EVALUATION OF SUPER-ELITE RICE (*Oryza sativa* L.) ACCESSIONS FOR PRODUCTIVITY AND ROOT CHARACTER UNDER AEROBIC CONDITION

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BENGALURU

2013

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Thesis submitted to the

University of Agricultural Sciences, Bengaluru

in partial fulfilment of the requirements for the award of the degree of

Master of Science (Agriculture)

in

PLANT BIOTECHNOLOGY

BENGALURU JULY, 2013


Affectionately
Dedicated to
My Beloved Father
Shri Surendra Pawar
& Mother
Smt. Chayya Pawar

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CERTIFICATE

This is to certify that the thesis entitled "**EVALUATION OF SUPER-ELITE RICE (*ORYZA SATIVA* L.) ACCESSIONS FOR PRODUCTIVITY AND ROOT CHARACTER UNDER AEROBIC CONDITION"** submitted in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture) in PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, Bengaluru, is a bonafide record of research work done by **Ms. PAWAR NAMRATA SURENDRA, ID No. PALB-1226** during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
July, 2013

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ACKNOWLEDGEMENT

I feel the inadequacy of words to express my deep sense of gratitude and profound indebtedness to ***Dr. H.E. Shashidhar****, Professor, Department of Plant Biotechnology and Chairperson of my Advisory Committee for his valuable guidance, constant supervision, constructive criticism, vivid encouragement and affectionate dealing throughout the period of investigation and during preparation of the manuscript. I confess that it has been a great fortune and proud privilege for me to be associated with him during my Master degree program. I am highly grateful to him for critically going through the manuscript and for his valuable suggestions for the improvement of the thesis.*

I wish to place my sincere and heartfelt gratitude to ***Dr. H. V. Vijayakumar Swamy,*** *Professor and Head, Department of Plant Biotechnology and* ***Dr. T. H. Ashok,*** *Professor, Department of Plant Biotechnology and* ***Dr. D. M. Gowda,*** *Professor and Head Department of Agricultural Statistics for their constant encouragement, suggestions and valuable guidance as member of my Advisory Committee during the course of my study and investigation.*

I thank all teaching faculty, ***Dr. Ramanjini Gowda, Dr. R. L. Ravikumar, Dr. Anitha Peter, Dr. Dayal Doss, Dr. N. Earanna, Dr. C.K. Suresh, Dr. Shyamalamma*** *and* ***Dr. Theertha Prasad,*** *and non-teaching staff of the Department of Plant Biotechnology for their kind co-operation and encouragement during my study and research.*

I express my whole hearted thanks to my lab mates ***Ms. Rakhi, Mr. Vimarsh, Mr. Pavan, Mr. Sumanth, Ms. Sowjanya, Mr. Rakesh, Mr. Naveen, Mr. Ravindra, Mr. Shashank, Ms. Suma, Ms. Rajeshwari***

*& **Mr. Ashwath** for their kind help, constructive suggestions and encouragement during my investigation.*

*I have been highly fortunate in having many affectionate friends whose helping hand was evident at every stage of my investigation. I thank **Ms. Pallavi, Ms. Snehal, Ms. Deepa** and **Mr. Sujeet** for their friendship, love and for making my period of study enjoyable.*

*I record my respectful indebtedness and gratitude to my father **Shri Surendra Pawar**, mother **Smt. Chayya Pawar**, Brother **Mr. Manoj** and Sister **Ms. Nisha** their blessings, encouragement, unquantifiable love and affection.*

*I thank all the field workers, **Raju, Tulasamma, Radhamma, Bailamma** who rendered their help during the course of my research work.*

The grant of DBT-HRD Fellowship during my studies is greatly acknowledged.

I finally thank all those who have helped me directly and indirectly to complete the research study to the best of my satisfaction.

Bengaluru

July, 2013 ***(PAWAR NAMRATA SURENDRA)***

"Evaluation of Super-Elite Rice (*Oryza sativa* L.) Accessions for Productivity and Root Character under Aerobic Condition"

THESIS ABSTRACT

Rice is the premier staple food of the world especially in the rainfed areas of Asia, where drought is a major production constraint. Recognizing this water scarce problem globally for rice production, the better option is aerobic rice cultivation which saves almost 50 per cent of water compared to puddled situation. Adaptation to aerobic condition is specific to varieties. Thus, selection of superior genotypes from diverse rice accessions through phenotyping for whole-plant architecture and molecular characterization in rice is an attractive strategy. Evaluation of the total 56 diverse genotypes were done in field as well as in PVC pipes under aerobic conditions for productivity and root characters. Periodic observations were recorded like plant height, tiller numbers, days to 50% flowering, maximum root length, root volume, biomass of root and shoot and grain yield. All the super-elite rice selections were screened for amplification with trait specific markers associated with biotic stress. Field evaluation revealed significant difference among the means of different genotypes for all the traits under aerobic condition. As root morphological traits were directly related to drought resistance. Different genotypes with higher root length and root number have been identified and the superior genotypes were selected on the basis of high grain yield and root characters. The genotypes Seeta sail, Champa Khushi, AM-FS-F7-163A, IRRI-44, AM-94B, NDR 2026, High Iron Rice, 196(M), 182(M) and Vandana were found to be superior for yield and drought resistance characters under aerobic condition in *Kharif*–2012.

Signature of the student
(Pawar Namrata S.)

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(H. E. Shashidhar)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES


INTRODUCTION

I. INTRODUCTION

About half the total world rice area is rainfed, and challenged by drought stress. The complex inheritance of drought resistance, encouraged breeders to adopt alternative strategies to improve stress tolerance. Fukai and Cooper, (1995) reported that under low-moisture stress, traits that help the plant gain access to additional moisture reserves were more important than traits associated with reducing moisture losses.

Rice is central to the lives of billions of people around the world being the staple food for 2.5 billion people. The demand for rice is estimated to be 2,000 million metric tons by year 2030 for which there is need to produce 40% more rice (Anon., 2002). Moreover it is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide. It plays a very important role in the food security of many nations (Khush, 2005). The Green Revolution led to a tremendous increase in tropical rice production. Much of these yield increases were seen in the irrigated and stress free ecosystems. To meet this demand, further improvement in production and productivity is essential. This is proving to be difficult as the yields have plateaued.

Drought is a major abiotic stress, affecting 20% of the total rice-growing area in Asia (Pandey and Bhandari, 2008). Roots are the principal plant organs for nutrient and water uptake. Therefore, improving our understandings on interactions between root function and drought tolerance in rice could have a significant impact on global food security.

Selection of rice lines with desirable root traits should be considered in drought resistance breeding. For screening the root traits

in rice, several techniques such as root box (Yoshida & Hasegawa, 1982), aeroponic culture (Carter, 1942), hydroponic culture (Ekanayake et al., 1985b) and root pulling force techniques (O'Toole & Saemartono, 1981; Shashidhar, 1990) have been developed. Moisture stress is the single most important factor limiting rice productivity in the rainfed habitats. Aerobic rice does not require standing water and use efficiently rain water in field. Therefore, farmer can skip irrigation if soil moisture status is sufficient for crop growth (Shashidhar, 2007, www.aerobicrice.in). It is time to save water even from the irrigated rice eco-system of rice cultivation by adopting the aerobic rice cultivation.

In rice grown ecosystem, biotic stresses like bacterial leaf blight, blast and brown plant hopper infestation is very high and utmost care should be taken for prevention of yield losses. These stresses are further influenced by drought stress.

India is facing the daunting challenge of sustaining food security even at the dawn of the new millennium. To attain this goal, utmost attention should be paid to enhance rice production and productivity where bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is still one of the most devastating diseases across the tropics and semi-tropics. Damages range from 6 to 60% in India, Japan and Indonesia.

Bacterial blight caused by *X. oryzae* pv. *oryzae* is one of the most serious diseases of rice worldwide. The disease was first reported in 1884 from Japan. In the 1960s, bacterial blight epidemics occurred in other Asian regions including Indian sub-continent as a result of the introduction of modern, high yielding but susceptible rice cultivar. While seed treatment and phytosanitation practices are recommended, breeding for varietal resistance has been and will continue to be the most eco-friendly and economical method of BB control (Devadath, 1989).

In addition to Bacterial blight, Blast disease is also a serious disease of rice in both the tropical and temperate regions. The rice blast disease is caused by the fungus *Pyricularia grisea*, which, in its sexual state is known as *Magnaporthe grisea*. The disease can strike all aerial parts of the plants in popular varieties, such as TN1 and IR8. This fungal disease is estimated to cause production losses of US$55 million each year in South and Southeast Asia. Molecular genetics of blast resistance have been extensively studied, and many useful DNA markers corresponding to major genes conferring race-specific resistance have been identified (Fjellstrom *et al.*, 2006).

Brown Plant Hopper (BPH) is a major insect pest of rice that causes serious hopper burn in conducive environmental conditions and damage to rice production in the tropics and subtropics other than Blast and Bacterial blight. It also transmits several viral diseases, including grassy stunt and ragged stunt. The genes Bph1, bph2, and Bph18 have been used for MAS of BPH resistance in temperate japonica and tropical indica rice cultivars (Jena *et al.*, 2006).

Brown Plant Hopper (BPH) known as *Nilaparvata lugens*. The symptoms of infection are hopper burn or yellowing, browning and drying of plant with ovipositional marks exposing the plant to fungal and bacterial infections. It was recorded that at a population density of more than 400-500 nymphs or 200 adults per plant, BPH can cause complete loss of rice plants (Santhanalakshmi *et al.*, 2010).

Keeping these points in view, the present investigation was taken up with the following objectives to create impact on drought tolerance and biotic stress tolerance:

- Characterization of super-elite rice from Indian Rice Biofortification Programme (IRBP) for root morphology and productivity
- Molecular characterization of these super-elite rice using informative markers for biotic stresses


REVIEW OF
LITERATURE

II. REVIEW OF LITERATURE

The available literature pertinent to the present investigation has been reviewed in detail in this chapter under the following headings.

2.1 Rice environments and drought

2.1.1 Rice growing ecosystem

2.1.2 Lowland rice and problems associated

2.1.3 Aerobic rice

2.1.4 Drought tolerant approaches and strategies

2.2 Genetic variation for root traits and their role in drought resistance

2.3 Genetic variation for biotic resistances i.e. blast, BLB and BPH

2.4 Genetic variability parameters and genetic divergence studies for root and shoot

2.4.1 Heritability and Genetic Advance

2.4.2 Genetic Diversity in Rice

2.5 Genes conferring drought tolerance

2.1 Rice environments and drought

2.1.1 Rice growing ecosystem

Across species, morphological and physiological changes in plant growth due to effects of hardpans on roots include a reduction in transpiration rate and leaf area expansion, and ultimately a decrease in dry matter accumulation (Masle and Passioura, 1987; Ludlow *et al.*, 1989; Assaeed *et al.*, 1990; Masle, 1992).

In addition to the presence of a hardpan and greater stratification of soil characteristics due to puddling, rainfed lowland soils differ from upland soils in that severe soil cracking occurs upon soil drying. Soil

cracking, which can penetrate hardpans, strongly influences rainfall infiltration and water evaporation processes (Tuong *et al.*, 1996).

Despite the direct link with development issues, there has been little success in developing drought-tolerant rice cultivars. A cultivar with high mean yield but a low degree of fluctuations in yield in diverse environments is considered stable and desirable for the rainfed ecosystem. However, rice breeders have not been able to select for such stable varieties because of high genotype by environment (G × E) interactions for grain yield (Cooper *et al.*, 1999).

Rice is produced in a wide range of locations under a variety of climatic conditions, occupying one-tenth of the global agricultural land. Rice is grown in four main ecosystems; irrigated lowland, rainfed lowland, deep-water and upland. As described by IRRI, in rainfed lowland, rice is transplanted or direct seeded in puddled soil, on level slightly sloping, bunded or dyked fields with variable depth and duration of flooding, depending of rainfall.

Altogether, there are some 11 million ha of flood-prone rice areas in the world, with average yield of around 1.5 t ha^{-1} (Maclean *et al.*, 2002).

Belder *et al.*, (2005) reported relatively low uptake of nitrogen under aerobic conditions as compared to flooded conditions which was reflected by the relatively low fertilizer N recovery under aerobic conditions.

In tropical and sub-tropical rice production systems in Asia, alternative irrigation methods have been shown to save up to 50% of the

water while maintaining yields at 70–80% of non-water-saving conditions (Belder *et al.*, 2005).

In flood-prone rice environments, the fields are periodically subjected to excess water and uncontrolled deep flooding. Deep-water and floating rice are found in this environment (Bouman *et al.*, 2006).

Rice cultivation in flooded 'paddy' fields has been the most widely used and highest-yielding cultivation system. With a 75% share of total rice production, irrigated lowland rice has been feeding billions of people mainly living in Asia and Africa. Unfortunately, this system consumes about 30% of all fresh water used worldwide (Peng *et al.*, 2006), thereby posing a huge sustainability problem.

Peng *et al.*, (2006) reported decline in soil organic matter as a possible reason for a decline in yield under aerobic cultivation, because total soil N at physiological maturity in the micro plots was not significantly lower in aerobic than in continuously flooded soil.

In the irrigated and deep-water rice ecosystems, water shortage does not usually occur, but in both the rainfed upland and lowland cultivation systems, drought stress is often the most important abiotic stress factor limiting yields (Atlin *et al.*, 2006).

In irrigated system, land is level, bunded with water control. One or more crops per year is taken with yields ranging from 3 to 9 t/ha. Out of total 147 m ha occupied by rice, about 30 m ha is grown as irrigated rice. In India out of 42.5 m ha under rice, irrigated rice occupies 19.66 m ha and in Karnataka, it is 0.86 m ha out of 1.17 m ha.

Deep water and tidal wetlands are highly flooded prone areas. Field may be prone to medium to very deep flooding (50 to more than 300 cm) from rivers and from tides in river mouth deltas. Around 10 m ha of Rice

area in South and Southeast Asia are under this situation. The average yields are 1.5 t/ha.

Different types of drought develop in rainfed rice fields and they vary according to the severity and timing of the drought in relation to the stage of crop development and they affect the yield potential differently. So defining the type of drought stress is important for targeted breeding strategy. There are three basic drought patterns affecting rice production viz-vegetative stage, intermittent and terminal drought stresses (Kamoshita *et al.*, 2008).

2.1.2 Lowland rice and problems associated

Root plasticity can be defined as the ability of a genotype to adjust its root growth phenotype according to environmental constraints (O'Toole and Bland, 1987). Some studies claim that root plasticity might be an important physiological trait in genotypic adaptation for drought stress under lowland conditions (Ingram *et al.*, 1994; Yamauchi *et al.*, 1996).

Large areas of rice are grown under lowland and upland rainfed conditions. These areas respectively occupy 31% and 11% of the global rice-growing area (IRRI, 2001).

Worldwide there are about 54 million ha of rainfed lowlands, which contribute 19% of the world's total rice production and 14 million ha of rainfed uplands, which contribute 4% of the world's total rice production. Average yields range from 2.3 t ha^{-1} in rainfed lowlands to 1 t ha^{-1} in uplands (Maclean *et al.*, 2002).

Rainfed lowland rice is grown in bunded fields, predominantly puddled, that are flooded with rainwater for at least part of the cropping season to water depths that exceed 100 cm for no more than 10 days.

The fields rely entirely on rainfall or drainage from higher lands in a watershed. Water seepage is limited by puddling and bunding. However rainfed upland rice is grown under dry land conditions with no standing water, without irrigation, little or no fertilizer application and without puddling, usually in non bunded fields (Bouman *et al.*, 2006).

Rainfed lowland rice is grown in bunded fields that are flooded for at least part of the cropping season to water depths that do not exceed 5-10 cm for more than 10 consecutive days. They are characterized by lack of water control, with floods and drought being the potential problems.

Global reduction in rice production due to drought averages 18 Mt annually was reported. This abiotic stress is therefore a major constraint to rice production in water-limited environments (Bernier *et al.*, 2008).

2.1.3 Aerobic rice

Aerobic rice is a new method of cultivating rice in less water than traditional flooded condition. Aerobic rice varieties are developed by crossing lowland varieties with upland varieties and cultivated in irrigated but non flooded and non puddle soils (Bouman *et al.*, 2002; Bouman et al., 2005).

Aerobic rice has efficient water use efficiency which saves about 45% of water utilization, decrease methane production, reduce production cost and eco-friendly compared to conventional irrigated rice (Shashidhar, 2007).

BI33 (ARB6) is an aerobic adapted high yielding rice variety developed from a cross between Buddha and IR64 in University of Agricultural Sciences, GKVK, Bangalore (www.aerobicrice.in). It has manifested high degree of drought resistance and gives high yield, comparable to improved varieties when sufficient moisture is available.

It has deep roots which are capable of extracting moisture from deeper layers of soil column (Gowda, 2010). It grows fast and has high biomass.

In upland fields during stress, the major sources of water for growth and development are rain that is retained by the soil and groundwater. A coarse and deep root system, for soil penetration and access to water reserves deep in the soil, is considered valuable for improved drought resistance under upland conditions (O'Toole and Chang, 1979; Ling *et al.*, 2002).

In upland conditions, Mumbani and Lal, (1983) reported a significant positive correlation between deep root growth and grain yield, and they clearly demonstrated that deep roots had a role in water uptake.

Rice cultivars adapted exclusively to upland conditions are typically characterized by a deep and coarse root system, tall stature, thicker stems, and fewer tillers (Ge, 1992; Ling *et al.*, 2002),whereas lowland rice cultivars have shallow and finer roots, a large number of roots, and many tillers (Lang *et al.*, 2003).

Drought resistance mechanisms that are appropriate for upland systems may not be suitable for rainfed lowland conditions and vice versa (Mackill *et al.*, 1996), mainly because of the unique hydrology of rainfed lowlands in which soil transitions from flooded and anaerobic to drought and aerobic (Wade *et al.*, 1999).

2.1.4 Drought tolerant approaches and strategies

Nature of anthesis and flowering date is another important integrative trait. Lafitte and Courtois, (2002) evaluated 45 rice cultivars in managed stress environments to relate cultivar X environment (C X E) interaction for yield to specific putative drought-adaptive mechanisms.

They found that early maturity was advantageous under drought over later flowering in terms of higher spikelet fertility, higher harvest index, and higher yield even when stress was applied at specific developmental stages for each cultivar. Other traits such as leaf percentage fresh weight, root pressure, leaf area, and rooting depth were also found to be associated with yield.

The global reduction in rice production due to drought averages 18 million tonnes annually (O'Toole, 2004). In the Eastern states of Jharkhand, Orissa, and Chhattisgarh alone, rice production losses in severe droughts which occur about once in five years is estimated to be about 40% of total production (Pandey and Bhandari, 2009). Severe droughts in recent years, such as those seen in 2002-03 and 2009-10in India had a great impact on rice production and hence food security. The declaration of drought/scarcity/drought-like situation in 334 districts by 14 State Governments during the year 2009-10 coincided with a drastic reduction in total rice production in India from 99.4 million tonnes in 2008-09 to 89.1 million tonnes in 2009-10 (Anon., 2010).

In Asia alone where majority of world's rice is grown and consumed, drought is a regular feature in approximately 23 million ha. (Pandey *et al.*, 2007). Severe droughts in recent years, such as those seen in 2002-03 and 2009-10 in India had a great impact on rice production and hence food security. This abiotic stress is therefore a major constraint to rice production in water-limited environments. Early droughts often result in delayed sowing or transplanting. Yield reductions from early droughts are minimal and result mainly from a reduction in tiller numbers. Breeding varieties suited to these conditions is an important element in reducing risk and increasing productivity in drought-prone environments, but progress in breeding for drought tolerance in rice has been slow (Bernier *et al.*, 2008).

Serraj *et al.*, (2009) suggested that development of rice cultivars by combining improved drought resistance with yield potential under favorable conditions would be the strategy to increase rice productivity in drought-prone areas.

Jeong *et al.*, (2010) demonstrated that root-specific overexpression of OsNAC10 enlarges roots, enhancing drought tolerance of transgenic plants, which increases grain yield significantly under field drought conditions.

Farooq *et al.*, (2010) reported that leaf growth is one of the first physiological processes affected by changes in plant water status under drought. Changes in leaf size and stomatal opening are potential adaptive mechanisms, which may help avoid drought by reducing transpiration rate, and can be used to improve rice genotypes in water-saving cultivation.

Drought is one of the most important abiotic stresses causing drastic reductions in yield in rainfed rice environments. The suitability of grain yield (GY) under drought as a selection criterion has been reported in the past few years. Most of the quantitative trait loci (QTLs) for GY under drought in rice reported so far has been in the background of low-yielding susceptible varieties (Vikram *et al.*, 2011).

Seo *et al.*, (2011) reported that Jasmonates play important roles in development, stress responses and defense in plants. They also reported the results of a study using a functional genomics approach that identified a rice basic helix-loop-helix domain gene, OsbHLH148 that conferred drought tolerance as a component of the jasmonate signaling module in rice.

Sanchez *et al.*, (2010) reported response of rice plants to inoculation with an arbuscular mycorrhizal (AM) fungus, *Azospirillum brasilense*, or combination of both microorganisms, was assayed under well-watered or drought stress conditions. AM colonization increased stomatal conductance, particularly when associated with *A. brasilense*, which enhanced this parameter by 80% under drought conditions and by 35% under well-watered conditions as compared to single AM plants.

Rice root growth encompasses a remarkable genetic diversity in terms of growth patterns, architecture, and environmental adaptations. The ability to grow deep roots is currently the most accepted target trait for improving drought resistance (Gowda *et al.*, 2011).

2.2 Genetic variation for root traits and their role in drought resistance

The ability to maintain water uptake at levels determined by atmospheric evaporative demand during a rainless period appears to be a major attribute that increases drought resistance in rice (O'Toole and Chang, 1979). A principal mechanism by which rice has become adapted to water deficiency is through the possession of a pronounced root system which maximizes water capture and allows access to water at depth (O'Toole, 1982).

The impact of drought in rice is influenced by cultivar, stage of drought imposition, and duration of stress. Rice's susceptibility to water stress is more pronounced at the reproductive stage and causes the greatest reduction in grain yield when stress coincides with the irreversible reproductive processes (Cruz and O'Toole, 1984).

The overall size and maximum depth of the rice root system and individual root thickness (measured in the field and hydroponics) have been positively related to field drought resistance. Development of a deep

and extensive root system is an adaptive strategy of plants for drought avoidance. Root characteristics such as thickness, depth of rooting, and root length density have been associated with drought avoidance in rice (Ekanayake *et al.*, 1985; O'Toole and Chang, 1979).

O'Toole and Bland, (1987) reviewed the genotypic variations in root systems and reported that plant root systems have capability of coping with the changes in environmental factors such as water status and temperature.

A significant genotypic variation for root penetration ability was reported by Yue *et al.*, (1995) by using the wax layer method. Using the same method, Babu *et al.*, (2001) found that *japonica* accessions have a higher root penetration index (number roots penetrating the wax layer/ total number nodal and seminal roots) than *indica* types, and these were used to develop double haploid mapping populations (CT9993/IR62266 and IR58821/IR52561) for mapping and tagging root traits, over the next decades.

Root to shoot ratio is a measure of the allocation of resources between different plant components. The allocation of resources toward the root is high at early vegetative stages but decreases markedly at flowering and is almost negligible after anthesis (Gregory *et al.*, 1996).

Root architecture has been suggested, as the main basis of variation, to help maintain plant water status under prolonged drought at vegetative stage (Nguyen *et al.*, 1997). In a field trial, Mitchell *et al.*, (1998) reported that plant size actually defines the extent and role of the root system in plants under drought.

Rooting depth and root thickness determined drought tolerance of rice varieties under rainfed lowland conditions. In experiments

simulating rainfed lowland conditions using pot plants, Wade *et al.*, (1999) noticed genotypic difference for water extraction and their relation with root distribution under drought stress.

Genotypic differences for root mass, root length and distribution across lowland rice varieties were reported by Azhiri-Sigari *et al.*, (2000). Similarly Thanh *et al.*, (1999) noticed large genotypic variation for root traits among upland varieties.

The constitutive root traits are expressed under favorable anaerobic conditions. The adaptive root traits require water stress for their expressions (e.g., promoted root elongation rate and plastic lateral root production; Banoc *et al.*, (2000) and enhanced aerenchyma under sudden waterlogged conditions; Justin and Armstrong, 1987).

The genotypic variation for root traits in different types of rice were studied by Lafitte *et al.*, (2001) and reported that *Indica* rice types had thin, highly branched superficial roots with narrow vessels and low root to shoot ratio, whereas *japonica* types had coarse roots with wider vessel, less branched long roots and a large root to shoot ratio and aus types had intermediate root diameter, with a root distribution profile similar to that of *japonica* but with thin roots.

Toorchi *et al.*, (2001) evaluated root morphology and related characters at three different stages and different moisture regimes using PVC tubes. They notices significant genotypic differences for all root and shoot traits measured across different sampling.

Venuprasad *et al.*, (2002) in a study involving simultaneous evaluation of root character and grain yield of under stress, and control conditions concluded that roots, that a rice plant produced prior to onset of stress, will enable a plant to tide through the stress situation and also

produce better yield that a genotype that did not have the capacity to produce roots prior to the onset of stress.

Rice plants require different root system development strategies for adaptation. The extent of the contribution of root plastic development is difficult to assess under fluctuating soil moistures, as both the constitutive and adaptive root trait responses are present and each interacts with both the existing conditions and the genotypic background of the diverse cultivars used (Kamoshita *et al.*, 2002).

Kamoshita *et al.*, (2004) evaluated six diverse rice genotypes selected from rainfed lowland germplasm to examine the development of a deep root system and osmotic adjustment, and their relationship with biomass production during drought and after rewatering, under two different drought durations (shorter and prolonged). Two genotypes NSG19 and KDML105 showed superior drought recovery even after a prolonged drought period in which they suffered a greater reduction in transpiration, water use efficiency and biomass production which was attributed to with the larger plant size by the end of the drought period rather than with plant water status during drought, such as osmotic adjustment or leaf water potential.

Genetic differences have also been observed in the ability of seminal roots to continue elongation and form aerenchyma under flooded conditions after drought (Suralta and Yamauchi, 2008; Suralta *et al.*, 2008, 2010).

Even for deep water rice, accounting for 11% of the world's rice area, and upland rice which is grown under aerobic conditions and rain-fed fields without standing water and accounts for 13% of the world's rice area, the productivity is threatened by the scarcity of water at the

seedling stage before the floods and a lack of standing water, respectively (Wang *et al.*, 2009).

Suralta *et al.*, (2010) examined root plastic development through aerenchyma formation and lateral root production, and their functions to maintain atmospheric O_2 transport and soil water uptake under prolonged transient soil moisture stresses. Such responses were also quantified in terms of their contribution to the maintenance of physiological responses such as stomatal conductance, transpiration, and photosynthesis and ultimately, shoot growth in terms of dry matter production.

Chromosome segment substitution lines (CSSLs) derived from Nipponbare and Kasalath crosses were evaluated by Kano *et al.*, (2011) under soil moisture gradients with line source sprinkler system up to around heading. CSSL50 was found consistently to show significantly higher shoot dry matter production than its parent Nipponbare. It showed phenotypic plasticity through greater total root length through promoted lateral root branching and elongation than Nipponbare, which was found to be key trait that effectively contributed to plant dry matter production through increased total root length and thus water uptake.

2.3 Genetic variation for biotic resistances i.e. BLAST, BLB and BPH

Rice blast, caused by the fungal pathogen *Magnaporthe grisea* (Herbert) Borr. (anamorph =*Pyricularia grisea*), is a haploid filamentous Ascomycete with a relatively small genome of~40 Mb divided into seven chromosomes. Although many resistant varieties have been developed, due to genetic plasticity in the pathogen genome, there is a continuous threat to the effectiveness of the developed cultivars (Hittalmani *et al.*, 2000).

As many as genes conferring resistance (bacterial blight) and some genes for resistance to blast have been identified to various races of the pathogen have been identified and utilized in rice breeding programs. The rice-rice blast interaction has long served as a model system to study plant-pathogen interaction. Two new resistance genes were reported *Pi-36* on chromosome 8 and *Pi-37* on chromosome 1. After that, a novel gene *Pi-40* linked to the DNA markers derived from the NBS-LRR motifs confers broad spectrum was reported. Currently, about 30 resistance genes or loci against to Xoo have been identified in cultivated and wild rice. So far, five dominant R genes, *Xa-21, Xa-1, Xa-26, Xa-27* and *Xa-3* and two recessive R genes, *xa-5* and *xa-13*, have been isolated by map-based cloning (Lang *et al.*, 2008).

Reddy and Kumar, (2009) studied saturation mapping of QTL region determine in resistance specificity to bacterial leaf blight pathogen in rice with molecular markers, ESTs and genes on sequences *in-silico* by knowing the quantitative nature of inheritance which reflect the additive effects on several genetic loci throughout the genome. For defense response they performed *in-silico* anchoring of the QTL genetic marker data to the rice physical map where the genes on sequence were classified based on their functional resistance pathway which showed many defense related protein which express during the disease resistance against the bacterial leaf blight and this would further helped for validation of the QTL as well as fine dissection for development of the blight resistance variety.

To breed rice varieties with more durable blast resistance, multiple resistance utilizing both qualitative and quantitative genes must be incorporated into individual varieties (Joshi *et al.*, 2009).

Zhai *et al.*, (2010) reported that rice blast resistance gene *Pik*, which is one of the five classical alleles located at the Pik locus on the

long arm of chromosome 11, confers high and stable resistance to many Chinese rice blast isolates.

Takahashi *et al.*, (2010) reported that R gene-mediated resistance is one of the most effective mechanisms of immunity against pathogens in plants. In this work they isolated the R gene *Pish*, and identified several other mutants involved in the signal transduction required for Pish-mediated resistance.

Fujita *et al.*, (2010) developed near-isogenic lines (NILs) and PYLs for GRH resistance by means of marker assisted selection (MAS), and to facilitate the use of the GRH-resistance genes and QTLs in future rice improvement. Six GRH-resistance genes (Grh1, Grh2, Grh3, Grh4, Grh5, and Grh6) and one quantitative trait locus (QTL; qGRH4) have been identified. We selected near-isogenic lines (NILs) carrying Grh1, Grh2, Grh4, Grh5, Grh6, and qGRH4 with the *japonica* genetic background (Taichung 65 cultivar) by means of marker-assisted selection using new simple sequence repeat markers flanking the GRH-resistance genes and QTL. We also developed three pyramided lines (PYLs; Grh2/Grh6-PYL, Grh4/Grh6-PYL, and Grh5/qGRH4-PYL) using each NIL that carried a GRH-resistance gene or QTL.

The severe damage and frequent outbreaks of BPH, along with the hazardous effects of pesticides, have prompted researchers to seek BPH-resistant germplasm from various sources and to utilize the resistance genes for rice improvement. To date, more than 24 major BPH-resistance genes have been reported in *indica* cultivars and five wild *Oryza* species (*O. australiensis, O. eichingeri, O. latifolia, O. officinalis, and O. minuta).* Several of the BPH-resistance genes have been assigned to rice chromosomes 2, 3, 4, 6 and 12. F2 population derived from a cross between ADR52 and a susceptible cultivar, Taichung 65 (T65), was used for quantitative trait locus (QTL) analysis. BPH25 cosegregated with

marker S00310 on the distal end of the short arm of chromosome 6, and BPH26 cosegregated with marker RM5479 on the long arm of chromosome 12 (Myint *et al.*, 2012).

Jiang *et al.*, (2012) identified a dominant R gene, *Pi2-2*, at the Pi2/9 locus from Jefferson, an elite U.S. rice cultivar, through genetic and physical mapping. Fine mapping delimited *Pi2-2* to a 270-kb interval between the markers AP5659-3 and RM19817, and this interval contains three nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes in the Nipponbare genome. Utilization of broad-spectrum resistance (R) genes is an effective and economical strategy to control the fungal pathogen *Magnaporthe oryzae*, the causal agent of the rice blast disease. Among the cloned blast resistance genes, *Pi9, Pi2* and *Piz-t* confer broad-spectrum resistance to diverse M. *Oryzae* isolates.

Hua *et al.*, (2012) isolated the *Pi1*, a gene conferring broad-spectrum resistance to rice blast. An extensive germplasm survey using this FNP reveals that *Pi1* is a rare allele in germplasm collections and one that has conferred durable resistance to a broad spectrum of pathogen isolates.

Breeding and the development of resistant cultivars carrying major resistance (R) genes have been the most effective and economical strategy to control BB disease. Molecular markers have made it possible to identify and pyramid valuable genes of agronomic importance in resistance rice breeding. Suh *et al.*, (2013) transferred three resistance genes (*Xa4+xa5+Xa21*) from an *indica* donor (IRBB57), using a marker-assisted backcrossing (MAB) breeding strategy, into a BB-susceptible elite *japonica* rice cultivar, Mangeumbyeo, which is high yielding with good grain quality.

2.4 Genetic variability parameters, and genetic divergence studies for root and shoot traits in rice

2.4.1 Heritability and Genetic Advance

Information on heritability and gene action of a plant trait is a prerequisite for a successful breeding program. Such studies rely heavily upon a relatively large number of plants to arrive at statistically significant results, thus making root traits difficult to incorporate. As such, information on heritability and gene action of rice root traits is still very limited. Earlier studies (Armenta-Soto *et al.*, 1983) using aeroponic systems found that dominant genes control root number, root depth, and root mass, whereas root diameter is controlled by both dominant and recessive genes.

Armenta-Soto *et al*, (1983) reported higher narrow-sense heritability estimates for root diameter (62%), root length (60%), and root number (44%).

Ekanayake *et al.*, (1985) using F1, F2, and F3 populations from a cross between IR20 (shallow, fine root system) and MGL-2 (deep, coarse root system), reported that root diameter, root dry weight, and root length are polygenic traits with substantial proportions of additive variation and with narrow-sense heritabilities greater than 50%. They suggested that selection for these root traits based on individual plant performance could be successful in early-segregating generations.

In another study, Ekanayake *et al.*, (1985) observed low inheritance in lowland rice for root pulling force, which is the vertical force required to remove one plant from the soil. Low heritability for some root traits is a common breeding concern, but the issue can be resolved by developing suitable selection methods that take full advantage of

genetic variability and also make possible rapid selection, by increasing both heritability and selection response (Richards and Passioura, 1981).

The genotypic variation in drought tolerance together with the genetic tools available for rice, such as marker maps, sequence information, and microarrays (Matsumoto *et al.,* 2005; Rensink and Buell, 2005) and the possibility to test the agronomic relevance of a scientific discovery (Xu *et al.*, 2006), make rice a most interesting model system for research in drought tolerance of grass crops

Manickavelu *et al.*, (2006) investigated grain yield and its components on drought situation of recombinant inbred population (IR 58821/IR 52561) under lowland managed stress situation and reported high phenotypic and genotypic coefficient of variation for leaf drying, drought recovery rate and productive tillers per plant. While the traits leaf rolling, grains per panicle, biomass yield and harvest showed high phenotypic and moderate genotypic coefficient of variation. Heritability in broad sense ranged from 9.80% to 83.75%.

Kumar *et al.*, (2007) evaluated doubled haploid lines with a narrow range of flowering dates, derived from the population CT9993/IR62266, under full irrigation and severe drought stress induced by draining the paddy before flowering and found similar broad sense heritability estimates under both the conditions.

Verulkar *et al.*, (2010) evaluated advanced-generation breeding lines of less than 100 days, 100–120 days, and greater than 120 days duration generated at eight national institutes and IRRI using diverse drought tolerant donors under stress imposed at the reproductive stage. Yield reduction of 34-53% under moderate stress and 65-88% under severe stress was achieved in comparison to irrigated controls. The study showed that grain yield, days to flowering, plant height and harvest index

were significantly influenced by genotypes (G) and genotype x environment interaction (G x E). Moderate to high heritability for grain yield under drought stress was observed *indica*ting selection for yield under drought stress is repeatable.

2.4.2 Genetic Diversity in Rice

Genetic diversity in root growth exists within the rice germplasm (O'Toole and Bland, 1987) and traits such as maximum root length, root thickness, root weight and root volume have been shown to have medium to high heritabilities (Armenta-Soto *et al.*, 1983; Chang *et al.*, 1982; Ekanayake *et al.*, 1985; Loresto *et al.*, 1983). There is, therefore, potential for improving root-related drought resistance in rice through breeding programmes.

Thanh *et al.*, (1998) evaluated genetic variation for root morphology in upland rice accessions and investigated their genetic diversity using microsatellite markers. Forty-one alleles were detected with 14 rice microsatellite primer pairs among all the rice accessions.

Garris *et al.*, (2005) genotyped a sample of 234 accessions of rice at 169 nuclear SSRs and two chloroplast loci. The data were analyzed to resolve the genetic structure and to interpret the evolutionary relationships between groups. Five distinct groups were detected, corresponding to *indica*, aus, aromatic, temperate *japonica* and tropical *japonica* rices.

Bose and Pradhan, (2005) assessed the nature and magnitude of genetic divergence among 35 deep water rice genotypes from India using Mahalanobis D^2 statistics. The genotypes were grouped into 10 clusters showing fair degree of relationship between geographic distribution and genetic divergence. Traits such as plant yield, days to 50% flowering and plant height were the major contributors to genetic divergence.

Chandra *et al.*, (2007) assessed fifty-seven upland rice genotypes including 32 local rice germplasm for the nature and magnitude of genetic divergence among them based of D^2 values, the 57 genotypes were grouped into five clusters. The most divergence clusters were III and IV (D^2=3387.9) followed by III and V (D^2=2808.2) and clusters II and III (D^2=1908.7). The clustering patterns of the genotypes were quite at random *indica*ting that the geographical origin and genetic diversity were not related. The characters contributing more towards the genetic divergence were 1000-grain weight, grain yield and biological yield.

Roy *et al.*, (2009) estimated genetic variability of root traits and physiological traits that confer drought resistance, and study the influence of moisture stress imposed through different osmotics of PEG-6000 to evaluate the drought stress effects on proline, protein and antioxidant enzyme catalase to screen the drought tolerant genotypes of rice.

The study was undertaken to assess the genetic diversity among aromatic rice genotypes using simple sequence repeat (SSR) and randomly amplified polymorphic DNA (RAPD) markers through marker aided selection (MAS). Based on Nei's genetic distance using the unweighted pair-group method with arithmetic means (UPGMA) dendrogram the SSR primers showed the highest genetic distance which was 2.306 and in case of RAPD marker it was 0.7634 (Kibria *et al.*, 2009).

Understanding genetic diversity, population structure, and the level and distribution of linkage disequilibrium (LD) in target populations is of great importance and a prerequisite for association mapping. In this study, 100 genome-wide simple sequence repeat (SSR) markers were used to assess genetic diversity, population structure, and LD of 416 rice accessions including landraces, cultivars and breeding lines collected

mostly in China. A model-based population structure analysis divided the rice materials into seven subpopulations (Jin *et al.*, 2010).

Li *et al.*, (2011) reported Nucleotide variation in 14 unlinked nuclear genes in species-wide samples of African rice (*Oryza glaberrima*) and its wild progenitor (*O. barthii*). About 70% less diversity was found in *O. glaberrimathan* in its progenitor *O. barthii*. Genealogical analyses showed that all *O. glaberrima* accessions formed a strongly supported cluster with seven *O. barthii* individuals that were sampled exclusively from the proposed domestication centers of African rice.

Molecular markers serve as a valuable tool to assess the genetic variation, varietal classification, and germplasm identification of rice. Random amplified polymorphic DNA fingerprinting was performed to assess the genetic diversity among rarely cultivated traditional *indica* rice (*Oryza sativa* L.) varieties (Rekha *et al.*, 2011).

Root length

Kawata and Soejima, (1974) indicated that roots produced after flowering may play an important role during the grain-filling period.

In rice, emphasis on root diameter, depth of rooting, and root:shoot dry weight ratio in relation to drought resistance appears justified based on the positive correlations between these characters and visual scores of plant vigor in upland field drought-screening trials both in the Philippines (O'Toole and Soemartono, 1981).

Advantages conferred by a deep root system depend on three major factors: duration of the drought period, availability of water at depth, and rate of water uptake. If water is not limited in upper layers of the soil, the plant may not benefit from the formation of deep roots. In upland conditions, Puckridge and O'Toole, (1981) found that deep-rooted cultivar

Kinandang Patong extracted more water at a depth of 40–70cm than shallow-rooted cultivars (IR20 and IR36).

Growth of the rice root, in terms of total dry matter, maximum root depth, and root length density, increases until flowering stage and then decreases sharply to maturity (Yoshida and Hasegawa, 1982).

The maximum depth that roots reach is genetically determined and differs substantially between cultivars grown under identical conditions, but at the same time is affected by environmental conditions in the field (Yoshida and Hasegawa, 1982). Maximum root depth of a particular genotype is achieved only when roots do not encounter a physical limit to growth.

While measurements of rooting depth and root length density alone may not predict the ability of a genotype to extract soil water, computer simulations designed to assess the consequences of increasing the root zone depth in soybean, sorghum and wheat have suggested that such a change was accompanied by increased leaf area, growth, photosynthesis, transpiration (Jones and Zur, 1984), and yield (Muchow and Sinclair, 1986).

It was reported that a high pulling force is associated with the plant's ability to develop deeper and larger diameter roots with great penetration ability. However, heritability of root pulling force was relatively low (Ekanayake *et al.*,1985) since many other soil factors in addition to root depth affect root pulling force.

Chang *et al.*, (1986) also found that rice with a deep root system avoided drought better than rice with a shallow root system.

Lateral roots, which comprise a greater proportion of the root system in total length and number (Yamauchi *et al.*, 1987) are

responsible for the greatest amount of water and nutrient absorption (Yoshida *et al.*, 1982).

Kato *et al.*, (2006) have reviewed the effects of various water regimes on deep root growth and biomass partitioning to roots in upland rice. The authors concluded that while many studies report an increase in root: shoot ratio and deep root growth in response to drought, conditions as timing of the drought at seedling stage, very severe drought stress, and presence of hardpans have reduced resource partitioning to roots.

Root dry weight

Root dry weight along with root thickness, root volume and number of thick roots were found to be significantly correlated to root pulling resistance (Ekanayake *et al.*, 1986). On imposition of stress, the root weight decreases which may be attributed to decrease in the associated traits (Cruz *et al.*, 1986). Okuyama and Colasante, (1987) indicated that root dry weight might increase with increase in duration of crop growth. Jeena and Mani, (1990) proposed root weight as a selection criterion in selecting drought tolerant genotypes.

Sorte *et al.*, (1992) reported 74 per cent reduction in root weight when soil moisture content was reduced by 44 per cent. Survival during stress reflects on the roots capacity to function. The drought tolerant genotypes should have greater root weight as compared to upland and drought susceptible cultivars (Vijayalakshmi and Nagarajan, 1994).

Asch *et al.,* (2004) reported that the proportion of total dry matter allocated to root or shoot parts depended on the rate of soil dry-down, with root shoot ratio averaging 0.05–0.1 at flowering in soil-filled PVC pipes.

Root Number

Mao, (1984) reported that multiple genes with additive and or positional effect governed root number along with root length. When IR54 was subjected to a gradient of soil moisture conditions using a line source sprinkler system, 19 days of mild water stress at vegetative stage resulted in decrease in the number of roots (Cruz *et al.*, 1986). A study on developmental changes in root mass was conducted by Suga and Yamazaki, (1998) where in root mass was correlated with leaf mass and the primary root number was found to increase exponentially with plant age expressed in terms of leaf number. The consistency in the production of roots was checked by Haque *et al.*, (1989). They used four varieties of the Aus type and four of the hill type along with a drought resistant and a susceptible check. These were grown in aeroponic and hydroponic culture. Root number of the genotypes differed in these two cultures revealing its inconsistent nature.

2.5 Genes conferring drought tolerance

Many quantitative trait loci (QTLs) associated with root characteristics under different water conditions have been reported based on genetic populations derived from crosses between low-land *indica* rice and upland *japonica* rice varieties, or between upland *indica* rice and upland *japonica* rice varieties (Champoux *et al.*, 1995; Price and Tomos, 1997).

Drought is a major abiotic stress of upland rice, and good root growth has been associated with drought avoidance. Price and Tomos, (1997) reported on the genetic mapping of root growth traits in an F2 population derived from two drought-resistant rice varieties, Bala and Azucena. One QTL for root volume and two QTLs for adventitious root thickness were detected.

In QTL mapping, the main factor limiting the precision of QTL localization is the number of progenies used in the study, more so than other factors such as using more molecular markers or using better statistical techniques (Kearsey and Farquhar, 1998).

Zheng *et al.*, (1999) Used a wax–petrolatum layer system simulated to compacted soil layers, root traits were evaluated in a doubled haploid (DH)population derived from the cross between 'IR64' and 'Azucena'. Twelve putative QTLs (quantitative trait loci) were detected by interval mapping comprising four QTLs for root-penetration ability, four QTLs for root thickness, two QTLs for penetrated root number, and two QTLs for total root number. The identified consistent QTLs could be used for marker-assisted selection for deep and thick roots with high root-penetration ability in rice.

At molecular level, large numbers of genes are known to be involved in plant responses in drought. These include genes involved in signal transduction and transcriptional regulation (Xiong and Zhu, 2002; Oh *et al.*, 2005), biosynthesis of osmotic and other protectors. Besides, the availability of rice genome sequence will permits now the identification of the function of each of rice genes through functional genomics, was published in 2002, serving as a gold standard for all future investigation.

Sharma *et al.*, (2002) identified tightly linked markers for root traits adopting bulked segregant analysis and found two markers (OPBH14 and RM201) to be cosegregating with maximum root depth in the IR64/Azucena mapping population and these were validated across different germplasm subspecies group, that is, *indica* × *indica* or *japonica* × *japonica* .

Rice high-density linkage maps and the positions of many genes or expressed sequence tags (ESTs) in the rice genome are available for the candidate-gene approach to clone QTLs for root traits under different water-supply conditions and different genetic backgrounds (Harushima *et al.*, 1998; Wu *et al.*, 2002).

Zheng *et al.*, (2003) used a recombinant inbred line (RIL) population from a cross between the lowland *indica* variety IR1552 and the upland *japonica* variety Azucena to examine QTLs for root growth under flooding and upland conditions at the early seedling stage. Eighteen QTLs were detected for seminal root length (SRL), adventitious root number (ARN), and lateral root length (LRL) and lateral root number (LRN) on the seminal root at a soil depth of from 3 to 6 cm.

Gene expression analysis helps in identifying the functionally important genes and pathways involved in root architecture under water-deficit conditions (Breyne *et al.*, 2003).

Yang *et al.*, (2004) conducted a tissue-specific gene expression study in drought-stressed rice roots, in which 66 transcripts were identified and cloned in roots of Azucena. Four transcripts were mapped within an interval containing QTLs for root growth under water deficit in the Azucena/IR1552 population.

Wang *et al.*, (2007) observed that the majority of genes expressed in upland rice and lowland rice are almost identical and that 13% of all the expressed sequence tags (ESTs) detected in leaves and 7% of those in roots were expressed differentially in transcripts between the two cultivar types.

Transgenic rice with the transcription factor AtDREB1A or its orthologue OsDREB1A (Dehydration-Responsive Element Binding gene)

tested in pots demonstrated improved resistance to simulated drought, high salt, and low-temperature stresses (Yamaguchi-Shinozaki, 2004).

Thanh *et al.*, (2006) mapped QTLs for root traits related to drought resistance (maximum root length, root thickness, root weight to shoot and deep root weight to shoot ratios) in upland rice using a recombinant inbreed (RI) population derived from a cross between Vietnamese upland rice accessions (one of which belong to *japonica* subspecies, another is *indica* once), and SSR and AFLP markers. These QTLs could be very useful for precise locating drought resistant gene(s) and marker-assisted selection.

Vinod *et al.*, (2006) identified candidate genes for root traits related to morphology and physiology. These were validated in the CT9993/IR62266 mapping population, which was evaluated for root traits under contrasting moisture regimes.

Candidate genes are sequenced genes of known biological action involved in the development or physiology of a trait. Vinod *et al.*, (2006) identified and designed polymerase chain reaction (PCR) primers for CGs directly related to drought resistance and productivity at the morphophysiological, phonological, biochemical, genetic and phenotypic levels.

Shen *et al.*, (2001) conducted a study for introgressing a root depth QTL from a deep-rooted variety, Azucena, into IR64 in three cycles of marker-assisted backcrosses. However, few lines with a significantly improved phenotype (deeper roots) resulted from that effort.

Steele *et al.*, (2006) introgressed four QTLs related to root length and diameter and one QTL related to aroma into an Indian upland variety, Kalinga III, in a 6-year marker-aided backcross program.

Most of the QTL mapping studies for rice root traits conferring drought tolerance have been conducted using progenies derived by crossing varieties belonging to a different subspecies group (*japonica* × *indica*; as reviewed in Kamoshita *et al.*, (2008) rather than by using progenies derived by crossing varieties belonging to the same.

Prabuddha *et al.*, (2008) identified near-isogenic lines for several root traits, and candidate genes that were found to be associated with a particular root trait were also validated with the near-isogenic lines.

Kamoshita *et al.*, (2008) have summarized the large number of studies in rice for drought resistance in QTL mapping studies of at least 15 different populations.

Rice root QTLs for drought have been compiled using QTL meta-analysis in multiple populations (Norton *et al.*, 2008; Courtois *et al.*, 2009). Multiple QTL studies from a single population (Bala × Azucena: Khowaja *et al.*, 2009) have also been conducted that identified several dense clusters of root QTLs. This integrative approach across a large number of studies conducted in multiple environments/phenotyping systems is more likely to identify important areas of the genome for rice root response to drought than any single QTL study alone.

Transgenic lines carrying the OsNAC045 transcription factor, whose functions include a role in the development of lateral roots, were reported to have a greater survival rate in rice after drought and salt treatments and OsNAC10 lines with a root-specific promoted showed greater yield than the wild type under drought in the field (Jeong *et al.*, 2010).

Improved performance under drought was obsersved in transgenic plants of the gene OsMT1a (metallothionein) that is predominantly expressed in roots and is induced by dehydration (Yang *et al.*, 2009).

Novel genes identified as useful in enhanced drought resistance will need to be transformed, most likely as a suite of genes. The general approach on transgenic rice for improving drought resistance has been recently reviewed (Herve and Serraj, 2009).

Association mapping is a promising method for complex trait dissection and it focuses on association within populations of unrelated individuals. Using association mapping, it is possible to locate QTLs with better precision than using a map-ping population (Courtois *et al.*, 2009).

To bring some light on functional gene response to drought stress Lang *et al.*, (2010) done cloning and sequencing to identify candidate gene response to drought stress condition and determining their relationship with identified candidate genes, providing some insights into the molecular basis of gene tolerant to drought in rice.

Uga *et al.*, (2011) and Obara *et al.*, (2010) reported a major QTL for rooting depth (Dro1) and root length (qRL6.1) using basket and solution culture (hydroponic) methods, respectively.

The number of QTLs identified for root traits has ranged from 1 to 19 and the amount of phenotypic variability explained among the progeny examined by any one QTL ranged from about 4% to as much as 66.6% (Uga *et al.*, 2011).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The details of the plant material used and the methods followed in field experiments conducted in the field of Department of Plant Biotechnology, GKVK campus, Bengaluru are described below. The information on protocols and statistical tools used for the analysis of data are presented under the respective experiments headings separately.

3.1 Experiment 1

Characterization of super-elite rice from mapping population for root morphology and productivity

The phenotypic studies were done in two parts, *viz.*, study of shoot parameters in the field during *Kharif* -2012 with three replications and post-harvest study of root morphology parameters in pipe experiment conducted in the same plot with three replication.

3.1.1 EVALUATION OF SHOOT MORPHOLOGY AND YIELD OF THE CROP

3.1.1.1 Experimental site

The experiment was carried out in the field of Department of Plant Biotechnology, UAS, GKVK, Bengaluru, during *Kharif* 2012. Study location represent eastern dry zone which is located at the latitude of 12°58' North; longitude 77°35' East and altitude of 930 meters above mean sea level (MSL). Randomized complete block design (RCBD) was adopted meeting statistical requirement with three replications (Plate 1 and 2).

Plate 1: Twenty one days old rice seedlings at GKVK field under aerobic condition during *Kharif*-2012

Plate 2: Different rice genotypes in field for evaluation of yield and its associated traits at maturity stage under aerobic condition during *Kharif*-2012

3.1.1.2 Plant materials

The study consisted samples collected from 10 locations across India by Directorate of Rice Research Hyderabad, under the project Rice "Rice Project Harvest Plus". Fifty four different superior genotypes were selected for high iron and zinc content from the previous study with 2 checks namely NDR-359 and IR-64. The decoded list and source location of RPHP genotypes as coded by DRR is given in Table1. Four five star lines were also included in the study that belonged to F7 generation of Azucena and Moro mutant cross developed at DPBT,GKVK.

3.1.1.3 Cultural Operations

Seeds were direct sown in the plots of size 3 X 1.5 cm^2. Four rows per genotype were sown at the spacing of 30 cm between rows and 15 cm between plants. One plant per hill was maintained by thinning the excess seedlings. Recommended cultural practices were carried out to ensure uniform crop stand as per the package of practices recommended by UAS, Bangalore. Soil moisture was maintained at optimum by irrigating the field 2-3 times a week. Irrigation was also given when plant were showing stress symptoms such as leaf wilting, due to high atmospheric temperature. Proper care was given to raise healthy plants. Observations were recorded at regular intervals and at harvest.

3.1.1.4 Method of sampling and recording of observations

Five plants were selected at random in each genotype for recording observations. Mean values of the traits were used for statistical analysis. The characters observed for eliciting the information are described in the following subheadings.

Table 1: Plant material used for the study

Sl. No.	DRR code	Variety name/cross	Co-op center
1	RPHP-7	AM-72	GKVK, Bengaluru, Karnataka
2	RPHP-9	BJ-23	GKVK, Bengaluru, Karnataka
3	RPHP-10	AM-27	GKVK, Bengaluru, Karnataka
4	RPHP-11	AM-143	GKVK, Bengaluru, Karnataka
5	RPHP-16	AM-65	GKVK, Bengaluru, Karnataka
6	RPHP-21	AM-94B	GKVK, Bengaluru, Karnataka
7	RPHP-27	Azucena	GKVK, Bengaluru, Karnataka
8	RPHP-29	BJ-5	GKVK, Bengaluru, Karnataka
9	RPHP-33	BJ-21	GKVK, Bengaluru, Karnataka
10	RPHP-36	TKM-9	GKVK, Bengaluru, Karnataka
11	RPHP-37	Mainpuri	IGKVV, Raipur, Chhattisgarh
12	RPHP-39	Jhelum	IGKVV, Raipur, Chhattisgarh
13	RPHP-42	Shalimar Rice -1	IGKVV, Raipur, Chhattisgarh
14	RPHP-44	BR- 2655	IGKVV, Raipur, Chhattisgarh
15	RPHP-45	Panvel -3	CRRI, Cuttack, Odisha
16	RPHP-47	Pathara	CRRI, Cuttack, Odisha
17	RPHP-48	Bindli	CRRI, Cuttack, Odisha
18	RPHP-51	Vandana	CRRI, Cuttack, Odisha
19	RPHP-52	Sebati	CRRI, Cuttack, Odisha
20	RPHP-53	PB-164	CRRI, Cuttack, Odisha
21	RPHP-55	Kalinga -3	CRRI, Cuttack, Odisha
22	RPHP-56	IRRI-44	CRRI, Cuttack, Odisha

23	RPHP-59	Taroari Basmati	CRRI, Cuttack, Odisha
24	RPHP-68	Subhdra	CRRI, Cuttack, Odisha
25	RPHP-80	24(K)	N.Sarla, Bio-Tech. DRR, Hyderabad
26	RPHP-81	233(K)	N.Sarla, Bio-Tech. DRR, Hyderabad
27	RPHP-84	51(B)	N.Sarla, Bio-Tech. DRR, Hyderabad
28	RPHP-87	140(M)	N.Sarla, Bio-Tech. DRR, Hyderabad
29	RPHP-90	182(M)	N.Sarla, Bio-Tech. DRR, Hyderabad
30	RPHP-91	185(M)	N.Sarla, Bio-Tech. DRR, Hyderabad
31	RPHP-92	196(M)	N.Sarla, Bio-Tech. DRR, Hyderabad
32	RPHP-93	Type-3	Dr. V.R. Babu, DRR, Hyderabad
33	RPHP-102	Kanchana	R. K.V. Rao, Oil Science, Hyderabad
34	RPHP-103	Pant sugandh dhan -17	R. K.V. Rao, Oil Science, Hyderabad
35	RPHP-104	Kasturi	R. K.V. Rao, Oil Science, Hyderabad
36	RPHP-105	Moirang phou	R. K.V. Rao, Oil Science, Hyderabad
37	RPHP-106	Akut phou	R. K.V. Rao, Oil Science, Hyderabad
38	RPHP-107	Improved Chitti Mutyalu	R. K.V. Rao, Oil Science, Hyderabad
39	RPHP-108	High Iron Rice	R. K.V. Rao, Oil Science, Hyderabad
40	ssRPHP-125	NDR 2026	R. K.V. Rao, Oil Science, Hyderabad
41	RPHP-129	Kamad	R. K.V. Rao, Oil Science, Hyderabad
42	RPHP-130	China 1007	R. K.V. Rao, Oil Science, Hyderabad
43	RPHP-134	NJA VORA	Aduthurai, Tamilnadu
44	RPHP-135	Kadamakudy Pokkali	Karaikal, Puducherry
45	RPHP-138	Edavankudi	Karaikal, Puducherry

		Pokkali	
46	RPHP-140	Vytilla Anakondan	Karaikal, Puducherry
47	RPHP-156 (ABCDE)	BPT 5204 /Chitti Mutyalu-SB	Dr. V.R. Babu, DRR, Hyderabad
48	RPHP-159	Radhuni Pagal	Chinsurah, West Bengal
49	RPHP-161	Champa Khushi	Chinsurah, West Bengal
50	RPHP-163	Seeta sail	Chinsurah, West Bengal
51	RPHP-165	Tilak kachari	Chinsurah, West Bengal
52	RPHP-166	NC 365	Chinsurah, West Bengal
53	AM-FS-F7-111	-	-
54	AM-FS-F7-57	-	-
55	AM-FS-F7-113B	-	-
56	AM-FS-F7-163A	-	-

Note- F7=F7generation**,** AM=Azusena/Moromutant,

FS=five-star, BJ=BI33/Jeergesanna.

3.1.1.4.1 Plant height

The height of the plant was recorded from the base of the plant to the tip of the main panicle, expressed in centimetres.

3.1.1.4.2 Tillers per plant

The total number of tillers per plant was counted at the time of harvest and recorded.

3.1.1.4.3 Productive tillers per plant (No of Panicles)

Among the total number of tillers per plant the productive tillers at the time of harvest was counted and recorded.

3.1.1.4.4 Days to flowering

The total number of days taken by each genotype from sowing to opening of first flower of the plants.

3.1.1.4.5 Days to maturity

The total number of days taken by each entry from sowing to maturity.

3.1.1.4.6 Grain yield per plant (g)

Total weight of all the filled grains per plant was considered as grain yield, recorded at harvest and expressed in grams.

3.1.1.4.7 Days to 50% flowering

The total number of days taken by each genotype from sowing to opening of half of the flower of the plants population.

3.1.1.4.8 Biomass per plant (g)

The total dry weight of plant (grain yield plus shoot biomass) is considered as plant biomass.

3.1.2 Evaluation for shoot and root morphology in PVC pipe experiment

Observations were recorded for maximum root length, shoot length, number of roots, total root volume, total shoot and root dry weight, total root wet weight and over all plant growth rates for plants grown in pipes.

3.1.2.1 Experimental Site

The experiment was carried out in the field of the Department of Plant Biotechnology, UAS, GKVK, Bengaluru, during *Kharif*-2012 in a randomized complete block design (RCBD) with three replications in standard sized PVC pipes of 68 cm length and 6 inch diameter.

3.1.2.2 Cultural Operations

Soil was filled in pipes and it was compacted by adding water over repeated time intervals across days. On the top layer, vermicompost was mixed and fertilizers were applied as basal dose of 50:50:50=N: P: K. Seeds were sown in soil filled PVC pipes. Two to three seeds were sown in different individual PVC pipes in three replications. After germination the seedlings were thinned to retain a single vigorous seedling and the observations were recorded at maturity.

3.1.2.3 Method of recording observations

Soon after the harvest of shoot, the pipes were soaked in water for 2 days to loosen the soil. The roots were washed thoroughly and the following observations were recorded for the genotypes.

3.1.2.3.1 Maximum root length (cm)

Root length was measured from crown to tip of the root and expressed in centimeter.

3.1.2.3.2 Total root number

Number of roots were counted two cm below the crown region of the root.

3.1.2.3.3 Total root volume

The total root volume of each genotype was taken by water displacement method. A standard graduated measuring cylinder was used to record data.

3.1.2.3.4 Total root dry weight (g)

The roots were dried in hot air oven for 2 days at 70 degree Celsius. The dry weight of three replications have been recorded in grams by using electronic balance.

1.2.3.5 Total root wet weight (g)

After the washing of roots, root wet weight was recorded in grams by using electronic balance.

3.2 Statistical analysis

The statistical analysis of the data on individual characters was performed using SPAR 2 statistical package. Statistical methods employed for the analysis are given below.

3.2.1 Analysis of Variance

The analysis of variance for different characters was carried using the mean data for aerobic condition in order to partition the variability

due to different sources following the method given by Panse and Sukhatme (1964).

ANOVA

Source of Variation	**Degree of freedom**	**Sum of squares**	**Mean sum of squares**	**F-ratio**
Replication	(r-1)	rSS		
Genotype	(g-1)	gSS	gSS -------- = eMS (g-1)	gMS ------- EMS
Checks	(c-1)	cSS	cSS ------- = cMS (c-1)	cMS ------ EMS
Lines	(v-1)	vSS	vSS -------- = vMS (v–1)	vMS ------ EMS
Line Vs Check	1	cvSS	cvSS -------- = cvMS 1	cvMS -------- EMS
Error	(r-1)(g-1)	ESS	ESS -------- = EMS c(b-1)	
Total	(rg-1)	TSS		

Where,

r = Number of replications

v = Number of lines

c = Number of checks

The significance was tested by comparing with the table values as given by Yates, (1965). Standard error of means (SEM) and Critical

difference (CD) were worked out using appropriate formula for comparing individual line means.

3.2.3 Phenotypic and Genotypic coefficient of variation (PCV and GCV)

The phenotypic and genotypic coefficient of variation was computed as per Burton and Dewane (1953) for low moisture stress.

$$PCV = \frac{P}{X} \times 100$$

$$GCV = \frac{G}{X} \times 100$$

Where,

P = Phenotypic standard deviation

G = Genotypic standard deviation

X = Grand mean of character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

PCV and GCV were classified according to Robinson *et al.*, (1949).

0-10% was considered as low, 10-20% as moderate and 20% and above as high.

3.2.4 Heritability (h^2)

Broad sense Heritability estimate as per cent mean was calculated using the formula (Hanson *et al.*, 1956).

$$h^2\% = \frac{Vg}{Vp} \times 100$$

Where,

$h^2\%$ =Heritability percentage

Vg = Genotypic variance

Vp = Phenotypic variance

Heritability percentage was categorized as follows (Robinson *et al.*, 1949).

0-30% was considered as low,

30-60% was considered as moderate,

60% and above as high.

3.2.5 Genetic advance (GA)

Genetic advance was calculated by using formula given by Johnson et al., (1955).

$$GA = h^2 x\ \sigma p\ x\ K$$

Where,

h^2= Heritability (Broad sense)

σp = Phenotypic standard deviation

K = Selection differential which is 2.06 at 5% intensity of selection (Lush, 1949).

3.2.6 Genetic advance as per cent mean

$$\text{GA as per cent mean} = \frac{GA}{X} \times 100$$

Where,

GA = Genetic advance and

X = Treatment mean for the character.

The GA as percent mean was classified (Johnson *et al.*, 1955) as given below.

0-10 % Low,

10-20 % Moderate,

20% and above as high.

3.2.7 Correlation analysis

To estimate the degree of association between the traits studied, phenotypic correlation was computed by using the formula given by Webber and Moorthy, (1952).

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

Where,

rp = phenotypic correlation co-efficient.

COV (X, Y) = Phenotypic covariance.

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

The significance of correlation co-efficient was tested by referring to the table value at n-2 df given by Snedecor and Cochran, (1967).

3.3 Experiment 2

Molecular characterization of superior accessions using informative loci for biotic stresses.

3.3.1 GENOMIC DNA EXTRACTION

Leaf samples of twenty five days old seedlings grown during *Kharif* -2012 were used for DNA extraction. DNA was isolated as per CTAB (Cetyl Trimethyl Ammonium Bromide) method. DNA extraction was done using CTAB method as the protocol described by Doyle and Doyle (1990) as below.

a) Approximately 2 g of leaf tissue was ground in liquid nitrogen to fine powder with the help of a sterile pestle and mortar.

b) 10 ml of preheated CTAB extraction buffer was added to the fine powder and then it is transferred to the 50 ml oak ridge tube.

Extraction buffer (1 per cent PVP, 2 per cent cTAB, 1.4 M Nacl, 20 mM EDTA, 0.2 per cent Mercapta ethanol, 100 mM Tris HCL at pH 8).

c) The tubes were incubated in a water bath maintained at 65^0C for 30 minutes with gentle shaking at every five minutes interval.

d) After incubation, tubes are cooled and centrifugation was done at 13,000 rpm for 10 minutes.

e) Supernatant was collected from the tubes and transferred to new tubes.

f) To the volume of supernatant collected, equal volume of C: I (Chloroform: Isoamyl alcohol, in the ratio 24: 1) was added to each tube.

g) Then the tubes were again centrifuged at 13,000 rpm for 15 minutes.

h) Upper aqueous phase was transferred into fresh tube with the help of a micropipette.

i) Chloroform: isoamyl alcohol extraction was repeated once again at 13,000 rpm for 10 minutes to remove protein impurities.

j) After centrifugation, upper aqueous phase of each tube was carefully pipetted in to fresh tube and the DNA was precipitated with equal volume of chilled isopropanol and half the volume of 1.4 M NaCl.

k) Tubes were kept at -20^o C overnight and centrifuged at 13,000 rpm for 15 minutes.

l) Supernatant was decanted and pellet was washed in 70 percent ethanol and air dried.

m) The DNA pellets were then dissolved in 300 µl of 1X TE buffer and DNA samples were stored in -20^o C.

3.3.2 Marker analysis

3.3.2.1 Simple Sequence Repeats (SSR)

Microsatellite or simple sequence repeat (SSR) markers have been successfully used for genomic mapping, DNA fingerprinting, and marker-assisted selection in many plant species. Microsatellites are tandem repeats of DNA sequences of few base pairs (1-6 bp) in length, the most abundant being the dinucleotide repeats (Litt and Lutty, 1989). Over time, these repeated sequences vary in length between one cultivar and another. These variations in length are easy to trace in the lab and allow us to track genotypic variation in breeding programs.

The different SSR markers used in the present study are mentioned in the Table 2 for investigation. These are already mapped and associated with various biotic stress resistance characters in different mapping population.

3.3.2.2 PCR reaction mixture

Normalization of DNA was done to bring all DNA concentrations to a relatively equal level (25ng/µl) by appropriate dilutions. Dilutions were done with Distilled water. PCR Reaction mixture consisted of 25 ng /µl of template DNA, 3 pM each of forward and reverse primers, 10 mM each of dNTPs, 1 U of Taq polymerase and 10X PCR buffer A (Tris with 15 mM MgCl2) in a volume of 20 µl.

Table 2: Biotic stress markers used

Sl. No.	Primers	Marker	Forward sequence 5' to 3'	Reverse sequence 5' to 3'	Expected Product Size (bp)	Chr. No.	Annealing temp.(^{0}c)
Bacterial Leaf Blight							
1	*Xa4*	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGG	140	11	53
2	*xa5*	RM122	GAGTCGAGGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC	250	5	55
3	*xa13*	RG136	TCCCAGAAAGCTACTACAGC	GCAGACTCCAGTTTGACTTC	500	8	55
4	*Xa21*	pTA248	AGACGCGGAAGGGTGGTTTCCCGA	AGACGCGGTAATCGAAAGATGAAA	850	11	53
Brown Plant Hopper							
5	*Bph3*	RM190	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCTTGATG	143	6	50.6
6	*Bph18*	RM7376	TCACCGTCTCCTCTTATGC	GGTGGTTGTGTTCTGTTTGG	194	11	50
7	*Bph20(t)*	B44	TCTCAAACCGGCTCTACCAG	TTACTGGTATGGCAGGAGCA	207	4	60
Blast							
8	*Pi1*	candidate gene	ATATACTGTAGGTCCATCCCATCCA	AGATAGTATAGCGAAGCAGC	155	11	56
9	*Pi2*	AP5659-5	CTCCTTCAGCTGCTCCTC	TAGGATGACTTCCAAACGGT	330	6	58
10	*Pik^h*	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	155	11	57

The amplification profile was as follows:

Sl. No.	Steps	Temperature	Time in min.
1	Initial denaturation	94^{o} C	5.0
2	Denaturation	94^{o} C	0.5
3	Primer annealing	56^{o} C	0.5
4	Primer extension	72^{o} C	1.0
5	Final extension	72^{o} C	6.0
6	Holding temperature	4^{o} C	until removed

Note: 2, 3, and 4 steps were repeated for 35 times

3.3.2.3 Biotic stress markers

Biotic stress is stress that occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Identification of both the DNA markers tightly linked to resistance genes and the plant materials carrying the resistance gene will open new strategies for the development of resistance varieties in rice and in other cereal crops.

Work has been done in present study on three aspect *viz.* BLB, Blast, and BPH. Bacterial blight caused by *X. oryzae* pv. *oryzae* is one of the most serious diseases of rice worldwide. The rice blast disease is caused by the fungus *P. grisea*, which, in its sexual state, known as *M. grisea*. The disease can strike all aerial parts of the plant. Brown plant hopper (BPH) known as *N. lugens* shows symptoms Hopper burn or yellowing, browning and drying of plant.

3.2.4 Gel electrophoresis

Agarose gel of 2.0 per cent was prepared using electrophoresis grade agarose in a volume of electrophoresis buffer (1X TAE) sufficient for constructing a gel. Ethidium bromide was added at concentration of 0.5 µg /ml. The gel was allowed to solidify before removing the comb and loading the samples. 8 µl of loading dye was added to 20 µl of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between wells. A voltage of 5.0 V/cm distance between electrode was given for a time period of one hour for separation of PCR fragments. After electrophoresis, gel was viewed under UV light and the DNA banding pattern was documented in gel documentation unit (Alpha biotech) and banding pattern was recorded directly or photographed.

3.3.2.5 Scoring and validation of markers

The product size obtained from the study by using various biotic resistance markers was compared with already established studies based on the same markers in rice. Amplified product size for resistant genotype and susceptible genotypes has been reported earlier based on this resistance and susceptible genotype was identified in the study. Also the candidate gene responsible for imparting resistance against all the three biotic stresses under study (BLB, Blast, BPH) has also been studied and compared with published literature.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results obtained from the present investigation on are presented under the following subheadings.

4.1 Performance of super-elite accessions of rice for yield and yield attributes under aerobic condition

4.1.1 Variability and genetic parameters estimated for growth, yield and yield components under aerobic condition.

4.1.1.1 Analysis of variance

Analysis of variance for yield traits and its related traits of super elite rice under aerobic condition is presented in Table 3. Significant differences were observed for plant height, number of tillers per plant, number of productive tillers per plant, days to flowering, days to 50% flowering, days to maturity, grain yield per plant and biomass.

4.1.1.2 Variability and genetic parameters estimated for growth, yield and yield components at aerobic conditions

The genetic variability parameters *viz.*, minimum, maximum, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (H^2), for the traits under aerobic conditions are presented in the Table 4. And graphical representation of GCV and PCV, and Heritability and genetic advance as percent mean is shown in figure 1 and 2 respectively.

4.1.1.2.1 Plant height (cm)

Average value of 60.7 cm with range of 39.6 cm (BPT 5204 /Chitti Mutyalu-SB) to 97.6 cm (Azucena) was observed for this trait under aerobic condition. High GCV (20.23) and PCV (23.78) and high

Table 3: Analysis of variance for yield and its associated traits in super-elite rice accessions under aerobic condition during *Kharif*-2012

Sl. No.	Source of variation	Df	MSS							
			DF	DFF	DTM	PHT	NT	NPT	BM	GY
1	Replications	2	106.90**	341.35**	8.78**	15.06**	17.90**	6.87**	9.48**	4.20**
2	Treatments	57	222.21**	329.76**	247.11**	510.33**	59.70**	33.39**	388.69**	32.55**
3	Error	114	53.98	58.10	64.65	57.55	9.32	8.31	35.54	1.96
4	SEm		4.24	4.40	4.64	4.38	1.76	1.66	3.44	0.81
5	CD at 1%		15.96	16.56	17.46	16.48	6.63	6.26	12.95	3.04
6	CD at 5%		12.00	12.45	13.13	12.39	4.99	4.71	9.73	2.28

* Significant at 5%
** Significant at 1%
PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
GY= Grain yield per plant (g)
DF= Days to initial flowering
DFF= Days to 50% flowering
DAM= Days to maturity

Table 4: Estimates of genetic parameters for different quantitative traits among super-elite rice accessions for yield and its associated traits under aerobic condition during *Kharif*-2012

Sl. No.	Character	Minimum	Maximum	Mean	PCV	GCV	H^2%	GAPM
1	Days to initial flowering	64.7	99	81.1	12.94	9.24	50.96	11.14
2	Days to 50% flowering	67.3	112	91.3	13.35	10.42	60.91	14.4
3	Days to maturity	116	154	135.8	8.25	5.74	48.48	6.67
4	Plant Height(cm)	39.6	97.6	60.7	23.78	20.23	72.39	32.03
5	Number of tillers	5.7	31.3	14	36.61	29.36	64.31	42.34
6	Number of productive tillers	5	20.6	10.9	37.38	26.47	50.16	31.55
7	Biomass(g)	16.8	49.8	28.5	49.13	43.06	76.81	38.31
8	Grain yield(g)	0.1	18.8	6	57.95	53.08	83.9	94.63

GCV = Genotypic coefficient of variation
PCV = Phenotypic coefficient of variation
H^2% = Heritability percentage in broad sense

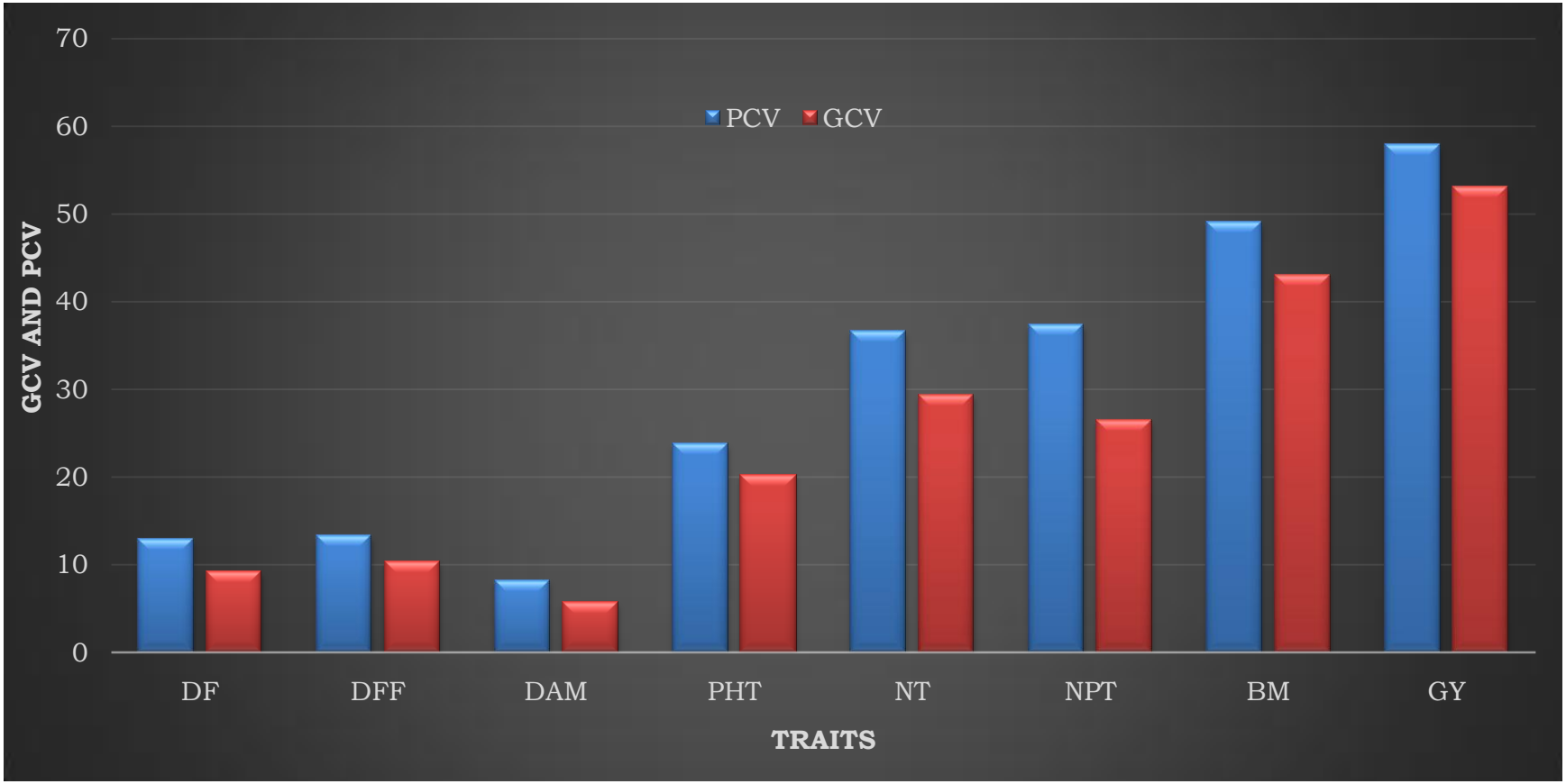


Fig. 1: Genotypic and phenotypic co-efficient of variability for yield and yield attributing traits among 56 super-elite rice genotypes evaluated in *Kharif*-2012 under aerobic condition

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
GY= Grain yield per plant (g)
DF= Days to initial flowering
DFF= Days to 50% flowering

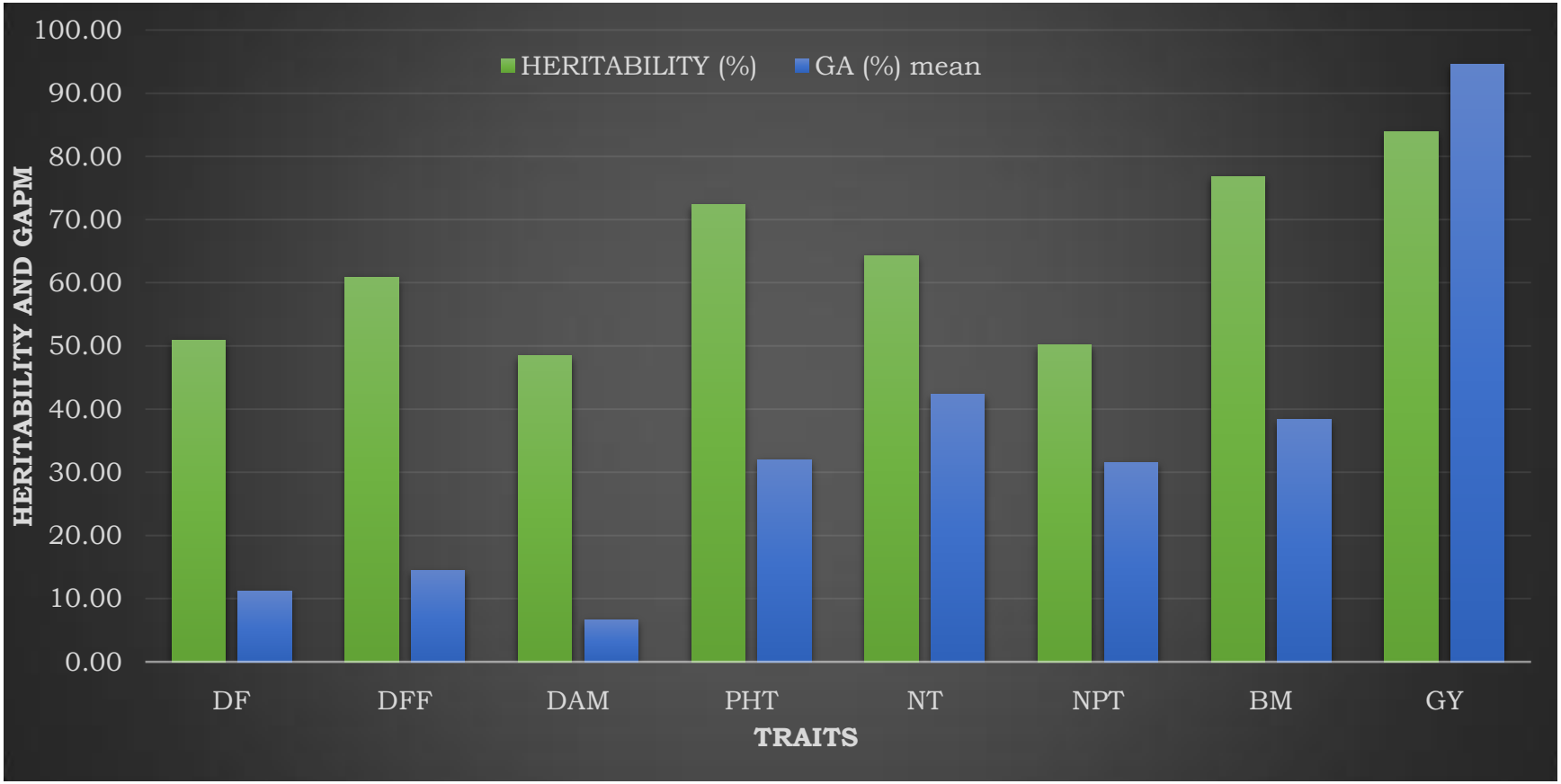


Fig. 2: Heritability % and genetic advance as percent mean for yield and yield attributing traits among 56 super-elite rice genotypes evaluated in *Kharif*-2012 under aerobic condition

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)

GY= Grain yield per plant (g)
DF= Days to initial flowering
DFF= Days to 50% flowering

heritability (72.39%) and high GA as per cent mean (32.03) were recorded for this trait.

4.1.1.2.2 Number of tillers

Average value of 14 with minimum of 5.7 (Azucena) and maximum of 31.3 (TKM-9) tillers were recorded under aerobic condition. High GCV (29.36), PCV (36.61) and high heritability (64.31%) and high GA as per cent mean (42.34) were observed for this trait.

4.1.1.2.3 Number of productive tillers

Average value of 10.9 with the range of minimum of 5 (Azucena) and maximum of 20.6 (TKM-9) has been found. High GCV (26.47) and PCV (37.38) have been recorded with high heritability (50.16 %) and high genetic advance per cent mean (31.55) have been noticed.

4.1.1.2.4 Days to initial flowering

Average value of 81.1 with the range of minimum of 64.7 (Bindli) and maximum of 99 (Kanchana) has been found. High GCV (9.24) and PCV (12.94) have been recorded with moderate heritability (50.96 %) and moderate genetic advance per cent mean (11.14) have been noticed.

4.1.1.2.5 Days to 50% flowering

Majority genotypes flowered with mean of 91.3 days, with a range of minimum67.3 days (Bindli) and maximum of 112 days (Panvel -3). Moderate GCV (10.42) and PCV (13.35) and high heritability (60.91 %) and moderate genetic advance per cent mean (14.40) have been noticed.

4.1.1.2.6 Days to maturity

Majority of the genotypes have been found to mature in average of 135.8 days with a range of minimum 116 days (Edavankudi Pokkali) to the maximum of 154 days (Shalimar Rice -1). Low GCV (5.74) and PCV

(8.25) have been noticed with moderate heritability (48.48 %) and low GA as per cent mean (6.67) have been observed for this trait.

4.1.1.2.7 Grain yield per plant (g)

Average yield of 6 grams per plant which ranged from minimum of 0.1 g (130) to 18.8 g (36). High GCV (53.08) and PCV (57.95), high heritability (83.90 %) and high GA as per cent mean (94.63) has been noticed for yield character.

4.1.1.2.8 Biomass per plant (g)

Average biomass of 28.5 grams per plant which ranged from minimum of 16.8 g (China 1007) to 49.8 g (TKM-9). High GCV (43.06) and PCV (49.13), high heritability (76.81 %) and high GA as per cent mean (38.31) has been noticed for biomass character.

4.3 Correlation studies among growth and yield components under aerobic condition

An attempt was made to understand the association among growth traits, yield traits and drought resistant traits through correlation under aerobic situation. The results of the correlation analysis are presented in Table 5.

A significant and positive correlation has been observed between Days to initial flowering and days to 50% flowering, days to maturity; Days to 50% flowering and days to maturity; Plant height and biomass, grain yield; Number of tillers and number of productive tillers, biomass, grain yield; Number of productive tillers and biomass, grain yield; Biomass and grain yield.

A significant and negative correlation had been noticed between Days to initial flowering and Plant height, number of tillers and number

Table 5: Phenotypic correlation coefficient among ten quantitative traits studied in super-elite rice accessions evaluated under aerobic condition

	DFF	DAM	PHT	NT	NPT	BM	GY
DF	0.820**	0.582**	-0.124	-0.059	-0.097	0.117	-0.084
DFF	1	0.589**	-0.060	-0.034	-0.055	0.147	-0.023
DAM		1	-0.079	-0.177	-0.137	0.139	-0.142
PHT			1	-0.164	-0.020	0.249*	0.273*
NT				1	0.816**	0.494**	0.491**
NPT					1	0.558**	0.547**
BM						1	0.654**

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
GY= Grain yield per plant (g)
DF= Days to initial flowering
DFF= Days to 50% flowering
DAM= Days to maturity

of productive tillers, grain yield; Days to 50% flowering and Plant height, number of tillers and number of productive tillers, grain yield; Days to maturity and Plant height, number of tillers and number of productive tillers, grain yield; Plant height and number of tillers and number of productive tillers.

4.2 Root experiment –*Kharif* 2012

4.2.1 Experimental site

The study was carried out at Department of Plant Biotechnology, UAS, GKVK, Bengaluru under aerobic condition during *Kharif*-2012 in a randomized complete block design (RCBD) with three replications in aerobic conditions (Plate 3). In this study, root sampling 56 genotypes (Table 1) were done. The results obtained in this experiment are presented below.

4.2.2 Variability and genetic parameters estimated for growth, root characters under aerobic condition

4.2.2.1 Analysis of variance

Analysis of variance for yield traits and its related traits of super elite accessions of rice under aerobic condition is presented in Table 6. As evident from Table, the analysis of variance indicated significant differences among the mean of different genotypes for, Maximum root length, total root number, total root volume, total root dry weight and total root wet weight.

4.2.2.2 Variability and genetic parameters estimated for drought resistance and root characters under aerobic conditions

The genetic variability parameters viz., minimum, maximum, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h2), genetic advance and

Table 6: Analysis of variance for root and its associated traits in super-elite rice accessions under aerobic condition during *Kharif*-2012

Sl. No.	Source of variation	Df	MSS									
			PHT	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
1	Replication	2	596.41**	8.73**	10.88**	47.77**	6.86**	276.47**	9.35**	21.37**	32.34**	30.71**
2	Treatment	57	444.40**	16.45**	12.48**	97.93**	10.92**	267.01**	25.34**	3840.40**	257.37**	52.50**
3	Error	114	273.37	4.58	6.12	18.68	4.07	132.89	12.76	521.90	62.39	19.47
4	SEm		9.55	1.24	1.43	2.50	1.17	6.66	2.06	13.19	4.56	2.55
5	CD at 1%		35.91	4.65	5.37	9.39	4.38	25.04	7.76	49.62	17.16	9.58
6	CD at 5%		27.00	3.50	4.04	7.06	3.30	18.83	5.83	37.31	12.90	7.21

* Significant at 5%
** Significant at 1%
PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
GY= Grain yield per plant (g)
RDW= Root dry weight
RWW= Root wet weight
RL = Root length (cm)
NOR=Number of root
TRV = Total root volume (ml)

Table 7: Estimates of genetic parameters for different quantitative traits among super-elite rice accessions for root morphological studies under aerobic condition during *Kharif*-2012

Sl No.	Character	Minimum	Maximum	Mean	PCV	GCV	H^2%	GAPM
1	Plant height (cm)	41.3	95	67.6	27.9	11.59	17.26	7.64
2	Number of tillers	6.7	15	10	29.36	19.98	46.32	28
3	Number of productive tillers	4.3	13	7.8	37.15	18.84	25.72	19.6
4	Biomass (g)	9.3	31.3	17.8	37.78	28.91	58.57	45.6
5	Grain yield (g)	1.5	10.3	5.3	47.25	28.31	35.89	35
6	Root length (cm)	30.9	75	50.3	26.51	13.3	25.17	13.8
7	Root dry weight (g)	9.7	22.9	18.2	22.61	11.24	24.72	11.5
8	Number of root	30	195	74.7	53.99	44.51	67.94	75.2
9	Total root volume (ml)	8.4	58	24.4	46.05	32.89	51.02	48.2
10	Root wet weight (g)	20.2	46.9	32.4	17.07	10.26	36.12	12.7

GCV = Genotypic coefficient of variation
PCV = Phenotypic coefficient of variation
H^2% = Heritability percentage in broad sense

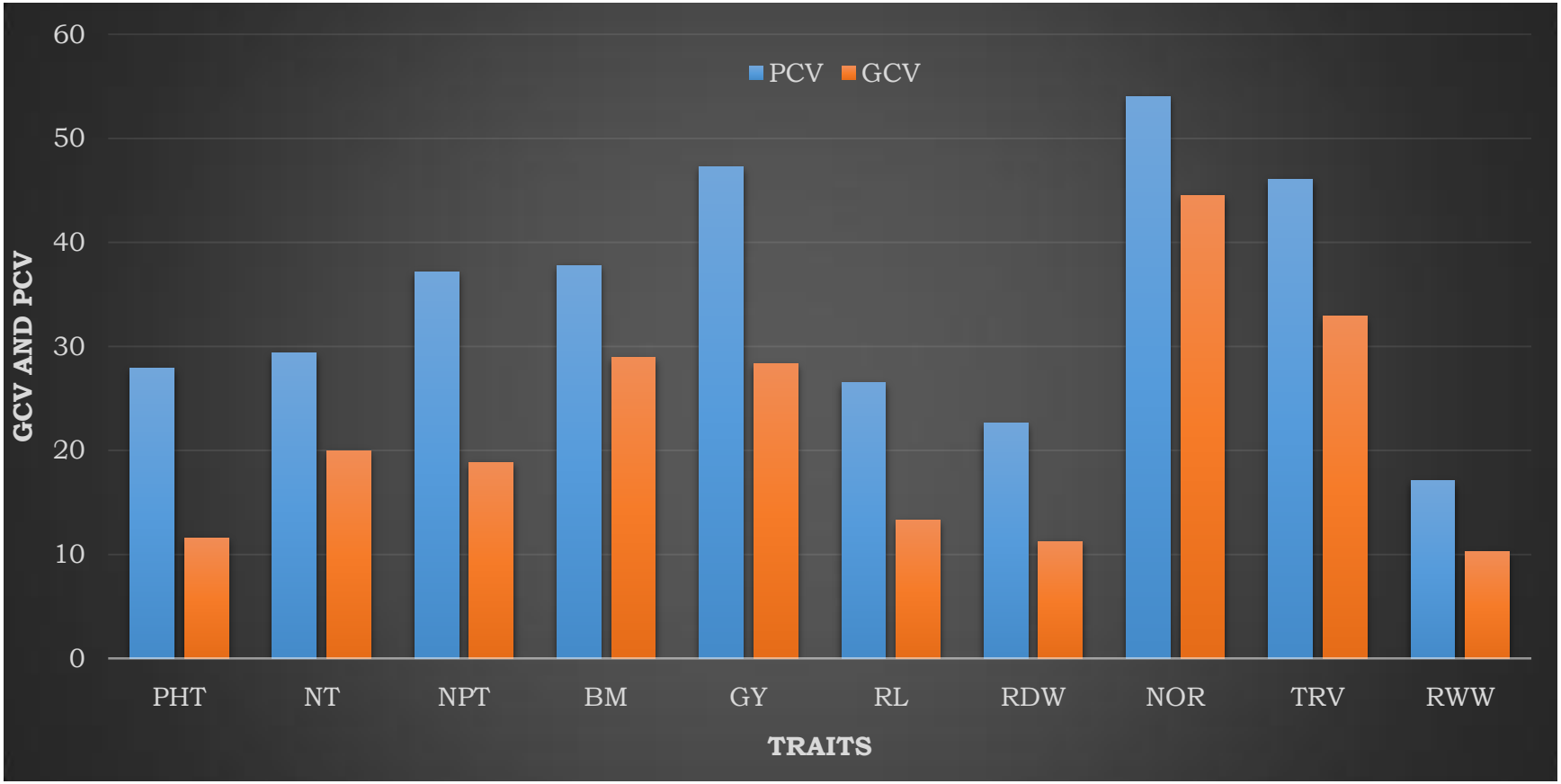


Fig. 3: Genotypic and phenotypic co-efficient of variability for root morphological traits among 56 super- elite rice genotypes evaluated in *Kharif*-2012 under aerobic condition

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
GY= Grain yield per plant (g)
RDW= Root dry weight
RWW= Root wet weight
RL = Root length (cm)

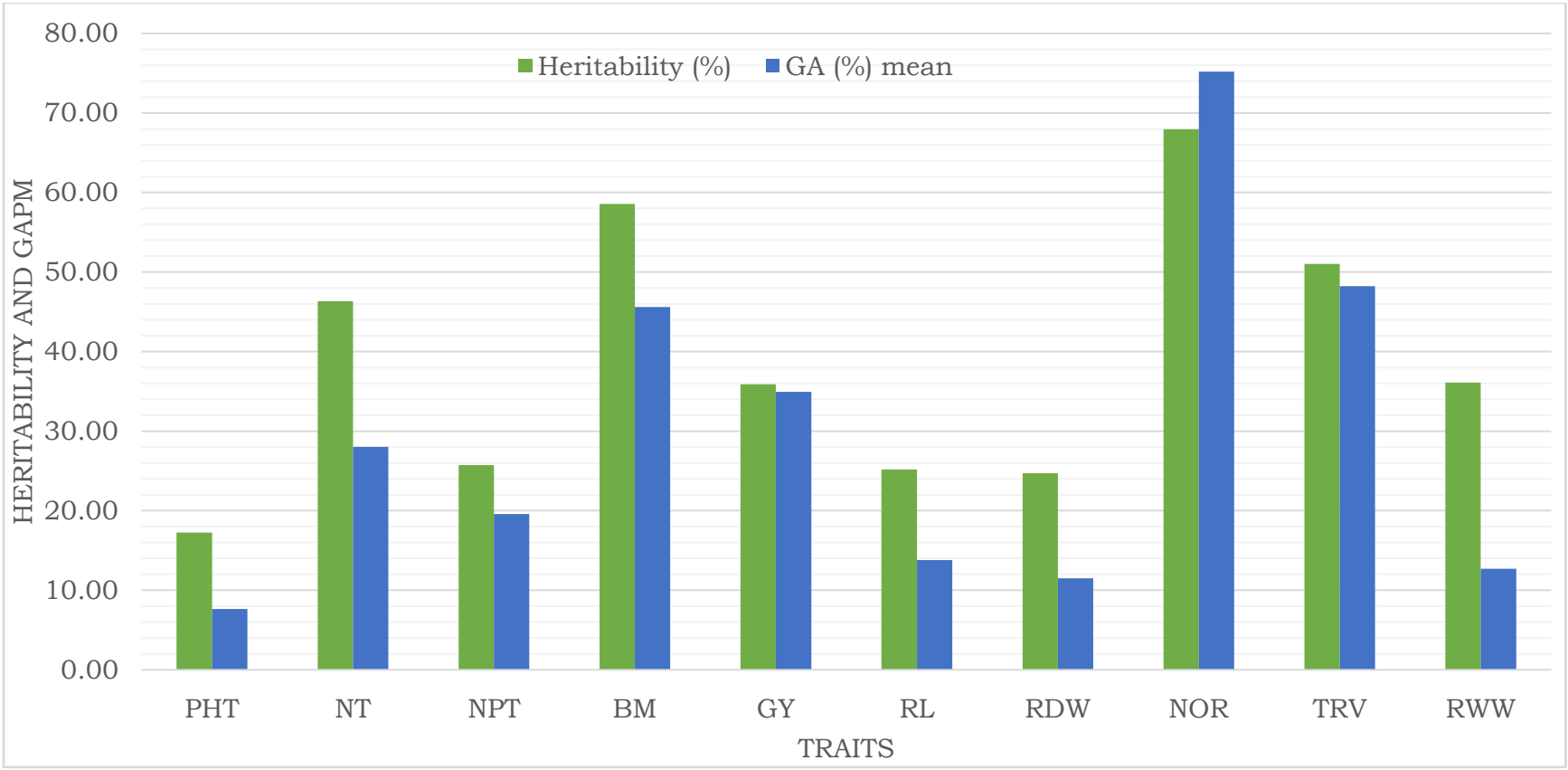


Fig. 4: Heritability % and genetic advance as percent mean for root morphological traits among 56 super-elite rice genotypes evaluated in *Kharif*-2012 under aerobic condition

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
GY= Grain yield per plant (g)
RDW= Root dry weight
RWW= Root wet weight
RL = Root length (cm)

genetic advance as per cent mean for the traits under aerobic conditions are presented in the Table 7. And graphical representation of GCV and PCV, and Heritability and genetic advance as percent mean is shown in figure 3 and 4 respectively.

4.2.2.2.1 Maximum root length (cm)

Average value of 50.3 cm with range of 30.9 cm (Jhelum) to 75 cm (Kamad) was observed for this trait under aerobic condition as shown in plate 7 and 8 respectively. Moderate GCV of (13.30) and PCV high (26.51) and low heritability (25.17 %) and moderate GA as per cent mean (13.79) were recorded for this trait (Plate 4).

4.2.2.2.2 Total root number

Average value of 74.7 with minimum of 30 (Jhelum) and maximum of 195(AM-FS-F7-57) roots were recorded under aerobic condition. High GCV (44.51), PCV (53.99), high heritability (67.94 %) and high GA as percent mean (75.19) have been observed for this trait.

4.2.2.2.3 Total root volume

Average value of 24.4 cm with the range of minimum of 8.4 (Shalimar Rice -1) and maximum of 58.57 (AM-FS-F7-57). High GCV (32.89) and PCV (46.05) have been recorded with moderate heritability (51.02 %) and high GA as percent mean (48.24) have been noticed for this character under aerobic condition.

4.2.2.2.4 Total root dry weight (g)

Average value of 18.2 grams with minimum of 9.7 (233(K)) and maximum of 22.9 (182(M)) were observed for this trait under aerobic condition, with moderate GCV (11.24) and high PCV (22.61) have been recorded with low heritability (24.72 %) and moderate GA as percent

Plate 3: Fifty six rice genotypes in PVC pipes for evaluation of root morphology and its associated traits at maturity stage under aerobic condition during *Kharif*-2012

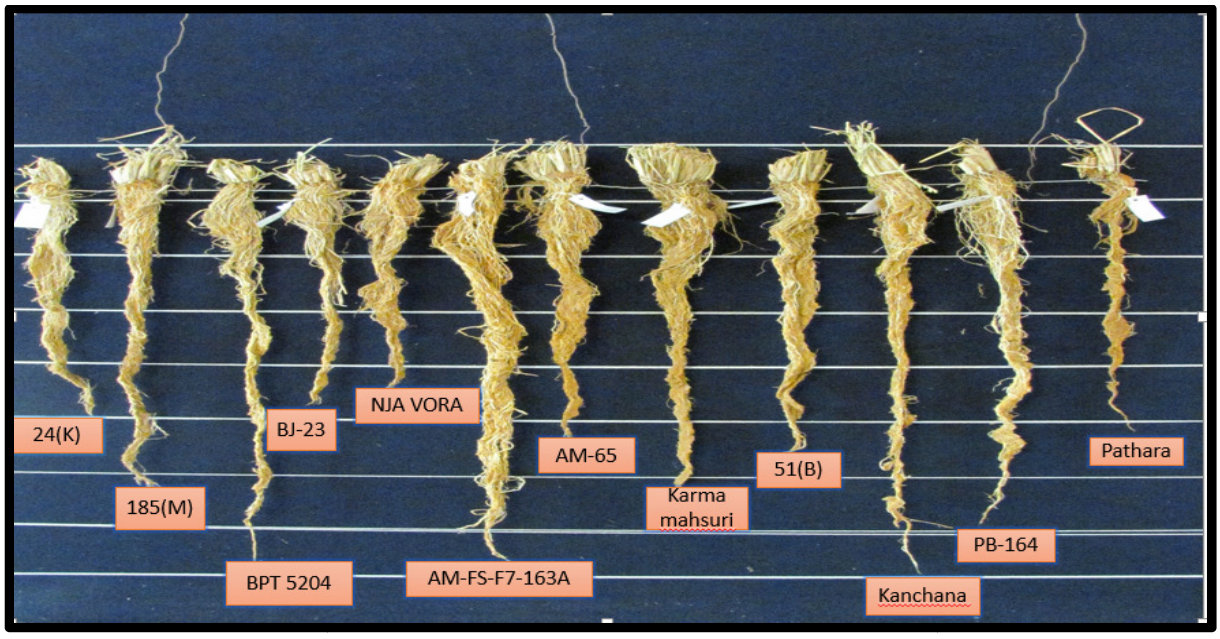


Plate 4: Different rice genotypes showing variation in root length grown in PVC pipes for evaluation of root morphology and its associated traits under aerobic condition during *Kharif*-2012.

mean (11.48) have been noticed for this plant character under aerobic condition.

4.2.2.2.5 Total root wet weight (g)

Average value of 32.4 grams with minimum of 20.2 (233(K)) and maximum of 46.9 (Sampada) were observed for this trait under aerobic condition, with moderate GCV (10.26) and PCV (17.07) have been recorded with moderate heritability (36.11 %) and moderate GA as percent mean (12.70) have been noticed for this plant character under aerobic condition.

4.1.2.2.6 Plant height (cm)

Average value of 67.6 cm with range of 41.3 cm (Mainpuri) to 95 cm (196(M)) was observed for this trait under aerobic condition. Moderate GCV (11.59) and high PCV (27.9) and low heritability (17.25%) and low GA as percent mean (7.64) have been observed for this trait.

4.1.2.2.7 Number of tillers

Average value of 10 with minimum of 6.7 (Mainpuri) and maximum of 15 (Taroari Basmati) tillers were recorded under aerobic condition. Moderate GCV (19.98), high PCV (29.36) and moderate heritability (46.32 %) and high GA as percent Mean (28.02) have been observed for this trait.

4.1.2.2.8 Number of productive tillers

Average value of 7.8 with the range of minimum of 4.3 (High Iron Rice) and maximum of 13 (AMFSF7-183B) has been found. Moderate GCV (18.84) and high PCV (31.15) have been recorded with low heritability (25.71 %) and moderate GA as percent mean (19.60) have been noticed.

4.1.2.2.9 Grain yield per plant (g)

Average yield of 6 grams per plant which ranged from minimum of 0.1 g (China 1007) to 18.8 g (TKM-9). High GCV (53.08) and PCV (57.95), moderate heritability (35.89 %) and high GA as percent mean (34.95) has been noticed for yield character.

4.1.2.2.10 Biomass per plant (g)

Average biomass of 17.8 grams per plant which ranged from minimum of 9.3 g (Azucena) to 31.3 g (AM-72). High GCV (28.91) and PCV (37.78), moderate heritability (58.57 %) and high GA as percent mean (45.59) has been noticed for biomass character.

4.2.3 Correlation studies among growth, yield and drought resistant components under aerobic condition

An attempt was made to understand the association among growth traits, yield traits and drought resistant traits through correlation under aerobic situation. The results of the correlation analysis are presented in Table 8.

A significant and positive correlation has been observed between Number of tillers and number of productive tillers, biomass; Number of productive tillers and biomass; Biomass and grain yield; Grain yield and root length, root dry weight, total root volume, root wet weight; Root length and root dry weight, number of root, total root volume, root wet weight; Root dry weight and number of root, total root volume, root wet weight; Number of root and total root volume, root wet weight; Total root volume and root wet weight.

A significant and negative correlation had been noticed between Number of productive tillers and total root volume.

Table 8: Phenotypic correlation coefficient among ten quantitative traits studied in super-elite rice accessions evaluated under aerobic condition

	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
PHT	0.100	0.098	0.233	0.173	0.107	0.176	0.142	0.094	0.187
NT	1	0.665**	0.341**	0.217	0.160	0.046	0.167	0.084	0.097
NPT		1	0.409**	0.247	0.239	0.132	0.046	-0.003	0.163
BM			1	0.465**	0.258	0.226	0.086	0.146	0.075
GY				1	0.559**	0.542**	0.167	0.277*	0.335*
RL					1	0.842**	0.376**	0.404**	0.687**
RDW						1	0.396**	0.477**	0.770**
NOR							1	0.765**	0.312*
TRV								1	0.324*

PHT = Plant height (cm)
NPT = Number of Productive tillers per plant
BM = Biomass per plant (g)
NT = Number of tillers per plant
NOR = Number of root
GY= Grain yield per plant (g)
RDW= Root dry weight
RWW= Root wet weight
RL = Root length (cm)
TRV = Total root volume (ml)

4.2 Molecular characterization of super elite rice accessions for biotic loci using informative markers.

Along with complete phenotypic characterization of all 56 super elite rice accessions genotypic characterization was also performed with the help of trait specific SSR markers. Total genomic DNA was extracted by CTAB method and PCR reaction was performed and product was run on agarose gel. All the marker used are already reported in literature along with the associated traits. Obtained band size was compared with already reported band size in GRAMENE database.

4.2.2 Biotic stress markers

10 biotic stress markers have been used to screen the genotypes for genotypic characterization of all the genotypes against biotic stress resistance and susceptibility. Specific band size showing resistance against a particular biotic stress (BPL, BLAST or BPH) has been reported in literature. Plate 6 shows the resistant and susceptible genotype for candidate gene *pi2* responsible for BLAST resistance, Plate 5 shows the banding pattern of all genotype when screened with *Xa5* for BLB resistance. Genotype showing resistant band is shown in table 9.

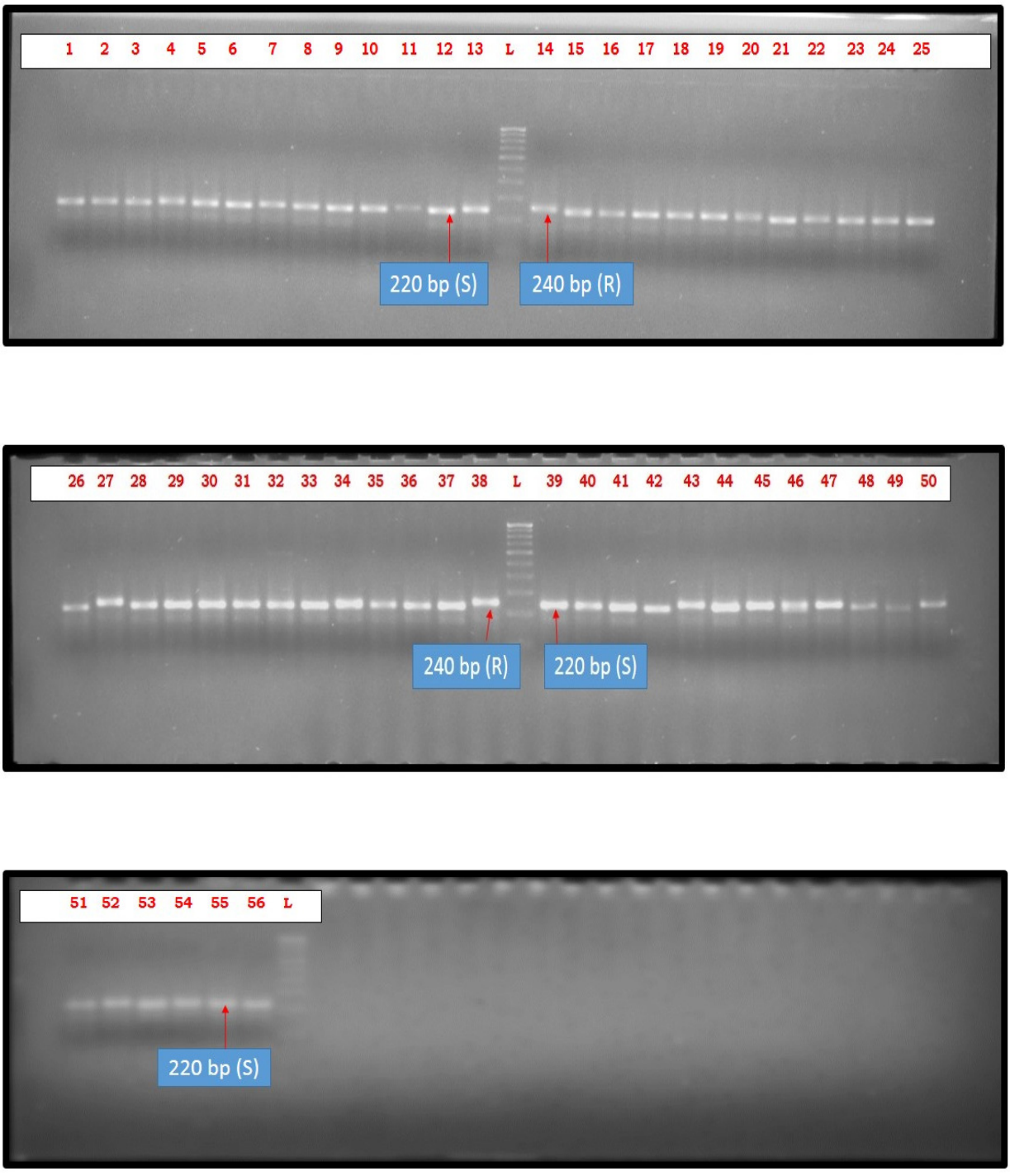
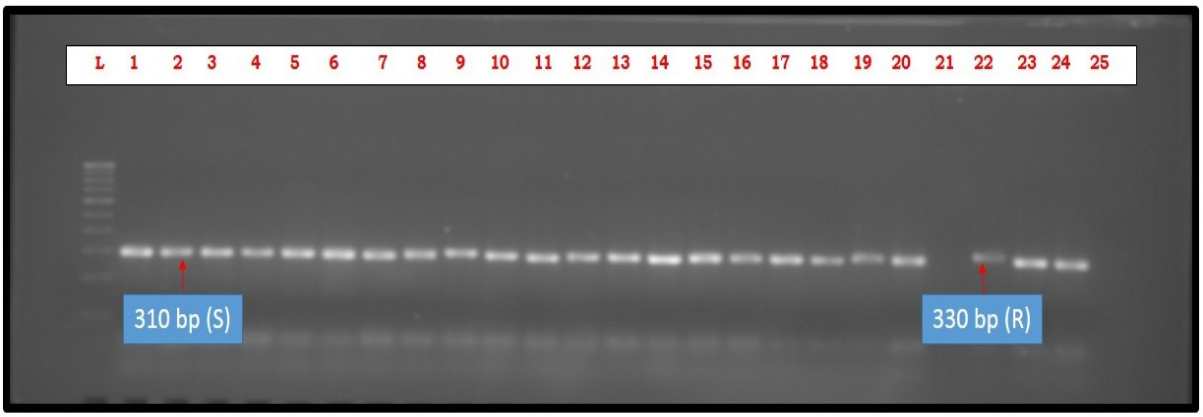


Plate 5: Scoring pattern and validation of biotic resistance marker *Xa5* associated with BLB resistance indicating polymorphism



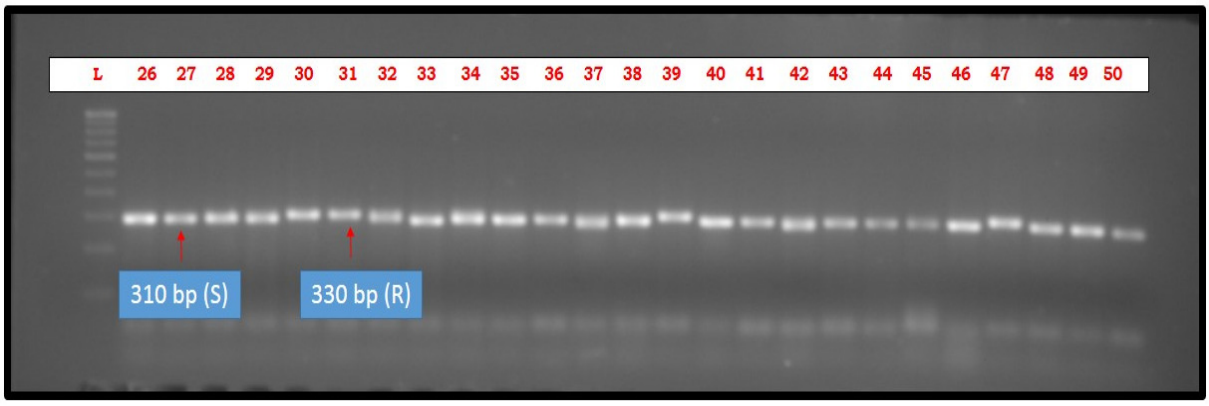


Plate 6: Scoring pattern and validation of biotic resistance candidate gene *pi2* associated with BLAST resistance indicating polymorphism

Table 9: Genotypes showing resistance to biotic stresses

Marker	Genotypes
Xa-21	RPHP-9, RPHP-10, Azucena, BJ-5, BJ-21, TKM-9, Mainpuri, Jhelum, BR- 2655, Pathara, Sebati, IRRI-44, Subhdra, 51(B), 140(M), 233(K), 185(M), Moirang phou, Akut phou, Improved Chitti Mutyalu, Nja Vora, Edavankudi Pokkali, Vytilla Anakondan, BPT 5204 /Chitti Mutyalu-MS, Champa Khushi, Seeta sail, AM-FS-F7-183B, AM-FS-F7-163A, AM-FS-F7-111, AM-FS-F7-57
Xa4	AM-143, Azucena, TKM-9, Shalimar Rice -1, Panvel -3, Pathara, Bindli, Sebati, PB-164, Taroari Basmati, Subhdra, 233(K), 140(M), 196(M), Kanchana, Kasturi, Moirang phou, Akut phou, High Iron Rice, China 1007, Nja Vora, Edavankudi Pokkali, NC 365, AM-FS-F7-111, AM-FS-F7-57
Xa-5	AM-143,Jhelum,Shalimar Rice -1,140(M),High Iron Rice,Kadamakudy Pokkali
xa-13	All 56 genotype monomorphic
Bph18	AM-65, AM-94B, Azucena, BJ-21, TKM-9, Mainpuri, Jhelum, BR- 2655, Pathara, Bindli, Sebati, IRRI-44, Taroari Basmati, Subhdra, 24(K), Champa Khushi, Seeta sail, Tilak kachari
Bph-3	BJ-23, AM-94B, Azucena, BJ-21, BR- 2655, Bindli, IRRI-44, Subhdra, 51(B), Type-3, High Iron Rice, NDR 2026, Kamad, BPT 5204 /Chitti Mutyalu-SB, Champa Khushi, Tilak kachari, AM-FS-F7-183B, AM-FS-F7-111
Bph20(t)	All 56 genotype monomorphic
Pi-1	AM-72, BJ-23, AM-27, AM-94B, BJ-21, TKM-9, Shalimar Rice -1, Jhelum, Panvel -3, Pathara, Bindli, Vandana, Sebati, PB-164, Kalinga -3, IRRI-44, Taroari Basmati, 24(K), 233(K), 51(B), 140(M), 182(M), Type-3, Pant sugandh dhan -17, Moirang phou, Improved Chitti Mutyalu, High Iron Rice, NDR 2026, Nja Vora, Kadamakudy Pokkali, AM-FS-F7-163A
sPi-2	IRRI-44, High Iron Rice, BPT 5204 /Chitti Mutyalu-SB, Champa Khushi, Tilak kachari, AM-FS-F7-163A, AM-FS-F7-111
Pik^h	AM-72, BJ-23, AM-27, AM-143, BJ-5, BJ-21, Jhelum, Shalimar Rice -1, Panvel -3, Vandana, Sebati, Kalinga -3, Subhdra, 140(M), 182(M), 185(M), Type-3, Kanchana, Pant sugandh dhan -17, Moirang phou, Akut phou, NDR 2026, Kamad, Kadamakudy Pokkali , BPT 5204 /Chitti Mutyalu-SB, Seeta sail, AM-FS-F7-163A, AM-FS-F7-111


DISCUSSION

V. DISCUSSION

The results of the present investigation are discussed separately under the following sub headings.

5.1 Variability and other genetic parameters for yield and growth parameters under aerobic condition

5.2 Variability and other genetic parameters for root growth and drought associated traits under aerobic condition

5.3 Correlation studies among growth and yield under aerobic condition

5.4 Correlation studies among growth, yield, and drought tolerance under aerobic condition

5.5 Molecular characterization of super-elite rice for biotic resistance (BPL, BLAST, BPH) using trait specific markers

5.6 Identification of superior rice accessions

5.1 Variability and other genetic parameters for yield and growth parameters under aerobic condition

Genetic variability is prerequisite for selection in any crop. The variability observed is the sum total of hereditary effects of concerned genes as well as the environmental influence. Hence the variability is partitioned into heritable and non-heritable components with suitable genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance (GA). The estimation of these genetic variability parameters helps in achieving the required crop improvement by selection.

The analysis of variance for ten quantitative traits of the genotypes showed significant difference among the means of different genotypes for all the traits under aerobic condition, thus indicating the scope for

selection. The differences in the mean performance of the genotypes under aerobic conditions can be attributed mainly to the environmental factors and interaction between genotypes and environment.

The range in the mean values reflects the extent of phenotypic variability present in the plant material. High range was noticed for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, Duration of growth stage III (days), and days to flowering.

High phenotypic coefficient of variability was observed for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, biomass per plant, days to initial flowering, whereas high genotypic coefficient of variability was observed for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, biomass per plant, days to initial flowering under aerobic conditions. Moderate phenotypic coefficient of variability and genotypic coefficient of variability was observed for days to 50% flowering. Low phenotypic coefficient of variability and genotypic coefficient of variability was observed for days to maturity. Thanh *et al.*, (1999) and Kondo *et al.*, (2003) noticed large genotypic variation for root traits among upland varieties.

The heritability of a trait within a population is the proportion of observable differences in a trait between individuals within a population that is due to genetic differences. Factors including genetics, environment and random chance can all contribute to the variation between individuals in their observable characteristics. Heritability thus analyzes the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. Heritability is an important concept in quantitative genetics, particularly

in selective breeding. However, heritability values with genetic advance give better response in selection program.

High heritability was observed for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, biomass per plant and days to 50% flowering. Whereas moderate heritability was observed for days to initial flowering and days to maturity. Armenta-Soto *et al*, (1983) reported higher narrow-sense heritability estimates for root diameter (62%), root length (60%), and root number (44%).

Genetic advance as per cent mean was high for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, biomass per plant. Moderate Genetic advance as per cent mean was noticed for days to 50% flowering, days to initial flowering whereas low Genetic advance as per cent mean was noticed for days to maturity.

5.2 Variability and other genetic parameters for root growth and drought associated traits under aerobic condition

The results of analysis of variance show that variability was significant for all ten traits. High phenotypic coefficient of variability was observed for plant height, number of tillers per plant, number of productive tillers per plant, grain yield per plant, biomass per plant, root length, number of roots and total root volume whereas moderate phenotypic coefficient of variability was observed for root wet weight. High genotypic coefficient of variability was observed for number of roots, total root volume, grain yield per plant, biomass per plant whereas moderate genotypic coefficient of variability was observed for plant height, number of tillers per plant, number of productive tillers per plant, root length, root wet weight.

In present study heritability in broad sense was calculated. Moderate heritability was observed for total root volume, root wet weight, grain yield per plant, biomass per plant and number of tillers per plant whereas high heritability was observed for number of roots. High Genetic advance as per cent mean was observed for grain yield per plant, biomass per plant and number of tillers per plant, total root volume, number of roots whereas moderate Genetic advance as per cent mean was observed for root length, number of productive tillers per plant, root wet weight indicating the suitability for improvement through selection.

5.3 Correlation studies among growth and yield under aerobic condition

In the present experiment a significant and positive correlation has been observed between Days to initial flowering and days to 50% flowering, days to maturity; Days to 50% flowering and days to maturity; Plant height and biomass, grain yield; Number of tillers and number of productive tillers, biomass, grain yield; Number of productive tillers and biomass, grain yield; Biomass and grain yield.

A significant and negative correlation had been noticed between Days to initial flowering and Plant height, number of tillers and number of productive tillers, grain yield; Days to 50% flowering and Plant height, number of tillers and number of productive tillers, grain yield; Days to maturity and Plant height, number of tillers and number of productive tillers, grain yield; Plant height and number of tillers and number of productive tillers.

5.4 Correlation studies among growth, yield and drought tolerance under aerobic condition

Correlation between various plant characters arises because of linkage, pleiotropy or developmentally included functional relationship.

To assess the extent and nature of association between yield, drought and their contributing components, knowledge of interaction of characters among themselves is very essential. In the present investigation, phenotypic correlation was worked out for ten quantitative traits under aerobic condition.

A significant and positive correlation has been observed between Number of tillers and number of productive tillers, biomass; Number of productive tillers and biomass; Biomass and grain yield; Grain yield and root length, root dry weight, total root volume, root wet weight; Root length and root dry weight, number of root, total root volume, root wet weight; Root dry weight and number of root, total root volume, root wet weight; Number of root and total root volume, root wet weight; Total root volume and root wet weight.

A significant and negative correlation had been noticed between Number of productive tillers and total root volume. In upland conditions, Mumbani and Lal, 1983 reported a significant positive correlation between deep root growth and grain yield, and they clearly demonstrated that deep roots had a role in water uptake.

5.5 Molecular characterization of super-elite rice for biotic resistance (BPL, BLAST, BPH) using trait specific markers

In the present study all the 56 genotypes were screened using trait specific biotic resistance markers and it was found that out of 10 markers, 8 markers were polymorphic. Resistant and susceptible band size were extracted from the already established literature and genotype sowing resistance to particular disease was noted based on the band size.

It was found that out of 56 genotype 25 showed resistant band for *Xa21* imparting resistance against BLB out of those 25 genotypes 14

were also found to have resistant band for *Xa4* marker and 2 genotypes have resistant band for *Xa5* marker. Thus it can be inferred that those 14 genotype contain two region imparting resistance against BLB. 2 genotypes was found to contain resistance band for all three markers. Currently, about 30 resistance genes or loci against to *Xoo* have been identified in cultivated and wild rice. So far, five dominant R genes, *Xa-21, Xa-1, Xa-26, Xa-27* and *Xa-3* and two recessive R genes, *xa-5* and *xa-13*, have been isolated by map-based cloning (Lang *et al.*, 2008).

Similarly candidate gene *Pi1* for BLAST resistance was found polymorphic and 31 genotypes showed resistant band size when screened with *Pi1* marker. Since, it is a candidate gene we can directly say that those 31 genotypes have an inbuilt genotypic ability to confer resistance against blast causing pathogen. 25 genotypes genotypes showed resistant band size when screen with Pik^h marker and 5 genotypes showed resistant band size when screen with *Pi2* marker. AM-FS-F7-163A was found to contain resistance band for all three markers against blast. Zhai *et al.*, 2010 reported that rice blast resistance gene *Pik,* which is one of the five classical alleles located at the Pik locus on the long arm of chromosome 11, confers high and stable resistance to many Chinese rice blast isolates.

Similarly marker *Bph18* for brown plant hopper resistance was found polymorphic and 18 genotypes showed resistant band size when screened with *Bph3* marker and 17 genotypes showed resistant band size when screened with *Bph18* marker.

5.6 Identification of superior rice accessions

The superior genotypes Seeta sail, Champa Khushi, AM-FS-F7-163A, IRRI-44, AM-94B, NDR 2026, High Iron Rice, 196(M), 182(M) and Vandana were selected based on high yield and maximum root length under aerobic condition during *Kharif*-2011. The graphical comparison among superior genotypes for yield and drought resistance character is given in figure 5.


SUMMARY

VI. SUMMARY

Rice is an important cereal food crop feeding more than one half of the world's population. Over 90% of rice is produced and consumed in Asia. It accounts for more than 65% of caloric intake providing 23% of global human per capita energy and 16% of per capita protein.

Drought is a major abiotic stress, affecting 20% of the total rice-growing area in Asia. The annual losses due to shortage of water are estimated to be of 134 kg ha-1 in Asia alone. Roots are the principal plant organ for nutrient and water uptake. Therefore, improving our understandings on interactions between root function and drought tolerance in rice could have a significant impact on global food security. Developing drought tolerant genotypes in rice can allow rice to be grown on marginal soils with limited water supply accompanied with maximum productivity.

In field all the genotypes were screened for number of characters and detailed root study was conducted during Kharif-2012. Correlation was determined. Not only root traits, same wise other traits have also been mentioned in this study viz., days to 50% flowering, days to maturity, plant height, number of tillers and number of productive tillers. These can also be used for further breeding improvements.

The results of the analysis of variance of evaluation of genotypes under aerobic situation indicated significant difference among the means of different genotypes for all the traits under aerobic condition. High to low heritability and GA as percent mean was noticed for various root and shoot characters.

A significant and positive correlation has been observed between Number of tillers and number of productive tillers, biomass; Number of

productive tillers and biomass; Biomass and grain yield; Grain yield and root length, root dry weight, total root volume, root wet weight; Root length and root dry weight, number of root, total root volume, root wet weight; Root dry weight and number of root, total root volume, root wet weight; Number of root and total root volume, root wet weight; Total root volume and root wet weight.

Genotypic study was done using SSR markers, biotic resistance markers and candidate genes. These polymorphic banding pattern is highlighted in the results. As shown by biotic resistance markers many genotypes are resistant to biotic stresses BLAST, BPH and BLB. These findings reflects that gene pool is rich in biotic resistance gene and knowledge of the resistance gene containing genotype can be a great help in breeding program.

Finally, the main aim of the investigation was detailed characterization of all genotype at phenotypic and genotypic level. All the 56 genotypes were having highly diverse genotypic composition showing capacity to confer biotic resistance to the plant. The genotypes Seeta sail, Champa Khushi, AM-FS-F7-163A, IRRI-44, AM-94B, NDR 2026, High Iron Rice, 196(M), 182(M) and Vandana were found to be superior for yield and drought resistance characters under aerobic condition during *Kharif* –2012. All this information can be further utilized for research and development work.

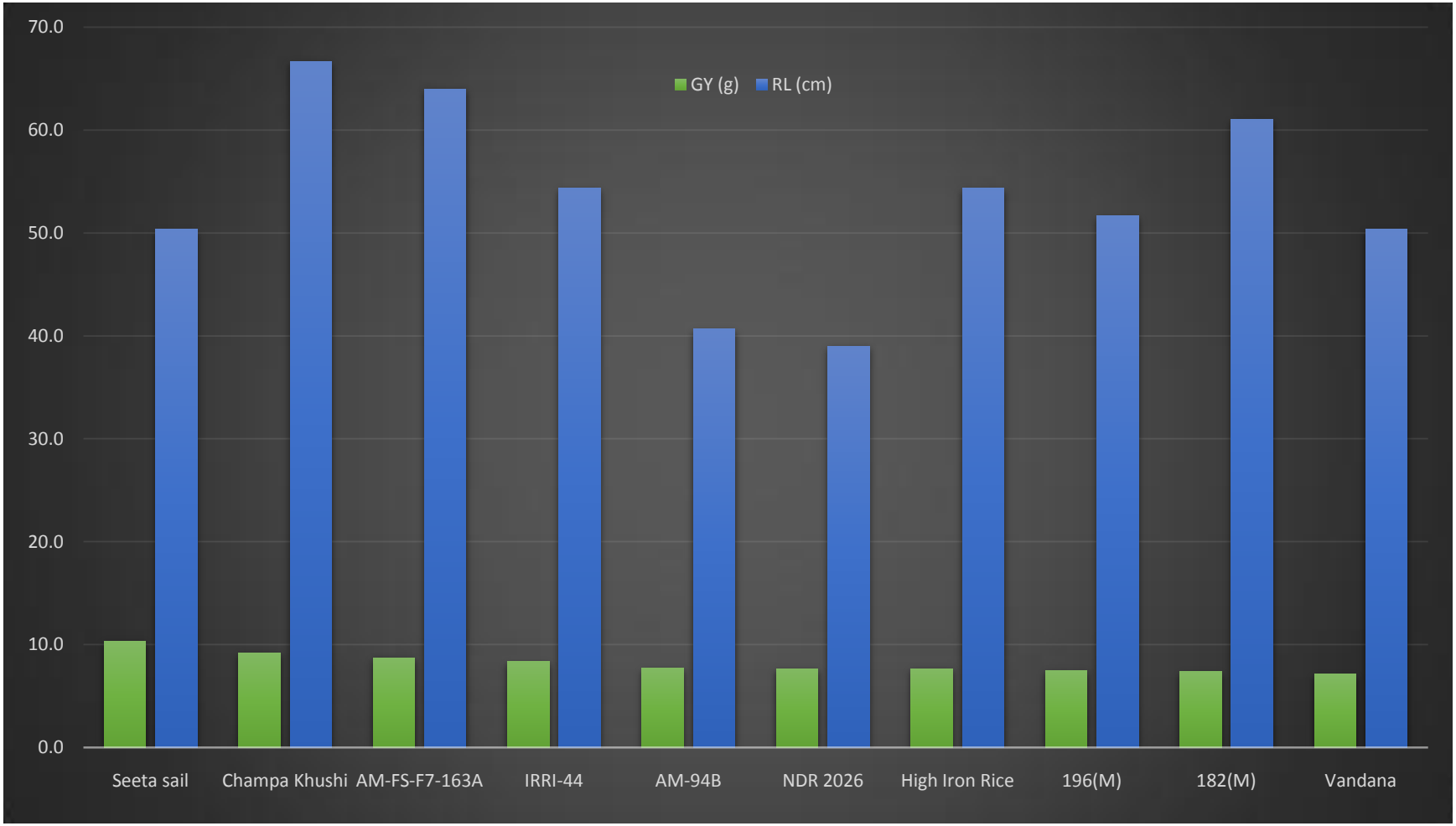


Fig. 5: Comparison among superior rice genotypes for yield and root length, selected from Kharif-2012 under aerobic condition

GY = Grain yield per plant (g) RL = Maximum root length (cm)

FUTURE LINE OF WORK

Combined results obtained from both the study reflected that genotype used in study was having high level of heritable variability in terms of root traits, yield, disease resistance and all genotypes were found to perform well under stress condition. All this information can be further utilized for research and development work. So as stated above that existing variability in rice is a great source of alleles related to biotic stress resistance and can be exploited for development of new disease resistant varieties of rice.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APPENDICES

Appendix : Mean value of ten quantitative traits observed in 56 rice genotypes for root and its associated traits evaluated under aerobic condition during Kharif-2012

Sl. No.	Genotype Name	PHT	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
1	AM-72	67.0	11.0	9.7	31.3	6.7	48.7	18.7	66.0	16.5	27.7
2	BJ-23	73.5	10.7	9.0	18.0	5.0	47.3	22.7	126.7	42.3	36.3
3	AM-27	68.0	7.7	6.3	16.7	6.7	62.3	20.7	60.0	16.7	32.0
4	AM-143	71.7	8.7	7.0	17.7	6.9	45.0	17.7	66.7	23.2	34.3
5	AM-65	90.3	8.0	6.0	20.7	3.4	55.0	22.3	95.0	26.2	35.3
6	AM-94B	52.7	10.0	8.3	18.0	7.7	40.7	19.0	41.3	15.0	32.0
7	Azucena	80.0	8.3	6.7	9.3	2.6	52.7	20.0	54.3	19.2	38.0
8	BJ-5	67.7	9.0	8.0	13.0	5.1	41.7	15.7	49.0	15.8	30.7
9	BJ-21	86.3	8.3	7.0	13.3	2.5	38.0	15.7	62.3	18.8	34.7
10	TKM-9	63.3	7.0	4.7	19.7	5.6	54.3	21.7	73.7	30.7	35.4
11	Mainpuri	52.0	6.7	4.7	12.0	1.8	30.9	10.9	30.0	10.1	22.0
12	Jhelum	41.3	7.0	5.3	15.0	3.8	46.3	14.4	42.7	17.3	28.5
13	Shalimar Rice -1	61.7	8.3	6.0	21.0	3.8	40.0	14.2	33.3	8.4	28.0
14	BR- 2655	73.0	9.7	5.7	11.7	3.2	48.3	18.2	52.7	20.5	32.3
15	Panvel -3	65.0	9.0	8.0	19.0	5.5	46.0	15.4	47.0	47.0	30.0
16	Pathara	55.0	10.0	9.3	14.7	4.4	44.3	17.2	71.0	17.7	28.7
17	Bindli	67.0	7.3	7.3	11.0	3.2	44.0	18.6	74.3	21.5	34.7
18	Vandana	68.7	9.3	8.3	23.3	7.1	50.3	19.5	50.7	25.7	39.0

Sl. No.	Genotype Name	PHT	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
19	Sebati	63.0	11.0	8.0	12.3	4.8	40.7	14.6	107.0	31.0	30.0
20	PB-164	78.7	8.0	6.7	13.3	4.0	57.7	21.0	78.0	30.7	40.0
21	Kalinga -3	70.7	14.3	10.3	28.3	6.7	55.0	19.6	87.0	30.0	34.2
22	IRRI-44	65.3	14.0	10.7	25.3	8.3	54.3	18.1	87.3	22.0	29.3
23	Taroari Basmati	85.0	15.0	12.7	25.3	5.0	45.7	15.8	116.0	27.3	28.7
24	Subhdra	63.3	9.7	8.7	29.0	4.7	48.3	18.7	76.7	33.7	30.8
25	24(K)	78.5	8.0	8.3	25.3	6.5	47.3	17.5	74.0	25.1	30.6
26	233(K)	47.8	8.0	5.3	13.3	1.5	34.3	9.7	34.0	10.9	20.2
27	51(B)	68.0	9.7	5.3	15.0	5.5	42.7	17.4	110.7	34.7	33.2
28	140(M)	53.3	11.7	8.0	17.0	5.2	56.0	22.0	141.3	37.5	34.2
29	182(M)	65.7	11.0	10.0	20.0	7.4	61.0	22.9	49.0	22.3	35.7
30	185(M)	84.3	10.3	6.7	22.7	5.2	51.0	17.6	75.7	30.4	30.3
31	196(M)	95.0	12.0	7.3	28.0	7.5	51.7	17.1	62.7	19.7	35.3
32	Type-3	85.3	8.3	6.0	22.7	6.3	47.3	17.1	74.0	19.7	29.3
33	Kanchana	81.7	9.0	6.7	15.7	6.8	51.7	22.7	92.7	44.0	34.3
34	Pant sugandh dhan -17	78.5	9.3	7.0	24.3	2.5	37.7	14.5	47.7	23.5	26.3
35	Kasturi	53.7	11.7	6.7	11.7	6.3	38.3	16.8	54.3	18.3	29.4
36	Moirang phou	61.0	11.7	8.0	14.3	4.0	36.3	15.4	41.7	13.9	29.8
37	Akut phou	72.0	7.3	6.3	13.3	5.2	50.7	18.8	124.3	24.3	31.3
38	Improved Chitti Mutyalu	47.7	7.3	6.0	10.0	2.6	33.3	13.6	48.3	15.2	28.4

Sl. No.	Genotype Name	PHT	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
39	High Iron Rice	65.0	6.7	4.3	14.7	7.7	54.3	18.9	86.7	33.0	31.1
40	NDR 2026	48.0	9.3	6.7	16.3	7.7	39.0	16.2	58.0	28.8	28.6
41	Kamad	58.3	11.0	10.0	24.3	5.8	75.0	22.8	65.3	28.0	35.7
42	China 1007	73.0	7.3	6.3	13.7	5.9	53.0	20.2	35.3	13.7	33.4
43	Nja Vora	73.7	6.7	5.7	11.0	4.4	63.0	20.4	118.0	40.7	32.9
44	Kadamakudy pokkali	82.5	9.3	10.0	16.0	3.7	45.0	16.9	46.7	19.3	30.0
45	Edavankudi Pokkali	58.5	9.3	6.3	13.0	5.8	53.3	18.7	81.3	32.2	32.0
46	Vytilla Anakondan	59.5	13.0	9.0	12.3	3.6	42.7	15.5	50.7	22.3	28.6
47	BPT 5204 /Chitti Mutyalu-SB	71.0	10.3	9.0	13.0	2.5	45.7	15.6	44.7	10.3	28.9
48	Radhuni Pagal	61.0	8.3	8.0	12.7	6.2	56.3	19.5	54.0	13.1	32.7
49	Champa Khushi	60.3	8.7	9.0	28.0	9.2	66.7	22.2	46.7	22.0	37.6
50	Seeta sail	73.3	13.3	8.0	16.7	10.3	50.3	18.8	57.3	22.3	33.6
51	Tilak kachari	73.3	12.3	4.3	13.7	6.3	59.7	20.2	100.7	30.0	32.7
52	NC 365	78.5	8.3	8.0	13.3	6.0	54.0	17.7	48.3	24.3	34.6
53	AM-FS-F7-183B	71.7	14.3	13.0	26.7	6.0	54.7	17.4	45.3	19.7	33.6
54	AM-FS-F7-163A	78.0	11.7	9.3	24.0	8.7	64.0	21.4	169.3	32.7	35.0
55	AM-FS-F7-111	58.7	14.3	10.0	21.3	5.6	54.3	19.1	120.0	34.0	32.3
56	AM-FS-F7-57	57.3	8.3	6.7	15.7	4.8	61.7	20.9	195.0	58.0	36.7

Appendix : Mean value of eight quantitative traits observed in 56 rice genotypes for yield and its associated traits evaluated under aerobic condition during Kharif-2012

Sl. No.	Genotype Name	DF	DFF	DAM	PHT	NT	NPT	BM	GY
1	AM-72	83.0	90.3	130.0	57.1	19.1	16.2	48.1	10.4
2	BJ-23	82.5	88.2	142.7	60.9	14.1	11.4	30.7	3.1
3	AM-27	73.0	93.0	136.0	61.7	14.7	11.8	28.6	6.8
4	AM-143	80.0	98.0	135.0	58.7	14.5	11.3	29.2	10.5
5	AM-65	83.0	97.0	141.7	96.3	8.9	6.9	47.8	10.0
6	AM-94B	78.3	87.0	139.0	47.1	14.0	15.5	31.7	5.4
7	Azucena	89.0	102.0	139.3	97.6	5.7	5.0	24.3	6.5
8	BJ-5	73.0	78.7	125.7	46.9	15.1	13.0	24.6	5.9
9	BJ-21	72.0	78.7	135.7	63.3	11.1	8.7	33.8	5.9
10	TKM-9	80.7	87.3	135.0	53.7	31.3	20.6	53.9	18.8
11	Mainpuri	83.7	87.0	134.3	43.2	14.3	13.5	16.8	6.5
12	Jhelum	80.3	85.3	122.7	47.8	14.3	11.3	27.8	5.4
13	Shalimar Rice -1	95.7	108.0	154.0	64.8	8.4	6.6	29.8	5.5
14	BR- 2655	91.3	109.0	143.0	48.5	19.5	12.7	25.7	5.0
15	Panvel -3	96.0	112.0	141.7	52.6	12.5	8.9	22.2	3.7
16	Pathara	70.3	76.0	131.0	53.7	16.9	13.3	23.3	6.6
17	Bindli	64.7	67.3	126.3	57.8	10.5	7.1	14.2	2.4
18	Vandana	65.0	74.3	120.0	60.8	17.4	10.6	20.6	6.1
19	Sebati	74.0	80.7	127.7	63.9	12.1	10.5	21.7	10.6
20	PB-164	80.0	104.3	144.0	66.0	12.3	10.9	21.5	7.3
21	Kalinga -3	68.7	76.3	130.3	76.8	12.9	10.7	23.4	8.4
22	IRRI-44	81.3	100.7	140.0	46.4	14.5	11.0	21.5	3.1
23	Taroari Basmati	80.7	90.5	144.0	64.9	7.5	6.3	11.2	1.3
24	Subhdra	69.3	79.7	133.7	49.2	11.5	8.4	11.3	2.9
25	24(K)	89.7	101.3	143.7	63.4	8.3	6.7	11.1	1.7
26	233(K)	71.0	80.0	122.7	55.0	12.7	9.7	29.7	3.4
27	51(B)	96.7	101.0	152.5	42.6	9.5	8.5	18.7	2.8
28	140(M)	92.3	103.3	151.0	70.0	11.3	10.3	41.2	4.9
29	182(M)	73.3	79.3	120.3	70.8	12.1	9.2	17.5	7.0

Sl. No.	Genotype Name	DF	DFF	DAM	PHT	NT	NPT	BM	GY
30	185(M)	75.3	80.3	123.7	71.3	12.1	9.7	22.1	6.3
31	196(M)	75.7	83.3	124.3	83.9	10.0	7.7	14.5	6.2
32	Type-3	71.3	83.3	136.3	72.0	13.6	11.7	25.2	6.4
33	Kanchana	99.0	102.7	143.7	41.5	12.3	8.6	16.9	2.8
34	Pant sugandh dhan -17	89.0	100.0	146.0	65.3	16.0	13.3	26.7	4.5
35	Kasturi	79.3	89.0	136.7	67.3	15.1	12.9	27.7	8.1
36	Moirang phou	70.7	80.3	121.0	75.8	13.9	11.9	25.2	8.4
37	Akut phou	90.7	108.0	145.7	60.3	6.9	6.0	15.3	6.3
38	Improved Chitti Mutyalu	80.3	93.3	136.3	44.9	14.3	11.7	16.9	4.3
39	High Iron Rice	81.3	89.3	140.3	41.2	12.0	10.7	19.7	4.7
40	NDR 2026	93.3	99.3	145.7	64.0	18.7	16.3	60.3	10.0
41	Kamad	80.5	92.2	147.0	76.9	11.0	9.1	14.4	5.3
42	China 1007	70.0	74.7	125.7	61.8	14.2	10.3	3.0	0.1
43	Nja Vora	84.3	93.0	141.0	44.0	9.3	6.3	8.1	0.5
44	Kadamakudy Pokkali	88.7	94.3	143.0	53.9	18.1	11.5	42.9	8.7
45	Edavankudi Pokkali	76.7	82.0	116.0	65.7	10.8	7.5	17.5	4.0
46	Vytilla Anakondan	72.3	86.3	125.7	42.4	11.2	7.7	12.8	3.2
47	BPT 5204 /Chitti Mutyalu-SB	88.7	98.7	133.7	39.6	26.0	9.2	15.5	3.2
48	Radhuni Pagal	80.3	86.0	140.0	65.8	10.9	8.1	20.1	2.2
49	Champa Khushi	85.0	97.7	139.0	71.8	17.6	14.3	38.6	11.3
50	Seeta sail	86.0	100.0	138.7	76.5	20.1	15.7	46.9	10.7
51	Tilak kachari	93.7	106.7	143.3	64.7	17.4	13.1	35.4	7.8
52	NC 365	79.3	93.7	120.3	61.8	10.5	7.3	15.7	6.3
53	AM-FS-F7-183B	73.0	84.7	130.0	47.5	20.3	18.4	23.5	9.9
54	AM-FS-F7-163A	74.7	86.3	133.0	74.2	15.1	13.7	29.2	10.8
55	AM-FS-F7-111	88.0	95.0	143.3	64.5	18.3	15.1	28.8	3.0
56	AM-FS-F7-57	76.3	91.0	129.3	75.0	18.9	17.3	28.6	6.1

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EVALUATION OF SUPER-ELITE RICE (*ORYZA SATIVA L.*) ACCESSIONS FOR ROOT CHARACTERS AND PRODUCTIVITY UNDER AEROBIC CONDITION

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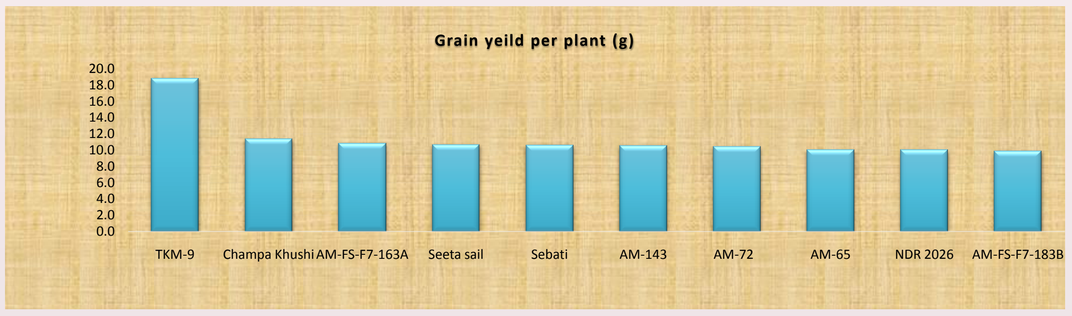
INTRODUCTION:

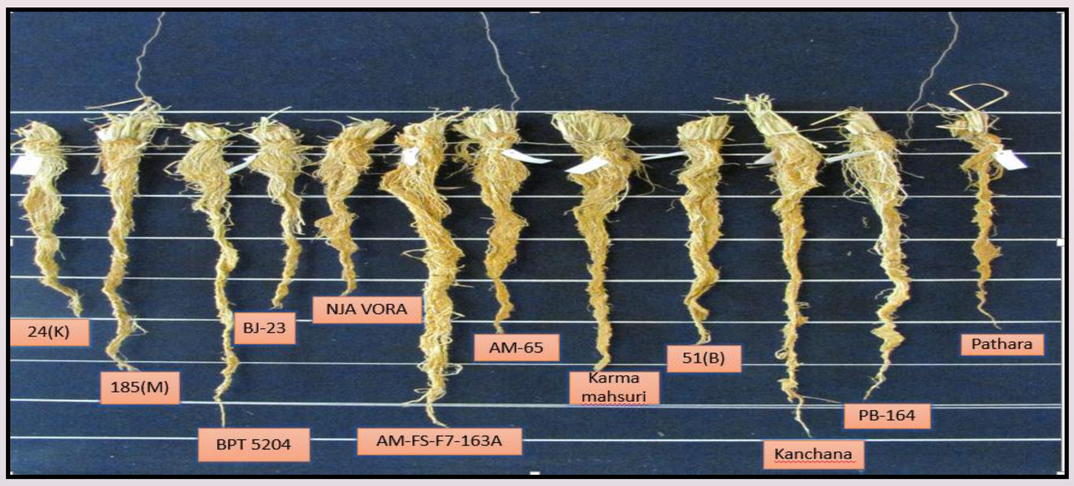
- Rice is central to the lives of billions of people around the world being the staple food for 2.5 billion people
- About half the total world rice area is rainfed, and challenged by drought stress.
- Roots are the principal plant organs for nutrient and water uptake.
- Therefore, improving our understandings on interactions between root function and drought tolerance in rice could have a significant impact on global food security.



Top ten rice varieties showing highest yield among 56 super-elite rice accessions evaluated in field during *Kharif*-2012

OBJECTIVES:

1. Characterization of super-elite rice from Indian Rice Biofortification Programme (IRBP) for root morphology and productivity
2. Molecular characterization of these super-elite rice using informative markers for biotic stresses



Different rice genotypes showing variation in root length grown in PVC pipes

METHODOLOGY:

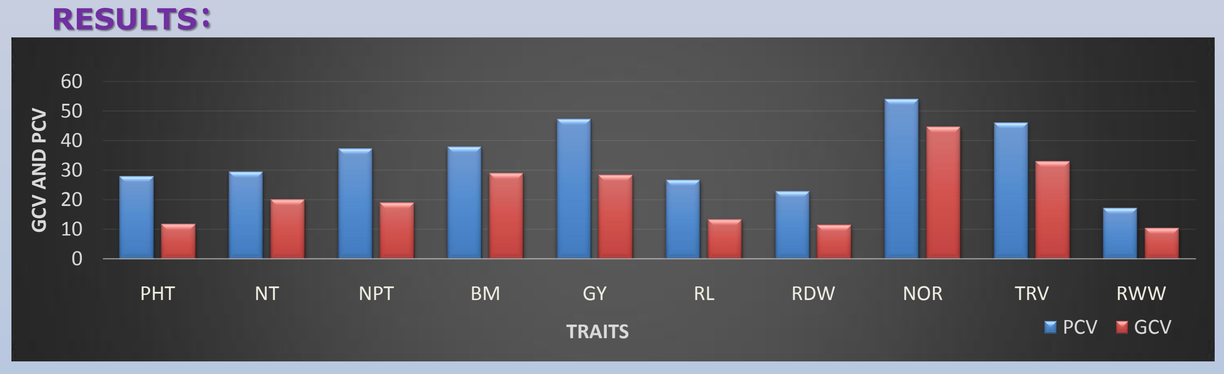
- The study consisted samples collected from 10 locations across India by Directorate of Rice Research Hyderabad, under the project "Rice Project Harvest Plus"..
- Four five star lines were also included in the study that belonged to F7 generation of Azucena and Moro mutant cross developed at DPBT,GKVK...
- The phenotypic studies were done in two parts, *viz.*, study of shoot parameters in the field during *Kharif* -2012 with three replications and post harvest study of root morphology parameters in pipe experiment conducted in the same plot with three replication.
- The analysis of data was performed with SPAR 2 software to calculate GCV, PCV, GAPM, heritability % and correlation between traits.
- Molecular characterization of these super-elite rice accessions were done using ten informative marker for biotic stresses.

TRAITS	NT	NPT	BM	GY	RL	RDW	NOR	TRV	RWW
PHT	0.100	0.098	0.233	0.173	0.107	0.176	0.142	0.094	0.187
NT	1	0.665**	0.341**	0.217	0.160	0.046	0.167	0.084	0.097
NPT		1	0.409**	0.247	0.239	0.132	0.046	-0.003	0.163
BM			1	0.465**	0.258	0.226	0.086	0.146	0.075
GY				1	0.559**	0.542**	0.167	0.277*	0.335*
RL					1	0.842**	0.376**	0.404**	0.687**
RDW						1	0.396**	0.477**	0.770**
NOR							1	0.765**	0.312*
TRV								1	0.324*

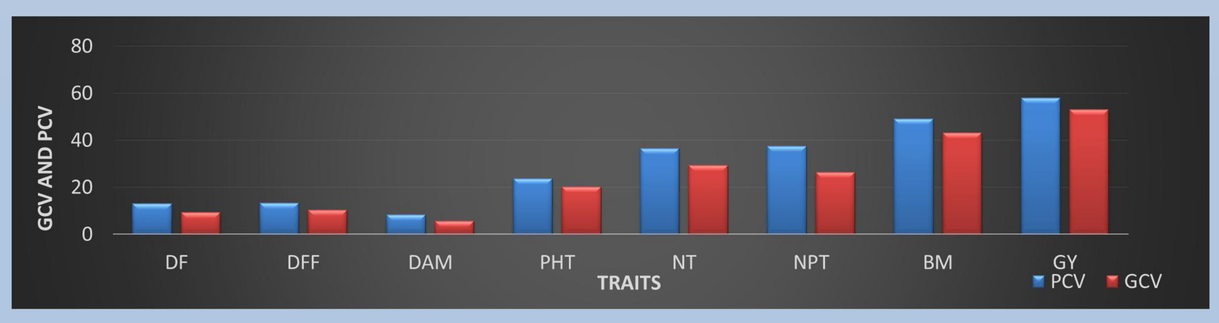
Phenotypic correlation among growth, yield and drough resistance components

Different rice genotypes grown in PVC pipes and field for evaluation of root character, yield and its associated traits

RESULTS:



PCV and GCV **for root morphological traits among 56 super-elite rice genotypes**



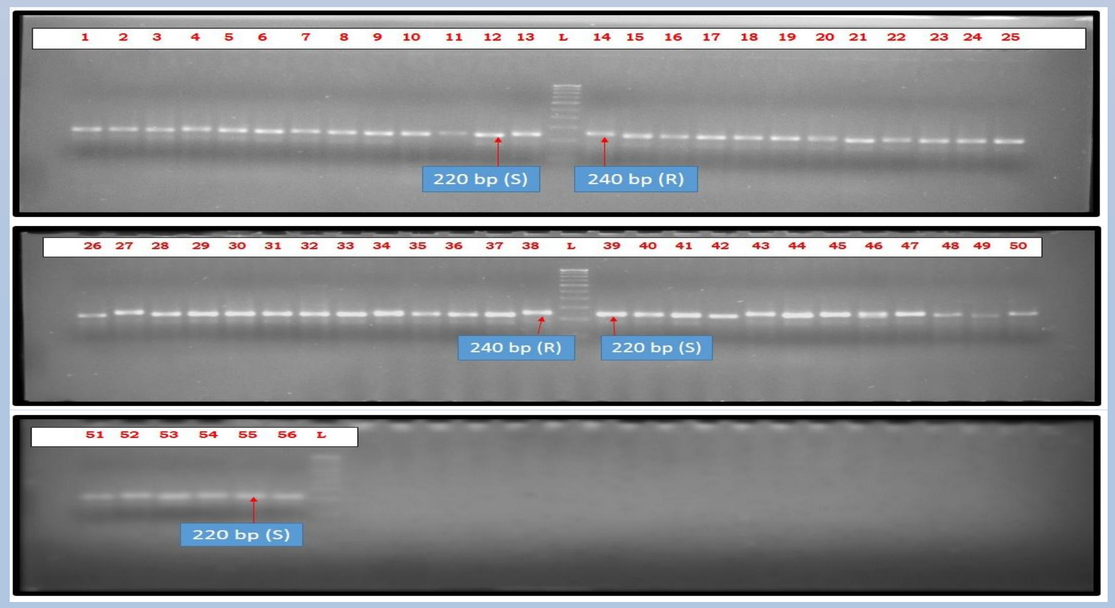
PCV and GCV **for morphological traits for yield and yield attributing traits among 56 super-elite rice genotypes**

Sl No.	Character	Minimum	Maximum	Mean	PCV	GCV	Heritability BS %	GAPM
1	Days to initial flowering	64.7	99.0	81.1	12.94	9.24	50.96	11.14
2	Days to 50% flowering	67.3	112.0	91.3	13.35	10.42	60.91	14.40
3	Days to maturity	116.0	154.0	135.8	8.25	5.74	48.48	6.67
4	Plant Height(cm)	39.6	97.6	60.7	23.78	20.23	72.39	32.03
5	Number of tillers	5.7	31.3	14.0	36.61	29.36	64.31	42.34
6	Number of productive tillers	5.0	20.6	10.9	37.38	26.47	50.16	31.55
7	Biomass(g)	16.8	49.8	28.5	49.13	43.06	76.81	38.31
8	Grain yield(g)	0.1	18.8	6.0	57.95	53.08	83.90	94.63

genetic parameters for different quantitative traits among super-elite rice accessions for yield and its associated traits

Sl. No.	Primers	Marker	Expected product size (bp)	Chr. No.	Annealing temperature(^{0}c)
Bacteril Leaf Blight					
1	Xa4	RM224	140	11	53
2	xa5	RM122	250	5	55
3	xa13	RG136	500	8	55
4	Xa21	pTA248	850	11	53
Brown Plant Hopper					
5	Bph3	RM190	143	6	50.6
6	Bph18	RM7376	194	11	50
7	Bph20(t)	B44	207	4	60
Blast					
8	Pi1	candidate gene	155	11	56
9	Pi2	AP5659-5	330	6	58
10	Pik^h	RM206	155	11	57

Biotic stress markers used



Scoring pattern and validation of biotic resistance marker *Xa5* associated with BLB resistance indicating polymorphism

CONCLUSIONS

- All the 56 genotypes were having highly diverse genotypic composition showing capacity to confer biotic resistance to the plant.
- Phenotypically, all the genotype under study reflected high vigour, and ability to withstand adverse situation and had shown high survivability under aerobic condition.
- All this information can be further utilized for research and development work.

ACKNOWLEDGEMENT:

Authors are thankful to **DBT-HRD** for providing facilities this research.