"MAPPING QTLs FOR IRON AND ZINC CONCENTRATIONS IN RICE (ORYZA SATIVA L.)"

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IN

PLANT MOLECULAR BIOLOGY & BIOTECHNOLOGY

BY

DHARA K. SAVSANI
M.Sc. (Plant Molecular Biology & Biotechnology)
Registration No. 04-2349-2014

DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

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"Mapping QTLs for iron and zinc concentrations in rice (*Oryza sativa* L.)"

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ABSTRACT

Rice (*Oryza sativa* L.) is nutritionally, one of the most important staple food crops for approximately half of the world population. It is rich in carbohydrate and the predominant dietary energy source for Asia, North and South America and Africa, re iterating its importance to human welfare.

The important micronutrients present in rice grain are lost during processing of rice grain for consumption and also due to non scientific storage practices. In this context, biofortification of rice (*Oryza sativa* L.) is of primary importance not only because it is consumed widely all over the world but also as a cheap source of energy if it can be enhanced with micronutrient such as Fe, Zn and vitamin A.

Elemental analysis was performed on mature seeds (R9 stage) of seventy two rice genotypes by Atomic Absorption Spectroscopy (AAS) for Iron and Zinc concentrations in grains. Fe concentration was found to be the highest in Pankhali-203 with 75 ppm, followed by Krishna Kamod and Sambha Masuri with 60 and 55 ppm respectively. For zinc concentrations also it was observed the highest in Pankhali-203 (61 ppm), followed by Krishna Kamod (55 ppm) and Gurjari (50 ppm). GR-11 is the female parent because of its high adaptability and characters like dwarf plant type with early maturity and medium sized white colored grain. High iron and zinc containing genotypes Pankhali-203, Krishna Kamod and Gurjari were selected as the donor parents for developing the F7 RIL mapping populations.

Three recombinant inbred line (RIL) mapping populations were developed by the Single Seed Descent (SSD) method till the F7 generation and evaluated for the agronomic traits, and grain Fe and Zn concentration to detect the genomic regions responsible for variation in trait expression. RIL populations based on the cross GR-11 X Pankhali-203, GR-11 X Krishna Kamod and GR-11 X Gurjari having 300, 250 and 300 RILs respectively, were phenotyped along with their parents.

Three hundred RIL populations for the cross based GR-11 X Pankhali-203 were analysed along with their parents for gain Fe and Zn concentration on AAS. The Fe concentration recorded in the 300 RIL populations ranged from 13.45 ppm to 140.70 ppm while Zn concentration ranged from 15.01 ppm to 98.11 ppm. In case of GR-11 X Krishna Kamod, Fe concentration ranged from 20.14 ppm to 88.24 ppm whereas Zn concentration ranged from 37.95 ppm to 99.15 ppm in the 250 RILs. For the cross based GR-11 X Gurjari, 300 RIL populations were developed in which Fe concentration ranged from 34.18 ppm to 100.69 ppm while Zn was at 11.92 ppm to 88.74 ppm.

The correlations between Fe and Zn were consistent across all crosses, suggested the possibility that at least some of the genes that control these traits are linked or have pleiotropic effect. Linkage maps were constructed with different marker systems to determine the position and effect of the genes controlling the traits, which could be used to conduct a search of QTLs throughout the genome.

Parental polymorphism survey revealed that out of 600 SSR markers, 229 (38.00%) were polymorphic and among the 52 gene specific markers, 33 (63.46%) were polymorphic. In all, 325 (51.34%) markers distributed on all the 12 chromosomes were polymorphic between the parents, indicating the possibility of constructing a linkage map.

Genotyping of all the RIL populations were done with the polymorphic markers. Of the 234 markers mapped, in the cross based GR-11 X Pankhali-203, segregation of 34 SSR and 7 gene specific markers were distorted, while in case of GR-11 X Krishna Kamod, 27

SSRs and 8 gene specific markers out of 258 markers were distorted from the Mendelian pattern of inheritance. In case of GR-11 X Gurjari, a total of 266 markers were mapped of which 37 were distorted.

A map length of 1370.4 cM which represented on average one marker on every 6.7 cM for the mapping population based on the cross GR-11 X Pankhali-203. In case of GR-11 X Krishna Kamod, 258 (226 SSR and 32 gene specific) markers were mapped at an distance of 2490.5 cM (Haldane) with an average length of 207.5 cM and an average marker loci of 21.5 on each linkage group. The total length of the map for the cross GR-11 X Gurjari was 2663.2 cM (Haldane), represented an average one marker at every 10.1 cM.

QTL analysis, GR-11 X Pankhali-203 base population identified 21 QTLs for Zn concentration, 20 QTLs for Fe concentration and 36 QTLs for the yield and yield related traits. The QTLs identified for both Fe and Zn on chromosome 8 (qZn8.3 and qFe8.1) were found to be co-localized for grain Fe and Zn concentrations. QTLs detected for grain Zn concentration (qZn12.2 and qZn6.2) were co-localize for plant height (qgp12.2) and number of filled grains per panicle (qph6.1). Similarly for the grain Fe concentrations QTLs were detected on both the chromosomes 9 and 12 which could be co-localized with the grain test weight (qtw9.1) and number of grains per panicle (qgp12.2).

In case of the cross GR-11 X Krishna Kamod, 23 QTLs were detected for the Zn concentration, 17 for the Fe concentration and 50 QTLs for the yield and yield related traits. QTLs located for both the Fe (qFe8.2) and Zn (qZn8.3) concentration were co-localized on chromosome 8. The QTLs identified for the grain Fe concentration (qFe1.4) and the test weight (qtw1.1) as well as the number of filled grains per panicle (qgp1.3) on chromosome 1. On chromosome 6 QTLs were co-localized for grain Fe concentration (qFe6.1) and test weight (qtw6.2). Chromosome 7 housed four QTLs which were co-localized for the Zn concentration (qZn3.2 and qZn3.3) and test weight (qtw3.2 and qtw3.3), whereas QTLs on chromosome 12 was also responsible for the two traits, Zn concentration (qZn12.2) and number of filled grains per panicle (qgp12.1).

Twenty two QTLs were identified for the Zn concentration, 22 for iron concentration and 49 QTLs for the yield and yield related traits in the cross based GR-11 X Gurjari. The QTLs identified for both Fe (qFe1.1) and Zn (qZn1.1) concentration on chromosome 1 were responsible for both the grain Fe and Zn concentrations. GR-11 showed the increased QTLs in both these alleles. On chromosome 3 the QTLs were co-localized for the Fe concentration (qFe3.4) and number of effective tillers per plant (qnt3.2), whereas on chromosome 8 it was localized for the Fe concentration (qFe8.2) and number of filled grains per panicles (qgp8.1). The QTLs for the grain Fe concentration (qFe6.2) and for grain yield (qgy6.1) were located on chromosome 6. Chromosome 11 could house 2 QTLs for the grain Zn concentration (qZn11.1) and for panicle length (qgl11.1).

QTLs for iron were co-localized with that of zinc on chromosomes 3, 5, 7 and 12. In all 18 candidate genes were known for iron and zinc homeostasis and yield and yield related traits underlie the QTLs. Thus the high priority candidate genes for iron and zinc and yields and yield related traits in seed are from the family YSL, NRAMP, FRO, ZIP and NAS were analysed *insilico*, based on the genetic mapping studies as these genes strictly underlie QTLs.

In case of cross GR-11 X Pankhali-203, 51 RILs had > 100 ppm and 42 line had >80 ppm Fe concentration. 30 lines had > 60 ppm Zn concentration. 30 elite lines had > 100 ppm Fe concentration and also > 60 ppm Zn concentration. 51 elite lines had > 70 ppm of Fe concentration and also > 60 ppm of Zn concentration for the cross based GR-11 X Krishna Kamod whereas for GR-11 X Gurjari, 60 elite lines had > 65 ppm of Fe concentration and > 55 ppm of Zn concentration. Several high Fe and high Zn lines with identified QTLs were obtained. Fe and Zn concentration were analysed using AAS in the mature grains of the selected high Fe and Zn lines. These will be used for variety development for gene discovery and also for use in bio fortification programs.

In all, 31 candidate genes related to iron and zinc concentrations were found to be present within the selected QTLs and beyond the distance of the left or right flanking markers. Spatio-temporal pattern of expression of these genes was examined *insilico* by analysing their expression in various tissues and organs using RiceXPro and TIGR database. *OsNAS1, OsNAS2, OsZIP6 and OsZIP7* genes expressed in endosperm. *OsIRT1, OsNAS3, OsNRAMP1, OsZIP6, OsZIP7 and OsZIP8* genes expressed in seeds and embryo. In addition these genes also expressed in other tissues such as leaves, anther, pistil, roots and shoots suggesting their role in uptake and transport of Fe and Zn throughout the plant and not just the seeds.

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CERTIFICATE

This is to certify that the thesis entitled **"MAPPING QTLs FOR IRON AND ZINC CONCENTRATIONS IN RICE (*Oryza sativa* L.)"** submitted by **Ms. Dhara K. Savsani (Reg. No. 04-2349-2014)** in partial fulfillment of the requirements for the award of the degree of **Ph.D of Science** in **Plant Molecular Biology and Biotechnology** of the Anand Agricultural University is a record of bonafide research work carried out by her under my personal guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Anand   **(Y. M. Shukla)**
Date: /   /2017   Major Advisor

DECLARATION

      This is to declare that the entire research work reported here in the thesis for the partial fulfillment of the requirements for the degree of **Ph.D. of Science** in **Plant Molecular Biology and Biotechnology**, by the undersigned is the results of investigation done by her under the direct guidance and supervision of **Dr. Y. M. Shukla**, Principal, College of Agriculture & Polytechnic in Agriculture, Anand Agricultural University, Vaso, and no part of the work has been submitted for any other degree so far.

Place: Anand

Date: /   /2017   **(Dhara K. Savsani)**

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CERTIFICATE

This is to certify that I have no objection for supplying a copy of my thesis or any part of it to scientists or workers for rendering service either in library or documentation centre.

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Place: Anand

Date :   /   /2017   (**Dhara K. Savsani**)

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ABBREVIATIONS AND ACRONYMS

%	Per cent
&	And
@	At the rate of
μg	Microgram
μl	Microliter
^{0}C	Degree Celsius
3'	3 prime end
5'	5 prime end
AAS	Atomic Absorption Spectroscopy
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
BAC	Bacterial Artificial Chromosome
BC	Back Cross
bp	Basepair
C.D.	Critical difference
C.V.	Coefficient of variation
C:I	Chloroform : Isoamyl-alcohol
Ca	Calcium
CCD	Charge Coupled Device
CIM	Composite Interval Mapping
cM	Centimorgan
CTAB	Cetyl di Methyl ethyl Ammonium Bromide
CV	Coefficient of Varaince
DAT	Days after Transplanting
DFF	Days to 50% Flowering
DHL	Double haploid Lines
DMA	2'-deoxymugenic acid
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribose Nucleotide Triphosphate
DRR	Director of Rice Research
ds	Double Stranded
DTPA	Diethylene Triamine Penta Acetic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
et al.	*et allii* ; and co-workers
EtBr	Ethidium Bromide
etc.	*Et cetera;* and rest, so on
FAO	Food and Agriculture Organization
Fe	Iron
Fig.	Figure
FRO	Ferric stripe like

g	Gram
GB	Grain breadth
GL	Grain length
GY	Grain yield
HCL	Hydrogen Chloride
i.e.	That is
IM	Interval Mapping
IRGSP	International Rice Genome Sequencing Project
K	Potassium
Kg	Kilogram
KJ	Kilo-Joule
L	Litre
L:B R	Length: Breadth Ratio
LG	Linkage Group
LOD	Log_{10} of the Likelihood ratio
M	Molar
MAB	Marker Assisted Breeding/Backcrossing
MAS	Marker Assisted Selection
Max.	Maximum
Mg	Magnesium
$MgCl_2$	Magnesium Chloride
Min.	Minimum
mL	Millilitre
Mn	Manganese
N	Normal
NAAT	Nicotinamine Aminotransferase
NaCl	Sodium Chloride
NAS	Nicotinamine Synthase
NETP	Number of effective tillers per plant
NFGP	Number of filled grains per panicle
ng	Nanogram
NIL	Near Isogenic Lines
NRAMP	Natural resistance associated macrophage protein
O D	Optical Density
P	Phosphorous
p	Probability
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
PH	Plant height
PIC	Polymorphism Information Content
PL	Panicle length

pmol	Picomol
ppm	Parts per million
PVP	Poly Vinyl Pyrrolidone
QTL	Quantitative Trait Loci
r	Correlation Coefficient
R3	Panicle emergence stage
R5	Grain filling stage
RAPD	Random Amplification Polymorphic DNA
RE	Restriction Enzyme
RIL	Recombinant Inbred Lines
RM	Rice Microsatellite Marker
RNA	Ribonucleic Acid
RNase	Ribonuclease
rpm	Revolution per minute
rRNA	Ribosomal RNA
RT	Real Time
SCAR	Sequenced Characterized Amplified Region
SD	Standard deviation
Sec	Second
SEm	Standard error of mean
SMA	Single Marker Analysis
Sr.	Serial
SSR	Simple Sequence Repeats
Taq	*Thermus Aquaticus*
TBE	Tris-Borate EDTA
TE	Tris- EDTA
Tm	Melting Temperature
TS	Transgressive Segregants
TWT	Test weight
U	Unit of Enzyme
UV	Ultra violet
V	Volts
v/v	Volume per volume
VIT	Vacuolar iron transporters
viz.,	Namely
WHO	World Health Organization
wt.	Weight
YSL	Yellow Stripe Like Protein
ZIP	Zrt/Irt-related proteins
Zn	Zinc
^{2}	Chi-Square

CONTENTS

I. INTRODUCTION

Micronutrients deficiency is a global burning health problem pervasive in both urban and rural areas. About three billion people in world are deficient in key vitamins and minerals, particularly vitamin A, iodine (I), iron (Fe), and zinc (Zn) (Dahiya *et al*., 2008). Poverty, lack of affordability to diverse and balanced foods, lack of awareness about optimal dietary practices, and high incidence of infectious diseases are some of the factors leading to micronutrient deficiency. In the developing countries, micronutrient deficiency is the major underlying causes of numerous human health problems, and therefore, the situation of nutrient deficiencies is more drastic industrialized countries (Welch and Graham 2004). Moreover, modern agricultural practices including the improved cultivars of crops following the green revolution have further contributed and aggravated the malnutrition in the resource-poor populations by greater removal and exhaustion of major- and micro-plant nutrients in soil. Hence, in the present genomic era, plant nutrition research needs a new paradigm for agriculture and nutrition to meet the global demand for sufficient food production with enhanced nutritional value (Cakmak 2004).

The consumption of less diverse and monotonous food leads to deficiencies in micronutrients, especially iron (Fe), zinc (Zn), iodine (I), selenium (Se), and vitamin A. Among trace elements, Fe and Zn are essential for a variety of metabolic processes (Underwood 1977; Prasad 1978). The main sources of Zn in poor population are staple cereals, starchy roots, tubers, and legumes which are low either in quantity or bioavailability of Zn (Gibson 1994). Cereals contribute up to 50 % of the Fe intake in the poorest households. This means that doubling the Fe or Zn density of food staples could increase total intakes by 50 % (Ruel and Bouis 1998). Fe deficiency is estimated to affect about 30% of the world population, making Fe by far the most deficient nutrient worldwide (Lucca *et al*., 2001). Zn deficiency has subsequently been reported from all over the world and could be ascribed to the removal of high amounts of Zn from the soil due to the intensive cultivation of high yielding varieties (Takkar and Walker 1993). The intake of Fe and Zn appears to be below the recommended dietary allowance for an average Indian adult; this was observed in particularly low-income rural households in the rice consuming regions. Fe deficiency is often accompanied by Zn deficiency as both of these nutrients are derived from

similar sources in the diet (Welch 2001). In addition, minerals are essential for plant growth and reproduction, and nutrient deficiencies can limit yield potential and plant products that represent an important source of minerals in the human diet.

Iron serves as an important cofactor for various enzymes performing basic functions in humans. Fe participates in various cellular events such as respiration, chlorophyll biosynthesis, and photosynthetic electron transport. Low chlorophyll content (chlorosis) of young leaves is the most obvious visible symptom of Fe deficiency. Fe deficiency also seems to trigger oxidative stress (Tiwari *et al.*, 2009 and Bashir *et al.*, 2007). Fe is also essential for the functioning of chloroplast and mitochondria. Iron deficiency (sideropenia or hypoferremia), is the most widespread nutritional disorder in the world. This condition impairs immunity, making humans susceptible to infection and increase the risk of complications during child-birth. Anaemia among children can impair health and development, limit learning capacity, impair immune systems and reduce adult work performance.

Zinc is the fourth important micronutrient after vitamin A, iron and iodine and is now receiving increasing global attention. Zinc is required as a cofactor for over 300 enzymes and plays critical structural roles in many protein and transcriptional factors. Zn plays diverse roles in different cellular processes (Ishimaru *et al.*, 2011). Protein, nucleic acid, carbohydrate, and lipid metabolism depend to a great extent on Zn (Rhodes and Klug, 1993 and Vallee and Falchuk, 1993). In plants, Zn deficiency results in the accumulation of starch and inactive RNases, suggesting that RNA degradation could be regulated by the availability of Zn in the cell (Suzuki *et al.*, 2012). Zinc deficiency is an important cause of morbidity due to infectious disease and growth-faltering among young children. Zinc deficiency causes most important health risk factors in developing countries and worldwide. Amongst children, zinc deficiency is commonly associated with diarrhoea, pneumonia, stunting and child mortality. Recent epidemiological studies reported that whole-grain intake (such as brown rice) is linked to disease prevention against cancer, cardiovascular disease, diabetes and obesity (Slavin, 2003).

The best way of combating the micronutrient malnutrition is to ensure consumption of a balanced diet that is adequate in every nutrition. The common yet effective approaches to addressing malnutrition and micronutrient deficiencies are

through dietary diversification (consumption of meat, vegetables, fish and fruits along with staple foods), supplementation (ingestion of micronutrients in tablets or sachet forms) and food fortification (addition of minerals to processed foods), but the long term effectiveness of such interventions depend on continued funding, infrastructure and a good distribution network. Alternatively a more efficient and a cost effective solution is to increase bioavailable concentrations of an element in edible portions of crop before harvesting (White and Broadley, 2005). Furthermore, plants that accumulate more micronutrients (e.g., Fe, Zn, and Mn) would contribute significantly to combat micronutrient deficiencies in humans.

To address the occurrence of mineral deficiencies in human populations, plant scientist are devising methods of applying fertilizers and/or using plant breeding strategies to increase the concentrations and/or bioavailability of mineral elements in agricultural produce (Cakmak, 2000, 2008; Pfeiffer and MacClafferty , 2007; White and Broadley, 2009). These approaches are termed as 'agronomic' and 'genetic' biofortification respectively. To combact the drawbacks of supplementation, "Biofortification" (breeding for increased mineral and vitamin content) is a promising innovation that could help fight hidden hunger especially in rural areas. It can complement the other approaches by providing a sustainable and low cost means of reducing the number of persons requiring treatment through supplementation and commercial fortification. The biofortification approach involves a set of one-times, fixed costs in developing breeding methodologies, breeding nutritional quality traits into current crop varieties, and adapting these varieties to diverse environment. No large recurrent investments, are required after nutritious varieties have been initially disseminated, and the cost do not increase with the number of people, and the benefits can be made available to all the developing countries in the world. Finally breeding for higher trace mineral density in the consumed plant parts will not incur a yield penalty (Graham and Welch, 1996).

To ameliorate this health problem agricultural scientists are working to improve the nutrient content of iron and zinc in the grains of staple cereal crops by breeding methods. This method is a low-cost, sustainable strategy to solve micronutrient malnutrition for people living in developing countries that cannot afford iron and zinc fortified foods, by supplementation of iron and zinc into their staple diets.

Cereals provide the most calories to humans and are by far the most important source of total food consumption in the developing countries. Rice (*Oryza sativa L.*) occupies the enviable prime place among the food crops, with approximately half of the world's population dependent on it for a significant proportion of their caloric intake, cultivated around the world. Therefore, it is one of the most important crop plants on Earth (Lucca *et al*., 2002). The area under its cultivation is 163.1 million hectares producing 481.5 million tons and a productivity of 3.0 tons per hectares in the world. India is the second largest rice producing country with a production of over 148 million tons, cultivation area of 45 million hectares, and mean productivity of 3.2 tons per hectares, next to China. In India, rice production was recorded 96 million tonnes in 2013–14, 103.4 million tonnes in 2014–15 and 100 million tonnes in 2015-16 (FAO, Agricultural Outlook and Situation Analysis Reports, United Nations, 2015-16). Rice growing states, in India include: West Bengal, Andhra Pradesh, Uttar Pradesh, Punjab (ranks first in productivity), Orissa, Tamil Nadu, Chhattisgarh, Karnataka and Haryana.

Rice (*Oryza sativa* L.) occupies the enviable prime place among the food crops cultivated around the world. It is known as the grain of life and is synonymous with food for Asians as it supplies majority of starch, protein and micronutrient requirements (Donald, *et al*., 2002). Rice being the second largest consumed cereal (after wheat), shapes the lives of millions of people. Over half of the world's population depend on rice for 80% of it's food caloric requirement. Nutritionally, rice provides 1,527 kJ (365 kcal) energy per 100 gm of seeds and consists of carbohydrates (80 gm), sugar (0.12 gm), dietary fibers (1.3 gm), fats (0.66 gm), protein (7.13 gm), water (11.61 gm), vitamins such as thiamine (vit. B1), riboflavin (vit.B2), niacin (vit.B3), pantotenic acid (vit.B5), vitamin B6 and ions such as Ca, Fe, Mg, Mn, P, K and Zn (Anonymous, 1999). The contribution of rice in terms productivity to the food basket is comparatively high.

The availability of large genetic variability in micronutrient concentration in grains of rice and its huge preference as a staple food by large populations, particularly resource poor people in the world, made it the best candidate for biofortification of food grains to enrich with crucial micronutrients (Graham, *et al.,* 1999). Biofortification is a genetic approach which aims at biological and genetic

enrichment of food stuffs with vital nutrients like vitamins, minerals and proteins (Bouis, 2002). This approach has multiple advantages. It capitalizes on the regular daily intake of a consistent and large amount of staple foods. After the one time investment to develop seeds that fortify themselves, recurrent costs are low, and germplasm can be shared internationally. Biofortified crop system is highly sustainable and the nutritionally improved varieties will continue to be grown and consumed year after year, even if government attention and international funding for micronutrient issues fades (Nestle *et al*., 2006). Ideally, once rice is biofortified with vital nutrients, the farmer can grow indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way. This is also the only feasible way of reaching the malnourished population in India (Nagesh, *et al*., 2012). Thus enhancing the availability of grain iron and zinc by biofortification strategy involving molecular breeding tools will help to reduce the problem of global micronutrient malnutrition in mankind.

Rice holds a unique position among domesticated crop species in that it is both a critical staple food and the first fully sequenced crop genome making it possible to analyzed the genes / QTLs involved in uptake, transport and loading of iron and zinc in rice grain and their coordinated expression depending on the deficiency or sufficiency of the elements in the cellular and root environment is only beginning to get clear. This information is being used in gene discovery and allele mining for good sources of germplasm (Banerjee *et al*., 2010).

Earlier studies were mostly confined to the production of high yielding varieties, but currently the focus has shifted to enrichment of micronutrients in staple food crops like rice and wheat which helps in ameliorating the problems of micronutrient deficiency in the form of hidden hunger in the human population. Also, in the past two decades, the major effort in breeding has changed from traditional phenotypic-pedigree based selection systems to molecular genetics with emphasis on QTL identification and Marker Assisted Selection (MAS). MAS is an excellent tool for selecting beneficial genetic traits that are difficult to measure, that exhibit low heritability and/or are expressed late in development (Davies *et al*., 2006., Wilde *et al*., 2007 and Ender *et al*., 2008).

QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. The markers are used to partition the mapping population in to different genotypic classes based on genotypes at the marker locus, and apply the correlative statistics to determine whether the individual of one genotype differ significantly with the individuals of other genotype with respect to the trait under study. A significant difference between phenotypic means of the two / more groups depending on the marker system and type of population indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait. QTL mapping is an integration of linkage mapping and traditional statistical and quantitative genetic approaches. QTLs are loci controlling quantitative traits that are governed by large number of genes with smaller contributions to the trait resulting in continuous rather than discrete variation (Liu, 1998). Fischer (1918) was first to provide an understanding of quantitative traits and their measurement. Even up to 1980's, the genetics of such traits were studied by using simple statistical approaches like means, variances, co-variances, heritabilties etc. The assumptions underlying such techniques are that there are several genes segregating in a given population and that these genes would share individual allelic contributions which are relative to environmental contribution (Sofi and Rather, 2007).

Current methods to locate QTLs include single marker approach, interval mapping, composite interval mapping and multiple interval mapping, that have been standardized using several mapping populations such as F2, backcross, RILs, NILs and double haploids. Among these, the first approach is based on ANOVA, or simple linear regression, and performs statistical tests based solely on single DNA marker information. Genetic map is not required for single marker analysis, and the calculations are based on phenotypic means and variances within each of the genotypic classes. The other approaches require genetic map construction to locate the QTL. So far, a large number of QTLs have been identified for most of the economic traits. Advances in QTL mapping helps in the genetic analysis of complex traits, improved selection efficiency with the aid of MAS, thereby making it possible to enrich and enhance existing germplasm. A number of molecular marker systems such as RFLP, AFLP, SSRs (Microsatellites), SNPs etc. coupled with a number of computer packages such as QTL Cartographer, MAPMAKER (Lander, *et al*., 1987)

have made it possible to construct high density maps where in quantitative traits can be associated with markers

A suitable mapping population generated from phenotypically contrasting parents is prerequisite for QTL mapping. The parental lines used in development of mapping population should be genetically diverse, which enhance the possibility of identifying a large set of polymorphic markers that are well distributed across the genome. The ability to defect QTL in F2 or F2 derived populations and RILs are relatively higher than other mapping population. The size of the mapping population for QTL analysis depends on several factors viz., type of mapping population used for QTL analysis, genetic nature of the target trait, objective of the study, and resources available for handling a sizable mapping population in terms of phenotyping and genotyping.

Mapping means placing the markers in order, indicating the relative genetic distance between them and assaying them to their linkage groups on the basis of recombination values from all pair wise combination between the markers. Linkage map indicates the position and relative genetic distance between markers along chromosomes. A vanity of molecular markers viz., RFLPs, RAPD, SSRs, AFLP, and SNPs etc. have been used to identify individual QTLs and to find out effects and position of these QTLs.

Microsatellites or Simple Sequence Repeats (SSRs) have been developed for major crop plants, which are predicted to lead to even more rapid advances in both marker development and implementation of breeding programs (Garland, *et al*., 2000). Microsatellite polymorphism is an important source of genetic diversity, providing support for map-based cloning and molecular breeding. Microsatellites or SSRs are polymorphic loci present in nuclear and organellar DNA that consist of repeating units of di / tri / tetra-nucleotides of 1-6 base pairs in length. They are typically neutral, co-dominant and are used as molecular markers which have wide ranging applications in the field of genetics including population studies. SSRs are locus-specific, and therefore it is possible to identify the chromosomal locations of the gene(s) controlling the traits, based on linked SSR markers (Neelu, *et al.,* 2006).

Thus, to facilitate breeding by MAS, identification of polymorphic markers between parents for high Fe and Zn is necessary and also knowledge on the genetic

basis of Zn and Fe concentration is important (Ghandilyan *et al.,* 2006). The genetic basis of accumulation of micronutrients in the grains and mapping of the QTL will provide the basis for preparing the strategies and improving grain micronutrient concentration through marker assisted selection (Gande *et al.,* 2014). The final permanent solution to micronutrient malnutrition is breeding staple foods that are dense in minerals and vitamins to provide a low-cost, sustainable strategy for reducing levels of micronutrient malnutrition (Babu, 2013). Hence, understanding the genetic basis of accumulation of Fe and Zn in rice grains and mapping of the QTL will provide the basis for devising plant breeding strategies and for improving grain micronutrient concentration (Fe and Zn) through marker-assisted selection.

In the present investigation attempts have been made to identify the genomic regions responsible for rice grain density of iron and zinc to improve the nutritional status of iron and zinc in grain of several elite rice hybrid parental line with the following objectives:

- ❖ To evaluate the promising rice genotypes for Fe/Zn
- ❖ To evaluate the Fe/Zn concentrations in F7/RIL populations of rice (Phenotyping of the F7/RIL populations)
- ❖ Identification of polymorphic microsatellite markers between parents
- ❖ Genotyping of the mapping population using polymorphic markers in the F7/RILs
- ❖ To map QTLs for Fe/Zn concentrations in F7/RIL populations of rice
- ❖ To study candidate genes underlying target QTLs

II. REVIEW OF LITERATURE

The link between food and health is long and well documented, but food access depends on income. The Green Revolution increased global grain production and helped improve food security. The world's population is projected to grow from 6.1 billion to 7.9 billion by 2025, and by 2050 some 84% of people live in countries that constitute the developing world (Khush, 2001). Therefore one of the most important challenges for agriculture in future, besides enhancing food production, will be to provide the essential minerals and organic nutrients required by humans for maintenance of health and proper organ function.

Billions of people worldwide, especially in developing countries, suffer from the sinister form of hunger called micronutrient malnutrition, a form of unnoticed under nutrition. Micronutrient deficiencies can exist in populations even when the food supply is adequate in terms of meeting energy requirements. The human body requires more than 22 mineral elements that can be supplied by an appropriate diet (Philip and Martin, 2005) for their normal growth and development. A mixture of carbohydrates, lipids and proteins (amino acids), 17 mineral nutrients and 13 vitamins are essential components of the human diet (Grusak, 1999) of which macronutrients are needed in large amounts whereas micronutrients are needed in smaller quantities.

Micronutrient malnutrition resulting from the dietary deficiency of critically important minerals such as iron (Fe) and zinc (Zn) has been reported to be a major food-related primary health problem among the populations of developing world- including those in India-that are heavily dependent on cereals and legume-based diets have limited access to meat, fruits and vegetable (Welch, 2002; Sandstead, 1991; Gibson, 1994). Individuals between 25 and 50 years of age require 10 to 15 mg Fe and 12 to 15 mg of zinc (Welch and Graham, 2004). Worldwide, one out of seven people suffer from hunger; and most of them are poor people, particularly women, infants and children. Micronutrient malnourishment persist as a major problem that not only affects the vital growth in children but also actively damages the cognitive development of children, resulting in blindness, lowering disease resistance and reducing the likelihood that mothers survive childbirth (Sharma *et al*., 2003).

2.1.RICE: A GLOBAL GRAIN

Taxonomically rice belongs to the genus *Oryza*, family *Poaceae* and subfamily *Oryzoidea*. The *indica* and *japonica* are subspecies of *Oryza sativa.* Rice is the most amenable crop for molecular genetic studies due to its small genome size of 400-430 Mbp, haploid chromosome number of n=12, model monocotyledonous self-pollinated crop, enriched genetic map, availability of entire genome sequence and relative ease of transformation (Gorantla *et al*., 2005).

Rice is known as the grain of life and is synonymous with the food of Asians as it supplies majority of starch, protein and micronutrient requirements. It is especially important in biofortification efforts because it is an indispensible staple food for more than half of the world's population. Improvement in agronomic traits of rice is bound to affect a sizeable population, since it is a primary source of sustenance. In countries where rice is used as staple food, the per capita consumption is very high ranging from 62 to 190 kg per year. Thus, even a small increase in the nutritive value of rice can highly contribute to human nutrition, mostly in developing countries (Graham *et al*., 1999). Hence, to combat micronutrient hidden hunger at low cost the HarvestPlus Challenge Program (www.harvestplus.org) of the CGIAR has included among its targets the genetic enhancement of rice grain iron (Fe) and zinc (Zn) contents to further enhance this crop's nutritional value. The major objectives of HarvestPlus Program are 1) to determine the extent of genetic diversity for grain Fe and Zn contents available to breeding programs, 2) developing and testing micronutrient dense germplasm, 3) determining trait heritability, 4) study of G×E interaction effects on micronutrient expression, 5) conducting genetic studies and developing molecular markers to facilitate breeding, 6) assessing the feasibility of breeding to achieve meaningful increments of nutrient in edible plant parts without yield penalty, and finally 7) investigating the bioavailability of the micronutrients.

2.1.1. Rice Genomics

Genomics is all about mapping, sequencing, and functional analysis of genomes, the entire genetic complement of an organism. Before high-throughput DNA sequencing was available, genomes were studied largely by mapping using genetic markers. With the complete genome sequencing of many organisms and the development of powerful methods of gene discovery, the identification of functions

for thousands of genes is proceeding rapidly (functional genomics). Rice has been at the forefront of plant genomics because of its small genome size and relatively low amount of repetitive DNA, its diploid nature, and its ease of manipulation in tissue culture. The sequencing of the rice genome will facilitate the identification of many important genes. The forward genetics approach for identifying functionally important genes derives from a known allelic difference conferring an improved phenotype. In such an approach, the objective is to identify a sequence change conferring the improved phenotype. Such a sequence change can then become the basis for a marker that is specific for that allele. These types of markers will always co-segregate with the trait of interest and should also be polymorphic in any cross. Such a marker will often be based on a single nucleotide polymorphism (SNP). Numerous assays are available to detect these SNPs (Kirk *et al*., 2002). The SNPs can be detected in high-throughput systems in such a way that large numbers of plants can be assayed for a particular allele. An example of a high throughput, non-gel based approach is the Taqman® system (Livak *et al*., 1995). Genes that can be mapped on the rice chromosomes will become easier targets for identification. Studies in *Arabidopsis* have shown the potential of using the DNA sequence to identify new microsatellite markers in particular regions for saturation mapping at high resolution (Casacuberta *et al*., 2000). High-throughput genetic mapping using multiplexed SSRs and small mapping populations can be used to rapidly map important genes and determine their sequence in relatively small positional cloning experiments (Lukowitz *et al.,* 2000).

Together with *Arabidopsis*, rice stands out as a model for plant genomics (Shimamoto and Kyozuka, 2001). The International Rice Genome Sequencing Project (IRGSP), with roots in the Japanese Rice Genome Project, sequenced the whole 430 Mb of the rice genome of the japonica variety, cultivar Nipponbare, based on a clone-by-clone approach, aiming at high accuracy of 10-4 (Eckardt, 2000 and Delseny *et al*., 2001). In parallel, the Beijing Genomics Institute (BGI) and Syngenta have separately published drafts of the rice genome based on a whole shotgun sequencing methodology. The Chinese team obtained coverage of 92% of the genome of the *indica* variety, cultivar 93-11, with a prediction of 46,022 to 56,615 genes (Yu *et al*., 2002). The Sygenta draft comprises 93% of the japonica variety genome, with an estimate of 32,000 to 50,000 genes (Goff *et al*., 2002).

Gross *et al*., (2003) performed an analysis of the rice genome searching for sequences related to the YS, ZIP, FRO, NRAMP and Ferritin protein families using the *indica* variety genome, the IRGSP data and the expressed sequence tags (ESTs) deposited in public databanks. The purpose of this study was to contribute to the understanding of Fe homeostasis dynamics in rice (focusing on gene families involved in uptake, intracellular targeting and storage) and to compare the molecular picture of Fe nutrition in this model grass species to *Arabidopsis thaliana*. They studied Fe homeostasis related genes in rice and assigned possible functions to thirty-nine new rice genes together with four previously reported sequences.

2.2. IMPORTANCE OF IRON AND ZINC IN HUMAN NUTRITION

A sufficient and balanced diet is possibly most important contribution to human health and prophylaxis. Diets should not be only rich with energy but should also supply the essential nutrients and minerals I, Fe, Zn and Se (Sautter *et al*., 2006). There is compelling evidence that persistent deficiency of minerals and vitamins are major underlying causes of numerous human health problems in developing countries reported to be at risk of malnutrition. In this view Fe and Zn can be considered as most essential for health concern. Fe has several vital functions in the human metabolism, viz., synthesis of the oxygen transport proteins (hemoglobin and myoglobin) and formation of heme enzymes and other Fe-containing enzymes, which are particularly important for energy production, immune defense, and thyroid function (Roeser 1986). The other key functions for the Fe-containing enzymes include the synthesis of steroid hormones and bile acids, the detoxification of foreign substances in the liver, and signal controlling in some neurotransmitters such as the dopamine and serotonin systems in the brain. With respect to the mechanism of absorption there are two kinds of dietary iron: haem and non haem (Halberg *et al*, 1991). In human diet, primary sources of haem Fe are the haemoglobin and myoglobin from consumption of meat, poultry and fish, whereas non haem Fe are generally plant diet based.

The body has three unique mechanisms for maintaining the Fe balance: 1) continuous reutilization of Fe from catabolizes erythrocytes, 2) access to specific Fe storage protein ferritin, and 3) regulation of absorption of Fe from the intestine. In case of increased Fe requirement or decreased bioavailability, the regulatory capacity

to prevent Fe deficiency is limited. Therefore, inadequate Fe absorption will first lead to mobilization of storage Fe, then to insufficient Fe transport to bone marrow and finally to lower hemoglobin levels or anemia.

Zinc is involved in the functioning of more than 300 enzymes and is an essential component of many Zn-dependent enzymes. Zn plays a major role in gene expression and acts as a stabilizer of membrane structures and cellular components (Palmgren *et al.*, 2008). Although body Zn homeostasis can be maintained over a wide range of Zn intakes by increasing or decreasing both intestinal Zn absorption and endogenous intestinal Zn excretion; ultimately low Zn intake and/or bioavailability results in Zn deficiency. Meat and seafood are good sources of Zn (Sanstead 1995). However, in many parts of the developing world, most Zn is provided by cereals and legume seeds. These plant foods are high in phytic acid, which is a potent inhibitor of Zn absorption (Navert *et al.*, 1985).

2.3. MICRONUTRIENTS DEFICIENCY: A GLOBAL CHALLENGE TO HEALTH

Deficiencies of micronutrients are a major global health problem. About three billion people are deficient in key vitamins and minerals, particularly Vitamin A, Iron (Fe), Zinc (Zn), and Iodine (I) (WHO, 2007). Micronutrient deficiency generally increases the risk of infectious illness and of dying from diarrhoea, measles, malaria and pneumonia in the people living in low income countries. Moreover, agriculture is partly responsible for the current high levels of malnutrition among the poor globally. Green revolution cropping systems have contributed to the growth in micronutrient deficiencies in resource poor populations as agriculture policies never made nutrient output an explicit goal of the food production system.

In industrialised societies, mineral deficiencies are addressed by ensuring that fresh fruits and vegetables are included in the diet; along with supplementation and fortification programmes to enhance the nutritional value of staple foods (Naqvi *et al.*, 2009). However developing countries lack similar provisions, so micronutrient deficiency is rife and contributes significantly to the poor socioeconomic conditions. In the present genomic era, plant nutrition research needs a new paradigm for agriculture and nutrition to meet the global demand for sufficient food production with increased nutritive value (Cakmak, 2002).

Poverty, lack of access to variety of food, lack of knowledge of optimal dietary practices and high incidence of infectious disease are some of factors that lead to micronutrient deficiency. Modern and improved agriculture practices have further aggravated malnutrition and narrowed the base of global food security.

Now-a-days, people consume diet less diverse than 30 years ago, leading to deficiencies in micronutrients such as Iron (Fe), Zinc (Zn), Iodine (I), Selenium (Se) and also Vitamin A (Genc *et al.*, 2005). These micronutrient deficiencies are common in children and even more common in women due to blood losses occurred during menstruation and child birth (Singh, 2009). Among trace elements, Fe and Zn are essential for a variety of metabolic processes (Underwood, 1977; Prasad, 1978). Zn and Fe deficiencies ranked fifth and sixth respectively, among the top ten risk factors contributing to disease burden globally.

The main sources of Zn for poor populations are staple cereals, starchy roots, tubers and legumes, which are low in either quantity or bioavailability of Zn (Gibson, 1994). Cereals contribute up to 50% of the Fe intake in poorest households (Bouis, 2002). Only 17-19% of total Zn intake was from animal sources. This means that doubling the Fe and/or Zn density of food staples could increase total intakes of these minerals by 50% (Ruel and Bouis, 1998).

The consequences of malnutrition are severe and long lasting, sometimes moving from generation to generation. Maternal malnutrition during pregnancy increases the risk of mortality, as well as affecting foetal growth, resulting in low birth weight (LBW), risking the survival of the child. The prevalence of malnutrition remains a public health problem in developing countries. Fe deficiency is estimated to affect about 30% of the world's population, making Fe by far the most deficient nutrient worldwide (Lucca *et al.*, 2001). Zn deficiency has been subsequently reported all over the world (Takkar and Walker, 1993). Zn deficiency in crops might be due to intensive cultivation since high yielding varieties remove relatively high amount of Zn from the soil at every harvest. The World Bank has shown the countries whose population suffer from micronutrient deficiencies encounter economic losses as high as 5% of gross domestic product (GDP) (Mannar and Shankar, 2004). The intake of Fe and Zn appears to be below the recommend dietary allowance for an average Indian adult. This was observed particularly for low-income rural households in rice

consuming regions. Fe deficiencies are often accompanied by Zn deficiency as both of these nutrients are derived from similar source in the diet (Welch, 2001). In addition, these minerals are essential for plant growth and reproduction, and nutrient deficiency can limit the yields of plant products that represent important source of minerals in human diets.

2.4. POVERTY AND MALNUTRITION: AN INTERLINKED SOCIAL PROBLEM

Poverty is the principle cause of hunger and malnutrition. The causes of poverty include poor people's lack of resource, extremely unequal income distribution, low purchasing power and hunger itself (Tenth Five Year Plan), consequently increasing the number of undernourished people in the world. Today poverty is the state for the majority of world's people and nation. Poverty is becoming more intensified with on-going economic crisis which may be attributed to fewer employment opportunities and lower earning to more volatile commodity prices and restricted access to food. Poor populations cope with higher food prices by shifting to less balanced diets, foregoing health care or education, selling assets or eating less-all of which are direct effects of poverty that increase the burden of malnutrition. Hence, that malnutrition is a function of poverty is self-evident.

According to the strategic document published by World Bank (2006), malnutrition leads to indirect losses in productivity from cognitive development and schooling. Consequently adults that grow up malnourished will be less able to work and therefore earn less, which results in their entire family consuming less food. This vicious cycle of malnutrition leading to poverty exacerbates hunger, poverty and ill-health. Hence, poor people are caught in vicious circle: poverty breeds ill-health, ill-health maintains poverty (Wagstaff, 2002). Moreover ill-health and medical expenses often compete with money needed for food. When financial concerns are present meal are often skipped and food that is purchased may not provide a nutritionally adequate diet.

Nutrition is one of the key components of the most fundamental assets-human health. Poor people are more exposed to the risk of malnutrition and less prepared to cope with them, are less informed with the benefits of healthy life style, and have less

access to quality health care, so they suffer from more illness, disability and malnutrition. Family welfare is significantly dependent on health and nutritional status, and the physical and intellectual capacity, of the adults in the family. As a result, deteriorating the nutritional status of the adults in poor households undermines the capacity of families to survive and ensure basic nutritional and health needs. Hence, malnutrition is a serious emerging social and public health problem that has been neglected by researchers/institutions because obesity and overweight is drawing more attention, especially in developed countries.

2.5. MICRONUTRIENT DEFICIENCY IN SOIL AND ITS IMPLICATIONS ON HUMAN HEALTH

In enhancing agricultural productivity and quality, micronutrient supply is of critical importance as both agricultural production and quality are constrained by the deficiencies of plant nutrients and nutrient imbalances. Therefore, the information on the micronutrient status of soil and crop edible tissues is crucial (Mahnaz *et al*., 2010; Sahrawat *et al*., 2010). Research has been conducted to address the relationship between soil micronutrient status and crop yield and quality (Welch and Graham 2004; Gupta 2005).

In fact, intensified land use, without the addition of fertilizers, has apparently resulted in substantial removal of minerals (Sahrawat *et al*., 2007). Instead of judicious application, the imbalanced use of fertilizers has the problems, especially in the developing countries. Furthermore, it is reported that soils are becoming Zn and Fe deficient worldwide (Ghorbani *et al*., 2009; Sahrawat *et al*., 2007). The low availability of Fe, Zn, and copper (Cu) in calcareous or alkaline soils is also considered as the cause for the low mineral concentrations in edible plant parts (White and Brown 2010; Sahrawat *et al*., 2008). Thus, a successful breeding program for the biofortification of crops with grains denser in minerals will very much depend on the size of plant available mineral pool in soil (Cakmak 2008).

Zinc deficiency is an increasingly important risk factor to the global agriculture and human health, especially in the arid and semiarid regions of world (Nayyar *et al*., 1990; Sahrawat *et al*., 2007). Among the cereals, wheat and rice in particular suffer from Zn deficiency. Duffy (2007) reported that 30% yield loss was common in wheat, rice, maize, and other staple crops grown on Zn-deficient soils.

Hence, the widespread deficiency of Zn has serious implications for human health in countries where dominant diet is cereal based and also equally important for all forms of life including plants and animals. Moreover, low solubility of Zn in soils rather than the total amount of Zn is the major reason for the widespread occurrence of Zn-deficiency problem in crops (Cakmak 2008; Sahrawat *et al*., 2007). Total mineral concentrations in many infertile soils are often sufficient to support mineral-dense crops, if only the minerals are in the plant available form (Graham *et al*., 1999).

It is indicated that soils on which cereals are regularly grown for human rations are actually low in native nutrient reserves, and thus, it may lead to a situation in which nutrient deficient crops will be food of poor people. Moreover, the availability of Fe is lower in soils of the arid and semiarid regions, and as a result, grains produced on these soils have lower Fe content (Singh 2009). Frequent application of herbicide glyphosate could lead to the shortage of energy needed to maintain root growth and initiate ferric-reductase activity, and this may lead to Fe deficiency. A likely reason for this is that glyphosate interferes with root uptake of Fe by inhibiting ferric-reductase activity in plant roots, required for Fe acquisition by dicot and non-grass species (Ozturk *et al*., 2008). Eventually, we have come to understanding of micronutrient deficiency in human is derived from the deficiencies of trace elements in soils and foods. Therefore, it is a multifaceted vicious cycle among the soil-plant-human system. Soil is the base medium for all living things; thus, sick soil means sick plants, sick animal, and sick people (http://www.ecoorganics.com/sick-soil/). It is simpler to cure the sick soils than the sick people. Nevertheless, not all soils are nutritionally sick; in such cases, an improvement in plant uptake and efficiency by genetic modulation is an imperative strategy to combat the mineral deficiency in plant as well as humans.

2.6. FE MALNUTRITION AND ITS CONSEQUENCES

Fe deficiency is the prevalent nutrient deficiency in the world and is recognized by WHO as one of the ten greatest global health risk in world (Murray and Beard, 2009). Fe deficiency anaemia affects about one billion people worldwide and is most prevalent in infants, children and women of reproductive age in developing countries, where some 50% or more of these population groups may be anaemic, recently estimated to affect about three billion people worldwide (Welch, 2001). The

consequences of Fe deficiency include both morbidity and mortality of mother and child at child birth, reduce physical capacity, poor immune functions, changes in cognition, emotions and behavior. The bulk of research has been carried out in infants and children, and most studies have reported altered mental and motor development in Fe deficient-anaemic children (Murray and Beard, 2009). In the recent finding Sorenson *et al.,* (2010) have reported that maternal Fe deficiency may increase the risk of schizophrenia spectrum disorder in offspring. Fe deficiency anaemia is widespread in more than 85% in women and children in India (Singh, 2009). Similarly, the prevalence rate of Fe deficiency anemia among school children is estimated at 53%. The highest prevalence rate is in Asia followed by Africa.

2.7. ZN MALNUTRITION AND ITS CONSEQUENCES

Zn is essential for micronutrient required for growth and development of higher plants. In 2002, Zn deficiency was reported as a major risk factor to global and regional burden of disease along with Vitamin A, Fe and Iodine (I) deficiencies. It is directly or indirectly essential for the formation of proteins in humans. In biological systems Zn is required by a large number of proteins. It has been estimated that nearly 2,800 human proteins are capable of associating Zn, which correspond to 10% of the human proteome. Almost 40% of the Zn binding proteins are the transcription factors needed for gene regulation and the remaining 60% are enzymes and proteins involved in ion transport. Zn is also the critical micronutrient required for structural and functional integrity of biological membranes and for detoxification of highly reactive free radicle oxidants. Zn deficiency in humans reduces sexual maturity. It is also reported Zn deficiency also causes hair loss and hypochromic anaemia. A panel of eight worldwide distinguished economist at the Copenhagen Consensus Conference has identified Zn deficiency as the top priority problem facing the world currently. Zn deficiency has been shown as the major cause of child death in the world, and is responsible for the death of nearly 450,000 children under 5 years of age.

2.8. BIOAVAILABILITY OF FE AND ZN

The nutritional quality of a diet can be determined based on the concentration of individual nutrients as well as by the interactions of other elements, promoters, and antinutrients, which affect the bioavailability of micronutrients (Khoshgoftarmanesh

et al., 2010). Bioavailability is a term used to describe the digestion, absorption, and subsequent utilization of dietary compounds (Linder 1991). Not all ingested minerals are completely absorbed and utilized in humans and livestock, leading to certain segments of vegetarian population at risk for Fe and Zn and other trace element deficiency (Grusak and Cakmak 2004). Thus, just producing the mineral-dense food does not mean an improved nutrient status of people as the bioavailability of micronutrients needs also to be improved. That is why, for effective biofortification of food, the understanding of bioavailability of minerals to humans is a prerequisite (Dahiya *et al.*, 2008).

The levels of bioavailable mineral in staple food crop seeds and grains are as low as 5 % and 25 %, respectively; thus, breeder should consider the bioavailability of micronutrients while considering breeding program (Bouis and Welch 2010). The micronutrients interact with various types of biochemical substances which promote or inhibit the bioavailability of minerals (Khoshgoftarmanesh *et al.*, 2010). Inhibitory substances, called antinutrients, reduce, whereas promotive substances, called promoters, enhance/stimulate micronutrient bioavailability to humans (Graham *et al.*, 2001). Amounts of both antinutrients and promoters in grains depend on genetic and environmental factors (Welch and Graham 2004; White and Broadley 2005).

The bioavailability of dietary Fe and Zn is generally impaired by the phytic acid, fiber, and possibly other constituents of some plant foods (Hunt 2002; Mendoza 2002), while oxalate (Sotelo *et al.*, 2010), polyphenolics (Ma *et al.*, 2010), and to certain extent calcium (Zamzam *et al.*, 2005) inhibit Fe absorption. Dietary phytate can influence the bioavailability of several minerals, because of its capacity to form insoluble precipitates which cannot cross the membrane transporters on the surface of enterocytes, making nutrients unavailable (Wise 1995). Negatively charged phytate is the primary storage form of phosphorus in most mature seeds and grains and complexes with positively charged Fe and Zn ions, inhibiting their uptake (Zhou and Erdman 1995). Being a monogastric creature, humans do not synthesize the phytate-degrading enzyme, phytase, as a result digestive tract cannot absorb, but can excrete (Lott *et al.*, 2000).

Zn bioavailability can be predicted by considering phytate-to-Zn molar ratios in foods and has been widely used for zinc bioavailability (International Zinc

Nutrition Consultative Group IZiNCG (IZiNCG) 2004; Gargari *et al*., 2007). Zinc absorption in the intestine is reduced at ratios above a value of around 20 (Frossard *et al*., 2000). A bioavailability model should be used to screen a large number of promising lines of micronutrient-enriched genotypes identified in breeding programs before advancing them as it is impractical to test the bioavailability of micronutrients in genotypes of staple plant foods generated in plant breeding programs (Welch and Graham 2004). Earlier reports in humans revealed that cysteine had a positive effect on mineral absorption, particularly Zn (Snedeker and Greger 1981, 1983; Martinez-Torres and Layrisse 1970), while Fe and copper were less affected by the sulfur-amino acids. Further research is required to focus on the effects of protein and sulfur containing amino acids on Zn and non-heme Fe bioavailability in diets.

2.9 BIOFORTIFICATION: A VITAL DEVICE TO ALLEVIATE MICRONUTRIENT MALNOURISHMENT

Addressing micronutrient malnutrition in the country, a combination of strategies involving food fortification, pharmaceutical supplementation and food diversification can be emphasized and implemented. Various complementary approaches are often implemented in different phases: i) to ensure relief to vulnerable group through supplementation ii) to improve micronutrient uptake across population in medium term through food fortification and iii) to ensure sustained long term outcomes through dietary diversity. These cost-effective strategies normally face logistic constrains, as in case of dietary modifications, which are promising but require behavioral changes that depend on intensive and costly education, communication and social marketing strategies and investment. Furthermore, fortification is difficult for each micronutrient as specially for Fe as fortification of Fe leads to its rapid oxidation as well as increase the loss of Iodine (I) (Poletti *et al*., 2004). Unfortunately none of the strategies *i.e.* food fortification, pharmaceutical supplementation and dietary diversification have been successful to fight hidden hunger (Bouis, 2003; Lyons, 2003).

Therefore, alternatively, problem can be tackled through agricultural methods of crop cultivation by adding fertilizers – agronomic fortification – in farming system (White and Brown 2010) known as fertifortification (Prasad 2010). Fertifortification depends upon sufficient amount of available minerals in the soil (Cakmak 2008).

Despite its success in Finland and Turkey, fertifortification is not practicable in the developing countries because of financial and ecological considerations (Ju *et al*., 2009) as well as it requires specific agricultural practices with regular application of nutrients. Additionally, they are not effective for Zn and Fe due to their limited mobility in phloem (Marschner 1995) and do not always increase mineral concentrations in edible or economic parts to the desired level and increase the cost of cultivation (Dai *et al*., 2004; White and Broadley 2005). Complementarily, agronomic fortification can be used as an approach to increase the mineral content in edible plant parts. A substitute approach, endogenous fortification is used by the accumulation of trace minerals directly in cereal grains using breeding. This complimentary solution termed "biofortification" by Bouis (2003).

Crop improvement through breeding has been the key in the past successes of agricultural production (Beddington 2010). Although, breeding based strategy for biofortification is unproven as yet, it has the potential to become sustainable and cost-effective and to reach remote rural populations (Mannar and Sankar 2004; Genc *et al*., 2005). It is argued that once mineral-dense lines have been developed, there will be little additional cost in incorporating them into ongoing breeding programs (Welch and Graham 2004). It has been reported that the seed of mineral-dense crops produce more vigorous seedlings on infertile soils (Rengel and Graham 1995). High trace mineral density in seed produces more viable and vigorous seedlings in the next generation, and the efficiency in the uptake of trace minerals improves disease resistance (Welch 1999; Yilmaz *et al*., 1997). Variety, land races, and wild species are being explored for their mineral levels, and this knowledge is further used to create new varieties with higher micronutrient content (Ghandilyan *et al*., 2009). Hence, plant-breeding approaches utilize existing genetic variation coupled with marker-/genomics-assisted selection.

2.10. RELATIONSHIP AMONG GRAIN MINERALS AND YIELDS

Biofortification to address nutrient deficiencies is an enticing concept, but there is much to understand about the potential impact on other important traits. For instance, it is not clear whether selection for increased mineral micronutrient content negatively affects yield or other important agronomic and end-use characters. This could occur if genes that increase mineral content are linked with genes that have a

deleterious effect on other desired traits, or it could occur as a consequence of trait associations. Correlation between grain Fe and Zn has been studied in several crops, with results showing similar trends. For instance, positive and highly significant correlation between Fe and Zn concentrations had been observed in many crops (Gregorio *et al.*, 2000; Ozkan *et al.*, 2007; Velu 2013). Such correlations among micronutrients indicate that improvement in one element may simultaneously improve the concentration of other element (Ozkan *et al.*, 2007). However, in few studies, negative correlations between the concentrations of Zn in grain and grain yield were reported in wheat (Oury *et al.*, 2006; Zhao *et al.*, 2009) and indicate the difficulty to breed wheat with high Zn concentration and high grain yield. Positive correlations among micronutrients suggest that similar transport and chelation process affect the accumulation of elements in seeds (Ding *et al.*, 2010). The correlations among different minerals implement pleiotrophy for genes controlling the accumulation of these minerals or have close linkage of genes (Wu *et al.*, 2008). Moreover, the positive correlation between Fe and Zn concentrations in grain is less affected by environment and can be combined with other agronomic traits (Banziger and Long 2000; Welch 2005).

2.11. EXPLOITING EXISTING MICRONUTRIENT VARIABILITY: PREREQUISITE FOR BIOFORTIFICATION

Genetic variation in wild, landraces, and cultivated species is the most important basic resource to generate new plant types with desirable traits for effective crop improvement programs (Vreugdenhil *et al.*, 2004). Observed variation among crop plants can either be qualitative, caused by one or two major loci, or quantitative, caused by the combined effects of multiple loci (Salt *et al.*, 2008). Germplasm of crops differs in the grain mineral content, and the selection followed by utilization of mineral rich germplasm for breeding is an important component of research for increasing the grain mineral content. Thus, genetic resources enable plant breeders to create novel plant gene combinations and select crop varieties more suited to the needs of diverse agricultural systems (Glaszmann *et al.*, 2010). With the aim to improve nutritional value of food for human beings, researchers in the past decade have shown much interest in developing cultivars of staple food with higher mineral content (Graham *et al.*, 1999; Grusak and DellaPenna 1999; White and Broadley 2005; Cakmak 2008; Tiwari *et al.*, 2009; Norton *et al.*, 2010), but very little attention

has been paid in breeding for grain mineral content (Vreugdenhil *et al*., 2004). The identification of "left behind" valuable alleles in the wild ancestors of crop plants and their reintroduction into cultivated crops is the target of modern plant breeding (Tanksley and McCouch 1997; Chatzav *et al*., 2010).

Dissecting the variation is prerequisite to utilize the natural diversity through molecular breeding for crop improvement. Therefore, research on the screening of natural genetic variability for seed mineral concentrations in various crop species in order to use selected lines for breeding has also been conducted (White and Broadley 2009). Identification of genotypes with differing nutrient efficiencies generally includes investigation of the potential morphological, physiological, and biochemical mechanisms involved therein (Khoshgoftarmanesh *et al*., 2010). Growing evidences indicate that the wild and primitive genotypes show large and useful genetic variation for grain concentrations of Zn and Fe (Ghandilyan *et al*., 2006). The genetic variations for Fe and Zn in major food crops are explained as followed.

Rice is a dominant cereal crop accounting for 50 % of the worldwide consumption in many developing countries (Lucca *et al*., 2001). However, currently polished rice is a poor source of essential micronutrients such as Fe and Zn (Bouis and Welch 2010) and contains average of only 2 parts per million (ppm) iron (Fe) and 12 ppm of zinc (Zn). Experts estimate that a rice-based diet should contain 14.5 $\mu g\ g^{-1}$ Fe in endosperm, the main constituent of polished grain, but breeding programs have failed to achieve even half of that value. Low mineral concentration in rice may be attributed to low level of minerals in endosperm and the loss during grain polishing as well. Since 1992, genetic difference for grain Fe has been explored by researchers at the IRRI (Gregorio *et al*., 2000; Graham *et al*., 1999). Gregorio *et al*., (2000) evaluated 1,138 brown rice genotypes for Fe and Zn content and reported that grain Fe and Zn contents ranged between 6.3 and 24.4mg kg^{-1} and 13.5–58.4 mg kg^{-1}, respectively. On the other hand, aromatic rice exhibited consistently more grain Fe (range 18–22 mg kg-1) and Zn (24–35mg kg-1) content than the nonaromatic rice genotypes. Research at the International Rice Research Institute (IRRI) showed that local varieties had iron content up to 2.5 times higher than that of the common high yielding varieties (Kennedy and Burlingame 2003). Glahn *et al*., (2002) evaluated 15 selected Fe-dense and normal genotypes of unpolished rice from the IRRI and

reported that the Fe concentration ranged from 14 to 39 mg kg^{-1}. These results indicated that "aromatic and brown rice germplasm" as a potential reservoir of micronutrients which can be harnessed to improve existing micronutrient levels in rice.

Ya-wen *et al*., (2004) estimated mineral elements content of 653 unpolished rice samples harvested by ICP-AES method. The K, Mg, Ca and Mn content in high-yielding varieties were high and other nutrients such as P, Fe, Zn and Cu were low, which is connected with the heredity and physiological mechanism of mineral nutrients. Anuradha *et al.,* (2012) conducted a study to quantify iron and zinc content in rice grain and analyze the correlation between Fe and Zn concentration and seed dimension. They opined that iron concentration ranged from 6.2 ppm to 71.6 ppm and zinc from 26.2 ppm to 67.3 ppm having a significant correlation between Zn concentration and grain elongation.

Wheat (Triticum spp.), a major staple food crop having significant impact on human health, contributes 28% of the world's edible dry matter and up to 60 % of the daily calorie intake in several developing countries (Grusak and Cakmak 2005; FAOSTAT 2008). To examine genetic variation for Fe and Zn with other trace minerals, 132 wheat germplasm accessions at the CIMMYT were screened (Monasterio and Graham 2000). The variability in grain Fe ranged from 28.8 to 56.5 mg kg^{-1} and from 25.2 to 53.3 mg kg^{-1} for Zn. In a set of 30 T. *tauschii*, Monasterio and Graham (2000) reported mean Fe concentration of 76 mg kg^{-1} and a maximum value of 99 mg kg^{-1}. Similarly, Oury *et al*., (2006) identified wheat cultivars with Zn concentration ranging from 15 to 35 mg kg^{-1}, but the grain Zn increased to 43 mg kg^{-1} in selected germplasm. Fe concentration ranged from 20 to 60 mg kg^{-1} and was 88 mg kg^{-1} in non-adapted material. A total of 154 genotypes, including wild emmer accessions were evaluated for Fe and Zn by Chatzav *et al*., (2010) and reported that Fe ranged from 36 to 69 mg kg^{-1} with a mean of 52 mg kg^{-1}. Similarly, grain Zn concentrations ranged from 35 to 90 mg kg^{-1} with a mean of 58 mg kg^{-1}. The results of other studies (Balint *et al*., 2001; Morgounov *et al*., 2007) clearly showed the existence of alleles for mineral diversity within wheat germplasm to improve the food value.

Maize is the world's leading staple food along with rice and wheat due to its diverse functionality as a food source for both humans and animals (Grusak and

Cakmak 2005; Nuss and Tanumihardjo 2010). Unfortunately, even though maize kernels supply many macro- and micronutrients necessary for human metabolic needs, the amounts of some essential nutrients with phytic acid are ill balanced or inadequate for consumers who rely on maize as a major food source (Grusak and Cakmak 2005; Nuss and Tanumihardjo 2010). The range of Fe and Zn in maize kernels is not as high as in other cereals, but considerable variation in the grain micronutrient content has been reported (Welch and Graham 2004). Menkir (2008) evaluated 149 lowland and 129 mid-altitude maize inbred lines at the IITA, Nigeria, and showed that the lines varied between 11 and 34 mg kg^{-1} in Fe and 14 and 45 mg kg^{-1} in Zn. The best-inbred line in each trial had a kernel Fe concentration that exceeded the average of all the inbred lines by 37% in trial-1, 32% in trial-2, 52% in trial-3, 39% in trial-5, 42% in trial-6 and 78% in trial-7. Similarly, the best-inbred line in each trial had 14–180 % greater concentrations of Zn and other mineral elements than the average of all inbred lines. This represents a broad range of variability in adapted maize germplasm available in the maize breeding program at the IITA. During an F4-mapping population, Simic *et al*., (2009) reported good range of Fe (17–34 mg kg^{-1}) and Zn (17–28 mg kg^{-1}).

Jambunathan (1980) reported an average Fe concentration of 59 mg kg^{-1} with a range of 26–96 mg kg^{-1}, while grain Zn varied between 19 and 57mg kg^{-1} with an average of 33mg kg^{-1} in the samples of 100 varieties of sorghum. At the ICRISAT, Reddy *et al*., (2005) screened 84 accessions of sorghum for grain Fe and Zn content. The grain Fe and Zn varied from 20.1 to 37 mg kg^{-1}, and grain Zn content varied from 13.4 to 31 mg kg^{-1}. Kayode *et al*. (2006) evaluated 76 farmers' varieties of sorghum for Fe and Zn concentrations. The Fe and Zn concentration of the grains ranged from 30 to 113 mg kg^{-1} and 11 to 44 mg kg^{-1}, respectively. These varieties exhibited fourfold range in grain Fe and Zn concentrations. In most genotypes, grain Fe was higher than Zn, the difference being one- to fivefold. The level of Fe found in the Kayode *et al*., (2006) study is in agreement with values reported in the literature. Waters and Pedersen (2009) also reported a wide range in grain Fe (24–73 mg kg^{-1}) and Zn (15–59 mg kg^{-1}) in sorghum.

Pearl millet is an important staple food in arid and semiarid regions of Asia and Africa and serving as a major source of dietary energy in these regions (Velu *et*

al., 2006). Like other cereals, no much work has been done on the genetic variation of Fe and Zn content and the potential to improve it through plant breeding in pearl millet. Preliminary studies were conducted by Jambunathan and Subramanian (1988) in 27 pearl millet genotypes and Hulse *et al*., (1980) which reported as high as 38 mg kg^{-1} of Fe and 16 mg kg^{-1} of Zn. Similar studies for genetic variation for grain Fe and Zn content have been reported by Khetarpaul and Chauhan (1990), Kumar and Chauhan (1993), and Abdalla *et al*., (1998). Higher micronutrient densities in African pearl millet landraces were comparable to those reported in improved varieties and hybrid lines. This demonstrated the potential of landraces for breeding pearl millet with grains denser in Fe and Zn (Buerkert *et al*., 2001). The genetic variation is presently being exploited in breeding program at different CGIAR centers under Harvest Plus program coordinated by IFPRI and CIAT (Bouis 2003).

2.12. GENETICS OF FE AND ZN CONTENT IN GRAIN

Understanding the nature of gene action and inheritance of seed mineral content is crucial to develop effective breeding strategies for micronutrients (Cichy *et al*., 2005). Very limited information has been generated on the inheritance of grain Fe and Zn content in crops. The genetic bases responsible for the uptake of some micronutrients, especially Fe uptake, in crop plants is now much better understood. Research on the genetics of kernel micronutrient density of maize described additive gene action in the 1960s and 1970s (Gorsline *et al*., 1964; Arnold and Bauman 1976). The recurring feature of micronutrient efficiency characters are single, major-gene inheritance (Epstein 1972). Weiss (1943) demonstrated this by detecting single major dominant gene while working with Fe efficiency in soybeans. Since Weiss's pioneering study, another study in soybean indicated that several minor additive genes contributed to Fe efficiency (Fehr 1982). Cichy *et al*., (2005) reported a single dominant gene controlling the high seed Zn in navy bean. Velu *et al*., (2006) found the prevalence of additive gene action in pearl millet, controlling grain Fe and Zn content. Based on the inheritance study, selection during breeding should be undertaken in a later generation (such as F5), where the dominance effect (unfixable genes) is not present.

2.13. MOLECULAR BREEDING: MAXIMIZING THE EXPLOITATION OF GENETIC VARIATION

Genetic diversity offers opportunity to utilize various genomic sources and technologies in an effort to manipulate mineral levels in crop edible parts (Grusak and Cakmak 2005). But characterization of genetic variation within natural populations and among breeding lines is crucial for effective conservation and exploitation of genetic resources for crop improvement programs (Varshney and Tuberosa 2007). The development of molecular marker techniques has lead to a great increase in our knowledge of rice genomics and our understanding of structure and behavior of the cereal genomes (Gupta and Varshney 2000). Renewed interest in the use of markers was generated when studies with maize and tomato demonstrated that some markers explained much of the phenotypic variance of complex traits (Anderson *et al*., 1993). DNA-based molecular markers having no known effects on phenotype, unaffected by environmental conditions and gene interactions, proved to be powerful and ideal tools for examining quantitative traits and genetic research (Beckmann and Soller 1986). A variety of genetic models and designs including the analysis of mating designs in segregating population are being used to study the quantitative traits to estimate the effective factors applying biometrical or molecular marker methods (Lynch and Walsh 1998; Zeng *et al*., 1990).

In this genomic era, molecular markers have been proven to be useful in characterization of the available germplasm and estimation of genetic diversity with the aim of using this information for the selection of parents for hybridization programs (Roy *et al*., 2002; Kalia *et al*., 2011). Furthermore, the recent development in quantitative genetics by employing of molecular markers allow the development of linkage map to determine the map position and effect of different loci/genes of metric characters known as quantitative trait loci (QTL). This development is to expedite the use of markers for tagging genes/QTLs for qualitative and quantitative traits and for marker-assisted selection (MAS) (Sharma 2001; Yadav *et al*., 2002). Thus, molecular breeding can enhance the pace of genetic variation exploitation.

2.14. MOLECULAR MARKERS AND ITS IMPORTANCE

During the last few decades, molecular markers have been immensely used in plant biotechnology and their genetic studies. They are used in assessment of genetic

variability and characterization of germplasm; estimation of genetic distance between population, inbreeds and breeding material; genetic mapping; detection of monogenic and quantitative trait loci (QTLs); marker assisted selection; increase the speed and quality of backcrossing to introgress desirable traits from closely related varieties to elite germplam and identification of sequences of useful candidate genes (Farooq and Azam, 2002., Rana and Bhat, 2004., Murtaza *et al*., 2005).

There is such an enormous amount of diversity in the DNA of higher plants that no two organisms are likely to be identical in their DNA sequences. Variations have been detected in restricted *(i.e.,* enzymatically digested) genomic DNA of plants and these restriction fragment length polymorphisms (RFLPs) have paved way for the development of molecular markers (Winter and Kahl, 1995). Genetic engineering and biotechnology hold great potential for application in plant breeding as they promise to reduce the time taken to produce crop varieties with desirable characters. With the use of molecular techniques, it would now be possible to hasten the transfer of desirable genes among varieties and to introduce novel genes from related species (Mohan *et al.,* 1997). Molecular markers detect unambiguous, single-site genetic differences that can easily be scored and mapped in most segregating populations. It is not difficult in populations of most crop species to identify and map 10-50 segregating molecular markers per chromosome pair (Kearsey, 1998). DNA markers can increase efficiency in breeding programs in a number of ways:

I.   The ability to screen in the seedling stage for traits that are expressed late in the life of the plant.

II.   The ability to more efficiently screen for traits that are extremely difficult, expensive, or time consuming to score phenotypically. Since DNA-based markers themselves have no known effects on the phenotype of the plant, they are ideal for studying quantitative traits (Stuber *et al.,* 1992).

III.   The ability to distinguish between the homozygous and heterozygous conditions of many loci in a single generation without progeny testing.

IV.   The ability to perform simultaneously, marker-aided selection to screen for a character or complex of characters that could not previously be included in the program because of cost or difficulty of conventional methods based on phenotypic screens.

Molecular markers can accelerate the generation of new varieties and allow association of phenotypic characters with the genomic loci responsible for them. However, the real advantage of using molecular markers is to permit backcross transfer and pyramiding of desirable alleles in a directed manner that would not be practical with conventional phenotypic selection procedures.

Polygenic characters that were previously very difficult to analyze using traditional plant breeding methods can now be readily studied and it is now relatively easy to establish genetic relationships between even sexually incompatible crop species (Mohan *et al.,* 1997). The ability to map genes contributing towards variation in complex traits with enough accuracy to be useful for plant breeding applications has been made possible through the development of comprehensive molecular markers-based genetic linkage maps (Jones *et al.,* 1997). DNA fingerprinting of the cereals has a very long scientific history.

When DNA profiling technology first came into use, restriction fragment length polymorphism (RFLP) markers were considered state-of-an-art. RFLP technology was followed by random amplification of polymorphic DNA (RAPD) method and later by the amplified fragment length polymorphism (AFLP) technique. Most recently microsatellite markers or simple sequence repeats (SSR) have become the preferred marker technology for many plant breeding applications. Advantages of SSR markers are:

❖ The method is relatively simple and can be automated;
❖ Most of the markers detect a single locus and show Mendelian inheritance;
❖ SSR markers are highly informative and reproducible;
❖ A large number of public SSR primers are available in most major crop species;
❖ Cost effective per genotype and primer, and avoid use of radioactive material.

2.14.1. Importance of Microsatellite (SSR) and Its Application

Microsatellites, alternatively known as simple sequence repeats (SSRs), short tandem repeats (STRs), simple sequence length polymorphisms (SSLPs), or variable

tandem repeats (VTRs), are tandem repeats of sequence units generally less than *5* bp in length, *e.g.* (TG)n or (AAT)n (Bruford and Wayne, 1993). SSRs have received considerable attention and are probably the current marker system of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya *et al.,* 1992; Chin *et al.,* 1996). These markers appear to be hypervariable, in addition to which their co-dominance and reproducibility make them ideal for genome mapping, as well as for population genetic studies (Dayanandan *et al.,* 1998). Inter-SSRs are a variant of the RAPD technique, although the higher annealing temperature probably means that they are more rigorous than RAPDs. Chloroplast microsatellites (cpSSRs), are similar to nuclear microsatellites but the repeat is usually only 1 bp, *i.e.* $(T)_n$ (Provan *et al.,* 1999).

The repeat regions are generally composed of di-, tri-, tetra- and sometimes greater length perfectly repeated nucleotide sequences (Tautz and Ranz, 1984) that exhibit a high degree of polymorphism (Weber and May, 1989).

Microsatellite variation results from differences in the number of repeat units. These differences are thought to be caused by errors in DNA replication (Moxon and Wills, 1999; Jarne and Lagoda, 1996; Edwards *et al.,* 1992); the DNA polymerase "slips" when copying the repeat region, changing the number of repeats (Jarne and Lagoda, 1996). Larger changes in repeat number are thought to be the result of processes such as unequal crossing over (Strand *et al.,* 1993). Such differences are detected on polyacrylamide gels, where repeat lengths migrate different distances according to their sizes or by capillary electrophoresis, where smaller repeat lengths migrate through the column in less time than do larger ones.

Simple sequence repeats are abundant in eukaryotic genomes. They provide a co-dominant, and usually highly polymorphic marker system (Bryan *et al.,* 1997; Tautz and Ranz, 1984). In plant genomes, the overall frequency of microsatellite repeats appears to be generally lower than animal genomes (Morgante and Olivieri, 1993; Wu and Tanksley, 1993). In general, plants have about 10 times less SSR than humans (Mohan *et al.,* 1997). The incidence of closely spaced repeats AC or TC is very common, but in plants AT is more common followed by AG or TC.

Microsatellites, which detect variation at individual loci, have been thought of as the "new allozymes". Consequently much of their use has been in studies where

allozymes have previously been used, *e.g.* diversity studies *(e.g.* Rossetto *et al.,* 1999), gene flow and mating systems (Chase *et al.,* 1996), and paternity analysis (Streiff *et al.,* 1999). Rossetto *et al.,* (1999) studied the partitioning of variation within and between populations of *Melaleuca alternifolia* (Myrtaceae) to facilitate the identification of genetic resources and assist in the conservation of genetic diversity. Chase *et al.,* (1996) studied the gene flow and mating patterns of *Pithecellobiurn eleguns* (Leguminosae) in a forest fragment in Costa Rica, whilst Aldrich *et al.,* (1998) analyzed the genetic structure and diversity of fragmented populations of *Symphonia globulifera* (Clusiaceae). However, there are few phylogenetic studies that use microsatellite markers, perhaps because few microsatellite markers are transferable across species (Sorrells *et al.,* 2003). Many microsatellite studies appear to be expansions of goups that have been studied using biochemical or molecular markers. Rossetto *et al.,* (1999) study on genetic structure in *Meluleuca alternifolia* is an expansion of allozyme studies by Butcher *et al.,* (1992), albeit Rossetto *et al.,* (1999) used a greater number of individuals and populations.

Unique sequences that flank the tandem repeats can be used as highly polymorphic probes or for making PCR primers. There are well-established methods of finding microsatellites by screening phage libraries with oligonucleotide probes. But a quicker, if limited, approach is to examine sequence data banks for their presence (Burr, 2001). SSR-based primers representing tri-, tetra- and penta-nucleotide repeats have been used successfully to generate distinct banding patterns that are resolvable on low resolution agarose gels using ethidium bromide staining (Gupta *et al.,* 1994; Weising *et al.,* 1995) on high-resolution polyaclylamide gels by silver staining (Buscot *et al.,* 1996). through primer radioactive labeling followed by autoradiography (Gupta *et al.,* 1994), or through primer labeling with fluorescent dyes and automated high resolution visualization of PCR products separated by PAGE or capillary electrophoresis. As would be predicted, better product size discrimination is obtained with polyacrylamide-based gel analysis although agarose gel is sufficient for many applications (Vogel and Scolnik, 1997). Further, automated high-resolution visualization of dye-labeled PCR products allows effective size discrimination of one base pair.

In any case, SSRs are generally among the most reliable and highly reproducible of molecular makers. Indeed SSRs are now widely recognized as the

foundation for many framework linkage maps. SSRs have played a critical role even in merging disparate linkage maps (Bell and Ecker, 1994; Akkaya *et al.,* 1995) since they define specific locations in the genome unambiguously (Young, 2001). These markers can require considerable investment to generate but are then inexpensive to use in mapping and MAS. The large startup costs for this technique should be justifiable for crops where large-scale mapping and MAS are a practical necessity (Hash and Bramel-Cox, 2000). Post-PCR multiplexing involves the simultaneous separation of PCR amplification products of several SSR loci in a single gel lane (Masi *et al.,* 2003). Simplex PCR conditions were optimized for each primer pair by first testing different cycling conditions and then varying (1) the amount of DNA template, (2) the concentration of primers, and (3) the concentration of $MgC1_2$, and (4) the amount of *Taq* DNA polymerase.

Many studies have reported significantly greater diversity of microsatellites over RFLPs (McCouch *et al*., 1997) and high number of alleles for rice microsatellite markers. It has been found that genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite for every 16-20 cM (Chen *et al*., 1997).

Rice microsatellites have been demonstrated to be polymorphic between (Wu and Tanskley, 1993 and Akagi *et al*., 1997) and within rice varieties (Olufowote *et al*., 1997). The hyper variable repeat regions which have been termed 'microsatellites' (Litt and Luty, 1989) are individually amplified by means of the Polymerase Chain Reaction (PCR), using a pair of flanking unique oligonucleotides as primers. SSR markers almost invariably showed extensive polymorphism due to site specific length variation, as a consequence of the occurrence of different number of repeat units (Litt and Luty, 1989). A search for di- and tri- nucleotide repeats from published DNA sequence databases revealed that PCR amplified microsatellites are frequent and widely distributed; they were uncovered in thirty-four species, with frequency of one in every 50 kb (Morgante and Olivieri, 1992).

Wu and Tanksley (1993) showed that SSRs such as (GA)n and (GT)n are not only present in mammalian genome but also in rice genome. Yang *et al.* (1994) reported that because of the greater resolving power and the efficient production of massive amounts of SSR data, it is choice of markers for germplasm assessment and

evolutionary studies of crop plants. It is found that the average percent polymorphism between *indica* and *japonica* accessions was 31, 35 and 76 %, for AFLP, RAPD and microsatellite markers, respectively (Mackill *et al*., 1995).

According to McCouch *et al*., (1997) microsatellite loci have a high level of allelic diversity of 2-25 alleles per SSLP locus compared to 2-4 alleles per RFLP locus in cultivated indica, and japonica germplasm making them reliable genetic markers. It is possible to tag markers adjacent to the targeted gene or QTL for the trait of interest when two alleles (i.e. a marker and the target gene) are more or less likely to appear together. However, developing perfect markers is very difficult; most of applied markers in plant breeding are positioned at a certain genetic distance from the gene of interest (Boersma *et al*., 2007).

Reddy *et al.,* (2002), in their review, have reported an Inter Simple Sequence Repeat (ISSR)-PCR technique that involves the use of microsatellite sequences as primers in polymerase chain reactions to generate multi-locus markers. It is a simple and quick method that combines most of the advantages of SSRs and amplified fragment length polymorphism to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and are potentially useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

Jiming *et al*., (2004) reported an interspecific advanced backcross population derived from a cross between *Oryza sativa* L. "V20A" (a popular male-sterile line used in Chinese rice hybrids) and *Oryza glaberrima* L. that was used to identify quantitative trait loci (QTL) associated with grain quality. A total of 308 BC3F1 hybrid families were evaluated for 16 grain-related traits under field conditions and the same families were evaluated for RFLP and SSR marker segregation. Eleven QTL, associated with improvements in grain shape and appearance, resulted in an increase in kernel length, transgressive variation for thinner grains, and increased length to width ratio.

Ge *et al*., (2005) performed QTL analysis of rice grain quality traits (cooked rice grain elongation, volume expansion and water absorption) using a recombinant inbred population derived from a cross between two indica cultivars, 'Zhenshan 97'

and 'Minguhi 63'. QTLs were detected on chromosome 1, 2, 3, 5, 6, 7, 8, 9 and 11. Among these, three QTLs for cooked rice grain length elongation on chromosome 2, 6 and 11, six QTLs for width expansion on chromosomes 1, 2, 3, 6, 9 and 11 and two QTLs for water absorption on chromosome 2 and 6 were detected, respectively. The use of SSR markers in genetic diversity analysis in differentiating rice cultivars according to their subspecies were indicated by Victoria *et al.*, (2007). Locus amplifying di-nucleotide repeat motifs were found to be more polymorphic than those of tri-nucleotide and tetra-nucleotide repeats. Among di-nucleotide repeat motifs, markers with GA-repeat motif showed the largest variability. The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The higher the PIC value of a locus, the higher the number of alleles detected.

Ilango and Sarla, (2010) assessed the parental polymorphism between the selected five rice varieties. The RM markers located on chromosome were screened for iron and zinc dense grain (Jalmagna, Madhukar and regional cultivars Swarna, BPT 5204, IR64) to map QTLs (Quantitative trait loci) for iron and zinc.

Chandel *et al.*, (2011) characterized five known QTLs, two (QTL qFE-1 and Qfe-9) governing Fe content and three (QTLs qZN-5, qZN-11) governing Zn content in rice. They indicated the abundance of microsatellites or SSR in the genomic, cDNA, exon, intron and UTR regions, and developed twenty six candidate gene based microsatellite markers from the genomic, cDNA, exon, intron and UTR region.

Tabkhkar *et al.*, (2012) performed genetic diversity analysis of rice cultivars by microsatellite markers, which were tightly linked to cooking and eating qualities. In their study, 48 rice genotypes were grouped using seven microsatellite (SSR) markers, linked to major QTLs controlling three major components of rice cooking qualities viz., amylose content, gelatinization temperature and gel consistency.

2.15 MAPPING POPULATIONS

The development of molecular marker technology has generated renewed interest in genetic mapping. The most critical decisions in constructing linkage maps with DNA markers are those made in developing the mapping population. In making these decisions, several factors must be kept in mind, the most important of which is the goal of the mapping project. Young (1994) reviewed the most important factors

for a mapping project the success or failure of which is mainly dependent on which parents are chosen for crossing, the size of the population, how the cross is advanced, and which generations are used for DNA and phenotypic analysis. Linkage maps of crop species are often constructed with segregating populations *i.e.* F2 populations or backcrosses (Sunil, 1999). Several types of interspecific cross derived populations, such as F2 (Xiong *et al*., 1999 and Yoon *et al.*, 2006), back cross population (Xiao *et al.,* 1998., Septiningsih *et al.,* 2003., Thomson *et al*., 2003 and Marri *et al*., 2005), introgression lines (Tian *et al*., 2006) and Recombinant Inbred Lines have been used for QTL mapping in rice. Doubled haploid lines (DHLs), Near Isogenic Lines (NILs) and recombinant inbred lines (RILs) have been used for molecular genetic mapping in rice. RILs were used to study not only yield related QTLs (Cao *et al*., 2010 and Bai *et al*., 2011) but also to study QTLs for micronutrient (Fe and Zn) variability (Bekele *et al*., 2013) in rice.

2.15.1. DNA Polymorphisms among Parents

Sufficient detectable DNA sequence polymorphism between parents must be present. This cannot be over-emphasized, for in the absence of detectable DNA polymorphism, segregation analysis and linkage mapping are virtually useless. However, in many allogamous species, any cross that does not involve related individuals will provide sufficient polymorphism for mapping (Helentjaris, 1987). Miller and Tanksley (1990) reported that in naturally inbreeding species the levels of DNA sequence variations are generally low and finding suitable DNA polymorphism can be more challenging. The requirement for sufficient DNA sequence polymorphism may preclude the use of DNA markers in some narrow-based crosses (Young, 1994). More recently developed technologies, like electrophoresis systems capable of separating DNA molecules with only a single base pair change (Riedel *et al.,* 1990), provide better methods for uncovering polymorphisms within narrow-based crosses, Probes based on mini-satellites (Dallas, 1988) or simple repeated tetra-nucleotide motifs (Weising *et al.,* 1989) can uncover polymorphisms between closely related individuals. Because these are so variable at the DNA sequence level, such sequences are likely to eventually provide markers useful for mapping in narrow-based crosses (Winter *et al.,* 1999; Choumane *et al*., 2000).

2.15.2. Choice of Segregating Population

Once suitable parents have been identified, the type of genetic population to be used for linkage mapping must be considered. Several different kinds of genetic populations are suitable. The simplest are the F2 population derived from a true FI hybrid, and their backcross populations. For most plant species, populations such as these are easy to construct, although sterility in the FI hybrid can limit some combinations of parents, particularly in wide crosses. The major drawback to F2 and backcross populations is that they are ephemeral that is seed derived from selfing these individuals will not breed true. It is difficult or impossible to measure characters as part of QTL mapping in several locations or over several years with F2 or backcross populations (Young, 1994). Soller and Beckmann (1990) describe advanced generation progeny-based phenotyping of F2- genotyped individuals. Based on this, Hash and Witcombe (1994) described a method for developing and maintaining a pearl millet mapping population based on F2 plants derived by selfing a single F, plant that will provide an "immortal" mapping population available for several seasons. The uses of inbred populations comprised of recombinant inbred lines (RTLs) derived from individual F2 plants are an excellent strategy to provide more permanent mapping resources (Burr *et al.,* 1988; Burr and Burr, 1991). Similar types of inbred populations, such as doubled haploids, can also be used for linkage mapping with many of the same advantage of FULs (Heun *et al*., 1991). A doubled haploid population is only a form of RIL population differing from conventional RIL populations in the procedure used to produce it.

2.15.2.1. Recombinant Inbred Lines (RILs)

Recombinant inbred lines are developed by single-seed selections from individual plants of an F2 population. Single-seed descent is repeated for several generations. At this point (F6 RILs), all of the seed from an individual plant is bulked. The result is a set of homogeneous, homozygous lines for which large amounts of seed can be produced for replicated trials. These lines are especially powerful for analyzing quantitative traits because replicated trials can be analyzed using identical genetic material. The quantitative trait data can then be used to determine if any molecular markers are closely associated with those traits. RIL populations are genetically true breeding with high homozygosity, stable and permanent and well

suited to QTL analysis. Further, RILs undergoes multiple round of meiosis before homozygosity is reached, there is a greater chance for linked gene to recombine, providing an opportunity for accurate detection of QTLs (Burr and Burr, 1991 and McCouch and Doerge, 1995).

2.16. LINKAGE MAPPING

Linkage mapping is putting marker loci (and QTLs) in order, indicating the relative distances among them, and assigning them to their linkage groups on the basis of their recombination values from all pair-wise and three-point combinations. The first linkage map of the human genome based on molecular markers (Botstein *et al.,* 1980) fuelled the development of molecular marker based genome maps in other organisms.

2.16.1. The Basis of Linkage Mapping

The theory of linkage mapping is same for DNA markers as in classical genetic mapping; however, several new considerations must be kept in mind. This is primarily a result of the fact that potentially unlimited numbers of DNA markers can be analyzed in a single mapping population. DNA-based maps can be related to existing cytogenetic maps through the use of aneuploid or substitution lines (Helentjaris *et al.,* 1986; Sharp *et al.,* 1989; Young *et al.,* 1987) or *in situ* hybridization (ISH) (Zhang *et al.,* 2000). Since DNA marker technology was first applied to plants, there has been an explosion in the development and application of genetic linkage maps (Mohan *et al.,* 1997). Using these new DNA based markers, scientists have constructed maps in species where only poorly populated classical maps existed before (Bonierbale *et al.,* 1988; Gebhardt *et al.,* 1991; Liu *et al.,* 1994), located genes governing quantitative characters, often in great detail, and gone on to attempt (sometimes successfully) gene cloning based on genetic map position. Detailed genetic linkage maps are also fundamental tools for studies on selection, identification and organization of plant genomes (Beckmann and Soller, 1986; Landry and Michelmore, 1987; Tanksley, 1993).

2.16.2. Computer Software Packages for Genetic Linkage Mapping

Advances in computer technology have been essential to progress in DNA marker-based genetic linkage maps. The theory behind linkage mapping with DNA markers is identical to mapping with classical genetic markers, but the complexity of

the problem has dramatically increased because of the larger numbers of markers that can and must be used. This increase in numbers of segregating loci (and the number of progenies in which they are segregating) relative to studies of classical genetic markers has necessitated the development of complex computer algorithms and software packages specifically for this purpose.

Construction of a genetic linkage map from a DNA marker data set requires computer software packages capable of running ^{2} contingency table analysis. The program LINKAGE-1 (Suiter *et al.,* 1983) carries out this type of analysis automatically and also compares the observed allelic distributions to expected distributions. In a different strategy for optimizing the use of DNA marker information, the computer program "Hyper Gene" converts genotypic data into a "graphical genotype" (Young and Tanksley, 1989a, b). In this complete genome of an individual from the mapping population is displayed.

MAPMAKEREXP is a linkage analysis software package for constructing primary linkage maps of markers segregating in experimental crosses. It performs full multipoint linkage analysis for dominant, recessive and co-dominant *(e.g.* RFLP-like) markers in BC, backcrosses, F_2 and F_3 (self) intercrosses and recombinant inbred lines (Lander *et al.,* 1987; Lincoln *et al.,* 1992). The software package Join Map (Stam, 1993; Stam and Van Ooijen, 1995) can be used to analyze all types of mapping populations and can combine maps of different mapping populations provided there are common markers. Another software for linkage mapping is G mendel from Oregon State University, USA (Holloway and Knapp, 1994). The package MapMaker, with different versions such as QTS, QTXP and QTX-Classic for Macintosh- and IBM-compatible computers (Manly, 1993; Manly and Olsen, 1999), can be used to analyses the results of genetic mapping experiments using backcrosses or recombinant inbred lines.

2.17. QTL MAPPING

Before the development of molecular markers QTLs were called as polygenes (Mather, 1941). Later, Gelderman, (1975) called them Quantitative Trait Loci (QTLs). The term QTL is not very old, but the concept of detecting QTLs are old and were developed more than 75 years ago by Sax (1923), who noted that seed size in bean, a complex trait was associated with seed coat pattern and pigment, a monogenic

trait. QTL analysis is based on association between trait values and marker alleles. Marker trait associations can be identified using Bulk Segregation Analysis (BSA) (Michelmore *et al*., 1991), but linkage maps (Tanksley, 1993) provide higher mapping resolution than do methods based on closely related meiosis events making them an important statistical tools for detecting DNA variants responsible for genetic traits.

Availability of molecular markers and understanding the genetic basis of accumulation of micronutrients in the grains has facilitated mapping of the QTL and tagging the genes responsible for the high zinc content, and use these markers for devising the plant breeding strategies and for improving grain micronutrient content through marker-assisted selection (Zimmerman and Hurrel, 2002., Lu *et al*., 2008., Tiwari *et al*., 2009). It is reported that grain Zn content in rice is governed by a number of QTLs located on different regions of the chromosome with different phenotypic effects (Avendano, 2000., Biradar *et al., 2007., Lu et al., 2008.*, Garcia-Olivera *et al*., 2009., Zhang *et al*., 2011 and Anuradha *et al*., 2012). But, there is no report indicating tight linkage of a marker to grain Zn content in the rice grains. Zn accumulation in seeds involves a polygenic inheritance which is attributed by the interactions between two or more genes and their environment (Grusak and Dellapenna, 1999). QTLs responsible for the trait of interest can be identified with closely linked molecular markers. Molecular markers have been used to identify the genetic regions involved in grain Zn content in plants including *Arabidopsis* (Vreugdenhil *et al*., 2004., Filatov *et al*., 2007), bean (Guzman-Maldonado *et al*., 2003), barley (Sadeghzadeh *et al*., 2010), wheat (Shi *et al*., 2008, Tiwari *et al*., 2009).

Sadeghzadeh *et al*., (2010) obtained one dominant microsatellite anchored fragment length polymorphism (MFLP) marker SZnR1 (seed Zn regulator 1) associated with increased accumulation of Zn in barley seed in a study done using 150 double haploid mapping populations derived from a cross between Clipper (low-Zn-accumulator) and Sahara 3771 (high-Zn accumulator). It showed that SZnR1 marker was 12 cM from Xbcd175 marker on the short arm of chromosome 2H. This marker showed a correlation with seed Zn concentration and content and explained 21 and 18 % increase in seed Zn concentration and content, respectively.

Single-marker analysis using 26 SSR markers on 176 RILs of Azucena X Moromutant showed that three markers RM263, RM152 and RM21 had association

with grain zinc concentration (Bekele *et al*., 2013). Genetic identification of QTLs for contents of Fe, Zn, Mn, Cu, Ca, Mg, P and K of 85 introgression lines (ILs) derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipogon*) were studied by Garcia Oliveira *et al*. (2009). For iron content, one QTL located on chromosome 2 accounted for 5% and 7% phenotypic variation in 2005 and 2006, respectively. During 2006, a minor QTL was also detected near marker RM296 on chromosome 9. The favorable allele for iron content at chromosome 2 was contributed by *O. rufipogon*, whereas recipient parent Teqing was accommodated at chromosome 9. Three QTLs for Zn content were identified on chromosomes 5, 8 and 12. The QTL near marker RM152 on chromosome 8 accounted for the largest proportion of phenotypic variation (11–19%) for Zn content over both years, whereas the QTL that was located on chromosome 12 accounted for 9% phenotypic variation and was detected only during 2005. The *O. rufipogon* alleles enhanced the Zn content at these loci with a range of 3.86–6.91ppm. On the other hand a minor QTL for Zn content was contributed by Teqing at chromosome 5 with an additive effect of 2.29–2.44 ppm.

Mapping of the chromosomal regions associated with zinc content involving the F2 populations derived from a cross between Samba Mahsuri and Ranbir Basmati using microsatellite markers derived from the genomic regions associated with zinc metabolism, was done by Lalasa, *et al*. (2012). Out of the 45 microsatellite markers used for the parental polymorphism studies, 16 markers were polymorphic, 8 markers were monomorphic and 21 were not amplified. Three polymorphic markers associated with cation uptake viz., SC 129, SC 135 and SC 141 were used to assay F2 individual plants. The linkage distance of these three markers, SC 129, SC 135 and SC 141 with candidate genes *OsZIP1, OsZIP8* and *OsNRAMP7* on chromosomes 3, 5 and 12 were found to be 47.8 cM, 15.2 cM and 44.6 cM, respectively.

Several researchers have mapped QTLs for Zn content in rice grain. Avendano (2000) reported the presence of a QTL on chromosome 5 between marker OSR35 and RM267 in a study on recombinant inbred lines (RILs) derived from Madhukar and IR26 for higher Zn content in grains. It is found that marker RM267 is 12.5 cM from the gene responsible for higher Zn content in grains. She also reported QTL analysis for zinc deficiency tolerance using the same mapping population and it was mapped on chromosome 5 between markers RM164 and RM87 showing a variation of 61.9%.

Table 2.1 QTLs related to Fe accumulation in rice reported by different groups

Sr. No.	Cross	No. of QTLs identified	Chromosome Location	Fe variation explained	Co-localized gene or previously identified QTL	References
1.	indica IR64 X japonica Azucena	3	2	17%	-	Stangoulis *et al.* (2007)
			8	18%	High Fe QTL OsNRAMP7	
			12	14%	-	
2.	indica Teqing X wild rice (Oryza rufipogon)	10	All chromosomes except 4, 7 and 11	6% (Chromosome 2)	-	Garcia-Oliveira *et al.* (2009)
3.	Multiple Crosses	3	7	19-30%	-	Gregorio *et al.*, 2000
			8	19-30%	-	
			9	19-30%	-	
4.	indica Zhengshan 97 X indica Minghui 63	1	1	26%	-	Lu *et al.* (2008)
			9	11%	-	
5.	indica Bala X japonica Azucena	4	1	16%	High Fe QTL	Norton *et al.* (2010)
			3	21%	-	
			4	10%	-	
			7	15%	-	
6.	indica Jalmagna X indica Swarna	7	1	69%	-	Anuradha *et al.* (2012)
			1	69.2%	High Fe QTL, *OsYSL1*	
			5	69.2%	*OsMTP1, OsZIP6, OsZIP7, OsYSL4*	
			7	69%	*OsNAS3*	
			7	69%	*OsNRAMP1,Heavy metal ion transport, OsZIP8*	
			12	72%	*APRT*	
			12	72%	-	

Biradar *et al.,* (2007) identified a total of six QTLs for Zn content in rice grain using single-marker analysis on chromosome 1, 4, 5, 8, 9 and 11 using 93 double haploid mapping populations obtained from IR64 X Azucena with 254 SSR and RFLP markers. These QTLs explained phenotypic variation ranging from 4.4 to 9.5 %. The maximum phenotypic variation (9.5 %) was explained by RZ536 marker present on chromosome 11. They have also reported the overlapping of markers RG908, RZ390 and RG556 for partial resistance to rice blast with both silicon and zinc content in rice grains.

Susanto (2009) in IRRI identified two QTLs controlling Zn content in polished rice on chromosome 6 (zn-vb6.1) and chromosome 12 (znvb12.1) and two QTLs controlling iron content on chromosome 3 (fevb3.1) and chromosome 6 (fe-vb6.1) from 115 BC1F1 population obtained from IR75862-206-2-8-3-B-B-B/IR64 parents. Shi *et al*. (2008) detected four and seven QTLs for Zn concentration and Zn content, respectively in wheat 119 doubled haploid (DH) population developed from a cross between two winter wheat varieties Hanxuan10 and Lumai 14 using 395 markers. All the four QTLs for Zn concentration co-located with the QTLs for Zn content on chromosome 4A, 4D, 5A and 7A suggesting a possibility to improve both grain Zn concentration and content simultaneously.

Zhang *et al*. (2011) identified two quantitative trait loci (QTLs) qZnc-4 qZnc-6 associated with grain Zn content using 127 doubled haploid population derived from a cross between *japonica* JX17 and *indica* ZYQ8 rice cultivars from genetic linkage map constructed using a total of 160 RFLP and 83 SSR markers. These QTLs accounted for 10.83 % and 12.38 % of the total phenotypic variation.

Gande *et al.* (2014) evaluated candidate gene markers in recombinant inbred lines (RIL) derived from IRRI38 X Jeerigesanna and validated putative candidate gene markers with rice accessions. Among twenty four candidate gene markers they used, eight showed polymorphism and out of three simple sequence repeats (SSR) markers, three showed polymorphism. Single marker analysis revealed that four (OsNAC, OsZIP8a, OsZIP8c and OsZIP4b) candidate gene markers showed significant variation among RILpopulation with a phenotypic variation of 4.5, 19.0, 5.1 and 10.2%, respectively.

2.17.1. QTL Analysis: Statistical Methods

Jayakar (1970) suggested mathematical-statistical methods for the detection and estimation of linkage between a qualitative marker gene and a locus influencing a quantitative character. Since then, experimental designs for determination of linkage between marker loci and QTL have been widely described (Elston and Stewart, 1971; Geldeman, 1975; Hill, 1975; Soller and Beckmann, 1983, 1990; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.,* 1990).

Marker-QTL association detection can be conducted through t-tests based on single markers (Soller *et al.,* 1976) or by means of likelihood ratio tests that involve that use of a pair of markers bracketing a QTL, a procedure termed 'Interval Mapping' (Weller, 1987; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.,* 1990), although simpler approaches are also possible (Thoday, 1961; Weller, 1987; Haley and Knott, 1992).

Lander and Botstein (1989) described a set of analytical methods that modify and extend the classical theory for mapping QTLs and that are implemented in the computer software package MAPMAKERIQTL. In this, interval mapping is applied to several population types. Each interval between adjacent pairs of markers along a chromosome is scanned and the likelihood profile of a QTL being at any particular point in each interval is determined. The genetic methods required to analyze possible associations between traits that are inherited in a quantitative manner using QTL analysis were reported by Prioul *et al*. (1997). Advantages and some limitations of QTL analysis over other methods then in use by plant physiologists to test associations between traits were also discussed. Particularly in the case of cross-pollinating crop populations, interval mapping has been enhanced to "all marker mapping'. To calculate the likelihood of a segregating QTL, the segregation information of all linked markers is employed. Each segregating marker may follow a different segregation type, with two to four alleles (Maliepaard and van Ooijen, 1994).

An alternate approach was developed for QTL analysis using regression by Knapp *et al.* (1990) and Haley and Knott (1992). It produces results very similar to interval mapping both in terms of accuracy and precision, but has the advantage of speed and simplicity of programming. This method used the coefficient of regression

of the phenotype on the genotype of the different markers (Martinez and Curnow, 1992; Wu and Li, 1994). A significant regression coefficient is indicative of an association between the marker locus and gene(s) contributing to phenotypic difference.

Two classical approaches used for QTL detection are marker-by-marker ANOVA and multiple marker methods. The principle of the ANOVA is to test whether there are significant differences between the phenotypic means of genotypes classes at a particular marker locus (Prioul *et al.,* 1997). Van Ooijen (1999) presented methods that provide reasonably accurate approximations to LOD significance thresholds for QTL analysis, which were obtained by large-scale simulations. Churchill and Doerge (1994) described an empirical method, based on the concept of permutation tests, for estimating threshold values for declaring significant QTL effects.

2.17.2. QTL mapping software

Normally all QTL mapping software require input of the data for
1. The quantitative trait value(s) for each progeny
2. The genotype (molecular markers) for each progeny

There are over one hundred genetic analysis software packages available. Here is the brief list of some commonly used software packages:

MAPMAKEWQTL (*ftp://genome.wi.mit.edu/pub/rnapmaker3/) is* the original QTL mapping software for Macintosh and IBM computers (Lincoln et *al.,* 1992). It is user-friendly, freely distributed, and runs on almost all platforms. It will analyze F2 or backcross data using standard interval mapping procedures.

MQTL is an IBM-compatible computer program for composite interval mapping in multiple environments (van Ooijen and Maliepaard, 1996). It can also perform simple interval mapping. Currently, MQTL is restricted to the analysis of the data from homozygous progeny (doubled haploids or recombinant inbred lines). Progeny types with more than two marker classes *(e.g.* F2) are not handled.

PLABQTL *(http://www.uni-hohenheim.de/-ipspwww/soft.html) is* a freely distributed IBM-compatible computer program for composite interval mapping and simple interval mapping of QTLs (Utz and Melchinger, 2000; Utz *et al.,* 2000). Its main purpose is to localize and characterize QTLs in mapping populations derived from a biparental cross by selfing or production of double haploids. Currently, this program is the easiest software to use for composite interval mapping.

QTL cartographer *(http://statgen.ncsu.edu/qtlcart/cartographer.html)* is a *Q*TL mapping package written for UNIX, Macintosh or Windows computer operating systems. It performs single-marker regression, interval mapping, and composite interval mapping. It permits analysis of F2 or backcross populations. It displays map positions of QTLs using the GNUPLOT software. QTL Cartographer was developed by the group of Zeng in USA (Zeng, 1993, 1994; Basten *et al.,* 1994, 1997). It allows markers to be chosen as cofactors to reduce the background genetic noise and increase the resolutions of QTL detection. This is an effective strategy for improving the ability to detect QTLs of small effect provided that the number of progenies in the mapping population is reasonably large.

MapQTL (http:/lwww.cpro.dlo.nl/cbwi/s) a similar composite interval mapping methods package has been developed by Jansen and co-workers (Jansen, 1993; Jansen and Stam, 1994) called multiple QTL modeling (MQM).

Multimapper (Sillanpaa and Arjas, 1988), based on Bayesian modeling and inference, treats the number of quantitative trait loci as an unobserved random variable using ideas similar to composite interval mapping. This method is introduced for inbred lines and it can be applied also in situations involving frequent missing genotypes.

Qgene is a QTL mapping and marker-aided breeding package written for Macintosh computer operating systems. It has a user-friendly graphical interface and produces graphical outputs. QTL mapping is conducted by either single-marker regression or interval regression.

QTLSTAT is based on interval mapping using nonlinear regression for F2, backcross, RIL and DH populations and outputs results in graphical form (Knapp *et al.,* 1992; Liu and Knapp, 1992). PGRI calculates based on the functions of t-test,

conditional t-test, linear regression, multiple QTL modeling, and permutation tests (Lu and Liu, 1995). It is for F2, backcross, RIL, heterozygous F1 and open-pollinated populations.

SAS (SAS, 1999) is a general statistical analysis software package. It can detect QTL by identifying association between marker genotype and quantitative trait by single marker analysis approaches such as ANOVA, t-test, and regression (e.g. PROC ANOVA, PROC GLM or PROC REG).

R/qtl is an extensible, interactive environment for mapping quantitative trait loci (QTL) in experimental crosses. It is implemented as an add-on package for the freely available and widely used statistical language/software R (www.r-project.org).vThe current version of R/qtl includes facilities for estimating genetic maps, identifying genotyping errors, and performing single-QTL genome scans and two- QTL, two-dimensional genome scans, by interval mapping (with the EM algorithm), Haley-Knott regression, and multiple imputation. All of this may be done in the presence of covariates (such as sex, age or treatment).One may also fit higher-order QTL models by multiple imputation and Haley-Knott regression (Broman *et al.,* 2003).

2.18. MAPPING QTLS ASSOCIATED WITH MINERALS (FE AND ZN) CONCENTRATIONS

Quantitative trait locus (QTL) analysis provides a powerful approach to understand the genetic factors and to unravel the genes underlying the natural variation for Fe and Zn concentrations (Ghandilyan *et al*., 2006). The identification and tagging of major QTLs for grain micronutrients with large effects would be helpful in the selection of the QTLs in early generations with MAS technique and will greatly accelerate wheat cultivar development for improving mineral concentration in grain (Ortiz-Monasterio *et al*., 2007). Using various populations, many QTLs for micronutrient concentration in grain/leaf have been mapped in recent years (Table 2.2). Brief results of various QTL studies in major staple crops are described in brief in the later sections.

Table: 2.2 QTL/s associated with concentrations of essential mineral elements in various crop species

Sr. No.	Crop Species	Tissue	Elements	Mapping Populations	Number of Lines	Number of Markers	Number of QTLs	References
1.	Rice (*Oryza sativa*)	Grain	Fe, Zn	DH	129	582	3Fe, 2Zn	Stangoulis *et al.*, (2007)
			Fe, Zn	RIL	241	221	3Zn, 2Fe	Lu *et al.*, (2008)
			Fe, Zn	BIL	85	179	2Fe, 3Zn	Garcia-Oliveira *et al.*, (2009)
			Fe, Zn	RIL	79	164	4Fe, 4Zn	Norton *et al.*, (2010)
			Zn	DH	127	243	2	Zhang *et al.*, (2011)
			Zn	DH	93	254	6	Biradar *et al.*, (2007)
			Zn, Fe	BC1F	115	93	2Zn, 3Fe	Susanto (2009)
			Fe, Zn	RIL	168	110	7Fe, 6Zn	Anuradha *et al.*, (2012)
2.	Wheat (*Triticum spp.*)	Grain	Zn	DH	119	39	11	Shi *et al.*, (2008)
			Fe, Zn	DH	90	470	4Zn, 1Fe	Genc *et al.*, (2009)
			Fe, Zn	RIL	152	690	6Zn, 11Fe	Peleg *et al.*, (2009)
			Fe, Zn	RIL	93	169	3Fe, 2Zn	Tiwari *et al.*, (2009)
			Fe, Zn, Mn	RIL	168	477	1Fe, 2Zn, 2Mn	Ozkan *et al.*, (2007)
3.	Barley (*Hordeum vulgare*)	Grain	Zn	DH	150	417	5	Lonergan *et al.*, (2009)
			Zn	DH	150	302	2	Sadeghzadeh *et al.*, (2010)
4.	Maize (*Zea mays*)	Grain	Fe	RIL	232	1338	3	Lungaho *et al.*, (2011)
			Fe, Zn	F4	294	121	3Fe, 1Zn	Simic *et al.*, (2012)
			Fe, Zn	RIL	113	47	7Fe, 11Zn	Beebe *et al.*, (2000)
			Fe, Zn	F2:3	218	240	4Zn, 1Fe	Jin *et al.*, (2013)

Sr. No.	Crop Species	Tissue	Elements	Mapping Populations	Number of Lines	Number of Markers	Number of QTLs	References
5.	Bean (*Phaseolus vulgaris*)	Seed	Zn	RIL	73	5	Two markers associated with Zn	Gelin *et al.*, (2007)
			Fe, Zn	RIL	87	236	13Fe, 13Zn	Blair *et al.*, (2009)
			Fe, Zn	RIL	77		6Fe, 4Zn	Cichy *et al.*, (2009)
			Fe, Zn	RIL	110	114	8Fe, 9Zn	Blair *et al.*, (2010)
			Fe, Zn	RIL	100	122	6Fe, 3Zn	Blair *et al.*, (2011)
			Fe, Zn	F2:3	120	57	1Zn, 2Fe	Guzman-Maldonado *et al.*, (2003)
6.	Soybean (*Glycine max*)	Seed	Ca	F2:3	178	148	4	Zhang *et al.*, (2009)
7.	Oilseed Rape (*Brassica napus*)	Seed	Fe, Zn	RIL	124	553	10Zn, 9Fe	Ding *et al.*, (2010)
8.	B. *oleracea*	Leaf	Ca, Mg	DH	90	547	11Mg, 17Ca	Broadley *et al.*, (2008)
9.	B. *rapa*	Leaf	Fe, Zn	DH	183	287	2Zn, 1Fe	Wu *et al.*, (2008)
10.	Pearl millet (*Pennisetum glaucum*)	Grain	Fe, Zn	RIL	106	305	1Fe, 1Zn	Kumar (2011)
			Fe, Zn	RIL	317	234	11Fe, 8Zn	Kumar (2011)

DH Double haploid, RIL Recombinant inbred line, BIL Backcross inbred lines, F2:3 F2 derived F3, BC1 First Backcross generation

2.18.1 Rice

In a rice double haploid (DH) population, two QTLs for phytate concentration (explaining 24 % and 15 % of total phenotypic variation), three QTLs for Fe concentration (explaining 17 %, 18 %, and 14 % of total phenotypic variation), and two QTLs for Zn concentration (explaining 15 % and 13 % of total phenotypic variation) were identified by Stangoulis *et al*., (2007) and reported that Zn concentration QTL co-localized with the Fe QTL. Garcia-Oliveira *et al*., (2009) reported 31 putative QTLs for eight mineral elements (Fe, Zn, Mn, Cu, Ca, Mg, P, and K) in seeds of introgression lines (IL) by single-point analysis, out of which, 17 QTLs were observed during both years. QTLs associated with Zn and Si content in rice was identified by Biradar *et al*., (2007) in DH population. Based on the interval mapping results, one QTL was detected for Si. Similarly, a total of 6 QTLS were detected for Zn content using SMA explaining 1–10 % of total phenotypic variation. Lu *et al*., (2008) reported ten QTLs for Cu, Ca, Mn, Zn, and Fe in a RIL population in grains and reported three QTLs for Zn content. Among these QTLs, the major QTL accounted for 19 % of phenotypic variation, whereas two QTLs for Fe accounted for 37 % phenotypic variation. Gregorio *et al*., (2000) and Avendano (2000) also detected QTL for Fe and Zn, respectively, on same chromosomes. Using a mapping population consisting of 85 backcross inbred lines (BIL), two QTLs for increasing grain cadmium (Cd) concentration were detected by Ishikawa *et al*., (2010). A major effect QTL accounted for 35% of all phenotypic variation. A putative QTL for grain Fe concentration explained 15 % of the phenotypic variation, whereas no QTL for grain Zn concentration was found. Three QTLs for straw Fe concentration and two QTLs for straw Zn concentration were found. Grain concentration QTL was not genetically related to any QTL for other mineral concentration or those for agronomic trait, suggesting that QTL was specific for Cd.

2.18.2 Wheat

Peleg *et al*., (2009) identified 82 significant QTLs for nine grain mineral nutrient concentrations including four secondary mineral nutrients and proteins. GEI was exhibited by 38 QTLs. A total of six significant QTLs were associated with Zn explaining 1–13% of variance with three QTLs showing significant GEI, while a total 11 significant QTLs were associated with Fe, explaining 2–18% variance with GEI

for five QTLs, out of which three QTLs for Zn were in agreement with the results reported in previous studies (Ozkan *et al*., 2007; Shi *et al*., 2008; Distelfeld *et al*., 2007; Genc *et al*., 2009). Similarly, two out of 11 QTLs have been mapped (Ozkan *et al*., 2007; Distelfeld *et al*., 2007). In another study, Tiwari *et al*., (2009) detected Zn concentration QTL on same region as reported by Shi *et al*., (2008). The QTL for grain Fe and Zn mapped in the study conducted by Tiwari *et al*., (2009) explained 25–30 % of the total phenotypic variation with significant correlation between both elements.

2.18.3 Maize

Three modest QTLs for grain Fe concentration (FeGC) were detected by Lungaho *et al*., (2011), indicating that FeGC was controlled by many small QTLs. Ten QTLs for FeGB were identified 54% of the variance observed in samples from a single year/location. Three of the largest FeGB QTLs were isolated in sister derived lines, and their effect was observed in three subsequent seasons in New York. The results indicated that iron biofortification of maize grain is achievable using specialized phenotyping tools and conventional plant breeding techniques. The analysis of variance indicated that environment played a strong role in influencing grain Fe concentration. By using 294 F4 lines of a biparental population taken from field trials of over 3 years, Simic *et al*., (2009) revealed 32 significant QTLs (three for Fe and one for Zn). Significant additive effects with no significant dominant effects suggested that biofortification traits in maize were predicted by a simple additive model and mostly controlled by numerous small-effect QTLs.

2.18.4 Pearl Millet

Using 106 RILs (ICMB 841-P3 _ 863B-P2), two co-localized QTLs for Fe and Zn concentrations on LG 3 were identified in pearl millet by Kumar (2011). Fe and Zn QTLs explained 19% and 36 % of observed phenotypic variation, respectively. Likewise, Kumar (2011) also detected 19 putative QTL for grain Fe and Zn concentration in ICMS 8511B _ AIMP 92901-derived-08 RIL population (317 RILs) on the base of single environment data, of which 11 were for Fe (66 % of phenotypic variation) and eight were for Zn (60% of phenotypic variation). LG 1 harbored two co-localized main effect putative QTLs for Fe and Zn concentrations.

2.19. CORRELATION ANALYSIS FOR FE AND ZN, YIELD AND RELATED TRAITS

Correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. But measure of correlation does not consider dependence of one variable over the other. Direct contribution of each component to the yield and the indirect effects it has through its association with other components cannot be differentiated from mere correlation studies. But this can be studied using path-coefficient analysis. It was first developed and described by Wright (1921). Kabir (2001) reported a positive correlation between the iron and zinc content of milled rice, further higher content of iron and zinc was recorded in local, aromatic varieties than the high-yielding, non-aromatic varieties.

Gregorio (2002) in IRRI reported the presence of the correlation for Fe and Zn accumulation in the grains of rice among 1,138 genotypes studied. It was shown that the highest grain Fe concentrations (i.e., ranging from ~18 – 22 µg g-1) were found in several aromatic rice varieties, such as Jalmagna, Zuchem and Xua Bue Nuo. These same aromatic lines also contained the highest grain/Zn concentrations (ranging from ~24 – 35 µg g-1). The positive correlation between Zn concentration and Zn content reported in wheat (Cakmak *et al*., 2000) explained that seed size did not affect micronutrients. Zeng *et al*., (2005) in 653 accessions from Yunnan rice found that there is no correlation between microelements and grain traits (grain length and breadth) except Fe, Zn with rice thickness. Shi *et al*. (2008) reported a negative correlation between grain yield and Zn concentration and content in doubled haploid (DH) population developed from winter wheat varieties Hanxuan10 and Lumai.

Kumar *et al*., (2009) showed significant positive correlation between grain Fe and Zn contents for sorghum lines indicating that either genetic factors for Fe and Zn contents are linked, or physiological mechanisms were interconnected for Fe and Zn uptake/translocation in the grains. On the other hand, they found that grain Fe and Zn contents showed significant negative correlation with grain yield but genetic enhancement for grain Fe and Zn contents does not have yield penalty. The high genetic correlations between grain characteristics and some mineral element contents can be used to conduct indirect selection of a grain characteristic for mineral element content in a breeding program.

The correlation coefficients between 100 grain weight (GW) and the grain Fe and Zn concentrations were found to be non-significant with r varying between 0.0 and 0.15 indicating that no relation between 100 grain weight and Fe and Zn concentrations in the grains of the RIL population of wild wheat (Tiwari *et al*., 2009). Morete *et al*. (2011) in rice genotypes also showed that rice grain zinc content and grain weight were inversely related indicating that there is a yield dilution effect.

Akinwale *et al*. (2011) observed significantly positive correlation of grain yield with the number of tillers per plant (r = 0.58), panicle weight (r = 0.60) and number of grains per panicle (r = 0.52) in rice. Therefore, the results suggest that these traits can be used for grain yield selection. Sadeghzadeh *et al*. (2010) found that barely (*Hordeum vulgare* L.) Zn-efficient genotypes can produce greater yield and accumulate more Zn in seed under Zn deficiency than standard (Zn-inefficient) genotypes in a study done on a population of 150 DH lines derived from a cross between Clipper (low-Zn-accumulator) and Sahara 3771 (high-Zn accumulator).

Anandan *et al*. (2011) reported a positive correlation of Fe, Zn, Mn, and Cu contents in rice grain but they showed a negative correlation between grain yield and mineral contents. They also observed a positive correlation between mineral element contents and cooking quality traits like, kernel length after cooking and kernel linear elongation ratio indicating a role of micronutrients in cooking quality traits. Nagesh *et al.* (2012) observed positive correlation between iron and zinc content but there is no correlation between grain iron and zinc content with grain yield in rice hybrids. It was also showed that positive correlation of grain yield with number of productive tiller per plant, test weight and number of grains per panicle. Path analysis also revealed the highest direct effect of test weight on grain yield followed by number of productive tillers per plant and iron content.

Significant positive correlations were observed for days to 50% flowering, days to maturity and plant height with yield. Path analysis revealed high and positive direct effects of days to maturity and plant height (Choudhury and Das, 1998). F2 generations of 21 crosses were evaluated for the genetic parameters as well as association of certain yield components in rice by Raju *et al.,* (2004). Among the yield components, productive tillers per plant and 100 grain weight had significant correlation as well as direct positive effects on grain yield per plant. Significant

positive association of plants height with grain yield per plant was reported by Rasheed *et al*., (2002); Rajeswari and Nadarajan (2004) and Khan *et al*., (2009).

Path analysis by Panwar *et al.* (2007) showed that, grain yield per panicle, days to fifty per cent flowering, number of productive tillers per plant had high positive direct effect on grain yield. Significant positive association of number of tillers per plant with number of productive tillers per plant was reported by Laxuman *et al*., (2011) and Nagesh *et al.,* (2012). According to Nagesh *et al.,* (2012) grain length, number of grains per panicle, test-weight had highest positive direct effect towards grain yield while L : B ratio, grain breadth and days to 50 per cent flowering had highest negative direct effect for grain yield. Other traits like days to maturity, plant height, panicle length, tillers per plant and grain zinc content had moderate to low direct effects on grain yield. Among indirect effects, grain breadth had highest indirect effect *via* length to breadth ratio.

In a study conducted by Bekele *et al*., (2013), 176 RILs of Azucena X Moromutant were used to evaluate genetic variability parameters, correlation that exist for grain Zn concentration and yield related traits in rice during wet seasons of 2010 and 2011. The study revealed significant genetic variability for all the traits. Grain yield per plant and grain zinc concentration showed higher phenotypic and genotypic co-efficient of variation. Significant positive correlation was observed for grain yield per plant with number of productive tillers per plant (r = 0.5) and number of tillers per plant (r = 0.4). Grain zinc concentration showed negative correlation with grain yield per plant (r = - 0.27). Grain zinc concentration showed negative direct effect on grain yield per plant (-0.186).

Garcia-Oliveira *et al*., (2009) reported that micro elements exhibited a weak correlation with each other. Fe content showed very weak correlation from negative Cu to positive Zn and Mn, respectively, whereas Zn showed a fragile correlation in a positive direction with Cu and Mn. Nagesh *et al.,* (2012) found highly significant positive correlation for grain yield per plant with number of productive tillers per plant (0.660 Genotypic (G), 0.653 Phenotypic (P)) followed by tillers per plant (0.566G, 0.552P), test-weight (0.473G, 0.472P), and number of grains per panicle (0.355G, 0.356P). Grain zinc content had significant negative correlation with grain yield at genotypic level (-0.312) but non-significant at phenotypic level (-0.270). They

also observed a positive correlation (0.908G, 0.487P) between grain iron content and zinc content. Iron content had non-significant correlation with grain yield. As there is no correlation between grain mineral content with grain yield, they have concluded that there can be separate breeding producer to enhancement of grain mineral content and grain yield.

In a study conducted on accessions of sorghum germplasm for genetic variability and plant character association of grain Fe and Zn by Kumar *et al*., (2009) there was significant positive correlation between grain Fe and Zn contents (r=0.75). Both grain Fe (-0.36) and Zn (-0.46) contents showed significant negative correlation with grain yield but numerically low indicating that genetic enhancement for grain Fe and Zn contents does not have yield penalty. Bekele *et al*., (2013) identified that the grain zinc concentration showed significant positive correlation with 100 grain weight (0.268). Number of tillers per plant had significant positive correlation with number of productive tillers per plant (0.906). Plants height exhibited a significant positive correlation with grain yield per plant (0.379). Assessment of the relationship between grain zinc concentration and grain yield per plant using linear regression showed the absence of correlation between these traits.

Gande *et al*., (2013) reported that grain yield per plant showed highly significant positive correlation with number of tillers (0.818, 0.813), number of productive tillers (0.462, 0.548), biomass of the plant (0.327, 0.587) and plant height (0.288, 0.360). Grain zinc content (-0.379, -0.221) showed highly significant negative correlation with grain yield per plant in both the seasons sown. Path coefficient analysis showed high positive direct effect for number of productive tillers (0.748) in 2011, harvest index (0.714) in *kharif* 2012 respectively, on grain yield per plant. Highest negative direct effect of number of tillers (0.532) in *kharif* 2011, grain zinc content (-0.102) in *kharif* 2012, respectively on grain yield were observed. Grain zinc content showed negative effect (-0.320, -0.102) on grain yield in two seasons consistently. Path coefficient analysis reveals direct and indirect effect on grain yield by all other traits. These results showed that grain zinc content has no contribution for grain yield indicating that grain zinc content could not be used as criteria for enhancement of grain yield.

III. MATERIALS AND METHODS

The study was conducted at the Plant Biotechnology Laboratory, Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand. The objectives of the investigation were to construct linkage map and find QTLs governing the iron and zinc concentrations in rice through mapping populations. The materials and methods adopted during the course of study, to fulfill these objectives, are described below:

3.1. SEED MATERIAL

Three mapping populations were used to construct the rice linkage map and detect QTLs for iron and zinc. The first population was comprised of 300 F7 Recombinant Inbred Lines (RILs) from the cross (GR-11 × Pankhali-203), while the second population was based on cross (GR-11 × Krishna Kamod), and consisted of F7 250 RILs, while the third population comprised 300 F7 RILs from the cross (GR-11 × Gurjari). All three populations were segregating for grain iron and zinc concentrations ([Fe] and [Zn], respectively), along with many other traits. The RILs and parental lines of both populations were used for phenotyping and genotyping studies. The characteristics of the parental lines are given in Table 3.1.

3.2. SALIENT FEATURES OF PARENTAL LINES OF THE MAPPING POPULATION

3.2.1. Pankhali-203

Pankhali-203 was derived from selection of the local cultivar Pankhali. It was released in 1955 from Main Rice Research Station, Nawagam, Gujarat. Pankhali-203 is a late maturing variety; tall plant type, golden yellow husk with purple apiculous, highly susceptible to blast and transplanted type of sowing practices are generally employed. The grain is white in color, medium in size and scented in nature.

3.2.2. Krishna Kamod

Krishna Kamod is a local selected variety from Gujarat. It is tall, late maturing variety with short bold, black colored kernel. It is a non-shattering variety showing moderate resistance to White Backed Plant Hopper. It is highly known for its grain quality of superior quality with strong aroma.

Table: 3.1 Characteristics of the parents used in crossing

Sr. No.	Characteristics	Pankhali-203	Krishna Kamod	Gurjari	GR-11
1.	Parentage	Selection from Pankhali	Local Variety	Asha/Kranti	Z-31/IR-8-246
2.	Year of release	1955	-	1997	1977
3.	Plant Height (cm)	140-150	160-180	110-115	100-110
4.	Days to 50% maturity	105-110	120-125	90-95	90-95
5.	Duration (Days)	135-140	145-150	120-125	125
6.	Plant Type	Tall	Tall	Erect	Tall
7.	Foliage	Pale Green	Green	Green with strong culm	Green
8.	Panicle	Well erected	Well erected and compact	Well erected and compact	Well erected
9.	Spikelet	Awnless	Awnless	Tip awned	Awnless
10.	Grain type	Fine	Short Bold, Black	Coarse	Fine
11.	1000-grain wt. (gms)	17.0-17.6	17.0-18.0	27.0-28.0	16.1
12.	Grain length (mm)	6.2-6.4	5.4	9.2-9.4	7.85-8.13
13.	Kernel type	White	Short bold	Long bold	White
14.	LB ratio	3.55-3.82	2.73-2.94	3.68-4.00	3.79-4.00
15.	Aroma	Mild	Strong	Absent	Absent
16.	Cooking	Good	Good	Good	Good
17.	Grain yield (kg/ha)	3400-3600	3500-4000	5000-8000	5000-6000

3.2.3. Gurjari

Gurjari derived from across between Asha and Kranti was identified in 1988 from a multi-resistant varietal trail of AICRIP at Nawagam location. It was tested in different trails of AICRIP foe a period of three years. Due its bold grain size, high yield performance, earliness and multiple resistances, it was further evaluated in Large Scale Varietal Trial Early during 1993 to 1995 and in district trials during 1994 to 1996 at various locations in the state of Gujarat.

3.2.4. GR-11

GR-11 was derived by a cross of Zinnia-31 and IR-8-246. It's a dwarf plant type with early maturity. Rice grain is white in color with medium sized grain. GR-11 is used as a female parent for all the three populations since it has all the desirable characteristics and having higher adaptability.

3.3. DEVELOPMENT OF MAPPING POPULATION

The mapping populations were developed at Main Rice Research Station, Nawagam, Gujarat. A single F1 plant produced after the initial plant × plant cross of GR-11 × Pankhali-203 was selfed to produce F2 seeds, and the progeny were then advanced to Single Seed Decent method (SSD) till the F7 generations, after which they have been maintained as self-bulks. Similarly, GR-11 × Krishna Kamod and GR-11 × Gurjari based populations were advanced to F7 generations to generate RILs. During generation advance hand sowing was done for each entry. All recommended agronomic practices and measures to control disease/pests were followed to have proper growth of the crop.

Seeds of the RILs were sown in seedbed and 30 day old seedlings were transplanted. Crop was raised under irrigated condition and standard agronomic practices were followed. Soil was fertilized with 5 t/ha of FYM and NPK in the ratio of 100: 25: 00 kg/ha. A physico-chemical property of soil in the experimental site of Nawagam was available as 7.16 ppm for Fe concentration, 6 ppm for Zn concentration (using DTPA, diethylenetriamine penta acetic acid extractable methods). Plants were tagged and leaf samples were collected for DNA isolation. Phenotypic data was recorded on yield and its component traits on these tagged

plants. Harvested seed was bulked for each line separately and used for Fe and Zn analysis.

3.4. EXPERIMENTAL TRIALS FOR PHENOTYPING

3.4.1. Phenotypic Data

The phenotyping of Fe and Zn was carried out at 'The Micronutrient Project', Anand Agriculture University, Anand.

3.4.1.1. Sample Preparation and Mineral Element Extraction from Grains

A representative sample from well-dried panicles of each plot was dehusked manually and cleaned to remove glumes and other possible contaminants, especially sand particles. The cleaned grain samples were placed in individual labeled paper bags and dried in an oven at 45^0C overnight. A 15 g sample was powered, stored in labeled paper bags and kept in an oven at 45^0C overnight. Fe and Zn composition of different RIL populations were estimated using Atomic Absorption Spectroscopy (AAS). A minimum of two replications from each of the genotypes were analyzed for the two micronutrients. The samples were estimated by its comparison with blank sample. Concentrations were expressed in unit parts per molar (ppm). The procedure for iron and zinc extraction from seed, performed by Di-acid method as described by Lindsay and Norvell, 1978 (Table 3.2.).

Table : 3.2 Preparation of reagents for iron and zinc extraction from seeds

Sr. No	Reagents	Method of preparation
1	Di-acid mixture (3:1) (1 Litre).	Taken 900 ml of Conc. Nitric Acid and mixed with 100 ml of Conc. Perchloric Acid. The solution was stored in a bottle at room temperature.

3.4.1.1.1. Procedure for extract preparation

1. Seeds were harvested from plants grown to full maturity and dehusked gently and powdered.

2. 1 g of powdered seed sample was taken in 250 ml conical flask to which 10 ml of Nitric acid was added.

3. The samples were kept for incubation overnight. These samples were allowed on hot plate for digestion.

4. Twenty ml of Di-acid Mixture (Nitric: Perchloric acid) at the ratio of 3:1 was added. The samples were digested on hot plate until it turned colorless and digested till 2-3 ml of sample remained.

5. Filter the sample and make up the volume up to 50 ml by double distilled water. These samples were used for iron and zinc estimation.

3.4.1.1.2. Iron and Zinc Measurement

An atomic Absorption Spectrophotometer (AAS) was used to measure the mineral element contents of each ground grain samples. Each sample was measured twice and the means of these replicate observations were used to samples mineral element content. For error control three blanks and two standard reference samples were taken into consideration. The mean observation for each sample was converted into the amount of mineral elements (ppm) in each sample using Microsoft Excel.

3.4.1.1.3. Preparation of Standard Solution

From the stock solutions of 10 mgL^{-1} of Fe and Zn, aliquots of 1, 2, 3, 4 and 5 ml were pipetted out into well labeled 25 ml volumetric flasks. For each flask the volume was made upto 25 ml with 0.5% H_2SO_4. This provided a set of standards for instrument calibration for both mineral elements.

3.4.1.1.4. Instrumental Parameters for Fe and Zn

Atomic Absorption Spectrophotometer working conditions were fixed as:

Element measured	Fe	Zn
Lamp	Current	Current
Fuel	Acetylene/Air	Acetylene/Air
Wavelength	248.3 nm	213.9 nm

3.4.1.1.5. Analysis

The values of sample obtained were subtracted from the blank value. Formula used for estimation of Zn and Fe content was

$$\text{ppm of mineral content} = (S-B) \times 50$$

Where, S= sample reading.

B=blank reading.

The observations for Fe and Zn content were taken in three replications for treatment and control, which were analyzed using completely randomized design at 5% level of significance (C.D.) (Compton, 1994).

3.4.1.2. Agro-Morphological Traits

The data on ten quantitative traits (*i.e.* 50% flowering time, Plant height, panicle length, number of productive tillers per plant, number of filled panicles per grain, 1000 grain weight, grain yield per plant, Grain length, Grain breadth and grain length breadth ratio) were recorded for each population in field experiment.

3.4.1.2.1. Days to 50% Flowering

The dates of flowering in 50% plants for each plot were noted and the number of days worked out from date of sowing in nursery to that of 50% flowering and recorded as number of days to 50% flowering

3.4.1.2.2. Plant height (cm)

Height of the plants in each plot was measured in centimeter from soil level to apical tip of the top panicle at the time of maturity.

3.4.1.2.3. Panicle length (cm)

The length of the main panicle of plants from the ciliate ring to the tip of the panicle in centimeter was measured at the time of harvesting.

3.4.1.2.4. Number of productive tillers per plant

The tillers producing panicle with grains were considered as productive tillers. Number of productive tillers of the selected plants in each plot was counted at the time of harvesting and means were worked out.

3.4.1.2.5. Number of filled grains per panicle

The filled grains of main panicle per plant were counted for plants as filled grains per panicle and the average was computed.

3.4.1.2.6. 1000 grain weight (Test Weight)

Thousand grains were randomly collected and weighed in grams upto two decimals.

3.4.1.2.7. Grain yield per plant (g)

All the grains were threshed, cleaned and weighed to workout grain yield per plant.

3.4.1.2.8. Grain length (mm)

From each sample ten healthy grains were selected and length was measured by Dial thickness guage meter in mm as distance from the base of lower most sterile lemma to the tip of fertile lemma or palea, whichever was long. In case of awned varities grain length was measured to point comparable to tip of apiculus

3.4.1.2.9. Grain breadth (mm)

Grain breadth was measured through Dial thickness gauge meter as the distance across the fertile lemma and palea at the widest point.

3.4.1.2.10. Grain Length Breadth ratio

The length breadth ratio was computed from length and breadth dimensions.

3.5. STATISTICAL ANALYSIS

3.5.1. Phenotypic Data Analysis

3.5.1.1. Correlation Coefficient

Standard Excel program of Microsoft Office was used to calculate mean, range and standard deviation of phenotypic data and Fe and Zn concentration collected from parents and RILs in all the three crosses. Frequency distribution curves were plotted for the phenotypic data and Fe and Zn concentration in rice. Skewness was calculated. Test of significance for all traits were analyzed. Pearson's Correlation analysis between character pairs were computed at $p < 0.05$, $p < 0.01$, $p < 0.005$ and $p < 0.001$ in Microsoft Excel using trait averages for yield and related traits and also for iron and zinc concentration in the mapping population among all the three crosses. Significance of correlation coefficients (r) at $P = 0.05$ or 0.01 or 0.005 or 0.001 is indicated by * or ** or ***, or **** respectively.

$$r_{xy} = \frac{Cov(x.y)}{\sqrt{V(x).V(y)}}$$

where, r_{xy} = Correlation coefficient between x and y,

$V(x)$ = variance of x,

$V(y)$ = variance of y.

3.6. GENOTYPING OF MAPPING POPULATION
3.6.1 Genomic DNA Extraction from Parents and RILs
3.6.1.1. Materials
3.6.1.1.1. Chemicals, Buffers and Reagents

All the chemicals and fine reagents used in the experiments were of molecular and analytical grade obtained from standard manufacturers', which are Applied Biosystems, Sigma, Amresco, Fermentas, Himidia, Sigma-Aldrich, Merck and Qualigenes (Table 3.3)

3.6.1.1.2. Glass-wares and Plastic-wares

Properly cleaned and neutral glassware (Borosil grade) were used. The glass-wares were sterilized in oven before use (Table 3.4). Plastic-wares used for the

experiment were compatible with molecular biology work (Table 3.5). All the plastic-wares like micropipette tips, PCR tubes, centrifuge tubes and eppendorf tubes were autoclaved before use (Table 3.6).

Table: 3.3 Chemicals and reagents

Chemicals and reagents	Manufacturer
Ethidium bromide	Himedia, India
Tris buffer	Himedia, India
CTAB buffer	Himedia, India
EDTA	Himedia, India
Chloroform	Himedia, India
Primers	Eurofins Scientific, USA

Table: 3.4 Glass-ware

Glass ware	Manufacturer
Reagent Bottles 250 ml, 500 ml, 1 litre	Borosil, india
Volumetric flask 500 ml	Borosil, india

Table: 3.5 Plastic-ware

Plastic ware	Manufacturer
Tips 10 , 100, 1000	Eppendorf, Germany
Research Pipette	Eppendorf, Germany
MicroAmp® Optical 96-Well Reaction Plate	Thermo fisher Scientific, USA
MicroAmp® Optical Adhesive Film	Thermo fisher Scientific, USA
0.2 ml PCR vials	Thermo fisher Scientific, USA
Micro centrifuge tubes 2 ml, 1.5ml, 0.5 ml	Eppendorf, Germany

Table: 3.6 Instruments

Instruments	Manufacturer
Applied Biosystem veriti thermal cycler	Thermo fisher Scientific, USA
Refrigerated Centrifuge	Eppendor, Germany
-80 New Brunswick Deep freezer	Eppendor, Germay
-20 deep freezer	Siemens, Germany
Gel electrophoresis unit	Hoefer, New England **&** Biorad, USA
Laminar flow	India
Distilled water unit	Millipore, USA
Water bath	India
Gel documentation unit	Syngene, USA

3.6.1.2. Preparation of Stock Solutions for Reagent and Buffer for DNA Extraction

The reagents and buffers for DNA isolation were prepared as per Sambrook *et al.,* (1989). The composition and procedure for preparation of various stock solutions and buffers are given in the Table 3.7 and Table 3.8.

3.6.1.3. Protocol for genomic DNA extraction

Total DNA was extracted from the leaves by Cetyltrimethyl ammonium bromide (CTAB) method (Zidani *et al*., 2005) with some minor modifications.

1. The leaves of rice seedlings (300 mg) from each genotype were powdered in liquid nitrogen using a pestle and mortar.

2. Pre warmed (65°C) CTAB buffer (1 ml) containing 1% (v/v - mercaptoethanol (added freshly) and 10 µl Proteinase K (Fermentas AG) was added to each micro centrifuge tube (2 ml) and vortexed to mix. The tubes were incubated at 60°C for 1 hr with frequent swirling.

3. An equal volume of chloroform: isoamylalcohol (24:1) was added and centrifuged at 10,000 rpm and 4°C for 15 min to separate the phases.

4. The supernatant was carefully decanted and transferred to a new tube. The above steps, beginning with the addition of chloroform: isoamylalcohol (24:1) and ending with decanting of supernatant, were repeated twice.

5. The supernatant was precipitated with 2/3 volume of absolute alcohol. The precipitated nucleic acids were collected and washed twice with the 80% ethanol.

Table: 3.7 Preparation of stock solutions for DNA extraction

Sr. No	Solution	Method of preparation
1	1M TrisHCl (pH 8.0)	Dissolved 12.11 g Tris base (Merck) in 80 ml distilled water and pH was adjusted to 8.0 by adding concentrated HCl. The volume was adjusted to 100 ml. Thereafter it was dispensed to reagent bottle and sterilized by autoclaving.
2	0.5M EDTA (pH 8.0)	7.306 g of EDTA di Sodium salt (SRL) was dissolved in 80 ml distilled water and pH was adjusted to 8.0 by adding NaOH pellets. Volume was adjusted to 100 ml. Thereafter it was dispensed to reagent bottle and sterilized by autoclaving.
3	5M NaCl	Weighed 29.92 g NaCl (Lobachem) and added to 50 ml of distilled water. When the salts get completely dissolved, the final volume was adjusted to 100 ml. Dispensed in to reagent bottle and autoclaved.
4	70% Ethanol, 500 ml	360 ml of ethanol was mixed with 140 ml of distilled water. Dispensed to reagent bottle and store at 4^0C.
5	Chloroform: Isoamyl alcohol (24:1), 500 ml	480 ml of chloroform was measured and added to 20 ml of isoamyl alcohol. Mixed well and stored into reagent bottle at room temperature.
6	Ethidium Bromide (10 mgl^{-1}), 10 ml	Added 0.1 g Ethidium Bromide to 10 ml of distilled water. Kept on magnetic stirrer to ensure that the dye has dissolved completely. Dispensed to amber colored reagent bottle and stored at 4^0C.

Table: 3.8 Preparation of buffers for DNA extraction

Sr. No	Buffer	Method of preparation
1	CTAB Extraction buffer (4%), 10 ml	Measured 1 ml of 1M TrisHCl (pH 8.0), 2.8 ml of 5M NaCl, and 1 ml of 0.5M EDTA (pH 8.0). Mixed with about 4 ml of hot distilled water, added 0.4 g (W/V) CTAB (AMRESCO) and 0.1 g (W/V) PVP (AMRESCO) to it. Dispensed to reagent bottle. Just before use, added 100 µl (1%) -mercaptoethanol.
2	TE buffer (0.1mM), 100 ml 10mM TrisHCl (pH 8.0) 0.1mM EDTA(pH 8.0)	Taken 1 ml of TrisHCl (1M), 200 µl of EDTA (0.5M) and mixed with 99 ml of sterile distilled water thoroughly in a reagent bottle, autoclaved and stored at room temperature.
3	TBE buffer 5X (1 liter)	Weighed 54 g of Tris base, 27.5g of boric acid, 20 ml of 0.5M EDTA (pH 8.0) to around 450 ml distilled water. Dissolved the salt and the volume was adjusted to 1 liter.

6.   The pellets were air dried and resuspended in 100 µl of 1X TE buffer (10 mM Tris-HCl pH 8.0, 0.1mM EDTA).

7.   5 µl of DNase free RNase A (Fermentas AG) was added to the dissolved DNA and incubated in a water bath at 37^0C for 1 hour followed by 60 ^{0}C for 10 minutes for enzyme inactivation.

8.   The samples were stored at -20 ^{0}C.

3.6.2.   Qualitative and Quantitative Assessment of DNA

In order to perform PCR based analysis, the DNA has to be quantified. Spectrophotometry was performed to determine DNA concentration at absorbance ratio 260/280 nm and the data were analyzed using Nanodrop N.D.1000 (Software V.3.3.0).

To check the DNA quality of isolated genomic DNA, electrophoresis was done using 0.8% agarose gel. In gel electrophoresis, good quality of DNA showed sharp compact single band, whereas, poor quality of DNA showed smear.

Dilutions of 50 ng/µl working solutions were prepared from stock solutions.

3.6.3. Agarose Gel Electrophoresis

Agarose is the polymer of galactose and it is used for separation of nucleic acid. DNA applied to an agarose gel, when exposed to an electrical field, migrates towards the anode, because of negative charge on DNA. The smaller molecules run faster than larger one through the gel matrix (Table 3.9).

Table: 3.9 Preparation of buffers and solutions for agarose gel electrophoresis

Ethidium bromide (10 mg ml^{-1})	1 g of ethidium bromide was added to 100 ml of double distilled water and was dissolved properly. The container was wrapped in aluminum foil or the solution was transferred to a dark bottle and stored at room temperature or 4^0C.
TBE buffer 5x (1 liter) pH 8.0	54.5 g of Tris base, 27.5 g of boric acid (biogene) were taken and 20 ml of 0.5 M EDTA (pH 8.0) was added. The final volume was adjusted to 1 liter by adding distilled water and the pH was adjusted to 8.0.
6X Gel loading dye	Ready to use from Bangalore Genei

3.6.3.1. Gel Preparation and Gel Running

Agarose powder (0.8 g) was dissolved in 1X TBE buffer (100 ml) by slowly boiling in a microwave oven. Agarose was allowed to cool down to 60°C (just cool enough to hold). 4 µl of Ethidium bromide (EtBr) was added to the gel at a concentration of 1mg/ml and mixed well. The casting tray was prepared by joining the clamps and inserted combs. The agarose was poured into the prepared gel tray. Then allowed it to solidify (20-30 minutes) and carefully removed the clamps and combs.

The DNA samples (10 µl) were loaded after mixing with bromophenol blue dye into the wells. The electrophoresis was conducted at a constant voltage of 80 V. The separated bands were visualized under UV transilluminator and photographed using Alpha EaseFC4.0.0 Gel Documentation System (Alpha Innotech Corporation, USA).

3.6.4. Assessing Parental Polymorphism through Microsatellite Markers

The parental lines of three mapping populations were initially surveyed for their polymorphism with a total 600 (RM Series) SSR marker primer pairs. The polymorhic SSR markers were then used for genotyping of all the individuals in RIL population (Table 3.10).

3.6.4.1. Development of Gene Specific Primers (*insilico*) for Iron and Zinc Transporter

To facilitate the PCR analysis of all the investigated genes under same reaction conditions, primers were designed using Primer Express® Software v3.0. The sequences of candidate genes were obtained from locus ID number of genes, according to the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu.). Searches were made using the TBLASTN tool (Altschul *et al*., 1997) against the query database with search specifications for *Oryza sativa* [Organism]. The BLAST server used was that of the National Center for Biotechnology Information (http:// www. ncbi. nlm. nih.gov/BLAST/). As selection criteria of BLAST hits for genomic sequences, a cutoff e-value of e-10 was previously set. The sequences were than compared to the reference sequences for the search. The algorithm of choice for the multiple alignments of sequences was ClustalW. Markers were designed from the conserved fragments or regions of similarity with Primer Express® Softwarev3.0 with the following parameters: Tm around 60^0C and amplicon length of 50 to 150 bp, yielding primer sequences with a length of 19 to 23 nucleotides with an optimum at 20 nucleotides, and a GC content of 45 to 60%. The primers were also screened for hairpins, homo dimmers, hetro dimers and target specificity using Oligo IDT software.

3.6.4.2. PCR Assay for SSR

Since the pipetting of small volumes is difficult and often inaccurate, a master mix was prepared, wherein, all the reactions components (except template DNA) were

Table: 3.10 Rice Microsatellite markers polymorphic between the RIL populations of rice

S. N.	Name of Marker	Ch No	Forward Primer	Reverse Primer
1.	RM562	1	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
2.	RM580	1	GATGAACTCGAATTTGCATCC	CACTCCCATGTTTGGCTCC
3.	RM272	1	AATTGGTAGAGAGGGGAGAG	ACATGCCATTAGAGTCAGGC
4.	RM302	1	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
5.	RM472	1	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG
6.	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTGTCTCTCATTATG
7.	RM259	1	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
8.	RM315	1	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
9.	RM1096	1	GTAGATGGAGTCGTGGTTGATCG	TGACAGACGAGGAAACAGGAAG
10	RM4959	1	GTACAATATTTTTGGTAGGA	CAACCAGCTTAACTAATTAA
11	RM312	1	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
12	RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
13	RM24	1	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
14	RM243	1	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
15	RM488	1	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
16	RM490	1	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG

17	RM595	1	CAATGGCAGAGACCCAA AAG	CTGGCATGTAACGACAGT GG
18	RM7	1	TTCGCCATGAAGTCTCTC G	CCTCCCATCATTTCGTTGT T
19	RM595	1	CCTTGACCCTCCTCTTACT T	TCCTATCAAAATTTGGCA AC
20	RM34	1	GAAATGGCAATGTGTGCG	GCCGGAGAACCCTAGCTC
21	RM237	1	CAAATCCCGACTGCTGTC C	TGGGAAGAGAGCACTAC AGC
22	RM493	1	TAGCTCCAACAGGATCGA CC	GTACGTAAACGCGGAAG GTG
23	RM3825	1	AAAGCCCCCAAAAGCAGT AC	GTGAAACTCTGGGGTGTT CG
24	RM428	1	AACAGATGGCATCGTCTT CC	CGCTGCATCCACTACTGT TG
25	RM5	1	TGCAACTTCTAGCTGCTC GA	GCATCCGATCTTGATGGG
26	RM8085	1	TGCGTTTCGATTTCTTTTT A	GGAAAGTTGTGTTCTTTG GC
27	RM3642	1	TCGTTTCCGAGATGTCAC TG	AATTCTCGGGAGAGGGTA CG
28	0sNRAMP 6	1	GAAAAGGGGTGCAAATA CGA	GGGAAGAACCAACAATC ACC
29	0sYSL18	1	CCACGAACGCACCTAAAA A	TCTCCAGCCCGAATAAAA AC
30	RM110	2	TCGAAGCCATCCACCAAC GAAG	TCCGTACGCCGACGAGGT CGAG
31	RM2486	2	CGTCTTCTCTGCAACATT AC	CGAACGCGTTTAGACTAA TA
32	RM13530	2	CATCGGGTTTCTGTTCTTG ACACG	AACCCAAGGAATCGGAC ACAGC
33	RM263	2	CCCAGGCTAGCTCATGAA CC	GCTACGTTTGAGCTACCA CG
34	RM3666	2	TGATTTTCAGGGCTGTAG GG	AGTAAAATGCTCCCCATG GC
35	RM6942	2	ACTAGAAAAATGCCCGTG CG	TTTGAGACGATCGAACTC CC

36	RM6843	2	GACAAATTCAGCTGTTGACC	ATAAACCACAATGAGCAAGC
37	RM106	2	CGTCTTCATCATCGTCGCCCCG	GGCCCATCCCGTCGTGGATCTC
38	RM71	2	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG
39	RM2634	2	GATTGAAAATTAGAGTTTGCAC	TGCCGAGATTTAGTCAACTA
40	RM279	2	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG
41	RM1920	2	CAAACACAGTGTTGACAGAA	GCTATTGACTTATCCGTTCA
42	RM3874	2	TGGGTGATCTTAGTTTGGCC	AATGTGCCTGCACATGTCAC
43	RM475	2	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
44	RM7451	2	TAATACGAGCAGCGATCGTG	GCTAATTGCAGCTTGTGTCG
45	RM3515	2	GGAAAGAAGATATGCCATGC	AGAGAGAATCAGAAACACCAAC
46	RM6318	2	TGCTGCTTCTGTCCAGTGAG	GGATCATAACAAGTGCCTCG
47	RM250	2	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
48	OsYSL8	2	CTCAAGCTAGCCTTCCATCG	TGCTACACCAGCTGCTTCTC
49	OsNAAT1	2	TTATCCAAGGTGGCAGAGGT	TCATGGATTCCCTCCAAAAG
50	OsYSL14	2	CCGGTTAGTCGTGCCATC	ATCTGGAAATACATTTGGAGGAG
51	OsNRAMP4	2	ACCCCACGATCAGACAAAAG	AGCTTGATCTCCCCAAAACA
52	OsYSL2	2	TGGAGAGAGTTGTGGGTTTCT	TGAAGTGGTAAAGGCCATCC
53	OsYSL7	2	CGTAGTGGTTGATTGGGAAA	GATGGAGATGATCGACAGGA
54	OsYSL15	3	TTGTTGGTGGTGGATTGGT	TCCTTTGGGCTCTGCTTTT
55	OsIRTI1	3	GCAATTCGCTGCATTGTTAG	GAAGTACATCAGTCACGAA
56	RM426	3	ATGAGATGAGTTCAAGGCCC	AACTCTGTACCTCCATCGCC
57	RM36	3	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTACC
58	OsNRAMP8	3	GTTTGGGGATGACCATTTTG	GTGCCTTTGCTCCATTCTGT

59	OsNAS1	3	GCGGGTTCCTGTACCCGA TCGT	AGCTCCTTGTTGGCGGCA AACTC
60	RM565	3	AGTAACGAGCATAGCAG GCG	GCAAAGCCTTCAGGAATC AG
61	RM16	3	CGCTAGGGCAGCATCTAA A	AACACAGCAGGTACGCGC
62	OsNAS2	3	TGCATAGTAATCCTGGCT GTGT	TCAGCACCTTACTCGTCG TT
63	RM251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGA TC
64	RM489	3	ACTTGAGACGATCGGACA CC	TCACCCATGGATGTTGTC AG
65	RM231	3	CCAGATTATTTCCTGAGG TC	CACTTGCATAGTTCTGCA TTG
66	RM514	3	AGATTGATCTCCCATTCC CC	CACGAGCATATTACTAGT GG
67	RM517	3	GGCTTACTGGCTTCGATT TG	CGTCTCCTTTGGTTAGTGC C
68	RM218	3	TGGTCAAACCAAGGTCCT TC	GACATACATTCTACCCCC GG
69	RM1600	3	TCTGTACTGAAATGGTCT CCAAGC	GCTATTGGTTCACCAAGC AAGG
70	RM81	3	GAGTGCTTGTGCAAGATC CA	CTTCTTCACTCATGCAGTT C
71	RM85	3	CCAAAGATGAAACCTGGA TTG	GCACAAGGTGAGCAGTCC
72	RM168	4	TGCTGCTTGCCTGCTTCCT TT	GAAACGAATCAATCCACG GC
73	RM119	4	CATCCCCCTGCTGCTGCT GCTG	CGCCGGATGTGTGGGACT AGCG
74	RM335	4	GTACACACCCACATCGAG AAG	GCTCTATGCGAGTATCCA TGG
75	RM16656	4	AACAGCAACCTGACAGAAGAATG	TATGTGGCTTCTCGTTGAGTTGG
76	RM6909	4	AAGTACTCTCCCGTTTCA AA	CCTCCCATAAAAATCTTG TC
77	RM7585	4	CCTCCTCCCTCGACTACCT C	GGTGTGTCGGTGTGATAT GC
78	RM551	4	AGCCCAGACTAGCATGAT TG	GAAGGCGAGAAGGATCA CAG
79	RM324	4	CTGATTCCACACACTTGT GC	GATTCCACGTCAGGATCT TC
80	RM341	4	CAAGAAACCTCAATCCGA GC	CTCCTCCCGATCCCAATC

81	RM470	4	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG
82	RM423	4	AGCACCCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC
83	RM17483	4	TAGCTTCGGTTCTTGATCGTTGG	AAACAGATTGCTCACCACCTTGG
84	OsYSL6	4	TGTGCATGTACTTCAAGCCATC	AAGAACAAAGTTACTGCACTTTTG
85	OsFRO1	4	ACACTGTCATCACCGATGATCC	GTGAGGAACACCGCCCACATGAG
86	OsYSL5	4	GCATAATCGCTCCACTCACA	CCGCATGAAAACTCCAAAG
87	OsYSL10	4	TTTTTGGTGGGACGAAGG	GCTGGGGTTCTTGATGTTGT
88	RM518	5	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
89	RM516	5	GTTTCCTGCATGCTTGGAAC	ATGTGATTGTATCAGGCTCG
90	RM3322	5	CTTCTCCACCCATGCCAC	CCTGCAACGAACACCCAC
91	RM3437	5	AACCACCTAGGTTTCTCCCC	TAGCAACGAGGTTATTGGGC
92	OsYSL4	5	GCAGGGCAAGAATCAAAAAG	GAAAAGTGTGTGCGTGGAAA
93	RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
94.	OsZIP7	5	TGCACAACAACGCATACAGA	GTCTCACGCCCATGAAAAA
95	RM153	5	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG
96	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
97	RM169	5	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
98	RM592	5	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA
99	RM5140	5	GACGAGGTTGTTTATTAGTTG	CTTATTTTCACGTGTACGTT
100.	OsZIP5	5	CAGGAATGGCAGGTTTTTGT	CCAAGATGAAGGACAGTGGTAG
101.	OsZIP6	5	TGCCAGGTGCTGAATGATAG	TTGCCCTCCAACAAGACA

102	RM574	5	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC
103.	RM122	5	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGA
104.	RM249	5	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC
105.	RM18589	5	CACACTCATCGTAAGGCTGAAGTC	GCAACCACTCTCTCCTTCCTTCC
106.	RM3695	5	TCAGTCCACTGCTCACCCC	CCAGAGCGGTTTTGTCCTAC
107.	RM8616	5	TCTAGGCAGTTGGTGTAACTCAGTGG	AACTCAAGTCTCAAGCCATCTACAGG
108.	RM8039	6	CGTACGTACTTATATCTCAT	AAATCTAATGTATCTGAGGT
109.	RM3575	6	CCTGGAATGATGATGGAAGG	GTTTTGCTTCCTGGAAGTGC
110.	RM103	6	CTTCCAATTCAGGCCGGCTGGC	CGCCACAGCTGACCATGCATGC
111.	RM340	6	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
112.	RM19660	6	TTTGTCCCTGCCGTACTTGC	AGCCACGTTGGGTGAAATTAGC
113.	RM586	6	ACCTCGCGTTATTAGGTACCC	GAGATACGCCAACGAGATACC
114.	RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
115.	RM19	6	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
116.	RM247	6	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
117.	RM415	6	CTTCGATCCATCATCCATGG	ATTGCTGTACGCAGTTCGG
118.	RM528	6	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
119.	RM7488	6	ACCTCCATAAGGGACAAATG	GATTTAGGAGGGTTTTGAGG
120.	RM276	6	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
121.	RM225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
122.	RM439	6	TCATAACAGTCCACTCCCCC	TGGTACTCCATCATCCCATG

123.	RM541	6	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG
124.	RM19697	6	AACAACCTGAGAACACCTCTTGG	GGACAAACACATGGTGATCTGC
125.	RM314	6	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACGC
126.	RM217	6	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
127.	RM400	6	ACACCAGGCTACCCAAACTC	CGGAGAGATCTGACATGTGG
128.	RM3	7	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT
129.	RM235	7	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
130.	RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
131.	RM248	7	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
132.	0sNRAMP1	7	CGGTGTTGGCTGGTTTTTAT	CATTCTGCCAATCTGCCAAT
133.	RM501	7	GCCCAATTAATGTACAGGCG	ATATCGTTTAGCCGTGCTGC
134.	RM1135	7	AGCCAACCAAGCAAGATAGC	ACACACATGTAAGCCTCCCC
135.	RM180	7	CTACATCGGCTTAGGTGTAGCAACACG	ACTTGCTCTACTTGTGGTGAGGGACTG
136.	RM21975	7	GCCATGAGGTAGGAAATTCATCG	ACTAGTACACTGCAGATCACGTA
137.	0sNRAMP5	7	GGATGCACTCAAAGAAACGA	CAAGGCAGAATGCAAGAACA
138.	RM21976	7	CTTCCTCCTACCTTCCTCCATCC	GCACCATCACCTCCATCTCTAGC
139.	RM346	7	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGAC
140.	RM21596	8	TTCTGCTCCACGTGTTCTTG	TAACCCATAGTCCCGTACGC
141.	RM21599	8	TGTTAGGCTGGAGATAGATACGC	GTTCCTACCTGTAGGTTGATTCG
142.	RM72	8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
143.	RM337	8	GCATCGGTCCTAACTAAGGG	CGATAGATAGCTAGATGTGGCC

144.	RM325	8	GACGATGAATCAGGAGAACG	GGCATGCATCTGAGTAATGG
145.	RM264	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
146.	RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
147.	RM1235	8	AGCAGAGGAGGAGATGATGG	GGACCAAAACGAAGCTATCC
148.	RM38	8	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCCAC
149.	RM230	8	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG
150.	RM331	8	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG
151.	RM3231	8	AACACGAAGACCGGCCTC	CAGGTAGGAGCATGAGAGCC
152.	RM544	8	TGTGAGCCTGAGCAATAACG	GAAGCGTGTGATATCGCATG
153.	RM8271	8	TCTTGAGAAATCTGCCATTC	ACTGATGTGCATTTCGTC
154.	RM3214	8	GTGGGGAGCAAGACAGAATC	TCAGTAACCAACCAGCATGG
155.	RM3644	8	GAAGAGAGTGGGAGGATGGG	AATTTGTGTGCTCCTCCACC
156.	RM6999	8	TTATCTGGGATCCATCGAGC	GTGAATTTCCTTGGAGGGAC
157.	RM152	8	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
158.	RM547	8	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
159.	RM6027	8	AAGCTCAACAGCTACCTCGG	CCCGTACACCACCGGAAAC
160.	RM3395	8	ACCTCATGTCCAGGTGGAAG	AGATTAGTGCCATGGCAAGG
161.	RM3215	8	CGGCGTAGCTAAATTTGGAC	ATGGCGAGCAAGGAAGTAAG
162.	RM3481	8	CTCGTCGCGTTCGTCAAC	CATCTCATCACCTCACGTCG

163.	RM1111	8	CCTCCTGTCGGATCTGGT AG	CTTATCCACTTGCCCTCTC G
164.	RM483	8	CTTCCACCATAAAACCGG AG	ACACCGGTGATCTTGTAG CC
165.	RM447	8	CCCTTGTGCTGTCTCCTCT C	ACGGGCTTCTTCTCCTTCT C
166.	OsZIP4	9	CTCGGCGCGTCACAGAAT CCGGAA	ATACCTGCACGATGCAGC CACC
167.	RM22565	9	TCCACGCGTTGTCGTAGA AATTT	AGCCCGAGCACCATGAAA CACC
168.	RM242	9	GGCCAACGTGTGTATGTC TC	TATATGCCAAGACGGATG GG
169.	RM219	9	CGTCGGATGATGTAAAGC CT	CATATCGGCATTCGCCTG
170.	OsVITI	9	GTGCCACTCCTACCCTAC A	TAAACGGGCCCTTGACAT AG
171.	RM215	9	CAAAATGGAGCAGCAAG AGC	TGAGCACCTCCTTCTCTGT AG
172.	RM434	9	GCCTCATCCCTCTAACCC TC	CAAGAAAGATCAGTGCGT GG
173.	RM410	9	GCTCAACGTTTCGTTCCT G	GAAGATGCGTAAAGTGA ACGG
174.	RM257	10	CAGTTCCGAGCAAGAGTA CTC	GGATCGGACGTGGCATAT G
175.	RM24382	10	TTTACCCTTTGGTACGGT GTGG	GTCCTAATCATGTTCGAT GAGACG
176.	RM484	10	TCTCCCTCCTCACCATTGT C	TGCTGCCCTCTCTCTCTCT C
177.	OsFER2	10	CAGCCGTGTCTATCTCCA AA	AATGCCAAGCGAACATCC
178.	RM496	10	GACATGCGAACAACGAC ATC	GCTGCGGCGCTGTTATAC
179.	RM258	11	TGCTGTATGTAGCTCGCA CC	TGGCCTTTAAAGCTGTCG C
180.	RM304	11	TCAAACCGGCACATATAA GAC	GATAGGGAGCTGAAGGA GATG
181.	RM216	11	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGC CA

182.	RM3605	11	GATGGACGACGAGTAGTGGG	CTCTCCATTTTTCCCCTTCC
183.	RM167	11	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
184.	RM209	11	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
185.	RM21	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
186.	RM287	11	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
187.	RM332	11	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC
188.	RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
189.	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
190.	RM254	11	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
191.	RM330	12	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC
192.	OsNAC5	12	CAGCAGCTGATGGTATTGTC	AGAGACCTGTTTGGCACGAA
193.	RM27172	12	GAAAGAAGGGATGTCTTGCATGA	GAACATCCTAACCACGTCGGAAGC
194.	RM17	12	TGCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
195.	RM463	12	TTCCCCTCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG
196.	RM512	12	CTGCCTTTCTTACCCCCTTC	AACCCCTCGCTGGATTCTAG
197.	RM7102	12	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG
198.	OsNRAMP 7	12	GCTGCCAAATCAGATCATCA	GCTTCAGGACGACACAGTCA

199.	RM28560	12	TTGTGCGTACTTGCTTGTC ATGG	CTGTTGTTGTTTGCCGCTA ATCC
200.	OsFER1	12	GGTTTCGTTTCTTCCATCC A	CGTGTAAATGCTCCCCAA A

combined in one tube and multiplying the volume for one reaction with total number of samples. Later, the appropriate amount of master mix was dispensed to each tube and template DNA was added separately in each tube. PCR reactions for SSR were carried out in a reaction volume of 25 µl (Table 3.11) in 200 µl thin walled PCR tubes. As per the above cocktail, millipore sterilized water was added first, followed by addition of DyNAzyme PCR Buffer (10X Tris with 15 mM $MgCl_2$), dNTPs, DyNAzyme II DNA polymerase and finally forward and reverse primers. The reagents were mixed gently by tapping against the tube and short spinning (~8,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler for cyclic amplification (Fig. 3.1).The genomic DNA was amplified using primers.

3.6.4.2.1. PCR Components

a) DyNAzyme PCR buffer (10 x) Finnzymes
b) Primers (10 pmoles μl^{-1}) (MWG)
c) dNTPs (2.5 mM) Bangalore Genei, India.
d) DyNAzyme II DNA polymerase (1U μl^{-1}) Finnzyme
e) Template DNA (50 ng μl^{-1})

Table: 3.11 PCR reaction mixture for Microsatellite (SSR) assay

Sr. No	PCR Components	Volume.
1	PCR buffer (10 x) with 15 mM $MgCl_2$	2.5 µl
2	Forward Primer (10 p moles μl^{-1})	0.5 µl
3	Reverse Primer (10 p moles μl^{-1})	0.5 µl
4	dNTPs mix (2.5 mM each)	0.5 µl
5	*Taq* DNA polymerase (1U μl^{-1})	0.5 µl
6	Template DNA (50 ng μl^{-1})	1.5 µl
7	Sterile distilled water	19.0 µl
Total volume		**25.0 ~l**

Fig. 3.1. Thermal cycling conditions for Microsatellite (SSR) assay (Joshi *et al*., 2006)
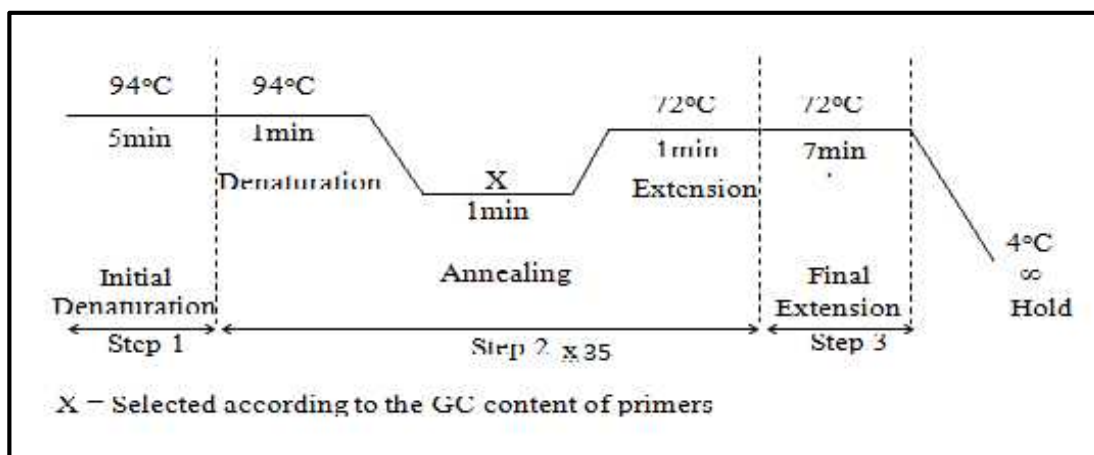
3.6.4.3. Gel electrophoresis

3.6.4.3.1. Reagents / Chemicals

(a) Metaphore Agarose (Lonza, Rocland, ME, USA)

(b) 100 bp DNA ladder (Fermentas)

(c) 50 bp DNA ladder (Fermentas)

Table: 3.12 Preparations of buffers and solutions for metaphore gel electrophoresis

Ethidium bromide (10mg ml^{-1})	1 g of ethidium bromide was added to 100 ml of double distilled water and was dissolved properly. The container was wrapped in aluminum foil or transferred the solution to a dark bottle and stored at room temperature or 4^{0}C.
TBE buffer 5x (1 liter) pH 8.0	54.5 g of tris base, 27.5 g of boric acid (biogene) were taken, 20 ml of 0.5 M EDTA (pH 8.0) was added. The final volume of 1 liter was adjusted by adding distilled water and the pH was adjusted to 8.0.
6X Gel loading dye	Ready to use from Bangalore Genei

3.6.4.3.2. Gel Preparation and Gel Running.

Metaphore agarose gel was prepared following the manufacture's protocol (Table 3.12). 3 g agarose powder was slowly sprinkled into the beaker containing chilled 100 ml 1X TBE buffer, while stirring on the magnetic stirrer. The beaker was heated on high power till the solution came to a boil. The casting tray was prepared by joining the clamps and inserted combs. The solution was cooled to 50-60°C prior to casting and 4 µl of ethidium bromide was added. Once the gel was cast molten agarose was allowed to cooled and solidify at room temperature. The gel was then placed at 4°C for 20 min. to obtain optimal resolution and gel banding characteristics. PCR amplified products (8 µl and 2 µl loading dye) were loaded in to the wells. To determine the length of the separated fragments in the gel, a molecular weight marker (50 bp and 100 bp) was loaded in, lane alongside the experimental material. The electrophoresis was conducted at a constant voltage of 80 V to separate the amplified bands. The separated bands were visualized under UV transilluminator and photographed using Alpha EaseFC4.0.0 Gel Documentation system (Alpha Innotech Corporation, USA).

3.6.5. Screening of the RIL Population with Polymorphic Markers

3.6.6. Data Scoring

The allelic data were scored as R/qtl readable data in order to facilitate mapping. Based on the amplicon sizes detected in the parents, data were scored for all optimized primers. Individuals having female parent allele homozygote were always scored as 'A' irrespective of the size of the amplicon. Similarly, those having the male parent allele homozygote were always scored as 'B' the lines having alleles present from both of the parents were scored as 'H' and missing data point were scored as'.'. In the present study three RIL mapping population were screened. Therefore, the allele scoring was as follows:

'A' Homozygous for allele of female parent

'B' Homozygous of allele of male parent

'H' Heterozygous (presence of both parental alleles)

'.' Missing Data (Failed amplification)

3.6.7. χ^2 Test

A standard χ^2 test was employed to test the segregation at each marker locus for deviation from the expected Mendelian Segregation. Karl person, an English Mathematician, applied statistics to biological problems of heredity and Evolution. He developed the chi-square test of statistical significance which is commonly used in Mendelian and population genetics. This is a test of statistical significance which is used to test the significance of difference between observed and expected frequencies or ratio, the general formula of χ^2 is as followed.

$$\chi^2 = \sum (O-E)^2 / E$$

Where, $\sum$ = Summation

O = Observed Frequencies

E = Expected Frequencies

3.6. Linkage Map Construction

Linkage map was developed for the molecular markers used in the analysis of rice RIL generation. Genotyping data was analyzed using the χ^2 test to assess the goodness-of-fit to the expected 1:1 Mendelian segregation ratio for each marker. In the preliminary analysis of the data markers were deleted that had high segregation distortion (p 0.01). The linkage map was developed using R software with map one programme. The distance between the markers have been demonstrated in the linkage map in cM unit. The data generated by map one programme were used for QTL mapping. The "sequence", "group" and "map" command were performed for linkage mapping and "build" command to place new markers from genotypic data set in the most appropriate position within the identified linkage group. Then software Mapchart was used to draw all linkage groups of the genetic linkage map. Linkage between the markers and the QTL was detected by a statistical test called the

Logarithm of Odds (LOD) score method. The LOD score was estimated according to the following formula:

$$\text{LOD score} = \log_{10} = \frac{\text{Probability of certain degree of linkage}}{\text{Probability of independent assortment}}$$

Generally there is a direct one to one transformation between LOD scores and Likelihood Ratio Statistic (LRS) scores. The conversion was calculated by the formula:

Likelihood Ratio Statistic (LRS) = 0.217 x LOD.

Each of the scored traits along with phenotypic means was subjected to QTL mapping. QTLs were detected by Single Marker Analysis (SMA), Interval Mapping (IM), (Lander and Botstein, 1989) and Composite Interval Mapping (CIM) procedure of Windows QTL Cartographer v.2.5 software.

Single marker analysis was done on RIL data by simple linear regression of phenotypic observations on marker genotypes using QTL cartographer 2.5 (Botstein *et al.* 1980). Single marker analysis was done using the software QTL Cartographer 2.5 version and Map-Disto version 1.7.7.0.1. The analysis followed simple linear regression model; $y = b0 + b1\ x + e$, (if b1 is significantly different from 0 then that marker is associated with the trait of interest) on excel work sheet which involved comparing traits for each marker where 'y' is the phenotypic value of a line, 'b0' is the population mean, 'b1' is the additive effect of the locus on the trait and 'e' is the residual error term. 'x' is directly related to the genotypic code at the locus being tested for the line considered, it is -1 (for female parent) or 1 (for donor or male parent). The proportion of the trait phenotypic variations explained by the QTL was calculated as R2 value. The percent of the total phenotypic variance for the trait that was accounted for by a marker was % R2 which was obtained by multiplying the R2 value provided in the ANOVA results by 100. Significance was tested at 5%, 1%, 0.1% and 0.01% levels which are indicated by *, **, *** and ****, respectively. Composite interval mapping (Zeng, 1994) was conducted using the default settings (e.g., Model 6, five cofactors selected automatically by forward regression with a 10 cM window) (http://statgen.ncsu.edu/qtlcart/cartographer.html).

3.7. Detection of QTLs

The objective of trait mapping is to identify simply inherited markers in close proximity to genetic factors affecting quantitative traits, which are commonly referred to as quantitative trait loci (QTLs). This localization relies on the process that creates a statistical association between markers and QTL alleles, and processes that selectively reduce that association as a function of marker distance from the QTL. Marker order and map distances inferred by R/qtl were used to find the candidate QTLs and to estimate their effects by composite interval mapping. These QTL analysis were performed using all the measured traits of the RILs among all the three populations along with their respective genotypic data sets. The method of composite interval mapping (CIM) with cofactors (Jansen and Stam, 1994) was used for detecting, mapping and characterizing QTLs. Once the model containing cofactor is built, the entire genome is rescanned using interval mapping. To declare a putative QTL as statistically significant, a minimum LOD score of 3 was fixed according to the Bonferroni correction.

Since the mapping populations comprise of the RILs, the additive model 'AA' was employed for analysis in which additive × additive epistatic effects were included. The point at which the LOD score has the maximum value in the interval was taken as the estimate of the QTL position. The proportion of the phenotypic variance explained by each QTL was determined by its partial coefficient of determination (R^2). Estimates of the additive effects of each detected putative QTLs were obtained by fitting a multiple linear regression model that simultaneously included all the detected putative QTLs for a trait in question. The additive effects were calculated as half the difference between genotypic values of the two parental homozygotes.

$$\text{Additive effect} = (\text{Parent2} - \text{Parent1})/2$$

3.9. QTL Nomenclature

Nomenclature for QTLs was as described by McCouch *et al.,* (1997), where a two or three letter abbreviation is followed by the number of the chromosome on which the QTL is found and a terminal suffix, separated by a period, providing a unique identifier to distinguish multiple QTL on a single chromosome.

IV. RESULTS AND DISCUSSION

The research experiment titled "Mapping QTLs for Iron and Zinc concentrations in rice (*Oryza sativa* L.)" was conducted at the Biotechnology laboratory of Department of Genetics and Plant Breeding, BACA, AAU, Anand during 2014-2017 to identify novel SSR markers associated to the QTLs related to grain micronutrient (Iron/Zinc) concentration in three RIL populations. Genotyping of the mapping populations and identification of QTLs for iron, zinc, yield and other yield related traits based on co-segregation with high grain Fe and Zn contents. The results are being presented and discussed here by comparing to recent literature pertaining to different experiments in the following sub headings:

4.1. Evaluate the promising rice genotypes for Fe and Zn

4.2. Phenotyping of the mapping populations

4.3. Molecular Analysis

4.4. Genotyping of the mapping populations

4.5. Constructing linkage maps

4.6. Mapping QTLs for iron, zinc, yield and yield related traits

4.1. EVALUATE THE PROMISING RICE GENOTYPES FOR FE AND ZN

Elemental analysis was performed on mature seeds (R9 stage) of seventy two rice genotypes by Atomic Absorption Spectroscopy (AAS). Both Fe and Zn were high in white rice and least in red rice. Fe concentration was found to be the highest in Pankhali-203 with 75 ppm, followed by Krishna Kamod and Sambha Masuri with 60 and 55 ppm respectively (Table 4.1). For zinc concentrations also it was observed the highest in Pankhali-203 (61 ppm), followed by Krishna Kamod (55 ppm) and Gurjari (50 ppm). The donor and the recipient parents for the development of the mapping populations was selected from the elemental analysis Fe and Zn concentrations in rice seed grains. GR-11 is the female parent because of its high adaptability and characters like dwarf plant type with early maturity and medium sized white colored grain. High iron and zinc containing genotypes Pankhali-203, Krishna Kamod and Gurjari were selected as the donor parents for developing the F7 RIL mapping populations.

Table: 4.1 Iron and Zinc concentration of 72 rice genotypes from seeds.

Sr.No.	Name of Genotypes	Fe content (ppm)	Zn content (ppm)
1	Madhukar	25.00	44.00
2	Jalmagna	35.00	48.00
3	PTB-8	24.50	23.50
4	ASD-9	32.50	25.00
5	PTB-49	29.00	36.50
6	PTB-28	33.00	27.50
7	PTB-13	20.00	40.00
8	PTB-39	11.50	28.50
9	Aathira	26.40	22.50
10	Sudha	15.00	21.00
11	Shah Sharang	36.00	26.00
12	Jyothi	23.00	23.50
13	Kanchana	26.50	20.50
14	Matta Triveni	14.00	29.00
15	Narmada	15.16	20.00
16	Ashoka 200F	38.00	38.00
17	Krishna Kamod	60.00	55.00
18	Pusa basmati	28.50	33.00
19	Dandi	19.00	19.50
20	Jaya	22.50	15.50
21	Masuri	29.16	18.50
22	Sambha Masuri	55.00	23.50
23	Gurjari	58.00	50.00
24	GAR-1	14.50	21.00
25	GAR-2	48.00	14.50
26	IR-64	13.16	22.50
27	SK-20	10.50	10.00
28	GR-3	15.00	28.00
29	GR-6	26.50	27.00

30	GR-11	32.00	31.00
31	GR-12	22.50	34.00
32	GR-104	11.50	36.00
33	GR-103	12.16	18.50
34	GR-102	13.60	31.50
35	GAUR-13	12.16	26.00
36	GR-7	9.50	20.50
37	AAUDR-1	14.50	26.00
38	Pankhali-203	75.00	61.00
39	IR-22	12.16	31.00
40	DDR-8	22.50	39.00
41	Mahisugandha	30.00	36.00
42	IET-17429	28.00	37.00
43	IRBB-7	32.00	29.00
44	NWGR-3003	12.11	14.00
45	TN-1	36.00	25.00
46	IR-59656	42.00	31.00
47	CRMAS-2231	38.00	29.00
48	NWGR-2002	15.10	11.00
49	IET-10750	17.80	16.20
50	IR-38	12.40	18.40
51	IR-72	19.40	23.00
52	IET-16804	21.00	25.40
53	IET-17905	12.60	25.00
54	IET-16810	16.20	27.00
55	Pusa Sugandha	16.40	39.00
56	GAUR-100	18.11	40.00
57	Mahisagar	11.40	22.00
58	Swarna	15.70	43.00
59	GR-9	19.40	39.00
60	GR-8	17.50	35.00
61	GR-101	25.90	27.00

62	SLR-51214	14.20	30.00
63	GR-4	12.60	29.00
64	GR-3	13.80	32.00
65	GAUR-10	11.20	41.00
66	GAR-3	16.10	37.00
67	Sathi-34-36	23.40	34.00
68	Nawagam-19	15.60	23.00
69	Zinnia-31	44.90	35.00
70	Jirasar-280	27.80	19.00
71	GR-5	33.30	36.50
72	SK-20	25.00	44.00
	SEm±	0.5	0.5
	C.D.$_{0.05}$	1.5*	1.5*
	C.V. %	5.2	3.4

There is a great variation for mineral and agronomic traits in rice. The phenotypic characterization of the traits was carried out with the objective of generating additional information on variation at the genetic and molecular level. The phenotypic characterization of RIL populations helps in broadening our genetic understanding of quantitative traits. The explanatory power of trait dissection and QTL analysis largely depends on reliable assessment of phenotypic variance for the traits under study. Such variances cannot be detected on single individuals but they can be estimated for whole populations. Experimental mapping populations are a basic resource to elucidate the genetic basis of quantitatively inherited multigenic traits. Therefore three recombinant inbred line (RIL) populations were evaluated for agronomic traits, and grain Fe and Zn mineral concentration to detect the genomic regions responsible for variation in studied traits expression. RIL populations based on crosses GR-11 X Pankhali-203, GR-11 X Krishna Kamod and GR-11 X Gurjari, having 300, 250 and 300 RILs respectively, were phenotyped along with their parents. The phenotypic data collected for all three crosses were analyzed statistically.

4.2. PHENOTYPING OF THE MAPPING POPULATION

300 F7 RIL populations were available for GR-11 X Pankhali-203, 250 for GR-11 X Krishna Kamod and 300 for GR-11 X Gurjari which were developed by Single Seed Descent Method at Main Rice Research Station, Nawagam, AAU, Anand. The RILs were analyzed for 12 different parameters such as Iron (Fe) concentration (ppm), Zinc (Zn) concentration (ppm), Days to 50% flowering (DFF), Plant height (PH) (cm), Panicle length (PL) (cm), Number of effective tillers per plant (NETP), Number of filled grains per panicle (NFGP), Test weight (TWT) (g), Grain yield (GY) (g), Grain length (GL) (cm), Grain breadth (GB) (cm) and Grain Length: Breadth ratio (L:B R). Data were analyzed and the overall mean, range standard deviation, transgressive segregation and skewness of all the traits were measured in all the RIL populations for all the three crosses are presented in the table 4.2, 4.3 and 4.4.

4.2.1. RILs Compared with Parents

RIL populations were analyzed for means and were compared with their respective parents in each population separately and the results were summarized.

4.2.1.1. Cross GR-11 X Pankhali-203

The mean of RIL populations was significantly higher as compared to P1 (GR-11) for Zinc concentration, Iron concentration, Plant height, Panicle length, number of filled grains per panicle, test weight, grain yield and grain breadth except for days to 50% flowering and L:B ratio. Days to 50 % flowering, number of effective tillers per plant, grain length and L:B ratio were not significantly related to the means of the RIL populations. The mean of Zn and Fe concentration in the selfed seeds of RIL population was lower in GR-11 whereas for Pankhali-203 it was significantly higher (Table 4.2).

The means of days to 50 % flowering for RIL populations was comparatively lower as compared to both the parents. Early initiation of flowering would be beneficial to get more number of grains and thereby higher grain yield. Hence, genotypes flowering in lower number of days are desirable. The same results were also obtained for the length breadth ratio. Panicle length (27.68 cm) was higher for RIL populations than both the parents (27.05 cm for GR-11 and 25.95 cm for Pankhali-203.

Table: 4.2 Phenotypic performance of yield and yield related traits in cross GR-11 and Pankhali-203.

S. No.	Traits	GR-11 (n=10)	Pankhali-203 (n=10)	RILs (n=300)	SD	SE	Range	TS(%)	Skew	CV(%)
1.	Zn	31.53	61.49	51.01	13.67	0.78	15.01-98.11	0.60	0.313	26.80
2.	Fe	32.49	75.87	71.53	26.16	1.51	13.45-140.70	0.60	0.261	36.57
3.	DFF	95.00	100.00	93.03	5.72	0.33	80.14-101.07	-	-0.065	6.15
4.	PH	112.06	137.11	115.51	13.95	0.80	80.04-150.89	-	-0.048	12.07
5.	PL	27.05	25.95	27.68	0.59	0.03	26.05-29.74	3.00	0.676	2.13
6.	NETP	8.43	7.63	8.01	0.84	0.04	6.14-10.65	-	-0.047	10.60
7.	NFGP	182.35	182.12	182.76	0.98	0.05	180.00-185.94	6.00	0.337	6.53
8.	TWT	15.67	16.39	16.13	1.12	0.06	13.02-18.94	-	-0.313	6.97
9.	GY	15.34	19.18	18.91	1.35	0.07	15.02-20.68	-	-1.250	7.14
10.	GL	8.64	8.13	8.36	0.91	0.05	6.01-10.94	3.00	0.141	10.94
11.	GB	2.32	2.14	2.66	0.38	0.02	2.00-3.75	2.00	0.809	14.33
12.	L:B R	3.79	3.73	3.20	0.57	0.03	2.01-5.21	1.50	0.175	18.00

Zn: Zinc, **Fe:** Iron, **DFF:** Days to 50% Flowering, **PH:** Plant height (cm), **PL:** Panicle length (cm), **NETP:** Number of effective tillers per plant, **NFGP:** Number of filled grains per panicle, **TWT:** Test weight (g), **GY:** Grain yield (g), **GL:** Grain length (cm), **GB:** Grain breadth (cm), **L:B R:** Length: Breadth ratio. RILs= Recombinant Inbred Lines. SD= Standard deviation. Range= Minimum-maximum. TS= Transgressive segregation. Skew= Skewness. n=Number of plants. CV=Coefficient of Variation, SE= Standard Error.

Table: 4.3 Phenotypic performance of yield and yield related traits in cross GR-11 and Krishna Kamod.

S. No.	Traits	GR-11 (n=10)	Krishna Kamod (n=10)	RILs (n=250)	SD	SE	Range	TS(%)	Skew	CV(%)
1.	**Zn**	31.53	55.29	55.44	11.45	0.72	20.14-88.21	6.00	-0.130	22.26
2.	**Fe**	32.49	60.75	74.95	12.35	0.77	37.95-99.15	5.00	-0.439	16.43
3.	**DFF**	95.00	100.00	90.96	9.94	0.62	62.34-120.32	-	-0.024	10.93
4.	**PH**	112.06	121.4	114.74	6.54	0.41	94.75-129.65	-	-0.365	5.70
5.	**PL**	27.05	24.9	26.09	1.04	0.06	21.04-26.57	-	-0.706	4.35
6.	**NETP**	8.43	7.82	8.29	0.76	0.04	5.02-8.98	-	-1.074	10.46
7.	**NFGP**	182.35	182.42	182.46	0.77	0.04	180.14-184.75	-	-0.031	0.42
8.	**TWT**	15.67	18.04	16.17	1.06	0.06	14.02-19.64	-	-0.893	6.22
9.	**GY**	15.34	18.57	16.64	1.08	0.06	14.02-19.65	-	-1.013	6.17
10.	**GL**	8.64	6.40	7.50	1.03	0.06	5.06-9.98	1.10	0.098	13.72
11.	**GB**	2.32	3.01	2.64	0.36	0.02	2.01-3.75	1.50	0.806	13.67
12.	**L:B R**	3.79	2.73	3.45	0.50	0.03	1.70-4.33	1.20	0.168	17.48

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio. RILs= Recombinant Inbred Lines. SD=Standard deviation. Range= Minimum-maximum. TS= Transgressive segregation. Skew= Skewness. n=Number of plants. CV=Coefficient of Variation, SE= Standard Error.

Greater length of panicles is associated with greater number of spikelets and therefore higher yield of the crop. The results of elemental analysis revealed that grain zinc content ranged from 15.01 ppm to 98.11 ppm with an average of 51.01 ppm in RIL populations whereas for iron it ranged from 13.45 ppm to 140.70 ppm with an average of 71.53 ppm in RIL population. Phenotypically for number of filled grains per panicle, more transgressive segregants were observed. The means of number of filled grains per panicle was 182.76, ranging from 180.00 to 185.54   (Table 4.2).

4.2.1.2. Cross GR-11 X Krishna Kamod

Mean phenotypic values of RIL population has significance with the parent GR-11 for most of the traits except for days to 50 % flowering, panicle length and length breadth ratio. Phenotypic performance of the RIL population was higher for the traits like zinc concentration, iron concentration and number of filled grains per panicle than both the parents. Higher percentage of transgressive segregant was observed for both the traits zinc content and iron content. For zinc content the concentration of iron ranged from 20.14 ppm to 88.24 ppm with an average of 55.44 ppm, which was higher than both the parents. Similarly, for zinc content it range from 37.95 ppm to 99.15 ppm with an average of 74.95 ppm (Table 4.3).

Number of filled grains per panicle was also significantly higher (182.46) in RIL populations as compared to the both the parents. Increase in number of grains per panicle would automatically improve grain yield of the crop. Number of effective tillers per plant was more in RILs than Pankhali-203. Effective tillers are branches that develop from the leaf axils at each elongated node of the main shoot or from other tillers during vegetative growth that bears panicles. Hence more the tillers more are the panicles and grains.

4.2.1.3. Cross GR-11 X Gurjari

The mean performances of RILs for days to 50 % flowering, plant height, panicle length, number of effective tillers per plant, number of panicles for plant, grain length and length breadth ratio were not significantly different from GR-11. Zinc concentration ranged from 11.92 ppm to 88.74 ppm with an average of 50.91 ppm in RIL population. Similarly, iron concentration ranged from 34.18 ppm to 100.69 ppm with an average of 72.24 ppm in RIL population which was significantly higher than both the parents (Table 4.4).

Table: 4.4 Phenotypic performance of yield and yield related traits in cross GR-11 and Gurjari.

S. No.	Traits	GR-11 (n=10)	Gurjari (n=10)	RILs (n=300)	SD	SE	Range	TS(%)	Skew	CV(%)
1.	Zn	31.53	50.47	50.91	13.56	0.78	11.98-88.74	-	0.000	26.64
2.	Fe	32.49	58.21	72.24	14.41	0.83	34.18-100.69	-	-0.439	19.95
3.	DFF	95.00	88.00	92.25	8.73	0.50	55.26-110.47	1.00	0.028	10.61
4.	PH	112.06	111.49	110.85	6.69	0.38	82.65-121.48	-	-0.166	6.38
5.	PL	27.05	22.7	25.35	0.75	0.04	20.13-24.63	-	-0.023	3.35
6.	NETP	8.43	6.62	7.21	0.82	0.04	5.02-8.98	-	-0.763	11.49
7.	NFGP	182.35	181.83	182.41	1.03	0.05	180.05-184.92	1.20	0.054	0.56
8.	TWT	15.67	28.09	18.52	4.62	0.26	10.48-48.06	1.50	0.887	21.49
9.	GY	15.34	20.93	17.48	1.46	0.08	14.02-21.78	-	-0.114	8.40
10.	GL	8.64	8.94	8.06	1.09	0.06	5.06-10.94	1.20	0.156	14.31
11.	GB	2.32	2.92	2.36	0.38	0.02	2.00-3.75	1.50	0.809	14.33
12.	L:B R	3.79	3.06	3.63	0.52	0.03	1.70-4.76	1.20	0.159	18.02

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio. RILs= Recombinant Inbred Lines. SD=Standard deviation. Range= Minimum-maximum. TS= Transgressive segregation. Skew= Skewness. n=Number of plants. CV=Coefficient of Variation, SE= Standard Error.

The parents of this RIL population showed statistically significant divergent phenotypes for all the traits except plant height. Plant height was significantly lower in RIL populations (110.85 cm) as compared to the parents (112.06 cm for GR-11 and 111.49 cm for Gurjari). Plant height is positively related with lodging of the plants after heading. Hence, plants with less height are desirable in this crop. A wide range of variation for all traits was detected among the RILs of mapping population.

The appearance of transgressive segregants is a common phenomenon in wide crosses. Though the parents taken in the present study are cultivated species, parent Pankhali-203 is a cultivar selected from landraces. So there is a possibility of occurrence of wide variations in the population. These new variations may be seen because of many genetic and epigenetic factors (Wang *et al.,* 2005 and Kovach and Mc Couch, 2008). One of the main advantages of wide crosses is the possibility to introgress useful genetic variability into elite cultivars. Wider variability was observed for all the traits, and this provides the breeder with more opportunities to select plants with different combinations of desirable traits. These transgressive segregants might have resulted due to the accumulation of favorable genes controlling grain characters or development of new combinations of genes controlling grain traits derived from the parents. Transgressive segregation is commonly observed in segregating populations for quantitative traits (De Vicente and Tanksley, 1993 and Xiao *et al*., 1996). There are several potential causes of transgression including *denovo* mutations and unmasking of recessive deleterious alleles due to inbreeding. However, occurrence of such transgressive segregants is possibly due to accumulation of complementary alleles from the parents at multiple loci in certain RILs (Tanksley, 1993) and G x G interactions (epistasis) (Lanceras *et al*., 2004).

In a nutshell the results of observations on the RILs and their parents indicate the existence of sufficient genetic variability between parents of all the three populations, provide good chances of recovering all the desirable recombinants, and opportunities for QTL mapping suggesting that scorable marker polymorphism was well distributed across the nuclear genome in all the three populations. Crop improvement depends on the magnitude of the genetic variability in the base population, as well as the population mean, population size that can be assessed and the reliability of the assessment of the selection units. The variability detected in the study can be exploited if the heritability of the observed trait is high, and the observed

traits are indeed relevant to the breeding target (in this case high yielding rice with high grain mineral micronutrient density).

4.2.2. Correlation among Quantitative Traits

Knowledge of correlations among different plant traits at phenotypic levels is required to determine the expected responses of other traits when selection is applied for the traits of greatest interest in breeding program (Falconer, 1989). Correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. But measure of correlation does not consider dependence of one variable over the other. Direct contribution of each component to the yield and the indirect effects it has through its association with other components cannot be differentiated from mere correlation studies. In the present study Pearson's correlation were computed for phenotypic traits for all the three RIL populations.

4.2.2.1. Correlation study in GR-11 X Pankhali-203

Days to 50 % flowering was negatively correlated to all the traits except for panicle length ($r= 0.042$) (Table 4.5). Plant height was positively correlated to all the traits except days to 50 % flowering, test weight ($r=-0.038$), grain yield (-0.026) and grain breadth ($r= -0.083$). Panicle length has higher positive correlations with the grain zinc ($r= 0.0217$) and iron concentrations ($r= 0.0201$) at 1 % significance level in rice. It was also found to be positively correlated with the test weight ($r= 0.148$) and negatively correlated with the grain yield and grain breadth. Grain Zn concentrations were positively and significantly correlated to the number of effective tillers ($r= 0.131$), grain yield ($r= 0.177$), grain length ($r= 0.126$) and panicle length ($r= 0.217$). Similarly, grain Fe concentration was also positively correlated to panicle length ($r= 0.201$), test weight ($r= 0.132$), grain yield ($r= 0.143$) and grain length ($r= 0.147$). Grain Fe and Zn were also positively correlated to each other in the population, suggesting the fact that there can be a simultaneous improvement of both the grain micronutrients in rice. Since the panicle length also correlates to the iron and zinc content, by increasing the panicle length the number of seed on the panicle increases which in turn leads to the increase in grain yield of the crop.

Table: 4.5 Pearson's Correlation coefficient analyses among yield and related traits and iron and zinc concentration in the grain studied in RIL population, derived from a cross between GR-11 and Pankhali-203.

Traits	Zn	Fe	DFF	PH	PL	NETP	NFGP	TWT	GY	GL	GB	L:B R
Zn	1.000	0.495	-0.078	0.0405	0.217	0.131	0.165	0.048	0.177	0.126	-0.003	0.085
Fe	0.495	1.000	-0.179	0.563	0.201	0.061	0.246	0.132	0.143	0.147	-0.012	0.096
DFF	-0.078	-0.179	1.000	-0.164	0.042	-0.022	-0.133	-0.034	-0.140	-0.199	0.119	-0.163
PH	0.0405	0.563	-0.164	1.000	0.255	0.160	0.190	-0.038	-0.026	0.095	-0.083	0.118
PL	0.217**	0.201**	0.042	0.255	1.000	0.046	0.071	0.148**	-0.078	0.045	-0.126	0.101
NETP	0.131**	0.061	-0.022	0.160	0.046	1.000	0.028	-0.008	0.057	0.020	-0.036	0.045
NFGP	0.165	0.246	-0.133	0.190	0.071	0.028	1.000	0.045	0.042	0.084	0.039	0.021
TWT	0.048	0.132*	-0.034	-0.038	0.148**	-0.008	0.045	1.000	0.120*	0.043	0.023	0.003
GY	0.177**	0.143*	-0.140	-0.026	-0.078	0.057	0.042	0.120*	1.000	0.078	-0.099	0.142*
GL	0.126*	0.147*	-0.199	0.095	0.045	0.020	0.084	0.043	0.078	1.000	-0.052	0.654
GB	-0.003	-0.012	0.119*	-0.083	-0.126	-0.036	0.039	0.023	-0.099	-0.052	1.000	-0.773
L:B R	0.085	0.096	-0.163	0.118*	0.101	0.045	0.021	0.003	0.142*	0.654	-0.773	1.000

*, **Significant at 5% and 1% levels respectively.

Zn: Zinc, Fe: Iron, DFF: Days to 50% Flowering, PH: Plant height (cm), PL: Panicle length (cm), NETP: Number of effective tillers per plant, NFGP: Number of filled grains per panicle, TWT: Test weight (g), GY: Grain yield (g), GL: Grain length (cm), GB: Grain breadth (cm), L:B R: Length: Breadth ratio.

4.2.2.2. Correlation study in GR-11 X Krishna Kamod

Grain zinc content was positively and significantly correlated to Fe content (r= 0.138), plant height (r= 0.154), panicle length (r= 0.136) and grain yield (r= 0.152). Grain Fe content had significant correlations with Zn content (r= 0.138), panicle length (r= 0.146) and grain yield (r= 0.126). The rest of the traits had no significant relations among the RIL populations (Table 4.6).

4.2.2.3. Correlation study in GR-11 X Gurjari

Panicle length was significantly and positively correlated to grain length (r= 0.087) and L:B ratio (r= 0.042). Grain length was also positively correlated to grain breadth (r= 0.107). Grain zinc concentration was highly and positively correlated to Fe concentration (r= 0.149), plant height (r= 0.173), panicle length (r= 0.180) and grain yield (r= 0.103). Grain iron content was positively correlated to plant height (r= 0.188), panicle length (r= 0.143) and grain yield (r= 0.146). The rest of the traits did not exhibit any significant correlations to the others (Table 4.7).

Consistent correlations between Fe and Zn across all crosses, suggested the possibility that at least some of the genes that control these traits are linked or have pleiotropic effect. To determine the position and effect of the genes controlling these traits, linkage maps were constructed with different marker systems that could be used to conduct a search of QTLs throughout the genome.

The results were in agreement with the earlier results reported by Anuradha *et al.* (2012b). The parental lines used by Anuradha *et al.* (2012b) had grain Zn concentration of 53.70 ppm (Madhukar) and 27.20 ppm (Swarna) in brown rice and the RIL population generated from this cross showed Fe concentration in the range of 0.20 to 224.00 ppm and Zn in the range of 0.40 to 104.00 ppm. The study conducted by Bekele *et al.* (2013b) on the estimation of genetic variability and correlation studies for grain Zn concentration in 64 rice genotypes revealed the range of variation for grain Zn concentration as 18.90 ppm to 36.90 ppm with an average value of 26.74 ppm. Wide variation was observed for Fe concentration among the population than Zn concentration at both locations and similar results were obtained earlier by Pandian *et al.* (2011) and Anuradha *et al.* (2012b). There could be several reasons for variations that may include effect of environment, genotype and environment interactions (Suwarto and Nasrullah, 2011), soil properties like pH, organic matter content, Fe and Zn levels in the soil etc. (Chandel *et al.*, 2010 and Pandian *et al.*, 2011).

Table: 4.6 Pearson's Correlation coefficient analyses among yield and related traits and iron and zinc concentration in the grain studied in RIL population, derived from a cross between GR-11 and Krishna Kamod.

Traits	Zn	Fe	DFF	PH	PL	NETP	NFGP	TWT	GY	GL	GB	L:B R
Zn	1.000	0.138*	0.009	0.154*	0.136*	0.000	0.002	0.041	0.152*	0.041	-0.095	0.103
Fe	0.138*	1.000	-0.163	0.305	0.146*	0.078	-0.103	-0.037	0.126*	0.061	0.088	-0.019
DFF	0.009	-0.163	1.000	-0.047	0.003	-0.102	0.109	0.002	-0.083	-0.146	-0.044	-0.076
PH	0.154*	0.305	-0.047	1.000	-0.062	0.005	-0.065	0.000	0.109	0.059	0.053	0.000
PL	0.136*	0.146*	0.003	-0.062	1.000	-0.025	0.034	-0.009	0.015	0.052	-0.152	0.160
NETP	0.000	0.078	-0.102	0.005	-0.025	1.000	0.105	-0.075	-0.016	0.079	-0.010	0.067
NFGP	0.002	-0.103	0.109	-0.065	0.034	0.105	1.000	-0.049	0.035	-0.016	0.014	-0.026
TWT	0.041	-0.037	0.002	0.000	-0.009	-0.075	-0.049	1.000	-0.025	-0.025	0.008	-0.044
GY	0.152*	0.126*	-0.083	0.109	0.015	-0.016	0.035	-0.025	1.000	0.020	-0.063	0.074
GL	0.041	0.061	-0.146	0.059	0.052	0.079	-0.016	-0.025	0.020	1.000	0.315	0.680
GB	-0.095	0.088	-0.044	0.053	-0.152	-0.010	0.014	0.008	-0.063	0.315	1.000	-0.619
L:B R	0.103	-0.019	-0.076	0.000	0.160*	0.067	-0.026	-0.044	0.074	0.680	-0.619	1.000

*, **Significant at 5% and 1% levels respectively.**

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio

The correlation between grain Fe and Zn concentrations has been studied in several crops, with the results, by a large, showing similar trends. In all three populations Fe and Zn concentrations were strongly and positively associated. This may point to common molecular mechanism controlling the uptake and metabolism of these minerals in grains or common transporters controlling the movement of these minerals within plants (Vreugdenhil *et al*., 2004; Ghandilyan *et al*., 2006). Co-segregation of these genes for traits might be the reason of strong association between the minerals in all populations. The direction and intensity of association suggest there are good opportunities for simultaneous genetic improvement of both micronutrients (Velu *et al*., 2008) by co-transferring superior alleles controlling these traits into the genetic backgrounds of elite lines.

4.2.3. Frequency Distribution

The frequency distribution evaluated for the traits of all the three populations are given in Fig. 4.1 to 4.36. Frequency distributions of the histogram of the RILs of all the three populations represent the frequency distribution of genotypes for all traits. The measurements were grouped into equally spaced classes on the X-axis, and the frequency of the individuals falling in each class was plotted on Y-axis. Continuous distribution of phenotypic frequency supported the quantitative inheritance of all observed traits, as expected for quantitative traits. Normal curves for the distributions are also indicated by the lines over the bars. The frequency distributions of all the traits showed continuous phenotypic variation and transgressive segregation (lines with lower values than the lowest parent or higher values than the highest parent) in both the directions suggesting multiple gene action.

In case of the GR-11 X Pankhali-203 population, the distribution of RILs for plant height, number of effective tillers per plant, number of filled grains per panicle, panicle length, grain length, grain breadth and L:B ratio were skewed towards GR-11 parental values, while for Fe concentration, Zn concentration, test weight and grain yield the distribution was skewed towards the Pankhali-203 values (Fig. 4.1 to 4.12). Transgression beyond the parental values was observed for all traits including those for which the parental values hardly differ such as panicle length, grain breadth and L:B ratio. For the latter, there was a non-significant difference between the parents, and hence a narrow range of the trait distribution of most of the RILs was significantly closer to the value of the parent.

Table: 4.7 Pearson's Correlation coefficient analyses among yield and related traits and iron and zinc concentration in the grain studied in RIL population, derived from a cross between GR-11 and Gurjari.

Traits	Zn	Fe	DFF	PH	PL	NETP	NFGP	TWT	GY	GL	GB	L:B R
Zn	1.000	0.149	0.007	0.173	0.080	0.077	-0.053	0.012	0.003	0.041	-0.044	0.060
Fe	0.149**	1.000	-0.015	0.488	0.043	0.044	0.021	0.016	0.046	0.005	-0.008	0.071
DFF	0.007	-0.015	1.000	0.014	-0.093	-0.088	0.164	-0.050	-0.014	-0.024	0.005	-0.019
PH	0.173**	0.188**	0.014	1.000	0.082	0.060	-0.007	0.027	-0.005	-0.057	-0.130	0.046
PL	0.180*	0.143*	-0.094	0.082	1.000	-0.021	-0.048	-0.008	0.006	0.087	0.033	0.042
NETP	0.077	0.044	-0.088	0.060	-0.021	1.000	0.042	0.006	0.017	-0.072	-0.003	-0.065
NFGP	-0.053	0.021	0.164	-0.007	-0.048	0.042	1.000	-0.049	-0.033	-0.073	0.018	-0.067
TWT	0.012	0.016	-0.050	0.027	-0.008	0.006	-0.049	1.000	-0.045	0.042	0.058	-0.010
GY	0.103*	0.146*	-0.014	-0.055	0.006	0.017	-0.033	-0.045	1.000	-0.053	0.131	0.061
GL	0.041	0.005	-0.024	-0.057	0.087*	-0.072	-0.073	0.042	-0.053	1.000	0.107*	0.666
GB	-0.044	-0.008	0.005	-0.130	0.033	-0.003	0.018	0.058	0.131	0.107*	1.000	-0.652
L:B R	0.060	0.071	-0.019	0.046	0.042*	-0.065	-0.067	-0.010	0.061	0.666	-0.652	1.000

*, **Significant at 5% and 1% levels respectively.**

Zn: Zinc, **Fe:** Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio
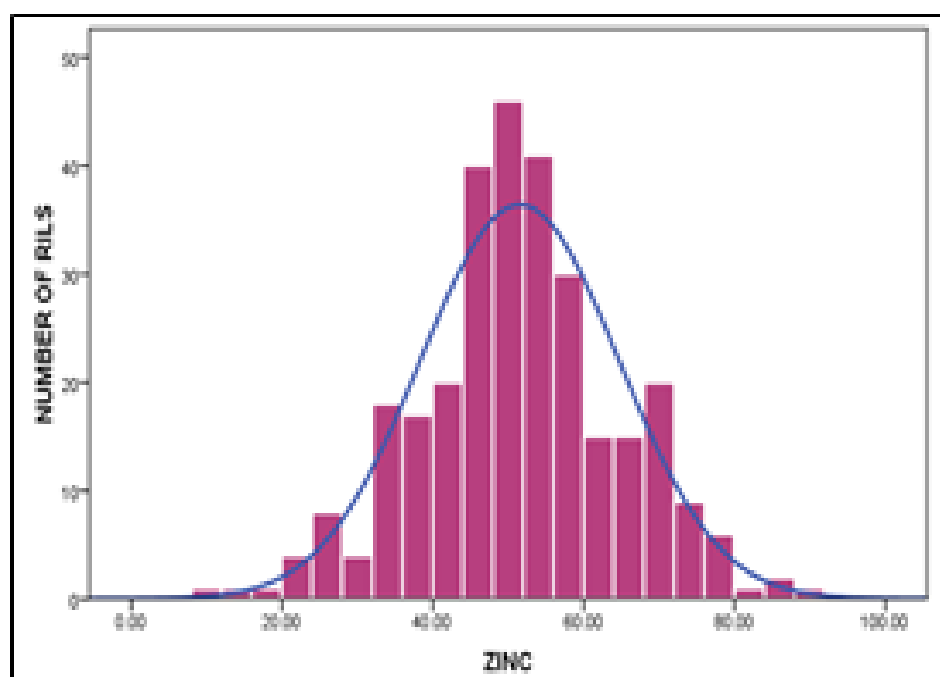
Fig 4.1 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Zinc concentration
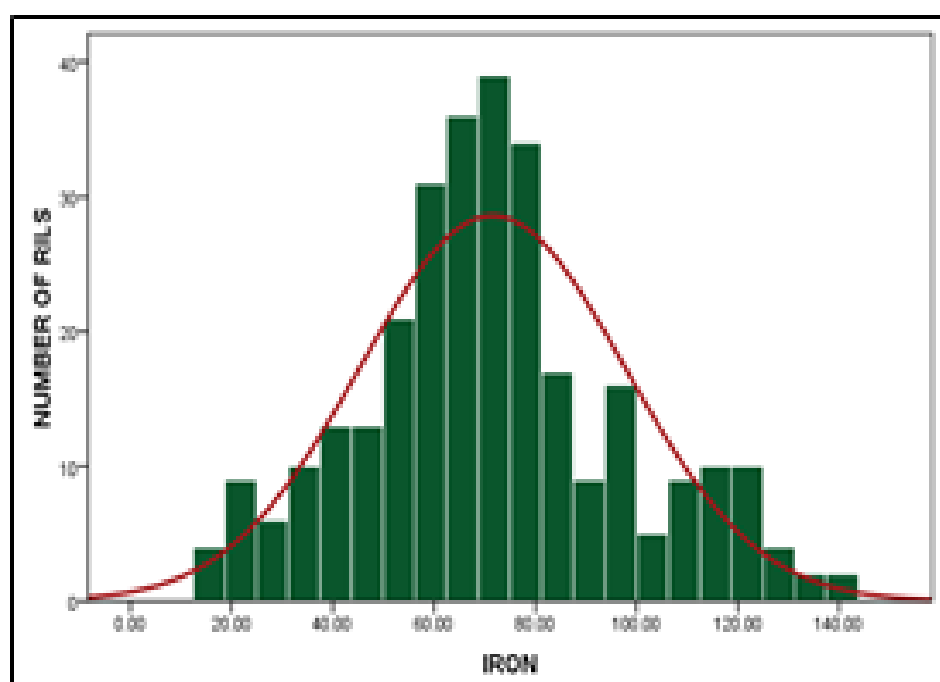
Fig 4.2 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Iron concentration
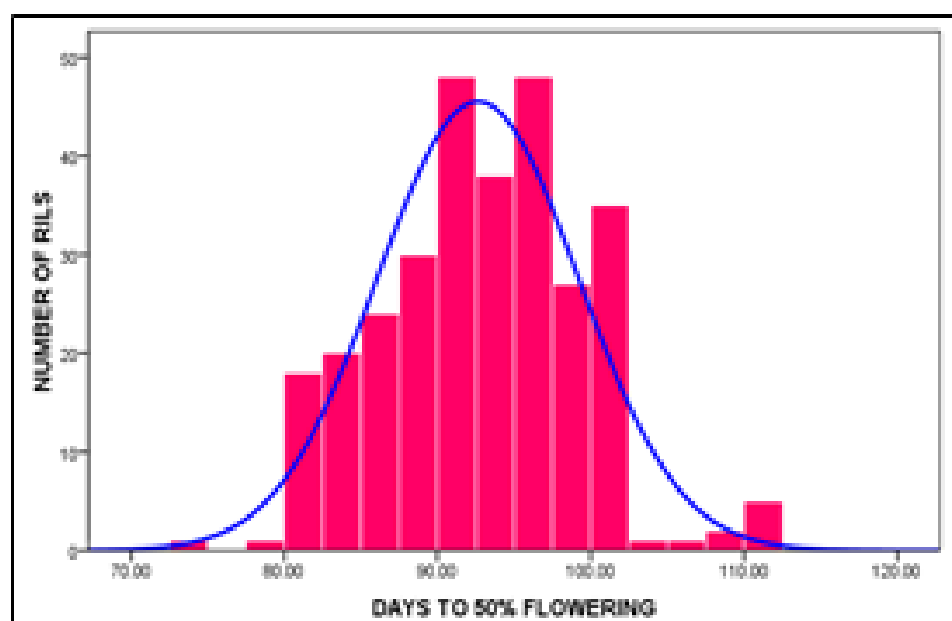
Fig.4.3 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Days to 50% flowering
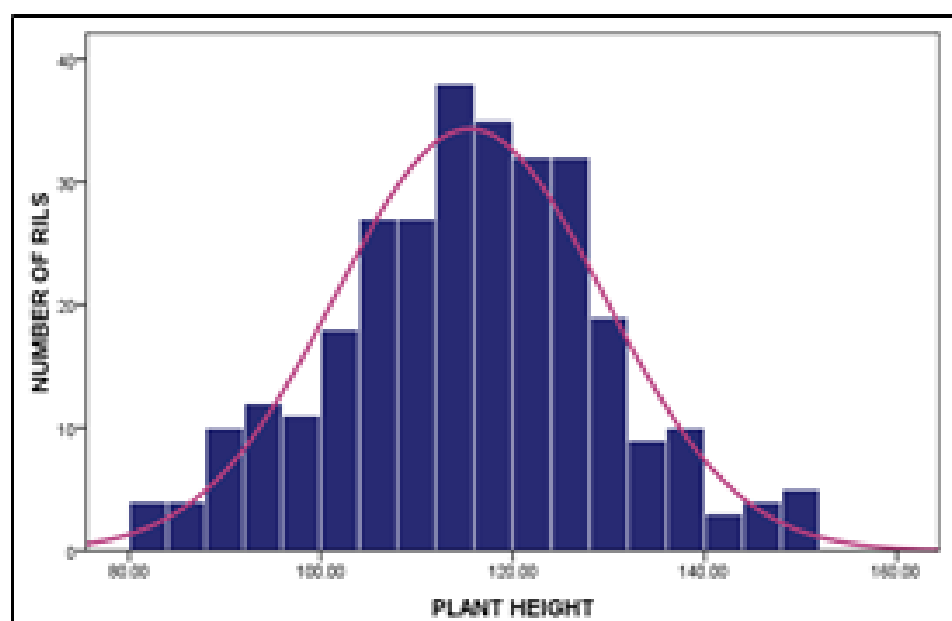
Fig.4.4 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Plant Height
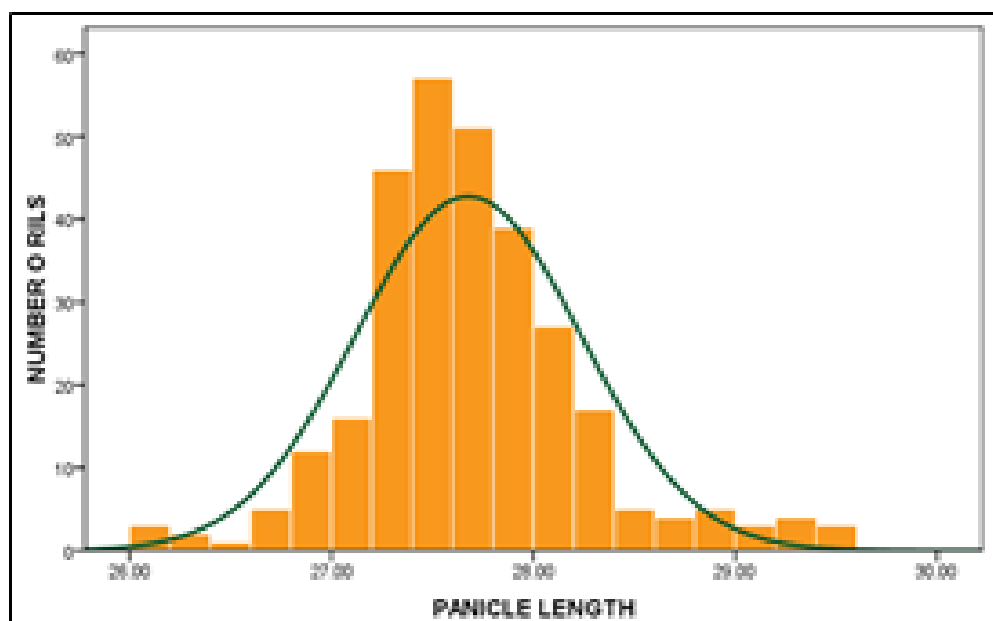
Fig.4.5 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Panicle Length
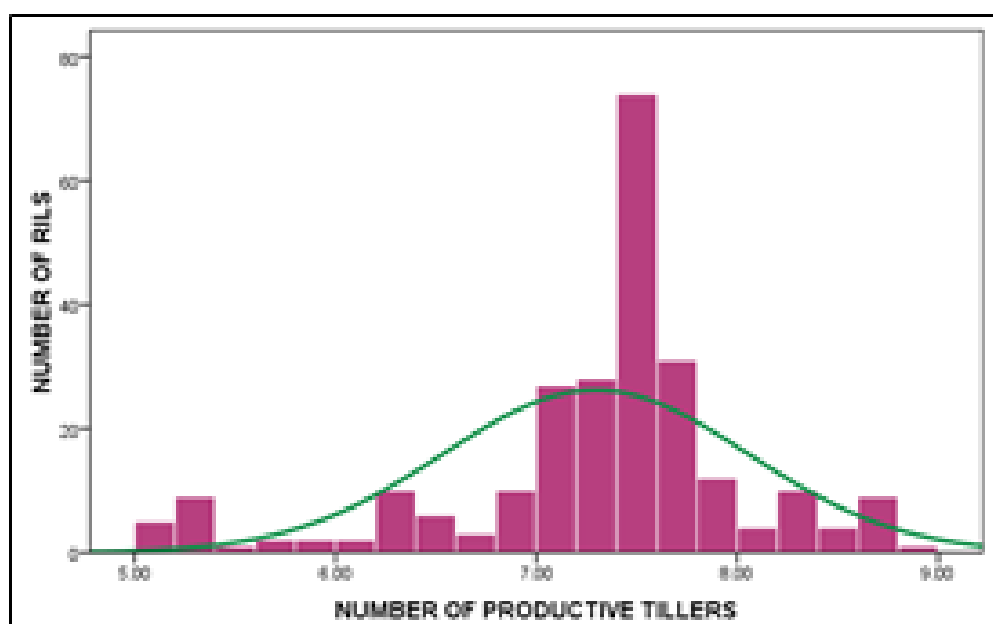
Fig.4.6 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Number of Productive Tillers
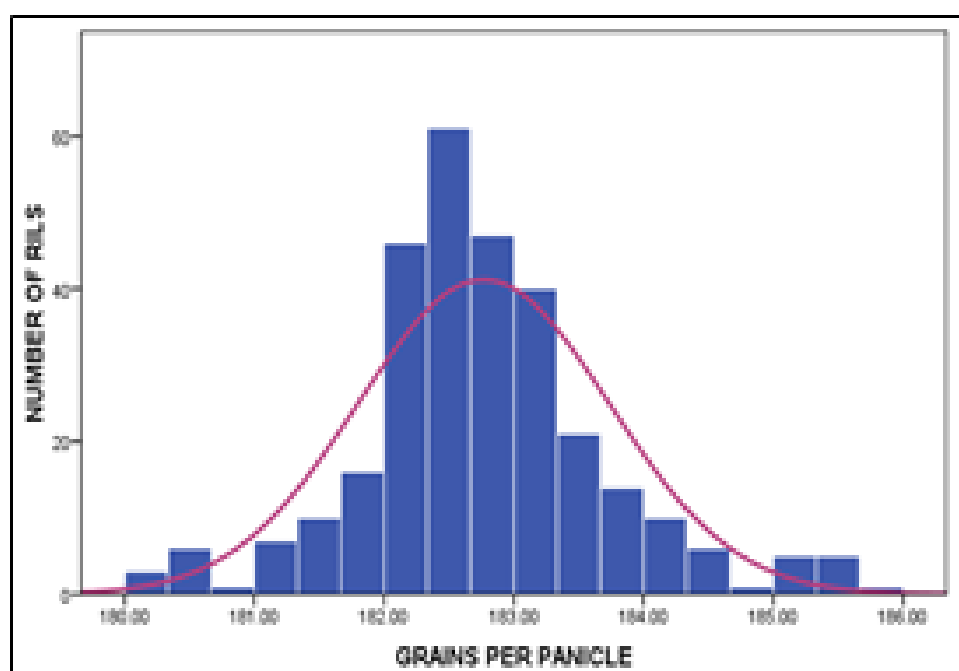
Fig.4.7 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Grains per Panicle
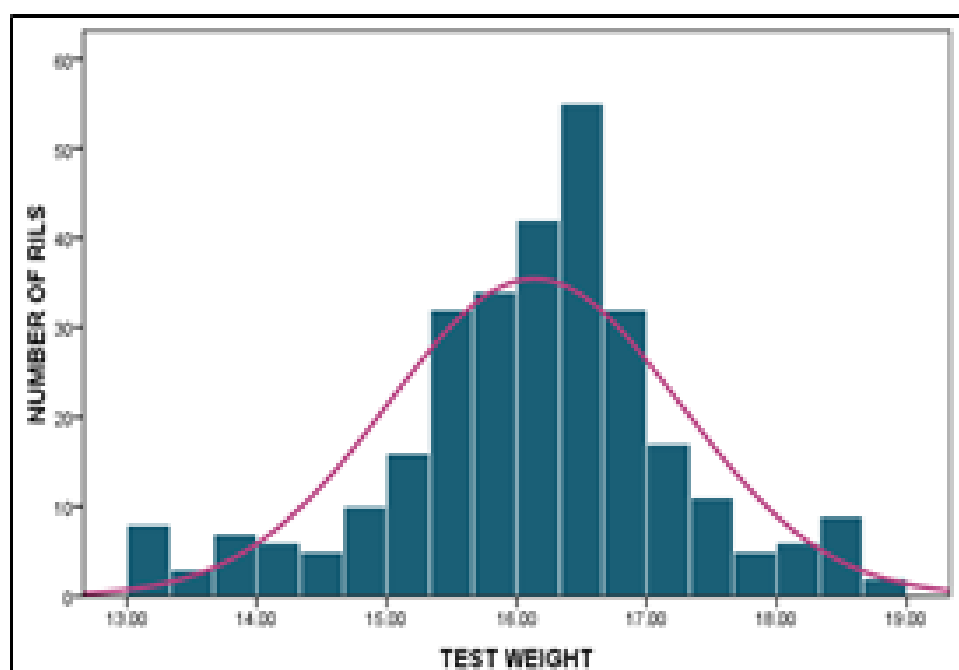
Fig.4.8 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Test Weight
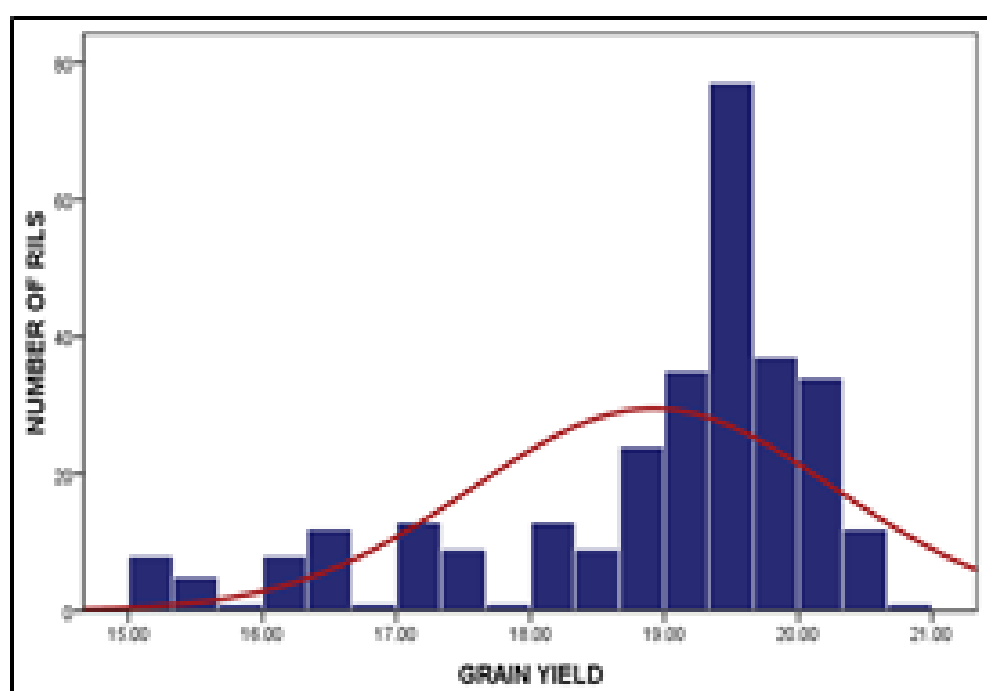
Fig.4.9 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Pankhali-203 for Grain Yield
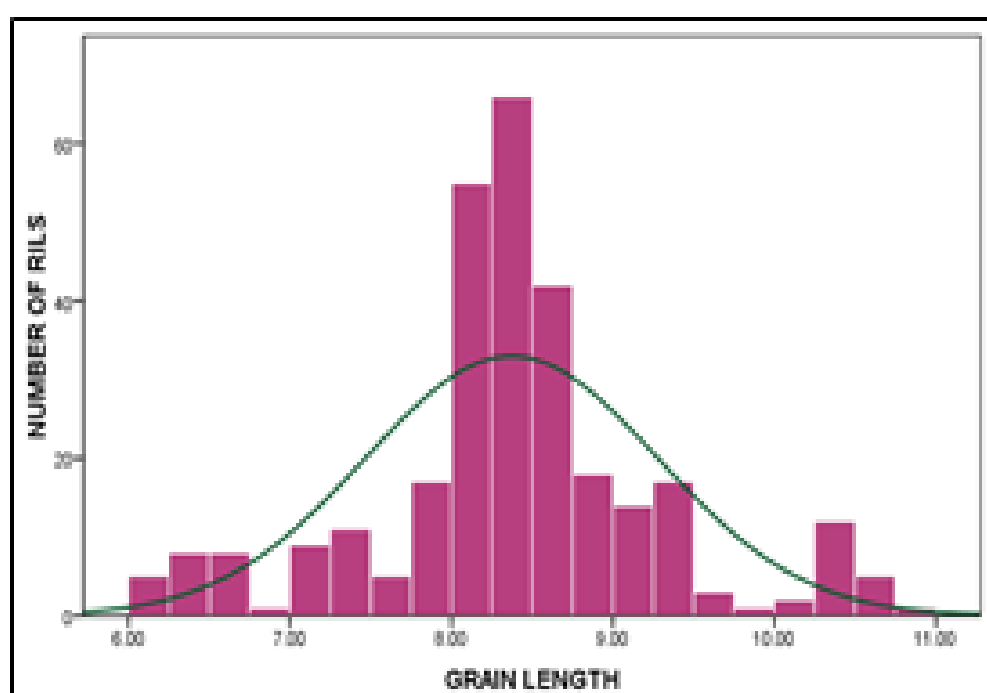
Fig.4.10 Frequency distribution of 300 RIL population derived from the cross GR-11 X Pankhali-203 for Grain Length
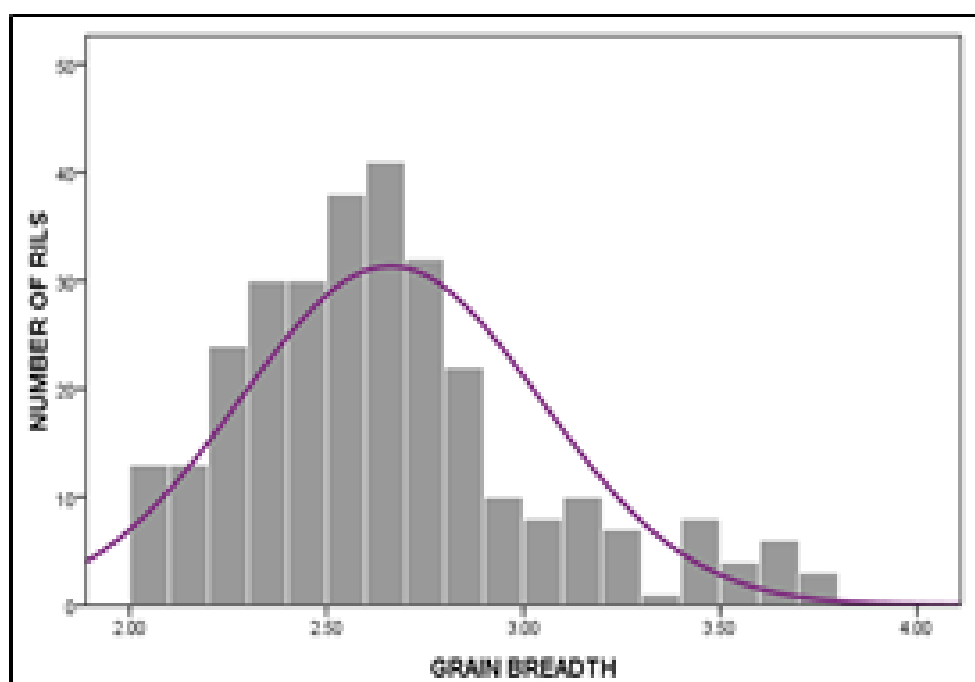
Fig 4.11 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Panbhali-203 for Grain Breadth
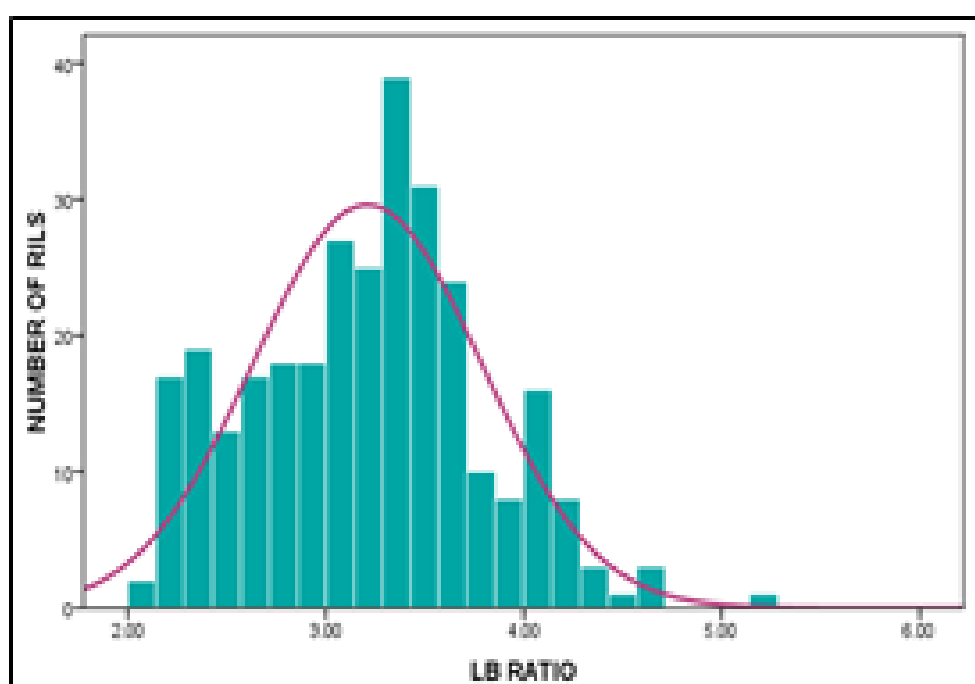
Fig 4.12 Frequency distribution of 300 RIL populations derived from the cross GR-11 X Panbhali-203 for L:B Ratio
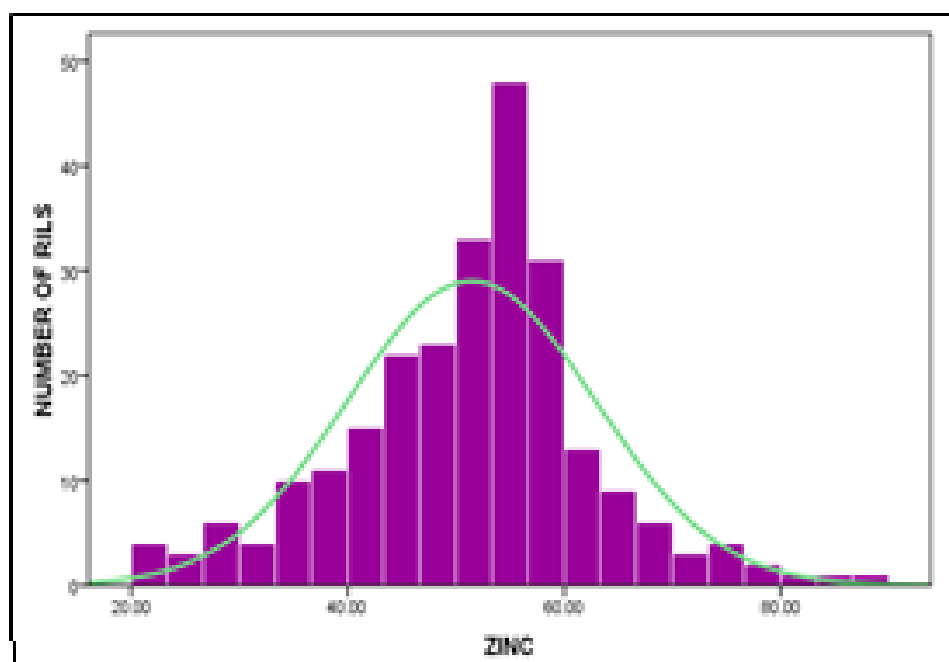
Fig.4.13 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Zinc concentration
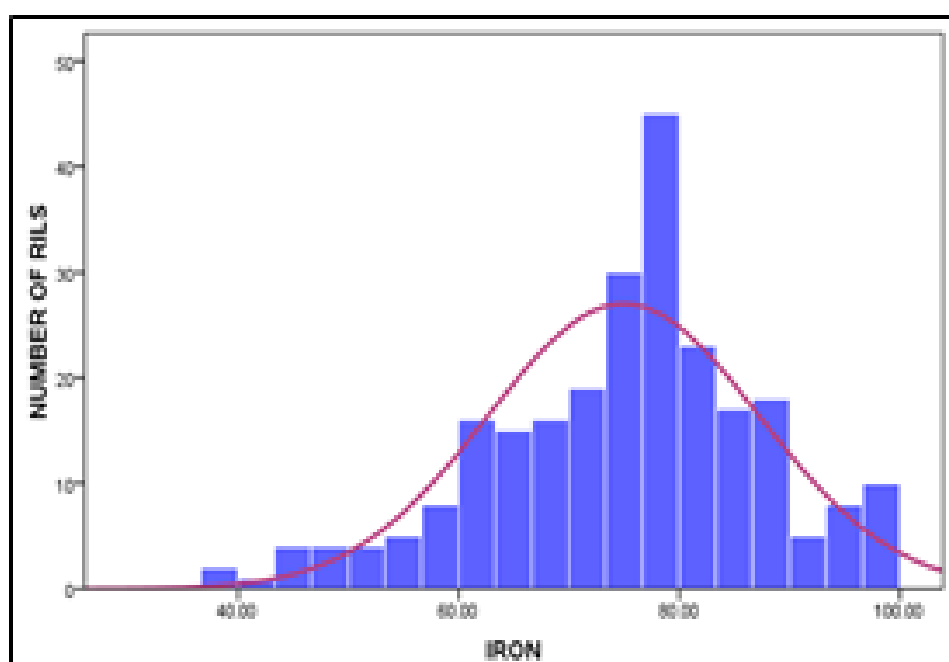
Fig.4.14 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Iron concentration
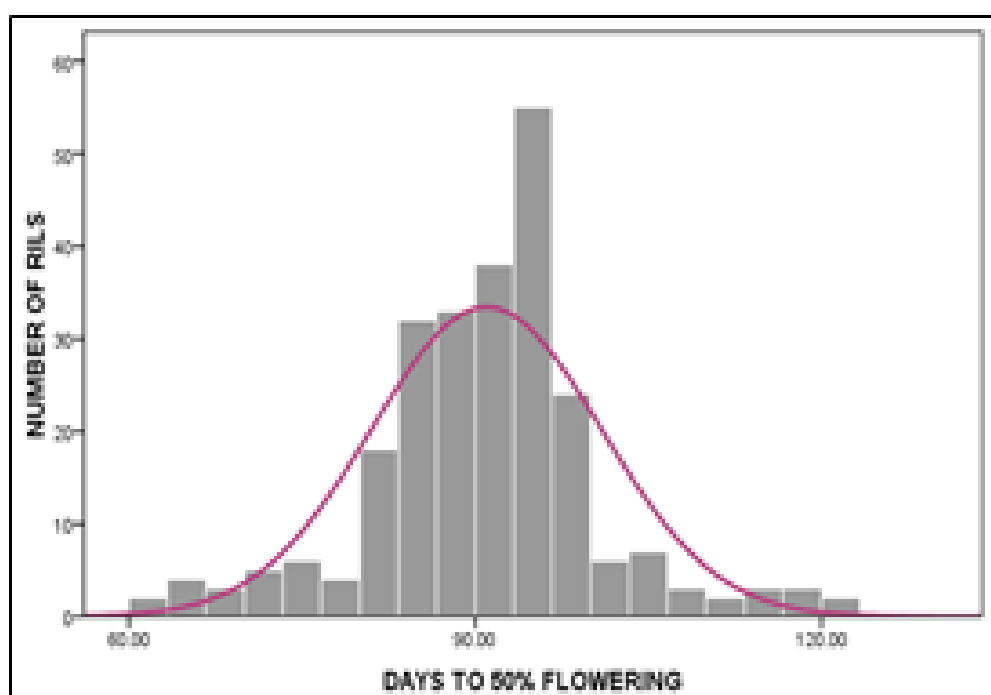
Fig.4.15 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishen Kamod for Days to 50% flowering
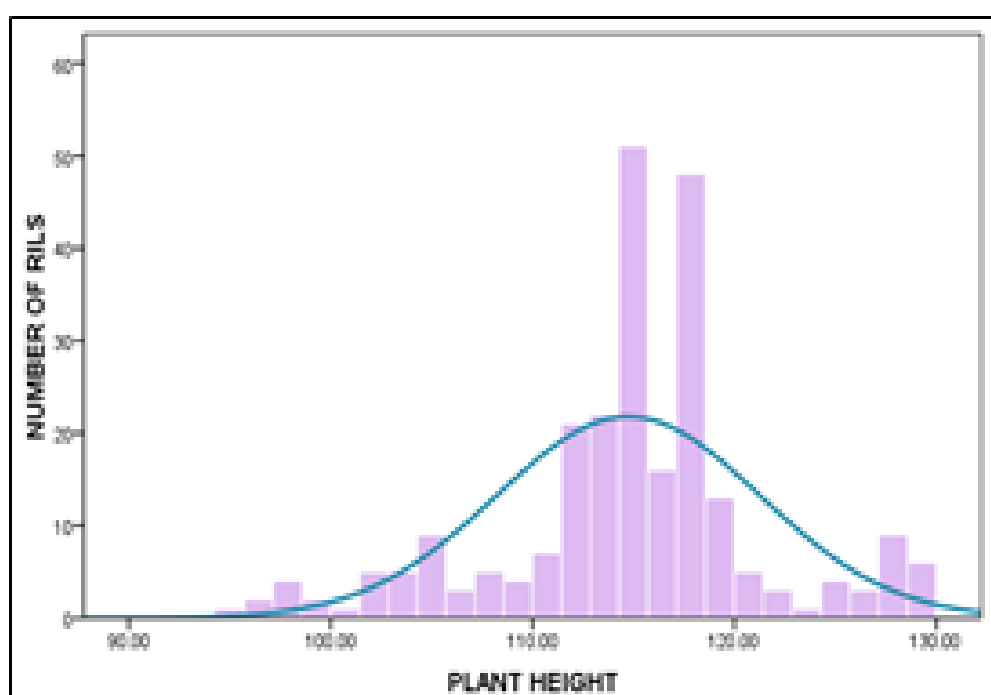
Fig.4.16 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishen Kamod for Plant Height
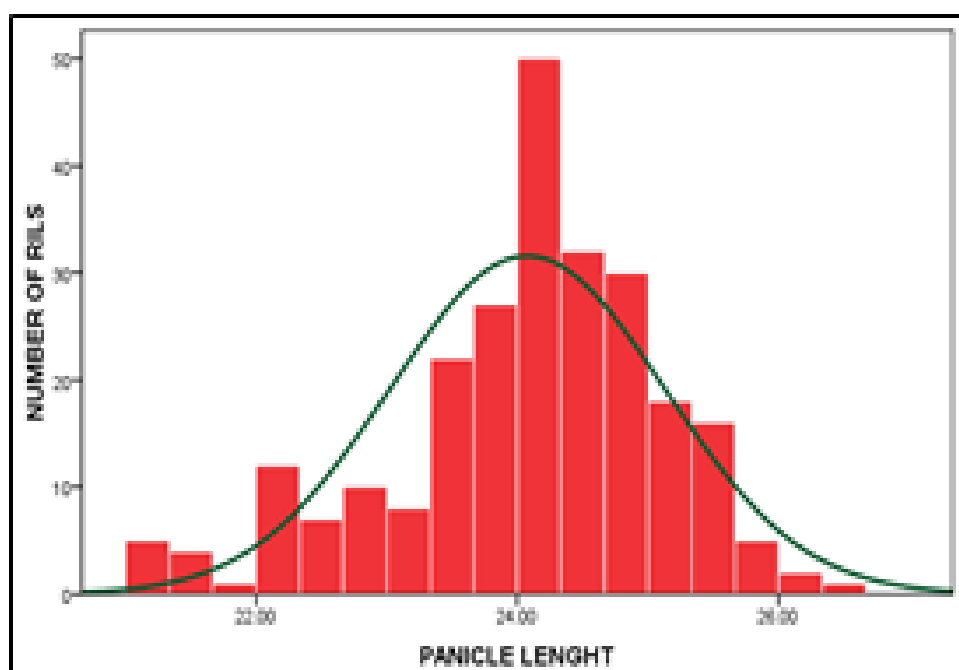
Fig.4.17 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Panicle Length
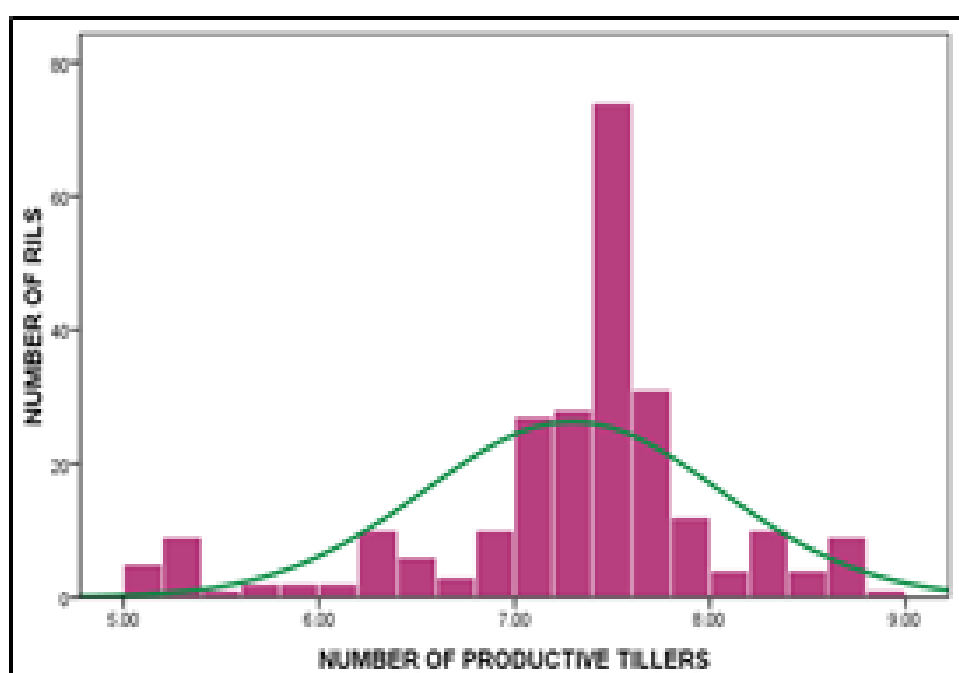
Fig.4.18 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Number of Productive Tillers
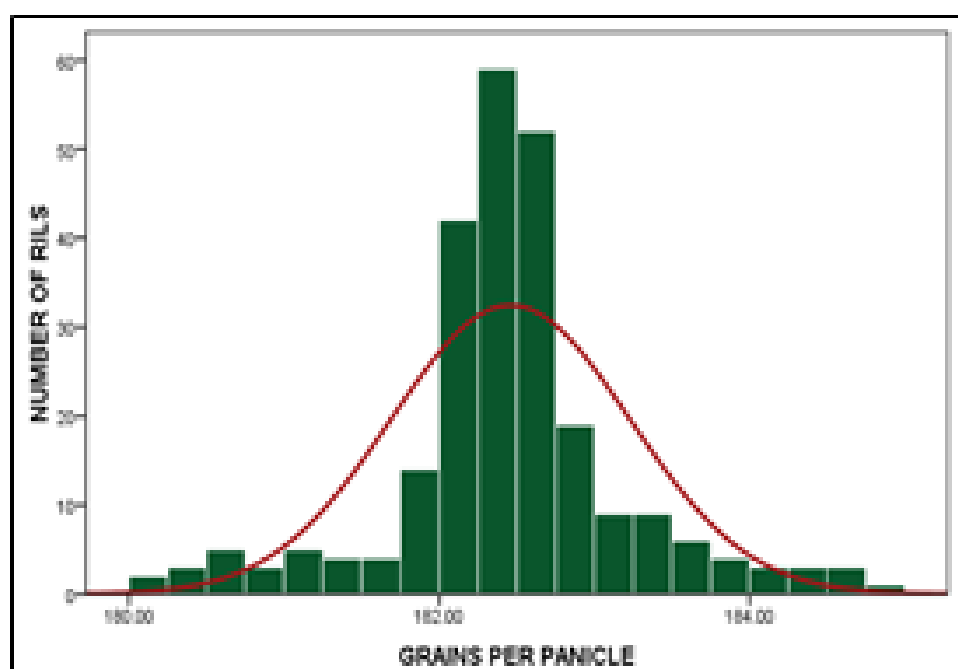
Fig.4.19 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Grains per Panicle
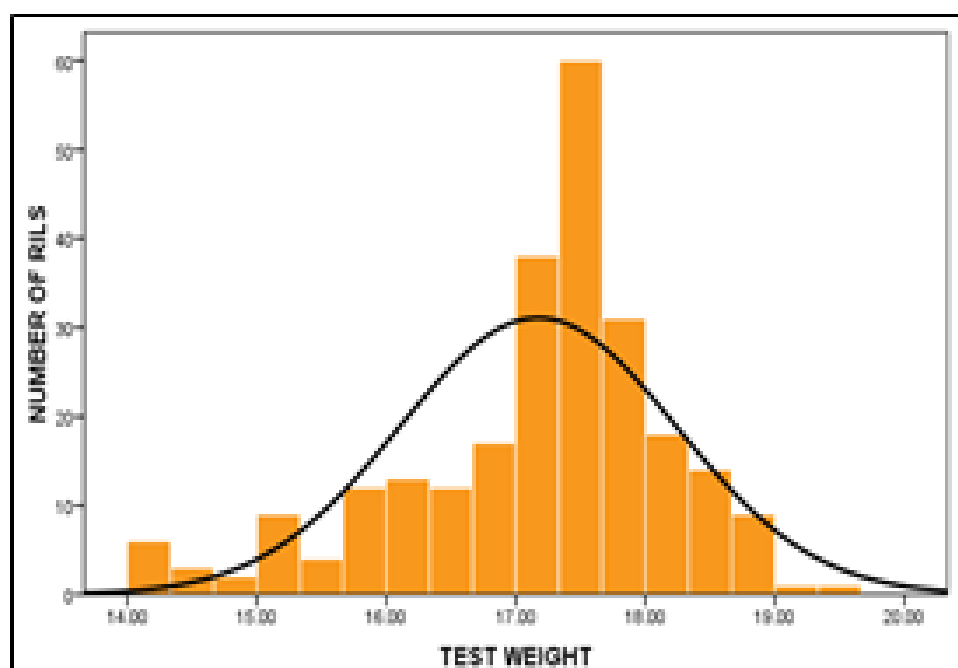
Fig.4.20 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Test weight
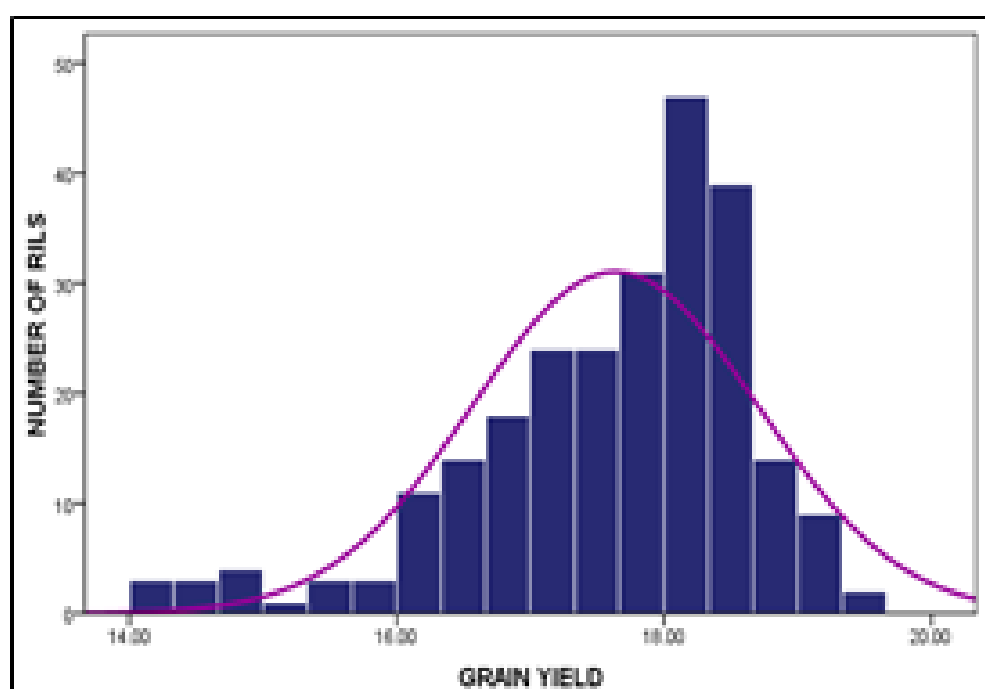
Fig.4.21 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Grain Yield
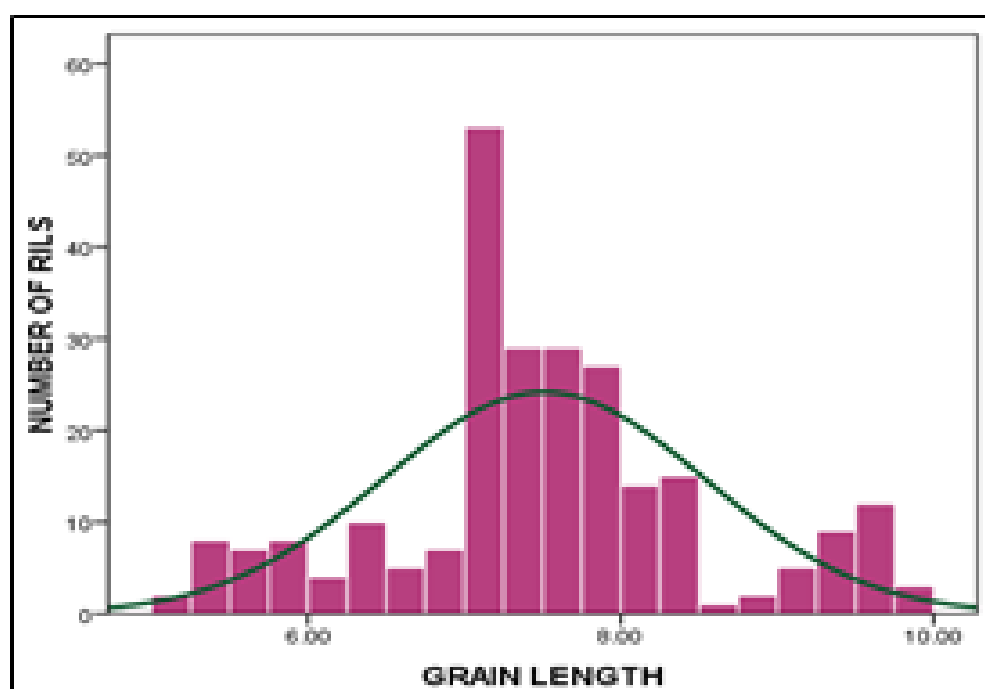
Fig.4.22 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Grain Length
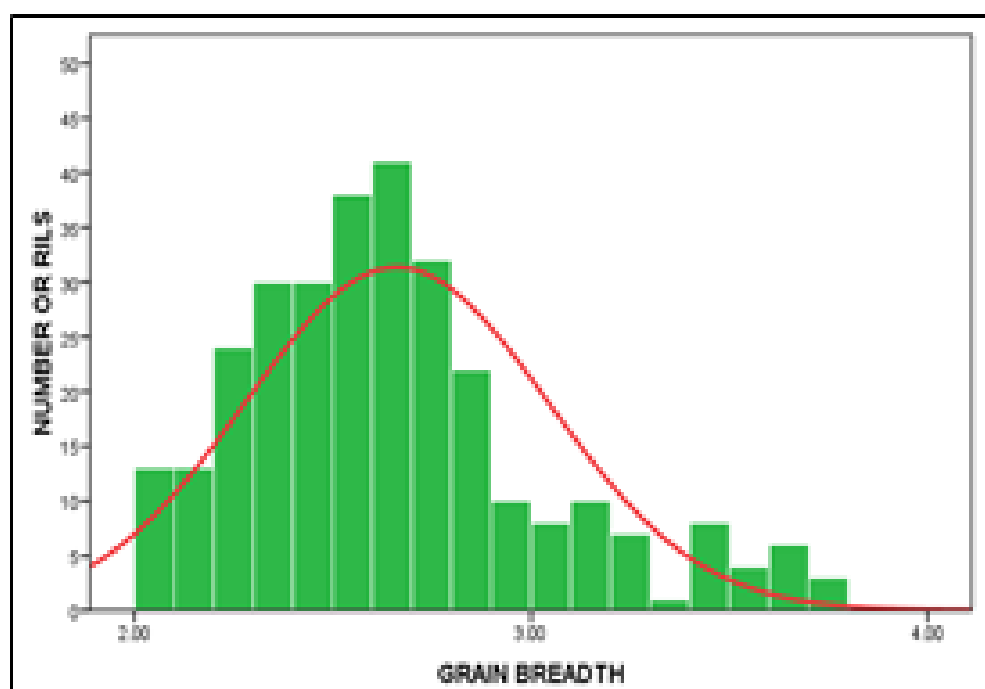
Fig 4.23 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for Grains Breadth
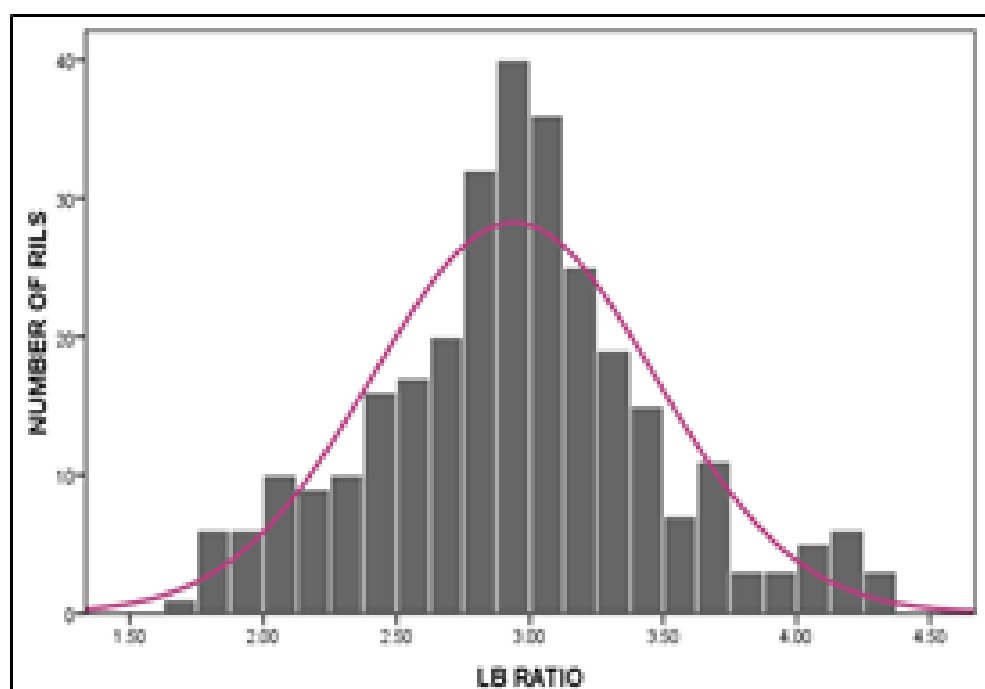
Fig.4.24 Frequency distribution of 250 RIL populations derived from the cross GR-11 X Krishna Kamod for L:B Ratio
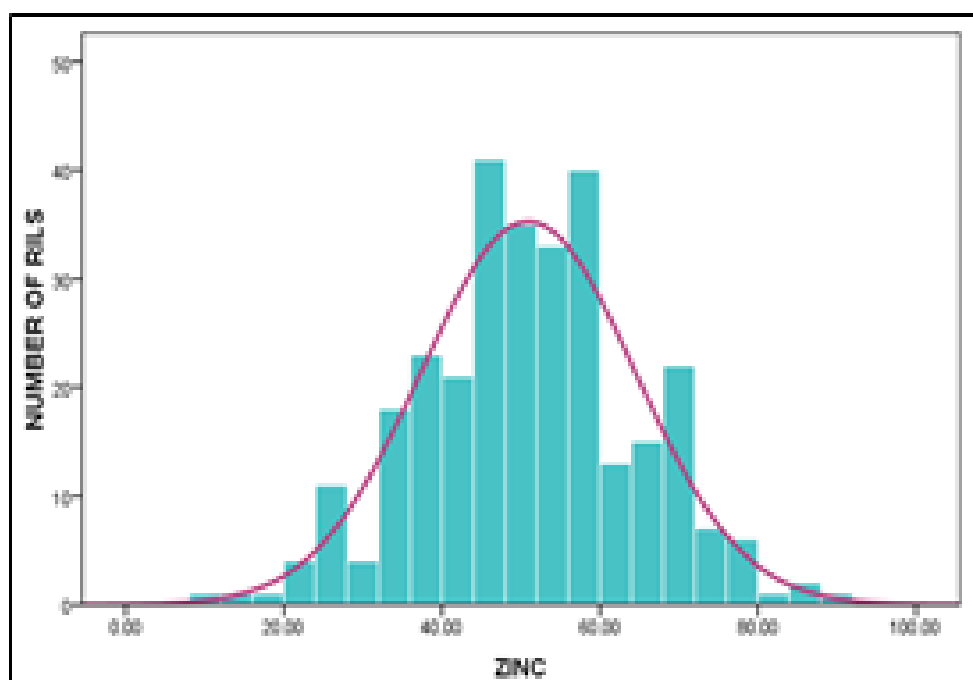
Fig.4.25 Frequency distribution of 300 RILs population derived from the cross GR-11 X Gurjari for Zinc concentration
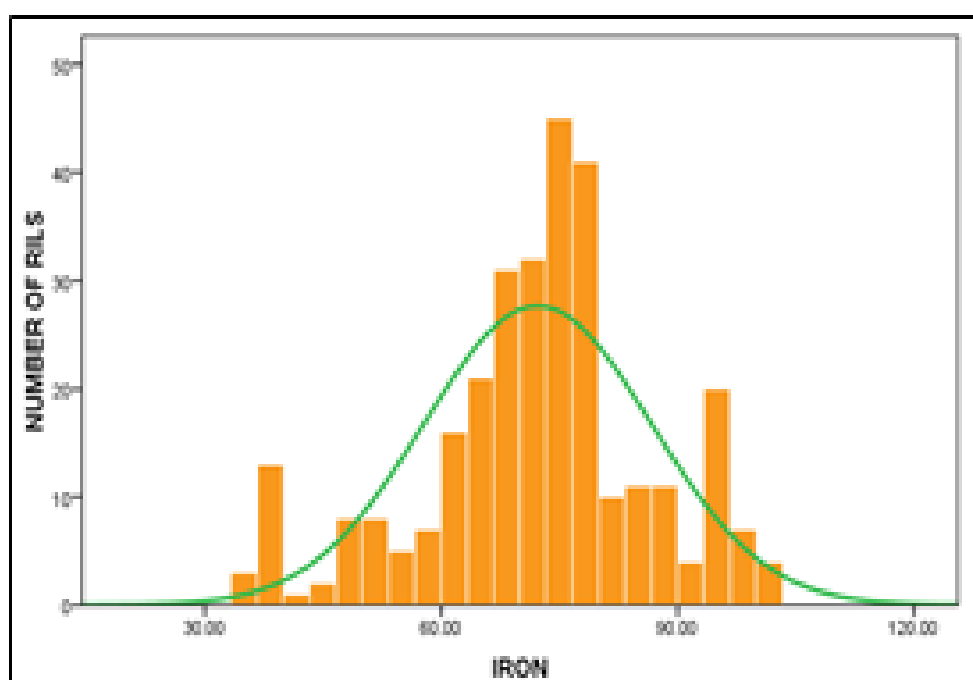
Fig.4.26 Frequency distribution of 300 RILs population derived from the cross GR-11 X Gurjari for Iron concentration
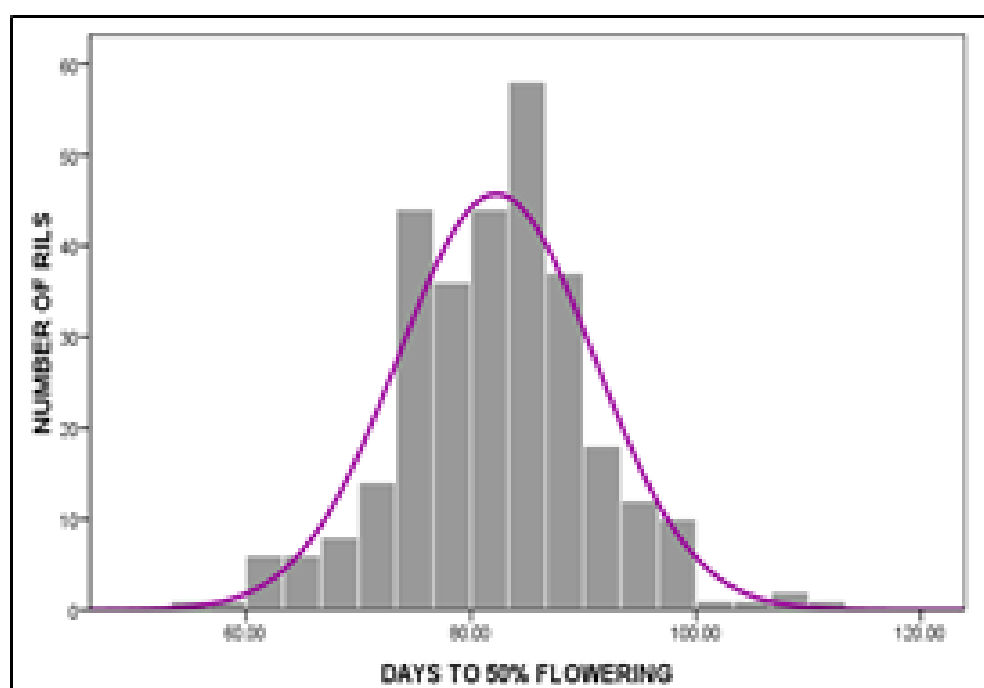
Fig.4.27 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Days to 50% flowering
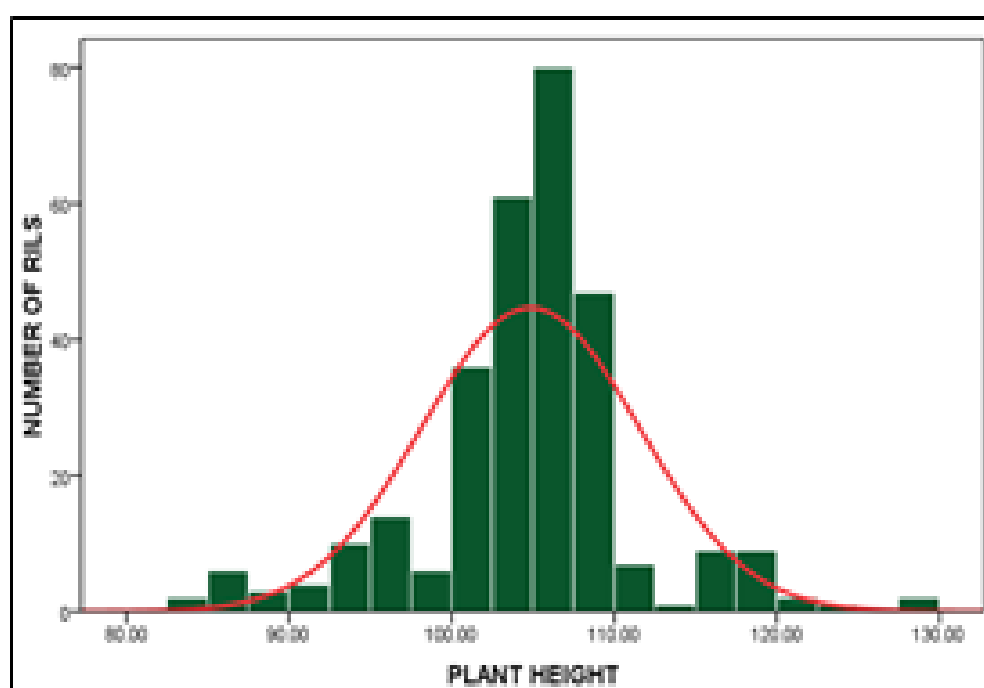
Fig.4.28 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Plant height
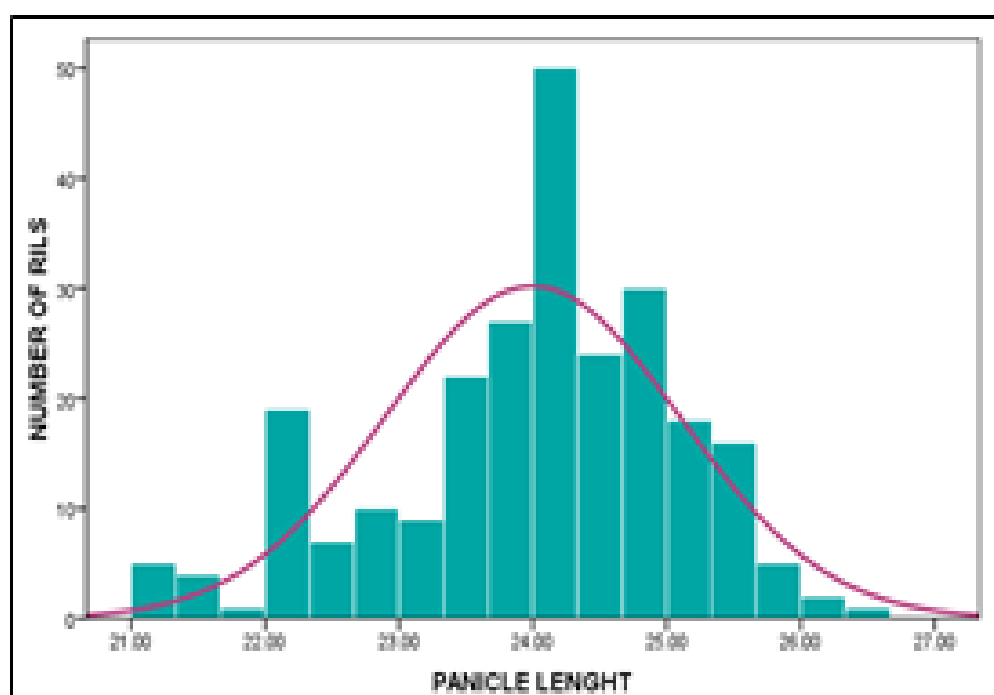
Fig 4.29 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Panicle length
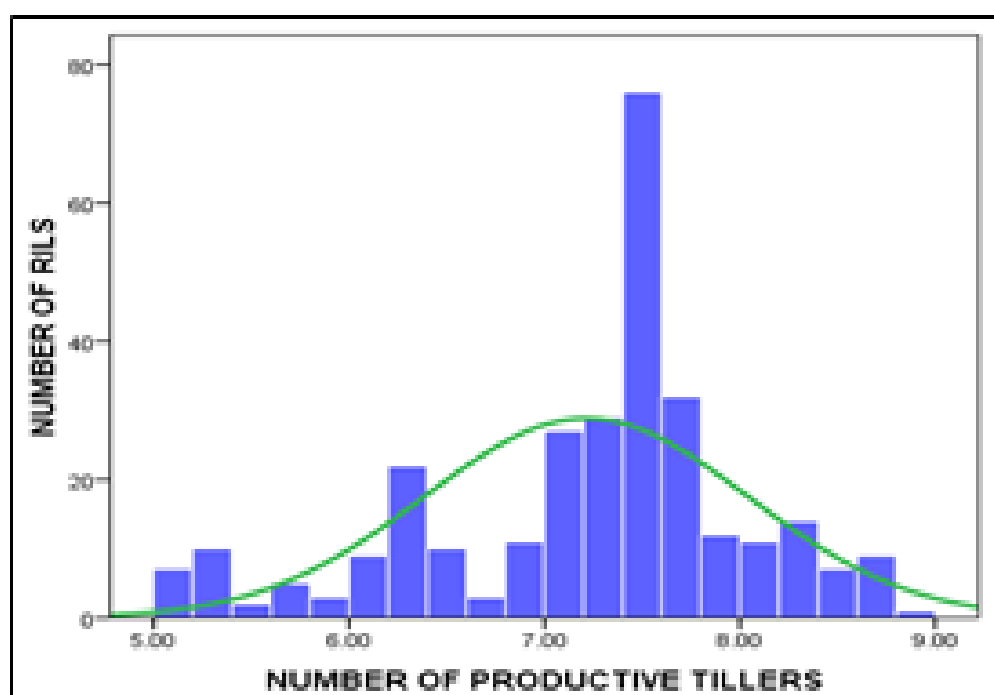
Fig 4.30 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Number of Productive Tillers
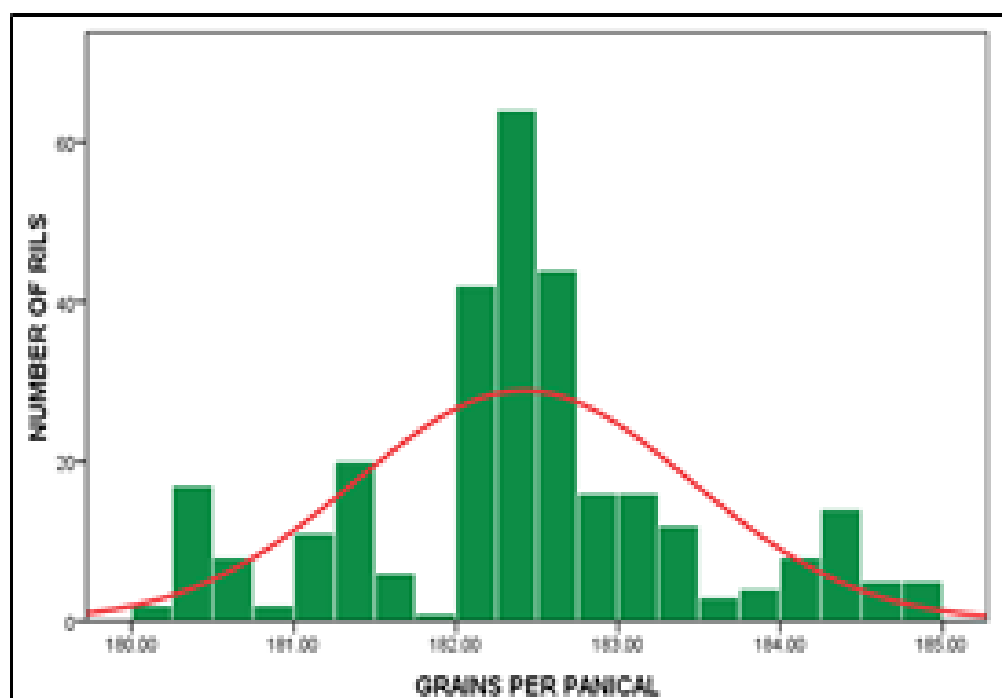
Fig.4.31 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Grains per panicle
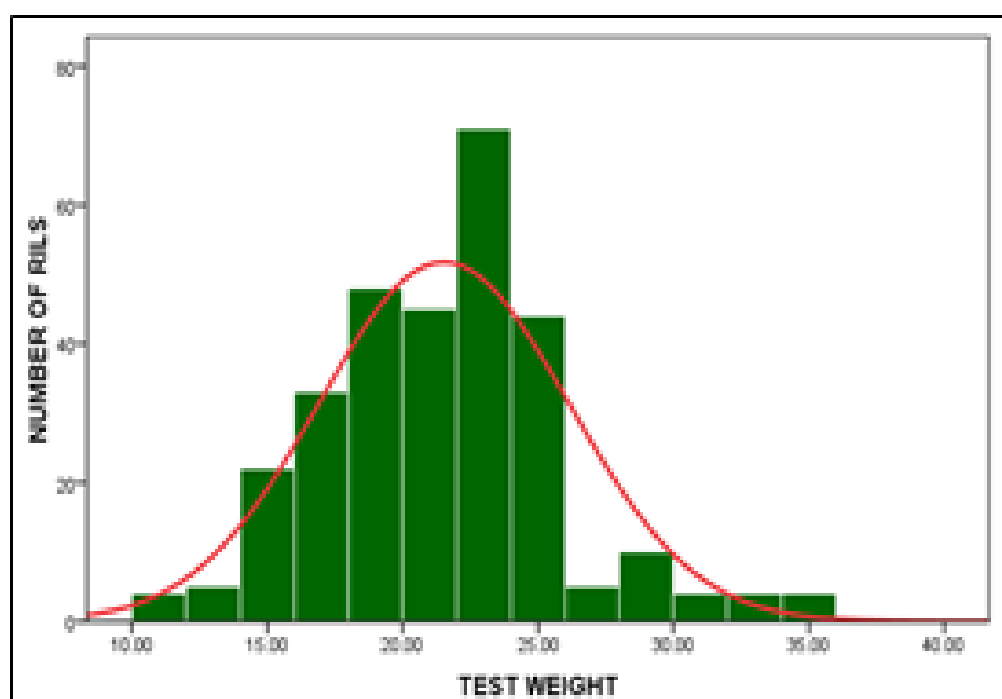
Fig.4.32 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Test Weight
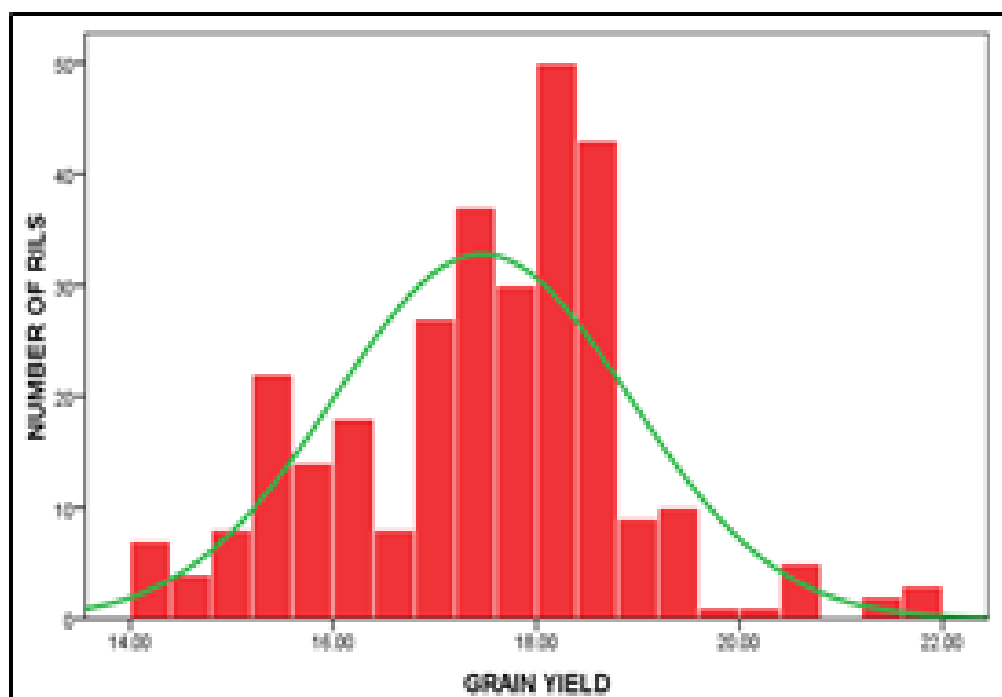
Fig.4.33 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Grain Yield
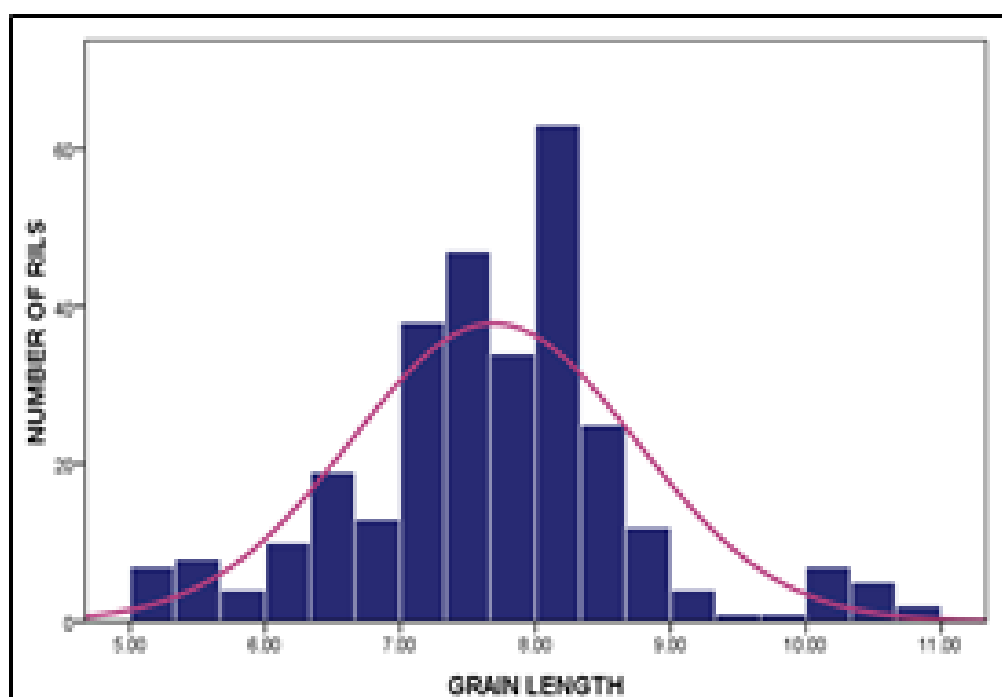
Fig.4.34 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Grain Length
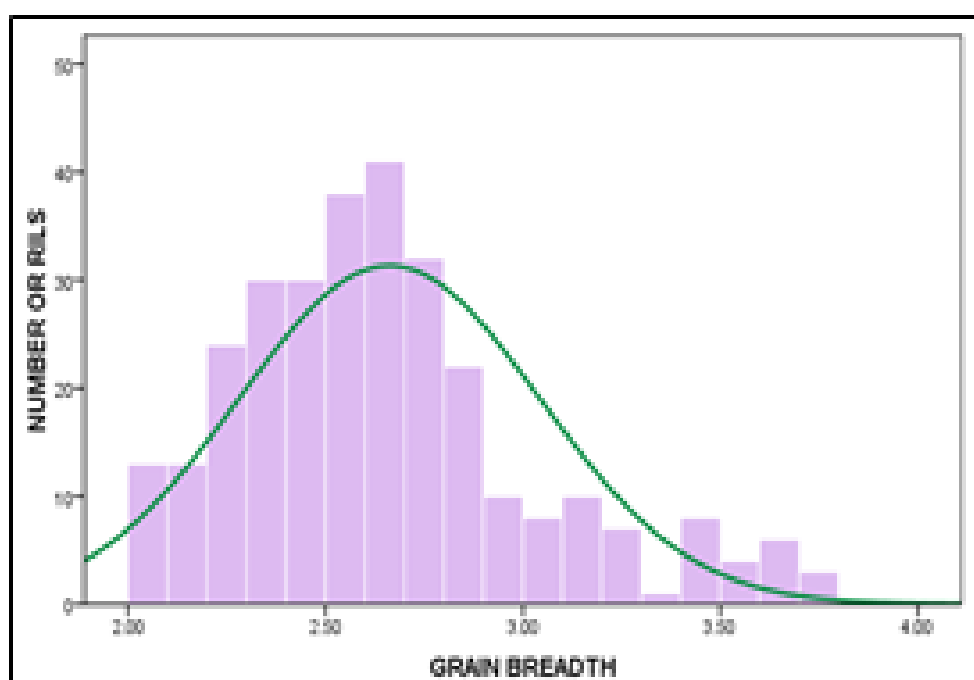
Fig.4.35 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for Grain Breadth
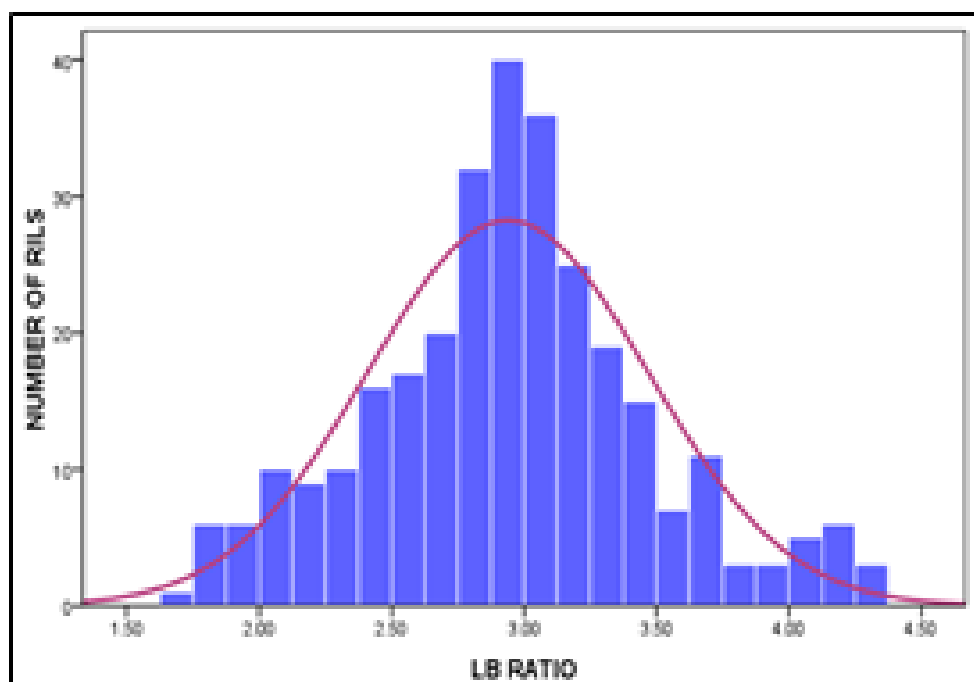
Fig.4.36 Frequency distribution of 300 RILs populations derived from the cross GR-11 X Gurjari for L-B Ratio

The days to 50 % flowering was roughly portioned into two phenotypic classes one with early flowering while the other showing late flowering. In this bi-model distribution of the trait both peaks were distributed normally each with almost similar numbers of genotypes even though the RILs were little bit skewed towards the GR-11 parental values. This bi-model distribution indicates the presence of one major genomic segment influencing the trait, with modifiers than contributing to quantitative variation around the two peaks of these distributions. In contrast for all the other observed traits the phenotypic normal distributions and transgressive segregations in the RIL population indicated polygenic inheritance.

For GR-11 X Krishna Kamod the distribution of RILs followed normal distribution for all the observed traits. All the traits where skewed towards GR-11, except for iron and zinc concentration which was skewed towards Krishna Kamod. Transgression beyond the parental values was observed for all traits. Similar kind of trend was seen as that of GR-11 X Pankhali-203 (Fig. 4.13 to 4.24).

In case of GR-11 X Gurjari, a normal distribution curve was observed for all the traits and the skewness patterns were similar except for Fe and Zn. The latter was again found to be skewed towards the Gurjari (Fig. 4.25 to 4.36). Transgressive segregation was also observed beyond the parental values, for all the traits observed. For plant height, grain length and grain breadth there was a non significant difference between the parents, and hence a narrow range of trait distribution in most of the RILs remained significantly closer to the values of the parents.

By using immortalized mapping populations known as recombinant inbred lines (RILs), derived from divergent accessions cross, individual genes can be resolved into homozygous progenies. Plotting a histogram using a phenotypic data of such a population, the number and the size of the phenotypic classes obtained is directly related to the number of genes influencing the observed trait (Rao *et al*., 2007). Hence, from the preliminary observations of the histogram the approximate number of genes responsible for each trait can be predicted. If the histogram is normally distributed it indicates the presence of additive effects with mid parental value as the mean of population while the presence of asymmetry in the distribution or skewness in plot with transgressive segregation is indicative of the epistatic interaction (Pooni and Jinks, 1982; Dickinsons *et al*., 2003). Presence of transgressive

segregation also indicates the occurrence of genetic recombination (Falconer 1989), which points out that both favorable and unfavorable alleles for the trait studied are already scattered between the parents.

A segregating population with heterozygosity can show effects of additive gene action with over dominance and epistasis (Cho *et al*., 2002), therefore superiority transgressive individuals of will not be maintained in successive generations. However, the populations used for this study were sets of nearly homozygous inbred lines after a large number of generations of selfing. Therefore, the transgressive segregation detected in these populations is more likely to be due to additive effects of polygenes for which favorable alleles were contributed from both parents. In all three populations, histogram revealed continuous distributions for the traits studied, which indicated the polygenes inheritance of traits.

4.3. MOLECULAR ANALYSIS

4.3.1. Parental Polymorphism

A total of 600 SSR markers (RM series) were chosen based on their distribution throughout genome and 52 gene specific markers designed from the genes associated with uptake, transport and remobilization of mineral nutrients in rice. Among these 600 SSR markers, 229 (38.00%) were polymorphic and among the 52 gene specific markers, 33 (63.46%) were polymorphic. In all, 325 (51.34%) markers distributed on all the 12 chromosomes were polymorphic between the parents, indicating the possibility of constructing a linkage map. The parental polymorphism was detected in the gel electrophoresis among the four parental lines used to develop the mapping populations (Plate 1).

In a study on microsatellite polymorphism in rice by Shankar and Sarla (2010), out of 112 markers selected for screening, 33 (29.4%) were found to be polymorphic. Several reports indicated the narrow genetic variability for mineral elements in cultivated rice, whereas a higher level of mineral elements was observed in wild rice *O. rufipogon* (Cheng *et al*., 2005). In earlier reports of mapping yield QTLs using single accession of *O. rufipogon* (IRGC105491) as donor parent and different *O. sativa* accessions revealed 60 to 90 % polymorphism (Xiao *et al*., 1998; Septiningsih *et al.,* 2003; Thomson *et al*., 2003 and Marri *et al*., 2005).
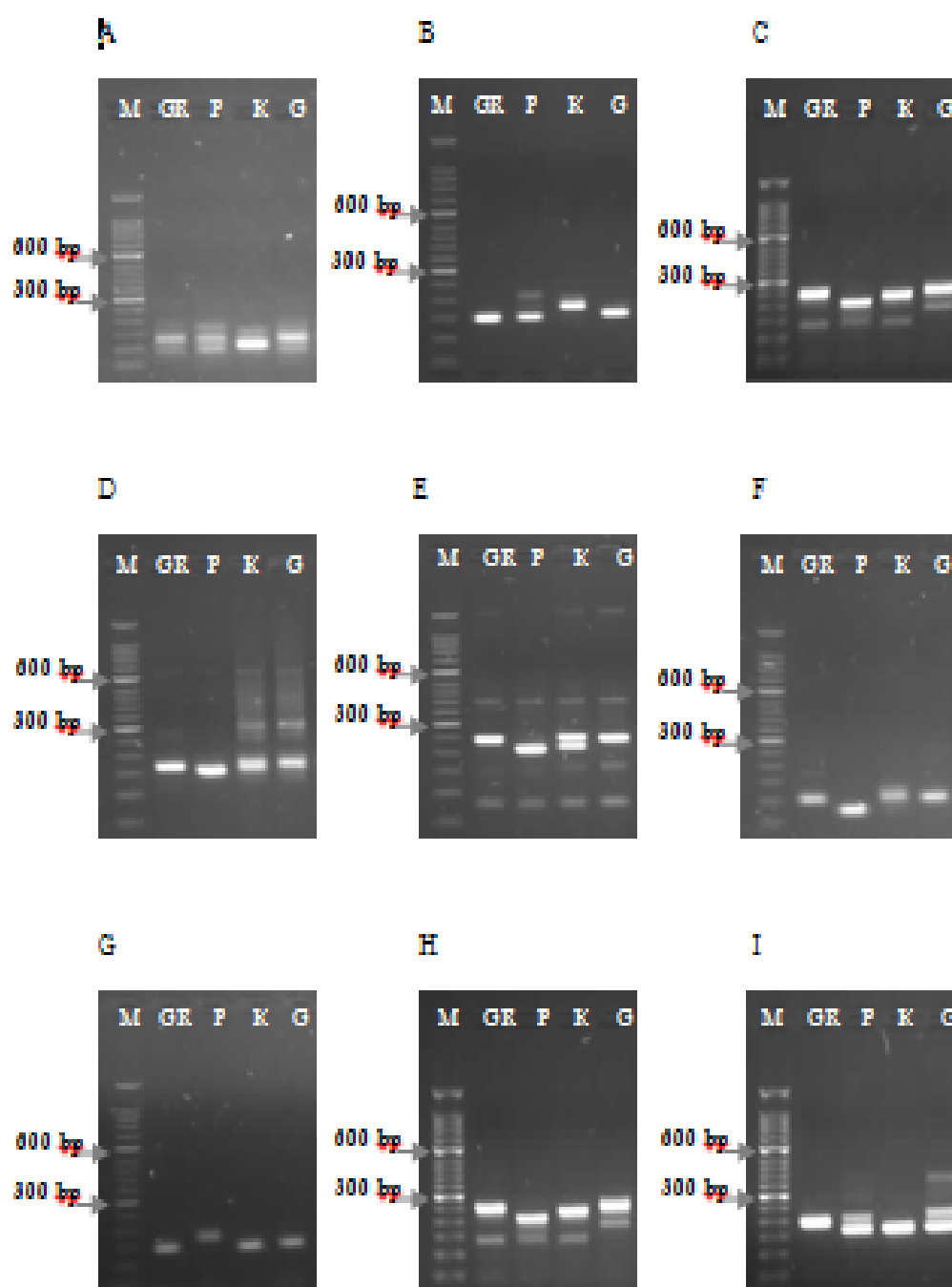
Plate 4.1: SSR markers showing polymorphism among four parents.

A. RM19 B. RM512 C. RM330 D. RM3331 E. RM496 F. RM22565 G. RM346 H. RM72 I. RM112

M- 50 bp ladder, GR: GR-11, P: Pankhali-203, K: Krishna Kamod, G: Gurjari

The pioneering work of Nilsson-Ehle (1909) almost 100 years ago gave an idea that continuous variation in trait performance is due to the joint segregation of several genes and the interaction of the environment, and all of the genes with a small but quasi-additive effects together produce a phenotype. The genes responsible for such traits were originally called polygenes by Mather (1941) but are now generally referred to as quantitative loci or QTLs (Gelderman, 1975). Till the birth of DNA based molecular markers in the late 1980s (Lander and Botstein, 1989), the determination of number, position and arrangements of genomic regions/QTLs controlling a polygenic trait and their effects with interaction were difficult. QTLs can also be mapped by following their co-segregation with molecular markers, which are responsible for much of the progress in the area of polygenic-controlled expression of the traits in the recent year. The advent of marker system to construct considerably dense linkage maps across nuclear genomes of segregating populations, combined by powerful biometric methods, has led to considerable progress in QTL mapping in plants as well as other animals. In the present study SSR and gene specific markers were used to construct three linkage maps for RIL populations and QTLs for studied traits were mapped.

4.4. GENOTYPING OF THE MAPPING POPULATIONS

Out of the 600 molecular markers, 232 were used for screening the entire mapping population derived from the cross GR-11 X Pankhali-203 along with parents which included 32 gene specific markers. Segregation pattern of 300 RILs with representative primers RM 1748, RM 180, RM512, RM484 and RM 423 were depicted in Plate 4.2A,B; 4.3A,B; 4.4A,B; 4.5A,B and 4.6A,B. Genotypic data was used for linkage analysis after scoring the bands obtained for each marker separately in the 300 RIL population.

For the 250 RIL population derived from the cross based GR-11 X Krishna Kamod, the mapping population was screened with the polymorphic 258 microsatellite and 32 gene specific markers. Segregation pattern of the 250 RILs along with the parental lines with the representative markers RM209, RM21, RM242, RM206, RM514, RM219, RM314, RM224, RM470 and OsYSL15 were depicted in the Plate 4.7A,B to Plate4.16A,B. Genotypic data was used for the linkage analysis

after scoring the bands obtained for each marker separately in the 250 RIL populations.

In case of the cross based on GR-11 X Gurjari, 262 markers were screened among all the 300 RIL populations of which 33 were genes specific markers while 229 were the microsatellite markers. Segregation pattern of the 300 RIL populations with the representative markers OsYSL7, OsNAC5, OsYSL5, RM501 and RM595 were depicted in the Plate 4.17A,B to Plate 4.21A,B. Genotypic data were then scored for the presence and absence of the bands among these 300 RIL populations separately for each markers and were used for linkage analysis.

4.4.1. Segregation Distortion

The deviation of genetic segregation ratios from their expected Mendelian fraction is known as segregation distortion (Lyttle, 1991). For each population, the 1:1 expected Mendelian Segregation ratios of SSR and gene specific markers were analyzed using χ^2 test in MapDisto software (Table 4.8 to Table 4.10). If the percentages of the more common alleles are higher than expected, they were assigned as skewed loci towards the favorable allele. In the absence of segregation distortion, equal frequencies of the parental classes are expected. In all the three populations a good number of loci were skewed towards either the male or the female parental alleles.

4.4.1.1. Segregation Distortion in the GR-11 X Pankhali-203 based cross

Out of 36 mapped markers on LG1, 6 SSR and 1 gene specific marker were skewed and 5 of which are skewed in favor of GR-11 allele. The 7 distorted markers were equally distributed across LG1. Out of 26 markers harbored by LG2, 4 exhibited significant deviations favoring GR-11 allele while the remaining 22 (17 SSR and 5 gene specific) of LG2 were normally segregating. In case of LG3, out of 25 mapped markers, 24 % (i.e. 4 SSRs and 2 genes specific) were skewed in favor of GR-11 allele.

Plate 4.2A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM1748 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Pankhali-203 allele homozygote
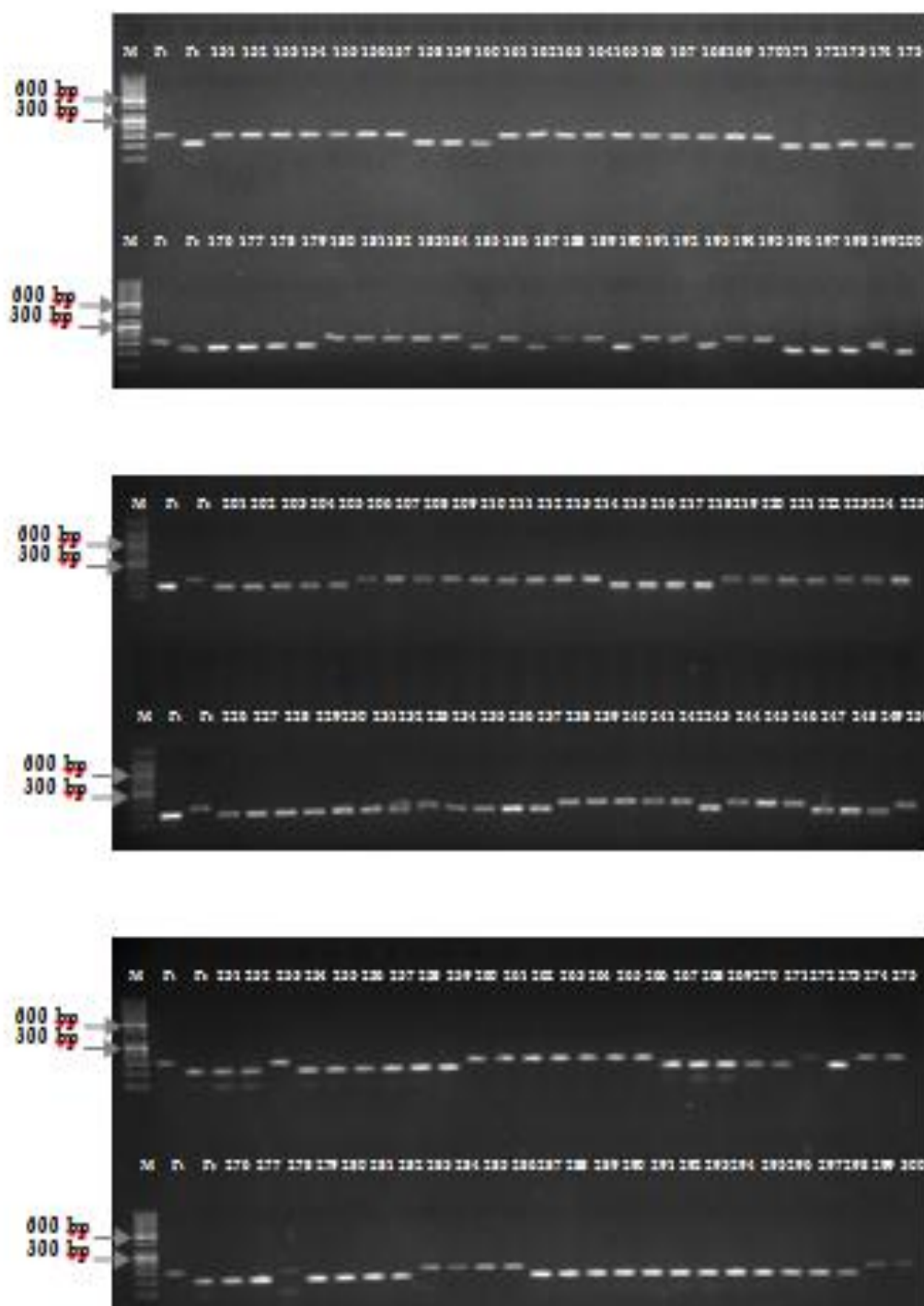
Plate 4.2B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Pankhali-203 with RM1748 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
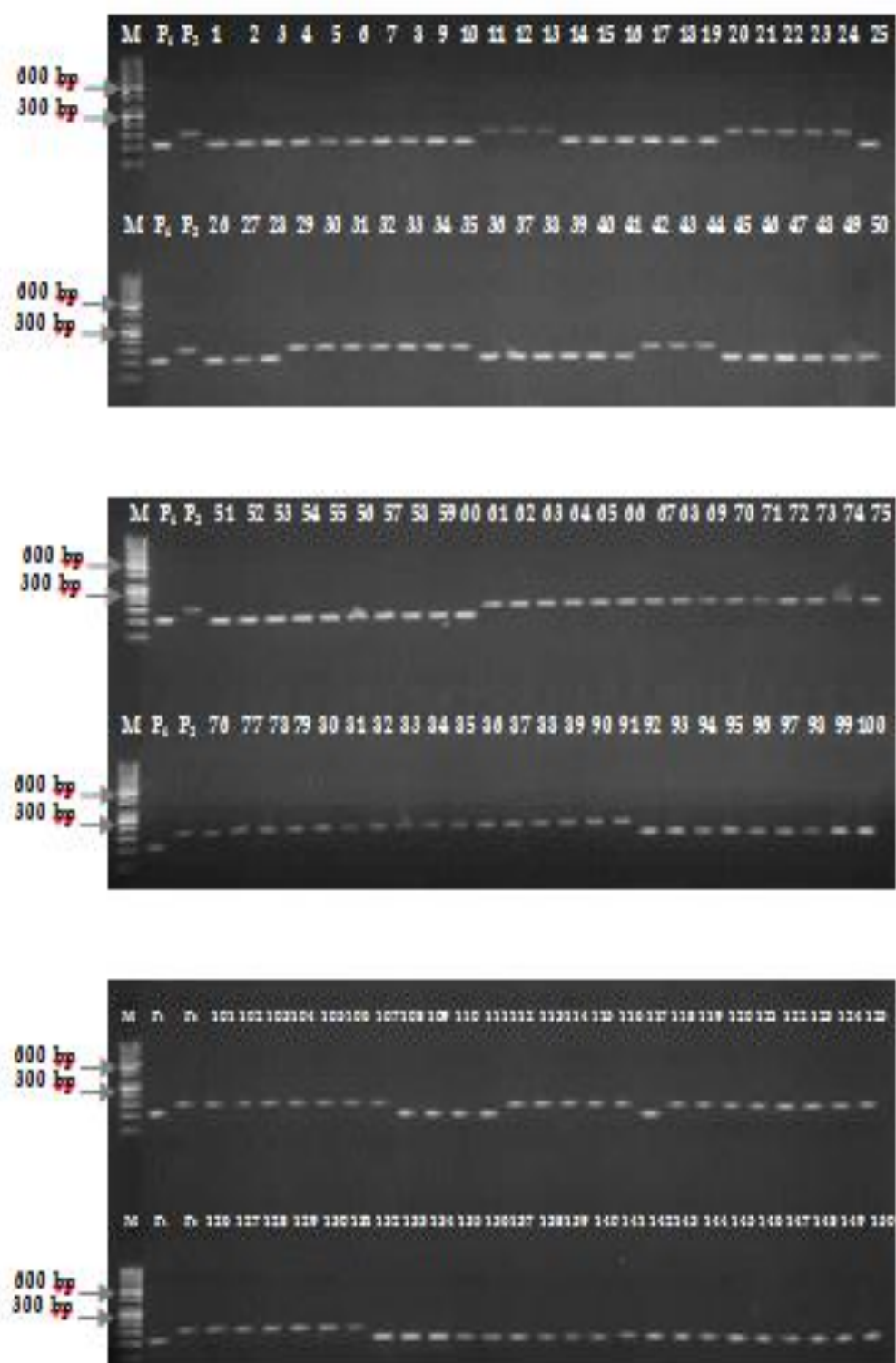
Plate 4.3A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM180 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
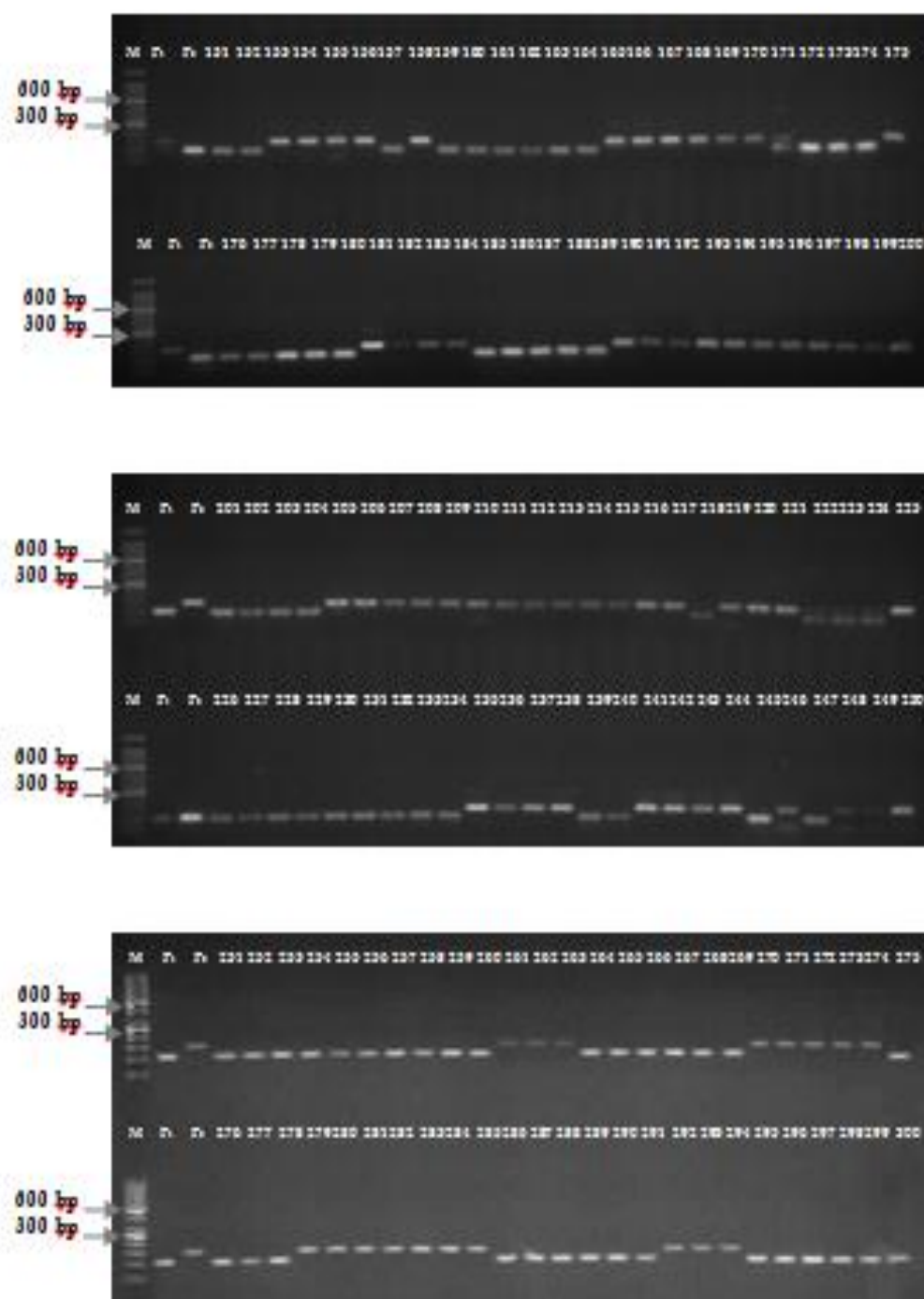
Plate 4.3B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Pankhali-203 with RM180 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
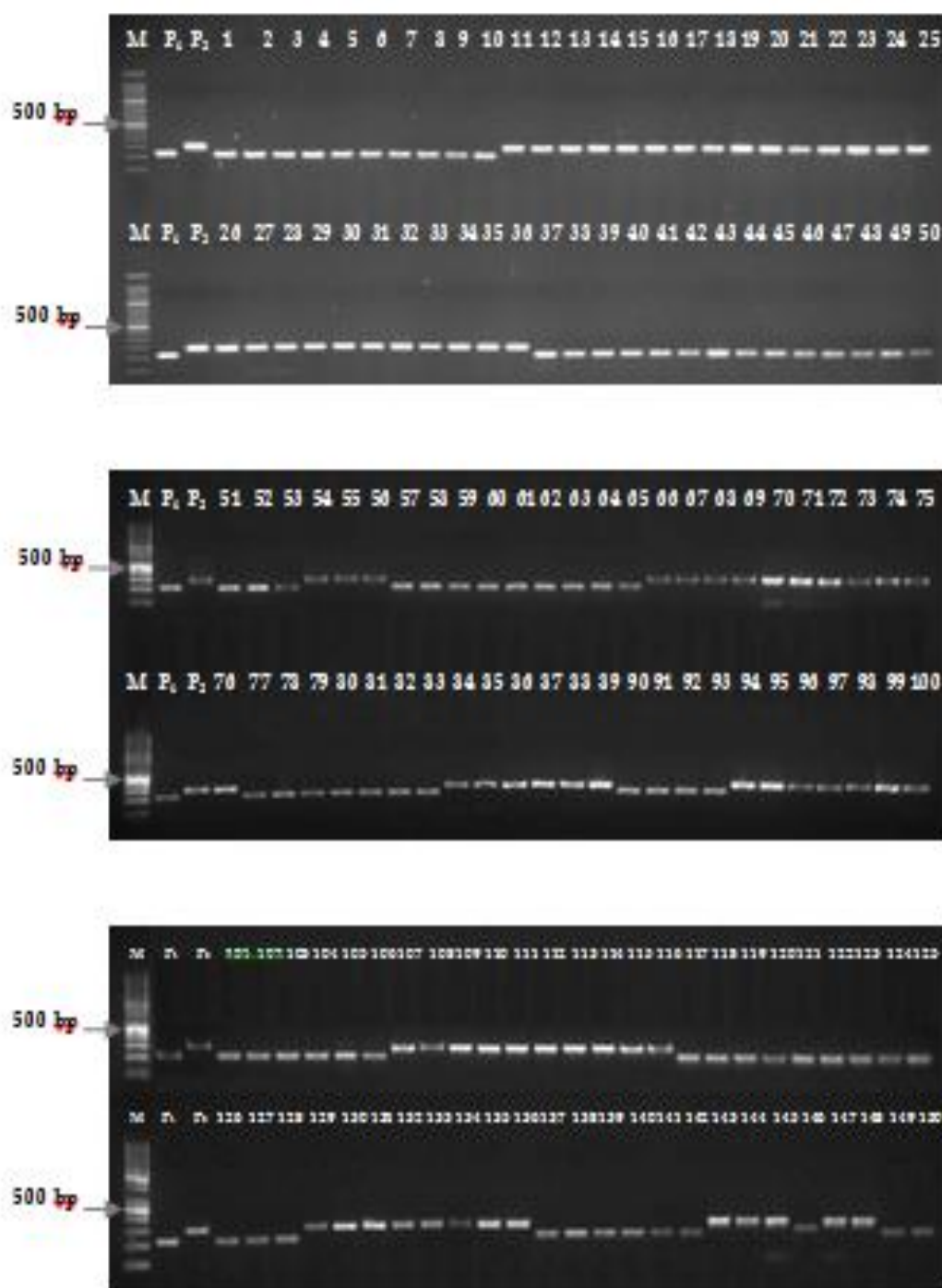
Plate 4.4A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM512 on 4.5 % metaphore gel

M: 100 bp marker; P₁:GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
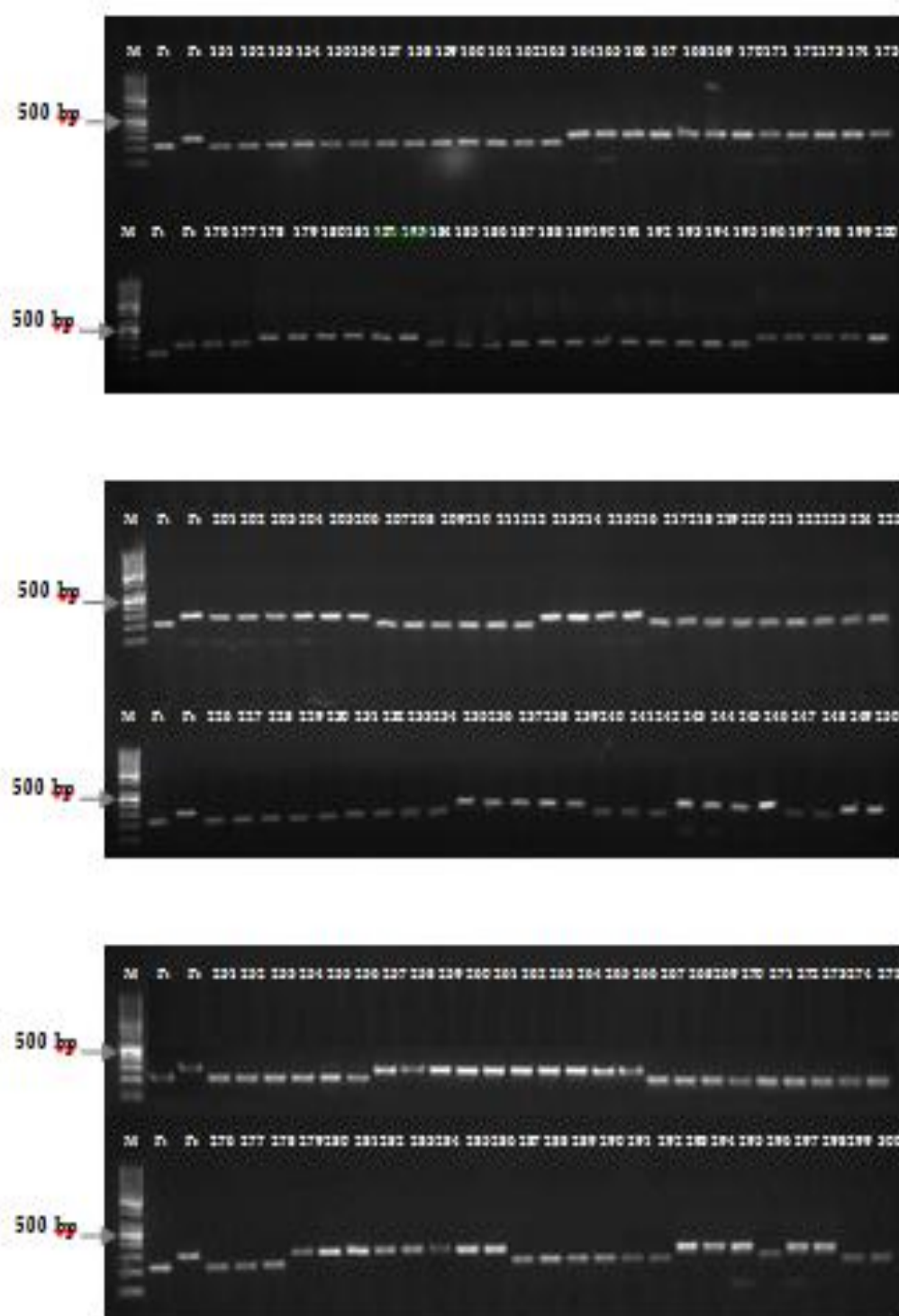
Plate 4.4E: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Pankhali-203 with RM512 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
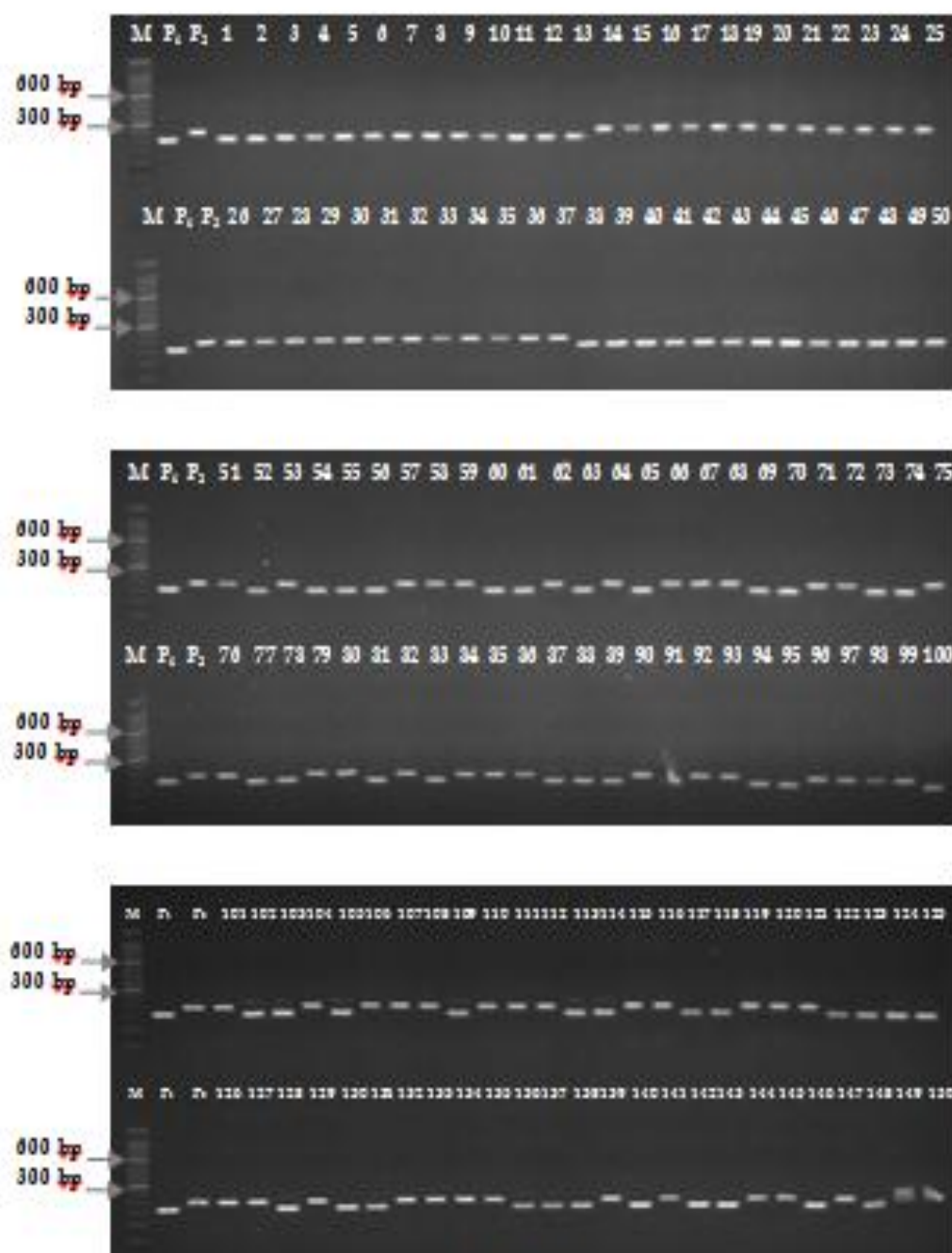
Plate 4.5A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM484 on 4.5 % metaphore gel

M: 50 bp marker; P₁:GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
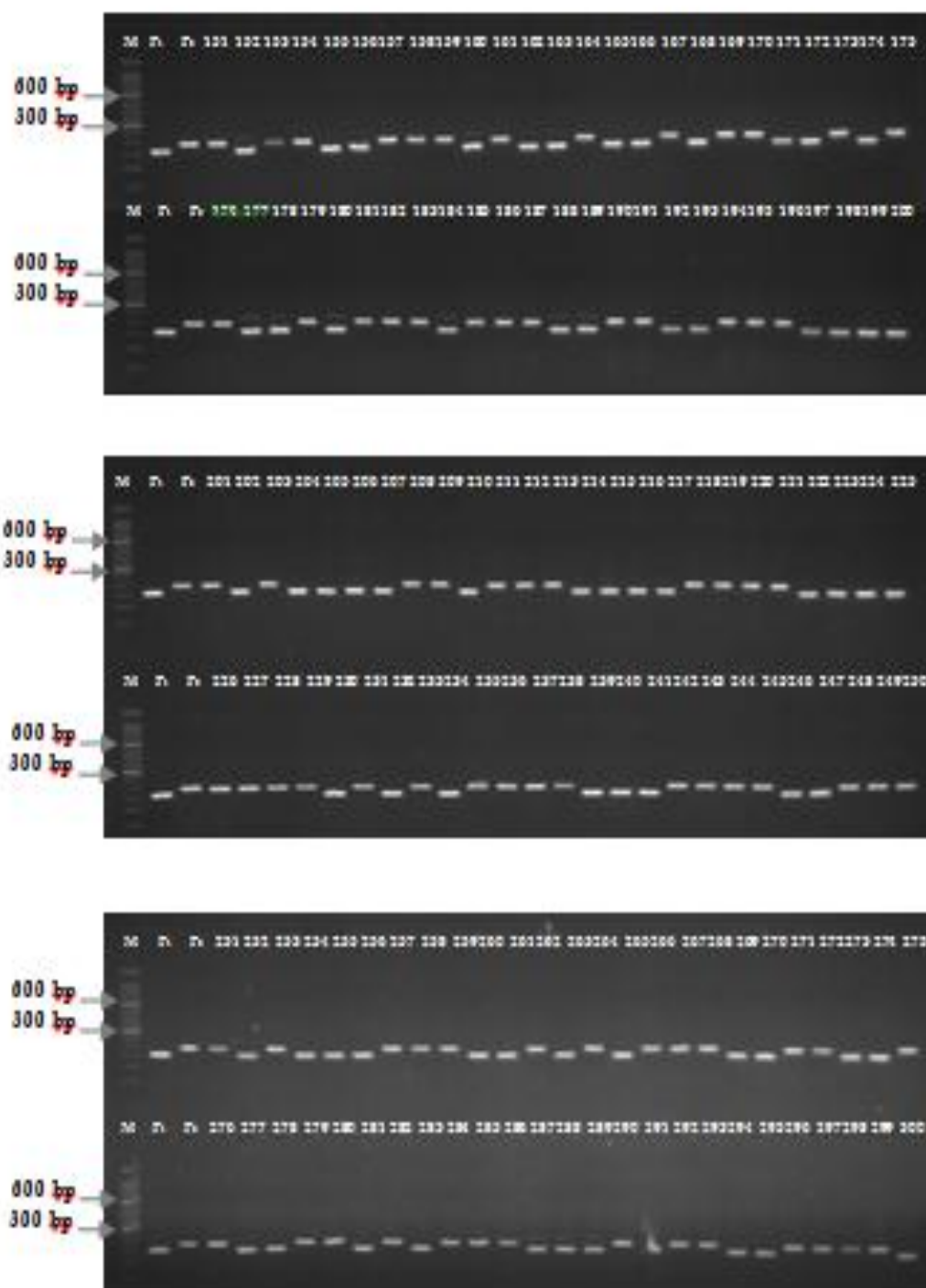
Plate 4.5B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Pankhali-203 with RM484 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Pankhali-203 allele homozygote
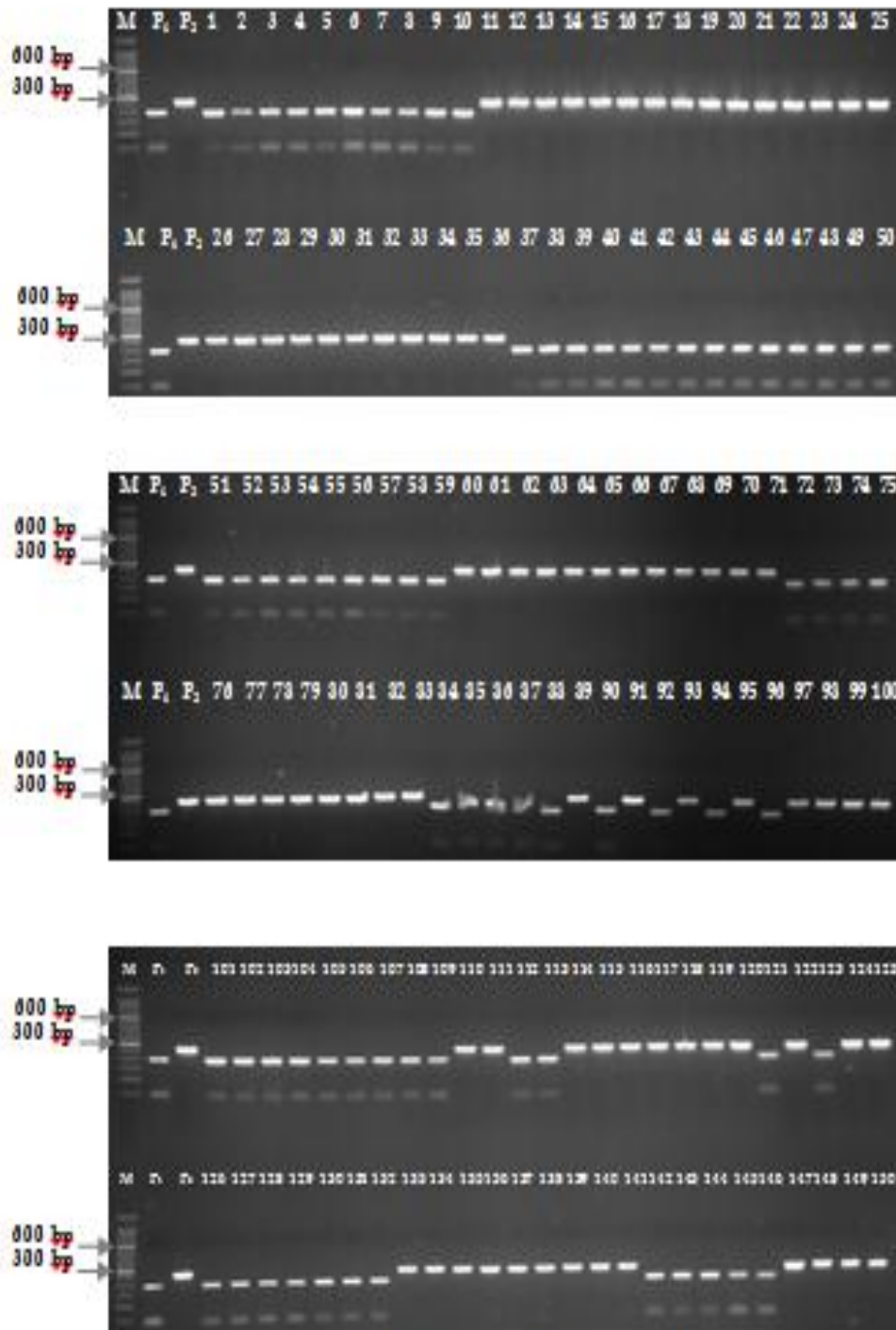
Plate 4.6A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM423 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Pankhali-203 allele homozygote
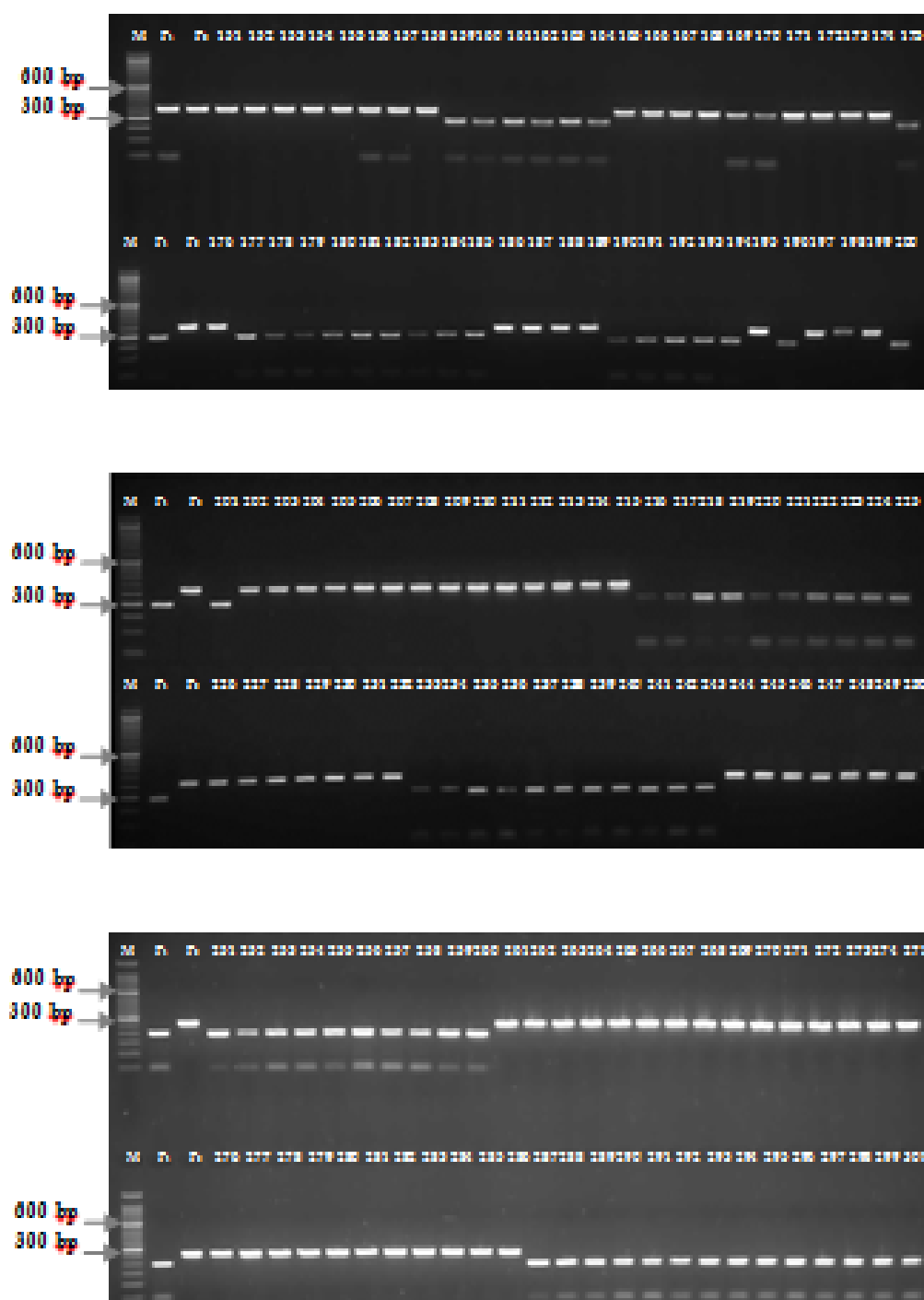
Plate 4.6B: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Pankhali-203 with RM423 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Pankhali-203 allele homozygote
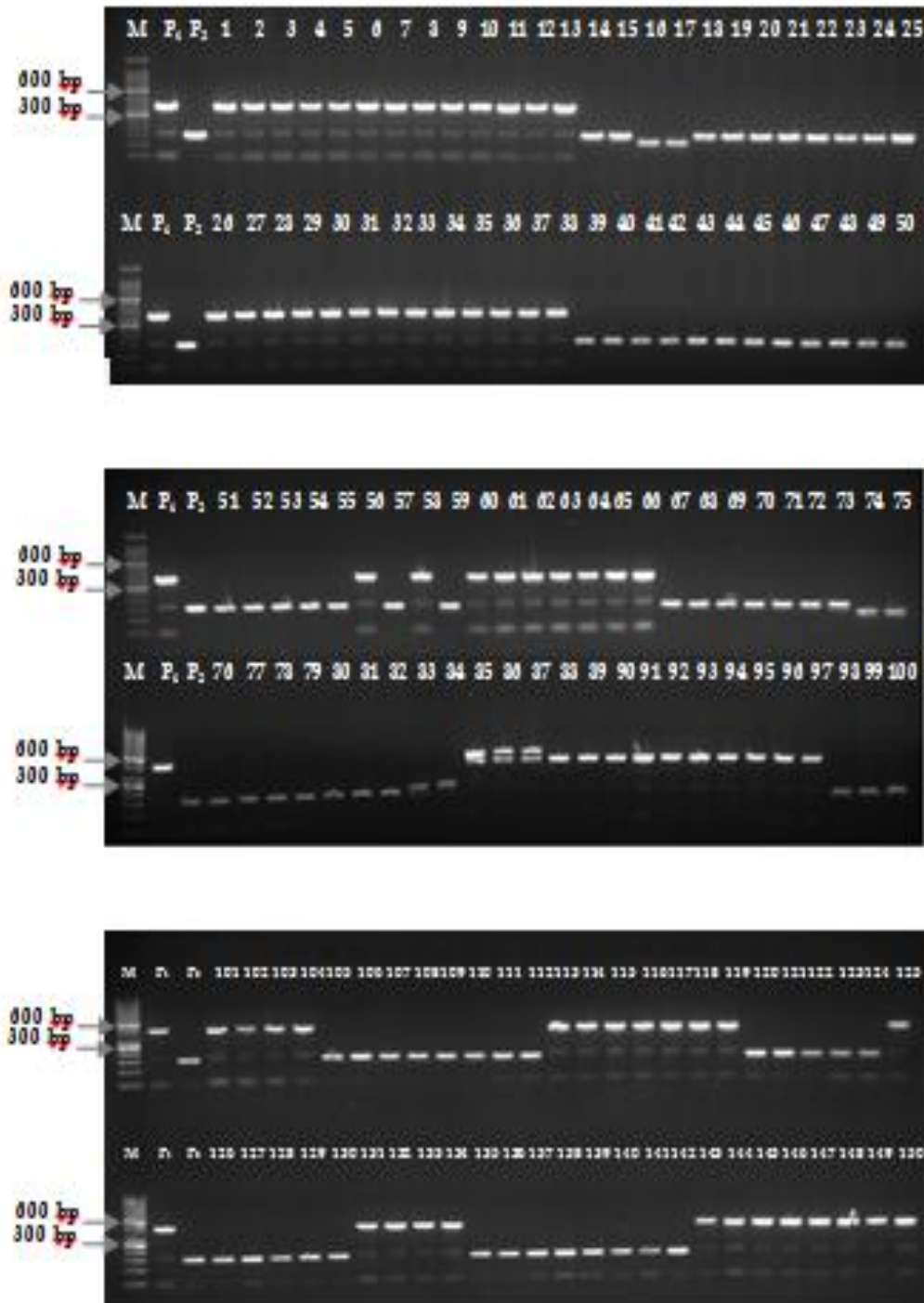
Plate 4.7A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM209 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Krishna Kamod allele homozygote
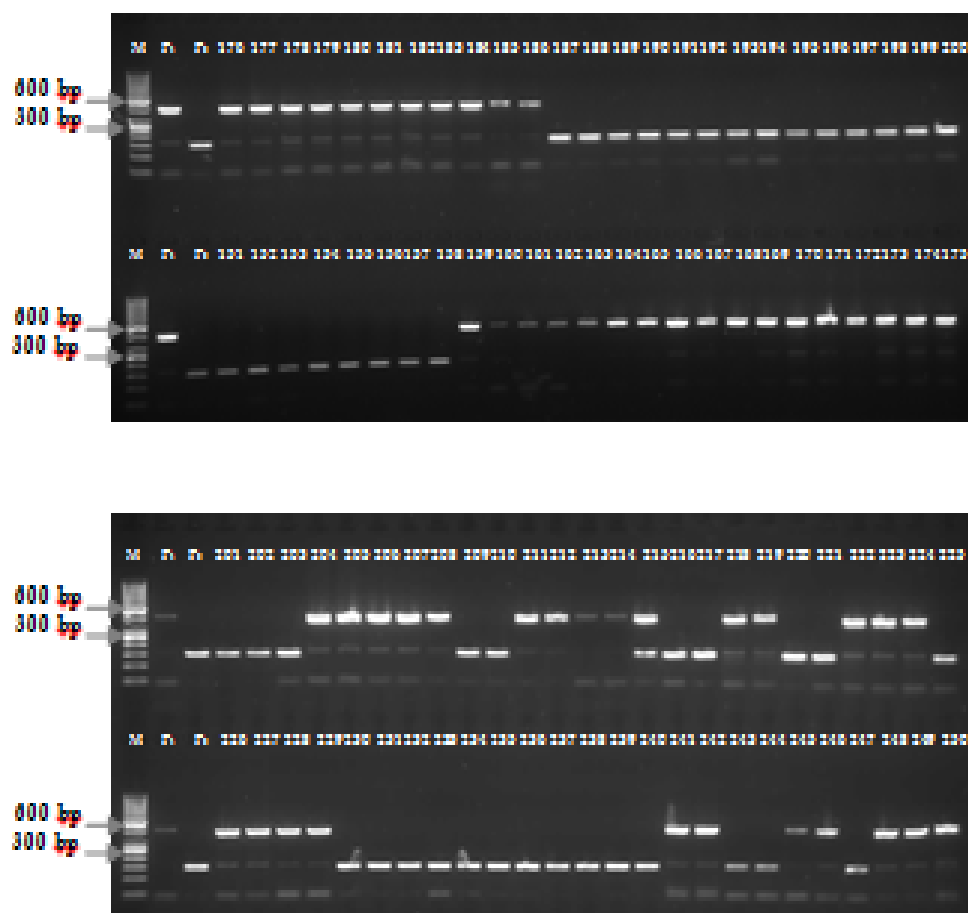
Plate 4.7B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with RM209 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote
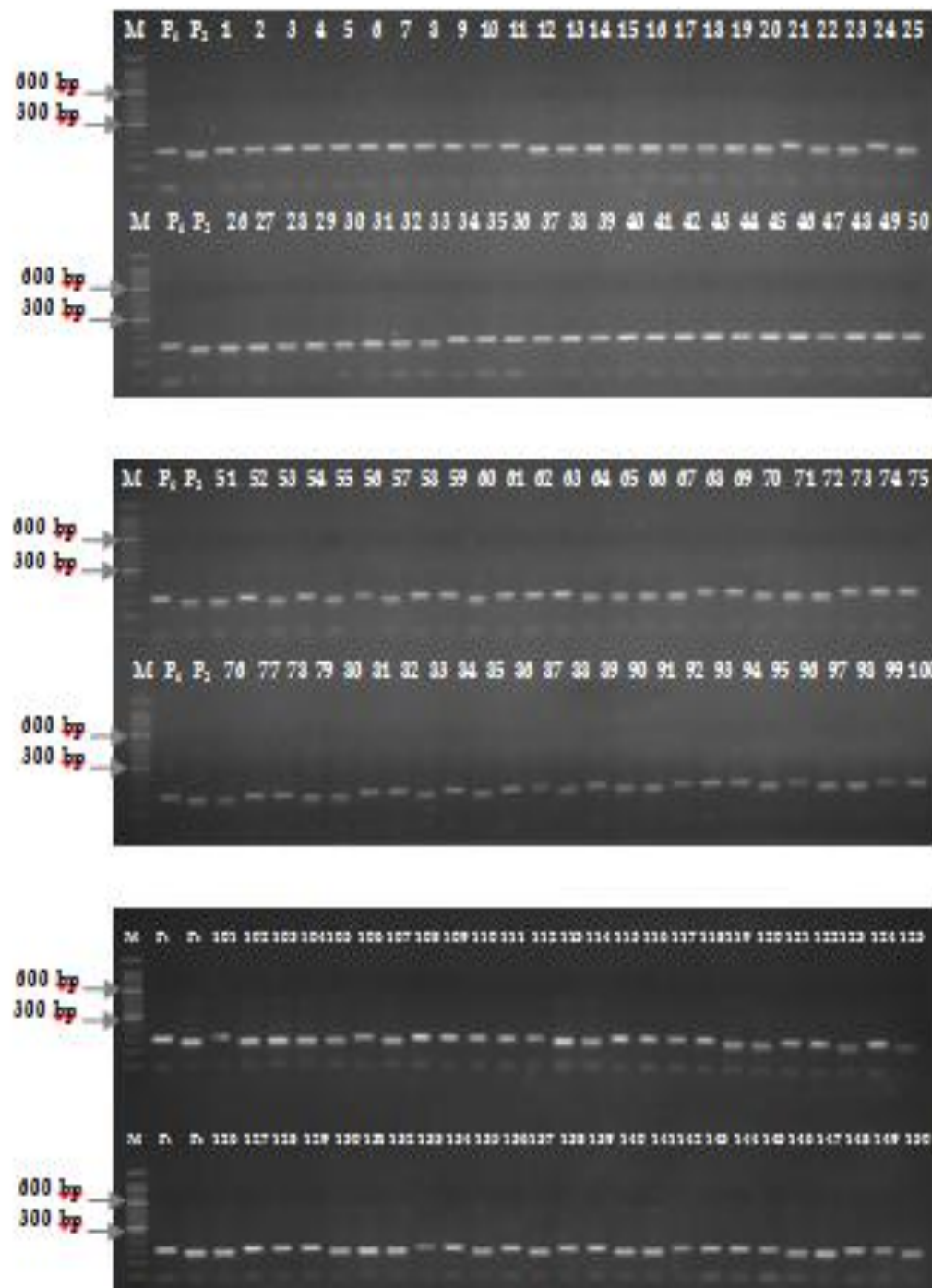
Plate 4.8A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM21 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.8E: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with RM21 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote
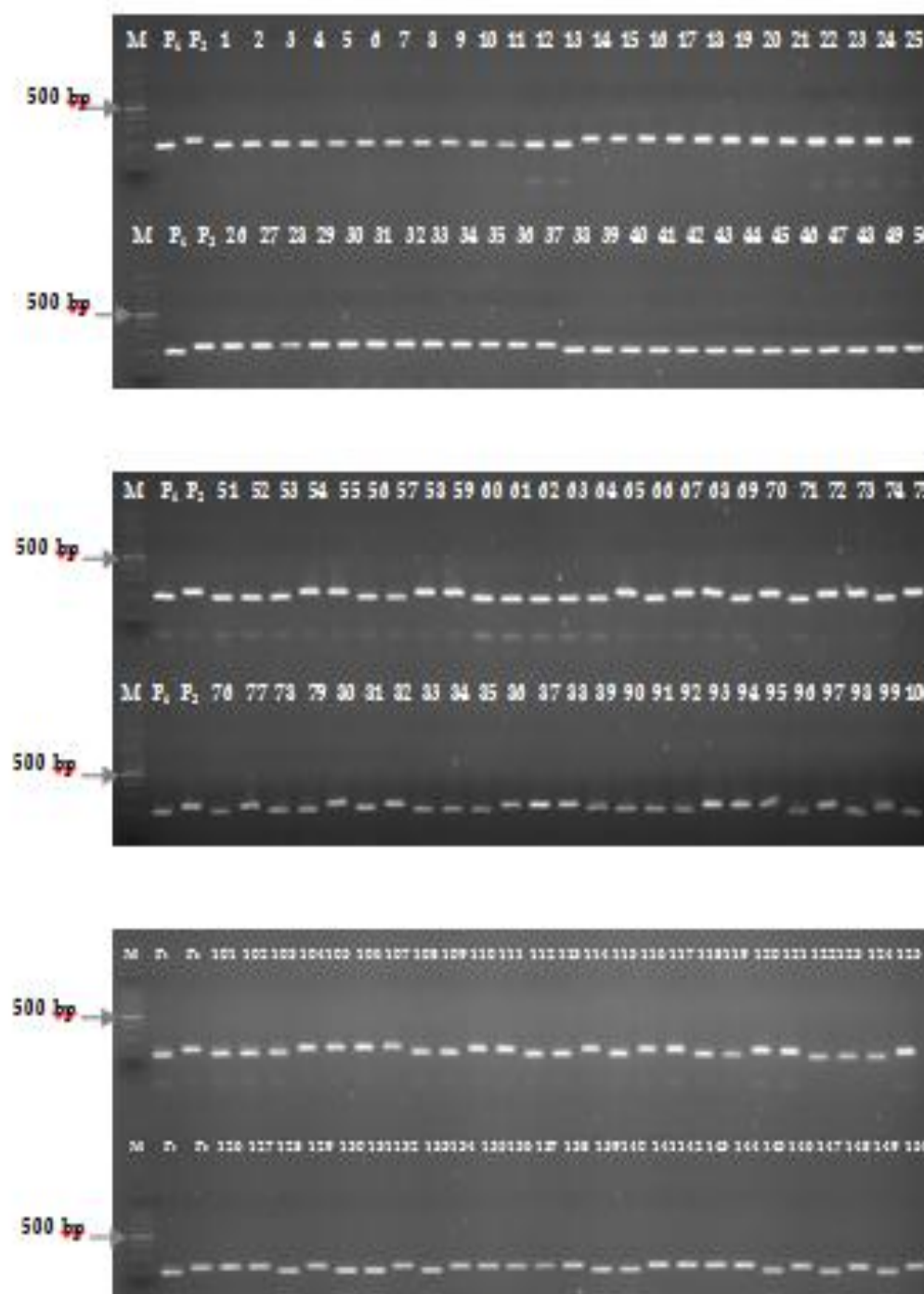
Plate 4.9A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM242 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.9B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with RM242 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote
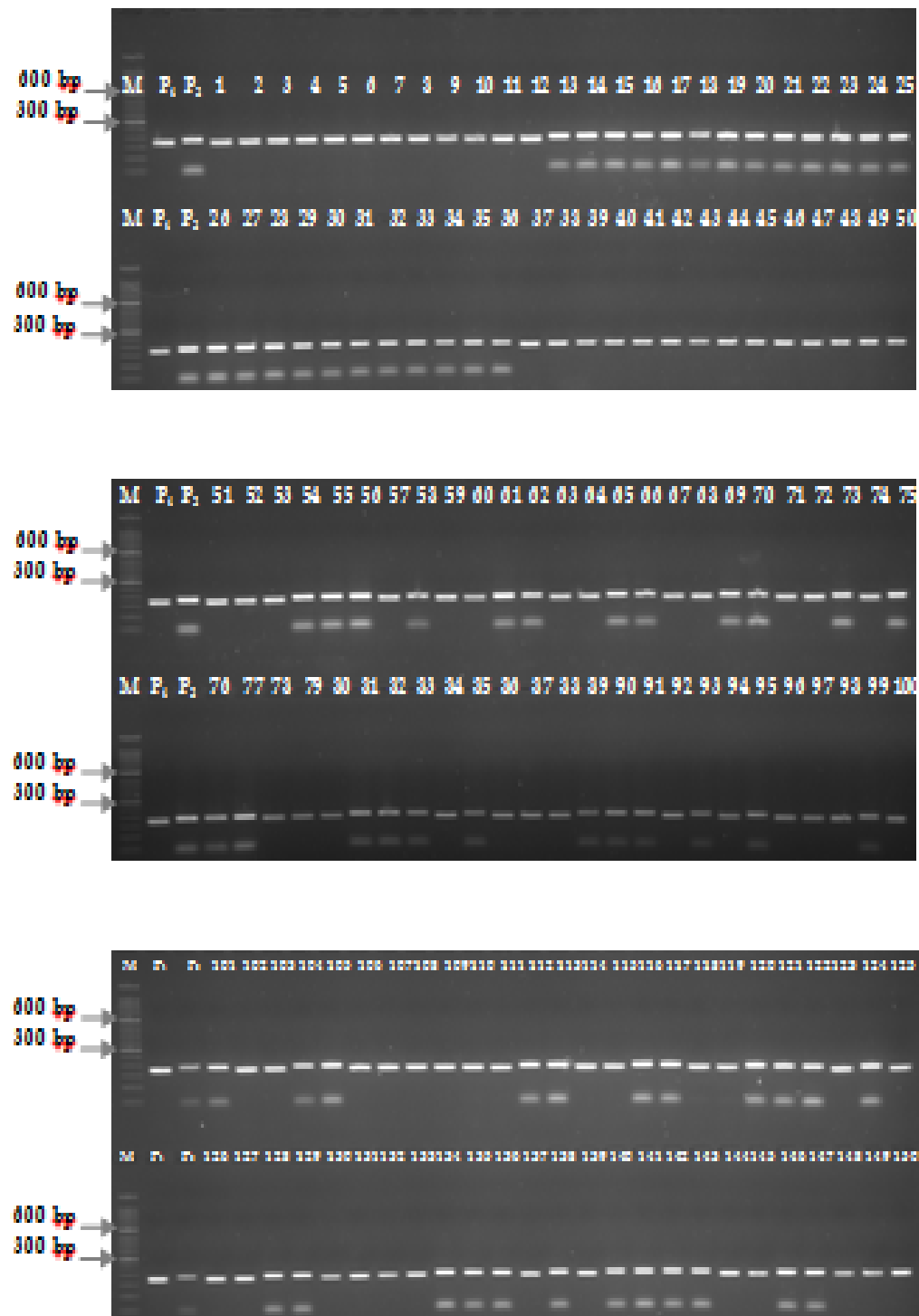
Plate 4.10A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM206 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.10B: Segregation pattern of 250 (150-250) RIL populations based on cross
GR-11 X Krishna Kamod with RM206 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele
homozygote
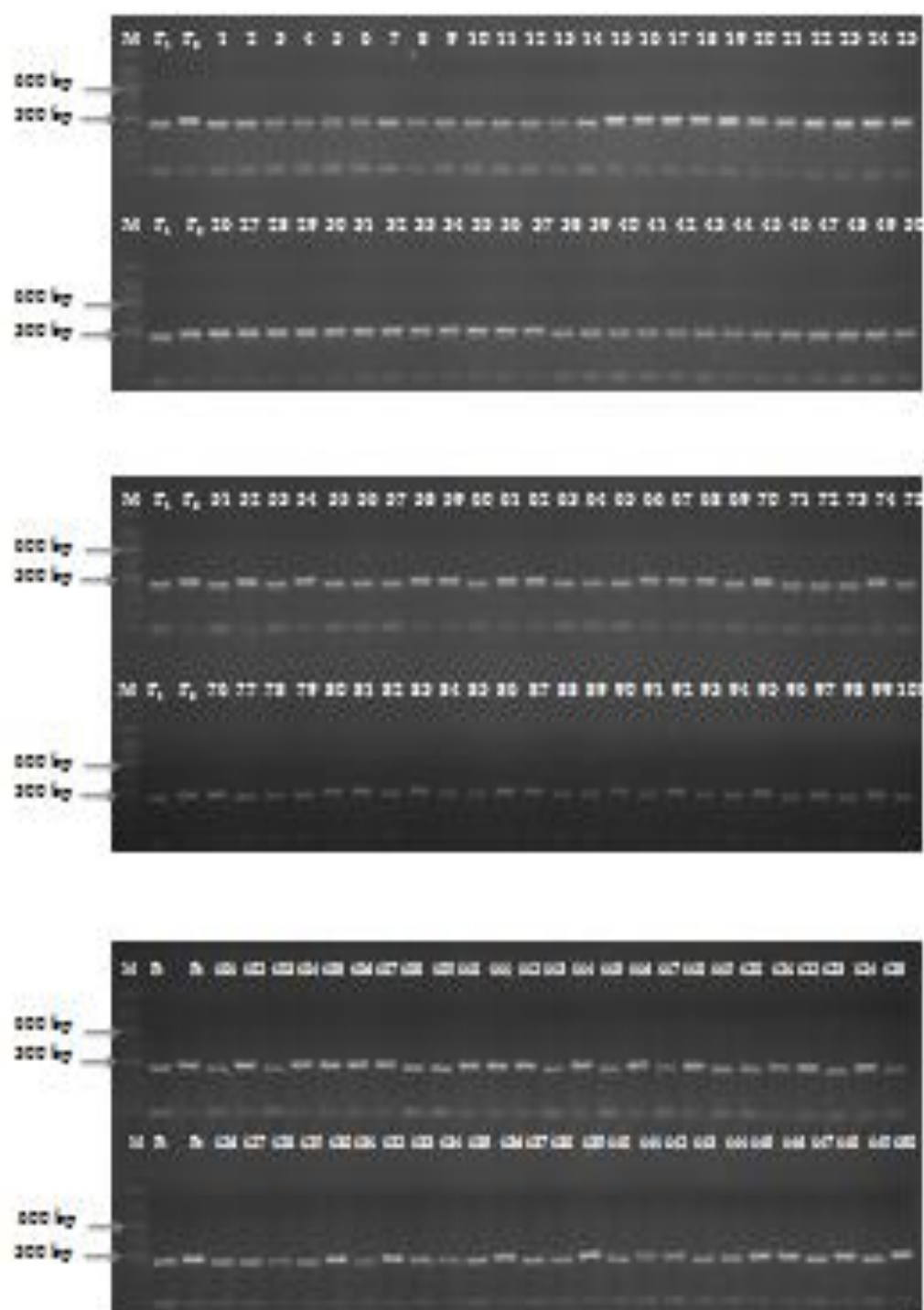
Plate 4.11A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Hamsa with RM514 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Krishna Hamsa allele homozygote

Plate 4.11B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Hamsa with RM516 on 4.5 % metaphor gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Hamsa allele homozygote
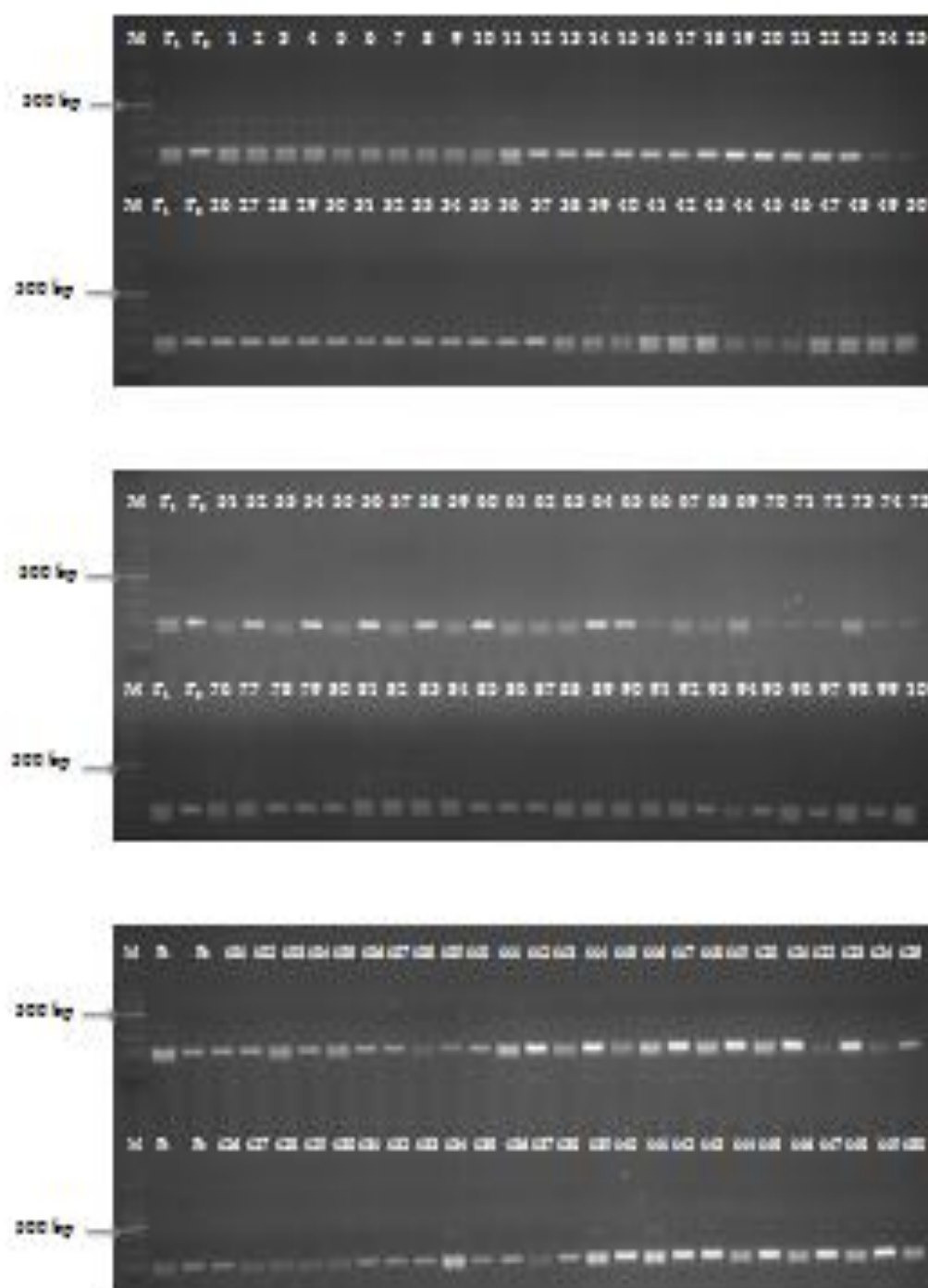
Plate 4.12A: Segregation pattern of 150 (1-150) RIL populations based on cross GR-11 X Krishna Hamod with RM219 on 4.5 % metaphore gel

M: 100 bp marker; P1: GR-11 allele homozygote; P2: Krishna Hamod allele homozygote

Plate 4.12B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Hamsa with RM219 on 4.5 % metaphore gel

M: 100 bp marker; P_1: GR-11 allele homozygous; P_2: Krishna Hamsa allele homozygous

Plate 6.12A: Segregation pattern of 250 (1-250) RIL populations based on cross GR-11 X Krishna Hamsad with RM514 on 6.5 % moraphore-gel

M: 50 bp marker; P_1: GR-11 allelic homozygote; P_2: Krishna Hamsad allelic homozygote

Plate 4.13B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with RM514 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.16A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM224 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.16B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Hamsa with RM224 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Hamsa allele homozygote

Plate 4.15A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with RM470 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote
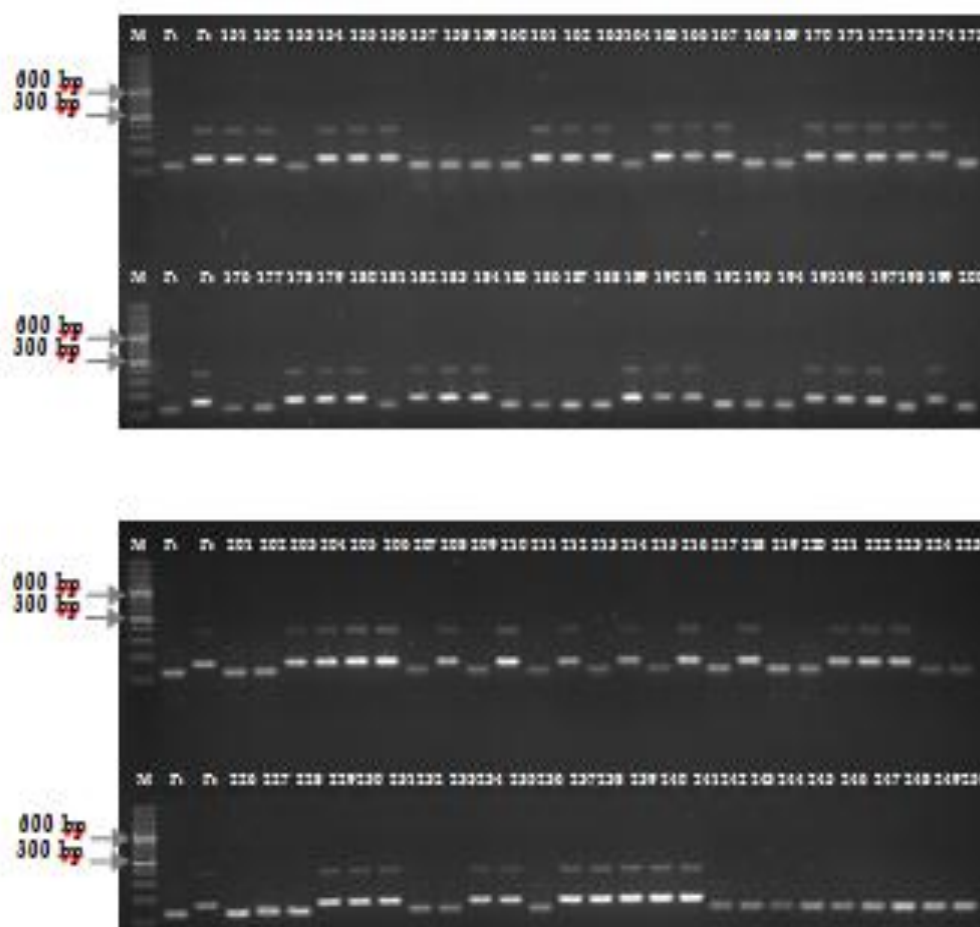
Plate 4.15B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with RM470 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Krishna Kamod allele homozygote

Plate 4.16A: Segregation pattern of 250 (1-150) RIL populations based on cross GR-11 X Krishna Kamod with OsYSL15 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.16B: Segregation pattern of 250 (150-250) RIL populations based on cross GR-11 X Krishna Kamod with OsYSL15 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Krishna Kamod allele homozygote

Plate 4.17A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Gurjari with OsYSL7 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote

Plate 4.17B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Gurjari with OsYSL7 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote

Plate 4.18A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Gurjari with OsNAC5 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote
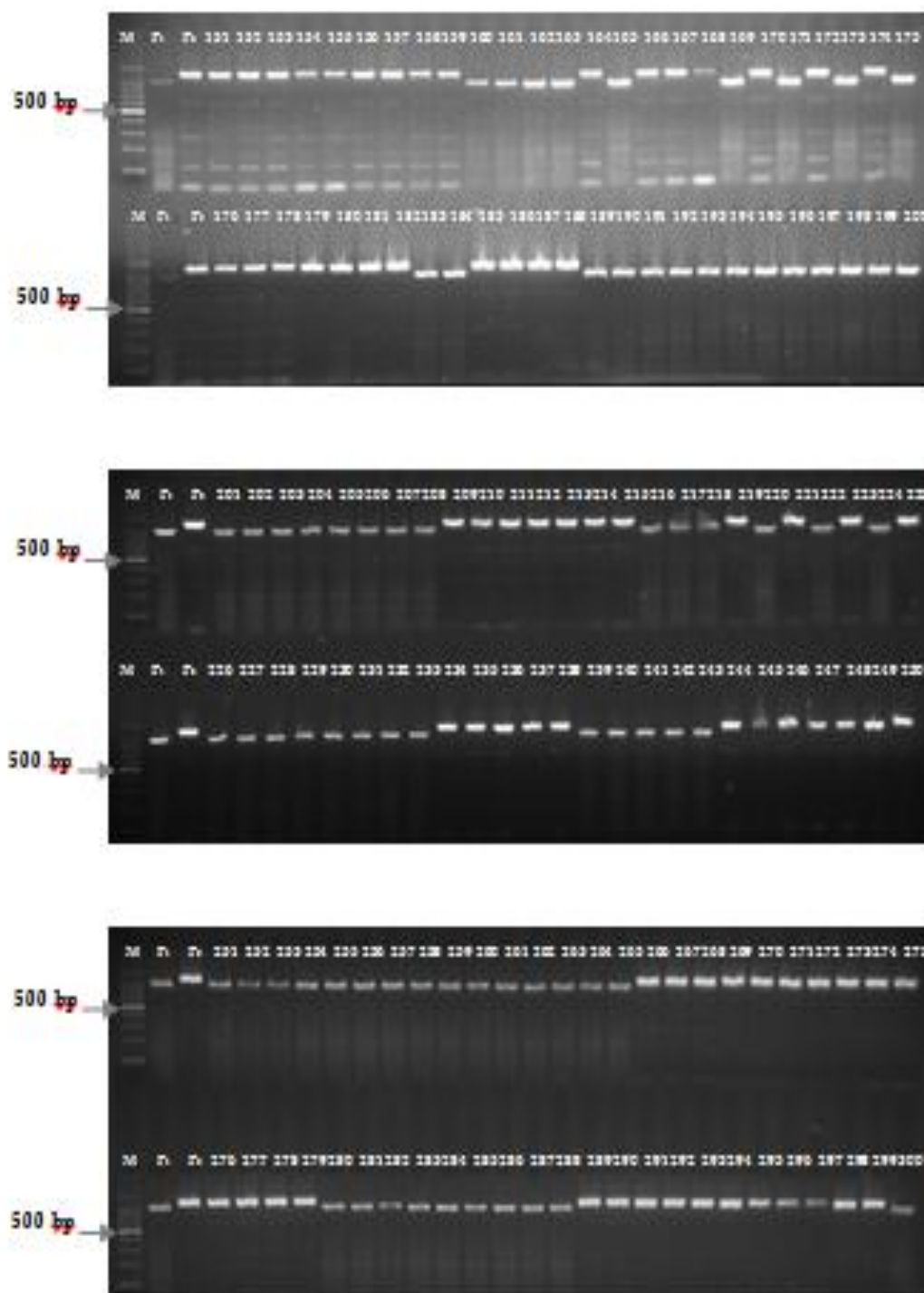
Plate 4.18B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Gurjari with OsNAC5 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote
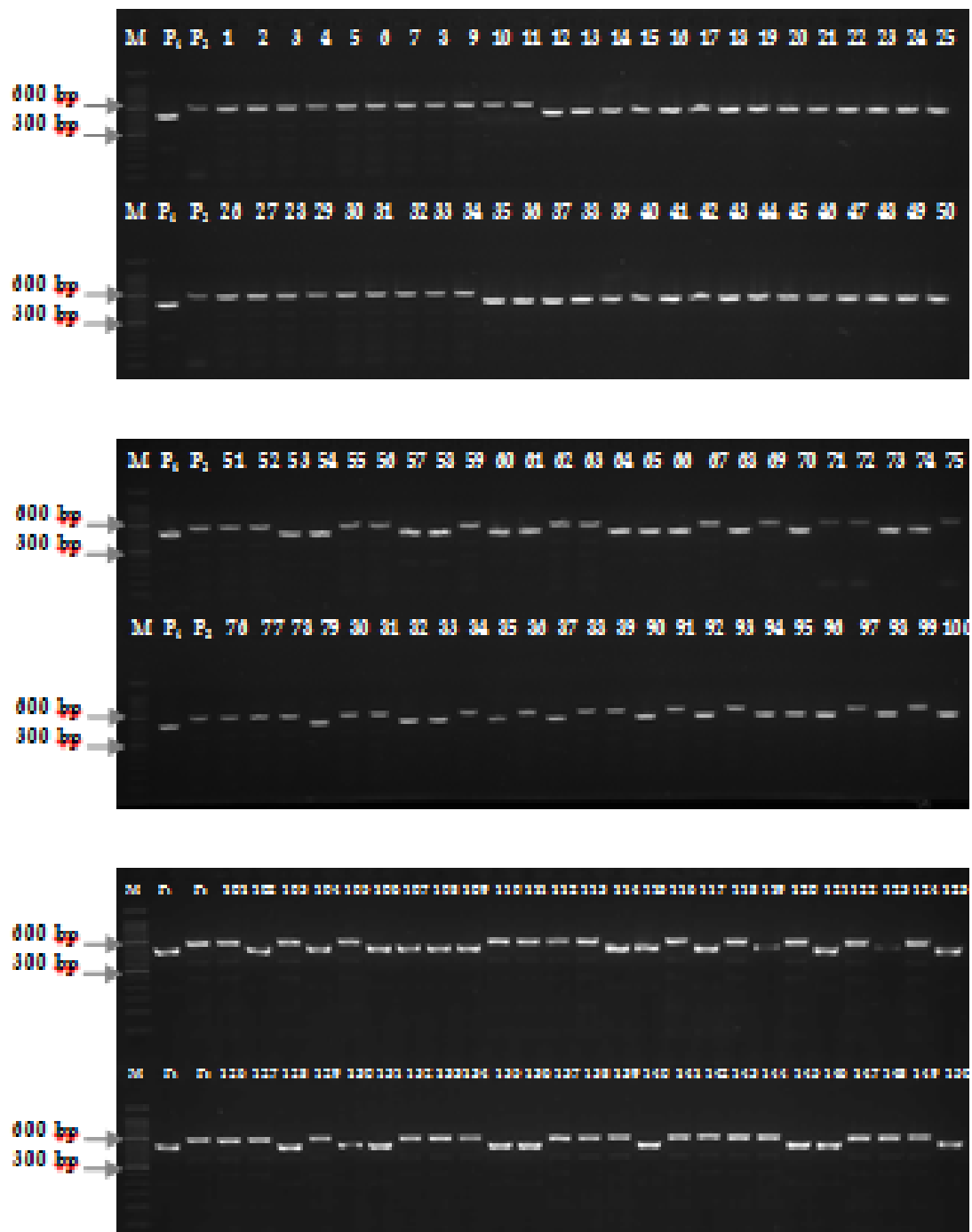
Plate 4.19A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Gujari with OsYSL5 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Gujari allele homozygote
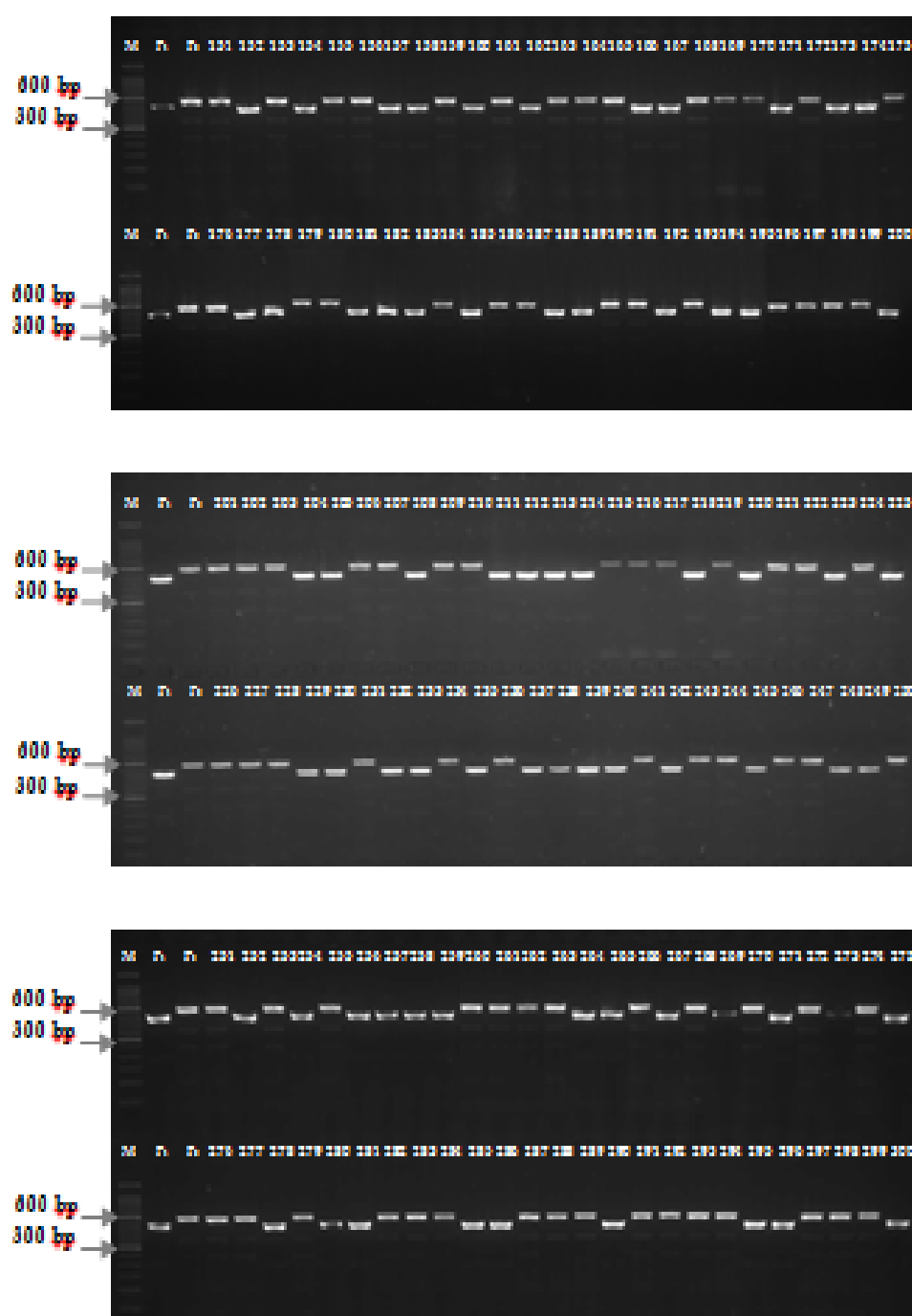
Plate 4.19B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Gurjari with OsYSL5 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote
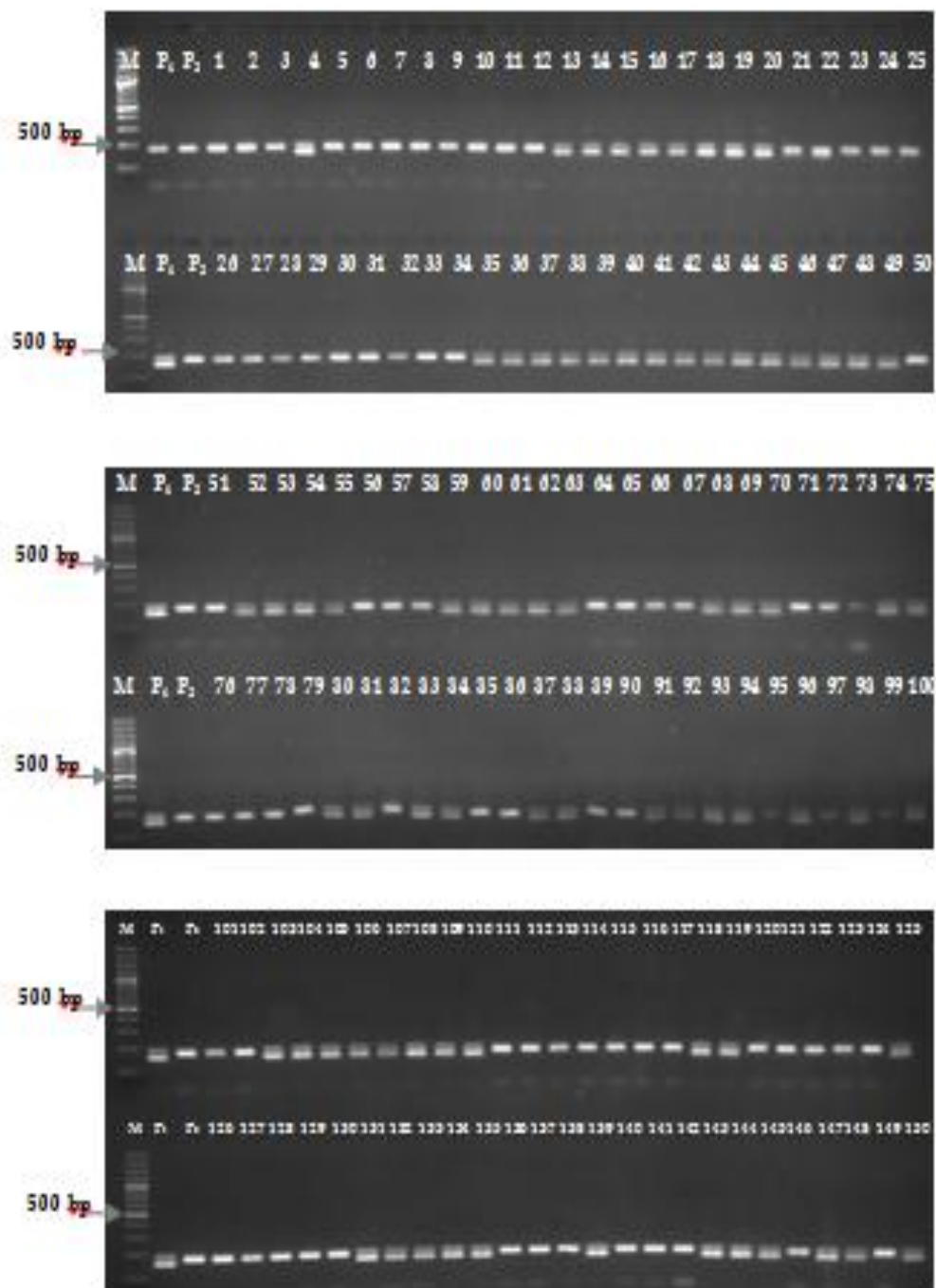
Plate 4.20A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Gurjari with RM501 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote
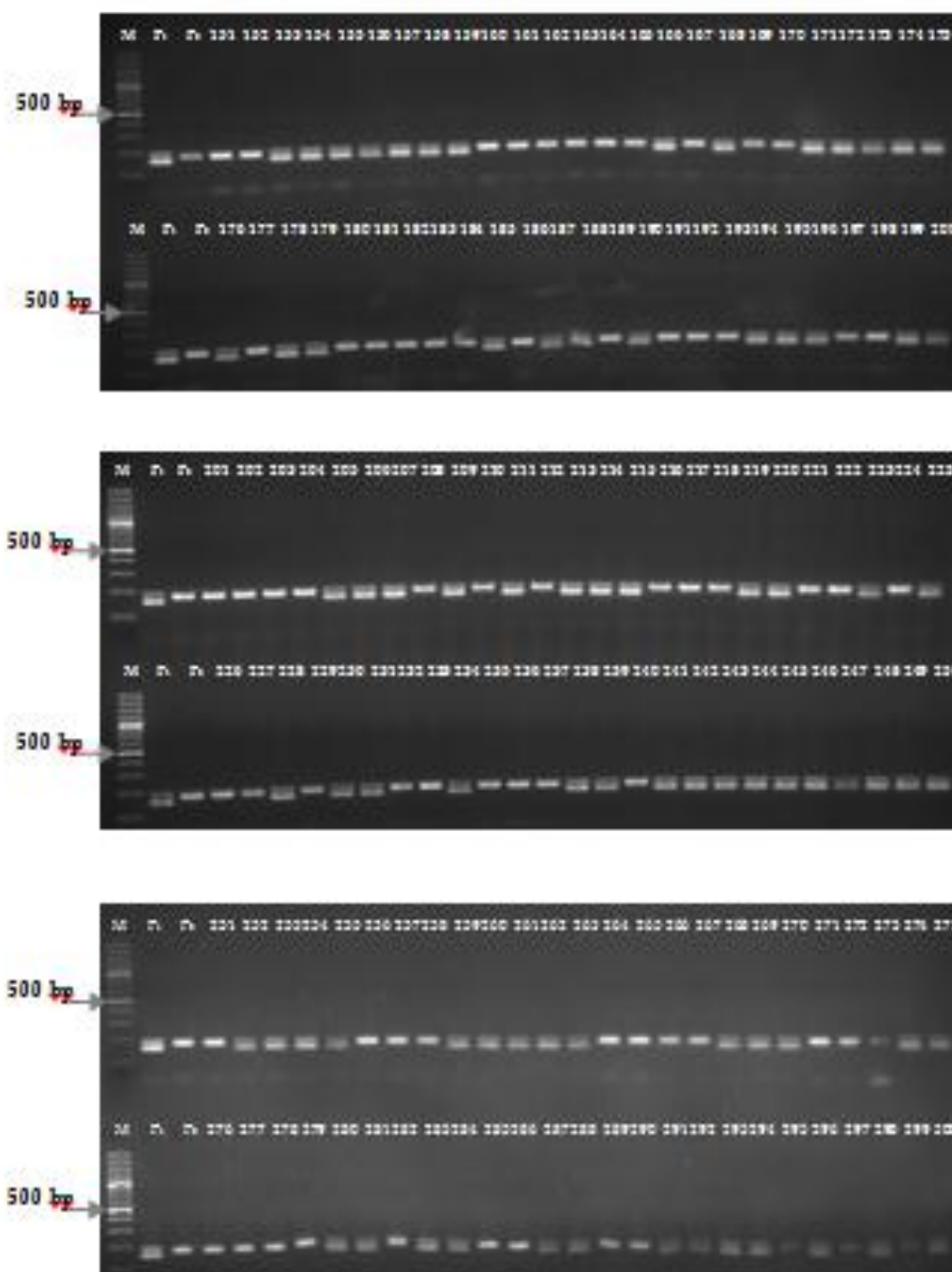
Plate 4.20B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Gurjari with RM501 on 4.5 % metaphore gel

M: 100 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote

Plate 4.21A: Segregation pattern of 300 (1-150) RIL populations based on cross GR-11 X Gusjaci with RM595 on 4.5 % metaphore gel

M: 50 bp marker; P_1: GR-11 allele homozygote; P_2: Gusjaci allele homozygote
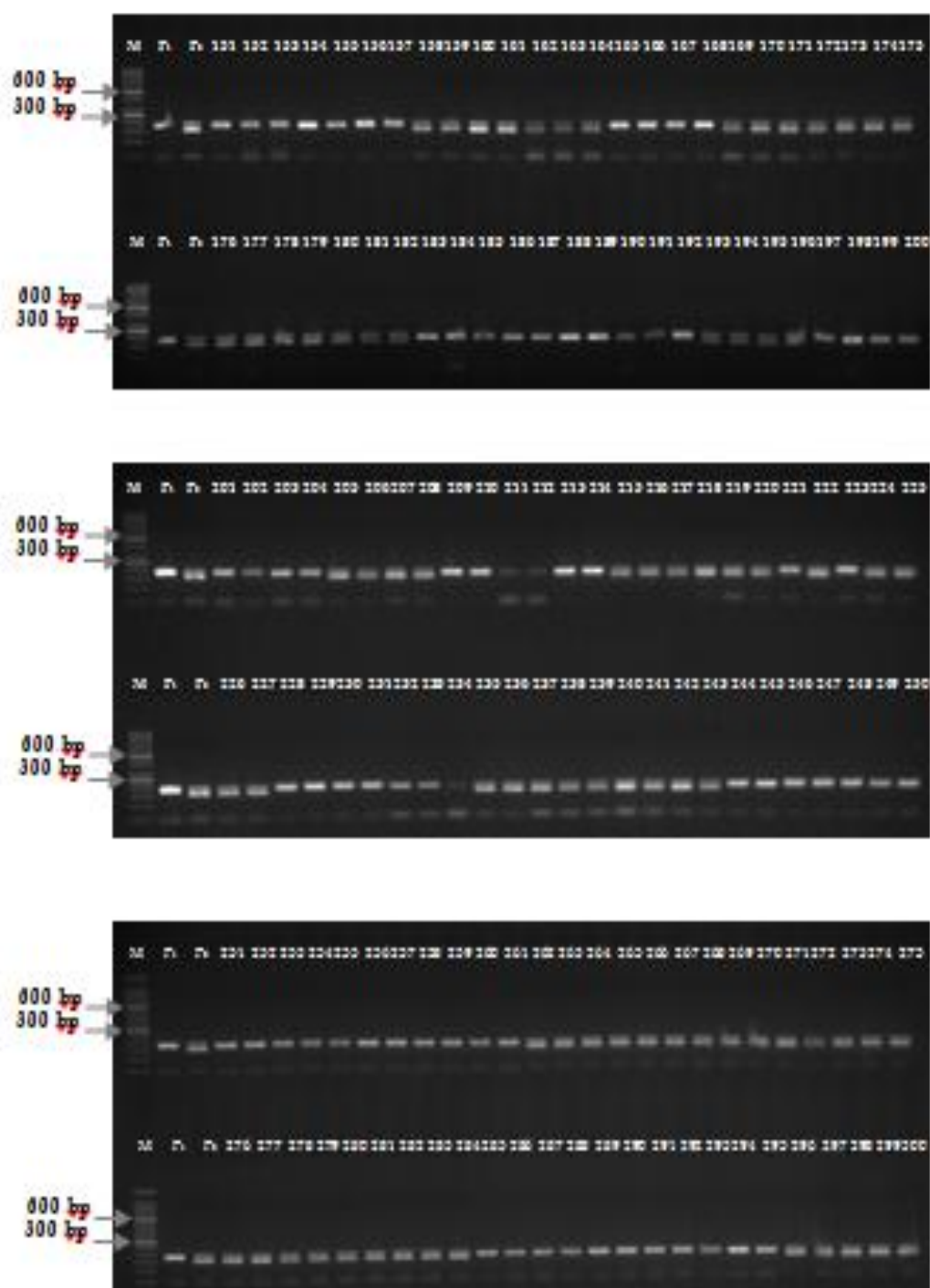
Plate 4.21B: Segregation pattern of 300 (150-300) RIL populations based on cross GR-11 X Gurjari with RM295 on 4.5 % metaphore gel

M: 50 bp marker; P₁: GR-11 allele homozygote; P₂: Gurjari allele homozygote

Table: 4.8 Chi square values of microsatellite markers that showing segregation distortion among 300 RILs of the cross GR-11 X Pankhali-203.

S. No.	Marker	Chr. No.	χ^2 1:1	*P* value	Significance	Skeweness
1	RM562	1	15.2	0.00	****	GR-11
2	RM580	1	7.8	0.00	**	GR-11
3	RM302	1	17.4	0.00	****	GR-11
4	RM71	2	9.9	0.00	**	Pankhali-203
5	RM475	2	11.4	0.00	***	GR-11
6	RM81	3	15.9	0.00	****	Pankhali-203
7	RM335	3	9.6	0.00	**	GR-11
8	RM119	4	7.2	0.00	**	GR-11
9	RM168	4	13.9	0.00	***	Pankhali-203
10	RM3322	5	16.8	0.00	****	Pankhali-203
11	RM16	5	13.4	0.00	***	GR-11
12	RM400	6	9.2	0.00	**	GR-11
13	RM103	6	11.6	0.00	***	Pankhali-203
14	RM3	7	16.2	0.00	****	GR-11
15	RM235	7	12.1	0.00	***	Pankhali-203
16	RM21596	8	7.5	0.00	**	GR-11
17	RM21599	8	15.8	0.00	****	GR-11
18	RM447	8	8.3	0.00	**	Pankhali-203
19	OsVITI	9	17.6	0.00	****	GR-11
20	RM410	9	12.7	0.00	***	GR-11
21	RM257	10	8.5	0.00	**	Pankhali-203
22	RM496	10	12.4	0.00	***	GR-11
23	RM206	11	16.4	0.00	****	Pankhali-203
24	RM167	11	11.8	0.00	***	GR-11
25	RM330	12	8.2	0.00	**	GR-11
26	RM3331	12	17.2	0.00	****	GR-11

P = probability; Chr = Chromosome number.

*Significance at 5 %, ** Significance at 1 %, *** Significance at 0.5 %, ****Significance at 0.1 %

In case of LG4, 2 of 18 markers were distorted, one skewed in favor of the each parent, were distorted from expected Mendelian ratios. Three SSR and one gene specific marker on LG5 were skewed towards Pankhlai-203 while the remaining while the remaining 22 markers segregated in Mendelian pattern. Out of 22 SSR markers mapped on LG6, four were distorted and skewed towards Pankhlai-203 parent allele, while the gene specific did not show any segregation distortion. Two of 12 SSR were distorted while 1 of 3 genes specific was skewed towards the GR-11 parental allele.

Segregation distortion was also prominent in the case of LG8. Out of 12 markers on LG8 a total of three markers didn't show any agreement with Mendelian segregation and were skewed towards GR-11. In case of LG9, out of 7 SSR 2 were distorted while the gene specific markers were normally distributed. LG10 had least number of markers mapped, and 2 of them were skewed and showing the tendency to favor Pankhali-203. Of the 14 SSR markers mapped on LG11 only 2 were distorted and skewed towards the Pankhali-203 parental allele. All gene specific markers mapped on LG12 were segregating in 1:1 ratio while 2 out of 11 distorted markers were clustered closely and skewed in favor of GR-11.

Out of 40 % distorted markers, the highest proportion was observed in the SSR markers as compared to the gene specific markers. In summary, out of 234 mapped markers, segregation of 34 SSR and 7 gene specific markers were distorted and of these distributions of only 18 were skewed towards GR-11.

4.4.1.2. Segregation Distortion in the GR-11 X Krishna Kamod based cross

Out of 32 SSR markers genotyped for this RIL population only 35 were skewed towards either of the two alleles. Twenty seven of 226 SSR markers and 8 of 32 gene specific markers were distorted and skewed towards both the parental alleles. Of the 37 markers on LG1, 2 SSR and 1 gene specific markers were distorted and skewed towards GR-11. Moreover, most of the skewed markers were residing at the end of this linkage group. Out of 28 markers, 18 % (SSR and gene specific) on LG2 were distorted and skewed towards Pankhali-203. Three SSR and 1 gene specific marker were skewed towards the GR-11 allele on LG3. In case of LG4, 2 of 19 markers both SSR and gene specific markers were skewed and distorted.

Table: 4.9 Chi square values of microsatellite markers that showing segregation distortion among 250 RILs of the cross GR-11 X Krishna Kamod.

S. No.	Marker	Chr. No.	χ^2 1:1	*P* value	Significance	Skeweness
1	RM312	1	15.7	0.00	****	GR-11
2	RM580	1	7.4	0.00	**	GR-11
3	RM562	1	12.8	0.00	***	GR-11
4	RM475	2	3.4	0.01	*	GR-11
5	RM6641	2	9.4	0.00	**	GR-11
6	RM5928	3	15.6	0.00	****	Krishna Kamod
7	RM85	3	17.1	0.00	****	GR-11
8	RM335	4	7.6	0.00	**	GR-11
9	RM470	4	13.6	0.00	***	Krishna Kamod
10	RM289	5	8.5	0.00	**	GR-11
11	RM516	5	16.2	0.00	****	GR-11
12	RM518	5	12.4	0.00	***	Krishna Kamod
13	RM8039	6	17.9	0.00	****	GR-11
14	RM3575	6	3.9	0.01	*	Krishna Kamod
15	RM3	7	8.1	0.00	**	GR-11
16	RM214	7	15.9	0.00	****	GR-11
17	RM2159	8	9.7	0.00	**	GR-11
18	RM21599	8	5.7	0.03	*	GR-11
19	RM2255	9	17.4	0.00	****	Krishna Kamod
20	RM321	9	4.6	0.02	*	GR-11
21	RM484	10	12.8	0.00	***	Krishna Kamod
22	RM257	10	4.9	0.02	*	GR-11
23	RM258	11	16.3	0.00	****	GR-11
24	RM216	11	4.7	0.02	*	Krishna Kamod
25	RM330	12	9.6	0.00	**	GR-11
26	RM27172	12	16.4	0.00	****	GR-11

P = probability; Chr = Chromosome number.

*Significance at 5 %, ** Significance at 1 %, *** Significance at 0.5 %, ****Significance at 0.1 %

Out of 26, two of 22 SSR markers and two of 4 genes specific did not follow the Mendelian segregation pattern on LG5. Out of 24 SSR markers 2 were distorted and skewed towards the GR-11 allele. Of the 24 SSR markers, 2 did not follow the Mendelian ratio of segregation on LG6. Out of 17 markers harbored by LG7, one each (SSR and gene specific) exhibited significant deviations favoring GR-11 allele while the remaining 15 (14 SSR and 3 gene specific) of LG7 were normally segregating. Segregation distortion was also prominent in the case of LG8. Out of 32 markers on LG8 a total of four SSR markers didn't show any agreement with Mendelian segregation and were skewed towards GR-11.

In case of LG9, only one marker was distorted and skewed towards the Pankhali-203 allele. LG10 has the least markers mapped, of which two were skewed for the favorable allele GR-11 located at the ends of the chromosomes. Three SSR on LG11 were skewed towards Pankhlai-203 while the remaining 12 markers segregated in Mendelian pattern. All gene specific markers mapped on LG12 were segregating in 1:1 ratio while 3 out of 11 distorted markers were clustered closely and skewed in favor of GR-11.

In summary out of 258 markers on the base map for this RIL population, segregations of 27 SSR and 8 gene specific markers were distorted and of these only 18 were skewed towards the parent GR-11.

4.4.1.3. Segregation Distortion in the GR-11 X Gurjari based cross

LG1 could accommodate 36 markers (32 SSR and 4 genes specific), of which 4 SSR were distorted and segregated towards GR-11 while 1 gene specific marker was skewed in favor of GR-11. In case of LG2 3 SSR markers were segregated and distorted towards the GR-11 allele while 2 among 6 markers got skewed in favor of GR-11. Out of 27 markers mapped on LG3, 4 SSR among 22 and 1 gene specific among 5 were distorted and skewed towards the GR-11 allele.

Out of 15 SSR, 2 did not follow the Mendelian pattern of inheritance and had segregation distortion. Three SSR and one gene specific marker on LG5 were skewed towards Pankhlai-203 while the remaining while the remaining 24 markers segregated in Mendelian pattern. Out of 26 SSR markers mapped on LG6, four were distorted and skewed towards Pankhlai-203 parent allele, while the gene specific did not show any segregation distortion.

Table: 4.10 Chi square values of microsatellite markers that showing segregation distortion among 300 RILs of the cross GR-11 X Gurjari.

S. No.	Marker	Chr. No.	χ^2 1:1	*P* value	Significance	Skeweness
1	RM272	1	17.4	0.00	****	GR-11
2	RM104	1	12.4	0.00	***	GR-11
3	RM431	1	3.7	0.04	*	Gurjari
4	RM6641	2	16.3	0.00	****	GR-11
5	RM279	2	3.5	0.04	*	Gurjari
6	RM22	3	2.4	0.05	*	GR-11
7	RM426	3	15.4	0.00	****	GR-11
8	RM119	4	15.2	0.00	****	Gurjari
9	OsYSL10	4	5.7	0.03	*	GR-11
10	RM161	5	7.5	0.00	**	GR-11
11	RM178	5	2.8	0.05	*	GR-11
12	RM3822	5	5.4	0.03	*	Gurjari
13	RM8039	6	9.8	0.00	**	GR-11
14	RM133	6	9.4	0.00	**	Gurjari
15	RM11	7	8.2	0.00	**	GR-11
16	RM455	7	8.6	0.00	**	Gurjari
17	RM25	8	8.2	0.00	**	GR-11
18	RM3481	8	5.0	0.03	*	Gurjari
19	RM321	9	11.9	0.00	***	GR-11
20	RM219	9	7.4	0.0	**	Gurjari
21	RM257	10	7.2	0.00	**	GR-11
22	RM271	10	15.4	0.00	****	GR-11
23	RM4601	11	3.4	0.05	*	Gurjari
24	RM552	11	5.6	0.03	*	GR-11
25	RM260	12	8.9	0.00	**	GR-11
26	RM27172	12	17.6	0.00	****	GR-11

P = probability; Chr = Chromosome number.

*Significance at 5 %, ** Significance at 1 %, *** Significance at 0.5 %, ****Significance at 0.1 %

Fifteen of 18 SSR and 3 gene specific markers were mapped on LG7, of which one SSR was distorted and skewed towards the GR-11 allele. Out of 30 SSR three markers were distorted and the remaining 27 SSR markers followed the 1:1 ratio of Mendelian inheritance. Among the 12 markers harbored on LG9, only one marker got distorted and favored the GR-11 parental allele.

Least number of markers was mapped on LG10, of which only 2 SSR markers were distorted. Of the 15 SSR markers mapped on LG11 only 2 were distorted and skewed towards the Pankhali-203 parental allele. All gene specific markers mapped on LG12 were segregating in 1:1 ratio while 2 out of 11 distorted markers were clustered closely and skewed in favor of GR-11.

In a nutshell, of 266 markers mapped for the RIL population of GR-11 X Gurjari, 37 were distorted and only 14 were skewed towards Pankhali-203. Among the markers mapped on the RIL population highest degree of distortion was observed in SSR markers as compared to the gene specific markers.

The ideal set of molecular marker data for linkage mapping has no missing values, no genotyping errors and the markers segregate in the expected ratios for that type of population (Hackett and Broadfoot, 2003). After first reported in maize by Mangelsdorf and Jones (1926) using morphological markers non-mendelian segregation of various kinds of markers is a common incidence in number of species and this phenomenon is believed to be an important evolutionary force. But segregation distortion affects the estimation of mapping distance, the order of markers and the overall trait mean of the population of DHs or RILs if QTLs are within this region (Wu, 2010; Cheng *et al.*, 1997). Lorieux *et al.*, (1995) indicated that the estimation of recombination fractions with the co-dominant markers is less affected by segregation distortion than that of the dominant markers. In the present study, markers in all the three populations showed the segregation distortion.

Many reasons of segregation distortion have been explained previously. The reason behind distortion is due to one or more segregation distortion loci (SDL) (Xu, 2008). These loci are subjected to genetic selection by competition of preferential fertilization (Lyttle, 1991; Faris *et al.*, 1998), zygotic selection (Harushima *et al.*, 1996) or both meiotic drive/preferential segregation, sampling/selection during

population development, and differential responses of parental lines in tissue cultures in response to DHs.

Type and size of the mapping populations used, is one of the reason suggested for segregation distortion. Segregation distortion can also be specific with respect to some markers in an otherwise normal mapping population. Yang *et al*. (2012) suggested that RILs usually show extreme segregation distortion, because during the RIL development process many recessive lethal genes become homozygous and their expression causes failure to contribute seeds to subsequent generations, and consequently results in a skewed population. Distorted segregation in RILs populations via single-seed descent (SSD) method represents the cumulative effects of both genetic and environment factors on multiple generation, and G X E interactions become more pronounced with the progress of selfing (Wang *et al*., 2009).

Quantitative trait loci (QTLs) mapping is an important application of genetic mapping. According to Hackket and Broadfoot (2003), segregation distortions has a very little effect both on marker order and map length but otherwise have reported reduction in map length due to the presence of loci with significant segregation distortion. Moreover, the effect of segregation distortion on QTL analysis can be neglected if a map of correct distance and marker order is constructed with proper linkage analysis. However, if the recombination fractions or the order of marker loci are inferred incorrectly, basic assumptions of QTL analysis do not hold and results will be imprecise at best. According, to He *et al*. (1999) due to stepwise regression, detection of QTLs using composite interval mapping would not be influenced by inclusion of distorted markers during QTL mapping. In a recent study, Zhang *et al*. (2011) suggested that if the distorted markers are not closely linked with any of the QTL, segregation distortion will not have a significant impact on QTL mapping results.

4.5. CONSTRUCTING LINKAGE MAPS

A genetic map or linkage map, an essential tool for QTL studies, is a map of frequencies of recombination that occur between the markers on homologous chromosomes during meiosis. Being a path to link the genetic region to a trait of interest it is also an important resource for fine mapping and cloning of genes. The present study set out to construct genetic linkage maps in three populations.

Therefore, it was important to develop reliable maps from large segregating populations that provided good genome coverage. The linkage map constructed in the present investigation was generated with co-dominant SSR and gene specific markers responsible for iron and zinc uptake, transport and their homeostasis.

4.5.1. Cross GR-11 X Pankhali-203

Out of 229 SSR markers, only 172 markers could be mapped in 300 selected RIL populations. Rest of the polymorphic markers could not be mapped due to dominant inheritance, lack of linkage, very high percentage of distortion towards one parent due to removal of RILs. Twenty eight out of 52 polymorphic gene specific markers were used to construct the maps as the remaining markers were unlinked. Hence, the results showed that out of 325 markers genotyped, 200 markers were assigned in 12 linkage groups with a LOD score of 3.0 to construct the genetic linkage map.

The total length of the map is 1370.4 cM (Haldane) which represents on average one marker on every 6.7 cM (Fig. 4.37 (a,b)). The individual linkage groups ranged from 102.8 cM for linkage group 8 with the highest number of markers (29) to 25.4 cM for linkage group 10 with 5 markers (Table 4.11).

162.4 cM of map length was observed on LG2 accommodating a total of 26 markers (20 SSR and 6 gene specific) with an average marker interval of 6.2 cM. LG3 could house a total of 25 molecular markers (20 SSR and 5 genes specific) on 112.4 cM with an average interval of 4.4 cM. The map length of LG5, LG6, LG7, LG8 and LG9 were 137.1 cM, 129.3cM, 106.3cM, 162.7cM, 123.0 cM respectively.

4.5.2. Cross GR-11 X Krishna Kamod

To construct the linkage map a small portion of gene specific and many polymorphic SSR markers were used for the genotyping of the 250 RIL populations. The map construction was started using the data of 258 molecular markers (226 SSR and 32 genes specific) on the 250 RIL populations using the R/qtl program. The results showed that all the SSR and gene specific markers were grouped to construct the linkage map.

Table: 4.11 Linkage group wise summary of markers exhibiting distorted segregation in rice RIL population based on cross GR-11 X Pankahli-203

S. No.	Linkage group	SSR loci	Skewed SSR loci	Gene specific loci	Skewed gene specific loci	Total marker loci	Total skewed loci	Skewed loci (%)	Total length (cM)[A]
1	1	32	6	4	1	36	7	19	232.9
2	2	20	3	6	1	26	4	15	162.4
3	3	20	4	5	2	25	6	24	112.4
4	4	14	1	4	1	18	2	11	99.7
5	5	19	3	4	1	23	4	17	137.1
6	6	22	4	0	0	22	4	18	129.3
7	7	12	2	3	1	15	3	20	106.3
8	8	27	3	0	0	27	3	11	162.7
9	9	7	2	2	0	9	2	22	123.0
10	10	4	2	1	0	5	2	40	72.6
11	11	14	2	0	0	14	2	14	102.4
12	12	11	2	3	0	14	2	14	95.4
13	Total	202	34	32	7	234	41	225	1536.2

[A] Map Length (Haldane Centimorgans)
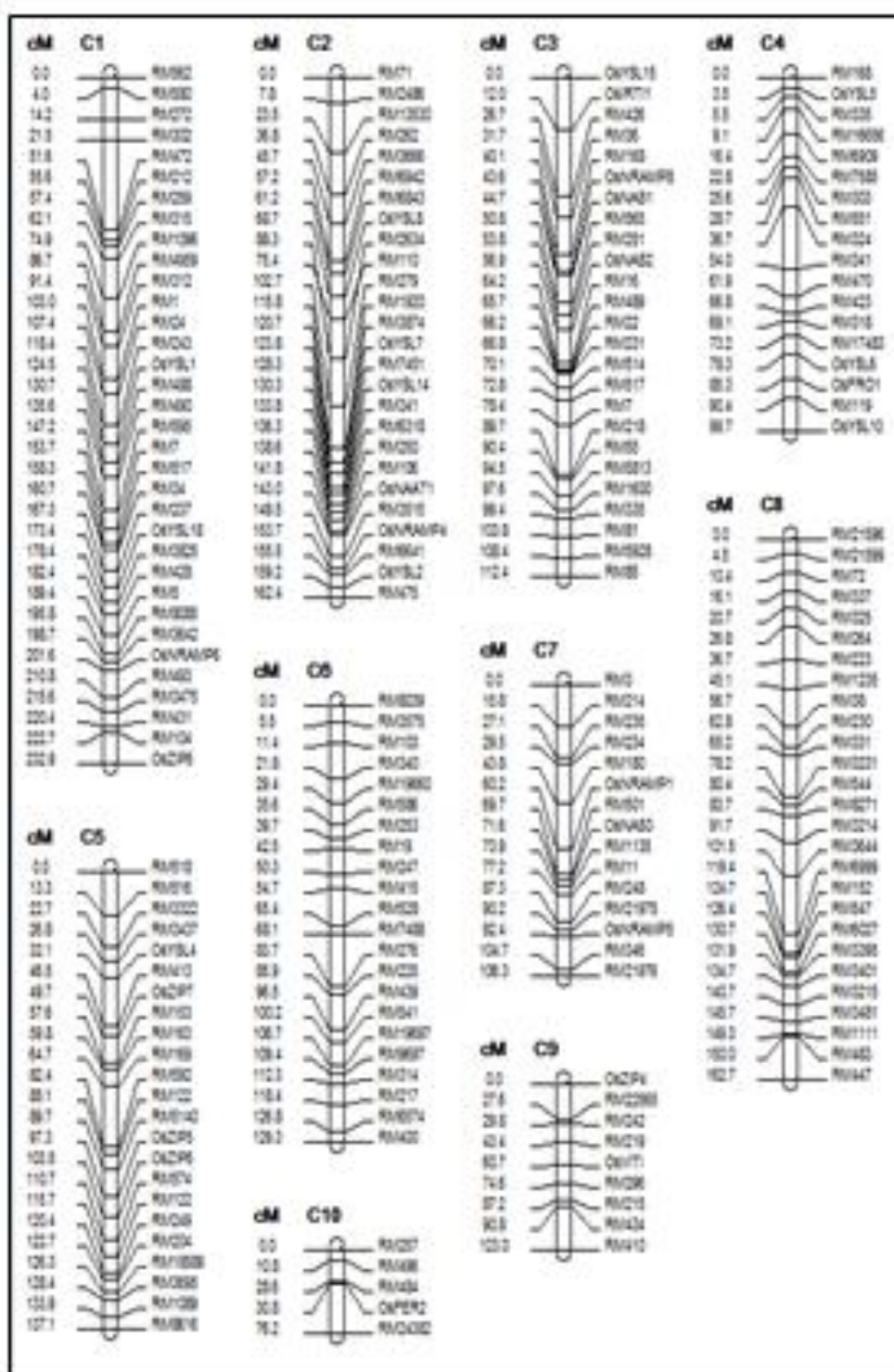
Fig. 4.37 (a): Linkage map (LG1 – LG10) of rice RIL (F7) population based on cross GR-11 X Pankhali-203
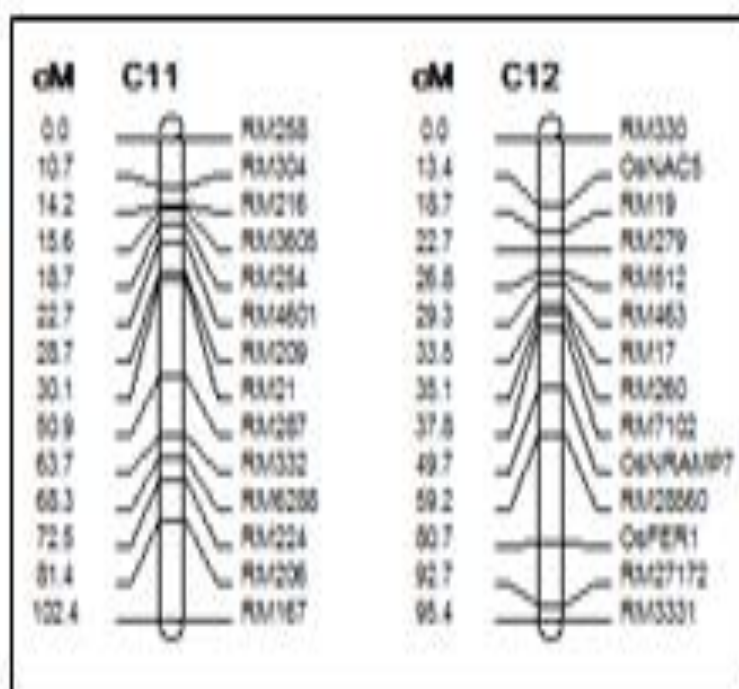
Fig. 4.37 (b): Linkage map (LG11 and LG12) of rice RIL (F7) population based on cross GR-11 X Pankhali-203
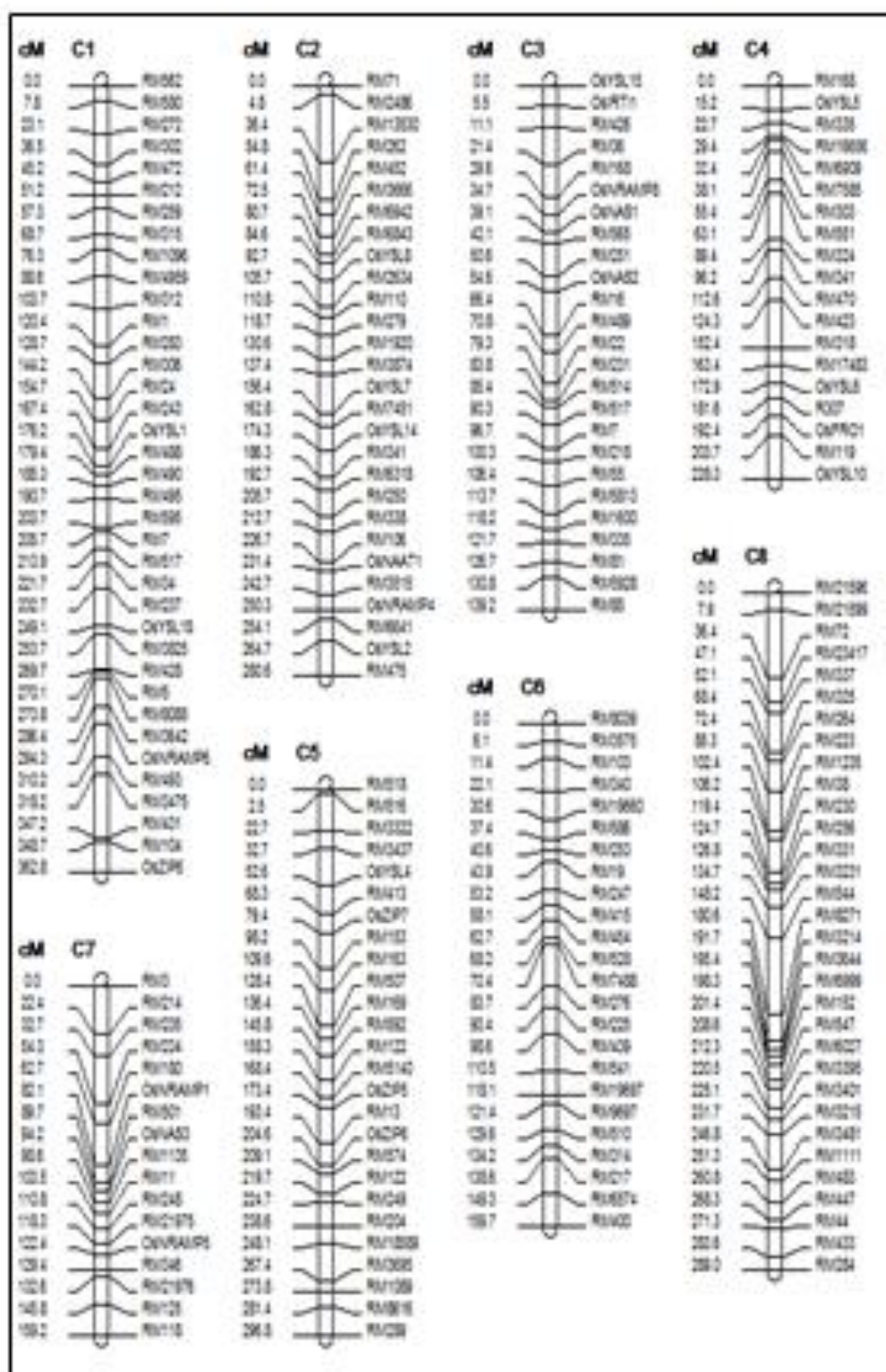
Fig. 4.38 (a): Linkage map (LG1- LG8) of rice RIL (F7) population based on cross GR-11 X Krishna Kamod
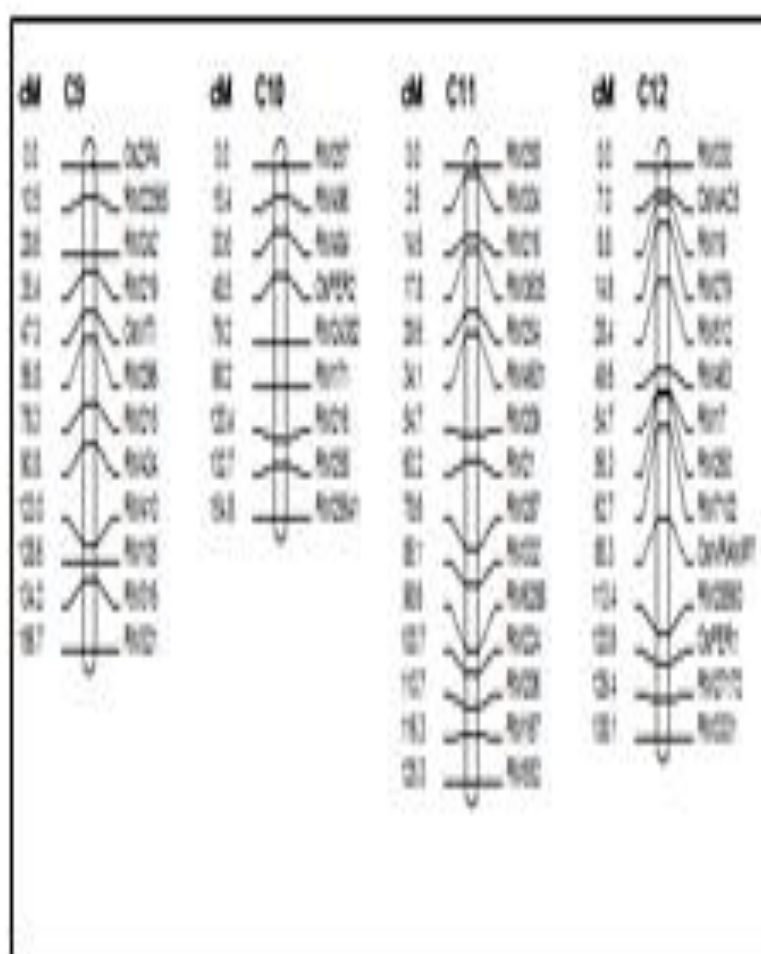
Fig. 4.38 (b): Linkage map (LG9 – LG12) of rice RIL (F7) population based on cross GR-11 X Krishna Kamod

The longest linkage group on the base map was LG1, which housed 37 marker loci within a length of 362.8cM, whereas the LG5 has 26 marker loci within the length of 296.8 cM (Fig. 4.38 (a,b)). In contrast, at 125.3 cM long LG11 was the smallest with 15 markers SSR markers, followed by LG12 of 138.1 cM length holding 11 SSR and 3 gene specific markers (Table 4.12).

LG6 (24 SSR), LG8 (32 SSR) and LG11 (15 SSR) could map only the SSR markers with the map length of 159.7 cM, 289.0 cM, 125.3 cM respectively. LG2 (280.6 cM), LG3 (139.2 cM), LG4 (228.3 cM), LG5 (296.8 cM), LG7 (159.2 cM) had a map interval or map gap of 10, 5.5, 12, 11.4, 9.3 respectively.

A total of 258 (226 SSR and 32 gene specific) markers were mapped at an distance of 2490.5 cM (Haldane) with an average length of 207.5 cM and an average marker loci of 21.5 on each linkage group. Large number of markers was grouped by LG1, followed by LG5, LG8 and LG2 with 37, 26, 32 and 28 markers respectively. Gaps of more than 10 cM marker interval between the two adjacent markers were detected in case of LG9 and LG10. LG1 was longest (362.8 cM) whereas LG11 was the shortest.

4.5.3 Cross GR-11 X Gurjari

To construct the linkage map a total of 262 (229 SSR and 32 gene specific) markers were used for the genotyping of 300 RIL populations in rice. The total length of the map is 2663.2 cM (Haldane), which represented an average one marker at every 10.1 cM (Fig. 4.39 (a,b)). The individual linkage groups ranged from 398.4 cM for LG1 with highest number of markers (34) to 134.6 cM for LG10 with the least number of markers (8) followed by LG12. The average linkage group length was 221.9 cM with an average loci of 21.8 on al the 12 linkage groups. 304.5 cM of map length was observed in LG2 with a total of 28 molecular markers (22 SSR and 8 genes specific), of which the distorted markers were mapped on the ends of the chromosome (Table 4.13).

LG6 (26 SSR and LG11 (15 SSR) could map only the SSR markers with the map length of 164.2 cM, 138.0 cM respectively. LG3 had a total of 27 markers mapped with a length of 180.6 cM, each with an average interval of 6.6. Similarly for LG4 (19 markers), LG7 (18 markers), LG8 (31 markers) had a map interval of 12, 10.7, 9.4 respectively.

Table: 4.12 Linkage group wise summary of markers exhibiting distorted segregation in rice RIL population based on cross GR-11 X Krishna Kamod

S. No.	Linkage group	SSR loci	Skewed SSR loci	Gene specific loci	Skewed gene specific loci	Total marker loci	Total skewed loci	Skewed loci (%)	Total length (cM)[A]
1	1	33	2	4	1	37	3	8	362.8
2	2	22	3	6	2	28	5	18	280.6
3	3	20	3	5	1	25	4	16	139.2
4	4	15	1	4	1	19	2	10	228.3
5	5	22	2	4	2	26	4	15	296.8
6	6	24	2	0	0	24	2	8	159.7
7	7	14	1	3	1	17	2	12	159.2
8	8	32	4	0	0	32	4	12	289.0
9	9	10	1	2	0	12	1	8	156.7
10	10	8	2	1	0	9	2	22	154.8
11	11	15	3	0	0	15	3	20	125.3
12	12	11	3	3	0	14	3	21	138.1
13	Total	226	27	32	8	258	35	170	2490.5

[A] Map Length (Haldane Centimorgans)

Table: 4.13 Linkage group wise summary of markers exhibiting distorted segregation in rice RIL population based on cross GR-11 X Gurjari

S. No.	Linkage group	SSR loci	Skewed SSR loci	Gene specific loci	Skewed gene specific loci	Total marker loci	Total skewed loci	Skewed loci (%)	Total length (cM)^A
1	1	32	4	4	1	36	5	14	389.4
2	2	22	3	6	2	28	5	18	304.5
3	3	22	4	5	1	27	5	18	180.6
4	4	15	2	4	1	19	3	16	229.4
5	5	24	3	4	1	28	4	14	318.4
6	6	26	4	0	0	26	4	15	164.2
7	7	15	1	3	0	18	1	6	192.6
8	8	30	3	1	0	31	3	10	294.1
9	9	10	1	2	0	12	1	8	147.0
10	10	7	2	1	0	8	2	25	134.6
11	11	15	2	0	0	15	2	13	138.0
12	12	11	2	3	0	14	2	14	170.4
13	Total	229	31	33	6	262	37	171	2663.2

^{A} Map Length (Haldane Centimorgans)
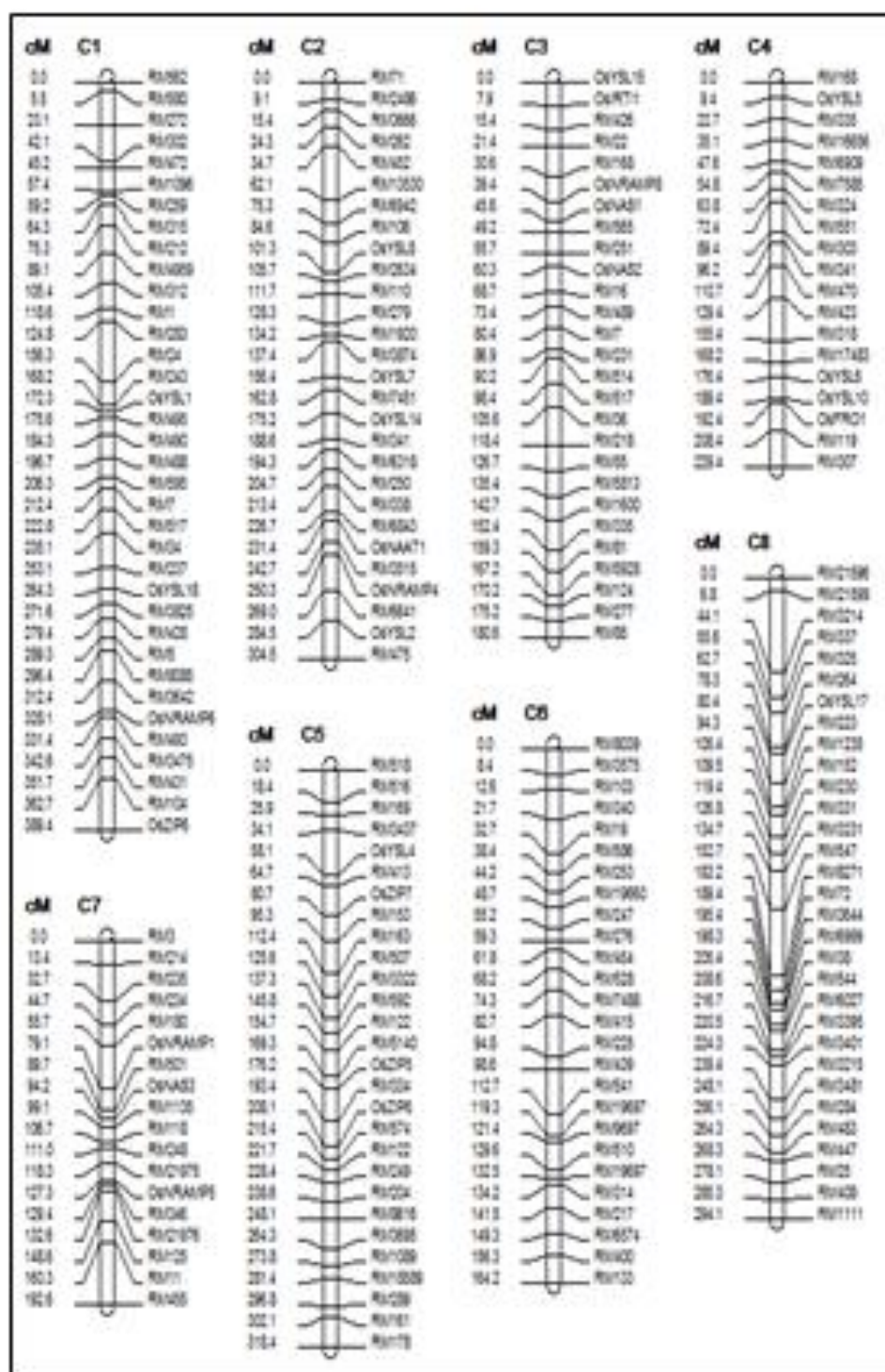
Fig. 4.39 (a): Linkage map (LG-1 – LG-8) of rice RIL (F7) populations based on cross GR-11 X Gurjari
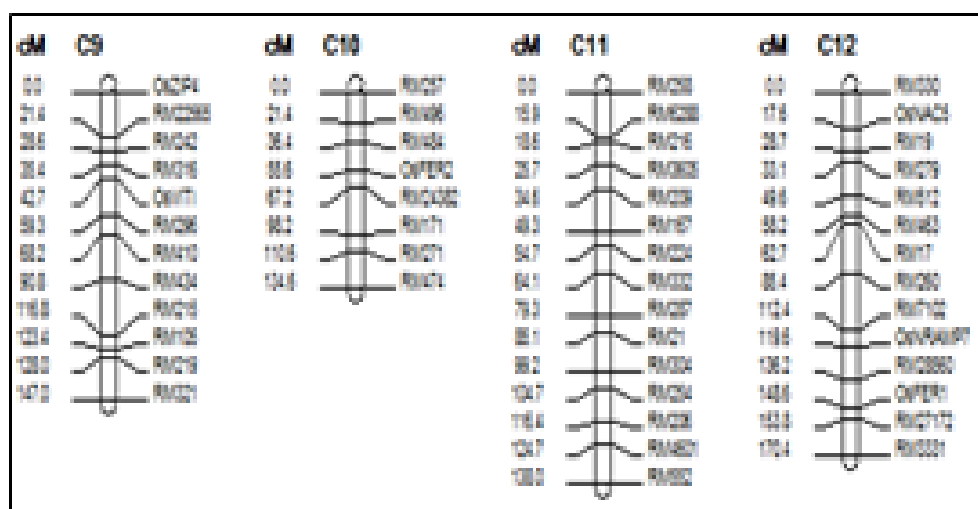
Fig. 4.39 (b): Linkage map (LG-9 – LG-12) of rice RIL (F7) populations based on cross GR-11 X Gurjari
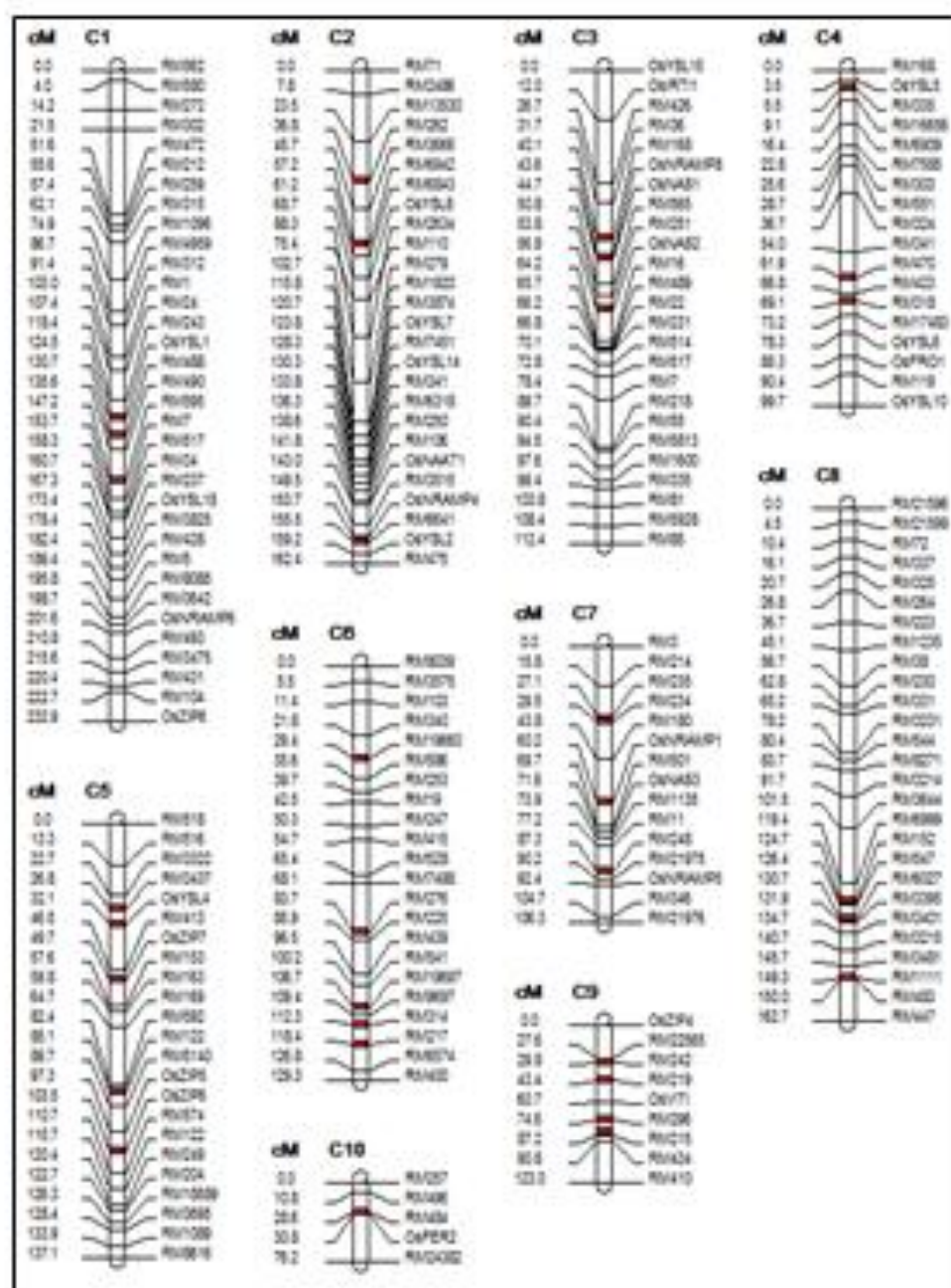
Fig. 4.40 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Pankhali-203 using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
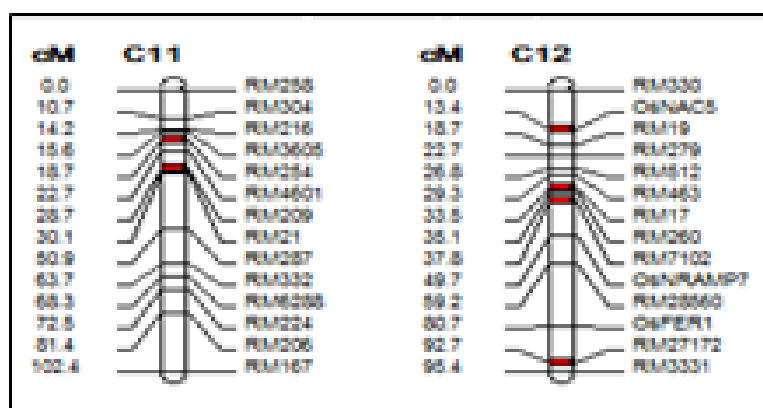
Fig. 4.40 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Pankhali-203 using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top. IM = Interval Mapping.
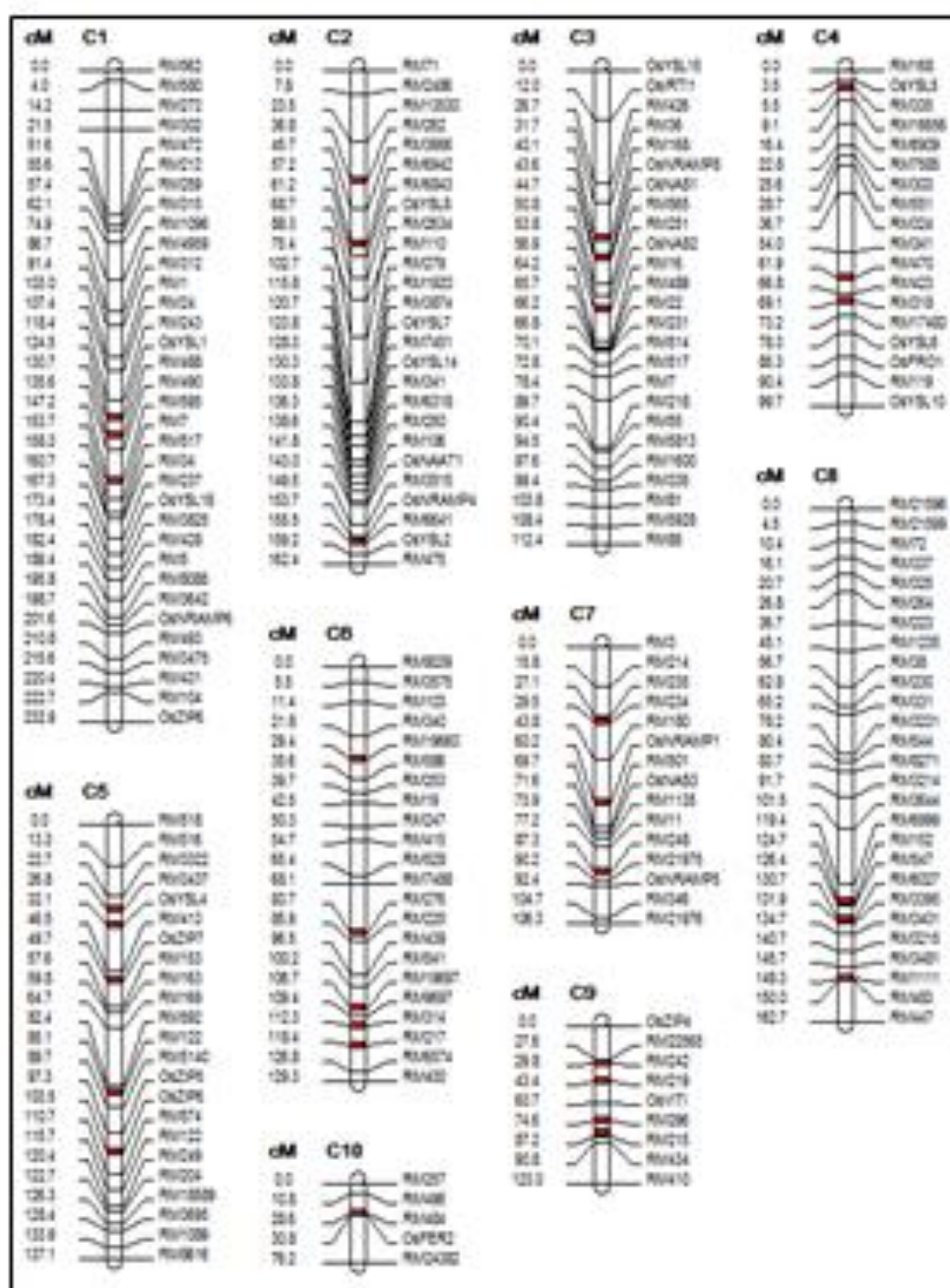
Fig. 4.41 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Pankhali-203 using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
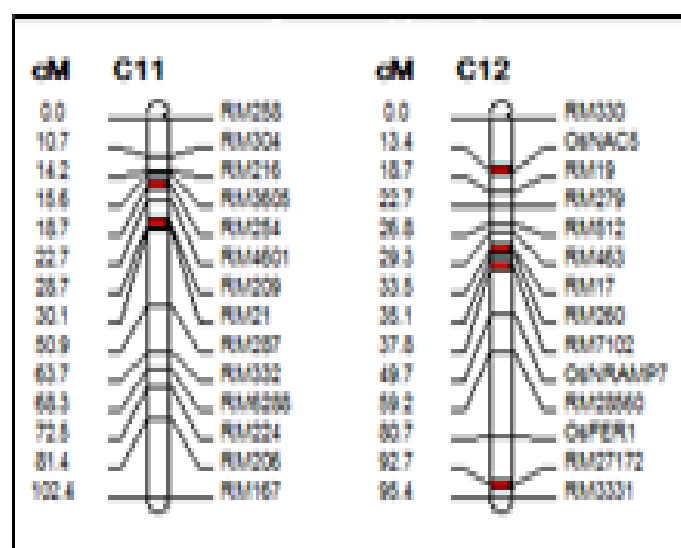
Fig. 4.41 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Pankhali-203 using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.

A large number of markers were mapped on LG1 with 34 (32 SSR and 4 gene specific) markers followed by the 31 (30 SSR and 1 gene specific) markers on LG2, 28 markers both on LG2 (22 SSR and 6 gene specific) and LG5 (24 SSR and 4 gene specific), while 26 (SSR) markers on LG6. Gaps of more than 10 cM were detected on LG10 and LG11. LG1 was the longest while the LG10 was the shortest.

4.6 Mapping Quantitative Trait Loci (QTLs)

Mapping populations derived from cross GR-11 X Pankhali-203, GR-11 X Krishna Kamod and GR-11 X Gurjari were used to map quantitative trait loci (QTLs) associated with traits under study. In order to detect the QTL positions for different traits, the available genotypic data and inter marker distances were used with the phenotypic data using Single Marker Analysis (SMA), Interval Mapping (IM) and Composite Interval Mapping (CIM). QTL positions were assigned to the point of maximum LOD score in the target regions. The details of QTLs detected on different linkage groups of each population are presented in table 4.14 to 4.16.

4.6.1. Single Marker Analysis (SMA)

Single Marker Analysis was done for each marker locus independent of information from other loci. It provides the information on whether the differences between the marker locus and genotype classes are significant or not.

4.6.1.1. Cross GR-11 X Pankhali-203

Ten markers were significantly linked to Zn content in the 300 RIL population of cross GR-11 X Pankhali-203 (Table 4.14). SSR and gene specific makers mapped across all the twelve chromosomes were significantly linked on chromosome 2, 5, 7, 8, 9 and 11. Two markers RM3666 and *OsYSL8* were significantly linked to chromosome 2 with a phenotypic variance of 4.26 % and 1.72 % respectively. Gene specific markers *OsZIP7* was linked to chromosome 5 with a phenotypic variance of 2.06 % while that of *OsNRAMP1* was linked to chromosome 7 at a significant variance of 2.10 %. RM337, RM230 and RM484 were significantly linked on chromosome 8 for zinc content in the 300 RIL populations for the cross GR-11 X Pankhali-203. RM410 was significantly linked to Zn content on chromosome 9.

Table: 4.14 Markers linked to Fe, Zn concentration and other yield related traits in unpolished rice of GR-11 x Pankhali-203 RIL population using Single Marker Analysis

S. No.	Trait	Chromosome	Marker	pr(F)	R^2 (%)
1.	Zn	2	RM3666	0.0003***	4.26
		2	OsYSL8	0.0229*	1.72
		5	OsZIP7	0.0127*	2.06
		7	OsNRAMP1	0.0119*	2.10
		8	RM337	0.0272*	1.62
		8	RM230	0.0494*	1.29
		8	RM484	0.0336*	1.50
		9	RM410	0.0357*	1.47
		11	RM3605	0.0255*	1.66
		11	RM254	0.0416*	1.39
2.	Fe	2	RM3666	0.0011**	3.50
		2	RM106	0.0283*	1.60
		3	RM36	0.0267*	1.64
		5	RM249	0.0416*	1.38
		11	RM332	0.0404*	1.40
		11	RM254	0.0138*	2.02
3.	DFT	1	RM562	0.0178*	1.87
		1	RM259	0.0356*	1.47
		1	RM490	0.0425*	1.37
		1	RM34	0.0356*	1.47
		1	OsYSL18	0.0022**	3.09
		2	RM110	0.0356*	1.47
		2	RM3666	0.0107*	2.17
		2	RM279	0.0167*	1.90
		5	RM3695	0.0447*	1.34
		7	RM21975	0.0491*	1.29
		8	RM331	0.0400*	1.41
		8	RM544	0.0044**	2.68

		9	RM434	0.0416*	1.39
		11	RM287	0.0348*	1.49
		12	RM512	0.0285*	1.60
4.	PH	1	RM580	0.0147*	1.98
		1	RM3825	0.0490*	1.29
		2	RM3666	0.0169*	1.19
		2	RM7451	0.0274*	1.62
		5	OsZIP7	0.0221*	1.74
		5	RM153	0.0481*	1.30
		5	RM5140	0.0415*	1.39
		5	RM122	0.0388*	1.42
		6	RM8039	0.0221*	1.42
		6	RM541	0.0221*	1.74
		7	OsNRAMP1	0.0439*	1.36
		9	OsZIP4	0.0288*	1.59
		11	RM206	0.0079**	2.34
5.	PL	1	RM562	0.0197*	1.81
		1	RM243	0.0403*	1.41
		1	RM3825	0.0170*	1.89
		2	RM6942	0.0337*	1.50
		2	OsNAAT1	0.0397*	1.41
		3	OsNRAMP8	0.0132*	2.04
		6	RM276	0.0443*	1.35
		8	RM72	0.0438*	1.36
		8	RM152	0.0142*	2.00
		8	RM483	0.0473*	1.31
6.	NETP	2	RM3874	0.0112*	2.00
		2	RM7451	0.0012**	3.44
		2	OsYSL7	0.0023**	3.07
		3	RM426	0.0392*	1.42
		3	RM251	0.0196*	1.81
		4	RM6909	0.0350*	1.48

		4	RM17483	0.0427*	1.37
		5	RM592	0.0018**	3.22
		6	RM247	0.0128*	2.06
		6	RM7488	0.0086**	2.99
		6	RM276	0.0369*	1.45
		8	RM21596	0.0353*	1.48
		11	RM304	0.0188*	1.84
		11	RM224	0.0102*	2.19
		12	RM463	0.0138*	2.02
7.	NFGP	1	RM212	0.0102*	2.19
		1	RM259	0.0189*	1.83
		1	RM34	0.0189*	1.83
		1	RM8085	0.0241*	1.69
		2	RM110	0.0189*	1.83
		2	RM1920	0.0301*	1.57
		2	RM7451	0.0119*	2.10
		3	RM514	0.0387*	1.43
		4	RM17483	0.0488*	1.34
		5	RM5140	0.0100*	2.20
		6	RM528	0.0214*	1.76
		6	RM3695	0.0123*	2.08
		7	RM3575	0.0455*	1.33
		7	RM103	0.0040**	2.74
		7	RM7488	0.0238*	1.70
		8	RM544	0.0437*	1.36
		8	RM8271	0.0032**	2.87
		8	RM3644	0.0356*	1.47
		8	RM547	0.0075**	2.37
		8	RM1111	0.0074**	2.38
		8	RM447	0.0188*	1.84
		11	RM3605	0.0233	1.71

		2	RM3666	0.0081**	2.32
8.	TWT	3	OsNAS1	0.0405*	1.40
		3	RM565	0.0023**	3.05
		4	RM335	0.0373*	1.45
		5	RM163	0.0191*	1.83
		5	RM3695	0.0380*	1.44
		9	RM434	0.0271*	1.63
		10	RM257	0.0143*	1.99
		10	OsFER2	0.0329*	1.52
		11	RM332	0.0448*	1.34
9.	GY	1	RM302	0.0101*	2.19
		2	RM3666	0.0000****	6.99
		2	RM2634	0.0461*	1.33
		2	OsYSL8	0.0108*	2.16
		4	RM6909	0.0022**	3.10
		4	RM341	0.0444*	1.35
		4	OsYSL6	0.0064**	2.46
		8	RM223	0.0212*	1.77
		8	RM6999	0.0297*	1.58
		8	RM3481	0.0402*	1.40
		8	RM447	0.0395*	1.41
		10	RM496	0.0155*	2.21
		11	RM167	0.0336*	1.51
		12	RM17	0.0231*	1.72
10.	GL	1	RM490	0.0265*	1.64
		2	RM3666	0.0054**	2.57
		2	RM212	0.0048**	2.64
		4	RM17483	0.0447*	1.31
		4	OsYSL5	0.0280*	1.61
		5	OsZIP6	0.0116*	2.12
		6	RM528	0.0312*	1.55
		7	OsNRAMP5	0.0090**	2.27

		8	RM337	0.0200*	1.80
		8	RM3481	0.0415*	1.99
		9	OsZIP4	0.0060**	2.50
		9	RM215	0.0446*	1.35
		9	RM434	0.0098**	2.22
		10	RM484	0.0026*	2.99
		12	RM27172	0.0358*	1.47
11.	GB	1	RM1	0.0391*	1.42
		2	RM250	0.0377*	1.44
		5	RM169	0.0338*	1.50
		6	RM276	0.0227*	1.73
		10	RM24382	0.0135*	2.03
		12	RM17	0.0147*	1.98
12.	L:B R	5	RM163	0.0496*	1.29
		6	RM253	0.0080**	2.33
		6	RM276	0.0174*	1.88
		8	RM21599	0.0308*	1.55
		8	RM337	0.0495*	1.29
		9	OsZIP4	0.0031**	2.89
		12	RM17	0.0212*	1.77

*Significance at 5 %, **Significance at 1 %, ***Significance at 0.5 %.

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio.

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Fig. 4.42 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Krishna Kamod using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
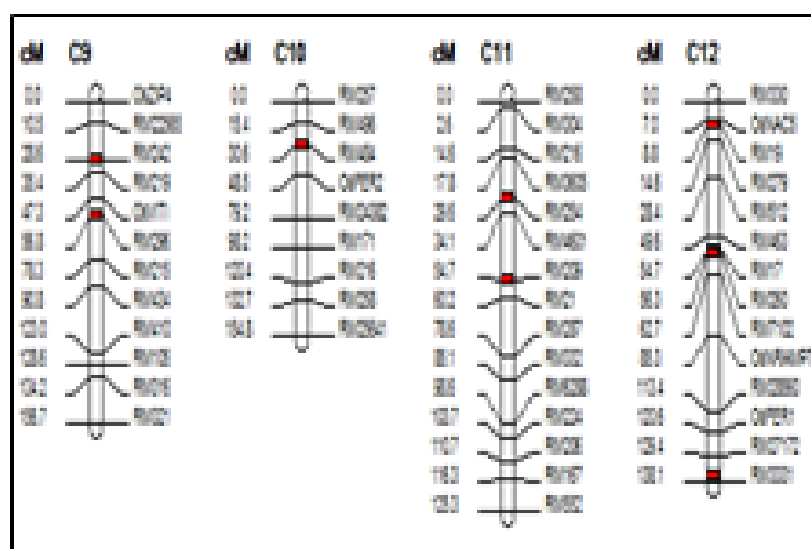
Fig. 4.42 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Krishna Kamod using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top. IM = Interval Mapping.
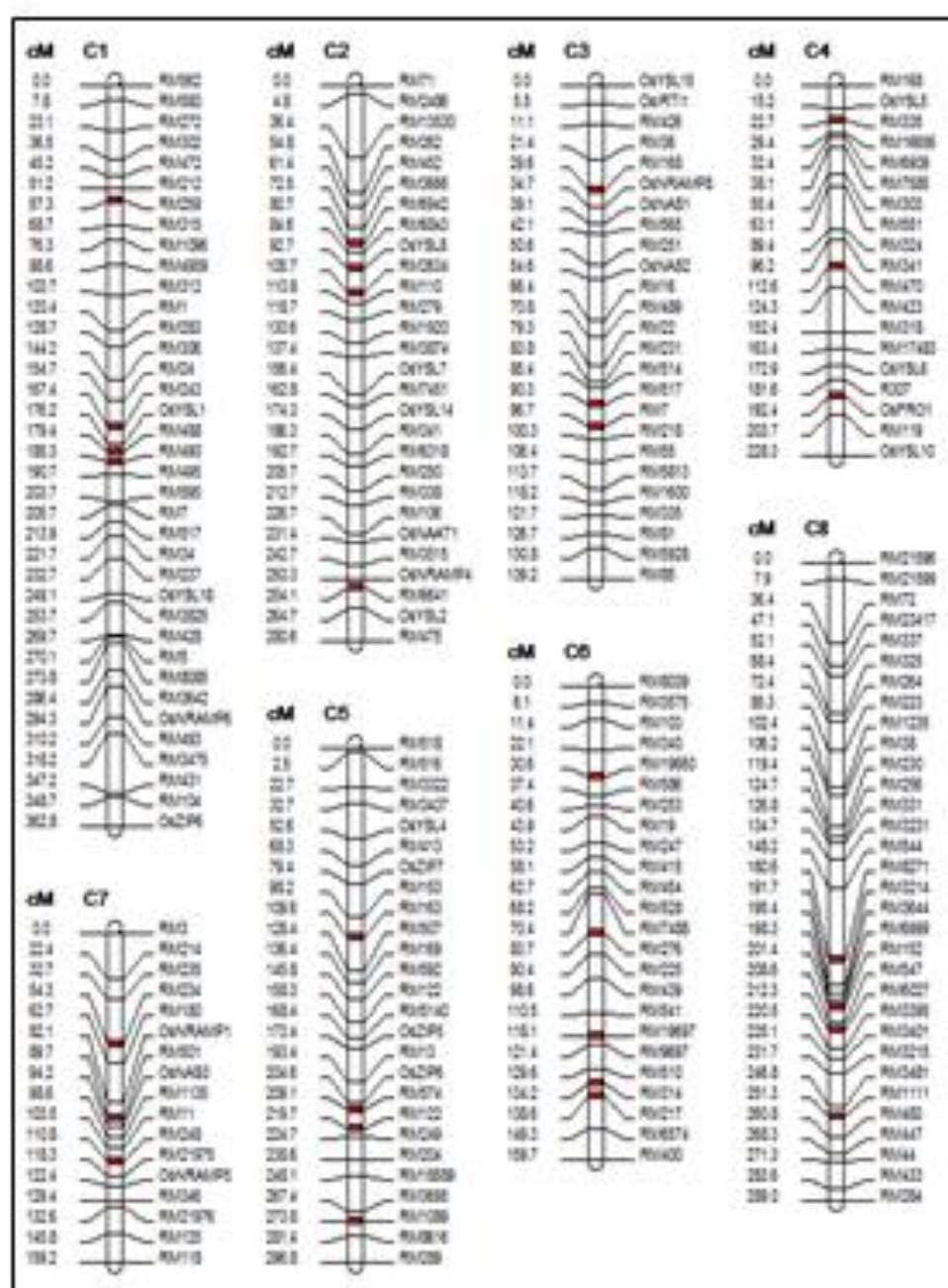
Fig. 4.43 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Krishan Kamod using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
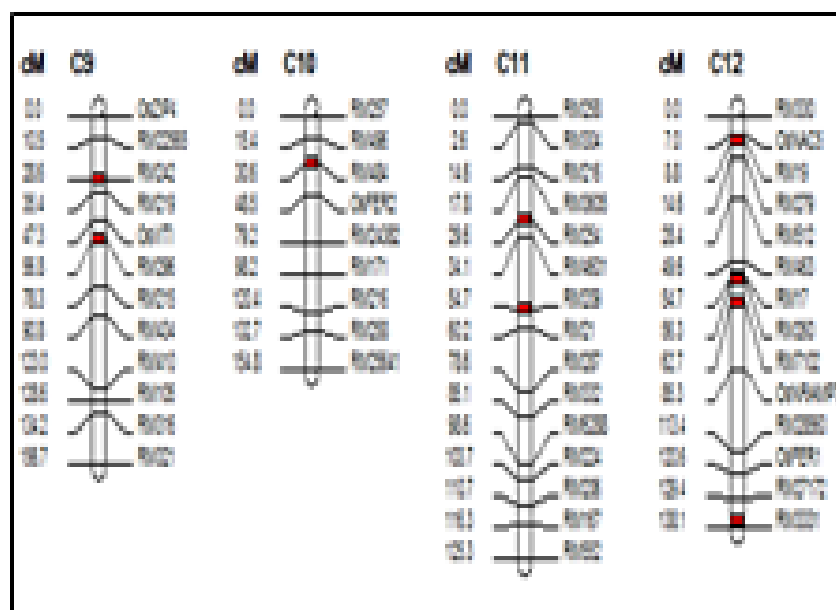
Fig. 4.43 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Krishna Kamod using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.

Six markers mapped across all the chromosomes, were significantly linked to iron concentration among all the RIL populations. RM3666 and RM106 were significantly linked to Fe concentration on LG2 with high phenotypic variance of 3.50 % and 1.60 % respectively. Lesser phenotypic variance was observed for the RM36 on LG3 and RM249 on LG5. In case of LG11, RM332 and RM214 were significantly linked to iron concentration with a higher degree of phenotypic variance. RM3666 was associated to both the traits i.e. zinc and iron concentrations in RIL populations.

Out of 234 markers mapped across all the linkage groups in 300 RIL population of GR-11 X Pankhali-203, 15 markers were significantly associated to days to 50 % flowering. Four SSR (RM562, RM259, RM490 and RM34) and one gene specific marker (*OsYSL18*) showed significant relevance for days to 50 % flowering on LG1. OsYSL18 was highly significant with the phenotypic variance of 3.09 %. Three markers were significantly correlated to LG2 with a lesser degree of phenotypic variance. The least phenotypic variance was obtained for RM21975 on LG7 followed by RM3695 on LG5. A higher liaison was observed for RM544 on LG8 with a phenotypic variance of 2.68 %.

In case of plant height, high significance bearing marker RM206 with phenotypic variation of 2.34 % was mapped on LG11 while the lowest (1.19 %) was on LG2 for RM3666. Gene specific marker *OsNRAMP7* was significantly linked on LG7 whereas *OsZIP4* made a significant relevance to LG9. Four markers were significantly marked to LG5 with less phenotypic variance on the other hand only two markers were in association to LG6.

A total of ten markers exhibited significant kinship across all the 12 linkage groups for panicle length in 300 RIL populations. The phenotypic variance ranged from 2.04 % for *OsNRAMP8* on LG3 to 1.35 % for RM276 on LG6. Three SSR markers were mapped on LG8 of which RM152 having highest variation.

Fifteen SSR markers were associated to number of effective tillers per plant across all the linkage group among the markers mapped. Phenotypic variance ranged from 3.07 % for OsYSL7 on LG2 to 1.37 % for RM17483 on LG4. Two marker were linked for each on LG3 (RM426 and RM251), LG4 (RM6909 and RM17483) and LG11 (RM304 and RM224). Only one marker was associated for this trait on both the LG5 and LG12, both having a high variation at the phenotypic level.
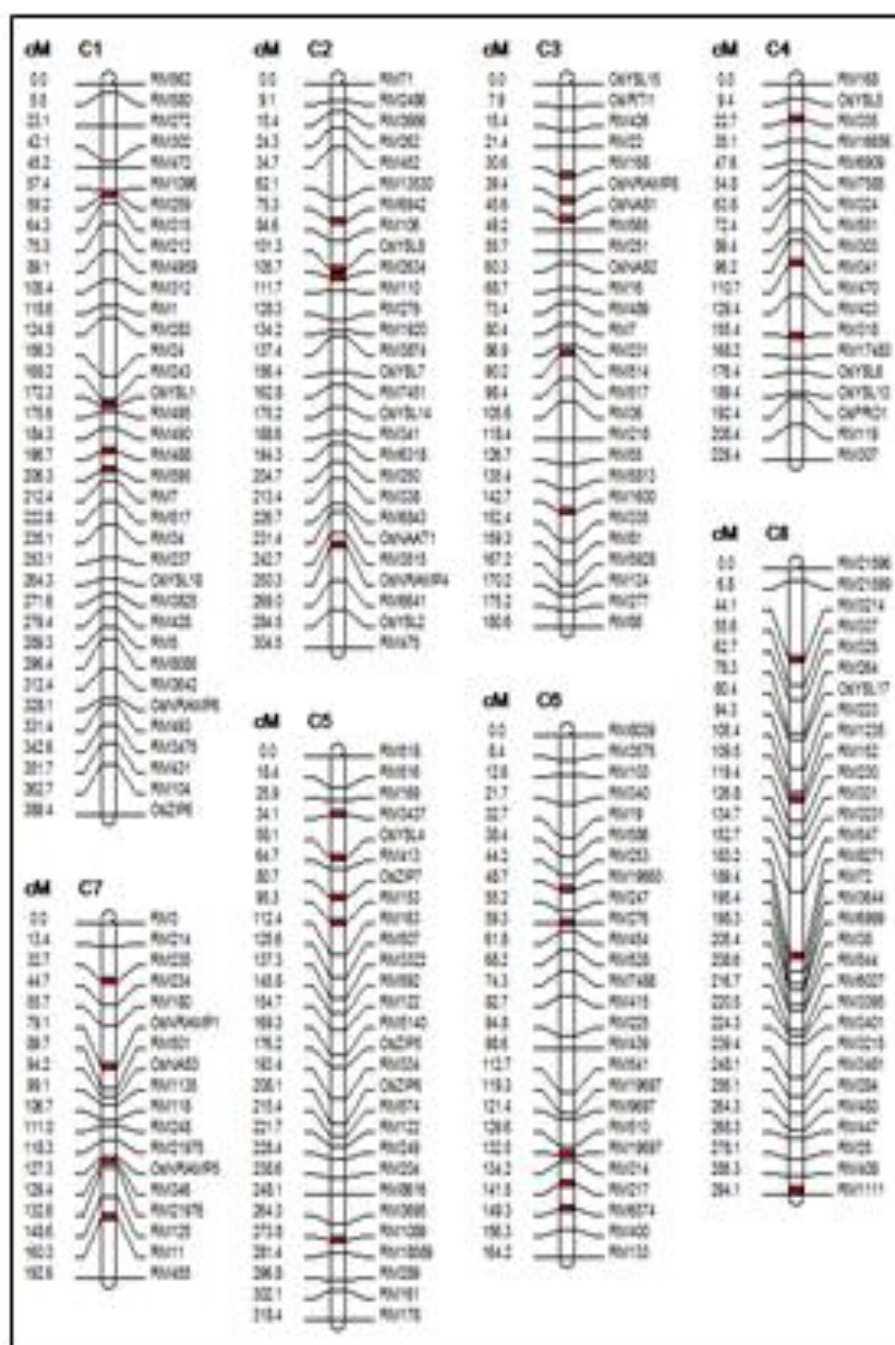
Fig. 4.44 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Gurjari using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
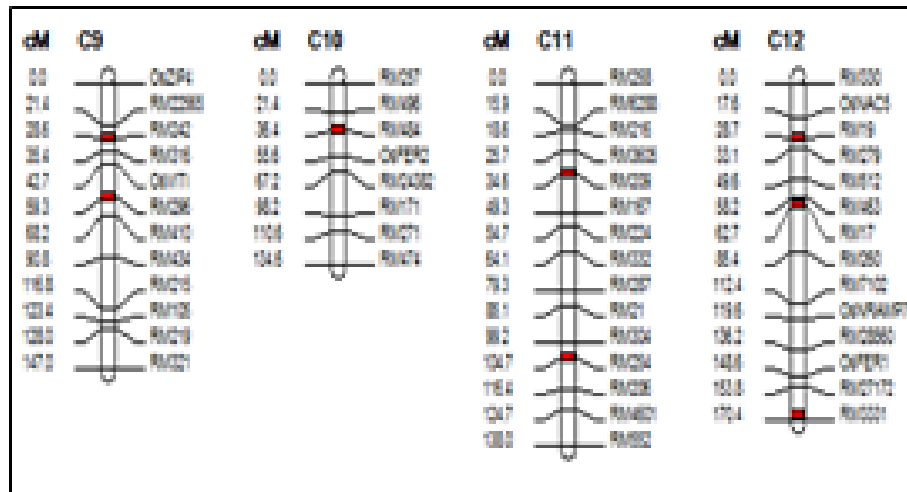
Fig. 4.44 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Gurjari using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
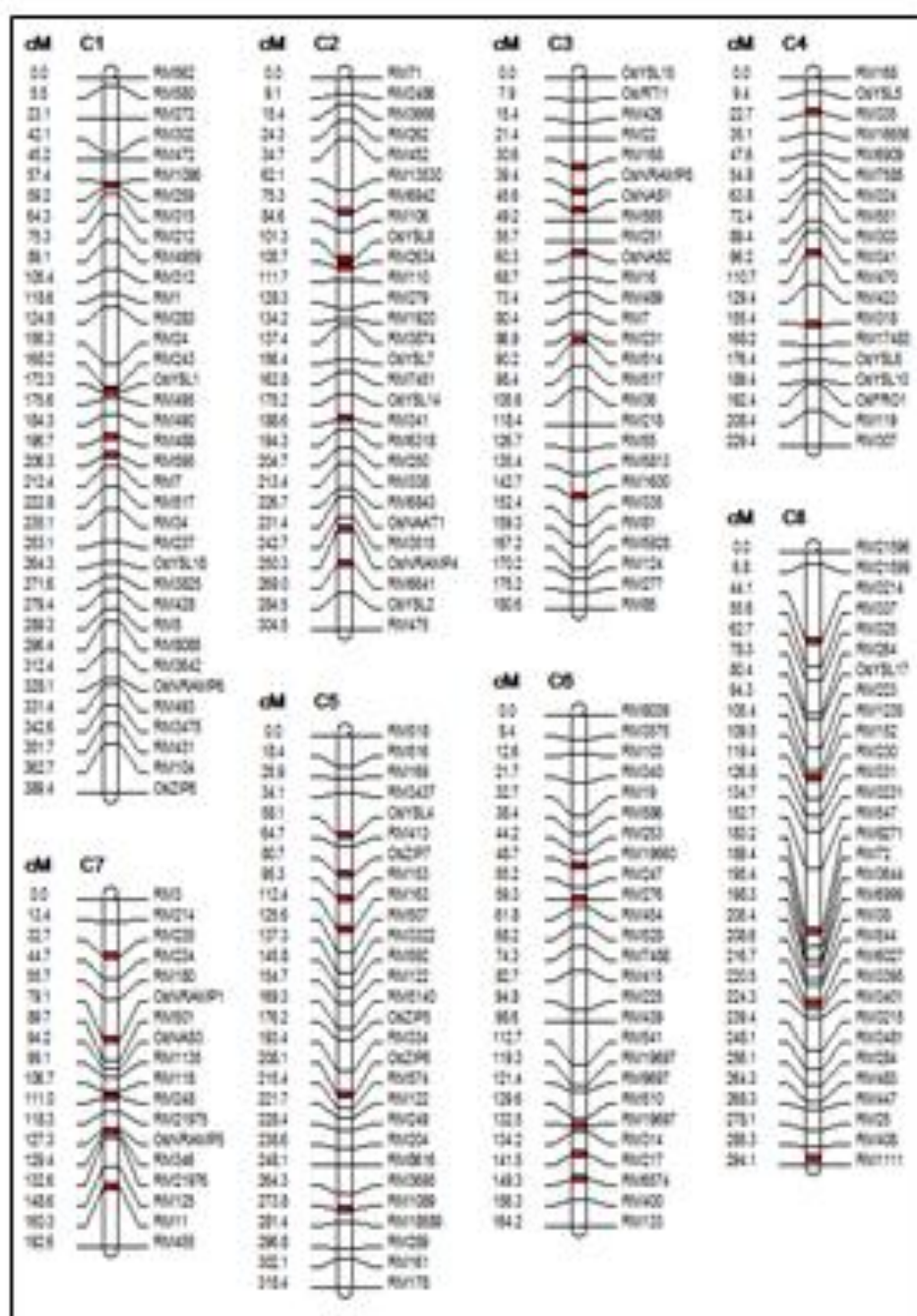
Fig. 4.45 (a): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Gurjari using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
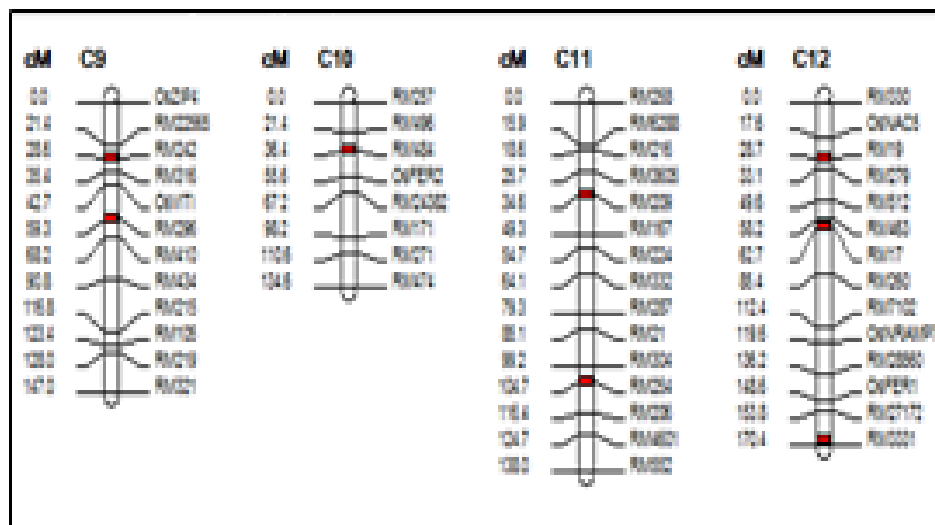
Fig. 4.45 (b): Distribution of QTLs for Fe and Zn in the molecular linkage map of GR-11 X Gunjan using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.

Number of filled grains per panicle had a phenotypic variance ranging from 2.87 % (RM8271) to 1.33 % (RM3575). A total of 20 markers were linked across all the linkage group for number of filled grains per panicle. Out of 27 markers mapped on LG8, 6 were significantly linked for the filled grains per panicle. Markers were linked to all the linkage groups except for the LG12. Highest number of markers were linked to LG8 (6), followed by LG1 (4), LG2 (3) and LG7 (3) while the rest of the linkage groups could harbor only one marker.

In all 10 markers located on chromosome 2, 3, 4, 5, 9, 10 and 11were linked with test weight. The range of phenotypic variance by these 10 loci ranged from 3.05 % (RM565) on LG3 to 1.34 % (RM332) on LG11. Two gene specific markers were linked to LG3 (*OsNAS1*) and LG10 (*OsFER2*). LG3, LG5 and LG10 could bear two markers on each chromosome.

Markers on chromosome 1, 2, 4, 8, 10, 11, 12 significantly correlated to grain yields for all the RIL population. On chromosome 2, RM3666 explained the highest phenotypic variance of 6.99 % while the lowest was observed on chromosome 4 for RM341 of 1.35 %. Four markers were linked to chromosome 8, while three loci on both the chromosome 2 and chromosome 4. Two gene specific markers were linked to chromosome 2 for OsYSL8 and on chromosome 4 for *OsYSL6*.

A total of 15 markers were linked across all the chromosomes for grain length. Four gene specific markers were linked to grain length on chromosome 4 (*OsYSL5)*, chromosome 5 (*OsZIP6*), chromosome 7(*OsNRAMP5*), chromosome 9 (*OsZIP4)* with a phenotypic variance of 16.1 %, 2.12 %, 2.27 % and 2.50 % respectively.

Six SSR markers were linked to grain breadth on 1, 2, 5, 6, 10 and 12 with the highest phenotypic variance observed on chromosome 10 (2.03 %). Least significant difference for the phenotypes was obtained on the RM1 on chromosome 1.

In all 7 markers, located on chromosome 5, 6, 8, 9 and 12 with the phenotypic variance of 1.29 %, 2.33 %, 1.88 %, 1.55 %, 1.29 %, 2.89 % and 1.77 respectively were significantly associated to grain length and breadth ratio. OsZIP4, a gene specific marker located on chromosome 9 has a been in kinship to grain L:B ratio with the phenotypic variation of 2.89 %.

4.6.1.2. Cross GR-11 X Krishna Kamod

Zinc content was significantly associated with the six loci with the highest phenotypic variance of 6.48 % on chromosome 1 for RM1 (Table 4.15).

Table: 4.15 Markers linked to Fe, Zn concentration and other yield related traits in unpolished rice of GR-11 x Krishna Kamod RIL population using Single Marker Analysis

S. No.	Trait	Chromosome	Marker	pr(F)	R^2 (%)
1.	Zn	1	RM495	0.0165*	2.29
		1	RM1	0.0000***	6.48
		2	RM263	0.0034**	3.40
		3	RM218	0.0075**	2.85
		5	RM516	0.0222*	2.09
		6	RM103	0.0415*	1.66
2.	Fe	1	RM472	0.0165*	2.29
		1	RM259	0.0075**	2.85
		1	RM315	0.0000****	6.48
		1	RM283	0.0034**	3.40
		1	RM24	0.0073**	2.86
		1	RM243	0.0415*	1.66
		2	RM452	0.0034**	3.40
		3	RM338	0.00344**	3.40
		3	OsNAS2	0.0222*	2.09
		3	RM1600	0.0222*	2.09
		4	RM324	0.0165*	2.29
		4	RM470	0.0075**	2.85
		4	RM423	0.0000****	6.48
		4	OsYSL10	0.0073**	2.86
		5	RM518	0.0415*	1.66
		7	OsNRAMP1	0.0165	2.29
		7	RM21975	0.0060**	3.00
		8	RM21596	0.0060**	3.00

		8	RM38	0.0165*	2.29
		8	RM331	0.0075**	2.85
		8	RM3231	0.0000****	6.48
		8	RM6999	0.0073**	2.86
		8	RM152	0.0415*	1.66
		8	RM483	0.0073**	2.86
		8	RM447	0.0415*	1.66
		9	RM22565	0.0222*	2.09
		9	RM410	0.0067**	3.00
		10	OsFER2	0.0060**	3.00
		11	RM21	0.0165*	2.85
		11	RM332	0.0075**	2.85
		11	RM224	0.0000****	6.85
		12	RM27172	0.0073*	2.86
		12	RM17	0.0415*	1.66
3.	DFT	1	RM8085	0.0458*	1.60
		2	RM3666	0.0458*	1.60
		2	RM6318	0.0165*	2.85
		3	OsYSL15	0.0067**	3.00
		5	OsZIP5	0.0415*	1.66
		6	RM8039	0.0075**	2.85
		6	RM276	0.0165*	2.85
		7	RM3	0.0415*	1.66
4.	PH	1	RM315	0.0383*	1.72
		1	RM237	0.0314*	1.85
		2	RM1920	0.0314*	1.85
		4	RM423	0.0383*	1.72
		5	RM153	0.0314*	1.85
		6	RM19	0.0314*	1.85
		7	RM21975	0.0469*	1.58
		8	RM21596	0.0469*	1.58

		8	RM3231	0.0383*	1.72
		9	RM410	0.0469*	1.58
		10	OsFER2	0.0469*	1.58
		11	RM224	0.0383*	1.72
5.	PL	1	OsNRAMP6	0.0132*	2.45
		2	OsYSL8	0.0132*	2.45
		3	RM338	0.0097**	2.66
		3	RM85	0.0133*	2.44
		5	RM574	0.0469*	1.58
		6	RM510	0.0121*	2.51
		6	RM439	0.0132*	2.45
		7	OsNRAMP5	0.0097**	2.66
		8	RM21599	0.0097**	2.66
		9	RM219	0.0133*	2.44
		10	RM257	0.0097**	2.66
		10	RM496	0.0097**	2.66
6.	NETP	1	RM1	0.0057**	3.04
		1	RM237	0.0009***	4.33
		2	RM1920	0.0009***	4.33
		3	RM16	0.0236*	2.05
		3	RM231	0.0411*	1.67
		3	RM218	0.0236*	2.05
		4	RM168	0.0411*	1.67
		4	OsYSL5	0.0057**	3.04
		5	RM153	0.0009***	4.33
		6	RM19	0.0009***	4.33
		8	RM3644	0.0057**	3.04
		9	OsZIP4	0.0236*	2.05
		9	OsVITI	0.0411*	1.67
		12	OsNAC5	0.0057**	3.04
7.	NFGP	1	RM495	0.0222*	2.09
		2	RM3666	0.0415*	1.66
		5	RM289	0.0165*	2.29

		6	RM3575	0.0075**	2.85
		8	RM433	0.0000****	6.48
		9	RM242	0.0034**	3.40
		12	RM330	0.0073**	2.86
8.	TWT	1	RM580	0.0043**	3.24
		4	RM6906	0.0043**	3.24
		5	RM507	0.0456*	1.60
		6	RM454	0.0456*	1.60
		7	RM235	0.0043**	3.24
		8	RM264	0.0456*	1.60
		11	RM3605	0.0043**	3.24
9.	GY	1	RM212	0.0421*	1.65
		1	RM315	0.0123*	2.50
		3	RM514	0.0426*	1.65
		3	RM517	0.0472*	1.58
		4	RM119	0.0426*	1.65
		4	RM35	0.0472*	1.58
		4	RM341	0.0421*	1.65
		4	RM423	0.0123*	2.50
		7	RM118	0.0029**	3.51
		7	RM501	0.0421*	1.65
		7	RM1135	0.0421*	1.65
		7	RM180	0.0472*	1.58
		8	RM230	0.0421*	1.65
		8	RM3231	0.0123*	2.50
		9	RM321	0.0019**	3.82
		9	RM215	0.0426*	1.65
		9	RM434	0.0472*	1.58
		11	RM287	0.0421*	1.65
		11	RM224	0.0123*	2.50
10.	GL	1	RM472	0.0425*	1.65
		1	RM212	0.0306*	1.87

		1	RM315	0.0349*	1.78
		1	RM493	0.0033**	3.42
		2	RM110	0.0328*	1.82
		2	RM279	0.0033**	3.42
		2	RM3874	0.0033**	3.42
		2	OsYSL14	0.0328*	1.82
		3	RM565	0.0033**	3.42
		4	RM324	0.0425*	1.65
		4	RM341	0.0306*	1.87
		4	RM423	0.0349*	1.78
		5	RM163	0.0033**	3.42
		5	RM249	0.0328*	1.82
		6	RM253	0.0033**	3.42
		6	RM247	0.0033**	3.42
		6	RM19697	0.0328*	1.82
		7	OsNRAMP1	0.0425*	1.65
		7	RM501	0.0306*	1.87
		7	OsNRAMP5	0.0102*	2.62
		8	RM433	0.0118*	2.53
		8	RM21599	0.0102*	2.62
		8	RM38	0.0425*	1.65
		8	RM230	0.0306*	1.87
		8	RM284	0.0349*	1.78
		10	RM257	0.0102	2.62
		10	RM496	0.0102*	2.62
		11	RM21	0.0425*	1.65
		11	RM287	0.0306*	1.87
		11	RM224	0.0349*	1.78
11.	GB	1	RM562	0.0378*	1.73
		4	RM16656	0.0378*	1.73
		8	RM44	0.0110*	2.58
		8	RM325	0.0378*	1.73

		11	RM216	0.0378*	1.73
12.	L:B R	1	RM1	0.0393*	1.70
		1	RM493	0.0394*	1.70
		2	RM279	0.0394*	1.70
		2	RM1920	0.0110*	2.58
		2	RM3874	0.0378*	1.73
		3	RM565	0.0306*	1.87
		4	OsYSL5	0.0394*	1.70
		5	RM13	0.0493*	1.55
		5	RM163	0.0393*	1.70
		6	RM253	0.0033**	3.42
		6	RM247	0.0493*	1.55
		8	RM3644	0.0328*	1.82
		12	OsNAC5	0.0366*	1.87

*Significance at 5 %, **Significance at 1 %, ***Significance at 0.5 %.

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio.

The range of phenotypic variance explained by these six loci ranged from 1.66 % for RM103 on chromosome 6 to 6.48 % for RM1 on chromosome 1.

Out of 258 markers mapped across all the 12 linkage groups in 250 RIL population of the cross GR-11 X Krishna Kamod, 33 markers were mapped on the chromosomes 1, 2, 3, 4, 5, 7, 8, 9 , 10, 11 and 12. Four gene specific markers (*OsNAS2, OsYSL10, OsNRAMP1* and *OsFER2*) on chromosome 3, 4, 7 and 10 respectively were associated to iron concentration in rice grain. Eight markers were linked on chromosome 8 followed by six on chromosome 1, 4 markers on chromosome 4, 3 markers each on chromosome 3 and 11, while 2 markers each on chromosome 7, 9 and12. The phenotypic variance ranged from 6.85 % for RM224 on chromosome 11 to 1.66 % for RM17 (chromosome 12), RM447 (chromosome 8), RM152 (chromosome 8) and RM518 (chromosome 5).
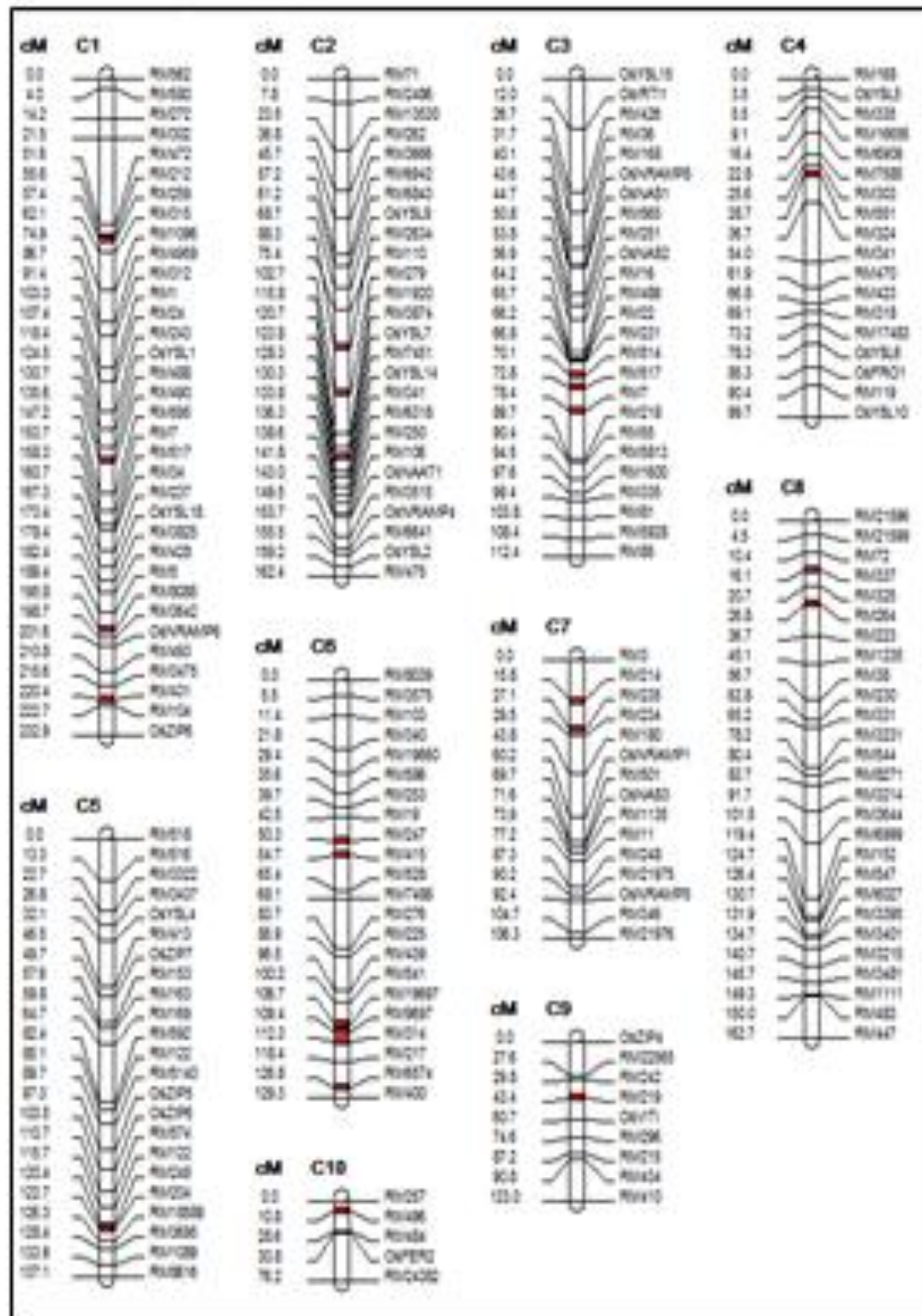
Fig. 4.46 (a): Distribution of QTLs for yield and related traits in the molecular linkage map of GR-11 X Pankhali-203 using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top. IM = Interval Mapping.

Fig. 4.46 (b): Distribution of QTLs for yield and related traits in the molecular linkage map of GR-11 X Pankhali-203 using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top. IM = Interval Mapping.

Fig. 4.47 (a): Distribution of QTLs for yield and related traits in the molecular linkage map of GR-11 X Pankhali-203 using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centimorgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.

Fig. 4.48 (b): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Krishna Kamod using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.

Eight markers were significantly linked to days to 50 % flowering. The phenotypic variance ranged from 2.85 % for RM6318 on chromosome 2, for RM8039 and RM276 on chromosome 6 to 1.60 % for RM3666 on chromosome 2. Two gene specific markers *OsYSL15* had the highest phenotypic variance 3.0 %, while *OsZIP5* on chromosome 5 had the lowest phenotypic variance.

Plant height had a significant correlation to 12 markers on 10 chromosomes. *OsFER2* had a significant correlation for plant height on chromosome 10 with a phenotypic variance of 1.58 %. Highest phenotypic variance was observed for RM237 on chromosome 1 while the least was in case of RM19 on chromosome 6. Two markers showed significant relevance on chromosome 1 and 8 with high level of phenotypic variance.

A total of 12 SSR markers were linked significantly to panicle length across all the chromosomes in the cross GR-11 X Krishna Kamod. Three gene specific markers on chromosome 1 (*OsNRAMP6*), chromosome 2 (*OsYSL8)* and chromosome 7 (*OsNRAMP5*) possessed a high phenotypic variance of 2.45 %, 2.45 % and 2.66 % respectively. RM338 was found to be highly significant on chromosome 3 with the phenotypic variance of 2.66 % and the same was also observed in case of RM21599 on chromosome 8 and RM257 on chromosome 10.

Fourteen markers were significantly associated to number of effective tillers per plant, of which four were gene specific and ten were SSR. The phenotypic variance observed for SSR markers were higher as compared to the gene specific marker. High significance at 0.5 % was observed among the markers RM237 on chromosome 1, RM1920 on chromosome 2, RM153 on chromosome 5 and RM19 on chromosome 6. Phenotypic variance ranged from 1.67 % on *OsVITI* to 4.33 % for RM920 on chromosome 2.

A total of seven SSR markers were linked to number of filled grains per panicle. The phenotypic variance ranged from 6.48 % for RM433 on chromosome 8 to 1.66 % for RM3666 on chromosome 2. RM3575, RM242 and RM330 had significant associated to number grains per panicle on chromosome 6, 9 and 12 respectively with a phenotypic variance of 2.85 %, 3.40 % and 2.86 % respectively.

Positive significant interrelations were observed among the markers mapped on chromosome with test weight. Out of seven markers, 4 markers *viz.* RM580 on chromosome 1, RM6906 on chromosome 4, RM235 on chromosome 7 and RM3605 on chromosome 11 possessed high phenotypic variation.

Nineteen markers were positively linked to the mapped chromosomes for grain yield. Two markers RM118 on chromosome 7 and RM321 on chromosome 9 were significantly correlated with grain yield with a high phenotypic variance. The phenotypic variance was highest for RM118, 3.51 % and least for RM517 on chromosome 3. Four markers were linked both on chromosome 4 and 7 having a significant variation on phenotypes.

A total of 30 markers were significantly linked to grain length for all the markers mapped across the 250 RIL populations in rice. Three gene specific markers *OsYSL14* on chromosome 2 and, *OsNRAMP1* and *OsNRAMP5* on chromosome 7 were linked to grain length with high phenotypic variance. The range of phenotypic variance explained by these 30 loci ranged from 3.42 % for RM493 on chromosome 1 to 1.65 % for RM21 on chromosome11.

Only five markers were associated with grain breadth. Highest phenotypic variance was 2.58 % observed for RM44 on chromosome 8, while the rest had the same variance at 0.1 % level of significance.

The markers mapped on all 12 chromosomes, only 12 were responsible for the grain L:B ratio. Two gene specific markers were linked to L:B ratio on chromosome 4 (*OsYSL5)* and chromosome 12 (*OsNAC5*). RM253 was highly significant at 5 % level of significance with the highest phenotypic variance of 3.42 %.

4.6.1.3. Cross GR-11 X Gurjari

A total of 15 markers were linked to Fe and Zn concentration related traits on all 300 RIL populations across all the 12 chromosomes (Table 4.16). Two gene specific markers were linked to zinc concentrations on chromosome 4 (*OsYSL6*) and chromosome 5 (*OsYSL4*) with the phenotypic variance of 2.18 % and 2.00 % respectively. The phenotypic variance observed for zinc content ranged from 2.34 % for RM302 on chromosome 1 to 1.30 % for RM447 on chromosome 8. RM3481 was

highly significant for zinc content on chromosome 8 with the phenotypic variance of 2.34 %.

Table: 4.16 Markers linked to Fe, Zn concentration and other yield related traits in unpolished rice of GR-11 x Gurjari RIL population using Single Marker Analysis

S. No.	Trait	Chromosome	Marker	pr(F)	R^2 (%)
1.	Zn	1	RM302	0.0079**	2.34
		1	RM312	0.0484*	1.30
		2	RM6318	0.0215*	1.76
		4	*OsYSL6*	0.0104*	2.18
		5	*OsYSL4*	0.0142*	2.00
		5	RM592	0.0104*	2.18
		6	RM247	0.0104*	2.18
		8	RM3481	0.0079**	2.34
		8	RM447	0.0484*	1.30
		12	RM28560	0.0215*	1.76
2.	Fe	1	RM495	0.0046**	2.66
		4	RM324	0.0186*	1.84
		8	RM325	0.0186*	1.84
		8	RM331	0.0046**	2.66
		11	RM224	0.0162*	1.92
3.	DFT	1	RM472	0.0215*	1.76
		2	RM2486	0.0104*	2.18
		2	RM13530	0.0142*	2.00
		5	RM289	0.0104*	2.18
		6	RM8039	0.0186*	1.84
		8	RM44	0.0046**	2.66
		8	RM21599	0.0162*	1.92
		9	*OsZIP4*	0.0215*	1.76
4.	PH	2	RM243	0.0365*	1.46
		4	RM324	0.0142*	2.00

		8	RM325	0.0142*	2.00
		12	RM7102	0.0365*	1.46
5.	PL	1	RM272	0.0165*	1.91
		1	RM7	0.0346*	1.49
		2	RM13530	0.0298*	1.57
		2	RM6942	0.0394*	1.57
		2	RM106	0.0155*	1.95
		2	RM7451	0.0499*	1.28
		2	*OsNRAMP4*	0.0213*	1.77
		3	*OsNAS2*	0.0194*	1.82
		4	*OsYSL6*	0.0333*	1.51
		5	RM13	0.0225*	1.73
		5	RM592	0.0333*	1.51
		6	RM340	0.0225*	1.73
		8	RM331	0.0165*	1.91
		10	RM484	0.0346*	1.49
		11	RM552	0.0394*	1.41
		11	RM209	0.0298*	1.57
		11	RM206	0.0155*	1.95
		12	RM7102	0.0499*	1.22
		12	*OsFER1*	0.0213*	1.77
6.	NETP	1	RM562	0.0375*	1.44
		1	RM472	0.0088**	2.28
		2	RM13530	0.0204*	1.79
		3	*OsNAS2*	0.0232*	1.72
		4	RM423	0.0259*	1.65
		7	RM1135	0.0232*	1.72
		8	RM230	0.0375*	1.44
		8	RM3231	0.0259*	1.65
		8	RM6999	0.0088**	2.28
		9	*OsZIP4*	0.0392*	1.42
		11	RM209	0.0204*	1.79

		1	RM562	0.0008***	3.37
7.	NFGP	1	RM272	0.0186*	1.84
		1	RM495	0.0153*	1.96
		1	RM490	0.0169*	1.90
		1	RM595	0.0153*	1.96
		1	RM428	0.0383*	1.43
		1	RM8085	0.0141*	2.00
		2	RM452	0.0153*	1.96
		2	RM13530	0.0001***	4.90
		3	RM36	0.0038**	2.77
		5	RM507	0.0233*	1.71
		6	RM415	0.0233*	1.71
		7	RM3	0.0038**	2.77
		8	RM230	0.0008***	3.71
		8	RM331	0.0186*	1.84
		8	RM3215	0.0153*	1.96
		8	RM1111	0.0169*	1.96
		9	*OsZIP4*	0.0153*	1.96
		9	RM22565	0.0141*	2.00
		9	RM316	0.0153*	1.96
		10	RM257	0.0383*	1.43
		11	RM304	0.0153*	1.96
		11	RM209	0.0001***	4.90
		2	RM13530	0.0007***	3.76
		2	RM3666	0.0234*	1.71
		2	RM250	0.0113*	2.13
		2	*OsYSL2*	0.0472*	1.31
8.	TWT	5	RM516	0.0210*	1.77
		5	RM289	0.0440*	3.15
		5	*OsYSL4*	0.0355*	1.47
		5	RM13	0.0000****	5.56
		6	RM3575	0.0210*	1.77

		6	RM340	0.0000****	5.56
		6	RM510	0.0440*	1.35
		6	RM247	0.0355*	1.47
		6	RM217	0.0472*	1.31
		8	RM1235	0.0440*	1.35
		8	RM3644	0.0440*	1.35
		9	RM215	0.0355*	1.47
		10	*OsFER2*	0.0440*	1.35
		11	RM209	0.0007***	3.76
		11	RM21	0.0234*	1.71
		12	RM512	0.0113*	2.13
9.	GY	1	RM7	0.0099**	2.21
		1	RM5	0.0330*	1.52
		2	RM13530	0.0000****	7.91
		2	RM250	0.0322*	1.53
		3	*OsNAS2*	0.0436*	1.36
		4	RM16656	0.0239*	1.70
		4	RM470	0.0083**	2.31
		5	RM413	0.0383*	1.43
		5	*OsZIP5*	0.0083**	2.31
		6	RM253	0.0383*	1.43
		7	*OsNRAMP1*	0.0436*	1.36
		8	RM72	0.0239*	1.70
		10	RM22565	0.0330*	1.52
		10	RM242	0.0099**	2.21
		11	RM209	0.0000****	7.79
		12	RM512	0.0322*	1.53
10.	GL	1	RM212	0.0105*	2.18
		1	RM283	0.0414*	1.39
		1	RM243	0.0068**	2.43
		3	*OsIRTI1*	0.0161*	1.93
		3	RM85	0.0161*	1.93

		5	RM163	0.0068**	2.43
		5	RM249	0.0068**	2.43
		6	RM3695	0.0161*	1.93
		8	RM433	0.0160*	1.93
		8	RM8271	0.0105*	2.18
		8	RM483	0.0410*	1.39
		8	RM447	0.0149*	1.97
		9	RM24382	0.0068**	2.43
11.	GB	1	RM3825	0.0313*	1.55
		2	RM110	0.0215*	1.76
		2	RM2486	0.0472*	1.31
		2	RM13530	0.0000****	5.06
		2	RM3666	0.0346*	1.49
		4	RM16656	0.0299*	1.57
		4	RM551	0.0313*	1.55
		8	RM72	0.0299*	1.57
		9	RM219	0.0313*	1.55
		11	RM216	0.0215*	1.76
		11	RM167	0.0472*	1.31
		11	RM209	0.0000****	5.06
		11	RM21	0.0346*	1.46
12.	L:B R	1	RM212	0.0284*	1.60
		1	RM283	0.0092**	2.25
		1	RM24	0.1555*	1.89
		1	RM3825	0.0073**	2.39
		2	RM3666	0.0356*	1.47
		3	*OsYSL15*	0.0220*	1.75
		3	RM517	0.0223*	1.74
		3	RM85	0.0220*	1.75
		4	RM551	0.0073**	2.39
		4	*OsYSL5*	0.0239*	1.70
		5	RM5140	0.0239*	1.70

		5	RM3695	0.0239*	1.70
		6	RM541	0.0223*	1.74
		6	RM400	0.0220*	1.74
		8	RM433	0.0223*	1.74
		8	RM8271	0.0284*	1.6
		8	RM483	0.0092**	2.25
		8	RM447	0.0170*	1.89
		9	RM219	0.0073**	2.39
		11	RM21	0.0356*	1.47

*Significance at 5 %, **Significance at 1 %, ***Significance at 0.5 %.

Zn: Zinc, **Fe**: Iron, **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:B R**: Length: Breadth ratio.

RM495 on chromosome 1 and RM331 on chromosome 8 were significantly correlated to iron concentration in RIL populations with a phenotypic variance of 2.66 %. The least phenotypic variance was observed for the marker RM324 on chromosome 4.

Seven SSR and one gene specific markers were significantly correlated to the days to 50 % flowering. *OsZIP4* on chromosome 9 had the highest phenotypic variance of 1.76 %. Among all these eight markers the phenotypic variance ranged from 1.76 % for RM472 to 2.66 % for RM44 on chromosome 4. RM44 was highly significant at 1 % level of significance and possessed the highest phenotypic variance for days to 50 % flowering.

Plant height was significantly related to four markers across all the markers mapped. The phenotypic variance ranged from 1.46 % to 2.00 %. Markers responsible for plant height were located on chromosome 2, 4, 8 and 12.

A total of 19 markers were responsible for panicle length with significant relationships. Four gene specific markers *OsNRAMP4* on chromosome 2, *OsNAS2* on chromosome 3, *OsYSL6* on chromosome 4 and *OsFER1* on chromosome 12 were linked to panicle length. The phenotypic variance ranged from 1.22 % for RM7102 on

chromosome 12 to 1.95 % for RM206 on chromosome 11. The rest of the markers linked were having significant relation to panicle length.

RM472 on chromosome 1 and RM6999 on chromosome 11 were highly significant for number of effective tillers per plant. Eleven markers could correlate to the number of effective tillers per plant. Two gene specific markers *OsNAS2* on chromosome 3 and *OsZIP4* on chromosome 9 had a high phenotypic variance. The phenotypic variance observed among all these 11 markers ranged from 2.28 % to 1.42 %.

Only one gene specific marker *OsZIP4* on chromosome 9 was linked to number of filled grains per panicle. Highly significant markers were observed on chromosome 1 (RM562), chromosome 2 (RM13530), chromosome 8 (RM230), chromosome 11 (RM209). Phenotypic variance for all these markers ranged from 4.90 % to 1.43 %. Highest phenotypic variance was observed for marker RM13530 on chromosome 2 and RM209 on chromosome 11.

A total of 20 markers were linked to test weight across all the markers mapped on 12 chromosomes. Highly significant interrelations were observed among the markers RM13530 on chromosome 2, RM13 on chromosome 5, RM340 on chromosome 6 and RM209 on chromosome 11. Three gene specific markers were associated with test weight on chromosome 2 (*OsYSL2*), chromosome 5 (*OsYSL4*) and chromosome 10 (*OsFER2)*. The phenotypic variance ranged from 5.56 % to 1.31 %. Chromosome 6 had a high relevance for test weight.

Three gene specific markers out of a total of fifteen markers were associated with grain yield. SSR markers had a high phenotypic variance for grain yield than gene specific markers. The phenotypic variance ranged from 7.79 % to 1.36 %. RM13530 on chromosome 2 had the highest phenotypic variance, while, *OsNAS2* on chromosome 7 had the least variance. RM470, *OsZIP5*, RM242 and RM249 had the highest significance for grain yield.

A total of 13 markers are linked to grain length. *OsIRIT1* was associated to chromosome 3 for grain length. Four markers exhibited high significance with higher phenotypic variance. RM243 on chromosome 1, RM163 and RM249 on chromosome 5, and RM24832 on chromosome 9 had a high phenotypic variance of 2.43 %.

Chromosome 8 had a 4 SSR markers linked to grain length, while 2 on each chromosome 3 and chromosome 5.

Grain breadth had high relevance to chromosomes 1, 2, 4, 8, 9 and 11. High phenotypic variance was observed in case of RM13530 on chromosome 2 and RM209 on chromosome 11. The phenotypic variance ranged from 1.31 % to 5.06 %. Gene specific markers were not associated to grain breadth. Chromosome 2 and chromosome 1 both had 4 markers each associated with grain breadth.

A total of 20 markers were linked to all the chromosomes except 7, 10 and 12 for L:B ratio in 300 RIL populations. Two gene specific markers, *OsYSL15* on chromosome 3 and *OsYSL5* on chromosome 4 were linked to the yield related traits. RM283 and RM3825 on chromosome 1, RM551 on chromosome 4, RM483 on chromosome 8 and RM219 on chromosome 9 had high phenotypic variance for the L:B ratio.

Anuradha *et al*., (2012b) also identified markers on chromosome 8 linked to Fe concentration through single marker analysis with 1.93 % to 4.58 % phenotypic variance. They also reported linked markers on chromosomes 3 and 5 for Zn concentration with a phenotypic variance of 2.49 % to 4.65 %. Bekele *et al*. (2013a) reported linked marker on chromosome 3 using single marker analysis for grain zinc concentration.

4.6.2. QTL mapping

Quantitative traits Loci (QTLs) are the locatable genetic markers that are closely linked to genes affecting biological or agronomic traits. The association between the markers and the trait is used to discover genetic locations of genes controlling the trait. The identification of marker trait association helps breeders to construct beneficial allelic combinations and accelerate breeding programs for cultivar development. The number of QTLs detected in any study depends upon the genetic diversity among parents, environmental conditions, number of markers in the map, and the type and size of the mapping populations (Brondani *et al*., 2002). The genetic architecture of a trait is characterized by the number of effective factors (Wright, 1968), and has an impact on both the power of QTL detection and the magnitude of the bias when estimating QTL effects (Melchinger *et al*., 1998). The

inconsistency observed in the present study due to appearance/loss of QTLs across environments could be explained by altered genes expression in response to environments (Sankaran *et al.*, 2009). The ability of a genotype to alter phenotypic expression in response to different environmental conditions is referred to as phenotypic plasticity (Ungerer *et al.*, 2003). Phenotypic plasticity of the quantitative traits arises in nature from interactions between QTLs and environments at the molecular level.

Mapping populations derived from cross GR-11 X Pankhali-203, GR-11 X Krishna Kamod and GR-11 X Gurjari (Fig.4.37 to Fig. 4.51) were used to detect the quantitative loci (QTLs) associated with the traits under study. In order to detect QTL positions for different the available genotypic data and the inter marker distance were used with the phenotypic data using Interval Mapping (IM) and Composite Interval Mapping (CIM). QTL positions were assigned to the point of maximum LOD score in the target regions. Phenotypic and genotypic data relating to RILs for all the three populations were subjected to QTL mapping using QTL Cartographer 2.5.

4.6.2.1. QTLs Detected for Zinc Concentration

4.6.2.1.1. Cross GR-11 X Pankhali-203

For zinc concentration, one QTL (qZn1.1) located on LG1 was detected using IM and CIM both with the phenotypic variance of 15.7 % and a LOD score of 3.4 (Table 4.17 and Table 4.18). The favorable allele for Zn concentration contributed towards the GR-11. On LG2, qZn2.1 and qZn2.2 were associated for Zn concentration with the phenotypic variance of 16.8 % and 17.2 % and was contributed by GR-11 and Pankhali-203 allele. A phenotypic variance of 21.8 % was observed for the qZn4.1 on LG4, contributed by the GR-11 allele and a LOD of 5.6. Three QTLs were detected both for LG6 and LG7 associated to zinc concentrations among all the populations. QTL (qZn5.3) was contributed by Pankhali-203, located on LG5 with a phenotypic variance of 18.3 % and a LOD score of 4.2. All the three QTLs on LG6 were contributed by GR-11 allele. The QTLs qZn7.1 and qZn7.2 were inherited by Pankhali-203 with a phenotypic variance of 17.2 % and 21.8 % respectively. LG8 had three QTLs qZn8.1, qZn8.2 and qZn8.3, of which qZn8.2 and qZn8.3 had a high amount of phenotypic variance of 32.1 %. Two QTLs were related to zinc concentration on LG9 both with a high phenotypic variance. Only one QTL, qZn10.1

was on LG10 with a marker interval of 10.8 to 30.8 cM. On LG11 also only one QTL was observed qZn11.1 for zinc concentration with a LOD of 5.2 and was inherited from the allele GR-11. QTLs, qZn12.1 and qZn12.2 were detected on LG12 of which qZn12.2 had a highest LOD score of 9.2 with the phenotypic variance of 32.1 %.

Table: 4.17 QTLs detected for Zinc concentration in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qZn1.1	1	124.5	RM565	RM243-RM488	3.4	-19.5	15.7	118.4	130.7	G
2		qZn2.1	2	57.2	RM6942	RM3666-RM6843	4.5	-17.2	16.8	45.7	61.2	G
3		qZn2.2	2	155.5	RM6641	OsNRAMP4-OsYSL2	4.7	17.3	17.2	153.7	159.2	P
4		qZn4.1	4	69.1	RM318	RM423-RM17483	5.6	-19.4	21.8	66.8	73.2	G
5		qZn5.1	5	49.7	OsZIP7	RM413-RM153	5.1	-18.3	21.0	46.5	57.6	G
6		qZn5.2	5	85.1	RM122	RM592-RM5140	4.3	-6.6	16.2	82.4	89.7	G
7		qZn5.3	5	103.5	OsZIP6	OsZIP5-RM574	4.2	18.3	16.2	97.3	110.7	P
8		qZn6.1	6	83.7	RM276	RM7488-RM225	7.4	-13.4	26.8	68.1	85.9	G
9		qZn6.2	6	112.3	RM314	RM9697-RM217	5.0	-12.0	21.8	109.4	118.4	G
10	Zn	qZn6.3	6	118.4	RM217	RM314-RM6574	4.3	-15.5	16.2	112.3	126.8	G
11		qZn7.1	7	60.2	OsNRAMP1	RM180-RM501	4.7	9.8	17.2	43.8	69.7	P
12		qZn7.2	7	87.3	RM248	RM11-RM21975	5.6	19.5	21.8	72.2	90.2	P
13		qZn8.1	8	124.7	RM152	RM6999-RM547	4.6	-10.2	17.2	119.4	126.4	G
14		qZn8.2	8	131.9	RM3395	RM6027-RM3401	9.8	-17.7	32.1	130.7	134.7	G
15		qZn8.3	8	149.3	RM1111	RM3481-RM483	9.2	20.1	32.0	145.7	150.0	P
16		qZn9.1	9	29.8	RM242	RM22565-RM219	4.2	-4.8	16.2	27.6	43.4	G
17		qZn9.2	9	87.2	RM215	RM296-RM434	9.8	-3.8	32.1	74.6	90.8	G
18		qZn10.1	10	28.6	RM484	RM496-OsFER2	9.2	-10.0	32.1	10.8	30.8	G
19		qZn11.1	11	18.7	RM254	RM3605-RM4601	5.2	-10.0	21.0	15.6	22.7	G
20		qZn12.1	12	33.5	RM17	RM463-RM260	4.2	-19.5	16.2	29.3	35.1	G
21		qZn12.2	12	37.8	RM7102	RM260-OsNRAMP7	9.4	10.2	32.1	35.1	49.7	P

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; G: GR-11; P: Pankhali-203

Table: 4.18 QTLs detected for Zinc concentration in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qZn1.1	1	124.5	RM565	RM243-RM488	4.2	-19.5	16.4	118.4	130.7	G
2		qZn2.1	2	57.2	RM6942	RM3666-RM6843	7.4	-17.2	27.0	45.7	61.2	G
3		qZn2.2	2	155.5	RM6641	OsNRAMP4-OsYSL2	5.0	17.3	21.8	153.7	159.2	P
4		qZn4.1	4	69.1	RM318	RM423-RM17483	4.3	-19.4	16.8	66.8	73.2	G
5		qZn5.1	5	49.7	OsZIP7	RM413-RM153	4.7	-18.3	17.2	46.5	57.6	G
6		qZn5.2	5	85.1	RM122	RM592-RM5140	5.6	-6.6	21.6	82.4	89.7	G
7		qZn5.3	5	103.5	OsZIP6	OsZIP5-RM574	4.6	18.3	17.2	97.3	110.7	P
8		qZn6.1	6	83.7	RM276	RM7488-RM225	9.8	-13.4	32.6	68.1	85.9	G
9		qZn6.2	6	112.3	RM314	RM9697-RM217	9.2	-12.0	32.0	109.4	118.4	G
10		qZn6.3	6	118.4	RM217	RM314-RM6574	4.2	-15.5	16.4	112.3	126.8	G
11	Zn	qZn7.1	7	60.2	OsNRAMP1	RM180-RM501	4.5	9.8	16.8	43.8	69.7	P
12		qZn7.2	7	87.3	RM248	RM11-RM21975	5.8	19.5	21.6	72.2	90.2	P
13		qZn8.1	8	124.7	RM152	RM6999-RM547	9.2	-10.2	32.0	119.4	126.4	G
14		qZn8.2	8	131.9	RM3395	RM6027-RM3401	6.4	-17.7	24.5	130.7	134.7	G
15		qZn8.3	8	149.3	RM1111	RM3481-RM483	9.8	20.1	32.6	145.7	150.0	P
16		qZn9.1	9	29.8	RM242	RM22565-RM219	9.2	-4.8	31.8	27.6	43.4	G
17		qZn9.2	9	87.2	RM215	RM296-RM434	5.2	-3.8	21.8	74.6	90.8	G
18		qZn10.1	10	28.6	RM484	RM496-OsFER2	4.2	-10.0	16.4	10.8	30.8	G
19		qZn11.1	11	18.7	RM254	RM3605-RM4601	9.4	-10.0	32.4	15.6	22.7	G
20		qZn12.1	12	33.5	RM17	RM463-RM260	8.5	-19.5	28.5	29.3	35.1	G
21		qZn12.2	12	37.8	RM7102	RM260-OsNRAMP7	5.3	10.2	21.6	35.1	49.7	P

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; G: GR-11; P: Pankhali-203

In summary the highest phenotypic variance of 32.1 % was observed for qZn8.2, qZn8.3, qZn9.2, qZn10.1 and qZn12.2 on LG8, LG9, LG10 and LG12 respectively. The majority of the QTLs were inherited by the GR-11 parent. The phenotypic variance observed among all these 21 QTLs ranged from 15.7 % to 32.1 %. The QTLs detected by the IM model were the same as that detected by CIM model with little more phenotypic variance.

4.6.2.1.2. Cross GR-11 X Krishna Kamod

A total of 23 QTLs were detected with the aid of CIM for Zn concentration in the 250 RIL population based cross GR-11 X Krishna Kamod, while only 21 QTLs were identified with the aid of IM (Table 4.19 and Table 4.20). Three QTLs, qZn1.1, qZn1.2 and qZn1.3 at 80.7 cM, 105.7 cM and 254.1 cM with the phenotypic variance of 16.8 %, 17.1 % and 21.9 % and a LOD of 4.3, 4.7 and 5.6 respectively. LG3 had three QTLs, qZn3.1 (29.6 cM), qZn3.2 (90.3 cM) and qZn3.3 (96.7 cM) had a LOD of 4.6, 9.8 and 9.2 with a phenotypic variance of 17.2 %, 32.1 % and 31.5 % respectively. A single QTL was identified at LG4 with the phenotypic variance of 15.3 % and a LOD of 3.4. A phenotypic variance of 16.8 % and 16.3 % was observed for qZn5.1 and qZn5.2 on LG5 with a LOD of 4.5 and 4.7 respectively. In case of LG6, three QTLs qZn6.1, qZn6.2 and qZn6.3 had a phenotypic variance of 21.9 %, 21.3 % and 16.8 % with a LOD of 5.6, 5.1 and 4.3 respectively. QTL, qZn6.2 was contributed by Krishna Kamod allele. Two QTLs were detected on LG7 at 89.7 cM and 110.8 cM with the LOD of 9.2 and 5.4 and a phenotypic variance of 31.5 % and 21.7 % respectively. Three QTLs on LG8 were inherited by GR-11 allele with a phenotypic variance of 17.2 %, 22.0 % and 28.3 % and a LOD of 4.8, 5.0 and 8.1 respectively. Single QTLs, qZn9.1 and qZn10.1 were observed on LG9 and LG10 at 28.6 cM and 30.6 cM with the phenotypic variance of 15.3 % and 17.3 % and a LOD of 3.4 and 4.5 respectively. Zinc concentrations on LG11 were governed by two QTLs, qZn11.1 and qZn11.2 at 29.6 cM and 54.7 cM with the highest phenotypic variance of 43.7 % and 35.6 % and a LOD of 12.4 and 10.6 respectively. A QTL, qZn12.2 on LG12 was inherited by the Krishna Kamod allele with a phenotypic

variance of 27.5 % and a LOD of 7.8. The other QTL, qZn12.1 was contributed by GR-11 allele and had a LOD of 9.8 and a phenotypic variance of 32.6 %.

Table: 4.19 QTLs detected for Zinc concentration in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qZn2.1	2	80.7	RM6942	RM3666-RM6843	7.8	-13.4	27.2	72.5	84.6	G
2		qZn2.2	2	105.7	RM2634	OsYSL8-RM110	9.2	-12.0	32.6	92.7	110.8	G
3		qZn2.3	2	254.1	RM6641	OsNRAMP4-OsYSL2	5.4	-15.5	22.4	250.3	264.3	G
4		qZn3.1	3	29.6	RM168	RM163-OsNAS1	4.8	9.8	17.3	21.4	34.7	K
5		qZn3.2	3	90.3	RM517	RM231-RM517	5.0	19.5	22.6	85.4	96.7	K
6		qZn3.3	3	96.7	RM7	RM514-RM7	8.1	-10.2	29.7	90.3	100.3	G
7		qZn5.1	5	109.6	RM163	RM153-RM507	3.4	-12.0	15.2	96.2	128.4	G
8		qZn6.1	6	83.7	RM276	RM7488-RM225	4.5	-13.0	16.8	70.4	90.4	G
9		qZn6.2	6	134.2	RM314	RM510-RM217	4.3	-17.5	16.4	129.6	138.6	G
10		qZn6.3	6	138.6	RM217	RM314-RM6574	4.7	11.2	17.1	134.2	149.3	K
11	Zn	qZn7.1	7	89.7	RM501	OsNRAMP1-OsNAS3	5.6	-17.5	22.5	82.1	94.2	G
12		qZn7.2	7	110.8	RM248	RM11-RM21975	4.6	-19.2	17.8	103.5	118.3	G
13		qZn8.1	8	180.6	RM8271	RM544-RM3214	9.8	17.9	31.6	148.2	191.7	K
14		qZn8.2	8	201.4	RM152	RM6999-RM547	9.2	-19.8	31.2	198.3	208.6	G
15		qZn8.3	8	251.3	RM1111	RM3481-RM483	3.4	-17.3	15.2	246.8	260.8	G
16		qZn9.1	9	28.6	RM242	RM22555-RM219	4.5	-6.8	16.9	10.5	35.4	G
17		qZn10.1	10	30.6	RM484	RM496-OsFER2	4.7	17.3	17.2	15.4	48.5	K
18		qZn11.1	11	29.6	RM254	RM3605-RM4501	5.6	-16.4	22.3	17.8	34.1	G
19		qZn11.2	11	54.7	RM229	RM4501-RM21	5.1	-12.0	21.8	34.1	60.2	G
20		qZn12.1	12	54.7	RM17	RM453-RM250	4.3	-16.5	17.6	49.6	56.3	G
21		qZn12.2	12	62.7	RM7102	RM260-OsNRAMP7	4.8	-19.2	17.9	56.3	85.3	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; G: GR-11; K: Krishna Kamod

Table: 4.20 QTLs detected for Zinc concentration in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qZn2.1	2	80.7	RM6942	RM3666-RM6843	4.3	-15.4	16.8	72.5	84.6	G
2		qZn2.2	2	105.7	RM2634	OsYSL8-RM110	4.7	-12.2	17.1	92.7	110.8	G
3		qZn2.3	2	254.1	RM6641	OsNRAMP4-OsYSL2	5.6	-16.5	21.9	250.3	264.3	G
4		qZn3.1	3	29.6	RM168	RM163-OsNAS1	4.6	10.8	17.2	21.4	34.7	K
5		qZn3.2	3	90.3	RM517	RM231-RM517	9.8	17.5	32.1	85.4	96.7	K
6		qZn3.3	3	96.7	RM7	RM514-RM7	9.2	-12.2	31.5	90.3	100.3	G
7		qZn4.1	4	22.7	RM335	OsYSL5-RM16656	3.4	-17.7	15.3	15.2	29.4	G
8		qZn5.1	5	109.6	RM163	RM153-RM507	4.5	-11.0	16.8	96.2	128.4	G
9		qZn5.2	5	273.8	RM1089	RM3695-RM8616	4.7	-12.0	16.3	267.4	281.4	G
10		qZn6.1	6	83.7	RM276	RM7488-RM225	5.6	-19.5	21.9	70.4	90.4	G
11		qZn6.2	6	134.2	RM314	RM510-RM217	5.1	10.2	21.3	129.6	138.6	K
12	Zn	qZn6.3	6	138.6	RM217	RM314-RM6574	4.3	-19.5	16.8	134.2	149.3	G
13		qZn7.1	7	89.7	RM501	OsNRAMP1-OsNAS3	9.2	-17.2	31.5	82.1	94.2	G
14		qZn7.2	7	110.8	RM248	RM11-RM21975	5.4	17.3	21.7	103.5	118.3	K
15		qZn8.1	8	180.6	RM8271	RM544-RM3214	4.8	-19.4	17.2	148.2	191.7	G
16		qZn8.2	8	201.4	RM152	RM6999-RM547	5.0	-17.3	22.0	198.3	208.6	G
17		qZn8.3	8	251.3	RM1111	RM3481-RM483	8.1	-6.6	28.3	246.8	260.8	G
18		qZn9.1	9	28.6	RM242	RM22555-RM219	3.4	18.3	15.3	10.5	35.4	K
19		qZn10.1	10	30.6	RM484	RM496-OsFER2	4.5	-15.4	17.3	15.4	48.5	G
20		qZn11.1	11	29.6	RM254	RM3605-RM4501	12.4	-13.0	43.7	17.8	34.1	G
21		qZn11.2	11	54.7	RM229	RM4501-RM21	10.6	-14.5	35.6	34.1	60.2	G
22		qZn12.1	12	54.7	RM17	RM453-RM250	9.8	-19.2	32.6	49.6	56.3	G
23		qZn12.2	12	62.7	RM7102	RM260-OsNRAMP7	7.8	15.8	27.5	56.3	85.3	K

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; G: GR-11; K: Krishna Kamod

To summarize, in all 23 QTLs were detected for the zinc concentration in 250 RIL populations across all the linkage groups. The highest phenotypic variance of 35.6 % was observed for qZn11.2. Among all the 23 QTLs identified for the zinc concentration only six were contributed by the Krishna Kamod the rest had an inheritance by GR-11 allele. The QTLs qZn2.4 on LG2 and qZn4.1 on LG 4 were only identified by the CIM method.

4.6.2.1.3. Cross GR-11 X Gurjari

In all 22 QTLs were detected for zinc concentration among all the 300 RIL populations (Table 4.21 and Table4.22). A single QTL was identified at 206.3 cM on LG1 with a LOD of 5.4 and a phenotypic variance of 22.1 %. Four QTLs were detected on LG2 with the phenotypic variance of 16.4 % (qZn2.1), 17.3 % (qZn2.2), 21.4 % (qZn2.3) and 22.6 % (qZn2.4) and a LOD of 4.3, 4.7, 5.6 and 5.1 respectively. Except for qZn2.4 that was inherited by Gurjari all the others contributed to GR-11. A single QTL governing the gene specific marker OsNRAMP8 had a phenotypic variance of 27.1 % and a LOD of 7.2 and was inherited by GR-11. On LG4, two QTLs, qZn4.1 and qZn4.2 at 22.7 cM and 155.4 cM had a phenotypic variance of 32.6 % and 31.0 % with a high LOD of 9.8 and 9.4 respectively. Three QTLs on LG5 related to Zinc concentration with a phenotypic variance of 32.4 % (qZn5.1), 22.0 % (qZn5.2) and 21.4 % (qZn5.3) and a LOD of 9.2, 5.2 and 5.1 respectively. In case of LG6 three QTLs, qZn6.1, qZn6.2 and qZn6.3 could relate to zinc concentration with the LOD of 4.3, 10.6 and 4.6 having a phenotypic variance of 17.6 %, 35.0 % and 17.0 %. Two QTLs on LG8 were inherited by GR-11 allele and had a phenotypic variance of 22.5 % (qZn8.1) and 21.3 % (qZn8.2) with a LOD of 5.0 and 5.2 respectively. Single QTL was detected each on LG9 and LG10 at 29.6 cM and 36.4 cM with a phenotypic variance of 27.3 % and 32.4 % and a LOD of 7.8 and 9.2 respectively. LG11 and LG12 were inherited by GR-11 allele and had a LOD of 5.4 and 4.8 with a phenotypic variance of 22.4 % and 17.6 %.

In a nutshell, the highest phenotypic variance of 42.6 % was observed for the qZn8.1 on LG8 with the highest LOD of 12.4. The phenotypic variance ranged from

18.2 % to 42.6 % for the zinc concentration in all the 300 RIL populations. Among all the QTLs detected were inherited by GR-11 except two qZn6.2 and qZn8.2 contributed to Gurjari. QTLs, qZn2.3 and qZn2.4 located on LG2 were only detected using CIM method.

Table: 4.21 QTLs detected for Zinc concentration in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qZn1.1	1	206.3	RM595	RM488-RM7	5.5	-18.4	21.4	196.7	212.4	GR
2		qZn2.1	2	75.3	RM6942	RM13530-RM106	5.2	-13.0	21.6	62.1	84.6	GR
3		qZn2.2	2	105.7	RM2634	OsYSL8-RM110	4.8	-11.5	18.2	101.3	111.7	GR
4		qZn3.1	3	39.4	OsNRAMP8	RM168-OsNAS1	6.1	-9.8	23.7	30.6	45.6	GR
5		qZn4.1	4	22.7	RM335	OsYSL15-RM16656	7.3	-11.7	24.5	9.4	35.1	GR
6		qZn4.2	4	155.4	RM318	RM423-RM17483	8.0	-14.8	26.1	129.4	168.2	GR
7		qZn5.1	5	80.7	OsZIP7	RM413-RM153	5.4	-9.9	21.4	64.7	95.3	GR
8		qZn5.2	5	95.3	RM153	OsZIP7-RM163	5.4	-19.7	21.4	80.7	112.4	GR
9		qZn5.3	5	112.4	RM163	RM153-RM507	5.6	-12.2	21.8	95.3	125.6	GR
10		qZn6.1	6	59.3	RM276	RM247-RM545	5.1	-19.7	21.6	55.2	61.8	GR
11	Zn	qZn6.2	6	141.5	RM217	RM314-RM6574	4.7	20.1	18.4	134.2	149.3	G
12		qZn6.3	6	149.3	RM5574	RM217-RM400	4.3	-4.8	18.3	141.5	156.3	GR
13		qZn7.1	7	32.7	RM235	RM214-RM234	4.8	-5.8	18.5	13.4	44.7	GR
14		qZn7.2	7	160.3	RM11	RM125-RM455	9.1	-17.5	20.7	148.6	192.6	GR
15		qZn8.1	8	109.5	RM152	RM1235-RM230	12.4	-17.2	42.6	105.4	119.4	GR
16		qZn8.2	8	183.2	RM8271	RM547-RM72	6.1	19.3	23.7	152.7	189.4	G
17		qZn9.1	9	28.6	RM242	RM2256-RM242	7.4	-15.4	24.2	21.4	35.4	GR
18		qZn10.1	10	36.4	RM484	RM496-OsFER2	5.4	-17.3	20.8	21.4	55.6	GR
19		qZn11.1	11	104.7	RM254	RM304-RM206	7.4	-6.8	24.4	99.2	116.4	GR
20		qZn12.1	12	28.7	RM19	OsNAC5-RM279	6.3	-18.9	23.5	17.6	33.1	GR

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; GR: GR-11; G: Gurjari

Table: 4.22 QTLs detected for Zinc concentration in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Composite Interval Mapping (CIM)

S. No.	QTL	Trait	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	qZn1.1		1	206.3	RM595	RM488-RM7	5.4	-16.4	22.1	196.7	212.4	GR
2	qZn2.1		2	75.3	RM6942	RM13530-RM106	4.3	-12.0	16.4	62.1	84.6	GR
3	qZn2.2		2	105.7	RM2634	OsYSL8-RM110	4.7	-10.5	17.3	101.3	111.7	GR
4	qZn2.3		2	188.6	RM341	OsYSL14-RM6318	5.6	-18.2	21.4	175.2	194.3	GR
5	qZn2.4		2	269.0	RM6641	OsNRAMP4-OsYSL2	5.1	17.9	22.6	250.3	284.5	G
6	qZn3.1		3	39.4	OsNRAMP8	RM168-OsNAS1	7.8	-9.8	27.1	30.6	45.6	GR
7	qZn4.1		4	22.7	RM335	OsYSL15-RM16656	9.8	-12.7	32.6	9.4	35.1	GR
8	qZn4.2		4	155.4	RM318	RM423-RM17483	9.4	-15.8	31.0	129.4	168.2	GR
9	qZn5.1	Zn	5	80.7	OsZIP7	RM413-RM153	9.2	-10.9	32.4	64.7	95.3	GR
10	qZn5.2		5	95.3	RM153	OsZIP7-RM163	5.2	-18.7	22.0	80.7	112.4	GR
11	qZn5.3		5	112.4	RM163	RM153-RM507	5.1	-12.2	21.4	95.3	125.6	GR
12	qZn6.1		6	59.3	RM276	RM247-RM545	4.3	-18.7	17.6	55.2	61.8	GR
13	qZn6.2		6	141.5	RM217	RM314-RM6574	10.6	19.1	35.0	134.2	149.3	G
14	qZn6.3		6	149.3	RM5574	RM217-RM400	4.6	-5.8	17.8	141.5	156.3	GR
15	qZn7.1		7	32.7	RM235	RM214-RM234	4.3	-6.8	17.3	13.4	44.7	GR
16	qZn7.2		7	160.3	RM11	RM125-RM455	6.5	-16.5	24.3	148.6	192.6	GR
17	qZn8.1		8	109.5	RM152	RM1235-RM230	5.0	-17.2	22.5	105.4	119.4	GR
18	qZn8.2		8	183.2	RM8271	RM547-RM72	5.2	19.8	21.3	152.7	189.4	G
19	qZn9.1		9	28.6	RM242	RM2256-RM242	7.8	-16.4	27.3	21.4	35.4	GR
20	qZn10.1		10	36.4	RM484	RM496-OsFER2	9.2	-18.3	32.4	21.4	55.6	GR
21	qZn11.1		11	104.7	RM254	RM304-RM206	5.4	-8.8	22.4	99.2	116.4	GR
22	qZn12.1		12	28.7	RM19	OsNAC5-RM279	4.8	-19.9	17.6	17.6	33.1	GR

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Zn: Zinc concentration; GR: GR-11; G: Gurjari
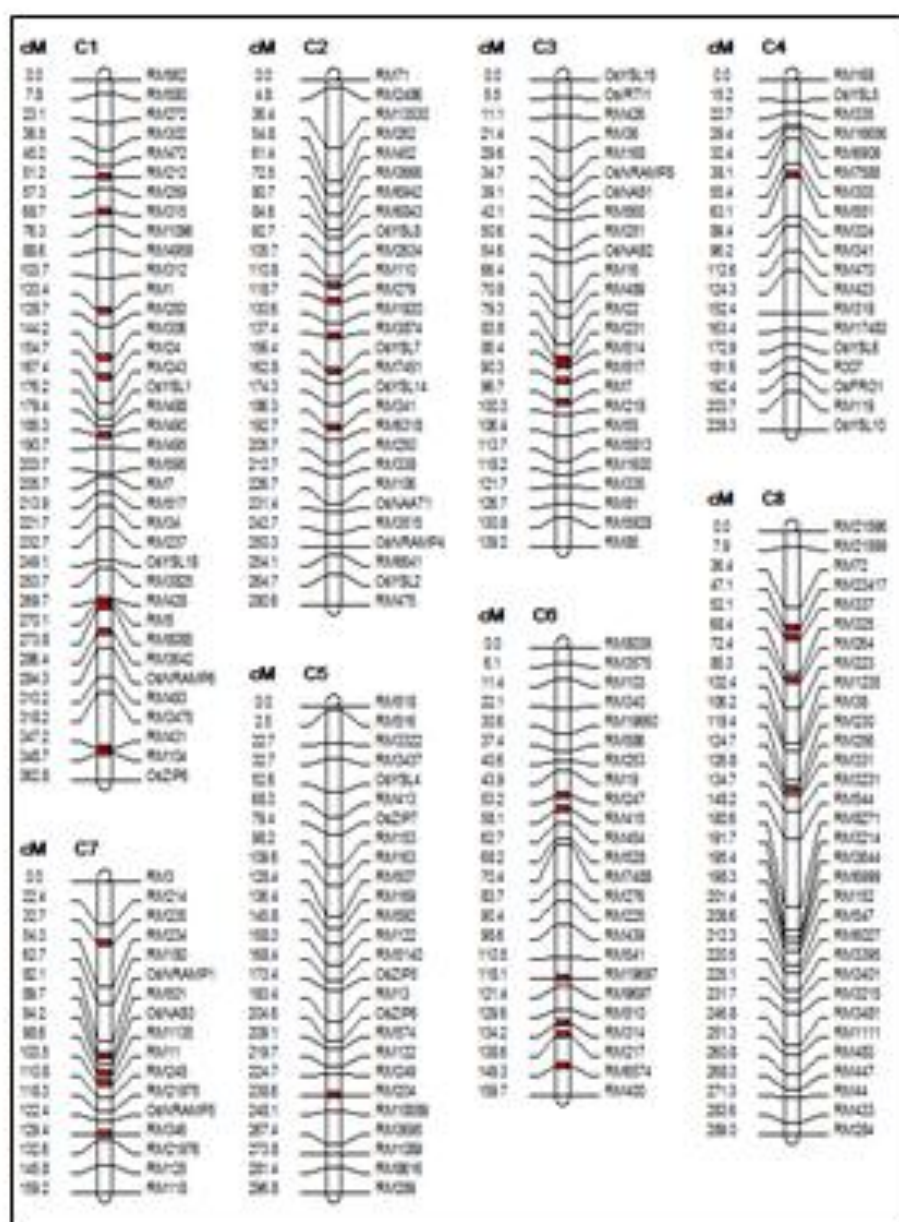
Fig. 4.49 (a): Distribution of QTLs for yield and yield related in the molecular linkage map of GR-11 X Krishna Kamod using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
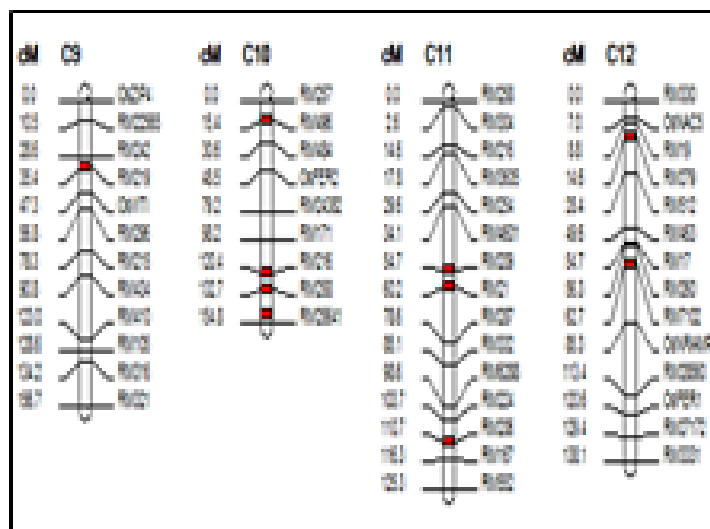
Fig. 4.49 (b): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Krishna Kamod using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
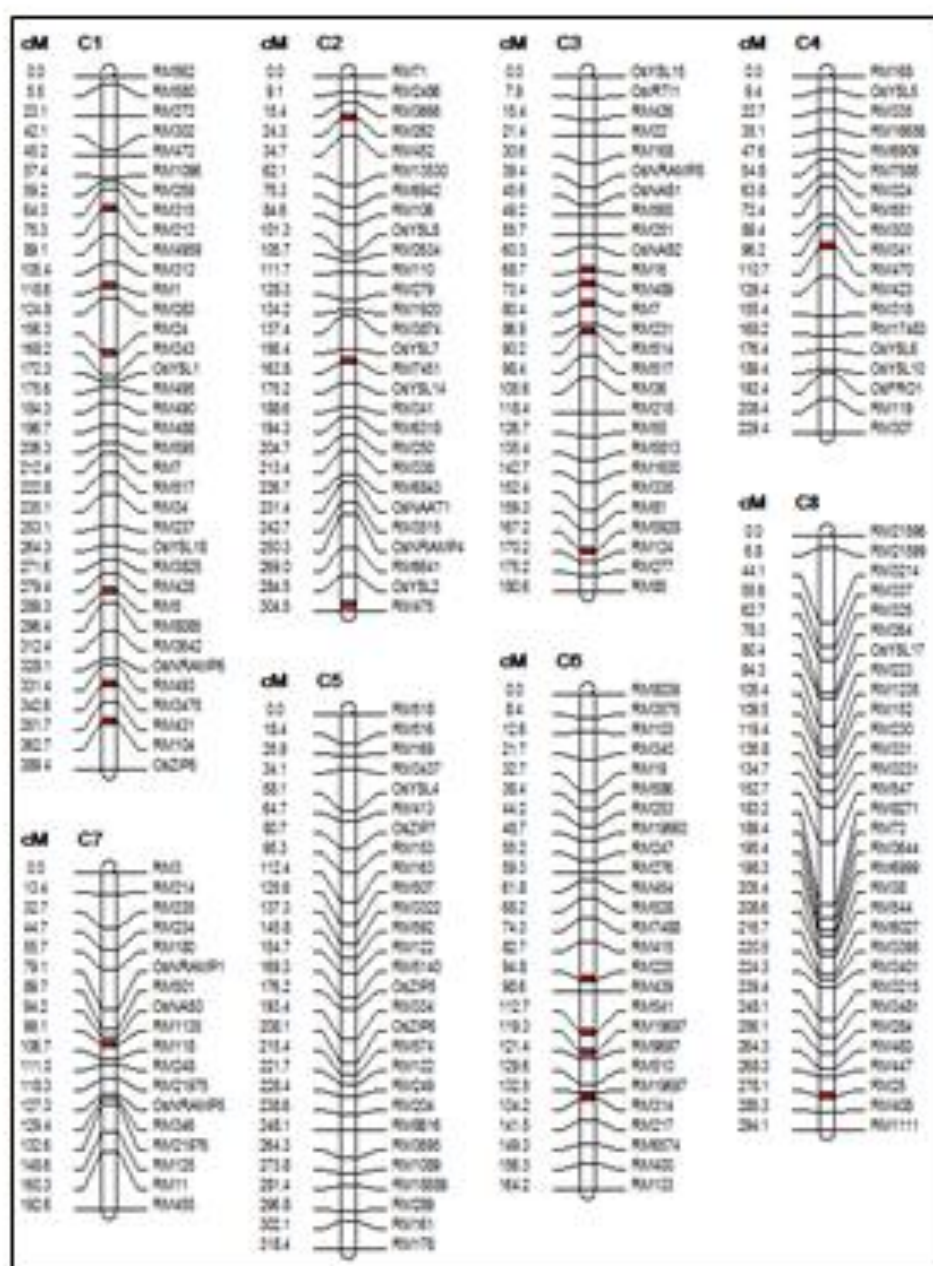
Fig. 4.50 (a): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Gurjari using IM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centimorgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
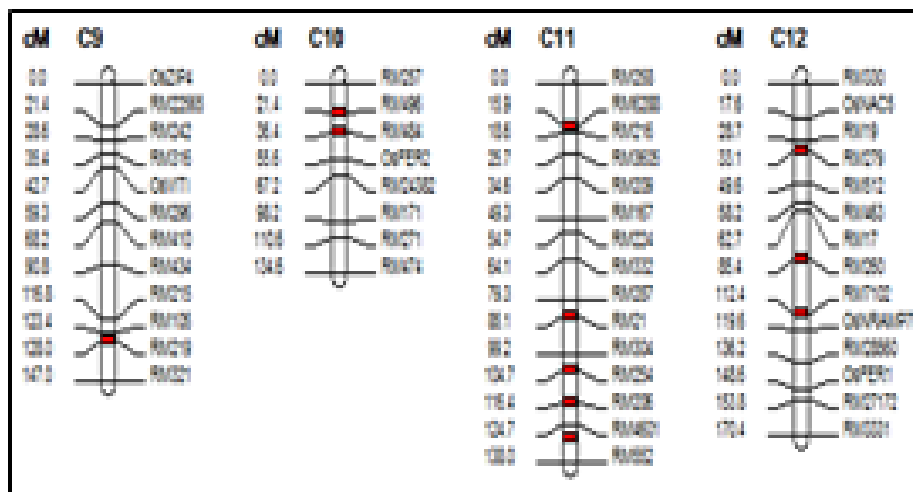
Fig. 4.50 (b): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Gurjari using IM.

QTLs are indicated in red colour, in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, IM = Interval Mapping.
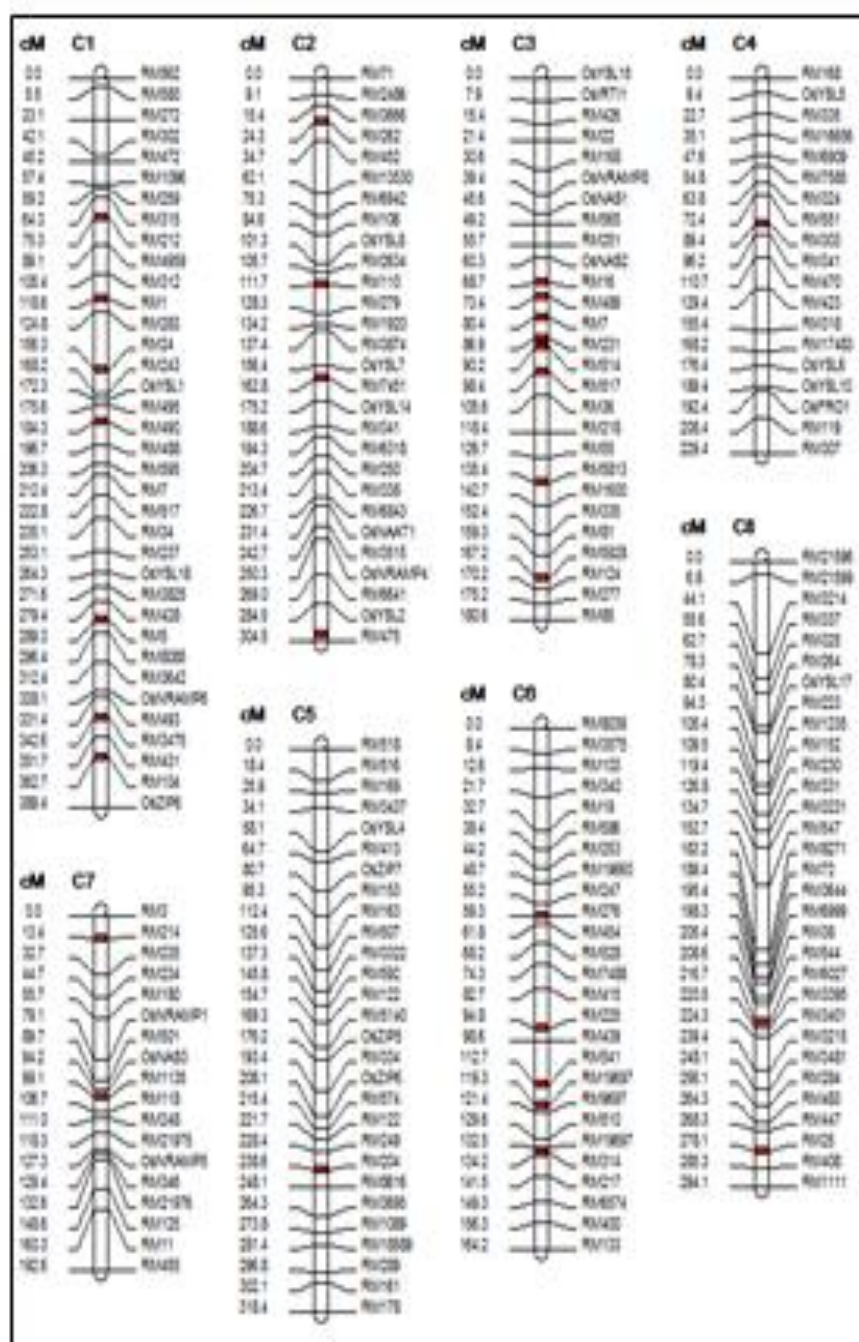
Fig. 4.51 (a): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Gurjari using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.
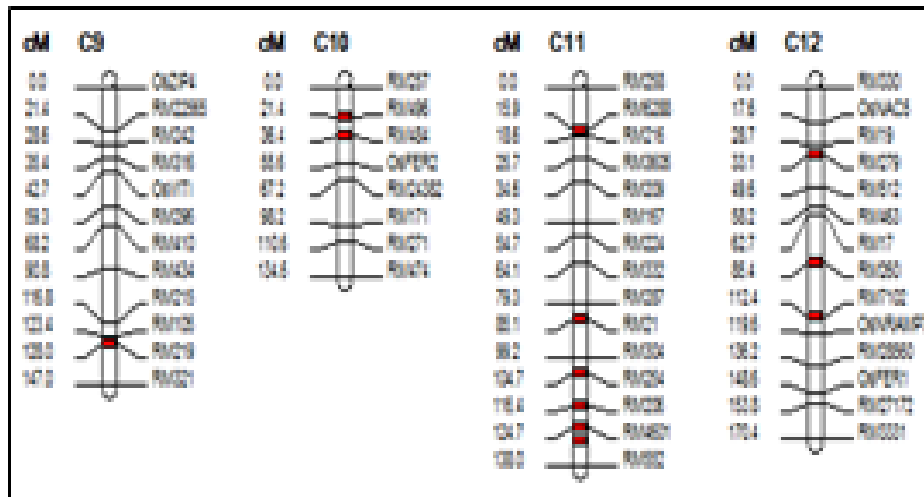
Fig. 4.51 (b): Distribution of QTLs for yield and yield related traits in the molecular linkage map of GR-11 X Gurjari using CIM.

QTLs are indicated in red colour in the linkage map. Names of the markers represented right side of the linkage map. Genetic distances between markers in centi morgans (cM) are represented on the left side of the linkage map. The number of the chromosome is mentioned on the top, CIM = Composite Interval Mapping.

QTLs for Fe and Zn in rice were reported earlier by Neelamraju *et al.* (2012) on chromosome 8 that explained more than 15 % phenotypic variation. Fe QTLs on chromosomes 2 and 9 with a phenotypic variation of 5 % and 7 % in two seasons was reported by Garcia-Oliveira *et al.* (2009). They also reported three QTLs for Zn on chromosomes 5, 8 and 12. The QTL on chromosome 8 accounted for 11-15 % phenotypic variation while the QTL on chromosome 5 was a minor effect QTL. QTL for Zn content on chromosome 12 was reported earlier by Stangoulis *et al.* (2007). They also reported Fe QTLs on chromosome 2, 8 using a double haploid population of IR64/Azucena. Gregorio *et al.* (2000) also previously reported three loci explaining 19–30% variation for Fe concentration on chromosomes 7, 8, and 9 in rice.

4.6.2.2. QTLs detected for Iron concentration

4.6.2.1. Cross GR-11 X Pankhali-203

A total of 20 QTLs were detected for grain iron content among 300 RIL populations for this cross based GR-11 X Pankhali-203. The phenotypic variance ranged from 16.2 % to 32.6 % for all the linkage groups. A higher LOD value was observed for this trait than zinc concentration. LG3 and LG12 had more QTLs than any other linkage groups (Table 4.23 and Table4.24).

Two QTLs, qFe1.1 and qFe1.2 were detected on LG1 with a high phenotypic variance of 32.6 % and a LOD score of 9.8. Only one QTL, qFe2.1 was associated with the iron concentration on LG2 at 36.8 cM and was inherited by Pankhali-203 allele. A total of three QTLs were associated with the iron concentration on LG3, of which two (qFe3.1 and qFe3.2) had favorable allele from GR-11 and one from Pankhali-203. QTLs, qFe4.1 and qFe4.2 were detected on LG4, inherited by GR-11 allele with the phenotypic variance of 16.2 % and 16.9 % respectively. Two QTLs were detected for iron concentration on LG5 with a high phenotypic variance and LOD value and were contributed by GR-11 parental allele. Only one QTL on LG7 at 29.5 cM was detected with a phenotypic variance of 16.2 % and a LOD of 4.3 inherited by GR-11 allele. On LG8, A QTL was detected at 149.3 cM with a LOD of 4.5 and contributed by GR-11 allele. Two QTLs were related for iron concentration on LG9 at 43.4 cM (qFe9.1) and 74.6 cM (qFe9.2) with a phenotypic variance of 22.6% and 16.9 % respectively. QTLs, qFe9.1 were inherited by Pankhlai-203 while qFe9.2 was inherited by GR-11.

Table: 4.23 QTLs detected for Iron concentration in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qFe1.1	1	124.5	OsYSL1	RM243-RM488	9.8	-15.5	32.6	118.4	130.7	G
2		qFe1.2	1	130.7	RM488	OsYSL1-RM490	9.2	9.8	32.6	147.2	158.3	P
3		qFe2.1	2	36.8	RM262	RM13530-RM3666	5.2	19.5	22.0	23.5	45.7	P
4		qFe3.1	3	40.1	RM168	RM3-OsNRAMP8	4.2	-10.2	17.8	31.7	43.6	G
5		qFe3.2	3	44.7	OsNAS1	OsNRAMP8-RM565	9.4	-17.7	32.6	43.6	50.8	G
6		qFe3.3	3	56.9	OsNAS2	RM251-RM16	5.6	20.1	22.3	53.8	64.2	P
7		qFe4.1	4	3.5	OsYSL5	RM168-RM335	4.2	-4.8	16.2	2.1	5.5	G
8		qFe4.2	4	61.9	RM470	RM314-RM423	4.7	-3.8	16.9	66.8	73.2	G
9		qFe5.1	5	26.8	RM3437	RM3322-OsYSL4	4.8	-10.0	17.0	22.7	32.1	G
10	Fe	qFe5.2	5	26.8	OsYSL14	RM3437-RM413	4.2	-10.0	16.8	26.8	46.5	G
11		qFe6.1	6	29.4	RM19660	RM340-RM586	7.4	-19.5	27.4	21.8	35.6	G
12		qFe6.2	6	106.7	RM19697	RM541-RM9697	5.0	10.2	22.0	100.2	109.4	P
13		qFe7.1	7	29.5	RM234	RM235-RM180	4.3	-19.5	16.2	27.1	43.8	G
14		qFe8.1	8	149.3	RM1111	RM3481-RM483	4.7	-17.2	16.9	145.7	150.0	G
15		qFe9.1	9	43.4	RM219	RM242-OsVITI	5.6	17.3	22.6	29.8	60.7	P
16		qFe9.2	9	74.6	RM296	OsVITI-RM215	4.6	-19.4	16.9	60.7	87.5	G
17		qFe11.1	11	28.7	RM21	RM209-RM287	9.8	-18.3	32.6	22.7	30.1	G
18		qFe12.1	12	13.4	OsNAC5	RM330-RM19	9.2	-6.6	32.6	12.1	18.7	G
19		qFe12.2	12	37.8	RM7102	RM260-OsNRAMP7	4.2	18.3	16.2	35.1	49.7	P
20		qFe12.3	12	92.7	RM27172	OsFER1-RM3331	4.3	-13.4	16.2	80.7	95.4	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; G: GR-11; P: Pankhali-203

Table 4.24: QTLs detected for Iron concentration in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qFe1.1	1	124.5	OsYSL1	RM243-RM488	5.4	-15.5	21.9	118.4	130.7	G
2		qFe1.2	1	130.7	RM488	OsYSL1-RM490	4.4	9.8	17.0	147.2	158.3	P
3		qFe2.1	2	36.8	RM262	RM13530-RM3666	4.2	19.5	16.8	23.5	45.7	P
4		qFe3.1	3	40.1	RM168	RM3-OsNRAMP8	7.4	-10.2	27.4	31.7	43.6	G
5		qFe3.2	3	44.7	OsNAS1	OsNRAMP8-RM565	5.0	-17.7	22.0	43.6	50.8	G
6		qFe3.3	3	56.9	OsNAS2	RM251-RM16	4.3	20.1	16.8	53.8	64.2	P
7		qFe4.1	4	3.5	OsYSL5	RM168-RM335	4.7	-4.8	16.8	2.1	5.5	G
8		qFe4.2	4	61.9	RM470	RM314-RM423	5.6	-3.8	21.9	66.8	73.2	G
9		qFe5.1	5	26.8	RM3437	RM3322-OsYSL4	4.6	-10.0	16.8	22.7	32.1	G
10	Fe	qFe5.2	5	26.8	OsYSL14	RM3437-RM413	9.8	-10.0	32.6	26.8	46.5	G
11		qFe6.1	6	29.4	RM19660	RM340-RM586	9.2	-19.5	32.0	21.8	35.6	G
12		qFe6.2	6	106.7	RM19697	RM541-RM9697	4.2	10.2	16.8	100.2	109.4	P
13		qFe7.1	7	29.5	RM234	RM235-RM180	9.8	-19.5	32.6	27.1	43.8	G
14		qFe8.1	8	149.3	RM1111	RM3481-RM483	9.2	-17.2	32.0	145.7	150.0	G
15		qFe9.1	9	43.4	RM219	RM242-OsVIT1	5.2	17.3	21.5	29.8	60.7	P
16		qFe9.2	9	74.6	RM296	OsVIT1-RM215	4.2	-19.4	16.8	60.7	87.5	G
17		qFe11.1	11	28.7	RM21	RM209-RM287	9.4	-18.3	31.5	22.7	30.1	G
18		qFe12.1	12	13.4	OsNAC5	RM330-RM19	5.5	-6.6	22.3	12.1	18.7	G
19		qFe12.2	12	37.8	RM7102	RM260-OsNRAMP7	4.9	18.3	17.3	35.1	49.7	P
20		qFe12.3	12	92.7	RM27172	OsFER1-RM3331	5.6	-13.4	21.9	80.7	95.4	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; G: GR-11; P: Pankhali-203

Highest phenotypic variance was observed for qFe11.1 with 32.6 % and a LOD of 9.8 and inherited by GR-11 allele. Three QTLs were detected for iron concentration on LG12, of which qFe12.2 was only inherited by the Pankhali-203 allele with a phenotypic variance of 16.2 %. qFe12.1 (13.4 cM) and qFe12.3 (92.7 cM) were detected with a LOD of 9.2 and 4.3 respectively, while the phenotypic variance was high in case of qFe12.1.

In summary the highest phenotypic variance of 32.6 % was observed for the qFe5.2, qFe7.1. Among all the QTLs detected, six were inherited by the Pankhali-203 while the rest had an inheritance from the GR-11 allele. The phenotypic variance ranged from 16.8 % to 32.6 %. All the QTLs were detected by IM and CIM method.

4.6.2.2. Cross GR-11 X Krishna Kamod

A total of 17 QTLs were detected for the iron concentration across all the linkage groups (Table 4.25 and Table4.26). Four QTLs were identified on LG1 with the phenotypic variance of 27.2 % (qFe1.1), 32.6 % (qFe1.2), 22.4 % (qFe1.3) and 17.6 % (qFe1.4) and a LOD of 7.8, 9.2, 5.4 and 4.8 respectively. Single QTL was detected for iron concentration on LG2 (qFe2.1) and LG3 (qFe3.1) with the phenotypic variance of 21.7 % and 29.4 % and a LOD of 5.0 and 8.1 respectively. On LG6, two QTLs, qFe6.1 and qFe1.2 were detected for the iron concentration at 30.6 cM and 118.1 cM and a phenotypic variance of 22.1 % and 16.9 % respectively. The phenotypic variance of 32.6 % was observed for the QTL, qFe7.1 on LG7 with a LOD of 9.8 and was inherited by the Krishna Kamod allele. Two QTLs on LG8, qFe8.1 and qFe8.2 at 212.3 cM and 251.3 cM and had a phenotypic variance of 32.0 % and 21.8 % respectively. QTL qFe8.1 was inherited by Krishna Kamod while qFe8.2 was contributed by GR-11 allele. LG9 and LG12 both had detected a QTL at 56.8 cM and 7.0 cM respectively. QTL qFe9.1 was contributed by the GR-11 allele whereas the qFe12.1 was inherited by the Krishna Kamod allele.

In a nutshell, a total of 17 QTs were identified for the iron concentration in 250 RIL populations across the linkage groups. The highest phenotypic variance of 32.6 % was observed for the qFe1.2 and qFe7.1. The phenotypic variance ranged from 15.3 % to 32.6 %. Six QTLs were inherited by the Krishna Kamod allele while the rest were contributed by the GR-11 allele. QTLs, qFe1.3 and qFe8.1 were only detected by the CIM method.

Table: 4.25 QTLs detected for Iron concentration in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qFe1.1	1	57.3	RM259	RM212-RM315	10.4	17.3	35.2	51.2	68.7	K
2		qFe1.2	1	167.4	RM243	RM24-OsYSL1	12.6	-19.4	43.8	154.7	176.2	G
3		qFe1.4	1	185.3	RM490	RM488-RM495	5.0	-6.6	22.0	179.4	190.7	G
4		qFe2.1	2	92.7	OsYSL8	RM6843-RM2634	5.7	18.3	22.8	84.6	105.7	K
5		qFe3.1	3	29.6	RM168	RM168-OsNAS1	7.8	-15.4	27.6	21.4	34.7	G
6		qFe4.1	4	112.5	RM470	RM341-RM423	9.2	-13.0	32.6	96.2	124.3	G
7	Fe	qFe5.1	5	209.1	RM574	OsZIP6-RM122	5.4	-14.5	22.5	204.6	219.7	G
8		qFe5.2	5	219.7	RM122	RM574-RM249	4.8	-19.2	17.2	209.1	224.7	G
9		qFe6.1	6	30.6	RM19660	RM340-RM586	5.0	15.8	21.5	22.1	37.4	K
10		qFe6.2	6	118.1	RM19697	RM541-RM9697	8.1	-8.2	28.6	110.5	121.4	G
11		qFe7.1	7	54.3	RM234	RM235-RM180	3.4	-10.5	15.4	32.7	62.7	G
12		qFe8.2	8	251.3	RM1111	RM3481-RM483	4.5	16.3	16.8	246.8	260.8	K
13		qFe9.1	9	56.8	RM296	OsVIT1-RM215	4.2	14.7	16.4	47.3	78.3	K
14		qFe12.1	12	7.0	OsNAC5	RM330-RM19	5.6	8.0	22.4	2.5	10.0	K

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; G: GR-11; K: Krishna Kamod

Table: 4.26 QTLs detected for Iron concentration in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1		qFe1.1	1	57.3	RM259	RM212-RM315	7.8	16.3	27.2	51.2	68.7	K
2		qFe1.2	1	167.4	RM243	RM24-OsYSL1	9.2	-18.4	32.6	154.7	176.2	G
3		qFe1.3	1	179.4	RM488	OsYSL1-RM490	5.4	-18.3	22.4	176.2	185.3	G
4		qFe1.4	1	185.3	RM490	RM488-RM495	4.8	-7.6	17.6	179.4	190.7	G
5		qFe2.1	2	92.7	OsYSL8	RM6843-RM2634	5.0	20.3	21.7	84.6	105.7	K
6		qFe3.1	3	29.6	RM168	RM168-OsNAS1	8.1	-16.4	29.4	21.4	34.7	G
7		qFe4.1	4	112.5	RM470	RM341-RM423	3.4	-11.0	15.3	96.2	124.3	G
8	Fe	qFe4.2	4	192.4	OsFRO1	RM307-RM119	4.5	-13.5	16.4	181.6	203.7	G
9		qFe5.1	5	209.1	RM574	OsZIP6-RM122	4.3	-21.2	16.8	204.6	219.7	G
10		qFe5.2	5	219.7	RM122	RM574-RM249	4.7	16.8	16.7	209.1	224.7	K
11		qFe6.1	6	30.6	RM19660	RM340-RM586	5.6	-9.2	22.1	22.1	37.4	G
12		qFe6.2	6	118.1	RM19697	RM541-RM9697	4.6	-11.5	16.9	110.5	121.4	G
13		qFe7.1	7	54.3	RM234	RM235-RM180	9.8	16.8	32.6	32.7	62.7	K
14		qFe8.1	8	212.3	RM6027	RM547-RM3395	9.2	14.9	32.0	208.6	220.5	K
15		qFe8.2	8	251.3	RM1111	RM3481-RM483	5.4	-14.2	21.8	246.8	260.8	G
16		qFe9.1	9	56.8	RM296	OsVIT1-RM215	5.5	-19.2	22.0	47.3	78.3	G
17		qFe12.1	12	7.0	OsNAC5	RM330-RM19	5.2	6.9	22.0	2.5	10.0	K

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; G: GR-11; K: Krishna Kamod

4.6.2.3. Cross GR-11 X Gurjari

In all a total of 22 QTLs were detected for the iron concentration (Table 4.27 and Table 4.28). Of these LG1 had a three QTLs, qFe1.1, qFe1.2 and qFe1.3 at 59.2 cM, 168.2 cM and 196.7 cM with the phenotypic variance of 27.6 %, 32.6 % and 22.0 % respectively. Two QTLs, qFe2.1 and qFe2.2 on LG2 were inherited by the GR-11 allele with a phenotypic variance of 17.6 % and 21.0 % and a LOD of .8 and 5.0. On LG3, qFe3.1, qFe3.2, qFe3.3, qFe3.4 and qFe3.5 were detected with the phenotypic variance of 29.7 %, 15.2 %, 16.8 %, 17.2 % and 21.6 % and were all inherited by the GR-11 allele. Single QTLs were identified on each LG4, qFe4.1 had a favorable allele for Gurjari while on LG5, qFe5.1 had a phenotypic variance of 17.8 % and a LOD of 4.3 and was inherited by the GR-11 allele. In case of LG6 two QTLs, qFe6.1 and qFe6.2 had a phenotypic variance of 17.3 % and 26.4 % and were both inherited by the GR-11 allele. Three QTLs, qFe7.1, qFe7.2 and qFe7.3 were identified on LG7 with a phenotypic variance of 21.9 %, 17.3 % and 17.9 % respectively. QTL, qFe7.2 was inherited by the Gurjari allele. On LG8, two QTLs, qFe8.1 and qFe8.2 were identified at 44.1 cM and 226.7 cM with the phenotypic variance of 21.6 5 and 16.9 5 respectively. Single QTLs on LG9 and LG11 which were favored by the Gurjari allele while on LG12, qFe12.1 was located at 58.2 cM with the phenotypic variance of 17.3 % and was contributed by the favorable allele GR-11.

In all, 22 QTLs were detected for the iron concentration for the 300 RIL populations mapped. The highest phenotypic variance of 32.8 % was observed for the qFe11.1. Among all the QTLs detected only five were inherited by the Gurjari allele the rest had the favorable allele of GR-11. The phenotypic variance ranged from 15.2 % to 32.8 %. The QTLs, qFe3.3 and qFe7.2 were only detected with the help of CIM.

4.6.2.3. QTLs detected for yield and yield related traits

4.6.2.3.1. Days to 50 % flowering

4.6.2.3.1.1. Cross GR-11 X Pankhali-203

Two QTLs qdft4.1 and qdft8, located on LG1 and LG8 were detected with the aid of CIM and IM with a phenotypic variance of 21.9 % and 17.0 % and a LOD score of 5.4 and 4.4 respectively for days to 50 % flowering. The favorable allele for days to 50 % flowering at LG4 and LG8 were contributed to GR-11 (Table 4.29 and Table 4.30).

Table: 4.27 QTLs detected for Iron concentration in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	Fe	qFe1.1	1	59.2	RM592	RM1096-RM315	4.3	-12.5	16.5	57.4	64.3	GR
2		qFe1.2	1	168.2	RM243	RM24-OsYSL1	4.7	-20.2	17.4	156.3	172.3	GR
3		qFe1.3	1	196.7	RM488	RM490-RM595	5.6	15.8	22.0	184.3	206.3	G
4		qFe2.1	2	101.3	OsYSL8	RM106-RM2634	4.6	-8.2	16.4	84.6	105.7	GR
5		qFe2.2	2	250.3	OsNRAMP4	RM3515-RM6641	9.8	-10.5	32.6	242.7	269.0	GR
6		qFe3.1	3	30.6	RM168	RM22-OsNRAMP8	9.2	-15.8	33.0	21.4	39.4	GR
7		qFe3.2	3	45.6	OsNAS1	OsNRAMP8-RM565	5.2	-7.8	21.8	39.4	49.2	GR
8		qFe3.4	3	90.2	RM514	RM231-RM517	4.2	-17.5	16.5	85.9	98.4	GR
9		qFe3.5	3	142.7	RM1600	RM5813-RM335	9.4	-8.2	32.6	135.4	152.4	GR
10		qFe4.1	4	110.7	RM470	RM341-RM423	8.1	-15.7	28.1	96.2	129.4	GR
11		qFe5.1	5	58.1	OsYSL1	RM3437-RM413	3.4	18.1	15.8	34.1	64.7	G
12		qFe6.1	6	48.7	RM19660	RM243-RM247	4.5	-5.8	17.0	44.2	55.2	GR
13		qFe6.2	6	132.5	RM19697	RM510-RM314	4.7	-4.8	17.4	129.6	134.2	GR
14		qFe7.1	7	79.1	OsNRAMP1	RM180-RM501	5.6	-15.5	22.0	55.7	89.7	GR
15		qFe7.3	7	127.3	OsNRAMP5	RM21975-RM346	5.1	-16.2	21.3	118.3	129.4	GR
16		qFe8.1	8	44.1	RM3214	RM21599-RM337	4.3	-17.3	16.5	6.8	55.6	GR
17		qFe8.2	8	216.7	RM6027	RM544-RM3395	4.2	-14.5	16.2	208.7	220.5	GR
18		qFe9.1	9	59.3	RM296	OsVIT1-RM410	7.4	10.8	27.1	42.7	68.2	G
19		qFe11.1	11	34.6	RM209	RM3605-RM167	5.0	17.5	21.0	25.7	49.3	G
20		qFe12.1	12	58.2	RM463	RM512-RM17	4.2	-11.2	16.3	49.6	62.7	GR

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; GR: GR-11; G: Gurjari

Table: 4.28 QTLs detected for Iron concentration in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	Fe	qFe1.1	1	59.2	RM592	RM1096-RM315	7.8	-13.5	27.6	57.4	64.3	GR
2		qFe1.2	1	168.2	RM243	RM24-OsYSL1	9.2	-21.2	32.6	156.3	172.3	GR
3		qFe1.3	1	196.7	RM488	RM490-RM595	5.4	16.8	22.0	184.3	206.3	G
4		qFe2.1	2	101.3	OsYSL8	RM106-RM2634	4.8	-9.2	17.6	84.6	105.7	GR
5		qFe2.2	2	250.3	OsNRAMP4	RM3515-RM6641	5.0	-11.5	21.0	242.7	269.0	GR
6		qFe3.1	3	30.6	RM168	RM22-OsNRAMP8	8.1	-16.8	29.7	21.4	39.4	GR
7		qFe3.2	3	45.6	OsNAS1	OsNRAMP8-RM565	3.4	-9.8	15.2	39.4	49.2	GR
8		qFe3.3	3	60.3	OsNAS2	RM251-RM16	4.5	-19.5	16.8	55.7	68.7	GR
9		qFe3.4	3	90.2	RM514	RM231-RM517	4.7	-10.2	17.2	85.9	98.4	GR
10		qFe3.5	3	142.7	RM1600	RM5813-RM335	5.6	-17.7	21.6	135.4	152.4	GR
11		qFe4.1	4	110.7	RM470	RM341-RM423	5.1	20.1	21.8	96.2	129.4	G
12		qFe5.1	5	58.1	OsYSL1	RM3437-RM413	4.3	-4.8	17.8	34.1	64.7	GR
13		qFe6.1	6	48.7	RM19660	RM243-RM247	4.2	-3.8	17.3	44.2	55.2	GR
14		qFe6.2	6	132.5	RM19697	RM510-RM314	7.4	-19.5	26.4	129.6	134.2	GR
15		qFe7.1	7	79.1	OsNRAMP1	RM180-RM501	5.0	-17.2	21.9	55.7	89.7	GR
16		qFe7.2	7	106.7	RM118	RM135-RM248	4.3	17.3	17.3	99.1	111.0	G
17		qFe7.3	7	127.3	OsNRAMP5	RM21975-RM346	4.7	-19.4	17.9	118.3	129.4	GR
18		qFe8.1	8	44.1	RM3214	RM21599-RM337	5.6	-18.3	21.6	6.8	55.6	GR
19		qFe8.2	8	216.7	RM6027	RM544-RM3395	4.6	-15.5	16.9	208.7	220.5	GR
20		qFe9.1	9	59.3	RM296	OsVIT1-RM410	9.8	9.8	32.6	42.7	68.2	G
21		qFe11.1	11	34.6	RM209	RM3605-RM167	9.2	19.5	32.8	25.7	49.3	G
22		qFe12.1	12	58.2	RM463	RM512-RM17	4.2	-10.2	17.3	49.6	62.7	GR

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; GR: GR-11; G: Gurjari

Table: 4.29 QTLs detected for yield and yield related traits in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	DFT	qdft4.1	4	36.7	RM551	RM303-RM324	5.4	-20.11	21.9	28.7	54.0	G
2		qdft8.1	8	26.8	RM264	RM325-RM223	4.4	-10	17.0	16.1	26.8	G
3		qph1.1	1	57.4	RM259	RM212-RM315	4.2	-10	16.8	55.6	62.1	G
4		qph1.2	1	195.8	RM8085	RM5-RM3642	7.4	3.8	27.4	189.4	198.4	P
5	PH	qph6.1	6	112.4	RM314	RM9697-RM217	5.0	-13.3	22.0	109.4	118.3	G
6		qph7.1	7	27.1	RM235	RM214-RM234	4.3	-12.4	16.8	15.8	29.5	G
7		qph11.1	11	30.1	RM21	RM209-RM287	4.7	-18.4	16.8	28.7	50.9	G
8		qpl6.1	6	54.7	RM415	RM217-RM528	5.6	19.2	21.9	50.3	65.4	P
9	PL	qpl7.1	7	27.1	RM235	RM214-RM234	4.6	-19.7	16.8	15.8	29.5	G
10		qpl11.1	11	81.4	RM206	RM224-RM167	9.8	-5.4	32.6	72.5	102.4	G
11		qpl11.2	11	28.7	RM209	RM4061-RM21	9.2	-6.8	32.0	22.7	30.1	G
12		qnt3.1	3	70.1	RM514	RM231-RM517	9.8	-2.3	32.6	66.8	72.8	G
13	NEFT	qnt3.2	3	72.8	RM517	RM514-RM7	9.2	18.3	32.6	70.1	78.4	P
14		qnt3.3	3	78.4	RM7	RM517-RM218	5.2	1.2	22.0	72.8	89.7	P
15		qnt10.1	10	10.8	RM496	RM257-RM484	4.2	-4.5	17.8	0.0	28.6	G
16		qgp1.1	1	135.6	RM490	RM488-RM595	9.4	-2.8	32.6	130.7	147.2	G
17		qgp2.1	2	115.8	RM279	RM279-RM3874	5.6	-15.5	22.3	102.7	120.7	G
18	NFGP	qgp6.1	6	126.8	RM6574	RM217-RM400	4.2	-18.7	16.2	118.4	129.3	G
19		qgp8.1	8	16.1	RM337	RM72-RM325	4.7	12.4	16.9	104.	20.7	G
20		qgp12.1	12	22.7	RM279	RM19-RM512	4.8	-16.4	17.0	18.7	26.8	G
21		qgp12.2	12	37.8	RM7102	RM260-OsNRAMP7	4.2	17.9	16.8	35.1	49.7	P
22	TWT	qtw1.1	1	135.6	RM490	RM488-RM595	4.2	-14.6	16.4	130.7	147.2	G

23		qtw5.1	5	122.7	RM204	RM249-RM18589	7.4	-10.4	27.0	120.4	126.3	G
24		qtw6.1	6	106.7	RM19697	RM541-RM9697	5.0	-5.4	21.8	100.2	109.4	G
25		qtw7.1	7	15.8	RM214	RM3-RM235	4.3	-6.8	16.8	0.0	27.1	G
26		qtw9.1	9	43.4	RM219	RM242-OsVIT1	4.7	-2.3	17.2	29.8	60.7	G
27		qgy1.1	1	220.4	RM431	OsNRAMP6-RM3475	5.6	-18.3	21.6	215.5	222.7	G
28	GY	qgy5.1	5	122.7	RM204	RM249-RM18589	4.6	-1.2	17.2	120.4	126.3	G
29		qgy6.1	6	54.7	RM415	RM217-RM528	9.8	-4.5	32.6	50.3	65.4	G
30		qgy8.1	8	16.1	RM337	RM72-RM325	9.2	-13.4	32.0	10.4	20.7	G
31		qgl2.1	2	75.4	RM110	RM2634-RM279	4.2	15.6	16.4	68.7	102.7	P
32	GL	qgl5.1	5	122.7	RM204	RM249-RM18589	7.4	18.7	27.4	120.4	126.3	P
33		qgl11.1	11	81.4	RM206	RM224-RM167	5.0	-10.8	22.0	72.5	102.4	G
34		qgl12.1	12	37.8	RM7102	RM260-OsNRAMP7	4.3	-13.7	16.2	35.1	49.7	G
35	GB	qgb6.1	6	50.3	RM247	RM19-RM415	4.7	-19.4	16.9	42.5	54.7	G
36		qgb6.2	6	109.4	RM9697	RM19697-RM314	5.6	-21.1	22.6	106.7	112.3	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; DFF: Days to 50% Flowering, PH: Plant height (cm), PL: Panicle length (cm), NETP: Number of effective tillers per plant, NFGP: Number of filled grains per panicle, TWT: Test weight (g), GY: Grain yield (g), GL: Grain length (cm), GB: Grain breadth (cm), G: GR-11; P: Pankhali-203

Table: 4.30 QTLs detected for yield and yield related traits in 300 RIL populations of rice based on cross GR-11 X Pankhali-203 employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	DFT	qdft4.1	4	36.7	RM551	RM303-RM324	4.2	-20.5	16.4	28.7	54.0	G
2	DFT	qdft8.1	8	26.8	RM264	RM325-RM223	7.4	-10.2	27.0	16.1	26.8	G
3		qph1.1	1	57.4	RM259	RM212-RM315	5.0	-10	21.8	55.6	62.1	G
4		qph1.2	1	195.8	RM8085	RM5-RM3642	4.3	4.8	16.8	189.4	198.4	P
5	PH	qph6.1	6	112.4	RM314	RM9697-RM217	4.7	-15.3	17.2	109.4	118.3	G
6		qph7.1	7	27.1	RM235	RM214-RM234	5.6	-12.8	21.6	15.8	29.5	G
7		qph11.1	11	30.1	RM21	RM209-RM287	4.6	-19.4	17.2	28.7	50.9	G
8		qpl6.1	6	54.7	RM415	RM217-RM528	9.8	19.8	32.6	50.3	65.4	P
9	PL	qpl7.1	7	27.1	RM235	RM214-RM234	9.2	-19.7	32.0	15.8	29.5	G
10		qpl11.1	11	81.4	RM206	RM224-RM167	4.2	-5.8	16.4	72.5	102.4	G
11		qpl11.2	11	28.7	RM209	RM4061-RM21	5.4	-8.8	21.9	22.7	30.1	G
12		qnt3.1	3	70.1	RM514	RM231-RM517	4.4	-4.3	17.0	66.8	72.8	G
13	NEFT	qnt3.2	3	72.8	RM517	RM514-RM7	4.2	19.3	16.8	70.1	78.4	P
14		qnt3.3	3	78.4	RM7	RM517-RM218	7.4	2.5	27.4	72.8	89.7	P
15		qnt10.1	10	10.8	RM496	RM257-RM484	5.0	-4.5	22.0	0.0	28.6	G
16		qgp1.1	1	135.6	RM490	RM488-RM595	4.3	-2.8	16.8	130.7	147.2	G
17		qgp2.1	2	115.8	RM279	RM279-RM3874	4.7	-15.8	16.8	102.7	120.7	G
18	NFGP	qgp6.1	6	126.8	RM6574	RM217-RM400	5.6	-18.7	21.9	118.4	129.3	G
19		qgp8.1	8	16.1	RM337	RM72-RM325	4.6	14.4	16.8	104.	20.7	G
20		qgp12.1	12	22.7	RM279	RM19-RM512	9.8	-16.4	32.6	18.7	26.8	G
21		qgp12.2	12	37.8	RM7102	RM260-OsRAMP7	9.2	17.9	32.0	35.1	49.7	P
22	TWT	qtw1.1	1	135.6	RM490	RM488-RM595	5.2	-12.6	22.0	130.7	147.2	G
23		qtw5.1	5	122.7	RM204	RM249-RM18589	4.2	-11.4	17.8	120.4	126.3	G

24		qtw6.1	6	106.7	RM19697	RM541-RM9697	4.7	-5.4	16.9	100.2	109.4	G
25		qtw7.1	7	15.8	RM214	RM3-RM235	9.8	-8.8	32.6	0.0	27.1	G
26		qtw9.1	9	43.4	RM219	RM242-OsVIT1	9.2	-3.3	32.6	29.8	60.7	G
27	GY	qgy1.1	1	220.4	RM431	OsNRAMP6-RM3475	5.2	-19.3	22.0	215.5	222.7	G
28		qgy5.1	5	122.7	RM204	RM249-RM18589	4.2	-2.2	17.8	120.4	126.3	G
29		qgy6.1	6	54.7	RM415	RM217-RM528	9.4	-4.5	32.6	50.3	65.4	G
30		qgy8.1	8	16.1	RM337	RM72-RM325	5.6	-13.4	22.3	10.4	20.7	G
31		qgl2.1	2	75.4	RM110	RM2634-RM279	4.2	15.6	16.2	68.7	102.7	P
32	GL	qgl5.1	5	122.7	RM204	RM249-RM18589	4.7	21.7	16.9	120.4	126.3	P
33		qgl11.1	11	81.4	RM206	RM224-RM167	4.8	-12.8	17.0	72.5	102.4	G
34		qgl12.1	12	37.8	RM7102	RM260-OsNRAMP7	4.2	-15.7	16.8	35.1	49.7	G
35	GB	qgb6.1	6	50.3	RM247	RM19-RM415	5.2	-20.4	22.0	42.5	54.7	G
36		qgb6.2	6	109.4	RM9697	RM19697-RM314	5.2	-20.1	22.0	106.7	112.3	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; DFF: Days to 50% Flowering, PH: Plant height (cm), PL: Panicle length (cm), NETP: Number of effective tillers per plant, NFGP: Number of filled grains per panicle, TWT: Test weight (g), GY: Grain yield (g), GL: Grain length (cm), GB: Grain breadth (cm); G: GR-11; P: Pankhali-203

4.6.2.3.1.2. Cross GR-11 X Krishna Kamod

A total of two QTLs could control the days to 50 % flowering located on LG4 (qdft4.1) and LG8 (qdft8.1) with the aid of IM and CIM with a phenotypic variance of 27.2 % and 32.6 % and a LOD of 7.8 and 9.2 respectively. The favorable alleles for days to 50 % flowering at both the linkage groups were inherited by GR-11 (Table 4.31 and Table 4.32).

4.6.2.3.1.3. Cross GR-11 X Gurjari

No QTLs were detected for the days to 50 % flowering across the cross GR-11 X Gurjari among all the 12 linkage groups with the aid of IM and CIM across 300 RIL populations (Table 4.33 and Table 4.34).

Anuradha *et al*, (2013) reported one QTL (*qdf2.1*) was identified using CIM explaining phenotypic variance of 15.9%. Only one marker (RM324) was common in QTLs detected using SMA, IM, and CIM for days to 50 % flowering.

4.6.2.3.2. Plant height

4.6.2.3.2.1. Cross GR-11 X Pankhali-203

Five QTLs were detected for plant height on LG1, LG6, LG7 and LG11. The phenotypic variance of qph1.1 (16.8 %), qph1.2 (27.8 %), qph6.1 (22.0 %), qph7.1 (16.8 %) and qph11.1 (16.8 %) with a LOD score of 4.2, 7.4, 5.0, 4.2 and 4.7 respectively (Table 4.29 and Table 4.30). The favorable allele for plant height was contributed to GR-11 for all of them except for qph1.2.

4.6.2.3.2.2. Cross GR-11 X Krishna Kamod

A total of seven QTLs were located on LG1, LG6, LG7, LG10 and LG11 for plant height (Table 4.31 and Table 4.32). Three QTLs were detected on LG1 with a phenotypic variance of 22.4 % (qph1.1), 17.6 % (qph1.2) and 21.6 % (qph1.3) and a LOD of 5.4, 4.8 and 5.0 respectively. Single QTLs were detected each on LG6 (qph6.1) with a phenotypic variance of 29.4 % and a LOD of 8.1, LG7 (qph7.1) having a phenotypic variance of 15.3 % and a LOD 3.4, LG10 (qph10.1) exhibiting a variance of 16.4 % and a LOD of 4.2, while LG11 (qph11.1) had a phenotypic variance of 16.8 % and a LOD of 4.3.

Table: 4.31 QTLs detected for yield and yield related traits in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	DFT	qdft4.1	4	63.1	RM551	RM303-RM324	7.8	-16.4	27.2	55.4	89.4	G
2		qdft8.1	8	72.4	RM264	RM325-RM332	9.2	-11.0	32.6	68.4	85.3	G
3		qph1.1	1	68.7	RM259	RM212-RM315	5.4	-13.5	22.4	57.3	76.3	G
4		qph1.2	1	154.7	RM241	RM24-OsYSL1	4.8	-21.2	17.6	144.2	167.4	G
5	PH	qph1.3	1	270.1	RM5	RM428-RM8085	5.0	16.8	21.7	269.7	273.8	K
6		qph6.1	6	134.2	RM314	RM510-RM217	8.1	-9.2	29.4	129.6	138.6	G
7		qph7.1	7	98.6	RM1135	OsNAS3-RM11	3.4	-11.5	15.3	94.2	103.5	G
8		qph10.1	10	120.4	RM216	RM171-RM258	4.5	-16.8	16.4	98.2	132.7	G
9		qph11.1	11	60.2	RM21	RM209-RM287	4.3	-9.8	16.8	54.7	78.6	G
10		qpl1.1	1	347.2	RM431	RM3475-RM104	4.7	-19.5	16.7	318.2	348.7	G
11		qpl2.1	2	156.4	OsYSL7	RM3874-RM7451	5.6	-10.2	22.1	137.4	162.8	G
12	PL	qpl8.1	8	47.1	RM23417	RM72-RM337	4.6	-17.7	16.9	36.4	52.1	G
13		qpl8.2	8	124.7	RM256	RM230-RM331	9.8	20.1	32.6	119.4	126.8	K
14		qpl11.1	11	54.7	RM209	RM4601-RM21	9.2	-4.8	32.0	34.1	60.2	G
15		qpl11.2	11	110.7	RM206	RM224-RM167	5.4	-3.8	21.8	103.7	116.3	G
16		qnt2.1	2	137.4	RM3874	RM1920-OsYSL7	5.5	-19.5	22.0	130.6	156.4	G
17	NEPT	qnt3.1	3	85.4	RM514	RM231-RM517	5.2	-17.2	22.0	83.3	90.3	G
18		qnt3.3	3	90.3	RM517	RM514-RM7	4.5	-15.4	16.8	85.4	96.7	G
19		qnt10.1	10	15.4	RM496	RM257-RM484	4.3	-17.3	16.4	0.0	30.6	G
20		qgp1.1	1	51.2	RM212	RM472-RM259	4.7	-8.8	17.1	45.2	57.3	G
21	NFGP	qgp1.2	1	120.4	RM1	RM312-RM283	5.6	-19.9	22.5	103.7	128.7	G
22		qgp3.1	3	83.8	RM231	RM22-RM514	4.6	-14.2	17.8	79.3	85.4	G
23		qgp6.1	6	149.3	RM6574	RM314-RM400	9.8	15.9	31.6	138.6	159.7	K

No.	Trait	QTL	Chr	Pos	LFM	RFM Interval	LOD	Add	PVE	LFM Pos	RFM Pos	Source
24		qgp7.1	7	98.6	RM1135	OsNAS3-RM11	9.2	-13.4	31.2	94.2	103.5	G
25		qgp8.1	8	52.1	RM337	RM23417-RM325	3.4	-17.3	15.2	47.1	68.4	G
26		qgp12.1	12	62.7	RM7102	RM260-OsNRAMP7	4.5	-12.5	16.9	56.3	85.3	G
27		qtw1.1	1	269.7	RM428	RM3825-RM5	4.3	17.5	16.8	253.7	270.1	K
28	TWT	qtw1.2	1	28.6	RM3642	RM8085-OsNRAMP6	4.7	-10.2	17.1	273.8	294.3	G
29		qtw6.1	6	118.1	RM19697	RM541-RM9697	5.6	20.1	21.9	110.5	121.4	K
30		qtw6.2	6	138.6	RM214	RM314-RM6574	4.6	-4.8	17.2	134.2	149.3	G
31		qtw7.1	7	103.5	RM11	RM1135-RM248	9.8	-4.8	32.1	98.6	110.8	G
32		qtw9.1	9	35.4	RM219	RM242-OsVIT1	9.2	12.6	31.5	10.5	47.3	K
		qgy1.1	1	347.2	RM431	RM3475-RM104	4.2	-11.2	15.2	56.3	130.6	G
	GY	qgy5.1	5	238.6	RM204	RM249-RM18589	3.2	-17.3	31.7	78.3	85.4	G
		qgy6.1	6	58.1	RM415	RM247-RM454	5.2	-21.7	17.2	94.2	85.3	G
33		qgl1.1	1	347.2	RM431	RM3475-RM104	3.4	-15.8	15.3	318.2	348.7	G
34	GL	qgl2.1	2	110.8	RM110	RM2634-RM279	4.5	-20.5	16.8	105.7	118.7	G
35		qgl5.1	5	238.6	RM204	RM249-RM18589	4.7	-15.7	16.3	224.7	248.1	G
36		qgl11.1	11	110.7	RM206	RM224-RM167	5.6	-3.8	21.9	103.7	116.3	G
37	GB	qgb2.1	2	137.4	RM3874	RM1920-OsYSL7	5.1	-5.9	21.3	130.6	156.4	G
38		qgb6.1	6	53.2	RM247	RM19-RM415	4.3	-17.5	16.8	43.9	58.1	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm); G: GR-11; K: Krishna Kamod

Table: 4.32 QTLs detected for yield and yield related traits in 250 RIL populations of rice based on cross GR-11 X Krishna Kamod employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	DFT	qdft4.1	4	63.1	RM551	RM303-RM324	3.4	-18.4	15.3	55.4	89.4	G
2		qdft8.1	8	72.4	RM264	RM325-RM332	4.5	-13.0	16.4	68.4	85.3	G
3		qph1.1	1	68.7	RM259	RM212-RM315	4.3	-11.5	16.8	57.3	76.3	G
4		qph1.2	1	154.7	RM241	RM24-OsYSL1	4.7	-20.2	16.7	144.2	167.4	G
5		qph1.3	1	270.1	RM5	RM428-RM8085	5.6	16.9	22.1	269.7	273.8	K
6	PH	qph6.1	6	134.2	RM314	RM510-RM217	7.8	-9.8	27.2	129.6	138.6	G
7		qph7.1	7	98.6	RM1135	OsNAS3-RM11	9.2	-11.7	32.6	94.2	103.5	G
8		qph10.1	10	120.4	RM216	RM171-RM258	5.4	-14.8	22.4	98.2	132.7	G
9		qph11.1	11	60.2	RM21	RM209-RM287	4.8	-9.9	17.6	54.7	78.6	G
10		qpl1.1	1	347.2	RM431	RM3475-RM104	5.0	-19.7	21.7	318.2	348.7	G
11		qpl2.1	2	156.4	OsYSL7	RM3874-RM7451	8.1	-12.2	29.4	137.4	162.8	G
12		qpl8.1	8	47.1	RM23417	RM72-RM337	3.4	-19.7	15.3	36.4	52.1	G
13	PL	qpl8.2	8	124.7	RM256	RM230-RM331	4.5	20.1	16.4	119.4	126.8	K
14		qpl11.1	11	54.7	RM209	RM4601-RM21	4.3	-4.8	16.8	34.1	60.2	G
15		qpl11.2	11	110.7	RM206	RM224-RM167	4.7	-5.8	16.7	103.7	116.3	G
16		qnt2.1	2	137.4	RM3874	RM1920-OsYSL7	5.6	-17.5	22.1	130.6	156.4	G
17		qnt3.1	3	85.4	RM514	RM231-RM517	4.6	-17.2	16.9	83.3	90.3	G
18	NEPT	qnt3.2	3	96.7	RM7	RM517-RM218	9.8	19.3	32.6	90.3	100.3	K
19		qnt3.3	3	90.3	RM517	RM514-RM7	9.2	-15.4	32.0	85.4	96.7	G
20		qnt10.1	10	15.4	RM496	RM257-RM484	5.4	-17.3	21.8	0.0	30.6	G
21		qgp1.1	1	51.2	RM212	RM472-RM259	5.5	-6.8	22.0	45.2	57.3	G
22		qgp1.2	1	120.4	RM1	RM312-RM283	5.2	-18.9	22.0	103.7	128.7	G
23	NFGP	qgp1.3	1	185.3	RM490	RM488-RM495	4.2	-6.8	16.4	179.4	190.7	G
24		qgp1.4	1	348.7	RM104	RM431-OsZIP6	4.3	6.2	16.8	347.2	362.8	K
25		qgp1.5	1	118.7	RM279	RM1110-RM1920	4.5	-15.5	16.8	110.8	130.6	G
26		qgp3.1	3	83.8	RM231	RM22-RM514	4.3	-16.2	16.4	79.3	85.4	G

No.	Trait	QTL	Chr	Position	LFM	RFM						Source
27		qgp6.1	6	149.3	RM6574	RM314-RM400	4.7	17.9	17.1	138.6	159.7	K
28		qgp7.1	7	98.6	RM1135	OsNAS3-RM11	5.6	-14.4	22.5	94.2	103.5	G
29		qgp8.1	8	52.1	RM337	RM23417-RM325	4.6	-19.3	17.8	47.1	68.4	G
30		qgp12.1	12	62.7	RM7102	RM260-OsNRAMP7	9.8	-13.5	31.6	56.3	85.3	G
31		qtw1.1	1	185.3	RM490	RM488-RM495	9.2	10.8	31.2	179.4	190.7	K
32		qtw1.2	1	269.7	RM428	RM3825-RM5	3.4	15.5	15.2	253.7	270.1	K
33	TWT	qtw1.3	1	28.6	RM3642	RM8085-OsNRAMP6	4.5	-11.2	16.9	273.8	294.3	G
34		qtw6.1	6	58.1	RM415	RM247-RM454	4.3	-19.7	16.8	53.2	62.7	G
35		qtw6.2	6	118.1	RM19697	RM541-RM9697	4.7	21.1	17.1	110.5	121.4	K
36		qtw6.3	6	138.6	RM214	RM314-RM6574	5.6	-5.8	21.9	134.2	149.3	G
37		qtw7.1	7	103.5	RM11	RM1135-RM248	4.6	-3.8	17.2	98.6	110.8	G
38		qtw9.1	9	35.4	RM219	RM242-OsVIT1	9.8	11.6	32.1	10.5	47.3	K
39		qgy1.1	1	347.2	RM431	RM3475-RM104	5.2	-9.2	16.2	56.3	130.6	G
40	GY	qgy5.1	5	238.6	RM204	RM249-RM18589	4.2	-15.3	32.7	78.3	85.4	G
41		qgy6.1	6	58.1	RM415	RM247-RM454	9.2	-19.7	15.2	94.2	85.3	G
42		qgl1.1	1	347.2	RM431	RM3475-RM104	9.2	-17.8	31.5	318.2	348.7	G
43		qgl1.2	1	348.7	RM104	RM431-OsZIP6	3.4	-9.8	15.3	347.2	362.8	G
44		qgl2.1	2	110.8	RM110	RM2634-RM279	4.5	-21.5	16.8	105.7	118.7	G
45	GL	qgl2.2	2	186.3	RM341	OsYSL14-RM6318	4.7	-11.2	16.3	174.3	192.7	G
46		qgl5.1	5	238.6	RM204	RM249-RM18589	5.6	-18.7	21.9	224.7	248.1	G
47		qgl7.1	7	32.7	RM235	RM235-RM180	5.1	20.1	21.3	22.4	54.3	K
48		qgl11.1	11	110.7	RM206	RM224-RM167	4.3	-4.2	16.8	103.7	116.3	G
49	GB	qgb2.1	2	137.4	RM3874	RM1920-OsYSL7	9.2	-3.9	31.5	130.6	156.4	G
50		qgb6.1	6	53.2	RM247	RM19-RM415	5.2	-18.5	22.0	43.9	58.1	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; Fe: Iron concentration; **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm); G: GR-11; K: Krishna Kamod

4.6.2.3.2.3. Cross GR-11 X Gurjari

Ten QTLs were detected for plant height on the linkage groups with the aid of IM and CIM. Three QTLs were detected in LG1 with a phenotypic variance of 32.6 % (qph1.1), 32.6 % (qph1.2) and 22.0 % (qph1.3) with a LOD of 9.8, 9.2 and 5.2 respectively (Table 4.33 and Table 4.34). The favorable allele for qph1.1 was inherited by Gurjari. Single QTL was located on LG2 (qph2.1) with a phenotypic variance of 17.8 5 and a LOD of 4.2. Two QTLs were associated with the LG3 (qph3.1 and qph3.2) with a relative high phenotypic variance and were inherited by both the parental alleles. Single QTL each on LG6 (qph6.1) and LG7 (qph7.1) were detected for plant height with a variance of 16.2 % and 16.9 % respectively. A phenotypic variance of 17.0 % and 16.8 % was observed for the QTLs located on LG11, qph11.1 and qph11.2 with a LOD of 4.8 and 4.2 respectively.

QTLs for plant height were earlier reported by Marri *et al.* (2005) and Anuradha *et al*. (2012b) on chromosome 1. The phenotypic variation explained by two QTLs they reported for plant height detected by Marri *et al.* (2005) on chromosome 1 was 17.48 % and 6.82 %. Anuradha *et al*. (2012b) reported 5 QTLs on chromosome 1 with the phenotypic variation ranging between 19-62.7 %.

4.6.2.3.3. Panicle length

4.6.2.3.3.1. Cross GR-11 X Pankhali-203

For panicle length, four QTLs were located on LG6, LG7 and LG11 which were inherited by the favorable allele of GR-11, only qpl6.1 was contributed by Pankhali-203. A phenotypic variance ranged from 22.9 5 % to 32.6 % among all the QTLs observed for all the linkage groups. qpl6.1 on LG6 was located at 54.7 cM, while qpl7.1 at a distance of 27.1 cM where as for LG11 (qpl11.1 and qpl11.2) was located at 21.7 and 81.7 cM (Table 4.29 and (Table 4.30).

4.6.2.3.3.2. Cross GR-11 X Krishna Kamod

A total of sic QTLs were detected for panicle length across the linkage groups in all the 300 RIL mapping population employing IM and CIM. Singe QTLs were located both on the LG1 (qpl1.1) and LG2 (qpl2.1) with a phenotypic variance of 16.7 % and 22.1 % with a LOD of 4.7 and 5.6 respectively and both were contributed by the GR-11 allele. Two QTLs were detected for panicle length on LG11, qpl11.1 at 54.7 cM and qpl11.2 at 110.7 cM with a LOD of 9.2 and 5.4 respectively (Table 4.31 and Table 4.32).

Table: 4.33 QTLs detected for yield and yield related traits in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Interval Mapping (IM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	PH	qph1.1	1	118.6	RM1	RM312-RM283	9.8	17.3	32.6	105.4	124.8	G
2		qph1.2	1	156.3	RM24	RM283-RM243	9.2	-17.4	32.6	124.8	168.2	GR
3		qph1.3	1	289.3	RM5	RM428-RM8085	5.2	-17.3	22.0	279.4	296.4	GR
4		qph2.1	2	162.8	RM7451	OsYSL7-OsYSL14	4.2	-7.9	17.8	156.4	175.2	GR
5		qph3.1	3	68.7	RM16	OsNAS2-RM489	9.4	21.3	32.6	60.3	73.4	G
6		qph3.2	3	73.4	RM489	RM16-RM7	5.6	-17.4	22.3	68.7	80.4	GR
7		qph6.1	6	134.2	RM314	RM19697-RM217	4.2	-12.0	16.2	132.5	141.5	GR
8		qph7.1	7	99.1	RM1135	OsNAS3-RM118	4.7	-11.5	16.9	94.2	106.7	GR
9		qph11.1	11	18.6	RM216	RM6288-RM3605	4.8	-22.2	17.0	15.9	25.7	GR
10		qph11.2	11	85.1	RM21	RM287-RM304	4.2	15.8	16.8	79.3	99.2	G
11	PL	qpl6.1	6	94.8	RM225	RM415-RM439	7.4	-10.2	27.4	82.7	98.6	GR
12		qpl11.1	11	104.7	RM254	RM304-RM206	5.0	-10.5	22.0	99.2	116.4	GR
13		qpl11.2	11	116.4	RM206	RM254-RM4601	4.3	14.8	16.2	104.7	224.7	G
14	NETP	qnt3.1	3	80.4	RM7	RM489-RM231	4.7	14.9	16.9	73.4	86.9	G
15		qnt3.2	3	98.4	RM514	RM231-RM517	5.6	-15.2	22.6	90.2	105.6	GR
16		qnt3.3	3	135.7	RM517	RM54-RM36	4.6	-21.2	16.9	126.7	142.7	GR
17		qnt10.1	10	21.4	RM496	RM257-RM484	9.8	7.9	32.6	0.0	36.4	G
18		qnt10.2	10	36.4	RM484	RM496-OsFER2	9.2	-19.4	32.6	21.4	55.6	GR
19	NFGP	qgp1.1	1	75.3	RM212	RM315-RM4959	4.2	-12.0	16.2	64.3	89.1	GR
20		qgp1.3	1	362.7	RM104	RM131-OsZIP6	4.3	-20.2	16.2	351.7	389.4	GR
21		qgp2.1	2	24.3	RM262	RM3666-RM452	5.6	17.8	22.3	15.4	34.7	G
22		qgp7.1	7	99.1	RM1135	OsNAS3-RM118	4.2	-12.5	16.2	94.2	106.7	GR
23		qgp11.1	11	124.7	RM4601	RM206-RM552	4.7	-10.8	16.9	116.4	138.0	GR

24		qgp12.1	12	33.1	RM279	RM19-RM512	4.8	-19.5	17.0	28.7	49.6	GR
25		qgp12.2	12	85.4	RM260	RM17-RM7102	4.2	-11.2	16.8	62.7	112.4	GR
26		qgp12.3	12	112.4	RM7102	RM260-OsNRAMP7	9.8	-16.7	32.6	85.4	119.6	GR
27		qtw1.1	1	184.3	RM490	RM495-RM488	9.2	18.1	32.6	175.6	196.7	G
28		qtw1.2	1	342.6	RM3475	RM493-RM431	5.2	-5.8	22.0	331.4	351.7	GR
29	TWT	qtw2.1	2	24.3	RM262	RM3666-RM452	4.2	-4.8	17.8	15.4	34.7	GR
30		qtw3.1	3	167.2	RM5813	RM55-RM1600	9.4	-18.5	32.6	159.3	170.2	GR
31		qtw4.1	4	89.4	RM303	RM551-RM341	5.6	-16.2	22.3	72.4	96.2	GR
32		qtw9.1	9	128.0	RM219	RM105-RM321	4.2	-14.3	16.2	123.4	147.0	GR
33		qgy3.1	3	167.2	RM5928	RM5928-RM124	4.7	-14.5	16.9	159.3	170.2	GR
34	GY	qgy6.1	6	119.3	RM19697	RM541-RM9697	4.2	10.8	16.2	112.7	121.4	G
35		qgy11.1	11	124.7	RM4601	RM206-RM552	4.7	18.5	16.9	116.4	138.0	G
36		qgl1.1	1	362.7	RM104	RM131-OsZIP6	4.8	-11.2	17.0	351.7	389.4	GR
37		qgl2.1	2	111.7	RM110	RM2634-RM279	4.2	-16.7	16.8	105.7	128.3	GR
38		qgl6.1	6	94.8	RM225	RM415-RM439	7.4	11.4	27.4	82.7	98.6	G
39	GL	qgl11.1	11	104.7	RM254	RM304-RM206	5.0	10.2	22.0	99.2	116.4	G
40		qgl11.2	11	116.4	RM206	RM254-RM4601	4.3	3.6	16.2	104.7	224.7	G
41		qgl12.1	12	85.4	RM260	RM17-RM7102	5.2	11.4	22.0	62.7	112.4	G
42		qgl12.2	12	112.4	RM7102	RM260-OsNRAMP7	4.8	9.7	17.0	85.4	119.6	G
43	GB	qgb6.1	6	112.7	RM541	RM439-RM19697	5.6	5.3	22.3	98.6	119.3	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm); **GR**: GR-11; **G**: Gurjari

Table: 4.34 QTLs detected for yield and yield related traits in 300 RIL populations of rice based on cross GR-11 X Gurjari employing Composite Interval Mapping (CIM)

S. No.	Trait	QTL	Chr. No.	Position (cM)	Maker	Marker Interval	LOD	Additive Effect	PVE(%)	LFM	RFM	Allelic Effect
1	PH	qph1.1	1	118.6	RM1	RM312-RM283	4.3	16.3	16.5	105.4	124.8	G
2		qph1.2	1	156.3	RM24	RM283-RM243	4.7	-18.4	17.4	124.8	168.2	GR
3		qph1.3	1	289.3	RM5	RM428-RM8085	5.6	-18.3	22.0	279.4	296.4	GR
4		qph2.1	2	162.8	RM7451	OsYSL7-OsYSL14	4.6	-7.6	16.4	156.4	175.2	GR
5		qph3.1	3	68.7	RM16	OsNAS2-RM489	9.8	20.3	32.6	60.3	73.4	G
6		qph3.2	3	73.4	RM489	RM16-RM7	9.2	-16.4	33.0	68.7	80.4	GR
7		qph6.1	6	134.2	RM314	RM19697-RM217	5.2	-11.0	21.8	132.5	141.5	GR
8		qph7.1	7	99.1	RM1135	OsNAS3-RM118	4.2	-13.5	16.5	94.2	106.7	GR
9		qph11.1	11	18.6	RM216	RM6288-RM3605	9.4	-21.2	32.6	15.9	25.7	G
10		qph11.2	11	85.1	RM21	RM287-RM304	8.1	16.8	28.1	79.3	99.2	G
11	PL	qpl6.1	6	94.8	RM225	RM415-RM439	3.4	-9.2	15.8	82.7	98.6	GR
12		qpl11.1	11	104.7	RM254	RM304-RM206	4.5	-11.5	17.0	99.2	116.4	GR
13		qpl11.2	11	116.4	RM206	RM254-RM4601	4.7	16.8	17.4	104.7	224.7	G
14	NETP	qnt3.1	3	80.4	RM7	RM489-RM231	5.6	14.9	22.0	73.4	86.9	G
15		qnt3.2	3	98.4	RM514	RM231-RM517	5.1	-14.2	21.3	90.2	105.6	GR
16		qnt3.3	3	135.7	RM517	RM54-RM36	4.3	-19.2	16.5	126.7	142.7	GR
17		qnt10.1	10	21.4	RM496	RM257-RM484	4.2	6.9	16.2	0.0	36.4	G
18		qnt10.2	10	36.4	RM484	RM496-OsFER2	4.2	-16.4	16.2	21.4	55.6	GR
19	NFGP	qgp1.1	1	75.3	RM212	RM315-RM4959	4.7	-11.0	16.9	64.3	89.1	GR
20		qgp1.2	1	184.3	RM490	RM495-RM488	4.8	-13.5	17.0	175.6	196.7	GR
21		qgp1.3	1	362.7	RM104	RM131-OsZIP6	4.2	-21.2	16.8	351.7	389.4	GR
22		qgp2.1	2	24.3	RM262	RM3666-RM452	7.4	16.8	27.4	15.4	34.7	G
23		qgp3.1	3	86.9	RM231	RM7-RM514	5.0	-9.2	22.0	80.4	90.2	GR
24		qgp7.1	7	99.1	RM1135	OsNAS3-RM118	4.3	-11.5	16.2	94.2	106.7	GR
25		qgp8.1	8	216.7	RM6027	RM544-RM3395	9.8	-16.8	32.6	208.6	220.5	GR
26		qgp11.1	11	124.7	RM4601	RM206-RM552	9.2	-9.8	32.6	116.4	138.0	GR

			Chr			LFM-RFM						
27		qgp12.1	12	33.1	RM279	RM19-RM512	5.2	-19.5	22.0	28.7	49.6	GR
28		qgp12.2	12	85.4	RM260	RM17-RM7102	4.2	-10.2	17.8	62.7	112.4	GR
29		qgp12.3	12	112.4	RM7102	RM260-OsNRAMP7	9.4	-17.7	32.6	85.4	119.6	GR
30		qtw1.1	1	184.3	RM490	RM495-RM488	5.6	20.1	22.3	175.6	196.7	G
31		qtw1.2	1	342.6	RM3475	RM493-RM431	4.2	-4.8	16.2	331.4	351.7	GR
32		qtw2.1	2	24.3	RM262	RM3666-RM452	4.7	-3.8	16.9	15.4	34.7	GR
33	TWT	qtw3.1	3	167.2	RM5813	RM55-RM1600	4.8	-19.5	17.0	159.3	170.2	GR
34		qtw4.1	4	89.4	RM303	RM551-RM341	4.2	-17.2	16.8	72.4	96.2	GR
35		qtw6.1	6	59.3	RM276	RM247-RM454	7.4	17.3	27.4	55.2	61.8	G
36		qtw7.1	7	13.4	RM214	RM3-RM235	5.0	-19.4	22.0	0.0	32.7	GR
37		qtw9.1	9	128.0	RM219	RM105-RM321	4.3	-18.3	16.2	123.4	147.0	GR
38		qgy3.1	3	167.2	RM5928	RM81-RM124	4.7	-15.5	16.9	159.3	170.2	GR
39	GY	qgy6.1	6	119.3	RM19697	RM541-RM9697	5.6	9.8	22.6	112.7	121.4	G
40		qgy11.1	11	124.7	RM4601	RM206-RM552	4.6	19.5	16.9	116.4	138.0	G
41		qgl1.1	1	362.7	RM104	RM131-OsZIP6	9.8	-10.2	32.6	351.7	389.4	GR
42		qgl2.1	2	111.7	RM110	RM2634-RM279	9.2	-17.7	32.6	105.7	128.3	GR
43		qgl5.1	5	238.6	RM204	RM249-RM8616	4.2	10.0	16.2	228.4	248.1	G
44	GL	qgl6.1	6	94.8	RM225	RM415-RM439	4.3	16.4	16.2	82.7	98.6	G
45		qgl11.1	11	104.7	RM254	RM304-RM206	5.6	8.2	22.3	99.2	116.4	G
46		qgl11.2	11	116.4	RM206	RM254-RM4601	4.2	5.6	16.2	104.7	224.7	G
47		qgl12.1	12	85.4	RM260	RM17-RM7102	4.7	10.4	16.9	62.7	112.4	G
48		qgl12.2	12	112.4	RM7102	RM260-OsNRAMP7	4.8	8.7	17.0	85.4	119.6	G
49	GB	qgb6.1	6	112.7	RM541	RM439-RM19697	4.2	6.3	16.8	98.6	119.3	G

Chr: Chromosome; PVE: Phenotypic variation; LFM: Left Flanking Marker position; RFM: Right Flanking Marker position; **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant, **NFGP**: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm); **GR**: GR-11; G: Gurjari

4.6.2.3.3.3. Cross GR-11 X Gurjari

Three QTLs were detected for panicle length with the aid of IM and CIM on LG6 and LG11. Two QTLs, qpl6.1 and qpl11.1 were inherited by GR-11 allele. Phenotypic variances of 27.4 % were observed for qpl6.1 while qpl11.1 and qpl11.2 had a phenotypic variance of 22.02 % and 16.2 % with a LOD of 5.0 and 4.3 respectively (Table 4.33 and Table 4.34).

4.6.2.3.4. Number of effective tillers per plant

4.6.2.3.4.1. Cross GR-11 X Pankhali-203

A total of four QTLs were detected for number of effective tillers per plant of which three were located on LG3 and one on LG10. Of the four, two QTLs were inherited by the allele GR-11 and the other two alleles by Pankhali-203. For qnt3.1, qnt3.2, qnt3.3 and qnt10.1 the phenotypic variance was 32.6 %, 32.6 %, 22.0 % and 17.8 % with a LOD sore of 9.8, 9.2, 5.2 and 4.2 respectively (Table 4.29 and Table 4.30).

4.6.2.3.4.2. Cross GR-11 X Krishna Kamod

In all five QTLs were observed for the number of effective tillers per plant with the aid of CIM across the linkage groups detected for the cross. QTL qnt2.1 was located at 137.4 cM with a LOD of 5.5 and was contributed by GR-11 allele. Three QTLs on LG3 were inherited by GR-11 with the phenotypic variance of qnt3.1 (22.0 %), qnt3.2 (32.6 %) and qnt3.3 (16.8 %) and a LOD of 4.6, 9.8 and 9.2 respectively (Table 4.31 and Table 4.32). QTL qnt3.2 was inherited by Krishna Kamod and was detected only with the aid of CIM. A phenotypic variance of 21.8 % was observed for the QTL, qnt10.1 located at 10.4 cM on LG10 with a LOD of 5.8.

4.6.2.3.4.3. Cross GR-11 X Gurjari

Five QTLs were detected with the aid of IM and CIM on LG3 and LG10. Three QTLs were inherited by GR-11 while two contributed by Gurjari. LG3 could harbor 3 QTLs at 80.4 cM (qnt3.1), 98.4 cM (qnt3.2) and 135.7 cM (qnt3.3) with the phenotypic variance of 16.9 %, 22.6 % and 16.9 % and a LOD of 4.7, 5.6 and 4.6 respectively (Table 4.33 and Table 4.34). High phenotypic variance of 32.6 % was observed on LG10 for both the QTLs with a LOD of 9.8 (qnt10.1) and 9.2 (qnt10.2).

Except for the QTL, qnt10.2 which was inherited by Gurjari the rest was contributed by GR-11.

Previous reports of Marri *et al.* (2005) revealed QTLs for number of tillers on chromosomes 2 and 5 with a phenotypic variation of 11.11 % and 5.9 % respectively and Anuradha *et al.* (2013) identified QTLs for number of tillers on chromosomes 3, 6 and 10 with a phenotypic variation ranging between 25.9 % to 34.5 %.

4.6.2.3.5. Number of filled grains per plant

4.6.2.3.5.1. Cross GR-11 X Pankhali-203

In case of number of filled grains per plant a total of 6 QTLs were detected with CIM and IM. A single QTL, qgp1.1 was located on LG1 with a phenotypic variance of 32.6 % and a LOD score of 9.4. A LOD with 5.6 was detected for qgp2.1 on LG2 with a phenotypic variance of 22.3 %. Single QTL, qgp6.1 and qgp8.1 was detected on LG6 and LG8 with a phenotypic variance of 16.2 % and 16.9 % and a LOD of 4.2 and 4.7 respectively. Two QTLs, qgp12.1 and qgp12.2 were located on LG12 with a phenotypic variance of 17.0 % and 16.8 % with a LOD of 4.8 and 4.2 respectively (Table 4.29 and Table 4.30).

4.6.2.3.5.2. Cross GR-11 X Krishna Kamod

A total of 10 QTLs were detected by the aid of CIM, while only 7 could be identified employing IM. Five QTLs were detected with the aid of CIM while only two were identified by IM. QTLs qgp1.1, qgp1.2, qgp1.3, qgp1.4 and qgp1.5 were identified using CIM with the phenotypic variance of 22.0 %, 22.0 %, 16.4 %, 16.8 % and 16.8 % with a LOD of 5.5, 5.2, 4.2, 4.3 and 4.5 respectively. Single QTL for number of filled grains per plant was detected each on LG3, LG6, LG7, LG8 and LG12 with the phenotypic variance of 16.4 %, 17.1 %, 22.5 %, 17.8 % and 31.6 % and a LOD of 4.3, 4.7, 5.6, 4.6 and 9.8 respectively. All the QTLs were inherited by GR-11 allele except for the qgp1.4 and qgp6.1 which were contributed by Krishna Kamod (Table 4.31 and Table 4.32).

4.6.2.3.5.3. Cross GR-11 X Gurjari

In all eleven QTLs were detected for number of filled grains per panicle using CIM while only eight were identified employing IM. Three QTLs were identified on

LG1, qgp1.1, qgp1.2 and qgp1.3 with the phenotypic variance of 16.9 %, 17.0 % and 16.8 % and a LOD of 4.7, 4.8, and 4.2 respectively. All the QTLs on LG1 were contributed by GR-11 (Table 4.33 and Table 4.34).

Marri *et al.* (2005) identified QTLs for grain number per panicle on chromosomes 2, 3 and 5 with a phenotypic variation ranging between 5.42 % and 16.65 %.

4.6.2.3.5. Test weight

4.6.2.3.5.1. Cross GR-11 X Pankhali-203

A total of 5 QTLs were detected for test weight with both CIM and IM. On LG1, qtw 1.1 was detected with a phenotypic variance of 16.4 % and a LOD of 4.2, while on LG5, qwt5.1 had a LOD of 7.4 and a phenotypic variance of 27.0 %. On LG6, qtw6.1 was located at 106.7 cM with a phenotypic variance of 21.8 % and a LOD of 5.0. The observed phenotypic variance for the detected QTLs on LG7 (qtw7.1) and LG9 (qtw9.1) were 16.8 % and 17.2 % with the LOD of 4.3 and 4.7 respectively. All the QTLs were inherited by the favorable allele of GR-11 (Table 4.29 and Table4.30).

4.6.2.3.5.2. Cross GR-11 X Krishna Kamod

Eight QTLs were identified with the aid of CIM, while only six with the help of IM in 250 RIL populations derived from the cross based GR-11 X Krishna Kamod. Three QTLs, qtw1.1, qtw1.2 and qtw1.3 were detected on LG1 with the help of CIM having a phenotypic variance of 31.2 %, 15.2 % and 16.9 % and a LOD of 9.2, 3.4 and 4.5 respectively. LG6 could also have three QTLs for test weight with a LOD of 4.3, 4.7 and 5.6 and a phenotypic variance of 16.8 %, 17.1 % and 21.9 % respectively. Single QTL was detected on LG7 and LG9, qtw7.1 at 103.5 cM with the phenotypic variance of 17.2 % and a LOD of 4.6 and was contributed by GR-11 while qtw9.1 was inherited by Krishna Kamod with a phenotypic variance of 32.1 % and a high LOD of 9.8 (Table 4.31 and Table 4.32).

4.6.2.3.5.3. Cross GR-11 X Gurjari

In all eight QTLs could govern the test weight on the linkage groups for rice employing CIM and IM. QTLs, qtw1.1 and qtw1.2 were located 184.3 cM and 342.6

cM respectively on LG1 with the LOD of 5.6 and 4.2 with the phenotypic variance of 22.3 % and 16.2 % respectively. Single QTLs were detected on the LG3, LG4, LG6, LG7 and LG9 with the phenotypic variance of 17.0 %, 196.8 %, 27.4 %, 22.0 % and 16.2 % and a LOD of 4.8, 4.2, 7.4, 5.0 and 4.3 respectively. All the QTLs were inherited by GR-11 allele except for the qtw1.1 and qtw6.1 were contributed by Gurjari (Table 4.33 and Table 4.34).

QTLs for grain test weight mapped on chromosome 6 were also reported earlier by Li *et al.* (1997), Xing *et al.* (2002), Ishimaru, (2003) and Guo *et al.* (2003). Three QTLs on chromosome 2 were identified for grain weight by Marri *et al.* (2005) with a phenotypic variation ranging between 7.16 % and 10.8 %. They also identified another QTL for grain weight on chromosome 9 with a phenotypic variation of 13.95 %. Two QTLs were reported for grain weight on chromosomes 3 and 6 in F2 population of rice by Guo *et al*. (2009).

Nine QTLs for 1,000-grain weight were detected in both generations were reported by Qiang *et al*, (2010). . Of the nine QTLs, a positive QTL, *kgw1.2* was located on chromosome 1, and the QTL, *kgw2.1*, with largest LOD value detected in both generations, explaining 9% and 6% of total variance, respectively, but the allele from *O. rufipogon* decreased 1,000-grain weight in the 93-11 genetic background.

4.6.2.3.6. Grain yield

4.6.2.3.6.1. Cross GR-11 X Pankhali-203

Four QTLs were observed for grain yield in the cross based GR-11 X Pankahli-203. Single QTL on LG1 (qgy1.1) was located at a distance 220.4 cM with the phenotypic variance of 21.6 % and a LOD of 5.6. On LG5 (qgy5.1) was detected with the phenotypic variance of 17.2 % and a LOD of 4.6. In case of LG6, single QTL (qgy6.1) was detected for grain yield with a LOD of 9.8 and a phenotypic variance of 32.6 %. At 16.2 cM, qgy8.1 was detected employing IM and CIM on LG8 with a phenotypic variance of 32.0 % and a LOD 9.2 (Table 4.29 and Table 4.30).

4.6.2.3.6.2. Cross GR-11 X Krishna Kamod

A total of three QTLs were identified for the grain yield on the LG3, LG6 and LG11. On LG1, qgy1.1 at 347.2 cM had a phenotypic variance of 16.2 % and a LOD of 5.2, while qgy5.1 on LG5 had a LOD of 4.2 with a phenotypic variance of 32.7 %.

QTL, qgy6.1 was mapped at 58.1 cM on LG6 had a phenotypic variance of 15.2 % and a LOD of 9.2 (Table 4.31 and Table 4.32).

4.6.2.3.6.3. Cross GR-11 X Gurjari

Three QTLs were detected for grain yield on LG3, LG6 and LG11 with the aid of IM and CIM having a high phenotypic variance of 16.9 %, 22.6 % and 16.9 % with a LOD of 4.7, 5.6 and 4.6 respectively. The favorable allele for all the three QTLs is GR-11 (Table 4.33 and Table 4.34).

Previous reports of Marri *et al.* (2005) indicated single plant yield QTLs on chromosome 2 and 9 with a phenotypic variation ranging between 7.05 % and 23.2 %. Anuradha *et al.* (2013) reported 3 QTLs for yield on chromosomes 2, 8 and 12 with a phenotypic variation ranging between 8.4 % and 18.5 %. Yield per plot QTLs were also identified by Marri *et al.* (2005) were on chromosomes 1, 2 and 8 with a phenotypic variation ranging between 3.98 % and 50. 47 %. Li *et al.* (2004) reported two QTLs for brown rice yield on chromosomes 7 and 12.

4.6.2.3.7. Grain length

4.6.2.3.7.1. Cross GR-11 X Pankhali-203

With the aid of IM and CIM, a total of four QTLs were detected for grain length in the cross GR-11 X Pankhali-203. Single QTLs were detected on LG2, LG5, LG11 and LG12. The phenotypic variance ranged from 16.2 % to 27.4 %. QTLs, qgl2.1 and qgl5.1 were inherited by the GR-11 allele (Table 4.29 and Table 4.30).

4.6.2.3.7.2. Cross GR-11 X Krishna Kamod

With the aid of IM four QTLs were identified, while employing CIM could detect a total of seven QTLs across all the linkage groups. Two QTLs, qgl1.1 and qgl1.2 located at 347.2 cM and 348.7 cM on LG1 with the phenotypic variance of 31.5 % and 15.3 % and a LOD of 9.2 and 3.4 respectively. In case of LG2, qgl2.1 and qgl2.2 had a LOD of 4.5 and 4.7 with a phenotypic variance of 16.8 % and 16.3 % respectively. Single QTLs were identified on LG5, LG7 and LG11 with the phenotypic variance of 21.9 %, 21.3 % and 16.8 % and a LOD of 5.6, 5.1 and 4.3 respectively (Table 4.31 and Table 4.32).

4.6.2.3.7.3. Cross GR-11 X Gurjari

Seven QTLs were detected with the aid of IM while CIM could identify eight across the linkage groups in 300 RIL populations of the cross based GR-11 X Gurjari. Single QTLs were detected on the LG1, LG2, LG5 and LG6 with the phenotypic variance of 32.6 %, 32.6 %, 16.2 % and 16.2 % with a LOD of 9.8, 9.2, 4.2 and 4.3 respectively. Two QTLs on LG11 at 104.7 cM (qgl11.1) and 116.4 cM (qgl11.2) had a phenotypic variance of 22.3 % and 16.2 % with a LOD of 5.6 and 4.2 respectively. LG12 had in all detected two QTLs for grain length with the aid of CIM having a phenotypic variance of 16.9 % and 17.0 % and a LOD of 4.7 and 4.8 respectively (Table 4.33 and Table 4.34).

4.6.2.3.8. Grain breadth

4.6.2.3.8.1. Cross GR-11 X Pankhali-203

Only two QTLs for grain breadth were detected for the cross GR-11 X Pankhali-203 on LG6 at the distance of 50.3 cM (qgb6.1) and 109.4 cM (qgb6.2). The phenotypic variance for qgb6.1 and qgb6.2 was 16.9 % and 22.6 % with a LOD of 4.7 and 5.6 respectively (Table 4.29 and Table 4.30).

4.6.2.3.8.2. Cross GR-11 X Krishna Kamod

In all two QTLs were detected for the grain breadth on LG2 and LG6 with the phenotypic variance of 31.5 % and 22.0 % and a LOD of 9.2 and 5.2 at 137.4 cM and 53.2 cM respectively. Both the QTLs were inherited by the GR-11 allele (Table 4.31 and Table 4.32).

4.6.2.3.8.3. Cross GR-11 X Gurjari

Single QTL was detected with the aid of IM and CIM on LG6 having a phenotypic variance of 16.8 % and a LOD of 4.2 at 112.7 cM and was inherited by Gurjari (Table 4.33 and Table 4.34).

A QTL on chromosome 4 for grain width was also reported by Wan *et al.* (2008) with a phenotypic variation of 6.6 %. They also identified five more QTLs for grain width on chromosomes 1, 5, 9, 10 and 12 with phenotypic variation ranging between 9 % and 25.9 %. Ten QTLs for grain shape traits like grain width were

previously reported by Bai *et al*. (2011) on chromosomes 2, 3, 5 and 9. Li *et al*. (2004) reported fine mapping of the grain width QTL on chromosome 3 using near isogenic lines. Two QTLs on chromosome 7 that explained 10.1 % and 18.9 % phenotypic variation were mapped for grain width that has been reported by Amarawathi *et al.* (2008). Redona and Mackill, (1998) mapped a major locus on chromosome 7 that explains 22 % phenotypic variation.

The high grain Fe and Zn concentration lines identified in the present study may be used as for marker assisted breeding programs.

4.7. Co Localization of QTLs

4.7.1. Cross GR-11 X Pankhali-203

The QTLs identified for both Fe and Zn on chromosome 8 (qZn8.3 and qFe8.1) were found to be co-localized for grain Fe and Zn concentrations. QTLs detected for grain Zn concentration (qZn12.2 and qZn6.2) were co-localize for plant height (qgp12.2) and number of filled grains per panicle (qph6.1). Similarly for the grain Fe concentrations. QTLs were detected on both the chromosomes 9 and 12 which could be co-localized with the grain test weight (qtw9.1) and number of grains per panicle (qgp12.2). On chromosome 5, three QTLs were localized for the traits grain length (qgl5.1), grain test weight (qtw5.1) and grain yield (qgy5.1). QTLs on chromosome 1, 6 and 11 were governed simultaneously for the traits grain test weight (qtw1.1) and number of filled grains per panicle (qgp1.1), grain yield (qgy6.1) and panicle length (qpl6.1) and grain length (qgl11.1) and panicle length (qpl11.1) respectively. QTLs on chromosome 8 were co-localized for the traits grain yield (qgy8.1) and number of filled grains per panicle (qgp8.1). Panicle length and plant height were governed by QTLs located on chromosome 7 (qpl7.1 and qph7.1).

4.7.2. Cross GR-11 X Krishna Kamod

QTLs located for both the Fe (qFe8.2) and Zn (qZn8.3) concentration were co-localized on chromosome 8. The QTLs identified for the grain Fe concentration (qFe1.4) and the test weight (qtw1.1) as well as the number of filled grains per panicle (qgp1.3) on chromosome 1. On chromosome 6 QTLs were co-localized for grain Fe concentration (qFe6.1) and test weight (qtw6.2). Chromosome 7 housed four QTLs which were co-localized for the Zn concentration (qZn3.2 and qZn3.3) and test weight

(qtw3.2 and qtw3.3), whereas QTLs on chromosome 12 was also responsible for the two traits, Zn concentration (qZn12.2) and number of filled grains per panicle (qgp12.1). QTLs located on chromosome 1 were co-localized for the panicle length (qpl1.1) and grain yield (qgy1.1) and also for the test weight (qtw1.1) and number of filled grains per panicle (qgp1.3). QTLs were also co-localized on chromosome 5 for grain yield (qgy5.1) and grain length (qgl5.1), on chromosome 6 for grain yield (qgy6.1) and test weight (qtw6.1) and on chromosome 11 for grain length (qgl11.1) and panicle length (qpl11.2).

4.7.3. Cross GR-11 X Gurjari

The QTLs identified for both Fe (qFe1.1) and Zn (qZn1.1) concentration on chromosome 1 were responsible for both the grain Fe and Zn concentrations. GR-11 showed the increased QTLs in both these alleles. On chromosome 3 the QTLs were co-localized for the Fe concentration (qFe3.4) and number of effective tillers per plant (qnt3.2), whereas on chromosome 8 it was localized for the Fe concentration (qFe8.2) and number of filled grains per panicles (qgp8.1). The QTLs for the grain Fe concentration (qFe6.2) and for grain yield (qgy6.1) were located on chromosome 6. Chromosome 11 could house 2 QTLs for the grain Zn concentration (qZn11.1) and for panicle length (qgl11.1). QTLs for plant height (qph7.1) and for number of filled grains per panicle (qgp7.1) were located on chromosome 7. Chromosome 6 and 11 harbored six QTLs for the panicle length (qpl6.1, qpl11.1 and qpl11.2) and grain length (qgl6.1, qgl11.1 and qgl11.2) respectively. The QTLs located on chromosome 1 were co-localized for the number of filled grains per panicle (qgp1.2) and test weight (qtw1.1). GR-11 allele favored for the QTLs on chromosome 1 for the QTLs number of filled grains per panicle (qgp1.3) and grain length (qgl1.1). Traits like number of filled grains per panicle (qgp2.1) and test weight (qtwtw2.1) can be simultaneously improved by the presence of the QTLs co-localized on the chromosomes 2. Four QTLs were located on chromosome 12 were co-localized for the number of filled grains per panicle (qgp12.2 and qgp12.3) and for the grain length (qgl12.1 and qgl12.2).

Co-localized QTLs were reported by Swamy *et al.* (2011) on chromosome 8. They also reported co-location of Fe and Zn QTLs on chromosomes 2, 3 and 12. Co-location of Zn QTL on chromosome 12 with Fe QTL was reported previously by

Stangoulis *et al*. (2007). Anuradha *et al.* (2012b) reported co-location of Fe and Zn QTLs on chromosomes 7 and 12. Co-location of QTLs may suggest the possibility of simultaneous selection of lines with high Fe and Zn using molecular markers in these regions.

4.8. High Fe and Zn Elite Lines with Fe and Zinc Enhancing QTLs

4.8.1. Cross GR-11 X Pankhali-203

Several lines were identified with high Fe alone, high Zn alone and both high Fe and high Zn (Table 4.35). 51 RILs had > 100 ppm and 42 line had >80 ppm Fe concentration. 30 lines had > 60 ppm Zn concentration. 30 elite lines had > 100 ppm Fe concentration and also > 60 ppm Zn concentration. 70 % of the RILs had QTL qZn5.1 and qFe3.1. The two common QTL for Fe and Zn were found in 55 % RILs. Individual lines with high Fe and Zn concentrations were analyzed for the presence of identified QTLs. Line 158 was found to have the highest number of trait improving QTLs identified either from the GR-11 or Pankhali-203 allele. More than 5 Pankhali-203 derived QTLs which increase Fe and Zn concentration were found in 12 lines. Line 157 had 3 traits increasing QTLs (qZn2.1, qFe1.2 and qFe7.1), 154 had 6 traits for increasing Fe and Zn concentration (qZn2.1, qZn5.1, qZn9.1, qZn7.2, qFe1.2 and qFe7.1), line 17 had 4 traits increasing QTLs for high Fe and Zn concentration (qZn2.1, qZn5.1, qZn9.1 and qFe7.1). Line 80 had two traits increasing QTLs (qZn2.1 and qZn5.1) for Zn concentration and line 100 had three traits increasing QTLs (qFe1.2, qFe7.1 and qFe5.1) for Fe concentration.

4.8.2. Cross GR-11 X Krishna Kamod

60 RILs had > 70 ppm Fe concentration and 55 lines had > 60 ppm Zn concentrations (Table 4.36). 51 elite lines had > 70 ppm of Fe concentration and also > 60 ppm of Zn concentration. 60 % of the RILs had QTL qZn5.1 and qFe3.1. The two common QTL for Fe and Zn were found in 45 % RILs. Individual lines with high Fe and Zn concentrations were analyzed for the presence of identified QTLs. Line 15 was found to have the highest number of trait improving QTLs identified either from the GR-11 or Pankhali-203 allele. More than 3 Pankhali-203 derived QTLs which increase Fe and Zn concentration were found in 15 lines. Line 15 had 6 traits increasing QTLs (qFe5.1, qZn5.1, qZn9.1, qZn7.2, qFe1.2 and qFe7.1), 13 had 6 traits

for increasing QTLs (qZn2.1, qFe3.3 and qFe7.1) for Fe and Zn concentration, line 32 had 4 traits increasing QTLs for high Fe and Zn concentration (qZn2.1, qZn5.1, qZn9.1 and qFe3.3). Line 34 had two traits increasing QTLs (qZn2.1 and qZn5.1) for Zn concentration and line 51 had three traits increasing QTLs (qFe5.1, qFe3.3 and qFe5.1) for Fe concentration.

4.8.3. Cross GR-11 X Gurjari

70 RILs had > 65 ppm of Fe concentration while 63 lines had > 55 ppm of Zn concentrations (Table 4.37). A total of 60 elite lines with > 65 ppm of Fe concentration and > 55 ppm of Zn concentration were selected. 73 % of the RILs had QTL qZn5.1 and qFe3.1. The two common QTL for Fe and Zn were found in 60 % RILs. Individual lines with high Fe and Zn concentrations were analyzed for the presence of identified QTLs. Line 11 was found to have the highest number of trait improving QTLs identified either from the GR-11 or Pankhali-203 allele. More than 6 Pankhali-203 derived QTLs which increase Fe and Zn concentration were found in 20 lines. Line 11 had 3 traits increasing QTLs (qZn2.1, qFe1.2 and qFe9.1), 10 had 6 traits for increasing Fe and Zn concentration (qZn2.1, qZn5.1, qZn9.1, qZn7.2, qFe1.2 and qFe7.1), line 19 had 4 traits increasing QTLs for high Fe and Zn concentration (qZn2.1, qZn5.1, qZn9.1 and qFe7.1). Line 20 had two traits increasing QTLs (qZn2.1 and qZn5.1) for Zn concentration and line 111 had three traits increasing QTLs (qFe9.1, qFe7.1 and qFe8.1) for Fe concentration.

Thus several high Fe and high Zn lines with identified QTLs were obtained. Fe and Zn concentration were analyzed using AAS in the mature grains of the selected high Fe and Zn lines. These will be used for variety development for gene discovery and also for use in bio fortification programs after evaluating their yield and other quality traits such as cooking quality.

4.9. Candidate Gene Analysis

Sixteen QTLs detected using CIM and IM were analyzed *insilico* for identifying candidate genes in the cross based GR-11 X Pankhali-203 (Table 4.38). In all, 10 genes governing Fe and Zn concentrations were found within the QTLs using various gene identification parameters.

Table: 4.35 Top ten lines with high Iron and Zinc concentration for GR-11 × Pankhali.

Sr No	Line No	Seed Image	Kernel Image	Iron Con. ppm	Line No	Seed Image	Kernel Image	Zinc Con. ppm
1.	158			120.14 (63.14 ppm Zn)	11			78.21 (112.01 ppm Fe)
2.	157			119.05 (68.47 ppm Zn)	16			76.48 (110.47 ppm Fe)
3.	154			117.15 (34.12 ppm Zn)	17			70.59 (108.21 ppm Fe)
4.	153			115.04 (65.48 ppm Zn)	80			69.24 (108.27 ppm Fe)
5.	123			108.01 (77.71 ppm Zn)	32			65.78 (102.11 ppm Fe)
6.	4			106.11 (48.05 ppm Zn)	29			62.17 (99.54 ppm Fe)
7.	46			86.34 (48.26 ppm Zn)	88			58.24 (89.14 ppm Fe)
8.	48			72.15 (46.18 ppm Zn)	89			57.41 (83.78 ppm Fe)
9.	105			67.24 (55.29 ppm Zn)	91			55.18 (80.47 ppm Fe)
10.	174			66.23 (39.41 ppm Zn)	100			53.17 (75.12 ppm Fe)

Table: 4.36 Top ten lines with high Iron and Zinc concentration for GR-11 × Krishna Kamod.

Sr No	Line No	Seed Image	Kernel Image	Iron Con. ppm	Line No	Seed Image	Kernel Image	Zinc Con. ppm
1.	15			86.79 (54.57 ppm Zn)	75			77.14 (80.21 ppm Fe)
2.	31			80.14 (56.25 ppm Zn)	102			74.12 (79.15 ppm Fe)
3.	13			79.51 (67.48 ppm Zn)	114			71.01 (79.35 ppm Fe)
4.	32			79.14 (55.57 ppm Zn)	233			65.38 (78.14 ppm Fe)
5.	26			77.12 (53.12 ppm Zn)	232			64.28 (77.14 ppm Fe)
6.	24			75.24 (55.24 ppm Zn)	245			61.20 (77.02 ppm Fe)
7.	34			73.05 (52.48 ppm Zn)	223			54.57 (75.12 ppm Fe)
8.	41			71.26 (51.24 ppm Zn)	219			52.79 (70.12 ppm Fe)
9.	51			68.21 (51.72 ppm Zn)	205			50.24 (69.32 ppm Fe)
10.	57			66.20 (50.14 ppm Zn)	182			48.26 (52.48 ppm Fe)

Table: 4.37 Top ten lines with high Iron and Zinc concentration for GR-11 × Gurjari.

Sr No	Line No	Seed Image	Kernel Image	Iron Con. ppm	Line No	Seed Image	Kernel Image	Zinc Con. ppm
1.	11			79.34 (48.24 ppm Zn)	4			60.48 (78.23 ppm Fe)
2.	10			78.62 (45.16 ppm Zn)	44			56.37 (68.32 ppm Fe)
3.	8			77.12 (68.32 ppm Zn)	45			54.02 (78.15 ppm Fe)
4.	19			75.48 (44.75 ppm Zn)	72			52.89 (75.46 ppm Fe)
5.	20			74.69 (15.98 ppm Zn)	64			51.69 (68.59 ppm Fe)
6.	22			70.91 (46.12 ppm Zn)	111			50.47 (61.28 ppm Fe)
7.	34			67.14 (45.21 ppm Zn)	112			50.18 (70.02 ppm Fe)
8.	31			65.32 (69.14 ppm Zn)	170			49.32 (60.12 ppm Fe)
9.	188			56.23 (59.62 ppm Zn)	168			46.78 (60.47 ppm Fe)
10.	204			48.26 (42.06 ppm Zn)	166			43.48 (59.47 ppm Fe)

For the cross base GR-11 X Krishna Kamod, 16 QTLs were analyzed *insilico* for identifying candidate genes of which only three genes were found to be within the QTLs (Table 4.39). Eighteen QTLs identified for the Fe, Zn and yield and yield related traits in the cross based GR-11 X Gurjari were analyzed *insilico* for identifying candidate genes of which only six were located within the QTLs (Table 4.40).The genes belong to the different classes of protein families 1. Yellow Stripe Like Proteins, 2. Cation efflux family, 3. Oligopeptide Transporter proteins, 4. Natural Resistance Associated Macro-Phage Protein, 5. Nicotianamine Synthase Protein, 6. Phosphoribosyl Transferase Domain family and 7. Iron regulated Metal Transporter. The other genes found at the distance away from left or right flanking markers of the QTLS.

The availability of rice genome sequence has opened up the possibility of identifying candidate genes involved in iron and zinc accumulation in grains. Genomic regions encompassing all the QTLs contributing to Fe and Zn in rice were analyzed for the presence of candidate genes. In all, ten genes related to iron and zinc concentrations were found to be present within selected QTLs on chromosomes 1, 3, 5, 7 and 12. These ten Fe and Zn related genes were *OsYSL1, OsIRT1, OsNAS1, OsNAS2, OsMPT1, OsNAS3* and *OsNRAMP1* belonging to different protein families. The OsYSL genes are known as components of Strategy II of metal transport found in cereals, encoding oligopeptide phytosiderophore transporter proteins (Curie *et al*., 2001; Gross *et al*., 2003). *OsNRAMP1* showed positive correlation with final Zn concentration in the seeds. *OsNRAMP1* functions as a metal efflux transporter participating in the export of metals from the vacuolar compartment to the cytosol, resulting in increased metal concentration available to be transported to the seeds (Sperotto *et al*., 2010). Nicotianamine synthase (NAS) is required for the biosynthesis of nicotianamine (NA), a non-peptidyl metal chelator that is believed to be a co-substrate of the YSL proteins (Schaaf *et al*., 2004). Recently, over-expression of *OsNAS1, OsNAS2* and *OsNAS3* genes in rice showed about two fold increases in Fe and Zn concentrations in unpolished seed (Johnson *et al*., 2011). Transgenic rice expressing *OsNAS2* showed 2.7 fold increase in Zn and 20 fold increase in NA level. It has been suggested that the higher amount of NA led to greater exudation of phytosiderophores (PS) from the roots, as well as stimulated Zn uptake, translocation and seed-loading (Lee *et al*., 2009).

Table: 4.38 Chromosome wise putative candidate genes for QTLs governing Fe, Zn and yield and yield related traits in the cross based GR-11 X Pankhali-203

S. No.	QTL	Gene Names	Locus names	Location
1	qZn2.2	OsNRAMP4	LOC_Os02g03900	0.2 Mb right of QTL
2	qZn2.2	OsYSL2	LOC_Os02g43370	2.3 Mb left of QTL
3	qZn5.1	OsZIP7	LOC_Os05g10940	Within QTL
4	qZn5.3	OsZIP6	LOC_Os05g07210	Within QTL
5	qZn5.3	OsZIP5	LOC_Os05g39560	0.4 Mb left of QTL
6	qZn7.1	OsNRAMP1	LOC_Os07g15460	Within QTL
7	qFe1.1	OsYSL1	LOC_Os01g13710	Within QTL
8	qFe3.1	OsNRAMP8	LOC_Os03g41070	1.5 Mb right of QTL
9	qFe3.2	OsNAS1	LOC_Os03g19427	Within QTL
10	qFe3.3	OsNAS2	LOC_Os03g19420	Within QTL
11	qFe4.1	OsYSL5	LOC_Os04g32060	Within QTL
12	qFe5.2	OsYSL14	LOC_Os02g42220	Within QTL
13	qFe12.1	OsNAC5	LOC_Os11g08210	Within QTL
14	qFe12.2	OsNRAMP7	LOC_Os12g39180	1.3 Mb right of QTL
15	qtw9.1	OsVITI	LOC_Os09g23300	2.6 Mb right of QTL
16	qgy1.1	OsNRAMP6	LOC_Os01g31870	Within QTL

The first digit after QTL (qFe or qZn) refers to chromosome number.

Iron and zinc content was elevated in shoots, roots and mature seeds of transgenic rice plants over-expressing *OsIRT1*. These plants showed enhanced tolerance to iron deficiency at the seedling stage and were sensitive to excess Zn and Cd, indicating that *OsIRT1* also transports these metals. Results demonstrated that *OsIRT1* can be used for enhancing micronutrient levels in rice grains (Lee and An, 2009). Chandel *et al*. (2011) identified candidate genes for grain Fe and Zn underlying the target QTL regions. They reported 8 genes related to Fe and Zn from different protein families. Consistent with their results, two related genes belonging to cation efflux family on chromosome 5 and heavy metal associated domain containing protein on chromosome 7 were observed in present study also.

Table: 4.39 Chromosome wise putative candidate genes for QTLs governing Fe, Zn and yield and yield related traits in the cross based GR-11 X Krishna Kamod

S. No.	QTL	Gene Names	Locus names	Location
1	qZn2.3	OsNRAMP4	LOC_Os02g03900	1.1 Mb left of QTL
2	qZn2.3	OsYSL2	LOC_Os02g43370	1.1 Mb right of QTL
3	qZn3.1	OsNAS1	LOC_Os03g19427	0.4 Mb right of QTL
4	qZn7.1	OsNRAMP1	LOC_Os07g15460	1.1 Mb left of QTL
5	qZn7.1	OsNAS3	LOC_Os07g48980	0.3 Mb right of QTL
6	qZn10.1	OsFER2	LOC_Os02g25641	5.2 Mb right of QTL
7	qZn12.2	OsNRAMP7	LOC_Os12g39180	0.4 Mb right of QTL
8	qFe1.2	OsYSL1	LOC_Os01g13710	1.3 Mb right of QTL
9	qFe2.1	OsYSL8	LOC_Os02g02460	Within the QTL
10	qFe4.2	OsFRO1	LOC_Os04g36720	Within the QTL
11	qgp1.4	OsZIP6	LOC_Os05g07210	0.4 Mb right of QTL
12	qtw9.1	OsVITI	LOC_Os09g23300	0.8 Mb right of QTL
13	qFe12.1	OsNAC5	LOC_Os11g08210	Within the QTL
14	qgb2.1	OsYSL7	LOC_Os02g02450	2.6 Mb right of QTL
15	qtw1.2	OsNRAMP6	LOC_Os01g31870	3.5 Mb right of QTL
16	qgl2.2	OsYSL14	LOC_Os02g42220	4.2 Mb left of QTL

The first digit after QTL (qFe or qZn) refers to chromosome number.

In addition, they also found genes belonging to *ZIP*, Zinc transporter family (zinc-regulated transporter/iron regulated transporter proteins), 2Fe–2S iron–sulfur cluster binding domain, and major facilitator super-family on chromosomes 1, 9 and 11. It was believed that mapping QTLs does not accurately position genes underlying polygenic traits on the genome, which limits the application of QTL analysis in marker-assisted selection and gene discovery. But now map based cloning or tagging of several plant QTLs shows that they are accurate to within 2 cM or less (Price, 2006).

Table: 4.40 Chromosome wise putative candidate genes for QTLs governing Fe, Zn and yield and yield related traits in the cross based GR-11 X Gurjari

S. No.	QTL	Gene Names	Locus names	Location
1	qZn2.2	OsYSL8	LOC_Os02g02460	0.2 Mb left of QTL
2	qZn3.1	OsNAS1	LOC_Os03g19427	3.4 Mb right of QTL
3	qZn4.1	OsYSL15	LOC_Os02g43410	0.4 Mb right of QTL
4	qZn3.1	OsNRAMP8	LOC_Os03g41070	Within the QTL
5	qZn5.1	OsZIP7	LOC_Os05g10940	Within the QTL
6	qZn10.1	OsFER2	LOC_Os02g25641	5.2 Mb right of QTL
7	qZn12.1	OsNAC5	LOC_Os11g08210	2.4 Mb left of QTL
8	qFe1.2	OsYSL1	LOC_Os01g13710	0.4 Mb right of QTL
9	qFe2.2	OsNRAMP4	LOC_Os02g03900	Within the QTL
10	qFe3.3	OsNAS2	LOC_Os03g19420	Within the QTL
11	qFe7.1	OsNRAMP1	LOC_Os07g15460	Within the QTL
12	qFe7.3	OsNRAMP5	LOC_Os07g15370	Within the QTL
13	qFe9.1	OsVITI	LOC_Os09g23300	4.2 Mb left of QTL
14	qph2.1	OsYSL7	LOC_Os02g02450	0.3 Mb right of QTL
15	qph2.1	OsYSL14	LOC_Os02g42220	0.2 Mb left of QTL
16	qgp1.3	OsZIP6	LOC_Os05g07210	2.1 Mb right of QTL
17	qgp7.1	OsNAS3	LOC_Os07g48980	4.5 Mb right of QTL
18	qgl12.2	OsNRAMP7	LOC_Os12g39180	0.3 Mb right of QTL

The first digit after QTL (qFe or qZn) refers to chromosome number.

In present study, 31 candidate genes were identified close to the QTLs beyond the distance of the left or right flanking markers. These genes in the vicinity of the QTLs belong to different protein families on chromosomes. *ZIP* family genes are known to participate in divalent metal transport in plants. Expression of OsZIP8 was correlated to high grain Fe concentration (Banerjee and Chandel, 2011). Transgenic rice lines were developed by activation tagging of *OsNAS2* expressing elevated levels of NA. These *OsNAS2* lines contained 20 fold more NA and 2.7 fold more zinc suggesting that higher amount of NA stimulated Zn uptake, translocation and seed loading.19 out of 50 QTLs showed the presence of putative candidate gene within QTLs for Fe, Zn and yield and yield related traits. It is possible that novel genes

underlie the other two QTLs. Thus several genes known to contribute to Fe and Zn in seed were found within or close to QTLs identified in my study. These can be used for map based cloning and functional analysis.

Insilico analysis was also carried out for expression profiling of the genes to predict the temporal and spatial pattern of expression. The genes were expressed in various tissues such as seed, callus, leaves, shoot, root and panicle (Table 4.41, 4.42, 4.43, 4.44, 4.45 and 4.46). Spatio-temporal pattern of expression of these genes was examined *insilico* by analyzing their expression in various tissues and organs using RiceXPro and TIGR database. *OsNAS1, OsNAS2, OsZIP6* and *OsZIP7* genes expressed in endosperm. *OsIRT1, OsNAS3, OsNRAMP1, OsZIP6, OsZIP7* and *OsZIP8* genes expressed in seeds and embryo. In addition these genes also expressed in other tissues such as leaves, anther, pistil, roots and shoots suggesting their role in uptake and transport of Fe and Zn throughout the plant and not just the seeds. Lee *et al*. (2009) showed that shoots and roots of *OsNAS3* activation-tagged plants accumulated more Fe and Zn. They suggested that the activation of OsNAS3 resulted in higher metal content in leaves and mature seeds. Over-expression of nicotianamine synthase 2 *(NAS2)*, resulted in 3-fold rise in Fe content in mature seeds (Lee *et al*., 2009). Overexpression of *OsNAS1* gene in rice-grain endosperm of an elite japonica rice variety showed that polished rice from *OsNAS1* transgenic lines had twice as much bioavailable iron as that of non-transgenic control line. Fe and Zn concentrations in unpolished grains of transgenic lines were 18.8% to 45.6% and 33.4% to 55.4% higher than control plants respectively (Zheng *et al*., 2010). Over-expression of *NAS* also has a positive effect on root to shoot transport, especially for Fe and Zn, as was found by over-expression of the HvNAS1 gene from barley that approximately doubled the Fe and Zn concentrations in young leaves of tobacco (Takahashi *et al*., 2003).

V. SUMMARY AND CONCLUSION

Rice is the staple food crop for more than half of the world's population. It is the predominant dietary energy source for Asia, North and South America and Africa. More than 90 percent of rice is produced and consumed in Asia. The important micronutrients present in rice grain are lost due to the processing of rice grain and storage practices. Biofortification of rice (*Oryza sativa* L.) is an important objective for crop improvement, because it is a staple food for more than half of the world population and rice grain contains low available levels of important micronutrient such as Fe, Zn and vitamin A.

The present investigation entitled **"Mapping QTLs for iron and zinc concentrations in rice (*Oryza sativa* L.)"** was conducted at the Plant Biotechnology Laboratory, Department of Genetics & Plant Breeding and Centre of Excellence in Biotechnology, B.A. College of Agriculture, Anand Agricultural University, Anand, during 2014-2017 with following objective of development of new mapping population and, characterizing and understanding the genetic architecture of grain Fe and Zn density and other traits in rice through QTL analysis. The experimental material for this study consisted of three RIL mapping populations. The female parents of all three populations were having low Fe and Zn concentrations as compared to their corresponding male parents. The population based on cross GR-11 X Pankhali -203 consisted of 300 RILs, while that based on GR-11 X Krishna Kamod was developed with 250 RILs and GR-11 X Gurjari consisted of 300 RIL populations.

The study was designed to construct three linkage map one for each population, to identify and map QTLs controlling grain Fe and Zn concentrations (ppm), days to 50 % flowering (d), plant height (cm), panicle length (cm) and grain yield (g). Both the populations were raised in fields at Main Rice research Station, Nawagam, Gujarat, using augmented design. Phenotypic data for days to 50 % flowering (d), plant height (cm), panicle length (cm), number of effective tillers per plants, number of filled grains per panicle, test weight (g), grain yield (g), grain length (cm), grain breadth (cm), L:B ratios Fe concentrations (ppm) and Zn concentrations (ppm) for all the three mapping populations. Phenotyping for Fe and Zn concentration in grain sample was carried out with di-acid digestion followed by Atomic Absorption Spectroscopy (AAS) reading. The phenotypic data were also used

to calculate the associations among the traits studied. For molecular marker studies genomic DNA was isolated from each of the RILs and their two parental lines from all the three populations. DNA quality was checked using 0.8 % agarose gel and was quantified by nanodrop. After marker polymorphism survey between the pairs of parental lines, polymorphic markers were used to genotype each of the RILs in each population. Employing R/qtl, a linkage map was constructed for the GR-11 X Pankhali-203 based RIL population with 200 marker loci, a linkage map of the second RIL population based on GR-11 X Krishna Kamod was constructed with 220 marker loci, while the third RIL population based on GR-11 X Gurjari was constructed using 222 marker loci. All the three maps contained both the co-dominant SSRs as well as the gene specific markers. Genotypic data marker order and distance were used to find the candidate QTLs and to estimate their effects by composite interval mapping (CIM) implemented in QTL Cartographer 2.0. The salient findings of the study are briefly summarized here:

Elemental analysis was performed on mature seeds (R9 stage) of seventy two rice genotypes by Atomic Absorption Spectroscopy (AAS) for Iron and Zinc concentrations in grains. Fe concentration was found to be the highest in Pankhali-203 with 75 ppm, followed by Krishna Kamod and Sambha Masuri with 60 and 55 ppm respectively. For zinc concentrations also it was observed the highest in Pankhali-203 (61 ppm), followed by Krishna Kamod (55 ppm) and Gurjari (50 ppm). GR-11 is the female parent because of its high adaptability and characters like dwarf plant type with early maturity and medium sized white colored grain. High iron and zinc containing genotypes Pankhali-203, Krishna Kamod and Gurjari were selected as the donor parents for developing the F7 RIL mapping populations.

Three hundred RIL populations for the cross based GR-11 X Pankhali-203 were analysed along with their parents for gain Fe and Zn concentration on AAS. The Fe concentration recorded in the 300 RIL populations ranged from 13.45 ppm to 140.70 ppm while Zn concentration ranged from 15.01 ppm to 98.11 ppm. In case of GR-11 X Krishna Kamod, Fe concentration ranged from 20.14 ppm to 88.24 ppm whereas Zn concentration ranged from 37.95 ppm to 99.15 ppm in the 250 RILs. For the cross based GR-11 X Gurjari, 300 RIL populations were developed in which Fe concentration ranged from 34.18 ppm to 100.69 ppm while Zn was at 11.92 ppm to 88.74 ppm.

For the cross based GR-11 X Pankhali-203, grain Zn concentrations were positively and significantly correlated to the number of effective tillers (r= 0.131), grain yield (r= 0.177), grain length (r= 0.126) and panicle length (r= 0.217). Similarly, grain Fe concentration was also positively correlated to panicle length (r= 0.201), test weight (r= 0.132), grain yield (r= 0.143) and grain length (r= 0.147). Since the panicle length also correlates to the iron and zinc content, by increasing the panicle length the number of seed on the panicle increases which in turn leads to the increase in grain yield of the crop.

In case of GR-11 X Krishna Kamod, grain zinc content was positively and significantly correlated to Fe content (r= 0.138), plant height (r= 0.154), panicle length (r= 0.136) and grain yield (r= 0.152). Grain Fe content had significant correlations with Zn content (r= 0.138), panicle length (r= 0.146) and grain yield (r= 0.126). The rest of the traits had no significant relations among the RIL populations.

Grain zinc concentration was highly and positively correlated to Fe concentration (r= 0.149), plant height (r= 0.173), panicle length (r= 0.180) and grain yield (r= 0.103) for the cross GR-11 X Gurjari. Grain iron content was positively correlated to plant height (r= 0.188), panicle length (r= 0.143) and grain yield (r= 0.146). The rest of the traits did not exhibit any significant correlations to the others.

Correlation studies indicated significant and strong positive correlation between grain Fe and Zn concentrations in all the three RIL populations However, a positive correlation was also observed for panicle length and grain yield with the mineral nutrient concentrations, which indicated the direct effect on uptake, metabolism and acquisition of both minerals. Likewise significant associations were also observed among all observed traits in all the three RIL populations. Consistent correlations between Fe and Zn across all crosses, suggested the possibility that at least some of the genes that control these traits are linked or have pleiotropic effect. To determine the position and effect of the genes controlling these traits, linkage maps were constructed with different marker systems that could be used to conduct a search of QTLs throughout the genome.

The frequency distribution of all the observed mineral related traits showed continuous phenotypic variation and transgressive segregation (lines with lower values than the lowest parent or higher value than the highest parent) in both

directions, suggesting multiple gene action. The recovery of the large proportion of the transgressive segregants suggested possible importance of epistasis and also the dispersed nature of favorable and unfavorable alleles for all traits among the parents.

Frequency distribution for most of the traits showed normal (Zinc concentration, Fe concentration, days to 50 % flowering, plant height, panicle length, number of productive tillers per plant, number of filled grains per plant, test weight, grain yield, grain length and L:B ratio) and near normal distribution (grain breadth) in all the three crosses. This clearly indicated that Zn concentration was probably governed by a QTL, while few other QTLs might be involved in controlling Fe concentration.

A total of 600 SSR markers (RM series) were chosen based on their distribution throughout genome and 52 gene specific markers designed from the genes associated with uptake, transport and remobilization of mineral nutrients in rice for parental polymorphism survey. Among these 600 SSR markers, 229 (38.00%) were polymorphic and among the 52 gene specific markers, 33 (63.46%) were polymorphic. In all, 325 (51.34%) markers distributed on all the 12 chromosomes were polymorphic between the parents, indicating the possibility of constructing a linkage map.

Genotyping of all the RIL populations were done with the polymorphic markers. Of the 234 markers mapped, in the cross based GR-11 X Pankhali-203, segregation of 34 SSR and 7 gene specific markers were distorted. Out of 40 % distorted markers, the highest proportion was observed in the SSR markers as compared to the gene specific markers.

In case of GR-11 X Krishna Kamod, 27 SSRs and 8 gene specific markers out of 258 markers were distorted from the Mendelian pattern of inheritance. Of 32 SSR markers genotyped for this RIL population only 35 were skewed towards either of the two alleles. Large numbers of markers were distributed on LG5 and LG6 amon all the other linkage groups and the least distortion was observed in case of the LG1.

Out of 266 markers mapped for the RIL population of GR-11 X Gurjari, 37 were distorted and only 14 were skewed towards Pankhali-203. Among the markers

mapped on the RIL population highest degree of distortion was observed in SSR markers as compared to the gene specific markers.

Using 200 marker loci and 300 RILs, a linkage map was constructed for the Gr-11 X Pankhali-203 based RIL population. The linkage map constructed for this population spanned 1370.4 cM (Haldane), which represented on average one marker for every 6.7 cM. The individual linkage groups ranged from 102.8 cM for linkage group 8 with the highest number of markers (29) to 25.4 cM for linkage group 10 with 5 markers

A total of 258 (226 SSR and 32 gene specific markers were mapped for the 250 RILs derived from the cross GR-11 X Krishna Kamod at an distance of 2490.5 cM (Haldane) with an average length of 207.5 cM and an average marker loci of 21.5 on each linkage group. Large number of markers was grouped by LG1, followed by LG5, LG8 and LG2 with 37, 26, 32 and 28 markers respectively. Gaps of more than 10 cM marker interval between the two adjacent markers were detected in case of LG9 and LG10. LG1 was longest (362.8 cM) whereas LG11 was the shortest.

To construct the linkage map a total of 262 (229 SSR and 32 gene specific) markers were used for the genotyping of 300 RIL populations developed from GR-11 X Gurjari. The total length of the map is 2663.2 cM (Haldane), which represents an average one marker at every 10.1 cM. The individual linkage groups ranged from 398.4 cM for LG1 with highest number of markers (34) to 134.6 cM for LG10 with the least number of markers (8) followed by LG12. The average linkage group length was 221.9 cM with an average locus of 21.8 on all the 12 linkage groups. 304.5 cM of map length was observed in LG2 with a total of 28 molecular markers (22 SSR and 8 genes specific), of which the distorted markers were mapped on the ends of the chromosome.

QTL analysis, GR-11 X Pankhali-203 base population identified 21 QTLs for Zn concentration, 20 QTLs for Fe concentration and 36 QTLs for the yield and yield related traits. Highest phenotypic variance of 32.1 % was observed for qZn8.2, qZn8.3, qZn9.2, qZn10.1 and qZn12.2 on LG8, LG9, LG10 and LG12 respectively. The majority of the QTLs were inherited by the GR-11 parent. Highest phenotypic variance of 32.6 % was observed for the qFe5.2, qFe7.1. Among all the QTLs detected for the iron concentration, six were inherited by the Pankhali-203 while the

rest had an inheritance from the GR-11 allele. The phenotypic variance ranged from 16.8 % to 32.6 %. The QTLs identified for both Fe and Zn on chromosome 8 (qZn8.3 and qFe8.1) were found to be co-localized for grain Fe and Zn concentrations. QTLs detected for grain Zn concentration (qZn12.2 and qZn6.2) were co-localize for plant height (qgp12.2) and number of filled grains per panicle (qph6.1). QTLs were detected on both the chromosomes 9 and 12 which could be co-localized with the grain test weight (qtw9.1) and number of grains per panicle (qgp12.2). On chromosome 5, three QTLs were localized for the traits grain length (qgl5.1), grain test weight (qtw5.1) and grain yield (qgy5.1). QTLs on chromosome 1, 6 and 11 were governed simultaneously for the traits grain test weight (qtw1.1) and number of filled grains per panicle (qgp1.1), grain yield (qgy6.1) and panicle length (qpl6.1) and grain length (qgl11.1) and panicle length (qpl11.1) respectively. QTLs on chromosome 8 were co-localized for the traits grain yield (qgy8.1) and number of filled grains per panicle (qgp8.1). Panicle length and plant height were governed by QTLs located on chromosome 7 (qpl7.1 and qph7.1).

In case of the cross GR-11 X Krishna Kamod, 23 QTLs were detected for the Zn concentration, 17 for the Fe concentration and 50 QTLs for the yield and yield related traits. Among all the 23 QTLs identified for the zinc concentration only six were contributed by the Krishna Kamod the rest had an inheritance by GR-11 allele. The QTLs qZn2.4 on LG2 and qZn4.1 on LG 4 were only identified by the CIM method. The highest phenotypic variance of 32.6 % was observed for the qFe1.2 and qFe7.1. The phenotypic variance ranged from 15.3 % to 32.6 %. Six QTLs were inherited by the Krishna Kamod allele while the rest were contributed by the GR-11 allele. QTLs, qFe1.3 and qFe8.1 were only detected by the CIM method. QTLs located for both the Fe (qFe8.2) and Zn (qZn8.3) concentration were co-localized on chromosome 8. The QTLs identified for the grain Fe concentration (qFe1.4) and the test weight (qtw1.1) as well as the number of filled grains per panicle (qgp1.3) on chromosome 1. On chromosome 6 QTLs were co-localized for grain Fe concentration (qFe6.1) and test weight (qtw6.2). Chromosome 7 housed four QTLs which were co-localized for the Zn concentration (qZn3.2 and qZn3.3) and test weight (qtw3.2 and qtw3.3), whereas QTLs on chromosome 12 was also responsible for the two traits, Zn concentration (qZn12.2) and number of filled grains per panicle (qgp12.1). QTLs located on chromosome 1 were co-localized for the panicle length (qpl1.1) and grain yield

(qgy1.1) and also for the test weight (qtw1.1) and number of filled grains per panicle (qgp1.3). QTLs were also co-localized on chromosome 5 for grain yield (qgy5.1) and grain length (qgl5.1), on chromosome 6 for grain yield (qgy6.1) and test weight (qtw6.1) and on chromosome 11 for grain length (qgl11.1) and panicle length (qpl11.2).

Twenty two QTLs were identified for the Zn concentration, 22 for iron concentration and 49 QTLs for the yield and yield related traits in the cross based GR-11 X Gurjari. Among all the QTLs detected were inherited by GR-11 except two qZn6.2 and qZn8.2 contributed to Gurjari. QTLs, qZn2.3 and qZn2.4 located on LG2 were only detected using CIM method. Among all the QTLs detected for the iron concentration, only five were inherited by the Gurjari allele the rest had the favorable allele of GR-11. The phenotypic variance ranged from 15.2 % to 32.8 %. The QTLs, qFe3.3 and qFe7.2 were only detected with the help of CIM. The QTLs identified for both Fe (qFe1.1) and Zn (qZn1.1) concentration on chromosome 1 were responsible for both the grain Fe and Zn concentrations. GR-11 showed the increased QTLs in both these alleles. On chromosome 3 the QTLs were co-localized for the Fe concentration (qFe3.4) and number of effective tillers per plant (qnt3.2), whereas on chromosome 8 it was localized for the Fe concentration (qFe8.2) and number of filled grains per panicles (qgp8.1). The QTLs for the grain Fe concentration (qFe6.2) and for grain yield (qgy6.1) were located on chromosome 6. Chromosome 11 could house 2 QTLs for the grain Zn concentration (qZn11.1) and for panicle length (qgl11.1). QTLs for plant height (qph7.1) and for number of filled grains per panicle (qgp7.1) were located on chromosome 7. Chromosome 6 and 11 harbored six QTLs for the panicle length (qpl6.1, qpl11.1 and qpl11.2) and grain length (qgl6.1, qgl11.1 and qgl11.2) respectively. The QTLs located on chromosome 1 were co-localized for the number of filled grains per panicle (qgp1.2) and test weight (qtw1.1). GR-11 allele favored for the QTLs on chromosome 1 for the QTLs number of filled grains per panicle (qgp1.3) and grain length (qgl1.1). Traits like number of filled grains per panicle (qgp2.1) and test weight (qtwtw2.1) can be simultaneously improved by the presence of the QTLs co-localized on the chromosomes 2. Four QTLs were located on chromosome 12 were co-localized for the number of filled grains per panicle (qgp12.2 and qgp12.3) and for the grain length (qgl12.1 and qgl12.2).

In case of cross GR-11 X Pankhali-203, 51 RILs had > 100 ppm and 42 line have >80 ppm Fe concentration. 30 lines had > 60 ppm Zn concentration. 30 elite lines had > 100 ppm Fe concentration and also > 60 ppm Zn concentration. 51 elite lines had > 70 ppm of Fe concentration and also > 60 ppm of Zn concentration for the cross based GR-11 X Krishna Kamod whereas for GR-11 X Gurjari, 60 elite lines had > 65 ppm of Fe concentration and > 55 ppm of Zn concentration. Several high Fe and high Zn lines with identified QTLs were obtained. Fe and Zn concentration were analyzed using AAS in the mature grains of the selected high Fe and Zn lines. These will be used for variety development for gene discovery and also for use in bio fortification programs.

In all, 31 candidate genes related to iron and zinc concentrations were found to be present within the selected QTLs and beyond the distance of the left or right flanking markers. Spatio-temporal pattern of expression of these genes was examined *insilico* by analyzing their expression in various tissues and organs using RiceXPro and TIGR database. *OsNAS1, OsNAS2, OsZIP6* and *OsZIP7* genes expressed in endosperm. *OsIRT1, OsNAS3, OsNRAMP1, OsZIP6, OsZIP7* and *OsZIP8* genes expressed in seeds and embryo. In addition these genes also expressed in other tissues such as leaves, anther, pistil, roots and shoots suggesting their role in uptake and transport of Fe and Zn throughout the plant and not just the seeds.

The high grain Fe and Zn concentration lines identified in the present study can be used as for marker assisted breeding programs. QTLs identified for more than one trait could suggest their role in the simultaneous improvement of more than one trait.

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APPENDICES

Appendix 1: Morphological data for the cross GR-11XPankhali-203

Sr. No.	Zn (ppm)	Fe (ppm)	DFT	PH (cm)	PL (cm)	NEFT
1	28.01	15.21	97.02	92.15	27.10	6.31
2	22.15	20.18	100.75	92.65	27.10	7.54
3	47.49	65.48	95.21	91.02	27.60	7.26
4	48.05	106.11	95.26	93.14	27.15	6.32
5	43.23	80.08	95.14	110.58	27.60	8.25
6	27.01	14.57	100.00	93.48	27.60	9.34
7	45.01	80.49	100.00	93.17	27.60	8.16
8	26.19	86.34	95.34	92.58	27.90	7.45
9	15.27	20.15	97.54	91.24	26.48	6.32
10	18.18	20.48	95.14	115.06	27.60	8.45
11	98.21	140.70	95.48	150.89	29.10	10.25
12	28.17	33.18	95.26	119.24	28.30	8.14
13	82.24	13.45	95.64	140.56	29.10	9.34
14	27.48	37.15	97.45	117.25	27.58	7.15
15	24.25	20.18	95.24	114.28	27.19	7.25
16	95.04	140.47	95.48	148.36	29.10	10.25
17	87.04	135.28	95.16	149.37	29.10	10.25
18	45.28	34.58	95.47	115.29	27.30	8.69
19	23.15	18.47	98.00	117.24	27.26	7.15
20	46.27	77.18	100.24	118.24	27.26	8.26
21	27.15	35.26	100.49	114.06	27.34	9.34
22	24.25	19.27	99.98	112.31	27.18	9.15

23	25.26	19.24	95.67	111.45	28.30	8.24
24	47.12	79.15	95.24	111.58	27.30	6.32
25	22.01	20.15	97.01	110.59	27.72	6.32
26	72.48	110.15	94.57	147.02	27.45	8.65
27	27.18	20.58	94.21	111.59	27.49	8.45
28	49.05	80.49	94.00	112.48	27.60	7.26
29	80.17	135.24	95.67	147.26	28.30	8.32
30	26.17	20.48	99.00	113.59	26.15	8.49
31	48.17	77.79	98.00	113.48	27.30	7.26
32	74.18	115.48	100.48	148.57	28.48	9.32
33	24.18	38.48	99.00	114.02	27.68	8.14
34	48.19	106.11	95.84	115.64	28.90	6.32
35	27.14	39.91	100.27	114.29	27.48	8.45
36	49.56	86.34	100.00	117.28	27.30	8.36
37	28.47	37.15	98.00	117.59	27.30	8.24
38	50.14	86.34	98.00	115.24	28.16	6.23
39	75.18	120.04	88.64	146.39	27.48	8.21
40	40.17	39.98	84.36	118.27	27.98	8.54
41	28.48	37.94	90.00	118.24	27.30	6.32
42	78.24	120.54	99.00	147.36	29.30	7.48
43	75.26	119.05	84.26	149.35	29.74	7.21
44	25.15	37.95	89.27	117.24	26.30	8.24
45	28.01	15.21	97.02	92.15	27.10	6.31
46	76.28	120.58	100.00	140.25	28.60	8.56
47	48.26	86.34	88.24	114.29	28.90	8.35
48	50.18	64.28	90.00	114.18	26.74	7.41

49	46.18	72.15	81.37	118.34	26.74	7.25
50	47.15	70.48	82.15	117.18	27.48	8.63
51	79.24	119.05	98.14	134.28	27.30	10.29
52	76.14	117.45	90.00	133.69	27.90	8.59
53	77.08	120.04	98.00	137.48	27.30	8.21
54	74.26	118.05	89.00	132.58	27.48	8.08
55	74.15	114.75	88.27	137.24	28.06	8.48
56	60.48	109.15	99.65	98.65	28.30	10.04
57	45.17	37.51	98.59	80.04	27.90	7.50
58	43.18	25.67	100.00	88.24	27.30	8.21
59	44.29	35.48	90.00	87.69	28.19	8.26
60	47.15	32.17	99.00	85.26	27.90	8.32
61	44.26	37.41	84.26	86.34	27.18	6.34
62	55.24	40.58	100.00	86.31	27.30	8.26
63	48.15	30.15	99.00	81.24	27.30	7.21
64	48.17	28.14	99.00	90.48	27.69	8.36
65	47.26	29.95	84.26	82.14	27.90	7.24
66	47.31	29.84	98.00	82.39	26.74	7.15
67	47.18	35.18	98.00	90.14	28.06	8.36
68	54.26	37.95	98.00	91.27	26.74	8.24
69	42.13	38.65	90.00	92.16	27.30	8.01
70	54.27	34.15	95.34	92.48	27.30	6.31
71	40.08	38.61	97.45	91.27	27.90	8.36
72	43.16	40.59	97.06	94.05	27.90	7.15
73	46.28	38.75	89.12	98.98	27.90	10.25
74	43.18	29.94	98.00	98.99	28.15	8.69

75	41.27	37.69	98.00	98.99	27.90	7.45
76	47.16	55.16	90.21	99.98	27.48	8.59
77	43.15	52.18	94.27	91.24	27.30	8.47
78	46.59	58.45	87.45	92.68	27.48	7.48
79	44.18	56.29	89.92	93.48	27.90	7.29
80	44.27	59.94	90.00	99.27	27.49	8.26
81	79.89	119.48	99.00	97.15	27.30	6.32
82	57.10	57.48	90.00	94.25	27.90	8.14
83	54.17	48.84	97.18	91.58	27.30	8.21
84	75.14	118.04	83.14	138.56	27.90	8.65
85	55.13	58.19	84.19	114.28	28.30	7.26
86	42.18	59.47	95.21	118.95	27.64	10.36
87	59.17	57.24	97.40	116.34	28.18	8.25
88	45.26	55.18	98.00	118.02	27.62	7.59
89	58.24	59.04	89.24	112.12	27.30	6.34
90	57.41	59.57	95.21	118.24	27.90	8.24
91	48.21	48.84	89.96	117.65	28.90	7.15
92	55.14	59.17	99.00	116.48	27.90	8.29
93	55.18	57.19	99.00	116.34	27.90	7.64
94	60.18	48.24	90.00	117.28	27.30	8.26
95	47.54	58.26	93.17	91.24	27.30	8.32
96	54.27	48.84	99.00	100.58	27.48	7.45
97	49.26	59.24	90.00	110.54	27.90	8.21
98	44.31	55.34	95.21	122.06	27.30	7.26
99	50.18	57.85	98.00	101.65	27.90	8.95
100	48.29	86.34	86.34	122.06	27.30	7.32

101	53.17	59.31	94.16	122.06	28.49	8.59
102	59.18	106.11	84.26	110.59	27.18	8.65
103	57.48	86.34	98.00	122.06	27.48	7.12
104	58.26	59.15	82.17	122.06	27.30	8.41
105	54.17	49.12	95.26	122.06	28.63	8.26
106	55.29	67.24	81.26	110.48	27.90	7.56
107	59.14	64.18	100.00	107.48	28.90	7.21
108	32.14	48.84	99.00	101.59	28.00	6.32
109	34.28	48.84	85.61	101.48	28.30	8.45
110	60.18	55.29	98.00	110.67	27.30	7.21
111	33.48	57.48	98.00	120.55	27.30	10.65
112	55.26	55.19	85.34	120.55	28.65	10.35
113	35.19	59.34	99.00	109.48	28.15	10.01
114	38.17	48.26	88.17	108.24	28.00	8.21
115	37.14	47.12	98.00	120.55	28.59	7.48
116	59.18	49.26	100.00	120.55	27.90	8.32
117	57.14	55.34	85.34	122.46	27.90	6.29
118	35.24	55.28	84.06	122.55	27.19	8.15
119	35.18	55.17	101.00	122.54	27.30	8.47
120	59.24	80.26	98.00	122.54	27.30	7.65
121	36.18	82.15	84.27	108.34	27.30	8.26
122	34.25	88.04	87.16	122.65	27.90	8.32
123	57.85	90.01	100.00	122.54	28.96	7.14
124	77.71	108.01	100.00	139.64	27.34	8.26
125	58.49	100.50	80.64	140.28	29.30	8.95
126	34.25	98.02	98.58	139.64	29.30	7.45

127	54.15	99.15	87.26	136.48	27.69	8.65
128	40.15	89.15	98.00	130.24	27.46	6.32
129	56.23	88.15	90.48	111.25	27.30	6.14
130	54.18	99.64	98.95	111.25	27.00	8.24
131	55.14	94.12	94.24	124.01	27.48	8.59
132	38.47	96.17	90.18	98.59	28.00	8.34
133	63.12	97.48	89.00	129.64	27.30	8.65
134	65.48	94.25	84.26	111.25	27.48	7.54
135	35.64	96.18	87.24	111.25	27.16	7.48
136	61.27	99.75	90.18	111.25	28.00	8.20
137	37.41	86.12	80.64	124.89	27.85	8.14
138	57.15	88.16	85.13	125.64	27.30	9.01
139	58.14	84.27	97.00	124.98	27.41	7.14
140	36.12	95.38	85.75	128.36	29.56	6.32
141	51.54	97.14	88.26	111.25	28.00	8.56
142	60.06	92.15	80.47	124.86	27.30	8.21
143	34.21	85.26	97.00	126.34	27.89	8.45
144	34.15	84.36	86.34	123.58	27.30	7.26
145	60.18	96.15	89.00	129.68	27.48	8.05
146	50.24	91.24	94.14	123.54	29.56	8.19
147	68.27	82.16	97.00	124.36	27.68	7.18
148	65.39	94.27	84.12	126.34	27.55	8.69
149	37.15	87.14	94.27	126.47	27.48	8.34
150	66.24	93.21	84.17	124.21	28.00	8.26
151	38.17	99.01	82.49	128.15	27.48	7.89
152	64.28	97.21	89.00	111.25	27.59	6.34

153	66.19	86.12	99.00	129.34	27.48	8.26
154	65.48	115.04	86.34	133.48	28.30	7.24
155	34.12	117.15	94.18	131.05	27.30	8.31
156	65.98	112.06	100.00	137.89	28.30	8.62
157	32.14	116.07	85.17	138.49	27.30	7.45
158	68.47	119.05	97.00	98.95	27.10	8.21
159	63.14	120.48	89.00	135.18	28.00	8.08
160	33.59	109.08	89.46	136.48	27.00	7.46
161	64.27	108.56	97.00	134.18	28.30	6.31
162	34.18	108.21	97.00	132.15	27.48	8.26
163	33.64	107.24	84.26	131.68	29.59	7.29
164	35.24	119.02	89.00	131.48	27.90	8.35
165	66.48	114.59	94.15	137.49	27.10	7.15
166	37.14	115.24	82.24	98.95	28.00	8.24
167	37.48	130.42	89.00	118.18	27.68	8.26
168	54.28	130.42	84.65	115.04	27.60	7.45
169	38.14	78.45	98.00	114.69	27.48	8.15
170	70.49	65.02	99.00	112.94	28.30	8.26
171	52.18	67.41	89.34	116.34	27.00	8.39
172	68.27	86.34	97.04	114.85	27.48	6.31
173	39.95	48.84	97.00	115.42	27.90	9.48
174	56.34	65.12	82.36	117.18	27.46	8.26
175	66.89	66.23	101.47	114.29	27.98	8.14
176	35.15	79.34	97.00	120.78	28.00	8.05
177	58.24	80.15	85.24	119.48	27.48	8.48
178	66.28	60.47	89.00	112.48	27.60	8.69

179	37.45	64.28	98.00	111.64	27.30	8.75
180	36.15	109.24	87.15	134.87	27.90	7.45
181	37.58	105.49	95.64	135.48	27.48	8.21
182	38.40	66.37	89.00	123.45	27.30	8.26
183	58.96	69.48	86.31	122.19	27.60	7.45
184	34.12	69.74	97.00	121.02	27.90	8.21
185	31.45	80.15	89.14	121.48	27.41	9.62
186	57.48	60.24	94.26	122.48	28.00	8.14
187	55.24	63.18	101.34	123.98	27.90	7.26
188	35.24	64.28	85.26	125.48	27.10	8.32
189	55.18	48.84	84.34	126.43	27.00	8.15
190	34.27	55.02	97.00	111.25	27.30	8.26
191	65.18	66.35	89.00	124.85	27.89	7.45
192	64.87	130.42	82.19	121.49	27.30	8.26
193	58.24	68.21	95.47	122.64	27.30	8.59
194	59.12	55.02	80.67	122.48	28.00	7.41
195	69.40	61.29	94.18	123.84	27.30	7.26
196	68.32	55.02	84.27	124.75	27.60	8.26
197	66.18	72.15	92.35	125.94	27.60	9.31
198	64.98	86.34	81.10	126.34	27.64	8.29
199	63.18	130.42	98.00	127.48	27.60	8.54
200	64.27	79.14	87.94	128.95	27.49	7.56
201	63.48	80.28	89.00	129.48	27.84	8.89
202	65.24	86.34	97.00	129.64	27.60	8.54
203	65.28	73.50	86.31	127.48	27.60	7.25
204	58.05	72.45	95.48	128.56	27.49	7.48

205	66.21	71.25	85.64	129.34	27.48	9.32
206	67.15	64.28	94.28	125.48	26.90	8.15
207	33.58	55.02	89.67	126.34	27.60	7.24
208	55.84	55.02	99.25	124.20	27.30	8.36
209	64.18	69.24	91.25	126.48	27.70	7.18
210	54.28	69.12	82.48	125.64	27.90	8.24
211	60.24	70.18	100.00	128.69	29.30	7.69
212	58.24	71.24	94.12	127.45	27.48	8.26
213	65.18	62.58	87.29	123.48	27.70	9.31
214	61.12	64.02	89.00	124.51	26.90	7.48
215	64.28	55.02	88.37	128.74	27.90	8.26
216	62.18	70.28	94.12	129.64	27.70	8.06
217	55.14	72.45	95.69	121.48	27.59	8.34
218	64.24	73.18	91.27	122.05	27.41	7.15
219	54.18	69.54	89.24	124.87	27.60	7.69
220	63.89	80.04	97.00	126.48	28.00	7.48
221	55.24	48.84	94.15	128.47	27.45	7.26
222	37.15	62.34	84.17	101.64	26.90	9.34
223	56.24	63.18	98.95	103.49	27.89	7.05
224	64.41	55.02	92.34	124.75	27.45	7.69
225	57.28	77.28	85.16	125.49	27.70	8.16
226	58.14	78.09	94.27	117.64	27.48	7.45
227	68.15	79.24	99.26	98.46	27.60	7.89
228	65.24	74.59	84.27	115.24	27.43	8.69
229	34.25	75.28	94.16	114.05	28.30	8.15
230	67.48	72.15	87.45	124.64	27.60	7.24

231	63.24	73.64	99.98	124.87	28.34	8.15
232	58.59	70.18	85.26	127.49	27.45	8.24
233	65.18	71.29	95.65	128.54	27.70	7.59
234	66.18	61.24	86.39	101.46	27.49	9.32
235	44.57	63.15	92.18	109.61	27.70	8.15
236	39.15	64.12	91.24	107.24	28.05	7.24
237	50.18	62.89	98.24	107.45	28.30	7.28
238	55.24	55.02	82.17	109.64	27.70	8.64
239	54.21	65.24	95.68	105.24	26.90	7.26
240	55.28	55.02	94.12	104.67	28.05	8.35
241	55.17	94.21	83.48	104.29	26.90	7.48
242	45.02	68.02	98.65	106.34	27.98	8.26
243	47.12	60.14	83.15	104.64	26.09	9.31
244	44.18	61.27	81.24	103.65	27.70	7.42
245	47.05	64.18	98.15	102.49	26.05	8.34
246	44.28	55.02	92.34	103.65	27.65	8.26
247	47.19	69.34	93.18	104.78	28.31	7.35
248	56.24	70.08	95.14	104.56	27.48	8.45
249	57.48	72.29	94.37	105.12	28.69	8.32
250	49.58	74.16	89.24	106.34	27.45	7.15
251	50.01	76.84	94.16	108.59	26.90	7.48
252	58.28	74.15	91.27	107.45	27.48	7.59
253	48.17	75.58	95.36	104.64	27.65	7.54
254	43.15	77.59	100.00	105.98	28.30	7.21
255	52.18	88.26	92.58	109.84	26.15	7.59
256	59.12	78.24	99.00	104.67	27.48	9.65

257	48.26	77.24	93.85	104.64	26.90	7.26
258	47.18	77.15	94.27	105.98	27.70	8.54
259	44.28	55.02	95.48	106.48	28.65	7.21
260	49.02	66.34	91.25	107.89	27.70	8.63
261	50.64	61.27	95.26	107.45	27.70	7.42
262	40.58	61.38	95.16	102.94	27.70	8.16
263	53.16	62.34	94.21	101.48	26.90	7.24
264	42.18	63.38	92.37	102.64	27.45	8.32
265	53.17	80.14	100.00	103.74	27.70	7.46
266	54.28	63.19	94.12	101.05	28.15	9.31
267	45.18	80.45	101.00	105.64	28.14	7.49
268	46.18	80.17	95.38	105.48	27.59	8.26
269	43.15	79.26	93.14	104.94	26.90	8.34
270	60.17	55.02	101.00	106.34	27.45	8.21
271	46.48	79.15	100.00	102.64	27.59	7.48
272	58.57	65.29	94.27	104.21	27.63	8.62
273	58.24	69.34	100.00	104.69	28.58	8.24
274	55.19	64.05	92.15	102.34	28.51	7.26
275	46.15	67.23	94.18	102.89	28.36	8.24
276	45.28	69.12	101.00	117.56	27.89	8.48
277	47.16	68.45	91.24	115.64	26.90	7.45
278	55.28	61.27	95.26	114.24	27.70	9.65
279	52.18	62.35	90.48	114.89	27.46	9.47
280	45.67	63.98	90.02	113.64	27.49	7.65
281	44.25	65.24	94.08	114.79	26.38	7.25
282	48.21	75.89	100.00	115.24	27.70	7.45

283	54.15	72.15	101.00	118.79	27.94	7.32
284	49.58	71.24	80.14	114.26	28.16	7.24
285	43.18	73.59	95.28	116.47	27.48	8.26
286	40.17	74.18	96.34	118.94	26.90	8.34
287	55.18	75.28	94.28	119.34	28.56	7.15
288	56.29	68.95	80.15	114.27	27.41	8.26
289	42.17	69.34	94.28	112.49	26.31	9.34
290	47.26	70.14	100.00	114.27	27.49	7.26
291	47.18	74.26	94.26	113.46	27.70	8.34
292	42.26	75.28	92.15	111.24	28.05	8.15
293	52.18	72.36	100.00	111.04	27.70	7.06
294	47.59	79.15	91.24	112.89	27.70	8.02
295	55.26	80.24	94.28	118.64	26.90	8.34
296	53.19	71.28	100.00	117.26	27.70	7.16
297	48.27	74.29	100.00	118.06	27.58	8.29
298	56.19	64.28	92.58	114.02	28.06	8.34
299	44.09	62.15	101.00	117.46	27.70	7.05
300	50.16	65.34	100.00	119.54	27.59	9.04

Zn: Zinc concentration (ppm); **Fe**: Iron concentration (ppm); **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant

Appendix 2: Morphological data for the cross GR-11XPankhali-203

Sr. No.	NFGP	TWT (g)	GY (g)	GL (cm)	GB (cm)	L:BR
1	181.24	13.25	15.26	6.03	2.59	2.33
2	182.36	13.65	15.48	8.04	2.64	3.05
3	182.90	13.92	19.64	8.04	2.87	2.80
4	182.85	16.38	19.92	6.18	2.68	2.31
5	182.48	16.90	15.26	6.15	2.54	2.42
6	182.06	16.34	15.48	7.89	2.89	2.73
7	185.24	16.90	19.34	8.04	2.89	2.78
8	182.90	17.28	19.92	8.25	2.59	3.19
9	182.94	16.34	19.78	6.60	2.47	2.67
10	180.02	16.21	15.63	6.65	2.58	2.58
11	185.59	13.93	15.24	10.28	2.89	3.56
12	185.35	13.06	15.28	8.29	2.28	3.64
13	182.00	16.38	19.92	8.34	2.05	4.07
14	185.12	16.21	15.24	8.91	2.89	3.08
15	185.23	14.06	19.05	8.24	2.04	4.04
16	185.47	17.89	19.34	10.45	2.59	4.03
17	182.96	17.89	15.62	7.89	2.68	2.94
18	185.34	16.90	19.34	8.24	2.57	3.21
19	182.96	15.60	15.68	8.02	2.77	2.90
20	185.48	16.34	19.34	8.06	2.98	2.70
21	182.91	14.02	15.02	6.60	2.89	2.28
22	180.45	14.90	19.67	7.89	2.01	3.93
23	180.00	15.91	15.28	7.89	2.89	2.73

24	182.90	14.06	19.92	8.64	2.48	3.48
25	180.69	14.05	15.64	8.97	2.47	3.63
26	182.92	13.28	19.34	8.64	2.59	3.34
27	180.54	16.90	15.24	8.34	2.65	3.15
28	182.05	16.34	16.39	6.01	2.77	2.17
29	180.24	16.21	19.92	8.26	2.98	2.77
30	182.90	15.56	16.34	6.34	2.89	2.19
31	180.45	15.56	19.92	8.29	3.69	2.25
32	185.65	16.34	16.34	7.89	2.98	2.65
33	182.05	17.02	19.25	6.60	2.57	2.57
34	182.95	14.90	16.20	7.89	2.59	3.05
35	180.65	16.21	16.27	8.24	2.69	3.06
36	182.95	16.34	16.34	6.60	2.56	2.58
37	180.24	16.21	19.92	8.34	2.48	3.36
38	180.34	16.38	16.78	7.89	2.89	2.73
39	182.91	13.93	19.25	6.98	2.89	2.42
40	181.90	16.90	16.02	6.34	2.34	2.71
41	182.94	15.62	16.58	8.25	2.58	3.20
42	181.56	15.56	16.07	8.67	2.47	3.51
43	182.91	16.34	16.48	6.34	2.49	2.55
44	181.48	13.05	20.02	8.26	2.57	3.21
45	182.37	15.56	20.58	8.91	2.28	3.91
46	181.48	17.24	20.48	6.38	2.67	2.39
47	182.94	16.21	19.05	8.02	2.54	3.16
48	182.91	15.56	19.06	8.60	2.58	3.33
49	181.56	16.91	20.14	6.38	2.34	2.73

50	182.91	14.57	19.92	8.26	2.77	2.98
51	182.19	16.21	20.54	6.34	2.50	2.54
52	182.94	15.56	19.92	8.94	2.68	3.34
53	182.19	16.34	19.64	6.37	2.89	2.20
54	182.95	15.56	20.59	10.25	2.98	3.44
55	182.34	16.34	19.92	6.60	2.89	2.28
56	182.19	16.21	20.54	8.34	2.64	3.16
57	181.57	16.90	20.65	9.23	2.89	3.19
58	182.19	16.34	20.48	8.36	2.54	3.29
59	181.56	16.21	20.68	6.60	2.89	2.28
60	182.19	16.02	19.92	10.25	2.77	3.70
61	182.19	15.23	16.38	8.24	2.89	2.85
62	181.00	16.90	19.92	6.60	2.89	2.28
63	182.00	13.02	16.37	6.15	2.47	2.49
64	182.00	14.90	16.05	10.46	3.05	3.43
65	181.04	15.64	16.38	8.24	3.06	2.69
66	182.19	16.35	19.64	10.36	2.77	3.74
67	181.90	15.56	16.35	8.54	2.77	3.08
68	182.19	16.35	19.92	8.92	2.28	3.91
69	181.56	15.56	16.32	8.61	3.75	2.30
70	182.19	16.90	19.92	8.54	2.05	4.17
71	182.69	16.34	16.34	10.05	2.98	3.37
72	183.25	16.21	19.27	8.78	3.75	2.34
73	182.56	14.90	16.48	8.59	2.48	3.46
74	182.48	16.90	16.28	8.42	2.65	3.18
75	182.64	16.91	16.28	10.26	2.89	3.55

76	183.24	15.56	20.48	8.54	2.75	3.11
77	182.47	16.90	19.65	8.69	2.48	3.50
78	182.65	15.64	20.48	8.16	2.35	3.47
79	182.48	15.23	20.56	8.47	2.48	3.42
80	182.19	14.90	19.92	10.26	2.54	4.04
81	182.20	16.21	20.48	8.34	2.23	3.74
82	182.19	16.90	19.65	8.29	2.32	3.57
83	182.47	18.65	20.48	8.48	2.28	3.72
84	182.56	16.88	18.90	8.37	2.26	3.70
85	182.05	15.23	19.92	10.26	2.35	4.37
86	181.90	13.35	19.92	8.64	2.54	3.40
87	182.45	14.53	18.90	8.34	2.77	3.01
88	182.65	15.46	19.62	6.35	2.28	2.79
89	182.19	15.23	18.90	8.16	2.98	2.74
90	182.47	16.90	20.23	10.94	3.75	2.92
91	182.19	16.35	19.92	8.26	2.68	3.08
92	181.24	18.24	20.23	8.75	2.54	3.44
93	182.19	16.35	19.34	8.19	2.28	3.59
94	182.19	16.88	19.36	8.69	2.57	3.38
95	181.47	13.24	20.23	8.34	2.34	3.56
96	182.90	16.92	19.92	8.26	2.28	3.62
97	182.90	16.12	19.35	10.48	2.29	4.58
98	181.24	17.12	18.90	10.67	2.64	4.04
99	182.90	15.56	19.05	10.49	2.28	4.60
100	182.90	15.56	20.23	8.97	2.48	3.62
101	181.47	18.93	19.64	10.68	2.05	5.21

102	182.90	18.52	18.90	10.49	2.50	4.20
103	181.47	16.34	19.25	10.64	2.28	4.67
104	182.34	14.53	19.02	8.35	2.31	3.61
105	182.91	16.90	17.05	8.29	2.35	3.53
106	181.27	15.68	18.90	8.47	2.28	3.71
107	182.91	15.23	18.90	8.31	3.54	2.35
108	182.94	16.38	19.65	8.26	3.24	2.55
109	185.47	16.21	20.23	10.64	2.77	3.84
110	182.91	15.56	19.35	6.64	2.77	2.40
111	182.65	16.35	17.05	8.27	2.05	4.03
112	181.27	16.21	19.64	8.69	2.28	3.81
113	182.05	14.53	18.90	10.64	2.54	4.19
114	183.55	18.59	19.21	8.26	2.35	3.51
115	182.36	18.64	17.89	8.34	2.48	3.36
116	182.34	16.90	19.21	8.19	2.47	3.32
117	183.55	16.22	20.23	8.74	2.59	3.37
118	182.35	15.56	19.02	9.25	2.65	3.49
119	182.36	16.32	19.92	9.64	2.54	3.80
120	182.91	16.22	18.90	8.12	2.38	3.41
121	182.91	14.05	19.31	9.02	2.69	3.35
122	184.65	17.24	17.45	9.14	2.67	3.42
123	182.59	13.54	19.21	10.48	2.28	4.60
124	182.47	16.88	19.65	8.06	2.57	3.14
125	182.59	16.92	18.90	8.19	2.48	3.30
126	184.59	15.23	19.92	8.47	2.48	3.42
127	182.34	18.54	17.20	8.59	2.59	3.32

128	182.47	16.32	18.90	9.65	2.28	4.23
129	184.67	16.21	19.65	8.24	2.63	3.13
130	182.35	15.56	19.92	8.62	2.34	3.68
131	182.47	18.26	17.25	9.34	2.77	3.37
132	183.26	16.34	19.34	8.16	2.77	2.95
133	182.65	13.24	19.27	9.31	2.77	3.36
134	182.47	16.90	17.48	9.25	2.51	3.69
135	183.24	18.59	19.34	8.64	2.34	3.69
136	182.02	16.32	17.24	9.02	2.75	3.28
137	184.57	15.91	19.35	8.64	2.35	3.68
138	182.02	15.23	17.02	9.12	2.89	3.16
139	184.65	18.65	19.04	8.14	2.64	3.08
140	182.34	16.34	17.56	8.64	2.77	3.12
141	182.91	16.90	19.35	9.20	2.77	3.32
142	183.25	16.32	17.02	8.63	2.36	3.66
143	184.05	15.91	19.92	9.01	2.47	3.65
144	183.55	13.21	17.05	9.45	2.28	4.14
145	183.55	16.01	19.65	9.87	2.47	4.00
146	182.16	17.02	17.45	8.65	2.51	3.45
147	183.24	16.35	19.35	9.24	2.61	3.54
148	182.90	15.69	19.02	8.16	2.34	3.49
149	182.90	16.92	18.90	9.24	2.58	3.58
150	182.91	15.64	19.65	8.59	2.47	3.48
151	185.94	18.29	19.92	8.64	2.69	3.21
152	182.97	16.34	17.20	9.34	2.34	3.99
153	182.34	15.91	19.24	8.24	2.28	3.61

154	184.00	16.34	17.45	8.15	2.50	3.26
155	182.00	16.94	19.35	9.64	3.21	3.00
156	184.00	15.91	17.02	8.26	3.65	2.26
157	182.91	17.24	19.64	8.34	2.98	2.80
158	182.94	17.95	18.90	9.14	2.77	3.30
159	182.91	14.89	19.62	8.26	2.28	3.62
160	182.34	16.35	17.45	8.05	2.48	3.25
161	182.65	14.89	19.24	9.34	2.68	3.49
162	183.55	16.34	19.20	8.61	2.59	3.32
163	182.90	15.91	19.92	9.34	2.28	4.10
164	182.18	14.05	19.68	8.25	2.28	3.62
165	184.27	17.26	17.24	9.04	2.65	3.41
166	182.15	16.34	19.92	8.26	2.77	2.98
167	184.27	16.02	19.34	9.34	2.17	4.30
168	182.29	15.91	17.05	8.14	2.48	3.28
169	184.05	15.56	18.90	8.16	2.36	3.46
170	182.05	16.34	19.34	9.45	2.28	4.14
171	184.27	17.25	17.41	8.02	2.48	3.23
172	182.60	15.56	19.42	8.16	3.05	2.68
173	182.31	15.91	19.92	9.02	3.04	2.97
174	184.05	15.56	18.90	8.45	3.28	2.58
175	182.47	17.24	19.21	8.16	2.65	3.08
176	182.06	15.91	19.62	9.14	2.17	4.21
177	182.34	14.89	18.90	8.26	2.18	3.79
178	184.27	16.34	19.24	9.01	2.64	3.41
179	182.05	17.48	17.64	9.42	2.48	3.80

180	183.26	16.34	19.28	8.16	3.56	2.29
181	182.34	15.91	19.34	9.34	2.77	3.37
182	183.23	14.40	17.24	8.16	3.40	2.40
183	182.14	16.34	19.28	8.26	2.15	3.84
184	183.24	16.91	19.48	9.47	2.68	3.53
185	182.05	17.24	18.05	8.16	2.53	3.23
186	182.47	17.69	19.64	9.24	2.52	3.67
187	182.36	16.32	18.90	8.60	2.22	3.87
188	182.41	15.56	19.63	8.92	2.77	3.22
189	182.90	16.20	18.02	9.34	3.24	2.88
190	184.57	15.91	19.64	8.06	2.35	3.43
191	183.24	17.48	18.90	8.65	2.15	4.02
192	182.15	17.24	19.68	9.31	2.36	3.94
193	184.26	16.34	18.02	8.26	2.56	3.23
194	182.34	15.91	19.64	9.34	2.14	4.36
195	184.57	13.93	18.05	8.06	2.31	3.49
196	183.26	17.45	19.64	8.26	2.54	3.25
197	182.57	16.32	18.05	8.94	3.15	2.84
198	182.59	15.91	18.06	9.34	2.77	3.37
199	183.90	14.98	18.05	7.89	3.58	2.20
200	182.54	16.35	19.64	8.16	2.64	3.09
201	182.02	15.91	18.90	7.89	2.89	2.73
202	183.90	17.48	19.92	8.24	2.77	2.97
203	183.02	17.48	18.06	8.06	3.24	2.49
204	183.90	16.34	19.92	8.24	2.49	3.31
205	183.90	15.91	18.90	7.89	2.37	3.33

206	182.64	16.34	19.25	8.45	2.77	3.05
207	183.05	13.93	18.11	8.29	3.64	2.28
208	183.90	16.38	18.59	8.34	2.75	3.03
209	183.04	13.93	18.47	7.15	2.64	2.71
210	182.49	15.91	19.65	8.02	2.77	2.90
211	182.64	13.93	19.34	8.46	2.75	3.08
212	183.90	15.68	18.54	8.14	2.69	3.03
213	182.34	17.24	19.20	7.89	2.34	3.37
214	182.75	16.88	18.90	7.14	2.77	2.58
215	183.90	14.89	19.57	7.00	3.48	2.01
216	182.45	16.88	18.65	8.34	3.24	2.57
217	182.06	16.88	19.24	8.04	2.68	3.00
218	183.90	15.23	20.23	8.59	2.54	3.38
219	182.47	17.02	19.65	7.41	2.77	2.68
220	183.90	16.38	20.23	7.02	2.68	2.62
221	182.45	15.23	20.23	7.89	2.64	2.99
222	183.09	16.32	18.05	8.24	2.84	2.90
223	182.59	15.05	20.23	8.69	2.75	3.16
224	183.90	15.56	19.65	8.84	2.61	3.39
225	183.00	15.59	19.48	8.79	3.28	2.68
226	183.90	17.24	18.05	8.59	3.49	2.46
227	182.56	17.42	19.64	8.59	2.77	3.10
228	183.04	15.91	20.23	7.54	2.68	2.81
229	182.45	15.34	19.34	8.62	2.16	3.99
230	183.04	15.23	19.87	8.16	2.34	3.49
231	182.69	15.02	20.23	7.45	2.18	3.42

232	183.04	18.94	20.23	8.39	2.35	3.57
233	183.04	16.34	19.37	8.90	2.14	4.16
234	183.05	15.91	19.05	7.48	2.57	2.91
235	182.69	15.23	20.23	8.26	2.16	3.82
236	183.90	18.26	19.34	8.14	2.30	3.54
237	182.47	15.91	20.23	8.27	2.77	2.99
238	183.90	17.15	19.78	7.46	3.48	2.14
239	182.00	17.26	18.05	7.89	3.47	2.27
240	183.00	15.91	19.60	8.29	3.60	2.30
241	181.90	16.34	18.90	7.89	2.35	3.36
242	182.64	15.91	20.23	8.29	2.36	3.51
243	183.90	16.32	20.23	8.34	2.14	3.90
244	181.90	15.91	19.63	7.05	2.98	2.37
245	183.47	18.54	20.23	8.02	3.65	2.20
246	183.25	15.91	19.64	8.36	2.34	3.57
247	183.64	17.48	19.25	8.14	3.15	2.58
248	183.00	16.32	20.23	7.89	2.31	3.42
249	183.00	15.91	19.64	8.27	2.65	3.12
250	182.00	15.62	20.23	7.05	3.15	2.24
251	182.00	15.91	19.64	7.08	2.15	3.29
252	183.48	18.05	20.23	8.69	3.14	2.77
253	181.90	16.34	19.34	8.14	2.35	3.46
254	183.65	15.91	18.54	8.24	3.65	2.26
255	182.47	16.38	19.67	7.59	3.15	2.41
256	183.65	15.91	19.24	8.26	3.48	2.37
257	181.90	17.69	18.87	8.34	2.68	3.11

258	183.47	18.64	18.08	7.89	2.47	3.19
259	181.90	15.91	18.88	8.88	2.61	3.40
260	182.95	16.34	19.60	8.24	3.08	2.68
261	183.64	17.45	19.34	7.65	3.01	2.54
262	181.90	15.91	19.92	8.16	2.75	2.97
263	183.24	16.34	20.23	7.89	2.48	3.18
264	181.90	15.91	19.35	7.29	2.00	3.65
265	183.48	16.34	20.23	8.35	2.63	3.17
266	183.24	15.29	19.32	8.48	2.01	4.22
267	183.47	15.34	19.65	7.26	2.45	2.96
268	182.34	17.58	20.23	8.35	2.61	3.20
269	181.90	17.62	19.65	7.29	2.85	2.56
270	183.24	18.23	19.34	8.34	2.69	3.10
271	183.04	17.48	19.25	8.69	2.34	3.71
272	183.17	16.35	19.34	7.48	2.15	3.48
273	183.04	15.28	19.57	7.21	3.16	2.28
274	183.59	16.25	18.59	7.59	2.48	3.06
275	183.14	15.34	19.64	8.59	2.95	2.91
276	183.24	16.35	18.57	8.54	2.61	3.27
277	183.48	15.28	19.64	8.21	2.04	4.02
278	181.90	16.37	19.35	7.49	3.15	2.38
279	181.90	16.21	20.23	8.32	3.47	2.40
280	181.90	16.34	19.35	8.47	2.05	4.13
281	182.57	16.28	20.23	7.29	2.64	2.76
282	183.24	15.68	19.63	8.59	2.01	4.27
283	183.05	16.32	20.23	8.79	2.45	3.59

284	183.47	16.88	19.34	7.59	2.89	2.63
285	182.00	16.88	20.23	8.64	2.97	2.91
286	182.00	16.21	19.34	7.28	2.64	2.76
287	183.64	16.38	20.23	8.15	3.15	2.59
288	183.57	16.24	19.34	7.24	3.02	2.40
289	181.90	16.32	20.23	8.69	3.48	2.50
290	183.26	15.48	19.64	8.49	2.57	3.30
291	183.24	16.35	20.23	8.34	2.04	4.09
292	183.59	16.88	19.64	8.15	3.19	2.55
293	182.54	16.21	19.21	8.27	2.47	3.35
294	182.14	16.34	18.54	8.54	3.56	2.40
295	183.26	16.28	19.63	8.64	3.05	2.83
296	183.00	16.21	18.56	8.27	2.47	3.35
297	182.00	16.34	19.32	8.06	2.64	3.05
298	181.90	16.29	20.23	8.04	3.15	2.55
299	183.02	16.21	17.40	8.75	3.48	2.51
300	183.47	16.34	19.30	8.94	2.01	4.45

NFGP: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:BR**: Length: Breadth Ratio

Appendix 3: Morphological data for the cross GR-11XGurjari

Sr. No.	Zn (ppm)	Fe (ppm)	DFT	PH (cm)	PL (cm)	NEFT
1	54.57	74.57	120.14	122.71	21.21	5.24
2	64.12	80.14	81.14	101.15	25.47	7.74
3	54.57	79.15	82.45	120.45	23.86	7.14
4	62.12	86.70	87.14	120.60	24.78	8.54
5	65.21	80.14	84.25	106.32	24.15	8.02
6	54.57	86.79	92.41	114.34	23.64	7.21
7	54.57	80.14	120.32	117.20	23.86	6.73
8	63.15	79.21	115.41	111.04	25.47	7.48
9	65.24	86.79	86.24	103.13	24.15	7.54
10	38.21	80.14	82.14	109.64	24.16	6.92
11	29.34	80.14	90.48	105.27	21.06	7.48
12	54.57	99.15	90.12	125.34	24.57	7.65
13	70.50	79.14	82.47	103.13	24.02	6.93
14	67.48	77.02	89.12	117.20	24.57	7.74
15	54.57	86.79	88.24	103.13	24.78	6.92
16	37.48	73.51	87.15	103.13	24.15	6.73
17	69.32	86.79	84.12	103.13	24.35	8.62
18	32.58	55.21	117.45	111.66	23.86	5.12
19	52.79	75.48	92.41	117.20	24.48	7.89
20	60.21	74.57	82.01	117.20	24.18	8.62
21	58.48	75.14	84.17	119.63	23.48	7.14
22	56.25	70.64	83.26	117.20	23.86	6.24
23	42.15	79.21	89.21	117.20	23.23	7.48

24	55.24	75.24	84.15	113.02	24.16	7.74
25	57.02	88.21	92.41	112.21	24.48	7.24
26	57.45	77.15	87.14	114.59	23.23	7.48
27	58.26	69.21	118.02	102.81	24.78	7.78
28	56.25	68.24	99.14	117.20	24.56	5.21
29	56.32	80.32	84.15	117.48	24.15	6.93
30	53.12	75.14	100.14	119.64	24.78	7.48
31	56.25	80.14	100.02	106.32	25.63	7.59
32	55.57	79.51	95.64	115.24	23.86	6.92
33	54.23	77.12	98.17	114.48	23.48	6.73
34	88.21	73.05	95.12	114.34	24.02	7.24
35	58.47	80.14	97.14	108.45	24.17	7.89
36	56.25	76.12	111.04	117.20	23.45	7.24
37	57.42	74.57	96.31	117.24	22.53	7.12
38	58.12	84.15	92.14	119.02	24.58	7.58
39	54.78	61.12	91.78	112.04	24.17	7.25
40	52.16	60.14	93.14	114.05	24.59	7.48
41	51.24	71.26	88.26	114.34	23.23	6.92
42	85.26	99.12	95.14	129.65	25.14	7.15
43	53.02	79.14	94.21	117.20	25.64	5.02
44	75.48	89.21	87.14	112.03	24.35	7.74
45	55.62	69.33	116.24	114.34	21.15	7.89
46	56.25	66.23	87.12	113.47	24.78	6.93
47	58.47	89.14	92.41	128.95	22.53	7.45
48	56.32	74.57	95.64	114.02	23.89	7.21
49	58.15	79.12	81.02	102.81	24.15	7.48

50	74.15	80.06	94.15	105.48	24.15	7.26
51	57.21	68.21	82.47	119.64	24.78	7.35
52	52.79	74.57	86.32	117.20	24.61	6.93
53	55.32	79.32	92.14	117.31	22.53	7.79
54	56.25	80.14	96.14	109.31	24.18	7.48
55	62.79	60.12	87.24	114.34	24.78	7.59
56	60.32	79.14	112.05	117.20	24.78	8.21
57	55.14	66.20	94.15	117.14	22.53	7.89
58	76.32	99.12	87.15	108.25	23.23	5.32
59	58.14	46.06	92.41	127.45	23.23	7.74
60	56.25	84.15	95.32	114.34	24.78	7.48
61	47.12	73.12	84.12	117.31	22.53	7.26
62	58.62	71.04	97.65	117.20	24.16	7.48
63	56.25	72.15	95.12	114.32	22.53	7.95
64	48.02	79.14	96.31	114.34	22.53	7.79
65	57.41	77.25	91.24	117.20	23.89	7.15
66	51.46	74.02	92.17	112.47	25.14	7.24
67	52.60	69.32	87.36	117.20	24.48	6.93
68	53.12	69.33	98.64	119.32	23.89	6.54
69	56.32	61.24	114.06	119.47	21.48	5.26
70	56.25	65.32	118.24	117.20	25.78	7.74
71	56.25	75.48	99.31	114.02	24.01	7.48
72	52.06	78.12	92.41	102.81	24.56	7.21
73	44.15	60.14	95.64	114.34	23.89	7.02
74	54.21	79.24	84.21	114.25	22.78	7.48
75	77.14	80.21	98.15	109.26	24.78	7.56

76	56.25	60.48	95.26	112.04	23.89	6.93
77	57.12	78.23	84.17	111.36	24.15	6.54
78	58.32	77.14	94.26	111.04	25.78	7.48
79	56.14	79.35	95.32	113.24	25.48	7.25
80	57.24	80.14	88.21	108.24	24.16	7.48
81	53.02	74.57	102.47	114.59	23.89	7.59
82	42.15	39.14	98.36	111.66	24.15	7.14
83	58.62	65.21	95.21	116.47	23.89	7.41
84	79.21	84.15	95.26	122.71	25.18	7.45
85	44.12	69.33	98.32	119.62	24.78	7.42
86	59.61	79.21	92.60	117.20	23.89	7.89
87	58.21	46.06	90.14	102.81	24.16	6.32
88	51.26	73.14	97.54	114.75	24.35	7.74
89	54.21	78.21	101.48	114.34	25.81	6.32
90	55.23	46.06	95.62	112.04	24.78	5.63
91	55.48	79.14	84.26	113.14	22.16	7.48
92	55.62	71.05	92.41	114.25	21.05	6.32
93	80.14	86.79	95.47	114.34	23.48	8.25
94	52.60	79.12	87.21	117.31	24.17	7.48
95	58.46	77.14	106.34	114.34	23.89	7.74
96	58.23	65.21	96.11	117.84	25.64	8.25
97	48.21	77.14	92.41	115.02	24.18	6.45
98	56.25	76.21	81.47	114.34	23.88	7.24
99	62.79	66.35	98.16	113.48	24.15	7.41
100	53.16	78.21	82.14	112.14	25.18	7.54
101	52.60	84.15	97.64	113.16	24.75	6.54

102	74.12	79.14	87.15	114.47	23.88	7.48
103	48.26	65.21	98.24	114.34	25.48	7.59
104	57.32	80.01	104.65	105.48	23.88	7.62
105	58.02	84.15	99.31	117.20	22.14	7.34
106	52.60	37.95	95.24	111.66	24.78	7.15
107	56.25	74.57	85.47	119.62	23.88	7.48
108	62.79	73.48	96.31	117.42	24.15	6.32
109	57.48	72.05	87.24	110.48	23.48	6.02
110	58.23	79.34	96.31	110.63	21.45	6.40
111	62.79	65.21	104.58	117.20	25.64	8.26
112	55.26	69.33	94.78	120.48	24.78	7.48
113	57.41	73.14	88.26	120.57	23.88	7.01
114	71.01	79.35	87.41	117.31	24.15	7.74
115	40.15	58.21	99.21	111.66	23.88	7.48
116	56.25	75.14	92.60	116.47	23.48	7.12
117	58.03	65.21	85.02	114.52	25.18	7.48
118	44.15	60.41	108.24	117.20	24.01	7.02
119	57.24	72.02	109.64	116.34	23.48	7.25
120	62.79	79.15	95.32	117.48	25.64	8.65
121	53.18	74.57	88.14	117.20	24.75	5.03
122	43.02	72.48	98.26	117.31	23.88	7.48
123	55.16	62.15	97.14	116.34	22.14	7.16
124	62.79	65.31	85.21	117.20	24.78	8.65
125	56.25	75.14	82.16	117.15	23.88	7.48
126	48.21	49.21	81.24	96.34	25.64	7.48
127	57.64	78.12	98.36	119.47	24.15	7.48

128	52.60	48.21	84.57	98.62	21.78	7.65
129	58.23	79.12	92.14	113.04	24.58	7.41
130	58.12	80.14	107.26	108.52	23.88	7.02
131	20.14	61.48	85.34	114.59	25.06	6.28
132	52.60	68.47	93.14	102.81	24.78	7.59
133	56.25	80.59	85.26	114.34	23.88	7.32
134	54.12	79.21	93.64	117.48	24.15	7.74
135	43.65	73.59	85.14	117.20	25.63	5.29
136	42.01	47.12	91.57	97.45	25.14	7.74
137	56.25	79.42	84.21	114.34	25.25	7.45
138	25.16	79.51	85.67	114.87	24.15	6.32
139	46.32	78.15	92.60	117.20	25.78	7.89
140	29.15	80.14	106.54	111.26	24.89	7.41
141	46.32	68.23	92.47	112.48	23.88	7.20
142	30.48	65.24	94.32	116.32	23.23	7.89
143	45.16	43.10	92.60	95.84	24.18	7.74
144	44.78	55.18	94.15	97.56	23.45	7.65
145	22.35	62.14	88.21	114.28	24.78	7.74
146	44.18	69.32	76.38	114.32	25.16	7.41
147	47.16	59.24	95.21	117.31	23.45	8.65
148	48.26	70.14	75.02	116.32	24.17	6.32
149	52.60	58.21	88.64	94.75	23.88	5.98
150	48.26	69.45	95.12	116.48	24.78	7.48
151	28.47	80.12	101.05	117.20	25.68	8.01
152	54.57	84.57	88.27	119.64	25.16	7.48
153	40.52	80.16	82.14	120.48	23.48	7.51

154	46.32	57.12	94.26	98.56	22.15	6.32
155	52.60	91.16	78.34	123.47	24.58	7.48
156	42.15	65.24	92.61	116.25	25.12	7.74
157	41.87	85.47	83.21	116.34	23.53	7.15
158	33.78	63.25	72.06	114.82	21.74	8.34
159	52.79	85.14	105.21	127.49	24.15	5.68
160	29.65	64.47	95.64	114.60	24.75	6.48
161	54.57	52.18	82.34	99.98	25.16	7.02
162	38.15	52.62	77.14	128.56	23.53	7.14
163	37.74	74.57	98.24	117.45	24.78	7.69
164	52.79	68.14	95.26	117.02	23.53	8.35
165	36.95	92.06	81.04	129.34	24.78	8.45
166	27.85	54.32	103.05	97.26	23.54	7.26
167	52.79	78.14	95.64	115.06	25.15	6.35
168	28.05	78.45	82.16	116.34	24.13	7.74
169	47.15	60.29	93.47	114.48	25.16	7.12
170	48.26	79.45	96.31	115.27	23.53	7.48
171	31.45	98.14	72.18	127.49	24.11	7.68
172	33.59	48.26	95.26	117.20	22.15	7.48
173	45.26	75.02	85.34	114.62	25.47	6.03
174	42.15	94.12	94.27	129.34	23.45	7.48
175	52.60	72.48	104.75	117.20	24.15	5.18
176	52.60	70.15	85.24	115.06	25.61	6.28
177	47.15	60.32	87.64	114.34	24.75	7.48
178	48.26	94.15	75.19	113.59	23.53	7.59
179	22.98	96.32	92.31	114.02	25.15	8.68

180	43.65	60.14	89.64	117.20	21.04	7.74
181	37.14	50.28	98.21	116.34	24.48	7.48
182	48.26	46.06	84.26	114.28	22.90	7.15
183	37.18	94.75	105.48	124.59	24.15	7.24
184	46.29	75.26	99.34	112.04	23.53	8.24
185	24.15	75.14	85.26	116.34	22.90	7.02
186	45.68	75.01	94.17	113.48	25.48	7.48
187	42.59	79.26	85.26	114.59	22.01	8.52
188	38.59	99.02	65.34	117.31	22.90	7.48
189	52.79	93.15	76.14	124.75	24.15	8.65
190	54.57	53.26	95.21	114.02	24.78	5.26
191	52.79	78.14	87.63	115.64	22.90	7.48
192	26.48	75.42	66.31	119.34	24.15	7.02
193	52.60	58.26	96.24	117.85	23.01	7.14
194	35.28	59.32	85.16	114.02	25.48	7.48
195	45.78	54.12	91.32	115.64	24.17	7.26
196	41.59	74.18	72.14	116.34	22.94	7.48
197	35.28	83.02	92.60	105.48	24.17	7.32
198	47.18	89.15	68.21	108.24	25.65	7.48
199	23.15	60.47	87.24	114.15	24.18	7.95
200	62.48	65.21	85.21	117.20	22.93	7.24
201	54.57	81.47	92.14	108.75	21.48	7.74
202	48.02	74.57	88.65	114.26	24.57	5.51
203	49.63	82.47	95.17	105.24	22.91	7.48
204	36.47	62.59	96.32	115.48	24.57	7.26
205	50.24	69.32	62.34	117.78	22.15	8.62

206	35.26	84.15	71.05	104.95	22.91	7.40
207	69.85	84.15	94.28	122.71	24.57	7.25
208	70.16	84.15	88.61	119.52	22.97	7.34
209	61.24	58.47	95.32	114.75	24.17	7.48
210	63.58	59.62	84.12	115.26	24.63	8.06
211	45.16	69.48	96.31	115.02	24.59	7.74
212	42.74	85.14	85.47	106.34	25.14	7.47
213	64.12	70.36	91.26	114.78	24.57	7.12
214	40.57	56.34	75.34	111.05	22.01	8.98
215	30.59	89.21	85.12	105.64	24.18	7.58
216	47.05	84.27	63.02	128.42	22.31	7.68
217	34.12	72.18	96.14	114.34	24.59	8.29
218	48.62	89.35	92.60	105.04	24.59	7.14
219	52.79	99.02	91.24	112.76	24.17	7.28
220	54.57	88.47	70.18	129.34	24.59	5.26
221	43.02	77.16	87.21	114.58	24.75	7.74
222	48.56	98.24	85.62	114.34	24.59	7.95
223	54.57	88.34	80.26	105.64	24.59	7.48
224	45.21	95.14	92.34	126.34	23.45	7.56
225	68.12	97.65	88.26	126.34	22.13	7.21
226	49.56	79.23	96.34	115.24	23.45	7.48
227	64.25	95.14	87.12	127.84	24.56	8.21
228	38.12	83.25	94.15	125.49	22.14	7.48
229	43.59	82.14	69.37	114.34	26.57	7.26
230	34.78	88.26	89.24	128.54	24.59	7.42
231	47.12	85.14	92.60	114.20	24.59	7.74

232	64.28	89.32	92.34	127.49	24.78	7.41
233	65.38	79.14	89.12	117.31	24.16	5.06
234	43.12	68.21	78.25	116.34	24.57	7.48
235	66.06	78.24	99.21	117.20	24.59	7.02
236	45.28	72.14	85.16	115.20	26.31	5.98
237	36.98	88.23	68.34	112.48	25.18	7.63
238	36.48	98.14	92.31	114.34	24.89	7.42
239	38.15	91.28	96.57	128.49	24.65	8.02
240	48.26	96.32	94.12	126.34	23.23	7.89
241	69.31	84.55	78.20	119.64	24.17	8.32
242	44.18	94.57	98.62	112.76	24.59	7.14
243	67.42	65.14	95.34	112.64	26.14	8.56
244	35.28	92.54	66.38	128.94	24.13	5.20
245	61.20	86.79	92.14	112.76	24.15	7.48
246	49.35	97.68	93.65	112.76	24.17	7.05
247	44.78	70.62	94.21	114.85	25.62	7.89
248	45.12	75.63	64.28	118.21	23.23	8.62
249	50.16	85.24	99.21	112.64	24.15	7.46
250	50.24	85.14	75.14	112.48	25.06	5.31

Zn: Zinc concentration (ppm); **Fe**: Iron concentration (ppm); **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant

Appendix 4: Morphological data for the cross GR-11XKrishna Kamod

Sr. No.	NFGP	TWT (g)	GY (g)	GL (cm)	GB (cm)	L:BR
1	180.21	14.56	14.50	5.51	2.59	2.13
2	182.46	17.21	18.26	8.60	2.64	3.26
3	182.54	18.24	14.88	7.23	2.87	2.52
4	182.31	17.49	19.26	7.21	2.68	2.69
5	182.15	15.02	18.26	8.26	2.54	3.25
6	182.06	16.91	14.88	8.21	2.89	2.84
7	180.14	18.89	17.15	7.32	2.89	2.53
8	183.95	18.64	14.62	7.24	2.59	2.80
9	182.34	17.48	17.90	7.23	2.47	2.93
10	182.04	16.91	19.23	8.21	2.58	3.18
11	182.54	17.21	14.26	5.63	2.89	1.95
12	183.95	17.90	19.56	8.26	2.28	3.62
13	182.47	16.98	19.23	7.23	2.05	3.53
14	182.02	17.48	19.23	7.21	2.89	2.49
15	182.54	15.20	19.10	7.01	2.04	3.44
16	182.47	18.64	18.26	7.23	2.59	2.79
17	183.95	14.02	19.34	8.02	2.68	2.99
18	182.04	17.21	19.23	5.32	2.57	2.07
19	182.95	17.90	19.10	5.29	2.77	1.91
20	182.47	17.48	17.24	8.32	2.98	2.79
21	183.95	18.50	19.65	8.14	2.89	2.82
22	182.04	17.48	17.94	7.29	2.01	3.63
23	180.65	17.59	17.90	8.05	2.89	2.79

24	182.04	17.90	17.26	8.26	2.48	3.33
25	182.47	17.21	19.23	7.23	2.47	2.93
26	182.59	14.05	17.26	7.23	2.59	2.79
27	184.67	18.45	19.21	5.26	2.65	1.98
28	180.47	18.94	18.56	8.01	2.77	2.89
29	182.04	17.90	14.88	8.32	2.98	2.79
30	182.06	17.48	18.89	8.24	2.89	2.85
31	182.47	17.52	14.02	7.23	3.69	1.96
32	182.64	14.02	18.65	6.70	2.98	2.25
33	182.02	17.90	14.88	5.06	2.57	1.97
34	182.47	16.91	19.23	7.23	2.59	2.79
35	182.65	17.90	14.28	8.17	2.69	3.04
36	184.21	17.21	18.26	7.44	2.56	2.91
37	182.04	18.24	16.88	8.26	2.48	3.33
38	182.47	18.02	18.57	8.21	2.89	2.84
39	180.65	18.27	16.88	8.16	2.89	2.82
40	180.45	17.90	15.62	8.34	2.34	3.56
41	182.47	17.90	16.88	6.70	2.58	2.60
42	180.65	17.48	18.24	7.44	2.47	3.01
43	182.45	17.49	18.92	7.23	2.49	2.90
44	180.65	17.90	15.62	8.32	2.57	3.24
45	184.57	17.90	18.24	6.70	2.28	2.94
46	182.54	17.90	16.88	6.70	2.67	2.51
47	182.34	18.05	18.26	8.26	2.54	3.25
48	182.47	17.21	16.90	8.31	2.58	3.22
49	180.65	17.21	18.21	7.23	2.34	3.09

50	182.14	17.21	16.90	7.23	2.77	2.61
51	182.04	17.78	16.90	7.44	2.50	2.98
52	182.05	17.49	18.34	6.90	2.68	2.57
53	182.57	18.05	15.68	8.26	2.89	2.86
54	182.64	17.21	15.68	8.98	2.98	3.01
55	182.47	17.51	18.00	7.23	2.89	2.50
56	184.57	17.92	18.69	8.12	2.64	3.08
57	182.54	14.02	16.90	8.26	2.89	2.86
58	180.26	16.91	18.54	7.56	2.54	2.98
59	182.02	17.41	18.69	6.90	2.89	2.39
60	182.47	18.51	16.90	8.21	2.77	2.96
61	182.12	18.84	16.90	7.58	2.89	2.62
62	182.64	17.91	18.65	7.48	2.89	2.59
63	182.34	17.91	15.89	5.24	2.47	2.12
64	182.57	16.91	18.64	7.23	3.05	2.37
65	182.04	17.40	16.90	6.90	3.06	2.25
66	182.65	17.21	18.59	8.32	2.77	3.00
67	182.98	17.24	18.64	8.46	2.77	3.05
68	182.47	17.59	16.90	7.23	2.28	3.17
69	184.27	14.62	18.67	7.50	3.75	2.00
70	184.20	16.91	16.90	5.64	2.05	2.75
71	182.05	17.45	18.97	6.90	2.98	2.32
72	182.47	17.21	18.64	7.48	3.75	1.99
73	182.64	17.91	16.90	7.48	2.48	3.02
74	182.95	17.48	18.59	7.50	2.65	2.83
75	182.65	18.64	15.64	6.90	2.89	2.39

76	182.57	17.45	18.34	7.48	2.75	2.72
77	182.47	17.21	15.26	7.59	2.48	3.06
78	182.47	17.05	17.90	7.24	2.35	3.08
79	184.27	18.59	16.05	7.60	2.48	3.06
80	182.95	16.91	16.34	7.50	2.54	2.95
81	184.27	17.24	18.56	6.90	2.23	3.09
82	182.61	18.02	18.24	7.50	2.32	3.23
83	182.32	14.68	17.90	6.90	2.28	3.03
84	182.47	17.45	18.29	7.50	2.26	3.32
85	182.05	15.50	17.90	7.23	2.35	3.08
86	182.06	17.90	18.20	7.23	2.54	2.85
87	182.34	18.02	16.25	7.23	2.77	2.61
88	182.05	17.45	18.98	7.23	2.28	3.17
89	182.95	17.91	18.54	7.23	2.98	2.43
90	184.62	17.02	17.90	7.60	3.75	2.03
91	182.47	16.98	18.52	7.90	2.68	2.95
92	182.06	17.48	17.90	7.50	2.54	2.95
93	182.45	18.02	18.02	7.90	2.28	3.46
94	182.06	15.90	18.54	7.23	2.57	2.81
95	182.00	14.86	16.34	7.50	2.34	3.21
96	184.24	15.90	18.21	7.60	2.28	3.33
97	184.75	15.90	16.23	8.24	2.29	3.60
98	182.45	17.48	18.75	8.26	2.64	3.13
99	182.95	15.90	16.95	7.60	2.28	3.33
100	182.04	18.57	17.90	7.23	2.48	2.92
101	182.47	14.65	18.25	7.50	2.05	3.66

102	182.95	17.48	18.05	7.50	2.50	3.00
103	182.95	17.90	16.34	7.90	2.28	3.46
104	182.75	17.48	18.14	5.62	2.31	2.43
105	182.41	18.01	16.29	7.60	2.35	3.23
106	182.47	17.48	16.84	7.50	2.28	3.29
107	182.59	18.05	16.12	8.98	3.54	2.54
108	182.47	18.02	18.48	7.50	3.24	2.31
109	182.04	18.95	16.95	7.90	2.77	2.85
110	182.95	17.48	16.34	7.25	2.77	2.62
111	183.65	17.07	18.02	7.50	2.05	3.66
112	182.47	15.90	16.02	7.90	2.28	3.46
113	182.47	17.35	17.90	7.50	2.54	2.95
114	182.05	17.21	18.29	7.50	2.35	3.19
115	182.47	15.90	16.21	7.28	2.48	2.94
116	182.04	17.45	18.20	7.64	2.47	3.09
117	182.96	15.90	17.90	7.50	2.59	2.90
118	182.34	15.90	18.87	7.23	2.65	2.73
119	182.78	17.48	17.90	7.98	2.54	3.14
120	182.95	18.29	16.47	7.20	2.38	3.03
121	182.64	17.45	16.42	7.90	2.69	2.94
122	182.54	14.02	16.78	7.90	2.67	2.96
123	182.34	18.98	18.98	7.90	2.28	3.46
124	182.05	17.46	18.02	7.90	2.57	3.07
125	182.47	17.21	18.24	7.23	2.48	2.92
126	182.65	17.21	16.20	7.58	2.48	3.06
127	182.47	17.02	18.48	7.23	2.59	2.79

128	182.04	15.90	18.59	7.44	2.28	3.26
129	183.47	17.48	16.21	7.90	2.63	3.00
130	182.04	15.90	16.34	7.90	2.34	3.38
131	182.95	17.48	14.02	7.90	2.77	2.85
132	182.64	17.45	16.48	7.22	2.77	2.61
133	182.47	18.95	18.24	5.62	2.77	2.03
134	182.74	19.32	16.48	7.22	2.51	2.88
135	182.64	17.04	18.02	7.90	2.34	3.38
136	183.02	15.90	16.34	7.22	2.75	2.63
137	182.04	16.95	16.48	5.68	2.35	2.42
138	182.94	17.98	17.94	5.32	2.89	1.84
139	182.65	17.48	16.34	7.90	2.64	2.99
140	183.04	14.06	16.02	7.90	2.77	2.85
141	182.47	18.49	16.48	7.50	2.77	2.71
142	182.05	18.03	16.04	5.26	2.36	2.23
143	182.15	19.64	18.24	7.90	2.47	3.20
144	183.06	15.02	16.48	7.02	2.28	3.08
145	182.47	17.21	18.27	7.90	2.47	3.20
146	183.04	17.06	17.90	7.60	2.51	3.03
147	183.45	17.21	17.90	7.22	2.61	2.77
148	182.47	16.91	18.24	7.50	2.34	3.21
149	182.04	18.59	18.05	7.90	2.58	3.06
150	182.65	17.48	17.96	7.90	2.47	3.20
151	182.48	15.64	18.65	7.25	2.69	2.70
152	183.47	16.91	17.24	5.68	2.34	2.43
153	183.02	17.48	18.56	7.14	2.28	3.13

154	182.64	16.91	17.24	7.20	2.50	2.88
155	182.05	17.48	18.59	5.47	3.21	1.70
156	183.47	15.02	17.92	7.90	3.65	2.16
157	183.04	18.59	18.20	7.20	2.98	2.42
158	183.59	17.46	17.93	7.44	2.77	2.69
159	183.47	18.54	18.20	5.46	2.28	2.39
160	183.64	17.91	18.79	7.44	2.48	3.00
161	183.47	17.91	18.24	7.22	2.68	2.69
162	181.93	17.89	18.02	5.41	2.59	2.09
163	182.65	15.50	17.90	7.48	2.28	3.28
164	182.47	18.54	18.34	7.44	2.28	3.26
165	182.61	17.21	17.05	7.22	2.65	2.72
166	182.05	17.49	18.64	7.22	2.77	2.61
167	181.93	16.32	17.04	7.22	2.17	3.33
168	182.64	17.48	18.56	7.48	2.48	3.02
169	182.57	15.02	17.90	6.02	2.36	2.55
170	182.60	16.38	18.26	7.22	2.28	3.17
171	181.93	17.48	17.90	7.22	2.48	2.91
172	181.93	18.06	18.45	9.05	3.05	2.97
173	180.91	17.21	17.90	7.22	3.04	2.38
174	180.91	17.48	18.57	7.26	3.28	2.21
175	182.47	17.91	17.91	7.44	2.65	2.81
176	180.91	15.08	18.79	7.90	2.17	3.64
177	181.93	18.98	17.21	7.44	2.18	3.41
178	182.47	16.34	18.79	6.32	2.64	2.39
179	181.93	17.48	17.46	6.48	2.48	2.61

180	182.06	15.68	17.24	7.96	3.56	2.24
181	181.93	17.21	17.89	7.96	2.77	2.87
182	182.65	16.98	18.24	9.87	3.40	2.90
183	181.93	18.94	17.92	6.21	2.15	2.89
184	181.93	16.02	17.02	6.35	2.68	2.37
185	182.65	17.48	17.24	7.48	2.53	2.96
186	182.34	16.32	17.93	7.48	2.52	2.97
187	181.93	17.21	18.24	9.32	2.22	4.20
188	182.65	17.45	17.06	7.90	2.77	2.85
189	181.93	15.02	17.94	9.02	3.24	2.78
190	182.04	18.65	18.56	6.34	2.35	2.70
191	182.54	17.21	17.06	6.25	2.15	2.91
192	181.74	16.57	17.96	5.90	2.36	2.50
193	182.65	17.48	18.24	7.44	2.56	2.91
194	181.45	16.30	17.04	7.34	2.14	3.43
195	182.05	17.21	17.89	7.90	2.31	3.42
196	182.47	17.89	18.65	9.34	2.54	3.68
197	181.78	16.34	17.96	5.90	3.15	1.87
198	182.04	18.24	18.65	7.96	2.77	2.87
199	182.59	17.91	17.48	6.32	3.58	1.77
200	181.64	17.21	18.65	6.15	2.64	2.33
201	182.57	18.20	17.24	5.90	2.89	2.04
202	181.24	18.79	18.98	6.14	2.77	2.22
203	181.93	15.64	17.24	6.70	3.24	2.07
204	182.57	17.48	18.02	9.58	2.49	3.85
205	182.47	16.20	17.45	9.12	2.37	3.85

206	183.47	17.35	18.62	9.34	2.77	3.37
207	183.47	17.21	17.03	9.54	3.64	2.62
208	182.47	18.05	18.27	9.98	2.75	3.63
209	182.45	16.34	17.48	9.68	2.64	3.67
210	183.65	16.49	18.65	5.90	2.77	2.13
211	183.04	17.28	17.48	9.57	2.75	3.48
212	182.54	17.19	17.24	6.32	2.69	2.35
213	183.47	17.03	17.48	6.32	2.34	2.70
214	182.34	16.98	18.24	5.90	2.77	2.13
215	182.31	16.38	17.46	6.34	3.48	1.82
216	182.95	17.48	17.24	7.12	3.24	2.20
217	182.65	16.02	17.24	9.65	2.68	3.60
218	183.14	17.89	18.64	7.12	2.54	2.80
219	182.51	16.34	17.58	5.90	2.77	2.13
220	183.65	15.02	18.02	9.56	2.68	3.57
221	182.95	17.48	17.56	9.54	2.64	3.61
222	182.64	16.98	17.45	9.63	2.84	3.39
223	183.62	16.34	17.02	5.90	2.75	2.15
224	182.54	17.12	17.48	7.15	2.61	2.74
225	183.14	16.54	18.29	7.26	3.28	2.21
226	182.47	17.89	17.56	9.65	3.49	2.77
227	181.93	16.32	17.52	7.56	2.77	2.73
228	182.54	17.48	18.45	7.24	2.68	2.70
229	181.02	16.32	17.59	6.30	2.16	2.92
230	181.74	16.25	18.64	7.45	2.34	3.18
231	182.65	17.48	17.24	8.02	2.18	3.68

232	181.47	15.02	18.26	5.90	2.35	2.51
233	182.47	17.48	18.41	7.24	2.14	3.38
234	182.64	16.21	17.46	7.12	2.57	2.77
235	181.24	17.48	18.59	9.32	2.16	4.31
236	182.95	16.24	17.59	9.65	2.30	4.20
237	182.36	17.48	17.34	9.21	2.77	3.32
238	181.45	16.20	17.48	9.54	3.48	2.74
239	182.57	16.94	18.20	9.60	3.47	2.77
240	181.24	17.48	17.56	7.23	3.60	2.01
241	182.65	16.32	18.24	7.02	2.35	2.99
242	181.34	17.48	17.48	9.45	2.36	4.00
243	181.02	17.91	18.24	9.26	2.14	4.33
244	182.49	17.87	17.49	9.14	2.98	3.07
245	181.59	16.34	18.65	9.35	3.65	2.56
246	182.47	17.42	18.24	9.87	2.34	4.22
247	182.65	17.91	17.05	7.26	3.15	2.30
248	182.47	16.48	17.46	9.34	2.31	4.04
249	182.65	17.28	18.26	9.26	2.65	3.49
250	182.18	17.48	17.34	7.26	3.15	2.30

NFGP: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:BR**: Length: Breadth Ratio

Appendix 5: Morphological data for the cross GR-11XGurjari

Sr. No.	Zn (ppm)	Fe (ppm)	DFT	PH (cm)	PL (cm)	NEFT
1	85.21	100.58	55.26	119.23	24.13	8.45
2	70.16	99.61	77.21	118.59	23.46	7.74
3	60.48	89.32	71.05	110.48	22.48	7.14
4	65.32	80.47	72.16	105.62	22.18	5.25
5	40.89	69.34	75.15	102.45	20.46	8.02
6	20.48	74.69	74.11	85.47	22.74	7.21
7	50.61	73.90	82.79	107.59	23.05	6.73
8	68.32	77.12	59.32	108.65	23.46	7.48
9	11.98	74.69	78.02	108.24	22.18	7.54
10	45.16	78.62	80.16	106.34	22.75	6.92
11	48.24	79.34	75.30	105.24	20.69	7.48
12	66.31	80.26	74.02	101.11	23.05	7.65
13	49.12	85.14	82.79	101.48	20.14	6.93
14	88.74	100.69	70.63	124.75	20.48	7.74
15	41.78	62.34	110.48	102.59	22.31	6.92
16	67.23	65.31	82.79	102.34	22.48	6.73
17	42.05	67.49	74.05	103.48	22.69	5.12
18	68.01	69.31	88.32	103.24	23.06	8.62
19	44.75	75.48	108.65	104.57	23.14	7.89
20	15.98	74.69	89.32	88.24	23.08	8.62
21	43.62	78.26	92.79	104.56	22.45	7.14
22	46.12	70.90	74.26	89.32	22.18	6.24
23	68.74	79.14	89.31	87.14	20.47	7.48

24	49.21	100.58	109.65	118.24	22.69	7.74
25	50.14	73.52	82.79	105.26	23.15	7.24
26	87.59	74.69	82.79	128.75	23.04	7.48
27	44.16	38.95	85.31	107.49	22.15	7.78
28	41.78	39.74	80.45	87.26	23.14	5.21
29	47.56	71.25	75.26	108.32	22.05	6.93
30	69.32	74.95	78.31	108.59	22.48	7.48
31	69.14	65.32	82.79	109.64	22.64	7.59
32	48.02	62.05	86.32	110.24	20.14	6.92
33	49.32	63.48	106.48	110.48	22.48	6.73
34	45.21	67.14	85.32	104.58	22.16	7.24
35	44.17	82.59	75.14	104.75	23.04	7.89
36	46.21	73.15	74.02	102.65	23.18	7.24
37	43.18	76.89	90.31	108.45	23.84	7.12
38	70.48	77.48	100.56	107.26	24.16	7.58
39	70.19	72.58	95.31	104.26	22.16	7.25
40	42.61	69.34	84.12	105.48	20.48	7.48
41	45.31	70.12	82.79	104.37	22.31	6.92
42	55.18	64.28	86.32	106.33	22.14	7.15
43	46.21	95.66	75.14	117.05	23.05	5.02
44	56.37	68.32	94.21	104.67	23.65	7.74
45	54.02	78.15	84.36	110.48	22.48	7.89
46	18.95	74.69	74.11	82.65	23.51	6.93
47	44.87	73.02	82.06	100.24	22.48	7.45
48	70.49	74.58	71.46	105.24	23.04	8.21
49	48.26	76.31	94.32	108.26	20.48	7.48

50	47.03	64.00	82.79	104.26	22.48	7.26
51	42.64	69.32	81.24	106.48	22.65	7.35
52	55.15	62.45	78.00	108.59	22.75	6.93
53	46.87	64.85	82.79	103.26	23.01	7.79
54	65.31	99.12	72.48	115.24	23.14	7.48
55	63.48	79.52	83.65	104.78	22.75	7.59
56	61.74	77.46	84.02	106.23	22.59	7.21
57	49.21	77.15	86.31	108.59	22.16	7.89
58	41.78	73.90	96.31	109.34	22.30	5.32
59	43.12	39.65	89.52	107.15	23.14	7.74
60	44.87	73.12	74.02	105.26	20.48	7.48
61	46.28	62.48	90.45	105.34	22.75	7.26
62	56.28	70.90	97.62	86.34	23.06	7.48
63	54.11	88.22	81.03	103.24	21.45	7.95
64	51.69	68.51	90.45	101.48	22.14	7.79
65	36.20	69.31	92.79	101.64	21.64	7.15
66	70.48	64.02	79.32	105.26	24.58	7.24
67	56.28	73.18	92.15	102.34	22.13	6.93
68	55.14	89.62	81.36	104.85	23.04	6.54
69	55.97	75.48	92.79	106.34	23.15	5.26
70	53.64	65.28	82.65	107.48	21.64	7.74
71	52.89	38.59	79.33	87.26	22.48	7.48
72	37.14	69.32	95.26	101.48	21.67	7.21
73	58.06	72.15	75.16	105.64	22.14	7.02
74	68.17	74.69	84.26	104.78	22.06	7.48
75	58.49	73.14	99.15	108.95	22.01	7.56

76	59.46	75.48	92.35	109.63	23.48	6.93
77	57.21	39.62	88.46	104.58	22.16	6.54
78	54.18	65.18	74.15	110.26	23.14	7.48
79	38.47	88.65	89.23	104.75	21.47	7.25
80	68.59	65.23	85.02	102.34	21.04	7.48
81	53.21	74.15	84.21	102.45	21.59	7.59
82	57.48	73.90	97.26	105.62	21.64	7.14
83	62.14	79.63	94.26	104.75	21.48	7.41
84	59.34	78.30	86.19	106.32	22.14	7.45
85	58.96	100.02	78.26	129.41	23.04	7.42
86	71.48	68.59	79.33	104.59	22.55	7.89
87	55.02	69.34	72.05	107.48	22.64	6.32
88	68.35	65.18	88.64	108.62	23.48	7.74
89	57.14	62.38	84.02	109.34	22.61	6.32
90	38.16	70.90	92.79	102.45	23.04	5.63
91	55.47	61.24	82.34	101.78	22.48	7.48
92	78.27	69.87	95.16	105.26	21.75	8.25
93	54.15	68.39	85.32	106.34	22.69	6.32
94	59.64	63.14	75.16	104.85	23.05	7.48
95	60.58	74.28	88.32	108.59	22.14	7.74
96	69.74	70.90	84.02	106.34	21.06	8.25
97	59.32	75.41	92.79	107.48	21.74	6.45
98	37.48	72.26	93.14	107.48	22.31	7.24
99	58.26	86.34	85.26	101.56	22.48	7.41
100	57.98	77.18	95.31	105.34	23.05	7.54
101	56.24	78.56	74.02	102.48	22.48	6.54

102	55.84	68.93	75.61	101.59	22.16	7.48
103	57.14	69.32	88.23	103.24	23.01	7.59
104	68.23	77.41	94.26	104.65	21.75	7.62
105	38.94	72.05	91.57	108.29	22.14	7.34
106	56.48	82.45	85.23	109.34	23.18	7.15
107	68.45	75.69	75.48	105.24	21.05	7.48
108	53.02	75.41	84.12	104.78	21.69	6.32
109	80.49	73.16	92.65	106.48	23.14	8.26
110	50.18	70.90	82.30	105.59	22.01	6.40
111	50.47	61.28	79.12	103.48	21.48	6.02
112	40.98	65.32	80.56	105.24	22.63	7.48
113	59.64	95.63	95.12	104.48	23.01	7.01
114	65.47	66.48	98.32	105.28	21.48	7.74
115	53.78	77.25	75.14	108.47	22.56	7.48
116	57.82	74.69	81.26	109.62	23.01	7.12
117	37.15	37.12	85.21	105.48	21.47	7.48
118	52.48	69.34	78.26	102.64	21.06	7.02
119	56.29	79.02	99.32	106.34	21.48	7.25
120	56.87	78.62	86.12	106.28	21.30	6.28
121	54.17	75.14	92.79	104.59	22.48	5.03
122	38.47	94.30	78.26	117.02	23.01	7.48
123	57.48	64.18	88.32	106.48	22.15	7.16
124	64.12	63.47	79.14	104.26	22.64	8.65
125	51.87	61.02	94.02	107.48	23.15	7.48
126	39.15	39.65	89.31	108.59	21.48	7.48
127	51.24	74.69	79.33	105.26	22.05	7.48

128	58.67	69.32	86.32	106.34	23.48	7.65
129	66.15	86.14	83.05	104.75	24.15	7.41
130	57.48	68.32	74.12	102.48	22.47	7.02
131	74.21	61.48	75.49	102.47	24.61	8.65
132	58.47	79.25	95.26	103.59	21.35	7.59
133	58.95	88.62	98.31	101.12	22.48	7.32
134	58.26	89.34	85.06	101.23	21.39	7.74
135	68.31	94.00	78.01	106.48	22.75	5.29
136	59.41	92.14	75.48	102.65	21.05	7.74
137	38.21	78.63	84.02	102.45	21.26	7.45
138	57.14	87.62	97.62	105.68	22.94	6.32
139	52.06	78.34	82.31	108.82	21.30	7.89
140	34.75	74.69	78.15	104.84	21.48	7.41
141	55.12	77.14	75.62	105.63	22.61	7.20
142	69.32	72.69	84.02	106.48	21.03	7.89
143	60.48	71.32	74.16	107.45	22.74	7.74
144	75.12	73.05	75.32	108.26	24.26	7.65
145	34.17	37.89	86.14	104.37	21.08	7.74
146	59.62	75.14	71.05	104.28	22.64	7.41
147	32.47	93.06	98.62	120.48	22.30	8.65
148	33.58	74.58	70.62	105.48	21.47	6.32
149	56.32	77.16	70.34	105.26	21.59	5.98
150	57.14	76.28	85.26	104.78	22.05	7.48
151	35.26	94.25	73.04	112.34	22.47	8.01
152	68.14	91.03	79.33	114.58	21.59	7.48
153	44.26	79.65	99.05	108.59	22.64	7.51

154	54.78	77.48	68.32	107.26	24.01	6.32
155	25.98	50.26	69.10	108.32	20.13	7.48
156	39.62	69.34	75.49	108.41	21.58	7.74
157	38.14	38.16	84.06	109.26	22.60	7.15
158	67.12	34.18	62.31	106.48	22.34	8.34
159	45.69	69.74	63.48	104.32	21.48	5.68
160	26.30	80.56	64.12	105.48	22.06	6.48
161	46.78	62.31	98.62	104.51	24.13	7.02
162	37.15	68.47	75.24	104.26	21.59	7.14
163	55.24	74.69	66.12	102.34	22.48	7.69
164	65.89	78.62	68.32	101.48	22.61	8.35
165	36.54	94.01	69.31	120.47	21.03	8.45
166	43.48	79.02	79.33	101.64	22.48	7.26
167	35.16	74.59	84.02	101.48	22.16	6.35
168	46.78	89.36	85.62	103.24	21.48	7.74
169	71.05	75.14	79.31	106.34	21.03	7.12
170	49.32	79.32	62.01	104.28	22.48	7.48
171	34.69	68.14	88.34	105.64	24.61	7.68
172	66.48	65.38	78.03	104.75	22.59	7.48
173	33.98	66.17	75.64	106.48	22.31	6.03
174	49.15	74.69	80.98	105.47	21.15	7.48
175	32.06	39.65	80.16	108.65	22.48	5.18
176	46.78	64.15	75.21	104.27	22.65	6.28
177	43.15	94.02	79.33	109.34	24.18	7.48
178	38.26	68.29	60.48	108.24	22.31	7.59
179	54.17	99.32	81.45	119.32	21.04	8.68

180	58.06	62.14	72.65	105.48	22.59	7.74
181	67.48	66.35	69.32	104.21	22.61	7.48
182	25.69	74.69	89.15	84.21	21.47	7.15
183	46.31	56.38	79.33	109.65	22.30	7.24
184	57.49	94.05	79.45	108.42	22.14	8.24
185	38.61	56.32	74.12	108.62	21.78	7.02
186	73.05	84.12	68.25	105.48	22.59	7.48
187	77.48	74.69	63.18	88.21	24.06	8.52
188	59.62	56.32	89.32	104.57	22.14	7.48
189	46.31	95.64	75.48	106.32	22.17	8.65
190	39.64	38.49	73.01	95.60	22.03	5.26
191	60.15	77.12	87.56	108.59	21.04	7.48
192	45.28	74.05	65.32	104.75	22.59	7.02
193	37.41	86.32	74.15	105.62	21.48	7.14
194	44.26	84.15	89.21	106.34	24.06	7.48
195	59.32	56.29	79.33	94.21	21.98	7.26
196	42.11	85.47	68.41	104.60	24.63	7.48
197	29.65	74.69	85.32	85.21	22.15	7.32
198	79.33	35.62	79.14	94.21	22.48	7.48
199	41.05	59.47	62.03	108.26	22.06	7.95
200	54.02	79.65	89.32	104.75	22.49	7.24
201	34.78	48.26	78.14	99.32	22.61	7.74
202	66.19	88.32	84.12	104.75	22.31	5.51
203	35.48	81.06	69.31	108.26	22.20	7.48
204	42.06	48.26	75.26	104.32	22.15	7.26
205	33.44	74.15	84.02	105.48	22.48	8.62

206	51.48	72.59	74.12	104.75	22.17	7.40
207	42.87	73.90	82.32	94.35	22.60	7.25
208	71.06	83.06	75.14	102.48	22.16	7.34
209	26.97	74.69	64.02	98.61	22.34	7.48
210	51.64	70.90	65.32	92.34	22.18	8.06
211	64.32	77.25	84.75	106.35	22.49	7.74
212	36.14	59.61	79.33	97.48	22.67	7.47
213	55.89	94.26	88.23	104.26	22.15	7.12
214	38.75	74.58	79.12	102.35	22.34	8.98
215	44.15	50.34	62.31	94.57	22.48	7.58
216	72.48	78.26	89.15	106.34	22.15	7.68
217	24.99	70.90	92.79	98.26	22.06	8.29
218	62.31	79.61	79.32	105.34	22.31	7.14
219	37.48	70.90	78.56	95.75	22.48	7.28
220	47.02	50.64	87.01	97.02	22.59	5.26
221	48.15	94.01	75.16	116.34	22.61	7.74
222	47.62	50.14	77.26	95.64	22.34	7.95
223	72.58	72.59	77.32	109.35	22.18	7.48
224	48.61	93.48	82.56	105.48	22.05	7.56
225	40.69	75.48	75.14	106.48	22.48	7.21
226	54.27	51.48	85.21	94.27	22.47	7.48
227	22.96	77.26	74.69	104.58	22.16	8.21
228	62.31	95.32	81.26	102.34	22.31	7.48
229	38.54	60.48	75.32	102.47	22.48	7.26
230	49.62	75.21	88.41	103.59	22.57	7.42
231	47.12	73.05	77.48	105.62	22.06	7.74

232	37.49	52.49	79.33	92.32	22.14	7.41
233	24.65	36.48	89.32	94.57	22.31	5.06
234	53.12	57.26	72.14	91.03	22.48	7.48
235	26.95	94.02	71.56	115.64	22.59	7.02
236	61.02	76.35	85.14	105.75	22.61	5.98
237	44.75	48.26	73.69	97.12	22.17	7.63
238	64.38	79.34	75.42	108.26	22.06	7.42
239	78.59	49.16	87.10	94.32	22.31	8.02
240	33.54	39.57	74.95	93.14	22.05	7.89
241	55.26	48.56	88.62	97.48	22.31	8.32
242	33.48	94.21	74.16	117.02	22.24	7.14
243	44.17	43.68	79.33	97.46	22.59	8.56
244	49.62	49.57	85.47	94.32	22.14	5.20
245	54.12	94.00	84.29	118.75	22.34	7.48
246	48.59	78.26	79.61	108.26	22.18	7.05
247	26.84	37.48	82.32	97.54	22.65	7.89
248	32.06	51.03	78.15	95.26	22.47	8.62
249	73.14	68.59	86.02	109.32	22.31	7.46
250	66.95	85.61	75.49	102.48	22.47	5.31
251	48.23	68.34	85.12	104.78	22.16	6.48
252	60.48	86.47	84.06	102.65	22.31	7.74
253	74.18	58.92	88.32	101.29	22.48	7.24
254	48.26	84.26	82.04	91.32	22.05	7.48
255	35.21	69.31	81.45	107.46	22.64	6.32
256	48.95	59.47	85.47	99.32	22.59	8.29
257	47.63	87.24	86.23	105.26	22.98	6.35

258	49.32	70.61	84.02	106.34	22.34	8.14
259	26.48	84.23	83.14	105.26	22.15	8.26
260	60.14	60.48	87.14	102.48	22.47	7.48
261	48.26	88.21	85.14	118.34	22.63	7.02
262	78.31	70.49	84.59	104.59	22.05	5.69
263	35.62	59.36	85.26	96.37	22.59	5.98
264	44.17	99.32	80.12	116.32	22.68	5.61
265	58.24	99.15	80.64	111.48	22.66	6.32
266	76.31	70.15	80.32	105.11	22.31	6.32
267	45.29	72.69	90.14	108.94	22.45	8.41
268	42.17	94.27	88.01	119.62	22.85	6.14
269	48.06	72.16	90.15	108.33	22.06	6.58
270	28.99	52.34	82.04	97.22	22.95	8.02
271	27.94	98.65	81.75	117.45	22.47	8.47
272	47.62	78.15	85.36	104.27	22.31	6.32
273	43.02	79.26	83.26	104.58	22.56	8.15
274	46.18	80.41	84.21	106.34	22.14	5.14
275	49.61	49.62	82.65	95.23	22.58	6.02
276	58.62	92.31	85.31	118.74	22.64	8.02
277	56.32	44.17	86.30	94.26	22.18	6.34
278	55.48	94.02	90.31	108.57	22.40	6.01
279	44.72	80.59	88.65	102.31	22.16	5.48
280	45.61	80.64	84.15	106.48	22.48	8.59
281	46.29	48.26	87.26	99.62	22.17	8.21
282	22.59	94.01	84.26	118.32	22.14	6.32
283	47.15	79.48	87.12	105.64	22.48	5.12

284	35.64	74.02	82.03	106.38	22.59	6.14
285	48.92	57.16	81.47	96.31	22.47	8.02
286	54.37	75.48	83.16	108.24	22.01	5.26
287	49.62	65.32	88.25	107.12	22.61	6.32
288	53.17	98.26	86.14	109.63	22.35	8.14
289	21.48	68.14	88.21	108.24	22.01	6.25
290	50.48	69.31	89.32	104.26	22.59	8.04
291	50.64	78.56	81.02	105.74	22.64	5.69
292	30.48	55.21	82.56	95.26	22.31	8.32
293	44.15	69.34	85.32	108.26	22.48	6.13
294	55.27	61.02	84.02	107.32	22.05	6.02
295	47.32	94.01	87.14	115.24	22.47	6.48
296	25.48	64.35	85.69	101.79	22.64	6.32
297	48.69	42.59	86.32	101.23	22.13	6.05
298	60.31	73.15	89.01	106.59	22.48	6.89
299	40.87	69.25	87.26	102.48	22.17	6.48
300	30.15	60.34	88.15	105.62	22.06	6.32

Zn: Zinc concentration (ppm); **Fe**: Iron concentration (ppm); **DFF**: Days to 50% Flowering, **PH**: Plant height (cm), **PL**: Panicle length (cm), **NETP**: Number of effective tillers per plant

Appendix 6: Morphological data for the cross GR-11XGurjari

Sr. No.	NFGP	TWT (g)	GY (g)	GL (cm)	GB (cm)	L:BR
1	184.92	11.02	19.56	10.45	2.59	2.13
2	184.37	15.47	18.26	10.25	2.64	3.26
3	183.95	15.64	19.56	8.02	2.87	2.79
4	182.49	21.78	19.26	8.02	2.68	2.99
5	180.47	17.01	18.26	8.26	2.54	3.25
6	182.47	16.98	14.88	8.21	2.89	2.84
7	180.56	16.34	17.15	8.02	2.89	2.53
8	180.47	22.04	14.62	5.51	2.59	3.10
9	180.65	13.48	17.90	8.02	2.47	3.25
10	182.47	12.74	19.23	8.21	2.58	3.18
11	182.79	23.47	14.26	5.63	2.89	1.95
12	182.47	18.04	19.56	8.26	2.28	3.62
13	180.65	19.64	19.23	8.02	2.05	3.91
14	182.48	20.48	19.23	8.02	2.89	2.78
15	180.47	20.18	19.10	7.01	2.04	3.44
16	182.64	17.48	18.26	8.02	2.59	3.10
17	180.78	14.26	19.34	5.32	2.68	2.99
18	180.32	24.31	19.23	8.02	2.57	2.07
19	182.78	24.01	19.10	5.29	2.77	1.91
20	180.41	25.48	17.24	8.32	2.98	2.79
21	182.75	15.26	19.65	5.24	2.89	2.82
22	182.95	20.48	17.94	7.29	2.01	3.63
23	182.74	21.47	17.90	8.05	2.89	2.79

24	180.65	21.06	18.56	8.21	2.48	3.33
25	184.14	17.48	19.23	8.02	2.47	3.25
26	184.27	18.26	17.26	8.02	2.59	2.79
27	182.94	22.48	14.50	5.26	2.65	1.98
28	182.47	19.47	14.50	5.21	2.77	2.89
29	182.65	17.02	14.88	8.32	2.98	2.79
30	184.37	18.64	18.89	8.24	2.89	2.85
31	182.45	16.32	14.02	8.02	3.69	2.17
32	184.67	26.48	18.65	6.70	2.98	2.25
33	184.27	25.48	14.88	5.06	2.57	1.97
34	184.05	11.64	19.23	8.02	2.59	3.10
35	184.67	22.01	14.28	8.17	2.69	3.04
36	180.46	16.34	18.26	7.44	2.56	2.91
37	180.31	14.89	16.88	8.26	2.48	3.33
38	184.27	48.06	18.57	8.21	2.89	2.84
39	184.06	23.47	16.88	8.16	2.89	2.82
40	180.46	18.47	15.62	8.34	2.34	3.56
41	180.74	12.64	16.88	6.70	2.58	2.60
42	180.56	24.59	18.24	7.14	2.47	3.01
43	182.47	25.48	18.92	5.21	2.49	3.22
44	182.03	15.26	15.62	8.32	2.57	3.24
45	180.65	17.48	18.24	6.70	2.28	2.94
46	182.75	21.48	16.88	5.64	2.67	2.51
47	180.14	22.21	18.26	8.26	2.54	3.25
48	180.47	18.97	16.90	8.31	2.58	3.22
49	180.36	18.41	18.21	8.02	2.34	3.43

50	182.15	18.47	16.90	8.02	2.77	2.90
51	183.48	17.98	16.90	7.44	2.50	2.98
52	183.14	10.48	18.34	6.90	2.68	2.57
53	183.06	23.64	15.68	8.26	2.89	2.86
54	182.47	16.48	15.68	8.98	2.98	3.01
55	183.45	25.48	18.00	7.23	2.89	2.50
56	182.47	18.34	18.69	8.12	2.64	3.08
57	183.06	23.47	16.90	8.26	2.89	2.86
58	182.47	18.97	18.54	7.56	2.54	2.98
59	183.04	17.46	18.69	5.27	2.89	2.39
60	182.79	19.34	16.90	6.90	2.77	2.96
61	183.04	16.24	16.90	7.58	2.89	2.62
62	183.26	16.34	18.65	7.48	2.89	2.59
63	183.47	23.48	15.89	8.21	2.47	2.12
64	183.65	22.49	18.64	7.23	3.05	2.37
65	183.04	17.21	16.90	5.24	3.06	2.25
66	180.47	22.48	18.59	8.32	2.77	3.00
67	180.59	14.68	18.64	8.46	2.77	3.05
68	182.47	18.29	16.90	7.23	2.28	3.17
69	182.64	11.98	18.67	7.50	3.75	2.00
70	180.47	21.47	16.90	5.64	2.05	2.75
71	180.29	19.64	18.97	6.90	2.98	2.32
72	180.47	15.20	18.64	7.48	3.75	1.99
73	182.47	18.97	16.90	7.48	2.48	3.02
74	180.26	21.48	18.59	7.50	2.65	2.83
75	180.05	16.48	15.64	6.90	2.89	2.39

76	180.49	22.84	18.34	7.48	2.75	2.72
77	180.75	20.47	15.26	7.59	2.48	3.06
78	180.26	12.69	17.90	7.24	2.35	3.08
79	184.75	23.48	16.05	7.60	2.48	3.06
80	184.02	20.48	16.34	7.50	2.54	2.95
81	184.75	20.47	18.56	6.90	2.23	3.09
82	184.26	18.62	18.24	7.50	2.32	3.23
83	184.75	19.34	17.90	6.90	2.28	3.03
84	182.06	23.14	18.29	7.50	2.26	3.32
85	182.45	17.89	17.90	7.23	2.35	3.08
86	184.20	16.02	18.20	8.02	2.54	2.85
87	182.47	22.48	16.25	7.23	2.77	2.61
88	184.26	17.48	18.98	7.23	2.28	3.17
89	184.32	18.06	18.54	7.23	2.98	2.43
90	184.59	15.23	17.90	7.60	3.75	2.03
91	182.47	19.87	18.52	7.90	2.68	2.95
92	184.02	23.47	17.90	7.50	2.54	2.95
93	184.26	14.68	18.02	7.90	2.28	3.46
94	182.04	18.21	18.54	7.23	2.57	2.81
95	184.59	23.47	16.34	7.50	2.34	3.21
96	182.06	19.67	18.21	7.60	2.28	3.33
97	184.03	17.04	16.23	8.24	2.29	3.60
98	184.57	22.59	18.75	8.26	2.64	3.13
99	182.49	15.68	16.95	7.60	2.28	3.33
100	184.15	16.48	17.90	7.23	2.48	2.92
101	184.79	22.48	18.25	7.50	2.05	3.66

102	184.26	18.79	18.05	7.50	2.50	3.00
103	182.06	19.62	16.34	7.90	2.28	3.46
104	182.47	14.02	18.14	5.62	2.31	2.43
105	184.29	15.64	16.29	7.60	2.35	3.23
106	184.26	21.47	16.84	7.50	2.28	3.29
107	184.29	15.63	16.12	8.98	3.54	2.54
108	184.37	22.48	18.48	7.50	3.24	2.31
109	183.02	15.64	16.95	7.90	2.77	2.85
110	183.14	16.32	16.34	7.25	2.77	2.62
111	183.04	23.48	18.02	7.50	2.05	3.66
112	183.47	14.07	16.02	7.90	2.28	3.46
113	183.49	13.64	17.90	7.50	2.54	2.95
114	183.25	18.96	18.29	7.50	2.35	3.19
115	183.04	21.47	16.21	7.28	2.48	2.94
116	183.47	15.62	18.20	7.64	2.47	3.09
117	183.56	14.03	17.90	7.50	2.59	2.90
118	183.02	19.87	18.87	7.23	2.65	2.73
119	183.95	30.45	17.90	7.98	2.54	3.14
120	182.63	14.78	16.47	7.20	2.38	3.03
121	183.95	24.69	16.42	7.90	2.69	2.94
122	183.95	15.60	16.78	7.90	2.67	2.96
123	182.47	25.47	18.98	7.90	2.28	3.46
124	182.59	23.48	18.02	7.90	2.57	3.07
125	181.00	21.48	18.24	7.23	2.48	2.92
126	181.45	32.47	16.20	7.58	2.48	3.06
127	181.47	21.22	18.48	7.23	2.59	2.79

128	182.59	23.59	18.59	7.44	2.28	3.26
129	181.47	22.64	16.21	7.90	2.63	3.00
130	181.26	16.02	16.34	7.90	2.34	3.38
131	182.05	18.98	14.02	7.90	2.77	2.85
132	181.47	35.47	16.48	7.22	2.77	2.61
133	182.69	22.68	18.24	5.62	2.77	2.03
134	181.47	19.25	16.48	7.22	2.51	2.88
135	181.02	21.48	18.02	7.90	2.34	3.38
136	182.49	19.06	16.34	7.22	2.75	2.63
137	182.56	14.28	16.48	5.68	2.35	2.42
138	182.47	23.48	17.94	5.32	2.89	1.84
139	182.36	22.89	16.34	7.90	2.64	2.99
140	182.26	34.17	16.02	7.90	2.77	2.85
141	182.59	21.06	16.48	7.50	2.77	2.71
142	181.47	25.34	16.04	5.26	2.36	2.23
143	182.95	24.89	18.24	7.90	2.47	3.20
144	181.04	19.60	16.48	7.02	2.28	3.08
145	182.65	18.26	18.27	7.90	2.47	3.20
146	181.47	29.48	17.90	7.60	2.51	3.03
147	182.06	31.47	17.90	7.22	2.61	2.77
148	182.05	32.95	18.24	7.50	2.34	3.21
149	181.49	22.58	18.05	7.90	2.58	3.06
150	182.47	21.47	17.96	7.90	2.47	3.20
151	181.59	23.15	18.65	7.25	2.69	2.70
152	181.26	22.48	17.24	5.68	2.34	2.43
153	182.48	17.05	18.56	7.14	2.28	3.13

154	181.47	18.95	17.24	7.20	2.50	2.88
155	182.05	22.64	18.59	5.47	3.21	1.70
156	181.49	22.48	17.92	7.90	3.65	2.16
157	182.05	18.21	18.20	7.20	2.98	2.42
158	181.47	19.34	17.93	7.44	2.77	2.69
159	183.02	21.48	18.20	5.46	2.28	2.39
160	183.56	21.59	18.79	7.44	2.48	3.00
161	182.49	22.05	18.24	7.22	2.68	2.69
162	183.06	31.47	18.02	5.41	2.59	2.09
163	183.47	22.95	17.90	7.48	2.28	3.28
164	182.06	22.48	18.34	7.44	2.28	3.26
165	183.04	17.05	17.05	7.22	2.65	2.72
166	182.59	19.64	18.64	7.22	2.77	2.61
167	183.04	23.48	17.04	7.22	2.17	3.33
168	182.79	21.01	18.56	7.48	2.48	3.02
169	182.06	23.47	17.90	6.02	2.36	2.55
170	183.48	32.10	18.26	7.22	2.28	3.17
171	183.47	25.48	17.90	7.22	2.48	2.91
172	182.79	25.64	18.45	9.05	3.05	2.97
173	183.06	17.48	17.90	7.22	3.04	2.38
174	182.45	21.04	18.57	7.26	3.28	2.21
175	183.49	25.49	17.91	7.44	2.65	2.81
176	181.56	22.14	18.79	7.90	2.17	3.64
177	182.47	34.78	17.21	7.44	2.18	3.41
178	181.49	32.06	18.79	6.32	2.64	2.39
179	182.64	23.48	17.46	6.48	2.48	2.61

180	181.20	21.75	17.24	7.96	3.56	2.24
181	181.47	24.05	17.89	7.96	2.77	2.87
182	182.44	24.18	18.24	9.87	3.40	2.90
183	182.65	21.69	17.92	6.21	2.15	2.89
184	182.94	23.48	17.02	6.35	2.68	2.37
185	182.33	19.74	17.24	7.48	2.53	2.96
186	181.47	19.06	17.93	7.48	2.52	2.97
187	182.47	21.47	18.24	9.32	2.22	4.20
188	182.05	34.05	17.06	7.90	2.77	2.85
189	182.66	31.78	17.94	9.02	3.24	2.78
190	182.34	25.06	18.56	6.34	2.35	2.70
191	181.59	25.14	17.06	6.25	2.15	2.91
192	182.06	20.48	17.96	5.90	2.36	2.50
193	182.45	26.31	18.24	7.44	2.56	2.91
194	181.06	23.04	17.04	7.34	2.14	3.43
195	181.29	18.79	17.89	7.90	2.31	3.42
196	182.47	17.45	18.65	9.34	2.54	3.68
197	182.56	22.64	17.96	5.90	3.15	1.87
198	181.49	28.19	18.65	7.96	2.77	2.87
199	182.74	28.74	17.48	6.32	3.58	1.77
200	181.49	17.08	18.65	6.03	2.64	2.28
201	182.65	18.65	17.24	8.04	2.89	2.78
202	182.04	21.48	18.98	8.04	2.77	2.90
203	181.59	23.14	17.24	6.18	3.24	1.91
204	182.74	16.98	18.02	6.15	2.49	2.47
205	182.04	18.24	17.45	7.89	2.37	3.33

206	181.50	17.04	18.62	8.04	2.77	2.90
207	182.59	17.69	17.03	8.25	3.64	2.27
208	181.62	19.32	18.27	6.60	2.75	2.40
209	182.47	27.48	17.48	6.65	2.64	2.52
210	181.04	22.45	18.65	10.28	2.77	3.71
211	182.59	23.14	17.48	8.29	2.75	3.01
212	182.47	21.48	17.24	8.34	2.69	3.10
213	181.06	15.62	17.48	8.91	2.34	3.81
214	182.59	24.78	18.24	8.24	2.77	2.97
215	181.24	29.64	17.46	10.45	3.48	3.00
216	182.04	25.31	17.24	7.89	3.24	2.44
217	182.35	18.24	17.24	8.24	2.68	3.07
218	181.04	23.24	18.64	8.02	2.54	3.16
219	181.27	19.62	17.58	8.06	2.77	2.91
220	182.59	25.16	18.02	6.60	2.68	2.46
221	181.04	18.75	17.56	7.89	2.64	2.99
222	181.75	20.49	17.45	7.89	2.84	2.78
223	182.49	17.54	17.02	8.64	2.75	3.14
224	182.60	21.79	17.48	8.97	2.61	3.44
225	181.24	24.79	18.29	8.64	3.28	2.63
226	182.49	18.24	17.56	8.34	3.49	2.39
227	182.56	28.65	17.52	6.01	2.77	2.17
228	182.47	16.30	18.45	8.26	2.68	3.08
229	182.06	23.14	17.59	6.34	2.16	2.94
230	182.56	18.24	18.64	8.29	2.34	3.54
231	182.03	18.95	17.24	7.89	2.18	3.62

232	182.48	19.64	18.26	6.60	2.35	2.81
233	182.47	25.14	18.41	7.89	2.14	3.69
234	182.62	19.32	17.46	8.24	2.57	3.21
235	182.55	21.78	18.59	6.60	2.16	3.06
236	182.49	22.21	17.59	8.34	2.30	3.63
237	182.36	24.05	17.34	7.89	2.77	2.85
238	182.45	28.95	17.48	6.98	3.48	2.01
239	182.01	29.61	18.20	6.34	3.47	1.83
240	182.75	23.48	17.56	8.25	3.60	2.29
241	182.06	21.05	18.24	8.67	2.35	3.69
242	182.50	21.48	17.48	6.34	2.36	2.69
243	182.64	22.47	18.24	8.26	2.14	3.86
244	182.55	24.89	17.49	8.91	2.98	2.99
245	182.48	25.61	18.65	6.38	3.65	1.75
246	182.02	23.48	18.24	8.02	2.34	3.43
247	182.64	21.04	17.05	8.60	3.15	2.73
248	182.74	24.78	17.46	6.38	2.31	2.76
249	182.03	25.16	18.26	8.26	2.65	3.12
250	182.49	22.04	17.34	6.34	3.15	2.01
251	182.06	21.89	21.64	8.94	2.15	4.16
252	182.64	23.14	15.79	6.37	3.14	2.03
253	182.75	24.17	15.65	7.32	2.35	4.36
254	182.31	25.60	15.27	6.60	3.65	1.81
255	182.49	21.68	15.48	8.34	3.15	2.65
256	182.11	23.15	21.78	9.23	3.48	2.65
257	182.49	25.74	15.29	8.36	2.68	3.12

258	182.34	22.48	15.34	6.60	2.47	2.67
259	182.55	21.69	21.48	7.21	2.61	3.93
260	182.04	21.47	15.02	8.24	3.08	2.68
261	182.65	23.05	15.98	6.60	3.01	2.19
262	182.47	24.78	15.64	6.15	2.75	2.24
263	182.47	22.15	21.74	10.46	2.48	4.22
264	182.06	21.04	21.35	8.24	2.00	4.12
265	182.57	25.96	20.48	10.36	2.63	3.94
266	182.31	23.14	20.14	8.54	2.01	4.25
267	182.05	25.78	15.62	8.92	2.45	3.64
268	182.65	22.01	15.34	8.61	2.61	3.30
269	182.47	23.04	15.08	8.54	2.85	3.00
270	182.03	24.79	20.47	10.05	2.69	3.74
271	182.54	21.04	15.68	8.78	2.34	3.75
272	182.15	22.65	15.34	8.59	2.15	4.00
273	182.04	22.14	15.29	8.42	3.16	2.66
274	182.79	22.78	15.04	10.26	2.48	4.14
275	182.44	22.47	15.48	8.54	2.95	2.89
276	182.05	24.89	20.49	8.69	2.61	3.33
277	182.64	26.15	15.64	8.16	2.04	4.00
278	182.49	24.17	15.27	8.47	3.15	2.69
279	182.32	21.68	20.47	10.26	3.47	2.96
280	182.02	21.78	15.60	8.34	2.05	4.07
281	182.47	24.05	15.32	8.29	2.64	3.14
282	182.05	20.48	15.48	8.48	2.01	4.22
283	182.61	22.26	15.94	8.37	2.45	3.42

284	182.03	23.15	15.26	10.26	2.89	3.55
285	182.94	23.21	15.47	8.64	2.97	2.91
286	182.33	23.24	20.48	8.34	2.64	3.16
287	182.45	23.15	14.59	6.35	3.15	2.02
288	182.79	27.14	15.26	8.16	3.02	2.70
289	182.05	25.89	15.34	10.94	3.48	3.14
290	182.45	24.61	14.78	8.26	2.57	3.21
291	182.65	24.53	14.29	8.75	2.04	4.29
292	182.04	24.78	14.02	8.19	3.19	2.57
293	182.03	23.05	15.29	8.69	2.47	3.52
294	182.65	23.14	15.37	8.34	3.56	2.34
295	182.47	21.79	15.49	8.26	3.05	2.71
296	182.06	25.61	15.08	10.48	2.47	4.24
297	182.41	25.34	15.29	10.67	2.64	4.04
298	182.65	28.95	15.34	10.49	3.15	3.33
299	182.04	28.74	14.08	8.50	3.48	2.44
300	182.05	28.79	20.15	6.80	2.01	3.38

NFGP: Number of filled grains per panicle, **TWT**: Test weight (g), **GY**: Grain yield (g), **GL**: Grain length (cm), **GB**: Grain breadth (cm), **L:BR**: Length: Breadth Ratio