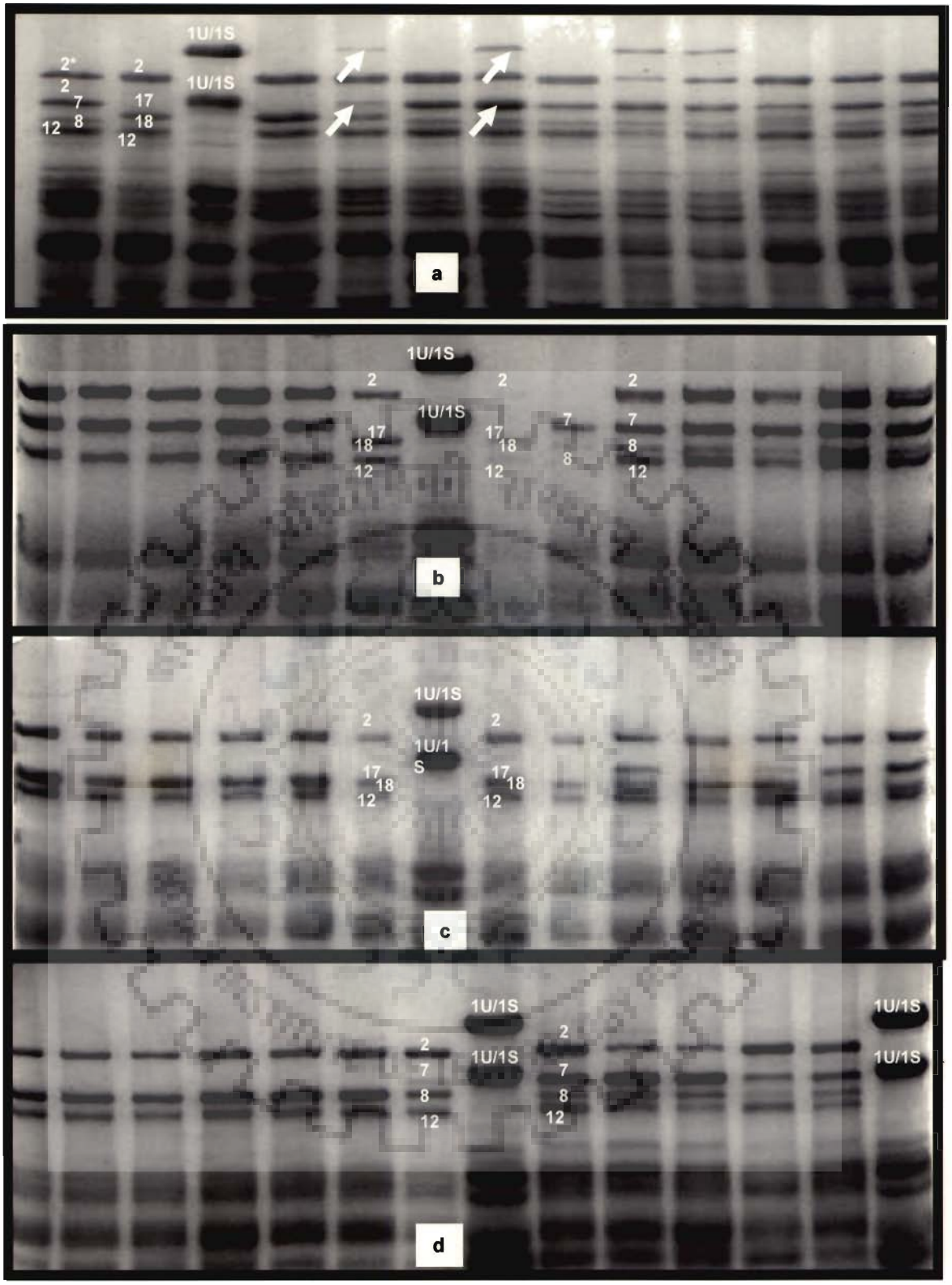
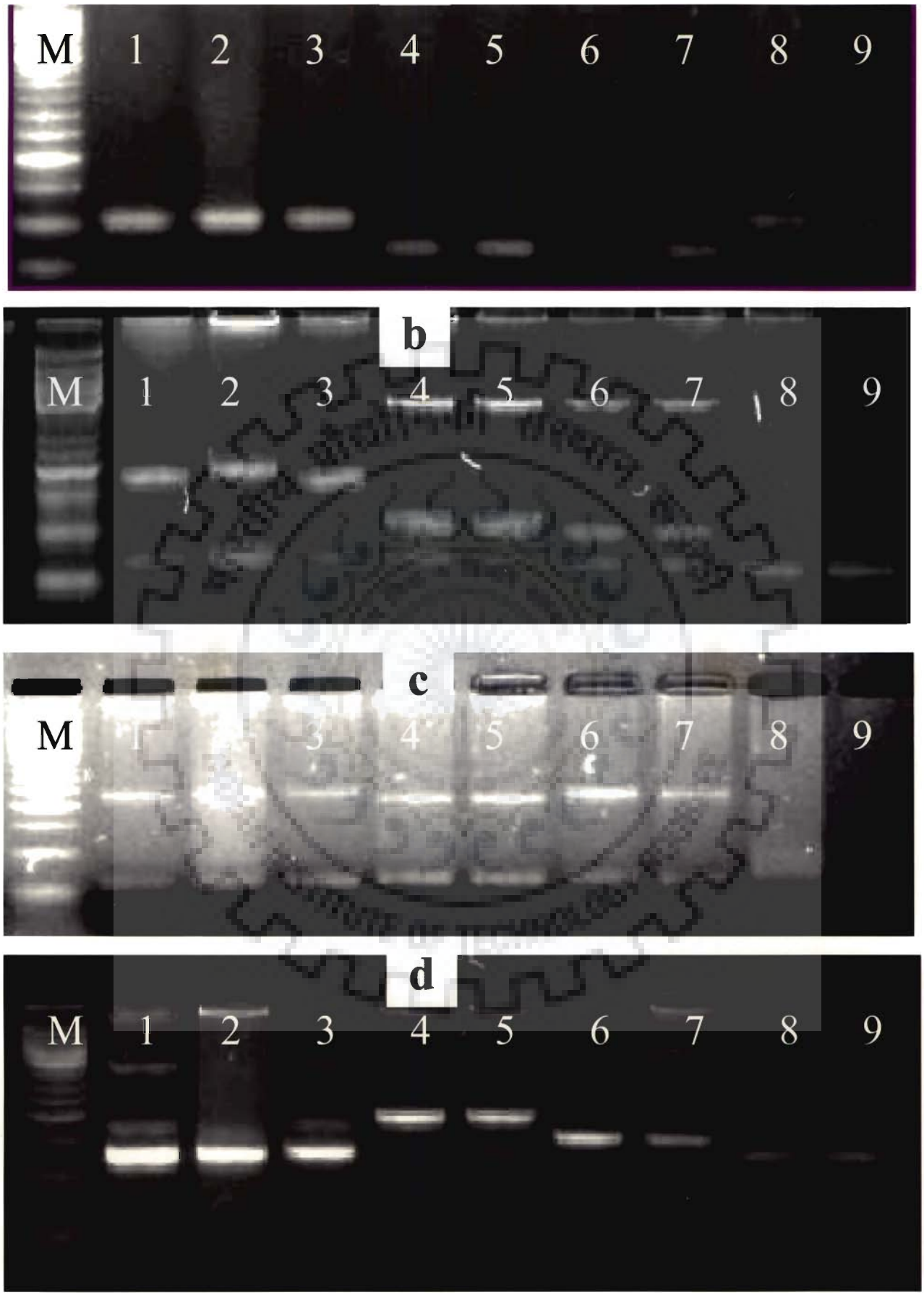


Fig:4.14 HMW Glutenin Subunit profile of some selected derivatives. (a) 58-11(bulk), (b) 66-1,pl-89, 63-2, pl-13,(c)117- 18,pl-17 and 117-18,pl-22, (d) 77-50,pl-8 and 77-50, pl-15



4.16 (a)Wmc 349, 4A ;(b) Wmc 809, 7A; (c) Cfd 69, 7D; (d) A, gdm33, 1A ; (1) CS(Ph^l) ;(2) WL-711;(3) UP-8; (4)*Ae.kot* 3790; (5) *Ae.kot* 396; (6)*Ae. Pereg* 3519; (7) *Ae. Pereg* 13772; (8) *Ae. long* 28; (9) *Ae. long* 3770

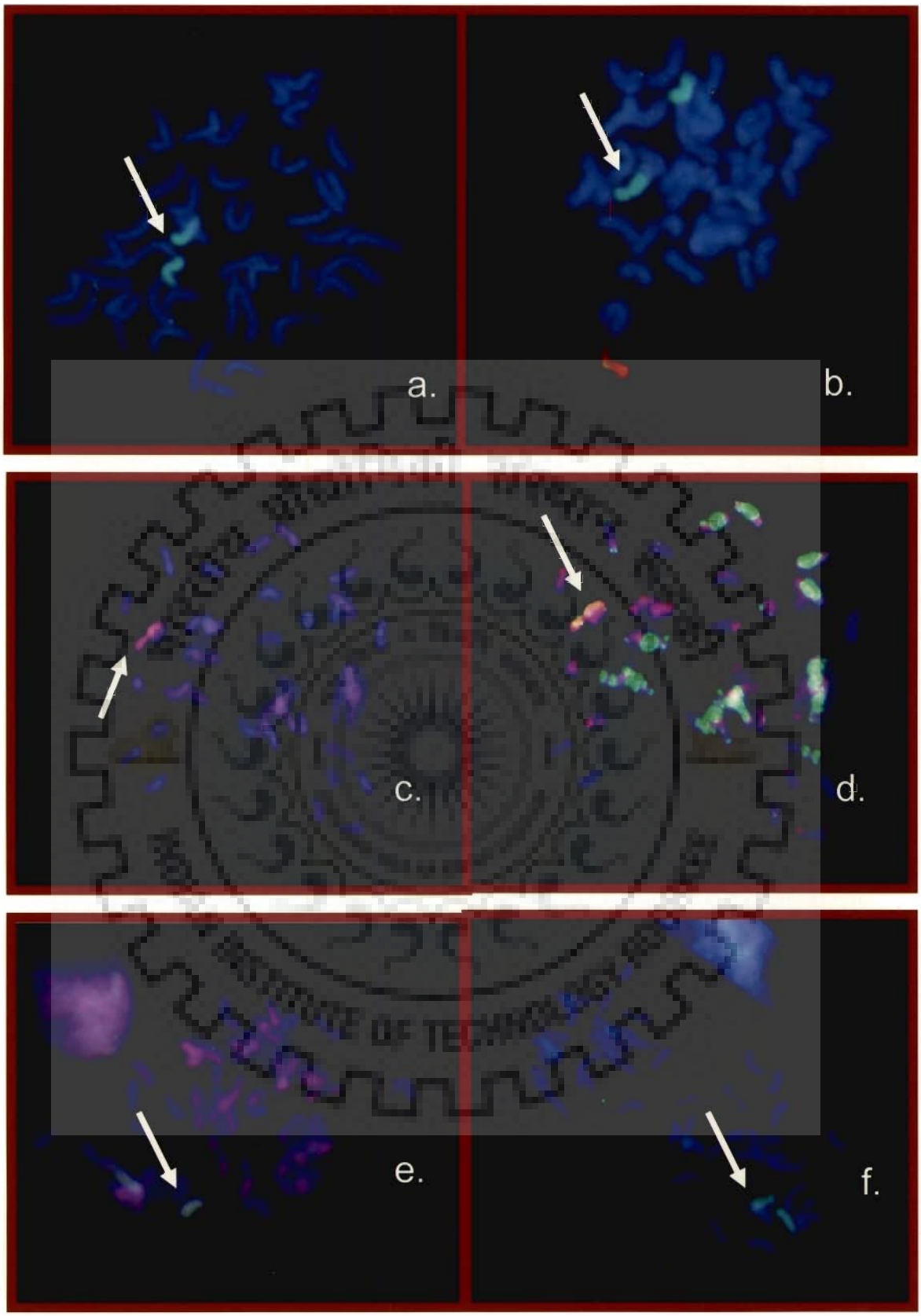


Figure 4.15. *in-situ* hybridization of few selected derivatives showing introgression of *Ae. kotschyi* (a) and (b); plant 63-2-13, (c) and (d); derivative 117-18-17. (e) and (f); derivative 77-50-15

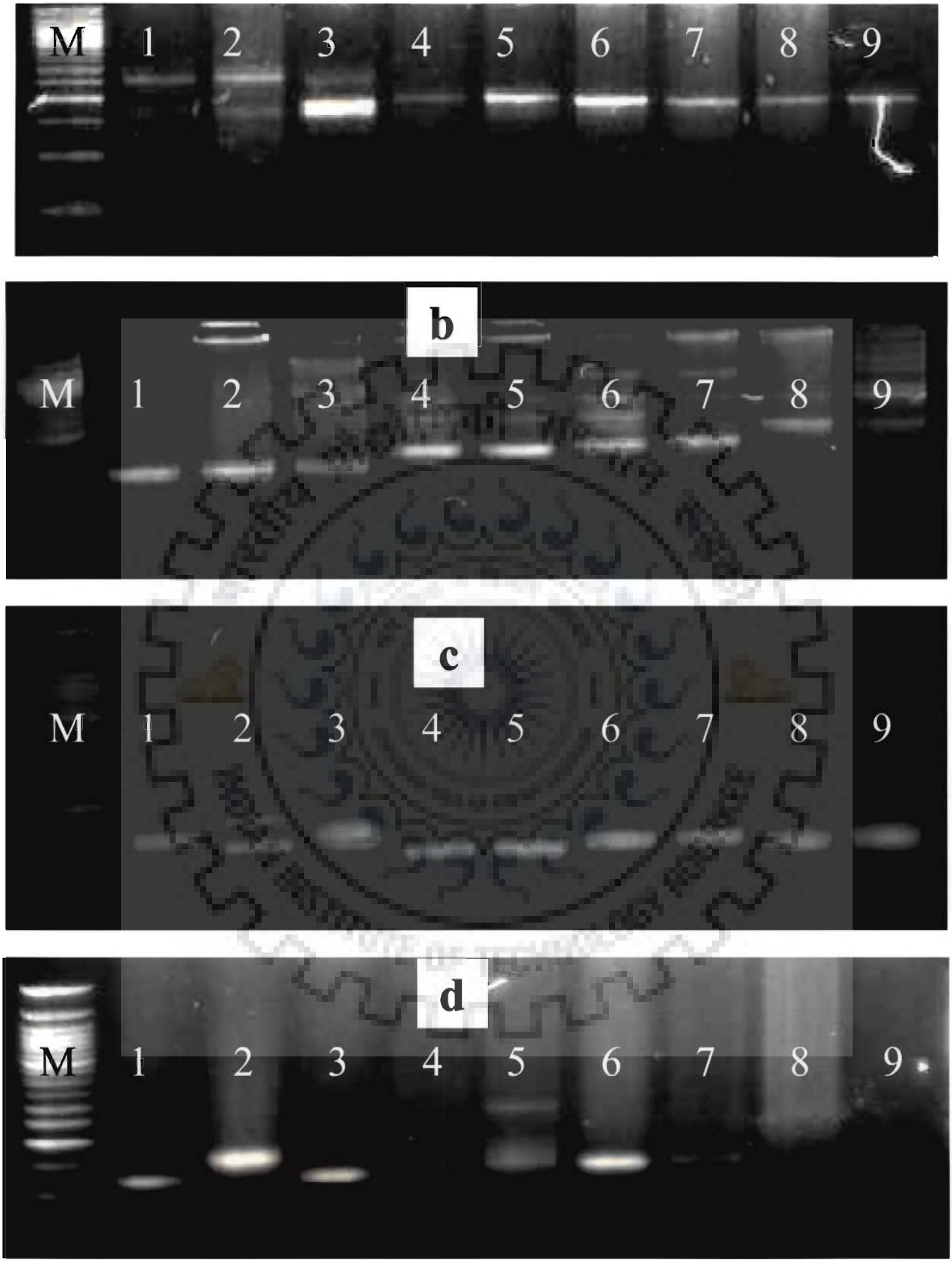


Fig 4.17 (a) 2 D cfd 56; (b) Gdm 148, 2D; (c) WmC 169, 3 A; (d) Wmc 533, 3D; (1) CS(*Ph*^{l}) ;(2) WL-711;(3) UP-233
(4)*Ae.kot* 3790; (5) *Ae.kot* 396; (6)*Ae. Pereg* 3519; (7) *Ae. Pereg* 13772; (8) *Ae. long* 28; (9) *Ae. long* 3770

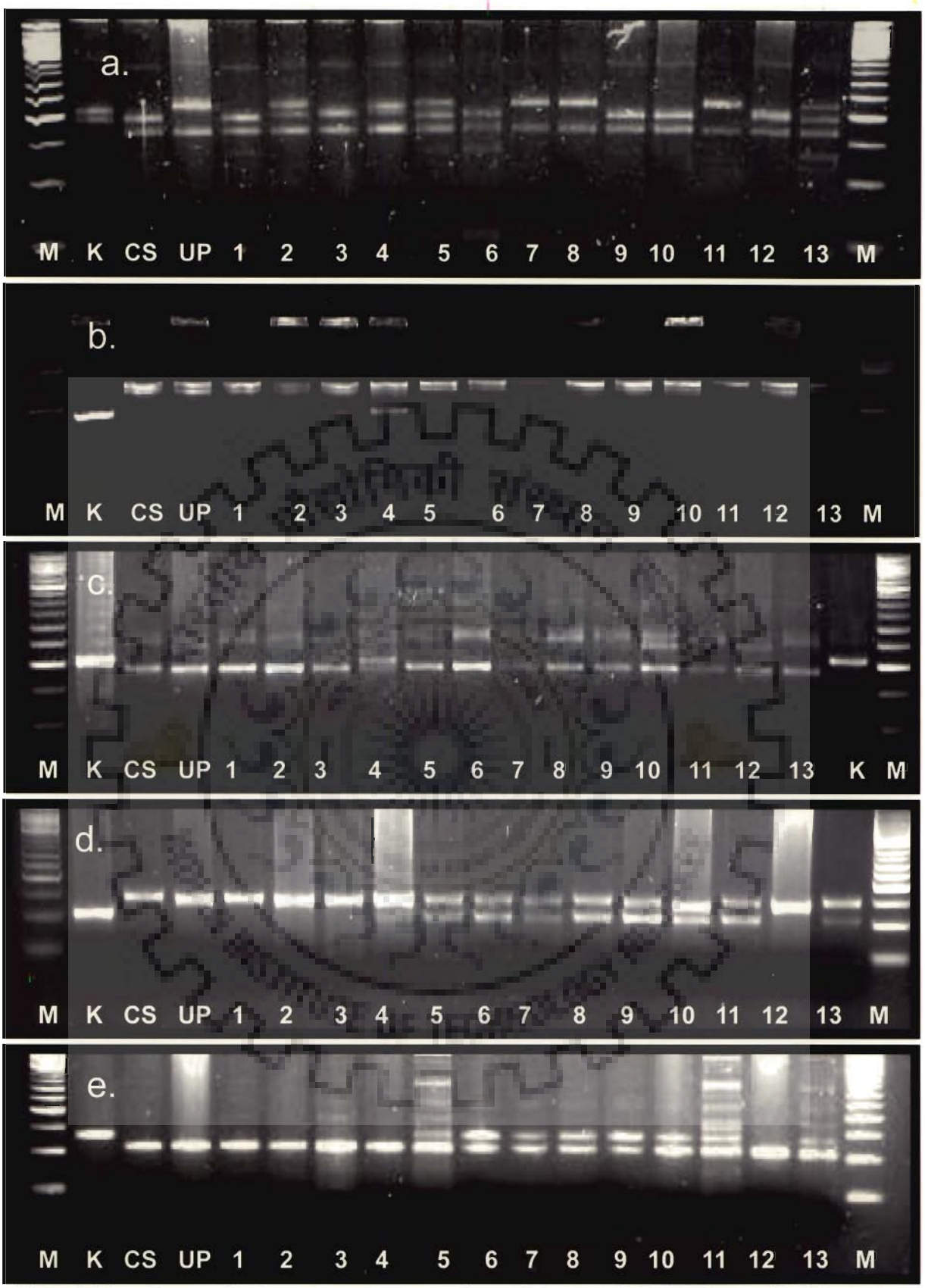


Fig. 4.18. Group 2 chromosome markers (a) wmc25, (b) barc 11, (c) wmc 601 (2DS) (d) gwm 539 and (e) gdm148 (2DL) on *Ae. kotschyi*, CS(*PhI*), UP2338, 1. 58-5 Pl-12, 2. 58-5 Pl-33, 3. 58-11 (Bulk), 4. 66-1 Pl-89, 5-77-33 Pl-2, 6. 77-36 Pl-6 ,7. 77-50 Pl-8, 8. 77-50 Pl-15, 9. 77-46 Pl-3, 10. 117-18 Pl-17, 11. 117-18 PL-22, 12. 63-2 Pl-13, 13. 77-23 Pl-1.

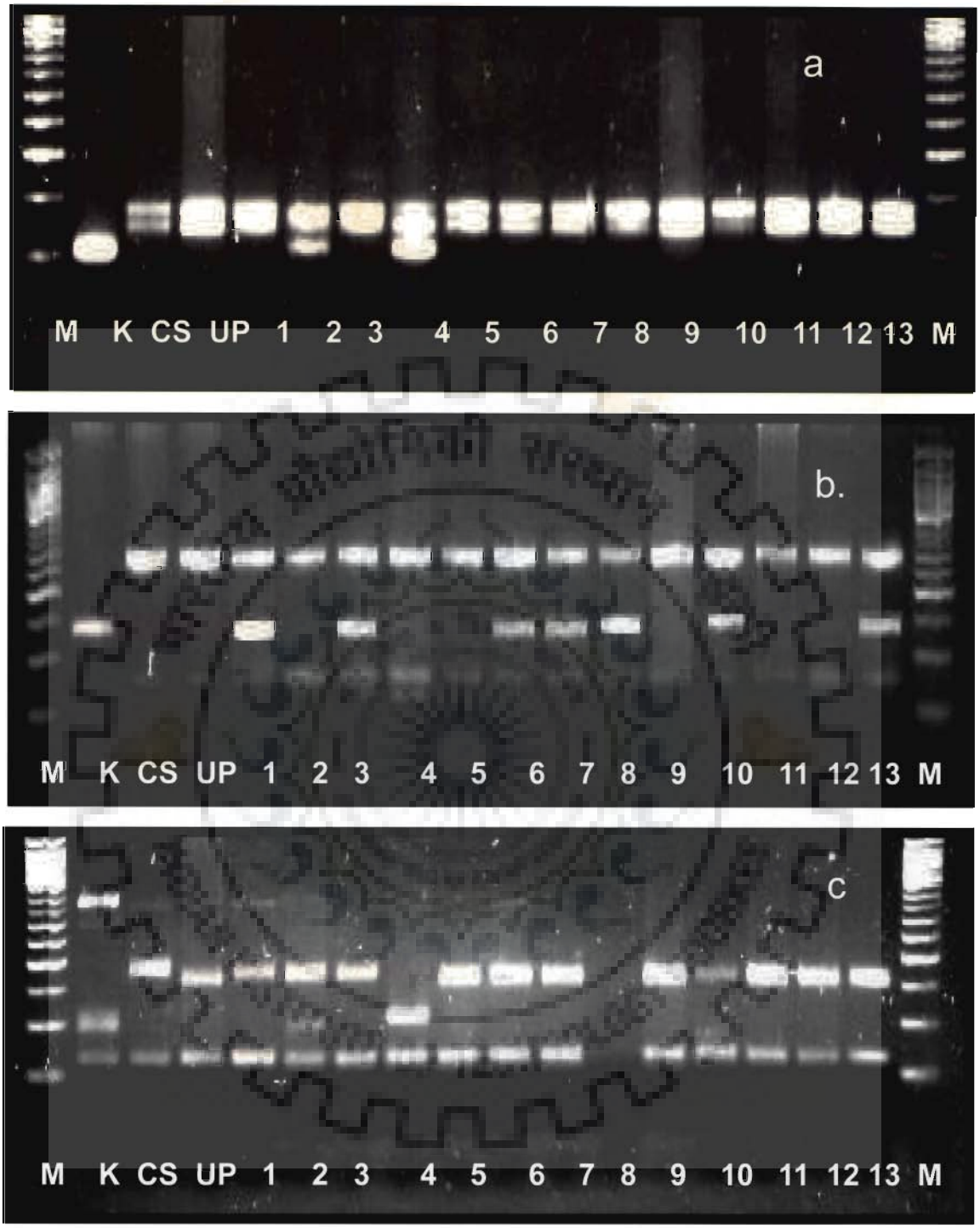


Fig 4.19. Group 7 chromosome markers (a) wmc488 (b) cfd 41 and (c) wmc809 on *Ae. kotschyi*, CS(*PhI*), UP2338, 1. 58-5 Pl-12, 2. 58-11 (Bulk), 3. 58-5 Pl-33, 4. 66-1 Pl-89, 5-77-33 Pl-2, 6. 77-36 Pl-6 ,7. 77-50 Pl-8, 8. 77-50 Pl-15, 9. 77-46 Pl-3, 10. 117-18 Pl-17, 11. 117-18 PL-22, 12. 63-2 Pl-13, 13. 77-23 Pl-1.

Table 4.10. Details of alien chromosome introgressed in selected BC_2F_2 and BC_1F_3 derivatives (yellow for group 2 and green for group 7

Group 2 chromosomes

I.D. No.	1AS	1AL	1BS	1BL	1DS	1DL	2AS	2AL	2BS	2BL	2DS	2DS	2DL	2D	2D	3AS	3AL	3BS	3BL	3DS	3DL	4AS
→ *SSR Markers used*	*gdm33*	*barc287*	*gwm403*	*Wmc500*	*cfd 61*	*wmc405*	*Cfd36*	*wmc63*	*barc318*	*wmc474*	*barc 11*	*Gwm539*	*Wmc25*	*wmc601*	*gdm148*	*barc12*	*wmc169*	*barc75*	*gwm340*	*cfd79*	*wmc552*	*barc206*
Ae. Kot.	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K
CS(*PhI*)	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
UP2338	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
58-5-12	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
58-5-33	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	W	W	W	W	W	W	W
58-11 (x)	W	W	W	W	W	W	W	W	W	W	K+W	W	W	W	W	W	W	W	W	W	W	W
66-1-89	W	W	W	W	W	K	W	W	W	W	K+W	K+W	W	K+W	K+W	W	W	W	W	W	W	W
77-33-2	W	W	W	W	W	W	W	W	W	W	W	K+W	K+W	W	K+W	W	W	W	W	W	W	W
77-36-6	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
77-50-8	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
77-50-15	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
77-46-3	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
117-18-17	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
117-18-22	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	K+W	W	W	W	W	W	W	W
63-2-13	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
77-23-1	W	W	W	W	W	W	W	W	W	W	W	K+W	K+W	W	K+W	W	W	W	W	W	W	W

Group 7 chromosome specific markers

I.D. No.	4AL	4BS	4BL	4DS	4DL	5AS	5AL	5BS	5BL	5DS	5DL	6AS	6AL	6BS	6BL	6DS	6DL	7AS	7AL	7BS	7BL	7DS	7DL
↑ *SSR Markers used*		*barc10*	*wmc349*	*barc225*	*wmc331*	*barc180*	*wmc415*	*cfd5*	*wmc386*	*gwm190*	*wmc630*	*wmc182*	*wmc446*	*gwm613*	*wmc486*	*cfd49*	*gdm98*	*Wmc809*	*wmc139*	*barc65*	*wmc396*	*Cfd41*	*wmc488*
Ae. kot.	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K
CS(*PhI*)	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
UP2338	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
58-5-12	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
58-5-33	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
58-11 (x)	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	W	W	K+W
66-1-89	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W	W	W	W	K+W
77-33-2	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
77-36-6	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
77-50-8	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
77-50-15	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
77-46-3	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
117-18-17	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
117-18-22	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
63-2-13	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W
77-23-1	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	K+W	W

Table 4.11 Effect of alien chromosome/chromosomes arm introgression on grain iron and zinc content

Derivatives ID	Group of introgressed alien chromosome	% increase in Iron	% increase in Zinc
58-5-12	7	67.6	75.7
58-5-33	2+7	71.6	80.5
58-11 (x)	7	118.2	107.1
66-1-89	2+7	117.7	145.5
77-33-2	2	74.7	75.4
77-36-6	2+7	96.6	126.2
77-50-8	2+7	80.0	119.2
77-50-15	2+7	102.1	148.0
77-46-3	2	111.6	114.8
117-18-17	2	74.6	132.8
117-18-22	2	84.0	133.3
63-2-13	7	89.8	93.0
77-23-1	2+7	81.8	77.8

Iron content of the selected derivatives showed 67-122% increase over the control and for zinc it ranged from 75.7-148% as compared to their wheat parents (Table 4.11). Out of these 13 selected derivatives 58-5-12 had minimum grain iron and zinc increase over control. Molecular marker data suggested that this plant had an introgression of short arm of group 7 chromosomes of *Ae. kotschyi.* However the highest increase for grain iron and zinc content was observed in derivatives 66-1-89 with 118% increase for iron and 146% increases for zinc content. On the basis of molecular data it was found that the derivative 66-1-89 had introgression of group 2 and group 7 chromosomes of *Ae. kotschyi.* Out of 13 derivatives only one had waxy on leaves and stems, a dominant

morphological trait of group 2 chromosomes. One the basis of waxy /non-waxy leaves and stem data it can be concluded that, with the exception of derivative 63-2-13 all the derivatives had introgression of either a complete group 2 chromosome(s) (2U/2S) or an arm introgression. Most of the plants had amber grains suggesting absence of group 3 *Aegilops* chromosomes and the same was supported by molecular markers. It was found that the derivatives with the introgression of either group 2 or group 7 of *Ae. kotschyi* had high grain iron and zinc content. However it was observed that the derivatives with introgression of group 2 and group 7 had very high grain iron and zinc content. On basis of morphological and molecular data it can be suggested that group 2 and group 7 of *Aegilops kotschyi* chromosomes had important gene(s) /QTL for high grain iron and zinc content.

Limited FISH and GISH analysis also confirmed the introgression of alien chromosomes of U/S genome in the selected derivatives.

4.2 Result:

Interspecific hybridization between *T. turgidum* ssp durum cv. 233, 274 and *Aegilops longissima* accessions 28, 3507, 3770 were made. The data on some plant morphological and spike characteristics of durum wheat cultivars and *Ae. longissima* parents, their F_1 hybrids and amphiploids is given in Table 4.12 and Fig. 4.20. The F_1 hybrids were intermediate between the parents for number of tillers per plant, plant height whereas they had spelta and glaucus heads with disarticulating spikes and hard threshing like that of *Ae. longissima* parents. All the *T. turgidum ssp. durum* x *Ae. longissima* F_1 hybrids were partially fertile (Table 4.13). The seeds set on the spelta heads of F_1 hybrids with disariculating spikes and hard threshing were bold and long. Chromosome pairing in the pollen mother cells (PMCs) of durum cultivars- *Ae. longissima* F_1 hybrids and putative amphiploids is given in Table 4.13, Fig. 4.21 and Fig. 4.22. The chromosome pairing in the F_1 hybrids (Table 4.13) range from 0-6 II (rod), 0-1 III and 9-21 I. The two F_1 hybrids with *T. turgidum* ssp. *durum* cv. PDW274 with two different *Ae. longissima* accessions 28 and 3770 gave low chromosome pairing (Fig. 4.21 a, b, e, f) and higher selfed seed set per spike (10-12 seeds/spike) whereas the F_1 PDW233/ *Ae. longissima* 28 with higher chromosome pairing and a trivalent (Fig. 4.21 c, d) had lower seed set rate (4.2 seeds/ spike).

Table 4.12. Morphological characteristics of *T. turidum, Ae. longissima*, their F1 hybrids and putative amphiploids

Plant material	No. of tillers	Plant height (cm)	Head type	Spike disarticulation	Threshability	1000 grain weight (g)
Triticum turgidum cv. PDW274	8-10	100	Waxy, Square	Tough	Free	42.6 ± 3.2
cv. PDW233	8-10	100	Waxy, Square	Tough	Free	40.3 ± 4.5
cv. PBW34	8-10	100	Waxy, Square	Brittle	Free	41.8 ± 3.8
Ae. longissima acc. 28	250-300	35	Nonwaxy, Spelta	Brittle	Hard	5.4 ± 1.8
Ae. longissima acc. 3770	250-300	33	Nonwaxy, Spelta	Brittle	Hard	5.2 ± 2.1
Ae. longissima acc. 3507	250-300	30	Nonwaxy, Spelta	Brittle	Hard	7.9 ± 1.2
F_1 PDW274/*Ae. longissima* 28	70-85	115	Nonwaxy, Spelta	Brittle	Hard	53.3 ± 2.6
F_1 PDW233/*Ae. longissima* 28	75-80	118	Nonwaxy, Spelta	Brittle	Hard	43.7 ± 1.7
F_1 PDW274/*Ae. longissima* 3770	75-80	120	Nonwaxy, Spelta	Brittle	Hard	44.5 ± 2.5
F_1 PDW233/*Ae. longissima* 3507	70-80	115	Nonwaxy, Spelta	Brittle	Hard	47.8 ± 1.9
Amph. PDW274-*Ae. longissima* 28	8-10	100	Nonwaxy, Spelta	Brittle	Hard	46.6 ± 2.8
Amph. PDW233-*Ae. longissima* 28	8-10	100	Nonwaxy, Spelta	Brittle	Hard	47.3 ± 2.3
Amph. PDW274-*Ae. longissima* 3770	10-12	98	Nonwaxy, Spelta	Brittle	Hard	48.0 ± 2.9
Amph. PDW233-*Ae. longissima* 3507	10-12	96	Nonwaxy, Spelta	Brittle	Hard	46.9 ± 2.3

Fig. 4.20. Spike and seed morphology of *T. turgidum* ssp. *durum*- *Ae. longissima* amphiploids as compared to the *T. turgidum* ssp. *durum* and *Ae. longissima*. a- PDW 274 (left), PDW233 (right), b- Amph. PDW274-*Ae. longissima* 28, c- Amph. PDW274-*Ae. longissima* 3770, d- Amph. PDW233-*Ae. longissima* 28, e- Amph. PDW233-*Ae. longissima* 3507, f- *Ae. longissima* 28 (left), *Ae. longissima* 3507 (right)

Table 4.13. Chromosomal pairing and seed set in F_1 hybrids and amphiploid plants

Cross/ Amphiploid	*No. of PMCs studied*	*No. of chromosomes*	*Univalent*	*Bivalent*	*Trivalent*	*Average Seed set per spike (± SE)*
			Mean ± S.D. (Range)	Mean ± S.D. (Range)	Mean ± S.D. (Range)	
F_1 PDW274/ *Ae longissima* 28	25	21	14.6 ± 2.7 (11-21)	3.2 ± 1.4 (0-5)	-	9.4 ± 3.4
F_1 PDW233/ *Ae longissima* 28	25	21	12.0 ± 2.5 (9-21)	4.2 ± 1.2 (0-6)	0.2 ± 0.1 (0-1)	4.2 ± 1.2
F_1 PDW274/ *Ae longissima* 3770	25	21	16.2 ± 1.5 (11-21)	2.4 ± 1.9 (0-5)	-	12.3 ± 1.5
F_1 PDW 233/ Ae. *longissima* 3507	25	21	15.4 ± 2.3 (11-21)	2.8 ± 1.6 (0-6)	-	11.5 ± 2.3
Amph. PDW233-*Ae. longissima* 28 Pl #15	25	42	3.2 ± 0.8 (0-6)	19.4 ± 0.4 (19-21)	-	22.7 ± 0.9
Amph. PDW274-*Ae. longissima* 28 Pl #35	25	42	2.8 ± 0.4 (0-4)	19.6 ± 1.7 (16-21)	-	26.2 ± 1.8
Amph. PDW274-*Ae. longissima* 3770 Pl #20	25	41	2.4 ± 1.2 (0-6)	18.4 ± 2.4 (18-21)	0.6 ± 0.1 (0-1)	38.2 ± 2.4
Amph. PDW233-*Ae. longissima* 3507 Pl #19	25	42	2.8 ± 0.8 (0-4)	19.6 ± 0.9 (18-21)	-	26.8 ± 2.8

Table 4.14. Grain iron and zinc content of PDW233, *Ae. longissima*, F_2 seeds and amphiploids

Plant material	Average grain micronutrient (mg/kg) ± SD	
	Iron	Zinc
T. durum PDW233	22.1 ± 1.0	21.7 ± 0.4
Ae. longissima 28	49.6 ± 1.1	44.7 ± 0.2
Ae. longissima 3770	45.5 ± 0.3	43.3 ± 0.1
Ae. longissima 3507	47.0 ± 0.4	44.2 ± 0.4
F_2 Seed		
PDW274/ *Ae. longissima* 28	56.1 ± 0.1	51.2 ± 0.3
PDW233/ *Ae. longissima* 28	49.7 ± 0.8	52.1 ± 0.8
PDW274/ *Ae. longissima* 3770	45.4 ± 0.4	56.2 ± 0.9
PBW233/ *Ae. longissima* 3507	47.8 ± 0.5	45.8 ± 0.6
Amphiploid seed		
Amph. PDW233-*Ae. longissima* 28	50.5 ± 0.5	45.6 ± 0.4
Amph. PDW274-*Ae. longissima* 28	55.9 ± 1.2	50.4 ± 0.2
Amph. PDW274-*Ae. longissima* 3770	52.2 ± 0.7	49.8 ± 0.2
Amph. PDW233-*Ae. longissima* 3507	54.2 ± 1.5	50.1 ± 0.3

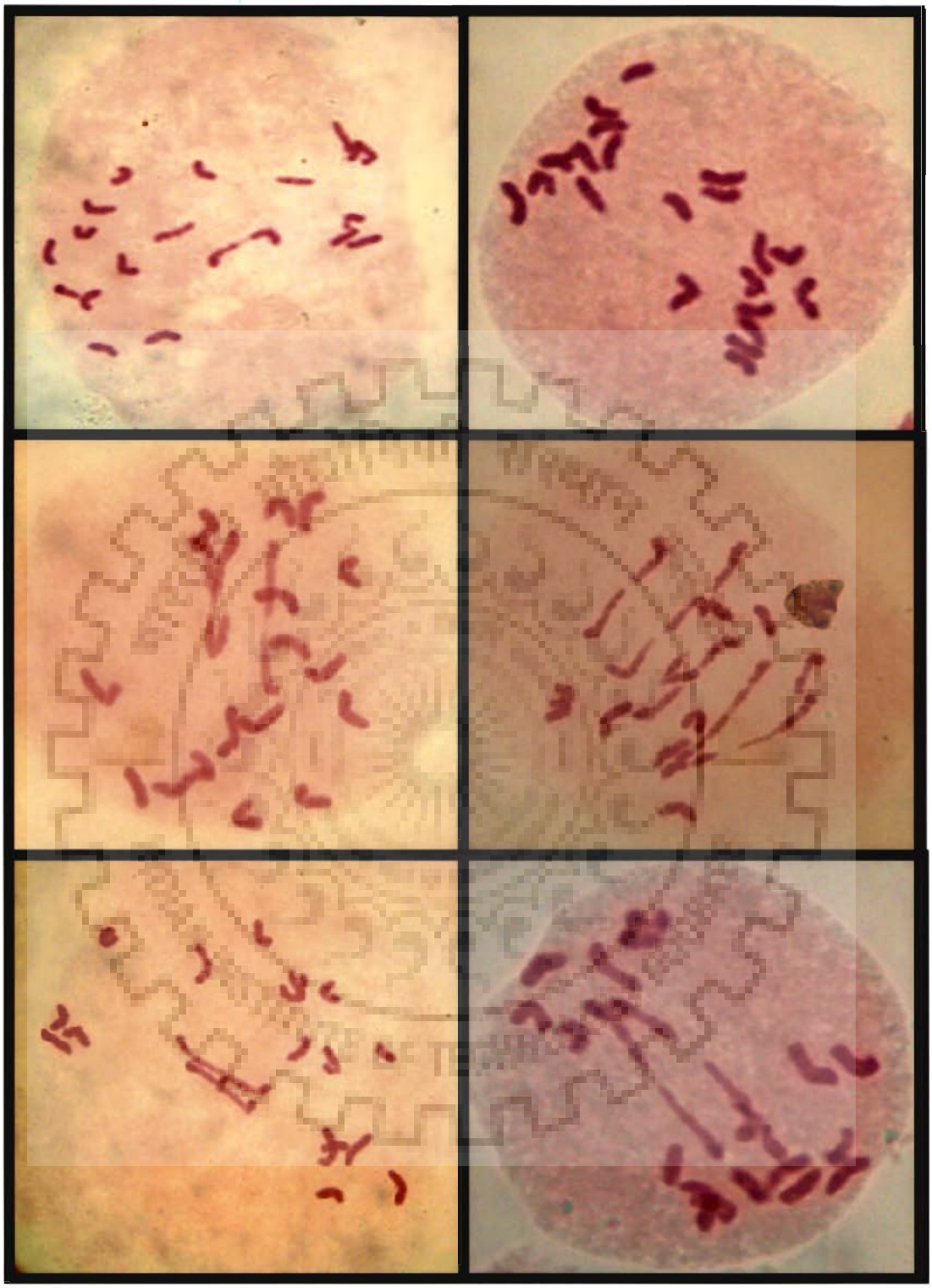


Fig. 4.21. Chromosome pairing at metaphase-I PMCs in *T. durum/ Ae. longissima* F_1 hybrids a. *T. durum* PDW274/ *Ae. longissima* 28 hybrid 1II + 19I, b. 21I, c. F1 PDW233/*Ae..longissima* 28 1III+ 1II + 16I, d. 6II + 9I and e. F1 PDW274/*Ae..longissima* 3770 2 II+17 I, f. 2 II+17 I.

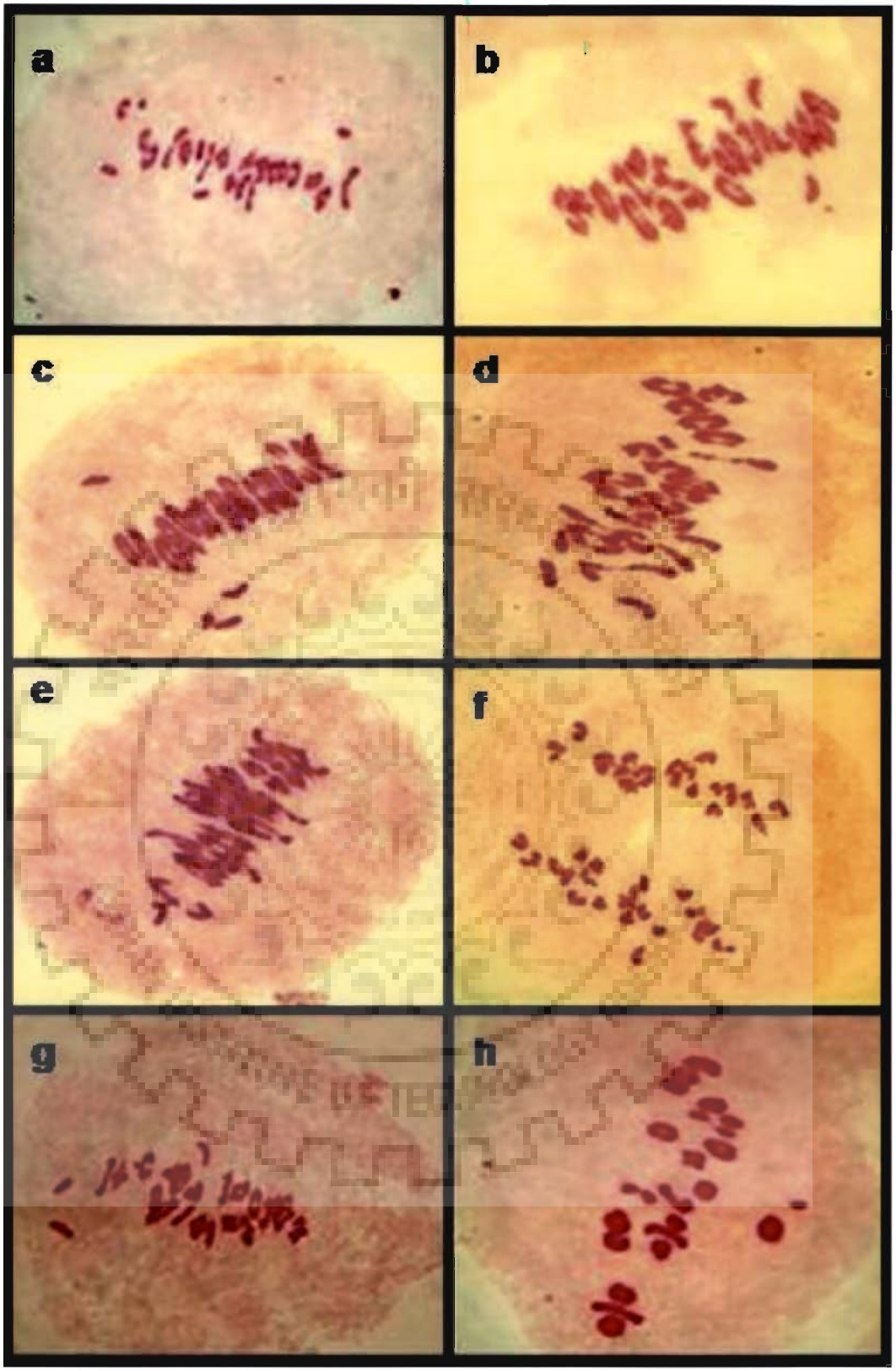


Fig. 4.22. Chromosome pairing at metaphase-I PMCs in *T. turgidum* ssp. *durum-Ae. longissima* amphiploids a. PDW233-*Ae.longissima* 28 19 II + 4 I, b. 21 II, c. PDW274-*Ae. longissima* 28 19 II + 4 I, d. 20 II + 2 I, e. PDW274-*Ae. longissima* 3770 20 II +1 I, f. 20 II +1 I, g. PDW233-*Ae. longissima* 3507 20 II + 2 I, h. 21 II

The second generation amphiploids with 40-42 chromosomes, however, were stable with high frequency of ring bivalents, no multivalent and nearly normal distribution of homologous chromosomes to poles (Fig. 4.12) and limited laggards or movement of univalents to metaphase I plate like that in the F_1 hybrids. Higher seed set in subsequent generations of putative amphiploids is attributed to normal chromosome pairing and regular meiosis whereas in the F_1 hybrids the seed set was dependent on the frequency of unreduced male and female gametes. However, in the *T. durum/ Ae. longissima* hybrids, we found that both the mechanisms operate simultaneously and contribute to the formation of unreduced viable gametes. In PMCs undergoing SDM, the paired chromosomes disjoin and move to different poles during a relatively prolonged metaphase (Fig. 4.23a) while all the univalents move to metaphase-I plate with visible sister chromatids and get subsequently equationally separated to two poles (Fig. 4.23b-d) with unreduced chromosome number and only dyads leading to fertile gametes (Fig. 4.23e). First division meiotic restitution was also observed in several PMCs where all the 21 univalent chromosomes align at the metaphase equatorial plate but fail to divide and form a restitution interphase nucleus. Metaphase-II would ultimately divide them equationally into dyads. The low fertility in the F_1 hybrids (Table 4.13, Fig. 4.21c) with higher chromosome pairing is due to failure of meiotic restitution and formation of unreduced gametes. The paired chromosomes undergo reduction division and move to two poles during metaphase-I to telophase-I, whereas the univalents come to the same metaphase plate I and their chromatids get divided equationally leading to duplications and deficiencies of several chromosomes in the dyads especially the paired chromosomes. The univalents of paired chromosomes disjoining at metaphase-I with

intact sister chromatids lead to meiosis-II for their equational division while the sister chromatids already separated during metaphase-I either move randomly to different poles or experience centromeric breakage and fusion leading to triads, tetrads with mini and micro nuclei and very low frequency of unreduced gametes (Fig. 4.23f). The grain iron and zinc content (mg/kg) in parents, F_1 hybrids and putative amphiploids is given in Table 4.14. *Ae. longissima* accessions used in the present study have up to twice as much higher grain iron and zinc content as that of the durum wheat cultivars.

The F_2 seeds (putative amphiploids) despite being bolder than both the parents had higher micronutrient content (Table 4.12, Fig. 4.20) suggesting that high iron and zinc content of seeds of *Ae. longissima* is not due to their smaller size and hence larger surface area but because of evidently its better genetic system(s) for higher uptake, translocation and sequestration into seeds which could be transferred to durum and bread wheat. All the amphiploids had grain iron and zinc content as high as *Ae. longissima* parent and nearly twice as high as the durum wheat cultivar PDW233. Calderini and Monasterio (2003) reported that the lower seed set in *T. durum-Ae. tauschii* was responsible for higher concentration of micronutrients in their seeds. The fertility of all the *T. turgidum* - *Ae. longissima* amphiploids was quite high (Table 4.13), their high iron and zinc content could be attributed to the better genetic system(s) of

Ae. longissima controlling micronutrient content. Grain ash and the ash iron and zinc content of *Ae. longissima*, durum wheat cultivar and the amphiploids (Table 4.15) showed that *Ae. longissima* not only has higher ash content but also higher Fe and Zn content in ash as compared to

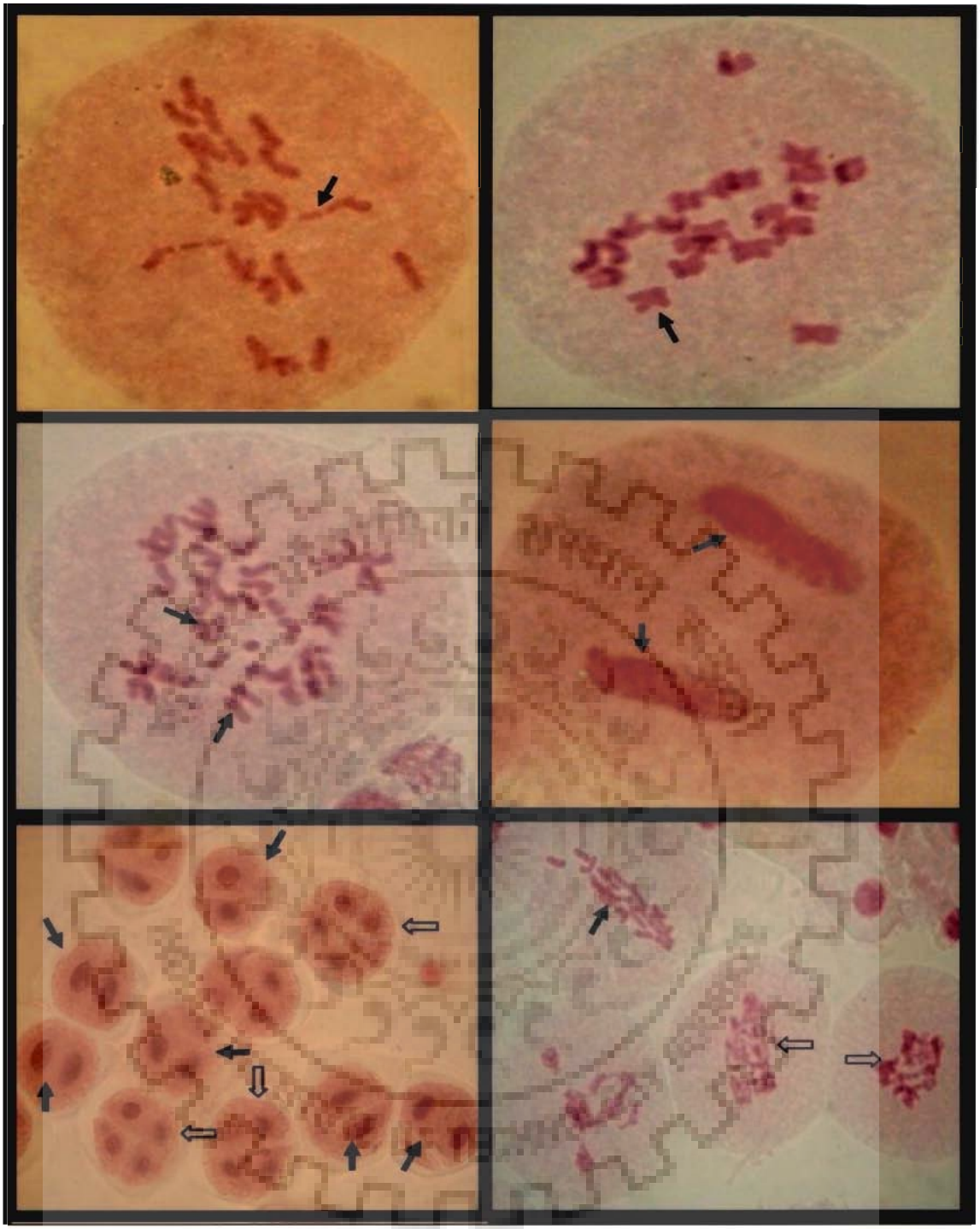


Fig. 4.23. Unreduced gamete formation in PMCs of F1 *T. durum* PDW 274/ *Ae. longissima* 3770 a. Early metaphase-I, 1 II (arrow) + 19 I (coming to metaphase plate), b. Late metaphase-I 2I on poles and 19 I with visible sister chromatids (arrow) on metaphase-I plate, c. Late metaphase-I, 2 I at each pole and 17 I equationally dividing at metaphase I (arrows), d. Telophase I with unreduced gametes (arrows), e. Dyads with unreduced gametes (arrows), tetrads and pentads with mini and micronuclei (open arrows), f. First division restitution univalent chromosomes aligned at the metaphase-I plate (arrow) and proceeding to interphase (open arrows).

Fig. 4.24 GISH study of *T. durum* / *Ae. longissima* amphiploids. *Ae. longissima* chromosomes are in pink colour.

Table 4.15. Grain ash and grain ash iron and zinc content of *T. turgidum* ssp. *durum* cultivar, *Ae. longissima* and *T. turgidum* ssp. *durum*- *Ae. longissima* amphiploids.

S. No.	Grain Material	Ash %	Fe (µg/g) of ash	% Increase in ash Fe content over PDW 233	Zn (µg/g) of ash	% Increase in ash Zn content over PDW 233
1	PDW 233	1.63	1396.32	-	1287.06	-
2	*Ae. longissima* 28	2.25	2679.11	91.9	2261.95	75.7
3	Amph. PDW233-*Ae. longissima* 28	1.98	2305.05	65.1	2091.36	62.5
4	Amph. PDW274-*Ae. longissima* 28	1.89	2030.69	45.4	2103.96	63.5
5	Amph. PDW274-*Ae. longissima* 3770	2.01	1989.71	42. 5	2350.16	82.6
6	Amph. PDW233-*Ae. longissima* 3507	1.94	2385.57	70.5	2184.46	69.7

the durum wheat cultivar. The amphiploids with grains bolder than the durum cultivars still had higher ash content and 42 -70% higher ash iron and 60-80% higher zinc content indicating that *Ae. longissima* possesses superior genetic system for micronutrient content irrespective of smaller seed size. Thus the 'S^l' genome amphiploids ($AABBS^lS^l$) can be used for biofortification of elite bread wheat cultivars with high micronutrient content

4.3 Mapping of QTL for high grain iron and zinc content in diploid wheat RIL population

4.3.1 Grain Fe and Zn content

Grain iron and zinc analysis of *T. monococcum* and *T. boeoticum* 5088, the parents of Recombinent Inbred Lines were performed in 4 replications using Atomic Absorption Spectrophotometer (AAS). Micronutrient analysis of the parental lines showed that *Tb5088* had relatively higher grain Fe and Zn concentration than *Tm14087* (Table 4.16). For *T5088*, grain Fe and Zn concentration, averaged over the four environments, were 40.1 and 44.6 mg/kg, respectively whereas *T. monococcum* had an average grain Fe and Zn concentration of 23.8 and 29.2 mg/kg. A wide range of variation was observed in the RIL population at both the locations for both the years with transgressive segregants in both directions for both Fe and Zn (Table 4.16, Fig. 4.25). The population showed continuous distribution but was skewed towards lower levels of micronutrients (Fig. 4.25), thereby indicating involvement of relatively less number of partially recessive genes governing accumulation of Fe and Zn in the grains.

4.3.2 Correlation analysis

To study the effect of the environment over grain Fe and Zn content in *T. boeoticum /T. monococcum* RIL population. Pearson's correlation coefficient was determined for all the data sets. Correlation analysis showed that the grain Fe content of all the four locations was highly consistent with correlation coefficient (r) ranging from 0.87-0.96 (Table 4.17). It was true for grain Zn content with correlation coefficient ranging from 0.82-0.97.

Table 4.16. Grain Fe and Zn content (mg/kg) of the parental accessions and *T. boeoticum*/*T. monococcum* RIL population in two environments over two years 2005 and 2006.

Environment	Parents		RILs (Range)	Mean	SE
	Tb5088	*Tm14087*			
Fe (mg/kg)					
IITR2005	39.6	22.5	17.5-70.9	30.3	1.2
IITR2006	37.8	24.5	16.3-69.2	30.0	1.2
PAU2005	38.9	23.6	16.6-69.0	33.3	1.2
PAU2006	44.1	24.6	13.1-69.4	32.9	1.3
Average	40.1	23.8	-	-	-
Zn (mg/kg)					
IITR2005	43.8	29.7	17.1-64.3	31.9	1.2
IITR2006	48.4	31.4	18.8-63.5	31.9	1.2
PAU2005	41.9	28.6	18.0-69.0	33.1	1.2
PAU2006	44.6	27.2	16.8-60.0	32.2	1.2
Average	44.6	29.2	-	-	-
100GW (g)					
PAU2005	0.64	1.08	0.30-2.16	1.1	-
PAU2006	1.16	1.32	0.45-2.41	1.5	-

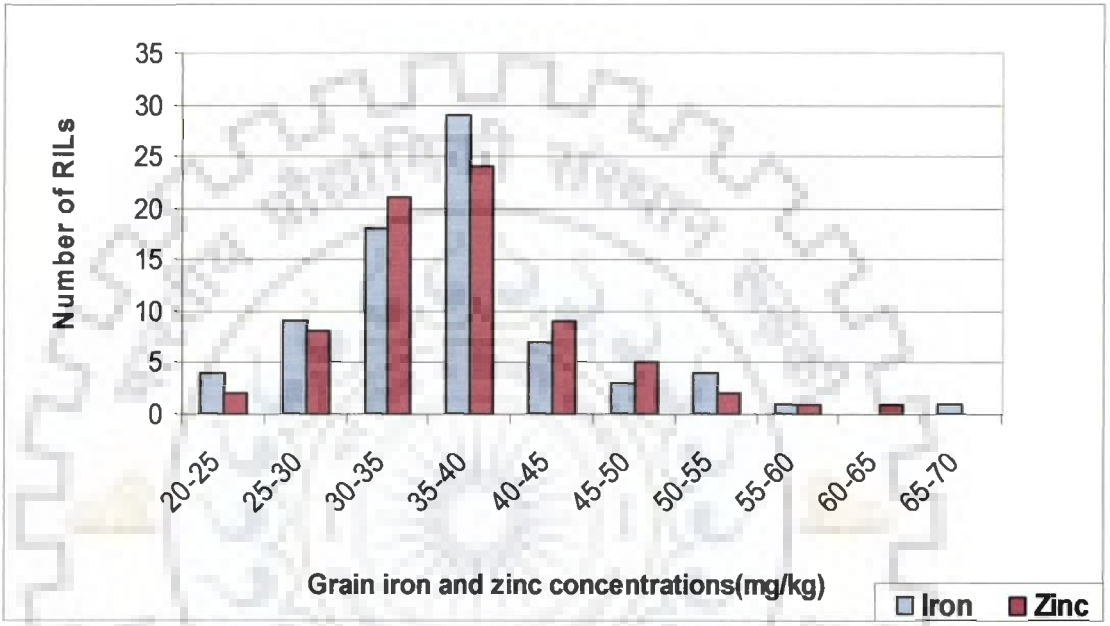


Figure 4.25. Distribution of *T. boeoticum*/*T. monococcum* RIL population for grain Fe and Zn concentration based on pooled data of four environments, PAU2005, PAU2006, IIT2005, IIT2006. Average grain Fe concentrations (mg/kg) in *Tb5088* and *Tm14087* were 40.2 and 23.8 respectively and the average grain Zn concentration (mg/kg) was 44.5 for *Tb5088* and 29.1 for *Tm14087*.

However, no significant correlation was observed between Fe and Zn content of the grains in this population indicating that grain Fe and Zn accumulation in grains may be controlled by different loci (Table 4.17).

Table 4.17. Correlation of Grain Fe and Zn in the *T. boeoticum/T. monococcum* RIL population

	ZnIIT2005	ZnIIT2006	ZnPAU2005	ZnPAU2006	FeIIT2005	FeIIT2006	FePAU2005	FePAU2006	GW2005
ZnIIT2005	1								
ZnIIT2006	0.97**	1							
ZnPAU2005	0.93**	0.88**	1						
ZnPAU2006	0.93**	0.92**	0.82**	1					
FeIIT2005	0.13	0.15	0.09	0.15	1				
FeIIT2006	0.17	0.18	0.15	0.19	0.93**	1			
FePAU2005	0.08	0.11	0.06	0.11	0.96**	0.87**	1		
FePAU2006	0.13	0.14	0.10	0.15	0.91**	0.91**	0.9**	1	
GW2005	0.03	0.04	0.00	0.09	0.15	0.10	0.14	0.12	1
GW2006	0.14	0.10	0.13	0.10	0.04	0.02	0.04	0.05	0.63

*, ** Significant at $P<0.05$ and $P<0.01$, respectively.

Micronutrients in the grains are concentrated in the aleurone layer and diploid primitive and wild wheats have a smaller grain size and there is a concern that any higher micronutrient content could be the result of a concentration effect rather than a real high micronutrient density. Correlations coefficients for 100-GW and grain Fe and Zn content were found to be non significant with 'r' ranging from 0.0-0.15 indicating that no significant correlation existed between 100GW and Fe and Zn concentrations in the grains of *T. boeoticum/T. monococcum* population.

4.3.3 QTL analysis for grain Fe and Zn concentration

A framework linkage map, based on 169 SSR and RFLP loci (Singh et al. 2007), was used for mapping the grain Fe and Zn QTL in a set of 93 RILs (Table 4.18).

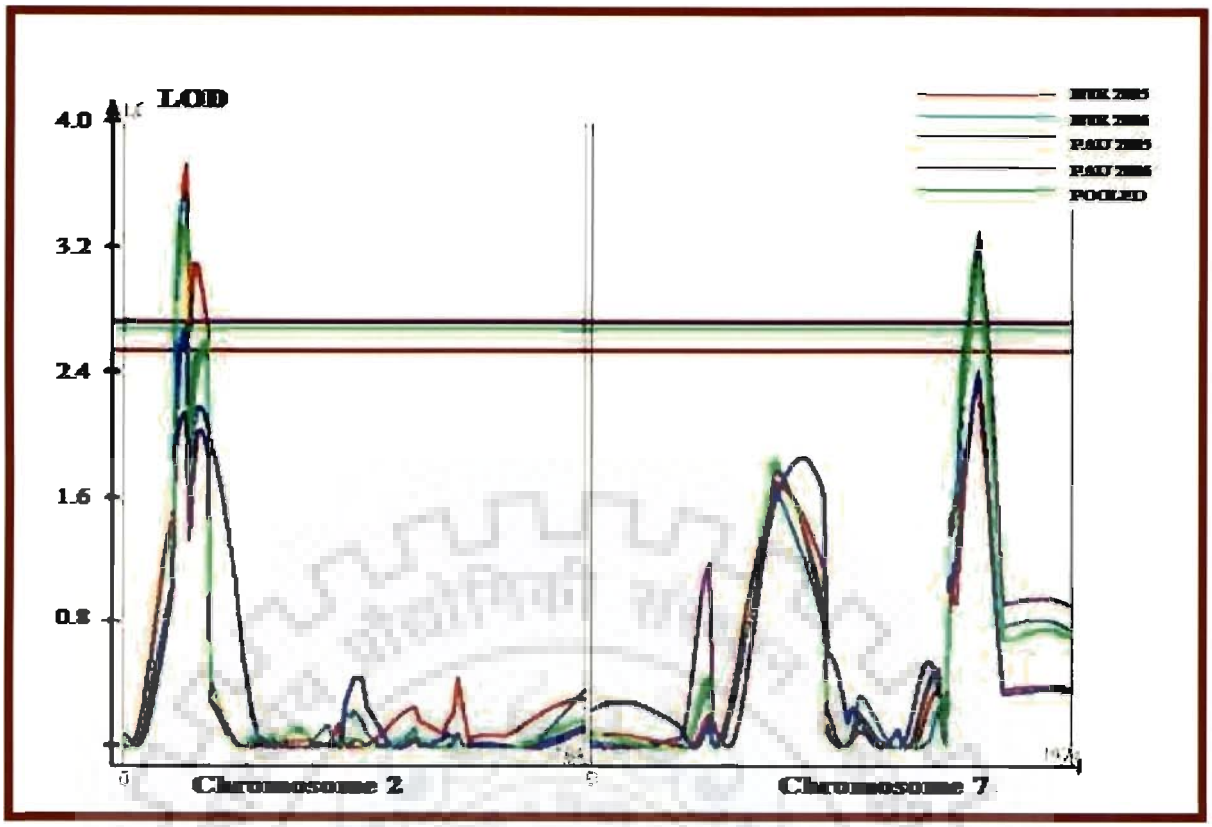


Figure 4.25. Composite interval mapping for Fe concentration in the grains in the RIL population based on environments IIT2005, IIT2006, PAU2005, PAU2006 and pool data.

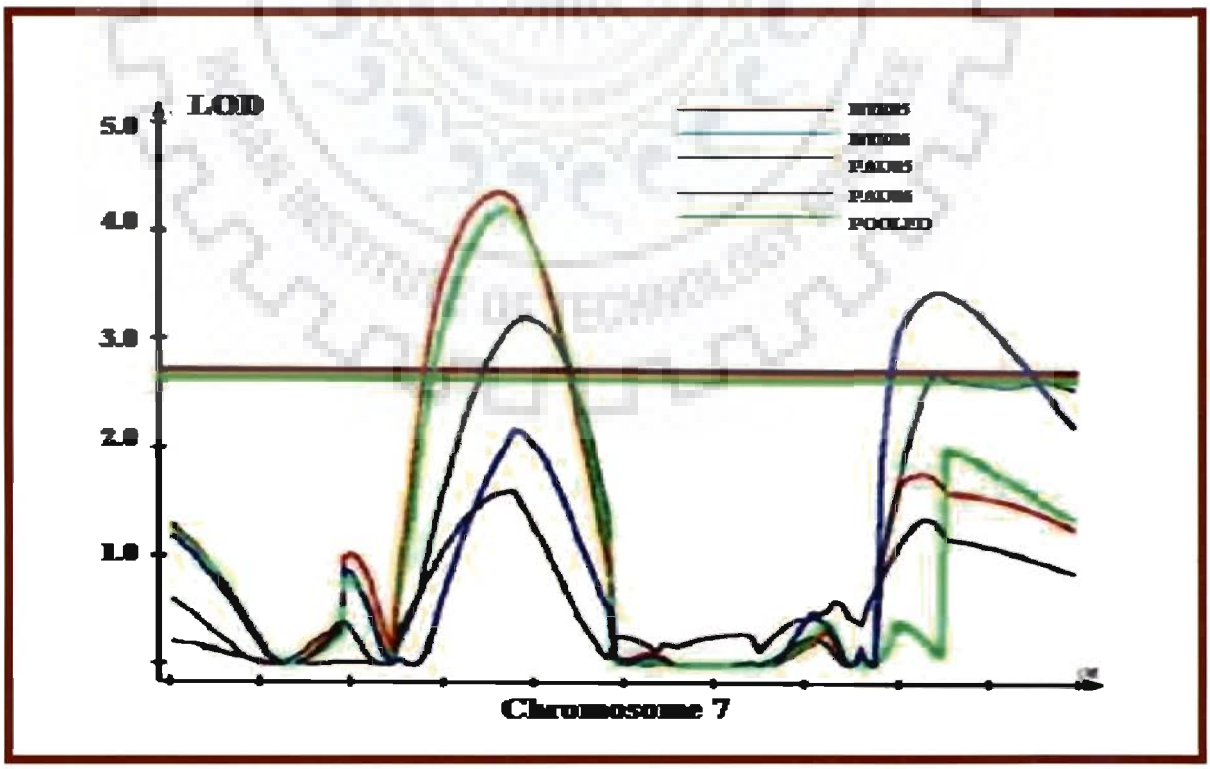


Figure 4.26. Composite interval mapping for Zn concentration in the grains in the RIL population based on environments IIT2005, IIT2006, PAU2005, PAU2006 and pool data.

Table 4.18. Summary of the QTLs for grain Fe and Zn In the *T. boeoticum/T. monococcum* population detected using composite Interval mapping

Chromosome	Marker interval	Position (CM)	IIT2005		IIT2006		PAU2005		PAU2006		Pool	
			LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
QTL for grain Fe												
2	Xwmc382 -Xbarc124	23.6	3.7	14.3	3.6	13.8	2.7	10.0	2.14	8.0	3.34	12.6
7	Xgwm473 -Xbarc29	153.8	2.3	8.0	3.3	12.6	2.40	10.0	3.3	11.7	3.2	11.7
7	Xcfd31-Xcfa2049	72.6	1.7	6.0	1.7	5.0	1.9	10.0	1.8	6.0	1.9	7.0
Threshold LOD values			2.6		2.7		2.7		2.7		2.7	
QTL for grain Zn												
7	Xcfd31-Xcfa2049	72.6	4.4	21.1	3.2	14.4	2.2	8.1	1.6	6.0	4.2	18.8
7	Xgwm473 -Xbarc29	153.8	1.8	7.0	2.7	11.8	3.5	14.7	1.4	6.0	2.0	9.0
Threshold LOD values			2.7		2.7		2.7		2.7		2.6	

The data for the individual environments IIT2005, IIT2006, PAU2005 and PAU2006 and the average of all the four environments was used for detection and mapping the QTLs controlling grain Fe and Zn concentrations. For grain Fe concentration, two significant and one suggestive QTL were detected (Table 4.18 and Fig. 4.26) whereas for grain Zn concentration one significant and one suggestive QTL were detected (Table 4.18, Fig. 4.27). The two significant QTL map on chromosomes 2A and 7A in the marker intervals *Xwmc382-Xbarc124* and *Xgwm373-Xbarc29*, respectively (Table 4.18, Fig. 4.28). The suggestive QTL was also detected on chromosome 7 in the marker interval *Xcfd31-Xcfa2049*. The QTL on 2A, designated as *QFe.pau-2A*, had a LOD score of 3.7, 3.6, 2.7, 2.1 and 3.3, based on the Fe content measured in the environments IIT2005, IIT2006, PAU2005, PAU2006 and the pool data, respectively, with an R2 value of 14.3, 13.8, 10.0, 8.0 and 12.6 (Fig. 4.26, Table 4.18). Likewise the QTL on chromosome 7A, designated as *QFe.pau-7A*, was detected at LOD scores of 2.3, 3.3, 2.4, 3.3 and 3.2 with R2 values of 8.0, 12.6, 10.0, 11.7 and 11.7, respectively for the environments IIT2005, IIT2006, PAU2005, PAU2006 and the pool data (Table 4.18). For grain Zn concentration, both the significant and the suggestive QTLs mapped on chromosome 7A in the marker intervals *Xcfd31-Xcfa2029*, and *Xgwm373-Xbarc29* respectively (Table 4.18, Fig 4.27). The QTL mapped in the marker interval *Xcfd31-XCfa2029* (Fig 4.28) was detected at LOD score of 4.4, 3.2 and 4.2 with R2 values 21.1, 14.4 and 18.8 for the environments IIT2005, IIT2006 and pooled data, respectively (Fig. 4.27, Table 4.18). This QTL is designated as *QZn.pau-7A*. The suggestive QTL for grain Zn concentration mapped in the same region where QTL for grain Fe concentration mapped and the major grain Zn QTL was observed to be suggestive QTL for grain Fe concentration (Table 4.18, Fig. 4.27).

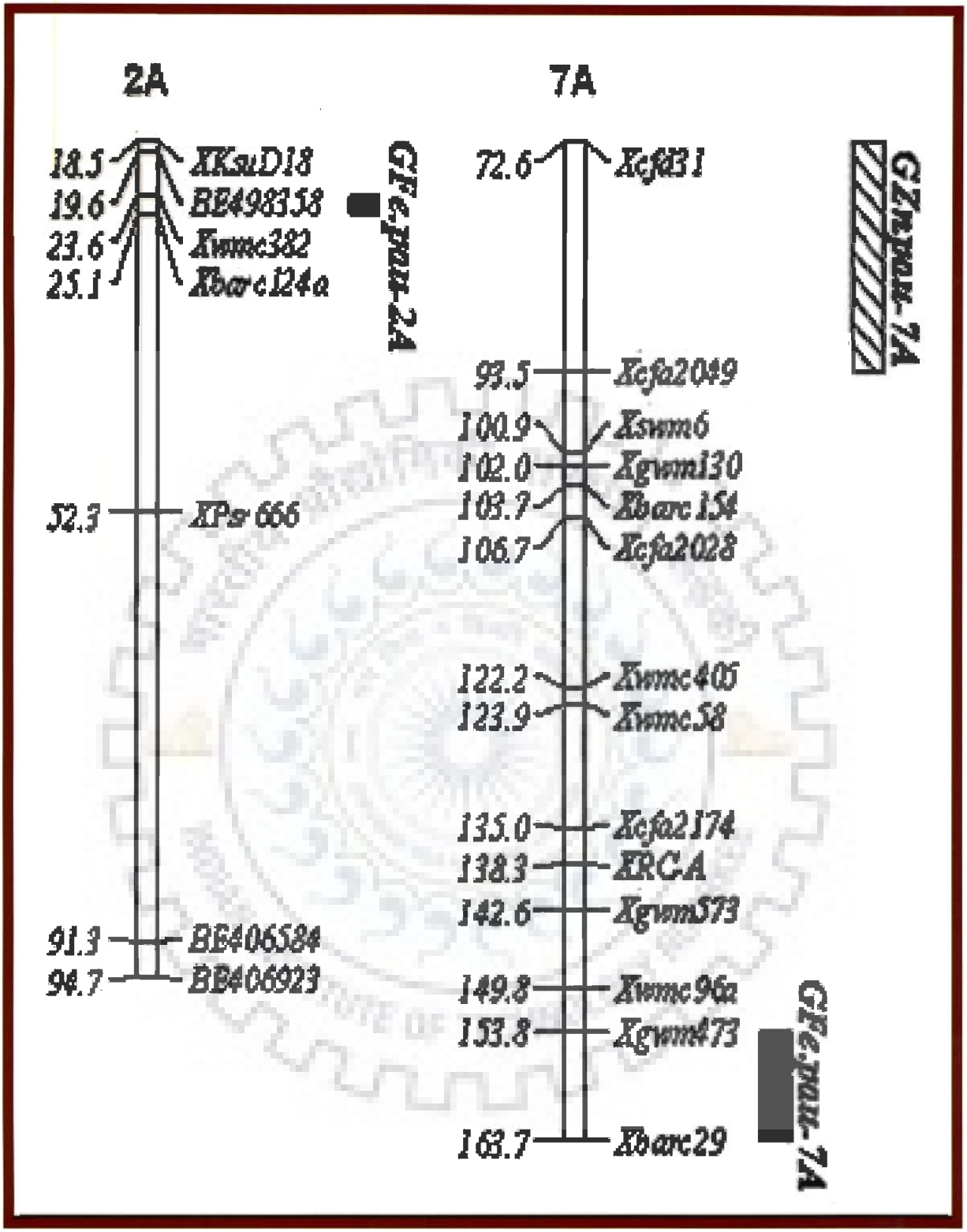


Fig. 4.27. Partial linkage maps of chromosomes 2A and 7A showing chromosomal location of significant QTL for grain Fe and Zn concentrations. Values on the left of the map are the Cm distances from terminal end of the short arm of the linkage map. (http://wheat.pw.usda.gov/report?class=mapdata&name=T.%20boeoticum%20x%20monococcum)

4.4 Genetic diversity analysis of wheat landraces from high hilly areas of himalayas of uttarakhand state

The data on different morphological traits and disease incidence of the pre green revolution cultivars, post green revolution cultivars along with landraces collected from hilly areas of Uttarakhand, India, has been summarized in Table 4.19. A detail of the morphological data has been summarized in annexure I. A set of 63 landraces along with 15 check wheat cultivars (pre and post green revolution) was evaluated for morphological data, grain iron and zinc content, grain hardness index, HMW subunit and diversity analysis using SSR markers.

4.4.1 Variability for grain iron and zinc content

Grain iron and zinc content of landraces varied from as low as 14.6mg/kg (IITR 99), to 43.60 mg/kg for the landrace IITR 66. IITR 66 showed nearly 75% increase in iron content as compared to the modern wheat cultivar PBW502 and WL711. Other landraces IITR 20, IITR 23, IITR 24, IITR25 IITR 26, IITR 27,IITR 28, IITR30, IITR 34 collected from Rudraprayag, Okhimath, Joshimath, Gopeshwar area of Uttarakhand also showed 40-60% increased iron content over wheat cultivars. Landraces IITR65, IITR 66, IITR 68 collected from Tehri Garhwal district of Uttarakhand, showed 55-70% increase in iron content.

Table 4.19 Mean and range of various morphological traits and quality characteristics of pre and post green revolution wheat cultivars and two sets of landraces collected from hilly areas of Uttarakhand

Morphological and quality characteristics	**Pre-green revolution indigenous cultivars**	**Post-green revolution modern cultivars**	**Set A (IITR 8-IITR 34)**	**Set B (IITR 65-IITR 103)**
No. of tillers/plant	9, (5-11)	6, (3-8)	11, (5-13)	18, (7-23)
Plant height (cm)	136.7 (125.6-145.2)	92.8, (85.4-107.5)	138.1, (96.6-155.0)	136.2, (100.3-156.7)
Yield per plant	15.0, (12.2-19.3)	17, (10.4-27.0)	5.5, (3.6-11.6)	6.1, (3.2-12.0)
No. of spikelets per spike	18 (17-19)	17, (15-19)	20, (16-23)	20, (11-23)
Spike length(cm)	8 (7-10)	11, (9-12)	9, (8-13)	9, (7-13)
Days to flowering	98 (87-102)	89, (64-103)	104, (82-138)	114, (63-135)
TKW (g)	42.6 (37.1-55.1)	42.5, (28.2-54.0)	22.7, (19.4-27.5)	26.4, (21.3-37.12)
Powdery Mildew	6-9	7-9	0-9	2-9
Iron content (mg/kg)	26.3, (19.9-30.1)	25.6, (18.9-28.7)	38.7, (20.8-55.0)	31.4, (14.6-44.4)
Zinc content (mg/kg)	20.81, (16.6-22.1)	18.3, (15.7-20.5)	21.6, (17.5-27.5)	22.7, (12.8-29.1)
Hardness Index (SKCS)	102.3, (96.7-107.7)	98.6, (85.0-103.4)	90.0, (43.0-116)	87.3, (33.64-112.5)

1. **Pre-green revolution indigenous cultivars (8A, 9D, C-273,NP-4, C-518, C591, C-306)**
2. **Post-green revolution modern cultivars (Kal.Sona, WG-357, UP-262, WL711, PBW 343, PBW 502**

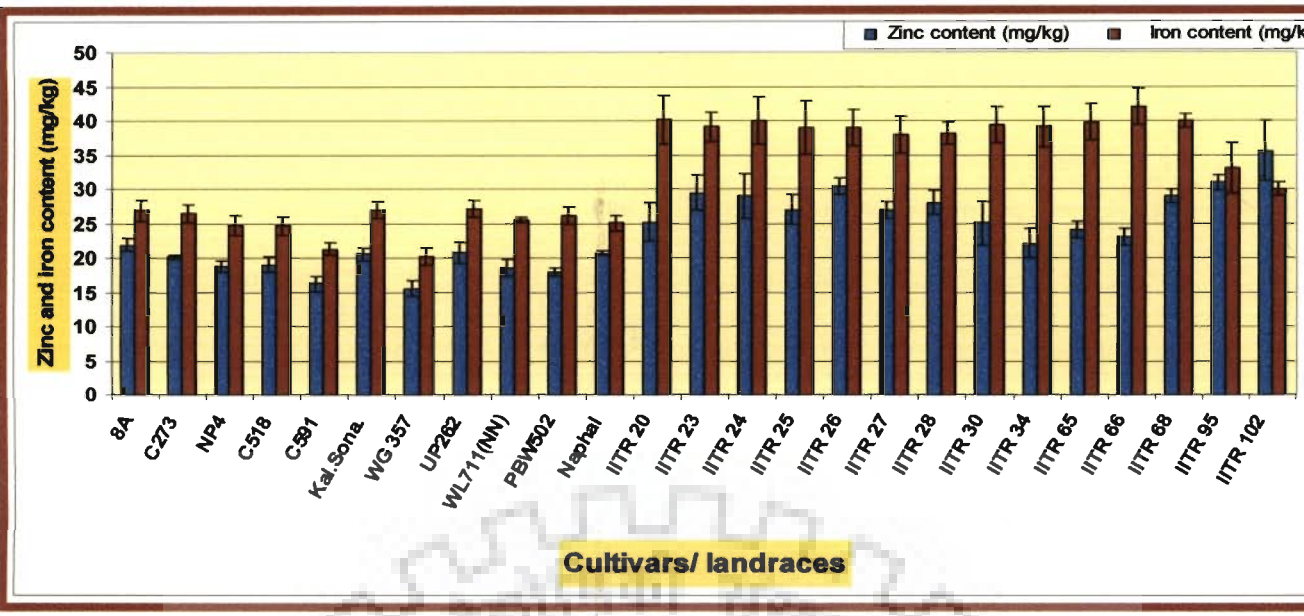


Fig 4.29 . Iron and zinc (Mean ± SD) content of selected landraces with few wheat cultivars

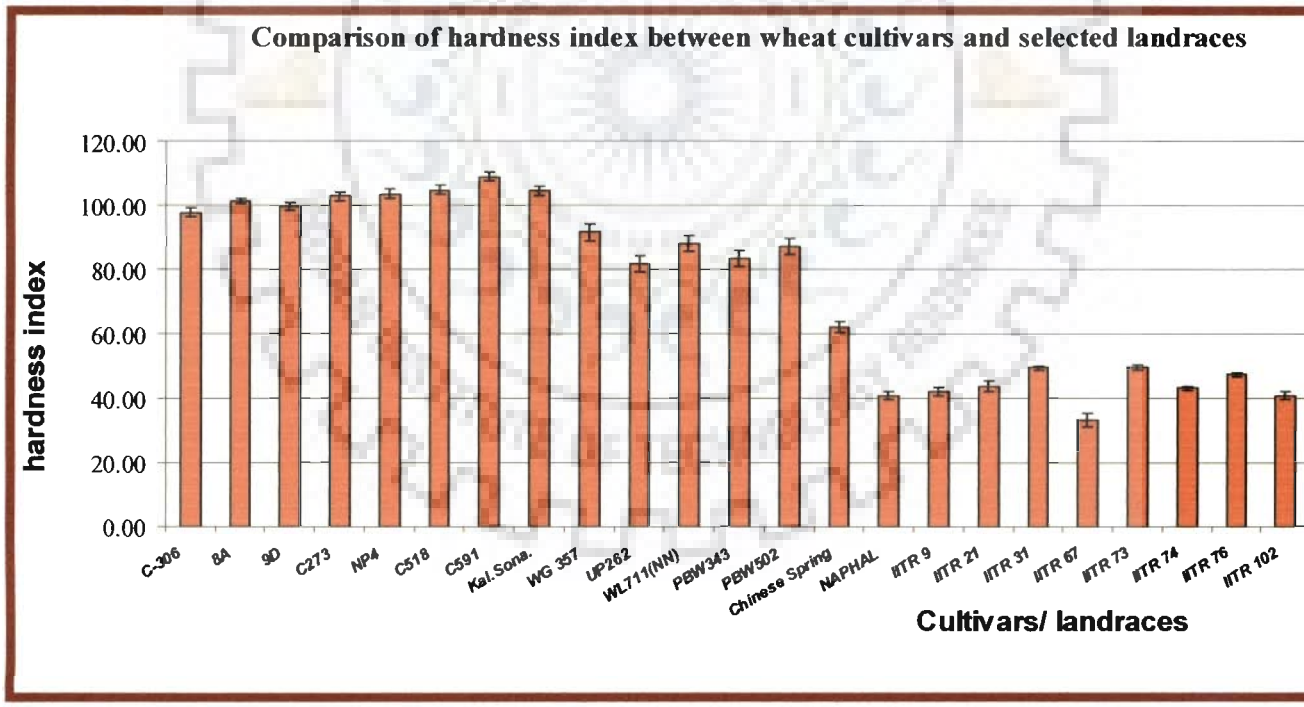


Fig 4.30. Hardness index ((Mean ± SD) of few wheat cultivars and selected landraces.

On the basis of micronutrient analysis few landraces with striking difference with respect to wheat cultivars were selected and are presented in fig.4.29. The zinc content in most of the landraces ranged between 15mg/kg to 35.6 mg/kg. Few landraces have showed 2 times higher zinc content over the wheat cultivar controls. Zinc content of landrace IITR 102, collected from Barkot village of Uttarkashi was found highest with 36.0 mg/kg where as IITR95, 68, IITR26 had relatively higher grain zinc content(Fig 4.29). Values for iron and zinc content of complete set of landraces along with 15 cultivars have been summarized in appendix II.

4.4.2 Grain hardness and softness index:

Both types of seeds were found among the set of landraces, hard as well as soft. The high hardness index of 103.8, 107.4, 114.9, 117.7, 109.9, 113.6, 109.3 and 110.9, 106.7 and106.9 was recorded for the landraces IITR 8, IITR 11, IITR 16, IITR 17, IIT R 20, IITR 31, IIT R 77, IITR 78, and IITR 92, IITR 93 and IITR 98, respectively. IITR 18, and IITR 32 had hardest texture and higher than most of the cultivars. The least value for the hardness index 33.64 was recorded for IITR 67. Other landraces also showed significantly less values as compared to the modern wheat cultivars, some lines with contrasting low value of kernel hardness were presented as bar diagram in Fig.4.30. The grain hardness of landraces IITR 21- (40.93), IITR 74 – (43.18), IITR 102 –(40.8) values are much lower than the landrace Chinese Spring-(63.4) and comparable to that of the landrace, Naphal with both *pina* and *pinb genes* and absence of HMW glutenin 1D subunits. These landraces can be used for biscuit making because of their extra soft seeds.

The hardness index values of all 63 landraces and 15 check wheat cultivars evaluated are shown in annexure II.

4.4.3 Genetic diversity for HMW-Glutenin Subunit composition

The landraces had higher diversity for HMW-glutenin subunits coded by Glu-B1 than within the wheat cultivars analyzed. They showed distinct subunit combinations (annexure III).

4.4.4 Genetic diversity on the basis of molecular marker data

On the basis of dendrogram based on molecular marker data (Fig 4.31) all the cultivars along with landraces were grouped in six different major groups with around 50 % similarity. With a few exception landraces collected from a given region grouped together, indicating their higher similarity among themselves and distinctness from the landraces of other regions. Cluster I included landraces IITR 86 to IITR 102, which were collected from hilly area of Uttarkashi district, whereas cluster II had landraces from IITR 67 to IITR 85 collected from nearby region. Again landraces collected from Rudraprayag, Chamoli, Joshimath, Guptkashi, were grouped into two clusters, III and IV with high similarity. Most of pre and post green revolution wheat cultivars were grouped together in cluster V along with some land races. The wheat cultivars PBW 343 and PBW 502 released for cultivation in NWPZ were more closely related with each other than any other cultivars or land races. Two landraces one from China (Chinese Spring) and other from Kumaom, India(Nephal) with very soft grains grouped together. Most of the land races were distinctly different than from the pre and post green revolution wheat cultivars released in the 20^{th} century. The land races with high iron and zinc content viz, IITR 23,

Fig 4.31. Dendrogram of 78 genotypes using UPGMA method using the matrix of Jaccard's coefficient from SSR marker data. The scale shown above is measure of genetic similarity according to Jaccard's similarity coefficient.

IITR24, IITR 26, IITR 28, IITR 30 and IITR 66 collected from Gopeshwar area of Uttarakhand grouped together indicating this similarity among themselves.

Chapter V

Discussion

Discussion

The low mean and range of grain iron and zinc content in elite wheat and durum cultivars used in this study strongly emphasize the need of screening and identification of useful variability among related wild species of wheat for an effective biofortification programme. Cakmak *et al.* (2000) also reported lower iron and zinc content among *T. durum* and *T. aestivum* cultivars as compared to the wild and primitive *Triticum* species. CIMMYT and HarvestPlus have already used *T. tauschii* and *T. dicoccoides* synthetics in wheat breeding programme for biofortification of wheat for iron and zinc content (Calderini and Monasterio, 2003). *Ae. longissima* (S), *Ae. kotschyi* (US), and *Ae. peregrina* (US) all having S genome gave consistently higher levels and range of both iron and zinc content. Extensive screening of a number of accessions of diploid and tetraploid wild *Triticum* and *Aegilops* species has shown that the non-progenitor S and U genomes may be the useful sources for transferring genes controlling high iron and zinc content to wheat. The S genome being closely related to the B genome of polyploid wheat (Faris *et al.*, 2002; Dvorak and Zhang, 1990; Daud and Gustaffson, 1996) can be effectively used for transferring useful variability for high iron and zinc content into wheat. *T. boeoticum* and *T. monococcum* with A^m genome most closely related to that of A genome of polyploid wheat, *T. tauschii* with D genome and *T. dicoccoides* having AB genomes have been reported to have high grain iron and zinc content (Monasterio and Graham 2000 and Cakmak *et al.* 2000). In addition to the progenitor genomes, some other non-progenitor genomes (U, M) also controlling high iron and zinc can be exploited for biofortification using Ph^l mediated induced homeologus chromosome pairing (Chen *et al.*, 1994; Aghaee-Sarbarzeh, *et al.*, 2002). Among the screened wild relatives in general and selected donors

in particular, accessions with high grain iron content were also found to have high grain zinc content, which strongly suggests similar mechanisms of uptake, translocation and deposition of the two micronutrients. Welch and Graham (2004) also reported high correlation between grain iron and grain zinc concentrations in wheat cultivars and related species. Phytosiderophores like mugineic acids are known to facilitate uptake of iron, zinc and other micronutrients (Takagi *et al.*, 1998; Marschner and Romheld, 1994). Some of the accessions of *Aegilops* species with high grain and leaf iron and zinc also possess high level of phytosiderophores (unpublished results HSD).

Leaves of wild species also have higher micronutrient content than the cultivars. The high positive correlation between flag leaf and grain iron and zinc (Rawat *et al.*, 2008) suggests the possibility of using flag or other leaves for the early selection of plants for high iron and zinc in the segregating generations of interspecific crosses. Garnett and Graham (2005) reported nearly 77%, 62% and 42 % remobilization of wheat shoot iron, copper and zinc, respectively in wheat into grains during anthesis to maturity under controlled experimental conditions. This supports our observations of higher content of leaf and grain iron than their zinc content. It appears that remobilization of zinc follows the same pattern as that of iron but in lower proportion to that of iron (Garnett and Graham, 2005). Wild allele of transcription factor NAM-B1 responsible for accelerated senescence during grain filling period of wheat (Uauy *et al.*, 2006) can be used for high level of translocation of iron and zinc from the biofortified leaves to grains.

Micronutrient analysis of the selfed seeds of fertile derivatives in advanced backcross generations was done for further confirmation of the 'proof of concept'. The iron and zinc

content of grains of fertile BC_2 and BC_1F_2 plants showed variation ranging from that of the wheat parent to the wild donors. The variation could be due to the presence of one or more chromosomes of the wild donors controlling the efficient uptake and translocation of the micronutrients. As the grain size of the fertile derivatives was almost similar or even greater than that of the wheat parent, the higher iron and zinc found in their seeds was not due to concentration effect, unlike the synthetic hexaploids where Calderini and Monasterio (2000) found lower grain yield to be a major contributing factor to their higher micronutrient contents. The recovery of fertile derivatives with high harvest index seeds as bold as that of the wheat cultivars with micronutrient content as high as that of the wild donors gives unequivocal 'proof of the concept' that *Aegilops kotschyi* possesses efficient genetic system for uptake and translocation of the micronutrients which could be effectively used for biofortification of wheat cultivars.

A few derivatives among BC_2F_2 and BC_1F_3 generations were selected on the basis of good harvest index, recovered background and iron and zinc content. The selected derivatives with high grain iron and zinc content were characterized for detection of alien introgression conferring the desired traits. On the basis of biochemical, cytological and molecular analysis presence of the group 2 and 7 alien chromosomes in the high grain iron and zinc derivatives was established. QTL for grain iron and zinc content have been mapped previously to homoeologous chromosomes 2 and 7 (Tiwari *et al.*, in press). Shi *et al.* (2008) detected as many as four QTL for grain Zn concentration (mg/kg) and seven for grain Zn content (µg/grain), however, four of these were common. The QTL detected by Shi *et al.* (2008) on chromosome 7A, explaining the highest level of phenotypic variation, maps in the same region where a suggestive QTL is mapping in the present study. In rice,

using a doubled haploid (DH) population Stangoulis *et al.* (2007) mapped three QTL for grain Fe accumulation on chromosomes 2S, 8L and 12L and two QTL for grain Zn on chromosomes 1L ad 12L. The region where grain Zn QTL was mapped on rice chromosome 1 is orthologous to wheat chromosome 7.

The selfed seed set on F_1 hybrids could be attributed to unreduced male and female gamete formation in the hybrids as reported in hybrids of several *T. turgidum* subspecies with *Ae. tauschii* (Kihara and Lilienfeld, 1949; Matsouka and Nasuda, 2004; Tanaka, 1961; Xu and Joppa, 1995; Xu and Dong, 1992). Vardi and Zohary (1967) also reported unreduced gamete formation in *T. durum* x *Ae. longissima* triploid hybrids but no selfed seed set was obtained because of complete male sterility. However no unreduced gamete formation was observed in *T. durum* x *T. boeoticum* and *T. durum* x *Ae. speltoides* triploid hybrids(Vardi and Zohary, 1967; Vardi, 1971). Variable chromosome pairing in different F_1 hybrids could be attributed to different gene interactions among genetic system(s) promoting or inhibiting homoeologous pairing as reported in numerous interspecific and intergeneric hybrids in Triticeae and related genera (Friebe *et al.*, 2000; Maan and Sasakuma, 1977; Sears, 1976). The reduced seed set in F_1 hybrids with more chromosome pairing may be due to lower frequency of unreduced gamete formation as the *Ph1* supressed homoeologous pairing is a prerequisite for the occurrence of FDR or SDM (Jauhar, 2007; Matsouka and Nasuda, 2004; Vardi, 1971). Some variation in chromosome number in amphiploids from 40 to 42 chromosomes and a few univalents could be due to failure of the paired chromosome in F_1 hybrids to go through FDR or SDM leading to fertile gametes with one deficient chromosome. Limited deficiency of a chromosome or an arm can be tolerated due to buffering by homoeologous chromosomes of other genomes in polyploids (Friebe *et al.*,

2005). Unreduced gamete formation has been reported in F_1 hybrids of *T. turgidum* or *T. aestivum* with *Aegilops tauschii* (Xu and Dong, 1992; Zhang *et al.*, 2007), *Ae. ovata* (David *et al.*, 2004), *Ae. speltoides* (Friebe *et al.*, 2000), *T. crassum* (Wagenaar, 1968), *Ae. heldreichii* (Maan and Sasakuma, 1977), *Hynaldia villosa* (Blanco *et al.*, 1983, Liu *et al.*, 1986), *Hordeum vulgare* (Islam and Shepherd, 1980), *Secale cereale* (Balatero and Darvey, 1993) as well as in haploid plants of *T. durum* (Jauhar *et al.*, 2000). Earlier workers attributed the mode of selfed seed set and fertility in F_1 hybrids of *Triticum* species to two strictly distinct mechanisms i.e. SDM and FDR taking place in different combinations of parents involved. Pignone (1993) reported SDM to be solely responsible for fertility of a *T. turgidum* x *Ae. longissima* hybrid. The interspecific hybrids with little or no chromosome pairing as that of *T. durum* x *Ae. tauschii* are expected to have high frequency of unreduced gamete formation due to meiotic restitution (FDR or SDM) and seed set (Jauhar, 2007, Matsouka and Nasuda, 2004). Low to high pairing in F_1 hybrids suggests that some transfer might have already occurred and included in the amphiploids which could be identified through molecular cytogenetics. These amphiploids with regular meiosis and high fertility have been backcrossed with durum and bread wheat cultivars for transfer of high grain iron and zinc content of *Ae. longissima*. Similar work for transferring high iron and zinc content from *Ae. tauschii* (DD) to bread wheat is being carried out at CIMMYT, Mexico in collaboration with HarvestPlus through the use of synthetic hexaploid wheat (Calderini and Monasterio, 2003, Poletti *et al.*, 2004, White and Broadley, 2005).

Although the grain Fe and Zn concentrations in *Tb5088* and *Tm14078* was in the same range as reported for the cultivated hexaploid wheat germplasm (Morgounov *et al.*, 2007) but the grain Fe and Zn concentration in *Tb5088* was almost double than in *Tm14087*.

Transgressive segregation in the RIL population is an indication of presence of different sets of genes in the parental lines for the target traits. A few transgressive RILs in the present study had grain Fe and Zn concentration 65mg/kg and 60mg/kg, respectively. Shi *et al.* (2008) detected as many as four QTL for grain Zn concentration (mg/kg) and seven for grain Zn content (µg/grain), however, four of these were common. The QTL detected by Shi *et al.* (2008) on chromosome 7A, explaining the highest level of phenotypic variation, maps in the same region where a suggestive QTL has been mapped. The interspecific derivative with high grain Fe and Zn generated from crosses with *Ae. kotschyi* (UUSS) introgression of group 2 and group 7 chromosome suggesting the presence of diploid wheat orthologs of *QFe-2A, QFe-pau-7A* in *Ae. kotschyi*.

There is a possibility that the QTL with major effect are not yet detected from this population. In several studies on the micronutrient analysis in different crops and populations high positive correlation has been reported between iron and zinc content in grains and other tissues. Accumulation of grain Fe and Zn, however, did not show any correlation in the present study. The notion, that accumulation of Fe and Zn in grains is positively correlated (Shi *et al.*, 2008) may not be true as all the studies were based on estimation of grain Fe and Zn in a set of fixed lines (Morgounov *et al.*, 2007), rather than in the segregating populations. Another notion, that the grain weight may affect the grain Fe and Zn concentrations may also not be true, as we did not observe any correlation between grain size in RILs and the Fe and Zn accumulation. The higher micronutrient concentration in the wild species has often been thought as a result of dilution/concentration effect due to smaller seeds but identification of some of the RILs with bolder seeds and higher micronutrient content (RIL11, 20, 38, 46 and

57 in the present study) refutes this concept as well. This, in fact, is encouraging for attempting to transfer the grain Fe and Zn QTL from wild species to the cultivated wheat.

Diversity analysis among wheat cultivars and landraces collected from different hills of Uttarakhand using microsatellite markers clearly indicated that the landraces in general were distinct from the pre and post green revolution wheat cultivars of northern India. The landraces collected from a given region were more similar among themselves then those from the other regions indicating their region specific adaptation and limited moment. The landraces had useful variability for HMW glutenin subunit profile, grain hardness and macronutrient content besides disease resistance. Some of the landraces from Gopeshwar region had nearly double the grain iron and zinc content which could be exploited directly or used in breeding programmes.

Resolution and mapping of QTL for grain micronutrient content in diploid wheat population on chromosomes 2A and 7A on one hand and confirmation of 2 and 7 chromosomes of *Ae. Kotschyi* in advanced backcross derivatives with two to three times higher grain iron and zinc content on the other clearly demonstrates that some of QTL / major grain genes for micronutrient content are on group 2 and 7 of Tritiaceae chromosomes. Precise transfer, tagging and pyramiding of the QTL / genes from these and other related species in elite wheat cultivars with more than double the existing level of the micronutrient would not only alleviate the micronutrient deficiency among human beings but also overcome the nutrient deficiency in wheat crop on nutrient deficient and calcareous soils.

Fine mapping and cloning of the putative QTL / genes for micronutrient content would help in better understanding of the genetics of uptake, transfer and sequestration of micronutrients in grains.

Chapter VI

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6. References

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Chapter VI

Annexures

Annexure-I

List of wheat SSR markers used

	Primer	Forward Sequence[5'-3']	Reverse Sequence[5'-3']	Tm
Chr1A				
1	gdm33	GGCTCAATTCAACCGTTCTT	TACGTTCTGGTGGCTGCTC	56
2	barc119	CACCCGATGATGAAAAT	GATGGCACAAGAAATGAT	48
3	barc263	GGAAGCGCGTCAGCACTAGGCAAC	GGCTTCTAGGTGCTGCGGCTTTTGTC	70
4	gwm136	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC	59
5	gwm11	GGATAGTCAGACAATTCTTGTG	GTGAATTGTGTCTTGTATGCTTCC	58
6	cfd16	GGATCCAAGGGAATCCAAAT	TCCTTCGGTTCCCATATCAC	56
7	wmc336	GTCTTACCCCGCGATCTGC	GCGGCCTGAGCTTCTTGAG	62
8	barc148	GCGCAACCACAATGTATGCT	GGGGTGTTTTCCTATTTCTT	54
9	barc162	GCGTTTAAAGACAAGGTGGTAGGTATT	GCGTGTCCCATCATGCATAGA	61
10	barc120	CCCCCTCTCTTCCTCAT	ATATAGCTCCCCCATTTCCT	55
11	barc213	GCGTAGATTCTCGGTTTGTTGGCTTGC	CCGTCCCTCCTTCCTGGTCT	64
12	barc83	AAGCAAGGAACGAGCAAGAGCAGTAG	TGGATTTACGACGACGATGAAGATGA	73
13	gwm164	ACATTTCTCCCCCATCGTC	TTGTAAACAAATCGCATGCG	54
14	gwm135	TGTCAACATCGTTTTGAAAAGG	ACACTGTCAACCTGGCAATG	57
15	barc240	AGAGGACGCTGAGAACTTTAGAGAA	GCGATCTTTGTAATGCATGGTGAAC	64
16	gwm357	TATGGTCAAAGTTGGACCTCG	AGGCTGCAGCTCTTCTTCAG	59
17	wmc716	CATTTATGTGCACGCCGAAG	CCATAAGCATCGTCACCCTG	58
18	wmc312	TGTGCCCGCTGGTGCGAAG	CCGACGCAGGTGAGCGAAG	64
19	barc158	TGTGTGGGAAGAAACTGAGTCATC	AGGAATACCAAAAGAAGCAAACCAAC	63
20	gwm99	AAGATGGACGTATGCATCACA	GCCATATTTGATGACGCATA	54
21	barc17	GCGCAACATATTCAGCTCAACA	TCCACATCTCGTCCCTCATAGTTTG	60
22	barc287	CGGATGGGTTACTTACTTAGGATG	CGCAACTCCATTTCAGAATCATT	59
23	wmc611	GGTTCGCTTTCAAGGTCCACTC	CGGGACACTAGTGCTCGATTCT	66
Chr1B				
24	gwm33	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	60
25	wmc134	CCAAGCTGTCTGACTGCCATAG	AGTATAGACCTCTGGCTCACGG	64
26	gwm403	CGACATTGGCTTCGGTG	ATAAAACAGTGCGGTCCAGG	52
27	gwm268	AGGGGATATGTTGTCACTCCA	TTATGTGATTGCGTACGTACCC	59
28	wmc719	TTGTGGGAATCTACATCAGAAGG	AACAGCCACGCTCTATCTTCAGT	61
29	wmc367	CTGACGTTGATGGGCCACTATT	GTGGTGGAAGAGGAAGGAGAGG	62
30	gwm259	AGGGAAAAGACATCTTTTTTTC	CGACCGACTTCGGGTTC	56
31	gwm140	ATGGAGATATTTGGCCTACAAC	CTTGACTTCAAGGCGTGACA	60
32	wmc419	GTTTCGGATAAAACCGGAGTGC	ACTACTTGTGGGTTATCACCAGCC	62
33	wmc269	GCACCTTCTAACCTTCCCCAGC	CCCTAATCCAGGACTCCCTCAG	66
34	barc137	GGCCCATTTCCCACTTTCCA	CCAGCCCCTCTACACATTTT	58
35	barc187	GTGGTATTTCAGGTGGAGTTGTTTTA	CGGAGGAGCAGTAAGGAAGG	63
36	barc61	TGCATACATTGATTCATAACTCTCT	TCTTCGAGCGTTATGATTGAT	55
37	barc188	CGTGAGATCATGTTATCAGGACAAG	GCGTTGAAAGGTGTTAGTGGGATGG	64
38	barc81	GCGCTAGTGACCAAGTTGTTATATGA	GCGGTTCGGAAAGTGCTATTCTACAGT AA	65
39	barc80	GCGAATTAGCATCTGCATCTGTTTGAG	CGGTCAACCAACTACTGCACAAC	65
40	wmc500	ATAGCATGTTGGAACAGAGCAC	CTTAGATGCAACTCTATGCGGT	60
41	wmc619	TTCCCTTTCCCCTCTTTCCG	TACAATCGCCACGAGCACCT	60
42	wmc626	AGCCCATAAACATCCAACACGG	AGGTGGGCTTGGTTACGCTCTC	62
Chr1D				
43	cfd61	ATTCAAATGCAACGCAAACA	GTTAGCCAAGGACCCCTTTC	52

44	gwm106	CTGTTCTTGCGTGGCATTAA	AATAAGGACACAATTGGGATGG	62
45	gwm232	ATCTCAACGGCAAGCCG	CTGATGCAAGCAATCCACC	52
46	gdm111	CACTCACCCCAAACCAAAGT	GATGCAATCGGGTCGTTAGT	58
47	wmc216	ACGTATCCAGACACTGTGGTAA	TAATGGTGGATCCATGATAGCC	60
48	cfd19	TACGCAGGTTTGCTGCTTCT	GGAGTTCACAAGCATGGGTT	58
49	wmc36	TTCTCTTTTCCTTTCGCACTCC	CATCAGTTGTGGGGTTTCTTCA	60
50	wmc93	ACAACTTGCTGCAAAGTTGACG	CCAACTGAGCTGAGCAACGAAT	60
51	cfd63	TCCTGAGGATGTTGAGGACC	GAGAGAGGCGAAACATGGAC	60
52	wmc813	TGTTGGATGCGTGCGAC	CCTCTCCCGGACTCCTGC	52
53	barc66	CGCGATCGATCTCCCGGTTTGCT	GGGAAGAGGACCAAGGCCACTA	66
54	wmc609	CATCCAGCCCATGTAGACGC	AACGGTGCCCATCATCTCCC	63
55	wmc222	AAAGGTGCGTTCATAGAAAATTAGA	AGAGGTGTTTGAGACTAATTTGGTA	59
56	wmc339	CCGCTCGCCTTCTTCCAG	TCCGGAACATGCCGATAC	52
57	wmc590	CGCACGAAGCTATCTGATACCA	GGAAAACCTAACCCTAGCCACC	62
58	barc152	CTTCCTAAAATCGGGCAACCGCTTGTTG	GCGTAATGATGGGAGTGGCTATAGGGC AGTT	70
59	barc229	GGCCGCTGGGGATTGCTATGAT	TCGGGATAAGGCAGACCACAT	61
60	barc99	CGCATTCTTTCGCATTCTCTGTCATA	CGCATACTGTGTCGTGTTCCTGGTTTAG A	65
61	barc169	CCGCGAACCATACAAAGGAAAC	GCTATAGAGGCGCCTTGGAGTACC	62
62	barc271	CGCACCTAATATCGTAAAACAATGTA	CGCTTTCCCAGAATATTATTTGTATTGT	62
63	barc346	ACCGCCTCAGCCTTATTCCTTG	TGGGCTCGGGTTGGTCTCT	62
64	gwm458	AATGGCAATTGGAAGACATAGC	TTCGCAATGTTGATTTGGC	52
65	wmc405	GTGCGGAAAGAGACGAGGTT	TATGTCCACGTTGGCAGAGG	60
Chr2A				
66	cfd36	GCAAAGTGTAGCCGAGGAAG	TTAGAGTTTTGCAGCGCCTT	56
67	gwm512	AGCCACCATCAGCAAAAATT	GAACATGAGCAGTTTGGCAC	54
68	barc1138	GCGATGTCATGCTCACCAATGTGT	GCGTGCTCCACTCAGAGACTATCATAA A	65
69	wmc382	CATGAATGGAGGCACTGAAACA	CCTTCCGGTCGACGCAAC	60
70	gwm359	CTAATTGCAACAGGTCATGGG	TACTTGTGTTCTGGGACAATGG	59
71	wmc602	TACTCCGCTTTGATATCCGTCC	GTTTGTTGTTGCCATCACATTC	58
72	wmc453	ACTTGTGTCCATAACCGACCTT	ATCTTTTGAGGTTACAACCCGA	58
73	barc124	TGCACCCCTTCCAAATCT	TGCGAGTCGTGTGGTTGT	54
74	wmc382	CATGAATGGAGGCACTGAAACA	CCTTCCGGTCGACGCAAC	60
75	gwm515	AACACAATGGCAAATGCAGA	CCTTCCTAGTAAGTGTGCCTCA	54
76	gwm473	TCATACGGGTATGGTTGGAC	CACCCCCTTGTTGGTCAC	58
77	wmc261	GATGTGCATGTGAATCTCAAAAGTA	AAAGAGGGTCACAGAATAACCTAAA	61
78	gwm47	TTGCTACCATGCATGACCAT	TTCACCTCGATTGAGGTCCT	56
79	wmc109	AATTCGGGAAGAGTCTCAGGGG	TTCGAAGGGCTCAAGGGATACG	64
80	barc231	GCGATCAATAACCGTGCCACCA	GCACTTGCGATGTCACTAAAATG	61
81	barc309	GCGAAAGCCCTAAAGTTACAA	AAGCCGCAGAGAAGGTCAGC	57
82	cfd168	CTTCGCAAATCGAGGATGAT	TTCACGCCCAGTATTAAGGC	56
83	barc353	GAAGTTCCCAAAATGCCTCTGTC	GCGGATCGAAGACCTAAGAAAAG	63
84	wmc181	TCCTTGACCCCTTGCACTAACT	ATGGTTGGGAGCACTAGCTTGG	62
85	barc279	GCGTTTTTTACCTAAAGAAAAGGTGATTG	CGCAACACACATTCCATTCCATTTCAC	65
86	gwm356	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	57
87	barc76	ATTCGTTGCTGCCACTTGCTG	GCGCGACACGGAGTAAGGACACC	61
88	wmc658	CTCATCGTCCTCCTCCACTTTG	GCCATCCGTTGACTTGAGGTTA	62
89	gwm311	TCACGTGGAAGACGCTCC	CTACGTGCACCACCATTTTG	58
90	gwm425	GAGCCCACAAGCTGGCA	TCGTTCTCCCAAGGCTTG	56
91	wmc407	GGTAATTCTAGGCTGACATATGCTC	CATATTTCCAAATCCCCAACTC	58
92	wmc177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	58
93	wmc63	GTGCTCTGGAAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	60

Chr2B				
94	wmc764	CCTCGAACCTGAAGCTCTGA	TTCGCAAGGACTCCGTAACA	58
95	barc318	CGACTAACAATTTTTCATTT	TGATTTCGCTAACAAGGAG	48
96	barc200	GCGATATGATTTGGAGCTGATTG	GCGATGACGTTAGATGCGGAATTGT	61
97	barc349	CGAATAGCCGCTGCACAAG	TATGCATGCCTTTCTTTACAAT	55
98	barc13	GCAGGAACAACCACGCCATCTTAC	GCGTCGCAATTTGAAGAAAATCATC	63
99	wmc154	ATGCTCGTCAGTGTCATGTTTG	AAACGGAACCTACCTCACTCTT	60
100	barc128	GCGGGTAGCATTTATGTTGA	CAAACCAGGCAAGAGTCTGA	56
101	gwm429	TTGTACATTAAGTTCCCATTA	TTTAAGGACCTACATGACAC	52
102	barc101	GCTCCTCTCACGATCACGCAAAG	GCGAGTCGATCACACTATGAGCCAATG	66
103	gwm148	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	57
104	barc183	CCCGGGACCACCAGTAAGT	GGATGGGGAATTGGAGATACAGAG	62
105	wmc272	TCAGGCCATGTATTATGCAGTA	ACGACCAGGATAGCCAATTCAA	58
106	wmc265	GTGGATAACATCATGGTCAAC	TACTTCGCACTAGATGAGCCT	57
107	gwm129	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	52
108	cfd73	GATAGATCAATGTGGGCCGT	AACTGTTCTGCCATCTGAGC	58
109	gwm501	GGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGCGCC	57
110	wmc332	CATTTACAAAGCGCATGAAGCC	GAAAACTTTGGGAACAAGAGCA	58
111	wmc149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG	66
112	wmc317	TGCTAGCAATGCTCCGGGTAAC	TCACGAAACCTTTTCCTCCTCC	62
113	gwm382	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	58
114	gwm526	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	58
115	gwm374	ATAGTGTGTTGCATGCTGTGTG	TCTAATTAGCGTTGGCTGCC	58
116	gwm410	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	58
117	wmc474	ATGCTATTAAACTAGCATGTGTCG	AGTGGAAACATCATTCCTGGTA	58
118	wmc445	AGAATAGGTTCTTGGGCCAGTC	GAGATGATCTCCTCCATCAGCA	62
119	wmc356	GCCGTTGCCCAATGTAGAAG	CCAGAGAAACTCGCCGTGTC	60
120	wmc592	GGTGGCATGAACTTTCACCTGT	TGTGTGGTGCCCATTAGGTAGA	62
Chr2D				
121	cfd56	TTGCATAATTACTTGCCCTCC	CTGGTCCAACTTCCATCCAT	57
122	barc297	GCGTAGGAGAGATGCCCCAAAGGTT	GCGTGCGGACTCGTGAATCATTACA	69
123	wmc25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	58
124	gwm261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	59
125	barc168	GCGATGCATATGAGATAAGGAACAAATG	GCGGCTCTAAGGCGGTTTCAAAT	65
126	wmc470	ACTTGCAACTGGGGACTCTC	TCCCCAATTGCATATTGACC	56
127	barc228	CCCTCCTCTCTTTAGCCATCC	GCACGTACTATTCGCCTTCACTTA	63
128	barc145	GCAGCCTCGAATCACA	GGGGTGTTGAAGATGA	48
129	barc11	GCGATGCGTGTAAAGTCTGAAGATGA	GCGTCCATGGAGCTCTGTTTTATCTGA	66
130	gwm249	CAAATGGATCGAGAAAGGGA	CTGCCATTTTTCTGGATCTACC	56
131	wmc601	ACAGAGGCATATGCAAAGGAGG	CTTGTCTCTTTATCGAGGGTGG	62
132	barc219	GCGATCCCACAATGCATGACAACTTC	GGACGTCCGATCGAATTGGTTT	62
133	barc159	CGCAATTTATTATCGGTTTTAGGAA	CGCCCGATAGTTTTTCTAATTTCTGA	62
134	wmc41	TCCCTCTTCCAAGCGCGGATAG	GGAGGAAGATCTCCCGGAGCAG	66
135	gwm608	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	55
136	gwm349	GGCTTCCAGAAAACAACAGG	ATCGGTGCGTACCATCCTAC	58
137	wmc175	GCTCAGTCAAACCGCTACTTCT	CACTACTCCAATCTATCGCCGT	62
138	wmc817	TGACGGGGATGATGATAACG	CGGTGAGATGAGAAAGGAAAAC	58
139	gwm301	GAGGAGTAAGACACATGCCC	GTGGCTGGAGATTCAGGTTC	60
140	gdm5	CTAGCCAGAAGGTTACTTTG	CAACATTAACATTAACGCAC	52
141	gwm455	ATTCGGTTCGCTAGCTACCA	ACGGAGAGCAACCTGCC	57
142	gwm484	ACATCGCTCTTCACAAACCC	AGTTCCGGTCATGGCTAGG	58
143	gwm102	TCTCCCATCCAACGCCTC	TGTTGGTGGCTTGACTATTG	58
144	gwm539	CTGCTCTAAGATTCATGCAACC	GAGGCTTGTGCCCTCTGTAG	60
145	gdm148	GATTTGACCGTCTGAGGTCG	AACTAGTTCTGTGGCAAGCT	56

146	gwm296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	55
Chr3A				
147	wmc11	TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	61
148	barc310	GGGCGGCGCATGTGCACCTA	GCGTGGAAGCGACTAAATCAACT	63
149	barc12	CGACAGAGTGATCACCCAAATATAA	CATCGGTCTAATTGTCAATGTA	57
150	gwm369	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	56
151	barc179	GCGTCGTCATAATTGCCTTTCACTTG	GCGAGCCCATATTGCCTTGTCTTCT	66
152	barc45	CCCAGATGCAATGAAACCACAAT	GCGTAGAACTGAAGCGTAAAATTA	60
153	wmc505	AGGGGAGGAAAACCTTGTAATC	ACGACCTACGTGGTAGTTCTTG	60
154	barc67	GCGGCATTTACATTTCAGATAGA	TGTGCCTGATTGTAGTAACGTATGTA	59
155	barc19	GCGACCCGAGTAGCCTGAA	GGTGGACCATTAGACGCTTACTTG	62
156	barc25	GCGGTGCATCAAGGACGACAT	GCGTAGTTCATCCATCCGTAAT	60
157	wmc428	TTAATCCTAGCCGTCCCTTTTT	CGACCTTCGTTGGTTATTTGTG	58
158	barc314	CTGTGGAAACCAATAAAAACAA	GTGCGCGAATAACTACAAGAAA	55
159	gwm494	ATTGAACAGGAAGACATCAGGG	TTCCTGGAGCTGTCTGGC	58
160	wmc96	TAGCAGCCATGCTTAGCATCAA	GTTTCAGTCTTTCACGAACACG	60
161	wmc173	TGCAGTTGCGGATCCTTGA	TAACCAAGCAGCACGTATT	53
162	wmc153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAG	60
163	cfa2076	CGAAAAACCATGATCGACAG	ACCTGTCCAGCTAGCCTCCA	56
164	gwm666	GCACCCACATCTTCGACC	TGCTGCTGGTCTCTGTGC	58
165	gwm480	TGCTGCTACTTGTACAGAGGAC	CCGAATTGTCCGCCATAG	56
166	barc284	GCGTCAGAAATGCAAGAAAAATAGG	GCGGAAGAAAAGGACGAAGACAAG	63
167	wmc289	CATATGCATGCTATGCTGGCTA	AGCCTTTCAAATCCATCCACTG	60
168	gwm155	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	56
169	wmc83	TGGAGGAAACACAATGGATGCC	GAGTATCGCCGACGAAAGGGAA	62
170	wmc169	TACCCGAATCTGGAAAATCAAT	TGGAAGCTTGCTAACTTTGGAG	57
Chr3B				
171	barc75	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTCTA	59
172	gwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	54
173	barc133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	50
174	wmc597	AACACACCTTGCTTCTCTGGGA	GACTAGGGTTTCGGTTGTTGGC	62
175	cfd28	TGCATCTTATTACTGGAGGCATT	CGCATGCCCTTATACCAACT	58
176	barc102	GGAGAGGACCTGCTAAAATCGAAGACA	GCGTTTACGGATCAGTGTTGGAGA	65
177	wmc679	TAGGGGACAGGAGGGAGGG	CGGATCCAGACCAGGAAGGT	63
178	barc218	GGTGAGGAGATGGCCCAAAGTAAC	GGGGGTGTGAGGAGAACGTATCAACTCT	67
179	wmc625	CACAGACCTCAACCTCTTCTT	AGTACTGTTCACAGCAGACGA	59
180	gwm77	ACAAAGGTAAGCAGCACCTG	ACCCTCTTGCCCGTGTTG	58
181	barc164	TGCAAACTAATCACCAGCGTAA	CGCTTTCTAAAACTGTTCGGGATTTCTAA	58
182	cfa2134	TTTACGGGGACAGTATTCGG	AAGACACTCGATGCGGAGAG	58
183	barc84	CGCATAACCGTTGGGAAGACATCTG	GGTGCAACTAGAACGTACTTCCAGTC	67
184	barc77	GCGTATTCTCCCTCGTTTCCAAGTCTG	GTGGGAATTTCTTGGGAGTCTGTA	64
185	gwm108	CGACAATGGGGTCTTAGCAT	TGCACACTTAAATTACATCCGC	58
186	wmc687	AGGACGCCTGAATCCGAG	GGGAGCGTAGGAGGACTAACA	58
187	wmc206	TTGTGCTCGTGAATTGCATACC	GCCAAAATGGCAGCTTCTCTTA	60
188	gwm114	ACAAACAGAAAATCAAAACCCG	ATCCATCGCCATTGGAGTG	57
189	gwm547	GTTGTCCCTATGAGAAGGAACG	TTCTGCTGCTGTTTTCATTTAC	57
190	wmc632	GTTTGATTGGTCGTTCCTGGTC	AACAGCGAATGGAGGGCTTTAG	62
191	gwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	56
192	gwm566	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	59
193	wmc231	CATGGCGAGGAGCTCGGTGGTC	GTGGAGCACAGGCGGAGCAAGG	70
194	gwm340	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	57

195	wmc307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	60
196	wmc471	GGCAATAATAGTGCAAGGAATG	GCCGATAATGGGCAATATAAGT	58
Chr3D				
197	cfd35	GGGATGACACATAACGGACA	ATCAGCGGCGCTATAGTACG	58
198	cfd141	CGTAAAGATCCGAGAGGGTG	TCCGAGGTGCTACCTACCAG	60
199	barc321	TGCACTTCCCACAACACATC	TTGCCACGTAGGTGATTTATGA	58
200	cfd79	TCTGGTTCTTGGGAGGAAGA	CATCCAACAATTTGCCCAT	53
201	gwm52	CTATGAGGCGGAGGTTGAAG	TGCGGTGCTCTTCCATTT	54
202	barc6	TTCGGTCGTTGAGGTGACCAATTATG	GACAAAGGATTAGCCCAAAGTAAGAG	65
203	barc135	ATCGCCATCTCCTCTACCA	GCGAACCCATGTGCTAAGT	57
204	wmc631	TTGCTCGCCCACCTTCTACC	GGAAACCATGCGCTTCACAC	60
205	cfd211	AGAAGACTGCACGCAAGGAT	TGCACTAAAGCATCTTCGTGTT	58
206	barc42	GCGACTCCTACTGTTGATAGTTC	GCGTTCTTTTATTACTCATTTTGCAT	60
207	gdm72	TGGTTTTCTCGAGCATTCAA	TGCAACGATGAAGACCAGAA	54
208	barc71	GCGCTTGTTCCTCACCTGCTCATA	GCGTATATTCTCTCGTCTTCTTGTTGGTT	67
209	barc270	GCGCATTGTGACAGGTGAAC	GGAGGGAGTACTTGGTTATTAGGGT	60
210	barc323	GCGAATCTGATGTGGCATGTTAGTT	GGCATATTTCCTTCACAGTTTT	57
211	gwm456	TCTGAACATTACACAACCCTGA	TGCTCTCTCTGAACCTGAAGC	58
212	gwm383	ACGCCAGTTGATCCGTAAAC	GACATCAATAACCGTGGATGG	58
213	gwm314	AGGAGCTCCTCTGTGCCAC	TTCGGGACTCTCTTCCCTG	59
214	gwm341	TTCAGTGGTAGCGGTCGAG	CCGACATCTCATGGATCCAC	59
215	gwm497	GTAGTGAAGACAAGGGCATT	CCGAAAGTTGGGTGATATAC	56
216	wmc552	ACTAAGGAGTGTGAGGGCTGTG	CTCTCGCGCTATAAAAGAAGGA	60
217	wmc533	AATTGGATCGGCAGTTGGAG	AGCAAGCAGAGCATTGCGTT	58
Chr4A				
218	barc206	GCTTTGCCAGGTGAGCACTCT	TGGCCGGGTATTTGAGTTGGAGTTT	63
219	barc138	CTCGATTCGCCGTCAG	GTGGGGGAAGAAGAAACC	53
220	barc106	GCCCTCAAATAATTACGCCAATCCCTATG	GCGTCAAGATCAGAAGGCATCCTATTATTG	69
221	barc170	CGCTTGACTTTGAATGGCTGAACA	CGCCCACTTTTTACCTAATCCTTTTGAA	64
222	gwm637	AAAGAGGTCTGCCGCTAACA	TATACGGTTTTGTGAGGGGG	58
223	wmc707	GCTAGCTGACACTTTTCCTTTG	TCAGTTTCCCACTCACTTCTTT	58
224	barc343	GGCCTAATTACAAGTCCAAAAG	GCTCAAAGTAAAGTTCACGAATAT	58
225	wmc718	GGTCGGTGTTGATGCACTTG	TCGGGGTGTCTTAGTCCTGG	60
226	wmc698	GTGAAGGGAGAGCTAGCAA	ACAGTTGGCCCAGCTAGTA	57
227	barc70	GCGAAAAACGATGCGACTCAAAG	GCGCCATATAATTCAGACCCACAAAA	63
228	gwm160	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC	57
229	barc78	CTCCCCGGTCAAGTTTAATCTCT	GCGACATGGGAATTTCAGAAGTGCCTAA	63
230	wmc219	TGCTAGTTTGTCATCCGGGCGA	CAATCCCGTTCTACAAGTCCA	59
231	barc52	GCGCCATCCATCAACCGTCATCGTCATA	GCGAGGAAGGCGGCCACCAGAATGA	72
232	barc315	CATCCAGGCGGGCGCACGAGA	CAAGCCTCCGTGCACACCGTAT	66
233	barc184	TTCGGTGATATCTTTTCCCCTTGA	CCGAGTTGACTGTGTGGGCTTGCTG	62
234	barc153	CGCGCCTTGCTTTATTAGTATTAGTATT	GCGGCATGCACATATAATTCTCATTGACT	64
235	gwm397	TGTCATGGATTATTTGGTCGG	CTGCACTCTCGGTATACCAGC	57
236	wmc513	TGAATTGAATCTGGTTGCGG	TGGCAATTCACAGGCACATA	56
237	wmc468	AGCTGGGTTAATAACAGAGGAT	CACATAACTGTCCACTCCTTTC	58
238	wmc283	CGTTGGCTGGGTTATATCATCT	GACCCGCGTGTAAGTGATAGGA	60
239	wmc313	GCAGTCTAATTATCTGCTGGCG	GGGTCCTTGTCTACTCATGTCT	62
Chr4B				
240	barc193	GCGCATCCATATTTTTCCAGCAAGCACTT	GCGTTCTTGTTTGTGGTTTCTATTTTTCT	69

241	barc10	GCGTGCCACTGTAACCTTTAGAAGA	GCGAGTTGGAATTATTTGAATTAAACA AG	63
242	wmc47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA	60
243	barc292	GCGTGTGAGTCAATCCGTGCTTTAT	GCGTTGGTTTTAAGAGGTGCCTGAA	66
244	barc163	GCGTGTTTTAAGGTATTTTCCATTTTCT	GCGCATCCTGTTCCTCCATTCATA	63
245	cfd22	GGTTGCAAACCGTCTTGTTT	AGTCGAGTTGCGACCAAAGT	56
246	barc60	CATGCTCACAAAACCCACAAGACT	CTCGAAAGGCGGCACCACTA	63
247	wmc546	CGGCTAAAATCGTACACTACACA	CTCACTTGCACGATTTCCCTAT	60
248	wmc710	GTAAGAAGGCAGCACGTATGAA	TAAGCATTCCCAATCACTCTCA	58
249	wmc617	CCACTAGGAAGAAGGGGAAACT	ATCTGGATTACTGGCCAACTGT	60
250	wmc42	GCCCTTGGTCCTGGGGTGAGCC	GCCTCATCCAGAGAGCCTGCGG	70
251	gwm149	CATTGTTTTCTGCCTCTAGCC	CTAGCATCGAACCTGAACAAG	59
252	gwm375	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC	60
253	gwm6	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	60
254	wmc125	ATACCACCATGCATGTGGAAGT	ACCGCTTGTCATTTCCTTCTGT	60
255	wmc349	ACACACACTCGATCGCAC	GCAGTTGATCATCAAAACACA	55
Chr4D				
256	wmc285	TGTGGTTGTATTTGCGGTATGG	TTGTGGTGCTGAGTTAGCTTGT	60
257	barc225	CGCAATAATTCAGTACTACTTCCCCGCAA TA	CGAAGGATTTGCATGGTACTGTGGGTG AT	70
258	gwm213	TGCCTGGCTCGTTCTATCTC	CTAGCTTAGCACTGTCGCCC	60
259	barc308	GCGATCTTGCGTGTGCGTAGGA	GCGTGGGATGCAAGTGAACAAT	62
260	barc288	GGGTTTTGCTTGGTTGACA	CGGGACGATTTTATTTAGGAGT	55
261	barc98	CCGTCCTATTCGCAAACCAGATT	GCGGATATGTTCTCTAACTCAAGCAAT G	63
262	cfd39	CCACAGCTACATCATCTTTCCTT	CAAAGTTTGAACAGCAGCCA	56
263	cfd84	GTTGCCTCGGTGTCGTTTAT	TCCTCGAGGTCCAAAACATC	58
264	wmc622	CAGGAAGAAGAGCTCCGAGAAA	CTTGCTAACCCGCGCC	56
265	barc48	GCGAGCTGCAGAGGTCCATC	GCGTTAGTCTTCTTGGTCAATCAC	64
266	wmc825	GCTAGCTGCTGGTTCCACTTG	TGTCCACTCCACTCCAGCATTAC	63
267	gwm624	TTGATATTAAATCTCTCTATGTG	AATTTTATTTGAGCTATGCG	50
268	gwm609	GCGACATGACCATTTTGTTG	GATATTAAATCTCTCTATGTGTG	56
269	wmc51	TTATCTTGGTGTCTCATGTCAG	TCGCAAGATCATCAGAACAGTA	58
270	wmc48	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	60
271	wmc52	TCCAATCAATCAGGGAGGAGTA	GAACGCATCAAGGCATGAAGTA	60
272	wmc89	ATGTCCACGTGCTAGGGAGGTA	TTGCCTCCCAAGACGAAATAAC	60
273	wmc331	CCTGTTGCATACTTGACCTTTTT	GGAGTTCAATCTTTCATCACCAT	59
Chr5A				
274	barc122	CCCGTGTATATCCAGGAGTG	CAGCCCTTGTGATGTGATG	56
275	barc316	GCGTCCCACCTGTCATTAACTGTC	GCGGGCCCACTCCTGTTAGATTA	70
276	barc180	GCGATGCTTGTTTGTTACTTCTC	GCGATGGAACTTCTTTTTGCTCTA	61
277	barc186	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	65
278	gwm443	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	54
279	barc360	GCGATGGCAAAAACTGTGACC	GCGCTCCAGCAGATACATAAGATAAC	61
280	barc40	GCCGCCTACCACAGAGTTGCAGCT	GCGGCATTGACAAGACCATAGC	64
281	cfa2104	CCTGGCAGAGAAAGTGAAGG	AGTCGCCGTTGTATAGTGCC	60
282	barc141	GGCCCATGGATAATTTTTGAAATG	CAATTCGGCCAAAGAAGAAGTCA	60
283	barc330	GCACTAAGCGCTCTTTATTTAC	CCTGCATCTGGTATGGAGA	57
284	gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	57
285	barc56	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	63
286	gwm186	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	58
287	wmc492	AGGATCAGAATAGTGCTACCC	ATCCCGTGATCAGAATAGTGT	57
288	gwm156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	59
289	barc230	CCCCTCCTCCTTCTCCCTCCTCCTA	GGCTCATGCGGGCGTGTTTGG	67

290	barc319	GCAGAGCTACGGCAATGT	GCGTAAGTCCCGGAAGTAACAGAA	56
291	barc151	TGAGGAAAATGTCTCTATAGCATCC	CGCATAAACACCTTCGCTCTTCCACTC	63
292	cfa2155	TTTGTTACAACCCAGGGGG	TTGTGTGGCGAAAGAAACAG	56
293	barc232	CGCATCCAACCATCCCCACCCAACA	CGCAGTAGATCCACCACCCCGCCAGA	71
294	cfa2185	TTCTTCAGTTGTTTTGGGGG	TTTGGTCGACAAGCAAATCA	54
295	wmc110	GCAGATGAGTTGAGTTGGATTG	GTACTTGGAAACTGTGTTTGGG	60
296	gwm126	CACACGCTCCACCATGAC	GTTGAGTTGATGCGGGAGG	58
297	wmc577	CTGTCCGACTCCCCAGATG	CCCTGTCAGAGGCTGGTTG	62
298	gwm595	GCATAGCATCGCATATGCAT	GCCACGCTTGGACAAGATAT	56
299	wmc727	CATAATCAGGACAGCCGCAC	TAGTGGCCTGATGTATCTAGTTGG	60
300	gwm291	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG	59
301	gwm154	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA	56
302	wmc415	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	60
303	wmc497	CCCGTGGTTTTCTTTCCTTCT	AACGACAGGGATGAAAAGCAA	58
Chr5B				
304	cfd5	TGCCCTGTCCACAGTGAAG	TTGCCAGTTCCAAGGAGAAT	56
305	wmc773	GAGGCTTGCATGTGCTTGA	GCCAACTGCAACCGGTACTCT	56
306	barc32	GCGTGAATCCGGAAACCCAATCTGTG	TGGAGAACCTTCGCATTGTGTCATTA	65
307	barc216	TGACGACCCAATCCATAGACA	GGTGATTATTCGTGAGTTCCCTGTG	59
308	barc340	GCAACCAAGGCAGCGTAAATG	GCGTGTAGCCGTCCATAAGCATCAT	61
309	barc4	GCGTGTTTGTGTCTGCGTTCTA	CACCACACATGCCACCTTCTTT	62
310	barc89	GGGCGCGGCACCAGCACTACC	CTCCGAGGCCACCGAAGACAAGATG	71
311	wmc728	GCAGGCTCTGCATCTTCTTG	CGCAGAGCTGAGCTGAAATC	60
312	cfa2121	TAAATGGCCATCAAGCAATG	GCTTGTGAACTAATGCCTCCC	54
313	gdm146	ATCCTGACGGCCACCAC	CAAAGCCTGCGATACATCAA	56
314	gwm66	CCAAAGACTGCCATCTTTCA	CATGACTAGCTAGGGTGTGACA	56
315	gwm274	AACTTGCAAAACTGTTCTGA	TATTTGAAGCGGTTTGATTT	50
316	barc140	CGCCAACACCTACCATT	TTCTCCGCACTCACAAAC	52
317	cfd2	GGTTGCAGTTTCCACCTTGT	CATCTATTGCCAAAATCGCA	54
318	barc156	CGCATCGAGGTCTTCCCCGCTGTCCAA	CGCACCCACACATGTATCTGAGTTTCCTA	70
319	barc142	CCGGTGAGAGGACTAAAA	GGCCTGTCAATTATGAGC	54
320	barc69	AGGCGGCGGTCGTGGAACA	GCGTACCGAGAAGTGATCAAGAACAT	64
321	gwm408	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	56
322	wmc118	AGAATTAGCCCTTGAGTTGGTC	CTCCCATCGCTAAAGATGGTAT	60
323	wmc640	AATTTATCTCGATCATGTGAGC	TGAGTAGTTCCCTTAGGACCTT	57
324	wmc783	AGGTTGGAGATGCAGGTGGG	TCTTCCTTCTCCTGCCGCTA	60
325	wmc258	GCGATGTCAGATATCCGAAAGG	ACCAGGACACCAGAACAGCAAT	62
326	wmc503	GCAATAGTTCCCGCAAGAAAAG	ATCAACTACCTCCAGATCCCGT	60
327	gwm234	GAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG	60
328	gwm499	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAAACTGCATAA	58
329	wmc386	ATCACTGAAACGAAATGAGCGG	TGGTTGGCGGTTTTTCTCTACA	60
330	wmc363	TCTGTAACGCATAATAGAATAGCCC	ATGATTGCGTTATCTTCATATTTGG	64
Chr5D				
331	barc130	CGGCTAGTAGTTGGAGTGTTGG	ACCGCCTCTAGTTATTGCTCTC	66
332	gwm190	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	58
333	barc205	GCGACAGTTGTAGCGGCAGTAGC	GAGCGTAGTAGAAGCAGAAGGAG	70
334	cfd81	TATCCCCAATCCCCTCTTTC	GTCAATTGTGGCTTGTCCCT	60
335	barc143	TTGTGCCAAATCAAGAACAT	GGTTGGGCTAGGATGAAAAT	54
336	barc44	CCCTACAAAATACGAACATGAAGTCAG	GGGTCCTACTCAGATAGTGACAGTCAAC	65
337	gdm136	CTCATCCGGTGAGTGCATC	CCCGCATGTCTACATGAGAA	58
338	cfd7	AGCTACCAGCCTAGCAGCAG	TCAGACACGTCTCCTGACAAA	59

339	gwm174	GGGTTCCTATCTGGTAAATCCC	GACACACATGTTCCTGCCAC	60
340	wmc215	CATGCATGGTTGCAAGCAAAAG	CATCCCGGTGCAACATCTGAAA	60
341	barc286	GCGAAGAAAACATTAGACCAAAA	GCGATATGTTTCCCGACAACTA	58
342	gwm654	TGCTGATGTTGTAAGAAGGC	TGCGTCAGATATGCCTACCT	56
343	barc347	GCGCACCTCTCCTCACCTTCT	GCGAACATGGAAATGAAAACTATCT	61
344	cfd86	TTAATGAGCGTCAGTACTCCC	GCAACCATGTTTAAGCCGAT	56
345	barc320	CGTCTTCATCAAATCCGAACTG	AAAATCTATGCGCAGGAGAAAC	58
346	wmc161	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	58
347	gwm469	CAACTCAGTGCTCACACAACG	CGATAACCACTCATCCACACC	61
348	barc93	GCCGGACGGATTTAGGTGGAGGAGA	CGCAACCTCACCATCACCGCCTCATC	71
349	wmc443	CCTCCTCTGTTTTCCCTCTGTT	CACACTCTGTGCTTCTGTTTGC	62
350	barc322	GAGAACATGAACGTGATTTACC	CGCAAACTTGTGTATCCTTATC	58
351	barc110	CCCGAACAATGGCTTTGGTGTCGTAAT	CATGGTGACGGCAAGTGTGAGGT	66
352	barc177	GCGATCCTGTTGTTGAGCGTTTGCATAA	TCCCGTTTTCCCGTGTGTTAGTCTA	66
353	barc144	GCGTTTTAGGTGGACGACATAGATAGA	GCGCCACGGGCATTTCTCATAC	66
354	gwm182	TGATGTAGTGAGCCCATAGGC	TTGCACACAGCCAAATAAGG	56
355	gdm63	GCCCCCTATTCCATAGGAAT	CCTTTTGATGGTGCATAGGA	56
356	wmc97	GTCCATATATGCAAGGAGTC	GTACTCTATCGCAAAACACA	54
357	wmc630	ATAATGCACGGTAGGACTGAGG	CATACTGAGACAATTTGGGGGT	60
Chr6A				
358	gwm459	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	57
359	gwm334	AATTTCAAAAAGGAGAGAGA	AACATGTGTTTTTAGCTATC	50
360	barc23	GCGTGAAATAGTGCAAGCCAGAGAT	GCGCTAACACCTCGGCAAGACAA	66
361	wmc182	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	58
362	barc37	CAGCGCTCCCCGACTCAGATCCTT	GCGCCATGTTTCTTTTATTACTCACTTT	64
363	wmc672	GGAGGAGCAAGCTAGGCAA	TTTATAGAGGGAGGGGAGGCAG	59
364	barc3	TTCCCTGTGTCTTTCTAATTTTTTTT	GCGAACTCCCGAACATTTTTAT	58
365	gwm570	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	56
366	wmc179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	64
367	barc195	CCCACATGTCATTGGCTGTTTAA	GCCCGGCCCAGAACGATTTAAATG	61
368	barc113	GCGCACAACAACGGACACTTAACAATT	GGGACTCATTTAGCTTCTACTCGCCATTA	67
369	barc204	CGCAGAAGAAAAACCTCGCAGAAAAACC	CGCAGTGTATCCAAATGGGCAAGC	67
370	gwm169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	62
371	wmc417	GTTCTTTTAGTTGCGACTGAGG	CGATGTATGCCGTATGAATGTT	58
372	wmc580	AAGGCGCACAACACAATGAC	GGTCTTTTGTGCAGTGAACTGAAG	58
373	gwm617	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	60
374	wmc621	GACGTAGGGCGGCGGATA	TGCGCCGTGTTTAATTGCTC	58
375	wmc254	AGTAATCTGGTCCTCTCTTCTTCT	AGGTAATCTCCGAGTGCACTTCAT	62
376	wmc59	TCATTCGTTGCAGATACACCAC	TCAATGCCCTTGTTTCTGACCT	60
377	wmc446	CCAGCTAGTACTCTATATCTACATC	TATTTGAACAAGAGTTATGTGG	55
378	wmc256	CCAAATCTTCGAACAAGAACCC	ACCGATCGATGGTGTATACTGA	60
379	wmc201	CATGCTCTTTCACTTGGGTTCG	GCGCTTGCAGGAATTCAACACT	62
Chr6B				
380	gwm613	CCGACCCGACCTACTTCTCT	TTGCCGTCGTAGACTGG	52
381	wmc487	CAAATTTGGCCACCATTTTACA	CGGTTCAATCCTTGGATTTACA	58
382	wmc104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	58
383	gwm518	AATCACAACAAGGCGTGACA	CAGGGTGGTGCATGCAT	55
384	barc198	CGCTGAAAAGAAGTGCCGCATTATGA	CGCTGCCTTTTCTGGATTGCTTGTCA	66
385	barc24	CGCCTCTTATGGACCAGCCTAT	GCGGTGAGCCATCGGGTTACAAAG	64
386	barc178	GCGTATTAGCAAAACAGAAGTGAG	GCGACTAGTACGAACACCACAAAA	62
387	barc134	CCGTGCTGCAAATGAACAC	AGTTGCCGGTTCCCATTGTCA	57
388	gwm219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	61

389	gwm132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCTTCCCC	58
390	wmc105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	60
391	wmc486	CCGGTAGTGGGATGCATTTT	ATGCATGCTGAATCCGGTAA	56
392	gwm133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	58
Chr6D				
393	cfd49	TGAGTTCTTCTGGTGAGGCA	GAATCGGTTCACAAGGGAAA	56
394	cfd135	GGATCTCGGGGATGTCCT	TAAGCACCTTCTTCATGGGG	56
395	barc173	GGGGATCCTTCAACAATAACA	GCGAGATGGCATTTTTAAATAAAGAGAC	57
396	cfd13	CCACTAACCAAGCTGCCATT	TTTTTGGCATTGATCTGCTG	54
397	wmc749	GGGTACAGGAGGATCTGACAGG	TCTCGTCTCCGTCTAGGTTCG	63
398	cfd132	CAAATGCTAATCCCCGCC	TGTAAACAAGGTCGCAGGTG	56
399	barc54	GCGAACAGGAGGACAGAGGGCACGAGAG	GCGCTTTCCCACGTTCCATGTTTCT	67
400	cfd287	TCAAGAAGATGCGTTCATGC	GGGAGCTTTCCCTAGTGCTT	56
401	wmc469	AGGTGGCTGCCAACG	CAATTTTATCAGATGCCCGA	52
402	wmc786	GGGTCACCAACCCGCTC	CGTGGGTGCAATTCTCAGG	59
403	barc1121	GCGAGCAAACTGATCCCAAAAAG	TATCGGTGAGTACGCCAAAAACA	61
404	barc175	GCGTAACAGAAGCGGAGAAAGC	GCGAATCATTTAGTGTTAGGTGGCAGTG	64
405	barc96	AAGCCTTGTTGTTCCGTATTATT	GCGGTTTATATTTTGTGGTTGAGCATTTT	58
406	gdm132	ACCGCTCGGAGAAAATCC	AGGGGGGCAGAGGTAGG	56
407	gdm98	CCATCCATGAAATGGCG	GCCCTTCACTAGCCTTCATG	50
Chr7A				
408	wmc158	AACTGGCATCATGTTTTGTAGG	AATGTAGTCAAAAGAGGTGGTG	60
409	gwm350	ACCTCATCCACATGTTCTACG	GCATGGATAGGACGCCC	54
410	gwm471	CGGCCCTATCATGGCTG	GCTTGCAAGTTCCATTTTGC	56
411	wmc479	GACCTAAGCCCAGTGTCATCAG	AGACTCTTGGCTTTGGATACGG	66
412	wmc168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG	58
413	gwm60	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG	58
414	cfa2049	TAATTTGATTGGGTCGGAGC	CGTGTCGATGGTCTCCTTG	56
415	barc127	TGCATGCACTGTCCTTTGTATT	AAGATGCGGGCTGTTTTCTA	56
416	cfa2028	TGGGTATGAAAGGCTGAAGG	ATCGCGACTATTCAACGCTT	56
417	barc64	GCG GAG TCT GCA ATT AGT ATA GGT AT	GCA TCC ACC TCC GCA GTC AGT	65
418	wmc826	GAGGTAGATGACCACGCCG	CACGATCCCCCAAGCAC	57
419	barc174	TGGCATTTTTCTAGCACCAATACAT	GCGAACTGGACCAGCCTTCTATCTGTTC	61
420	barc108	GCGGGTCGTTTCCTGGAAATTCATCTAA	GCGAAATGATTGGCGTTACACCTGTTG	68
421	barc121	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	59
422	barc29	GCACGCAGGAGCACCACCACGAC	GCGAGAGTAAGCAGCACCGAGGCACGAC	72
423	gwm282	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	52
424	wmc633	ACACCAGCGGGGATATTTGTTAC	GTGCACAAGACATGAGGTGGATT	63
425	wmc525	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT	60
426	cfa2040	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT	56
427	wmc809	CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA	57
428	barc275	GCG TTT GGT CAG AAT AGG GAA GAT	GCG TAT GTT CGT GTT AGT GTT GGT TAT GC	64
429	gwm130	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	60
430	wmc9	AACTAGTCAAATAGTCGTGTCCG	GTCAAGTCATCTGACTTAACCCG	61
431	gwm332	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	56
432	wmc139	TGTAACTGAGGGCCATGAAT	CATCGACTCACAACTAGGGT	56
433	wmc603	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	62

Chr7B				
434	gwm569	GGAAACTTATTGATTGAAAT	TCAATTTTGACAGAAGAATT	48
435	barc65	CCCATGGCCAAGTATAATAT	GCGAAAAGTCCATAGTCCATAGTCTC	54
436	barc72	CGTCCTCCCCCTCTCAATCTACTCTC	CGTCCCTCCATCGTCTCATCA	63
437	barc176	GCGAAAGCCATCAAACACTATCCAACT	GGTAACTAAGCACGTCACAAGCATAAA	65
438	barc278	GCATGCACTACGCTCAGAATAAAC	TAAAAGGCCCGTCAACATACAAGTA	63
439	gwm68	AGGCCAGAATCTGGGAATG	CTCCCTAGATGGGAGAAGGG	56
440	barc85	GCGAACGCTGCCCGGAGGAATCA	GCGTCGCAGATGAGATGGTGGAGCAAT	70
441	wmc476	TACCAACCACACCTGCGAGT	CTAGATGAACCTTCGTGCGG	60
442	gwm333	GCCCGGTCATGTAAAACG	TTTCAGTTTGCGTTAAGCTTTG	54
443	cfa2106	GCTGCTAAGTGCTCATGGTG	TGAAACAGGGGAATCAGAGG	58
444	wmc540	CGGGGTCCTAACTACGGTGA	CCTGTAATGGAGGACGGCTG	63
445	wmc517	ATCCTGACGTTACACGCACC	ACCTGGAACACCACGACAAA	58
446	wmc792	GGATGCAGTAGCAGTCAGGGA	CTCCATCGCTAGGCAGGG	61
447	barc20	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTCAGCCTTTT	63
448	wmc557	GGTGCTTGTTCATACGGGCT	AGGTCCTCGATCCGCTCAT	59
449	barc123	GGCCGAATTGAAAAAGCC	CCTGCCGTGTGCCGACTA	52
450	gwm146	CCAAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG	56
451	gwm344	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	52
452	wmc398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	56
453	wmc273	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	61
454	wmc323	ACATGATTGTGGAGGATGAGGG	TCAAGAGGCAGACATGTGTTCG	62
455	wmc396	TGCACTGTTTTACCTTCACGGA	CAAAGCAAGAACCAGAGCCACT	60
456	wmc10	GATCCGTTCTGAGGTGAGTT	GGCAGCACCCTCTATTGTCT	58
457	wmc526	TCCCATTGGTTCACAAACTCG	GATGGTATCGCATTCATCGGT	59
458	wmc70	GGGGAGCACCCTCTATTGTCTA	TAATGCTCCCAGGAGAGAGTCG	64
Chr7D				
459	wmc646	GGAGTAAATGGAGACGGGGAC	GCCAGTGTGATGCATGTGAC	60
460	barc154	GTAATTCCGGTTCCACTTGACATT	GGATGGGCAGCTTCAAGGTATGTT	62
461	barc352	CCCTTTCTCGCTCGCCTATCCC	CTGTTTCGCCCAATCTCGGTGTG	66
462	wmc450	GCAGGACAGGAGGTGAAGAAG	AGGCGTTGCTGATGACACTAC	61
463	barc126	CCATTGAAACCGGATTTGAGTCG	CGTTCCATCCGAAATCAGCAC	61
464	cfd41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC	60
465	barc214	CGCTTTCGGGACAGTGAAGGTGTAT	CGGTACGCGCGAGGAGGAAGAAGG	67
466	gdm88	TCCCACCTTTTTGCTGTAGA	AAGGACAAATCCCTGCATGA	56
467	wmc606	CCGATGAACAGACTCGACAAGG	GGCTTCGGCCAGTAGTACAGGA	64
468	barc26	GCGCTGGGTAAAAAGTGAAATTC	TGCAAGTGGAGGGGGAGGCGAGAG	61
469	barc87	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC	65
470	barc172	GCGAAATGTGATGGGGTTTATCTA	GCGATTTGATTTAACTTTAGCAGTGAG	62
471	barc105	CAGGAAGAAAAGGAAAGCATGCGACAA	GCGGTGTGGCAATAATTACTTTTT	60
472	barc111	GCGGTCACCAGTAGTTCAACA	GCGTATCCCATTGCTCTTCTTCACTAAC	61
473	wmc488	AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG	60
474	gwm121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	54
475	barc235	GCGCTCACCCTCCTACACTTCCTA	GCGCAAGTCTGTCAAAGCCTAA	62
476	cfd25	CATCGCTCATGCTAAGGTCA	CGTGTCTGTTAGCTGGGTGG	58
477	wmc824	CCGATGAACTTAAAAGTACCACCTG	CATGGATTGACACGATTGGC	58
478	barc53	GCGTCGTTCCTTTGCTTGTACCAGTA	GCGCGTCCTTCCAATGCAGAGTAGA	68
479	cfd69	AAATACCTTGAATTGTGAGCTGC	TCTGTTCATCCCCAAAGTCC	58
480	wmc14	ACCCGTCACCGGTTTATGGATG	TCCACTTCAAGATGGAGGGCAG	64
481	cfd175	TGTCGGGGACACTCTCTCTT	ACCAATGGGATGCTTCTTTG	56
482	gdm86	GGTCACCCTCTCCCATCC	GGCGCTCCATTCAATCTG	54
483	gwm295	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG	60

Annexure II

Grain iron and zinc content of *T. monococcum/ T. boeoticum* RIL population

RIL NO	IITR 2005	IITR 2006	PAU2005	PAU 2006	POOLED
RIL-1	17.16	18.69	20.08	16.73	18.17
RIL-2	29.91	30.24	34.98	35.55	32.67
RIL-3	29.05	27.14	33.97	32.44	30.65
RIL-4	36.73	38.12	42.44	39.12	39.10
RIL-6	69.10	71.11	69.01	69.44	69.67
RIL-8	36.62	35.87	42.83	40.66	38.99
RIL-9	28.22	29.42	24.22	24.55	26.60
RIL-10	30.68	32.89	35.88	31.42	32.72
RIL- 11	39.01	40.33	45.62	54.75	44.93
RIL-13	33.07	39.79	38.68	46.41	39.49
RIL-14	32.13	31.60	37.58	45.10	36.60
RIL-15	37.40	38.44	43.74	52.49	43.02
RIL-16	37.41	46.34	43.75	52.50	45.00
RIL-17	26.73	33.97	31.26	37.51	32.37
RIL-18	39.99	44.84	46.78	51.23	45.71
RIL-19	25.55	26.11	29.88	26.77	27.08
RIL-20	45.60	44.48	51.02	48.89	50.94
RIL-22	31.27	30.65	36.58	32.44	32.73
RIL-23	48.87	46.92	50.82	58.92	55.48
RIL-24	28.13	28.62	32.90	33.67	30.83
RIL-26	22.68	23.78	26.53	19.98	23.24
RIL-28	26.77	22.00	31.31	30.12	27.55
RIL-29	24.22	22.28	16.62	13.11	19.06
RIL-30	28.35	28.78	33.16	27.12	29.35
RIL-32	23.17	17.08	27.10	25.47	23.20
RIL-33	20.97	21.75	24.53	23.06	22.58
RIL-34	18.62	14.36	21.78	20.47	18.81
RIL-38	31.97	25.54	37.39	35.15	32.51
RIL-40	36.42	30.51	42.60	40.04	37.39
RIL-41	51.00	54.61	58.80	55.27	54.92
RIL-42	49.90	47.71	51.80	55.27	51.17
RIL-44	37.19	34.37	43.50	40.89	38.99
RIL-46	30.07	36.56	35.17	33.06	33.72
RIL-47	51.56	43.69	53.87	50.66	49.95
RIL-48	34.82	34.83	40.70	38.26	37.15
RIL-49	29.15	21.31	27.87	29.90	27.06
RIL-50	32.91	32.29	38.50	36.19	34.97
RIL-52	31.97	28.06	29.76	32.67	30.62
RIL-53	25.83	30.04	30.22	28.40	28.62

RIL-55	29.38	33.83	34.37	32.31	32.47
RIL-56	30.90	29.78	36.14	33.98	32.70
RIL-57	31.89	28.77	37.30	35.06	33.25
RIL-58	32.47	36.65	37.98	35.70	35.70
RIL-60	31.86	28.50	28.89	27.12	29.09
RIL-61	28.83	26.61	33.72	31.70	30.21
RIL-62	24.73	28.13	28.92	27.18	27.24
RIL-64	21.29	29.33	19.23	18.08	21.98
RIL-65	24.30	24.56	28.42	26.71	26.00
RIL-66	19.29	22.84	22.56	21.78	21.62
RIL-68	38.48	38.97	45.00	41.90	41.09
RIL-70	26.81	29.53	31.36	37.23	31.23
RIL-72	23.57	29.44	27.56	29.12	27.42
RIL-73	25.62	20.91	29.96	22.78	24.82
RIL-75	26.35	20.37	30.82	31.89	27.36
RIL-77	33.25	33.25	30.99	32.66	32.54
RIL-78	27.16	24.79	31.76	25.46	27.29
RIL-79	27.00	26.45	31.58	23.44	27.12
RIL-80	22.46	28.86	26.27	27.90	26.37
RIL-82	27.55	30.74	32.22	30.42	30.23
RIL-83	25.25	20.19	29.53	21.87	24.21
RIL-84	22.38	16.05	26.17	19.98	21.14
RIL-86	28.23	33.59	33.01	29.12	30.99
RIL-87	27.85	32.61	32.57	31.45	31.12
RIL-88	22.12	20.36	24.42	17.42	21.08
RIL-90	30.53	30.96	35.71	31.23	32.11
RIL-91	29.87	27.89	24.44	30.33	28.13
RIL-92	34.70	34.35	40.58	38.17	36.95
RIL-96	25.11	28.26	29.36	27.62	27.59
RIL-99	30.12	35.50	30.65	35.52	32.95
RIL-100	26.98	26.30	31.56	29.68	28.63
RIL-102	32.71	36.04	38.26	35.98	35.75
RIL-103	26.11	23.21	25.66	27.11	25.52
RIL-104	36.22	44.28	21.12	44.12	36.44
RIL-105	36.75	35.79	42.98	40.83	39.09
RIL-106	28.71	31.45	33.58	31.90	31.41
RIL-107	42.44	42.87	49.90	51.20	46.60
RIL-108	32.13	32.35	37.58	35.70	34.44
RIL-110	37.88	37.55	38.99	42.11	39.13
RIL-113	32.13	32.88	37.58	35.70	34.58
RIL-115	31.55	33.05	36.90	35.06	34.14
RIL-117	41.22	38.88	43.44	45.22	42.19
RIL-118	28.82	30.01	33.71	32.02	31.14
RIL-121	28.85	24.54	33.74	32.06	29.80

RIL-122	29.95	25.63	35.03	33.28	30.97
RIL-124	30.47	27.84	35.64	33.86	31.95
RIL-127	35.88	38.44	40.64	39.22	38.55
RIL-128	30.34	32.84	35.48	38.56	34.31
RIL-129	55.89	57.34	54.12	56.44	55.95

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13. **Tiwari, V.K.**, Rawat, N., Singh, N., Randhawa, G.S., Singh, K., Chhuneja, P., Dhaliwal, H.S. 2008. Preferential elimination of group 1 chromosome in sitopsis species wheat-*Aegilops* amphiploids (Communicated).

Publications

Annexure III

Morphological data of landraces along with wheat cultivars

Accessions	Plant height	Tiller number	No. of SpikletS	Grain color	Spike color	Powdery meildew	Leaf ru
8D	81.66	4	15.66	Amber		9	10
9D	86.5	3.5	16.25	Amber		9	10
C273	96.2	4.6	17.2	Amber	White	9	20
NP4	84.75	4	16.25	Amber	White	S	40
C518	79	5.75	16.2	Amber		9	10
C591	93.4	5.4	18.4	Amber		9	20
Chinese Spring	104	4.6	15.6		White	S	20
C306	100.8	5.8	16.8	Amber	White	9	60
Kalyan Sona	80.4	3.2	20	Amber	White	9	80
W6357	81.8	4	14.4	Amber		9	60
UP262	75	3.8	15	Amber		9	10
WL711	81.4	2.8	17	Amber		9	10
PBW343	68.75	3.25	17	Amber	White	9	40
PBW502	72.25	4.25	18	Amber	White	9	40
IITR-7	75	2	14	Amber	White	5	
IITR-8	80.5	3.8	18	Amber	White	9	10
IITR-9	124.4	5.2	16.8	Amber		9	60
IITR-11	100.2	3.8	16		White	7	80
IITR-13	105.2	4.4	16.6			6	40
IITR-15	99.6	3.4	16.8			9	40
IITR-16	105.8	3.8	18.8		White	5	60
IITR-17							.
IITR-18	105.4	4.8	18.2	/White	White	7	60
IITR-19	111.4	3.6	16.6			9	60
IITR-20	125.4	3.4	19.8		White	7	40
IITR-21	115	2.8	19.6	White	White	9	80
IITR-22	85	6.5	13.5		White	9	60
IITR-23	114	4.2	18.2	Amber/White		6	40
IITR-24	96	4	17.6	/White		7	60
IITR-25	105.8	4.6	18.6		White	6	60
IITR-26	106	4.2	18	White	White	8	20
IITR-27	93	4.2	16.6			9	80
IITR-28	110.6	5	18.4	White	White	9	20
IITR-29	100.6	5.8	15.8		White	6	20
IITR-30	89.4	6.6	14.2		White	5	40
IITR-31	109.6	5.4	18.75	White	White	6	80
IITR-32							
IITR-33							
IITR-34	102	9	16.4	White	White	4	40
IITR-65	104.8	9.6	18	White		2	80
IITR-66	86.66	7.33	14.66		White	2	80
IITR-67	99.6	7.2	16.6			7	80
IITR-68	100.75	6	17		White	4	60

IITR-69	99.8	4.6	15.6	White		4	40
IITR-70	98.2	6.4	16.6	White	White	9	60
IITR-71	100.4	5.2	16.6		White	6	60
IITR-72	120.6	6.4	17.6	White	White	7	40
IITR-73	93	5	15.2	White	White	6	60
IITR-74	95	4	16.2	White		5	60
IITR-75	96.4	4.2	16.4	White	White	2	60
IITR-76	106.8	4.2	17.8	White	White	2	60
IITR-77	87.5	4.5	14.5	White		2	40
IITR-78	104.4	5.2	16.6		White	7	40
IITR-79	93.4	4.4	17.2		White	6	40
IITR-80	70.6	5.75	15	Amber	White	7	40
IITR-81	87..75	4	16.75		White	5	20
IITR-82	100.2	4.6	16.2	/White	White	6	40
IITR-83	95.2	4	15.4	/White		8	20
IITR-84	91.8	4.2	15.8			8	40
IITR-85	122.6	4.2	22	White	White	9	20
IITR-86	122.6	4.4	20.6	White	White	8	40
IITR-87	94	4	18.8	White	White	9	10
IITR-88	115	4.6	18.8		White	2	40
IITR-89	111.6	7	20.2		White	8	40
IITR-90	91.2	6	16.6	White	White	9	40
IITR-91	84.6	5.8	15.8		White	7	60
IITR-92	102.8	10.6	18			0	20
IITR-93	90.2	9.8	16		White	5	20
IITR-94	94.8	7	16.6		White	8	20
IITR-95	85.8	6.2	15.4	White	White	8	60
IITR-97	122.8	6.4	17.8	White	White	7	0
IITR-98	103	7.6	17.2	White	White	8	40
IITR-99	91.2	3	17.6	White		1	0
IITR-100	84.66	9.66	16.33	White	White	9	40
IITR-101	80.33	5.2	17.33	White	White	7	0
IITR-102	88	6.4	14.8	White	White	4	80
IITR-103	97.4	5.2	16.6	Amber		5	40

Annexure IV

Grain iron, zinc content and hardness index of a set of landraces along with a set of wheat cultivars

Accession/cultivar/landrace	Mean± SD. Zinc content (mg/kg)	Mean± SD. Iron content (mg/kg)	Hardness index SKCS
T. aestivum cv C-306	21.61 ± 0.34	32.21 ± 1.49	97.68
T. aestivum cv 8A	21.89 ± 0.87	26.86 ± 1.24	101.11
T. aestivum cv 9D	21.52 ± 0.34	32.10 ± 1.48	99.45
T. aestivum cv C273	20.15 ± 0.32	26.43 ± 1.22	102.85
T. aestivum cv NP4	18.78 ± 0.90	24.72 ± 1.14	103.43
T. aestivum cv C518	18.78 ± 1.12	24.61 ± 1.14	104.67
T. aestivum cv C591	16.23 ± 1.11	21.29 ± 0.98	108.83
T. aestivum cv Kal.Sona.	20.54 ± 0.87	26.96 ± 1.25	104.42
T. aestivum cv WG 357	15.35 ± 0.99	20.22 ± 0.94	91.52
T. aestivum cv UP262	20.73 ± 1.45	27.18 ± 1.26	81.64
T. aestivum cv WL711(NN)	18.50 ± 1.22	25.52 ± 0.42	87.77
T. aestivum cv PBW343	20.05 ± 0.96	29.00 ± 1.34	83.34
T. aestivum cv PBW502	18.00 ± 0.66	26.00 ± 1.20	87.22
T. aestivum lr Chinese Spring	18.29 ± 0.34	30.50 ± 1.41	62.13
T. aestivum lr Naphal	20.64 ± 0. 33	25.04 ± 1.16	40.90
T. aestivum lr IITR 8	17.12 ± 0.27	22.26 ± 1.03	101.50
T. aestivum lr IITR 9	22.13 ± 0.31	29.75 ± 1.38	42.10
T. aestivum lr IITR 11	20.81 ± 0.30	33.60 ± 1.55	113.87
T. aestivum lr IITR 13	22.44 ± 0.44	29.64 ± 1.37	107.41
T. aestivum lr IITR 15	21.32 ± 0.30	33.71 ± 1.56	99.41
T. aestivum lr IITR 16	18.67 ± 0.26	23.54 ± 1.09	100.12
T. aestivum lr IITR 17	28.05 ± 1.12	36.54 ± 1.23	114.85
T. aestivum lr IITR 18	19.71 ± 2.40	28.46 ± 2.64	117.74

T. aestivum lr IITR 19	26.52 ± 1.98	33.28 ± 2.18	94.42
T. aestivum lr IITR 20	25.18 ± 3.10	40.12 ± 3.75	109.87
T. aestivum lr IITR 21	22.08 ± 1.10	33.53 ± 1.33	43.70
T. aestivum lr IITR 22	21.98 ± 1.50	28.46 ± 1.82	100.82
T. aestivum lr IITR 23	29.43 ± 2.10	39.11 ± 2.54	96.28
T. aestivum lr IITR 24	28.30 ± 3.33	39.98 ± 4.33	108.86
T. aestivum lr IITR 25	29.66 ± 2.11	38.64 ± 2.74	103.37
T. aestivum lr IITR 26	30.34 ± 1.99	38.82 ± 2.59	90.81
T. aestivum lr IITR 27	26.83 ± 2.78	37.74 ± 3.61	63.76
T. aestivum lr IITR 28	27.51 ± 3.10	38.22 ± 4.03	84.88
T. aestivum lr IITR 29	23.89 ± 2.44	29.61 ± 2.64	101.73
T. aestivum lr IITR 30	24.90 ± 3.21	39.38 ± 3.47	87.40
T. aestivum lr IITR 31	22.19 ± 0.99	34.65 ± 1.07	49.40
T. aestivum lr IITR 32	23.45 ± 1.12	25.43 ± 1.21	113.59
T. aestivum lr IITR 33	27.29 ± 0.83	28.46 ± 1.74	95.96
T. aestivum lr IITR 34	22.13 ± 2.12	39.12 ± 3.77	99.75
T. aestivum lr IITR 65	24.09 ± 1.22	39.38 ± 2.56	53.74
T. aestivum lr IITR 66	23.65 ± 1.24	48.84 ± 2.60	88.27
T. aestivum lr IITR 67	17.82 ± 4.12	28.60 ± 4.52	33.30
T. aestivum lr IITR 68	28.49 ± 1.18	39.96 ± 2.48	79.35
T. aestivum lr IITR 69	26.07 ± 2.66	31.80 ± 3.51	102.14
T. aestivum lr IITR 70	16.28 ± 1.05	24.49 ± 0.98	60.35
T. aestivum lr IITR 71	26.40 ± 1.70	41.66 ± 1.67	91.21
T. aestivum lr IITR 72	22.88 ± 1.47	31.91 ± 1.28	80.40
T. aestivum lr IITR 73	25.30 ± 1.63	32.97 ± 1.32	49.65
T. aestivum lr IITR 74	23.43 ± 1.51	27.35 ± 1.09	43.18
T. aestivum lr IITR 75	26.10 ± 1.98	30.42 ± 1.22	75.29
T. aestivum lr IITR 76	27.22 ± 2.06	39.86 ± 1.60	47.35
T. aestivum lr IITR 77	26.54 ± 2.01	31.16 ± 1.25	62.13
T. aestivum lr IITR 78	27.66 ± 2.10	36.57 ± 1.46	109.65

T. aestivum lr IITR 79	23.07 ± 1.75	33.07 ± 1.32	98.42
T. aestivum lr IITR 80	24.86 ± 1.88	29.47 ± 1.18	58.65
T. aestivum lr IITR 81	24.30 ± 1.84	25.28 ± 0.36	72.99
T. aestivum lr IITR 82	23.86 ± 1.81	31.56 ± 0.46	60.29
T. aestivum lr IITR 83	29.90 ± 4.80	27.74 ± 0.17	73.27
T. aestivum lr IITR 84	24.53 ± 1.86	29.50 ± 0.43	80.76
T. aestivum lr IITR 85	23.74 ± 1.80	24.01 ± 0.35	84.82
T. aestivum lr IITR 86	21.84 ± 1.65	27.64 ± 0.40	86.02
T. aestivum lr IITR 87	28.78 ± 2.18	33.42 ± 0.48	99.59
T. aestivum lr IITR 88	22.51 ± 1.71	23.13 ± 0.33	87.39
T. aestivum lr IITR 89	17.79 ± 2.18	17.93 ± 0.26	95.64
T. aestivum lr IITR 90	21.78 ± 2.67	20.19 ± 0.29	74.63
T. aestivum lr IITR 91	27.47 ± 3.37	20.97 ± 0.30	94.06
T. aestivum lr IITR 92	25.29 ± 3.10	23.91 ± 0.35	105.24
T. aestivum lr IITR 93	20.69 ± 2.54	21.66 ± 0.31	106.76
T. aestivum lr IITR 94	22.26 ± 2.73	21.74 ± 0.73	106.14
T. aestivum lr IITR 95	30.25 ± 1.11	32.87 ± 3.71	56.23
T. aestivum lr IITR 96	27.71 ± 0.84	25.10 ± 3.40	84.58
T. aestivum lr IITR 97	27.47 ± 0.94	27.83 ± 3.37	83.81
T. aestivum lr IITR 98	20.81 ± 1.00	29.72 ± 2.55	110.30
T. aestivum lr IITR 99	16.94 ± 0.52	15.33 ± 2.08	79.98
T. aestivum lr IITR 100	22.99 ± 2.82	19.95 ± 0.67	96.38
T. aestivum lr IITR 101	27.23 ± 3.34	27.09 ± 0.91	80.81
T. aestivum lr IITR 102	35.21 ± 4.32	29.61 ± 1.00	40.82
T. aestivum lr IITR 103	25.53 ± 3.13	24.36 ± 0.82	86.77