

**Genetic characterization and identification of Molecular
markers associated with Root traits and WUE through
LD based association mapping in rice germplasm
accessions (*Oryza sativa* L.)**

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**DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES,
BENGALURU
2010**

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**Dedicated to my beloved
family and teachers**

**DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BENGALURU**

C E R T I F I C A T E

This is to certify that the thesis entitled “**Genetic characterization and identification of Molecular markers associated with Root traits and WUE through LD based association mapping in rice germplasm accessions (*Oryza sativa* L.)**” submitted in partial fulfillment of **Doctor of Philosophy in Crop Physiology** to the University of Agricultural Sciences, Bengaluru, is a record of research work carried out by **Mohan Kumar M V, PAK 5036**, under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

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ABSTRACT

The hoisting population and the unfavorable climate change have reported massive depletion of irrigation water in many parts of the world. Saving water has become one of the prime areas of research in recent years. Rice is a staple food crops for more than half of the human community. With the receding water availability, saving water and improving rice productivity under upland conditions is essential. Pyramiding two or more traits through trait introgression breeding on to single genetic background would be most conceivable approach to achieve the above objective. Among several traits, water acquisition and efficient utilization have paramount significance in improving productivity under water limited conditions. The above being multi-genic in inheritance, a comprehensive molecular breeding program is therefore essential.

A set of diverse germplasm accessions were extensively phenotyped in two seasons i.e 2007 and 2008 for root traits and Water Use Efficiency (WUE). WUE was phenotyped based on carbon isotope discrimination and Root phenotyping was done on a specially constructed root structure. ANOVA revealed a significant genetic variability among root traits, WUE and other associated traits. Regressions analysis revealed a significant consistency between the experiments for most of the physiological traits.

The same set of germplasm accessions was genotyped by locus specific SSR markers on a capillary based electrophoresis system. Molecular analysis revealed adequate genetic diversity among the germplasms. Model based population structure analysis revealed the presence of four distinct sub-populations among the germplasms. This structure was confirmed by distance based neighbor-joining analysis. Marker-trait association analysis was carried out using the software TAssEL MLM strategy considering both population structure and relatedness. Several QTLs were discovered for root traits, WUE and associated traits. The markers RM223, RM163 and RM 224 have been identified to be associated with root trait and WUE as well and also as overlapping markers for several traits.

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ABBREVIATIONS

CID	- Carbon Isotope Discrimination
DAS	- Days After Sowing
HI	- Harvest Index
IRGA	- Infrared Gas Analyzer
IRMS	- Isotopic Ratio Mass Spectrometer
ISSR	- Inter Simple Sequence Repeats
LA	- Leaf Area
LAD	- Leaf Area Duration
LAI	- Leaf Area Index
LOD	- Likelihood of Odds
MTR	- Mean Transpiration Rate
NAR	- Net Assimilation Rate
QTL	- Quantitative Trait Loci
R	- The molar ratio of heavy to the lighter stable isotope
RAPD	- Random Amplified Polymorphic DNA
RFLP	- Restricted Fragment Length Polymorphism
RuBisCO	- Ribulose 1,5- Bis Phosphate Carboxylase/Oxygenase
RWC	- Relative Water Content
SLA	- Specific Leaf Area
SSR	- Simple Sequence Repeats
BM	- Total Biomass
TE	-Transpiration Efficiency
VPD	-Vapour Pressure Deficit
WUE	-Water Use Efficiency
‰	-Parts per thousand
$\delta^{18}\text{O}$	-Oxygen isotope enrichment
$\Delta^{13}\text{C}$	- Discrimination against ^{13}C

Introduction

I. INTRODUCTION

Rice (*Oryza sativa* L.) is staple food for more than half of the human community. More than three billion people, most of them in Asia, depend on rice for their major source of calories. Rice is the only major cereal crop that is consumed almost exclusively by human beings. It is cultivated worldwide in 114 of the total 193 countries of the world. Rice occupies about 11 percent of the world's arable land and grown annually on more than 150 million hectares with an annual production of around 600 million tons. Rice is probably the most diverse of all food crops. Rice is grown at more than 3,000 meters elevation in Bhutan and three meters below sea level in Kerala (Anderson, 2003). About 90% of the global rice area is in the Asian continents, where more than 90% of the world's rice is produced and consumed. It is home for 60% of the world's population and two-thirds of the world's poor reside here.

Since majority of the rice grown in the irrigated lands, rice alone consumes roughly about one half of the total fresh water used for irrigation. Rice stands first followed by sugarcane in terms of thousand litres of water used to produce one million ton of economic yield. With the global fresh water reserves rapidly depleting and the burgeoning population demands a strategic shift in crop cultivation. In India total food grain demand will grow from 198 MT in 2009 to 241 MT in 2050 (UN report, 2006). Among this rice being a major food crop, accounts for about 55% of the total food grain production. This implies that the fresh water requirement for agriculture will hike by 68.5 Trillion litres between 2000 and 2025 in India. Thus evolving strategies that can save water from rice ecosystem should get the utmost emphasis.

Several technologies have been developed to explore the possibility of cultivating rice which reduces water requirement. Water requirement can be lowered by reducing loss by seepage, percolation and evaporation. Promising technologies include saturated soil culture, intermittent irrigation, and the system of rice intensification (SRI). However these technologies still use prolonged periods of flooding, so water loss remains high. A fundamentally different approach is to grow rice like an upland crop such as wheat on non-flooded aerobic soil. A new approach is called Aerobic approach has been practiced which has shown a great potential to save water, but with a severe yield compromise

(Bouman *et al*, 2000). Thus it is imminent that rice cultivars must be genetically improved to suit aerobic practice for stabilizing productivity.

From the physiologist's perspective, plant water relations play a very curial role in determining the level of drought adaptation in plants. Root occupies the pivotal position in maintaining tissue water relations through its role in extracting water from deeper layer of soil profile (Sheshshayee *et al*, 2010). A deeper root system has been shown to allow upland rice to extract more water from the soil, resulting in a higher yield potential (Manbani and Lal, 1983). Individual root characteristics, such as thickness, depth of rooting, and the ability to penetrate compacted soils have been associated with drought avoidance (O'Toole and Chang, 1979; Yoshida and Hasegawa, 1982; Ekanayake *et al*, 1985a). Plants with deep root system hence have the ability to supply water to support a higher transpiration demand, thereby enhancing total biomass (Li *et al.*, 2005; Yadav *et al.*, 1997). These studies emphasized the relevance of breeding to improve root traits to achieve better productivity under water limited conditions (Reynolds *et al.*, 2007; Reynolds and Tuberosa, 2008).

Plants are thought to have evolved numerous strategies for coping with limited water availability including changes in phenological, developmental and physiological traits (Schulze *et al.*, 1987; Ludlow, 1989; Passioura, 1996; Araus *et al.*, 2002). Such traits which are evolved under water limited condition are adaptive traits and have only survival significance with little or no advantage for crop growth and productivity (Udayakumar and Prasad, 1994, Collins, *et al.* 2008). Any trait would have relevance only when it is associated with superior crop growth rates. From this context, water mining traits associated with roots, efficient use of water for biomass production (WUE), water conservation strategies have great relevance.

Water Use Efficiency (WUE) is defined as the ratio of carbon gained per unit water lost. As per the Passioura's yield model (Passioura, 1986), WUE is an important determinant of crop growth, besides being an adaptive trait under water limited conditions. However, for a comprehensive important in a drought tolerance and productivity, Root traits and WUE need to be pyramided on to a single elite genetic background. The combination of these relevant physiological traits like root and WUE must be pyramided on to a single genetic background in order to achieve an overall

improvement in drought tolerance as well as enhanced productivity under such conditions. However, breeding for these physiological traits is complex as these traits have polygenic inheritance and are also difficult to quantify. In this direction much progress has been made in understanding the genetic basis of complex traits through Quantitative Trait Loci (QTL) mapping strategy. QTL mapping starts from the construction of populations that originate from homozygous, inbred parental lines with different phenotypic distribution. The resulting F_1 lines will tend to be heterozygous at all loci. From the F_1 population, crosses are made to develop several mapping populations and the segregation of markers is statistically modelled. Phenomenal success has been made towards identifying QTLs for several such traits through traditional bi-linkage mapping. However, development of mapping populations for various complex traits has been a major limitation for identifying QTLs through Bi-parental linkage mapping. Successful identification of QTLs for Quantitative traits depends on the selection of contrasting parents and the extent of complex trait segregation in the recombinants. Furthermore, improving drought tolerance requires a simultaneous engineering of several diverse traits, which renders linkage mapping a very difficult proposition. In this context, association mapping has emerged as an attractive alternative approach for traditional bi-parental QTL mapping.

The higher mapping resolution, the ability for identifying rare alleles, the possibility of associating markers for very diverse traits render LD mapping a potential approach for QTL tagging. This strategy scores over the conventional linkage mapping in being able to screen for multiple alleles while linkage mapping is limited to scoring only two alleles. The greatest advantage of association mapping lies in the time taken for establishing an association mapping panel compared to the time and cost required for developing a stable association mapping population.

The availability of genome information in rice has greatly complimented the process of genome mapping. Large numbers of SNP and SSR markers in rice are being extensively used for whole genome as well as candidate gene association mapping. Furthermore, association mapping is best suited for identifying markers to diverse traits such as those necessary for drought tolerance. The major objective of this investigation was to exploit the advantages of Linkage disequilibrium (LD) based association mapping for tagging genomic regions that govern the variations in drought tolerance traits.

With this background, present study was under taken to identify the QTLs associated with drought tolerance traits such as Root traits, WUE and related traits in rice with the following specific objectives

- 1) Phenotyping for variability in Root traits and Water use efficiency (WUE) in diverse rice germplasm accessions.
- 2) Genotyping rice germplasm with SSR markers to determine population structure.
- 3) Identification of Marker-trait association through LD based association mapping.

Review of Literature

II. REVIEW OF LITERATURE

India receives an annual precipitation of 4,000 Trillion litres. About 19.4% of total precipitation is non-usable water. Domestic and industrial activities consume about 11% of total water. Percolation or ground water account for 36 % and remaining 64% is used for agricultural needs. On the other hand the annual precipitation is on a decrease and the Indian population is expected to increase from 1.13 billion in 2005 to 1.66 billion by 2050. This is coupled with increase in urbanization, industrialization and per capita income. Also the production of water intensive crops is expected to increase by 80% between 2000 and 2050. This water scarcity posed a severe threat to grain production to feed the increasing population.

Rice being a hydrophilic crop is the staple food of south Asia for its high carbohydrate and protein levels. Although India accounts for 21.9% of total world rice production, to feed the growing population rice production has to be increased from 178 million ton in 2000 to 241 million ton in 2050. This increase in the production level is expected from the same net cropped area which also has to cater to other crops. Out of total 47 M Ha under rice cultivation in India, 22.4 M Ha is under irrigated rice cultivation and the rest of the rice productivity come from upland rainfed conditions, which accounts for 45 % of national rice production.

Rice relies strictly on the rainfall for its water supply under rainfed condition; drought is a serious constraint to rice production and yield due to unpredictable, insufficient and uneven rainfall during the growing period. Droughts have been frequent in different parts of India and are responsible for many famines and rural poverty. Due to the severe and unprecedented drought conditions during the year 2002-03, the total annual rice production reduced to 76 million tonnes, 17 million tonnes less than the record rice production of 93 million tonnes during 2001-02 (Mishra, 2004). In the rainfed lowland ecosystem of eastern India, drought stress causes 1.2 million tonnes of rice yield loss annually. It has been estimated that the rice demand in India at 2010 will be 100 million tonnes and in 2025, the demand will be 140 million tonnes (Mishra, 2004). This increased demand for rice can no longer be met only from irrigated areas. Greater efforts are needed to enhance the contribution of rainfed areas to overall agricultural production.

As agriculture still accounts for at least 70 percent of world's total water usages, water supply is the major limiting factor in producing more food in future.

South-west as well as south-east monsoon are the major rainfall currents which bring about 95 % of the rainfall in India. South-west monsoon accounts for about 80 % of the total rainfall are major source of agricultural water resource in south and central India. Recent abrupt increase in global warming and also spreading desertification has caused frequent and unpredictable monsoon in recent years. This has resulted in serious drought damage in rice growing areas in many upland and low land rice growing areas that rely solely on rain to grow rice. Due to increasing water scarcity, exploring ways to produce more rice with less water is essential for food security and sustaining environmental health in Asia.

2.1 Importance of roots as drought tolerance traits

Drought tolerance in rice is physiologically and genetically complex. Drought tolerance may be defined as the ability of a crop to withstand suboptimal water availability by utilizing adaptations that permit metabolism to occur at low water potential and produce sustainable yields. Different types of drought are described based on the period of occurrence. They are

- 1) Terminal drought
- 2) Vegetative drought
- 3) Intermittent drought

Terminal drought occurs towards the end of the crop season from anthesis to grain filling. Among all these types of drought, terminal drought has been very devastating in south India due to the cessation of monsoon rain before maturity. It is said that about majority of the total rice yield reduction are caused by terminal drought (Kamoshita *et al* 2008).

There are a number of traits which are thought to contribute to drought tolerance mechanisms. The ability of root systems to provide for evapotranspirational demand from deep soil moisture and capacity for osmotic adjustment forces to consider roots as major drought tolerance traits in rice (Passioura 1982, Nguyen and Chandrababu, 1997).

Root traits being constitutive traits, provide the ability to the plant to meet the evapotranspirational demand during the period terminal drought stress by extracting water from deeper layers of soil (Lilley and Fukai, 1994).

Yoshida and Hasegawa, (1982) demonstrated that under upland conditions genotype which has thicker and deeper roots will extract large amount of water from deeper layers and maintain high plant water status.

Genotype or cultivars with deep root development will maintain higher leaf water potential, provided that the genotype has high hydraulic conductance. If the genotype has high root restriction the genotype suffers from low leaf water potential status. This shows that a well developed root system will help the plant in maintaining high plant water status (Kato *et al*, 2007).

Tardieu and Davies, (1993) showed that root signals such as ABA play an important role in stomatal regulation under mild drought conditions and further the hydraulic force becomes important under severe drought. However Siopongco *et al* 2008 showed that evidences for genotypic difference in leaf water potential in rice is very poor.

Umayal *et al* (2001) showed that drought tolerant *indica* land races in south India has thicker and wider xylem vessels compare to susceptible types. These wider xylem vessels may decrease the chance of cavitation and increase the ability of root to conduct water.

In some regions which has very less interval (7 to 10 days) of terminal drought, conservative use of water by shallow root types are more beneficial. Pantuwan *et al* (2002) showed that in shallow root types when a terminal drought occurs for a short period the soil water from 0 to 45 cm soil depth tend to be extracted water slowly during the first 7 days, since these smaller root system that have reduces water demand. These genotypes produce higher yield under such conditions.

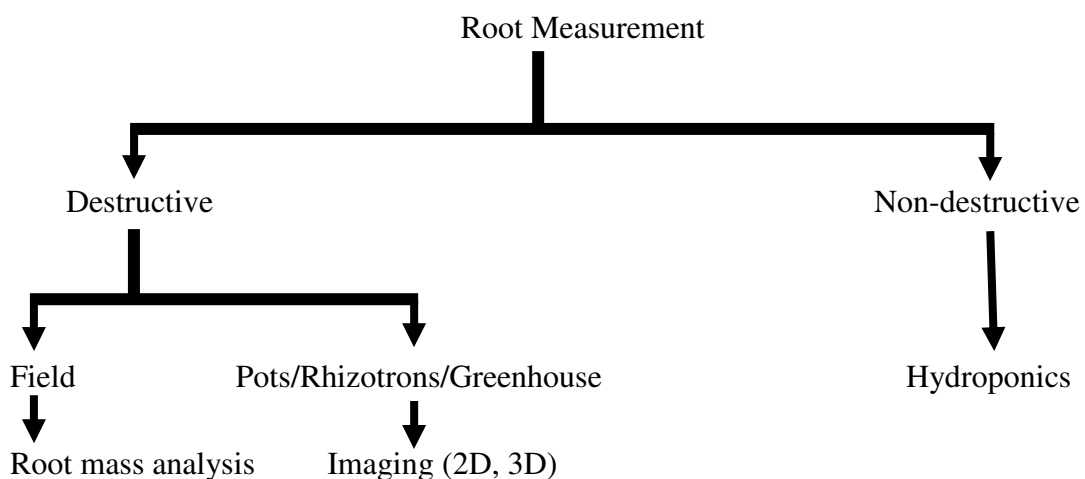
However, in areas where there is sufficient water availability, water extraction would exhaust the soil water resources resulting in the end season stress (Condon *et al.*, 2002). The deeper root system would significantly increase the total biomass as well as yield (Sinclair and Muchow, 2001).

2.1.1 Measurement of root traits

Majority of the above ground traits which play an important role in drought tolerance has been exploited to the maximum extent. This resulted in the reduction of the variability of majority of crop plants. Root being below ground traits play an important role in water mining and thereby overcoming the drought. Determination of genetic variability in root traits represents the most difficult challenge in crop improvement programs.

Several methodologies have been adopted for quantification of root traits (Fig. 1).

Fig. 1: The flow diagram depicts different methods used for root analysis



Tuberosa *et al.*, (2002), used hydroponics for studying root traits, which involves rising of plants in suitable tanks filled with a nutrient solution.

Attempts were made to raise the plants in soil columns/soil pipes of similar length. Soil column was removed carefully without disturbing the roots by destroying the soil pipe. Soil was washed and root mass analysis was carried out (Venuprasad *et al.*, 2002; Ayappa, 2004; Giuliani *et al.*, 2005).

The technique of scanning the root system was evolved for cotton where sampling was done for every 10 cm row for a dept of 0 – 100 cm of soil using a root sampler. The sampled soil was washed and scanned using a hand scanner at 200 dpi. The resultant image was analysed using DT-SCAN software (version 1.0; Delta Inc, UK) to measure root length, average diameter and surface area (Bouman *et al.*, 2000; Zhang *et al.*, 2005).

Junichi *et al* 2006 assessed the genetic variability in a mini core collection of chick pea. The variability in root traits was investigated using a cylinder culture system during two consecutive growth systems.

Significant genetic variability was observed for seven root associated traits in a Double haploid population from a cross between two *japonica* cultivars. The evaluation was done in two different conditions (Lowland and upland). PCV pipes of 1.5M length were used to assess the root variability in upland conditions (Li *et al.*, 2005).

2.1.2 Genetic variability for Root traits in Rice

O'Toole and Bland, (1987) reviewed the genotypic variations in root systems and reported that plant root systems is an important trait that needs to be exploited to enhance the drought tolerance in crop plants.

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

Significant genetic variability in some of these root traits have been demonstrated and implicated for improved drought tolerance in crop plants O'Toole and De Datta, 1986; Thangaraj *et al.*, 1990; Sharma *et al.*, 1994; Sinclair and Muchow, 2001).

Drought tolerant genotype in wheat has significantly more roots in the crown region with the nodal and seminal roots concentrated close to the soil surface. The susceptible genotypes had fewer roots in the crown regions with the roots concentrated away from the soil surface (Kinyua *et al.*, 2003)

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.

Genetic variability in many root related traits demonstrated variation in root hair density, hydraulic conductivity, root length density in chickpea (Kashiwagi *et al.*, 2006)

Maximum root length, root diameter, root dry weight, root length density, root number, root-shoot ratio, root dry weight, thick root number below 30 cm, and root mass density at different depths of soil layers are a few such traits which influences drought tolerance. Significant genetic variability in these traits have also been reported (Armenta-Soto *et al.*, 1883; Chang *et al.*, 1982; Ekanayake *et al.*, 1985a ; Sharma *et al.*, 1994)

2.1.3 Genetic Parameters of Root Traits

Kashiwagi *et al.*, 2006 observed a significant genetic variability at 35 days after sowing for root length density (RLD) (heritability, $h^2 = 0.51$ and 0.54) across seasons, and followed by the ratio of plant dry weight to root length density with h^2 of 0.37 and 0.47 for first and second season, respectively

Significant genetic variability was observed among fifty-nine Asian cultivated rice for root characteristics. The genotypes showed high variability for four root traits (root thickness, stele transversal area, total transversal area and number of late metaxylem vessels) and two morphological traits (root length index and ratio of deep rooting). PCA analysis of root traits among varietal groups showed that *japonica* accessions were scattered more widely than those of the *indica* groups, whereas the PC2 scores of *indica* groups were scattered more widely than those of the *japonica* accessions. This suggested that the *japonica* and *indica* groups contain different genetic diversity with respect to their root characteristics (Uga *et al.*, 2009).

2.2 Importance of WUE in crop productivity

Apart from roots other important traits which can has stimulated interest in the prospect of gaining yield increase under water limited condition is Water use Efficiency (WUE). At whole plant level water use efficiency is defined as the ratio of biomass produced to water used. According to Passioura (1996) crop yield is given by

$$\text{Seed yield (Y)} = \text{Water Use (T)} \times \text{Water Use Efficiency (WUE)} \times \text{Harvest Index (HI)}$$

Where T is the water transpired, HI is the harvest index, ratio of the economical production to total biomass produced which has already stagnated in most of the crops. Therefore at a given level of T, breeding for improved water use efficiency may be beneficial for improved productivity under water drought prone environments.

Most yield improvements have achieved by increasing the transpirational component through management and breeding, and by increasing HI in certain grain crops (Austin *et al.*, 1978). C₄ species typically have a WUE of 4 to 5.5 g. kg⁻¹ compared to the C₃ species (1.5 – 3.0 g. kg⁻¹). Perhaps because of this trait, the C₄ species have significantly higher productivity compared to the C₃ species under arid and semi-arid tropics where water availability is the major constraint. These observations suggest that improving WUE of our crop species have relevance with crop productivity.

2.2.1 Approaches to study Genetic variability of WUE in various crops

2.2.1.1 Gas exchange studies

Physiological WUE can be evaluated by measuring CO₂ fixation and transpiration rate. These measurements are usually performed on a single leaf over a limited period of time.

Biomass produced is a function of photosynthetic rate. Hence WUE at a single leaf level is the ratio of carbon assimilation rate (A) to transpiration (T). Transpiration rate is determined by the intrinsic stomatal conductance and the existing leaf to air vapour pressure difference (V). If the plants being studied are grown under a very similar environmental condition, it can be expected that the leaf to air vapour pressure will be similar and hence, the major factor that determines transpiration would be the intrinsic stomatal conductance (g_s). Therefore,

$$\text{WUE} = \frac{A}{g_s}$$

Though not strong and statistically significant, a positive association between WUE measured by gravimetry and A/g_s was noticed which suggests that A/g_s could be a useful indicator of variation in whole plant WUE (Boominathan, 2001)

Poor relationship between intrinsic WUE (A/g_s) and WUE measured gravimetrically was found in Cowpea suggesting that instantaneous measure of WUE, though dynamic but not stable, hence do not correlate with season long WUE (Bindumadhava, 2000).

2.2.1.2 Carbon Isotope Discrimination

Plants discriminate against the heavy isotope of carbon (^{13}C) during photosynthesis resulting in the depletion of the ^{13}C content in the biomass (O' Leary, 1981). This deviation of the carbon isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) of biomass from that of air, called discrimination ($\Delta^{13}\text{C}$) is related to the ratio of the partial pressures of CO_2 inside the leaf to that in ambient air (P_i/P_a) (O' Leary, 1981; Farquhar *et al*, 1982; Farquhar *et al*, 1989a; Hubick and Farquhar, 1989) as follows;

$$\Delta^{13}\text{C} = a + (b - a) P_i / P_a,$$

Where, a and b are fractionation against ^{13}C during diffusion through stomata and carboxylation by RuBisCO respectively. P_i and P_a are partial pressures of CO_2 in air and inside the leaf tissue, respectively.

Since WUE is also related to the CO_2 partial pressures, at a given VPD, a strong inter-relationship between $\Delta^{13}\text{C}$ and WUE is expected (Hubick and Farquhar, 1989; O'Leary, 1993).

There are two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C . Most of the carbon is ^{12}C (98.9%) with 1.1% being ^{13}C . The isotopes are unevenly distributed among and within different compound and this isotopic distribution can reveal information about the physical, chemical and metabolic processes involved in carbon transformations.

The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric CO_2 , indicating that carbon isotope discrimination occurs during the incorporation of CO_2 into plant biomass. Because the isotopes are stable and non-radioactive in nature, the information is inherent in the ratio of abundance of carbon isotopes, presented by convention as $^{13}\text{C}/^{12}\text{C}$, is invariant as long as carbon is not lost.

The $\Delta^{13}\text{C}$ in plant samples is generally determined using a sophisticated analytical instrument called Isotope Ratio Mass Spectrometer (IRMS) specially designed for high precision measurements of the ratio R, defined as:

$$R = ^{13}\text{CO}_2/^{12}\text{CO}_2$$

The plant material is converted to CO_2 by combustion to determine the isotope composition. In general R is low in organic sample. The atmosphere has a relatively higher fractionation value of around -7.8 per mil (‰), which is in comparison with a standard PDB (Pee Dee Belemnite, from North Carolina, USA). The R in this standard is 0.0124 and in many of the plant material it is approximately 0.012, suggesting a very minor changes in the R value, and hence R in a sample can be compared with that of standard and expressed as ^{13}C in units per mil or parts per thousand (‰).

$$\delta^{13}\text{C} = R_{\text{sample}} - R_{\text{standard}}/R_{\text{standard}} * 1000$$

Since the organic sample has less R-value than the standard, $\delta^{13}\text{C}$ of organic material is more negative, i.e., less ^{13}C content hence more discrimination and vice versa (O'Leary, 1984).

Importance and biochemical basis of $\Delta^{13}\text{C}$ and the relationship of Δ with WUE have extensively been studied (Farquhar *et al.*, 1982; Hubick *et al.*, 1988; Condon *et al.*, 1990; Read *et al.*, 1991). The range of $\Delta^{13}\text{C}$ values across the crop types, having different photosynthetic pathways, is given below:

Range of $\delta^{13}\text{C}$ composition

1. Atmosphere (Air) = -6.4 to -7.0 ‰
2. C_3 plants = -22 to -44 ‰
3. C_4 plants = -9 to -19 ‰
4. CAM plants = -11 ‰ (approximate)

2.2.1.3 Carbon isotope discrimination ($\Delta^{13}\text{C}$) at different steps during photosynthesis

The fractionation of carbon isotope during photosynthesis involves several distinct biochemical and physical processes. These processes have different tendencies to discriminate between ^{12}C and ^{13}C , and the overall discrimination of a particular plant will be a function of the mechanism it uses for CO_2 fixation and the relative balance of the processes that participate in photosynthesis. During photosynthesis, CO_2 must diffuse from the atmosphere to the chloroplast stroma. Since $^{12}\text{CO}_2$ diffuses faster than $^{13}\text{CO}_2$, several fractionation processes occurs along this diffusion path, so that the CO_2 available at the sites of carboxylation is always significantly depleted in ^{13}C compared to the atmosphere (Table 1) (Farquhar *et al.*, 1989; Bragnoli and Farquhar, 1998).

Table 1: Fractionation of carbon isotope during photosynthesis.

Process	Discrimination (‰)	Reference
Diffusion of CO_2 in air through the stomatal pore	4.4	Craig, 1953
Diffusion of CO_2 in air through the boundary layer to the stomata	2.9	Farquhar, 1983
Diffusion of dissolved CO_2 through water	0.7	O’Leary, 1984
Fixation of gaseous CO_2 by RuBisCO	29	Guy and Hoering, 1987

The principal components of photosynthesis that influence discrimination are diffusion of CO_2 through the stomata and the carboxylation process mediated by RuBisCO (O’Leary, 1988, 1993). Irrespective of the photosynthetic sub component-determining discrimination, $\Delta^{13}\text{C}$ is related to P_i (see above)

2.2.1.4. Relationship between $\Delta^{13}\text{C}$ and WUE

As seen above, carbon isotope discrimination is a function of P_i/P_a . Both stomata conductance (g_s) and chloroplast capacity determine the differences in P_i and hence, these two parameters would also control the variability in $\Delta^{13}\text{C}$. Therefore, a

strong relationship between $\Delta^{13}\text{C}$ and WUE can be expected and explained by the following equation (Farquhar *et al.*, 1989b).

$$\text{WUE} = \{(1 - \theta)(b - d - \Delta)\} / 1.6V(b - a)$$

Where, θ is the proportion of fixed CO_2 lost in respiration, V is the leaf-air vapor pressure gradient.

An inverse relationship between A/g_s and $\Delta^{13}\text{C}$ (Meinzer *et al.*, 1990; Richards and Tieszen, 1993) and a positive relationship between P_i/P_a and $\Delta^{13}\text{C}$ signify that P_i determines the variability in $\Delta^{13}\text{C}$ (Hubick *et al.*, 1988; Gutterrez and Meinzer, 1994).

Although WUE and $\Delta^{13}\text{C}$ are related through the ratio of P_i/P_a , as well as with A/g_s (Condon *et al.*, 1990), because of diurnal and seasonal fluctuations in 'A' and g_s , these parameters will not give an integrated estimate of WUE over a period of time (Hall *et al.*, 1993 and Udayakumar and Prasad, 1994). From, this context, $\Delta^{13}\text{C}$ is a dependable parameter as it is a reflection of time integrated estimate of carbon gain per unit transpiration, especially in C_3 plants. $\Delta^{13}\text{C}$ in whole plant dry matter appears to be reliable indicator of plant WUE in pot grown sunflower and negative relationship was obtained between these two traits in structural carbon both in well watered and drought conditions (Johnson *et al.*, 1993). In wheat, as in other C_3 species, genetic variability in $\Delta^{13}\text{C}$ is reflected in variation in WUE at both the leaf and at the whole-plant level (Condon and Richards, 1993).

Such a relationship between $\Delta^{13}\text{C}$ and WUE in several crop species as depicted (Table 2) was not altered even when plants were subjected to abiotic stresses. Maintenance of the relative ranking of genotypes in control and stress implies that for WUE and $\Delta^{13}\text{C}$, genotype and environment interaction is low and the broad sense heritability is high (Hubick *et al.*, 1988; and Wright *et al.*, 1993). Due to these distinct advantages, $\Delta^{13}\text{C}$ appear to be a very reliable parameter for the identification of variability in WUE. This led to the initiation of several breeding programs to improve WUE using carbon isotope discrimination technique (Hall *et al.*, 1993; White, 1993).

Table 2: Relationship between $\Delta^{13}\text{C}$ and WUE in crop species

Species	Relationship			Reference
	r	p value	Number of genotypes	
Cereals and millets				
wheat	-0.75	0.01	12	Farquhar and Richards, 1984
Wheat	-0.74	0.01	16	Condon <i>et al.</i> , 1990
well watered plant				
Watered stressed plant				
Rice	-0.47	0.01	39	Nadaradjan <i>et al.</i> , 2005
Rice	-0.815	0.05	6	Impa <i>et al.</i> , 2005
pulses				
cowpea	-0.93	0.05	5	Ismail and Hall, 1992
Soybean	-0.68	-	-	White <i>et al.</i> , 1996
Irrigated				
Water stressed				
Navy bean	-0.80	0.05	-	Wright,1996
Chickpea	-0.83	0.001	12	Gangadhara, 1995
Cowpea	-0.68	0.02	11	Bindumadhava, 2000
Oilseeds				
Peanut	-0.81	0.01	34	Wright, 1996
Peanut	-0.64	0.05	8	Roy Stephen, 1995
Peanut	-0.69	0.01	17	Shashidhar, 2002
Others				
Kentucky Bluegrass	-0.55	0.01	11	Ebdon <i>et al.</i> , 1998
Crested wheat grass	-0.87	0.05	14	Read <i>et al.</i> , 1991

2.2.2 Stable Isotopes of Oxygen

The existence of natural isotopes of oxygen and hydrogen has been known for more than half a century. The earliest references for the measurements of $\delta^{18}\text{O}$ in organic matter were way back in 1970s. At the leaf however, the oxygen isotope ratio of leaf water increases because of VPD effect (Ferhl and Letolle, 1997). The extent of enrichment of oxygen isotope in water in leaf surfaces depends on, among other factors a few specific physiological traits of the species. Thus, for instance, enrichment of ^{18}O in water from CAM plants doesn't occur during the day, but it does in C_3 plants (Epstein *et al.*, 1977).

Craig and Gordon, (1965) originally developed a model of isotopic fractionation for process occurring during the evaporation of water from the water bodies. They discovered that during evaporation, water molecules with the lighter isotope of oxygen ($\text{H}_2\ ^{16}\text{O}$) tend to diffuse and evaporate faster than their heavier isoform ($\text{H}_2\ ^{18}\text{O}$). This,

they showed, resulted in an enrichment of H_2^{18}O at the evaporating surface of the water body. As an explanation, they studied the isotopic effects of oxygen in water.

Oxygen isotope compositions are expressed as parts per thousand (per mil) as that of carbon isotopes, i.e.,

$$\delta^{18}\text{O} = (\text{R}_{\text{sample}} - 1) / (\text{R}_{\text{standard}} * 1000)$$

Where, R is molar ratio of heavy to light isotope. All water sample compositions are expressed relative to SMOW (Standard Mean Oceanic Water). The absolute ratio for SMOW used in the calculations were $^{18}\text{O}/^{16}\text{O} = 0.0020052$ (Ehleringer and Osmond, 1989).

^{18}O signals can be utilized to measure the rate of transpiration and stomatal conductance (g_s) in plants. The stomatal conductance showed a strong positive correlation with ^{18}O composition of leaf water suggesting that oxygenic isotopic enrichment occurs in leaf water due to g_s .

2.2.3 Oxygen isotope ($\delta^{18}\text{O}$) related with Root biomass

No significant fractionation seems to occur during uptake of water by the roots and the subsequent transfer of water from the roots to the leaves (White, 1993). Much of the $\delta^{18}\text{O}$ enrichment takes place from the leaf due to transpiration. A model has been developed to describe this effect.

$$\delta_l \approx (1 - h) (\delta_s - \delta_v + \epsilon_k) + \epsilon_e + \delta_v.$$

Where $\delta_l = \delta^{18}\text{O}$ of leaf water, $\delta_s = \delta^{18}\text{O}$ of soil water. $\delta_v = \delta^{18}\text{O}$ of atmospheric water vapour, ϵ_e = Equilibrium fractionation due to the change of phase from liquid to vapour, ϵ_k = Kinetic fractionation due to the diffusion of water vapour into under saturated air, h = Relative humidity.

It has been confirmed that the mean transpiration rate is related to the ^{18}O enrichment in leaf biomass and the genetic variability in T and g_s can be quantified by the ^{18}O enrichment technique also it is also observed that the difference in the ^{18}O enrichment can also be used to ascertain whether stomatal factor or the mesophyll capacity brings about the genetic variability in WUE which can significantly contribute for WUE (Sheshayee *et al*, 2005.)

2.2.4 Specific Leaf Area (SLA)

Specific leaf area is the ratio of leaf area to leaf dry weight expressed as cm²/g. SLA has been proposed as an indirect selection criterion for $\Delta^{13}\text{C}$ and WUE (Table 3).

Table 3: Relationship of SLA with $\Delta^{13}\text{C}$ and WUE in a few crop species

Species	Relationship		Reference
	With WUE	With $\Delta^{13}\text{C}$	
Ground nut	-0.70 (p<0.001)	0.75(p<0.001)	Craufurd <i>et al.</i> , 1999
Navybean	-0.86 (p<0.05)	-	Wright, 1996
Soybean	-0.70 (p<0.05)	-	White <i>et al.</i> , 1996
Groundnut	-0.77 (p<0.05)		Sheshshayee <i>et al.</i> , 2006

Improvement of WUE of field crops through conventional breeding programs is not practical because of the complexity and difficulty of measuring WUE of large number of breeding lines under field conditions (Ismail and Hall, 1992). Thus, there is a need for finding an alternative to the conventional approach for the improvement of WUE of field crops. Indirect selection for improved WUE through molecular markers linked to QTL conditioning WUE of a crop plant may prove to be a useful approach in this respect. Molecular marker maps can help us to resolve this complex character into its contributing quantitative trait loci. The identification of QTL allows the analysis and selection of this complex quantitative trait as a set of single gene traits.

2.2.5 Genetic variability of WUE

Characterization of natural variation in WUE is an important prerequisite to application of the assembled body of molecular genetic knowledge associated with different crop species to a broader understanding the genetic control of this phenomenon. Genetic variation in WUE in different crop species can be measured at different technique (Table 4).

At single level, gas exchange efficiency is defined as the ratio of the rates of net CO₂ uptake (A) and transpiration (T) besides agronomic approaches (Gregory 2004). Both uptake and transpiration occur almost exclusively through the stomatal pore in the surface of the Leaf and can be described as diffusion processes. WUE can be improved at the physiological level (Boyer 1996, Parry *et al.* 2005), where it is often approached using the “intrinsic” WUE, i.e. the ratio of net assimilation (A_N) to stomatal conductance (g_s),

which was introduced to compare photosynthetic properties at a common evaporative demand (Osmond *et al.* 1980). Differences between the genotypes for the value of the ratio C_i/C_a and WUE have been reported to have a genetic basis (Martin *et al.* 1989, Masle *et al.* 2005), and breeding for high WUE has become a main objective for many crops (Condon *et al.* 2004). However, as pointed out by Blum (2005), genotypic variations in WUE are normally expressed mainly due to variations in water use or g_s . Because Net Assimilation (A_N) shows a direct, curvilinear dependency of g_s , this increase in WUE often results in reduced photosynthesis and yield.

Heritability serves as a good index for transmission of characters from one generation to next and it should be considered in terms of selection concept (Hanson 1959).

Ray *et al.*, (1999) have demonstrated the broad sense heritability among the physiological traits viz., WUE, delta, canopy temperature and yield in alfalfa cultivars grown in water stressed conditions in two cropping seasons. Heritability in WUE traits and $\Delta^{13}C$ was reported in cowpea (Menendez and Hall, 1995).

Large G x E interactions associated with WUE was observed in Recombinant Inbred lines of *Arabidopsis thaliana*. Relatively low heritability of WUE of 0.18 indicated suggested that selection for WUE will be effective when evaluated in uniform environments (Nienhuis *et al.*, 1994)

In contrast, Hubick *et al.*, 1988 showed a heritability value of 81% in field grown peanut F_2 plants for delta, which is a surrogate estimate for WUE with no significant G x E interactions. They also reported that nuclear genes control the WUE and high WUE exhibited the partial dominance.

Table 4: Genetic variability in WUE across different crop species

Species	Genetic Variability	Reference
Cereals and Millets		
Rice	3.94 – 5.47 g/Kg	Nadaradjan, 2004
Rice	2.5 – 5.4 g/Kg	Impa <i>et al.</i> , 2005
Wheat	4.60 – 5.27 g/Kg	Condon <i>et al.</i> , 1990
Pulses		
Cow pea	2.74 – 3.00 g/Kg	Ismail and Hall, 1992
Cow pea	2.08 – 3.29 g/Kg	Bindumadhava, 2000
Soybean	1.66 – 2.44g/Kg 2.03 – 2.78g/Kg	White <i>et al.</i> , 1996
Irrigated		
Water stress		
Oilseeds		
Groundnut	1.57- 2.66 g/Kg	Hebbar,1990
Ground nut	2.46 – 3.71g/Kg 1.81 – 3.15 g/Kg	Wright, 1996
Irrigated		
Stressed		
Ground nut	1.41 – 3.30g/Kg	Shashidhar, 2002
Ground nut	2.55 – 4.02g/Kg	Roy Stephen, 1995
Others		
Arabidopsis	1.86 – 2.40mg/g	Nienhuis <i>et al.</i> ,1994

Ideal trait is the one which has high genetic variability and importantly it should be less influenced by the environment. *i.e* it should have less G x E interactions. Low G x E for transpiration efficiency has, however been reported in legume crops such as peanut (Wright *et al.*, 1993; Rao and Wright, 1994), cowpea (Hall *et al.*, 1993) and soybean (White, 1998). Significant relationship between the K (WUE adjusted for the VPD) of cowpea in two experiments conducted in two entirely different seasons and soil volumes Thus indicating a low G x E interaction when WUE was adjusted for the prevailing VPD and the relative ranking of the genotypes between pot and minilysimeter also showed similar trend.

Further Wright *et al.*, 1993 showed that a significant variability among peanut cultivars in WUE under well-water and water limited conditions in isolated plants grown in glasshouse and in small canopies in field. Further it was also shown that the G x E interaction for WUE, $\Delta^{13}\text{C}$ and SLA was shown to be very low, while heritability of $\Delta^{13}\text{C}$ was high, indicating that these traits could be used for selecting high WUE in peanut breeding programs.

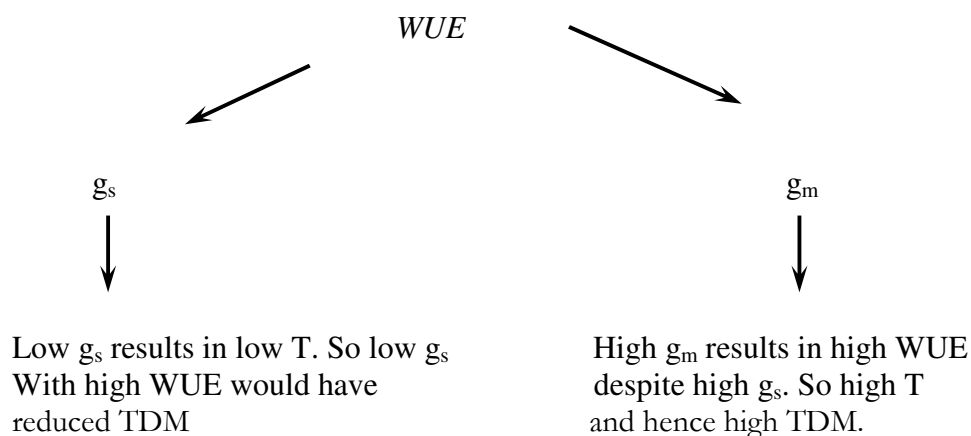
2.2.6 Genetic Advance, Coefficient of Variations for WUE

In an investigation consisting of 16 genotypes rice, carried out by Anasuya, (2003) the genetic parameters like genetic advance, GCV and PCV were assessed. The GCV for WUE was about 32.39% and PCV was about 34%. The genetic advance over the percent mean was high (63.13%).

2.2.7 Intrinsic factors affecting WUE

WUE is predominantly regulated by the stomatal diffusive factor (g_s) and /or mesophyll efficiency (g_m) that regulated carbon assimilation. Therefore, the variability in WUE brought about by intrinsic differences in g_m is desirable. In such cases WUE and T will be less dependent on each other.

In capacity types, the mesophyll factors associated with A will determine the variability in WUE. So, in these types, WUE is independent of g_s and hence T will not be associated with WUE. Selection for high WUE from these types will result in high CGR (Hubick and Gibson, 1993). WUE as well as yield levels can be enhanced only when capacity types selected and are used in breeding program.



Inter relationship between WUE, T and mesophyll conductance in determining biomass production

2.3 Root traits and Water use efficiency in Aerobic Rice

Aerobic rice is a new upcoming strategy for saving water in rice cultivation. It is a fundamental approach to reduce water inputs in rice and is to grow the crop like an irrigated upland crop such as wheat or maize (Bouman and Tuong 2001). Upland rice is already grown in Asia aerobically with minimal inputs in upland environments, but with a low yielding subsistence crop. Achieving high yields under irrigated aerobic conditions requires new varieties of “aerobic rice” that respond to inputs, use water efficiently and resist to lodging. Many strategies have been developed to save water in rice cultivation. A comparison of different rice growing strategy is given in table 5. Although considerable work has been done on aerobic rice, a lot of questions have remained unanswered like what is the extent of water use and water use efficiency in aerobic condition? What is the contribution of root system for maintaining plant water status? What is the actual trade off between water uptake from root system, water use efficiency and Yield? Our basic understanding of Aerobic rice is the genotype which has high root with high water use efficiency will maintain high plant water status and will give sustainable yields under water limited conditions. Traits like high root, low water use efficiency and high yield appears to be tightly linked. Dissecting this linkage and bringing them to a common genetic background is very much essential for development of aerobic rice.

In Brazil, a breeding program on upland rice has resulted in improved varieties with a yield potential of up to 6 t ha⁻¹. Farmers grow these varieties commercially on an estimated 250,000 ha under sprinkler irrigation (Guimaraes and Stone 2000).

In the tropics, upland rice grown under favourable conditions typically reaches maximum yield of over 4 t ha⁻¹ (George, *et al.*, 2001 & 2002). IRRI started to develop tropical aerobic rice systems in 2001, using existing improved upland and lowland germplasm. In upland rice Yields reached a maximum of 5–6 t ha⁻¹, and water savings were 50–60% compared with flooded rice (Castaneda *et al* 2002). As in China, researchers identified specially bred aerobic rice varieties and proper management of water and nutrients as keys to obtaining high yields

Table 5: Classification of rice Production systems by water management strategies

Lowland (Anaerobic)					Aerobic	
	Flooding	Saturated Soil culture	Alternate wetting and Drying		Aerobic Rice	Upland rice
			Irrigated	Rainfed		
Hydrology	Flooded	Saturated	Flooded, Aerobic	Flooded, Aerobic	Aerobic	Aerobic
Irrigation	Irrigated	Irrigated	Irrigated	Rainfed	Irrigated, Rainfed	Irrigated, rainfed
Tillage	Puddled, Bunded	Puddled, Bunded	Puddled, Bunded	Puddled, Bunded	Nonpuddled Bunded	Nonpuddled Nonbunded
Environment	Favourable lowland	Favourable lowland	Favourable lowland	Unfavourable lowland	Favorable upland or Unfavourable lowland	Unfavorable upland
Germplasm	Lowland	Lowland	Lowland	Lowland	Improved Upland×Lowland	Upland
Yield Levels	High	High	High-Medium	Medium-Low	High-Medium	Low

Source: Adopted from Bouman *et al* (2001)

2.4 Quantitative traits

In crop plants a greater proportion of drought tolerant traits are governed by a large number genes. Each gene has smaller contribution to the trait which results in continuous variation rather than discrete variation (Liu 1998). Identification and analysis of such genes is of prime importance because of their complex features such as large number of genes, smaller individual gene effects and high susceptibility to environmental influences. These drought tolerant traits do not provide complete insight on the trait as these are greatly depend on *genetic* $\times$ *environmental* ($G \times E$) interactions (J. Ross *et al.* Collard *et al.*, 2005).

Initial understanding of Quantative traits (QTs) and their measurement was carried out by Fisher (1918). A lot of understanding and experimental research has been made after Fisher in understanding Quantitative traits (Kearsey, 2002). Recent advances in biometrics have made it possible to get finer details of QTs. But these advances help in understanding the multi-gene trait in terms of means, variances and co-variances. however the precise nature of individual gene action in far from satisfactory. The major barrier in dissecting the QTLs is the interference of phenotypic effects of smaller gene, whose actual number and position cannot be determined.

2.5 Molecular Markers and their relevance in Quantitative Trait Loci (QTL) Mapping

Molecular marker is a sequence of nucleic acid molecules which represents genetic differences between individual organisms or species. Molecular markers act as the tags for the target gene. Those markers which are in close proximity to the target gene are referred as tightly linked marker or gene tags. These markers themselves do not affect phenotype of the trait since these regions are usually neutral genomic regions which are closely linked to the gene. Over several years different molecular marker system has evolved and utilized in several applications (Table 6).

Table 6. DNA marker systems that have been developed over the years.

Sl No	Acronym	Technique	Reference
1	AFLP	Amplified Fragment Length polymorphism	Vos <i>et al.</i> , 1995
2	AP-PCR	Arbitrary primed PCR	Welsh and McClelland, 1990
3	AS-PCR	Allele Specific PCR	Sarkar <i>et al.</i> , 1990
4	CAPS	Cleaved Amplified Polymorphic Sequence	Lyamichev <i>et al.</i> , 1993
5	DAF	DNA Amplification Fingerprinting	Caetano-Anolles <i>et al.</i> , 1991
6	ISSR	Inter Simple Sequence Repeats	Zietkiewicz <i>et al.</i> , 1994
7	MP-PCR	Microsatellite Primed PCR	Meyer <i>et al.</i> , 1993
8	RAMS	Randomly Amplified Microsatellite	Ender <i>et al.</i> , 1996
9	RAPD	Random Amplified Polymorphic DNA	Williams <i>et al.</i> , 1990
10	REMAP	Retrotransposon Microsatellite Amplified Polymorphism	Kalendar <i>et al.</i> , 1999
11	RFLP	Restriction fragment Length Polymorphism	Botstein <i>et al.</i> , 1980
12	SAP	Specific Amplicon Polymorphism	Williams <i>et al.</i> , 1991
13	SCAR	Sequence Characterized Amplified Region	Williams <i>et al.</i> , 1991
14	SRAP	Sequence Related Amplification Polymorphism	Li and Quiros, 2001
15	SNP	Single Nucleotide Polymorphism	Nikiforov <i>et al.</i> , 1994
16	SSCP	Single Stranded Conformation Polymorphism	Orita <i>et al.</i> , 1989
17	SSLP	Microsatellite Simple Sequence Length Polymorphism	Rongwen <i>et al.</i> , 1995
18	SSR	Simple Sequence Repeat	Hearne <i>et al.</i> , 1992
19	STMS	Sequence Tagged Microsatellite Sites	Beckmann and Soller, 1990
20	STS	Sequence Tagged Sites	Fakuoka <i>et al.</i> , 1994
21	TRAP	Target Region Amplification Polymorphism	Hu and Vick, 2003

2.5.1 SSR Markers

The genomes of higher organisms are repeated with multiple copies of simple DNA in arrays of sequences. All arranged in arrays of vastly differing in size (Hancock, 1999). A good thumb rule is that bigger the repeat unit is longer the array tend to be. These DNA sequences are of 3 types:

1. Major satellite DNAs: Long tracts up to several Mb, of well known families of repeat sequence elements.
2. Minisatellites: Blocks of repeated core elements longer than 10 to 15bp in tandem arrays up to 30Kb or longer.
3. Microsatellites: The smallest class of simple sequence repeat elements in tracts less or much less than 1Kb.

The microsatellites are also called as short tandem repeats (STR) or simple sequence repeats (SSR) or simple sequence length polymorphisms. Typically they may be dinucleotides (AC)_n; trinucleotides (TCT)_n; tetranucleotide (TATG)_n and so on, where

n is the number of repeating units within the microsatellites locus. In addition to occurring at many different loci they can also be polyallelic. The (AT)_n dinucleotides are the most abundant type of SSR in plants (Ma *et al*, 1996). The methodology used to isolate an SSR at particular locus starts with the construction of the small insert genomic library. PCR amplification is used to generate DNA banding patterns on a gel and to reveal the polymorphism based on different number of repeats at the two alleles of a locus (Fig. 2).

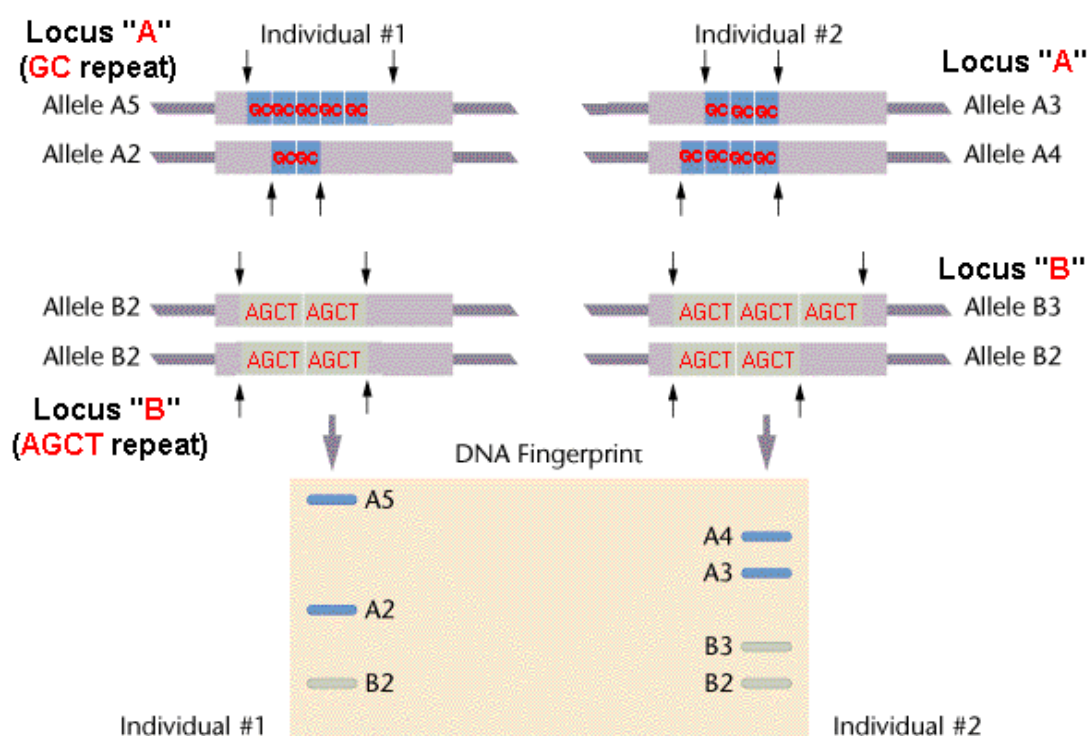


Fig. 2: Schematic representation of SSR protocol

The marker thus has the advantage of being co-dominant as well as multi-allelic. In addition they are simple, PCR based and extremely polymorphic, and highly informative due to large number and high frequency of alleles detected and to their ability to distinguish between closely related individuals. They find application as marker for mapping, cultivar identification, protecting germplasm, determination of hybridity, analysis of gene pool variation and as diagnostic markers for traits of economic value (Powell *et al.*, 1996). Table 7 represents a comparison of different PCR based markers widely used for plant DNA fingerprinting.

In plant systems, microsatellites or simple sequence repeats (SSRs) have become popular as DNA molecular markers. These sequences are abundant, ubiquitous, hyper

variable, co-dominant and have been used for a variety of studies including genome mapping in several animal, insects and plant species (Gupta *et al.*, 1996; Gupta and Varshney, 1999). All these molecular marker systems are compared in the following table 7 (Muller and Wolfenbarger, 1999).

Multi-allelic SSRs markers have been used to characterize population structure in maize (Flint-Garcia *et al.* 2003; Remington *et al.* 2001) and rice (Garris *et al.* 2003, Agrama *et al.*, 2007, 2008); and LD-based associations in wheat, (Kruger *et al.* 2004), barley (Maccaferri *et al.* 2005), and Lolium (Skot *et al.* 2005).

Table 7: Comparison of different molecular marker systems

Criterion	RAPD	ISSR	AFLP	SSR
Quantity of information	High	High	High	High
Replicability	Variable	Moderate	High	High
Resolution of genetic differences	Moderate	Moderate	High	High
Ease of use and development	Easy	Easy	Moderate	Difficult
Development time	Short	short	Short	Long

2.6 Genetic basis of complex traits

Understanding the genetic basis of complex traits is the foremost challenge facing modern geneticists. It's been a big challenge because of the following nature of complex traits. First, instead of one major gene as in the Mendelian inherited traits, they are controlled by many genes, and the effect of each individual gene is usually small and there are many gene-gene interactions. Second, the gene effects are also sensitive to the environment, which means large gene-environment interactions are involved here. There are 2 main approaches to achieve this goal. One is called linkage mapping (also known as QTL mapping in plant and animal breeding studies), which tests for marker-trait associations based on segregation in experimental crosses or pedigrees. Another approach is called Association mapping (as known as Linkage disequilibrium mapping), which looks for marker-trait associations in a population of “unrelated” individuals.

2.7 QTL and linkage mapping

Quantitative trait loci are loci controlling Quantitative traits. Any chromosomal region associated with QT and a marker is defined as a QTL. QTL mapping is a hybrid of statistical genetics and Quantitative genetics. Procedure of QTL mapping involves two basic steps. Identifying and locating the QTL respectively. Sax (1923) first elucidated the concept of detecting QTLs. Further Thoday (1961) developed statistical methods for locating a QTL. Standard QTL mapping approaches such as Composite Interval Mapping (Zeng, 1994) can be used, but power and precision may be compromised by limited recombination in segregating/recombinant inbred lines and by relatively small sample size of most mapping populations (Kearsey and Farquhar, 1998; Beavis, 1998).

2.7.1 Basic Problems in linkage QTL analysis

Even though QTL mapping has been standardized to achieve a fair level of precision yet they suffer from certain constraints arising out of complexity of QTs.

- 1) Lack of recombination causes large confidence intervals, up to 40cM (Chen and Churchill, 2001). The basic inference of confidence interval is that the region 10cM contains an average of 130 genes in rice. Thus in order to sample large number of meiosis a large number of populations has to be analysed. Thereby using a large number of samples and several number of recombination is impracticable.
- 2) Another basic problem in precise QTL location is to identify the true number of QTLs governing a trait, (Parvex sofi and Rather, 2007). Hyne *et al.*, 1995 reported that it is difficult to locate more than 12 QTLs in a given population at one time. Due to this only QTL with significant effects which is biased towards higher value will be reported. Thus the QTL mapping over all results in underestimation of true QTLs with over estimation of their dominance and additive effects.
- 3) Kearsey (2002) reported that it is not possible to detect QTLs which are less than 20cm apart. This result in underestimation of QTL which are in repulsion phase or a ghost QTL will be inserted in between two true QTLs in coupling phase. This greatly declines the power and precision of QTL mapping in precise location of QTLs.
- 4) QTL mapping involves the development of mapping population, which is a tedious process and involves time and more human resource.
- 5) In a given population analysis of only two alleles in consideration is possible. Thereby the chance of association of a marker with a trait is less.

2.8 QTL Mapping in Segregating Populations

Both linkage analysis and association studies rely on co-inheritance of functional polymorphism and neighbouring DNA variants. The difference is that in linkage analysis, there are only a few opportunities for recombination to occur within families and pedigrees with known ancestry, resulting in relatively low mapping resolution; where as in association mapping historical recombination and natural genetic diversity were exploited for high resolution mapping (Fig. 3). Linkage disequilibrium mapping between a functional locus and molecular markers is low except for those within very short distance.

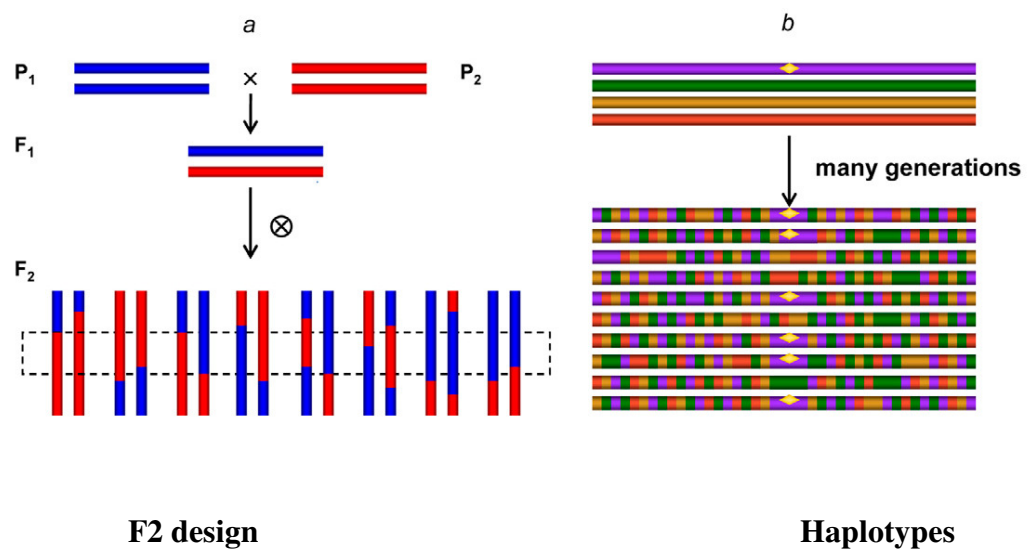


Fig. 3: Schematic representation of Linkage and association mapping.

Mapping population like Double haploids (Li *et al.* 2003; Hittalmani *et al.* 2002) recombinant inbreds (Ge *et al.* 2005; Guo *et al.* 2005) or advanced backcross lines (Li *et al.* 2004; Jing *et al.* 2005) derived from an F₁ cross between different cultivars were used for QTL mapping for different traits. The mapping population used in conventional QTL mapping requires high linkage disequilibrium between the linked loci (Mather *et al.* 2004) and there should be no population structure in the population. Presence of population structure and linkage disequilibrium between non-linked loci may result in false positive results.

2.9 Association mapping/ Linkage disequilibrium mapping/ Gametic phase linkage disequilibrium mapping

Association mapping is an alternative approach to standard QTL methods that is routinely used in human studies and one that is gaining support in the plant research community (Fig. 4). The aim of Association studies is to map phenotype influencing genes by identifying alleles whose presence or absence in individuals correlates with phenotype (Risch and Merikangas 1996; Clark., 2003). Association mapping identifies QTLs by examining the marker-trait associations, and enables researchers to use modern genetic technologies to exploit natural diversity and locate valuable genes in the genome (Zhu *et al.* 2008, Yan *et al.*, 2009). Fig. 5 shows a comparative advantage of association mapping over other mapping strategies.

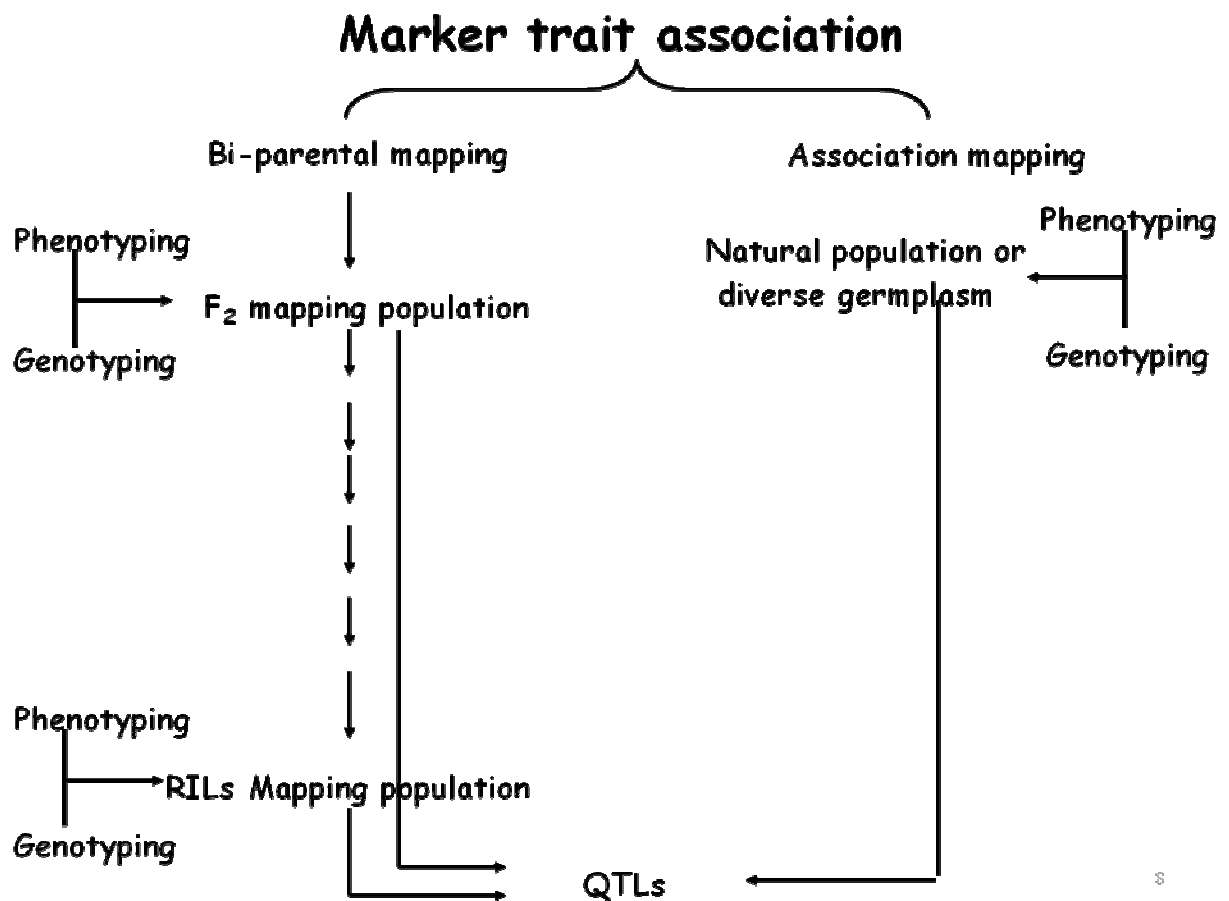


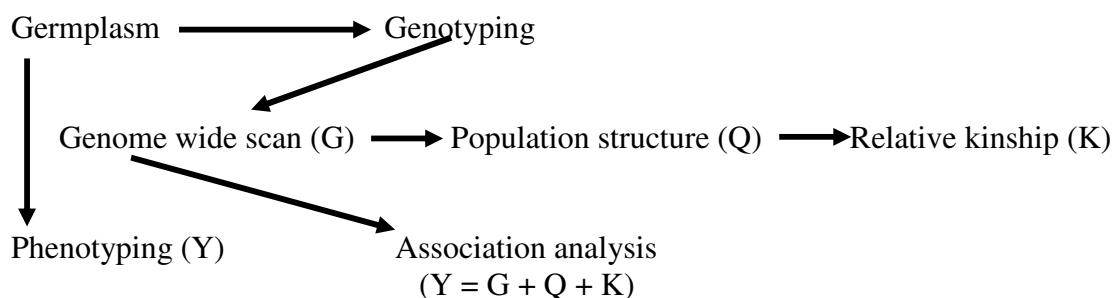
Fig. 4: Comparison of marker trait association with Bi-parental mapping and Association mapping

Association studies rely on the fact that remnants of the ancestral genome in which a phenotypically important allele originated can be shared among descendants who carry the phenotype. The primary advantage of this approach is based on the ability to rapidly query informative regions of the genome among unrelated individuals that have generated numerous meiotic events over multiple generations. Linkage disequilibrium studies have been conducted for various marker-trait associations in maize (Yu and Buckler, 2006), rice (Garris *et al.*, 2003), potato (Simko *et al.*, 2004), barley (Cockram *et al.*, 2008; Malysheva-Otto *et al.*, 2006; Rostoks *et al.*, 2006) and wheat (Breseghello and Sorrells, 2006). Few studies have validated results in separate test populations.

2.9.1 Requirements of Association mapping

1. A suitable natural population which have diverse genetic background
2. Linkage disequilibrium map of natural population
3. Population structure (Q matrix) of the given natural population
4. Kinship Co-efficient (K matrix) of the given population
5. Reliable phenotypic screening methods and generation of phenotypic data
6. Mapping programmes for determining population structure and marker-trait association.

2.9.2 Step in Association mapping



2.9.3 Natural populations and their relevance in Association studies

The choice of natural population is crucial for discovery of marker-trait association. The populations should cover the full range of phenotypic variance. To get higher number of alleles sampled genetically diverse set of populations should be selected

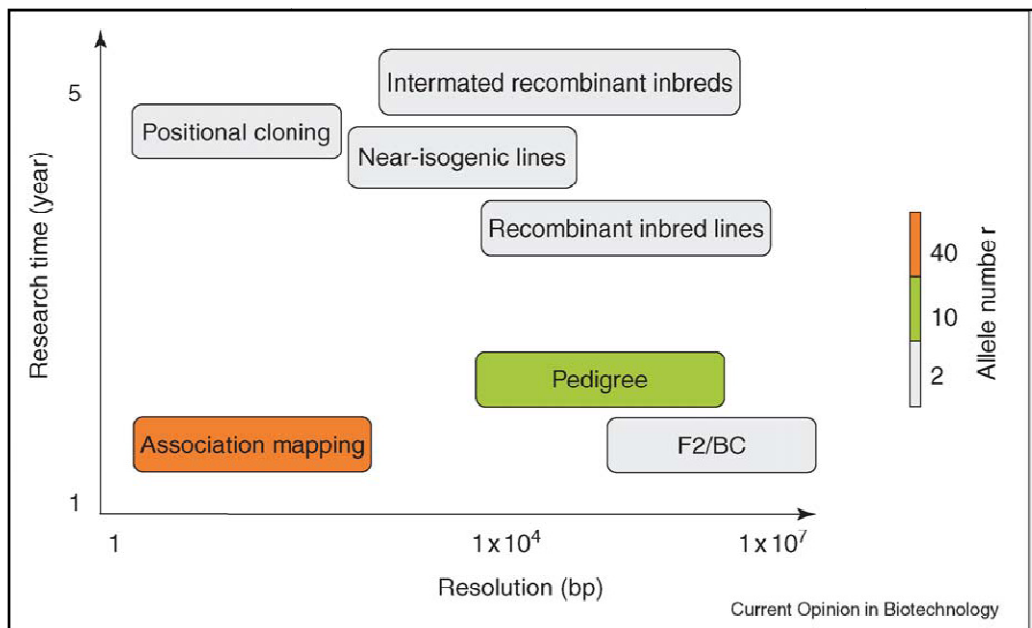


Fig. 5: Advantage of Association mapping compared with other Mapping approach

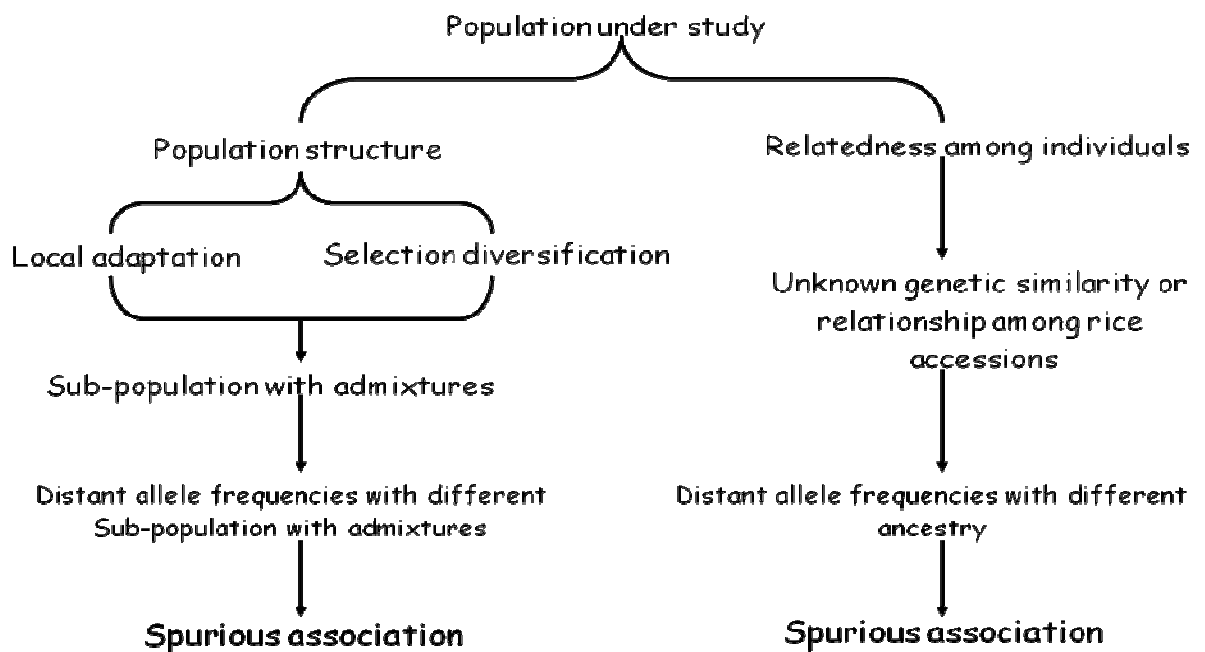


Fig. 6: Confounding effect caused by Population structure and familial relatedness

2.10 Linkage Disequilibrium

The term LD refers to the non-random association between two markers or two genes/QTLs or between a gene/ QTL and a marker locus (Gupta *et al* 2005). LD is commonly found in natural populations between loci which have sufficient time to dissipate the initial disequilibrium. When a population in LD mates at random, the amount of disequilibrium progressively declines with succeeding generations. Thereby the degree of LD is therefore dependant on both the recombination fraction and the time in generations. LD of a given population depends on a very large number of factors (Table 8)

Table 8. Factors that affect LD in a population

	Factor	Effect
1	Recombination rate	Higher recombination lowers LD
2	Mating systems:	Selfing species High LD
3	Mating systems:	out-crossing species Low LD
4	Genetic isolation between lineages	Increases LD
5	Population subdivision	Increases LD
6	Population admixture	Increases LD
7	Natural and artificial selection Locally	increases LD
8	Population size	Small populations have more LD
9	Balancing selection	increases LD
10	Mutation rate	High mutation rate decreases overall LD but LD around newly created mutated allele remains high until dissipated by recombination
11	Epistatic interactions with significant phenotypic effects	Increase LD
12	Genomic rearrangements	Rearrangements suppress local recombination

2.10.1 Linkage Disequilibrium in plants

LD varies significantly among self and cross pollinated crops. LD decay is very high in cross when compared to self pollinated crops (Oraguzie *et al*, 2007). LD values and patterns in maize were found to vary substantially among populations with different selection and developmental histories (Tenaillon *et al.*, 2001; Clark *et al.*, 2004).

Remington *et al.*, (2001) reported that LD declined rapidly within a 1500 bp intra-genic region of four candidate genes for height and flowering among a diverse

collection of maize inbred lines. SSR markers produced larger LD regions than SNP markers across the genome.

Using elite maize inbred lines, Ching *et al.* (2002) found, in contrast to diverse germplasm, little or no reduction in LD values over a 300-500 bp range for 18 candidate genes. *A. thaliana* as a self pollinating species produced allelic associations over a much greater region (~ 250 kb) than in outcrossing species such as maize (Hagenblad and Nordborg, 2002; Nordborg *et al.*, 2002).

LD mapping was used recently to identify a serine substitution in the candidate CRY2 photoperiod receptor gene in *A. thaliana*, presumably responsible for the A(S) early flowering phenotype (Olsen *et al.*, 2004).

Garris *et al.* (2003) characterized LD in the candidate region of *xa5*, a recessive gene conferring race-specific resistance to bacterial blight disease in rice. Thirteen segments from a 70-kb candidate region in 114 landrace accessions were sequenced along with five additional segments from an adjacent 45-kb region in resistant accessions. The results showed significant LD up to 100 kb between sites that suggested genome-wide scanning may be feasible for markers that are associated with simple and complex traits.

The candidate gene approach was recently employed in LD mapping of QTLs for disease and maturity traits in tetraploid potato (Gebhardt *et al.*, 2004; Simko *et al.*, 2004). A list of several association mapping studies in several crops is given in Table 9.

Table 9. List of Association and Linkage disequilibrium studies in plants

Plant	Study	Sailent features	Reference
Barley	Nucleotide sequence diversity between two alcohol dehydrogenase genes (<i>adh3</i> and <i>adh1</i>) in wild barley	Strong geographic pattern of diversity at “ <i>adh3</i> ” despite geographic uniformity at <i>adh1</i> . evidence of intralocus recombination in the “ <i>adh3</i> ” data, despite the high level of self-fertilization characteristic of wild barley	Lin <i>et al.</i> , 2000
Barley	Patterns of nucleotide sequence diversity analyzed for three duplicate alcohol dehydrogenase loci (<i>adh1–adh3</i>) within a species-wide sample of 25 accessions of wild barley	The <i>adh1</i> and <i>adh2</i> loci are tightly linked (RF=0.01) while the <i>adh3</i> locus is inherited Independently. Data reveal heterogeneity in patterns of nucleotide sequence diversity and levels of linkage disequilibrium that are indistinguishable from those observed at <i>adh1</i> in maize, an outbreeding grass species	Lin <i>et al.</i> , 2001
Barley	LD near the Ror1 gene	LD extended >100 bp. Regions of highly localized recombination events around Ror1 (close to the 1H centromere) was observed	Collins <i>et al.</i> 2002
Barley	Association mapping of partitioning loci in barley	<i>VRN-H1</i> and the candidate <i>VRN-H2</i> are identified using association Mapping. This provided the proof of concept for feasibility of association mapping in barley, even within highly structured populations.	Cockram <i>et al</i> 2008
Maize	SSR and AFLP markers were compared for extent of LD in elite European maize inbred lines.	Results indicated that SSRs should be favoured over AFLPs but the opposite applies to populations with a long history of recombination	Stich <i>et al.</i> , 2005
Maize	Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci	Maize phytonene synthase Y1 was associated with endosperm color. Nucleotide diversity was found to be equivalent at PSY2, another putative phytonene synthase, but was 19-fold less in yellow maize inbred lines than in white	Palaisa <i>et al.</i> , 2003
Maize	Genetic structure and diversity among 260 maize inbred lines partitioned into five groups.	Diversity was high among tropical lines; significant LD in 66% of SSR pairs was attributed to linkage (structure) within groups	Liu <i>et al.</i> 2003
Sorghum	Genetic Diversity and Association Mapping in Sorghum for Brix and Height.	Three significant associations for height were detected. Two of these, on chromosomes 9 and 6, support published QTL studies. One significant association for brix, on chromosome 1, 12kb from a glucose-6-phosphate isomerase homolog, was detected	Murray <i>et al.</i> , 2009
Arabidopsis	Genome wide association mapping for pathogen resistance and flowering time.	Feasibility of Association mapping was tested with 95 accessions. In spite of an extremely high rate of false positives due to population structure, we were able to identify known major genes for all phenotypes tested, thus demonstrating the potential of genome-wide association mapping in <i>A. thaliana</i> and other species with similar patterns of variation	Aranzana <i>et al.</i> , 2005

Arabidopsis	Linkage Disequilibrium Mapping of Arabidopsis <i>CRY2</i> Flowering Time Alleles	<i>CRY2</i> DNA sequences reveal strong LD and the existence of two highly differentiated haplogroups (<i>A</i> and <i>B</i>) across the gene. <i>CRY2</i> indicate that these haplogroups are limited to ~65 kb genomic region around <i>CRY2</i> . Whereas the <i>B</i> haplogroup cannot be delimited to ≤ 16 kb around <i>CRY2</i>	Olsen <i>et al.</i> , 2004
Rice	Population structure and its effect on haplotype diversity and LD surrounding xa5 locus using genotypic data from 114 accessions for 21 SSRs.	Populations were highly structured. In 75 kb region of xa5 locus, and additional 45 kb adjoining region, significant LD was observed between sites up to 100 kb apart (r^2 approached 0.1 only after 100 kb) . This is comparable to the level of LD in flowering time gene FRIGIDA in <i>A. thaliana</i> , but differs markedly from that of dwarf8 (d8) and other genes in maize	Garris <i>et al.</i> 2003
Rice	Association mapping of stigma and spikelet characteristics in rice	109 Core rice collections were used in study and a mixed linear model combining PC matrix and k matrix was adopted for mapping. Resultant associations were adjusted using false discover rate technique.	Yan <i>et al.</i> , 2009
Rice	Association mapping for yield and yield components in rice	A set of 92 blast resistant rice genotypes were genotyped with 123 SSR markers. Population structure analysis identified eight main clusters for the accessions which corresponded to the major geographic regions,	Agrama <i>et al.</i> , 2007
Rice	A survey of extent of LD in rice.	LD in five 500-kb regions of the rice genome in three major cultivated rice varieties <i>indica</i> , tropical <i>japonica</i> , and temperate <i>japonica</i> and in the wild ancestor of Asian rice, <i>Oryza rufipogon</i> was characterised. Extent of LD is greatest in temperate <i>japonica</i> (probably >500 kb), followed by tropical <i>japonica</i> (~150 kb) and <i>indica</i> (~75 kb). LD extends over a shorter distance in <i>O. rufipogon</i> (≤ 40 kb) than in any of the <i>O. sativa</i> groups.	Mather <i>et al.</i> , 2007
Rice	Genetic diversity and population structure using 169 nuclear loci & 2 chloroplast loci in 236 <i>O. sativa</i> and 93 SSR loci in 198 <i>O. glaberrima</i> accessions	Accessions were partitioned into five groups each overlapping the partitions based on phenotype; the study suggested that a higher degree of resolution of population structure is needed to effectively utilize LD for association mapping	McCouch <i>et al.</i> 2004

Source: Adopted from Gupta P K *et al.*, 2005

2.11 Population structure and spurious allelic association

It is hoped that all alleles observed to be associated with a phenotype have either direct or indirect associations. However, in an instance of the general problem of statistical confounding the existence of an unmeasured variable associated with both genotype and phenotype can be an additional cause of genotype–phenotype associations (Rosenberg and Nordborg, 2006).

The status of an individual for such a variable affects both the genotype and the phenotype of the individual, inducing a genotype–phenotype relationship. The allele of an individual provides information about the individual's ancestry, which in turn provides information about the phenotype of the individual. This gain in knowledge about phenotype from knowledge of genotype is a statistical association. However, because alleles across the genome have the potential to have this type of association with the phenotype, identifying these associations does not assist in localizing phenotype influencing alleles. Consequently, an important problem in association mapping is the separation of the spurious associations due to population structure from the indirect and direct associations (Lander and Schork 1994; Ewens and Spielman 1995; Risch 2000; Pritchard and Donnelly 2001; Thomas and Witte 2002; Ziv and Burchard 2003).

2.11.1 Population Structure

The primary variable that produces genotype-phenotype misleading relationship can be due to population structure or genetic background. Depending upon the crop's genetic origin, mutation rate, or admixtures can affect its probability of having any allele that varies in frequency across groups, as well as the probability of having any varying phenotype.

Undetected population stratification can cause spurious genotype-phenotype associations in candidate-gene approaches and indirect association via linkage disequilibrium mapping as well (Jorde LB, 1995).

Further spurious associations between genotype and phenotype caused by population stratification must be detected among unrelated individuals in association

studies to reduce Type I errors. Clustering techniques are one approach to identify stratified populations. For example, the model-based clustering “Structure” software program identifies putative population structure and assigns individuals to subgroups based on genotype frequencies (Pritchard *et al.*, 2000).

Population structure is an important component of Association analysis, since it can reduce both type I and type II errors between DNA markers and traits in autogamous species e.g. Rice and Barley (Agrama *et al.* 2007; Agrama and Eizenga 2008; Breseghello and Sorrells 2006; Yu and Buckler, 2006, Yan *et al.*, 2009)

Breseghello and Sorrells (2005) inferred four subpopulations based on 36 unlinked markers. These four sub populations captured relevant subdivisions in populations.

Population structure was investigated among 234 rice accessions genotypes SSRs. The data revealed five distinct groups corresponding to *indica*, aus, aromatic, temperate *japonica* and tropical *japonica* types. Close evolutionary relationship between the *indica* and the aus and among the tropical *japonica*, temperate *japonica* and aromatic groups (Amanda *et al.*, 2005). Analysis of population structure among a mini core collection of 90 accession using 109 SSR markers revealed four populations. This agreement validated the results from the PCA (Yan *et al.*, 2009).

2.11.2 Kinship Co-efficient (K Matrix)

Kinship describes the probability that two homologous genes are identical by decent in a given sample. However, kinship relationships have not been considered in most plant mapping or marker-assisted selection strategies. Mixed models using variance component approaches that account for kinship estimates have been exploited in animal research for over two decades (Henderson, 1984; George *et al.*, 2000). Arbelbide *et al.*, (2006) developed a mixed model for self pollinating plants that accounted for multiple location effects and kinship based on pedigree records. Arbelbide and Bernardo (2006) applied single and multiple marker analyses in the mixed model format for candidate loci and genes associated with bread quality traits in wheat (*Triticum aestivum* L.).

Mapping Software

a) Structure:

Structure is a model-based clustering method for using multilocus genotype data to infer population structure and assign individuals to populations. We assume a model in which there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned (probabilistically) to populations, or jointly to two or more populations if their genotypes indicate that they are admixed (Jonathan Pritchard *et al* 2000). The software allows for admixed individuals by introducing a vector Q to denote the admixed proportions for each individual. The software used for this article is available from <http://www.stats.ox.ac.uk/zpritch/home.html>.

b) SPAGeDi (Spatial Pattern Analysis of Genetic Diversity):

SPAGeDi software which estimates genetic distances between populations or relatedness coefficients between individuals using data from co-dominant genetic markers (Hardy and Vekemans, 2002). SPAGeDi was used to estimate relative kinship coefficient (Ritland., 1996) using our SSR data set.

c) TAssEL (Trait analysis by association, evolution and linkage)

TASSEL is a software package to evaluate marker traits associations, and linkage disequilibrium. The strength of this software is that it provides a number of new and powerful statistical approaches to association mapping such as General Linear Model (GLM) and Mixed Linear Model (MLM). The TASSEL software program (<http://www.maizegenetics.net>) incorporates population structure and kinship estimates into a mixed model for association genetics of unrelated individuals (Yu *et al.*, 2006). The TASSEL mixed model was used recently in association studies of a complex agronomic traits (Rostoks *et al.*, 2006., Agrama *et al* 2007., Yan *et al* 2009.,).

2.11.3 Power, Precision and Accuracy of LD based Association Mapping compared to Conventional QTL mapping

Both QTL and association mapping identify QTLs by detecting co-segregating polymorphic markers with genes underlying trait variation. However association genetics has much more advantages than QTL mapping in detecting closely associated QTL. Table 10 summarises some of the important key properties of conventional QTL and Association mapping.

Table 10. Comparison of QTL and Association mapping

Characteristic	QTL mapping	Association Mapping
Detection Goals	Quantative trait locus, Wide region around the gene location ~ 10 to 30 cM	Quantative trait nucleotide. Physically as close as possible to the gene ~1 to 10 cM.
Resolution	Low to moderate	Moderate to high
Experimental Populations	Defined pedigrees, e.g., backcross, F2, RI, three and two generation pedigrees/families, half-sib families,	Natural populations, unrelated individuals/ unstructured populations.
Marker discovery rates	Moderate	High

2.12 QTLs associated with several Quantative traits

2.12.1 QTL studies for and $\Delta^{13}\text{C}$

Mian *et al.*, (1996) reported that a restriction fragment length polymorphism (RFLP) map was constructed from a soybean [*Glycine max* (L) Merr.] population of 120 F₄ derived lines from cross of ‘Young’ x PI 416937. A total of four and six independent RFLP markers were associated with WUE and Leaf Ash (LASH) and if combined each group of marker would explain 38 and 53 % of the variability in the respective traits, One marker locus (cr 497 –1), on USDA linkage Group J, explained 13.2% of the variation in WUE indicating the presence of major QTL, The LASH was negatively correlated with WUE and QTL were associated with both WUE and LASH, For each of these QTL, the allele for increased WUE was associated with reduced LASH.

In another study by Mian *et al.*, (1998) reported that a restriction fragment length polymorphism (RFLP) linkage map was created from 142 marker and 116 F₂ derived lines of the population. The map covered about 1100 cM of the soybean genome with

120 markers linked on 25 linkage groups. Two independent markers were associated with WUE in this soybean population. There was no interaction between the two markers. One (A063E) of the two independent markers was also associated with WUE in the Young x PI 41697 population. Marker A489N, on Linkage Group (LG) L, was unique to the S 100-x Tokyo population and explained 14% of the variation in WUE in this soybean population.

Carbon isotope discrimination ($\Delta^{13}\text{C}$) is currently the subject of intense interest in relation to crop drought resistance, as it is an effective integrated measure of long-term plant water use efficiency (Farquhar *et al.*, 1989b). Although Δ is a complex trait, Handley *et al.*, (1994) identified a major effect of barley chromosome 4 for $\Delta^{13}\text{C}$ in wheat-barley addition lines.

The composition of stable isotopes of plant carbon ($\delta^{13}\text{C}$) was earlier shown to be an excellent indicator of WUE in Tomato and other species. Martin *et al.*, (1989) reported that $\delta^{13}\text{C}$ could be satisfactorily predicted from three-restriction fragment length polymorphism (RFLP) that is genetic markers for discrete DNA sequences within the genome. An additive type of gene action was observed for all three RFLP markers, and for one of them there also was a significant non-additive component. Combining the $\delta^{13}\text{C}$ and RFLP technologies may aid in identifying genes associated with WUE and in mechanisms contributing to WUE. These technologies will also be useful for crop improvement.

Zhang and Xu (2002) and Zhang (2003), reported that in Wheat 2 QTLs controlling WUE, 10 QTLs significantly affecting per plant WUE (Total dry matter/amount of water used per plant); 6 QTLs significantly controlling leaves and stem WUE (dry weight of roots /amount of water used per plant), 3 pairs of interaction QTL influencing root WUE were associated. There were gene clusters constituted by 2 or 4 QTLs linking closely together found on 1A, 3B, 4A and 6D chromosomes.

Nadaradjan *et al.*, (2005), identified markers overlapping for WUE at whole plant level and $\Delta^{13}\text{C}$ in a DH population of rice. Five markers were found to be overlapping

for both WUE and $\Delta^{13}\text{C}$ in experiment 1 and four in experiment 2, out of which marker OPZ1_1 was found to be consistent across the experiments.

Juenger *et al.*, (2005), identified five QTL for flowering time and five QTL affecting $\delta^{13}\text{C}$ in RIL population of the cross Ler X Cvi in *Arabidopsis thaliana*. Two QTL for flowering time overlapped in genomic position with two $\delta^{13}\text{C}$ QTL.

ERECTA a putative leucine rich receptor like kinase was identified as a major contributor to a locus for $\Delta^{13}\text{C}$ Arabidopsis chromosome 2 (Masle *et al.*, 2005). They also showed that this gene can modify transpiration efficiency by acting on epidermal and mesophyll development, stomatal density and the porosity of the leaves.

2.12.2 QTLs associated with root traits

Identifying molecular markers associated with roots would strongly augment the breeding efforts to both improve this trait and also to introgress the root traits with other agronomically desirable characteristics. To this end, several investigators have attempted to identify markers to the QTL regions conditioning root traits. Table 11 summarizes the literature in this area of research.

Table 11. QTLs associated with root traits in different crop species.

Trait	Species	Type	QTL#	Reference
Root morphology	Rice	DHL	29	Li <i>et al.</i> , 2005
Root growth and Architecture	Arabidopsis	IL	13	Olivier <i>et al.</i> , 2005
Root penetration ability & root thickness	Rice	RIL	20	Zheng <i>et al.</i> , 2000
Root length	Rice	DHL	2	Toorchi <i>et al.</i> , 2002
Root traits	Rice	DHL	8	Kamoshita <i>et al.</i> , 2002
Root number, thickness & Dry weight	Rice	RIL	18	Champoux <i>et al.</i> , 1995
Root length, No., thickness, penetration	Rice	RIL	28	Ali <i>et al.</i> , 2000
Root traits and yield	Maize	F ₃	11	Tuberosa <i>et al.</i> , 2002
Total and penetrated root number, ratio	Rice	RIL	18	Price <i>et al.</i> , 2000
Root growth traits	Rice	RIL	24	Price <i>et al.</i> , 2002
Root traits	Rice	DHL	29	Zichao <i>et al.</i> , 2005
Root morphological traits	Rice	DHL		Shashidhar <i>et al.</i> , 1999
Root morphology and root distribution	Rice	DHL	39	Yadav <i>et al.</i> , 1997
Root morphology, root cell length	Rice	F ₂	24	Price and Tomos 1997
Root thickness, root penetration index	Rice	DHL	5	Zhang <i>et al.</i> , 2001
Tiller and root number, penetration ability	Rice	RIL	39	Ray <i>et al.</i> , 1996
Root thickness, root penetration index	Rice	DHL	12	Zheng <i>et al.</i> , 2000
Yield and root traits under limited water	Rice	DHL		Venuprasad <i>et al.</i> , 2002

Much research on drought resistance in rice has focused on the root systems since it has been shown that drought avoidance is associated with the possession of a thick, deep root system (Yoshida and Hasegawa, 1982; Ahmadi, 1983; Loresto *et al.*, 1983; Ekanayake *et al.*, 1985 a & 1985 b).

2.12.3 Markers associated to some of the physiological traits in different crops.

Research experience in understanding the drought stress responses of plants, several plant physiological processes have been enumerated as important traits through which plants acquire tolerance or resistance to water stress situations. Most of these

traits have also been shown to be quantitative in inheritance and hence efforts have been made to identify DNA markers linked with the QTLs influencing these traits.

Mian *et al.*, (1998) identified six putative independent RFLP markers associated with specific leaf weight in soybean. Out of these six markers four of the marker loci were consistent across the environments. They also identified three putative independent RFLP markers associated leaf size out of which was consistent across the environment.

Misawa *et al.*, (2000) conducted QTL analysis for cold tolerance at the seedling stage by subjecting the rice plants to a temperature of 4 °c for 3 days. They identified 5 QTLs that were associated with low temperature response, one each on chromosome 1,9, and 11, two on chromosome 3.

Andaya and Mackill, (2003) identified a major QTL designated as qCTSI2a, that was closely associated with cold induced necrosis and wilting tolerance in RILs of rice derived from the cross of M-202 X IR50. This QTL accounted for 41% of phenotypic variation. The recent and relevant literature regarding the QTLs associated with different physiological traits is summarized in the Table 12.

The use of LD for mapping of QTLs for a quantitative trait is more problematic, but is also more rewarding, because it allows more precise location of the position of a QTL that controls the trait of interest. When comparing linkage analysis and LD mapping or QTL detection, it has been shown that LD mapping gives more precise location of an individual QTL (Gupta *et al.*, 2005). Ultimately the effectiveness of any MAS procedure will depend on the accuracy of the phenotypic classification of trait expression and the degree of linkage between the markers and the traits of interest. Although MAS may increase gain from selection when compared to phenotypic selection, marker utility in plant improvement programs will ultimately be determined by cost/unit information (Edwards and Page, 1994). Clearly, laboratory costs associated with MAS applications are decreasing and more effective and efficient molecular markers are being developed (Gu *et al.*, 1995). This progress will make MAS more attractive and will foster its prudent implementation as a tool for plant breeding.

Table 12. : QTLs associated with various physiological traits

Trait	Species	Population	QTL	Reference
Osmotic adjustment and dehydration tolerance	Rice	RIL	7	Lilley <i>et al.</i> , 1996
Osmoregulation under drought	Wheat	RIL	1	Morgan and Tam 1996
Osmotic adjustment under drought	Barley	RIL	12	Teulat <i>et al.</i> , 1998
Osmotic adjustment under drought	Rice	DHL	5	Zhang <i>et al.</i> , 2001
Flowering parameters under drought, ASI	Maize	F ₂	7	Ribaut <i>et al.</i> , 1996
Plant height, ear number, days to silking, yield	Maize	F ₃	5	Agrama and Moussa, 1996
Morphological and physiological traits	Rice	DHL	15	Hemamalini <i>et al.</i> , 2000
Dehydration avoidance traits	Rice	RIL	17	Price <i>et al.</i> , 2002
Grain yield and components under stress	Pearl millet	various		Yadav <i>et al.</i> , 2002
Yield, biomass, Osmotic adjustment, roots	Rice	DHL	47	Chandra Babu <i>et al.</i> , 2003
Absciscic acid concentration	Wheat	DHL F ₂	1	Quarrie <i>et al.</i> , 1994
Leaf ABA concentration	Maize	F ₃	16	Tuberosa <i>et al.</i> , 1998
Leaf rolling and stomatal conductance	Rice	F ₂	8	Price <i>et al.</i> , 1997
WUE, RWC, Dry matter, carbon discrimination	<i>S. scabra</i>	F ₂	36	Thumma <i>et al.</i> , 2001
Photosynthesis and water status traits	Sunflower	RIL	19	Herve <i>et al.</i> , 2001
Dry mass, Na ⁺ , K ⁺ uptake, concentration and ratio	Rice	RIL	25	Koyama <i>et al.</i> , 2001
Na ⁺ K ⁺ absorption	Rice	RIL	2	Bonilla <i>et al.</i> , 2002
Growth and germination	Arabidopsis	RIL	11	Quesada <i>et al.</i> , 2002
Cold tolerance at the booting stage	Rice	DHL	3	Takeuchi <i>et al.</i> , 2001
Chilling tolerance for spikelet sterility	Rice	RIL	2+	Andaya & Mackill, 2003
Root trait	Rice	DHL	18	Kanbar <i>et al.</i> , 2002
Heat tolerance in grain filling	wheat	F ₁ .F ₂ .F ₃	2	Yang <i>et al.</i> , 2002
Stay green, chlorophyll content	Sorghum	RIL	13	Xu <i>et al.</i> , 2000
Stay green under drought	Sorghum	RIL	5	Tao <i>et al.</i> , 2000
Stay green, chlorophyll content	Sorghum	RIL	7	Crasta <i>et al.</i> , 1999
Stay green, pre-flowering drought stress	Sorghum	RIL	9	Kebede <i>et al.</i> , 2001
Stay green	Sorghum	RIL	4	Sanchez <i>et al.</i> , 2002

Material and Methods

III. MATERIAL AND METHODS

Drought is one of the main constraints for high yield in rainfed rice. Exploring ways to produce more rice with less water is essential for food security and sustaining environmental health in Asia. The present research exploits integrated approach using physiology, genetics and integrated resource management to increase rice yield and reduce water demand and water input for rice production

WUE is the biomass produced per unit amount of water transpired (Passioura's 1996). WUE is a yield determining factor, thereby Improving WUE would reduce the water requirement for a specific yield potential and thus can save considerable amount of irrigation water. Root traits are key components of plant adaptation to drought environments. Root depth and extension into deep soil is crucial for crop performance under limited water supply if there is moisture available at deep soil level. The expression of deep rooting capacity does not require stress conditions. Roots do not grow into deeper soil in pursuit of the receding wetting front. The potential capacity for deep roots is expressed constitutively and can be phenotyped under non-stress conditions.

Root development can be modified by soil conditions such as soil moisture status and soil strength. Topsoil moisture conditions and strength can modify root distribution in the soil. However, the basic difference between shallow and deep-rooted genotypes will be expressed irrespective of stress conditions. The capacity of roots to penetrate hard soil is not necessarily linked to deep rooting capacity. Thus, WUE and deep root systems have significant implications for enhanced biomass and yield. All, these traits are complex in nature and show a quantitative inheritance. Locating the segments of the genome which contributes to these drought adaptive traits by the use of molecular mapping promises to increase our understanding of drought resistance and to produce markers for the strategic crop improvement by marker assisted breeding.

In this circumstance an investigation was carried out to test the feasibility of genome wide association mapping in rice germplasm accessions. Phenotyping was carried out using 204 rice germplasm accessions with an objective of associating markers for root traits and WUE.

3.1 Phenotyping for WUE and root traits

Diverse rice germplasm accessions were obtained from Central Rice Research Institute, Cuttack. Geographical distribution information of these germplasm accessions was unavailable. All the accessions were phenotyped for WUE, Root traits and other associated physiological traits in two years (2007 and 2008) in kharif season (Appendix I and II). Selected contrasts from the above experiments were again phenotyped in 2009 kharif (Appendix III, Raju, 2009). Phenotyping for roots were carried out in the field facility, Department of Crop physiology, GKVK, UAS, Bengaluru. Plate 1 depicts the extent of genetic diversity for grain size. Grain size among germplasm lines was considered as a passport data for assessing the genetic diversity.

3.1.1 Phenotypic Evaluation of the germplasm

3.1.1.1 Measurement of Roots in Root structures

Root structures of height 2.0m, length 19m and width of 3.0m were constructed above ground (Plate. 2). The wall of root structure was built with cement blocks. Fresh soil was collected and filled to the root structures. Soil was mixed with farm yard manure at the rate of 15 t ha⁻¹. Three root structures were constructed to replicate 192 genotypes twice in Randomised Complete Block Design (RCBD) design.

Rice seeds were soaked in water and treated with bavistin overnight. Seeds were directly sown on the root structure with two seeds per hill with the spacing of 25cm×25cm. Thinning was subsequently done to maintain one healthy plant per hill. The plants were provided with adequate nutrients through fertilizers at the rate of 100:50:50 kg NPK per hectare. The amount of fertilizer was calculated on weight basis and accurate amount of Urea, Single super phosphate (SSP) and Murate of Potash (MOP) were added to the soil of each root structures. Periodic plant protection measures were taken to develop healthy seedlings devoid of any pests and diseases. Root structures were irrigated once a day so as to maintain field capacity as per the definition of aerobic rice. Iron deficiency poses a serious threat to aerobic rice cultivation. In order to avoid Iron deficiency an equimolar concentration of Fe-EDTA solution was prepared at the concentration of 2gm/lt and 10 liters for each structure was added at an interval of every 10 days.

3.1.1.2 Root structure dismantling

Root structure was dismantled by removing each brick units at 90 days after sowing (50% flowering). High pressure of water was applied to the soil in the structure and roots were taken without damaging along with the whole plant (Plate. 3). Roots were separated from the shoot at the node between the shoot and the root.

3.1.1.3 Observation recorded

Total dry biomass

Plants were separated for leaf, stem and roots. The plant parts were dried at 80⁰C for five days in a hot air oven and weight was recorded.

Root length: Root was separated from each plant at the internodal position. Length of the root was recorded in centimeters in a graduated scale.

Root volume: A known volume of water was taken in a graduated beaker, and the separated roots were immersed and then the increase in the volume of water was recorded which actually represents the actual root volume (ml)

Root weight: Roots collected from each plant were oven dried at 80⁰C for four to five days in a hot air oven and the weight was recorded in grams.

Specific leaf area (SLA): Specific leaf area is the ratio of leaf area to leaf dry weight and was computed as follows.

$$SLA = \text{leaf area in cm}^2 / \text{leaf dry weight in g}$$

Total Leaf Area: Three leaves from three plants were sampled from each canopy positions and this leaf area was determined by measuring the maximum length and mean breadth. The same leaves were oven dried and their dry weight was determined. The



Plate 1. Genetic variability in grain size among rice germplasm accessions



Plate 2. Layout of root structures



Plate 3. Root washing in root structure

High root types



Low root types



Plate 4. Variability in root morphology among rice germplasm accessions

remaining leaves of the plant were harvested separately and their oven dry weight was recorded.

Area of 9 leaves

————— X Total leaf dry weight

Weight of 9 leaves

Days to 50 % flowering: Number of days taken from date of sowing to flowering of 50 % of the plants was recorded. This experiment was conducted in a different location (VC Farm, Mandya).

Yield per plant: Panicles were harvested after attaining maturity and sterile seeds were separated and the weight of total filled seeds was recorded in grams. This experiment was conducted in a different location (VC Farm, Mandya).

3.1.2 Water use efficiency

Genetic variability in WUE was determined both at single leaf level by gas exchange and a time integrated whole plant measurement based on $\Delta^{13}\text{C}$.

3.1.2.1 Determination of WUE at single leaf level by Gas exchange

Gas exchange traits such as net CO_2 assimilation rate (A), stomatal conductance (g_s) intercellular CO_2 concentration (C_i) etc., were measured using portable photosynthetic system Li- 6400 (LiCor 6400, Lincoln, Nebraska, USA).

It consists of a main console that harbors separate CO_2 and H_2O IRGAs, an internal air supply unit and the necessary software for the computation of the gas exchange parameters and a leaf chamber to clamp the leaves. LiCor uses four independent infrared gas analyzers, 2 each for CO_2 and H_2O . One pair of CO_2 and H_2O analyzers has a common inlet/outlet and is defined as the reference and similarly second pair as analysis. Measurements are expressed as absolute concentrations of the reference, and as the difference between the reference and the analysis concentrations.

3.1.2.2 The Leaf Chamber (PLC)

It accommodates a leaf area of 6.25 cm^2 and is equipped to measure the light intensity in the PAR range by a quantum sensor, relative humidity by a thermocouple and temperature of the air by a thermostat. Butyl rubber tubing is used to carry air from the leaf chamber into the IRGA.

The leaf chamber air to leaf VPD can be regulated by altering the chamber Relative Humidity (RH) and temperature. The RH is normally regulated by altering the flow rates of dry air into the cuvette or by regulating the RH of the input air. The system used this investigation has specially designed software that automatically regulates the input RH. At a fixed chamber temp, a specific leaf to air VPD can thus be maintained. This option is used in all the experiments where gas exchange parameters are recorded.

The instrument measures the following primary parameters:

1. Volume flow rate of dry air into the cuvette ($\text{cm}^3 \text{ s}^{-1}$)
2. Photon flux density incident on cuvette ($\mu \text{ moles m}^{-2} \text{ s}^{-1}$)
3. CO_2 concentration and water vapour pressure of air entering and leaving the leaf cuvette.
4. Leaf area (cm^2)
5. Boundary layer resistance to water vapour ($\text{m}^2 \text{ s}^{-1} \text{ mol}^{-1}$)

Using several physical constants and measured parameters, the equipment computes the following gas exchange parameters.

1. Stomatal conductance to water vapour ($\text{m mol m}^{-2} \text{ s}^{-1}$)
2. Photosynthetic rate ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)
3. Transpiration Rate ($\text{m mol m}^{-2} \text{ s}^{-1}$)
4. Sub-stomatal cavity CO_2 concentration (ppm)
5. Leaf temperature ($^{\circ}\text{C}$)
6. Saturated vapour pressure at leaf temperature (bars)
7. Vapour pressure of water entering the cuvette (bars)
8. Vapour pressure of water in the cuvette

3.1.2.3 Recording gas exchange parameters

Second fully expanded leaf was clamped to the leaf chamber and observations were recorded after A , g_s , and C_i values reached a steady state level. All gas exchange

measurements were recorded between 9 AM and 12 noon on bright sunny days. The temperature of the leaf chamber and the air inside were maintained near to that of the ambient levels. The leaf chamber of LiCor is equipped with a peltier cooling system that can maintain the chamber temperature.

Intrinsic WUE: At a single leaf level, WUE is the ratio of the mmoles of carbon fixed per mole of water transpired. The intrinsic WUE was computed by determining the ratio of assimilation rate to stomatal conductance (A/g_s) or Assimilation to transpiration rate per unit leaf area (A/T).

Mesophyll efficiency: Photosynthesis is a function of CO_2 supply controlled by stomatal factors and the efficiency of Carbon fixation in the chloroplast. Though A and g_s can be measured using Infra Red Gas Analyzers, there are no direct measures of mesophyll efficiency. A number of indirect estimates of mesophyll efficiency have been developed that demand the generation of the assimilation response to CO_2 concentrations. A simple method to determine the in-vivo mesophyll efficiency was developed by Sheshshayee *et al.*, (1996). The intercellular CO_2 concentration decreases if mesophyll consumption of the substrate is higher at a given supply function. Accordingly, the ratio of C_i to stomatal conductance (C_i/g_s) has been shown to be a fairly accurate estimate of mesophyll efficiency (Krishnaprasad *et al.*, 1996). Mesophyll efficiency was arrived at by computing the ratio of C_i to g_s for all the rice accessions in this investigation.

3.1.3 Stable isotope studies

3.1.3.1 Determination of genetic variability in WUE based on Carbon Isotope Discrimination ($\Delta^{13}C$)

During the fixation of carbon by photosynthesis, the naturally occurring heavy stable isotope, ^{13}C , is discriminated against ^{12}C . Therefore, plants have a smaller ratio of ^{13}C to ^{12}C than does the CO_2 of air that feeds them. Farquhar *et al.*, (1982) developed the theory, which predicted that this discrimination would be least in those C_3 plants that fix the most carbon per unit amount of water transpired i.e. in those that have the greatest water use efficiency. Ever since then, the $\Delta^{13}C$ technique has been widely adopted as a powerful surrogate estimate of WUE.

3.1.3.2 Determination of $\Delta^{13}\text{C}$ in leaf samples

The Carbon Isotope Discrimination ($\Delta^{13}\text{C}$) was determined with an objective to study how far $\Delta^{13}\text{C}$ is associated with the observed genetic variability in WUE in rice accessions.

1. Third fully expanded leaves that were developed during the experimental period were collected (5-6 for each genotype)
2. Kept at 80°C for 3 days to completely dry the samples.
3. Dried samples were finely powdered in a mortar and pestle. Care was taken to prevent any mixing of different samples by washing the pestle and mortar with alcohol after grinding each sample
4. One gram of powdered leaf sample was put in glass vial and properly labelled.

The $\Delta^{13}\text{C}$ of the leaf samples was determined using Continuous-Flow Isotope Ratio Mass Spectrometer (IRMS) at the National Facility for Stable Isotopes studies in Biological Sciences, Department of Crop Physiology, UAS, and Bengaluru (Plate 5). The details of the methodology are described below.

3.1.3.3 Mass spectrometric analysis

The IRMS installed at our center works on the continuous flow basis interfaced with an elemental analyzer (CN1112, CarloErba, Italy) for sequential combustion of biomass samples to generate CO_2 gas.

Finely powdered leaf samples were accurately weighed in the range 0.8 to 1.2 mg into silver capsules. The crimped capsules with the sample were placed sequentially in the carousel of the Autosampler. The samples were dropped at precise times along with an injection of pure O_2 into the oxidation reactor.

The Combustion (oxidation) reactor contains chromium oxide and silvered cobaltous-cobaltic-oxide in a quartz column heated to 1080°C . The biomass is completely



Plate 5. Isotope Ratio Mass Spectrometer (IRMS) facility for measuring stable isotope discrimination in plants

oxidized to produce CO₂, N₂O and H₂O. These gases were swept into the reduction furnace using helium carrier gas (purity 99.995%). The reduction furnace contains reduced copper in quartz tubes heated to 680°C. In this reaction, the N₂O is reduced to N₂ and the excess O₂ is absorbed. The resultant gases are then flushed through scrubbers to trap water. The pure CO₂ and N₂ gases are then passed through a GC column (5°A molecular sieve). The N₂ gas elutes faster through the GC and hence gases can be effectively separated before introduction into the ion source of the IRMS. At the ion source, CO₂ is ionized by electron impacts ionization to produce molecular radicals (CO₂[•]). These CO₂ radicals are accelerated under the influence of high voltage potential (3000 volts) through a strong magnetic field. When accelerated radicals pass through a strong magnetic field, the radicals are deflected with the radius of deflection being proportional to the molecular mass of the radicals. Faraday cups collect these deflecting ¹²CO₂ and ¹³CO₂ and the signal is amplified and transmitted to the computer.

3.1.3.4 Determination of Oxygen isotopic enrichment ($\Delta^{18}\text{O}$)

Stable oxygen isotope has generated considerable interest in plant carbon and water relations in recent years. Water vapour molecules containing the lighter isotope of oxygen (¹⁶O) diffuse relatively faster than do molecules with the heavy isotope of oxygen. Further the isotopic enrichment occurs during evaporation also, because the vapour pressures of ¹⁸O water is lower compared to that of ¹⁶O water, which favors faster evaporation of H₂¹⁶O(water) leading to ¹⁸O enrichment. Transpiration being an evaporative process would result in the enrichment of H₂¹⁸O in leaf water. This oxygen isotopic signature subsequently finds its path into the leaf biomass. Thus, $\delta^{18}\text{O}$ in leaf biomass has emerged as a surrogate for transpiration rate (Bindumadhava *et al.*, 1999, Bindumadhava, 2000).

3.1.3.5 Protocol for quantification of ¹⁸O composition in leaf biomass

The oxygen isotopic composition of biomass is generally determined by quantitative pyrolysis of the sample at high temperature in the complete absence of oxygen. The dried leaf powder (0.8- 1.2mg) was taken in silver capsules and placed in sample carousel of the Autosampler. The Autosampler drops the sample at precisely designated times sequentially into the pyrolysis column. The pyrolysis column contains a

graphite tube filled with glassy carbon catalyst. The graphite tube is placed in a ceramic column heated to 1400 °C. The pyrolysis of organic matter resulted in the production of carbon monoxide (CO) and nitrogen (N₂) gas. CO₂ is generally <5 per cent. These gases were swept by a Helium (He) carrier gas (purity >99.996%), into a GC column and then into the ion source of IRMS.

Since the mass to charge ratio (m/z) of CO and N₂ is same, it is essential to separate these two gases before introducing into the ion source for isotopic ratio determination. These gases were passed through a GC- column containing 5⁰A molecular sieve heated to 90°C. The CO travels slower than N₂ gas through the molecular sieve hence the two gas species can be quantitatively separated. The m/z ratio for 28 and 30 masses corresponding to C¹⁶O and C¹⁸O, respectively was determined by the IRMS. An appropriate standard (Craig-corrected against v SMOW) was also introduced in the run to determine the accuracy of mass detection and standard deviation of the run. The ¹⁸O enrichment over and above the source water was computed as follows:

$$\Delta^{18}\text{O} (\text{‰}) = \delta^{18}\text{O}_{\text{sample}} - \delta^{18}\text{O}_{\text{water}}$$

The $\delta^{18}\text{O}$ was determined by CO₂ equilibration technique using the gas bench coupled to the IRMS. The $\delta^{18}\text{O}$ of irrigation water was found to be -3.73‰.

3.2 Statistical analysis

The data obtained from experiments were analyzed using statistical software packages like MSTATC and MS EXCEL etc.

3.2.1 Analysis of Variance

The genotypic variability for Root traits, WUE and associated physiological traits were assessed using Analysis of Variance as per Fisher's method. The level of significance was tested at 0.05 and 0.01 probability level in 'F' test. The genotypic means were compared with the critical difference values. This analysis was performed using MSTATC.

3.3 Genetic studies

3.3.1 Phenotypic and genotypic coefficient of variations

The phenotypic and genotypic coefficients of variations were computed as per Burton and Dewane (1953).

$$PCV \% = (P/\bar{X}) \times 100$$

$$GCV \% = (G/\bar{X}) \times 100$$

Where,

P = Phenotypic standard deviation

G = Genotypic standard deviation

X = Grand mean of the character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

GCV and PCV were classified as given below.

0 –10 % - low

10- 20% - moderate

20% and above – high

3.3.2 Heritability

Broad sense heritability was estimated using the following formula (Hanson *et al.*, 1956).

$$H \% = (V (g) / V (p)) \times 100$$

Where,

H = broad sense heritability

V (g) = Genotypic variance

V (p) = Phenotypic variance

Heritability was categorized into following three classes.

0-30 % - low heritability

30-60% - medium heritability

60% and above- high heritability

Genetic advance

Genetic advance was calculated by the formula given by Johnson *et al*, 1955

$$GA = H \times \sigma_p \times k$$

Where,

H is the broad sense heritability

σ_p = phenotypic standard deviation

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

Genetic advance as per cent mean

$$GA \text{ as per cent mean} = (GA / \bar{X}) \times 100$$

Where GA = Genetic advance

Genetic advance as per cent means (Johnson *et al.*, 1955) is as given below:

0-10% - low

10-30% - moderate

20% and above – high

All these analysis were done using the software GENERES

Correlation analysis

Correlation coefficients were computed to find the association among the traits using the formula given by Sunderraj *et al.*, 1972.

$$r_{(x, y)} = \frac{\text{Cov (x/y)}}{\sqrt{V (x) * V (y)}}$$

Where,

$r_{(x, y)}$ = correlation coefficient between x and y

V (x) = variance of x

V (y) = variance of y

Significance of coefficient of correlation was tested at (n-2) degree of freedom “t-table from Fisher’s table at 5% and 1% significance. All these analysis were done using the software Statistica

3.4 Molecular Characterization

The young leaves of the rice germplasm were collected in liquid nitrogen for DNA extraction. DNA was extracted from all the 192 germplasm lines using CTAB method explained by Saghai - Maroof *et al.*, (1984).

3.4.1 Protocol for extraction of genomic DNA

One gram of fresh young leaf sample was taken in a mortar and powdered well using liquid nitrogen.



The ground tissue was transferred to a 50 mL sterile centrifuge tube by adding 15mL of CTAB buffer (Extraction buffer- 2%CTAB, 1.4 M NaCl, 20 mM EDTA (Disodium) & 10mMTrisbase (pH 8)). Then 50 μ L of β - mercaptaethanol was added to each tube and the extract was mixed well by inverting the tubes several times. The tubes were incubated in a water bath maintained at 65 $^{\circ}$ C for an hour with constant stirring at an interval of 15 minutes.



After an hour, 15 mL of chloroform: isoamyl alcohol (24:1) was added to the incubated sample and mixed well by inverting. The tubes were then centrifuged at 6000 rpm for 20 minutes.



The aqueous upper phase was carefully transferred using the 1mL cut tips into fresh sterile centrifuge tubes. To this supernatant, 0.7 volume (10.5mL) of cold isopropanol was added.



The tubes were carefully inverted and kept for 5 min on ice. DNA precipitation was seen in the form of strands. The tubes were then centrifuged at 6000 rpm for 20 minutes and sedimentation of DNA as a hard pellet was seen.



Further the supernatant was decanted gently and the tubes were inverted on a clean filter paper.



The pellet was washed twice by suspending in one mL of 70% ethanol for 5 to 10 minutes and the DNA was centrifuged at 6000 rpm for two minutes.



Ethanol was drained off slowly and the pellet was vacuum dried in a desiccator for 5 to 10 minutes. The pellet was then dissolved in 500 μ L of TE buffer by flicking the tubes. (TE buffer = 0.1mM Tris + 0.05mM EDTA)



To remove the RNA 5 μ L of RNase (10mg/mL) was added into the DNA solution and incubated at 37 $^{\circ}$ C in a water bath for 1 hour.



Again the DNA solution was further purified by washing with equal volume (500 µL) of phenol: chloroform:Isoamyl alcohol (25:24:1) by invert mixing several times and centrifuging at 6000 rpm for 15 minutes to separate the two phases.



The aqueous upper phase was transferred into a clean 1.5 mL eppendorf tube and twice the volume of 100% ethanol was added to precipitate the DNA.



The pellet was washed with 70% ethanol twice and dissolved in 500 µL TE.

The extracted DNA was quantified using both spectrophotometer and by 0.8% agarose gelelectrophoresis.

3.4.2 Checking DNA purity and quantification

Using spectrophotometer, the ratios between the reading at 260nm and 280nm provide an estimate of DNA purity. Pure DNA samples will have a ratio of 1.8. If the sample is contaminated by protein the ratio will be significantly less. Ratio of 2.0 or more indicates a high proportion of RNA in the sample. DNA has maximum absorbance at 260nm. An absorbance of 1.0 corresponds to 40µg /mL for double stranded DNA.

Procedure

Two µL of DNA isolated from rice leaves were diluted to one mL with TE buffer and the absorbance at 260 and 280nm were recorded against a buffer blank. A 260/280 nm ratio for all the samples was calculated to check the purity. DNA was quantified using the formula

$$\mu\text{g ds DNA}/\mu\text{L} = (A_{260} * 40)/2$$

Further all the samples were diluted to a final concentration of 10 ng/µL.

DNA quantification on agarose gel

Agarose gel submersible electrophoresis is used to separate the DNA based on size (0.1 to 2.5 kb). Agarose gel electrophoresis is usually conducted in a submerged horizontal tray. The tray is chosen in such way that its length should allow required band resolution.

Preparation of agarose gel

0.8g of agarose was weighed and taken in to a clean 100mL conical flask. To this 100 mL of 1x TBE buffer was added. (TBE buffer (0.89M Tris base, 0.02MEDTA, 0.89M Boric acid: pH = 8))



Agarose was dissolved by heating. After agarose completely melts, it was cooled to 60°C and 3.5 µL of ethidium bromide was added (10mg/mL) and mixed.



The ends of gel casting trays were sealed with tapes. Agarose was poured; comb was inserted and allowed to solidify.



After solidification the tape on either side was removed and the gel was immersed in electrophoresis tray having 1x TBE buffer. Then the comb was removed.



To 5 µL of DNA samples 3µL of 6x loading buffer was added and mixed well and loaded into the well. 3µL of standard uncut Lambda DNA was used as marker. (Loading buffer or tracking dye 6x – 40% sucrose, 0.025% bromophenol blue, 0.25% xylene cyanol)



Electrophoresis was carried out at 50V for 2 to 3 hours until the bromophenol blue dye migrated to two-third of the gel. The gel tray was removed and the gel was observed under UV transilluminator and documented using BioRAD Gel Doc system. The quantity of the DNA was determined based on the intensity of the band relative to lambda uncut band.

3.5 Molecular Marker Systems Used

3.5.1 Simple sequence repeats (SSRs)

SSR markers are short sequences of nucleotides (2-6 units in length) that are repeated in tandem:

Di-nucleotide repeat: CACACACA

Tri-nucleotide repeat: ATGATGATGATG

It is believed that when DNA is being replicated, errors occur in the process and extra sets of these repeated sequences are added to the strand. Over time, these repeated sequences vary in length between one cultivar and another. Example of allelic variation in SSRs:

Allele A: CACACACA (4 repeats of the CA sequence)

Allele B: CACACACACACA (6 repeats of the CA sequence)

These variations in length are easy to trace in the lab and allow us to track genotypic variation in breeding programs.

3.5.2 Marker selection from the database

All the markers used in the study were selected from the gramene database (www.gramene.com). Based on the gel profile presented in the data base for the respective markers, only polymorphic informative markers were selected for screening rice germplasm accessions. The genetic distance on the chromosome was also obtained from the same database using the Cornell University. List of SSR markers selected from the database is given in Appendix VI.

3.6 Standardization of annealing temperature for SSR primers

The annealing temperature was standardized using gradient PCR technique. In this method different annealing temperatures ($T_m \pm 5^{\circ}\text{C}$) were set to each block and amplification was carried as per below mentioned reaction conditions. After standardization of annealing temperature the germplasm lines were amplified using the same protocol.

3.6.1 SSR primer amplification condition

Initial denaturation 5 min at 94°C	
Denaturation 1 min at 94°C	
Annealing 1 min at $50^{\circ}\text{C} - 68^{\circ}\text{C}$ (primer specific)	} 30 cycles
Extension 2min at 72°C	
Final extension 8 min at 72°C	

This takes about 3hrs for PCR amplification.

3.7 Fluorescent dye labelled PCR

Genotyping was performed by polymerase chain reaction (PCR) with defined oligonucleotide primers. To analyze the length of the PCR products by electrophoresis with a laser detection system, one of these primers is fluorescent dye labelled i.e

6-carboxy-fluorescein (FAM), Hexachloro-6-carboxy-fluorescein (HEX), 6-carboxy-X-rhodamine (ROX). Direct primer labelling with these fluorescent dyes are more expensive. Therefore to reduce the cost of primer dye labelling, we followed fluorescent dye labelled M13 tailed PCR. This method is more economic method compared to direct primer dye labelling.

3.7.1 Steps in fluorescent dye labelled M13 tailed PCR

M13 tailed PCR consists of two steps of separate PCR. In the first step, the SSR regions are amplified using locus specific forward and reverse primer. In the second step the amplified products are re-amplified using M13 fluorescent dye labelled primer (Figure 7).

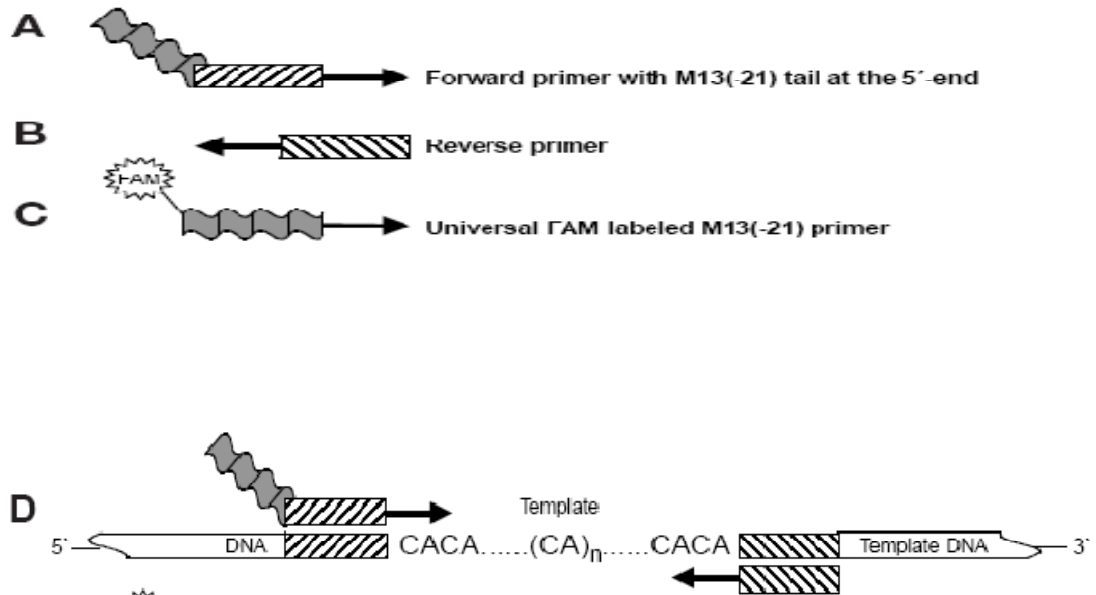
Primers Used

Three primers are used in M13 PCR,

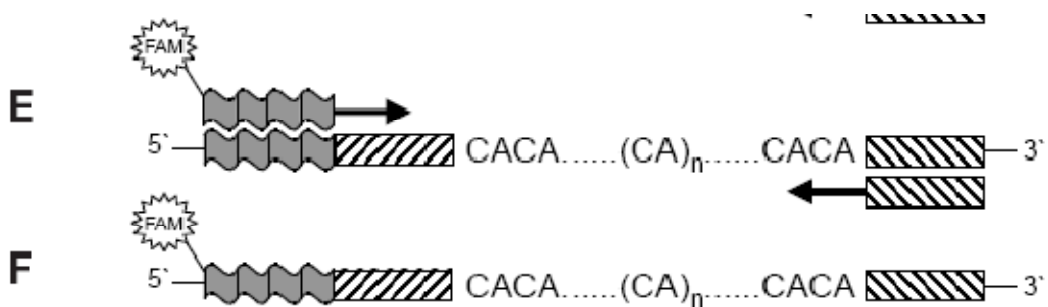
-  Forward primer attached with M13 (-21) tail at 5' end
-  Reverse primer
-  Universal M13 primer labelled with HEX or FAM at 5' end

Fig. 7: Schematic diagram of M13 labelled PCR

PCR Step 1:



PCR Step 1:



3.7.2 The PCR components for Fluorescent dye labelled M13 tailed PCR

PCR Step one:

The reaction mixture was set up in sterile PCR plates. The reaction mixture volume per reaction is as follows:

10x Taq Polymerase buffer	1.0 μ L
2 mM dNTPs	1.0 μ L
MgCl ₂	0.2 μ L(2mM)
Forward primer (1pMole/ μ L)	0.4 μ L
Reverse primer (1pMole/ μ L)	1.0 μ L
Taq Polymerase (1U)	0.2 μ L
Template DNA (25-50ng)	2 μ L(10ng/ μ L)
Sterile water	4.20 μ L
Total volume	10.0 μ L

SSR primer amplification condition

Initial denaturation for 5 min at 94 ⁰ C	
Denaturation 1 min at 94 ⁰ C Primer AT 50-70 ⁰ C Extension 2min at 72 ⁰ C	20 Cycles
Final extension 10 min at 72 ⁰ C	

PCR Step two: The following reaction volume is added to the amplified product. The volume of PCR components per reaction is as follows:

Fluorescent dye labelled M13 primer	0.2 μ L(3pm)
10x Taq Polymerase buffer	0.2 μ L
2 mM dNTPs	0.2 μ L
MgCl ₂	0.05 μ L(2mM)
Taq Polymerase (1U)	0.2 μ L

SSR primer amplification condition

Initial denaturation for 2 min at 94 ⁰ C	
Denaturation 45 sec at 94 ⁰ C Primer AT 53 ⁰ C Extension 2min at 72 ⁰ C	20 Cycles
Final extension 8 min at 72 ⁰ C	

3.8 Capillary based Electrophoresis

Amplified products were separated by capillary based electrophoreses using MegaBACE500 (Amersham Bioscience).

Materials required for SSR Genotyping using MegaBACE

- 1) De-ionized water
- 2) LPA Buffer
- 3) LPA Martix
- 4) Sample plate

LPA Buffer: Linear Poly-acrylamide Buffer 1X is prepared from 10X.

LPA matrix: Three Matrix tubes tubes, provided by GE health care is used directly to fill the capillaries.

Sample Plate: Each sample plate contains

Sample loading solution,

ET-ROX-400

PCR products

3.8.1 Multiplexing of Primers

Multiplexing of primers was done based on both dye and size. Dye multiplexing was done with FAM and HEX florescent dyes. Size multiplexing was done with primers differing by at least 50bp.

3.8.2 Preparation of sample plate

Each well contains Sample loading solution mixed with ET-ROX-400 and PCR products in the following proportion so that the total volume is 10 µl.

Sample Loading Solution: 5.75µl

ET-ROX: 0.25 µl

PCR products: 4 µl (1 µl from each four primers)

The samples were mixed thoroughly and spun down the sample plate at 1500 rpm. Further the samples were denatured at 95⁰C for one minute, immediately cooled on samples on ice for several minutes and stored at -20⁰C until they are loaded for capillary electrophoresis using the MegaBACE 500.

3.8.3 Protocol for performing runs with MegaBACE

Capillary electrophoresis was carried in MegaBACE following the protocol given in the instrument control manager using genotyping option. All the materials were prepared and kept ready before the start of the run.

1) Materials required for Rinse tips:

- A squirt filled with fresh deionised filtered water
- A clean water filled with fresh deionised filter water
- One 2 ml tube for each array installed in the instrument, each containing 1.8 ml fresh deionised filtered water

2) Materials required for the Matrix fill and Prerun protocol

- A plate containing 100 µl of LPA buffer (1x) per well
- One 2-ml tube for each array installed in the instrument, each containing 0.7ml LPA matrix. Matrix tube was spin down at 1000 rpm for one minute.

3) Materials required for Inject sample and run

- Sample plate containing 48 samples
- Before using Inject sample and run, plate set up window was used to create plate definition which includes plate ID, electrophoresis parameters, chemistry parameters and sample names.

3.9 Analysis of the sample and Product size scoring

Fragments were sized and binned into alleles using Genetic Profiler. The PCR product size was manually scored by selecting for the highest peak and the corresponding fragment size in base pairs was recorded. If two peaks of a locus had different intensities, the highest intensity was scored as required peak size. If the two

peaks of a locus had similar intensities, the peak was scored as heterozygous. If the peak was absent, the locus was considered missing. Scoring was carried out manually by recording the peak size.

3.10 Linkage disequilibrium (LD)

LD is defined as the non-random association of alleles or haplotypes within unrelated populations with a distinctly shared ancestry. The concept was initially described by Lewtonin (1964). Several quantities are used to measure LD which involves quantities like D' , r^2 , D^2 , D^* , F , $X(2)$, and δ . However D' and r^2 are most commonly used measures of LD. D' is the difference between the observed gametic frequencies of haplotypes and the expected gametic haplotype frequencies under linkage equilibrium. Squared correlation coefficient (r^2) is affected by both mutation and recombination while D' is affected by more mutational histories. Considering the objective, the most appropriate LD quantification measure needed for association mapping is r^2 , which is also an indicative of marker trait association. In the present study r^2 was used to estimate Linkage Disequilibrium (LD) between each pair of marker loci. Power marker v 3.25 was used to assess the extent of LD between intra-chromosomal marker loci. Location of the marker on the chromosome in terms of genetic distance was obtained from gramene database (www.gramene.com). The size of the LD block was estimated plotting r^2 values with the genetic distance. Genetic distance of the marker loci was obtained from the www.gramene.com database.

3.11 Determination of Population structure

3.11.1 Distance based neighbor joining method

Population structure was constructed based on neighbor joining (Saitou and Nei, 1987) method using DARWIN 5.0. Boots strap of 500 was set for constructing the cluster tree.

3.11.2 Model based Population structure using “STRUCTURE” (Q matrix)

Model-based Bayesian clustering programme “STRUCTURE” (Pritchard *et al* 2000) was used to infer population structure using multi-locus genotype data and assign individuals to populations. Model with admixture assumes that each individual is assumed to have inherited some proportion of its ancestry from each one of K population. The length of simulation (burn-in length) was set to 150,000. The software STRUCTURE provides an option to use both admixture model as well as no admixture model. Since the pedigree of the rice accessions was unknown we used admixture linkage model with correlated allele frequency for identification of population structure.

To identify the number of sub-groups (K) among the rice accessions is vital for effective association mapping. To do this K (sub-groups) was allowed to vary from 2 to 10. Exact K was determined based on Estimated log probability of data “ $LnP(D)$ ” and alpha value. Further it was confirmed with DeltaK (ΔK) as suggested by Evanno *et al*, 2005. After identifying the number of population “STRUCTURE” was again run with a run length of 1, 00,000. Output from STRUCTURE (Q matrix was used for TASSEL software).

3.11.3 Determination of Kinship Co-efficient (K-Matrix)

SPAGeDi (Spatial Pattern Analysis of Genetic Diversity): program was used to determine K-matrix. Allele frequencies were calculated by GenAlEx 6.0. Output was obtained as square matrix. Resultant K-matrix was used for TASSEL.

3.12 Discovering marker trait association using TASSEL (Trait analysis by association evolution and linkage)

Marker trait association was determined using TASSEL. Mixed linear Model was adopted to discover marker trait association which incorporated information from both population structure estimates (Q matrix) and family relatedness (K Matrix) with in population (Yu, *et al.*, 2006).

Results

VI. RESULTS

Developing drought tolerant rice varieties is the most efficient way to stabilize rice production and alleviate food insecurity and poverty in India and Asia. But past breeding efforts to combine high yield with drought tolerance have not been successful, largely because of our poor understanding of the physiological basis underlying both high yield potential and drought tolerance in rice. Considerable efforts have recently been taken to understand the Physiological and genetic basis of drought tolerance in rice by mapping quantitative trait loci (QTLs) associated with traits putatively related to drought tolerance (DT) in rice (Price *et al.* 2002; Zhang *et al.*, 2001). Unfortunately most of these QTLs have not been used in breeding program because of poor resolution. The advent of advanced genomic technologies combined with stringent statistical methods has made it possible to identify QTLs with more resolution and minimum research time.

One such approach is the adoption of linkage disequilibrium (LD) mapping or association mapping. This approach relies on the determination of allele frequency of marker loci among a panel of diverse germplasm accessions. Since the diverse germplasm accessions represents a wide range of phenotypic as well as molecular diversity, this approach has greater resolution than other mapping approaches. A diverse germplasm accessions comprising of germplasm lines and a few promising cultivars were extensively phenotyped for relevant drought tolerant traits. Molecular diversity of this set of germplasm was assessed using SSR markers.

Among several traits that have relevance in improving growth under water limited aerobic conditions, traits that associated with maintenance of tissue water relation and metabolism under lower water potential are the most important. From this context, root traits water use efficiency and water conservation traits deserve emphasis. Furthermore, for a comprehensive improvement of drought tolerance, it is also essential that genotypes possess a reasonably, high degree of cellular level tolerance. Thus assessing the variability in several of these parameters has paramount significance in breeding. Most of the traits that have relevance in breeding exhibit a complex Quantative inheritance besides a varied extent of genotype $\times$ environment interaction. To assess the $G \times E$ interaction or stability of the trait across season, two root structure experiments were carried out in the year 2007 and 2008. Genotypic co-efficient of variation (GCV)

and Phenotypic co-efficient of variation (PCV) represents the variability among the population and are fairly good estimates of stability or heritability in broad sense (H^2). In the present study GCV, PCV and h^2 were calculated for various parameters. Results of various experiments are described in this chapter.

4.1 Genetic variability in biometric traits among rice germplasm accessions

Canopy cover and biomass accumulation are the most prominent determinants of growth rates. Total biomass and leaf area were recorded on all the accessions in the two experiments. Results are presented in this chapter. Significant genetic variability was observed among all shoot traits. Plant height varied significantly ranging from 17.50 cm in PS-276 to as high as 112 cm in CTH-1 with a mean of 56.55 cm in 2007. In the 2008 experiment plant height ranged from 34 cm in JBT-37/4 to 108.25 cm in Dodabatta with a mean of 72.30 cm (Table 13). Plant height showed a high heritability of 0.75 with a GCV and PCV of 20.74 % and 23.9 % respectively in experiment 2007 (Table 17). These parameters were similar in the 2008 experiment with a high heritability of 0.77 and GCV and PCV values of 17.7 % and 20.08 % respectively was recorded in the experiment 2008 (Table 18). Plant height revealed moderate to high genetic advance of 37.07 % and 32.15 % was observed in experiment 2007 and experiment 2008 respectively (Table 17 & Table 18).

Shoot weight in experiment 2007 ranged from 6.98 g.plant⁻¹ (AC-35513) to 58.25 g.plant⁻¹ (AC-35612) with a mean of 24.18 g.plant⁻¹ whereas in 2008 a range of 3.13 g.plant⁻¹ (JBT-36/119) to 46.25 g.plant⁻¹ (AC-39020) was observed with a mean of 15.01 g.plant⁻¹ (Table 13). GCV and PCV values of 31 % and 43.13 % respectively was observed in experiment 2007 with a moderate heritability of 0.53 (Table 17), whereas a GCV and PCV of 40.93 % and 47.76 % respectively was recorded in experiment 2008 with a high heritability of 72.26 (Table 18). Genetic advance of 37.07 % and 32.15 % was observed in experiment 2007 and 2008 respectively (Table 17 & Table 18). Leaf weight varied from a minimum of 2.27 g.plant⁻¹ to a maximum of 21.98 g.plant⁻¹ with a mean of 8.99 g.plant⁻¹ in the experiment 2007, whereas in the experiment 2008 the leaf weight ranged from 1.53 g.plant⁻¹ to 30.2 g.plant⁻¹ with a mean of 11.45 g.plant⁻¹ (Table 13). Moderate heritability (0.64) was observed with a GCV and PCV of 34.82 % and 43.54 % respectively in the experiment 2007 (Table 17) and a comparable heritability of

0.66 was observed with a GCV and PCV of 38.44 % and 47.26 % respectively in the experiment of 2008 (Table 18).

Total dry matter (TDM) varied significantly ranging from 14.34 g (AC-35351) to 94.60 g.Plant⁻¹ (AC-35612) with a mean of 41.05 g.Plant⁻¹ in 2007. Genetic variability in the 2008 experiment was also significantly ranging from 12.68 g.Plant⁻¹ (PS-319) to 84.10 g.Plant⁻¹ (AC-35310). High heritability was observed for TDM in both the experiment with GCV and PCV values of 37.62 % and 45.58 % respectively were observed in experiment of 2007 (Table 17), whereas the heritability was 0.71 in the experiment of 2008 with GCV and PCV values of 45.19 % and 53.5 % respectively (Table 18). Genetic advance of 63.95 % and 78.81 % was observed in experiment 2007 and 2008 respectively (Table 17 & Table 18). Continuous variability in both the seasons was noticed for TDM. A positively skewed mesokurtic distribution was observed in 2008, whereas in experiment 2007 a positively skewed platykurtic distribution towards lower values was observed (Fig 8 & Fig 9).

Total leaf area (TLA) is an important parameter in determining the canopy photosynthesis, transpiration and growth. The canopy architecture is an important biological parameter that determines the light interception, and therefore the photosynthetic rate and transpiration rate. Thus the knowledge of plant total leaf area is important for understanding the vegetative growth and water loss by the plant. Leaf area ranged varied significantly among the rice accessions ranging between 0.04 m²plant⁻¹ (AC-35017) and 0.71 m²plant⁻¹ (AC-39002) with a mean of 0.27 m²plant⁻¹ in 2007. Significant variability was also noticed in the experiment of 2008 that ranged from 0.053 m².plant⁻¹ (JBT-36/66) to 0.53 m²plant⁻¹ (PS-229) with a mean of 0.21 m²plant⁻¹ (Table 13). Both the experiment showed a continuous variability for TLA. A positively skewed mesokurtic distribution was observed towards lower values in both the experiments (Fig 8 & Fig 9). High heritability was observed for TLA in both the experiments. A genetic advance of 63.95 % and 78.81 % was observed in the experiments in 2007 and 2008 respectively (Table 17 & Table 18).

4.2 Genetic variability in root traits among rice germplasms accessions

Ability to extract water from deeper soil profiles is an extremely important determinant of crop growth under water limited conditions. Presence of genetic diversity among the accessions under study holds the key for rice crop improvement under aerobic condition. The rice germplasms accessions were screened for various root traits in two seasons *viz*, kharif 2007 and kharif 2008 in specially built root structures.

Significant genetic variability was observed for various root traits like root length, root volume, root weight, ratio of root to shoot and ratio of root to leaf area in both the experiments. Plate 4 represents the genetic variability in root morphology. Root length varied from 15 cm (AC-35043) to 94.75 cm (JBT-39/17) in 2007, whereas it showed a range of 22.83 cm (AC-35382) to 78 cm (JBT-36/41) in 2008. However the mean across the experiments was on par (Table 14). High broad sense heritability of 0.62 was observed for root length over the mean in experiment 2007. The experiment of 2008 also recorded a high broad sense heritability of 0.67. A genetic advance of 41% and 28.71% was observed in experiments 2007 and 2008 respectively. A GCV and PCV of 25.28 % and 32.09 % respectively were observed in 2007. Similarly, GCV and PCV of 17.08 % and 20.91 % was observed in 2008 respectively was observed (Table 17 & Table 18). Root length showed a continuous variability in both the experiment. A positively skewed mesokurtic distribution towards the lower values was observed in both the experiments (Fig 8 & Fig 9).

In experiment 2007 root volume varied between a minimum of 12.5 mL (JBT-37/130) to a maximum of 100 mL (PS-329) with a mean of 50.09 mL whereas in experiment of 2008 the values of root volume varied between 5.67 mL (JBT-36/139) to 100 mL (AC-39020) with a mean of 32.05 mL (Table 14). In experiment of 2007 the GCV and PCV values for root volume 34.98 % and 42.24 % respectively was observed with a high broad sense heritability of 0.69. Whereas in the experiment of 2008 GCV and PCV values of 43.88 % and 48.88 % respectively was observed with a heritability of 0.8. Genetic advance of 59.68 % and 81.11 % was observed in experiment 2007 and experiment 2008 respectively (Table 17 & Table 18). Continuous variability was observed for root volume among germplasm accessions. Root volume was positively

Table 13: Genetic variability in biometric traits among rice germplasm accessions

Experiment I (2007)					
Traits	Mean	SE	Minimum	Maximum	CD_(0.05)
Plant height (cm)	56.55	0.89	17.5	112	1.69
Shoot weight (g. Plant⁻¹)	24.18	0.57	6.98	58.25	0.91
Leaf weight (g. Plant⁻¹)	11.45	0.29	1.53	30.2	0.451
TDM (g. Plant⁻¹)	41.05	0.85	14.34	94.6	1.292
TLA (m²)	0.21	0.023	0.06	0.53	0.056
Experiment II (2008)					
Traits	Mean	SE	Minimum	Maximum	CD_(0.05)
Plant height (cm)	72.3	0.97	34	112.83	0.676
Shoot weight (cm)	14.94	0.47	3.13	46.25	0.435
Leaf weight (g. Plant⁻¹)	8.99	0.27	2.27	21.98	0.22
TDM (g. Plant⁻¹)	40.79	1.07	12.68	84.1	1.482
TLA (m²)	0.27	0.076	0.04	0.71	0.012

TDM : Total dry matter (g)

TLA : Total leaf area (m²)**Table 14: Genetic variability in root traits among rice germplasm accessions**

Experiment I (2007)					
Traits	Mean	SE	Minimum	Maximum	CD_(0.05)
Root Length (cm)	48.25	0.92	15	94.5	1.71
Root volume (ml)	50.09	1.05	12.5	100	2.01
Root Weight (g. Plant⁻¹)	5.42	0.14	1.68	12.30	0.209
Experiment II (2008)					
Traits	Mean	SE	Minimum	Maximum	CD_(0.05)
Root Length (cm)	45.38	0.61	22.83	78	0.797
Root volume (ml)	32.05	1.06	5.67	100	0.781
Root Weight (g. Plant⁻¹)	8.55	0.23	1.88	16.29	0.188

skewed towards lower value in both the experiment. Both the experiments showed mesokurtic distribution (Fig 8 & Fig 9).

Root weight, one of the important root traits that determine root system architecture varied from 1.68 g.plant⁻¹ (Jyothi) to 12.30 g.plant⁻¹ (PS-369) with a mean of 5.42 g.plant⁻¹ in 2007. In 2008 the root weight ranged from 1.88 g.plant⁻¹ (JBT-36/139) to 16.29 g.plant⁻¹ (IET-16348) with a mean of 8.55 g.plant⁻¹ (Table 14). High Broad sense heritability (0.69) was observed with a GCV and PCV of 36.32 and 43.75 respectively in the experiment of 2007 (Table 17) whereas in experiment 2008 a high heritability of 0.75 with a PCV and GCV of 35.35 % and 41.08 % respectively was observed (Table 18). Genetic advance of 62.11 % and 63.01 % was observed in experiment 2007 and 2008 respectively over the means (Table 17 & Table 18). Root weight showed a positively skewed mesokurtic distribution towards lower values; however experiment 2008 showed a normal mesokurtic distribution (Fig 8 & Fig 9).

4.3 Genetic variability in surrogates for water use efficiency $\Delta^{13}\text{C}$ and SLA among rice germplasm accessions

Plants discriminate against heavy isotope of carbon (¹³C) during photosynthesis. This discrimination has been shown to occur during the diffusion of CO₂ through stomata and during the primary carboxylation in the chloroplast. Since both these processes of CO₂ diffusion and carboxylation also determine the difference in WUE, theory linking carbon isotope discrimination ($\Delta^{13}\text{C}$) and WUE has been well worked and accepted. Several experiments under field and pot conditions have validated the relationship between $\Delta^{13}\text{C}$ and WUE resulting in this technique being used extensively to assess genetic variability in WUE. $\Delta^{13}\text{C}$ was determined for all the genotypes in the panel of rice germplasms for the two experiments.

$\Delta^{13}\text{C}$ varied from 17.24 ‰ (AC-39000) to 21.38 ‰ (AC-39012) with a mean of 19.25 ‰ in 2007, whereas it varied from 18.15 ‰ (JBT-37/112) to 22.7 ‰ (PS-369) with a mean of 20.14 ‰ in 2008 (Table 15). Both the experiments showed high broad sense heritability of 0.87 and 0.64 in 2007 and 2008 respectively. Genetic advance of 8.53 % and 6.57 % was observed for $\Delta^{13}\text{C}$ in 2007 and 2008 (Table 17 & Table 18).

Table 15: Genetic variability in Water Use Efficiency (WUE) surrogates among rice germplasm accessions

Experiment I (2007)					
Traits	Mean	SE	Minimum	Maximum	CD _(0.05)
$\Delta^{13}\text{C}$ (‰)	19.25	0.05	17.24	21.38	0.013
SLA (cm ² .g ⁻¹)	235.9	2.27	151.7	345.5	2.299
Experiment II (2008)					
Traits	Mean	SE	Minimum	Maximum	CD _(0.05)
$\Delta^{13}\text{C}$ (‰)	20.14	0.06	18.15	22.79	0.0109
SLA (cm ² .g ⁻¹)	239	2.03	153.35	428.04	5.335

$\Delta^{13}\text{C}$: Carbon Isotope Discrimination (‰)
SLA : Specific leaf area (cm².g⁻¹)

Table 16: Genetic variability in DM/LA, Root to Shoot ratio, and Root to leaf area ratio among rice germplasm accessions

Experiment I (2007)					
Traits	Mean	SE	Minimum	Maximum	CD _(0.05)
DM/LA (g. m ⁻²)	164.8	47	72.3	848.4	8.334
Root/Shoot (g.g ⁻¹)	0.16	0.09	0.03	0.51	0.104
Root/Leaf area (g. m ⁻²)	22.95	1.21	4.5	130.59	3.01
Experiment II (2008)					
Traits	Mean	SE	Minimum	Maximum	CD _(0.05)
DM/LA (g.m ⁻²)	205.1	36	95.1	525.6	11.624
Root/Shoot (g.g ⁻¹)	0.378	0.13	0.161	0.894	0.27
Root/Leaf area (g. m ⁻²)	43.52	1.62	12.44	111.64	2.83

DM/LA: Ratio dry mater to leaf area (g.m⁻²)

Fig. 8: Continuous variability observed for various traits among rice germplasm accessions in experiment 2007

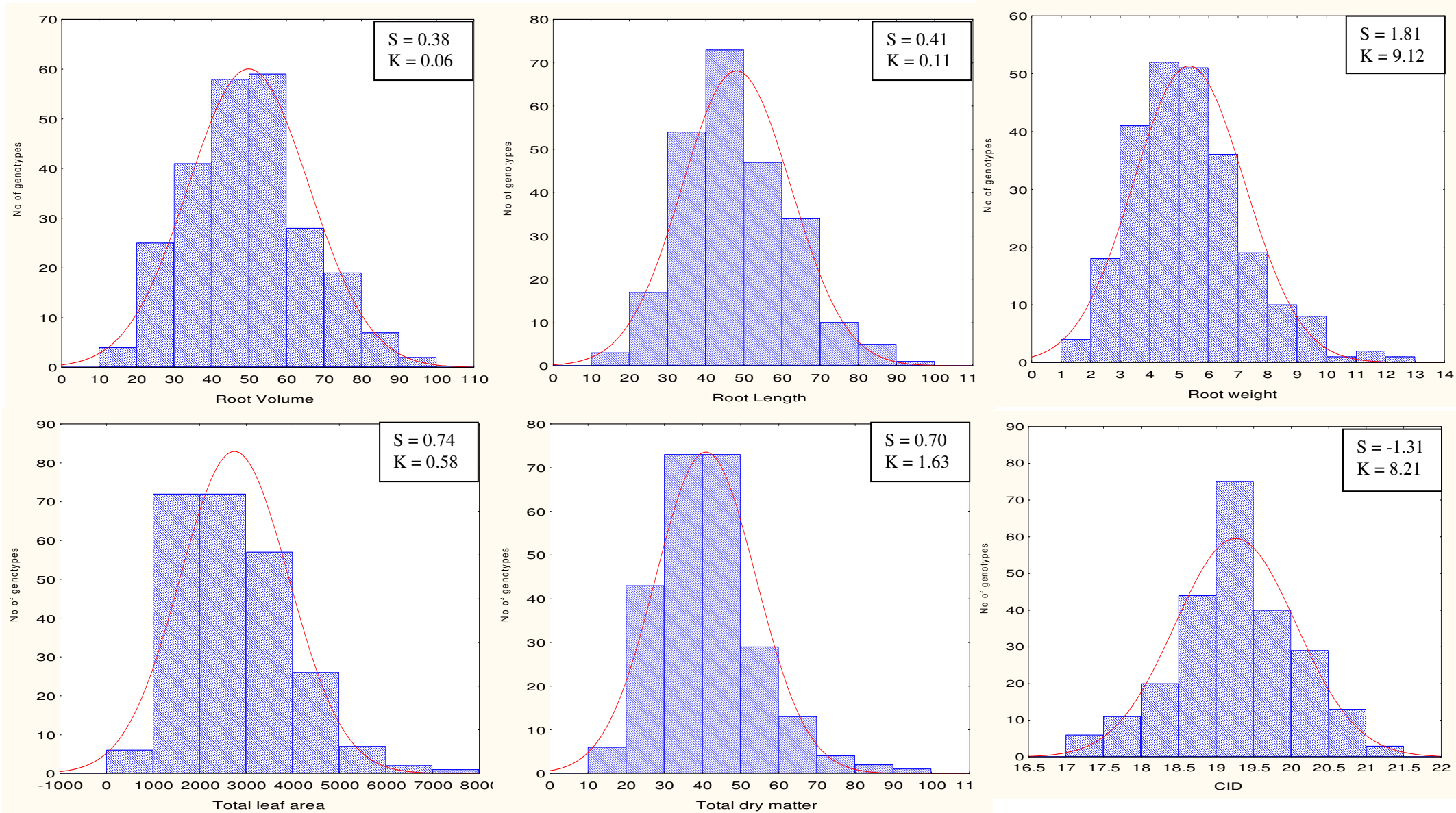


Fig. 9: Continuous variability observed for various traits among rice germplasm accessions in experiment 2008

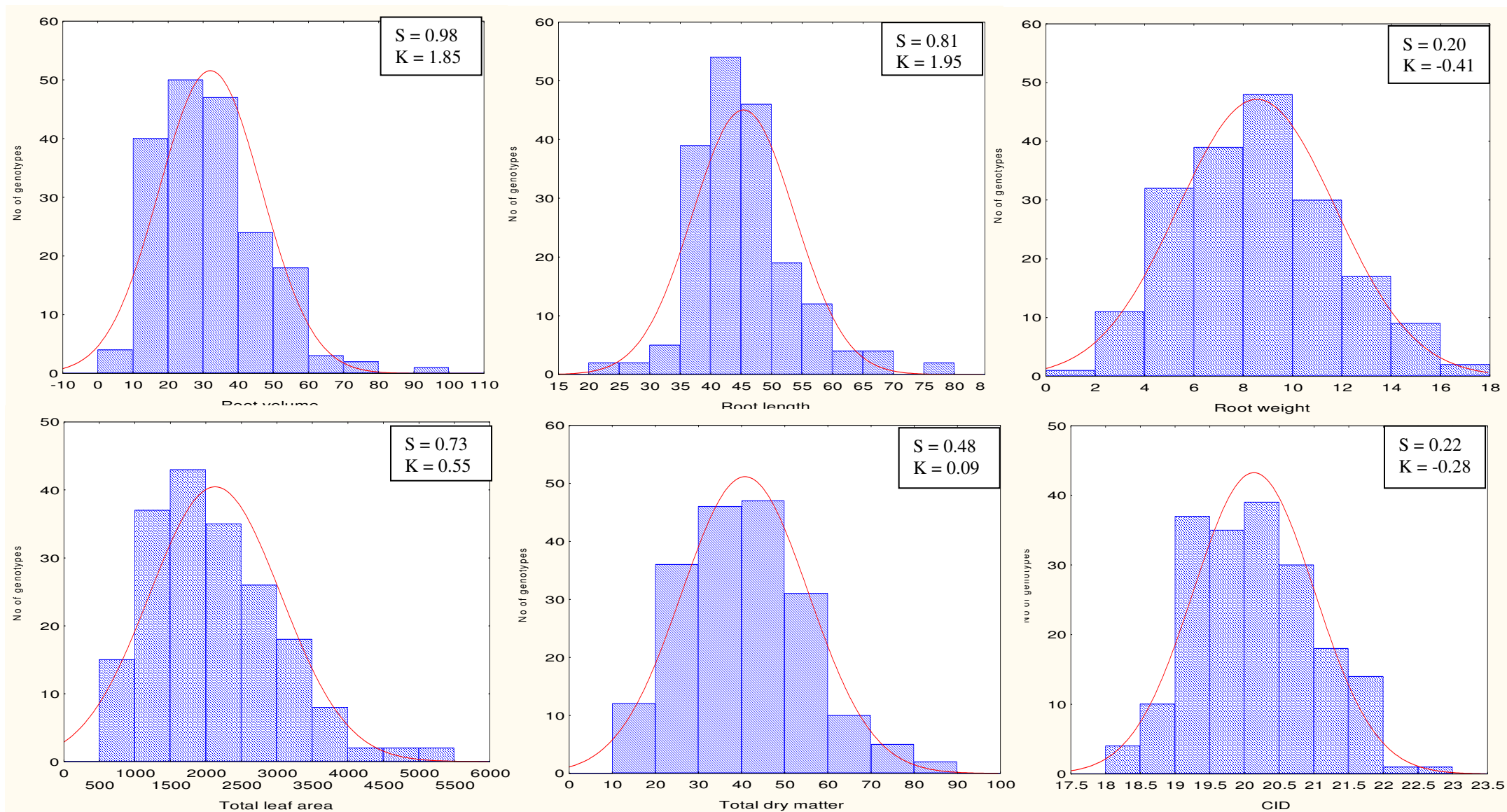


Table 17: Genetic parameters in relevant physiological traits among rice germplasms accessions in experiment 2007

Experiment I (2007)				
Trait	GCV (%)	PCV (%)	H ²	GA (%)
Root Length	25.28	32.09	0.62	41.01
Root Volume	34.98	42.24	0.69	59.68
Root Weight	36.32	43.75	0.69	62.11
Plant height	20.74	23.9	0.75	37.07
Shoot Weight	31	43.13	0.52	45.91
Leaf Weight	34.82	43.54	0.64	57.38
TDM	26.85	31.03	0.75	47.86
SLA	10.49	12.96	0.66	17.49
TLA	37.62	45.58	0.68	63.95
$\Delta^{13}\text{C}$	4.44	4.78	0.87	8.53

TDM :Total dry matter (g.Plant⁻¹)

SLA : Specific leaf area (g.cm⁻²)

TLA : Total leaf area (cm² Plant⁻¹)

$\Delta^{13}\text{C}$: Carbon Isotope Discrimination (‰)

Table 18: Genetic parameters in relevant physiological traits among rice germplasms accessions in experiment 2008

Experiment II (2008)				
Trait	GCV (%)	PCV (%)	H ²	GA (%)
Root Length	17.08	20.92	0.67	28.71
Root Volume	43.88	48.88	0.8	81.11
Root Weight	35.45	41.08	0.74	63.01
Plant height	17.7	20.08	0.77	32.15
Shoot Weight	40.93	47.76	0.73	72.26
Leaf Weight	38.44	47.26	0.66	64.4
TDM	34.43	39.32	0.76	62.12
SLA	17.72	21.6	0.67	29.94
TLA	45.19	53.4	0.71	78.81
$\Delta^{13}\text{C}$	3.97	4.95	0.64	6.57

TDM :Total dry matter (g. Plant⁻¹)

SLA : Specific leaf area (g.cm⁻²)

TLA : Total leaf area (cm² Plant⁻¹)

$\Delta^{13}\text{C}$: Carbon Isotope Discrimination (‰)

Table 19: Genetic variability in gas exchange parameters

Traits	Mean	Minimum	Maximum	CD_(0.05)
A	21.67	11.51	33.31	2.48
g_s	0.75	0.2	2.15	0.13
Ci	308.47	242.5	461.83	0.04
T	8.91	4.56	16.18	0.13
A/T	2.48	1.22	3.4	0.79
Ci/gs	476.47	159.24	1356.17	0.07
A/gs	32.34	10.41	71.38	0.48
Fv'/Fm'	0.533	0.367	0.777	-

Ci – Intercellular CO₂ concentration (ppm)

A – Assimilation rate ($\mu\text{ mol m}^{-2}\text{ s}^{-1}$)

T – Transpiration rate ($\text{mmol m}^{-2}\text{ s}^{-1}$)

g_s - Stomatal conductance ($\text{mmol m}^{-2}\text{ s}^{-1}$)

A/T – Ratio of Assimilation to transpiration rate ($\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$)

A/gs – reflection of WUE at single leaf level ($\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$)

Ci/gs – reflection of mesophyll efficiency at single leaf level

Fv'/Fm' – Light adapted quantum efficiency

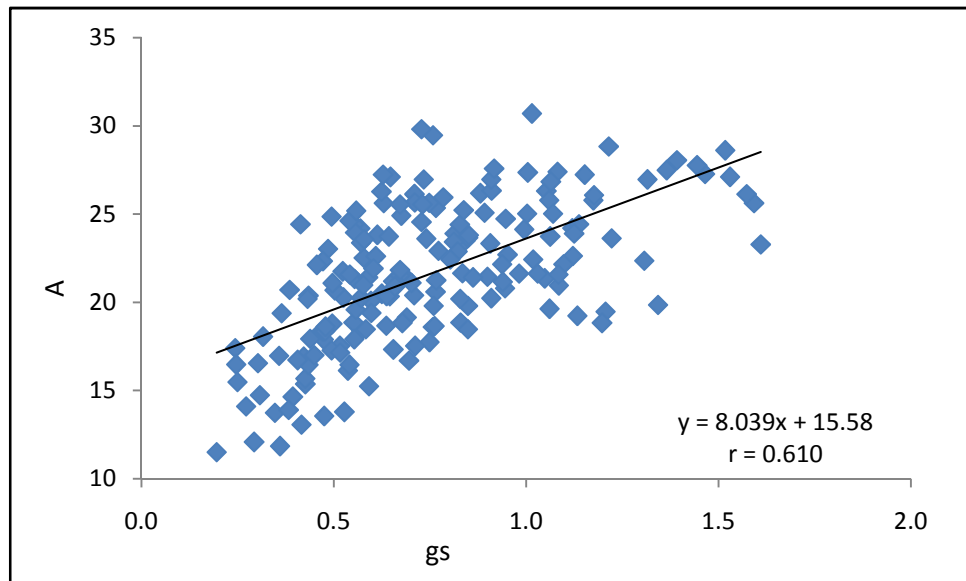


Fig. 10: Relationship between assimilation rate (A) and stomatal conductance (gs) among rice germplasm accessions

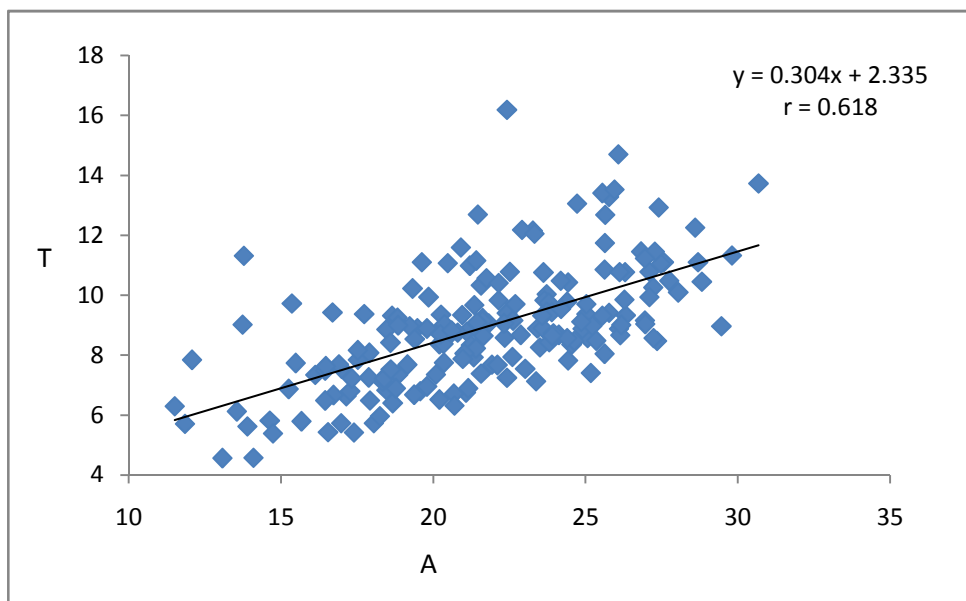


Fig. 11: Relationship between assimilation rate (A) and transpiration (T) among rice germplasm accessions

Experiment 2007 showed a normal mesokurtic distribution, whereas experiment 2008 showed a negatively skewed leptokurtic distribution (Fig 8 & Fig 9).

Though $\Delta^{13}\text{C}$ is an unequivocal surrogate for WUE, non availability of infrastructure to measure carbon isotope ratios and the cost involved in its determination have forced scientists to explore other cheaper alternative methods. Specific Leaf Area (SLA), an indirect reflection of leaf spread is often considered as one such alternate estimate of water use efficiency (WUE). SLA showed a significant variability ranging from $153.4 \text{ cm}^2 \text{ g}^{-1}$ in (JBT-36/2) to $428.0 \text{ cm}^2 \text{ g}^{-1}$ (Hamsa) in 2007 with a mean of $235.9 \text{ cm}^2 \text{ g}^{-1}$. Similarly, in the experiment of 2008 also, SLA varied significantly between $151.7 \text{ cm}^2 \text{ g}^{-1}$ (JBT-37/112) to $345.5 \text{ cm}^2 \text{ g}^{-1}$ (S-9) with a mean of $235.9 \text{ cm}^2 \text{ g}^{-1}$ (Table 15). High heritability of 0.66 in 2007 and 0.67 in 2008 was observed. Comparatively less genetic advance of 17.49 % was observed on 2007 compared to the genetic advance of 29.94 % in the experiment of 2008.

4.4 Genetic variability in DM/LA, Root to Shoot ratio, and Root to leaf area ratio among rice germplasm accessions

Though root traits and WUE are important determinants of drought tolerance, these traits have limited advantage if genotypes with higher values for these traits are not associated with superior genotype crop growth rates. Hence, the genotype identification criteria should necessarily consider growth rates. Simple parameters have been suggested to assess growth rates among large number of accessions. The ratio of total biomass to the total leaf area (DM/LA) is one such parameter. This ratio is an indirect measure of net photosynthetic carbon assimilation. DM/LA ratio is an important physiological parameter that indicated the net photosynthetic ability of the plant. Significant Genetic variability was observed in DM/LA in both the experiments among rice germplasm accessions. In 2007 DM/LA ranged from a minimum of 72.3 g.m^{-2} to as high as 848.4 g.m^{-2} with a mean of 164.8 g.m^{-2} . In 2008 a range of 95.1 g.m^{-2} to 525.6 g.m^{-2} was observed with a mean of 205.1 g.m^{-2} .

These results indicate significant variation in photosynthetic capacity. It can be visualised that high DM/LA types when coupled with higher root traits would result in a more comprehensive improvement in drought tolerance. Nevertheless, it is also essential that high root types possess adequate biomass accumulation. To assess this, the ratio of

root to shoot biomass and root to leaf area ratio were computed. A twofold higher variability was observed for root to shoot ratio in both years. Root to shoot ratio varied from a minimum of 0.03 g.g^{-1} in Jyothi to a maximum of 0.51 g.g^{-1} in PS-369 with a mean of 0.16 g.g^{-1} in 2007, whereas it varied from 0.161 g.g^{-1} in JBT-36/179 to 0.894 g.g^{-1} in AC-35290 with a mean of 0.378 g.g^{-1} in 2008. Similarly the ratio of root to leaf area varied from 4.5 g.m^{-2} (AC-35476) to 130.59 g.m^{-2} (PS-369) with a mean of 22.95 g.m^{-2} in the experiment of 2007 whereas it varied from 12.44 g.m^{-2} (PS-253) to 111.64 g.m^{-2} (PS-369) with a mean of 43.52 g.m^{-2} (Table 16).

4.5 Variability in gas exchange parameters among rice germplasm accessions

At a single leaf level, photosynthesis is often considered as a diffusive process. CO_2 diffuses from atmosphere to the leaf down the concentration gradient, while water vapour diffuses out in to a drier atmosphere. Though diffusion of CO_2 and H_2O are controlled by respective concentration gradients, the diffusive resistance for the two gaseous molecules significantly alters gas exchange between leaf and atmosphere. Stomata are the prominent structures that have profound influence on overall diffusion of gases. In other words stomata significantly control both CO_2 diffusion for photosynthesis and evaporation of water during transpiration. Gas exchange parameters like A, g_s , C_i and T were measured using a portable IRGA and the results are described.

Assimilation rate (A) varied from $11.51 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ to $33.3 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ with a mean of $21.7 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ representing a significant genetic variability among rice germplasm accessions. Transpiration rate (T) also exhibited a significant genetic variability ranging from $4.56 \text{ mmol.m}^{-2}.\text{s}^{-1}$ to $16.18 \text{ mmol.m}^{-2}.\text{s}^{-1}$ with a mean of $8.91 \text{ mmol.m}^{-2}.\text{s}^{-1}$. Similarly stomatal conductance (g_s) varied from $0.2 \text{ mmol.m}^{-2}.\text{s}^{-1}$ to $2.15 \text{ mmol.m}^{-2}.\text{s}^{-1}$ with a mean of $0.7515 \text{ mmol.m}^{-2}.\text{s}^{-1}$ (Table 19). A significant positive correlation was observed between photosynthetic rate (A) and stomatal conductance (g_s) (Fig 10) suggesting stomata strongly controls photosynthetic rate. Photosynthetic rate is also found to have a strong positive correlation with Transpiration (Fig 11) and hence though higher g_s would result in higher Carbon fixation with inadvertent increase in transpiration. This is not a good strategy under water limited condition. Hence the ratio of assimilation rate to stomatal conductance (A/g_s) and the ratio of assimilation rate to

transpiration (A/T) were computed. This ratio of gas exchange trait is fairly good estimate of WUE at a single leaf level.

The calculated ratios like A/T and A/g_s showed a significant genetic variability among germplasm accessions. A/T varied from a minimum of 1.22 $\mu\text{mol of CO}_2\text{mmol}^{-1}\text{H}_2\text{O}$ to a maximum of 3.4 with a mean of 2.48 $\mu\text{mol of CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$, whereas a range of 32.34 $\mu\text{mol of CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$ to 71.38 $\mu\text{mol of CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$ with a mean of 32.34 $\mu\text{mol of CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$ (Table 19). Florescence emission from the reaction centre provides an extremely useful tool to assess the status of photochemical efficiency. Light adopted variable to the maximum florescence ratio was measured using a pulse amplitude modulated florescence system (LICOR-6400-40) fitted to work in simultaneous mode with LICOR-6400. Fv'/Fm' is therefore an estimate of apparent quantum efficiency of PSII. The rice germplasm accessions AC-35417 showed the lowest apparent quantum efficiency of 0.367 while JBT-36/14 recorded the maximum Fv'/Fm' of 0.777. The mean quantum efficiency of the entire panel of germplasm was 0.533 (Table 19).

4.6 Consistency of Drought tolerance traits across the experiments

Phenotypic expression is a dynamic interaction between genotype and the prevailing environmental conditions. Quantative traits are controlled by several genes exhibiting a string modulation by the environment. However an inconsistent phenotype expression would not be very useful in crop improvement through breeding. Therefore it becomes essential that environmental modulation and genetic control of any given trait is essential for exploitation. The mean and range values of a few important traits are compared to assess the modulation of a trait by the environment. Stability of the phenotypic expression across environment is a good indicator of strong genetic control of the trait. Heritability of such traits will be generally high and hence assessing stability of traits becomes a pre-requisite of any breeding programme. Most of the traits exhibited continuous variation indicating a Quantative inheritance pattern. Further a regressions analysis was carried out to assess the consistency of a trait across the seasons. The results of such analysis are coated under this section.

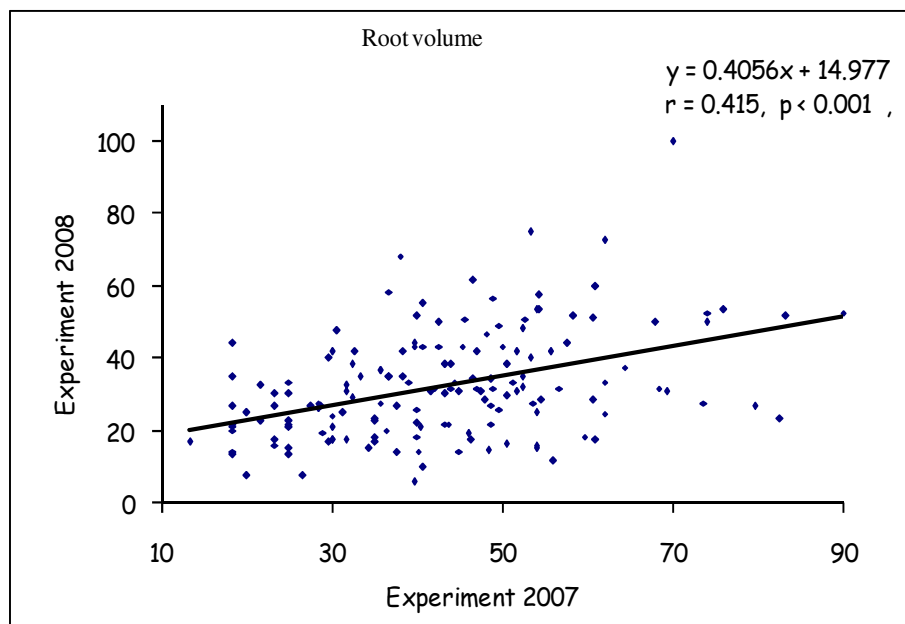


Fig. 12: Relationship of Root volume measured in two separate experiments

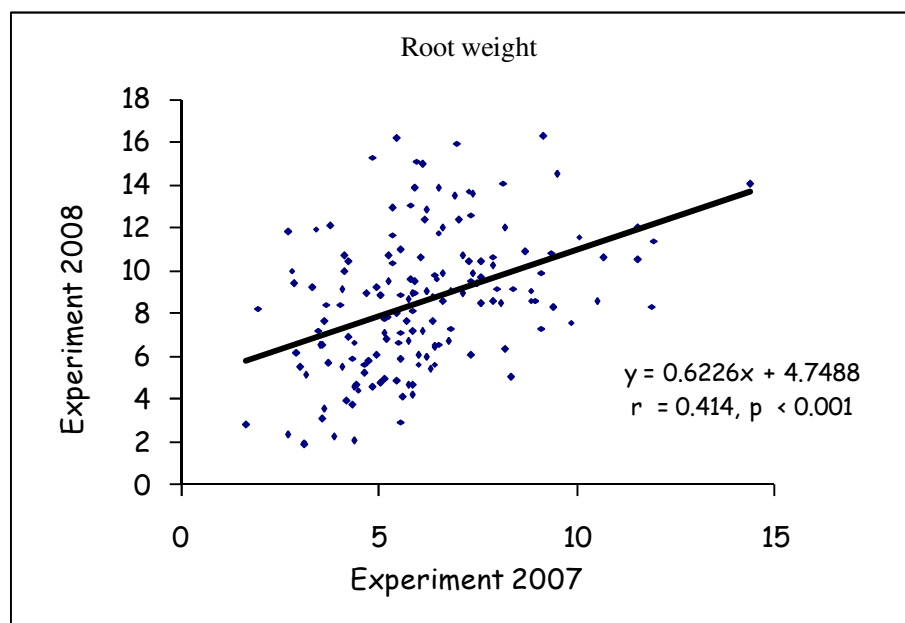


Fig. 13: Relationship of Root weight measured in two separate experiments

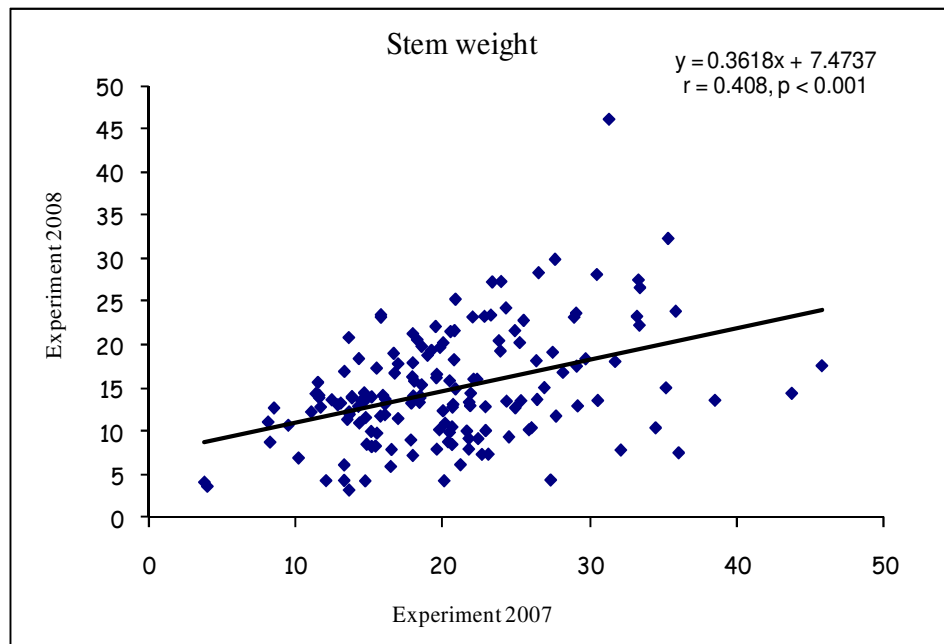


Fig. 14: Relationship of Stem weight measured in two separate experiments

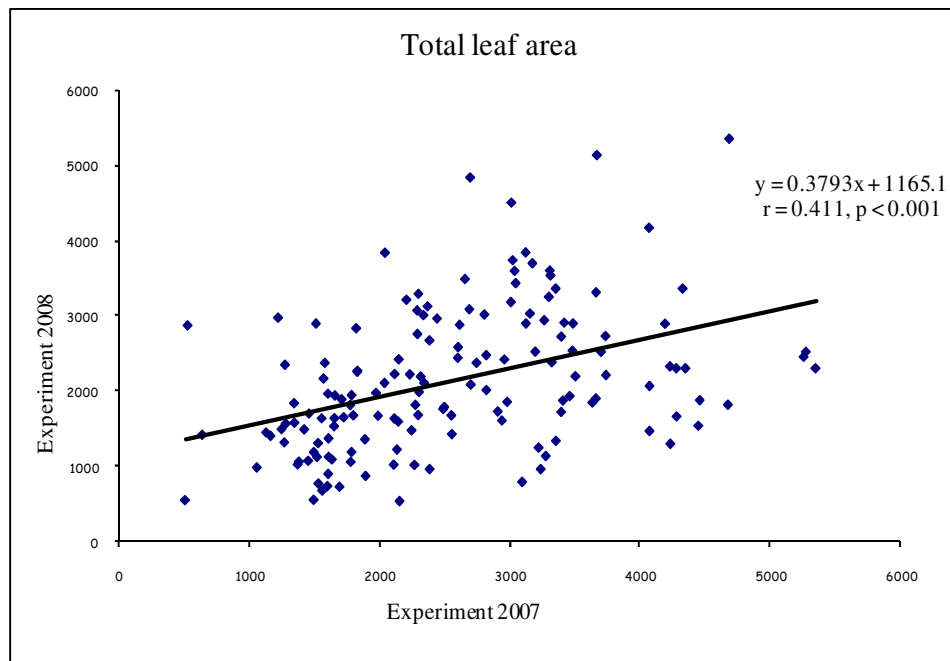


Fig. 15: Relationship of Total leaf area measured in two separate experiments

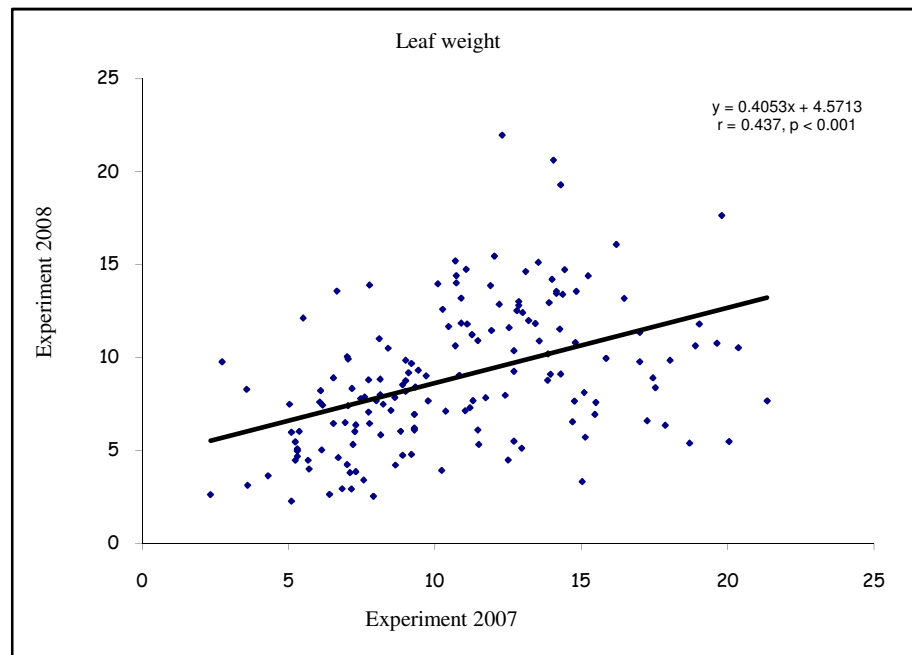


Fig. 16: Relationship of Leaf weight measured in two separate experiments

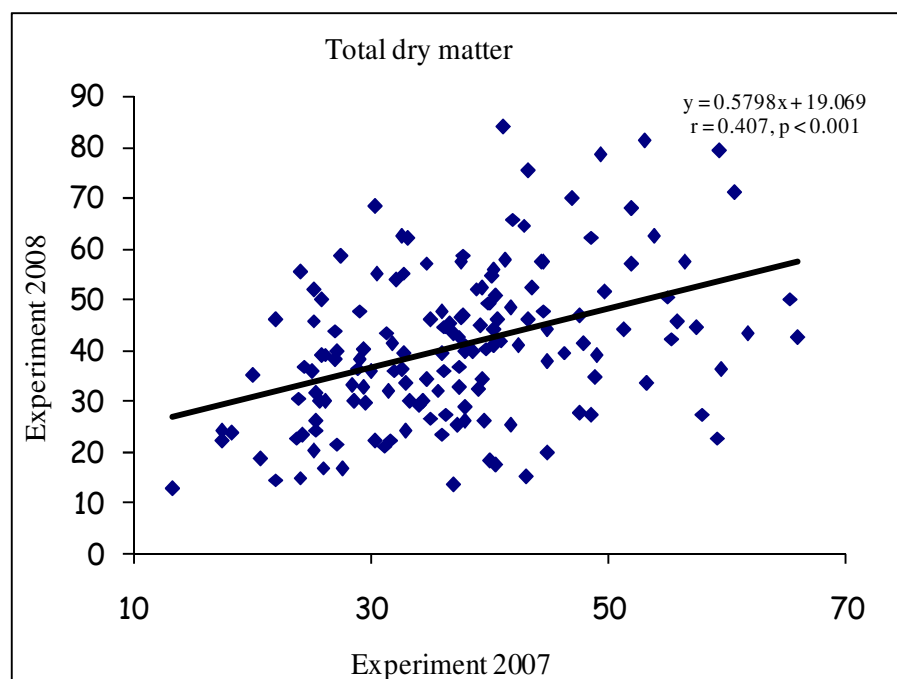


Fig. 17: Relationship of Total Dry Matter measured in two separate experiments

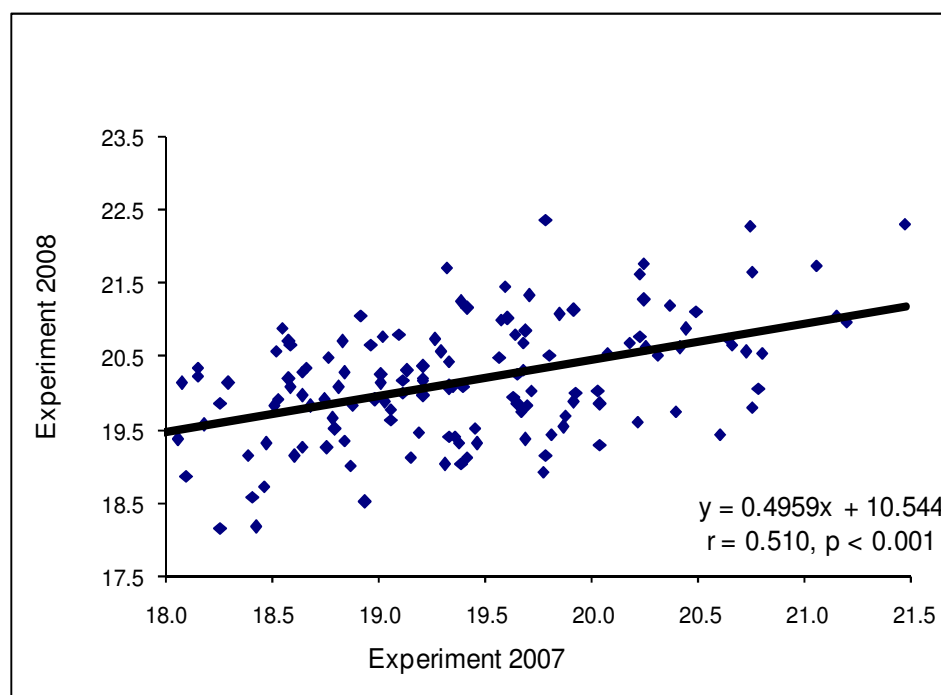


Fig. 18: Relationship of $\Delta^{13}\text{C}$ measured in two separate experiments

A significant correlation of root volume ($r = 0.415$) and root weight ($r = 0.414$) across the seasons (Fig 12 & Fig 13) indicated a low $G \times E$ interaction for root weight and volume. Leaf characters such as leaf area and leaf weight also showed a significant consistency across the season with an r value of 0.411 and 0.437 respectively indicating a lower effect of environment on these traits (Fig 15 & Fig 16). Similarly shoot weight also exhibited a significant consistency across the experiment (Fig 14). Several previous experiments have shown that yield is a function of TDM in many crops and increasing TDM at a given optimized harvest index. In the present study TDM also exhibited significant consistency across the seasons with an r value of 0.407 (Fig 17). Stable carbon isotope discrimination ($\Delta^{13}\text{C}$) has been demonstrated as a time averaged surrogate for water use efficiency. $\Delta^{13}\text{C}$ also showed a significant consistency among rice germplasm accessions across experiments ($r = 0.510$) (Fig 18).

4.7 Selection of contrasts and trait validation

Crop growth rate under aerobic condition is normally associated with better water mining and superior water use efficiency. Plants allocate various proportions of biomass ranging between 10 % to as high as 40 % to roots. Though roots are extremely important for water mining, a reasonable amount of biomass in above ground parts is essential to realise higher productivity. Relevant statistical strategy was adopted for selecting germplasm lines where a weighted average for root biomass, WUE (estimated by $\Delta^{13}\text{C}$), root to shoot ratio and leaf area to dry matter ratios was calculated and contrasting lines were identified (Appendix III).

A set of 21 genotypes representing high TDM, high root weight, high WUE and high root to shoot ratios along with 10 low types were selected (Appendix III). The major interest of selecting these genotypes was to examine the consistence of the traits that have relevance to drought tolerance. These traits that were considered most crucial namely root dry weight, WUE, root to shoot ratio and total biomass, showed significant variability in the experiment conducted during kharif 2009 (Raju, 2009). Besides genetic variability, consistency of the trait across seasons or location is essential for transferring traits through breeding. Among the traits evaluated $\Delta^{13}\text{C}$ showed the strongest regression for values determined between two different experiments. $\Delta^{13}\text{C}$ of the two years was related significantly ($r = 0.589$) compared with a more complex trait such as TDM ($r = 0.523$), (Fig. 19). These values reveal that the strategic approach of attempting to

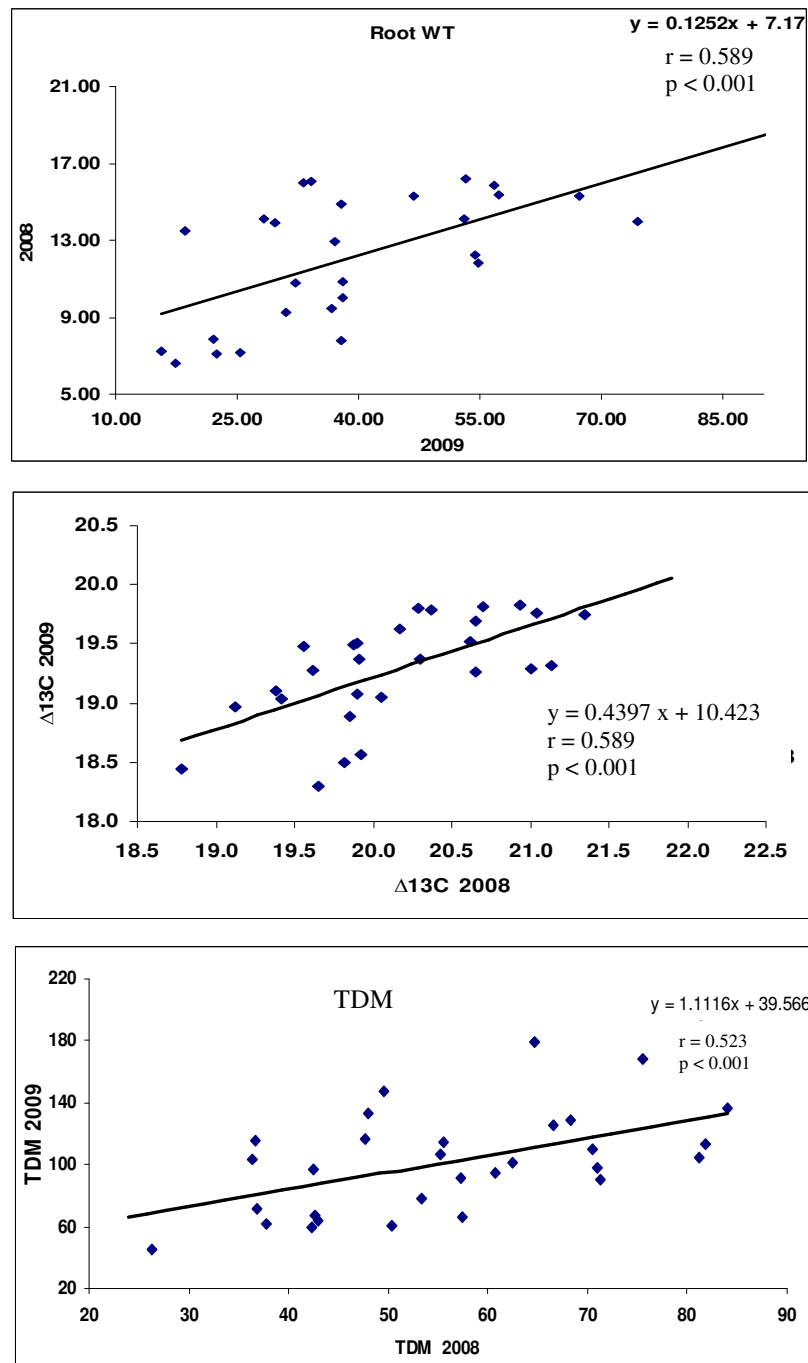


Fig. 19: Consistency of different traits among selected genotypes grown in root structure

improve constitutive drought tolerance trait is possible.

The major outcome of the Phenotyping experiment are given below-

- Significant genetic variability in drought tolerance trait revealed among the rice germplasms lines.
- Most of the trait showed consistency across seasons indicating a strong genetic control over the trait. However, presence of normal distribution of these traits indicated a polygenic Quantative inheritance.
- The promising lines identified can be used as trait donor lines to transfer specific trait in to a recipient genotype.
- The contrasting lines will be useful in developing trait specific mapping population as well as for greater understanding of molecular regulation of these traits.

4.8 Molecular characterization and genetic diversity analysis

4.8.1 Molecular markers system used

4.8.1.1 Simple Sequence Repeats (SSR markers)

Among the several marker system used for genotyping, SSR markers are one the most suitable markers for genotyping natural populations. Due to the co-dominant and multi-allelic nature of SSR markers, they reveal maximum information among the genotypes. Known genetic position of these SSR markers will help to assess the level of linkage disequilibrium (LD) among rice accession. In the present study a total of 109 SSR markers were used. Markers distributed on all chromosomes were selected from gramene database (www.gramene.com). Based on the gel picture in the database, only polymorphic informative SSR markers were selected. Of the 109 markers, 78 gave locus specific amplification (Plate 6). SSR Markers bands were resolved and scored using a capillary based DNA fragment analyser (MegaBACE-500). Fig 20 represents the electropherogram of SSR primer multiplexing based on dye colour as well as product size. Markers which had more than 20 % missing data were removed from the analysis. A total of 69 markers, resolved and scored using Fragment analyser were used for analysis (78 locus specific amplification - 9 missing data). In addition to this, 45 markers used in a previous work (Diwakara; 2007) run and scored using 3% agarose for the same germplasm accessions were also added to the analysis. A total of 113 markers were used for association analysis. The common genotypes among the two experiments were selected for association analysis. A total of 151 germplasm lines were found to be

common among the two experiments. On screening the polymorphic 113 markers with a total of 151 rice accessions, a total of 678 alleles were resolved. Among these 69 primers which were scored using MegaBACE resulted with a total of 546 alleles with an average of 7.6 alleles per marker locus and 45 primers scored using 3% agarose produced a total of 132 alleles with an average of 2.97 alleles per marker locus. Though a significant amount of information may have been most by scoring on agarose gels, the allelic diversity would still be very useful in analysing the molecular diversity among the population.

4.8.2 Chromosome wise distribution of SSR markers and their allelic diversity

Allelic diversity was analysed using power marker software (Liu and Muse 2005). SSR markers were chosen such that they were equally distributed on the entire genome. Highest number of 17 markers was present on chromosome 2, whereas Chromosome 12 had the least of 3 markers. An average of 10.27 markers was present on every chromosome except chromosome 10. The number of alleles per locus varied from 2 (RM 411 on chromosome 3) to 19 (RM 317 on chromosome 4). Maximum allelic points were observed on chromosome 2 (129) and minimum on chromosome 12 (14) with an average of 61.45 alleles per chromosome (Table 20).

4.8.2.1 Gene diversity or Expected Heterozygosity (H_{Exp})

Gene diversity is often referred to as expected heterozygosity and is defined as the probability that two randomly chosen alleles from the population are different. Gene diversity gives a representation of the genetic diversity present in the natural population. Gene diversity ranged from 0.43 in chromosome 3 and 7 to 0.68 on chromosome 11 and 12. Mean allelic diversity was observed to be 0.55 across the entire genome (Table 20).

4.8.2.2 Heterozygosity

Heterozygosity is the proportion of heterozygous individual in the population. In the present study heterozygosity ranged from 0.03 on chromosome 7 and 4 to 0.14 on

Plate 6: Gel profile depicting annealing temperature standardization of SSR primers

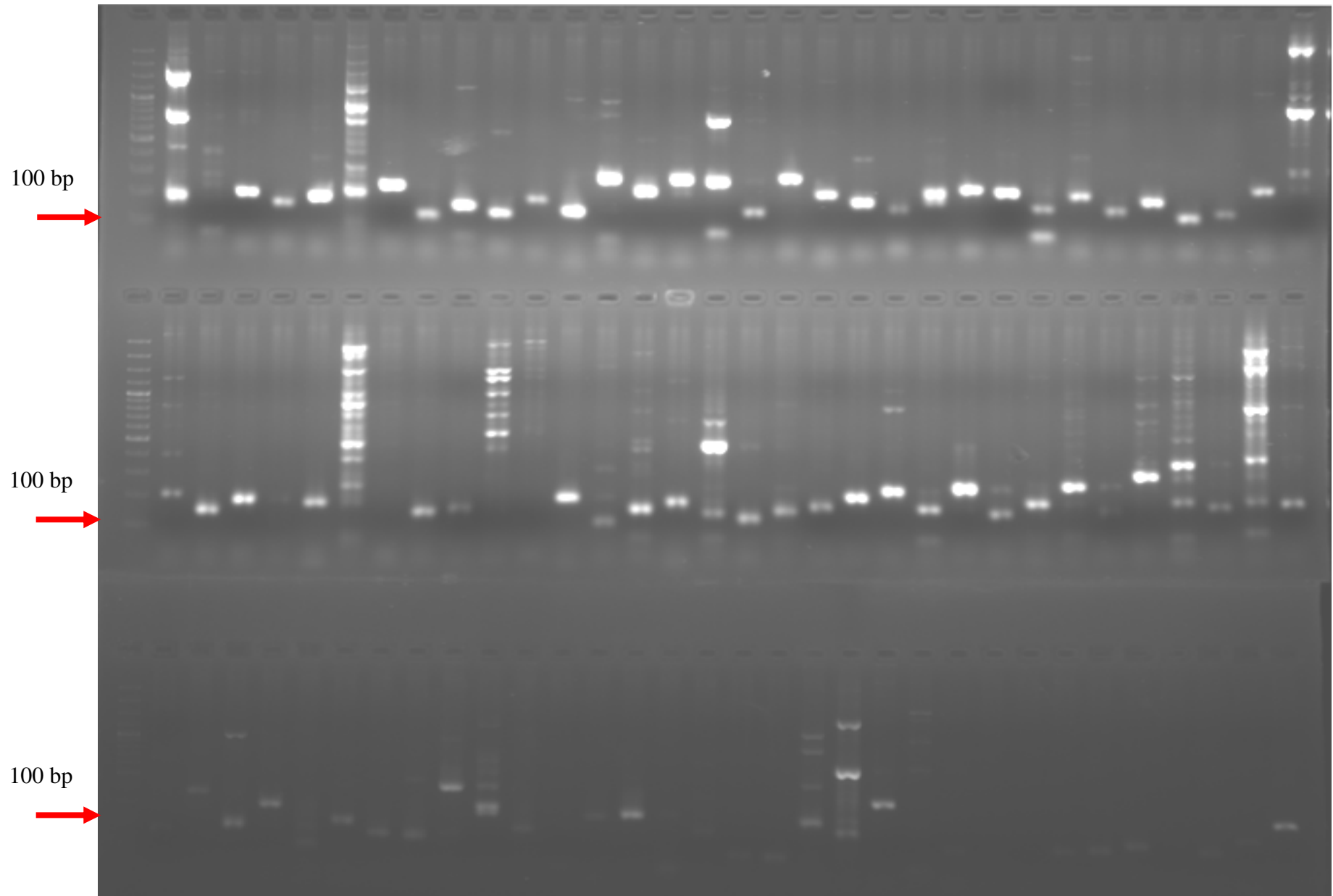
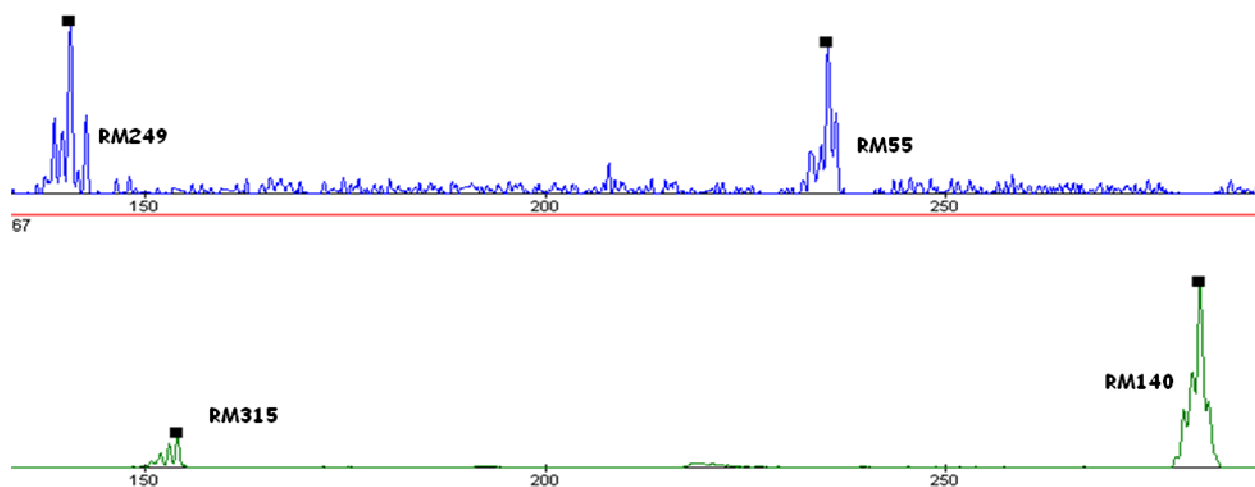


Figure 20. Electropherogram depicting Multiplexing of SSR primers based on size as well as florescent dye color in MegaBACE-500



FAM (Blue)		
Marker	EXPECTED SIZE	OBSERVED SIZE
RM249	139	140
RM55	226	235
HEX (Green)		
	EXPECTED SIZE	OBSERVED SIZE
RM315	141	154
RM140	279	282

chromosome 12. Mean heterozygosity over the entire genome was observed to be 0.06 (Table 20). The low level of heterozygosity clearly suggests that the germplasm used in this study were real inbreds, such a population would be ideal for association genetic analysis.

4.8.2.3 Polymorphism information content (PIC)

Polymorphism information (PIC) content of each locus was analysed to determine the extent of polymorphism explained by these markers using Power marker software (Botstein *et al.*, 1980). PIC value indicated a measure of close relativity among individuals. The polymorphic information content of markers exhibited a significant variation ranging from 0.03 for marker RM124 on the chromosome 4 to as high as 0.91 for the marker RM317 located also on chromosome 4. While chromosome 4 had marker with the highest range of PIC values (0.03 to 0.91), the lowest range of PIC values was seen for markers located on chromosome 12 (0.55 to 0.67). The mean PIC value of all markers on the genome was 0.51 which ranged between 0.39 for marker on the chromosome 3 and 0.63 for markers on chromosome 11 and 12 (Table 20).

4.8.3 Molecular variance

Analysis of molecular variance (AMOVA) gives an estimation of molecular genetic variation among and within the clusters. AMOVA revealed a 91.97 % of the total variance among clusters whereas 8.03% of the variation was within the clusters (Table 21, Fig 21).

4.9 Population structure analysis and determination of Q matrix

Population structure was analysed with model based programme “STRUCTURE” proposed by Pritchard *et al.* (2000), Falush *et al.* (2003).

4.9.1 Determination of the number of subgroups (K) in rice germplasm accessions

The power of LD mapping depends on the accurate resolution of the number of sub-population in a population. The model proposed by Jonathan Pritchard (Pritchard,

Table 20: Chromosome wise distribution of SSR markers and their allelic diversity

Chromosome	Markers	Avg. Allele No.	Total Allelic points	Allele diversity	Heterozygosity	Mean PIC	Range
1	13	6	74	0.53	0.04	0.49	0.05-0.86
2	17	8	129	0.59	0.05	0.55	0.04-0.80
3	7	5	36	0.43	0.04	0.39	0.20-0.64
4	8	8	63	0.63	0.03	0.60	0.03-0.91
5	11	7	72	0.53	0.06	0.50	0.08-0.81
6	14	5	73	0.52	0.09	0.48	0.07-0.83
7	12	5	63	0.43	0.03	0.40	0.07-0.73
8	8	6	50	0.59	0.04	0.55	0.21-0.78
9	14	5	71	0.50	0.04	0.45	0.18-0.88
11	6	6	33	0.68	0.05	0.63	0.28-0.88
12	3	5	14	0.68	0.14	0.63	0.55-0.67
Mean	10.27	5.92	61.64	0.55	0.06	0.51	

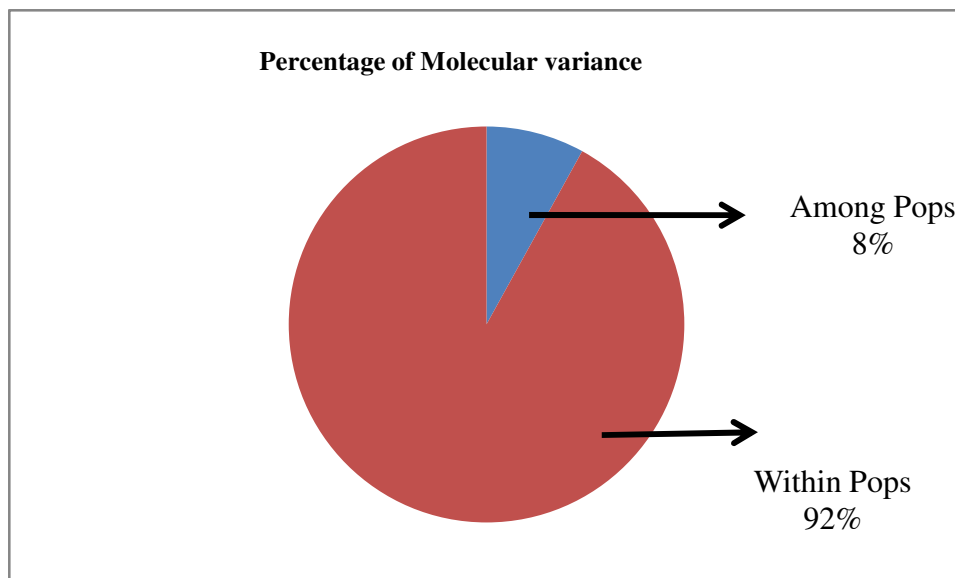


Fig. 21: Pie diagram depicting Analysis of Molecular variance

Source	d.f.	Sum of Squares	Variance components	Percentage variance	Value	Prob
Among Clusters	3	1360.391	9.764	8%		
Within Clusters	147	16443.417	111.860	92%	0.080	0.010
Total	150	17803.809	121.624			

Table 21: Summary AMOVA Table

Note: Analysis of Molecular Variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about such differentiation. AMOVA was calculated using the software Genetic analysis in Excel (GenAlEx 6.0).

2000) accurately estimates the ancestry vector, and can extract more information from the data which is useful for admixture mapping. Markov chain Monte Carlo (MCMC) algorithm has been included in the newer version which integrates over the possible chunk sizes and break points. It reports the overall ancestry for each individual, taking account of the linkage.

In the present study we adopted the complex ancestry model which takes in to account the possibility of genetic material originating from different populations (migration and linkage models) allowing for admixture and correlated allele frequency. When individuals have mixed ancestry, it indicates that each genotype comes from one or other of the K populations.

Since any set of germplasms may have certain extent of genetic similarity, an estimate of admixture would be essential. The estimate “ α ” (alpha) represents the extent of admixture while computing the admixture. The K values were allowed to vary for each K ranging from 2 to 10 with an MCMC of 100,000 and a burnin of 150,000. Independent runs for each K was done with 6 iterations. The likelihood estimates runs yielded consistent results, accordingly these genotypes were classified under different sub-groups.

Estimated log probability ($LnP(D)$) represents the extent of genetic diversity hidden in the population. ($LnP(D)$) of data varied from -35202 at K=2 to -30974 at K=10. The distribution of $LnP(D)$ with the number of sub-population (K) did not show a clear mode for the true value of K (Fig 22). To overcome difficulties in interpreting the real value of K, another *ad hoc* quantity ΔK was used (Evanno *et al.*, 2005). ΔK , is related to the second order rate of change of the log probability of data with respect to the number of clusters, and is a good predictor of the real number of clusters. ΔK is given by

$$\Delta K = \frac{m (|L(K + 1) - 2 L(K) + L(K - 1)|)}{s [L (K)]}$$

Where

- K = subpopulation considered
- m* = mean of LnP(D) estimated for individual Subgroups (K)
- L (K) = average of LnP(D) and half the variance is subtracted with the mean
- s* = standard deviation for LnP(D) estimates among individual subgroups (K)

Unlike *LnP(D)*, ΔK did show a clear mode for true value of K and the distribution of ΔK with K clearly indicated that the number of sub-population was four (Fig 23). This shows that the maximum diversity among the rice germplasm accessions was at K=4. Alpha, another measure of the extent of admixture and genetic diversity also indicated four presences of four major clusters. The distribution of alpha value versus K also confirmed that the total number of sub-population was at 4 (Fig 24). Alpha value ranged from 0.0500 at K=10 to 0.09595 at K=4 (Table 22). The range indicated that the alpha value did not vary significantly during the course of run indicating that the individuals had least admixture.

4.10 Tree construction

Assessment of population structure is important for genetic association studies. Presence of high degree of cryptic structure may cause confounding effects in population based association studies leading to false positive results. Two major approaches have been proposed to detect population structure: distance based clustering methods and model based methods. Model based programmes like structure uses standard statistical methods and is being used widely to assess the population structure. Distance based approach used the proportion of alleles shared between the individuals. Although model based programmes are used to assess the population structure, confirmation by distance based methods would substantiate the results of model based analysis.

DARwin 5.0 software developed for phylogenetic analysis was used for tree construction by the neighbour – joining method (Saitou and Nei, 1987). This method is based on the relative neighbourhood and weighted average for dissimilarity studies.

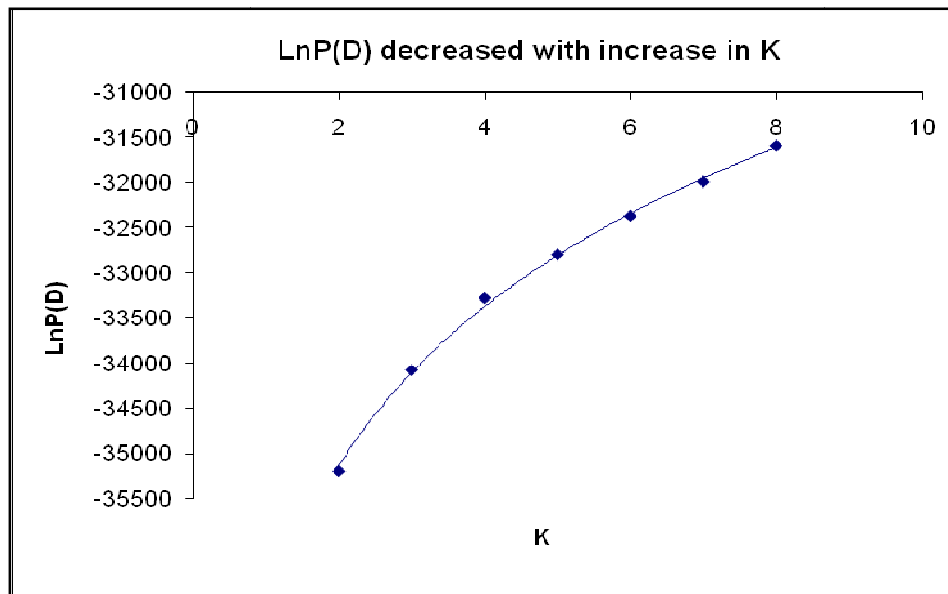


Fig. 22: Population Structure Analysis with model based program.

Note: Scatter plot of Estimated Logn Probability of Data (LnP(D) vs number of subpopulation assumed (K). The plot did not reveal a clear mode for the true value of K. Estimated Logn Probability of Data (LnP(D) was calculated using the programme “STRUCTURE” assuming the subpopulation from 2 to 10.

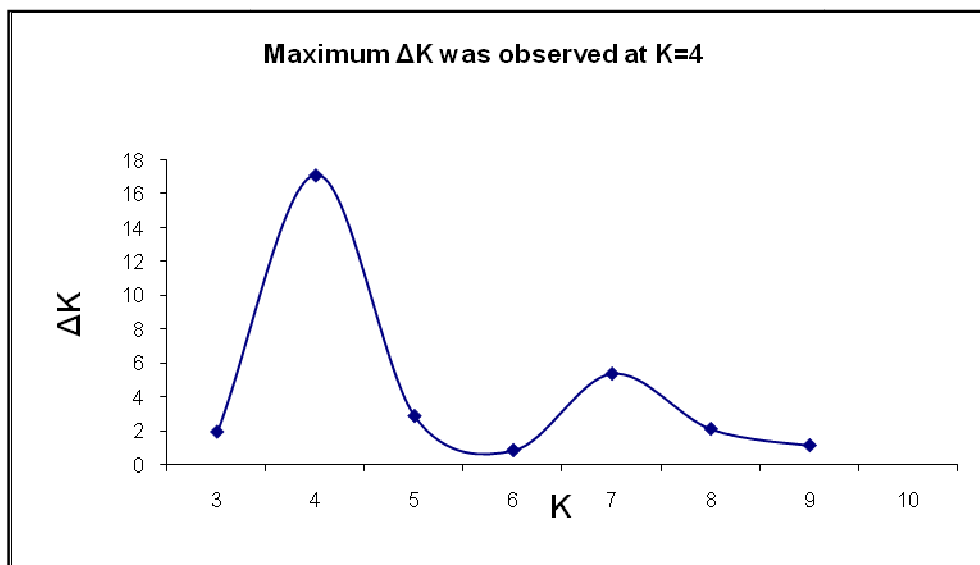


Fig. 23: Determination of number of Sub-population (K)

Note: Plot representing distribution of Delta K (ΔK) with the number of subpopulation. ΔK is related to the second order rate of change of the log probability of data with respect to the number of clusters. ΔK clearly revealed the presence of four subgroups among rice accessions. ΔK was calculated from LnP(D) (revealed from STRUCTURE) as per Evanno *et al*, 2005.

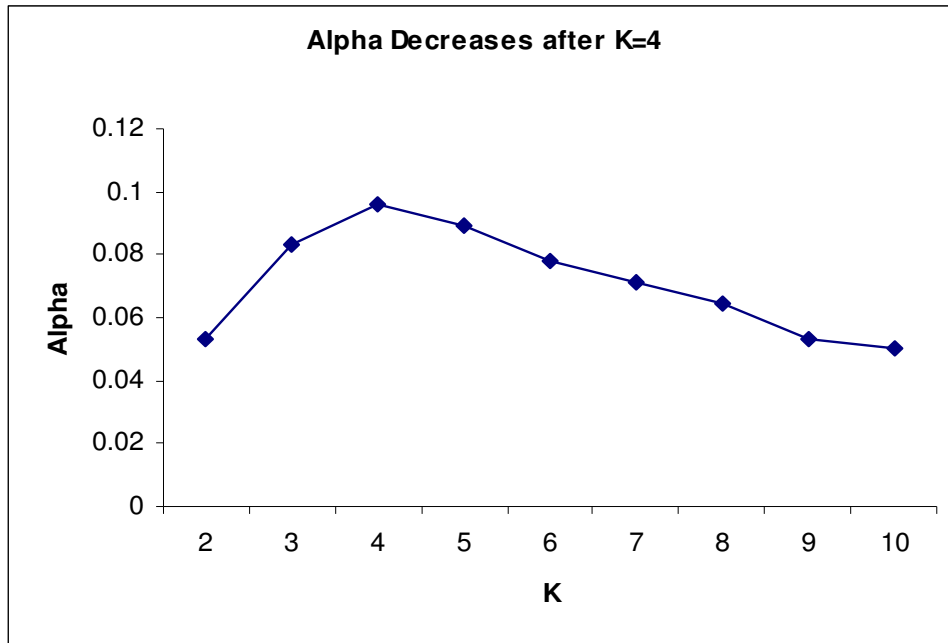


Fig. 24: Degree of admixture (α) present in a range of subpopulation

Note: Scatter plot of α (degree of admixture) vs the number of subpopulation. Plot revealed that the maximum genetic diversity was at $K=4$. α was estimated using the programme “STRUCTURE”.

Table 22: Variation in alpha values in each assumed sub-groups

K	Mean Alpha
10	0.0500
9	0.0533
2	0.0536
8	0.0642
7	0.0713
6	0.0784
3	0.0791
5	0.0892
4	0.0960

Neighbor-joining method is used for structured populations in which each of these subsets has followed its own independent evolution process.

A statistical resampling strategy called bootstrapping was performed while analysing the diversity, the highest possible limit of bootstrap value of 500 was fixed for the diversity analysis in Darwin 5.0. NJ analysis also revealed the presence of four major clusters among the rice accessions. The colour in the phylogenetic tree corresponds to the genetic populations revealed by “STRUCTURE” (Fig 25).

4.11 Determination of admixture among germplasm (Q matrix)

The determination of admixture among the germplasms is crucial for providing the necessary correlations to avoid false positive necessary correlations to avoid false positive associations. Based on the genetic distance and phylogenetic associations, the software “STRUCTURE” computes the admixture matrix called Q matrix. This matrix would be the input for computing marker-trait association using the software TASSEL (Trait analysis by association, evolution and linkage). Among four sub-groups identified by structure analysis (Fig 26), sub-group 1 included highest number of 64 germplasm, while sub-group 2 included the lowest number of 11 germplasm accessions. Sub-group 3 and sub-group 4 included 42 and 34 accessions respectively. The number of rice accessions present in each accessions given in Table 23. A pictorial representation of genomic admixture as revealed by STRUCTURE is given in Fig 27.

4.12 Determination of extent of relatedness (K matrix) among rice germplasm accessions

Association of markers with LD mapping is achieved using advanced statistical algorithms. These methods are designed to avoid erroneous association of markers and traits, the most common errors that can completely destroy the power of association are

- a) Considering a marker as tightly linked with a trait though in reality it may not be the case. This is often referred to as “false positive results”
- b) Rejecting a marker despite its association with a trait in reality. This error is called type II error or “false negative association” is less common than type I error.

Table 23: List of rice germplasm present in each sub-group as revealed by STRUCTURE

Cluster I	Cluster II	Cluster III	Cluster IV
AC-35004, AC-35067, AC-35187, AC-35256, AC-35298, AC-35313, AC-35321, AC-35331, AC-35361, AC-35367, AC-35375, AC-35382, AC-35388, AC-35389, AC-35392, AC-35421, AC-35516, AC-35517, AC-35534-1, AC-39004, AC-39014, AC-39016, BPT-5204, BR-2655, "CHECK-4, CR143-20-2" IET-15924, JAYA, JBT-27/110, JBT-3/60, JBT-36/126, JBT-36/139, JBT-36/3, JBT-37/111, JBT-37/154, JBT-37/170, JBT-37/4, JBT-37/91, JBT-37/92, JBT-38/108, JBT-38/130, JBT-38/19, JBT-38/25, JBT-38/39, JBT-38/88, JBT-39/6, JYOTHI, MTU-1010, PS-221, PS-242, PS-253, PS-267, PS-276, PS-306, PS-307, PS-319, PS-329, PS-339, PS-369, PS-379, PS-406, PS-408, PS-91, SAMRAT, THANU, CHECK-IVANDANA	AC-35611, AC-39002, AC-39010, AC-39012, JBT-36/119, JBT-36/76, JBT-37/118, JBT-37/126, JBT-37/85, PS-250, PS-367	AC-35006, AC-35027, AC-35059, AC-35119, AC-35135, AC-35198, AC-35310, AC-35417, AC-35484, C-35607, AC-39000, AC-39019, JBT-36/14, JBT-36/41, JBT-36/79, JBT-37/100, JBT-37/112, JBT-37/128, JBT-37/134, JBT-37/164, JBT-37/29, JBT-37/5, JBT-37/95, JBT-38/10, JBT-38/119, JBT-38/138, JBT-38/17, JBT-38/26, PS-226, PS-259, PS-273, PS-284, PS-329, PS-332, PS-346, PS-350, PS-353, PS-370, PS-399, PS-419, PS-427, C-2 VAVAPRAVA	AC-35003, AC-35256, AC-35290, AC-35325, AC-35389, AC-35406, AC-35438, IET-16348, IR-50, JBT-36/104, JBT-36/114, JBT-36/64, JBT-37/37, JBT-38/61, PS-224, PS-225, PS-226, PS-229, PS-303, PS-305, PS-316, PS-320, PS-330, PS-336, PS-360, PS-362, PS-366, PS-389, PS-397, PS-398, PS-401, RASI, CHECK-3 SELVIPIKIT.

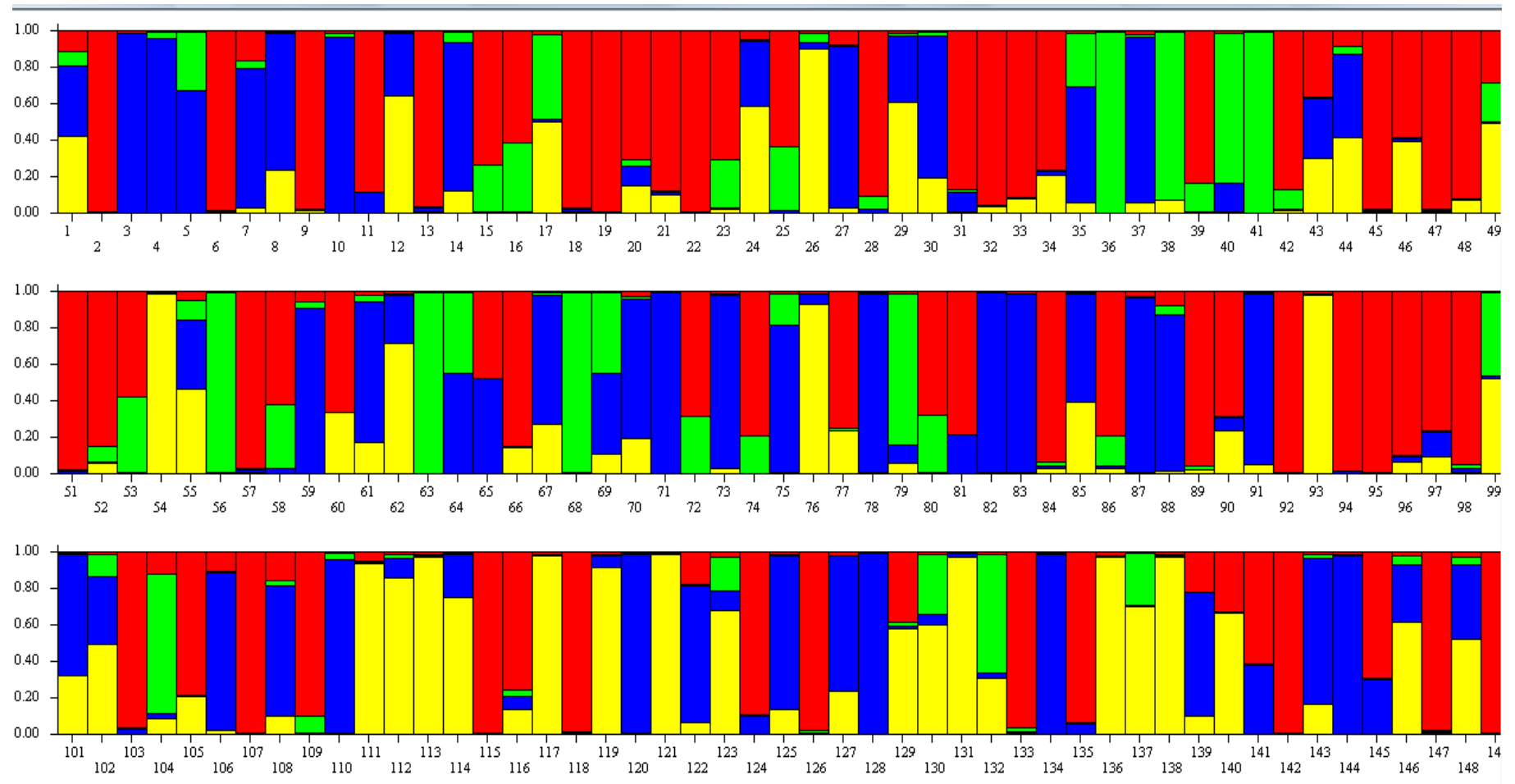


Fig 27: Pictorial representation of admixtures in the rice germplasm accessions at genomic level.

Note: The figure represents the extent of admixture present in individual genotype at genomic level as revealed by SSR markers. The diagram was generated by the software: STRUCTURE”

In marker-trait association studies, avoiding false positive (type I error) is most crucial. However it is proposed to compute the extent of relatedness among the germplasms lines (McArdle *et al*, 2007).

A statistical estimate K is computed measure of relatedness among the germplasms. A software SPAGEDI was employed to compute the K matrix for the panel of rice germplasm. This K matrix and the Q matrix were the major inputs to the TASSEL programme while computing marker-trait association. A multiple linear model was adopted in this study to associate marker with trait

4.13 Assessment of genome wide linkage disequilibrium decay

Linkage disequilibrium (LD) and linkage equilibrium are population genetics terms used to describe the co-occurrence of alleles at different loci in a population. LD is defined as the non-random association of alleles on different chromosomes. Assessment of LD was carried out using squared correlated allele frequency (r^2), which summarises both mutational and recombinational history. The extent of genome-wide LD among the entire set of rice accessions was evaluated through pairwise comparison of 113 SSR loci using Power marker software. This analysis yielded an estimate of 439 pairwise LD values from selected 99 markers. Fig 28 shows the distribution of r^2 (Linkage disequilibrium) values as a function of genetic distance in centimorgans for loci pairs located on entire chromosome. The distribution of data points in the plot of LD (r^2) decay against distance (cM) within the intra chromosomal region (Fig 4.17). Most of the r^2 observed was < 0.15 indicating a moderately less LD decay among rice accessions. Out of all 439 intra chromosomal loci pairs assessed, only 29 loci pairs had r^2 more than 0.15. However, the r^2 values decreased as the genetic distance between loci pairs increased. LD decayed with distance and most of the LD was observed within $r^2 = 0.1$. Average LD blocks of 40 cM to 50 cM over entire genome was observed at $r^2 = 0.1$. An average LD block size of 20 cM was observed with an r^2 of 0.2.

4.14 Association of Markers/QTLs with various physiological traits

The population stratification (Q matrix) and family relatedness (K Matrix) among rice accessions were used for association analysis using the software TASSEL by

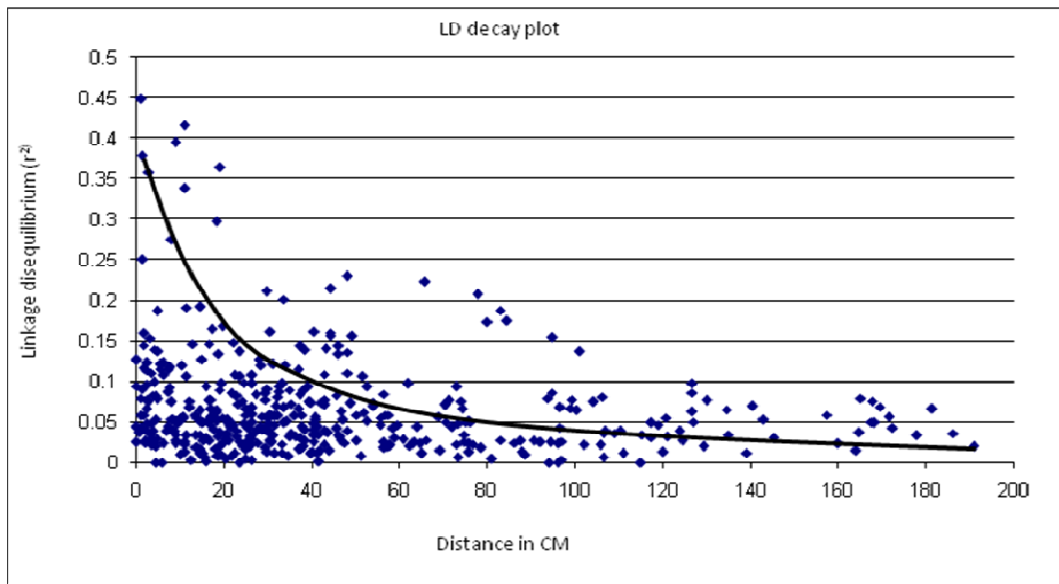


Fig. 28: Extent of average Linkage disequilibrium decay in rice germplasm accessions

Note: Scatter plot of pair wise LD (r^2) vs the genetic distance (cM). The plot suggests that the average genome wide LD decay at $r^2 = 0.1$ is around 40 to 50 cM. LD values were calculated using the programme Powermarker ver. 3.25. Genetic distance of the respective Rice markers was obtained from the database www.gramene.com.

Multi-Linear Model (MLM). In the following section, the results on the association of markers with the traits are presented. Markers which are associated significantly ($P < 0.05$) are selected and analysed.

4.15 Marker associated with various biometric traits

4.15.1 Markers associated with Root traits

A total of 18 markers were associated with various root traits (Table 24). Among them 4 markers were associated with root length, 6 markers associated with root volume and 8 markers with root weight.

Root length

Among markers associated with root length RM223 described the highest phenotypic variance of 27.77 % which was located on the chromosome 2 with a p-value of 0.0046. The marker RM166 explained the least Phenotypic variance of 9.56 % (Table 24).

Root volume

Six SSR markers were associated with root volume explaining a phenotypic variance ranging from 13.06 % in RM319 located on chromosome 1 to 73.17 % in RM224 located on chromosome 11. Lowest p-value was observed in RM135 which explained a phenotypic variance of 33.56 % and was located on chromosome 3. Highest p-value of 0.0456 was documented in RM307 located on chromosome 4 (Table 24).

Root weight

A total of 8 markers were associated with root weight. The marker RM163 described the highest phenotypic variance of 55.46 % located at a genetic distance of 75.4 cM on chromosome 5. In contrast to this marker, the marker RM319 documented the phenotypic variance of 15.22 % on chromosome 1. The marker RM135 recorded least Phenotypic variance of 16.17 % located on chromosome 3 at distance of 157.3 cM whereas the marker RM307 recorded highest p-value of 0.037 on chromosome 2 (Table 24).

Table 24: QTLs associated with root traits like, root length , root weight and root volume

Trait	Locus	Chromosome	Genetic Position (cM)	Allele number	p-value	R ² %
Root Length	RM166	2	183	3	0.0046	9.56
Root Length	RM223	8	80.5	14	0.0012	27.77
Root Length	RM240	2	158	5	0.0124	12.75
Root Length	RM447	8	124.6	8	0.0006	20.73
Root Volume	RM135	3	157.3	3	0.0057	33.56
Root Volume	RM224	11	120.1	4	0.0215	73.17
Root Volume	RM263	2	127.5	14	0.0419	52.82
Root Volume	RM307	4	0	9	0.0456	49.56
Root Volume	RM319	1	150.5	3	0.0452	13.06
Root Volume	RM411	3	127.9	2	0.043	44.5
Root weight	RM135	3	157.3	3	0.0055	16.17
Root weight	RM138	2	196.8	8	0.0166	21.27
Root weight	RM163	5	75.4	13	0.0105	55.46
Root weight	RM240	2	158	5	0.037	18.41
Root weight	RM319	1	150.5	3	0.0181	15.22
Root weight	RM432	7	43.5	5	0.0055	19.88
Root weight	RM435	6	2.2	4	0.0126	16.89
Root weight	RM571	3	205.4	7	0.0134	28.22

4.15.2 Markers associated with biometric traits like Plant height (PH), Total leaf area (TLA), Total leaf weight

The markers associated with various biometric traits are given in Table 25. A total of 5 markers were associated with plant height. The phenotypic variance varied from a minimum of 16.03 % (RM112 on chromosome 2) to a maximum of 64.9 % (RM187 located on chromosome 11). The SSR markers associated with leaf traits are presented in Table 25. A total of 11 SSR markers associated with several leaf traits like Total leaf weight and Total Leaf Area (TLA). Six SSR markers were associated with TLA. Among these markers, RM163 showed a maximum phenotypic variance of 37.69 % with a p-value of 0.0279 and least phenotypic variance of 11.74 % was explained by RM165 with a p-value of 0.0276. RM202 located on 11th chromosome located at genetic distance of 54 cM showed lowest p-value of 0.0069 with a phenotypic variance of 13.93 % whereas the marker RM223 located on chromosome 8 located with at a genetic distance of 80.5 cM showed a highest p-value of 0.0359 explaining the phenotypic variance of 34.61 %. Among five markers associated with TLW, the marker RM163 located on chromosome 5 explained highest phenotypic variance of 50.7 % with a p-value of 0.0408. The marker RM106 with a p-value of 0.0214 explained a least Phenotypic variance of 7.08 % which is located on chromosome 2. Lowest p-value was observed in RM106 whereas highest p-value was observed in RM510 on chromosome 6 which explained a phenotypic variance of 11.22 %. None of the markers were associated with shoot weight and total dry matter.

4.16 Markers associated water use efficiency surrogates like $\Delta^{13}\text{C}$ and SLA

Out of eight markers associated with $\Delta^{13}\text{C}$, five markers explained more than 20 % of the phenotypic variance. The marker RM224 explained the highest phenotypic variance of 73.37 % which was located on chromosome 11 at 120.1 cM with a p-value of 0.0001. Least Phenotypic variance was observed in RM319 on chromosome 1 with a phenotypic variance of 8.03 % with a p-value of 0.0077 located at genetic distance of 150.5 cM. The marker RM224 which explained highest phenotypic variance also

Table 25: QTLs associated with various biometric parameters like plant height (PH), total leaf area (TLA), total leaf weight (TLW)

Trait	Locus	Chromosome	Genetic Position (cM)	Allele number	p-value	R² %
PH	RM112	2	166	2	0.0191	16.03
PH	RM240	2	158	5	0.0455	23.56
PH	RM225	6	26.2	6	0.0152	28.17
PH	RM186	3	168.2	3	0.0378	45.24
PH	RM187	11	79.6	6	0.0275	64.9
TLA	RM202	11	54	3	0.0069	13.93
TLA	RM472	1	171.6	9	0.0212	23.54
TLA	RM165	1	197.8	3	0.0276	11.74
TLA	RM163	5	75.4	13	0.0279	37.69
TLA	RM201	9	81.2	11	0.0292	19.93
TLA	RM223	8	80.5	14	0.0359	34.61
TLW	RM106	2	123.2	2	0.0213	7.08
TLW	RM164	5	80.1	9	0.0373	26.87
TLW	RM192	7	0.8	3	0.0395	8.47
TLW	RM163	5	75.4	13	0.0408	50.7
TLW	RM510	6	20.8	4	0.0492	11.22

PH: Plant height

TLA: Total leaf area

TLW: Total leaf weight

Table 26: QTLs associated water use efficiency surrogates like carbon isotope discrimination ($\Delta^{13}\text{C}$) and Specific leaf area (SLA)

Trait	Locus	Chromosome	Genetic Position (cM)	Allele number	p-value	R² %
$\Delta^{13}\text{C}$	RM224	11	120.1	4	0.0001	73.37
$\Delta^{13}\text{C}$	RM263	2	127.5	14	0.0022	47.86
$\Delta^{13}\text{C}$	RM319	1	150.5	3	0.0077	8.03
$\Delta^{13}\text{C}$	RM141	6	143.7	11	0.0134	53.8
$\Delta^{13}\text{C}$	RM223	8	80.5	14	0.0281	54.31
$\Delta^{13}\text{C}$	RM186	3	168.2	3	0.0296	45.43
$\Delta^{13}\text{C}$	RM178	5	118.5	11	0.0344	19.55
$\Delta^{13}\text{C}$	RM314	6	33.6	10	0.0386	19.56
SLA	RM13	5	30	5	0.0026	13.78
SLA	RM201	9	81.2	11	0.0136	16.96
SLA	RM38	8	28	3	0.0209	8.11
SLA	RM571	3	205.4	7	0.021	41.04
SLA	RM222	9	11.3	3	0.0219	5.9
SLA	RM125	7	24.8	3	0.027	54.73
SLA	RM420	7	115.8	3	0.0361	18.55
SLA	RM228	9	110.7	4	0.0381	8.92
SLA	RM175	3	18.9	3	0.0402	25.73

$\Delta^{13}\text{C}$: Carbon isotope discrimination
SLA: Specific leaf area

explained lowest p-value of 0.0001 whereas the marker RM314 located on the chromosome 6 at a genetic distance of 33.6 cM explained a phenotypic variance of 19.56 % (Table 26). A total of 9 SSR markers were associated with SLA. The microsatellite marker RM125 explained maximum phenotypic variance of 54.73 %, whereas RM222 explained a least Phenotypic variance of 5.9 %. Marker RM13 had the lowest p-value of 0.0026 with a phenotypic variance of 13.78 %. In contrasting to that marker RM 175 had a p-value of 0.0402 with explaining a phenotypic variance of 25.73 %.

4.17 Markers associated with biomass derived parameters DM/LA, R/S and R/LA

A total of 15 markers associated with derived parameters. Among them 7 markers were associated with DM/LA ratio, 2 markers associated with R/S ratio and 6 markers were associated with R/LA ratio. The SSR marker RM2 81 associate with DM/LA ratio described a highest phenotypic variance of 46.66 % ($p = 0.0094$) located on the chromosome 8 at genetic distance of 128.6 cM. The marker RM116 explained the lowest phenotypic variance of 6.35 % located on the chromosome 11 at a genetic distance of 41.4 cM. Similarly the maker RM281 explained a phenotypic variance of 46.66 % with a p-value of 0.0313 on the chromosome 8. Among several markers associated with R/LA ratio the marker RM454 explained a highest phenotypic variance of 25.55 % with a p value of 0.0046. Least phenotypic variance explained by the marker RM136 with a phenotypic variance value of 2.45 % (Table 27).

4.18 Makers associated with gas exchange parameters

All the markers associated with gas exchange parameters like A, g_s , C_i and T are given in the Table 28. Several markers were significantly associated with gas exchange parameters. The marker RM576 described the highest phenotypic variance of 42.7 % with a p-value of 0.0367 located at genetic distance of 52 cM on the chromosome 1. The marker RM444 associated with C_i explaining a phenotypic variance of 45.4 % ($p=0.000004$) located on the chromosome 9 at a genetic distance of 3.3 cM. Only two markers were associated with g_s , the marker RM152 explained the phenotypic variance of 64.4 %. Similarly the marker RM152 explained the phenotypic variance of 44.7 %

Table 27: QTLs associated with biomass derived parameters like Dry matter to leaf area ratio (DM/TLA), root to shoot ratio (R/S) and root to leaf area ratio (R/LA)

Trait	Locus	Chromosome	Genetic Position (cM)	Allele number	p-value	R² %
DM/LA	RM116	11	41.5	2	0.0094	6.35
DM/LA	RM228	9	110.7	4	0.0458	8.98
DM/LA	RM165	1	197.8	3	0.006	9.95
DM/LA	RM225	6	26.2	6	0.0389	12.25
DM/LA	RM141	6	143.7	11	0.034	16.54
DM/LA	RM178	5	118.5	11	0.003	20.52
DM/LA	RM351	7	95	11	0.0066	24.43
R/S	RM154	2	4.8	12	0.0012	55.59
R/S	RM281	8	128.6	6	0.0313	46.66
R/LA	RM165	1	197.8	3	0.00008	14.58
R/LA	RM454	6	99.3	10	0.0046	25.55
R/LA	RM224	11	120.1	4	0.0062	10.58
R/LA	RM154	2	4.8	12	0.0206	19.61
R/LA	RM166	2	183	3	0.0208	6.46
R/LA	RM136	6	51.2	2	0.0575	2.45

DM/LA: Drymatter to Leaf area ratio

R/S: Root to shoot ratio

R/LA: Root to leaf area ratio

Table 28: QTLs associated with gas exchange parameters like assimilation rate (A), stomatal conductance (g_s), and intercellular CO₂ concentration (Ci) and transpiration rate (T)

Trait	Locus	Chromosome	Genetic Position (cM)	Total Alleles	p-value	R² %
A	RM106	2	123.2	2	0.0044	8.3
A	RM152	8	9.4	3	0.0256	9
A	RM303	4	116.9	11	0.0279	30.56
A	RM241	4	106.2	4	0.0283	12.45
A	RM235	12	120.1	4	0.0308	14.86
A	RM576	1	52	11	0.0368	42.67
A	RM119	4	76.1	3	0.0477	6.87
Ci	RM106	2	123.2	2	0.0008	8.34
Ci	RM184	9	58.3	2	0.0219	11.77
Ci	RM187	11	79.6	6	0.0026	18.61
Ci	RM207	2	191.2	13	0.0328	35.86
Ci	RM263	2	127.5	14	0.0192	20.71
Ci	RM444	9	3.3	16	4E-06	45.39
g_s	RM5	1	94.9	4	0.0393	64.42
g_s	RM501	1	32.4	13	0.0192	27.66
T	RM152	8	9.4	3	0.0095	44.68
T	RM501	1	32.4	13	0.0183	28.59

Ci – Intercellular CO₂ concentration (ppm)

A – Assimilation rate ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)

T – Transpiration rate ($\text{mmol m}^{-2} \text{ s}^{-1}$)

g_s - Stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$)

Table 29: QTLs associated with derived gas exchange parameters like, A/T, A/gs, Fv'/Fm' and Ci/gs

Trait	Locus	Chromosome	GP (cM)	No of alleles	p-value	R² %
A/gs	RM13	5	30	5	0.0108	13.06
A/gs	RM178	5	118.5	11	0.049	16.04
A/gs	RM209	2	32.7	8	0.0127	27.43
A/gs	RM221	2	143.7	4	0.0108	25.12
A/gs	RM303	4	116.9	11	0.0291	60.8
A/gs	RM9	9	92.4	6	0.0053	17.38
A/T	RM455	7	65.7	5	0.0231	14.83
A/T	RM6	2	154.7	4	0.0419	9.95
Ci/gs	RM114	3	203.1	11	0.0046	46.65
Ci/gs	RM175	3	18.9	3	0.0428	6.54
Ci/gs	RM303	4	116.9	11	0.0004	49.97
Ci/gs	RM444	9	3.3	16	0.000004	75.37
Fv'/Fm'	RM503	3	153.9	7	0.014	9.15

GP: Genetic position

A/T – Ratio of Assimilation to transpiration rate ($\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$)

A/gs – reflection of WUE at single leaf level ($\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$)

Ci/gs – reflection of mesophyll efficiency at single leaf level

Fv'/Fm' – Light adapted quantum efficiency

with a p-value of 0.0095. Totally 12 markers were associated with the gas exchange derived parameters like A/g_s , A/T and Ci/g_s . Among these markers 6 were associated

with A/g_s and 4 markers were associated with Ci/g_s and only two markers were associated with A/T . The markers RM303 explained the highest phenotypic variance of 60.8 %. Similarly the marker RM444 explained the highest phenotypic variance (75.4 %) for Ci/g_s located on the chromosome 9 with a p-value of 0.000006 (Table 29). Among the two markers associated with A/T the marker RM455 explained the highest phenotypic variance of 14.8 % which was located on the chromosome 2 at genetic distance of 65.7 cM.

Discussion

V. DISCUSSION

More than 80 % of the rice is grown and consumed in Asia, wherein China and India significantly contribute both in terms of area and production. However, to achieve food security in India, rice production should increase to 128 million tons in 2025 from the present production of 98 million tons, with limited scope for expanding area under rice cultivation. It is estimated that the unit area productivity needs to be increased from 1930 kg ha⁻¹ at present to 3000 kg ha⁻¹ in the next decade. The annual growth rate of cereal production in India needs to be increased from 0.66 % to 1.92 % in order to meet this demand.

Cultivation of rice is a water intensive process requiring 3000 to 5000 litres of water to produce once kg of grain. With the looming crises for irrigation water, developing strategies to reduce water requirement of rice is a national agenda. This has been the driving force for developing several water saving strategies including transplanted rice, direct sown rice (wet seeded), alternate wetting and drying method (AWD), system of rice intensification (SRI) and aerobic rice. However SRI method and AWD also required puddle condition before transplanting leading to water loss. The concept of aerobic rice has been developed to suit for water limited areas. Aerobic rice cultivation where fields remain unsaturated throughout the season like an upland crop offers an opportunity to produce rice with less water. This practice has the potential to save 20 % to 50 % water depending on the soil type. However this water saving in aerobic practice is concomitantly associated with a yield penalty up to 20 – 50 % (Bouman *et al.*, 2002). This necessitates the development of suitable rice cultivars that show less yield penalty under aerobic condition. Though direct selection for yield is expected to lead to the identification of such cultivars, a very narrow variability in yield among existing cultivars may severely limit the possibility of identifying aerobic suitable rice cultivars. From the ongoing global efforts it is apparent that specific component trait needs to be improved for evolving suitable rice cultivars for aerobic conditions.

A number of physio-morphological traits are exploited in improving drought resistance (Yu *et al.*, 1995). Among these traits, active accumulation of compatible solutes antioxidant agents, heat shock proteins, molecular chaperones, Leaf expansion, parahelionastic movements, development of pubescence, rapid phenological

development, sensitive stomata etc have been studied extensively. But all these traits have only survival significance and are largely counterproductive. Such types of traits which have significance only under stress conditions are called “Adaptive traits”. Genotypes which have adaptive traits can maintain higher leaf water potential and relative water content under water limited condition and hence can tolerate stress better. On the other hand “Constitutive traits” are inherent traits expressed all the times irrespective of the stress occurrence. Constitutive traits such as root traits, water use efficiency, water conservation, cellular level tolerance, flowering time, and stay green traits can be routinely screened without a drought challenge. These traits have implications for drought protection and survival (Kamoshita *et al* 2008).

These constitutive traits besides improving drought tolerance, significantly contribute to growth rates under any given condition. Therefore exploiting such traits has great relevance in improving productivity under water limited conditions (Udayakumar and Prasad, 1994; Sheshshayee *et al*, 2003). The water saving aerobic cultivation practice has intervals of period when water can be limiting. To increase growth rates under such conditions, abilities to mine water associated with roots and WUE becomes crucial.

A more comprehensive improvement of growth rates can be achieved only when constitutive traits are introgressed on to a genetic background with reasonably high cellular level tolerance. In other words, it is important to pyramid “constitutive QTL” on to a background of superior adaptive traits. Clearly, the challenge of evolving rice cultivars with high yield potential under aerobic conditions appears to be a formidable approach. Besides water availability, a few other factors further exacerbate crop growth rate under aerobic condition. Availability of nutrients, especially Iron (Fe), Zinc (Zn), and Nitrogen (N), and Phosphorous (P) significantly reduce growth rates under aerobic conditions (Dobermann and Fairhurst, 2000; Ponnampereuma, 1975).

As per the Passioura model, crop growth rate is a function of the ability of the plant to harness water from deeper soil profile to meet the evaporational demand of the plant (Roots) and the efficiency of the plant to use water for biomass production (WUE) (Passioura, 1986, 1977). Though plants have evolved naturally increase WUE, it is generally achieved through a reduction in transpiration, because of a strong link between

transpiration and photosynthesis, any reduction in transpiration would have an adverse effect on growth rates (Richards *et al* 2002, Sheshshayee *et al*, 2003; Condon *et al*, 2004). Hence increase in WUE must be achieved without a substantial reduction in transpiration. For comprehensive improvement in rice productivity under aerobic conditions requires introgression of both WUE and root traits on to a simple elite genetic background. The advantage of these constitutive traits can be that achieved only under a reasonably high cellular level tolerance. The yet another constraint for crop growth under aerobic condition is the non availability of a few essential nutrients like Nitrogen (N), Phosphorous (P) and Iron (Fe). Thus, evolving a rice variety to sustain productivity under aerobic condition, diverse traits need to be pyramided to a single background.

However, all these traits are quantitatively inherited polygenic traits and their introgression can be effectively achieved only through a focussed molecular breeding approach. Conventionally DNA based molecular markers flanking QTL are discovered using a bi-parental mapping population segregating for a specific trait. Since diverse trait are needed for improving drought tolerance, the bi-parental linkage mapping strategy become very cumbersome and laborious. Thus attempts to increase root traits and WUE have great relevance.

Besides genetic variation, consistency of the trait across seasons and or environment is extremely essential for achieving success in crop improvement. The phenotype is a dynamic interaction between the genotype and environment (G× E). Most of the traits are highly heritable in terms of broad sense. Broad sense heritability is defined as the ratio of genotypic variance to phenotypic variance. However phenotypic variance includes both additive as well as non-additive variance. Non-additive variance is comprised of dominance and epistatic effect which are not inherited. Thereby estimating only broad sense heritability among the rice accessions trait donor lines is not sufficient to confirm and validate the traits. A set of selected genotypes (Appendix III) were raised once again in root structure during 2009 (Raju, 2009) to verify the consistency of traits. Comparison of specific traits among a set of contrasting lines revealed a significant consistency for $\Delta^{13}\text{C}$, root biomass, etc (Fig 19).

The precision with which QTL can be located depends, among other factors, on the number of meioses that are sampled. In populations of lines derived from F₂s and backcrosses, unless very large numbers of lines are grown, the number of meiosis can be

quite small. As a result, particularly for traits of lower heritability and for smaller QTL effects, this precision is often poor. Recent effort in human genetics has, therefore, looked for alternative methods to locate genes. A method showing great promise is to exploit unobserved, historical recombination's through linkage disequilibrium mapping, also called association mapping, or association genetics.

5.1 Association mapping/Association genetics

Association mapping relies on historical events, particularly mutations and population bottlenecks, to generate strong associations between QTL and markers. Over successive generations, these associations decline at a rate proportional to the recombination fraction between them, such that in the present, strong marker-trait associations imply a close proximity of the marker to a QTL.

Association mapping without consideration of population structure would give a high rate of false positive type-I errors (Mather *et al.* 2004). A unified mixed-model approach to account for multiple levels of relatedness simultaneously, as detected by genetic markers, has resulted in improved control of both type-I and type-II error rates (Yu *et al.* 2006). Structured population and relatedness characteristics in Korean assayed rice landrace accessions suggest the need to consider population structure and kinship in conducting population-based association mapping in rice germplasm (Pritchard *et al.* 2000; Yu *et al.* 2006). Hence, an attempt was made to identify QTLs conditioning drought traits using mixed linear model (MLM) approach of Yu *et al.* (2006), considering both population structure (Q) and kinship (K) to eliminate possible spurious associations.

To achieve the envisaged objective of closely associated makers with complex physiological traits, 204 diverse rice germplasm accessions (*Oryza sativa L sp.indica*) were procured from the Central Rice Research Institute (CRRI). Since *indica* sub-species processes more genetic variability than *japonica* sub-species (Negrao *et al.*, 2008). Further association analysis within the species results in highest power to detect QTLs by reducing population stratification (Pritchard, *et al.* 2000). Due to these reasons only *indica* sub-species types were used in the study.

5.2 Molecular Characterisation and assessment of genetic diversity among rice germplasm accessions

Diverse rice accessions were genotyped with 113 polymorphic SSR markers. SSR markers being co-dominant and multi-allelic in nature are the most suited marker system for assessment of genetic diversity and association analysis. (Agrama *et al*, 2007, Semon *et al*, 2005; Cockram *et al*, 2008; Stich *et al*, 2005).

Distribution of SSR markers on the entire genome is necessary for accurate discovery of population structure. Keeping this in view markers were selected from gramene database so that they are distributed evenly on all the chromosomes. Genotyping analysis revealed an average marker number of 10.27 on each chromosome except on chromosome 10 with an average 5.92 alleles per locus. A similar allelic frequency was also reported in rice by Reif *et al*, 2005 (5.9 alleles). However, average number of alleles per locus in the present study was lower than reported by Agrama *et al*, 2007 (8.2). The marker RM317 showed maximum 19 alleles per locus. Least number of alleles of 2 per locus was observed in RM411. Total gene diversity of the entire rice germplasm accessions in our survey was 0.55 (Table 20). Similar kind of analysis was carried out by Agrama *et al*, 2007, which revealed a total gene diversity of 0.635 among diverse rice germplasm which consists of various *japonica*, *indica* and Aus types. Though, the total gene diversity in the present study was low compared to the above studies, the gene frequency reported in our investigation is the first of its kind in *indica* types. The molecular diversity within the *indica* is expected to be much lower compared to the diversity across all *oryza sativa* sub-species. Despite this, the gene frequency among *indica* types studied revealed a considerably high gene frequency.

Another measure of diversity among the population is the extent of polymorphism information content value (PIC) of markers. (Smith *et al.*, 1997). In the present study the average PIC value was 0.51 suggesting that sufficient genetic diversity exists among the rice germplasm accessions. The molecular diversity indicated that the set of germplasm represents sufficient variation and can be exploited for association analysis. A low level of heterozygosity (0.06) further reiterated the inference that the molecular diversity can be effectively exploited as most loci on the genome existed in the homozygous condition (Table 20)

5.2.1 Identification of population structure

Existence of genetic diversity among the population is a primary requirement for accurate association mapping based on Linkage disequilibrium. But collection of such diverse materials results in high level of population structure as well as familial relatedness (Yu *et al.*, 2005). Each sub-population is known to have distinct allele frequency caused by unique genetic and social history. This asymmetrical distribution of allele frequency between sub populations result in increased Type I error (false positive) and type II error (false negative) rates (Cardon and Palmer, 2003). These type I error is further confounded by the presence of large number of admixtures (Pritchard *et al.*, 2000). The programme STRUCTURE implements a model based bayesian clustering method for inferring population structure using genotyping data consisting of unlinked markers (Pritchard *et al.*, 2000). Estimated Log probability of Data obtained from structure although represents the extent of genetic diversity existing in the population and the model does not assume a particular mutation process and, in most cases, the estimated 'log probability of data' does not provide an accurate estimation of the number of clusters, K (Evanno *et al.* 2005) (Fig 22). In the present study also the distribution of estimated 'log probability of data' with the number of sub-populations did not show a clear mode for the true value of K . But ΔK did show a clear mode for the true value of K at $K=4$ (Fig 23) (Evanno *et al.*, 2005). This result was confirmed with the distribution of Alpha with K which also indicated the number of sub-population was four (Fig 24). The neighbour joining based tree clustering also indicated the presence of four sub-groups among the population (Fig 25). The relatively small value of alpha ($\alpha = 0.071$) indicated that most accessions originated form one primary ancestor, with few admixed individuals (Table 22). The analysis revealed that some accessions with partial ancestry probably had a complex breeding history involving intercrossing and introgression between germplasm from diverse backgrounds, overlaid with strong selection pressure for abiotic and biotic (Mather *et al.*, 2004). Model-based analyses of population structure, such as those conducted here, may be helpful in providing information that could be incorporated into association mapping analyses. A Bayesian clustering analysis using STRUCTURE software carried out by Semon *et al.*, 2004 indicated the presence of at least five cryptic sub-populations among 198 accessions of *Oryza glaberrima*. Similar reports were given by Yan *et al.*, (2009), the model based programme STRUCTURE identified four sub-

groups among a core collection which consists of 1,794 accessions collected from 114 countries.

5.2.2 Allelic diversity using neighbour joining method

In neighbour joining method the distance between clusters are determined by the distance of the two closest objects in the different clusters. In the present study tree clustering using Neighbour joining method revealed the presence of four sub-groups among the germplasm accessions indicating the presence of sufficient genetic diversity among the population. This result confirms the result revealed by model based analysis by “STRUCTURE”. The colours in the cluster tree correspond to the population structure identified by “STRUCTURE”. Ni *et al*, (2002) assessed the differences in pattern of diversity within the two rice sub-species *indica* and *japonica* and UPGMA based cluster analysis clearly differentiated the sub-species to *indica*, *tropical japonica*, *temperate japonica* and wild species.

Diversity assessment based on both distance based and model based analysis revealed the population structure of rice accessions as four. Using population structure with K=4, the rice accessions were significantly differentiated to four subgroups using structure software (Fig 26). The inferred Q-matrix from structure was further used as a correction factor for the confounding effect of population structure (Type I error) in association studies.

Association analysis among structured population is highly prone for false positive association (Type I error). This is one of the major drawbacks of association analysis, which occurs because of elevated confounding population structure. An ideal population for association should have minimal population structure or familial relatedness thus resulting in greatest statistical power, provided that the trait of interest is well distributed among the population (Yu *et al*, 2005). In this regard in the present study AMOVA was carried to reveal the diversity among the sub-groups as revealed by structure. AMOVA explain a variation of 8% among the clusters and a variation of 92% within the clusters, thus indicating the suitability of rice accessions for association mapping

Successful mapping of genes requires suitable modelling of genetic effects. Such modelling is often performed within a regression framework where the effect of genotype on phenotype can be assessed. Such modelling strategy becomes more complicated when the study sample includes families and the trait is correlated among related individuals by virtue of their shared genetic background. Use of any association models ignoring familial relatedness would lead to false positive results (type I error) (McArdle *et al*, 2007).

5.3 Linkage disequilibrium Decay plot

Successful MAS depends upon the frequency of crossing over between the marker and the QTL. If the frequency of crossing over between the marker and QTL is high, there is a high likelihood of breakage of association between the marker-trait association is also high leading to failure of MAS. Therefore identification of genomic regions which are segregated without any recombination within the region in which the marker and the QTL are localized has a great relevance in association mapping. This chromosomal region which is inherited is called LD block. However, these LD blocks decays with generation and varies from one population to other. Identification of these LD blocks in rice germplasms accessions is of a great interest in association mapping which simplifies the LD mapping efforts of complex traits. LD blocks are very useful in association mapping when sizes are calculated. Furthermore, identification of LD block size also suggests the needs for the minimum number of markers to efficiently cover the genome-wide haplotype blocks in association mapping.

To estimate the size of these LD blocks, the r^2 values (alternatively, D' can also be used) usually plotted against the genetic (cM) or weighted (bp) distance referred to as a “LD decay plot”. Extent of LD decay in the population gives a measure of marker resolution in association mapping. D' and r^2 are the most common measures of LD (Gupta *et al*, 2005). r^2 , represents the square of the correlation coefficient between the two loci, has more reliable sampling properties than D' in cases with low-allele frequencies (Abdurakhmonov and Abdugarimov 2008). The r^2 is affected by both mutation and recombination, while D' is affected more by mutational history (Gaut and Long 2003; Gupta *et al*. 2005). In the sets of rice landraces from Korea that were examined here, linkage disequilibrium as r^2 , which is also an indication of marker–trait

correlations, is the most appropriate LD quantification measure for association mapping (Abdallah *et al.* 2003; Gupta *et al.* 2005).

Knowledge of rate of LD decay with physical/genetic distance is necessary to detect significant associations between marker polymorphisms and particular phenotypes (Remington *et al.*, 2001; Pritchard *et al.*, 2000). In the present study the average intra-chromosomal LD decayed around 40-50 cM at $r^2 = 0.1$ (Fig 28). This indicates that adequate level of LD persists among rice germplasm accession. However, many other studies have reported a lower LD values between 20 and 30 cM, suggesting it should, nevertheless, be possible to achieve resolution down to the 25 cM level in germplasm lines (Agrama *et al.*, 2007). Long range LD has also been reported in many other self pollinated species like rice and Barley. Malysheva-Otto *et al.* (2006) reported an extensive intra-chromosomal LD up to 50 cM at $r^2 = 0.05$ in a collection 953 diverse barley accessions. While a LD of 4 cM persists in cross pollinated crops such as Sorghum (Jun *et al.*, 2008). In some local populations of arabidopsis a long distance LD has been reported up to 50-100 cM at $r^2 = 0.2$. (Nordborg *et al.*, 2002). Kraakman *et al.*, 2004 reported a long range of LD extending up to 100 cM ($r^2 = 0.1$) among European two-row spring barley populations. Many other studies have examines the rate of LD decay within the gene (Semon *et al.*, 2004) as well as whole genome LD (Garris *et al.* 2003; Semon *et al.* 2005; Rakshit *et al.* 2007; Mather *et al.* 2007; Agrama *et al.* 2007; Agrama and Eizenga 2008).

The major objective of association mapping is to identify simply inherited markers that are in close proximity with the trait of interest through LD based association mapping strategy. The software TASSEL was used for identification of marker-trait association. Mixed Linear Model (MLM) was used for association which incorporates both Q matrix as well as K matrix. Analysis of marker-trait association using TASSEL resulted in several tightly associated markers.

The conceptual frame work provided by Passioura emphasized the importance of total transpiration, WUE, and Harvest index as the most important physiological traits that determine crop growth and productivity. The diverse germplasm accessions were extensively phenotyped to assess the variability in these traits. Significant variation in these traits was noticed among germplasm lines (Table 13, 14, 15, 16). These traits also

showed significant consistency across seasons (Fig 19). The mean phenotypic value of the two experiments was used to generate marker-trait association by LD mapping. Several markers showed significant association with the traits. While the total of 17 markers was associated with root traits, 8 markers associated with $\Delta^{13}\text{C}$, a surrogate for WUE. The marker RM 224 located on the chromosome on 11 at a distance of 10.1 cM explained a maximum phenotypic variance of 72.1 % with a p-value of 0.02 for root volume. Interestingly the same marker RM 224 also showed strong association with $\Delta^{13}\text{C}$ explaining a phenotypic variance of 73.41 %. The marker RM 209 located on the chromosome 2 at a distance of 32.7 explained a variance of 75 % for A/T. the ratio of assimilation to transpiration (A/T) is often considered as a rapid estimation of WUE at single leaf level.

Root phenotyping is generally considered as a tough challenge. Assessing variation in root traits is generally achieved by growing plants in hydroponics (Tuberosa *et al*, 2002) and pipes (Kanbar *et al*, 2002; Ayappa, 2004; Giuliani *et al*, 2005). These two strategies have serious limitations and hence may not represent a proper phenotypic expression. We developed a novel strategy of raising the seedling in specially designed root structures. Since the plants were grown under the normal recommended population, the phenotypic expression was close to natural condition and hence it is of great significance. The entire set of germplasm was phenotyped in these root structures to reveal the genetic variability.

Conventionally markers flanking the QTL region governing the variation in these traits were accomplished by using bi-parental mapping population and linkage analysis segregating for the trait of interest (Impa, 2006; Impa *et al*, 2005; Impa *et al*, 2003, Venuprasad *et al*, 2002; Courtis *et al*, 1995; Xiao *et al*, 1996; Zhuang *et al*, 1997 and Girishkumar, 1998).

One of the major factors that determine the success of molecular breeding is the association of marker with the trait of interest. The strategy adopted in the investigation represents a precise phenotyping strategy; hence the marker identified would be potentially useful in Marker Assisted Breeding (MAB). These markers are further being validated.

The major advantage of phenotyping lies in its ability to identify superior trait donor genotype. It can be either used as parents for crossing in conventional plant breeding programmes or as recipients of validated genes through transgenic approach.

5.4 Overlapping markers among traits

The other major advantage of phenotyping the germplasm is the discovery of contrasts for specific traits. Such contrasts can be effectively used for developing trait specific mapping population. Besides they also serve an excellent material for understanding the molecular and physiological basis of variation in the traits. These contrasts would also frame the basis for the subsequent discovery of genes through allele mining. Some of these contrasts and promising trait donors are given below (Table 30).

Association analysis accounts for the allelic variation in the germplasm. The marker identified by through this strategy would be robust and can be effectively used for trait introgression through QTL pyramiding.

In the present study some of the markers co-segregated with several important physiological traits. RM223 co-segregated with root length, $\Delta^{13}\text{C}$ and TLA. Similarly RM163 co-segregated with Root weight, TLW and TLA. RM319 co-segregated with Root volume, Root weight and $\Delta^{13}\text{C}$ (Table 32). Similarly overlapping markers were also reported by Impa (2006). She found an AFLP marker TC_CAG5 to be overlapping for WUE at single leaf level as well as whole plant level. Several overlapping markers were identified for WUE at whole plant level and $\Delta^{13}\text{C}$ in doubled haploid population of Rice (Nadaradjan *et al.* 2005). A co-dominant marker RM110 was identified in rice which was found to be associated with TDM in both single marker analyses as well as in composite interval mapping (Shashidhar G Parsi, 2009).

5.5 Markers associated with the various traits in previous study

Although several markers have been identified using QTL mapping strategy, not many of them are used in Marker assisted selection. The major constrain for this is the low resolution of the marker with the QTL. The QTLs identified with low resolution

Table 30: Trait donor lines identified for trait introgression

Trait	Genotypes	Average value	Population Mean of 2007 and 2008 experiment
Roots	AC-35310; AC39020; IET 16348; PS-339; JBT 36/14	10.49	7.15
WUE	AC39020; JBT 37/4; IET 16348; JBT-36/14; AC-35310	19.58	19.7

Table 31: Markers associated with root and other morphological traits in previous studies

Marker	Present study	Previous studies	Reference
RM166	Root Length	Root Number	Hemamalini <i>et al</i> , 2000
RM223	Root Length	Root Length	Nguyen <i>et al</i> , 2003
RM223	Root Length	Root dry weight	Nguyen <i>et al</i> , 2003
RM243	Root Length	Root dry weight	Xu <i>et al</i> , 2004
RM447	Root Length	Root Thickness	Hemamalini <i>et al</i> , 2000
RM224	Root Volume	Root Length and Root Dry weight	Xu <i>et al</i> , 2002
RM224	Root Volume	Root Dry weight	Lian <i>et al</i> . 2005
RM224	Root Volume	Root to shoot ratio	Xu <i>et al</i> , 2004
RM224	Root Volume	Root activity	Yan <i>et al</i> , 2003
RM224	Root Volume	Root dry weight and branching	Horii <i>et al</i> ,2006
RM263	Root Volume	Root thickness	Kamoshita <i>et al</i> , 2002
RM138	Root weight	Root number	Hemamalini <i>et al</i> , 2000
RM168	Root weight	Root activity	Yan <i>et al</i> , 2003
RM240	Root Weight	Root weight	Xu <i>et al</i> , 2004
RM163	TLW	Leaf width	Mei <i>et al</i> , 2003
RM13	SLA	Leaf length	Mei <i>et al</i> , 2003
RM201	SLA	Leaf area	Cui <i>et al</i> , 2003
RM223	TLA	Leaf width, leaf length	Cui <i>et al</i> , 2003
RM187	Shoot length	Plant height	Akagi <i>et al</i> , 1996
RM225 RM240,	Shoot length	Plant height	Yang <i>et al</i> , 2003

tend to break the linkage between the marker and the QTL when used across the population. The low resolution QTLs are also known to bring substantial amount of linkage drag. Therefore there is a need to identify high resolution QTLs. Keeping this in view, a survey was undertaken to search for markers which have been consistently associated with the similar QTLs in previous studies.

In the present study more than a few markers associated consistently with several important physiological traits. Of the total 96 markers associated with several traits, 15 of them consistently associated with one or more similar traits in previous studies. Interestingly one of the marker RM224 which is associated with Root volume, $\Delta^{13}\text{C}$ and 50% flowering in the present study associated with root traits in previous five other independent studies (Table 31). The marker RM224 can be putative marker and has emerged as a potential marker used for crop improvement through Marker assisted selection.

Table 32. Overlapping markers for several important physiological traits

Trait	Locus	Chromosome	Genetic Position (cM)	p-value	R² %
Root weight	RM163	5	75.4	0.0105	55.46
Total Leaf weight				0.0408	50.7
Total Leaf Area				0.0279	37.69
Root Length	RM223	8	80.5	0.0012	27.77
Delta				0.0281	54.31
Total Leaf Area				0.0359	34.61
Root Length	RM240	2	158	0.0124	12.75
Root weight				0.037	18.41
Shoot length				0.0455	23.56
Root Volume	RM319	1	150.5	0.0452	13.06
Root weight				0.0181	15.22
Δ¹³C				0.0077	8.03

Summary

VI. SUMMARY

The advantage of water saving aerobic cultivation practice can be best exploited only when suitable rice genotypes are evolved. Taking the leads from the enormous amount of research information accruing in the literature, it is evident that rice genotypes that have the ability to harness water from deeper soil profile, efficient use of water for biomass production and cellular level tolerance (CLT) have great relevance for improved productivity under aerobic cultivation. It is also evident that for a comprehensive improvement in productivity, it is essential to pyramid several of these traits in to a single genetic background. Each of these traits is quite complex and have polygenic inheritance. This has rendered conventional breeding for trait introgression almost impossible. Considering the inevitability of trait pyramiding, a highly focused molecular breeding is the most important need of the hour.

Though markers linked with traits are conventionally discovered using linkage mapping strategy, this approach would be highly cumbersome and laborious in achieving the objective of introgression of several drought tolerance traits. The power of association mapping is being exploited for plant trait improvement in recent years. This strategy involves the use of diverse germplasm accessions, which would have undergone infinite recombinations. The major advantage of association mapping involves increase in the power of mapping besides resulting in higher resolution of map distance. However for a successful marker - trait association through LD based association mapping it is essential to determine the relatedness among rice accessions.

With this background, the major focus of the present study was to assemble a set of diverse germplasm accessions and determine the population structure. Further the other major goal of the investigation was to identify robust markers associated with drought tolerance traits such as roots, WUE, etc through LD based association mapping.

Rice accessions revealed a significant genetic variability for roots, WUE ($\Delta^{13}\text{C}$). Further significant genetic variability in WUE at single level was also observed. A significant consistency for these traits between seasons indicated that the traits are stable

and are hence suitable for selection. Most of the traits showed continuous variability indicating the quantitative nature of the traits.

Although association mapping has very high resolution, the major disadvantage is the very high rate of False positive associations (type I error rates) caused by the confounded effects of population structure. To reduce the Type I error, Pritchard *et al.*, (1999) developed “Structured Associations” (SA) using the programme STRUCTURE. Further, recently, Yu *et al.* 2007 developed new methodology, namely the a mixed linear model (MLM) that combines both population structure information (Q-matrix) and level of pair wise relatedness coefficients - “kinship” (K-matrix) in the analysis.

A set of diverse rice germplasm accessions were genotyped with 113 co-dominant, multi-allelic SSR markers. Sufficient genetic diversity among the rice germplasm accessions was noticed indicating the suitability of the population for association mapping. The average Linkage disequilibrium (LD) blocks in the germplasm was revealed by plotting intra chromosomal LD with genetic distance (cM). An LD block of size 40 – 50cM was identified by LD decay plot. Model based Population structure analysis was carried out using both distance based approach and model based approaches. Population structure analysis revealed the presence of four subgroups in the population which was confirmed by distance based analysis using neighbour joining method. Q-matrix was calculated assuming four subgroups. K-matrix was calculated using SPAGEDI software according Ritland, (1996). Both Q-matrix and K-matrix were used as inputs for assessing marker-trait association using the software TASSEL by Mixed Linear Model (MLM model).

Marker-trait analysis using TASSEL revealed several DNA markers associated with different traits. Some of them co-segregated with more than one trait. . RM223 co-segregated with root length, $\Delta^{13}\text{C}$ and Total leaf area. Similarly RM163 co-segregated with Root weight, Total leaf weight and Total leaf area. RM319 co-segregated with Root volume, Root weight and $\Delta^{13}\text{C}$. One of the ways to validate the marker is to search for markers which have been consistently associated with the similar QTLs in previous studies. This study revealed several markers which were consistently associated with same or similar trait in previous studies. Interestingly, one marker RM224 associated

with Root volume and $\Delta^{13}\text{C}$ in the present study associated with similar root traits in previous five other independent studies.

The salient findings can be summarized as follows

- Significant genetic variability in drought tolerance trait was revealed among the rice germplasm lines.
- Most of these traits showed consistency across seasons indicating a strong genetic control over the trait. However, presence of normal distribution of these traits indicated a polygenic Quantitative inheritance.
- The promising lines identified can be used as trait donor lines to transfer specific trait into a recipient genotype.
- The contrasting lines will be useful in developing trait specific mapping population as well as for greater understanding of molecular regulation of these traits.
- Population structure of rice germplasm accessions was revealed indicating the presence of four sub-groups among the population.
- QTLs have been identified for various physiological traits including Root traits and $\Delta^{13}\text{C}$ an important trait under aerobic conditions.
- Several overlapping markers have been identified for physiological traits.

Future Line of Work

The present investigation involves the extensive characterization of a diverse set of *indica* rice genotypes for variation in physiological traits relevant for drought tolerance such as roots, WUE, etc. Furthermore the genotype was characterized for molecular diversity using SSR markers

The major outcomes of the investigation are

- 1) Contrasting genotypes varying in specific traits
- 2) Validated trait donor genotypes
- 3) Genome wide LD decay
- 4) Marker-trait association by LD mapping

The investigations leading to the identification of molecular markers associated with specific drought tolerant traits, is the first of its kind in *indica* species. This study paves the way for several investigations which have both basic research and translational research potential.

- The contrasting genotypes differing in specific traits forms the basis to develop greater understanding on the factors that cause the variations in the trait both at the physiological level as well as molecular level.
- A number of genes regulating root growth, leaf epidermal patterning, Epicuticular wax biosynthesis, Cellular level tolerance, etc have been cloned and characterised. Mining superior alleles of these genes is extremely crucial in developing focussed translational research activities either through breeding or transgenics. The contrast groups of genotypes for these traits form an excellent material for allele mining.
- Development of segregating populations for specific traits is still the most effective strategy for identifying novel genes and alleles by map based cloning or positional cloning. The contrasts for specific traits are hence an excellent source for developing such trait specific populations, which can be used for QTL mapping as well as gene discovery.

While these form the basic research components, the following would be the translational research activities.

- LD mapping in *indica* rice resulted in the identification of a few robust SSR markers linked with a few important drought tolerant traits. Some of these

markers have been validated *in silico*. Such validated markers can be effectively be used for traits introgression through Marker Assisted Selection (MAS). The trait donor parents can be crossed with elite genetic background to introgress drought tolerant traits. The progenies can be screened using these markers for traits introgression through MAS.

- Effectiveness of MAS almost entirely depends on the identification of perfect markers and the determination of chromosome wise LD. Both of these can only be achieved by significant increasing the density of markers and recomputing LD decay.
- Genotyping the panel of germplasm within large number of SSR markers is hence very essential.

Some of the promising trait donor lines have already been crossed with a few agronomically elite cultivars for trait introgression. The progenies are being evaluated.

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Appendix I. Physiological parameters recorded among rice germplasms during experiment 2007

Sl No	Genotype name	RL	RW	RV	TLA	TDM	R/S	DM/LA	R/LA	Delta
1	AC-35303	57.33	4.27	62.33	3294.8	51.85	0.0897	157.4	12.95	19.332
2	JBT-38/17	94.75	6.43	73.33	2859.6	45.62	0.1642	159.5	22.50	19.696
3	AC-35043	15.00	2.20	61.00	1939.2	40.32	0.0577	207.9	11.34	18.794
4	AC-39020	51.83	6.75	48.33	3614.5	42.25	0.1901	116.9	18.67	19.136
5	AC-35389	38.75	5.35	42.50	4502.9	52.98	0.1123	117.7	11.88	20.674
6	JBT-38/61	47.50	8.35	56.75	4065.2	59.80	0.1623	147.1	20.54	19.209
7	AC-35119	53.50	10.58	71.50	4124.7	63.43	0.2001	153.8	25.64	19.051
8	AC-35611	44.75	7.37	88.83	4248.9	56.44	0.1501	132.8	17.34	18.867
9	PS-290	65.80	5.48	45.83	1929.9	28.07	0.2428	145.4	28.41	19.315
10	PS-389	32.97	4.88	34.17	2958.0	36.42	0.1546	123.1	16.48	19.353
11	PS-305	37.46	6.13	61.83	3031.4	41.72	0.1721	137.6	20.20	18.876
12	PS-350	65.33	6.50	71.33	4693.6	47.33	0.1592	100.8	13.85	18.301
13	PS-316	51.33	9.05	49.83	3178.6	48.68	0.2284	153.1	28.47	20.048
14	JBT-38/10	29.75	6.13	49.17	2158.8	42.43	0.1687	196.6	28.37	18.582
15	PS-370	47.50	5.50	39.17	2401.6	29.43	0.2299	122.5	22.90	20.231
16	PS-226	29.83	3.72	56.00	2313.8	38.02	0.1084	164.3	16.06	17.265
17	AC-35310	42.50	5.33	29.17	2067.9	33.43	0.1894	161.7	25.75	18.988
18	JBT-36/8	57.33	3.85	26.67	1250.1	33.88	0.1282	271.0	30.80	19.706
19	PS-353	38.83	4.32	48.83	2171.5	34.43	0.1433	158.6	19.88	17.945
20	IR-20	25.92	4.25	48.92	1678.4	35.41	0.1364	211.0	25.32	19.335
21	PS-302	45.83	5.58	43.67	3026.4	57.53	0.1073	190.1	18.42	19.192
22	RASI	48.83	8.03	45.83	2074.4	38.40	0.2645	185.1	38.73	18.153
23	JBT-36/66	50.08	6.58	40.67	3096.2	55.31	0.1349	178.6	21.24	19.012
24	PS-336	44.17	11.21	62.00	5384.6	86.51	0.1488	160.7	20.82	19.420
25	PS-396	57.25	5.15	32.75	2495.5	36.00	0.1669	144.3	20.64	19.576
26	AC-39012	33.17	4.90	45.50	1958.1	30.14	0.1941	153.9	25.02	21.377
27	JBT-37/29	43.00	3.50	34.00	1248.3	27.53	0.1456	220.6	28.04	19.518
28	AC-35415	49.17	5.73	41.33	1594.3	24.19	0.3106	151.7	35.96	20.228
29	AC-35006	33.54	4.78	33.33	1901.9	25.41	0.2314	133.6	25.11	19.564
30	JBT-38/119	68.08	4.65	30.75	2379.4	42.41	0.1232	178.2	19.54	19.805
31	JBT-37/112	31.25	8.55	47.83	2353.6	34.80	0.3257	147.9	36.33	19.397
32	JBT-36/104	31.08	8.95	41.67	2933.6	61.14	0.1715	208.4	30.51	19.453
33	PS-398	40.67	3.53	52.83	2456.9	36.75	0.1061	149.6	14.35	18.590
34	PS-224	45.08	7.08	66.33	3082.6	48.91	0.1691	158.7	22.95	19.668
35	JBT-37/130	58.75	2.20	12.50		23.08	0.1054			19.537
36	JBT-38/148	42.33	5.45	46.33	3059.2	46.17	0.1339	150.9	17.81	20.475
37	AC-36110	57.50	3.70	49.17	890.5	24.86	0.1749	279.1	41.55	19.292
38	JAYA	31.50	2.47	35.33	1701.5	23.76	0.1159	139.6	14.50	19.362
39	AC-35367	40.50	4.94	51.00	2861.0	39.92	0.1413	139.5	17.27	18.661
40	JBT-36/119	46.92	3.78	29.00	1288.1	23.33	0.1931	181.1	29.31	20.105
41	JBT-37/118	56.92	3.65	25.83	1809.5	35.08	0.1161	193.8	20.17	18.967
42	AC-35309	40.50	5.08	20.83	1781.3	41.73	0.1385	234.2	28.49	19.259
43	AC-35109	28.33	3.45	78.33	2042.6	27.27	0.1449	133.5	16.89	17.473
44	PS-225	68.67	7.48	64.50	4375.7	61.58	0.1382	140.7	17.08	17.730
45	AC-35380	49.50	4.43	65.75	3951.1	57.87	0.0828	146.5	11.20	18.655
46	JBT-36/64	35.67	4.59	56.17	2204.5	38.56	0.1352	174.9	20.83	19.636
47	AC-35086	61.25	9.05	40.00	3067.3	54.38	0.1996	177.3	29.50	19.140
48	AC-35135	46.50	4.25	22.50	1075.7	18.67	0.2948	173.5	39.51	19.717
49	JBT-36/14	39.50	4.28	29.00	1855.9	31.52	0.1573	169.8	23.08	18.586
50	JBT-37/134	43.00	4.68	62.33	1769.4	45.42	0.1150	256.7	26.47	

Sl No	Genotype name	RL	RW	RV	TLA	TDM	R/S	DM/LA	R/LA	Delta
51	JBT-38/26	38.00	5.43	39.50	2153.6	36.42	0.1754	169.1	25.23	20.253
52	JBT-36/112	40.33	4.20	43.67	3548.0	44.88	0.1033	126.5	11.84	19.093
53	PS-338	65.70	3.78	35.50	3943.5	45.00	0.0916	114.1	9.57	18.477
54	AC-35364	48.75	4.65	24.67	1893.2	40.71	0.1290	215.0	24.56	19.056
55	AC-35513	32.25	5.40	40.83	2235.7	20.14	0.3663	90.1	24.15	18.868
56	AC-35585	37.79	6.45	53.17	2661.7	26.83	0.3164	100.8	24.23	18.708
57	AC-35059	52.25	3.90	31.67	2097.5	28.47	0.1588	135.7	18.59	20.422
58	AC-35027	51.00	7.30	58.67	2824.9	45.47	0.1913	161.0	25.84	19.117
59	JBT-37/164	56.25	9.45	67.83	2377.1	55.15	0.2068	232.0	39.76	18.866
60	AC-39001	15.00	3.90	99.33	1778.6	45.40	0.0940	255.3	21.93	18.522
61	AC-39000	25.00	3.55	52.67	1846.7	26.89	0.1521	145.6	19.22	17.244
62	AC-35438	50.33	5.90	65.50	3824.8	55.03	0.1201	143.9	15.43	19.591
63	PS-229	71.33	7.46	32.50	2768.2	47.20	0.1877	170.5	26.94	18.686
64	AC-39010	43.00	1.91	29.33	1708.2	26.30	0.0782	154.0	11.17	20.841
65	AC-35573	56.67	9.50	83.33	4465.7	61.70	0.1820	138.2	21.27	18.641
66	IR-50	44.00	4.79	38.33	1943.5	41.76	0.1296	214.9	24.65	19.890
67	AC-35607	59.00	5.71	63.25	2983.4	41.78	0.1582	140.1	19.13	18.218
68	JBT-38/37	69.00	7.20	55.00	1216.0	47.50	0.1787	390.6	59.21	20.680
69	PS-400	34.50	5.43	35.83	2835.5	42.33	0.1470	149.3	19.13	17.672
70	JBT-36/26	48.42	5.13	54.17	3319.7	32.78	0.1857	98.8	15.46	19.905
71	JBT-38/46	40.33	2.05	58.67	1904.6	22.92	0.0982	120.3	10.76	19.204
72	JBT-36/60	50.90	5.15	61.33	2296.4	29.92	0.2079	130.3	22.43	19.047
73	AC-35284	49.50	3.93	35.00	2115.7	34.13	0.1300	161.3	18.55	19.147
74	JBT-37/6	31.70	2.70	15.00	1614.1	23.77	0.1282	147.2	16.73	19.906
75	PS-329	52.17	11.55	100.00	5265.6	55.80	0.2610	106.0	21.93	19.387
76	AC-35198	50.00	7.60	23.33	3041.3	40.55	0.2307	133.3	24.99	19.228
77	PS-273	84.00	7.30	48.67	2825.3	37.60	0.2409	133.1	25.84	20.054
78	PS-342	38.50	6.45	61.33	4884.7	57.29	0.1269	117.3	13.20	18.691
79	AC-35484	53.67	5.93	41.00	2252.0	38.01	0.1847	168.8	26.31	17.850
80	PS-419	35.42	5.35	44.58	2435.4	43.55	0.1401	178.8	21.97	19.050
81	JBT-37/37	34.50	5.95	54.67	1968.2	22.78	0.3536	115.7	30.23	17.615
82	AC-35281	55.00	6.05	78.33	3891.7	62.25	0.1077	160.0	15.55	18.079
83	AC-35534-1	50.00	6.00	79.00	2305.3	40.35	0.1747	175.0	26.03	
84	PS-346	59.00	4.43	59.83	3011.4	43.89	0.1121	145.8	14.69	18.830
85	JBT-36/169	37.67	4.47	62.50	1275.6	32.47	0.1595	254.5	35.02	19.949
86	AC-35140	35.00	4.70	86.00	4528.6	51.58	0.1002	113.9	10.38	19.539
87	PS-250	60.58	5.88	56.17	2779.2	35.22	0.2006	126.7	21.17	19.073
88	AC-35341	40.37	4.45	58.67	1983.1	30.67	0.1697	154.6	22.44	19.333
89	AC-39019	75.50	12.30	47.92	4795.6	69.30	0.2158	144.5	25.65	18.123
90	JBT-37/126	34.17	4.58	51.50	1709.5	31.65	0.1693	185.1	26.81	20.238
91	PS-369	60.67	9.10	90.00	1531.5	59.40	0.5076	387.9	130.59	18.700
92	AC-35481-1	31.08	5.45	65.33	2896.1	45.23	0.1370	156.2	18.82	18.908
93	JBT-37/95	89.17	6.40	50.50	3464.4	66.00	0.1074	190.5	18.47	19.312
94	JBT-36/81	46.67	6.43	48.33	3529.6	41.17	0.1852	116.6	18.23	17.707
95	AC-35599	78.50	5.80	56.17	3673.4	48.52	0.1358	132.1	15.79	19.702
96	PS-330	64.00	3.03	25.00	1489.2	18.30	0.1987	122.9	20.37	19.003
97	AC-35170	45.00	6.50	80.00	4430.4	78.20	0.0907	176.5	14.67	17.757
98	PS-266	51.58	1.88	23.33	1946.0	37.04	0.0533	190.3	9.64	19.197
99	AC-35291	28.75	5.68	56.67	2462.4	37.23	0.1799	151.2	23.05	20.696
100	JBT-38/138	35.92	7.88	67.67	1874.7	52.55	0.1763	280.3	42.01	18.104

Sl No	Genotype name	RL	RW	RV	TLA	TDM	R/S	DM/LA	R/LA	Delta
101	AC-35417	60.00	9.05	73.67	4656.3	64.60	0.1629	138.7	19.44	19.030
102	JBT-37/85	57.00	5.98	75.83	3098.1	43.46	0.1594	140.3	19.29	19.165
103	JBT-38/25	55.83	5.18	37.60	3680.3	44.33	0.1322	120.5	14.06	19.015
104	JBT-38/39	64.00	2.73	31.67	2311.0	24.15	0.1276	104.5	11.83	21.158
105	AC-39014	36.00	5.12	29.17	1707.9	36.06	0.1654	211.1	29.96	18.630
106	AC-35031	36.38	8.23	79.50	4675.8	59.98	0.1589	128.3	17.59	19.201
107	PS-259	41.62	4.88	50.00	3142.5	40.14	0.1382	127.7	15.51	18.762
108	PS-284	45.50	5.18	48.33	2715.3	30.72	0.2026	113.1	19.06	19.690
109	AC-39016	64.50	8.33	63.42	3976.7	56.56	0.1726	142.2	20.93	18.903
110	JBT-36/114	49.83	3.19	33.92	1300.7	33.24	0.1062	255.6	24.54	19.415
111	JBT-30/137	33.77	3.70	35.00	3710.2	50.70	0.0787	136.6	9.97	20.620
112	AC-35563	56.67	3.60	32.67	3034.4	28.73	0.1433	94.7	11.86	19.807
113	PS-303	25.53	3.08	42.50	1722.1	25.72	0.1362	149.3	17.90	19.348
114	AC-35506	71.00	5.13	57.50	3662.0	41.30	0.1417	112.8	13.99	19.049
115	AC-35548	44.67	3.75	38.67	3623.0	42.48	0.0968	117.2	10.35	19.475
116	AC-39018	37.00	4.90	36.67	3216.8	42.93	0.1289	133.4	15.23	18.839
117	Seluimpikit	49.17	5.78	34.17	1807.7	34.73	0.1995	192.1	31.95	19.310
118	PS-397	58.00	7.30	45.00	1727.5	42.50	0.2074	246.0	42.26	20.311
119	AC-39002	64.50	4.20	54.00	7138.8	64.75	0.0694	90.7	5.88	20.413
120	JBT-36/76	28.97	4.13	51.17	1479.0	34.27	0.1372	231.7	27.95	18.464
121	PS-367	56.75	3.50	25.00	2054.4	33.68	0.1160	163.9	17.04	19.930
122	AC-35003	34.50	4.11	43.08	2535.2	40.86	0.1118	161.2	16.21	19.286
123	JBT-37/24	34.00	7.35	67.67	2142.1	41.35	0.2162	193.0	34.31	19.955
124	PS-399	51.50	5.90	48.33	1121.1	27.20	0.2770	242.6	52.63	18.880
125	AC-35327	57.50	5.13	45.00	1869.0	40.83	0.1436	218.4	27.42	18.314
126	PS-355	38.08	3.30	25.83	2264.1	34.98	0.1042	154.5	14.58	19.021
127	JBT-36/2	62.33	5.05	43.33	1099.0	25.12	0.2517	228.5	45.95	20.082
128	AC-35000	48.00	4.00	38.33	1112.5	21.75	0.2254	195.5	35.95	19.586
129	Vanaprava	33.63	2.98	45.33	2011.9	41.28	0.0777	205.2	14.79	18.744
130	AC-39008	54.58	4.48	33.17	1939.0	35.58	0.1439	183.5	23.08	19.634
131	PS-380	45.17	5.75	72.00	2792.2	40.27	0.1666	144.2	20.59	18.512
132	JBT-37/128	60.42	5.73	51.25	3052.0	45.76	0.1430	149.9	18.76	18.767
133	AC-35122	44.00	3.40		6132.4	74.80	0.0476	122.0	5.54	18.474
134	PS-362	46.50	5.02	54.83	2659.7	38.32	0.1507	144.1	18.86	20.744
135	AC-35044	43.50	2.98	20.00	1395.4	16.08	0.2271	115.2	21.32	19.119
136	Nivara	35.33	4.55	46.33	3702.6	38.31	0.1348	103.5	12.29	19.937
137	AC-35351	43.50	3.03	31.33	856.4	14.34	0.2673	167.5	35.32	20.002
138	PS-427	46.17	7.93	74.00	3588.1	50.53	0.1860	140.8	22.09	19.076
139	JBT-37/5	52.67	5.98	56.17	4772.6	57.78	0.1153	121.1	12.52	19.392
140	AC-35308	56.50	5.73	58.33	3475.0	45.52	0.1439	131.0	16.48	19.355
141	AC-35534	20.13	3.90	47.00	698.8	33.15	0.1333	474.4	55.81	18.709
142	AC-35361	29.73	6.82	49.33	2747.5	44.84	0.1793	163.2	24.81	19.692
143	JBT-38/27	61.33	6.73	50.33	3643.5	64.38	0.1166	176.7	18.46	18.462
144	143-20-2	68.29	9.60	52.50	1696.2	39.45	0.3216	232.6	56.60	21.194
145	PS-366	28.67	4.99	74.17	2482.7	28.83	0.2094	116.1	20.11	19.936
146	JBT-37/89	66.67	9.10	87.00	3793.8	46.83	0.2412	123.4	23.99	20.405
147	AC-35116	54.00	6.70	35.00	1727.3	32.95	0.2552	190.8	38.79	19.016
148	JBT-36/79	60.96	4.96	73.00	3224.4	48.28	0.1144	149.7	15.38	20.031
149	AC-35330	39.75	4.35	23.33	2319.5	41.30	0.1177	178.1	18.75	20.170
150	AC-35067	80.67	7.90	51.67	2929.4	47.00	0.2020	160.4	26.97	20.253

Sl No	Genotype name	RL	RW	RV	TLA	TDM	R/S	DM/LA	R/LA	Delta
151	AC-35017	45.00	7.45	44.83	632.6	33.90	0.2817	535.9	117.77	19.083
152	JBT-36/41	53.58	5.43	50.67	2565.6	36.35	0.1754	141.7	21.15	18.513
153	AC-35383	30.00	5.55	76.00	4286.8	48.57	0.1290	113.3	12.95	17.473
154	AC-35290	35.25	6.54	56.50	3452.0	42.23	0.1833	122.3	18.95	18.151
155	PS-320	67.83	5.15	50.67	2308.9	40.37	0.1462	174.8	22.31	18.079
156	JBT-37/90	35.00	3.65	26.67	1940.1	31.65	0.1304	163.1	18.81	19.748
157	AC-35220	33.33	3.90	72.83	5884.5	46.05	0.0925	78.3	6.63	19.101
158	AC-3516	50.67	6.35	41.83	2159.4	39.48	0.1917	182.8	29.41	20.474
159	PS-332	66.42	4.75	35.50	1619.4	31.21	0.1795	192.7	29.33	19.100
160	AC-35406	45.08	5.78	59.17	4596.6	52.52	0.1238	114.3	12.58	20.786
161	PS-360	72.03	7.73	49.17	4572.7	46.53	0.1991	101.8	16.89	19.059
162	AC-35098	44.83	6.20	53.83	2582.8	34.15	0.2218	132.2	24.01	18.491
163	AC-35616	40.47	3.60	52.15	1633.5	19.56	0.2256	119.7	22.04	19.749
164	AC-35392	35.83	6.32	29.17	1553.0	30.39	0.2624	195.7	40.67	18.643
165	AC-35388	33.50	3.95	59.50	4042.3	56.53	0.0751	139.9	9.77	19.314
166	PS-401	20.50	3.83	45.00	2281.9	32.03	0.1356	140.4	16.76	19.774
167	PS-402	61.83	5.43	33.83	2190.6	41.71	0.1495	190.4	24.76	19.401
168	PS-384	43.33	5.23	79.67	3608.0	43.23	0.1377	119.8	14.50	20.217
169	JBT-36/139	36.17	4.06	59.67	2187.0	36.88	0.1237	168.6	18.56	19.377
170	AC-35612	68.50	6.15	48.67	6283.7	94.60	0.0695	150.5	9.79	18.955
171	JBT-37/170	42.00	4.83	18.33	959.9	24.65	0.2439	256.8	50.35	17.584
172	AC-35475	80.67	3.70	28.33	1813.8	32.05	0.1305	176.7	20.40	
173	PS-349	48.33	5.85	48.00	3193.5	40.40	0.1693	126.5	18.32	18.004
174	PS-404	36.33	2.55	49.83	1335.7	27.81	0.1010	208.2	19.09	18.388
175	PS-445	72.75	4.38	35.67	2565.4	51.39	0.0931	200.3	17.05	20.289
176	AC-35038	25.00	5.03	62.33	3516.9	54.70	0.1013	155.5	14.31	19.260
177	JBT-36/126	33.92	3.60	55.67	3040.3	36.90	0.1081	121.4	11.84	19.401
178	JBT-37/195	40.58	2.03	49.33	2701.6	22.98	0.0967	85.0	7.50	20.274
179	AC-35207	43.00	4.60	40.00	1997.7	43.60	0.1179	218.3	23.03	20.340
180	JBT-37/100	63.17	7.62	41.67	2902.8	43.67	0.2113	150.4	26.24	18.146
181	JBT-38/2	62.33	3.40	30.00	1548.2	21.23	0.1907	137.2	21.96	19.366
182	AC-35507	45.00	3.00	38.00	1671.9	19.70	0.1796	117.8	17.94	
183	PS-276	74.17	4.15	48.33	2340.0	44.80	0.1021	191.5	17.73	19.212
184	PS-207	38.00	4.77	37.50	1574.4	28.22	0.2033	179.2	30.28	19.204
185	AC-35574	42.17	3.58	54.58	1884.2	28.54	0.1432	151.5	18.97	19.185
186	JBT-37/91	66.45	6.15	47.83	2950.1	42.57	0.1689	144.3	20.85	20.336
187	JBT-38/116	84.17	4.25	46.33	4474.5	37.95	0.1261	84.8	9.50	19.250
188	AC-35321	47.25	4.70	63.00	3443.8	35.88	0.1507	104.2	13.65	18.641
189	AC-35024	52.67	3.88	33.17	1899.9	29.82	0.1494	156.9	20.40	18.334
190	PS-339	51.67	4.38	57.50	2413.1	32.01	0.1583	132.6	18.13	20.246
191	AC-3538	43.92	6.35	51.50	3173.5	45.32	0.1630	142.8	20.01	18.922
192	JBT-38/53	31.17	4.70	43.50	393.4	33.38	0.1639	848.4	119.48	20.288
193	PS-319	73.92	5.73	36.00	2792.8	55.68	0.1146	199.4	20.50	19.656
194	AC-35526	15.00	2.05	71.00	3274.1	41.40	0.0521	126.4	6.26	19.741
195	Vandana	30.25	2.78	48.17	1755.1	34.65	0.0871	197.4	15.81	18.262
196	JBT-37/113	45.00	2.80	57.67	1445.5	27.45	0.1136	189.9	19.37	18.641
197	JBT-38/18	49.08	5.14	59.33	2507.6	32.89	0.1853	131.2	20.50	18.952
198	AC-35256	66.00	6.80	30.00	4163.2	89.20	0.0825	214.3	16.33	19.654
199	AC-35025	47.67	6.85	68.50	2084.1	47.08	0.1703	225.9	32.87	19.190
200	AC-35476	23.33	1.95	73.67	4334.2	47.60	0.0427	109.8	4.50	19.570

Sl No	Genotype name	RL	RW	RV	TLA	TDM	R/S	DM/LA	R/LA	Delta
201	AC-35517	43.08	2.27	50.58	1169.1	20.70	0.1230	177.1	19.39	18.842
202	AC-35375	39.58	6.98	53.17	2249.5	35.10	0.2480	156.0	31.01	20.041
203	JBT-38/130	24.42	4.35	66.50	2136.5	39.28	0.1246	183.8	20.36	18.584
204	AC-35271	29.00	6.13	43.25	4059.0	58.60	0.1167	144.4	15.09	17.882
205	AC-35325	43.17	8.95	85.17	5538.4	72.18	0.1416	130.3	16.16	19.464
206	JBT-36/15	31.00	4.03	50.17	1601.4	21.44	0.2311	133.9	25.13	19.241
207	AC-39009	38.88	8.25	69.92	2623.4	42.85	0.2384	163.3	31.45	18.144
208	AC-39021	42.67	2.87	30.00	1817.9	38.83	0.0797	213.6	15.77	19.782
209	JBT-37/154	41.17	5.15	38.33	1352.8	25.49	0.2532	188.4	38.07	19.367
210	PS-291	49.83	2.15	25.00	1435.9	22.43	0.1060	156.2	14.97	19.618
211	AC-35004	63.33	6.75	69.33	4145.8	53.47	0.1445	129.0	16.28	20.753
212	JBT-37/140	76.00	4.95	57.83	1672.4	27.80	0.2166	166.2	29.60	19.544
213	JBT-37/111	48.25	5.33	69.17	3250.4	43.13	0.1409	132.7	16.38	18.800
214	PS-261	69.92	7.58	38.00	2735.9	35.91	0.2677	131.2	27.72	18.827
215	PS-408	33.33	2.88	50.83	3216.7	32.83	0.0960	102.0	8.94	19.301
216	AC-35187	51.87	6.11	42.67	2959.1	43.71	0.1625	147.7	20.64	19.210
217	AC-35381	42.78	6.93	64.83	2224.7	41.95	0.1977	188.6	31.13	17.411
218	HAMSA	78.33	4.85	40.50	5564.5	40.23	0.1371	72.3	8.72	20.566
219	MTU-1010	42.50	4.38	31.67	3060.8	36.30	0.1370	118.6	14.29	19.812
220	IET-16348	43.92	7.40	60.33	4263.4	57.60	0.1474	135.1	17.36	19.551
221	JYOTHI	39.42	1.68	31.33	2520.8	51.83	0.0334	205.6	6.64	20.724
222	KCP-1	62.50	9.08	56.50	5424.7	71.86	0.1447	132.5	16.74	19.614
223	IET-15924	60.35	3.78	36.58	3109.1	36.44	0.1156	117.2	12.14	20.660
224	THANU	58.17	4.75	54.58	4912.6	48.40	0.1088	98.5	9.67	19.877
225	IET-8116	65.50	6.83	80.17	2410.8	35.98	0.2341	149.2	28.31	19.262
226	CTH-3	44.25	4.78	54.83	2580.2	60.98	0.0850	236.3	18.51	19.333
227	KRH-2	31.07	8.03	54.83	3512.0	49.20	0.1949	140.1	22.85	17.858
228	BPT-5204	49.00	6.25	44.83	1811.9	24.68	0.3392	136.2	34.49	19.475
229	CTH-1	58.00	3.33	58.08	4559.6	65.65	0.0533	144.0	7.29	19.253
230	KR-1	56.50	6.35	59.00	3779.5	38.02	0.2005	100.6	16.80	18.692
231	MTU-1045	70.00	3.70	62.00	1605.2	25.90	0.1667	161.3	23.05	20.798
232	S-9	53.00	3.48	30.00	1006.6	27.98	0.1418	277.9	34.52	20.366
233	ES-18	48.00	6.59	43.17	5286.1	63.84	0.1151	120.8	12.47	20.601
234	IET-15963	57.67	3.93	38.33	1716.1	31.22	0.1438	181.9	22.87	19.102
235	BR-2655	54.17	4.98	59.33	3778.5	37.93	0.1512	100.4	13.19	19.333
236	MANGALA	46.75	4.43	59.50	3466.4	37.74	0.1331	108.9	12.79	18.597
237	KRH-3	39.33	6.13	45.33	1865.3	32.41	0.2330	173.7	32.84	19.446
238	SAMRAT	51.17	6.79	54.17	3212.9	45.75	0.1743	142.4	21.14	18.528
239	NAGENA	38.50	6.58	49.25	1229.6	31.70	0.2617	257.8	53.47	19.321

Appendix II. Physiological parameters recorded among rice germplasms during experiment 2008

Sl No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
1	JBT-36/66	6	8	4	10	28	46.83	20.83	5.85	26.02	535	0.45	486.6	109.41	20.14	81	14.48	27.40	1.081	323.1	12.93	2.12	298.8	25.34	0.586
2	AC-39010	3	4	4	3	14	37.67	14.17	2.83	18.62	1059	0.33	175.9	26.76	19.33	82	16.28	23.50	0.842	312.5	8.26	2.85	371.0	27.90	0.546
3	JBT-37/140	6	6	6	0	18	54.33	26.67	3.95	25.33		0.40			20.04			27.94	0.64	276.0			429.8	43.51	
4	AC-35170	21	10	8	5	38	42.00	50.00	11.77	48.32	1815	0.55	266.2	64.82	21.23	104	23.44	23.60	0.740	309.8	8.90	2.65	418.4	31.88	0.590
5	PS-242	12	10	6	4	32	45.50	35.00	8.02	40.67	1843	0.33	220.7	43.51	19.18	103	18.04	23.63	1.222	331.3	10.75	2.20	271.0	19.33	0.627
6	AC-35450	12	12	6	3	33	50.17	33.33	7.78	46.82	2878	0.27	162.7	27.05	20.15	105	19.36	23.28	1.610	339.5	12.14	1.92	210.9	14.46	0.587
7	JBT-36/79	3	6	2	3	14	38.33	11.67	2.33	21.62	1221	0.16	177.1	19.11	20.03	83	29.82	26.33	0.911	313.0	9.33	2.82	343.8	28.91	0.592
8	PS-261	9	10	14	1	46	47.17	12.92	5.47	40.19	2211	0.43	181.8	24.73											
9	JBT-37/85	15	12	8	3	38	38.83	28.33	9.82	45.22	2721	0.38	166.2	36.07	18.52	80	15.24	21.33	0.554	299.3	7.93	2.69	540.2	38.49	0.485
10	AC-39019	12	14	4	4	34	45.33	52.50	8.33	50.20	2301	0.26	218.2	36.18	19.32	104	27.14	21.46	0.685	308.6	12.69	1.69	450.6	31.34	0.499
11	AC-35187	6	4	8	3	21	38.00	17.92	4.20	18.18	1019	0.33	178.4	41.20	20.16	117	8.04	25.78	1.060	318.3	9.41	2.74	300.2	24.32	0.546
12	JBT-37/134	12	6	10	2	30	40.92	14.58	7.10	26.20	1973	0.37	132.8	35.99	20.13	96	14.28	27.10	1.530	332.5	10.78	2.51	217.3	17.71	0.576
13	AC-39052	6	8	6	4	24	49.33	35.00	4.97	29.00	1352	0.57	214.5	36.74	19.83			25.38	0.98	315.3			320.1	25.77	
14	PS-398	9	6	12	5	32	36.83	17.33	6.53	22.23	960	0.56	231.6	68.05	21.10	93	29.86	21.10	0.702	317.0	8.78	2.40	451.6	30.06	0.497
15	AC-39018	6	4	8	3	21	43.00	38.34	4.58	17.62	1135	0.39	155.3	40.40	20.28	102	34.26	19.23	1.133	335.0	8.96	2.15	295.7	16.97	0.499
16	PS-305	15	10	8	3	36	45.83	51.67	9.88	39.91	2438	0.37	163.7	40.54	19.83	102	21.46	20.09	0.597	307.5	7.35	2.73	515.1	33.64	0.429
17	AC-35122	21	12	10	6	49	56.00	37.50	11.96	44.60	1446	0.48	308.4	82.70	21.87	101	23.24	30.69	1.015	295.6	13.73	2.24	291.2	30.23	0.491
18	PS-290	15	10	8	5	38	42.67	22.50	8.55	40.13	1675	0.40	239.6	51.06	19.77	102	16.84	13.08	0.416	313.8	4.56	2.87	754.2	31.43	0.584
19	AC-35633	12	12	6	3	33	42.33	28.33	8.02	48.08	2696	0.29	178.4	29.74	19.48	86	22.98	28.70		340.0	11.10	2.59	165.0	13.93	0.568
20	AC-35585	9	8	8	4	29	42.83	18.33	5.99	28.75	1536	0.30	187.2	39.01	19.83	78	13.98	18.35	0.472	290.8	7.12	2.58	616.0	38.88	0.418
21	JBT-36/169	18	14	6	5	43	26.17	5.67	10.70	57.17	2163	0.33	264.3	49.46	21.74	81	11.2	17.00	0.450	300.8	7.54	2.26	668.7	37.80	0.468
22	AC-35325	18	10	8	3	39	35.75	17.50	10.59	39.28	2380	0.45	165.1	44.51	19.31	106		19.63	1.061	333.8	11.10	1.77	314.7	18.51	0.545
23	JBT-37/91	6	12	2	3	23	42.67	15.83	4.77	43.49	2937	0.20	148.1	16.23	19.39	83	18.34	19.85	1.343	336.3	9.93	2.00	250.5	14.79	0.547
24	JBT-38/10	9	10	6	4	29	40.00	29.17	6.07	36.28	1810	0.28	200.4	33.51	20.19	107	10.9	21.08	0.497	298.3	6.75	3.12	600.7	42.45	0.590
25	JBT-38/39	21	14	8	3	46	39.58	32.50	11.83	55.50	3288	0.34	168.8	35.98	19.38	106	14.66	22.33	0.472	277.9	9.05	2.47	589.2	47.34	0.511

Sl No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
26	JBT-38/19	24	12	12	3	51	44.25	55.00	14.98	47.66	3014	0.49	158.1	49.69	20.62	111	32.52	25.63	1.593	334.3	10.85	2.36	209.9	16.09	0.547
27	ES-18	9	10	4	3	26	54.75	33.33	4.12	27.47	2064	0.25	133.1	19.94	19.26	79		17.53	0.516	303.5	8.16	2.15	587.9	33.95	0.611
28	JBT-3/60	15	16	4	7	42	49.84	26.67	9.60	51.95	1698	0.44	306.0	56.54	20.60	93	29.02	24.43	0.414	258.4	7.82	3.12	624.3	59.02	0.456
29	AC-35406	15	8	10	2	35	49.75	22.08	9.68	27.19	2325	0.49	117.0	41.66	20.06	82	21.94	20.38	0.435	278.0	9.03	2.26	639.4	46.87	0.520
30	IET-17616	18	14	6	5	43	45.67	61.67	10.58	54.62	2380	0.28	229.4	44.46	20.22	105	7	22.60	0.610	303.8	7.94	2.85	498.4	37.08	0.508
31	JBT-38/61	6	8	2	5	21	43.67	25.00	3.73	34.82	1296	0.24	268.7	28.81	20.21	77	30.5	17.90	0.554	309.3	8.07	2.22	558.7	32.34	0.559
32	JBT-38/148	12	10	6	4	32	40.67	33.33	7.20	37.72	1898	0.31	198.7	37.92	20.65	104	21.62	24.43	1.138	326.8	10.43	2.34	287.3	21.47	0.572
33	MANGALA	15	10	10	7	42	43.83	38.34	9.00	33.48	1016	0.65	329.6	88.60	21.02	82	7.58	18.36	0.477	302.7	7.22	2.54	634.1	38.46	0.467
34	AC-35059	9	4	20	3	36	32.00	13.50	6.65	14.95	869	0.87	172.0	76.51	19.43	90	23.04	23.73	1.063	326.0	10.03	2.36	306.8	22.32	0.638
35	PS-384	15	12	6	2	35	38.50	26.67	9.52	46.15	2728	0.28	169.2	34.89	19.10	117	20.84	23.73	0.643	301.8	9.43	2.52	469.1	36.88	0.541
36	JBT-37/92	6	4	10	4	24	41.42	20.00	4.02	14.17	808	0.54	175.4	49.73	18.77	99	19.4	24.55	0.728	306.0	8.35	2.94	420.1	33.71	0.538
37	JBT-37/154	12	14	4	3	33	75.17	30.00	6.80	50.07	2869	0.26	174.5	23.71	19.13	82	21.84	24.86	0.494	272.1	9.11	2.73	550.7	50.32	0.501
38	PS-389	15	8	12	3	38	38.17	26.25	8.63	30.09	1725	0.40	174.4	50.04	20.55	104	11.52	21.93	0.604	299.3	7.68	2.86	495.9	36.33	0.506
39	AC-35382	12	4	20	4	40	22.83	19.17	7.33	15.27	790	0.78	193.2	92.71	21.06	118	13.78	25.18	0.558	290.5	7.41	3.40	520.4	45.10	0.541
40	JBT-37/112	15	10	8	5	38	43.50	30.83	9.53	41.39	1475	0.40	280.6	64.62	18.15	105	19.16	23.89	0.815	309.3	9.43	2.53	379.7	29.33	0.510
41	PS-391	12	6	10	5	33	36.50	12.50	7.33	27.32	1051	0.63	259.9	69.77	20.11	102	15.92	19.33		347.0	10.22	1.89	183.8	10.24	0.569
42	AC-35534-1	15	6	8	3	26	51.33	16.25	5.66	27.48	1986	0.26	138.4	28.49	19.09	104	16.3	25.35	0.765	308.0	8.48	2.99	402.5	33.13	0.584
43	JBT-30/135	9	10	6	6	31	48.17	37.50	5.97	35.06	1136	0.32	308.5	52.51	18.32	102	17.32	23.81	0.613	302.0	8.43	2.82	492.6	38.84	0.518
44	JBT-36/41	12	10	16	5	52	78.00	31.67	7.15	32.83	1425	0.42	230.3	50.17	19.82	86	10.62	20.48	0.625	300.5	11.06	1.85	480.7	32.75	0.444
45	PS-408	18	14	6	3	41	41.17	30.83	10.53	57.38	3838	0.26	149.5	27.45	20.81	116	23	20.35	0.637	312.0	7.74	2.63	489.8	31.95	0.599
46	AC-35484	12	8	8	5	33	40.17	38.33	7.60	31.83	1417	0.34	224.7	53.64	19.34	118		13.74	0.347	298.0	9.01	1.52	858.9	39.61	0.377
47	AC-35198	15	12	6	3	36	51.17	41.67	8.47	50.65	3430	0.25	147.7	24.69	22.03	112	12.08	25.03	1.070	324.0	9.70	2.58	302.8	23.39	0.601
48	AC-35046	12	8	8	4	32	44.92	31.67	7.88	31.61	1641	0.38	192.6	47.99	19.24			24.90	0.676	300.5	8.88	2.80	444.7	36.85	0.525
49	AC-35599	12	10	6	4	32	26.92	41.67	7.74	38.85	1801	0.42	215.7	42.99	19.49			22.73	1.14	332.5			292.3	19.98	
50	AC-35573	24	12	14	4	54	62.67	51.67	14.48	43.33	2209	0.58	196.2	65.57	20.29	104	23.34	24.73	0.947	308.9	13.05	1.89	326.1	26.11	0.525

Sl No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv/Fm'
51	AC-35256	15	12	6	4	37	50.00	24.17	9.05	49.33	2891	0.36	170.6	31.30	19.64	103	19.5	27.10	0.648	283.8	9.93	2.73	438.1	41.85	0.515
52	AC-35006	24	18	8	3	53	50.50	51.67	13.07	62.50	4501	0.37	138.9	29.03	21.14	104	29.36	20.24	0.571	308.1	8.70	2.33	539.6	35.44	0.500
53	JBT-37/6	12	14	4	5	35	56.33	28.33	7.88	47.10	2332	0.50	202.0	33.80	18.95			19.25	0.37	277.5			760.3	52.74	
54	AC-39040	9	6	10	8	31	47.17	22.50	4.48	29.41	1473	0.28	199.6	30.37	20.26	70	11.32	20.40	0.709	313.3	8.54	2.39	442.0	28.78	0.531
55	AC-35331	12	10	6	4	32	56.33	30.00	7.33	35.41	1672	0.35	211.8	43.82	19.09	105	19.52	24.19	0.569	290.5	10.48	2.31	510.4	42.50	0.492
56	PS-353	9	6	6	4	25	36.67	23.33	5.23	25.97	1138	0.39	228.1	45.98	19.11	96	16.8	16.98	0.358	285.5	5.73	2.97	798.6	47.48	0.528
57	AC-35611	12	10	6	5	33	51.33	23.33	6.56	39.53	1678	0.32	235.5	39.08	18.87	92	20.5	21.35	1.048	331.0	9.67	2.21	315.8	20.37	0.511
58	AC-35392	6	8	4	4	22	35.17	17.50	4.64	33.19	1634	0.26	203.1	28.38	19.96	104	21.52	23.43	0.812	312.5	8.89	2.64	384.9	28.85	0.574
59	AC-35607	18	14	6	3	41	41.33	43.33	10.69	55.76	3696	0.34	150.9	28.93	20.80	104	7.2	21.25	0.767	318.8	8.33	2.55	415.9	27.72	0.543
60	PS-350	15	14	6	4	39	52.50	28.33	10.42	56.93	2519	0.27	226.1	41.36	20.14	102	19.92	17.74	0.749	325.5	9.37	1.89	434.7	23.69	0.390
61	PS-362	21	12	10	4	47	51.83	56.67	12.38	49.37	2417	0.37	204.3	51.24	21.81	112	14.48	27.24	0.629	292.5	8.57	3.18	465.3	43.33	0.507
62	AC-35388	21	10	14	4	49	49.33	40.00	12.40	36.36	1872	0.58	194.3	66.25	21.74	111	11.72	12.08	0.293	294.6	7.84	1.54	1004.7	41.20	0.453
63	PS-329	18	12	10	4	44	47.17	20.00	12.01	45.77	2451	0.50	186.7	48.99	21.25	105	25.66	21.15	0.939	325.0	6.89	3.07	346.1	22.52	0.601
64	PS-369	21	8	10	8	38	60.50	52.50	8.58	30.01	768	0.68	390.7	111.64	22.79	84	11.36	20.98	0.576	305.3	7.87	2.66	529.7	36.40	0.570
65	PS-224	12	10	4	3	29	37.33	27.50	6.72	41.95	2373	0.30	176.8	28.31	19.74	81	9.7	18.48	0.583	311.8	6.83	2.71	534.5	31.68	0.492
66	JBT-38/88	18	14	8	3	43	40.00	38.33	10.93	52.88	2914	0.39	181.5	37.49	20.22	105	40.34	25.08	0.892	313.8	8.57	2.93	351.9	28.13	0.599
67	AC-39006	21	16	6	4	47	48.67	40.00	11.78	57.45	3058	0.35	187.9	38.51	21.66	102	22.32	19.80	0.759	321.0	8.88	2.23	422.8	26.08	0.573
68	PS-336	18	10	10	5	43	43.50	34.17	11.42	41.45	1813	0.47	228.5	62.95	21.16	104	17	16.45	0.541	311.5	6.48	2.54	576.1	30.42	0.575
69	JBT-36/104	15	10	8	2	35	41.12	26.67	8.63	38.90	3179	0.35	122.4	27.13	19.53	109	17.94	24.40	0.553	280.9	9.77	2.50	508.3	44.15	0.498
70	JBT-38/109	24	12	12	2	50	67.33	46.66	13.87	46.17		0.44			21.90	138	6.36	25.95	0.785	302.8	13.53	1.92	385.7	33.06	0.525
71	PS-276	18	12	8	4	42	52.83	43.34	10.70	44.03	2104	0.34	209.3	50.86	19.97	112	23.98	25.64	0.748	298.8	11.74	2.18	399.2	34.26	0.498
72	MTU-1045	15	10	8	4	37	38.17	33.33	8.43	38.88	1962	0.40	198.2	42.99	20.56	104	8.62	20.95	1.085	335.5	9.34	2.24	309.2	19.31	0.555
73	AC-35067	18	18	4	7	47	45.00	30.83	10.67	69.93	2009	0.37	348.0	53.09	20.62	100	14.14	26.08	1.177	323.0	14.70	1.77	274.5	22.16	0.554
74	AC-35003	6	2	14	5	27	36.83	16.67	4.43	13.63	553	0.68	246.6	80.20	21.27	91	15.06	18.65	0.762	325.8	9.32	2.00	427.6	24.48	0.591

Sl No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
75	AC-35044	21	18	6	4	49	42.50	55.00	12.20	66.40	2972	0.29	223.4	41.05	21.67	103	25.98	25.03	1.002	325.5	9.37	2.67	324.8	24.97	0.588
76	CTH-1	21	16	6	6	49	48.50	50.00	12.83	61.62	2103	0.48	293.0	61.03	20.07										
77	JBT-37/113	18	14	6	4	42	36.67	44.17	10.00	58.67	2894	0.29	202.7	34.56	19.98	94	27.32	19.58	0.558	307.3	6.79	2.88	550.9	35.10	0.541
78	SAMRAT	15	8	14	4	41	25.00	33.34	9.85	29.30	1490	0.62	196.6	66.09	21.57	103	18.2	17.15	0.517	302.8	6.63	2.59	586.2	33.20	0.570
79	PS-221	15	10	8	4	37	51.25	21.67	9.41	41.19	2104	0.36	195.8	44.72	19.83	119		16.55	0.303	274.8	5.43	3.05	906.4	54.60	0.485
80	PS-226	6	6	6	5	23	44.42	15.00	4.58	23.57	896	0.35	263.1	51.08	19.07	102	14.56	21.58	0.542	292.4	10.32	2.09	539.3	39.80	0.534
81	THANU	6	6	6	3	21	37.58	13.75	4.65	27.71	1659	0.25	167.0	28.02	18.58	113	19.4	27.35	1.005	313.0	8.47	3.23	311.6	27.23	0.549
82	AC-35375	15	18	4	4	41	60.17	57.50	8.58	64.67	2905	0.24	222.6	29.54	19.85	103	25.68		0.738	280.4	12.06	2.76	380.0	45.15	0.547
83	JBT-37/37	15	12	8	3	38	44.33	22.50	9.50	45.90	3084	0.42	148.8	30.80	20.96	97	23.8	23.58	0.579	282.9	9.31	2.53	488.7	40.73	0.540
84	JBT-27/110	15	8	12	4	39	37.25	14.17	9.18	31.69	1541	0.60	205.7	59.55	20.55	84	13.1	22.15	0.938	324.5	9.83	2.25	346.1	23.63	0.559
85	PS-302	6	4	8	2	20	35.00	16.00	4.85	19.92	1488	0.34	133.9	32.60	19.47	92	16.2	22.63	1.121	321.5	9.15	2.47	286.7	20.18	0.522
86	PS-253	6	12	4	2	22	27.83	21.67	7.99	46.88	1675	0.37	279.9	47.71	19.26	104	20.24	27.58	0.917	310.3	11.10	2.48	338.3	30.07	0.545
87	AC-39012	3	4	4	5	16	37.33	7.50	2.07	16.88	675	0.29	250.2	30.63	20.56	80	11	21.58	1.084	323.0	7.39	2.92	298.0	19.91	0.470
88	JBT-36/64	12	10	6	3	31	47.33	20.83	7.88	36.80	2267	0.33	162.3	34.73	19.07	103	22.88	18.40	0.572	299.8	7.22	2.55	524.0	32.17	0.519
89	PS-346	18	14	8	3	43	50.83	50.84	11.67	52.23	3068	0.35	170.2	38.03	20.70	104		13.55	0.475	318.8	6.12	2.22	671.1	28.53	0.537
90	JBT-37/130	6	4	6	3	19	49.17	15.83	3.12	18.70	1338	0.61	139.8	23.30	19.97			27.13	0.69	295.0			427.4	39.30	
91	JBT-36/119	3	4	4	2	13	32.33	13.33	2.02	14.60	1124	0.27	129.9	17.95	18.81	80	12.2	22.43	1.018	325.8	9.40	2.39	320.0	22.03	0.561
92	JBT-38/96	18	10	10	3	41	46.67	26.67	10.54	39.82	2639	0.81	150.9	39.94	19.87			27.25	1.17	319.8			273.3	23.29	
93	IET-15924	12	6	12	4	34	36.00	20.00	8.74	25.23	1336	0.61	188.9	65.43	20.67	99	13.56	18.48	0.849	330.5	8.85	2.09	389.5	21.77	0.561
94	JBT-37/29	6	6	4	4	20	38.67	25.00	3.53	24.28	1083	0.32	224.2	32.55	18.17	99	27.76	18.68	0.636	309.3	6.39	2.92	486.1	29.35	0.499
95	PS-400	6	4	8	2	20	38.83	12.50	4.18	18.03	1337	0.68	134.8	31.28				20.78	0.38	261.9			695.3	55.16	
96	AC-35548	15	10	8	3	36	36.33	43.34	8.43	40.43	2533	0.36	159.6	33.26	19.65	91	19.74	18.05	0.316	272.3	5.73	3.15	860.9	57.08	0.553
97	AC-35438	21	12	10	3	46	39.17	21.67	12.85	51.47	3599	0.33	143.0	35.71	21.14	123		21.83	0.673	308.8	9.06	2.41	459.1	32.45	0.540
98	AC-39014	9	8	6	6	29	38.00	21.25	5.65	29.73	1123	0.34	264.7	50.32	20.68	89	27.04	21.63	1.030	330.5	9.23	2.34	320.9	21.00	0.596
99	CR143-20-2	9	8	4	7	28	57.50	18.33	5.02	34.20	1022	0.35	334.7	49.09	19.91	80	10.26	16.45	0.435	289.0	7.48	2.20	665.1	37.86	0.502
100	PS-303	9	8	6	3	26	38.50	21.67	6.52	31.48	1886	0.34	166.9	34.55	20.10	104	17.5	22.43		343.0	16.18	1.39	159.2	10.41	0.565

SI No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
101	AC-35313	6	6	4	3	23	40.42	15.83	4.60	27.17	1543	0.20	176.1	29.82	19.24	117	8.04	26.14	0.710	295.5	8.67	3.02	416.4	36.84	0.500
102	AC-35389	21	20	4	4	49	52.17	61.67	12.90	81.22	4166	0.28	195.0	30.97	20.37	103	18.84	20.60	0.765	321.0	8.82	2.34	419.6	26.93	0.583
103	JBT37/24	9	6	8	3	26	38.17	14.17	6.08	25.27	1592	0.41	158.7	38.22	19.88	104	12.1	23.80	0.851	316.5	9.74	2.44	371.9	27.97	0.567
104	JBT-37/164	18	18	4	7	47	51.67	50.00	9.90	67.97	1929	0.27	352.3	51.32	21.85	104	19.96	17.53	0.711	326.5	7.84	2.23	459.1	24.64	0.594
105	RASI	15	12	10	4	37	37.83	25.83	8.48	44.68	1758	0.40	254.1	48.25	20.23	118	25.2	27.75	1.445	326.8	10.47	2.65	226.2	19.21	0.479
106	AC-35321	15	10	6	2	30	46.67	41.67	9.63	36.03	2254	0.42	159.8	42.71	19.26	105		13.78	0.528	318.5	11.31	1.22	603.4	26.11	0.486
107	AC-35119	9	6	12	5	32	50.33	24.58	6.41	22.72	960	0.49	236.7	66.77	18.44	94	26.34	21.20	0.654	308.6	10.97	1.93	471.9	32.42	0.502
108	JBT-37/5	9	10	4	4	27	42.17	27.08	6.58	42.27	2299	0.28	183.8	28.63	18.78	100	15.62	21.63	0.982	328.3	9.15	2.36	334.4	22.03	0.586
109	VAVAPRAVA	15	16	4	10	45	48.83	41.67	9.23	57.27	1089	0.33	525.7	84.74	19.91	93	18.86	25.55	0.672	293.0	13.40	1.91	435.8	38.01	0.541
110	APS-397	6	8	4	4	22	47.17	22.50	4.48	29.41	1515	0.46	194.1	29.53	20.26			17.98	0.46	300.0			651.1	39.01	
111	AC-36110	15	16	6	4	41	45.75	50.00	9.95	58.80	3028	0.30	194.2	32.86	20.56	105	30.94	28.05	1.392	322.8	10.09	2.78	231.9	20.15	0.551
112	JBT-38/26	15	8	12	5	40	58.67	51.25	9.18	32.67	1368	0.50	238.9	67.13	21.56	92	14.88	22.91	0.772	312.0	12.18	1.88	404.1	29.67	0.535
113	AC-35024	15	8	10	4	37	54.67	36.67	9.13	36.48	3834	0.44	95.2	23.82	19.82	103	23.66	15.36	0.427	304.4	9.72	1.58	713.7	36.02	0.483
114	SELVIPIKIT	12	12	4	6	34	69.92	35.00	7.63	44.65	1668	0.29	267.7	45.76	19.03	100	19.26	23.33	0.907	324.0	12.05	1.94	357.2	25.72	0.505
115	JBT-38/17	24	10	12	3	46	42.75	21.67	13.59	36.90	2219	0.77	166.3	61.25	19.55	97	18.26	26.18	0.881	309.8	9.00	2.91	351.5	29.70	0.546
116	IR-50	24	10	20	5	59	47.58	17.50	15.11	39.14	1565	0.77	250.2	96.56	21.17	88		21.40	0.862	317.3	8.22	2.60	368.0	24.83	0.566
117	PS-367	12	8	6	4	30	40.67	25.00	5.70	30.08	1605	0.24	187.5	35.52	20.01	81	9.14	22.88	0.822	312.0	8.67	2.64	379.7	27.84	0.499
118	AC-35476	12	10	6	4	32	42.50	27.50	8.23	41.07	2299	0.32	178.6	35.80	20.49	100	4.8	18.83	1.198	340.5	9.25	2.03	284.3	15.72	0.597
119	JBT-38/108	12	10	6	4	32	43.83	17.25	6.87	39.83	1876	0.25	212.3	36.60	19.93			27.48	1.365	327.5	11.05	2.49	239.9	20.13	0.571
120	PS-404	6	8	2	7	23	47.58	14.17	2.92	30.45	979	0.38	311.1	29.80				25.21	0.81	299.3			370.2	31.19	
121	BPT-5204	15	6	20	4	45	47.33	75.00	9.43	23.42	1306	0.71	179.3	72.25	21.65	119	11.26	11.51	0.196	265.6	6.30	1.83	1356.2	58.79	0.415
122	JAYA	9	6	12	5	32	44.25	32.50	7.21	22.72	1056	0.70	215.2	68.29	19.41	117	15.22	25.63	0.630	292.3	8.04	3.19	464.1	40.69	0.557
123	PS-225	15	14	4	3	36	35.67	35.00	8.60	57.80	3249	0.25	177.9	26.47	20.42	105	27.28	22.70	0.952	321.0	9.70	2.34	337.2	23.84	0.615
124	JBT-37/118	9	6	10	6	31	49.50	7.50	5.48	22.43	725	0.43	309.5	75.65	20.65	80	13.54	20.34	0.645	318.8	8.37	2.43	494.0	31.52	0.506
125	JBT-38/130	21	14	8	4	47	48.17	31.67	12.00	54.65	2961	0.33	184.6	40.53	21.33	84	23.34	23.90	1.125	327.3	8.64	2.77	290.9	21.24	0.585

Sl No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
126	PS-427	18	12	10	3	43	48.83	43.34	10.92	44.10	2896	0.37	152.3	37.70	21.46	116		28.83	1.215	321.3	10.45	2.76	264.4	23.72	0.583
127	PS-379	12	6	10	2	30	45.71	27.92	6.25	22.02	2135	0.40	103.1	29.25	19.92	104	24.66	20.70	0.386	269.3	6.31	3.28	698.0	53.66	0.483
128	JBT-37/100	15	12	6	5	38	48.50	43.34	8.91	46.01	1784	0.37	257.8	49.92	21.62	104	26.92	18.61	0.479	305.0	7.52	2.47	637.4	38.90	0.512
129	PS-399	3	6	4	9	22	51.83	31.67	2.95	24.18	548	0.22	440.9	53.80	20.32	83	9.5	13.90	0.383	309.8	5.62	2.47	808.2	36.27	0.525
130	JBT-36/14	27	20	6	3	56	44.50	68.33	15.28	75.50	5131	0.34	147.1	29.79	21.35	105	40.44	20.23	0.910	324.8	8.88	2.28	357.1	22.24	0.777
131	PS-316	12	8	8	4	32	48.33	40.00	8.27	34.23	1943	0.38	176.2	42.54	19.15	104	20.48	25.78	1.175	332.6	13.28	1.94	283.1	21.94	0.565
132	IET-16348	27	18	8	4	57	53.83	50.83	16.29	70.98	3534	0.34	200.9	46.10	19.65	104	7.62	15.25	0.592	318.5	6.87	2.22	538.5	25.78	0.554
133	PS-91	9	8	6	4	27	40.17	18.33	6.17	33.02	1620	0.35	203.8	38.06	19.14	105	12.58	23.03	0.485	281.5	7.55	3.05	580.4	47.47	0.561
134	PS-332	15	12	6	5	38	47.67	26.67	8.92	46.00	1836	0.45	250.5	48.56	20.80	102	27.82	20.20	0.432	288.0	6.51	3.10	666.7	46.76	0.437
135	AC-35361	27	16	10	3	56	44.83	49.17	15.96	57.33	3485	0.43	164.5	45.80	20.85	105	9.84	20.20	0.829	321.5	8.36	2.42	388.1	24.38	0.537
136	JBT-36/114	9	8	6	5	28	35.83	15.00	5.92	30.58	1447	0.36	211.3	40.88	19.13			26.10	0.709	297.4	8.88	2.94	419.2	36.79	0.618
137	AC-35516	21	12	10	3	46	43.25	46.67	12.75	49.87	3352	0.42	148.8	38.04	21.18	107	20.82	26.83	1.065	320.5	11.45	2.34	301.1	25.20	0.546
138	S-9	3	4	4	4	15	36.33	30.83	3.08	20.20	972	0.30	207.9	31.74	20.86	81	11.06	23.38	0.571	296.0	7.12	3.28	518.8	40.97	0.543
139	AC-35517	9	8	4	5	26	36.50	14.17	5.15	34.99	1315	0.27	266.1	39.16	19.34	103	19.68	29.47	0.758	461.8	8.96	3.29	609.4	38.88	0.557
140	PS-267	9	10	8	3	26	56.83	48.30	8.20	41.80	2853	0.29	146.5	28.74	19.75	121	15.12	24.20	1.120	321.3	9.54	2.54	287.0	21.62	0.447
141	AC-35135	18	12	8	7	45	55.67	27.50	10.40	43.90	1401	0.39	313.4	74.24	20.03	105	19.2	21.41	0.589	292.8	11.15	1.92	496.7	36.33	0.502
142	JBT-36/126	12	10	6	3	31	51.08	31.67	7.63	39.33	2581	0.31	152.4	29.57	20.10	100	6.76	26.95	0.909	308.3	9.16	2.94	339.0	29.64	0.556
143	PS-396	24	18	8	4	54	50.50	47.50	13.83	65.81	3357	0.37	196.0	41.20	21.00	94	24.6	21.45	0.899	327.5	9.09	2.36	364.2	23.85	0.559
144	AC-35341	24	14	10	4	52	43.17	40.42	15.12	55.32	2739	0.77	201.9	55.18	21.23			27.58	1.72	335.8			195.5	16.06	
145	PS-250	9	10	10	3	26	43.42	25.00	4.93	38.92	3966	0.21	98.1	12.44	20.75	91	18.44	21.53	0.672	307.8	8.76	2.46	458.0	32.03	0.503
146	AC-39002	6	6	6	4	22	42.33	15.00	3.95	23.93	1282	0.33	186.6	30.80	19.77	85	17.32	19.80	0.849	325.0	6.95	2.85	382.8	23.32	0.559
147	JBT-37/89	12	12	6	3	33	44.00	23.33	7.33	44.07	2534	0.57	173.9	28.94	19.32			27.24	0.84	305.4			362.7	32.35	
148	JBT-36/76	15	16	4	8	43	63.45	35.00	9.20	62.16	1637	0.45	379.7	56.20	20.20	84	14.72	19.38	0.364	276.8	6.67	2.90	759.8	53.19	0.478
149	JBT-38/138	15	14	6	3	38	46.00	37.50	9.08	50.52	2191	0.25	230.6	41.47	20.08	110	13.38	18.85	0.830	328.8	8.99	2.10	396.2	22.72	0.556
150	AC-35027	15	12	6	3	36	45.33	38.34	9.18	44.75	2475	0.31	180.8	37.07	19.56	107	30.6	17.40	0.244	242.5	5.42	3.21	994.9	71.38	0.496

SI No	Genotype Name	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
151	AC-39004	6	8	4	3	21	42.17	13.33	4.42	29.37	1977	0.32	148.5	22.34	20.17	83	12.8	22.43	0.803	292.8	7.25	3.10	364.6	27.93	0.574
152	MTU-1010	9	6	8	4	27	40.33	20.83	5.76	26.68	1246	0.41	214.2	46.23	19.43	96	17.2	18.25	0.464	298.0	5.97	3.06	642.6	39.35	0.425
153	AC-35367	18	8	16	4	46	38.08	53.34	10.62	29.96	2106	0.55	142.3	50.42	20.35	125		20.80	0.945	324.8	8.75	2.38	343.7	22.01	0.574
154	JBT-37/126	12	14	4	5	35	41.67	33.34	7.12	54.94	2082	0.28	263.9	34.19	18.83	101	7.22	19.48	1.206	333.3	8.90	2.19	276.3	16.15	0.525
155	AC-35066	15	10	8	7	40	54.67	50.00	9.10	39.47	1217	0.76	324.3	74.78	19.94			9.21	0.22	280.8			1272.5	41.76	
156	JBT-37/111	18	12	8	3	41	45.42	30.83	10.38	47.83	3309	0.31	144.6	31.38	19.51	107	15.92	17.88	0.474	289.0	7.25	2.47	609.7	37.73	0.397
157	BR-2655	24	10	14	4	52	47.83	32.08	13.47	42.72	2419	0.50	176.6	55.67	20.06	118	23.74	28.60	1.518	328.8	12.25	2.33	216.6	18.85	0.562
158	JBT-36/139	3	4	2	5	14	37.00	10.00	1.88	16.70	982	0.24	170.1	19.18	19.33	79	14.06	18.85	0.552	309.0	7.34	2.57	559.8	34.15	0.464
159	JBT-38/116	15	10	10	4	39	52.67	76.67	9.01	33.69	1879	0.72	179.3	47.94	19.90			11.45	0.26	298.3			1130.8	43.41	
160	CH5-IR20	15	10	8	3	36	48.08	41.67	8.90	38.32	2222	0.40	172.4	40.05	19.41	104	12.24	27.23	1.152	320.3	10.25	2.66	278.0	23.63	0.600
161	PS-259	12	12	6	6	36	47.67	33.33	13.17	40.87		1.18			20.60			26.30	1.053	319.3	10.77	2.44	303.3	24.98	
162	AC-35298	18	20	4	4	46	46.50	33.33	10.38	78.44	4156	0.25	188.8	24.99	20.28	100	23.54	21.65	0.835	316.3	8.64	2.51	378.9	25.94	0.594
163	JBT-38/119	18	14	6	4	42	47.00	40.00	10.17	54.27	2518	0.40	215.5	40.38	20.53	103	27.44	25.55	0.731	304.3	9.32	2.74	416.4	34.96	0.578
164	Vandana	9	8	6	7	30	56.67	35.00	6.13	36.13	1070	0.27	337.6	57.23	19.86	104	16.4	22.51	0.579	294.9	10.78	2.09	509.7	38.92	0.508
165	PS-330	9	6	8	4	27	44.58	30.00	5.48	23.68	1185	0.32	199.8	46.27	19.63	110	3.08	20.68	0.503	297.5	6.70	3.08	591.5	41.10	0.527
166	PS-397	3	6	2	3	14	34.50	16.67	2.28	24.25	1188	0.17	204.2	19.23	20.52	74	14.74	17.93	0.439	295.3	6.48	2.77	673.3	40.88	0.564
167	PS-320	15	12	6	3	36	47.17	29.59	7.77	44.03	2756	0.25	159.8	28.19	20.13	103	28.78	19.40	0.598	310.5	8.54	2.27	519.4	32.46	0.516
168	JBT-37/4	18	14	8	3	43	68.00	33.33	10.80	50.35	3411	0.30	147.6	31.66	19.42	106	22.58	26.28	0.624	288.6	9.85	2.67	462.4	42.09	0.550
169	PS-349	12	10	6	3	31	56.67	28.33	8.16	41.05	2518	0.38	163.0	32.40	19.79	90	23.78	29.81	0.728	282.1	11.32	2.63	387.3	40.93	0.539
170	PS-370	9	6	4	7	30	41.50	21.67	5.63	22.15	2190	0.59	101.2	25.73	20.77	95	10.18	18.78	0.496	296.4	6.89	2.73	597.1	37.82	0.516
171	JBT-37/128	15	14	6	4	39	41.50	25.83	9.04	52.40	2520	0.27	207.9	35.88	20.50	99	32.5	24.63	0.541	285.8	8.47	2.91	528.7	45.56	0.432
172	PS-406	12	10	6	4	32	59.50	26.67	6.87	40.75	2108	0.29	193.3	32.58	19.12	102	31.76	16.90	0.422	284.8	7.69	2.20	675.4	40.09	0.445
173	KMR-3	27	16	10	5	58	42.83	57.50	14.72	54.52	2803	0.45	194.5	52.50	21.59	105	12.4	20.91	0.659	312.9	11.59	1.80	475.1	31.74	0.514
174	AC-35310	27	20	6	3	56	49.83	58.34	16.18	84.10	4833	0.33	174.0	33.47	19.90	105	22.8	22.15	1.098	328.8	10.41	2.13	299.5	20.18	0.629
175	JBT38/2	12	10	6	4	32	36.75	25.00	7.82	42.53	2112	0.56	201.4	37.02	19.62	103	22.68	25.33	0.77	304.5			393.9	32.76	

Sl No	Genotype	RW	TDM	R/S	DM/LA	CW	RL	RV	RW	TDM	TLA	R/S	DM/LA	R/LA	Delta	F	Yld	A	gs	Ci	T	A/T	Ci/gs	A/gs	Fv'/Fm'
176	PS-307	12	12	4	4	32	44.08	15.00	5.92	40.52	2456	0.28	165.0	24.09	18.71	98	28.38	19.15	0.690	318.5	7.68	2.50	461.9	27.77	0.502
177	JBT-39/6	15	8	20	5	49	40.42	18.33	7.72	35.20	2407	0.39	146.3	32.06	19.96	101	17.36	16.73	0.407	299.8	6.66	2.51	737.4	41.14	0.532
178	Doddabatta	27	18	8	5	58	64.00	73.33	15.28	68.28	2783	0.66	245.3	54.91	20.17			15.91	0.26	258.6			978.3	60.19	
179	AC-35004	9	8	6	5	28	44.33	30.83	6.68	33.45	1467	0.27	228.0	45.55	19.80	95	16.26	27.28	1.465	331.8	11.45	2.38	226.5	18.62	0.614
180	JBT-37/170	6	8	4	2	20	45.17	16.67	4.64	29.93	2346	0.29	127.6	19.79	19.41	84	20.84	14.10	0.272	280.8	4.57	3.09	1031.2	51.79	0.546
181	JYOTHI	27	12	16	4	59	43.25	41.67	14.02	43.20	1650	0.70	261.8	84.95	20.56	100	18.5	24.40	0.828	309.5	8.57	2.85	373.7	29.46	0.588
182	PS-319	6	2	18	3	29	35.25	22.50	4.92	12.68	734	0.74	172.9	67.02	19.85	86	16.5	15.68	0.426	304.3	5.79	2.71	714.6	36.82	0.547
183	AC-35383	21	18	6	4	49	49.83	53.33	10.98	62.22	3358	0.26	185.3	32.70	20.47	106	17	21.03	0.667	312.3	8.05	2.61	468.5	31.55	0.536
184	PS-366	21	14	8	4	47	42.17	31.67	12.62	53.78	2877	0.42	187.0	43.86	20.17	104	16.86	23.95	0.556	287.8	8.73	2.74	517.3	43.06	0.490
185	AC-39016	24	16	8	4	52	49.33	48.34	14.10	57.37	2895	0.35	198.2	48.71	20.70	112	27.08	17.33	0.655	316.0	7.22	2.40	482.3	26.44	0.562
186	PS-306	12	10	8	3	33	44.17	30.00	6.98	34.16	2369	0.27	144.2	29.45	19.61	105	14.66	20.26	0.525	291.1	9.35	2.17	554.4	38.59	0.587
187	PS-360	9	8	6	3	26	34.83	31.67	6.33	32.13	1854	0.32	173.3	34.11	19.79	101	31.7	23.65	0.849	316.5	9.83	2.41	372.8	27.86	0.596
188	AC-39021	15	14	4	4	37	45.50	17.50	9.42	52.07	2831	0.28	183.9	33.27	19.29	99	18.04	11.85	0.360	313.5	5.71	2.08	870.8	32.91	0.484
189	PS-339	27	14	10	3	54	49.00	72.50	15.32	55.23	3209	0.44	172.1	47.73	20.93	102	38.6	25.23	0.838	308.0	8.97	2.81	367.5	30.10	0.573
190	PS-401	9	12	4	6	31	48.50	44.17	6.05	39.68	1357	0.32	292.4	44.59	18.91	103	13.08	21.75	0.524	292.3	10.57	2.06	557.9	41.52	0.451
191	KRH2	18	10	10	5	43	41.50	35.00	10.25	41.08	1719	0.39	239.0	59.63	20.77	104	16.3	18.83	0.679	318.3	9.10	2.07	469.0	27.75	0.512
192	AC-39000	21	12	12	4	49	49.83	40.83	13.03	46.53	2144	0.52	217.0	60.75	20.64	101	13.42	26.13	1.574	333.0	10.77	2.43	211.6	16.60	0.561
193	AC-35417	18	18	6	4	46	65.15	60.00	10.85	62.45	3123	0.29	200.0	34.74	19.87			16.47	0.246	249.2	7.62	2.16	1011.7	66.86	0.367
194	AC-39020	27	20	6	5	58	46.50	100.00	13.73	78.50	3007	0.22	261.1	45.65	20.30	104	12.94	15.49	0.250	254.8	7.73	2.00	1021.0	62.07	0.458
195	AC-35415	21	14	8	4	47	41.83	41.67	12.00	47.85	2370	0.50	201.9	50.62	21.63	103	34.42	16.70	0.696	325.0	9.42	1.77	467.0	23.99	0.527
196	JBT-37/95	9	10	4	3	26	39.67	16.25	6.42	42.47	2193	0.26	193.6	29.26	18.84	98	13.68	24.13	0.995	324.3	8.67	2.78	325.9	24.25	0.609
197	PS-284	9	8	6	5	28	49.17	27.50	6.65	36.00	1632	0.28	220.5	40.74	19.37	104	19.52	25.65	0.711	296.4	12.68	2.02	417.0	36.09	0.513
198	AC-35290	24	8	18	6	53	37.17	34.17	11.58	32.28	1076	0.89	299.9	107.60	20.36	105	21.12	16.13	0.537	316.5	7.34	2.20	589.9	30.06	0.568
199	CR-149/20/2	9	10	6	10	35	39.08	11.67	3.58	28.97	1432	0.28	202.2	25.02	20.71	108	16.56	22.35	1.307	331.0	8.58	2.61	253.3	17.10	0.632
200	JBT-36/3	15	12	12	4	58	46.67	30.00	8.85	35.98	1938	0.41	185.7	45.66	20.15	114		26.95	1.315	324.8	11.23	2.40	247.0	20.49	0.541
201	JBT-38/25	15	10	8	5	38	44.92	26.67	7.64	38.13	1529	0.34	249.4	49.93	20.25	105	26.1	22.11	0.456	260.8	7.67	2.88	572.0	48.51	0.495
202	AC-35421	21	14	8	3	46	58.67	35.42	12.38	56.93	3952	0.30	144.1	31.33	21.39	104	15.98	14.74	0.308	281.4	5.39	2.74	913.6	47.85	0.489
203	PS-419	15	10	8	4	37	49.83	26.67	8.91	41.12	1845	0.36	222.8	48.28	19.37	101	21.86	14.64	0.394	288.6	5.81	2.52	732.8	37.17	0.385
204	PS-229	18	20	4	4	46	59.00	44.17	11.41	79.22	5348	0.24	148.1	21.33	21.08	100	23.52	18.60	0.758	324.8	8.41	2.21	428.3	24.53	0.521

Appendix III. List of selected high root type germplasms lines

Sl No	Name of Germplasm	Root length	Root volume	Root weight	TDM	R/S	TLA(M2)	DM/LA(g/m2)	R/LA (g/m2)	Delta	50% flowering	garin yd/pl
1	AC-35006	50.50	51.66	13.06	62.50	0.369	0.450	138.86	29.02	21.13	104	29.36
2	AC-35027	45.33	38.33	9.17	44.75	0.307	0.248	180.79	37.06	19.55	107	30.6
3	AC-35310	49.83	58.33	16.17	84.10	0.328	0.483	174.00	33.46	19.90	105	22.8
4	AC-35375	60.17	57.50	8.58	64.67	0.244	0.291	222.57	29.54	19.85	103	25.68
5	AC-35389	52.17	61.66	12.90	81.22	0.275	0.417	194.95	30.96	20.36	103	18.84
6	AC-35417	65.15	60.00	10.85	62.45	0.293	0.312	199.97	34.74	19.87		
7	AC-35573	62.67	51.66	14.48	43.33	0.584	0.221	196.16	65.56	20.28	104	23.34
8	AC-39000	49.83	40.83	13.02	46.53	0.522	0.214	217.03	60.74	20.64	101	13.42
9	AC-39016	49.33	48.33	14.10	57.37	0.350	0.289	198.18	48.71	20.69	112	27.08
10	AC-39020	46.50	100.00	13.72	78.50	0.223	0.301	261.09	45.65	20.30	104	12.94
11	BR-2655	47.83	32.08	13.47	42.72	0.498	0.242	176.59	55.67	20.05	118	23.74
12	C-2 vavaprava	48.83	41.67	9.23	57.26	0.331	0.109	525.66	84.73	19.91	93	18.86
13	Doddabatta	64.00	73.33	15.28	68.28	0.66	0.278	245.32	54.90	20.17		
14	IET-16348	53.83	50.83	16.29	70.98	0.336	0.353	200.85	46.10	19.65	104	7.62
15	JBT-36/14	44.50	68.33	15.28	75.50	0.340	0.513	147.14	29.78	21.35	105	40.44
16	JBT-37/4	68.00	33.33	10.80	50.35	0.298	0.341	147.59	31.65	19.41	106	22.58
17	JBT-38/116	52.67	76.67	9.01	33.69	0.72	0.188	179.31	47.94	19.90		
18	JBT-38/19	44.25	55.00	14.97	47.66	0.494	0.301	158.14	49.69	20.62	111	32.52
19	JBT-38/39	39.58	32.50	11.83	55.50	0.340	0.329	168.77	35.98	19.38	106	14.66
20	PS-339	49.00	72.50	15.31	55.23	0.443	0.321	172.11	47.72	20.93	102	38.6
21	PS-396	50.50	47.50	13.83	65.81	0.369	0.336	196.00	41.20	21.00	94	24.6
	Population mean	45.51	32.10	8.49	40.51	0.40	0.21	205.3	43.52	20.12	100.00	18.76

List of selected low root type germplasms lines

Sl No	Name of Germplasm	Root length	Root volume	Root weight	TDM	R/S	TLA(M2)	DM/LA(g/m2)	R/LA (g/m2)	Delta	50% flowering	garin yd/pl
1	AC-35024	54.67	36.66	9.13	36.48	0.437	0.383	95.15	23.82	19.81	103	23.66
2	JBT-36/64	47.33	20.83	7.87	36.80	0.335	0.227	162.29	34.73	19.07	103	22.88
3	JBT-37/126	41.66	33.33	7.11	54.94	0.277	0.208	263.92	34.18	18.83	101	7.22
4	JBT-37/134	40.91	14.58	7.10	26.20	0.371	0.197	132.81	35.99	20.12	96	14.28
5	JBT-37/5	42.16	27.08	6.58	42.26	0.278	0.230	183.82	28.63	18.77	100	15.62
6	JBT-38/10	40.00	29.16	6.06	36.28	0.278	0.181	200.42	33.51	20.19	107	10.9
7	JBT-38/148	40.66	33.33	7.20	37.71	0.315	0.190	198.66	37.92	20.65	104	21.62
8	JBT38/2	36.75	25.00	7.82	42.53	0.56	0.211	201.42	37.01	19.62	103	22.68
9	PS-379	45.70	27.91	6.24	22.01	0.396	0.214	103.11	29.24	19.91	104	24.66
10	PS-406	59.50	26.66	6.86	40.75	0.288	0.211	193.34	32.58	19.12	102	31.76
	Population mean	45.51	32.10	8.49	40.51	0.40	0.21	205.3	43.52	20.12	100.00	18.76

Appendix 4. List of Simple Sequence Repeat (SSR) markers used for genotyping rice accessions

SI No	Marker name	Chr no	Map position	Repeat Motif	Prod size (bp)	AT(°C)
1	RM165	1	197.8	(CT)13	185	63
2	RM522	1	33.9	(AAT)6	143	63
3	RM446	1	78.4	(AGA)7	169	63
4	RM283	1	31.4	(GA)18	151	63
5	RM237	1	115.2	(CT)18	130	63
6	RM315	1	165.3	(AT)4(GT)10	133	63
7	RM148	1	176.3	(TG)12	129	56.1
8	RM158	1	53.4	(GGC)9	231	NA
9	RM472	1	171.6	(GA)21	296	63
10	RM129	1	78.4	(CGG)8	205	63
11	RM576	1	51-54.2	(AT)10(GT)14	241	63
12	RM140	1	78.4	(CT)12	261	63
13	RM499	1	0	(TA)22	116	63
14	RM319	1	150.5	(GT)10	134	63
15	RM113	1	69.6	(CA)8	151	63
16	RM8135	1	181.8	(AT)123	364	55.1
17	RM113	1	81.9	(CA) ₈	151	64.9
18	RM9	1	92.4	(GA) ₁₅ GT(GA) ₂	136	54.8
19	RM5	1	94.9	(GA) ₁₄	113	55.8
20	RM128	1	134.8	(GAA) ₉	157	61.1
21	RM525	2	143.7	(AAG)12	131	63
22	RM526	2	136.3	(TAAT)5	240	63
23	RM240	2	158	(CT)21	132	63
24	RM263	2	127.5	(CT)34	199	63
25	RM221	2	143.7	(TC)4T3C3(TC)(CT)2	192	63
26	RM209	2	32.7	(CT)18	134	63
27	RM207	2	191.2	(CT)25	118	63
28	RM208	2	186.4	(CT)17	173	63
29	RM191	2	13.36	(AG)7	227	63
30	RM318	2	150.8-154.7	(GT)15	140	56.1
31	RM174	2	47.5	(AGG)7(GA)10	208	63
32	RM138	2	196.8	(GT)14	233	63
33	RM145	2	49.8	(GA)31	215	54.8
34	RM154	2	4.8	(GA)21	183	63
35	RM233	2	16.3	(CT)20	162	54.8
36	RM497	2	150.8	(CAC)11	213	63
37	RM166	2	183	(T)12	321	55.1
38	RM110	2	6.9	(GA) ₅	156	61.1
39	RM106	2	123.2	(GAA) ₅	297	65.9
40	RM6	2	154.7	(AG) ₁₆	163	55.8
41	RM112	2	166	(GAA) ₅	128	64.2
42	RM503	3	153.9	(CA)59(TA)26	268	55.1
43	RM442	3	224.2	(AAG)10	257	63
44	RM203	3	122.4	(AT)21	203	NA
45	RM183	3	38.2	(GA)8	222	NA
46	RM571	3	205.4	(GT)11(AG)13	188	63
47	RM114	3	203.1	(GA)7	209	63
48	RM55	3	168.2	(GA)17	226	54.8
49	RM347	3	0	(GGC)5(AT)7	207	54.8
50	RM175	3	15.7-23.9	(CCG) ₈	95	62.9
51	RM411	3	127.9	(GTT) ₇	110	55.9
52	RM135	3	157.3	(CGG) ₁₀	131	65.9
53	RM186	3	168.2	(CGG) ₅	124	65.9
54	RM559	4	155.8	(AACA)6	160	63

Sl No	Marker name	Chr no	Map position	Repeat Motif	Prod size (bp)	AT(°C)
55	RM307	4	0	(AT)14(GT)21	174	63
56	RM335	4	21.5	(CTT)25	104	61.2
57	RM317	4	118.3	(GC)4(GT)18	155	63
58	RM349	4	160	(GA)16	136	63
59	RM303	4	116.9	[AC(AT)2-10]9(GT)7(ATGT)6	200	54.8
60	RM185	4	50.8	(AGG) ₉	197	64.9
61	RM119	4	76.1	(GTC) ₆	166	64.2
62	RM252	4	99	(CT) ₁₉	216	54.8
63	RM124	4	150.1	(TC) ₁₀	271	65.9
64	RM241	4	106.2	(CT) ₃₁	138	52.7
65	RM163	5	75.4	(GGAGA)4(GA)11C(GA)20	124	63
66	RM440	5	92.7	(CTT)22	169	63
67	RM164	5	80.1	(GT)16TT(GT)4	246	63
68	RM169	5	77.1	(GA)12	167	63
69	RM274	5	126.6	(GA)15-7-(CGG)5	160	63
70	RM173	5	99.8	(GA)9	186	NA
71	RM178	5	118.5	(GA)5(AG)8	117	63
72	RM305	5	96.9	(GT)4+DEGENER	203	63
73	RM430	5	78.7	(GA)25	173	63
74	RM249	5	50.2	(AG)5A2(AG)14	121	63
75	RM334	5	141.8	(CTT)20	182	NA
76	RM153	5	1.15	(GAA) ₉	201	56.8
77	RM13	5	30	(GA) ₆ -(GA) ₁₆	141	54.8
78	RM146	5	78.7	(CT) ₁₁ -(CT) ₇	345	59.8
79	RM161	5	96.9	(AG) ₂₀	187	65.9
80	RM435	6	2.2	(ATG)7	166	63
81	RM528	6	121.6	(AGAT)9	232	63
82	RM461	6	139.9	(AAAC)6	195	63
83	RM454	6	99.3	(GCT)8	268	63
84	RM30	6	125.4	(AG)9A(GA)12	105	63
85	RM204	6	25.1	(CT)44	169	63
86	RM225	6	26.2	(CT)18	140	63
87	RM103	6	143.7- 14907	(GAA)5	336	63
88	RM121	6	43.8	(CT)7	170	63
89	RM141	6	143.7	(CT)12	136	63
90	RM133	6	0	(CT)8	230	55.1
91	RM314	6	33.6	(GT)8(CG)3(GT)5	118	63
92	RM510	6	20.8	(GA)15	122	63
93	RM133	6	0	(CT) ₈	230	64.9
94	RM115	6	33.6-35.3	(AG) ₇	190	64.9
95	RM111	6	35.3	(GA) ₉	124	64.9
96	RM136	6	51.2	(AGG) ₇	101	64.9
97	RM162	6	108.3	(AC) ₂₀	229	65.9
98	RM501	7	30.1-34.7	(TC)10(TA)21	179	63
99	RM432	7	43.5	(CATC)9	187	63
100	RM455	7	65.7	(TTCT)5	131	63
101	RM420	7	115.3-116.6	(AAAT)7	197	63
102	RM351	7	95	(CCG)9(CGAAG)4	134	NA
103	RM298	7	35.6	(GT)10	135	55.1
104	RM172	7	115.3	(AGG)6	159	63
105	RM325A	7	33.3	(CAT)4TAG(CAT)5	201	63
106	RM192	7	0.8	(TGG)5	267	63
107	RM180	7	5.4	(ATT)10	110	63
108	RM248	7	144.1	(CT)25	102	63

Sl No	Marker name	Chr no	Map position	Repeat Motif	Prod size (bp)	AT(°C)
109	RM182	7	61	(AT) ₁₆	346	63
110	RM320	7	62.4	(AT) ₁₁ GTAT(GT) ₁₃	167	63
111	RM473	7	124.6	(TCTA) ₁₄	97	55.1
112	RM82	7	4.4-17.7	(TCT) ₁₁	186	49.7
113	RM125	7	24.8	(GCT) ₈	127	64.9
114	RM195	7	80.7	(GA) ₉ -(CT) ₈	311	63.9
115	RM118	7	96.9	(GA) ₈	156	64.9
116	RM447	8	124.6	(CTT) ₈	111	63
117	RM281	8	128.6	(GA) ₂₁	138	63
118	RM264	8	128.6	(GA) ₂₇	178	63
119	RM531	8	90.3	(AT) ₁₅	128	63
120	RM310	8	57	(GT) ₁₉	111	NA
121	RM223	8	80.5	(CT) ₂₅	165	55.1
122	RM152	8	9.4	(GGC) ₁₀	151	50.8
123	RM38	8	28	(GA) ₁₆	250	56.1
124	RM149	8	103.7	(AT) ₁₀	253	62.1
125	RM316	9	1.8	(GT) ₈ -(TG) ₉ (TTTG) ₄ (TG) ₄	192	63
126	RM219	9	11.7	(CT) ₁₇	202	63
127	RM434	9	57.7	(TC) ₁₂	152	63
128	RM444	9	3.3	(AT) ₁₂	162	56.1
129	RM201	9	81.2	(CT) ₁₇	158	63
130	RM105	9	32.1	(CCT) ₆	134	57.8
131	RM242	9	73.3	(CT) ₂₆	225	54.4
132	RM205	9	114.7	(CT) ₂₅	122	56.4
133	RM215	9	99.4	(CT) ₁₆	148	61.4
134	RM271	10	59.4	(GA) ₁₅	101	57.5
135	RM311	10	25.2	(GT) ₃ (GTAT) ₈ (GT) ₅	179	NA
136	RM222	10	11.3	(CT) ₁₈	213	53.4
137	RM216	10	17.6	(CT) ₁₈	146	50.4
138	RM184	10	58.3	(CA) ₇	219	59.8
139	RM171	10	73	(GATG) ₅	328	63.9
140	RM228	10	93-96.3	(CA) ₆ (GA) ₃₆	154	54.1
141	RM286	11	0	(GA) ₁₆	110	63
142	RM287	11	68.6	(GA) ₂₁	118	55.1
143	RM229	11	77.8	(TC) ₁₁ (CT) ₅ C ₃ (CT) ₅	116	63
144	RM536	11	55.1	(CT) ₁₆	243	63
145	RM552	11	40.6	(TAT) ₁₃	195	NA
146	RM206	11	102.9	(CT) ₂₁	147	NA
147	RM116	11	26.5	(CT) ₉	258	64.9
148	RM202	11	54	(CT) ₃₀	189	54.0
149	RM224	11	120.1	(AAG) ₈ (AG) ₁₃	157	54.0
150	RM187	11	85.7-93.6	(AT) ₂₉ (GT) ₇	146	55.8
151	RM512	12	21.7	(TTTA) ₅	214	63
152	RM519	12	62.6	(AAG) ₈	122	63
153	RM453	12	28.2	(TC) ₁₀	178	63
154	RM313	12	65.5	(GT) ₆ CA(CG) ₅ -6-(GT) ₈	111	63
155	RM28048	12	11.31	(CGC) ₈	93	56.1
156	RM179	12	49.5	(TG) ₇	190	64.9
157	RM235	12	102.8	(CT) ₂₄	124	54.0
158	RM12	12	109.1	(GA) ₂₁	184	54.0