TRANSFER OF GENES FOR HIGH GRAIN Fe AND Zn CONTENT OF GROUP 7 CHROMOSOMES OF *Aegilops* TO WHEAT

Ph.D. THESIS

by

SATISH KUMAR

DEPARTMENT OF BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE - 247 667 (INDIA)
SEPTEMBER, 2013

TRANSFER OF GENES FOR HIGH GRAIN Fe AND Zn CONTENT OF GROUP 7 CHROMOSOMES OF *Aegilops* TO WHEAT

A THESIS

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

of

DOCTOR OF PHILOSOPHY

in

BIOTECHNOLOGY

by

SATISH KUMAR

DEPARTMENT OF BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE - 247 667 (INDIA)
SEPTEMBER, 2013

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"TRANSFER OF GENES FOR HIGH GRAIN Fe AND Zn CONTENT OF GROUP 7 CHROMOSOMES OF *Aegilops* TO WHEAT"** in partial 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 the period from January, 2009 to September, 2013 under the supervision of Dr. R. Prasad, Professor & Head and Dr. H. S. Dhaliwal, Professor (Retd.), Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, India.

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.

(SATISH KUMAR)

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

(H. S. Dhaliwal)
Supervisor

(R. Prasad)
Supervisor

Date:

The Ph.D Viva-Voce Examination of **Mr**. **SATISH KUMAR** Research Scholar has been held on ……

Signature of Supervisors Signature of Chairman, SRC Signature of External Examiner

Head of the Department/Chairman, ODC

ABSTRACT

More than 2 billion people in the developing world are affected by iron (Fe) and zinc (Zn) deficiency. Fe deficiency often leads to anemia, impaired physical growth, mental retardation, weak learning capacity and ability to do physical labour. Zn acts as necessary component in more than 200 enzyme systems for normal growth and development, maintenance of body tissue, sexual function, brain development, cognitive ability, vision and immune system. Micronutrient deficiency can be alleviated by supplementation, diet diversification, fortification and biofortification. Biofortification is the most sustainable, targeted and cost effective approach for improving nutritional quality of staple crops. There are several approaches to biofortify crops, including agronomic biofortification, conventional or molecular breeding and genetic engineering. Wheat is the second most consumed cereal in Asia after rice. The polyploid nature of wheat provides considerable genetic buffering thus allowing interogression of useful variability from related species. High yielding cultivars of wheat are the poor sources of important micronutrients especially Fe and Zn. Wheat is also rich in anti- nutritional compounds like phytic acid and fibres which reduces the bioavailability of the micronutrients.

In 2002, the Consultative Group for International Agricultural Research (CGIAR) and HarvestPlus initiated a program to develop biofortified crops with focus on three critical micronutrients Fe, Zn and vitamin A. The related wild *Triticum* and *Aegilops* species with useful variability for high grain Fe and Zn content can be utilized for biofortification of wheat. Several wild progenitor and non-progenitor species of wheat were used for development of alien addition, translocation and substitution lines for transfer of useful variability. The quantitative trait loci (QTL) for grain Fe and Zn were mapped on chromosome 2A and 7A in diploid wheat. Introgression of chromosome 2S and 7U from *Aegilops kotschyi* to wheat led to high grain Fe and Zn content. Several *Aegilops* alien addition and substitution lines of group 2 and 7 with high grain Fe and Zn are also available.

Transfer of useful variability from non-progenitor species can be easily achieved by induced homoeologous pairing, through *ph1b* deletion and 5B defciency. The wheat ph1b mutation, which promotes meiotic pairing between homoeologous chromosomes, was employed to induce recombination between *Ae. kotschyi* 396 chromosome 7S and 7U and their wheat homoeologue. Radiation hybrid is also very useful approach for gene transfer and gene localization. Presence of high density microsatellite maps of wheat and modern cytological techniques like GISH and FISH can be used for precise transfer and tagging of genes responsible for grain micronutrient content. GISH is of potentially wide application in plant

breeding programmes involving alien translocations. This study was undertaken to reduce linkage drag in substitution lines of 7S and 7U chromosomes of *Ae. kotschyi* 396 and *Ae. kotschyi* 3790, respectively through fine transfer of genes for high grain iron and zinc content.

Anchored wheat SSR markers of group 7 of wheat were used for transferability and polymorphism between *Aegilops* donor and recipient wheat cultivars. A total of 173 markers of group 7 chromosomes were screened using PCR. 77.45 % (134 markers) of these 174 markers were found to be transferable. All the markers which were transferable were not polymorphic among wheat and *Aegilops* species. Polymorphic proportion varied for 41-70 % of 7A, 7B and 7D chromosome markers, from the long and short arms. A total of 51.49 % (69 markers) markers were found to be polymorphic out of 134 transferable markers of group 7 of wheat. Polymorphic markers between wheat and *Aegilops* species were analysed for transferability to 7S and 7U chromosomes using 7S substitution and 7U addition line as the genes for Fe and Zn are mostly located on these chromosomes supported by micronutrient data of substitution and addition lines of 7S and 7U in wheat and Chinese Spring background. Out of 69 polymorphic markers of group 7 chromosomes of wheat 36 52.29% were transferable to 7S and 7U chromosome, 28.98% specific to 7S, 14.49% for 7U and 8.69% for both the chromosomes. The tentative consensus map of 7S and 7U chromosomes of *Ae. kotschyi* was prepared by Join Map using markers which were found polymorphic between *Triticum aestivum* and *Ae. kotschyi* and transferable to 7S and 7U chromosomes. These 7S and 7U specific markers were used for molecular characterization of introgressed derivatives.

In the present study, two kinds of radiation hybrid approaches, seed irradiation and pollen irradiation were used for precise gene transfer. Wheat-*Aegilops* substitution line CS(*Ph*^{I})/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6⊗ of 7S for 7D chromosome was seed irradiated at 40 krad of gamma radiation. These irradiated seeds were grown in the field and the plants were crossed with recurrent parent WL711 to get SRH_1 plants. For pollen irradiation, spikes of wheat-*Aegilops* 7S and 7U substituted lines CS(*Ph*^{I})/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6⊗ and CS(*Ph*^{I})/*Ae. kotschyi* 3790//UP2338-2///WL711(63)-2-13⊗**,** respectively were irradiated at 2 krad of gamma radiation and used for pollination of recipient wheat cultivar PBW343 with *Lr24* and *GPC1*. PRH_1 plants were selfed and screened for micronutrients content and transfer of small fragment/genes with high grain Fe and Zn using polymorphic marker of group 7 chromosome and (GISH). Nitric acid digested seed samples of SRH_1, SRH_3, PRH_1, PRH_2, BC_1F_2, (*ph1b*) BC_1F_3 (*ph1b*) and BC_2F_2 were analysed for micronutrients using AAS and ICP-MS. The SRH_2 plants had Fe and Zn concentrations in the range of 46.8 to 127.4 mg/kg and 41.25 to 110.10 mg/kg, respectively,

The SRH_2 plants had Fe and Zn concentrations in the range of 23.18 to 92.34 mg/kg and 27.15 to 72.90 mg/kg, respectively, as compared to 32.20 mg/kg Fe and 40.56 Zn for the wheat cultivar WL711. The plant SRH_3-28-2 had 70% increase in grain Fe and 15% increase in grain Zn and plant no. SRH_3-14-2-⊗ and SRH_2-28-6-⊗ had 187% and 97% increase in grain Fe and 40% and 47% in grain Zn content, respectively. These plants had short arm and terminal transfers of 7S. In some plants of PRH_1 of 48-41-6⊗ 20-125% increase in grain Fe content and 40-140% increase of grain Zn content or 40-60 % increase of both the elements was observed over PBW343. The plants of PRH_2 of 48-41-6⊗ had Fe and Zn content in the range of had Fe and Zn concentrations in the range of 13.89 to 150.52 mg/kg and 27.11 to 192.48 mg/kg respectively. PRH_1-82 and PRH_1-124 had 7S chromosome translocations. Grain Fe concentration varied between 18.9 mg/kg to 77.45 mg/kg and grain Zn concentration varied between 23.32 mg/kg to 164.9 mg/kg. for the plants of PRH_2 of 48-41-6⊗**.** The plants of PRH_2 of 63-2-13⊗ had Fe and Zn concentrations in the range of 4.04 to 133.16 mg/kg and 22.12 to 124.15 mg/kg respectively. PRH_1-312 had short arm translocation. It was found that Fe content of PRH_2 plants varied in the range of 19.3 mg/kg to 71.5 mg/kg and Zn content varied in the range of 22.4 mg/kg to 48.03 mg/kg.

For an alternative strategy for gene transfer, 7S substitution lines of *Aegilops* were also crossed with *ph1bph1b* deletion to obtain F_1 plants (*ph1bph1*//7S/7D) and again backcrossed with *ph1b* mutant plants. The BC_1F_1 plants were screened for homozygous *ph1bph1b* through *Ph1* locus specific marker psr574 and 7S monosomic 7S by wheat anchored 7S specific SSR The plant with *ph1bph1b* and 7S monosomic were selected selfed to get BC_1F_2. Backcross derivative were further screened for high grain Fe and Zn content. The seeds of BC_1F_2 were mostly shriveled because of *ph1bph1b* and leaf yellowing. The shriveled seeds and leaf yellowing seems to be associated with the absence of *ph1* locus. Only a few plants obtained had equivalent harvest index to that of the cultivar and 40-60% increase of the Fe or Zn or both the element. Plant BC_1F_2-471 and BC_1F_2-487 had multiple translocations and long arm of 7S chromosome, respectively. The BC_1F_3 had Fe and Zn concentrations in the range of 22.7 to 53.95 mg/kg and 16.58 to 62.12 mg/kg respectively.

The derivatives of all three hybrids approaches i.e. seed irradiation, pollen irradiation and *ph1b* hybrids, which had high grain Fe and Zn content were also found resistant to powdery mildew and had 7S short arm transferred, indicating that the genes for micronutrient uptake and powdery mildew resistance might be linked on short arm of 7S chromosome. Plant PRH_2 -124 had translocation of 7S chromosome telomeric region, was resistant to powdery mildew and plant PRH_2 -82 had 7S chromosome without telomeric region, was found

susceptible to the powdery mildew, indicating that powdery mildew resistance gene could be present in sub-telomeric region of the 7S chromosome. Powdery mildew resistance might be linked to SSR markers wmc405 and barc126 as indicated by SSR marker data on seed irradiated hybrids. Genes for micronutrient uptake were also linked to these markers, further proving the linkage of powdery mildew and micronutrient uptake genes.

Mono 5B plants of *Triticum aestivum* cv. Pavon were cytologically identified and crossed with *Aegilops* 3790 as the male parent. The F_1 plants were screened by molecular markers psr574. The absence of these markers indicated the absence of 5B i.e. 34 chromosomes in total. The ABDUS hybrids were also confirmed absence of 5B by cytological analysis at meiosis. The F_1 plants with 34 chromosomes (without 5B) showed high chromosome pairing up to, 2V+4III+2II+1I, while the plants with 35 chromosomes (with 5B) had reduced homoeologous pairing, with 6II+23I. Plants with 34 chromosomes (without 5B) were selected and backcrossed extensively with wheat cultivar PBW343 with *Lr24* and *GPC1* for transfer of useful variability of *Aegilop*s for micronutrients biofortification. Fertile derivative were further screened for high grain Fe and Zn content. Fe and Zn content of mono 5B BC_2F_2 plants ranged from 43-114 mg/kg and 141-238 mg/kg due to concentration effect. The chromosome number of BC_2F_1 plants varied 42-48 with 2-7 univalents.

The Derivatives of all types of hybrids i.e. SRH, PRH, *ph1b* induced and 5B deficiency induced, with very high Fe and Zn content had poor tillering, seed set, and low harvest index, indicated that micronutrient content was negatively correlated with yield and harvest index. This negative correlation might be due to distribution of fixed amount of micronutrient per plant among less number of seeds the plants. Plants with shrivelled seeds in the hybrid progenies also had high Fe and Zn content suggesting that the negative correlation between seed size and micronutrient concentration, could be due more aleurone area per unit mass of shrivelled seeds as compared to the bold seeds.

All the selected plants with chromosomal translocations had better genetic system for Fe and Zn uptake from the soil and transport within the plants but the overall concentrations of these micronutrients in the seeds was however less than the donor *Aegilops* species. The biofortification of wheat for Fe and Zn content could be achieved up to 40-50% without any linkage drag. Pyramiding of these introgressed genes/QTLs from different sources through molecular breeding can be done to achieve enhanced biofortification of these micronutrients.

ACKNOWLEDGEMENTS

Having attained the completion of this research work, I would like to express my sincere gratitude and thankfulness to my supervisors Dr. H.S. Dhaliwal Retd. Professor, Department of Biotechnology, IIT Roorkee (Presently Vice Chancellor, Eternal University, Baru Sahib) and Dr. R. Prasad, Professor and Head, Department of Biotechnology, IIT Roorkee for their thought provoking guidance, constant encouragement, supporting presence and meticulous help throughout the course of this study. Dr. Dhaliwal has not only been my Ph.D guide, but also a source of inspiration and my role model to whom I shall be indebted forever for being like a guardian to me. I would like to thanks Dr. Prasad for giving me work freedom during my PhD tenure.

I would like to thanks to Dr. G.S. Randhawa, Professor and chairman DRC, Department of Biotechnology for moral support, inspirations and caring nature of him. His lecture "How to be Successful" had proved to be a booster dose to me. I am grateful to the members of my student research committee, Dr. P. Roy, Associate Professor, Department of Biotechnology and Dr. S.K. Sondhi, Professor, Department of Chemistry for constant help and kind cooperation throughout my thesis work. I owe my gratitude and sincere thanks to all faculty members of the department who provided an environment of intellectual and academic freedom at IIT Roorkee that made this work an enjoyable learning experience.

The valuable help of Dr. Sundip Malik, Associate professor, Dept of Biotechnology, GBPUA&T, for GISH analysis and moral support is also duly acknowledged. Help provided by Priyanka Mathpal, Sachin and Dr. Upendera Balyan is acknowledged. I am highly obliged to Dr. P. Chhuneja, Associate Professor, PAU Ludhiana and Dr. Kuldeep Singh, Director, School of Biotechnology, PAU Ludhiana for valuable suggestions and help in every possible way to make this work a reality. I would like to thank Dr. K.S Gill, Washington State University USA, Dr. P. K. Gupta and Dr. H.S. Balyan, CCS University, Meerut for important discussions during VIGS Workshop in Meerut .

I am thankful to my friends at IIT Roorkee in the Biotechnology Department, for informal milieu, friendly and lively atmosphere. Special thanks are due to my dear labmates Dr. Anjali Awasthi, Dr. Priyanka Paul, Shailender Kumar Verma and Rajbala for their invaluable help, constant support and providing family environment since my joining, without their helping and caring nature completion of this thesis would have just like impossible. I would be shirking in my duty if I do not express my thankfulness towards my seniors Dr. Deepak Rajpurohit, faculty member at Rajsthan University, Dr. Javed Ansari faculty at King South University Riyad, Dr. Neelam faculty at PAU, Dr. Durga Panigarhi, Dr. Santosh kumar Shrivastav , Parmesh Ramlu Lambari, Dr. Supriya Deepak patil and Alok kumar Jha. I acknowledge the help provided by my friends and colleagues Dr. Nagesh, Umesh Tanwar, Swati Verma, Shilpi Kumari, Manisha, Shalini Pareek, Pallavi, Navneet Kaur, Deepa Dewan Savita Budania and Raghavendra. Pradeep, Sanjay, Khursheed and Shahanwaj proved to be of great assistance in successful completion of field and lab work.

I would like to thank Dr. Prabhjot Kaur Gill, Dr. Parash Sharma, Dr. Vishal Chugh, Dr. Dharmendra , Dr. Vinod Sangwan, Dr. Punesh Sangwan and Er. Rajveer Singh, faculty members of Akal School of Biotechnology, Eternal University for their invaluable help, cooperation and moral support. I am thankful to my friends at Eternal University, Baru Sahib, help provided by Anoop Thakur and Sanjeev Kumar, Imran Shekh, Prachi Sharma, Navdeep Boparai, Ravi, Preeti, Ravinder Singh, Harpreet and Kiran cannot be valued.

I cannot forget the help, moral support and inspirations of Praveen Kaushik my senior at Kurukshetra University Kurukshetra. Inspiration of Vikas Sharma, PhD scholar at AIIMS is acknowledeged. Help and best wishes of Netrapal Shekhawat, Schain Gulati, Devender Redu ,Kapil Punia , Satish Master, Mahender Singh and Pradeep kumar, my friends at Kurukshetra University Kurukshetra is acknowledged.

The motherly affection of Mrs. Dhaliwal will remain fresh forever in my memory. Special thanks are due to her for hosting the many 'makkey di roti and sarson da saag' parties at their home. I can never forget her caring nature.

I would like to thank Mr. R. Juyal for his valuable help in AAS analysis throughout the term of this work. I am also thankful to Mr. Handa, Technical Officer, Institute Instrumentation Centre for his help in ICP-MS analysis of samples.

To make my stay pleasant in the department and completing this work, the help provided by non-teaching staffs of the department are duly acknowledged.

Financial support in the form of JRF and SRF from Council of Scientific and Industrial Research (CSIR) Delhi, India and DBT, India for Research funding is highly acknowledged.

I am indebted to my Mama's family, my parents, brothers and sisters for their love, patience, care, encouragement and untiring support. The love and affection of my mama's family members always refreshed me in testing times. This work has taken the present shape only due to the love and support of my Mama's family.

I dedicate this dissertation to my Naani ***Chander Nain****, who dreamt of me getting all success and to instill into me, the value of hard work.*

Date: ***Satish Kumar***

ABBRIVATIONS USED

%	Percentage
⊗	Selfing
µ	micron
µg	Microgram
AAS	Atomic absorption spectrometer
AFLP	Amplified fragment length polymorphism
BC	Back Cross
bp	Base Pair
CAPS	Cleaved amplified polymorphic sequence
cDNA	Complimentary DNA
cM	Centimorgan
CTAB	Cetyl Trimethyl Ammonium Bromide
cv.	Cultivated Variety
DNase	Deoxyribonuclease
DMA	2-Deoxy mugenic acid
dNTPs	Deoxy Nucleotide Triphosphates
EDTA	Ethylene Diamine Tetraacetic Acid
EST	Expressed sequence tag
EtBr	Ethidium Bromide
F_1	First Filial Generation
F_2	Second Filial Generation
F_3	Third Filial Generation
FAO	Food and Agriculture Organisation
Fig.	Figure
FISH	Fluorescent in situ hybridization
GISH	Genome in situ hybridization
g	Gram
Epi-HDMA	epihydroxy -2hydroxy muginneic acid
Epi-HMA	3-epi-hydroxy muginneic acid

ICP-MS	Inductively coupled plasma mass spectrometer
IRT	Iron regulatory transporters proteins
Kb	Kilobase pairs (10^3 bps
kg	Kelogram
L	Litre
M	Molarity
MA	Mugenic acid
MAS	Marker Assisted Selection
Mb	Megabase pairs (10^6 bp)
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimole
MTP	Metal tolerance proteins
ng	Nanogram
NRAMP	Natural resistance associated macrophage protein
^oC	^{o}Centigrade
PAGE	Poly-acrylamide Gel Electrophoresis
SDS PAGE	Sodium dodecyl sulphate PAGE
PCR	Polymerase Chain Reaction
Ph 1	Pairing homogous 1
ph 1b	*Ph 1* deletion
Ph^I	Pairing homogous inhibitor
PMC	Pollen mother cell
ppm	Parts per million
PRH	Pollen irradiated hybrids
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
RDA	Recommended dietary allowance
RFLP	Restriction Fragment Length Polymorphism

RIL	Recombinant inbred line
RNase	Ribonuclease
SDS	Sodium dodecyl sulphate
sec	Second
SNPs	Single Nucleotide Polymorphism
SRH	Seed irradiated hybrids
SSC	Sodium Citrate
SSCP	Single strand conformation polymorphism
SSR	Simple Sequence Repeats
SSRs	Simple Sequence Repeats
STMS	Sequence tagged microsatellite site
STS	Sequence Tagged Sight
TAE	Tris Acetate
TBE	Tris Borate
TE	Tris EDTA
TEMED	Tetramethylene diamine
T_m	Melting Temperature
U	Units
v/v	Volume/Volume
w/v	Weight/Volume
WHO	World health organisation
ZIP	Zinc regulated- iron regulated transporter proteins

Table of Contents

S. No.	LIST OF TABLES	Page No.

S. No.	LIST OF FIGURES	Page No.

1. INTRODUCTION

Over two billion people of the world are suffering from deficiency of important micronutrients like Fe and Zn leading to malnutrition (Poletti *et al.*, 2004; Welch and Graham, 2004; Bouis, 2007). Their dietary deficiency known as hidden hunger has serious implications on human health especially in the developing countries (Demment *et al.*, 2003; Holtz and Brown, 2004; Gitlin, 2006; Bhaskaram, 2008). The most common consequences of low intake of Fe 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 (Barbin *et al.*, 2001, Stein, 2005). Zn is also an important element of human diet and acts as cofactor of various important enzymes.

In the 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) whereas vegetables, fruits, animal and fish products with high mineral content form negligible proportion. All these staple food crops have very low content of these micronutrients. For better Zn or Fe nutrition for human beings, cereal grains should contain around 40-60 mg/kg Zn or Fe but in the present diet available amount is in the range of 10-30 mg/kg (Cakmak *et al.*, 2000). Dietary diversification and other nutritional interventions like supplementation, fortification and biofortification are some of the major approaches which are suggested for the alleviation of micronutrient malnutrition (Zimmerman and Hurrel, 2007). Among the various interventions to improve nutritional status of deprived human beings, biofortification of the crops is the most promising, widely accepted, cost-effective and easily affordable (Zimmerman and Hurrel, 2002; Lonnerdal, 2003). Micronutrient enriched cereals involving higher efficiency of uptake and translocation of the micronutrients to the grains is the first and foremost requirement for biofortification of cereal crops. Dicotyledonous plants adopt reduction based strategy-I, Fe^{3+} is reduced to Fe^{2+} by proton excretion into the rhizosphere (Olsen, 1981). Graminaceous plants including wheat utilize strategy –II, to solubilize soil Fe by secreting Fe(III) chelators, called mugenic acid (MA) family phytosiderophores (Marschner *et al.*, 1986; Ueno *et al.*, 2009).

Wheat is the main sources of carbohydrates in human diet all over the world. The three major cereal crops which account for more than 85% of all grain production worldwide and more than half of all the food calories are wheat, rice and maize (http://faostat.fao.org/site/567/default.aspx#ancor). Cultivated wheat genotypes have very low Fe and Zn contents 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 Fe and Zn content than the related wild

Triticum and *Aegilops* species (Cakmak *et al.*, 2000; Monasterio and Graham 2000; Chhuneja *et al*., 2006; Rawat *et al*., 2008). The related non-progenitor wild species with S, U and M genomes have up to 3–4 fold higher grain Fe and Zn content as compared to bread and durum wheat cultivars (Rawat *et al*., 2009). QTLs for Fe and Zn were mapped on 2A and 7A chromosomes of *T. monococcum* (Tiwari *et al*., 2009) Substitution and addition derivatives of 2S and 7U of *Ae. kotschyi* and group 4S and 7S chromosomes of *Ae. peregrina* had high grain Fe and Zn concentration (Kumari *et al*., 2011; Tiwari *et al.*, 2010).

Precise transfer of useful variability from the alien chromosomes can be done by induced homoeologous pairing and recombination through absence of 5B chromosome, *ph1b* mutation and Ph^I of *Ae. Speltoides*. Disomic-5D nullisomic-5B (5D(5B)-substitution-disomic) line of durum wheat (*Triticum turgidum* L.) was used for homoeologous pairing (Joppa and Williams, 1988). The *ph1b* mutation was also used for transfer of stem rust resistance gene (*Sr39* (Yu *et al*., 2010). Radiation induced transfer is a recent approach which does not rely on meiotic recombination and can be used for fine transfer of alien genes into wheat. It has been used for transferring a leaf rust resistance gene (*Lr9*) from *Ae. umbellulata* Zhuk to wheat (Sears, 1956) and powdery mildew resistance *Pm21* locus transferred from *Haynaldia villosa* to wheat using female gamet irradiation induced transfer (Chen *et al.*, 2012).

Germplasm of related wild *Aegilops* species 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 (Schneider *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 (Friebe *et al.*, 1996; Marais *et al.*, 2005; McIntosh *et al.*, 2005). Various cytological and molecular techniques have been used to analyse the alien chromomatin introgressed from wild germplasm to wheat. Among these, molecular markers are the most powerful diagnostic tools to detect DNA parental polymorphism. These are often associated with specific genes or specific chromosomes and act as "signposts" to those genes and chromosomes. SSR markers were used extensively for crop improvement in addition to RFLP, AFLP, RAPD, SSR, EST, SNP and DArT. Further cytology is an evergreen technique to study chromosomal compliment. Standard C-banding karyotypes of many wild relatives of wheat have been developed and used for monitoring alien introgressions (Friebe, 1995a; 1995b). Genomic *in situ* hybridization (GISH) involves labelling total genomic DNA for using as a probe to identify alien chromosomes in a wheat background (Le *et al*., 1989; Heslop-Harrison *et al*., 1992). Addition and substitution lines of 2S, 4S, 7S and 7U of *Ae. kotschyi* and *Ae. peregrina* for high grain Fe and Zn contents were screened by using GISH (Tiwari *et al*., 2011; Kumari *et al*., 2011).

The present study aimed at precise transfer of genes controlling high grain Fe and Zn from wheat-*Aegilops* alien addition and substitution derivatives was undertaken with the following objectives:-

- Identification of group 7 *Aegilops* chromosome introgression lines with high grain Fe and Zn content using molecular and cytological techniques.
- Induced homoeologous pairing between group 7 *Aegilops* and wheat chromosomes for precise transfer of useful variability.
- Radiation induced transfer of genes for high grain Fe and Zn content.
- Biochemical analysis of introgressed derivatives with high grain Fe and Zn content.
- Molecular mapping and tagging of introgressed *Aegilops* genes for high grain Fe and Zn content.

Table of Contents

2. REVIEW OF LITERATURE

2.1 Wheat an important cereal crop

Wheat is the main source of carbohydrates in human diet all over the world. The three major cereal crops wheat, rice and maize account for more than 85% of total grain production worldwide and more than half of the total food calories (http://faostat.fao.org/site/567/default.aspx#ancor). Hexaploid wheat (*Triticum aestivum* L.) is the second most important crop after maize of the world and is the single largest traded crop having global annual production exceeding 700 million tonnes from a cultivated area of 215.26 million hectare. India is the second largest wheat producer after china in the world. Hexaploid wheat (bread wheat or common wheat) is generally used for making bread, cookies, pastries and noodles, whereas durum wheat is used for making pasta and semolina products.

The growing season for *Triticum aestivum* and *T. durum* is from November to April. Various studies and researches show that wheat and wheat production play an important role in the management of India's economy. The world wheat production was about 702 million tonnes in year 2013, 93.6 million tones of which was produced in India (FAO, 2013).

2.2 The wheat plant

Wheat (*Triticum* spp.) is a member of the grass (Poaceae) family and one of the first cereals known to have been domesticated. According to archaeological findings, wheat first occurred in the region known as the Fertile Crescent and the Nile Delta. The Fertile Crescent region is often considered the cradle of civilization as it saw the development of many of the earliest human civilizations, and is one of the earliest sites to use both written language and the wheel. The modern-day countries with significant territory within the Fertile Crescent are Iraq, Israel, Jordan, Lebanon, Palestine and Syria, besides the southeastern fringe of Turkey and the western fringe of Iran (Belderok, 2000). The wheat plant has long, slender leaves, stems that are hollow in most varieties, and heads composed of varying numbers of florets, ranging from 20 to 100. The florets are grouped together in spikelets, each having two to six florets. In most spikelets, two or three of the flowers are fertilized, producing grains. Of the numerous varieties of wheat known, the most important is *Triticum aestivum*, is used to make bread; *T. durum*, used in making pasta (alimentary pastes) such as spaghetti and macaroni; and *T. compactum* or club wheat, a softer type, used for making cake, crackers, cookies, pastries, and family flours. The major cultivated species of wheat are summarized in table 2.1. Within a species, wheat cultivars are further classified by growing season, such as winter wheat vs.

spring wheat, by gluten content, such as hard wheat (high protein content) vs. soft wheat (high starch content) or by grain colour (red, white or amber).

2.3 Wheat evolutionary history

The evolution of bread wheat occurred about place 0.008-0.01 millions of years ago. The hexaploid wheat, *Triticum aestivum* (2n=6x=42) has three different genomes designated as A, B and D. Evolution of allohexaploid wheat involved two separate natural amphiploidization events. Approximately 0.5 million years ago, two wild diploid species crossed in nature and by spontaneous chromosome doubling, leads to evolution of wild tetraploid species *Triticum turgidum* ssp. *dicoccoides* (BBAA) also known as wild emmer wheat. The A genome donor of common wheat was the wild diploid species *Triticum urartu*, (Dvörak *et al*., 1993), while *Aegilops speltoides* (SS) is considered as the potential B genome donor of common wheat (Maestra and Naranjo, 1998). Bread wheat (*Triticum aestivum* L.) arose 0.008-0.01 millions of years ago (Feldman, 1995) from the spontaneous hybridization of the tetraploid wheat *T. turgidum* L. (2n= 4x= 28, BBAA) with diploid goat grass *Triticum tauschii* Coss. (2n= 2x= 14, DD) (Kihara, 1944; Huang *et al.*, 2002; Jauhar, 2007)

2.4 Biology of Wheat Plant

Wheat is broadly categorised into two groups; wild wheat and cultivated wheat. Wild wheat are commonly divided into two sub-groups; goat grasses belonging to the genus *Aegilops* (goat grass I; *Aegilops speltoides*, goat grass II; *A. tauschii, A. squarrosa*) and wild wheat of the genus *Triticum* (*Triticum urartu,T monococcum* and *T.boeoticum*). Wild species of *Aegilops* are found as diploid (2x), tetraploid (4x) and hexaploid (6x) whereas that of *Triticum* are found as diploid and tetraploid based on the basic number of chromosomes (N=7). Cultivated wheat are included in the genus *Triticum* and exists in three ploidy levels, diploid (einkorn wheat), tetraploid (emmer wheat) and hexaploid (bread/spelt wheat). Emmer wheat may have been domesticated earlier than einkorn wheat was domesticated.

The predominant type of wheat cultivated throughout Europe and Mediterranean for thousands of years before durum wheat (Ozkan *et al.*, 2005). The evolution of polyploid wheat was the formation of modern hexaploid breadwheat (*Triticum aestivum*), in the region of Transcaucasia, as a consequence of several spontaneous hybridizations between a cultivated form of the tetraploid emmer wheat (AABB) and one of the wild goat grass II with a DD genome. Therefore, breadwheat has three genomes A, B and D of three original parental diploid species (Belderok, 2000; Gustafson *et al.*,2009) resulting in large genome size of 17 Gb (Pennisi, 2008). The reason for calling *T. aestivum* as ‘breadwheat’ is based on the

qualities such as light, easily chewable and readily digestible products which were not provided by any other cereal. The diverse environmental conditions and food habits in India support the cultivation of three species of wheat *viz. T. aestivum, T. durum* and *T. diococcum*. Among these *T. aestivum* contributes approximately 95% to the total production while rest comes from *T. durum* (4%) and *T. diococcum* (1%). Genome organization of grasses can be studied by using *Lolium perenne/Festuca pratensis* hybrids. This system can also be usefull for studing genetic control and marker association of the trait. (King *et al.*, 2007). The genome consititution of different species of *Triticum and Aegilops* is sumrized in table 2.1 where as the genome size of cereals and related non progenitor is given in table 2.2.

2.5 Micronutrient malnutrition in human

Worldwide three billion people are suffering from deficiency of key micronutrient like Fe and Zn (Stoltzfus , 2003; Poletti *et al.*, 2004; Welch and Graham, 2004; Bouis, 2007; Pfeiffer *et al.*, 2007) leading to malnutrition, also known as hidden hunger. Recent reports showed prevalence of hidden hunger in the developing countries have serious implications on human health (Demment *et al.*, 2003; Holtz and Brown, 2004; Bhaskaram, 2008; Ramakrishnan *et al.*, 2009). According to FAO/WHO, 2001 reports over 30 % of the world population was severely affected by Fe deficiency, and mostly found in 47.4% of preschool children, 41.8 % pregnant women and 30.2% non pregnant women (In south Asia and Africa a high number of maternal deaths were reported due to Fe deficiency anemia (Monasterio *et al.*, 2007, Stoltzfus, 2004). Severing of micronutrient deficiency in India is evident from, prevalence of anemia which was found to be 70-80% in children, 70 % in pregnant women and 24% in adult men (WHO, 2007).

Table-2.1 The genome constitutions of different species of *Triticum and Aegilops*

Species	Genomic constitution
Cultivated	
Triticum aestivum L. (Bread wheat)	ABD
Triticum turgidum L. (Pasta wheat)	AB
Triticum monococcum (Einkorn wheat)	A^m
Triticum zhukovskyi Menabde & Ericz.	A^tA^mG
Triticum timopheevii (Zhuk.) Zhuk. (cultivated form)	A^tG
Wild	
Triticum urartu ex Gamdilyan (wild form)	A^u
Aegilops speltoides Tausch	S
Aegilops longissima Schweinf. & Muschl.	S^1
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 caudata 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.	$D^{c1}M^c(D^{c1}X^C)$
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)
Aegilops geniculata Roth (syn. *Ae. ovata)*	UM (UM^0)
Aegilops biuncialis Vis.	UM (UM^0)
Aegilops kotschyi Boiss.	US (US^1)
Aegilops peregrina (Hack. in J. Fraser) Maire & Weiller (syn. *Ae. variabilis*)	US (US^1)

Source: Gill and Friebe, 2002, FAO Document repository

Table-2.2 The genome size of cereal and progenitor species

Hordeum vulgaris 2n=2x =14	5,550Mb
Secale cereale (rye) 2n=2x =14	8,280Mb
Triticum monoccocum 2n=2x =14	6,230Mb
Triticum urartu 2n=2x =14	4,940Mb
Aegilops tauschii 2n= 2x =14	5,010Mb
Ae. speltoides 2n=2x =14	5,800Mb
T. durum 2n=4x =28	12,030Mb
T. aestivum 2n=6x =42	17,330Mb

Zn is also an important element of human diet. It is a cofactor of various important enzymes. According to WHO reports on the major risk factor causing disease burden on humans, Zn deficiency ranks 11th among the 20 most important risk factors in the world and

5th among the 10 most important risk factors in developing countries (Cakmak, 2008). It causes serious health implications such as impairment of physical growth, immune system learning ability, cancer development and other adverse effect during pregnancy. Approximately 800,000 child deaths are reported worldwide due to Zn deficiency (Micronutrient Initiative, 2006).

2.5.1 Role of Fe in human

Fe is an essential element for most of the living organisms and present in all cell types of an organism. It is an important component of proteins involved in oxygen transport and metabolism. Haemoglobin (Hb) contains almost two-thirds of the total Fe in human body. Hb is present in red blood cells (RBCs) that carries oxygen to different body part. Comparatively smaller amounts of Fe are found in myoglobin (Mb) protein that helps muscle cells to store oxygen and in enzymes that assist biochemical reactions in different cells (Beard, 2001). Fe is also involved in electron transport chain and essential for ATP production, therefore without Fe energy currency cannot be properly synthesized. Additionally Fe also helps to increase resistance to stress and wide range of diseases. It is present in many foods and vegetables that after consumption absorbed into the body through stomach and stored in liver, spleen and bone-marrow. About 15% of body's Fe is stored for future needs and mobilized under inadequate diets (Beard, 2001). Fe also contributes significantly in functioning of immune system. Adjacently essential for physical and mental growth specifically during childhood and pregnancy where the development of foetus solely depends on maternal Fe supplies. It was observed that Fe supplementation in deficient mother improves the pregnancy outcome (Scholl, 2005). Fe is lost from the body through a various ways such as urination, defecation, sweating and exfoliating of old skin cells. Bleeding is the major cause of Fe loss and in women due to monthly periods its percentage is comparatively higher than men, therefore women's Fe demand is higher than men. Under Fe deficiency, normal Hb production get restricted, transport of oxygen is diminished, thus leads to symptoms such as fatigue, dizziness and weak immunity (Beard, 2001). Consumption of food deficient in Fe for prolonged duration can lead to Fe deficiency anemia (IDA). The genetic disorder called hemochromatosis affects regulation of Fe absorption within body, thus resulted in accumulation of high Fe content in body than normal. Its treatment comprised of low-Fe diet, no Fe supplements and phlebotomy (blood removal) on a regular basis (Kirking, 1991). The amount of Fe requrement in each person vary depending on their age, gender and body storage therefore, dietary requirement of Fe is different for different age groups and sex. Fe found in food in two different forms: haeme Fe and non-haeme Fe. Haeme Fe, generally found in fish, meat and poultry and are best sources for increasing or maintaining healthy Fe levels. Non-

haeme Fe: commonly found in fruits, vegetables, nuts and grains products. Non-haeme Fe is not easily absorbed compared to haeme Fe. Some commonly used food material include: Eggs, dried beans, breads, spinach, broccoli, mustard greens, kale, radish and dried fruits (Kim *et al.*, 2007).

2.5.2 Role of Zn in human

Zn is an essential trace element for all organisms. It is required for many important biological functions and has crucial role in functioning of more than 300 enzymes involved in synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids along with the metabolism of other micronutrients. Furthermore, Zn has an essential role in transcription of mRNA or in gene expression (Hambidge, 1987). Its importance mostly accounted during pregnancy, for the growth of foetus whose cells are rapidly dividing as well as to avoid congenital abnormalities and pre-term delivery. It also play vital role in height, weight and bone development in infants, children and teenagers, thus contribute to active growth (Shankar and Prasad, 1998). Zn has central role in the immune system, affecting number of aspects with respect to cellular and humoral immunity (Shankar and Prasad, 1998). Zn deficiency causes growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system and the appearance of behavioral changes (http://www.fao.org). A study of 25 intervention trials comprising 1834 children under 13 years of age, with a mean duration of approximately 7 months and a mean dose of Zn 14 mg/day (214 mM/day), showed a small but significant positive effect of Zn supplementation on height and weight increases (Brown *et al.*, 1998). Results suggested that a low Zn status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (Black, 1998).

About 30 mM Zn (2-3 gm) is present in an adult human body, 90% of which is present in muscles and bones. Concentration dependent absorption of Zn occurs throughout the small intestine. Zn when administered through aqueous solutions to fasting persons absorbed efficiently (60-70 percent) compared to absorption from solid diets. Among solid diet absorption of Zn varies with respect to Zn content of the diet and its composition (Sandstrom, 1997). Zn is lost from the body through kidneys, skin and intestine (King and Turnlund, 1989). Lean red meat, whole-grain cereals, pulses and legumes provide the highest concentrations of Zn 25-50 mg/kg (380-760 mmol/kg) of raw weight. Processed cereals with low extraction rates, polished rice and meat with high fat content have a moderate Zn content 10-25 mg/kg (150-380 mmol/kg) (Sandstrom, 1989). Earlier isotope studies revealed two factors which were major determinants of absorption and utilization of dietary Zn, the content of inositol hexaphosphate phytate and the dietary protein content. Phytates are present in large amount in

whole-grain cereals and legumes and reported to be anti nutritional because of strong potential for binding divalent cations (Sandstrom and Lonnerdal, 1989).

2.5.3 Role of Fe in plants

Fe is one of 16 essential elements required for plant growth, development and reproduction. It plays important role in cholorophyll development, plant respiration, plant metabolism and nitrogen fixation. Since it is constituent of certain enzymes and proteins involved in electron transport chain such as cytochromes with heme proteins, thus crucial for energy production in chloroplasts and mitochondria. Fe is also associated with certain non-heme proteins such as ferredoxin plays role in many oxidation reduction reactions within plants (Hochmuth, 2011). Most annual plants have a requirement for Fe on the order of 1-1.5 lb per acre, compared with nitrogen (N) at 80-200 lb per acre (Hochmuth, 2011). There are many factors which affect the Fe availability to plants, among them pH of soil is most important. High soil pH reduces Fe availability while an acidic soil increases the Fe availability. The high pH effect is increased in waterlogged, compact and poorly aerated soils. Organic matter and related compounds were able to form Fe complexes that improve availability.

Fe deficiency causes interveinal chlorosis of young leaves. However severe deficiencies may progressively affect the entire plant leading to symptoms where all the leaves changed colour from yellow to bleached-white. Excess Fe can result in dark green foliage, stunted growth of tops and roots, dark brown to purple leaves on some plants. Fe also play important role in cereal defence mechanisms. Monocot plants challenged by pathogenic fungi showed redistribution of cellular Fe to the apoplast in a controlled manner to activate both intracellular and extracellular defences (Greenshields *et al.*, 2007).

2.5.4 Role of Zn in plants

Zn is one of the essential micronutrient required for optimum plant growth however its deficiency causes adverse effect on growth and yield of crops. Zn is an important component of various enzymes which are responsible for driving many metabolic reactions in plants. The Zn deficiency can lead to > 40 % yield losses in crops (Alloway, 2007). Zn is involved in formation of chlorophyll and carbohydrate as it is present in several dehydrogenases, proteinase and peptidase enzymes. It promotes growth hormones (auxin) and starch formation, thus plays very important role in grain formation and availability of nutrition to endosperm (Alloway. 2007). Zn is involved in regulation of wide range of metabolic processes such as carbohydrate, lipid, protein and nucleic acid synthesis and degradation through large mosaic of Zn binding motifs (Auld, 2001). The ZIP (ZRT, IRT like Protein) and CDF (Cation Diffusion

Facilitator) families are important in Zn transport. The ZIP transporters were well characterized in Arabidopsis (Grotz *et al.,* 1998), soybean (Moreau *et al.,* 2002) and rice (Ramesh *et al.,* 2003; Ishimaru *et al.,* 2005). Studies based on Zn mobility in different plants revealed that Zn mobility in phloem was relatively high in case of wheat (Haslett *et al.,* 2001; Riesen and Feller, 2005). Zn had high phloem mobility from roots to leaves, stems and developing grain, and also from one root to another (Rengel, 2001). Loading of Zn into developing wheat grain occurs mostly through phloem, where the Zn in xylem transferred to phloem in the rachis and the peduncle of wheat (Pearson *et al.,* 1995).

Zn efficiency is defined in terms of ability of plants to maintain high yield in soils with low Zn availability. A number of mechanisms may be responsible for Zn efficiency (Rengel, 2001), therefore depending on experimental conditions and plant species, the most important mechanisms may be Zn uptake from roots (Genc *et al.,* 2006) and Zn utilisation in tissues (Hacisalihoglu and Kochian, 2003). Under Zn deficiency, Zn-efficient genotypes exhibited high activity of Cu/Zn-SOD (Cakmak *et al.,* 1997; Hacisalihoglu *et al.,* 2003 and 2004; Yu *et al.,* 1999) and carbonic anhydrase (Hacisalihoglu *et al.,* 2003; Rengel, 1995).

Under Zn deficiency plants failed to develop normally and certain characteristic deficiency symptoms were appeared. With corn, these symptoms usually appear in the first 2-3 weeks of the growing season. If the deficiency of Zn was severe, these symptoms may persist throughout the cropping season (Alloway, 2007). Zn deficiency in wheat and rice causes brown spots on the leaves of young plants leading to reduced photosynthesis, reduced plant growth and finally reduced yield of the crops.

2.6 Reasons for micronutrient malnutrition

In developing countries, major proportion of the daily calorie intake of poor people came from carbohydrate rich cereal such as rice, wheat and maize (FAO, 2004) however, vegetables, fruits, animal and fish products with high mineral content contribute negligible proportion. The micronutrient content in staple crops are very low and further reduced by various processing methods of crops such as milling and polishing during which nutrients rich layer (aleurone) get removed. Consequently diet based on only staple cereals is not sufficient to provide recommended dietary allowance (RDA). According to Impa *et al.* (2013), it was found that polished rice contains an average of only 2 mg Fe kg^{-1} and 12 mg Zn kg^{-1} of whereas RDA for Fe is 10-15 mg and 12-15 mg for Zn. For better Zn or Fe nutrition cereal grains should contain about 40-60 mg/kg Zn or Fe whereas at present scenario, available amount is in the range of 10-30 mg/kg (Cakmak *et al.,* 2000). Fe is the fourth most abundant mineral in earth crust and is not readily available to the plants since it present in complex form

of hydroxides, oxides and phosphates, hence its normal concentration in plants is only 0.005% (Welch and Graham, 2002; Meng *et al.,* 2005). Cakmak reported that nearly half of the world's cereal growing area was affected by Zn deficiency while one third is Fe deficient due to high soil pH (Mori, 1999). Inefficient uptake of these metals in calcareous or salt stressed alkaline soil resulted in severe yield loss and poor nutritional quality of grains (Brown, 1961; Cakmak, 2008).

In cereals, most of the important nutrients, reside in husk, aluerone layer and embryo. Major constrain in utilization of these stored micronutrients was the issue of bioavailability for human as mineral absorption was very low from plant food sources. Presence of anti nutritional factor such as phytic acid, polyphenols, fibers, certain tannins and haemagglutinins in plant based diets potentially reduces the absorption of micronutrients. The *myo*-inositol-(1, 2, 3, 4, 5, 6)-hexakisphosphate accounts for about 1% of the seed weight (Lott *et al.,* 2000; Cosgrove, 1996) and stores 50-80% of total seed phosphorus. Phytate ion has strong tendency to chelate metal cations in seeds since it has high negative charge density and leads to formation of stable metal salts (Brinch-Pederson, 2002). Monogastric animals (like humans, poultry, pigs and fish) unlike ruminants are unable to utilize phytic acid due to the absence of microbial flora in their gut capable of degrading phytic acid. Thus presence of phytic acid further aggravates micronutrient deficiency in human diet and animal feeds and acts as a strong antinutrient. It is considered to be the single most important anti-nutritional factor in food (Bouis, 2000). There are certain organic acids, heme-protein, some aminoacids, long chain fatty acids, β-carotene, that promotes Fe and Zn bioavailability (Graham *et al.,* 2001). Bioavailability of minerals also dependent on the available forms of micronutrients present in body such as Fe exist as Fe^{+3} ions within ferrites protein which is largely localized in leaves and in amyloplasts of seeds. Ferritin bound Fe has relatively high bioavailability. The profile of soil also play significant role in micronutrient availability to plants. If the soil is deficient in Fe and Zn, the crop grown on such soil also observed to be deficient of micronutrients. It was also observed that growth of wheat plants was effected negatively by concentration of Zn in soil and among wheat, durum wheat was affected mostly when grown on calcareous soil (Cakmak *et al.,* 1996)**.** Major area under Zn deficient soil was confined to India, Pakistan, Australia and China (Alloway, 2007). Another study revealed that in calcareous or salt stressed alkaline soil, plants showed 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. These observations may also be attributed to inefficient uptake of metals from such problematic soils, ultimately resulted in severe loss of yield and poor nutritional quality of grains (Brown, 1961; Cakmak, 2008). The micronutrient

content in grains depends on the uptake of micronutrients by roots during grain development followed by remobilization and redistribution to grain from vegetative tissue via phloem. The mobility of each element through phloem differs greatly. It has been found that Zn showed good remobilization; Fe had intermediate mobility whereas Cu and Mn have lower phloem mobility (Pearson and Rengel, 1995; Kochian, 1991). In wheat and rice, only 4-5% of the shoot Fe was translocated to the grain at maturity (Impa *et al.*, 2013).

2.7 Strategies for alleviating micronutrient malnutrition

Dietary diversification and other nutritional interventions like supplementation, fortification and biofortification are some of the major approaches which were suggested for the alleviation of micronutrient malnutrition (Zimmerman and Hurrel, 2007). Supplementation refers to additional supply of Fe, Zn and other micronutrients in the form of capsules and tablets. Individuals with chronic deficiency of Fe could be given Fe in the form of Fe^{+2} Fe salts *i.e.* ferrous fumarate, ferrous sulphate and ferrous gluconate since these were reported to be the best absorbed forms (Hoffmann, 2000). Likewise Zn could be provided as Zn gluconate, Zn sulphate and Zn acetate. Fortification of foods involves addition of minerals to the food materials. Various examples of fortification are iodination of salt or fluro fortification of toothpaste, fortification of flour with Zn oxide (20-50 mg/kg) and copper gluconate (1.0–3.0 mg/ kg) (Rosado unpublished results, 2000). These methods have reported several difficulties such as fortified foods with high Fe are very sensitive to the oxidation process and leads to increased loss of iodine. Similarly fortified rice with vitamin foliate reported to lost during boiling due to higher solubility. Field spray of micronutrients was also reported to be not feasible method due to deployment of sophisticated techniques and high recurring costs (Cakmak, 2002). Although consumption of diversified diet including meat, fish, fruits, vegetables, legumes were sustainable approach however change of dietary practices and preferences were difficult and expensive. Moreover, such practices were impractical in developing countries where poverty prevails and over three billion people earn less than US$ 2 per day (Zhu *et al.*, 2007). Thus, the most effective strategy might be the use of biofortified cereals in food material. Consequently micronutrient enriched cereal grains involving higher efficiency of uptake and translocation of the micronutrients to the grains are the priority and foremost requirement for removing micronutrient deficiency through biofortification of cereal crops.

2.8 Metal uptake from soil

The uptake of minerals from soil, transport within plant and finally deposition to the edible part was the mechanism responsible for high grain Fe and Zn content. Despite high Fe and Zn in soils, plants were not able to readily used these as these were present in complex Fe^{+3} and Zn^{+3} salts form. Fe was present exclusively in Fe^{+3}oxidized form in soil having very low solubility in water, affected by both pH and oxygen. Plants require approximately 10^{-8} M Fe, but in calcareous or high pH soils total soluble Fe was below 10^{-10} M. Consequently without active mechanisms for extracting and uptaking Fe from soil, most plants exhibited iron-deficiency symptoms, such as leaf interveinal chlorosis (Kim and Geurinot, 2007). Similarly very limited free Zn^{2+} ions occur in soils, again specific uptake strategies were required for absorbing Zn from soil (Haydon and Cobbett, 2007; Palmgren *et al.,* 2008). Zn concentration varied in different tissue of wheat seed, Zn concentration in endosperm tissue cannot be increased by altering or increasing the external supply of the element but considerable amount of Zn could be increased in other tissue of seed such as aleurone layer (Stomph *et al.,* 2011).

Plants have developed sophisticated and strongly regulated mechanisms for acquiring metals from soil, which can be grouped into two strategies (I and II). Dicotyledonous plants adopted reduction based strategy-I whereas monocots (grasses) adopted chelation based strategy-II for uptake of metal ions under deficiency conditions (Romheld and Marschner, 1986; Kim and Geurinot, 2007).

In strategy-I, non-graminaceous plants under deficiency condition directed for enhanced excretion of protons from roots to surrounding rhizosphere that resulted in lowering of soil pH, thus at low pH Fe^{+3} was reduced to more soluble Fe^{+2} form at the root surface. Fe^{3+} was reported to be 1000 times more soluble when reduced to Fe^{2+} (Garrido *et al.,* 2006). Many genes of strategy I have been investigated identified and isolated for understanding of the molecular mechanism of the uptake of metals. Three ferric- chelate reductase genes *AtFRO2*, *PsFRO1*, *Le FRO1* have been isolated from *Arabidopsis*, pea and tomato, respectively (Robinson *et al.,* 1999; Li L *et al.,* 2004; Waters *et al.,* 2008). Transgenic rice with induced expression of ferric chelate activity showed 7.9 fold increase in Fe uptake ability in calcareous soil (Kim and Guerinot, 2007; Ishimaru *et al.,* 2007). Similarly, transgenic soyabean with tenfold higher heterologus expression of *Arabidopsis* ferric chelate rductase activity showed increased tolerance to chlorosis, increased chlorophyll concentration and higher Fe content in shoots (Vanconcelos *et al.,* 2006). Fe^{2+} is transported into the root by metal transporters of the ZIP (Zn regulated- Fe regulated transporter Proteins) family. Fe regulated transporter 1 and 2 (IRT1 and IRT2) were the representatives of this family and were

located in the plasma membrane of epidermal of roots. IRT1 can transport many divalent metals such as Fe, Zn, Mn and Cd (Curie and Briat, 2003). Ishimaru *et al.* (2007) over expressed *OsZIP4,* Zn transporter in rice and found 10 times higher concentration of Zn in the roots of transgenic rice. Ramesh *et al.* (2004) reported that overexpression of ZIP1, an *Arabidopsis* Zn transporter, leads to 2-folds higher Zn concentrations in seeds and shoots of transgenic wheat. Many (IRTs) from the Zn and Fe transporter family (ZIP) have been isolated in various plants such as *AtIRT1, AtIRT2* from *Arabidopsis thaliana* (Vert and curie, 2001), *LeIRT1, LeIRT2* from tomato (Eckhardt *et al.,* 2001) and *PsITR1* from pea (Cohen *et al.,* 1998) Transgenic tomato with over expression of *IRT1* could accumulate more cadmium and Zn than wild type under Fe starved condition (Connolly *et al.,* 2000). The *Arabidopsis irt1* mutants exhibit severe chlorosis and impaired growth (Henriques *et al.,* 2002; Vert *et al.,* 2002) indicating the role of *IRT* family protein in uptake of metals. Another class of metal transporters encoded by natural resistance associated macrophase proteins (*NRAMP*) family transporters were also present in various plants, animals, fungi and are found to be involved in transport of divalent cations (Hall and Williams, 2003). They also reported to facilitate mobilization of vacuolar Fe for seed germination on low Fe (Lanquar *et al.,* 2005).

In strategy–II, graminaceous plants solubilize soil Fe by secreting Fe^{+3} chelators, called Mugenic acid family phytosiderophores (MA) (Marschner, *et al.,* 1986; Ueno *et al.,* 2009). The resulting Fe^{+3}-MA complexes were reabsorbed into the roots through a specific transporter. Under Fe and Zn deficiency, production and secretion of mugineic acids increased significantly in wheat, rice, maize, sorghum and other graminaceous plants and was well correlated with the tolerance ability of plants to Fe deficiency chlorosis and necrosis (Brown and Jolley, 1989; **C**akmak *et al.,* 1994; Curie *et al.,* 2001). Mugineic acid family phytosiderophores include 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 Fe, Zn and other micronutrients has been reported by various workers (Zhang *et al.,* 1989; Mori *et al.,* 1991; Kanazawa *et al.,* 1994). Biosynthesis of mugineic acid involves trimerization of three molecules of S-adenosylmethionine molecules to nicotinamine by the enzyme nicotinamine synthase (NAS) which later 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 was the first MA synthesized. Subsequent hydroxylation of DMA produced other forms of MAs depending on the plant species (Bashir *et al.,* 2006). Two barley cDNA clones particularly expressed in Fe 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.,* 2001), 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 was 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. Similarly, transgenic rice with either *NAAT-A* or *NAAT-B* genes from barley showed 1.8 times higher production of DMA under Fe limiting conditions over non-transformants (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. Transgenic plants with NAAT showed remarkable tolerance to Fe deficiency in calcareous soil and produced higher shoot dry weight. Transgenic lines with IDS3 inserts secreted MAs in addition to DMA which further contributed to enhance Fe availability in calcareous soil. Masuda *et al.* (2008) also reported increase in grain Fe by 1.40 times and Zn concentration by 1.35 times in transgenic lines of rice with IDS3 inserts. Nearly two times increase in Fe and Zn concentration has also been found in transgenic tobacco (*Nicotiana tabacum*) with higher expression of HvNAS1 genes (Takahasi *et al.,* 2003). After the chelation of Fe^{3+} by phytosiderophores (PS), the metal-PS complex was taken up by the *YSL1* (Yellow-Stripe 1) transporters located in the plasma membrane of root cells (Roberts *et al.,* 2004). *YSL1* was the first transporter of a metal ion-ligand identified in plants (Curie *et al.,* 2001). The *ysl1* maize mutants were defective in uptake of Fe-PS leading to interveinal necrosis (Curie *et al.,* 2001) depicting their role in transport of minerals.

Qualitative and quantitative differences in MAs production has been observed among graminaceous plants. Rice, wheat and maize secreted only 2-deoxymugineic acid (DMA) in relatively low amounts, thus reported susceptible to low Fe availability. In contrast, barley secreted large amounts of different types of MAs, including MA, 3-hydroxymugineic acid and 3-epi-hydroxymugineic acid therefore reported more tolerant to low Fe availability (Singh *et al.,* 1993). After reaching the root cells, 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 were specific transporters

of Zn and Cd whereas for Fe or Mn, YSL2 and AtIREG1 were suggested transporters (Kim and Geurinot, 2007; Colangelo and Geurinot, 2006). Long distance transport through the xylem sap having pH 5.5-6 involved chelation of metal ions with highly mobile ligands of low molecular weight e.g. Fe was transported as Fe (III)-citrate complexes in the xylem to aerial parts over long distances (Hell and Stephan, 2003).

Table-2.3 Metal transporters proteins, their cellular localization and tissue of their expression.

Proteins	Tissue expression	Cellular localization	Inducing conditions	Proposed/ known substrates	Reference(s)
(A) Metal efflux proteins					
P1B-ATPase					
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					
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					
ZmYSL1	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					
AtNRAMP3/4	Root, shoot, seed	Vacuolar Membrane		Fe	Lanquar *et al.*,.2005
TjNRAMP4		Plasma membrane		Ni	Mizuno *et al.*, 2005
ZIP					
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					
AtCOPT1	Root, pollen, embryo, stomata, trichome		Downregulated by Cu	Cu	Sancenon *et al.*, 2004

From xylem vessels, micronutrients undergo active transport to the leaf mesophyll tissue using metal transporters of the parenchyma cells where further movement followed symplastic pattern within the leaf cells. Later the transportation of micronutrients to the developing grains occured either by direct upake from soil, or from remobilization of stored minerals in the senescing leaves (Uauy *et al.,* 2006). YSL transporters were suggested to play role in this transport (Waters and Grusak, 2008). Metal transporters proteins, their cellular localization and tissue of their expression are given in table 2.3.

2.9 Biofortification

Among the various interventions to improve nutritional status of deprived human beings, biofortification of the crops is the most promising, widely accepted, cost-effective and easily affordable method (Zimmerman and Hurrel, 2002; Lonnerdal, 2003). Biofortification refers to the process of developing genetically improved food crops that were rich in bioavailable micronutrients, either through conventional breeding or genetic modification (Johns and Eyzaguirre, 2007). Various micronutrient initiative programmes are running worldwide. HarvestPlus had started biofortification challenge programme with the objective of improving nutritional status in staple food crops with Zn, Fe and vitamin A by using plant breeding strategy (Lucca *et al.,* 2006; Pfeiffer and McClafferty, 2007). During the first phase, priorty was given to rice, wheat, maize, sweet potato, cassava and beans while in the second phase potato, barley, cowpeas, groundnuts, lentils, millets, plaintains, sorghum, pigeon peas and yams will be targeted. The biofortification of cereals achieved through combined techniques of conventional breeding, molecular breeding and genetic engineering ((Bouis, 1999; DellaPena, 1999; Nestel *et al.,* 2006; Hirschi, 2008). Simplest method of fortification 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, in plant and its accumulation site. Two other approaches involved genetic engineering and conventional molecular breeding methods for nutritional enhancement of cereals.

2.9.1 Genetic engineering for biofortification of cereals

Various transgenic strategies for nutritional fortification of cereals included alteration in metabolic pathway for either increasing the amount of desirable compound, decreasing the amount of competitive compounds or extension of the biosynthetic pathway for the production of novel product (Capell and Christou, 2004). It also involved expression of recombinant proteins that make minerals to be stored in trivalent form such as ferritin. This is an Fe storage protein consisting of 24 subunit shell around a 4500-atom Fe core (Theil, 2004). Ferritin

resisted the denaturation during gastrointestinal digestion and also protected it from chelators during digestion, thus enhances Fe absorption. 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 Fe level than wild type has been reported (Goto *et al.,* 1999; Vasconcelos *et al.,* 2003). In another study use of constitutive promoter resulted in elevated Fe level in the leaves of transgenic rice and wheat plants (Drakakaki, *et al.,* 2000). Six fold Fe and 1.6 fold Zn content was increased by transgenic approach for over expression of the Fe storage protein ferritin *soyferH2*, overexpression of *HvNAS1* for the over production of the natural metal chelator nicotianamine, and iron(II)-nicotianamine transporter *OsYSL2* under the control of an endosperm-specific promoter and sucrose transporter promoter (Masuda *et al.,* 2012). Two to Six fold increase in Fe content of endosperm of rice seed were observed in genetically transformed rice where nicotianamine synthase genes (NAS) and ferritin genes were expressed independently or in conjugation for this increase(Johnson *et al.,* 2011).

Another aspect of biofortification and alleviation of malnutrition is bioavailability. Even after achieving higher micronutrient content in edible tissue, how much of these get absorbed by human gut. It was found that phytic acid present in food chelates metal cation's such as Fe^{2+}, Zn^{2+} and Ca^{2+} and forms phytin, thus reduces their absorption in the human gut and acts as an antinutrtional factor (Raboy, 2001). Reduction in phytic acid could be achieved through development of low phytic acid mutants and development of thermostable phytase enzyme for solublizing phytic acid. Low phytic acid mutants have been identified in rice (Liu *et al.,* 2007), barley (Larson, 1998), maize (Pilu, 2003; Shi *et al.,* 2007), wheat (Guttieri, 2004) and soyabean (Wilcox *et al.,* 2000; Yuan, 2007). Expression of phytase and reduction of phytate biosynthesis increased the bioavailability of Fe and Zn in cereals grain (Brinch-pederson *et al.,* 2007). Nearly 55 to 60 % reduction in phytic acid phosphorus was reported 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). Stable transgenics may be used for hybrid production in maize, rice with improve phosphorus availability. In rice, the gene controlling MIPS was under the control of *RINO1* gene expressed in developing rice seeds specifically in aleurone and embryo. Using antisense *RINO1* technology, transgenic rice with 68% lower phytic acid with normal seed weight, germination and plant growth had been produced (Kuwano *et al.,* 2008).

Production of transgenic seeds with higher phytase activity might also resulted in enhanced minerals absorption. Maize seeds expressing phyA2 gene showed 2,200 units of phytase activity per kg seeds which was nearly 50 fold increase over non-transgenic maize

(Chen *et al.,* 2008). Transgenic crops containing phytase genes from various *Aspergillus* species have been produced in tobacco (Ullah *et al.,* 1999), soybean and alfalfa (Ullah *et al.,* 1999; Denbow *et al.,* 2000), wheat, rice and canola seeds (Brinch-Pedersen *et al.,* 2000; Zhang *et al.,* 2000; Lucca *et al.,* 2001; Ponstein *et al.,* 2002; Hong *et al.,* 2004). Differential gene expression, coding sequence and copy numbers resulted in postzygotic sterility **(**Walia *et al.,* 2009). 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 for biofortification programme. Other problem refers to various socio-economical and socio-political concerns related with the acceptance of transgenic crops by farmers and common people. Issue associated with licensing and intellectual property rights also creates troubles in popularization of these biofortified crops and ultimately they didn't reach to the neediest people.

2.9.2 Molecular breeding techniques

Molecular breeding is a very useful tool for crop improvement and shortening crop breeding program. It had been utilized for almost all crops. Cereals such as rice, wheat, maize and millets are very poor source of micronutrients. Among cereals, polished rice had lowest grain Fe content *i.e.* 5- 6 mg/ kg (Gregorio *et al.,* 2002). Wheat cultivars had very low Fe and Zn content in grains which largely distributed in embryos and the peripheral tissue of bran (Welch and Graham, 1999). In contrast, non progenitor wheat species had good genetic variability for Fe and Zn concentration, which ranged 2-3 fold higher than that of wheat cultivars (Cakmak *et al.,* 2000; Monasterio and Graham, 2000; Chhuneja *et al.,* 2006; Rawat *et al.,* 2008). Genetic variability for grain Fe and Zn concentrations was present in various wild relatives of rice (Lott *et al.,* 2004). The Fe concentration in brown rice samples ranged from 6.3-24.4 ppm with a mean value of 12.2 ppm where for Zn, the range was 13.5-58.4 ppm with a mean of 25.4 ppm. Some traditional varieties of rice such as Jalmagna, Zuchem, Xua Bue Nuo, Madhukar were reported to have twice the Fe and Zn content than that of elite cultivars. This variability was utilized for developing biofortified varieties through plant breeding. Bänziger and Long (2000), reported potential variability in white grained tropical maize germplasm having Fe and Zn concentrations 16.4 – 22.9 μg/g (mean 19.6 μg/g) and 14.7 – 24.0 μg/g (mean 19.8 μg/g) respectively.

Useful variability of *Ae. kotschii* and other non progenitor wheat was screened (Chhuneja *et al.,* 2006) and used for wheat biofortification for Fe and Zn by molecular breeding (Tiwari *et al.,* 2010). Addition and substitution derivatives of *Ae. kotschyi* group 1, 2 and 7 chromosomes had been developed for high grain micronutrients (Fe and Zn) by molecular breeding (Rawat *et al.,* 2011). Variability from *Ae. Peregrina* for high Fe and Zn

was utilized by Kumari *et al.,* 2011. Scientists at CIMMYT, Mexico have used synthetic hexaploid wheat from crosses between *T. durum* and *Ae. tauschii* with high Fe and Zn contents in breeding programmes and developed wheat lines with higher level of these micronutrients which were tested at agricultural fields in India, Pakistan and other countries (Calderini and Monasterio, 2003). However the level of enhancement of Fe and Zn using wheat synthetics has not been very impressive because of the limited variability for Fe and Zn in the progenitor wild parents. Therefore, screening of non-progenitor species for additional variability for micronutrients is required and considered to be very important.

2.10 Biofortification of wheat for Fe and Zn

Wheat (*Triticum* sp.) is the second major staple food crop of the world in terms of cultivated area and food source. According to FAO (2013), nearly 700 million tones human consumption of wheat is estimated in the year 2013/14. It alone contributes 28% of world's edible dry matter and up to 60 % of daily calorie intake in several developing countries (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. The related wild *Triticum* and *Aegilops* species with useful variability for high grain Fe and Zn content can be utilized for biofortification of wheat (Cakmak *et al.,* 2000; Chhuneja *et al.,* 2006; Rawat *et al.,* 2009). Amphiploids ($AABBS^lS^l$) of *Ae longissima* and *T. turgidum* were generated and had high Fe and Zn content (Tiwari *et al.,* 2008). QTL of Fe and Zn were localised on chromosome 2A and 7A (Tiwari *et al.,*2010). *Ae. kotschyi* possesses a distinctive genetic system for the micronutrient uptake, translocation and sequestration than wheat cultivars. Synthetic amphiploids between *Triticum aestivum* landrace Chinese Spring (Ph^I) and cultivar WL711 with different accessions of *Aegilops kotschyi* (UUSS) were developed through colchicine treatment of sterile hybrids (Rawat *et al.,*2009). The related non-progenitor wild species with S, U and M genomes have upto 3–4 folds higher grain Fe and Zn content as compared to bread and durum wheat (Rawat *et al.,* 2008). *Aegilops* species have 3-4 times higher release of PS than that of wheat cultivars under both nutrient-sufficient and -deficient conditions (Kumari *et al.,* 2011). Zn content of wheat-*Aegilops* addition lines was found to be ranging between the wheat and *Aegilops* Zn content (Schlegel *et al.,* 1998**).** Another study reported chromosome 2S and 7U addition and substitution derivatives of *Ae. kotschii 3790* had increased grain Fe and Zn concentration compared to elite wheat cultivar (Tiwari *et al.,* 2010). *Ae. peregrina* derived addition and substitution lines of group 4 and 7 chromosomes were developed and reported to have high grain Fe and Zn concentration (Kumari *et al.,* 2011). Additionally 5B, 6A and 6B chromosome

substitution lines had high grain Fe and Zn content compared to their recipient lines (Cakmak *et al.*, 2004). In wheat, Fe and Zn concentrations have positive correlation where the positive correlation was also observed for Zn with S, P, Fe and Na and Cu with K, Mg, Ni, P (Caballero, 2002). Durum wheat was fortified for Fe and Zn content using wild and synthetic parents (Cakmak *et al.*, 2010).

2.10.1 Use of mono 5B line

The 5B chromosome of wheat contain locus for Ph1 gene on long arm (5BL) that was reported to suppress homoeologous pairing (Holm, 1988). Both homologous and homoeologous chromosomes reported to pair randomly and formed multivalent in the absence of 5BL (Hobolth, 1981). Thus, 5B deficiency allows the pairing and recombination between the chromosomes of wheat and those of the related species and this may be a useful wheat breeding tool to introduce alien variation into wheat. The study based on hybrids between *Ae. peregrina* and chineese spring (CS) substitution lines showed that chromosome 5B in hybrids was replaced by either 5B of *Triticum turgidum* or 5G of *Triticum timopheevii* ssp. *timopheevii*.(Ozkan and Feldman, 2001). Homoeologous pairing was observed in mono5B wheat and the relative species where pairing was upto 37% in case of wheat-rye crosses and 50% in case of wheat-*Ae. columnaris* crosses (Lacadena, 1967). Joppa and Williams, 1988 developed lines of durum wheat in which B-genome chromosomes were replaced by their respective D-genome homoeologues. Disomic-5D nullisomic-5B (5D (5B)-substitution-disomic) line of durum wheat (*Triticum turgidum* L.) was used for homoeologous pairing. This line was fertile, vigorous, and had homoeologous pairing because of the absence of the *Ph* gene on chromosome 5B (Joppa and Williams, 1988). Homoeologous recombination was observed in triploids of *Festuca arundinacea* var. glaucescens (GGG′G′) and tetraploid *Lolium multiflorum* (*LmLmLmLm*) hybrids using GISH (Morgan *et al.*, 2001).

2.10.2 Use of *ph1b* mutant

The *ph1* gene of wheat allows pairing of homologous chromosomes but prevent homoeologous pairing. In wheat there were several options for manipulating the *ph1* gene for induced homoeologous recombination. One approch involved elimination of the *Ph1* gene either through use of nullisomy for 5B or using deletion mutants spanning the *Ph1* locus such as *ph1b* and *ph1c* (Sears, 1977; Giorgi, 1983). *Ph* ^{I} genes was another option, transfered from *T. speltoides* (syn *Ae. speltoides*) to *T. aestivum* (Chen *et al.*, 1994). Mutant for *ph* was developed by irradiating the pollen by X-ray and pollinating them on mono5B plants (Sear, 1977). The *ph1* gene was fine mapped relative to the breakpoints of various deletion and mutant lines (Gill *et al.*, 1993). Two mutant lines, *ph1b* and *ph1c* mutants were generated in

hexaploid wheat cultivar Chinese Spring (Sears, 1977), and in tetraploid wheat cultivar Cappelli (Jampates and DvoĺÁk, 1986) respectively for the *Ph1* gene. Both mutants resulted from interstitial deletions covering the *ph1*gene-containing regions of the chromosome (Gill *et al.,* 1993). The ph1 locus was flanked by the breakpoints of two deletions (5BL-1 and ph1c) and marked by a DNA probe (XksuS1). The *phb* deletion was linked to Xpsr128, Xpsr2120, Xpsr574 and Xksu75 probes. The deletion size in *ph1c* was about 0.89 mm and was smaller than that in *ph1b*, which is 1.05 mm in length (Gill *et al.,* 1993). The *ph1* gene was further localized to a much smaller region within the GRR (*Ph1* gene region). The ph1 region is syntenic to rice chromosome 9 and 7 (Sidhu *et al.,* 2008). Alien additions, substitutions, translocations, deletions, monosomes, ditelosomes and nullisomes of wheat were developed using *ph1b* line. The wheat *ph1b* mutation, which promotes meiotic pairing between homoeologous chromosomes, was employed to induce recombination between wheat chromosome 2B and goat grass 2S chromatin using a backcross scheme favorable for inducing and detecting the homoeologous recombinants with introgression of small goatgrass chromosome segments. (Niu *et al.,* 2011). Pairing was observed between non-homologous *Th. bessarabicum* chromosomes in presence of *ph1c* mutation (King *et al.,* 1993). Translocation lines of wheat were developed with stem rust resistance that have *Sr39* gene confering resistance to seven stem rust races (Yu *et al.,* 2010).

2.10.3 Radiation hybrid mapping and gene transfer

Hybrid sterility and lack of recombination between wheat and alien chromosomes are the major barriers in alien gene transfer in wheat. Different strategies have been used for transferring alien segments that were smaller than the complete chromosome arms. Radiation hybrid mapping was important physical mapping approach for plants and other organisms and can be used for fine gene transfer (Michalak *et al.,*2008). Gene transfer has been obtained in wheat by pollen irradiation (Snape et al. 1983). Radiation treatment was used to transfer leaf rust resistance gene *lr9* from *Ae. umbellulata* Zhuk. to wheat (Sears, 1956). X-ray irradiation at the dose of 2, 3 and 5 Krad was used for transfer of genes or chromosome fragments in wheat (Snape *et al.,* 1983). Gama rays at the rate of 10, 20, 30 and 40 Krad were used for mutagenesis of wheat for grain quality improvements and reduced plants height (Singh and Balyan, 2009). Radiation hybrid mapping was reported to be 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 point (Hossain *et al.,* 2004) and were able to locate an alien scs^{ae} gene of *Ae. longissima* in wheat.

A very high resolution physical map of wheat chromosome 3B has been recently generated using radiation hybrid mapping along with other approaches of mapping (Paux *et al.,* 2008). Pollen irradiated transfer of gene was more precise and depends on irradiation dose where higher the dose, smaller the fragment transferred. Powdery mildew resistance locus *Pm21* was transfered from *Haynaldia villosa* to wheat using female gametes irradiation induced transfer (Chen *et al.,* 2012).

2.11 Alien introgression for wheat improvement

Aegilops germplasm has been utilized extensively for the wheat improvement and various addition, substitution, translocation lines for different chromosomes of *Aegilops* species have already been reported by many workers (Schneider *et al.,* 2008). Several wild progenitor and non-progenitor species of wheat were used for development of alien addition, translocation and substitution lines for transfer of useful variability (Friebe *et al.,* 2000; Raupp *et al.,* 1995; Qi *et al.,* 2007). A number of genes for resistance against various wheat diseases have been introgresed into wheat from related progenitor and non- progenitor species (Friebe *et al.,* 1996; Marais *et al.,* 2005; McIntosh *et al.,* 2005) 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), and *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). Alien introgression of *Lr57/Yr40* from *Ae. geniculata* and *Lr58* from *Ae. triuncialis* to wheat was achieved without linkage drag.(Gill *et al.,*2008)

2.12 Assesment of different strategies of crop improvement

Various cytological and molecular techniques have been deployed to analyse the introgressed alien chromosome during chromatin transfers from wild germplasm to wheat.

2.12.1 Development and use of molecular markers

Molecular techniques are very useful in detecting differences in the DNA of individual plants and have many applications for crop improvement. Molecular markers are the most

powerful diagnostic tools to detect DNA polymorphism both at the level of specific loci and at the whole genome level. Molecular markers are often associated with specific genes or specific chromosomes and act as "signposts" to respective genes and chromosomes. Such markers can be linked to genes of interest and used to screen or select indirectly for the presence of desirable allele or trait in test samples and known as marker assisted selection (MAS). MAS was used to accelerate back-crossing of such allele and in pyramiding several desirable alleles (Rajpurohit *et al.*, 2011). EST and STS markers linked to tillering gene (*tin3*) were mapped in *Triticum monococcum* on 3AL chromosome(Kuraparthy *et al.*, 2008). EST based SSR markers were developed and used for transferability and polymorphic suvey *Triticum–Aegilops* (Bandhopandhyay *et al.*, 2004; Balyan *et al.*, 2005). Expressed sequence tags (ESTs) were also isolated analysed for environmental stress related genes (Zhang *et al.*, 2004)

Genetic diversity among different cultivars, within populations and among related species can be studied by using molecular markers. The applications of such evaluations include varietal fingerprinting for identification and protection, understanding relationships among the taxa under study, efficiently managing genetic resources, facilitating introgression of chromosomal segments from alien species, and tagging of specific genes. In addition, map-based cloning and gene isolation is based on markers and comparative mapping of various genes on different chromosomes. Previously DNA based markers were developed either based on DNA restriction digestion and hybridization, RFLPs and or based on PCR amplification of genomic DNA (RAPDs) or on both (AFLP). However recent addition of high throughput data on genomic DNA sequences and cDNA sequences (ESTs) in the public databases made marker development more direct and cost effective.

PCR-based markers includes Random Amplified Polymorphic DNA (RAPDs), AFLPs (amplified fragment length polymorphism), STSs (sequence tagged sites), SNPs (single nucleotide polymorphisms) and microsatellites.

In RAPD (Williams *et al.*, 1990) markers short (10 mer) random oligonucleotides as primers were used to amplify genomic DNA sequences. RAPDs inherited as dominant markers and show presence/absence polymorphisms. RAPDs lacks reproducibility and locus specificity restricted, so they were less useful in polyploid species like wheat.

AFLPs were DNA fragments (80-500 bp) obtained from restriction enzyme digestion, followed by ligation of oligonucleotide adapters to the fragments and selective PCR amplification of the ligated fragments, thus based on southern hybridization and PCR. AFLP markers were also scored as dominant markers. AFLP technique is very useful because of its higher degree of polymorphism and reproducibility, thus widely used in plant genetic mapping

(Vos *et al.,* 1995). Earlier genetic diversity studies in wheat and related species had been conducted using AFLPs (Heun *et al.,* 1997).

Simple sequence repeats (SSRs) or microsatellites markers were based on di-, tri-, or tetra-nucleotide repeats and DNA sequences flanking the repeats are used for designing forward and reverse primers for PCR amplification. The amplified product showed polymorphism due to variable number of repeats in different species, generated during evolution (Gupta *et al.,* 1996). SSRs can discriminate between homozygote and heterozygote i.e. attributed to its codominant characteristic and exhibited high locus specificity. Hence, these were used extensively to develop genetic maps in wheat (Röder *et al.,* 1998; Somers *et al.,* 2004; Singh *et al.,* 2007). Wheat anchor SSR markers were mapped on different chromosomes of wheat using W7984 × Opata 85 (ITMI*pop*) as mapping population (Gupta *et al.,* 2002). A QTL, *QGpc.ccsu-2D* for grain protein content was cosegregated with *wmc41* (Prasad *et al.,* 1999).

SSRs were used for detecting polymorphism in bread wheat and polymorphic information content was found 0.473 for the SSRs (Singh *et al.,* 2006). SSR Markers were further used for dissecting polygenic traits into their Mendelian components or quantitative trait loci (QTL) for high Fe and Zn content, thus increasing understanding of the inheritance and gene action for such traits (Tiwari *et al.,* 2009). A mutation encoding brittle culm was mapped on long arm of 5A chromosome in *T. monococum* using anchored SSR marker of A genome of wheat (Ansari *et al.,* 2012). Leaf rust resistance was found to be linked to gwm136 locus on chromosome of *T. monococcum* as confirmed by BSA and transferred to wheat (Kuraparthy *et al.,* 2001). SSR markers were used for genotyping and preparation of dendrogram of wheat (Prasad *et al.,* 2000, Routray *et al.,* 2007). Smut resistance was linked to *Xgwm234* and *Xgwm443* (SSR marker) and a SCAR marker (*Utd1*) on 5BS chromosome of durum wheat (Randhawa *et al.,* 2009).

Single Nucleotide Polymorphism (SNP) markers were based on single base differences within a given segment of DNA between any two individuals. These were created by point mutation. SNPs are identified by sequence alignments of the target sequence among different accessions of the plant material. Highly variable frequencies of SNPs were observed in European barley cultivars (Rostoks *et al.,* 2005). SNPs were ideal markers for identifying genes associated with complex diseases for two main reasons. Firstly, SNPs were densely located on the human genome at about one SNP per 500–1000 bp approximately. Secondly, large numbers of commercial platforms were available for semi-automated or fully automated SNP genotyping. These platforms serve different purposes since they differ in SNP selection,

reaction chemistry, signal detection, throughput, cost and assay flexibility (Ding and Jin *et al.*, 2009).

2.12.2 Cytology

Cytololy was basic and essential technique to study chromosomal compliment of any organism. The chromosome behavior was analyzed at different stages of cell cycle, like pairing at metaphase plate. Cytology was also useful for detecting euploidy and aneuploidy in an organism. Further modification in cytological procedure could be used for specific purposes. Standard C-banding karyotypes of many wild relatives have been developed and used for monitoring alien introgressions (Friebe, 1995a; 1995 b). Later C-banding was used to develop and identify complete set of wheat-*Ae. geniculata* addition lines (Friebe *et al.,* 1999). Pairing affinities between Aegilops and wheat genomes have been analysed from meiotic associations at metaphase I in low and high homoeologous pairing hybrid plants as well as from different meiotic configurations (bivalents and multivalents) in those hybrids with a high pairing mutant (phib). Such distinguishable associations revealed the same relative order: AD-UM > A-D> U-M > AD-B > UM-B in both low and high homoeologous pairing hybrids (Fernandez-Calvin and Orellana, 1992). Metaphase chromosomal pairing behaviour of various wide hybrids of *Triticum aestivum* and *Agilopos kotschhii* was studied by meiotic preparations at IIT Roorkee (Kumari *et al.,* 2011, Tiwari *et al.,* 2010, Rawat *et al.,* 2011). Monosomic and substitution lines of *L. perenne/F. pratensis*system and the Pooideae cereals were development and analysed cytologically (Harper *et al.,* 2011). Various disomic and ditelosomic addition lines were developed by crossing wheat with *Leymus racemosus* (2n= 4x=28, JJNN) and studied by C-banding (Qi *et al.*,1997). Random chromosome elimination of *Aegilops kotschyi* was observed cytologically in synthetic amphiplois of *Triticum aestivum L.–Aegilops kotschyi Boiss* (Tiwari *et al.,* 2010).

2.12.3 GISH

GISH (Genomic *in situ* hybridization) was a modified cytological application which works on the principle of fluorescence. It involved labeling of total alien genomic DNA and further used as a probe to identify alien chromosomes in wheat background by in situ hybridization (Le *et al.,* 1989; Heslop-Harrison *et al.,* 1992). GISH had wide application in plant breeding programmes involved alien translocations (Mukai and Gill, 1991; Heslop-Harrison *et al.,* 1992). This technique has been used to identify the parental origin of each chromosome in hybrids of *Hordeum chilense* and *H. vulgare* and in hybrids of *H. vulgare* X *H. bulbosum* L. (Schwarzacher *et al.,* 1992; Leitch *et al.,* 1990). Alien chromosomes and chromosome segments from *S. cereale* and *H. vulgare* in hexaploid wheat cultivars (Mukai

and Gill, 1991) and triticale were also identified by GISH (Le and Armstrong, 1991). Addition and substitution line of *Agilopos kotschii* for high grain Fe and Zn content were screened by using GISH (Tiwari *et al.*, 2010; kumari *et al.*, 2011). C-banding and GISH were used for developing hexaploid *Secale cereal* x *Triticum aestivum* derivatives (Wang *et al.*, 1993).

Table of Contents

3. MATERIALS AND METHODS

3.1 Plant Materials

The experimental material used for polymorphic survey comprising three wheat (*Triticum aestivum*) cultivars WL711, PBW343, Chinese spring and six accessions non progenitor *Aegilops* species i.e. *Ae. kotschyi* 3790, 396, 3573, *Ae. peregrina* 13772, 3519 and *Ae. longissima* 28 (Table 3.1) which were used for wheat biofortification for Fe and Zn was obtained from the Wheat Germplasm Collection maintained at the Punjab Agricultural University, Ludhiana, India. The related wild species and bread wheat cultivars were grown at the experimental fields of the IIT, Roorkee in 2009-10, 2010-11, 2011-12 and 2012-13.

The experimental material which was used for seed and pollen irradiation was developed by Prof. H.S Dhaliwal, Department of Biotechnology, Indian Institute of Technology, Roorkee. A 7S substitution line (CS(Ph^I)/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6⊗ in which 7D was substituted by 7S of *Aegilops kotschyi* 396, 7U substitution line CS(Ph^I)/*Ae. kotschyi* 3790//UP2338-2///WL711(63)-2-13⊗ of 7U of *Aegilops kotschyi* 3790 were used for seed and pollen irradiation. The related wild species, substitution lines, wheat and irradiated materials were grown at the experimental fields of the IIT, Roorkee, Roorkee for four consecutive seasons of 2009-10, 2010-11, 2011-12 and at Eternal University, baru Bahib, in 2012-13 as single row of 1.5 meter length with plant to plant distance was kept 15 cm with a row to row spacing of 30 cm along with recommended fertilizers and irrigation for wheat crop. Grains, spikelets and spikes were harvested and threshed from cultivars, derivatives and wild accessions at physiological maturity. Due to frequent shattering of spikes in various wild species, collection of mature spikelets and spikes were carried out repeatedly at different intervals over two-three weeks. Due to tough glumes and hard threshing in wild species the grains were taken out manually.

Table-3.1 Name, genome and micronutrient content of *Triticum aestivum* and its wild relatives included in polymorphic survey of SSR markers.

S. No.	Species name	Genome	Micronutrient content (Fe and Zn)
1	*Triticum aestivum*-cv WL711	AABBDD	Low
2	*Triticum aestivum*-cv PBW343	AABBDD	Low
3	*Triticum aestivum*-line Chinese spring	AABBDD	Low
4	*Aegilops kotschyi* 396	UUSS	High
5	*Aegilops kotschyi* 3790	UUSS	High
6	*Aegilops kotschyi* 3573	UUSS	High
7	*Aegilops peregrina* 3519	UUSS	High
8	*Aegilops peregrina* 13772	UUSS	High
9	*Aegilops longissima* 28	SS	High
10	7S substitution line 48-41-6⊗ Cs(Ph^I)/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-69(X)	AABBDD/7SS	High
11	7U addition line of *Ae. Paringrina* species	AABBDD/7U	High

3.2 Methods

3.2.1 Radiation hybrids

For fine transfer of useful variability for higher concentration of Fe and Zn from selected substitution line 48-41-6⊗ to wheat, crosses were made using wheat cultivar WL711 as the female parent and substitution line as the male parent. Substitution line was seed irradiated at 35 Krad of ^{60}Co (BRIT, GC-5000, Gama Chamber, at INMAS, Delhi) gamma radiation for chromosome breakage and reunionin in season 2009-10. (Fig. 3.2.1)

In next season i.e. 2010-11, selected substitution lines 48-41-6⊗ and 63-2-13⊗ were used for pollen irradiation. Irradiation was done at a low dose of 2 Krad of ^{60}Co (Blood Irradiation, GC-2000, Gama Chamber) at PAU, Ludhiana, India. Crosses were made using wheat cultivar PBW343 having *GPC* and *Lr24* genes as the female parent and substitution lines as the male parent, the pollen of which were irradiated at 2 Krad gamma irradiation at dehiscence stage for chromosome breakage and reunion (Fig. 3.2.2). Spikes were detached in the evening which were going to be dehiscence at next morning, irradiated and kept in water till the next morning. Pollination of spikes emasculated two days earlier was done at next morning when ovary was receptive and look feathery. Seed set was checked at regular intervals. The crossed seed were harvested carefully and stored properly till next sowing. These seeds were germinated in Petri dishes on clean tissue paper. The roots of these plantlets were fixed for doing GISH after micronutrient analysis and the plantlets were transplanted in field. Extensive care was taken for survival of these plants in the field.

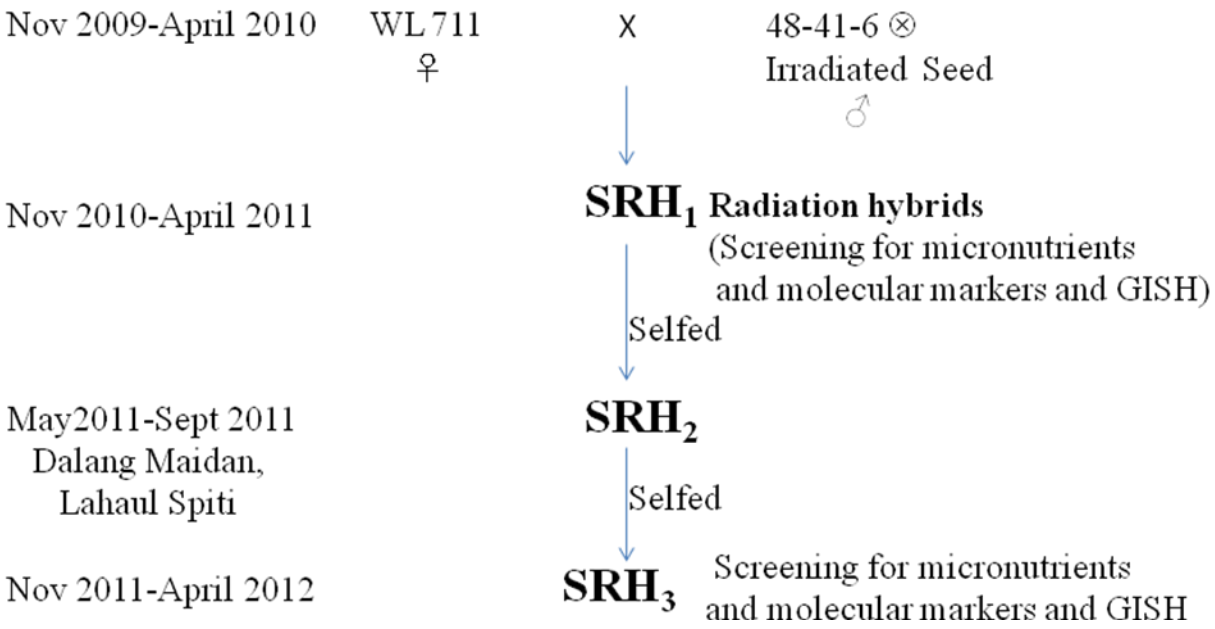

Fig. 3.2.1 Schematic presentation of seed irradiation induced transfers.

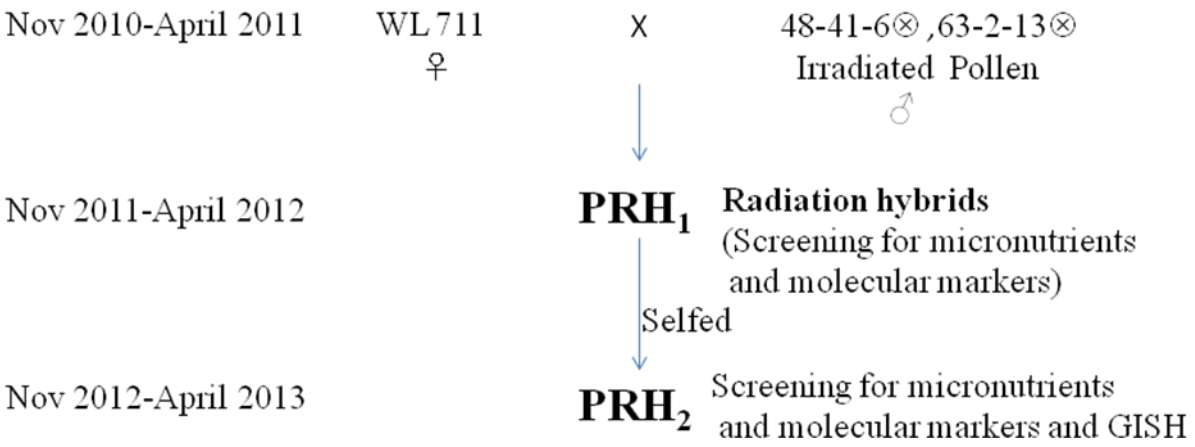

Fig. 3.2.2 Schematic diagram of pollen irradiation induced transfers.

3.2.2 *Ph1b* induced homoeologous chromosome pairing

The 7S substitution line 48-41-6⊗ was crossed with *ph1bph1b* deletion stock received from Dr. Adam J. Lukaszewski, Professor of Genetics, Dept. of Botany & Plant Sciences, University of California (Fig. 3.2.3). *Ph1b* is a recessive deletion mutant of *ph1* for homoeologous pairing in wheat and it must be homozygous to induce homoeologous paring and alien introgression. The F1 hybrids are further backcrossed with *ph1b* again to get *ph1b* homozygous plants. The *ph1b* homozygous plants were selected by a linked marker among the BC_1F_1 and the selfed F_2. The homozygous plants for *ph1b* were further analysed for the presence of 7S chromosome carring the gene of high grain micronutrients. These plants were selfed for getting BC_1F_2 in 2011-12 growing season. Powdery mildew data of each plant was

recorded at regular intervals for transfer of alien genes due to *ph1b* induced homoeologous pairing.

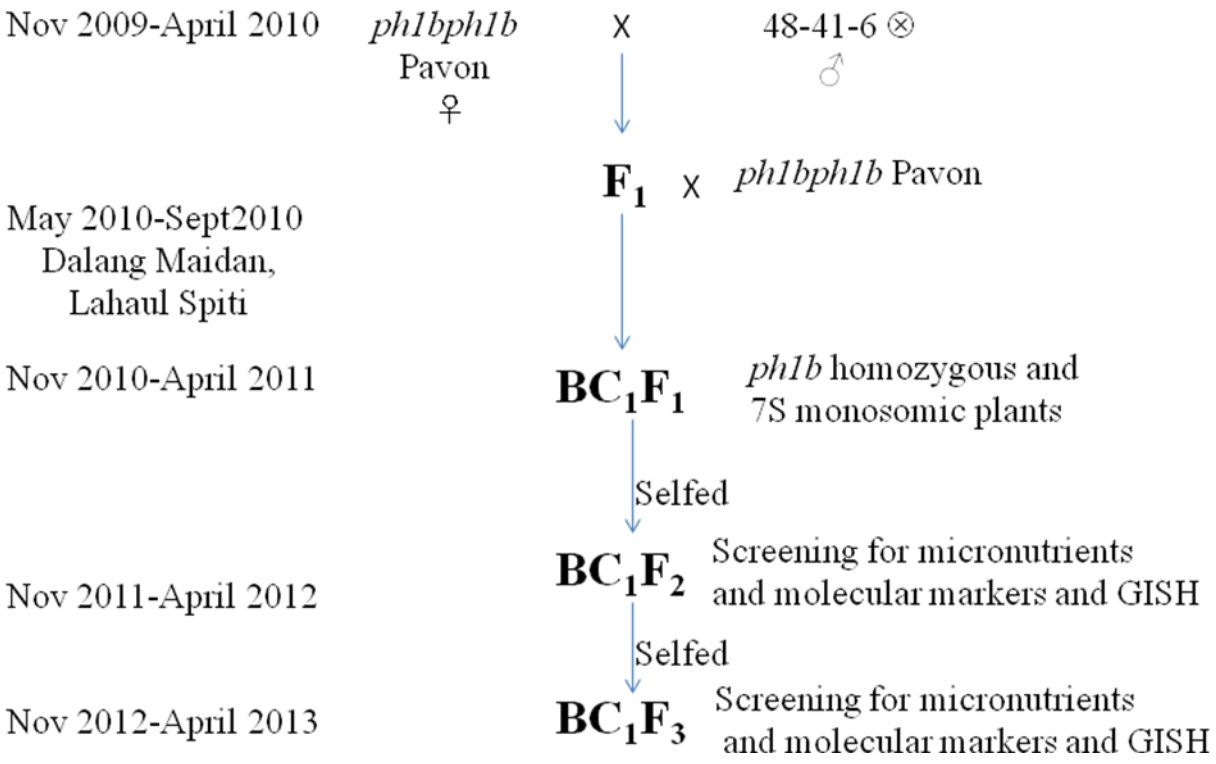

Fig. 3.2.3 Schematic diagrm of the Ph1b induced homoeologous pairing .

3.2.3 5B deficiency induced homoeologous pairing

Crosses were made between *T. aestivum* cv. Pavon Mono 5B wheat line, received from Dr. Adam J. Lukaszewski and the donor species *Ae. kotschyi* 3790 in 2009-10 (Fig. 3.2.4). The pentaploid (ABDSU) F_1 plants were completely male sterile. Two types of F_1 plants were obtained with 5B (35) chromosomes and without 5B (34) chromosomes. The F_1 plants having 34 chromosomes were selected by meiotic analysis and molecular marker (psr574) linked to *ph1b*. The F_1 plants were backcrossed with wheat cultivar PBW343 with *GPC* and *Lr24* genes in 2010-11. The BC_1F_1 plants were selfed to get BC_1F_2 and backcrossed furthur with wheat cultivar PBW343 (with *GPC* and *Lr24*) to get BC_2F_1. The BC_1F_2 and BC_2F_1 seeds were germinated in the lab, their roots were fixed in alcohol and glacial acetic acid solution and plantlets were transplanted in field.

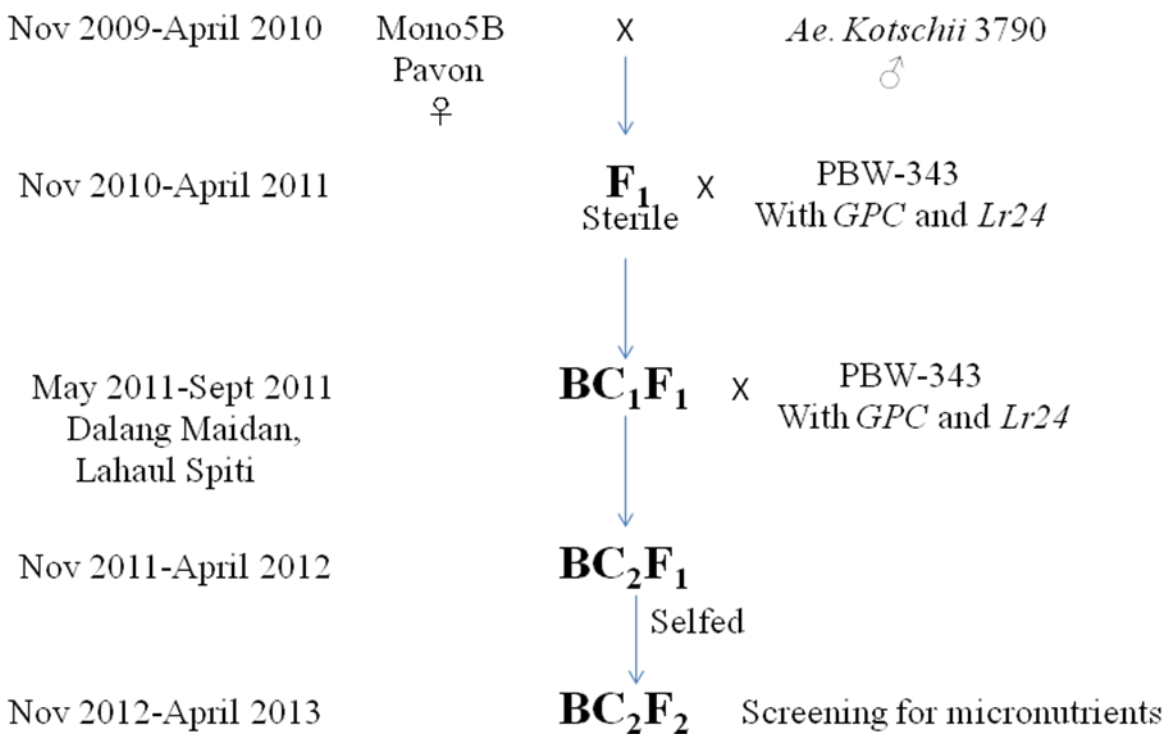

Fig. 3.2.4 Schematic diagram of the development of wheat-*Aegilops* introgressive derivatives

3.2.4 Grain micronutrient Analysis

For micronutrient analysis whole grain samples (0.5gm) from wheat cultivars PBW343, WL711 and the derivatives developed by seed and pollen irradiation and *ph1b* homeologus pairing, were digested on hot plate at 120ºC temperature in concentrated nitric acid (Merck), (5 ml each sample) and hydrogen peroxide (2 ml each time) was added at regular interval for 3-4 times for complete oxidation of grain sample. Digestion was continued till the clear water soluble solution was obtained. Required volume was made after the completion of digestion and the digests were analyzed by Atomic Absorption Spectrophotometer; (GBC- Avanta Garde M) and by Inductively Coupled Plasma Mass Spectrometer (ICPMS, Perkin Elmer). A minimum of three replications of micronutrient analysis was made for each of the cultivars and derivatives.

3.2.5 Cytological Studies

For meiotic analysis spikes of interspecific F_1 hybrids 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 of such slides 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_2-KI). Deep blue, round and fully developed pollen were fertile due to synthesis of starch and yellowish, smaller pollen were sterile due to improper synthesis of starch.

3.2.6 Genomic *In situ* hybridization

Genomic *in situ* hybridization was done in order to finally visualize the alien introgression in the selected derivatives using the method described by Dou *et al.* (2006). Seeds were germinated at room temperature. Root tips were collected at a length of 0.5–2 cm, pretreated in ice-water for 24 hours, and fixed in 99% ethanol–glacial acetic acid (3:1). Squashes were prepared by squashing in 45% acetic acid. Genomic DNA of *Ae. longissima* and *Ae. umbelullata* were used as the probes in GISH.

3.2.7 Isolation and purification of genomic DNA from leaf tissues

DNA was extracted from young leaves of the parents and selected irradiation hybrids and *ph1b* induced homoeologous pairing plants using CTAB method described by Murray and Thompson (1980) with slight modifications (table-3.2 and 3.3)

Table 3.2 Composition of DNA Extraction buffer

S.No.	*Name of the reagent*	*Composition*
1	Tris (pH 8.0)	200mM
2	EDTA (pH 8.0)	20mM
3	NaCl	140mM
4	CTAB	2%
5	β mercaptoethanol	0.01%

All chemicals used were of HiMedia (Analytical and Molecular biology grade).

Table 3.3 DNA isolation and purification reagents

S.No.	*Name of the reagent*	*Composition*
1	Tris buffer (pH 8.0)	10 mM
2	EDTA (pH 8.0)	1mM
3	RNAse solution	10 mg/ml
4	Phenol:Chloroform: Isoamyl alcohol	25:24:1
5	Ethanol, Isoproponal	Absolute
6	Ethanol	70%

About 5-7g of young, healthy and disease free leaves from each plant were collected and kept in the plastic bags on ice. One or two leaves were frozen in liquid nitrogen and crushed to fine powder using autoclaved and pre-chilled mortar and pestle. The powder was transferred to 2 ml centrifuge tubes containing pre-warmed (65°C) DNA extraction buffer (1 ml for approximately 0.3g of leaves). It was gently mixed and incubated at 65°C water in bath for 1.5 hour, mixing gently every 15 min. Equal volumes of phenyl: chloroform: isoamyl alcohol (25:24:1) solution was added to the samples followed by gentle mixing for 15 min to ensure emulsification of phases. The samples were centrifuged at 10,000 rpm for 20 min at 25°C. Supernatants were transferred to the fresh centrifuge tubes with the help of micropipettes. Equal volume of ice cold isopropanol was added and left overnight at 4°C for complete precipitation of DNA. DNA was precipitated out by centrifuging at 5000 rpm for 5 min. Supernatant was discarded and pellet was washed with 600µl 70% ethanol. It was centrifuged at 5000rpm for 5 min, washing was done twice. Ethanol was drained out, pellets were air dried and resuspended in 200µl TE buffer. Subsequently RNAse treatment at final concentration of 100 µg/ml was given 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 absorbance at 260nm and 280nm was checked as a measure of DNA purity. At wavelength 260 nm, the concentrations of DNA (OD 260 x 50x dilution factor) were determined and subsequently samples were diluted to a concentration of 50ng/µl. Electrophoresis was carried out finally for the qualitative and quantitative analysis in 0.8% agarose gel with standard protocol (Sambrook, 2001).

3.2.8 Application of microsatellite markers

Wheat microsatellite markers (173 in number) representing all the 3 chromosomes of group 7 of wheat covering both chromosomal arms were selected from the publications of Röder *et al*. (1998), Pestsova *et al*. (2000) and Somers *et al*. (2004). A list of the markers used has been given in Annexure-I. Parental polymorphism between wheat cultivars and *Aegilops* species was done. PCR was carried out according to Röder *et al*. (1998) with some modifications (Table-3.4). The primers were synthesized from Hysel India (Pvt.) Ltd. Transferable polymorphic markers of each chromosome arm were used to identify the introgressed chromosome fragment in the finally selected derivatives.

Table 3.4 Composition of PCR reaction mix, in a volume of 20 µl.

S.No.	*Name of the reagent*	*Composition*
1	DNA(25ng/ µL)	2µl
2	PCR Buffer (10X)	2µl
3	dNTP mix (1mM each)	4µl
4	Primer F(5mM)	1µl
5	Primer R(5mM)	1µl
6	Taq polymerase	1 unit
7	$MgCl_2$ (25mM)	1.2 µl
8	Water	7.8 µl

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 10 min.

3.2.9 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 (AMERCO) having 0.5 µg/ml ethidium bromide (10 mg/ml) and prepared with 1X TBE buffer. The gels were visualized and photographed using BioRad Gel Documentation system.

3.2.10 Polyacrylamide gel electrophoresis of amplified DNA

Preparation of 40% Acrylamide-bis acrylamide solution

Acrylamide (38g) and bis acrylamide (2g) were weighed and dissolved in 50 ml of double distilled water. The volume was made up to 100 ml by double distilled water.

Preparation of 10X TBE

The composition of TBE buffer is given in table 3.5

Table 3.5 Composition of 10X TBE buffer

S.No.	*Name of the reagent*	*Composition*
1	Tris	10.9g
2	Boric acid	5.56g
3	EDTA	0.98g
4	Distilled water	100 ml

Procedure

The composition of PAGE gel is given in table 3.6

Table 3.6 Composition of 8% PAGE gel

S.No.	*Name of the reagent*	*Composition for 150 ml*
1	40% acrylamide-bis acrylamide solution	30 ml
2	10X TBE	7.5 ml
3	Ammonium persulphate	0.105g
4	TEMED	125µL
5	Double distilled water	92.38 ml

All the constituents as mentioned in Tabel 4.2 were taken. Ammonium persulphate and TEMED were added just before pouring. After pouring the comb was fixed in gel and allowed to solidify for about 1 hour.

3.2.11 Silver staining

Preparation of Solution 1 (Fixative solution)

The composition of fixative solution is given in table 3.7

Table-3.7 Composition of fixative solution

S.No.	*Name of the reagent*	*Composition*
1	Methanol	20 ml
2	Glacial acetic acid	1 ml
3	Distilled water	179 ml

Preparation of Solution 2 (Staining solution)

The composition of staining solution is given in table 3.8

Table-3.8 Composition of staining solution

S.No.	***Name of the reagent***	***Composition***
1	Methanol	20 ml
2	Glacial acetic acid	1ml
3	$AgNO_3$	0.2g
4	Distilled water	179 ml

Preparation of Solution 3 (Developing solution)

The composition of developing solution is given in Table 3.9

Table 3.9 Composition of developing solution

S.No.	***Name of the reagent***	***Composition***
1	NaOH	5.1g
2	Formaldehyde	600µl
3	Distilled water	199.4 ml

After gel electrophoresis, PAGE plates were disassembled. The gel was carefully placed in the staining tray. The gel was treated with fixative solution for 5 min with gentle rocking. After fixing the DNA the fixing solution was decanted. Similarly, the gel was incubated in staining solution for 5 min. The staining solution was decanted and the gel was washed gently with distilled water to remove excess silver nitrate on the gel and tray. The gel was then treated with developing solution for visualizing the bands. After the visualization of bands, the developing solution was replaced by fixative solution for increasing the depth and sharpness of bands.

Table of Contents

4. RESULTS

4.1 Transferability of molecular markers and Polymorphic survey

4.1.1 Transferability

In the present study, the polymorphic survey of anchored wheat SSR markers was done on three wheat cultivars, six *Aegilops* species and two addition lines of 7S and 7U of *Aegilops* (Table 3.1.1). A total of 173 markers of group 7 chromosomes were screened using PCR. The 77.45% (134 markers) of these 173 markers were found to be transferable, while remaining were found non-transferable to related *Aegilops* species. A total of 52 among 174 markers were specific to chromosome 7A. Out of these 52 markers 69.23% (36 markers) were found to be transferable to *Aegilops* species. 25 transferable markers were of short arm and 27 were of long arm. Out of 57 markers of chromosome 7B, 44 (77.19%) were found to be transferable to related *Aegilops* species. Among these 13 were of short arm and 9 (69.23%) were transferable. 79.54% (35) were found transferable out of 44 markers of 7B long arm. 64 marker of 7D were screened, 32 from the long arm and 32 from the short arm, of which 26 (81.25%) of the short arm and 28 (87.50%) of the long arm were found transferable to *Aegilops* species (Fig. 4.1.1).

4.1.2 Polymorphism

All the markers which were transferable were not polymorphic among wheat and *Aegilops* species, polymorphism varies 41-70% of 7A, 7B and7D chromosome markers of the long and short arms. A total of 51.49% (69 markers) markers were found to be polymorphic out of 134 transferable markers of group 7 of wheat. A list of these 69 polymorphic markers along with transferability and polymorphic status among individual lines is given in table 4.1.3 Out of 36 transferable markers of 7A, 17 markers (47.22%), 10 (52.63%) from short arm and 7 (41.17%) from long arm were polymorphic. A total of 20 markers (45.45%) were polymorphic among transferable markers of 7B, 5 (55.55%) were from short arm and 15 (42.85%) were from long arm. 7D had highest number of transferable markers and polymorphic markers as compared to 7A and 7B markers. 32 (59.25%) markers were polymorphic out of 54 transferable markers, 18 (69.25%) were from 7D short arm and 14 (50%) were from 7D long arm. The chromosome arm wise percentage of transferable and polymorphic markers is given in table 4.1.1.

Table 4.1.1 Transferable and polymorphic SSR markers of group 7 of wheat

Chromosome (Chr.) Group	Chr.	Chr. location	Marker Screened	Transferable (%)	Polymorphic (%)	Marker Screened	Transferable (%)	Polymorphic (%)
GROUP7	7A	7AS	52	36 (69.23%)	17 (47.22%)	25	19 (76%)	10 (52.63%)
		7AL				27	17 (62.96%)	7 (41.17%)
	7B	7BS	57	44 (77.19%)	20 (45.45%)	13	9 (69.23%)	5 (55.55%)
		7BL				44	35 (79.54%)	15 (42.85%)
	7D	7DS	64	54 (84.37%)	32 (59.25%)	32	26 (81.25%)	18 (69.25%)
		7DL				32	28 (87.50%)	14 (50%)
Total			**173**	**134**	**69**			

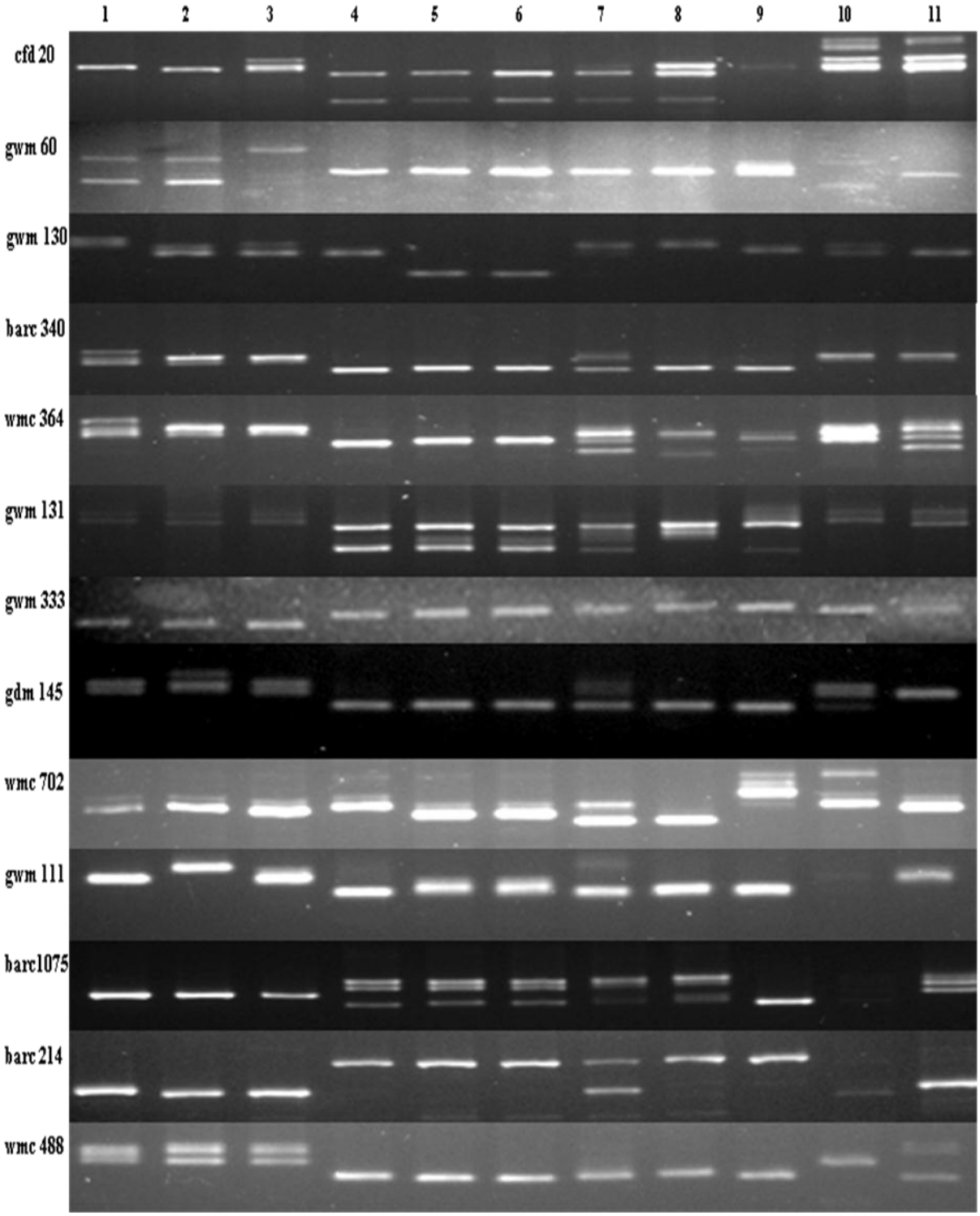

Fig. 4.1.1 Polymorphism of SSR markers of wheat *Triticum aestivum* among wheat cultivars and *Aegilops* species, Lanes 1-WL711, 2-PBW343, 3-Chinese Spring, 4-*Ae. kotschyi 396*, 5-*Ae kotschyi3790*, 6-*Ae. kotschyi 3573*, 7-*Ae. peregrina 3519*, 8-*Ae. peregrina 13772*, 9-*Ae. longissima 28*, 10-7S substitution line of *Ae. kotschyi 396*, 11-7U addition line of *Ae. peregrina* species

4.1.3 Transferability of group 7 markers of wheat to 7S and 7U of *Aegilops* species

Polymorphic markers between wheat and *Aegilops* species were analysed for transferability to 7S and 7U chromosomes by using 7S substitution and 7U addition lines of wheat. The genes for Fe and Zn are located on these chromosomes as supported by micronutrient data of substitution and addition lines of 7S and 7U in Chinese spring and other background. Out of 69 polymorphic markers of group 7 chromosomes of wheat 36 markers (52.29%) were transferable to 7S and 7U chromosome, 28.98% specific to 7S, 14.49% for 7U and 8.69% for both the chromosomes. Polymorphic markers from 7A chromosome were found transferable to 7S, 7U and both in a proportion of 41.17%, 5.88% and 5.88%, respectively, where as for chromosome 7B this proportion was 20%, 15% and 15%. 28.12% markers were found transferable to 7S, 18.75% to 7U and 6.25% to both of these from Chromosome 7D polymorphic markers. Chromosome arm wise transferability to 7S and 7U chromosomes is given in table 4.1.2.

Table 4.1.2 Transferability of group 7 SSR markers of wheat to 7S and 7U of *Aegilops* species

Chr. group	Chr.	Chr. Arm	Specific to 7S	Specific to 7 U	Specific to both	Specific to 7S	Specific to 7U	Specific to both
Group 7	7A	7AS	7(41.17%)	1(5.88%)	1(5.88%)	5(50%)	1(10%)	-
		7AL				2(28.57%)	-	1(14.28%)
	7B	7BS	4(20%)	3(15%)	3(15%)	1(20%)	1(20%)	1(20%)
		7BL				3(20%)	2(13.33%)	2(13.33%)
	7D	7DS	9(28.12%)	6(18.75%)	2(6.25%)	6(33.33%)	2(11.11%)	-
		7DL				3(21.42%)	4(28.57%)	2(14.28%)
Total			**20**	**10**	**6**			

Table 4.1.3 Transferability and polymorphism of SSR markers of group 7 chromosomes of wheat among wheat and *Aegilops* species and specificity to 7S and 7U chromosomes of *Aegilops* species

Marker name	Chr. location	Polymorphism in wheat	Transferability to *Ae. kotschyi*	Polymorphism in *Ae. kotschyi* and wheat	Transferability to *Ae. peregrina*	Polymorphism in *Ae. peregrine* and wheat	Transferability to *Ae. longissima*	Polymorphism in *Ae. longissima* and wheat	Specificity to 7S or 7U genome
gwm350	7AS/DS	Y	Y	Y	Y	Y	Y	Y	7S
wmc479	7AS	Y	Y	Y	Y	Y	Y	Y	-
gwm60	7AS	Y	Y	Y	Y	Y	Y	Y	7U
Cfd242*	7AS	N	Y	Y	Y	Y	Y	Y	7S
gwm573	7AS	Y	Y	Y	Y	Y	Y	Y	-
gwm260	7AS	N	Y	Y	Y	Y	Y	Y	7S
barc1005	7AS	Y	Y	Y	Y	Y	Y	N	-
wmc596	7AS	Y	Y	Y	Y	Y	Y	Y	7S
gdm152	7AS	N	Y	Y	Y	Y	Y	Y	7S
barc1025	7AS	Y	Y	Y	Y	Y	Y	Y	-
cfa2040	7AL	Y	Y	Y	Y	Y	Y	Y	7S
wmc809	7AL	Y	Y	Y	Y	Y	Y	Y	-
wmc9	7AL	Y	Y	Y	Y	Y	Y	Y	-
Gwm4*	7AL	N	Y	Y	Y	Y	Y	N	-
barc49	7AL	Y	Y	Y	Y	Y	Y	Y	7S,7U
cfd20	7AL	Y	Y	Y	Y	Y	N	N	-
cfd2019	7AL	Y	Y	Y	Y	Y	Y	Y	7S
wmc405	7AS/ BS/DS	Y	Y	Y	Y	Y	Y	Y	7S
barc340	7BS	Y	Y	Y	Y	Y	Y	Y	-
gwm537	7BS	Y	Y	Y	Y	Y	Y	Y	7U
gwm68*	7BS	N	Y	Y	Y	Y	Y	Y	-
gwm43*	7BS	Y	Y	Y	Y	Y	Y	Y	7S,7U
barc65	7BL	Y	Y	Y	Y	Y	Y	Y	7S
gwm333	7BL	N	Y	Y	Y	Y	Y	Y	7S,7U
wmc273	7BL	Y	Y	N	Y	Y	Y	Y	7U
wmc396	7BL	N	Y	Y	Y	Y	Y	Y	7S

Marker name	Chr. location	Polymorphism in wheat	Transferability to *Ae. kotschyi*	Polymorphism in *Ae. kotschyi* and wheat	Transferability to *Ae. peregrina*	Polymorphism in *Ae. peregrine* and wheat	Transferability to *Ae. longissima*	Polymorphism in *Ae. longissima* and wheat	Specificity to7S or 7U genome
wmc76*	7BL	Y	Y	Y	Y	Y	Y	Y	7S
wmc311	7BL	Y	Y	N	Y	Y	Y	Y	-
barc182	7BL	Y	Y	Y	Y	Y	Y	Y	-
barc63	7BL	N	Y	Y	Y	N	Y	N	-
wmc364	7BL	Y	Y	Y	Y	Y	Y	Y	7S,7U
barc315*	7BL	N	Y	Y	Y	Y	Y	Y	-
gwm131	7BL	N	Y	Y	Y	Y	Y	Y	-
wmc435	7BL	Y	Y	Y	Y	Y	Y	Y	-
barc1073	7BL	N	Y	Y	Y	Y	Y	Y	-
gwm344	7BL	N	Y	N	Y	Y	N	N	7U
wmc792	7BL	N	Y	Y	Y	Y	Y	N	-
gwm130	7DS	Y	Y	Y	Y	N	Y	N	-
wmc646	7DS	Y	Y	Y	Y	Y	Y	Y	7S
barc126	7DS	Y	Y	Y	Y	Y	Y	Y	7S
cfd41	7DS	Y	Y	Y	Y	Y	Y	Y	7S
barc214	7DS	Y	Y	Y	Y	Y	Y	Y	-
gdm88	7DS	Y	Y	Y	Y	Y	Y	Y	7U
gdm145	7DS	Y	Y	Y	Y	Y	Y	Y	7S
cfd31*	7DS	N	Y	Y	Y	Y	Y	Y	7U
cfd26	7DS	Y	N	N	Y	Y	N	N	-
barc5	7DS	Y	Y	Y	Y	N	Y	N	-
cfd21	7DS	N	Y	Y	Y	Y	Y	Y	7S
wmc827	7DS	N	Y	Y	Y	N	Y	N	-
gwm44	7DS	N	Y	Y	Y	Y	Y	N	7S
gwm111	7DS	Y	Y	Y	Y	Y	Y	Y	
cfd66*	7DS	N	Y	Y	Y	Y	Y	N	-
barc125	7DS	N	Y	Y	Y	Y	Y	Y	-
cfd46	7DS	N	Y	N	Y	Y	Y	Y	-
wmc702	7DS	N	Y	Y	Y	Y	Y	Y	-
wmc488	7AL/DL	N	Y	Y	Y	Y	Y	Y	7U

Marker name	Chr. location	Polymorphism in wheat	Transferability to *Ae. kotschyi*	Polymorphism in *Ae. kotschyi* and wheat	Transferability to *Ae. peregrina*	Polymorphism in *Ae. peregrine* and wheat	Transferability to *Ae. longissima*	Polymorphism in *Ae. longissima* and wheat	Specificity to7S or 7U genome
cfd175	7DL	N	Y	Y	Y	Y	Y	Y	7U
gdm86	7DL	N	Y	Y	Y	Y	Y	Y	-
gwm37	7DL	Y	Y	Y	Y	Y	Y	Y	-
wmc634	7DL	Y	Y	Y	Y	Y	Y	Y	7S
gdm46	7DL	N	Y	Y	Y	Y	Y	N	-
barc1075	7DL	N	Y	Y	Y	Y	Y	Y	7U
gwm437	7DL	Y	Y	Y	Y	Y	Y	Y	7S
wmc150	7DL	N	Y	Y	Y	Y	Y	Y	7U
gdm84	7DL	N	Y	Y	Y	Y	Y	N	-
gdm150	7DL	N	Y	Y	Y	Y	Y	Y	7S,7U
gdm142	7DL	Y	Y	Y	Y	Y	Y	Y	-
barc184	7DL	N	Y	Y	Y	Y	Y	Y	7S,7U
wmc94	7DL	N	Y	Y	Y	Y	Y	Y	7S

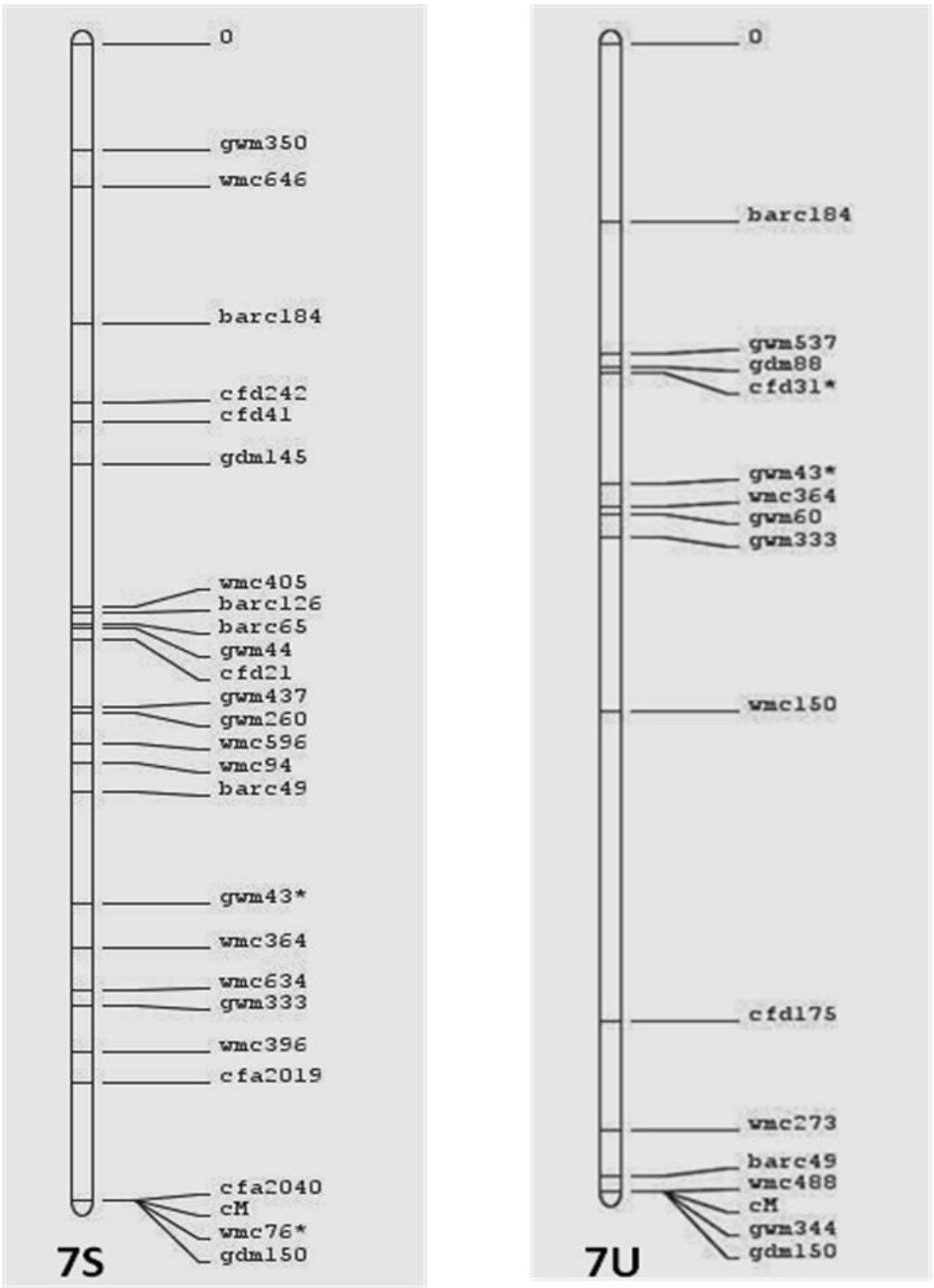

Fig. 4.1.2 Consensus map of 7S and 7U chromosomes made from 7A, 7B and 7D SSR marker found specific to 7S and 7U

4.1.4 Preparation of consensus map of 7S and 7U chromosomes

Consensus map of 7S and 7U was made using join map program. The markers from 7A, 7B and 7D which were found specific to 7S and 7U were mapped by join map on the consensus map. A total of 25 markers were mapped on 7S and 9 markers were mapped on 7U chromosome (Fig. 4.1.2).

4.2 Confirmation of 7S substitution in wheat–*Ae. kotschyi* substitution line CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗

Disomic substitution of 7S chromosome of *Ae kotschyi 396* was confirmed by GISH (Fig. 4.2.2) and molecular markers of group 7 of wheat i.e. wmc 405 and barc126 in plants of 48-41-6⊗ (Fig. 4.2.1). The Fe and Zn content of 48-41-6⊗was found to be 51.65 mg/kg and 48.42 mg/kg, respectively.

4.2.1 Radiation induced transfer of chromosome fragments

Pollens from seed irradiated plants of wheat -*Ae. kotschyi* substitution line CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ were used for pollination elite wheat cultivar WL711, and 45 seeds were obtained as SRH_1. Irradiated pollen of two wheat -*Ae. kotschyi* substitution lines CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗ and CS(*Ph*^{I})/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗ at 2 Krad of gama irradiation were used for pollination of PBW343 cultivar having *Lr24* and *GPC* genes. 410 and 500 PRH_1 seeds were obtained for the above two substitution lines, respectively.

4.2.2 Seed irradiation of Cs(*Ph*^{I})/ *Ae. kotschyi* 396//PBW343-3///PBW373-41-6⊗

The seeds of 48-41-6⊗ line were irradiated at 35 Krad after optimization of radiation dose by seed germination data. The irradiated seeds were sown and the plants were crossed with wheat cultivar WL711. The chromosomal breakage and pollen viability of some of the seed irradiated plants was recorded for confirming the effect of radiation (Fig. 4.2.3) The SRH_1 plants were screened for micronutrient data and molecular marker retention.

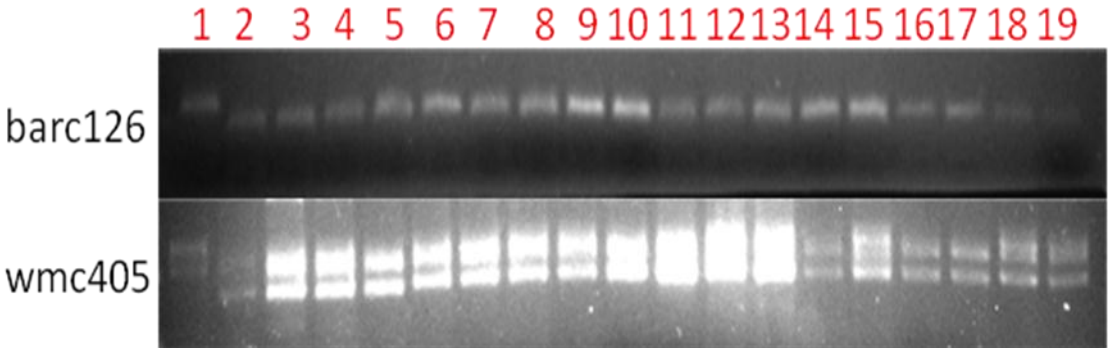

Fig. 4.2.1 PCR with Molecular markers barc126 and wmc405 in parents and 7S substitution line 48-41-6⊗ Lanes. 1 PBW343, 2 *Ae. kotschyi 396* and 3-19 individual plants of 48-41-6⊗

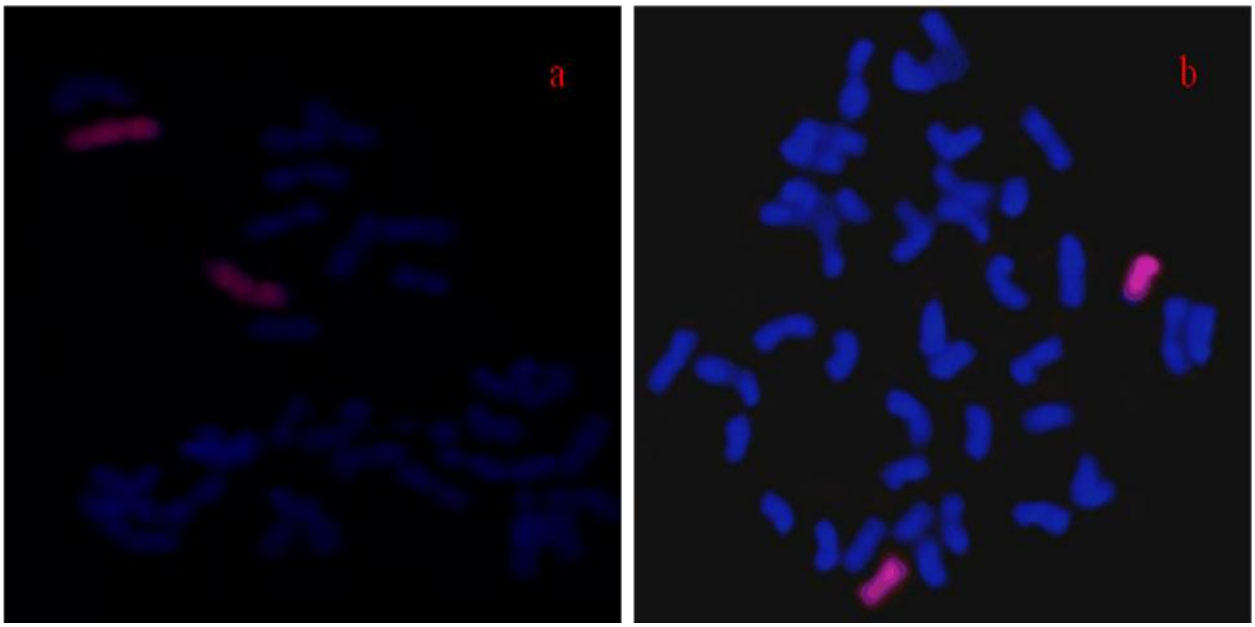

Fig. 4.2.2 Confirmation of disomic substitution by GISH a) CS(*Ph*^{I})/ *Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6⊗b) CS(*Ph*^{I})/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗

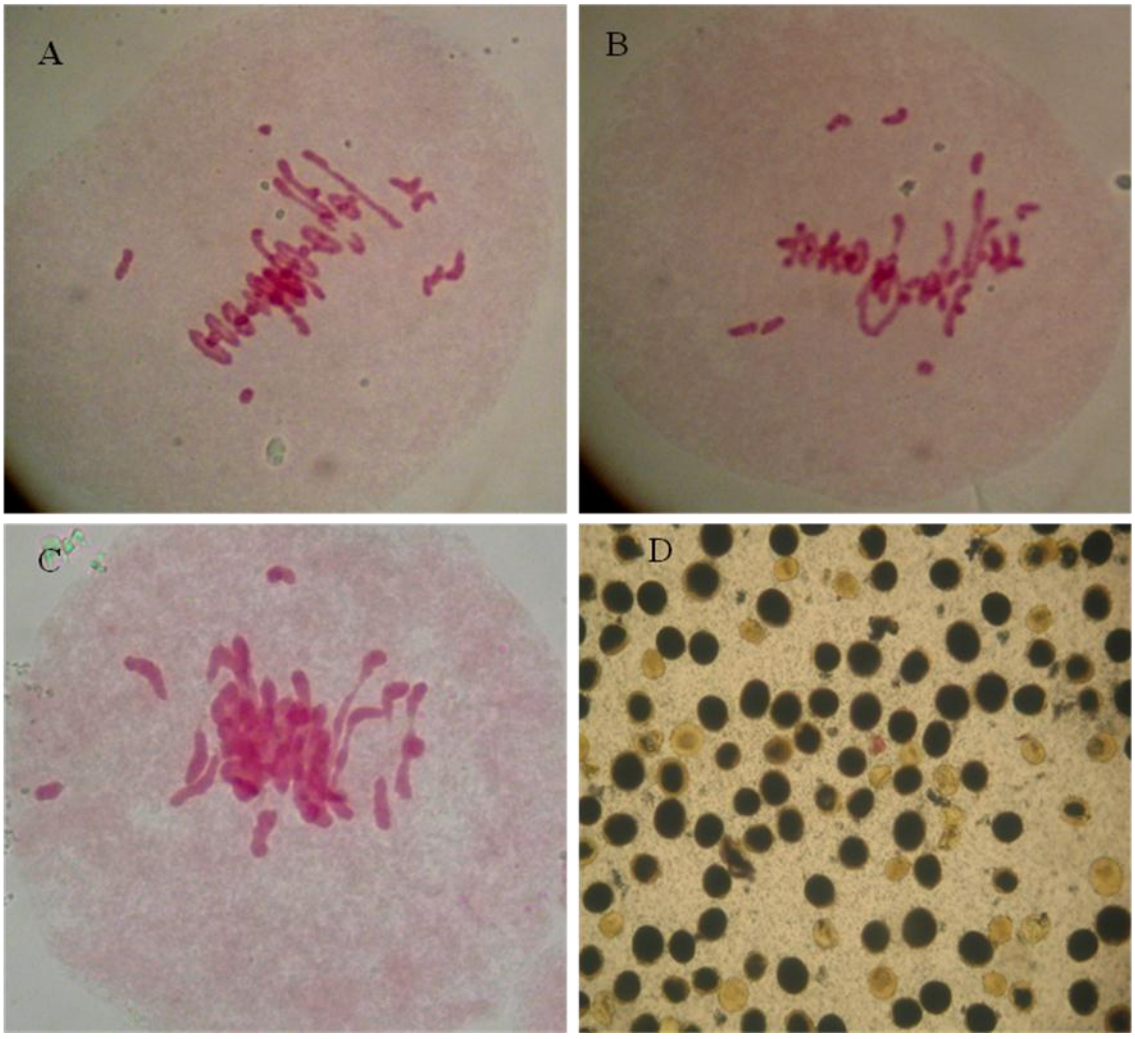

Fig. 4.2.3 Chromosome breakage and pollen viability after gamma irradiation at 35 Krad of 48-41-6⊗ A; P-1, B; P-2, C; P-3 and D; I_2-KI stained pollen of 48-41-6⊗

4.2.3 Morphological and micronutrient characterization of SRH_1 plants

The SRH_1 plants resembled the recurrent parent in tiller number, plant height, head type and seed colour (table 4.2.1). The SRH_1 plants having high tillering, yield and harvest index were analysed for their grain Fe and Zn content. The plants which were still sterile or partially fertile with very low seed set were discarded. Seeds of these plants were as bold as or even bolder than their wheat parents and had Fe and Zn concentrations in the range of 46.8 to 127.4 mg/kg and 41.25 to 110.10 mg/kg, respectively, compared to 49.3 and 49.5 mg/kg of Fe and Zn, respectively, for the WL711. Fe and Zn concentration had negative correlation (r) -0.54 and -0.76, respectively with the harvest index. So concentration effect was there. Susceptibility to the powdery mildew was recorded on a scale of 0-9, where 0 showing no disease symptoms, whereas in plants given score 9, the disease had reached up to heads. 0-3 score was taken as resistant and 4-6 as medium susceptible and 7-9 as highly susceptible. It was observed that the plants having high grain Fe and Zn contents were resistant to powdery mildew, indicating that the genes of Fe and Zn contents might be linked to powdery mildew resistance. Molecular marker data of selected SRH_1 plants is given in table 4.2.2. SSR marker wmc405 co segregated with the high grain micronutrient content (Fig. 4.2.4).

Table 4.2.1 Micronutrient and morphological data of seed irradiated SRH_1 plants of CS(Ph^I)/ *Ae. kotschyi 396//*PBW343-3///PBW373(48)-41-6⊗ X WL711 grown at IITR

Plant Id	Fe mg/kg	Fe % increase over WL711	Zn mg/kg	Zn % increase over WL711	Harvest index	1000 grain weight	Powdery mildew	No. of Tiller/Plant	Plant height in cm
SRH_1-1	**122.45**	**148.38**	**73.65**	**48.79**	**37.32**	**43.3**	**0**	**19**	**108**
SRH_1-2	58.00	17.65	51.65	4.34	44.63	35.2	8	20	105
SRH_1-3	87.05	76.57	65.90	33.13	43.74	48.1	0	23	111
SRH_1-4	58.80	19.27	53.10	7.27	50.97	33.1	9	27	94
SRH_1-5	76.90	55.98	62.95	27.17	41.59	45.2	0	21	110
SRH_1-6	89.65	81.85	97.45	96.87	8.34	30.0	2	7	105
SRH_1-7	107.75	118.56	100.00	102.02	30.19	40.0	0	12	103
SRH_1-8	**96.20**	**95.13**	**77.05**	**55.66**	**45.63**	**46.5**	**0**	**22**	**108**
SRH_1-9	74.00	50.10	59.65	20.51	48.55	47.5	0	25	98
SRH_1-10	54.50	10.55	61.70	24.65	46.05	37.1	2	30	110
SRH_1-11	67.65	37.22	53.10	7.27	48.17	40.2	2	28	112
SRH_1-12	69.80	41.58	61.60	24.44	50.77	38.5	0	29	112
SRH_1-13	60.70	23.12	55.35	11.82	51.39	43.7	0	32	110
SRH_1-14	**96.25**	**95.23**	**78.80**	**59.19**	**51.31**	**39.0**	**0**	**35**	**102**
SRH_1-15	**72.85**	**47.77**	**57.35**	**15.86**	**48.79**	**39.2**	**9**	**26**	**105**
SRH_1-16	76.55	55.27	79.15	59.90	32.35	47.2	0	14	105
SRH_1-17	98.25	99.29	76.65	54.85	30.37	43.3	0	11	118
SRH_1-18	52.75	7.00	54.60	10.30	43.66	30.0	5	23	100
SRH_1-19	83.35	69.07	84.60	70.91	33.92	44.5	3	18	108
SRH_1-20	**46.80**	**-5.07**	**53.55**	**8.18**	**52.61**	**41.0**	**0**	**29**	**110**
SRH_1-21	**93.05**	**88.74**	**68.40**	**38.18**	**44.51**	**43.1**	**0**	**33**	**106**
SRH_1-22	89.25	81.03	67.80	36.97	34.85	46.1	0	18	106

Plant Id	Fe mg/kg	Fe % increase over WL711	Zn mg/kg	Zn % increase over WL711	Harvest index	1000 grain weight	Powdery mildew	No. of Tiller/Plant	Plant height in cm
SRH_1-23	**80.10**	**62.47**	**70.70**	**42.83**	**47.46**	**49.6**	**0**	**27**	**105**
SRH_1-24	89.90	82.35	78.95	59.49	39.52	49.0	0	22	100
SRH_1-25	61.70	25.15	53.50	8.08	43.96	36.7	9	23	98
SRH_1-26	**105.25**	**113.49**	**95.45**	**92.83**	**29.53**	**36.5**	**0**	**19**	**104**
SRH_1-27	67.50	36.92	57.00	15.15	36.36	42.7	0	24	108
SRH_1-28	**89.30**	**81.14**	**56.65**	**14.44**	**53.04**	**42.1**	**2**	**37**	**110**
SRH_1-29	56.90	15.42	42.05	-15.05	49.29	37.9	9	31	102
SRH_1-30	**127.45**	**158.52**	**114.10**	**130.51**	**20.00**	**43.2**	**0**	**5**	**108**
SRH_1-31	90.15	82.86	84.05	69.80	36.22	41.4	0	13	107
SRH_1-32	48.80	-1.01	41.25	-16.67	51.02	32.9	6	33	104
SRH_1-33	**114.05**	**131.34**	**87.65**	**77.07**	**40.80**	**40.5**	**0**	**28**	**106**
WL711	49.3	0	49.5	0	42.52	41.9	9	22	92

Table 4.2.2 Molecular data of SRH_1 plants CS(Ph^I)/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711

Plant Id	gwm350	wmc388	barc184	gdm145	gwm130	wmc 405	barc126	wmc76	barc 65	wmc634
RH_1-1	W+K	W	W	W+K	W+K	W+K	W+K	W+K	W+K	W+K
RH_1-8	W+K	W	W+K	W+K	W	W+K	W+K	W+K	W+K	W+K
RH_1-14	W	W	W	W	W	W	W	W+K	W+K	W+K
RH_1-15	W	W	W	W	W+K	W	W	W	W	W
RH_1-20	W	W	W	W	W	W	W	W	W	W
RH_1-21	W+K	W	W	W+K	W+K	W	W+K	W+K	W+K	W+K
RH_1-23	W+K	W	W	W	W+K	W+K	W+K	W+K	W+K	W+K
RH_1-26	W+K	W	W	W+K	W+K	W	W+K	W+K	W+K	W+K
RH_1-28	W	W	W	W	W	W	W	W+K	W+K	W
RH_1-30	W+K	W+K	W	W+K	W+K	W+K	W+K	W+K	W+K	W
RH_1-33	W+K	W+K	W	W+K	W+K	W+K	W+K	W+K	W	W

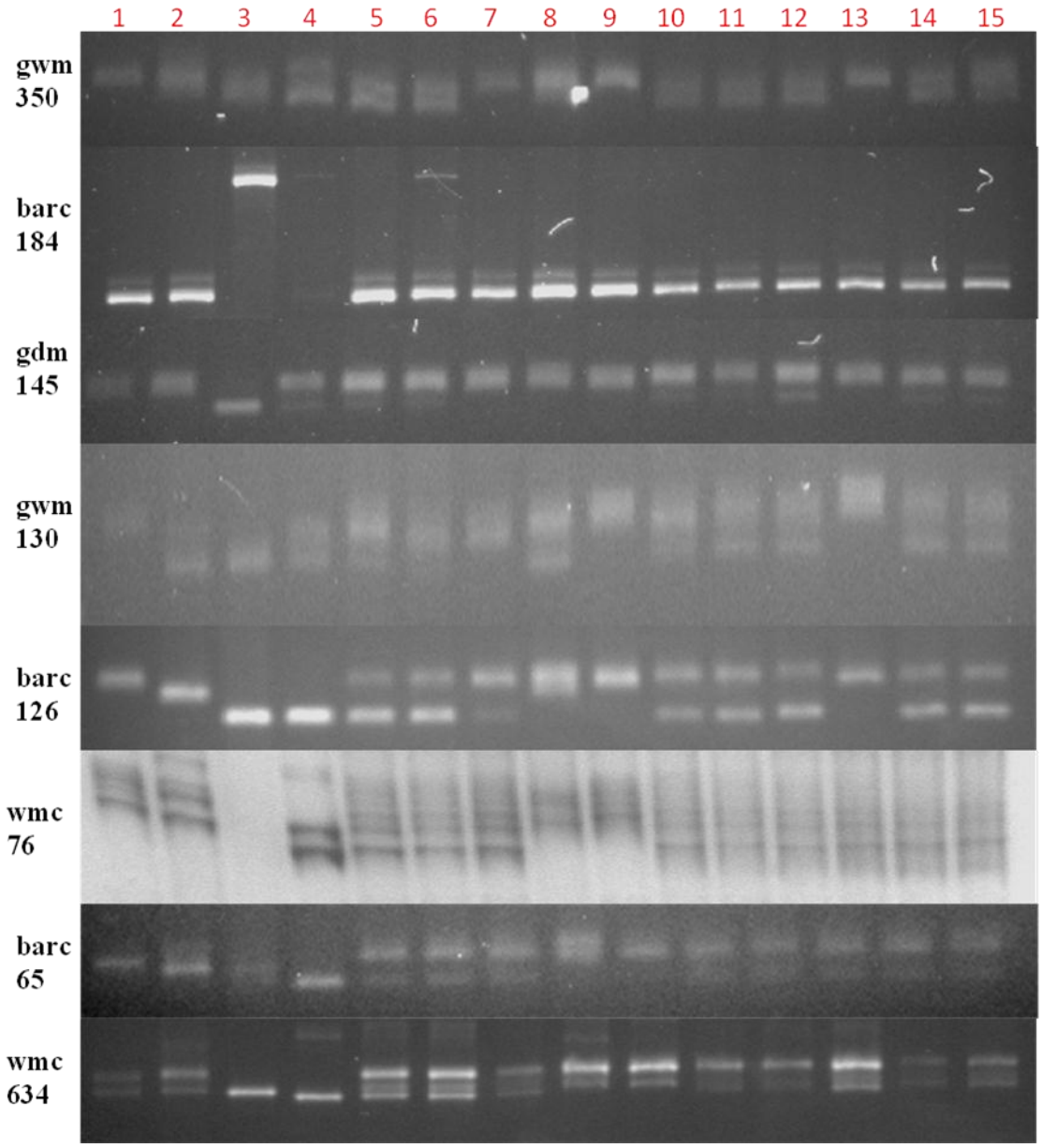

Fig. 4.2.4 Molecular analysis of selected seed irradiation hybrid plants with SSR markers. Lanes 1. Chinese Spring, 2. PBW343, 3. *Ae. Kotschyi 396* , 4. 48-41-6⊗, 5. SRH_1-1, 6. SRH_1-8, 7. SRH_1-14, 8. SRH_1-15, 9. SRH_1-20, 10. SRH_1-21, 11. SRH_1-23, 12. SRH_1-26, 13. SRH_1-28, 14. SRH_1-30, 15. SRH_1-33. Marker wmc 76 was resolved on PAGE

4.2.4 Micronutrient and morphological characterization of SRH_3 plants seed irradiated plants CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711

The SRH_1 plants were selfed to SRH_2 at Dalang Maidan, Kelong (HP). The generated SRH_2 were selfed to SRH_3 at IITR, Roorkee, analysed for micronutrients after harvesting as line bulk. The morphological and micronutrients analysis data of selected SRH_3 plants in given in table 4.2.3. These plants resembled recurrent parent in tiller number, plant height, head type however the seed colour was amber for most of the derivatives. The SRH_3 plants with high tillering, good biomass and fair seed set were analysed for their grain Fe and Zn content (Table 4.2.3), whereas the sterile plants and the plants with very low seed set were discarded. Seeds of selected plants were as bold as of their wheat parents and had Fe and Zn concentrations in the range of 23.18 to 92.34 mg/kg and 27.15 to 72.90 mg/kg, respectively and higher compared to 32.20 mg/kg, and 40.56 Fe and Zn, respectively for the WL711 cultivar (Fig. 4.2.5). Concentration effect was there in seed irradiated SRH_2 plants. Susceptibility to powdery mildew was recorded on a scale of 0-9, where 0 score represents disease free and 9 score describe highly severe infection. 0-3 score was taken as resistant and 4-6 as medium susceptible and 7-9 as highly susceptible plants (Fig. 4.2.7). Plants with high Fe and Zn content were found resistant to powdery mildew, indicating the linkage between the genes. Selected SRH_2 plants were analysed for alien chromosome fragment transfer by GISH. Plant SRH_3-14-2-⊗, SRH_3-28-6-⊗ had the short arm and telomere transfer of 7S chromosome (Fig. 4.2.6).

Table 4.2.3 Micronutrient morphological data of SRH_3 of seed irradiated plants CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711, grown at IITR

Plant ID	Fe mg/kg	Fe % increase over WL711	Zn mg/kg	Zn % increase over WL711	1000 grain weight	Powdery mildew	Plant height in cm
WL711	32.20	-8.35	40.56	-3.46	41.34	9	94
PBW343	35.13	0.00	42.01	0.00	40.56	9	102
SRH_3-1-2-⊗	52.74	50.12	49.77	18.47	45.44	0	104
SRH_3-1-3-⊗	27.00	-23.15	42.08	0.17	36.36	0	105
SRH_3-1-4-⊗	**51.34**	**46.14**	**68.77**	**63.68**	**37.08**	**0**	**100**
SRH_3-8-2-⊗	**47.58**	**35.44**	**50.40**	**19.97**	**51.45**	**3**	**110**
SRH_3-8-3-⊗	**87.11**	**147.96**	**48.87**	**16.32**	**44.26**	**0**	**104**
SRH_3-8-4-⊗	**70.70**	**101.24**	**72.90**	**73.53**	**36.86**	**0**	**98**
SRH_3-8-5-⊗	24.23	-31.04	54.35	29.36	47.71	6	96
SRH_3-8-6-⊗	**51.16**	**45.63**	**48.41**	**15.23**	**47.96**	**6**	**105**
SRH_3-8-7-⊗	40.45	15.13	57.42	36.68	47.56	2	108
SRH_3-14-1-⊗	30.76	-4.13	57.11	37.59	52.32	3	108
SRH_3-14-2-⊗	**92.34**	**187.80**	**58.15**	**40.09**	**51.15**	**0**	**102**
SRH_3-14-3-⊗	27.41	-14.56	40.62	-2.13	42.06	0	102
SRH_3-14-4-⊗	46.75	45.69	39.89	-3.91	40.71	0	109
SRH_3-17-1-⊗	**64.21**	**82.76**	**65.32**	**55.48**	**41.92**	**0**	**102**
SRH_3-17-3-⊗	**60.46**	**72.10**	**72.19**	**71.84**	**37.25**	**0**	**95**
SRH_3-17-4-⊗	**64.77**	**84.37**	**63.41**	**50.93**	**49.21**	**0**	**98**
SRH_3-17-6-⊗	55.90	59.10	52.66	25.34	38.86	2	102
SRH_3-17-7-⊗	60.71	72.81	36.59	-12.91	45.47	4	104

Plant ID	Fe mg/kg	Fe % increase over WL711	Zn mg/kg	Zn % increase over WL711	1000 grain weight	Powdery mildew	Plant height in cm
SRH_3-20-1-⊗	43.57	35.78	28.25	-31.95	47.52	6	112
SRH_3-20-2-⊗	23.18	-27.76	34.36	-17.21	42.93	7	108
SRH_3-20-3-⊗	36.61	14.09	32.88	-20.78	50.27	7	112
SRH_3-20-4-⊗	32.16	0.25	31.03	-25.24	47.53	7	102
SRH_3-20-5-⊗	19.00	-40.79	38.65	-6.89	46.01	7	103
SRH_3-20-6-⊗	25.94	-19.17	38.80	-6.52	48.84	7	106
SRH_3-20-7-⊗	23.20	-27.68	41.78	0.67	46.17	7	98
SRH_3-21-1-⊗	35.31	10.05	37.85	-8.82	41.61	0	97
SRH_3-21-2-⊗	24.71	-22.99	46.74	12.59	46.84	4	140
SRH_3-21-3-⊗	46.60	45.23	30.75	-25.93	47.09	5	145
SRH_3-21-5-⊗	26.64	-16.97	32.99	-20.52	35.11	0	98
SRH_3-22-1-⊗	24.69	-23.06	39.09	-5.83	36.71	0	102
SRH_3-22-2-⊗	32.17	0.26	44.86	8.08	44.04	2	105
SRH_3-22-3-⊗	40.98	27.71	59.46	43.25	44.91	4	108
SRH_3-22-4-⊗	**41.34**	**28.83**	**47.00**	**13.24**	**45.99**	**0**	**108**
SRH_3-22-5-⊗	29.50	-8.07	34.68	-16.46	43.53	0	105
SRH_3-22-6-⊗	42.51	32.49	33.66	-18.90	47.92	0	106
SRH_3-23-2-⊗	**56.21**	**75.19**	**29.73**	**-28.38**	**45.77**	**0**	**102**
SRH_3-23-3-⊗	49.88	55.45	28.54	-31.24	41.34	0	104
SRH_3-23-4-⊗	50.06	56.01	27.15	-34.58	50.12	0	98
SRH_3-23-5-⊗	44.30	38.06	34.93	-15.85	36.21	0	95
SRH_3-23-6-⊗	51.70	61.12	32.05	-22.79	41.99	0	142
SRH_3-24-1-⊗	47.95	49.43	29.00	-30.13	43.32	2	107
SRH_3-24-2-⊗	38.45	19.83	28.02	-32.49	45.61	7	109

Plant ID	Fe mg/kg	Fe % increase over WL711	Zn mg/kg	Zn % increase over WL711	1000 grain weight	Powdery mildew	Plant height in cm
SRH_3-24-3-⊗	38.48	19.94	36.38	-12.36	41.58	0	109
SRH_3-24-4-⊗	44.51	38.73	53.14	28.03	46.41	0	110
SRH_3-24-5-⊗	20.79	-35.22	41.05	-1.09	44.05	0	102
SRH_3-24-6-⊗	56.80	77.03	42.14	1.51	39.88	3	112
SRH_3-26-1-⊗	48.63	51.57	37.91	-8.67	48.18	3	108
SRH_3-26-3-⊗	14.59	-54.52	29.94	-27.87	42.85	0	108
SRH_3-26-4-⊗	26.14	-18.52	39.89	-3.89	41.95	3	104
SRH_3-26-5-⊗	24.91	-22.37	34.30	-17.37	43.83	6	103
SRH_3-26-6-⊗	48.57	51.37	43.20	4.08	53.53	3	110
SRH_3-28-1-⊗	27.64	-13.87	45.52	9.66	47.98	2	138
SRH_3-28-2-⊗	**54.95**	**71.26**	**47.90**	**15.40**	**47.96**	**0**	**102**
SRH_3-28-3-⊗	51.10	59.27	47.62	14.72	41.48	0	97
SRH_3-28-5-⊗	48.95	52.57	43.79	5.50	43.48	0	107
SRH_3-28-6-⊗	**63.31**	**97.31**	**61.10**	**47.21**	**55.06**	**0**	**96**
SRH_3-28-7-⊗	39.71	23.75	47.47	14.37	53.11	0	110

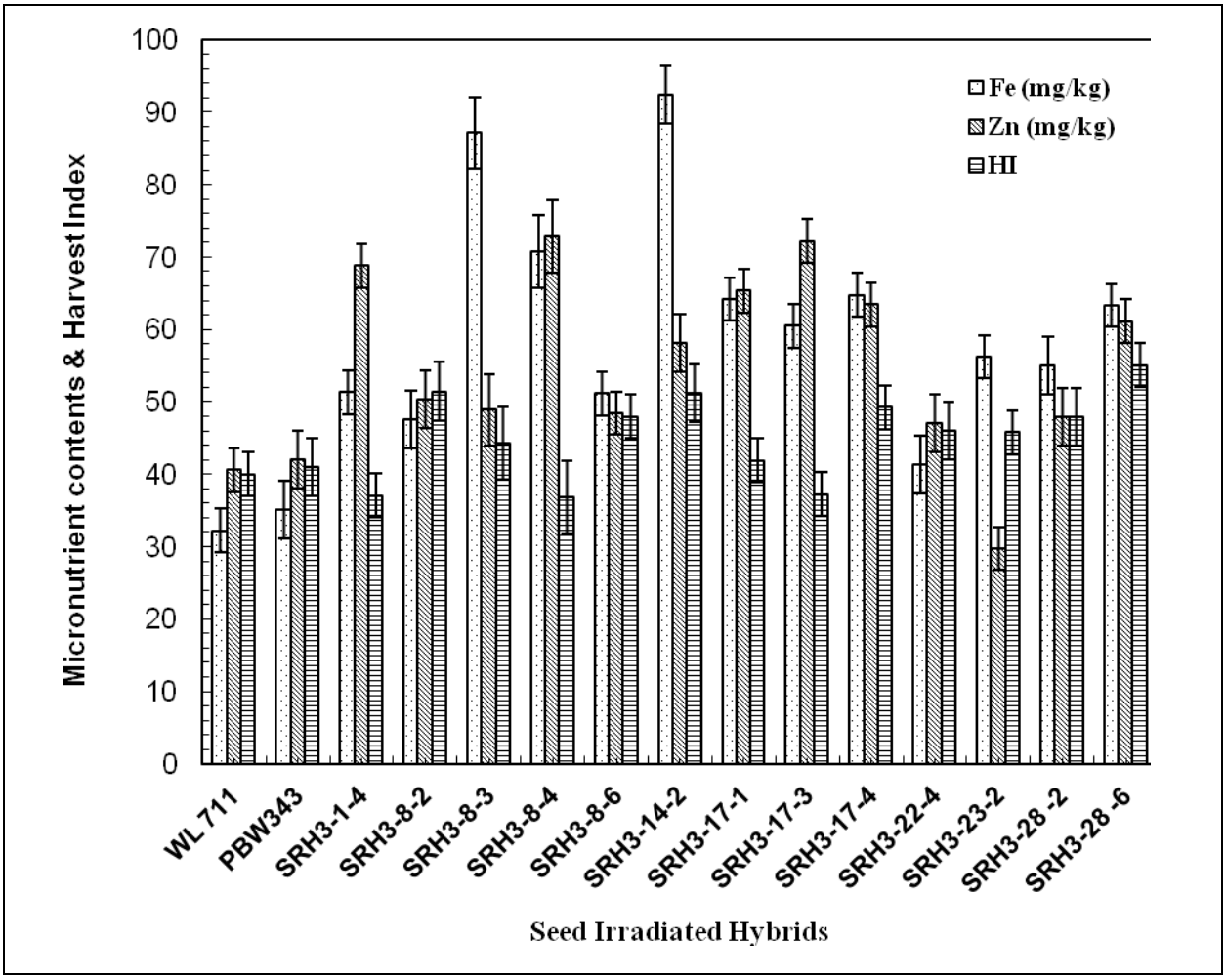

Fig. 4.2.5 Graphical representation of micronutrient characterization of selected SRH_3 of seed irradiated CS(Ph^I)/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711

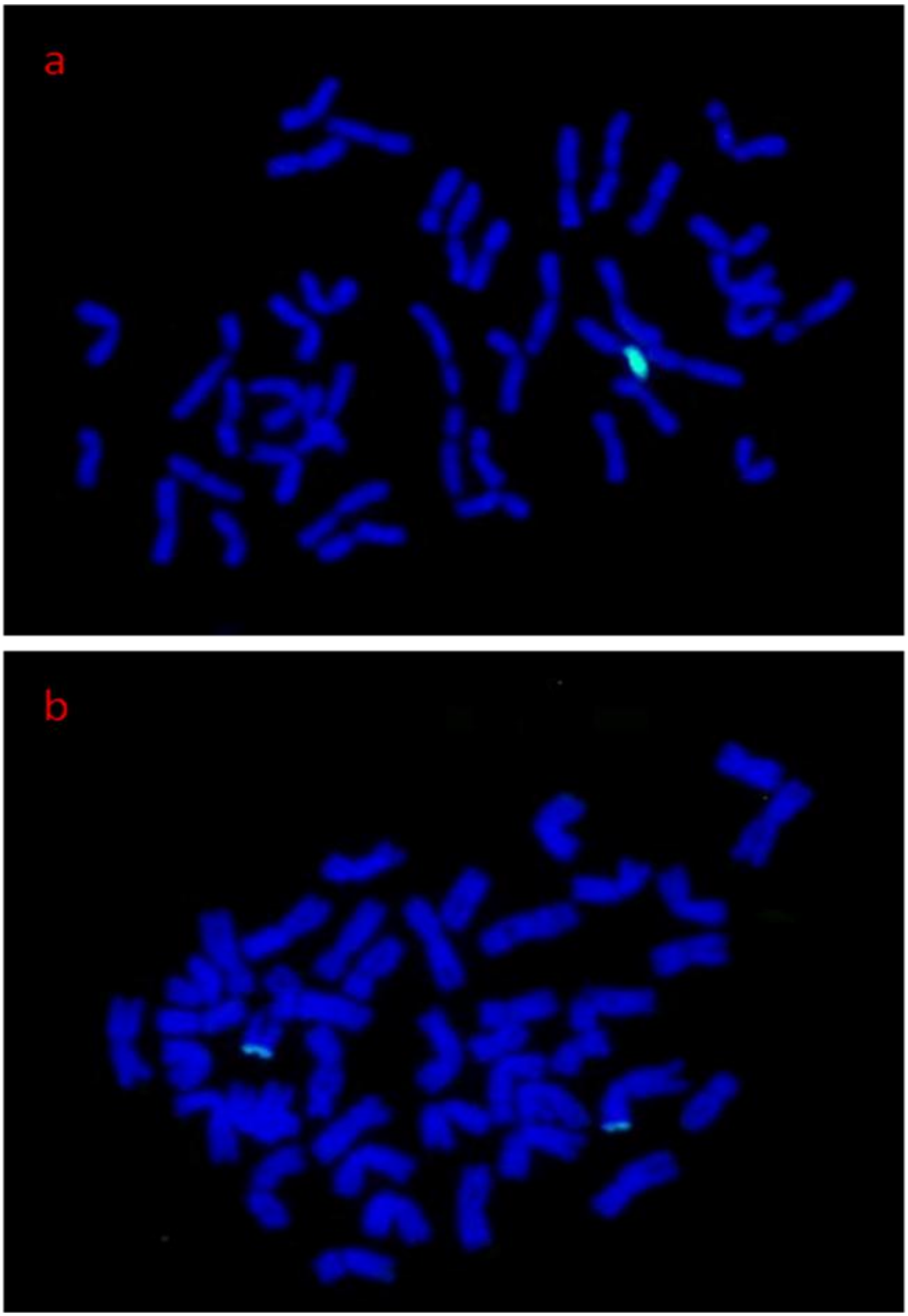

Fig. 4.2.6 GISH showing introgression of alien chromosome fragment (green) in SRH_3 of seed irradiated CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711. a) SRH_3-14-2, b) SRH_3-28-6

Fig. 4.2.7 Incidence of powdery mildew and leaf rust of SRH_3 plants of CS(Ph^I)/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗ X WL711

4.2.5 Pollen irradiation of CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗

Pollen of derivative 48-41-6⊗were irradiated in the following year i.e. 2011 and used for pollinating PBW343 (*Lr24+GPC*) line. 410 PRH_1 seeds were germinated in Petri dishes, out of which 230 seeds were germinated and transferred to the field. Only 170 out of 230 plants were survived. Day to day observation showed considerable variation for plant growth habits and morphological characters like leaf surface area, spreading or erect growth habit, number of tillers per plant and susceptibility to diseases like powdery mildew and sterility. The sterility ranged for 1-2% to 100%. 70 fertile plants were harvested at maturity, while the remaining 100 plants were sterile indicating the pollen irradiation at 2 Krad has been more effective for radiation induced transfer of genes. The shrivelled SRH_1 seeds were 10-15 days late to germinate in Petri dish and the plants had higher sterility (70-100 %). The bold seeds germinated quickly and grow fertile plants. The seeds of these 70 PRH_1 plants were analysed for Fe and Zn content.

4.2.6 Micronutrient and morphological characterization of PRH_1 of CS(*Ph*^{I})/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6(⊗/PBW343 (*GPC+ Lr24*)

The PRH_1 plants resembled recurrent parent in tiller number, plant height, head type etc. Susceptibility to the powdery mildew was recorded in same way as previously, in the case of seed irradiation (Fig. 4.2.10). It is observed that the plants having high grain Fe and Zn content were resistant to powdery mildew like that of seed irradiated plants. The susceptibility to regional pathotype of leaf rust was recorded, all plant were resistant to leaf rust except two or three plants due to *Lr24* from PBW343 (*Lr24+GPC*) line taken as the female parent.

The PRH_1 plants having high tillering and seed set were analysed for their grain Fe and Zn content (Table 4.2.4). The seeds of these selected plants were as bold as or even bolder than the seed of their wheat parents and had Fe and Zn concentrations in the range of 13.89 to 150.52 mg/kg and 27.11 to 192.48 mg/kg respectively, compared to 37.72 mg/kg, and 43.29 Zn of PBW343 (Fig. 4.2.8).

Table 4.2.4 Micronutrient and morphological data of PRH_1 pollen irradiated plants CS(*Ph^I*)/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC+ Lr24*) (IITR)

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield In grams	Harvest index	Powdery mildew
WL711	36.29	-3.78	44.33	2.40	15	41.34	20.0	46.51	9
PBW343	37.72	0.00	43.29	0.00	14	40.56	23	45.10	9
PRH_1-5	25.40	-32.66	41.02	-5.25	28	43.36	55.0	55.26	0
PRH_1-6	37.27	-1.17	47.79	10.38	10	41.14	17.5	50.80	0
PRH_1-10	26.71	-29.18	43.00	-0.67	14	36.28	21.5	51.81	3
PRH_1-12	17.93	-52.46	41.94	-3.12	12	42.88	23.0	48.94	7
PRH_1-14	34.55	-8.38	48.18	11.30	14	37.11	66.5	49.44	7
PRH_1-15	45.05	19.45	40.36	-6.79	17	42.71	47.0	54.02	7
PRH_1-17	**50.04**	**32.68**	**42.00**	**-3.00**	**15**	**47.81**	**35.5**	**54.20**	**3**
PRH_1-18	26.93	-28.58	41.44	-4.28	30	39.16	59.5	53.36	7
PRH_1-19	37.36	-0.94	44.42	2.60	21	39.96	48.5	53.59	9
PRH_1-20	39.36	4.37	42.84	-1.04	22	41.72	52.0	48.15	9
PRH_1-22	32.57	-13.65	42.38	-2.11	27	38.64	59.5	54.34	9
PRH_1-24	71.00	88.24	110.83	156.00	18	41.49	3.0	8.11	7
PRH_1-28	28.08	-25.56	38.90	-10.15	27	39.44	34.0	45.95	9
PRH_1-30	35.65	-5.49	42.43	-1.99	31	38.03	50.5	44.89	9
PRH_1-35	97.92	159.63	114.54	164.56	12	45.82	10.0	16.67	7
PRH_1-37	81.55	116.23	122.81	183.67	38	37.78	16.0	10.53	0
PRH_1-39	58.28	54.52	113.59	162.37	42	35.85	9.0	11.39	0

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Fe % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
PRH_1-44	122.34	224.38	140.28	224.02	11	41.23	2.5	5.88	7
PRH_1-46	24.68	-34.56	39.63	-8.47	30	36.44	76.5	51.35	7
PRH_1-49	32.54	-13.73	35.47	-18.08	36	41.31	61.0	51.26	6
PRH_1-50	33.31	-11.68	39.06	-9.79	8	31.11	14.5	50.00	6
PRH_1-51	85.11	125.66	88.78	105.07	22	31.28	3.5	10.45	0
PRH_1-53	46.26	22.65	43.22	-0.17	15	45.58	2.0	7.14	0
PRH_1-54	93.04	146.69	145.16	235.30	5	36.71	0.6	6.05	0
PRH_1-56	29.22	-22.53	37.64	-13.06	31	38.18	54.5	48.44	7
PRH_1-57	36.73	-2.61	40.42	-6.65	38	45.94	64.0	49.23	7
PRH_1-59	43.60	15.60	40.15	-7.25	33	33.18	48.5	48.98	7
PRH_1-67	101.76	169.81	135.33	212.59	6	30.05	0.5	7.69	7
PRH_1-68	**47.35**	**25.55**	**45.60**	**5.33**	**18**	**42.99**	**40.0**	**51.28**	**7**
PRH_1-69	45.85	21.57	47.57	9.89	27	40.81	55.5	52.61	7
PRH_1-72	**45.51**	**20.67**	**56.15**	**29.69**	**32**	**49.02**	**88.0**	**55.70**	**7**
PRH_1-73	40.54	7.49	44.68	3.20	27	47.61	74.0	56.92	6
PRH_1-74	23.71	-37.14	36.74	-15.14	10	44.71	18.5	56.92	3
PRH_1-75	**51.97**	**37.79**	**46.08**	**6.43**	**41**	**43.51**	**74.0**	**48.68**	**7**
PRH_1-76	40.98	8.66	41.01	-5.27	19	44.95	42.5	51.52	7
PRH_1-78	20.82	-44.79	28.70	-33.70	21	40.64	35.5	52.59	7
PRH_1-79	**46.31**	**22.80**	**48.35**	**11.69**	**29**	**45.22**	**56.0**	**49.12**	**7**
PRH_1-82	**65.76**	**74.37**	**47.32**	**9.30**	**23**	**47.19**	**61.0**	**53.04**	**9**
PRH_1-85	93.22	147.18	142.58	229.33	27	35.87	10.0	14.71	0

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
PRH_1-86	120.11	218.46	140.90	225.44	13	40.62	3.0	7.32	0
PRH_1-87	**52.91**	**40.30**	**50.54**	**16.74**	**13**	**43.42**	**41.0**	**51.90**	**7**
PRH_1-89	92.10	144.19	111.14	156.72	15	36.73	3.0	11.11	5
PRH_1-91	**81.42**	**115.87**	**116.94**	**170.11**	**28**	**41.51**	**33.5**	**22.71**	**0**
PRH_1-93	35.62	-5.56	38.76	-10.48	31	42.92	66.0	49.25	2
PRH_1-94	67.97	80.21	116.91	170.03	18	35.61	3.0	7.32	9
PRH_1-97	29.82	-20.92	44.34	2.41	17	43.02	41.5	52.20	3
PRH_1-98	30.26	-19.77	39.29	-9.25	15	48.39	44.0	56.41	3
PRH_1-99	58.99	56.41	84.86	96.01	18	45.62	6.0	23.08	0
PRH_1-101	38.15	1.15	40.19	-7.16	29	37.02	79.0	56.03	3
PRH_1-103	13.89	-63.18	27.11	-37.38	39	41.18	80.5	49.54	7
PRH_1-104	21.86	-42.05	31.20	-27.93	19	43.03	44.0	55.00	3
PRH_1-109	150.52	299.10	192.48	344.60	27	33.18	1.5	2.80	0
PRH_1-114	**39.43**	**4.55**	**56.35**	**30.15**	**26**	**46.56**	**72.0**	**55.38**	**3**
PRH_1-115	32.69	-13.33	44.05	1.75	26	46.48	56.0	52.83	3
PRH_1-124	**52.93**	**40.33**	**75.62**	**74.66**	**23**	**37.43**	**25.5**	**34.69**	**0**
PRH_1-128	40.31	6.89	42.07	-2.83	44	34.41	88.0	50.00	3
PRH_1-132	**51.83**	**37.42**	**47.43**	**9.55**	**36**	**38.15**	**78.0**	**46.99**	**5**
PRH_1-133	**46.97**	**24.54**	**42.95**	**-0.80**	**43**	**43.31**	**116**	**55.77**	**4**
PRH_1-138	20.64	-45.27	46.82	8.14	26	42.21	54.5	52.00	5
PRH_1-141	68.41	199.77	73.57	137.88	8	40.38	3.0	8.57	0
PRH_1-143	25.73	12.74	35.45	14.63	66	34.11	114.0	47.50	3

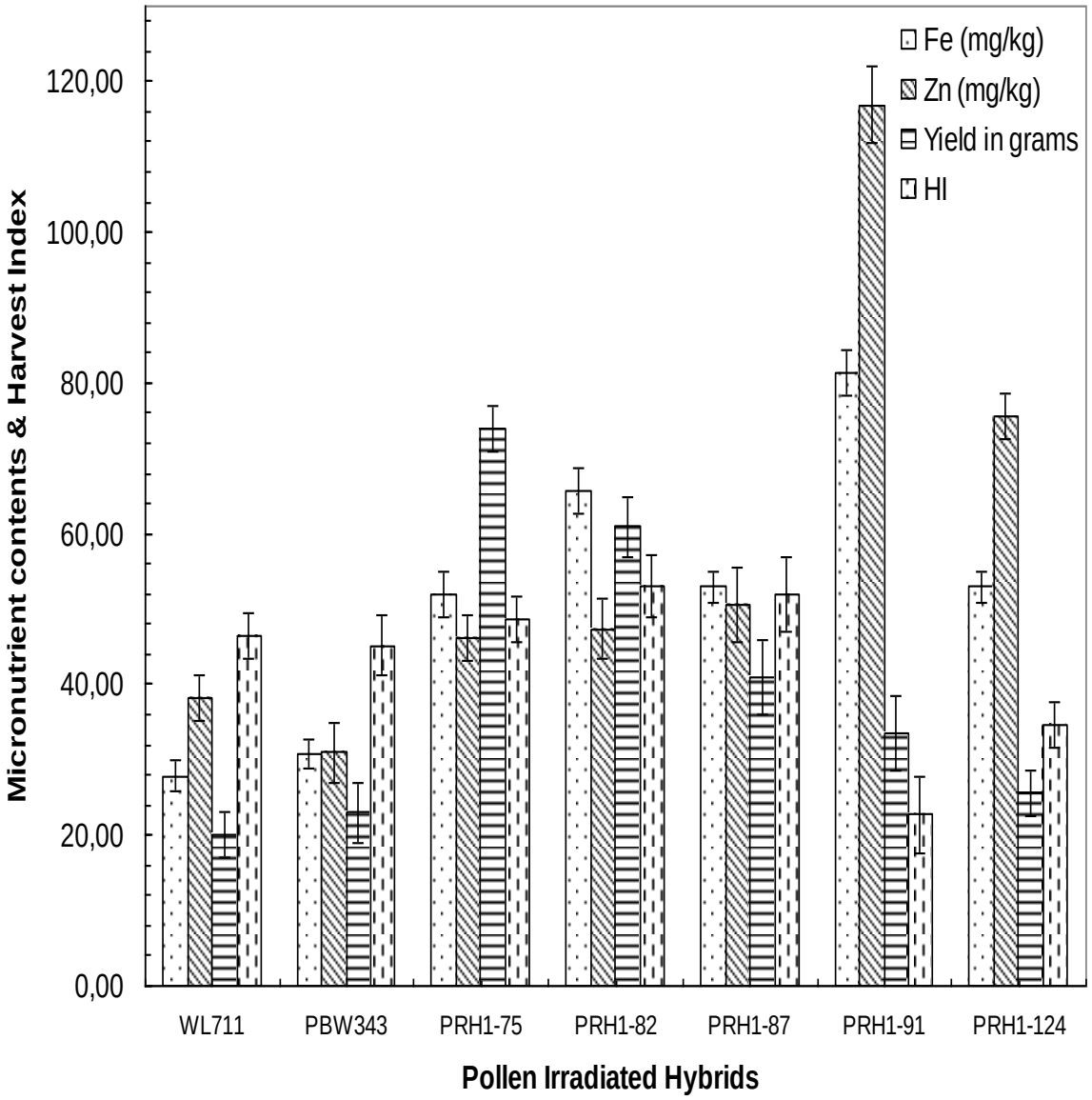

Fig. 4.2.8 Graphical representation of micronutrient content of selected PRH_1 of pollen irradiated plants of CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC +Lr24*)

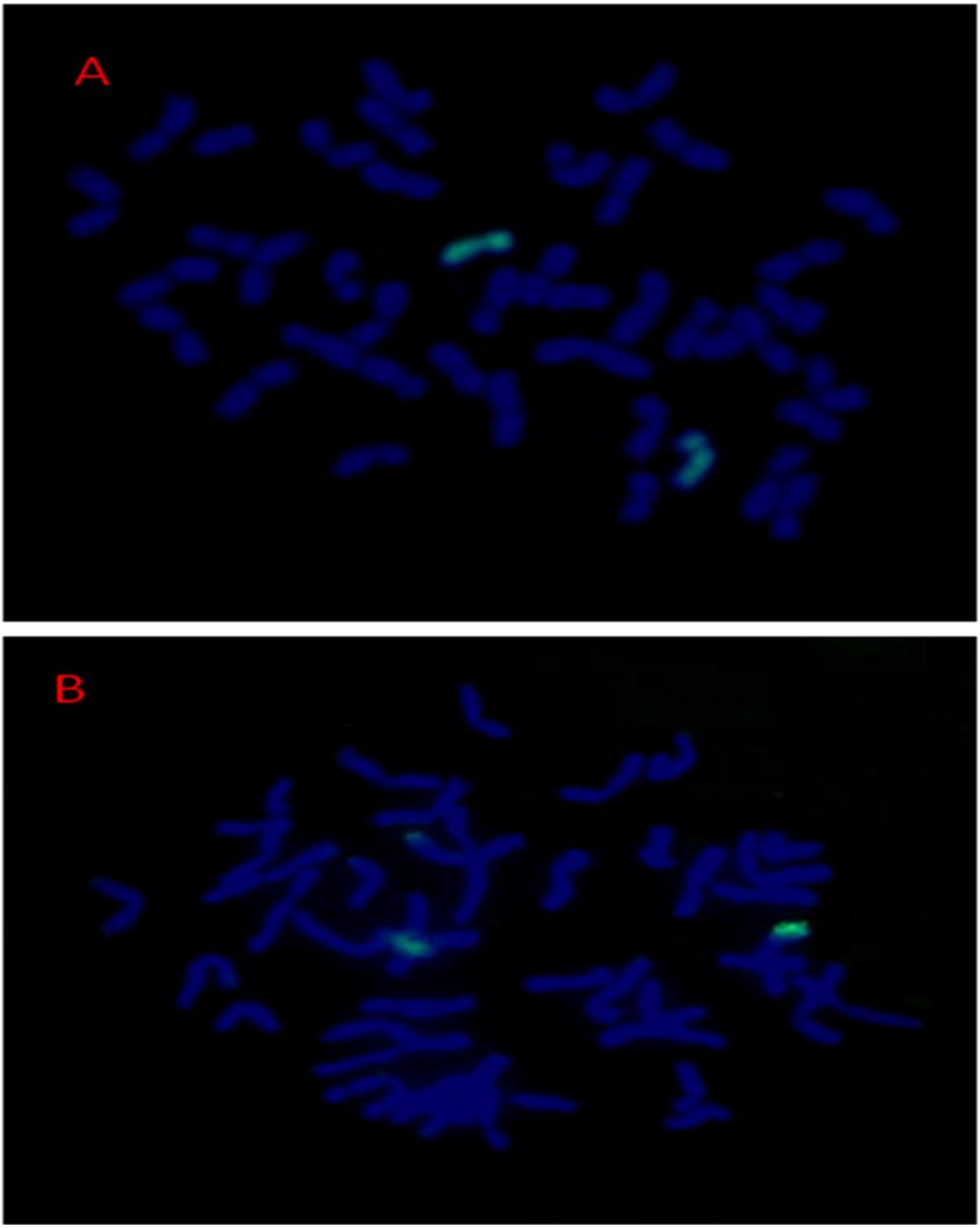

Fig. 4.2.9 GISH showing introgression of alien chromosome fragment (green) of PRH_1 of pollen irradiated plants of CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗/PBW343 (*GPC+ Lr24*) A; PRH_1-82, B; PRH_1-124

Fig. 4.2.10 Plant morphology and incidence of powdery mildew of PRH_1 of pollen irradiated plants of CS(*Ph*^{I})/ *Ae.kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC+ Lr24*). a) 48-41-6⊗ a; powdery mildew resistant and leaf rust susceptible, b; PRH_1-124 resistant to both powdery mildew and leaf rust, c; PRH_1-82 susceptible to both powdery mildew and leaf rust, d; PBW343(*GPC+ Lr24)* resistant to leaf rust and susceptible to powdery mildew

Table 4.2.5 Micronutrient content and morphological data of PRH_2 plants of CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC+ Lr24*). (Eternal University, Baru Sahib, 2012-13)

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-17-1	31.25	3.72	32.66	10.82	5	9	21.8	54.77
PRH_2-17-2	34.85	15.67	32.48	10.21	4	9	19.8	49.75
PRH_2-17-4	**36.35**	**20.64**	**36.32**	**23.26**	**2**	**14**	**26.1**	**51.08**
PRH_2-17-5	34.90	15.83	36.17	22.73	3	9	21.3	51.70
PRH_2-68-1	32.40	7.53	28.78	-2.33	6	13	26.2	51.07
PRH_2-68-3	26.10	-13.38	29.13	-1.16	2	14	25.1	46.92
PRH_2-68-5	18.90	-37.27	28.56	-3.07	2	14	19.9	39.48
PRH_2-68-18	27.35	-9.23	29.53	0.20	3	13	26.8	49.26
PRH_2-72-1	29.95	-0.60	30.89	4.82	6	8	17.8	52.51
PRH_2-72-2	24.85	-17.52	30.00	1.81	4	17	35.2	49.86
PRH_2-72-3	5.00	-83.41	34.66	17.61	2	23	44.7	53.92
PRH_2-72-5	28.70	-4.75	29.84	1.27	3	8	16.6	53.38
PRH_2-75-2	30.20	0.23	30.27	2.71	0	10	20.1	51.80
PRH_2-75-6	30.05	-0.27	29.60	0.45	3	15	22.3	50.80
PRH_2-75-7	26.00	-13.71	29.09	-1.28	0	12	23.2	50.54
PRH_2-75-9	26.20	-13.04	31.56	7.09	4	12	23.4	50.43
PRH_2-75-10	26.55	-11.88	33.79	14.66	1	19	39.2	49.12
PRH_2-75-14	-22.35	-174.18	30.66	4.04	2	11	20.0	50.76
PRH_2-75-15	20.65	-31.46	27.97	-5.09	0	9	14.2	48.80
PRH_2-79-1	26.55	-11.88	30.01	1.83	7	9	20.5	58.57
PRH_2-79-2	36.30	20.48	35.60	20.80	6	12	20.0	54.20
PRH_2-79-3	31.80	5.54	33.02	12.04	3	9	22.7	54.96

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2 -79-4	31.35	4.05	32.20	9.28	2	17	34.2	52.86
PRH_2 -79-5	29.45	-2.26	30.58	3.78	2	13	28.8	55.49
PRH_2 -82-1	26.50	-12.05	29.22	-0.83	3	10	22.4	49.67
PRH_2 -82-2	30.85	2.39	31.15	5.70	0	9	18.7	52.68
PRH_2 -82-4	25.80	-14.37	30.61	3.88	4	10	19.5	50.26
PRH_2 -82-5	32.10	6.54	33.30	13.01	5	8	19.9	52.79
PRH_2 -82-6	31.35	4.05	33.18	12.60	6	16	39.0	57.44
PRH_2 -82-8	31.10	3.22	33.41	13.37	4	15	28.5	50.71
PRH_2 -82-9	29.45	-2.26	32.26	9.47	2	15	30.7	52.21
PRH_2 -82-10	34.30	13.84	33.12	12.40	5	13	31.2	53.89
PRH_2 -82-14	33.75	12.01	32.78	11.23	3	14	27.3	50.28
PRH_2 -82-15	31.90	5.87	32.28	9.53	3	21	48.6	53.88
PRH_2 -82-17	32.35	7.37	32.89	11.62	6	15	27.7	51.30
PRH_2 -82-18	25.40	-15.70	29.22	-0.83	7	11	20.3	54.72
PRH_2 -82-19	19.10	-36.61	26.20	-11.08	2	10	18.1	48.79
PRH_2 -82-20	30.20	0.23	31.78	7.85	2	20	30.6	50.92
PRH_2 -87-1	22.70	-24.66	31.27	6.12	3	12	26.6	50.47
PRH_2 -87-3	28.35	-5.91	33.16	12.52	6	15	26.4	51.06
PRH_2 -87-4	**77.45**	**157.05**	**31.03**	**5.29**	**4**	**16**	**34.5**	**55.11**
PRH_2 -87-6	27.65	-8.23	29.38	-0.29	6	11	19.0	48.72
PRH_2 -87-7	23.00	-23.66	26.50	-10.07	0	10	23.0	51.34
PRH_2 -87-8	27.05	-10.22	29.65	0.61	3	11	21.6	48.43
PRH_2 -87-10	**35.60**	**18.15**	**34.69**	**17.71**	**2**	**12**	**23.9**	**51.31**
PRH_2 -87-11	29.10	-3.42	29.47	0.01	7	16	32.7	54.32

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2 -87-13	26.80	-11.05	34.10	15.73	1	10	31.0	57.09
PRH_2 -87-14	22.35	-25.82	31.77	7.80	7	14	27.6	51.78
PRH_2 -87-15	24.95	-17.19	30.54	3.64	7	15	34.7	53.63
PRH_2 -87-16	28.60	-5.08	28.30	-3.96	3	14	29.2	49.74
PRH_2 -91-5	**67.80**	**125.02**	**78.06**	**164.90**	**2**	**8**	**10.8**	**34.29**
PRH_2 -91-6	**32.70**	**8.53**	**44.75**	**51.87**	**0**	**15**	**16.4**	**35.42**
PRH_2 -114-1	23.25	-22.83	23.82	-19.18	0	9	17.0	48.99
PRH_2 -114-2	27.80	-7.73	26.51	-10.05	0	15	22.6	48.60
PRH_2 -114-3	35.60	18.15	30.94	5.00	0	14	21.0	48.39
PRH_2 -114-4	40.75	35.25	26.73	-9.29	2	12	25.1	50.30
PRH_2 -114-5	34.95	16.00	27.67	-6.10	5	11	26.8	53.92
PRH_2 -124-1	28.20	-6.41	33.89	15.01	0	9	20.8	54.88
PRH_2 -124-5	**41.00**	**36.08**	**39.33**	**33.46**	**4**	**11**	**25.4**	**51.94**
PRH_2 -124-6	27.10	-10.06	36.83	24.97	1	13	28.7	55.19
PRH_2 -124-9	**32.85**	**9.03**	**41.68**	**41.45**	**1**	**11**	**18.1**	**43.51**
PRH_2 -124-11	36.90	22.47	33.38	13.28	1	17	38.6	51.74
PRH_2 -124-13	**77.10**	**155.89**	**27.29**	**-7.40**	**2**	**17**	**46.5**	**56.57**
PRH_2 -124-14	31.90	5.87	33.02	12.04	2	7	14.7	55.68
PRH_2 -124-15	**38.05**	**26.29**	**39.07**	**32.59**	**2**	**7**	**22.9**	**56.40**
PRH_2 -124-16	29.90	-0.76	35.12	19.19	3	10	27.0	56.72
PRH_2 -124-17	27.05	-10.22	29.37	-0.33	2	12	33.7	52.33
PRH_2 -124-18	**34.85**	**15.67**	**40.05**	**35.90**	**0**	**9**	**29.2**	**74.49**
PRH_2 -132-1	33.85	12.35	34.94	18.56	0	11	28.0	56.11
PRH_2 -132-2	26.65	-11.55	27.35	-7.20	3	13	30.7	53.77

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-132-3	33.75	12.01	27.89	-5.35	4	19	45.6	58.24
PRH_2-132-4	28.45	-5.58	39.34	33.49	0	12	28.2	54.55
PRH_2-132-5	28.10	-6.74	33.87	14.95	1	13	28.3	51.83
PRH_2-132-6	33.40	10.85	28.32	-3.91	0	12	25.5	50.00
PRH_2-132-7	34.90	15.83	30.22	2.56	2	16	36.8	53.10
PRH_2-132-8	25.50	-15.37	29.92	1.54	1	15	29.8	54.68
PRH_2-132-9	26.40	-12.38	28.15	-4.47	1	6	13.1	51.57
PRH_2-132-10	26.75	-11.22	27.34	-7.22	2	12	22.7	51.83
PRH_2-133-1	23.55	-21.84	28.34	-3.82	2	18	30.6	50.00
PRH_2-133-2	25.85	-14.21	27.58	-6.40	2	14	34.6	58.45
PRH_2-133-3	29.95	-0.60	32.30	9.62	2	12	31.2	58.65
PRH_2-133-4	23.80	-21.01	29.83	1.22	4	14	40.8	52.78
PRH_2-133-5	24.90	-17.36	29.26	-0.70	1	17	34.0	51.91
PRH_2-133-6	22.70	-24.66	31.19	5.83	0	11	25.8	52.87
PRH_2-133-8	18.95	-37.11	29.51	0.13	7	15	30.2	52.16
PRH_2-133-9	22.80	-24.33	26.79	-9.10	5	12	33.1	59.64
PRH_2-133-10	23.00	-23.66	29.99	1.78	2	13	22.5	49.78
PRH_2-143-1	**38.15**	**26.62**	**35.38**	**20.07**	**1**	**11**	**26.5**	**56.14**
PRH_2-143-2	33.10	9.86	28.31	-3.94	2	10	21.2	52.87
PRH_2-143-3	33.20	10.19	29.32	-0.51	2	17	40.1	52.83
PRH_2-143-4	35.85	18.98	23.33	-20.84	4	15	25.5	54.72
PRH_2-143-5	33.45	11.02	25.98	-11.85	2	12	23.1	51.91
PBW343	30.13	-	29.46	-	9	13	25.24	52.19

There is a negative correlation (r) -0.82 and -0.88 for Fe and Zn content, respectively with harvest index. The correlation between Fe and Zn content with yield per plant was found (r) -0.56 and -0.64, respectively, indicating high micronutrient content could be due to concentration effect and is further linked to seed shape, shrivelled seed having higher grain Fe and Zn content compared to the bold seeds probably because of aleurone layer ratio to the total seed mass. But there are some plants in the table 4.2.4 i.e. plant PRH_1-75, PRH_1-82, PRH_1-87, PRH_1-124, PRH_1-132 which have high harvest index and bold seeds as indicated by 1000 grain weight, had up to 98% fertility and still have 20-74% increase in Fe or Zn or both the elements as compared to the cultivars. These plants were selected for molecular marker analysis and GISH for identification of the fragment of chromosome of *Ae. kotschyi* controlling the micronutrient. Plant PRH_1-82, PRH_1-124 had the alien transfer as shown in Fig. 4.2.9.

4.2.7 Micronutrient content and morphological data of PRH_2 plants of CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC+ Lr24*)

Micronutrient and morphological data of PRH_2 plants of CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗/PBW343 is given in table 4.2.5. Plants were selected on the basic of good morphology and equivalent harvest index over the control i.e. PBW343 (*GPC+Lr24*). Grain Fe concentration varied between 18.9 mg/kg to 77.45 mg/kg and grain Zn concentration varied between 23.32 mg/kg to 164.9 mg/kg. Fe and Zn content of PBW343 (control) were 30.13 mg/kg and 29.46 mg/kg, respectively. The 63-2-13 ⊗ had 55.6 mg/kg Fe and 68.4 mg/kg Zn content. The Fe content had no correlation with harvest index and yield but Zn had negative correlation (r) -0.17 and -0.25 with yield and harvest index. It was observed that the plants having high grain Fe and Zn content were resistant to powdery mildew in PRH_2 plants.

4.2.8 Pollen irradiation of CS(*Ph*^{I})/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗

Pollen grain of 63-2-13⊗ derivative were irradiated at 2 Krad and used for pollination of PBW343 (*Lr24+GPC*). 500 PRH_1 seeds were obtained, out of which 243 germinated under lab condition. 230 out of 243 survived in the field, showing lot of variation for leaf surface area, growth habit, number of tillers per plant, head type and susceptibility to powdery mildew. Nevertheless 95 out of 230 plants were fertile, remaining were sterile.

4.2.9 Micronutrient content and morphological data of PRH_1 of pollen irradiation of CS(*Ph*^{I})/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*)

The plants resembled recurrent parent in tiller number, plant height, head type etc . whereas seed colour was amber for most of the derivatives. All PRH_1 plants were susceptible to powdery mildew indicating that the genes for powdery mildew resistance could be present on 7S not on 7U chromosome of the *Ae. kotschyi*. The PRH_1 plants having fair seed set were analysed for their grain Fe and Zn content (Table 4.2.6), whereas the plants which were still sterile or partially fertile with very low seed set were discarded. Seeds of some plants were as bold as or even bolder than their wheat parents and had Fe and Zn concentrations in the range of 4.04 to 133.16 mg/kg and 22.12 to 124.15 mg/kg respectively, as compared to 30.82 mg/kg, and 30.93 mg/kg Zn for PBW343 the elite cultivar.

Again Fe and Zn contents were negatively correlated with harvest index and yield indicating concentration effect. The correlation of Fe was (r) - 0.43 and -0.28 with harvest index and yield per plant, respectively. The correlation of Zn was (r) - 0.53 and -0.28 with harvest index and yield per plant, respectively. Some of the plants had 20-125% increase in grain Fe content and 40-140% increase of grain Zn content or 40-60% increase of both elements over PBW343 while high harvest index. Plant PRH_1-201, 237, 258, 312 and 368 were selected for GISH. PRH_1-312 had the translocation of 7U chromosome of *Ae. kotschyi 396* (Fig. 4.2.12), good morphology (Fig. 4.2.13), harvest index and yield (Fig. 4.2.11).

Table 4.2.6 Micronutrient content and morphological data of PRH_1 plants of pollen irradiation of CS(*Ph*^{I})/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*)

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
WL711	27.83	-9.71	38.13	23.30	15	41.34	20.0	46.51	9
PBW343	30.82	0.00	30.93	0.00	14	40.56	23.0	45.10	9
PRH_1-201	**59.29**	**92.37**	**43.35**	**40.15**	**18**	**43.23**	**26.0**	**48.15**	**7**
PRH_1-204	27.25	-11.60	45.90	48.40	16	38.43	36.5	56.59	7
PRH_1-205	**30.48**	**-1.11**	**87.93**	**184.31**	**33**	**39.61**	**27.5**	**28.80**	**8**
PRH_1-206	18.59	-39.70	37.17	20.17	9	29.51	11.0	44.00	5
PRH_1-207	18.89	-38.71	42.79	38.36	4	42.74	6.5	61.90	3
PRH_1-208	19.23	-37.59	38.94	25.92	23	46.13	35.0	43.21	3
PRH_1-209	23.57	-23.54	38.62	24.86	15	48.41	37.5	51.02	2
PRH_1-210	23.20	-24.74	40.88	32.18	27	48.31	68.0	54.84	3
PRH_1-211	32.53	5.53	51.96	67.99	16	42.58	25.5	53.68	6
PRH_1-212	28.72	-6.82	93.27	201.59	20	43.53	46.0	54.76	6
PRH_1-214	40.93	32.81	50.17	62.20	14	29.41	1.0	3.45	9
PRH_1-215	133.16	332.04	124.15	301.42	22	35.91	2.5	7.69	8
PRH_1-217	43.46	41.00	85.32	175.88	15	40.56	6.5	17.81	7
PRH_1-219	46.57	51.10	31.37	1.43	40	37.71	83.0	49.70	2
PRH_1-221	34.34	11.41	45.96	48.60	27	39.51	61.0	54.95	8
PRH_1-222	20.19	-34.50	48.82	57.86	13	37.21	25.0	53.19	7
PRH_1-223	28.45	-7.71	40.90	32.25	19	31.07	22.0	42.31	8
PRH_1-224	15.10	-51.01	39.89	28.99	19	38.68	29.5	49.58	6
PRH_1-225	25.60	-16.94	47.04	52.09	21	44.99	42.0	56.76	6

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
PRH_1-226	40.63	31.82	22.12	-28.47	18	40.31	29.0	49.15	6
PRH_1-227	41.99	36.23	38.56	24.68	27	41.12	45.5	50.84	6
PRH_1-228	31.83	3.28	30.22	-2.30	18	41.93	36.0	52.94	5
PRH_1-229	16.85	-45.32	32.21	4.16	16	37.74	30.0	51.72	6
PRH_1-230	30.38	-1.43	44.73	44.63	18	35.67	15.0	36.59	8
PRH_1-231	**44.41**	**44.09**	**75.21**	**143.18**	**11**	**34.24**	**15.5**	**39.24**	**8**
PRH_1-232	24.55	-20.36	42.02	35.86	13	38.75	24.5	52.69	7
PRH_1-233	24.74	-19.74	42.14	36.25	20	38.48	43.0	58.90	6
PRH_1-234	23.13	-24.96	38.13	23.28	26	38.29	45.0	49.45	6
PRH_1-236	8.19	-73.42	50.13	62.08	17	45.46	18.5	43.53	5
PRH_1-237	**69.30**	**124.84**	**26.27**	**-15.05**	**25**	**45.96**	**62.0**	**55.36**	
PRH_1-238	**48.82**	**58.40**	**87.04**	**181.43**	**17**	**36.68**	**21.0**	**33.33**	**7**
PRH_1-239	25.82	-16.22	43.64	41.11	31	40.29	55.0	50.46	6
PRH_1-240	44.52	44.44	61.94	100.28	14	40.11	10.0	29.41	8
PRH_1-241	26.05	-15.47	49.21	59.13	30	42.46	90.0	50.00	6
PRH_1-245	23.47	-23.87	41.43	33.97	27	40.23	56.0	53.85	7
PRH_1-246	95.83	210.93	155.39	402.43	4	37.76	1.5	100.00	7
PRH_1-247	**65.95**	**113.98**	**52.38**	**69.35**	**20**	**42.36**	**27.5**	**32.93**	**8**
PRH_1-248	19.29	-37.40	80.87	161.48	12	41.51	4.5	21.95	8
PRH_1-249	25.94	-15.85	39.15	26.57	18	41.44	37.5	55.56	6
PRH_1-251	29.80	-3.32	47.88	54.80	20	39.15	51.5	57.54	2
PRH_1-252	76.63	148.62	124.17	301.50	14	48.74	6.0	12.50	8
PRH_1-253	28.54	-7.41	52.86	70.93	17	48.51	40.0	54.05	7
PRH_1-255	38.28	24.19	32.37	4.67	30	37.51	64.5	52.65	6

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
PRH_1-256	63.74	106.79	73.75	138.47	16	45.42	11.0	22.45	7
PRH_1-257	20.57	-33.28	34.55	11.72	6	35.31	8.0	50.00	8
PRH_1-258	**37.52**	**21.72**	**56.29**	**82.01**	**24**	**40.61**	**52.0**	**49.06**	**6**
PRH_1-261	27.85	-9.64	56.94	84.12	6	42.83	5.5	41.00	6
PRH_1-262	28.45	-7.70	40.96	32.43	18	40.06	30.5	52.14	7
WL711	23.92	-14.72	32.31	-19.04	15	41.34	20.0	46.51	9
PBW343	28.05	0.00	39.91	0.00	14	40.56	23.0	45.10	9
PRH_1-263	22.45	-19.96	59.52	49.14	8	36.38	7.5	41.00	6
PRH_1-264	45.30	61.49	35.51	-11.00	32	37.49	70.5	51.65	7
PRH_1-266	**71.52**	**154.98**	**72.05**	**80.55**	**5**	**41.31**	**3.0**	**33.33**	**6**
PRH_1-267	15.31	-45.42	44.29	10.98	3	23.31	2.5	50.00	7
PRH_1-269	4.04	-85.58	41.87	4.92	5	44.45	12.0	54.55	7
PRH_1-270	30.68	9.36	32.68	-18.10	37	38.22	90.0	53.57	2
PRH_1-272	20.94	-25.35	47.32	18.58	17	34.56	44.0	56.41	3
PRH_1-273	6.37	-77.29	32.07	-19.64	20	40.86	44.5	52.66	3
PRH_1-274	13.62	-51.43	40.83	2.32	6	39.55	4.5	31.03	7
PRH_1-276	**80.37**	**186.54**	**86.22**	**116.05**	**15**	**29.56**	**3.0**	**35.03**	**7**
PRH_1-277	26.57	-5.27	48.03	20.35	27	42.04	79.0	56.83	6
PRH_1-279	26.59	-5.22	68.80	72.39	20	46.06	16.0	34.78	5
PRH_1-283	27.28	-2.75	54.33	36.15	9	40.69	14.5	59.18	6
PRH_1-286	34.52	23.08	81.56	104.39	18	31.82	3.0	9.68	7
PRH_1-288	24.04	-14.30	64.26	61.02	9	35.53	7.0	41.18	5
PRH_1-290	49.54	76.62	119.07	198.37	26	26.41	3.5	7.64	7
PRH_1-297	7.41	-73.58	47.98	20.23	10	43.55	13.5	23.48	7

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	No. of Tiller/Plant	1000 grain weight	Yield in grams	Harvest index	Powdery mildew
PRH_1-298	23.56	-16.02	66.00	65.38	27	39.22	27.5	40.06	8
PRH_1-301	44.58	58.92	111.18	178.59	24	34.51	2.0	8.12	5
PRH_1-309	49.40	76.10	39.73	-0.44	33	40.44	58.5	52.94	8
PRH_1-310	81.25	189.65	72.01	80.45	10	43.37	2.5	13.51	8
PRH_1-311	13.70	-51.16	40.18	0.69	29	38.14	62.5	49.41	8
PRH_1-312	**47.85**	**70.58**	**65.44**	**64.00**	**28**	**45.88**	**70.0**	**51.47**	**6**
PRH_1-315	48.82	74.05	80.54	101.82	19	44.71	6.0	14.29	6
PRH_1-316	**52.11**	**85.76**	**74.93**	**87.76**	**21**	**39.45**	**5.5**	**11.58**	**6**
PRH_1-317	27.64	-1.47	41.64	4.36	34	34.13	55.5	49.78	7
PRH_1-318	72.47	158.36	84.54	111.84	10	42.13	3.0	15.79	7
PRH_1-320	**37.46**	**33.55**	**56.28**	**41.04**	**27**	**41.71**	**31.0**	**40.26**	**7**
PRH_1-322	28.43	1.34	99.10	148.33	12	25.04	2.0	8.33	7
PRH_1-323	**64.17**	**128.76**	**66.49**	**66.61**	**7**	**37.83**	**5.5**	**25.58**	**7**
PRH_1-327	34.20	21.92	48.39	21.27	31	37.64	71.0	53.38	5
PRH_1-328	20.65	-26.39	42.63	6.83	41	44.71	84.5	50.75	7
PRH_1-331	**78.16**	**178.65**	**113.90**	**185.43**	**29**	**43.81**	**17.0**	**21.52**	**7**
PRH_1-338	77.95	177.89	115.48	189.38	23	45.44	10.0	15.63	8
PRH_1-339	71.63	155.37	122.53	207.05	25	39.88	9.0	11.69	8
PRH_1-340	18.44	-34.27	67.84	70.01	60	41.61	16.5	41.00	8
PRH_1-346	29.98	6.89	43.19	8.23	50	43.09	114.0	52.29	3
PRH_1-349	103.02	267.26	112.71	182.44	16	35.56	4.5	7.44	2
PRH_1-351	38.52	37.32	37.85	-5.14	31	43.36	63.5	56.44	1
PRH_1-354	35.17	25.39	131.91	230.56	24	30.44	7.0	22.58	5
PRH_1-368	**55.32**	**97.20**	**69.72**	**74.72**	**17**	**43.16**	**17.5**	**40.00**	**4**

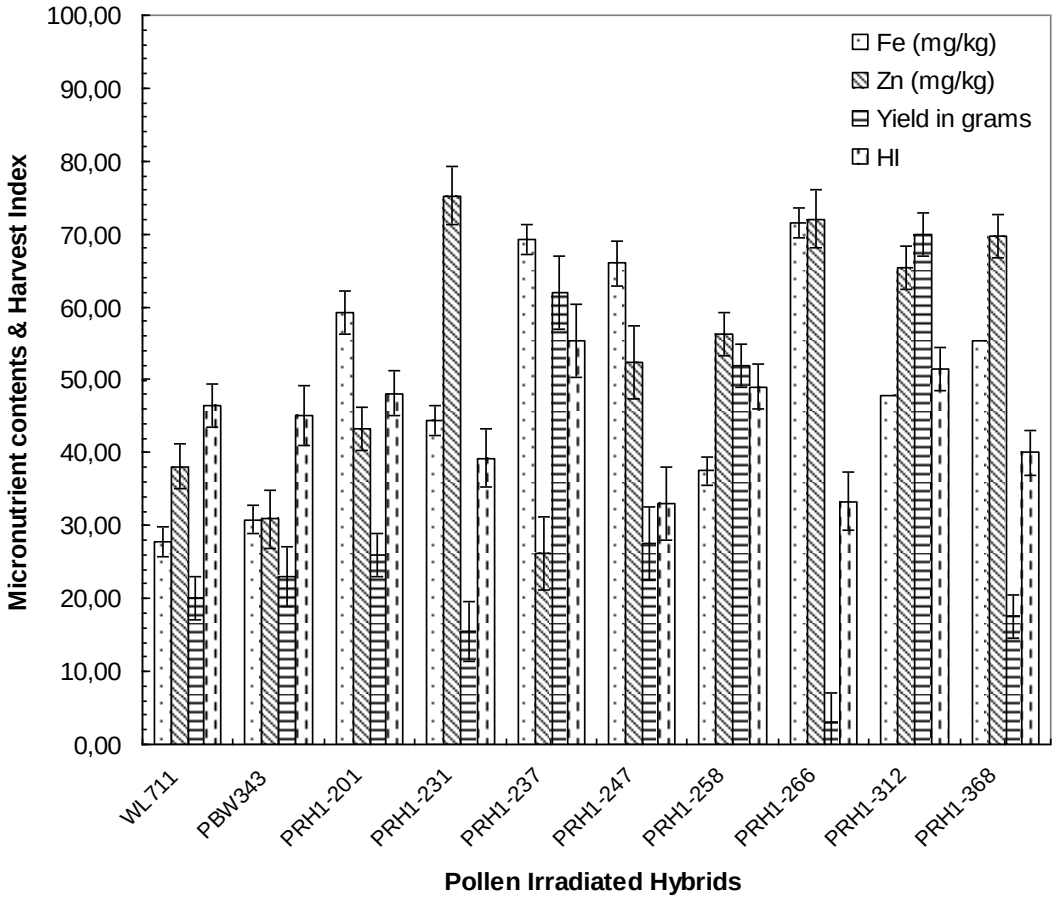

Fig. 4.2.11 Graphical representation of micronutrient data of PRH_1 plants CS(Ph^I)/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*) after pollen irradiation

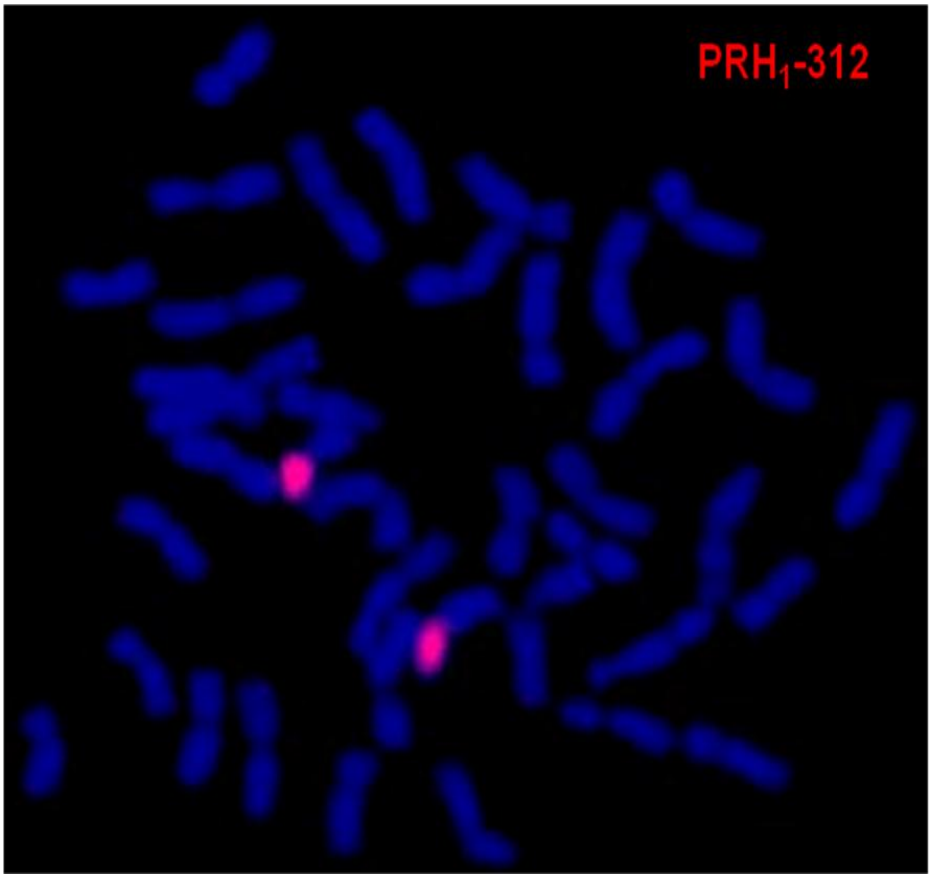

Fig. 4.2.12 GISH showing translocation (red) in PRH_1-312 after pollen irradiation of CS(*Ph*^{I})/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*)

Fig. 4.2.13 Morphology of PRH_1-312 CS(*Ph*^{I})/ *Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*), with parents

Table 4.2.7 Micronutrient and morphological data of PRH_2 of pollen irradiation of CS(*Ph*^{I})/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*) (Eternal University, Baru Sahib, 2012-13)

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-201-2	29.95	-0.60	29.92	1.54	2	12	30.2	61.26
PRH_2-201-3	37.40	24.13	30.94	5.00	1	19	24.2	54.63
PRH_2-201-5	29.80	-1.10	35.33	19.90	2	14	26.8	51.74
PRH_2-201-6	**42.45**	**40.89**	**39.75**	**34.90**	**3**	**13**	**17.7**	**52.68**
PRH_2-201-8	**41.10**	**36.41**	**46.69**	**58.45**	**2**	**10**	**22.2**	**49.01**
PRH_2-205-1	35.75	18.65	36.27	23.09	0	14	29.3	52.79
PRH_2-205-3	37.25	23.63	28.78	-2.33	3	6	14.5	57.09
PRH_2-205-5	35.85	18.98	27.26	-7.50	0	14	28.2	51.09
PRH_2-205-6	28.80	-4.41	32.77	11.21	0	10	21.9	57.18
PRH_2-205-7	39.65	31.60	26.79	-9.10	0	11	16.7	50.00
PRH_2-205-8	31.45	4.38	31.28	6.14	0	9	13.6	45.03
PRH_2-205-9	36.85	22.30	38.47	30.56	6	8	15.7	48.16
PRH_2-231-1	25.20	-16.36	30.91	4.90	5	9	18.9	54.94
PRH_2-231-3	27.35	-9.23	32.67	10.87	2	10	23.2	53.70
PRH_2-231-6	22.90	-24.00	25.94	-11.98	3	9	17.1	51.51
PRH_2-231-7	25.25	-16.20	28.14	-4.52	4	9	19.5	54.02
PRH_2-231-8	28.00	-7.07	31.63	7.34	3	16	34.0	52.15
PRH_2-231-10	24.30	-19.35	25.06	-14.95	4	9	16.9	52.98
PRH_2-231-11	10.85	-63.99	29.18	-0.97	0	14	28.2	54.97
PRH_2-231-12	24.70	-18.02	27.39	-7.05	4	8	16.8	52.83
PRH_2-231-13	22.20	-26.32	27.31	-7.32	2	11	21.0	56.00

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-231-14	24.95	-17.19	28.11	-4.60	0	10	20.6	54.21
PRH_2-231-15	21.15	-29.80	30.26	2.68	3	10	20.7	51.11
PRH_2-237-1	**37.30**	**23.80**	**47.16**	**60.03**	**7**	**12**	**18.1**	**40.31**
PRH_2-237-2	**37.50**	**24.46**	**40.23**	**36.53**	**4**	**9**	**23.9**	**47.90**
PRH_2-237-5	32.00	6.21	40.39	37.06	7	11	7.9	23.51
PRH_2-238-1	**59.65**	**97.98**	**54.91**	**86.35**	**6**	**12**	**26.3**	**100.00**
PRH_2-238-2	**46.60**	**54.66**	**37.74**	**28.06**	**6**	**12**	**22.3**	**48.48**
PRH_2-238-3	**46.40**	**54.23**	**29.35**	**-0.39**	**3**	**10**	**26.2**	**48.97**
PRH_2-238-4	37.85	25.62	37.46	27.11	8	13	28.2	50.63
PRH_2-238-5	33.45	11.02	36.92	25.28	0	11	21.9	50.34
PRH_2-238-6	33.00	9.53	30.13	2.25	7	13	22.0	51.16
PRH_2-247-3	**40.90**	**35.75**	**47.63**	**61.63**	**6**	**15**	**19.2**	**42.95**
PRH_2-247-4	**45.80**	**52.01**	**40.65**	**37.96**	**7**	**13**	**18.0**	**45.00**
PRH_2-258-1	33.50	11.18	31.50	6.89	0	16	31.1	55.04
PRH_2-258-3	39.50	31.10	27.93	-5.23	0	12	26.4	51.66
PRH_2-258-4	**71.50**	**137.31**	**26.83**	**-8.95**	**0**	**17**	**28.8**	**53.43**
PRH_2-258-5	33.05	9.69	26.64	-9.61	0	11	22.7	50.00
PRH_2-258-6	32.10	6.54	26.23	-11.00	2	9	16.5	50.77
PRH_2-258-7	29.85	-0.93	27.42	-6.96	0	12	25.9	50.19
PRH_2-258-9	29.60	-1.76	28.66	-2.74	0	10	19.1	51.48
PRH_2-258-10	31.30	3.88	27.15	-7.88	2	33	59.0	53.44
PRH_2-258-11	33.90	12.51	25.68	-12.87	3	18	22.0	50.69
PRH_2-258-12	32.90	9.19	27.05	-8.20	0	12	22.0	51.64

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-258-16	35.20	16.83	28.32	-3.89	4	16	30.6	59.77
PRH_2-258-18	34.35	14.01	29.19	-0.95	3	15	26.5	50.38
PRH_2-258-20	32.45	7.70	26.65	-9.57	4	10	17.5	51.17
PRH_2-266-1	31.10	3.22	31.78	7.85	4	17	48.8	54.04
PRH_2-276-1	30.40	0.90	26.84	-8.93	3	19	33.8	50.52
PRH_2-276-2	**36.55**	**21.31**	**42.73**	**45.00**	**6**	**8**	**16.9**	**46.81**
PRH_2-312-1	31.74	5.34	36.38	23.48	2	16	32.2	53.14
PRH_2-312-3	26.34	-12.58	26.88	-8.78	4	11	15.8	50.48
PRH_2-312-4	28.70	-4.75	26.84	-8.93	0	9	17.4	49.57
PRH_2-312-5	24.40	-19.02	27.25	-7.52	2	14	34.1	57.99
PRH_2-312-6	34.40	14.17	24.37	-17.31	2	9	14.7	51.94
PRH_2-312-7	28.20	-6.41	35.45	20.31	2	8	20.1	50.12
PRH_2-312-8	28.55	-5.24	35.22	19.51	3	8	13.5	53.15
PRH_2-312-10	19.30	-35.94	26.10	-11.42	0	6	9.7	51.32
PRH_2-312-11	**39.40**	**30.77**	**34.34**	**16.54**	**3**	**23**	**30.5**	**53.89**
PRH_2-312-12	32.35	7.37	27.41	-6.99	3	17	34.2	57.29
PRH_2-312-13	38.40	27.45	32.87	11.54	4	13	25.9	51.59
PRH_2-312-14	27.55	-8.56	34.37	16.63	3	11	19.6	50.39
PRH_2-312-15	39.55	31.26	34.21	16.10	3	8	16.5	52.22
PRH_2-312-17	**35.15**	**16.66**	**35.18**	**19.37**	**5**	**6**	**12.0**	**52.17**
PRH_2-312-18	**40.90**	**35.75**	**28.64**	**-2.80**	**4**	**13**	**11.8**	**54.63**
PRH_2-312-19	39.60	31.43	27.66	-6.15	4	5	8.3	58.04
PRH_2-312-20	27.95	-7.24	35.28	19.71	4	4	19.6	65.77

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	Powdery mildew	No. of Tiller/Plant	Yield in grams	Harvest index
PRH_2-312-21	31.90	5.87	38.72	31.41	3	6	9.5	50.80
PRH_2-312-22	**48.05**	**59.48**	**33.41**	**13.38**	**2**	**5**	**11.1**	**52.11**
PRH_2-316-1	**45.40**	**50.68**	**39.82**	**35.14**	**4**	**9**	**16.2**	**48.21**
PRH_2-316-2	36.25	20.31	42.41	43.91	2	13	32.5	50.39
PRH_2-316-3	**38.35**	**27.28**	**41.22**	**39.89**	**5**	**8**	**17.1**	**43.73**
PRH_2-320-1	28.65	-4.91	39.77	34.97	0	17	39.8	53.86
PRH_2-320-5	30.65	1.73	36.68	24.48	7	18	29.5	45.60
PRH_2-331-1	**34.90**	**15.83**	**36.15**	**22.67**	**4**	**17**	**29.9**	**47.46**
PRH_2-331-2	**53.70**	**78.23**	**41.31**	**40.18**	**6**	**18**	**34.5**	**46.00**
PRH_2-331-3	**39.65**	**31.60**	**43.14**	**46.39**	**6**	**14**	**26.2**	**44.56**
PRH_2-331-4	**44.95**	**49.19**	**48.03**	**63.00**	**8**	**23**	**22.7**	**40.75**
PRH_2-368-2	33.85	12.35	33.04	12.11	4	15	24.0	50.85
PRH_2-368-6	33.65	11.68	37.71	27.98	5	24	35.4	52.14
PRH_2-368-7	28.70	-4.75	25.89	-12.14	7	16	45.8	53.69
PRH_2-368-9	24.85	-17.52	30.28	2.76	7	22	30.7	48.65
PBW343	30.13	-	29.46	-	9	13	25.24	52.19

4.2.10 Micronutrient and morphological data of PRH_2 of pollen irradiation of CS(*Ph*^{I})/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24*)

PRH_2 plants of Cs(*Ph*^{I})/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 were analysed for micronutrient content after doing selection on the basis of morphology and harvest index. It was found that Fe content of PRH_2 plants varied in the range of 19.3 mg/kg to 71.5 mg/kg and Zn content varied in the range of 22.4 mg/kg to 48.03 mg/kg. On the other hand Fe and Zn content of PBW343(*GPC+ Lr24*) was 30.13 mg/kg and 29.46 mg/kg respectively (Table 4.2.7). No correlation was found for Fe and Zn concentration with harvest index and yield per plant.

4.3 The *ph1b* induced transfer of genes for high grain Fe and Zn content from 7S substitution line CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗

CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373**(**48)-41-6⊗ derivative having uniform substitution 7D/7S was crossed with Pavon (*ph1bph1b*) and back crossed again with Pavon (*ph1bph1b*) to get homozygous *ph1b* deletion. The BC_1F_1 seedlings were screened for presence of the *ph1b* linked marker psr574 and the absence of amplification indicated homozygous *ph1b*. Wheat anchored SSR markers, wmc 405 and barc126, polymorphic between wheat and *Ae. kotschyi* were used for the identification of 7S chromosome monosomic (Fig. 4.3.1, 4.3.2). The plant which were homozygous for *ph1b* and monosomic for 7S chromosome were selected and sown for getting BC_1F_2.

BC_1F_1 plants were selected for *ph1b* homozygous and 7S monosomics. Plant BC_1F_1-13, BC_1F_1-14, and BC_1F_1-43 had *ph1b* homozygous and 7S monosomics (Table 4.3.1). These plants were selfed to BC_1F_2 as bulk. The remaining ph1b homozygous plant were either disomic or nullisomic for 7S chromosome.

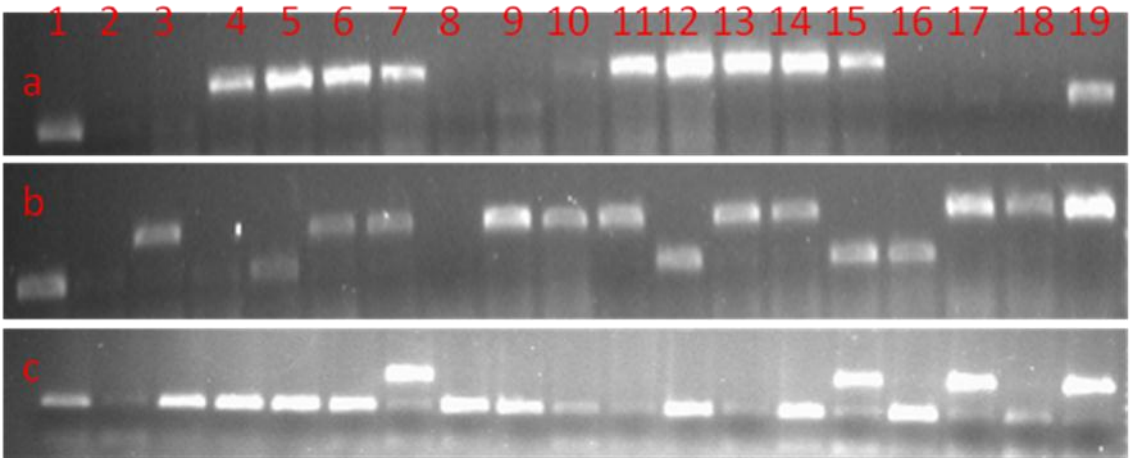

Fig. 4.3.1 Molecular marker analysis (psr574) linked to *ph1b* BC_1F_1 plants. Identification of ph1b homozygous plants in BC_1F_1 of CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/*ph1bph1b//ph1bph1b* using dominant psr574 marker linked to ph1b deletion. a) Lanes. 1. PBW343, 2. Pavon(*ph1b*) 3-19. (1-17) BC_1F_1 plants., b) Lanes. 1. PBW343 , 2. Pavon (*ph1b*) 3-19. (18-34) BC_1F_1 plants., c) Lanes. 1. PBW343, 2. Pavon(*ph1b*) 3-19. (35-51) BC_1F_1 plants

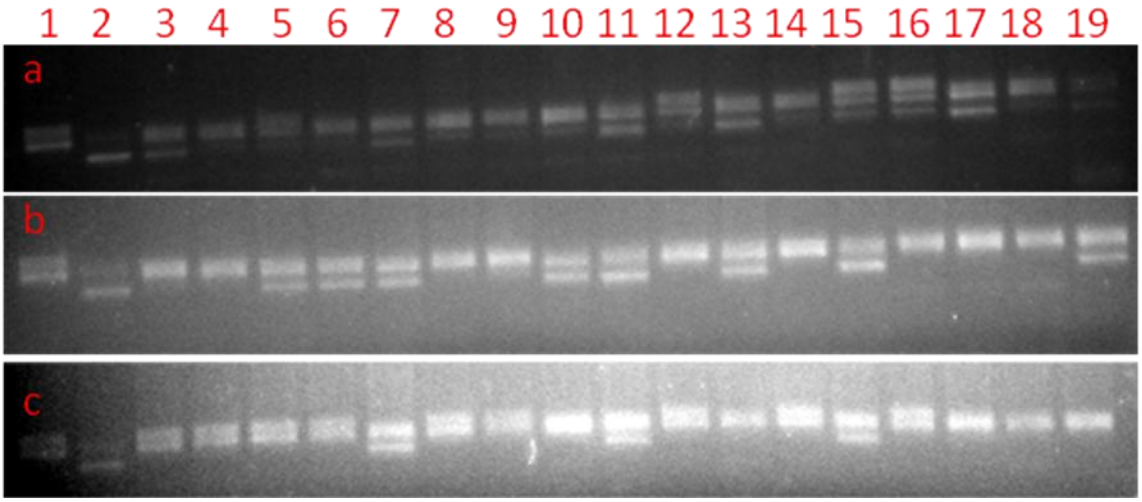

Fig. 4.3.2 Identification of 7S chromosome using wmc405 ,7S specific molecular marker on BC_1F_1 plants of Cs(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/*ph1bph1b//ph1bph1b*, a) Lanes. 1. PBW343 , 2. 48-41-6⊗ 3-19. (1-17) BC_1F_1 plants., b) Lanes. 1. PBW343, 2. Pavon(*ph1b*) 3-19. (18-34) BC_1F_1 plants., c) Lanes. 1. PBW343, 2. Pavon(*ph1b*) 3-19. (35-51) BC_1F_1 plants

Table 4.3.1 Molecular markers data of psr574 (*ph1b*) and wmc405 (7S) of BC_1F_1 plants CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗/*ph1bph1b*//*ph1bph1b*

Plant ID	*ph1b* Homozygous(absence of amplification)	7S Monosomic
PBW343	-	-
Pavon(*ph1b*)/	+	
48-41-6⊗		-
BC_1F_1-1	+	-
BC_1F_1-2	+	-
BC_1F_1-3	+	-
BC_1F_1-4	+	-
BC_1F_1-5	+	-
BC_1F_1-6	+	-
BC_1F_1-7	-	-
BC_1F_1-8	+	-
BC_1F_1-9	+	-
BC_1F_1-10	+	-
BC_1F_1-11	+	-
BC_1F_1-12	+	-
BC_1F_1-13	**+**	**+**
BC_1F_1-14	**+**	**+**
BC_1F_1-15	+	-
BC_1F_1-16	+	-
BC_1F_1-17	-	-
BC_1F_1-18	+	-
BC_1F_1-19	+	-
BC_1F_1-20	-	-
BC_1F_1-21	+	-
BC_1F_1-22	+	-
BC_1F_1-23	+	-
BC_1F_1-24	+	-
BC_1F_1-25	+	-
BC_1F_1-26	+	-
BC_1F_1-27	-	-
BC_1F_1-28	+	-
BC_1F_1-29	+	-
BC_1F_1-30	-	-
BC_1F_1-31	-	-
Plant ID	***ph1b* Homozygous(absence of**	**7S Monosomic**

	amplification)	
BC_1F_1-32	+	-
BC_1F_1-33	+	-
BC_1F_1-34	+	-
BC_1F_1-35	-	-
BC_1F_1-36	-	-
BC_1F_1-37	-	-
BC_1F_1-38	-	-
BC_1F_1-39	+	-
BC_1F_1-40	-	-
BC_1F_1-41	-	-
BC_1F_1-42	-	-
BC_1F_1-43	**+**	**+**
BC_1F_1-44	-	-
BC_1F_1-45	+	-
BC_1F_1-46	-	-
BC_1F_1-47	+	-
BC_1F_1-48	-	-
BC_1F_1-49	+	-
BC_1F_1-50	-	-
BC_1F_1-51	+	-

4.3.1 Morphological and micronutrient data of CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗/*ph1bph1b//ph1bph1b* homozygous for ph1b and monosomic for 7S

The morphological and micronutrients data of BC1F2 plants is given in table 4.3.2. The BC_1F_2 plants having high tillering, were analysed for their grain Fe and Zn content. Whereas the plants which were still sterile or partially fertile with very low seed set were discarded. The seeds of BC_1F_2 were shrivelled mostly because of *ph1bph1b* and leaf yellowing. The shrivelled seeds and leaf yellowing seems to be associated with the absence of *ph1bph1b*. Fe concentration varied between 5-72 mg/kg and Zn concentration varied between 34-90 mg/kg. Fe and Zn concentration for PBW343 (control) was 20.05 mg/kg and 39.91 mg/kg respectively. Only a few plants were having equivalent harvest index to that of the recipient cultivar and have 40-60% increase of the Fe or Zn or both the elements (Fig. 4.3.3). The selection was done, based on 40-60% increase in either one of the element, seed boldness (1000 grain weight) and harvest index. The selected plants were screened for the molecular markers and GISH. Plant BC_1F_2 -471, BC_1F_2 -487 had the alien transfer (Fig. 4.3.4). The

correlation of Fe was (r) - 0.08 and -0.17 with harvest index and yield per plant, respectively. The correlation of Zn was (r) - 0.18 and -0.19 with harvest index and yield per plant, respectively.

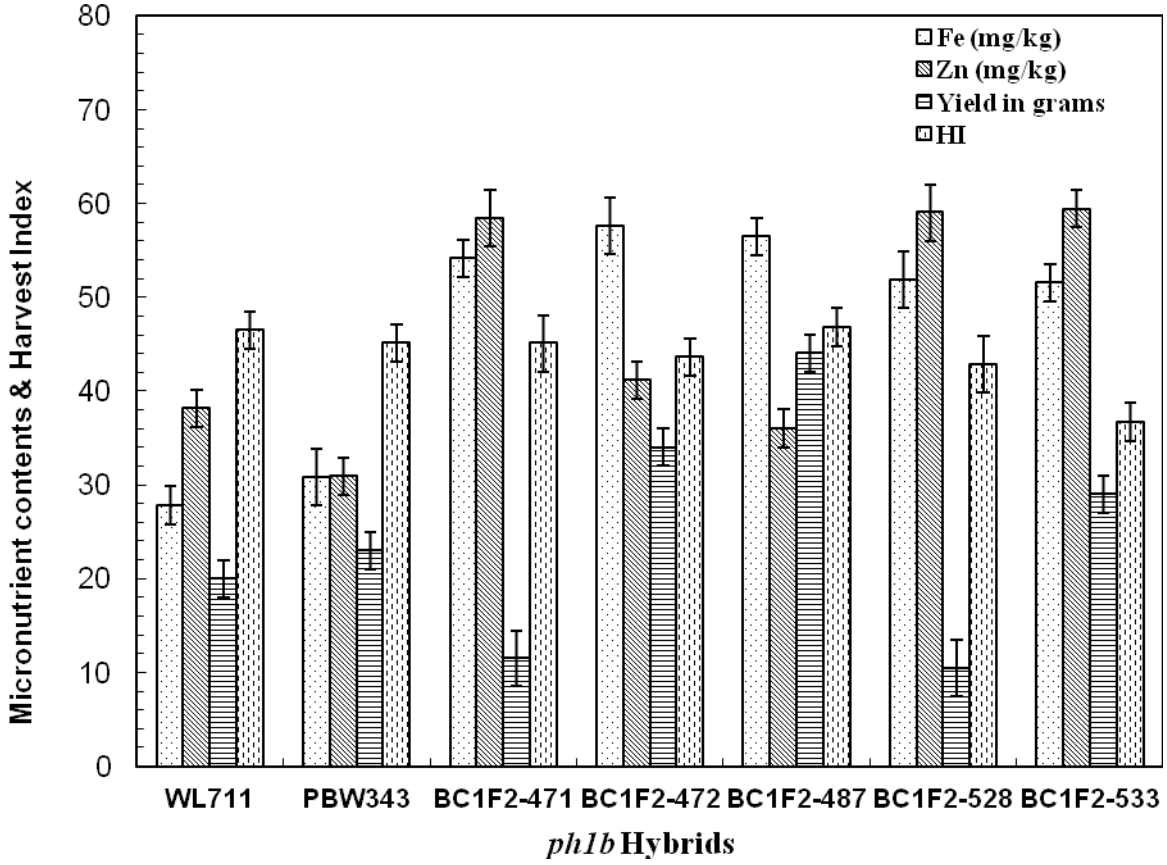

Fig. 4.3.3 Graphical representation micronutrients data along with harvest index and yield of BC_1F_2 plants CS(*Ph*^{1})/ *Ae. kotschyi 396//*PBW343-3///PBW373(48)-41-6⊗/*ph1bph1b//ph1bph1b* homozygous for *ph1b* and monosomic for 7S

Table 4.3.2 Morphological and micronutrient data of BC_1F_2 plants CS(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/*ph1bph1b//ph1bph1b* homozygous for ph1b and monosomic for 7S

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
WL711	23.92	-14.72	32.31	-19.04	15	41.34	20	46.51	N	9
PBW343	28.05	0.00	39.91	0.00	14	40.56	23	45.10	N	9
BC_1F_2-401	**27.94**	**-0.38**	**33.98**	**-14.86**	**14**	**22.55**	**8.5**	**27.87**	**Y**	**0**
BC_1F_2-402	21.37	-23.82	49.28	23.49	6	44.01	10	38.46	N	0
BC_1F_2-403	31.28	11.52	35.64	-10.69	11	29.98	13.5	42.86	N	0
BC_1F_2-404	5.87	-79.06	33.03	-17.22	30	43.71	68	52.31	N	0
BC_1F_2-405	35.88	27.91	36.21	-9.27	11	36.76	5.5	100.00	N	0
BC_1F_2-406	27.60	-1.61	34.19	-14.33	21	40.96	57.5	48.94	N	0
BC_1F_2-407	52.91	88.63	66.65	67.03	18	35.76	7.5	18.99	Y	0
BC_1F_2-408	28.40	1.25	49.32	23.60	12	11.25	3	15.79	Y	0
BC_1F_2-409	15.63	-44.28	43.11	8.02	8	14.15	2	20.00	Y	0
BC_1F_2-410	9.89	-64.74	55.29	38.54	5	22.64	3	27.27	Y	0
BC_1F_2-411	30.79	9.79	38.71	-3.00	13	21.11	13	33.33	N	0
BC_1F_2-412	45.41	61.88	66.50	66.64	20	27.76	8	14.81	N	0
BC_1F_2-413	24.13	-13.96	58.03	45.41	36	27.39	7	10.14	N	0
BC_1F_2-414	-25.48	9.16	2.63	-93.42	20	51.17	1.5	2.80	N	0
BC_1F_2-415	29.69	5.85	42.48	6.44	12	32.41	7	25.93	N	0
BC_1F_2-416	35.80	27.65	70.07	75.58	10	27.36	5	20.00	N	0
WL711	27.79	-29.63	36.46	-11.89	15	41.34	20	46.51	N	9
PBW343	39.50	0.00	41.38	0.00	14	40.56	23	45.10	N	9
BC_1F_2-417	29.06	-26.42	51.66	24.84	24	42.93	56	44.44	N	9

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
BC_1F_2-418	34.93	-11.57	48.26	16.61	16	31.99	23	37.70	N	9
BC_1F_2-419	44.90	13.67	41.79	0.99	8	40.25	16	44.44	N	9
BC_1F_2-420	33.47	-15.26	55.19	33.37	8	41.82	8	33.33	N	0
BC_1F_2-421	**70.36**	**78.15**	**90.92**	**119.71**	**4**	**41.91**	**2**	**14.29**	**N**	**0**
BC_1F_2-422	44.58	12.88	43.49	5.10	17	18.73	4	11.76	Y	0
BC_1F_2-423	33.70	-14.67	42.49	2.67	11	25.94	10.5	32.31	N	8
BC_1F_2-424	44.10	11.64	43.94	6.17	20	29.26	17	29.82	N	9
BC_1F_2-425	38.86	-1.61	38.60	-6.72	14	35.86	22	40.74	N	9
BC_1F_2-426	18.77	-52.48	39.12	-5.48	7	29.76	5.5	35.48	N	9
BC_1F_2-427	32.16	-18.58	52.03	25.74	34	39.11	50.5	42.62	N	9
BC_1F_2-428	44.90	13.69	55.26	33.53	11	15.26	4.5	27.27	N	0
BC_1F_2-429	27.99	-29.14	52.84	27.69	14	26.95	30.5	48.80	N	9
BC_1F_2-430	41.17	4.22	49.85	20.46	50	36.59	46	30.26	N	9
BC_1F_2-431	47.08	19.20	56.27	35.99	23	13.87	6.5	17.81	Y	9
BC_1F_2-433	**68.16**	**72.57**	**42.93**	**3.74**	**10**	**31.07**	**19**	**38.78**	**N**	**8**
BC_1F_2-434	41.23	4.39	45.91	10.93	18	37.25	31	39.24	N	7
BC_1F_2-435	**72.04**	**82.39**	**45.96**	**11.06**	**11**	**29.81**	**13.5**	**36.00**	**N**	**2**
BC_1F_2-436	44.41	12.43	112.28	171.33	15	27.84	2	7.14	N	0
BC_1F_2-437	56.24	42.38	34.46	-16.73	20	23.87	18.5	29.60	N	9
BC_1F_2-438	23.18	-41.31	44.95	8.62	21	33.24	45	41.00	N	9
BC_1F_2-440	39.91	1.05	33.09	-20.05	23	35.11	42	43.75	N	0
BC_1F_2-442	66.52	68.42	57.21	38.24	34	25.11	26	28.89	N	0
BC_1F_2-443	38.29	-3.07	32.00	-22.67	3	31.81	7	53.85	N	9
BC_1F_2-444	31.67	-19.83	60.25	45.60	14	39.41	15.5	39.24	N	9

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
BC_1F_2-445	44.18	11.86	55.39	33.84	10	38.84	19	40.43	N	9
BC_1F_2-446	57.74	46.20	44.93	8.56	16	35.71	15	25.42	N	7
BC_1F_2-447	**56.36**	**42.69**	**43.25**	**4.52**	**10**	**30.18**	**20.5**	**37.61**	**N**	**0**
BC_1F_2-448	30.54	-22.67	42.28	2.18	14	34.35	14	43.75	N	9
BC_1F_2-449	10.32	-73.86	46.63	12.67	21	30.94	28	42.42	N	9
BC_1F_2-450	25.38	-35.75	39.25	-5.16	26	35.44	15	30.61	N	9
BC_1F_2-451	31.63	-19.91	48.55	17.32	22	28.76	20.5	40.59	N	9
BC_1F_2-452	47.64	20.62	44.15	6.70	7	27.86	6.5	44.83	N	2
BC_1F_2-453	59.09	49.61	36.46	-11.91	8	21.88	9.5	28.36	N	3
BC_1F_2-454	37.76	-4.39	55.98	35.27	14	35.74	23	40.35	N	2
BC_1F_2-455	21.53	-45.50	48.10	16.24	14	33.44	37	39.78	N	9
BC_1F_2-456	51.25	29.74	52.70	27.34	23	32.73	22	36.67	N	9
BC_1F_2-457	32.84	-16.85	52.93	27.90	17	38.17	28	42.42	N	9
BC_1F_2-458	38.78	-1.82	54.41	31.48	11	30.71	27.5	38.46	N	9
BC_1F_2-459	**62.32**	**57.77**	**43.78**	**5.80**	**7**	**34.65**	**5**	**33.33**	**N**	**6**
BC_1F_2-460	42.08	6.53	48.97	18.32	19	33.87	11.5	32.39	N	3
BC_1F_2-461	28.37	-28.18	40.75	-1.52	31	25.81	44.5	34.63	N	3
BC_1F_2-462	9.67	-75.51	38.59	-6.74	16	34.38	16.5	40.74	N	9
BC_1F_2-463	41.96	6.24	47.44	14.63	9	20.19	5	33.33	Y	3
BC_1F_2-464	39.81	0.79	66.73	61.24	3	28.55	3.5	36.84	N	9
BC_1F_2-465	65.16	64.97	45.84	10.78	9	25.65	7.5	29.41	N	0
BC_1F_2-466	**59.09**	**49.61**	**49.35**	**19.26**	**26**	**39.18**	**16**	**32.00**	**N**	**9**
BC_1F_2-467	36.90	-6.57	46.05	11.27	11	41.19	13.5	38.03	N	9
BC_1F_2-468	51.86	31.30	83.52	101.83	6	32.03	3.5	25.93	N	9

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
BC_1F_2-469	23.58	-40.29	56.31	36.07	14	23.45	7.5	29.41	N	5
BC_1F_2-470	48.17	21.95	63.34	53.06	12	22.38	2.5	17.24	N	9
BC_1F_2-471	**54.11**	**36.99**	**58.47**	**41.30**	**7**	**36.46**	**11.5**	**45.10**	**N**	**0**
BC_1F_2-472	**57.58**	**45.78**	**41.17**	**-0.51**	**21**	**37.71**	**34**	**43.59**	**N**	**0**
BC_1F_2-473	65.64	66.19	45.87	10.85	14	21.93	13	33.33	N	9
BC_1F_2-474	**76.23**	**93.00**	**46.06**	**11.29**	**3**	**33.62**	**2**	**33.33**	**N**	**2**
BC_1F_2-476	46.50	17.72	42.04	1.59	14	38.42	28.5	48.72	N	9
BC_1F_2-477	34.22	-13.36	40.52	-2.08	9	33.22	10.5	36.84	N	9
BC_1F_2-478	32.76	-17.05	52.17	26.06	32	43.55	66	45.21	N	9
BC_1F_2-480	24.09	-39.02	53.38	29.00	13	23.36	16.5	42.86	N	9
BC_1F_2-481	45.32	14.74	57.18	38.17	31	30.41	41.5	45.36	N	9
BC_1F_2-482	27.71	-29.84	49.14	18.74	8	35.67	2.5	31.00	N	9
WL711	32.20	-8.35	40.56	-3.46	15	41.34	20	46.51	N	9
PBW343	35.13	0.00	42.01	0.00	14	40.56	23	45.10	N	9
BC_1F_2-483	31.87	-9.29	42.66	1.54	7	29.31	26	72.22	N	2
BC_1F_2-485	16.58	-52.82	38.64	-8.03	12	39.71	16.5	40.74	N	9
BC_1F_2-486	**62.74**	**78.59**	**46.30**	**10.21**	**24**	**29.78**	**10.5**	**30.43**	**N**	**9**
BC_1F_2-487	**56.50**	**60.83**	**36.04**	**-14.22**	**19**	**38.01**	**44**	**46.81**	**N**	**0**
BC_1F_2-488	32.55	-7.36	52.53	25.03	8	32.97	8.5	32.08	N	9
BC_1F_2-489	**55.12**	**56.90**	**50.28**	**19.69**	**17**	**32.21**	**21**	**41.18**	**N**	**9**
BC_1F_2-490	7.97	-77.32	55.13	31.23	4	40.44	3.5	36.84	N	9
BC_1F_2-491	5.07	-85.58	39.67	-5.56	3	32.16	3	42.86	N	9
BC_1F_2-492	48.49	38.02	34.19	-18.61	7	29.12	7.5	42.86	N	9
BC_1F_2-493	28.35	-19.30	50.91	21.18	7	32.01	13.5	49.09	N	9

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
BC_1F_2-494	**64.18**	**82.67**	**49.76**	**18.44**	**8**	**31.64**	**9.5**	**28.36**	**N**	**9**
BC_1F_2-495	55.94	59.23	36.24	-13.75	17	22.45	15	38.46	N	9
BC_1F_2-496	14.71	-58.12	39.13	-6.87	23	28.47	56.5	49.34	N	9
BC_1F_2-497	15.66	-55.42	47.34	12.67	17	38.18	21	41.18	N	9
BC_1F_2-498	9.73	-72.29	41.18	-1.98	25	38.51	42.5	37.78	N	9
BC_1F_2-499	30.26	-13.88	53.77	27.98	19	28.61	21	35.59	N	9
BC_1F_2-500	39.41	12.18	58.08	38.24	14	28.12	26.5	42.40	N	9
BC_1F_2-501	57.19	62.78	47.90	14.02	14	28.78	15	29.41	N	9
BC_1F_2-502	**48.12**	**36.95**	**52.27**	**24.43**	**13**	**29.98**	**10**	**35.71**	**N**	**9**
BC_1F_2-503	12.86	-63.38	48.17	14.67	10	35.98	14	41.18	N	9
BC_1F_2-504	26.97	-23.22	60.56	44.16	31	35.87	37.5	37.69	N	0
BC_1F_2-505	23.58	-32.87	51.96	23.68	15	33.44	16	34.78	N	9
BC_1F_2-506	**61.76**	**75.78**	**59.08**	**40.62**	**5**	**45.27**	**7.5**	**18.07**	**N**	**9**
BC_1F_2-507	**56.76**	**61.55**	**62.21**	**48.07**	**8**	**36.33**	**6.5**	**35.14**	**N**	**9**
BC_1F_2-509	38.31	9.05	42.85	1.99	23	26.38	31	41.33	N	9
BC_1F_2-510	60.36	71.81	41.12	-2.12	24	25.91	27.5	38.46	N	9
BC_1F_2-511	38.61	9.90	58.80	39.95	22	25.94	30	36.59	N	9
BC_1F_2-512	18.44	-47.52	44.01	4.75	44	35.63	65	40.88	N	9
BC_1F_2-513	60.61	72.52	40.24	-4.21	17	34.16	18	40.91	N	9
BC_1F_2-514	22.05	-37.23	67.17	59.88	7	33.48	4	33.33	N	9
BC_1F_2-515	48.03	36.71	52.92	25.95	12	27.92	7.5	34.88	N	9
BC_1F_2-516	64.42	83.37	40.03	-4.71	17	21.75	9	31.03	N	9
BC_1F_2-517	**57.07**	**62.45**	**49.58**	**18.01**	**33**	**41.45**	**33.5**	**30.59**	**N**	**9**
BC_1F_2-519	37.52	6.79	61.29	45.89	-	26.61	6.5	31.00	N	9

Plant ID	Fe mg/kg	Fe % Increase over PBW343	Zn mg/kg	Zn % Increase over PBW343	No. of Tillers/Plant	1000 Grain weight	Yield in grams	Harvest Index	Leaf yellowing	Powdery mildew
BC_1F_2-520	35.23	0.27	54.46	29.62	7	30.95	4.5	31.03	N	9
BC_1F_2-521	40.33	14.80	45.52	8.36	13	30.71	5	23.81	N	9
BC_1F_2-522	37.76	7.48	36.62	-12.84		34.72	9.5	41.00	N	9
BC_1F_2-525	**55.67**	**58.46**	**47.49**	**13.04**	**20**	**30.41**	**37.5**	**48.39**	**N**	**9**
BC_1F_2-526	47.37	34.83	45.53	8.38	8	36.87	15.5	46.27	N	9
BC_1F_2-527	46.11	31.26	52.25	24.37	5	29.12	6	37.50	N	9
BC_1F_2-528	**51.92**	**47.77**	**59.04**	**40.52**	**9**	**34.46**	**10.5**	**42.86**	**N**	**9**
BC_1F_2-529	34.00	-3.24	56.69	34.95	42	30.46	17.5	19.13	N	9
BC_1F_2-530	54.95	56.42	40.21	-4.29	11	27.11	9	39.13	N	9
BC_1F_2-531	76.47	117.65	49.34	17.45	7	22.03	3	33.33	N	9
BC_1F_2-532	69.56	98.01	45.10	7.36	17	20.25	14	31.00	N	9
BC_1F_2-533	**51.56**	**46.77**	**59.42**	**41.44**	**18**	**41.91**	**29**	**36.71**	**N**	**0**

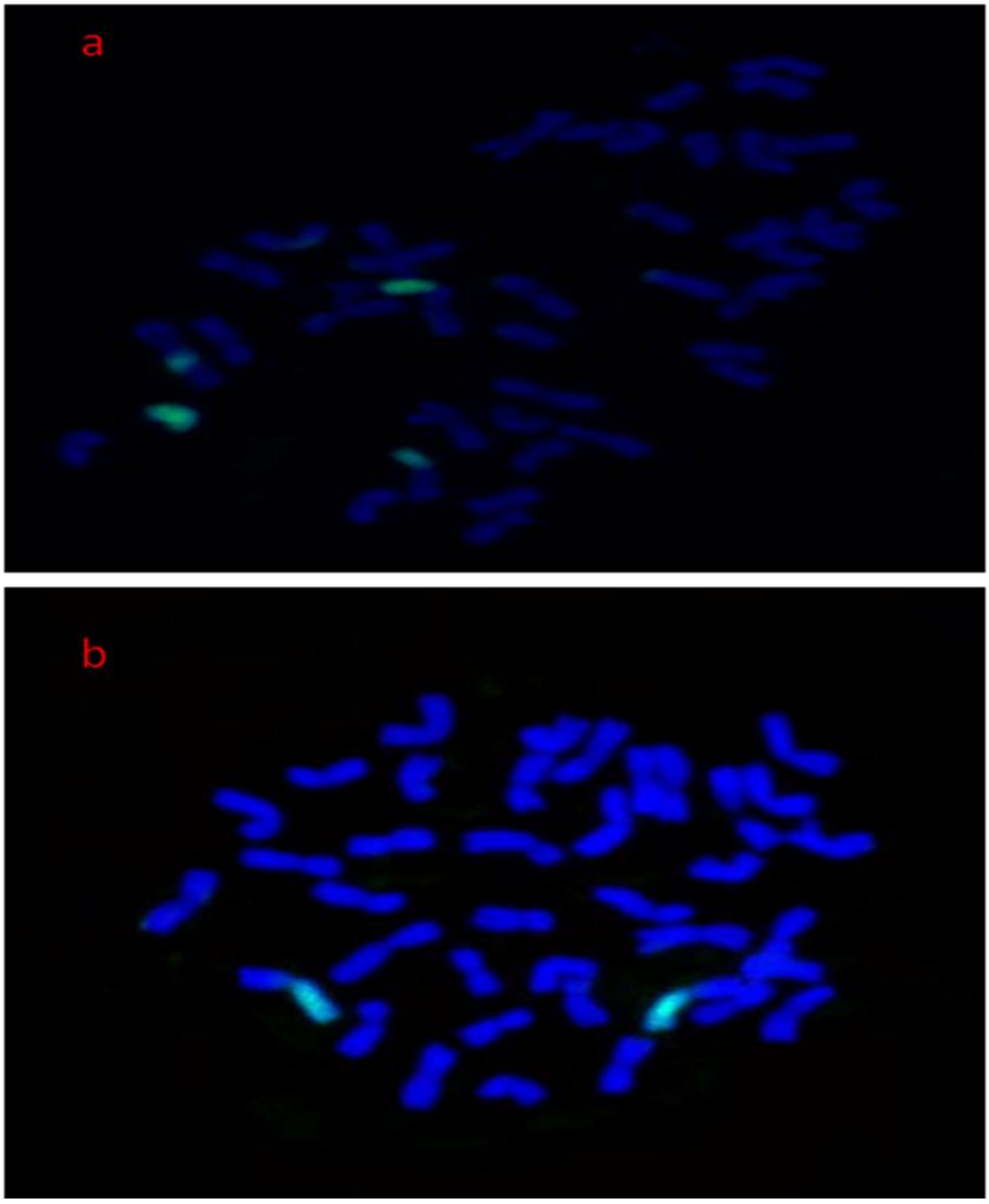

Fig. 4.3.4 GISH of BC_1F_2 plants CS(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/*ph1bph1b//ph1bph1b* homozygous for *ph1b* and monosomic for 7S showing translocation (green) of 7S chromosome a) BC_1F_2-471, b) BC_1F_2-487

4.3.2 Morphological and micronutrient data of BC_1F_3 plants Cs(*Ph*^{I})/ *Ae. kotschyi 396*//PBW343-3///PBW373-41-6⊗/*ph1bph1b//ph1bph1b* homozygous for *ph1b* and monosomic for 7S

The data of the morphology and micronutrients of some BC_1F_3 plants were given in table 4.3.3. The plants resembled recurrent parent in tiller number, plant height, head type etc. The BC_1F_3 having high tillering, good biomass and fair seed set were analysed for their grain Fe and Zn content. The plants which had very low seed set were discarded. The BC_1F_3 had Fe and Zn concentrations in the range of 22.7 to 53.95 mg/kg and 16.58 to 62.12 mg/kg respectively, compared to 30.13 mg/kg, and 29.46 mg/kg Zn for the PBW343(*GPC+Lr24*). Concentration effect was prominent in BC_1F_3 plants. Plants which had high grain Fe and Zn content were also resistant to powdery mildew, indicating that genes for high micronutrient content and powdery mildew resistance could be linked, as was indicated in case of irradiated hybrids. No correlation was found for Fe and Zn concentration with harvest index and yield per plant.

Table 4.3.3 Morphological and micronutrient data of BC_1F_3 plants Cs(*Ph*^{I})/*Ae. kotschyi 396*//PBW343-3///PBW373(48)-41-6⊗/ph1bph1b//ph1bph1b homozygous for *ph1b* and monosomic for 7S. (Eternal University, Baru Sahib, 2012-13)

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	Powdery mildew	No. of Tillers/ Plants	Yield in grams	Harvest index
BC_1F_3-407-1	40.75	35.25	34.74	17.88	0	16	46.5	52.90
BC_1F_3-407-2	**53.95**	**79.06**	**62.13**	**110.84**	**0**	**14**	**45.0**	**59.29**
BC_1F_3-407-6	26.60	-11.72	32.52	10.35	0	14	28.8	50.00
BC_1F_3-433-1	35.75	18.65	28.39	-3.65	0	13	24.3	44.83
BC_1F_3-433-2	30.85	2.39	33.24	12.79	0	12	20.5	48.58
BC_1F_3-433-4	30.45	1.06	29.69	0.76	0	14	18.3	45.98
BC_1F_3-433-5	35.85	18.98	30.15	2.30	0	18	23.8	42.12
BC_1F_3-435-1	24.80	-17.69	34.56	17.27	0	12	10.6	48.62
BC_1F_3-435-2	22.70	-24.66	33.13	12.43	0	13	24.2	46.90
BC_1F_3-435-4	23.15	-23.17	28.18	-4.36	0	6	7.9	45.40
BC_1F_3-471-5	**26.46**	**-12.18**	**35.44**	**20.28**	**0**	**14**	**21.2**	**41.90**
BC_1F_3-471-6	28.70	-4.75	30.76	4.37	0	14	17.4	44.96
BC_1F_3-471-7	31.35	4.05	34.24	16.20	0	15	18.5	42.92
BC_1F_3-471-8	**40.65**	**34.92**	**31.03**	**5.31**	**0**	**13**	**28.5**	**49.48**
BC_1F_3-471-11	28.15	-6.57	30.37	3.07	0	8	13.8	49.11
BC_1F_3-471-12	36.75	21.97	25.16	-14.63	0	13	24.6	49.00
BC_1F_3-471-13	31.40	4.22	30.38	3.08	0	10	14.3	44.00
BC_1F_3-471-14	**37.15**	**23.30**	**32.45**	**10.13**	**2**	**16**	**31.8**	**43.21**
BC_1F_3-471-15	31.95	6.04	29.92	1.54	0	10	24.3	48.80
BC_1F_3-471-17	36.55	21.31	30.53	3.61	0		23.7	45.66
BC_1F_3-471-18	31.45	4.38	27.39	-7.06	0	10	16.4	50.15

Plant Id	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	Powdery mildew	No. of Tillers/ Plants	Yield in grams	Harvest index
BC_1F_3-472-1	32.90	9.19	30.89	4.82	0	11	20.2	46.12
BC_1F_3-472-3	35.70	18.49	26.34	-10.61	0	15	26.8	51.34
BC_1F_3-472-4	31.90	5.87	28.28	-4.04	0	20	37.1	46.96
BC_1F_3-472-5	32.75	8.70	33.14	12.47	0	18	36.9	48.36
BC_1F_3-472-8	33.95	12.68	29.58	0.39	0	15	31.6	48.92
BC_1F_3-472-9	32.70	8.53	29.63	0.54	0	13	30.7	49.84
BC_1F_3-472-10	36.15	19.98	30.20	2.47	0	9	15.8	50.64
BC_1F_3-487-1	34.10	13.18	42.91	45.63	0	11	25.1	41.28
BC_1F_3-487-2	**54.68**	**82.62**	**51.45**	**72.03**	**0**	**12**	**27.2**	**42.08**
BC_1F_3-487-5	43.25	43.54	31.31	6.24	0	13	18.0	34.75
BC_1F_3-487-6	**43.35**	**43.88**	**34.65**	**17.59**	**0**	**8**	**23.1**	**46.19**
BC_1F_3-487-8	32.10	6.54	35.09	19.07	0	12	22.1	40.93
BC_1F_3-487-9	29.05	-3.58	30.42	3.22	0	-	13.0	43.77
BC_1F_3-487-10	36.15	19.98	30.59	3.80	0	8	16.4	42.38
BC_1F_3-487-11	32.00	6.21	36.46	23.72	0	8	18.3	44.31
BC_1F_3-487-13	33.20	10.19	30.85	4.68	0	6	16.3	51.91
BC_1F_3-487-15	31.75	5.38	35.95	22.01	0	10	18.5	46.60
BC_1F_3-487-18	**37.65**	**24.96**	**37.45**	**27.08**	**0**	**10**	**20.1**	**44.87**
BC_1F_3-489-1	29.00	-3.75	28.74	-2.48	0	-	9.8	45.58
BC_1F_3-489-2	24.15	-19.85	32.90	11.64	0	10	12.5	42.66
BC_1F_3-489-4	26.65	-11.55	33.13	12.42	0	7	14.7	49.16
BC_1F_3-517-2	27.90	-7.40	32.49	10.26	0	13	32.2	48.06
BC_1F_3-517-4	26.90	-10.72	33.43	13.44	0	13	26.0	52.31

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn % increase over PBW343	Powdery mildew	No. of Tillers/ Plants	Yield in grams	Harvest index
BC_1F_3-517-5	25.50	-15.37	26.83	-8.95	0	15	33.0	48.89
BC_1F_3-517-6	25.40	-15.70	27.80	-5.67	0		31.3	49.37
BC_1F_3-517-7	25.10	-16.69	24.25	-17.72	0	12	26.4	47.06
BC_1F_3-517-8	25.60	-15.03	24.59	-16.56	0	11	26.1	47.98
BC_1F_3-517-9	27.70	-8.07	27.23	-7.61	0	14	26.3	52.39
BC_1F_3-517-10	24.80	-17.69	27.90	-5.33	0	11	18.5	100.00
BC_1F_3-525-2	33.00	9.53	24.22	-17.80	0	9	14.3	42.69
BC_1F_3-525-5	34.60	14.84	26.06	-11.56	0	10	14.9	45.29
BC_1F_3-528-1	30.35	0.73	31.65	7.41	0	12	14.3	42.69
BC_1F_3-528-2	30.40	0.90	40.00	35.73	0	12	12.5	43.55
BC_1F_3-528-3	26.65	-11.55	33.42	13.42	0	11	13.0	39.39
BC_1F_3-528-4	27.20	-9.72	32.17	9.16	1	8	10.4	42.62
BC_1F_3-528-6	27.40	-9.06	32.06	8.79	0	20	22.4	39.23
BC_1F_3-533-1	29.45	-2.26	24.88	-15.56	0	7	12.7	44.25
BC_1F_3-533-2	22.45	-25.49	16.59	-43.71	0	8	10.6	42.74
BC_1F_3-533-3	35.35	17.32	34.17	15.95	0	16	24.6	41.77
BC_1F_3-533-6	28.85	-4.25	24.19	-17.91	0	8	15.2	41.87
BC_1F_3-533-7	28.40	-5.74	22.30	-24.32	0	5	11.5	46.56
PBW343	30.13	-	29.46	-	9	14	19.7	52.19

4.4 5 B deficiency induced transfer of genes for Fe and Zn

Mono 5B plants of Pavon were crossed with *Ae. Kotschyi 3790*, and F_1 plants with and without 5B were selected. The F_1 plants were screened by *ph1b* linked molecular marker psr574 (Fig. 4.4.1), the absence of band in the gel indicated the absence of 5B ie 34 chromosomes in total. Some of these plants were further confirmed by homoeologous pairing by cytological analysis (Fig. 4.4.2). Plants with 34 chromosome were back crossed with PBW343 (*Lr24+GPC*) to get BC_1F_1. Three plants survived out of 23 plants of BC_1F_1. The BC_1F_1 were backcrossed again with PBW343(*Lr24+GPC*) making BC_2F_1. Out of 40 BC_2F_1 plants 18 were found fertile and analysed for micronutrients and cytology.

4.4.1 Micronutrient analysis of BC_2F_1

Grain Fe and Zn content and their % increase is given in table 4.4.1. Fe content varied from 43.47 mg/kg to 70.88 mg/kg as compared to 33.05 mg/kg for PBW343(*Lr24+GPC*). Zn concentration varied between 53.3 mg/kg to 84.42 mg/kg as compared to 24.96 mg/kg. Fe and Zn content negatively correlated to yield. Most of the plant with high grain Fe and Zn content had very less seed set.

4.4.2 Cytological analysis BC_2F_1

BC_2F_1 plants had variable number of chromosomes as confirmed by meiotic preparations. Most of the plants had one to eight univalents and nineteen to twenty three bivalents. The number of chromosomes, bivalents and univalents are given in table 4.4.1 and fig. 4.4.3. Some of the plants were not analysed due to maturation of spikes, the meiosis had advanced to pollen development.

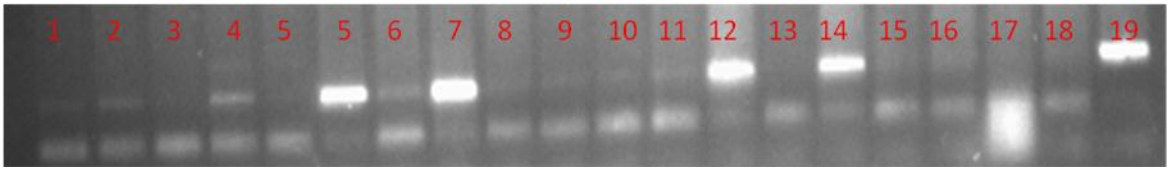

Fig. 4.4.1 The *ph1b* linked SSR marker psr574 on mono 5B/*Ae kotschyi 3790* lane no.-1) 55.14, 2)55.15 , 3) 55.16, 4) 55.17, 5) 55.18, 6) 55.19, 7) 55.20, 8) 55.21, 9) 61.1, 10) 61.2, 11) 61.3, 12) 61.5, 13) 14) 61.6, 15) 61.7, 16) 61.8, 17) 61.9, 18) 61.10, 19) mono 5B.

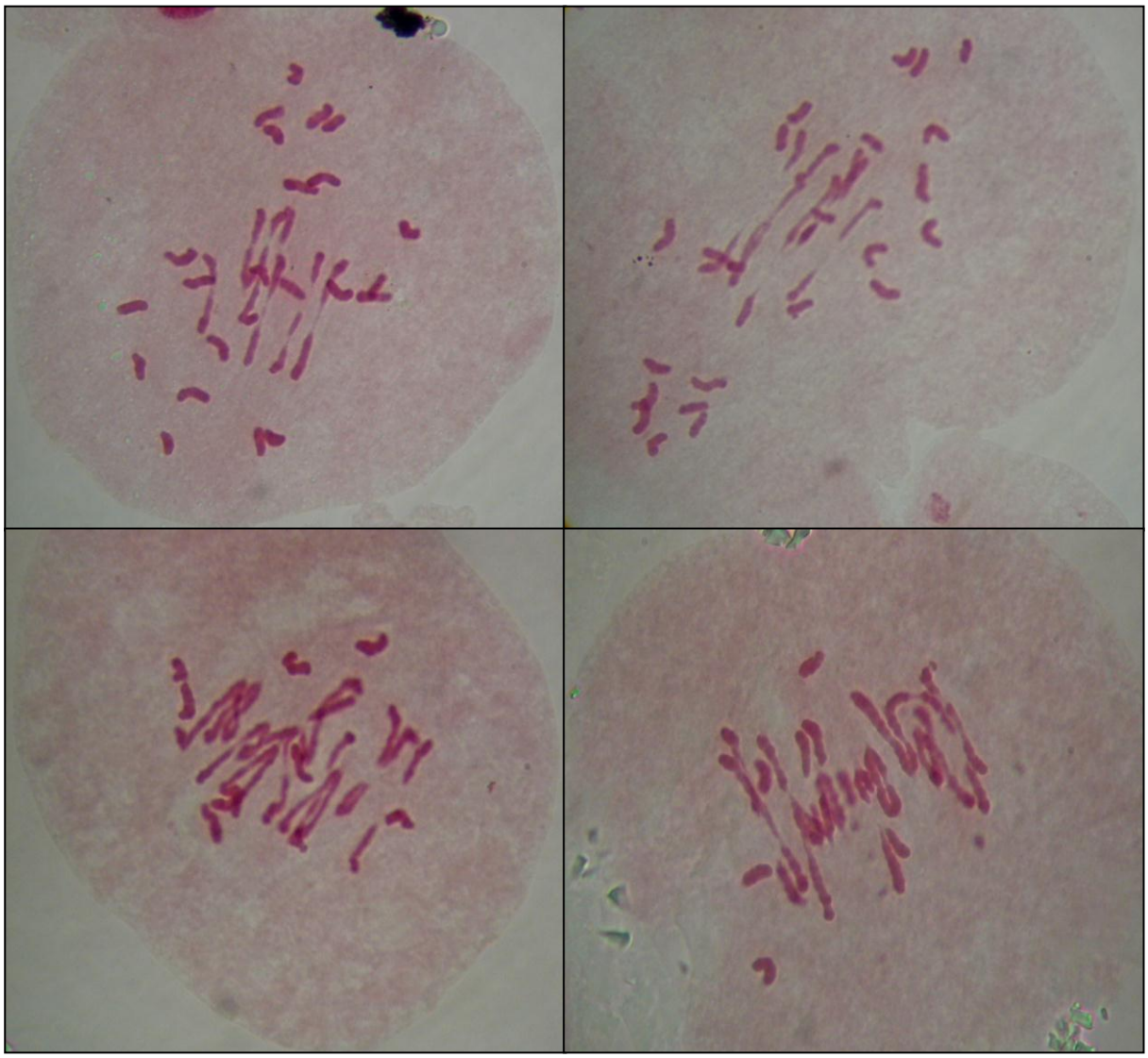

Fig. 4.4.2 Chromosome number and pairing at metaphase 1 of *Triticum aestivum* cv. Pavon Mono 5B/*Ae kotschyi 3790* with and without chromosome 5B, F1 hybrid (2n=35) with chromosome 5B: a (6 II + 23 I), b (6 II + 23 I); F1 hybrid (2n=34) without chromosome 5B: c (2 V + 4 III + 2 II + 8 I), d (1 V + 2 III + 9 II + 5 I).

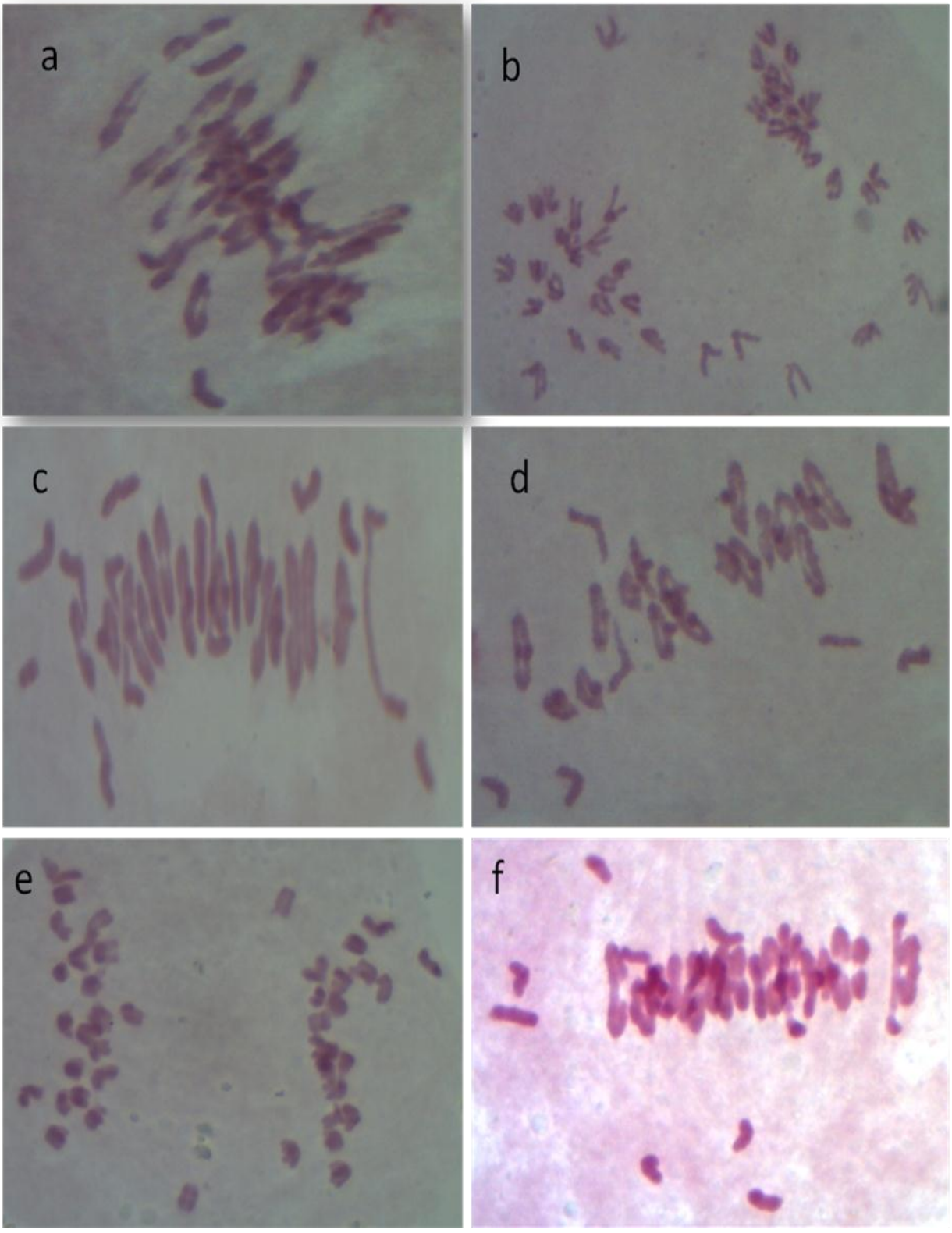

Fig. 4.4.3 Chromosome number and pairing at meosis 1 of *Triticum aestivum* cv. Pavon Mono 5B/*Ae. kotschyi 3790*//PBW343(*GPC+Lr24*)/// PBW343 (*GP +Lr24*) a- MB-2(23 II + 2 I), b -MB-6(20II + 4 I), c-MB7 (20II + 6 I), d - MB-13(19 II + 4 I) , e- MB-27 (20 II + 2 I), f –MB-31(21 II + 6 I)

Table 4.4.1 Micronutrient and cytological data of BC_2F_2 Pavon mono 5B/*Ae. kotschyi 3790*//PBW343(*GPC+ Lr-24*)///PBW343(*GPC+ Lr-24*)

Plant ID	Fe mg/kg	Fe % increase over PBW343	Zn mg/kg	Zn% increase over PBW343	Yield in grams	No. and pairing of Chromosome
BC_2F_1-2	43.55	31.77	60.17	141.08	15.70	48(23II + 2I)
BC_2F_1-3	**47.63**	**44.10**	**64.84**	**159.77**	**14.61**	**42(19II + 4I)**
BC_2F_1-4	44.12	33.49	68.58	174.75	11.34	-
BC_2F_1-6	56.83	71.95	74.03	196.59	15.32	44(20II +4I)
BC_2F_1-7	52.26	58.14	72.08	188.79	15.94	46(20II +6I)
BC_2F_1-11	57.62	74.33	53.30	113.56	0.97	-
BC_2F_1-12	43.47	31.53	53.83	115.67	11.73	46(21II + 4I)
BC_2F_1-13	**55.40**	**67.61**	**70.48**	**182.36**	**16.07**	**42(19II + 4I)**
BC_2F_1-15	55.23	67.11	63.84	155.77	6.13	44(20II + 4I)
BC_2F_1-16	57.41	73.69	70.17	181.12	19.98	-
BC_2F_1-17	70.88	114.46	63.47	154.29	6.11	-
BC_2F_1-19	60.99	84.52	75.90	204.09	14.29	45(19II + 7I)
BC_2F_1-20	61.47	86.00	80.11	220.94	10.91	
BC_2F_1-21	63.85	93.18	79.43	218.21	5.79	44(20II + 4I)
BC_2F_1-26	65.15	97.11	81.89	228.08	11.33	-
BC_2F_1-27	**65.02**	**96.74**	**80.47**	**222.39**	**5.71**	**42(20II + 2I)**
BC_2F_1-29	68.59	107.52	84.42	238.22	7.51	43(19II + 5I)
BC_2F_1-31	65.57	98.41	82.73	231.46	3.15	48(21II + 6I)
Pavon mono 5B	29.39	-11.08	32.34	29.55	14.56	41(20II + 1I)
PBW 343	33.05	0.00	24.96	0.00	19.42	42(21II)
Ae. kotschyi 3790	50.00	51.29	63.36	153.83	-	28(14II)

Table of Contents

5. DISCUSSION

Nearly one third of the world population is suffering from deficiency of important micronutrients like Fe and Zn (Stoltzfus, 2003; Poletti *et al.,* 2004; Welch and Graham, 2004; Bouis, 2007; Pfeiffer *et al.,* 2007) leading to malnutrition. Cultivated wheat genotypes have very low Fe and Zn contents in grains, which are largely distributed in embryos and the peripheral tissues of bran (Welch and Graham, 1999). The related non-progenitor wild species with S and U genomes have up to 3–4 fold higher grain Fe and Zn content as compared to bread and durum wheat cultivars (Rawat *et al*., 2009). The derivative of *Ae. kotschyi* 396, *Ae. kotschyi* 3790, and *Ae. peregrina* 13772 had linkage drag involving low harvest index and reduced grain yield. (Tiwari *et al.,* 2010; Rawat *et al.,* 2010; Kumari *et al.,* 2011). This study was undertaken to reduce linkage drag in substitution lines of 7S and 7U chromosomes of *Ae. kotschyi* 396 and *Ae. kotschyi* 3790, respectively through fine transfer of genes for high grain Fe and Zn content.

Among the wheat group 7 anchored SSR markers, the D genome specific markers had the highest transferability to the donor *Aegilops* species followed by B genome and A genome specific SSR markers. Polymorphism Among the transferable markers between *Aegilops* species and wheat cultivars followed the same pattern. High transferability of D genome specific markers compared to the B genome markers suggested that *Ae. tauschii* (donor of D genome) is more close to S and U genome of *Ae. kotschyi* than *Ae. speltoides* (donor of B genome). The A genome specific markers had least transferability suggesting that *Triticum urartu* (donor of A genome) is distant to *Ae. kotschyi*. Among the polymorphic markers, 20 markers were transferable to 7S chromosome, 10 markers to 7U and 6 markers were transferable to both of the chromosomes. The remaining 33 markers were transferable to any of the 7S and 7U chromosome, indicating that genomes of related nonprogenitors species might have been reorganised. The tentative consensus map of 7S and 7U chromosomes was prepared by Join Map. A high density consensus map of wheat was also prepared by using Join Map (Somers *et al.,* 2004). The D genome is more similar to S and U genomes compared to B and A genomes (Golovnina *et al.,* 2007). The B genome of wheat is more close to S genome of *Aegilops* species compared to the A genome (Dvorak and Zhang, 1990; Daud and Gustaffson, 1996; Faris *et al.,* 2002).

Molecular markers and GISH analysis of parents and the two derivatives confirmed the substitution of chromosome 7S for 7D in 48-41-6⊗ as reported previously (Rawat *et al.,* 2010). The substitution of 7U in 63-2-13⊗ was confirmed by GISH, but could not be confirmed by molecular markers as was reported previously (Tiwari *et al.,* 2010). Both the derivatives had consistently high grain micronutrients that the recipient wheat cultivars over

the years and locations confirming the presence of *Ae. kotschyi* substitution. In the derivatives stable meiosis of 63-2-13⊗ derivative with 21II indicated disomic substitution. Hydroponic culture of 63-2-13⊗ under Fe deficient conditions led to grain maturity without any sterility, further suggesting the Ae. *kotschyi* chromosome substitution (Sundip Kumar unpublished results, 2013).

Both the derivatives 48-41-6⊗ and 63-2-13⊗ had constitutively high but variable amount of grain Fe and Zn content, over different years and locations, indicating the presence of superior genes/QTLs for micronutrient uptake/translocation or deposition on 7S and 7U chromosomes of *Ae. kotschyi 396.* QTLs for high grain Fe and Zn have been reported on 7A chromosome (Tiwari *et al.,* 2009). Shi *et al.,* (2008) detected as many as 4 QTL for grain Zn concentration (milligrams/kilograms) and 7 (including these 4) for grain Zn content (micrograms/grain) on 7A chromosome. One more QTL was reported on 7A chromosome by Peleg *et al.* (2009). 7S and 7U substitution lines were also reported previously (Rawat *et al.,* 2010; Tiwari *et al.,* 2010; Kumari *et al.,* 2011).

Both the derivatives 48-41-6⊗ and 63-2-13⊗ had comparatively reduced grain yield and harvest index as compared to the recipient wheat cultivars indicating that part of the high micronutrient content could be attributed to the concentration effect through distribution of a given pool of micronutrients among fewer grains. Because of the associated linkage drag none of the addition line or substitution line of wheat for complete chromosome or complete chromosome arms (Friebe *et al.* 1996b; Conner *et al.,* 1998; Dhaliwal *et al.* 2002) could be used as cultivar, except 1RS.1BL translocation of *Secale cereal* (Yan *et al.,* 2005).

Both the approaches for precise transfer of useful variability from alien substitution lines including induced homoeologous pairing and irradiation induced transfer were found effective. The wheat *ph1b* deletion, which promotes meiotic pairing among homoeologous chromosomes, was employed to induce recombination between wheat chromosome 2B and goat grass 2S chromatin using a backcross scheme favorable for inducing and detecting the homoeologous recombinants with small goat grass chromosome segments. (Niu *et al.,* 2011). The *Agropyron intermedium* leaf rust resistance gene *Lr38* was transferred to wheat by seed irradiation and were analyzed by C-banding and GISH (Friebe *et al.,* 1993). Pollen irradiation transfer of gene is more precise and depends on irradiation dose, more the dose, smaller the fragment transferred. Powdery mildew resistance *Pm21* locus was transferred from *Haynaldia villosa* translocation line T6VS/6AL to wheat using female gamete irradiation induced transfer (Chen *et al*., 2012).

Seed irradiation Hybrids (SRH) induced precise transfer in 7S substitution line 48-41-6⊗ at 35 Krad was found effective. SRH_1 plants of CS(Ph^I)/ *Ae. kotschyi 396*//PBW343-

3///PBW373(48)-41-6⊗ X WL711 with high micronutrients, yield and harvest index were isolated. GISH analysis and molecular marker data of some of The SRH_1 -1, -8, -14, -23 and -28 plants confirmed the transfer of 7S chromosome fragments. Molecular marker data also indicated that the SSR markers wmc405 and barc126, tentatively mapped on short arm of 7S chromosome, were associated with high grain Fe and Zn content. Plant SRH_3 -14-2-⊗ had short arm translocations and SRH_3 -28-6-⊗ had short arm telomeric transfer. Both these derivatives had high Fe and Zn content, indicating that the short arm of 7S had the genes for Fe and Zn uptake/sequestration. Other plants with high micronutrients without any GISH signal might have smaller transfer, beyond the limits of GISH. Gama rays at 10, 20, 30 and 40 Krad were used for mutagenesis of wheat for grain quality improvements and reduced plants hight (Singh and Balyan, 2009). Selfed seeds from a tetraploid *H. vulgare* x *H. bulbosum* hybrid were irradiated for transferring powdery mildew-resistance (Pickering *et al.*, 1995).

Pollen irradiation hybrids (PRH) of both 7S and 7U (?) substitution derivatives, 48-41-6⊗ and 63-2-13⊗**,** respectively at 2 Krad was equally effective for precise transfer as that of seed irradiation hybrids. Plants PRH_1 -75, -82, -87, -91 and -124 of CS(Ph^I)/*Ae. kotschyi* 396//PBW343-3///PBW373(48)-41-6⊗/PBW343 (*GPC+ Lr24)* had high grain Fe and Zn content and good yield and harvest index. Plant no. PRH_2 -124 had translocation of 7S chromosome telomeric region, indicating that the genes for Fe and Zn uptake might be present on 7S short arm but not in the telomere, and QTLs for Fe and Zn uptake were present on the long arm of 7S chromosome as was indicated by *ph1b* hybrid plant BC_1F_2-487.

The Plants PRH_1 -201, -231, -237, -247, -258, -266, -312 and -368 of CS(Ph^I)/*Ae. kotschyi 3790*//UP2338-2///WL711(63)-2-13⊗/PBW343 (*GPC+ Lr24)* were isolated based on high grain Fe and Zn content, grain yield and harvest index. PRH_1 -312 had the 7U short arm transferred, indicating that short arm of 7U also had the genes for Fe and Zn uptake/sequestration, which might be orthologous to the genes on 7S chromosome. A positive correlation was observed in Fe and Zn concentration among advanced progenies of each type of hybrid derivatives. Positive correlation was also observed for Zn with S, P, Fe and Na and Cu with K, Mg, Ni, P in common wheat (Caballero, 2002). The wheat-*A. cristatum* disomic addition was used as bridge material to produce wheat-*A. cristatum* translocation lines for good agronomic traits, such as high grain number per spike, powdery mildew resistance and stress tolerance induced by ^{60}Co-γ irradiation (Song *et al.*, 2013).

Among the advance generation BC_1F_2 of three selected *ph1bph1b* homozygous and 7S/7D monosomic plants, plants BC_1F_2-407, -433, -435, -471, -472, -487, -489, -517, -525, and -533 with high yield, harvest index and micronutrients have been isolated. GISH analysis

of some of these plants confirmed the 7S chromosomal translocations. In some of the selected *ph1bph1b* homozygous plants, BC_1F_2-471 with high grain Fe and Zn content, there were found multiple transfers as per GISH indicating simultaneous transfer from 7S to various group 7 chromosomes through multivalent formation. The BC_1F_2-487 plant had long arm of 7S chromosome substituted for 7DL. Both these plants had high Fe and Zn content, indicating the QTLs for Fe and Zn uptake are present on both the arms of the 7S chromosome. Tiwari *et al.*, (2009) reported that QTLs for Fe and Zn content were present on both the arms of 7A chromosome. In BC_1F_3 generation of *ph1b* homozygous and 7S/7D monosomic plants, BC_1F_2-407-2, -471-5, -471-8, -471-14, -487-2, -487-6 and -487-18 had high grain Fe and Zn content along with high grain yield and harvest index. These plants had 40-60% increase in Fe and Zn content over the wheat cultivar. Translocation lines of wheat were developed with stem rust resistance that had *Sr39* gene conferring resistance to at least seven stem rust races using *ph1b* (Yu *et al.*, 2010). Naranjo and Fernández-Rueda (1996) found homoeologous pairing and recombination between individual chromosomes of wheat and rye in their hybrids carrying the *ph1b* mutation.

The 7S/7D substitution line, 48-41-6⊗ with high grain micronutrient was also highly resistant to powdery mildew suggesting that the genes for powdery mildew resistance and genes for micronutrients uptake might be linked on 7S chromosome. The derivatives of all three hybrids approaches i.e. seed irradiation, pollen irradiation and *ph1b* hybrids, which had high grain Fe and Zn content were also found resistant to powdery mildew and had 7S short arm transferred, indicating that the genes for micronutrient uptake and powdery mildew resistance might be linked on short arm of 7S chromosome. Plant PRH_2-124 had translocation of 7S chromosome telomeric region, was resistant to powdery mildew and plant PRH_2-82 had 7S chromosome without telomeric region, was found susceptible to the powdery mildew, indicating that powdery mildew resistance gene could be present in sub-telomeric region of the 7S chromosome. Powdery mildew resistance might be linked to SSR markers wmc405 and barc126 as indicated by SSR marker data on seed irradiated hybrids. Genes for micronutrient uptake were also linked to these markers, further proving the linkage of powdery mildew and micronutrient uptake genes. *Aegilops variabilis* and various other *Aegilops* species have genes for powdery mildew resistance. These genes have been transferred to wheat by molecular breeding (Spetsov *et al.*, 1997, Schneider *et al.*, 2008). Powdery mildew resistance *Pm21* locus was transferred from *Haynaldia villosa* to wheat using female gamete irradiation induced transfer (Chen *et al.*, 2012). But no powdery mildew resistance gene has been reported so far in *Ae. kotschyi* 396, indicating that this gene might be new gene for powdery mildew resistance, which can be used for wheat improvement.

During development of wheat-Aegilops derivatives for high grain Fe and Zn content (Rawat *et al.,* 2010; Tiwari *et al.,* 2010; Kumari *et al.,* 2011) Chinese Spring with Ph^I (Chen *et al.,* 1994) was used to induce homeologuos pairing. None of the derivatives had any transfer to wheat chromosomes indicating that either the Ph^I gene is ineffective for inducing homoeologous pairing or the Ph^I stock was not stable. It was therefore decided to initiate fresh cross of high micronutrient *Aegilops* species with mono 5B wheat (*Triticum aestivum* cv. Pavon) for homoeologous pairing in hybrids without 5B chromosome.

5B deficiency allows the pairing and recombination between the chromosomes of wheat and those of the related species. Plants of Pavon mono 5B/*Ae. kotschyi* 3790 without 5B, had high chromosome pairing and multivalent formation. Chromosome pairing and multivalent formation in 5B deficient plants indicated the effectiveness of the use of mono 5B in wide hybridization. The BC_2F_1 plants of Pavon mono 5B/*Ae. kotschyi* 3790//PBW343(*GPC+Lr-24*)///PBW343(*GPC+Lr-24*) had high Fe and Zn content. The chromosome number of these plants varied form 42-48 and with 2-7 univalents. These plants are expected to have high frequency of recombination between wheat and *Ae. kotschyi* chromosomes which can be selected through molecular markers and GISH. Both homologous and homoeologous chromosomes formed multivalent in the absence of 5BL (Hobolth, 1981). Deficiency of 5B chromosome allowed the painring and recombination in *T. aestivum* cv. Chinese Spring x *Aegilops columnaris* hybrids (Lacadena *et al.,* 1967).

The Derivatives of all types of hybrids i.e. SRH, PRH, *ph1b* induced and 5B deficiency induced, with very high Fe and Zn content had poor tillering, seed set, and low harvest index indicated that micronutrient content was negatively correlated with yield and harvest index. This negative correlation might be due to distribution of fixed amount of micronutrient per plant among less number of seeds the plants. Plants with shrivelled seeds in the hybrid progenies also had high Fe and Zn content suggesting that the negative correlation between seed size and micronutrient concentration, could be due more aleurone area per unit mass of shrivelled seeds as compared to the bold seeds.

All the selected plants with chromosomal translocations had better genetic system for Fe and Zn uptake from the soil and transport within the plants but the overall concentrations of these micronutrients in the seeds was however less than the donor *Aegilops* species. The biofortification of wheat for Fe and Zn content could be achieved up to 40-50% without any linkage drag. Pyramiding of these introgressed genes/QTLs from different sources through molecular breeding can be done to achieve enhanced biofortification of these micronutrients.

Table of Contents

6. REFERENCES

1. Abdel-Ghany S.E., Muller-Moule P., Niyogi K.K., Pilon M. and Shikanai T. Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. *Plant Cell.* **17**:1233-1251 (2005).
2. Alloway B.J. Zinc in Soils and Crop Nutrition. IZA Publications. International Zinc Association, Brussels (2007).
3. Andres-Colas N., Sancenon V., Rodriguez-Navarro S., Mayo S., Thiele D.J., Ecker J.R., Puig S. and Penarrubia L. The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. *Plant J.* **45**:225-236 (2006).
4. Ansari M.J., Kumar R., Singh K. and Dhaliwal H.S. Characterization and molecular mapping of EMS-induced brittle culm mutants of diploid wheat (*Triticum monococcum* L.). *Euphytica*. **186**:165-176 (2012).
5. Auld D.S. Zinc coordination sphere in biochemical zinc sites. Biometals **14**:271-313 (2001).
6. Balyan H.S., Gupta P.K., Rustgi S., Bandopadhyay R., Goyal A., Singh R., Kumar A. Kumar N. and Sharma S. Development and use of SSRs of bread wheat for genetic and physical mapping and transferability to the species of *Triticum-Aegilops* complex. *Czech J. Genet. Plant Breed*. **41**:141-144 (2005).
7. Bandhopadhyay R., Sharma S., Rustgi S., Singh R, Gupta A, Balyan H.S. and Gupta P.K. DNA polymorphism among 18 species of *Triticum- Aegilops* complex using wheat EST-SSRs. *Plant Sci.* **166**:349-356 (2004).
8. Bänziger M. and Long J. The potential for increasing the iron and zinc density of maize through plant breeding. *Food Nutr. Bull.* **21**:397-400 (2000).
9. Bashir K., Inoue H., Nagasaka S., Takahashi M., Nakanishi H., Mori S. and Nishizawa N.K. Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. *J. Biol. Chem.* **43**:32395-32402 (2006).
10. Beard J.L. Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. *J. Nutr.* **131**:568-580 (2001).
11. Belderok B. Development in bread-making processes. Plant Foods for Human Nutrition **55**:1-14 (2000).
12. Bhaskaram P. Micronutrient malnutrition, infection and immunity: an overview. *Nutr. Rev*. **60**:40-45 (2008).
13. Black M.M. Zinc deficiency and child development. *Am. J. Clin. Nutr.* **68**:464S-469S (1998).
14. Bouis H.E. Special issue on improving human nutrition through agriculture. *Food Nutr. Bull*. **21**:351-576 (2000).
15. Bouis H.E. The potential of genetically modified food crops to improve human nutrition in developing countries. *J. Dev. Stud.* **43**:79-96 (2007).
16. Bouis H.E. Economics of enhanced micronutrient density in food staples. *Field Crop Res*. **60**:165-173 (1999).

17. Brabin B.J., Hkimi M. and Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. *J Nutr*. **131**:604-614 (2001).
18. Brinch-Pederson H., Borg S., Tauris B. and Holm P.B. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J Cereal Sci.* **46**:308-326 (2007).
19. Brinch-Pederson H., Olesen A., Rasmussen S.K. and Holm P.B. Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. *Mol. Breed*. **6**:195-206 (2000).
20. Brinch-Pederson H., Sørensen L.D. and Holm P.B. Engineering crop plants: getting a handle on phosphate. *Trends Plant Sci.* **7**:118-125 (2002).
21. Brown J.C. and Jolley V.D. Plant metabolic responses to iron-deficiency stress. *Bio Science.* **39**:546-551 (1989).
22. Brown J.C. Iron chlorosis in plants. *Adv. Agron*. **13**:329-369 (1961).
23. Brown K., Peerson J.M. and Allen L.H. Effects of zinc supplementation on children's growth. In: *Role of trace elements for health promotion and disease prevention.* Sandström, B., Walter, P., eds. *Bibliotheca Nutritio et Dieta*, Basel: Karger. **54**:76-83 (1998).
24. Caballero B. Global patterns of child health: the role of nutrition. *Ann. Nutr and Metabol.* **46**:3-7 (2002).
25. Cakmak I., Pfeiffer W.H. and McClafferty B. Biofortification of durum wheat with zinc and iron. *Cereal Chem.* **87**:10-20 (2010).
26. Cakmak I. Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant Soil.* **247**:3-24 (2002).
27. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil*. **302**:1-17 (2008).
28. Cakmak I., Öztürk L., Eker S., Torun B., Kalfa H.I. and Yilmaz A. Concentration of zinc and activity of copper/zinc superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. *J. Plant Physiol*. **151**:91-95 (1997).
29. Cakmak I., Yilmaz A., Ekiz H., Torun B., Erenoglu B. and Braun H.J. Zinc deficiency as a critical nutritional problem in wheat production in Central Anatolia. *Plant and Soil.* **80**:165-172 (1996).
30. Cakmak I., Derici R., Torun B., Tolay I., Braun H.J. and Schlegel R. Role of rye chromosomes in improvement of zinc efficiency in wheat and triticale. *Plant Soil.* **196**:249-253 (1997).
31. Cakmak I., Gulut K.Y., Marschner and Graham R.D. Effect of Zinc and iron deficiency on phytosiderophore release in wheat genotypes differing in zinc efficiency. *J. Plant. Nutr.* **17**:1-17 (1994).
32. Cakmak I., Ozkan H., Braun H.J., Welch R.M. and Romheld V. Zinc and iron concentrations in seeds of wild, primitive and modern wheats. *Food. Nutr. Bull*. **21**(4):401-403 (2000).

33. Cakmak I., Torun A., Millet E., Feldman M., Fahima T., Korol A., Nevo E., Braun H.J. and Ozkan H. *Triticum dicoccoides*: An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci. Plant Nutr.* **50**:1047-1054 (2004).
34. Cakmak I., Yilmaz A., Ekiz H., Torun B., Erenoglu B. and Braun H.J. Zinc deficiency as a critical nutritional problem in wheat production in Central Anatolia. *Plant and Soil.* **80**:165-172 (1996).
35. Calderini D.F. and Monasterio I.O. Grain position affects grain macronutrient and micronutrient concentrations in wheat. *Crop Sci.* **43**:141-151 (2003).
36. Capell T. and Christou P. Progress in plant metabolic engineering. *Curr. Opin. Biotechnol.* **15**:148-154 (2004).
37. Ceoloni C., Signore D., Pasquini G. and Testa A. Transfer of mildew resistance from *Triticum longissimum* into wheat by *ph1* induced homoeologous recombination. In: TE Miller and RMD Koebner (*Eds*) *Proc. 7th Int. Wheat Genet Symp*. Cambridge P-221-226 (1988).
38. Chen P.,You C., Hu Y., Chen S., Zhou B., Cao A. and Wang X. (2012) Radiation-induced translocations with reduced Haynaldia villosa chromatin at the Pm21 locus for powdery mildew resistance in wheat. *Mol Breeding* **31**:477-484 (2013).
39. Chen Q., Tsujimoto H. and Gill B.S. Transfer of Ph^I gene promoting homoeologous pairing from *Triticum speltoides* into common wheat and their utilization in alien genetic introgression. *Theor. Appl. Genet*. **88**:97-101 (1994).
40. Chen R., Xue G., Chen P., Yao B., Yang W., Ma Q., Fan Y., Zhao Z., Tarczynski M.C. and Shi J. Transgenic maize plants expressing a fungal phytase gene. *Trangenic Res.* **17**:633-643 (2008).
41. Chhuneja P., Dhaliwal H.S., Bains N.S. and Singh K. *Aegilops kotschyi* and *Ae. tauschii* are the sources for high grain iron and zinc. *Plant Breed.* **125**:1-3 (2006).
42. Cohen C.K., Gavin D.F. and Kochian L.V. The role of iron deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiol*. **116**:1063-1072 (1998).
43. Colangelo E.P. and Geurinot M.L. Put the metal to the petal: metal uptake and transport throughout plants. *Curr. Opin. Plant Biol.* **9**:322-330 (2006).
44. Conner J.A., Conner P., Nasrallah M.E. and Nasrallah, J.B. Comparative mapping of the Brassica S locus region and its homeolog in Arabidopsis: implications for the evolution of mating systems in the Brassicaceae. *Plant Cell* **.10**:801-812 (1998).
45. Connolly E.L., Fett J.P. and Guerinot M.L. Expression of the IRTI metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell*. **4**:1347-1357 (2002).
46. Cosgrove D.J. The chemistry and biochemistry of inositl phosphates, *Rev. Pure Appl. Chem*. **6**:209-224 (1996).
47. Curie C. and Briat J.F. Iron transport and signaling in plants. *Annu. Rev. Plant. Biol*. **54**:183-206 (2003).

48. Curie C., Panaviene Z., Loulergue C., Dellaporta S.L., Briat J.F. and Walker E.L. Maize yellow stripe 1 encodes a membrane protein directly involved in Fe (III) uptake. *Nature.* **409**:346-349 (2001).
49. Daud H.M. and Gustafson J.P. Molecular evidence of Triticum speltoides as a B-genome progenitor of wheat (Triticum aestivum). *Genome.* **39**:543-548 (1996).
50. Davila-Hicks P., Theil E.C. and Lonnerdal B. Iron in ferritin or in salts (ferrous sulphate) is equally bioavailable in nonanemic women. *Am J Clin Nutr*. **80**:936-940 (2004).
51. DellaPena D. Nutritional genomics: manipulating plant micronutrients to improve human health. *Science.* **285**:375-379 (1999).
52. Demment M.W., Young M.M. and Sensenig R.L. Providing micronutrients through food-based solutions: A key to human and national development. *J. Nutr.* **133**:3879-3885 (2003).
53. Denbow D.M., Grabau E.A., Lacy G.H., Kornegay E.T., Russell D.R. and Umbeck P.F. Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. *Poult Sci.* **77**(6):878–881 (1998).
54. Dhaliwal G.S. and Arora R. Estimation of losses due to insect pests in field crops In: B. Sarath Babu, K.S. Varaprasad, K. Anitha, R.D.V.J. Prasada Rao, S.K. Chakrabarty and P.S. Chandukar (eds) Resources Management in Plant Protection. Vol. 1. Plant Protection Association of India, Hyderabad. 11-23 (2002).
55. DiDonato R.J.J., Roberts L.A., Sanderson T., Eisley R.B. and Walker E.L. *Arabidopsis* YELLOW STRIPE-LIKE2 (YSL2) a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant J*. **39**:403-414 (2004).
56. Ding C. and Jin S. High throughput methods for SNP genotyping. *Methods Mol Bio™.* **578**: 245-254 (2009).
57. Dou Q. W., Tanaka H., Nakata N. and Tsujimoto H. Molecular cytogenetic analyses of hexaploid lines spontaneously appearing in octoploid Triticale. *Theor. Appl. Genet.* **114**:41-47 (2006).
58. Drager D.B., Desbrosses-Fonrouge A.G., Krach C., Chardonnens A.N., Meyer R.C., Saumitou-Laprade P. and Kramer U. Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co segregate with zinc tolerance and account for high MTP1 transcript levels. *Plant J.* **39**:425-439 (2004).
59. Drakakaki G., Christou P. and Stöger E. Constitutive expression of soybean ferritin cDNA intransgenic wheat and rice results in increased iron levels in vegetative tissues but not in seeds. *Transgenic Res.* **9**:445-452 (2000).
60. Dvorak J. and Zhang H.B. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proc Natl Acad Sci USA.* **87**:9640–9644 (1990).
61. Dvorak J., Di-Terlizzi P., Zhang H.B. and Resta P. The evolution of polyploid wheats: identification of the A genome donor species. *Genome.* **36:**21-31 (1993).
62. Eckhardt U., Marques A. and Buckhout T.J. Two iron- regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. *Plant Mol. Biol*. **45**:437-448 (2001).

63. Eren E. and Arguello J.M. *Arabidopsis* HMA2, a divalent heavy metaltransporting P(IB)-type ATPase, is involved in cytoplasmic Zn2+ homeostasis. *Plant Physiol*. **136**:3712-3723 (2004).
64. FAO. Cereals and other starch-based staples: are consumption patterns changing? Joint Meeting of The Intergovernmental Group on Grains (30TH Session) And The Intergovernmental Group on Rice (41ST Session) Rome, Italy (2004).
65. Faris J.D., Friebe B. and Gill B.S. Wheat Genomics: Exploring the Polyploid Model. *Current Genomics*. **3**(15):577-591 (2002).
66. Feldman M., Lupton F.G.H., Miller T.E. Wheats. *Triticum* spp. (*Gramineae-Triticinae*). In: Smartt J, NW Simmonds (eds) Evolution of crop plants, 2nd edn. Longman Scientific & Technical Press, London, UK. 184-192 (1995).
67. Fernandez-Calvin B. and Orellana J. Relationship between pairing frequencies and genome affinity estimations in *Aegilops ovata* ×*Triticum aestivum* hybrid plants. *Heredity*. **68**:165–172 (1992).
68. Friebe B., Jiang J., Gill B.S. and Dyck P.L. Radiation-induced nonhomoeologous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust. *Theor. Appl. Genet.* **86**:141-149 (1993).
69. Friebe B., Jiang J., Tuleen N. and Gill B.S. Standard karyotype of *Triticum umbellulatum* and the characterization of derived chromosome addition and translocation lines in common wheat. *Theor. Appl. Genet.* **90**:150-156 (1995a).
70. Friebe B., Tuleen N.A. and Gill B.S. Standard karyotype of *Triticum searsii* and its relationship with other S-genome species and common wheat. *Theor. Appl. Genet.* **91**: 248-254 (1995b).
71. Friebe B., Jiang J., Raupp W.J., McIntosh R.A. and Gill B.S. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica*. **91**:59-87 (1996).
72. Friebe B.R., Tuleen N.A. and Gill B.S. Development and identification of a complete set of *Triticum aestivum-Aegilops geniculata* chromosome addition lines. *Genome*. **42**:374-380 (1999).
73. Friebe B., Qi L.L., Nasuda S., Zhang P., Tuleen N.A. and Gill B.S. Development of complete set of *Triticum aestivum- Aegilops speltoides* chromosome addition lines. *Theor. Appl. Genet.* **101**:51-58 (2000).
74. Garrido M.S., Morikawa K.C., Nakanishi H. and Saigusa M. Strategies for iron mobilization and uptake in plant roots. *Tohoku Journal of Agricultural research*. **56**:3-4 (2006).
75. Genc Y., McDonald G.K. and Graham R..D. Contribution of different mechanisms to zinc efficiency in bread wheat during early vegetative stage. *Plant Soil*. **281**:353-367 (2006).
76. Gill B.S. and Friebe B. Cytogenetics, phylogeny and evolution of cultivated wheats. In: Curtis BC, Rajaram S, Gómez Macpherson H, editors. Bread Wheat: improvement and production. Food and Agriculture Organization of the United Nations, Rome, Italy. (2002).
77. Gill K.S., Gill B.S., Endo T.R. and Mukai Y. Fine physical mapping of Ph1, a chromosome pairing regulator gene in polyploid wheat. *Genetics*. **134**:1231-1236 (1993).

78. Gill B.S., Huang L., Kuraparthy V., Raupp W.J., Wilson D.L. and Friebe B. Alien genetic resources for wheat leaf rust resistance, cytogenetic transfer, and molecular analysis. *Australian Journal of Agricultural Research.* **59**(3):197–205 (2008).
79. Gitlin J.D. Distributing nutrition. *Science.* **314**:1252-1253 (2006).
80. Golovnina K.A., Glushkov S.A., Blinov A.G., Mayorov V.I., Adkison L.R. and Goncharov N.P. Molecular phylogeny of the genus Triticum L. *Pl. Syst. Evol.* **264**:195-216 (2007).
81. Goto F., Yoshihara T., Shigemoto N., Toki S. and Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotech*. **17**:282-286 (1999).
82. Graham R.D., Welch R.M. and Bouis H.E. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Adv. Agron.* **70**:77-142 (2001).
83. Greenshieldsn D.L., Liu G. and Wei Y. Roles of iron in plant defense and fungal virulence. *Plant Signal Behav.* **2**:300-302 (2007).
84. Gregorio G.B. Progress in breeding for trace minerals in staple crops. In Symposium: Plant breeding: A new tool for fighting micronutrient malnutrition. *J. Nutr.* **132**:500S-502S (2002).
85. Grotz N., Fox T., Connolly E., Park W., Guerinot M.L. and Eide§ D. Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. *Proc. Natl. Acad. Sci. USA.* **95**:7220-7224 (1998).
86. Gupta P.K., Balyan H.S., Edwards K.J., Isaac P., Korzun V., Roder M., Gautier M.F., Jourdrier P., Schlatter A.R., Dubcovsky J., de la Pena R.C., Khairallah M., Hayden M.J., Keller B., Wang R.C.C., Hardouin J.P., Jack P. and Leroy P. Genetic mapping of 66 new SSR loci in bread wheat. *Theor. Appl. Genet.* **105**:413-422 (2002).
87. Gupta P.K., Balyan H.S., Sharma P.C. and Ramesh B. Microsatellites in plants: A new class of molecular markers. *Curr. Sci*. **70**:45-54 (1996).
88. Gustafson P., Raskina O., Ma X. and Nevo E. Wheat Evolution, Domestication, and Improvement, in Wheat Science and Trade (ed B. F. Carver), Wiley-Blackwell, Oxford, UK. (2009).
89. Guttieri M.J., Bowen D., Dorsch J.A., Raboy V. and Souza E. Identification and characterization of a low phytic acid wheat. *Crop Sci*. **44**:418-424 (2004).
90. Hacisalihoglu G., Hart J.J., Vallejos C.E. and Kochian L.V. The role of shootlocalized processes in the mechanism of Zn efficiency in common bean. *Planta.* **218**:704-711 (2004).
91. Hacisalihoglu G., Hart J.J., Wang Y.H., Cakmak I. and Kochian L.V. Zinc efficiency is correlated with enhanced expression and activity of zinc-requiring enzymes in wheat. *Plant Physiol*. **131**:595-602 (2003).
92. Hacisalihoglu G. and Kochian L.V. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytologist.* **159**:341-350 (2003).
93. Hajjar R. and Hodgkin T. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica.* **156**:1-13 (2007).
94. Hall J.L. and Williams L.E. Transition metal transporters in plants. *J. Exp Bot.* **54**:2601-2613 (2003).

95. Hambidge K.M. Zinc. In: Trace elements in human and animal nutrition. Mertz, W., ed. 5th, Vol. 1. Academic Press, Inc. Orlando, Florida (1987).
96. Harper J., Armstead I., Thomas A., James C., Gasior D., Bisaga M., Roberts L., King I. and King J. Alien introgression in the grasses *Lolium Perenne* (Perennial Ryegrass) and *Festuca Pratensis* (Meadow Fescue): The development of seven monosomic substitution lines and their molecular and cytological characterization *Annals Of Botany*. **107**(8):1313-1321 (2011).
97. Haslett B.S., Reid R.J. and Rengel Z. Zinc mobility in wheat: Uptake and distribution of zinc applied to leaves or roots. *Ann. Bot*. **87**:379-386 (2001).
98. Haydon M.J. and Cobbett C.S. Transporters of ligands for essential meal ions in plants. *New Phytologist*.**174**:499-506 (2007).
99. Hell R.. and Stephan U.W. Iron uptake, trafficking and homeostasis in plants. *Planta* **216**:541-551 (2003).
100. Henriques R., Jasik J., Klein M., Martinoia E., Feller U., Schell J., Pais M.S. and Koncz C. Knock-out of Aabidopsis metal transporter gene IRT1 results in irn deficiency accompanied by cell differentiation defects. *Plant Mol Biol*. **50**:587-597 (2002).
101. Heslop-Harrison J.S., Harrison G.E. and Leitch I.J. Reprobing of DNA: DNA in situ hybridization preparations. *Trends Genet.* **8**: 372-373 (1992).
102. Heun M., Schafer-Prehl R., Klawan D., Castagna R., Accerbi M., Borghi B. and Salamini F. Site of einkorn wheat domestication identified by DNA fingerprinting. *Science.* **278:** 1312-1314 (1997).
103. Higuchi K., Suzuki K., Nakanishi H., Yamaguchi H., Nishizawa N.K. and Mori S. Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. *Nature Biotechnology*. **19**:466-469 (2001).
104. Hirschi K. Nutritional improvements in plants: time to bite on biofortified foods. *Trends in Plant Science.* **13**:459-463 (2008).
105. Hobolth P. Chromosomal pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalent to bivalnets, a mechanism of exclusive bivalent formation. carlsberg res. *Comuni.* **46**:129-173 (1981).
106. Hochmuth G. J. Iron (Fe) nutrition of plants (SL353), A Series of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. (2011).
107. Hoffman R., Benz E., Shattil S., Furie B., Cohen H., Silberstein L. and Glave M.P. Hematology: basic principles and practice, 3rd ed. ch 26: disorders of iron metabolism: iron deficiency and overload. Churchill Livingstone, Harcourt Brace & Co, New York (2000).
108. Holm P.B. Chromosomal pairing and synaptonemal comlex formation in hexaploid wheat, Monosomic for 5B. carlsberg res. *Comuni.* **53**:57-89 (1988).
109. Holtz C. and Brown K.H. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* **25**: 94-204 (2004).

110. Hong C.Y., Cheng K.J., Tseng T.H., Wang C.S., Liu L.F. and Yu S.M. Production of two highly active bacterial phytases with broad pH optima in germinated transgenic rice seeds. *Transgenic Res.* **13**:29-39 (2004).
111. Hossain K.G., Riera-lizarazu O., Kalavacharla V., Vales M.I., Maan S.S. and Kianian S.F. Radiation hybrid mapping of the species cytoplasm-specific (*scs*^{ae}) gene in wheat. *Genetics***. 168:**415-423 (2004).
112. Biannual reports on global food markets. Food Outlook, June 2013. http://www.globefish.org/upl/Publications/Food%20Outlook%20June%202013.pdf
113. http://faostat.fao.org/site/567/default.aspx#ancor
114. http://www.fao.org/docrep/004/y2809e/y2809e0m.htm
115. Huang S., Sirikhachornkit A., Su X., Faris J., Gill B., Haselkorn R. and Gornicki P. Gene encoding plastids acetyl-CoA carboxylase and 3 phosphoglycerate kinase of the *Triticum*/ *Aegilops* complex and the evolutionary history of polyploid wheat. *Proc. Natl. Acad. Sci. USA.* **99**:8133-8138 (2002).
116. Hurtado P., Olsen K.M., Buitrago C., Ospina C., Marin J., Duque M., de Vicente C., Wongtiem P., Wenzel P., Killian A., Adeleke M. and Fregene M. Comparison of simple sequence repeat (SSR) and diversity array technology (DArT) markers for assessing genetic diversity in cassava (Manihot esculenta Crantz). *Plant Genetic Resouces*. **6**:208-214 (2008).
117. Impa S.M., Morete M.J., Ismail A.M., Schulin R. and Johnson-Beebout S.E. Zn uptake, translocation and grain Zn loading in rice (*Oryza sativa* L.) genotypes selected for Zn deficiency tolerance and high grain Zn. *J Exp Bot*. **64**:2739-2751 (2013).
118. Ishimaru Y., Masuda H., Suzuki M., Bashir K., Takahashi M., Nakanishi H., Mori S. and Nishizawa N.K. Overexpression of the OsZIP4 zinc transporter confers disarrangement of zinc distribution in rice plants. *J. Exp Bot.* **58**:2909-2915 (2007).
119. Ishimaru Y., Suzuki M., Kobayashi T., Takahashi M., Nakanishi H., Mori S. and Nishizawa N.K. OsZIP4, a novel zinc-regulated zinc transporter in rice. *J. Exp. Bot.* **56:**3207-3214 (2005).
120. Jampates R. and Dvorak J. Location of the *Ph1* locus in the metaphase chromosome map and the linkage map of the 5Bq arm of wheat. *Can J Genet Cytol.* **28**:511-519 (1986).
121. Jauhar P.P. Meiotic Restitution in Wheat Polyhaploids (Amphiploids): A Potent Evolutionary Force. *J. Hered.* **98:**188-193 (2007).
122. Johns T. and Enzaguirre P.B. Biofortification, biodiversity and diet: A search for complementary applications against poverty and malnutrition. *Food Policy.* **32**:1-24 (2007).
123. Johnson A., Kyriacou B., Callahan D., Carruthers L., Stangoulis J., Lombi E. and Tester M. Constitutive overexpression of the *OsNAS* gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One.* **6**(9):24476 (2011).
124. Joppa L.R. and Williams N.D. Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. *Genome*. **30**:222-228 (1988).
125. Kalavacharla V., Hossain K., Gu Y., Riera-Lizarazu O., Vales M.L., Bhamidimarri S., Gonzalez-Hernandez J.L., Maan S.S. and Kianian S.F. High-resolution radiation hybrid map of wheat chromosome 1D. *Genetics.* **173**:1089-1099 (2006).

126. Kanazawa K., Higuchi K., Nishizawa N.K., Fushiya S., Chino M. and Mori S. Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient conditions, in Gramineae. *J Exp Bot.* **45**:1903-1906 (1994).
127. Kerber E.R. and Dyck P.L. Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops speltoides* x *Triticum monococcum*. *Genome.* **33**:530-537 (1990).
128. Kihara H. Discovery of the DD-analyser, one of the ancestors of Triticum vulgare (Japanese). *Agric Horticulture (Tokyo).* **19:**13-14 (1944).
129. Kim C.S., Hong H., Lee J.S., Kim J.Y and Maeng W.J. A Study on Nutrient Intake Status and Food Sources of Iron by Dietary Iron Density of High School Girls in Seoul. *Korean J Nutr*. **40**:371-384 (2007).
130. Kim D., Gustin J.L., Lahner B., Persans M.W., Baek D., Yun, D.J. and Salt D.E. The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae. Plant J*. **39**:237-251 (2004).
131. Kim S.A. and Guerinot M.L. Mining iron: Iron uptake and transport in plants. *FEBS letters*. **581**:2273-2280 (2007).
132. King I.P., Purdie K.A., Orford S.E., Reader S.M. and Miller T.E. Detection of homoeologous chiasma formation in *Triticum durum* X *Thinopyrum-bessarabicum* hybrids using genomic in-situ hybridization. *Heredity.* **71**:369-372 (1993).
133. King J., Armstead I.P., Donnison I.S., Harper J.A., Roberts L.A., Thomas H., Ougham H., Thomas A., Huang L. and King I.P. Introgression mapping In The grasses. *Chromosome Research: An International Journal On The Molecular, Supramolecular And Evolutionary Aspects Of Chromosome Biology*. **15**(1): 105-113 (2007).
134. King J.C. and Turnlund J.R. Human zinc requirements. In: Mills C.F., ed. Zinc in Human Biology. London: Springer-Verlag. UK (1989.).
135. Kirking M.H. Treatment of chronic iron overload. *Clin Pharm*. **10**:775-783 (1991).
136. Kobae Y., Uemura T., Sato M.H., Ohnishi M., Mimura T., Nakagawa T. and Maeshima M. Zinc transporter of *Arabidopsis thaliana* AtMTP1is localized to vacuolar membranes and implicated in zinc homeostasis. *Plant Cell Physiol*. **45**:1749-175 (2004).
137. Kobayashi T., Nakanishi H., Takahashi M., Mori S. and Nishizawa N. Generation and field trials of transgenic rice tolerant to iron deficiency. *Rice.***1**:144-153 (2008).
138. Kochian L.V. Mechanisms of micronutrient uptake and translocation in plants. In: Mortvedt J.J., Cox F.R., Shuman L.M., Welch R.M., eds. Micronutrients in agriculture. Madison: *Soil Science Society of America.* 229-296 (1991).
139. Koike S., Inoue H., Mizuno D., Takahashi M., Nakanishi H., Mori S. and Nishizawa N.K. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J*. **39**:415-424 (2004).
140. Kumari N., Rawat N., Tiwari V.K., Kumar S., Chhuneja P., Singh K., Randhawa G.S. and Dhaliwal H.S. Introgression of group 4 and 7 chromosomes of *Ae. peregrina* in wheat enhances grain iron and zinc density. *Molecular Breeding.* **28**:623-634 (2011).

141. Kuraparthy V., Chhuneja P., Dhaliwal H. S., Kaur S., Bowden R.L. and Gill B.S. Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. *Theor. App. Genet.* **114**:1379-1389 (2007a).
142. Kuraparthy V., Chhuneja P., Dhaliwal H. S., Kaur S., Bowden R.L. and Gill B.S. Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. *Theor. App. Genet.* **114**:1379-1389 (2007b).
143. Kuraparthy V., Singh H., Singh S., Chunneja P. and Dhaliwal H.S. Microsatellite marker linked to a leaf rust resistance gene from *Triticum monococcum* transferred to bread wheat. *Journal of Plant Biochemistry & Biotechnology*. **10**:127-132 (2001).
144. Kuraparthy V., Sood S., Chhuneja P., Dhaliwal H.S., Kaur S., Bowden R.L. and Gill B.S. A cryptic wheat-*Aegilops triuncialis* translocation with leaf rust resistance gene *Lr58*. *Crop Science*. **47**:1995–2003 (2007b).
145. Kuraparthy V., Sood S. and Gill B.S. Genomic targeting and mapping of tiller inhibition gene (*tin3*) of wheat using ESTs and synteny with rice. *Functional Integrative Genomics.* **8**:33-42 (2008).
146. Kuwano M., Mimura T., Takaiwa F. and Yoshida K.T. Generation of stable 'low phytic acid' transgenic rice through antisense repression of the 1**D**-***myo***-inositol 3-phosphate synthase gene (*RINO1*) using the 18-kDa oleosin promoter. *Plant Biotechnol. J.* **7**:96-105 (2008).
147. Lacadena J.R. Introduction of allien variation into wheat by gene recombination. I. Crosses between mono V (5B) Triticum aestivum L. and Secale cereale L. and Aegilops columnaris zhuk. *Euphytica*, **16**(2):221-230 (1967).
148. Lanquar V., Lelievre F., Bolte S., Hames C., Alcon C., Neumann D.,Vansuyt G., Curie C., Schroder A., Kramer U., Barbier-Brygoo H. and Thomine S. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO J.* **24**:4041-4051 (2005).
149. Larson S.R., Young K.A., Cook A., Blake T.A. and Raboy V. Linkage mapping of two mutations that reduce phytic acid content of barley grain. T*heor Appl Genet*. **97**: 141-146 (1998).
150. Le H.T. and Armstrong K.C. In-situ hybridization as a rapid means to assess meiotic pairing and detection of alien DNA transfers in interphase cells of wide crosses involving wheat and rye. *Mol. Gen. Genet.* **225**:33-37 (1991).
151. Le H.T., Armstrong K.C. and Miki B. Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Mol. Biol. Reporter*.**7**:150-158 (1989).
152. Le Jean M., Schikora A., Mari S., Briat J. F. and Curie C. A loss-of function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. *Plant J*. **44**:769-782 (2005).

153. Leitch A.R., Mosgoller W., Schwarzacher T., Bennett M.D. and Heslop-Harrison J.S. Genomic in-situ hybridization to sectioned nuclei shows chromosome domains in grass hybrids. *J. Cell Sci.* **95**:335-341 (1990).
154. Li L., Cheng X.D. and Ling H.Q. Isolation and characterization of Fe (III) chelate reductase gene LeFRO1 in tomato. *Plant Mol. Biol***. 54**:125-136 (2004).
155. Liu Q.L., Xu X.H., Ren X.L, Fu H.W., Wu D.X. and Shu Q.Y. Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). *Theor Appl Genet.* **114**:803-808 (2007).
156. Lonnerdal B. Genetically modified plants for improved trace element nutrition. *J. Nutr.* **133:**1490S–1493S (2003).
157. Lopez-Millan A.F., Ellis D.R. and Grusak M.A. Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. *Plant Mol Biol* . **54**:583-596 (2004).
158. Lott J.N.A., Liu J.C., Ockenden I., Truax M. and Lott J.N.A Phytic acid-phosphorus and other nutritionally important mineral nutrient elements in grains of wild-type and low phytic acid (lpa1–1) rice. *Seed Sci. Res.* **14**:109-116(2004).
159. Lott J.N.A., Ockenden I., Raboy V., Baten G.D. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Sci. Res.* **10** :11-33 (2000).
160. Lucca P., Hurrell R. and Potrykus I. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theor. Appl. Genet.* **102**:392-397 (2001).
161. Lucca P., Poletti S. and Sautter C. Genetic engineering approaches to enrich rice with iron and vitamin A. *Physiol plant.* **126**:291-303 (2006).
162. Lukac R.J., Aluru M.R. and Reddy M.B. Quatification of ferrtin from staple food crops. *J Agric Food Chem.* **57**:2155-2161 (2009).
163. .Goto F., Yoshihara T., Shigemoto N., Toki S. and Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotechnol*. 282–286.**57:** 2155-2161 (1999).
164. Maestra A.B. and Naranjo T. Homoeologous relationships of Aegilops speltoides chromosomes to bread wheat. *Theor Appl Genet.* **97**:181-186 (1998).
165. Maestra B. and Naranjo T. Homoeologous relationships of Aegilops speltoides chromosomes to bread wheat. *Theor Appl Genet.* **97**:181-186 (1988).
166. Marais G.F., McCallum B., Snyman J.E., Pretorius Z.A. and Marais A.S. Leaf rust and stripe rust resistance genes *Lr54* and *Yr37* transferred to wheat from *Aegilops kotschyi*. *Plant Br.* **124:**538-541 (2005).
167. Marschner H., Römheld V. and Kissel M. Different strategies in higher plants in mobilization and uptake of iron. *Journal Plant Nutrition.* **9**:695-713 (1986).
168. Masuda H., Ishimaru Y., Aung M.S., Kobayashi T., Kakei Y., Takahashi M., Higuchi K., Nakanishi H. and Nishizawa N.K. Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Scientific Reports.* **2**:1-7 (2012).
169. Masuda H., Suzuki M., Morikawa K.C., Kobayashi H.T., Nakanishi M., Takahashi M., Saigusa M. and Nishizawa N. K. Increase in iron and zinc concentrations in rice grains via

the introduction of barley genes involved in phytosiderophore synthesis. *Rice.* **1**:100-108 (2008).

170. McIntosh R.A., Devos K.M., Dubcvsky J., Rogers W.J., Morris C.F. supplement Appels R, Anderson O.D. Catalogues of gene symbols for wheat: (2005).
171. Mendoza C., Viteri F.E., Lönnerdal B., Young K.A., Raboy V. and Brown K.H. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr*. **68**:1123-1127(1998).
172. Meng F., Wei Y. and Yang X. Iron content and bioavailability in rice. *Trace Elements in Medicine and Biology*. **18**:333-3338 (2005).
173. Michalak M., Kumar A., Riera-Lizarazu O., Paux E., Gu Y., Choulet F., Feuillet C., Kumar S., Goyal A., Tiwari V., Dogramaci M., Hegstad J., Peckrul A., Kalavacharla V., Hossain K., Balyan H.S., Dhaliwal H.S., Gupta P.K., Randhawa G.S., Maan S.S. and Kianian, S.F. High-resolution radiation hybrid mapping in wheat: an essential tool for the construction of the wheat physical maps. *XI International Wheat Genetic Symposium*, Brisbane, Australia 2008.
174. Micronutrient Initiative, Controlling vitamin and mineral deficiencies in India: Meeting the Goal. Micronutrient Initiative, New Delhi (2006).
175. Mills R.F., Francini A., Ferreira da Rocha P.S., Baccarini P.J., Aylett M., Krijger G.C. and Williams L.E. The plant P1B-type ATPase AtHMA4 transports Zn and Cd and plays a role in detoxification of transition metals supplied at elevated levels. *FEBS Lett*. **579**:783-791 (2005).
176. Miranda L.M., Murphy J.P., Marshall D., Cowger C. and Leath S. Chromosomal location of Pm35, a novel *Aegilops tauschii* derived powdery mildew resistance gene intogressed into common wheat (*Triticum aestivum* L.) *Theor. Appl. Genet.* **114**:1451-1456 (2007).
177. Mizuno T., Usui K., Horie K., Nosaka S., Mizuno N. and Obata H. Cloning of three ZIP/Nramp transporter genes from a Ni hyperaccumulator plant *Thlaspi japonicum* and their Ni^{2+}-transport abilities. *Plant Physiol. Biochem.* **43**:793-801 (2005).
178. Monasterio I. and Graham R.D. Breeding for trace minerals in wheat. *Food. Nutr. Bull.* **21**:392-396 (2000).
179. Mori S. and Nishizawa N. Methionine as a dominant precursor of phytosiderophores in Graminaceae plants. *Plant Cell Physiology.* **28**:1081-1092 (1987).
180. Monasterio I., Rojas P.N., Meng E., Pixley K., Trethowan R. and Peña R.J. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J. Cereal Sci*. **46**:293–307 (2007).
181. Moreau S., Thomson R.M., Kaiser R.N., Trevaskis B., Guerinot M.L., Udvardi M.K., Puppo A. and Day D.A. GmZIP1 encodes a symbiosis-specific zinc transporter in soybean. *J. Biol. Chem.* **277**:4738-4746 (2002).
182. Morgan W.G., King I.P., Koch S., Harper J.A. and Thomas H.M. Introgression of chromosomes of *Festuca arundinacea* Var. glaucescens into *Lolium multiflorum* revealed by genomic in situ hybridisation (Gish). *Theor Appl Genet*. **103**(5): 696-701 (2001).

183. Mori S, Nishizawa N., Hayashi H., Chino M., Yoshimura E., Ishihara J. Why young rice plants highly susceptible to iron deficiency. *Plant and Soil.* **130**:143-156 **(**1991).
184. Mori S. (1999) Iron acquisition by plants. *Curr. Opin. Plant Biol*. **2**: 250–253.
185. Mori S., Nishizawa N. and Fujigaki K. Identification of rye chromosome 5R as a carrier of the genes for mugineic acid and related compounds. *Jpn. J. Genet.* **102**:373-378 (1990).
186. Mukai Y. and Gill B.S. Detection of barley chromatin added to wheat by genomic in-situ hybridization. *Genome.* **34**:448-452 (1991).
187. Murray M.G., Thomson W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic acid Res.* **8**: 4321-4325.
188. Nakanishi H., Okumura N., Umehara Y., Nishizawa N.K., Chino M., Mori S. Expression of a gene specific for iron deficiency (*Ids*3) in the roots of *Hordeum vulgare*. *Plant Cell Physiol*. **34**:401-410 (1993).
189. Nakanishi H., Yamaguchi H., Sasakuma T., Nishizawa N.K. and Mori S. Two dioxygenase genes, Ids3 and Ids2, from Hordeum vulgare are involved in the biosynthesis of mugineic acid family phytosiderophores. *Plant Mole Bio* **44**:199-207 (2000).
190. Naranjo T. and Fernández-Rueda P. Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the *ph1b* mutation. *Theor. Appl. Genet*. 93(1-2): 242-248 (1996).
191. Nestel P., Bouis H.E., Meenakshi J.V. and Pfeiffer W. Biofortification of Staple Food. *Crops. J. Nutr.* **136:**1064-1067 (2006).
192. Nishizawa N.K., Nakanishi H. and Mori S. Effect of iron deficiency on S-adenosylmethionine synthetase in barley roots. *Plant Nutri.* **19**:1189-1200 (1996).
193. Niu Z., Klindworth D.L., Friesen T.L., Chao S., Jin Y., Cai X., Xu S.S. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. *Genetics.* **187**(4):1011-1021 (2011).
194. Okumura N., Nishizawa N.K., Umehara Y., Ohata T, Nakanishi H., Yamaguchi T., Chino M. and Mori S. A dioxygenase gene (Ids2) expressed under iron deficiency conditions in the roots of *Hordeum vulgare*. *Plant Mol. Biol.* **25**:705-719 (1994).
195. Olsen R.A., Clark R.B. and Bennet J.H. The enhancement of soil fertility by plant roots. *Am. Scientist*. **69**:378-384 (1981).
196. Özkan H., Brandolini A., Pozzi C., Effgen S., Wunder J. and Salamini F., reconsideration of the domestication geography of tetraploid wheats. *Theor and App Genet* **110**:1052-60 (2005).
197. Ozkan H. and Feldman M. Genotypic variation in tetraploid wheat affecting homoeologous pairing in hybrids with *Aegilops peregrina. Genome.* **44**:1000–1006 (2001).
198. Palmgren M.G., Clemens S., Williams L.E., Krämer U., Borg S., SchjØrring J.K. and Sanders D. Zinc biofortification of cereals: problems and solutions.Trends in plant. *Science* **13**:464-473 (2008).
199. Paux E., Sourdille P., Salse J., Saintenac C., Choulet F., Leroy P., Korol A., Micahalak M., Kianian S., Spielmeyer W., Lagudah E., Somers D., Kilian A., Alaux A., Vaurin S., Bergès

H., Eversole K., Appels R., Safar J., Simkova H., Dolezel J., Bernerd M. and Feuillet C. A physical map of the 1-Gigabase bread wheat chromosome 3B. *Science.* **322:**101-104 (2008).

200. Pearson J.N. and Rengel Z. Uptake and distribution of ^{65}Zn and ^{54}Mn in wheat grown at sufficient and deficient levels of Zn and Mn II. During grain development. *J. Exp. Bot.* **46**:841-845 (1995).
201. Pearson J.N., Rengel Z., Jenner C.F. and Graham R.D. Transport of zinc and manganese to developing wheat grains. *Physiol. Plant.* **95**:449-455 (1995).
202. Peleg Z., Cakmak I., Ozturk L., Yazici A., Jun Y., Budak H., Korol A.B., Fahima T. and Saranga Y. Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theor. Appl. Genet.* **119**:353-369 (2009).
203. Peng J.H., Zadeh H., Lazo G.R., Gustafson J.P., Chao S., Anderson O.D., Qi L.L., Echalier B., Gill B.S., Dilldirligi M., Sandhu D., Gill K.S., Greene R.A., Sorreles M.E., Akhunov E.D., Dvorˇa´k J., Linkiewicz A.M., Dubcovsky J., Hossain K.G., Kalavacharla V., Kianian S.F., Mahmoud A.A., Miftahudin, Conley E.J., Anderson, J. A., Pathan M.S., Nguyen H.T., McGuire P.E., Qualset C.O. and Lapitan N.L.V. Chromosome Bin Map of Expressed Sequence Tags in Homoeologous Group 1 of Hexaploid Wheat and Homoeology With Rice and Arabidopsis. *Genetics* **168**:609-623 (2004).
204. Pennisi E. Deciphering the Genetics of Evolution. *Science.* **321**:760-763 (2008)
205. Pestsova E., Ganal M.W. and Röder M.S. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome.* **43**:689-697 (2000).
206. Pfeiffer W.H. and McClafferty, B. Harvest plus: breeding crops for better nutrition 2007. *Crop Sci.* **47**:S-88 (2007).
207. Pickering R.A., Hill A.M., Michel M. and Timmerman-Vaughan G.M. The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L to barley (*H. vulgare* L.) chromosome 2 (2I). *Theor Appl Genet.* **91**:1288-1292 (1995).
208. Pilu R., Panzeri D., Gavazzi G., Rasmussen S.K., Consonni G. and Nielsen E. Phenotypic, genetic and molecular characterization of a maize low phytic acid mutant (lpa241). *Theorl Appl Genet.* **107**:980-987 (2003) .
209. Poletti S., Gruissem W. and Sautter C. The nutritional fortification of cereals. *Curr. Opin. Biotechnol*. **15**: 162-165 (2004).
210. Ponstein A.S., Bade J.B., Verwoerd T.C., Molendijk L., Storms J., Beudeker R.F. and Pen J. Stable expression of Phytase (*phyA*) in canola (*Brassica napus*) seeds: towards a commercial product. *Mol Breeding.* **10**:31-44 (2002).
211. Prasad M., Varshney R.K., Kumar A., Balyan H.S., Sharma P.C., Edwards K.J., Singh H., Dhaliwal H.S., Roy J.K., Gupta P.K. A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. *Theor. Appl. Genet*. **99**:341-345 (1999).
212. Prasad M., Varshney R.K., Roy J.K., Balyan H.S. and P. K. Gupta The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theor. Appl. Genet*. **100**:584-592 (2000).

213. Qi L., Friebe B., Zhang P. and Gill B.S. Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res.* **15**:3-19 (2007).
214. Qi L.L., Wang S.L., Chen P.D., Liu D.J, Friebe B. and Gill B.S. Molecular cytogenetic analysis of *Leymus racemosus* chromosome added to wheat. *Theor Appl Genet.* **95**:1084-1091 (1997).
215. Raboy V. Seeds for a better future : 'low phyate' grains help to overcome malnutrition and reduce pollution. *Trends in Plant Sci*. **6:**458-462 (2001).
216. Rajpurohit D., Kumar R., Kumar M., Pal P., Avasti A., Basha O.P., Puri A., Jung T., Singh K. and Dhaliwal H.S. Pyramiding of two bacterial blight resistance and semi dwarfing gene in Type 3 basmati using marker assisted selection. *Euphytica.* **178**(1):111-126 (2011).
217. Ramakrishnan U., Nguyen P. and Martorell R. Effects of micronutrients on growth of children under 5 y of age: meta-analyses of single and multiple nutrient interventions. *Am J Clin Nutr.* **89**:191-203 (2009).
218. Ramesh S.A., Choimes S. and Schachtman D.P. Over-Expression of an *Arabidopsis* Zinc transporter in *Hordeum Vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Mol. Bio.* **54**:373-385 (2004).
219. Ramesh S.A., Shin R., Eide D.J. and Schachtman D.P. Differential metal selectivity and gene expression of two zinc transporters from rice. *Plant Physiol.* **133**:126-134 (2003).
220. Randhawa H.S., Popovic Z., Menzies J.G., Knox R.E. and Fox S.L. Genetics and identification of molecular markers linked to resistance to loose smut (*Ustilago tritici*) race T33 in durum wheat. *Euphytica.*, **169**(2):151-157 (2009).
221. Raupp W.J., Gill B.S., Friebe B., Wilson D.L., Cox T.S. and Sears R.G. The Wheat Genetic Resource Center: Germ-plasm conservation, evaluation and utilization. In: Li ZS, Xin ZY (eds) *Proceedings of the 8th international wheat genet symposium,* China Agricultural Scientech Press, Beijing, China p 469-475 (1995).
222. Rawat N., Neelam K., Tiwari V.K., Randhawa G.S., Friebe B., Gill B.S. and Dhaliwal H.S. Development of molecular characterization of wheat *Aegilops kotschyi* addition and substitution lines with high grain protein, iron and zinc. Genome **54**(11):943-953 (2011).
223. Rawat N., Tiwari V.K., Singh N., Randhawa G.S., Singh K., Chhuneja P. and Dhaliwal H.S. Evaluation and utilization of *Aegilops* and wild *Triticum species* for enhancing iron and zinc content in wheat. *Genet Resour Crop Evol*. **56**:53-64 (2009).
224. Rawat N., Tiwari V.K., Singh N., Randhawa G.S., Singh K., Chhuneja P., Dhaliwal H.S. Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. *Genet. Resour. Crop Evol*. **56**:53-64 (2009).
225. Rawat, N., Tiwari, V.K., Neelam K., Randhawa, G.S., Singh, K., Chhuneja, P. and Dhaliwal, H.S. Development and characterization of wheat- *Aegilops kotschyi* amphiploids with high grain iron and zinc. *Plant Genet Resour*. **7**(3):271-280 (2009).
226. Rengel Z. Carbonic anhydrase activity in leaves of wheat genotypes differing in Zn efficiency. *J. Plant Physiol.* **147**:251-256 (1995).
227. Rengel Z. Genotypic differences in micronutrient use efficiency in crops. *Comm. Soil Sci Pl. An.* **32**:1163-1186 (2001).

228. Riesen O. and Feller U. Redistribution of nickel, cobalt, manganese, zinc, and cadmium via the phloem in young and maturing wheat. *J. Plant Nutr*. **28**:421-430 (2005).
229. Riley R., Chapman V. and Johnsson R. The incorporation of alien disease resistance to wheat by genetic interference with regulation of meiotic chromosome synapsis. *Genet. Res. Camb.* **12**: 199-219 (1968).
230. Roberts L.A., Pierson A.J., Panaviene Z. and Walker E.L. Yellow stripe1, Expanded roles for the maize iron-phytosiderophore transporter. *Plant Physiol*. **135**:112-120 (2004).
231. Robinson N.J., Procter C., Connolly E., Guerinot M. A ferric- chelate reductase for iron uptake from soils. *Nature.* **397**:694-697 (1999).
232. RÖder M.S., Korzun V., Wandehake K., Planschke J., Tixier M.H., Leroy P. and Ganal M.W. A microsatellite map of wheat. *Genetics.* **149**:2007-2023 (1998).
233. Römheld V. and Kissel M. Different strategies in higher plants in mobilization and uptake of iron. *Plant Nutri.* **9**:695-713 (1986).
234. Rostoks N., Mudie S., Cardle L., Russell J., Ramsay L., Svensson J.T., Wanamaker S., Walia H., Rodriguez E., Hedley P., Liu H., Close T.J., Marshall D. and Waugh R., SNP-based barley integrated linkage map developed from genes responsive to abiotic stress identifies regions of conserved synteny in barley and rice. *Mol Genet Genomics.* **274**(5):515-527 (2005).
235. Routray P., Basha O., Garg M., Singh N.K. and Dhaliwal H.S. Genetic diversity of landraces of wheat (*Triticum aestivum* L.) from hilly areas of Uttaranchal, India. *Genet Resour and Crop Evolution.* **54:**1315-1326 (2007).
236. Sancenon V., Puig S., Mateu-Andres I., Dorcey E., Thiele D.J. and Penarrubia L. The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. *J Biol. Chem.* **279**:15348-15355 (2004).
237. Sandström B. Dietary pattern and zinc supply. In: *Zinc in Human biology*. Mills C.F. ed. p. 350-363. Devon , U.K., Springer-Verlag (1989).
238. Sandström B. and Lönnerdal B. Promoters and antagonists of zinc absorption. In: *Zinc in Human biology*. Mills C.F. ed. p.57-78. Devon , U.K., Springer-Verlag (1989).
239. Sandström B. Bio-availability of zinc. *Eur. J. Clin. Nutr.*, 51(suppl. 1): S17-S19 (1997).
240. Schlegel R., Cakmak I., Torun B., Eker S., Tolay I., Ekiz H., Kalayci M. and Braun H.J. Screening for zinc efficiency among wheat relatives and their utilisation for alien gene transfer. *Euphytica.* **100**:281-286 (1998).
241. Schneider A., Molnár I. and Molnár-Láng M. Utilisation of *Aegilops*(goatgrass) species to widen the genetic diversity of cultivated wheat. *Euphytica.* **163**:1-19 (2008).
242. Scholl T.O. Iron status during pregnancy: setting the stage for mother and infant. *Am J Clin Nutr.* **81**:1218-1222 (2005).
243. Schwarzacher T., Heslop-Harrison J.S., Anamthawat-Jonsson K., Finch R.A. and Bennett M.D. Parental genome separation in reconstructions of somatic and premeiotic metaphases of *Hordeum vulgare x H. bulbosum. J. Cell Sci*. **101**:13-24 (1992).
244. Sears E. The transfer of leaf rust resistance from Aegilops umbellulata to wheat. *Brookhaven Symp. Biol.* **9**:1-22 (1956).

245. Sears E.R. and Gustafson J.P. Use of radiation to transfer alien chromosome segments to wheat crop. *Sci.* **33**:897-901 (1993).
246. Sears E.R. An induced mutant with homoeologous pairing in common wheat. *Can J Genet Cytol.* **19**:585-593 (1977).
247. Sebesta E.E., Young H.C. and Wood E.A. Wheat streak mosaic virus resistance, *Ann. Wheat Newsletter.* **18**:136-140 (1972).
248. Seigneurin-Berny D., Gravot A., Auroy P., Mazard C., Kraut A., Finazzi G., Grunwald D., Rappaport F., Vavasseur A. and Joyard J. HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. *J. Biol. Chem.* **281**:2882-2892 (2005).
249. Shankar A.H. and Prasad A.S. Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr.* **68**:447S-463S (1998).
250. Shi J., Wang H., Schellin K., Li B., Faller M., Stoop J.M., Meeley R.B., Ertl D.S., Ranch J.P. and Glassman K. Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nature Biotechnology.* **25**:930-937 (2007).
251. Shi R., Li H., Tong Y., Jing R., Zhang F. and Zou C. Identification of quantitative trait locus of zinc and phosphorus density in wheat (Triticum aestivum L.) grain. *Plant Soil* **306**:95-104 (2008).
252. Sidhu G.K., Rustgi S., Shafqat M.N., Wettstein D.V. and Gill K.S. Fine structure mapping of a gene-rich region of wheat carrying *Ph1*, a suppressor of crossing over between homoeologous chromosomes. Proc Natl Acad Sci. USA. **105**(15):5815–5820 (2008).
253. Singh K., Chino M., Nishizawa N.K., Ohata T. and Mori S. Genetic aspects of plant mineral nutrition. In Randall RJ, Delhaize E, RichardsRA, Munns R (eds) Kluwer Academic, pp. 335–-339 (1993).
254. Singh K., Ghai M., Garg M., Chhuneja P., Kaur S., Schnurbusch T., Keller B. and Dhaliwal H.S. An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* X *T. monococcum* RIL population. *Theor. Appl. Genet.* **115**:301-312 (2007).
255. Singh N.K. and Balyan H.S. Induced mutations in bread wheat (*Triticum aestivum* L.) CV. 'Kharchia 65' for reduced plant height and improve grain quality traits. *Advances in Biological Research*. **3**(5-6):215-221 (2009).
256. Singh R., Kumar N., Bandopadhyay R., Rustgi S., Sharma S., Balyan H.S. and Gupta P.K. Development and use of anchored-SSRs to study DNA polymorphism in bread wheat (*Triticum aestivum* L.). *Mol Ecol Notes.* **2**:296-299 (2006).
257. Snape J.W., Parker B.B., Simpson C.C., Ainsworth C.C., Payne P.I. and Law C.N. The use of irradiated pollen for differential gene transfer in wheat (*Triticum aestivum*). *Theor Appl Genet.* **65**:103-111 (1983).
258. Somers D.J., Peter I. and Edwards K. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor Appl Genet.* **109**:1105-1114 (2004).
259. Song L, Jiang L, Han H, Gao A, Yang X, Li L. and Liu W. Efficient Induction of Wheat-*Agropyron cristatum* 6P Translocation Lines and GISH Detection. *PLoS ONE* **8**(7):1-7 (2013).

260. Spetsov P., Mingeot M., Jacquemin J.M., Samardjieva K. and Marinova E. Transfer of powdery mildew resistance from Aegilops variabilis into bread wheat. *Euphytica* **93**:149-154 (1997).
261. Stein A.J., Meenakshi J.V., Qaim M., Nestel P., Sachdev H.P.S. and Bhutta Z.A. Analysing health benefits of biofortified staple crops by means of the disability-adjusted life years approach a handbook focusing on iron, zinc and vitamin A. HarvestPlus Technical Monograph No. 4. HarvestPlus, Washington, D.C. (2005).
262. Stoilova T. and Spetsov P. Chromosome 6U from *Aegilops geniculata* Roth. carrying powdery mildew resistance in bread wheat. *Breeding Sci.* **56**:351-357 (2006).
263. Stoltzfus R. J., Chway H M., Montresor A., Tielsch J. M., Jape K.J., Albonico M. and Savioli L. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelminthic treatment improves growth, appetite and anemia in Zanzibari preschool children. *J. Nutr*. **134**:348–356 (2004).
264. Stoltzfus R.J. Iron deficiency: global prevalence and consequences. *Food Nutr Bull.* **24**(4):99-103 (2003).
265. Stomph T., Choi E-Y., Stangoulis J. Temporal dynamics in wheat grain zinc distribution: is sink limitation the key? *Annals of Botany.* **107**(6):927-937 (2011).
266. Takahashi M. Overcoming Fe deficiency by a transgenic approach in rice. *Plant cell, Tissue and Organ culture.* **72**:211-220 (2003).
267. Takahashi M., Nakanishi H., Kawasaki S., Nishizawa N.K. and Mori S. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat Biotechnol.* **19**:466-469 (2001).
268. Takahashi M., Yamaguchi H., Nakanishi H., Shioiri T., Nishizawa N.K. and Mori S. Cloning two genes for nicotianamine aminotransferase, a criticalenzyme in iron acquisition (Strategy II) in graminaceous plants. *Plant Physiol.* **121**:947-956 (1999).
269. Takizawa R., Nishizawa N., Nakanishi H. and Mori S. Effect of iron deficiency on S-adenosylmethionine synthetase in barley roots. *J Plant Nutr.* **19**:1189-1200 (1996).
270. Tenaillon M.I., Sawkins M.C., Long A.D., Gaut R.L., Doebley J.F. and Gaut B.S. Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.) *Proc Natl Acad Sci U S A.* **98**(16):9161-9166 (2001).
271. Theil E.C. Iron, ferritin, and nutrition. *Annl Rev Nutr.* **24**:327-343 (2004).
272. Tiwari V.K. Inrogression and molecular mapping of high Fe and Zn content from *Aegilops* into wheat. Ph.D Thesis. Indian Institute of Technology Roorkee, Roorkee, India (2008).
273. Tiwari V.K., Rawat N., Chhuneja P., Neelam K., Aggarwal R., Randhawa G.S., Dhaliwal H.S., Keller B. and Singh K. Mapping of quantitative trait loci for grain iron and zinc concentration in diploid A genome wheat. *J Heredity.* **100**:771-776 (2009).
274. Tiwari V.K., Rawat N., Kumari N., Kumar S., Randhawa, G.S. and Dhaliwal H.S. Substitutions of 2S and 7U chromosomes of *Aegilops kotschyi* in wheat enhance grain iron and zinc concentration. *Theor and Appl Genet.* **121**:259-269 (2010).
275. Tiwari V.K., Rawat N., Neelam K., Kumar S., Randhawa G.S. and Dhaliwal H.S. Random chromosome elimination in synthetic Triticum-Aegilops amphiploids leads to development

of a stable partial amphiploid with high grain micro- and macronutrient content and powdery mildew resistance. *Genome*. **53**(12):1053-1065 (2010).

276. Tiwari V.K., Rawat N., Neelam K., Randhawa G.S., Singh K., Chhuneja P. and Dhaliwal H.S. Development of *T. turgidum* ssp. *durum- Aegilops longissima* amphiploids with high iron and zinc content through unreduced gamete formation in F_1 hybrids. *Genome.* **51**:757-766 (2008).
277. Uauy C., Distelfeld A., Fahima T., Blechl A. and Dubcovsky J. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science.* **314**:1298-1301 (2006).
278. Ueno D., Yamaji N. and Ma J.F. Further characterization of ferric—phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley. *Journal of Experimental Botany*. **60**:3513-3520 (2009).
279. Ullah A.H., Sethumadhavan K., Mulaney E.J., Zeigelhoffer T. and Austin-Phillips S. Characterization of recombinant fungal phytase (*phy*A) expressed in tobacco leaves, *Biochem. Biophys. Res. Commun*. **264**:201-206 (1999).
280. Vasconcelos M., Datta K., Oliva N., Khalekuzzaman M., Torrizo L., Krishna S., Oliveira, M., Goto F. and Datta S.K. Enhanced iron and zinc accumulation in transgenic rice with the *ferritin* gene. *Plant Sci*. **164**:371-378 (2003).
281. Vasconcelos M., Eckert H., Arahana V., Graef G., Grusak M.A. and Clemente T. Molecular and phenotypic characterization of transgenic soybean expressing the*Arabidopsis* ferric chelate reductase gene,*FRO2*. *Planta.* **224**:1116-1128 (2006).
282. Vert G. and Curie J.F. Arbidopsis IIRT2 gene encodes a root periphery iron transporter. *Plant J.* **26**:181-189 (2001).
283. Vert G., Grotz N., Dedaldechamp F., Gaymard F., Geurinot M.L., Briat J.F. and Curie C. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and plant growth. *Plant Cell.* **14**:1223-1233 (2002).
284. Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot, J., Peleman J. and Kuiper M. AFLP: a new technique for DNA fingerprinting. *Nucl Acid Res.* **23**:4407-4414 (1995).
285. Walia H.**,** Josefsson C., Dilkes B., Kirkbride R., Harada J. and Comai L. Dosage-dependent deregulation of an AGAMOUS-LIKE GENES cluster contributes to interspecific incompatibility. *Current Biology*. **19**(13):1128-1132 (2009).
286. Wang G., Ji J., Wang Y.B., Hu H., King I.P. and Snape J.W. The genetic-characterization of novel multi-addition doubled haploid lines derived from triticale X wheat hybrids *Theor And Appl Genet*. **87**(5):531-536 (1993).
287. Waters B.M. and Grusak M.A. Whole-plant mineral partitioning through the life cycle in *Arabidopsis thaliana* ecotypes Columbia, Landsberg erecta, Cape Verde Islands and the mutant line *ysl1ysl3*. *New Phytol*. **177**:389-405 (2008).
288. Welch R.M. and Graham R. D. A new paradigm for world agriculture: meeting human needs – productive, sustainable, and nutritious. *Field Crops Res*. **60**:1-10 (1999).

289. Welch R.M. and Graham R.D. Breeding crops for enhanced micronutrient content. *Plant and Soil.* **245**:205-214 (2002).
290. Welch R.M. and Graham R.D. Breeding for micronutrient in staple food crops from a human nutrition prospective. *J. Exp. Botany.* **55**:353-364 (2004).
291. Welch R.M. and House W.A. Factors affecting the bioavailability of mineral nutrients in plant foods. In : Welch RM, Gabelman WH, eds. Crops as sources of nutrients for humans. *American Society of Agronomy.* pp37-54 Madison, WI (1984).
292. Wilcox J., Premachandra G., Young K. and Raboy V. Isolation of high seed inorganic P, low-phytate soybean mutants. *Crop Sci.* **40**:1601–1605 (2000).
293. Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acid Res.* **18**:6531-6535 (1990).
294. The world health report 2007 - A safer future: global public health security in the 21st century World Health Organization, (2007).
295. Mori S., Nishizawa N., Hayashi H., Chino M., Yoshimura E. and Ishihara J. Why are young rice plants highly susceptible to iron deficiency? In: Chen, Y. and Hadar, Y., eds., Iron Nutrition and Interactions in Plants, Kluwer Academic Publishers, Dordrecht, p. 175-188 (1991).
296. Yan B.J., Zhang H.Q., Ren Z.L. and Yi Chuan. Molecular cytogenetic identification of a new 1RS/1BL translocation line with secalin absence. *Hereditas* **27**:513-517 (2005).
297. Yu G.T., Zhang Q., Klindworth D.L., Friesen T.L., Knox R., Jin Y., Zhong S., Cai X. and Xu S.S. Molecular and cytogenetic characterization of wheat introgression lines carrying the stem rust resistance gene *Sr39* **50**:1393-1400 (2010).
298. Yu Q., Worth C. and Rengel Z. Using capillary electrophoresis to measure Cu/Zn superoxide dismutase concentration in leaves of wheat genotypes differing in tolerance to zinc deficiency. *Plant Sci.* **143**:231-239 (1999).
299. Yuan F.J., Zhao H.J., Ren X.L., Zhu S.L., Fu X.J. and Shu Q.Y. Generation and characterization of two novel low phytate mutations in soybean (*Glycine max* L. Merr.). *Theor Appl Genet.* **115**:945-957 (2007).
300. Zhang D., Choi D.W., Wanamaker S., Fenton R.D., Chin A., Malatrasi M., Turuspekov Y., Walia H., Akhunov E.D., Kianian P., Otto C., Simons K., Deal K.R., Echenique V., Stamova B., Ross K., Butler G.E., Strader L., Verhey S.D., Johnson R., Altenbach S., Kothari K., Tanaka C., Shah M.M., Chingcuanco D.L., Han P., Miller R.E., Crossman C.C., Chao S., Lazo G.R., Klueva N., Gustafson J.P., Kiannian S.F., Dubcovsky J., Walker-Simmons M.K., Gill K.S., Dvořák J., Anderson O.D., Sorrells M.E., McGuire P.E., Qualset C.O., Nguyen H.T. and Close T.J. Construction and evaluation of cDNA libraries for large-scale expressed sequence tag sequencing in wheat (*Triticum aestivum* L.) *Genetics.* **168**(2):595-608 (2004).
301. Zhang H., Nasuda S. and Endo T.R. Identification of AFLP markers on the satellite region of chromosome 1BS in wheat. *Genome*. **43**:729–735 (2000).

302. Zhang F.S. Release of zinc and iron mobilizing root exudates by zinc deficient wheat. Z. Pflanzenernaehr. Bodenkd. **152**:205-210 (1989).
303. Zhu C., Naqvi S., Galera S.G., Pelacho A.M., Capell T. and Christou P. Transgeneic straegies for the nutritional enhancement of plants. *Trends in Plant Sci*. **12**:1360- 1385 (2007).
304. Zimmerman M.B. and Hurrel R.F. Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. in Biotechnol*. **13**:142-145 (2002).
305. Zimmerman M.B. and Hurrel R.F. Nutritional iron deficiency. *The Lancet*. **370**:511-519 (2007).

Table of Contents

Annexure-I

List of wheat SSR markers used

Primer	Forward Sequence[5'-3']	Reverse Sequence[5'-3']
Chr7A		
wmc158	AACTGGCATCATGTTTTGTAGG	AATGTAGTCAAAAGAGGTGGTG
gwm350	ACCTCATCCACATGTTCTACG	GCATGGATAGGACGCCC
gwm471	CGGCCCTATCATGGCTG	GCTTGCAAGTTCCATTTTGC
wmc479	GACCTAAGCCCAGTGTCATCAG	AGACTCTTGGCTTTGGATACGG
wmc168	AACACAAAAGATCCAACGACAC	CAGTATAGAAGGATTTTGAGAG
gwm60	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG
cfa2049	TAATTTGATTGGGTCGGAGC	CGTGTCGATGGTCTCCTTG
barc127	TGCATGCACTGTCCTTTGTATT	AAGATGCGGGCTGTTTTCTA
cfa2028	TGGGTATGAAAGGCTGAAGG	ATCGCGACTATTCAACGCTT
barc64	GCG GAG TCT GCA ATT AGT ATA GGT AT	GCA TCC ACC TCC GCA GTC AGT
wmc826	GAGGTAGATGACCACGCCG	CACGATCCCCCAAGCAC
barc174	TGGCATTTTTCTAGCACCAATACAT	GCGAACTGGACCAGCCTTCTATCTGTTC
barc108	GCGGGTCGTTTCCTGGAAATTCATCTAA	GCGAAATGATTGGCGTTACACCTGTTG
barc121	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG
barc29	GCACGCAGGAGCACCACCACGAC	GCGAGAGTAAGCAGCACCGAGGCACGAC
gwm282	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC
wmc633	ACACCAGCGGGGATATTTGTTAC	GTGCACAAGACATGAGGTGGATT
wmc525	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT
cfa2040	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT
wmc809	CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA
barc275	GCG TTT GGT CAG AAT AGG GAA GAT	GCG TAT GTT CGT GTT AGT GTT GGT TAT GC
gwm130	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC
wmc9	AACTAGTCAAATAGTCGTGTCCG	GTCAAGTCATCTGACTTAACCCG
gwm332	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC
wmc139	TGTAACTGAGGGCCATGAAT	CATCGACTCACAACTAGGGT
wmc603	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC
gwm233	TCAAAACATAAATGTTCATTGGA	TCAACCGTGTGTAATTTTGTCC
wmc388	TGTGCGGAATGATTCAATCTGT	GGCCATTAGACTGCAATGGTTT
wmc593	GGGGAGAAGCAGCAGGG	CGCGCGGTTGCCGGTGG
cfd242	CCAGTTTGCAGCAGTCACAT	CAGACCTTAACGGGGTTGAA
barc154	GTAATTCCGGTTCCACTTGACATT	GGATGGGCAGCTTCAAGGTATGTT
barc222	AAATCCGGCATCTGCTGTATCCATA	GTCCGGCCGCTGAATACTGTT
cfd6	ACTCTCCCCCTCGTTGCTAT	ATTTAAGGGAGACATCGGGC
barc1167	CGCTAGCACTATCGCCTCCTGACT	GGTTCGGTTCAAAGCTGCAATAC
gwm573	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACTAC
gwm260	GCCCCCTTGCACAATC	CGCAGCTACAGGAGGCC

wmc17	ACCTGCAAGAAATTAGGAACTC	CTAGTGTTTCAAATATGTCGGA
wmc65	TGGATGGGAAGGAGAATAAGTG	ATCCAACCGGAACTACCGTCAG
wmc596	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT
wmc422	GGACTACTGAACTGGAGAGTGTG	GCATTAGAATTTGGAGTTTGGAG
barc49	GTCCCACCAAATTAACAGCTCCTA	AGGCGCAGTGCTCGAAGAATATTAT
wmc607	ATATATGCCCATGAAGCTCAAG	GATCGAGCTAAAGCTGATACCA
gwm4	GCTGATGCATATAATGCTGT	CACTGTCTGTATCACTCTGCT
gwm276	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG
cfa2257	GATACAATAGGTGCCTCCGC	CCATTATGTAAATGCTTCTGTTTGA
cfd20	TGATGGGAAGGTAATGGGAG	ATCCAGTTCTCGTCCAAAGC
gwm63	TCGACCTGATCGCCCCTA	CGCCCTGGGTGATGAATAGT
cfa2019	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT
gwm554	TGCCCACAACGGAACTTG	GCAACCACCAAGCACAAAGT
wmc809	CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA
gdm152	ATAACATGCACACAAATTTT	GCCAGTGCCAAGCTTGC
barc1088	TGCGACATTGCCAACATCTTAGTT	AAGGCAGTGGTTTTCGGTTTTCTA
barc1025	GCGCTCATGTTCACGGGTATACGGTCTA	TAACCAACACATAAACGCACGTACA
barc1034	GCGACGCTTAAATGGAAGTCATACTCTAT	GCGCATCAATACAACAAGGTCAGACA
barc1005	CGCGTTTGCCTCTCTTGTGCTATAC	CGCGAGATACCCGAAAGTTTTGAT
barc192	GCGAATAGCACTATGGTAAACATTGAGGTAC	GCGGGTTCAATTATCAAAAGGCACAG
Chr7B		
gwm569	GGAAACTTATTGATTGAAAT	TCAATTTTGACAGAAGAATT
barc65	CCCATGGCCAAGTATAATAT	GCGAAAAGTCCATAGTCCATAGTCTC
barc72	CGTCCTCCCCCTCTCAATCTACTCTC	CGTCCCTCCATCGTCTCATCA
barc176	GCGAAAGCCATCAAACACTATCCAACT	GGTAACTAAGCACGTCACAAGCATAAA
barc278	GCATGCACTACGCTCAGAATAAAC	TAAAAGGCCCGTCAACATACAAGTA
gwm68	AGGCCAGAATCTGGGAATG	CTCCCTAGATGGGAGAAGGG
barc85	GCGAACGCTGCCCGGAGGAATCA	GCGTCGCAGATGAGATGGTGGAGCAAT
wmc476	TACCAACCACACCTGCGAGT	CTAGATGAACCTTCGTGCGG
gwm333	GCCCGGTCATGTAAAACG	TTTCAGTTTGCGTTAAGCTTTG
cfa2106	GCTGCTAAGTGCTCATGGTG	TGAAACAGGGGAATCAGAGG
wmc540	CGGGGTCCTAACTACGGTGA	CCTGTAATGGAGGACGGCTG
wmc517	ATCCTGACGTTACACGCACC	ACCTGGAACACCACGACAAA
wmc792	GGATGCAGTAGCAGTCAGGGA	CTCCATCGCTAGGCAGGG
barc20	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTCAGCCTTTT
wmc557	GGTGCTTGTTCATACGGGCT	AGGTCCTCGATCCGCTCAT
barc123	GGCCGAATTGAAAAAGCC	CCTGCCGTGTGCCGACTA
gwm146	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG
gwm344	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA
wmc398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG
wmc273	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT
wmc323	ACATGATTGTGGAGGATGAGGG	TCAAGAGGCAGACATGTGTTCG

wmc396	TGCACTGTTTTACCTTCACGGA	CAAAGCAAGAACCAGAGCCACT
wmc10	GATCCGTTCTGAGGTGAGTT	GGCAGCACCCTCTATTGTCT
wmc526	TCCCATTGGTTCACAAACTCG	GATGGTATCGCATTCATCGGT
wmc70	GGGGAGCACCCTCTATTGTCTA	TAATGCTCCCAGGAGAGAGTCG
barc340	GCAACCAAGGCAGCGTAAATG	GCGTGTAGCCGTCCATAAGCATCAT
gwm46	GCA CGT GAA TGG ATT GGA C	TGA CCC AAT AGT GGT GGT CA
gwm537	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTCCT
gwm400	GTGCTGCCACCACTTGC	TGTAGGCACTGCTTGGGAG
wmc426	GACGATCGTTTCTCCTACTTTA	ACTACACAAATGACTGCTGCTA
gwm43	CACCGACGGTTTCCCTAGAGT	GGTGAGTGCAAATGTCATGTG
wmc335	TGCGGAGTAGTTCTTCCCCC	ACATCTTGGTGAGATGCCCT
gwm297	ATCGTCACGTATTTTGCAATG	TGCGTAAGTCTAGCATTTTCTG
wmc475	AACACATTTTCTGTCTTTCGCC	TGTAGTTATGCCCAACCTTTCC
wmc662	AGTGGAGCCATGGTACTGATTT	TGTGTACTATTCCCGTCGGTCT
gwm644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC
wmc364	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC
barc267	GCGTGCTTTTTATTTTTGTGGACATCTT	GCGAATAATTGGTGGGTGAAACA
wmc218	TCTCCTGTCGGCTGAAAGTGTT	CCATGGAGGTTCACCTAGCAAA
wmc435	GCACTATACTTATTGGATTGTCA	CATGGTATCCCTAGTAAGTTTTT
barc258	AGCGGACTGGTAATTAGCAACAAAG	GATCGGCCTCTAGTAAGCTCCT
barc315	CATCCAGGCGGGCGCACGAGA	CAAGCCTCCGTGCACACCGTAT
gwm112	CTAAACACGACAGCGGTGG	GATATGTGAGCAGCGGTCAG
wmc76	CTTCAGAGCCTCTTTCTCTACA	CTGCTTCACTTGCTGATCTTTG
cfd22	GGTTGCAAACCGTCTTGTTT	AGTCGAGTTGCGACCAAAGT
gwm131	AATCCCCACCGATTCTTCTC	AGTTCGTGGGTCTCTGATGG
gwm302	GCAAGAAGCAACAGCAGTAAC	CAGATGCTCTTCTCTGCTGG
wmc723	CTCGCTCGATCCCCTTTC	CGAGGTGGAGTCCCGTCTAT
wmc311	GGGCCTGCATTTCTCCTTTCTT	CTGAACTTGCTAGACGTTCCGA
wmc613	ACAACTGTGAAACGAGACGGTG	GTGAGTGTGAAAACCAAGACGC
gwm611	CATGGAAACACCTACCGAAA	CGTGCAAATCATGTGGTAGG
gwm577	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT
wmc581	CATGTTGCCATCAAACTCGC	GCTATTGACATGCAACTATGGACCT
wmc276	GACATGTGCACCAGAATAGC	AGAAGAACTATTCGACTCCT
barc182	CCATGGCCAACAGCTCAAGGTCTC	CGCAAAACCGCATCAGGGAAGCACCAAT
barc50	GCGTAGGGAGTCACAAATTAGTATAGGT	TGCGCCTTCCCTTTCTTGACTCT
barc259	CGCAGCCTAGTCGGAAGATTTATTTTT	CGCTTAGTGGGTTTTATTGTATCAGTAGAA
barc63	GCGTTATAATTCGGTCCCATCAGAT	GCCCGCAAAAAGTAACATTAAT
barc82	CGACCCGAACACCTTTGATGACGAG	CCACCCTTGCCCCTTCTTTGCTTTAT
barc1073	GCGGGCACAATATTCTAATGGACAAAGT	GCGCAGATGCAGAGGCCAGGGGTCA
barc1181	GCCACGACTTCCTAGACCTC	TCCCCTAATCTATTTTTCTGCTTCT
Chr7D		
wmc646	GGAGTAAATGGAGACGGGGAC	GCCAGTGTGATGCATGTGAC

barc154	GTAATTCCGGTTCCACTTGACATT	GGATGGGCAGCTTCAAGGTATGTT
barc352	CCCTTTCTCGCTCGCCTATCCC	CTGTTTCGCCCAATCTCGGTGTG
wmc450	GCAGGACAGGAGGTGAAGAAG	AGGCGTTGCTGATGACACTAC
barc126	CCATTGAAACCGGATTTGAGTCG	CGTTCCATCCGAAATCAGCAC
cfd41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC
barc214	CGCTTTCGGGACAGTGAAGGTGTAT	CGGTACGCGCGAGGAGGAAGAAGG
gdm88	TCCCACCTTTTTGCTGTAGA	AAGGACAAATCCCTGCATGA
wmc606	CCGATGAACAGACTCGACAAGG	GGCTTCGGCCAGTAGTACAGGA
barc26	GCGCTGGGTAAAAAGTGAAATTC	TGCAAGTGGAGGGGGAGGCGAGAG
barc87	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC
barc172	GCGAAATGTGATGGGGTTTATCTA	GCGATTTGATTTAACTTTAGCAGTGAG
barc105	CAGGAAGAAAAGGAAAGCATGCGACAA	GCGGTGTGGCAATAATTACTTTTT
barc111	GCGGTCACCAGTAGTTCAACA	GCGTATCCCATTGCTCTTCTTCACTAAC
wmc488	AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG
gwm121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG
barc235	GCGCTCACCCTCCTACACTTCCTA	GCGCAAGTCTGTCAAAGCCTAA
cfd25	CATCGCTCATGCTAAGGTCA	CGTGTCTGTTAGCTGGGTGG
wmc824	CCGATGAACTTAAAAGTACCACCTG	CATGGATTGACACGATTGGC
barc53	GCGTCGTTCCTTTGCTTGTACCAGTA	GCGCGTCCTTCCAATGCAGAGTAGA
cfd69	AAATACCTTGAATTGTGAGCTGC	TCTGTTCATCCCCAAAGTCC
wmc14	ACCCGTCACCGGTTTATGGATG	TCCACTTCAAGATGGAGGGCAG
cfd175	TGTCGGGGACACTCTCTCTT	ACCAATGGGATGCTTCTTTG
gdm86	GGTCACCCTCTCCCATCC	GGCGCTCCATTCAATCTG
gwm295	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG
wmc506	CACTTCCTCAACATGCCAGA	CTTTCAATGTGGAAGGCGAC
gwm635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG
cfd41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC
barc125	GCGTCGAGGGTAAAACAACATAT	GTAGCGTCAGTGCTCACACAATGA
gdm145	TGAAGGACAAATCCCTGCAT	TCCCACCTTTTTGCTGTAGA
cfd31	GCACCAACCTTGATAGGGAA	GTGCCTGATGATTTTACCCG
cfd26	TCAAGATCGTGCCAAATCAA	ACTCCAAGCTGAGCACGTTT
cfd66	AGGTCTTGGTGGTTTTGGTG	TTTTCACATGCCCACAGTTG
wmc629	TTTGTGTGTTGGATGCGTGC	AATAAAACGCGACCTCCCCC
wmc827	ACGGTGACCTCAGTGCTCAC	ATGCTTGCCTCAGCAAAACC
cfd30	AATCGCACAACAATGGTTCA	GCCTCTCCTCTCTGCTCCTT
barc5	GCGCCTGGACCGGTTTTCTATTTT	GCGTTGGGAATTCCTGAACATTTT
wmc463	GATTGTATAGTCGGTTACCCCT	ATTAGTGCCCTCCATAATTGTG
gwm44	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG
cfd21	CCTCCATGTAGGCGGAAATA	TGTGTCCCATTCACTAACCG
wmc702	GAATCACATCGAATGGATCTCA	GAGGCCTTTTTCGATATTCTGC
wmc438	GACCGTTGGGCTGTATAGCATT	CTCTGACAGTGGTGGAGCTTGA
cfd46	TGGTGGTATAGTCGTTGGAGC	CCACACACACACACCATCAA

wmc121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA
gwm111	TCT GTA GGC TCT CTC CGA CTG	ACC TGA TCA GAT CCC ACT CG
wmc653	AGTGTTTTAGGGGTGGAAGGGA	CGGAACCCTAAACCCTAGTCG
wmc489	CGAAGGATTTGTGATGTGAGTA	GGACAACATCATAGAGAAGGAA
gwm437	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA
wmc221	ACGATAATGCAGCGGGGAAT	GCTGGGATCAAGGGATCAAT
cfd14	CCACCGGCCAGAGTAGTATT	TCCTGGTCTAACAACGAGAAGA
wmc473	TCTGTTGCGCGAAACAGAATAG	CCCATTGGACAACACTTTCACC
wmc94	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA
wmc488	AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG
wmc150	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG
gdm67	AAGCAAGGCACGTAAAGAGC	CTCGAAGCGAACACAAAACA
wmc671	GTACGTCAAAGAAAGAGAATTACCTC	CTCAGAGATATATCTTCGTTGTCAGT
gwm428	CGAGGCAGCGAGGATTT	TTCTCCACTAGCCCCGC
gwm37	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT
wmc634	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT
wmc166	ATAAAGCTGTCTCTTTAGTTCG	GTTTTAACACATATGCATACCT
gdm46	TGTGTTGGCCTTGTGGTG	CTACCCAATGCATCCCCTTA
gdm84	GGGATGAATTGTGTGCTCG	CGCACAATCTCTTCGTGAAA
gdm130	CCATCCAAGTACACCCGC	CGGAGGAGGAATGACGG
gdm150	ACTAGCCTGGCAGTTGATGC	CCGACCGGTTCACTTCC
gdm142	TGTGCCATGGAACAGGG	TGAAGCGCCGATTAGGAG
barc1033	GTCGGAGATCCAACGCCCATGT	CCCTGTAAAATCTTCACCCCGCAAAA
barc97	GCGCCAACTACGGAGCTCGGAGAAT	GCAGGATCAAACGTAGCCATGGTG
barc1046	GCGGAAGTCCAAAATTAGTATAGGTAG	ACTCCAATGGCAAATACTCAACA
barc1075	GCCTCTAGAAAAATCTTCCCCACGAC	GCCCTGAATCCGACACTCTTCCATA

LIST OF PUBLICATIONS

1. Deepak Rajpurohit, Anjali Awasthi, Priyanka Paul, Satish Kumar, Rahul Kumar, Kuldeep Singh, Harcharan S. Dhaliwal (Registered at NBPGR) Registration of high yielding pyramid lines of Type 3 Basmati with two bacterial leaf blight resistance genes and a semidwarfing gene using marker-assisted backcross breeding

2. Anjali Awasthi, Priyanka Paul, Satish Kumar, Shailendra Kumar Verma, R. Prasad, H.S. Dhaliwal, Abnormal endosperm development causes female sterility in rice insertional mutant OsAPC6 , Plant Science 183(2012)167-174

3. Priyanka Paul, Anjali Awasthi, Satish Kumar, Shailendra Kumar Verma, R. Prasad, H.S. Dhaliwal, Development of multiple embryos in polyembryonic insertional mutant OsPE of Rice. Published in Plant Cell Report . ISSN 0721-7714 Plant Cell Rep DOI 10.1007/s00299-012-1291-3

Abstracts

1. Shailender Kumar Verma, Satish Kumar, R.Prasad& H.S Dhaliwal,Powdery Mildew Resistnce in wheat-Aegilops Derivatives .Presented in conference on Recent Advances in fungal Biotechnology (22-23 Sept,2011) at Forest Research Institute, Dehradun.

2. Shailender Kumar Verma, Satish Kumar, Nisha S.Khalko, Harsh Chaudhary R.Prasad& H.S Dhaliwal, Utilization of Wild Wheat Relatives For Improvement of Nutritional traits In Wheat to Combat With Hidden-Hunger oh Human Beings. Presented in 1st WORLD CONGERSS FOR MAN AND NATURE. At Gurukula Kangri University, Haridwar.