DEVELOPMENT AND MOLECULAR ANALYSIS OF HIGH GRAIN IRON AND ZINC WHEAT-*AEGILOPS* DERIVATIVES

A THESIS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **DEVELOPMENT AND MOLECULAR ANALYSIS OF HIGH GRAIN IRON AND ZINC WHEAT-*AEGILOPS* DERIVATIVES** 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 August 2009 under the supervision of Dr. H.S. Dhaliwal, Professor, Department of Biotechnology and Dr. S.K. Tripathi, Professor, Department of Water Resources Development and Management, 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.

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Abstract

1. ABSTRACT

Biofortification of crops is the most feasible and economical approach for overcoming the global challenge of iron and zinc deficiency which currently affects over 3 billion people of the world. Wheat is the staple food crop of about one-third of the world population, but popular wheat cultivars have very low grain micronutrient content indicating the importance of exploration and utilization of its gene pools for transfer of useful variability for biofortification for grain iron and zinc. Eighty accessions of nine species of wild *Triticum* and *Aegilops*, namely *Ae. peregrina* (US), *Ae. longissima* (S^l), *Ae. kotschyi* (US), *Ae. ovata* (UM), *Ae. cylindrica* (CD), *Ae. ventricosa* (DN), *T. dicoccoides* (AB), *T. araraticum* (AB), and *T. boeoticum* (A) were evaluated for their grain micronutrient contents. *Aegilops* species showed up to 2-3 folds higher grain iron and zinc contents than the bread and durum wheat cultivars. Interspecific hybridization was done between a wheat cultivar and selected accessions of *Ae. peregrina* (1155-1-1, 13772, 3519, 3477 and 1155-5-3). The F_1 hybrids with the expected chromosome number 35, had little chromosomal pairing, high male and fermale sterility and had no seed set. Therefore, extensive backcrossing was done to get some BC_1 seeds. The BC_1 plants were further backcrossed as they also did not set seeds. The resulting fertile BC_2 plants were screened for high iron and zinc content. Twelve BC_2F_2 plants with recovered wheat background and high grain iron and zinc content were further characterized using cytology, HMW- glutenin profiles, anchored wheat microsatellite markers and GISH/ FISH. Application of SSR markers showed introgression of chromosomes of group 4 and 7 of *Ae. peregrina* in the selected derivatives. Further application of GISH/ FISH revealed translocation of 7U in one of the derivatives whereas in others addition of 7U and 4S and 7S was seen. Thus it may

be concluded that chromosomes of group 4 and 7 of *Ae. peregrina* had genes/ QTL for high grain iron and zinc content.

Two synthetic amphiploids of *Triticum aestivum* (AABBDD) landrace Chinese Spring (*Ph*^{I}) with *Ae. peregrina* (UUS^{I}S^{I}) accessions 3477 and 1155-5-3 were developed through colchicine treatment of sterile F_1 hybrids. The F_1 hybrids and amphiploid plants were intermediate between the parents for plant morphology and spike characteristics. The amphiploids of advanced generations, however, had variable frequency of univalents at meiotic metaphase-I. HMW glutenin subunits of amphiploids along with the parents showed the additive presence and expression of the parental genomes in the amphiploids. The amphiploids with bolder seeds had higher grain iron and zinc content than the wheat parents and comparable to those of their *Ae. peregrina* parents suggesting that *Ae. peregrina* possesses distinctive genetic system for the micronutrient uptake and/or translocation than the wheat cultivars. The variable chromosome number in PMCs in advanced generation of amphiploids suggests somatic chromosome elimination in the amphiploids. The amphiploids can be used for transfer of high grain iron and zinc content to wheat and development of alien addition and substitution lines.

A few *T. aestivum* cultivars and accessions of the *Aegilops* species were investigated for the release of phytosiderophores *in vitro* under iron and zinc sufficient and deficient media and root and shoot iron and zinc conent was estimated. All the *Aegilops* species had significantly higher mean and range with 3-4 times higher release of phytosiderophores than the wheat cultivars under both nutrient sufficient and deficient conditions throughout the investigation. The wheat cultivars as well as *Aegilops* species had comparable rate of increase of phytosiderophores in iron or zinc deficient media with peak at 11th and 14th days, respectively which

leveled off rapidly among the wheat cultivars and continued to be high among *Aegilops* species. The absolute amount of iron and zinc expressed on the shoot and root dry weight basis after 18th days on iron and zinc deficient media showed nearly three times higher concentration in both roots and shoots of *Aegilops* species than that of the wheat cultivars. *Aegilops* species secrete higher amounts of phytosiderophores than *T. aestivum* cultivars under micronutrient deficient as well as sufficient media. Further more the deficiency symptoms like chlorosis started to appear later in the *Aegilops* species than the cultivars. Fertile introgressive derivatives of *Ae. peregrina* were also found to secrete 2-3 times higher amount of phytosiderophores than the wheat cultivars. The higher grain iron and zinc content in the *Aegilops* species reported earlier may be attributed to their diverse and efficient mechanism for phytosiderophore mediated micronutrient uptake and translocation system which could be exploited for biofortification of wheat.

Wheat-*Aegilops* addition lines for chromosomal identification of the genes controlling high grain iron and zinc content and also for the release of phytosiderophores. Addition lines of group 2 (U/S), 4 U and 7 U of *Ae. peregrina*, 1S, 2S and 5S, 6S, 7S of *Ae. longissima* and 2 U and 5U of *Ae. umbellulata* in Chinese spring background were found to harbour genes for high grain iron whereas for zinc content, mainly group 7 chromosomes was found responsible. Higher release of phytosiderophores was observed in addition lines of group 2 (U/S), 4 (U) and 7(S) of *Ae. peregrina*, 2S^{l} and 6 S^{l} of *Ae. longissima* and 2U and 5U of *Ae. umbellulata.* Addition lines with high grain iron and zinc content also showed higher phytosiderophore release. Significant positive correlation (r^2=0.56) between grain iron and zinc content and phytosiderophore release under iron deficient media strongly suggests that higher release of phytosiderophores may be responsible for higher grain

iron and zinc in these addition lines. These addition lines with two to three fold high grain iron and zinc content could be used for precise introgression of genes to the elite wheat cultivars for the improvement in their grain micronutrient content and enhanced efficiency of uptake of these minerals in problematic soils

A diploid wheat Recombinant Inbred Line (RIL) population of *T. monococcum* accession pau14087 x *T. boeoticum* accession pau5088 was studied for phytosiderophore release under iron deficiency conditions over 3 years. The amounts of phytosiderophore released varied from 3.0 – 22.1 µg Fe mobilized per plant. A linkage map with 169 molecular markers available for the RIL population was used for mapping QTL for phytosiderophore release. The QTL mapping led to the identification of two highly linked QTL for phytosiderophore release on chromosome 6A. These two QTL were mapped in the marker intervals *Xbarc113- Xgwm670* and *Xgwm670 - Xgwm1017* explaining 15.8 % and 21.6 % contribution of the total phenotypic variation respectively. The chromosomal locations of the QTL on group 6 in the RIL population is also supported by the facts that *Ae. longissima* addition line with $6S^l$ chromosome has high phytosiderophore release and in barley also the genes for hydoxy-mugineic acid synthesis are located on chromosome 6H which is syntenous to wheat chromosome 6.

The precise transfer of *Ae. peregrina* 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 phytosiderophore release will lead to thorough understanding of the pathways for their uptake from soil and translocation to grains.

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Kumari Neelam.

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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
HMA2	Heavy Metal Transporting ATPase2
HMW-GS	High Molecular Weight glutenin subunit
HPLC	High Performance Liquid Chromatography
ICPMS	Inductively Coupled Plasma Mass Spectrometer
IRT	Iron regulatory transporter protein
MA	Mugineic acid
mg/kg	Milligram per kilogram
MTP	Metal tolerance proteins
NRAMP	Natural Resistance Associated Macrophage Proteins
NAAT	Nicotinamine Amino Transferase
NAS	Nicotinamine Amino Synthase
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

Deficiency of micronutrients iron and zinc also known as hidden hunger leads to increased morbidity and mortality rates in humans (Bouis, 2007; Welch and Graham, 2004). Presently about half of the world population is suffering from iron and zinc malnutrition (WHO, 2007). Iron deficiency leads to anemia, lower birth weight of infants, impairment in cognitive skills and physical activity, chronic blood loss due to hook worm infestation and malaria in addition to poor neuropsychological function (Stein, 2005). Zinc deficiency too has serious health implications like impairment of physical growth, lower immunity, sterility in males, poor learning ability, development of cancers besides adversely affecting pregnancy (Palmgren, 2008; Cakmak, 2008; Stein, 2007). Not only humans, iron and zinc are also essential for plants for photosynthesis and respiration, chlorophyll synthesis, nitrogen fixation and as cofactors for numerous enzymes (Kim and Guerinot, 2007; Garrido *et al.*, 2006). Iron and zinc are unavailable to plants in soils with high pH.

Out of the various approaches to address micronutrient deficiency like supplementation, fortification, biofortification and dietary diversification, biofortification has been recognized as the most promising strategy (Zimmerman and Hurrel, 2007). Biofortification refers to the process of developing genetically improved food crops that are rich in bioavailable micronutrients, either through molecular breeding or genetic engineering (Johns and Eyzaguirre, 2007). Wheat is currently the primary staple food for almost one-third of the world's population (FAO, 2004). However, the popular bread wheat and durum wheat cultivars grown worldwide have low micronutrient content

(Rawat *et al.*, 2009; Cakmak *et al.*, 2000). Therefore biofortification of wheat is very crucial for enhancing its nutritional value.

Recommended cultivars of wheat have low micronutrient content whereas wild relatives of wheat have been reported to be rich source of useful variability for grain iron and zinc content (Rawat *et al.*, 2009; Monasterio and Graham, 2000) and can be effectively utilized for enhancing the micronutrient status of the elite cultivars. A practical approach to transfer the desirable trait to the latter would be wide hybridization with wild relatives having high iron and zinc content followed by precise transfer and selection of the desirable trait without linkage drag. Many genes for pest and disease resistance, abiotic stresses and quality traits have been transferred to wheat from wild relatives through interspecific hybridization (Jiang *et al.*, 1994; Friebe *et al.*, 1999; Marais, 2005). *Ph*^{l} gene transferred from *Ae. speltoides* (Chen *et al.*, 1994) has been frequently used successfully for induction of homoeologous chromosome pairing between wheat and non-progenitor genomes for precise transfer of useful variability (Aghaee-Sarbarzeh *et al.*, 2002). Application of GISH, FISH, C- banding, HMW glutenin profiles and molecular markers has been done for characterizing the transfers of the desired traits. The useful variability from related germplasm can be combined immortally with wheat genomes as amphiploids which may be used to develop translocation, addition and substitution lines to study and transfer desirable traits (Jiang *et al.*, 1994).

Graminaceous plants secrete metal chelators called phytosiderophores (PS) of mugineic acid (MA) family in the rhizosphere for taking up iron and zinc under their deficiency in soil (Curie *et al.*, 2001: Mori, 1990). The quantities and types of PS secreted by plants determine their tolerance to low mineral concentration in soil, for

example, rice, wheat, and maize secrete only 2-deoxymugineic acid (DMA) in relatively low amounts and are thus susceptible to low iron availability whereas barley secretes large amounts of many types of MAs, including MA, 3-hydroxymugineic acid, and 3-epi-hydroxymugineic acid and hence survives successfully under low iron availability conditions (Singh *et al.*, 1993). Evaluation of wild relatives of wheat for higher amounts of PS secreted will be highly useful for transfer to cultivars for enhancing their tolerance under physiological deficiency of iron and zinc.

Alien addition lines are important genetic resources for dissection, introgression, mapping and expression of useful variability from related species (Endo, 1990; McIntosh *et al.*, 2003; Endo and Gill, 1996). Wheat-barley and wheat-rye addition lines have been used for chromosomal mapping of genes involved in biosynthesis of various phytosiderophores (Ma *et al.*, 1999; Mori, 1990)

Genetics of phytosiderophores release has not been studied in wheat so far. No major locus or QTLs have been mapped for amount of phytosiderophores released in wheat. Understanding the genetic basis of phytosiderophore release will provide the basis for devising the plant breeding strategies for improving tolerance of wheat plants to physiological deficiency of iron and zinc by marker assisted selection and biofortification in wheat grains.

Investigation for the evaluation of several non-progenitor *Aegilops* species, wheat cultivars and landraces, for higher grain iron and zinc content and higher amounts of phytosiderophores released; their utilization for wheat biofortification through molecular breeding and mapping of phytosiderophore release 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. peregrina* into wheat cultivars.
3. Molecular and cytological characterization of introgressive derivatives with high grain iron and zinc content.
4. Development and characterization of *T. aestivum- Ae. peregrina* amphiploids with high grain iron and zinc content.
5. Analysis of the variability in amounts of phytosiderophores released under iron and zinc deficiency conditions of some popular wheat cultivars and related wild *Triticum* and *Aegilops* species.
6. Evaluation and identification of wheat-*Aegilops* addition lines cotrolling high grain iron and zinc content and phytosiderophore released.
7. Mapping of QTL for phytosiderophores released in diploid wheat *T. monococcum* x *T. boeoticum* RIL population.

Chapter II

Review of Literature

2. REVIEW OF LITERATURE

2.1 Micronutrient malnutrition in humans

Over three billion people of the world suffer from micronutrient malnutrition (Bouis, 2007; Welch and Graham, 2004) caused particularly by deficiency of iron and zinc in their diets (White and Broadley, 2005; Poletti *et al.*, 2004). Dietary deficiency of micronutrients such as iron, zinc, selenium, iodine and vitamin A, is most commonly known as hidden hunger, has serious implications on human health especially in developing countries (Ramakrishnan *et al.*, 2009; Bhaskaram, 2008; Holtz and Brown, 2004; Demment *et al.*, 2003). Over 30 % of the world population is severely affected by Iron deficiency anemia and is mainly prevalent in preschool aged children (47.4 %), pregnant women (41.8 %) and non pregnant women (30.2%) (FAO/ WHO, 2001, Mclean *et al.*, 2008). In south Asia and Africa relatively higher percentage (43% and 37%, respectively) of maternal deaths are caused due to iron deficiency anemia (Monasterio *et al.*, 2007, Stoltzfus, 2004). In India, prevalence of anemia was found to be 70-80% in children, 70 % in pregnant women and 24% in adult men (WHO, 2007). The most common consequences of low intake of iron are increase in morbidity and mortality rates, lower birth weight of infants, impairment in cognitive skills and physical activity, chronic blood loss due to hook worm infestation and malaria (Stein, 2005). It also adversely affects neuropsychological function.

A large proportion of world population (25%) is also affected by dietary deficiency of zinc (Palmgren, 2008). According to WHO reports on the major risk factor causing disease burden on humans, zinc deficiency ranks 11th among the 20 most important factors in the world and 5th among the 10 most important factors in developing

countries (Cakmak, 2008). In India, loss due to zinc deficiency is very high *i.e* 2.8 million disability-adjusted life years (Stein, 2007). Approximately 800,000 child deaths are reported worldwide due to zinc deficiency (Micronutrient Initiative, 2006). Zinc deficiency causes serious health implications such as impairment of physical growth, immune system, learning ability, cancer development and also has adverse effects during pregnancy.

2.2 Iron and zinc deficiency in cereals

Iron is also essential micronutrient for plant metabolism as it is involved in most of the redox processes of electron transport of photosynthesis and respiration, chlorophyll synthesis and nitrogen fixation (Kim and Guerinot, 2007; Garrido *et al.*, 2006). Though iron is the fourth most abundant mineral in the earth crust Most of this iron is unavailable to the plants as it is present in the form of hydroxide, oxide, phosphate and other complex form (Meng *et al.*, 2005; Graham and Welch, 2002). Zinc is involved as cofactor in nearly 300 enzymes and plays critical role in the structure of many proteins and transcription factors (Hershfinkel, 2006; Krämer and Clemens, 2000). Nearly half of the world's cereal growing area is affected by soil zinc deficiency (Cakmak, 2002) whereas one third area is iron deficient due to high soil pH (Mori, 1999). Inefficient uptake of these metals in calcareous or salt stressed alkaline soil by the plants, results into severe yield loss and poor nutritional quality of grains (Brown, 1961; Mori, 1990; Cakmak, 2008). Thus, micronutrient enriched cereal grains involving higher efficiency of uptake and translocation of the micronutrients to the grains is the first and foremost requirement for biofortification of cereal crops.

2.3 Reasons for micronutrient malnutrition

In developing countries, most of the daily calorie intake (59%) of poor people comes from carbohydrate rich cereals such as rice, wheat and maize (FAO, 2004) where vegetables, fruits, animal and fish products with high mineral content forms negligible proportion. All these staple food crops have very low content of micronutrients. This situation is further aggravated by various processing methods such as milling, polishing etc during which nutrients rich layer i.e. aleurone gets removed. As a consequence of this diet, based on only staple cereals is not sufficient to provide recommended dietary allowance (RDA). According to an IRRI report (2006), it is found that polished rice contains an average of only 2 mg kg^{-1} Fe and 12 mg kg^{-1} of Zn whereas RDA for Fe is 10-15 mg and 12-15 mg for zinc. For a better Zn or Fe nutrition for human beings, cereal grains should contain around 40-60 mg/kg each of Zn and Fe but in the present conditions available amount is in the range of 10-30 mg/kg (Cakmak *et al.*, 2000).

In cereals, most of the important nutrients, reside in the husk, aluerone layer and embryo. Major constraint in their utilization for human growth is their bioavailability. Mineral absorption from plant food source is very low. Presence of anti-nutritional factors such as phytic acid, polyphenols, fibers, certain tannins and haemagglutinins remarkably reduce the absorption of these micronutrients. Phytic acid chelates with metal cations (Fe^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+} etc) and renders them unavailable for humans as non ruminant animals have no or very limited phytase activity in their digestive track (Campion *et al.*, 2009; Zimmerman and Hurrel, 2007). There are certain organic acids, heme-protein, some aminoacids, long chain fatty acids, β-carotene, that promote Fe and Zn bioavailability (Graham *et al.*, 2001).

The deficiency of micronutrients in cereal grains is further aggravated by growing crops on potentially Zn and Fe deficient soils. Nearly half of the world's cereal growing area is affected by soil zinc deficiency (Cakmak, 2002) while one third is iron deficient (Mori, 1999). In calcareous soil or salt stressed alkaline soil, plants shows high degree of susceptibility to environmental stress such as drought stress, pathogenic infections, and development of deficiency symptoms like leaf necrosis, chlorosis and stunting growth. Consequently, inefficient uptake of these metals in problematic soils results into severe yield loss and poor nutritional quality of grains (Brown, 1961; Mori, 1990, Cakmak, 2008). The micronutrient content in grains also depends on the amount taken up by the roots during grain development and their remobilization and redistribution to the grain from vegetative tissue via phloem. The mobility of each element through phloem differs greatly. It has been found that zinc showed good remobilization; iron had intermediate mobility whereas Cu and Mn have lower phloem mobility (Pearson and Rengel, 1994; Kochian, 1991). In wheat and rice, only 4 – 5 % of the shoot iron is translocated to the grain at maturity (Marr *et al.*, 1995; Hocking, 1994). It necessitates the further detail studies of the pathways involved in the metal uptake and transport.

2.4 Strategies for alleviating micronutrient malnutrition

Dietary diversification and other nutritional interventions like supplementation, fortification and biofortification are some of the major approaches which have been suggested for the alleviation of micronutrient malnutrition (Zimmerman and Hurrel, 2007). Supplementation refers to additional supply of iron, zinc or other micronutrients in the form of capsules and tablets. Individuals with chronic deficiency of iron could be given iron in the form of ferrous iron salts *i.e* ferrous fumarate, ferrous sulphate and

ferrous gluconate which are best absorbed forms (Hoffman, 2000). Likewise zinc could be provided as zinc gluconate, zinc sulphate and zinc acetate. Fortification of foods involves addition of minerals to the food stuffs. Various examples of fortification are iodination of salt or fluro-fortification of toothpaste, fortification of flour with zinc oxide (20-50 mg/kg) and copper gluconate (1.0 – 3.0 mg/ kg) (Rosado, 2000). These methods have faced several difficulties such as fortified foods with high Fe are very sensitive to the oxidation process and it also increases loss of iodine. Similarly fortified rice with folate tends to be getting lost during boiling due to higher solubility. Field management of micronutrients is also not feasible due to use of sophisticated techniques, requirement of product control and recurring costs every time (Cakmak, 2002). Though consumption of diversified diet including meat, fish, fruits, vegetables, legumes is a sustainable approach, but change of dietary practices and preferences is difficult and expensive. Moreover, it is impractical in developing countries where poverty prevails and over three billion people earn less than US$ 2 per day (Zhu, *et al.*, 2007).

2.5 Biofortification:

Among the various intervention approaches to improve nutritional status of deprived human beings, biofortification of crops is the most promising, widely accepted, cost-effective and easily affordable (Zimmerman and Hurrel, 2002; Lonnerdal, 2003). Biofortification refers to the process of generating genetically improved food crops through conventional breeding or genetic modification that are rich in bioavailable micronutrients (Johns and Eyzaguirre, 2007). Various micronutrient initiative programmes are running worldwide. HarvestPlus had started biofortification programme

(www.harvetsplus.org) aiming at improving nutritional status of stable food crops with Zn, Fe and vitamin A by using plant breeding strategy (Lucca *et al.*, 2006; Pfeiffer and McClafferty, 2007). The biofortification of cereals combines techniques of conventional breeding, molecular breeding and genetic engineering (Hirschi, 2008; Nestel *et al.*, 2006; Bouis, 1999; DellaPena, 1999). Simplest method of biofortification relies on the addition of the required micronutrient as an inorganic compound to the fertilizer but its applicability depends on various factors such as soil composition, mineral mobility in soil and also within plant and its accumulation site. This strategy is useful for biofortification of some minerals (iodine and selenium), but could not be applied in general to all minerals (Hartikainen, 2005).

2.5.1 Genetic engineering for biofortification of cereals

Various transgenic strategies for nutritional fortification of cereals includes alteration in metabolic pathway for either increasing the amount of desirable compound, decrease in the amount of competitive compounds or extension of the biosynthetic pathway for the production of novel product (Capell and Christou, 2004). It also involves expression of recombinant proteins that makes minerals to be stored in bioavailable form such as ferritin which is an iron storage protein consisting of 24 subunit shell around a 4500-atom iron core (Theil, 2004). Ferritin resists denaturation during gastrointestinal digestion and it also protects itself from chelators during digestion and thus enhances iron absoption (Hicks, 2004). Ferritin gene expression has been demonstrated in a variety of plants including *Arabidopsis*, soybeans, beans, cowpeas, peas, and maize (Lukac *et al.*, 2009). Transgenic rice with 3 to 4.4 times higher grain iron level than wild type has been

reported (Vasconcelos *et al.,* 2003; Goto *et al.*, 1999). Use of constitutive promoter resulted in elevated iron level in the leaves of transgenic rice and wheat plants (Drakakaki, *et al.*, 2000).

Along with higher content of minerals in edible tissue, one important aspect is how much of these minerals get absorbed by human gut. Phytic acid being an antinutritional factor, poses major threat to the micronutrient bioavailability. It binds to the metal cation's such as Fe^{2+}, Zn^{2+} and Ca^{2+} and forms phytin and thus reduces their absorption to the human gut (Raboy, 2001). Reduction in phytic acid could be achieved through development of low phytic acid mutants in cereals and also by the development of thermostable phytase enzyme. Low phytic acid mutants have been identified in rice (Liu *et al.*, 2006), barley (Larson, 1998), maize (Shi *et al*; 2007; Pilu, 2003), wheat (Guttieri, 2004) and soybean (Yuan, 2007; Wilcox *et al.*, 2000). Nearly 55 to 60 % reduction in phytic acid phosphorus was found in these low phytic acid mutants. In tortillas made by *lpa* maize, 49% increase in Fe bioavailabilty has been observed as compared with wild type maize (Mendoza *et al.*, 1998).

Production of transgenic seeds with higher phytase activity will also lead to enhance minerals absorption. Maize seeds expressing *phyA2* gene showed 2,200 units of phytase activity per kg seeds which is nearly 50 fold increase over non transgenic maize (Chen *et al.*, 2008). Transgenic plants containing phytase genes from various *Aspergillus* species have been produced in tobacco (Pen *et al.*, 1993; Ullah *et al.*, 1999), soybean and alfalfa (Denbow *et al.*, 2000; Ullah *et al.*, 1999), wheat, rice and canola seeds (Hong *et al.*, 2004; Ponstein *et al.*, 2002; Lucca *et al.*, 2001; Brinch-Pedersen *et al.*, 2000; Zhang *et al.*, 2000). Stable transgenic could be used for hybrid production in maize and rice with

improved phosphorus availability. In rice, the gene myo-inositol phosphate synthase (*MIPS*) is under the control of *RINO1* gene which is specifically expressed in developing rice seeds in aleurone and embryo. Using RNAi technology, transgenic rice with 68% lower phytic acid and normal seed weight, germination and plant growth have been produced (Kuwano *et al.*, 2008).

In spite of some advances with transgenic approach, there are certain constraints associated with it. Stability in the expression of transgenic plants from one generation to next generation is a key concern of biofortification programme. There are various socio-economical and socio-political concerns related with the acceptance of transgenic crops by farmers and consumers.

2.5.2 Molecular breeding techniques

Cereals such as rice, wheat, maize and millets are very poor source of micronutrients. Among cereals, rice had lowest grain iron content *i.e* 5- 6 mg/ kg (Gregorio *et al.*, 2002). Genetic variability for grain iron and zinc concentrations are presents in various wild relatives of rice. 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 ppm with a mean of 25.4 ppm. Some traditional varieties of rice such as Jalmagna, Zuchem, Xua Bue Nuo, Madhukar, were found to have twice the iron and zinc content than elite cultivars. This variability can be utilized for developing biofortified varieties through plant breeding. Bänziger and Long (2000), while working with maize, reported potential variability presents in white grained tropical maize germplasm, land races for the improvement of recommended cultivars. 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.

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). 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 important.

2.6 Wheat biofortification

Wheat (*Triticum* spp) is the second major staple food crop of the world in terms of cultivated area and food source. According to FAO (2009), nearly 456 million tonnes

of wheat for human consumption is estimated in the year 2009. It alone contributes 28% of world's edible dry matter and up to 60 % of daily calorie intake in several developing countries (Distelfield *et al.*, 2007; Welch and Graham 2004). As wheat is staple food in more than 40 countries and for over 35 % of the global population (Peng *et al.*, 2004), its biofortification will help in combating the threat of hidden hunger.

2.7 Evolution of wheat

The hexaploid wheat, *Triticum aestivum* (2n=6x=42) has three different genomes designated as A, B and D. Evolution of this allohexaploid wheat involves two separate natural amphiploidization events (Fig. 2.1). Diploid einkorn types of wheat are the oldest while the hexaploid wheats including the bread wheat, *T. aestivum,* constitute the most recent and latest step in the evolution of the wheat complex. Approximately 0.5 million years ago, two wild diploid species crossed in nature and through spontaneous chromosome doubling, a wild tetraploid species (BBAA) got created The A genome donor of common wheat was the wild diploid species *Triticum urartu* (AA), (Dvörak *et al.*, 1993) while *Aegilops speltoides* (SS) is considered as the potential B genome donor of common wheat (Maestra & Naranjo, 1998). These two diploids crossed and produced the wild tetraploid species *Triticum turgidum* ssp. *dicoccoides* (genomes BBAA), also known as wild emmer wheat. Bread wheat (*Triticum aestivum* L.) arose 8000- 10,000 years ago (Feldman, 1995) from the spontaneous hybridization of the tetraploid wheat *T.turgidum* L. (2n= 4×= 28, BBAA, genomes), with diploid goat grass *Triticum tauschii* Coss. (2n= 2×= 14, DD genomes) (Jauhar, 2007; Huang *et al.,* 2002; McFadden and Sears, 1946; Kihara, 1944).

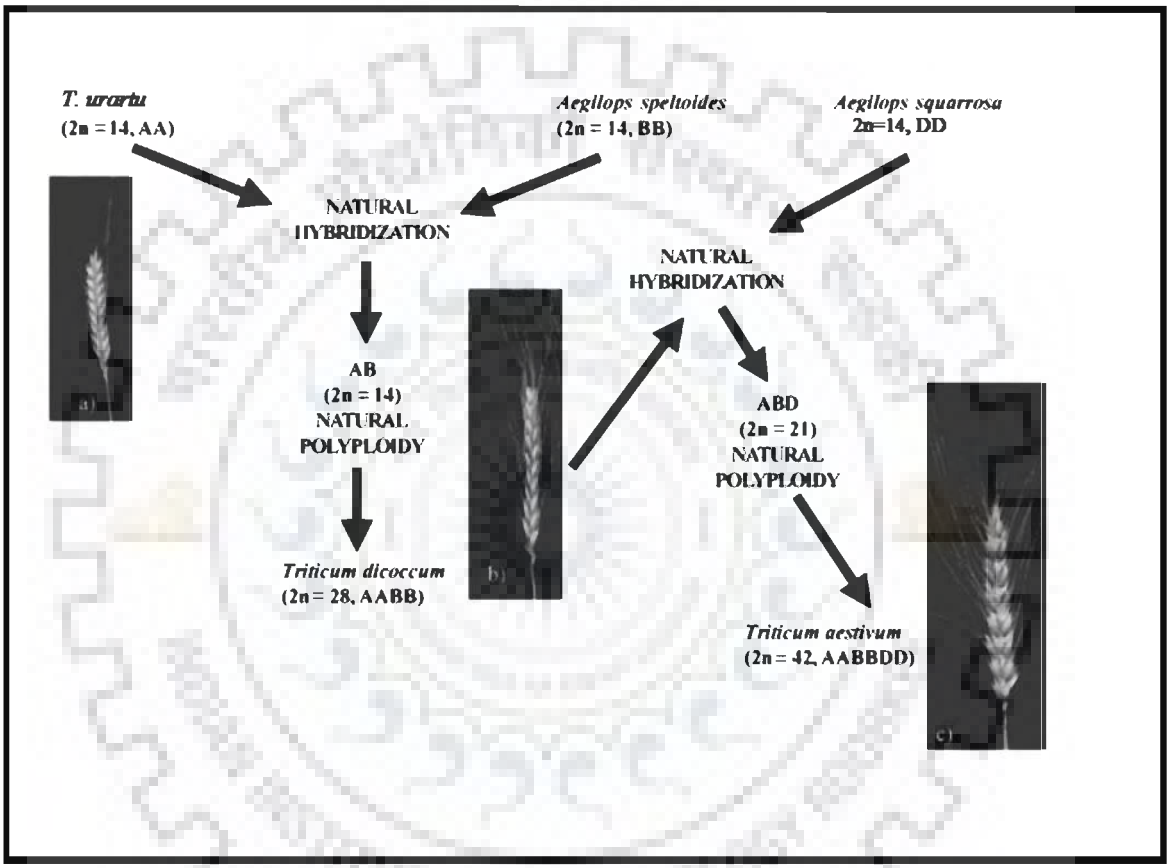

Fig. 2.1 Origin of wheat

2.8 Wheat taxonomy and its gene pool:

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; Feuillet *et al.*, 2007). Detailed information of classification of *Triticum* and *Aegilops* genera can be archived by following link: http://www.kstate.edu./wgrc/Taxonomy/taxaeg .html.

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 homologous recombination with conventional breeding approaches. The secondary gene pool includes polyploid *Triticum* and *Aegilops* species, which share one genome with any of the three genomes of wheat. 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 homoeologous genomes. Embryo rescue is a complementary aid for obtaining hybrids. Diploid and polyploid species with non-progenitor genomes constitute the tertiary gene pool; hence, gene transfers require special techniques that assist homoeologous exchanges (Kazi and Rajaram, 2002).

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

Species	Genomic constitution
Triticum aestivum L. (common or bread wheat)	ABD
Triticum turgidum L. (pollard wheat) ssp. *carthlicum* (Nevski)	AB
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^l)
Aegilops peregrina (Hack. in J. Fraser) Maire & Weiller (syn. *Ae. variabilis)*	US (US^l)

2.9 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.*, 2008). A number

of genes for resistance against various wheat diseases have been introgresed into wheat from related progenitor and non- progenitor species (McIntosh *et al.*, 2005; Marais *et al.*, 2005; Friebe *et al.*, 1996) and commercially exploited. Sears (1956) transferred *Lr9* from *Ae. umbellulata* to wheat using irradiation. Since then various workers have utilized wild wheat germplasm for different purposes of wheat improvement. Some of the examples are *Yr8* from *Ae. camosa* (Riley *et al.*, 1968), wheat streak mosaic resistance from *Agropyron elongatum* (Sebesta *et al.*, 1972), *Pm13* from *Ae. longissima* (Ceoloni *et al.*, 1988), *Lr35* and *Sr39* from *Ae. speltoides* (Kerber and Dyck, 1990), *H21* and *H25* (Hessian Fly resistance) from rye (Friebe, 1990), *Pm29* from *Ae. geniculata* (Stoilova and Spetsov, 2006), *Lr57* and *Yr40* from *Ae. geniculata* (Kuraparthy *et al.*, 2007a), *Lr58* from *Ae. truncialis* (Kuraparthy *et al.*, 2007 b), *Pm19* and *Pm35* from *Ae. tauschii* (Miranda *et al.*, 2007). Genes for yield and quality improvement have also been transferred from wild species to cultivars (Hajjar and Hodgkin, 2007).

2.10 Study of Introgression of Alien Chromosomes

Characterization and identification of alien introgressions conferring useful traits have been done using various cytological and molecular techniques out of which some have been summarized in the following section.

2.10.1 HMW-Glutenin Subunits of wheat storage proteins

The genes controlling High Molecular Weight (HMW) subunits of glutenin proteins are located on long arm of group 1 homoeologous chromosomes of wheat (Payne, 1987; Payne *et al.*, 1980). In bread wheat these loci have been named *Glu-A1*,

Glu-B1 and *Glu-D1*. Each locus includes two genes linked together encoding two different types of HMW-GS, x- and y-type subunits (Shewry *et al.*, 1992; Payne, 1987). The x-type subunits have generally lower electrophoretic mobility in SDS-PAGE and higher molecular weight than the y-type subunits. Electrophoresis studies show appreciable polymorphism in the number and mobility of HMW-GS coded by loci of different genomes. As such the HMW glutenin subunits have been used to monitor group 1 alien addition/ substitution to wheat (Dou *et al.*, 2006; Koebner and Shepherd, 1985). Koebner and Shepherd (1987) used HMW-GS to study allosyndetic recombination between a chromosome of *Aegilops umbellulata* and wheat chromosomes.

2.10.2 Chromosome C-Banding and *in situ* Hybridization

C-banding karyotypes of many related wild species have been developed and used for monitoring alien introgressions (Friebe, 1995a; 1995 b). Genomic *in situ* hybridization (GISH) involves labelling total genomic DNA and using it as a probe to identify alien chromosomes in a wheat background (Heslop-Harrison *et al.*, 1992; Mukai and Gill, 1991; Le *et al.*, 1989). GISH has been used to identify the parental origin of each chromosome in hybrids of *Hordeum chilense* and *H. vulgare* and in a *H. vulgare* X *H. bulbosum* L. hybrid (Schwarzacher *et al.*, 1992; Leitch *et al.*, 1990), 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).

Fluoresence *in situ* hybridization (FISH) has been extensively used to characterize alien introgression in various plant species (William and Mujeeb-Kazi, 1995; Cai *et al.*, 1996). Badaeva *et al.* (2004) used GISH and C-banding to study genome differentiation

in *Aegilops* and evolution of the U-genome species. BACs have also been 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).

2.10.3 Molecular markers

Molecular markers have been extensively used as a tool for identification of alien chromatin in addition and substitution lines (Schneider *et al.*, 2008; Ma *et al.*, 1994). Characterization of addition lines in wheat has been frequently done using Randomly Amplified Polymorphic DNA (RAPD) markers. Addition lines of *Hordeum vulgare* (Devos and Gale, 1993), *Thinopyrum bessarabicum* (King *et al.*, 1993), *Hordeum chilense* (Hernandez *et al.*, 1995), *Aegilops searsii* (Diaz-Salazar and Orellana, 1995), *Dasypyrum villosum* (Qi *et al.*, 1996) and *Aegilops markgrafii* (Peil *et al.*, 1998) are some such examples. Restriction Fragment Length Polymorphism (RFLP) markers were used by Francki *et al.* (1997) and Qi *et al.* (1997) to identify addition lines from *Th. intermedium* and addition and substitution lines of *Leymus racemosus* respectively. Kuraparthy *et al.* (2007a and 2007b) used RFLP markers in combination with GISH to identify cryptic translocations in wheat from *Ae. geniculata* and *Ae. triuncialis*. With the discovery of PCR based marker systems like SSR, AFLP the molecular maps became denser and identification of alien translocations became much easier (Somers, 2004; Röder *et al.*, 1998). With the increase in number of expressed sequence tag (EST) databases, EST-based microsatellite markers are being used to assay functional diversity in introgressive populations (Varshney *et al.*, 2005). These markers are useful because

they represent transcribed genes and a putative function can often be deduced by homology search. Dou *et al.* (2006) used HMW-GS, GISH and SSR markers to study molecular cytogenetics of hexaploid lines spontaneously appearing in octoploid *Triticale*.

DArT (Diversity Array Technology) is the latest technique based on microarray to analyse DNA polymorphisms. The DArT technology was basically developed for rice, with a small genome of 430 Mbp (Jaccoud *et al.*, 2001) and now being subsequently applied to a range of other crops (19 plant species and three fungal plant pathogens). A DArT molecular map for wheat with 339 markers across all the 21 chromosomes is already developed by Akbari *et al.* (2006). Peleg *et al.* (2008) have developed a high density molecular map using 197 microsatellite and 493 DArT markers in a tetraploid wheat RIL population.

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 (RH) is a recent approach which does not rely on meiotic recombination and can be used in generating high resolution radiation hybrid maps of wheat (Michalak *et al.*, 2008). 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 of *Ae. longissima* (Species Cytoplasm-Specific) in wheat by using radiation hybrid mapping approach. A very high resolution physical map of wheat chromosome 3B has been generated using radiation hybrid mapping along with other approaches of mapping (Paux *et al.*, 2008).

2.10.5 QTL mapping

Quantitative traits refer to the traits whose phenotypic characteristic vary in degree and are influenced by the interaction between two or more genes and their environment. QTL mapping is useful for understanding of the genetics of the polygenic traits. It provides information regarding degree of association of a specific region on the genome to the inheritance of the trait of interest. Shi *et al.* (2008), reported seven QTLs on chromosome 1A, 2D, 3A, 4A, 4D, 5A and 7A for zinc content in a double haploid wheat population of Hanxuan10 and Lumai 14. Four zinc QTLs were also identified on chromosome 3D, 4B, 6B and 7A in a double haploid wheat population by Genc *et al.* (2009). Stangoulis *et al.* (2007), found three QTLs for grain iron content in rice on chromosome 2, 8 and 12 explaining 17%, 18% and 14% of the total phenotypic variation, respectively and for zinc concentration two QTLs were found on rice chromosome 1 and 12 explaining 15 % and 13% variation, respectively.

2.11 Metal Acquisition in plants

Uptake of minerals from soil, transport within plant and finally deposition to the edible part are the important steps determining high grain iron and zinc content of grains.

2.11.1 Metal uptake from soil

Despite its abundance in soils, much Fe is present in the soil in insoluble form Fe (III) and zinc as Zn (III) and not readily available to the plants. Therefore plants have developed sophisticated and strongly regulated mechanism for acquiring metals from soil, which can be grouped into two strategies which have been summarized

diagrammatically in Fig. 2.2. In strategy I, non-graminaceous plants lower the soil pH by enhancing proton excretion into the rhizosphere thus reducing iron to more soluble ferrous form at the root surface which includes the expression of ferric-chelate reductase activity. Fe^{3+} is 1000 times more soluble when reduced to Fe^{2+} (Olsen, 1981). In strategy -II, graminaceous plants solubilize soil iron by secreting Fe (III) chelators, called Mugenic acid family phytosiderophores (MA) (Marschner, *et al.*, 1986; Ueno and Ma, 2009).

Many genes of strategy I have been investigated identified and isolated for enzymes and transporters of iron for understanding of the molecular mechanism of the uptake of metals. Three ferric-chelate reductase genes (*AtFRO2*, *PsFRO1*, *LeFRO1*) have been isolated from *Arabidopsis*, pea and tomato (Robinson *et al.*, 1999; Waters *et al.*, 2002; Li *et al.*, 2004). Fe^{2+} is transported into the root by metal transporters of the ZIP (Zinc regulated- Iron regulated transporter Proteins) family. Iron regulated transporter 1 and 2 (IRT1 and IRT2) are representatives of this family and are located in the plasma membrane of epidermal cells of roots. Many (IRTs) from the zinc and iron transporter family (ZIP) have been isolated in various plants such as *AtIRT1*, *AtIRT2* from *Arabidopsis thaliana* (Vert *et al.*, 2001), *LeIRT1*, *LeIRT2* from tomato (Eckhardt *et al.*, 2001) and *PsIRT1* from pea (Cohen *et al.*, 1998). The *Arabidopsis irt1* mutants exhibit severe chlorosis and impaired growth (Vert *et al.*, 2002; Henriques *et al.*, 2002) indicating the role of *IRT* family protein in uptake of metals. Another class of metal transporters of natural resistance associated macrophage proteins (*NRAMP*) family are also present in various plants, animals, fungi and is found to be involved in transport of divalent cations (Hall and Williams, 2003). They also facilitate mobilization of vacuolar

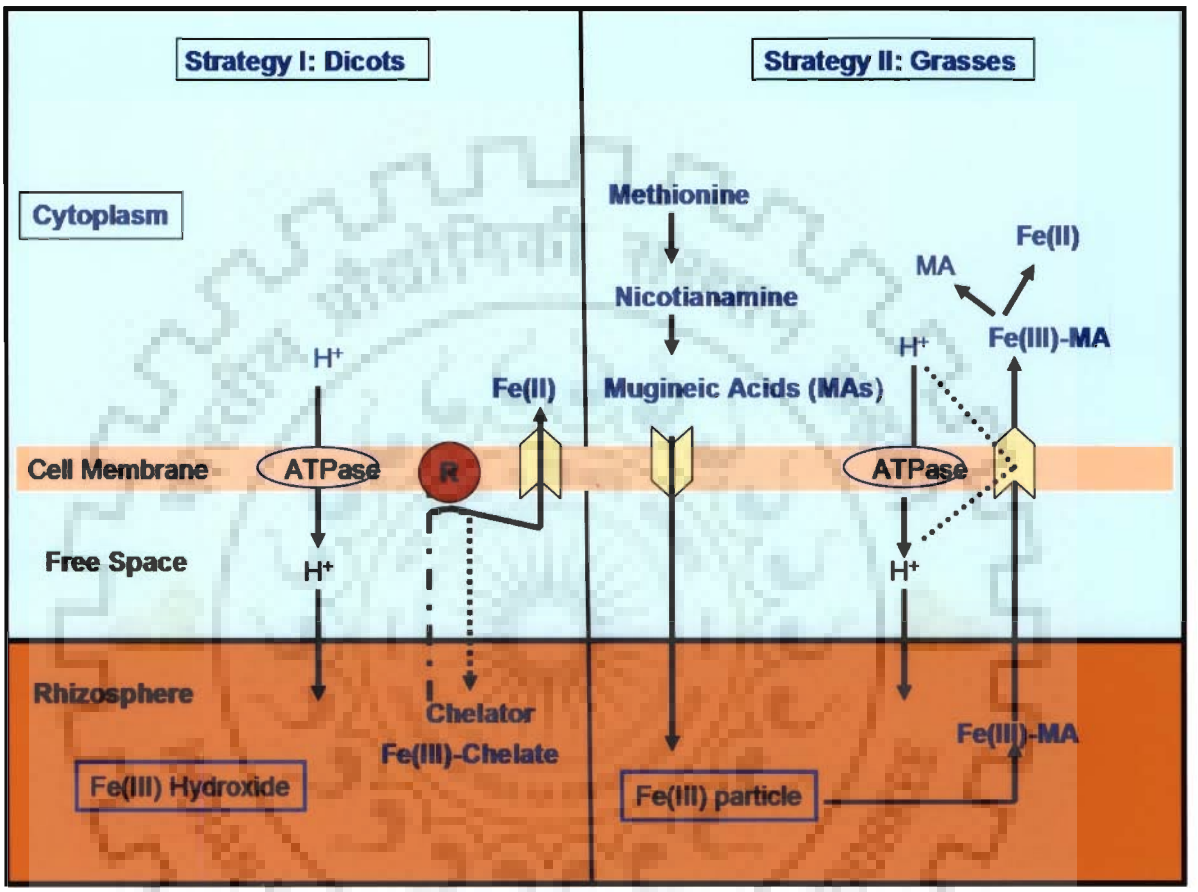

Fig. 2 Strategies for metal uptake in dicot and monocot plants

iron for seed germination on low iron (Lanquar *et al.*, 2005). Table 2.2 summarizes the tissue expression, cellular localizations, factors affecting expression and known substrates of metal transporter proteins in *Arabidopsis.*

In graminaceous plants, mobilization of ions takes place by the release of phytosiderophores (PS) of mugineic acid family which is composed of mugineic acid (MA), 2'-deoxymugeneic acid (DMA), 3 epihydroxymugeneic acid (epi-HMA) and 3-epihydroxy-2hydroxy mugineic acid (epi-HDMA). The phenomenon of significant release of phytosiderophores by graminaceous species (Strategy II plants) under deficiency of iron, zinc and other micronutrients has been reported by various workers (Kanazawa *et al.*, 1995; Mori *et al.*, 1991; Zhang *et al.*, 1989). Biosynthesis of mugineic acid involves trimerization of 3 molecules of S-adenosylmethionine molecules to nicotinamine by the enzyme nicotinamine synthase (NAS). This is then converted into a 3-keto intermediate by the transfer of an amino group by nicotianamine aminotransferase (NAAT). The subsequent reduction of the 3 carbon of the keto intermediate produces DMA. DMA is the first MA synthesized. Subsequent hydroxylation of DMA produces other forms of MAs depending on the plant species (Bashir *et al.*, 2006). Two barley cDNA clones particularly expressed in iron deficient roots, *Ids2* and *Ids3*, were shown to encode dioxygenases involved in hydroxylation of DMA to epiHMA and epiHDMA (Nakanishi, 2000). The genes for SAM synthase (Takizawa *et al.*, 1996), NAS (Higuchi *et al.*, 1999), NAAT (Takahashi *et al.*, 1999), DMAS (Bashir, 2006), IDS2 (Okumura *et al.*, 1994) and IDS3 (Nakanishi *et al.*, 1993) have been cloned and characterized. The expression of genes encoding synthesis of NAAT enzymes is most crucial in biosynthetic pathway of MAs as it hastens the production of DMA (Curie and Brait, 2003). Higuchi *et*

al. (2001), reported 20-30% higher NAS activity in the transgenic rice plants with *HvNAS1* genomic fragment from barley under Fe deficient condition. This leads to 50 % increase in NAS protein in transgenic rice plants. Similarly, transgenic rice with either *naat-A* or *naat-B* genes from barley showed 1.8 times higher production of DMA under iron limiting conditions over non transformant (Takahashi, 2003). Transgenic plants with *naat* genes remains green for longer time and produce 4.2 times higher shoot mass in alkaline soil (Takahashi, 2001).

Kobayashi *et al.* (2008) produced transgenic rice plants with enhanced tolerance to Fe deficiency by introducing barley HvNAS1, HvNAAT-A, HvNAAT-B, and/or IDS3 genes. After the chelation of the Fe^{3+} by phytosiderophores (PS), the metal-PS complex is taken up by the YS1 (Yellow-Stripe 1) transporters located in the plasma membrane of root cells (Roberts *et al.*, 2004). YS1 was the first transporter of a metal ion-ligand identified in plants (Curie *et al.*, 2001). The *ys1* maize mutants were defective in uptake of Fe-PS leading to interveinal necrosis (Curie *et al.*, 2001) depicting their role in transport of minerals. Thus increasing the role of iron, zinc and other minerals from soil is significant prerequisite for increasing the amount of iron and zinc in edible portion of grain.

2.11.2 Metal transport within plants

Once inside the root cells the metal ions undergo symplastic diffusion between interconnected root cells towards the stele. Movement across the xylem parenchyma to the vessels is brought about by HMA2 (Heavy Metal Transporting ATPase2) and HMA4, which pump metal ions into the root vascular system. HMA2 and HMA4 are specific

transporters of Zn and Cd but not of Fe or Mn, for which YSL2 and AtIREG1 are suggested to be responsible (Kim and Geurinot, 2007; Colangelo and Geurinot, 2006). Thus *hma2hma4* double mutants suffer from inadequate Zn supply to the shoot, resulting in stunted growth and chlorosis (Hussain *et al.*, 2004). Tissue expression, cellular localizations, factors affecting expression and known substrates of metal transporter proteins in Arabidopsis has been summarized in Table 2.2. Long distance transport through the xylem sap where pH is around 5.5-6 involves chelation of metal ions with mobile low molecular weight ligands. For example Fe is present as Fe (III) - citrate complexes in the xylem for transport to aerial parts over long distances (Hell and Stephan, 2003).

From xylem vessels, micronutrients undergo active transport to the leaf mesophyll tissue using metal uptake transporters of the parenchyma cells. Their further movement within the leaf cells is symplastic. The transport to the developing grains takes place either by direct upake from the soil, or from remobilization of stored minerals in the senescing leaves (Uauy *et al.*, 2006). YSL transporters have been suggested to be involved in this transport (Waters and Grusak, 2008).

Table 2.2 Tissue expression, cellular localizations, factors affecting expression and known substrates of metal transporter proteins. The number of family members known in *Arabidopsis* is given in parentheses. Source: Colangelo and Geurinot, 2006.

Proteins	Tissue expression	Cellular localization	Inducing conditions	Proposed/ known substrates	Reference(s)
(A) Metal efflux proteins					
P1B-ATPase (8)					
AtHMA2/HMA4	Vasculature of root and shoot, anther	Plasma membrane		Zn, Cd	Eren *et al.*, 2004; Mills *et al.*, 2005
AtHMA5	Root, flower		+Cu	Cu	Andres-Colas *et al.*, 2006
AtHMA6(PAA1)	Root, shoot	Plastid envelope		Cu	Abdel-Ghany *et al.*, 2005
AtHMA8 (PAA2)	Shoot	Thylakoid membrane		Cu	Abdel-Ghany *et al.*, 2005
AtHMA1	Root, shoot	Chloroplast envelope		Cu	Seigneurin-Berny *et al.*, 2005
CDF (12)					
AtMTP1	Root, shoot, flower	Vacuolar Membrane		Zn	Kobae *et al.*, 2004
AhMTP1	Root	Vacuolar Membrane	+Zn	Zn	Drager *et al.*, 2004
TgMTP1		Plasma membrane		Zn	Kim *et al.*, 2004
(B) Metal Uptake Proteins					
YSL (8)					
ZmYS1	Root, shoot		-Fe	Fe^{3+}-PS, Fe^{3+}, Fe-, Ni-, Cu-NA,	Roberts *et al.*, 2004
AtYSL1	Silique, leaf (xylem parenchyma), flower		+Fe	Fe-NA	Le Jean *et al.*, 2005
AtYSL2	Root (endoderm pericycle), shoot	Plasma membrane	+Fe, downregulated by –Zn		Di Donato *et al.*, 2004
OsYSL2	Leaf (phloem), root, seed	Plasma membrane	-Fe	Fe-, Mn-NA	Koike *et al.*, 2004
NRAMP (6)					
AtNRAMP3/4	Root, shoot, seed	Vacuolar Membrane		Fe	Lanquar *et al.*, 2005
TjNRAMP4		Plasma membrane		Ni	Mizuno *et al.*, 2005
ZIP (16)					
OsZIP4	Root, shoot (phloem meristem)		-Zn	Zn	Ishimaru *et al.*, 2005
MtZIP1	Root, leaf		-Zn	Zn	Lopez-Millan *et al.*, 2004
MtZIP3	Root, leaf		Downregulated by -Mn, -Fe	Fe	Lopez-Millan *et al.*, 2004
MtZIP4	Root leaf		-Zn	Mn	Lopez-Millan *et al.*, 2004
MtZIP5	Leaf		-Zn, -Mn	Zn, Fe	Lopez-Millan *et al.*, 2004
MtZIP6	Root, leaf			Zn, Fe	Lopez-Millan *et al.*, 2004
MtZIP7	Leaf			Mn	Lopez-Millan *et al.*, 2004
TjZNT1				Ni, Cd, Mn, Zn	Mizuno *et al.*, 2005
COPT (5)					
AtCOPT1	Root, pollen, embryo, stomata, trichome		Downregulated by Cu	Cu	Sancenon *et al.*, 2004

Chapter III

Materials and Methods

3. MATERIALS AND METHODS

3.1 Plant Materials

3.1.1 Plant material for development of introgressive derivatives

The experimental material comprising nearly 80 accessions of nine species of wild *Aegilops* and *Triticum* namely, *Ae. peregrina* (US), *Ae. longissima* (S^l), *Ae. kotschyi* (US), *Ae. ovata* (UM), *Ae. cylindrica* (CD), *Ae. ventricosa* (DN), *T. dicoccoides* (AB), *T. araraticum* (AB), and *T. boeoticum* (A) and popular semi-dwarf cultivars of bread wheat from different geographical regions was 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.

F_1 hybrids

For transfer of useful variability for higher concentration of iron and zinc from selected wild donors (*Ae. peregrina*), interspecific crosses were made using wheat cultivars as the maternal parent. A bread wheat line Chinese Spring with Ph^l transferred from *Ae. speltoides* obtained from Dr. B.S. Gill of Kansas State University, Kansas was

used for making crosses for induced homoeologus pairing whereas interspecific crosses were also made with wheat and durum cultivars without Ph^I gene.

Backcross derivatives

In the following season of 2006-07 the F_1 hybrids CS(Ph^I)/*Ae. pergerina* 13772, CS(Ph^I)/*Ae. peregrina* 1155-1-1 and CS(Ph^I)/*Ae. pergerina* 3519 were backcrossed with elite cultivars. The BC_1 plants were crossed with recurrent parent next year as they were self sterile. The BC_2 plants were allowed to self and their seeds were analyzed for their micronutrient content and the selected progenies were sown next year. Finally BC_2F_2 seeds were put to rigorous chemical analysis to select a few derivatives with exceptionally higher micronutrient content than the control wheat cultivar WL711. Fig. 3.1 shows schematic presentation of the development of wheat- *Aegilops pergerina* backcross derivatives. These selected derivatives were characterized on the basis of morphology, cytology, HMW-glutenin subunit profiles, microsatellite markers and finally Genomic *in situ* Hybridization (GISH).

Period	Generation	Remarks
Nov, 2005-April, 2006	CS(Ph^I) ♀ X *Ae. peregrina* ♂	
-	↓	
Nov, 2006-April, 2007	F_1 X Wheat cultivar	F_1 hybrids sterile
	↓	
Nov, 2007-April, 2008	BC_1 X Wheat cultivar	Partially fertile plants allowed to self
	↓	
Nov, 2008-April, 2009	BC_2F_1	Sufficient selfed seed set
	↓	
	selfed	High Fe and Zn derivatives sown
	↓	
	BC_2F_2 and BC_1F_3 seeds	Selected derivatives analysed

Fig. 3.1 A schematic presentation of the development of introgressive derivatives

3.1.2 Synthetic wheat-*Aegilops peregrina* amphiploids

Seven F_1 hybrids were produced using CS(Ph^l) or WL711 as the female parent and two *Ae. peregrina* accessions (acc. no. 3477 and 1155-5-3) as the male parent. In the following year, the F_1 seeds were sterilized with 1% sodium hypochlorite for five minutes, washed thrice with distilled water and germinated on two layers of sterilized moist filter paper in Petri plates. The chromosomes of the F_1 hybrids were doubled by treating coleoptiles of germinating seeds with 0.25% of colchicine (in 5 % DMSO solution) for 5 hours. The colchicine treated seedlings were transplanted in the field.

During flowering the spikes with dehiscing viable pollen grains and seed set, evidently due to chromosome doubling were identified and tagged. Seeds (C_0 generation of amphiploids) from the doubled sectors of the tagged spikes were harvested carefully before shattering of spikes. The C_1 generation of these amphiploids was grown in the field during 2007-2008. Collection of mature spikelets and spikes of the F_1 hybrids and synthetic amphiploids had to be done repeatedly at different intervals over two-three weeks because of frequent shattering of spikes. Due to tough glumes and hard threshing in the amphiploids and wild donors, the grains were threshed manually. Mean number of seeds per spike was determined for each amphiploid by taking average of seeds of 10 spikes.

3.1.3 Plant material for phytosiderophore release study

The experimental material comprised of a total of 24 accessions of *Aegilops* species i.e *Ae. peregrina* (7), *Ae. kotschyi* (7), *Ae. ventricosa* (3), *Ae. geniculata* (3) and *Ae. longissima* (4) and five *Triticum aestivum* L. cultivars. Seeds were surface sterilized

with 1% sodium hypochlorite solution for 15 minutes, washed thrice with distilled water followed by soaking in distilled water overnight and were germinated on filter paper saturated with distilled water in dark at 20°C. After 3 days, seedlings were transferred to Saran net floating on 0.5mM $CaCl_2$ solution at pH 5.6 in a plastic container. Seven days old seedlings were transferred to 10 liter plastic trays with floating sterofoam having 15 holes each of 2 cm diameter with 5 seedlings in each hole, with continuous aeration of the nutrient solution containing half strength Hoagland's solution (Ma and Nomoto, 1993) which was replaced later on with full strength.

3.1.4 Plant material for study of phytosiderophore release in addition lines

The plant material consisted of a complete set of disomic or monosomic addition lines for chromosomes 1-7 U and 1-7 S of *Ae. peregrina*, 1-7 S^l chromosomes from *Ae. longissima* and 1, 2, 4, 5 and 6 U of *Ae. umbellulata*, in Chinese Spring (CS) background. The seeds of addition line 3 and 7U of CS- *Ae. umbellulata* were not available. The seeds of these addition lines were kindly provided by Dr. B. S. Gill of Kansas State University, USA. These addition lines along with Chinese Spring were grown at the experimental fields of the Indian Institute of Technology Roorkee, India for two consecutive seasons of 2006–2007 and 2007–2008 in two replications of 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 (50:25:25 kg/acre N, P_2O_5, K_2O) and irrigation as that of wheat.

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

The plant materials used for mapping of the QTL for amount of phytosiderophore released per plant consisted of a set of 88 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). Seeds of the RIL population over 3 years and 6 locations along with their parents were analyzed for phytosiderophore released.

3.2 Chemical Analyses

3.2.1 Grain analysis:

For chemical analysis whole grain samples from cultivated and wild accessions were washed with N/10 HCl (Merck), dried in hot air oven at 80°C for 4 hours. Grain samples (0.5 g) 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 (AAS); (GBC- Avanta Garde M). A minimum of five replications of chemical analysis were made in each of cultivars and wild accessions. Grain iron and zinc status of selected donors and selected derivatives were also

reconfirmed by Inductively Coupled Plasma Mass Spectrometer (ICPMS) (Perkin Elmer).

3.2.2 Flag leaf analysis

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.

3.3 Cytological Studies

For meiotic analysis spikes of interspecific F_1 plants and subsequent generations 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 pairing in all the crosses. Photographs were taken with a digital camera (Canon PC1049, No. 6934108049). Pollen stainability was measured by staining the pollen grains after squashing the anthers in Iodine-Potassium Iodide solution (I-KI).

3.4 Protein analysis

HMW gluentenin subunit proteins were analysed using method described by Smith and Payne (1984) with some modifications.

3.4.1 HMW glutenin subunit extraction reagents and procedure

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

3.4.2 Extraction procedure:

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

3.4.3 HMW glutenin SDS PAGE reagents

Acrylamide 40%: Acrylamide (SRL) 100g; total volume was made upto 250ml with distilled water.

Bis-acrylamide 2%: 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 setting pH to 8.3.

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

Tetramethylene diamine (TEMED, HiMedia)

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 (5ml)

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

3.4.4 HMW glutenin SDS PAGE procedure:

The 10% running gel was poured in the preset gel casting unit (Atto, Japan), overlaid with butanol and allowed to polymerize for 40 minutes. Butanol was drained off and thoroughly washed with distilled water. Stacking gel was poured over the running gel and comb was inserted. It was left for 15 minutes for polymerizing. Comb was pulled and gel was washed by pushing distilled water with mild pressure. Chilled 1X tank buffer was poured in the assembly, gel was inserted into it and samples (10 µl) were loaded in the wells.

3.5 Genomic *In situ* hybridization

The genomic DNAs of *Ae. longissima* (SS) and *Ae. umbellulata* (UU) were used to prepare genomic probes for subsequent utilization in genomic *in situ* hybridization

experiments. However, two of the clones PAS1 and pHvG38 were used in sequencial fluorescent in situ hybidization (FISH) experiments. The barley clone pHvG38 contains a 900-bp GAA-satellite sequence (Pedersen *et al.*, 1996) which has multiple FISH sites on the B-genome chromosomes, some minor sites on A- and D-genome chromosomes of wheat and uniformly distributed sites in S- and U-genome chromosomes. However, the FISH pattern of this repeat is distinguishable in hexaploid wheat (A-, B-, D-genome chromosomes) and S- and U-genome chromosomes. Clone pAs1 contains a 1-kb fragment which was isolated from *Ae. tauschii* (Rayburn and Gill, 1986) which permits identification of the D-genome chromosomes (Rayburn and Gill, 1986). Using both pHvG38 and pAs1 clones, all 21 chromosomes of hexaploid wheat can be identified (Pedersen and Langridge, 1997).

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).

3.6.1 DNA Extraction buffer:

200mM Tris (pH 8.0), 20mM ethylene diamine tetra-acetic acid (pH 8.0)
140mM NaCl, 2% CTAB (Cetyl-trimethyl ammonium bromide), 0.01% β mercaptoethanol. All chemicals used were of HiMedia (Molecular biology grade).

3.6.2 DNA isolation and purification reagents:

TE buffer (10 mM Tris (pH 8.0) 1mM EDTA (pH 8.0)), RNAse solution (10mg/ml), Phenol: Chloroform: Isoamyl alcohol (25:24:1), Isoproponal, Absolute Ethanol, 70% ethanol

3.6.3 DNA isolation procedure

About 5-7g 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 10,000rpm for 20 minutes at 25°C. Supernatants were transferred to the falcon tubes with the help of micropipettes. Equal volume of ice cold propon-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 8000 rpm 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, 2001) 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

Wheat microsatellite markers (401 in number) representing all the 21 chromosomes of wheat covering both chromosomal arms were selected from publications of Röder *et al.* (1998), Pestsova *et al.* (2000) and Somers *et al.* (2004). Details of the markers used have been given in Annexure-I. Transferability of markers and parental polymorphism between wheat cultivars and *Ae. perregrina* was checked. PCR was carried out according to Röder *et al.* (1998) with some modifications. The primers were got synthesized from Hysel India (Pvt.) Ltd. Distal transferable polymorphic markers of each chromosome arm were used in the finally selected derivatives to identify the introgressed chromosome(s). Finally the introgression was characterized by using additional polymorphic markers of the entire introgressed chromosome(s) to the selected derivatives.

3.7.1 Composition of PCR 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:

4µl of 6X gel loading dye (New England Biolabs) 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.

3.8 Phytosiderophore estimation

3.8.1 Nutrient media composition

The Hoagland's nutrient media consisted of 0.7 mM K_2SO_4, 0.1 mM KCl, 2mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 1µM H_3BO_3, 0.5 µM $MnSO_4$, 0.2 µM $CuSO_4$ and 0.01 µM $(NH4)_6Mo_7O_{24}$. Nutrient media was supplemented with 0.5 µM $ZnSO_4$ and 100 µM Fe- EDTA for Zn and Fe sufficient plants. For Fe deficiency treatment, plants were grown in nutrient solution without Fe but with 0.5 µM $ZnSO_4$ and for Zn deficiency treatment, nutrient media were supplemented with 100 µM Fe- EDTA but without Zn. Deionized water was used for preparation of nutrient media and assay solution. Nutrient media were changed at 3 days intervals. The pH of the nutrient media was not adjusted and it was found to be between 6 and 7. All experiments were carried under controlled environmental conditions (light/day) regime of 16/8 h at 23°C - 25°C with relative humidity of 65- 75 percent. Each experiment was performed in five replications in a completely randomized design.

3.8.2 Collection of root exudates

Intact plants were removed from the nutrient media and the roots were repeatedly washed in deionized water. The root exudates were collected in glass beakers having 20 ml Milli-Q water and collection was done after 2 hours of onset of light period and further continued for three hours. Collection was done in three replications of 5 plants each of wheat cultivars and related wild species. Exudates were collected on 6^{th}, 11^{th} and 17^{th} days after transfer of plants in Fe deficient media while for Zn deficient media root exudate collections were made on 10^{th}, 14^{th} and 18^{th} days after transfer to deficient media. To reduce microbial degradation of phytosiderophores in the collections Micropur was added and the samples were stored at -80°C till further processing.

3.8.3 Determination of Phytosiderophores (PS)

The quantity of PS in the root exudates was determined indirectly by modified Fe binding assay of Gries *et al.* (1995). Freshly prepared, 0.5ml of $FeCl_3$ (1.0 mM) solution with pH 2.1 was added to 10 ml of the collected solution and shaken for 15 min followed by addition of 1 ml of sodium acetate buffer (1 M, pH 7) by shaking for 10 minutes and the content was filtered through Whatman # 42 paper into 0.25 ml 6 N HCl. Ferric ions were reduced by addition of 0.5 ml 8 % hydroxylamine hydrochloride solution and then heated at 60 °C for 20 min. To this solution, 0.25 ml of 0.25 % ferrozine, and 1ml of Na-acetate buffer (pH 4.7) were added. The concentration of ferrous ion was determined colorimetrically at 562 nm wavelength.

For determination of PS under zinc deficiency, Zn-CAS method was used. For preparation of CAS, 6 ml volume of 10mM HDTMA (Hexadecyl trimethyl amine) solution was added in 100 ml flask followed by addition of 1.5 ml of 1mM $ZnCl_2$ and 1.5

ml of 10mM HCl. 7.5 ml of 2mM aqueous CAS was also added in the same flask followed by addition of solution containing 4.2 g of anhydrous piperazine dissolved in 6.25 ml of 12N HCl (pH 5.6). Final volume was made to 100 ml after addition of 5-sulfosalicylic acid (4mM). Determination of phytosiderophores was done by addition of 0.5 ml of aliquot of phytosiderophore exudate and 0.5 ml of Zn CAS solution. Change in the color from blue to orange was observed and absorbance was recorded at 630 nm.

3.9 QTL mapping

The amount of phytosiderophore released per plant 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 $P \leq 0.05$ (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.

Chapter IV

Results

4. RESULTS

Results of this study are presented in the following sections

1. Development of introgressive derivatives of *Aegilops peregrina* with high grain iron and zinc and their cytological and molecular characterization.
2. Development of wheat- *Ae. peregrina* synthetic amphiploids with high grain iron and zinc content.
3. Screening of mugineic acid release among *Aegilops* species and advanced backcrossed derivatives
4. Evaluation and identification of wheat- *Aegilops* addition lines controlling high grain iron and zinc content and mugineic acid
5. QTL mapping of phytosiderophore content in diploid *T. monococcum* X *T. boeoticum* RIL population

4.1 Development of introgressive derivatives of *Aegilops peregrina* with high grain iron and zinc content and their cytological and molecular characterization

4.1.1 Screening of germplasm

Mean grain iron and zinc content of bread and durum wheat cultivars and accessions of ten different progenitor and non- progenitor *Triticum* and *Aegilops* species is given in Fig. 4.1. Narrow range of variability for grain iron (20-25 mg/kg) and zinc content (15- 20 mg/kg) has been observed in all of the bread and durum wheat cultivars. Wild *Triticum* and *Aegilops* species had shown significant increase in grain iron and zinc content of over elite wheat cultivars. Among *Triticum* species, *T. boeoticum* had shown maximum increase in grain iron content (47.74 %) whereas for zinc maximum increase was observed in *T. dicoccoides* (88.52 %). Nearly 2.5 to 3 fold higher grain iron and zinc content was found in various non- progenitor *Aegilops* species *i.e. Ae. kotschyi* (US), *Ae. peregrina* (US), *Ae. ventricosa* (DN), *Ae. longissima* (S), *Ae. geniculata* (UM) and *Ae. cylindrica* (CD).Variation for micronutrient content was also found within and among various *Aegilops* species. Among *Aegilops* species, *Ae. peregrina* had shown highest iron (68.73 mg/ kg) and zinc content (45.14 mg/ kg). Within *Ae. peregrina*, accessions 13772, 3519, 3477, 1155-1-1 and 1155-5-3 had higher micronutrient content than other accessions (Fig. 4.2). In general, all accessions of *Ae. peregrina* had higher grain iron content than that of zinc. Based on highly consistent results, these accessions were selected as a donor for transfer of useful variability for high grain iron and zinc content to *T. aestivum* and for development of amphiploids.

4.1.2. The F_1 hybrids

The F_1 hybrids between wheat and *Ae. peregrina* were developed using *T. aestivum* Chinese Spring (*Ph*^{l}) as female parent and selected *Ae. peregrina* accessions as male parents. The wheat x *Ae. peregrina* F_1 hybrids were completely sterile. Morphologically they were intermediate between two parents. They had awn-less lemma and glumes like CS (*Ph*^{l}) and had spelta heads with brittle rachis as that of *Ae. peregrina*.

4.1.2.1. Cytology

The details of fertility and chromosome pairing of F_1 hybrids between CS (*Ph*^{l}) and five accessions of *Ae. peregrina* are given in Table 4.1. There was very limited intergenomic pairing in the F_1 hybrids (Table 4.1 and Fig. 4.3) with very high frequency

Table 4.1 Mean and range (within parentheses) of induced homoeologous pairing of F_1 hybrids between Chinese Spring (*Ph*^{l}), and different accessions of *Ae. peregrina*

Cross	Chr No.	Number of PMCs studied	Mean ± S.D. (Range)	Mean ± S.D. (Range)	Mean ± S.D. (Range)	Pollen Stainability %
			Univalent (I)	Bivalent (II)	Trivalent (III)	
CS (*Ph*^{l}) X *Ae. peregrina* 1155-1-1	35	100	30.12 ± 0.32 (21-35)	2.14 ± 0.38 (0-6)	0.20 ± 0.02 (0-2)	15.39
CS (*Ph*^{l}) X *Ae. peregrina* 1155-5-3	35	100	30.28 ± 0.22 (25-35)	2.09 ± 0.24 (0-5)	0.18 ± 0.04 (0-1)	18.22
CS (*Ph*^{l}) X *Ae. peregrina* 13772	35	100	28.31 ± 0.68 (24-35)	3.12 ± 0.33 (0-7)	0.15 ± 0.08 (0-1)	20.28
CS (*Ph*^{l}) X *Ae . peregrina* 3519	35	100	28.35 ± 0.46 (26-35)	3.04 ± 0.21 (0-3)	0.19 ± 0.05 (0-3)	19.71
CS (*Ph*^{l}) X *Ae. peregrina* 3477	35	100	30.70 ± 0.35 (26-35)	2.0 ± 0.26 (0-4)	0.10 ± .05 (0-1)	18.73

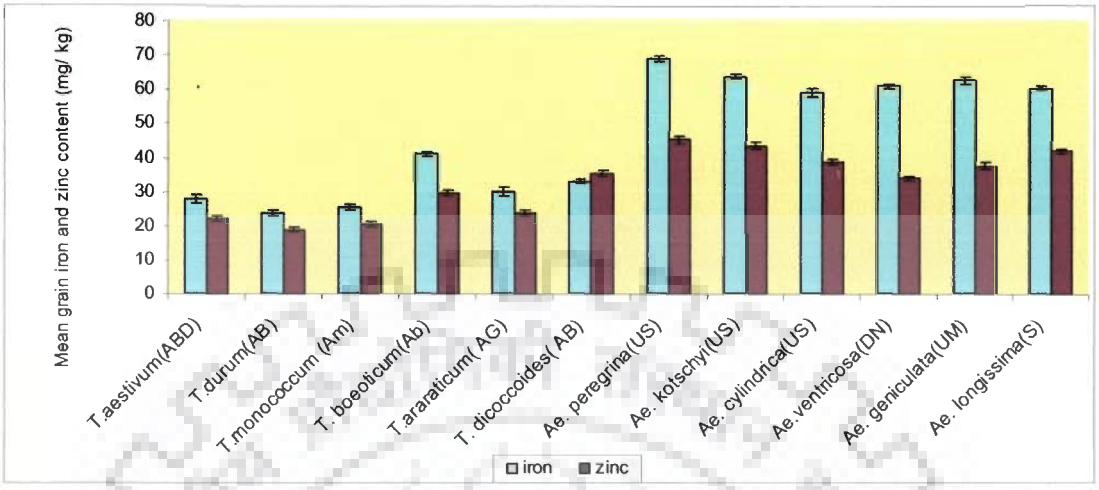

Fig. 4.1 Mean grain iron and zinc content of bread and durum wheat cultivars, wild *Triticum* and *Aegilops* species over 2 years. Error bars represent standard error of mean.

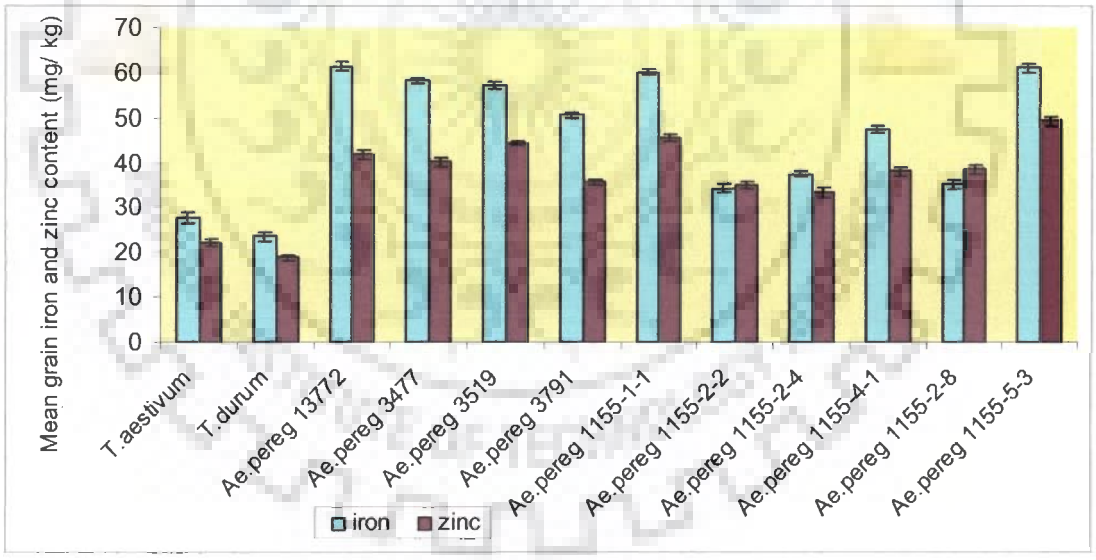

Fig. 4.2 Mean grain iron and zinc content of bread and durum wheat cultivars and *Aegilops peregrina* accessions over 2 years. Error bars represent standard deviation for the trait.

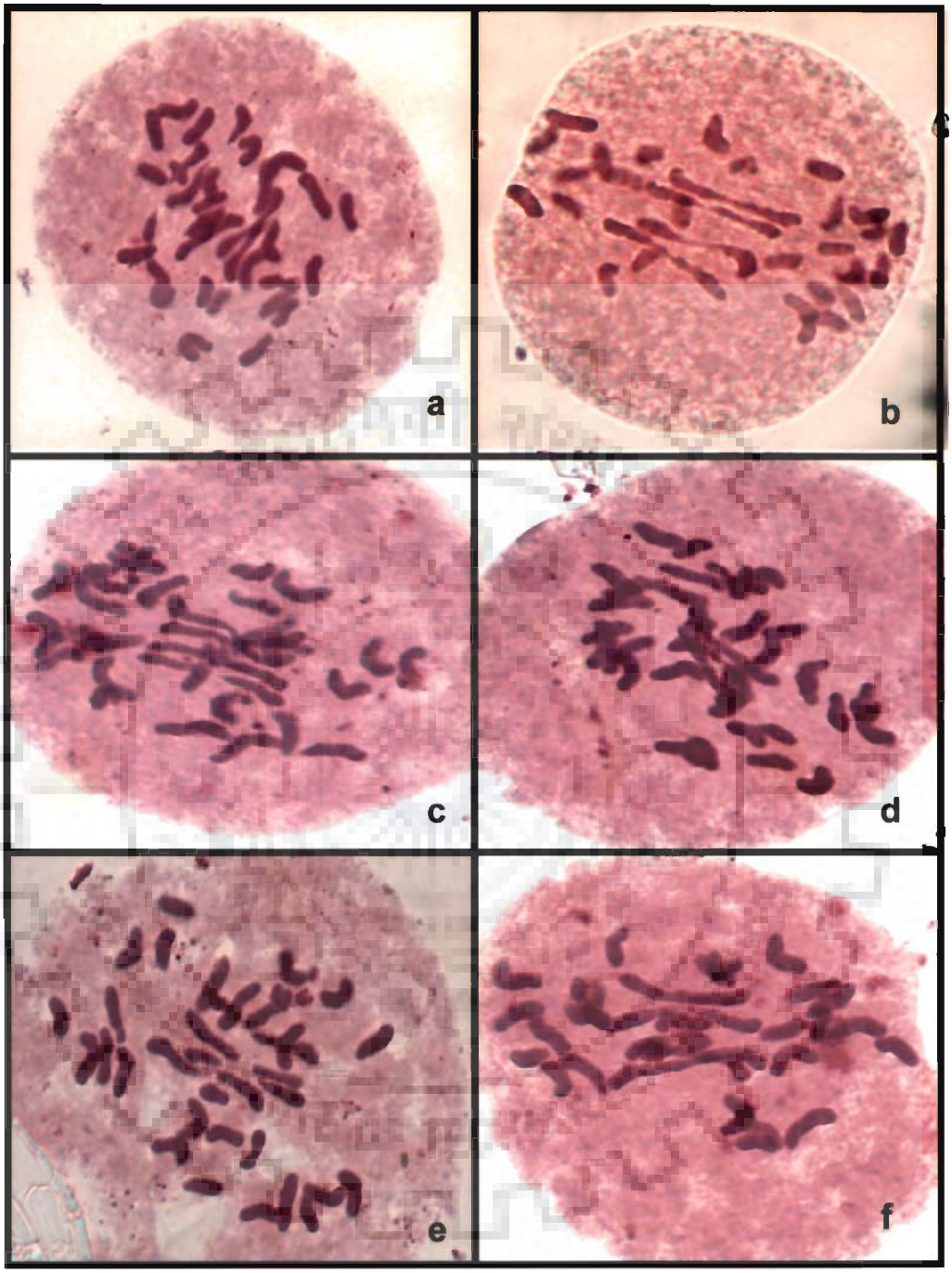

Fig. 4.3 Chromosomal pairing at metaphase-I of CS (*Ph*^{I})/ *Ae. peregrina* F_1 hybrids. a- F_1 CS(*Ph*^{I})/ *Ae. peregrina* 1155-1-1 (5 II + 25 I), b- CS(*Ph*^{I})/ *Ae. peregrina* 1155-5-3 (4 II + 27 I), c- F_1 CS (*Ph*^{I})/*Ae. peregrina* 13772 (5 II + 25 I) , d- F_1 CS (*Ph*^{I})/*Ae. peregrina* 13772 (1 II + 1 III + 30 I), e- F_1 CS (*Ph*^{I})/*Ae. peregrina* 3519 (4 II + 27 I) and f- F_1 CS (*Ph*^{I})/*Ae. peregrina* 3477 (5 II + 25 I)

of univalents (28.31-30.97), low frequency of bivalents (2.0- 3.12), and an occasional trivalent (0.10-0.20). Occurrence of induced homoeologous pairing between CS (Ph^I) and accessions of *Ae. peregrina* may be attributed to epistatic effect of Ph^I gene on *Ph1* gene on the long arm of chromosome 5B (Aghaee-Sarbarzeh *et al.*, 2002; Chen *et al.*, 1994; Riley and Chapman 1958). High frequency of bivalents was observed in F_1 CS *(Ph^I)* X *Ae. peregrina* 13772 and CS *(Ph^I)* X *Ae. peregrina* 3519 as compared to other F_1 hybrids (Table 4.1). Very low pollen stainability was observed among all F_1 hybrids (Table 4.1). There was no anther dehiscence in any of the hybrids.

4.1.2.2. Flag leaf analysis

The F_1 hybrids between CS (Ph^I) and *Ae. peregrina* were either sterile with no seed set. Positive correlation between leaf iron content and grain iron content (r=+0.82) and between leaf zinc and grain zinc content (r=+0.92) has been reported by Rawat *et al*; 2008.Therefore flag leaves of F_1s along with both parents were analyzed for their iron and zinc content. In general all accessions of *Ae. peregrina* had nearly 2 to 3 folds higher iron and zinc content in their flag leaves over wheat cultivars. The F_1 hybrids of CS (Ph^I) with *Ae. peregrina* accessions 13772, 3519 and 1155-1-1 had iron content approaching as that of wild parents while with accessions 3477 and 1155-5-3 had shown higher iron content than both of the parents (Fig. 4.4). Similar results were obtained for zinc content of flag leaves of F_1 hybrids. These findings indicate presence of distinct genetic system of uptake of micronutrients in *Ae. peregrina* accessions which could be transferred to wheat cultivars.

4.1.3 BC_1 plants

F_1 hybrids between CS (*Ph*^{l}) and *Ae. peregrina* accessions were completely sterile. Therefore, extensive backcrossing was done to get some BC_1 seeds. The morphology, chromosome number, fertility, and iron, zinc status in the flag leaves of some of the BC_1 plants has been given in Table 4.2. The growth habit of BC_1 plants varied from erect as that of wheat parent, spreading or intermediate between two parents. Variation in ear morphology was also observed among BC_1 plants such as square or spelta heads, awned or awnless ears and waxy or non waxy plants.

4.1.3.1 Cytology

Chromosome number in various BC_1 plants varied from 30 to 56. Some of the plants were sterile, some were partially fertile and only few plants among hundreds of BC_1 seeds planted were fertile (Table 4.2). Cytological details of meiotic pairing among BC_1 plants are given in Fig. 4.5. Some of the BC_1 plants had still shown presence of higher number of univalents while some had shown higher number of bivalents (up to 20) and trivalents (up to 2) indicating increase in the stability of the backcross derivatives (Fig. 4.5).

4.1.3.2 Micronutrient analysis

Only a few BC_1 plants had seed set. Therefore, micronutrients analysis was done using flag leaves of these plants. Iron and zinc content in the flag leaves of some BC_1 plants is given in Table 4.2. Some of the BC_1 plants such as CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1 and CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 373-1, had higher iron content than both of the parents reflecting the probability of getting transgressive

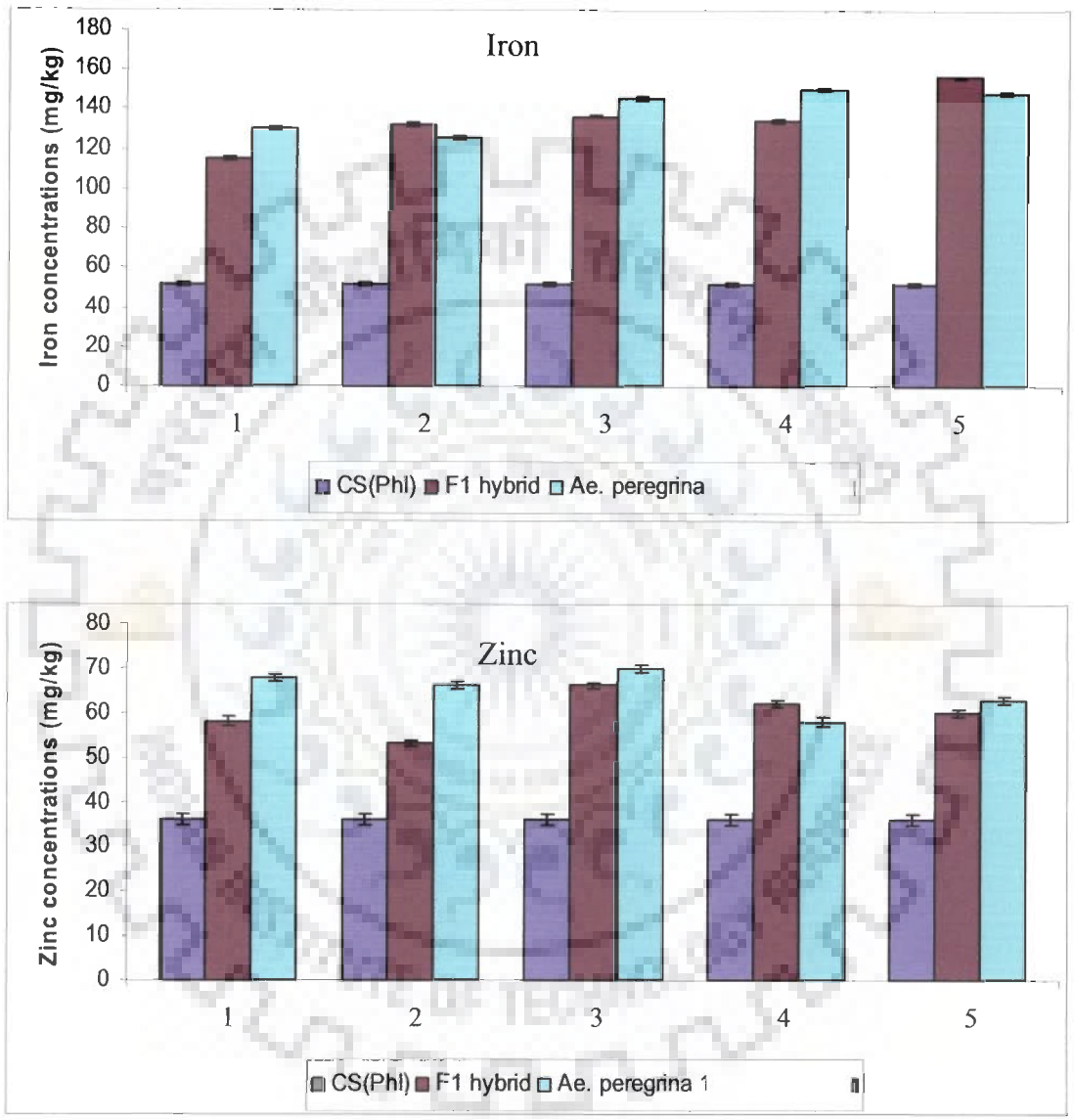

Fig. 4.4 Iron and zinc concentrations in flag leaves of CS (*Ph*^{l}) cultivars, their F_1 hybrids and *Aegilops peregrina* parents; 1-*Ae. peregrina* 13772, 2-*Ae. peregrina* 3477, 3-*Ae. peregrina* 3519, 4-*Ae. peregrina* 1155-1-1, 5-*Ae. peregrina* 1155-5-3. Error bars represent standard deviation for the trait.

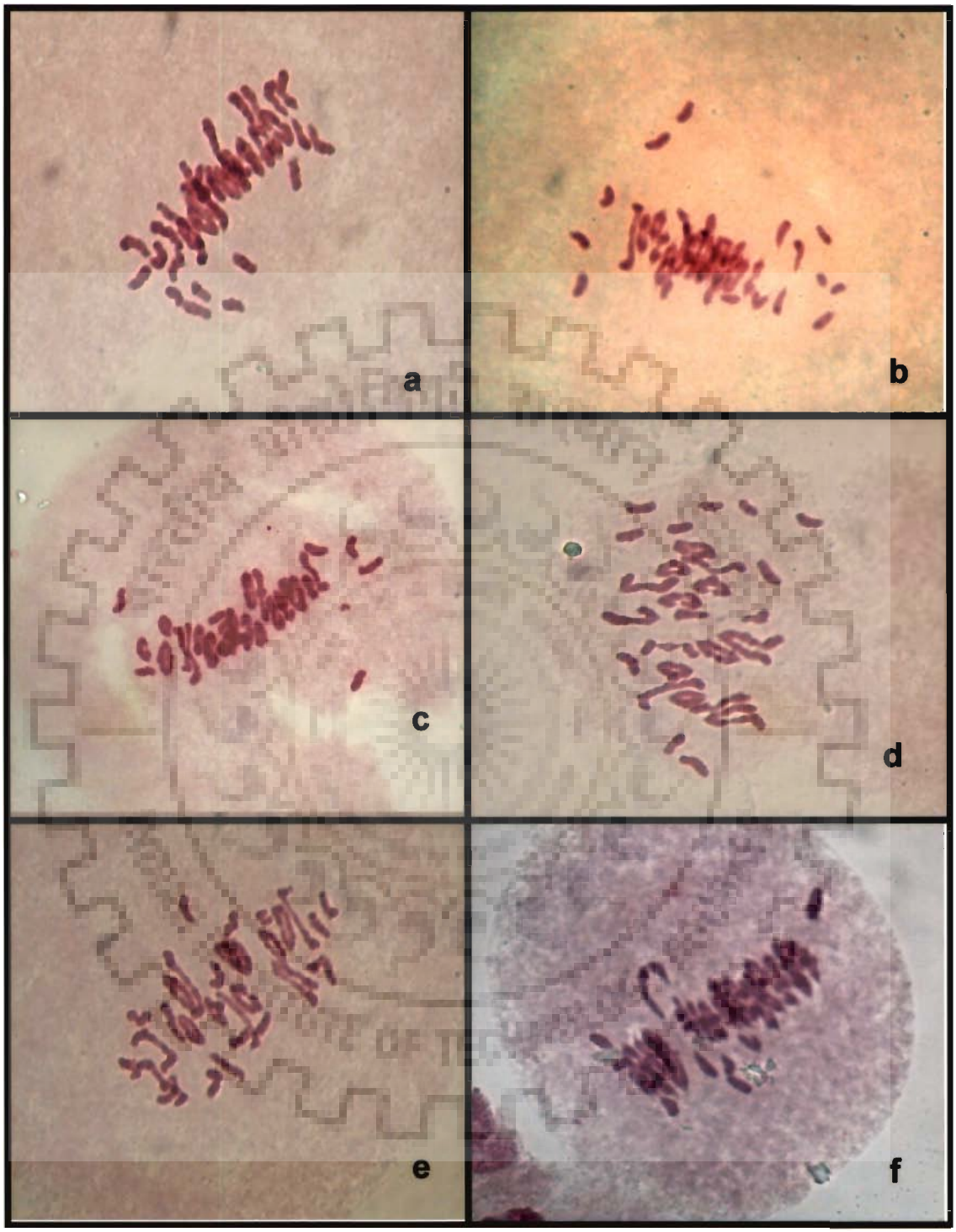

Fig. 4.5 Chromosomal pairing at metaphase-I of some BC_1 plants. a- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772 // PBW 373-1 45 Chr (1III+17II+8I), b- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772 // WL 711-1 44 Chr (17II+10I), c- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 1155-1-1//PBW 373-1 50 Chr (21II+7I+ 1 mini chr), d- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 3519 // WL 711- 2 44 Chr (2III+13II +12I), e- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 3519 // WL 711- 3 44 Chr (1III+15II+11 I) and f- BC_1 CS (*Ph*^{I})/ *Ae. peregrina* 1155-5-3 // PBW 373- 7 (50 Chr, 20II+14I)

Table 4.2 Morphology, chromosome number, fertility, iron and zinc content in the flag leaves of BC_1 plants

Parentage/ Pedigree	No. of Tillers	Height (cm)	Plant waxiness and awnness	Chr. No.	% Pollen stainability	Female Fertility	Iron (mg/ kg)	Zinc (mg/ kg)
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1	22	65	Awned, waxy	45	76.20	Fertile	219.95	48.42
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -2	10	35	Awned, waxy	53	8.31	Sterile	67.35	33.02
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -5	14	46	Awned, waxy	51	9.36	Sterile	30.19	38.14
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// WL 711-3	6	80	Awnless, waxy	48	10.53	sterile	50.48	26.04
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// WL 711-8	5	61	Awnless, waxy	54	8.92	sterile	49.31	18.40
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// UP2338-2	26	79	Awnless, waxy	42	68.49	Fertile	60.30	34.07
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// UP2338- 10	27	46	Awned, waxy	39	9.42	Sterile	70.28	20.38
CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// UP2338- 15	8	112	Awned, waxy	54	10.4	Sterile	75.03	43.61
CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1	25	70	Awned, waxy	44	80.07	Fertile	195.04	49.83
CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-3	23	70	Awnless, waxy	56	7.54	Sterile	152.17	58.01
CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-7	12	55	Awnless, waxy	48	18.52	Sterile	32.62	19.38
CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 343-1	37	73	Awned, waxy	53	8.06	Sterile	122.47	37.06
CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 343-13	42	46	Awned, waxy	49	14.64	Sterile	93.91	41.53
CS (*Ph*^{l})/ *Ae. peregrina* 3519// WL 711- 1	16	93	Awnless, nonwaxy	37	74.27	Fertile	167.41	45.72
CS (*Ph*^{l})/ *Ae. peregrina* 3519// WL 711- 2	26	73	Awned, nonwaxy	44	76.72	Fertile	47.42	35.08
CS (*Ph*^{l})/ *Ae. peregrina* 3519// WL 711- 3	22	80	Awned, waxy	44	85.63	Fertile	145.30	50.25
CS (*Ph*^{l})/ *Ae. peregrina* 3519// WL 711- 13	12	36	Awned, nonwaxy	52	13.18	Sterile	78.02	23.02
CS (*Ph*^{l})/ *Ae. peregrina* 3519// PBW 373- 5	7	90	Awned, waxy	45	8.28	Sterile	129.04	38.66
CS (*Ph*^{l})/ *Ae. peregrina* 3519// PBW 373- 14	12	110	Awnless, nonwaxy	45	10.35	Sterile	74.31	28.66

Table 4.2 Continued…

Parentage/ Pedigree	No. of Tillers	Height (cm)	Plant waxiness and awnness	Chr. No.	% Pollen stainability	Female Fertility	Iron (mg/ kg)	Zinc (mg/ kg)
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 5	12	55	Awnless, nonwaxy	40	8.18	Sterile	137.31	48.26
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 7	7	40	Awned, waxy	39	12.40	Sterile	46.19	67.41
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 8	30	84	Awned, waxy	52	9.15	Sterile	44.73	18.37
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 10	15	77	Awned, nonwaxy	39	16.19	Fertile	38.04	33.54
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 14	5	94	Awnless, waxy	42	8.24	Fertile	33.29	18.36
CS (*Ph*^{I})/ *Ae. peregrina* 3477// WL 711- 10	12	65	Awnless, waxy	40	9.38	Sterile	134.44	34.52
CS (*Ph*^{I})/ *Ae. peregrina* 3477// PBW343- 7	16	45	Awnless, waxy	45	68.34	Fertile	67.35	40.73
CS (*Ph*^{I})/ *Ae. peregrina* 3477// PBW 343- 9	20	102	Awned, waxy	54	14.38	Sterile	74.33	18.83
CS (*Ph*^{I})/ *Ae. peregrina* 3477// PBW 343- 12	24	90	Awned, waxy	43	12.05	Sterile	134.28	40.38
CS (*Ph*^{I})/ *Ae. peregrina* 1155-5-3// PBW 373- 7	45	30	Awned, nonwaxy	50	12.83	Sterile	123.37	38.51
CS (*Ph*^{I})/ *Ae. peregrina* 1155-5-3// PBW 373- 9	23	93	Awned, waxy	45	8.42	Sterile	52.09	23.65
CS (*Ph*^{I})/ *Ae. peregrina* 1155-5-3// PBW 373- 12	17	80	Awned, waxy	53	55.74	fertile	119.31	18.57
CS (*Ph*^{I})/ *Ae. peregrina* 1155-5-3// PBW 373- 14	15	83	Awned, nonwaxy	48	16.25	Sterile	45.25	36.05
T.aestivum CS (*Ph*^{I})	20	118	Awnless, nonwaxy	42	84.32	Fertile	52.27	30.25
T.aestivum WL 711	19	100	Awned, waxy	42	80.91	Fertile	56.50	32.02
Ae. peregrina 1155-5-3	200	36	Awned, nonwaxy	28	85.38	Fertile	155.46	63.47
Ae. peregrina 1155-1-1	250	38	Awned, waxy	28	78.60	Fertile	167.35	67.45
Ae. peregrina 3519	230	35	Awned, nonwaxy	28	80.12	Fertile	172.04	58.09

segregants. Some backcross derivatives had even lower micronutrient content than the recipient parents.

Some BC_1 plants were self fertile giving sufficient BC_1F_2 seeds while others with partial fertility were further backcrossed with recurrent wheat cultivars to get BC_2F_1 seeds.

4.1.4 BC_2F_1 plants

Data for various morphological characters, cytological details and micronutrients content of BC_2F_1 plants were recorded (Table 4.3). Most of the BC_2F_1 plants had nearly recovered background as that of recurrent wheat parent. Variation for tiller number, plant height, head type, seed colour, seed shape and waxiness was observed among BC_2F_1 derivatives. They had comparable tiller number, plant height, and head type with good seed set. Some of the derivatives had red grain colour like *Aegilops* parents. Sterile or partially fertile plants with low seed sets were discarded. Derivatives with fair seed set and good harvest index were analysed for grain iron and zinc content.

4.1.4.1 Cytology

Cytological details of BC_2F_1 derivatives were given in Fig. 4.6. Chromosome number in these backcross derivatives varied from 39 to 54 (Table 4.3). Reduction in univalent frequency and corresponding increase in bivalent frequency (BC_2F_1 17-1-2, 21II) was observed in BC_2F_1 derivatives (Fig. 4.6). BC_2F_1 derivatives with nearly 42 chromosomes and high grain iron and zinc content were selected for further advancement.

Table 4.3 Morphology, Chromosome number and grain Fe and Zn content of some BC_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 1-1-1	CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-1	32	85	Spelta, waxy, awned,	45	Amber, bold	30.17	17.25
BC_2 1-1-2	CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2	40	96	Square, nonwaxy, awned	46	Amber, round	43.24	38.94
BC_2 1-1-7	CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7	24	90	Square , waxy, awned	46	Red, plump, slender	47.08	50.41
BC_2 1-1-9	CS (*Ph*^{l})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-9	31	115	Spelta , waxy, awned	50	Amber, bold	39.72	49.33
BC_2 14-1-1	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-1	23	110	Square, waxy, awnless	44	Red, slender, plump	41.04	47.16
BC_2 14-1-8	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-8	28	130	Square, waxy, awned	43	Amber, bold	52.27	54.31
BC_2 14-1-9	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-9	30	100	Square, waxy, awnless	44	Amber, bold	52.3	46.97
BC_2 14-1-10	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-10	16	120	Square, waxy, awnless	42	Amber, bold, slender	32.74	42.25
BC_2 14-1-11	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-11	10	120	Square, waxy, awnless	42	Red, round	38.81	46.42
BC_2 14-1-12	CS (*Ph*^{l})/ *Ae. peregrina* 13772// WL 711-1///WL 711-12	17	119	Square, waxy, awnless	44	Red, shrivelled	45.7	38.72
BC_2 16-1-5	CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-5	14	105	Square, waxy, awned	44	Amber, plump	32.19	44.03
BC_2 16-1-8	CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-8	39	95	Square, waxy, awned	43	Red, bold	60.28	60.37
BC_2 16-1-9	CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-9	7	65	Square, waxy, awned	45	Red, shrivelled	23.83	34.02
BC_2 16-1-10	CS (*Ph*^{l})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-10	14	47	Square, waxy, awned	40	Red, round	28.16	17.35

BC_2 17-1-2	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-2	30	83	Spelta, waxy, awnless	44	Amber, bold	63.39	54.25
BC_2 17-1-3	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-3	46	150	Square, waxy, awned	43	Red, shrivelled	39.42	27.31
BC_2 17-1-5	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-5	24	138	Square, waxy, awnless	40	Amber, bold	58.31	45.14
BC_2 17-1-9	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-9	5	55	Spelta, waxy, awned	39	Red, shrivelled	38.24	16.33
BC_2 17-2-2	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-2	17	94	Square, waxy, awned	39	Amber, shrivelled	28.15	20.92
BC_2 17-2-8	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-8	22	65	Square, nonwaxy, awned	42	Amber, plum	60.28	60.37
BC_2 17-2-9	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-9	16	110	Spelta, waxy, awned	44	Red, shrivelled	34.15	18.32
BC_2 17-2-14	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-14	6	93	Spelta, waxy, awned	42	Amber, slender	24.72	35.84
BC_2 17-2-15	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-15	4	79	Square, nonwaxy, awnless	45	Red, bold	35.21	17.93
BC_2 17-3-4	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-4	69	120	Spelta, waxy, awned	43	Red, slender	21.36	21.30
BC_2 17-3-8	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-8	29	120	Spelta, waxy, awned	41	Amber, shrivelled	29.20	15.03
BC_2 17-3-9	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-9	52	100	Square, waxy, awned	38	Red, bold	27.59	38.26
BC_2 17-3-13	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-13	55	105	Spelta, waxy, awned	48	Amber, slender	30.14	23.57
BC_2 17-3-17	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-17	15	125	Square, waxy, awned	45	Amber, bold	27.52	32.05
BC_2 17-3-32	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-32	23	110	Square, waxy, awned	42	Amber, shrivelled	43.20	18.30
BC_2 17-3-37	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-37	19	105	Square, waxy, awned	44	Amber, bold	58.37	44.73
BC_2 17-3-38	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-38	17	82	Spelta, waxy, awned	45	Amber, bold	45.36	48.09

4.1.4.2 Grain iron and zinc content

Backcross derivatives with good seed set were analyzed for grain iron and zinc content. Nearly 200 BC_2F_1 plants were analyzed for micronutrient contents. Some of the backcross derivatives such as BC_2F_1 1-1-7, BC_2F_1 1-1-9, BC_2F_1 14-1-1, $BC_2F_1$14-1-8, $BC_2F_1$14-1-9, BC_2F_1 16-1-8, $BC_2F_1$17-1-2, and BC_2F_1 17-2-8 had nearly two to three folds higher grain iron and zinc content whereas some derivatives such as BC_2F_1 14-1-10, BC_2F_1 14-1-11 and 17-2-14 had only higher zinc content (Table 4.3). Some of the derivatives had even lower content of these micronutrients than control. Derivatives with high grain iron, zinc content and with good background recovery were selected and kept for further generation advancement.

4.1.5 BC_2F_2 derivatives

Seeds of selected progenies were sown in 10 rows at two locations. Morphological, chemical, cytological and molecular characterizations of a large number of plants from each selected plant progenies were done.

4.1.5.1 Morphology

BC_2F_2 plants of different progenies were morphologically very near to recurrent wheat parent. Morphological variation among plants of different progenies was observed for height, waxiness, head type, no of tillers, grain colour, seed shape and brittleness. Most of the plants in the selected progenies were waxy (Table 4.4). Some of the plants of 17-3-37 progenies were non-waxy which indicates, absence of the homoeologous group 2 chromosome of *Ae. peregrina*. Some of the plants such as BC_2F_2

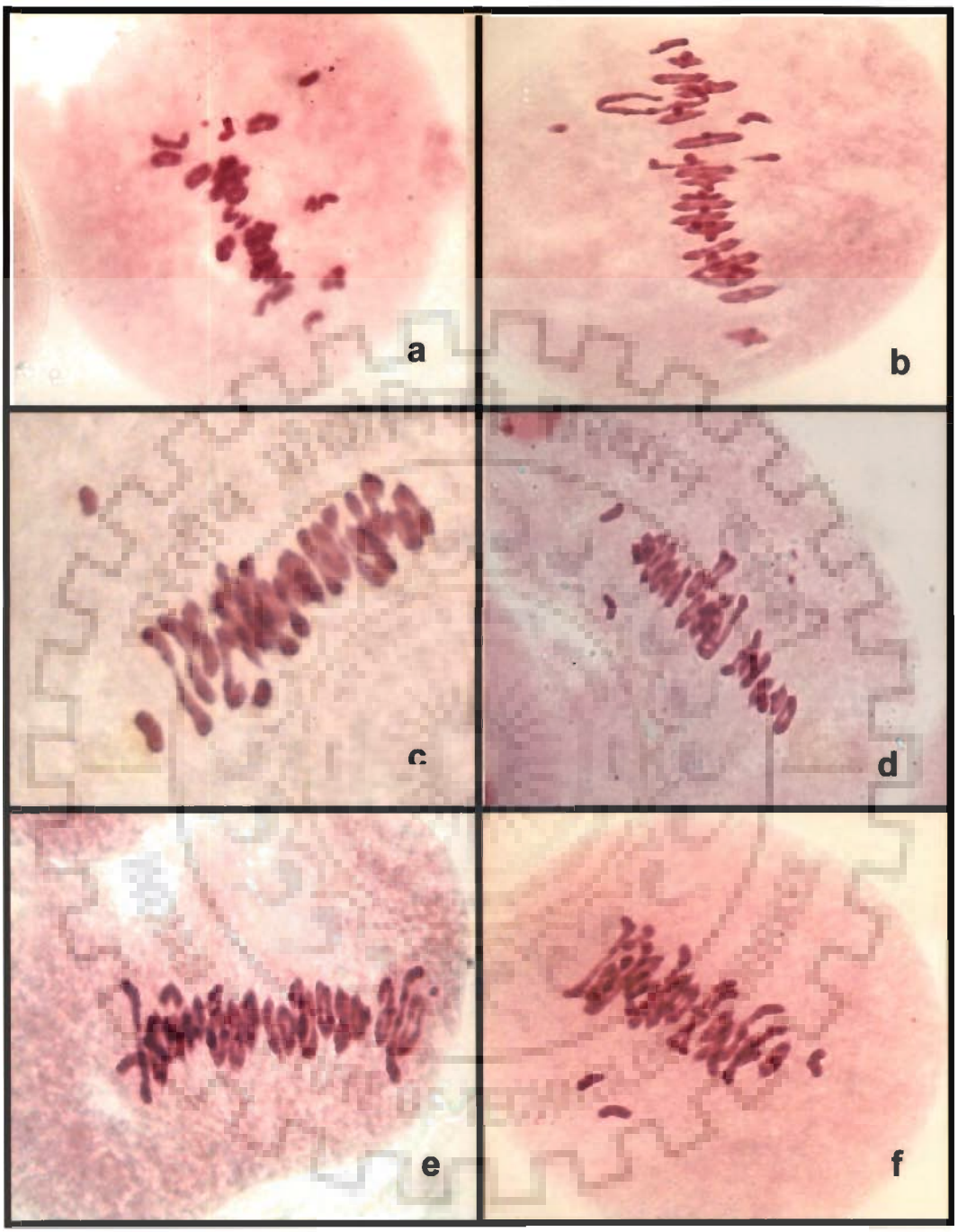

Fig. 4.6 Chromosomal pairing at metaphase-I of some BC_2F_1 plants. a- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772// WL 711-1///WL 711-12 43 Chr (18II+6I), b- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-8 43 Chr (1IV+18II+3I), c- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 3519// WL 711-1///WL 711-2 44 Chr (21II+2I), d- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-10 43 Chr (1 III+ 19II+2I), e- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 13772// PBW 373-1///WL 711-10 42 Chr (20II+2I) and f- BC_2F_1 CS (*Ph*^{I})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL711-7 45 Chr (21II+3I)

Table 4.4 Morphological characteristics of some representative plantsof BC_2F_2 derivatives. Selected derivatives are in bold

ID. No.	Pedigree	No. of tillers/ plant	Plant Height (cm)	Head type and waxyness	Rachis	Grain color	Harvest Index (%)
BC_2F_2 1-1-2-1	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2-1	19	100	Awned, square, waxy	NB	Amber, slender	12.6
BC_2F_2 1-1-2-2	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2-2	17	97	Awned, square, waxy	NB	Red, bold	8.2
BC_2F_2 1-1-2-9	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2-9	6	80	Awned, square, waxy	NB	Amber, small	25.3
BC_2F_2 1-1-2-16	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2-16	7	60	Awned, square, waxy	NB	Amber, bold	21.0
BC_2F_2 1-1-2-19	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-2-19	9	68	Awned, square, waxy	NB	Amber, small	13.5
BC_2F_2 1-1-7-4	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7-4	14	84	Awned, square, waxy	NB	Amber, bold	25.0
BC_2F_2 1-1-7-6	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7-6	11	67	Awned, square, waxy	NB	Amber, bold	25.5
BC_2F_2 1-1-7-8	**CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7-8**	**23**	**95**	**Awned, square,waxy**	**NB**	**Amber, bold**	**27.3**
BC_2F_2 1-1-7-13	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7-13	19	83	Awned, square,waxy	NB	Red ,bold	40.3
BC_2F_2 1-1-7-18	**CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-7-18**	**17**	**85**	**Awned, square,waxy**	**NB**	**Red, bold**	**25.6**
BC_2F_2 1-1-9-4	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-9-4	7	105	Awned, square, waxy	NB	Amber, bold	8.5
BC_2F_2 1-1-9-14	CS (*Ph*^{1})/ *Ae. peregrina* 1155-1-1// PBW 373 -1///WL 711-9-13	9	106	Awned, square, waxy	NB	Red, bold	10.4
BC_2F_2 14-1-1-51	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-1-51	9	65	Awned, square, waxy	NB	Amber, shrivelled	36.5
BC_2F_2 14-1-1-58	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-1- 58	15	89	Awnless, square, waxy	NB	Amber, shrivelled	29.2
$BC_2F_2$14-1-8-11	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-8- 11	4	100	Awned, square, waxy	NB	Amber, bold	7.5
BC_2F_2 14-1-8-18	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-8- 18	7	66	Awned, square, waxy	NB	Amber, bold	24.3
BC_2F_2 14-1-8-36	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-8- 36	20	95	Awnless, square, waxy	NB	Amber, bold	20.4
BC_2F_2 14-1-9-5	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-9- 5	7	96	Awned, square, waxy	B	Red, small	12.4
BC_2F_2 14-1-9-8	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-9- 8	11	100	Awnless, square, waxy	NB	Red, small	18.0
BC_2F_2 14-1-9-11	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-9- 11	7	110	Awnless, square, waxy	NB	Red, slender	10.3
BC_2F_2 14-1-11-6	CS (*Ph*^{1})/ *Ae. peregrina* 13772// WL 711-1///WL 711-11- 6	10	80	Awned, square, waxy	B	Amber, bold	14.0

ID. No.	Pedigree	No. of tillers/ plant	Plant Height (cm)	Head type and waxyness	Rachis	Grain color	Harvest Index (%)
BC_2F_2 14-1-11-11	CS (*Ph^l*)/ *Ae. peregrina* 13772// WL 711-1///WL 711-11- 11	7	97	Awned, square, waxy	NB	Amber, bold	38.9
BC_2F_2 14-1-12-12	**CS (*Ph^l*)/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 12**	**18**	**80**	**Awned, square, waxy**	**NB**	**Amber, plumped**	**33.3**
BC_2F_2 14-1-12-27	CS (*Ph^l*)/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 27	5	110	Awned, square, waxy	B	Red, bold	12.0
BC_2F_2 14-1-12-44	**CS (*Ph^l*)/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 44**	**16**	**84**	**Awned, square, waxy**	**NB**	**Amber, bold**	**27.1**
BC_2F_2 14-1-12-48	**CS (*Ph^l*)/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 48**	**19**	**110**	**Awned, square, waxy**	**NB**	**Red, bold**	**28.4**
BC_2F_2 16-1-3-33	CS (*Ph^l*)/ *Ae. peregrina* 13772// PBW 373-3///WL 711-3-33	5	56	Awnless, square, waxy	B	Amber, bold	16.9
BC_2F_2 16-1-3-42	CS (*Ph^l*)/ *Ae. peregrina* 13772// PBW 373-3///WL 711-3-42	15	90	Awnless, square, waxy	B	Amber, bold	19.4
BC_2F_2 16-1-8-4	**CS (*Ph^l*)/ *Ae. peregrina* 13772// PBW 373-3///WL 711-8-4**	**16**	**91**	**Awned, square, waxy**	**NB**	**Amber, bold**	**30.1**
BC_2F_2 16-1-8-32	CS (*Ph^l*)/ *Ae. peregrina* 13772// PBW 373-3///WL 711-8-32	5	103	Awned, square, waxy	B	Red, bold	14.4
BC_2F_2 16-1-8-56	**CS (*Ph^l*)/ *Ae. peregrina* 13772// PBW 373-3///WL 711-8-56**	**15**	**92**	**Awned, square, waxy**	**NB**	**Red, bold**	**25.0**
BC_2F_2 17-1-2-1	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-2-1	9	80	Awned, square, waxy	NB	Red, bold	8.5
BC_2F_2 17-1-2-2	**CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-2-2**	**14**	**76**	**Awned, square, waxy**	NB	**Red, bold**	**28.5**
BC_2F_2 17-1-2-5	**CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-2-5**	**16**	**87**	**Awned, square, waxy**	NB	**Amber, bold**	**29.4**
BC_2F_2 17-1-3-28	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-3-28	13	65	Awned, square, waxy	NB	Amber, shrivelled	9.7
BC_2F_2 17-1-3-37	**CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-3-37**	**15**	**79**	**Awned, square, waxy**	**NB**	**Red, not plumped**	**25.4**
BC_2F_2 **17-1-3-38**	**CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 1///WL 711-3-38**	**18**	**83**	**Awnless, square, waxy**	**NB**	**Red, bold**	**28.3**
BC_2F_2 17-2-8-4	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-8-4	12	82	Awned, square, waxy	NB	Amber, slender	11.0
BC_2F_2 17-2-8-19	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-8-19	17	46	Awned, square, nonwaxy,	B	Amber, bold	9.5
BC_2F_2 17-2-8-22	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 2///WL 711-8-22	7	95	Awnless, square, waxy,	B	Amber, bold	20.4
BC_2F_2 **17-3-37-16**	**CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-37-16**	14	105	**Awned, square, waxy**	**NB**	**Red, bold**	**15.1**
BC_2F_2 17-3-37- 27	CS (*Ph^l*)/ *Ae. peregrina* 3519// WL 711- 3///WL 711-37-27	6	110	Awned, square, waxy	B	Amber, bold	6.4

1-1-7-18, BC_2F_2 14-1-12-48, BC_2F_2 16-1-8-56, BC_2F_2 17-1-2-2 had red grain colour. This may be due to presence of group 3 chromosome of *Ae. peregrina* in these progenies. Harvest index of each plant was recorded. Plants with good harvest index were analysed for their grain iron and zinc content and other cytological and molecular characterisation.

4.1.5.2 Cytology

Detailed cytological studies of BC_2F_2 derivatives were done. In most of the derivatives chromosome number varied from 41 to 43 (Table 4.5). Only BC_2F_2 1-1-7-8 had shown 45 chromosome number. Nearly 42 chromosomes numbers were observed in selected BC_2F_2 derivatives with bivalent frequency ranging from 18.90 (BC_2F_2 16-1-8-4) to 21.37 ($BC_2F_2$14-1-12-12) and univalent frequency of 0.77 (BC_2F_2 17-3-37-16) to 3.15

Table 4.5 Chromosome number and pairing at metaphase-I of selected BC_2F_2 derivatives

Derivative I.D. No.	2n	PMCs	Mean ± S.D Univalent (I)	Mean ± S.D Bivalent (II)	Mean ± S.D Trivalent (III)
BC_2F_2 1-1-7-8	45	25	2.50 ± 0.13	19.00 ± 0.34	1.50 ± 0.27
BC_2F_2 1-1-7-18	42	25	2.00 ± 0.36	19.01 ± 0.19	0.66 ± 0.14
$BC_2F_2$14-1-12-12	43	25	1.64 ± 0.28	20.68 ± 0.22	0.00
BC_2F_2 14-1-12-44	43	25	2.53 ± 0.31	20.23 ± 0.25	0.00
BC_2F_2 14-1-12-48	42	25	1.82 ± 0.21	20.09 ± 0.34	0.00
BC_2F_2 16-1-8-4	42	25	1.20 ± 0.18	18.90 ± 0.13	1.00 ± 0.10
BC_2F_2 16-1-8-56	42	25	1.00 ± 0.26	20.5 ± 0.15	0.00
BC_2F_2 17-1-2-2	43	25	2.42 ± 0.17	19.29 ± 0.40	1.00 ± 0.14
BC_2F_2 17-1-2-5	42	25	1.37 ± 0.32	20.31 ± 0.31	0.00
BC_1F_3 17-1-3-37	43	25	3.15 ± 0.29	19.92 ± 0.37	0.00
BC_1F_3 17-1-3-38	42	25	2.19 ± 0.34	19.90 ± 0.19	0.00
BC_2F_2 17-3-37-16	42	25	0.77 ± 0.23	20.61 ± 0.11	0.00

(BC_1F_3 17-1-3-37). Some of the plants had also revealed presence of trivalents in their PMCs (Table 4.5, Fig. 4.7, Fig. 4.8). Mini or dot chromosome was observed in BC_2F_2 14-1-12-12 plant (Fig. 4.8).

4.1.5.3 Grain iron and zinc content

Approximately twenty to thirty plants from each selected progeny with good harvest index and good background recovery were analysed for grain iron and zinc content by inductively coupled plasma spectrometer. Among BC_2F_2 1-1 derivatives, 1-1-2-9, 1-1-7-8, 1-1-7-18 and 1-1-9-4 had shown 40 to 60 percent increase in grain iron and zinc content (Fig. 4.9). Iron and zinc content was also calculated on seed basis. Increase of 106.63 and 76.4 percent in iron and 73.4 to 94.0 in zinc content was observed in BC_2F_2 1-1-7-8 and BC_2F_2 1-1-7-18. Except few plants among 14-1 derivatives, in general all had high grain iron content over control. BC_2F_2 14-1-1-90, 14-1-8-36, 14-1-8-18, 14-1-9-8 and 14-1-11-6 had shown increase of 61 to 123 percent in grain iron concentration whereas only 14-1-8-18 and 14-1-11-6, also had 70 % increase in grain zinc concentration (Fig. 4.10). Among 14-1 progenies, 14-1-12-12, 14-1-12-44 and 14-1-12-48 had approximately 2 to 3 fold higher grain iron and zinc concentration than control and therefore selected for cytological and molecular analysis (Table 4.3). Similarly, with in $BC_2F_2$16-1 progeny, BC2F2 16-1-8-4 and 16-1-8-56 had both high iron and zinc concentrations (Table 4.6, Fig 4.11). Some plants with only high zinc concentration such as BC_2F_2 17-1-3-37 and 17-3-37-16 were also selected for molecular analysis (Table 4.6, Fig 4.12). Some of the plants had high grain and zinc content but had lower harvest index therefore they were discarded. Low seed set may lead to the concentration effect of micronutrients in few grains. The selected derivatives were as fertile as the wheat parent

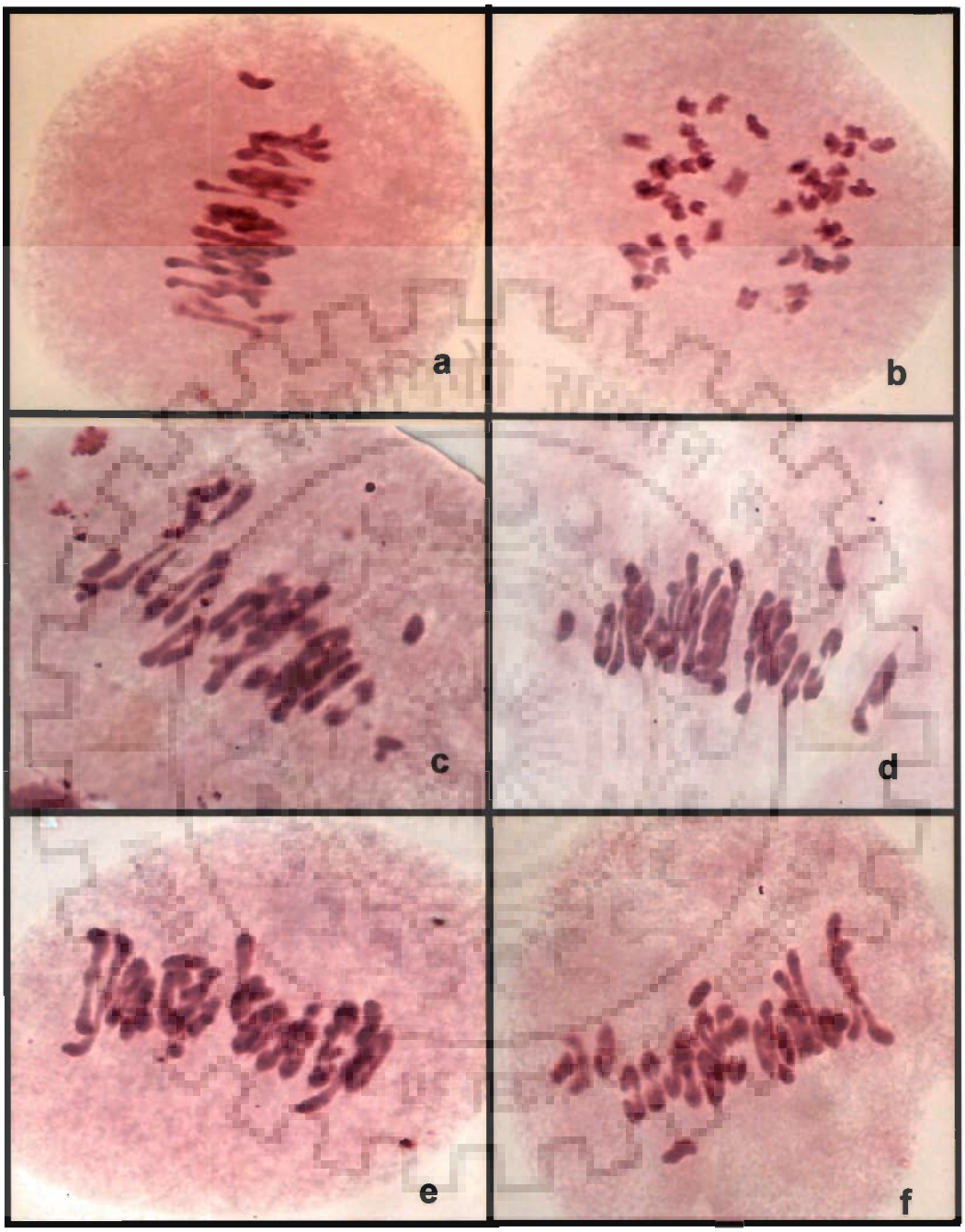

Fig. 4.7 Chromosomal pairing at metaphase-I in some BC_2F_2 plants. a, b- BC_2F_2 CS (*Ph*^{I})/ *Ae. peregrina* 1155-1-1// PBW 373-1///WL 711-7-8 45 Chr (22II+1I), c, d- BC_2F_2 CS (*Ph*^{I})/ *Ae. peregrina* 1155-1-1// PBW 373-1///WL 711-7-18 42 Chr (1 III +18 II+ 3I), e, f- CS (*Ph*^{I})/ *Ae. peregrina* 13772// PBW 373-3///WL 711-8-56 42 Chr (20 II + 2I)

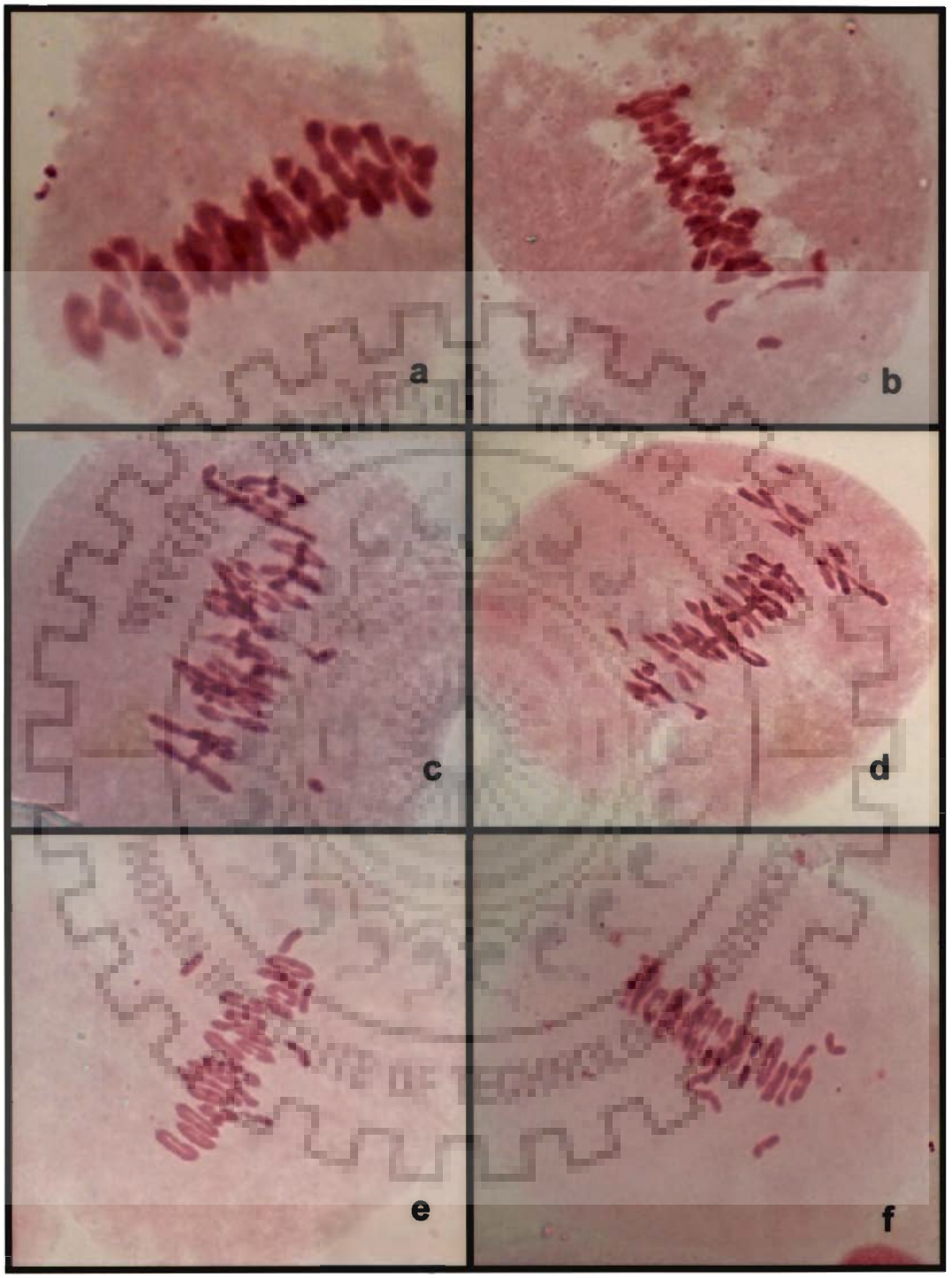

Fig. 4.8 Chromosomal pairing at metaphase-I of some BC_2F_2 plants a, b- BC_2F_2 CS (*Ph*^{I})/ *Ae. peregrina* 13772// PBW 373-3///WL 711-8-4 42 Chr (21 II, 20 II+ 2I respectively), c, d- BC_2F_2 CS (*Ph*^{I})/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 12 43 Chr (21II+ 1I), e, f- BC_2F_2 CS (*Ph*^{I})/ *Ae. peregrina* 13772// WL 711-1///WL 711-12- 44 43 Chr (20II+ 3I)

Table. 4.6 Grain iron and zinc concentration and content of selected derivatives

ID.NO.	Iron concentration (mg/kg) Mean ± S.E	% Increase over WL 711	Iron content per seed (mg) Mean ± S.E	% Increase over WL711	Zinc concentration (mg/ kg) Mean ± S.E	% Increase over WL 711	Zinc content per seed (mg) Mean ± S.E	% Increase over WL 711
WL 711	27.2 ±1.2	-	1.8 ± 0.1	-	19.0 ± 0.8	-	1.3 ± 0.1	-
BC_2F_2 1-1-7-8	39.6 ±0.82	46.0	3.7 ± 0.3	106.3	23.8 ± 1.4	25.5	2.2 ± 0.1	73.7
BC_2F_2 1-1-7-18	43.1 ± 1.5	58.8	3.3 ± 0.1	76.4	33.0 ± 1.0	73.9	2.5 ± 0.1	94.0
BC_2F_2 14-1-12-12	51.3 ±0.45	88.1	3.5 ± 0.2	87.5	33.3 ± 0.4	75.4	2.3 ± 0.0	75.6
BC_2F_2 14-1-12-44	54.7 ±1.0	101.6	3.7 ± 0.2	101.3	34.1 ± 0.3	79.2	2.3 ± 0.1	79.4
BC_2F_2 14-1-12-48	63.8 ±0.33	134.9	4.5 ± 0.1	142.1	37.0 ± 0.5	95.2	2.6 ± 0.0	102.2
BC_2F_2 16-1-8-4	49.0 ±1.4	80.6	3.3 ± 0.0	79.9	66.7 ± 1.5	251.2	4.6 ± 0.3	251.9
BC_2F_2 16-1-8-56	34.2 ±1.0	25.9	2.3 ± 0.1	25.6	48.7 ± 0.9	156.3	3.3 ± 0.2	156.8
BC_2F_2 17-1-2-2	44.4 ±0.5	63.6	3.8 ± 0.2	105.9	35.1± 0.9	84.9	3.0 ± 0.2	133.8
BC_2F_2 17-1-2-5	44.3 ±1.1	63.2	4.0 ± 0.1	114.3	39.4± 1.2	107.4	3.5 ± 0.1	173.4
BC_2F_2 17-1-3-37	53.4 ±1.1	96.6	3.4 ± 0.1	83.3	43.3 ± 0.7	127.7	2.7 ± 0.1	113.3
BC_2F_2 17-1-3-38	55.7 ±0.6	105.1	5.8 ± 0.5	212.8	35.1 ± 0.5	84.9	3.7 ± 0.2	182.9
BC_2F_2 17-3-37-16	53.8 ±0.6	98.0	3.4 ± 0.8	84.63	43.0 ± 0.4	126.4	2.7 ± 0.1	112.1

(Fig. 4.13) and had seeds as bold as or even bolder than the cultivar seeds (Fig. 4.14). Therefore they had true increase in grain micronutrients and thus subjected to molecular and GISH characterisation.

Three other elements in addition to iron and zinc *viz.*, manganese, copper and calcium were also analysed in the digested seed samples using ICPMS. Nearly three fold high grain Mn, Cu and Ca concentration were found in BC_2F_2 seeds of these derivatives (Table 4.7)

Table 4.7 Grain mineral content in selected BC_2F_2 seeds

ID. No.	Mn (mg/kg)	Cu (mg/kg)	Ca (mg/kg)
WL 711	19.5	4.6	40.6
BC_2F_2 1-1-2	42.5	21.4	170.7
BC_2F_2 1-1-9	45.2	25.0	105.4
BC_2F_2 1-1-7	60.5	22.1	158.5
BC_2F_2 14-1-8	62.8	14.4	122.7
BC_2F_2 14-1-12	65.6	13.3	120.1
BC_2F_2 16-1-8	38.5	25.3	115.0
BC_2F_2 16-1-5	44.1	14.2	132.1
BC_2F_2 17-2-8	68.5	17.3	108.3
BC_2F_2 17-1-3	47.4	17.5	120.4
BC_1F_3 17-3-37	44.1	19.2	94.2

4.1.6 Molecular and cytogenetic analysis of introgressive derivatives

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

The HMW glutenin subunit profile of some of the selected derivatives is given in Fig. 4.15. SDS-PAGE profiling of single seeds from each progeny was done for the tracking of group 1U/1S from *Ae. peregrina* in the introgressive derivatives. None of the derivatives had shown introgression of group 1 chromosomes from *Ae. peregrina*. This also suggests that group 1 chromosome of *Ae. peregrina* 1155-1-1,13772 or 3519 either

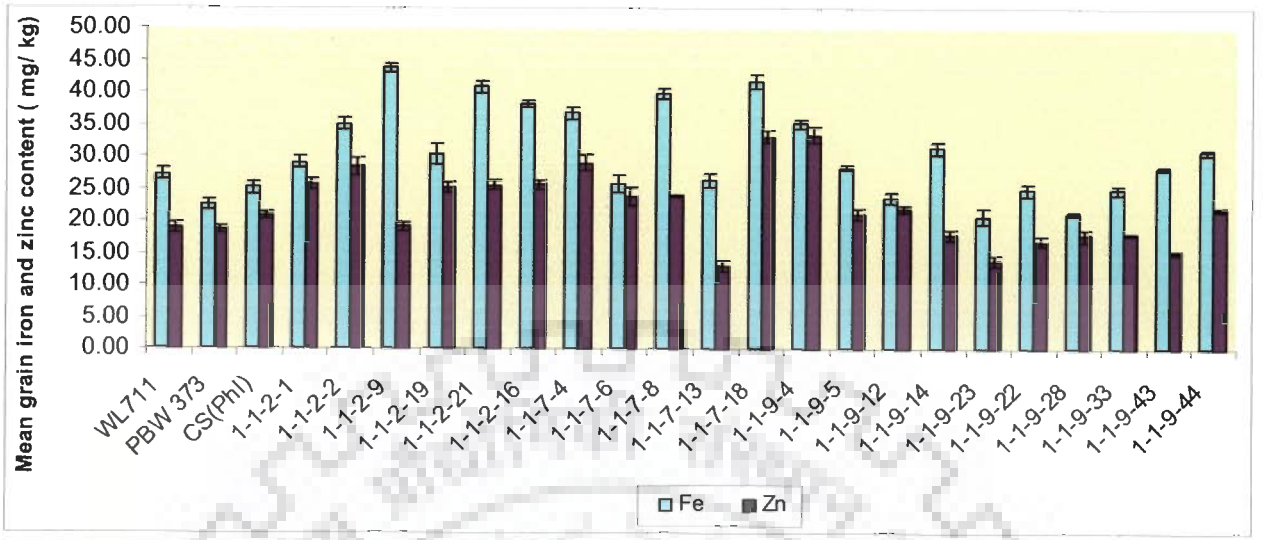

Fig. 4.9 Mean grain iron and zinc contents of BC_2F_2 1-1 progeny

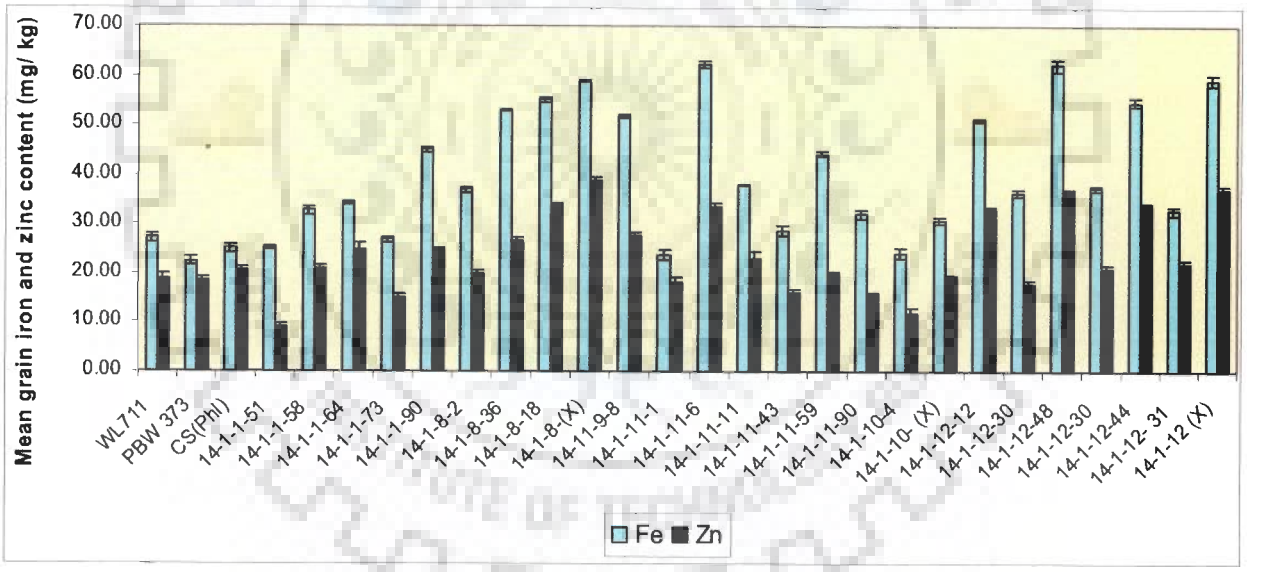

Fig. 4.10 Main iron and zinc contents of BC_2F_2 14-1 progeny

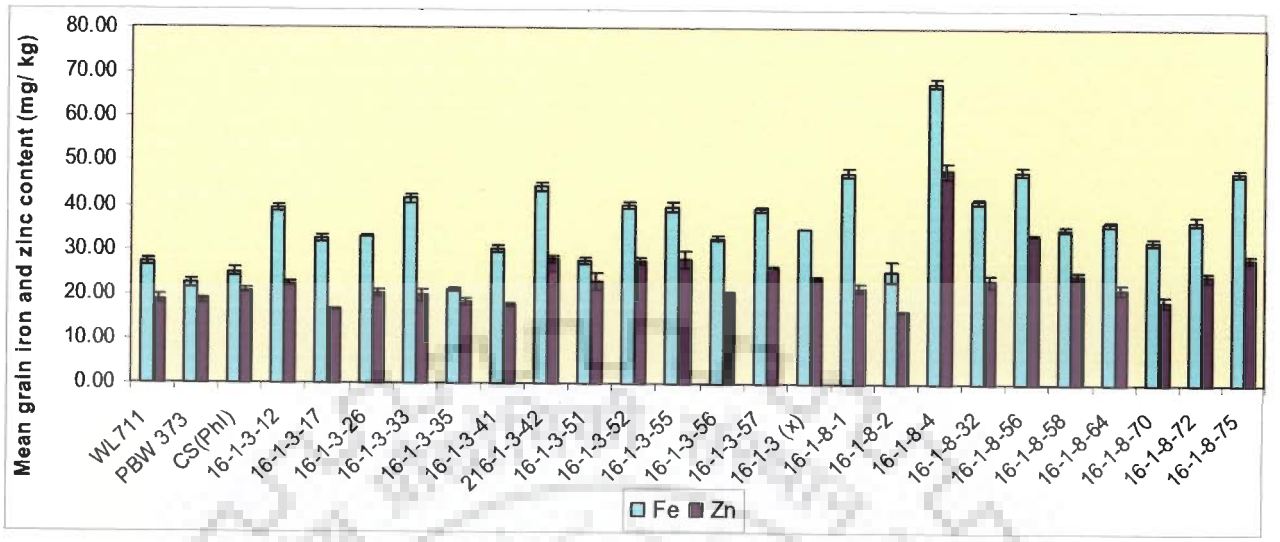

Fig. 4.11 Mean grain iron and zinc contents of BC_2F_2 16-1 progeny

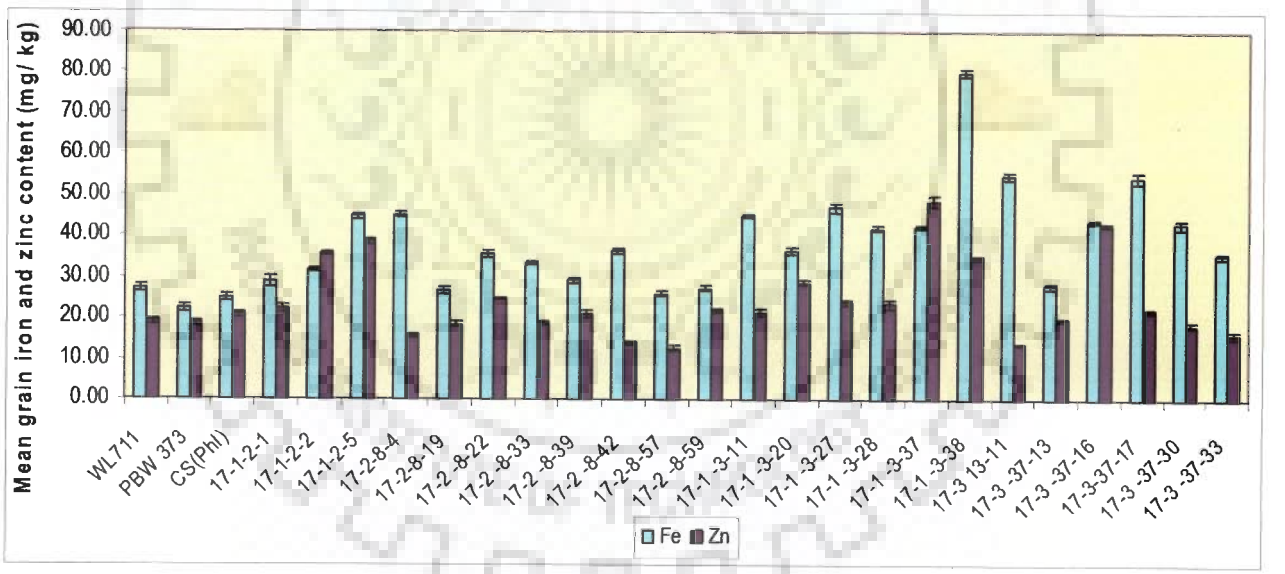

Fig. 4.12 Mean grain iron and zinc concentrations of BC_2F_2 17-1, 17-2, 17-3 progeny

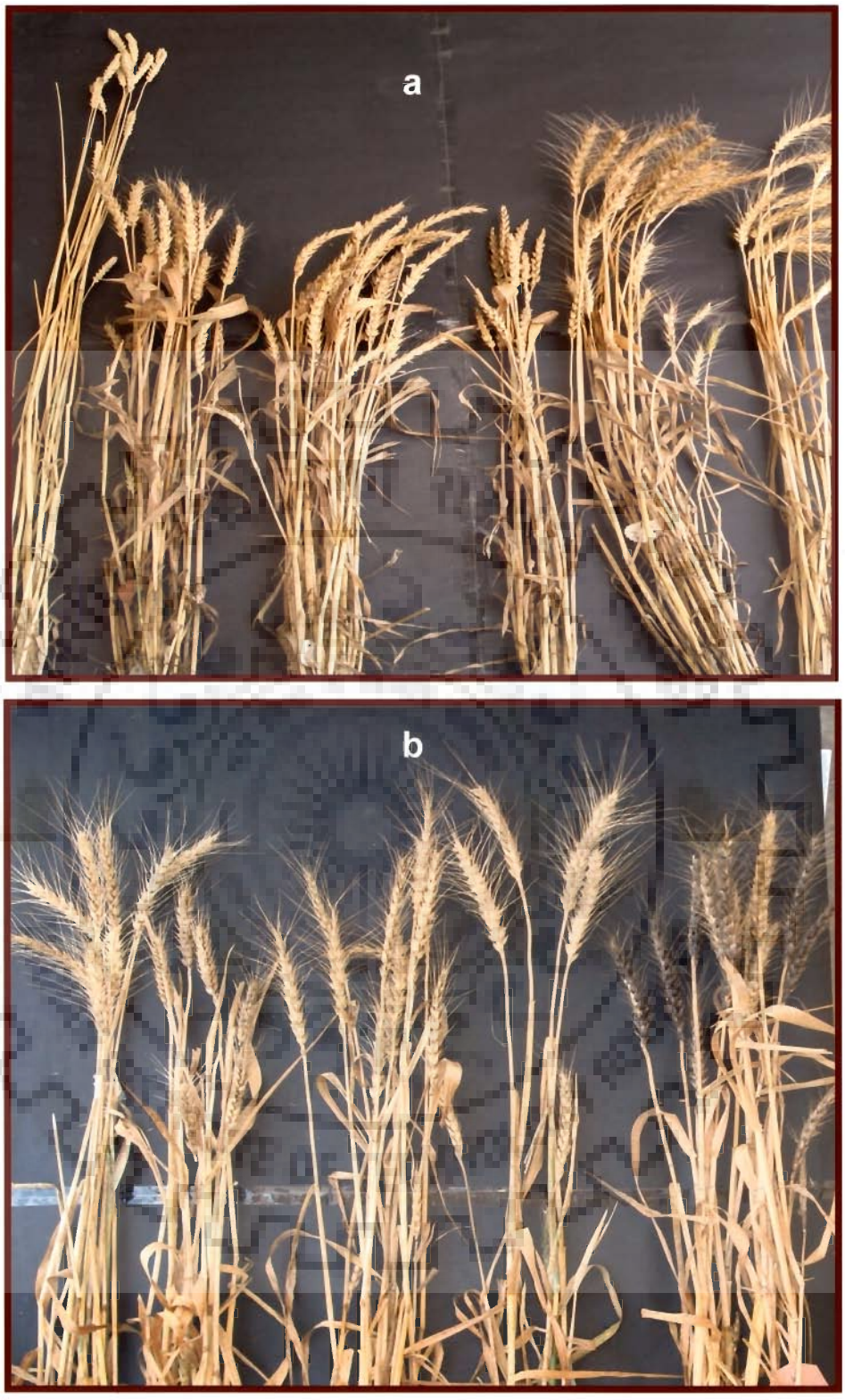

Fig. 4.13 Plant morphology of two BC_2F_2 progenies a- left to right CS (*Ph*^{l}), BC_2F_2 16-1-8-4: Pl-4, Pl-43, Pl-48, Pl-56, WL711, b. left to right WL 711, BC_2F_2 1-1-7-8: Pl-4, Pl-8, Pl-12, Pl-13

Fig. 4.14 Seeds of selected BC_2F_2 derivatives. 1. WL 711, 2. BC_2F_2 1-1-7-8, 3. BC_2F_2 14-1-8-18, 4. BC_2F_2 16-1-8-56, 5. BC_2F_2 17-1-2-5, 6. BC2F2 17-1-3-38 and 7. *Ae. peregrina* 13772

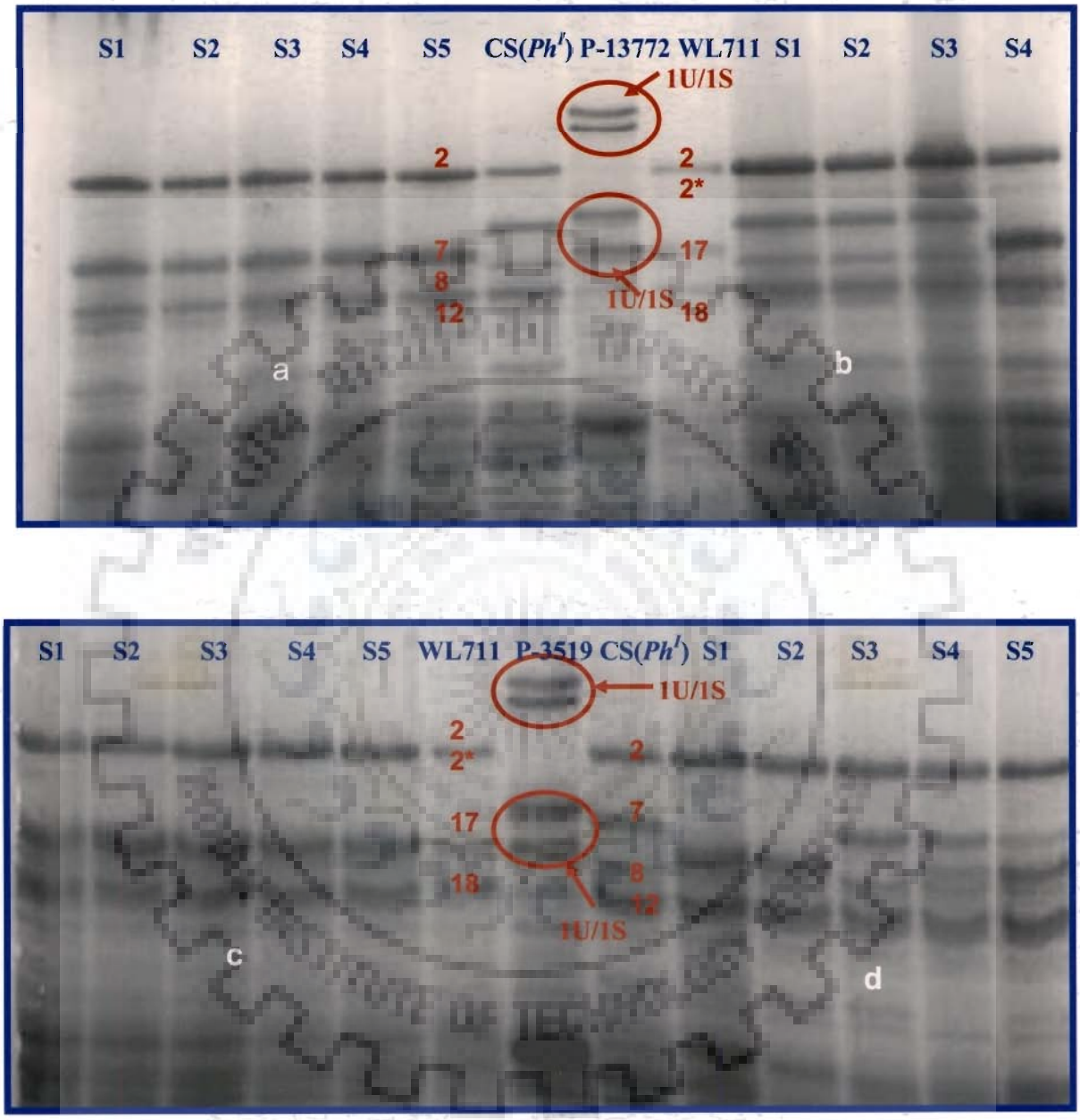

Fig. 4.15 High Molecular Weight- Glutenin Subunit profile of some selected BC_2F_2 plants a- BC_2F_2 14-1-12-12, b- BC_2F_2 14-1-12-44, c- BC_2F_2 17-1-2-5 and d- BC_2F_2 17-1-3-37

do not have gene(s) /QTLs for high grain iron and zinc content or have been preferentially eliminated.

4.1.6.2 Molecular markers analysis

Anchored wheat microsatellite markers (STMS) were applied to the selected introgressive derivatives with high grain iron and zinc content. Terminal markers of each chromosome i.e from 1A, B, D to 7 A, B, D were used to characterise the selected derivatives. 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 parents. Total number of applied markers, transferable to the *Ae. peregrina* and those found polymorphic for the parents are given in Table 4.8. Few transferable and polymorphic markers are shown in Fig 4.16 and 4.17. Out of 420 markers 65 % markers were found transferable and of these transferable markers 43.22 % were found polymorphic between wheat and *Aegilops* parent. Among A, B and D genome markers, B genome markers had shown maximum transferability (69 %) which may be due to closeness of B genome of wheat to S genome of *Ae. peregrina*.

Firstly anchored wheat SSR markers at distal positions of each of the 42 chromosome arms transferable to *Ae. peregrina* with distinct polymorphism between wheat and the *Aegilops* parent were applied on the selected derivatives to examine the alien chromosome/chromosome arm introgressed. The details of alien chromosome introgression in selected derivatives are given in the Table 4.9. Introgression of group 4 and 7 chromosomes of *Ae. peregrina* (U/S) was observed in the derivatives with high grain iron and zinc content. Introgression of these alien chromosomes was further

Table 4.8. Transferability and polymorphism of anchored wheat microsatellite markers between wheat and *Aegilops* species

Homoeologous genome	Chromosome	Markers Tested	Markers Transferable	Markers Polymorphic	Transferable (%)	Polymorphism in transferable markers (%)	Genome wise transferability of markers
A	1A	23	15	7	65.2	46.7	62.6
	2A	28	18	9	64.3	50.0	
	3A	20	14	6	70.0	42.9	
	4A	19	9	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	24	17	8	70.8	47.1	69.0
	2B	27	20	9	74.1	45.0	
	3B	18	12	3	66.7	25.0	
	4B	18	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	23	14	7	60.9	50.0	63.2
	2D	26	18	7	69.2	38.9	
	3D	15	9	5	60.0	55.6	
	4D	17	12	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		420	273	118	63.6	46.3	

confirmed by using additional distinct polymorphic markers of group 4 and 7 chromosomes.

The details of the introgressed chromosomes identified are given in Table 4.10 and Fig 4.18, 4.19. Out of 3 genomes of group 4, anchored markers of wheat 4A and 4B showed introgressed *Ae. peregrina* (U/ S) chromosome. Introgression of group 4 and 7 chromosome were observed in the derivatives BC_2F_2 1-1-7-8, BC_2F_2 1-1-7-18, BC_2F_2 16-1-8-56, BC_2F_2 17-1-3-38, BC_2F_2 17-1-2-2, 17-1-2-5 whereas BC_2 F_2 17-1-3-37, BC_2 F_2

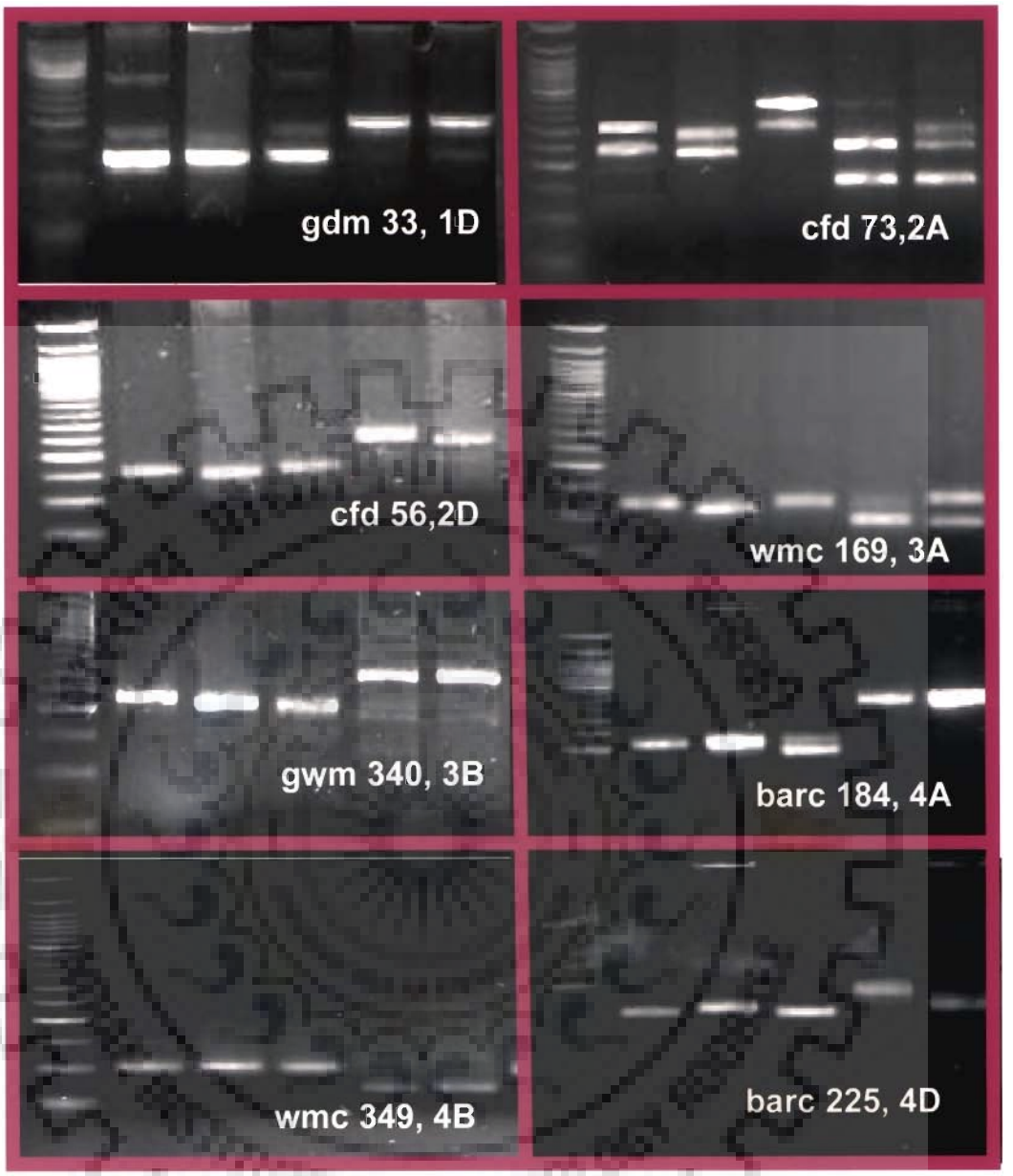

Fig. 4.16 Wheat SSR markers showing polymorphism among wheat and *Aegilops* parents. 1. WL 711, 2. CS (*Ph*^{l}), 3. PBW 373, 4. *Ae. peregrina* 1155-1-1 and 5. *Ae. peregrina* 13772

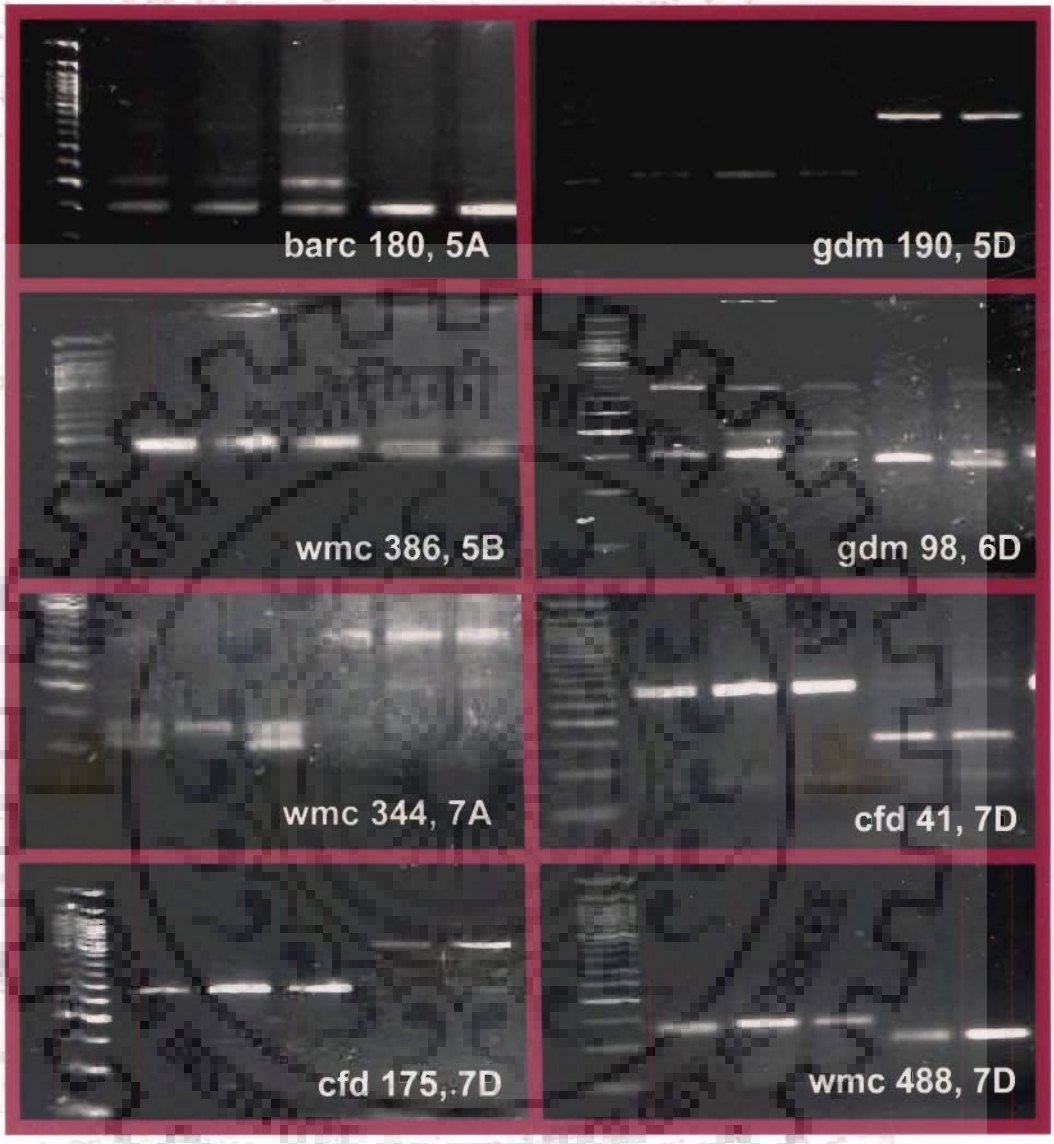

Fig. 4.17 Wheat SSR markers showing polymorphism among wheat and *Aegilops* parents. 1. WL 711, 2. CS (*Ph*^{I}), 3. PBW 373, 4. *Ae. peregrina* 1155-1-1 and 5. *Ae. peregrina* 13772

17-1-2-2, BC_2 F_2 14-1-12-12, BC_2 F_2 14-1-12-48 and BC_2 F_2 17-3-37-16 had shown introgression of only group 4. Only one of the derivatives 17-1-3-38 showed introgression of group 5 chromosome. Percentage increase by the addition of group 4 and group 7 is given in the Table 4.9

Fig 4.9 Application of anchored polymorphic SSR markers of group 4, 5 and 7 chromosomes of wheat on selected derivatives

Derivatives' ID	Group of introgressed alien chromosome	% Increase in iron content	% Increase in zinc content
BC_2 F_2 1-1-7-8	4, 7	106.3	73.7
BC_2 F_2 1-1-7-18	4, 7	76.4	94.0
BC_2 F_2 16-1-8-4	7	80.6	251.2
BC_2 F_2 16-1-8-56	4,7	25.9	156.3
BC_2 F_2 17-1-3-37	4	96.6	127.7
BC_2 F_2 17-1-3-38	4,5, 7	105.1	84.9
BC_2 F_2 17-1-2-2	4	63.6	84.9
BC_2 F_2 17-1-2-5	4,7	63.2	107.4
BC_2 F_2 14-1-12-12	4	88.1	75.4
BC_2 F_2 14-1-12-44	-	101.6	79.2
BC_2 F_2 14-1-12-48	4	134.9	95.2
BC_2 F_2 17-3-37-16	4	98.0`1	126.4

Table 4.10 Details of alien chromosome introgression in the selected BC_2F_2 and BC_1F_3 derivatives with high grain iron and zinc content.

I.D. No.	1AS	1AL	1BS	1BL	1DS	1DL	2AS	2AL	2BS	2BL	2DS	2DL	3AS	3AL	3BS	3BL	3DS	3DL	4AS	4AL	4BS
→ *SSR Markers used*	*gdm33*	*barc287*	*gwm403*	*Wmc500*	*cfd 61*	*wmc405*	*Cfd36*	*wmc63*	*barc318*	*wmc474*	*cfd56*	*gdm148*	*barc12*	*wmc169*	*barc75*	*gwm340*	*cfd79*	*wmc552*	*gdm 6*	*Wmc698*	*barc10*
CS(Ph^1)	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
WL 711	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
PBW343	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Ae. pereg 1155-1-1	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Ae. pereg 13772	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1-1-7-8	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	P+W	W
1-1-7-18	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W
16-1-8-4	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
16-1-8-56	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W	W
17-1-3-37	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W	W
17-1-3-38	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W	W
17-1-2-2	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W
17-1-2-5	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	P+W	W
14-1-12-12	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W	W
14-1-12-44	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
14-1-12-48	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W
17-3-37-16	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W	W

CONTINUED...

I.D. No.	4BL	4DS	4DL	5AS	5AL	5BS	5BL	5DS	5DL	6AS	6AL	6BS	6BL	6DS	6DL	7AS	7AL	7BS	7BL	7DS	7DL
→ *SSR Markers used*	*wmc349*	*barc225*	*wmc331*	*barc180*	*wmc415*	*cfd5*	*wmc386*	*gwm190*	*wmc630*	*wmc182*	*wmc446*	*gwm613*	*wmc486*	*cfd49*	*gdm98*	*gwm350*	*wmc809*	*barc65*	*wmc396*	*cfd41*	*wmc488*
CS(Ph^1)	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
WL 711	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
PBW343	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
Ae. pereg 1155-1-1	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Ae. pereg 13772	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1-1-7-8	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W
1-1-7-18	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	P+W
16-1-8-4	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W
16-1-8-56	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	P+W
17-1-3-37	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
17-1-3-38	P+W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	P+W	W
17-1-2-2	P+W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
17-1-2-5	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
14-1-12-12	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
14-1-12-44	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
14-1-12-48	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
17-3-37-16	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W

4.1.6.3 *In situ* hybridization:

Fluorescent *in situ* hybridization (FISH) of selected derivatives was carried out to confirm alien introgression. Probes pAS1 (D genome specific) and pHvG38 (B genome specific probe) were used. The pAS1 clone contains 1kb repetitive DNA sequence from *T. tauschii* (Rayburn and Gill, 1986) whereas clone pHvG38 contains the GAA- satellite sequence (Pedersen, 1966). GISH using *Ae. umbellulata* and *Ae. longissima* genomic DNA was also done to identify the introgressed U and S genome chromosomes, respectively. U genome chromsomes appeared pink and S genome chromosomes were green in colour. Introgression of single S genome choromosome from *Ae. peregrina* had been observed in two derivatives BC_2F_2 1-1-7-18 and BC_2F_2 14-1-8-18 (Fig 4.20 a, b). Derivative 16-1-8-4 had shown introgression of U genome from *Ae. peregrina* whereas in BC_2F_2 17-1-3-38 a translocation of one the U genome was observed. Photographs taken at anaphase stage confirm introgression of single U chromosome (Fig. 4.20). It has been observed that the derivatives with introgresssed S geome had higher Zn content whereas backcrossed derivatives 16-1-8-4 and BC_2F_2 17-1-3-38 with introgressed U genome resulted in the enhancement of both iron and zinc content.

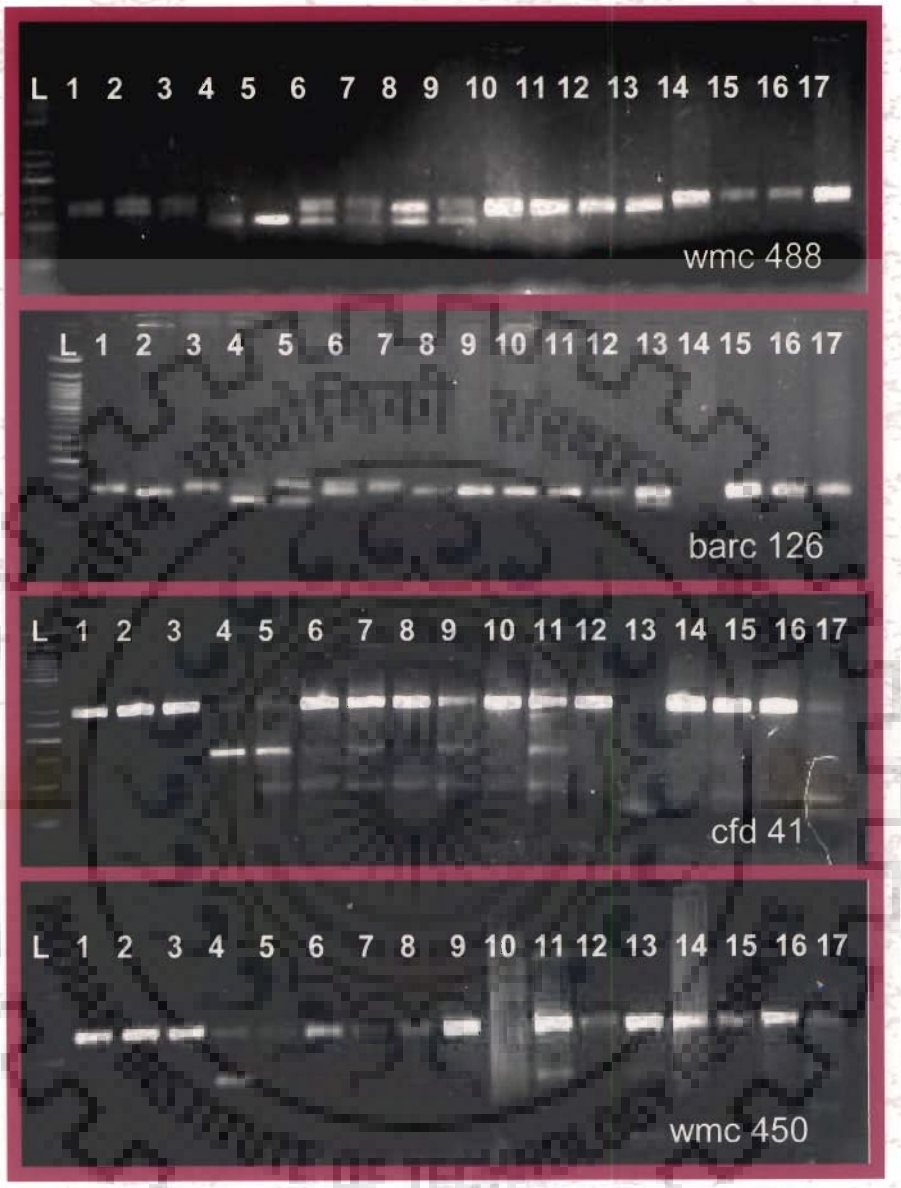

Fig 4.18 Application of anchored polymorphic SSR markers of wheat group 7 chromosomes on selected derivatives. 1. WL 711, 2. CS (*Ph*^{I}), 3. PBW 373, 4. *Ae. peregrina* 1155-1-1, 5. *Ae. peregrina* 13772, 6. BC_2F_2 1-1-7-8, 7. BC_2F_2 1-1-7-18, 8. BC_2F_2 16-1-8-4, 9. BC_2F_2 16-1-8-56, 10. BC_2F_2 17-1-3-37, 11. BC_2F_2 17-1-3-38, 12. BC_2F_2 17-1-2-2, 13. BC_2F_2 17-1-2-5, 14. BC_2F_2 14-1-12-12, 15. BC_2F_2 14-1-12-44, 16. BC_2F_2 14-1-12-48, 17. BC_2F_2 17-3-37-16

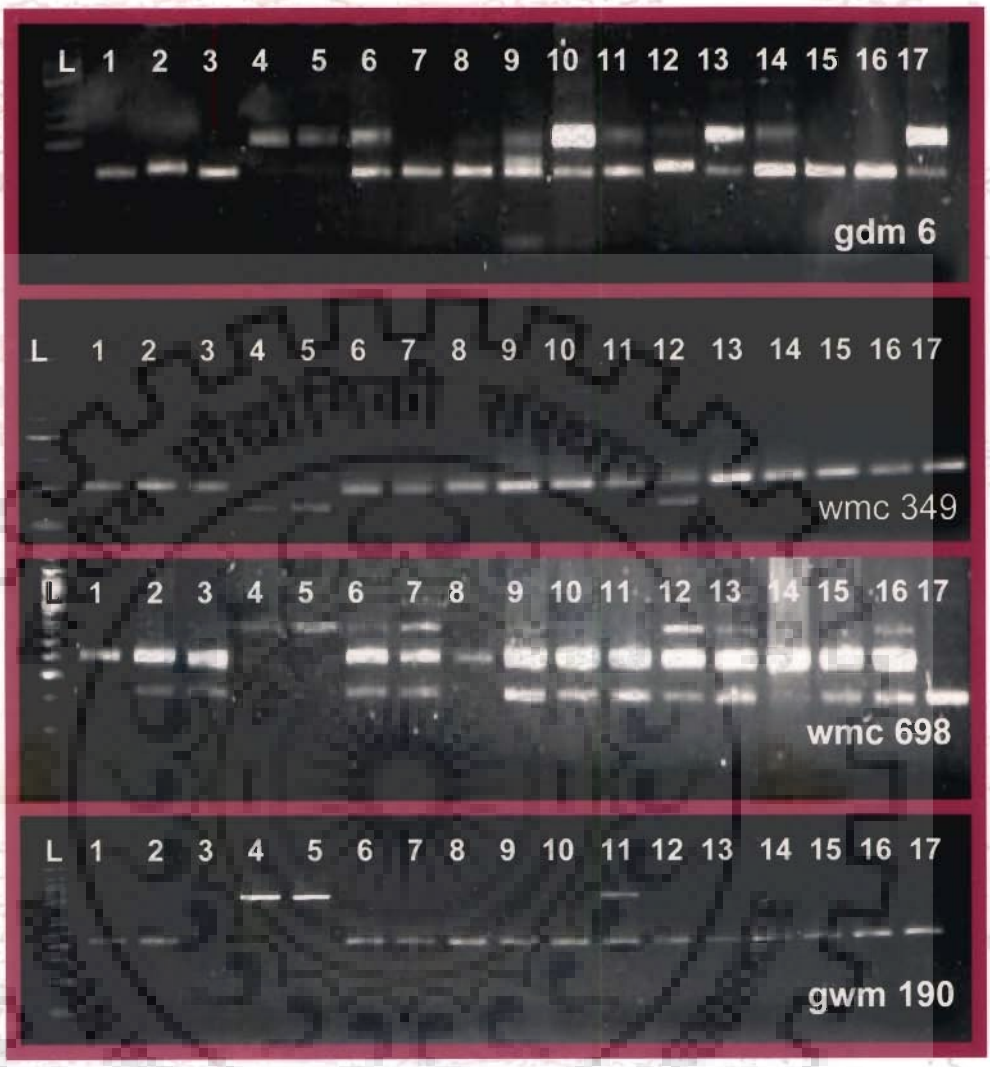

Fig. 4.19 Application of anchored polymorphic SSR markers of wheat group 4 (gdm6, wmc349, wmc698) and 5 (gwm190) chromosomes on selected derivatives. 1. WL 711, 2. CS (Ph^I), 3. PBW 373, 4. *Ae. peregrina* 1155-1-1, 5. *Ae. peregrina* 13772, 6. BC_2F_2 1-1-7-8, 7. BC_2F_2 1-1-7-18, 8. BC_2F_2 16-1-8-4, 9. BC_2F_2 16-1-8-56, 10. BC_2F_2 17-1-3-37, 11. BC_2F_2 17-1-3-38, 12. BC_2F_2 17-1-2-2, 13. BC_2F_2 17-1-2-5, 14. BC_2F_2 14-1-12-12, 15. BC_2F_2 14-1-12-44, 16. BC_2F_2 14-1-12-48, 17. BC_2F_2 17-3-37-16

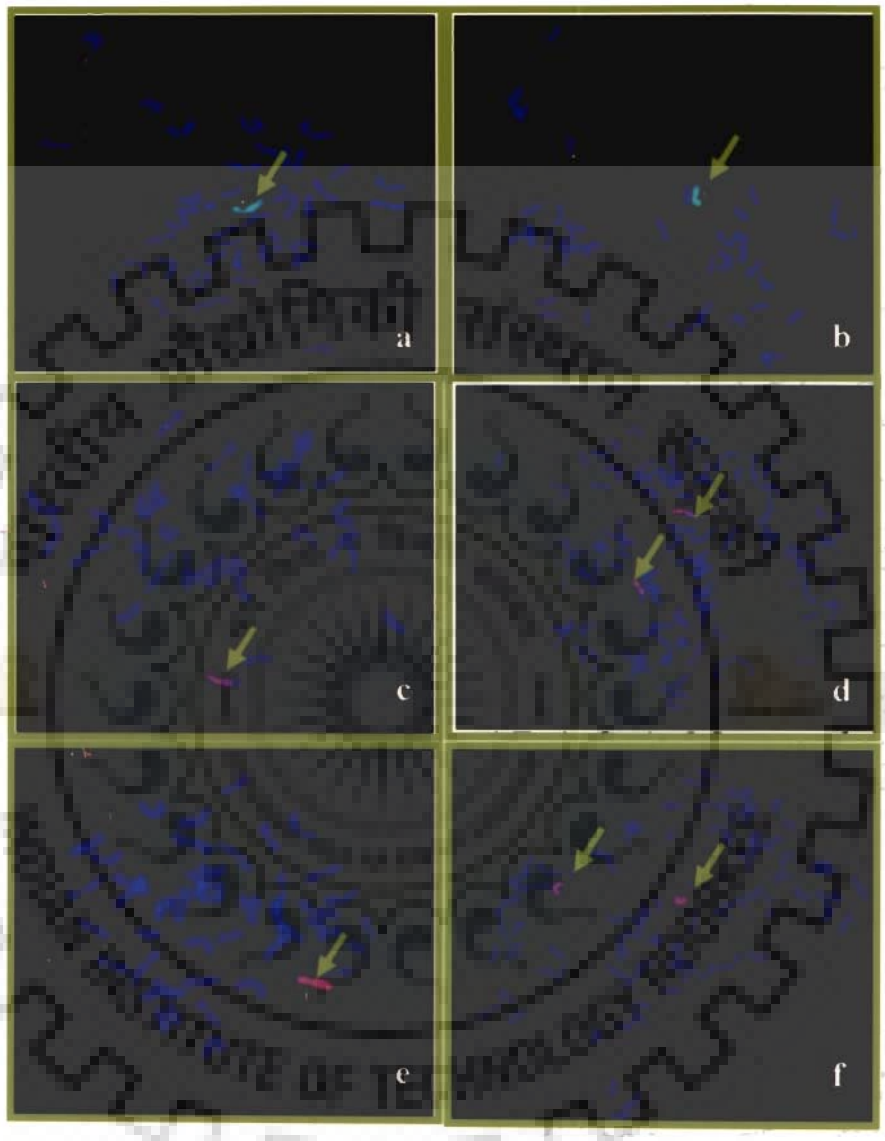

Fig. 4.20 Genomic *in situ* Hybridization of some of the selected derivatives showing introgressed alien chromosomes a. BC_2F_2 1-1-7-18, b. BC_2F_2 14-1-8-18, c. BC_2F_2 17-1-3-38 (metaphase), d. BC_2F_2 17-1-3-38 (anaphase), e. BC_2F_2 16-1-8-4 (metaphase), f. BC_2F_2 16-1-8-4 (anaphase)

4.2 Development of wheat- *Ae. peregrina* synthetic amphiploids with high grain iron and zinc content.

4.2.1 Colchicine treated C_0 generation

The colchicine treated F_1 hybrids were morphologically intermediate between the *Aegilops* and wheat parent. After colchicine treatment, they were not completely sterile but had some doubled sectors or spikes with dehiscing anthers. The doubled sectors with dehiscing anthers had high seed set whereas no seed set was observed in the other F_1 spikes without chromosome doubling. The seeds thus obtained were designated as the synthetic amphiploids (C_0 generation) and were used for further studies.

4.2.2 Morphology and fertility of the synthetic amphiploids

The C_0 generation seeds were advanced and studied for their morphological, cytological, seed storage proteins and grain micronutrients. These C_1 generation amphiploids were morphologically similar to the F_1 hybrids having intermediate growth habit, plant height, and tiller number. The plants were very vigorous with profuse tillering but the tillering ability was less than the F_1 hybrids. Morphological characteristics of *T. aestivum*, *Ae. peregrina,* their F_1 hybrids and amphiploids is given in Table 4.11. Both of the amphiploids had head characteristic as that of *Aegilpos* parents such as spelta head and brittle rachis. Other characters were intermediate between two parents. *Ae. peregrina* had 4-5 glume awns whereas *T. aestivum* CS (Ph^I) had single toothed glume (Fig. 4.21). Both of the amphiploids had a single toothed glume and lemma. Seeds of amphiploids were bolder than *Aegilops* parents and seed weight was comparable to the wheat parent. Early dehiscing anthers had low pollen stainability but the spike with late dehiscing

Table 4.11 Morphological characteristics of *T. aestivum*, *Ae. peregrina*, their F_1 hybrids and amphiploids

Plant material	No. of tillers per plant	Plant height (cm)	Ear shape	Spikelets per spike	Glume awn	Lemma awn	Rachis	1000 grain weight (g)	Average seed set per spike
Parents									
T. aestivum lr. CS(*Ph*^{l})	18.3	100.3	Square	16.6	0	0	Tough	29.6	53
Ae. peregrina acc. 1155-5-3	262.5	35.7	Spelta	7.5	5-7	2 small	Brittle	13.8	12
Ae. peregrina acc. 3477	250.4	32.8	Spelta	8.2	5-7	2 small	Brittle	16.3	8.2
F_1 hybrids									
F_1 CS(*Ph*^{l})/ *Ae. peregrina* 1155-5-3	82	95.1	Spelta	18.4	1	1	Brittle	N.A.*	-
F_1 CS(*Ph*^{l})/ *Ae. peregrina* 3477	78	102.3	Spelta	19.2	1	1	Brittle	N.A.	-
Amphiploids									
Amphi. CS(*Ph*^{l})- *Ae. peregrina* 1155-5-3	60.5	80.1	Spelta	16.3	1	1	Brittle	25.3	7.5
Amphi CS(*Ph*^{l})-*Ae.peregrina* 3477	68.6	82.8	Spelta	17.5	1	1	Brittle	28.9	12.3

* N.A. data not available due to sterility of F_1 hybrids

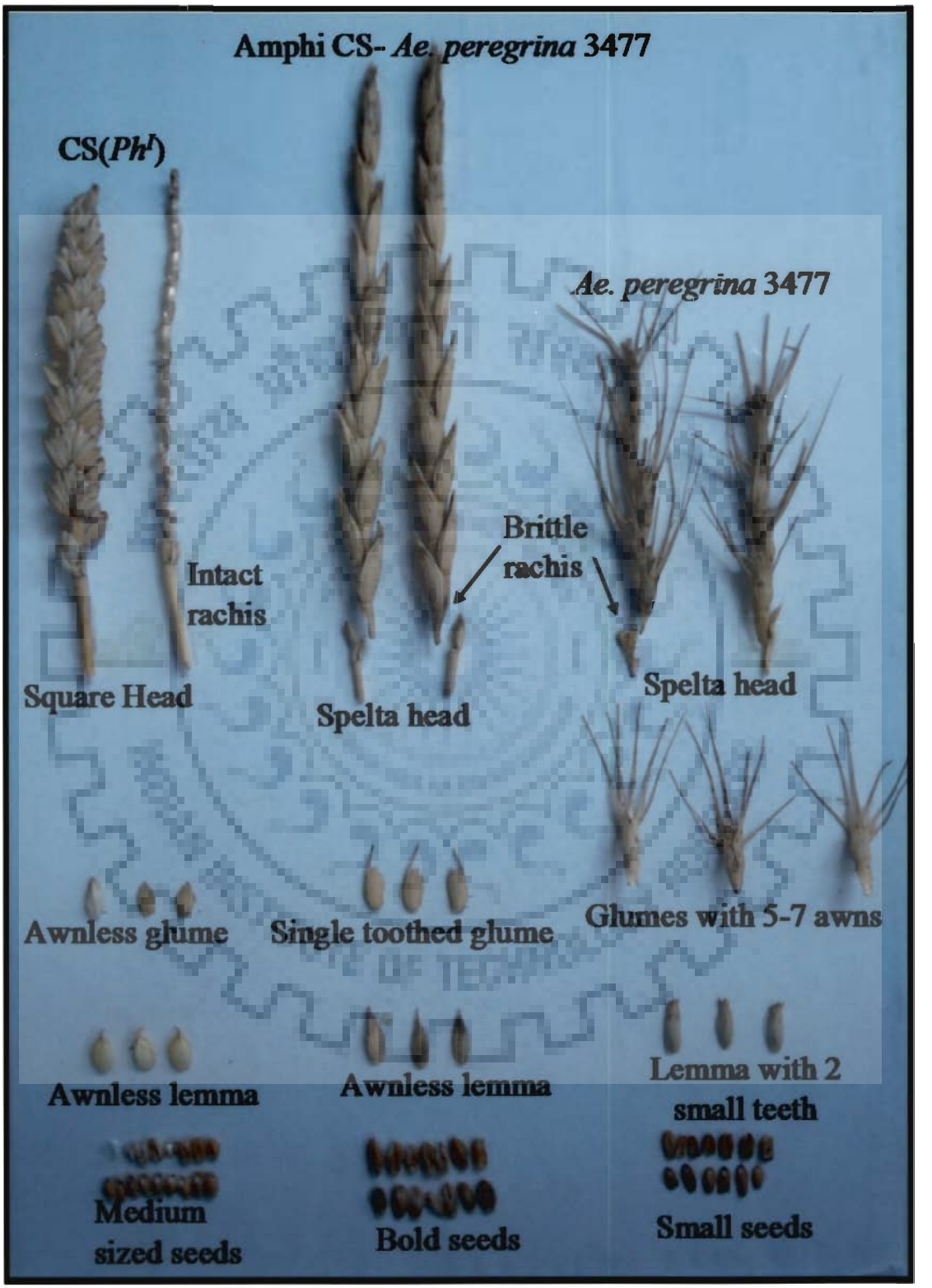

Fig. 4.21 Spike and grain characteristics of amphiploid CS (Ph^l) – *Ae. peregrina* 3477 (center) along with parents CS(Ph^l) (left) and *Ae. peregrina* 1155-5-3 (right).

anthers had good pollen stainability and seeds set. Pollen stainability varied from 65.8 to 80.4 percent. The seeds of amphiploids were red in colour as that of *Aegilops* parents.

4.2.3 Cytology of the synthetic amphiploids

Chromosome number in the amphiploids was highly variable (Table 4.12, Fig. 4.22). In C_1 generation chromosome number varied from 61 to 70. Chromosome elimination had taken place during the process of stabilisation. As a consequence of this wide range (35- 70) of chromosome number was observed in C_2 generation (Fig. 4.23). Some of the PMCs had chromosome number as low as 31 whereas some other cells in the same anther had chromosome number from 66 to 68 (Table 4.12). Amphiploids CS (*Ph*^{I})-*Ae.peregrina* 3477 had higher pollen stainability and higher seed set. Mean univalent frequency in C_1 generation ranged from 3.60 to 5.4 with higher number of bivalent frequency (29.2 to 30.80). Higher univalents frequency was observed in the PMCs of C_2 generation with chromosome elimination.

Table 4.12 Chromosome number, meiotic pairing and seed set in *T. aestivum lr* CS (*Ph*^{I}) - *Ae. peregrina* synthetic amphiploids (C_1 and C_2)

Amphiploid	No. of PMCs studied	Chromo -some number (Range)	Mean ± S.D. (Range) Univalent (I)	Mean ± S.D. (Range) Bivalent (II)	Mean ± S.D. (Range) Quadrivalent (IV)	Pollen Stain-ability %
Amphi. C_1 CS (*Ph*^{I})-*Ae .peregrina* 1155-5-3	25	62 - 70	5.4 ± 1.03 (2-10)	29.2 ± 1.91 (2-34)	0.77 ± 0.17 (0-1)	65.8
Amphi. C_1 CS (*Ph*^{I})-*Ae .peregrina* 3477	25	62 - 68	3.60 ± 0.84 (1-8)	30.80 ± 0.28 (28-34)	-	70.6
Amphi. C_2 CS (*Ph*^{I})-*Ae .peregrina* 1155-5-3	25	41 - 66	14.40 ± 0.75 (10-17)	24.31 ± 1.06 (21-29)	-	74.2
Amphi. C_2 CS (*Ph*^{I})-*Ae .peregrina* 3477	25	31 - 68	11.92 ± 0.61 (1-14)	27.80 ± 0.68 (25-32)	0.25 ± 0.15 (0-1)	80.4

4.2.3 HMW glutenin subunit profiles of amphiploids

The SDS-PAGE profiles of the HMW glutenin subunits of CS(*Ph*^{I}), *Ae. peregrina* accessions 1155-5-3 and 3477 and the CS(*Ph*^{I})-*Ae. peregrina* amphiploids is given in Fig. 4.24. CS (*Ph*^{I}) shoewed HMW glutenins subunit pattern of *Glu 1B* controlled 7+8 subunits and *Glu 1D* controlled 2+12 subunits. Both of the accessions of *Ae. peregrina* (UUSS) expressed 4-5 novel subunits of high molecular weight glutenin subunits. All of the glutenin subunits from wheat and *Ae. peregrina* parents were present in the amphiploids except one slower migrating group of *Ae.peregrina* in C_2 generation of amphiploids (Fig. 4.24 a). SDS-PAGE analysis from 10 single seeds of CS(*Ph*^{I})- *Ae. peregrina* 3477 amphiploid revealed uniform elimination of one or the other HMW glutenin subunit from *Aegilops* parent in C_2 generation amphiploids (Fig.4.24 b).

4.2.4. Grain iron and zinc content

Both *Ae. peregrina* accessions used for amphiploids development had 2-3 fold high grain iron and zinc content than the CS (*Ph*^{I}). Iron and zinc content was analysed in the flag leaves of amphiploids. *Aegilops* parent had nearly 2 to 2.5 high iron as well as zinc content in their flag leaves over CS (*Ph*^{I}). Increase of 200 % in iron and zinc content in the flag leaves of amphiploids was observed (Fig. 4.25). This indicates distincy mechanism of uptake and transport of these micronutrients to the flag leaves and ultimately to the grains in *Aegilops* and amphiploids. Nearly 2 to 2.5 folds increase in grain iron and zinc concentrations was found in amphiploids (Table 4.13). *Ae. peregrina* 1155-5-3 and *Ae. peregrina* 3477 had shown 150 % and 137 % increase in grain iron concentration, respectively and 122.39 %, 98.95 % increase in grain zinc concentration,

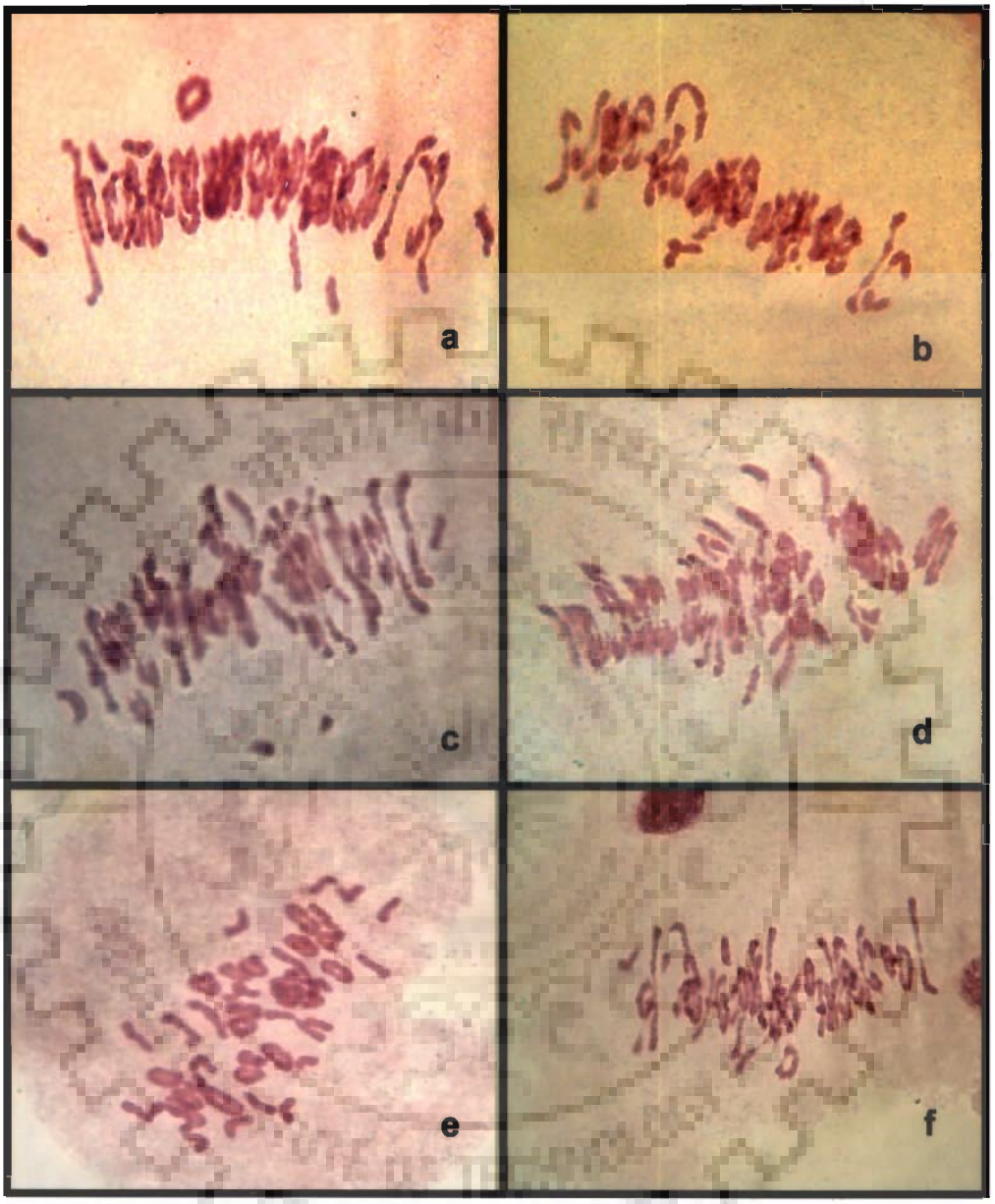

Fig. 4.22 Chromosomal pairing in CS (*Ph*^{l})- *Ae. peregrina* amphiploids a- CS (*Ph*^{l})-*Ae. peregrina* 3477 Chr- 67 (31II +5I), b- Chr-63 (1IV+ 1III+27 II + 2I), c- Chr-62 (29 II + 4 I); d- CS (*Ph*^{l})-*Ae. peregrina* 1155-5-3 Chr-62 (31 II), e- Chr-61 (26II+9I) and f- Chr- 62 (30 II + 2 I)

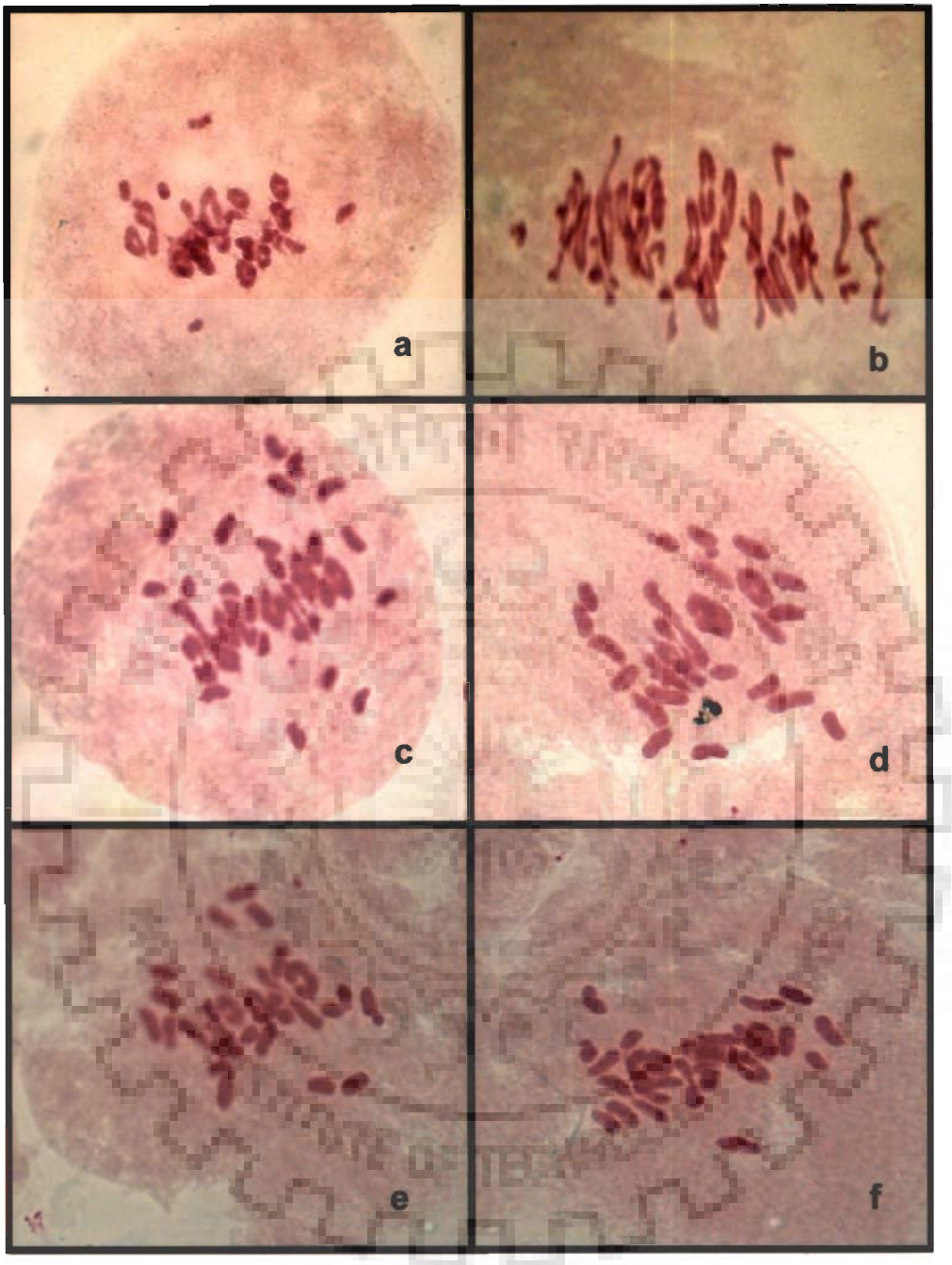

Fig. 4.23 Chromosome elimination in amphiploids a. CS (*Ph*^{I}) - *Ae. peregrina* 3477 Chr- 41 (16 II+ 1IV +5I), b. Chr-58 (28 II + 2I), c. Chr-37 (11 II + 15 I), d. CS (*Ph*^{I})- *Ae. peregrina* 1155-5-3 Chr-32 (9II+ 14 I), e. Chr 31 (16II+15I), f. Chr-32 (8 II + 16 I)

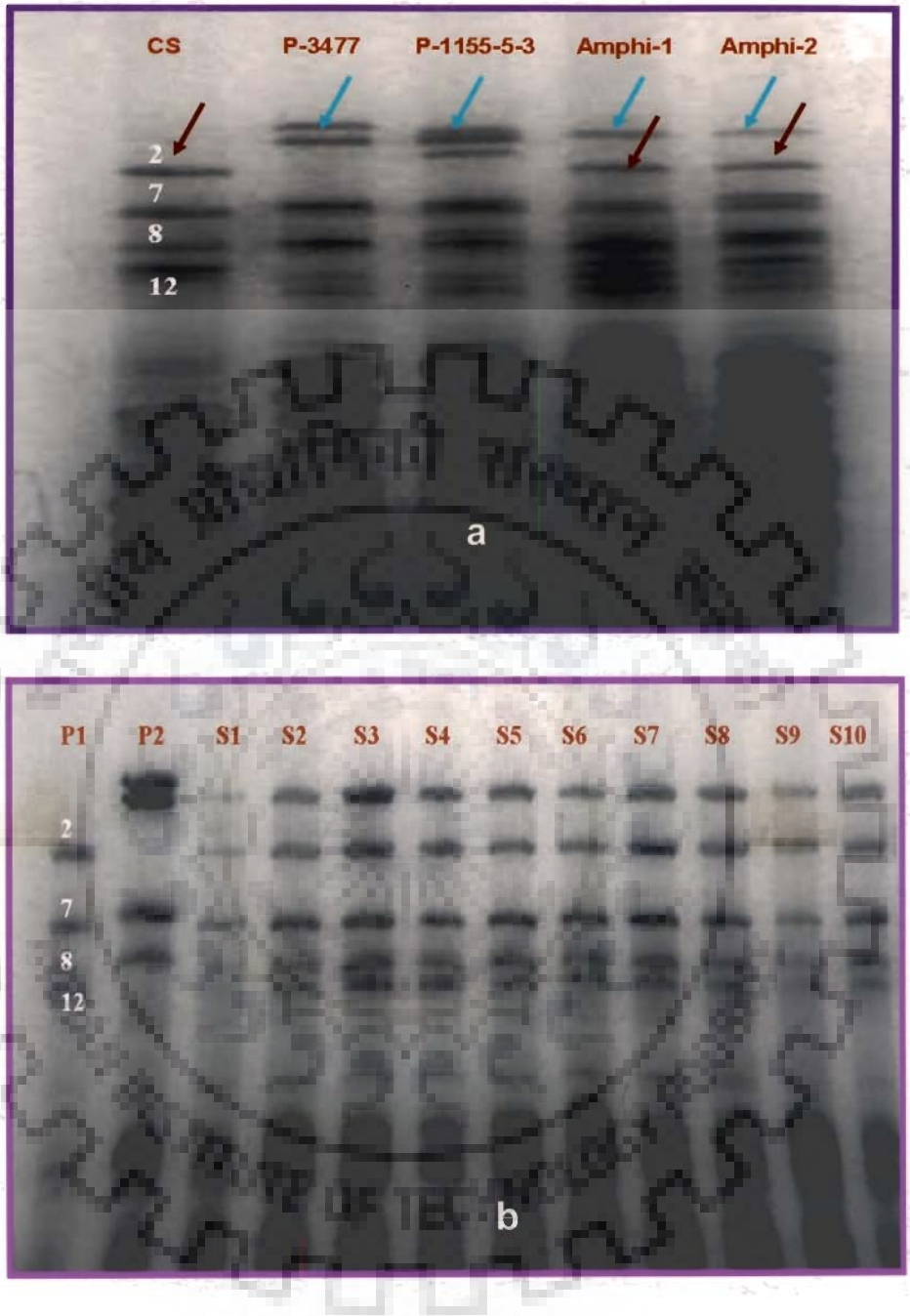

Fig. 4.24 (a) HMW glutenin subunit profile of *T. aestivum* lr CS (Ph^I), accessions *Ae. peregrina* 3477 and *Ae. peregrina* 1155-5-3 and their amphiploids, (b) HMW glutenin subunit profile of 10 single seeds from amphiploids CS(Ph^I)- *Ae. peregrina* 3477: P1- CS(Ph^I), P2- *Ae. peregrina* 3477, seeds S1 to S10

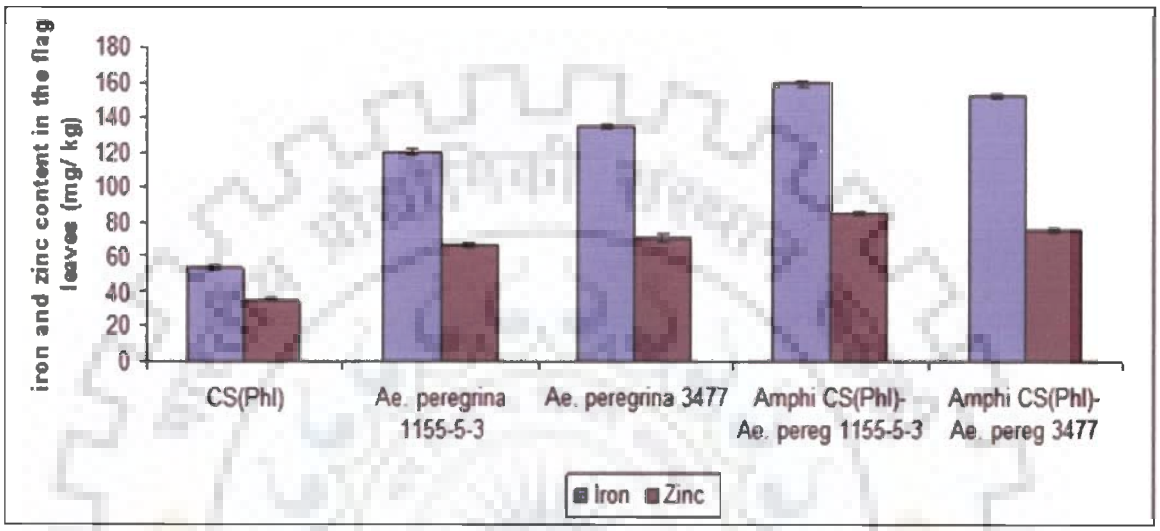

Fig.4.25 Iron and zinc contents in the flag leaves of parents and their amphiploids

respectively (Table 4.13). The micronutrient contents of amphiploids were comparable to those of *Ae. peregrina.* Two folds increase in grain iron and zinc concentration was observed in both of the amphiploids CS (Ph^I)-*Ae. peregrina* 1155-5-3 and CS (Ph^I)-*Ae. peregrina* 3477.

Table 4.13 Grain iron and zinc concentrations of parents and their amphiploids

Amphiploids	Iron (ppm) Mean ± S.D	% increase over CS (Ph^I)	Zinc (ppm) Mean ± S.D	% increase over CS (Ph^I)
CS (Ph^I)	25.41 ± 0.94		19.25 ± 0.58	
Ae. peregrina 1155-5-3	63.73 ± 0.48	150.78	42.71 ± 0.85	122.39
Ae. peregrina 3477	60.35 ± 0.57	137.40	38.20 ± 0.66	98.95
Amphi. CS (Ph^I)-*Ae .peregrina* 1155-5-3	58.21± 0.62	129.13	35.94 ± 0.71	86.97
Amphi. CS (Ph^I)-*Ae .peregrina* 3477	55.27 ± 0.71	117.32	33.41 ± 0.64	73.95

4.3 Evaluation and utilization of *Aegilops* species for high phytosiderophore release under iron and zinc deficient and sufficient conditions

4.3.1 Evaluation of *Aegilops* species for high phytosiderophore release

4.3.1.1 Plant response to iron and zinc deficiency stress

Typical visual iron deficiency symptoms in the wheat cultivars were observed only after 7 day of transfer of plants to iron deficient media (Fig 4.26). Under Fe deficiency, severe yellowing in the younger leaves of cultivars was observed much earlier as compared to *Aegilops* species. Among *Aegilops* species *Ae. kotschyi*, *Ae. peregrina*, *Ae. geniculata*, *Ae. ventricosa* stayed green till fifteen days of growth in deficient medium as compared to *Ae. longissima* where slight yellowing was observed. Under zinc deficiency, white necrotic spots on leaves were observed in cultivars at the end of the experiment whereas no such deficiency symptoms were observed among the *Aegilops* species.

4.3.1.2 Release of phytosiderophores on iron sufficient and deficient media

The amount of phytosiderophores released by roots of wheat cultivars and various accessions of different species at different days on iron sufficient and deficient media is given in Table 4.14. The amount of phytosiderophores among wheat cultivars on sufficient media on 6^{th} day of transfer of plants varied from 0.89 to 1.26 µg Fe mobilized per gram dry root weight whereas it was 3.5 times higher among *Aegilops* species (3.56-

Fig 4.26 Comparison of deficiency symptoms between some *Aegilops* species and wheat cultivars on 11th day in iron deficient medium 1- *Ae. kotschyi*, 2- *Ae. peregrina*, 3- *Ae. longissima*, 4- *T. aestivum* lr Chinese Spring (*Ph*^{I}), 5- *T. aestivum* cv. WL 711

Table 4.14. Phytosiderophores released from the roots of wheat cultivars and *Aegilops* species under iron and zinc sufficient and deficient conditions

Species	No. of accessions	Genome	Phytosiderophores released under iron sufficient and deficient media (µg of Fe mobilized / g dry root wt / 3 h)						Phytosiderophores released under zinc sufficient and deficient media (Activity units of phytosiderophores / g dry root wt/ 3 h)					
			6th day		11th day		17th day		10th day		14th day		18th day	
			Sufficient	Deficient	Sufficient	Deficient	Sufficient	Deficient	Sufficient	Deficient	Sufficient	Deficient	Sufficient	Deficient
			Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E	Average ± S.E
			Range	Range	Range	Range	Range	Range	Range	Range	Range	Range	Range	Range
T. aestivum	5	ABD	1.06 ± 0.09	8.23 ± 0.28	1.79 ± 0.19	9.67 ± 0.34	0.80 ± 0.25	4.59 ± 0.64	0.08 ± 0.01	0.12 ± 0.03	0.11 ± 0.04	0.24 ± 0.02	0.07 ± 0.04	0.1 ± 0.01
			(0.89 - 1.26)	(7.70 - 8.85)	(1.88 - 2.11)	(8.75- 10.42)	(0.66-0.86)	(3.97-5.21)	(0.05-0.10)	(0.07 – 0.15)	(0.05- 0.15)	(0.11 – 0.18)	(0.04 – 0.10)	(0.06– 0.15)
Ae. kotschyi	7	US	4.66±0.28	25.26 ± 0.89	8.08 ± 0.46	39.29 ± 1.33	5.85 ± 0.31	19.58 ± 1.24	0.20 ± 0.05	0.30 ± 0.04	0.31 ± 0.27	0.74 ± 0.01	0.28 ±0. 06	0.56± 0.04
			(3.56 – 5.82)	(21.92-28.20)	(6.55 – 8.51)	(35.91- 44.48)	(4.96- 7.13)	(20.13- 29.02)	(0.08- 0. 25)	(0.08 - 0.35)	(0.07- 0.38)	(0.51 – 0.91)	(0.14 – 0.32)	(0.35- .68)
Ae. peregrina	7	US	4.88 ± 0.38	24.09 ± 1.32	10.5 ± 0.89	36.86 ± 1.55	5.74 ± 0.45	19.58 ± 0.93	0.18 ± 0.04	0.28 ± 0.05	0.22 ± 0.05	0.75 ± 0.04	0.26 ± 0.05	0.49 ± 0.03
			(3.86 – 5.38)	(20.51 – 28.27)	(6.06 -3.62)	(30.94– 42.94)	(4.31 – 7.97)	(17.55 –2.72)	(0.12 – 0.21)	(0.19 – 0.31)	(0.18– 0.36)	(0.74– 0.81)	(0.25 – 0.38)	(0.42- 0.58)
Ae. ventricosa	4	DN	4.06 ± 0.38	21.06 ± 1.98	8.96 ± 0.82	29.05 ± 1.85	5.53 ± 0.58	18.04 ± 1.04	0.14 ± 0.04	0.21 ± 0.02	0.19 ± 0.04	0.62 ± 0.04	0.18 ± 0.02	0.51 ± 0.04
			(3.59 – 5.04)	(18.56 – 27.19)	(7.8 – 10.64)	(25.86 – 31.74)	(4.73 – 6.05)	(15.74 – 20.42)	(0.11 – 0.19)	(0.19 – 0.25)	(0.14 – 0.25)	(0.56 – 0.67)	(0.16 – 0.24)	(0.40 -0. 59)
Ae. geniculata	3	UM	4.45 ± 0.35	20.08 ± 2.01	8.73 ± 0.47	36.86 ± 1.85	6.83 ± 1.07	20.45±1.06	0.20 ± 0.01	0.27 ± 0.04	0.29 ± 0.01	0.69 ± 0.02	0.22 ± 0.03	0.42 ± 0.05
			(3.71- 5.04)	(17.21 – 24.18)	(7.81 – 9.35)	(34.38 - 40.42)	(5.39 – 8.94)	(19.68 – 22.27)	(0.18 – 0.22)	(0.25 – 0.32)	(0.25– 0.33)	(0.65 – 0.72)	(0.19 - 0.24)	(0.39-.0.45)
Ae. longissima	5	S^l	4.43 ± 0.28	21.38 ± 1.56	7.03 ± 0.86	33.21 ± 1.36	5.91 ± 0.35	20.52 ± 1.63	0.15 ± 0.04	0.19 ± 0.04	0.28 ± 0.03	0.61 ± 0.03	0.23 ± 0.01	0.40 ± 0.03
			(3.71 – 5.05)	(17.47 – 25.01)	(5.05 – 8.43)	(28.18 – 36.33)	(4.03 – 5.92)	(15.54–20.47	(0. 12– 0.18)	(0.16 – 0.25)	(0.25- 0.32)	(0.58– 0.67)	(0.19 – 0.28)	(0.36- 0.47)

5.82 µg Fe mobilized/ g dry root weight/ 3 h). The amount almost doubled by the 11th day and decreased nearly to the amounts observed on the 6th day after 17th day of treatment in sufficient media. All the wheat cultivars and various accessions of *Aegilops* species had 5-7 times higher phytosiderophore release on the 6th day on Fe deficient medium as compared to that on sufficient medium (Table 4.14) which increased to all time high on 11th day followed by reduction on 17th day. The rate of release of phytosiderophores among *Aegilops* species from 6th to 11th day was much higher than that of the wheat cultivars which was also subsequently reduced by 17th day at a rate higher than the wheat cultivars. The absolute amount of phytosiderophores among the *Aegilops* species under all conditions of experiment remained several fold higher than among wheat cultivars.

4.3.1.3 Release of phytosiderophores on zinc deficient medium

The response of wheat phytosiderophores among wheat and *Aegilops* species on zinc deficient media was almost similar to that on iron deficient medium. The release of phytosiderophores was delayed by four days under Zn deficiency. There was hardly any release of phytosiderophores on zinc deficient medium till 6th day (data not shown) which started increasing by 10 days with maximum amount on 14th day followed by slower reduction on 18th day of the experiment (Table 4.14). As in the case of Fe deficient medium all the *Aegilops* species had several fold higher phytosiderophores released than the wheat cultivars under various conditions and durations of the experiments.

4.3.1.4 Iron and zinc content in shoots and roots of wheat cultivars and *Aegilops* species

All the *Aegilops* species had significantly lower (2-3 times) mean and range of root dry weight per plant as compared to that of the wheat cultivars (Table 4.15) under both iron as well as zinc deficient media. The shoot dry weight per plant of *Aegilops* species was nearly 2/3 of that of wheat suggesting higher efficiency of *Aegilops* roots for nutrient uptake and translocation to the shoots. Various *Aegilops* species had highly significant and 2-3 times higher iron and zinc content in both shoots and roots expressed on dry weight basis after 18th days of growth on iron deficient medium (Table 4.15). The

Table 4.15 Shoot and root dry matter production and iron concentrations in the shoots and roots of wheat cultivars and *Aegilops* species grown for 17 days in iron deficient media

Species	Iron Deficiency				Zinc Deficiency			
	Mean dry wt ± S.E (mg / plant) Range		Iron concentrations ± S.E (mg/kg root dry wt) Range		Mean dry wt ± S.E (mg / plant) Range		Zinc concentrations ± S.E (mg/kg root dry wt) Range	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
T. aestivum	59.2 ± 1.6 (30- 44)	32.1 ± 1.8 (6- 9.3)	8.4 ± 3.5 (7- 16)	12.4 ± 2.8 (44-62)	52.5 ± 2.1 (30 - 44)	37.4 ± 1.8 (6 - 9.3)	5.6 ± 3.2 (4 - 8)	6.8 ± 2.7 (4 - 11)
Ae. kotschyi	37.7 ± 1.9 (32- 44)	12.5 ± 2.5 (6- 17)	25.8 ± 2.2 (21- 32)	32.6 ± 3.4 (28- 35)	40.4± 0.9 (30 - 48)	14.2 ± 2.5 (8 - 18)	11.3 ± 2.2 (8 - 14)	20.4 ±4.4 (13 - 25)
Ae. peregrina	42.4 ± 3.8 (31- 46)	17.9 ± 1.8 (12-22)	26.1 ± 1.9 (20- 33)	30.4 ± 4.2 (28- 35)	36.7 ± 2.8 (28 - 46)	19.1 ± 1.8 (16 - 24)	14.2 ± 4.1 (9 - 21)	21.0 ± 1.8 (16- 25)
Ae. ventricosa	35.8 ± 1.5 (30 - 41)	11.3 ± 4.1 (8- 14)	21.3 ± 2.1 (17- 25)	27.5 ± 3.7 (21- 36)	30.4 ± 3.9 (25 - 34)	15.6 ± 4.1 (9 - 20)	12.2 ± 3.5 (8 - 15)	18.9 ± 2.6 (13- 20)
Ae. geniculata	45.1 ± 3.5 (40.5 - 48)	13.2 ± 1.9 (7- 18)	29.1 ± 3.1 (22- 32)	35.3 ± 2.2 (30- 36)	39.2 ± 3.6 (35 - 42)	18.4 ± 2.0 (16- 20)	18.4 ± 2.6 (17 - 20)	24.5± 2.5 (22- 28)
Ae. longissima	44 ± 3.2 (34.6 - 46)	14. 8 ± 2.8 (8-18)	28.4 ± 1.2 (21- 34)	30.2± 3.1 (23- 36)	37.1 ± 2.2 (32 - 39)	13.5 ± 2.8 (10 - 17)	14.1 ± 1.6 (12 - 18)	22.1 ± 3.6 (13-26)

roots of both wheat cultivars and *Aegilops* species had invariably higher Fe and Zn content than in their shoots.

4.3.2.1 Phytosiderophore release in wheat-*Aegilops* derivatives with high grain iron and zinc content

Ae. peregrina acc. no. 1155-5-3, acc. no. 3519, CS(Ph^l), WL711 and five backcross derivatives BC_2F_2 1-1-7-8, BC_2F_2 14-1-8-36, BC_2F_2 14-1-12-44, BC_2F_2 16-1-8-4, BC_2F_2 14-1-12-48, BC_2F_2 17-1-3-37, BC_2F_2 17-1-2-5 were grown in hydroponics in iron deficient media and iron sufficient media. Typical visual iron deficiency symptoms in the wheat cultivars, CS(Ph^l) and WL711 were first observed on 7th day of experiment (Fig 4.27) while in case of derivatives, iron deficient chlorosis appeared after 11th days in deficient media. Variation in the amount of phytosiderophores released was observed in BC2F2 derivatives under iron deficient media. Among all the derivatives, BC_2F_2 14-1-12-12 and BC2F2 14-1-12-48 remained green in deficient media for the longest time (Fig 4.28).

Phytosiderophore estimation was done on 7th, 10th and 14th day after transfer of plants to hydroponic culture. Nearly 180 to 190 % increase in phytosiderophores release was found in *Aegilops* species over control on 7th days. In case of wheat cultivars, an organized pattern of phytosiderophores released had been observed. Maximum release of PS was observed on day 11th (mean 5.27 µg Fe mobilized/ g dry root weight/3hr) and as days in deficiency medium increase, reduction in PS release was noticed. In derivatives, amount of PS released was higher than the wheat cultivars over 14 days in iron deficient media. At the end of the experiment (on day 14), nearly 4 to 5 times higher PS released

Fig. 4.27 Comparison of deficiency symptoms between wheat cultivars and backcross derivatives on 7th day in iron deficient medium 1. WL711, 2. CS (*Ph*^{l}), 3. 14-1-12-44, 4. 14-1-12-48, 5. 16-1-8-4, 6. 17-1-2-5

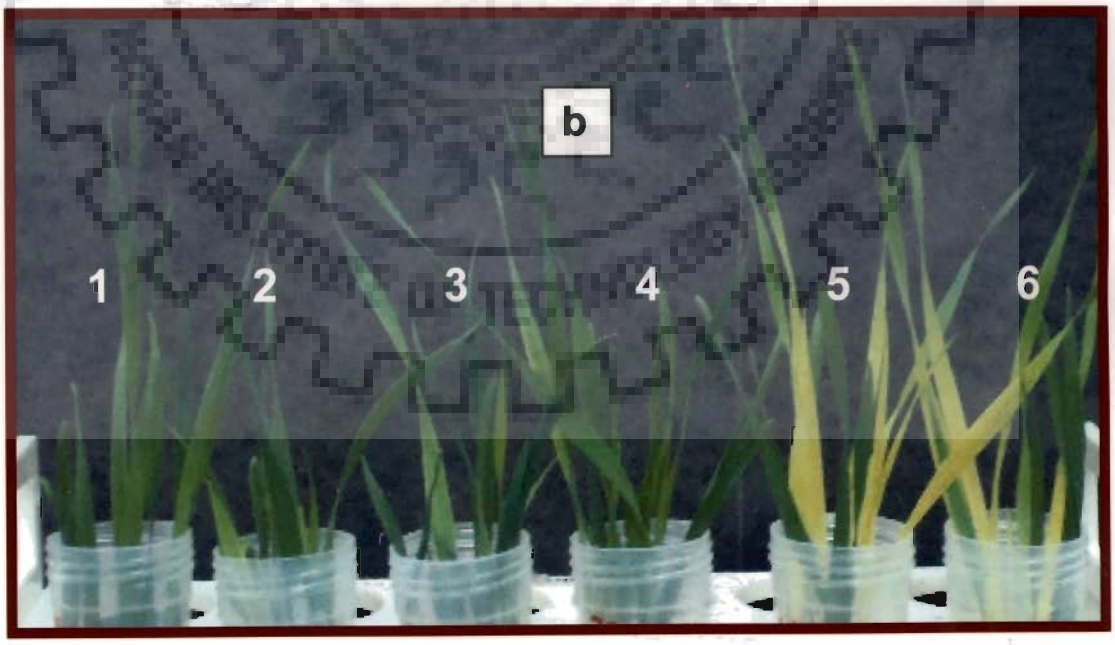

Fig. 4.28 Comparison of deficiency symptoms between wheat cultivars and backcross derivatives on 11th day in iron deficient medium1. 14-1-12-44, 2. 14-1-8-36, 3. 17-1-3-37, 4. 17-1-2-5, 5. WL711, 6. CS (*Ph*^{l})

was observed in all the derivatives over control. Till 14th day, higher rate of phytosiderophore release was observed in derivatives than control. Maximum release of phytosiderophore was found in the two of the derivatives $BC_2F_2$14-1-12-44 and $BC_2F_2$14-1-12-48. As days in deficiency media increases, reduction in phytosiderophore release was also observed in derivatives

Table 4.16 Phytosiderophore released (µg Fe mobilized/ g dry root weight/3hr) in iron deficient media by parent and backcross derivative plants

Accessions	Days in iron deficient media			
	7th day	11th day	14th day	17th day
PBW 343	2.9 ± 0.46	5.43 ± 0.26	2.10 ± 0.53	0.56 ± 0.38
WL711	2.3 ± 0.36	5.02 ± 0.19	1.93 ± 0.33	0.62 ± 0.53
BC_2F_2 1-1-7-8	2.68 ± 0.80	4.10 ±0.74	3.15 ±0.72	1.86 ± 0.22
BC_2F_2 14-1-8-36	3.43 ± 1.03	6.10 ± 0.54	7.42 ± 0.58	5.01 ± 0.64
$BC_2F_2$14-1-12-44	4.05 ± 0.75	7.19 ±0.85	10.58 ± 0.44	7.04 ± 0.47
BC_2F_2 16-1-8-4	5.35 ± 0.44	7.27 ±0.48	6.12 ± 0.37	4.68 ± 0.93
$BC_2F_2$14-1-12-48	6. 42 ± 0.29	8.16 ±0.35	10.05 ± 0.64	8.46 ± 0.82
$BC_2F_2$17-1-3-37	3.04 ± 0.52	6.06 ±0.46	3.02 ± 0.55	2.31 ± 0.44
BC_2F_2 17-1-2-5	4.01 ± 0.47	6.51 ± 0.55	3.21 ±0.74	2.53 ± 0.38
Ae. pereg 1155-5-3	8.41 ±0.67	11.53 ± 0.73	12.03 ± 0.47	8.09 ± 0.57
Ae. pereg 3477	8.56 ± 0.58	12.15 ± 0.83	13.40 ± 0.63	7.52 ± 0.86

4.4 Evaluation and identification of wheat-*Aegilops* addition lines for high grain iron and zinc content and phytosiderophores production

4.4.1 Grain Fe and Zn content

Addition lines of *Ae. peregrina*, *Ae. longissima* and *Ae. umbellulata* were analyzed for grain iron and zinc concentration along with Chinese Spring (CS) as control. CS had very low concentration of iron (mean 24.63 mg/kg) and zinc (mean 20.44) in grains (Table 4.17). Addition of chromosome of 2S (TA 7595) or 2 U (TA 7615) from *Ae. peregrina* to CS resulted in increase of 74.83% and 71.75% of grain iron concentration, respectively (Table 4.17). No significant increase in grain zinc concentration was observed by the addition of chromosomes of group 2 of *Ae. peregrina.* Fifty percent increase in grain iron concentration was also observed in chromosome 4U addition line. The addition line of group 7 had shown remarkable increase in grain zinc concentration over control. The addition line 7U (TA 7620) led to enhancement of both grain iron (76.84 %) and zinc concentration (61.53%) over control (Table 4.17). The CS-*Ae. longissima* addition line $2S^l$ had nearly 74 % increase in grain iron content whereas enhancement of 60 to 65% iron was also observed by addition lines $1S^l$, $6S^l$ and $7S^l$ (Table 4.17). Similarly, considerable differences for grain iron and zinc concentrations were observed among CS- *Ae. umbellulata* addition lines. Addition line for 2U led to 80% increase over control for grain iron content whereas addition of the chromosome 5 U led to 100% enhancement in grain zinc content (Table 4.17). A complete set of all the addition lines of *Ae. umbellulata* could not be screened due to lack of seeds.

Table 4.17 Grain iron and zinc concentrations of CS- *Ae. peregrina* , CS -*Ae. longissima* and CS- *Ae. umbellulata* addition lines

S.N		Addition lines	Fe (mg/ kg) Mean ± S.E	Zn(mg/ kg) Mean ± S.E
1	Chinese Spring	-	24.63 ± 0.62	20.44 ± 0.32
2	TA 7594	CS.*Ae. pereg* DA 1S^	20.60 ± 0.91	18.01 ± 0.93
3	TA 7595	CS.*Ae. pereg* DA 2S^	43.07 ± 1.75	23.63 ± 1.10
4	TA 7596	CS.*Ae. pereg* DA 3S^	24.27 ±1.55	22.35 ± 0.73
5	TA 7597	CS.*Ae. pereg* DA 4S^	21.80 ± 1.06	25.36 ± 1.04
6	TA 7598	CS.*Ae. pereg* DA 5S^	21.92 ± 1.43	16.19 ± 0.84
7	TA 7599	CS.*Ae. pereg* DA 6S^	19.96 ± 1.41	23.55 ± 1.35
8	TA 7600	CS.*Ae. pereg* DA 7S^	34.58 ± 0.73	27.92 ± 0.54
9	TA 7614	CS.*Ae. pereg* DA 1U^	18.17 ± 0.52	17.65 ± 0.63
10	TA 7615	CS.*Ae. pereg* DA 2U^	42.30 ± 1.11	19.41 ± 0.98
11	TA 7616	CS.*Ae. pereg* DA 3U^	19.17 ± 1.05	22.05 ± 0.80
12	TA 7617	CS.*Ae. pereg* DA 4U^	37.66 ± 1.03	25.16 ± 0.98
13	TA 7618	CS.*Ae. pereg* DA 5U^	31.76 ± 2.42	18.21 ± 2.26
14	TA 7619	CS.*Ae. pereg* DA 6U^	24.13 ± 1.21	24.53 ± 0.84
15	TA 7620	CS.*Ae. Pereg* DA 7U^	43.56 ± 1.53	33.01 ± 1.17
16	TA 7543	CS *Ae. long* DA 1Ŝ l^	37.82 ± 0.48	22.36 ± 0.74
17	TA7544	CS *Ae. long* DA 2Ŝ l^	40.85 ± 0.86	27.18 ± 0.91
18	TA 7545	CS *Ae. long* DA 3Ŝ l^	20.20 ± 0.94	20.86 ± 0.37
19	TA 7546	CS *Ae. long* DA 4Ŝ l^	25.30 ± 0.33	18.24 ± 1.17
20	TA 7547	CS *Ae. long* DA 5Ŝ l^	38.70 ± 1.38	22.51 ± 0.94
21	TA 7548	CS *Ae. long* DA 6Ŝ l^	34.23 ± 0.58	31.21 ± 1.77
22	TA 7549	CS *Ae. long* MA 7Ŝ l^	37.39 ± 1.82	34.17 ± 1.37
23	TAC BOW 0045	CS. *Ae. umbell.* 21"+ 1" 1U	22.01 ± 0.34	10.85 ± 0.18
24	TAC BOW 0046	CS. *Ae. umbell.* 21"+ 1" 2U	45.07 ± 0.20	29.35 ± 0.29
25	TAC BOW 0047	CS. *Ae. umbell.* 21"+ 1" 4U	13.00 ± 0.23	26.65 ± 0.48
26	TAC BOW 0048	CS. *Ae. umbell.* 21"+ 1" 5U	33.61 ± 0.29	39.85 ± 0.36
27	TAC BOW 0049	CS. *Ae. umbell.* 21"+ 1" 6U	29.46 ± 0.61	30.41 ± 0.27

4.4.2 Mugineic acid production

The addition lines were analyzed for release of mugineic acids, the mugineic acids, on 6^{th}, 11^{th}, 14^{th} and 17^{th} days after transfer to Fe deficient and sufficient media. Release of mugineic acids was expressed indirectly as µg of Fe mobilized/dry root wt/3 hour (Table 4.18). On day sixth, less differences were observed in phytosiderophore production between sufficient and deficient media in CS as well as in addition lines. Fe deficiency induced chlorosis was observed in Chinese Spring on 6^{th} day onwards while in addition lines of group 2 (U/S), 4 (U) and 7(S) of *Ae. peregrina* (Fig.4.29 a), 2 and 6 S^l of *Ae. longissima* (Fig. 4.29 b) and 2 and 5Uof *Ae. umbellulata* symptoms appeared late. The CS-*Ae. peregrina* addition lines 2S and 2U had highest mugineic acids production in terms of Fe mobilized on 6^{th} day under both iron deficient as well as sufficient media. Three to four times higher amount of mugineic acids were released by 11^{th} day in Chinese Spring and also in all addition lines as compared to that of 6^{th} day in Fe deficient media which leveled off by 14^{th} to 17^{th} day. Besides the 2S and 2U addition lines which continued to release higher amount of mugineic acids by 11^{th}, 14^{th} and 17^{th} days in iron deficient and sufficient media addition line 4U had more than 10 times higher phytosiderophore released by 11^{th} day on Fe deficient media as compared to that on 6^{th} day suggesting that 4U carries genes for higher level of phytosiderophore production under iron deficiency. Addition lines of 2S and 6S of *Ae. longissima* showed consistently higher release of mugineic acids over control and over other lines (Table 4.18) under both conditions. Nearly 10 times increase from 6^{th} day to 11^{th} day was observed by the addition of 6S of *Ae. longissima*. Remarkable increase (12 times) in mugineic acids release on 11^{th} day under iron deficient condition as compared to 6^{th} day was seen in 2U

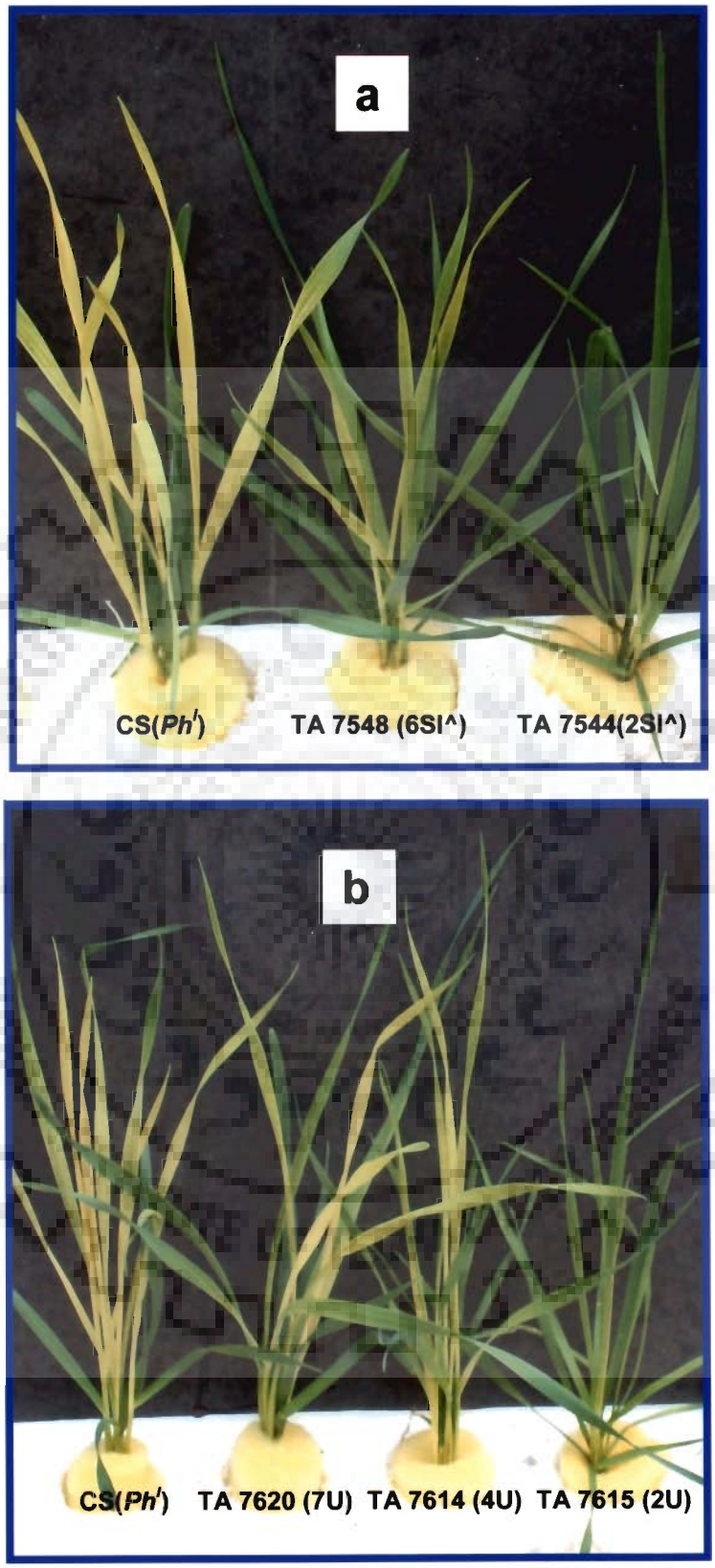

Fig. 4.29 Iron deficiency symptoms in the leaves of addition lines on 17th day in iron deficient medium. a- *Ae. longissima* addition lines 1.CS (*Ph*^{l}) 2. TA7548 (6 Sl^), 3 .TA 7544 (2Sl^). b- *Ae. peregrina* addition lines 1.CS (*Ph*^{l}) 2. TA 7620 (7U), 3.TA 7614 (4U), 4.TA 7615 (7U^)

Table 4.18. Phytosiderophores released (µg / g dry root weight/ 3 hour/) Fe mobilized in CS-*Ae. peregrina* ,CS- *Ae. longissima* and CS- *Ae. umbellulata* addition lines under iron deficient and sufficient media up to 17 days of growth

Addition lines	6th day		11th day		14th day		17th day	
	deficient	sufficient	deficient	sufficient	deficient	sufficient	deficient	sufficient
Chinese Spring	0.35	0.22	1.06	0.74	0.86	0.20	0.28	0.18
TA 7594 (1S^)	0.39	0.19	1.62	0.63	0.89	0.50	0.40	0.25
TA 7595 (2S^)	0.56	0.40	2.55	1.80	1.08	0.95	0.60	0.34
TA 7596 (3S^)	0.48	0.31	1.78	0.54	0.83	0.41	0.43	0.30
TA 7597 (4S^)	0.20	0.21	1.52	1.05	0.78	0.31	0.34	0.31
TA 7598 (5S^)	0.37	0.21	1.60	1.00	0.56	0.23	0.31	0.21
TA 7599 (6S^)	0.47	0.35	1.74	0.45	0.76	0.35	0.36	0.30
TA 7600 (7S^)	0.43	0.41	2.28	1.77	0.79	0.86	0.46	0.38
TA 7614 (1U^)	0.50	0.39	1.67	0.69	0.85	0.24	0.78	0.18
TA 7615 (2U^)	0.81	0.56	3.30	2.24	1.88	0.93	0.71	0.27
TA 7616 (3U^)	0.32	0.38	1.68	0.48	0.88	0.20	0.35	0.24
TA 7617 (4U^)	0.28	0.18	3.32	0.87	1.42	0.67	0.56	0.31
TA 7618 (5U^)	0.47	0.27	1.50	0.62	0.52	0.24	0.45	0.21
TA 7619 (6U^)	0.29	0.19	1.37	0.52	0.85	0.26	0.57	0.14
TA 7620 (7U^)	0.27	0.21	0.99	0.50	0.75	0.27	0.20	0.16
TA 7543 (1Ŝ l^)	0.35	0.15	2.11	0.18	1.24	0.23	0.22	0.18
TA7544 (2Ŝ l^)	0.55	0.39	3.32	0.35	2.31	0.31	0.43	0.32
TA 7545 (3Ŝ l^)	0.28	0.18	1.93	0.20	0.94	0.15	0.21	0.12
TA 7546 (4Ŝ l^)	0.32	0.14	2.35	0.19	1.00	0.19	0.31	0.16
TA 7547 (5Ŝ l^)	0.38	0.30	1.87	0.38	1.03	0.31	0.24	0.25
TA 7548 (6Ŝ l^)	0.47	0.37	4.57	0.49	2.06	0.42	0.48	0.38
TA 7549 (7Ŝ l^)	0.35	0.23	1.82	0.27	0.87	0.14	0.57	0.12
TAC BOW 0045 (1U)	0.28	0.25	2.88	0.43	2.58	0.31	0.48	0.28
TAC BOW 0046 (2U)	0.45	0.38	5.62	0.79	4.81	0.53	1.03	0.48
TAC BOW 0047 (4 U)	0.20	0.17	2.93	0.35	2.34	0.21	0.43	0.19
TAC BOW 0048 (5U)	0.33	0.30	3.75	0.51	2.80	0.46	0.99	0.31
TAC BOW 0049 (6U)	0.26	0.23	2.07	0.38	1.73	0.28	0.33	0.25

addition line of *Ae. umbellulata* followed by 5 U suggesting the presence of genes for higher mugineic acids release on these chromosomes (Table 4.18).

4.4.3 Chlorophyll content

Total chlorophyll content was estimated at the end of the experiment (17^{th} day) and expressed as mg per gram of leaf tissue. Addition lines 2S, 4S, 2U and 7U of *Ae. peregrina* had significantly higher chlorophyll content than the control and other addition lines indicating indirectly their higher grain iron content or its availability (Fig. 4.29 a). Similarly, CS- *Ae. longissima* addition line $2S^{l}$ and CS- *Ae. umbellulata* addition lines 2U had also higher chlorophyll content (Table 4.19). These addition lines stayed green for longer time than the rest of the addition lines and Chinese Spring. As the days in iron deficient media increase, these addition lines also developed chlorosis.

4.4.4 Iron concentration in the root and shoots of addition lines

At the end of experiment, shoots and roots of Chinese Spring and addition lines were separated and digested. In homoeologous group 2 addition lines of *Ae. peregrina*, *Ae. longissima*, *Ae. umbellulata* nearly two fold higher concentration of iron was found in roots under iron deficient condition over control (Table 4.19). Similar results for shoots iron concentration were also observed. Even under iron sufficient condition, this increase was 1.5 to 2 times in 2U and 2S addition lines. Also, chromosome 4S and 7U of *Ae. peregrina*, $1S^{l}$, $6S^{l}$ and $7S^{l}$ of *Ae. longissima* and 5U of *Ae.umbellulata* had higher concentration of iron in roots as well in shoots under both conditions. Higher shoot iron concentration in 4 U addition line of *Ae. peregrina* revealed better translocation ability

Table 4.19 Chlorophyll in leaves and iron content in roots and shoots of CS-*Ae. peregrina*, CS -*Ae. longissima* and CS- *Ae. umbellulata* addition lines under iron deficient and sufficient condition on 17th day of iron deficient media

Addition Lines	**Chlorophyll content (mg/ g) of leaf tissue**	**Iron content (mg/ kg)**			
		Roots		Shoots	
	Deficient	Deficient	Sufficient	Deficient	Sufficient
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Chinese Spring	31.85 ± 1.43	45.7 ± 1.04	195.58 ± 1.64	10.24 ± 1.07	21.04 ± 1.33
TA 7594 (1S^)	46.48 ± 1.63	61.42 ± 1.95	250.19 ± 2.03	10.12 ± 0.57	18.93± 0.73
TA 7595 (2S^)	66.50 ± 1.49	108.1 ± 1.72	377.53 ± 2.19	14.33 ±0.78	28.52 ± 1.29
TA 7596 (3S^)	42.46 ± 1.73	58.42 ± 1.66	243.91 ± 1.02	12.76 ± 1.05	22.16 ± 1.32
TA 7597 (4S^)	61.37 ± 1.68	74.03 ± 1.73	317.54 ± 2.88	28.64 ± 0.83	33.65 ± 1.05
TA 7598 (5S^)	41.47 ± 1.59	59.91 ± 1.04	155.72 ± 2.06	15.08 ± 0.91	24.61 ± 0.95
TA 7599 (6S^)	45.94 ± 1.55	70.64 ± 0.73	212.51 ± 1.44	9.49 ± 1.14	20.71 ± 0.44
TA 7600 (7S^)	48.15 ± 1.42	89.29 ± 1.47	238.41 ± 2.16	18.92 ± 1.28	32.94 ± 0.52
TA 7614 (1U^)	42.88 ± 1.66	58.04 ± 1.04	208.83 ± 1.65	15.33 ± 1.36	25.62 ± 0.48
TA 7615 (2U^)	83.08 ± 1.34	84.09 ± 1.61	394.61 ± 1.88	13.45 ± 1.06	28.96 ± 0.71
TA 7616 (3U^)	56.72 ± 1. 22	62.18 ± 2.09	217.48 ± 1.99	14.38 ± 1.09	19.1 ± 0.75
TA 7617 (4U^)	60.93 ± 1.52	73.49 ± 1.10	233.37 ± 2.01	21.98 ± 0.62	26.36 ± 0.21
TA 7618 (5U^)	41.39 ± 0.72	57.28 ± 1.38	102.82 ± 2.08	10.02 ± 0.72	18.53 ± 0.49
TA 7619 (6U^)	58.71 ± 0.83	60.13 ± 1.42	217.98 ± 1.83	12.05 ± 0.81	15.48 ± 1.47
TA 7620 (7U^)	68.43 ± 1.21	54.15 ± 1.28	210.34 ±1.32	13.28 ± 1.17	15.79 ± 1.27
TA 7543 (1Ŝ l^)	32.41 ± 1.03	52.98 ± 0.92	325.35 ± 1.29	21.78 ± 1.48	27.74 ±1.04
TA7544 (2Ŝ l^)	50.62 ± 0.87	91.54 ± 1.86	367.56 ± 0.83	28.71 ± 1.02	32.25 ± 0.88
TA 7545 (3Ŝ l^)	27.38 ± 0.72	48.09 ± 1.29	209.13 ± 0.53	10.94 ± 0.96	14.4 ± 1.04
TA 7546 (4Ŝ l^)	24.79 ± 0.93	55.09 ± 2.07	212.05 ± 1.40	11.09 ± 1.53	15.44 ± 1.47
TA 7547 (5Ŝ l^)	31.68 ± 1.04	64.73 ± 2.01	267.06 ± 1.22	23.41 ± 2.01	16.04 ± 1.93
TA 7548 (6Ŝ l^)	46.42 ± 0.71	83.47 ± 2.31	376.79 ± 1.38	25.83 ± 0.94	31.16 ± 1.30
TA 7549 (7Ŝ l^)	46.72 ± 0. 92	78.04 ± 1.53	345.76 ± 0.84	20.29 ± 1.04	27.74 ± 0.52
TAC BOW 0045 (1U)	33.73 ± 1.42	37.31 ± 0.84	238.05 ± 1.28	11.59 ± 1.31	16.04 ±0. 91
TAC BOW 0046 (2U)	45.02 ± 1.74	87.22 ± 1.04	288.33 ± 1.05	13.43 ± 1.42	18.33 ± 1.42
TAC BOW 0047 (4U)	31.04 ± 1.39	41.66 ± 0.73	369.21 ± 1.43	25.04 ± 1.32	35.44 ± 0.58
TAC BOW 0048 (5U)	38.93 ± 1.58	68.02 ± 1.38	269.05 ± 1.06	17.04 ± 1.22	22.19 ± 1.65
TAC BOW 0049 (6U)	32.01 ± 1.63	51.04 ± 1.72	302.95 ± 1.12	19.04 ± 0.95	30.04 ± 1.20

of 4U. This indicates that these chromosomes harbor genes for higher uptake.

4.4.5 Correlation between grain and root iron and phytosiderophores released

To confirm whether the accessions with high grain iron concentration also had higher release of phytosiderophores, correlation between grain iron and mugineic acids released on 11th day was calculated. Significant correlation between these two parameters was observed among CS- *Ae. peregrina* (0.73) and CS- *Ae. umbellulata* (0.73) addition lines with the exception of CS- *Ae. longissima* where it was non significant (0.27). This could be explained on the basis of the fact that in *Ae. longissima*, higher grain iron and zinc concentration was found on chromosome 1, 2, 5,6 and 7 whereas higher release of mugineic acids on 11th day was induced only in addition lines with 2S and 6S of *Ae. longissima.* Correlation between root iron concentration and phytosiderophores released was also found significant on 11th day in CS- *Ae. peregrina* (0.59), CS- *Ae. longissima* (0.64) and CS- *Ae. umbellulata* (0.86) addition lines.

4.5 Mapping of QTL for phytosiderophores release in diploid wheat RIL population

4.5.1 Phytosiderophores release in diploid wheat RIL population

Phytosiderophore release was estimated in diploid *T. monococcum14087* X *T. boeoticum 5088* RIL population on 11th day in iron deficient media. Estimation was done in 4 replications. Variation in phytosiderophores release was evident between Tm 14087 and Tb 5088. For Tb 5088, phytosiderophores released was averaged over the five environments and was found 8.51µg of Fe mobilised /plant whereas Tm 14087 had an average of 4.81 µg of Fe mobilised /plant. A wide range of variation was observed in the RIL population over three years and six environments (Table 4.20).

Table 4.20. Phytosiderophore released (µg of Fe mobilised / plant) of the parental accessions and *T. boeoticum/T. monococcum* RIL population in five environments over three years 2007, 2008 and 2009.

Environment	Parents		RILs (Range)	Mean ± SE
	Tb5088	*Tm14087*		
	Phytosiderophore released (µg of Fe mobilized / plant)			
New_ TT1	7.2	4.5	3.8-18.3	6.3 ± 0.5
IITR 2007	7.4	4.6	3.2-25.6	9.4 ±0.5
WRDI 2008	7.6	5.1	3.5-21.3	7.8 ± 0.4
MF 2008	8.9	4.1	2.9-21.3	7.9 ± 0.4
GH 2009	9.3	4.9	3.1-17.4	6.9 ± 0.3
DH 2009	9.2	5.5	3.2-23.7	8.7 ± 0.5
Pool	8.5	4.8	3.0-22.1	8.1 ± 0.4

Minimum phytosiderophore release was observed in RIL 17 (2.9 µg of Fe mobilised / plant) and whereas maximum release of phytosiderophore was found in RIL 86 (25.6 µg of Fe mobilised / plant) under iron deficient media.

4.5.2 QTL analysis for high phytosiderophore release

A framework linkage map, based on 169 SSR and RFLP loci (Singh *et al.*, 2007), was used for mapping the phyotosiderophore QTL in a set of 88 RILs (Table 4.18). The data for the individual environments New-TT-1, IITR2007, WRDI 2008, MF 2008, GH 2009 and DH 2009 and the average of all the six environments was used for detection and mapping the QTLs controlling phytosiderophore production. For phytosiderophore production under iron deficient media, two significant QTL were detected (Fig. 4.28). The two significant QTL were mapped on chromosomes 6A in the marker intervals *Xbarc113-Xgwm*670 and *Xgwm670-Xgwm 1017*, respectively (Table 4.21, Fig. 4.30). The QTL on 6A, designated as *QIITRa-6A*, had a LOD score of 3.7, 3.8, 4.1, 3.9, 3.7, 3.5 and 4.0, based on the phytosiderophore released under iron deficient media, measured in all six environments, New-TT-1, IITR2007, WRDI 2008, MF 2008, GH 2009 and DH 2009 and the pool data, respectively, with an R^2 value of 13.2, 21.8, 22.3, 21.8, 16.2, 13.6 and 15.8 (Fig. 4.30, Table 4.21). Likewise, the other QTL was also on chromosome 6A and designated as *QIITRb-6A* was detected at LOD scores of 5.0, 5.2, 4.3, 4.2, 5.3 and 5.1 and 5.4 on the pool data, respectively, with R^2 values of 29.5, 21.2, 14.8, 20.6, 20.1 and 16.2 and 21.6 on pooled data, respectively for the six environments. With further saturation of markers between the intervals of the two QTL, only a single major QTL may be detected within Xbarc113- Xgwm1017 interval of chromosome 6A of *T. boeoticum.*

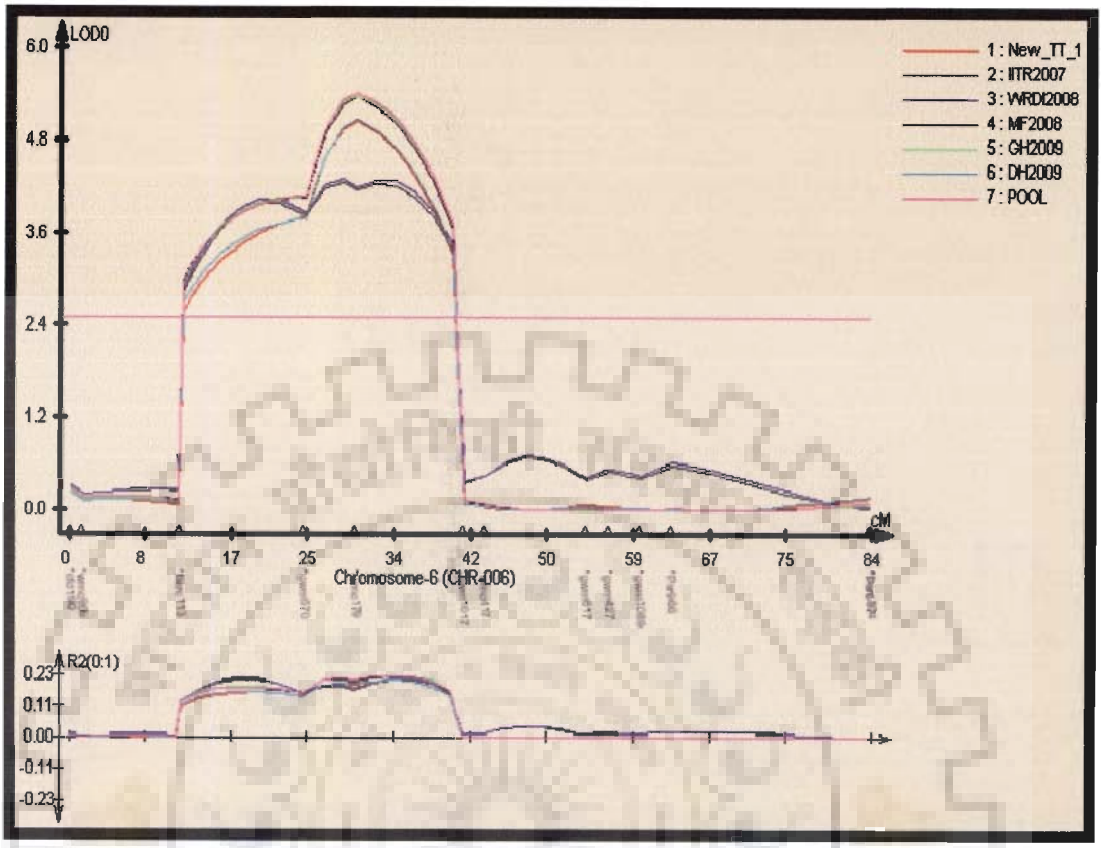

Fig. 4.30 Composite Interval Mapping of phytosiderophore release under iron deficient media in RIL population based on environments New-TT-1, IITR 2007, WRDI 2008, MF 2008, GH 2009, DH 2009 and Pool data

Table 4. 21. Summary of the QTLs for phytosiderophore released in the *T. boeoticum*/*T. monococcum* RILpopulation detected using Composite Interval Mapping

Chromos-ome	Marker interval	Position (CM)	New-TT-1		IITR 2007		WRDI 2008		MF 2008		GH 2009		DH 2009		Pool	
			LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
QTL for phytosiderophore released																
6	Xbarc113-Xgwm 670	21.4	3.7	13.2	3.8	21.8	4.1	22.3	3.9	21.8	3.9	16.2	3.5	13.6	4.0	15.8
6	Xgwm 670-Xgwm 1017	29.5	5.0	15.2	5.2	21.2	4.3	14.8	4.2	20.6	5.3	20.1	5.1	16.2	5.4	21.6

Chapter V

Discussion

5. DISCUSSION

Dietary deficiency of iron and zinc commonly known as micronutrient malnutrition affects more than three billion people of the world (Bouis, 2007). According to WHO recommended dietary allowances (RDA) for iron in the age group of 25-50 years is 10 mg for men and 15 mg for women. For zinc the RDA in the same age group is 15 mg and 12 mg for men and women, respectively (FAO/WHO, 2000). Cereals are poor sources of micronutrients. Further due to the presence of antinutritional factors in cereals and other dietary components, bio-available micronutrients lag far behind the required levels.

Among cereals, wheat alone provides 28% of world's edible dry matter production and 60 % of daily calorie intake in several developing countries (Distelfield *et al.*, 2007). Most of the *Triticum aestivum* L. and *T. turgidum* L. ssp. d*urum* (Desf.) cultivars have lower grain iron (18-25 mg/kg) and zinc content (15-22 mg/kg). It necessitates the search for variability for grain micronutrient content. Wheat germplasm comprising traditional cultivars, landraces and related progenitor and non-progenitor species harbour useful variability for several traits including micronutrient content. Several *Aegilops* species, such as *Ae. kotschyi*, *Ae. peregrina*, *Ae. longissima*, *Ae. ventricosa*, *Ae. cylindrica* were found to have 2-3 folds higher concentrations of grain iron and zinc content. These findings were also supported by the work of Rawat *et al.*, 2008 and Chhuneja, 2006. Synthetic wheat lines developed by scientists at CIMMYT, various wild *Triticum* species such as *T. boeoticum* L., *T. turgidum* L. ssp. *dicoccoides*, *T. turgidum* L. ssp. *dicoccon, T. tauschii* L. were found to have higher iron and zinc content than the elite wheat cultivars (Rawat *et al.,* 2008; Chhuneja, 2006, Calderini and Monasterio, 2003a, 2003 b; Monasterio and Graham, 2000). This variability could be utilized for the improvement of elite wheat cultivars.

Biofortification of wheat could be achieved either by genetic engineering or through molecular breeding approaches. Due to various problems associated with genetic engineering such as low acceptance of transgenic crops by farmers and common people, various socio-economical and socio-political concerns, problems in licensing of transgenic crops and recurring cost, this approach is not very popular whereas biofortification through molecular breeding is cost-effective, easily affordable and widely accepted (Lonnerdal, 2003; Zimmerman and Hurrel, 2002).

Micronutrient analysis of sterile F_1 hybrids and sterile BC_1 derivatives reveled significant increase in iron and zinc content of flag leaves. Higher content of the micronutrients in the flag leaves of these fertile backcrossed gives unequivocal 'proof of the concept' that *Aegilops peregrina* possesses distinct genetic system for uptake and translocation of the micronutrients which could be effectively used for biofortification of wheat cultivars. Rawat *et al.* (2009) also reported higher content of micronutrients in the flag leaves of sterile F_1 hybrids and BC_1 derivatives of *Ae. kotschyi.* Micronutrient analysis of the selfed seeds of fertile derivatives in advanced backcross generations showed two to three folds high grain Fe and Zn content. This variation could be associated with the presence of one or more chromosomes of the wild donors controlling the distinct uptake and translocation of the micronutrients.

A few derivatives among BC_2F_2 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 identification of alien introgression. Cytological studies revealed chromosome number 42 to 43 in these selected backcross derivatives indicating nearly recovered wheat background. These fertile BC_2F_2 backcross derivatives with one or more chromosomes from *Ae. peregrina* had shown 100 to 200 % increase in grain iron

and zinc concentrations. On the basis of comprehensive molecular analysis it could be suggested that the group 7 chromosomes followed by group 4 chromosomes from *Ae. peregrina* had genes for high grain iron and zinc content. Successful introgression of chromosomes of group 2 and 7 from two different accessions of *Ae. kotschyi* with 2-3 times higher grain iron and zinc content into wheat cultivars strongly supports the above said findings (Rawat *et al.*, 2009; Tiwari *et al.*, 2009). Tiwari *et al.* (2009), reported major QTLs for grain iron content on chromosomes 2A and 7A and one major QTL on 7 A for grain zinc content in *T. boeoticum* × *T. monococcum* RIL population. Grain size of the fertile backcross derivatives was almost similar or even greater than that of the wheat parent. Therefore, 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.

These selected backcrossed derivatives also had high content of other minerals such as Ca, Mn and Cu, without any selection for these minerals. It could be suggested that the genes on group 4 and 7 chromosomes which controlled high iron and zinc content also had pleiotropic effect on uptake and transport of other minerals to the grains.

The CS(Ph^l)-*Ae. peregrina* F_1 hybrids as well as the amphiploids were morphologically intermediate between the wheat and *Ae. peregrina* parents for plant height, growth habit, tiller numbers per plant etc. However other characters like ear shape, glume awns, hard threshing and brittle rachis and red grain colour were more like their *Ae. peregrina* parents. The genes controlling brittle rachis (*Br*), tenacious glumes (*Tg*) of *Ae. peregrina* appear to be epistatic over the *Q* locus in controlling

square head, tough rachis and free threshing in *T. aestivum* (Li and Gill, 2006 and Endo and Gill, 1996) as the amphiploids resembled their *Ae. peregrina* parents. The intermediate morphology of the F_1 hybrids and their synthetic amphiploids has been reported in several studies (Oliver *et al.*, 2005; Sharma *et al.*, 1987; Martin and Laguna, 1982; Sears, 1954).

Medium to highly fertile synthetic amphiploids ($AABBDDUUS^{l}S^{l}$) with nearly expected chromosome number (2n=10x=70) were obtained in C_0 generation. Variable chromosome number, pollen fertility, seed set and HMW glutenin subunit profiles of individual seeds of amphiploids in advanced generation indicated chromosomal instability among them. Some of the chromosomes from any of the parents got eliminated during the process of stabilisation. As a result of this process, amphiploids may get stabilised as complete or partial segmental amphiploids. Somatic chromosome elimination leading to variable chromosome number had also been reported in several wheat-*Thinopyrum* amphiploids and also in newly synthesised amphiploids up to several generations (Yang *et al.*, 2006; Ozkan, 2001; Feldman *et al.*, 1997; Cauderon *et al.*, 1973). Elimination of either one or the other 1U/ 1S HMW-Glutenin subunit from *Ae. peregrina* was also observed in both of the amphiploids. No elimination of group 1 D controlled HMW- Glutenin subunits were observed in both of the amphiploids against the report of preferential elimination of group 1D controlled HMW- Glutenin subunits in wheat- *Ae. kotschyi* amphiploids, as observed by Rawat *et al.* (2009). Garg *et al.* (2007) also reported loss of whole or a part of 1 D chromosome in $1S^{v}$ and $1S^{l}$ *Ae. peregrina* and *Ae. longissima* addition lines.

Both of the amphiploids had grain iron and zinc content comparable to that of *Aegilops* species. This may be due to distinct genetic system of *Ae. peregrina* which leads to higher grain high iron and zinc content in the amphiploids. Rawat *et al.*

(2009) also reported high grain micronutrient contents in several wheat- *Ae. kotschyi* amphiploids. Tiwari *et al.* (2009) also reported high grain Fe and Zn content in natural amphiploids of *T. durum* - *Ae. longissima*. Thus it may be concluded that *Aegilops* species are good reservoir for variability for grain micronutrient contents. This variability for grain micronutrient contents present in amphiploids could be utilized through recurrent backcrossing and development of alien addition and substitution lines in wheat background.

Enhanced rate of release of phytosiderophore under micronutrient deficient condition was observed in wheat cultivars and *Aegilops* species. The phenomenon of significant release of phytosiderophores by graminaceous species (Strategy II plants) under deficiency of iron, zinc and other micronutrients has been reported by various workers (Kanazawa *et al.*, 1995; Cakmak *et al.*, 1994; Mori *et al.*, 1991; Zhang *et al.*, 1989). *Aegilops* species released higher amounts of phytosiderophores under all conditions throughout the experiment. 2'-deoxymugineic (DMA) is the first phytosiderophore to be synthesized in the mugineic acid pathway as a result of trimerization of 3 molecules of S-adenosylmethionine molecules to form nicotinamine by the enzyme nicotinamine synthase (NAS) followed by transfer of an amino group leading to the formation of 3'-keto intermediate. The subsequent reduction of these 3'-keto intermediate by deoxymugineic acid synthase forms DMA. In rice under micronutrient deficiency, the amounts of various amino acids such as valine, histidine and methionine have been found to decrease to one third or one fourth as compared to that in sufficient media (Mori *et al.*, 1991) as they are utilized in the biosynthesis of 2'-deoxymugineic acid. The activity of NAS is markedly enhanced in order to meet the requirements of nicotinamine in rice under iron deficiency (Kanazawa *et al.*, 1995; Higuchi *et al.*, 2001). In graminaceous plants, enhanced expression of genes involved

in 2'- deoxymugineic acid biosynthesis was observed under both iron and zinc deficiency (Higuchi *et al.*, 2001).

In *Aegilops* species, nearly 2-4 times higher amount of phytosiderophores was released under iron and zinc deficiency as compared to *Triticum* species. Even under nutrient sufficient conditions all *Aegilops* species showed higher rate of release of phytosiderophores than the wheat cultivars. Difference in the rate of phytosiderophores released by wheat varieties under deficiency of iron and zinc has been found by Tolay *et al.* (2001). Variation in zinc efficiency among hexaploid wheat, hexaploid oats, diploid rye, triticale, *Aegilops* and wheat-alien chromosome addition lines has been studied by Schlegel *et al.* (1998). Greater sensitivity of durum wheat to zinc deficiency was attributed to its low capacity for uptake of adequate zinc under deficient conditions and also less secretion of zinc mobilizing phytosiderophores to the rizosphere from their roots (Cakmak *et al.*, 1996; Rengel and Graham, 1996). In addition to quantity of phytosiderophores released by the roots, the composition of MAs also affects the ability of plants to tolerate micronutrient deficiency (Mori, 1987). In rice, wheat, maize and sorghum only 2′deoxymugineic acid (DMA) is secreted making these plants are susceptible to micronutrient deficiency (Masuda *et al.*, 2008; Kobayashi *et al.*, 2008). In barley along with DMA, 3-hydroxymuginiec acid (HMA) and 3-epihydroxy mugineic acids (3- epi HMA) are secreted and hence it is more efficient in uptake of micronutrients under low mineral availability (Mori *et al.*, 1987; Singh *et al.*, 1993; Ma *et al.*, 1999). It is possible that more than one type of mugineic acids are secreted by *Aegilops* species also, as a result of which they have higher absolute amount of phytosiderophores enabling them to sustain the deficiency of micronutrients for longer time as compared to wheat.

Thus high genetic diversity in non-progenitor *Aegilops* species for high grain iron and zinc concentration (Chhuneja *et al.*, 2006; Rawat *et al.*, 2009) is most likely attributed to their diverse and efficient phytosiderophore release systems for micronutrient uptake which could be used for biofortification of wheat through interspecific hybridization.

The BC_2F_2 derivatives with high grain iron and zinc content also had 2-3 folds higher release of phytosiderophores under iron deficient medium. This may be due to transfer of group 4 and 7 chromosomes from *Ae. peregrina* to these fertile derivatives. Addition of these chromosomes probably led to higher release of phytosiderophores in introgressive derivatives. Higher expression of SAM, NAS and NAAT enzymes under iron deficient medium in BC_2F_2 derivatives strongly supports the above findings.

Addition lines of chromosome 2U, 2S, 4U and 7U of *Ae. peregrina*, 1S, 2S and 7S of *Ae. longissima* and 2U of *Ae. umbellulata* were found to have high grain iron content. Similarly, genes controlling high zinc concentration were found on 7U of *Ae. peregrina,* 7S of *Ae. longissima* and 5U of *Ae. umbellulata* . Shi *et al.* (2008), reported seven QTLs on chromosome 1A, 2D, 3A, 4A, 4D, 5A and 7A for zinc content in a double haploid wheat population of Hanxuan10 and Lumai 14. Four zinc QTLs were also identified on chromosome 3D, 4B, 6B and 7A in a double haploid wheat population by Genc *et al.* (2009). Stangoulis *et al.* (2007) found three QTLs for grain iron content in rice on chromosome 2, 8 and 12 explaining 17%, 18% and 14% of the total phenotypic variation, respectively and for zinc concentration two QTLs were found on rice chromosome 1 and 12 explaining 15 % and 13% variation, respectively. Rice chromosome 2 has been found syntenic and collinear with chromosome 3, 6 and 7 of wheat, chromosome 8 and 12 found syntenic to wheat 7S

and 5S, respectively and chromosome 1 of rice to wheat choromosome 3 (Ahn *et al.*, 1993). This supports the role of chromosome 7 and 5 of wheat in controlling grain iron and zinc content in wheat. In rye, chromosomes 7R and 1R were found to be responsible for higher tolerance to zinc deficiency (Cakmak *et al.*, 1997; Graham, 1984). Devos *et al.* (1995) reported complete homoeologous relationship between 1R of rye and group 1 chromosomes of wheat while rye 7R has synteny with the wheat homoeologous group 2. As collinearity is conserved among genomes of different genera across the tribe Triticeae, where the *Triticum- Aegilops* alliance belongs (Akhunov *et al.*, 2003), it could be assumed that the genes for high zinc efficiency might be present on homoeologous group 1 and 2 of *Aegilops species*. Lonergan, (2001), identified leaf zinc QTLs on chromosome 4HS and three QTLs for grain zinc concentration on 2HS, 2HL and 5HL in barley. A QTL on choromosome 6B was found to affect grain iron, zinc and protein content in *Triticum. turgidum ssp. dicoccoides* (Korn) Thell (Distelfeld *et al.*, 2007).

Addition lines 2S, 7S, 2U and 4U of *Ae. peregrina*, $2S^{l}$ and $6S^{l}$ from *Ae. longissima*, 2U and 5U from *Ae. umbellulata* had shown higher amount of mugineic acids released as compared to CS suggesting that the genes for mugineic synthesis and induction are localized on these chromosomes. The maximum increase in mugineic acids released on 11^{th} day was found by the addition of chromosome 4 U of *Ae. peregrina* which indicates presence of genes for higher mugineic acids release or different types of mugineic acids on chromosome 4. Almost all the addition lines with higher level of mugineic production under iron deficient and sufficient media had also higher grain iron and zinc content suggesting that their higher level of micronutrient content could be attributed to their ability to release higher amount of mugineic acids for higher uptake of the micronutrients. The genes controlling hydroxylation of

mugineic acids were located on different chromosomes of barley. Ma *et al.* (1999) reported that the long arm of barley chromosome 4H and long arm of chromosome 7H in wheat (*Triticum aestivum* L. cv. Chinese Spring)-barley (cv. Betzes) ditelosomic addition lines were responsible for hydroxylation of DMA to MA and from MA to epi-HMA, respectively. Barley chromosomes 2H, 3H, 4H, 5H, 6H and 7H are syntenic to wheat chromosome groups 2, 3, 4, 7, 6 and 1, respectively (Deynze *et al.*, 1995; and Gale and Devos 1998, Cho, 2005). Therefore it could be assumed that genes for synthesis of MA and epi-HMA might be present on chromosome 2, 4, and 7 in *Ae. peregrina.* Mori *et al.* (1990), identified rye chromosome 5R as a carrier of the genes for mugineic acids synthetase and 3- hydroxymugineic acids synthetase using wheat- rye addition lines. Devos *et al.* (1993) studied chromosomal rearrangements in the rye genome relative to that of wheat and found that short arm and most of the long arm of rye showed homoeology with wheat chromosome 5B and 5D. This supports our finding of higher release of mugineic acids by addition line of 5U of *Ae. umbellulata.* Approximately, 25% to 30 % of the world's agricultural area is potentially under iron and zinc deficiency (Mori *et al.*, 1990; Cakmak *et al.*, 1996). Significant positive correlation between grain micronutrient content and phytosiderophore was observed, hence these addition lines could be used for introgression of genes for tolerance to zinc and iron deficiency through broader spectrum and higher production of mugineic acids.

The addition lines for homoeologous group 2 followed by group 7 and 4 maintained higher chlorophyll content till the end of experiment suggesting indirectly that the addition lines had higher grain iron content and or availability. As the chlorophyll content is a good measure of iron nutrition of the plant it could be assumed that due to higher grain iron and zinc concentration in these addition lines

they provide mineral nutrition to the plant for longer time. These results are very much in accordance with Shen *et al.* (2002). While working with two wheat genotypes, one N85021 with high seed Fe concentration and another Z181 with low seed Fe concentration, when grown under Fe deficient condition, N85021 had better seedling vigour, chlorophyll content, shoot and root dry matter production and better uptake of iron as compared to Z181. He also reported higher mugineic acids release in N85021 then Z181.

Major QTLs for phytosiderophore was observed on chromosome 6 in *T. monococcum* X *T. boeoticum* RIL population. Patterson *et al.* (2007) also observed choromosome 6H of barley responsible for higher HMA production and boron tolerance. This is very helpful in understanding of the genetics of phytosiderophore production.

The fertile BC_2F_2 derivatives with addition, substitution or translocation of *Ae. peregrina* chromosomes could be further utilized for precise transfer of high grain iron and zinc content genes through radiation hybrid mapping or induced homoeologous pairing .The identification of more than one addition lines of group 2, 4 and 7 chromosomes in each of the investigated species *Ae. peregrina, Ae. longissima* and *Ae. umbellulata* each with more than 70% higher grain iron concentration and group 7 chromosomes for both higher grain iron and zinc clearly demonstrates that these chromosomes carry major genes for micronutrient biofortification. Most of these addition lines had also higher level of mugineic acids production under iron deficient and sufficient media than Chinese Spring the recipient wheat line further suggesting that the higher grain iron and zinc in the addition lines and their parental *Aegilops* donor species could be attributed to efficient micronutrient uptake and subsequent translocation and deposition. These addition lines and their

parental donor species could be outstanding sources for precise introgression of genes for effective biofortification of elite wheat cultivars through induced homoeologous pairing; molecular cytogenetics and molecular breeding with 2-3 times higher grain micronutrient content and concentration.

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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	AGGGAAAAGACATCTTTTTTTTC	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	GCGGTTCGGAAAGTGCTATTCTACAGTAA	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	GCGTAATGATGGGAGTGGCTATAGGGCAG TT	70
59	barc229	GGCCGCTGGGGATTGCTATGAT	TCGGGATAAGGCAGACCACAT	61
60	barc99	CGCATTCTTTCGCATTCTCTGTCATA	CGCATACTGTGTCGTGTTCCTGGTTTAGA	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	GCGTGCTCCACTCAGAGACTATCATAAA	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	GCGTTTTTTACCTAAAGAAAAGGTGATT G	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	CTGCAGGAAAAAAAAGTACACCC	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	GCGAGTTGGAATTATTTGAATTAAACAAG	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	CGCAATAATTCAGTACTACTTCCCCGCA ATA	CGAAGGATTTGCATGGTACTGTGGGTGAT	70
258	gwm213	TGCCTGGCTCGTTCTATCTC	CTAGCTTAGCACTGTCGCCC	60
259	barc308	GCGATCTTGCGTGTGCGTAGGA	GCGTGGGATGCAAGTGAACAAT	62
260	barc288	GGGTTTTGCTTGGTTGACA	CGGGACGATTTTATTTAGGAGT	55
261	barc98	CCGTCCTATTCGCAAACCAGATT	GCGGATATGTTCTCTAACTCAAGCAATG	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	CGCAGAAGAAAAACCTCGCAGAAAAA CC	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	CCAAAAAAACTGCCTGCATG	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	CAGGAAGAAAAGGAAAGCATGCGACA A	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

Phytosiderophores released (µg of Fe mobilized / g dry root wt / 3 hour) in *T. monococcum* × *T. boeoticum* RIL population

RIL No	NEW _TT1	IITR2007	WRDI 2008	MH 2008	GH 2009	DH 2009	POOL
RIL-1	4.51	5.20	4.29	4.34	3.86	4.81	4.50
RIL-2	4.04	4.63	3.82	3.86	3.44	4.29	4.01
RIL-3	5.62	5.23	4.32	4.36	3.88	4.84	4.52
RIL-4	4.48	5.18	4.27	4.32	3.84	4.79	4.48
RIL-6	8.82	10.18	8.40	8.48	7.55	9.41	8.80
RIL-8	7.43	7.89	6.51	6.57	5.85	7.30	6.82
RIL-9	5.55	5.04	4.16	8.57	7.63	9.52	6.98
RIL-10	8.38	7.75	8.30	8.38	7.46	3.48	7.07
RIL- 11	7.37	8.68	7.16	7.23	6.44	8.03	7.51
RIL-13	7.42	8.64	7.12	7.20	6.40	7.99	7.47
RIL-14	4.12	4.82	3.98	4.02	3.58	4.46	4.17
RIL-15	5.37	5.03	4.15	4.19	3.73	4.65	4.35
RIL-16	11.46	12.59	10.38	10.49	9.33	11.64	10.89
RIL-17	3.03	3.54	2.92	2.95	2.63	3.28	3.06
RIL-18	6.06	6.76	5.58	5.63	5.01	6.25	5.85
RIL-19	4.54	5.47	4.51	4.56	4.06	5.06	4.73
RIL-20	3.55	4.42	3.65	3.69	3.28	4.09	3.83
RIL-22	7.24	8.64	7.12	7.20	6.40	7.99	7.47
RIL-23	4.05	4.42	3.68	3.69	3.28	4.09	3.83
RIL-24	7.04	7.30	6.07	6.08	5.41	6.75	6.32
RIL-26	4.75	5.85	4.86	4.87	4.34	5.41	5.06
RIL-28	6.05	6.40	5.32	5.34	4.75	5.92	5.55
RIL-29	10.37	10.82	9.00	9.02	8.02	10.01	9.37
RIL-30	3.8	4.29	3.56	3.57	3.18	3.96	3.71
RIL-32	3.18	4.23	3.52	3.52	3.14	3.91	3.66
RIL-33	7.04	8.64	7.18	7.20	6.40	7.99	7.48
RIL-34	4.56	5.96	4.95	4.96	4.42	5.51	5.16
RIL-38	5.83	6.77	5.63	5.64	5.02	6.26	5.87
RIL-40	5.25	6.85	5.69	5.71	5.08	6.33	5.93
RIL-41	4.63	5.47	4.55	4.56	4.06	5.06	4.74
RIL-42	5.74	6.45	5.37	5.38	4.79	5.97	5.59
RIL-44	5.04	5.95	4.95	4.96	4.41	5.50	5.16
RIL-46	8.04	8.79	7.31	7.32	6.52	8.13	7.61
RIL-47	4.02	4.73	3.93	3.94	3.51	4.37	4.10
RIL-48	18.57	20.16	16.76	16.80	14.95	18.64	17.46
RIL-49	7.58	8.98	7.46	7.48	6.66	8.30	7.78

RIL-50	9.04	11.70	9.73	9.75	8.67	10.82	10.13
RIL-52	5.04	5.88	4.89	4.90	4.36	5.44	5.10
RIL-53	12.48	13.93	11.59	11.61	10.33	12.89	12.07
RIL-55	6.64	7.43	6.18	6.19	5.51	6.88	6.44
RIL-56	3.84	5.15	4.28	4.29	3.82	4.76	4.46
RIL-57	5.37	5.45	4.53	4.54	4.04	5.04	4.72
RIL-58	3.95	4.21	3.50	3.51	3.12	3.89	3.65
RIL-60	4.84	5.96	4.96	4.97	4.42	5.52	5.17
RIL-61	9.37	10.53	8.75	8.77	7.81	9.74	9.12
RIL-62	4.74	5.68	4.72	4.73	4.21	5.25	4.92
RIL-64	8.82	10.86	9.03	9.05	8.05	10.05	9.41
RIL-65	15.03	14.20	11.81	11.84	10.53	13.14	12.31
RIL-66	16.94	18.03	15.00	15.03	13.38	16.68	15.62
RIL-68	16.03	18.03	15.00	15.03	13.38	16.68	15.62
RIL-70	13.04	14.82	12.33	12.35	10.99	13.71	12.84
RIL-72	8.45	9.58	7.96	7.98	7.10	8.86	8.30
RIL-73	5.39	6.99	5.81	5.82	5.18	6.46	6.05
RIL-75	15.38	16.19	13.46	13.49	12.01	14.97	14.02
RIL-77	12.46	13.85	11.52	11.54	10.27	12.81	12.00
RIL-78	4.74	5.38	4.47	4.48	3.99	4.97	4.66
RIL-79	10.48	11.20	9.31	9.33	8.31	10.36	9.70
RIL-80	14.93	16.19	13.46	13.49	12.01	14.97	14.02
RIL-82	3.38	4.48	3.72	3.73	3.21	4.14	3.86
RIL-83	4.62	6.03	5.02	5.03	4.32	5.58	5.20
RIL-84	14.92	15.81	13.15	13.18	11.33	14.63	13.62
RIL-86	26.42	25.67	21.35	21.39	18.40	23.75	22.11
RIL-87	8.59	10.34	8.60	8.62	7.41	9.57	8.91
RIL-88	5.04	6.80	5.65	5.67	4.87	6.29	5.86
RIL-90	8.04	9.93	8.26	8.28	7.12	9.19	8.56
RIL-91	11.74	12.73	10.59	10.61	9.13	11.78	10.97
RIL-92	5.84	6.52	5.42	5.43	4.67	6.03	5.62
RIL-96	19.03	21.14	17.58	17.61	15.15	19.55	18.20
RIL-99	6.93	6.68	5.56	5.57	4.79	6.18	5.75
RIL-100	8.05	10.03	8.34	8.36	7.19	9.28	8.64
RIL-102	18.42	22.55	18.75	18.79	16.16	20.86	19.42
RIL-103	5.25	6.68	5.56	5.57	4.79	6.18	5.75
RIL-104	22.39	24.33	20.23	20.27	17.43	22.50	20.95
RIL-105	8.94	9.00	7.48	7.50	6.45	8.32	7.75
RIL-106	15.19	17.10	14.22	14.25	12.26	15.82	14.73
RIL-107	8.84	9.00	7.48	7.50	6.45	8.32	7.75
RIL-108	12.03	11.49	9.56	9.58	8.24	10.63	9.90
RIL-110	6.04	6.41	5.33	5.34	4.59	5.93	5.52
RIL-113	6.04	8.23	6.85	6.86	5.90	7.61	7.09

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1. Tiwari, V.K., Rawat, N , **Neelam K** , Randhawa, G.S., Singh, K., Chhuneja, P. and Dhaliwal, H.S. 2008. Development of *Triticum turgidum* ssp. *durum*-*Aegilops longissima* amphiploids with high iron and zinc content through unreduced gamete formation in F1 hybrids. *Genome*. 51: 757-766. (Shared first co authorship)
2. Rawat, N., Tiwari, V.K., **Singh, N.**, Randhawa, G.S., Singh, K., Chhuneja, P. and Dhaliwal, H.S. 2008. Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content In wheat. *Genet. Res. Cr. Evol.* DOI: 10.1007/s10722-008-9344-8 (Shared first co authorship)
3. Tiwari, V.K., Rawat, N, Chhuneja P, **Neelam K**, Aggarwal R, Randhawa G.S, Dhaliwal H.S, Keller B, Singh K. 2009. Mapping of Quantitative Trait Loci for Grain Iron and Zinc Concentration in A Genome Diploid Wheat. *J. Heredity*. (JOH)-2008-154.R3.
4. Rawat, N., Tiwari, V.K., **Neelam K.**, Randhawa, G.S., Singh, K., Chhuneja, P., Dhaliwal, H.S. 2009. Development and characterization of wheat- *Aegilops kotschyi* amphiploids with high grain iron and zinc. *Plant Genet. Resour.* DOI: 10.1017/s147926210935 (Shared first co authorship)
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7. Dhaliwal, H.S., Rawat, N., **Neelam, K.**, Tiwari, V. K. 2009. Development and characterization of Wheat-*Aegilops* amphiploids and interspecific derivatives with high grain iron and zinc content. In Proceedings of *International Wheat Quality Conference-IV.*

8. Tiwari, V.K., Rawat, N., **Singh, N.**, Randhawa, G.S. and Dhaliwal, H.S. 2006. *Aegilops* and wild *Triticum* species: a good reservoir of useful variability for higher iron and zinc content, in International Conference on Biotechnology Approach for Alleviating Malnutrition and Human Health. Bangalore, India .

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10. Rawat, N., Tiwari, V.K., **Neelam K.**, Randhawa, G.S., and Dhaliwal, H.S 2009. Characterisation and SEM-EDX studies of low phytic acid mutant of *Triticum monococcum* and germinating wheat grains. In Proceedings of *International Wheat Quality Conference-IV.*

Articles submitted to refereed journals

1. **Neelam, K**[1]., Rawat, N. [1], Tiwari, V.K. [1], Chhuneja, P., Randhawa, G.S., Singh, K., and Dhaliwal, H.S. 2009. Evaluation and characterization of addition lines of *Ae. peregrina* and *Ae. longissima for* grain micronutrients. *Euphytica.*
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4. Tiwari, V.K., Rawat, N., Chhuneja, P., **Neelam, K**., Randhawa, G.S., Singh, K., and Dhaliwal, H.S. 2009. Molecular and cytological characterization of introgressive derivatives high grain and zinc concentrations. *Theor. Appl. Genet.*

RIL-115	8.01	8.23	6.85	6.86	5.90	7.61	7.09
RIL-117	5.48	6.13	5.10	5.11	4.40	5.67	5.28
RIL-118	12.03	14.28	11.88	11.90	10.24	13.21	12.30
RIL-121	5.04	6.33	5.26	5.27	4.53	5.85	5.45
RIL-122	7.04	8.91	7.41	7.43	6.39	8.25	7.68
RIL-124	17.28	22.85	19.00	19.04	16.37	21.13	19.68
RIL-127	3.24	3.22	2.68	2.69	2.31	2.98	2.78
RIL-128	12.06	12.65	10.52	10.55	9.07	11.70	10.90
RIL-129	10.62	12.84	10.68	10.70	9.12	11.88	11.06