GENETIC ANALYSIS AND IDENTIFICATION OF GENOTYPES WITH NOVEL SOURCES OF RESISTANCE FOR GALL MIDGE (Biotype 3) IN RICE *(Oryza sativa* L.*)*

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B.Sc. (Hons.) Ag.

MASTER OF SCIENCE IN AGRICULTURE
(GENETICS AND PLANT BREEDING)

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

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2023

DECLARATION

I, **ANIL KUMAR,** hereby declare that the thesis entitled **"GENETIC ANALYSIS AND IDENTIFICATION OF GENOTYPES WITH NOVEL SOURCES OF RESISTANCE FOR GALL MIDGE (Biotype 3) IN 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.

Place: Hyderabad (**ANIL KUMAR)**

Date: **I. D. No: - RAM/2020-68**

CERTIFICATE

Mr. ANIL KUMAR has satisfactorily prosecuted the course of research and that the thesis entitled **"GENETIC ANALYSIS AND IDENTIFICATION OF GENOTYPES WITH NOVEL SOURCES OF RESISTANCE FOR GALL MIDGE (Biotype 3) IN RICE *(Oryza sativa* L.*)*"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any university.

Place: Hyderabad **(Dr. J. Suresh)**

Date:

CERTIFICATE

This is to certify that the thesis entitled **"GENETIC ANALYSIS AND IDENTIFICATION OF GENOTYPES WITH NOVEL SOURCES OF RESISTANCE FOR GALL MIDGE (Biotype 3) IN RICE *(Oryza sativa* L.*)*"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the **Professor Jayashankar Telangana State Agricultural University, Hyderabad** is a record of the bonafide original research work carried out by **Mr. ANIL KUMAR** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

(Dr. J. Suresh)
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(ANIL KUMAR)

LIST OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
Ml	:	Microliter
<	:	less than
>	:	more than
0 c	:	Degree Celsius
ANOVA	:	Analysis of variance
bp	:	base pair
C	:	Chloroform
cm	:	centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
CV	:	Coefficient of variation
DFF	:	Days to fifty per cent flowering
DNA	:	Deoxyribo Nucleic Acid
dNTP	:	Dinucleotide tri-phosphate
EDTA	:	Ethylene diamine tetra acetic acid
et al.	:	and others
etc	:	and more
GA	:	Genetic advance
GCV	:	Genotypic coefficients of variation
GMB	:	Gall Midge Biotype
GPP	:	Number of grains per panicle
h^2 (bs)	:	Heritability in broad sense
ha	:	Hectare
HCL	:	Hydrochloric acid
i.e.	:	that is
IAA	:	Iso amyl alcohol
INDELs	:	Small Insertions and Deletions
kg	:	kilogram
m	:	meter
M	:	molar
mA	:	milli ampere
m ha	:	million hectare

min	:	minutes
ml	:	millilitre
mM	:	milli molar
NaCl	:	Sodium chloride
PCR	:	Polymerase chain reaction
PCV	:	Phenotypic coefficient of variation
PH	:	Plant height
PL	:	Panicle length
PT	:	Number of productive tillers
PVP	:	Poly vinyl pyrrolidone
QTL	:	Quantitative trait loci
r	:	Correlation coefficient
Rpm	:	Revolutions per minute
RT	:	Room Temperature
SPY	:	Grain yield per plant
SSR	:	Simple Sequence Repeat
SNP	:	Single nucleotide polymorphism
TBE	:	Tris Borate EDTA
TE	:	Tris EDTA
TGW	:	Thousand grain weight
viz.	:	Namely
w/v	:	Weight by volume

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ABSTRACT

In the present study, 42 rice entries comprised of land races, released varieties and advanced breeding lines including susceptible check (TN 1) and resistant check (Aganni) were evaluated in a replicated trial against gall midge biotype, GMB3 at Regional Agricultural Research Station (RARS), Jagtial under field conditions during *kharif* 2021. Based on field screening, selected entries were genotypically characterized by using *Gm3, Gm4* and *Gm8* based functional SSR markers for presence/absence of resistant gene(s). Further these 42 entries were subjected to genetic divergence, correlation and path analysis during *rabi*, 2021-22 at RARS, Jagtial. Among 42 entries, four entries *viz.,* Kakai, WGL-1145, WGL-1147 and WGL-1127 had nil damage and they had shown high resistant against GMB3 in field condition. Two entries *viz.,* IR72476-B-P-9-3-1-1 and RP-5332-54-11-8-2-13 were found resistant against GMB3.

Promising test lines when genotypically characterized by using *Gm3, Gm4* and *Gm8* gene linked molecular markers for presence/absence of resistant gene(s), revealed in presence of *Gm1* gene in two genotypes like IR72476-B-P-9-3-1-1 and WGL-1145; *Gm4* gene in four genotypes like Kakai, WGL-1145, WGL-1147 and WGL-1127 and *Gm8* gene in in five genotypes like RP-5332-54-11-8-2-13, Kakai, WGL-1145, WGL-1147 and WGL-1127.

Analysis of variance revealed significant variability among the genotypes for all the traits studied indicating presence of high variability among the rice genotype. The genotypic coefficients of variation for all the characters studied were lesser than the phenotypic coefficients of variation indicating the modifying effect of the environment in association with the characters at genotypic level. High PCV coupled with high GCV observed for number of grains per panicle, 1000 grains weight, grain yield per plant, head rice recovery and kernel L/B ratio suggesting the presence of wide variability among the genotypes with regard to these characters. High heritability coupled with high genetic advance as per cent of mean was observed for the characters like plant height, number of

grains per panicle, 1000-grains weight, grain yield per plant, head rice recovery, kernel length, kernel breadth and kernel L/B indicated that these traits were controlled by additive genes and can be further improved by following simple selection procedure

The genetic divergence was high among the 42 rice genotypes and was grouped into seven different clusters. Kernel breadth, plant height and kernel length showed maximum contribution towards genetic divergence.

Divergence studies through D^2 statistics indicated the presence of substantial diversity by forming large number of clusters with wide range of inter-cluster distances. The highest inter cluster distance was observed between cluster II and VII (418.05) followed by cluster IV and VII (350.95), cluster II and III (298.15) and cluster II and V (281.16). The lowest inter cluster distance was observed between cluster V and VI (54.44). The greater the distance between two clusters, the greater the genetic diversity between the genotypes belonging those clusters. The genotypes belonging to most divergent clusters may exploit the maximum amount of heterosis. High intra cluster distance was observed in cluster III (85.85) followed by cluster II (56.94) and cluster I (55.10) revealing that some genetic divergence still existed among the genotypes of these cluster. Keeping in view, it is concluded that crossing between the genotypes of cluster II and VII, cluster VI and VII, cluster II and III and cluster II and V could results in evolution of desirable transgressive segregants.

Based on cluster mean analysis it can be concluded that the genotypes of cluster II can be used in breeding program for generating gall midge resistant lines with less plant height and fine grain nature. Similarly, crosses between genotypes of cluster II and VII could results in development of coarse grain varieties with gall midge resistance. Didianga (Cluster V) with good yield potential can be crossed with lines from diverse groups for generating high yielding lines. The genotypes namely, Didianga (Cluster V), Kakirekkalu (Cluster VI) and Geetanjali (Cluster IV) were found to be good sources for development of lines with good head rice recovery. The percent contribution towards total genetic divergence was greater for kernel breadth (37.39%) followed by plant height (14.52 %) and kernel length (11.03 %). Therefore, these characters should be given importance during hybridization and selection.

Character association studies revealed that grain yield per plant exhibited significant and positive association with panicle length, number of grains per panicle, 1000-grains weight, plant height and kernel breadth indicating that the simultaneous selection for these characters could improve the yield.

Path coefficient analysis revealed that 1000 grains weight has exhibited the highest positive direct effect on grain yield per plant followed by number of grains per panicle and milling percentage 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 higher yield.

Chapter I

Introduction

Chapter I

INTRODUCTION

Rice *(Oryza sativa* L.*)* is the leading food crop and staple food for 40% of the world population. Worldwide, rice is cultivated in 164 million hectares with an annual production of 756.7 million tonnes (FAOSTAT. 2022). More than 90% of rice is produced and consumed in Asia. India stands first in area with 45 million hectares and second in production with 178.3 million tonnes, constituting up to 23.5% of global rice production (FAOSTAT. 2022). In India, rice is the most important cereal food crop and more than 65% of population dependent on rice. India has the largest area among the rice growing countries and cover about one-fourth of its total cultivated area under rice crop. A variety of topographic and hydrologic conditions ranging from rainfed highland to lowland as well as deep water condition is well suited for rice cultivation. However, as high yielding varieties progressively becoming susceptible to a wide range of pests and diseases, stability in rice productivity could not be sustained. Approximately half of global rice production is lost annually owing to the damage caused by biotic stress factors, of which 25% is attributed to the attack by insect pests (Yarasi *et al.,* 2008). Major insect pests of rice that cause huge economic losses in South Asia are stem borer, brown plant hopper and gall midge. Of these, gall midge alone is responsible for a worldwide damage of more than US $ 700 million annually (Herdt, 1991).

So far, two species of the rice gall midge have been identified, the Asian rice gall midge, (*Orseolia oryzae*) and the African rice gall midge*, (Orseolia oryzivora)*. Both of the species belong to the common family *Cecidomyiidae* of the order *Diptera*. The Asian gall midge, *Orseolia oryzae* is one of the serious insect pests of rice responsible for causing an estimated annual yield loss worth more than US $ 700 million (Herdt, 1991). In India gall midge damage causes an average annual yield loss of about 4.77 lakh tonnes of grain or 0.8% of the total production amounting to US $ 80 million (Bentur *et al.,* 2003). It has been reported as a serious pest of wet season in rice in several South and Southeast Asian countries including Bangladesh, China, India, Indonesia, Myanmar, Sri Lanka and Vietnam. In India, it is rated as third most important pest of rice in terms of spread and severity of damage and yield loss, next to stem borer and plant hopper. Asian rice gall midge is prevalent in almost all the rice growing states in India except the Western Uttar Pradesh, Uttarakhand, Punjab, Haryana and hill states like Himachal Pradesh and Jammu and Kashmir (Bentur *et al.,* 1992).

Using chemical in controlling this pest is not effective and results in environmental pollution. Hence cultivation of resistant cultivar is considered as an environmental friendly and economical approach for managing this pest. However, gall midge resistant genes must be identified and utilized in rice breeding programmes. Identification of resistant genes will be helpful in marker-assisted selection (MAS) to pyramid the resistant genes and develop a durable resistant variety against rice gall midge in areas with frequent outbreaks.

So far 12 gall midge resistant genes *(Gm1* through *Gm12)* and seven biotypes (GMB1 through GMB6 and GMB4M) of gall midge have been identified (Leelagud *et al.,*2020 and Himabindu *et al.,* 2010) based on a set of host plant gene differentials. Interestingly, none of the identified genes confers resistance to all the gall midge biotypes, while none of the gall midge biotypes is virulent against all the resistance (R) genes (Anusha *et al.,* 2022). However, cultivation of varieties containing a single resistant gene for a long time has resulted in frequent breakdown of resistance due to emergence of virulent biotypes of the insect across many regions in India. It is thus imperative to identify new resistance sources and find novel genes and deploy them effectively.

Screening of diverse genotypes like land races, high yielding varieties, pre released cultures etc. in hotspot locations result in identification of new resistant sources with novel genes or gene combinations. In Telangana State, Jagtial region for gall midge biotype 3 and Warangal for biotype 4M are identified as hotspots based on its severe incidence at regular intervals. Identified resistant lines with promising agronomic traits can be released as varieties after station and multilocation yield trials. The doners with poor agronomic characters either diversly used as parents in breeding programmes or can be used for developing pyramided lines with durable resistance.

Identification of transgressive segregants with durable gall midge resistance and one or other promised agronomic characters is possible from the present investigation and which can be used in further breeding programmes.

Before launching any breeding programme, a thorough knowledge of the nature and magnitude of genetic variability, heritability, genetic advance, genetic divergence and association between different characters with yield in a crop essential. Even in the association of the characters, information regarding the direct and indirect effects contributed by each character to yield will be an added advantage for further crop improvement.

Assessment of variability present in any crop species is an essential prerequisite for formulating an effective breeding programme. The existing variability can be used to

further enhancement of yield level of the cultivars by following an appropriate breeding strategy. As the estimation of genetic variability alone does not give a clear indication of the possible improvement that can be achieved through simple selection it should be used in conjunction with heritability and genetic advance. Studies on genotypic coefficient of variation enable the breeder to compare the amount of variability present in different characters. Heritability is the measure of transmission of characters from generation to generation and estimates of heritability are helpful to the breeder in selecting superior individuals and successfully utilizing them in breeding programme(s). The correlation studies may help the plant breeder to know how the improvement in one character will bring simultaneous improvement in other characters. Direct selection for yield is not a reliable approach since it is influenced by the environment. Therefore, it is essential to identify the component characters through which yield can be improved. The nature and magnitude of genetic divergence available in a species is essential for selection of desirable parents for use in hybridization programme. Mahalanobis' D^2 statistics is an essential tool in quantifying the degree of genetic divergence present in the material at genotypic level. It provides a quantitative measure of association between geographical distribution and genetic diversity based on generalized distance (Mahalanobis, 1936).

Keeping this in view, the present investigation entitled **"Genetic analysis and identification of genotypes with novel sources of resistance for gall midge (Biotype 3) in rice *(Oryza sativa L.)"*** was taken up with the following objectives.

1. Identification of gall midge (Biotype 3) resistant lines through field screening and molecular confirmation using SSR markers.
2. Identification of diverse parental lines for yield and quality traits through genetic divergence studies.
3. To study the character association between yield, yield components and quality traits through correlation and path analysis.

Chapter II

Review of Literature

Chapter II

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is the staple food for almost half of the world's population. More than 90% of rice is produced and consumed in Asia. As the total rice demand continues to rise day by day, it is insufficient to meet the food demand for the estimated nine billion people in 2050 (Khush, 2005 and Ray *et al.*, 2013). The International Rice Research Institute (IRRI), Philippines developed the first semi-dwarf high yielding rice variety, IR 8 using the semi-dwarfism (*sd1*) and photoperiod insensitive traits of Dee-Geo-Woo Gen. This led to the green revolution in rice production in the 1960s because of the increased yield potential of the developed cultivars. However, as high yielding varieties progressively becoming susceptible to a wide range of pests and diseases, stability in rice productivity could not be sustained.

Major insect pests of rice that cause huge economic losses in South Asia are stem borer, brown plant hopper and gall midge. Of these, gall midge alone is responsible for a worldwide damage of more than US $ 700 million annually (Herdt, 1991). In India gall midge damage causes an average annual yield loss of about 4.77 lakh tonnes of grain or 0.8% of the total production amounting to US $ 80 million (Bentur *et al.,* 2003). Testing of diverse genotypes at hot spot location (Jagtial) for gall midge biotype 3 could results in identification of novel resistant sources. Through diversity analysis, genetically diverse parents for yield and gall midge resistance will be identified for further use in breeding programmes.

A brief review of literature pertaining to the present study **"Genetic analysis and identification of genotypes with novel sources of resistance for gall midge (Biotype 3) in rice *(Oryza sativa L.)"*** is presented under the following heads:

2.1 Genes governing gall midge resistance in rice

2.2 Heritability and Genetic advance

2.3 Genetic divergence

2.4 Correlation and Path coefficient analysis

2.1 Genes governing gall midge resistance in rice

Gall midge resistant in rice is mostly governed by dominant genes. Cultivation of varieties containing a single resistant gene has resulted in frequent breakdown of resistance due to emergence of virulent biotypes of the insect across many regions in

India. Thus, there is a need to study the inheritance and allelic relationship of the resistance of various donors in order to find novel genes and deploy them effectively. Pyramiding of two or more effective genes in a single cultivar may lead to durable gall midge resistance.

2.1.1 Genes conferring resistance to gall midge

So far 12 non allelic gall midge resistant genes *(Gm1 through Gm12)* and seven biotypes (GMB1 through GMB6 and GMB4M) of gall midge have been identified (Leelagud *et al.,*2020 and Himabindu *et al.,* 2010). Of these, ten genes *viz., Gm1 through Gm8, Gm11* and *Gm12* have been mapped to different rice chromosomes using molecular markers. However, the remaining two resistant genes (*Gm9* and *Gm10*) have not yet been assigned to specific chromosomes (Leelagud *et al.,* 2020) and further effort is needed to identify and precisely map additional resistance genes that will facilitate the marker assisted breeding of gall midge resistant rice varieties.

The *Gm1* gene found in 'Samridhi' and *Gm2* gene found in 'Surekha' were the first resistant genes discovered (Chaudhary *et al*., 1985). Subsequently several nonallelic gall midge resistant genes and the donor cultivar carrying the resistant gene were identified: *gm3* from RP2068-18-3-5 (Kumar *et al*., 1998 b), *Gm4* from 'Abhaya' (Shrivastava *et al*., 1993), *Gm5* from ARC 5984 (Kumar *et al.*, 1998 a), *Gm6* from 'Duokang 1' (Zhang *et al*., 1997), *Gm7* from RP2333-156-8 (Kumar *et al*., 1999).

Kumar *et al.* (2000) crossed Jhitpiti with susceptible parents (Kranti and TN1) as well as parents with known resistant genes. The gene for gall midge resistance in Jhitpiti was non-allelic to *Gm1*-*Gm7*, as revealed by F_2 segregation ratio. Therefore, this gene was designated as *Gm8*.

Shrivastava *et al.* (2003) investigated the mode of inheritance and allelic relationship of the resistant genes in resistant donor Line 9, a sib of the susceptible cultivar 'Madhuri.' The presence of a single dominant gene for resistance was established by the segregation behaviour of F_1, F_2 and F_3 populations of the cross between Line 9 and susceptible cultivar MW10. Allelism tests with all known genes associated with resistance in this population revealed that Line 9 harbored a novel gene identified as *Gm9*.

Kumar *et al*. (2005) studied the inheritance patterns of F_1, F_2 and F_3 populations derived from crossings of BG 380-2 and Gurmatia with susceptible cultivars and differentials, harboring the known resistance genes. In both BG 380-2 and Gurmatia, inheritance studies revealed a single dominant gene for gall midge resistance. Allelic

analysis showed that the gall midge resistance gene present in BG 380-2 was nonallelic to all currently known genes *(Gm1, Gm2, gm3, Gm4, Gm5, Gm7, Gm8, and Gm9)* that confer resistance to the gall midge biotype 1 population, and was designated *Gm10*. The gall midge resistant gene found in Gurmatia was allelic to the previously discovered *Gm1* gene.

Himabindu *et al.* (2009) evaluated F_3 families derived from crossing CR57-MR1523 with the susceptible check TN1, as well as rice varieties with known resistant genes and other resistant sources with unknown gene(s) in the field against gall midge biotypes 1, 3, and 4. MR1523 was resistant to biotypes 1 and 4, but vulnerable to biotype 4M. This indicated that the single dominant gene in MR1523 that confers biotype 4 resistance is not allelic to *gm3, Gm4, or Gm8*. *Gm11* is the proposed name for this novel gene.

Leelagud *et al.* (2020) identified a novel genetic locus for resistance to Rice gall midge, designated as *Gm12*, on the short arm of rice chromosome 2 in the Thai rice landrace MN62M. The locus was identified using linkage analysis in 144 F_2 plants derived from a cross between susceptible cultivar KDML105 and RGM-resistant cultivar MN62M with single nucleotide polymorphism (SNP) markers and $F_{2:3}$. This finding yielded SNP and SSR markers that can be used in MAS to develop cultivars with broad-spectrum resistance to Rice gall midge. The new resistant gene provides important information for the identification of Rice gall midge biotypes in Thailand and Southeast Asia.

Table 2.1. Gall midge resistant genes identified and designated

Gene	Source variety/germplasm	Reference
Gm1	Usha Samridhi Bd6-1 R302-111 R335-2 Eswarakora W1263 R30-300 Kakatiya	Chaudhary *et al*. (1985) Chaudhary *et al*. (1985) Chaudhary *et al*. (1985) Kumar *et al*. (1994) Kumar *et al*. (1994) Reddy *et al*. (1997) Reddy *et al*. (1997) Kumar *et al*. (1998 b) Motiramani *et al*. (1999)
Gm2	Surekha IET6286 R435-107 Calrose 70 Siam29, Phalguna CR410-3225, CR392-5088-2	Chaudhary *et al*. (1985) Chaudhary *et al*. (1985) Kumar *et al*. (1994) Shrivastava (1998) Mohan *et al.* (1994) Motiramani *et al*. (1999)
gm 3	RP2068-18-3-5 (TET9244), Swarnadhan/Velluthacheera	Kumar *et al.* (1998 b)
Gm4	Abhaya Balam MNP76	Shrivastava *et al.* (1993) Shrivastava (1992) Shrivastava (1992)
Gm5	ARC5984	Kumar *et al.* (1998 a)
Gm6	Duokang #1	Tan *et al.* (1996)
Gm7	RP2333-156-8 (TET13404), Ratna/ARC10659	Kumar *et al.* (1999)
Gm8	Jhitipiti Aganni	Kumar *et al.* (2000) Sama *et al.* (2012)
Gm9	Line 9 (Sister selection of Madhuri, Jaya/Dubraj)	Shrivastava *et al.* (2003)
Gm10	BG380-2	Kumar *et al.* (2005)
Gm11	CR57-MR1523	Himabindu *et al.* (2010)
Gm12	MN62M	Leelagud *et al.* (2020)

2.1.2 Field screening for identification of gall midge resistant lines

Screening of rice entries for gall midge biotypes has been reported by several workers, some of which are described below:

Kudagamme and Gunawardena (1989) evaluated eight varieties for resistance to Asian rice gall midge in Sri Lanka and found that ARC 6650 and IR 8 were susceptible and RP 1551 was resistant to gall midge.

Muthuswami and Gunathilagaraj (1989) screened 794 entries for resistance against rice gall midge among which 24 entries showed resistance.

Kandalkar *et al.* (1991) evaluated 56 genotypes for resistance to gall midge, among which Neela, Samalie and Sarasa genotypes were found resistant with 0-5 per cent silver shoots.

Harinkhere and Pophaly (1991) screened nine rice varieties for resistance against rice gall midge in Madhya Pradesh, India and found that five varieties *viz.,* WGL 28712, CR 95-181-2, CR 151-17, Sureka and Phalguna were resistant and remaining four varieties *viz.,* R 44-3012, CR 127-3, R 2700-34001 and Asha were moderately resistant when compared with the susceptible check Jaya.

Bentur *et al.* (1994) screened rice germplasm under field conditions to gall midge biotypes 1, 2 and 4 and found that 22 entries were resistant to all three biotypes, 13 were resistant to biotypes 1 and 2 and IR 9828-2-3-1 was resistant to biotypes 1 and 4.

Sain and kalode (1994) screened a total of 2256 germplasm accessions, 3000 advanced generation breeding lines and 150 released varieties of rice under artificial infestation in the greenhouse. Among these, 15 new germplasm accessions, 50 breeding lines derived from 16 crosses and 15 released varieties were resistant against gall midge.

Mukherjee *et al.* (1998) evaluated 11 rice entries against gall midge population under field conditions at northern hills of Chattisgarh and identified Mahamaya (R 320-298), as highly resistant to gall midge.

Meher *et al.* (2009) conducted field screening of 87 genotypes under natural condition in order to know the reaction of popular high yielding varieties (HYVs) and few improved genotypes to gall midge. With 0 % silver shoot, genotypes such as Ananga, Annada, Kharavela and Shaktiman showed highly resistant reaction. Cultivar Jajati, Suraksha and Chaitanya showed moderately resistant reaction. Other cultivars had a high

gall midge infestation, as shown by the presence of silver shoots, and were classified as susceptible or highly susceptible.

Archana *et al.* (2012) carried out field screening of 49 rice hybrids against gall midge during *kharif* season of 2009-10 at Agricultural Research Station, Sirsi (Uttar Kannada) under rainfed ecosystem. Among 49 rice hybrids screened, 42 rice hybrids showed susceptible reaction [11-25% silver shoots (SS)]; two hybrids showed highly susceptible (>25% SS) and remaining five hybrids showed highly resistant (0% SS).

Sumathi and Manickam (2013) carried out field trial on screening of rice accessions for resistance/susceptible to rice gall midge at RRS Tirur, Tiruvallur. Among 17 entries screened in gall midge biotype monitoring trial (GMBT), the entries ARE 6605 and INRC 3021 were found resistant to gall midge, INRC 202 and INRC 1997 were found moderately susceptible and remaining entries were found susceptible to highly susceptible to gall midge damage.

Suvendhu *et al.* (2014) screened about 100 gall midge resistant rice genotypes under field condition against gall midge biotype GMB4M at Warangal and GMB1 in greenhouse at DRR Hyderabad. Furthermore, these entries were genotyped by using five gene specific markers which can detect the presence of *Gm1*, *gm3, Gm4, Gm8* and *Gm11* genes with high degree of success.

Anusha *et al.* (2017) evaluated pre-breeding lines with known source of Gall midge resistance through *Gm1* gene in the elite backgrounds in a replicated trial under greenhouse conditions at IIRR, Rajendranagar, Hyderabad against biotype 1 and in field at Jagtial and Warangal against biotype 3 and 4, respectively. Among 38 pre-breeding lines, chosen with phenotypic acceptability, 12 lines showed "nil" damage crosses against biotype 1 and 6 lines against biotype 3. Of these RNR17927-1 (Tellahamsa X JGL11690) and RNR19872, RNR19875, RNR19880, RNR19881 and RNR19883 (MTU1010/JGL3855) were found resistant against both biotype 1 and 3 but susceptible to biotype 4M.

Pandey *et al.* (2018) conducted investigation at S.G. College of Agriculture and Research Station, Jagdalpur, Chhattisgarh using 97 genotypes during *Kharif 2016* against different insect pests, fifty-five genotypes were free, one only exhibited 1 score, 20 and 21 were 3 and 5 score respectively against gall midge.

Seni and Naik (2019) evaluated 137 rice germplasm against rice gall midge, in the experimental farm of Regional Research and Technology Transfer Station *(*OUAT*)*,

Chiplima, Sambalpur, Odisha, during *Kharif,* 2016. It was observed that among 137 rice entries screened against gall midge, the entries *viz.,* WGL 1164, WGL 1127, RP 5925, RP 1, INRC 3021, IBT R4, IBT GM (1, 2, 3, 4, 7, 9, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 46) exhibited highly resistant, they can be developed as varieties or can be used in breeding programme as a source of gall midge resistance.

Kumar *et al.* (2020) screened 173 entries against gall midge for resistance at Regional Agricultural Research Station, Warangal, Telangana during wet season (*Kharif)* of 2019 under delayed planting situation. Three entries *viz.,* IBT MRR 18, IBT MRR 23 and IBT MRR 24 were found highly resistant and six entries *viz.,* IBT MRR 17, IBT MRR 19, IBT MRR 20, IBT MRR 21, IBT MRR 22 and IBT MRR 28 had shown resistant reaction against gall midge. These entries can be used in breeding programmes as a source of gall midge resistance or could be released as varieties, if found promising in yield traits.

Anusha *et al.* (2022) evaluated 52 rice germplasm lines along with resistant and susceptible checks against rice gall midge, biotype1 under greenhouse conditions at the ICAR-IIRR, Hyderabad and under field conditions against biotype 3 at RARS, Jagtial and biotype 4M at RARS, Warangal during Wet seasons 2014 and 2015. Five germplasm lines, *viz.,* CR 2613- 1-1-1-5-1, CR 2615-1, JGL 11470, JGL 11727 and Sukaradidhan 1 were found resistant against GMB1. Intivadlu was identified as a source of resistance against GMB3. None of the tested lines exhibited consistent resistant against biotype 4M.

2.2 Heritability and Genetic Advance

Heritability and Genetic advance are important selection parameters in crop improvement programme. In plant breeding, only genetic component of variation is important because only this component is transmitted to the next generation. The ratio of genotypic variance to total variance or phenotypic variance is called heritability. It denotes contribution of genotype to the phenotypic variation and expressed in terms of per cent. Such estimates are valid for homozygous lines or populations but in case of segregating generations, genetic variance consists of additive, dominance and epistatic components of variance. Thus, for segregating generation, the ratio of additive component of variance to the total phenotypic variance is more appropriate estimates of heritability; called “Narrow sense heritability”. Heritability serves as useful guide to the breeders. The breeder is able to appreciate the proportion of variation that is due to genotypic (broad sense heritability) or additive (narrow sense heritability) effects, i.e., the heritable portion

of variation in first case, and the proportion of genetic variation that is fixable in purelines in latter case.

Heritability estimates along with genetic advance are normally more helpful in predicting the genetic gain under selection than heritability estimates alone. Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection which is depend on three factors *viz;* genetic variability, heritability and selection intensity.

Chongkid (1997) evaluated eight rice varieties and their 28 F_1's for reaction to the yellow orange leaf virus (YOLV), based on differences in percent leaf yellowing, plant height, number of productive tillers, and grain yield per plant. ARCll554 and GS10719 were classified as resistant, SPR60 and RD9 as moderately resistant, RDl and RD23 as moderately susceptible, and RD7 and Inl as susceptible varieties. For each character heritability and genetic advances were studied. The results indicated that all four characters were heritable with slight effects of environmental factors.

Bharadwaj *et al.* (2007) studied heritability and genetic advance in three New Plant Type (NPT) based crosses of rice for thirteen characters in two environments of normal and high dose of nitrogen. High heritability estimates coupled with high genetic advance as per cent of mean was seen in all the crosses for days to 50% flowering, days to maturity, plant height, panicle length, L:B ratio and 1000 grain weight, while high heritability with moderate genetic advance was seen in average grain length and grain breadth. Spikelets per panicle and filled grains per panicle had moderate heritability and high genetic advance. Productive tillers per plant, spikelet sterility and grain yield per plant showed low to moderate heritability coupled with low to moderate genetic advance as per cent of mean.

Padmaja *et al.* (2008) studied genetic variability, genotypic and phenotypic coefficients of variation, heritability and genetic advance in 150 genotypes for 11 characters. Except days to 50% flowering and panicle length, heritability and genetic advance were high for all the characters indicating additive type of gene action governing these characters.

Bisne *et al.* (2009) conducted trial with four CMS lines, eight testers and 32 hybrids to estimate genetic parameters for yield and its correspondent characters in rice. High heritability coupled with high genetic advance was exhibited by harvest index, total number of chaffy spikelets per panicle, grain yield per plant, total number of filled

spikelets per panicle and spikelet fertility percentage and selection may be effective for these characters.

Ahmadikhah (2010) conducted a study with four generations, including two parents, BC_1 and BC_1S_1 populations to estimate selection effect, genetic advance, heritability and selection success in rice. High general heritability was observed for most traits except grain yield showed a relatively low specific heritability. High expected genetic advance was obtained for tiller number, followed by grain yield and plant height, while the highest real genetic advance was obtained for heading date and tiller number.

Lal and Chauhan (2011) evaluated 48 rice genotypes in RBD to estimate genetic parameters for yield and its component characters in rice. High heritability was estimated for all the characters. High genetic advance was estimated for plant height, number of panicles per plant, stem length, L /B ratio, 1000 grain weight and number of grains per panicle, however moderate genetic advance was estimated for leaf breadth and days to flowering.

Islam *et al.* (2015) conducted an experiment to study genetic variability, heritability and genetic advance for yield and yield associated traits in rice. High heritability was obtained for grain width, days to 50% flowering, thousand grain weight, grain length, days to maturity and number of filled grains per panicle. High to medium estimates of heritability and genetic advance were obtained for number of filled grains per panicle, days to 50% flowering, days to maturity indicating the roles of additive gene action and a good scope of selection using their phenotypic performance.

Sumanth *et al.* (2017) investigated 23 rice genotypes and concluded that plant height, flag leaf length, biological yield per plant, spikelets per panicle and panicles per plant exhibited high estimates of heritability. High genetic advance was observed for number of spikelets per panicle and plant height, indicating predominance of additive gene effects and possibilities of effective selection for the improvement of these characters.

Srujana *et al.* (2017) carried out investigation consisting 29 rice genotypes to study genetic variability, heritability and genetic advance. High estimates of heritability were observed for spikelets per panicle, days to maturity, biological yield, grain yield per hill, panicles per hill and tillers per hill. High estimates of heritability along with moderate to low estimates of genetic advance were observed for spikelets per panicle, seed yield per plant, tillers per plant, panicles per plant and biological yield per hill.

Yadav *et al.* (2017) evaluated 35 genotypes of rice under sodic soil and concluded plant height and grain yield per plant had high estimates heritability coupled with high genetic advance as per cent of mean indicating the role of additive gene action governing these traits.

Prasad *et al.* (2017) evaluated 50 bororice genotypes to estimate variability, heritability and genetic advance in yield and yield contributing characters. High heritability coupled with high genetic advance as per cent of mean was observed for plant height, number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, number of unfilled grains per panicle, 1000 grain weight and grain yield per plant which indicated that these traits were governed by additive gene action.

Abebe *et al.* (2017) evaluated 36 rice genotypes to study genetic parameters for grain yield and yield associated traits. The highest heritability was recorded for culm length followed by plant height, biomass yield and panicle length. High to medium heritability coupled with high GCV and high genetic advance as per cent of means were exhibited for plant height, biomass yield, grain yield and number of unfilled grains per panicle. High genetic advances as per cent of mean were recorded by plant height, culm length, biomass yield, grain yield and number of unfilled grains per panicle.

Ajmera *et al.* (2017) evaluated 37 rice genotypes to estimate heritability and genetic advance in per cent of mean for yield and yield component traits. Except days to fifty percent flowering, all the characters under study exhibited high heritability associated with high genetic advance as per cent of mean indicating that these traits were controlled by additive type of gene action.

Adhikari *et al.* (2018) conducted to estimate the genotypic and phenotypic variability, heritability, genetic advance and correlation on yield and yield associated traits. High heritability was estimated for days to flowering, maturity, thousand grain weight and plant height indicating that, these traits are under high genetic control.

Sandeep *et al.* (2018) carried out experimental in order to estimate genetic variability, heritability and genetic advance. The characters *viz.,* spikelet fertility, plant height, grain yield per plant, number of grains per panicle, number of tillers per hill, pollen viability, number of productive tillers per hill, panicle length and 1000 grain weight exhibited high heritability estimates coupled with high genetic advance as per cent of mean which suggested that these traits were amenable for further improvement by following simple selection methods.

Lingaiah *et al.* (2019) carried out an experiment with the material comprised of 31 elite mid early group genotypes of rice. The characters *viz.,* number of grains per panicle, yield, test weight and plant height had high genetic advance as per cent of mean along with high heritability.

Acevedo-Siaca *et al.* (2021) characterized photosynthetic and morphological traits in *indica* rice to examine natural variation and the potential for hybridization in the future. Additionally, broad-sense heritability was calculated for photosynthetic traits, including, for the first time, biochemical limitations to photosynthesis. Heritability was high for CO_2 assimilation in saturating light and CO_2, the maximum rate of carboxylation, the maximum rate of electron transport, and triosephosphate utilization. Estimated genetic advance was medium, suggesting that it would be possible to not only select for the improvement of biochemical components of photosynthesis but also achieve significant gains in one generation.

Genetic variability, heritability and genetic advance were studied by Dubey *et al.* (2022) in 74 diverse rice genotypes during *kharif,* 2020. A collection of diverse genotypes was evaluated for certain physiological traits *viz.,* days to 50% flowering, plant height, flag leaf area, biological yield per plant, harvest index, chlorophyll content, leaf nitrogen, leaf temperature and grain yield per plant. A high estimate of heritability coupled with high genetic advance was found for majority of parameter indicating the preponderance of additive gene action. Therefore, these traits can be improved through direct selection. The moderate broad sense heritability with low genetic advance in per cent of mean were observed for leaf temperature indicating presence of non-additive gene action suggesting heterosis breeding may be useful for rice improvement in sodic soil.

Gupta *et al.* (2022) evaluated 32 rice genotypes and three check verities at Crop Research Station Masodha from July to November 2019 to estimate genetic variability, heritability and genetic advance for grain yield and yield associated traits. The highest heritability was recorded for days to 50 percent flowering followed by number of seeds per panicle, plant height, days to maturity and seed yield per plant. High to medium heritability coupled with high GCV and high genetic advance as per cent of means were exhibited for plant height, number of tillers per plant, length of panicle and test weight.

Jasmine *et al*. (2022) carried out an investigation to study the genetic variability, heritability, correlation and path analysis of yield and yield components parameters in maintainer and restorer lines of rice. Except days to 50 % flowering and panicle length,

all the characters had high heritability coupled with high genetic advance as per cent of mean indicating that these traits were controlled by additive type of gene action.

2.3 Genetic divergence

Variability present in population of a species is known as genetic divergence. Variability differs from diversity in the sense that the former has observable phenotypic differences, whereas the latter may or may not have such an expression. Genetic diversity may arise due to geographical separation or due to genetic barriers to crossability. For the study of genetic diversity present within a population either D^2 statistic or metroglyph analysis is used.

Metroglyph analysis developed by Anderson in 1957 is a semigraphic method of studying variability which is based on mean value of different traits. On the other hand D^2 statistic is numerical approach proposed by Mahalanobis in 1928 which measure genetic divergence at two levels namely intracluster and intercluster levels and thus helps in selection of genetically divergent parents for exploitation in hybridization programmes.in addition to the aiding in the selection of divergent parent for use in breeding programme it measures the degree of divergence and determines the relative proportion of each component character to the total divergence. The D^2 statistic provides reliable estimates of genetic divergence than Metroglyph analysis.

Singh *et al.* (2006) in the genetic divergence study of 52 traditional lowland rice genotypes from five states of North Eastern Region of India using Mahalanobis D^2 statistic, concluded that the geographical origin was not found to be a good parameter of genetic divergence.

Pandey *et al.* (2009) carried out an experiment with 40 rice genotypes to estimate genetic diversity in different accessions of rice for various agro-economically important characters and found that sufficient amount of variability was present in the entire gene pool for all traits studied.

Banumathy *et al.* (2010) evaluated 53 rice entries using D^2 analysis to study the diversity pattern among the genotypes. Based on the analysis, the genotypes were grouped into 11 clusters. Maximum number of 16 and 15 genotypes were grouped under cluster XI and I respectively, while clusters II, IV, V, VI, VIII, IX and X had only two genotypes each and clusters III and VII consisting of 3 and 5 genotypes respectively. Maximum inter cluster D^2 value was observed between cluster I and X followed by cluster I and IV. The

greater the distance between the two clusters indicates wider the genetic diversity between genotypes.

Garg *et al.* (2011) carried out an investigation with the 48 genotypes of rice to study genetic divergence using D^2 statistics. Days to maturity, gel consistency and days to 50 per cent flowering contributed 74.55 per cent of total divergence. Maximum inter-cluster distance was recorded between clusters II and III followed by clusters III and IV indicating wide genetic diversity and it may be used in rice hybridization programme for improving grain yield.

Ashfaq *et al.* (2012) carried out study to assess the genetic diversity for various rice traits and their association with yield and found that the highest genetic variability was observed in plant height, spikelets per panicle, panicle length, days to heading and days to maturity. Panicle length, seeds per panicle and seed weight per panicle showed significant positive correlation with grain yield.

Karuppaiyan *et al.* (2013) estimated genetic divergence among 27 genotypes of rice through Mahalanobis D^2 statistics. The genotypes were grouped into four clusters. Yield/ha contributed maximum towards divergence followed by plant height, grain breadth and grain length.

Medhabati *et al.* (2013) based on twelve agromorphological characters, grouped 37 germplasms into five clusters following Tocher's method of cluster formation. The characters which contributed maximum towards total genetic divergence in the study were grain yield/plant, spikelets/panicle, 100 grain weight, grain length, days to 50% flowering, ear bearing tillers/plant and flag leaf length.

Ramanjaneyulu *et al.* (2014) evaluated 10 popular low land rice genotypes for variability, genetic divergence and stability analysis. The pattern of constellations into 4 clusters indicated the existence of a significant amount of variability.

Soni *et al.* (2014) evaluated 36 rice lines comprising of 16 Tropical *japonica* and same number of *indica*, to assess nature and magnitude of genetic divergence. Clustering pattern of genotypes showed no definite relationship between genetic divergence and geographical distribution of genotypes.

Ahamed *et al.* (2014) estimated genetic divergence of indigenous and exotic genotypes of rice under sodic soil. Various clusters showed considerable differences in inter-cluster group means for 12 characters. Therefore, crosses between members of

clusters having high cluster means for important characters coupled with high inter-cluster distance between them are likely to be more rewarding.

Bhati *et al.* (2015) investigated genetic divergence using D^2 statistics with 52 genotypes of rice. Maximum genetic divergence among genotypes was observed in cluster IV. Number of spikelets per panicle, plant height, grain per panicle and grain yield per plant contributed maximum towards genetic divergence. Maximum inter-cluster distance was observed between cluster IV and VIII followed by cluster IV and cluster VII, cluster II and cluster VIII and cluster II and cluster VII.

Mohan *et al.* (2015) evaluated 43 genotypes derived through pedigree method of breeding involving 11 divergent parents for genetic divergence using D^2 studies revealed that highest inter cluster distance was observed between cluster II & V followed by cluster II & III suggesting wide diversity between parents. Among the traits studied, test weight and days to flowering contributed highest. The genotypes grouped in cluster II (JGL 20668, JGL 20779, JGL 21057, JGL 21062, JGL 21088, JGL 21106, JGL 3855) could be crossed with JGL 21002 and JGL 21005 of cluster III to derive transgressive segregants for improvement of grain yield, number of grains per panicle and test weight.

Chandramohan *et al.* (2016) found that days to 50% flowering and 1000 grain weight manifested highest contribution towards total divergence as revealed from study of D^2 analysis with 44 genotypes of rice, thus, these traits could be given due importance for further crop improvement.

Srinivas *et al.* (2016) evaluated 18 rice genotypes for genetic parameters and genetic divergence for yield contributing characters and gall midge resistance at RARS, Jagtial, Telangana and found that the characters *viz.,* 1000 grain weight and days to 50% flowering were contributed maximum (75.16%) to the total divergence which could be given due importance during hybridization and selection in segregating population.

Tripathi *et al.* (2017) studied on genetic divergence of 32 genotypes of rice using Mahalanobis's D^2 statistic and grouped the genotypes into 7 clusters. Cluster II had maximum number (16) of genotypes. Maximum inter cluster distance was found between cluster IV and VII. However, intra cluster distance was maximum in cluster III.

Sruthi *et al.* (2019) characterized 96 parental lines with respect to their morphological traits (n = 12) using Mahalanobis D^2 and molecular markers using a set of 50 SSR markers. Strong correspondence was observed between the pedigree of parental

lines with molecular genotyping-based grouping than morphological trait-based grouping.

Dhakal *et al.* (2020) on the basis of Mahalanobis genetic divergence study with 30 rice landraces, concluded that landraces from clusters V and VI or clusters III and VI or clusters IV and VI can be used in the hybridization program to develop the superior hybrids by exploiting heterosis in segregating generation.

2.4 Correlation and path analysis

The statistics that measure the degree and direction of association between two or more variables is known as correlation. A positive value of correlation indicates that the changes in two variables are in the same direction. Path analysis is simply standardized partial regression coefficient, which splits correlation coefficient into measure of direct and indirect effects and measures the direct and indirect contribution of each independent variable on the dependent variable. In addition, path analysis also measures the residual effect i.e., the effect of other possible independent variable which are not included in the study.

Zahid *et al.* (2006) conducted correlation coefficient and path coefficient analysis in 14 genotypes of basmati rice. Plant height had negative correlation with yield whereas grain per panicle had positive and significant genotypic and phenotypic correlation with yield.

Ashfaq *et al.* (2012) evaluated 40 rice genotypes for yield and yield attributing traits. The study indicated that the traits *viz.,* panicle length, number of seeds per panicle and seed weight per panicle had significant positive correlation with grain yield.

Pratap *et al.* (2012) evaluated 100 high yielding rice genotypes to determine character association, variability and diversity for grain yield, yield components and quality characters. Majority of the traits showed significant and positive associations between yield and yield components like biological yield/hill followed by harvest-index as most important traits which need due consideration at the time of devising selection strategy.

Kiani (2012) studied the relationship between grain yield and yield components in 20 rice genotypes. Results showed that total number of grains per panicle and filled grains per panicle correlated significantly with grain yield. Path coefficient analysis revealed that grain yield associated with number of panicles per plant and the total number of grains per panicle with the direct effects of 0.765 and 0.718, respectively. Information

obtained in this study revealed that traits the number of panicles per plant and total number of grains per panicle could be used as selection criteria for grain yield improvement in rice.

Babu *et al.* (2012) carried out an investigation to study the correlation and path analysis in 21 popular hybrids of rice. Character association of the yield attributing traits revealed significantly positive association of grain yield per plant with number of productive tillers per plant. Hence, selection for these traits can improve yield. Path coefficient analysis revealed that panicle length and number of productive tillers per plant exhibited positive direct effect on yield. Among these characters, number of productive tillers per plant possessed both positive association and high direct effects. Hence, selection for this character could bring improvement in yield and yield components.

Idris *et al.* (2012) conducted an experiment to study genetic variability and correlation between yield, yield components in rice genotypes. Positive phenotypic and genotypic correlation coefficient was detected between grain yield and number of filled grains per panicle, harvest index, panicle length and number of grains per panicle.

Correlation and path coefficient analysis were carried out by Bhadru *et al.* (2012) for yield and yield components in 21 gall midge resistant rice genotypes. Number of grains per panicle, days to 50% flowering and panicle length had a significant positive association with yield and also manifested a positive direct effect on grain yield. Thus, selection for these characters helps in selection of superior gall midge resistant genotypes in rice.

Veni *et al.* (2013) assessed 70 rice entries for genetic variability and correlations between yield and yield components and quality parameters. Days to 50 per cent flowering, productive tillers per plant, panicle length, Head Rice Recovery (HRR) and volume expansion ratio manifested significant positive association with grain yield indicating that simultaneous improvement of all the characters is possible. Productive tillers, panicle length, kernel breadth and L/B ratio manifested positive direct effect on grain yield per plant.

Ramanjaneyulu *et al.* (2014) evaluated 10 popular low land rice genotypes for yield and yield attributing traits. A significant and positive correlation of grain yield was manifested with total number of tillers per plant, number of productive tillers per plant, number of grains per spikelet and spikelet length, indicating that grain yield and these traits had the same physiological basis for expression.

Lakshmi *et al.* (2014) evaluated 70 genotypes of rice to study the correlation among yield and yield contributing characters. The study revealed that grain yield per plant was positively and significantly associated with days to maturity, number of productive tillers per plant, plant height and kernel length indicating importance of these traits as selection criteria in breeding programmes.

Premkumar *et al.* (2015) investigated forty-three rice genotypes for grain yield and kernel characters to understand the nature and extent of correlation among yield contributing traits and their direct and indirect influence on Grain yield per plant. The result of correlation analysis registered positive significant association of characters *viz.,* number of productive tillers per plant, kernel breadth, hundred grain weight and number of filled grains per panicle on grain yield per plant. Path-coefficient analysis revealed that days to 50% flowering, panicle length, kernel L/B ratio, hulling % and number of productive tillers per plant had positive direct effect on grain yield.

Mohan *et al.* (2015) evaluated 43 genotypes derived through pedigree method of breeding for variability and genetic divergence and genetic parameter studies. Grain yield recorded significantly positive correlation with plant height, test weight and days to flowering. Test weight had significant positive correlation with gall midge incidence indicating fine grain varieties were relatively tolerant and bold grain varieties were susceptible.

Dhurai *et al.* (2016) conducted field experiment using 32 rice genotypes to estimate genetic parameters for grain yield and yield attributing traits. Correlation analysis showed significant positive association of grain yield per plant with independent variables *viz.,* harvest index, days to maturity and number of grains per panicle. A positive direct effect on grain yield were shown by traits like kernel elongation ratio, kernel length, harvest index and days to maturity.

Rathod *et al.* (2017) studied correlation and path analysis in 56 high iron and zinc rice genotype. Character association studies revealed that grain yield per plant showed significant positive association with number of productive tillers per plant, panicle length, number of filled grains per panicle and grain iron concentration. Path coefficient analysis revealed direct influence of the characters *viz.,* 1000 grain weight, numbers of filled grains per panicle, number of productive tillers per plant, grain iron concentration, grain zinc concentration, days to 50% flowering and plant height on grain yield per plant.

Manjunatha *et al.* (2017) carried out an investigation to study the character association and path analysis in 65 genotypes of rice under organic management. For the trait *viz.,* chlorophyll content of flag leaf, chlorophyll content of third leaf and number of tillers per plant at 30 DAT, the genotypic correlation coefficients were higher than phenotypic correlation coefficients, suggesting the less influence of environment on these characters. However, in the case of parameters namely, number of tillers per plant at 60 DAT, number of tillers per plan at 90 DAT and number of tillers per plant at harvest, the phenotypic correlation coefficients were higher than genotypic correlation coefficients, which indicates that the influence of environment on these characters was high. Among these characters, number of tillers at harvest and number of tillers at 60 DAT possessed both positive association and direct effects. Hence, selection for these characters could bring improvement in organic rice yield improvement programmes.

To find the traits attributing the yield and its effect on yield, an experiment was done with 34 rice genotypes using correlation and path coefficient analysis (Mukesh *et al.*, 2018). The characters *viz.,* effective number of tillers per plant, number of spikelets per plant, number of fertile grains per plant and biological yield per plant were found to be strong positively correlated with grain yield while plant height, days to 50% flowering, panicle length and flag leaf length had revealed negative correlation with grain yield.

Vengatesh (2018) carried out an experiment to study the correlation and path analysis in 25 rice genotypes. Among the traits studied, six root traits, four physiological traits and six yield related traits showed significant positive genotypic correlations with grain yield. Path analysis revealed a very high magnitude of positive direct effect was exerted by root length on grain yield.

With a view to select better yield attributes in rice, Saha *et al.* (2019) estimated genetic parameters of 13 yield and yield attributing traits in 40 landraces of rice. Study revealed that the traits like days to 50% flowering, days to maturity, flag leaf area, number of total tillers per hill, number of effective tillers per hill, pollen fertility, number of grains per panicle, number of filled grains per panicle were significantly and positively associated with grain yield per plant indicating selection of these characters for yield improvement may be rewarding. The characters *viz.,* days to 50% flowering, flag leaf area, number of effective tillers per hill, pollen fertility, panicle length, number of grains per panicle and 100 seed weight had direct positive effect on yield per plant both at phenotypic and genotypic level indicating importance of these characters during selection in yield improvement program.

Correlation study of 32 rice genotypes showed significant positive correlation on yield per plant by independent variables *viz.,* leaf length, leaf width, number of panicles per plant, number of grains per panicle, 1000 grain weight and time to maturity (Francis *et al.*, 2020). Path analysis revealed that significant high direct effect on yield per plant were registered by traits like number of panicles per plant, number of grains per panicle, 1000 grain weight, days to 50 per cent flowering and time to maturity. Number of grains per panicle via number of panicles per plant showed significant high association on grain yield per plant.

Singh *et al.* (2020) evaluated character association for 16 yield and yield contributing traits for 112 rice genotypes. Correlation and path coefficient analysis envisaged characteristics such as spikelets per panicle, grains per panicle, grain weight per panicle, biomass yield per plot, first flowering, 50 per cent flowering and days to maturity showed positive direct effect and very strong correlation with grain yield per plot, indicating that these characters could be exploited in future breeding programmes.

An investigation was carried out by Meena *et al.* (2020) to examine the character association and path coefficient analysis for grain yield and its attributing traits in 25 rice genotypes. The result of character association revealed that the traits like number of productive tillers per plant, 1000-grains weight, panicle length, plant height, days to maturity and days to 50 % flowering exhibited significant and positive correlation with grain yield per plant at phenotypic as well as genotypic level indicating that grain yield per plant could be improved by selecting genotypes containing higher values for these traits. The path analysis result showed that the maximum direct positive effect on the grain yield per plant was exerted by number of productive tillers per plant followed by 1000-grain weight, panicle length, plant height, days to maturity and days to 50 per cent flowering.

Jeke *et al.* (2021) carried out correlations and path coefficient analysis for yield and yield component traits in some KAFACI doubled haploid and other rice genotypes. Grain yield showed significant positive correlation with number of productive tillers per plant and only positive in panicle length and spikelets per panicle. Path Coefficient analysis revealed that grain yield per plant was directly influenced by the characters like days to maturity, panicle length and number of spikelets per panicle.

Begum *et al*. (2021) carried out an investigation to understand the interrelationship and degree of dependence of grain yield on its components and gall midge incidence. Correlation coefficient analysis suggested that grain yield per plant had

significant and positive association with panicle length, number of grains per panicle and number of productive tillers per plant and negative non-significant association with gall midge incidence. Panicle length and number of grains per panicle had positive direct effect on grain yield per plant. Gall midge incidence showed a negative direct effect at the phenotypic level and a positive direct effect at the genotypic level on the grain yield per plant.

Character association and path analysis for yield and yield component traits in some maintainer and restorer line of rice were studied by Jasmine *et al*. (2022). The study revealed that, there was a significant positive correlation between Fe and Zn content, indicating the improvement of both mineral elements could be achieved simultaneously. A positive significant correlation of grain yield per plant were shown with thousand grain weight indicating that yield can be improved through improvement of this trait. The traits thousand grain weight, number of spikelets per panicle, number of productive tillers per plant and grain iron concentration had a high positive direct effect as revealed by path analysis.

Chapter III

Material and Methods

Chapter III

MATERIAL AND METHODS

The present investigation entitled **"**Genetic analysis and identification of genotypes with novel sources of resistance for gall midge (biotype 3) in rice *Oryza sativa* L.*)"* was carried out during *kharif, 2021* and *rabi, 2021-22* at Regional Agricultural Research Station, Professor Jayashankar Telangana State Agricultural University, Polasa, Jagtial to identify the gall midge (Biotype 3) resistant lines through field screening and confirmation at molecular level and also to identify the diverse parental lines based on genetic diversity and to estimate correlation, direct and indirect effects of various yield contributing traits on yield.

The experimental site is located at an altitude of 243.4 m above mean sea level on 18º49'40" N latitude and 78º56'45" E longitudes in Northern Zone of Telangana State and was quite uniform in respect of topography and fertility with clay loamy soil. The experiment was irrigated with open well source located adjacent to the experimental site with proper drainage facility.

3.1 Experimental material

The experimental material for the present study includes 42 entries comprised of land races, IRRI lines, released varieties and advanced breeding lines along with susceptible check (TN1) and resistant check (Aganni) for gall midge screening studies and genetic analysis. The experimental material was obtained from Regional Agricultural Research Station, Polasa, Jagtial and details are furnished in appendix 3.1.

3.2 Methods

3.2.1 Identification of gall midge resistant lines through field screening

The experimental material for field screening against gall midge (Biotype 3) was sown on delayed situations *i.e.,* on 17. 07. 2021 which favours more incidence of gall midge under natural conditions. Test entries were transplanted in the main field at 34 days after sowing and each entry was transplanted in two rows of 20 hills per row with spacing of 20×15 cm. Susceptible check (TN 1) was planted as border row around the experimental material for increasing the gall midge pest load. All the recommended agronomic practices were followed to raise the crop. No plant protection measures were followed to allow the pest population build up under natural conditions.

3.2.1.1 Scoring of Damage Symptoms

The test entries were scored for plant damage at 30 and 50 days after transplanting (DAT). Observations were recorded on total number of plants, number of damaged plants (with silver shoots), total number of tillers and number of silver shoots. Per cent damaged plants with silver shoots and percent silver shoots were calculated. Scoring was done as per Standard Evaluation System (SES) (IRRI, 2013) (Table 3.1.).

Table 3.1. Standard Evaluation System (SES) for rice gall midge (IRRI, 2013)

Score	Percent silver shoot damage	Category
0	No damage	Highly Resistant
1	< 1	Resistant
3	1-5	Moderately Resistant
5	6-10	Moderately Susceptible
7	11-25	Susceptible
9	> 25	Highly Susceptible

$$\text{Per cent damaged plants(\%DP)} = \frac{\text{Damaged plants}}{\text{Total number of plants}} \times 100$$

$$\text{Per cent silver shoots(\%SS)} = \frac{\text{Number of silver shoots}}{\text{Total number of tillers}} \times 100$$

3.2.2 Confirmation of gall midge resistant lines through molecular confirmation using SSR markers

Molecular screening was carried out for selected samples (those which had nil galls) of each line using functional SSR markers to identify allelic status of target gene in these plants. The details of the markers employed for molecular analysis are mentioned in Table 3.2.

Table 3.2. Details of markers used for molecular analysis

Gene	Marker	Sequence of Marker	Resistant allele (bp)	Susceptible allele (bp)	References
gm3	gm3del3	F-5'CTGCCAGAGATGGGCCTTCCA3' R-5'CGTACAAATTCCTGTACCACTC3'	250	550	Sama *et al*. (2014)
Gm4	LRR-del	F-5'GTGGATCGAGAGAAGACAAG3' R-5'CTTGAGGACGATATTCAAGC3'	350	600	Divya *et al*. (2015)
Gm8	PRP	F-5'TCATGTTGTGCAGATCAACC3' R-5'AGCCATATGAAAACCACCAA3'	300	350	Divya *et al*. (2013)

3.2.2.1 Preparation of stocks and buffer solutions

a) ***1M TrisHCl (pH 8.0)****:* Tris base of 30.28 g was dissolved in 200 ml distilled water. The pH was adjusted to 8.0 with 10.5ml of concentrated HCl and the solution was cooled to room temperature. After cooling, the final volume was made to 250ml with distilled water and the solution was sterilized by autoclaving at 121^0C temperature and 15psi pressure for 20 minutes and stored in a transparent reagent bottle.

b) ***0.5M EDTA (pH 8.0):*** Disodium Ethylene Diamine Tetra Acetate (EDTA) of 46.53g was added to 200ml of distilled water and the pH adjusted to 8.0 by adding 4g of NaOH pellets and stirred vigorously on a magnetic stirrer. The total volume was adjusted to 250ml with distilled water and sterilized by autoclaving and stored in transparent reagent bottle.

c) ***5M NaCl:*** 73.05g of NaCl was dissolved in 200ml of distilled water and the final volume made upto 250 ml with distilled water. It was sterilized by autoclaving and stored at room temperature in transparent reagent bottle.

d) ***2% CTAB:*** 2g of Cetyl Trimethyl Ammonium Bromide (CTAB) was dissolved in 100ml of sterile distilled water and stored at room temperature.

e) ***Tris EDTA (TE) Buffer:***

TrisHCl (pH 8.0) : 10mM
EDTA : 1mM

f) ***Ice-cold Isopropanol***

g) ***Chloroform: Isoamylalcohol*** (24: 1 V/V)

h) ***3M Sodium acetate*** (pH 5.2 adjusted using glacial acetic acid)

i) ***Ethanol*** (100% and 70%)

j) ***Polyvinylpolypyrrolidine*** (PVP).

k) *Extraction buffer*

Table 3.3. Details of buffer components

Component	Stock concentration	Working concentration	Volume/quantity of stock solution/chemical required to prepare for 100ml working solution
Tris- HCl (pH 8.0)	1M	100 mM	10ml
EDTA (pH 8.0)	0.5M	20mM	4ml
NaCl	5M	1.4M	28ml
PVP		3%	3g
CTAB		2%	2g
Distilled water	To make up the final volume		
Total			100ml

Note: 0.1% v/v Mercaptoethanol was added just before the use

3.2.2.2 Leaf sample collection and isolation of genomic DNA

Leaf samples of test lines were collected from the plants grown under field conditions at RARS, Jagtial during *rabi*, 2021-22; for isolation of genomic DNA by following the protocol of Zheng *et al.* (1995)

This protocol is useful when small quantities (~1 µg) of DNA is to be isolated rapidly. There will be a slight shearing (breakage) of DNA, but the quality obtained is sufficient for the most common PCR applications. The turnaround time (time required for completion) is under 4 hours.

1. Healthy leaf sample (2 cm long) was collected in a sterile microcentrifuge tube (1.5 ml), labelled with the plant number/variety name and placed on ice.
2. In the laboratory, the leaf tissue was cut into 0.5 cm long segments and placed in a well of Spot Test Plate (Thomas Scientific).
3. 300 µl of DNA extraction buffer was added immediately using micropipette (1000 µl capacity) and grinded the tissue with a thick glass rod till the buffer turns dark green (a sign of cell breakage and release of chlorophyll).

4. 300 µl more of DNA extraction buffer was added, mixed and transferred the entire content in the well into the original 1.5 ml micro centrifuge labeled with the plant number/variety name.
5. 600 µl of chloroform was added, mixed well and spin for about 15 min at 13,000 rpm in a micro centrifuge. The top (aqueous) phase (~500-600 µl) was transferred to another 1.5 ml labeled micro centrifuge tube using a 200 µl or 1000 µl capacity micropipette using cut tips (tips cut at the bottom using sterile scissors).
6. Equal volume of chilled isopropanol was added and mixed well. Spined for 10 min at 10,000 rpm. DNA settled at the bottom as a pellet after centrifugation.
7. To the pellet 200 µl of 70% ethanol (70% EtOH and 30% sterile double distilled water, V/V) was added, centrifuged at 10,000 rpm for 5 min and then the supernatant was discarded. The micro centrifuge tubes containing the DNA pellet was kept open at room temperature (air drying) for about 1-2 hours.
8. After the pellet was completely dried (with no smell of alcohol coming from the tube), resuspended the DNA pellet in 100-200 µl of TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH 8.0 prepared using sterile double distilled water). The DNA was stored at -20^0C.
9. 2-3 µl of DNA (25 – 50 ng) was used per PCR reaction.

3.2.2.3 DNA Extraction procedure

Total genomic DNA was isolated from the plant materials using the CTAB method.

3.2.2.4 Quantification of DNA by Agarose Gel Electrophoresis

The DNA extracted was quantified on 1% agarose gels in a submarine horizontal electrophoresis unit Major Science (MS). Agarose (2g) was weighed and transferred into a reagent bottle containing 200 ml 1X TAE (Tris Acetate EDTA) buffer and mixed well. The content was then boiled gently in a microwave oven with intermittent mixing. The process was followed until the agarose melts completely and the solution became clear. In the meanwhile, the gel-casting tray was washed with water and wiped with ethanol. Then, it was sealed with cello tape. The agarose gel solution was cooled down to bearable temperature (~40^0C) and ethidium bromide (5µl/50ml) was added to it. The content was mixed thoroughly and poured into the gel-casting tray, where the combs were arranged in the slots on the gel-casting tray. The gel was allowed

to solidify at room temperature for 20-30 min. Care was taken so that no air bubble was present.

Later, the gel was transferred to the electrophoresis unit containing 1X TAE buffer. DNA samples were mixed with 1/6th volume of gel loading dye (40% sucrose with 0.25% bromophenol blue) and loaded into the wells using pipette. The electrodes were connected to power pack and samples were run at 60V for 1hr. Un-cut λ DNA (100ng/µl) diluted to 100ng, 200ng, 300ng, 400ng and 500ng concentrations and loaded in the first five wells to determine the DNA concentration in the samples. The gel was visualized under UV Transilluminator and documented in gel documentation system (Syngene International Ltd., USA). Based on the intensity and thickness of genomic DNA bands when compared with λ DNA the concentration and quality of individual DNA samples was determined. Finally, all samples were adjusted to a uniform concentration of approximately 5ng/µl using sterile water for further processing.

3.2.2.5 Polymerase Chain Reaction (PCR)

The genomic DNA was subjected to PCR amplification as per the standardized procedure. PCR was carried out using a programmable thermocycler. The PCR conditions used were as follows (Table 3.4 and 3.5).

Table 3.4. Components of PCR reaction

S.No.	PCR Components	Concentration	Volume
1	Genomic DNA	50-100 ng/µl	3.0 µl
2	Forward primer	5 Pico moles	0.5µl
3	Reverse primer	5 Pico moles	0.5µl
4	Buffer and $MgCl_2$	10 X	1.0 µl
5	dNTPs	2.5 mM	0.4 µl
6	Taq DNA polymerase enzyme	5U/µl	0.1µl
7	Sterile distilled water	-	2.0µl
	Total volume		**10.0 µl**

Table 3.5. PCR temperature regime

S. No.	Steps	Temperature	Duration	Cycles
1	Initial denaturation	94℃	5 minutes	1
2	Denaturation	94℃	30 seconds	35
3	Annealing	55℃	30 seconds	
4	Elongation	72℃	1 minute	
5	Final extension	72℃	7 minutes	1
6	4℃	-	∞	-

The PCR products were stored at 4℃ for short period and at -20℃ for long duration.

3.2.2.6 Agarose Gel Electrophoresis of PCR Products

The PCR products were analyzed by electrophoresis using an agarose gel. The percentage of the Agarose gel depends on the size of the DNA fragments, because higher concentrations of agarose facilitate separation of small DNA fragments, while low agarose concentrations allow resolution of larger DNA fragments. In the present study, 1.2% Agarose gel was used for gm3del3 and LRR-del markers and 3% Agarose gel was used for Proline Rich Protein (PRP) marker (due to only 50 bp difference in resistant and susceptible allele).

The materials used were:

- ✓ Tris acetate buffer (TAE) – 1X (Tris base, Acetic Acid, 0.5 M EDTA)
- ✓ Agarose (SeaKem LE Agarose, Lonza company)
- ✓ Gel loading dye (40% Sucrose, 0.25% Bromo phenol Blue, 0.25% xylene cylon)
- ✓ Ethidium Bromide solution (5 µl/50ml)

Procedure:

- 2.4 g of agarose was dissolved in 300 ml of 1X TAE buffer and was melted in a microwave oven.
- And then poured into a gel cast fixed with appropriate gel combs and was allowed to solidify at room temperature for 1 hr.
- Later the gel was transferred into a horizontal electrophoresis unit (Thermo Scientific company) containing 1X TAE buffer solution.

- Before loading the PCR product in the wells of Agarose gel; the PCR amplified products were mixed with 3µl of loading dye.
- Then the mixture (PCR product +loading dye) was loaded into each well of the gel along with 100bp DNA ladder (Fermentas Company) in the first or last well and run at a constant voltage of 80 volts for 1.5 hrs - 2.0 hrs.

3.2.2.7 Gel Documentation

After completion of the gel run, the gels were visualized in a UV gel documentation system (Syngene, USA) for analysis of DNA bands. The gel was exposed to UV light for about 20 seconds until the bands appeared clearly. Then, the gel image was captured and saved.

3.2.3 Genetic analysis

The experiment for genetic analysis studies was carried out at RARS, Polasa, Jagtial (Plate 3.1) during *rabi, 2021-22*. 30 days age old nursery of 42 entries was transplanted in randomized block design. Each genotype was planted in three rows of 4m length with three replications. A spacing of 20 cm between rows and 15 cm between plants were maintained. The normal recommended package of practice was followed for raising good and healthy crop.

Plate 3.1. Satellite view of experimental site

3.3 Observations

Observations were recorded on the five randomly selected plants except for days to 50% flowering (recorded on plot basis) from each entry in every replication for the statistical analysis. The plants were selected from middle row, excluding border plants to minimize the border effect. Observations were recorded on following yield and yield contributing trains.

Observations recorded on yield and yield contributing characters:

3.3.1 Days to 50% Flowering

Number of days counted from the date of sowing in nursery to the day on which 50 % of the plants initiate flowering in a genotype was recorded as days to 50 % flowering.

3.3.2 Plant height(cm)

The plant height was measured in centimeters from the ground level to the tip of tallest panicle (awn excluded) of the mother tiller, at the time of the maturity.

3.3.3 Panicle length(cm)

Panicle length was measured in centimeters from panicle base (neck node of the panicle) to the tip at the upper most spikelet, excluding awns.

3.3.4 Number of productive tillers per plant

Out of the total number of tillers per plant, number of panicles bearing tillers in plants was counted at maturity.

3.3.5 Number of grains per panicle

Presence of endosperm in spikelet was considered as filled grains and total number of filled grains in five random panicles were counted and averaged.

3.3.6 1000 grain weight(g)

One thousand grains were counted from a random sample of each entry in each replication weighed on an electronic balance. The weight of 1000-grain of rice were recorded and expressed in gram (g).

3.3.7 Grain yield per plant(g)

Five randomly selected plants in each entry were harvested at maturity, threshed, cleaned and dried to 12-14 per cent moisture content individually. The grains so obtained were weighed in the grams and mean was worked out.

3.3.8 Hulling (%)

100g of paddy weighed and subjected to dehusking in a standard dehusker/sheller. After cleaning, the dehusked kernels (brown rice) are weighted and hulling (%) was determined with the following formula.

$$\text{Hulling (\%)} = \frac{\text{Weight of Dehusked kernel (g)}}{\text{Weight of Paddy (g)}} \times 100$$

3.3.9 Milling (%)

Dehusked kernels (brown rice) are then put into a standard polisher/miller. Total polished kernels (white rice) are weighed and then milling (%) was calculated by the following formula.

$$\text{Milling (\%)} = \frac{\text{Weight of polished kernel (g)}}{\text{Weight of paddy (g)}} \times 100$$

3.3.10 Head rice recovery (%)

It is the percentage of full rice kernel (excluding broken grains) obtained from a sample of paddy after milling and polishing. The milled rice obtained from the rice miller was separated into broken and non-broken in a sizing device and head rice recovery was calculated as the ratio of non-broken to the initial weight of the paddy sample and expressed as percentage (%).

$$\text{Head rice recovery (\%)} = \frac{\text{Weight of whole polished grain (g)}}{\text{Weight of paddy (g)}} \times 100$$

3.3.11 Kernel length (mm)

Ten dehusked paddy grains were selected randomly from each replication and kernel length was measured by using dial micrometer and expressed in mm.

3.3.12 Kernel breadth (mm)

Ten dehusked paddy grains were selected randomly in each replication and the breadth was recorded in millimeters using dial micrometer.

3.3.13 L/B ratio

The kernel L/B ratio was calculated by using the following formula. Ten randomly selected filled kernels were used.

$$\text{L/B ratio} = \frac{\text{Mean length of kernel (mm)}}{\text{Mean breadth of kernel (mm)}}$$

3.4 Statistical Analysis

The experimental data were compiled by taking mean value over randomly selected plants for all the three replications and subjected to following statistical analysis:

3.4.1 Analysis of variance for the design of experiment

3.4.2 Selection parameter

A. Mean and range
B. Phenotypic and genotypic coefficient of variance
C. Heritability
D. Genetic advance

3.4.3 Genetic divergence (D^2) analysis

3.4.4 Correlation and path analysis

3.4.1 Analysis of variance (ANOVA)

The mean of five plants of each entry in each replication for all the studied characters were used for analysis of variance following Panse and Sukhatme, (1985). Analysis of variance for each quantitative trait was carried out on the basis of mean values for partitioning of total variance into variance due to replication, treatment and error. The test of significance of difference between replications and among the genotypes was done by F-test.

The model for analysis of variance is given below:

$$\mathbf{Y_{ij} = \mu + g_i + r_j + e_{ij}}$$

Where,

Y_{ij} = Phenotypic observation in the i^{th} Genotype in the j_{th} Block

μ = Grand Mean

g_i = Effect of i^{th} Genotypes

r_j = Effect of j^{th} Replication

e_{ij} = Random Error

Here,

i = 1,2,3…….. 42.

j = 1,2,3.

Table 3.6. Analysis of variance (ANOVA)

Source	d.f	SS	MSS	F value (Calculated)
Replication	r-1	RSS	RSS/(r-1) = RMS	RMS/EMS
Treatment	t-1	TrSS	TrSS/ (t-1) = TMS	TMS/EMS
Error	(r-1) (t-1)	ESS	ESS/(r-1) (t-1) = EMS	
Total	(rt-1)	TSS		

Where,

r = No. of replication

t = No. of treatments

d.f = Degree of freedom

RSS = Replication sum of square

TrSS = Treatment sum of square

ESS = Error sum of square

TSS = Total sum of square

RMS = Replication mean sum of square

TMS = Treatment mean sum of square

EMS = Error mean sum of square

The test of significance of difference between replications and among the genotypes was done by comparing the calculated "F" value with the table value of F at 5 and 1 percent value of significance at (r-1) and (r-1) (t-1) degree of freedom respectively.

3.4.2 Selection parameter

A. Mean and range

Mean

The mean value of each character was determined as follows:

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

Where,

$\bar{X}$ = Mean

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

N = Number of observations

Range

Lowest and highest value of each trait were taken as range.

Estimation of genetic parameter

B. Genotypic and phenotypic variance:

These are obtained as below:

Error variance, σ^2_e = M_e

Genotypic variance, σ^2_g = $\frac{M_t - M_e}{r}$

Phenotypic variance, σ^2_p = $\sigma^2_g + \sigma^2_e$

Standard error of difference between two means [S.E.d.(M)]

S.E.d.(M) was calculated as follows:

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

Where,

r = Number of replications

EMS = Mean sum of square due to error

Coefficient of variation (CV)

Phenotypic and genotypic coefficient of variation was calculated by the method suggested by Burton and Devane (1953):

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

= $\frac{\sigma_p}{\bar{X}} \times 100$

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

= $\frac{\sigma_g}{\bar{X}} \times 100$

Table 3.7. Scale for PCV and GCV were classified by Robinson *et al.* (1949)

S. No.	Value of GCV and PCV (percent)	Scale
1.	0 to 10	Low
2.	10 to 20	Moderate
3.	More than 20	High

C. Heritability(h^2):

Heritability is an index of transmission of character from one generation to next generation. The ratio of genotypic variance to phenotypic variance or total variance expressed in percentage is called as the broad sense heritability. It was calculated by the formula given by Johnson *et al.* (1955) as follows:

$$h^2\ (\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

h^2 = Heritability (Broad sense)

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance or total variance

Table 3.8. Scale for heritability were classified by Robinson *et al.* (1949)

S. No.	Value of heritability in broad sense (%)	Scale
1.	0 to 30	Low
2.	30 to 60	Medium
3.	More than 60	High

D. Genetic advance (GA)

It is measure of genetic gain under selection. Genetic advance for each character was calculated by using the formula (Allard, 1960):

$$\textbf{Genetic advance (GA)} = \mathbf{H} \times \sqrt{\sigma_p^2} \times \mathbf{k}$$

Where,

H = Heritability coefficient

$\sqrt{\sigma_p^2}$ = Phenotypic standard deviation

K = Selection differential in standard units which is 2.06 at 5% selection intensity

Genetic advance as percent of mean was calculated by following formula:

$$\text{GA as per cent of mean} = \left[\frac{\text{GA}}{\bar{\text{X}}}\right] \times 100$$

Where,

GA = Expected genetic advance

$\bar{X}$ = General mean of characters

Table 3.9. Scale for genetic advance as per cent mean were classified by Johnson *et al.* (1955)

S. No.	Value of genetic advance (%)	Scale
1.	Less than 10	Low
2.	10-20	Medium
3.	Above 20	High

3.4.3 Genetic divergence by Mahalanobis D^2– Statistics

The data collected on different characters was analyzed using Mahalanobis' D^2 analysis to determine the genetic divergence among the genotypes (Mahalanobis, 1936). D^2 value between i^{th} and j^{th} genotypes for 'P' characters was calculated as follows:

$$\mathbf{D^2_{ij}} = \sum_{\mathbf{t=1}}^{\mathbf{P}} \left(\mathbf{Y_i^t} - \mathbf{Y_j^t}\right)^{\mathbf{2}}$$

Where,

Y_i^t = uncorrelated mean value of i^{th} genotype for 't' characters

Y_j^t = uncorrelated mean value of j^{th} genotype for 't' characters

D^2_{ij} D^2 between i^{th} and j^{th} genotypes.

The various steps involved in estimation of D^2 values are given below

3.4.3.1 Test of significance

Variances were calculated for all the characters investigated and test of significance was done. Analysis of covariance for the character pairs was estimated on the basis of mean values (Panse and Sukhatme, 1967). After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences in the mean values of a number of correlated variables with regard to the pooled effect of characters was carried out using 'V' statistic, which in turn utilizes Wilk's criterion. The sum of squares and sum of products of error and error + variety, variance and covariance matrix were used for this purpose. The estimation of Wilk's criterion was done using the following relationship.

$$\hat{} = \frac{(E)}{(E+V)} = \frac{\text{Determinant of error matrix}}{\text{Determinant of error + Variety matrix}}$$

Where,

^ = Wilk's criterion

(E) = Determinant of error matrix and

(E+V) = Determinant of error + variety sum of squares and sum of product matrix

The significance of '^' was tested by

$$\chi^2 pq = V_{(Stat)} = -m \log_e \hat{} = -[n-(p+q+1)/2] \log_e \hat{}$$

Where,

m = n-(P+Q+1)/2

n= Degrees of freedom for error + varieties

$\log_e \hat{} = 2.3026 \log_{10} \hat{}$

P = Number of variables or characters

Q = Number of variables – 1(or d.f for varieties)

V (Stat) can be approximately modelled as χ^2pq, and if the derived 'V' value from the formula exceeds χ^2pq'K', the hypothesis is rejected at the K level of significance; otherwise it is not.

3.4.3.2 Transformation of correlated variables

In the present model, computation of D^2 values were reduced to simple summation of the differences in the mean values of various characters of the two genotypes i.e., $\sum d^2$. Therefore, transformation of the correlated variables into uncorrelated ones was done before working out the D^2 values. Transformation was done using pivotal condensation method.

3.4.3.3 Computation of D^2 values

For the given combination of i and j genotype, the mean deviation i.e., Y_i^t-Y_j^t for t=1,2…p variables are computed and the D^2 values were calculated as:

$$D^2_{ij} = \sum_{t=1}^{P} \left(Y_i^t - Y_j^t\right)^2$$

where,

Y_i^t uncorrelated mean value of i^{th} genotype for 't' characters

Y_j^t uncorrelated mean value of j^{th} genotype for 't' characters

D^2_{ij} D^2 between i^{th} and j^{th} genotype

3.4.3.4 Testing the significance of D^2 values

The D^2 value obtained for a pair of population is taken as calculated value of χ^2 and is tested against the tabulated value of χ^2 for P degrees of freedom where P is the number of characters considered.

3.4.3.5 Contribution of individual characters towards divergence

In all combinations, each character was ranked on the basis of its contribution towards divergence between two entries ($d_i = Y_i^t - Y_j^t$). Rank 1 is given to the highest mean difference and the rank P to the lowest difference, where, P is the total number of characters. Percentage contribution towards genetic divergence was calculated using the following formula.

$$\text{Percentage contribution of the character} = x = \frac{N \times 100}{M}$$

Where,

N = Number of genotype combinations where the character was ranked first

M = All possible combinations of number of genotypes considered

3.4.3.6 Grouping of genotypes into various clusters

The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1952). The criterion was that the two varieties belonging to the same cluster at least on an average show a smaller D^2 value than those belongingto different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described by Singh and Chaudhary (1977). To start with, two populations having the closest distance from each other were considered, to which the third population having the smallest D^2 value from the first two populations was added. Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage when it was felt that after adding a particular population there was an abrupt increase in the average D^2, that population was not considered for including in that cluster. The genotypes of the first cluster were then eliminated and the rest were treated in a similar way. This procedure was continued till all the genotypes were included into one or other cluster.

3.4.3.7 Average intra- cluster distance

For the measurement of intra-cluster distances, the formula used was $\Sigma D_i^2/n$ where, ΣD^2_i was the sum of distances between all possible combinations (n) of the populations included in a cluster.

3.4.3.8 Average inter- cluster distance

Clusters were taken one by one and the distances from other clusters were calculated. The distance between two clusters was the sum of D^2 values between the members of one cluster to each of the members of the other clusters divided by the product of number of genotypes in both the clusters under consideration.

$$\text{Average inter-cluster distance} = \frac{D^2}{n_1 \times n_2}$$

Where,

n_1 and n_2 are number of genotypes of two clusters.

D^2 = Difference in mean value between two populations when all the characters are considered simultaneously

Category	'D' Values
Closely related	<22
Moderately divergent	22 to 30
Highly divergent	>30

3.4.4 Correlation and Path analysis

Correlations

Phenotypic and genotypic correlations were worked out by using the formulaesuggested by Falconer (1964).

Phenotypic coefficient of correlation (r_p)

$$r(x_i.x_j)p = \frac{Cov(x_i x_j)p}{\sqrt{\sigma^2(x_i)_p.\sigma^2(x_j)_p}}$$

Where,

$r(x_i.x_j)p$ = Phenotypic correlation between i^{th} and j^{th} character

$Cov(x_i.x_j)p$ = Phenotypic covariance between i^{th} and j^{th} character

$\sigma^2(x_i)p$ = Phenotypic variance of i^{th} character

$\sigma^2(x_j)p$ = Phenotypic variance of j^{th} character

Genotypic coefficient of correlation (r_g)

$$r(x_i.x_j)g = \frac{Cov(x_i x_j)g}{\sqrt{\sigma^2(x_i)_g.\sigma^2(x_j)_g}}$$

Where,

$(x_i.x_j)g$ = Genotypic correlation between i^{th} and j^{th} character

$Cov(x_i.x_j)g$ = Genotypic covariance between i^{th} and j^{th} character

$\sigma^2(x_i)g$ = Genotypic variance of i^{th} character

$\sigma^2(x_j)g$ = Genotypic variance of j^{th} character

Z transformation for genotypic correlation

Since the t table can be used only for testing the null hypothesis $p = 0$. It is unsuited for testing other null hypothesis, such as $p = 0.5$, or p1 =p2, or for making confidence statements about p. when $p \neq 0$ the shape of the distribution of r changes, becoming skew.

A solution to these problems was quoted by Snedecor and Cochran (1981) who devised a transformation from r to a quantity z, distributed normally with standard error.

$$\sigma_z = \frac{1}{\sqrt{n-3}}$$

Practically independent of the value of the correlation in the population from which the sample is drawn, the relation of r to z is given by

$$Z = 1/2[\log_e (1+r) - \log_e (1+r)]$$

Test of significance

Significance of correlation coefficients was tested by comparing phenotypic correlation coefficients with the table values (Fisher and Yates, 1963) at (n-2) degrees of freedom at 5% and 1% level where 'n' denotes the number of paired observations used in the calculation.

Path coefficient analysis

Path coefficient analysis, suggested by Wright (1921) and elaborated to Dewey & Lu (1959) was used to calculate the direct and indirect contribution of various traits to yield.

For estimation of various direct and indirect effects, a set of simultaneous equations were formed:

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

$$r_{2y} = r_{12} P_{1y} + P_{2y} + r_{23} P_{3y} + \ldots\ldots + r_{2n} P_{ny}$$

.

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

Where,

1,2,3…….n	=	Independent variables
y	=	Dependent variable
$r_{1y}, r_{2y}……. r_{ny}$	=	Coefficient of correlation between casual factors '1' to 'n' on dependent character y.
$P_{1y}, P_{2y} …… P_{ny}$	=	Direct effect of character 1 to n on character y

Indirect effects were calculated as :

The indirect effect of the X_1 variable *via* X_2 variable	=	$r_{12}P_{2y}$
The indirect effect of the X_1 variable *via* X_3 variable	=	$r_{13}P_{3y}$
The indirect effect of the X_1 variable *via* X_n variable	=	$r_{1n}P_{ny}$

Residual effect

In plant breeding, it is very difficult to have complete knowledge of all component traits of yield. The residual effect permits precise explanation about the pattern of interaction of other possible components of yield. In other words, residual effect measures a role of other possible independent variables which were not included in the study on the dependent variable. The residual effect estimated with the help of direct effects and simple correlation coefficients.

$$Pry = \sqrt{1 - (P_{1y}.r_{1y} + P_{2y}.r_{2y} + \ldots\ldots\ldots\ldots + P_{ny}.r_{ny})}$$

where,

Pry	=	Residual effect
P_{ny}	=	Direct effect of x_n on y
r_{ny}	=	Correlation coefficient of x_n on y.

Table 3.10. The Scales for path coefficients (Lenka and Mishra, 1973)

Value for Direct or indirect effect	Rate
0.00-0.09	Negligible
0.10-0.19	Low
0.20-0.29	Moderate
0.30-0.99	High
>1.00	Very high

Chapter IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

Results of the present investigation entitled **"GENETIC ANALYSIS AND IDENTIFICATION OF GENOTYPES WITH NOVEL SOURCES OF RESISTANCE FOR GALL MIDGE (BIOTYPE 3) IN RICE *(Oryza sativa* L.*)"*** are presented below, wherein 42 rice entries comprised of land races, released varieties and advanced breeding lines along with susceptible check (TN1) and resistant check (Aganni) were screened. The entries for genotyping were selected based on field screening against Gall Midge Biotype 3 (GMB3) at Regional Agricultural Research Station (RARS), Polasa, Jagtial during *kharif,* 2021. These lines were further genotypically characterized by using *gm3, Gm4* and *Gm8* based functional SSR markers for presence or absence of resistant gene(s). Further all the 42 entries were subjected to genetic divergence studies, correlation and path analysis. The findings of the present investigation are presented under the following sub headings.

4.1 Screening of rice genotypes under field condition against Gall Midge Biotype 3.

4.2 Molecular confirmation for presence of *gm3, Gm4* and *Gm8* genes using functional SSR markers.

4.3 Analysis of variance.

4.4 Mean performance.

4.5 Phenotypic and genotypic coefficient of variance (GCV and PCV), Heritability (h^2) and Genetic advance as percent of mean.

4.6 Genetic divergence (D^2) studies among yield and yield component traits analysis.

4.7 Correlation studies.

4.8 Path coefficient analysis.

4.1 Screening of rice genotypes under field condition against Gall Midge Biotype 3

Rice gall midge is one of the major pests of rice in Jagtial, Telangana. The 42 test entries were screened and assessed their reaction against gall midge biotype 3 as per the standard evaluation system of IRRI (SES, 2013) by recording the damage score at 50 DAT (Appendix 4.1). In the 42 test entries screened against rice gall midge, per cent

of silver shoots ranged from 0 (Kakai, WGL-1145, WGL-1147 and WGL-1127) to 84.18 (IRBL-T-K59). “Nil” damage was noticed in four entries *viz.,* Kakai, WGL-1145, WGL-1147 and WGL-1127 followed by RP-5332-54-11-8-2-13 and IR72476-B-P-9-3-1-1 which recorded 0.52% and 0.65 % of silver shoots respectively at 50 DAT. Remaining entries showed more per cent of silver shoots ranged from 9.29 % (IR-74101-3R-1-1) to 84.18 % (IRBL-T-K59). Between the checks, the resistant check, Aganni had shown 0% silver shoots at 30 DAT and 50 DAT exhibited highly resistance reaction whereas, the susceptible check TN 1 had recorded 9.25% and 79.09% sliver shoots at 30 DAT and 50 DAT respectively and found to be highly susceptible to gall midge biotype 3.

Based on reaction of resistant and susceptible checks against gall midge biotype 3, the 4 entries *viz.,* Kakai, WGL-1145, WGL-1147 and WGL-1127 were identified as highly resistant (Score 0) cultures and IR72476-B-P-9-3-1-1 and RP-5332-54-11-8-2-13 as resistant (Score 1). The entry IR-74101-3R-1-1 (Score 5) was found to be moderately susceptible, whereas, three entries *viz.,* Dular, HH-25-SAL-10-D2-DT-1 and Laicha were found susceptible (Score 7). The remaining entries (30) were identified as highly susceptible cultures (Score 9).

The gall midge resistant lines *viz.,* Kakai, WGL-1145, WGL-1147, WGL-1127, IR72476-B-P-9-3-1-1 and RP-5332-54-11-8-2-13 could be used as donor in breeding program for the development of gall midge resistant varieties after their further screening under field conditions over the seasons or can be directly released as varieties based on their performance in yield trials over the seasons and locations.

Similar screening work was carried out by Seni and Naik (2019) with 137 rice germplasm against rice gall midge. Among 137 rice entries screened, the entries *viz.,* WGL-1164, WGL-1127, RP-5925, RP 1, INRC 3021, IBT R4, IBT GM IBT GM 1, IBT GM 2, IBT GM 3, IBT GM 4, IBT GM 7, IBT GM 9, IBT GM 11, IBT GM 12, IBT GM 13, IBT GM 16, IBT GM 17, IBT GM 18, IBT GM 19, IBT GM 20, IBT GM 21, IBT GM 22, IBT GM 23, IBT GM 25, IBT GM 26, IBT GM 27, IBT GM 28, IBT GM 29, IBT GM 30, IBT GM 31, IBT GM 32, IBT GM 33, IBT GM 34, IBT GM 35, IBT GM 36, IBT GM 37, IBT GM 38, IBT GM 39, IBT GM 40, IBT GM 41, IBT GM 42 and IBT GM 46 exhibited highly resistant against gall midge.

Sumathi and Manickam (2013) screened 45 GMS cultures, of which RP 4683-29- 2-645, RP 4683-30-1-648, RP 4686-49-1-943, RP 4687-52-2-1197, RP 4688-53-2-1258, RP 4688-53-2-1259, JGL17025, JGL17183, JGL17187, JGL17189, Kavya,

JGL17190, JGL17196, JGL 17198, JGL17211 and JGL17221 did not record any damage against gall midge biotype 3 at Tirur, Tamil Nadu.

Kumar *et al.* (2020) screened 173 entries against gall midge and identified IBT MRR 18, IBT MRR 23 and IBT MRR 24 as highly resistant and six entries *viz.,* IBT MRR 17, IBT MRR 19, IBT MRR 20, IBT MRR 21, IBT MRR 22 and IBT MRR 28 as resistant reaction against gall midge.

After extensive research of host-plant differentials it was found that biotype 3 is present in Jagtial district of Telangana State. Beside this biotype 3 was also present in Ranchi (Jharkhand) and Wangbal (Manipur) (Seni *et al.,* 2017). Although gall midge biotype 3 is present at Jagtial but from last few years it infested RP 2068 variety containing *gm3* gene and Abhaya variety containing *Gm4* gene so, possibility of another biotype could not be excluded.

Sources of resistance that have been identified can be further investigated for known genes. It is crucial to characterize these donors that have consistently shown resistant reactions in order to identify the genes giving resistance using the functional SSR markers that have already been established. This will allow the proper donors for the resistance breeding programme to be selected. These resistant lines have a better potential to be used as donors in the rice breeding programme for the development of gall midge resistant varieties as well as for monitoring virulence in gall midge populations. In different rice cultivars, the use of molecular markers for the improvement of gene pyramids in the desired combination is now being investigated, and DNA markers for the selection of resistant plants for gene pyramiding has been accepted as an established tool. (Sundaram *et al.,* 2008; Suvendhu *et al.,* 2014).

4.2 Molecular confirmation for presence of *gm3, Gm4* and *Gm8* genes using functional SSR markers

Molecular screening for gall midge resistance was done for those lines which were found resistant phenotypically under field screening. Genotyping was done for knowing the presence of already reported gall midge resistance genes (g*m3, Gm4 and Gm8)* or new resistance genes.

Four entries, *viz.,* Kakai, WGL-1145, WGL-1147 and WGL-1127 showed “nil” (score 0) damage against GMB3. Two entries namely, IR72476-B-P-9-3-1-1 and RP-5332-54-11-8-2-13 were found resistant (score 1) against GMB3. So, these six lines were used for genotyping for gall midge resistance with functional SSR markers.

For control of gall midge, development of resistant rice varieties using marker assisted selection can be a sustainable and cost-effective approach (Dutta *et al.,* 2014). Gene pyramiding with two or additional active genes in a single variety may lead to strong gall midge resistance rice varieties. Nowadays, the use of molecular markers for improvement of gene pyramids in desired combination is being monitored in different rice cultivars and presently using DNA markers for selection of resistant plants for gene pyramiding has been accepted as an established tool (Sundaram *et al.,* 2008; Dutta *et al.,* 2014).

4.2.1 gm3del3 functional marker for g*m3* gene:

gm3del3: This marker amplified a band size of 250 bp in the resistant source RP2068 and 550 bp in susceptible source TN1. Out of six genotypes, two genotypes, IR72476-B-P-9-3-1-1 and WGL-1145 were observed to be positives for *gm3* gene (Plate 4.1), when screened by using gm3del3 functional marker. The amplification pattern of the marker is in confirmation with Dutta *et al.* (2014) and Sama *et al.* (2014).

The functional marker, gm3del3 marker designed based on sequence polymorphism of the NB-ARC gene (candidate gene) (Sama *et al*., 2014) was used to confirm the presence of the *gm3* resistance gene in these test entries. The functional marker, gm3del3 showed an amplicon size of 550 bp for susceptible allele and 250 bp for resistant allele (Sama *et al*., 2014). This gene has been recently validated through RT-PCR studies (Divya, 2015). Similarly, gm3del3 marker has been used by Venkanna *et al*. (2018) to screen gene pyramided lines (*Gm1, gm3* and *Gm8*) for the presence of *gm3* gene.

4.2.2 LRR del functional marker for *Gm4* gene:

LRR del: This marker amplified a band size of 350 bp in the *Gm4* resistant source Abhaya and 600 bp in susceptible source TN1. Out of six genotypes, four genotypes namely, Kakai, WGL-1145, WGL-1147 and WGL-1127 were observed to be positives for *Gm4* gene (Plate 4.2), when screened by using LRR del functional marker. The amplification pattern of the marker is in agreement with Divya *et al.* (2014) and Dutta *et al.* (2014).

The functional marker, LRR-del, targeting the deletion region of the candidate gene (Divya *et al*., 2015) was used to confirm the presence of resistance gene, *Gm4* in these test entries. The *Gm4* functional marker LRR-del showed an amplicon size of 350 bp for resistant allele and 600 bp for susceptible allele (Divya *et al*., 2015). Similarly, LRR-del marker has been used by Kumar *et al*. (2017) to screen ICF4 (inter crossed F4)

lines possessing *Gm4*, *Gm8* and *Xa21* genes for the presence of *Gm4* gene and also by Kalpana (2015) to screen backcross derived lines possessing *Gm4, Gm8* and *Xa21* genes for the presence of *Gm4* gene.

4.2.3 PRP-del functional marker for *Gm8* gene:

PRP: *Gm8* functional SSR marker PRP amplified a band size of 300 bp in resistant source Aganni and 350 bp in susceptible source TN1. This marker amplified resistant specific allele (*i.e.,* Aganni allele) in all the entries except IR72476-B-P-9-3-1-1 (Plate 4.3). The amplification pattern of the marker is in confirmation with Divya *et al.* (2013) and Dutta *et al.* (2014).

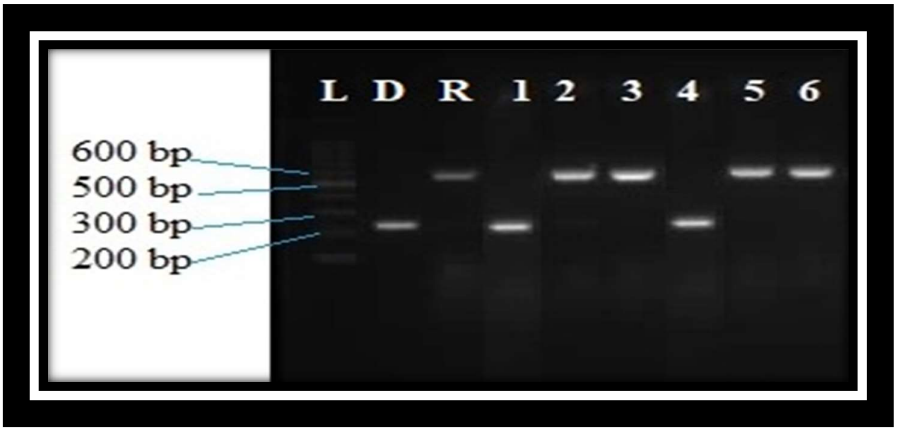
The functional marker, PRP encoding Proline Rich Protein (Divya *et al*., 2013) was used to confirm the presence of resistance gene *i.e.*, *Gm8* in these test entries. The *Gm*8 functional marker PRP showed an amplicon size of 300 bp for resistant allele and 350 bp for susceptible allele (Divya *et al*., 2013). Function of this gene has been recently validated in RT-PCR studies (Divya *et al*., 2015). Similarly, PRP marker have been used by Kumar *et al*. (2017) to screen ICF4 (inter-crossed F4) lines possessing *Gm4*, *Gm8* and *Xa21* genes for the presence of *Gm8* gene and by Kalpana (2015) to screen backcross derived lines possessing *Gm4*, *Gm8* and *Xa21* genes for the presence of *Gm8* gene and by Venkanna *et al*. (2018) to screen gene pyramided lines (*Gm1, gm3* and *Gm8*) for the presence of *Gm8* gene.

Hence, the markers used in the present study were most appropriate to confirm the presence of the resistance genes with 100 per cent efficiency as they are within the genes.

Table 4.1. Genotypes used for molecular screening

Sl. No.	Genotypes
1.	IR72476-B-P-9-3-1-1
2.	RP-5332-54-11-8-2-13
3.	Kakai
4.	WGL-1145
5.	WGL-1147
6.	WGL-1127

Plate: 4.1. PCR Amplification pattern of selected genotypes with *gm3del3 functional* marker, screening for presence of *gm3* gene

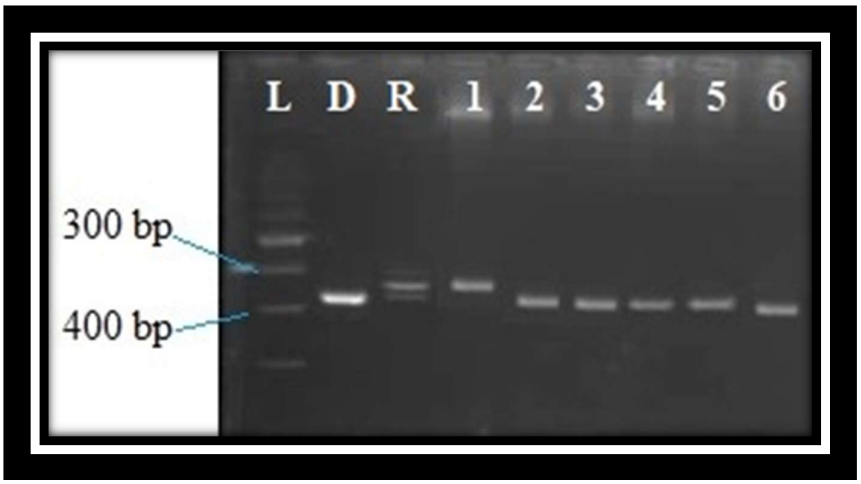


L- 100bp ladder, D- RP2068 (donor parent for g*m3* gene), R-TN1 (susceptible parent for gm3 gene), The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 4.1.

Plate: 4.2. PCR Amplification pattern of selected genotypes with *LRR Del functional* marker, screening for presence of G*m4* gene

L- 100bp ladder, D- TN1(susceptible parent), R-*Abhaya* (donor parent for *Gm4* gene). The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 4.1.

Plate: 4.3. PCR Amplification pattern of selected genotypes with *PRP-del functional* marker, screening for presence of *Gm8* gene



L-100 bp ladder, D- Aganni (donor parent) R- TN1 (susceptible parent for *Gm8* gene). The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 4.1.

4.3 Analysis of variance

The analysis of variance for thirteen characters was carried out to partition the total variation into variation due to genotypes, variation due to replication and variation due to other sources. The analysis of variance for thirteen traits of 42 rice genotypes is presented in Table 4.2. 'F' test indicated that mean sum of squares due to genotypes were highly significant for all the characters suggesting the presence of considerable amount of genetic variability in the material and there is ample scope of improvement with regards to the traits under investigation.

4.4 Mean performance

The mean performance of 42 genotypes for all the thirteen characters is presented in Table 4.3.

4.4.1 Days to 50 % flowering

The days to 50 % flowering ranged from 91 to 120 days, with a general mean of 108 days. Among all tested genotypes Dular was recorded least number of days to 50% flowering (91 days) followed by IR-74101-3R-1-1 (100 days), Kamar samba, Njavara, Laicha and Kalabunt (101 days) and IR72476-B-P-9-3-1-1, WGL-1145, WGL-1147 (102 days) while the entry Calotoc recorded highest number (120 days). The genotypes Dular, IR-74101-3R-1-1, Kamar samba, Njavara, Laicha and Kalabunt had shown mean values in desirable direction.

4.4.2 Plant height (cm)

Plant height ranged from 85.8 cm to 163.8 cm with a general mean of 117.05 cm. The shortest genotype was WGL-1127 (85.8 cm) followed by KNM-604 (94.53 cm), WGL-1145 (95.47 cm) and CGZR 2 (96.40 cm) while the tallest genotype was Didianga (163.8 cm). Genotypes KNM-604, Kapurala vadlu, CGZR 2, WGL-1145 and WGL-1127 were found to be preferable based on mean values.

4.4.3 Panicles length(cm)

The range of variation observed for panicle length was from 19.8 cm to 28.4 cm with a general mean of 24.67 cm. The genotype NDLR-7 had shorter panicle length of 19.8 cm. Manipuri black recorded longest panicle length of 28.4 cm followed by Aganni (27.40 cm), CGZR 1 (27.07 cm), JGL 34594 (26.90 cm) and Kudel (26.67 cm). Preferable genotypes were Manipuri black, Aganni, Kudel, JGL 34594 and CGZR 1.

4.4.4 Number of productive tillers per plant

The number of productive tillers per plant was ranged from 6 to 11 with general mean of 9. Highest number of productive tillers per plant was recorded in CGZR 1, Didianga, Geetanjali and TN1 (11) followed by MUT NS 1, IR-04A-216, Kapurala vadlu and Njavara (10). Preferable genotypes were CGZR 1, Didianga, Geetanjali and TN1 with regard to this character.

4.4.5 Number of grains per panicle

The range of variation observed for this trait was from 83 to 222 grains per panicle with a mean value of 127. The highest number of grains per panicle was recorded in genotype Didianga (222) followed by Kakirekaalu (221) and JGL 34594 (192), whereas, the minimum number of grains per panicle was recorded in Manipuri black (83) followed by Dular and TN 1 (88) and IR-04A-216 (89). Genotypes Didianga and Kakirekaalu were found to be desirable.

4.4.6 1000-grain weight (g)

The 1000-grain weight ranged from 11.26 g (NDLR-7) to 28.29 g (HH-25-SAL-10-D2-DT-1) with the general mean of 20.53 g. The entries JGL 34594 (11.54 g), KNM-604 (12.38 g), WGL-1127 (12.68 g), RP-5332-54-11-8-2-13 (12.88) and Chittimutyalu (13.13 g) also exhibited low test weight. HH-25-SAL-10-D2-DT-1 recorded high test weight of 28.29 g followed by Dular (28.06 g), Kudel (27.97), Calotoc (27.40) and TN1 (25.57). Therefore, HH-25-SAL-10-D2-DT-1, Dular, Kudel, Calotoc and TN1 found to be preferable.

4.4.7 Grain yield per plant (g)

The grain yield per plant ranged from9.50 g (Laicha) to 24.67 g (HH-25-SAL-10-D2-DT-1) with general mean of 14.08 g. The entries Manipuri black (9.57 g) CGZR 2 (9.60 g) and Kapurala vadlu (10.07g) also exhibited low grain yield per plant. HH-25-SAL-10-D2-DT-1 recorded high grain yield per plant of 24.67 g followed by Didianga (22.03), KNM-630 (19.93) and Kamar samba (19.50). The Most desirable genotypes were HH-25-SAL-10-D2-DT-1, Didianga, KNM-630 and Kamar samba.

4.4.8 Hulling (%)

The hulling per cent ranged from 75.33 % (Burma Black and NSN 1/296-2016) to 85.67 % (RP-5332-54-11-8-2-13) with general mean of 78.95%. The entries Mahamaya (82.67 %), KNM-604 (82.33 %), Umleng-1 and WGL-1147 (82.00 %) also

recorded high hulling per cent values. Accordingly, the most desirable genotypes with regard to hulling % were Burma Black and NSN 1/296-2016.

4.4.9 Milling (%)

The milling per cent values ranged from 42.67 % (Kudel) to 79.33 % (RP-5332-54-11-8-2-13) with general mean of 64.78%. The entries NDLR-7 (74 %) and KNM-604 (70.33) also recorded high milling per cent values. Genotypes which are found to be good are RP-5332-54-11-8-2-13, NDLR-7 and KNM-604.

4.4.10 Head rice recovery (%)

The values of head rice recovery were ranged from23.00 % (WGL-1145) to 62.33 % (Didianga) with general mean of 39.61 %. The entries KNM-604 (57.67%), RP-5332-54-11-8-2-13 (55.67%), Kakirekaalu (55.00%) and JGL 34594 (53.33%) also recorded high head rice recovery values.

4.4.11 Kernel length (mm)

The range of variation observed for this character was from 4.05 mm (Umleng-1) to 6.82 mm (Burma Black) with a general mean of 5.40 mm. The entries Burma Black (6.82 mm), Geetanjali (6.62 mm), HH-25-SAL-10-D2-DT-1 (6.28 mm), Manipuri black (6.21 mm) and RDR 1210 (6.19 mm) recorded high kernel length, whereas Umleng-1 (4.05 mm), Calotoc (4.58 mm), WGL-1127 (4.59 mm) and Kakirekaalu (4.63 mm) recorded lowest kernel length.

4.4.12 Kernel breadth (mm)

The range of variation observed for this character was from 1.19 mm (NDLR-7) to 2.47 mm (Calotoc) with a general mean of 1.81 mm. The highest kernel breadth was recorded in Calotoc (2.47 mm) followed by Kalabunt (2.36 mm), Kudel (2.27 mm), Dular (2.22 mm) and Mahamaya (2.19 mm), whereas, the lowest kernel breadth was recorded in NDLR-7 (1.19 mm) followed by CGZR 2 (1.28 mm), JGL 34594 (1.34 mm) and KNM-630, Kapurala vadlu (1.40 mm).

4.4.13 L/B ratio

Higher the value of kernel L/B ratio, more the slenderness and more preferable**.** The range of variation observed for this character was from 1.85 (Calotoc) to 4.45 (Geetanjali) with a general mean of 3.08. Among all tested genotypes Geetanjali (4.45) was recorded highest value of L/B ratio followed by RDR 1210 (4.11) and CGZR 2 (4.01). The most suitable genotypes were Geetanjali, CGZR 2 and RDR 1210.

Table 4.2. Analysis of variance (ANOVA) for 13 yield and yield attributing characters for 42 genotypes of rice

Source of Variations		Replication	Treatment	Error
d.f		**2**	**41**	**82**
Mean Sum of Squares	**DFF**	15.01	113.91 **	2.57
	PH	4.11	1187.18 **	14.17
	PL	2.59	11.44**	1.12
	PT	0.45	3.47**	1.38
	GPP	162.50	3693.22**	337.77
	TGW	0.51	67.82**	1.13
	SPY	17.28	42.61**	5.69
	H (%)	1.17	17.28*	11.061
	M (%)	46.02	149.60**	15.14
	HRR (%)	60.17	301.65**	8.39
	KL	0.06	0.99**	0.02
	KB	0.01	0.3**	0.003
	L/B	0.001	1.34**	0.012

*** Significant at 5% level, ** Significant at 1% level**

DFF- Days to 50% flowering, **PH -** Plant height (cm), **PL -** Panicle length (cm), **PT -** Number of productive tillers, **GPP -** Number of grains per panicle, **TGW -** 1000 grains weight (g), **SPY –**Grain yield per plant (g), **H (%) -** Hulling (%), **M (%) -** Milling (%), **HRR (%) -** Head rice recovery (%), **KL -** Kernel length (mm), **KB -** Kernel breadth (mm), **L/B -** L/B ratio.

Table 4.3. Mean performance of yield and yield attributing characters and physical grain quality parameter

S.No.	Genotypes	DFF	PH (cm)	PL (cm)	PT	GPP	TGW (g)	SPY (g)	H (%)	M (%)	HRR (%)	KL (mm)	KB (mm)	L/B
1	IR72476-B-P-9-3-1-1	102.00	106.67	24.73	9.00	123.00	22.79	15.12	78.67	65.33	34.00	5.50	1.90	2.90
2	IR-74101-3R-1-1	100.00	101.70	26.00	9.00	159.00	23.48	12.00	78.67	65.33	42.67	5.44	1.93	2.83
3	IR-72049-B-R-22-3-1-1	109.00	114.80	25.27	9.00	113.00	20.90	12.73	80.00	68.00	40.00	5.63	1.73	3.25
4	Calotoc	120.00	148.67	26.20	9.00	109.00	27.40	10.47	81.00	49.00	34.67	4.58	2.47	1.85
5	Dular	91.00	115.53	24.20	6.00	88.00	28.06	11.87	75.67	66.00	32.67	5.52	2.22	2.49
6	KNM-630	107.00	106.60	23.27	8.00	195.00	14.88	19.93	80.00	63.66	49.00	5.25	1.40	3.76
7	KNM-604	114.00	94.53	22.53	8.00	156.00	12.38	10.50	82.33	70.33	57.67	5.13	1.47	3.48
8	NDLR-7	116.00	104.80	19.80	9.00	116.00	11.26	11.03	77.33	74.00	48.00	4.61	1.19	3.87
9	Sampada	116.00	105.40	24.33	9.00	129.00	15.70	11.23	76.00	68.00	44.67	5.38	1.71	3.14
10	Dukong-1	113.00	99.60	24.90	9.00	146.00	21.80	14.37	75.67	66.33	38.67	5.70	1.82	3.15
11	CGZR 1	108.00	155.47	27.07	11.00	144.00	19.08	16.43	78.00	65.67	29.00	5.29	1.83	2.89
12	RP-5332-54-11-8-2-13	116.00	110.93	25.27	8.00	153.00	12.88	16.03	85.67	79.33	55.67	4.81	1.70	2.85
13	HH-25-SAL-10-D2-DT-1	106.00	145.07	23.27	8.00	156.00	28.29	24.67	78.00	70.00	35.67	6.28	1.95	3.22
14	MUT NS 1	112.00	112.53	26.27	10.00	119.00	19.36	14.20	80.67	66.00	52.00	5.59	1.50	3.72
15	IR-04A-216	109.00	97.87	24.87	10.00	89.00	22.27	15.37	80.67	59.33	29.67	5.86	1.73	3.40
16	NSN 1/296-2016	105.00	97.60	22.13	8.00	160.00	18.10	10.47	75.33	62.33	37.00	5.66	1.79	3.16
17	Betangamblin	111.00	118.33	25.07	9.00	96.00	21.37	14.53	79.00	65.67	36.67	5.73	1.50	3.81
18	Chittimutyalu	102.00	131.87	26.33	9.00	148.00	13.13	11.67	81.33	70.00	30.00	5.54	1.92	2.89
19	IRBL-T-K59	114.00	118.50	24.93	7.00	134.00	22.13	18.77	80.67	70.00	49.33	5.45	2.07	2.63
20	IRTON 270	105.00	102.40	25.53	9.00	121.00	22.00	16.00	80.33	69.67	49.67	5.95	1.63	3.66
21	Kapurala vadlu	107.00	96.53	22.40	10.00	94.00	17.40	10.07	81.67	61.00	37.33	5.55	1.40	3.74
22	Mahamaya	114.00	110.93	24.30	8.00	150.00	25.00	13.60	82.67	68.67	34.67	5.86	2.19	2.67
23	CGZR 2	119.00	96.40	21.80	9.00	108.00	14.65	9.60	78.00	66.30	43.33	5.14	1.28	4.01
24	JGL 34594	117.00	97.80	26.90	9.00	192.00	11.54	11.93	80.33	69.00	53.30	4.66	1.34	3.48
25	RDR 1210	113.00	104.53	25.70	9.00	107.00	18.58	13.10	81.00	66.30	24.67	6.19	1.51	4.11
26	Kamar samba	101.00	129.10	25.53	8.00	92.00	22.17	19.50	77.67	71.00	29.00	5.35	2.18	2.46
27	Kudel	103.00	134.60	26.67	9.00	105.00	27.97	12.37	79.30	42.67	29.33	4.86	2.27	2.16

S.No.	Genotypes	DFF	PH (cm)	PL (cm)	PT	GPP	TGW (g)	SPY (g)	H (%)	M (%)	HRR (%)	KL (mm)	KB (mm)	L/B
28	Njavara	101.00	120.53	22.80	10.00	101.00	25.80	13.33	76.67	43.66	27.00	4.94	2.10	2.35
29	Laicha	101.00	126.80	24.93	9.00	92.00	20.62	9.50	75.33	52.67	27.33	5.19	2.20	2.36
30	Kalabunt	101.00	144.00	26.20	8.00	120.00	25.32	22.7	77.67	61.67	33.00	4.68	2.36	1.99
31	Kakirekaalu	114.00	142.27	22.60	8.00	221.00	22.92	15.53	77.33	64.33	55.00	4.63	2.17	2.14
32	Manipuri black	109.00	118.4	28.40	8.00	83.00	24.67	9.57	76.67	62.67	41.33	6.21	1.69	3.68
33	Burma Black	109.00	146.67	26.67	9.00	103.00	21.34	10.00	75.33	63.00	44.33	6.82	1.74	3.92
34	Didianga	105.00	163.77	26.40	11.00	222.00	22.32	22.03	78.33	69.67	62.33	5.28	2.01	2.62
35	Geetanjali	103.00	104.47	24.53	11.00	94.00	20.76	15.13	80.33	68.67	51.67	6.62	1.49	4.45
36	Umleng-1	106.00	136.27	24.80	7.00	130.00	18.53	11.40	82.00	70.00	28.67	4.05	2.15	1.88
37	Kakai	109.00	138.33	26.60	10.00	98.00	23.91	17.20	78.33	66.33	27.67	5.22	2.07	2.52
38	Aganni	109.00	140.33	27.40	10.00	118.00	24.37	18.33	77.67	59.67	46.67	5.29	2.14	2.47
39	WGL-1145	102.00	95.47	20.87	8.00	102.00	20.67	13.40	76.00	66.33	23.00	5.69	1.48	3.85
40	WGL-1147	102.00	97.20	20.73	9.00	108.00	17.90	11.43	82.00	67.00	40.00	5.65	1.50	3.76
41	WGL-1127	106.00	85.80	22.60	9.00	143.00	12.64	11.20	76.67	63.00	36.00	4.59	1.48	3.10
42	TN1	114.00	96.80	24.80	11.00	88.00	25.57	13.27	80.00	63.33	41.67	5.63	2.00	2.82
Range Lowest		91.33	85.80	19.80	6.33	83.00	11.26	9.50	75.33	42.67	23.00	4.05	1.19	1.85
Range Highest		119.67	163.77	28.40	11.33	222.33	28.29	24.67	85.67	79.33	62.33	6.82	2.47	4.45
Mean		108.16	117.04	24.66	9.00	126.78	20.53	14.08	78.95	64.78	39.62	5.41	1.82	3.08
C.V. (%)		1.48	3.22	4.29	13.15	14.49	5.17	16.92	4.21	6.00	7.31	2.72	3.02	3.61
C.D. (at 5%)		2.60	6.11	1.72	1.91	29.85	1.72	3.87	5.40	6.32	4.70	0.24	0.09	0.18

DFF- Days to 50% flowering, **PH -** Plant height (cm), **PL -** Panicle length (cm), **PT -** Number of productive tillers, **GPP -** Number of grains per panicle, **TGW -** 1000 grains weight (g), **SPY -**Grain yield per plant(g).**H% -** Hulling (%), **M% -** Milling (%), **HRR -** Head rice recovery (%), **KL -** Kernel length (mm), **KB -** Kernel breadth(mm), **L/B -** L/B ratio.

4.5 Phenotypic and genotypic coefficient of variance (GCV and PCV), Heritability and Genetic advance as percent of mean

The result of estimated genotypic and phenotypic coefficient of variation, heritability and genetic advance as percent of mean for 42 rice genotypes sown during *rabi, 2021-22* for all the thirteen traits are furnished in Table 4.4. and Fig 4.1. and 4.2. The characters studied in the present investigation exhibited low, moderate and high PCV and GCV values.

4.5.1 Days to 50% flowering

The genotypic and phenotypic coefficient of variation estimate observed for this trait were low (*i.e.,* 5.63% and 5.82% respectively) indicating low variation among the genotypes for this trait. The results are in conformity with Padmaja *et al.* (2008) and Rita *et al*. (2009). The observed heritability estimates for this character were high (93.5%) with moderate genetic advance as percent of mean (11.22 %) indicating low environmental effect and it is governed by both additive and non-additive gene action. Seyoum *et al.* (2012) reported similar results for high heritability coupled with moderate genetic advance.

4.5.2 Plant height (cm)

The genotypic and phenotypic coefficient of variation estimate observed for this trait was moderate *i.e.,* 16.89% and 17.20%, respectively. The results are in conformity with Rita *et al.* (2009) and Rohit *et al*. (2017). The observed heritability estimates for this character was high (96.5%) with high genetic advance as percent of mean (34.19 %), indicating that character is governed by additive gene action and selection is highly useful for improvement of the trait. Padmaja *et al*. (2008), Kumar *et al.* (2012), Seyoum *et al.* (2012), Lingaiah (2015), Sumanth *et al.* (2017), Rohit *et al.* (2017) and Ranjith *et al*. (2018) observed high heritability coupled with high genetic advance for plant height.

4.5.3 Panicle length (cm)

Genotypes were less variable for this trait as genotypic and phenotypic coefficients of variation estimate observed for this trait were low *i.e.,* 7.52% and 8.66%, respectively. The observed heritability estimates for this character was high (75.5%) with moderate genetic advance as percent of mean (13.46%) indicating that character is little influenced by environment and governed by both additive and non-additive gene action. The similar results were reported by Adhikari *et al.* (2018) for low genotypic

and phenotypic coefficient of variation, Lingaiah *et al.* (2019), Sankar *et al*. (2006), Abebe *et al.* (2017) for high heritability.

4.5.4 Number of productive tillers per plant

The genotypic and phenotypic coefficient of variation estimate observed for this trait were low (9.35%) and moderate (16.14%) respectively indicating low genotypic and moderate phenotypic variability. The observed heritability estimates for this character were moderate (33.6%) with moderate genetic advance as percent of mean (11.17 %). The similar results were reported by Yadav *et al.* (2010), Edukondalu *et al*. (2017) and Babu *et al.* (2012) for moderate genotypic and phenotypic coefficient of variation, Chakraborty and Chakraborty (2010) for medium heritability.

4.5.5 Number of grains per panicle

The high genotypic (26.38 %) and phenotypic coefficient of variation (30.10 %) estimate were observed for this trait coupled with high heritability (76.8 %) and genetic advance as per cent of mean (47.62 %) suggesting the presence of wide variability among the genotypes and presence of additive gene action governing trait indicating selection for this trait may be effective. These results are comparable with the findings of Chandramohan *et al*. (2016), Edukondalu *et al*. (2017) and Singh and Verma (2018) for high genotypic and phenotypic coefficient of variation, Akinwale *et al.* (2011), Edukondalu *et al*. (2017), Sandeep *et al*. (2018) and Singh and Verma (2018) for high heritability coupled with high genetic advance as percent of mean.

4.5.6 1000-grain weight (g)

A high genotypic (22.97%) and phenotypic coefficient of variation (22.97%) estimate observed for this trait coupled with high heritability (95.2%) and genetic advance as per cent of mean (46.16%) suggesting wide variability and selection for this trait would be effective. Prasad *et al.* (2001), Padmaja *et al.* (2008), Seyoum *et al.* (2012), Lingaiah (2015) and Sala and Shanthi (2016) observed high heritability with high genetic advance as percent of mean for this trait.

4.5.7 Grain yield per plant (g)

The genotypic and phenotypic coefficient of variation estimates observed for grain yield was high (24.90% and 30.11% respectively) indicating high variability among the genotypes for this trait. The results are in conformity with Prasad *et al.* (2001), Padmaja *et al.* (2008), Kumar *et al.* (2012), Lingaiah (2015), Sala and Shanthi, (2016), Sumanth *et al.* (2017), Abebe *et al.* (2017), Rohit *et al.* (2017), Venkatesan *et*

al. (2017). The observed heritability estimate for this character was high (95.2%) with high genetic advance as per cent of mean (46.16%). It showed higher role of additive gene action in the inheritance of the trait and selection would be effective. Prasad *et al*. (2001), Padmaja *et al.* (2008), Rita *et al.* (2009), Seyoum *et al.* (2012), Lingaiah (2015), Sala and Shanthi (2016), Rohit *et al.* (2017), Abebe *et al.* (2017) and Venkatesan *et al.* (2017) reported similar results of high heritability coupled with high genetic advance as per cent of mean for grain yield.

4.5.8 Hulling (%)

The genotypic and phenotypic coefficient of variation estimate observed for hulling (%) was low *i.e.,* 1.82% and 4.59%, respectively. The observed heritability estimate for this character was low (15.8%) with low genetic advance as per cent of mean (1.49%). It implies very low variability among the genotypes and the trait is highly influenced by the environment and selection would be ineffective. Similar results were reported by Rakesh *et al.* (2013), Rukmini *et al.* (2014), Gampala *et al*. (2015), Edukondalu *et al*. (2017) and Sahu *et al*. (2017) for low genotypic and phenotypic coefficient of variation and Nagajyothi (2001) for low heritability coupled with low genetic advance as per cent of mean.

4.5.9 Milling (%)

The genotypic and phenotypic coefficient of variation estimate observed for milling (%) was moderate *i.e.,* 10.33% and 11.95%, respectively. The results are in conformity with Asish Binodh *et al.* (2007). The observed heritability estimate for this character was high (74.8%) with moderate genetic advance as per cent of mean (18.41%) indicating that character is little influenced by environment and probably governed by both additive and non-additive gene action. Chaudhary and Motiramani (2003) reported similar results of high heritability coupled with moderate genetic advance as per cent of mean for milling per cent.

4.5.10 Head rice recovery (%)

The genotypic and phenotypic coefficient of variation estimate observed for head rice recovery was high *i.e.,* 24.95% and 26%, respectively. The observed heritability estimate for this character was high (92.1%) with high genetic advance as per cent of mean (49.34%). The result indicating that genotypes are highly variable for this trait and the trait is governed by additive gene action. Similar results were reported by Edukondalu *et al*. (2017) and Rukmini *et al.* (2019) for high heritability.

4.5.11 Kernel length (mm)

The genotypic and phenotypic coefficient of variation estimate observed for kernel length were moderate (10.49% and 10.48%) indicating moderate variability among the genotypes for this trait. The observed heritability estimate for this character was high (93.7%) with high genetic advance as per cent of mean (20.91 %) indicating the heritability is due to additive gene effects and selection may be effective. These results were akin to the reports of Rakesh *et al.* (2013), Vanisree *et al*. (2013), Edukondalu *et al*. (2017), Sahu *et al*. (2017), Khan *et al.* (2019) and Radha *et al.* (2019).

4.5.12 Kernel breadth (mm)

A moderate genotypic (17.74 %) and phenotypic (17.99 %) coefficients of variation was observed for this trait indicating moderate variability among the genotypes for this trait. The heritability estimate was high (97.2 %) with a high genetic advance as per cent of mean (36.03 %) indicating the heritability is due to additive gene action and selection is highly useful for improvement of the trait. Similar results were reported by Rukmini *et al.* (2014), Edukondalu *et al*. (2017), Sahu *et al*. (2017).

4.5.13 L/B ratio

The genotypic and phenotypic coefficient of variation estimate observed for kernel L/B ratio were high *i.e.,* 21.63% and 21.93%, respectively. The observed heritability estimates for this character was high (97.3%) with high genetic advance as per cent of mean (43.95%). The result indicating that genotypes are highly variable for this trait and the trait is governed by additive gene action. These results are in accordance with findings Rakesh *et al.* (2013), Edukondalu *et al*. (2017), Sahu *et al*. (2017), Kumar *et al*. (2018), Brijesh *et al.* (2019), Chinnapa *et al.* (2019) and Radha *et al.* (2019) for high heritability and high genetic advance as per cent of mean.

Table 4.4. Estimates of variability, heritability and genetic advance for yield and quality parameters in rice

S. No	Characters	Mean	Range		Phenotypic Variance	Genotypic Variance	PCV (%)	GCV (%)	Heritability in broad sense (h^2) (%)	Genetic Advance as % of mean
			Min	Max						
1	Days to 50% flowering	108.00	91.00	120.00	39.68	37.11	5.82	5.63	93.50	11.22
2	Plant height (cm)	117.04	85.80	163.80	405.17	391.00	17.20	16.89	96.50	34.19
3	Panicle length (cm)	24.66	19.80	28.40	4.56	3.44	8.66	7.52	75.50	13.46
4	Number of productive tillers	9.00	6.00	11.00	2.08	0.70	16.14	9.35	33.60	11.17
5	Number of grains per panicle	127.00	83.00	222.00	1456.25	1118.48	30.10	26.38	76.80	47.62
6	1000 grains weight (g)	20.52	11.26	28.29	23.36	22.23	23.54	22.97	95.20	46.16
7	Grain yield per plant(g)	14.08	9.50	24.67	17.99	12.31	30.11	24.90	68.40	42.43
8	Hulling (%)	78.95	75.33	85.67	13.13	2.07	4.59	1.82	15.80	1.49
9	Milling (%)	64.78	42.67	79.33	59.96	44.82	11.95	10.33	74.80	18.41
10	Head rice recovery (%)	39.61	23.00	62.33	106.14	97.75	26.00	24.95	92.10	49.34
11	Kernel length (mm)	5.40	4.05	6.82	0.34	0.32	10.84	10.49	93.70	20.91
12	Kernel breadth (mm)	1.81	1.19	2.47	0.11	0.10	17.99	17.74	97.20	36.03
13	L/B ratio	3.08	1.85	4.45	0.46	0.44	21.93	21.63	97.30	43.95

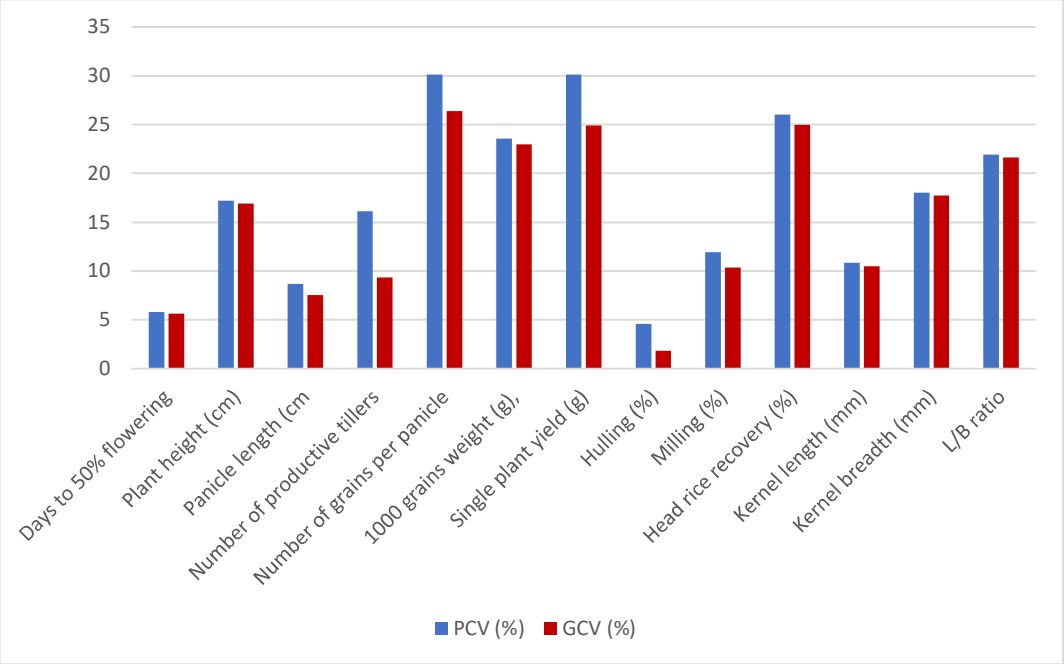


Figure 4.1. Graphical representation of PCV and GCV

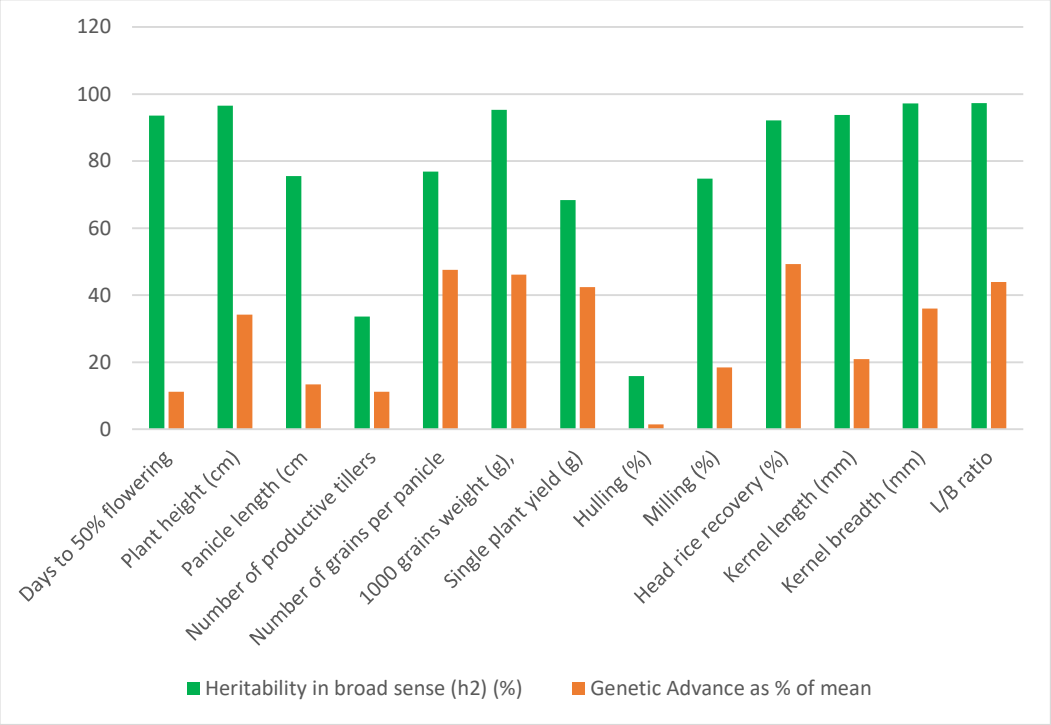


Figure 4.2. Graphical representation of Heritability in Broad sense and Genetic Advance as % mean

4.6 Genetic divergence (D^2) studies among yield and yield component traits analysis

D^2 analysis is one of the potent techniques of measuring genetic divergence in the germplasm collections. Genetic diversity analysis helps in assessing nature of diversity among the genotypes. In order to identify the genetically diverse genotypes for their uses in breeding programme, Mahalanobis' generalized distance (D^2) analysis was carried out using 13 characters in 42 genotypes. The test entries were grouped into different clusters based on inter and intra cluster distance. The results obtained from D^2 analysis are discussed under following heads:

4.6.1 Mahalanobis generalized distance D^2 values

In order to assess the genetic diversity among 42 genotypes, D^2 statistic was used following the procedure given by Rao (1952). Since the entire yield component characters were correlated, they were transformed into uncorrelated linear combination through pivotal condensation method.

4.6.2 Wilk's 'V' Criterion Test

Wilk's 'V' (statistic) criterion was used to test the significant differences between the genotypes for individual characters and later the significant differences between the genotypes based on the pooled effects of all the characters were determined. The significance of 'V' (statistic) value was tested at 533 degrees of freedom for all the characters. The 'V' statistic value was highly significant indicating that the genotypes differed significantly when all the characters were considered simultaneously. Analysis of variance for dispersion showed significant pooled effects of all the characters under study among the genotypes.

4.6.3 Grouping of genotypes into various clusters

The analysis of variance (Table 4.2.) for yield and its components revealed highly significant differences among the genotypes for all the 13 traits indicating the existence of genetic variability among the experimental material. The rice genotypes were grouped into seven clusters based on D^2 values using Tocher's method (Rao, 1952). The composition is presented in Table 4.5. Cluster I comprising of 18 genotypes was the largest group followed by the cluster III comprising of 9 genotypes and cluster II comprising of 7 genotypes, whereas, the clusters IV, V, VI and VII, were represented by single genotype indicating high degree of heterogeneity among the genotypes (Fig. 4.3). Gall midge resistant genotypes *viz.,* WGL – 1147, IR72476-B-P-9-3-1-1and WGL-1145 fall in cluster I, RP-5332-54-11-8-2-13 and WGL-1127 fall in cluster II whereas

kakai and Aganni falls in cluster III. Remaining clusters does not include any resistant genotypes.

4.6.4 Average inter and intra cluster distances

The average intra and inter cluster D^2 values among seven clusters are presented in Table 4.6. Intra cluster D^2 values are zero for clusters IV, V, VI and VII as these were mono genotypic clusters and genotypes in these clusters were more divergent and they could be utilized as parents for hybridization. High intra cluster distance was observed in cluster III (85.85) followed by cluster II (56.94) and cluster I (55.10) revealing that some genetic divergence still existed among the genotypes of these cluster. The intra cluster distances were lower than inter cluster distances, indicating the existence of genetic diversity among the genotypes of different clusters, which could be made use of in the yield improvement through recombination breeding. The highest inter cluster distance was observed between cluster II and VII (418.05) followed by cluster IV and VII (350.95), cluster II and III (298.15) and cluster II and V (281.16). The lowest inter cluster distance was observed between cluster V and VI (54.44). The greater the distance between two clusters, the greater the genetic diversity between the genotypes of those clusters. Using the genotypes belonging to most divergent clusters as parents in breeding program may exploit the maximum amount of heterosis. It is indicated that hybridization between the genotypes of clusters II and VII, clusters IV and VII, clusters II and III and clusters II and V could result in desirable transgressive segregants. Since cluster I, II and III contain some gall midge resistant genotypes so, these genotypes can be crossed with genotypes of most divergent clusters to generate breeding material with high genetic diversity and gall midge resistance. Similar study was carried out by Dhakal *et al.,* (2020) with 30 rice landraces to identify the potential parents for hybridization using Mahalanobis distance (D^2) and concluded that the Landraces from clusters V (Pakhe Sali, Chiniya, Mansara) and VI (Marsi Local, Pahele) or clusters III (Pahelo Anadi, Pudke Dhan, Gokule Mansuli, Rato Anadi) and VI (Marsi Local, Pahele) or clusters IV (Rato Masino, Seto Anadi, Indrabeli) and VI (Marsi Local, Pahele) can be used in the hybridization program to develop the superior hybrids by exploiting heterosis in segregating generation. Genetic diversity in 40 traditional boro rice genotypes was studied by Siddique *et al.,* (2013) through Mahalanobis D^2 statistic for grain yield and yield contributing characters. The inter-cluster distances were higher than intra-cluster distances indicating wider genetic diversity among the clusters. The genotypes from cluster II (Kali boro 80/9) could be hybridized with the genotypes of other clusters for producing transgressive segregants.

Keeping this in view, it is concluded that genotypes from cluster II and VII, cluster II and III, cluster II and V may be used as parents in the breeding programmes to obtain desirable segregants with gall midge resistance. Crossing the genotypes from cluster VI and VII could results in desirable segregants with promising yield and yield attributing traits.

Table 4.5. Clustering pattern of rice genotypes (Tocher's method)

Cluster number	Number of genotypes	Name of genotypes
Cluster I	18	Kapurala vadlu, WGL-1147, IRTON 270, Betangamblin, IR-72049-B-R-22-3-1-1, MUT NS 1, Sampada, Dukong-1, NSN 1/296-2016, IR-04A-216, RDR 1210, Manipuri black, IR72476-B-P-9-3-1-1, WGL-1145, TN1, IR-74101-3R-1-1, IRBL-T-K59, Mahamaya
Cluster II	7	KNM-604, JGL 34594, KNM-630, CGZR 2, Chittimutyalu, RP-5332-54-11-8-2-13, WGL-1127
Cluster III	13	Kamar samba, kakai, Laicha, Njavara, Kudel, Kalabunt, Aganni, CGZR 1, Umleng-1, Chittimutyalu, Dular, HH-25-SAL-10-D2-DT-1, Burma Black
Cluster IV	1	Geetanjali
Cluster V	1	Didianga
Cluster VI	1	Kakirekaalu
Cluster VII	1	Calotoc

Table 4.6. Intra (diagonal) and inter cluster distances (D^2 values) of rice genotypes

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**	**Cluster VII**
Cluster I	55.10	142.77	126.53	99.78	201.21	161.21	278.83
Cluster II		56.94	298.15	137.32	281.16	203.98	418.05
Cluster III			85.85	218.65	154.07	146.14	182.15
Cluster IV				0.00	202.94	216.96	350.95
Cluster V					0.00	54.44	190.36
Cluster VI						0.00	111.37
Cluster VII							0.00

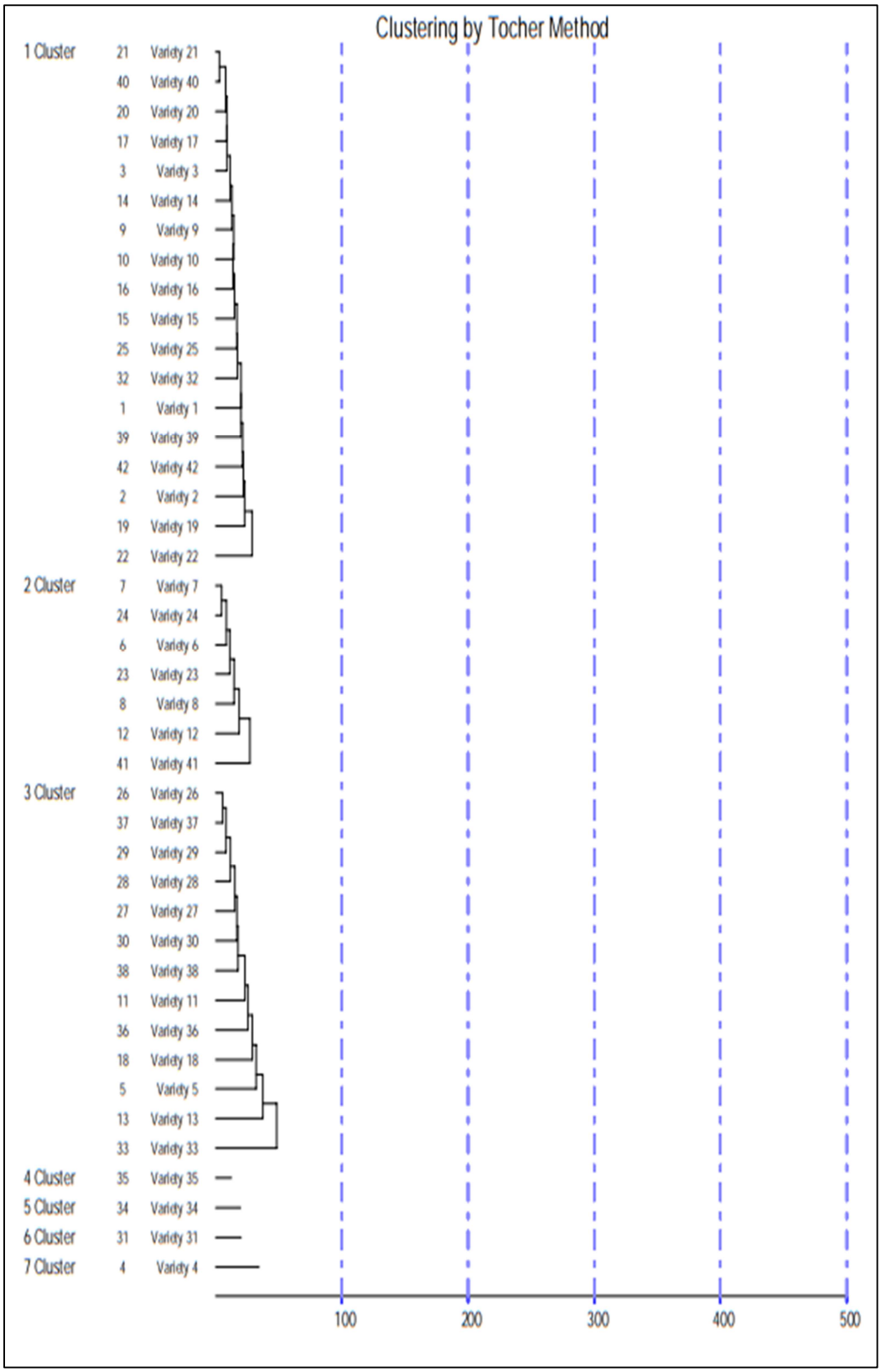


Figure 4.3. Clustering Pattern of rice genotypes by Tocher's Method

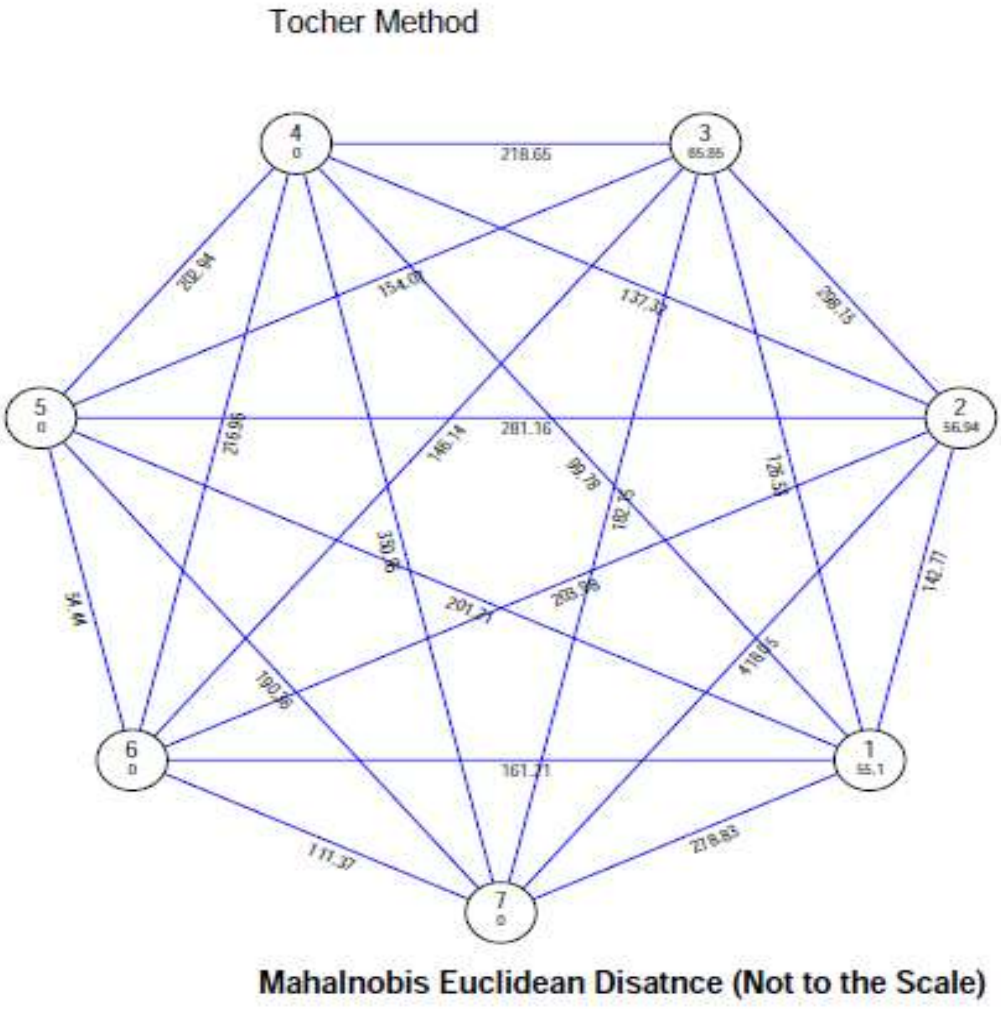


Figure 4.4. Average inter and intra cluster distance between 7 clusters by Tocher's method

4.6.5 Cluster means of the characters

The cluster means of 13 characters are presented in Table 4.7. The data indicated that the cluster means for days to 50% flowering was highest in cluster VII (119.67) and lowest in cluster IV (103.33). So, genotype of cluster IV can be used in breeding programme to develop varieties with early maturity.

Plant height was highest in cluster V (163.77 cm) and lowest in cluster II (99.55 cm). Since cluster II contain some gall midge entries (RP-5332-54-11-8-2-13 and WGL-1127) so these entries can be crossed with genotypes of most divergent clusters (cluster VII, III and V) to generate lodging resistant coupled with gall midge resistant breeding material as dwarfness is one of the important traits helps in preventing the lodging.

Panicle length was highest in cluster V (26.40 cm) and lowest in cluster VI (22.60 cm). Number of productive tillers per plant was highest in cluster VI and V (10.67) and lowest in cluster VI (7.67). Number of grains per panicle was highest in cluster V (222.33) and lowest in cluster IV (94.00). Hence the genotype, Didianga (Cluster V) could be crossed with genotypes from diverse clusters for developing varieties with compact panicle having more number of grains.

1000-grain weight was highest in cluster VII (27.40 g) and lowest in cluster II (12.89 g). Calotoc of cluster VII can be crossed with gall midge resistant genotypes of cluster II (RP-5332-54-11-8-2-13 and WGL-1127) to get segregants with gall midge resistance and coarse grain nature. Cluster II with low mean test weight (12.89 g) and having accommodated with gall midge resistant entries, genotypes from this cluster can be crossed with genotypes from cluster III for development of fine grain rice varieties with gall midge resistance.

Grain yield per plant was highest in cluster V (22.03 g) and lowest in cluster VII (10.47 g). Therefore, Didianga of cluster V can be crossed with gall midge resistant genotypes of either cluster I, II or III to generate breeding material with gall midge resistant and high yield.

Hulling (%) was highest in cluster VII (81.00 %) and lowest in cluster VI (77.33 %). Milling (%) was highest in cluster V (69.67 %) and lowest in cluster VII (49.00 %). Head rice recovery was highest in cluster V (62.33 %) followed by cluster VI (55.00%) and cluster IV (51.67%) and lowest in cluster III (32.33 %). Hence the genotypes,

Didianga, Kakirekkalu and Geetanjali could be used in breeding programs for developing varieties with high head rice recovery which is a very important quality character. Kernel length was highest in cluster IV (6.62 mm) and lowest in cluster VII (4.58 mm). Kernel breadth was highest in cluster VII (2.47 mm) and lowest in cluster II (1.41 mm). Kernel L/B ratio was highest in cluster IV (4.45) and lowest in cluster VII (1.85).

4.6.6 Relative contribution of characters towards genetic divergence

The number of times that each of the 13 characters appeared in first rank and its respective percent contribution towards genetic divergence is presented in Table 4.8. and Fig 4.5. The results showed that the contribution of kernel breadth was highest (37.39%) towards total genetic divergence by 322 times ranking first followed by plant height (14.52 %) by 125 times, kernel length (11.03 %) by 95 times, head rice recovery (10.10 %) by 87 times, L/B ratio (9.29 %) by 80 times,1000 grains weight (7.55 %) by 65 times, days to 50% flowering (5.57 %) by 48 times, milling (%) (3.25 %) by 28 times, panicle length (0.58 %) by 5 times, number of grains per panicle (0.46 %) by 4 times and grain yield per plant (0.23 %) by 2 times to the genetic divergence. Ramanjaneyulu *et al.* (2014) evaluated 10 low land rice genotypes for genetic divergence and found that the yield attribute 1000 grains weight exhibited the maximum contribution to total genetic divergence (57.78%), followed by grain yield (15.56%), number of grains/spikelet (11.11%), straw yield (6.67%) and spikelet length (4.44%). On the other hand, the traits such as tillers/plant and harvest index failed to contribute toward genetic divergence. Nayak *et al.* (2004) and Bardhan and Thangavel (2011) also reported that the choice of parents mainly depends on the contribution of characters toward divergence.

Hence, it is noted that in the present experimental material, the traits kernel breadth, plant height, kernel length, head rice recovery and L/B ratio together contributed 82.30 % towards total divergence. Therefore, these characters should be given importance during hybridization and selection. The experimental material has nil contribution towards divergence pertaining to number of productive tillers per plant and hulling (%).

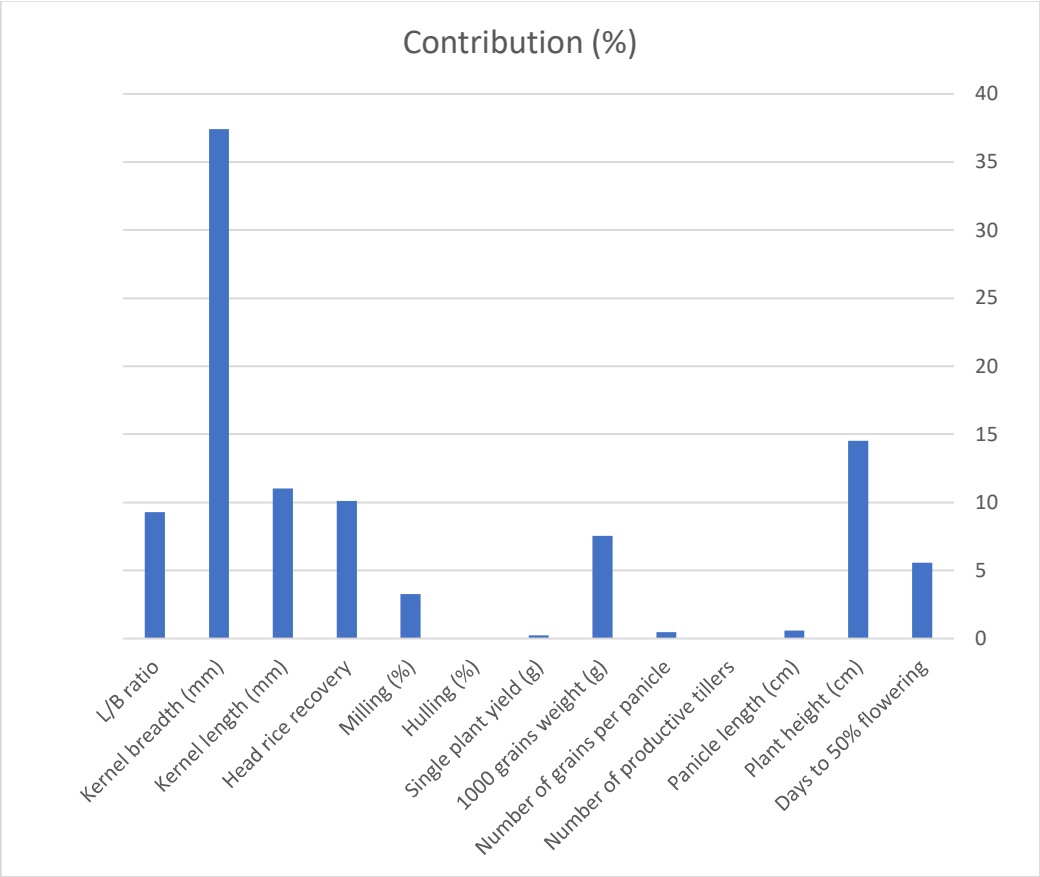
Table 4.7. Cluster means of rice genotypes for yield, yield components and quality traits

S. No	Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
1	Days to 50% flowering	108.85	113.52	103.56	103.33	105.00	114.33	119.67
2	Plant height (cm)	105.30	99.55	135.74	104.47	163.77	142.27	148.67
3	Panicle length (cm)	24.52	23.18	25.57	24.53	26.40	22.60	26.20
4	Number of productive tillers	8.98	8.76	8.79	10.67	10.67	7.67	8.67
5	Number of grains per panicle	117.76	151.90	115.05	94.00	222.33	220.67	109.33
6	1000 grains weight (g)	21.11	12.89	22.97	20.76	22.32	22.92	27.40
7	Grain yield per plant(g)	13.29	12.89	15.31	15.13	22.03	15.53	10.47
8	Hulling (%)	79.20	80.05	77.92	80.33	78.33	77.33	81.00
9	Milling (%)	65.63	69.38	61.72	68.67	69.67	64.33	49.00
10	Head rice recovery	38.72	49.00	32.33	51.67	62.33	55.00	34.67
11	Kernel length (mm)	5.71	4.88	5.31	6.62	5.28	4.63	4.58
12	Kernel breadth (mm)	1.73	1.41	2.09	1.49	2.01	2.17	2.47
13	L/B ratio	3.35	3.51	2.59	4.45	2.62	2.14	1.85

Table 4.8. Relative contribution of 13 characters to genetic diversity in 42 rice genotypes

Sl. No	Characters	Times ranked first	Contribution (%)
1	Days to 50% flowering	48	5.57
2	Plant height (cm)	125	14.52
3	Panicle length (cm)	5	0.58
4	Number of productive tillers	0	0.00
5	Number of grains per panicle	4	0.46
6	1000 grains weight (g)	65	7.55
7	Grain yield per plant(g)	2	0.23
8	Hulling (%)	0	0.00
9	Milling (%)	28	3.25
10	Head rice recovery	87	10.10
11	Kernel length (mm)	95	11.03
12	Kernel breadth (mm)	322	37.39
13	L/B ratio	80	9.29

Figure 4.5. Relative contribution of 13 characters towards total genetic divergence in 42 rice genotypes



4.7 Correlation studies

Correlation coefficient analysis was carried out among thirteen characters to find out positive and negative association of yield attributing traits towards the grain yield per plant. In general, the sign of genotypic and phenotypic correlation coefficient was same but the magnitude of genotypic correlation was higher than the phenotypic correlation, which indicate that though there is strong inherent association between characters studies, phenotypic expression is lessened due to masking effect of environmental factors. This is also in confirmation with the findings of Radhidevi *et al.* (2002), Najeeb and Wani (2004), Sarkar *et al.* (2007), Anbanandan *et al.* (2009), Sabesan *et al.* (2009), Jayasudha and Sharma (2010), Parimala *et al.* (2020), Adjah *et al.* (2020) and Chukwu *et al.* (2022). Considering the importance of phenotypic correlation, results pertaining to the correlation studies at phenotypic level only are described below. The phenotypic and genotypic correlation coefficient for yield and yield attributing traits are presented in (Table 4.8.). The result pertaining to correlation at phenotypic level between grain yield and other related characters are presented first followed by correlation among the yield attributes.

4.7.1 Correlation of grain yield with other characters

At phenotypic level, grain yield exhibited significant and positive association with panicle length (0.200^{*}), number of grains per panicle (0.314^{**}), 1000-grains weight (0.266^{**}), plant height (0.382^{**}) and kernel breadth (0.248^{**}), hence, direct selection for these characters would improve the yield wherein, other yield components *viz.,* productive tillers (0.044), hulling % (0.045), milling %(0.163), head rice recovery (0.095) and kernel length (0.033) had shown positive non-significant association. Significant and negative association at phenotypic level was found between grain yield and kernel L/B ratio (-0.196^{*}), while days to 50% flowering (-0.143) had shown negative and non-significant association with grain yield per plant. Similar results were reported by Rajamadhan *et al.* (2011), Limbani *et al.* (2017), Priya *et al.* (2017), Edukondalu *et al*. (2017) and Kumar and Sonali (2018) for grain yield per plant with days to 50 % flowering, plant height, number of productive tillers per plant, panicle length and 1000-grain weight; Singh *et al.* (2020) and Sadimantara *et al.* (2021) for kernel breadth.

4.7.2 Correlation among yield components

4.7.2.1 Days to 50% flowering

Days to 50% flowering showed positive and significant correlations at phenotypic level with number of grains per panicle (0.179*), 1000-grains weight (0.327**), hulling % (0.191*), milling % (0.180*), head rice recovery (0.397**) and kernel L/B ratio (0.185*) and negative and significant association with kernel breadth (-0.288**). These results was in accordance with the results of Bhadru *et al*. (2012), Meena *et al.* (2020), Sadimantara *et al.* (2021) and Chukwu *et al.* (2022) for days to 50% flowering with number of productive tillers per plant, panicle length, grains per panicle, 1000-grain weight and grain yield per plant.

4.7.2.2 Plant height (cm)

Plant height exhibited significant positive correlation with panicle length (0.451**), 1000grainsweight (0.446**), grain yield per plant (0.382**) and kernel breadth (0.632**) while non-significant positive correlation with number of grains per panicle (0.152). It had also shown significant negative correlation with kernel L/B ratio (-0.554**). The results are in collaboration with the findings of Kumar and Kannan Bapu (2005), Krishna *et al.* (2009), Veni *et al.* (2013) Singh and Verma (2018) and Rukmini *et al.* (2019).

4.7.2.3 Panicle length (cm)

Panicle length exhibited significant positive correlation with plant height (0.451**), kernel breadth (0.358**), grain yield per plant (0.200**) and 1000-grain weight (0.298**). A significant and negative correlation of this trait was observed with kernel L/B ratio (-0.258**). Sharma and Sharma (2007), Veni *et al.* (2013) and Krishna *et al.* (2008), Islam *et al.* (2019), Seneega *et al.* (2019), Kiruthikadevi *et al.* (2020) and Begum *et al.* (2021). also reported similar findings.

4.7.2.4 Number of productive tillers per plant

Number of productive tillers per plant exhibited significant positive correlation with kernel L/B ratio (0.180*) and recorded significant and negative correlation with none of the other traits. Bhadru *et al.* (2012), Minnie *et al.* (2013), Ashok *et al.* (2016), Meena *et al.* (2016), Siddi (2018), Hossain *et al.* (2020), Lakshmi *et al.* (2020) and Begum *et al.* (2021) also reported similar results.

4.7.2.5 Number of grains per panicle

Number of grains per panicle exhibited significant positive correlation with days to 50% flowering (0.179*), milling % (0.180*), grain yield per plant (0.314**) and head rice recovery (0.479*) and significant negative correlation with 1000-grain weight (-0.270**) and kernel length (-0.278**).

These results were in consonance with the earlier reports of Krishna *et al.* (2005), Akhtar *et al.* (2011), Dhurai *et al.* (2016), Saha *et al.* (2019), Hossain *et al.* (2020) and Begum *et al.* (2021).

4.7.2.6 1000-grain weight (g)

1000-grain weight exhibited significant positive correlation with days to 50% flowering (0.327**), plant height (0.446**), kernel length (0.236**), kernel breadth (0.703**), panicle length (0.298**) and grain yield per plant (0.266**). It has also shown significant negative correlation with number of grains per panicle (-0.270**), milling % (-0.432**), head rice recovery (-0.314**) and kernel L/B ratio (-0.447**).

Similar results were reported by Tara *et al*. (2001), Madhavilatha *et al*. (2002), Satish *et al*. (2003), Ashok *et al.* (2016), Panigrahi *et al.* (2018), Siddi (2018), Islam *et al.* (2019), Hossain *et al.* (2020), Lakshmi *et al.* (2020), Singh *et al.* (2020) and Rukmini Devi *et al.* (2022).

4.7.2.7 Hulling (%)

Hulling % exhibited significant positive correlation with milling % (0.407 **), days to 50% flowering (0.191*) and head rice recovery (0.229**). It has not shown significant negative correlation with any of the character studied. Similar results were reported by Prem *et al*. (2010), Begum *et al.* (2021) and Rukmini Devi *et al.* (2022).

4.7.2.8 Milling (%)

Milling % exhibited significant positive correlation with days to 50% flowering (0.180*), number of grains per panicle (0.180*), hulling % (0.407 **), head rice recovery (0.422**) and kernel L/B ratio (0.331**). However, it has also shown significant negative correlation with 1000 grains weight (-0.432**) and kernel breadth (-0.370**).

These results are in accordance with the findings of Ekka *et al.* (2011), Abdala *et al.* (2016), Prem Kumar *et al.* (2017), Rukmini Devi *et al.* (2017), Hemalatha (2018), Begum *et al.* (2021) and Rukmini Devi *et al.* (2022).

4.7.2.9 Head rice recovery (%)

Head rice recovery exhibited significant positive correlation with days to 50% flowering (0.397**), number of grains per panicle (0.479**), hulling % (0.229**) kernel L/B ratio (0.240**) and milling % (0.422**). However, it had shown significant negative correlation with 1000 grains weight (-0.314**) and kernel breadth (-0.316**). Abdala *et al.* (2016), Prem Kumar *et al.* (2017), Rukmini Devi *et al.* (2017), Hemalatha (2018), Begum *et al.* (2021) and Rukmini Devi *et al.* (2022) also reported similar results for this trait.

4.7.2.10 Kernel length (mm)

Kernel length exhibited significant positive correlation with 1000-grain weight (0.236**) and kernel L/B ratio (0.596 **). It had also shown significant negative correlation with number of grains per panicle (-0.278**) and kernel breadth (-0.215*).

Similar results were reported by Asha Christopher and Backiyarani (1999), Nayak *et al*. (2001), Lakshmi *et al.* (2020), Singh *et al.* (2020) and Rukmini Devi *et al.* (2022).

4.7.2.11 Kernel breadth (mm)

The association of kernel breadth was positive and significant with plant height (0.632**), panicle length (0.358**), grain yield per plant (0.248**) and 1000 grains weight (0.703**), whereas it had shown negative and significant correlation with days to 50% flowering (-0.288**), milling % (-0.370**), kernel length (-0.370**) and kernel L/B ratio (-0.899**).

Similar results were reported earlier by Meena *et al.* (2016), Santhi Priya *et al.* (2017), Lakshmi *et al.* (2020), Singh *et al.* (2020) and Rukmini Devi *et al.* (2022).

4.7.2.12 L/B ratio

Kernel L/B ratio exhibited significant positive correlation with days to 50% flowering (0.185*), head rice recovery (0.240**), number of productive tillers per plant (0.180*), milling % (0.331**) and kernel length (0.596 **) but had shown significant negative correlation with plant height (-0.554**), panicle length (-0.258 **), 1000 grains weight (-0.447**), grain yield per plant (-0.196*) and head rice recovery (-0.899**).

Similar results were reported earlier in rice by Santhi Priya *et al.* (2017), Siddi (2018), Lakshmi *et al.* (2020), Singh *et al.* (2020) and Rukmini Devi *et al.* (2022).

Table 4.9. Phenotypic (P) and Genotypic (G) correlation coefficients of yield, its components and quality traits in rice

Character		DFF	PH	PL	PT	GPP	TGW	H%	M%	HRR	KL	KB	L/B	SPY
DFF	**G**	**1.000**	-0.123	-0.016	0.210*	0.213*	-0.351**	0.513**	0.224*	0.431**	-0.142	-0.298**	0.196*	-0.172
	P	**1.000**	-0.115	0.016	0.102	0.179*	0.327**	0.191*	0.180*	0.397**	-0.139	-0.288**	0.185*	-0.143
PH	**G**		**1.000**	0.537**	0.003	0.174*	0.459	-0.263**	-0.205*	-0.085	-0.133	0.655**	0.569**	0.488**
	P		**1.000**	0.451**	-0.021	0.152	0.446**	-0.079	-0.165	-0.072	-0.132	0.632**	-0.554**	0.382**
PL	**G**			**1.000**	0.283**	-0.054	0.370**	0.114	-0.145	-0.016	0.148	0.437**	-0.310**	0.199*
	P			**1.000**	0.127	-0.029	0.298**	0.044	-0.092	-0.003	0.116	0.358**	-0.258 **	0.200*
PT	**G**				**1.000**	-0.263**	-0.040	-0.020	-0.317**	0.123	0.215**	-0.274**	0.301**	-0.012
	P				**1.000**	-0.085	-0.039	0.051	-0.113	0.0001	0.136	-0.154	0.180*	0.044
GPP	**G**					**1.000**	-0.308**	0.084	0.326**	0.575**	-0.316**	-0.027	-0.160	0.328**
	P					**1.000**	-0.270**	0.141	0.180*	0.479**	-0.278**	- 0.030	-0.134	0.314**
TGW	**G**						**1.000**	-0.426**	-0.509**	-0.341**	0.256**	0.737**	-0.467**	0.366**
	P						**1.000**	-0.155	-0.432**	-0.314**	0.236**	0.703**	-0.447**	0.266**
H%	**G**							**1.000**	0.199*	0.306**	-0.205*	-0.203*	0.087	0.049
	P							**1.000**	0.407 **	0.229**	-0.060	-0.087	0.051	0.045
M%	**G**								**1.000**	0.414	0.136	-0.433**	0.366**	0.252**
	P								**1.000**	0.422**	0.149	-0.370**	0.331**	0.163
HRR	**G**									**1.000**	-0.025	-0.339**	0.254**	0.148
	P									**1.000**	-0.008	-0.316**	0.240**	0.095
KL	**G**										**1.000**	-0.234**	0.600**	0.056
	P										**1.000**	-0.370**	0.596 **	0.033
KB	**G**											**1.000**	-0.907**	0.298**
	P											**1.000**	-0.899**	0.248**
L/B	**G**												**1.000**	-0.233**
	P												**1.000**	-0.196*

*** Significant at 5 percent level; ** Significant at 1 percent level**

DFF- Days to 50% flowering, **PH -** Plant height (cm), **PL -** Panicle length (cm), **PT -** Number of productive tillers, **GPP -** Number of grains per panicle, **TGW -** 1000 grains weight (g), **SPY -**Grain yield per plant (g), **H% -** Hulling (%), **M% -** Milling (%), **HRR -** Head rice recovery (%), **KL -** Kernel length (mm), **KB -** Kernel breadth (mm), **L/B -** L/B ratio.

4.8 Path coefficient analysis

The correlation coefficient between grain yield per plant and various component characters were partitioned into measure of direct and indirect effects through path coefficient analysis.

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 attributing characters. As discussed in correlation studies based on the importance of phenotypic effects, the results of phenotypic path coefficient of yield and yield contributing characters which are presented in Table 4.9, Figure 4.6 and 4.7 discussed under here.

4.8.1 Days to 50 % flowering

Days to 50% flowering had exhibited negligible direct phenotypic negative effect (-0.1421) on grain yield per plant and accordingly showed negative non-significant correlation with grain yield. The indirect and positive effect on grain yield was exhibited by days to 50% flowering through panicle length (0.0011), number of productive tillers per plant (0.0139), number of grains per panicle (0.0661), milling% (0.0641), kernel length (0.0316) and kernel L/B ratio (0.0674), whereas it showed indirect and negative effect through plant height (-0.0232), 1000-grain weight (-0.1456), hulling % (-0.0111), head rice recovery (-0.0174) and kernel breadth (0.0478).

Similar results were reported by Kavitha and Sree Rama Reddi (2001), Nayak *et al.* (2001), Keya Debnath *et al.* (2015), Meena *et al.* (2016), Bitew *et al.* (2018), Saha *et al.* (2019), Kiruthikadevi *et al.* (2020) and Rukmini Devi *et al.* (2022).

4.8.2 Plant height (cm)

Plant height had exhibited direct phenotypic positive effect (0.2012) and indirect positive effect through days to 50% flowering (0.0164), panicle length (0.0309), number of grains per panicle (0.0559), 1000-grain weight (0.1984), hulling % (0.0046), head rice recovery (0.0032), kernel length (0.0301) and kernel breadth (0.1050) on grain yield per plant and accordingly it showed positive correlation with grain yield per plant. Further, it has shown negative indirect effect on grain yield per plant through number of productive tillers per plant (-0.0029), milling % (-0.0588) and kernel L/B ratio (-0.2020).

Similar results were reported by Nandan *et al*. (2010), Rukmini *et al.* (2014), Rukmini Devi *et al.* (2017), Archana *et al.* (2018), Hossain *et al.* (2018) and Rukmini Devi *et al.* (2022) for positive direct effect of plant height on grain yield per plant.

4.8.3 Panicle length (cm)

Panicle length had exhibited direct phenotypic positive effect (0.0686) on grain yield per plant. The trait had positive and significant correlation (0.2000*) with grain yield. Further, it has shown positive indirect effect on yield through plant height (0.0908), number of productive tillers per plants (0.0173), 1000-grain weight (0.1326), head rice recovery (0.0002) and kernel breadth (0.0595) whereas negative indirect effect on grain yield per plant through days to 50% flowering (-0.0023), number of grains per panicle (-0.0109), hulling % (-0.0026), milling % (-0.0328), kernel length (-0.0266) and kernel L/B ratio (-0.0939). Similar results were reported by Bala (2001), Chakraborty *et al*. (2001), Kavitha and Sree Rama Reddi (2001), Tara *et al*. (2001), Nayak *et al*. (2004), Raju *et al.* (2004), Tulasi Guru *et al.* (2016), Patra and Das (2018), Siddi (2018), Kiruthikadevi *et al.* (2020) and Begum *et al.* (2021) for positive direct effect of panicle length on grain yield per plant.

4.8.4 Number of productive tillers per plant

Number of productive tillers per plant had exhibited low direct phenotypic positive effect (0.1367) on grain yield per plant. Whereas, the trait recorded positive non-significant correlation (0.0438) with grain yield. Further, it had positive indirect effect on yield through panicle length (0.0087), kernel L/B ratio (0.0657) and negative indirect effect through most of the characters *viz.,* days to 50% flowering (-0.0145), plant height (-0.0042), number of grains per panicle (-0.0311), 1000-grains weight (-0.0176), hulling % (-0.0030), milling % (-0.0402), kernel length (-0.0311) and kernel breadth (-0.0256).

These results are in line with the earlier reports of Edukondalu *et al*. (2017), Archana *et al*. (2018), Rachana *et al*. (2018), Kumar *et al.* (2018), Nanda *et al.* (2019) and Begum *et al.* (2021).

4.8.5 Number of grains per panicle

Number of grains per panicle exhibited direct phenotypic and positive effect (0.3682) on grain yield per plant and accordingly showed positive and significant correlation (0.3144**) with grain yield per plant. Moreover, the contribution of number

of grains per panicle on grain yield per plant through other traits was negligible in both positive and negative direction.

These results are in broad conformity with the earlier findings of Satish chandra *et al.* (2009), Nandan and Singh (2010), Meena *et al.* (2016), Prem kumar *et al.* (2017), Hemalatha (2018), Nanda *et al.* (2019), Saha *et al.* (2019), Hossain *et al.* (2020), Sudeepthi *et al.* (2020) and Rukmini Devi *et al.* (2022).

4.8.6 1000-grain weight (g)

1000-grain weight exhibited low direct phenotypic positive effect (0.4453) on grain yield per plant and accordingly contributed positive significant correlation (0.2657**) with yield. It has shown positive indirect effects on yield through days to 50% flowering (0.0465), plant height (0.0897), panicle length (0.0204), hulling % (0.0090), head rice recovery (0.0138), and kernel breadth (0.1167), whereas indirect negative effect through number of productive tillers (0.1167), number of grains per panicle (-0.0995), milling % (-0.1538), kernel length (-0.0539) and kernel L/B ratio (-0.1630).Similar results were reported by Nayak *et al*. (2001), Tara *et al.* (2001), Satish *et al.* (2003), Latha *et al*. (2003), Nayak *et al*. (2004), Siddi (2018) and Hossain *et al.* (2020) for positive direct effect of 1000-grain weight on grain yield per plant.

4.8.7 Hulling (%)

Hulling % has exhibited non-significant positive correlation (0.0447) on grain yield per plant, whereas, it has also shown direct negative effect (-0.0580). Further, it has shown positive indirect effect on yield through panicle length (0.0030), number of productive tillers per plant (0.0070), number of grains per panicle (0.0520), milling % (0.1447), kernel length (0.0138) and kernel L/B ratio (0.0187) and negative indirect effect through days to 50% flowering (-0.0271), plant height (-0.0160), 1000-grain weight (-0.0689), head rice recovery (-0.0100) and kernel breadth (-0.0144).

Nandan *et al* (2010), Prem Kumar *et al.* (2017), Begum *et al.* (2021) and Rukmini Devi *et al.* (2022) reported similar results for hulling %.

4.8.8 Milling (%)

Milling % exhibited low direct phenotypic positive effect (0. 1635) on grain yield per plant, whereas, it showed non-positive significant correlation with grain yield. However, the contribution of milling % on grain yield per plant through other traits was

negligible in both positive and negative direction. Begum *et al.* (2021) and Rukmini Devi *et al.* (2022) reported similar results for milling %.

4.8.9 Head rice recovery (%)

Head rice recovery exhibited negligible direct phenotypic positive effect (0.0952) on grain yield per plant; however, it showed negative non-significant correlation with grain yield. The contribution of head rice recovery on grain yield per plant through other traits was negligible in both positive and negative direction. Similar results were reported by Ekka *et al*. (2011) and Edukondalu *et al*. (2017) for positive direct effect of head rice recovery percentage on grain yield per plant. Similar results were reported by Ekka *et al*. (2011), Srinivas *et al.* (2016) and Edukondalu *et al*. (2017) for positive direct effect of head rice recovery percentage on grain yield per plant.

4.8.10 Kernel length (mm)

Kernel length had exhibited negligible direct phenotypic negative effect (-0.0326) on grain yield per plant, whereas, it had positive non-significant correlation with grain yield. Further, it showed positive indirect effect on yield through days to 50% flowering (0.0197), panicle length (0.0080), number of productive tillers (0.0186), 1000-grain weight (0.1051), hulling % (0.0035), milling% (0.0531), head rice recovery (0.0003) and kernel L/B ratio (0.2171). These results are in accordance with the findings of Rajeswari *et al*. (2010), Rukmini *et al.* (2014), Edukondalu *et al*. (2017) and Singh *et al.* (2020), for negative direct effect of kernel length on grain yield per plant.

4.8.11 Kernel breadth (mm)

Kernel breadth exhibited negligible direct phenotypic positive effect (0.1661) on grain yield per plant accordingly recorded significant positive correlation (0.2483**) with grain yield per plant. Further, it had indirect positive effect on grain yield per plant through days to 50% flowering (0.0409), plant height (0.1271), panicle length (0.0246), 1000-grain weight (0.3129), hulling % (0.0050), head rice recovery (0.0139) and kernel length (0.0491). Meena *et al.* (2016), Santhi Priya *et al.* (2017), Lakshmi *et al.* (2020) and Rukmini Devi *et al.* (2022) also reported similar findings for kernel breadth.

4.8.12 L/B ratio

Kernel L/B ratio exhibited negligible direct phenotypic positive effect (0.3643) on grain yield per plant whereas, it showed negative significant correlation (-0.1963*) with grain yield. However, the contribution of kernel L/B ratio on grain yield per plant through other traits was negligible in both positive and negative direction. These results

are in agreement with the findings of Ashok *et al.* (2016), Lakshmi *et al*. (2020), Begum *et al*. (2021), and Rukmini Devi *et al.* (2022).

4.8.13 Residual Effect

Residual effect was 0.7971 for phenotypic and 0.6227 for genotypic path coefficient analysis. High value of residual effect could be due to the influence of other traits which were not included in this study. The association of different component characters among themselves and with yield is quite important for devising 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 sometimes 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 on overall improvement in grain yield per plant directly. Path coefficient analysis revealed that 1000-grains weight has exhibited the highest positive direct effect on grain yield per plant followed by number of grains per panicle and milling % indicating that the selection for these characters may 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 potential for higher yield in rice genotypes.

Table 4.10. Phenotypic (P) and Genotypic (G) path coefficients of yield attributing traits in rice

Character		DFF	PH	PL	PT	GPP	TGW	H%	M%	HRR	KL	KB	L/B	SPY
DFF	**G**	**-0.8058**	-0.0276	0.0114	0.3089	0.2751	-0.592	0.562	0.3519	-0.3478	0.249	-0.8586	0.702	-0.1716
	P	**-0.1421**	-0.0232	0.0011	0.0139	0.0661	-0.1456	-0.0111	0.0641	-0.0174	0.0316	-0.0478	0.0674	-0.1429
PH	**G**	0.099	**0.2246**	-0.3804	0.0047	0.2246	0.7747	-0.2883	-0.3221	0.0688	0.2325	1.8873	-2.037	0.4883**
	P	0.0164	**0.2012**	0.0309	-0.0029	0.0559	0.1984	0.0046	-0.0588	0.0032	0.0301	0.1050	-0.2020	0.3820**
PL	**G**	0.013	0.1207	**-0.7078**	0.4165	-0.0701	0.6236	0.1252	-0.2282	0.0126	-0.258	1.2582	-1.1073	0.1986*
	P	-0.0023	0.0908	**0.0686**	0.0173	-0.0109	0.1326	-0.0026	-0.0328	0.0002	-0.0266	0.0595	-0.0939	0.2000*
PT	**G**	-0.1691	0.0007	-0.2002	**1.4723**	-0.3391	-0.0671	-0.0228	-0.4976	-0.0992	-0.3759	-0.7887	1.0751	-0.0116
	P	-0.0145	-0.0042	0.0087	**0.1367**	-0.0311	-0.0176	-0.0030	-0.0402	0.0000	-0.0311	-0.0256	0.0657	0.0438
GPP	**G**	-0.172	0.0391	0.0385	-0.3875	**1.2884**	-0.52	0.0916	0.5118	-0.4642	0.5517	-0.0784	-0.571	0.3280**
	P	-0.0255	0.0306	-0.0020	-0.0116	**0.3682**	-0.1203	-0.0082	0.0947	-0.0210	0.0635	-0.0050	-0.0490	0.3144**
TGW	**G**	0.2829	0.1032	-0.2618	-0.0586	-0.3974	**1.686**	-0.4671	0.5118	0.2749	-0.4469	2.121	-1.6705	0.3658**
	P	0.0465	0.0897	0.0204	-0.0054	-0.0995	**0.4453**	0.0090	-0.1538	0.0138	-0.0539	0.1167	-0.1630	0.2657**
H%	**G**	-0.4133	-0.0591	-0.0809	-0.0307	0.1077	-0.7188	**1.0957**	0.3129	-0.2474	0.3576	-0.5847	0.3105	0.0495
	P	-0.0271	-0.0160	0.0030	0.0070	0.0520	-0.0689	**-0.0580**	0.1447	-0.0100	0.0138	-0.0144	0.0187	0.0447
M%	**G**	-0.1804	-0.046	0.1028	-0.4661	0.4196	-0.8582	0.2181	**1.5716**	-0.3346	-0.2384	-1.2469	1.311	0.2524**
	P	-0.0256	-0.0333	-0.0063	-0.0155	0.0981	-0.1926	-0.0236	**0.3556**	-0.0185	-0.0341	-0.0614	0.1207	0.1635
HRR	**G**	-0.3472	-0.0191	0.0111	0.1809	0.7409	-0.5742	0.3359	0.6514	**-0.8072**	0.0433	-0.9762	0.9087	0.1482
	P	-0.0564	-0.0146	-0.0002	0.0000	0.1764	-0.1397	-0.0133	0.1501	**-0.0438**	0.0017	-0.0526	0.0875	0.0952
KL	**G**	0.1148	-0.0299	-0.1045	0.3167	-0.4069	0.4313	-0.2243	0.2145	0.0200	**-1.7471**	-0.6747	2.1461	0.0561
	P	0.0197	-0.0265	0.0080	0.0186	-0.1025	0.1051	0.0035	0.0531	0.0003	**-0.2282**	-0.0357	0.2171	0.0326
KB	**G**	0.2403	0.1472	-0.3093	-0.4033	-0.0351	1.2422	-0.2225	-0.6807	0.2737	0.4094	**2.879**	-3.2429	0.2979**
	P	0.0409	0.1271	0.0246	-0.0211	-0.0111	0.3129	0.0050	-0.1315	0.0139	0.0491	**0.1661**	-0.3276	0.2483**
L/B	**G**	-0.1582	-0.1279	0.2191	0.4425	-0.2057	-0.7874	0.0951	0.576	-0.2051	-1.0482	-2.6101	**3.5769**	-0.2328**
	P	-0.0263	-0.1115	-0.0177	0.0247	-0.0495	-0.1992	-0.0030	0.1178	-0.0105	-0.1360	-0.1494	**0.3643**	-0.1963*

*** Significant at 5 percent level; ** Significant at 1 percent level; Genotypic Residual effect = 0.6227; Phenotypic Residual effect = 0.7971; Bold values are direct effects**

DFF- Days to 50% flowering, **PH -** Plant height (cm), **PL -** Panicle length (cm), **PT -** Number of productive tillers, **GPP -** Number of grains per panicle, **TGW -** 1000 grains weight (g), **SPY -**Grain yield per plant (g), **H% -** Hulling (%), **M% -** Milling (%), **HRR -** Head rice recovery (%), **KL -** Kernel length (mm), **KB -**Kernel breadth (mm), **L/B -** L/B ratio.

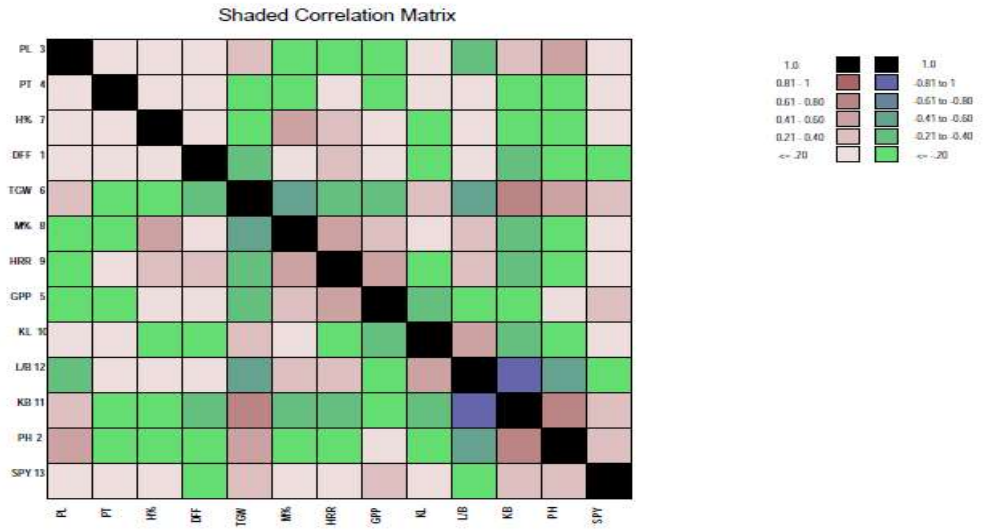


Figure 4.6. Correlation studies for 13 characters of rice lines during *Rabi*, 2021-22

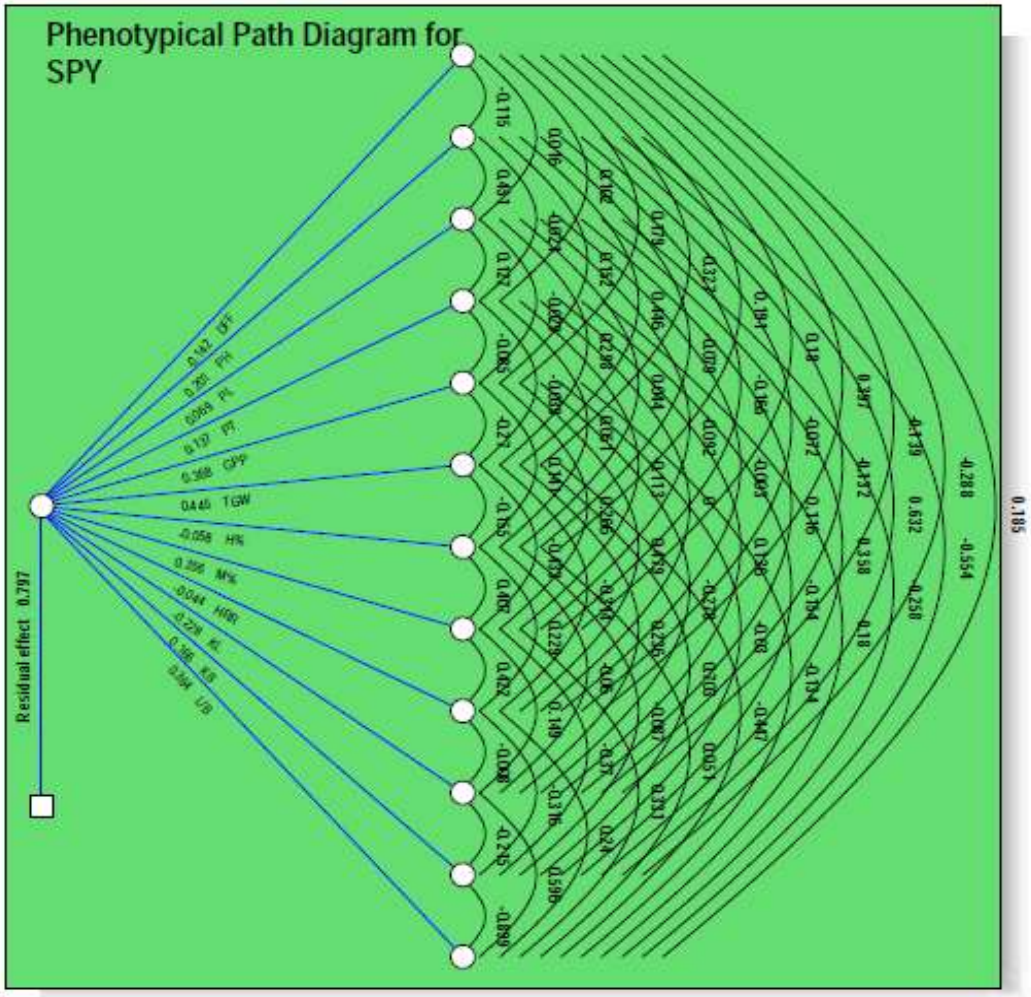


Figure 4.7. Phenotypical Path diagram for grain yield per plant

Chapter V

Summary and Conclusion

Chapter V

SUMMARY AND CONCLUSION

The Asian gall midge, *Orseolia oryzae* is one of the serious insect pests of rice responsible for causing significant annual yield loss in several South and Southeast Asian countries including India, Bangladesh, China, Indonesia, Myanmar, Sri Lanka and Vietnam. In India, it is rated as third most important pest of rice in terms of spread and severity of damage and yield loss, next to stem borer and plant hopper. Since chemical control of the pest is not economical, cultivation of rice varieties possessing one or more gall midge resistance genes has been recommended. However, genotypes with one or more gall midge resistance genes and new sources of resistance must be identified and utilized in rice breeding programs.

The present study entitled **"Genetic analysis and identification of genotypes with novel sources of resistance for gall midge (Biotype 3) in rice *(Oryza sativa* L.*)"*** was carried out utilizing 42 rice entries comprised of land races, released varieties and advanced breeding lines. The entries for genotyping were selected based on field screening against gall midge biotype 3 (GMB3) during *kharif*, 2021 at RARS, Polasa, Jagtial. These test lines were further genotypically characterized by using *gm3, Gm4* and *Gm8* based functional SSR markers for presence or absence of resistance gene(s). Further these 42 entries were subjected to genetic divergence, correlation and path analysis during *rabi*, 2021-22 at RARS, Polasa, Jagtial. The results of the present study are summarized as follows:

Field evaluation and molecular conformation of test entries

Among 42 entries screened, per cent of silver shoots ranged from 0 to 84.18 per cent. "Nil" gall midge incidence was noticed in four test entries *viz.,* Kakai, WGL-1145, WGL-1147 and WGL-1127 and they had shown high resistance (score 0) and two entries namely RP-5332-54-11-8-2-13 and IR72476-B-P-9-3-1-1 found to be resistant with 1 score. In total, 6 entries were found promising against gall midge under field conditions. The resistant check, Aganni recorded "Nil" damage and susceptible check TN1 found to be highly susceptible with 79.09 per cent silver shoots at 50 DAT. Genotyping of six gall midge resistant lines with functional markers "gm3del3" for *gm3* gene, "LRR del" for *Gm4* gene and "PRP" for *Gm8* gene revealed that Kakai showed the presence of *Gm4* and *Gm8* genes, WGL-1145 showed the presence of all three genes, WGL-1147 and WGL-1127 showed the presence of *Gm4* and *Gm8* genes, RP-

5332-54-11-8-2-13 showed the presence of *Gm8* gene and IR72476-B-P-9-3-1-1 showed the presence of g*m3* gene.

Genetic analysis

Observations were recorded on five randomly selected plants in each plot in each replication for the characters plant height (cm), panicle length (cm), number of productive tillers per plant, number of grains per panicle, grain yield per plant (g). However, observation on days to 50% flowering were made on whole plot basis and random sample in each plot was used for recording observations of 1000-grain weight (g), kernel length (mm), kernel breadth (mm), kernel L/B ratio, hulling %, milling % and head rice recovery %.

Analysis of variance revealed significant variability among the genotypes for all the traits studied indicating presence of high variability among the rice genotype. Thus, there is ample scope for selection of different quantitative and qualitative characters for improvement with regards to the traits under investigation.

A perusal of genetic parameters revealed that phenotypic and genotypic coefficients of variation were high for characters namely number of grains per panicle, 1000grains weight, grain yield per plant, head rice recovery and kernel L/B ratio suggesting the presence of wide variability among the genotypes with regard to these characters. Whereas moderate values were registered for plant height, number of productive tillers, milling %, kernel length and kernel breadth suggesting moderate variability among genotypes with regard to these characters. The values of genotypic and phenotypic coefficients of variation were low for days to 50% flowering, panicle length and hulling% indicating the traits are highly influenced by the environment and selection would be ineffective.

High heritability coupled with high genetic advance as per cent of mean was observed for plant height, number of grains per panicle, 1000 - grains weight, grain yield per plant, head rice recovery, kernel length, kernel breadth and kernel L/B ratio indicated that these traits were controlled by additive genes and can be further improved by following simple selection procedure. The high estimates of heritability coupled with moderate genetic advance as per cent of mean were observed for days to 50% flowering, panicle length, number of productive tillers and milling (%) indicating that these characters are little influenced by environment and governed by both additive and non-additive genes. Low estimates of heritability coupled with low genetic advance as

per cent of mean was observed for hulling (%) indicated the presence of non- additive gene effects in addition to influence of environment to some extent.

The genetic divergence was high among the 42 rice genotypes and was grouped into seven different clusters. Highest number (18) of genotypes were grouped in cluster I followed by the cluster III (13) and cluster II (7). However, cluster IV, V, VI and VII were represented by single genotype indicating high degree of heterogeneity among the genotypes.

Divergence studies through D^2 statistics indicated the presence of substantial diversity by forming large number of clusters with wide range of inter-cluster distances. The highest inter cluster distance was observed between cluster II and VII (418.05) followed by cluster IV and VII (350.95), cluster II and III (298.15) and cluster II and V (281.16). The lowest inter cluster distance was observed between cluster V and VI (54.44). The greater the distance between two clusters, the greater the genetic diversity between the genotypes belonging those clusters. The genotypes belonging to most divergent clusters may exploit the maximum amount of heterosis. High intra cluster distance was observed in cluster III (85.85) followed by cluster II (56.94) and cluster I (55.10) revealing that some genetic divergence still existed among the genotypes of these cluster. The intra cluster distances were lower than inter cluster distances, indicating the existence of genetic diversity among the genotypes of different clusters, which could be made use of in the yield improvement through recombination breeding. Keeping this in view, it is concluded that crossing between the genotypes of cluster II and VII, cluster VI and VII, cluster II and III and cluster II and V could results in evolution of desirable transgressive segregants.

Based on cluster mean analysis it can be concluded that the genotypes of cluster II can be used in breeding program for generating gall midge resistant lines with less plant height and fine grain nature. Similarly, crosses between genotypes of cluster II and VII could results in development of coarse grain varieties with gall midge resistance. Didianga (Cluster V) with good yield potential can be crossed with lines from diverse groups for generating high yielding lines. The genotypes namely, Didianga (Cluster V), Kakirekkalu (Cluster VI) and Geetanjali (Cluster IV) were found to be good sources for development of lines with good head rice recovery. The per cent contribution towards total genetic divergence was greater for the character kernel breadth (37.39%) followed by plant height (14.52 %) and kernel length (11.03 %). Therefore, these characters should be given importance during hybridization and selection.

Character association studies revealed that grain yield per plant showed significant and positive association with panicle length, number of grains per panicle, 1000-grains weight, plant height and kernel breadth indicating the simultaneous selection for these characters could improve the yield.

Path coefficient analysis revealed that 1000-grains weight exhibited the highest positive direct effect on grain yield per plant followed by number of grains per panicle and milling % 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 higher yield.

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APPENDICES

Appendix 3.1. List of genotypes utilized for the research programme in rice during *kharif, 2021*

Sl. No.	Entry name	Sl. No.	Entry name
1.	IR72476-B-P-9-3-1-1	22.	Mahamaya
2.	IR-74101-3R-1-1	23.	CGZR 2
3.	IR-72049-B-R-22-3-1-1	24.	JGL 34594
4.	Calotoc	25.	RDR 1210
5.	Dular	26.	Kamar Samba
6.	KNM-630	27.	Kudel
7.	KNM-604	28.	Njavara
8.	NDLR-7	29.	Laicha
9.	Sampada	30.	Kalabunt
10.	Dukong-1	31.	Kakirekaalu
11.	CGZR 1	32.	Manipuri black
12.	RP-5332-54-11-8-2-13	33.	Burma Black
13.	HH-25-SAL-10-D2-DT-1	34.	Didianga
14.	MUT NS 1	35.	Geetanjali
15.	IR-04A-216	36.	Umleng-1
16.	NSN 1/296-2016	37.	KAKAI
17.	Betangamblin	38.	AGANNI
18.	Chittimutyalu	39.	WGL-1145
19.	IRBL-T-K59	40.	WGL-1147
20.	IRTON 270	41.	WGL-1127
21.	Kapurala vadlu	42.	TN1

Appendix 3.2. List of equipments used in molecular analysis

Equipment	Company or Supplier
Autoclave	Equitron
Thermal cycler	Master Cycler (Eppendorf)
Gel electrophoresis units	Biorad
Freezer-20 ℃ and -80℃	Sanyo biomedical freezer
Gel documentation system	Biorad
Ice maker	Sanyo
Magnetic stirrer	Genei
Microwave oven	LG
Centrifuge	Eppendorf
Pipettes	Thermo scientific
pH meter	Thermo orion
UV absorbance spectrophotometer	Thermo electronic corporation
UV Transilluminator	Vilber Lourmat
Vortex mixer	Genei
Water bath	Cintex

Appendix 3.3. List of Chemicals used in molecular analysis

Chemical	Make
Agarose	Sigma
6X loading dye	Genei
Chloroform	Qualigens
CTAB	Himedia
dNTP's (Deoxy nucleotide triphosphates)	Biogene
EDTA (Ethylene diamino tetra acetic acid)	Himedia
Ethidium bromide	Sigma
Ethyl alcohol	Hayman
Isoamyl alcohol	Qualigens
Isopropanol	Qualigens
NaCl (Sodium chloride)	Qualigens
NaOH (Sodium hydroxide)	Qualigens
Poly vinyl pyrrolidone	Sigma
Taq polymerase	Ras lifesciences
Trizma base	Sigma
50 bp ladder	NEB
$MgCl_2$ buffer	Ras lifesciences
Primers	Sigma

Appendix 3.4. List of genotypes utilized for the research programme in rice during *rabi*,2021-22

Sl. No.	Entry name	Sl. No.	Entry name
1.	IR72476-B-P-9-3-1-1	22.	Mahamaya
2.	IR-74101-3R-1-1	23.	CGZR 2
3.	IR-72049-B-R-22-3-1-1	24.	JGL 34594
4.	Calotoc	25.	RDR 1210
5.	Dular	26.	Kamar Samba
6.	KNM-630	27.	Kudel
7.	KNM-604	28.	Njavara
8.	NDLR-7	29.	Laicha
9.	Sampada	30.	Kalabunt
10.	Dukong-1	31.	Kakirekaalu
11.	CGZR 1	32.	Manipuri black
12.	RP-5332-54-11-8-2-13	33.	Burma Black
13.	HH-25-SAL-10-D2-DT-1	34.	Didianga
14.	MUT NS 1	35.	Geetanjali
15.	IR-04A-216	36.	Umleng-1
16.	NSN 1/296-2016	37.	KAKAI
17.	Betangamblin	38.	AGANNI
18.	Chittimutyalu	39.	WGL-1145
19.	IRBL-T-K59	40.	WGL-1147
20.	IRTON 270	41.	WGL-1127
21.	Kapurala vadlu	42.	TN1

Appendix 4.1. Reaction of different rice entries against rice gall midge

Sl. No.	Name of Entry	Silver Shoot (SS) %		Reaction
		30 DAT	50 DAT	
1	IR72476-B-P-9-3-1-1	2.14	0.65	R
2	IR-74101-3R-1-1	1.26	9.29	MS
3	IR-72049-B-R-22-3-1-1	6.18	67.43	HS
4	WGL-1127	0.00	0.00	HR
5	WGL-1145	0.00	0.00	HR
6	WGL-1147	0.00	0.00	HR
7	Calotoc	9.09	58.51	HS
8	Dular	4.35	24.66	S
9	KNM-630	4.72	69.92	HS
10	KNM-604	9.41	72.41	HS
11	NDLR-7	8.39	73.36	HS
12	Sampada	11.25	65.08	HS
13	Dukong-1	9.79	64.43	HS
14	RP-5332-54-11-8-2-13	1.44	0.52	R
15	HH-25-SAL-10-D2-DT-1	5.26	24.88	S
16	MUT NS 1	5.63	55.21	HS
17	IR-04A-216	2.73	45.23	HS
18	NSN 1/296-2016	4.67	39.75	HS
19	Betangamblin	10.91	66.98	HS
20	Chittimutyalu	1.53	67.63	HS
21	IRBL-T-K59	12.18	84.18	HS
22	IRTON 270	16.29	70.90	HS
23	Kapurala vadlu	5.71	70.41	HS
24	Mahamaya	9.63	74.15	HS
25	CGZR 1	10.000	43.12	HS
26	CGZR 2	6.25	42.59	HS
27	JGL 34594	11.76	69.6	HS
28	RDR 1210	7.32	62.11	HS
29	Kamer samba	3.00	50.78	HS
30	Kudel	6.71	66.04	HS
31	Njavara	2.67	36.80	HS
32	Laicha	6.57	24.51	S
33	Kalabunt	12.96	64.86	HS
34	Kakirekaalu	2.65	52.67	HS

35	Manipuri black	5.98	57.94	HS
36	Burma Black	9.94	40.83	HS
37	Kakai	0.00	0.00	HR
38	Didianga	5.24	53.5	HS
39	Geetanjali	13.57	70.65	HS
40	Umleng-1	4.93	56.11	HS
41	Aganni (R. Check)	0.00	0.00	HR
42	TN 1 (S. Check)	9.25	79.09	HS

HR-Highly resistant, R-Resistant, MR- Moderately resistant, MS- Moderately susceptible, S- Susceptible, HS- Highly susceptible