“IDENTIFICATION OF ELITE PARENTAL POOL FOR BREEDING ZINC RICH RICE VARIETIES USING MODERN BREEDING APPROACHES”

M.Sc. (Ag) Thesis

by

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CERTIFICATE – I

This is to certify that the thesis Viva-voce in respect of **Mr. Subham Sharma** student of M.Sc. (Ag.) **Department of Plant Molecular Biology and Biotechnology,** has been conducted under the chairmanship of Head of the Department/Dean (in case of out campii) alongwith Advisory Committee on.18-09-2020. The necessary corrections have also been made as per comments/suggestions made by the Advisory Committee and Head of the Department/Dean.

Date: 18-09-2020

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CERTIFICATE – II

This is to certify that the thesis entitled **"Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches"** submitted by **SUBHAM SHARMA** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Plant Molecular Biology and Biotechnology** has been approved by the external evaluator and Student's Advisory Committee after oral examination, under the chairmanship of head of the Department/Dean (in case of out campii)

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LIST OF ABBREVIATIONS

Abbreviation	Details
%	Percent
µg	Microgram
µg/gm	Microgram per gram equivalent to ppm
µl	Microlitre
µM	Micromolar
º C	Degree Celsius
AAS	Atomic Absorption spectrophotometry
bp	Base pair
cm	Centimetre
PH	Plant Height
DFF	Days to 50% Flowering
ET	No. of Effective Tillers
GY	Grain Yield
DNA	Deoxyribo nucleic acid
dNTPs	Deoxynucleotide triphosphates
e.g.	Example
et al.	And others
EtBr	Ethidium bromide
Fe	Iron
Zn	Zinc
FERs	Ferritin
FROs	Ferric reductase oxidases
gm ppm	Gram Parts per million
i.e.	That is
M	Molar
mg	Milligram
$MgCl_2$	Magnesium chloride
Min.	Minute
ml	Millilitre
NA	Nicotinic acid
NCBI	National Center for Biotechnology Information
ng	Nanogram
nm	Nanomolar
BLUP	Best Linear Unbiased Prediction
PCR	Polymerase chain reaction

HRR	Head Rice Recovery
SNP	Single nucleotide polymorphism
SSR	Single sequence repeats
Tm	Melting temperature
VIT	Vacuolar membrane transporter
viz.	As follows
YSLs	Yellow stripe protein like
ZIPs	Zrt/Irt- related Proteins

THESIS ABSTRACT

a) Title of Thesis: Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches

b) Full name of Student: Subham Sharma

c) Major Subject: Plant Molecular Biology and Biotechnology

d) Name and Address of the Major Advisor: Dr. Girish Chandel (Professor) Dept. of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur.

e) Degree to be awarded: M.Sc. (Ag.) Plant Molecular Biology and Biotechnology

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Signature of the Major Advisor

Signature of the Student

Date: 18-09-2020

Signature of the Head of the Department

Zinc is a one of the vital micronutrients plays a significant role in human body by boosting our immune system, feeding oxygen to muscles, supporting nervous system, reproductive health, combating skin injuries and various respiratory ailments. As far as the present scenario is concerned regular outburst of severe infections has posed a threat to mankind. However, these can be avoided or managed by maintaining the intake of healthy balanced diets so that the immune system could be boost up to combat such ailments. Apart from macronutrients, micronutrients (zinc, iron, magnesium, vitamins etc.) are also considered to be the main component of balanced diet which requires in minuscule amounts with infinite positive impacts on human body. One of the best ways to supply micronutrients *ie* Zn is through intake of global staple food crops like rice, wheat and maize. Looking to the importance of Zn in human health, a study entitled "**Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches**" study was conducted to investigate and develop zinc enriched staple crop such as rice. One of the strategies to develop zinc rich rice varieties is Biofortification- breeding rice with enhanced grain Zn contents.

A panel of 75 advanced breeding lines (ABL) have been selected for the present study to analyse the grain zinc content in milled rice as well as to significantly correlate the phenotypic traits with the Zn content in order to analyse the variability and for the selection of superior lines. Field phenotyping of the ABL have been performed based on a few agronomic qualities, for example, DF,

and so on so as to examine the Genotype x Environment association on yield and grain zinc content. By considering yield as a fundamental boundary the genotype IR14V1020 having the greatest GY (76.8 Q/hec) while the base GY displayed by IR 45427-2B-2-2B-1-1::G1 (25.6 Q/hec) the mean GY to be about 48.52 Q/hec with a coefficient of variance of 5.9%. Processing traits have likewise been considered to propose an assortment having a higher estimation of HR so as to favour by the purchaser. Because of HR the genotype IRRI 174 having the most extreme HR (71.68%) while the base HR showed by IR 45427-2B-2-2B-1-1::G1 (14.56%) the mean HR to be about 53.02% with a coefficient of variance of 8.5%. Natural investigation uncovered the grain zinc content present in the ABL, the genotype IR 45427-2B-2-2B-1-1::G1 having the most extreme GZC (grain zinc content) (29.1 ppm) while the base GZC displayed by IR13N115 (12.3 ppm) the mean GZC to be about 17.9 ppm having a coefficient of variance of 5.9%.

The results of statistical operations revealed that the genotypes exhibit a broader sense of variability suggesting there is ample scope for selection of different quantitative and qualitative characters for rice improvement.Phenotypic correlation coefficient analysis of 75 ABLs showed significant correlation between mineral content (zinc) and grain yield related attributes. Grain zinc content and grain yield showed significant positive correlation with all the studied traits. Genomic studies performed at Intertek Laboratory, Hyderabad also revealed Pita and Pi9 genes confer resistance to blast in selected screened genotypes. BLUPs (Best Linear Unbiased Prediction) values for ABLs was generated for yield obtained at Raipur and five different locations. The yield BLUPs were substantially similar at station and multilocation trials 4 lines with variable zinc content were selected and 8 trait based SNPs for those selected lines were generated by KASP based SNP genotyping assay. The genotype IR14A150 has been found to have most extreme breeding values of 6.64 which predicts that the offspring will have higher yields. The results obtained from this study definitely paved a way for the creation of an elite pool having diverse high grain zinc content and high yield in order to develop varieties with such superior traits.

शोध सार

शोध शीर्षक: आधुनिक प्रजनन दृष्टिकोणों का उपयोग करते हुए ज़िंक समृद्ध चावल किस्मों के प्रजनन के लिए कुलीन माता- पिता पूल की पहचान

छात्र का नाम: सुभम शर्मा

प्रमुख विषय: पौध आणविक जीव विज्ञान और जैव प्रौद्योगिक

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प्रमुख सलाहकार के हस्ताक्षर

छात्र के हस्ताक्षर

दिनांक: 18-09-2020

विभागाध्यक्ष का हस्ताक्षर

जिंक महत्वपूर्ण सूक्ष्म पोषक तत्वों में से एक है जो हमारी प्रतिरक्षा प्रणाली को बढ़ाकर, मांसपेशियों को ऑक्सीजन खिलाकर, तंत्रिका तंत्र का समर्थन, प्रजनन स्वास्थ्य, त्वचा की चोटों और विभिन्न श्वसन रोगों का मुकाबला करके मानव शरीर में एक महत्वपूर्ण भूमिका निभाता है। जहां तक वर्तमान परिदृश्य का संबंध है, गंभीर संक्रमणों के नियमित प्रकोप ने मानव जाति के लिए खतरा पैदा कर दिया है। हालांकि, स्वस्थ संतुलित आहार के सेवन को बनाए रखने से इनसे बचा जा सकता है या प्रबंधित किया जा सकता है ताकि इस तरह की बीमारियों से निपटने के लिए प्रतिरक्षा प्रणाली को बढ़ावा मिल सके। मैक्रोन्यूट्रिएंट्स के अलावा, माइक्रोन्यूट्रिएंट्स (जस्ता, लोहा. मैग्नीशियम, विटामिन आदि) को भी संतुलित आहार का मुख्य घटक माना जाता है जिसकी आवश्यकता मानव शरीर पर अनंत सकारात्मक प्रभावों के साथ माइनसक्यूल मात्रा में होती है। सूक्ष्म पोषक तत्वों की आपूर्ति करने के सर्वोत्तम तरीकों में से एक Zn चावल, गेहूं और मक्का जैसी वैश्विक प्रधान खाद्य फसलों का सेवन है। मानव स्वास्थ्य में Zn के महत्व को देखते हुए, चावल में अनाज जस्ता सामग्री के साथ जुड़े " **आधुनिक प्रजनन दृष्टिकोणों का उपयोग**

में से एक Zn चावल, गेहूं और मक्का जैसी वैश्विक प्रधान खाद्य फसलों का सेवन है। मानव स्वास्थ्य में Zn के महत्व को देखते हुए, चावल में अनाज जस्ता सामग्री के साथ जुड़े " **आधुनिक प्रजनन दृष्टिकोणों का उपयोग करते हुए ज़िंक समृद्ध चावल किस्मों के प्रजनन के लिए कुलीन माता-पिता पूल की पहचान**" का अध्ययन चावल के रूप में जस्ता समृद्ध प्रधान फसल की जांच और विकसित करने के लिए किया गया था। जिंक से भरपूर चावल की किस्मों को विकसित करने की रणनीतियों में से एक है बायोफोर्टिफिकेशन- उन्नत अनाज ज़ेन सामग्री के साथ चावल प्रजनन।

वर्तमान अध्ययन के लिए मिल्ड राइस में अनाज जस्ता सामग्री का विश्लेषण करने के साथ-साथ परिवर्तनशीलता का विश्लेषण करने और Zn सामग्री के साथ फेनोटाइपिक लक्षणों को महत्वपूर्ण रूप से सहसंबंधित करने के लिए 75 उन्नत प्रजनन लाइनों (ABL) के एक पैनल का चयन किया गया है। बेहतर लाइनें। एबीएल के फील्ड फेनोटाइपिंग को कुछ एग्रोनोमिक गुणों के आधार पर प्रदर्शित किया गया है, उदाहरण के लिए, डीएफ, पीएच, पीटी, ईटी, जीवाई, एचआर और इसलिए पैदावार और अनाज जस्ता सामग्री पर जीनोटाइप एक्स पर्यावरण एसोसिएशन की जांच करना। एक मौलिक सीमा के रूप में पैदावार पर विचार करने से जीनोटाइप IR14V1020 में सबसे बड़ा GY (75.1 Q / hec) है, जबकि IR 45427-2B-2-2B-1-1::G1 (25.6 Q / hec) द्वारा प्रदर्शित बेस GY का अर्थ है कि YY लगभग 48.52 Q / hec के साथ है। 5.9% के विचरण का गुणांक। इसी तरह प्रसंस्करण गुण एचआर का एक उच्च अनुमान होने का आश्वासन देने का विचार किया गया है ताकि क्रेता का पक्ष लिया जा सके। HR के कारण जीनोटाइप IRRI 174 में सबसे चरम HR (71.68%) है, जबकि IR द्वारा दर्शाए गए आधार HR 45427-2B-2-2B-1-1 :: G1 (14.56%) का मतलब HR का लगभग 53%% होना है। 8.5% के विचरण का गुणांक। प्राकृतिक जाँच ने ABL में मौजूद अनाज जस्ता सामग्री, जीनोटाइप IR 45427-2B-2-2B-1-1 :: G1 सबसे चरम GZC (अनाज जस्ता सामग्री) (29.1 पीपीएम) का खुलासा किया, जबकि आधार GZC IR13N115 द्वारा प्रदर्शित किया गया (12.3 पीपीएम) मतलब GZC लगभग 17.9 पीपीएम है जिसमें 5.9% के विचरण का गुणांक है।

सांख्यिकीय परिचालनों के परिणामों से पता चला कि जीनोटाइप परिवर्तनशीलता की एक व्यापक भावना को प्रदर्शित करता है जिसमें सुझाव दिया गया है कि चावल में सुधार के लिए विभिन्न मात्रात्मक

और गुणात्मक वर्णों के चयन की पर्याप्त गुंजाइश है। 75 ABL के सहसंबंध गुणांक विश्लेषण ने खनिज सामग्री (जस्ता) और अनाज के बीच महत्वपूर्ण सहसंबंध दिखाया है। उपज संबंधित गुण। अनाज जस्ता सामग्री और अनाज की उपज ने सभी अध्ययन किए गए लक्षणों के साथ महत्वपूर्ण सकारात्मक सहसंबंध दिखाया। इंटरटेक लेबोरेटरी, हैदराबाद में किए गए जीनोमिक अध्ययनों से यह भी पता चला है कि चयनित स्क्रीन वाले जीनोटाइप में ब्लास्ट के लिए पीटा और पीआई 9 जीन को प्रतिरोध प्रदान करता है। ABL के लिए BLUPs (सर्वश्रेष्ठ रैखिक निष्पक्ष भविष्यवाणी) मूल्य रायपुर और पांच अलग-अलग स्थानों पर प्राप्त उपज के लिए उत्पन्न हुए थे। उपज BLUPs स्टेशन पर समान रूप से समान थे और बहुपरत परीक्षणों में चर जस्ता सामग्री के साथ 4 लाइनों का चयन किया गया था और उन चयनित लाइनों के लिए 8 विशेषता आधारित एसएनपी को KASP आधारित SNP जीनोटाइपिंग परख द्वारा उत्पन्न किया गया था। IR14A150 के जीनोटाइप में 6.64 के सबसे चरम प्रजनन मूल्य पाए गए हैं जो भविष्यवाणी करता है कि संतानों की पैदावार अधिक होगी। इस अध्ययन से प्राप्त परिणामों ने निश्चित रूप से इस तरह के श्रेष्ठ लक्षणों के साथ किस्मों को विकसित करने के लिए विविध उच्च अनाज जस्ता सामग्री और उच्च उपज वाले कुलीन पूल के निर्माण का मार्ग प्रशस्त किया।

CHAPTER - I

INTRODUCTION

Rice being the staple nourishment for the greater part of the total populace is nutritionally rich in 90 percent sugar, 8 percent protein and 2 percent fat is widely developed all through the tropics and subtropics. It likewise fills in as a significant wellspring of dietary vitality, proteins, nutrients and fundamental unsaturated fats, micronutrients for poor people and helpless society. Be that as it may, with the continuous financial turn of events and improved expectation for everyday comforts, improving nourishing quality, for example, micronutrient substance in grains has become another objective so as to comprehend the "shrouded hunger." Micronutrients, for example, iron (Fe), zinc (Zn), and selenium (Se), are basic dietary components for human wellbeing. These micronutrients are likewise significant for crop efficiency. Simultaneously, deficient admission of micronutrients, for example, iron (Fe), zinc (Zn), and selenium (Se) from food even causes serious medical issues, particularly for youngsters and ladies in the creating nations (Bouis *et al*., 2011). It has been seen that the insufficiencies of Iron, Zinc and Vitamin in human populace is common in creating nations. Zinc inadequacy fills in as the fifth significant reason for sicknesses that incorporates anorexia, dwarfism, powerless safe framework, skin sores, hypogonadism and looseness of the bowels and passings in nations (Kavya *et al*., 2020). The bioavailability of Zinc is regularly diminished because of high phylate substance of oat protein. Thusly, Biofortification, i.e., improving the grouping of supplements (smaller scale and full scale) in the consumable pieces of staple yields, is accordingly seen as the most savvy arrangement particularly in creating nations (Mayer *et al*., 2008).

Rice grain contains the accompanying: (I) structure which incorporates lemma and palea, and (ii) the rice caryopsis, which is known as brown colored rice. The hulled rice comprises of 6-7% wheat, 90% endosperm and 2-3% incipient organism and further expulsion of grain layer yields in polished (white) rice. It has been seen that, the more the measure of rice brown colored is expelled from grain during cleaning progressively mineral supplement is lost. The level of processing and cleaning lays a critical impact on the healthful parts of polished rice, particularly on minerals, because of a non-uniform dissemination of supplements in the bit. The general groupings of Zn in the diverse grain portions were; bran > hull > whole grain > brown rice > polished rice. For the most part, the substance of Zn in cleaned rice is a normal of just 12 mg kg/1, though the suggested dietary admission of Zn for people is 12–15 mg kg/1. Grain zinc content extended from 0.4 to 104 mg/kg in rice germplasm promotions and Bekele *et al*., (2013) revealed 16.1 to 88.6 mg/kg for the RIL populace in rice.

Zinc homeostasis in plants requires a tight and composed guideline of different transporters for cell take-up, send out and subcellular compartmentalization, and furthermore for chelation of free particles by little natural atoms, peptides and proteins (Ricachenevsky *et al*., 2015). Zn (II) is the most favored structure for take-up in roots, while Zn-NA (nicotianamine) or Zn DMA (deoxymugineic corrosive) are the significant structures for Zn translocation in rice (Bashir *et al*., 2012). Transporters associated with the take-up and transport of Fe and Zn incorporate protein (ZIP) family which have been distinguished as transporters for both Fe (II) and Zn (II) take-up with various substrate selectivity while other recognized ZIP individuals from OsZIP1 to OsZIP8 (aside from OsZIP6) are described as particular Zn transporters. ZIP transporters are viewed as one of the essential course for Zn take-up into the cytoplasm in plants, while MTP and HMA families are pivotal for Zn organelle sequestration/prohibition or entire plant apportioning (Palmer *et al*., 2009).

In the previous years, two basic investigations were directed; in the main evaluation, Descalsota *et al*., [2019] recorded phenotypic information of two DH populaces for two seasons and created genotypic information by utilizing a 6 K SNP chip and recognized a sum of 15 QTLs for agronomic characteristics and 50 QTLs for grain component fixation which incorporates 8 QTLs clarifying 8.6–27.7% PVE for grain zinc. They additionally contemplated the joined impact of QTL in the two populaces. Among the single-QTL lines, those with qZn9.1 displayed most noteworthy mean grain Zn of 18.1 and 19.1 mg kg−1 for two back to back seasons, separately. They announced that the Zn content increments by the expansion in number of QTLs and watched most noteworthy grain Zn of 28.2 and 24.3 mg kg−1 in two seasons, individually, in four QTLs (qZn2.1 + qZn5.1 + qZn5.1 + qZn11.1). Their outcomes demonstrated the likelihood of QTL pyramiding for improving the zinc content in rice. In the subsequent investigation, Kumar *et al*., distinguished one QTL for Zn and five QTLs for Fe having PVE 25% and 34.6–95.2%, separately, utilizing F4 populace (579 people) got from a combination of PAU 201 and Palman. These recognized QTLs can altogether upgrade the viability of rearing projects to improve the Zn and Fe thickness in rice.

SNPs serves as the major source of genetic variability across germplasm and their wild relatives therefore widely used in breeding programs for marker assisted and genomic selection, association and QTL mapping, positional cloning, haplotype and pedigree analysis, seed purity testing and variety identification (Bernardo 2008, Eathington *et al*., 2007, Jannink *et al*., 2010, Lorenz *et al*., 2010, Moose and Mumm 2008). Yamamoto *et al*., (2010) arranged an investigation in which a lot of 2,688 SNPs were utilized to genotype 151 Japanese rice cultivars discharged in the course of the most recent 150 years. At first, entire genome re-sequencing of the tip top Japanese cultivar, Koshihikari, was attempted and the short grouping peruses were adjusted to the Nipponbare genome. An aggregate of 67,051 SNPs were found, and from this disclosure pool, a lot of 2,688 well distributed SNPs (each 100–200 kb) were utilized to build up an Illumina GoldenGate SNP-discovery measure. This measure was utilized to genotype the Japanese rice cultivars, and in the wake of dispensing with ineffectively performing markers, 1,917 excellent SNPs were recognized. These SNPs were utilized to build up a blend of 768-SNP OPAs for use on the Illumina GoldenGate BeadArray stage. This SNPs can likewise be screened utilizing focused on genotyping frameworks that grant clients to settle on choices on-the-fly about which SNPs to remember for a test. These frameworks might be specifically compelling to reproducers and scientists who are keen on dissecting few explicit loci in countless examples. Hereditary portrayal of grain Zn in different Recombinant Inbred Lines (RILs) and furthermore in rice germplasm assortments has demonstrated noteworthy Phenotypic Co-proficient of Variation (PCV), Genotypic Co-effective of Variation (GCV), expansive sense Heritability and Genetic Advance (GA). Among the various investigations PCV and GCV for grain Zn focus shifted from 9.3 % to 40 % and from 9.2 % to 36 % separately, while heritability fluctuated from 41 % to 99.4 % and GA differed from 18.6 % to 66.6 %. Therefore the following study entitled **"Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches**" has been planned with the following major objectives:

1. Phenological characterization of diverse set of rice genotypes for various yield & quality attributes and grain mineral content.
2. Assessment of agronomically important genes in elite parental lines.

CHAPTER- II

REVIEW OF LITERATURE

All around the world, just about 800 million people are unremittingly energetic, inferring that they are rejected from real food to the extent calories (FAO *et al*., 2017). Practically about more than 2 billion people face the issue of covered wanting, suggesting that they experience the evil impacts of micronutrient needs (WHO, 2006). In spite of the fact that major advances have been taken in diminishing these issues for instance completing yearning in the whole of its structures – as referenced in the Sustainable Development Goals (SDGs) – remains an overall test (FAO *et al*., 2017; Stokstad *et al*., 2015; Obersteiner *et al*., 2016). The target of fulfillment hunger in the whole of its structures incorporates a wide significance of craving, including calorie needs (endless craving), micronutrient deficiencies (covered appetite), and related clinical issues. Disregarding the way that the goal to show up at Zero Hunger by 2030 is one of the fundamental objective of the Sustainable Development Goals, our hard-won increments are at present under peril or being convoluted. The 2019 GHI (Global Hunger Index) uncovered that various countries have higher needing levels now than in 2010, and around 45 countries are set to disregard to achieve low degrees of craving by 2030. Battle, isolation, and the effects of natural change have all additional to consistently raised degrees of yearning and food helplessness around the world. Outrageous climatic changes, savage conflicts, wars, and financial interruption and crises continue driving craving in various bits of the world. In like manner at the overall level a specific instrument called Global Hunger Index (GHI) to widely evaluate and follow hunger at around the world, regional, and national levels. GHI generally incorporates four pointers to be explicit undernourishment, kid wasting, youth thwarting and child mortality (Grebmer, Bernstein *et al*., 2019). In spite of the way that old style plant raising techniques like backcrosses and assurance of the perfect predominant rice cultivars with high sound advantage had laid a colossal impact on improving biofortification of rice cultivars yet by benefitting the progressing developments, for instance, DNA markers, inherited structure and allele mining allows to use them as an instruments to recognize the allelic assortment in characteristics essential these attributes and related QTLs/characteristics to improve the capability of conventional plant replicating through marker helped choice (MAS) (Pandit *et al*., 2016). As such staple food crops are centered around generally with the ultimate objective of biofortification so the people could be given the perfect enhancements in real add up to crush the issue of debilitated wellbeing and fight hid longing. The enhancement substance of noteworthy harvests is referenced in the Table 2.1 underneath.

Table 2.1: Nutrient content of major staple foods per 100 g portion

Nutrient Components	**Carbohydrtaes (g/100g)**	**Protein (g/100g)**	**Zinc (mg/100g)**	**Iron (mg/100g)**	**βcarotene (µg/g)**
Maize/ Corn	74	2.71	2.21	9.4	97
Rice (white)	80	0.8	1.09	7.1	0
Wheat	71	3.19	2.65	12.6	5
Potatoes	17	0.78	0.29	2	1
Cassava	38	0.27	0.34	1.4	8
Soybeans (Green)	11	3.55	0.99	13	0
Sweet Potatoes	20	0.61	0.3	1.6	8509
Yams	28	0.54	0.24	1.5	83
Sorghum	75	4.4	0	11.3	0
Plantain	32	0.6	0.14	1.3	457
RDA	130	8	11	50	10500

Source: "Nutrient data laboratory" (http://ndb.nal.usda.gov/ndb/search/list). United States Department of Agriculture. 2016

Rice being one of the critical staple food crops expect an extensive situation in improving the overall food security. The progression of new high yielding rice varieties having improved disorder impediment, strength to abiotic stresses, and unequivocal quality characteristics ought to be the critical objective towards achieving overall food security. The enthusiasm for rice is depended upon to augment radically and thusly basic yield updates are required for fulfilling the solicitations. The pace of genetic increment for grain yield has been assessed to stuck at generally 1% consistently which includes real concern, since it would not be satisfactory to the fulfill foreseen requirements for rice (Cobb JN *et al*., 2019). Biofortification, i.e., improving the gathering of micronutrients in the consumable staple yields, is in this way picked as the most monetarily wise course of action especially in making countries (Mayer *et al*., 2008). Biofortification fills in as a forthcoming, promising, monetarily keen, and acceptable technique for passing on micronutrients to a masses that has limited access to different eating systems and other micronutrient interventions. Attempts have been made for the assurance of refreshingly rich varieties of rice so the sustenance needs of the masses can be met. Advances in the field of genomics during the latest two decades are extraordinarily expected to accelerate the improvement of grain cultivars with high bio-available micronutrient obsession in the palatable parts. Now, a couple of stages have been adopted in understanding the strategies to grow the micronutrient intermingling of the cleaned and unpolished rice through genetic structure approach/ordinary raising philosophy. The degree of preparing and cleaning had been known to impact the feeding portions of white rice, especially on minerals, in view of a non-uniform movement of enhancements in the bit. The cleaned or cleaned rice when in doubt incorporated the rice grain endosperm, and the wheat contains most of the lacking creature and aleurone layer (Kavya HP *et al*., 2020). Along these lines, CGIAR began biofortification program through its Harvest Plus action with which overall cultivating and assessment centers are been allowed to make improved collections of staple yields which are overwhelmingly copious in supplements and minerals. In order to meet the dietary requirements of masses new collect groupings are to be made which have the significant enhancements content upto perfect level. Henceforth, Crop Improvement method incorporates screening germplasm for existing innate respectable assortment, making lines satisfactorily improved in other agronomic ascribes to fill in as guardians in duplicating venture, making and testing micronutrient thick germplasm, driving inherited examinations and making nuclear markers to cut down the costs and resuscitate the pace of raising. The availability of the rice genome courses of action and the data on bioinformatics procedures update the pertinence of the allele mining strategy to analyze the inherited reason of the assortment of micronutrients in the grain, planning of the quantitative trademark loci (QTL) and conspicuous confirmation of characteristics will give the reason to setting up the systems and improving the grain micronutrient obsession in rice. In this section an undertaking is made to review the open composition on biofortification, QTL examination and usage of availabel markers having high phenotypic vacillation quality that can be utilized for marker helped assurance.

2.1 Necessity of Micronutrients for Human Health:

Micronutrients are required in follow sums anyway their satisfactory deftly improves supplement availability and insistently impacts the physiology that is reflected in their yield and productivity. Exactly when micronutrients are lacking in soil the turn of events and yield of harvests are genuinely debilitated. The closeness of micronutrients inadequacy renders it boundless for the plants to increment most outrageous benefit by NPK manures application. In spite of the way that these enhancements are successfully connected with various plant advancement frameworks, yet their specific occupation for yield improvement in crops is still ought to be avowed. Supplements and minerals are essential dietary parts which are required in little adds up to finish all metabolic development, including cell hailing, motility, extension, partition and apoptosis that oversee tissue advancement, limit and homeostasis. Iron and zinc micronutrients are the most huge parts, absence of which is a critical explanation behind debilitated wellbeing. Iron fills in as a crucial piece of oxygen-moving proteins viz. hemoglobin and myoglobin and moreover huge part various synthetics related with imperativeness creation and the upkeep of immune limits (Stoltzfus *et al*., 2001). Fe deficiency in human prompts extended peril of maternal mortality, shortcoming, unfavorable births, low birth weight, and ruined scholarly and engine improvement (Bouis *et al*., 2003).

Zn expect a key job in physical turn of events and improvement, working of safe system, regenerative prosperity, respiratory limits, unmistakable limit, reduces infant demise rate, decreases degenerative changes in adults and neurobehavioural progression (Hotz *et al.*, 2004). Zinc inadequacy prompts distinctive clinical issues, for instance, incapacitation of pubescence advancement, incident in longing for, sufficiency of pregnant and lactating women, defers wound recovering, pneumonia. Zinc homeostasis in plants requires extraordinarily close and significantly arranged rule of transport for cell take-up, convey and subcellular compartmentalization, and besides helps in chelation of free particles by minimal normal molecules, peptides and proteins (Ricachenevsky *et al.*, 2015).

2.2 Impact of micronutrient deficiency on human health:

Micronutrients expect a critical job in assimilation similarly as in the help of tissue work. Very nearly 30 supplements and minerals that our body can't create in satisfactory totals in solitude are called as "fundamental micronutrients" which we genuinely get from a fitting eating routine. Their openness in the perfect sums and their privilege blends can over the long haul make differentiate between a sound nearness and one that is trouble by unforeseen shortcoming. All through the world, micronutrient needs (MNDs) exist, with pregnant women and their children under 5 years of age are all things considered important risk. Iron, iodine, folate, supplement and zinc insufficiencies are the most notable unfathomable MNDs, and all these MNDs are critical supporters of helpless turn of events, academic weakenings, perinatal bothers, and extended risk of repulsiveness and mortality (Bailey *et al.*, 2015).

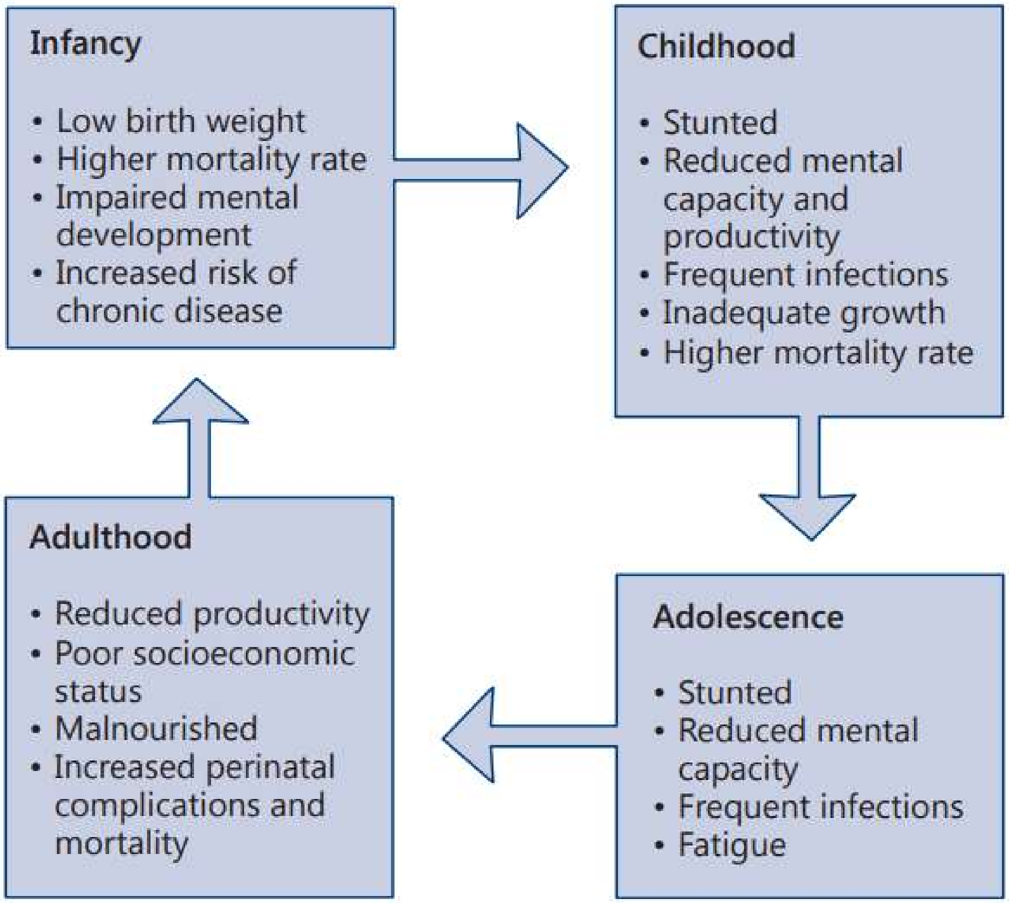


Figure 2.1: Source: National Institutes of Health (NIH). Annals of Nutrition and Metabolism published by S. Karger AG, Basel

Iron deficiency being the most notable MND on earth, which has been affecting over 30% of the absolute people, a normal 2 billion people. Iron deficiency prompts defenselessness and upsets perfect limit of both the endocrine similarly as safe structures. Iron deficiency is fundamental during pregnancy considering extended requirements for fetal turn of events and headway. It has been evaluated that iron insufficiency adds to 20% of maternal passings. All around, the World Health Organization (WHO) assessed that 25% of the masses (1.62 billion people; CI 1.50–1.74 billion) has shortcoming. Preschool kids (47.4%) and pregnant women (41.8%) have the most essential prevalence when all is said in done. Need zinc is seen as one of the fundamental drivers of grimness in making countries and, yet, incredibly especially less is contemplated the status of the world. . Universally, it has been assessed that 17.3% of the populace are zinc insufficient, with the most raised measures in Africa (23.9%) and Asia (19.4%). Pregnant females and their little adolescents has a spot with the most raised danger packs for zinc need. Starting at now, the WHO and UNICEF propose game plan of zinc supplements for 10–14 days close by oral rehydration treatment for extraordinary free entrails (Bailey *et al*., 2015).

2.3 Worldwide predominance of hidden hunger

Around 800 million people comprehensive are continually excited, which suggests that they are without real admission of calories (FAO *et al*., 2017). Helpless food to the extent micronutrient insufficiencies for instance disguised longing for has been found more than 2 billion people wherever all through the world. Despite the fact that fundamental advances have been taken to fight these issues, completing craving in the aggregate of its structures – as referenced in the Sustainable Development Goals (SDGs) – remains a general test (Allen *et al*., 2018; FAO *et al*., 2017; Obersteiner *et al*., 2016; Stokstad *et al*., 2015). The prime objective of fulfillment hunger in the sum of its points of view incorporates a wide importance of wanting, including calorie deficiencies (consistent longing for), micronutrient needs (covered appetite), and other related clinical issues. While inspecting for longing and food security, starvation is normally explained using varieties of UNICEF's applied framework for "explanations behind absence of sound food and destruction" (Black *et al*., 2008; Smith and Haddad, 2015).

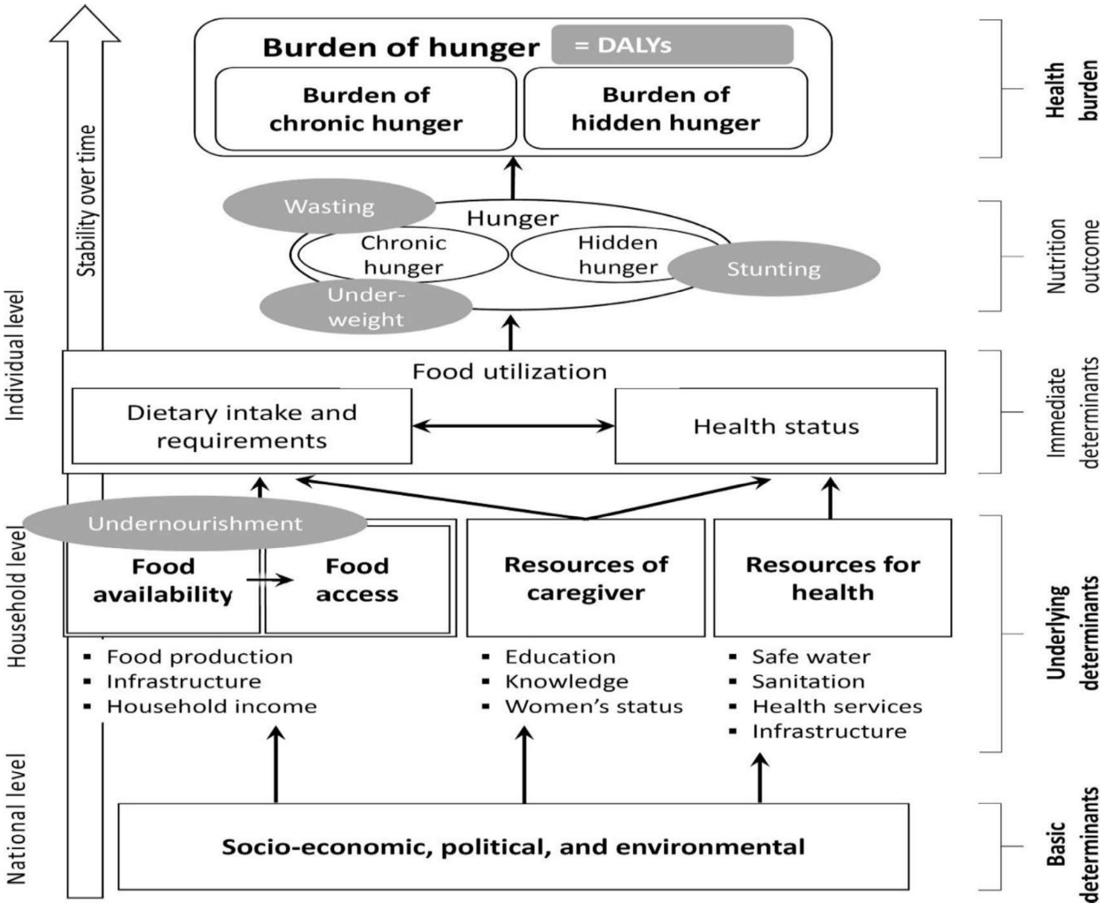


Figure 2.2. Determinants of hunger including related proxy measures. The conceptual framework is based on (UNICEF, 1990), with the elements in grey showing which indicators are used in the literature to analyze hunger and measure food insecurity. DALYs, Disability-Adjusted Life Years.

Feeble wellbeing has been seen as one of the noteworthy causes which constrains India's overall monetary potential. The no matter how you look at it weight of feeble wellbeing is hesitantly high, with about portion of all going of children under five years identified with helpless food (Black *et al*., 2013). An overall appraisal on kid ruining and money related outcomes communicated that a 1 cm increase in height was associated with a 4% development in pay for men and a 6% extension in pay for women (McGovern *et al*., 2017). Despite the imperative money related advancement in India over last continuous decades, consistent absence of sound food (upsetting) among adolescents having a spot with five years of age has diminished by just 33% some place in the scope of 1992 and 2016 and remains incredibly high, with 38.4% of children ruined in the country (NFHS, 1992; IIPS, 2017).

2.4 Approach to gear up micronutrient malnutrition

Micronutrient insufficiency for the most part happens when there is nonattendance of nutritious food in diet. The supplements and minerals expected to thwart micronutrient sickly wellbeing are acquired from a variety of nutritious staples. Courses of action and activities are to be made and realized to ensure better all year access to their relentless deftly and use of an adequate grouping and measure of safe, micronutrient-rich sustenances. Micronutrient-rich sustenances incorporates food sources that are copious in supplement A (retinol) and its antecedent (beta-carotene), iron (both haem and non-haem) and iodine and moreover sustenances which contain factors that redesign micronutrient ingestion (e.g., dietary fat and supplement C).

Four strategies have been designed to improvise the consumption of micronutrients in adequate quantities they are:

2.4.1. Introduction of Heterogeneity in Diet:

It incorporates improving the availability, receptiveness and utilization of sustenances well off as central micronutrients. It in like manner helps in improvising the physiological conditions of an individual by giving the fundamental nutritious food to the extent quality similarly as sum. Thusly for fulfilling the necessities of the masses we need to move to better social practices, better food storing and deftly chain, similarly as attempt should be settled on to improve food decision models and traditional methods for food getting ready (Hotz *et al*., 2001).

2.4.2. Promoting use of essential dietary supplements:

Direct supplementation of basic micronutrients to powerless subpopulations, this can be accomplished through an essential human services framework or social insurance conveyance framework like an inoculation program which has demonstrated to be viable and very financially savvy for little youngsters for nutrient. It principally works just if the enhancements are accessible and available and just constrained people really take them. These days much consideration has been dedicated towards utilization of numerous micronutrient supplementations, as opposed to the indigenous iron and folic corrosive just, for pregnant ladies; at times, noteworthy focal points has been seen (Novotny *et al*., 2010).

2.4.3 Fortification of essential foods:

Fortress of food suggests to the development of micronutrients to dealt with and packaged food things. A significant part of the time, this mediation had incited decently quick upgrades in the micronutrient status of a people, and at a genuinely reasonable cost, especially if points of interest can be cultivated from "Codex General Principles for the Addition of Essential Nutrients to Foods" in that it moreover unites world class general clinical favorable circumstances that may be gotten from extended micronutrient confirmations (as opposed to simply obvious focal points), considering new and creating consistent data. Food fortress can be of following sorts, for instance, mass stronghold, coordinated stronghold and market-driven fortress (Lindsay *et al*., 2006).

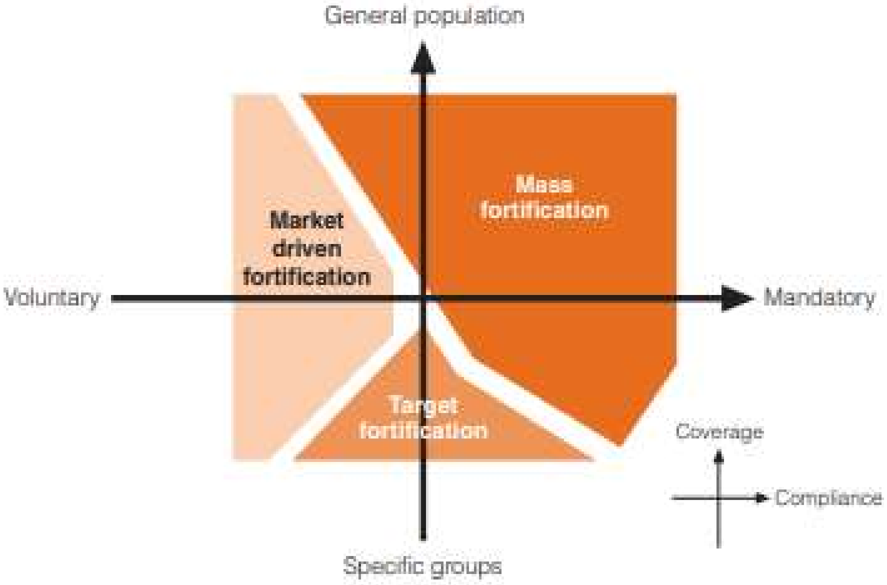


Figure 2.3: The interrelationships between the levels of coverage and compliance and the different types of food fortification

The critical purpose of food fortification joins headway of person's prosperity instead of deficiencies' expectation (Dwyer *et al*., 2015). So to fight all of these troubles, nutritious and unobtrusive sustenances are made from the locally available sustenances. Measures are being taken by introducing food-to-food stronghold. Food-to-food fortress is a technique that relies upon using an available, and open neighborhood resource (plant or animal) to fortify another food (Uvere *et al*., 2010). In any case, it's an irksome technique to find an advantage which meets the availability and moderate receptiveness conditions. It is in like manner significant that the fortress of food most likely won't impact the substantial properties of the food that ought to be supported.

2.4.4. Postharvest Processing:

It helps in keeping up and growing the time period of practical ease of use of perishables and decreasing food setbacks. Postharvest adversities are critical and abatement of such hardships would be the least requesting, more affordable, and best method instead of boosting food creation. They serve to diminish the breath rates, thwarts maturing, cuts down ethylene creation, and at the same time block senescence, hinder absence of hydration, and widen the time span of practical ease of use henceforth securing produce quality. Nowadays different systems have been performed for instance the use of transcriptomics, proteomic, and metabolic stages, broadened with various biochemical assessment similarly as phenotyping are being performed to grasp pressure physiology and its astounding rule at various degrees of cell control (e.g., epigenetic control, post-transcriptional, post-translational) in order to make and improve current imaginative techniques (Pedreschi *et al*., 2015).

2.4.5. Biofortification:

Generally speaking estimation predicts that by 2050 anyway there will be adequate calories made to deal with the overall people, yet there won't be adequate enhancements to take care of it. A multithemed approach is fundamental to not simply ensure that calorie affirmation is smoothed out rather to avoid both preventing and heftiness (the twofold weight), moreover to ensure that the sustenances exhausted contain enough micronutrients (Costa *et al*., 2019). Thusly biofortification can be described as the path toward raising food respects make crops that are progressively indulgent in micronutrients so as to improve the enhancement status of food. The bounty of biofortified staple reaps mainly depends upon whether they are recognized and eaten up by centered masses or not. A couple of examinations have been acted over the latest 8 years to understand purchasers' affirmation of sustenances made with biofortification. Purchaser affirmation is analyzed similarly as their unmistakable evaluation and financial valuation of biofortified varieties inverse conventional ones. Collections made under biofortification have appealing agronomic attributes, for instance, critical returns, affliction ressistant and usage properties that organize or beat those varieties farmers at present create. These purchaser affirmation peruses were performed for various harvests, joining those with a conspicuous food property (i.e., supplement An improved biofortified crops which changes concealing from white/cream to yellow/orange in view of the extended beta carotene content, for instance, OSP, supplement A maize and supplement A cassava, similarly as those yields with an imperceptible sustenance quality (i.e., mineral, for instance, iron or zinc, propelled harvests that don't change concealing with biofortification, for instance, iron pearl millet and iron beans.

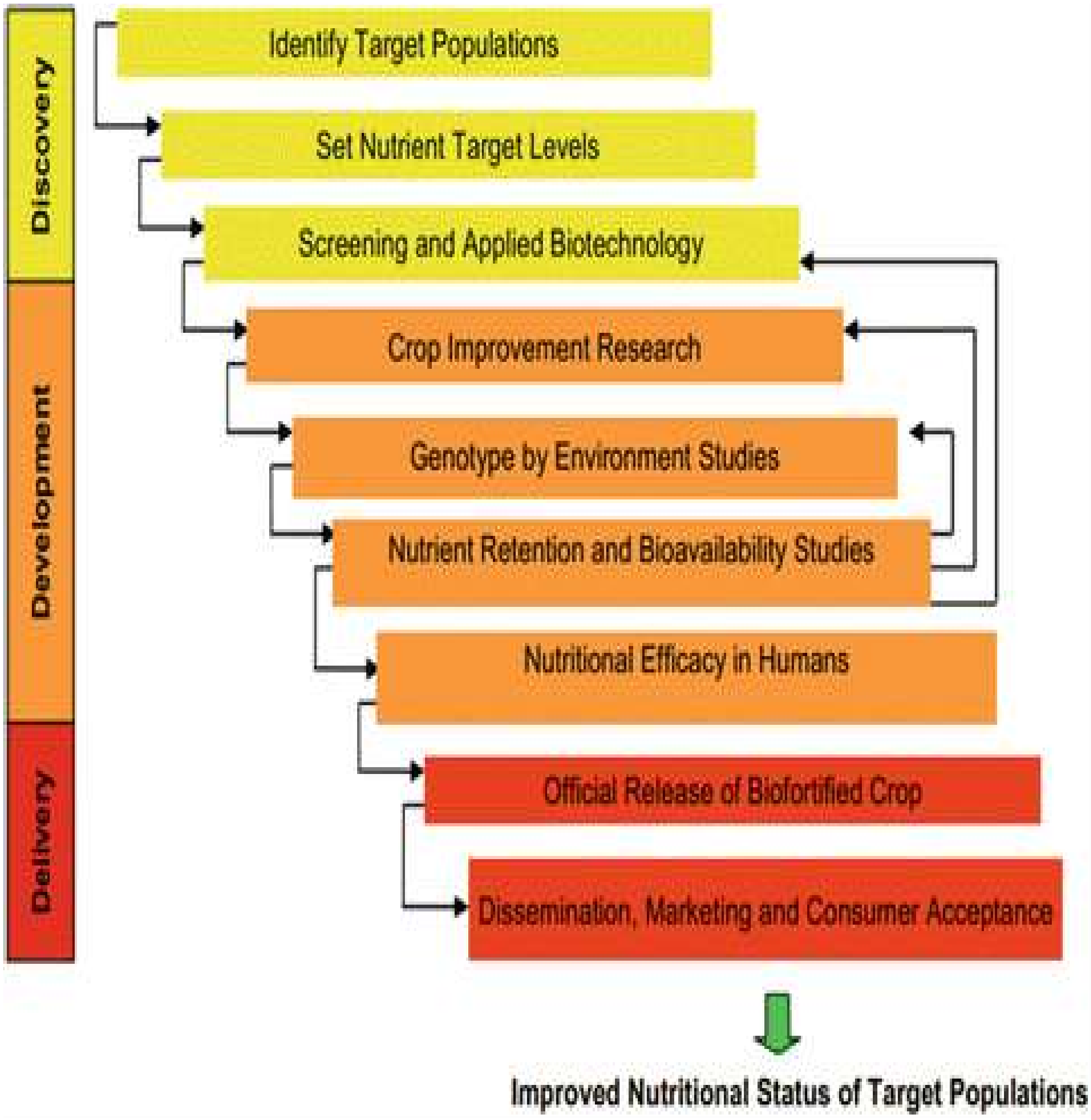


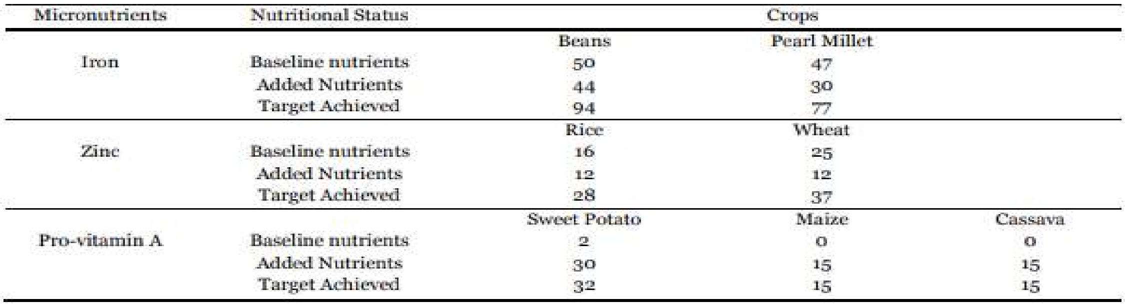
Figure 2.4: Pathway Showing Strategy of Biofortification & Product Development.

Source: Biofortification: Pathway Ahead and Future Challenges, Springer.

For example, going among IR8 and Taichung (Native)- 1 provoked the headway of new rice cultivar, having exceptional yield properties and are semi prevail in nature. This rice grouping produces 21 µg/g (2-cover) of iron obsession in earthy colored shaded rice. Moreover, IR68144 can hold breaking point of the iron substance (deduced 80%) in the wake of cleaning for 15 minutes diverged from various arrangements. Besides, use of IR68144 was raised to improve the degrees of iron in woman. Moreover approaches have been made in iron biofortification to improve the statement of ferritin by introducing soybean ferritin (SoyferH1 and SoyferH2) characteristics into rice (Xian Kok *et al*., 2018). A transgenic approach has been passed on to continually manufacture the Zn substance of oat grains which may incorporate the usage of transporters related in Zn translocation. As to take-up, translocation and declaration, people from the ZIP family expect a mind-boggling work (Sharma *et al*., 2012).

2.5. Goals and Objectives achieved through Biofortification:

Biofortification of staple yields fills in as a sufficient option for giving enhancement rich quality food to colossal amounts of nation poor dispersed across isolated domains of Africa and South Asia. Notwithstanding the way that before all else it demands some greater budgetary and intelligent data sources, yet the discontinuous costs are immaterial. Biofortification of staple harvests, for instance, rice, wheat, vegetables, etc is a sharp procedure to show up at an immense number of people on a pragmatic reason. Notwithstanding the way that earlier ordinary rearing strategies were performed to redesign supplement status of staple harvests, anyway nowadays it has been replaced by new advances, for instance, biofortification since they were dull and repetitive. The table surrendered underneath entireties the fundamental enhancement content in specific yields.



Micronutrients	Nutritional Status	Crops		
		Beans	Pearl Millet	
Iron	Baseline nutrients	50	47	
	Added Nutrients	44	30	
	Target Achieved	94	77	
		Rice	Wheat	
Zinc	Baseline nutrients	16	25	
	Added Nutrients	12	12	
	Target Achieved	28	37	
		Sweet Potato	Maize	Cassava
Pro-vitamin A	Baseline nutrients	2	0	0
	Added Nutrients	30	15	15
	Target Achieved	32	15	15

Table 2.2: Biofortification targets achieved through Conventional breeding (Parts per Million)

Source: International Journal of Biosciences, Vol. 15, No. 3, p. 206-218, 2019

In the wake of examining the limit of yield biofortification for achieving country's dietary security, ICAR moved a Consortia Research Platform (CRP) to ICAR-Indian Institute of Rice Research (IIRR), during the time of 2014–17. This has been done to enhance the sustaining status of huge staple food yields of the country, for instance, rice, wheat, maize, sorghum, pearl millet and little millets through biofortification moves close (Neeraja *et al*., 2017). The procedure of harvest improvement includes germplasm assortment, screening of germplasm, Pre reproducing of germplasm, testing and assessment of that germplasm for specific supplements for which it would be used in future without trading off the qualities and yield. Markers are being created which ought to be financially savvy and monetary for distinguishing the attributes. These markers are approved under various areas to check the Genotype × Environment connection so the impact of condition on the supplements that are presented in that harvest can be known (Bouis *et al*., 2017). In rice reproducing, assortments with high zinc and iron are chosen and screened, later on these chose genotypes are joined with the mineral attributes (Garg *et al*., 2018).

In the previous years a few a large number of rice germplasm lines have been examined for Fe and Zn content in both earthy colored and cleaned grain over the world, subsequently many promising givers have being distinguished. In any case, in lion's share of conditions, 90% of Fe and 40% of Zn are lost during cleaning. In spite of the fact that few QTL for grain Zn focus and different qualities related with Zn digestion have been distinguished in rice. Utilizing giver from the HarvestPlus program, 'DRRDhan 45' was discharged by IIRR. Two additional assortments with high Zinc content in cleaned rice were discharged as 'Chattisgarh zincrice-1' for the territory of Chattisgarh by Indira Gandhi Krishi Viswavidyalaya (IGKV) and as 'Mukul' (CRRDhan 311) for the province of Odisha by ICAR-National Rice Research Institute (NRRI) (Neeraja *et al*., 2017).

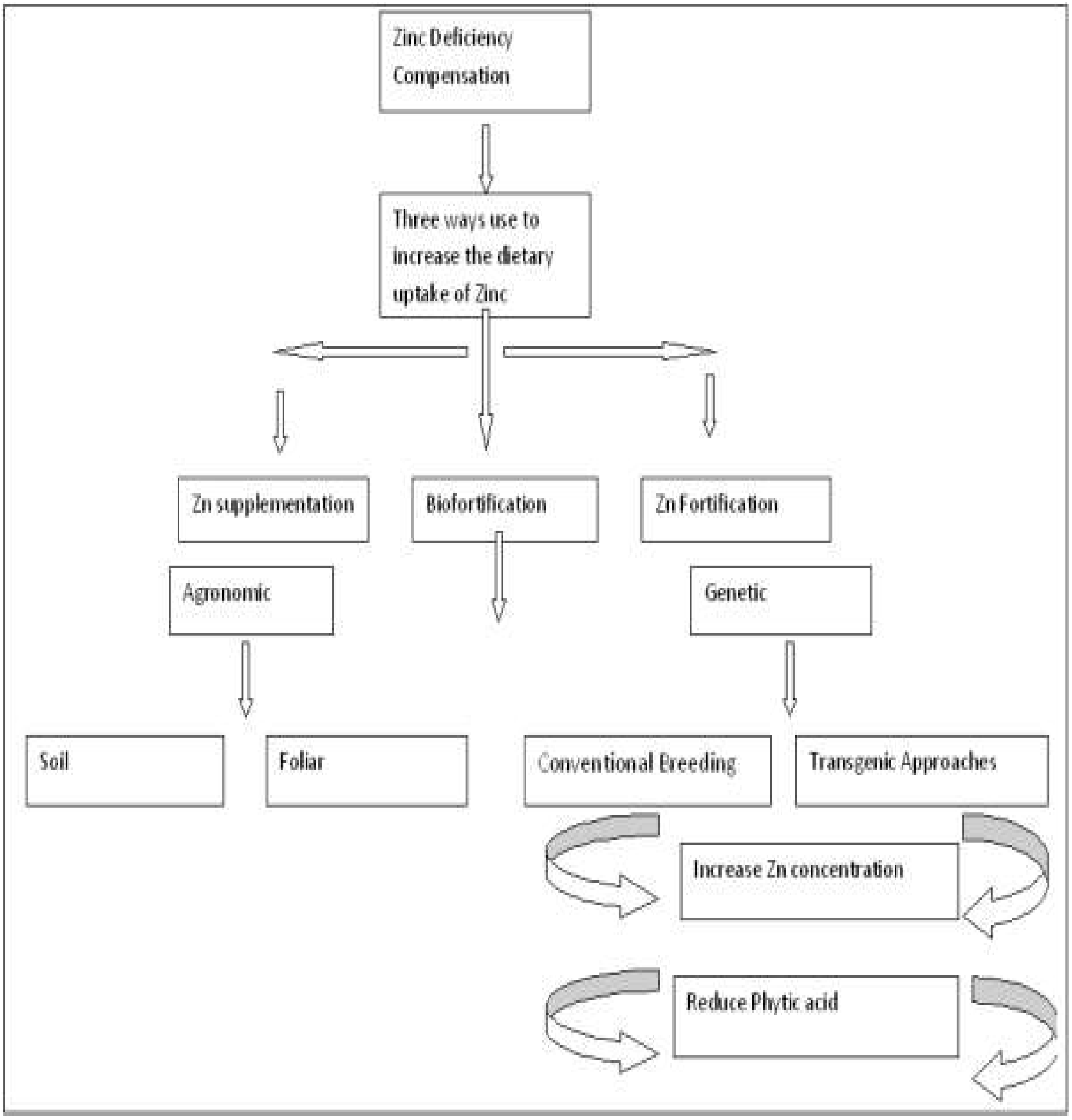


Figure 2.5: Different approaches to overcome dietary Zn deficiency (Nakandalage *et al*., 2016).

2.6. Rice as an essential source of dietary nutrients:

Rice (Oryza sativa) build up a major piece of diets and asset for over 3.5 billion people wherever all through the world. Cleaned rice has been essentially eaten up as staple food, satisfying step by step essentialness demands similarly as the noteworthy wellspring of various dietary enhancements required for advancement and food of an individual. Earthy colored shaded rice is correspondingly continuously nutritious, containing more lipids, minerals, supplements, dietary fiber, micronutrients, and bioactive blends. The sound advantage of cleaned rice is finally constrained by the rice endosperm, which essentially contains starch (70–80%), with proteins and lipids in little degrees, around 7–10% and under 1%, independently (Yang *et al.*, 2019). It has been understood that the cleaning improves rice cooking and eating quality, and overhauls its time range of ease of use; regardless, it moreover diminishes the enhancement substance in light of the fact that the early life form and the wheat layers are affluent in lipids, proteins, dietary fiber, and various parts. Covered yearning inconceivably wins in low-and focus pay countries and holds significant individual, social, and money related outcomes (Ruel-Bergeron *et al.*, 2015). Since 2003, HarvestPlus and its assistants have outlined that raising undertakings have made basic improvement of a couple of staple food crops having imperative degrees of the three micronutrients that are commonly obliging in keeps away from food: zinc, iron, and supplement A (Bouis and Saltzman 2017; HarvestPlus 2019).

Nutrient proximates	Brown rice	White rice
energy	82 calories	68 calories
protein	1.83 g	1.42 g
total lipid (fat)	0.65 g	0.15 g
carbohydrates	17.05 g	14.84 g
fiber, total dietary	1.1 g	0.2 g
sugars, total	0.16 g	0.03 g
calcium	2 milligrams (mg)	5 mg
iron	0.37 mg	0.63 mg
sodium	3 mg	1 mg
fatty acids, total saturated	0.17 g	0.04 g
fatty acids, total trans	0 g	0 g
cholesterol	0 mg	0 mg

Table 2.3: Nutrient content of White rice and Brown rice.

Source: https://www.healthline.com/health/food-nutrition/brown-rice-vs-white-rice

It has been considered that polished rice of some basic assortments around contain 2 µg/g Fe and 16 µg/g Zn on a normal. In any case, the significant exertion has been made to accomplish Fe-and Zn-enhanced rice by expanding these qualities by around six and two creases individually, with the goal that the objective focuses (Fe: 13 µg/g; Zn: 28 µg/g) can be achieved in biofortifed rice, which will permit individuals to jump on a normal about 30% of the evaluated normal every day necessities (Trijatmiko *et al*., 2016). A few quantitative quality loci (QTLs) reads have been directed for getting high Fe and Zn rice utilizing biparental populaces, and three meta-QTLs for grain Fe and two for grain Zn were identifed dependent on various examinations (Dixit *et al*., 2019). The recognizable proof of these QTLs have encouraged the reproducing procedure for high grain Fe and Zn rice assortments by utilizing marker-helped choice (MAS).

2.7 Phenological characterization of diverse set of rice genotypes for various yield & quality attributes:

In order to meet the demand of the growing population it is very crucial to select plants which are potentially sound to provide high yield. Therefore, phenotyping is essential in establishing the relationship between genes and traits.

Rahman *et al*., (2015) investigated that because of a gigantic increment in the utilization of food, feed, fuel and to meet the basic necessities of worldwide food security for taking care of the quickly developing human populace, so there is an interest to raise high yielding harvests that can without much of a stretch adjust to the worldwide climatic changes, especially in creating nations. To determine these worldwide difficulties, novel methodologies are required to recognize the quantitative phenotypes and furthermore to clarify the hereditary premise of horticulturally significant qualities. These advances helps in encouraging the screening of germplasm with superior qualities in asset restricted conditions. As of late, plant phenomics has given a set-up of new innovations, and we are on a way for updating the portrayal of complex plant phenotypes. High-throughput phenotyping stages have additionally been developed that helps in assortment of phenotypic information from plants in a non-ruinous way. Many plant scientists have been making endeavor to propose proper procedures for plants that would be impervious to ecological pressure, creepy crawlies and illnesses, while still have high supplement productivity (Zhang *et al*., 2007; Ahmed *et al*., 2013). For improving the supplement substance of oat crops, a great deal of exertion has been placed into utilitarian genomics considers which had chap prompted advancement of high-throughput genomic instruments (Huang *et al*., 2013; Pallotta *et al*., 2014; Valluru *et al*., 2014). Still more exertion has been required to coordinate genotype-phenotype connections for the worldwide improvement of yield reproducing (Tester *et al*., 2010). Because of the quick headway in the field of useful genomics and hereditary innovations, particularly in the field of high-throughput sequencing innovation many plant genomes are currently distinguished. Their utilitarian examination has entered the high-throughput stage, which empowers them for giving the accessible hereditary data just as empowering the genomic investigation.

Chandel *et al*., (2017) made an endeavor by assessing set of 96 rice genotypes including landraces, propelled rearing lines and discharged rice cultivars for grain micronutrient (Fe/Zn) and protein substance followed by perceiving different sub-atomic markers related with the above attributes. More extensive hereditary changeability for grain nutritive qualities i.e., iron, zinc and protein content in cleaned grain was recorded, which went from 14.3 ppm (Bisni) to 4.8 ppm (an ABR line got from IR681444/Morobarakan) for iron substance, 34.8ppm (Dullar) to 13.6ppm (GP-145-138) for Zinc and 10.28% (Kalam Gurmatia) to 5.78% (CGR-1539) for grain protein content among a few tried rice genotypes. Further, so as to identify the different sub-atomic markers related with grain micronutrient and grain protein levels rice genotypes were portrayed into a few gatherings utilizing SSR, QTL explicit and quality explicit markers. Out of the all out arrangement of 42 markers, 18 markers apparently was displaying polymorphism. It was seen that a sum of 36 alleles were delivered by 18 SSR preliminary set that are utilized in this examination. On performing single marker examination it was uncovered that eight DNA markers i.e., 7 RM marker and one QTL explicit marker Crm33-1 were related with grain iron, zinc and protein substance. The benefactor rice genotypes which were related to high grain Fe, Zn and protein substance and DNA markers related with three attributes will be of proficient use in the prompt improvement of nutri-rich rice assortments to battle ailing health. It was likewise seen that an aggregate of 8 huge markers were found related with the cleaned grain iron, zinc and protein content ($P<0.05$). The entirety of the 8 critical SSR loci were recognized for the iron, zinc and protein content, with the R2, level of the all out variety disclosed running from 4.04 to 9.1 %.

Descalsota *et al*., (2019) endeavored to build the convergences of components in the grain so as to mitigate the lack of healthy sustenance. It has been focused to create rice assortments having higher supplement content with exceptional returns in a savvy way. Sub-atomic markers were produced for high grain convergences of fundamental components, especially Zn, for its utilization in marker-helped choice (MAS) which can without much of a stretch quicken rearing endeavors to create rice assortments with supplement thick grain. We made an endeavor of QTL planning for four agronomic qualities: days to half blossoming, plant tallness, number of turners, grain yield, and 13 grain components: As, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn, in two multiplied haploid populaces got from the crosses IR64 × IR69428 and BR29 × IR75862. Phenotyping under these populaces were led during 2015DS and 2015WS at IRRI, Los Baños, The Philippines, and genotyping was performed utilizing a 6 K SNP chip. Comprehensive composite span planning showed 15 QTL for agronomic characteristics and 50 QTL for grain component fixation. Out of these, eight QTL displayed phenotypic fluctuation of >25% and 11 QTL were reliable across seasons. Indeed, even it was seen that there were seven QTL co-limitation locales containing QTL for multiple characteristics. Twenty five epistatic collaborations were additionally identified for two agronomic characteristics and seven mineral components. It likewise prompted the recognizable proof of different DH lines with high Fe and Zn. These lines can be made to use as benefactors for reproducing high-Zn rice assortments. A portion of the major QTL can likewise be additionally approved and utilized in MAS to improve the convergences of basic nutritive components in rice grain.

Wissuwa *et al*., (2013) examined that higher root-efflux paces of citrate (Hajiboland *et al*., 2005; Hoffland *et al*., 2006) and malate (Hajiboland *et al*., 2005; Gao *et al*., 2009; Rose *et al*., 2011a) have been dissected in Zn-productive upland and marsh rice genotypes enduring Zn inadequacy, hence two issues should be enthusiastically settled before one could come full circle that this efflux straightforwardly expands Zn take-up. Right off the bat, the efflux of LMWOAs regularly agrees with radical oxygen stress which prompts root spillage and may subsequently be an aftereffect of its root film burst instead of a functioning pressure reaction (Chen *et al*., 2009; Rose *et al*., 2011a, 2012). While the second uncertain inquiry identifies with the measure of LMWOAs required in the rhizosphere which can altogether discharge measures of Zn. In this way next to no data has been accessible with respect to how the watched establishes exudate efflux rates in supplement arrangements when contrasted with their focus in rhizosphere soil. From certain accessible direct estimations of rhizosphere LMWOA focuses, we can presume that malate might be available in fixations in the significant degree 0.1–1 mM, having a more extensive territory quantifiable in a citrate test showing 0.01–1 mM (Gao *et al*., 2009). This data suggested an extremely wide scope of rhizosphere root exudate fixations among various kinds of exudates. While the two soil hatching tests have suggested that the grouping of citrate and malate discharged from the underlying foundations of Zn-lacking rice plants were insufficient to activate adequate measure of Zn from the two Zn-inadequate soils (Gao *et al*., 2009; Rose *et al*., 2011a), the viability of a particular exudate is probably going to shift under various soil types (Impa and Johnson-Beebout, 2012), so further examination must be directed to figure out which root exudates are conceivably the most valuable quality.

Rao *et al*., (2020) led a test under which numerous germplasm sets of a few national establishments were described at IIRR for their zinc content in earthy colored rice by utilizing vitality dispersive X-beam fluorescence spectroscopy which gave the scope of zinc to be 7.3 to 52.7 mg/kg. A lot of various planning populaces including wild germplasm, landraces, and assortments for their zinc content were assessed to know the practicality of good recombination of high zinc substance and yield. A lot of ninety-nine genotypes from germplasm and 344 lines from planning populaces showed zinc substance of 28 mg/kg in cleaned rice fulfilling the objective zinc content set by the HarvestPlus. Since 2013, 170 test passages were designated under AICRIP biofortification preliminary, by different national establishments until 2017, and four biofortified rice assortments were discharged. Just the test section which have the objective zinc substance, yield, and quality boundaries is elevated to the following year; in this manner, each test passage is analyzed for a long time across 17 to 27 areas for their presentation. Multilocation preliminaries of two planning populaces and AICRIP biofortification preliminaries demonstrated that the zinc content is exceptionally affected by condition. The bioavailability of a discharged biofortified rice assortment, viz., DRR Dhan 45 was inspected to have twice that of control IR64. The four discharged assortments created through traditional rearing extended from 48 to 75% with zinc admission of 38 to be 47% and 46 to 57% of the RDA for male and female, individually.

Jeng *et al*., (2012) made an endeavor to create rice assortments biofortified with iron (Fe) and zinc (Zn) so as to mitigate healthful lacks in creating nations, where cleaned rice is devoured as the staple food. In this examination, we have assessed the substance of a few large scale and miniaturized scale minerals in cleaned rice grains of cultivar IR64 and its 254 sodium azide-initiated freaks (M8 age). Their outcomes gave that the substance of potassium, phosphorus, calcium, magnesium, iron, manganese, copper, and zinc displayed varieties among this tried freaks. The cleaned rice grains of freaks M-IR-75 and M-IR-58 were found to gather more Fe (28.10 and 27.26 mg kg−1, separately) than cultivar IR64 (3.90 mg kg−1). It was contemplated that the freak M-IR-75 likewise created more significant returns (normal of 8.65 ton ha−1 more than two harvest seasons) than cultivar IR64 (normal of 7.27 ton ha−1). It was likewise contemplated that freaks M-IR-180, M-IR-49 and M-IR-175 contained more Zn (26.58, 28.95 and 26.16 mg kg−1, individually) than cultivar IR64 (16.00 mg kg−1), yet just among these freaks just M-IR-180 indicated a grain yield similar to cultivar IR64. Accordingly, the freak M-IR-75 can be prescribed to ranchers to deliver Fe-rich rice grains. Moreover, the high-Fe (M-IR-75 and M-IR-58) and high-Zn (M-IR-180, M-IR-49 and M-IR-175) freaks can likewise be utilized as hereditary assets for rice improvement programs.

Boonchuay *et al*., (2013) arranged an investigation to assess how zinc (Zn) grouping of rice (Oryza sativa L.) seed might be expanded and resulting seedling development can be improved by foliar Zn application. A lot of eight foliar Zn medicines of 0.5% zinc sulfate ($ZnSO_4 \cdot 7H_2O$) were applied to the rice plant at various development stages. The subsequent seeds were saved for germination with the goal that impacts of Zn on seedling development can be assessed. It was seen that the foliar Zn shower expanded paddy Zn focus just when it was applied in the wake of blooming, and it was expanded to additionally content when applications were rehashed. The most extreme addition of up to ten-folds were in the husk, and littler increments in earthy colored rice Zn. In the underlying long periods of germination, seedlings from seeds having 42 to 67 mg Zn kg−1 had longer roots and coleoptiles than those from seeds having 18 mg Zn kg−1, however this impact vanished later. The upside of high seed Zn in seedling development can likewise be demonstrated by a positive connection between's Zn fixation in growing seeds and the joined roots and shoot dry weight ($r = 0.55$, $p < 0.05$). Zinc in rice grains can likewise be adequately raised by foliar Zn application subsequent to blossoming, which gave an expected advantage to rice eaters showed by up to 55% increments of earthy colored rice Zn, and agronomically in increasingly quick early development and foundation.

2.8 Assessment of agronomically important genes in elite parental lines.

Kanyange *et al*., (2019) performed an investigation to check the presence of Pita and Pi9 genes for blast resistance and also to check the presence of the badh2 gene for aroma in the screened F2 plants using molecular markers. Genotyping was performed in 103 F2 plants which were made to mature by using the KASP genotyping method with SNP markers (snpOS0007, snpOS0006, and snpOS0022) screening the Pita and Pi9 genes for blast resistance and the badh2 gene for aromatic fragrance. Genotyping results revealed that 38 F2 plants were found to have the Pita gene present in both alleles, 31

F2 plants having the Pita gene in one allele, and there were only one plant (3B1) with the Pi9 gene in one of its allele. The badh2 gene for aroma was identified in 27 F2 plants on both the alleles and on only one allele in 57 F2 plants. Thirteen plants possess both the Pita gene and the badh2 gene for aroma, and only one plant (3B1) have a combination of the three genes (Pita, Pi9, and badh2). Seven plants were found to be resistant for blast disease (2H2, 2H4, 1G2, 1C12, 1E13, 1B12, and 1C5) with the Pita and badh2 genes were found, and only one resistant plant (3B1) possess all the three genes Pi9, Pita, and badh2 which was recommended to be bulked for the development of the Supa aromatic rice variety resistant to blast disease.

Thakur *et al*., (2014) performed a study, by using the Illumina GoldenGate assay to validate and genotype SNPs in a set of six major rice blast resistance genes, viz. *Pi-ta*, *Piz(t)*, *Pi54*, *Pi9*, *Pi5(1)* and *Pib*, which were distributed over five chromosomes, for understanding their functional relevance and also tostudy the population structure in rice. All the screened SNPs loci (96) of six blast (*Magnaporthe oryzae*) resistance genes were utilised in successful genotyping of 92 rice lines with an overall genotype call rate of 92.0 % and minimum GenTrain cutoff score of ≥0.448. The highest number of SNPs were genotpyed in *japonica* type (97.1 %) rice lines, followed by *indica* (92.12 %), *indica* basmati (91.84 %) and minimum present in case of wild species (82.0 %). Among the genotyped loci, *Piz(t) was found to have* the highest score (98.68 %), followed by *Pi-ta*, *Pi5(1*), *Pib*, *Pi54* and *Pi9*. Polymorphism was clearly observed in 87.5 % SNPs loci producing 7,728 genotype calls. It was also observed that the minor allele frequency ranged from 0.01 to 0.49 providing them good differentiating power for distinguishing different rice accessions. A panel of genotypes from four rice subpopulations possessing “admix” ancestry (>26 %) with more than one genetic background of *indica*, *japonica* and wild types were revealed from the population structure analysis. SNPs markers were made to validate in a panel of 92 rice lines and transformed them into CAPS markers which could be used for blast resistance breeding programme.

Wettewa *et al*., (2014) made a study which revealed that the Betaine aldehyde dehydrogenease (badh2) gene is responsible for fragrance in rice. It was studied that an 8-bp deletion and three single nucleotide polymorphisms in the exon 7 of badh2 gene, named as badh2.1 allele, was responsible for accumulation of a major aromatic compound, 2-acetyl 1-pyrroline (2AP) in fragrant rice. Although badh2.1 is the predominant allele present in all fragrant varieties, however involvement of another genetic loci or allele had been significantly reported by several researchers exceptionally. This investigation was carried out with an objective of detecting presence or absence of badh2.1 allele in popular traditional Sri Lankan fragrant rice varieties. Also a 463-bp DNA fragment was amplified covering the 7th exon region and sequenced. The results revealed that “Lanka Samurdi”, the fragrant high yielding variety, possesses the 8bp deletion while highly fragrant three traditional rice varieties do not exhibited such particular mutation. Hence it was confirmed that the aroma in most of Sri Lankan traditional fragrant varieties is not resulted by the badh2.1 allele but by another genetic factor.

Jeong *et al*., (2020) explored a double haploid populace which was derived from a cross between the Korean japonica cultivars Goami 2 and Hwaseonchal and phenotyping was finished during the 2018 wet season (POP2018) and 2019 wet season (POP2019). The polished rice grains of POP2018 and POP2019 were utilized to discover the GIC-and GZC-related QTL. Digenic interactions and attributes which influences GIC and GZC were likewise distinguished. Paird sample t test revealed that the GIC of POP2018 was different as that of POP2019 ($t = 0.24$, $p = 0.81$), while the GZC of POP2018 was essentially unique in relation to that of POP2019 ($t = -4.35$, $p = 0.00$). Three moderate-impact (phenotypic difference [PVE] < 10.00%) GIC-and GZC-related QTL that were predictable somewhere in the range of POP2018 and POP2019 were recognized through comprehensive composite span planning: qFe9, qFe11, and qZn7. Each of the three QTL began from the sub-par parent Hwaseonchal. Epistasis investigation uncovered that the GIC and GZC of POP2018 and POP2019 were influenced by 79 noteworthy digenic interactions with moderate and minor impacts. On the other hand, GZC was unaffected by digenic interactions. An association between loci on chromosomes 1 and 5 gave the most elevated commitment to the PVE (13.72%) for GIC. Up-and-comer quality examination uncovered that 12 qualities identified with iron and zinc homeostasis or that require iron or zinc as ligands colocated with qFe9, qFe11, and qZn7. The QTL and epistatic areas distinguished here might be valuable for creating rice assortments with high GIC and GZC in their processed grains.

CHAPTER – III

MATERIALS AND METHODS

The present study entitled **“Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches”** was carried out at Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India. This chapter provides information regarding the experimental material and methods adopted during the course of the analysis. The detail of the materials used, methods adopted were presented as under the individual headings and sub headings.

3.1. Experimental site

The experiment was conducted at the Research cum Instructional Farm, IGKV, Raipur(C.G.) and at the Laboratory of Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur (C.G.).

3.2. Experimental material

The experimental material for the investigation is given in table 3.1 includes panel of diverse set of homozygous advance breeding lines (ABLs) provided by IRRI under Transformative Rice Breeding project (TRB) and developed by nutrition group under CRP & HP project of Dept. of PMBB, IGKV, Raipur.

Table 3.1: Genotypes used in present study

SN	Genotypes	SN	Genotypes
1	**IR16A1957**	39	**IR14F711**
2	**IRRI 193**	40	**IR15A4029**
3	**IR13N102**	41	**IRRI 186**
4	**IR11A293**	42	**IRRI 174**
5	**IR16A2332**	43	**PIR-26>C0-2071-1-4-2-1**
6	**IR09A228**	44	**APO::C1**
7	**IR15A2983**	45	**IR 64**
8	**IR13A514**	46	**IRRI 164**
9	**IR14A216**	47	**IR 72**
10	**IR16A2225**	48	**IR 58025 B**
11	**IR 93339:29-B-7-7-B-B-B-16**	49	**IR03A550**
12	**IR12A329**	50	**IRBB 60**
13	**IR12N231**	51	**IR10F379**
14	**IR10F559**	52	**IRRI 216**
15	**IR12A173**	53	**IR02A127**
16	**IR10N134**	54	**IR 45427-2B-2-2B-1-1::G1**
17	**IR05N341**	55	**IR09N496**
18	**IR13N115**	56	**IR10F365**
19	**IR09N516**		**IR 21015-72-3-3-3-1::IRGC**
20	**IR14A185**	57	**117004-1**
21	**IR14A150**	58	**MTU 1010**
22	**IR 54447-3B-10-2**	59	**IRRI 154**
23	**IR16A1070**	60	**IRRI 168**
24	**IR14V1020**	61	**R-RHP-IM-84**
25	**IR09A235**	62	**R-RHZ-AS-43**
26	**IR13N134**	63	**R-RGP-SB-114**
27	**IR12M112**	64	**R-RGP-CM-114**
28	**IR12A136**	65	**R-RGM-ATN-47**
29	**IR15A2442**	66	**R-RGM-AS-45**
30	**IR10N271**	67	**R-RHZ-KS-104**
31	**IR 110615-C1-B-B-B-1-1**	68	**R-RHZ-IA-94**
32	**IR14N153**	69	**R-RGP-RH-116**
33	**IR16A2261**	70	**R-RHZ-MI-95**
34	**IR13A153**	71	**IR-64**
35	**IR15A2373**	72	**IET-24780**
36	**IR11N121**	73	**CGZR-1**
37	**IR12A229**	74	**R- 56**
38	**IR 122310:7-2-2**	75	**IGKVR-1**

3.3 Experimental Details:

The diverse 75 ABLs was evaluated under irrigated field conditions under N:P:K dose of 100:60:40 kg ha^{-1} in the form of diammonium phosphate, single super phosphate and muriate of potash, respectively. The entire experiment was laid out in randomized complete block design (RCBD) with two replication. Field view of experiment is in **Fig 3.1**.

Zn ABLs from IRRI

Zn ABLs from CRP & HP projects from IGKV

Fig: 3.1 A view of experimental field

3.3.1 Cultural practices

3.3.1.1 Raising the seedling

Well pulverized raised nursery beds were prepared. The size of each nursery beds were 1 x 25 cm drainage channel of 30 cm width was provided between the beds. The dose of fertilizers was @ 100 kg N_2, 60 kg P_2O_5 and 40 kg K_2O ha^{-1} in the form of DAP, SSP and MOP. The seeds were sown in the raised nursery bed by hand drilling methods in rows. Light and frequent irrigation were given until the seedlings were transplanted.

3.3.1.2 Field preparation

The final preparation of the field was done by two criss-cross tractor ploughing followed by harrowing. The soil surface was labelled and the field was divided for the respective treatments and replication.

3.3.1.3 Fertilizer application

The fertilizers were applied as per the recommended package of practice. The ratio of 100:60:40 kg ha^{-1} N: P: K was employed in the form of diamonium phosphate , single super phosphate and murate of potash, respectively. Nitrogen was applied in 3 splits, viz. 50% of the total N as basal dose, 25% at panicle initiation and the remaining 25% at flowering. The whole amount of phosphorus and potash was applied as basal during transplanting.

3.3.1.4 Transplanting

Twenty three days old seedlings and single seedling per hill was transplanted in the field. Flexible rope marked at specific interval with the help of coloured cloth strips for maintaining the distance between plant and rows.

3.3.1.5 Weed management

The weeds were removed from the plot manually as per requirement; four hand weeding was done at 15 days interval.

3.3.1.6 Water management

After transplanting the soil was kept saturated until seedlings got established in transplanted field. After establishment of seedling 5±2cm standing water was maintained throughout the growing period.

3.3.1.7 Plant protection

Plant protection measures were adopted when required during the entire crop growth period.

3.4 Observations recorded in present study

To assess the genotypic differences among all the studied ABLs, observations for yield, quality and biochemical attributes were recorded periodically at all the phonological stages of crop. In order to investigate genotypes with absolute accuracy five representative samples were selected. Standard procedures as mentioned in IRRI manual for standard evaluation system (SES), physiological studies in rice were amalgamated for recording the precise data on various parameters. Additionally, digitization involving barcode creation, tagging the rice genotypes with barcode tag and electronic data capture was done (fig. 3.2). Observations recorded are given below:

1. **Plant Height (cm)**: was measured from soil surface to tip of the tallest panicle (awns excluded) of the rice plant.

2. **Effective tiller per plant (no.)**: was counted for each five randomly selected plant. These are number of viable tillers relates to those tillers which bears panicle.

3. **Panicle Length (cm)**: Enter actual measurements in centimetres from panicle base to tip at growth stage: Eight Dough stage.

4. **Head Rice Recovery (%)**: It is a processing quality property that is exceptionally persuasive toward the market cost of rice. It is characterized as the extent of paddy rice that holds 75% of its length in the wake of processing. For another rice variety to be acknowledged and received by farmers, the new variety's HRR ought to fulfil customer necessities of in any event 55% or above.

$$\text{Head rice (\%)} = \frac{\text{Weight of whole grains}}{\text{Weight of paddy samples}} \times 100$$

5. **Grain yield (q/hec)**:

   The actual yield of grain in gram per plot was recorded and later converted to q/hec for further analysis.

6. **Days to 50% flowering (days):**

Days from sowing to flowering period of half of the plants were recorded on plot basis by visual means.

7. **Grain Zinc Content (%)**

Before analyzing the rice samples for micronutrient analysis, the rice grain of the genotypes was subjected to dehusking and polishing. Approximately 200g seeds of each sample were hand dehusked using polyurethane coated hand dehusker unit to avoid metal contamination. The dehusked rice sample was polished using electronic polisher (Kett Mill) for 45-60 second. Whiteness range reading 35-50 of polished grains was measured using refractometer (Fig. 3.3).

Elemental analysis:

Estimation of micronutrient concentrations in rice genotypes was done using Energy-dispersive X-ray fluorescence (ED-XRF, S2 Ranger or Oxford Instruments X-Supreme 8000). The samples were processed as given below before analyzing iron and zinc concentration followed by estimation of elements as per protocol given by Paltridge *et al*., 2012. XRF steps are given in fig. 3.3 and detailed below:

a. 20g sample of polished rice sample is taken for the estimation of both grain iron and zinc concentration.

b. Iron and Zinc concentration was estimated by using Energy-dispersive X-ray fluorescence spectrometer (ED-XRF, S2 Ranger or Oxford Instruments X-Supreme 8000).

c. Samples were filled in sample cups assembled from 21mm diameter cups combined with polypropylene inner cups sealed at one end with 4 µm Poly-4 XRF sample film. Cups containing samples were gently shaken to evenly distribute grains followed by putting samples into tray for analysis.

d. Analysis time for each set of sample was 186 s which included 60s acquisition time for the separate Zn and Fe conditions as well as 66 s dead time“ during which the XRF establishes each measurement condition. Concentration was expressed in microgram per gram (µg/g).

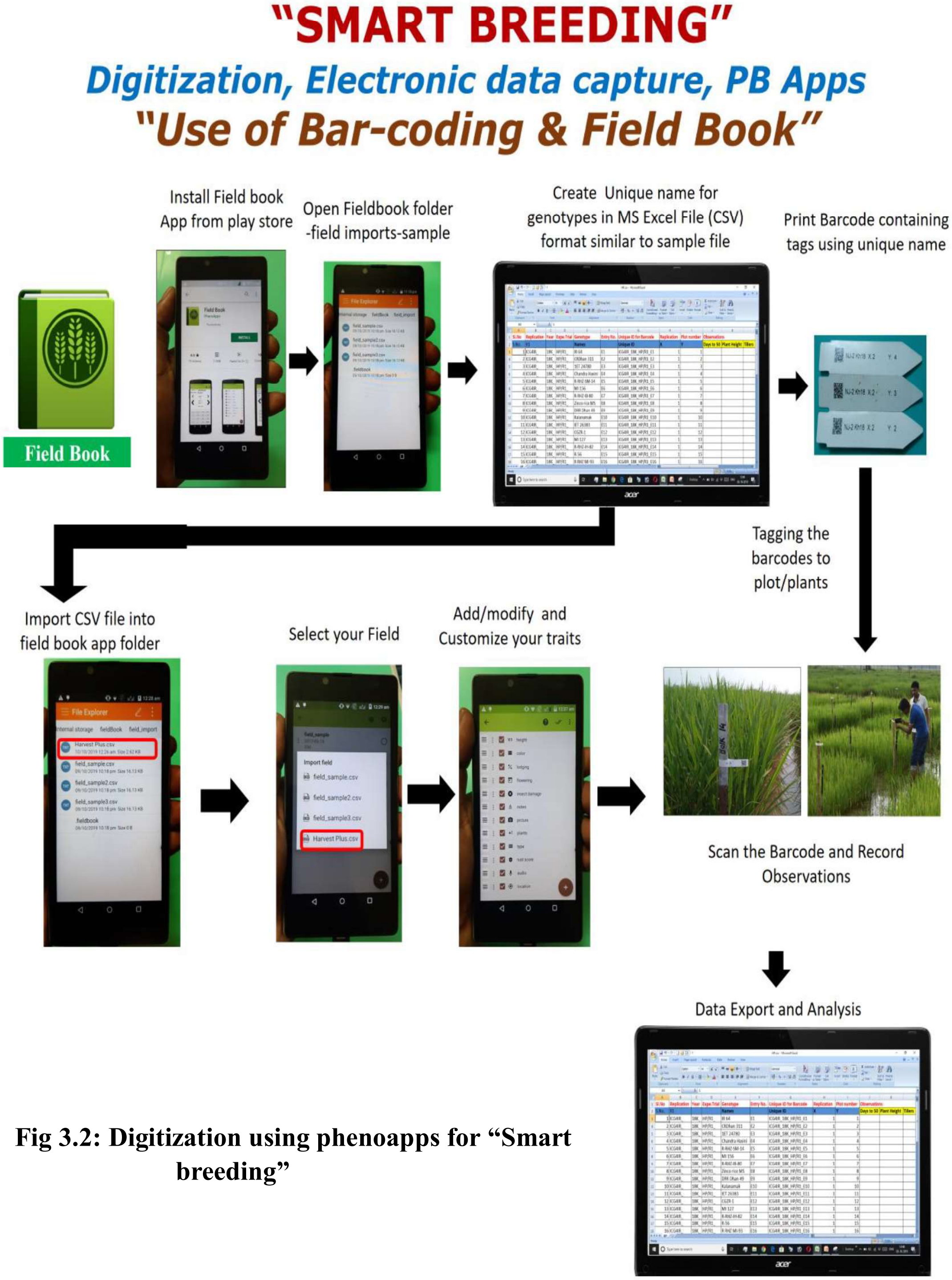


Fig 3.2: Digitization using phenoapps for "Smart breeding"

Fig 3.3: Schematic representation of various steps involved in elemental analysis.

Step 1: Dehusking of brown rice with the help of Dehusking machine and obtaining milled or polished rice.

Step 2: Obtained polished/milled rice is further graded using grader so that head rice can be recovered.

Step 3: Head rice is washed for 1 minute using Distilled Water to remove the dirt and dust if any.

Step 4: Air drying of sample.

Step 5: ED XRF Machine (S2 RANGER) for Fe and Zn content analysis.

3.5. Molecular Work

For SNP genotyping leaf sample was taken from 14 days old tender seedlings. Two leaf disc each with 5.5 mm diameter were punched/clipped from each entry with sterilized punching machine (Kangaro DP-800 make). The punching machine was sterilized using 70% ethanol after each sample collection to avoid any contaminants, extraneous tissue and residues of previous samples. The collected discs were placed in 96 well skirted plates and kept in ice before transporting sample to laboratory to avoid any tissue desiccation and significant DNA degradation of collected samples. The 96 well plates containing samples was incubated at 45± 1^0C in hot air oven for drying to remove excess moisture from procured samples. The plates were tightly sealed and sent to Intertek laboratory, Hyderabad for extraction of DNA and SNP genotyping using the Kompetitive Allele Specific PCR (KASP) genotyping technique.

3.5.1 DNA extraction from leaf disc:

High quality genomic DNA was extracted from the dried leaf discs using Sbeadex maxi plant kit according to the manufacturer *i.e.* LGC Ltd. instructions at Intertek laboratory, Hyderabad.

3.5.2 Genotyping using SNP markers

Kompetitive Allele Specific PCR (KASP) technique was used for genotyping the extracted genomic DNA samples in the Intertek laboratory in Hyderabad as per Smith and Maughan (2015). The gene specific KASP markers used in present study were designed by IRRI, Philippines (Table 3.3). Ten SNPs known to influence the biotic stress (4 BB genes [*Xa-21, xa-5, xa-13* and *Xa-4*] and Blast 3 genes [*Pi-ta, Pi-9* and *Pi-54*]), 2 genes for quality traits (*chalk-5* for non-chalkiness and *BADH-2* for aroma) and one gene (*Sub-1*) for abiotic stress submergence tolerance in rice were used for genotyping of 8036 lines. A total volume of 10ul for SNP-specific KASP assay mix, the DNA sample and universal Master mix (genotyping mixture) was prepared and used for all the PCR reactions. For PCR, in 96-well plates one well contained 5 µl of 50 ng DNA from each sample and a mixture of 5 µl genotyping mixture (4.4 µl of 2x KASP Master mix and 0.6 µl of KASP assay mix). Each KASP assay mix comprised of SNP marker specific three non labelled oligo-nucleotides encompassing two allele specific forward primers and one common reverse primer. Each primer harbored a unique tail sequence corresponding with a universal fluorescence resonant energy transfer (FRET) cassette and a primer-tail was labelled with FAM dye while on the tail of the second primer was labelled with HEX dye. The KASP Master mix on the other hand in a optimized buffer solution contained two universal FRET cassettes (HEX and FAM), ROX (passive reference dye), nucleotides, $MgCl_2$ and Taq polymerase. The Kompetitive Allele Specific PCR genotyping was performed in the following conditions according to Devran *et al.* (2016); one cycle for hot activation at 94° C for 15 min and the DNA denaturation was performed in 10 cycles at 94° C for 20 sec. The primer annealing and elongation were performed in 10 cycles for 60 seconds by dropping the temperature from 61 to 55° C at a rate of 0.6° C per each cycle. After, the temperature was raised to 94° C for 20 secs in 26 cycles to allow new denaturation and then lowered to 55° C for 60 seconds during annealing and elongation. When the amplification reactions were completed, 5 µl of the amplified products was transferred into the 384-well plates and detected on a BMG PHERA Star plate reader with a FRET using the genotype cluster analysis Kraken caller software from LGC Genomics assigning a genotype to each produced color. Genotypes was scored subsequently.

3.5.3 Statistical Analysis:

Based on the genotypic traits numerical scoring was done and these scores were used for calculation of major allele frequency, heterozygocity for each locus, gene flow and Analysis of Molecular Variance (AMOVA) in the studied population utilizing the GenAlex 6. (Peakall and Smouse, 2006).

3.5.4 Structure runs

`Most of parameters were set to their default values as advised in the user's manual of structure 2.0 (Pritchard and Wen 2003). The admixture model was chosen with the option of correlated allele frequencies between populations, as this configuration is considered best by Falush *et al.* (2003) in cases of subtle population structure. Lambda, the parameter of the distribution of allelic frequencies, was set to one, as the manual advised. From a pilot study, we found that a length of the burn-in of 50000 and MCMC (Markov chain Monte Carlo) of 100000 each was sufficient. For each data set 10 runs were carried out in order to quantify the amount of variation of the likelihood for each K. The final population was determined by using online Structure harvester programme. Delta K was plotted against K values; the best number of clusters was determined following the method proposed by Evanno *et al.* (2005) and obtained via the **Structure Harvester** platform (http://taylor0.biology.ucla.edu/structureHarvester).

Table 3.2. The genetic material: Different populations and number of genotypes used in present study

Institutes	State	Population 1	Population 2	Population 3	Total number of genotypes
AAU	Assam	3	1490	95	1588
IGKV	Chhattisgarh	65	1721	996	2782
NRRI	Odisha	313	1491	1862	3666
Total		381	4702	2953	8036

AAU: Assam Agricultural University, Jorhat, Assam; **IGKV**: Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh; **NRRI:** National Rice Research Institute, Cuttack, Odisha; **Population 1:** Released varieties, **Population 2:** Advanced breeding materials, **Population 3:** Germplasm/ Gene bank collections.

Table 3.3 : SNP marker details

S. No.	SNP IDs	Genes/traits	Chr #	Un-favourable Allele	Favourable Allele
1	snpOS0061	Xa21	11	G (Susceptible)	C (Resistant)
2	snpOS0054	xa5	5	TC (Susceptible)	AG (Resistant)
3	snpOS0002	xa13	8	A (Susceptible)	G (Resistant)
4	snpOS0052	Xa4	11	C (Susceptible)	A (Resistant)
5	snpOS0006	Pita	12	A (Susceptible)	C (Resistant)
6	snpOS0007b	Pi9	6	CGATGGTTTC (Susceptible)	- (Resistant)
7	snpOS0015	Pi54	11	C (Susceptible)	T (Resistant)
8	snpOS0022	BADH2	8	AAAAGATTATGGC(No Frag)	TATAT (Frag)
9	snpOS0024	chalk5	5	A (Chalk Type 1)	G (Non-Chalk type 5)
10	snpOS0040	Sub1	9	C (No-Sub1)	T (Sub1)

3.6 Statistical analysis

Statistical analysis of data was done on a personal computer using software packages such as MS-EXCEL, SPSS for different analysis. All data was analyzed without any transformation.

Analysis of variance (ANOVA)

Firstly, mean values were worked out for the traits for each lines and check variety. These mean data were utilized to calculate variability parameters *viz*., range, standard deviation, and coefficient of variation. The procedure of Panse and Sukhatme (1967) was adapted to analysis the data statistically.

Table 3.4: Skeleton of analysis of variance

Source of Variation	**Degree of Freedom**	**Sum of Square**	**Mean Sum of Square**	**F calculated**
Replication	(r-1)	SS_r	MS_r	MS_r / MS_e
Treatments	(t-1)	SS_t	MS_t	MS_t / MS_e
Error	(r-1)(t-1)	SS_e	MS_e	
Total	(rt-1)	SS_{total}		

Where,

r = Replication t = Treatments

SSR= Replication sum of squares SSt = Treatment sum of squares

SSe = Error sum of squares MSt = Treatment mean sum of

squares MSr= Replication mean sum of squares MSe= Error mean sum of

squares

The significance of treatment differences was determined by comparing the calculated value of F with the tabulated value at five per cent and or one per cent level of significance. If calculated value of F was greater than the tabulated value of F, then the F value will be significant or vice versa.

Assessment of variability:

1. **Range:** The lower and higher value of a character determines its range, which is expressed as follows:

   Range = Highest value – Lowest value

2. **Mean:** The mean is calculated by the following formula:

$$X = \Sigma Xi / N$$

Where,

ΣXi = Summation of all the observation

N = Total number of observation

3. **Standard deviation (SD):** Standard deviation is the root of sum of squares of deviation divided by their number, calculated by the formula:

Standard deviation = $\sqrt{\frac{\Sigma d^2}{n}}$

4. **Standard error (SE):**

Standard error = $\frac{S}{\sqrt{n}}$

5. **Correlation analysis:** To determine the degree of association between five physiological traits, the correlation co-efficient were calculated using the following formula:

$$r_{(xy)} = \frac{\mathrm{Cov}_{(xy)}}{\sqrt{\mathrm{Var}_{(x)} \times \mathrm{Var}_{(y)}}}$$

Where,

$r_{(xy)}$ = Correlation co-efficient between variable x and y
$\mathrm{Var}_{(x)}$ = Variance of x variable
$\mathrm{Var}_{(y)}$ = Variance of y variable
$\mathrm{Cov}_{(xy)}$ = Covariance between variable x and y

3.7. Estimation of best linear unbiased prediction (BLUP) for determining Genetic Gain in the Advance Breeding Lines:

Genetic Gain is an idea in rearing and traditional biometrical hereditary qualities where the pace of execution is contrasted with a pattern. It is typically assessed in the wake of propelling one age to the cutting edge that is finished by counterfeit choice (Falconer and Mackay 1996). In any case, long haul assessments of hereditary addition are estimated by looking at assortments together, including base or predominant assortments (Peng *et al.*, 1999). Yield and grain micronutrient content are complex traits and exhibit an unpredictable attribute and relies upon various hereditary and ecological elements. Estimation of the heritable segment should be possible by utilizing a strategy called best linear unbiased prediction (BLUP) that boosts the relationship among valid and anticipated rearing qualities through limiting prescient blunder change (Robinson 1991; Rutkoski *et al.*, 2014). BLUPs are utilized to choose the best ingrained lines to frame new reproducing populaces when joined with hereditary relatedness or connection data (for example coefficient of coancestry), once in a while assigned as family BLUPs which can be utilized to give evaluated rearing qualities. This is a proportion of the hereditary ability of a parent to deliver better offspring dependent on the parent's own presentation, pedigree records and progeny data. Evaluated rearing qualities are very valuable for parent determination for expanding hereditary addition (Bernardo 2018).

CHAPTER – IV

RESULTS AND DISCUSSION

In an era of recurring viral infection outbreaks that are capable of threatening lives, livelihoods, societies, and economies the most immediate frontline defence for people against infections is a healthy immune system. Scientific research have proven that micronutrients *i.e.* Zinc can be considered as a gatekeeper of the immune system, since the adequate function of virtually all immune cells is highly zinc-dependent. Furthermore, billions of people around the world rely largely on less nutritious, staple crops therefore agricultural research systems are working to develop and deliver staple crops that are biofortified to contain higher levels of zinc. Additionally, screening of diverse rice cultivars for enhanced grain micronutrients and mapping the related QTLs will help in selection of parental lines or donors for developing zinc rich hybrids or varieties. Despite having knowledge on different genes involved for iron and zinc uptake to final sequestration in grain (Grotz and Guerinot, 2006; Krohling *et al*., 2016), mapping for these traits is limited in most of the cereal crops. Hence development of micronutrient dense varieties or hybrids of rice and their consumption would meet the required recommended dietary allowance (RDA) for the Zn and also would pave way to development of biofortified rice. Considering these above facts the present study entitled **"Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches"** was planned and executed. The findings of the present study is presented and discussed below in this chapter.

4.1 ANOVA for experimental factors

Table 4.1 shows the analysis for the grain yield, related attributes and grain Zn content in the polished rice. Analysis of variance revealed highly significant differences among the genotypes for all the recorded characters, indicating presence of high variability among the rice genotypes in present study. Thus, there is ample scope for selection of different quantitative and qualitative characters for rice improvement.

Wang *et al*., (2014) in their paper on improved yield and Zn accumulation for rice grain by Zn fertilization and optimized water management reported the significant differences in evaluated grain yield and grain Zn content parameters. Fukrei *et al*., (2011) studied the genetic variability in yield and its components in upland rice grown in acid soils of north east India.

Their analysis of variance revealed presence of high variability among the genotypes for all the traits.

Table 4.1: ANOVA for yield, quality attributes and grain Zn content

S.No.	Characters	Replication	Treatment	F-test
1	GY	181.375	231.449	28.240**
2	GZCm	182.585	16.773	14.944**
3	PH	70.285	234.779	4.993**
4	DFF	0.107	125.678	10.199**
5	PL	0.034	5.394	0.461*
6	ET	1.707	1.261	0.392*
7	HRR	60.170	142.947	6.901**

***Significant at 5% and 1% level of significance**

GY=grain yield (q/hec.), GZCm=grain zinc content in milled rice (ppm), HRR= Head Rice Recovery (%), PH=Plant height (cm), ET/P= Effective tiller per plant (no.), DTF= Days to 50 % flowering (days), PL=Panicle Length (cm).

4.2 Mean performance of the genotypes

Large phenotypic variations were observed among the genotypes for all the studied traits. The results are depicted in table 4.2. The coefficient of variation (CV) ranged from 3.37 % to 5.92 % for the evaluated traits. Genotypic means for GY ranged from 25.63 q/hec to 75.13 q/hec, the genotype **IR14V1020** exhibited the maximum GY (75.13 q/hec) while the minimum value was shown by **IR 45427-2B-2-2B-1-1::G 1** (25.63 q/hec) the mean GY to be about 48.52 q/hec. The GZCm as depicted in table 4.3 ranged from 12.3 ppm to 29.1ppm, the genotype **IR 45427-2B-2-2B-1-1::G 1** have the maximum GZC (29.1 ppm) while the minimum GZC was observed in **IR13N115** (12.3 ppm) and the mean GZCm values was about 17.9 ppm. The PH ranged from 83.6 cm to 139.2 cm, the genotype **IR13N115** have the maximum PH (139.2cm) while the minimum PH was exhibited by **R-RHZ-IA-94** (83.6 cm) & the average values for PH was 111.12cm.

DFF in the panel of homozygous ABL lines exhibited a bimodal distribution. The DF ranging from 79 to 126 days, in which the genotype **IR14A216** exhibited the maximum DF (126 days), while **R-RGP-CM-114** exhibited minimum DF (79 days) the mean DF was about 104.5 days. The values for PL ranged from 17.1 cm to 33.7 cm, the genotype **IR12N231** showed the maximum PH (33.7 cm) while the minimum PL exhibited by **IR 45427-2B-2-2B-1-1::G 1** (17.1 cm) and the genotypic mean for PL was about 25.4 cm. The ET ranged from 5 to 11, the genotype **IR15A2442** have the maximum ET (11) while the minimum ET revealed by **IR 110615-C1-B-B-B-1-1** (5) and the mean ET was about 8. The Genotypic mean for HRR was 59.01%. HRR values ranged from 14.56% to 71.68%, the genotype **IRRI 174** have the maximum HRR (71.68%) while the minimum HRR was exhibited by **IR 45427-2B-2-2B-1-1::G 1** (14.56%) .

Similarly, Pippal *et al*., (2018) has done phenotyping for grain mineral contents (iron and zinc) and yield related attributes in PAU201 × PALMAN 579 F5 and BC1F4 populations in rice (*Oryza sativa* L.). The significant variation obtained by them for mineral content in F5 and BC1 F4 population suggested the existence of genetic potential to increase the concentrations of these micronutrients in rice grain. Over the last decade, several efforts have been made to biofortify food crops with micronutrients, which has led to a significant understanding of the physiological, genetic and molecular basis of high iron and zinc accumulation in grains and also the influence of agronomic management and environmental factors on iron and Zinc uptake, translocation and loading into grains (Impa *et al*., 2012). Additionally, a thorough study is still required for evaluating the grain mineral contents and yield attributes to achieve the biofortification goals.

Table 4.2: Mean performance of rice genotypes for all the evaluated traits during wet season 2019

Genotypes	DFF		PH		PL		ET		GY		HRR	
	Mean	**S.E.**	**Mean**	**SEm**	**Mean**	**SEm**	**Mean**	**SEm**	**Mean**	**SEm**	**Mean**	**SEm**
1	107.0	3.0	112.0	0.8	24.0	0.8	7.0	1.0	37.3	1.7	54.7	2.4
2	106.0	1.0	115.8	9.2	25.3	0.9	8.0	0.0	53.5	2.5	57.6	4.1
3	108.0	2.0	114.2	1.5	24.1	1.4	8.0	2.0	54.8	1.9	53.3	1.2
4	110.0	2.0	111.9	16.1	27.8	0.3	8.0	2.0	59.6	1.2	64.4	1.0
5	106.0	4.0	128.9	3.3	24.2	0.5	7.0	2.0	55.7	1.6	28.3	0.5
6	109.0	3.0	107.2	0.7	25.8	0.1	7.0	0.0	54.9	3.5	56.9	1.5
7	109.0	1.0	110.8	2.8	25.2	3.9	8.0	1.0	57.8	4.4	60.5	1.5
8	101.5	1.5	112.4	3.7	26.9	0.5	7.0	2.0	49.9	2.2	46.6	2.1
9	125.0	1.0	105.1	3.1	27.1	4.4	8.0	1.0	45.1	1.2	52.5	2.0
10	103.0	2.0	104.1	2.4	25.3	3.8	8.0	1.0	41.3	0.6	55.0	1.5
11	119.0	1.0	106.5	8.9	26.6	1.1	6.0	1.0	58.5	0.6	52.5	1.7
12	108.0	2.0	132.0	3.1	26.7	0.7	6.0	1.0	65.4	2.0	54.2	1.0
13	118.0	2.0	124.8	5.4	29.4	4.3	6.0	2.0	41.6	1.9	52.5	2.1
14	116.0	2.0	127.9	4.3	26.5	3.0	8.0	1.0	65.3	1.3	50.2	0.2
15	100.0	1.0	108.7	3.3	24.8	0.6	7.0	0.0	43.1	1.9	59.7	1.0
16	108.0	5.0	121.7	9.1	26.2	2.9	9.0	0.0	65.5	2.8	60.7	3.0
17	102.0	1.0	116.4	3.5	25.1	0.3	8.0	1.0	57.7	1.8	60.8	0.5
18	109.5	0.5	136.5	2.7	29.2	4.0	8.0	2.0	56.3	3.6	59.8	0.9
19	99.0	1.0	109.3	4.1	25.5	0.9	9.0	1.0	54.1	0.5	60.0	2.4
20	110.0	1.0	113.6	8.9	27.1	0.4	10.0	1.0	46.3	3.3	61.1	0.6

21	113.0	2.0	112.5	2.7	25.9	1.9	9.0	0.0	70.1	2.2	59.7	1.9
22	102.5	1.5	111.3	3.2	25.9	1.0	6.0	0.0	60.2	2.7	48.5	1.8
23	107.5	0.5	106.0	2.5	27.4	2.0	8.0	1.0	31.2	1.4	57.5	0.7
24	116.5	1.5	134.4	2.8	24.8	1.3	8.0	2.0	75.1	1.7	49.8	1.2
25	100.5	1.5	118.1	7.5	29.0	2.8	8.0	0.0	58.9	3.0	59.0	1.8
26	108.0	1.0	116.9	2.9	27.1	2.0	8.0	1.0	52.6	2.9	55.0	1.7
27	114.0	2.0	108.0	5.5	26.5	0.4	8.0	2.0	42.9	2.0	60.0	0.1
28	104.5	0.5	114.5	6.9	25.2	1.3	8.0	1.0	69.8	3.0	61.1	1.3
29	101.5	0.5	131.9	2.2	26.9	5.6	9.0	2.0	55.6	2.7	50.4	1.6
30	108.0	1.0	111.7	3.8	26.4	2.1	7.0	0.0	51.1	1.3	55.4	1.9
31	106.0	1.0	95.5	4.0	24.1	3.6	6.0	1.0	42.2	1.3	53.8	1.4
32	105.0	5.0	125.7	11.8	26.0	0.8	8.0	0.0	62.7	1.4	53.6	2.3
33	101.5	2.5	114.5	5.8	25.4	4.1	8.0	1.0	63.7	0.9	55.8	1.3
34	108.0	1.0	110.4	3.2	26.6	1.8	9.0	2.0	59.6	1.6	60.9	1.4
35	113.0	1.0	115.2	2.4	25.8	0.3	8.0	0.0	46.8	3.3	63.5	1.1
36	103.0	1.0	108.4	6.8	23.8	0.6	8.0	2.0	44.5	0.8	46.5	2.2
37	105.0	1.0	113.5	1.0	24.9	0.8	8.0	2.0	45.0	1.7	42.7	1.2
38	112.0	3.0	110.0	8.7	26.7	1.6	9.0	1.0	50.8	1.3	52.5	1.9
39	102.0	3.0	109.3	1.7	27.2	2.9	8.0	1.0	46.5	1.1	42.4	2.1
40	111.0	2.0	113.7	7.5	27.6	1.9	8.0	1.0	41.6	0.8	55.3	2.2
41	107.0	2.0	117.8	1.9	26.4	3.4	8.0	2.0	67.8	2.1	61.9	2.5
42	106.0	2.0	115.4	5.6	23.1	3.4	8.0	0.0	41.8	0.7	69.7	2.0
43	100.5	1.5	111.3	2.2	26.2	2.2	8.0	0.0	43.9	0.6	56.3	1.8

44	100.0	2.0	109.4	3.8	24.1	5.4	7.0	1.0	51.5	0.6	36.5	5.9
45	101.5	0.5	98.0	2.1	24.4	1.9	8.0	2.0	41.0	1.1	61.9	5.1
46	104.5	0.5	114.3	6.5	25.7	0.6	7.0	2.0	48.8	1.1	46.0	6.4
47	104.0	3.0	92.8	2.8	23.7	0.8	8.0	1.0	42.5	1.2	54.3	3.9
48	98.0	4.0	111.5	3.7	24.7	1.8	8.0	1.0	49.1	2.1	36.6	11.8
49	114.0	0.0	100.8	5.0	24.3	1.0	7.0	1.0	42.2	1.7	67.2	3.8
50	98.5	1.5	104.1	2.0	27.2	0.7	8.0	1.0	47.3	1.1	40.2	5.1
51	100.5	1.5	118.4	4.7	25.8	3.2	8.0	0.0	41.1	0.8	43.0	13.2
52	104.5	4.5	116.2	3.4	26.9	2.2	7.0	1.0	46.5	0.8	44.2	5.4
53	108.5	0.5	109.5	2.1	24.7	1.0	8.0	1.0	54.6	1.0	51.7	0.9
54	96.0	2.0	93.2	4.0	21.7	4.6	8.0	0.0	25.6	12.1	20.0	5.4
55	111.0	2.0	120.3	2.8	25.5	0.4	8.0	2.0	54.3	2.0	45.7	2.1
56	110.0	3.0	117.6	2.2	28.0	2.5	8.0	1.0	35.2	0.3	61.7	5.0
57	102.0	2.0	105.3	2.1	25.9	1.5	8.0	0.0	40.1	0.2	46.1	2.7
58	98.0	2.0	116.2	2.7	25.6	0.6	7.0	0.0	47.7	0.9	57.7	0.9
59	113.0	3.0	108.6	1.3	26.5	3.1	8.0	1.0	44.0	0.8	58.3	2.5
60	108.0	1.0	105.0	3.4	24.4	3.7	7.0	0.0	49.6	0.9	49.1	2.3
61	89.0	3.0	94.5	4.2	25.0	0.5	8.0	1.0	40.2	0.3	44.5	4.3
62	106.0	3.0	111.4	5.1	26.3	1.4	8.0	2.0	28.1	1.3	41.3	1.5
63	103.0	4.0	133.1	5.6	24.2	2.4	8.0	2.0	25.9	2.5	50.7	1.9
64	84.0	5.0	94.2	4.4	24.1	3.2	8.0	1.0	38.3	0.7	53.5	1.7
65	95.0	3.0	91.5	0.8	22.8	2.3	7.0	2.0	42.0	0.7	56.8	3.2
66	96.0	5.0	93.1	4.5	24.2	0.4	7.0	2.0	43.2	2.4	60.4	0.7

67	99.0	2.0	94.7	3.5	21.9	1.6	6.0	1.0	35.5	2.6	48.3	4.5
68	96.0	5.0	87.2	3.6	21.9	0.5	7.0	1.0	45.9	1.1	51.3	1.5
69	99.0	4.0	104.1	2.8	24.1	2.0	7.0	1.0	30.1	0.2	55.4	2.2
70	84.0	3.0	92.7	1.1	22.7	1.0	7.0	0.0	34.4	3.1	53.5	2.5
71	99.0	2.0	100.4	3.0	24.5	2.7	7.0	1.0	54.3	0.7	49.9	2.1
72	91.0	3.0	118.2	4.5	25.9	5.2	8.0	2.0	45.5	1.6	58.8	1.7
73	81.0	3.0	93.7	2.7	22.9	1.3	7.0	1.0	29.1	1.5	60.8	1.4
74	93.0	3.0	103.2	2.5	24.0	0.3	7.0	1.0	46.0	0.3	48.7	1.7
75	95.0	3.0	114.5	4.3	23.2	3.5	7.0	1.0	51.21	0.7	51.2	1.1
SE(m)	2.48		4.84		2.41		1.26		2.02		3.21	
SE(d)	3.51		6.85		3.42		1.79		2.86		4.55	
C.V.	3.37		6.18		13.44		23.40		5.90		8.58	

Table 4.3: Zinc concentration in milled rice grains of 75 ABLs

S. No.	Genotypes	Zinc Concentration (ppm)
1	**IR16A1957**	17.8±1.3
2	**IRRI 193**	15.4±1.1
3	**IR13N102**	14.8±0.4
4	**IR11A293**	13.6±1.1
5	**IR16A2332**	18.9±1.3
6	**IR09A228**	19.5±1.2
7	**IR15A2983**	16.4±0.9
8	**IR13A514**	18.4±1.4
9	**IR14A216**	15.4±2.0
10	**IR16A2225**	17.9±1.5
11	**IR 93339:29-B-7-7-B-B-B-16**	14.9±0.3
12	**IR12A329**	16.1±0.4
13	**IR12N231**	25.4±0.6
14	**IR10F559**	16.6±1.4
15	**IR12A173**	25.7±1.4
16	**IR10N134**	18.7±0.9
17	**IR05N341**	24.1±0.2
18	**IR13N115**	12.3±1.9
19	**IR09N516**	18.5±1.2
20	**IR14A185**	17.6±1.2
21	**IR14A150**	17.6±0.5
22	**IR 54447-3B-10-2**	17.5±1.3
23	**IR16A1070**	16.2±0.3
24	**IR14V1020**	16±1.6
25	**IR09A235**	17.9±1.1
26	**IR13N134**	20.1±0.7
27	**IR12M112**	17.6±0.7
28	**IR12A136**	17.5±1.7
29	**IR15A2442**	14.6±0.7
30	**IR10N271**	16.2±0.9
31	**IR 110615-C1-B-B-B-1-1**	22.6±0.8

32	**IR14N153**	15.2±1.0
33	**IR16A2261**	15±0.4
34	**IR13A153**	16.4±1.3
35	**IR15A2373**	17.6±1.8

S.No.	Genotypes	Zinc Concentration (ppm)
36	**IR11N121**	16.7±0.6
37	**IR12A229**	17.2±1.5
38	**IR 122310:7-2-2**	16.2±1.3
39	**IR14F711**	17.4±1.2
40	**IR15A4029**	15.6±1.3
41	**IRRI 186**	14.3±2.5
42	**IRRI 174**	15.3±2.1
43	**PIR-26>C0-2071-1-4-2-1**	16.0±1.2
44	**APO::C1**	21.2±1.4
45	**IR 64**	16.6±3.0
46	**IRRI 164**	18.0±0.2
47	**IR 72**	18.7±2.3
48	**IR 58025 B**	14.9±1.5
49	**IR03A550**	25.5±1.3
50	**IRBB 60**	22.2±1.6
51	**IR10F379**	22.8±1.4
52	**IRRI 216**	16.15±1.8
53	**IR02A127**	14.2±0.6
54	**IR 45427-2B-2-2B-1-1::G1**	29.1±1.3
55	**IR09N496**	16.2±0.8
56	**IR10F365**	17.05±1.1
57	**IR 21015-72-3-3-3-1::IRGC 117004-1**	19.35±1.2
58	**MTU 1010**	17.05±0.6
59	**IRRI 154**	15.5±1.2
60	**IRRI 168**	16.6±1.4
61	**R-RHP-IM-84**	19.2±1.4
62	**R-RHZ-AS-43**	18.4±1.0
63	**R-RGP-SB-114**	21.3±1.2

64	**R-RGP-CM-114**	23.4±2.1
65	**R-RGM-ATN-47**	18.9±1.6
66	**R-RGM-AS-45**	17.4±1.2
67	**R-RHZ-KS-104**	19.8±1.6
68	**R-RHZ-IA-94**	24.3±1.2
69	**R-RGP-RH-116**	23.2±1.8
70	**R-RHZ-MI-95**	18.9±2.0
71	**IR-64**	18.7±1.1
72	**IET-24780**	20.1±1.0
73	**CGZR-1**	23.4±1.3
74	**R- 56**	22.8±1.1
75	**IGKVR-1**	21.2±0.6

4.3 Variation of Grain Zn content in milled rice (GZCm) and Grain yield (GY)

Phenotyping for the mineral nutrients is one of the major factors for crop improvement and previous studies suggested that there is significant genetic diversity in rice genome to increase zinc concentration in rice grain by combining the high Zn traits with high yielding traits (Graham *et al.*, 1999; Gregorio *et al.*, 1999; Brar *et al.*, 2011; Nachimuthu *et al.*, 2014). At the same time identification of micronutrient-rich genotypes opens up the possibilities for the linkage mapping of genomic regions or QTLs responsible for mineral uptake and translocation, which can be subsequently used as a donor for developing nutrient enriched varieties. In the present investigation, we evaluated 75 ABLs showing contrasting behaviour for grain yield and grain zinc content as depicted in fig.4.1. Highest and lowest values for GY and GZCm is depicted in variation chart. These results indicate that the studied rice genotypes shows high variation for the grain yield component traits as well as zinc content. Previously, large variation for iron and zinc content has been reported by several workers in different sets of rice germplasm and populations. Brar *et al.*, (2011) reported large variation in iron and zinc contents of dehusked grains in a collection of 220 rice genotypes for Fe (5.1- 441.5 µg/g) and Zn (2.12 - 39.4 µg/g). Anuradha *et al.*, (2012) described variation for iron and zinc contents in brown rice samples of 126 rice accessions. Garcia-Oliveria *et al.*, (2009) reported variation for iron (4.9-20 µg/g, Tequing-7.5 µg/g) and zinc (13.3-60.1 µg/g, Tequing-16.6 µg/g) in a set of

85 introgression lines (ILs) derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipogon*).

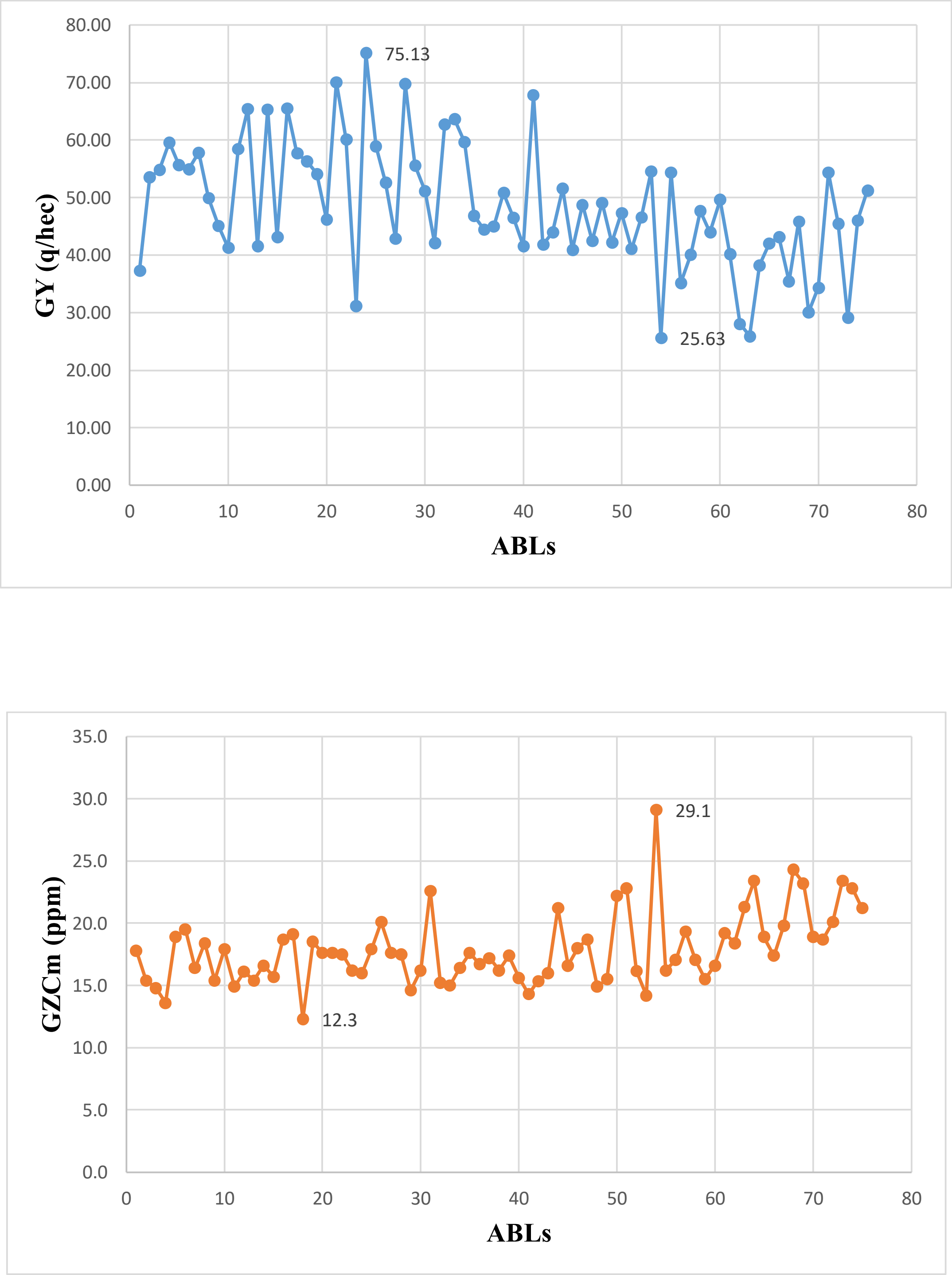


Figure 4.1: a) Variation of GY b) Variation of GZCm in a set of ABL lines.

4.4 Pearson's Correlation Coefficients

Phenotypic correlation coefficient analysis of 75 ABLs showed significant correlation between mineral content (zinc) and grain yield related attributes (fig. 4.2). Grain zinc content and grain yield showed significant positive correlation with all the studied traits. Correlation analysis offers an opportunity of simultaneous improvement of multiple traits in crop improvement programmes. Similar correlation results were obtained by Pipal *et al*., (2018) and Laxmi *et al*., (2019) who carried correlation studies for various yield and biochemical attributes.

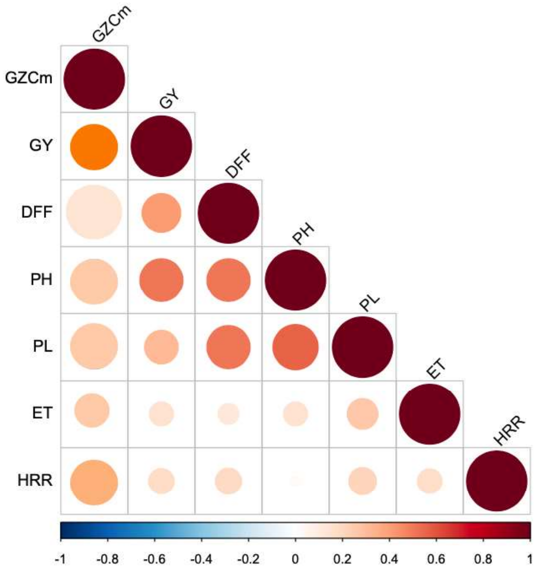


Fig 4.2: Correlation analysis between yield, quality and biochemical attributes

4.5 Molecular Screening

4.5.1. Best linear unbiased prediction (BLUP)

The key step in crop breeding is selection, and conventional breeding is based on phenotypic selection. Breeders choose good offspring using their experience and the observed phenotypes of crops, so as to achieve genetic improvement of target traits. However, both the development of new cultivars as well as the recommendation of newly released varieties require a selection to be made among a larger set of candidate genotypes, so estimation of genotypic values is at the heart of any breeding effort. Best linear unbiased prediction (BLUP) is a standard method for estimating random effects of a mixed model. BLUP is the good predictive method as compared to other procedure.

BLUPs values for ABLs from IRRI (ICP panel) was generated for yield obtained at Raipur and five different locations. The yield BLUPs were substantially similar at station and multilocation trials. The variable mineral content 4 lines were selected and 8 trait based SNPs for those selected lines were generated by KASP based SNP based genotyping methodology (fig. 4.6). In this study, Pita and Pi9 genes conferring blast resistance in selected screened genotypes are obtained making it suitable for diverse agro-ecological zones. Similarly, Kangaye *et al*., (2019) conducted genotyping for Blast (*Pyricularia Oryzae*) resistance genes in F_2 Population of super aromatic rice (*Oryza sativa* L.) using KASP based SNP genotyping. Pipheo *et al*., (2008) reviewed the role of BLUPs for phenotypic selection in plant breeding and variety testing.

These results pave a way for development of national elite pool for enhancing grain Zn content through population breeding based modern breeding strategies where thousands of minor genes & favorable alleles responsible for Zn trait are pooled through recurrent selection provides opportunity for making exemplary shift in genetic gain for complex trait *i.e.* Zn in rice.

Table 4.5: BLUP value for yield at Raipur and Multiple locations

S.N.	DESIGNATION	YLD_BLUP (Raipur)	YLD_BLUP (Multilocation)
1	APO::C1	5.480	5.049
2	IR 110615-C1-B-B-B-1-1	4.966	5.001
3	IR 122310:7-2-2	5.294	4.792
4	IR 21015-72-3-3-3-1::IRGC 117004-1	4.772	5.064
5	IR 45427-2B-2-2B-1-1::G1	3.514	4.986
6	IR 54447-3B-10-2	5.796	4.803
7	IR 58025 B	5.307	4.703
8	IR 64	4.609	4.732
9	IR 72	4.731	4.636
10	IR 93339:29-B-7-7-B-B-B-16	5.558	4.684
11	IR02A127	5.562	5.009
12	IR03A550	4.641	4.946
13	IR05N341	5.557	4.938
14	IR09A228	5.261	4.714
15	IR09A235	5.602	5.075
16	IR09N496	5.650	4.794
17	IR09N516	5.352	4.764
18	IR10F365	4.439	5.185
19	IR10F379	4.710	5.451
20	IR10F559	5.998	4.828
21	IR10N134	5.957	5.269
22	IR10N271	5.379	4.990
23	IR11A293	5.596	4.859
24	IR11N121	5.127	4.826
25	IR12A136	6.471	5.074

26	IR12A173	5.088	5.519
27	IR12A229	4.952	4.907
28	IR12A329	5.957	5.128
29	IR12M112	4.796	5.022
30	IR12N231	4.947	5.164
31	IR13A153	5.800	5.059
32	IR13A514	5.327	4.947
33	IR13N102	5.516	5.131
34	IR13N115	5.647	4.626
35	IR13N134	5.595	5.030
36	IR14A150	6.649	4.950
37	IR14A185	4.916	5.083
38	IR14A216	5.025	4.987
39	IR14F711	5.047	4.984
40	IR14N153	5.990	5.012
41	IR14V1020	6.484	5.332
42	IR15A2373	5.054	4.779
43	IR15A2442	5.529	4.787
44	IR15A2983	5.586	5.006
45	IR15A4029	4.724	5.052
46	IR16A1070	4.463	5.279
47	IR16A1957	4.729	5.145
48	IR16A2225	4.852	4.824
49	IR16A2261	5.919	5.125
50	IR16A2332	5.530	4.778
51	IRBB 60	5.053	4.683
52	IRRI 154	4.864	4.765
53	IRRI 164	5.335	4.794
54	IRRI 168	5.447	5.034

55	IRRI 174	4.851	5.244
56	IRRI 186	6.683	4.831
57	IRRI 193	5.458	4.833
58	IRRI 216	5.088	4.919
59	MTU 1010	5.329	4.758
60	PIR-26>C0-2071-1-4-2-1	4.956	4.569

Table 4.6: Trait based SNP of selected ABLs from IRRI

Designation	**YLD_BLUP**	**FLW_BV**	**PHT_BV**	**AMY_BV**	**Xa21**	**Xa5**	**Xa13**	**Pita**	**Pi9**	**Pi54**	**GZCm**
IR05N341	52.6	55.0	9.7	5.18	S	S	S	S	R	S	24.1
IR03A550	46.7	42.2	10.1	4.76	S	S	S	S	S	S	25.5
IIR12N231	52.0	41.0	12.4	5.02	S	R	S	R	S	S	25.4
IR12A173	59.7	43.0	12.6	5.05	S	R	S	R	S	S	25.7

CHAPTER – V

SUMMARY AND CONCLUSION

Scientific research have shown that the building block for good immune system is a balance and stable diet which allows people to meet the requirement of essential nutrients—particularly those nutrients that have the capability to boost immune system strength. Certain vitamins and minerals *i.e* .Zn can be singled out for their well-known antiviral properties. Billions of people around the world are not being reached by supplementation programs (or food fortification, for that matter), and rely largely on less-nutritious, staple-based foods. Lack of essential micronutrients in their diets leave these people vulnerable to disease and infection. That's why agricultural research systems to develop and deliver staple crops that are biofortified to contain higher levels of iron, vitamin A, or zinc.

Considering the above facts the present study entitled **"Identification of elite parental pool for breeding zinc rich rice varieties using modern breeding approaches**" was planned. A panel of diverse 75 homozygous ABLs were screened for analyzing the grain zinc content and their variability in the entire set. Also attempt has been made to study the correlation between the phenotypic traits and grain zinc content as well as considering the quality attributes of grain so that better rice variety can be released on the basis of their breeding values and milling parameters in order to meet the global food and micronutrient requirements.

CONCLUSIONS:

The above study provided a significant result and also provided us evidences regarding the existence of variability, also helps in proposing variety which could preferred and easily accepted by the consumer.

The findings of the study are as follows;

- Phenotypic data analysis such as DF, PH, PL, ET and GY revealed significant genetic diversity in rice genome to increase zinc concentration in rice grain by combining the high Zn traits with high yielding traits.
- Phenotypic correlation coefficient analysis of 75 ABLs showed significant correlation between mineral content (zinc) and grain yield related attributes. Grain zinc content and grain yield showed significant positive correlation with all the studied traits.

- Analysis of variance among set of 75 diverse ABLs revealed highly significant differences among the genotypes for all the recorded characters, exhibiting presence of high variability among the rice genotypes suggesting ample scope for selection of different quantitative and qualitative characters for rice improvement.
- BLUPs values for ABLs from IRRI (ICP panel) was generated for yield obtained at Raipur and five different locations this values revealed similar values at all locations thus providing a basis for selecting superior parents having high yield and zinc content.
- Genomic studies also revealed that Pita and Pi9 genes conferring blast resistance in selected screened genotypes are obtained making them suitable for diverse agro-ecological zones.
- Depending on the phenotypic and genotypic data generated and analyzing the variability an initiative has to be taken for development of elite pool of superior parents for paving a way to maximize genetic gain in superior off-springs rich in complex trait i.e. Zn in rice.

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