MOLECULAR CHARACTERIZATION AND GENETIC STUDIES FOR YIELD, YIELD COMPONENTS, ZINC AND IRON IN SWARNA X TYPE 3 RIL POPULATION OF RICE *(Oryza sativa* L.*)*

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MASTER OF SCIENCE IN AGRICULTURE
(GENETICS AND PLANT BREEDING)

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BY

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B.Sc. (Ag.)

THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILLMENTOF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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DECLARATION

I, **D. SHIVANI ,** hereby declare that the thesis entitled **""MOLECULAR CHARACTERIZATION AND GENETIC STUDIES FOR YIELD, YIELD COMPONENTS, ZINC AND IRON IN SWARNA X TYPE 3 RIL POPULATION OF RICE (*Oryza sativa* L.)"** submitted to the **Professor Jayashankar Telangana State Agricultural University** for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is the result of the original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Date: **(D. SHIVANI)**

Place: Hyderabad **ID.NO. RAM/16-40**

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With ever regardful memories……

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(D. SHIVANI)

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	: Analysis of variance
C. D.	: Critical difference
C. V.	: Coefficient of variation
Cm	: Centimeter
CTAB	: Cetyl Trimethyl Ammonium Bromide
d. f.	: Degrees of freedom
DFF	: Days to fifty percent flowering
DNA	: Deoxy Ribose Nucleic Acid
dNTPs	: Deoxyribo Nucleotide Tri Phosphate
e.g.	: For example
EDTA	: Ethylene Diamine Tetra-acetic Acid
et al.	: And others
etc	: And more
Fig .	: Figure
g	: Gram
GA	: Genetic advance
GCV	: Genotypic coefficients of variation
h^2	: Heritability in broad sense
ha	: Hectare
i.e.	: That is
kg ha-1	: Kilogram per hectare
M	: Molar
m.ha	: Million hectare

m.t	: Million tonnes
Min	: Minute
ml	: Millilitre
mM	: Milli Molar
mm	: Milli Meter
MSS	: Mean Sum of Squares
ng/µl	: Nanogram per micro litre
No.	: Number
ºC	: Degree Celsius
PCR	: Polymerase Chain Reaction
PCV	: Phenotypic coefficient of variation
PIC	: Polymorphic Information Content
pmole/ml	: Pico mole per micro litre
PVP	: Poly vinyl pyrollidone
R	: Correlation coefficient
RM	: Rice Markers
S.E (d)	: Standard Error deviation
S.E	: Standard Error
S.Em	: Standard Error of mean
SSR	: Simple Sequence Repeats or Microsatellites
Taq	: Thermus aquaticus
TBE	: Tris Boric Acid EDTA Buffer
TE	: Tris EDTA Buffer
U	: Unit
UV	: Ultra Violet light

viz.	: Namely
Vs.	: Versus
XRF	: X-ray crystallography
λ DNA	: Lambda DNA
$\sigma^2 g$	: Genotypic variance
$\sigma^2 p$	: Phenotypic variance

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ABSTRACT

The present investigation entitled "Molecular characterization and genetic studies for yield, yield components, zinc and iron in Swarna X Type 3 RIL population of rice (*Oryza sativa L.*)" was undertaken at Indian Institute of Rice Research farm, ICRISAT campus, Ramachandrapuram, Hyderabad in Augmented Block Design with four checks (Swarna, Type 3, BPT-5204, Chittimutyalu) during *kharif* 2017. The objective of study was to characterize the parents of RIL population using SSR markers, to study the variability, heritability, genetic advance as percent of mean, character association, path analysis for yield, yield attributing and nutritional traits in RIL population of rice.

A total of 171 SSR markers were analyzed with two parents and of which 56 markers showed polymorphism and 115 markers are found to be monomorphic. Among the 56 polymorphic markers, 7 SSR primer pairs RM 11744, RM 11743, RM 11741, RM 10361, RM 11307, RM 243, RM 10018 were observed to be polymorphic on chromosome 1, 3 SSR primer pairs RM13347, RM 14181, RM 3515 were polymorphic on chromosome 2, 8 SSR primer pairs RM 16097, RM 15630, RM 257, RM 282, RM 517, RM 282, RM 232, RM 16097 were polymorphic on chromosome 3, 6 SSR primer pairs RM348, RM 261, RM 17209, RM 241, RM 413, RM 16427 were polymorphic on chromosome 4, 5 SSR primer pairs RM 169, RM 142, RM 3486, RM 413, RM 164 on chromosome 5, 9 SSR primer pairs RM 340, RM 19341, RM 19620, RM 3431, RM 253, RM 111, RM 276,

RM 402, RM 190 were polymorphic on chromosome 6, two SSR primer pairs RM 20844, RM 11 were polymorphic on chromosome 7, two SSR primer pairs RM22885, RM 22524 were polymorphic on chromosome 8, 7 SSR primer pairs RM 3912, RM 296, RM 257, RM 24035, RM 24085, RM 296, RM 3912 were polymorphic on chromosome 9, One SSR primer pair RM 5095 was polymorphic on chromosome 10, two SSR primer pairs RM 27289, RM 206 were polymorphic on chromosome 11, 4 SSR primer pairs RM 27962, RM 28607, RM 235, RM 5313 were polymorphic on chromosome 12. Among all more number of polymorphic primers are observed on chromosome 6.

The data were recorded on quantitative characters like days to 50 per cent flowering, plant height, panicle length, number of productive tillers per plant, panicle weight, number of filled grains per panicle, test weight, grain iron content and grain zinc content. Analysis of variance indicated the existence of significant difference among treatments for all the characters. High GCV and PCV values were observed for number of filled grains per panicle, panicle weight, grain yield per plant.

High heritability coupled with high genetic advance as percent of mean was observed for plant height, number of filled grains per panicle, panicle weight, 1000-seed weight, grain yield per plant, grain iron and zinc concentration.

Character association studies revealed that the grain yield per plant showed positive significant association with plant height and 1000-seed weight. Path analysis revealed that the traits days to 50% flowering, number of productive tillers per plant, number of filled grains per panicle, plant height and test weight were directly influenced the grain yield per plant.

CHAPTER I

INTRODUCTION

Rice is a major staple food for the world's population and also a model crop for a major group of flowering plants. Asia is the leader in rice production accounting for about 90% of the world's production. The demand for rice is expected to increase further, as the world population is increasing in alarming rate. In India rice occupies nearly 44.50 million hectares with an annual production of 108.8 million tones (IIRR, 2017).

Producing nutritious and safe foods sufficiently and sustainably is the present ultimate goal of modern agriculture. Past efforts were focussed only on increasing crop yields, but at present enhancing the concentrations of mineral micronutrients has become an urgent task. Micronutrient malnutrition has been designed as the most serious challenge to humanity (Copenhagen Consensus, 2008) as two-third of the world's population is at risk of deficiency in one or more essential mineral elements (Cakmak, 2002; White and Broadley, 2009; and Stein, 2010). The mineral elements most commonly lacking in the human diets are Iron and Zinc (White and Bradely, 2009) which ranked fifth and sixth, respectively, among the top ten risk factors contributing to burden disease.

Malnutrition is the most common cause of Zinc deficiency (Ronaghy, 1987). About 25% of the world's population is at risk of zinc deficiency (Maret and Sandstead, 2006). In Asia and Africa, it is estimated that 500-600 million people are at risk for low Zinc intake (HarvestPlus, 2010). The reliance on cereal based diets may induce Zinc deficiency related health problems in humans, such as impairments in physical development, immune system, brain function, pneumonia, weight loss, growth retardation and delayed puberty in adolescents, poor appetite, delayed wound healing (WHO, 1996 and Cakmak, 2008).

Iron is an important component in human diet because it regulates enzyme activity and plays an important role in the immune system (Lynch, 2003). It is also an important component of human blood because it is the central atom of hemoglobin (Tuman *et al*. 1978). Humans require 10-15 milligrams (mg) of iron per day and deviation from these levels may cause deficiency symptoms like mental and psychomotor impairment in children and increased levels of both morbidity and mortality of mother and child during childbirth (Frossard *et al*. 2000).

With increasing population and pressure on food crops, now breeders are focusing more on breeding for nutritional enhancement. The range of iron and zinc concentration in brown rice is 6.3-24.4 μg^{-1} and 13.5-28.4 μg^{-1}, respectively. There is approximately a four fold difference in Iron and Zinc concentration, suggesting some genetic potential to increase the concentration of these micronutrients in rice grains (Gregorio, 2002).

Biofortification is a new approach that complements the existing "toolbox" of interventions and it aims at biological and genetic enrichment of food stuffs with vital nutrients (vitamins, minerals and proteins). Ideally, once rice is biofortified with vital nutrients, the farmer can grow the variety indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way. This is the only feasible way of reaching the malnourished population in India.

Exploiting the genetic variance in crop plants for micronutrient content is one of the most powerful tools to change the nutrient balance of a given diet on a large scale. Genetic improvement mainly depends upon the amount of genetic variability present in the population. Information on the nature and degree of genetic divergence would help the plant breeder in choosing the right parents for breeding programme (Vivekanandan and Subramanian, 1993).

Estimates of phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), heritability and genetic advance will help in knowing the nature of gene action affecting the concerned trait. In a rice improvement programme, it is the germplasm, which virtually determine the success and nature of end product. The development of superior rice population involved the intelligent use of available genetic variability, both indigenous as well as exogenous, to cater the need of various farming situations of rice.

The present research study was conducted to find out the genetic variability for different plant traits, direct and indirect contribution of these parameters towards rice yield and to identify better combinations as selection criteria for developing high yielding genotypes.

Most of the characters of interest to the breeders are complex and are the result of the interaction of a number of components. Understanding the relationship between yield and its components is of paramount importance for making the best use of these relationships

in selection. Character association derived by correlation coefficient, forms the basis for selecting the desirable plant, aiding in evaluation of relative influence of various component characters on grain yield. Path coefficient analysis discerns correlation into direct and indirect effects (Ekka *et al.* 2011).

The use of correlation coefficient and path analysis is to establish the extent of association between yield, its yield components and others characters, for fixing up the characters which are having decisive role in influencing the yield.

Keeping in view the present day requirements, the investigation was undertaken with the following objectives:

OBJECTIVES OF INVESTIGATION

1) To characterize parents of RIL population by using SSR markers.
2) To study the genetic variability for yield, yield attributes and nutritional traits (Zinc and Iron).
3) To study the character association between yield, yield attributing and nutritional traits (Zinc and Iron) through correlation and path analysis.

Chapter II

REVIEW OF LITERATURE

A brief review encompassing relevant, up to date published literature from various sources on topic 'Molecular characterization and genetic studies for yield, yield components, zinc and iron in Swarna x Type 3 RIL population of rice *(Oryza sativa* L.*)* is presented under the following heads.

2.1 Biofortification of rice with Zinc and Iron

2.2 Characterization of parents of RIL population using SSR markers

2.3 Genetic Variability

2.4 Heritability and Genetic Advance

2.5 Character association

2.6 Path Coefficient Analysis

2.1 Biofortification of rice with Zinc and Iron

Biofortification is the development of nutrient dense staple varieties in a crop using best traditional breeding practices and modern biotechnology methods. It makes food more nutritious as plants grow rather than having nutrients added to plant foods during processing.

Rice (*Oryza sativa* L.) feeds more than half of the world's population and is used as a staple food in many parts of Asia. It fulfils most of the requirements for the status of 'convenience food' by virtue of its qualities viz. nutritional fulfillment when complemented with other foods, higher yield, longer grain, less time and energy consumption during preparation and easy digestibility even by babies and sick people. Thus, the slogan "Rice is Life" aptly suits to the present day scenario where roughly half of the planet's population depends on rice.

Micronutrient malnutrition in rice has been designed as the most serious challenge to humanity (Copenhagen Consensus, 2008) as two-third of the world's population is at risk of deficiency in one or more essential mineral elements (Cakmak, 2002; White and Broadley, 2009; and Stein, 2010). The mineral elements most commonly lacking in the

human diets are Iron and Zinc (White and Bradely, 2009; Stein, 2010) which ranked fifth and sixth, respectively, among the top ten risk factors contributing to burden disease.

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Producing nutritious and safe foods sufficiently and sustainably is the present ultimate goal of modern agriculture. Biofortification is the idea of breeding crops to increase their nutritional value. This can be done either through conventional selective breeding, or through genetic engineering. Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. This is an improvement on ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially fortified foods.

Biofortified staple crops, when consumed regularly, will generate measureable improvements in human health and nutrition. As such, biofortification is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the developing world. The evidence and building blocks for scale are in place; with sufficient institutional leadership, biofortification is poised to reach one billion people by 2030.

Cumulatively, more than 150 biofortified varieties of 10 crops have been released in 30 countries. Candidate biofortified varieties across 12 crops are being evaluated for release in an additional 25 countries.

Some of the biofortified rice varieties (Nutrient Rich Quality Rice- A Journey to Healthy Life) are

1) BRRI Dhan 62 – worlds first Zinc rich variety released in 2013.
2) BRRI Dhan 64 – worlds second variety with higher Zinc concentration and yield.
3) BRRI Dhan 72 – worlds third variety with higher Zinc concentration.

DRR Dhan 45 (IET 23832) (Nirmala *et al*., 2016). It is the first high Zinc rice variety notified at national level with overall mean Zinc content of 22.6ppm.

2.2 Molecular characterization of parents of RIL population by using SSR markers

Molecular markers are landmarks located near genetic locus controlling a trait of interest and are usually co-inherited with the genetic locus in segregating population across generations. They are used to 'flag' the position of a particular gene or the inheritance of a particular characteristic. In a genetic cross, when a trait of interest is linked with the molecular markers, individuals can be selected in which the molecular marker is present, since the presence of the marker indicates the presence of the desired trait.

Molecular markers offer a chance for breeders to carry out indirect selection without assessing the phenotype or morphology of the plant directly. Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes (Joshi *et al.,* 1999). Since molecular markers assess variations in the genetic material directly, they are not influenced by environment and hence can predict accurately the trait phenotype.

Types of Molecular Markers

Molecular markers can be classified into three different categories depending on what is being assessed for genetic polymorphism.

They are:

1) Morphological markers: these visually characterize phenotypic traits or characters,

2) Biochemical markers: these include allelic variants of enzymes called isozymes and

3) DNA (or molecular) markers: these reveals the sites of variation in DNA.

Among these three types of markers, morphological and biochemical markers are limited, often unstable and are influenced by environmental conditions. DNA markers on the other hand are unlimited in number, stable and will not be influenced by environmental conditions. Hence DNA based markers are useful in marker assisted selection.

2.2.1 DNA based markers

DNA based markers assess polymorphism at the level of DNA, the genetic material. Hence amongst the molecular markers used, DNA markers are more suitable and ubiquitous to most of the living organisms (Joshi *et al.,* 1999). DNA markers reveal neutral sites of variation at the DNA sequence level. They represent genetic differences between individual organisms or species. Generally DNA markers themselves do not affect the phenotype of the trait of interest because they are located only near to or 'linked' to the genes controlling the trait. Use of DNA markers allows for the most efficient comparison between two individuals, because they are detectable at all stages of development of the organism (Mohan *et al*., 1997). Such markers have several advantages over the traditional phenotypic markers and thus offer great scope for improving the efficiency of conventional plant breeding. DNA markers are used in construction of linkage maps and assessing the level of genetic diversity within germplasm.

While it is extremely difficult to find a molecular marker, which would meet all the criteria for an efficient marker, depending on the type of study to be undertaken, a marker system can be identified that would fulfill most of the required characteristics. DNA based markers can be classified broadly into two types as (i) Hybridization based markers and (ii) PCR based markers. Generally PCR based Markers are routinely used than hybridization based markers in MAS. Since the advent of PCR technology a variety of PCR methods have been developed for various molecular biological applications. In particular, several PCR based markers were developed, which immensely simplified the DNA finger printing work. Among the PCR based markers, microsatellites are most commonly used.

2.2.2 Simple sequence repeats (SSR) or microsatellites

Microsatellites or simple sequence repeats (SSRs) are among the most commonly used DNA marker types for a wide range of purposes (diversity, genome mapping, varietal identification, etc.). The first report of microsatellites in plants was made by Condit and Hubbel (1991) and suggested that SSRs are abundant in plant system. Any number of tandem repeats of a certain nucleotide combination may be regarded as a microsatellite. These repeats are found in both coding and non coding regions and are usually characterized by a high degree polymorphism (Zane *et al*., 2002). Microsatellite loci are inherently unstable with high mutation rates, a phenomenon that was reported to be caused by DNA polymerase slippage or unequal recombination.

SSRs are highly polymorphic even between closely related lines, require low amounts of DNA, easily automated, allow high throughput screening, exchanged between laboratories and highly transferable between populations. Rice microsatellites have been demonstrated to be polymorphic between and within rice varieties (Wu and Tanksley, 1993). SSR Markers can detect a significantly higher degree of polymorphism in rice which becomes ideal or studies on genetic diversity and intensive genetic mapping (Cho *et al*., 2000). SSR markers can estimate genetic diversity between cultivars e.g. between parents of a gene pool or between plants extracted from a population or between populations.

Microsatellites are powerful for the identification of variations within cultivars implying that SSR markers can detect finer levels of variations among closely related breeding lines than RAPD (Ravi *et al*., 2003). Microsatellites have average polymorphism at least 1.5 times higher than AFLP and RAPD markers (Mackill *et al*., 1996).

Microsatellites offer several advantages compared to other molecular markers. They are highly reproducible, highly polymorphic, PCR based and readily portable with in a species. All these positive attributes coupled with multi allelic mature, co-dominant transmission, relative abundance, extensive genome coverage and requirement of only a small amount of template DNA have contributed to the extraordinary increase of interest in SSRs in many organisms (Zane *et al*., 2002).

2.2.3 A brief review of literature on characterization of parents of RIL population by using SSR markers in rice is presented here under

Rice microsatellite (RM) markers were used to study the parental polymorphism between the selected 5 rice varieties by Shankar Ilango and Sarla (2010). 112 RM markers located in rice chromosomes based on the reported distribution with an approximate genetic distance of 10 cM (centimorgan) between them. Out of 112 RM primers used 33 RM primers showed polymorphism which is found to be less. Therefore more RM primers are to be used for identifying the Microsatellite markers, as the polymorphic Rice Microsatellite markers can be used in the fine mapping for the Iron and Zinc rich micronutrient gene and to study the mapping populations of crosses obtained form these parents.

Gangaprasad Chowdary *et al.* (2013) studied genetic diversity in representative sets of high yielding varieties of rice released in India between 1970 and 2010 at molecular level employing hypervariable microsatellite markers. Of 64 rice SSR primer pairs studied, 52 showed polymorphism, when screened in 100 rice genotypes (81.25%). All the 52 markers revealed high PIC (Polymorphic Information Content) values the range being between 0.67 (RM16416) and 0.97 (RM14735) with more than 50% loci in the range of 0.8 to 0.9.

Mallikarjuna *et al*. (2013) used GPP 2 as donor parent for *Xa13, Xa21, Gm4* resistance to bacterial blight, gall midge and NLR 145 as another donor parent for "*Pi-kh* "gene resistance to blast and JGL 1798 as recurrent parent and investigated using 128 simple sequence repeat (SSR) primers covered on chromosome number 1-12. The results revealed that 36 HRM primers showed distinct polymorphism among the donor and recurrent parents studied indicating the robust nature of microsatellites in revealing polymorphism.

Kiranmayi *et al*. (2014) selected Jalamagna, an aromatic, lodging resistant variety with higher concentrations of Iron and Zinc in its grains and crossed to a high yielding local variety Swarna which has got less concentration of micronutrients. They reported that the cross resulted in production of 126 breeding lines/ recombinant inbred lines (RILs). Parental polymorphism was conducted with the aid of Polymerase Chain Reaction. Among 400 Rice Microsatellite primers used, 90 (22.5%) were polymorphic and among 71 gene

specific markers only 13 (18.3%) were polymorphic. These markers were used for screening RIL population and the data obtained will support in mapping Quantitative Trait Loci (QTLs) associated to high Fe and Zinc concentration.

Raj Kumar *et al*. (2016) conducted parental polymorphism survey among popular rice varieties of Andaman & Nicobar Islands *viz*., C 14-8, CARI Dhan 5 and donor IRBB 60 (carrying four genes for bacterial blight resistance viz., Xa4, Xa5, Xa13 and Xa21) by using 200 highly variable SSR markers. The results revealed that 36 and 48 SSR markers displayed polymorphism for C14-8 and CARI Dhan 5 respectively when compared to IRBB 60. The chromosome 4 showed highest polymorphic percentages in the C14-8 (53%) as well as CARI Dhan 5 (41 %) as compared to IRBB 60. whereas chromosome number 10 did not exhibit polymorphism for the markers tested.

Pradhan *et al*. (2016) studied polymorphism between two contrasting parents for drought tolerance and phenotyping for reproductive stage drought tolerance traits. A highly tolerant germplasm line CR 143-2-2 and a susceptible high yielding popular variety of Andhra Pradesh state of India, Krishnahamsa. Seventy seven markers (38.3%) distributed in all chromosomes except chromosome 4 and 5 showed polymorphism between the two genotypes. The markers like RM 12091, RM 279, RM 104, RM 263 and RM 523 were linked to multiple traits. The polymorphic markers obtained used to validate the developed recombinant inbred lines (RILs) and mapping of QTLs for drought tolerance.

To improve the efficiency of breeding for total grain protein in rice, Shashidara *et al*. (2017) has identified promising local indica rice HPR14, which possess relatively higher protein than cultivated rice. The rice protein normally posses 7-8 percent while the donor genotype identified had an average of 14.1 per cent total protein. The initial results on the segregation for protein content indicated 3.5-18 per cent of protein variation among the 1267 F_2 segregating lines. In order to transfer these valuable trait into popular rice variety BPT –5204, crosses were made and F2 segregating lines were developed. The parental plants were surveyed using 402 rice SSR markers, out of which 69 (17.20%) showed polymorphism on agrose gel, 81 (20.00%) on PAGE and 252 were monomorphic (indicating homology between the parents).

Haque *et al.* (2012) used thirty two rice lines of BC1F1 population (Binadhan-7/FL 378) to identify introgressed rice lines for salt tolerance using SSR markers at the Plant

Breeding and Biotechnology Divisions of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Parental polymorphism survey was assayed by 8 SSR markers and three polymorphic SSR markers viz., RM296, RM585 and OSR30 were selected to evaluate BC1F1 rice lines for salt tolerance. The polymorphism information content (PIC) values ranged from 0.3290 to 0.3671 with an average of 0.3544. Among the lines, all the loci were polymorphic and clearly distinct and the cluster analysis showed nearly similar pattern of variation which could be used for improvement of salt tolerant rice lines of rice through SSR markers.

Kumari *et al*. (2010) studied a total of 268 F7 RILs of IR50 and Rathu Heenati were phenotyped for their level of resistance against BPH by the standard seedbox screening test (SSST) in the greenhouse. A total of 53 SSR primers mapped on the chromosome 3 were used to screen the polymorphism between the parents IR50 and Rathu Heenati, The eleven primers that have shown polymorphism between the IR50 and Rathu Heenati parents were genotyped in a set of five resistant RILs and five susceptible RILs along with the parents for co-segregation analysis. Among the eleven primers, two primers namely RM3180 and RM2453 showed complete co-segregation with resistance.

Sruthi *et al*. (2016) used Rice microsatellite (RM) markers to study the parental polymorphism between the selected two parents APMS-6B a popularly used maintainer line with low stigma exertion (14.95%) and BF-16B, another maintainer line with high stigma exertion (80.25%). The two parents were screened for parental polymorphism using 454 SSR markers, of which 118 markers exhibited polymorphism. The overall polymorphism level for the surveyed SSR markers was 25.99 % across the 12 chromosomes. Construction of a Linkage Map could be ensued procuring the generated genotyping data which could further avail QTL analysis and identification of markers linked to stigma exertion trait.

Ravi *et al*. (2003) conducted genetic diversity studies among 40 cultivated varieties and five wild relatives of rice, Oryza sativa L. involving simple sequence repeat (SSR). The accessions were evaluated for polymorphism after amplification with 36 decamer primers and 38 SSR primer pairs. Out of 38 SSR primer pairs used, only one locus viz., RM115 was monomorphic. The average Polymorphism Information Content (PIC) value was 0.578 and it ranged from a low of zero (RM 115) to a high of 0.890 (RM 202). SSR analysis resulted in a more definitive separation of clusters of genotypes indicating a higher level of

efficiency of SSR markers for the accurate determination of relationships between accessions.

2.3 GENETIC VARIABILITY

Genetic variability in any crop is pre requisite for selection of superior genotypes over the existing cultivars. There are three main sources of genetic variability *viz.*, natural crossing over, spontaneous mutations and recombinations. Plant characters are of two types *viz*., quantitative or polygenic and qualitative or oligogenic. Almost all plant parts and functions exhibit difference in quantitative nature.

According to Allard (1960), yield is polygenically controlled quantitative character and is highly influenced by environment. Polygenic variation present in plant population is of three types *viz*., phenotypic, genotypic and environmental variation. Analysis of variance provides estimates of phenotypic, genotypic and environmental variances, which are used in estimation of phenotypic, genotypic and environmental coefficients of variation.

Partitioning of observed variability into heritable and non-heritable components is very much essential to get a true indication of the genetic coefficient of variability as a useful measure of the magnitude of genetic variance present in the population.

2.3.1 A brief review of literature on genotypic and phenotypic coefficients of variation in rice is presented in tabular form.

S.No.	Character	Range	Reference
1.	Days to 50 per cent flowering	High	Jayasudha and Sharma (2010) Nandan *et al*. (2010) Bisne and Sarorogi (2011) Seyoum *et al*. (2012) Thippeswamy *et al*. (2016)

S.No.	Character	Range	Reference
		Moderate	Selvaraj *et al.* (2011) Mohanty *et al.* (2012) Singh *et al.* (2012) Bekele *et al.* (2013) Sravan *et al.* (2014) Shrivastava *et al.* (2015)
		Low	Singh *et al.* (2011) Rahman *et al.* (2012) Sravan *et al*. (2012) Gangashetty *et al*. (2013) Singh *et al*. (2013) Vanisree *et al*. (2013) Dhurai *et al.* (2014) Rahman *et al.* (2014) Suresh *et al*. (2014) Mohan *et al.* (2015) Sameera *et al.* (2015) Shekawat *et al.* (2015) Devi *et al*. (2016) Lakshmi *et al. (*2017)

S.No.	Character	Range	Reference
2.	Plant height (cm)	High	Akhtar *et al.* (2011) Seyoum *et al*. (2012) Gangashetty *et al*. (2013) Rahman *et al.* (2014) Biswash *et al.* (2015) Islam *et al*. (2015) Thippeswamy *et al*. (2016)
		Moderate	Selvaraj *et al.* (2011) Sravan *et al*. (2012) Vanisree *et al*. (2013) Dhurai *et al.* (2014) Sravan *et al.* (2014) Shrivastava *et al*. (2015) Devi *et al*. (2016) Lakshmi *et al.* (2017)
		Low	Subbaiah *et al*. (2011) Pandey and Reddy (2012) Rajendar *et al*. (2013) Mohan *et al*. (2015) Sameera *et al.* (2015)

S.No.	Character	Range	Reference
3.	Number of productive tillers per plant	High	Akhtar *et al*. (2011) Padmaja *et al.* (2011) Singh *et al*. (2012) Basavaraja *et al*. (2013) Bekele *et al.* (2013) Gangashetty *et al*. (2013) Vanisree *et al*. (2013) Chakraborty and Chaturvedi (2014) Dhurai *et al.* (2014) Allam *et al.* (2015) Biswas *et al*. (2015) Sameera *et al.* (2015) Shekawat *et al*. (2015) Lakshmi *et al.* (2017)
		Moderate	Mohanty *et al*. (2012) Sravan *et al*. (2012) Dhanwani *et al*. (2013) Devi *et al*. (2016)
		Low	Garg *et al*. (2010).

S.No.	Character	Range	Reference
			Yadav *et al*. (2010) Mohan *et al.* (2015)
4.	Panicle length	High	Nayudu *et al.* (2007) Rahman *et al*. (2014) Sachan (2015) Thippeswamy *et al*. (2016)
		Moderate	Singh *et al*. (2002) Yadav *et al*. (2010) Dhanwani *et al*. (2013) Gangashetty *et al*. (2013) Singh *et al*. (2013) Dhurai *et al.* (2014) Lakshmi *et al.* (2017)
		Low	Padmaja *et al.* (2008) Singh *et al*. (2011) Mohanty *et al*. (2012) Pandey *et al*. (2012) Sravan *et al*. (2012) Vanisree *et al*. (2013) Sravan *et al.* (2014)

S.No.	Character	Range	Reference
			Suresh *et al*. (2014) Mohan *et al* . (2015) Sameera *et al*. (2015) Devi *et al*. (2016)
5.	Number of filled grains per panicle.	High	Padmaja *et al*. (2008) Chandra *et al*. (2009) Yadav *et al*. (2011) Singh *et al*. (2012) Basavaraja *et al*. (2013) Singh *et al*. (2013) Dhurai *et al*. (2014) Lingaiah *et al*. (2014) Patel *et al* . (2014) Suresh *et al*. (2014) Mohan *et al*. (2015) Sameera *et al*. (2015) Shekawat *et al*. (2015) Devi *et al*. (2016) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017)

S.No.	Character	Range	Reference
		Moderate	Satyavathi *et al.* (2001) Parray and shikari (2008) Pandey *et al*. (2012) Sravan *et al*. (2012)
		Low	Nath and Talukdar (1997)
6.	1000 grain weight (g)	High	Nayudu *et al.* (2007) Chandra *et al*. (2009) Seyoum *et al*. (2012) Chakraborty and Chaturvedi (2014) Rahman *et al*. (2014) Bhati *et al*. (2015) Gampala *et al*. (2015) Islam *et al.* (2015) Karande *et al.* (2015) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017)
		Moderate	Dhurai *et al.* (2014) Patel *et al*. (2014) Suresh *et al*. (2014)

S.No.	Character	Range	Reference
			Shekawat *et al.* (2015) Devi *et al*. (2016)
		Low	Satyavathi *et al*. (2001) Yadav *et al*. (2010) Rajender reddy *et al*. (2013) Sravan *et al.* (2014)
7.	Grain yield per plant (g)	High	Padmaja *et al.* (2008) Chandra *et al*. (2009) Yadav *et al*. (2010) Singh *et al*. (2011) Mohanty *et al*. (2012) Singh *et al*. (2012) Sravan *et al*. (2012) Dhanwani *et al*. (2013) Gangashetty *et al*. (2013) Singh *et al*. (2013) Vanisree *et al*. (2013) Dhurai *et al.* (2014) Patel *et al*. (2014) Rahman *et al*. (2014)

S.No.	Character	Range	Reference
			Sravan *et al.* (2014) Suresh *et al*. (2014) Bhati *et al*. (2015) Gampala *et al*. (2015) Islam *et al.* (2015) Karande *et al.* (2015) Devi *et al*. (2016) Thippeswamy *et al*. (2016) Lakshmi *et al.* (2017)
		Moderate	Satyavathi *et al*. (2001) Parray and shikari (2008) Adilakshmi *et al*. (2012) Pandey *et al*. (2012) Sameera *et al.* (2015)
		Low	Babu *et al*. (2012) Rajendar Reddy *et al*. (2013)
8	Panicle weight	High	Subudhi *et al*. (2009) Satish Vangaru *et al*. (2017)
		Low	Nandeshwar *et al*. (2015)

S.No.	Character	Range	Reference
9	Grain Iron and Zinc concentration	High	Gangashetty *et al*. (2013)
		Moderate	Kalaimaghal *et al.* (2011) Sala *et al.* (2015)

2.4 HERITABILITY AND GENETIC ADVANCE

Heritability and genetic advance are the most important selection parameters. Heritability estimates along with genetic advance are helpful in predicting the gain under selection than heritability estimates alone. However, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Jonson *et al.*1955).

Heritability is the ratio of genotypic variance to the phenotypic variance or total variance. It is a good index of transmission of characters from parents to their off spring (Falconer, 1989). The estimates of heritability help the plant breeder in selection of elite genotypes from diverse populations.

Heritability is of two types *viz*., broad sense heritability and narrow sense heritability. Broad sense heritability is the ratio of genotypic variance to total or phenotypic variance. It plays an important role in animal breeding than in plant breeding. Whereas narrow sense heritability is the ratio of additive or fixable genetic variance to total or phenotypic variance. It plays an important role in selection process in plant breeding.

Genetic advance refers to the improvement in the mean genotypic value of the selected plants over the parental population**.** It is the measure of genetic gain under selection. According to Allard (1960) the success of genetic advance under selection depends on three factors *viz.*, genetic variability, heritability and selection intensity.

2.4.1 A brief review of studies on heritability and genetic advance in rice is presented here under in tabular form.

S.No	Characters	Range		Reference
		Heritability	**Genetic Advance**	
1.	Days to 50 percent flowering	High	High	Nayak *et al*. (2002) Sankar *et al*. (2006) Bharadwaj *et al*. (2007) Kishore *et al*. (2008) Gampala *et al.* (2015) Sameera *et al*. (2015)
		High	Moderate	Singh *et al*. (2011) Singh *et al*. (2012) Rajender Reddy *et al.* (2013) Dhurai *et al.* (2014) Patel *et al.* (2014) Devi *et al*. (2016) Thippeswamy *et al*. (2016)
		High	Low	Yadav *et al*. (2010) Dhanwani *et al*. (2013) Karande *et al*. (2015) Shekawat *et al*. (2015) Mohan *et al.* (2016)

				Lakshmi *et al.* (2017)
		Moderate	Moderate	Singh *et al*. (2013)
2.	Plant height	High	High	Chandra *et al*. (2009) Mohanty *et al.* (2012) Singh *et al.* (2012) Sravan *et al*. (2012) Gangashetty *et al*. (2013) Chakraborty and Chaturvedi (2014) Dhurai *et al.* (2014) Lingaiah *et al.* (2014) Rahman *et al*. (2014) Sravan *et al.* (2014) Bhati *et al*. (2015) Sameera *et al*. (2015) Devi *et al*. (2016) Lakshmi *et al.* (2017)
		High	Moderate	Patel *et al.* (2014) Biswash *et al.* (2015)

				Shekawat *et al*. (2015) Thippeswamy *et al*. (2016)
		Moderate	Low	Mohan *et al*. (2015) Mohan *et al.* (2016)
		Low	Low	Vijayalakshmi *et al.* (2008) Rajender *et al.* (2013) Islam *et al*. (2015)
3.	Number of productive tillers per hill.	High	High	Jaiswal *et al*. (2007) Selvaraj *et al.* (2011) Gangashetty *et al.* (2013) Chakraborty and Chaturvedi (2014) Dhurai *et al*. (2014) Rahman *et al.* (2014) Karande *et al.* (2015) Lakshmi *et al*. (2017)
		Moderate	Moderate	Singh *et al*. (2011)

		Low	Low	Rajender *et al.* (2013) Singh *et al.* (2013) Mohan *et al*. (2015) Mohan *et al.* (2016) Thippeswamy *et al*. (2016)
4.	Panicle length	High	High	Nayak *et al*. (2002) Nayudu *et al.* (2007) Dhurai *et al.* (2014) Sameera *et al.* (2015) Lakshmi *et al*. (2017)
		High	Moderate	Yadav *et al*. (2010) Parikh *et al*. (2011) Singh *et al.* (2011) Biswash *et al.* (2015) Shekawat *et al*. (2015) Mohan *et al.* (2016)
		High	Low	Rajender *et al.* (2013)
		Moderate	Low	Thippeswamy *et al*. (2016)

5.	Number of filled grains per panicle	High	High	Padmaja *et al.* (2008) Chandra *et al*. (2009) Yadav *et al*. (2010) Pandey *et al.* (2012) Singh *et al.* (2012) Sravan *et al*. (2012) Kumar and senapathi *et al*. (2013) Singh *et al*. (2013) Chakraborty and Chaturvedi (2014) Lingaiah *et al*. (2014) Patel *et al.* (2014) Rahman *et al.* (2014) Devi *et al*. (2016) Mohan *et al*. (2016) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017)
		Moderate	Moderate	Singh *et al*. (2011)

6.	1000 grain weight	High	High	Chakraborty and Chaturvedi (2014) Dhurai *et al.* (2014) Lingaiah *et al.* (2014) Patel *et al*. (2014) Rahman *et al*. (2014) Suresh *et al*. (2014) Gampala *et al*. (2015) Sameera *et al.* (2015) Devi *et al*. (2016) Mohan *et al.* (2016) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017)
		High	Moderate	Seyoum *et al.* (2012)
		High	Low	Rajender *et al*. (2013) Mohan *et al.* (2015)
7.	Grain yield per plant	High	High	Chakraborty and Chaturvedi (2014) Dhurai *et al.* (2014) Lingaiah *et al.* (2014) Patel *et al*. (2014) Choudary *et al.* (2016)

				Devi *et al*. (2016) Suresh *et al*. (2014) Biswash *et al*. (2015) Karande *et al*. (2015) Lakshmi *et al.* (2017)
		Moderate	Moderate	Thippeswamy *et al*. (2016)
		Moderate	Low	Mohan *et al.* (2015)
		High	High	Dhawani *et al.* (2013) Vanisree *et al.* (2013) Sameera *et al*. (2015)
8	Panicle weight	High	High	Lestari *et al.* (2015) Satish Vangaru *et al*. (2017)
		Low	High	Nandeshwar *et al*. (2015)
		High	Low	Subudhi *et al*. (2009)
9	Grain Zinc and Iron	High	High	Gangashetty *et al.* (2013)

2.5 CHARACTER ASSOCIATION

Most of the characters of interest to breeders are complex and are the result of the interaction of a number of components. Correlation coefficient is a statistical measure which is used to find out the degree and direction of relationship between two or more variables. In plant breeding, correlation coefficient analysis measures the mutual relationship between various plant characters and it determines the component characters on which selection can be based for genetic improvement in yield.

A brief review of literature on the character association in rice is presented here under:

2.5.1 Association of yield component characters with grain yield/plant in rice

Character	Nature of Association	Reference
Days to 50per cent flowering	Positive significant	Kuldeep *et al.* (2004) Sankar *et al.* (2006) Krishna *et al*. (2008) Ekka *et al*. (2011) Reddy *et al.* (2013) Rashid *et al.* (2014) Sarker *et al.* (2014) Thippeswamy *et al*. (2016) Priya *et al*. (2017)
	Positive non – significant	Madhavilatha *et al*. (2005) Chandra *et al*. (2009) Nandan *et al.* (2010) Rao *et al.* (2014)
	Negative significant	Garg *et al*. (2010) Babu *et al*. (2012) Gangashetty *et al*. (2013) Anil Kumar *et al*. (2015) Tejaswini *et al.* (2016) Lakshmi *et al.* (2017)

		Ajmera *et al*. (2017)
	Negative non – significant	Seyoum *et al.* (2012) Nagaraju *et al.* (2013) Nikhil *et al*. (2014) Kalyan *et al.* (2017)
Plant height	Positive significant	Krishna *et al.* (2008) Nandan *et al.* (2010) Ekka *et al*. (2011) Mohanty *et al*. (2012) Chaudary *et al*. (2013) Reddy *et al*. (2013) Patel *et al.* (2014) Biswash *et al*. (2015) Thippeswamy *et al*. (2016) Kalyan *et al.* (2017) Priya *et al*. (2017)
	Positive non – significant	Madhavilatha *et al.* (2005) Tejaswini *et al.* (2016)
	Negative significant	Babu *et al*. (2012) Nagaraju *et al*. (2013) Nikhil *et al*. (2014) Rao *et al.* (2014) Rashid *et al.* (2014) Sarker *et al.* (2014)

		Anil kumar *et al*. (2015) Ratna *et al*. (2015) Ajmera *et al*. (2017)
	Negative non – significant	Seyoum *et al.* (2012) Rahman *et al.* (2014)
No. of productive tillers per plant	Positive significant	Garg et al. (2010) Padmaja et al. (2011) Gangashetty *et al*. (2013) Nagaraju *et al.* (2013) Naseem *et al*. (2014) Patel *et al.* (2014) Rao *et al.* (2014) Rashid *et al.* (2014) Sarker *et al.* (2014) Anil kumar *et al*. (2015) Ratna *et al.* (2015) Ashok *et al*. (2016) Thippeswamy *et al*. (2016) Kalyan *et al.* (2017) Priya *et al*. (2017)
	Positive non – significant	Seyoum *et al*. (2012) Nikhil *et al*. (2014) Rahman *et al.* (2014)

	Negative non – significant	Nandan *et al.* (2010)
Panicle length	Positive significant	Chandra *et al*. (2009) Garg *et al*. (2010) Ekka *et al*. (2011) Padmaja *et al.* (2011) Mohanty *et al*. (2012) Sravan *et al*. (2012) Nagaraju *et al.* (2013) Reddy *et al*. (2013) Patel *et al.* (2014) Rahman *et al.* (2014) Rao *et al.* (2014) Biswash *et al*. (2015) Tejaswini *et al.* (2016) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017) Priya *et al*. (2017)
	Positive non – significant	Madhavilatha *et al*. (2005) Krishna *et al*. (2008) Seyoum *et al.* (2012)
	Negative significant	Nandan *et al*. (2010)

		Nikhil *et al*. (2014) Rao *et al.* (2014) Rahman *et al.* (2014) Rashid *et al.* (2014) Anil Kumar *et al*. (2015)
No. of filled grains per panicle	Positive significant	Garg *et al*. (2010) Nandan et *al*. (2010) Padmaja *et al.* (2011) Mohanty *et al*. (2012) Sravan *et al*. (2012) Nagesh *et al*. (2013) Reddy *et al*. (2013) Naseem *et al*. (2014) Patel *et al.* (2014) Rao *et al.* (2014) Sarker *et al.* (2014) Biswash *et al*. (2015) Ashok *et al*. (2016) Kalyan *et al.* (2017) Lakshmi *et al*. (2017) Priya *et al.* (2017)

	Positive non – significant	Rahman *et al.* (2014) Rashid *et al.* (2014)
1000 – grain weight	Positive significant	Chandra *et al*. (2009) Basavaraja *et al.* (2011) Yadav *et al*. (2011) Rangare *et al*. (2012) Chakraborty and Chaturvedi (2014) Naseem *et al*. (2014) Patel *et al.* (2014) Rahman *et al.* (2014) Rao *et al.* (2014) Rashid *et al.* (2014) Anil kumar *et al*. (2015) Ashok *et al*.(2016) Kalyan *et al.* (2017) Lakshmi *et al.(*2017) Priya *et al*. (2017)
	Positive non – significant	Rahman *et al.* (2014) Tejaswini *et al.* (2016)

	Negative non – significant	Seyoum *et al*. (2012) Nikhil *et al*. (2014)
Panicle weight	Positive significant	Subudhi *et al*. (2009) Nandeshwar *et al*. (2015) Satish Vangaru *et al*. (2017) Prasad *et al.* (2017)
Grain Iron concentration	Negative significant	Sala and Geetha (2015)
	Negative non-significant	Nagesh *et al.* (2013)
Grain Zinc concentration	Positive non-significant	Sala and Geetha (2015)
	Negative non-significant	Nagesh *et al.* (2013)

2.5.2 Association among the yield component traits with Days to 50 percent flowering

Character	**Nature of Association**	**References**
Plant height (cm)	Positive significant	Madhavilatha *et al.* (2002) Babu *et al*. (2012) Anil Kumar *et al*. (2015) Ajmera *et al*. (2017)
	Positive non – significant	Babu *et al*. (1999) Sarker *et al.* (2014)
	Negative non – significant	Nandan *et al.* (2010) Rao *et al.* (2014)

Panicle length (cm)	Positive significant	Madhavilatha (2002) Chandra et al. (2009) Babu *et al* . (2012) Anil kumar *et al*. (2015) Ajmera *et al*. (2017)
	Negative significant	Nandan *et al*. (2010)
	Negative non – significant	Rao *et al.* (2014)
No. of productive tillers per plant	Positive significant	Sakthivel *et al.* (2001) Rao *et al.* (2014)
	Positive non – significant	Raju *et al*. (2002) Chandra *et al*. (2009) Sarker *et al.* (2014)
	Negative significant	Padmaja *et al*. (2011) Babu *et al*. (2012) Anil kumar *et al*. (2015)
No. of filled grains per panicle	Positive significant	Padmaja *et al*. (2011) Seyoum *et al*. (2012) Nagesh *et al*. (2013) Reddy *et al*. (2013) Rao *et al.* (2014) Ajmera *et al*. (2017)
	Positive non – significant	Nandan *et al.* (2010) Sarker *et al.* (2014)

1000 – grain weight	Positive significant	Yadav *et al*. (2011) Sarker *et al.* (2014) Anil Kumar *et al*. (2015)
	Positive non – significant	Rao *et al.* (2014)
	Negative significant	Bhadru *et al.* (2012)
Panicle weight	Positive significant	Subudhi *et al*. (2009)
Grain Iron concentration	Negative significant	Sala and Geetha (2015)
	Negative non significant	Ajmera *et al*. (2017)
Grain Zinc concentration	Positive significant	Sala and Geetha (2015)
	Negative non significant	Ajmera *et al*. (2017)

2.5.3 Association among the yield component traits with Plant height

Character	**Nature of Association**	**References**
No. of productive tillers per plant	Positive significant	Raju (2002) Chandra *et al*. (2009) Rahman *et al.* (2014)
	Positive non – significant	Rao *et al.* (2014)
Panicle weight	Positive non-significant	Prasad *et al.* (2017)

Panicle length	Positive significant	Yadav *et al*. (2010) Yadav *et al*. (2011) Babu *et al.* (2012) Sravan *et al*. (2012) Reddy *et al*. (2013) Rao *et al.* (2014) Sarker *et al.* (2014) Anil kumar *et al*. (2015) Ratna *et al.* (2015) Lakshmi *et al.* (2017)
	Positive non-significant	Raju (2002) Sala and Geetha (2015)
	Negative significant	Padmaja *et al.* (2011)
	Negative non – significant	Rahman *et al.* (2014)
No. of filled grains per panicle	Positive significant	Chandra *et al*. (2009) Sravan *et al*. (2012) Reddy *et al*. (2013) Rahman *et al.* (2014) Sarker *et al.* (2014)
	Negative significant	Akhtar *et al*. (2011)

		Rao *et al.* (2014)
	Negative non – significant	Nandan *et al*. (2010)
1000- grain weight	Positive significant	Krishna *et al.* (2008) Babu *et al*. (2012) Biswash *et al*.(2015)
	Positive non-significant	Dhurai *et al*. (2016)
	Negative significant	Rao *et al*. (2014) Rahman *et al*. (2014) Sarker *et al.* (2014) Ratna *et al.* (2015)
Grain Iron concentration	Positive significant	Gangashetty *et al.* (2013)
	Negative significant	Sala and Geetha (2015)
Grain Zinc concentration	Positive significant	Sala and Geetha (2015)
	Negative significant	Nagesh *et al*. (2013)

2.5.4 Association among the yield component traits with panicle length

Character	Nature of Association	Reference
No. of productive tillers per plant	Negative significant	Babu *et al*. (2012) Rahman *et al.* (2014)
	Negative non-significant	Dhurai *et al*. (2016)
No. of filled grains per panicle	Positive significant	Padmaja *et al*. (2011) Ajmera *et al*. (2017)
	Positive non – significant	Rahman *et al.* (2014)
	Negative significant	Rao *et al.* (2014)
	Negative non – significant	Nandan *et al*. (2010)
1000 – grain weight	Positive significant	Babu *et al*. (2012) Biswash *et al*. (2015) Lakshmi *et al*. (2017)
	Positive non – significant	Nandan *et al*. (2010) Rahman *et al.* (2014)
	Negative significant	Rao *et al.* (2014) Ratna *et al.* (2015)

	Negative non-significant	Dhurai *et al.* (2016)
Grain Iron concentration	Negative significant	Sala and Geetha (2015)
	Negative non – significant	Ajmera *et al*. (2017)
Grain Zinc concentration	Positive significant	Sala and Geetha (2015)
	Positive non-significant	Ajmera *et al*. (2017)
	Negative non-significant	Nagesh *et al*. (2013)

2.5.5 Association among the yield component traits with number of productive tillers/plant

Character	**Nature of Association**	**References**
Panicle length	Positive significant	Padmaja *et al*. (2011) Yadav *et al.* (2011) Nagaraju *et al.* (2013)
	Positive non – significant	Rao *et al.* (2014)
	Negative significant	Babu *et al.* (2012) Rahman *et al*. (2014) Ratna *et al*. (2015)

Plant height	Positive significant	Rahman *et al.* (2014)
No. of filled grains per panicle	Positive significant	Nagaraju *et al.* (2013)
	Positive non – significant	Rahman *et al.* (2014) Sarker *et al.* (2014)
	Negative significant	Rao *et al.* (2014) Thippeswamy *et al*. (2016) Lakshmi *et al*. (2017)
1000 – grain weight	Positive significant	Anil kumar *et al.* (2015)
	Negative significant	Padmaja *et al*. (2011) Rahman *et al.* (2014)
	Negative non – significant	Rao *et al.* (2014)
Grain Iron concentration	Positive significant	Gangashetty *et al.* (2013) Sala and Geetha (2015) Ajmera *et al*. (2017)
	Negative non-significant	Nagesh *et al.* (2017)
Grain Zinc concentration	Negative significant	Sala and Geetha (2015)

2.5.6 Association among the yield component traits with number of filled grains per panicle

Character	Nature of Association	References
1000 – grain weight	Positive significant	Rao *et al.* (2014)
	Positive non-significant	Biswash *et al.* (2015) Thippeswamy *et al*. (2016) Lakshmi *et al.* (2017)
	Negative significant	Rahman *et al.* (2014)
	Negative non – significant	Nandan *et al*. (2010)
Grain Iron concentration	Positive non-significant	Sala and Geetha (2015) Ajmera *et al*. (2017)
Grain zinc concentration	Negative significant	Sala and Geetha (2015) Ajmera *et al*. (2017)
	Negative non-significant	Nagesh *et al*. (2013)

2.5.7 Association among the yield component traits with panicle weight

Character	Nature of Association	Reference
Days to 50% flowering	Negative significant	Prasad *et al*. (2017

Plant height	Positive significant	Subudhi *et al*. (2009) Prasad *et al*. (2017)
1000 grain weight	Positive significant	Prasad *et al.* (2017)

2.5.8 Association of 1000 grain weight with grain iron and zinc concentration

Character	**Nature of Association**	**Reference**
Grain Iron concentration	Negative non-significant	Nagesh *et al.* (2013)
Grain Zinc concentration	Negative non-significant	Nagesh *et al.* (2013)

2.5.9 Association of grain iron concentration with grain zinc concentration

Character	**Nature of Association**	**Reference**
Grain zinc concentration (ppm)	Positive significant	Gangashetty *et al.* (2013) Nagesh *et al.* (2013)
	Positive non - significant	Patil (2008)
	Negative significant	Sala and Geetha (2015)

2.6 PATH COEFFICIENT ANALYSIS

The concept of path coefficient analysis was originally developed by Wright in 1921, but the technique was first used for plant selection by Dewey and Lu in 1929. It is defined as a standardized partial regression coefficient which splits the correlation coefficient into the measures of direct and indirect effects. In other words, it measures the direct and indirect contribution of various independent characters on dependent character. It measures the cause of association between two variables.

In path analysis, a line diagram is constructed with the help of simple correlation co efficient among various characters included under study is referred to as path diagram. According to Dewey and Lu (1959) path analysis consists of three steps *viz.*, calculation of direct effects, indirect effects and residual effects.

A brief review of literature on the association of characters in rice is presented here under:

2.6.1 Direct effects of yield contributing characters on grain yield per plant

Character	**Positive direct effect on grain yield**	**Negative direct effect on grain yield**
Days to 50 percent flowering	Chandra *et al*. (2009) Nandan *et al.* (2010) Ekka *et al*. (2011) Padmaja *et al*. (2011) Basavaraja *et al*. (2012) Mohanty *et al.* (2012) Seyoum *et al.* (2012) Nikhil *et al*. (2014) Ratna *et al. (*2015) Tejaswini *et al.* (2016) Kalyan *et al.* (2017) Priya *et al*. (2017)	Garg *et al*. (2010) Yadav *et al*. (2010) Yadav *et al*. (2011) Babu *et al.* (2012) Pandey *et al*. (2012) Patel *et al*. (2014) Lakshmi *et al*. (2017)

Plant height	Garg *et al*. (2010) Nandan *et al.* (2010) Padmaja *et al.* (2011) Yadav *et al*. (2011) Babu *et al.* (2012) Basavaraja *et al*. (2012) Mohanty *et al.* (2012) Seyoum *et al.* (2012) Nagaraju et al. (2013) Rahman *et al*. (2014) Ashok *et al*. (2016) Kalyan *et al.* (2017) Lakshmi *et al.* (2017) Priya *et al*. (2017)	

Number of productive tillers per plant	Garg *et al*. (2010) Ekka *et al*. (2011) Padmaja *et al.* (2011) Babu *et al.* (2012) Pandey *et al.* (2012) Gangashetty *et al*. (2013) Nagaraju *et al.* (2013) Nagesh *et al*. (2013) Sarkar *et al.* (2014) Lingaiah *et al*. (2014) Naseem *et al.* (2014) Rahman *et al*. (2014) Rao *et al*. (2014) Anil kumar *et al.* (2015) Ratna *et al. (*2015) Ashok *et al*. (2016) Kalyan *et al.* (2017) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Seyoum *et al*. (2012) Nikhil *et al*. (2014)

Panicle length	Ekka *et al*. (2011) Babu *et al.* (2012) Mohanty *et al*. (2012) Biswash *et al*. (2015) Ashok *et al*. (2016) Tejaswini *et al.* (2016)	Garg *et al*. (2010) Padmaja *et al*. (2011) Patel *et al*. (2014) Thippeswamy *et al*. (2016) Kalyan *et al.* (2017) Lakshmi *et al.* (2017) Priya *et al*. (2017)
Number of filled grains per panicle	Garg *et al*. (2010) Nandan *et al.* (2010) Padmaja *et al.* (2011) Yadav *et al*. (2011) Mohanty *et al.* (2012) Nagaraju *et al*. (2013) Naseem *et al*. (2014) Rahman *et al*. (2014) Rao *et al*. (2014) Sarker *et al*. (2014) Biswash *et al*. (2015) Ashok *et al*. (2016) Thippeswamy *et al*. (2016) Kalyan *et al*. (2017) Lakshmi *et al*. (2017)	Akhtar *et al.* (2011) Babu *et al.* (2012)

	Priya et al. (2017)	
1000 – grain weight	Suman (2003) Chandra *et al.* (2009) Yadav *et al.* (2011) Akhtar *et al. (*2011) Padmaja *et al*. (2011) Chakraborty and Chaturvedi (2014) Lingaiah *et al*. (2014) Nikhil *et al*. (2014) Rahman *et al*. (2014) Rao *et al*. (2014) Anil kumar *et al.* (2015) Ratna *et al.* (2015) Ashok *et al*. (2016) Thippeswamy *et al*. (2016) Tejaswini *et al.* (2016) Kalyan *et al.* (2017) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Garge *et al*. (2010) Basavaraja *et al*. (2011) Seyoum *et al*. (2012) Babu *et al*. (2012) Patel *et al*. (2014)
Panicle weight	Satish Vangaru *et al*. (2017)	Prasad *et al*. (2017)

Grain Iron concentration	Bekele *et al.* (2013)	Nagesh *et al*. (2013)
Grain Zinc concentration	Bekele *et al.* (2013)	Nagesh *et al*. (2013)

2.6.2 Indirect effects

2.6.2.1 Indirect effects of days to 50 per cent flowering on grain yield through

Character	Positive indirect effect	Negative indirect effect
Plant height	Madhavilatha (2002) Padmaja *et al*. (2011) Bhadru *et al.* (2012) Priya *et al*. (2017)	Kole *et al.* (2008) Chandra *et al*. (2009) Babu *et al*. (2012)
Number of filled grains per panicle	Nayak *et al*. (2001) Madhavilatha (2002) Sandhyakishore (2005) Bhadru *et al.* (2012) Kalyan *et al.* (2017) Priya *et al*. (2017)	Padmaja *et al*. (2011) Babu *et al*. (2012)
Panicle length	Kalyan *et al.* (2017) Lakshmi *et al.* (2017)	Tejaswini *et al.* (2016)

1000 – grain weight	Basavaraja *et al*. (2011) Tejaswini *et al.* (2016) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Padmaja *et al*. (2011) Babu *et al*. (2012)
No, of productive tillers per plant	Kalyan *et al* . (2017) Priya *et al*. (2017)	
Panicle weight	Prasad *et al.* (2017)	

2.6.2.2 Indirect effects of plant height on grain yield through

Character	**Positive indirect effect**	**Negative indirect effect**
Days to 50 percent flowering	Yadav *et al*. (2010) Babu *et al*. (2012) Tejaswini *et al.* (2016) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Padmaja *et al*. (2011) Bhadru *et al.* (2012) Yadav and Kumar (2012)
Number of productive tillers per plant	Yadav *et al*. (2010) Bhadru *et al.* (2012) Mohanty *et al*. (2012) Pandey *et al*. (2012) Reddy *et al*. (2013) Rahman *et al*. (2014) Kalyan *et al.* (2017)	Padmaja *et al.* (2011) Babu *et al*. (2012)

Number of filled grains per panicle	Madhavilatha *et al*. (2005) Chandra *et al.* (2009) Padmaja *et al*. (2011) Babu *et al*. (2012) Rahman *et al*. (2014) Lakshmi *et al.(*2017) Priya *et al*. (2017)	Bhadru *et al.* (2012)
1000 – grain weight	Bhadru *et al.* (2012) Rahman *et al*. (2014) Tejaswini *et al.* (2016) Priya *et al*. (2017)	Padmaja *et al*. (2011) Pandey *et al.* (2012) Babu *et al*. (2012)
Panicle weight	Prasad *et al*. (2017)	

2.6.2.3 Indirect effects of number of productive tillers per plant on grain yield through

Character	**Positive indirect effect**	**Negative indirect effect**
Days to 50 percent flowering	Nayak *et al.* (2001) Madhavilatha (2002) Sandhyakishore (2005) Padmaja *et al*. (2011) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Kavitha and Reddi (2001) Yadav *et al*. (2010) Babu *et al*. (2012)
Plant height	Yadav *et al*. (2010)	Babu *et al*. (2012)

	Rahman *et al*. (2014) Kalyan *et al. (*2017) Priya *et al*. (2017)	
Panicle length	Padmaja *et al*. (2011) Gangashetty *et al*. (2013) Rahman *et al*. (2014)	Babu *et al*. (2012)
Panicle weight	Prasad *et al*. (2017)	

2.6.2.4 Indirect effects of panicle length on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 percent flowering	Babu *et al*. (2012) Bhadru *et al.* (2012) Lakshmi *et al. (*2017) Priya *et al*. (2017)	Padmaja *et al*. (2011) Kalyan *et al.* (2017)
Plant height	Padmaja *et al*. (2011) Babu *et al*. (2012) Bhadru *et al.* (2012) Rahman *et al*. (2014) Biswash *et al*. (2015) Priya *et al*. (2017)	Yadav *et al*. (2010) Kalyan *et al.* (2017)

Number of productive tillers per plant	Padmaja *et al*. (2011) Mohanty *et al*. (2012) Reddy *et al*. (2013) Rahman *et al*. (2014) Lakshmi *et al. (*2017)	Babu *et al*. (2012) Bhadru *et al.* (2012)
Number of filled grains per panicle	Yadav *et al*. (2010) Rahman *et al*. (2014) Biswash *et al*. (2015)	Pandey *et al*. (2012) Reddy *et al*. (2013)
Number of filled grains per panicle	Lakshmi *et al.* (2017) Priya *et al*. (2017)	
1000 – grain weight	Basavaraja *et al*. (2011) Padmaja *et al*. (2011) Babu *et al*. (2012) Priya *et al*. (2017)	Bhadru *et al*. (2012) Pandey *et al*. (2012)
Panicle weight	Kumar and Senapathi (2013) Naseem *et al.* (2014)	Manna *et al.* (2006) Yadav *et al.* (2010) Basavaraja *et al.* (2011)

2.6.2.5 Indirect effects of number of filled grains per panicle on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 percent flowering	Padmaja *et al*. (2011) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Bhadru *et al*. (2012)
Plant height	Akhtar *et al*. (2011) Padmaja *et al*. (2011) Bhadru *et al*. (2012) Lakshmi *et al. (*2017) Priya *et al*. (2017)	Rahman *et al*. (2014)
Number of productive tillers per plant	Akhtar *et al*. (2011) Rahman *et al*. (2014) Kalyan *et al.* (2017) Priya *et al*. (2017)	Padmaja *et al*. (2011)
Panicle length	Padmaja *et al*. (2011) Rahman *et al*. (2014)	Bhadru *et al*. (2012)
1000 – grain weight	Akhtar *et al*. (2011) Padmaja *et al*. (2011) Rahman *et al*. (2014) Priya *et al*. (2017)	
Panicle weight	Prasad *et al*. (2017)	

2.6.2.6 Indirect effects of 1000-grain weight on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 percent flowering	Nayak *et al*. (2001) Madhavilatha (2002) Kole *et al*. (2008) Chandra *et al*. (2009) Priya *et al*. (2017)	Padmaja *et al*. (2011) Bhadru *et al*. (2012) Babu *et al*. (2012) Kalyan *et al.* (2017)
Number of filled grains per panicle	Padmaja *et al*. (2011) Priya *et al*. (2017)	Kalyan *et al.(*2017) Rahman *et al*. (2014)
Plant height	Babu *et al*. (2012) Rahman *et al*. (2014) Priya *et al*. (2017)	Yadav *et al*. (2010) Padmaja *et al*. (2011) Bhadru *et al*. (2012) Kalyan *et al.* (2017)
Number of productive tillers per plant	Bhadru *et al*. (2012) Rahman *et al*. (2014) Lakshmi *et al.* (2017) Priya *et al*. (2017)	Padmaja *et al*. (2011) Kalyan *et al. (*2017)
Panicle length	Padmaja *et al*. (2011) Rahman *et al*. (2014) Lakshmi *et al.* (2017)	Babu *et al*. (2012) Kalyan *et al.* (2017)
Panicle weight	Prasad *et al.* (2017)	

Chapter III
MATERIAL AND METHODS

EXPERIMENT : 1

The present investigation was undertaken at the Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad with the objective,

1) To characterize the parents of RIL population using SSR markers

3.1 Materials

3.1.1 Materials for parental polymorphism

The experimental plant material for molecular parental polymorphism comprised of donor parent Type 3 aromatic rice variety and recipient parent Swarna which were collected from the Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad.The details of parentage were described below.

Table 1. Parentage and their salient features.

S.No	Parents	Parentage	Salient features	Year of Release
1	Swarna	Vasistha / Mahsuri	High yielding adaptable variety, long duration, Medium resistant to Bacterial leaf blight and Sheath blight	1979
2	Type 3	Selection from Basmati Dehradun	Aromatic with high iron content	1973

3.2.Methods

3.2.1 Molecular Characterization

Two parents were grown in pots at Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad in order to get enough leaf material for extraction of DNA.

3.2.1.1 Isolation of Genomic DNA for Molecular Characterization: Extraction of bulk DNA was carried out by using maxi prep DNA isolation protocol described by Sambrook (1989) with some modification as given below. The chemicals, buffers and instruments used for DNA isolation are described in Appendix A.

1. The pooled leaf samples from 20-25 days old seedlings were taken and cut into small pieces and placed in an alcohol sterilized mortar.

2. Approximately 700µl of extraction buffer (100mM Tris HCl, pH 8.0; 20mM EDTA; 1.4M NaCl, 2% CTAB and 2% PVP) for each leaf samples was added and the leaf sample was ground with the help of alcohol sterilized pestle till it was completely homogenized.

3. Using a micropipette of 1 ml capacity (the tip was cut at the bottom using a sterile scissor), 700µl of crushed leaf sample was transferred to a fresh 1.5 ml capacity micro centrifuge tube.

4. Equal volume of (700µl) chloroform was added; the contents were mixed well by inversion for about 10 min and centrifuged at 13000 rpm for 15min at room temperature.

5. After the centrifugation, the supernatant (= 400µl) was aliquated from the micro centrifuge tube without disturbing the intermediate layer into a fresh 1.5 ml micro centrifuge tube.

6. To this clear supernatant, equal volume (= 400µl) of chilled isopropanol was added and kept in 4 °C for 10 minutes. (Isopropanol helps in the precipitation of DNA. The DNA appears as white sediment. For better precipitation keep the tubes at –20 ° C over night).

7. The contents were mixed gently and centrifuged at13000 rpm for 10 min at room temperature 21° C.

8. The supernatant was drained gently without disturbing the DNA pellet. About 200 µl of 70 per cent ethanol was added to the pellet and the centrifuge tube was tapped in order to dispense the pellet in ethanol. The contents were centrifuged at 13000 rpm for 5 minutes. (Ethanol helps in removal of other salts left over).

9. The supernatant was discarded and the DNA pellets were air-dried at room temperature overnight.

10. Then the DNA pellets were dissolved in 50-100 µl of TE buffer (10 mM Tris- HCl, pH 8.0 and 1 mM EDTA, pH

3.2.1.2 Quality and Quantity check of isolated DNA: The purity and concentration of the isolated bulk DNA sample was estimated by UV-absorption spectrophotometer (Beckman DU 650 model) as per the procedure described by Sambrook *et al.* (2001). The ratio of absorbance at 260 nm and 280 nm was used as an indicator of DNA purity. The ratio between 1.4 and 1.9 was considered as relatively pure DNA sample as it did not show any effect on PCR reaction. The bulk genomic DNA concentration was measured by using the formula**:**

Bulk genomic DNA concentration in µg/µl = OD 260 x 50 µg x dilution factor .

The isolated DNA was also quantified based on visual examination, which was done by analyzing the pure DNA on one per cent agarose gel with diluted uncut ladder DNA as standard. Based on the intensity and thickness of genomic DNA bands when compared to λ (lambda) DNA, the concentration and quality of DNA in individual samples was determined.

3.2.1.3 Analysis using SSR markers:

To detect polymorphism among the parents, a total of **171 polymorphic SSR primer pairs** were used for PCR amplification. These primer pairs were selected based on their polymorphic information content and uniform distribution across the 12 rice chromosomes.

3.2.1.4 Amplification of genomic DNA using Polymerase Chain Reaction (PCR):

The PCR plates were labeled with respect to sample number and 4 µl (i.e. 50-100 ng) of template DNA was added to the respective wells. The master mix consisted of 1.0 µl forward primer (2.5pmoles), 1.0 µl reverse primer (2.5pmoles), 0.5 µl dNTP's (2.5 mM), 0.3 µl *Taq* DNA polymerase (3U/µl; Bangalore Genei Pvt. Ltd.), 1.0 µl of 10 x PCR buffer (Tris with 1.5 mM Mg Cl2, Bangalore Genei Pvt. Ltd.) and 2.2 µl of sterile distilled water was added to make up the volume to 10 µl. Then the master mix (6.0 µl) was dispensed to the PCR plate with template DNA. The PCR plate was covered with a sealing mat. It was then placed in a programmable thermal cycle (M/s Applied Biosystem, USA) for DNA amplification. The temperature specification for the denaturation of DNA strands, annealing of primers and extension steps were followed as given in table 2.

Table 2. PCR thermal profile for amplification.

S.No	Step	Temperature	Time	Cycles
1	Initial Denaturation	95° C	5 minutes	1
2	Denaturation	94° C	30 seconds	35 cycles
3	Annealing	59° C	30 seconds	
4	Extension	72° C	1 minute	
5	Final extension	72° C	7 minutes	1
6	4° C		∞	

Step II was repeated for 30 cycles for proper amplification of DNA. After completion of the PCR, the plate was stored at –20° C and later resolved on a 3% ethidium bromide stained agarose gel.

3.2.1.5 Electrophoresis of PCR products in Agarose gel:

The PCR products were analyzed by electrophoresis using a 3 % agarose gel (M/s SeaKem LE Agarose, Lonza USA) in a Submarine Horizontal Electrophoresis Unit (M/S. CBS Scientific, USA). About 3.0 g of agarose was weighed and transferred into reagent bottle containing 100 ml of 0.5 x TBE (Tris borate) buffer and mixed well. The content was then boiled gently in a microwave oven with intermittent mixing. The process was followed until complete melting of agarose and the solution becomes crystal clear. In the meanwhile, the gel-casting tray was washed with water and wiped with ethanol. Then the gelcasting tray was sealed with cello tape. When the boiled agarose cool down substantially (when the heat is bearable, ≈ 40° C), 5 µl of ethidium bromide (10 mg/ml) was added to the melted agarose, mixed thoroughly and poured into the gel-casting tray and the combs were arranged in the slots on the gel-casting tray and allowed to solidify at room temperature for 20-30 minutes. Care was taken so that no air bubble was present. Later the gel was transferred to Submarine Horizontal Electrophoresis Unit containing 0.5 x TBE buffer. Before loading on to the gel, PCR amplified products were mixed with $1/6^{th}$ volume of gel loading dye (40% sucrose; 0.25% bromophenol blue) and loaded into the wells with the help of micro tips. The electrodes were connected to power pack. The samples were run at 180 V for 1 to 1½ hours. 50 bp ladder (Fermentas Generuler - 0.1µg/µl) was added in one well for each primer pair to determine the size of amplified fragments. The DNA fragments were then visualized under UV transilluminator and

documented using ALPHA IMAGER gel documentation system (M/S. Alpha Innotech) which was stored for further scoring and permanent records.

EXPERIMENT : 2

The experiment to study the genetic variability, yield, yield components, iron and zinc among RIL population of rice was conducted at Indian Institute of Rice Research Farm, Ramachandrapuram, Hyderabad, India, during *kharif*, 2017. The material used, experimental design adopted, the mode of data collection and the statistical procedures followed are outlined below.

3.3 EXPERIMENTAL MATERIAL

The material used for the present study comprise of 4 checks (Swarna, Type 3, BPT-5204, Chittimutyalu) and 100 RILs of F_7 population derived from Swarna X Type 3.

3.4 DESIGN AND LAYOUT

The experiment was based on Augmented design as suggested by **Federer (1956, 1961)** with the purpose of evaluating, and doing statistical analysis, of a large number of population. The yield of population is adjusted for block differences, which are measured by check varieties in every block. 100 RIL population were planted in an augmented block design in same year along with the four check varieties viz.,, Swarna, Type 3, BPT-5204, Chittimutyalu. Check varieties were allocated in each block and the germplam lines are then randomly allocated to the remaining plots of each block.

Details of technical programme

1. Site of experiment : Indian Institute of Rice Research Farm, Ramachandrapuram, Hyderabad
2. Number of genotypes : 104 (100 + 4 Checks)
3. Experimental design : Augmented Design
4. No. of blocks : 5
5. Standard checks (Four) : Swarna , Type 3, BPT-5204, Chittimutyalu.

3.5 RECORDING OF OBSERVATIONS

Observations were recorded for yield, yield attributing characters and nutritional characters on five randomly selected competitive plants for each entry . The mean data obtained was considered for final statistical analysis. Days to 50% flowering was recorded on plot basis. The details of observations recorded and techniques adopted to record each of them are furnished below.

3.5.1 QUANTITATIVE CHARACTERS

Data was recorded on nine metric parameters on five randomly selected plants in each plot, while the data on days to 50% flowering were noted on plot basis. These characters were measured as per the standard techniques and the mean of the five plants for all the characters except days to 50% flowering was utilized for carrying statistical analysis.

3.5.1.1 Recording of phenotypic observations

The readings were taken from each plant separately. The information for characters observed are described below.

3.5.1.2 Days to 50% flowering (DFF)

The total number of days taken from the date of sowing to complete exertion of the panicle in fifty per cent of the total plants in the plot.

3.5.1.3 Plant height at maturity (cm)

Plant height was measured in centimeters from base of the plant to the tip of the mother panicle in each plant at the time of harvest.

3.5.1.4 Number of productive tillers per plant

Number of ear bearing tillers per plant was counted at the time of harvest.

3.5.1.5 Panicle length (cm)

It was measured in centimeters at the time of plant maturity from the base of panicle (neck node) to the tip of last spikelet prior to harvesting.

3.5.1.6 No.of Filled grains per panicle

Number of filled grains was counted from five panicles in each selected plant and the mean was taken.

3.5.1.7 Grain yield per plant (g)

The weight of filled grains harvested from each plant was recorded in grams after bringing the grains to required moisture content.

3.5.1.8 1000 seed weight (g)

One thousand well filled grains were counted from a random sample of each entry in each replication and weighed with the help of electronic top pan balance in grams.

3.5.1.9 Panicle weight

The total Panicle weight was measured in grams.

3.5.1.10 Grain Iron and Zinc Concentrations

Grain iron and zinc concentration were determined by X – Ray fluorescence Spectrometry (XRF) (EDXRF, model- X-supreme 8000). In XRF the preselected wavelength of incident X – rays expel the electrons from the inner most orbit followed by the transfer of one of the electrons from the outermost orbit to the inner most orbit leading to release of specific wavelength of X – rays. The energy of the emitted radiation is specific for a particular atom. Therefore, it is simultaneously identified and quantified by the detector. This instrument is quite useful in non – destructive determination of relative iron and zinc concentrations in rice samples with more ease.

3.6 STATISTICAL ANALYSIS

The mean values recorded on different traits were subjected to the following statistical analysis.

1. Analysis of variance
2. Genotypic and phenotypic coefficients of variation
3. Heritability and genetic advance
4. Estimation of correlation coefficient
5. Direct and indirect effects of characters using path coefficient analysis

Table 3. Strcture of ANOVA as per Augmented design:

Source of variation	Degrees of freedom	Mean squares	M.S.S	"F" ratio
Blocks	(b-1)	Bss	bMSS	bMSS/EMS
Entries	(e-1)	eSS	eMSS	eMSS/EMS
Checks	(c-1)	cSS	cMSS	cMSS/EMS
Varieties	(v-1)	vSS	vMSS	vMSS/EMS
Checks vs.Varieties	1	cvSS	cvMSS	cvMSS/EMS
Error	(c-1)(b-1)	ESS	EMSS	
Total	(N-1)	TSS		

where,

b = number of blocks

v = number of genotypes

e = number of entries

c = number of checks

The significance of "F" value was tested by comparing the computed value with the table value as given by Fisher and Yates (1963). Further, standard error of means (SEm+), critical difference (CD) and co-efficient of variation (CV) were worked out using appropriate formulae to facilitate the comparison of adjusted means of the genotypes.

3.6.1 Estimation of genetic parameters

Mean

The mean value of each character was determined by summing up all the observations and dividing them by corresponding number of observations.

$$\overline{X} = \frac{\sum_{i=1}^{N} X_i}{N}$$

Where,

$\overline{X}$ = mean,

$\sum_{i=1}^{N} X_i$ = Sum of all observations

N = Number of observations

Range

The lowest and highest mean values for each character were taken as the range.

Standard Error of Difference between Two Means [SE.d.(m)]

SE.d.(m) was calculated with the help of error mean square from ANOVA table.

$$S.E.d.(M) = \sqrt{\frac{2M.S.E.}{r}}$$ Where,

r = Number of replications, MSE = Mean sum of square due to error

Variance

The genotypic and phenotypic variances were calculated as per the formulae (Burton and Devane, 1953).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{(Mean sum of squares due to treatments – Mean sum of squares due to error)}}{\text{Number of replications}}$$

Phenotypic variance $(\sigma^2 p) = \sigma^2 g + \sigma^2 e$

$(\sigma^2 e)$ = Error variance

3.6.2 Genotypic and phenotypic coefficients of variance

The genotypic and phenotypic coefficients of variation were calculated according to the formula given by Falconer (1981).

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\text{Genotypic standard deviation}}{\text{Mean}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\text{Phenotypic standard deviation}}{\text{Mean}} \times 100$$

Categorization of the range of variation was done as proposed by Sivasubramanian and Madhavamenon (1973).

< 10%	:	Low
10-20%	:	Moderate
> 20%	:	High

3.6.3 Heritability and genetic advance

Heritability

Heritability in the broad sense refers to the proportion of genotypic variance to the total observed variance in the total population. Heritability (h^2) in the broad sense was calculated according to the formula given by Allard (1960).

$$h^2_{(bs)} = \frac{\sigma^2 g}{\sigma^2 p}$$

Where,

h^2	=	heritability in broad sense
$\sigma^2 g$	=	genotypic variance
$\sigma^2 p$	=	phenotypic variance ($\sigma^2 g + \sigma^2 e$)
$\sigma^2 e$	=	environmental variance

As suggested by Johnson *et al.* (1955) (h^2) estimates were categorized as:

Low	:	0-30%
Medium	:	30-60%
High	:	above 60%

Genetic advance (Expected)

Genetic advance refers to the expected gain or improvement in the next generation by selecting the superior individuals under certain amount of selection pressure. From the heritability estimates the genetic advance was estimated by the following formula given by Burton (1952).

$$GA = K.\ h^2\ (b).\ \sigma p$$

Where,

GA	=	expected genetic advance
K	=	Selection differential, the value of which is 2.06 at 5 per cent selection intensity
σp	=	phenotypic standard deviation
h^2 (b)	=	heritability in broad sense

In order to visualize the relative utility of genetic advance among the characters, genetic advance as per cent for mean was computed.

$$\text{Genetic advance as per cent of mean} = \frac{GA}{\text{Grand mean}} \times 100$$

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

Low	=	Less than 10%
Moderate	=	10-20 %
High	=	More than 20%

3.6.4 Estimation of correlation coefficients

Simple correlation coefficients were calculated for grain yield and its components using the formulae given by Webber and Moorthy (1952).

$$rp = \frac{COV(X, Y)}{[V(X).\ V(Y))]^{1/2}}$$

Where,

rp = Phenotypic correlation co-efficient.

COV (X, Y) = Phenotypic covariance between trait X and Y

V(X) and V(Y) = Phenotypic variances of the traits X and Y

3.6.5 Path coefficient analysis

The direct and indirect effects at genotypic level were estimated by taking seed yield as dependent variable, using path coefficient analysis suggested by Wright (1921) and Dewey and Lu (1959). The following equations were formed and solved simultaneously for estimating the various direct and indirect effects.

$$r_{1y} = P_{1y} r_{11} + P_{2y} r_{12} + P_{3y} r_{13} \ldots\ldots\ldots\ldots + P_{ny} r_{1n}$$

$$r_{2y} = P_{1y} r_{21} + P_{2y} r_{22} + P_{3y} r_{23} \ldots\ldots\ldots\ldots + P_{ny} r_{2n}$$

. . . . .

. . . . .

$$r_{ny} = P_{1y} r_{n1} + P_{2y} r_{n2} + P_{3y} r_{n3} \ldots\ldots\ldots\ldots + P_{ny} r_{nn}$$

Where,

1, 2 . . . . . n	=	Independent variable
y	=	Dependent variable (yield per plant)

$r_{1y}, r_{2y} \ldots \ldots \ldots \ldots r_{ny}$ = Coefficient of correlation between causal factors '1' to 'n' on dependent character 1

$p_{1y}, P_{2y} \ldots \ldots P_{ny}$ = Direct effect of characters 1 to n on character Y

The above equations can be written in matrix form as:

$$A = C \cdot B$$

$$\begin{pmatrix} r_{1y} \\ r_{2y} \\ \vdots \\ \vdots \\ r_{ny} \end{pmatrix} = \begin{pmatrix} 1 & r_{12} & r_{13} & \ldots\ldots\ldots & r_{1n} \\ r_{21} & 1 & r_{23} & \ldots\ldots\ldots & r_{2n} \\ & \vdots & \vdots & \vdots & \\ & \vdots & \vdots & \vdots & \\ r_{n1} & r_{n2} & r_{n3} & \ldots\ldots\ldots & 1 \end{pmatrix} \begin{pmatrix} p_{1y} \\ p_{24} \\ \vdots \\ \vdots \\ p_{ny} \end{pmatrix}$$

Then

$B=[C]^{-1} A$ where C^{-1} =

$$\begin{pmatrix} c_{11} & c_{12} & c_{13} & \ldots\ldots & c_{1n} \\ c_{21} & c_{22} & c_{23} & \ldots\ldots & c_{2k} \\ \vdots & \vdots & \vdots & & \\ \vdots & \vdots & \vdots & & \\ c_{n1} & c_{n2} & c_{n3} & \ldots\ldots & c_{nn} \end{pmatrix}$$

Direct effects were as follows:

$$p_{1y} = \sum_{i=1}^{k} C_{1i}\ r_{iy}$$

$$p_{2y} = \sum_{i=1}^{k} C_{2i}\ r_{iy}$$

p_{ny} = $\sum_{i=1}^{k} c_{nj}\ r_{iy}$

Residual effect, which measures the contribution of characters not considered, was obtained as:

$$p_{ry} = \sqrt{1-(p_{1y}\, r_{iy} + p_{2y}\, r_{iy} + ... + p_{ny}\, r_{ny}}$$

Where, p_{ny} = Direct effect of x_n on Y

r_{iy} = Correlation coefficient of x_n

Chapter IV

RESULTS AND DISCUSSION

The results obtained from the present experimental study on evaluation of 100 RIL population of rice are furnished under the following heads.

4.1 Characterization of parents of RIL population using SSR markers

4.2 Mean performance

4.3 Genetic variability, heritability and genetic advance

4.4 Character association

4.5 Path coefficient analysis

4.1 Characterization of parents of RIL population using SSR markers

Microsatellites or simple sequence repeats (SSRs) are among the most commonly used DNA marker types for a wide range of purposes (diversity, genome mapping, varietal identification, etc.). These markers can detect a significantly higher degree of polymorphism in rice (Ni *et al*., 2002) and are powerful for the identification of variations within cultivars implying that SSR markers can detect finer levels of variations among closely related breeding lines than RAPD (Ravi *et al*., 2003). Microsatellites have average polymorphism atleast 1.5 times higher than AFLP and RAPD markers (Mackill *et al*., 1996).

Parental SSR marker polymorphism survey was carried out between donor Type 3 and recipient Swarna. A total of 171 SSR primer pairs distributed across the 12 chromosomes of rice genome were used for molecular characterization of two parents mentioned above, selected for the study.

Of these, 115 were monomorphic and 56 SSR primer pairs were found to be polymorphic across the 12 chromosomes of rice genome given below.

Table 4. Parental polymorphic survey in 100 RIL population of rice using SSR markers

S.NO	Markers used	Amplification status	Chromosome no
1	RM 11744	pp	1
2	RM 11743	PP	1
3	RM 11747	mp	1
4	RM 11741	pp	1
5	RM 12276	mp	1
6	RM 10361	pp	1
7	RM 1	mp	1
8	RM 5	mp	1
9	RM 10936	mp	1
10	RM 7075	mp	1
11	RM 11307	pp	1
12	RM 246	mp	1
13	RM 243	pp	1
14	RM 315	mp	1
15	RM 10936	mp	1
16	RM 12276	mp	1
17	RM 10018	pp	1
18	RM 11570	mp	1
19	RM 3738	mp	1
20	RM 10782	mp	1
21	RM 13347	pp	2
22	RM 13021	mp	2
23	RM 14140	mp	2

24	RM 14181	pp	2
25	RM 13075	mp	2
26	RM 262	mp	2
27	RM 3515	pp	2
28	RM 573	mp	2
29	RM 221	mp	2
30	RM 13075	mp	2
31	RM 14181	mp	2
32	RM 13347	mp	2
33	RM RM12469	mp	2
34	RM 6361	mp	2
35	RM 13354	mp	2
36	RM 14250	mp	3
37	RM 15855	mp	3
38	RM 85	mp	3
39	RM 15580	mp	3
40	RM 16097	pp	3
41	RM 5748	mp	3
42	RM 15630	pp	3
43	RM 257	pp	3
44	RM 282	pp	3
45	RM 517	pp	3
46	RM 85	mp	3
47	RM 282	pp	3
48	RM 232	pp	3

49	RM 15372	mp	3
50	RM 16097	pp	3
51	RM 348	pp	4
52	RM 273	mp	4
53	RM 261	pp	4
54	RM 16913	mp	4
55	RM 17209	pp	4
56	RM 17680	mp	4
57	RM 241	pp	4
58	RM 413	pp	4
59	RM 16427	pp	4
60	RM 18799	mp	5
61	RM 169	pp	5
62	RM 142	pp	5
63	RM 18704	mp	5
64	RM 3486	pp	5
65	RM 413	pp	5
66	RM 421	mp	5
67	RM 19159	mp	5
68	RM 164	pp	5
69	RM 440	mp	5
70	RM 18222	mp	5
71	RM 18704	mp	5
72	RM 170	mp	6
73	RM 3	mp	6

74	RM 20710	mp	6
75	RM 20746	mp	6
76	RM 340	pp	6
77	RM 225	mp	6
78	RM 20695	mp	6
79	RM 19981	mp	6
80	RM 19341	pp	6
81	RM 19620	pp	6
82	RM 3431	pp	6
83	RM 253	pp	6
84	RM 111	pp	6
85	RM 276	pp	6
86	RM 402	pp	6
87	RM 19697	mp	6
88	RM 190	pp	6
89	RM 20625	mp	6
90	RM 20672	mp	6
91	RM 19981	mp	6
92	RM 20583	mp	6
93	RM 225	mp	6
94	RM 340	mp	6
95	RM 541	mp	6
96	RM 234	mp	7
97	RM 21097	mp	7
98	RM 21693	mp	7

99	RM 20844	pp	7
100	RM 21679	mp	7
101	RM 21539	mp	7
102	RM 11	pp	7
103	RM 21097	mp	7
104	RM 21693	mp	7
105	RM 6389	mp	7
106	RM 172	mp	7
107	RM 22054	mp	7
108	RM 22885	pp	8
109	RM 23578	mp	8
110	RM 22655	mp	8
111	RM 22418	mp	8
112	RM 22524	pp	8
113	RM 23244	mp	8
114	RM 339	mp	8
115	RM 284	mp	8
116	RM 22622	mp	8
117	RM 23244	mp	8
118	RM 477	mp	8
119	RM 1381	mp	8
120	RM 23669	mp	9
121	RM 6543	mp	9
122	RM 3912	pp	9
123	RM 219	mp	9

124	RM 1026	mp	9
125	RM 296	pp	9
126	RM 23914	mp	9
127	RM 257	pp	9
128	RM 24035	pp	9
129	RM 24839	mp	9
130	RM 160	mp	9
131	RM 23669	mp	9
132	RM 6543	mp	9
133	RM 24085	pp	9
134	RM 24516	mp	9
135	RM 189	mp	9
136	RM 296	pp	9
137	RM 3912	pp	9
138	RM 25930	mp	10
139	RM 5095	pp	10
140	RM 25754	mp	10
141	RM 24849	mp	10
142	RM 25355	mp	10
143	RM 24941	mp	10
144	RM 26402	mp	10
145	RM 25151	mp	10
146	RM 24954	mp	10
147	RM 1375	mp	10
148	RM 26213	mp	11

149	RM 26402	mp	11
150	RM 27289	pp	11
151	RM 27387	mp	11
152	RM 27310	mp	11
153	RM 26239	mp	11
154	RM 206	pp	11
155	RM 27323	mp	11
156	RM 26797	mp	11
157	RM 20513	mp	11
158	RM 27172	mp	11
159	RM 27564	mp	12
160	RM 27877	mp	12
161	RM 27962	pp	12
162	RM 28130	mp	12
163	RM 3747	mp	12
164	RM 28607	pp	12
165	RM 27973	mp	12
166	RM 247	mp	12
167	RM 235	pp	12
168	RM 27814	mp	12
169	RM 5313	pp	12
170	RM 27406	mp	12
171	RM 27440	mp	12

mp = monomorphic primer pp = polymorphic primer

Among the 56 polymorphic markers, 7 SSR primer pairs (RM 11744, RM 11743, RM 11741, RM 10361, RM 11307, RM 243 and RM 10018) were polymorphic on chromosome 1, 3 SSR primer pairs (RM13347, RM 14181 and RM 3515) were polymorphic on chromosome 2, 8 SSR primer pairs (RM 16097, RM 15630, RM 257, RM 282, RM 517, RM 282, RM 232 and RM 16097) were polymorphic on chromosome 3, 6 SSR primer pairs (RM348, RM 261, RM 17209, RM 241, RM 413 and RM 16427) were polymorphic on chromosome 4, 5 SSR primer pairs (RM 169, RM 142, RM 3486, RM 413 and RM 164) on chromosome 5, 9 SSR primer pairs (RM 340, RM 19341, RM 19620, RM 3431, RM 253, RM 111, RM 276, RM 402 and RM 190) were polymorphic on chromosome 6, 2 SSR primer pairs (RM 20844, RM 11) were polymorphic on chromosome 7, 2 SSR primer pairs (RM22885, RM 22524) were polymorphic on chromosome 8, 7 SSR primer pairs (RM 3912, RM 296, RM 257, RM 24035, RM 24085, RM 296 and RM 3912) were polymorphic on chromosome 9, one SSR primer pair RM 5095 was polymorphic on chromosome 10, 2 SSR primer pairs (RM 27289, RM 206) were polymorphic on chromosome 11, 4 SSR primer pairs (RM 27962, RM 28607, RM 235 and RM 5313) were polymorphic on chromosome 12.

Among all the chromosomes more number of polymorphic primers i.e., 9 were observed on chromosome 6. It indicates there may exist more diversity between donor and recipient parents on chromosome 6.

The number of parental polymorphic SSR markers used in the present study is much higher than those used by earlier workers. In the assessment of polymorphism, Kiranmayi *et al*. (2014) used 71 gene specific markers only 13 (18.3%) were polymorphic while Gangaprasad Chowdary *et al.* (2013) used 64 rice SSR primer pairs, and 52 showed polymorphism whereas Shankar Ilango and Sarla (2010) used 112 RM markers and 33 RM primers were found to be polymorphic.

The parental polymorphic 56 SSR markers identified in the present study to be polymorphic between donor-recipient parent combinations will be highly useful for identification of variations within cultivars and also for diversity, genome mapping etc.

4.2 MEAN PERFORMANCE

Analysis of variance showed significant differences for all the characters studied in the present investigation. The results of analysis of variance are presented in Table 7. The mean values for 9 characters for 100 RIL population are given in Table 11.

4.2.1 QUANTITATIVE CHARACTERS

4.2.1.1 Days to 50 per cent flowering

Days to 50 per cent flowering ranged from 101 to 125 with a general mean of 114.1 days. Among all the RIL's P-76, P-78 and P-119 were earliest (101 days) while C1 (Swarna) was found late to flowering (125 days).

4.2.1.2 Plant height (cm)

Plant height ranged from 77 to 167 cm with a general mean of 134.6 cm. The shortest genotype was P-65 (77 cm) while the tallest genotype was P-77 (167 cm).

4.2.1.3 Number of productive tillers per plant

The mean productive tillers per plant ranged from 8.2 to 13.4 tillers per plant with a general mean of 10.3 tillers per plant. Genotypes P-32, P-33 and P-34 had less number of tillers, whereas P-100 had more number of productive tillers per plant.

4.2.1.4 Panicle length (cm)

The panicle length ranged from 16.8 to 28.2 cm with a general mean of 23.9 cm. The genotype P-70 had shorter panicle length of 16.8 cm and the genotype P-99 exhibited longest panicle length of 28.2 cm, which was almost 11.4 cm longer than P-70 .

4.2.1.5 Number of filled grains per panicle

The mean values for number of filled grains per panicle ranged from 32.30 in P-60, P-69, P-106 to 171 in C4 (Chittimutyalu), with general mean value of 105.4.

4.2.1.6 Panicle weight (g)

The panicle weight ranged from 2.23 to 6.01 g, with general mean of 4 g. Highest panicle weight was observed in genotypes P-87 and P-99, whereas less panicle weight was recorded in case of P-67.

4.2.1.7 1000-grain weight (g)

The general mean of 1000-grain weight was 18.8 g and the mean values ranged from 9.00 in P-34 to 24.5 g in P-26. Among the genotypes P-26 recorded the highest grain weight and P-34 recorded the lowest grain weight.

4.2.1.8 Grain yield per plant (g)

The mean for grain yield per plant was 21.7 g and the mean values ranged from 11.7 C2 (Type 3) to 33.2 g in P-7 . The check variety Type 3 recorded the lowest grain yield/plant while the genotype P-7 exhibited highest grain yield/plant.

4.2.2 NUTRITIONAL CHARACTERS

4.2.2.1 Grain iron concentration (ppm)

The mean for grain iron concentration was 9.77 ppm and the mean values ranged from 5.20 in P-101 to 16.10 ppm in C2 (Type 3). The genotype P-101 recorded the lowest grain iron concentration while the check variety C2 (Type 3) exhibited highest grain iron concentration.

4.2.2.2 Grain zinc concentration (ppm)

The mean for grain zinc concentration was 21.3 ppm and the mean values ranged from 14.0 in check variety BPT-5204 to 28.5 ppm in P-45. The check variety BPT-5204 recorded the lowest grain zinc concentration while the genotype P-45 exhibited highest grain zinc concentration.

4.3 GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE

The genotypic and phenotypic coefficients of variation, heritability and genetic advance as per cent of mean were estimated for 100 RIL population and results are furnished in Table 8.

4.3.1 QUANTITATIVE CHARACTERS

4.3.1.1 Days to 50 per cent flowering

The genotypic and phenotypic coefficients of variation were low i.e., 2.93 and 3.36, respectively. The results were in conformity with Singh *et al.* (2011), Rahman *et al*. (2012), Sravan *et al*. (2012), Gangashetty *et al*. (2013), Singh *et al*. (2013), Vanisree *et al*. (2013), Dhurai *et al*. (2014), Rahman *et al*. (2014), Suresh *et al.* (2014), Mohan *et al*. (2015), Sameera *et al*. (2015), Shekawat *et al*. (2015), Devi *et al*. (2016), Lakshmi *et al*. (2017) for low GCV and PCV.

The heritability observed for this trait was high (76.03%) with low genetic advance as per cent of mean (5.26%) indicating expression of trait is under the control of non – additive gene action, and its response to selection would be poor. In such cases hybridization programme is rewarded. Yadav *et al.* (2010), Dhanwani *et al.* (2013), Karande *et al.* (2015), Shekawat *et al.* (2015) Mohan *et al.* (2016) and Lakshmi *et al.* (2017) reported similar results for high heritability coupled with low genetic advance as percent of mean.

4.3.1.2 Plant height (cm)

The genotypic and phenotypic coefficients of variation estimates observed for this trait were moderate i.e., 12.566 and 12.645, respectively. The results were in conformity with Selvaraj *et al.* (2011), Sravan *et al*. (2012), Vanisree *et al*. (2013), Dhurai *et al.* (2014), Sravan *et al.* (2014), Shrivastava *et al*. (2015) Devi *et al*. (2016), Lakshmi *et al.* *(*2017) for moderate GCV and PCV.

The observed heritability estimates for this character was high (98.74%) with high genetic advance as per cent of mean (25.72%) indicating preponderance of additive gene

action in controlling of the trait. Hence direct selection of the plant height would be rewarding. Chandra *et al*. (2009), Mohanty *et al.* (2012), Singh *et al.* (2012), Sravan *et al*. (2012), Gangashetty *et al*. (2013), Chakraborty and Chaturvedi (2014), Dhurai *et al.* (2014), Lingaiah *et al.* (2014) , Rahman *et al*. (2014), Sravan *et al.* (2014), Bhati *et al*. (2015), Sameera *et al*. (2015), Devi *et al*. (2016), Lakshmi *et al. (*2017) for high heritability coupled with high genetic advance as per cent of mean.

4.3.1.3 Number of productive tillers per plant

The genotypic and phenotypic coefficients of variation for number of productive tillers per plant were low and moderate i.e., 9.168 and 10.385, respectively. The similar findings were reported by Garg *et al*. (2010), Yadav *et al*. (2010), Mohan *et al.* (2015) for low GCV , Mohanty *et al*. (2012), Sravan *et al*. (2012), Dhanwani *et al*. (2013) and Devi *et al*. (2016) for moderate PCV.

The observed heritability estimate was high (77.93%) with moderate genetic advance as per cent of mean (16.67%) indicating simple selection would be effective for this trait improvement. Jaiswal *et al.* (2007), Selvaraj *et al.* (2011), Singh *et al.* (2011), Gangashetty *et al.* (2013) , Chakraborty and Chaturvedi (2014), Dhurai *et al.* (2014), Rahman *et al.* (2014), Karande *et al.* (2015) and Lakshmi *et al.* (2017) observed high heritability coupled with moderate genetic advance as per cent of mean.

4.3.1.4 Panicle length (cm)

The genotypic and phenotypic coefficients of variation for this trait were low i.e., 7.10 and 7.66 respectively. Padmaja *et al*. (2008), Singh *et al.* (2011), Mohanty *et al.*. (2012), Pandey *et al.* (2012), Sravan *et al.* (2012), Vanisree *et al.* (2013), Sravan *et al.* (2014), Suresh *et al.* (2014), Mohan *et al.* (2015), Sameera *et al.* (2015), Devi *et al.* (2016) reported low GCV and PCV.

The heritability observed for this trait was high (85.84%) with moderate genetic advance as per cent of mean (13.56%) indicating simple selection would be effective for this trait improvement. Yadav *et al*. (2010), Parikh *et al*. (2011), Singh *et al.* (2011) ,

Biswash *et al. (*2015), Shekawat *et al*. (2015) and Mohan *et al.* (2016) observed high heritability and moderate genetic advance as percent of mean.

4.3.1.5 Number of filled grains per panicle

The genotypic and phenotypic coefficients of variation for this trait were high i.e., 33.21 and 33.58, respectively. The results were in conformity with Padmaja *et al*. (2008), Chandra *et al*. (2009), Yadav *et al*. (2011), Singh *et al*. (2012), Basavaraja *et al*. (2013), Singh *et al*. (2013), Dhurai *et al.* (2014), Lingaiah *et al.* (2014), Patel *et al .(*2014), Suresh *et al*. (2014), Mohan *et al. (*2015), Sameera *et al*. (2015), Shekawat *et al*. (2015), Devi *et al*. (2016), Thippeswamy *et al*. (2016) and Lakshmi *et al*. (2017) for high GCV and PCV.

The heritability estimate for number of filled grains per panicle was high (97.79%) with high genetic advance as per cent of mean (67.65%) indicating presence of high genetic variability and preponderance of additive gene action. Hence, this trait could be effectively improved by simple selection. Padmaja *et al.* (2008), Chandra *et al.* (2009), Yadav *et al*. (2010), Pandey *et al.* (2012), Singh *et al.* (2012), Sravan *et al*. (2012), Kumar *et al*. (2013), Singh *et al*. (2013), Chakraborty and Chaturvedi (2014), Lingaiah *et al.* (2014), Patel *et al*. (2014), Rahman *et al*. (2014) , Devi *et al*. (2016), Mohan *et al. (*2016), Thippeswamy *et al*. (2016) and Lakshmi *et al. (*2017) for high heritability and genetic advance as per cent of mean.

4.3.1.6 Panicle weight

The genotypic and phenotypic coefficients of variation for this trait were high i.e., 22.10 and 22.18, respectively. The results were in conformity with Subudhi *et al*. (2009), Satish vangaru *et al.* (2017) for high GCV and PCV.

The observed heritability estimate was high (99.23%) with high genetic advance as percent of mean (45.35%) indicating presence of high genetic variability and preponderance of additive gene action. Hence this trait could be effectively improved by simple selection. Lestari *et al.* (2015), Satish vangaru *et al*. (2017) reported high heritability and genetic advance as per cent of mean.

4.3.1.7 1000-grain weight (g)

The genotypic and phenotypic coefficients of variation for this trait were moderate i.e., 14.29 and 15.05, respectively. The results were in conformity with Dhurai *et al.* (2014), Patel *et al*. (2014), Suresh *et al*. (2014), Shekawat *et al.* (2015), Devi *et al*. (2016) for moderate GCV and PCV.

The observed heritability estimate was high (90.14%) with high genetic advance as per cent of mean (27.95) indicating preponderance of additive gene action in controlling of the traits. Hence direct selection of the characters would be effective in improving the seed yield. Chakraborty and Chaturvedi (2014), Dhurai *et al.* (2014), Lingaiah *et al.* (2014), Patel *et al*. (2014), Rahman *et al*. (2014), Suresh *et al*. (2014), Gampala *et al*. (2015), Islam *et al.* (2015), Sameera *et al.* (2015), Devi *et al*. (2016), Mohan *et al. (*2016) , Thippeswamy *et al*. (2016) and Lakshmi *et al. (*2017) reported for high heritability coupled with high genetic advance per cent of mean.

4.3.1.8 Grain yield per plant (g)

The complex character grain yield showed high GCV (20.22) and PCV (20.53) respectively. The results are in conformity with Padmaja *et al.* (2008), Chandra *et al.* (2009), Yadav *et al*. (2010), Singh *et al*. (2011), Mohanty *et al*. (2012), Singh *et al*. (2012), Sravan *et al*. (2012), Dhanwani *et al*. (2013), Gangashetty *et al*. (2013), Singh *et al*. (2013), Vanisree *et al*. (2013), Dhurai *et al.* (2014), Patel *et al*. (2014), Rahman *et al* . (2014), Sravan *et al.* (2014), Suresh *et al*. (2014) , Bhati *et al*. (2015), Gampala *et al*. (2015), Islam *et al.* (2015), Karande *et al. (*2015) , Devi *et al*. (2016), Thippeswamy *et al*. (2016) and Lakshmi *et al.(*2017) for high GCV and PCV.

High heritability estimate (96.98%) coupled with high genetic advance as per cent of mean (41.03) indicating that simple direct selection in the present material may be effective for getting high yield. Chakraborty and Chaturvedi (2014), Dhurai *et al.* (2014), Lingaiah *et al.* (2014), Patel *et al*. (2014), Choudary *et al. (*2016), Devi *et al*. (2016), Suresh *et al.* (2014) , Biswash *et al*. (2015), Karande *et al*. (2015) and Lakshmi *et al. (*2017) reported for high heritability coupled with high genetic advance per cent of mean.

4.3.2 NUTRITIONAL CHARACTERS

4.3.2.1 Grain iron concentration (ppm)

The genotypic and phenotypic coefficients of variation for grain iron concentration were moderate i.e., 14.00 and 16.43, respectively. Kalaimaghal *et al.* (2011), Sala *et al.* (2015) also reported moderate GCV and PCV.

The observed heritability estimate was high (72.56%) with high genetic advance as per cent of mean (24.57) indicating preponderance of additive gene action in controlling of the traits. Hence direct selection of the characters would be effective in getting lines with high iron concentration in grain. Gangashetty *et al.* (2013) also reported high heritability coupled with genetic advance as per cent of mean.

4.3.2.2 Grain zinc concentration (ppm)

The genotypic and phenotypic coefficients of variation for grain zinc concentration were moderate i.e., 12.44 and 13.56, respectively. Kalaimaghal *et al.* (2011), Sala *et al.* (2015) also reported moderate GCV and PCV.

The observed heritability estimate was high (84.18%) with high genetic advance as per cent of mean (23.515) indicating preponderance of additive gene action in controlling the grain zinc concentration. Hence direct selection of the characters would be effective in getting high yield. The results are in conformity with Gangashetty *et al*. (2013) for high heritability coupled with genetic advance as per cent of mean.

The knowledge of genetic variability present in a given crop species for the character under improvement is of paramount importance for the success of any plant breeding programme. Information on coefficient of variation is useful in measuring the range of variability present in the characters. Heritability and genetic advance are important selection parameters. Genotypic coefficient of variation (GCV) along with heritability estimates would provide a better picture of the amount of genetic advance to be expected by phenotypic selection (Burton, 1952). It is suggested that genetic gain should be

considered in conjunction with heritability estimates (Johnson *et al*., 1955). Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. Coefficients of variation studies indicated that the estimates of PCV were slightly higher than the corresponding GCV estimates for all traits indicating less influence of environment on the traits under study.

The estimates of heritability act as predictive instrument in expressing the reliability of phenotypic value. Therefore, high heritability helps in effective selection for a particular character. High heritability for quantitative characters indicates the scope of genetic improvement of these characters through selection. All the characters exhibited high degree of broad-sense heritability, which revealed that these characters are less influenced by environment and there could be greater correspondence between phenotypic and breeding values.

The genetic advance as per cent of mean is a useful indicator of the progress that can be expected as a result of exercising selection on the pertinent population. The traits which recorded high heritability and high genetic advance indicate the control of additive gene action and selection may be effective for these characters.

4.4 CHARACTER ASSOCIATION

Crop yield is the end product of the interaction of a number of other often interrelated attributes. A thorough understanding of the interaction of characters among themselves had been of great use in plant breeding. The efficiency of selection for yield mainly depends on the direction and magnitude of association between yield and its component characters and also among themselves. Character association provides information on the nature and extent of association between pairs of metric traits and helps in selection for the improvement of the character. Correlation studies were worked out on yield and yield contributing characters among 100 RIL population and are discussed here under the results presented in Table 9.

4.4.1 Days to 50 per cent flowering

The character days to 50 per cent flowering recorded a non-significant positive correlation with grain yield per plant (0.0807), test weight (0.1433), panicle weight (0.1232), number of filled grains per panicle (0.0690). It showed negative and significant correlation with plant height (-0.2523**) and non–significant negative for panicle length (-0.0135), number of productive tillers per plant (-0.0674), grain iron concentration (-0.0597), grain zinc concentration (-0.1333).

The Similar findings were recorded by Nandan *et al*. (2010), Sarker *et al*. (2014) for number of filled grains per panicle, Rao *et al.* (2014) for 1000 seed weight, Madhavilatha *et al.* (2005), Chandra *et al.* (2009), Nandan *et al.* (2010), Rao *et al.* (2014) for single plant yield, Rao *et al.* (2014) for panicle length and Ajmera *et al*. (2017) for grain iron and zinc concentration.

4.4.2 Plant height (cm)

The trait plant height had shown a significant positive correlation with single plant yield (0.2150*). It had positive non-significant correlation with plant height (0.1145), number of productive tillers per plant (0.0901), panicle weight (0.0103), test weight (0.0233), negative significant correlation with grain iron concentration (-0.2269*), grain zinc concentration (-0.1885*), days to 50 % flowering (-0.2523**) and negative non-significant correlation with number of filled grains per panicle (-0.0247).

The results are in accordance with Rao *et al.* (2014) for number of productive tillers per plant, Nandan *et al*. (2010) for number of filled grains per panicle, Raju (2002), Sala and Geetha (2015) for panicle length, Dhurai *et al*. (2016) for 1000 seed weight, Rajendra Prasad *et al*. (2017) for panicle weight, Sala and Geetha (2015) for grain iron concentration, Nagesh *et al*. (2012) for grain zinc concentration, Chaudary *et al*. (2013), Reddy *et al*. (2013), Patel *et al.* (2014), Biswash *et al*. (2015), Thippeswamy *et al*. (2016), Kalyan *et al.* (2017) and Priya *et al*. (2017) for single plant yield.

From the above it can be concluded that plant height could be considered as criterion for selection of higher yield as they were inter related among themselves showing

significant positive correlation.

4.4.3 Number of Productive Tillers per Plant

Number of productive tillers per plant exhibited non-significant positive correlation with plant height (0.0901), grain yield per plant (0.0378). It had negative non-significant correlation with days to 50% flowering (-0.0674), panicle length (-0.0059), number of filled grains per panicle (-0.0551), panicle weight (-0.0282), test weight (-0.0406), grain Iron concentration (-0.0578), grain Zinc concentration (-0.0705).

The results were in conformity with Rao *et al*. (2014) for plant height, Dhurai *et al*. (2016) for panicle length, Seyoum *et al*. (2012), Nikhil *et al*. (2014), Rahman *et al.* (2014) for single plant yield, Rao *et al.* (2014) for 1000 seed weight and Nagesh *et al.* (2012) for grain iron concentration.

4.4.4 Panicle Length (cm)

Panicle length registered non-significant positive correlation with plant height (0.1145), number of filled grains per panicle (0.0153), grain yield per plant (0.0306) and non-significant negative correlation with days to 50 % flowering (-0.0135), 1000 grain weight (-0.0399), number of productive tillers per plant (-0.0059), panicle weight (-0.0874), grain zinc concentration (-0.1104) and grain iron concentration (0.0973).

Similar results were reported by Rao *et al.* l. (2014) for days to 50 % flowering, Raju (2002), Sala and Geetha (2015) for plant height, Rahman *et al.* (2014) for number of filled grains per panicle, Ajmera *et al*. (2017) for grain iron concentration, Nagesh *et al.* (2012) for grain zinc concentration, Dhurai *et al*.. (2016) for 1000 seed weight, Dhurai *et al.* (2016) for number of productive tillers per plant, Madhavilatha *et al.* (2005), Krishna *et al.* (2008), Seyoum *et al.* (2012) for single plant yield.

4.4.5 Panicle weight

Panicle weight exhibited significant positive correlation with 1000 grain weight (0.2880**), non-significant positive correlation with days to 50% flowering (0.1232), plant

height (0.1034), single plant yield (0.1099), non-significant negative correlation with plant height (-0.0874), number of productive tillers per plant (-0.0282), filled grains per panicle (-0.0229), grain iron concentration (-0.0909), grain zinc concentration (-0.0183). It can be concluded that panicle weight and 1000 grain weight are dependent on each other, hence improvement made in panicle weight helps in simultaneous improvement of 1000 seed weight.

Prasad *et al.* (2017) also reported similar results for 1000 seed weight and plant height.

4.4.6 Number of filled Grains per Panicle

Number of filled grains per panicle exhibited a non-significant positive correlation with days to 50 % flowering (0.0690), panicle length (0.0153), grain yield per plant (0.1027) and 1000 seed weight (0.0203) whereas significant negative correlation with grain iron concentration (-0.1966*) and non-significant negative correlation with plant height (-0.0247), number of productive tillers per plant (-0.0551), panicle weight (-0.0229) and grain zinc concentration (-0.0803).

Similar findings were reported by Nandan *et al.* (2010), Sarker *et al.* (2014) for days to 50 % flowering, Nandan *et al*. (2010) for plant height, Rahman *et al.* (2014) for panicle length, Biswash *et al*. (2015), Thippeswamy *et al*. (2016), Lakshmi *et al. (*2017) for 1000 seed weight, Rahman *et al.* (2014), Rashid *et al.* (2014) for single plant yield, Nagesh *et al.* (2012) for grain zinc concentration.

4.4.7 1000 Grain Weight (g)

Thousand grain weight showed highly significant positive correlation with panicle weight (0.2888**), grain yield per plant (0.3937**) and non-significant negative correlation with panicle length (-0.0399), number of productive tillers per plant (-0.0406), grain iron concentration (-0.1288) and grain zinc concentration (-0.0414) and positive non-significant correlation with days to 50% flowering (0.1433), plant height (0.0233), number

.of filled grains per panicle (0.0203). This trait acts as an selection criterion for improvement of grain yield per plant.

Chandra *et al.* (2009), Basavaraja *et al.* (2011), Yadav *et al.* (2011)Rangare *et al*. (2012), Chakraborty and Chaturvedi (2014), Naseem *et al*. (2014), Patel *et al.* (2014), Rahman *et al.* (2014), Rao *et al.* (2014), Rashid *et al.* (2014), Anil kumar *et al*. (2015) , Ashok *et al*.(2016), Kalyan *et al.* (2017), Lakshmi *et al.* (2017) , Priya *et al*. (2017) for grain yield per plant and Nagesh *et al.* (2013) for grain zinc and iron concentrations reported similar results.

4.4.8 Grain Zinc concentration

Grain zinc concentration showed a negative significant correlation with grain yield per plant (-0.4243**), and positive significant correlation with grain iron content (0.6691**). This indicates that this trait does not acts as an selection criterion for improvement of grain yield per plant.

The results were in accordance with Nagesh *et al.* (2013) for grain yield per plant and Gangashetty *et al.* (2013) and Nagesh *et al.* (2013) for grain iron concentration.

4.4.9 Grain Iron concentration

Grain Iron concentration showed a negative significant correlation with grain yield per plant (-0.4059**), significant positive correlation with grain zinc concentration (0.6691**). With increase in the grain iron concentration there is decrease in the grain yield per plant, hence for the improvement of grain yield per plant grain iron content does not acts as an selection criteria.

Similar findings were reported by Nagesh *et al.* (2013) for grain yield per plant and Gangashetty *et al.* (2013) and Nagesh *et al.* (2013) for grain iron concentration.

4.4.10 Grain Yield per Plant

Grain yield per plant had significant positive association with plant height (0.21500*), 1000 seed weight (0.3937**). The trait recorded a non- significant positive association with

days to 50 per cent flowering (0.0807), panicle length (0.0306), number of productive tillers per plant (0.0378), panicle weight (0.1099), number of filled grains per panicle (0.1027) and showed significant negative correlation with grain iron concentration (-0.4059**) and grain zinc concentration (-0.4243**).

Grain yield per plant showed positive significant association with plant height and 1000 seed weight. It indicated that these characters are important for yield improvement. Similar kind of association was revealed by Madhavilatha *et al.* (2005), Chandra *et al.* (2009), Nandan *et al.* (2010), Rao *et al.* (2014) for days to 50% flowering, Krishna *et al.* (2008), Nandan *et al*. (2010), Ekka *et al*. (2011), Mohanty *et al*. (2012) Chaudary *et al*. (2013), Reddy *et al*. (2013), Patel *et al.* (2014), Biswash *et al*. (2015) , Thippeswamy *et al*. (2016), Kalyan *et al.* (2017), Priya *et al*. (2017) for plant height, Seyoum *et al*. (2012), Nikhil *et al*. (2014), Rahman *et al.* (2014) for number of productive tillers per plant, Madhavilatha *et al.* (2005), Krishna *et al.* (2008), Seyoum *et al.* (2012) for panicle length, Rahman *et al.* (2014), Rashid *et al.* (2014) for number of filled grains per panicle, Chandra *et al.* (2009), Basavaraja *et al.* (2011), Yadav *et al.* (2011), Rangare *et al*. (2012), Chakraborty and Chaturvedi (2014), Naseem *et al*. (2014), Patel *et al.* (2014), Rahman *et al.* (2014), Rao *et al.* (2014), Rashid *et al.* (2014), Anil kumar *et al*. (2015), Ashok *et al*. (2016), Kalyan *et al.* (2017), Lakshmi *et al.(*2017) and Priya *et al*. (2017) for 1000 seed weight, Sala and Geetha (2015) for grain iron concentration.

From the study it can be concluded that plant height and 1000 seed weight are very crucial for higher yields, as they exhibited significant positive correlation with grain yield per plant.

4.5 PATH COEFFICIENT ANALYSIS

Correlation gives only the relation between two variables whereas path coefficient analysis allows separation of the direct effect and their indirect effects through other attributes by partitioning the correlations (Wright, 1921). Hence, this objective was undertaken in the present investigation.

Based on the data recorded on the genotypes in the present investigation, the

genotypic and phenotypic correlations were estimated to determine direct and indirect effects of yield and yield contributing characters. If the correlation coefficient between a causal factor and the effect is almost equal to its direct effect, it explains the true relationship and a direct selection through this trait may be useful. If the correlation coefficient is positive, but the direct effect is negative or negligible, the indirect effects appear to be the cause of that positive correlation. In such situation the other factors are to be considered simultaneously for selection. However if the correlation coefficient is negative but direct effect is positive and high, a restriction has to be imposed to nullify the undesirable indirect effects in order to make use of direct effect.

As discussed in character association based on the importance of phenotypic effects the present results of phenotypic path coefficient of yield and yield contributing characters are discussed here under which were presented in table 10 and figure 3.

4.5.1 Days to 50 percent flowering

The direct contribution of days to 50 percent flowering to grain yield per plant was positive (0.0147) . These results are in agreement with Chandra *et al*. (2009), Nandan *et al.* (2010), Ekka *et al*. (2011), Padmaja *et al*. (2011), Basavaraja *et al*. (2012), Mohanty *et al.* (2012), Seyoum *et al.* (2012), Nikhil *et al*. (2014), Ratna *et al. (*2015), Tejaswini *et al.* (2016), Kalyan *et al.* (2017), Priya *et al*. (2017).

The positive non-significant correlation of days to 50% flowering (0.0807) with grain yield per plant was mainly due to indirect positive effects of this trait *via* panicle weight (0.0018), number of filled grains per panicle (0.0010), test weight (0.0021)

This trait exhibited negative indirect effects on grain yield per plant through panicle length (-0.0002), plant height (-0.0037), number of productive tillers per plant (-0.0010), grain iron concentration (-0.0009), grain zinc concentration (-0.0020).

4.5.2 Plant height (cm)

The direct contribution of this character to grain yield per plant was positive (0.1298). These results are in agreement with Garg *et al*. (2010), Nandan *et al.* (2010),

Padmaja *et al.* (2011), Yadav *et al*. (2011), Babu *et al.* (2012), Basavaraja *et al*. (2012), Mohanty *et al.* (2012), Seyoum *et al.* (2012), Nagaraju et al. (2013), Rahman *et al*. (2014), Ashok *et al*. (2016), Kalyan *et al.* (2017), Lakshmi *et al.* (2017), Priya *et al*. (2017).

The positive significant correlation of plant height (0.1614) with grain yield per plant was mainly due to indirect positive effects of this trait *via* panicle length (0.0149), number of productive tillers per plant (0.0117), panicle weight (0.0134), test weight (0.0030) and indirect negative effects of this trait on grain yield through days to 50% flowering (-0.0327), number of filled grains per panicle (-0.0032), grain iron concentration (-0.0294), grain zinc concentration (-0.0245).

4.5.3 Number of productive tillers per plant

The direct contribution of this character to grain yield per plant was positive (0.0161) . These results are in agreement with Garg *et al*. (2010), Ekka *et al*. (2011), Padmaja *et al.* (2011), Babu *et al.* (2012), Pandey *et al.* (2012), Gangashetty *et al*. (2013), Nagaraju *et al.* (2013), Nagesh *et al*. (2013), Sarkar *et al.* (2014), Lingaiah *et al*. (2014), Naseem *et al.* (2014), Rahman *et al*. (2014), Rao *et al*. (2014), Anil kumar *et al.* (2015), Ratna *et al. (*2015), Ashok *et al*. (2016), Kalyan *et al.* (2017), Lakshmi *et al.* (2017), Priya *et al*. (2017).

The indirect effects through plant height (-0.0006) was negative and days to 50% flowering (-0.0011), panicle length (-0.0001), panicle weight (-0.0005), number of filled grains per panicle (-0.0009), test weight (-0.0007), grain iron concentration (-0.0009), grain zinc concentration (-0.0011) showed negative indirect effect. This trait had non-significant positive association with grain yield per plant (0.0378).

This trait had positive direct effect on grain yield, it can be considered as selection criteria for improvement of yield.

4.5.4 Panicle length (cm)

The direct contribution of this character to grain yield per plant was negative (-0.0053). These results are in agreement with Garg *et al*. (2010), Padmaja *et al*. (2011),

Patel *et al*. (2014), Thippeswamy *et al*. (2016), Kalyan *et al*. (2017), Lakshmi *et al*. (2017), Priya *et al*. (2017) for grain yield per plant.

Panicle length has indirect positive effects on grain yield per plant *via* days to 50% flowering (0.0001), panicle weight (0.0005), test weight (0.0002), grain zinc concentration (0.0006) and exhibited negative indirect effects on grain yield per plant through plant height(-0.0006), number of filled grains per panicle (-0.0001). However non-significant positive correlation was observed for grain yield per plant (0.0306).

4.5.5 Number of filled grains per panicle

Positive direct effect (0.0502) was exhibited and it was reported by Garg *et al*. (2010) Nandan *et al.* (2010) Padmaja *et al.* (2011), Yadav *et al*. (2011), Mohanty *et al.* (2012), Nagaraju *et al*. (2013), Naseem *et al*. (2014), Rahman *et al*. (2014), Rao *et al*. (2014), Sarker *et al*. (2014), Biswash *et al*. (2015), Ashok *et al*. (2016), Thippeswamy *et al*. (2016), Kalyan *et al*. (2017), Lakshmi *et al*. (2017), Priya et al. (2017).

The indirect positive effects through days to 50% flowering (0.0035), panicle length (0.0008), test weight (0.0010) were expressed by this trait. Plant height (-0.0012), number of productive tillers per plant (-0.0028), panicle weight (-0.0012), grain iron concentration (-0.0099), grain zinc concentration (-0.0040) showed negative indirect effect. This trait had non-significant positive association with grain yield per plant (0.1028).

4.5.6 Panicle weight

Panicle weight exhibited negative direct effect (-0.0268). The indirect effects via days to 50% flowering (-0.0033), plant height (-0.0028), test weight (-0.0077) were negative. Panicle length (0.0023), number of productive tillers per plant (0.0008), number of filled grains per panicle (0.0006), grain iron concentration (0.0024), grain zinc concentration (0.0005) showed positive indirect effect. However a positive non-significant correlation was observed with grain yield per plant (0.1099).

Prasad *et al*. (2017) reported negative direct effect of panicle length on grain yield.

4.5.7 1000-grain weight (g)

This character expressed positive direct effect (0.3679) on grain yield and the indirect effects through days to 50% flowering (0.0527), plant height (0.0086), panicle weight (0.1063), number of filled grains per panicle (0.0075) were positive. Panicle length (-0.0147), number of productive tillers per plant (-0.0150), grain iron concentration (-0.0473), grain zinc concentration (-0.0153) showed negative indirect effect. This trait had a positive significant association with yield per plant (0.3937**).

Suman (2003), Chandra *et al.* (2009), Yadav *et al.* (2011), Akhtar *et al. (*2011), Padmaja *et al*. (2011), Chakraborty and Chaturvedi (2014), Lingaiah *et al*. (2014), Nikhil *et al*. (2014), Rahman *et al*. (2014), Rao *et al*. (2014), Anil kumar *et al.* (2015), Ratna *et al.* (2015), Ashok *et al*. (2016), Thippeswamy *et al*. (2016), Tejaswini *et al.* (2016), Kalyan *et al.* (2017), Lakshmi *et al.* (2017), Priya *et al*. (2017) also reported positive direct effect of 1000-grain weight with grain yield.

Thousand grain weight considered as important attribute in formulating selection criterion for achieving desired target due to its positive direct effect and significant genetic correlation with grain yield.

4.5.8 Grain iron concentration

The direct contribution of this character to grain yield per plant was negative (-0.1206)). These results are in agreement with Nagesh *et al*. (2013). This trait exhibited non-significant negative correlation (-0.4059**) with grain yield per plant.

Grain Iron concentration has indirect positive effects on grain yield per plant *via* days to 50% flowering (0.0072), plant height (0.0274), panicle length (0.0001), number of filled grains per panicle (0.0237), 1000-grain weight (0.0155), no.of productive tillers per plant (0.0070) and exhibited negative indirect effects on grain yield per plant through grain zinc concentration (-0.0807).

4.5.9 Grain zinc concentration

The direct contribution of this character to grain yield per plant was negative (-

0.2978). These results are in agreement with Nagesh *et al*. (2013). This trait exhibited negative non-significant correlation (-0.4243**) with grain yield per plant.

Grain Zinc concentration has indirect positive effects on grain yield per plant *via* days to 50% flowering (0.0397), plant height (0.0562), panicle length (0.0329), number of filled grains per panicle (0.0239), panicle weight (0.0055), number of productive tillers per plant (0.0210), 1000-grain weight (0.0124) and exhibited negative indirect effects on grain yield per plant through grain iron concentration (-0.1993).

The association of different component characters among themselves and with yield is quite important for designing an efficient selection criterion for yield. The total correlation between yield and component characters may be some times misleading, as it might be an over-estimate or under-estimate because of its association with other characters. Hence, indirect selection by correlated response may not be some times fruitful. When many characters are affecting a given character, splitting the total correlation into direct and indirect effects of cause as devised by Wright (1921) would give more meaningful interpretation to the cause of association between the dependent variable like yield and independent variables like yield components. This kind of information will be helpful in formulating the selection criteria, indicating the selection for these characters is likely to bring about an overall improvement in single plant yield directly.

Path coefficient analysis revealed that test weight exerted the highest positive direct effect on grain yield followed by plant height, number of filled grains per panicle, days to 50% flowering and number of productive tillers per plant indicating that the selection for these characters was likely to bring about an overall improvement in grain yield per plant directly. Therefore, it is suggested that preference should be given to these characters in the selection programme to isolate superior lines with genetic potentiality for high yield in rice genotypes. Negative direct effect on grain yield was exhibited by panicle length, panicle weight, grain iron and zinc concentration.

Table No. 5 List of polymorphic SSR markers with their expected base pair

MARKERS	Chromosome no.	Forward primer	Reverse primer	Expected base pair
RM11744	1	CCACCCGTATAGGACCAGTCG	TAGAGTCTCCAGGCAGTCTCACC	149
RM10018	1	ACTAGTACACCTCAACTTCACTCC	CCTTTAGTTTGCTTGTGACC	148
RM11307	1	AAAGCTCTGCAATCTTCTCTCC	GAATACGACATCAGAACAGTGC	148
RM243	1	CAGACTGCAGTTGCACGATACTACG	GAAAGCTGCAACGATGTTGTCC	112
RM14181	2	AGTACCACCACCATTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG	286
RM13347	2	TCCTTCTCCTTGAACAGCGACAGC	AGAGGCGAGAGGCATGGAGTGC	171
RM16097	3	CGCCTCGTAAGGTTGAGATCG	TGCCCTGTTCTTTCCATCTTGC	383
RM15630	3	AACTCGAAGGATCTCGCCCAACC	ACCCACCTCCTCACGCTGTACG	286
RM517	3	CAGCTCCTTCCTATCCGTCTCC	TCAGATCTAGCCGAGAAATCAAGG	190
RM13482	4	CCCTGCTGATGCAACTACGG	GCGGATTAGGAGCGTTTGTAGG	172
RM241	4	GTTCATTTCGTGATCTCTGAGC	GCAGATTTACAGGTTTGCTAGG	264
RM413	4	CCAATCTTGTCTTCCGGATCTTGC	AGATAGCCATGGGCGATTCTTGG	84
RM16427	4	CTCCTCATGTCGCTGATTCTTGG	CCGAGATCTACCTCTTGCTGTCC	292
RM142	5	TCTTCCTCTCCACTTCCATTTCC	AAGAAGCTCGGGATCTTCACC	188
RM3486	5	GGAGGTCGGCACGTAGTAGAGG	GTCGGTACTATTCCTGCCATCG	356
RM413	5	CCAATCTTGTCTTCCGGATCTTGC	AGATAGCCATGGGCGATTCTTGG	84
RM19341	6	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC	144
RM19620	6	GCGACGAGGAAGAAGATTAGTTCG	GCGGCACTTCGAGCAGTACG	168
RM3431	6	AAGGGAACATTCTGGAAGACACG	ACACATTGCGTGTAGTGTGAAGC	195
RM402	6	CATCTCTGCTAGGTGGTGAATGG	CTCAGCTGGCCTATGACAATGG	92
RM20844	7	GAGAGGGAAGGAGTTTCTTAGC	TAGTTTACACGTACCCATGTGC	404
RM22885	8	ACTGGGTGTGATCCTTTCTGATGC	GTGATCCCAGATACACGATGTAGGG	128
RM22524	8	GACTTGTGGTTGTTGCTTGTTGG	ACTGCCATATGCATTTCCCTAGC	176
RM3912	9	CACTCAGATTTGGCCGATCC	GCTGATCCAGATCTACCTGACACC	263
RM24035	9	GCTCCAGTTTCTAGTGGGCTTGC	ATGCGGCAGTCAATCAACAGG	231
RM24085	9	CGACGAACTCCTCTACCGTTTACC	CTGCGTGTATCCAATCCCAAGG	130

RM27962	11	GGGAGTCGTGGATTCTGAGACG	ATCCCACGCCAGGAGATAATAAGG	135
RM28607	12	AGCATTACAGTGTCCAGGTAGGG	CCTCCCTTCTTATATGCCTTTCC	200
RM5313	12	TCTTCTACTCCTCGTCTTCGTTCG	CATGCAGAGCAGAGACTTCTTGG	189

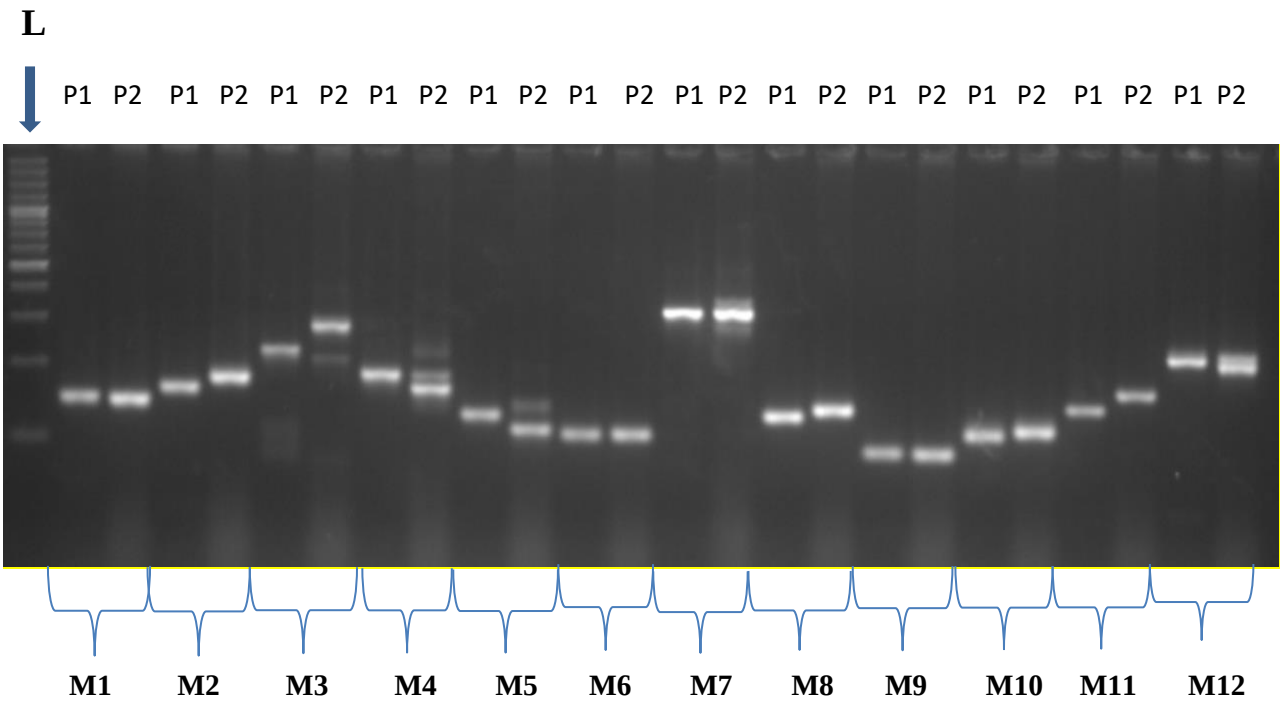

Plate No.1 Representative gel picture of parental polymorphism survey with SSR markers

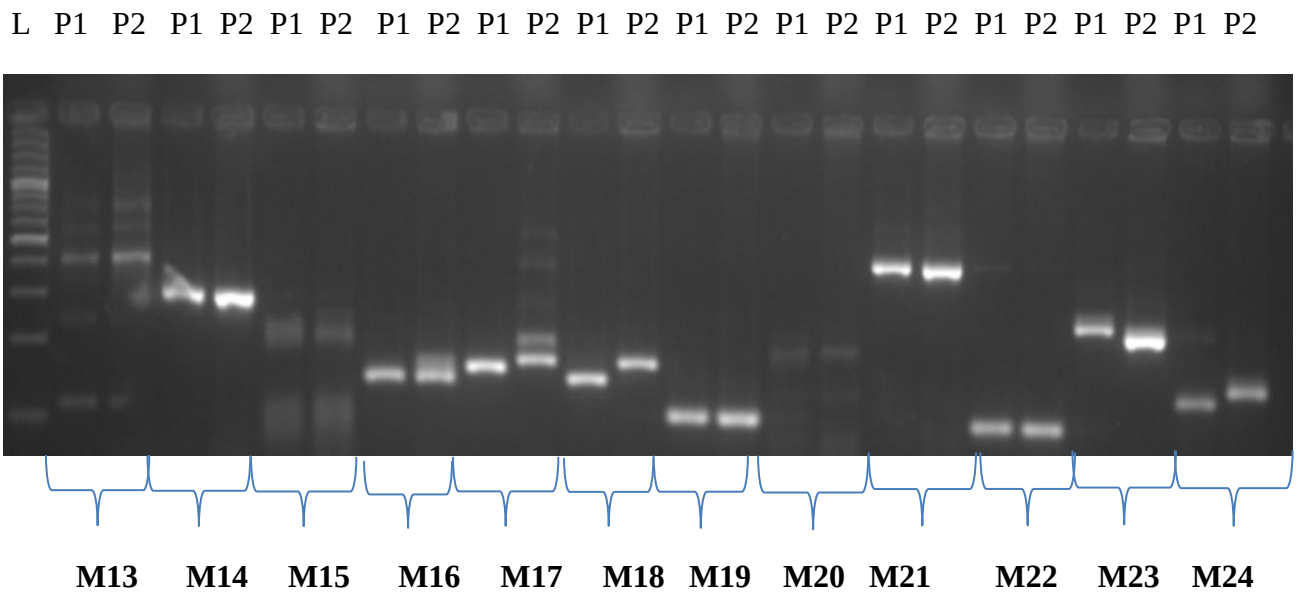

Plate No.2 Reprenetative gel picture of parental polymorphism survey with SSR markers

L- Ladder (100bp), P1 – Parent 1 (Swarna), P2 – Parent 2 (Type 3)

Table No. 6 Representative SSR markers with their amplification status

S.No	Markers	Amplification status
M1	RM11744	pp
M2	RM11743	pp
M3	RM11741	pp
M4	RM12276	mp
M5	RM10361	pp
M6	RM5	mp
M7	RM7075	mp
M8	RM11307	pp
M9	RM 246	mp
M10	RM 243	pp
M11	RM13347	pp
M12	RM13021	mp
M13	RM14140	mp
M14	RM114181	pp
M15	RM13075	mp
M16	RM 262	mp
M17	RM 3515	pp
M18	RM13347	mp
M19	RM85	mp
M20	RM15580	mp
M21	RM16097	pp
M22	RM5748	mp
M23	RM15630	pp
M24	RM257	pp

pp- polymorphic primer, mp- monomorphic primer

Table 8. Analysis of variance for yield and yield attributing traits.

Source of variation	d.f	MSS					
		DFF	PH	PL	NT	PW	FGP
Blocks (Eliminating check+Gen.)	4	6.500	4.315	0.927	0.722	0.009	2.640
Entries (Ignoring blocks)	103	26.242**	565.741**	3.947**	1.276**	0.967**	1694.255**
Checks	3	354.533**	3697.502**	5.938**	0.951*	2.063**	1220.556**
Genotypes	99	16.553**	359.398**	3.777**	1.282**	0.937**	1209.952**
Checks vs. Genotypes	1	0.540	11598.410**	14.852**	1.643*	0.623**	51061.300**
Error	12	3.533	3.904	0.470	0.251	0.006	23.056

Source of variation	d.f	SPY	TW	Fe	Zn
Blocks (Eliminating check+Gen.)	4	0.378	0.576	0.352	0.781
Entries (Ignoring blocks)	103	36.560**	10.468**	5.970**	14.499**
Checks	3	66.284**	49.612**	10.978**	183.64**
Genotypes	99	26.543**	9.301**	2.457**	9.520**
Checks vs. Genotypes	1	939.001**	8.532**	338.701**	0.054**
Error	12	0.692	0.800	0.603	0.546

* Significance at p = 0.01 **Significance at p = 0.05

DFF = Days to 50% flowering PH = Plant height (cm) PL = Panicle length
NT = Number of tillers plant^{-1} PW = Panicle weight (g) FGP = Filled grains per panicle
SPY = Single plant yield (g) TW = Test weight (g) Fe = Iron
Zn = Zinc

Table 9. Estimates of range, mean and genetic parameters for yield and yield attributing traits in rice

Characters	Mean	Range		Coefficient of variability		Heritability (%) broad sense	Gen.Adv as per cent of Mean (at 5%)
		Min.	Max.	PCV (%)	GCV (%)		
Days to 50% Flowering	114.1	101.0	125.0	3.36	2.93	76.03	5.26
Plant Height (cm)	134.6	77.0	167.0	12.64	12.57	98.74	25.72
Panicle Length(cm)	23.9	16.8	28.2	7.67	7.11	85.84	13.56
No. of productive tillers/ plant	10.3	8.2	13.4	10.39	9.17	77.93	16.67
Panicle weight (g)	4.0	2.2	6.0	22.19	22.10	99.23	45.35
Number of filled grains/ panicle	105.4	32.3	171.0	33.58	33.21	97.79	67.65
1000 grain weight(g)	18.8	9.0	24.5	15.51	14.30	90.14	27.95
Grain Iron conc (ppm)	9.7	5.2	16.1	16.44	14.00	72.56	24.57

Grain Zinc con (ppm)	21.3	14	28.5	13.47	13.02	93.47	25.93
Grain yield/ plant (g)	21.7	11.7	33.2	20.54	20.226	96.98	41.03

Table 10. Estimates of correlation coefficient for grain yield, yield components and nutritional traits in rice

	DFF	PH	PL	NT	PW	FGP	TW	Fe	Zn	SPY
DFF	1.0000	-0.25237**	-0.01350	-0.06742	0.12325	0.06909	0.14333	-0.05973	-0.13330	0.08072
PH		1.0000	0.11451	0.09012	0.10340	-0.02478	0.02336	-0.22693*	-0.18858*	0.21500*
PL			1.0000	-0.00590	-0.08740	0.01530	-0.03999	-0.00078	-0.11041	0.03064
NT				1.0000	-0.02825	-0.05510	-0.04068	-0.05784	-0.07053	0.03782
PW					1.0000	-0.02298	0.28880**	-0.09093	-0.01831	0.10992
FGP						1.0000	0.02035	-0.19668*	-0.08036	0.10278
TW							1.0000	-0.12862	-0.04148	0.39373**
Fe								1.0000	0.66919**	-0.40593**
Zn									1.0000	-0.42433**
SPY										1.0000

** Significance at p = 0.01 *Significance at p = 0.05

DFF = Days to 50% flowering
PH = Plant height (cm)
PL = Panicle length
NT = Number of tillers plant^{-1}
PW = Panicle weight (g)
FGP = Filled grains per panicle
SPY = Single plant yield (g)
TW = Test weight (g)
Fe = Iron
Zn = Zinc

Table 11. Phenotypic path analysis for yield, yield attributing and nutritional traits in rice

	DFF	PH	PL	NT	PW	FGP	TW	Fe	Zn
DFF	**0.0147**	-0.0037	-0.0002	-0.0010	0.0018	0.0010	0.0021	-0.0009	-0.0020
PH	-0.0327	**0.1298**	0.0149	0.0117	0.0134	-0.0032	0.0030	-0.0294	-0.0245
PL	0.0001	-0.0006	**-0.0053**	0.0000	0.0005	-0.0001	0.0002	0.0000	0.0006
NT	-0.0011	0.0014	-0.0001	**0.0161**	-0.0005	-0.0009	-0.0007	-0.0009	-0.0011
PW	-0.0033	-0.0028	0.0023	0.0008	**-0.0268**	0.0006	-0.0077	0.0024	0.0005
FGP	0.0035	-0.0012	0.0008	-0.0028	-0.0012	**0.0502**	0.0010	-0.0099	-0.0040
TW	0.0527	0.0086	-0.0147	-0.0150	0.1063	0.0075	**0.3679**	-0.0473	-0.0153
Fe	0.0072	0.0274	0.0001	0.0070	0.0110	0.0237	0.0155	**-0.1206**	-0.0807
Zn	0.0397	0.0562	0.0329	0.0210	0.0055	0.0239	0.0124	-0.1993	**-0.2978**
SPY	0.0807	0.2150	0.0306	0.0378	0.1099	0.1028	0.3937	-0.4059	-0.4243

Residual effect = 0.8050

DFF = Days to 50% flowering
PH = Plant height (cm)
PL = Panicle length
NT = Number of tillers plant^{-1}
PW = Panicle weight (g)
FGP = Filled grains per panicle
SPY = Single plant yield (g)
TW = Test weight (g)
Fe = Iron
Zn = Zinc

Table 7. Mean performance of yield and its components of RIL population along with checks

Genotypes	DFF	PH	PL	NT	PW	FGP	SPY	TW	Fe	Zn
C1	125	101.8	24.0	10.2	4.30	159.6	21.00	21.33	13.0	18.00
C2	115	100.6	26.8	10.4	2.93	128.0	13.00	16.67	15.0	27.00
C3	120	96.00	24.2	10.4	4.27	159.3	16.33	20.17	13.4	14.00
C4	103	156.6	26.4	11.0	3.93	157.0	13.00	17.33	17.0	28.00
P 1	118	145.8	25.2	11.0	4.79	89.33	27.17	21.83	7.50	22.00
P 2	119	140.6	21.8	10.4	4.15	87.33	27.67	21.83	9.20	23.50
P 3	114	136.0	25.8	10.8	3.32	113.0	20.57	19.17	8.80	27.50
P 4	115	102.8	23.6	9.40	3.17	91.67	25.33	18.33	7.50	17.70
P 5	113	148.0	25.8	10.6	5.02	89.67	31.83	22.83	10.4	22.00
P 7	116	138.0	25.2	10.2	5.52	70.67	33.20	20.83	7.50	20.20
P 8	116	142.4	25.6	9.00	5.17	63.00	28.67	21.00	7.40	16.30
P 9	120	127.2	24.6	10.0	5.05	49.00	21.67	19.50	8.50	21.50
P 11	116	142.4	24.0	10.4	3.60	79.67	22.60	20.50	8.70	21.70
P 12	117	153.4	26.6	10.2	3.10	51.67	21.83	19.83	10.8	21.30
P 16	117	148.4	25.8	10.0	3.00	47.33	31.67	20.83	8.90	18.30
P 18	112	150.8	25.4	9.20	5.07	90.33	30.67	20.17	9.70	23.80
P 19	115	153.4	24.0	11.6	3.23	73.67	25.00	19.83	9.40	24.10
P 21	118	106.6	24.6	9.00	3.58	41.33	16.33	20.17	8.80	16.50
P 22	105	103.2	21.8	9.00	3.50	60.00	31.50	20.50	9.50	21.10
P 23	117	159.4	23.6	10.0	5.10	154.6	24.33	20.83	8.40	20.80
P 24	115	152.4	25.8	10.0	4.73	143.0	22.00	21.00	9.40	22.90
P 25	116	144.8	24.2	10.0	4.42	82.33	29.50	11.67	10.1	24.60
P 26	114	154.4	25.0	9.60	3.74	115.0	29.67	24.50	9.30	22.40
P 27	115	148.2	23.6	9.20	4.53	80.33	19.17	21.00	8.40	20.40

C1	120	101.4	23.2	10.0	4.23	161.3	20.33	20.33	12.5	19.00
C2	110	99.20	26.6	9.20	2.93	131.6	12.67	13.33	16.1	26.00
C3	115	96.80	23.6	10.4	4.17	161.0	16.67	22.00	13.0	15.00
C4	105	154.2	24.2	11.0	3.90	152.3	12.67	16.67	12.1	27.00
P 28	116	147.4	22.8	9.80	3.11	63.00	14.67	17.83	9.10	21.50
P 29	118	124.0	22.4	9.00	3.50	52.33	23.17	20.33	7.80	16.40
P 30	116	119.4	21.4	8.60	5.74	151.3	15.17	15.83	8.00	18.50
P 31	116	102.6	22.0	8.60	4.16	103.3	26.00	21.17	8.30	17.90
P 32	117	113.4	20.2	8.20	2.57	81.00	17.33	12.50	7.20	19.20
P 33	118	143.4	25.6	8.20	3.77	101.6	15.83	19.83	9.50	25.90
P 34	117	127.0	23.6	8.20	3.54	122.0	22.67	9.000	8.70	19.70
P 35	116	135.8	25.6	9.40	4.25	52.33	20.00	21.17	6.80	17.60
P 36	115	109.2	19.8	9.00	4.22	101.6	31.43	19.00	7.20	18.60
p 37	117	150.8	22.2	9.80	4.01	51.00	19.67	21.50	11.2	23.60
P 38	113	161.8	26.0	9.20	3.65	52.33	23.83	21.50	7.30	22.60
P 39	116	152.6	22.8	9.60	4.32	70.67	21.67	19.50	9.20	25.80
P 40	107	164.2	25.0	8.80	2.40	43.33	22.17	20.00	10.3	21.50
P 41	116	143.4	24.2	10.6	3.55	54.00	21.17	20.83	7.90	18.30
P 42	114	88.80	27.6	12.0	3.44	92.33	20.17	19.17	8.20	22.60
P 43	116	153.0	23.6	9.60	4.83	147.3	27.77	22.50	8.40	22.30
P 44	113	150.6	24.6	10.8	3.10	45.00	20.47	12.50	9.80	24.00
P 45	116	151.4	25.0	10.4	3.68	92.00	28.33	20.83	9.40	28.50
P 46	116	164.6	22.8	13.2	3.55	141.0	16.20	12.50	9.00	21.20
P 48	117	138.2	23.2	11.0	5.75	148.3	25.23	23.83	7.10	15.40
C1	125	103.0	23.4	9.80	4.27	155.0	20.67	20.67	12.5	17.00
C2	110	98.60	24.6	9.60	3.03	128.0	11.67	13.67	15.0	25.00
C3	115	96.20	24.0	11.4	4.27	156.0	16.67	20.33	12.8	15.00

C4	103	147.6	26.2	11.2	3.90	165.6	14.00	17.67	12.3	26.00
P 49	115	116.2	18.8	8.40	4.62	79.00	23.67	20.67	7.70	19.60
P 50	117	151.6	24.4	10.4	4.03	93.33	25.77	21.83	8.40	15.40
P 51	117	106.6	25.2	8.80	5.10	142.3	22.17	20.00	7.60	17.50
P 52	115	135.6	24.6	10.8	4.57	120.0	23.33	19.67	7.80	18.30
P 53	116	110.4	23.4	10.0	3.53	88.00	17.17	20.50	7.00	16.50
P 54	114	142.0	21.6	12.4	5.77	122.3	30.67	21.00	9.40	20.40
P 55	117	147.6	24.2	10.6	5.54	147.6	22.30	19.67	7.40	19.80
p 56	115	144.6	22.4	10.2	3.42	49.00	22.17	10.83	8.50	18.60
P 57	117	166.4	23.0	8.80	6.02	104.6	14.00	16.17	9.30	27.10
P 58	109	147.2	21.2	10.2	4.18	75.00	31.87	20.17	10.1	19.60
P 59	120	154.8	22.4	12.8	3.10	92.67	13.17	16.17	9.90	21.60
P 60	114	155.2	23.0	10.6	3.52	32.33	22.00	20.50	10.8	20.60
P 61	102	149.0	22.6	9.40	3.39	117.6	15.17	21.08	10.0	22.50
P 62	116	149.8	25.4	9.60	2.98	82.33	32.67	18.83	8.20	20.50
P 64	115	119.6	23.0	10.2	2.96	127.6	25.33	15.17	7.10	20.20
P 65	110	77.00	19.4	9.20	2.42	96.67	22.00	19.83	9.50	20.90
P 67	116	111.2	19.6	9.40	2.23	80.00	15.17	12.50	7.40	19.90
P 68	115	114.2	25.8	11.2	3.61	92.33	16.40	20.83	7.60	21.90
P 69	115	151.6	23.4	11.2	3.04	32.33	23.50	20.33	8.30	19.40
P 70	117	109.6	16.8	12.0	3.54	115.0	32.83	21.75	8.70	22.10
C1	125	100.8	23.8	11.4	4.47	159.0	21.00	21.00	15.0	18.50
C2	110	101.0	26.2	11.0	2.80	125.0	12.67	14.33	16.1	26.00
C3	120	95.20	24.2	12.0	4.37	157.0	16.00	20.00	13.0	14.00
C4	104	152.4	25.8	10.8	4.07	171.0	12.67	17.00	11.5	28.00
P 71	112	108.2	24.2	11.2	4.28	150.0	19.67	18.50	10.4	21.30
P 72	114	153.2	25.6	10.8	4.63	42.33	24.17	19.50	10.5	23.50

P 73	113	152.0	25.0	12.0	2.55	71.00	25.17	20.33	9.80	25.90
P 74	110	164.4	27.4	11.4	3.13	92.33	30.47	18.50	9.00	22.50
P 75	105	149.2	24.8	11.6	5.52	102.0	23.17	20.50	8.30	20.10
P 76	101	143.4	24.4	10.8	4.11	137.3	27.17	21.00	8.50	21.00
P 77	114	167.0	25.8	10.6	4.17	93.33	25.80	20.00	6.90	17.30
P 78	101	142.4	23.4	11.4	4.50	158.3	21.33	19.00	9.90	23.60
P 79	114	145.2	21.6	11.6	4.10	157.6	26.17	20.17	9.80	23.10
P 80	116	153.6	23.2	10.8	3.53	128.6	15.33	16.50	14.9	22.00
P 82	103	153.8	22.6	11.0	5.40	162.3	19.90	13.83	8.50	17.90
P 83	115	113.2	20.6	10.8	3.37	82.33	31.50	16.67	6.40	16.00
P 84	115	135.6	21.6	10.8	5.42	51.67	18.33	21.17	9.40	23.80
P 85	116	151.2	22.6	11.6	5.39	91.33	19.60	22.33	10.2	22.90
P 86	114	158.0	23.0	11.4	3.47	142.3	21.17	16.50	5.90	15.80
P 87	112	102.6	21.8	10.2	6.00	147.6	20.67	20.17	11.0	23.40
P 88	115	158.0	26.4	12.0	3.72	82.33	25.90	16.42	8.20	21.30
P 91	116	148.2	24.4	10.6	3.54	132.3	20.33	16.17	10.9	25.10
P 92	115	149.8	24.4	11.4	5.03	119.0	20.57	13.33	11.8	26.30
P 93	112	165.8	24.8	10.4	3.55	102.3	22.10	20.50	13.5	22.80
C1	123	102.2	23.2	10.2	4.23	159.6	20.33	20.67	15.0	17.60
C2	110	99.80	25.0	10.4	2.93	128.3	12.33	13.33	15.4	26.00
C3	117	96.80	24.2	10.4	4.23	159.6	16.67	21.00	12.8	15.00
C4	105	156.6	24.2	11.0	3.90	157.3	16.00	16.67	11.8	26.00
P 94	117	156.4	24.4	10.0	3.22	102.6	15.33	13.00	8.40	22.30
P 95	110	154.4	23.4	9.80	4.10	90.33	16.17	14.17	14.1	20.60
P 96	114	131.4	20.0	11.6	3.54	119.0	19.50	12.50	9.40	26.80
P 97	111	145.0	24.4	9.80	2.45	82.67	31.17	22.33	10.2	26.30
P 98	117	136.6	25.0	9.40	5.20	114.3	21.10	19.83	8.10	22.00

P 99	114	152.8	28.2	11.0	6.00	168.3	21.83	19.83	11.2	26.30
P 100	114	150.0	23.6	13.4	3.86	76.00	21.00	20.83	9.20	20.40
P 101	117	104.0	22.6	8.60	3.11	102.3	19.50	17.42	5.20	14.60
P 102	117	136.2	23.2	10.4	5.30	82.33	20.33	18.50	8.50	19.00
P 103	114	152.8	24.2	9.20	2.43	84.67	25.17	19.17	10.7	25.50
P 104	116	131.4	25.2	8.80	3.53	152.3	23.17	22.17	8.40	18.80
P 105	117	132.6	23.6	10.0	4.53	162.3	28.33	16.50	10.4	22.20
P 106	103	125.8	24.8	11.8	2.99	32.33	19.17	16.17	7.30	17.10
P 107	114	145.6	24.2	10.0	3.33	119.6	22.00	18.17	8.50	22.90
P 108	114	132.6	23.8	9.20	5.92	117.0	17.83	17.83	8.30	20.20
P 109	117	135.2	23.2	11.4	5.50	112.3	28.17	19.75	10.3	25.40
P 112	117	138.8	26.8	11.6	5.10	92.33	26.33	21.00	9.70	22.40
P 119	101	157.0	25.2	10.0	4.05	60.33	29.00	20.17	10.3	21.40
P 134	117	139.6	25.2	10.8	3.25	91.67	29.87	15.83	10.2	25.80
P 136	114	149.0	23.4	8.60	3.66	85.67	34.17	21.17	10.7	27.10
Avg	114	133.7	23.84	10.3	4.01	106.0	21.7956	18.9023	9.88	21.158
Max	125	167.0	27.6	13.2	6.02	171.0	33.2	24.5	16.1	28.5
Min	101	77.00	16.8	8.20	2.23	32.33	11.67	9	5.9	14

Chapter V

SUMMARY AND CONCLUSIONS

Rice (*Oryza sativa* L.*)* is the most important food crop of the world and is popularly called as 'Global grain'. It feeds more than half the human population worldwide, most of whom live in developing countries and many have no other or virtually no other diet.. In the last two decades, new research findings generated by the nutritionists have brought to light the importance of micronutrients, vitamins and proteins in maintaining good health, adequate growth and even acceptable levels of cognitive ability apart from the problem of protein energy malnutrition.

The present investigation Molecular characterization and genetic studies for yield, yield components, zinc and iron in Swarna x Type 3 RIL population of rice *(Oryza sativa* L.*)* was carried out to study parental polymorphism, genetic variability, character association and direct and indirect effects of yield components and quality traits on grain yield with 100 RIL population along with 4 checks at Indian Institute of Rice Research Farm, Ramachandrapuram, Hyderabad, during *kharif*, 2017.

The parental polymorphism survey was carried out between donor Type 3 and recipient Swarna. A total 171 SSR primer pairs distributed across the 12 chromosomes of rice genome were used for molecular characterization of two parents Swarna and Type 3. Of these, 115 were monomorphic and 56 SSR primer pairs were found to be polymorphic across the 12 chromosomes.

Among all the chromosomes more number of polymorphic primers i.e., 9 were observed on chromosome 6. It indicates there may exist more diversity between donor and recipient parent on chromosome 6.

The observed parental polymorphic SSR markers in the present study between donor-recipient parent combinations will be highly useful for identification of variations within cultivars and also for diversity, genome mapping etc…

Analysis of variance revealed significant differences for all the traits studied indicating the presence of sufficient amount of genetic variability among the RIL population. A perusal of genetic variability parameters revealed that high variability was observed for number of filled grains per panicle, panicle weight and grain yield per plant, low variability was noticed for days to 50% flowering, panicle length and number of

productive tillers per plant whereas moderate variability was noticed for plant height, 1000 – grain weight, grain iron concentration, grain zinc concentration.

High heritability coupled with high genetic advance as percent of mean was observed for plant height, panicle weight, number of filled grains per panicle, 1000-grain weight, grain yield per plant, grain iron and zinc concentration indicating the influence of additive gene action, as such simple selection would likely be effective for improvement of these traits. Although heritability estimates are high, panicle length and no.of productive tillers per plant showed low variability and moderate genetic advance as percent of mean, whereas days to 50% flowering showing low variability and low genetic advance as percent of mean indicating the influence of non-additive gene action and hence hybridization or mutation followed by selection in later generations would improve this traits.

The character association studies revealed that grain yield per plant had significant positive association with plant height, test weight indicating that this characters are very important for yield improvement. Positive non- significant association of grain yield per plant was observed with days to 50 per cent flowering, panicle length, number of filled grains per panicle and significant negative correlation was observed for grain iron and zinc concentration.

Path coefficient analysis revealed that test weight exerted the highest positive direct effect on grain yield followed by plant height, no.of filled grains per panicle, days to 50% flowering and number of productive tillers per plant indicating that the selection for this characters was likely to bring about an overall improvement in grain yield per plant directly.

Critical analysis of results obtained from character association and path analysis indicated that days to 50% flowering, number of productive tillers per plant, no. of filled grains per panicle exerted positive direct effect on grain yield per plant but it had Positive non- significant association with yield which might be due to positive indirect effects manifested through other component traits. But plant height and test weight displayed significant positive correlation as well as positive direct effect on grain yield per plant. The positive direct effect of these traits on yield resulted in strong genetic correlation. Hence, due emphasis should be given to these traits in formulating selection criteria to bring yield as well as grain quality improvement.

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The pattern of “Literature Cited” presented above is in accordance with the with the guidelines for the thesis presentation for Professor Jayashankar Telangana State Agricultural University, Hyderabad.

APPENDIX A

I. DNA ISOLATION

Materials / Equipments used

1. Spot test plate (30 well) – available from Thomas scientific, USA.
2. Alcohol sterilized glass rod
3. Micropipettes (1 ml, 20 µl and 200 µl range)
4. 1.5 ml centrifuge tubes (autoclaved and dried)
5. 1 ml and 200 µl tips (autoclaved and dried)

Chemicals used

1. Extraction buffer (CTAB Buffer)

Preparation of stock solutions for CTAB Buffer:

i) 1M Tris HCl (pH-8):

60.57 g of 1M Trizma base (Tris hydroxyl methyl amino methane) of MW 121.14 was dissolved in about 400 ml distilled water and initial pH was basic (10.5 to 11.5) which is adjusted to 8 using 1N HCl and 1N NaOH and finally the volume was made to 500 ml. This stock solution was autoclaved and stored at room temperature.

ii) 0.5M EDTA:

93.06 g of Ethylene Diamine Tetra Acetic acid (EDTA) of MW 372.24 was dissolved in about 400 ml of distilled water and then the initial pH of the solution is adjusted to 8.0 with NaOH and the volume was made to 500 ml. This stock solution was autoclaved and stored at room temperature.

iii) 5M NaCl:

146.10 g of NaCl (MW 58.44) was dissolved in 500 ml of distilled water. The solution was autoclaved and stored at room temperature.

Master Mix

Components	Stock concentration	Quantity require for one reaction (µl)	Quantity required for 18reactions (µl)
Sterile distilled water	-	2	39.6
PCR buffer	10 x	1	18
dNTP's	2.5 mM	0	9
Primer F	2.5pmoles	1	18
Primer R	2.5pmoles	1	18
Taq DNA Polymerase	3 U / µl	0	5.4
Template DNA		4	

III. AGAROSE GEL ELECTROPHORESIS OF GENOMIC SSR AMPLIFIED PCR PRODUCTS

Materials / Equipments used

1) Gel casting unit, gel trays

2) Power supply unit

3) Microwave oven

4) Reagent bottle

5) Electronic balance

6) Gel documentation system including UV transilluminator

Chemicals used

1) Agarose

2) 10 X TBE buffer, 1000 ml: - 108 g Tris (0.9 M) base was added to 500 ml double distilled water. Then 55 g of boric acid was added and mixed well by stirring. 40 ml of 0.5 M EDTA was added and the volume was made to 1000 ml by using double distilled

water. 100 ml of 10 X TBE buffer was mixed with single distilled water and volume made to 2000 ml to obtain 0.5 X TBE buffer which was used further.

3) Ethidium bromide (10 mg/ml)

4)Bromophenol blue dye