MOLECULAR MARKER ANALYSIS ON MAXIMUM ROOT LENGTH IN RICE (*Oryza sativa* L.)

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MOLECULAR MARKER ANALYSIS ON MAXIMUM ROOT LENGTH IN RICE (*Oryza sativa* L.)

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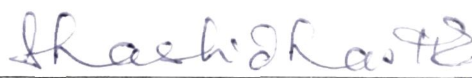

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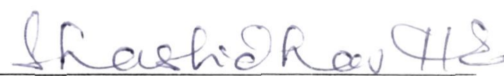

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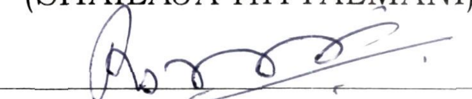

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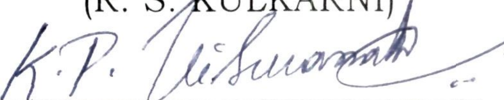

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Introduction

I. INTRODUCTION

Rice (*Oryza sativa*) is among the important staple food crops in the world. Although rice is grown across hundred countries, more than 90 per cent is produced and consumed in Asia. India ranks first in area (44.8 m ha) and second in production (131.2 mt) among rice producing countries in the world. However, India ranks ninth in terms of productivity (2.9 t/ha) (Anon., 2000).

Rapid increase in production and productivity of rice is needed to meet the ever increasing demand owing to unchecked population growth and increased purchasing power. With rapid urbanization, shrinking of irrigated areas and reduced ground water levels, there is an increasing reliance on less-than-favorable habitats for growing more rice. Rainfed lowlands, in which 28 per cent of world rice is grown, is characterized by uncertain and erratic rainfall which destabilizes the yields resulting in yearly fluctuations. Drought is, therefore, one of the most important constraints of rice production in many rice-producing areas of the world (Herdt, 1991). A large-scale survey individually listed drought at seedling period, vegetative period, and anthesis stage among the top 20 constraints of rice production (Lin and Shen, 1993).

With global shortages of water now emerging, reducing water consumption in crop production has now been generally recognized as an essential strategy for sustainable agriculture. It has also been gradually recognized as an important strategy for rice production, even for areas where water supplies are currently abundant. In addition, reduced levels of irrigation will decrease levels of water contamination and energy consumption, thus producing a significant positive impact for our environmental conservation efforts.

Plants have evolved different adaptive mechanisms to escape, avoid or tolerate water stress. Root systems form one of the most important

components of drought resistance and hence determine yield. Despite significant genetic variability in root traits, genetic improvement for root traits using conventional selection based on phenotype is difficult (O' Toole, 1989).

The advent of molecular markers has provided a means of genetical research. One of the most extensive applications of molecular makers is the development of molecular genetic maps of crop species. Today, around 3,500 DNA based markers are available for gene mapping and breeding in rice. These provide an average of less than 300 markers for each of the 12 chromosomes of rice, corresponding to markers at approximately every 200 kb length on average or every 30-50 genes (Anon., 2001). The markers can be used to detect and map quantitative trait loci (QTL) for economically important traits. Since drought resistant traits are quantitative in nature, molecular mapping technology and QTL mapping have emerged as powerful tools to accelerate breeding research. Tagging of root traits with molecular markers allows breeders to screen the segregating population of a selected parental cross combination contrasting for root morphology. Molecular marker-assisted selection (MAS) offers an excellent selection criterion, which is independent of phenotype for traits, which are difficult to evaluate such as root traits.

Keeping all these things in view, the present investigation was carried out with the following objectives:

1. Identification of molecular markers linked to maximum root length in rice
2. Phenotyping a subset of world germplasm for root morphology
3. Confirmation of marker-trait association with diverse genetic material.

Review of Literature

II. REVIEW OF LITERATURE

Roots play an important role in maintaining a good crop stand and combating drought through their distribution and dynamic responses. Plants need to have a well-endowed root system with ability to adjust to soil moisture perturbations. Understanding its inheritance has proved to be a difficult task. Root morphological characters, being interrelated to each other, add to the complexity. Literature on these aspects *vis-a-vis* rice are presented under the following headings.

2.1 Habitats

2.2 Concept of drought resistance.

2.3 Root morphology- Growth and development.

2.4 Genetic parameters of root and shoot characters.

2.5 Molecular markers

2.6 Bulked segregant analysis

2.1 Rice Habitats

Rice (*Oryza sativa* L.), a semi-aquatic monocot cereal crop is grown over an array of climatic conditions. Irrigated rice occupies about 30 m ha area out of 148 m ha the world over. Irrigated rice is grown in bunded and puddled fields. Research accomplishments have contributed significantly to irrigated rice cultivation by means of developing fertilizer responsive, high-yielding varieties resistant to biotic and abiotic stresses. Annually one or more crops are taken up with yield ranging from 3 to 9 t ha^{-1} (Yuan *et al.*, 1989).

As this study is pertaining to rainfed lowland, the characteristics of this habitat are briefed here. About half the area in the world under rice is occupied by rainfed rice. This is taken up only once in a year in bunded fields, which are flooded at least for part of the growing season and in lands which are unbunded without surface water accumulation. Owing to this, these fields are likely to face drought at one or the other stage of crop growth (O'Toole and Moya, 1978). These lands are characterized by lack of water control and hence, more often exposed to problems of floods and drought (Anon., 1995). As there are no improved varieties for these conditions, traditional varieties that are less productive, requiring relatively less inputs are being grown. Hence, it is imperative to develop improved varieties for these conditions in addition to retaining the drought tolerance capacity of traditional varieties.

2.2 Concept of Drought Resistance

Blum (1982) has related drought resistance to yield as a major economic consideration and as an integrator of the effects of plant drought stress in time and space and also opined that drought resistance is process-specific and its definition is linked to plant processes at any given level of organization.

Drought resistance is classified into drought escape and drought tolerance (Levitt, 1980). Drought escape is reflected by the plant's ability to mature before soil water deficit becomes a serious problem. This involves developmental plasticity and rapid phenological development.

Again, drought tolerance involves dehydration as well as dehydration tolerance mechanisms. Plant's ability to sustain the minimum injury to crop growth at lower levels of tissue water status or turgor, reflects its dehydration tolerance capacity. Dehydration

avoidance, also called as dehydration postponement, represents plant's ability to maintain high level of tissue water status or turgor under conditions of increasing soil water deficits. It is the ability of plant to maintain tissue dehydration for some period of time during moisture stress.

2.3 Root morphology- Growth and development

Rice has a high rooting density in the surface soil compared to other crops, which is attributed to tillering habit of rice. Banba and Ohkuba (1989) reported that 80 per cent of root dry matter was concentrated in the top six cm of soil in a study on root distribution of upland rice. Compared to maize and sorghum, rice has a shallow root system that makes it susceptible to water stress (Inthapan and Fukai, 1988; Fukai and Inthapan, 1988).

A number of physiological and morphological traits have been reported to improve the performance of crops affected by drought. Root system morphology is one of the important components of drought resistance (Passioura, 1982)

Yoshida and Hasegawa (1982) presented a detailed analysis of tillering and rooting pattern of rice plant. The fibrous root system of a rice plant consists mainly of numerous nodal roots and their laterals. A tiller and its roots come simultaneously from the same node i.e., when the leaf emerges, a tiller and its roots start emerging from (n-3)th node. They also reported significant differences in the root length density between upland and lowland rice.

Robertson *et al.* (1985) commented that the available techniques for evaluating root traits are tedious and time consuming. Cruz *et al.* (1986) reported that root length was maximum after heading stage and subsequently declined until maturity. Beyrouty *et al.* (1988) reported root growth to be most rapid during vegetative growth with

maximum root length at panicle initiation and it plateaued off or declined during reproductive stage.

Rice root growth exhibits plasticity in response to external factors *viz.*, soil profile characters, soil moisture, air temperature and growth stage. Aerobic conditions in dryland conditions favors root hair formation while, flooded soils impair it (Weaver and Himmer, 1930; Yoshida and Hasegava, 1982; Beyrouty, *et al.*, 1988).

The final configuration of a root system under field conditions is largely determined by factors such as chemical gradients, moisture content, mechanical impedance and aeration (Lynch, 1995), which are in turn affected by, soil, climate and cropping system. Large genetic variation in root morphology has been reported in germplasm adapted to different agroecological conditions (O'Toole and Bland, 1987). Courtois *et al.* (1996) reported that cultivars of upland origin are more deeply rooted and have a larger diameter of main axes compared with cultivars of lowland origin.

Price *et al.* (1997) and Thanh *et al.* (1999) opined that root growth is an important component of the adaptation of rice to drought prone environments. Drought is a major constraint to the productivity of rice in upland ecosystems and the rice root system plays an important role in the regulation of water uptake and extraction from deep soil layers.

2.3.1 Root parameters and drought resistance.

Nasiruddin and Haque (1981) observed low positive and or negative correlations among the root traits namely root length, root weight, root number and drought tolerance. Armento-Soto *et al.* (1983) attributed differential response of rice (*O. sativa* L.) genotypes to soil and atmospheric water stress to the root system characteristics.

Ekanayake *et al.* (1985) reported that root length, root thickness, number of thick roots and root volume were significantly correlated to the field recovery from drought. They also opined that resistance to or tolerance to water stress in crop plants is the combined result of many interacting morphological and physiological characters.

Chang *et al.* (1986) investigated genetic variability in root characters among cultivators and reported that deep thick root systems avoid drought better than those with shallow thin root systems.

O'Toole and Bland (1987) reviewed the genotypic variations in root systems and reported that plant root systems have the capability of coping with changes in environmental factors such as water status and temperature. Gomathinayagam *et al.* (1988) reported significant genotypic differences with respect to root length, root dry weight, leaf water potential.

Jeena and Mani (1990) studied root characters and grain yield on some upland rice varieties and indicated that apart from high root length density and root weight, the duration of crop was important for selecting drought tolerant genotypes.

A deep and thick root system with high ratio of deep root weight to shoot weight and high deep root length density are factors contributing to resistance to intermittent drought stress in upland rice (Fukai and Cooper 1995).

Ray *et al.* (1996) opined that root penetrating ability is an important factor for rice drought resistance in areas with soils subjected to both compaction and periodic water deficits. Breeding for root penetration ability is inhibited by difficulties associated with measuring root traits. Lilley *et al.* (1996) found rice variety 'Bala'

possessing considerable drought resistance mechanisms by virtue of leaf related drought mechanisms inspite of having poor root system.

Thanh *et al.* (1999) after finding positive correlations between the root traits concluded that, selection based on any of the root traits especially the easily measurable one, may provide breeders an opportunity to develop drought resistant upland rice varieties.

Root length

Puckridge and O'Toole (1981) reported that a deep-rooted rice cultivar 'Kinangdang Patang' extracted more water at 40-70 cm depth than two shallow rooted cultivars namely IR20 and IR36. O'Toole (1982) opined that for relatively large soil water reservoir (deep soils), increase in rooting depth, conductance and root to shoot ratio (by weight) results in increased soil water uptake capacity. Passioura (1982) reported that in deep wet soils, large root density at depth is necessary to extract water from deeper layers. O'Toole and Datta (1986) opined that increased rooting depth and density would increase the plant's capacity to extract water in rice.

Mumbani and Lal (1983) reported that rice plants with deep root systems maintain high leaf water potential, delaying leaf drying or death. Deep roots may also reduce the production of chemical signals from roots under drought conditions, which may otherwise reduce leaf growth and expansion and stomatal conductance (Turner *et al.,* 1986).

When plants are subjected to a moderate stress, they are supposed to put forth longer roots to absorb moisture from deeper layers but, Cruz *et al.* (1986) and Thangaraj *et al.* (1990) in their studies on line-source-sprinkler system with induced moisture gradient, observed decreased root length. They attributed this decrease in root length due to increased soil mechanical impedance.

Root studies are arduous under actual field conditions. For convenience some scientists have conducted root studies in aeroponic or hydroponics. Gomathinayagam *et al.* (1988b) studied seminal roots of rice seedlings in solution culture and suggested association of long seminal roots with drought resistance. Similar association between total root length and drought tolerance was observed in aeroponic study by Gomathinayagam *et al.* (1992).

Sorte *et al.* (1992) quantified reduction in root length under water stress. They imposed water stress for five days at 30 days after sowing and observed nineteen per cent reduction in root length when the reduction in moisture content was 44 per cent. Rao *et al.* (1994) studied the root systems under stress. They supplied the roots with water at deeper zones to relieve stress but stress was not relieved. This led them conclude that water deficit occurring upto a depth of 30-40 cm was critical in determining the drought tolerance and not the length of the root system.

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). Okuyama and Colasante (1987) speculated that root dry weight might increase with increase in duration of crop growth. On imposition of stress, the root weight decreases which may be attributed to decrease in the associated traits (Cruz *et al.*, 1986). 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).

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 (1988) wherein 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.

Root to shoot ratio

Banba and Ohkuba (1989) reported that root to shoot ratio increased under water stress in rice. They opined that the root to shoot ratio increases under stress. This increase was due to absolute increase in root growth during drought (water) stress.

Cruz *et al.* (1986) reported that percent reduction in shoot dry mass was less than that of total root dry mass, thereby decreasing root to shoot ratio under mild stress conditions during vegetative stage in rice. They attributed this to high soil strength or soil mechanical impedance, which decreased root length.

Haque *et al.* (1989) conducted root studies in both aeroponic and hydroponic cultures. They estimated all the root parameters required for determining drought reaction. They reported variation in

root to shoot ratio between the two cultures. Sorte *et al.* (1992) differed from the general hypothesis of increase in root growth during stress.

Root volume

Ekanayake *et al.* (1985) found predominantly additive gene effects for root volume along with root thickness. But, Price *et al.* (1997) failed to detect any significant additive or dominance gene effects for these traits.

Zuno-Altoveros *et al.* (1990) conducted an experiment to determine the root volume of some selected upland and lowland rice varieties. They found that Rikuto Norin12, a Japanese upland variety had very high root volume and a lowland variety, IR20 had low root volume. Correlation of root volume with root length and shoot length was positive and significant.

2.4 Genetic parameters for root and shoot characters

2.4.1 Heritability, genetic advance and coefficients of variation

Heritability estimates indicate its potential for selection of traits. There are numerous heritability estimates for root traits reported in several crop species. Heritability estimates (narrow sense) were moderately high for root length (61 %) and root tip thickness (62 %) and, moderately low for root number (44 %) and root dry weight (43 %) (Anon., 1980).

Chang *et al.* (1982) reported moderately high heritability for maximum root length (61 %), root tip and root base diameters (62 %), moderately low for root number (44 %) and root weight (43 %). Hemamalini (1997) reported similar results. Ekanayake (1985) reported high narrow sense heritability estimates for root dry weight,

for root dry weight, root thickness and root length density. They also reported moderate heritability for root volume, thick root number and low heritability for maximum root length. Armento-Soto *et al.* (1983) reported moderate to high heritability for root length, root thickness, root dry weight and root length density.

In a study on root systems under aeroponic culture it was reported that root length and root thickness exhibited high heritability (65 per cent to 75 per cent) whereas, root number and root weight showed 50 per cent to 65 per cent heritability (Anon., 1984).

Ekanayake *et al.* (1985) studied inheritance of root traits. Heritability estimates computed were higher for root dry weight, root thickness and root length density, in comparison to other traits. Heritability estimates for maximum root length and number of thick roots were low to intermediate ranging from 33 per cent to 51 per cent. Root volume had low heritability of 18 per cent. Based on their studies on F_2 plants obtained from a cross between IR-20 and MGL-2, they reported unimodal and continuous distributions for all the root characteristics. They observed slightly skewed distribution towards higher values for thick root number, root dry weight and root length. They also found high heritability for root number. Similar results were reported by Anon. (1980). Hemamalini (1997) reported higher heritability for root number and low heritability for root thickness.

Shashidhar *et al.* (1990) reported high heritability estimates for five root characters in a study on twenty four rice cultivars. High heritability was also reported for root length and root thickness (Das *et al.*, 1991). They also observed high environmental co-efficient of variability in case of root length, root dry weight, shoot dry weight, root to shoot ratio and tiller number.

High heritability and high genetic advance were noted for root

dry weight (Gomathinayagam *et al.,* 1990; Shashidhar *et al.,* 1990 ; Shahid *et al.,* 1994). Root dry weight was found to be more stable as compared to root length and total root number from the PCV and GCV estimates. Hajra and Hajra (1988) observed high variability for root systems with moderately high heritability for primary root length. Moderate heritability with moderate genetic advance was observed for root length and number (Gomathinayagam *et al.,* 1990).

Zheng *et al.* (1996) observed significant differences for root traits such as, number of roots, root length, root thickness and root growth ability in eight varieties they studied. Latha (1997) reported high heritability for root dry weight (94.05 %), shoot dry weight (87.26 5), root number (86.52 %), root volume (80.50 %), number of tillers (77.63 %) and root length (72.35 %) and, moderate heritability for other traits. She also reported high and low expected genetic advance as percent of mean for root dry weight and root thickness respectively. Hemamalini (1997) in her study, reported the highest expected genetic advance as per cent of mean for root volume and the lowest for root diameter.

Price *et al.* (1997) observed root length to be controlled by a combination of additive and dominant gene effects and its heritability to be moderately high. They also found high heritability for root thickness and moderate heritability for root volume. They reported that populations from crosses between genotypes 'Bala x Azucena' and 'Azucena x Maratelli' displayed unimodal segregation for maximum root length and adventitious root thickness.

Gireesha *et al.* (2000) observed root length to be controlled by additive gene action and its heritability to be high.

2.4.2 Correlations among root and shoot parameters

Significant positive correlation between root length and root

diameters (r=0.57 **) was reported. Correlation co-efficients between root characters and resistance to drought was studied and reported that root length, root number and root to shoot ratio were not significantly associated with field reaction to drought during vegetative stage (Anon., 1982).

Anon. (1984) reported positive association between plant height and shoot dry weight, root length and root thickness, root number and root dry weight. Tiller number was highly and positively correlated with root number and shoot dry weight. Ekanayake *et al.* (1985) reported that root thickness and root number were correlated with plant height, tiller number and shoot weight. They also found that root length, thickness and root volume were significantly correlated with recovery from drought. They observed positive association amongst root characters and reported significant correlation between plant height and root characters.

Salam and Subramanian (1988) reported positive correlation between root dry weight and plant characters such as plant height tillers production and dry matter production of rice at all growth stages. Zuno-Altoveros *et al.* (1990) studied root volume of selected upland and lowland rice varieties and reported that root volume was positively correlated with root and shoot length. Root volume was found to be negatively associated with damage caused by drought in the reproductive phase (r=-0.85**).

Shashidhar (1990) reported significant association of root weight with root length (r=0.69**) and root volume (r=0.62**). Shahid *et al.* (1994) reported positive correlations between root length, root dry weight, shoot dry weight, stomatal frequency and drought tolerance.

Genotypic correlation between root dry weight and root length

density in different horizons was found to be extremely high indicating that root dry weight though easy to measure can act as excellent predictor of root length density (Yadav *et al.,* 1995). They also noticed heritability of 58 per cent for root to shoot ratio and 84 per cent for root thickness. Yadav *et al.* (1997) reported moderate to high broad sense heritability for root traits with positive correlations amongst themselves.

Latha (1997) in her study, found highest correlation between shoot dry weight and total dry weight and she has also reported significant correlations between other root traits. Hemamalini (1997) found positive correlation between all root characters under well watered conditions, with significant ones ranging from (r=0.25*) between total root number and root length to (r=0.86**) between root dry weight and root volume.

Price *et al.* (1997) based on their study on rice cultivars in hydroponic system, reported positive and significant correlation between root length and root thickness. Lorseto *et al.* (1983) observed very good agreement between root growth in liquid culture and previously recorded data on root growth in both pots and field conditions. They concluded that maximum root length and thickness are important attributes contributing to drought resistance in rice.

Thanh *et al.* (1999) studied thirty three upland rice accessions and observed significant, positive correlations among all root traits except root number. In their study, the highest correlation among root characters was observed between maximum root length and total root dry weight (r=0.65**). In addition plant height was also found to be significantly correlated with root thickness, maximum root length and total root dry weight (r =0.80**). Similar results were reported by Champoux *et al.* (1995).

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Gireesha *et al.* (2000) found significant correlation between plant height and root traits *viz.,* total root number, root length, root dry weight and total dry weight. He also found that root volume, total root number, root length, root dry weight, shoot dry weight and total dry weight were interrelated positively and significantly. They also found high expected genetic advance for root dry weight and root to shoot ratio.

2.5 Molecular Markers

Over the last few decades, technological advances in the field of genetics and molecular biology have provided new tools to plant breeders for detailed analysis of genomes. Molecular markers have greatly facilitated genetic investigation of complex traits and their manipulation for productivity enhancements at a faster rate.

Among the molecular markers, two classes are recognized *viz.,* isozymes and DNA markers. Sax (1923) first reported the association of morphological marker with a quantitative trait in *Phaseolus vulgaris*. Thoday (1961) put forth the idea of using single gene markers to characterize and map individual polygene of quantitative traits.

2.5.1 Biochemical markers

Polymorphic isozymes and storage proteins have been used as molecular markers. Isozymes have been used in genome mapping, population and evolutionary studies, quantification of interrelationship with quantitative traits, identification of varieties, genetic resource management and breeding (Tanksley and Orton, 1983). However, non-availability of suitable range of isozymes over different genotypes and lack of neutrality to environmental conditions or management practices restrict their use.

2.5.2 DNA-based molecular markers

DNA based genetic markers are the next offshoot in molecular markers. These markers have been used in characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics.

2.5.2.1 Properties desirable for ideal DNA markers

- Highly polymorphic nature
- Co-dominant inheritance (determination of homozygous and heterozygous states of diploid organisms)
- Frequent occurrence in genome
- Selective natural behaviour (the DNA sequences of any organism *are neutral to environmental conditions or management practices*)
- Easy access (availability)
- Easy and fast assay
- High reproducibility
- Easy exchange of data between laboratories

Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers *viz.,* RFLP, and polymerase chain reaction (PCR)-based markers *viz.,* RAPD. In the former, DNA profiles are visualized by restriction enzyme-digested DNA, to a radio labelled probe, which is a DNA fragment to known origin or sequence. PCR-based markers involve *in vitro* amplification of particular DNA sequences or loci, with

the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme.

2.5.2.2 Types of DNA markers

2.5.2.2.1 Restriction Fragment Length Polymorphism (RFLP)

RFLPs are simply inherited naturally occurring Mendelian characters. They have their origin in the DNA rearrangements that occur due to evolutionary processes, point mutations within the restriction enzyme recognition site sequences, insertions or deletions within the fragments, and unequal crossing over.

2.5.2.2.2 Randomly amplified polymorphic DNA markers

In 1990, Welsh and McClelland developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within amplifiable range of each other, a discrete DNA product is formed through thermocyclic amplification.

2.5.2.2.3 Microsatellites

Microsatellites are multilocus probes creating complex banding patterns and are usually non-species specific occurring ubiquitously. Microsatellites consist of 1 to 6 bp long monomer sequence that is repeated several times. Microsatellites thus form an ideal marker system creating complex banding patterns by simultaneously detecting multiple DNA loci. Some of the prominent features of microsatellites are that they are dominant fingerprinting markers and co-dominant STMS (sequence tagged microsatellites) markers.

2.6 Bulked Segregant Analysis (BSA)

Molecular markers (DNA, isozyme) have become indispensable tools in plant breeding and genetics particularly in marker-aided selection and gene mapping. DNA markers for simply inherited traits can be readily identified by the synthesis of DNA pools from individuals sharing a common phenotype (Michelmore *et al.*, 1991). Bulked segregant analysis is a rapid procedure for identifying markers in specific regions of the genome. The method involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool or bulk, the individuals are identical for the traits or gene of interest but are arbitrary for all other genes. Two pools contrasting for a trait (e.g., resistant and susceptible for a particular disease) are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools (Michelmore *et al.*, 1991 and Wang and Paterson, 1994).

Different types of molecular markers have been used to develop trait specific markers *viz.*, Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Amplicon Fragment Length Polymorphisms (AFLPs) and Microsatellites. RAPD technique developed by Williams *et al.* (1990) relies on the differential enzyme amplification of small DNA fragments using PCR with arbitrary primers. Polymorphism results from either chromosomal changes in the amplified regions or base changes that alter primer binding (Wang and Paterson, 1994). The procedure is rapid, requires only small amounts of DNA, which need not be of high quality and involves no radioactivity.

Michelmore *et al.* (1991) identified 3 RAPD markers in lettuce linked to a gene for resistance to downy mildew. Martin *et al.* (1991),

using BSA in conjugation with NILs for *Pseudomonas* resistance identified RAPD marker linked to *Pseudomonas* resistance in tomato. Using 520 random primers Nair *et al.* (1995) screened 5420 loci on the genomic DNA of ARC 6650 and Phalguna of rice and identified two RAPD fragments viz., $F8_{1700}$ and $F10_{600}$ that could recognize susceptible (ARC 6650) and resistant (Phalguna) parents for gall midge resistant biotype 1 / resistant gene (*gm* 2).

Yoshimura *et al.* (1995) identified two RAPD markers linked to *Xa-1,* which confers resistance to bacterial blight in rice. Of these two primers, recombination was detected between *Xa-1* and YO_{3700}, but UO_{8750} was linked to *Xa-1* at a distance of 1.5 cM.

Monna *et al.* (1995) used 80 primers, either singly or pair wise, and tested 2,404 primer-pairs and established 14 markers tightly linked to the photoperiod sensitivity gene in F_2 individuals derived from a cross between Nipponbare and Kasalath. They further converted the markers into sequence-tagged sites by cloning and sequencing.

Sheng *et al.* (1995) screened about 300 random primers against bulked DNA samples of genotyped individuals from the F_2 segregating population of the cross CT9993/KDML105 for polymorphic DNA fragments linked to the aromatic gene. A single primer, Jas 1.5, was used to amplify DNA from each of the aromatic and non-aromatic plants in the F_2 bulks. The 1.5 kb polymorphic fragment co-segregated with the scent scores.

KeNong *et al.* (1996) used 624 RAPD primers to screen submergence tolerance using BSA in population of 169 F_2 plants (IR40931-26 X PI543851). Five primers were found to be linked with the quantitative trail loci (QTL), *Sub1*, which accounted for 69 per cent of the phenotypic variance for the trait

Using bulked segregant analysis Khush (1996) could successfully tag *gm-6(t)* by RAPD marker OPM06 and later identified that resistance gene mapped between RFLP RG 214 and RG163 on chromosome 4 in rice.

Koh *et al.* (1996) mapped super-giant embryo character in rice F_2 population (Hwacheongbeo-ges X Milyang 23) with two microsatellite markers RM10 and RM18. These markers were linked with *ges* gene at a distance of 9.6 and 7.7 cM, respectively.

Nair *et al.* (1996) extracted DNA from gall midge resistant variety Tulsi and their F_3 hybrid progeny bulks and screened using 500 random primers. They identified primer E20 amplified bands $E20_{570}$ and $E5_{538}$ were linked to resistant and susceptible genes, respectively. Later, Sequence Characterized Amplified Region (SCAR) markers were developed which amplified phenotype specific bands of 538 bp on susceptible F_3 lines and 570 bp in resistant F_3 lines.

Maheswaran *et al.* (1999) tagged photoperiod sensitivity gene, *Se-3(t)*. Out 435 markers screened, a single primer, OPA19 was linked to the gene in the cross Sac Nau/Nam Saugi 19.

Dioh *et al.* (2000) identified genetically independent avirulence genes, AVR1-Irat7, AVR1-MedNoi and AVR1-Ku86, in a cross involving isolates Guy11 and 2/0/3 of the rice blast fungus, *M. grisea*. Using 76 random progeny, they constructed a partial genetic map with restriction fragment length polymorphism (RFLP) markers revealed by probes such as the repeated sequences MGL/MGR583 and Pot3/MGR586, cosmids from the *M. grisea* genetic map, and a telomere sequence oligonucleotide. Avirulence genes AVR1-MedNoi and AVR1-Ku86 were closely linked to telomere RFLPs such as marker TelG (6 cm from AVR1-MedNoi) and TelF (4.5 cm from AVR1-Ku86). Avirulence gene AVR1-Irat7 was linked to a cosmid RFLP located on

chromosome 1 and mapped at 20 cM from the avirulence gene AVR1-CO39. Using BSA, 11 random amplified polymorphic DNA (RAPD) markers closely linked (0 to 10 cM) to the avirulence genes segregating in this cross were identified.

Dong *et al.* (2000) performed BSA using AFLP to tag thermosensitive genic male sterility (TGMS) gene in an F_2 population developed from a cross between a TGMS indica mutant, TGM-VN1 and a fertile inbred line, CH1. From this survey of 200 AFLP primer combinations, four markers (E2/M5-600, E3/M16-400, E5/M12-600, and E5/M12-200) linked to the TGMS gene were identified.

Using BSA, Shashidhar *et al.* (2000) screened about 500 RAPD markers and 83 microsatellite markers in F_2 bulks of DH population of IR64/Azucena. One RAPD primer, OPBH14, and a microsatellite primer, RM201, were found to be linked with maximum root length.

Material and Methods

III. MATERIAL AND METHODS

The details of the plant material used and the methods followed in the two experiments are presented separately. Information on the protocols and statistical tools employed for analysis is also presented in the respective experiments.

3.1 Experiment I. Identification of markers linked to root length in rice doubled haploids using random primer-pairs.

3.1.1 Plant material

Twenty doubled haploid lines which showed extreme phenotypes for root length (Shashidhar *et. al.*, 2000) and their parents from a doubled haploid population derived from a cross between IR64 and Azucena genotypes were used in the study (Table 1). These lines were sown in the greenhouse for DNA extraction.

3.1.2 Methods

3.1.2.1 DNA extraction

Thirty day old seedlings were used for DNA extraction . DNA was prepared as per the modified hexadecetyl methyl ammonium bromide (CTAB) method (Cao and Oard, 1997).

1. Young and healthy leaves (5 g) of about 25 days were collected from plants and brought to laboratory in ice.

2. The leaves were cut into pieces and homogenized completely with liquid nitrogen using pestle and mortar.

Table 1. List of DH lines belonging to each bulk and their root length

Sl. No.	Bulk A	Root Length (cm)	SD	Sl. No.	Bulk B	Root Length (cm)	SD
1	333	72.63	(+2)	1	488	32.1	
2	107	68.98	(+2)	2	272	31.9	
3	192	65.5	(+2)	3	78	31.53	
4	210	64.47	(+2)	4	284	29.87	(-1)
5	12	56.35	(+2)	5	467	28.45	(-1)
6	391	55.35	(+1)	6	124	28.2	(-1)
7	7	54.65	(+1)	7	163	27.43	(-1)
8	1564	54.55	(+1)	8	35	27.03	(-1)
9	336	53.33	(+1)	9	442	22.67	(-1)
10	290	52.15	(+1)	10	463	21.43	(-1)
Parents	**Azucena**	87.37	(+1)		**IR-64**	32.8	

3. Leaf powder was transferred to 50 ml Falcon tube containing 6 ml ice-cold extraction buffer.

4. The samples were incubated for 30 min. at 65°C after the addition of 0.8 ml 10 per cent SDS. During incubation, the samples were shaken at regular intervals.

5. After incubation, 2 ml of ice cold 5/3 potassium acetate was added and samples were vigorously rotated and incubated on ice for 30 minutes.

6. Samples were centrifuged for 10 minutes at 4000 rpm.

7. The supernatant was filtered through mira cloth into another centrifuge tube.

8. Six ml of Isopropanol was added to samples and then they were incubated at room temperature for 5 minutes for the precipitation of DNA.

9. The tubes were centrifuged for 10 minutes at 4000 rpm.

10. Supernatant was discarded and DNA pellet was air-dried.

11. Dried DNA pellet was dissolved in 200-400 μl T.E. buffer depending on size of the DNA pellet.

12. Dissolved DNA was transferred to 2.2 ml eppendorf tube and kept for incubation for 20 minutes at 37°C after adding RNAse and mixing.

13. Four hundred μl of CTAB buffer was added and tubes were incubated for 15 minutes at 65°C during which they were periodically shaken.

14. Eight hundred μl chloroform/isoamylalochol was added and tubes were centrifuged in minifuge.

15. To the supernatant was transferred to fresh 2.2 ml eppendorf tube, 1.4 ml of ethanol (96%) was added and mixture was incubated at room temperature for 15 min. for DNA precipitation.

16. The tubes were centrifuged for 10 min. in a minifuge and supernatant was discarded.

17. The DNA pellet obtained was washed with 70 per cent ethanol then ethanol was removed and pellet was dried.

18. Finally, the DNA pellet was dissolved in 200 μl of T.E. buffer and stored at –20°C until use.

3.1.2.2 Bulked segregant analysis (BSA)

BSA was employed to detect markers linked to root length. The methods involved are discussed here under:

3.1.2.2.1 Quantitation of DNA

The genomic DNA was quantified spectrophotometrically (UV-VIS Spectrophotometer, ELICO, India) both at 260 nm and 280 nm wavelengths (Table 2). The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 μg /ml of double stranded DNA. A

Table 2. DNA concentration calculated using UV Spectrophotometer values

Sl. No.	Bulk A	OD(260)	OD(280)	OD(260/280)	Concentration(μg/ml)	Sl. No.	Bulk B	OD(260)	OD(280)	OD(260/280)	Concentration(μg/ml)
1	7	0.051	0.027	1.86	2.55	1	35	0.100	0.058	1.71	5.00
2	12	0.013	0.007	1.79	0.65	2	78	0.048	0.027	1.75	2.40
3	107	0.017	0.010	1.75	0.85	3	124	0.058	0.033	1.77	2.90
4	192	0.028	0.016	1.76	1.40	4	163	0.026	0.014	1.90	1.30
5	210	0.023	0.013	1.82	1.15	5	272	0.081	0.049	1.65	4.05
6	290	0.029	0.015	1.90	1.45	6	284	0.071	0.041	1.75	3.55
7	333	0.030	0.018	1.65	1.50	7	442	0.131	0.071	1.85	6.55
8	336	0.071	0.044	1.60	3.55	8	463	0.071	0.040	1.79	3.55
9	391	0.037	0.022	1.70	1.85	9	467	0.127	0.068	1.87	6.35
10	1564	0.046	0.026	1.80	2.30	10	488	0.034	0.019	1.77	1.70
Parents	**Azucena**	0.041	0.023	1.82	2.05		**IR-64**	0.057	0.031	1.83	2.85

* OD values expressed in nano meters

pure sample of DNA shows the ratio of $OD_{260/280}$ as 1.8. Ratios less than 1.8 indicate contamination in the preparation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the preparation.

3.1.2.2.2 Agarose gel electrophoresis for detection of genomic DNA

Required amount of agarose was weighed out (0.8 per cent for genomic DNA) and melted in 1 x TBE buffer (10 x TBE : 0.89 M Tris, 0.89 M Boric acid, 0.11 M EDTA, pH 8.3) or 1 x TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) as per the requirement. After melting, the gel was cooled to about 50°C, ethidium bromide was added to a final concentration of 0.5 µg /ml. The mixture was poured immediately on a template with appropriate comb. After gelling the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank. To the DNA sample, required volume of loading buffer (40 % sucrose, 0.25 % bromophenol blue) was added and the samples were loaded onto the gel. Electrophoresis was performed at 5 volts/cm, until the tracking dye migrated to the end of the gel.

Ethidium bromide stained DNA bands were viewed under UV Transilluminator and photographed for documentation.

3.1.2.2.3 Pooling of DNA

Individuals were grouped in either bulk A (RTL +) or bulk B (RTL -) based on the phenotypic data (Table 1). The two bulks contained 10 individuals each. An equal concentration of DNA from each individual was added to each pool.

3.1.2.2.4 Conjugate primers

One hundred and ten decanucleotide primers (Operon Technologies, USA) were used in RAPD analysis (Table 3). Two primers were used in each RAPD reaction mixture.

3.1.2.2.5 Randomly Amplified Polymorphic DNA (RAPD) analysis

The RAPD reaction mixture consisted of 50 ng of template DNA, 10 ng of each of decanucleotide primer-pairs, 100 μM of dNTPs, 1 U of *Taq* DNA polymerase (Bangalore Genei), 1 X PCR buffer (10 mM Tris pH 8.0, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) in a volume of 20 μl. One drop of mineral oil (Sigma) was overlaid on the reaction mixture. The list of primer-pairs used in the study is given in Table 4.

Amplification was carried out on a Programmable Thermal Controller (PTC-100, M J Research). The amplification profile was as follows:

Initial denaturation temperature	:	94°C	-	5 min.
Denaturation	:	94°C	-	1 min.
Primer annealing	:	38°C	-	1 min.
Primer extension	:	72°C	-	2 min.

Latter three stages were repeated 35 times

Complete primer extension	:	72°C	-	5 min.
Soak temperature	:	4°C	-	Until removed

The amplified products were electrophoresed on 1.4 per cent agarose gels (3.1.2.2.2) and photographed for documentation.

Table 3. List of RAPD primers used and their sequences (5' -3')

Sl. No.	Primer	Sequence (5' - 3')	Sl. No.	Primer	Sequence (5' - 3')	Sl. No.	Primer	Sequence (5' - 3')	Sl. No.	Primer	Sequence (5' - 3')
1	OPA03	AGTCAGCCAC	31	OPAD06	AAGTGCACGG	61	OPAE19	GACAGTCCCT	91	OPBE12	GGTTGTTCCC
2	OPA05	AGGGGTCTTG	32	OPAD08	GGCAGGCAAG	62	OPAE20	TTGACCCCAG	92	OPBE13	TCGGTGAGTC
3	OPA08	GTGACGTAGG	33	OPAD09	TCGCTTCTCC	63	OPAF06	CCGCAGTCTG	93	OPBE14	CTTTGCGCAC
4	OPA09	GGGTAACGCC	34	OPAD10	AAGAGGCCAG	64	OPAF10	GGTTGGAGAC	94	OPBE15	TTCGGCGATG
5	OPA10	GTGATCGCAG	35	OPAD11	CAATCGGGTC	65	OPAF11	ACTGGGCCTC	95	OPBE17	GGGAAAAGCC
6	OPA13	CAGCACCCAC	36	OPAD13	GGTTCCTCTG	66	OPAF12	GACGCAGCTT	96	OPBE18	CCAAGCCGTC
7	OPA16	AGCCAGCGAA	37	OPAD14	GAACGAGGGT	67	OPAF13	CCGAGGTGAC	97	OPBE19	AGGCCAACAG
8	OPA18	AGGTGACCGT	38	OPAD15	TTTGCCCCGT	68	OPAF16	TCCCGGTGAG	98	OPBE20	CAAAGGCGTG
9	OPA19	CAAACGTCGG	39	OPAD16	AACGGGCGTC	69	OPAF19	GGACAAGCAG	99	OPBF01	GGAGCTGACT
10	OPAB01	CCGTCGGTAG	40	OPAD17	GGCAAACCCT	70	OPAF20	CTCCGCACAG	100	OPBF02	GACACACTCC
11	OPAB03	TGGCGCACAC	41	OPAD18	ACGAGAGGCA	71	OPAH12	TCCAACGGCT	101	OPBF03	TCCCTTGACC
12	OPAB06	GTGGCTTGGA	42	OPAD19	CTTGGCACGA	72	OPAK08	CCGAAGGGTG	102	OPBF06	TCCACGGGCA
13	OPAB07	GTAAACCGCC	43	OPAD20	TCTTCGGAGG	73	OPAK09	AGGTCGGCGT	103	OPBF08	CCTGGGTCCA
14	OPAB08	GTTACGGACC	44	OPAE01	TGAGGGCCGT	74	OPAK13	TCCCACGAGT	104	OPBF13	CCGCCGGTAA
15	OPAB09	GGGCGACTAC	45	OPAE02	TCGTTCACCC	75	OPBB05	GGGCCGAACA	105	OPBF15	ACGCGAACCT
16	OPAB11	GTGCGCAATG	46	OPAE03	CATAGAGCGG	76	OPBB09	AGGCCGGTCA	106	OPBF16	AGGGTCCGTG
17	OPAB12	CCTGTACCGA	47	OPAE04	CCAGCACTTC	77	OPBC11	TTTTGCCCCC	107	OPBF18	AGCCAAGGAC
18	OPAB13	CCTACCGTGG	48	OPAE05	CCTGTCAGTG	78	OPBC12	CCTCCACCAG	108	OPBF20	ACCCTGAGGA
19	OPAB14	AAGTGCGACC	49	OPAE06	GGGGAAGACA	79	OPBD19	GGTTCCTCTC	109	OPN07	CAGCCCAGAG
20	OPAB15	CCTCCTTCTC	50	OPAE07	GTGTCAGTGG	80	OPBE01	CACTCCTGGT	110	OPP18	GGCTTGGCCT
21	OPAB16	CCCGGATGGT	51	OPAE08	CTGGCTCAGA	81	OPBE02	ACGCCTGTAG			
22	OPAB17	TCGCATCCAG	52	OPAE09	TGCCACGAGG	82	OPBE03	TGGACTCGGT			
23	OPAB18	CTGGCGTGTC	53	OPAE10	CTGAAGCGCA	83	OPBE04	CCCAAGCGAA			
24	OPAB20	CTTCTCGGAC	54	OPAE11	AAGACCGGGA	84	OPBE05	GGAACGCTAC			
25	OPAC06	CCAGAACGGA	55	OPAE12	CCGAGCAATC	85	OPBE06	CAGCGGGTCA			
26	OPAC12	GGCGAGTGTG	56	OPAE13	TGTGGACTGG	86	OPBE07	CCGTCCTATG			
27	OPAD01	CAAAGGGCGG	57	OPAE14	GAGAGGCTCC	87	OPBE08	GGGAAGCGTC			
28	OPAD02	CTGAACCGCT	58	OPAE15	TGCCTGGACC	88	OPBE09	CCCGCTTTCC			
29	OPAD03	TCTCGCCTAC	59	OPAE16	TCCGTGCTGA	89	OPBE10	AAGCGGCCCT			
30	OPAD04	GTAGGCCTCA	60	OPAE17	GGCAGGTTCA	90	OPBE11	GTCCTGCTGT			

Table 4. List of primer-pairs used in this study

Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs
1	OPA03 / OPA05	31	OPA05 / OPA08	61	OPAB01 / OPA08	91	OPAD02 / OPAB03	121	OPAD15 / OPAB06
2	OPA03 / OPA08	32	OPA05 / OPA09	62	OPAB01 / OPA09	92	OPAD02 / OPAB06	122	OPAD15 / OPAB08
3	OPA03 / OPA09	33	OPA05 / OPA10	63	OPAB01 / OPA10	93	OPAD02 / OPAB07	123	OPAD15 / OPAB09
4	OPA03 / OPA10	34	OPA05 / OPA13	64	OPAB01 / OPA13	94	OPAD02 / OPAB08	124	OPAD15 / OPAB12
5	OPA03 / OPA13	35	OPA05 / OPA16	65	OPAB01 / OPA16	95	OPAD02 / OPAB09	125	OPAD15 / OPAB13
6	OPA03 / OPA16	36	OPA05 / OPA18	66	OPAB01 / OPA18	96	OPAD02 / OPAB11	126	OPAD15 / OPAB14
7	OPA03 / OPA18	37	OPA05 / OPA19	67	OPAB01 / OPA19	97	OPAD02 / OPAB12	127	OPAD15 / OPAB16
8	OPA03 / OPA19	38	OPA05 / OPAB01	68	OPAB01 / OPAC06	98	OPAD02 / OPAB13	128	OPAD15 / OPAB17
9	OPA03 / OPAB01	39	OPA05 / OPAC06	69	OPAB01 / OPAF06	99	OPAD02 / OPAB14	129	OPAD15 / OPAB20
10	OPA03 / OPAC06	40	OPA05 / OPAF06	70	OPAB01 / OPAF10	100	OPAD02 / OPAB16	130	OPAD15 / OPAD03
11	OPA03 / OPAF06	41	OPA05 / OPAF10	71	OPAB01 / OPAF11	101	OPAD02 / OPAB17	131	OPAD15 / OPAD04
12	OPA03 / OPAF10	42	OPA05 / OPAF13	72	OPAB01 / OPAF13	102	OPAD02 / OPAB20	132	OPAD15 / OPAD09
13	OPA03 / OPAF11	43	OPA05 / OPAF16	73	OPAB01 / OPAF16	103	OPAD02 / OPBE01	133	OPAD15 / OPAD11
14	OPA03 / OPAF13	44	OPA05 / OPAF19	74	OPAB01 / OPAF19	104	OPAD02 / OPBE02	134	OPAD15 / OPAD13
15	OPA03 / OPAF16	45	OPA05 / OPAF20	75	OPAB01 / OPAF20	105	OPAD02 / OPBE03	135	OPAD15 / OPAD16
16	OPA03 / OPAH12	46	OPA05 / OPAH12	76	OPAB01 / OPAK08	106	OPAD02 / OPBE05	136	OPAD15 / OPAD17
17	OPA03 / OPAK08	47	OPA05 / OPAK08	77	OPAB01 / OPAK13	107	OPAD02 / OPBE06	137	OPAD15 / OPAD18
18	OPA03 / OPAK09	48	OPA05 / OPAK13	78	OPAB01 / OPBB05	108	OPAD02 / OPBE07	138	OPAD15 / OPAD19
19	OPA03 / OPAK13	49	OPA05 / OPBB05	79	OPAB01 / OPBB09	109	OPAD02 / OPBE08	139	OPAD15 / OPBE05
20	OPA03 / OPBB09	50	OPA05 / OPBB09	80	OPAB01 / OPBC12	110	OPAD02 / OPBE09	140	OPAD15 / OPBE06
21	OPA03 / OPBC12	51	OPA05 / OPBC12	81	OPAB01 / OPBD19	111	OPAD02 / OPBE10	141	OPAD15 / OPBE08
22	OPA03 / OPBD19	52	OPA05 / OPBD19	82	OPAB01 / OPBE04	112	OPAD02 / OPBE11	142	OPAD15 / OPBE09
23	OPA03 / OPBE04	53	OPA05 / OPBE04	83	OPAB01 / OPBF01	113	OPAD02 / OPBE12	143	OPAD15 / OPBE10
24	OPA03 / OPBF02	54	OPA05 / OPBF02	84	OPAB01 / OPBF02	114	OPAD02 / OPBE13	144	OPAD15 / OPBE12
25	OPA03 / OPBF03	55	OPA05 / OPBF03	85	OPAB01 / OPBF03	115	OPAD02 / OPBE14	145	OPAD15 / OPBE13
26	OPA03 / OPBF06	56	OPA05 / OPBF06	86	OPAB01 / OPBF06	116	OPAD02 / OPBE15	146	OPAD15 / OPBE14
27	OPA03 / OPBF08	57	OPA05 / OPBF08	87	OPAB01 / OPBF08	117	OPAD02 / OPBE17	147	OPAD15 / OPBE15
28	OPA03 / OPBF13	58	OPA05 / OPBF13	88	OPAB01 / OPBF13	118	OPAD02 / OPBE18	148	OPAD15 / OPBE17
29	OPA03 / OPBF15	59	OPA05 / OPBF15	89	OPAB01 / OPBF15	119	OPAD02 / OPBE19	149	OPAD15 / OPBE18
30	OPA03 / OPBF18	60	OPA05 / OPBF18	90	OPAB01 / OPBF18	120	OPAD02 / OPBE20	150	OPAD15 / OPBF16

Table 4 (..contd.). List of Primer-pairs used in this study

Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs
151	OPAE01 / OPAE02	181	OPAF11 / OPA05	211	OPAF20 / OPAB03	241	OPAF20 / OPBF20	271	OPBE18 / OPAD06
152	OPAE01 / OPAE03	182	OPAF11 / OPA08	212	OPAF20 / OPAB09	242	OPBC11 / OPAB06	272	OPBE18 / OPAD08
153	OPAE01 / OPAE04	183	OPAF11 / OPA09	213	OPAF20 / OPAB11	243	OPBC11 / OPAB07	273	OPBE18 / OPAD10
154	OPAE01 / OPAE05	184	OPAF11 / OPA10	214	OPAF20 / OPAB12	244	OPBC11 / OPAB08	274	OPBE18 / OPAD11
155	OPAE01 / OPAE06	185	OPAF11 / OPA13	215	OPAF20 / OPAB13	245	OPBC11 / OPAB09	275	OPBE18 / OPAD13
156	OPAE01 / OPAE07	186	OPAF11 / OPA16	216	OPAF20 / OPAB14	246	OPBC11 / OPAB12	276	OPBE18 / OPAD16
157	OPAE01 / OPAE08	187	OPAF11 / OPA18	217	OPAF20 / OPAB15	247	OPBC11 / OPAB13	277	OPBE18 / OPAD18
158	OPAE01 / OPAE09	188	OPAF11 / OPA19	218	OPAF20 / OPAB16	248	OPBC11 / OPAB14	278	OPBE18 / OPAD19
159	OPAE01 / OPAE10	189	OPAF11 / OPAC06	219	OPAF20 / OPAB17	249	OPBC11 / OPAB17	279	OPBE18 / OPAD20
160	OPAE01 / OPAE11	190	OPAF11 / OPAF06	220	OPAF20 / OPAB18	250	OPBC11 / OPAB18	280	OPBE18 / OPBE01
161	OPAE01 / OPAE12	191	OPAF11 / OPAF10	221	OPAF20 / OPAB20	251	OPBC11 / OPAB20	281	OPBE18 / OPBE02
162	OPAE01 / OPAE13	192	OPAF11 / OPAF13	222	OPAF20 / OPAD14	252	OPBC11 / OPAD04	282	OPBE18 / OPBE03
163	OPAE01 / OPAE14	193	OPAF11 / OPAF16	223	OPAF20 / OPAD18	253	OPBC11 / OPAD06	283	OPBE18 / OPBE06
164	OPAE01 / OPAE15	194	OPAF11 / OPAF19	224	OPAF20 / OPAD19	254	OPBC11 / OPAD08	284	OPBE18 / OPBE07
165	OPAE01 / OPAE16	195	OPAF11 / OPAF20	225	OPAF20 / OPAD20	255	OPBC11 / OPAD16	285	OPBE18 / OPBE08
166	OPAE01 / OPAE17	196	OPAF11 / OPAH12	226	OPAF20 / OPBE01	256	OPBC11 / OPAD20	286	OPBE18 / OPBE10
167	OPAE01 / OPAE19	197	OPAF11 / OPAK08	227	OPAF20 / OPBE02	257	OPBC11 / OPBE01	287	OPBE18 / OPBE11
168	OPAE01 / OPAE20	198	OPAF11 / OPAK09	228	OPAF20 / OPBE03	258	OPBC11 / OPBE03	288	OPBE18 / OPBE12
169	OPAE01 / OPAF06	199	OPAF11 / OPAK13	229	OPAF20 / OPBE05	259	OPBC11 / OPBE09	289	OPBE18 / OPBE13
170	OPAE01 / OPAF10	200	OPAF11 / OPBB05	230	OPAF20 / OPBE06	260	OPBC11 / OPBE11	290	OPBE18 / OPBE14
171	OPAE01 / OPAF13	201	OPAF11 / OPBB09	231	OPAF20 / OPBE07	261	OPBC11 / OPBE13	291	OPBE18 / OPBE17
172	OPAE01 / OPAF16	202	OPAF11 / OPBC12	232	OPAF20 / OPBE08	262	OPBE18 / OPAB07	292	OPBE20 / OPA05
173	OPAE01 / OPAF19	203	OPAF11 / OPBD19	233	OPAF20 / OPBE09	263	OPBE18 / OPAB11	293	OPBE20 / OPA08
174	OPAE01 / OPAF20	204	OPAF11 / OPBE04	234	OPAF20 / OPBE10	264	OPBE18 / OPAB12	294	OPBE20 / OPA09
175	OPAE01 / OPAK08	205	OPAF11 / OPBF03	235	OPAF20 / OPBE11	265	OPBE18 / OPAB14	295	OPBE20 / OPA10
176	OPAE01 / OPBB05	206	OPAF11 / OPBF06	236	OPAF20 / OPBE12	266	OPBE18 / OPAB15	296	OPBE20 / OPA13
177	OPAE01 / OPBB09	207	OPAF11 / OPBF08	237	OPAF20 / OPBE13	267	OPBE18 / OPAB18	297	OPBE20 / OPA16
178	OPAE01 / OPBD19	208	OPAF11 / OPBF13	238	OPAF20 / OPBE14	268	OPBE18 / OPAD01	298	OPBE20 / OPA18
179	OPAE01 / OPBF13	209	OPAF11 / OPBF15	239	OPAF20 / OPBE15	269	OPBE18 / OPAD03	299	OPBE20 / OPA19
180	OPAE01 / OPBF18	210	OPAF11 / OPBF18	240	OPAF20 / OPBE17	270	OPBE18 / OPAD04	300	OPBE20 / OPAC06

Table 4 (..contd.). List of Primer-pairs used in this study

Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs	Sl. No.	Primer-pairs
301	OPBE20 / OPAF06	331	OPBF01 / OPAF16	361	OPBF02 / OPBF18	391	OPBF20 / OPBE17
302	OPBE20 / OPAF10	332	OPBF01 / OPAF19	362	OPBF20 / OPAB03	392	OPP18 / OPN07
303	OPBE20 / OPAF13	333	OPBF01 / OPAF20	363	OPBF20 / OPAB11		
304	OPBE20 / OPAF16	334	OPBF01 / OPAK08	364	OPBF20 / OPAB12		
305	OPBE20 / OPAF19	335	OPBF01 / OPAK13	365	OPBF20 / OPAB13		
306	OPBE20 / OPAF20	336	OPBF01 / OPBB05	366	OPBF20 / OPAB14		
307	OPBE20 / OPAH12	337	OPBF01 / OPBB09	367	OPBF20 / OPAB15		
308	OPBE20 / OPAK08	338	OPBF01 / OPBD19	368	OPBF20 / OPAB16		
309	OPBE20 / OPBB05	339	OPBF01 / OPBE04	369	OPBF20 / OPAB17		
310	OPBE20 / OPBB09	340	OPBF01 / OPBF02	370	OPBF20 / OPAB18		
311	OPBE20 / OPBC12	341	OPBF01 / OPBF13	371	OPBF20 / OPAB20		
312	OPBE20 / OPBD19	342	OPBF02 / OPAC06	372	OPBF20 / OPAD01		
313	OPBE20 / OPBE04	343	OPBF02 / OPAE09	373	OPBF20 / OPAD03		
314	OPBE20 / OPBF01	344	OPBF02 / OPAE10	374	OPBF20 / OPAD09		
315	OPBE20 / OPBF02	345	OPBF02 / OPAF06	375	OPBF20 / OPAD11		
316	OPBE20 / OPBF03	346	OPBF02 / OPAF10	376	OPBF20 / OPAD13		
317	OPBE20 / OPBF08	347	OPBF02 / OPAF11	377	OPBF20 / OPAD14		
318	OPBE20 / OPBF13	348	OPBF02 / OPAF13	378	OPBF20 / OPAD18		
319	OPBE20 / OPBF15	349	OPBF02 / OPAF16	379	OPBF20 / OPAD19		
320	OPBE20 / OPBF16	350	OPBF02 / OPAF19	380	OPBF20 / OPAD20		
321	OPBE20 / OPBF18	351	OPBF02 / OPAF20	381	OPBF20 / OPBE01		
322	OPBF01 / OPAC06	352	OPBF02 / OPAH12	382	OPBF20 / OPBE02		
323	OPBF01 / OPAC12	353	OPBF02 / OPAK08	383	OPBF20 / OPBE03		
324	OPBF01 / OPAE09	354	OPBF02 / OPAK13	384	OPBF20 / OPBE05		
325	OPBF01 / OPAE10	355	OPBF02 / OPBB05	385	OPBF20 / OPBE06		
326	OPBF01 / OPAF06	356	OPBF02 / OPBB09	386	OPBF20 / OPBE07		
327	OPBF01 / OPAF10	357	OPBF02 / OPBC12	387	OPBF20 / OPBE08		
328	OPBF01 / OPAF11	358	OPBF02 / OPBD19	388	OPBF20 / OPBE09		
329	OPBF01 / OPAF12	359	OPBF02 / OPBE04	389	OPBF20 / OPBE10		
330	OPBF01 / OPAF13	360	OPBF02 / OPBF13	390	OPBF20 / OPBE13		

3.2. Experiment II. Confirmation of marker-trait association with Core and Donor lines and local varieties.

3.2.1 Field Experiment

3.2.1.1 Material

Ninety-three Core and Donor lines (C and D lines, obtained from International Molecular Breeding Programme, IRRI, Phillipines) along with checks were used for evaluation (Table 5).

3.2.1.2 Methods

3.2.1.2.1 Experimental site and design

The experiment was carried out at MASLAB greenhouse, UAS, Bangalore, which is located at a latitude of 13° 0' North; longitude of 77° 35' East and an altitude of 930 m above mean sea level (MSL). The experiment was conducted in pipes of 1 m long with a diameter of 16 cm during summer season (2001).

The experiment was laid out in RCBD design with 3 replications. The pipes were filled with a mixture of sandy clay loam and FYM in 1:4 proportion. The soil was fertilized according to the recommended package of practices. Seeds were direct seeded in each PVC pipe and after their germination, only one seedling was allowed to grow in one pipe. The plants were well watered daily through out the experiment.

Sampling was done on 70th day. The pipes were removed carefully and put in water overnight to loosen the soil. The next day, roots were cleaned thoroughly and carefully. The intact root system and separated roots of each plant were collected, labelled

Table 5. List of Core and Donor lines used for the study and their origin

Sl. No.	ID No	Variety Name	Origin	Sl. No.	ID No	Variety Name	Origin
1	C-02	BR24	Bangladesh	51	D-18	Yu-Qui-Gu	China
2	C-03	93072	China	52	D-19	Zhong 123	China
3	C-05	C418	China	53	D-20	Giza 159	Egypt
4	C-07	Cheng-Hui 448	China	54	D-23	Khao Daeng	GRC
5	C-08	Feng-Ai-Zan	China	55	D-24	Madhukar	GRC
6	C-09	Gang 16	China	56	D-25	Milagrosa, Zawa Banday	GRC
7	C-11	Hua-Gen-Xian 74	China	57	D-29	Bhavani	India
8	C-13	R644	China	58	D-30	IR50	India
9	C-14			59	D-31	Jhona 349	India
10	C-15	Shen-Nong 89366	China	60	D-32	Karnal Local	India
11	C-16	Y134	China	61	D-35		
12	C-17	Yunhui 290	China	62	D-36	TB154E-TB-2	Indonesia
13	C-18	Yuanjing 7	China	63	D-37	Binam	Iran
14	C-19	Yu-Xiang-Zan	China	64	D-38	Domsiah	Iran
15	C-20	Zao-Xian 14	China	65	D-39	Taron Molaii	Iran
16	C-21	Zhong 413	China	66	D-42	MR 167	Myanmar
17	C-22	Zhong-You-Zao 81	China	67	D-44	Innmayebaw	Myanmar
18	C-24	Bg 90-2	GRC	68	D-47	Theehtatyin	Myanmar
19	C-25	Basmati 370	India	69	D-48	Basmati 385	Pakistan
20	C-26	Co 43	India	70	D-49	IR6	Pakistan
21	C-29	Rasi	India	71	D-51	At 354	Sri Lanka
22	C-30	TKM 9	India	72	D-52	Bg 304	Sri Lanka
23	C-31	Cisanggarung	Indonesia	73	D-55	CS94	Vietnam
24	C-33	Amol 3(Sona)	Iran	74	D-56	OM1706	Vietnam
25	C-34	Khazar	Iran	75	D-57	OM1723	Vietnam
26	C-35	IR64a*	IRRI	76	D-58	X21	Vietnam
27	C-36	Nipponbare	Japan	77	D-59	X22	Vietnam
28	C-37	Gayabyeo	Korea	78	D-60	X23	Vietnam
29	C-38	Iksan 438	Korea	79	D-61	C71	Vietnam
30	C-39	Ilmibyeo	Korea	80	D-62	C70	Vietnam
31	C-40	Milyang 23	Korea	81	D-63	Q5	Vietnam
32	C-42	MR 84	Malayasia	82	D-66	Pahk maw peuhn meuang	Thailand
33	C-44	Manawathukha	Myanmar	83	D-67	Haoannong	China
34	C-45	Shwe Thwe Yin Hyy	Myanmar	84	D-73	Pokhreli	Nepal
35	C-46	Bg 300	Sri Lanka	85	D-75	Khole marshi	Nepal
36	C-47	Bg 94-1	Sri Lanka	86	D-76	Jumli marshi	Nepal
37	C-48	CR203	Vietnam	87	D-77	Govind	India
38	C-49	OM997	Vietnam	88	D-78	UPR191-66	India
39	C-50	PSB RC28	Phillippine	89	D-79	ASD18	India
40	C-51	PSB RC66	Phillippine	90	D-82	Phalguna	India
41	C-52	IR64	IRRI	91	D-85	Budda	India
42	C-53	TEQING	IRRI	92	D-86	Doddabyranellu	India
43	C-55	IR66897B	IRRI	93	D-87	Doddi	India
44	C-58	Dhan4	India	94	Check	IR20	
45	C-59	Pusa (Basmati1)	India	95	Check	IR36	
46	D-01	NAN29-2	China	96	Check	IR42	
47	D-03	Babaomi	China	97	Check	Jaya	
48	D-04	Diantun 502	China	98	Check	Moroberekan	
49	D-08	Jiangxi-Si-Miao	China	99	Check	C0-39	
50	D-13	Peng-Shan-Tie-Gan-Zan	China	100	Check	Azucena	

C = Core lines D= Donor lines

and stored in poly bags containing water for recording observations.

3.2.1.2.2 Recording of observations

The following observations were recorded on sample plants of each genotype.

1. **Plant height**: At 70 th day from sowing, the height of the plant from ground level to the tip of the longest leaf was measured in centimeters.

2. **Number of tillers per plant**: Number of tillers were counted on 70th day from sowing.

3. **Root length**: Root length was measured from collar region to the tip of the longest root in cm.

4. **Total root number**: Total number of roots per plant at crown region were counted and recorded after sampling.

5. **Root volume**: Root volume was determined in ml by water displacement method.

6. **Dry weight**: The root and shoot of each plant after separation were oven dried at 50°C for 144 hours. Average dry weights of samples were recorded in grams.

7. **Computed observations**: Total dry weight was obtained by adding shoot and root dry weight.

8. **Root to shoot ratio**: Root to shoot ratio was derived by using root and shoot dry weights.

3.2.1.2.3 Statistical analysis

The data on individual characters were analyzed using SAS v6.12 (SAS, 1989) at UAS, GKVK, Bangalore. Different statistical methods employed for the analysis are:

3.2.1.2.3.1 Analysis of variance (ANOVA)

Fisher's method of analysis of variance was carried out using general linear models (GLM) procedure of SAS for different characters in order to assess the variability among the genotypes. Treatment means were compared with critical difference values.

3.2.1.2.3.2 Phenotypic and genotypic coefficients of variation

The phenotypic and genotypic coefficients of variation were computed by adopting the formulae given by Burton and Devane (1953).

$$PCV = P/X \times 100$$

$$GCV = G/X \times 100$$

Where ,

PCV and GCV were classified (Robinson *et al.,* 1949) as given below

0-10%	Low
10-20%	Moderate
20 % and above	High

3.2.1.2.3.3 Heritability (Broad sense)

Heritability estimated as per cent of mean were computed for different characters using formula (Hanson *et al.*, 1956):

$$H (\%) = Vg/Vp \times 100$$

Where,

H = Heritability

Vg and Vp are additive genotypic variance and phenotypic variance respectively (Hanson *et al.*, 1956).

3.2.1.2.3.4 Genetic Advance (GA)

Genetic advance was calculated by the formula given by Johnson *et al.* (1955).

$$GA = H \times P \times k$$

Where,

GA = Genetic Advance

H =Heritability

P = Phenotypic standard deviation

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

3.2.1.2.3.5 Genetic Advance as per cent of mean

$$\text{GA as per cent of mean} = GA/\overline{X} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = Treatment mean for the character.

The genetic advance as per cent of mean was classified (Johnson *et al.*, 1955) as given below:

0-10%	Low
10-20%	Moderate
20% and above	High

3.2.1.2.3.6 Correlation analysis

Correlation coefficients were computed to find the association amongst characters using the formula given by Sunderraj *et al.*(1972).

$$r_{xy} = \frac{COV\ (x1y)}{V_x .\ V_y}$$

Where,

r_{xy} = Correlation coefficient between x and y

V_x = variance of x

V_y = Variance of y

Phenotypic and genotypic correlation were calculated using the formula (Singh and Chaudhary, 1985).

$$rg(x,y) = \frac{\sigma_{gx\ gy}}{Vgx.\ Vgy}$$

$$rP(x,y) = \frac{\sigma_{Px\ Py}}{V_P\ x.\ V_{Py}}$$

Where, σ_{gxgy} = genotypic covariance of x and y

V_{gx} = genotypic variance of x

V_{gy} = genotypic variance of y

σ_{PxPy} = phenotypic covariance of x and y

P_{gx} = phenotypic variance of x

Pgy = phenotypic variance of y

$r_{g(x,y)}$ = genotypic correlation of x and y

$r_{p(x,y)}$ = phenotypic correlation of x and y

Significance of correlation coefficients was tested at (n-2) df 't' table from Fisher and Yates table at 5% and 1% significance.

3.2.1.2.3.7 Contrast analysis

Contrast analysis of genotypes based on country of origin was carried out using 'contrast' option of GLM procedure (SAS).

3.2.2 Screening with molecular markers

3.2.2.1 DNA extraction (Mini Prep)

Twenty five day old seedlings were used for DNA extraction. DNA was prepared as per the method given by Zheng *et al.* (1995).

1. A healthy leaf sample (2 cm) was collected in a 1.5 ml eppendorf tube, labelled and brought to the laboratory on ice.

2. The leaf tissue was cut on into 0.5 cm long segments and placed in a well of spot test plate.

3. Four hundred micro liter of DNA extraction buffer was added. The tissue was ground with thick glass rod until the buffer turned dark green.

4. Another 400 μl of DNA extraction buffer was added, mixed and allowed to stand for some time.

5. The supernatant (about 400 μl) was transferred back to 1.5 ml eppendorf tube.

6. To this, 400 μl of chloroform was added, thoroughly mixed and centrifuged for 3 minutes at 14000 rpm.

7. The top (aqueous phase) was transferred to another 1.5 ml tube. To this tube, 800μl of 96 per cent ethanol was added, mixed thoroughly and centrifuged for 3 minutes at 14000 rpm. The supernatant was decanted.

8. The DNA pellet was washed with 70 per cent ethanol, air-dried and re-suspended in 50 μl T.E. buffer and stored at -20° C.

The quality and concentration of DNA was estimated as described earlier (3.1.2.2.1 and 3.1.2.2.2).

3.2.2.2 RAPD marker analysis

The reaction mixture used was same as described in 3.1.2.2.5 except that 20 ng of a single decanucleotide primer (OPBH14) was used instead of conjugate primers. The amplification profile was same as described in 3.1.2.2.5 except that the annealing temperature was increased from 38° C to 44° C. The amplicons were electrophoresed on 1.4 % gel (3.1.2.2.2) and the gel documented.

3.2.2.3 Microsatellite marker analysis

The microsatellite marker RM201 which is linked to root length (Shashidhar *et al.*, 2000) was used for screening of C and D lines. The details of RM201 are presented in the table below:

Locus	Clone	Chr. No.	Size Range (bp)	Forward primer	Reverse primer
RM201	CT6	9	144-158	5′ctcgtttattacc tacagtacc 3′	5′ctacctcctttc tagaccgata 3′

The PCR reaction mixture consisted of 50 ng of template DNA 10 ng each of primers, 100 μM dNTPs, 0.8 U of *Taq* DNA polymerase and 1 X PCR buffer in a volume of 20 μl.

The amplification profile was as follows

Initial denaturation temperature	:	94°C	-	5 min.
Denaturation	:	94°C	-	1 min.
Primer annealing	:	56°C	-	0.5 min.
Primer extension	:	72°C	-	1 min.

Latter three stages were repeated 35 times

Complete primer extension	:	72°C	-	5 min.
Soak temperature	:	4°C	-	Until removed

3.2.2.3.1 Polyacrylamide gel electrophoresis

The microsatellite amplicons were separated by non-denaturing polyacrylamide gel electrophoresis on a 5 per cent gel.

The polyacrylamide gel was prepared as described by Bassam *et al.* (1991). The polyacrylamide gel was prepared with 1 X TBE as mentioned in appendix 1.

Required volume of gel mixture containing 30 per cent acrylamide: bis-acrylamide (29: 1) was prepared in double distilled water, filtered and stored. Just prior to casting 10 x TBE (0.89 M Tris, 0.89 M Boric acid, 0.11 M EDTA, pH 8.3), ammonium persulphate (10 % APS) and N, N, N^1, N^1, tetramethyl ethylene diamine (TEMED) were added at the concentrations of 10 per cent and 0.05 per cent respectively. After thorough mixing the gel mixture was poured into a template set by clean glass plates separated by spacer strips of 1.0 mm thickness. Immediately after pouring a 1.0 mm thick comb was inserted and the gel was allowed to polymerize for 30 minutes. After removing the comb, the gel set up was mounted on electrophoresis apparatus. After flushing the wells with running buffer (1 x TBE), the gel was subjected to a pre run with 1 X TBE as electrode buffer. Samples were prepared for loading by adding 3 ml of loading dye to 10 ml each of PCR amplified sample. After flushing the wells, again the amplification products were loaded onto gel along with 100 bp ladder as marker in separate lanes. The electrophoresis was at 8 V/cm for 4 hours. The gel was removed from the plates and silver stained.

3.2.2.3.2 Silver staining of DNA gels

Polyacrylamide gels were fixed using Buffer A solution (washed twice for 3 minutes) stained with Buffer B for 10 min. rinsed twice with distilled water and developed in an alkaline solution *i.e.,* Buffer C for 10-30 min. The gel was rinsed again with distilled water to remove any trace of the developing

solution. Developing was done until clear bands appeared and then gel was kept in a fixer. The stained gels were packed in covers and used for scoring of bands and photography. The details of silver staining reagents are given in appendix 2.

3.2.2.3.3 One-way analysis of variance

One-way ANOVA was carried out using GLM procedure of SAS to find out the association of markers with root length and R^2 values were worked out to find the amount of variability explained by these markers.

Experimental Results

IV. EXPERIMENTAL RESULTS

The experimental results of this investigation have been presented under the following sub-headings

1. Identification of molecular markers linked to maximum root length in rice.

2. Evaluation of rice genotypes for root morphological characters in the field.

3. Confirmation of marker-trait association with diverse genetic material.

4.1 Identification of molecular markers linked to maximum root length in rice.

In this study, 110 primers were randomly chosen. Two RAPD primers were used in a single reaction. Three hundred and ninety two primer-pairs were used for screening the bulks out of 5,995 possible primer combinations. Distinct amplified products were amplified from 335 primer-pairs. A total of 2,851 discrete bands were recorded. An average of 8.5 bands were produced per primer-pair. One hundred and twenty seven bands were scored as polymorphic between the two bulks from 47 primer-pairs. Confirmation of these putative polymorphic primer-pairs resulted in monomorphism between the bulks except for one primer-pair OPBF02/OPAF20 which showed polymorphism between the two bulks. Two bands were produced in bulk B (1.2kb and 0.8 kb bands) while bulk A had no such bands at that level. However, on re-confirmation for three time, this primer-pair failed to produce the same results.

4.2 Evaluation of rice genotypes for root morphological characters in the field.

This experiment was conducted during summer 2001. Ninety three Core and Donor lines along with six checks were used in the study. These genotypes were evaluated in PVC pipes measuring 1 m in length and 18 cm in diameter in a RCBD design with three replications. The results obtained in this experiment are presented below.

4.2.1 Mean values of various characters

4.2.1.1 Plant height (PHT)

Variety D-86 recorded maximum PHT (122.33 cm) while, D-59 recorded minimum (45.00 cm). The mean across the genotypes studied was 75.51 cm. The means of hundred varieties for nine different characters studied are presented in appendix 3.

4.2.1.2 Number of Tillers (NOT)

The range for this trait was 20.00 with C-34 recording maximum (24) and D-59 recording minimum number of tillers (5.33) respectively (Table 6.). The mean for this trait was 12.36.

4.2.1.3 Shoot dry weight (SDW)

The mean for SDW was 13.68 g. The range for this trait was 35.4 g with D-19 recording maximum (34.67 g) and D-13 recording minimum SDW (1.71 g) respectively. A range of 35.4 g was recorded.

4.2.1.4 Root length (RTL)

The maximum RTL observed for Moroberekan (85.67 cm)

Table 6. Descriptive statistics and genetic parameters for ten characters studied in diverse rice accessions at 70 DAS.

Trait	Mean	SE	Range		Heritability(%)	GCV (%)	PCV (%)	GA (%) mean
			Min.	Max.				
Plant height (cm)	75.51	6.30	45.00	122.33	77.20	18.66	21.23	33.76
Number of tillers	12.36	0.72	5.33	24.33	93.30	27.00	27.95	53.72
Shoot dry weight (g)	13.68	8.11	1.71	34.67	94.50	41.61	42.79	83.33
Root length (cm)	38.72	4.04	22.33	85.67	78.30	24.33	27.49	44.34
Root volume (cc)	18.95	7.50	5.00	80.00	48.60	47.55	68.18	68.34
Total root number	55.08	8.60	21.67	101.33	64.90	26.04	32.32	43.21
Root dry weight (g)	2.49	0.62	0.37	14.53	90.80	65.79	70.50	147.39
Total dry weight (g)	16.17	9.91	2.85	39.94	95.40	44.74	45.80	90.04
Root to shoot ratio	0.18	0.08	0.05	0.82	66.00	62.53	69.28	122.22

followed by Azucena (79.00 cm) and the minimum RTL was observed for D-13(22.33 cm). The mean was 38.72 cm. D-82 and D-31 recorded means of 59.33 and 58 cm, respectively.

4.2.1.5 Root volume (RTV)

Highest RTV was recorded for Moroberekan (80 cc) while, lowest was recorded for D-63 (5.00 cc). The mean and range for RTV was 18.95 cc and 157 cc, respectively.

4.2.1.6 Total root number (TRN)

D-79 recorded highest mean for TRN (101.33) and D-52 showed lowest mean (21.67). The mean across all varieties was 55.08. The range for this trait was 135.

4.2.1.7 Root dry weight (RDW)

Highest and lowest root dry weights were recorded for D-32 (14.53 g) and D-59 (0.37 g), respectively. The mean and range for RDW were 2.49 g and 14.80 g, respectively.

4.2.1.8 Total dry weight (TDW)

TDW was highest in D-19 (39.94 g) and lowest in D-13 (2.85 g). The mean was 16.17 g and range was 39.5 g.

4.2.1.9 Root to shoot ratio (R/S)

Investment in root per gram of shoot was highest in Moroberekan (0.82) and lowest in D-75 (0.05). The mean and range across the varieties studied were 0.18 g and 1.58 g, respectively.

Table 7. Analysis of variance for nine characters studied in rice

Source	D F	PHT	NOT	SDW	RTL	RTV	TRN	RDW	TDW	R/S
Genotypes	99	653.9 **	34.23 **	99.08 **	290.8 **	329.5 **	728.3 **	17.58 **	159.5 **	0.06 **
Replication	2	149.4	2.50	1.83	26.47	130.1	11.83	0.62	2.05	0.01
Error	198	58.69	0.79	1.87	24.59	85.76	111.3	0.57	2.50	0.01
SE		6.30	0.72	8.11	4.04	7.50	8.60	0.62	9.91	0.08
CD (5%)		12.33	1.44	2.20	7.98	14.91	16.98	1.22	2.55	0.15
CD (1%)		16.26	1.90	2.91	10.53	19.67	22.4	1.61	3.36	0.20
CV (%)		10.14	7.22	10.00	12.81	28.86	19.15	20.41	9.78	22.06

* Significant at p=0.05
** Significant at p=0.01

PHT = Plant height (cm)
NOT = Number of tillers
SDW = Shoot dry weight (g)
RTL = Root length (cm)
RTV = Root volume (cc)

TRN = Total root number
RDW = Root dry weight (g)
TDW = Total dry weight (g)
R/S = Root to shoot ratio

4.2.2 Analysis of variance

Treatment mean sum of squares were significantly differing for all the characters studied (≤0.01p) (Table 7.).

4.2.3 Genotypic and phenotypic correlations

Plant height was found to have significant and positive correlation with total root length (0.39, ≤0.01p), root volume (0.51, ≤0.01p), root dry weight (0.43, ≤0.01p), shoot dry weight (0.55, ≤0.01p) total dry weight (0.58) and root to shoot ratio (0.22, ≤0.05p) (Table 8.).

Number of tillers showed significant and correlation with root volume (0.31, ≤0.01p), total root number (0.45, ≤0.01p), root dry weight (0.26, ≤0.05p), shoot dry weight (0.48 ≤0.01p) and total dry weight (0.46 ≤0.01p).

Root volume was found to have positive and significant correlation with total root number (0.51 ≤0.01p), root length (0.73, ≤0.01p), root dry weight (0.93, ≤0.01p), shoot dry weight (0.55, ≤0.01p), and total dry weight (0.74, ≤0.01p).

Significant and positive correlation was found for total root number with root length (0.37, ≤0.01p), root dry weight (0.55,≤0.01p), shoot dry weight (0.40, 0.01p), total dry weight (0.49, ≤0.01p) and root to shoot ratio (0.49, ≤0.01p).

Root length showed positive and significant correlation with root dry weight (0.59 ≤0.01p), shoot dry weight (0.48, ≤0.01p) total dry weight (0.57, ≤0.01p) and root to shoot ratio (.38, ≤0.01p).

Root dry weight showed positive and significant correlation with shoot dry weight (0.53, ≤0.01p), total dry weight (0.74 ≤0.01p) and

Table 8. Genotypic and phenotypic correlations among nine characters in rice

		PHT	NOT	SDW	RTL	RTV	TRN	RDW	TDW
Number of tillers	P	-0.02							
	G	-0.06							
Shoot dry weight (g)	P	0.47 **	0.45 **						
	G	0.55 **	0.48 **						
Root length (cm)	P	0.34 **	0.15	0.42 **					
	G	0.39 **	0.15	0.48 **					
Root volume (cc)	P	0.38 **	0.25 *	0.38 **	0.64 **				
	G	0.51 **	0.31 **	0.55 **	0.73 **				
Total root number	P	0.12	0.36 **	0.31 **	0.37 **	0.52 **			
	G	0.08	0.45 **	0.40 **	0.37 **	0.51 **			
Root dry weight (g)	P	0.36 **	0.24 *	0.49 **	0.51 **	0.63 **	0.44 **		
	G	0.43 **	0.26 *	0.53 **	0.59 **	0.93 **	0.55 **		
Total dry weight (g)	P	0.50 **	0.44 **	0.96 **	0.50 **	0.51 **	0.39 **	0.72 **	
	G	0.58 **	0.46 **	0.96 **	0.57 **	0.74 **	0.49 **	0.74 **	
Root to shoot ratio	P	0.16	0.05	0.02	0.24 *	0.42 **	0.35 **	0.78 **	0.28 **
	G	0.22 *	0.06	0.05	0.34 **	0.73 **	0.49 **	0.80 **	0.30 **

* Significant at p=0.05

** Significant at p=0.01

root to shoot ratio (0.80, 0.01p). Shoot dry weight showed positive correlation with total dry weight (0.96, 0.01p). Total dry weight also showed positive correlation with root to shoot ratio (0.30, 0.05P).

4.2.4 Genetic variability

Root dry weight has recorded highest genotypic (65.79 %) and phenotypic (70.5 %) co-efficient of variation followed by root to shoot ratio (62.53 % and 69.28 %)(Table 6). The lowest genotypic (18.66 %) and phenotypic (21.23 %) co-efficient of variation were recorded for plant height. Root volume, root length, total dry weight, shoot dry weight and root to shoot ratio also showed higher genotypic and phenotypic co-efficients of variation (Fig. 1).

4.2.5 Heritability and genetic advance

Heritability (narrow sense) was higher for total dry weight (95.4 %), shoot dry weight (87.2 %), number of tillers (93.3 %) and root dry weight (90.8 %). Moderately high heritability was recorded for root length (78.3 %) and plant height (77.2 %). Lowest heritability was recorded for root volume (51.35 %) (Table 6) (Fig. 2).

Maximum expected genetic advance as per cent of mean was recorded for root dry weight (147.39 %) followed by root to shoot ratio (122.22 %), total dry weight (90.04 %). Lowest expected genetic advance as per cent of mean was observed for plant height (33.76 %).

4.2.6 Contrast analysis

Highly significant differences were observed between C and D lines, between Indian varieties and varieties from other countries for plant height (Table 9). Genotypes from GRC recorded highest mean for plant height (85.47 cm) followed by Indian varieties (81.44cm). Korean genotypes recorded lowest plant height (62.17 cm) (Table 10).

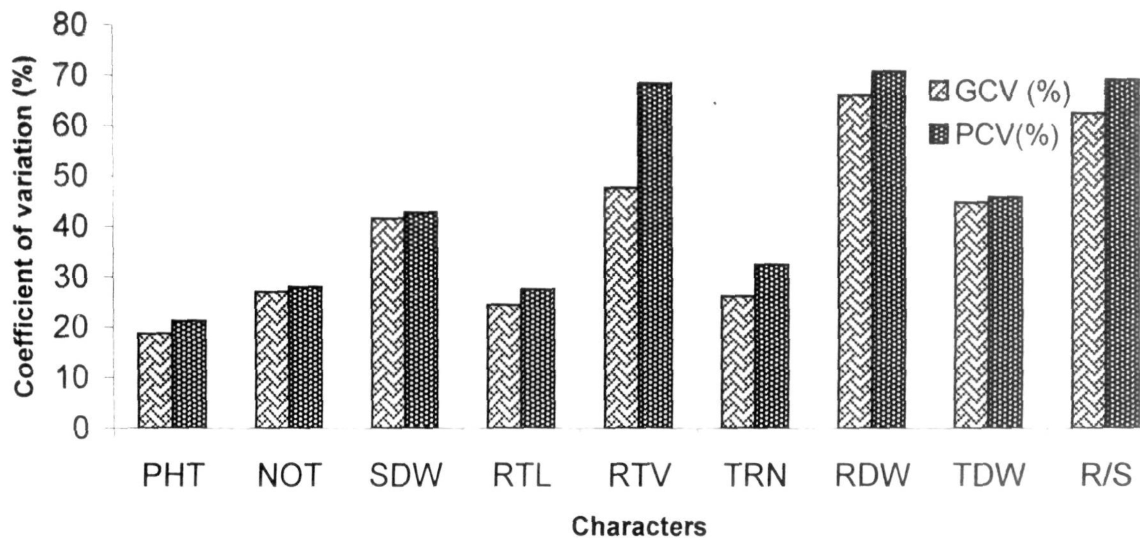

Fig 1. Phenotypic and genotypic coefficients of variation for nine characters of rice

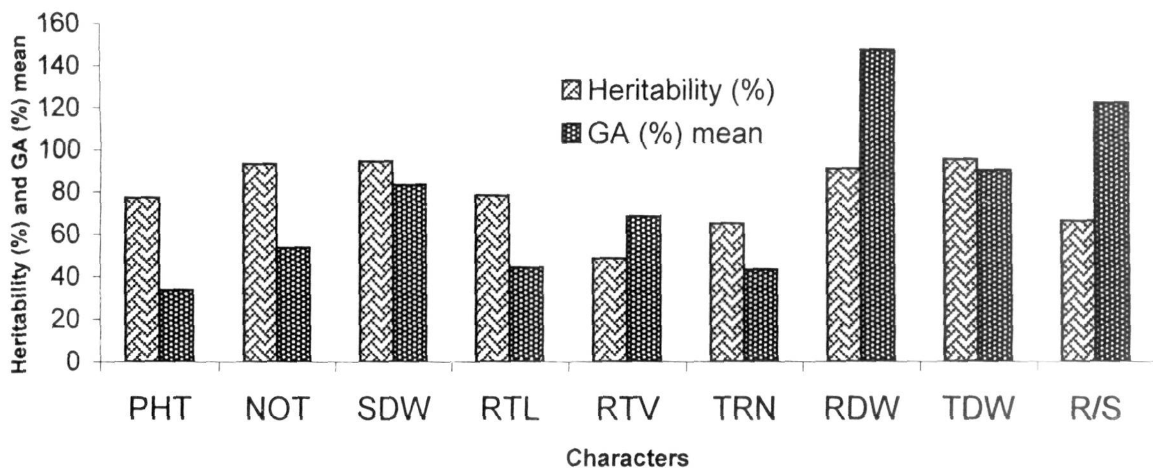

Fig 2. Heritability and genetic advance (as percent mean) for nine characters of rice

PHT = Plant height (cm)
NOT = Number of tillers
SDW = Shoot dry weight (g)
RTL = Root length (cm)
RTV = Root volume (cc)
TRN = Total root number
RDW = Root dry weight (g)
TDW = Total dry weight (g)
R/S = Root to shoot ratio

Table 9. Interesting contrasts for eight characters among diverse rice accessions.

Contrast	D.F.	Mean Sum of Squares							
		PHT	NOT	SDW	RTL	RTV	TRN	RDW	TDW
Core *vs* Donor lines	1	9014.87 **	12.59 **	72.93 **	399.41 **	14.49 *	13.79	3.00 *	105.49 **
India *vs* China	1	457.95 **	102.01 **	80.12 **	518.37 **	494.30	518.46 *	14.01 **	161.15 **
India *vs*. Iran	1	238.78 *	45.68 **	22.30 **	18.79 *	31.61	1548.03 **	0.23	27.06 **
India *vs* Vietnam	1	794.16 **	25.53 **	182.61 **	473.85 **	20.70	0.22	20.82 **	326.75 **
India *vs* GRC	1	776.57 **	2.27	122.55 **	20.08 **	97.22	122.66	3.06 *	164.35 **
India *vs* Korea	1	1670.79 **	1.54	28.11 **	1.44	34.61	875.75 **	1.12	40.45 **
India *vs* IRRI	1	81.41	186.51 **	274.79 **	13.7 **	1174.77 **	441.45 *	11.27 **	397.34 **
China *vs* Iran	1	851.79 **	0.29	0.66	18.79	67.92	664.19 *	3.47 *	7.17
China *vs* Vietnam	1	121.61	189.43 **	40.91 **	0.43	198.80	0.22 *	2.40 *	63.14 **

* Significant at p=0.05
** Significant at p=0.01

Table 10. Means values of some interesting contrasts for eight characters

	PHT	NOT	SDW	RTL	RTV	TRN	RDW	TDW
India	81.44	13.33	15.60	45.41	28.00	58.93	3.98	19.59
China	76.76	10.25	13.27	36.21	14.99	54.61	1.90	15.17
Iran	74.27	13.27	13.26	40.20	19.27	55.47	2.55	15.81
Vietnam	73.76	14.27	12.75	35.79	18.70	58.76	2.17	14.93
GRC	85.47	14.40	15.01	39.33	19.07	50.27	3.27	18.27
Korea	62.17	12.25	12.00	38.83	20.25	61.75	2.12	14.12
IRRI	68.29	13.00	15.31	39.78	17.43	60.10	2.62	17.94
Core lines	68.09	12.08	12.77	38.47	17.05	53.93	2.19	14.96
Donor lines	81.20	12.61	14.09	37.28	18.92	54.50	2.46	16.55

PHT = Plant height (cm)
NOT = Number of tillers
SDW = Shoot dry weight (g)
RTL = Root length (cm)
RTV = Root volume (cc)
TRN = Total root number
RDW = Root dry weight (g)
TDW = Total dry weight (g)
R/S = Root to shoot ratio

Significant differences were observed between genotypes of different countries for number of tillers except for Indian genotypes *vs* GRC genotypes, Indian *vs* Korean and Chinese *vs* Iranian genotypes. Maximum number of tillers was observed in the genotypes from GRC (14.40) and Vietnam (14.27).

Indian genotypes showed highest mean for root dry weight (3.98 g) while Chinese genotypes showed lowest root dry weight (1.98 g). Significant contrasts for different countries were observed for this trait.

The mean of Indian genotypes was highest for root length (45.41 cm) while Vietnamese genotypes showed lowest root length (35.79). Significant contrasts were obtained for Indian *vs* Chinese, and Indian *vs* IRRI varieties.

Most of the contrasts were non-significant for root volume. C lines were significantly different from D lines for root volume (≤0.05p) while Indians were significantly different from IRRI varieties (≤0.01p).

Maximum number of tillers was recorded in Korean varieties (61.8) followed by IRRI genotypes (60.1). Significant contrasts for different countries were observed for this trait.

Significant contrasts were observed for root dry weight. Indian varieties showed maximum mean values for root dry weight (3.98 g) Chinese genotypes recorded lowest for root dry weight. Contrasts were significant for most of the countries except for Indian *vs* Iranians and Indian *vs* Korean genotypes.

Except contrast for Chinese *vs* Iranian varieties, all other contrasts were significant for total dry weight. Significant contrasts for different countries were observed for this trait (≤0.01p). Indian varieties showed maximum mean values for total dry weight (19.59 g)

while Korean varieties recorded lowest total dry weight (14.12 g).

4.2.7 Confirmation of marker-trait association with diverse genetic material

The marker data obtained from screening of the two markers OPBH14 and RM201 on 100 genotypes and their mean values are presented in table 11.

Extreme genotypes for root length i.e., ±1 standard deviation form the mean were compared with marker data. RAPD marker OPBH14 recorded 8 individuals out of 11 phenotypically short rooted individuals as short rooted while RM201 recorded 9 genotypes as short rooted. Similarly, out of 9 phenotypically long rooted individuals, 5 were recorded by OPBH14 as long rooted while 4 individuals were recorded as long rooted by RM201 (Table 12).

Out of 100 varieties screened with OPBH14, 61 individuals showed IR64 type band (1.5kb) and their mean was 37.05 cm. Thirteen individuals showed heterozygous band i.e., both IR64 and Azucena type bands, and their mean was 34.59 cm while the rest showed Azucena type bands (1.47 kb) with a mean of 44.7 cm. The mean of individuals showing Azucena type band was significantly differently different from individuals showing IR64 type band and heterozygous band (<0.01p). But there was no significant difference between the means of the latter two groups

Similarly, out of 100 varieties screened with microsatellite marker RM201, 70 individuals showed IR64 type band (158 bp) and their mean was 36.89 cm, 4 individuals showed heterozygous band i.e., both IR64 and Azucena type bands, and their mean was 41.17 cm while 26 genotypes showed Azucena type bands (1.47 kb) with a mean of 43.27 cm. The mean of individuals showing Azucena type band was significantly different from individuals showing IR64 type band

Details of Plate 1.

M - Marker

1 - IR64

2 - Azucena

3 - BR24

4 - Hua-Gen-Xian

5 - Yunhui 290

6 - Bg 90-2

7 - CO-43

8 - MR 84

9 - Bg 300

10 - CR 203

11 - IR64

12 - Azucena

M - Marker

Details of Plate 2.

M - Marker

1 - Azucena

2 - IR64

3 - BR24

4 - 93072

5 - C418

6 - Feng-Ai-Zan

7 - Gang 16

8 - Hua-Gen-Xian

9 - R644

10 - Sheng-Nong 89366

11 - Yuanjing 7

12 - Yu-Xiang-Zan

13 - Zao-Xian 14

14 - Basmati 370

15 - TKM 9

16 - Cisanggarung

17 - Azucena

18 - IR64

M - Marker

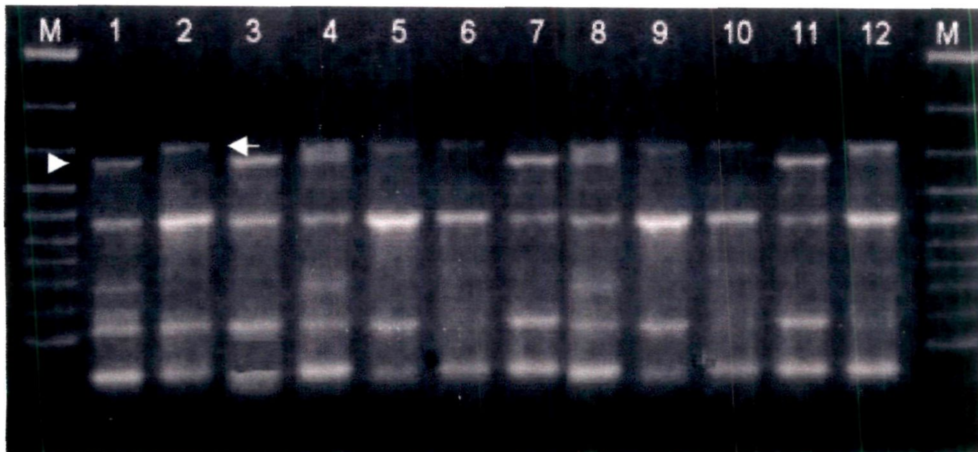

Plate 1. Detection of root length specific bands in rice accessions generated by RAPD primer OPBH14

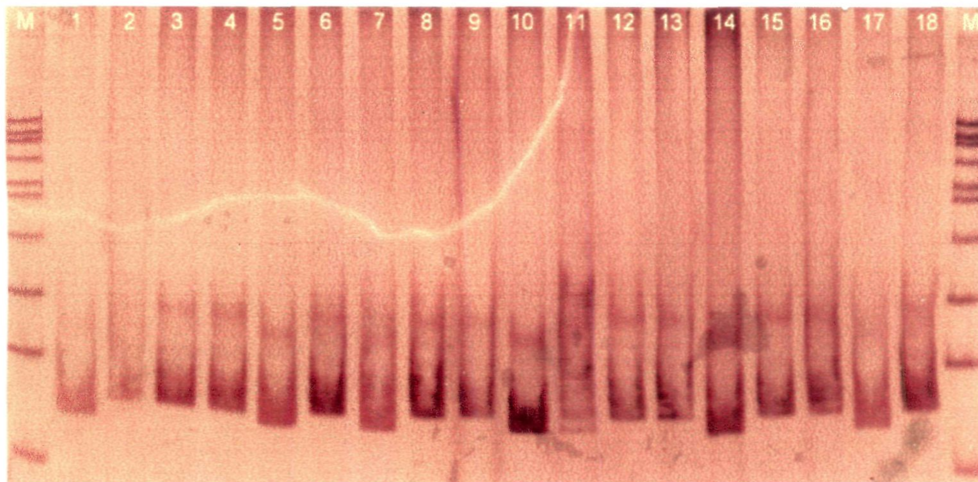

Plate 2. Detection of root length specific bands in rice accessions generated by microsatellite marker RM201

Table 11. Means of root length along with marker data in diverse accessions of rice.

Sl.No.	Line	OPBH14	RM201	RTL	Sl.No.	Line	OPBH14	RM201	RTL
1	BR24	1	1	34.00	51	Yu-Qui-Gu	1	1	27.67
2	93072	1	1	32.00	52	Zhong 123	3	1	46.33
3	C418	3	3	45.33	53	Giza 159	2	1	22.67
4	Cheng-Hui 448	1	1	55.00	54	Khao Daeng	1	1	32.67
5	Feng-Ai-Zan	1	1	41.00	55	Madhukar	3	3	42.33
6	Gang 16	1	3	37.67	56	Milagrosa, Zawa Banday	3	3	40.67
7	Hua-Gen-Xian 74	2	1	42.00	57	Bhavani	1	1	34.67
8	R644	1	1	27.67	58	IR50	1	1	27.33
9		1	1	29.00	59	Jhona 349	3	3	58.00
10	Shen-Nong 89366	1	3	40.00	60	Karnal Local	3	3	39.67
11	Y134	1	3	41.00	61		1	1	38.67
12	Yunhui 290	3	3	31.00	62	TB154E-TB-2	3	1	33.67
13	Yuanjing 7	2	2	34.67	63	Binam	3	3	45.67
14	Yu-Xiang-Zan	1	1	36.33	64	Domsiah	3	3	30.67
15	Zao-Xian 14	1	1	31.67	65	Taron Molaii	3	3	47.00
16	Zhong 413	1	1	33.00	66	MR 167	1	1	45.33
17	Zhong-You-Zao 81	1	1	30.00	67	Innmayebaw	1	1	41.00
18	Bg 90-2	3	3	46.33	68	Theehtatyin	2	3	40.67
19	Basmati 370	3	3	43.00	69	Basmati 385	3	3	36.67
20	Co 43	1	1	39.33	70	IR6	1	1	28.50
21	Rasi	1	1	30.50	71	At 354	2	1	36.33
22	TKM 9	1	1	39.33	72	Bg 304	2	1	26.67
23	Cisanggarung	1	1	44.00	73	CS94	2	1	37.33
24	Amol 3(Sona)	1	3	23.67	74	OM1706	1	1	37.00
25	Khazar	3	2	54.33	75	OM1723	2	1	41.33
26	IR64a*	1	1	33.33	76	X21	1	1	33.33
27	Nipponbare	3	3	30.33	77	X22	1	1	33.00
28	Gayabyeo	3	3	46.00	78	X23	1	1	40.67
29	Iksan 438	3	3	32.00	79	C71	1	1	31.00
30	Ilmibyeo	3	3	44.00	80	C70	2	1	30.00
31	Milyang 23	1	1	33.33	81	Q5	2	1	30.33
32	MR 84	2	1	42.67	82	Pahk maw peuhn meuang	1	1	46.33
33	Manawathukha	1	1	41.33	83	Haoannong	1	1	36.67
34	Shwe Thwe Yin Hyy	1	1	34.00	84	Pokhreli	1	3	40.33
35	Bg 300	1	1	40.67	85	Khole marshi	1	1	38.00
36	Bg 94-1	1	1	37.67	86	Jumli marshi	1	1	41.00
37	CR203	1	1	33.00	87	Govind	1	1	34.67
38	OM997	1	1	37.67	88	UPR191-66	1	1	47.33
39	PSB RC28	1	1	39.33	89	ASD18	3	1	27.00
40	PSB RC66	1	1	46.00	90	Phalguna	1	1	59.33
41	IR64	1	1	50.50	91	Budda	1	1	35.33
42	TEQING	1	1	37.00	92	Doddabyranellu	1	1	50.00
43	IR66897B	1	1	41.00	93	Doddi	3	2	46.33
44	Dhan4	3	3	50.67	94	IR20	1	1	27.67
45	Pusa (Basmati1)	1	1	38.67	95	IR36	1	1	48.00
46	NAN29-2	2	2	29.33	96	IR42	2	1	35.67
47	Babaomi	1	3	27.67	97	Jaya	1	1	33.00
48	Diantun 502	3	1	33.67	98	Moroberekan	3	3	85.67
49	Jiangxi-Si-Miao	3	1	47.00	99	C0-39	1	1	33.00
50	Peng-Shan-Tie-Gan-Zan	1	1	22.33	100	Azucena	3	3	79.00

1- IR64 type band ·2- Heterozygous band 3- Azucena type band RTL- Root length (cm)

Table 12. Per cent individuals (±1 SD) showing tight association with the markers and root length

Line	OPBH14	RM201	RTL	SD
D-13	1	1	22.33	-1
D-20	2	1	22.67	-1
C-33	1	3	23.67	-1
D-52	2	1	26.67	-1
D-79	3	1	27.00	-1
D-30	1	1	27.33	-1
C-13	1	1	27.67	-1
D-03	1	3	27.67	-1
D-18	1	1	27.67	-1
IR20	1	1	27.67	-1
D-49	1	1	28.50	-1
Percentage	72.73	81.82		
Line	OPBH14	RM201	RTL	SD
D-86	1	1	50.00	+1
C-52	1	1	50.50	+1
C-58	3	3	50.67	+1
C-34	3	2	54.33	+1
C-07	1	1	55.00	+1
D-31	3	3	58.00	+1
D-82	1	1	59.33	+1
Azu	3	3	79.00	+1
Moro	3	3	85.67	+1
Percentage	55.56	44.44		

1- IR64 type band   2- Heterozygous band
3- Azucena type band   RTL- Root length (cm)

and heterozygous band (<0.01p). But there was no significant difference between the means of the latter two groups (Fig 3).

Forty-nine genotypes were recorded as having IR64 type band for both markers with a mean of 37.07 cm. Two genotypes (C-18 and D-01) showed heterozygous bands for both markers. They recorded 34.67 cm and 29.33 cm, respectively. Fifteen individuals showed Azucena type bands and the mean was 46.09 cm, which was significantly higher than the other two groups (Fig 4 and 5)

Ten genotypes were identified by BH14 as IR64 type but RM201 showed Azucena type band in them (mean = 37.28 cm) and 11 individuals were identified by OPBH14 as Azucena type but RM201 recorded them as IR64 type. (Mean =40.12) (Fig 6).

4.2.7.1 Correlation between markers and root length

Significant and positive association was observed between OPBH14 and root length (0.28, $\leq 0.01p$). RM201 showed positive and significant correlation with root length(0.24, $\leq 0.05p$) (Table 13).

Table 13. Correlation between markers and root length

Trait	RM201	OPBH14
Root length (cm)	0.24 *	0.28 **

* Significant at p=0.05
** Significant at p=0.01

4.2.7.2 One-way analysis of variance

One way ANOVA showed highly significant association of RAPD marker OPBH14 and microsatellite marker RM201 with root length ($\leq$ 0.01p and $\leq$0.05p, respectively). OPBH14 recorded an R^2 of 0.1378 and RM201 recorded and R^2 of 0.0829 (Table 14).

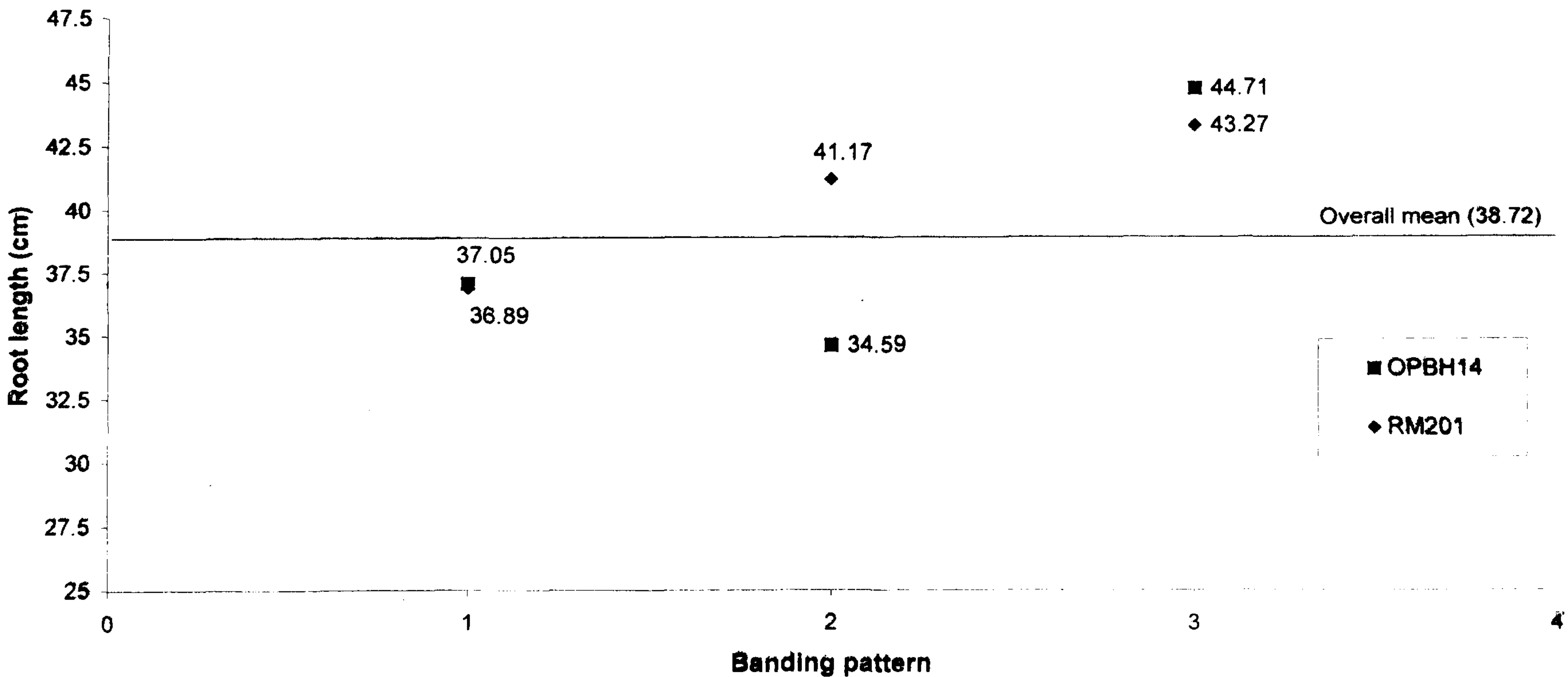

Fig 3. Means of individuals showing different banding patterns for OPBH14 and RM201

1 = IR64 type band 2 = Heterozygous band 3 = Azucena type band

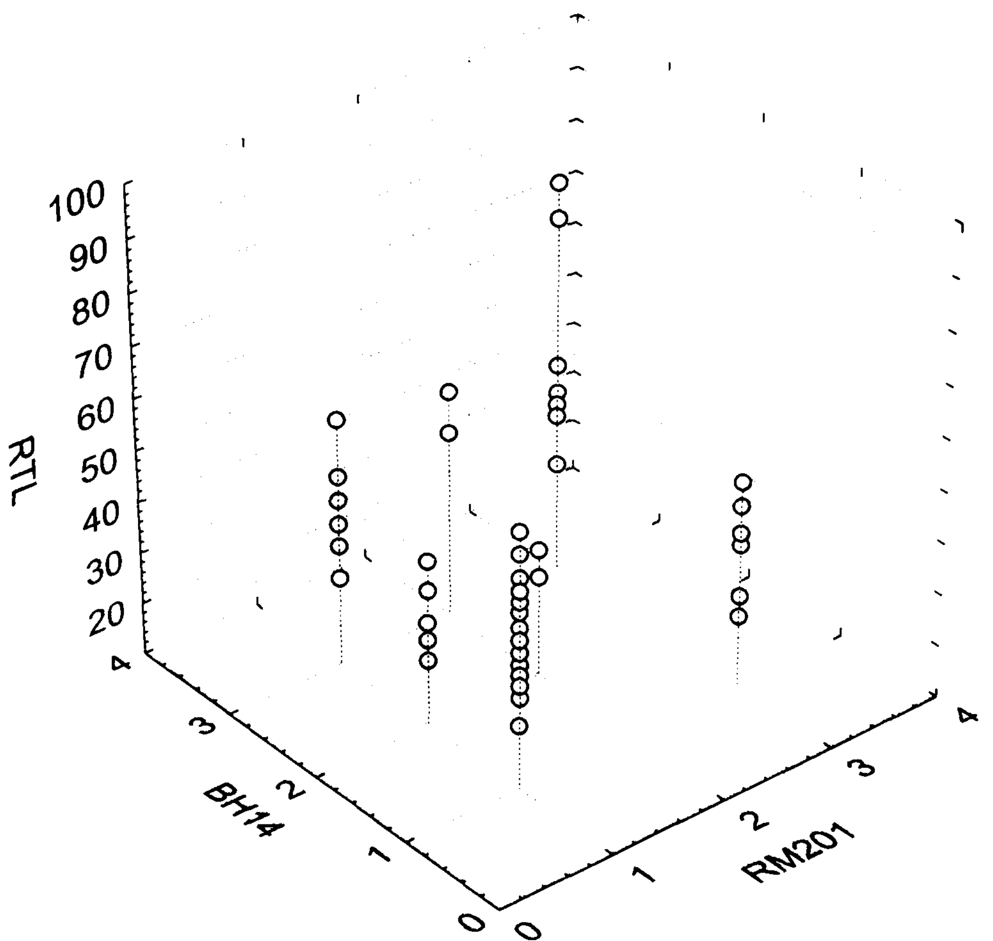

Fig 4. Three dimensional scatterplot of markers with root length.

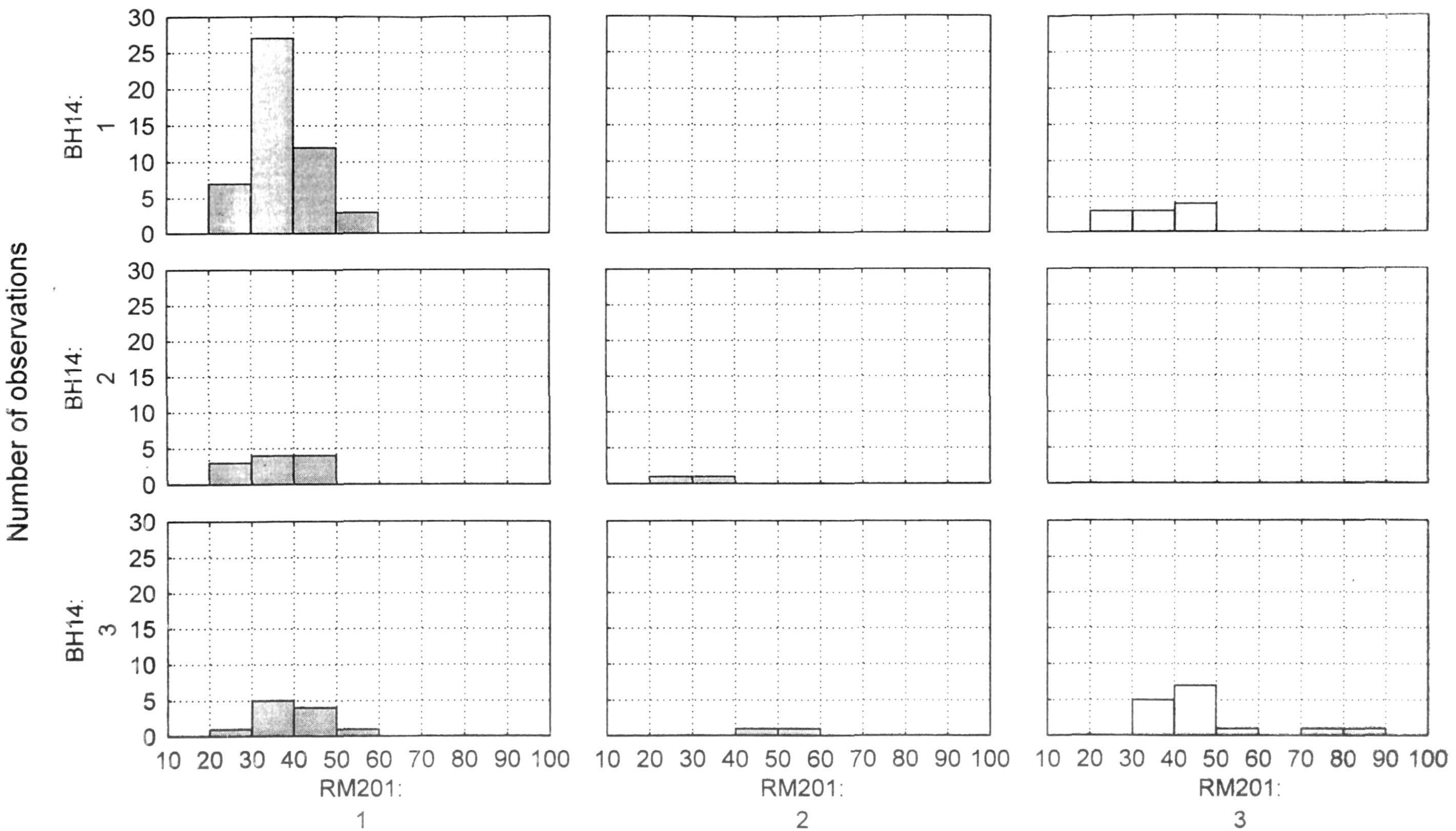

Fig 5. Histogram for root length categorized by RM201 and OPBH14 markers

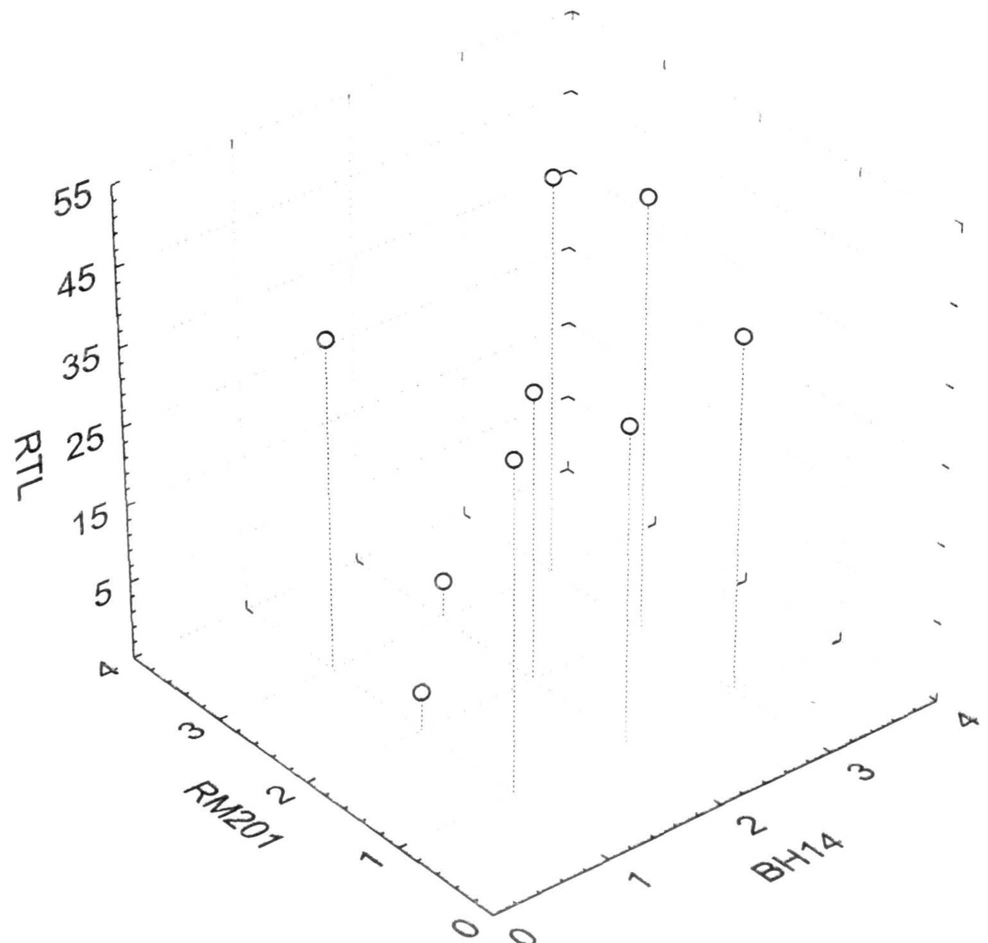

Fig 6. Means of individuals categorized by OPBH14 and RM201

Table 14. One way analysis of variance

Source	D.F.	Root length (cm)
OPBH14	2	661.45 **
Error	97	85.28
SE		5.33167
R^2		0.1378
RM201	2	397.93 *
Error	97	90.72
SE		5.49909
R^2		0.0829

* - Significant at p=0.05

** - Significant at p=0.01

Discussion

V. DISCUSSION

Food security in India could be under threat in the next two decades unless rice scientists achieve a major technological breakthrough and usher on a second green revolution. In other words, agricultural scientists should not be complacent after having achieved green revolution during 1970's because, complacency only leads to disaster. The main idea behind this thought is to forecast the challenges that India has to face in the forthcoming years, mainly, to increase the food production to feed thousands of new mouths that arrive in the country everyday.

In pursuit of increased food production, the country has to find new land as a result of good agricultural land being gobbled up by urbanization and dwindling water resources, a critical resource for agriculture, due to uncertain and erratic rainfall coupled with non-judicious usage of water. In this context, growing rice under drought-prone areas with sustainable yield is one of the promising solutions for increasing food production and productivity.

Possessing a well endowed root system is one of the vital mechanisms of crop plants to grow under water deficit conditions. The same inference was drawn by Passioura (1982); Armento-Soto *et al.* (1983); Ekanayake *et al.* (1985); Chang *et al.* (1986); O'Toole and Bland(1987); Shashidhar (1990); Hemamalini (1997); Price *et al.* (1997); Thanh *et al.* (1999). The advent of molecular markers has been a great boon to plant breeders for crop improvement. Tagging of markers for drought resistance parameters can help in generating tools for marker-assisted selection which in turn helps in accelerating the crop improvement.

The results of present study are discussed under the following headings

1. Identification of molecular markers linked to maximum root length in rice.

2. Evaluation of rice genotypes for root morphological characters in the field.

3. Confirmation of marker-trait association with diverse genetic material.

5.1 Identification of molecular markers linked to maximum root length in rice.

DNA markers have an indispensable tool in plant breeding and are of particular value in gene mapping and marker-assisted selection. Two types of molecular markers have been used to develop trait specific marker, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD). DNA markers for simply inherited traits can be readily identified by thc synthesis of DNA pools from individuals sharing a common phenotype (Miche1more *et al.*, 1991) bulk segregant analysis (BSA) or genotypes at an interval delineated by two RFLPs (Giovanoni *et al.*, 1991). Bulk segregant analysis is a rapid procedure for identifying markers in specific regions of the genome. The principle involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool or bulk, the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. Two pools contrasting for a trait (e.g., resistant and susceptible to blast disease in rice) are analyzed to identify markers that distinguish them. The minimum size of the bulk will be determined by the frequency with which unlinked loci might be detected as polymorphic between the bulked samples. This in turn will depend on the type of marker being screened (dominant -RAPD or codominant -RFLP) and the type of population used to generate the bulks (F_2, back

cross etc.). To identify dominant RAPD marker segregating in an F_2 population, the constructed bulks should be PCR analyzed using arbitrary primers.

The RAPD markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools (disease resistance). Using DNA pooling method -BSA - Michelmore *et al.,* (1991) identified three RAPD markers in lettuce linked to a gene for resistance to downey mildew for the first time. Monna *et al.* (1995) used 80 primers, either singly or pair-wise, and tested 2,404 primer-pairs and established 14 markers tightly linked to the photoperiod sensitivity gene in F_2 individuals derived from a cross between Nipponbare and Kasalath using bulk segregant analysis.

In the present investigation, two bulks were constituted for root length. Critically phenotyped data on root length in F_2 DH population from a cross between IR64 and Azucena was the basis to constitute the bulks (Shashidhar *et al.,* 2000). Bulk A (Long roots) consisted of DH lines showing long roots and bulk B (short roots) consisted of individuals showing short root length.

Preliminary screening of the bulks with conjugate primers resulted in 47 putative polymorphic primer-pairs. The bulks were once again screened for these primer-pairs for confirmation and the banding patterns were critically observed. But we failed to reproduce the results in 46 of the 47 primer-pairs as they turned out to be spurious. Only one primer-pair OPBF2/OPAF20 was of some interest since it produced two short root specific bands. However, on repeated screening of the bulks with this primer-pair we failed to obtain the same results and it turned out to be monomorphic. This can be attributed to non-reproducibility of RAPD, especially if the band is produced because of non-specific primer annealing. The second

reason for this could be due to chance factor of not getting any annealing sites for the primers screened at the target locus.

5.2 Evaluation of rice genotypes for root morphological characters in the field

The present study was undertaken to evaluate root morphological characters and some of the shoot traits among selected set of C and D lines and checks. In this experiment, seven genotypes were used as checks: IR20, IR36, IR42, Jaya, Moroberekan CO-39 and Azucena were evaluated to for root morphological and related characters. The findings are discussed below.

5.2.1 Root characters studied:

The following are the root characters studied with their relevance of study:

Root length:

Root length reflects the depth of soil from which plants can absorb water and is influenced by root penetrability. Higher root lengths play vital role under moisture deficit conditions so as to extract water from deeper layers. Similar impacts of root length on growth and development of rice were reported by Puckridge and O'Toole (1981); Mumbani and Lal (1983); Ekanayake *et al.* (1985); Cruz *et al.* (1986); Ray *et al.* (1996).

Root volume:

Root volume reflects the total space occupied by the roots in the soil. Higher root volume helps plants to get more water. Its role under water deficit conditions was reported by Ekanayake *et al.* (1985).

Total root number :

This trait is presumed to have influence on root volume. Along with other good root characters, this trait will strengthen plant's ability to get more water.

Root dry weight

Root dry weight reflects the amount of photosynthates diverted to root system. It is an associative trait as it depends on other root characters. Ekanayake *et al.* (1986) reported significant correlation of root dry weight with root pulling resistance while, Jeena and Mani (1990) mentioned its importance as selection criteria in selecting drought tolerant genotypes. Vijayalakshmi and Nagarajan (1994) stressed that drought tolerant genotypes should have greater root dry weight compared to upland and drought susceptible cultivators.

Root to shoot ratio

Root to shoot ratio gives unit investment on roots by plants per unit investment on shoot. Higher the root to shoot ratio, higher will be root dry weight. Banba and Ohkuba (1989) supposed that, root to shoot ratio increases under water stress owing to increase in root growth.

No doubt, root morphological characters reflects the crop plant's ability to tackle different water status conditions to a considerable extent. But, root studies is an arduous job when studied in soil which may act as an impediment for breeders to breed the genotypes for drought resistance as, all breeders cannot avail the infrastructure required for studies in hydroponic and aeroponic systems. Robertson *et al.* (1985) opined similar difficulties.

5.2.2 Genetic variability, heritability and genetic advance

ANOVA in the present experiment revealed that genotypes differed significantly for all the characters studied (P 0.01).

Various genetic parameters like percent genotypic and phenotypic coefficients of variation [GCV (%), PCV (%)], heritability (narrow sense) and expected genetic advance (GA) as per cent of mean were found out for all the characters studied.

Among the characters studied, root dry weight root to shoot ratio and root volume were found to have higher GCV (%) and PCV(%). While plant height, root length, shoot dry weight and total dry weight were found have moderate GCV (%) and PCV (%). Plant height had lower GCV (%) and PCV (%). Latha (1997) reported higher GCV (%) and PCV (%) for root dry weight, root volume. Gireesha (1999) reported higher GCV (%) and PCV(%) for root dry weight and root to shoot ratio, and lower GCV(%) and PCV(%) for plant height.

Highest heritability (broad sense) was found for total dry weight followed by shoot dry weight, number of tillers, root dry weight and root length and hence amenable for selection. In these characters, higher heritability is reflected in narrow differences between GCV (%) and PCV(%) for these traits. Here, additive gene action is the contributor for genotypic variance because most of the loci in straight varieties will be homozygous. Higher heritability for these traits was reported by Latha (1997) and Gireesha (1999).

Chang *et al.* (1982), reported moderately high heritability for root length but lower heritability for total and root dry weight. Anon.(1980) and Armento-Soto *et al.* (1983), reported moderately high heritability for root length and dry weight. But, Ekanayake *et al.* (1992) obtained low heritability for maximum root length and moderate for root volume. Ekanayake *et al.* (1985) and Hemamalini

(1997) got higher heritability for root number. Shashidhar *et al.* (1990) reported very high heritability for root dry weight and similarly by Gomathinayagam *et al.* (1990) and Shahid *et al.* (1994). Moderate heritability for root volume was reported by Price *et al.* (1997). Moderately high heritability was found for shoot dry weight by Gireesha (1999).

Higher expected genetic advance (GA) as percent of mean were found for root dry weight and root to shoot ratio, anticipating good response to selection for these characters. The results are in accordance with Shashidhar *et al.* (1990), Gomathinayagam *et al.* (1990), Shahid *et al.* (1994), Latha (1997) and Gireesha (1999). Hemamalini (1997) obtained higher expected GA as percent of mean for root volume.

Moderate expected GA as percent of mean were found for root volume. Lowest GA as percent of mean was found for plant height followed by total root number. Gireesha (1999) reported moderate GA as per cent of mean for root volume and root length, and lowest GA as per cent of mean for plant height.

Even though the traits total dry weight and root dry weight showed higher heritabilities, higher expected GA as percent of mean was recorded for root dry weight compared to total dry weight. This is because, total dry weight, inspite of having higher heritability, showed lower variability. Similarly, root length showed higher expected GA as percent of mean than both root length inspite of having lower heritability than root length. This is once again because, root volume showed higher variability than root length.

Since heritability and genetic advance are high for most of the traits selection efficiency will be high.

5.2.3 Genotypic and Phenotypic correlation.

Plant height was found to have significant and positive correlation with shoot dry weight, root length, root volume, root dry weight and total dry weight (P=0.01). The results are in conformity with Anon. (1984) and Gireesha (1999) except for the correlation between plant height and root number which was non-significant in our study. Ekanayake *et al.* (1985); Salam and Subramanian (1988); Latha (1997); Gireesha (1999) and Thanh *et al.* (1999) had reported similar correlations between plant height and root dry weight.

Number of tillers was significantly correlated with shoot dry weight, root length, root volume, root dry weight and total dry weight (at 0.05 P only). The results are in consonance with Anon. (1984); Ekanayake *et al.* (1985); Latha (1997) and Gireesha (1999) except for root length, which was non-significant in the present study.

Root volume, total root number, root length, root dry weight, shoot dry weight and total dry weight were found to be interrelated, positively and significantly. The results are in consonance with Anon. (1982); Anon. (1984); Ekanayake *et al.* (1985); Zuno-Altovers *et al.* (1990); Yadav *et al.* (1997); Hemamalini (1997); Latha (1997); Gireesha (1999) and Thanh *et al.* (1999).

Finding significant correlation between these traits indicates that increase in any one of these traits will increase the other correlated traits providing for selection advantage of many traits simultaneously through selection of one trait.

Root to shoot ratio showed significant and positive correlation with root volume and root dry weight but, showed negative and significant correlation with shoot dry weight and total dry weight. Latha (1997) reported positive and significant correlation between root to shoot ratio and root dry weight.

Root length has significant correlation with all the characters. Selection for this trait will greatly increase the selection efficiency for other traits as well resulting in improved drought tolerance.

5.2.4 Contrast analysis

Contrast analysis revealed significant differences between genotypes from different countries for most of the characters studied. This was expected because of greater diversity of the genotypes used in this study.

Indian genotypes showed significantly higher means for root length, root volume total dry weight, shoot dry weight, root dry weight and root to shoot ratio. Means were also higher for plant height and number of tillers. This indicates that Indian varieties are more drought tolerant than the genotypes from other countries. Such genotypes can be used to improve the superior high yield varieties for drought resistance.

5.3 Confirmation of marker-trait association with diverse genetic material.

The development of molecular genetics and associated technology has facilitated a quantum leap in our understanding of the underlying genetics of the traits sought through plant breeding. The usefulness of DNA markers for germplasm characterization and of marker-assisted selection - the manipulation through DNA markers of genomic regions that are involved in the expression of traits of interest - for single-gene transfer has been well demonstrated. However, when several genomic regions must be manipulated, marker-assisted selection has turned out to be less useful.

Currently, marker-assisted selection of single alleles is perhaps the most powerful approach that use DNA markers effectively. But,

most traits of agronomic importance are complex and regulated by several genes, with drought resistance and yield among the most polygenic and complex compared to a monogenic trait, the improvement of polygenic traits through marker-assisted selection has raised more questions (Lande and Thomson 1990). The difficulty of manipulating quantitative traits is related to the genetic complexity *i.e.*, the number of genes involved and the interactions between them.

Shashidhar *et al.* (2000) identified two markers; a microsatellite marker, RM201 and a RAPD marker, OPBH14 linked to root length in IR64 X Azucena doubled haploid population of rice. The present study was conducted to find the utility of these markers across diverse accessions of rice.

Since mean for root length is not a criterion for categorizing the plants as long or short rooted, individuals having root length of 1 standard deviation on either sides of the mean were considered. Both markers were able to identify more than 70 % of the individuals as short rooted but they could identify about 50 % of the individuals as long rooted. This indicates that the probability of that OPBH14 identifies a randomly chosen individual as long rooted or sort rooted is 64 % while the probability that RM201 identifies it correctly is 63%.

Both markers, RM201 and OPBH14 recorded IR64 type band in D-82, C-07, C-52 and D-86, but these individuals recorded significantly higher root length. The failure of these markers to identify the genotypes as long rooted indicates the following:

- There is recombination between the markers and the gene influencing root length.

- More number of genes are involved in the expression of this trait.

OPBH14 recorded heterozygous bands in 13 genotypes while RM201 recorded heterozygous bands in 4 genotypes. This can be due to outcrossing in these genotypes *i.e.,* these genotypes are segregationg for these marker loci. This could be verified by screening their progeny.

Some genotypes were showing Azucena type bands with primer OPBH14 but were showing IR64 type band with RM201 and vice-versa. This could be due to the following reasons:

- There is some recombination between either of these markers and the gene influencing the root length.

- RM201 is located on chromosome 9 (Zhang *et al.,* 2001) and OPBH14 is located on chromosome 10 (Shashidhar *et al.,* 2000). The individuals showing Azucena type band with RM201 and IR64 type band with OPBH14 could have received chromosome 9 from long rooted parent and chromosome 10 from short rooted parent. Similarly, the individuals showing IR64 type band with RM201 and Azucena type band with OPBH14 could have received chromosome 9 from short rooted parent and chromosome 10 from long rooted parent.

5.3.1 One-way analysis of variance.

One-way analysis of variance revealed significant association of OPBH14 with root length at 1 per cent F-probability threshold level. OPBH14 could explain 13.78 of variability in root length. Similarly, One-way ANOVA revealed significant association of RM201 with root length at 5 per cent F-probability threshold level and RM201 could explain 8.29 per cent of the variability in root length. These results are in consonance with Shashidhar *et al.* (2000).

The above study suggests that OPBH14 is more reliable than RM201 as a marker for root length. Lower variability explained by these markers owing to the complexity of the trait necessitates the identification of more markers for efficient marker-assisted selection programs.

The future line of work is given below

1. Evaluation of promising varieties over different locations for their rooting performance under moisture stress and well watered conditions so as to identify genotypes performing well under varied conditions of moisture.

2. Evaluation of promising C and D lines for yield.

3. Utilization of genotypes contrasting for different root traits in hybridization.

4. Use of RM201 and OPBH14 in marker-assisted selection.

Summary

VI. SUMMARY

Experiments were conducted to evaluate root morphological and associated shoot traits in Core and Donor lines. Experiments involved evaluation for root morphological traits in PVC pipes measuring 1 m length and 18 cm diameter, filled with clay soil and FYM in 1:4 proportion at MASLAB green house UAS, Bangalore, India. The results of these experiments are summarized below.

Wide range of variation was observed for the 9 characters considered for the study. Analysis of variance showed that genotypes exhibited highly significant differences for all the traits *viz.*, plant height, number of tillers, root length, root volume, root thickness, total root number, root dry weight, shoot dry weight, total dry weight and root to shoot ratio (by weight).

Root dry weight and plant height showed highest and lowest genotypic (70.5% and 18.66 %) coefficients of variation respectively. Highest heritability was found for total dry weight (95.4 %) followed closely by shoot dry weight while, lowest was found for root volume (48.6 %). Expected genetic advance as percent of mean was highest for root dry weight (147.39 %) followed by root to shoot ratio while, lowest was found for plant height (33.76 %).

All the root characters studied, showed significant and positive correlation among each other. Highest correlation among root characters was found between total dry weight and shoot dry weight (0.96, 0.01p).

Contrast analysis showed significant differences between Indian varieties and varieties from other countries for all the characters studied. Indian varieties recorded significantly higher means than varieties from other countries for all the characters studied except for

plant height, number of tillers and total root number. Core lines were significantly different from Donor lines for all the characters except for total root number.

The same genotypes were screened for root length with RAPD primer, OPBH14 and a microsatellite marker, RM201. One-way analysis was significant for both markers (< 0.01p and 0.05p, respectively). OPBH14 was able to explain 13.78 % of the variability in root length while RM201 was able to explain 8.29 % of the variability.

Bulk segregant analysis carried out in IR64 X Azucena DH population using 392 conjugate RAPD primers could not yield new markers for root length.

References

VII. REFERENCES

Anonymous, 1980, Annual report for 1979. IRRI, Los Banos, Philippines : 77-99.

Anonymous, 1982, Annual report for 1981. IRRI, Los Banos, Philippines : 77-99.

Anonymous, 1984, Annual report for 1983. IRRI, Los Banos, Philippines : 99-120.

Anonymous, 1995, *Challenges and opportunities in a less favourable ecosystem : Rainfed lowland rice.*, IRRI, Los Banos, Philippines pp. 8-12.

Anonymous, 2000, Rice facts index. http://www.riceweb.org/Rice_tabindex.htm.

Anonymous, 2001. Rice Simple sequence repeats. http://www.rice-research.org/rice_ssr.html

Armento –Soto, J., Chang, T.T., Lorseto, G.C. and O' Toole, J.C., 1983, Genetic analysis of root characters in rice (*Oryza sativa* L.). *Soc. Adv. Breed. Res. Asia Oceania, J.,* **15** : 103-16.

• Banba, H. and Ohkuba, T., 1989, Relationship between root distribution of upland crops and their yield. III. Influence of soil moisture levels on root distribution and root dry matter of upland cultured paddy rice, cross bed rice of paddy rice and upland rice. *Jap. J. Crop. Sci.,* **50** : 1-7.

Bassam, B, J., Caetano-Anolles, G. and Gresshoff, P. M., 1991, Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.*, **80**: 81-84.

Beyrouty, C.A., Wells, B.R., Norman, R.J., Marvel, J.N. and Pillow, J.A., 1988, Root growth dynamics of a rice cultivar grown at two locations. *Agron. J.,* **80** : 1001-1004.

Blum, A., 1982, Evidence for genetic variability in drought resistance and its far implications for plant breeding In : *Drought resistance in crops with emphasis on rice,* IRRI, Philippines : 53-68.

Burton, G.W. and Dewane, E.M., 1953, Estimating heritability in tall Fesue (*Fistula arundanaceae*) from replicated clonal material. *Agron. J.,* **45**:478-481.

Cao, D. and Oard, J.H., 1997, Pedigree and RAPD-based DNA analysis of commercial US Rice cultivars. *Crop Sci.,* **37**:1630-1635.

Champoux, M.C., Wang, G., Sarkarung., Mackill., O'Toole, J.C., Huang, N. and McCouch, S.R., 1995, Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theor. Appl. Genet.,* **90** : 969-981.

Chang, T.T., Armento-Soto, J.L., Mao, C.X., Peiri, R . and Loresto, G.C., 1986. Genetic studies on the components of drought resistance in rice. In : *Rice Genetics.,* IRRI, Los Banos, Philippines : 389-398.

Chang, T.T., Loresto, G.S. and Tagumay, O., 1982, Agronomic and growth characteristics of upland and lowland varieties. In : *Rice breeding.,* International Rice Research Institute Los Bonos, Philippines pp,648-661.

Courtois, B., Chaitep, W., Prasad, K., Moolsri, S., Sinha, P.K., Trebuil, G. and Yadav, R., 1996, Drought resistance and germplasm

improvement. Ongoing research in the Upland Rice Consortium In: *Proceedings of the Upland Rice Consortium Workshop,* Padang, Indonesia, 10-12 Jan 1996, IRRI, Los Banos, Philippines.

Cruz, R.T., O'Toole, J.C., Dingkuhn, M., Yambao, E.B., Thangaraj, M. and De Datta, S.K., 1986, Shoot and root responses to water deficits in rainfed lowland rice. *Aus. J. Plant Physiol.,* **13** : 567-575.

Das, R.K., Miah, N.M. and Loresto, G.C., 1991, Genetic variability in root and shoot characters of selected rice genotypes. *Int. Rice. Res. Newsl.,* **16** (5) : 5.

Dioh, W., Tharreau, D., Notteghem, J.L., Orbach, M. and Lebrun, M.H., 2000, Mapping of avirulence genes in the rice blast fungus, *Magnaporthe grisea,* with RFLP and RAPD markers. *Mol. Plant Microbe Interactions,* **13**: 217-227.

Dong, N.V., Subudhi, P.K., Luong, P.N., Quang, V.D., Quy, T.D., Zheng, H.G., Wang, B. and Nguyen, H.T., 2000, Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. *Theor. Appl. Genet.,* **100**: 727-734.

Ekanayake, I. J., Garrity, D.P. and O'Toole, J.C., 1986, Influence of deep root density on root pulling resistance in Rice. *Crop Sci.,* **26** : 1181-1186.

Ekanayake, I.J., O' Toole, J.C., Garrity, D.P. and Masajo, T.N., 1985, Inheritance of root characters and their relations to drought resistance in rice. *Crop Sci.,* **25** : 927-933.

Ekanayake, I.J., 1992, Inheritance of root characters in rice and their relation to drought resistance.

Fukai, S. and Cooper, M., 1995, Development of drought resistant cultivars using physio-morphological traits in rice. *Field Crops Res.*, **40** : 67-87.

Fukai, S. and Inthapan, P., 1988, Growth and yield of rice cultivars under sprinkler irrigation in southeastern Queensland. III. Water extraction and plant water relations-comparison with maize and grain sorghum. *Aust. J. Exp. Agric.*, **28** : 249-252.

Giovanoni, J.J., Wing, R.A., Ganal, M.W., and Tanksley, S.D., 1991, Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. *Nucl. Acids Res.,* 19: 6553-6558.

Gireesha, T. M., Shashidhar, H.E. and Hittalmani. S., 2000, Genetics of root morphology and related traits in an *indica-indica* based mapping population of rice (*Oryza sativa* L.). *Res. Crops*, **1**: 208-215.

Gireesha, T. M., 1999, Genetic investigations on root morphological characters in a double haploid population and crosses in rice (*Oryza sativa* L.). M. Sc. Thesis submitted to University of Agricultural Sciences, Bangalore. India.

Gomathinayagam, P., Ingram, K.T. and Maguling, M., 1988, A Pot screening for drought tolerance in rice. *Int. Rice Res. Newsl.,* ***13***(6) : 23-26

Gomathinayagam, P., Ingram, K.T. and Maguling, M.A., 1992, Screening upland rice cultivars for early drought. *Madras Agric. J.,* **79** (11) : 658-662.

Gomathinayagam, P., Natarajan, S. and Subramanian, M., 1990, Genetic variability in drought tolerant genotypes of rice. *Oryza,* **27** (3) : 328-330.

Gomathinayagam, P., Natarajan, S., Subramanian, M. and Nagarajan, M., 1988, b., A Low cost rapid screening technique for seminal root elongation *Int. Rice. Res. Newsl.,* **13** (5) : 17.

Hajra, N.G. and Hajra, S.G., 1988, Heritability of primary root length. *Int. Rice. Res. Newsl.,* **13** (5) : 7.

Hanson, C.H., Robinson, H.F. and Comstock, R.E., 1956, Biometrical studies of yield in segregating population of Korean population Lespedeza. *Agron. J.,* **48**: 267-262.

Haque, M.E., Lorseto, G.C., Chang, T.T. and Tepora, N., 1989, A comparative study of rice root characters related to the mechanisms of drought resistance under aeroponic and hydroponic cultures. *SABRAO Journal,* **21** (2) : 111-112.

Hemamalini, G.S., 1997, Molecular mapping of low-moisture stress induced response in rice (*Oryza sativa* L.) roots at peak vegetative stage. M.Sc. Thesis submitted to the University of Agricultural Sciences, Bangalore. India.

Herdt, R.W., 1991, Research priorities for rice biotechnology. In: G.S. Khush and G.H. Toenniessen (ed.) Rice Biotechnology. CAB International, Wallingford, UK. pp. 19-54.

Inthapan, P. and Fukai, S., 1988, Growth and yield of rice cultivars under sprinkler irrigation in southeastern Queensland II. Comparison with maize and grain sorghum under wet and dry conditions. *Aust. J. Exp. Agric.,* **28** : 243-248.

Jeena, H.S. and Mani, S.C., 1990, Studies on root characters and grain yield of some upland rice varieties. *Oryza*, **27**(2) : 214-216

Johnson, H.W., Robinson, H.F. and Comstock, R.E., 1955, Genotypic and phenotypic correlations in soybean and their implications in selection. *Agron. J.*, **47**:477-483.

KeNong, X., MacKill, D.J. and Xu, K.N., 1996, A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breeding*, **2**: 219-224.

Khush, G.S., 1990, Rice breeding accomplishments and challenges. *Plant Breed. Abstr.*, **60** (5) : 461-469.

Khush, G.S., 1996, Strategies for increasing crop productivity In: 2nd International Crop Science Congress Plenary Lecture. 17-24. November, 1996, New Delhi.

Koh, H.J., Heu, M.H. and McCouch, S.R., 1996, Molecular mapping of the *ges* gene controlling the super-giant embryo character in rice (*Oryza sativa* L.). *Theor. Appl. Genet.*, **93**: 257-261.

Lande, R. and Thomson, R. 1990, Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics*, **124**: 743- 756.

Latha, J., 1997, Genetic purification and characterization of traditional lowland rice accessions. M.Sc Thesis submitted to University of Agricultural Sciences, Bangalore. India.

Levitt, T., 1980, Stress terminology In :*Adaptation of plants to water and high temperature stress* (Eds. N.C. Turner and P.J. Kramer) : 437-439. Wiley, New York.

Lilley, J.M. and Ludlow, M.M., 1996, Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crop Res.,* **48** : 185-197.

Lin, J.Y. and Shen, M., 1993, Rice production constraints in China. In: R.E. Everson (ed.) Rice Production in Asia: Progress and Priorities. CAB International, Wallingford, UK. p. 161-178.

Lorseto, G.C., Zhang, W.X., Chaudhary, D. and Chang, T.T., 1983, Aeroponic technique for screening the drought avoidance mechanism of rice genotypes by root characters. *Garcia De Orta.,* **10** : 77-82.

Lush, J.L., 1949, Heritability of quantitative characters in farm animals. Proc 8th Congr. Genet., *Hereditas*, **35**:Suppl., pp. 356-375.

Lynch, J., 1995, Root architecture and plant productivity. *Plant Physiol.,* **109** : 7-13.

Maheswaran, M., Mackill, D.J., Ning, H., Sreerangasamy, S.R., McCouch, S.R. and Huang, N.,1999, Identification of RAPD markers linked to *Se-3(t)*, a gene enhancing the level of photoperiod sensitivity in rice. *J. Genet. Breed.,* **53**: 263-270.

Martin, G.B., Williams, J.K. and Tanksley, S.D., 1991, Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. *Proc. Natl. Acad. Sci.,* **88**: 2236-2237.

Mao, C.X., 1984, Inheritance of root characters in crosses among deep-rooted and shallow -rooted rice varieties. University of Phillipines at Los Banos, College, Laguna

Michelmore, R.W., Paran, I. and Kesseli, R.V.,1991, Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci.,* **88**: 9828-9832.

Monna, L., Miyao, A., Zhong, H.S., Sasaki, T. and Minobe, Y., 1995, Screening of RAPD markers linked to the photoperiod sensitivity in rice chromosome 6 using bulked segregant analysis. *DNA Res.,* **2**: 101-106.

Mumbani, B. and Lal, R., 1983, Response of upland rice varieties to drought stress. II. Screening rice varieties by means of variable moisture regimes along a toposequence. *Plant and Soil,* **73** : 73-94.

Nair, S., Bentur, J.S., Rao, U.P. and Mohan, M., 1995, DNA markers tightly linked to gall midge resistance gene (*gm* 2) are potentially useful for marker-aided selection in rice breeding. *Theor. Appl. Genet.,* **91**: 68-73.

Nair, S., Kumar, A., Srivastava, M.N. and Mohan, M., 1996, PCR-based DNA markers linked to a gall midge resistance gene, *gm 4(t)* has potential for marker-aided selection in rice. *Theor. Appl. Genet.,* **92**: 660-665.

Nasiruddin, M. and Haque, E., 1981, Root characteristics and drought tolerance in rice [Bangladesh]. *Bangladesh J. Botany,* **10** : 39-42.

O'Toole, J.C., 1982, Adaptation of rice to drought–prone environments In: *Drought resistance in crops with emphasis on rice.* IRRI, Philippines : 195-213.

O'Toole, J.C. and Bland, W.L., 1987, Genotypic variation in crop plant root systems. *Adv. Agron.,* **41** : 91-145.

O'Toole, J.C. and Datta, S.K., 1986, Drought resistance in rainfed lowland rice. In : *Progress in rainfed lowland rice research.* IRRI, Manila, Philippines: 145-158.

O'Toole, J.C. and Moya, J.B., 1978, Genotypic variation in maintenance of leaf water potential in rice. *Crop Sci.,* **18:** 873-876.

O'Toole, J.C., 1989, Breeding for drought resistance in cereals: Emerging new technologies. In: Baker, F.W.G. (ed.), Drought resistance in cereals. CAB International, Wallingford, UK, pp. 107-116.

• Okuyama, L.A. and Colasante, L.O., 1987, Root system distribution of dryland rice cultivated in dystrophic red latosal. *Pesquisa Agropeuaria Brasileira,* **22** (8) : 803-807.

Passioura, J.B., 1982, The role of root system characteristics in the drought resistance for crop plants. In : *Drought resistance in cereals crops with emphasis on rice,* IRRI, Los Bonos, Philippines 71-82.

Price, A.H., Tomos, A.D. and Virk, D.S., 1997, Genetic dissection of root growth in rice (*Oryza sativa* L.) I : a hydroponic screen. *Theor. Appl. Genet.,* **95**: 132-142.

Puckridge, D.W. and O'Toole, J.C., 1981, Dry matter and grain production of rice using a line source sprinkler I. Drought studies. *Field Crop Res.,* **3**: 303-319.

Rao, M.L.N., Reddy, K.J. and Venkateswarlu, B., 1994, Comparative studies on deep and shallow rooting rice cultivars with reference to sustenance and recovery from drought. *Indian J. Plant Physiol.,* **37** (2): 89-94.

Ray, J.D., Yu, L., McCouch, S.R., Champoux, M.C., Wang, G. and Nguyen, H., 1996, Mapping quantitative trait loci associated with root penetration ability in rice *Theor. Appl. Genet.,* **92** : 627-633.

Robertson, B.M., Hall, A.E. and Foster, K.W., 1985, A field technique for screening for genotypic differences in root growth. *Crop Sci.,* **25**: 1084-1090.

Robinson, H.F., Comstock, R.E. and Harvey, P.H., 1949, Estimates of heritability and degree of dominance in corn. *Agron. J.,* **41**: 353-359.

Salam, M.A. and Subramanian, S., 1988, Relationship between root weight and plant characters in rice in different seasons. *Indian. J. Agril. Sci.,* **58** (9): 710.

SAS Institute, Inc., 1989, SAS/STAT user's guide. Version 6, 4th edition. Cary, NC.

Sax, K., 1923, The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris.* *Genetics,* **8**: 552-560.

Shahid, M., Latif, T., Iqbal, M. and Khan, M.A., 1994, Genetic studies on drought tolerance in rice. *Sarhad J. Agri.,* **10** (6): 671-674.

Shashidhar, H.E., 1990, Root studies and stability of quantitative traits in rice (*Oryza sativa* L.) under Punji (Dry-cum wet

cultivation) and irrigated habitats. Ph.D. thesis submitted to University of Agricultural Sciences, Bangalore, India.

Shashidhar, H.E., Shailaja Hittalmani and Shivashankar, G., 1990, Heritability for root and shoot characters in drought tolerant rice cultivars. *Curr. Res.,* **19** (2): 200-201.

Shashidhar, H.E., Sharma, N. and Hittalmani, S., 2000, Identification of molecular markers associated with maximum root length in rice (*Oryza sativa* L.). 3rd International Crop Science Congress. 17-22 August, Hamburg, Germany.

• Sheng, J.Q., Qin, Q.B., Chao, W.Y., Bi, L.R., Jin, Q.S., Qiu, B.Q, Yan, W.C. and Luo, R.B., 1995, Tagging of a gene for aroma in rice by RAPD and RFLP. *Acta Agriculturae Zhejiangensis,* **7**: 439-442.

Singh, R.C. and Chaudhary, B.D., 1985, *Biometrical Methods in Quantitative Genetic Analysis*, Kalyani Publishers, New Delhi, pp.2-30.

Sorte, N.V., Deotale, R.D., Patankar, M.N., Narkhede, A.H., Golliwar, V.J. and Katole, B.D., 1992, Root and shoot physiology as influenced by short-term water stress in upland paddy. *J. Soils Crops,* **2** (1): 86-91.

• Suga, T. and Yamazaki, K., 1988, Development changes in root characters and growth correlation between root mass and leaf mass in rice. *Japanese J. Crop Sci.,* **57** (4): 671-677.

Sunderraj, N., Nagaraju, S., Venkataramu, M.N. and Jaganath, M.K., 1972, Designs and analysis of field experiments. University of Agricultural Sciences. Hebbal, Bangalore.

Tanksley, S.D. and Orton, T.J.D., 1983, Isozymes in Plant Breeding, part A. Elsevier, Amsterdam.

Thangaraj, M., O'Toole, J.C. and DeDatta, S.K., 1990, Root response to water stress in rainfed lowland rice. *Exptl. Agric.*, **26**: 287-296.

Thanh, N.O., Zheng, H.G., Dong, N.V., Trinh, L.N., Ali, M.L. and Nguyen, H.T., 1999, Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. *Euphytica*, **105** : 43-51.

Thoday, J.M., 1961, Location of polygenes. *Nature*, 191: 368-370.

Turner, N.C., O'Toole, J.C., Cruz, R.T., Namuco, O.S. and Ahmad, S., 1986, Responses of seven diverse rice cultivars to water deficits. *Field Crops Res.*, **13**: 257-271.

Vijayalakshmi, C. and Nagarajan, M., 1994, Effect of rooting pattern on rice productivity under different water regimes. *J. Agron.*, **173** (2) : 113-117.

Wang, G.G. and Paterson, A.H., 1994, Assessment of DNA pooling strategies for mapping of QTLs. *Theor. Appl. Genet.*, **88**:355-361.

Weaver, J.E. and Himmer, W.J., 1930, Relation of increased aeration to root development of hydrophytes. *Plant Physiol.*, **5**: 52-69.

Welsh, J. and McClelland, M., 1991, Fingerprinting genomes using PCR with arbitrary primers. *Nucl., Acids Res.*, **18**: 7213-7218.

Williams, J.G.K., Kubelik, A.R., Refalski, J.A. and Tingey, S.V., 1990, DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.,* **18**:6531-6531.

Yadav, R., Courtois, B., Huang, N. and McLaren, G., 1997, Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theor. Appl. Genet.,* **94**(5): 619-632.

Yadav, R., Courtois, B. and Huang, N., 1995, RFLP mapping of genes controlling root morphology in an *Indica* x *Japonica* DH population of rice.

Yoshida, S. and Hasegawa, S., 1982, The rice root system: Its development and function In: *Drought resistance in cereals crops with emphasis on rice,* IRRI, Philippines : 53-68.

Yoshimura, S., Yoshimora, A., Iwata, N., McCouch, S.R., Abenes, M.L., Baraoidan, M.R., Mew, T. and Nelson, R.J., 1995, Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Mol. Breed.,* **1**: 375-387.

Yuan, L.P., Virmani, S.S. and Mao, C.X., 1989, Hybrid rice : achievements and outlook. In: *Progress in irrigated rice research,* IRRI, P.O. Box 933, Manila 1099, Philippines : 219-236.

Zhang, J., Zheng, H.G., Aarti, A., Pantuwan, G., Nguyen, T.T., Tripathy, J.N., Sarial, A.K., Robin, S., Babu, R.C., Nguyen, Sarkarung, S., Blum A. and Nguyen, H.T., 2001, Locating genomic regions associated with components of drought resistance in rice: comparing mapping within and across species. *Theor. Appl. Genet.,* (In press).

* Zheng, J., Yuan, Z., Yin, G., He, G., Yang, Z., Jiang, K., Zheng, J.K., Yuan, Z.L., Yin, G.D., He, G.H., Yang, Z.L. and Jiang, K.F., 1996, Genetic studies on root growth ability in seedling stage of rice. *Chinese J. Rice Sci.,* **10** (1): 51-53.

Zheng, K., Huang, N., Bennett, J. and Khush, G.S., 1995, Rapid micro-level extraction of DNA in Rice, IRRI discussion paper. Series No. 12, pp. 24.

Zuno-Altoveros, C., Lorseto, G.C., Obien, M. and Chang, T.T., 1990, Differences in root volume of selected upland and lowland rice varieties. *Int. Rice. Res. Newsl.,* **15** (2): 8.

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Appendices

Appendix 1. Composition of Polyacrylamide gel

Distilled water	27.5 ml
10 x TBE	3.75 ml
30 % polyacrylamide	6.25 ml
10 % APS	250 ml
TEMED	32.5 ml

Appendix 2. Composition of reagents used in silver staining of polyacrylamide gels

	Reagent	**For two gels**
Buffer A	Ethanol (10%)	56 ml
	Acetic acid (0.5%)	2.8 ml
	dd. Water	500 ml
Buffer B	Silver nitrate (0.1 %)	0.25 g
	dd. water	250 ml
	(wash twice in dd. H_2O)	
Buffer C	NaOH (1.5 %)	3.75 g
	$NaBH_4$ (0.1 %)	0.025 g
	Formaldehyde (0.15 %)	1 ml
	dd. H_2O	250 ml

Appendix 3. Mean values of rice genotypes for nine morphological characters

No	Line	RTL	RTV	PHT	NOT	TRN	TDW	SDW	RDW	R/S
1	C-02	34.00	14.00	71.67	13.33	43.33	14.24	11.77	2.47	0.21
2	C-03	32.00	7.33	80.33	5.33	39.00	6.44	5.44	1.00	0.18
3	C-05	45.33	23.00	61.00	10.67	95.00	14.14	10.14	4.00	0.39
4	C-07	55.00	20.00	74.33	10.00	56.33	17.51	14.71	2.80	0.19
5	C-08	41.00	10.67	69.67	7.00	57.67	15.14	14.04	1.10	0.08
6	C-09	37.67	13.00	77.67	10.67	41.67	14.91	13.27	1.63	0.12
7	C-11	42.00	10.00	54.50	11.33	58.33	12.51	11.61	0.90	0.08
8	C-13	27.67	10.00	77.00	8.33	32.33	7.44	6.91	0.53	0.08
9	C-14	29.00	14.33	55.33	11.67	38.33	7.17	6.51	0.67	0.10
10	C-15	40.00	16.67	74.00	7.33	62.67	20.74	18.71	2.03	0.11
11	C-16	41.00	15.00	70.33	11.00	55.00	10.47	9.74	0.73	0.08
12	C-17	31.00	13.33	81.00	7.00	65.33	12.81	10.37	2.43	0.23
13	C-18	34.67	8.33	68.00	10.00	36.67	10.74	10.07	0.67	0.07
14	C-19	36.33	17.33	71.00	14.67	64.67	20.01	16.81	3.20	0.19
15	C-20	31.67	20.67	69.67	12.67	53.33	15.84	13.37	2.47	0.19
16	C-21	33.00	15.33	77.67	6.67	38.33	13.91	12.01	1.90	0.17
17	C-22	30.00	12.33	51.33	11.33	52.67	8.74	7.31	1.43	0.20
18	C-24	46.33	28.33	86.00	15.33	66.67	19.17	13.84	5.33	0.39
19	C-25	43.00	7.33	66.67	8.33	28.33	11.41	10.57	0.83	0.08
20	C-26	39.33	18.33	72.33	10.00	50.67	14.74	12.71	2.03	0.17
21	C-29	30.50	14.67	71.00	7.33	25.00	8.24	7.57	0.67	0.09
22	C-30	39.33	15.67	66.67	18.33	60.67	21.24	19.47	1.77	0.09
23	C-31	44.00	18.33	76.00	12.33	60.33	26.27	19.87	6.40	0.32
24	C-33	23.67	14.33	67.33	8.00	27.67	8.48	6.88	1.60	0.23
25	C-34	54.33	28.33	53.33	24.33	90.00	27.77	21.97	5.80	0.26
26	C-35	33.33	16.00	65.67	10.33	43.00	16.51	12.74	3.77	0.32
27	C-36	30.33	13.00	59.00	10.67	54.33	9.84	8.74	1.10	0.13
28	C-37	46.00	28.33	71.33	17.33	93.00	25.31	19.24	6.07	0.32
29	C-38	32.00	23.67	64.33	10.33	50.67	10.57	9.77	0.80	0.08
30	C-39	44.00	15.67	60.00	10.33	65.00	12.41	11.51	0.90	0.08
31	C-40	33.33	13.33	53.00	11.00	38.33	8.17	7.47	0.70	0.09
32	C-42	42.67	12.00	63.67	11.33	42.33	8.94	8.47	0.47	0.05
33	C-44	41.33	11.33	54.67	12.33	64.33	9.64	8.84	0.80	0.09
34	C-45	34.00	21.67	70.67	14.67	53.67	6.87	5.61	1.27	0.22
35	C-46	40.67	17.33	70.00	14.00	49.33	17.57	15.44	2.13	0.14
36	C-47	37.67	27.67	66.67	15.00	61.67	18.34	16.44	1.90	0.11
37	C-48	33.00	12.33	77.67	14.33	51.67	14.57	13.34	1.23	0.09
38	C-49	37.67	33.33	74.67	20.67	65.33	22.57	19.11	3.47	0.18
39	C-50	39.33	11.67	60.00	12.67	35.33	10.24	8.54	1.70	0.20
40	C-51	46.00	35.00	80.33	16.67	58.00	27.54	20.41	7.13	0.35
41	C-52	50.50	13.33	73.33	13.33	47.33	20.31	19.11	1.20	0.06
42	C-53	37.00	16.00	64.67	12.67	48.33	12.64	11.81	0.83	0.07
43	C-55	41.00	16.00	60.00	14.00	81.33	23.87	20.94	2.93	0.14
44	C-58	50.67	24.33	69.33	14.67	78.33	23.61	20.07	3.53	0.18
45	C-59	38.67	18.67	61.33	14.33	45.33	13.47	11.24	2.23	0.20
46	D-01	29.33	18.67	68.67	10.00	50.33	14.17	11.77	2.40	0.20
47	D-03	27.67	9.33	96.67	12.33	62.33	20.71	19.27	1.43	0.07
48	D-04	33.67	13.00	72.33	13.33	46.33	14.34	12.74	1.60	0.13
49	D-08	47.00	15.00	85.67	11.67	45.33	15.01	12.64	2.37	0.19
50	D-13	22.33	13.33	74.00	12.00	56.67	2.85	1.71	1.13	0.69

Appendix (contd.) .Mean values of rice genotypes for nine morphological characters

No	Line	RTL	RTV	PHT	NOT	TRN	TDW	SDW	RDW	R/S
51	D-18	27.67	23.00	88.33	11.67	50.00	27.14	25.41	1.73	0.07
52	D-19	46.33	21.67	117.00	13.00	67.67	39.94	34.67	5.27	0.15
53	D-20	22.67	8.33	79.67	8.33	49.33	9.94	8.54	1.40	0.16
54	D-23	32.67	18.33	94.00	15.67	41.67	23.87	19.54	4.33	0.22
55	D-24	42.33	22.67	94.00	16.67	51.67	23.51	20.24	3.27	0.17
56	D-25	40.67	11.67	86.00	9.67	41.33	14.67	12.34	2.33	0.19
57	D-29	34.67	14.33	67.33	14.67	50.00	10.14	9.07	1.07	0.12
58	D-30	27.33	13.00	59.33	17.67	52.67	13.87	12.87	1.00	0.08
59	D-31	58.00	35.33	85.33	11.33	55.33	22.54	16.07	6.47	0.40
60	D-32	39.67	50.00	90.00	15.67	83.33	33.51	18.97	14.53	0.77
61	D-35	38.67	19.00	103.67	11.33	27.33	11.44	10.54	0.90	0.09
62	D-36	33.67	17.67	71.33	12.00	59.33	12.34	10.74	1.60	0.15
63	D-37	45.67	14.00	87.33	9.33	50.00	16.67	15.11	1.57	0.10
64	D-38	30.67	15.00	88.00	7.33	49.00	9.34	7.04	2.30	0.33
65	D-39	47.00	15.00	90.67	15.67	60.67	21.64	19.94	1.70	0.09
66	D-42	45.33	23.67	72.00	11.00	50.00	11.81	10.47	1.33	0.13
67	D-44	41.00	15.67	78.00	5.33	47.33	9.14	8.44	0.70	0.08
68	D-47	40.67	12.67	54.33	14.33	36.67	15.94	14.24	1.70	0.12
69	D-48	36.67	16.33	79.00	14.67	44.00	17.51	16.21	1.30	0.08
70	D-49	28.50	13.67	76.33	10.33	44.33	13.94	13.11	0.83	0.06
71	D-51	36.33	20.00	74.67	17.33	61.67	23.07	20.41	2.67	0.13
72	D-52	26.67	7.00	65.67	10.67	21.67	8.04	7.04	1.00	0.14
73	D-55	37.33	16.67	77.67	11.00	53.33	11.31	9.31	2.00	0.21
74	D-56	37.00	16.67	83.00	15.33	60.00	22.77	19.64	3.13	0.16
75	D-57	41.33	19.00	70.00	12.00	57.33	13.84	12.67	1.17	0.09
76	D-58	33.33	24.33	73.67	14.67	66.67	6.74	5.81	0.93	0.16
77	D-59	33.00	9.33	45.00	10.67	45.00	3.74	3.37	0.37	0.11
78	D-60	40.67	22.67	77.00	17.67	81.67	8.51	6.61	1.90	0.29
79	D-61	31.00	14.67	64.33	11.67	60.33	7.64	6.84	0.80	0.12
80	D-62	30.00	21.00	76.00	14.67	67.33	20.54	18.31	2.23	0.12
81	D-63	30.33	5.00	69.00	10.33	31.00	15.34	13.51	1.83	0.14
82	D-66	46.33	27.33	101.00	15.00	60.00	27.93	21.09	6.83	0.32
83	D-67	36.67	21.67	71.67	12.67	73.00	19.78	15.38	4.40	0.29
84	D-73	40.33	17.67	105.33	7.67	68.33	13.34	12.51	0.83	0.07
85	D-75	38.00	13.33	95.00	7.33	38.33	11.97	11.44	0.53	0.05
86	D-76	41.00	16.00	110.00	8.00	37.67	17.77	16.84	0.93	0.06
87	D-77	34.67	11.67	61.33	12.00	52.67	8.94	8.41	0.53	0.06
88	D-78	47.33	18.33	85.00	14.67	59.00	16.14	13.97	2.17	0.16
89	D-79	27.00	17.33	60.00	14.00	101.33	13.17	11.31	1.87	0.16
90	D-82	59.33	41.67	103.67	16.67	55.00	38.37	29.04	9.33	0.32
91	D-85	35.33	18.67	70.00	13.33	58.33	12.87	10.87	2.00	0.19
92	D-86	50.00	36.67	122.33	15.67	84.67	24.04	19.01	5.03	0.26
93	D-87	46.33	50.00	95.33	15.33	61.67	29.07	25.77	3.30	0.13
94	IR20	27.67	10.00	63.33	14.33	42.00	10.11	9.54	0.57	0.06
95	IR36	48.00	26.67	74.33	14.33	70.00	19.17	15.91	3.27	0.21
96	IR42	35.67	20.67	75.00	16.33	58.33	20.24	18.74	1.50	0.08
97	Jaya	33.00	13.33	65.00	10.00	72.33	12.83	7.96	4.87	0.64
98	Moro	85.67	80.00	107.00	5.67	86.67	31.24	17.14	14.10	0.82
99	C0-39	33.00	17.33	91.00	14.00	59.33	25.97	23.67	2.30	0.10
100	Azucena	79.00	43.00	95.00	14.67	64.00	23.07	19.74	3.33	0.17