

**VARIABILITY STUDIES FOR EARLY VIGOUR,
ANAEROBIC GERMINATION, YIELD
COMPONENTS IN RICE (*Oryza sativa* L.)
GENOTYPES**

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DECLARATION

I, **Ms. M. SRILAKSHMI CHAITANYA**, hereby declare that the thesis entitled “**VARIABILITY STUDIES FOR EARLY VIGOUR, ANAEROBIC GERMINATION, YIELD COMPONENTS IN RICE (*Oryza sativa* L.) GENOTYPES**”, submitted to **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** in the major field of **Genetics and Plant Breeding** is the result of original research work done by me. I also declare that any material in the thesis has not been published earlier.

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CERTIFICATE

Ms. M. SRILAKSHMI CHAITANYA has satisfactorily prosecuted the course of research and that the thesis entitled **“VARIABILITY STUDIES FOR EARLY VIGOUR, ANAEROBIC GERMINATION, YIELD COMPONENTS IN RICE (*Oryza sativa* L.) GENOTYPES”** submitted is the result of original research work and is of sufficiently high standard to warrant it's presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by her for a degree of any university.

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This is to certify that thesis entitled “**VARIABILITY STUDIES FOR EARLY VIGOUR, ANAEROBIC GERMINATION, YIELD COMPONENTS IN RICE (*Oryza sativa* L.) GENOTYPES**” submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture in the major field of **Genetics and Plant Breeding** of the Acharya N. G. Ranga Agricultural University, Guntur, is a record of the bonafied research work carried out by **Ms. M. SRILAKSHMI CHAITANYA** under our guidance and supervision. The subject of the thesis has been approved by the student’s advisory committee.

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

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LIST OF SYMBOLS AND ABBREVIATIONS

$\bar{X}$	:	Grand mean
μl	:	Micro litre
μg	:	Micro gram
nm	:	Nano metre
pM	:	Pico mole
σ^2	:	Variance
%	:	per cent
$^{\circ}\text{C}$	:	Degree centigrade
AG	:	Anaerobic germination
ATP	:	Adenosine triphosphate
BC ₂	:	Back cross 2 with parent 2
BC3	:	Back cross 3 with parent 3
BPT	:	Bapatla
Conc.	:	Concentration
CD	:	Critical difference
cm	:	Centimeter
CTAB	:	Cetyl Tris Methyl Ammonium Bromide
CV	:	Coefficient of variation
DNA	:	Deoxy Ribo Nucleic acid
DAS	:	Days after sowing
dNTP	:	Deoxy nucleotide triphosphate
df	:	Degrees of freedom
<i>et al.</i>	:	and coworkers
EDTA	:	Ethidium diamino tri acetate
ESS	:	Error sum of squares
F1	:	First filial generation of a cross
F2	:	Second filial generation of a cross
F3	:	Third filial generation of a cross
F ₄	:	Fourth filial generation of a cross
Fig	:	Figure
JGL	:	JagthIyal
g	:	Grams
G	:	Genotypic
GCV	:	Genotypic coefficient of variation
GA	:	Gibberilic acid
GAM	:	Genetic advance as per cent of mean
h^2	:	Heritability
h^2 (b)	:	Heritability in broad sense
HCl	:	Hydrochloric acid

H ₂ SO ₄	:	Sulphuric acid
<i>i.e.</i>	:	That is
kg	:	Kilogram
m	:	Meter
m ²	:	Square meter
M	:	Molar
mM	:	milli molar
mg	:	milli gram
mg g ⁻¹	:	milligram per gram
mha	:	million hectares
ml	:	milli litre
mt	:	million tons
MgCl ₂	:	Magnesium chloride
MSS	:	Mean sum of squares
MTU	:	Maruteru
ng	:	Nano grams
N	:	Normality
NS	:	Non-significant
NaCl	:	Sodium chloride
NLR	:	Nellore
P	:	Phenotypic
<i>Per se</i>	:	As such with mean
PCV	:	Phenotypic coefficient of variation
PCR	:	Polymerase Chain Reaction
PIC	:	Ploymorphic information content
rpm	:	Revolutions per minute
RGL	:	Ragole
RSS	:	Replication sum of squares
S	:	Significant
SS	:	Sum of squares
SSR	:	Simple sequence repeats
TE	:	Tris EDTA
TAE	:	Tris acetate EDTA
TrSS	:	Treatment sum of squares
V	:	Volts
<i>viz.,</i>	:	Namely

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ABSTRACT

Name of the Author	: M. SRILAKSHMI CHAITANYA
Title of the thesis	: VARIABILITY STUDIES FOR EARLY VIGOUR, ANAEROBIC GERMINATION, YIELD COMPONENTS IN RICE (<i>Oryza sativa</i> L.) GENOTYPES
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The present investigation was carried out during *kharif*, 2016 at Agricultural College Farm, Bapatla, with 48 genotypes of Rice (*Oryza sativa* L.) to elicit information on the nature and extent of variability, heritability, genetic advance for early vigour & yield components and character association, the magnitude of direct and indirect effects of early vigour traits under anaerobic conditions and molecular diversity at RRU, Bapatla and APRRI & RARS, Maruteru.

The analysis of variance results revealed significant differences among the genotypes for all the characters studied indicating that the data generated from the above diverse material shall represent wide variability. The results of genetic parameters among yield components revealed high heritability coupled with high genetic advance as percent of mean for plant height, number of ear bearing tillers, grain yield and test weight. Under field conditions, shoot length at 7 & 14 days after sowing, root length at 7 & 14 days after sowing, dry matter at 7 & 14 days after sowing and vigour index manifested high heritability and coupled with high genetic advance as percent of mean. Germination percentage after 7 and 14 days of submergence and total soluble sugars also recorded high heritability and genetic advance as percent of mean indicating the operation of additive gene action in the inheritance of the trait. Hence good response to selection can be attained for improvement of these trait.

Among all genotypes studied NLR 4002, BPT 2673, NLR 33892, MTU 5182, MTU 1075, BPT 2740 and JGL 17004 recorded high grain yield under dry direct sowing. Among the genotypes studied, 28 exhibited resistance to anaerobic conditions upto one week of submergence suggesting their suitability for direct sowing and their tolerance to submergence for shorter period immediately after sowing. When we consider both grain yield per plant and anaerobic germination percentage the genotypes viz., BPT 2740, MTU 5249, MTU 2716, IR 85961-23-1-2-1, MTU 1140, BPT 2595, BPT 2782, BPT 2411, BPT 2573 and MTU 1064 can be recommended for direct sowing under anaerobic conditions also. Under 14 days of submergence

only 13 genotypes exhibited their resistance to anaerobic germination with > 75% germination. Hence these genotypes can be recommended for direct seeding or utilized as donors for incorporating this trait into other cultivars/varieties.

Under 21 days of submergence in 10cm depth, the genotype AC 39416A recorded 100% germination. Other genotypes viz., AC 39416A, AC 39397, IR 85961-23-1-2-1, BPT 2411, MTU 5249, MTU 1140 and BPT 2595 also exhibited more than 50 % germination under 21 days of submergence. The morphological adjustment enabled these cultivars to access oxygen at the water surface earlier which supported the growth of organs such as the primary leaf and root. also possess high amount of total soluble sugars and free amino acids. All these genotypes exhibited high values for shoot length, vigour index and anaerobic response index, root length, dry matter and number of leaves indicating the cumulative effect of all these traits which contributed for high anaerobic germination.

The character association studies among the yield components revealed that plant height, panicle length and test weight manifested non-significant and positive association with grain yield whereas number of ear bearing tillers showed significant positive association with grain yield. Likewise plant height also exhibited significant and positive correlation with panicle length, test weight, dry matter at 7 days after sowing and vigour index under field conditions indicating simultaneous improvement of these traits. The association studies among early vigour traits under field conditions revealed that dry matter 14 at days after sowing and germination percentage exhibited negative and significant association with grain yield. Shoot length at 7 DAS showed positive and significant association with shoot length at 14 days after sowing, dry matter at 7 and 14 days after sowing and vigour index. Similarly under anaerobic conditions also shoot length at 7 days of submergence manifested positive and significant association with shoot length after 14 days of submergence, root length after 7 days of submergence, dry matter after 7 days of submergence, anaerobic germination percentage under 7 and 14 days of submergence, vigour index under 7 and 14 days of submergence and total soluble sugars suggesting that the genotypes possessing high shoot length, root length, vigour index, dry matter and total soluble sugars would lead to better crop establishment under anaerobic conditions. Free amino acids and total soluble sugars also exhibited positive association with grain yield.

Studies of path analysis among early vigour related traits under anaerobic conditions with grain yield revealed that the characters, shoot length after 7 days of submergence, root length after 14 days of submergence, vigour index after 14 days of submergence, free amino acids and total soluble sugars manifested positive correlation coefficient coupled with positive direct effect on grain yield. Hence simple selection of these traits would improve the grain yield.

Twelve reported microsatellite (SSR) markers for anaerobic germination in rice were used to study the molecular diversity among the 48 genotypes under study. Among these, 8 markers exhibited polymorphism for this trait. The number of alleles detected per primer ranged from 2 to 5 with a mean of 2.91. The cluster analysis studies by the SSR markers classified the genotypes into two major clusters at 62% similarity level. Cluster I comprised of 18 genotypes which were further sub-divided into 2 clusters. Most of the elite breeding lines were grouped in cluster II with 30 genotypes which was further sub grouped into 2 clusters. The results indicates the genotypes expressed divergence based on ancestry and place of origin of genotypes.

Three genotypes viz., BPT 2411, MTU 1064, RGL 1414 with higher anaerobic germination percentage at 2 weeks and 3 weeks were grouped in cluster I. The genotype BPT

2411, manifested similar amplification pattern for qAG-2 linked alleles RM 6318. It clearly indicates gene/ QTL responsible for anaerobic germination are specific to genotypes which are to be identified by further QTL mapping.

The present investigation is aimed at identifying varieties/cultivars tolerant to anaerobic conditions combining high grain yield which can be recommended for direct sowing in rainfed and irrigated lowland areas. Further studies are recommended to test the survival rate and establishment of seedlings after removal of water from direct sown paddy field and to estimate the growth parameters and yield potential also.

Chapter I

INTRODUCTION

Rice is one of the most important food crops in the world and staple food for more than half of the global population. Looming water crisis, water intensive nature of rice cultivation and escalating labour costs drive the search for alternative management methods to increase water productivity in rice cultivation. Recent trend in rice production is marked with the shift from transplanting to direct seeding notably in areas where there is scarcity of irrigation water and high cost of labour. In India, rice is cultivated over an area of 43.95 Mha with a production of 104.80 Mt of milled rice and productivity of 2390 kg ha⁻¹ (Indiastat, 2014-15). In Andhra Pradesh the area, production and productivity of rice are 23.94 lakh ha, 88.70 lakh tonnes of milled rice and 3520 kg ha⁻¹ respectively (Statistical Abstracts of AP, 2015).

Of the total rice area in India, 49.5 % is irrigated, 13.5 % is upland and 32.4 % is rainfed lowland. In rainfed areas, waterlogging and shallow floods hinder adoption of direct seeded rice, especially when heavy rains occurs just after sowing. Poor emergence and mortality of young seedlings caused by submergence result in poor crop establishment and low grain yield (Ismail *et al.* 2009). Developing varieties that can germinate in flooded soils could be beneficial for direct seeded systems in these areas and even for intensive irrigation systems, where early flooding can suppress weeds (Ismail *et al.* 2012). This will consequently result in enormous savings in production costs in comparison to transplanted paddy. It can also reduce the costs of manual or mechanical weeding or the use of hazardous chemicals for weed control.

Despite being a semi-aquatic species, rice is sensitive to various types of flooding stress and this sensitivity varies with the genotypes, stages of development, duration and depth of flooding (Mackill *et al.* 2012). Even shallow flooding of the soils caused by heavy rainfall soon after seeding can reduce germination and result in poor crop establishment, since rice is sensitive to flooding during germination (Yamauchi *et al.* 1993; Ismail *et al.* 2009). Rice seeds can germinate and to some degree, extend their coleoptiles under hypoxic and even anoxic conditions, but fail to develop roots and leaves (Biswas and Yamauchi, 1997). Only tolerant genotypes have the ability of fast elongation of coleoptiles and root formation under submerged conditions in the field (Ismail *et al.* 2009). Tolerance of anaerobic conditions at these early stages is a prerequisite for effective direct seeded rice in rainfed and flood-affected areas.

Early seedling vigour is very important in direct sown varieties as the varieties possessing high early seedling vigour comes out from submerged fields, maintain sufficient crop stand and also suppresses weed growth which is a major problem in direct sown cultivation. Seedling vigour is a complex trait and is found to be associated with germination and seedling growth (Diwan *et al.*, 2013). The increasing importance of direct seeding in many Asian countries has made it critical to improve the seedling vigour of rice cultivars (Dingkuhn *et al.*, 1992).

Breeding for better germination, greater seedling vigour and higher tolerance of water logging in rice has been attempted before but with limited success because genotypes with sufficient tolerance were not available. The use of molecular markers (DNA markers) provides effective selection technique for crop improvement and has an added advantage to selection based on phenotype and have been extensively used for genetic dissection of agriculturally important traits in rice.

The estimation of genotypic variation alone may not provide necessary information on heritable variation. Hence, information on heritability along with genetic advance will be of immense help to the breeder in selecting superior individuals for a desired trait and successfully utilizing them in breeding programme. Correlation analysis is very essential to know the mutual relationship between two variables. It measures the degree of closeness and the linear relationship between two variables. The study of correlations may help the plant breeder to know how the improvement of one character will bring simultaneous improvement in other characters.

Path coefficient analysis is a standardized regression coefficient and measures the direct influence of one variable upon the other. 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. Selection would be more effective for the trait, which has got high positive direct effect and high correlation with grain yield per plant. Keeping in view of the above, the present research work is formulated with the following objectives:

Objectives of investigation

1. To study the variability parameters for root and shoot characters under anaerobic conditions.
2. To study the variability for seedling vigour and yield components under dry direct sowing.
3. To study the association of early vigour traits with anaerobic germination
4. To study the molecular diversity of genotypes using reported SSR markers.

Chapter II

REVIEW OF LITERATURE

Review of earlier reports pertaining to the present investigation in rice is presented under the following headings:

- 2.1 Early vigour and anaerobic germination
- 2.2 Variability, heritability and genetic advance as per cent of mean
- 2.3 Correlation and path analysis
- 2.4 Total soluble sugars and free amino acids
- 2.5 Molecular diversity on anaerobic germination

2.1. EARLY VIGOUR AND ANEROBIC GERMINATION

Early vigour is important in direct sown rice systems (Balasubramanian and Hill, 2002 and Cairns *et al.*, 2009). Rapid early growth increases stand establishment and weed competitiveness, both of which are important components for high yields in Direct sown rice (Zhao *et al.*, 2006). Traits like root length, shoot length, seedling dry weight and vigour index helps in assessing early vigour of rice genotypes under dry direct sowing.

Direct-seeding is becoming more important in rainfed lowlands because of its lower cost compared with transplanting. However, this approach is hindered by the risk of early flooding as rice is very sensitive to submergence during germination and early growth stages. Hence, identification of new sources of tolerance to submergence during germination (anaerobic germination) is of immense value in the development of improved lines with submergence tolerance at this stage (Reddy *et al.*, 2009).

Uma *et al.* (2017) evaluated a set of 253 BC₃F₄ lines derived from cross between Swarna and Moroberekan for early vigour and 8 related traits viz., early uniform emergence, shoot length, stem length, shoot fresh weight, total fresh weight, shoot dry weight, total dry weight and root dry weight in field as well as in the glass house. Trait relationships among the agro-physiological traits were examined to select the best genotypes for the given traits for use in future breeding programs.

Divya *et al.* (2016) reported that seed germination decreased significantly with increase in the grade of discolouration and storage period.

Yadav (2015) studied fifteen genetically diverse and promising varieties of rice for the characters like coleoptile length, root length, shoot length, seedling vigour index, root volume and seed yield under four agro-ecosystems in order to identify their suitability under direct seeding.

Adachi *et al.* (2015) compared four lines selected for anaerobic germination (AG lines). The α -amylase activity in germinating seeds was significantly higher in IR06F459 than in IR42 and identified that IR06F459, an AG line with a long coleoptiles has high α -amylase activity, high sucrose and glucose concentrations in germinating seeds.

Kretschmar *et al.* (2015) identified a trehalose-6-phosphate phosphatase gene, OsTPP7, as the genetic determinant in qAG-9-2, a major quantitative trait locus (QTL) for anaerobic germination tolerance. OsTPP7 is involved in trehalose-6-phosphate (T6P) metabolism, central to an energy sensor that determines anabolism or catabolism depending on local sucrose availability. OsTPP7 activity may increase sink strength in proliferating heterotrophic tissues by indicating low sugar availability through increased T6P turnover, thus enhancing starch mobilization to drive growth kinetics of the germinating embryo and elongating coleoptile, which consequently enhances anaerobic germination tolerance.

Frederickson *et al.* (2014) screened 343 accessions for anaerobic germination under green house conditions. Germination and seedling survival were measured 14 days after sowing and dry weights of the emerging seedlings were assessed at 21 days after sowing.

Garud *et al.* (2014) reported that characters like germination, vigour index, shoot length and seedling dry weight were high at physiological maturity. Harvesting of soybean at physiological maturity significantly increased the germination percentage, shoot length and seedling dry weight

Mohanty *et al.* (2012) identified patterns established from the global analysis of cis-element distribution for upregulated and downregulated genes and their associations with potential cognate regulatory transcription factors indicating the significant roles of

ethylene and abscisic acid mediated signaling during coleoptile elongation under anoxia. The regulation of genes encoding enzymes in the glycolytic and fermentative metabolism could be associated with abscisic acid and auxin in rice coleoptiles to maintain sugar and ATP levels for longer survival. The authors also speculated that GA may be related to inhibition of the coleoptile response to submergence during germination.

Maiti *et al.* (2009) found considerable amount of genotypic variability in 9 rice hybrids and their parents at the seedling stage under control and saline conditions with reference to the variables of shoot length, root length, shoot and root dry weights.

Manighbas *et al.* (2008) developed screening methods for rice genotypes tolerant to anaerobic conditions and good seedling vigour and identified the genotype, PR33-176-1-3, as tolerant to continuous submergence for 14 days in puddled soil, while the genotypes, NSIC RC146, PR32220-16-1-B-1-2, PR32749-10-1-B-B and PR32750-2-9-2 were intermediate to anaerobic germination. The genotypes, PR33176-1-3 and PR30536-B-48-1, showed better seedling vigour than the check varieties with good seedling vigour.

Rajendra Kumar and Christopher (2005) evaluated eleven cultivars for anaerobic tolerance and identified three tolerant cultivars viz., Co-43, ASD19 and ADT38, suitable for anaerobic seeding as they recorded higher seedling establishment, root growth, shoot growth, dry matter production and vigour index.

Li *et al.* (1990) observed heterosis for root length in a study involving parents and F₁ hybrids of five crosses of *indica* type; all the F₁ showed better root length compared to their respective parents.

Siddique (1986) reported that high vigour seeds when compared with medium and low vigour, yield levels were reduced by 7.89 and 14.4 % whereas Reddy *et al.* (1996) studied the effect of initial germination levels on vigour, growth and yield in rice and observed decrease in grain yield with a decrease in initial germination.

Lasanthi *et al.* (2007) analysed the expansin gene families and revealed that *Os-EXP7* and *Os-EXP12* were observed to be highly expressed in elongating coleoptiles under anoxia. Another 3 expansins were also reported to be induced under submergence by Takahashi *et al.* (2011). These expansins *EXPA1*, *EXPB11*, and *EXPB17* were

differentially expressed in the *rad* mutant in comparison with the control, and were affected by deficiency in ADH1. Therefore, these expansins may also be related to elongation under submergence.

Sadiq *et al.* (2011) observed that action of tubulin α -1 chain (TUBA1) and actin depolymerizing factor 4 (ADF4) were involved in fast coleoptile elongation under anaerobic conditions, as both genes are upregulated under anoxia during germination in rice, tubulins are involved in the formation of microtubules and are important for both cell division and elongation, and ADF4 is involved in regulating actin assembly (Mayer and Jürgens, 2002; Augustine *et al.*, 2008;) therefore both are speculated to play a role in fast growth of the coleoptiles under low oxygen.

Under anaerobic conditions coleoptile elongation was also reported to be regulated by ethylene (Atwell *et al.*, 1982; Van der Straeten *et al.*, 2001; Zhou *et al.*, 2002). However, an ethylene independent anoxia response was proposed (Pearce and Jackson, 1991; Pearce *et al.*, 1992), since ethylene and its precursor 1-aminocyclopropane-1-carboxylic-acid (ACC) would not function without oxygen (reviewed in Bailey-Serres *et al.*, 2010).

Under submerged conditions germination of rice coleoptile only results in the emergence and growth of a white coleoptiles (Kordan 1976). If during elongation under water, this organ reaches the atmosphere above the water, oxygen can be transported to the embryo with subsequent growth of radicle and leaf (Kordan 1976, Takahashi 1978). The anaerobic germination of a rice coleoptile is sustained by continuous translocation of carbohydrates, amino acids and salts from the endosperm (Atwell *et al.* 1982). Amino acids among which alanine and 4-amino butyric acid are the most representative (Reggiani *et al.* 1988), are important in maintaining the osmotic pressure in the anaerobic coleoptile.

2.2. VARIABILITY, HERITABILITY AND GENETIC ADVANCE AS PERCENT OF MEAN IN RICE

Improvement in any crop species depends upon the amount of variation present in a given population. The variability expressed by a genotype can be partitioned into genotypic and phenotypic components. The genotypic component being the heritable part of the total variability, its magnitude for yield and its component characters influences the selection strategies to be adopted by the breeders.

Hanson *et al.* (1956) defined heritability in broad sense as the ratio of genotypic variance to the total variance in the non-segregating populations. Phenotype is the expression of a genotype in a given environment, sometimes when the environmental influence is less; the true heritability becomes much higher. Heritability (h^2) measures the relative amount of the heritable portion of variability, while the genetic advance (GA) helps to measure the amount of progress that could be expected with selection in a character. So, only heritability will not give a true picture of inherited variance and high heritability is not always an indication of true genetic gain. Hence there is need to estimate genetic advance, which is a relative measure and reliable indication of true genetic gain. High heritability estimates together with high genetic advance indicates additive gene effect (Johnson *et al.*, 1955). So, high heritability estimates coupled with high genetic gain could successfully be improved by direct selection. Low genetic advance irrespective of high or low heritability leads to non-additive gene action (Panse, 1957) and improvement of that trait by simple selection may not be rewarding.

2.2.1 Early Vigour and anaerobic germination

Lakshmi *et al.* (2016) reported that root length at 10th day and 14th day has low PCV and GCV. Shoot length at 10th day and 14th, seedling dry weight, anaerobic germination percent has high heritability coupled with high genetic advance as percent of mean indicating predominance of additive gene effects in their expression and selection would be effective for improvement of these characters.

Pavanshankar (2015) studied on 15 early vigour traits and the results indicated that shoot length, root length at 10 DAS & 20 DAS in field has low PCV, GCV with low to moderate heritability coupled with genetic advance as percent of mean. Whereas dry weight and vigour index has moderate PCV, GCV with high heritability coupled with genetic advance as percent of mean but under lab conditions root length at 10 DAS has low PCV, GCV with high heritability coupled with moderate genetic advance, shoot length and dry weight has moderate PCV, GCV with high heritability coupled with high genetic advance as percent of mean.

Rajendra *et al.* (2015) crossed between BPT5204/ IR88633-1-136-B2 (good donor for early seedling vigour) and derived F₃ population and studied on characters plant height, leaf number, tiller number, biomass and vigor index at 15 and 30 days after

sowing. The results indicated that leaf number has low GCV at 15 DAS, moderate GCV at 30 DAS and high PCV 15 DAS and at 30 DAS, low heritability at both 15 and 30 DAS coupled with low genetic advance as percent of mean whereas vigour index has moderate and high GCV at 15 and 30 DAS with high PCV at 15 and 30 DAS respectively along with low heritability and low genetic advance as percent of mean.

Sathya and Jebaraj (2013) reported the traits viz., productive tillers per plant, filled grains per panicle, dry root weight, dry shoot weight, root: shoot ratio and root length had high heritability accompanied with high genetic advance indicating lesser environmental influence and were under the control of additive gene effect.

Hosseini *et al.* (2012) estimated heritability and genetic advance for 18 diverse rice genotypes under salt stress conditions. High heritability estimates associated with high genetic advance were obtained for plant height, root dry weight and shoot length characters and concluded that selection for these characters would be more effective because it has high heritability and high genetic advance.

Khriedinuo *et al.* (2011) reported that the traits viz., flag leaf area, plant height, number of tillers per plant, number of ear bearing tillers, number of filled grains per panicle, panicle weight, root length, straw weight and grain yield had high GCV and heritability except for flag leaf .

Genetic studies conducted by Allah *et al.* (2009), using F₂ and F₃ material derived from drought resistant varieties, IET 1444, Moroberekan and Gaori crossed with susceptible varieties Sakha 101 and Sakha 102. Their results shown that heritability estimates were high for days to heading, moderate for root-shoot ratio and low for root length.

Okelola *et al.* (2007) conducted a study to assess the genetic parameters for eleven seed vigour traits and seed yield in 24 genotypes of West African rice. Germination percentage manifested high PCV, GCV, heritability and genetic advance as percent of mean whereas vigour index has moderate PCV, GCV with high heritability coupled with high genetic advance as percent of mean.

Makhasi (2001) reported high heritability and moderate genetic advance for vigour index trait in upland rice germplasm. High phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and heritability for vigour index trait was observed by Ramadevi (1998).

Jiakui *et al.* (1996) studied heterosis, genetic variance and heritability of root characters and observed significant heterosis for these characters and inferred that they are mainly controlled by non-additive gene effects.

According to Redona and Mackill (1996), heritability value differed with different populations. Both high (0.80) and low (0.26) heritability values were obtained in two populations derived from different crosses.

Pandey *et al.* (1992) reported high variability for shoot length whereas moderate heritability for shoot length and seedling dry weight has high heritability was reported by Subbarao *et al.* (1996).

Akram *et al.* (1993) confirmed the role of additive and non additive type of gene actions in inheritance of seedling shoot length. However, additive gene effects were more prominent leading to high narrow sense heritability.

In a study by Sharma and Sharma (1993) seedling dry weight exhibited high heritability where as Chen *et al.* (1986) reported considerable variation for seedling dry weight in a study involving 242 rice varieties.

Lee *et al.* (1986) found that dry weight of 12 days old rice-seedling was under the control of additive and dominance effects, with additive effects being higher. High narrow sense heritability value of 65.24% was observed for these traits.

Li and Rutger (1980) studied F₁ and F₂ generations under cool temperature (18 °C) and results revealed that shoot length of seedling grown for 14 days was regulated by additive and dominance effects.

2.3 CORRELATION AND PATH ANALYSIS

Correlation coefficient is a measure of the degree of closeness and the linear relationship between two variables. Studies on correlation between yield and its components in population helps the breeder in understanding the shifts associated with different components to yield. The study of association between different characters may help the plant breeder to know how the improvement of one character will bring simultaneous changes in other characters. Character

association studies indicate the magnitude of association between pairs of characters and are useful for selecting genotypes with desirable combination of characters thereby aiding the plant breeder in the improvement of the concerned trait. If the correlation is strong between a set of desirable traits then if we make selection for one character, the other character will be taken care automatically. If unfavourable association exists between desirable and undesirable traits, selection may result in genetic slippage and limit genetic advance.

Path coefficient analysis as devised by Wright (1921) is standardized as partial regression coefficient. It is an important tool for partitioning the correlation coefficient into direct and indirect effects of an independent variable and dependent variable. Though correlation gives information about the component traits associated with the characters, they could not provide an exact picture of relative importance of the direct and indirect contribution of the component characters. Thus, correlation in combination with path analysis would give a better insight into the cause and effect relationship between different pairs of characters.

2.3.2 Early vigour and anaerobic germination

Yusuke *et al.* (2015) investigated morpho-physiological traits of rice during the germination and post-germination phases to explore avoidance of hypoxic condition and compared four lines selected for anaerobic germination (AG lines) with the variety IR42. The germination capacity of AG lines was higher than that of IR42. The germination percentages and coleoptile elongation differed among the four AG lines; IR06F459 showed the fastest germination and rapid coleoptile elongation. The coleoptiles of IR06F459 were significantly longer than those of IR42.

Pavanshankar (2015) studied the association of grain yield with early vigour traits under both field and laboratory conditions and the results revealed that under field conditions root length, shoot length, seedling dry weight at 10 DAS, shoot length at 20 DAS and shoot length at 10 DAS, seedling dry weight at 10 DAS in lab showed positive and significant association with grain yield whereas root length at 20 DAS showed negative and non-significant association with grain yield. Seedling dry weight, shoot length at 10 DAS (lab), root length at 10 DAS (lab) showed positive and significant association with root length and shoot length at 10 DAS whereas vigour index and seedling dry weight (lab) exhibited significant and positive association with shoot length, root length, seedling dry weight at 10 days after sowing in field. He also reported that seedling dry weight at 10 DAS showed positive indirect effect with root length at 10 DAS, shoot length at 10 DAS, vigour index, seedling dry weight at 10 DAS (lab). Whereas it has negative indirect effect *via* root length and shoot length at 20 DAS (field) and root length at 10 DAS (lab) manifested positive indirect effect through root length at 10 DAS and shoot length at 10 DAS (lab).

Subhas *et al.* (2014) investigated on 84 land races for anaerobic germination by two methods i.e., test tube method (for 7 days) and water logged soil method (for 14 days) and recorded data on plume length, radical length and vigour index. Some cultivars showed significant difference in anaerobic germination under submergence conditions. Correlation analysis was conducted among the cultivars using three parameters by two bioassay methods and the test tube method and water-logged soil method showed significant correlation with all other parameters.

Nitika *et al.* (2013) in a study involving two mapping populations, concluded that root length is positively and significantly correlated with panicle length, seeds per panicle, dry root weight and grain yield in Masarb 25 x Pusa Basmati 1460 cross derived population. In another population of HKR 47 x MAS 26, he also observed positive and significant relationship between shoot dry weight and panicle length, tiller number, dry root weight and grain yield.

Augustina *et al.* (2013) obtained a high and significant coefficient of regression on weight of plant, weight of shoot, weight of roots, weight of panicles, yield per plant, number of grains per plant and yield index ($p < 0.001$) when these traits were regressed against yield.

Zulqarnain *et al.* (2012) studied the morphological and yield related traits of twenty genotypes to ascertain the genetic and phenotypic correlation among some drought related and morphological traits and contribution of these traits to the yield under drought stress directly and indirectly in rice. The results indicated that root length, root shoot ratio, thousand grain weight, grains per panicle, spikelet fertility and drought response index showed positive and significant association with yield per plant under drought stress at genotypic level; whereas, leaf drying had significantly negative correlation with yield. Higher phenotypic correlation values for all traits indicated that the environmental effects on traits under stress are high under drought stress. Root shoot length ratio showed highest positive direct effect on yield per plant under drought stress followed by drought response index and thousand grain weight.

In an experiment by Haider (2012) morphological and yield traits of twenty genotypes were studied to ascertain the genetic and phenotypic correlation among some drought related and morphological traits and contribution of these traits to the yield under drought stress directly and indirectly in rice. They found that root length, root-shoot ratio, thousand grain weight, grain per panicle and spikelet fertility showed positive and significant association with grain yield at genotypic level.

Prasad *et al.* (2011) reported that days to maturity exhibited positive and significant correlation with plant height, days to 50% flowering, coleoptile length, seedling growth and seedling vigor. Early seedling vigor recorded a positive and significant correlation with seedling maturity after transplanting.

Adebisi *et al.* (2010) conducted a study to examine the relationships between seed vigour traits and field performance in 24 interspecific rice (*Oryza sativa* L.) genotypes. The study revealed that standard germination, energy of germination, seedling vigour index-11, seedling vigour index-1 and speed of germination index exhibited significant positive relationships with field emergence, seedling establishment, plant height, seedling dry weight and seed yield/plant.

Ability of rice seeds to germinate in flooded soils, produce and elongate roots and shoots has been associated with ability to breakdown starch stored in seeds and use it for the growing embryo under low oxygen stress (Ismail *et al.*, 2009).

Manickevelu *et al.* (2006) investigated the grain yield and its components under drought situation in a recombinant inbred population (IR 58821/ IR 52561) under low land managed stress situation. Correlation and path analysis indicated that relative water content, panicle length, grains per panicle, biomass yield, harvest index, root-shoot ratio, root length in positive direction and showed positive association with grain yield.

Kuldeep *et al.* (2004) reported that seedling length, number of leaves per seedling, number of productive tillers per plant, panicle length, number of days to maturity showed positive and significant association with grain yield .

Vaishali (2003) studied the NILs of IR64 and observed the positive association between root volume and root number, root fresh weight, shoot fresh weight and total dry weight under well watered conditions. Significant association between root volume and root-shoot ratio and grain yield with root length under low moisture stress conditions was noticed.

Singh *et al.* (2000) carried out association and estimated direct and indirect effects of various physiological parameters on seedling vigour in wheat with 10 genotypes. The results indicated that germination percentage exhibited significant positive correlation with shoot length, test weight, germination efficiency percentage and it is negatively correlated with root length and seedling dry weights. Path analysis revealed root length and shoot length had positive and indirect effect on dry weight of 100 seedling. Germination percentage has positive indirect effect on dry weight of 100 seedlings.

Chunhai *et al.* (1996) reported pleiotropic effects of *sd 1*, semi dwarf gene on root traits and observed direct and indirect effects of this gene on some of the seedling traits.

Seedling vigour has been associated with seed size and density as well as other parameters of the germinating seed and number of other seed related traits with the field emergence in 51 cultivars of rice. Among those, first and final counts of standard germination, germination rate, vigour index, germination at 15⁰C, germination in the presence of non-anoic acid and amylase activity showed significantly positive correlation with field emergence by Shenoy *et al.* (1990).

Krishnaswamy and Seshu (1989) studied 68 genotypes of rice and reported that oxygen uptake, dehydrogenase activity and free amino acids was positively correlated with germination rate and path coefficient analysis indicated that the germination rate was associated negatively with 100 seed weight largely due to negative indirect contribution through free amino acids.

2.4 TOTAL SOLUBLE SUGARS AND FREE AMINO ACIDS

Free amino acids form the initial source for polysome formation and protein synthesis that is essential for the radicle growth and protrusion (Bewely and black, 1983). Availability and supply of soluble sugars to the developing embryo results in faster and higher germination rate during submergence.

Lal *et al.* (2016) carried out anaerobic germination screening and its emerging coleoptiles were taken for the study of soluble sugar and starch content determination. The results indicated that starch content decreased at slower rate in susceptible genotypes, whereas in tolerant genotypes, it is in a faster rate. At the same time when the duration of flooding was longer, the depletion became progressively slower. For the intolerant genotypes, IR64 and IR64-Sub1, starch depletion was much slower and was partial even after shorter flooding. The same pattern was followed by soluble sugars.

Divya *et al.* (2016) investigated on changes in biochemical constituents between healthy and discoloured grains. The results indicated that significantly higher concentration of starch, total sugars, reducing and non-reducing sugars and lower concentrations of total phenols were observed in healthy grains than in discoloured grains.

Soluble sugar concentration in seed and its transport from the endosperm to the coleoptile are reported to be closely associated with coleoptile elongation under anoxia (Furuhata *et al.* 2006;Kato-Noguchi *et al.*2010).

Atwell *et al.* (1982) reported positive association of anaerobic elongation of rice coleoptiles by continuous translocation of amino acids, carbohydrates and salts from endosperm.

2.5 MOLECULAR DIVERSITY ON ANAEROBIC GERMINATION

Toledo *et al.* (2015) performed Marker Assisted Back Crossing to introgress the QTL for tolerance to anaerobic germination (*AG1*) to the previously developed Ciherang-Sub1 with the use of IR64 NILs containing *SUB1* or *AG1* as donors in order to produce an improved cultivar containing tolerance to both submergence and AG. They carried both phenotypic and genotypic evaluation.

Hsu and Tung (2015) demonstrated the value of combining genome-wide association analysis and biparental QTL mapping approaches to identify chromosomal

regions regulating coleoptile elongation in submerged rice plants. The significant genomic regions detected in this study are potential candidates for incorporation into elite cultivars to improve seedling survival during anaerobic germination.

Reddy *et al.* (2015) screened 48 genotypes for anaerobic germination. Nine genotypes were found to be resistant (75-95%) for 14 days of anaerobic germination. Among them, three genotypes Isukaravalu, IR 85956/23-1-2, MTU 1140 co segregated with markers RM 341 to qAG2 on chromosome 2, RM 206 to qAG11 on chromosome 11 indicating presence of genes pertaining to anaerobic germination.

Baltazar *et al.* (2014) identified 3 QTLs associated with tolerance of flooding during germination from a F₂ mapping population derived from the cross of IR64 and the tolerant **aus** landrace Nanhi. A major QTL for AG derived from Nanhi, qAG7 located on chromosome 7 with an LOD of 13.93 and 22.3% of the phenotypic variance.

Hendawy *et al.* (2014) studied 53 contrasting genotypes sown in soil and watered normally (control) or submerged with 10 cm of water for 17 days. Subsequently, the plants kept under normal rice cultivation conditions for a further 7 days. Among these genotypes, nine lines had been developed for anaerobic germination and submergence tolerance (AG + Sub1) by IRRI. Length and dry weight of shoot and root were measured during submergence and recovery periods (10,13 and 17 days after sowing).

Bharat *et al.* (2014) carried out molecular screening of 122 rice genotypes for anaerobic germination and other abiotic stress and identified gene specific alleles. Out of all the markers studied highest PIC value with RM324 marker for drought tolerance (0.67) and the lowest with RM8094 for salinity tolerance (0.26) were obtained in these genotypes.

Septiningsih *et al.* (2013) studied a population derived from the cross between IR42 (a susceptible variety) and Ma-Zhan Red (tolerant landrace from China.) Phenotypic data was collected based on the survival rates of the seedlings at 21 days after sowing of dry seeds under 10 cm of water. QTL analysis of the mapping population consisting of 175 F₂:3 families genotyped with 118 SSR markers identified six significant QTLs on chromosomes 2, 5, 6 and 7, and in all cases the tolerant alleles were

contributed by Ma-Zhan Red. By using a BC₂F₃ population largest QTL (qAG-7.1) was confirmed on chromosome 7 with LOD score of 14.5.

Abdolhamid *et al.* (2010) carried out screening of rice genotypes for anaerobic germination for over 8,000 gene bank accessions and breeding lines and detected five putative QTLs on chromosomes 1 (qAG-1-2), 3 (qAG-3-1), 7 (qAG-7-2) and 9 (qAG-9-1 and qAG-9-2) explaining 17.9 to 33.5% of the phenotypic variation with LOD scores of 5.69 to 20.34.

Angaji (2008) crossed KHAIYAN (tolerant variety) with IR 64 (sensitive to anaerobic conditions) and molecular screening was done using BC₂ F₂ population and detected four putative QTLs, one each on chromosomes 1 (qAG-1), 2 (qAG-2-1), 11 (qAG-11), and 12 (qAG-12). The LOD value of these QTLs ranged from 3.66 to 5.71.

Ling *et al.* (2006) identified two QTLs for anaerobic germination (AG) by using F₂ individuals derived from the cross USSR5×N22. USSR5 located on chromosomes 5 and 11, respectively, at both of which the USSR5 alleles acted to increase AG.

Ling *et al.* (2004) detected quantitative trait loci (QTLs) conferring AG by using 81 recombinant inbred lines derived from a cross of Kinmaze (japonica)/DV85 (indica). A total of five QTLs for AG in the recombinant inbred population were detected on chromosomes 1, 2, 5 and 7, respectively. Phenotypic variations explained by each QTL ranged from 10.5% to 19.6%. Based on the directions of the additive effects, the alleles at three loci qAG-1, qAG-2 and qAG-7 from Kinmaze increased AG, while alleles at loci qAG-5a and qAG-5b from DV85 increased AG.

Chapter III

MATERIAL AND METHODS

EXPERIMENT I: To study the variability parameters for early vigour related traits and yield components under dry direct sowing

The present experiment was carried out during *kharif*, 2016 in the Northern block of Agricultural college farm, Bapatla, Andhra Pradesh. The material used and methods followed are presented below.

3.1 MATERIAL

The experimental material consisted of forty eight genotypes including three donors obtained from Rice Research Unit, Bapatla and Andhra Pradesh Rice Research Institute, Maruteru. The information on pedigree of the genotypes is given in Table 3.1 and layout has been furnished in fig 3.1

3.2 METHODS

All the genotypes were sown separately in each experimental unit having two replications by following dry direct sowing method through manual dibbling method.

Experimental Details

The main field was laid out in a randomized block design with two replications. Each experimental unit consisted of 1.8 m² and the spacing adopted was 20 cm between rows and 15 cm between plants. The fertilizers were applied at the rate of 120 kg N, 40 kg P₂O₅, and 40 kg K₂O per hectare. One third dose of nitrogen and entire dose of P₂O₅ and ½ of the K₂O were applied as basal dose. The remaining nitrogen was applied in two equal split doses one at 30 days after sowing and another at 60 days after sowing. Remaining 1/2 of the K₂O applied along with last dose of Nitrogen. The intercultural operations like weeding was done at regular intervals and necessary plant protection measures were adopted during the growth of crop. The detailed experimental technique for the present investigation is furnished in Table 3.2 .

Table 3.2 Experimental details of evaluation of genotypes of rice (*Oryza sativa* L.)

Location	Agricultural College Farm, Bapatla, Andhra Pradesh
Season	<i>kharif</i> , 2016
Genotypes	48
Design	Randomized complete block design
Replications	2
Plot size	1.8 m ²
Spacing	20×15 cm
Row length	3 m
No. of rows	3
Number of plants per replication	60
Fertilizers	120 N: 40 P ₂ O ₅ : 40K ₂ O kg per ha

3.2.1. Collection of Data

Single plant observations were recorded on five plants selected at random per genotype per replication and their means were used for statistical analysis.

Field assessed parameters of vigour through destructive sampling method under dry direct sowing

3.2.1.1. Root length (7 DAS) and Shoot length (7 DAS) of five randomly selected seedlings per genotype per replication were measured through destructive sampling method in field condition and expressed in centimeter.

3.2.1.2. Root length (14 DAS) and Shoot length (14 DAS) of five randomly selected seedlings per genotype per replication were measured through destructive sampling method in field condition and expressed in centimeter.

3.2.1.3. Seedling dry weight (7&14 DAS) of five seedlings per genotype were dried in hot air oven to constant weight and weighed. The total weight of seedlings divided by number of seedlings taken for observation gave average weight of each seedling and this was expressed in milligrams per seedling.

3.2.1.4. Vigour Index (mass): Vigour index (VI) was calculated by multiplication of germination percentage with mean of seedling dry weight (Vijay *et al.*, 2010).

$$VI = \text{germination percentage} \times \text{seedling dry weight}$$

3.2.1.5 Germination Percentage: Germination percentage was calculated by using the formula

$$\text{Germination Percentage} = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds}} \times 100$$

Quantitative Characters

3.2.1.6 Plant Height (cm)

The plant height was measured in centimetres from ground level to the tip of the panicle of the mother tiller excluding awns at the time of harvest.

3.2.1.7 Number of Ear Bearing Tillers Plant⁻¹

Total number of ear bearing tillers in each randomly selected plant was counted at maturity.

3.2.1.8 Panicle Length (cm)

Length of panicle was measured from the neck of panicle to the tip of the top most spikelet excluding awns.

3.2.1.9 Test Weight (g)

One thousand well filled grains from each treatment were counted and weighed to nearest milligram at 12% moisture level.

3.2.1.10 Grain Yield Plant⁻¹ (g)

After thorough cleaning and sun drying up to 12% moisture level, grain yield was weighed.

EXPERIMENT II: To study the variability of rice genotypes for shoot and root characters under anaerobic germination (in polyhouse)

The present experiment was carried out during *kharif*, 2016 at Andhra Pradesh Rice Research Institute, Maruteru, Andhra Pradesh. The material used was same which was used in experiment I and methods followed are presented below.

Screening for tolerance to submergence during germination (anaerobic germination): Screening for anaerobic germination tolerance was done as per Reddy *et al.* (2015). Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30 °C for 48 h for germination. Fifteen pre-germinated seeds at 3 days after sowing were sown into seedling trays (35.5×10×4.5cm) at about 1 cm soil depth in two replications per treatment in C.R.D. Each trays consists of three rows and 10 holes (2.5 cm) in each row. After sowing, the tray were submerged carefully in concrete tanks filled with 10 cm depth of water above the soil surface in the trays and was maintained at that depth for 7 days, 14 days and 21 days and represented in plate 3.1 and 3.2.

Table 3.3 Experimental details of evaluation of rice genotypes for 7 and 14 days under anaerobic conditions

Location	APRRI & RARS, Maruteru, Andhra Pradesh
Season	<i>Kharif</i> , 2016
Genotypes	48
Design	Complete randomized block design
Replications	2
Number of plants per replication	15

The genotypes which recorded >75% germination under submergence for 14 days were screened for anaerobic germination under submergence for 21 days.

Table 3.4 Experimental details for evaluation of rice genotypes for 21 days under anaerobic conditions

Location	APRRI & RARS, Maruteru, Andhra Pradesh
Season	<i>Kharif</i> , 2016
Genotypes	13
Design	Complete randomized block design
Replications	2
Number of plants per replication	30

COLLECTION OF DATA

3.2.1.11 Root length (7 DAS) and Shoot length (7 DAS) of five randomly selected seedlings per genotype per replication were measured through destructive sampling method collected from trays and expressed in centimeter .

3.2.1.12 Root length (14 DAS) and Shoot length (14 DAS) of five randomly selected seedlings per genotype per replication were measured through destructive sampling method collected from trays and expressed in centimeter.

3.2.1.13 Root length (21 DAS) and Shoot length (21 DAS) of five randomly selected seedlings per genotype per replication were measured through destructive sampling method collected from trays and expressed in centimeter.

3.2.1.14 Seedling dry weight (7, 14 & 21 DAS) of five seedlings per genotype were dried in hot air oven at 72-80° C to constant weight and weighed. The total weight of seedlings divided by number of seedlings taken for observation gave average weight of each seedling and this was expressed in milligrams per seedling.

3.2.1.15 Vigour Index (mass):

Vigour index (VI) was calculated by multiplication of germination percentage with mean of seedling dry weight (Vijay *et al.*, 2010).

$$VI = \text{germination percentage} \times \text{seedling dry weight}$$

3.2.1.16 Anaerobic Germination Percentage: Plants survived after submergence was counted and germination percentage was calculated by using the formula

$$\text{Germination Percentage} = \frac{\text{No. of plants survived under submergence}}{\text{Total no. of seeds under treatment}} \times 100$$

The genotypes which recorded more than 75% of germination under anaerobic conditions are considered as resistant to anaerobic germination (Reddy *et al.* 2015).

3.2.1.17 Anaerobic response index: As per Hsu and Tung (2015) the difference in coleoptile length between control plants and those submerged in water is calculated as anaerobic response index .

3.2.1.18 Estimation of total sugars (mg g⁻¹)

Sugar concentration in seeds was estimated by using anthrone method described by Hedge and Hofreiter (1962).

Reagents

- a. 2.5 N Hydrochloric acid (HCl)
- b. Anthrone reagent: 200 mg of anthrone dissolved in 100 mL of ice cold 95% H₂SO₄ solution.
- c. Standard glucose :
 - 1) Stock standard: 100 mg of glucose dissolved in 100 mL of distilled water.
 - 2) Working standard: 10 mL of stock solution was stored in refrigerator after adding a few drops of toluene.

Procedure

100 mg of dried and powdered sample was weighed, placed in a boiling tube and subjected to hydrolysis by keeping it in a boiling water bath for 3 hours with 5 mL of 2.5 N HCl and cooled to room temperature. Then it was neutralised with sodium carbonate until the effervescence ceased and made the volume to 100 mL and centrifuged at 2000 rpm for about 2 min. The supernatant was collected and 1 mL of aliquot was taken for analysis.

The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution. "0" serves as blank. Afterwards the volume was made up to 1 mL in all tubes including the sample tubes by adding distilled water. Then 4 mL of anthrone reagent was added and heated for 8 min in a boiling water bath. Further the sample tubes were cooled rapidly and red green to dark green at 630 nm using spectrophotometer.

A standard curve was drawn by plotting concentration of the standard on X-axis versus absorbance on Y-axis. From the curve, the amount of carbohydrate present in the sample was calculated by using the following formula.

$$\frac{\text{Amount of carbohydrate present in 100 mg of sample}}{100} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

3.2.1.19 Total free amino acids (mg g⁻¹)

Amino acid concentration in seeds was estimated by using ninhydrin method described by Moore and Stein (1948).

Reagents

- a. Ninhydrin: 20 g of ninhydrin was dissolved in 500 mL of methyl cellulose. 0.8 g of stannous chloride (SnCl₂.2H₂O) was dissolved in 500 mL of 0.2 M citrate buffer (p^H 5.0). This stannous chloride solution was mixed with ninhydrin solution.
- b. 0.2 M Citrate Buffer p^H 5.0
- c. Diluent Solvent : Water and *n*-propanol were mixed in equal volumes.
- d. Standard : 50 mg of leucine was dissolved in 50 mL of distilled water in a volumetric flask. 10 mL of this stock standard was diluted to 100 mL in another volumetric flask for working standard solution. A series of volumes from 0.1 to 1 mL of this standard solution gives a concentration range 10 µg to 100 µg. Proceeded as that of sample and read the colour.

Procedure

Extraction of amino acids

500 mg of plant sample was weighed and ground in a pestle and mortar with a small quantity of acid-washed sand. To this homogenate, 5 to 10 mL of 80 per cent ethanol was added and filtered. The filtrate was saved. The extraction procedure was repeated twice with the residue and the filtrate was pooled. It was allowed for evaporation to reduce the volume and used for the quantitative estimation of total free amino acids.

Estimation

To 0.1 mL of extract, 1 mL of ninhydrin solution was added and the volume was made to 2 mL with distilled water. The tubes were heated in a boiling water bath for 20 min. 5 mL of the diluent solvent was added and the contents were mixed. After 15 min, the intensity of the purple colour was read against a reagent blank in a colorimeter at 570 nm. The colour is stable for 1 h. The reagent blank was prepared as above by taking 0.1 mL of 80 per cent ethanol instead of the extract.

3.3. MOLECULAR CHARACTERIZATION USING MICROSATELLITE MARKERS

3.3.1. DNA Extraction and Quantification

DNA was extracted from leaf tissue from all the genotypes using the modified Cetyl Tris Methyl Ammonium Bromide (CTAB) method of Dellaporta *et al.* (1983) as described under.

Reagents

CTAB Extraction Buffer, composition and concentration of CTAB extraction buffer is given in Table 3.5

Table 3.5 Composition and concentrations of CTAB extraction buffer

S. No.	Components	Stock concentration	Working concentration	Volume for 500 ml
1	Tris Hcl	1 M	200 mM	100 ml
2	EDTA	0.5 M	20 mM	20 ml
3	Nacl	5 M	1.4 mM	140 ml
4	CTAB	2%	2 g	10 g

- RNase-10 mg/ml
- Absolute ethanol
- Agarose gel (3%)
- 1X TAE Buffer which contains
 - Tris acetate (pH 8.0)-40 mM
 - Acetic acid-20 mM
 - EDTA-1mM

- Ethidium bromide-10 mg/ml
- 10X DNA Tracking dye which contains
 - Glycerol-5 ml
 - 10X Tris acetate EDTA Buffer-1 ml
 - Bromophenol blue-1 ml
 - Double distilled water-3 ml

Methodology

- Healthy leaf samples of 12 day old seedling were collected in a sterile plastic bag or cover and was labelled with plant number. It was then sealed and placed on ice or freezer.
- The leaf tissue was cut into 0.5 cm long pieces.
- 400 µl DNA Extraction buffer was added using micropipette and the tissue was grinded using quiagen tissue lyzer until the buffer turns dark green.
- The dark green colour solution obtained was transferred into a micro centrifuge tube labelled with the plant number or variety name.
- To this tube 200 µl of Tris saturated phenol and 200 µl of chloroform: isoamyl alcohol mixture (24:1) was added and centrifuged at 10,000 rpm for about 10 min in a cooling centrifuge.
- The supernatant thus obtained was transferred into another labelled tube using a micropipette.
- To the aqueous supernatant, 10 µl of RNase was added and incubated 37 °C for about 30-45 min.
- After incubation, 400 µl of chloroform: isoamyl alcohol mixture (24:1) was added and mixed gently and centrifuged at 10,000 rpm for 10 min.
- The top aqueous phase *i.e.*, supernatant was transferred to another labelled tube.
- To this solution, equal volume of chilled isopropanol was added, mixed well and centrifuged at 10,000 rpm.
- After centrifugation the supernatant was discarded and the DNA pellet settled at the bottom of the tube. 200 µl of 70% ethanol was added and centrifuged at 10,000 rpm for 10 min.

- The supernatant was discarded and the tubes containing DNA pellet were air dried at room temperature for about 1-2 hr till the smell of ethanol completely goes off.
- After the pellet was completely dried with no smell of alcohol the DNA pellet was suspended in 100-200 µl of T₁₀E₁ Buffer and stored at -20 °C.
- The DNA thus obtained was subjected to quantification.

3.3.2. Estimation of Quality and Quantity of DNA

DNA quantification was done by spectrophotometric technique using Thermo scientific Nano drop 8000 (spectrophotometer). The measurement of absorbance was done at 260 nm. DNA has maximum absorbance at 260 nm, one optical density (OD) corresponded approximately to 50 µl/ml of double stranded DNA.

An aliquot of 10 µl of the DNA was diluted in TE buffer in a ratio of 1:1000 in a microcuvette, mixed well and absorbance was determined at 260 nm against TE buffer blank. The DNA concentration was calculated using the following formula:

$$\text{Amount of DNA (}\mu\text{g/ml)} = \text{Dilution factor} \times \text{standard (50 }\mu\text{l/ml)} \times \text{OD at 260 nm}$$

The ratio of OD 260 to OD 280 provided information on the purity of the DNA

- 1) If the ratio is 1.8 to 2.0 the sample of DNA is relatively pure
- 2) If the ratio is < 1.8 the sample of the DNA is either contaminated with phenol or protein

3.3.3. Polymerase Chain Reaction

The *in vitro* amplification of DNA by repeated cycles of strand separation and polymerization, by DNA polymerase activity is called polymerase chain reaction (PCR). This technique was developed by noble laurate Mullis of California in 1985. The polymerase chain reaction technique is used for generating large quantities of a specified DNA segment of interest. A total of 10 µl reaction mixture was used for each amplification. The PCR components are presented in Table 3.6.

Table 3.6 Components of Polymerase Chain Reaction

PCR Components	1X
Taq buffer (10X) with MgCl ₂	1 micro litre
dNTPs mix (2.5 mM each)	1 micro litre
Forward primer (5 pM)	1 micro litre
Reverse primer(5 pM)	1 micro litre
Template (50 ng / µl)	3 micro litre
Taq polymerase (1u / µl)	1 micro litre
Sterile distilled Water	2 micro litre
Total	10 micro litres

3.3.4 PCR with Microsatellite Markers

SSR markers are very powerful tools in Marker Assisted Selection (MAS) due to their hyper polymorphism, abundance in the genome, co-dominant inheritance, simplicity of assay, most important being already mapped both physically and genetically in the rice genome. Amplification of single SSR involves two different primers- F (Forward) and R (Reverse) primers. SSR primers are usually in the size range of 18 to 25 bases. *Taq* DNA polymerase is the most vital ingredient of a PCR reaction. *Taq* DNA polymerase is a special category of DNA polymerase, which is obtained from a thermophilic bacterium, *Thermus aquaticus* and is highly thermo stable. The steps involved in PCR were given below

- Master Mix consisting of PCR buffer, dNTPs, Forward primer, Reverse primer, Milli Q water, *Taq* DNA polymerase was prepared.
- In order to avoid minor pipetting errors, a little extra quantity of master mix was prepared to ensure that adequate quantity was available for the samples. Empirically, for every 10 reactions, one extra reaction was taken.
- The tube containing master mix was spun for about 10 to 15 seconds and kept in ice until added to PCR tubes.
- Master mix was added equally to all the wells in the PCR plate, given a spin and kept inside the thermocycler (Applied Biosystem's Veriti PCR machine).

The actual technique of PCR involves repeated cycles for amplification of target DNA.

Each cycle has 3 stages.

1. Denaturation
2. Annealing
3. Extension

On raising the temperature to 94 °C for about 5.0 minutes, the DNA gets denatured and the two strands were separated. When the temperature of mixture was slowly cooled to about 58 °C for 0.5 minutes the primers base pair with the complementary regions flanking the target DNA segments. The initiation of DNA synthesis occurs at 3-hydroxyl end of each primer by the enzyme *Taq* DNA polymerase. The primers were extended by joining the bases complementary to DNA strands. However, the temperature has to be kept optimal as required by the enzyme *Taq* DNA polymerase, the optimum temperature was around 72 °C for 1 minute. It was estimated that the end of 35 cycles of PCR, about a million fold target DNA was synthesized. The thermo profile for PCR is given in Table 3.7.

Table 3.7 The Thermo Profile for Polymerase Chain Reaction

Activity	Temperature (°C)	Duration (minutes)	Number of cycles
Initial Denaturation	94	5.0	1
Denaturation	94	0.5	35
Annealing	58	0.5	35
Extension	72	1.0	35
Final Extension	72	7.0	1

After completion of 35 cycles, PCR plate was taken out and stored at 4 °C.

3.3.5 Agarose Gel Electrophoresis

The migration of charged particles under the influence of direct electric field is known as electrophoresis. Many important biological molecules such as amino acids, peptides, proteins, nucleic acids and nucleotides possess ionizable groups. When they are subjected to electric field the charged molecules migrate towards either cathode or anode under the influence of electric field. Agarose gel electrophoresis is useful in identification and separation of DNA molecules.

3.3.6 Gel Preparation

- Prepared 1X TAE buffer by diluting 50X TAE buffer.
- 3 % Agarose gel was prepared by adding 15 g agarose to 500 ml of 1X TAE buffer (3%) and boiled till agarose dissolves completely and a clear solution is formed.
- The electrophoresis apparatus and the combs were washed with distilled water and the combs were placed approximately 2 cm away from the cathode.
- When the temperature of molten Agarose was around 50 °C, ethidium bromide was added at a concentration of 15 µl/500 ml mixed and poured into the central part of the tank without any air bubbles.
- The gel casting set was kept undisturbed till the Agarose solidified.
- Upon solidification, 1X TAE buffer was poured into the gel tank till the level reached 0.6-0.8 cms above the gel surface.
- The combs were lifted gently ensuring that the wells remained intact

3.3.7 Loading DNA

PCR plate was taken out from the refrigerator and tracking dye, bromophenol blue (0.25% bromophenol blue and 40% sucrose mixed in water) was added and given one spin for uniform mixing of bromophenol blue. The samples were then loaded carefully into the wells.

3.3.8 Electrophoresis

- After loading all DNA samples into the wells electrophoresis unit was made air tight with a lid and power supply was switched on.
- DNA molecules with in an Agarose gel matrix were subjected to steady electric field; it will migrate through the gel towards the positive electrode, anode since DNA has a strong negative charge at neutral pH. The pores in the gel separate the linear fragments of DNA according to their size.
- The gel was run at 80 to 120 V till the dye front reached 3/4th of the gel.

3.3.9 Gel documentation

The resolved PCR bands were documented using Syngene gel doc system.

3.3.10 Molecular Data Analysis

Molecular markers data analysis: PCR amplicons were scored for presence of allele as 1 and absence as 0 for each marker over 48 genotypes. Genetic similarities of the genotypes were estimated from the matrix of the binary data using Jaccard's similarity coefficient. Cluster analysis of elite lines was performed utilizing NTSYS PC version 2.02 (Rohlf, 1999).

Polymorphic information content (PIC) was calculated, according to the method of Anderson *et al.* (1993).

$$PIC_i = 1 - \sum_{j=1}^n P_{ij}^2$$

Where P_{ij} is the frequency of the j th allele for the i th marker, and is summed over n alleles. The calculation was based on the number of alleles per locus.

A high degree of polymorphism of microsatellite markers allows rapid and efficient identification of rice breeding lines. In the present study, a total of 12 reported anaerobic germination gene linked microsatellite markers were used, which covered most of the genome of rice (chromosomes 1, 2, 3, 6, 7, 9, 11,) and the microsatellite markers used in the present study were shown in Table 3.8.

Table 3.8 Details of anaerobic germination linked markers used for diversity study

Marker	Chromosome no.	Gene/QTL	Reference
RM 10694	1	<i>qAG-1-1</i>	Angaji <i>et al.</i> (2010)
RM 263	2	<i>qAG-2-2</i>	Septiningsih <i>et al.</i> (2013)
RM 6318	2	<i>qAG-2-2</i>	Angaji <i>et al.</i> (2010)
RM 520	3	<i>qAG-3-1</i>	Angaji <i>et al.</i> (2010)
RM 253	6	<i>qAG-6</i>	Septiningsih <i>et al.</i> (2013)
RM 478	7	<i>qAG-7-1</i>	Angaji <i>et al.</i> (2010)
RM 210	8	<i>qAG-8-1</i>	Angaji <i>et al.</i> (2010)
RM 149	8	<i>qAG-8-1</i>	Angaji <i>et al.</i> (2010)
RM 8303	9	<i>qAG-9-1</i>	Angaji <i>et al.</i> (2010)
RM 104	1	<i>qAG-1-2</i>	Angaji <i>et al.</i> (2010)
RM 105	9	<i>qAG-9</i>	Septiningsih <i>et al.</i> (2013)
RM 206	11	<i>qAG-11</i>	Angaji (2008)

3.4. STATISTICAL ANALYSIS:

The mean data in respect of various characters were subjected to the following statistical techniques.

3.4.1.1 Analysis of variance for randomized block design

The analysis of variance for each character was found as per the standard statistical procedure given by Panse and Sukhatme (1967).

$$Y_{ij} = \mu + b_i + t_j + e_{ij}$$

Where,

Y_{ij} = Performance of the j^{th} genotype in the i^{th} block

μ = general mean

b_i = True effect of i^{th} block

t_j = True effect of j^{th} genotype

e_{ij} = random error associates with j^{th} genotype and i^{th} block

Analysis of variance was carried out for each character as indicated below.

Source of variation	d.f.	SS	MSS	Expected MSS	'F' calculated
Replications	(r-1)	RSS	M_r	$\sigma^2_e + t \sigma^2_r$	M_r / M_e
Treatments	(t-1)	TrSS	M_t	$\sigma^2_e + r \sigma^2_g$	M_t / M_e
Error	(r-1) (t-1)	ESS	M_e	σ^2_e	
Total	(rt-1)	TSS			

Where,

r = Number of replications

g = Number of genotypes

M_r = Mean sum of square of replication

M_t = Mean sum of square of treatment

M_e = Mean sum of square of error

σ_e^2 = Environmental variance

σ_r^2 = Variance due to replications

σ_g^2 = Variance due to genotypes

Test of significance for each character was carried out against the corresponding error degrees of freedom using 'F' table values given by Fisher and Yates (1963).

3.4.1.2. Analysis of variance for completely randomized design

For honeydew experiment and seed box screening scores, analysis of variance was computed based on completely randomized design separately as per standard statistical procedure (Panse and Sukhatme, 1985).

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

Where,

Y_{ij} = phenotypic observation of i^{th} genotype and j^{th} replication

μ = general mean

g_i = effect of i^{th} genotype

e_{ij} = random error associated with i^{th} genotype and j^{th} replication

ANOVA for completely randomized design

Source of variation	d.f	SS	MSS	Expected MSS	'F' calculated
Treatments (Genotypes)	(t-1)	TrSS	M_t	$\sigma_e^2 + r \sigma_g^2$	M_t / M_e
Error	(r-1)(t-1)	ESS	M_e	σ_e^2	
Total	(tr-1)	TSS	TMSS		

The analysis of variance for each character was carried out as indicated below:

Where,

g = Number of genotypes

M_t = Mean sum of square of treatment

M_e = Mean sum of square of error

σ_e^2 = Environmental variance

σ_g^2 = Variance due to genotypes

Test of significance for these was carried out against the corresponding error degrees of freedom using 'F' table values given by Fisher and Yates (1963).

3.4.2 Estimation of genetic parameters

3.4.2.1 Phenotypic and genotypic variance

This was estimated according to the method given by Lush (1940).

$$\text{Genotypic variance } (\sigma_g^2) = \frac{M_t - M_e}{r}$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + M_e = \frac{M_t - M_e}{R} + M_e$$

3.4.2.2 Coefficient of variation

Phenotypic and genotypic coefficients of variation (PCV and GCV) were computed according to Burton and Devane (1953).

$$\text{PCV} = \frac{\text{Phenotypic standard deviation } (\sigma_p)}{\text{General mean } (\bar{X})} \times 100$$

$$\text{GCV} = \frac{\text{Genotypic standard deviation } (\sigma_g)}{\text{General mean } (\bar{X})} \times 100$$

As suggested by Subramanian and Menon (1973), GCV and PCV were categorized into

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

3.4.2.3 Heritability in broad sense [h^2 (b)]

Heritability in broad sense was estimated as per Allard (1960).

$$h^2 (b) = \frac{\text{Genotypic variance } (\sigma_g^2)}{\text{Phenotypic variance } (\sigma_p^2)} \times 100$$

As suggested by Johnson *et al.* (1955), h^2 (b) estimates were categorized into

Low	=	0 – 30 %
Moderate	=	31-60 %
High	=	more than 60 %

3.4.2.4 Genetic advance as per cent of mean (GAM)

Genetic advance was estimated as per the formula proposed by Lush (1940) and Johnson *et al.* (1955).

$$\text{Genetic Advance (GA)} = K \times \sigma_p \times h^2 (b)$$

Where,

K = Selection differential at 5% selection intensity (2.06).

h^2 (b) = heritability in broad sense.

σ_p = phenotypic standard deviation.

$$\text{GAM} = \frac{\text{GA}}{\text{Grand mean } (\bar{X})} \times 100$$

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

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

3.4.3 Correlations

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

Phenotypic coefficient of correlation (r_p)

$$r(x_i, x_j)_p = \frac{Cov(x_i, x_j)_p}{\sqrt{V(x_i)_p \cdot V(x_j)_p}}$$

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

$V(x_i)_p$ = Phenotypic variance of i^{th} character

$V(x_j)_p$ = Phenotypic variance of j^{th} character

Genotypic coefficient of correlation (r_g)

$$r(x_i, x_j)_g = \frac{Cov(x_i, x_j)_g}{\sqrt{V(x_i)_g \cdot V(x_j)_g}}$$

Where,

$r(x_i, x_j)_g$ = Genotypic correlation between i^{th} and j^{th} character

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

$V(x_i)_g$ = Genotypic variance of i^{th} character

$V(x_j)_g$ = Genotypic variance of j^{th} character

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.

3.4.4 Path Coefficient Analysis

Path coefficient analysis suggested by Wright (1921) and elaborated by Dewey and 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:

$$\begin{aligned} r_{1y} &= P_{1y} + r_{12} P_{2y} + r_{13} P_{3y} + \dots + r_{1k} P_{ky} \\ r_{2y} &= r_{21} P_{1y} + P_{2y} + r_{23} P_{3y} + \dots + r_{2k} P_{ky} \\ r_{iy} &= r_{i1} P_{1y} + r_{i2} P_{2y} + r_{i3} P_{3y} + \dots + r_{ik} P_{ky} \\ r_{ky} &= r_{k1} P_{1y} + r_{k2} P_{2y} + r_{k3} P_{3y} + \dots + r_{kk} P_{ky} \end{aligned}$$

Where,

r_{1y} to r_{ky} = Coefficient of correlations between causal factors 1 to k and dependent character y.

r_{12} to $r_{k-1,k}$ = Coefficient of correlations among causal factors.

P_{1y} to P_{ky} = Direct effects of characters 1 to k on character y

The above equations were written in a matrix form as under:

$$\begin{matrix} & \text{A} & & \text{C} & & \text{B} \\ \begin{matrix} p_{1y} \\ p_{2y} \\ \vdots \\ r_{iy} \\ \vdots \\ p_{iy} \end{matrix} & \begin{pmatrix} r_{1y} \\ r_{2y} \\ \vdots \\ \vdots \\ r_{iy} \\ \vdots \end{pmatrix} & = & \begin{pmatrix} \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \end{pmatrix} & & \begin{pmatrix} 1 & r_{12} & r_{i1} \\ r_{21} & 1 & r_{i2} \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \\ r_{i1} & r_{i2} & i \\ \vdots & \vdots & \vdots \end{pmatrix} \end{matrix}$$

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

Where

$$[C]^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & \dots & C_{1i} \\ C_{21} & C_{22} & C_{23} & \dots & C_{2i} \\ C_{i1} & C_{i2} & C_{i3} & \dots & C_{ii} \end{bmatrix}$$

The direct effects were calculated as follows

$$P_{1Y} = \sum_{i=1}^k C_{1i} r_{iY}$$

$$P_{2Y} = \sum_{i=1}^k C_{2i} r_{iY}$$

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

Residual effect

The residual effect was estimated with the help of direct effects and simple correlation coefficients using following formula.

$$I = P^2 R_y + \sum P_{iy} r_{iy}$$

Where, $P^2 R_y$ is the square of the residual effect.

Chapter IV

RESULTS AND DISCUSSION

Direct seeding of rice is being adopted in irrigated low land ecosystem also because it reduces labour costs in addition to other benefits. Wide adoption of direct seeded rice practise has been hindered by poorly levelled fields, heavy rainfall and poor drainage which cause accumulation of water in the fields shortly after sowing leading to poor crop establishment. Hence, tolerance of anaerobic condition during germination is an essential trait for direct seeded rice cultivation both in rainfed as well as in irrigated ecosystems. Hence, the present investigation was undertaken to study the variability parameters for early vigour traits in field and under anaerobic conditions and for yield and yield components. In addition, association of early vigour traits under anaerobic conditions and molecular diversity of genotypes using reported SSR markers for anaerobic germination. The experimental findings of the present investigation conducted during *kharif* 2016 with 48 rice genotypes are presented and discussed below.

Analysis of variance

The data collected for all the characters were subjected to statistical analysis as detailed under materials and methods. Analysis of variance was carried out for thirty three characters viz., plant height (cm), ear bearing tillers per plant, panicle length (cm), test weight (g), grain yield per plant (g), root length at 7 DAS in field (cm), shoot length at 7 DAS in field (cm), dry matter at 7 DAS in field (mg), vigour index, root length at 14 DAS in field (cm), shoot length at 14 DAS in field (cm), dry matter at 14 DAS in field (mg), germination percentage under field conditions, root length after 7 days of submergence (cm), shoot length after 7 days of submergence (cm), dry matter after 7 days of submergence, anaerobic germination percentage under 7 days of submergence, number of leaves under 7 days of submergence, vigour index under 7 days of submergence, shoot length after 14 days of submergence, root length after 14 days of submergence, number of leaves under 14 days of submergence, anaerobic germination percentage under 14 days of submergence, vigour index under 14 days of submergence, dry matter after 14 days of submergence, shoot length after 21 days of submergence, root length after 21 days of submergence, dry matter after 21 days of

submergence, number of leaves under 21 days of submergence, anaerobic germination percentage under 21 days of submergence, vigour index under 21 days of submergence, total soluble sugars, free amino acids and the results are given in Table 4.1, 4.2 & 4.3. The results revealed significant differences for all 33 characters studied.

The results obtained are presented and discussed under the following headings.

4.1 Mean, variability, heritability and genetic advance as per cent of mean

4.2 Correlation

4.3 Path coefficient analysis

4.4 Molecular diversity

4.1 MEAN, VARIABILITY, HERITABILITY AND GENETIC ADVANCE AS PER CENT OF MEAN

4.1.1 Mean, Variability, Heritability and Genetic Advance as Percent of Mean for Yield and Early Vigour Traits in Field

The mean performance of genotypes is presented in Table 4.4. The estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2 broad sense) and genetic advance as per cent of mean (GAM) are presented in Table 4.6 and described character wise here under.

4.1.1.1 Plant height (cm)

Plant height exhibited a wide range of variation from 80.50cm (Binadhan -11) to 155.95cm (AC39416A) with a mean value of 102.54 cm. Under dry direct sowing conditions in irrigated areas, genotypes with short stature and non lodging traits are suitable. From the present study, genotypes identified with short stature include BPT 5204 (80.7cm), MTU 3626 (80.80cm), BPT 3291 (85.60cm) and BPT 2675 (86.20cm). This trait exhibited moderate genotypic (14.70) and phenotypic (15.18) coefficients of variation. High heritability (93.73) coupled with high genetic advance as per cent of mean (29.31) was observed for this trait. Mean performance of plant height is represented in fig 4.1.

The estimates of both GCV and PCV were moderate indicating that variation among the genotypes are medium. This results are in agreement with Sravan *et al.* (2012), Swarnajit *et al.* (2015) and Pavanshankar *et al.*(2016). High heritability coupled with high genetic advance as per cent of mean was observed for this trait indicating the operation of additive gene action in the inheritance of this trait. These findings are in agreement with the results reported by Pavanshankar *et al.* (2016), Ria *et al.* (2015) and Rukminidevi *et al.* (2016).

4.1.1.2 Number of ear bearing tillers

The trait ear bearing tillers per plant ranged from 5.50 (Binadhan-11) to 18.10 (BPT 2595) with a mean value of 12.98. This trait exhibited high phenotypic (23.46) and genotypic (21.03) coefficients of variation, high heritability (80.39) coupled with high genetic advance as percent of mean (38.85).

High estimates of PCV, GCV for this trait was previously reported by Ria *et al.* (2015), Revathi *et al.* (2016) and Onyia *et al.* (2017). High heritability coupled with high genetic advance as per cent of mean was observed for this trait indicating the operation of additive gene action in the inheritance of this trait. These findings are in agreement with results reported by Ria *et al.* (2015), Rukminidevi *et al.* (2016) and Rajendra *et al.* (2017)

4.1.1.3 Panicle length (cm)

Panicle length among all the genotypes varied from 19.40 cm (RGL 2537) to 27.80 cm (RGL 1414) with a mean value of 23.67 cm. Low phenotypic (9.02) and genotypic (7.42) coefficient of variation with high heritability (68.99) and moderate genetic advance as percent of mean (12.81) was observed for this trait.

Paikhomba *et al.* (2014), Swarnajit *et al.* (2015), Bagati *et al.* (2016), Rajendra *et al.* (2017) also reported low estimates of PCV and GCV for panicle length. High heritability coupled with moderate genetic advance as per cent of mean was observed for this trait indicating the operation of both additive and non additive gene action in the inheritance of this trait which are in agreement with the results reported by Shobarani *et al.* (2013), Swarnajit *et al.*(2015) and Rukminidevi *et al.* (2016).

4.1.1.4 Test weight (gm)

Test weight exhibited a wide range of variation from 15.90g (BPT 5204) to 32.45g (AC39397) with a mean value of 21.55g. Both genotypic (17.18) and phenotypic (17.66) coefficients of variation manifested moderate estimates for test weight. Similar results were reported previously by Swarnajit *et al.* (2015), Gampala *et al.* (2015), Pavanshankar *et al.* (2016). High heritability (94.66) coupled with high genetic advance as percent of mean (34.43) was manifested by test weight which are in accordance with the results reported by Kumar and Saravanan (2012), Revathi *et al.* (2016), Bitew (2016) and Rajendra *et al.* (2017).

4.1.1.5 Grain yield per plant (gm)

Among the genotypes studied grain yield per plant varied from 17g to 47g with a mean value of 35.05g. Minimum grain yield was recorded in NLR 4001 and MTU 2067 and maximum grain yield was recorded in NLR 4002 (47 g) followed by BPT 2673 (46.5 g), NLR 33892 (45g), MTU 5182 (44g), MTU 1075 (43.5g), BPT 2740 (43 g) and JGL 17004 (43 g). Other genotypes which recorded high grain yield/ plant include MTU 5293 (42 g), BPT 2660 (41 g), BPT 2593 (41 g), BPT 1768 (41 g), and BPT 2743 (41g). All these genotypes manifested high values for ear bearing tillers, panicle length and test weight which contributed to high grain yield. Phenotypic (21.50) and genotypic (20.81) coefficients of variation was observed high for this trait. Similar findings were reported by Seyoum *et al.* (2012), Tushara *et al.* (2013), Kishore *et al.* (2015), Rukminidevi *et al.* (2016) and Rajendra *et al.* (2017).

This trait recorded high heritability (93.73) coupled with high genetic advance as percent of mean (41.51) indicating the operation of additive gene action in inheritance of this trait which are in agreement with the findings reported by Sameera *et al.* (2015) and Rukminidevi *et al.* (2016).

4.1.1.6 Shoot length at 7 DAS in field (cm)

The character shoot length at 7 DAS is an important trait under direct sowing situations for suppression of weed growth. Genotypes for this trait varied from 5.71 cm (MTU 3626) to 12.02 cm (BPT 2740) with a mean value of 7.18 cm. High phenotypic (20.79) and moderate genotypic (18.12) coefficient of variation was observed for this trait indicating that variation among genotypes is high. High heritability (76.01)

coupled with high genetic advance as percent mean (32.25) indicates the predominance of additive gene action in inheritance of the trait. These findings were reported earlier by Lakshmi *et al.* (2016).

4.1.1.7 Root length at 7 DAS in field (cm)

Under dry direct sowing conditions in field minimum value of 1.55 cm was recorded by BPT 2782 for root length at 7 DAS while Binadhan-11 manifested maximum root length of 6.55 cm and the overall mean was 3.4 cm. Four entries viz., NLR 4001 (4.63 cm), MTU 5182 (4.63 cm), BPT 2507 (4.08 cm) and BPT 2593 (4.01 cm) exhibited maximum root length followed by Binadhan-11.

All the genetic parameters studied manifested high value for this trait. High phenotypic (26.02) and genotypic (22.97) coefficient of variation along with high heritability (77.90) and high genetic advance as percent of mean (41.76) were manifested by this trait. These findings are in agreement with the results reported by Lakshmi *et al.* (2016).

4.1.1.8 Dry matter at 7 DAS in field (mg)

The range of variation for this trait is high which varied from 12 mg (MTU 2067) to 26 mg (NLR 33892) with a mean value of 18.48 mg. This trait manifested moderate genotypic (13.42) and phenotypic (16.98) coefficients of variation coupled with high heritability (62.42) and high genetic advance as per cent of mean (21.84).

The estimates of both GCV and PCV were moderate indicating that the variation among the genotypes are moderate. These findings are in agreement with the results reported by Chen *et al.* (1986). High heritability coupled with high genetic advance as per cent of mean was recorded for this trait suggesting the role of additive gene action in the inheritance of this trait. These findings are in accordance with the results reported by Subba rao *et al.* (1996).

4.1.1.9 Shoot length at 14 DAS in field (cm)

Variability for this character ranged from 8.74 (MTU 1061) to 19.19 (BPT 2782) with a mean value of 12.42. This trait showed moderate genotypic (18.83) and high phenotypic (20.20) coefficient of variation. These findings were earlier reported by Pavanshankar (2015). High heritability (86.87) and high genetic advance as percent

of mean (36.15) were manifested by this trait. These findings were earlier reported by Akram *et al.* (1993). Mean performance of shoot length at 14 DAS in field is represented in fig 4.2.

4.1.1.10 Root length at 14 DAS in field (cm)

Genotypes for this trait varied from 2.44 cm (BPT 2595) to 7.4 cm (BPT 4358) with a mean value of 4.89 cm under field conditions. Both genotypic (26.95) and phenotypic (28.45) coefficient of variation were high indicating the variation among genotypes is high. The estimates of heritability (89.73) and genetic advance as percent of mean (52.60) were high for this trait which are in agreement with the results reported by Sathya and Jebraj (2013).

4.1.1.11 Dry matter at 14 DAS in field (mg)

Wide range of variation was observed for dry matter at 14 DAS under field condition which ranged from 19mg (NLR 33892, BPT 2573 and BPT2660) to 35mg (AC39416A) with a mean value of 24.69 mg. The estimates of genotypic (13.76) and phenotypic (17.11) coefficients of variation were moderate while the estimates of heritability (64.70) and genetic advance as per cent of mean (22.80) were high for this trait.

The estimates of both GCV and PCV were moderate indicating that the variation among the genotypes are moderate. These findings are in agreement with the results reported by Pavanshankar (2015). High heritability coupled with high genetic advance as per cent of mean was recorded for this trait suggesting the role of additive gene action in the inheritance of this trait. These findings are in accordance with the results reported by Lakshmi *et al.* (2016).

4.1.1.12 Germination percentage under dry direct sowing in field

Germination percentage in the genotypes studied range from 82 (BPT 2573) to 100 (BPT 2507 and MTU 1001) with a mean value of 92.28. Low genotypic (7.68) and phenotypic (8.43) coefficients of variation were recorded for this trait. Low GCV and PCV indicated less variation among the genotypes studied.

High heritability (82.98) and moderate genetic advance as percent of mean (14.41) was recorded for this trait indicating the involvement of both additive and non additive gene action. Similar findings were previously reported by Lakshmi *et al.* (2016) and Okelola *et al.* (2007).

4.1.1.13 Vigour index under field conditions

Vigour index recorded a mean value of 1.67 with a range of 1.04 (BPT 2660) to 2.45 (AC39416A). Moderate genotypic (15.33) and phenotypic (18.45) coefficients of variation, high heritability (69.04) and high genetic advance as per cent of mean (26.24) were recorded for this trait.

High heritability accompanied with high genetic advance as per cent of mean recorded for this trait indicating that most likely heritability is due to additive gene action and hence selection may be effective. High heritability coupled with moderate GCV and PCV indicates both additive and non additive gene effects in the inheritance of this trait. These findings are in agreement with the results reported by Ramadevi (1998) and Okelola *et al.* (2007).

4.1.2 Mean, variability, heritability and genetic advance as percent of mean for early vigour traits under anaerobic conditions

The mean performance of genotypes is presented in Table 4.5 The estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2 broad sense) and genetic advance as per cent of mean (GAM) are presented in Table 4.7 and described character wise here under.

4.1.2.1 Shoot length after 7 days of submergence (cm)

Variability for this character ranged from 0 cm (MTU 5293) to 19.10 cm (BPT 2743) with a mean value of 7.53 cm. This trait showed high estimates of genotypic (53.59) and phenotypic (54.65) coefficients of variation, high heritability (96.14) and low genetic advance as per cent of mean (8.54). These results are in accordance with Pandey *et al.* (1992). High heritability and low genetic advance indicated the role of both additive and non additive gene action and provides limited scope for improvement of traits through selection.

4.1.2.2 Root length after 7 days of submergence (cm)

Root length after 7 days of submergence recorded a mean value of 2.79 cm with a range of 0 cm (MTU 5293) to 8.04 cm (BPT 2573). High genotypic (44.24) and phenotypic (45.46) coefficient of variation. High heritability (94.74) and low genetic

advance as percent of mean (2.48) was recorded for this trait. Previous findings by Sathya and Jebaraj (2013) and Hosseini *et al.* (2012) also confirmed the present results. Mean performance of this trait is represented in fig 4.3.

4.1.2.3 Dry matter after 7 days of submergence (mg)

Under anaerobic conditions, dry matter varied from 0 mg (MTU 5293) to 23 mg (BPT 2673) with a mean value of 13.31mg. Both genotypic (29.87) and phenotypic (32.75) coefficient of variation were high indicating the variation among genotypes is high. These results are in agreement with the findings reported by Lakshmi *et al.* (2016). High heritability (83.14) with low genetic advance as percent of mean (7.47) was recorded for this trait suggesting the role of both additive and non-additive gene effects in controlling this trait.

4.1.2.4 Number of leaves under 7 days of submergence

Number of leaves among genotypes varied from 0.00 (MTU 5293) to 2 (BPT 2740, BPT 4358 and MTU 5249) with a mean value of 1.07. High genotypic (26.57) and phenotypic (29.47) coefficient of variation were recorded along with high heritability (81.25) and low genetic advance as percent of mean (0.53) indicating the role of both additive and non additive gene action in controlling this trait.

4.1.2.5 Anaerobic germination percentage under 7 days of submergence

With a mean value of 78.5% the anaerobic germination percentage at 7 DAS varied from 0.00 (MTU 5293) to 100 (BPT 2411, AC 39397 and AC 39416A). Among the forty eight genotypes studied 28 recorded more than 80% germination under 7 days after sowing under anaerobic conditions indicating that all these genotypes can tolerate anaerobic conditions up to one week and can be recommended for direct sowing in upland and irrigated areas also, because these genotypes can tolerate sowing under anaerobic conditions immediately after sowing for one week. All the variability parameters studied viz., GCV (24.37), PCV (25.35), heritability (92.43) and genetic advance as per cent of mean (37.88) manifested high values indicating that the variation among the genotypes are high and additive gene action is prevalent for the inheritance of this trait. Similar results were reported earlier by Lakshmi *et al.* (2016).

4.1.2.6 Vigour index under 7 days of submergence

The results revealed that vigour index recorded a mean value of 1.41 with a range of 0.00 (MTU 5293) to 3.18 (NLR 20084). This character manifested high genotypic (48.74) and phenotypic (49.99) coefficient of variation. High heritability (95.06) and low genetic advance as per cent of mean (1.38) were observed for this parameter which indicated the operation of both additive and non additive gene actions in controlling the expression of this trait. These findings are in agreement with the results reported by Rajendra *et al.* (2015).

4.1.2.7 Shoot length after 14 days of submergence (cm)

A mean value of 14.83cm was recorded by this parameter exhibiting the range between 0.00 cm (BPT 2571) and 28.1 cm (BPT 2573). High genotypic (39.88) and phenotypic (40.8) coefficient of variation were exhibited by this trait. This character manifested high heritability (95.52) and moderate genetic advance as percent of mean (11.91) suggesting the expression of this trait under the control of additive and non additive gene actions. Hosseini *et al.* (2012) also reported high heritability for this trait.

4.1.2.8 Root length after 14 days of submergence (cm)

Variability for this trait ranged between 0.00 cm (BPT 2571) and 8.25 cm (BPT 2573) attaining a mean value of 3.78cm. High estimates for genotypic (40.77) and phenotypic (43.13) coefficient of variation were recorded. High heritability (89.36) and low genetic advance as percent of mean (3.00) were exhibited by this character suggesting that the expression of root length 14 DAS under anaerobic conditions is under the control of both additive and non additive gene effects. These findings are in agreement with the results reported by Khriedinuo *et al.* (2011).

4.1.2.9 Dry matter after 14 days of submergence (mg)

It exhibited a wide variation with the range between 0.00 mg (BPT 2660) and 36 mg (NLR 20084) attaining a mean value of 19.16mg. It recorded high phenotypic (42.22) and genotypic (40.62) coefficient of variation indicating high variability among the treatments for this trait. High heritability (92.59) and moderate genetic advance (15.43) was recorded for this trait and represented in fig.4.4. These results are in agreement with the findings reported by Sharma and Sharma (1993).

4.1.2.10 Vigour index under 14 days of submergence

Vigour index 14DAS under anaerobic conditions in all genotypes range from 0.00 (BPT 2571) to 2.8 (BPT 2411) with a mean value of 0.87. High genotypic (66.41) and phenotypic (67.37) coefficients of variation indicated more variability among the 48 genotypes studied. It manifested high heritability (97.59) and low genetic advance as per cent of mean (1.18) suggesting that this trait is under both additive and non additive gene effects. Similar findings were reported by Rajendra *et al.* (2015).

4.1.2.11 Number of leaves under 14 days of submergence

Variability for this character ranged from 0.00 (BPT 2571) to 2 (BPT 2411) with a mean value of 1.3. Among the genotypes taken under study 19 entries exhibited more than mean value 1.3 for this trait. High estimates of phenotypic (47.68) and genotypic (47.03) were recorded along with high heritability (97.30) and low genetic advance as percent of mean (1.24). These findings are in agreement with the findings reported by Rajendra *et al.* (2015) in a study under aerobic conditions.

4.1.2.12 Anaerobic germination percentage under 14 days of submergence

Wide range of variation was observed for this trait under anaerobic conditions. Among the genotypes studied BPT 2571 (0) manifested low value for this trait while AC39416A (100) exhibited the maximum value with a mean value of 57.07. Three genotypes viz., BPT 2571, BPT 2660, BPT 2743 recorded no germination (0%) under 14 days after sowing under anaerobic conditions. BPT 2411 (93.3%), BPT 2782 (86.6%), BPT 2507 (86.6%), BPT 2740 (83.3%), MTU 1064 (83.3%) and BPT 2595 (80.0%) exhibited more than 80% germination under 14 days of submergence also. Other genotypes which recorded > 75% germination under 14 days of submergence include BPT 5204, BPT 2573, MTU 5249, MTU 1061, MTU 2716, NLR 4002 and IR 85961-23-1-2 showing resistance to anaerobic conditions upto 14 days submergence. The genotypes which recorded resistance to anaerobic germination under 14 days of submergence combining high grain yield / plant include BPT 2740, MTU 5249, MTU 2716, IR 85961-23-1-2-1, MTU 1140, BPT 2595, BPT 2782, BPT 2411, BPT 2573 and MTU 1064. Hence, these genotypes can be recommended for direct sowing under anaerobic conditions also.

Rajendra kumar and Christopher (2005) also studied 11 genotypes for their tolerance to anaerobic germination and identified 3 genotypes viz., ASD 19, Co 43, ADT 38 suitable for direct seeding. All the genetic parameters studied for this trait manifested high estimates. High genotypic (47.17) and phenotypic (48.27) coefficients of variation along with high heritability (95.48) and high genetic advance as per cent of mean (54.19) indicated that variation among the genotypes was high and the predominance of additive gene action in the inheritance of this trait. Hence, simple selection will be highly rewarding for improving this character which are in agreement with the results reported by Lakshmi *et al.* (2016).

4.1.2.13 Free amino acids (mg g⁻¹)

In the present study, the trait Free amino acids recorded a minimum value of 0.50 in MTU 2716 while AC39416A registered a maximum value of 4.12 with a mean value of 1.82 and the mean values are shown in fig.4.5. High estimates for genotypic (57.3) and phenotypic (57.19) coefficients of variation were recorded for this trait. The heritability estimates were high (100) while genetic advance as per cent of mean (2.14) was low for this trait.

The genotypes viz., IR 85961-23-1-2, AC 39397, BPT 2411, BPT 2595, MTU 5249 which recorded high values for free amino acids manifested high anaerobic germination percentage also indicating positive relationship between these two traits. Under anaerobic conditions, germination in rice caryopsis only results in the emergence and growth of coleoptile (Kordan 1976). The anaerobic elongation of rice coleoptile is sustained by continuous translocation of carbohydrates, amino acids and salts from endosperm (Atwell *et al.* 1982). The presence of free amino acids in the endosperm will enhance coleoptile elongation or germination of genotypes which is evident in the present study also.

4.1.2.14 Total soluble sugars (mg g⁻¹)

With a mean value of 21.75, genotypes for this trait showed wide range of variation from 9.44 (BPT 2295) to 41.66 (AC39416A). In the present study, 7 other genotypes viz., MTU 5249, BPT 2411, MTU 2716, BPT 2507, BPT 2782 also manifested higher values for total soluble sugars. All these genotypes exhibited high anaerobic germination percent at 7 and 14 days of submergence. Under 21 days of

anaerobic conditions also AC 39397, recorded maximum total soluble sugars (41.66) exhibited 100% germination. Like wise, AC 39397, MTU 5249 and BPT 2411 also recorded high values for total soluble sugars and manifested above 50% anaerobic germination suggesting that high concentration of total soluble sugars is associated with tolerance to anaerobic germination. Soluble sugar concentration in seed and its transport from the endosperm to the coleoptile are reported to be closely associated with coleoptile elongation under anoxia (Furuhata *et al.* 2006; Kato-Noguchi *et al.* 2010). Hence, presence of higher concentration of soluble sugars indicates tolerance of genotypes to anaerobic germination.

This trait observed high estimates for both GCV (51.88) and PCV (51.95) indicating that variation among the genotypes are high. High heritability (100) coupled with high genetic advance as per cent of mean (23.22) was observed for this trait indicating the operation of additive gene action. Hence simple selection would be effective for improving this character.

Most of the characters under study, showed high heritability estimates ranging from 80-90% reflecting ample scope for their improvement through appropriate breeding procedures. The estimates of phenotypic coefficient of variation for all the characters under study were high than the estimates of genotypic coefficient of variation. This close correspondence between the estimates of phenotypic coefficient of variation and genotypic coefficient of variation was also reflected by their high heritability values and indicated meagre influence of environment on the expression of these traits.

Based on the study, it may be concluded that among yield components high heritability coupled with high genetic advance as percent of mean was recorded for plant height, number of ear bearing tillers, grain yield and test weight. Under field conditions shoot length at 7 and 14 days after sowing, root length at 7 and 14 days after sowing, dry matter at 7 and 14 days after sowing and vigour index manifested high heritability coupled with high genetic advance as percent of mean. Germination percentage after 7 and 14 days of submergence and total soluble sugars also recorded high heritability and genetic advance as percent of mean indicating the operation of additive gene action in the inheritance of these traits. Hence good response to selection can be attained for improvement of these traits.

The estimates of high heritability and moderate to low genetic advance as percent of mean was recorded for panicle length, shoot length after 7 and 14 days of submergence, root length after 7 and 14 days of submergence, dry matter after 7 and 14 days of submergence, vigour index under 7 and 14 days of submergence, number of leaves under 7 and 14 days of submergence, germination percentage under field conditions and free amino acids. The traits exhibiting high heritability and low to moderate genetic advance as percent of mean indicates the involvement of both additive and non-additive gene action and provides limited scope for improvement of traits through simple selection.

4.1.3 Mean Performance And Variability Parameters For Vigour Traits Under Anaerobic Germination at 21 Days of Submergence

Use of cultivars tolerant to anaerobic conditions with high seedling vigour would improve anaerobic seedling establishment and stabilize the direct seeding method. Hence, an attempt was made to identify the cultivars which are tolerant to anaerobic seeding under flooded conditions for 21 days also. Among the 48 genotypes under study, only 13 genotypes showed resistance to anaerobic germination *i.e.*, > 75% anaerobic germination under 14 days of submergence indicating their tolerance to anaerobic conditions. Hence these 13 genotypes were screened for anaerobic germination under 21 days of submergence also. In this experiment only 13 genotypes were screened and hence the data generated was used for estimation of mean performance and genetic parameters (Table 4.8) and was not included in the estimation of correlation coefficient and path coefficient analysis.

The donors for anaerobic germination included in the present study *viz.*, AC 39416A recorded 100 % germination followed by AC39397 (90%) and IR 85961-23-1-2-1 (74.9%). The other genotypes which exhibited more than 50% germination under 21 days of sowing under anaerobic conditions include MTU 1140 (58.3%), BPT 2411 (54.95%) and MTU 5249 (56.65%). Hence, the above genotypes could be used as donors for anaerobic germination trait in crossing programme for developing suitable varieties for dry/wet direct sowing.

4.1.3.1 Shoot length after 21 days of submergence (cm)

Among the genotypes under study, shoot length after 21 days of submergence varied from 8.80cm (MTU 2716) to 28cm (MTU 1064) with a mean value of 17.63cm. The other genotypes which recorded high shoot length after 21 days of submergence include AC 39397, IR 85961-23-1-2-1, BPT 2411, AC 39416A and BPT 2595 which recorded more than 15cm shoot length and exhibited > 50% anaerobic germination and > 30 mg g⁻¹ of total soluble sugars suggesting that the ability of rice seeds to germinate under flooded soil and fast elongation of shoot length has been associated with the ability to breakdown starch stored in seeds and use it for the growing embryo under low oxygen stress (Ismail *et al* 2009). All the variability parameters studied viz., GCV (33.65), PCV (35.56), heritability (89.58) and genetic advance as per cent of mean (65.61) manifested high values for this trait indicating that the variation among the genotypes is high and the prevalence of additive gene action in controlling this trait. Akram *et al.* (1993) also reported similar findings.

4.1.3.2 Root length after 21 days of submergence (cm)

A mean value of 3.30cm was recorded by this parameter exhibiting a range between 1.95cm (RGL 1414) and 4.4cm (BPT 2411). Under 21 days submergence, BPT 2411 followed by MTU 1064, AC39397, AC 39416A, BPT 2595 and MTU 1140 recorded high root length along with high shoot length and dry matter also which resulted in high anaerobic germination percent. Ismail *et al.* (2009) also reported that the genotypes which can elongate their roots fastly by breaking the stored starch under low oxygen stress will manifest high germination. Both genotypic (25.42) and phenotypic (27.66) coefficient of variation recorded high estimates for this trait. This character manifested high heritability (84.47) coupled with high genetic advance as percent of mean (48.13) suggesting the expression of this trait is controlled by additive gene action. Similar findings were previously reported by Jiakui *et al.* (1996).

4.1.3.3 Dry matter after 21 days of submergence (mg)

The results revealed that dry matter 21 days under anaerobic conditions recorded a mean value of 14.69 with a range of 6 mg (NLR 4002) to 27 mg (AC39397). IR 85961-23-1-2-1, AC 39416A and BPT 2411 manifested more than 20mg dry matter under 21 days submergence. This character manifested high genotypic

(44.70) and phenotypic (45.49) coefficient of variation. High heritability (96.56) coupled with high genetic advance as per cent of mean (90.48) were observed for this parameter which indicated the operation of additive gene actions in controlling the expression of this trait. These findings are in accordance with the results reported by Subba rao *et al.* (1996).

4.1.3.4 Vigour index under 21 days of submergence

This trait recorded high genotypic (74.49) and phenotypic (74.81) coefficient of variation along with high heritability (99.38) and high genetic advance as percent of mean (214.57). It has a mean value of 0.88 with a range of 0.04 (NLR 4002) to 2.61 (AC39397). This indicated that variation among the genotypes is high and the predominance of additive gene action in the inheritance of this trait. These findings are in agreement with the results reported by Ramadevi (1998).

4.1.3.5 Number of leaves under 21 days of submergence

The range of variation for this trait varied from 1 (BPT 2595, BPT 2411, BPT 2595, BPT 2573, BPT 2740, MTU 5249, MTU 1064, MTU 1140, MTU 2716, RGL 1414 and IR85961-23-1-2-1) to 2 (AC 39416A and AC 39397) with a mean value of 1.21. This trait manifested high genotypic (33.04) and phenotypic (34.02) coefficient of variation coupled with high heritability (94.34) and high genetic advance as per cent of mean (66.11) suggesting the role of additive gene effects in the inheritance of this trait .

4.1.3.6 Anaerobic germination percentage after 21 days of submergence

Under anaerobic conditions, germination percentage at 21 days of submergence recorded a wide range of variation. The minimum value was observed in NLR 4002 (6.6) while the genotype AC39416A (100) recorded the maximum value followed by AC 39397 (90%), IR 85961-23-1-2-1 (74.95%), MTU 1140 (58.3%), MTU 5249 (56.65%) and BPT 2411 (54.95%). The improved germination and emergence in these lines were also associated with the ability to mobilize starch and maintain higher soluble sugar supply to the developing embryo under low oxygen stress (Ismail *et al.* 2009). The mean value of all the genotypes is 44.47. It manifested high PCV (72.4), GCV (71.65) along with high heritability (98.01) and high genetic advance as percent of mean (146.17) suggesting the expression of this trait is controlled by additive gene

action. These findings are in agreement with the findings of Okelola *et al.* (2007) in a study involving 24 genotypes under aerobic conditions.

4.1.3.7 Anaerobic response index

Variability for this character ranged from -3.8 (MTU 2716) to 15.4 (MTU 1064) with a mean value of 5.03. All the variability parameters viz., PCV (64.10), GCV (63.43), heritability (97.19) and genetic advance as percent of mean (129.31) recorded high values for this trait indicating that the variation among the genotypes is high and the prevalence of additive gene action in controlling this trait.

The coleoptiles and mesocotyl elongation of a rice seedling contribute to the emergence ability of dry seeded rice. Under flooding conditions, rapid elongation of coleoptiles and mesocotyl could facilitate contact with air and subsequent aeration of the growing radical and leaves (Magneschi *et al.*, 2009 and Alibu *et al.*, 2011).

Under 21 days of submergence in 10cm depth of water, the genotype AC 39416A recorded 100% germination. This genotype also exhibited high values for shoot length, vigour index, anaerobic response index, root length, dry matter and number of leaves indicating the cumulative effect of all these traits which contributed for high anaerobic germination. Other genotypes which manifested relatively high values for shoot length, root length, dry matter and vigour index also showed high anaerobic germination percent *i.e.* above 50%. Previous studies conducted by Yamauchi *et al.* 1994; Yamauchi and Biswas, 1997 proved that coleoptiles of flooding tolerant cultivars grew faster and longer in flooded soil. The morphological adjustment enabled the superior cultivars to access oxygen at the water surface earlier which supported the growth of organs such as the primary leaf and root. In addition, the genotypes viz., AC 39416A, AC 39397, IR 85961-23-1-2-1, BPT 2411, MTU 5249, MTU 1140 and BPT 2595 also possess high amount of total soluble sugars and free amino acids which might have contributed for faster elongation of root and shoot under anaerobic conditions.

Kretzschmar *et al.* (2015) reported enhanced starch availability increases anaerobic germination tolerance while Atwell *et al* (1982) studied the importance of free amino acids in the coleoptile elongation under anaerobic situation. Rajendra Kumar and Christopher (2005) also studied eleven cultivars and identified ASD 19, Co

43 and ADT 38 as anaerobic tolerant cultivars as they recorded higher seedling establishment, root growth, shoot growth, dry matter production and vigour index which are in agreement with the present results.

Further, based on this study, it may be concluded that all the vigour related traits studied under 21 days of submergence manifested higher estimates for all the genetic parameters under study indicating the role of additive gene effects in controlling these traits. Hence, simple selection can bring improvement in all these characters.

The present investigation is aimed at identifying varieties/cultivars tolerant to anaerobic conditions combining high grain yield which can be recommended for direct sowing in rainfed and irrigated lowland areas. Further studies are recommended to test the survival rate and establishment of seedlings germinated under anaerobic conditions and to estimate the growth parameters and yield potential also.

4.2 ASSOCIATION OF YIELD COMPONENTS AND EARLY VIGOUR TRAITS WITH GRAIN YIELD

A major shift in rice establishment methods towards direct seeding using tolerant anaerobic germination lines combined with good management practices will result in enormous savings in production costs as opposed to transplanted rice. The association studies among yield components and early vigour traits under anaerobic conditions are very limited. Hence, in the present investigation an attempt was made to study the relationships between yield components and vigour traits under anaerobic conditions.

Direct selection for yield and vigour traits is not effective as they are complex quantitative characters and highly influenced by environment. High genotype and environment interaction will restrict improvement, if selection is based on yield *per se*. The effective improvement in yield may be brought through selection of yield component characters and vigour traits. The aim of correlation studies is primarily to know the suitability of various characters for indirect selection because any particular trait may bring about changes in other associated characters (Singh, 1998).

Yield component characters show associations among themselves and also with yield. Unfavorable associations between the desired attributes under selection may limit genetic advance. Hence, knowledge of associations between the yield and vigour

components and also among the yield components is essential for planning a sound selection programme (Falconer, 1964).

The phenotypic and genotypic correlation coefficients for different grain yield components and early vigour parameters are presented in Tables 4.9 and 4.10 and discussed here under.

4.2.1 Plant Height (cm)

Plant height showed significant and positive association with panicle length (0.5947 and 0.5080), test weight (0.3868 and 0.3545), dry matter at 7 DAS in field (0.3595 and 0.2556), vigour index (0.2394 and 0.1746), anaerobic germination percentage under 7 days of submergence (0.2623 and 0.2530), anaerobic germination percentage under 14 days of submergence (0.2283 and 0.2141), vigour index under 7 days of submergence (0.2779 and 0.2707), vigour index under 14 days of submergence (0.3814 and 0.3672), free amino acids (0.2283 and 0.2189) and total soluble sugars (0.2191 and 0.2137) both at phenotypic and genotypic levels respectively. This trait showed significant negative genotypic association with shoot length at 14 DAS in field conditions (-0.1858). The results indicated that the tall genotypes manifested high percentage of anaerobic germination. It is also reflected by the significant positive association of this trait with free amino acids and total soluble sugars which are essential for faster shoot elongation under anaerobic conditions.

This trait showed non significant positive phenotypic and genotypic association with grain yield. These findings are in agreement with the results reported by Rangare *et al.* (2012), Uday *et al.* (2015), Kishore *et al.* (2015) and Onyia *et al.* (2017).

4.2.2 Number of Ear Bearing Tillers

It exhibited positive and significant association with panicle length (0.1759) at genotypic level and significant positive correlation with shoot length after 7 days of submergence (0.2446 and 0.1909), total soluble sugars (0.1867 and 0.1729) both at phenotypic and genotypic levels. Hence, selection of genotypes with high shoot length under anaerobic conditions will result in isolation of genotypes with more number of ear bearing tillers which ultimately result in high grain yield.

It showed significant negative association with test weight (-0.3614 and -0.3106), root length at 7 DAS in field (-0.2311 and 0.2127), dry matter at 14 DAS in

field (-0.3633 and -0.2800), root length after 14 days of submergence (-0.3060 and -0.2582), dry matter after 14 days of submergence (-0.3627 and -0.3096), vigour index under 7 days of submergence (-0.2331 and -0.1924) at both the levels. Careful selection of genotypes possessing with more number of ear bearing tillers and anaerobic germination is needed for development of genotypes for direct sowing.

This trait showed significant positive association at both genotypic and phenotypic levels with grain yield per plant which is in accordance with the results obtained by Ramanjaneyulu *et al.* (2014), Saikumar *et al.* (2014) and Onyia *et al.* (2017).

4.2.3 Panicle Length (cm)

The results revealed that panicle length showed significant and positive association with dry matter under 7 days of submergence (0.2482 and 0.1815), anaerobic germination percentage under 7 days of submergence (0.2559 and 0.2289), vigour index under 7 days of submergence (0.2894 and 0.2541), vigour index under 14 days of submergence (0.2515 and 0.1868) suggesting that indirect selection for genotypes with long panicles may result in isolation of genotypes with high anaerobic germination under 7 days of submergence. This trait also showed significant and genotypic negative association with shoot length 14 DAS in field (-0.2164) and dry matter at 14 DAS in field (-0.1982) indicating that tall genotypes used in the present investigation possessed shorter panicle length. This trait showed nonsignificant positive association with grain yield. These results are in agreement with Seyoum *et al.* (2012), Dhurai *et al.* (2014), Pradhan *et al.* (2015) and Kishore *et al.* (2015).

4.2.4 Test Weight (g)

It showed positive and significant association with root length at 7 DAS in field (0.2538 and 0.2595), root length at 14 DAS in field (0.3067 and 0.2751), dry matter 7 at DAS in field (0.4714 and 0.3665), dry matter at 14 DAS in field (0.5199 and 0.3947), germination percentage (0.1820 and 0.1725), vigour index (0.5017 and 0.4378), dry matter after 7 days of submergence (0.3549 and 0.3041), dry matter after 14 days of submergence (0.2486 and 0.2220), anaerobic germination percentage under 7 days of submergence (0.2791 and 0.2603), anaerobic germination percentage under 14 days of submergence (0.3597 and 0.3423), vigour index under 7 days of

submergence (0.2330 and 0.2171), vigour index under 14 days of submergence (0.4404 and 0.4212), free amino acids (0.1874 and 0.1820) and total soluble sugars (0.2380 and 0.2301) at both the levels. Results showed that bolder genotypes have better root anchorage and seedling establishment under dry direct sowing and with anaerobic germination percentage and selection of this trait is rewarding.

This trait showed positive and non-significant phenotypic (0.0042) and genotypic (0.0108) association with grain yield. Similar findings were reported by Basavaraja *et al.* (2013), Ratna *et al.* (2015), Kishore *et al.* (2015) and Hosseini *et al.* (2015).

4.2.5 Shoot Length at 7 DAS in Field (cm)

Shoot length at 7 DAS in field exhibited positive and significant association with shoot length at 14 DAS in field (0.9317 and 0.7563), dry matter at 7 DAS in field (0.2837 and 0.2634) and dry matter at 14 DAS in field (0.4462 and 0.3308). These findings are in accordance with the results reported by Pavanshankar (2015). It also showed positive association with vigour index (0.3520 and 0.2702), free amino acids (0.2855 and 0.2495) at genotypic and phenotypic levels. Indirect selection for this trait is desirable for seedling establishment under direct sowing.

This trait showed non-significant and negative association with grain yield at both genotypic (-0.0516) and phenotypic (-0.0461) level indicating the independent inheritance of this trait with grain yield

4.2.6 Shoot Length at 14 DAS in Field (cm)

It exhibited positive and significant genotypic and phenotypic association with dry matter at 14 DAS in field (0.4715 and 0.3911), germination percentage (0.1993 and 0.1634), vigour index (0.2354 and 0.1767), anaerobic germination percentage under 14 days of submergence (0.1853 and 0.1690) and free amino acids (0.2967 and 0.2753). This trait showed negative and non significant association with grain yield at both genotypic (-0.1366) and phenotypic (-0.1424) levels indicating independent inheritance of this trait with grain yield per plant.

Careful indirect selection of this trait would help in identification of suitable genotype for direct sowing possessing early vigour and anaerobic germination. Atwell

et al. (1982) also reported the positive association of rice coleoptiles elongation with translocation of amino acids from endosperm.

4.2.7 Root Length at 7 DAS in Field (cm)

Under field conditions root length at 7 DAS exhibited significant and positive association with root length at 14 DAS in field (0.5034 and 0.4189), shoot length after 14 days of submergence (0.1689 and 0.1779), root length after 14 days of submergence (0.2280 and 0.1842) at both genotypic and phenotypic levels.

This trait showed negative and non-significant association with grain yield indicating that it is independent of the trait in inheritance at both genotypic (-0.1171) and phenotypic levels (-0.1207). These findings are in agreement with the results reported by Pavanshankar (2015) for root length at 20 DAS in field.

4.2.8 Root Length at 14 DAS in Field (cm)

In the present study this trait showed positive and significant association with vigour index under 14 days of submergence (0.1840 and 0.1851) at both genotypic and phenotypic levels. It also showed negative and significant association with shoot length after 7 days of submergence (-0.4263 and -0.3996), root length after 7 days of submergence (-0.2111 and -0.2056) at both levels indicating that increase in root length at 14 DAS in field would lead to decrease in shoot length after 7 days of submergence and root length after 7 days of submergence. It exhibited negative non-significant association with grain yield at genotypic (-0.0576) and phenotypic levels (-0.0451). Similar findings were reported by Pavanshankar (2015) who studied 15 early vigour traits in 22 genotypes.

4.2.9 Dry matter at 7 DAS in Field (mg)

The results revealed that dry matter at 7 DAS in field showed positive and significant association with vigour index (0.8477 and 0.8127), number of leaves under 14 days of submergence (0.1830 and 0.1643), anaerobic germination percentage under 7 days of submergence (0.4396 and 0.3355), anaerobic germination percentage under 14 days of submergence (0.3411 and 0.2840), vigour index under 7 days of submergence (0.4262 and 0.3539), vigour index under 14 days of submergence (0.2951 and 0.2351), total soluble sugars (0.2550 and 0.1971), root length after 14 days of submergence (0.2356 and 0.2079), dry matter after 7 days of submergence (0.3143 and

0.1803), dry matter after 14 days of submergence (0.4334 and 0.3401) at both genotypic and phenotypic levels. Similar findings are reported by Pavanshankar (2015) in a study involving 22 rice genotypes sown under dry direct sowing.

It exhibited positive and non-significant association with grain yield at both genotypic (0.0127) and phenotypic (0.0619) levels. Pavanshankar (2015) reported positive and significant association of this trait with grain yield for this trait.

4.2.10 Dry matter at 14 DAS in Field (mg)

It exhibited positive and significant association with germination percentage (0.4132 and 0.2941), vigour index under field conditions (0.4760 and 0.2708), vigour index under 14 days of submergence (0.2722 and 0.2096) and free amino acids (0.2383 and 0.1915). It also showed negative significant genotypic and phenotypic association with shoot length after 14 days of submergence (-0.3481 and -0.2922). Similarly it manifested negative and significant association with grain yield at both phenotypic (-0.2473) and genotypic (-0.3155) levels. These findings are in agreement with Zulquarnain *et al.* (2012).

4.2.11 Germination Percentage in Field

This trait showed significant and negative association with grain yield at both genotypic (-0.2199) and phenotypic (-0.2067) level. It showed positive and significant association with vigour index at 14 DAS in field (0.5144 and 0.4459), dry matter after 7 days of submergence (0.1871 and 0.2037), number of leaves under 14 days of submergence (0.2017 and 0.1725), anaerobic germination percentage under 14 days of submergence (0.3393 and 0.3082), vigour index under 14 days of submergence (0.3544 and 0.3213) at both genotypic and phenotypic levels and test weight only at genotypic level which are in accordance with the findings of Singh *et al.*(2000). The results indicated that poor seedling survival leads to reduced grain yield due to lack of vigour traits under anaerobic conditions.

4.2.12 Vigour Index in Field

Vigour index in field showed positive and significant genotypic and phenotypic association with dry matter after 7 days of submergence (0.3756 and 0.2456), dry matter after 14 days of submergence (0.3959 and 0.2959), number of leaves under 7

days of submergence (0.3644 and 0.1762), number of leaves under 14 days of submergence (0.2225 and 0.1971), anaerobic germination percentage under 7 days of submergence (0.4542 and 0.3537), anaerobic germination percentage under 14 days of submergence (0.4542 and 0.3801) vigour index under 7 days of submergence (0.3934 and 0.3258), vigour index under 14 days of submergence (0.4249 and 0.3394) and total soluble sugars (0.3292 and 0.2703). The results indicated that genotypes with high vigour index under aerobic conditions also express high anaerobic germination percentage at 7 and 14 days of submergence.

Vigour index under field conditions showed negative and non-significant correlation both at genotypic (-0.1340) and phenotypic (-0.1003) levels with grain yield whereas positive and significant association between these two traits was reported by Adebisi *et al.* (2010). The discrepancy in results may be due to different sets of genotypes under study.

4.2.13 Shoot Length After 7 Days of Submergence (cm)

Under anaerobic conditions shoot length at 7 days of submergence showed significant and positive association with shoot length after 14 days of submergence (0.1975 and 0.1899), root length after 7 days of submergence (0.5396 and 0.5295), dry matter after 7 days of submergence (0.3969 and 0.3687), number of leaves under 7 days of submergence (0.2194 and 0.1804), anaerobic germination percentage under 7 days of submergence (0.2817 and 0.2588), anaerobic germination percentage under 14 days of submergence (0.2051 and 0.1941), vigour index under 7 days of submergence (0.3215 and 0.3067), vigour index under 14 days of submergence (0.1898 and 0.1827) and total soluble sugars (0.2908 and 0.2827). It manifested positive and non-significant association with grain yield (0.1576 and 0.1538) suggesting that it is independent in its inheritance. These findings were in agreement with the result reported by Zulqarnain *et al.* (2012).

4.2.14 Shoot Length After 14 Days of Submergence (cm)

The results revealed that this trait showed significant positive relationship with root length after 7 days of submergence (0.3784 and 0.3486), root length after 14 days of submergence (0.7597 and 0.7082), dry matter after 7 days of submergence (0.4756 and 0.3984) and dry matter after 14 days of submergence (0.6818 and 0.6504) which

are in agreement with the findings reported by Pavanshankar (2015) in a study of 22 genotypes under dry direct sowing. It also manifested positive association with number of leaves under 7 days of submergence (0.2815 and 0.2494), number of leaves under 14 days of submergence (0.7058 and 0.6719), anaerobic germination percentage under 7 days of submergence (0.3814 and 0.3477), anaerobic germination percentage under 14 days of submergence (0.5448 and 0.5148), vigour index under 7 days of submergence (0.3146 and 0.3019), vigour index under 14 days of submergence (0.4544 and 0.4354), free amino acids (0.2040 and 0.2013) and total soluble sugars (0.2258 and 0.2213). This trait showed negative and non-significant genotypic (-0.0232) and phenotypic (-0.0239) association with grain yield.

The results revealed that genotypes possessing high shoot length along with anaerobic germination percentage, vigour index, free amino acids, total soluble sugars would help in better seedling establishment under anaerobic conditions which is required for direct sowing in rainfed and low land areas.

4.2.15 Root Length After 7 Days of Submergence (cm)

It exhibited positive and significant association with root length after 14 days of submergence (0.4784 and 0.4465), number of leaves under 7 days of submergence (0.3689 and 0.2997), number of leaves under 14 days of submergence (0.2859 and 0.2702), anaerobic germination percentage under 7 days of submergence (0.1828 and 0.1734), anaerobic germination percentage under 14 days of submergence (0.2706 and 0.2553). This trait showed non-significant and negative association at genotypic (-0.0201) and phenotypic (-0.0143) level with grain yield. Similar findings were reported by Nitika *et al.* (2013) and Haider *et al.* (2012). Careful selection of genotypes is needed with this trait as it is negatively correlated for grain yield.

4.2.16 Root Length After 14 Days of Submergence (cm)

It showed positive and significant genotypic and phenotypic association with dry matter after 7 days of submergence (0.2703 and 0.2416), dry matter after 14 days of submergence (0.5731 and 0.5186), number of leaves under 7 days of submergence (0.3686 and 0.3028), number of leaves under 14 days of submergence (0.6688 and 0.6201), anaerobic germination percentage under 7 days of submergence (0.4706 and 0.4286), anaerobic germination percentage under 14 days of submergence (0.5956 and 0.5566), vigour index under 7 days of submergence (0.2040 and 0.1888) and vigour

index under 14 days of submergence (0.2464 and 0.2315). The results indicated that genotypes with better root anchorage possess high anaerobic germination percentage at 7 and 14 days and vigour index under anaerobic conditions.

This trait showed negative and non-significant genotypic (-0.1366) and phenotypic (-0.1138) association with grain yield which are in accordance with the results reported by Pavanshankar (2015) for root length at 14 DAS in field conditions.

4.2.17 Dry matter After 7 Days of Submergence (mg)

Dry matter after 7 days of submergence manifested significant and positive association with dry matter after 14 days of submergence (0.5931 and 0.5303), number of leaves under 7 days of submergence (0.3732 and 0.3053), number of leaves under 14 days of submergence (0.2261 and 0.1970), anaerobic germination percentage under 7 days of submergence (0.5798 and 0.4972), anaerobic germination percentage under 14 days of submergence (0.4692 and 0.4293), vigour index under 7 days of submergence (0.5477 and 0.4991), vigour index under 14 days of submergence (0.5911 and 0.5586), free amino acids (0.3366 and 0.3077) and total soluble sugars (0.4118 and 0.3758) at both the level. The results showed that genotype with high dry matter possess high anaerobic germination percentage at 7 and 14 days and vigour index under anaerobic conditions.

It showed positive and non-significant genotypic (0.0993) and phenotypic (0.0790) association with grain yield. Zulqarnain *et al.* (2012) also reported positive and significant association of this trait with grain yield per plant.

4.2.18 Dry matter After 14 Days of Submergence (mg)

Under anaerobic conditions, dry matter exhibited significant and positive association with number of leaves under 7 days of submergence (0.2635 and 0.2228), number of leaves under 14 days of submergence (0.6275 and 0.5887), anaerobic germination percentage under 7 days of submergence (0.5584 and 0.5245), anaerobic germination percentage under 14 days of submergence (0.5254 and 0.4906), vigour index under 7 days of submergence (0.7611 and 0.7351), vigour index under 14 days of submergence (0.4580 and 0.4453) and total soluble sugars (0.1797 and 0.1714) at both genotypic and phenotypic levels.

It showed significant and negative association with grain yield at both genotypic (-0.1939) and phenotypic (-0.1637) levels. Careful indirect selection for this trait is desirable for increasing grain yield.

4.2.19 Number of Leaves Under 7 Days of Submergence

It manifested non-significant and negative association with grain yield at both genotypic (-0.0095) and phenotypic (-0.0216) levels whereas Kuldeep *et al.* (2004) reported positive and significant association with grain yield. It showed significant and positive association with number of leaves under 14 days of submergence (0.4306 and 0.3835), anaerobic germination percentage under 7 days of submergence (0.4464 and 0.4005), anaerobic germination percentage under 14 days of submergence (0.2751 and 0.2439), vigour index under 7 days of submergence (0.2722 and 0.2075), vigour index under 14 days of submergence (0.2473 and 0.2278) at both the levels suggesting that high germination percent and vigour index under anaerobic conditions will bring simultaneous improvement in this trait.

4.2.20 Number of Leaves Under 14 Days of Submergence

The character number of leaves showed significant and positive genotypic and phenotypic association with anaerobic germination percentage under 7 days of submergence (0.5014 and 0.4747), anaerobic germination percentage under 14 days of submergence (0.5770 and 0.5565), vigour index under 7 days of submergence (0.3665 and 0.3565), vigour index under 14 days of submergence (0.3293 and 0.3144) suggesting that selection of genotypes with more vigour index and anaerobic germination percent will improve this trait. While it showed negative and non-significant association with grain yield at genotypic (-0.0940) and phenotypic (-0.0854) levels.

4.2.21 Anaerobic Germination Percentage Under 7 Days of Submergence

This trait exhibited positive and significant genotypic and phenotypic association with anaerobic germination percentage under 14 days of submergence (0.6853 and 0.6368), vigour index under 7 days of submergence (0.5860 and 0.5620), vigour index under 14 days of submergence (0.5399 and 0.5001), free amino acids (0.2649 and 0.2528) and total soluble sugars (0.4322 and 0.4172) suggesting that cultivars possessing high concentration of free amino acids and total soluble sugars

would record high germination percentage which are in agreement with the findings reported by Krishnaswamy and Seshu (1989) and Ismail *et al.* (2009). It showed negative and non-significant association both at genotypic (-0.0806) and phenotypic (-0.0794) levels with grain yield per plant.

4.2.22 Anaerobic Germination Percentage Under 14 Days of Submergence

It exhibited positive and significant association with vigour index under 7 days of submergence (0.3913 and 0.3688), vigour index under 14 days of submergence (0.7240 and 0.7164), free amino acids (0.2668 and 0.2606), total soluble sugars (0.3842 and 0.3770) at both the levels. Similar findings are reported by Singh *et al.* (2000) in wheat. The genotypes with high anaerobic germination percentage, better shoot length, root length, vigour index may result in tolerance to submergence at seedling stage under direct sowing.

This trait showed negative and significant genotypic (-0.1707) and non-significant phenotypic (-0.1553) association with grain yield.

4.2.23 Vigour Index Under 7 Days of Submergence

Under anaerobic conditions vigour index showed positive and significant association with vigour index under 14 days of submergence (0.3521 and 0.3363) and total soluble sugars (0.3325 and 0.3260) indicating that genotypes with high vigour index and total soluble sugars can emerge better under anaerobic conditions. This trait recorded negative and non-significant association at genotypic (-0.0674) and phenotypic (-0.0438) levels with grain yield. Adebisi *et al.* (2010) in a study conducted under aerobic conditions reported positive association of this trait with seed yield.

4.2.24 Vigour Index Under 14 Days of Submergence

It showed positive and significant association with free amino acids (0.4287 and 0.4239) and total soluble sugars (0.3468 and 0.3413). It exhibited non-significant and positive relationship at genotypic (0.0057) and phenotypic (0.0066) levels with grain yield. Prasad *et al.* (2011) reported positive and significant association of this trait with grain yield.

4.2.25 Free Amino Acids (mg g⁻¹)

The results revealed that free amino acids manifested positive and significant genotypic and phenotypic association (0.3876 and 0.3867) with total soluble sugars. Similarly it showed positive and significant genotypic (0.1802) and phenotypic (0.1722) association with grain yield suggesting simultaneous improvement of both the traits. It is desirable to identify genotypes with high free amino acids content which will aid in high anaerobic germination and plant survival under anoxia. Similar findings was previously reported by Atwell *et al.* (1982).

4.2.26 Total Soluble Sugars (mg g⁻¹)

It showed significant and positive association with grain yield at both genotypic (0.2907) and phenotypic (0.2823) levels. While it showed negative and non-significant association with root length at 14 DAS in field (-0.0526 and -0.0527), dry matter at 14 DAS in field (-0.0293 and -0.0276), root length at 7 DAS in field (-0.2080 and -0.1880) at genotypic and phenotypic levels. It has positive association with anaerobic germination, total soluble sugars and α -amylase activity under anoxia conditions helps in mere plant survival.

Among the yield components, plant height, panicle length and test weight manifested non-significant and positive association with grain yield whereas number of ear bearing tillers showed significant and positive association with grain yield. Plant height exhibited significant and positive correlation with panicle length, test weight, dry matter at 7 DAS in field and vigour index in field conditions indicating simultaneous improvement of these traits.

The results of association studies among early vigour traits under field conditions revealed that shoot length at 7 DAS, shoot length at 14 DAS, root length at 7 DAS, root length at 14 DAS and vigour index manifested negative and non-significant association with grain yield whereas dry matter at 14 DAS and germination percentage exhibited negative and significant association with grain yield. Dry matter at 7 DAS showed positive and non-significant association with grain yield. Vigour index showed positive and significant association with shoot length at 7 and 14 DAS, dry

matter at 7 and 14 DAS and germination percentage suggesting that genotypes possessing more shoot length and dry matter has early seedling establishment nature.

Under anaerobic conditions, the study of correlation coefficients among early vigour traits revealed that shoot length after 14 days of submergence, root length after 7 days of submergence, root length after 14 days of submergence, dry matter after 14 days of submergence, number of leaves under 7 days of submergence, number of leaves under 14 days of submergence, anaerobic germination percentage under 7 days of submergence, anaerobic germination percentage under 14 days of submergence and vigour index under 7 days of submergence manifested negative and non significant association with grain yield suggesting that careful indirect selection of early vigour traits is essential for improvement of anaerobic germination, grain yield. Early vigour is needed for good seedling establishment under direct sowing. However, the simultaneous improvement of these negatively associated traits might be possible through random matings or paired matings in F_2 and subsequent segregating generations. Free amino acids and total soluble sugars exhibited positive and significant association with grain yield whereas shoot length after 7 days of submergence, dry matter after 7 days of submergence and vigour index under 14 days of submergence exhibited positive and non-significant association with grain yield.

4.3 PATH COEFFICIENT ANALYSIS

The observed correlation between vigour traits and its component character is the net result of the direct and indirect effects of the component character through other vigour attributes. The correlation coefficients observed among vigour traits may sometimes be misleading. Since, it may be over or under estimate because of its association with other characters. Hence, direct selection on correlation may not be worthwhile. The correlation coefficient needs to be split into direct and indirect effects, using path coefficient analysis for critical evaluation. Thus, the correlation and path analysis in combination, can give a better insight, into cause and effect relationship between different pairs of characters.

In the present investigation, to study the relationship of early vigour traits among themselves under anaerobic conditions and with grain yield. Path analysis was carried out for 14 vigour related traits. The direct and indirect effects of different early

vigour traits on grain yield at phenotypic and genotypic levels are presented in Tables 4.11 and 4.12.

4.3.1 Shoot Length After 7 Days of Submergence (cm)

This character manifested positive direct effect (0.1472) on grain yield and its correlation is positive (0.1512) at phenotypic level. It had positive indirect effect *via* root length after 14 days of submergence (0.0045), dry matter after 7 days of submergence (0.0302), number of leaves under 14 days of submergence (0.0221), vigour index under 7 days of submergence (0.0375), vigour index under 14 days of submergence (0.0438), free amino acids (0.0084), total soluble sugars (0.0946) and prominent negative indirect effects *via* shoot length after 14 days of submergence (-0.0018), dry matter after 14 days of submergence (-0.0247), anaerobic germination percentage under 7 days of submergence (0.0055), anaerobic germination percentage under 14 days of submergence (-0.0972) and number of leaves under 14 days of submergence (-0.0214).

At genotypic level this character manifested positive association (0.1652) with grain yield coupled with positive direct effect (0.0904) on grain yield. Along with direct effects it also exhibited prominent positive indirect effects *via* root length after 14 days of submergence (0.0065), dry matter after 7 days of submergence (0.1287), number of leaves under 14 days of submergence (0.0527), vigour index under 7 days of submergence (0.0863), vigour index under 14 days of submergence (0.0651), free amino acids (0.0075), total soluble sugars (0.0910) and negative indirect effects *via* shoot length after 14 days of submergence (-0.0108), dry matter after 14 days of submergence (-0.0562) and number of leaves under 7 days of submergence (-0.0185).

This trait recorded significant positive association and positive direct effect on grain yield at both levels (Laxuman *et al.*, 2011; Pavanshankar 2015). Hence, indirect selection for this trait would improve grain yield.

4.3.2 Shoot Length After 14 Days of Submergence (cm)

Under anaerobic conditions, this character manifested non-significant and phenotypic negative association (-0.0253) with grain yield and negative direct effect (-0.0097) on grain yield. It had positive indirect effects *via* root length after 14 days of

submergence (0.1970), dry matter after 7 days of submergence (0.0326), number of leaves under 14 days of submergence (0.0973), vigour index under 7 days of submergence (0.0381), vigour index under 14 days of submergence (0.1051), free amino acids (0.0146), total soluble sugars (0.0740) and negative indirect effects *via* dry matter after 14 days of submergence (-0.2401) and number of leaves under 7 days of submergence (-0.0061).

It manifested non-significant and genotypic negative association (-0.0216) with grain yield and negative direct effect (-0.0549) on grain yield. It had positive indirect effects through root length after 14 days of submergence (0.3275), number of leaves under 14 days of submergence (0.2331), vigour index under 7 days of submergence (0.0821), vigour index under 14 days of submergence (0.1567), free amino acids (0.0130), total soluble sugars (0.0707) and negative indirect effects through number of leaves under 7 days of submergence (-0.0237), dry matter after 14 days of submergence (-0.5055) and anaerobic germination percentage under 7 days of submergence (-0.0837). These results are in conformity with the findings of Laxuman *et al.* (2011) and Pavanshankar (2015).

4.3.3 Root Length After 7 Days of Submergence (cm)

At phenotypic level, this trait manifested non-significant and positive association (-0.0178) with grain yield and negative direct effect (-0.1359) on grain yield. Along with direct effect it also exhibited prominent positive indirect effects *via* root length after 14 days of submergence (0.1242), number of leaves under 14 days of submergence (0.0391), vigour index under 7 days of submergence (0.0038), vigour index under 14 days of submergence (0.0072), total soluble sugars (0.0414) and prominent negative indirect effects *via* dry matter after 14 days of submergence (-0.0083), anaerobic germination percentage under 7 days of submergence (-0.0248) and number of leaves under 7 days of submergence (-0.0074) and free amino acids (-0.0095).

This trait showed non-significant negative association (-0.0661) with grain yield and negative direct effect (-0.1557) on grain yield at genotypic level. Along with direct effect it also exhibited prominent positive indirect effects *via* root length after 14 days of submergence (0.2067), number of leaves under 14 days of submergence (0.0944), vigour index under 7 days of submergence (0.0104), vigour index under 14 days of

submergence (0.0141), total soluble sugars (0.0401) and prominent negative indirect effects *via* dry matter after 14 days of submergence (-0.0253), number of leaves under 7 days of submergence (-0.0311) and free amino acids (-0.0085).

This trait recorded non-significant negative association and negative direct effect on grain yield at both phenotypic and genotypic levels indicating the true relationship between correlation coefficients and direct effects. Negative direct effect of root length for grain yield was previously reported by Chunhai *et al.*, (1996), Pavanshankar (2015).

4.3.4 Root Length After 14 Days of Submergence (cm)

This trait showed non-significant negative association (-0.1066) with grain yield and positive direct effect (0.2782) on grain yield at phenotypic level. Along with direct effect it also exhibited prominent positive indirect effects *via* number of leaves under 14 days of submergence (0.0898), dry matter after 7 days of submergence (0.0197), vigour index under 7 days of submergence (0.0234), vigour index under 14 days of submergence (0.0544), total soluble sugars (0.0390) and prominent negative indirect effects *via* anaerobic germination percentage under 7 days of submergence (-0.0614), dry matter after 14 days of submergence (-0.1914), number of leaves under 7 days of submergence (-0.0075) and free amino acids (-0.0057).

At genotypic level this trait manifested non-significant and negative association (-0.1461) and positive direct effect (0.4311) on grain yield. Along with direct effect it also exhibited prominent positive indirect effects *via* number of leaves under 14 days of submergence (0.2209), vigour index under 7 days of submergence (0.0535), vigour index under 14 days of submergence (0.0852), total soluble sugars (0.0380) and prominent negative indirect effects through free amino acids (-0.0052), number of leaves under 7 days of submergence (-0.0311) and dry matter after 14 days of submergence (-0.4249). Pavanshankar (2015) in a study of early vigour traits under dry direct sowing reported negative direct effect of this trait on grain yield. The discrepancy in results may be due to different set of genotypes under study.

This trait recorded negative correlation coefficient but the direct effect is positive. Under these circumstances a restricted simultaneous selection model is to be followed *i.e.*, restrictions to be imposed to nullify the undesirable indirect effects in order to make use of the direct effect for yield improvement.

4.3.5 Dry matter Under 7 Days of Submergence (mg)

At phenotypic level, this character showed non-significant positive (0.0769) association and contributed positive direct effect (0.0819) on grain yield. This trait also showed predominant indirect positive effects through number of leaves under 14 days of submergence (0.0285), vigour index under 7 days of submergence (0.0642), vigour index under 14 days of submergence (0.1377), free amino acids (0.0223), total soluble sugars (0.1257) and negative indirect effects through dry matter after 14 days of submergence (-0.1958), number of leaves under 7 days of submergence (-0.0075) and shoot length after 14 of submergence (-0.0038).

The direct contribution of this trait was positive (0.3243) and the association was positively significant (0.1039) at genotypic level with grain yield. Apart from that, this trait showed prominent positive indirect effects on grain yield *via* number of leaves under 14 days of submergence (0.0746), vigour index under 7 days of submergence (0.1393), vigour index under 14 days of submergence (0.2002), free amino acids (0.0215), total soluble sugars (0.1289) and negative indirect effects *via* dry matter under 14 days of submergence (-0.4397), number of leaves under 7 days of submergence (-0.0315) and shoot length after 14 days of submergence (-0.0261). Similar results were obtained by Pavanshankar (2015).

4.3.6 Dry matter After 14 Days of Submergence (mg)

It exhibited significant and phenotypic negative association (-0.2405) with grain yield and negative direct effects (-0.3692) on grain yield. It had positive indirect effects *via* number of leaves under 14 days of submergence (0.0852), vigour index under 7 days of submergence (0.0095), vigour index under 14 days of submergence (0.1105), free amino acids (0.0051), total soluble sugars (0.0573) and negative indirect effects *via* number of leaves under 7 days of submergence (-0.0055), anaerobic germination percentage under 7 days of submergence (-0.0752) and shoot length after 14 days of submergence (-0.0063).

It manifested significant and genotypic negative association (-0.2018) with grain yield and negative direct effects (-0.7414) on grain yield. It had positive and indirect effect through number of leaves under 14 days of submergence (0.2072), vigour index under 7 days of submergence (0.1924), vigour index under 14 days of submergence (0.1579), free amino acids (0.0048), total soluble sugars (0.0562) and

negative indirect effects through number of leaves under 7 days of submergence (-0.0222) and shoot length after 14 days of submergence (-0.0374). Pavanshankar (2015) also reported positive direct effect of this trait on grain yield in a study involving 22 rice genotypes under aerobic conditions.

4.3.7 Number of Leaves Under 7 Days of Submergence

Under anaerobic conditions, this character manifested non-significant and phenotypic negative association (-0.1711) with grain yield and negative direct effects (-0.0248) on grain yield. It had positive indirect effects *via* number of leaves under 14 days of submergence (0.0555), root length after 14 days of submergence (0.0842), vigour index under 7 days of submergence (0.0326), vigour index under 14 days of submergence (0.0564), free amino acids (0.0081) and total soluble sugars (0.2130). Negative indirect effects *via* anaerobic germination percentage under 7 days of submergence (-0.0574) and dry matter under 14 days of submergence (-0.0822).

It manifested non-significant and genotypic negative association (-0.1030) with grain yield and negative direct effect (-0.0843) on grain yield. It had positive and indirect effect through number of leaves under 14 days of submergence (0.1422), vigour index under 7 days of submergence (0.0577), vigour index under 14 days of submergence (0.0866), free amino acids (0.0080), total soluble sugars (0.0225) and negative indirect effects through dry matter after 14 days of submergence (-0.1953) and shoot length after 14 days of submergence (-0.0154).

As this character recorded negative direct effect, the indirect causal factors have to be considered simultaneously while exercising selection to improve grain yield.

4.3.8 Number of Leaves Under 14 Days of Submergence

It showed non-significant negative phenotypic correlation (-0.0836) with grain yield and positive direct phenotypic effect (0.1448). This trait showed prominent phenotypic positive indirect effects *via* vigour index under 7 days of submergence (0.0452), vigour index under 14 days of submergence (0.0775), free amino acids (0.0023), total soluble sugars (0.0536), root length after 14 days of submergence (0.1725) and negative indirect effects through dry matter after 14 days of submergence

(-0.2173), root length after 7 days of submergence (-0.0367) and number of leaves under 7 days of submergence (-0.0095).

It also showed non-significant negative genotypic correlation (-0.0952) with grain yield and positive direct phenotypic effect (0.3302). This trait showed prominent positive indirect effects *via* vigour index under 7 days of submergence (0.0958), vigour index under 14 days of submergence (0.1174), free amino acids (0.0021), total soluble sugars (0.0508), dry matter after 7 days of submergence (0.0733) and prominent negative indirect effects through number of leaves under 7 days of submergence (-0.0363), dry matter after 14 days of submergence (-0.4653) and shoot length after 14 days of submergence (-0.0387).

4.3.9 Anaerobic Germination Percentage Under 7 Days of Submergence

At 7 days after submergence, anaerobic germination percentage exhibited negative direct effect (-0.1434) on grain yield along with phenotypic negative correlation (-0.0711). This exhibited prominent indirect positive effects *via* vigour index under 7 days of submergence (0.0737), vigour index under 14 days of submergence (0.1246), root length after 14 days of submergence (0.1192), number of leaves under 14 days of submergence (0.0687), free amino acids (0.0183), total soluble sugars (0.1396) and negative indirect effects *via* number of leaves under 7 days of submergence (-0.0099), root length after 7 days of submergence (-0.0235) and dry matter after 14 days of submergence (-0.1936).

It exhibited negative direct effect (-0.2194) on grain yield along with genotypic negative correlation (-0.0932). This exhibited prominent indirect effects *via* number of leaves under 14 days of submergence (0.1656), vigour index under 7 days of submergence (0.1448), vigour index under 14 days of submergence (0.1946), free amino acids (0.0109), total soluble sugars (0.1353) and negative indirect effects through number of leaves under 7 days of submergence (-0.0376), dry matter after 14 days of submergence (-0.4140) and shoot length after 14 days of submergence (-0.0209). These are in accordance with the findings of Pavanshankar (2015) indicating indirect selection through such trait will be effective in yield improvement.

4.3.10 Anaerobic Germination Percentage Under 14 Days of Submergence

It exhibited negative and non-significant association (-0.1510) with grain yield and negative direct effect on grain yield (-0.5029) at phenotypic level. Along with direct effect it also manifested positive indirect effects through vigour index under 7 days of submergence (0.0473), vigour index under 14 days of submergence (0.1789), free amino acids (0.0188), total soluble sugars (0.1261) and negative indirect effects *via* dry matter after 14 days of submergence (-0.1811), number of leaves under 7 days of submergence (-0.0060) and anaerobic germination percentage under 7 days of submergence (-0.0913).

It manifested negative and significant association (-0.1726) and negative direct effect (-0.6425) on grain yield at genotypic level. Apart from direct effect, it also exhibited positive indirect effect *via* number of leaves under 14 days of submergence (0.1905), vigour index under 7 days of submergence (0.0998), vigour index under 14 days of submergence (0.2500), free amino acids (0.0170) and total soluble sugars (0.1203).

4.3.11 Vigour Index Under 7 Days of Submergence

It showed non-significant and phenotypic negative association (-0.0408) with grain yield and positive direct effect (0.1302). Apart from direct effect it also showed positive indirect effects *via* vigour index under 14 days of submergence (0.0842), number of leaves under 14 days of submergence (0.0503), free amino acids (0.0091), total soluble sugars (0.1057) and negative indirect effect *via* number of leaves under 7 days of submergence (-0.0060), dry matter after 14 days of submergence (-0.2699) and shoot length after 14 days of submergence (-0.0028).

It showed non-significant and genotypic negative association (-0.0757) with grain yield and high positive direct effect (0.2498). Apart from direct effects, it also showed positive indirect effects *via* vigour index under 14 days of submergence (0.1267), number of leaves under 14 days of submergence (0.1267), free amino acids (0.0089), total soluble sugars (0.1067) and negative indirect effects *via* number of leaves under 7 days of submergence (-0.0194), dry matter after 14 days of submergence (-0.5711) and shoot length after 14 days of submergence (-0.0180). Pavanshankar

(2015) reported negative direct effect on grain yield. The discrepancy in results may be due to difference in the material under study.

4.3.12 Vigour Index Under 14 Days of Submergence

It showed non-significant and phenotypic positive association (0.0123) with grain yield and positive direct effect (0.2590). Apart from direct effects it also showed positive indirect effects *via* free amino acids (0.0308), total soluble sugars (0.1090), vigour index under 7 days of submergence (0.0437), number of leaves under 14 days of submergence (0.0447) and negative indirect effect through dry matter after 14 days of submergence (-0.1626), anaerobic germination percentage under 14 days of submergence (-0.3586) and number of leaves under 7 days of submergence (-0.0055).

It showed significant and genotypic positive association (0.072) with grain yield and high positive direct effect (0.3411). Apart from direct effects it also showed positive indirect effects *via* free amino acids (0.0286), total soluble sugars (0.1084), vigour index under 7 days of submergence (0.0928), number of leaves under 14 days of submergence (0.1136) it also showed negative indirect effects *via* dry matter after 14 days of submergence (-0.3432), number of leaves under 7 days of submergence (-0.0214) and shoot length after 14 days of submergence (-0.0252).

This character had positive direct effect and positive correlation at both phenotypic and genotypic level on grain yield. These results indicated that the directional selection of direct causal factors would lead to improvement in grain yield. Similar findings were reported by Pavanshankar (2015).

4.3.13 Free Amino Acids (mg g^{-1})

Free amino acids recorded positive phenotypic significant association (0.1694) and positive direct effect (0.0722) on grain yield followed by positive indirect effect *via* total soluble sugars (0.1294), vigour index under 14 days of submergence (0.1070), vigour index under 7 days of submergence (0.0165), number of leaves under 14 days of submergence (0.0046), dry matter under 7 days of submergence (0.0252) and manifested negative indirect effects through number of leaves under 7 days of submergence (-0.0028), dry matter after 14 days of submergence (-0.0264) and root length after 14 days of submergence (-0.0219).

It recorded positive and significant association (0.1822) and positive direct effect (0.0638) on grain yield followed by positive indirect effects *via* total soluble sugars (0.1214), vigour index under 14 days of submergence (0.1531), vigour index under 7 days of submergence (0.0349), number of leaves under 14 days of submergence (0.0108) and negative indirect effects *via* root length after 14 days of submergence (-0.0353), dry matter after 14 days of submergence (-0.0557) and number of leaves under 7 days of submergence (-0.0106).

4.3.14 Total Soluble Sugars (mg g⁻¹)

The trait total soluble sugars manifested positive direct effect (0.3346) combined with phenotypic positive correlation (0.2803) on grain yield followed by positive indirect effects *via* free amino acids (0.0279), vigour index under 14 days of submergence (0.0818), vigour index under 7 days of submergence (0.0411), number of leaves under 14 days of submergence (0.0232), root length after 14 days of submergence (0.0324) and negative indirect effects *via* dry matter after 14 days of submergence (-0.0632), number of leaves under 7 days of submergence (-0.0015) and shoot length after 14 days of submergence (-0.0021) at phenotypic level.

This trait manifested positive direct effect (0.3131) and genotypic positive and significant correlation (0.2937) on grain yield followed by positive indirect effects *via* free amino acids (0.0247), vigour index under 14 days of submergence (0.1181), vigour index under 7 days of submergence (0.0851), number of leaves under 14 days of submergence (0.0536), root length after 14 days of submergence (0.0524) and it also exhibited negative indirect effect *via* dry matter after 14 days of submergence (-0.1332), number of leaves under 7 days of submergence (-0.0060), shoot length after 14 days of submergence (-0.0124). Hence, directional selection of this trait would bring simultaneous improvement in grain yield / plant.

Therefore, based on above results of path coefficient analysis among early vigour related traits under anaerobic conditions it may be concluded that the traits, shoot length after 7 days of submergence, root length after 14 days of submergence, vigour index under 14 days of submergence, free amino acids, total soluble sugars manifested positive correlation coefficient coupled with positive direct effect on grain yield. Hence simple selection of these traits would improve the grain yield *i.e.*, genotypes possessing high shoot length, root length, vigour index, free amino acids and

total soluble sugars would lead to better crop establishment under anaerobic conditions and improves the grain yield. Whereas shoot length after 14 days of submergence, root length after 7 days of submergence, dry matter after 7 and 14 days of submergence, number of leaves under 7 and 14 days of submergence and vigour index under 7 days of submergence are the characters which manifested negative direct effect and negative association with grain yield. Hence, it may be concluded that selection based on these characters will not aid improvement in yield and careful indirect selection has to be taken up for yield improvement. The negative direct effects and positive associations as observed in root length after 7 days of submergence explained the indirect effects seem to be the cause of positive correlation and the indirect causal factors have to be considered simultaneously while exercising selection for improvement of dry matter after 14 days of submergence under anaerobic conditions which ultimately improves the plant stand and grain yield.

4.4.1 Variability for Anaerobic Germination/Phenotyping for Anaerobic Germination Resistance

Rice is an essential food crop for billions of people. It can cope with a wide range of environmental conditions and, in particular, with low (hypoxia) or absent oxygen (anoxia), experienced during direct sowing in paddy field as a consequence of soil flooding (Yamauchi *et al.*, 2000). Soil flooding is one of the most important abiotic constraints on rice yield, with complete sowing under anaerobic conditions of plants being particularly serious for rice farmers in the rainfed lowlands of humid and semi-humid tropics of Asia (Jackson and Ram, 2003).

Although seeds of higher plant species fail to germinate under anaerobic conditions, rice germinates successfully even when deprived of oxygen completely (anaerobiosis) to create the metabolic state of anoxia (Perata and Alpi, 1993).

Vigorous seed germination and coleoptile growth is necessary when rice seeds are sown in flooded paddy fields. Rice is one of the few crops whose seeds can germinate and whose coleoptiles can elongate under flooded conditions (Alpi and Beevers, 1983; Perata *et al.*, 1992, 1993). Many physiological processes, including starch and sugar metabolism are involved in the seed germination and coleoptile elongation of rice in hypoxic conditions. Tolerant genotypes produce longer coleoptiles

and their mesocotyl and coleoptile elongate faster than those of sensitive genotypes (Setter *et al.*, 1994; Biswas *et al.*, 2002).

In the present study 48 rice genotypes were screened for tolerance to anaerobic germination for 7 days, 14 days and 21 days. Thirty genotypes showed >80 % of germination after 7 days sowing under anaerobic conditions in 10 cm submergence. Anaerobic germination percentage for 7 days submergence ranged from 0 (MTU 5293) to 100 (AC 39397, AC 39416A) indicating wide range of variability for the genotypes (table 4.8) suggesting that all these 30 genotypes can tolerate anaerobic conditions during germination up to 7 days. Hence, it may be concluded that these genotypes may be suitable for direct sowing and can withstand immediate submergence after sowing for 4-5 days due to unpredicted rainfall or uneven land levelling. Anaerobic germination percentage under 14 days submergence showed variability ranging from 0 (BPT 2660) to 100 (AC 39416A). Under 14 days of anaerobic conditions, only 11 genotypes showed more than 80 % of germination which include BPT 2782, BPT 2411, BPT 2595, BPT 2507, MTU 1064, BPT 2740, MTU 1075, MTU 1140, MTU 2716, RGL 1414, AC39397 and AC39416A and these genotypes can be used for direct seeding except AC 39397, AC 39416A which have tall plant stature and can be used for crossing programme. Reddy *et al.* (2015) also reported that nine genotypes viz., Nandi, Sabita, PSRC sub 82, Isukaravalu, PLA720, MTU 1140 (MTU PLA 109-1-5-1), MTU 1158 (MTUPLA106-1-1-1), Chaithanya and IR85956/23-1-2-1 exhibited tolerance/resistance (75-95%) for 14 days of anaerobic germination.

In the present study the genotypes which showed resistance to anaerobic germination with more than 75 % of germination under 14 days of submergence were screened for 21 days of anaerobic germination (Table 4.13). Among the 13 genotypes which were screened for germination under anaerobic conditions for 21 days, only 6 genotypes showed more than 50 % of anaerobic germination viz., BPT 2411, MTU 5249, MTU 1140, AC39397, AC 39416A, IR85961-23-1-2-1. Hence all these genotypes can be recommended for direct sowing and genotypes with higher anaerobic germination for 3 weeks viz., AC 39397 (90%), AC 39416A (100 %) and IR85961-23-1-2-1 (74.95%) could be used as donors for transferring anaerobic germination into the desired genotypes to make them suitable for direct seeding in rainfed as well as under irrigated lowland conditions also.

4.4.2 Genetic Variability For Known Anaerobic Germination Loci Using SSR Markers

Breeding for increased seed and seedling vigor as well as for enhanced tolerance of flooding or anaerobic conditions during germination has been previously attempted by several workers (Yamauchi *et al.* 1993 ; Yamauchi and Winn 1996; Redona and Mackill 1996; Biswas and Yamauchi 1997), but progress has been slow due to the lack of suitable donors with high level of tolerance. Limited knowledge on the genetics of tolerance, the involvement of several complex mechanisms (Seshu *et al.* 1988), and complex genetic backgrounds and methods used for measurement (Ling *et al.* 2004). Identifying molecular markers that are linked to QTLs or genes controlling tolerance of flooding during germination would facilitate selection for this trait, which has low heritability. Later several breeders identified QTLs that control the tolerance of anaerobic germination which include qAG-1-1, qAG-2-1 (Angaji *et al.* 2008), qAG-11, qAG -12 (Angaji 2008), qAg-6, qAG-7-1 (Septiningsih *et al.* 2013).

As per Reddy *et al.* (2015) with markers RM 341 to qAG-2 on chromosome 2 and RM 206 to qAG-11 on chromosome 11, the three genotypes viz., Isukaravalu, IR 85961/23-1-2, MTU 1140 are co-segregated. These results indicated the presence of genes pertaining to anaerobic germination contributing for tolerance in these genotypes.

Bharat *et al.* (2014) studied with SSR marker RM341 linked with qAG-2-1 associated with submergence tolerance during seed germination, 3 types of alleles were documented with PIC value at 0.45.

Results of molecular variability using gene/QTL linked markers for anaerobic germination among 48 genotypes are presented in Table 4.14. Number of alleles ranged from 2 to 5 with a mean of 2.91. Rice microsatellite marker RM 10694 exhibited maximum of 5 alleles followed by 4 (RM 6318), 3 (RM 210, RM 8303, RM 253, RM 478, RM 149, RM 104) and 2 alleles for rest of the markers. Polymorphic information content (PIC) of these markers ranged from 0.30 to 0.81 with an average of 0.64 indicated potentialities of these markers for assessing genetic diversity represented in Table 4.14. Markers RM 10694 (0.81), RM 6318 (0.81), RM 149 (0.79) and RM 478 (0.77) showed higher polymorphic information content. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Virk *et al.*, 1995).

The results of amplification pattern with the marker RM 10694 on chromosome 1 showed significant polymorphism among the genotypes studied (Plate 4.1). The bands that are amplified by the genotypes possessing anaerobic germination resistance viz., MTU 5249, AC39397, BPT 2411, MTU 2716, BPT 5204, MTU 1140, BPT 2573, MTU 4870, NLR 33892, BPT 2675, gave similar amplification size of 180bp. The phenotypic data results of these genotypes revealed >75% of germination under 7 days of submergence. The results of co-segregation patterns of RM 10694 in these ten genotypes with phenotypic data revealed that a QTL (*qAG-1*) might be present in all these lines on chromosome 1. The association of this marker with anaerobic germination QTL on chromosome 1 was reported by Angaji *et al.* (2010).

The amplification pattern with the primer RM 6318 on chromosome 2 manifested a clear polymorphism among the 48 genotypes under study (plate 4.2). The bands amplified by five genotypes viz., BPT 2411, BPT 2595, BPT 2507, MTU 1064, AC39416A recorded similar amplicon size of 175 base pairs and differed with the banding patterns manifested by the remaining genotypes. In phenotypic studies, these genotypes exhibited >75% of germination at both 7 and 14 days of sowing under anaerobic conditions *i.e.* under anaerobic conditions indicating the co-segregation between phenotyping and genotyping. The association of this marker with anaerobic germination QTL (*qAG-2-2*) was confirmed previously by Angaji *et al.* (2010) when it was screened for polymorphism on BC₂F₂ population of cross between IR64/Khao Hlan. These results indicated that expression of alleles for anaerobic germination are specific to genotypes. Hence, selection of sources for anaerobic germination is needed for crossing programme after thorough validation both genotypically and phenotypically.

Cluster analysis and dendrogram construction was done by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) profound based on Jaccard's similarity coefficient using NTSYS 2.0 version was shown in the figure 4.6.

Similarity index (Jaccards similarity coefficient) values ranged from 0.00 to 1.00 (Table 4.15). Lowest similarity value genotypes are most divergent. AC 39416A highly tolerant genotype for anaerobic germination for 3 weeks is distantly related to most of genotypes.

Among the genotypes studied MTU 1061 exhibited lowest similarity value of zero with genotypes BPT 2660, BPT 2644, BPT 2782, BPT 2590, BPT 2593, MTU 2077 and NLR 4001 indicating these genotypes expressed divergence for the markers used and also molecular diversity for anaerobic germination. Similarly, NLR 4002 also exhibited lowest similarity value of zero with MTU 4870, NLR 33892, MTU 5249, MTU 7029, BPT 2571 and NLR 20084 indicating these genotypes are distantly related at molecular level. BPT 4358 expressed lower similarity index values with BPT 2595 (0.14), BPT 2507 (0.14), BPT 1768 (0.10), MTU 2716 (0.15), BPT 5204 (0.20), MTU 1064 (0.10), MTU 1061 (0.20), MTU 1075 (0.33). JGL 17004 expressed lower similarity index with genotypes BINDHAN-11 (0.11), MTU 2077 (0.11), MTU 3626 (0.11), NLR 4001 (0.00). The results indicate the genotypes expressed divergence based on ancestry and place of origin of genotypes.

Genotypes with low similarity values are more divergent. In cluster analysis (Table 4.16), 48 genotypes were grouped into two major clusters at 62 % similarity level. Cluster I comprised of 18 genotypes which were further sub divided into 2 sub clusters. Sub cluster IA comprise of 7 genotypes and I B 11 genotypes

Most of the elite breeding lines were grouped in Cluster II with 30 genotypes. It was further sub grouped into 2 clusters. Twenty eight genotypes were grouped in sub cluster IIA and 2 in IIB.

All the genotypes which manifested anaerobic germination percentage > 80 under 21 days submergence and anaerobic germination percentage > 80 under 2 weeks were grouped in cluster IIA indicating that these genotypes have more co- segregation for anaerobic germination linked alleles. These genotypes can be exploited for future breeding purpose for anaerobic germination.

Few genotypes with higher anaerobic germination percentage at 2 weeks, 3 weeks were grouped in cluster I and which include BPT 2411, MTU 1064, RGL 1414. the genotype BPT 2411, manifested similar amplification pattern for qAG-2 linked alleles RM 6318. It clearly indicates gene/ QTL responsible for anaerobic germination are specific to genotypes which are to be identified by further QTL mapping.

Table 4.14. Total number of alleles, polymorphism information content (PIC) value of 12 SSR markers assayed in 48 rice genotypes

S.Number	Marker name	Numberof alleles	PIC for anaerobic germination for molecular diversity
1	RM 10694	5	0.815
2	RM 263	2	0.662
3	RM 6318	4	0.815
4	RM 520	2	0.301
5	RM253	3	0.522
6	RM478	3	0.77
7	RM 210	3	0.508
8	RM 149	3	0.799
9	RM 8303	3	0.713
10	RM 104	3	0.638
11	RM 105	2	0.426
12	RM 206	2	0.728
	Mean	2.91	0.64

Table 4.16 Distribution of genotypes into different clusters based on molecular genetic diversity

S. Number	Number of genotypes	Name of the cluster	Genotypes
1.	7	Cluster IA	MTU 4870, BPT 2411, BPT 2595 JGL 3828, BPT 2507, MTU 2067, MTU 7029
2.	11	Cluster I B	NLR 33892, NLR 4002, MTU 2716, BPT 3291, BPT 1768, MTU 1061, MTU 1064, BPT 5204 BPT 2231, JGL 17004, RGL 1414
3.	28	Cluster IIA	MTU 5249, AC39397, BPT 2675, MTU 1140, BPT 2573, BPT 2571, MTU 1075, BPT 2660, BPT 2295, RGL 2537, BPT 2270, BINADHAN-11, BPT 2644, IR 85961-23-1-2-1, NLR 4001, BPT 2782, BPT 2745, MTU 5182, MTU 1166, MTU 5293, BPT 4358 BPT 2590, BPT 2593, BPT 2740, MTU 2077, MTU 3626, MTU 1001, AC39416A
4	2	Cluster II B	BPT 2673, NLR 20084

Chapter – IV

Summary and Conclusions

Direct seeding is becoming a popular method in Southeast Asian countries to mitigate shortage of labour and water. However, poor seedling establishment has been a major obstacle preventing large scale adaption of direct seeding methods. Many environmental conditions like flooding due to rainfall or uneven agricultural land creates unfavourable conditions for rice to germinate and to develop strong and uniform seedlings. This is due to the inability of most rice varieties to germinate and reach the water surface under complete submergence. Hence, tolerance of anaerobic condition during germination is an essential trait for direct seeded rice cultivation not only in low land areas but also for intensive irrigated systems where early flooding can suppress weeds and use of herbicides will be reduced under direct seeding.

Hence the present investigation was undertaken during *Kharif*, 2016 at Agriculture college farm, Bapatla to study the variability parameters for early vigour traits in field under anaerobic conditions, yield and yield components. In addition, intercorrelation of early vigour traits under anaerobic conditions and molecular diversity of genotypes using reported SSR markers for anaerobic germination was also carried out.

The analysis of variance results revealed significant differences among the genotypes for all the characters indicating the presence of sufficient genetic variability in the studied material. The genotypic coefficients of variation for all the characters under study were lesser than the phenotypic coefficients of variation but the difference is very less indicating the low environmental influence. Among the genotypes studied NLR 4002, BPT 2673, NLR 33892, MTU 5182, MTU 1075, BPT 2740 and JGL 17004 were identified as superior and suitable for dry direct sown rice by considering the yield potential. Among the 48 genotypes studied in the present investigation, 28 exhibited resistance to anaerobic conditions upto one week suggesting their suitability for direct sowing and all these genotypes can tolerate the submergence conditions for shorter period immediately after sowing. When we consider both grain yield / plant and tolerance to

anaerobic conditions the genotypes viz., BPT 2740, MTU 5249, MTU 2716, IR 85961-23-1-2-1, MTU 1140, BPT 2595, BPT 2782, BPT 2411, BPT 2573 and MTU 1064 can be recommended for direct sowing under anaerobic conditions also. Under 14 days of submergence also 13 genotypes exhibited their resistance to anaerobic germination with > 75% germination. Hence these genotypes can be utilized as donors for incorporating this trait into other cultivars/varieties.

Under 21 days of submergence, the genotype AC 39416A recorded 100% germination. Under 10 cm water depth for 21 days this genotype exhibited high values for shoot length, root length, dry matter, number of leaves, vigour index and anaerobic response index indicating the cumulative effect of all these traits which contributed for high anaerobic germination. Other genotypes which manifested relatively high values for shoot length, root length, dry matter and vigour index also showed high anaerobic germination percent *i.e.* above 50%. The morphological adjustment might have enabled these superior cultivars to access oxygen at the water surface earlier which supported the growth of organs such as the primary leaf and root. In addition, the genotypes viz., AC 39416A, AC 39397, IR 85961-23-1-2-1, BPT 2411, MTU 5249, MTU 1140 and BPT 2595 also recorded high amount of total soluble sugars and free amino acids which might have contributed for increased germination under anaerobic conditions.

The study of genetic parameters showed high heritability estimates ranging from 80-90% for most of the characters reflecting ample scope for their improvement through appropriate breeding procedures. The estimates of phenotypic coefficient of variation for all the characters were high than the estimates of genotypic coefficient of variation. This close correspondence between the estimates of phenotypic coefficient of variation and genotypic coefficient of variation was also reflected by their high heritability values and indicated meagre influence of environment on the expression of these traits.

Among the yield components high heritability coupled with high genetic advance as percent of mean was recorded for plant height, number of ear bearing tillers, grain yield and test weight. Under field conditions shoot length at 7 and 14 days after sowing, root

length at 7 and 14 days after sowing, dry matter at 7 & 14 days after sowing and vigour index manifested high heritability coupled with high genetic advance as percent of mean. Anaerobic germination percentage after 7 and 14 days of submergence and total soluble sugars exhibited high heritability and high genetic advance as percent of mean. Under 21 days of submergence all the vigour traits studied viz., shoot length, root length, dry matter, number of leaves, anaerobic germination percentage, anaerobic response index and vigour index also recorded high heritability and genetic advance as percent of mean indicating the operation of additive gene action in the inheritance of the traits. Hence good response to selection can be attained for improvement of these traits.

High heritability estimates and moderate to low genetic advance as percent of mean was recorded for panicle length, shoot length after 7 and 14 days of submergence, root length after 7 and 14 days of submergence, dry matter after 7 and 14 days of submergence, vigour index under 7 and 14 days of submergence, number of leaves under 7 and 14 days of submergence, germination percentage in field and free amino acids suggesting the involvement of both additive and non-additive gene action and provides limited scope for improvement of these traits through selection.

The character association studies among the yield components revealed that plant height, panicle length and test weight manifested non-significant and positive association with grain yield whereas number of ear bearing tillers showed significant positive association with grain yield. Likewise plant height also exhibited significant and positive correlation with panicle length, test weight, dry matter at 7 days after sowing and vigour index under field conditions indicating simultaneous improvement of these traits. The association studies among early vigour traits under field conditions revealed that dry matter at 14 days after sowing and germination percentage exhibited negative and significant association with grain yield. Shoot length at 7 DAS showed positive and significant association with shoot length at 14 days after sowing, dry matter at 7 and 14 days after sowing and vigour index. Similarly under anaerobic conditions also shoot length at 7 days of submergence manifested positive and significant association with shoot length after 14 days of submergence, root length after 7 days of submergence, dry

matter after 7 days of submergence, anaerobic germination percentage under 7 and 14 days of submergence, vigour index under 7 and 14 days of submergence and total soluble sugars suggesting that the genotypes possessing high shoot length, root length, vigour index, dry matter and total soluble sugars would lead to better crop establishment under anaerobic conditions. Free amino acids and total soluble sugars also exhibited positive association with grain yield.

Studies of path analysis among early vigour related traits under anaerobic conditions with grain yield revealed that the characters *viz.*, shoot length after 7 days of submergence, root length after 14 days of submergence, vigour index after 14 days of submergence, free amino acids and total soluble sugars manifested positive correlation coefficient coupled with positive direct effect on grain yield. Hence simple selection of these traits would improve the grain yield.

The reported microsatellite (SSR) markers for anaerobic germination in rice were used to study the molecular diversity among the 48 genotypes under study. The number of alleles detected per primer ranged from 2 to 5 with a mean of 2.91. The SSR marker RM 10694 on chromosome number 1, linked to qAG-1-1 and RM 6318 on chromosome 2, linked to qAG-2-2 were identified as useful markers with high PIC values to distinguish genotypes for anaerobic germination.

All the genotypes which manifested anaerobic germination percentage of more than 80 under 21 days submergence and anaerobic germination percentage of more than 80 under 2 weeks of submergence were grouped in cluster IIA indicating that these genotypes have more co-segregation for anaerobic germination linked alleles. These genotypes can be exploited for future breeding purpose for anaerobic germination. Three genotypes *viz.*, BPT 2411, MTU 1064 and RGL 1414 with higher anaerobic germination percentage at 2 weeks and 3 weeks were grouped in cluster I. The genotype BPT 2411 showed similar amplification pattern for qAG-2 linked alleles RM 6318. It clearly indicates the gene/ QTL responsible for anaerobic germination are specific to genotypes which are to be identified by further QTL mapping.

The present investigation was aimed at identifying varieties/cultivars tolerant to anaerobic conditions combining high grain yield which can be recommended for direct sowing in rainfed and irrigated lowland areas. Further studies followed by demonstrations are recommended to test the survival rate and establishment of seedlings after removal of water under direct sown paddy field and to estimate the growth parameters and yield potential also.

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***Original not seen**

Note: The literature is cited as per the “Thesis Guidelines” prescribed by Acharya N. G. Ranga Agricultural University, Guntur.



Plate 3.1 Screening for anaerobic germination



Plate 3.2 Anaerobic germination after desubmergence

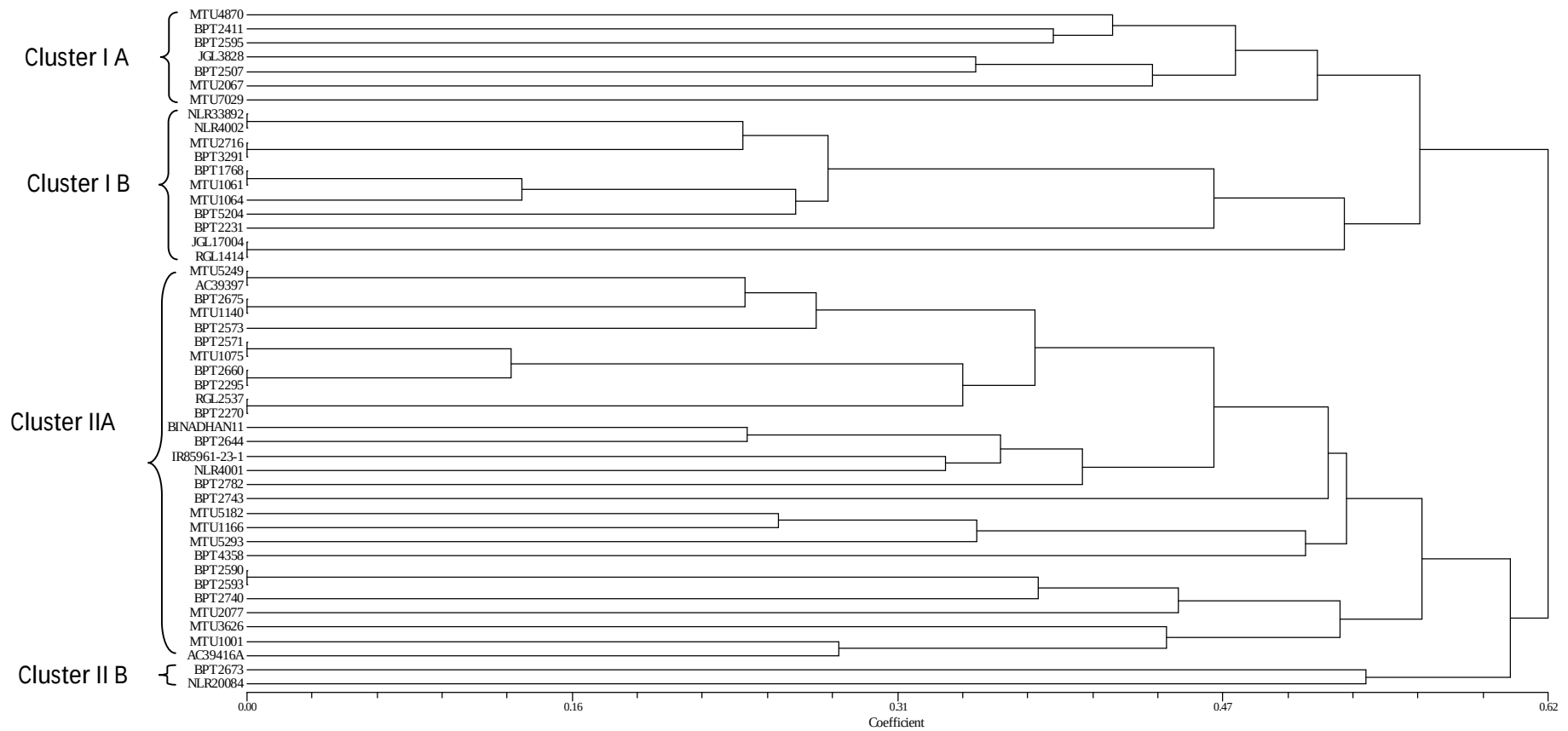


Fig. 4.6 Dendrogram showing genetic diversity among 48 rice genotypes using reported anaerobic germination molecular markers using NTSYS

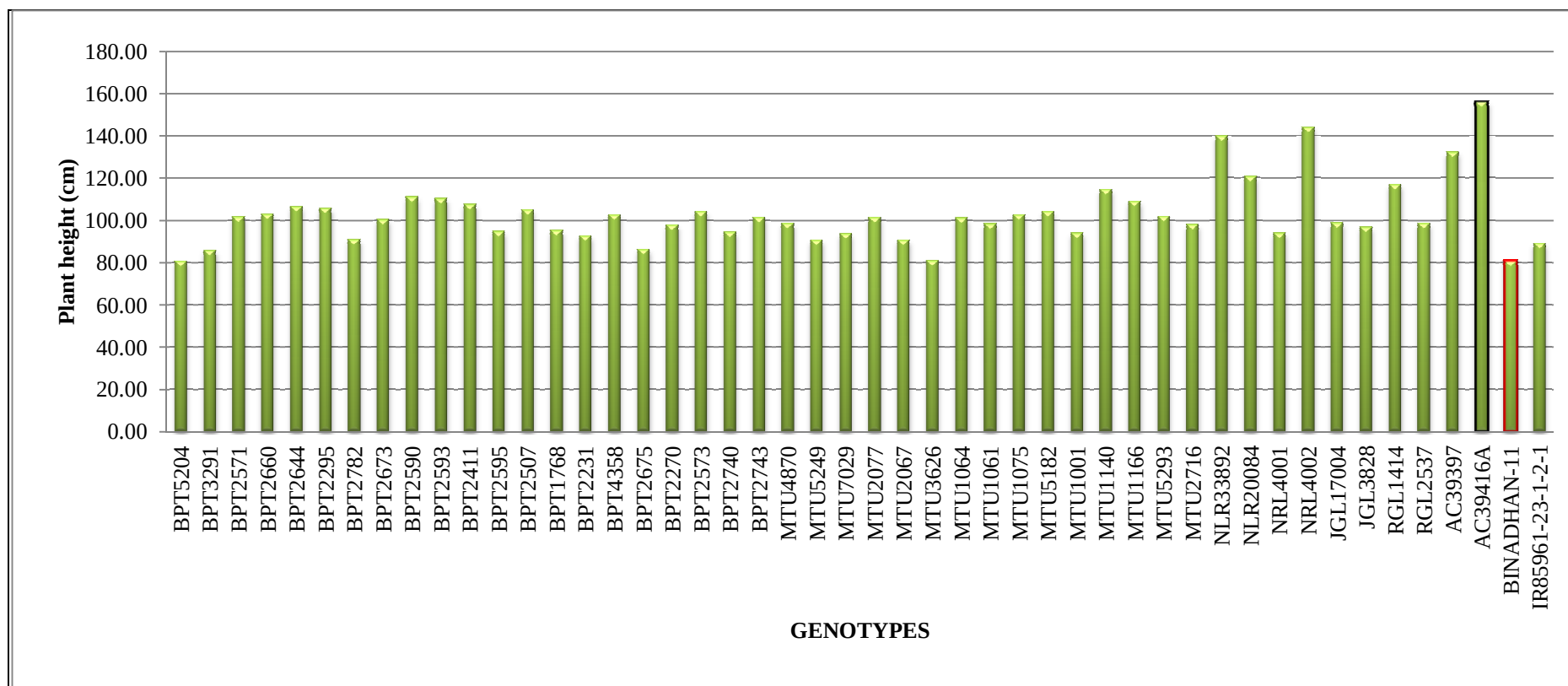


Fig. 4.1 Mean performance of 48 rice genotypes for plant height (cm)

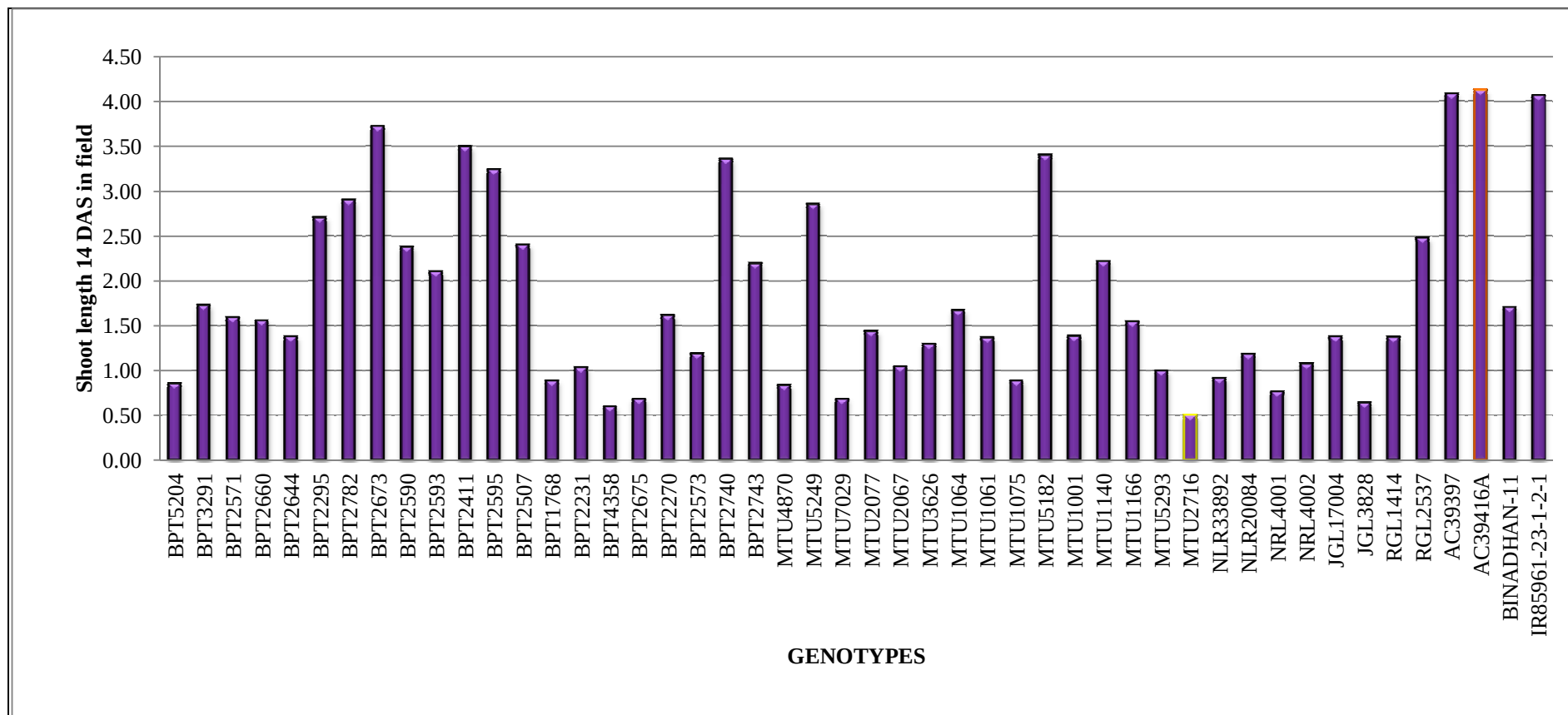


Fig. 4.2 Mean performance of 48 genotypes for shoot length at 14 DAS in field (cm)

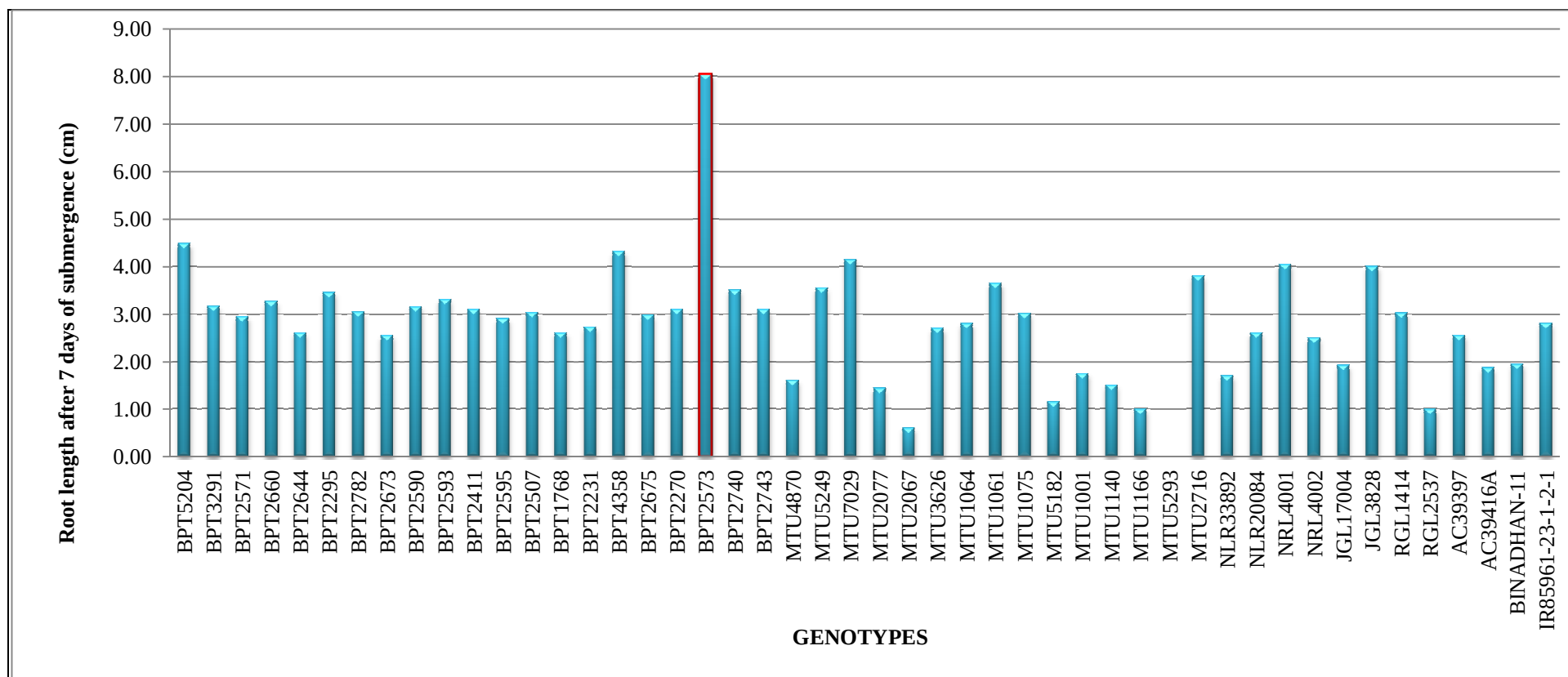


Fig. 4.3 Mean performance of 48 rice genotypes for root length after 7 days of submergence (cm)

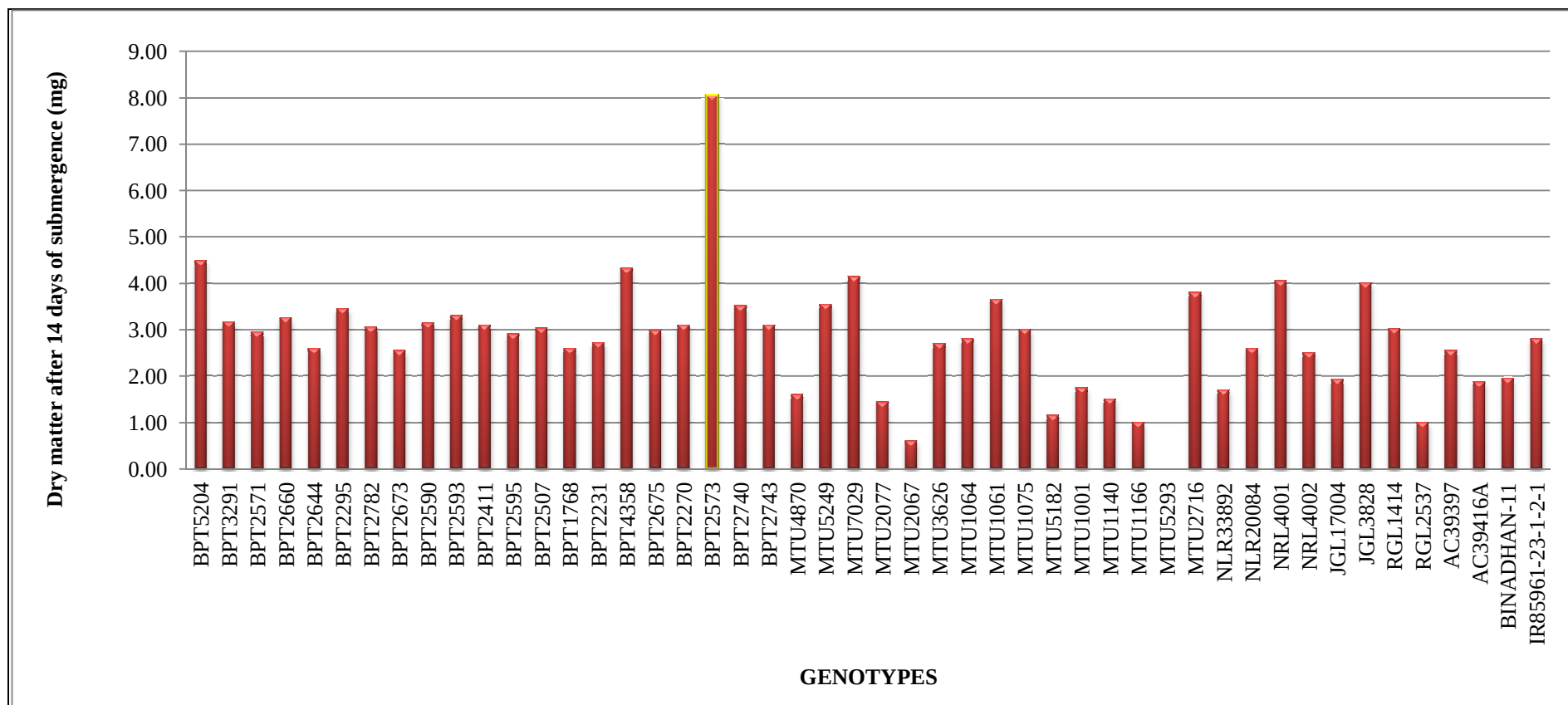


Fig. 4.4 Mean performance of 48 rice genotypes for dry matter after 14 days of submergence (mg)

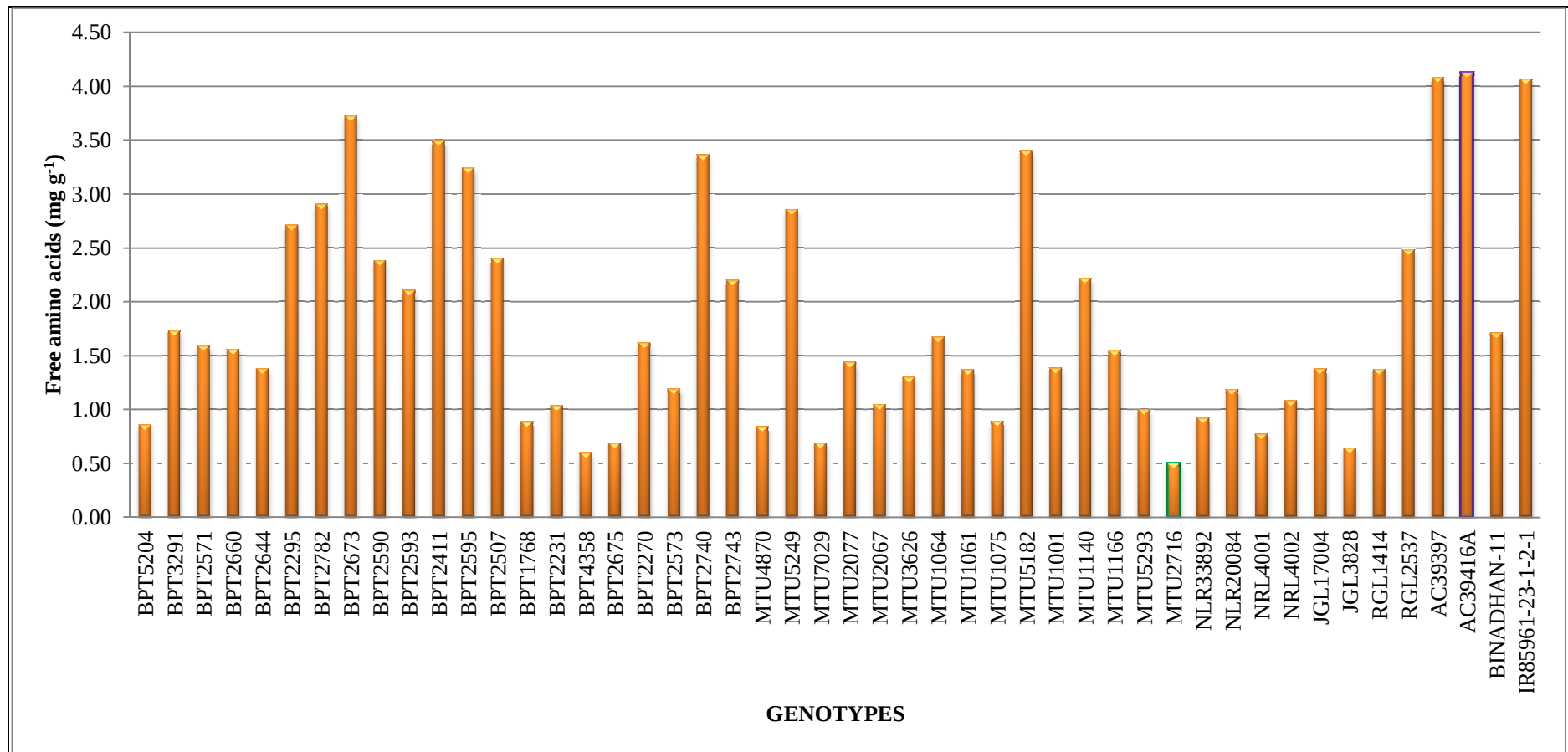


Fig. 4.5 Mean performance of 48 genotypes for free amino acids (mg g⁻¹)

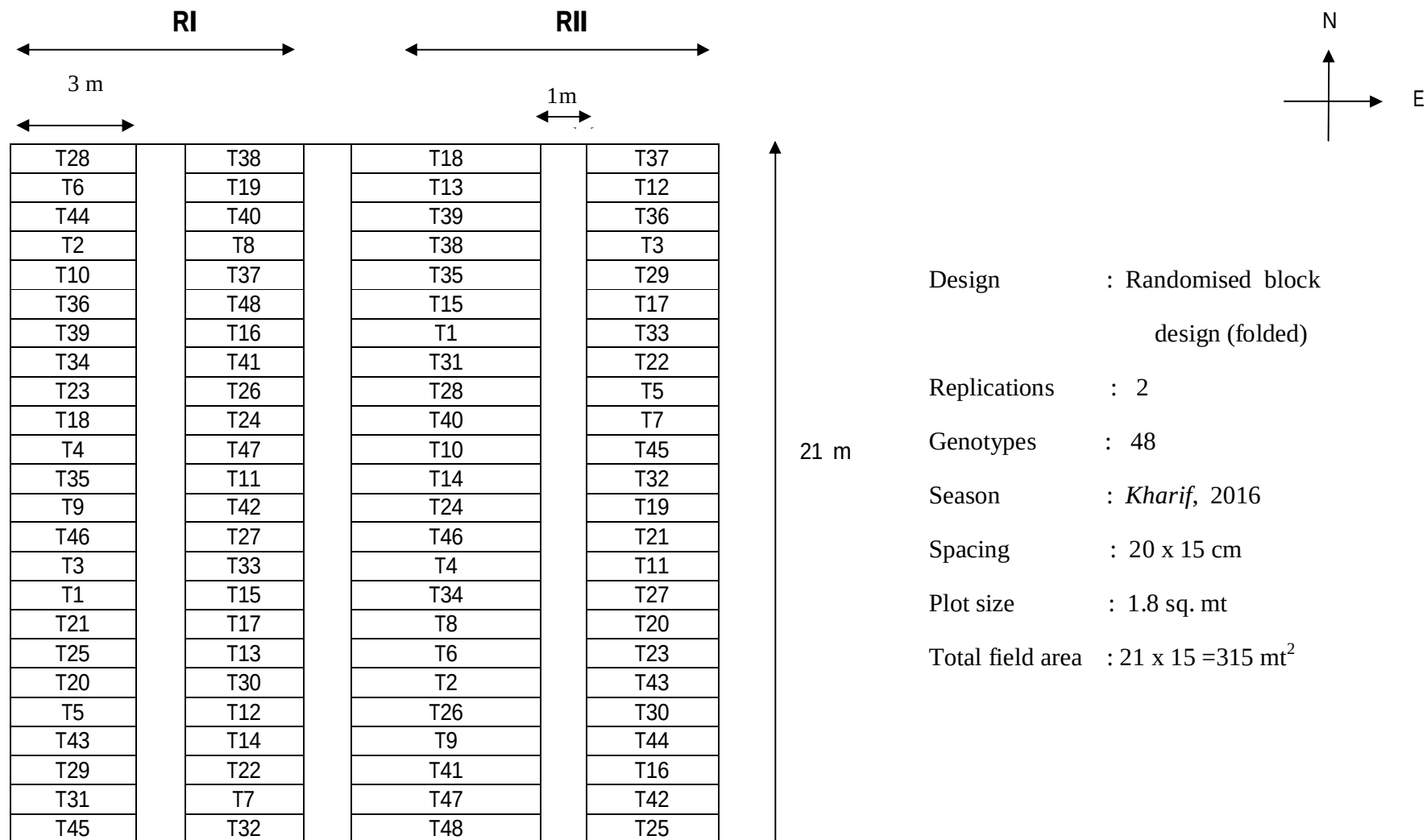


Fig 3.1 Layout of the Experimental plot

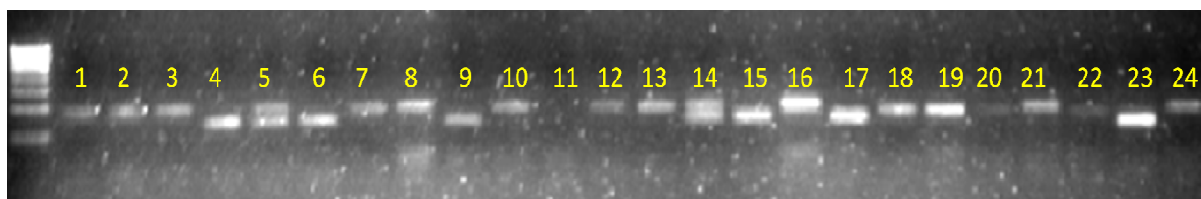


Plate 4.1 Amplification profile of the DNA of 48 rice genotypes using the primer RM 10694

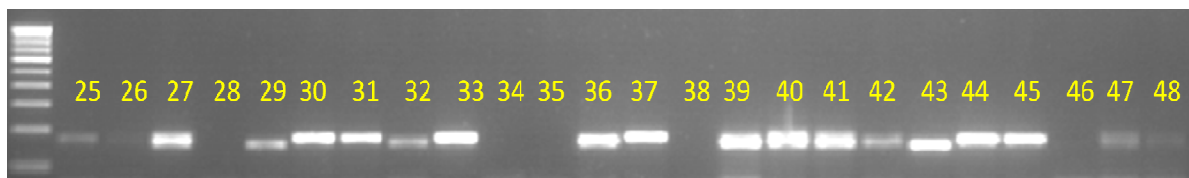


Plate 4.2 Amplification profile of the DNA of 48 rice genotypes using the primer RM 6318

1	MTU4870	13	NLR20084	25	MTU2067	37	BPT4358
2	NLR33892	14	BPT2590	26	JGL17004	38	NLR4002
3	MTU5249	15	BPT2593	27	RGL1414	39	MTU1001
4	MTU7029	16	MTU2077	28	BPT1768	40	BPT2675
5	BPT2571	17	MTU3626	29	MTU1064	41	BPT2270
6	BPT2660	18	IR85961-23-1-2-1	30	MTU2716	42	AC39416A
7	BINADHAN-11	19	NLR4001	31	BPT3291	43	MTU1140
8	BPT2644	20	AC39397	32	BPT2231	44	BPT2573
9	BPT2295	21	JGL3828	33	BPT5204	45	MTU1166
10	BPT2782	22	BPT2411	34	MTU1061	46	BPT2740
11	RGL2537	23	BPT2595	35	MTU1075	47	MTU5293
12	BPT2673	24	BPT2507	36	MTU5182	48	BPT2743

