MOLECULAR CHARACTERIZATION OF ROOT GENES IN RICE (*Oryza sativa* L.) AND SORGHUM (*Sorghum bicolor* L.)

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PALB 4061

DEPARTMENT OF PLANT BIOTECHNOLOGY

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Affectionately
Dedicated To,
My Family

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU – 560 065

CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR CHARACTERIZATION OF ROOT GENES IN RICE (*ORYZA SATIVA* L.) AND SORGHUM (*SORGHUM BICOLOR* L.)"** submitted in partial fulfillment of the requirement for the award of degree of **DOCTOR OF PHILOSOPHY (Agriculture)** in **PLANT BIOTECHNOLOGY** of the University of Agricultural Sciences, GKVK, Bengaluru is a bonafide record of research work done by **Ms. KRUPA, K. N., ID No. PALB 4061**, during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
October, 2017

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October, 2017 ***(Krupa, K. N.)***

"**Molecular characterization of root genes in rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.)**."

KRUPA, K. N.

THESIS ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important cereal crop, and a primary source of food for more than half the world's population. As a very important part of rice plant, root system plays multiple roles in rice growth. Five genotypes each from rice and sorghum were grown in the field during *Kharif*-2015 and Summer-2016. A novel PVC pipe-root experimentation for two seasons revealed significant differences among genotypes for several traits wherein MTU1001, AM72, Moroberekan in rice; MLHT, Roagro and CSH-14 in sorghum, exhibited better performance. The association among root characters was stronger during both season in both crops. Grain yield showed highly significant and positive association with maximum root length and root number indicating their important role for increased yield. Highest values for root length was 97.75 cm (MTU1001) in rice and 154.25 cm (Roagro) in sorghum. Highest values for grain yield/p was 22.40 g (AM72) in rice and 121.92 g (SJH-1) in common bean. The path coefficient analysis revealed importance of direct effects of root length and indirect contributions of total plant height which resulted in positive association of high order with grain yield. Molecular marker analysis was done with sixty two markers, as expected, most of them showed amplicons in all the genotypes of rice. However, some markers are amplified only in some sorghum genotypes. This indicates, rice SSR primers are operating efficiently in sorghum. All rice primers were monomorphic at agarose gel level in sorghum. Sequencing the amplicons of primers RM166 and RM149 showed the variation at nucleotide level.

October, 2017

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Signature of major advisor
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ಭತ್ತ (ಒರೈಜಾ ಸಟೈವಾ) ಮತ್ತು ಜೋಳ (ಸೋರ್ಗಮ್ ಬೈಕಾಲರ್)ದ ಬೇರಿನ ವಂಶವಾಹಿಗಳ ಆಣ್ವಿಕ ಲಕ್ಷಣಗಳು

ಕೃಪಾ. ಕೆ. ಎನ್.,

ಪ್ರಭಂದ ಅಮೂರ್ತ

ಭತ್ತವು (ಒರೈಜಾ ಸಟೈವಾ) ಅತ್ಯಂತ ಮುಖ್ಯವಾದ ಏಕದಳ ಬೆಳೆಯಾಗಿದ್ದು ಮತ್ತು ವಿಶ್ವದ ಜನಸಂಖ್ಯೆಯ ಅರ್ಧಕ್ಕಿಂತ ಹೆಚ್ಚು ಜನರ ಮೂಲ ಆಹಾರ ಪದಾರ್ಥವಾಗಿದೆ. ಭತ್ತ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಬೇರಿನ ವ್ಯವಸ್ಥೆಯು ಅನೇಕ ಪಾತ್ರವಹಿಸಿದ್ದು ಎಲ್ಲಾ ರೀತಿಯ ಭತ್ತ ಸಂಶೋಧನೆಗಳಿಗೂ ಇದು ಸಂಬದ್ದಿಸಿದ್ದಾಗಿದೆ. ಭತ್ತ ಮತ್ತು ಜೋಳದ ತಲಾ ಐದು ತಳಿಗಳನ್ನು ೨೦೧೫ ರ ಮುಂಗಾರು ಮತ್ತು ೨೦೧೬ ರ ಬೇಸಿಗೆಯಲ್ಲಿ ಭತ್ತ ಮತ್ತು ಜೋಳವನ್ನು, ಬೇರಿನ ಹೊಸ ರೀತಿ ಸಂಶೋಧನೆಯಾದ ಪಿ.ವಿ.ಸಿ ಪೈಪ್ ನಲ್ಲಿ ಬೆಳೆಯುವ ಪ್ರಯೋಗ ಮಾಡಲಾಯಿತು. ಎರಡು ಋತುಗಳ ಪ್ರಯೋಗದಿಂದ ಭತ್ತದ ತಳಿಗಳಾದ ಎಮ್ ಟಿ ಯು ೧೦೦೧, ಎ ಎಮ್ ೭೨, ಮೊರೋಬೇರಿಕನ್ ಗುಣಲಕ್ಷಣ ಗಳಲ್ಲಿ ಗಮನಾರ್ಹ ವ್ಯತ್ಯಾಸ ಕಂಡುಬಂದಿತು, ಜೋಳದ ತಳಿಗಳಾದ ಎಮ್ ಎಲ್ ಹೆಚ್ ಟಿ, ರೋಆರ್ಗೋ ಮತ್ತು ಸಿ ಎಸ್ ಹೆಚ್ ೧೪ ಉತ್ತಮ ವಾಗಿ ಬೆಳೆಯಲು ಅರ್ಹ ಎಂದು ತಿಳಿದು ಬಂದವು. ಎರಡು ಋತುಗಳಲ್ಲೂ ಸಹ ಎರಡು ಬೆಳೆಗಳ ಬೇರಿನ ಸಂಭದವು ಬಹಳ ಪ್ರಭಲವಾಗಿತ್ತು. ಧಾನ್ಯದ ಇಳುವರಿಯು ಹೆಚ್ಚಿನ ಸಂಖ್ಯೆಯ ಬೇರು ಮತ್ತು ಹೆಚ್ಚು ಉದ್ದ ಇರುವ ಬೇರಿಗೆ ಸಕಾರಾತ್ಮಕ ಸಂಭಂದ ಇದ್ದು ಹೆಚ್ಚಿನ ಇಳುವರಿಗೆ ಸಹಾಯಕ ವಾಗಿವೆ ಎಂದು ಕಂಡುಬಂದಿತು. ಅತೀ ಉದ್ದವಾದ ಬೇರು ಭತ್ತದ ಎಮ್ ಟಿ ಯು ೧೦೦೧ ಅಲ್ಲಿ ೯೭.೭೫ ಸೆಂ.ಮೀ ಕಂಡುಬಂದರೆ ಜೋಳದ ರೋಆರ್ಗೋ ೧೫೪. ಸೆಂ.ಮೀ ಕಂಡುಬಂದಿತು. ಹೆಚ್ಚಿನ ಧಾನ್ಯದ ಇಳುವರಿಯು ಭತ್ತದ ಎ ಎಮ್ ೭೨ ಅಲ್ಲಿ ೨೨.೪೦ ಗ್ರಾಂ ಇದ್ದರೇ ಜೋಳದ ಎಸ್ ಟಿ ಹೆಚ್-೧ ಅಲ್ಲಿ ೧೨೧.೯೨ ಗ್ರಾಂ ಇತ್ತು. ಮಾರ್ಗ ಗುಣಾಂಕ ವಿಶ್ಲೇಷಣೆಯಿಂದ ನೇರವಾಗಿ ಸಸ್ಯದ ಬೇರಿನ ಉದ್ದ ಪರೋಕ್ಷವಾಗಿ ಸಸ್ಯದ ಎತ್ತರ ಧಾನ್ಯದ ಉನ್ನತ ಇಳುವರಿಗೆ ಧನಾತ್ಮಕವಾಗಿ ಸಂಭಂದ ಪಟ್ಟಿವೆ ಎಂದು ತಿಳಿದುಬಂದಿತು. ಅರವತ್ತೆರಡು ಗುರುತುಕಾರಕಗಳಿಂದ ಆಣ್ವಿಕ ಗುರುತುಕಾರಕಗಳ ವಿಶ್ಲೇಷಣೆ ನಡೆಸಲಾಯಿತು ಸಾಮಾನ್ಯವಾಗಿ ಭತ್ತದ ಎಲ್ಲ ತಳಿಗಳಲ್ಲಿ ಯಾಂಪ್ಲಿಕಾನ್ ಗಳು ಕಂಡುಬಂದವು ಆದರೆ ಜೋಳದ ಕೆಲವು ತಳಿಗಳಲ್ಲಿ ಮಾತ್ರ ವರ್ಧನೆ ಕಂಡುಬಂದಿತು.ಇದರಿಂದ ಭತ್ತದ ಎಸ್ ಎಸ್ ಆರ್ ಪ್ರೈಮೆರ್ ಗಳು ಜೋಳದಲ್ಲಿ ಸಮರ್ಥವಾಗಿ ಕಾರ್ಯ ನಿರ್ವಹಿಸುತ್ತವೆ ಎಂದು ತಿಳಿದುಬಂದಿತು. ಭತ್ತದ ಎಲ್ಲಾ ಪ್ರೈಮರ್ ಗಳು ಜೋಳದ ಅಗರೊಸ್ ಜೆಲ್ ಹಂತದಲ್ಲಿ ಏಕರೂಪವಾಗಿರುವುದು ಕಂಡುಬಂದಿತು. ಭತ್ತದ ಅರ್ ಎಮ್ ೧೬೬ ಮತ್ತು ಆರ್ ಎಮ್ ೧೪೯ ಗಳ ಯಾಂಪ್ಲಿಕಾನ್ ಅನುಕ್ರಮಣಿಕೆಯಲ್ಲಿ ನುಕ್ಲಿಯೋಟೈಡ್ ಮಟ್ಟದಲ್ಲಿ ವ್ಯತ್ಯಾಸ ಹೊಂದಿದ್ದು ತಿಳಿದುಬಂದಿತು .

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ಗಾ. ಕೃ. ವಿ. ಕೆ. ಬೆಂಗಳೂರು -೬೫

ಪ್ರಧಾನ ಸಲಹೆಗಾರರ
(ಹೆಚ್. ಈ. ಶಶಿಧರ್)

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I INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important and nutritious cereal crop belonging to the family Poaceae, which contributes significantly to global food security and is the staple food for more than half of the world's population. Rice was probably first domesticated in the Yangtze River Valley in China, about 7,000 years ago, after which it spread to other parts of Asia (Khush, 1997). More than 90 % of rice produced in Asia, where China and India being the lead producer (Kumar *et al*., 2011). It has occupied the central position in Indian agriculture with 24 % of gross cropped area of the country. It contributes 42 % of total food grain production and 45 % of total cereal production of the country (Chethana *et al*., 2016).

The slogan 'Rice is life' is most appropriate for India as it is the means of livelihood for millions of rural households of India. India has the largest acreage under rice with area of 44.1 million hectare and with a production of about 105.5 million tonnes stands second after China [208.2 million tons (FAO, 2014)], and productivity is 2391 Kg/ha. In Karnataka is one of the major rice growing states in India. In Karnataka rice is cultivated in an area of 10 lakh ha with a production of 25.73 lakh tonnes and productivity of 2573 Kg/ha (Indiastat, 2014-15).

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

Water is the critical and most important factor in rice production. The 70 per cent of the world's food growing areas turn increasingly parched (IRRI, 2009), so that main challenge for rice producers is to develop a strategy to raise yields of the water-intensive crop. The situation is more aggravated due to the huge loss of crop yield as a result of

different abiotic stresses. Water stress, one of the top most abiotic stresses, imposes limitation to the growth and development of rice plant causing yield losses of more than 50 per cent (Das and Rao, 2015).

Plants have evolved different adaptive mechanism to escape, avoid/tolerate water stress. Root system form one of the most important components of drought resistance and determine yield. Despite significant genetic variability in root traits, genetic improvement for root traits using conventional selection based on phenotype is difficult (O'Toole, 1989)

Rice is the obvious choice for the first whole genome sequencing of a cereal crop. The rice genome is well mapped and well characterized, and it is the smallest of the major cereal crop genomes at an estimated 400 to 430 Mb. The next largest genome of an important cereal crop is that of sorghum, at 750 to 770 Mb, and the wheat genome is nearly 37 times the size of the rice genome at close to 16,000 Mb (Chapman *et al*., 2015).

Sorghum and rice belongs to family Poaceae. *Sorghum bicolor* is a widely grown cereal crop, particularly in Africa, ranking 5th in global cereal production. Sorghum's genome is relatively small (~730 M) and simple (10 chromosomes, diploid) compared to other C_4 crops in the Poaceae subfamily, such as maize and sugarcane (Dillon *et al*., 2007 and Luo *et al*., 2016).

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major crops of the world. It is known under variety of names such as, great millet and guinea corn in West Africa, Kafir corn in South Africa dura in Sudan and jowar in India. It is originated in Africa. The largest diversity of cultivated and wild sorghum is in Africa (Doggett, 1988). According to Vavilov, Indian subcontinent is considered to be secondary centre of origin of sorghum (Dorofeev, 1992).

Sorghum performance under water-limited conditions would be more effective if drought tolerance traits such as stay-green, waxy nature, water use efficiency (WUE), prolific root system are selected in addition to grain yield (Van Oosterom *et al.*, 1996). It is an ideal crop for growing on marginal lands for food, feed, and energy needs of

millions of resource poor people. Therefore, it is an important crop for food security in the semi-arid and tropical zones including East-West Africa and Asia. Global cultivation of sorghum covers an area of 43.73mha with annual production of 60mt (Sasaki and Baltazar, 2009).

Sorghum is usually grown in areas with low rainfall, where it is difficult to grow other food crops and feed grains. Its ability to adapt to a challenging situation makes sorghum an important food and feed crop in the arid and semi-arid regions of the world (Crasta *et al*., 1999). Adaptation of sorghum crop to a range of environmental conditions in semi- arid regions has resulted in the evolution of extensive genetic variation for drought tolerance (Blum, 1979; Doggett, 1988), which makes it an excellent model crop of choice for studying the genetic and physiological mechanism of drought tolerance. Its relatively small genome size and extensive genome synteny with other cereals were main considerations to sequence its genome (Blum, 2005).

It has been estimated that rice diverged from the common ancestor of sorghum and maize approximately 50 million years ago (Paterson, 2004). Sorghum-rice alignments based on the completely sequenced *S. bicolor* and *O. sativa* genomes, demonstrate high levels of DNA conservation between the two species. In addition, the number and sizes of sorghum gene families are similar to those of Arabidopsis and rice. It has been observed that 39.9 % of rice sorghum aligned sequences are conserved at the 70 %/100 bp level, and 77.5 % of the length of sorghum exon sequences overlap with those of rice (Paterson and Bowers, 2009; Yu *et al*., 2002; Matsumoto *et al*., 2005).

Drought is a major abiotic stress that limits rice productivity in rainfed, upland ecosystems (Bimpong *et al*., 2011) and worldwide, drought affects approximately 27 million ha of rainfed (IRRI, 2011). In India, area under rice cultivation remained stagnant and even declined in the recent years due to water availability. Drought reduces yield by 15-50 per cent depending on the stress intensity and crop growth period at which the stress occurs in rice (Srividhya *et al*., 2011 and Verulkar *et al*., 2010). Eastern India, comprising Jharkhand, Orissa, and Chhattisgarh alone accounts loss of about 40 per cent of the total rice production due to severe drought (Pandey and Bhandari, 2009).

Developing high yielding and drought resistant varieties for rainfed area is priority for improving rainfed rice production.

Sorghum is considered as a drought tolerant crop with deep roots that are assumed to play a key role in its drought adaptation. Although, several drought-related studies have been carried out with sorghum, surprisingly limited work has been done on the roots. Studies that have presented evidence of genotypic variation for root traits (Mayaki *et al*., 1976; Jordan *et al*., 1979), focused on only a few breeding lines with a limited genetic base. Genotypic variations for root traits have been found in other studies using solution culture (Blum *et al*., 1977), or in small pots (Nour *et al*., 1978). Salih *et al*. (1999) showed that a drought tolerant sorghum line possessed roots at least 40 cm deeper than a drought sensitive one. Due to the important role played by roots in plant growth, there is a need to conduct more studies on sorghum roots.

Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants (Zhang *et al*., 2012). The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress (Wang *et al*., 2011). A better understanding of the complex physiological mechanisms underlying drought response is important to improve rice yields under water-limited environments.

Roots are the hidden half of the plant and are essential organs for exploring and exploiting soil resources, such as water and mineral nutrients (MacMillan *et al*., 2006). Root traits are key component in rice plant adaptation to drought stress (Courtois *et al*., 2009). Root traits related to drought response are complex and controlled by many genes, each with a small genetic effect (Sharma *et al*., 2011). Different root architecture ideotypes that are adapted to different soil mineral nutrient balances or water statuses have been proposed. Roots develop primarily underground and are inaccessible to direct observation. This limitation partly explains why root architecture is not a widespread breeding objective. Because of this lack of past investment, there is room for improvement of rice root systems.

An improved root system can be obtained more easily using indirect selection methods, such as marker-assisted selection. It is therefore necessary to identify the main genetic determinants governing root development. Root development is a complex process that involves constitutive and adaptive mechanisms and regulatory correlations with the shoot part of the plant.

Evolution of roots was a fundamental development that enabled plants to migrate from aquatic to terrestrial habitats. In spite of great importance of roots and long history of root research, there remains much to be learned about development and function.

Root system morphology is one of the important components of drought resistance. The root system plays an important role in the regulation of water uptake and extraction from deep soil layers. Large genetic variation in root morphology has been reported in germplasm adapted to different agro-ecological conditions. The difference between shallow-rooted and deep-rooted varieties lies in root penetration of the soil layers deeper than 30cm from the surface. Root characteristics are cumbersome to evaluate when compared to shoot or above-ground characters and sometimes results in their damage while phenotyping, leading to erroneous values (Chaitra *et al*., 2006).

Root system consists of different component such as seminal, nodal and lateral roots with a characteristic branching order. These hierarchical components differ in age, morphological/physiological features and thus respond developmentally to various soil conditions. Yamauchi *et al*. (1996) pointed out that phenotypic plasticity in the root system structure plays a key role in stress tolerance of crop plants. Fukai and Cooper (1995) proposed that genotypic variation in the root system as an avenue to improving rice drought tolerance whereby the water capturing ability of the plants could be enhanced.

Adventitious roots constitute the bulk of the fibrous root system in cereals. Compared with the current understanding of shoot development, knowledge of the molecular mechanisms of development of the adventitious roots of cereals is limited (Liu *et al*., 2005).

Recent studies have demonstrated that plants are able to respond to temporally and spatially dynamic changes in resource availabilities (Shemesh *et al*., 2010). The development of a deep and extensive root system is a drought-adaptation strategy for plants. Upland rice usually has a deep and thick root system, which allows the crop to satisfy its water requirements under upland conditions (Nguyen *et al*., 1997). Therefore, it may be possible to enhance the drought tolerance of rice by introducing such root traits to increase rice production under upland/aerobic growth conditions. It is difficult to measure root traits under field conditions and thus these have rarely been used as parameters in breeding programs. Marker-aided selection (MAS) based on the accurate mapping of root traits may make it possible to develop a water-stress tolerant root system-oriented breeding program (Zheng *et al*., 2003). The work published by Steele *et al*. (2006) is an example, where marker-assisted back-crossing (MABC) breeding programme has been employed to improve the root morphological traits to increase drought tolerance in Kalinga III, an Indian upland rice variety.

Selection and breeding for desirable root traits associated with drought tolerance have been practiced in rice and the differential response of rice genotypes to drought has been related to root system characters (Steele *et al*., 2006, Kanbar and Shashidhar, 2011). It is recognized that a deeper, thicker and more branched root system with a high root to shoot ratio can enhance the tolerance of rice to water deficits (Gowda *et al*., 2011). Among the root morphological traits, maximum root length, root dry weight, root volume, root to shoot weight and length ratios are associated with drought tolerance in upland rice (O'Toole, 1981; Yoshida and Hasegawa, 1982; Babu *et al*., 2003 and Kanbar *et al*., 2009).

It has been a very difficult task to breed varieties with improved root traits related to drought resistance due to labour-intensive phenotypic procedures (Ingram *et al*., 1994). To circumvent the screening problems, molecular genetic markers could be employed to identify the genotypes having desired root characteristics in a breeding program aiming at development of varieties with improved drought resistance. The developments in genome mapping, sequencing, and functional genomic research have provided powerful tools for investigating the genetic and molecular bases of these quantitative traits. Dissection of the genetic bases of these traits based on molecular marker linkage maps resolved

hundreds of quantitative trait loci (QTLs) for these traits. Development of molecular genetic markers and their use in quantitative trait loci (QTLs) analysis has become a powerful approach for studying the inheritance of complex traits and helps for improving drought resistance in crop plants (Suji *et al*., 2012).

Molecular markers are now widely used to track loci and genome regions in crop breeding programs, as large numbers of molecular markers that are tightly linked to specific traits are available in most major crop species. Molecular markers are essential for mapping genes of interest, marker-assisted breeding, and cloning genes using mapping-based cloning strategies, of the various classes of existing markers, microsatellites or Simple sequence repeats (SSR) have emerged as the markers of choice for plant breeding applications (Miah *et al.*, 2013).

Microsatellites are simple repeated motifs consisting of 1 to 6 base pairs, and they can be found in both coding and non-coding regions. The primary advantage of microsatellites (SSR) as genetic markers is that they are inherited in a Mendelian fashion as codominant markers. Furthermore, high polymorphism rates, high abundance and a broad distribution throughout the genome have made microsatellites one of the most popular genetic markers for use in plant breeding programs (Miah *et al.*, 2013).

Keeping this in view, the present investigation entitled "**Molecular characterization of root genes in rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.)**." The experiments will be conducted at the fields of Aerobic Rice Research Laboratory at the Department of Plant Biotechnology, University of Agricultural Sciences, GKVK campus, Bangalore, during 2015-16 with the following objectives.

Objectives of investigation

1. Discern the extent of variation for root morphological characters in Rice and Sorghum,
2. Compare sequence variation for root related genetic materials across crops
3. Establish associations between variations at the sequence with phenotypic data and mine multiple alleles.

II REVIEW OF LITERATURE

In this chapter available literature pertaining to the present investigation has been reviewed and presented under the following headings.

1. Rice statistics and production
2. Aerobic rice
3. Importance of root system
4. Importance of phenotyping
5. Genetic architecture of root system
6. Importance of root genetic improvement
7. Molecular genetics of rice root
8. Molecular markers studies
9. Statistical tools for morphological data and SSR Analysis
10. Sorghum
11. Association among root and shoot parameters in rice and other crops
12. Bioinformatics resources

2.1 Rice statistics and production

Rice is one of the main pillars of food security in India. Its improvement for higher yield in sustainable agriculture system is also vital to provide energy and nutritional needs of growing world population, expected to reach more than 9 billion by 2050 Agarwal *et al*. (2016). Rice has been cultivated for more than 7,000 years (Yunfei *et al*., 2007; Zong *et al*., 2007 and Todaka *et al*., 2012). Rice is the major staple crop of nearly half of the world's population and accounts for around 23 % of the global calorie intake (Bernier *et al*., 2009). It supplies 35 to 60 % of the total calorie intake at any given day in Asia, where approximately 90 % of world's rice is produced and consumed (Zeigler and Barclay, 2008; Khush, 2004).

Rice is the most important crop for human consumption, with production on over 161 million hectares yielding almost 600 million tons annually (FAO, 2010). Global rice production has risen steadily in the last five decades from 150 million tons in 1960 to 678 million tons in 2009, thanks in large part to the rice Green Revolution in Asia. But most of this increase came from irrigated areas, where yield growth is now stagnating. Overall, there is an estimated global need for an additional 116 million tons of rice by 2035 as compared to 439 million tons production in 2010 (Seck *et al*., 2012). India is among the top three producers with China and Indonesia.

Rice is grown in more than a hundred countries, with a total harvested area in 2009 of approximately 158 million hectares, producing more than 700 million tons annually (IRRI, 2011). Rapid growth in human population throughout the world is boosting demand for a corresponding increase in grain yield and there is need to increase production 50 per cent more by 2025 (Khush, 2001 and Liang *et al*., 2010). For rice consuming countries there is need to produce 40 per cent more rice by 2030 (Khush, 2005 and Zhu *et al*., 2010).

2.2 Aerobic rice

The term “Aerobic rice”, was used for the first time by International Rice Research Institute (IRRI). Aerobic rice refers to a cultivation system where rice is seeded in well tilled levelled fields with uniform slope under unpuddled conditions. Crop is cultivated under aerobic conditions with no standing water throughout the season. Aerobic rice combines the drought-resistant characteristics of upland varieties (grown on non-flooded sloping islands) with the high-yielding traits of lowland varieties (grown in irrigated, flooded fields). Therefore, aerobic rice is considered "improved upland rice" in terms of yield potential and "improved lowland rice" in terms of drought tolerance. It is responsive to high inputs, can be rainfed or irrigated and can tolerate occasional flooding also (Bouman and Tuong, 2001). It can be spaced relatively wide enabling each plant to establish and flourish well. The crop can be irrigated once in 5-10 days, depending on the water holding capacity of the soil.

Aerobic rice is an important concept that aims at developing drought resistant and drought tolerant rice varieties. The variety, Anagha (BI 33) is one such product which has been carefully selected over years of phenotyping from the cross of Buddha and IR64 (www.aerobicrice.in).

Rice is a semi-aquatic cereal crop grown in diverse climatic conditions. It is one of the most water consuming crops which receive an estimated 34-43 % of the total world's irrigation water, or 24-30 % of the total world's freshwater withdrawals (Bouman *et al*., 2007). Aerobic rice is a new method of cultivating rice in less water than traditional flooded condition. Aerobic rice varieties are developed by crossing lowland varieties with upland varieties and cultivated in irrigated but non-flooded and non-puddle soils (Bouman *et al*., 2002; 2005).

Aerobic rice varieties have the ability to maintain rapid growth in soils with moisture content at or below field capacity, and can produce yields of 4-6 t/ha with a moderate application of fertilizers under such soil water conditions. Aerobic rice can save as much as 50 % of irrigation water in comparison to lowland rice (Parthasarathi *et al*., 2012) and is highly productive (George *et al*., 2002; Kato *et al*., 2009).

BI 33 (Anagha), a drought tolerant high yielding aerobic rice variety (up to 6.9 t/ha.), is one such outcome of a study developed in University of Agricultural Sciences, GKVK, Bangalore (www.aerobicrice.in). It has manifested high degree of drought resistance and gives better yield, comparable to improved varieties when sufficient moisture is provided. Main properties of tolerance was due to its impactful root traits such as deep roots, high root numbers and dense root hairs which are capable of extracting moisture from deeper layers of soil column and from the micropores of soil. Its growth rate and biomass accumulation efficiency is recorded to be high (Gowda, 2010).

Root characteristics, such as, density, length, and thickness (Yadav *et al.*, 1997) and greater root penetration (Clark *et al*., 2000) are important for aerobic rice varieties. In aerobic rice varieties, roots grow deeper and more profusely in comparison to shallow

roots in lowland rice varieties, which help in better absorption of water thereby eliminating the need for water logging and non-methane emitting capabilities.

Aerobic rice has efficient water use efficiency (WUE) which saves about 45 % of water utilized as compared to normal varieties. As anaerobic respiration is avoided, the loss of soil carbon as methane is also reduced. Methane acts as a greenhouse gas. Any reduction in quantity of methane emission will favour the environment. Methane production is very high in puddled water-logged rice fields. Hence, adaption of aerobic rice practices are considered eco-friendly as the methane emission is significantly reduced (Shashidhar, 2007).

It is well-known that weeds are the most severe constraints to widespread adoption of aerobic rice (Rao *et al*., 2007). Weed pressure in dry direct-seeded aerobic rice is significantly greater than that recorded in transplanted rice (Singh *et al*., 2008). Weeds in plots with a lower seeding rate have more chances to emerge, grow, and build up a strong population and thus pose a serious crop–weed competition. Mahajan *et al*. (2010) recommended a higher seeding rate to reduce weed biomass in dry direct-seeded aerobic rice.

Zhao *et al*. (2006) showed highest weed suppression capacity in selected aerobic rice genotypes. Forty rice cultivars and breeding lines used in International Rice Research Institute (IRRI) upland rice breeding programme were evaluated in adjacent weed-free and weedy trials in aerobic soil conditions during the wet seasons of 2001, 2002 and 2003. The objectives of this study were to investigate genetic variability in weed suppression and yield and to identify traits that could be used as selection criteria for improved weed competitiveness. Correlations among and heritability of agronomic traits and early vigor were estimated in weedy & weed-free trials.

Yang *et al.* (2005) and Bouman *et al.* (2006) reported data on yield, water use, and water productivity of aerobic rice cultivars grown under different irrigation regimes in a field experiment near Beijing in 2001-2002. Rice variety, HD297, showed yields of 3-3.5 t ha-1 with 450-500 mm total water input (rainfall plus irrigation) and 4.7-5.3 t ha-1

with 650 mm water input and more. Using the same variety in field experiments near Kaifeng, Feng *et al.* (2007) reported yields of 2.4-3.6 t ha-1, using 750-1,100 mm total water input. Bouman *et al.* (2007) reported yields of aerobic rice obtained by farmers around Kaifeng of up to 5.5 t ha-1 with sometimes as little as 566 mm of total water input, with only one or two supplementary irrigation applications.

Belder *et al*. (2005) showed that aerobic rice is irrigated only when the soil water potential reach below the threshold capacity of field. Atlin *et al*. (2006) reported aerobic rice yields of 3-4 t ha-1 using recently developed aerobic rice varieties in farmers' fields in rainfed uplands in the Philippines.

Xue *et al*. (2007) reported yield maxima of 3.6-4.5 t ha-1 with 688 mm of total water input in 2003, and 6.0 t ha-1 with 705 mm of total water input in 2004. Lampayan *et al*. (2010) evaluated the effects of amount and timing of fertilizer nitrogen application and of row spacing on the yield of aerobic rice under rainfed conditions in the 2004 and 2005 wet seasons in 3 and 2 locations, respectively, in Central Luzon, Philippines. Nitrogen timing and management were also evaluated under irrigated conditions at one location in the dry season in 2005. Yields were 3.1-4.9 t ha-1 with 60-150 kg ha-1 of applied nitrogen. Yields increased with nitrogen rate, up to rates of 60-150 kg ha-1 depending on site and season, but at rates beyond 90 kg ha-1 the risk of lodging increased, especially in the wet season.

Gandhi *et al*. (2011) reported that grain yield obtained by growing MAS 946-1 (aerobic rice variety), under aerobic situation was on par with submerged rice. The farmers in different districts harvested an average grain yield of 28.00q/acre with the highest grain yield of 41.00q/acre and the lowest grain yield of 18.60q/acre with a yield advantage of 29.87 per cent over the existing variety (Rasi) in Bangalore Urban district.

2.3 Importance of root system

Roots are essential organs for exploring and exploiting soil resources, such as water and mineral nutrients. Different root architecture ideotypes that are adapted to different soil mineral nutrient balances or water statuses have been proposed. As a very

important part of rice plant, root system plays multiple roles in rice growth, anchorage of the plant, acquisition of water and nutrient elements, and biosynthesis of amino acids and hormones, etc. Almost all of the hot spots about rice research are associated with rice root: drought tolerance, lodging resistance, and efficient use of nutrition, the goal is to increase the grain yield with desirable seed quality.

Although the understanding about rice root has been expanded in the last decades, there remain much to be done about root morphology and physiology, especially in root genetics. Rice root research is an exciting and focusing field in recent years. More and more researches on rice root genetics have been made. There is a close relation between above ground traits and underground roots, providing an alternative approach for rice genetic improvement. A number of genes associated with root architecture and physiological functions have been identified, or cloned. Root traits improvement should be taken into account in future breeding programs in all important cereal crops.

2.3.1 Role of root system in drought tolerance

Roots are mainly use full during drought, the mode of drought resistance with which roots are most likely associated is drought avoidance. Genotypes that have deep, coarse roots with a high ability of branching and penetration, higher root to shoot ratio, elasticity in leaf rolling, early stomatal closure, and high cuticular resistance are reported as component traits of drought avoidance (Blum *et al*., 1988; Wang and Yamauchi, 2006).

Nasiruddin and Haque (1981) observed low positive or negative correlations among the root traits namely root length, root weight, root number and drought tolerance. Ekanayake *et al*. (1985) reported that root length, root thickness and root volume were significantly correlated to the field recovery from drought. Further they also opined that resistance to tolerance of water stress in crop plants is the combined result of many interacting morphological and physiological characters.

The ability to grow deep roots is currently the most accepted target trait for improving drought resistance, but genetic variation has been reported for a number of

traits that may affect drought response. Gowda *et al*., (2011) reviewed variation in rice root response to drought from a physiological perspective in terms of morphology and function with respect to the different growth environments commonly used by farmers.

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

Nguyen *et al*., (1997) proposed that the genotypic variations in the root system in order to enhance the water-capture ability of the plant could be an avenue for improving the drought tolerance of rice. Penetration ability of root system is one of the important traits to confer the deep root system to rice plants.

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.

Terminal drought severely curtails rice yield, particularly under upland growing conditions. Hence, a deep root system capable of extracting additional soil moisture should positively impact yield in drought-prone areas where residual moisture is available in deeper soil layers. Several studies have investigated QTLs for root traits in rice and their associated effects on other drought-related traits and also grain yield (Tuberosa and Salvi, 2006).

Various Root related characters were found to confer drought resistance; especially root length and thickness are positively correlated with drought resistance (Fukai and Cooper, 1995). A dynamic root system is fine-tuned to soil moisture status and is known to regulate the amount of water available to the plant depending on its distribution in the soil. Among various root morphological traits, maximum root length, root diameter and root:shoot dry weight ratio were found to be associated with drought resistance (O'Toole and Soemartono, 1981).

Several rice root characteristics are considered to play an important role in drought avoidance. Depth of rooting, root thickness, and deep root to shoot ratio have been found to be associated with this mechanism (O'Toole and Chang, 1979; Yoshida and Hasegawa, 1982 and Ekanayake *et al*., 1985a).

Chang *et al*., (1986) also found that rice with a deep root system avoided drought better than rice with a shallow root system. Advantages conferred by a deep root system depend on three major factors: duration of the drought period, availability of water at depth, and rate of water uptake.

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

Gowda *et al*. (2011) found that rice root growth encompasses a remarkable genetic diversity in terms of growth patterns, architecture, and environmental adaptations. In order to harness this valuable diversity for improving rice response to drought, understanding of key root traits and effective drought response mechanisms is necessary. A trait-based approach with precise understanding of the target environment, including temporal and spatial heterogeneity, is a possible path toward the use of roots and dehydration avoidance traits for improved drought resistance in rice. The ability to grow deep roots is currently the most accepted target trait for improving drought resistance, but genetic variation has been reported for a number of traits that may affect drought response.

Haider *et al*. (2012), found the significant correlation among most of the root traits observed in their study which showed that these traits are interrelated with each other. In normal condition, almost all the traits are significantly positively correlated with each other except few one. They were interested in those traits that perform better under stress condition i.e. root length positively significantly correlated with shoot length, root

fresh weight and shoot fresh weight (r=0.340, r=0.350 and r=0.412). A similar result of root shoot traits was also reported by Wang *et al*., 2009.

2.3.2 Association of root traits with yield in rice

The relationships between root growth and grain yield are complex. Positive associations between root length and grain yield have been documented in rice (Mambani and Lal, 1983; Lilley and Fukai, 1994). In contrast, Ingram *et al*. (1994) found no significant association between the two traits. It may be that a simple correlation between root growth and yield could be expected only in well-defined target environments (Mambani and Lal, 1983; Ekanayake *et al*., 1985a).

Venuprasad *et al*. (2002), in a study involving simultaneous evaluation of root character and grain yield, concluded that genotypes with a deep rooting habit had an advantage in stress conditions and that those genotypes that had produced deep roots prior to the onset of stress showed improved productivity compared with a genotype that did not have the capacity to produce roots prior to the onset of stress. Subsequently, Toorchi *et al*. (2006) and Kanbar *et al*. (2009), based on canonical correlation studies conducted under contrasting moisture regimes, suggested that maximum root depth, root-shoot ratio, and root dry weight conferred an advantage to grain yield under stress. Root traits and their functional characteristics are mentioned in table 1.

2.4 Importance of phenotyping:

Although there are many comprehensive reviews of methods for root study that describe methods commonly used e.g., soil cores, monolith, minirhizotrons, pots, and solution culture (Bohm, 1979), some methods have been particularly used for rice.

In Rice, the PVC cylinder system (typically > 15 cm diameter and 1m height) is considered an improvement over pot culture since root depth is less restricted, and soil moisture with depth and soil drying are more representative of field conditions (Upchurch and Taylor, 1990).

Table 1: Root traits and their functional characteristics

Sl. No.	Root traits	Functional characteristics
1	Maximum root depth	Potential for absorption of soil moisture and nutrients in deeper soil layer
2	Root to shoot ratio	Assimilate allocation
3	Root volume	The ability to permeate a large volume of soil
4	Root number	Physical strength, potential for root system Architecture
5	Root diameter	Potential for penetration ability, branching, hydraulic conductivity
6	Deep root to shoot ratio	Vertical root growth, potential for absorption of soil moisture and nutrient in deeper soil layers
7	Root length/weight Density	Rate of water and nutrient uptake
8	Root branching	Power of soil exploration (the major contribution to total root length)
9	Total root length	Total root system size: the size of contact with soil (major determinant for water and nutrient uptake as an entire root system)
10	Specific root length	Degree of branching, density of root materials, porosity due to aerenchyma development

Steele *et al*. (2007) suggested that the most notable contrast among rice root QTL studies is the vast array of growth media and observation methods used. Since the G×E effect on root growth is particularly important for rice under drought conditions, with low land soils being a complex layering of disaggregated soil over a hardpan and ranging from flooded paddies to dry cracked soils over the same season, understanding how growth and observation methods affect root QTL studies is key for using our knowledge of QTLs to improve drought resistance in rice.

Shashidhar *et al*. (2012) had devised a strategy to characterize root and shoots to complement field plant productivity studies wherein, the plants were grown in PVC tubes that were filled with soil. Generally for rice, 20cm wide and 100cm long tubes were suitable for root growth in the soil medium. The PVC tubes were of same height and filled with equal medium which ensured that they were at the same level of vapour pressure deficit. A completely randomized design was used for this study. He emphasized several traits to be recorded, which includes plant height, number of tillers per plant, maximum root length, root number, root volume, grain yield per plot etc. He mentioned that a plant with a well-endowed root system will have a better access to water from a deeper and larger volume of soil. It was also mentioned that the genetic differences that exist for root parameters might manifest improved grain yield.

Shashidhar *et al*. (2012), reviewed several methods of field experiments for root studies in rice under drought conditions *viz*., field with soil cores and monolith, raised bed system and deep root restriction system.

In addition, for laboratory and greenhouse-based methods, plant growth is evaluated without intra- or interspecific competitions, which may be relevant for studying root traits in the field. Examples of these methods include: Growth and Luminescence Observatory for Roots (GLO-Roots), X-Ray Computed Tomography and the clear pots method in greenhouse. On the other hand, methods with high practical and physiological relevance use destructive assays. To a certain extent, shovelomics, soil coring methods and rhizolysimeters can minimize the loss of root structures (Khan *et al*., 2016). All field-

based methods are labour intensive and are subject to the effects of environmental variabilities in the field.

Paez-Garcia *et al*. (2015) reported that, the method chosen for culturing plants for root imaging will depend on a suite of factors including the specific root trait of interest (e.g., primary roots vs. crown roots), the desired timescale for sampling (hours vs. days/months), infrastructure capacity and costs. Representative methods for growing plants for subsequent root imaging and phenotyping are presented in Table 2.

2.5 Genetic architecture of root system

Roots are the principal plant organ for nutrient and water uptake. Therefore, improving our understanding of the interactions between root function and drought in rice could have a significant impact on global food security.

Kawata and Soejima (1974) indicated that roots produced after flowering may play an important role during the grain-filling period. The form of the rice root system also varies with cultivation methods. Growth of the rice root, in terms of total dry matter, maximum root depth, and root length density, increases until flowering stage and then decreases sharply to maturity (Yoshida and Hasegawa, 1982).

Rice is characterized by a shallow root system compared with other cereal crops (Angus *et al*., 1983), having limited water extraction below 60 cm (Fukai and Inthapan, 1988). The shape of the root system differs greatly with soil texture (particle size), soil water status, and soil compaction (Hoshikawa, 1989). Tuong and Bouman (2002) they suggested that in upland conditions with direct sowing, the root. System generally develops deeper than in transplanted plantings in lowland conditions.

Studying the root system is one of the most important part because water uptake in rice depends mainly on the root system (Nguyen *et al*., 1997). Drought resistance in rice can be improved through an improved root system. Developing cultivars with high root-penetration ability have been recognized as an important breeding objective for drought resistance improvement in rice (Hanson *et al*., 1990), and will have great impact

Table 2: Strategies and approaches for root phenotyping

Sl. No.	Plant Cultivation System	Growth Media	Description	References
1	Growth and luminescence observatory for roots (GLO-Roots)	Soil (lab)	This method combines custom-made growth vessels and new image analysis algorithms to non-destructively monitor RSA development over space (2-D) and time. The technique allows information on soil properties (e.g., moisture) to be integrated with root growth data. The system makes use of luminescence imaging of roots expressing plant codon-optimize luciferase.	Rellan-Alvarez *et al*. (2015)
2	X-Ray computed tomography	Soil (lab and greenhouse)	Non-destructively visualizes opaque root structures by measuring the attenuation of ionizing radiation as it passes through the root. A series of projections are acquired and combined to reconstruct a 3D image of the root system.	Mairhofer *et al*. (2013) Mooney *et al*. (2012)
3	Rhizophonics	Liquid media (lab)	Combines hydroponics and rhizotrons. System is made of a nylon fabric supported by an aluminum frame. The set-up is immersed in a tank filled with liquid media. Allows non-destructive, 2-D imaging of root architecture while simultaneously sampling shoots.	Mathieu *et al*. (2015)
4	Clear pot method	Soil (greenhouse)	Uses transparent pots filled with soil or other potting media. Seeds are planted close to the pot wall to enable high- throughput imaging of roots along the clear pot wall. To prevent light exposure, the clear pot is placed in black pots while roots are developing.	Richard *et al*. (2015)
5	Rhizoslides	Paper-based (lab, greenhouse)	The set-up consists of a plexiglass sheet covered with moistened germination paper. Seeds are planted on the slit of the plexiglass. The system allows separation of crown roots from embryonic roots.	Le Marie *et al*. (2014)

Table 2: conti…..

Sl. No.	Plant Cultivation System	Growth Media	Description	References
6	Shovelomics	Soil (field-based)	Involves manual excavation of plants and separating roots from the shoots. Washed roots are then placed on a phenotyping board for root trait quantification. New algorithms allow extraction of several root traits in a high throughput manner.	Bucksch *et al*. (2014) Trachsel *et al*. (2011)
7	Soil coring	Soil (field-based)	Uses a tractor-mounted, hydraulic soil corer to drive steel alloy sampling tubes into the soil. When combined with novel planting configurations (e.g., hill plots), this method allows for phenotyping deep rooted crop varieties.	Wasson *et al*. (2014)
8	Rhizolysimeters	Soil (field-based)	Elaborate facility consisting of an underground corridor and concrete silos and pipes to house soil-containing soil cores for direct root observation.	Eberbach *et al*. (2013)
9	Minirhizotrons	Soil (field-based)	A transparent observation tube permanently inserted in the soil. Images of roots growing along the minirhizotron wall at particular locations in the soil profile can be captured over time.	Maeght *et al*. (2013) Iversen *et al*. (2011)
10	PVC cylinder system	Soil (field and greenhouse based)	The PVC tubes were of same height and filled with equal medium which ensured that they were at the same level of vapour pressure deficit. Plant with a well-endowed root system will have a better access to water from a deeper and larger volume of soil	Shashidhar *et al*. (2012)

to boost rice production and sustain yield stability in rainfed lowland areas. However, incorporation of selection criteria into breeding programs has been difficult due to lack of reliable and efficient screening techniques and the laborious, time-consuming nature of measuring root traits such as root-penetration ability and root thickness (Garrity *et al*., 1986; Ingram *et al*., 1994; O'Toole, 1989).

Like in other cereals, root architecture in rice is composed of several embryonic and postembryonic types of roots. The rice root system is comprised of five root types, including the embryonic roots as well as the postembryonic roots: the radicle, the embryonic crown roots, the postembryonic crown roots, the large lateral roots, and the small lateral roots (Zhao *et al*., 2009). Adventitious postembryonic crown roots, also called nodal roots, emerge from the nodes on the stem and tillers arranged in one or two rows. Root ramification is also partially responsible for this fibrous architecture. Indeed, lateral rice roots can appear on any primary root, including embryonic and crown roots, and can be classified into two main anatomical types (Zheng *et al*., 2003). The small lateral roots elongate laterally, whereas the large lateral roots elongate downward, suggesting that the small lateral roots do not respond to gravity. Higher orders of branching can also be observed in the large lateral roots of the crown roots that emerge at later growth stages. Small lateral roots exhibit determinate growth and never bear lateral roots, whereas all other root types have indeterminate growth and bear numerous lateral roots, suggesting a different function for these two types of lateral roots.

Rice was domesticated approximately 10000 years ago (Sweeney and McCouch, 2007) and has since adapted to a large variety of ecosystems (irrigated, rainfed lowland, upland, flood prone, and mangrove) and production systems (from traditional low-input systems to intensive high-input systems). More than 1, 00, 000 rice genotypes are available in the Gene bank of the International Rice Research Institute (IRRI), Philippines. The phenotypic evaluation of a small sample of this diversity showed that the tested genotypes exhibited a great deal of root architecture variation (O'Toole and Bland, 1987 and Lafitte *et al*., 2001).

Lateral roots differentiate from the root pericycle and, in part, from the endoderm. The radial structure of the rice roots comprises the following tissues, from the center to periphery: the stele, including phloem and xylem vessels and the pericycle; the endoderm; the cortex, whose cells can undergo apoptosis to constitute the aerenchyma; the sclerenchyma; the exodermis; and the epidermis. This radial structure reflects the capacity of rice roots to grow in aerobic as well as anaerobic conditions. The remaining unexplored extensive variability constitutes a very significant opportunity for the identification of new genes involved in root development. (Rebouillat *et al*., 2009 and Orman-Ligeza *et al*., 2013).

Kato *et al*. (2009) studied root growth dynamics in rice grown in aerobic and flooded conditions to characterize the root growth and stomatal behavior of four rice cultivars grown in flooded and aerobic culture for 2 years. In aerobic culture, where the soil water potential at 20 cm depth averaged between -15 and -30 kPa, total root biomass was significantly lower than in flooded culture for the whole growth period, owing to a reduction in root biomass in the surface layer. Dry-matter partitioning to roots decreased, but the ratio of deep root biomass to total root biomass tended to be higher in aerobic culture than in flooded culture. Stomatal closure was distinct at the vegetative stage in aerobic culture, even when the soil water potential was near field capacity, partly because of the poor rooting vigour. When the soil water potential at 20 cm depth was below -50 kPa, the stomatal behaviour reflected the root growth in the subsurface layer suggesting the role of vigorous root growth in soil water uptake and hence, in maintaining transpiration in aerobic rice culture.

Kato *et al*. (2010) compared the estimates of root length by the Comair root length scanner and a flatbed scanner and image analysis software in rice grown in aerobic, near saturated and flooded fields. They reported that fine roots (diameter < 0.2mm) accounted for >80 % of root length in all hydrological conditions. Although root length measurement by image analysis software is still under development, this new tool will facilitate the phenotyping of root system architecture and shed light on the roles of fine roots in water saving rice cultivation.

Kato and Okami (2011) studied performance of root morphology, hydraulic conductivity and plant water relations of high-yielding rice grown under aerobic conditions and reported that unstable performance of rice in water-saving cultivations is often associated with reduction in leaf water potential. Lower leaf water potential reduces soil water conductivity that is related with water uptake capacity of roots under aerobic conditions, so rice performance in aerobic culture might be improved through genetic manipulation that promotes lateral root branching and rhizogenesis as well as deep rooting.

Reduced nutrient uptake, especially of nitrogen and phosphorus, has been the most important factor for lower yield in direct seeded cultivation systems compared with flooded systems of rice cultivation (Kumar and Ladha, 2011), and is a particular concern in low-input rainfed systems. This emphasizes the urgency of improving the rice root system so that plants are able to capture nutrients more efficiently. Strategies of efficient nutrient acquisition include root morphology for exploring nutrients in soils through the growth of axial roots with shallow angles and more dispersed lateral roots (Lynch, 2011), and the development of longer lateral roots with more root hairs (Rose *et al*., 2012).

Niones *et al*. (2013) analysed the quantitative trait loci (QTL) associated with the plasticity in aerenchyma development under TD-W stress using a mapping population of 60 F2 genotypes of chromosome segment substituted lines (CSSL) derived from CSSL47 and Nipponbare crosses. qAER-12 mapped on the short-arm of chromosome 12 was found to be significantly associated with the increase in lateral root elongation and branching which resulted in greater root system development as expressed in total root length and consequently contributed to higher dry matter production.

2.6 Importance of root genetic improvement

2.6.1 Increasing rice productivity

The low percentage of nutrients and water, among this the essential nutrient elements required for rice growth, inorganic carbon is absorbed mainly by leaves in the form of carbon dioxide, the other essential mineral elements are all absorbed mainly

through root surface from the soil. Root is the foundation of rice development (Russell, 1977). As described filled grains was closely associated with a quick decreased root activity during grain filling. It is the roots that absorb most in the reported by Zhang *et al*. (2009), the high grain yield was mainly due to a larger sink size (total number of spikelets) as a result of a larger panicle. Further research is needed to understand the mechanism involved in the low percentage of filled grains and yield fluctuation and to improve the yield performance in elite hybrid lines. The yield of elite varieties can be further increased by an increase in filled grains through enhancing root activity during grain filling (Yang, 2011 and Cheng *et al*., 2007c).

2.6.2 Enhancing tolerance to abiotic stresses

Drought is one of the most severe abiotic stresses limiting rice productivity in the world, and poses a serious threat to the sustainability of rice yields in rainfed agriculture. Development of drought resistant rice is one of the objectives in the water-saving agriculture programs (Dat, 1986). The Soil salinity is another severe abiotic stress in agriculture worldwide about 20 % of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Rhoades and Loveday, 1990).

Zhu *et al*. (2001) also summarize the high salt stress disrupts homeostasis in water potential and ion distribution. This disruption of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. Acquisition of more water from soil is a mechanism for drought tolerance in rice. For plants growing in saline soils, the exclusion of Na^+ and Cl^- by roots is of paramount importance (Barrett-Lennard, 2003). High-affinity K+ transport systems are also essential for preventing Na^+ toxicity. Under $NaCl^-$ dominated salt stress, the key to plant survival is maintaining a low cytosolic Na^+ level or Na^+/K^+ ratio. Therefore, one way to engineer plant cells with improved salt tolerance is to enhance K^+ uptake activity of the cells, while keeping Na^+ out during salt stress (Horie *et al*., 2011). Developing salt tolerance varieties is also a task for rice breeders in the future.

Improving the understanding of the interaction between root function and drought in rice could have a significant impact on global food security Therefore, improving root

system with deep root and high water uptake ability would be the key to developing elite rice varieties suitable for water-saving farming system Gowda *et al*. (2011).

2.6.3 Roots for improvement of drought resistance in rice

Chang *et al*. (1986) found that rice with a deep root system avoided drought better than rice with a shallow root system. The relationships between root growth and grain yield under drought are complex. Mambani and Lal, 1983a; Lilley and Fukai, 1994 have been documented Positive associations between root length and grain yield in rice. In contrast, Ingram *et al*. (1994) found no significant association between the two traits. It may be that a simple correlation between root growth and yield could be expected only in well-defined target environments.

Rice cultivars adapted exclusively to upland conditions are typically characterized by a deep and coarse root system, tall stature, thicker stems, and fewer tillers. In upland fields during stress, the major sources of water for growth and development are rain that is retained by the soil and groundwater. A coarse and deep root system, for soil penetration and access to water reserves deep in the soil, is considered valuable for improved drought resistance under upland conditions (Ge, 1992; O'Toole and Chang, 1979 and Ling *et al*., 2002). Whereas lowland rice cultivars have shallow and finer roots, a large number of roots, and many tillers (Lang *et al*., 2003). The authors noticed large variation among upland rice cultivars for root length density below 30cm and suggested that the effect of drought stress depends on the ability of plants to develop a deep root system.

Venuprasad *et al*. (2002) concluded that in a study involving simultaneous evaluation of root character and grain yield, genotypes with a deep rooting habit had an advantage in stress conditions and those genotypes that had produced deep roots prior to the onset of stress showed improved productivity compared with a genotype that did not have the capacity to produce roots prior to the onset of stress. They also suggested, based on QTL mapping, that the loci for productivity traits were not congruent with those related to root morphology, except at one locus. Subsequently, Toorchi *et al*. (2006) and Kanbar *et al*. (2009), based on canonical correlation studies conducted under contrasting

moisture regimes, suggested that maximum root depth, root–shoot ratio, and root dry weight conferred an advantage to grain yield under stress. Rice cultivation has two major land management systems, commonly referred to as upland and lowland. These two systems differ greatly in their yield potentials because of soil characteristics that affect root growth and plant response to drought.

2.7 Molecular genetics of rice root

2.7.1. Root development

Rice bears a shallow root system that is comprised of one seminal root (radicle), numerous adventitious roots (crown roots) arising from successive nodes, and large and small lateral root emerging from primary roots. Early in 1980s, Chang *et al*. (1982) and Ekanayake *et al*. (1985) have made some genetic researches on rice root. Fustuhara and Kitano in 1985 reported a crown root inhibiting gene RT1. To date, advances have been made in root genetics. Through the utilization of several mapping populations (including DH, RI, and F2 population, such as Akihikari/IRAT109, CT9993/IR6226, Bala/Azucena, IR64/Azucena), more than 700 QTLs related to rice root architecture (root length, root number, and root thickness etc.) have been mapped by Kamoshita *et al*. (2002), Price *et al*. (2002), Nguyen *et al*. (2004) and Horii *et al*. (2006).

Dissecting genetic and molecular mechanisms controlling rice root development is critical for the development of new rice ideotypes that are better adapted to adverse conditions and for the production of sustainably achieved rice yield potential (Rebouillat *et al*., 2009).

2.7.2 Adventitious root formation

There are four reported genes related to radicle development in rice. The ral1 is the first mutant impaired in both procambial development and vascular patterning to be isolated in a monocot species, which produce normal adventitious roots after germination. Hong *et al*. (1995) they presented the both ral2 and ral3 mutants also radicle inhibition. Scarpella *et al*. (2003) reported that the knockdown of RADICLELESS1 (RAL1) gene results in distinctive vascular pattern defects.

Inukai *et al*. (2001) they showed the crown rootless1 (crl1) mutant is defective in crown root formation. Because the auxin-related abnormal phenotypic traits in the roots, such as decreased lateral root number, auxin in sensitivity in lateral roots (LRs) formation, and impaired root gravitropism, whereas aboveground organs were normal.

Kamiya *et al*. (2003) they were showed that SCR (SCARECROWN) and SHR (SHORT-ROOT) genes related with root initiation and root elongation in plants. OsSCR1 and OsSCR2 are involved in root development. They are essential for the asymmetric division of the cortex/endodermis progenitor cell in the root, and expressed in the endodermal cell layer and down-regulated in the daughter cortex cell after asymmetric division in the root tip. A SHR homolog from rice is a moving transcription factor essential for endodermis specification. SHR movement is limited to essentially one cell layer. SCARECROW (SCR) blocks SHR movement by sequestering it into the nucleus through protein-protein interaction and a safeguard mechanism that relies on a SHR/SCR- dependent positive feedback loop for SCR transcription (Cui *et al*., 2007).

ARL1 is the same gene with CRL1 (Liu *et al*., 2005). CRL1 encodes a protein with a LOB domain. It expresses in lateral and adventitious root primordia, tiller primordia, vascular tissues, scutellum, and young pedicels. CRL2 is involved in crown root formation, the initiation and subsequent growth of adventitious roots primordia of mutant are impaired suggested by Inukai *et al*. (2005).

Kitomi *et al*. (2008a) were studied the CRL3 is exclusively involved in the formation of the crown root primordia, but not in the formation of other types of root primordial and shoot apical meristem. Kitomi *et al*. (2008b) also reported the CRL4 encodes OsGNOM1, which expressed in AR (adventitious root) primordia, vascular tissues, LRs, root tips, leaves, anthers and lemma veins CRL4 is mediated by OsPINs family, such as OsPIN2, OsPIN5b, OsPIN9, and affected the formation of ARs through regulating PAT (polar auxin transport) (Liu *et al*., 2009).

Kitomi *et al*. (2011) reported that the CRL5 encodes a member of the large AP2/ERF transcription factor family protein. The auxin-induced CRL5 promotes crown

root initiation through repression of cytokinin signaling by positively regulating type-A RR, OsRR1.

Luo *et al*. (2012) were reported that the OsKSR2 is responsible for root development; because of the OsKSR2 a dwarf phenotype and the elongation of primary roots, adventitious roots and lateral roots are severely impaired.

Wang *et al*. (2011) was analysed OsCAND1 is an orthologue of Arabidopsis CAND1, required for crown root emergence. The defect of visible crown root in the Oscand1 mutant is the consequence of a cessation of the G2/M cell cycle transition in the crown root meristem. During crown root primordium development, the expression of OsCAND1 is confined to the root cap after the establishment of fundamental organization.

2.7.3 Root elongation

SRT1 is a short root gene. The srt1 shows shorter length of root due to defective cell elongation (Ichii and Ishikawa, 1997). Subsequently, srt2, srt3, srt4 and srt5 were characterized (Liang and Ichii, 1995; Yi *et al*., 2002; Ichii and Ishikawa, 1997 and Yao *et al*., 2002). The mutant srt5 shows extreme inhibition of seminal root, crown root and lateral root elongation, and alter root hair formation at the seedling stage, due to the reduced cell size and cell number. SRT6 is restricted specifically to the development of primary roots. Its expression is phase-specific, and greatly reduced primary root length and diameter (Yao *et al*., 2002).

Inukai *et al*. (2001) reported that the root elongation is essentially driven by stem cells localized in apical meristems of roots. The mutants rrl1, rrl2 both show shorter root, RRL1, RRL2 inhibit the maintenance of root apical meristem and cell elongation. The mutant rrl3 with short roots is highly sensitive to mechanical stimulus. RRL3 specifically regulates the cell production process in the root meristematic zone under mechanically impeded condition, and does not regulate the sensitivity to ABA (abscisic acid), IAA (indoleacetic acid) and ethylene (Inukai *et al*., 2003). OsCKI1 encodes a casein kinase,

and plays an important role in formation and growth of adventitious root in rice (Liu *et al*., 2003).

ADP-ribosylation factor (ARF) proteins, which mediate vesicular transport, have little or no intrinsic GTPase activity. They rely on the action of GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) for their function. OsAGAP encodes a protein with predicted structure similar to ARF-GAP. Transgenic Arabidopsis with OsAGAP constitutively expression shows reduced apical dominance, shorter primary roots, increas- ing number of longer adventitious roots (Zhuang *et al*., 2005). Glu receptors are known to function as Glu-activated ion channels that mediate mostly excitatory neurotransmission in animals. Glu receptor-like genes have also been reported in higher plants, although their function is largely unknown. GLR3.1 is a Glu receptor-like gene in rice, the root meristematic activity of mutant is distorted and accompanied by enhanced programmed cell death. GLR3.1 is essential for the maintenance of cell division and individual cell survival in the root apical meristem at the early seedling stage (Li *et al*., 2006).

OsGNA1 is involved in de novo UDPN-acetylglucosamine biosynthesis. It encodes a glucosamine-6-P acetyltransferase. The gna1 mutant exhibited a temperature-sensitive defect in root elongation. The aberrant root morphology of the mutant includes shortening of roots, disruption of microtubules, and shrinkage of cells in the root elongation zone (Jiang *et al*., 2005). OsCyt-inv1 codes an alkaline/neutral invertase and is an ortholog of Arabidopsis gene AtCyt-inv1. The mutant showed short root under normal growth condition, the cell length along the longitudinal axis was reduced and the cell shape in the root elongation zone shrank. Map-based cloning revealed that a nucleotide substitution causing an amino acid change from Gly to Arg occurred in the predicted gene (Jia *et al*., 2008). The major QTL, qRL6.1, greatly promoted root elongation under NHþ4 condition, and was localized to the long-arm of chromosome 6 (Obara *et al*., 2010).

KSR1 is a short root gene, it is mapped to a 155 kb region, flanked by the InDel marker 4–24,725 K and the SSR marker RM17182 (Ning *et al*., 2010). OsSPR1 encodes

a mitochondrial protein with the Armadillo-like repeat domain. Osspr1 mutant exhibited decreased root cell elongation (Jia *et al*., 2011). The iron and zinc content of the mutant shoots was significantly altered. A homolog of KOR1 of rice, OsGLU3, encodes a putative membrane-bound endo-1,4-bglucanase. OsGLU3 can affect root cell wall cellulose synthesis to modulate root elongation (Zhang *et al*., 2012).

As an orthologue of Arabidopsis DGL1, human OST48 and yeast WBP1, OsDGL1 encodes the dolichyldiphospho oligosaccharide protein glycosyltransferase 48 kDa subunit precursors (Qin *et al*., 2013). The osdgl1 displayed a change of matrix polysaccharides in its root cell wall, shorter root cell length, smaller root meristem and cell death in the root.

Uga *et al*. (2013) was reported a large number of QTLs related to root development in rice have been detected, but just few major QTLs have been cloned. DEEPER ROOTING 1 (DRO1), a rice quantitative trait locus controlling root growth angle, was mapped and sequenced. DRO1 is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and downward bending of the root in response to gravity.

Wang *et al*. (2013) analysed the qRL7 is located between markers InDel11 and InDel17, which delimit a 657.35 kb interval in the reference cultivar Nipponbare. qRL7 plays a crucial role in root length.

2.7.4 Lateral root and root hair development

Chhun *et al*. (2003) developed Arm1 and Arm2 function in different processes in the auxin response pathways leading to lateral root formation. The arm1 displays a variety of morphological defects including reduced lateral root formation, increased seminal root elongation, reduced root diameter, and impaired xylem development in roots, while the arm2 reduces slightly lateral root formation, impaired xylem development in roots and an enhanced plant height.

The mutant lrt2 fails to form lateral roots and exhibits altered root response to gravity too (Wang *et al*., 2006). LRT2 is localized to a 10.8 cM interval on the short arm of chromosome 2, flanked by two sequence-tagged site (STS) markers Lrt2P1 and Lrt2P2. Root hairs differentiate from epidermal cells of root, and serve to acquisition of nutrients and water from the rhizosphere. Eight genes related to root hair development have been reported in last decade. The mutant root hairless 1 (rth1) shows absence of root hair (Yuo *et al*., 2009).

OsWOX3A plays important roles in organ development, including lateral axis outgrowth and vasculature patterning in leaves, lemma and palea morphogenesis in spikelet, and the numbers of tillers and lateral roots. OsWOX3A is encoded by NARROW LEAF2 (NAL2) and NAL3, a pair of duplicated genes. It also acts in the control of root hair formation (Cho *et al*., 2013). Mutation in the 9th exon of OsORC3 (Origin Recognition Complex subunit 3) is responsible for the mutant (orc3) phenotype. OsORC3 is strongly expressed in regions of active cell proliferation, including the primary root tip, stem base, lateral root primordium, emerged lateral root primordium, lateral root tip, young shoot, anther and ovary (Chen *et al*., 2013).

It is caused by a mutation of *OsAPY*, an important gene for root hair elongation and plant growth in rice. Root hairless 2 (*rh2*) is complete absence of root hairs, and with a strong reduction in root length among all root types, as well as a strong reduction in plant height. The mutation does not affect the number of crown roots or the morphology of leaves (Suzuki *et al*., 2003). *OsCSLD1* is required for hair elongation but not initiation (Kim *et al*., 2007). It expresses in only root hair cells, and is the only member of the four rice *CSLD* genes that shows root-specific expression. *OsRHL1* is a novel basic helix-loop-helix (bHLH) transcription factor involved in the regulation of plant root hair development, and belongs to subfamily C of the rice bHLH. It is highly homologous to members of subfamily 17 of the bHLH family in Arabidopsis (Ding *et al*., 2009).

OsSRH1 and *OsSRH3* are two short hair genes. *OsSRH1* was mapped between markers T1757 and T1768 with a distance of 115 kb on chromosome 6. *OsSRH3* flanked by markers S38, 978 and S39, 016 on chromosome 1. The elongation of root hairs in both

mutants is severely impaired (Ding *et al*., 2011, 2012). *OsEXPB5* is a root hair-specific *EXPB* gene that contains root hair-specific cis-elements (RHEs). It is thought to encode proteins that function more efficiently on cell wall modification during root hair morphogenesis (Won *et al*., 2010). *OsEXPA17* expresses in root hair cells. *OsexpA17* contains a point mutation, causing a change in the amino acid sequence. The mutant has short root hairs (Yu *et al*., 2011).

2.8 Molecular markers studies

Molecular marker based studies in rice have been carried out with several traits such as root characters, osmotic adjustment, cell membrane stability, relative water content, leaf rolling, stomatal conductance and grain yield (Lanceras *et al*., 2004).

Simple sequence repeat is an important tool for genetic variation identification of germplasm. SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhang *et al*., 2007 and Ma *et al*., 2011), construction of fingerprints (Xiao *et al*., 2006 and Ma *et al*., 2011), genetic purity test (Peng *et al*., 2003), analysis of germplasm diversity (Zhou *et al*., 2003; Jin *et al*., 2010; Ma *et al*., 2011) utilization of heterosis, especially in identification of species with closer genetic relationship. SSR markers can distinguish different alleles of a locus that make it more powerful.

Molecular markers are of great value in applying genetic technologies to crop improvement. One of the most important applications of DNA markers and molecular linkage maps is to dissect the genetic variation of quantitative traits into individual Mendelian factors through QTL mapping analyses (Li, 2001). In QTL mapping, genes controlling genetic variation of quantitative traits in segregating populations are resolved into individual Mendelian factors by detecting marker-trait associations. Another major purpose of QTL mapping is to identify DNA markers diagnostic for particular phenotypes of interest so that marker aided selection (MAS) can be used to efficiently manipulate progenies carrying alleles for target traits grown under non target environments (Li, 2001).

Analysis of the completed rice genome sequence provided the identification of thousands of new targets for DNA markers, especially SSRs. Using publically available BAC and PAC clones, more than 200 validated SSRs were released in 2002 (McCouch *et al*., 2002). This was soon followed by 18828 Class I (di-,tri-, tetra-repeats) SSRs that were released after the completion of the Nipponbare genome sequence in 2005 (Matsumoto *et al*., 2005). The extremely high density of SSRs (~51 SSRs per Mb) has provided a considerable "tool kit" for map construction and MAS for numerous applications.

Li *et al*. (2005) reported several QTL for root traits including basal root thickness, root number, root length and root biomass in a study involving evaluation of a DHL population from a cross between upland and lowland japonica rice in three environments.

Steele *et al*. (2006) conducted a marker-assisted back-crossing (MABC) breeding programme to improve the root morphological traits, and thereby drought tolerance, of the Indian upland rice variety, Kalinga III. The donor parent was Azucena, an upland japonica variety from Philippines. Selection was made in three backcross (BC) generations and two further crosses between BC3 lines to pyramid (stack) all five target segments. Twenty-two near-isogenic lines (NILs) were evaluated for root traits in five field experiments in Bangalore, India. The target segment on chromosome 9 (RM242-RM201) significantly increased root length under both irrigated and drought stress treatments, confirming that this root length QTL from Azucena functions in a novel genetic background.

Brondani *et al*. (2006), aimed his studies on the genetic variability of 30 elite genotypes of the upland rice, using 25 SSR markers. During their studies one hundred and thirty one alleles were obtained, an average of 5.2 alleles per locus, and mean PIC equal to 0.61. The number of alleles per locus, rare alleles and PIC (Polymorphism Information Content) were estimated using the software GDA (Lewis and Zaykin, 2000). The dendrograms were constructed based on the genetic distance matrix obtained by the distance coefficient of Rogers, modified by Wright (1978), henceforth called Rogers-W,

and the lines clustered by the UPGMA method, available in software NTSYS (Rohlf, 1989).

Toorchi *et al*. (2007) used selective genotyping strategy and STMs markers for mapping QTL for maximum root length in rainfed lowland rice. Total of 69 extreme plants were selected from P124 × IR64 mapping population for selective genotyping because of good combination of genetic factors conferring drought tolerance and yielding ability. Forty-two pairs of STMS primer pairs that were earlier found to be polymorphic between the IR64 and Azucena were selected and used in the study. RM215 on chromosome 9 showed co-segregation with QTL controlling MRL under both Well watered (WW) and Low Moisture Stress (LMS) conditions. This marker explained 2.5 % of the total variation in MRL under WW condition, while contribution of this QTL in total variability of MRL under LMS condition was 16 %. Under LMS condition, RM215 was the only microsatellite marker co-segregating with a QTL contributing to MRL. This QTL explained a high proportion (16 %) of the variation in MRL. When mean environment was taken into account as phenotype expression of MRL, only RM215 and RM6 showed a significant linkage with QTLs of interest.

Lin *et al*. (2007) reported five drought-related QTL associated with yield, RM136 (y6.1, chromosome 6), RM537 (y4.1, chromosome 4), RM5443 (y1.1, chromosome 1), RM3231 (y8.1, chromosome 8), and RM3 (y6.2, chromosome 6), using F2 mapping population generated from the cross between Taichung 189 (japonica type), a local drought susceptible rice, and Milyang 23 (indica type), a drought tolerant line.

Ikeda *et al*. (2007) studied twenty polymorphic SSR markers, from four hot spots chromosomal regions on chromosomes 1, 3, 7 and 9, were used to screen the entries of which RM302, RM212, RM265, RM315 an RM472 were located on chromosome 1; RM7, RM218, RM251, RM563 and RM282 were located on chromosome 3; RM182, RM455, RM234, RM248 and RM420 were located on chromosome 7; and RM434, RM257, RM242, RM278 and RM201 were located on chromosome 9. They found that QTLs for maximum root length (MRL) and root dry weight showed co-segregation with RM472, RM7 and RM201.

Liu *et al*. (2008) identified a major QTL for basal root thickness, designated brt4, on chromosome 4 using two early segregating populations derived from crosses between IRAT109 (a japonica upland cultivar) and two low-land rice cultivars.

Qu *et al*. (2008) used a mapping population of 120 recombinant inbred lines (RILs) derived from a cross between japonica upland rice 'IRAT109' and japonica low-land rice 'Yuefu' for mapping QTL of developmental root traits and detected a total of 84 additive effect QTL (14 for basal root thickness, 13 for root number, 14 for maximum root length, 11 for root fresh weight, 12 for root dry weight and 22 for root volume).

Courtois *et al*. (2009) extracted information from 24 published papers on QTL controlling 29 root parameters including root number, maximum root length, root thickness, root/shoot ratio, and root penetration index. A web-accessible database of 675 root QTL detected in 12 populations was constructed. This database includes also all QTL for drought resistance traits in rice published between 1995 and 2007.

Gomez *et al*. (2010) used a subset of 250 recombinant inbred lines of F8 generation derived from two indica rice lines (IR20 and Nootripathu) with contrasting drought-resistance traits to map the QTL for morpho-physiological and plant production traits under drought stress in the field in target environment. A genetic linkage map was constructed using 101 polymorphic PCR-based markers distributed over the 12 chromosomes covering a total length of 1,529 cM in 17 linkage groups with an average distance of 15.1 cM.

Kanagaraj *et al*. (2010) carried out bulk segregate analysis (BSA) to identify markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20/Nootripathu, two indica ecotypes with extreme drought response. The parents were screened for polymorphism using 1206 rice microsatellite primer pairs. Out of 134 SSR polymorphic primers between parents, three primers showed polymorphism between bulks. These three primers (RM212, RM302 and RM3825) co-segregated among the individual RI lines constituting the respective bulks. The genomic regions flanked by these markers have been reported to be associated with several drought resistance

component traits and will be useful in marker assisted breeding for drought resistance in rice.

Kanbar and Shashidhar (2011), studied F_2 population derived from the cross between deep-rooted variety ‘‘Moroborekan’’ with shallow-rooted variety ‘‘IR20’’ to identify and validate SSR markers associated with root morphological traits.

Uga *et al*. (2011) reported a new major quantitative trait locus (QTL) controlling ratio of deep rooting (RDR) on chromosome 9 by using 117 recombinant inbred lines (RILs) derived from a cross between the lowland cultivar IR64, with shallow rooting, and the upland cultivar Kinandang Patong (KP), with deep rooting which QTL explained 66.6 % of the total phenotypic variance in RDR in the RILs.

Vikram *et al*. (2011) identified a major QTL for grain yield under reproductive-stage drought stress, qDTY1.1, on rice chromosome 1 flanked by RM11943 and RM431 in all three populations under study. In combined analysis over two years, qDTY1.1 showed an additive effect of 29.3 %, 24.3 %, and 16.1 % of mean yield in N22/Swarna, N22/IR64, and N22/MTU1010, respectively.

Dixit *et al*. (2012) conducted fine-mapping studies on four QTL, qDTY2.1, qDTY2.2, qDTY9.1 and qDTY12.1, for grain yield (GY) under drought using four different backcross derived populations. They found the presence of a donor allele at RM262 within the earlier identified QTL region of qDTY2.1 (RM521–RM262) and RM24334 within qDTY9.1 (RM464–RM24421) showing a negative effect on GY under drought, indicating the necessity of precise fine mapping of QTL regions before using them in marker-assisted selection (MAS).

Swamy *et al*. (2013) identified four major-effect QTL for grain yield - one each on chromosomes 2, 4, 9 and 10 from six BC4F3 mapping populations produced by crossing the +QTL BILs with the 2QTL BILs and IR64. BC4F3-derived lines with the QTL conferred yield advantages of 528 to 1875 kg ha-1 over IR64 under reproductive-stage drought stress in the targeted ecosystems of South Asia. Meta-analysis of transcriptome data from the +QTL/2QTL BILs identified differentially expressed genes

(DEGs) significantly associated with these QTL on and the enrichment of DEGs associated with root traits points to differential regulation of root development and function as contributing to drought tolerance in these BILs.

Sandhu *et al*. (2013) reported two QTL (qGY8.1 with an R2 value of 34 % and qGY8.2 with an R2 value of 22.8 %) and one QTL (qGY2.2 with an R2 value of 43.2 %) for grain yield under aerobic conditions in the mapping populations MASARB25 × PB1460 and HKR47 × MAS26, respectively. Co-localization of QTL for yield, root traits and yield related agronomic traits indicate that the identified QTL may be exploited in marker-assisted breeding to develop novel varieties with high yield and aerobic traits.

Sandhu *et al*. (2014) reported a total of 26 QTL associated with 23 traits and 20 QTL associated with traits were mapped in the Aus276/3*IR64 and Aus276/3*MTU1010 BC2F4 populations, respectively. qGY6.1, qGY10.1, qGY1.1, and qEVV9.1 were found to be effective in both populations under a wide range of conditions. QTL for several seedling-stage traits collocated with QTL for grain yield, including early vegetative vigour and root hair length. On chromosome 5, several QTL for nutrient uptake co-located with QTL for root hair density and nematode gall rating.

2.8.1 SSR linked to traits and QTL's

Collard *et al*. (2005) mentioned that, regions within genome that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs).

Root traits are generally controlled by many genes through quantitative trait loci (QTL). Since the first study by Champoux *et al*. (1995) to locate genes controlling rice root traits with molecular markers, many QTLs related to root traits have been identified in rice using 12 different mapping populations with QTLs, identified and analysed in rice for more than 30 root morphological parameters. The most studied root traits in all QTL mapping studies are maximum root depth, root diameter, and root to shoot ratio. The most notable contrast among rice root QTL studies is the vast array of growth media and observation methods used.

Price *et al*. (2002) showed that, molecular markers can be used to identify linkage to quantitative trait loci (QTL) for rooting ability and these can be selected more easily in a breeding programme than the traits themselves.

Several mapping studies have identified QTL for root traits in segregating populations and have identified QTLs in the variety Azucena that increased root length, thickness and penetration ability (Price *et al*., 2000).

Microsatellite map of studied population was used with 232 markers to detect the linkage to the target traits. A linkage map thus constructed exhibits markers associated with drought tolerance were located mostly on chromosomes 2, 3, 4, 8, 9, 10 and 12. QTL mapping was used to determine effects of loci associated with drought tolerance traits (Nguyen and Buu, 2008).

Haider *et al*. (2012) screen out the diverse parents on the basis of single molecular marker (RM201) that linked to the drought tolerance in rice and morphological seedling traits.

Babu *et al*. (2010) found that three SSR markers RM212, RM302 and RM 3825 showed complete co-segregation among individual RI lines constituting the bulk and is located on chromosome 1 of rice between 135.8 and 143.7cM. This region was reported to be associated with RWC under stress in CT9993/IR62266 doubled haploid (DH) lines and root length, root thickness and root weight in Bala/Azucena RI lines of rice (Price *et al*., 2000).

Kamoshita *et al*. (2002) reported QTLs for root depth, penetrated root thickness, deep root to shoot ratio, deep root dry weight, deep root per tiller and deep root mass to be associated with RM212 on chromosome 1 in CT9993/IR62266 DH lines and also a QTL for osmotic adjustment was reported to be close to this region in IR62266/IR60080 back cross progenies (Robin *et al*., 2003). This region was found to be associated with root volume and basal root thickness in IRAT109/Yuefi RI lines (Li *et al*., 2005) and leaf drying in Zhenshen/IRAT109 RI lines in rice (Yue *et al*., 2006).

Xing *et al*. (2002) reported the QTLs for biomass and root dry weight to be associated with RM212 in Zh97/Ming63 RI lines. Thus, RM212 may be linked to drought-resistance traits and plant production under water stress in rice. QTLs on chromosome 4 and 9 influenced yield favourably through drought avoidance strategy, primarily due to deeper roots. A total of 675 QTLs linked to 29 root parameters from 12 populations were subjected to meta-QTL analysis recently (Courtois *et al*., 2009).

MAS to introgress QTLs controlling root traits into an Indian upland rice variety was successfully demonstrated by Steele *et al*. (2006). They introgressed five QTL regions associated with root traits from Azucena into Kalinga III. The target QTL on chromosome 9 (RM242-RM201) significantly increased root length under drought stress and non-stress conditions confirming that this QTL from Azucena functions in a novel genetic background. This QTL was found to improve root penetration ability as well (Clarke *et al*., 2008).

Large chromosomal segments corresponding to QTLs associated with root length from an upland variety, Azucena were introgressed into the IR64 background. Most of the lines carrying the desired introgressions failed to have deeper roots than IR64 (Shen *et al*., 2001). The lack of effect of the QTLs on root length may be because those QTLs were responsible for a small proportion of the total phenotypic variation (6-18 %) and had not been fine mapped (Bernier *et al*., 2009).

2.9 Statistical tools for morphological data and SSR Analysis

Nguyen *et al*. (1997), performed analysis of variance and simple correlation for root and shoot traits using the SAS Program (SAS Institute, Inc., Cary, NC, 1990). The band patterns were scored for each microsatellite primer pair in each rice accession starting from the small size fragment to the large ones. Presence and absence of each band in each rice accession was coded as 1 and 0, respectively.

Microsatellite scores and morphological data were used to create data matrix to analyze genetic relationship using the NTSYS-pc program (Rohlf, 1990). Dendrograms were created based on Jaccard's similarity/dissimilarity coefficients using microsatellite

DNA data and morphological data from selected rice accessions with an unweighted pair group method (UPGMA) (Jaccard, 1908).

Cluster analysis on the marker and physiological data was done separately based on the similarity matrix obtained in each case, using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973).

Neeraja *et al*. (2005) characterized the tall landraces of rice using gene-derived SSRs and compared the level of polymorphism of gene-derived SSRs with other SSRs of rice. Each fragment that was amplified using SSR primers was scored as 1 or 0 depending on its presence or absence. Genetic similarities were calculated using Dice similarity index. The similarity matrices were used to determine genetic relationships by cluster analysis using UPGMA (unweighted pair grouping with arithmetic means). Polymorphism information content (PIC) was calculated using the formula of Anderson *et al*. (1993).

2.10 Sorghum

Sorghum (*Sorghum bicolor* (L.) *Moench*), a C_4 grass, is the fifth most economically important cereal crop grown worldwide behind wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*) (CGIAR, 2009). It is a photoperiod sensitive plant that can grow in rainy as well as semiarid areas making it an important crop in areas too dry for maize production. C4 cereals, like sorghum, originated from the tropics and are generally more heat and drought tolerant than C3 plants, like wheat, which originated from temperate regions (Blum *et al*., 1990). The mean optimum temperature range for grain sorghum is 21 to 35°C for seed germination, 26 to 34°C for vegetative growth and development, and 25 to 28°C for reproductive growth (Maiti, 1996). Sorghum-producing regions often experience daytime/nighttime temperatures of >32/22°C (Prasad *et al*., 2006).

Sorghum bicolor is diverse and has been classified into five races (bicolor, guinea, caudatum, kafir, and durra) based on spikelet morphology (Harlan and deWet, 1972). Due to within race variability and the existence of race intermediates, Dahlberg *et al*.

(2004) established a classification with working groups (sub-races) which integrates Harlan and deWet's classification. These working groups explain the variation that exist within a given race.

Sorghum is only the second grass species that has been genetically mapped and with only less than one percent increase in grain yields specifically in grain sorghum in the last 50 years, potential room exists for continued breeding to improve nutrient-use efficiency and crop yields (Schupska, 2009). The diverse germplasm of sorghum possesses great potential for improved performance through breeding and genomic investigation (Salon, 2010).

2.10.1 Sorghum production and food security

In 2010 the leading world producers of sorghum included Nigeria (19.3 %), United States (16.3 %), India (11.7 %) and Mexico (10.5 %) (Agricultural Marketing Resource Center, 2011). According to the U.S Grains Council (2012), world sorghum production has risen slightly from 60 million metric tons (2.4 billion bushels) to 65 million metric tons (2.6 billion bushels) over the past decade.

With prediction of less water available for crop production as a result of climate change, sorghum's adaptability to dry environments suggests that it may play a larger role in global food security. Sorghum has a variety of uses including food for human consumption, feed grain for livestock and industrial applications such as ethanol production. Sorghum grain is a staple diet in Africa, the Middle East, Asia and Central America. China and India account for almost all of the food use of sorghum in Asia. Several million tonnes of sorghum are used across Africa for traditional beer brewing. Since sorghum does not contain gluten, it is considered a safe food for people with celiac disease (Ciacci *et al*., 2007).

In other parts of the world, sorghum grain is used mainly as an animal feed. Such use is concentrated in Mexico, many South American countries, the United States, Japan, and the Commonwealth of Independent States (CIS) which include Azerbaijan, Armenia, Belarus, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Turkmenistan,

Uzbekistan and Ukraine. The stover of sorghum also is used as fodder for animals. Brown midrib (BMR) varieties of sorghum have been developed for use as forage sources for livestock because of their reduced lignin content and higher digestibility of the stover (Aydin *et al*., 1999; Oliver *et al*., 2004).

Sorghum is usually grown in arid and semi-arid parts of the tropics and sub-tropics where it is affected by drought during various growth stages. This problem is intensified by the possible global climate change (Dillon *et al*., 2016) reported that recurring drought due to deficit of rainfall in semi-arid and dry sub-humid areas of the country is one of the major causes of underproduction of sorghum.

Drought is the major abiotic stress factor limiting crop productivity worldwide, and understanding the genetic and biochemical mechanisms which control drought tolerance is a central question in plant biology. Breeding for drought tolerance can increase long-term productivity in drought-prone environments. Breeding approaches utilizing physiological and morphological traits have been proposed to improve selection efficiency for superior drought-tolerant genotypes and to supplement the selection on the basis of yield (Blum, 1988). Root systems are important plant parts for taking up water and nutrients from the soil and to communicate with shoots to maintain integrated overall plant growth and health. Root responses when soil moisture dries out are important mechanisms for drought avoidance.

An important feature of the root system response to soil drying is the ability of some roots to continue elongation at water potentials that are low enough to inhibit shoot growth completely. This occurs in nodal (adventitious) roots of maize, which must penetrate through dry surface soil (Sharp and Davies, 1979; Westgate and Boyer, 1985). Venuprasad (1999) reported significant positive association of grain yield per plant with plant height, productive tiller number, panicle length, straw yield, total dry matter, harvest index and dry matter per day per plant both at phenotypic and genotypic levels for the rice genotypes. A thorough knowledge on the genetic diversity among cultivars is of importance for any plant breeding programme to be conducted to improve the efficiency of cultivars.

Blum, (1988); Levitt (1980) reported that several traits have been considered important in adaptation to stress. Drought related traits have been categorized as those for drought escape, drought avoidance and drought tolerance. Drought escape is through shorter growth duration to escape terminal drought. Drought avoidance is by either maintaining a more favourable water balance or by protecting the cellular functions from dehydration. Such traits include small plant size, deep root system and thick cuticle. Drought tolerance is through mechanisms such as osmotic adjustment, dehydration tolerance.

2.10.2 Characteristics of Root

Roots are the primary plant organ affected by drought stress and other environmental stresses of the soil (Prince *et al*., 2002). Sorghum crown roots grow about 2 to 3 cm per day (Routley *et al*., 2003) and root growth is mainly affected by the amount of carbon partitioned to the roots, although it varies with environmental and genetic factors (Blum, 2004). Sorghum roots may grow to depths of 1 to 2 m by the booting stage, and can efficiently extract water to a lateral distance of 1.6 m from the plant (Routley *et al*., 2003). Root growth in sorghum terminates at flowering stage; however, it is more prominent in a senescent than in no senescent sorghum genotypes (Robertson *et al*., 1993).

The root system of a sorghum plant can be divided into a primary and a secondary root system. The primary system develops first in the germinated seedling and supplies the seedling with water and nutrients. However, primary roots have a limited growth and their function is taken over by the secondary root system. The secondary roots develop from the root crowns which are located just under or just above the soil surface (Fig 1). The roots of an adult sorghum plant are all secondary adventitious roots. Lateral roots develop from the secondary adventitious roots and penetrate the soil in all directions. The root system of the sorghum plant spreads to at least 1.5 metres around the plant and is most dense in the top 90 centimetres of the soil.

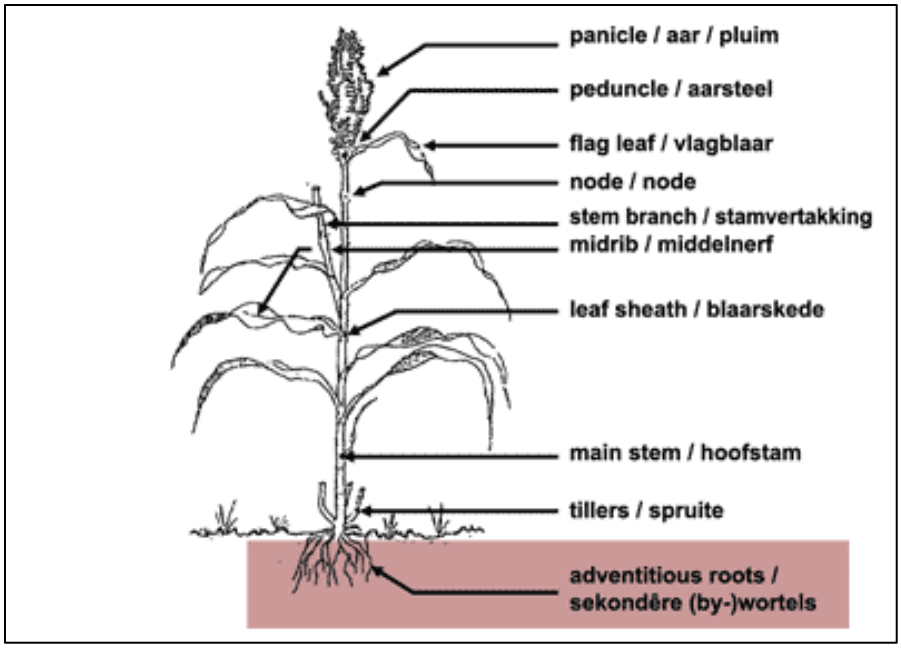


Fig. 1: Diagram of the sorghum plant and its components

Bawazir and Idle (1989) reported variation in root anatomy and morphology, among sorghum genotypes. Genotypes that have large number of seminal roots, large vessel diameter in both seminal and nodal roots showed better survival rate under drought stress conditions. Similarly, Habyarimana *et al*. (2004) found that the drought tolerance traits displayed by the genotypes were related to drought avoidance mechanisms. These, in turn, are associated with deep root system, which enables plants to exploit moisture from the deeper soil horizons.

The root has received less attention than the shoot in the search for characters of use for screening or selection for drought resistance. Esau (1965) remarked that in dry soil the restriction of adventitious root growth makes the efficiency of water transport depend more upon the conductivity of the seminal roots. Camacho *et al*. (1974) showed that plants with efficient water transport systems avoid dehydration of the leaf tissue during periods of atmospheric drought.

Meyer and Ritchie (1980) showed that, the root contributes more resistance than the shoot at least at high transpiration rates. Richards and Passioura (1981) demonstrated variation in vessel size related to climatic factors in some wheat accessions, and proposed

a selection and breeding programme for small vessels in the expectation of improving the performance of wheat under conditions of limited water supply. Passioura (1986) reports encouraging signs in this programme.

Ekanayake *et al*. (1985) indicated that drought stress tolerance was found to be highly associated with root characteristics such as root thickness, root length density, number of thick roots, root volume, and root dry weight. It was also found that number of thick root, root thickness, and root length density were highly associated with leaf water potential and field visual drought scoring using drying leaf. Drought stress adapted plants are often characterized by deep and vigorous root systems (Blum, 1997). Nour *et al*. (1978) also reported root weight is the best and easiest attribute to determine drought tolerance in grain sorghum. Matsuura *et al*. (1996), on the other hand, reported a positive correlation between drought tolerance and root length in sorghum and millet (*Pennisetum glaucum*). Moreover, Plaut *et al*. (1996) and Pace *et al*. (1999) reported that seedlings under water stress caused an increase in root length with reduced diameter. Root depth, root length density, root distribution were reported as drought tolerance contributing traits (Taiz and Zeiger, 2006).

Drought is often associated with nutrient availability and the capacity of roots to absorb the available nutrients. Ludlow and Muchow (1990) indicated that greater root activity under intermittent drought should enhance crop stability by reducing the incidence of water deficits. Egilla *et al*. (2001) and Hongbo *et al*. (2006) reported the significance of potassium (K) in improving drought resistance and root longevity. Shao *et al*. (2005) also reported the importance of mineral elements, such as K+ and Na+ for root signal transduction function. Shangguan *et al*. (2005) further denoted that the hydraulic conductivity of roots can be mainly affected by nitrogen and phosphorous nutrients. Hydraulic conductance in sorghum is primarily dependent on the number of fully functional nodal roots (Blum *et al*., 1977). In moisture stress conditions, plants with sufficient P supply exhibited higher hydraulic conductivity than P deficient plants. Therefore, plants with sufficient P are found to be more droughts tolerant, and also have a higher ability to recover after drought.

2.10.3 Morphological and architectural development of root systems in sorghum

The capacity of plant roots to access available soil water is critical to crop adaptation in water-limited environments (Ludlow and Muchow 1990). This is especially important in sorghum because it is a crop that is frequently grown in such environments. The importance of root system attributes in sorghum also has been implicated in modelling studies that quantified the relative adaptive advantage of sorghum over maize in water-limited conditions (Sinclair and Muchow, 2001). Extensive genetic variation has been observed in sorghum root systems (Jordan *et al*. 1979) and studies on other species have highlighted the critical role of root system architecture in crop adaptation.

Esau (1967) reported that, the temporal development of root system architecture is determined by the nature of the root system and its rate of progression into the soil. In cereals, such as sorghum and maize, the root system is formed from the seminal roots that appear at germination and the nodal, crown or adventitious roots that arise later from nodes of the shoot.

Seminal roots play an important part in initial water and nutrient uptake and establishment of seedlings, whereas nodal roots dominate during the later stages of growth. Studies in sorghum indicates that the root system grows into the soil at about 2–3 cm day−1 (Dardanelli *et al*., 1997; Manschadi *et al*., 2008; Robertson *et al*., 1993; Whish *et al*., 2005). Roots have been variously described as primary, seminal, nodal, or axile and have often been associated with descriptors of their point of origin, such as radicle, coleorhiza, scutellar node, coleoptilar node, and mesocotyl (Cahn *et al*. 1989; Ho *et al*., 2003; Hochholdinger *et al*., 2004; Hund *et al*., 2007; Mollier and Pellerin 1999; Tillich 1977).

Water supplied to the plant by the root contributes to the overall water balance of the shoot. There is much evidence that the force driving water into roots usually is provided by the tension (negative pressure) created by transpiration from the shoot and extending to root xylem (Steudle, 1997). Despite the important functions performed by roots, relatively little is known about the processes that govern root water uptake.

It may be possible to associate mature plant root system architecture with the nature of root system development in young plants. This would provide an opportunity for rapid and effective phenotypic screening of breeding populations for desirable root system properties. A number of studies across species (Kato *et al*., 2006; Lynch and van Beem 1993; Manschadi *et al*., 2008; Nakamoto and Oyanagi 1994) have indicated that the angle at which root axes appeared at the seedling stage was associated with subsequent root system architecture and acquisition of soil resources.

An improved understanding of the development and growth of root systems in young plants of sorghum is required in order to develop specifications for a rapid phenotyping system for root architecture. Comparison of development of root systems in sorghum and other related cereal crops would inform this process for sorghum.

2.11 Association among root and shoot parameters in rice and other crops

Anon (1984), reported a significant positive association between plant height or shoot dry weight and root length, root thickness, root number and root dry weight. Tiller numbers was highly and positively correlated with root number and shoot dry weight.

Ekanayake *et al*. (1985) reported that root thickness and root number was correlated with height, tiller number and shoot weight. It was also found that root length, thickness and root volume were significantly correlated with recovery from drought and root characters showed positive association amongst themselves. Altoveros *et al*. (1990) studied root volume of selected upland and lowland rice varieties and reported that volume was positively correlated with both root and shoot length.

Landi *et al*. (2001) compared the performance of five maize populations synthesized on the basis of number of mesocotyl roots and degree of root branching over a diverse set of environments. They reported that populations selected for a high number of mesocotyl roots and root branching out yielded other populations in all environments, but no significant differences were observed among populations for various morphological components.

Venuprasad *et al*. (2002) reported in rice that the correlation between maximum root length and grain yield was positive under stress and negative in non-stress. Genotypes with thicker and deeper roots, manifested higher biomass and grain yield under stress. Only one QTL found to increase days to flowering in non-stress was also found to influence root volume and dry weight negatively under stress. The study suggests that loci enhancing grain yield and related traits were not pleiotropic with loci for desirable root morphological traits studied under low-moisture stress at vegetative stage, in the genetic material used in the study. It is thus possible to combine higher grain yield and desirable root morphological traits, favourably, to enhance productivity of rice under low-moisture stress.

Hund *et al*. (2004) reported in maize that there was close relation with early field growth and length of main roots and total length and number of first order laterals of the seminal roots. In pearl millet, shoot length was significantly correlated to root length (Haryanto *et al*., 1998).

The significant correlation between shoot and root dry weights under controlled low temperature is consistent with the reports in maize and sorghum (Yu *et al*., 2004). Compared to shoot dry weight, collecting root dry weight is time consuming.

Kumar *et al*. (2012) examined the extent of genotypic variability for root traits in a diverse set of maize inbred lines. Root traits were measured in maize lines grown up to 6, 10 and 14 days in the growth chamber on a germination paper. Combined analysis of variance revealed intermediate to high heritability values (range = 0.6-0.9) for all measured traits, indicating consistency across experiments. Root DW was significantly correlated with other root traits, indicating that direct selection based on root DW might be sufficient to improve other root traits.

Bai *et al*. (2013) genetic relationships between plant height and root morphology were investigated in a diverse set of wheat germplasm (*Triticum aestivum* L.) to investigate whether *Rht* genes controlling shoot height also control seedling root growth. The results indicated that there is a close relationship between root traits and PH, as some

genes, or closely linked genes, control both root traits and PH; however, other genes only influence root traits or PH.

2.12 Bioinformatics resources

Localisation of QTLs through wet-lab based techniques is a laborious method and also time consuming. To overcome the disadvantages of wet-lab based methods, bioinformatics can be employed. Bioinformatics is nothing but the application of statistics and computer science in the field of molecular biology. Application of computers in the field of molecular biology will reduce lot of time required in performing some of the wet-lab works.

There are many databases available for public use which contains enormous quantity of data which can be used in the field of molecular biology and hence in MAS to improve any trait. Some of such databases are NCBI, Gramene and GenBank.

2.12.1 NCBI (National Centre for Biotechnology Information)

The National Centre for Biotechnology Information (NCBI) is a part of United States National Library of Medicine (NLM), a branch of National Institute of Health (NIH). The NCBI is located in Bethesda, Maryland. Established in 1988 through legislation sponsored by Senator Claude Pepper. Initially NCBI was established as a national information resource for molecular biology information. NCBI creates public databases, conducts research in computational biology, develops software tools for analysing genome data and finally disseminates biomedical information-all for the better understanding of molecular processes affecting human health and disease.

NCBI houses a series of databases which have relevance to biotechnology and also biomedicine. Some of the important databases of NCBI include Genbank for DNA sequences and a bibliographic database of the biomedical literature called PubMed. All these databases are available online to public use through Entrez search engine (www.wikipedia.com).

2.12.2 GenBank

The GenBank sequence database is an annotated collection of all publicly available nucleotide sequences and their protein translations. This database is produced at National Center for Biotechnology Information (NCBI) as part of an international collaboration with the European Molecular Biology Laboratory (EMBL), Data Library from the European Bioinformatics Institute (EBI) and the DNA Data Bank of Japan (DDBJ). The NCBI has had the responsibility for making the available GenBank DNA sequences since 1992 (www.ncbi.nlm.gov).

GenBank and its collaborators receive sequences produced in laboratories throughout the world from more than 100,000 distinct organisms. GenBank continues to grow at an exponential rate, doubling every 10 months. Release 134, produced in February 2003, and contained over 29.3 billion nucleotide bases in more than 23.0 million sequences. GenBank is built by direct submissions from individual laboratories, as well as from bulk submissions from large-scale sequencing centers.

Since 1992, NCBI has grown to provide other databases in addition to GenBank. NCBI provides Online Mendelian Inheritance in Man, the Molecular Modelling Database (3D protein structures), dbSNP a database of single-nucleotide polymorphisms, the Unique Human Gene Sequence Collection, a Gene Map of the human genome, a Taxonomy Browser, and coordinates with the National Cancer Institute to provide the Cancer Genome Anatomy Project. The NCBI assigns a unique identifier (Taxonomy ID number) to each species of organism.

The NCBI has software tools that are available by www browsing or by FTP. For example, BLAST is a sequence similarity searching program. BLAST can do sequence comparisons against the GenBank DNA database in less than 15 seconds.

2.12.3 Gramene

Out of many databases available for QTL localisation on chromosomes, Gramene is a very important one. Gramene (http://www.gramene.org) is a community source for rice and a comparative genome mapping database for grasses. Rice is a model monocot

plant for understanding the other economically important crops. Gramene uses a relational database based on Oracle, replacing the existing AceDB database "RiceGenes". Gramene provides curated and integrative information about maps, sequence, genes, genetic markers, mutants, QTLs, controlled vocabularies and publications. Its aims are to use the rice genetic, physical and sequence maps as fundamental organizing units, to provide a common denominator for moving from one crop grass to another and are to serve as a portal for interconnecting with other web-based crop grass resources (Ware *et al*., 2002).

It is a collaborative project of 'cold spring harbour laboratory', Cornell University and rice community. The main core base of Gramene is the MySQL database management systems, make it a user friendly front end system and it is a stable and well supported. Rice is used as a framework genome to organised information for other grass species due to its smaller genome to organised information for other grasses such as maize (2400mb) and wheat (16000mb) having considerable larger genome.

The goals of Gramene are to (i) establish a database utilising the rice genome as a framework for identifying and characterising genes in other grasses (ii) provide, comparative maps between rice and other grasses based upon orthologous sequence and a wealth of genetic and phenotypic information available among the grasses (iii) develop a pilot study to assign gene ontology (GO) functional classification of 4000 confirmed or predicted rice genes (iv) curate information on major mutants, strains, phenotypes, polymorphisms and quantitative trait loci (QTLs) utilising a structural controlled vocabulary and (v) integrated with other plant databases to allow comparisons of conversed syntenic relationship and mutant phenotypes (Ware *et al*., 2002).

Gramene is currently a hybrid system. Ligancy data including traits, QTLs, strains and literature citation, are maintained in rice genes AceDB databases. New data, including nucleotide sequence, sequence annotation, physical maps and new genetic marker are maintained in a completely redesigned system. The new system uses a pen object model based on the bio Perl (www.bioperl.org) and ensemble code bases. The back end consists of a set of pen script running in an apache/mod Perl environment. The

best feature of ‘QTL selection’ is to be a user friendly with mapped data. It facilitates the comparative study of QTL and their mapped regions to investigate collinear regions found to carry genes identified in rice genome. For convenience of searching the traits are grouped in eight major families related to biotic and abiotic stress, fertility, anatomy, development, vigour, quality and yield (Ware *et al*., 2002).

2.12.4 ClustalW2

It is a general purpose DNA or protein multiple sequence alignment program for three or more sequences. For the alignment of two sequences please instead use our pairwise sequence alignment tools.

ClustalW2 is a multiple sequence alignment tool for the alignment of DNA or protein sequences. ClustalW2 calculates the best match for the input sequences based on the parameters entered and generates an easy to interpret report. This sequence alignment report displays the optimal alignment score, the alignment between sequences in a form such that the identities, similarities and differences can be clearly seen and a guide tree of the evolutionary relationships of aligned sequences.

III MATERIAL AND METHODS

This chapter deals with the concise description of the materials used and techniques adopted during the investigation. The particulars of the materials used, methods protocols followed and statistical tools used for analysis, in different experiments are presented under the respective experiment separately.

Experiment I

3.1 Discern the extent of variation for root morphological characters in Rice and Sorghum

3.1.1 Field Experiment – *Kharif*-2015 and summer-2016

3.1.1.1 Plant Material

Material used for the study is the bio-fortified rice accessions, the genotypes represent subspecies of *O. sativa* namely indica and japonica (Table 3a) and sorghum genotypes were suggested by the breeder, VC farm, Mandya (Table 3b).

3.1.1.2 Experimental site

The present study was carried out during *Kharif* and summer season of 2015 and 2016 at aerobic rice research laboratory of Department of Plant Biotechnology, University of Agricultural Sciences, GKVK Campus, Bengaluru, India, to generate the phenotypic data under managed field conditions. The experimental site is located at a latitude of 12^{o} 58' North, longitude of 77^{o} 35' East and altitude of 930 meters above mean sea level (MSL). Climatic conditions at experimental sites during *Kharif*-2015 and Summer-2016 presented in Figure 2. Physico-chemical properties of soil at experimental site are listed in Table 4.

3.1.1.3 Experimental Design and Crop Management

All the five genotypes each from rice and sorghum were planted in field in *kharif*-2015 and summer-2016 for field evaluation of all the genotypes and data was recorded at regular basis, the genotypes were planted in four replication. The design used is RCBD.

Table 3a: List of different rice genotypes used for the genotyping and phenotyping during *Kharif*-2015 and summer -2016

Sl. No.	Genotype	Origin	Race	Parentage	History
1	AM 65	UAS (B), India	Japonica	Azucena × Moroberekan	Improved
2	AM 72	UAS (B), India	Japonica	Azucena × Moroberekan	Improved
3	ARB 6	UAS (B), India	Indica	Buddha × IR 64	Improved
4	Moroberekan	Republic of Guinea	Tropical japonica	IR8-24-6 (M307H5)	Traditional
5	MTU1001	Maruteru, India	Japonica	Vajram × MTU 7014	Improved

Table 3b: List of different sorghum genotypes used for the genotyping and phenotyping during *Kharif*-2015 and summer-2016

Sl. No	Genotype
1	CSH-14
2	Dhanvi
3	MLHT
4	Roagro
5	SJH-1

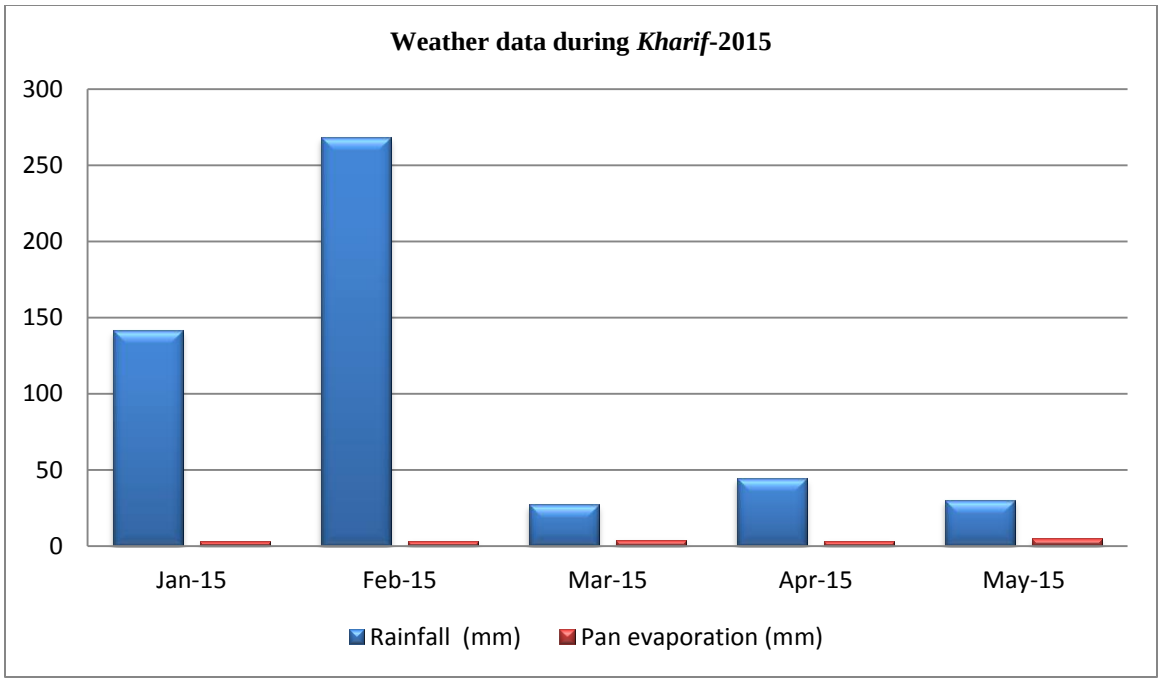


Fig. 2(a): Weather data during *Kharif*-2015

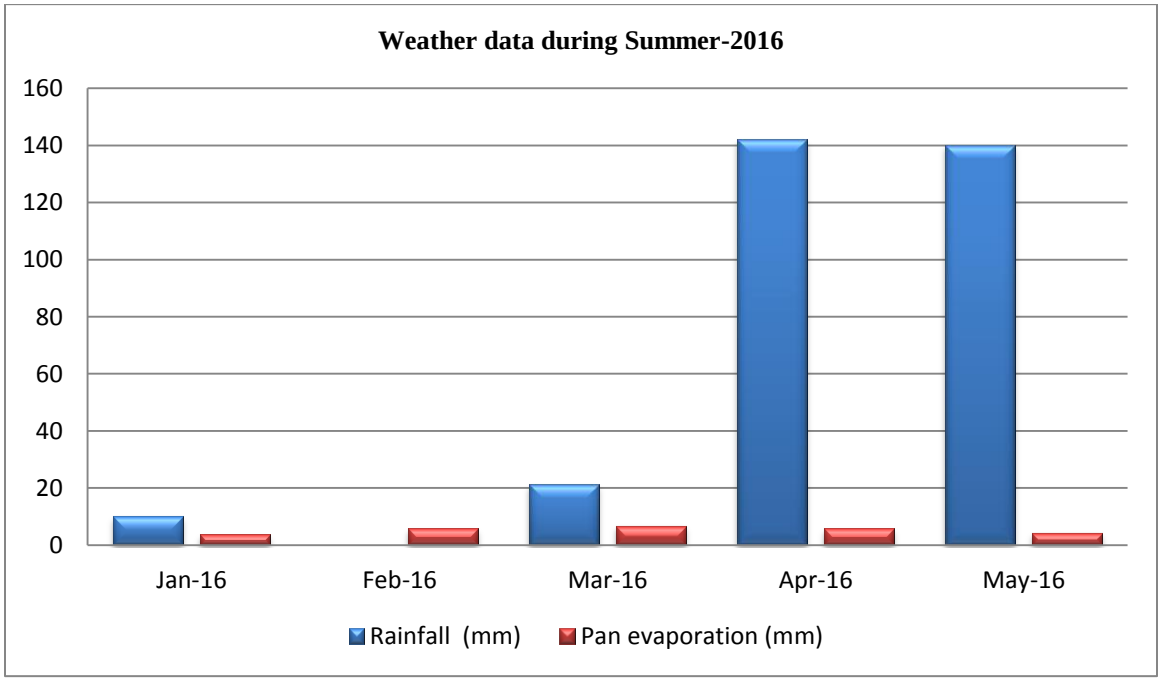


Fig. 2(b): Weather data during Summer-2016

Fig. 02: Weather parameters at experimental site during field and PVC pipe experiment

Table 4: Physico-chemical properties of soil at experimental site

Parameters	Value
Particle size distribution	
Sand (%)	79.0
Silt (%)	8.8
Clay (%)	13.6
Textural class	Sandy loam
pH	6.1
Electrical conductivity (dS m-1)	0.22
Organic carbon (g kg-1)	6.8
Cation exchange capacity {cmol kg-1}	9.1

Rice: Total area used for the experiment was 46.8 m sq, each plot contains 5 lines with 10 plants/line for each genotype. Fertilizers Basal dose of 100:50:50=N: P: K for rice was given and top dressing of 50 N was given after thirty days.

Sorghum: Total area used for the experiment was 123.75 m sq, each plot contains 5 lines with 10 plants/line for each genotype. Thus, one replication covers four lines. In case of sorghum fertilizers Basal dose of 90:45:45=N: P: K were given and top dressing of 50, 25, 25 N was given after thirty days intervals.

3.1.1.4 Cultural Operations

Seeds were sown directly in field at the spacing of 30 cm between rows and 10 cm between seeds within a row for rice and 60 cm between rows and 30 cm between seeds within a row for sorghum. One plant per hill was maintained. Recommended cultural practices were carried out to ensure uniform crop stand as per the package of practice (Anon, 2003). A view of the experimental plot is given in Plate 1. Irrigation was given when the leaves starts drying with puckered symptoms till required. Observations were recorded at regular intervals and at harvest.

3.1.1.5 Method of sampling and recording of observations

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

3.1.1.5.1 Observations recorded from rice and sorghum

3.1.1.5.1.1 Days to 50 per cent flowering

This is total number of days taken by each genotype from sowing to opening of first flower in 50 percent of the plants.

3.1.1.5.1.2 Days to maturity (d)

This is total number of days taken by each entry from sowing to maturity plants harvested only when fully mature.

Plate 01: Sorghum and Rice genotype grown in field condition

3.1.1.5.1.3 Plant height at maturity (cm)

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

3.1.1.5.1.4 Number of tillers per plant at maturity

Number of tillers (both productive and non-productive) was counted at the time of harvest.

3.1.1.5.1.5 Number of productive tillers per plant at maturity

Numbers of productive tillers were counted at the time of harvest.

3.1.1.5.1.6 Panicle length (cm)

Three panicle from each selected five plants were recorded at the time of harvest

3.1.1.5.1.7 Earhead length (cm)

The length from the base of the panicle (first whorl node) to the tip of the panicle was measured.

3.1.1.5.1.8 Shoot length (cm)

Shoot length was recorded from base of the plant to the upper most node of the plant.

3.1.1.5.1.9 Stem girth (cm)

This was measured at the middle of the first internode above the ground level with the help of vernier calipers

3.1.1.5.1.10 Biomass per plant (g)

The total dry weight of the plant parts above the ground comprising of grain & straw were recorded.

3.1.1.5.1.8 Grain yield per plant (g)

The total weight of all the filled grains of a plant was measured in grams.

3.1.1.5.1.11 Harvest index (%)

The ratio between the grain yield and biomass of the plant was recorded as harvest index.

3.1.2 PVC pipe experimentation during *Kharif*-2015 and summer-2016

All the genotypes were grown in polyvinyl chloride (PVC) pipes of 150 cm length and 18 cm diameter. Uniform soil mixture was prepared with the mix of manure and basal dose of NPK fertilizers and compacted into PVC pipes. Soil was compacted by frequent watering. Direct sowing was done and seeds were germinated in aerobic condition for five days (Plate 2). Ten days after germination, the seedlings were thinned, leaving only one seedling in each pipe. The plants were watered thrice weekly at equal intervals. At maturity stage, the shoots were cut at the base and the pipes were filled with water for overnight to loosen the soil. The following day, soil was eased out of the pipes and the roots were thoroughly cleaned with a jet of water and observations were recorded (Plate 3 and 4).

The observations were recorded on following root morphological traits in rice and sorghum.

3.1.2.1 Root Length (RL): It was recorded as length of the longest root (centimeter) from the crown.

3.1.2.2 Root Number (RN): Total number of roots per plant at crown region was recorded.

3.1.2.3 Root Volume (RV): Root volume was determined in cm^3 by water displacement method (Plate 5). Mean was calculated from values of all the replication.

Plate 02: Pictorial representation of root experiment in PVC pipe

Plate 03: Steps involved in washing of PVC pipe and collection of roots

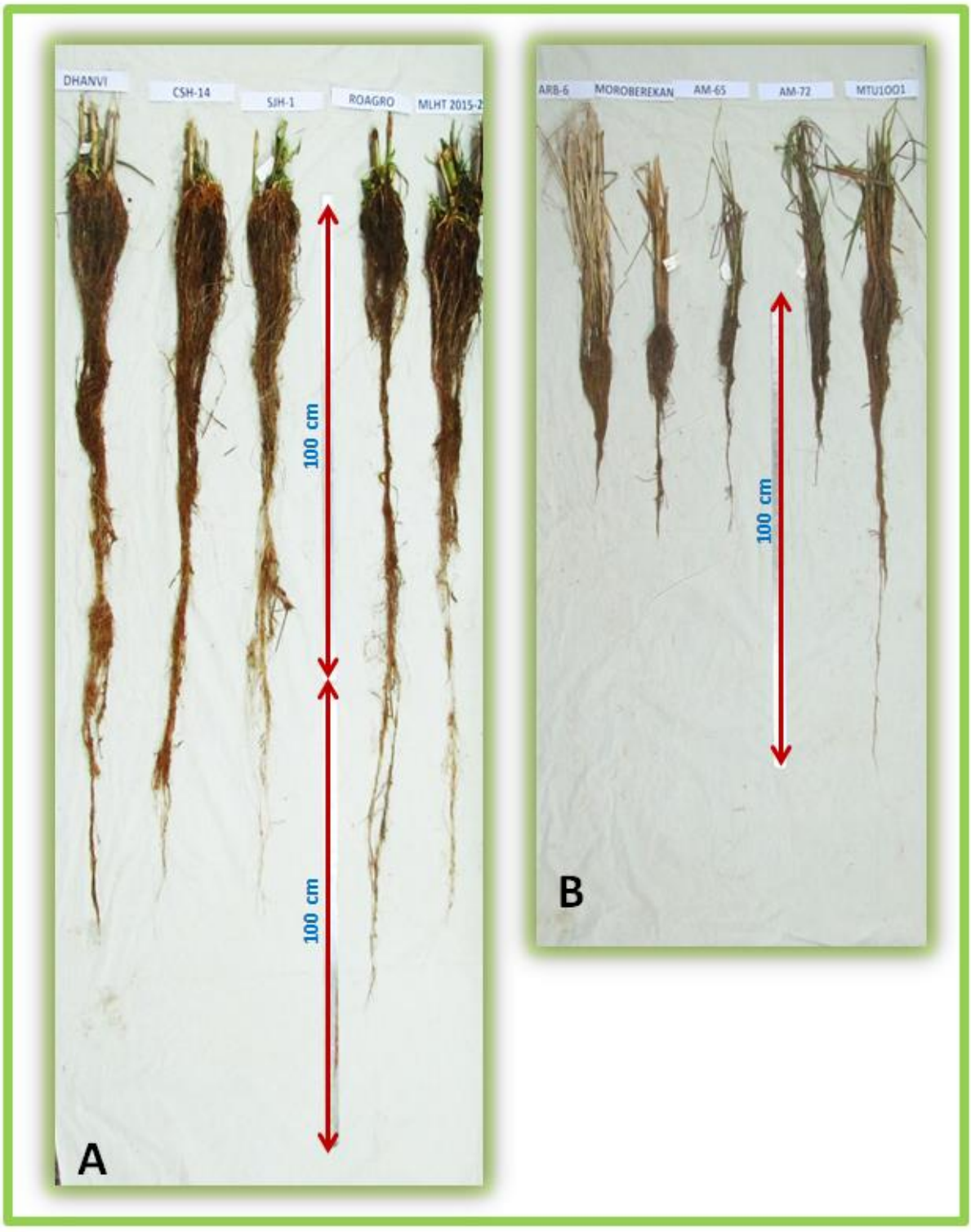


Plate 04: Roots of different Sorghum and Rice genotypes

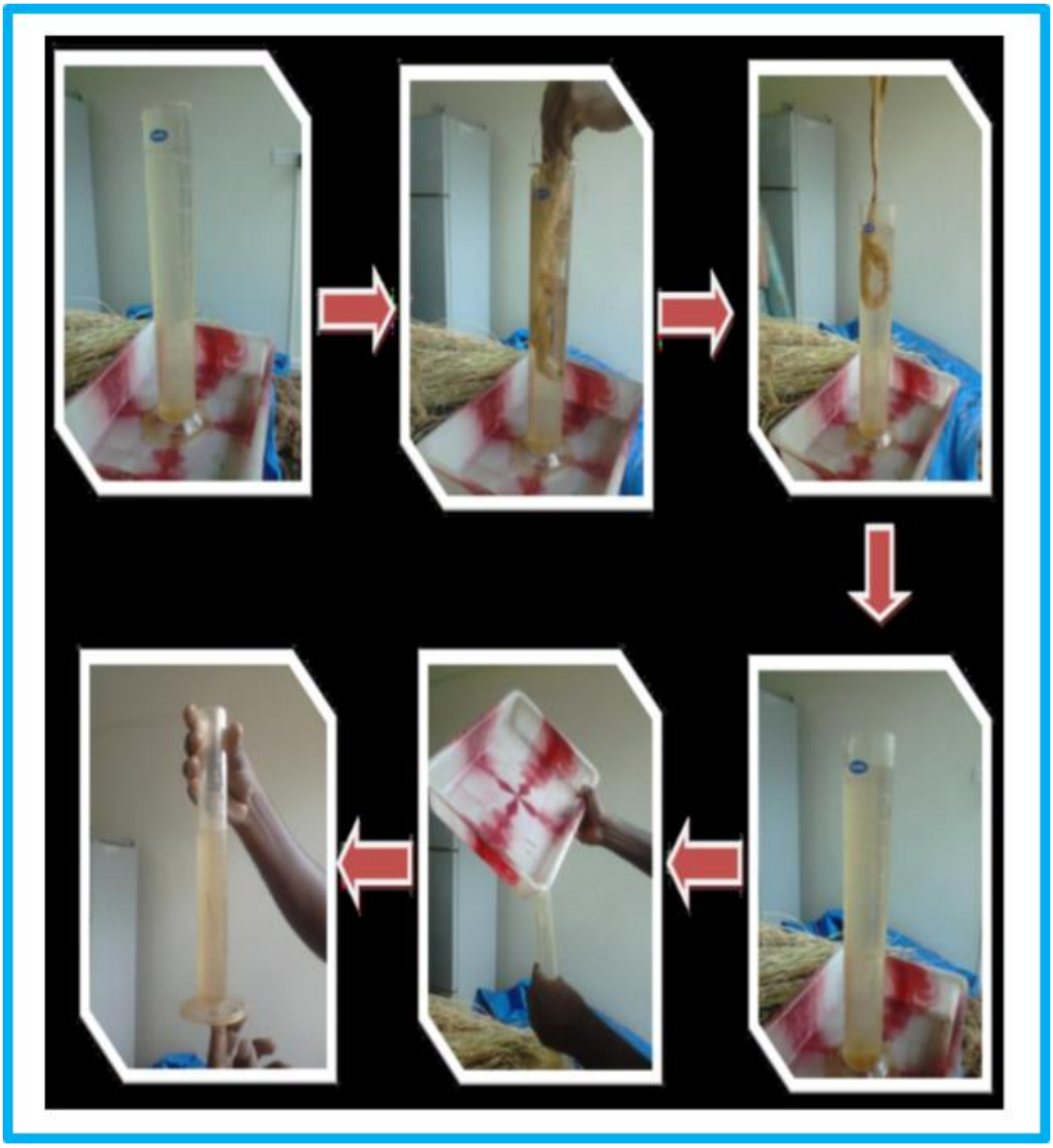


Plate 05: Measurement of root volume by water displacement method

3.1.2.4 Fresh Root Weight (FRW, g): The roots were cleared and blotted with filter paper for removal of excess water and the fresh weight was recorded in grams, using a digital balance.

3.1.2.5 Dry Root Weight (DRW, g): The roots, after cleaning were dried in the oven at 80^0 C for 72 hours, and the dry weight of roots was recorded in grams, using a digital balance.

3.1.2.6 Root: Shoot Length Ratio (RSLR): The ratio of maximum root length to plant height was computed and recorded.

3.1.3 Statistical analysis of field and pipe data from *Kharif*-2015 and summer 2016:

The observed field and PVC experiment from rice and sorghum were analysed by RBD (Panse and Sukhmate, 1964) and data were subjected to statistical analysis by using statistical software *viz*., SAS 9.4 and WINDOSTAT

3.1.3.1 Statistical analyses

Mean values of five plants used for recording the observations were computed for different plant characters for each of the genotypes. The phenotypic data for all the genotypes for each character was subjected to statistical analysis.

The observations recorded in respect of all the above quantitative traits were subjected to following standard statistical analysis.

3.1.3.1.1 Descriptive statistics

The following descriptive statistics were estimated as per Sunderaraj *et al.* (1972).

3.1.3.1.1.1 Mean

Mean is the sum of all observations in a sample divided by the number of observations.

3.1.3.1.1.2 Range

Range is the minimum and maximum values of the observations in a sample.

3.1.3.1.1.3 Standard error

It is the measure of uncontrolled variation present in a sample which is estimated by dividing the standard deviation by the square root of number of observations in the sample and is denoted by SE.

3.1.3.1.1.4 Variance

Variance is defined as the average of the squared deviations of individual observation from the mean or it is the square of the standard deviation. It is expressed as the sum of squares of the deviations of all observations of a sample from its mean and divided by (n-1), where n is the number of observations.

3.1.3.1.1.5 Analysis of variance

The analysis of variance for different characters was used to partition the variance due to different sources following the method given by Panse and Sukhatme (1964).

Source of Variation	Degree of freedom	Sum of squares	Mean sum of squares	F-ratio
Replication	(r-1)	rSS		
Genotype	(g-1)	gSS	$\frac{gSS}{(g-1)} = eMS$	$\frac{gMS}{EMS}$
Error	(r-1)(g-1)	ESS	$\frac{ESS}{(r-1)(g-1)} = EMS$	
Total	(rg-1)	TSS		

Wherein,

r = Number of Replications

g = Number of Genotypes

The significance was tested by comparing with the table values as given by Yates (1965). Standard error of means (SE_M) and Critical difference (CD) were worked out using appropriate formula for comparing individual line means.

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

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

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

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

Wherein,

P = Phenotypic standard deviation

G = Genotypic standard deviation

X = Grand mean of character

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

PCV and GCV were classified according to Robinson *et al.,* (1949). 0-10 % was considered as low, 10-20 % as moderate and 20 % and above as high.

3.1.3.1.1.7 Heritability (h^2)

Broad Sense Heritability was calculated using the formula (Hanson *et al.,* 1956).

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

Wherein,

h^2 % =Heritability percentage

Vg= Genotypic variance

Vp= Phenotypic variance

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

0-30 % was considered as low,

30-60 % was considered as moderate

60 % and above as high

3.1.3.1.1.8 Genetic advance (GA)

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

$$GA = h^2 \times \sigma_p \times K$$

Wherein,

h^2 = Heritability (Broad sense)

σ_p =Phenotypic standard deviation

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

3.1.3.1.1.9 Genetic advance as per cent mean

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

Wherein,

GA = Genetic advance and

X =Treatment mean for the character.

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

0-10 % Low;

10-20 % Moderate

20% and above as high

3.1.3.1.1.10 Correlation analysis

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

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

Wherein,

r_p = phenotypic correlation co-efficient.

COV (X, Y) = Phenotypic covariance.

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

3.1.3.1.1.11 Path-coefficient analysis

Path-coefficient analysis was carried out using phenotypic correlation values of different characters as suggested by Wright (1921) and as illustrated by Dewey and Lu (1959) was carried out to know the direct and indirect effect of the morphological traits and root traits on yield. Standard path-coefficients which are the standardized partial regression coefficients were obtained using statistical software package Genres 1. These values were obtained by solving the following set of 'P' simultaneous equations by using the above package.

$$P_{01} + P_{02}\, r_{12} + \text{----------} + P_{0P}\, r_{1P} = r_{01}$$

$$P_{01} + P_{12}\, r_{02} + \text{----------} + P_{0P}\, r_{2P} = r_{02}$$

$$\downarrow$$

$$P_{01} + r_{1P} + P_{02}\, r_{2P} \text{----------} + P_{0P} = r_{0P}$$

Wherein,

P_{01}, P_{02}, ------- P_{0P} are the direct effects of variables 1, 2, -------- p on the dependent variable 0 and r_{12}, r_{13} ,---------- r_{1P} -------- $r_{P\,(P-1)}$ are the possible correlation coefficients between various independent variables and r_{01}, r_{02}, r_{03} ------- r_{0P} are the correlations between dependent and independent variables. The indirect effect of the i^{th} variable *via* j^{th} variable is attained as ($P_{oj} \times r_{ij}$). The contribution of remaining unknown factor is measured as the residual factor, which is calculated and given below.

$$P^2ox = 1 - [P^2_{01} + 2P_{01}\, P_{02}\, r_{12} + 2\, P_{01}\, P_{03}\, r_{13} + \text{-------} + P^2_{02} + 2P_{02}\, P_{03}\, r_{13} + \text{-----} + P^2_{0P}]$$

Residual factor = $(P^2_{ox})^{½}$.

EXPERIMENT II

3.2 Compare sequence variation for root related genetic materials across crops

3.2.1. Molecular analysis in rice and sorghum

3.2.1.1 Plant material

Leaves were harvested from 21 days seedlings and DNA was extracted using CTAB method from young leaves tissues.

Requirements

- Leaf samples were collected from 21 days old seedlings and stored immediately at -70^{o}C.
- Cetyltrimethyl ammonium bromide (CTAB) extraction (100ml)

CTAB	2% (w/v)
TrisHCl (pH 8.0)	100 mM
Sodium Chloride	4 M
EDTA	20 mM

(Tris, sodium Chloride and EDTA were autoclaved and 2 % CTAB was added after autoclaving and preheated before using the buffer).

0.2 % β - Mercaptoethanol (added freshly).

- Tris EDTA (TE) buffer

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

(This was dissolved and made up to 100ml, autoclaved and stored at 4^{o}C)

- 2 % Polyvinyl poly pyrrolidone (PVP)
- Ice cold Isopropanol
- Chloroform: Isoamyl alcohol 24:1 (v/v)

- Sodium acetate (3.0 M, pH 7.0) (pH adjusted using glacial acetic acid)
- Absolute ethanol and 76 % ethanol (stored at -20^{o}C)
- RNase A: 10 mg/ml (RNase A was dissolved in TE buffer and stored at -20^{o}C).

Protocol for extraction of genomic DNA

- Leaf samples (approximately 2 g) were cut into small bits with sterile scissors and transferred to prechilled mortar.
- The leaf tissues were frozen using liquid nitrogen and ground to fine powder.
- The fine powder was allowed to thaw in 10ml of preheated extraction buffer in polypropylene centrifuge tubes and incubated for 30 minutes at 65^{o}C in water bath with occasional mixing.
- The tubes were removed from the water bath and equal volume of chloroform: Isoamyl alcohol mixture (24:1 v/v) was added and mixed by inversions for 15 minutes.
- The contents of polypropylene centrifuges tubes were centrifuged at 10000 rpm for 10 minutes at room temperature.
- The clear aqueous phase was transferred to a new sterile tube and equal volume of ice cold isopropanol was added and mixed gently by inversion and then kept in the freezer at 4^{o}C for the precipitation of DNA.
- Using blunt end tips, the precipitate was pooled out into a micro centrifuge tube and air dried after removing the supernatant by brief spin.
- 500 µl of chloroform: Isoamyl alcohol mixture was added and centrifuged at 10000 rpm for 10 minutes at room temperature.
- Aqueous phase was transferred to another micro centrifuge tube without disturbing the inner phase.
- To this, 2.5 volume of absolute alcohol and 1/10 volume of sodium acetate were added and kept for incubation overnight.

- It was centrifuged and the supernatant was discarded. To this 500 µl of each 70 % and 100 % ethanol was used subsequently to wash the DNA by centrifugation.
- The alcohol was discarded and DNA was completely air dried and DNA pellet was dissolved in 250 µl of TE and stored at 4^{o}C.

3.2.1.2 DNA quality assay using agarose gel electrophoresis

Materials

- 3X loading dye

5 M NaOH	200 µl
95 % formamide (v/v)	95 ml
50 % bromophenol blue (w/v)	50 g
0.5 % xylene cyanol (w/v)	50 mg

(Dissolved in sterile double distilled water and made up the volume to 100ml)

- 10X Tris Borate EDTA buffer (TBE)

Tris base	107.8 g
Boric acid	55.2 g
EDTA ($Na_2.2H_2O$)	9.2 g

(Dissolved in 800 ml of sterile double distilled water and made up to 1000 ml)

- 50X Tris Acetate EDTA buffer (TAE)

Tris base	242 g
Glacial acetic acid	57.1 ml
EDTA ($Na_2.2H_2O$) (0.5M, pH 8.0)	18.6 g in 100 ml of double distilled water

(Dissolved in 800 ml of sterile water and made up to 1000 ml)

- 100bp Ladder

  40 µl loading dye

  10 µl 100bp ladder

120 µl sterilized double distilled water

(Total volume made up to 170 µl)

Protocol

- The pyrex gel casting plate's open ends were sealed with cello tape and the comb was placed properly in casting plate kept on a perfectly horizontal platform
- 0.8 % (0.8 g/100 ml) agarose was added to 1X TBE, boiled until the agarose dissolved completely and then allowed to cool. Ethidium bromide (DNA intercalating agent) was added when temperature reaches to 55 - 60 ^{o}C as the staining agent.
- Then it was poured into the gel mould with combs placed and allowed to solidify. The comb and the cello tape were removed carefully after solidification of the agarose.
- The casted gel was placed in the electrophoresis unit with wells towards the cathode and submerged with 1X TBE to a depth of about 1 cm.

3.2.1.3 Loading the DNA samples

- 1 µl of the crude DNA sample was pipetted onto a parafilm and mixed well with 3 µl of 3X loading dye by pipetting up and down gently several times.
- The mixed contents were transferred to the wells of agarose gel placed inside the 1X TBE buffer.

3.2.1.4 Quantification of DNA

The genomic DNA was quantified Nano spectrophotometer both at 260 nm and 280 nm wavelengths. The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg of double stranded DNA. A pure sample of DNA shows the ratio of OD 260/280 as 1.8. Ratios less than 1.8 indicate contamination in the isolation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the isolation.

3.2.1.5 Normalization of the DNA concentration

Normalization of the DNA concentration was done to bring all the DNA concentrations to a relatively equal level (25ng/µl) by appropriate dilutions for PCR reaction. Dilution was done with double distilled sterile water.

3.2.2 Marker analysis in rice and sorghum

3.2.2.1 Use of primers for root related genes

The details of primer sequences for seven known candidate genes obtained from literature survey are given in Table 5

3.2.2.2 Simple Sequence Repeats (SSR)

Microsatellite or simple sequence repeat (SSR) markers have been successfully used for genomic mapping, DNA fingerprinting, and marker assisted selection in many plant species. Basically, SSR are comprised of tandom arrays of 2 to 5bp monomeric repeat units. Polymorphism appears because of variation in the number of tandom repeats in a given repeat motif. They are co dominant in their expression. 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. These variations in length are easy to trace in the lab and allow us to track genotypic variation in breeding programs.

SSRs provide fairly comprehensive genomic coverage, they are amenable to automation, they have locus identity, and they are multiallelic. Many agronomic and quality traits show quantitative inheritance, and the genes determining these traits have been quantified using quantitative trait locus (QTL) tools. Our objective is to find amplification of rice SSR markers in sorghum.

A total of 62 SSR markers widely distributed on 12 rice chromosomes were used in this study. The sequence information of these primers is available at http://www.gramene.org. The chromosome location and base sequence of forward and reverse primers of SSR markers used in the present study is mentioned in the Table 6.

Table 5: The list of root genes involved in different functions

Sl. No.	Gene	Chr. number	Function	Reference
1.	*EXP15*	3	Root Hair Development	Zhiming *et al*. (2011)
2.	*OsGLU3*	4	Root Elongation	Zhang *et al*.(2012)
3.	*OSCSLD*	10	Root Hair Development	Kim *et al*. (2007)
4.	*GLR3.1*	4	Root Elongation	Li *et al*. (2006)
5.	*EXP17*	3	Root Hair Development	Zhiming *et al*, (2011)
6.	*OsIAA11*	3	Lateral Root Development	Zhu *et al*. (2011)
7.	*OsIAA13*	3	Lateral Root Development	Kitomi *et al*. (2012)

Table 6: List of markers and their sequences used in the present investigation

Sl. No.	Marker	Chr. number	Amplicon Size (bp)	Annealing Temp (^{0}C)	Forward Sequence	Reverse Sequence
1.	RM212	1	136	52.5	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
2.	RM3810	1	105	55.4	ACGAAGGAACTACCCGTGTG	CGCACATGTTACTCTAGCGG
3.	RM3825	1	147	58.5	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
4.	RM157	1	106	60	CCTCCTCCTCACGAATCCCGCC	GGGCTTCTTCTCCGCCGGCTTC
5.	RM128	1	148	63.9	AGCTTGGGTGATTTCTTGGAAGCG	ACGACGAGGAGTCGCCGTGCAG
6.	RM140	1	261	64.6	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG
7.	RM543	1	98	55	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC
8.	RM5	1	113	55	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
9.	RM428	1	266	55	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTTG
10.	RM213	2	139	57	ATCTGTTTGCAGGGGACAAG	AGGTCTAGACGATGTCGTGA
11.	RM221	2	186	57	ACATGTCAGCATGCCACATC	TGCAAGAATCTGACCCGG
12.	RM250	2	153	59.5	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
13.	RM262	2	154	58.3	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC
14.	RM263	2	199	53.5	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
15.	RM315	2	133	53.5	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
16.	RM 7278	2	175	54.5	TGCTAGTCTGATGAATGCCG	TTAGAACACCACATGGCAGC
17.	RM211	2	161	60	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG
18.	RM166	2	321	60.3	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG
19.	RM324	2	175	60	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
20.	RM318	2	140	60.3	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC

Table 6: Cont….

Sl. No.	Marker	Chr. number	Amplicon Size (bp)	Annealing Temp (^{0}C)	Forward Sequence	Reverse Sequence
21.	RM53	2	182	55	ACGTCTCGACGCATCAATGG	CACAAGAACTTCCTCGGTAC
22.	RM48	2	204	55	TGTCCCACTGCTTTCAAGC	CGAGAATGAGGGACAAATAACC
23.	RM168	3	116	57	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC
24.	RM231	3	168	58.5	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
25.	RM60	3	165	55	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC
26.	RM563	3	185	60	CGACCCTAGGGTTTCTCC	CTCGACGTCGTGGAAAGC
27.	RM148	3	129	59.9	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG
28.	RM36	3	192	55	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTACC
29.	RM185	4	197	64.6	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC
30.	RM252	4	216	68.4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG
31.	RM518	4	171	60	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
32.	RM1153	4	114	60.3	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC
33.	RM348	4	136	50.4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
34.	RM194	5	250	68.7	GCCCTGCTTCTTGCCCACCACC	TCCAGGGAGGGCAAGGCTGAGC
35.	RM146	5	345	60	CTATTATTCCCTAACCCCCATACCCTCC	AGAGCCACTGCCTGCAAGGCCC
36.	RM5844	5	195	60	TGACTAACGTGGCATCCATG	GCTAGGAGCCATTGTCGAAG
37.	RM169	5	167	67	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
38.	RM170	6	121	55	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC
39.	RM248	7	102	59.2	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
40.	RM420	7	197	57.5	GGACAGAATGTGAAGACAGTCG	ACTAATCCACCAACGCATCC
41.	RM234	7	156	60.3	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG

Table 6: Cont….

Sl. No.	Marker	Chr. number	Amplicon Size (bp)	Annealing Temp (^{0}C)	Forward Sequence	Reverse Sequence
42.	RM223	8	145	56.3	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
43.	RM331	8	176	55	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG
44.	RM38	8	250	55	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCCAC
45.	RM195	8	311	63.9	AGAAAGAGAGGCCGTCGGCGGC	GGGCTCACCCCCAAACCTGCAG
46.	RM506	8	123	55	CGAGCTAACTTCCGTTCTGG	GCTACTTGGGTAGCTGACCG
47.	RM72	8	176	55	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
48.	RM407	8	172	55	GATTGAGGAGACGAGCCATC	CTTTTTCAGATCTGCGCTCC
49.	RM201	9	158	52	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
50.	RM205	9	122	59	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
51.	RM215	9	148	59	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
52.	RM242	9	225	58	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
53.	RM296	9	123	60	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG
54.	RM316	9	192	56.7	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
55.	RM328	9	172	55	CATAGTGGAGTATGCAGCTGC	CCTTCTCCCAGTCGTATCTG
56.	RM107	9	189	67	AGATCGAAGCATCGCGCCCGAG	ACTGCGTCCTCTGGGTTCCCGG
57.	RM2125	10	142	48.5	TACCTCCTAGCTTTACTTAT	ACTGATCTCTATCTCATTGT
58.	RM304	10	160	55	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
59.	RM206	11	147	52	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
60.	RM229	11	116	57.5	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
61.	RM4	11	159	55	CGTTGATTCGAAGGGTGTATCC	GAGGTCAGCACTGACGAGTTAAGC
62.	RM144	11	237	55	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG

3.2.2.3 PCR reaction mixture

PCR for candidate markers was performed in a total volume of 20 µl containing 1X PCR buffer (contains 10 mM Tris-HCl, pH 8.0 at 25 ^{0}C, 50 mM KCl, 1.5 mM $MgCl_2$), 0.25 µM of each forward and reverse primers (Sigma Aldrich, USA), 50 ng rice genomic DNA, 0.5 mM dNTPs mix and 1.2 units of *Taq* polymerase (Bangalore Genei, India). The PCR reaction in thermal cycler (Bioscience) was programmed as details given in Table 7 and pictorial representation of PCR programme used was depicted in Figure 3.

3.2.2.4 Agarose gel electrophoresis

Agarose gel (3.0 %) was prepared using electrophoresis grade agarose (Bangalore Genei, India) in a volume of electrophoresis buffer (1X TAE) sufficient for constructing a gel (220 ml for 20 × 20 cm gel). Ethidium bromide was added at concentration of 10 mg/ml of gel. The gel was allowed to solidify fully before removing the combs and loading the sample. 7 µl of 3X loading dye was added to 20 µl of PCR products, and mixed well before loading into the well. Care was taken to prevent mixing of samples between the wells. A voltage of 5 V/cm was given for a time period of three hours for separation of PCR fragments. The gel was viewed under UV trans-illuminator and the DNA banding pattern was recorded directly and later with Alpha Innotech gel documentation instrument

3.2.3 Screening of SSR markers for polymorphism

3.2.3.1 Scoring of bands

The bands generated by SSR primers were scored as ‘0’ for absence, 1, 3, 5 for same size single band and ‘2’ for those showing both the bands.

3.2.4 Sequencing the amplified products and establishing associations with the phenotype

3.2.4.1 Sequencing

Sequencing of the amplified product(s) was outsourced to "Indus biosolutions Pvt Limited", Bangalore.

Table 7: Temperature profile used for PCR amplification using SSR primers

Profile	Activity	Temperature (°C)	Duration	Cycles
1	Initial denaturation	95	5 mins	1
2	Denaturation	95	30 secs	
3	Annealing	55*	30 secs	35
4	Extension	72	1 mins	
5	Final extension	72	10 mins	1
6	Storage	4	∞	1

* = Temperature changes with different primers

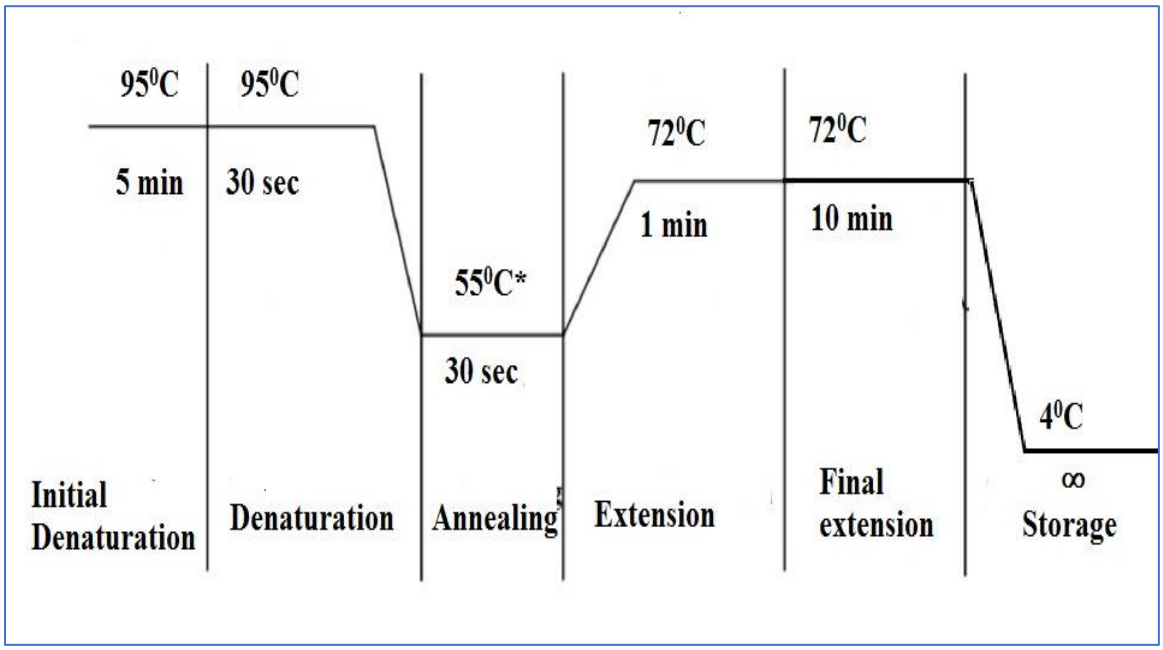


Figure 3: Pictorial depiction of PCR cycling used in the present study

* = Temperature changes with different primers

3.2.4.2 Alignment

Sequences obtained were then aligned using 'clustalw' tool http://www.ebi.ac.uk/Tools/msa/clustalw2 (Larkin *et al.,* 2007). All sequences of nine genotypes were aligned in this tool to discern the variations existing in the sequences.

3.2.4.3 Identification of variations

Jalview tool (Waterhouse *et al.,* 2009) of the clustalw website was used for the identification of the variations present in these sequences. Sequence of all ten genotypes for each and every marker chosen was aligned and variations were recorded at each base. Any changes in any position of these sequences with any base pair or 'in-dels' were recorded.

EXPERIMENT III

3.3 Establish associations between variations at the sequence with phenotypic data and mine multiple alleles

3.3.1 Associating the variations

Once the sequences were aligned, change at each base was recorded, statistical analysis was done to establish association(s) if any. Each possible change at any position of the sequence was scored. For sequences having same nucleotide as the consensus sequences was given code "1", any change was scored as 2, 3 or 4 depending upon the nucleotide substituted. In case of deletion at that locus, it was scored as 0. These scores were then used for the analysis using SPSS for associating these variations in the nucleotide level with the phenotypic variation for root traits.

3.3.2 Single-Marker Analysis (SMA)

To detect associations between molecular markers and traits of interest, data analysis approaches include single marker analysis, simple interval mapping (SIM), multiple interval mapping (MIM), and composite interval mapping (CIM). Although these approaches are designated for QTL analysis, they are also typically employed

whenever a trait's method of genetic control is unknown. In this study SMA was used to detect association between marker and trait.

Single-marker analysis was done with the help of student's t-test distribution given by Gosset in 1908.

$$t = \frac{X_1 - X_2}{\sqrt{SP^2\,(1/n_1 + 1/n_2)}}$$

Wherein,

X_1: mean of trait of interest under investigation in individuals of marker class I

X_2: mean of trait of interest under investigation in individuals of marker class II

SP^2: Pooled variance

$n_{1:}$ number of genotypes in marker class I

$n_{2:}$ number of genotypes in marker class II

$$SP^2 = \frac{S_1^2\,(n_1\text{-}1) + S_2^2\,(n_2\text{-}1)}{n_1 + n_2 - 2}$$

Where in,

S_1^2: variance of trait of interest for marker class I

S_2^2: variance of trait of interest for marker class II

Calculated t-value was compared with table t-value at $(n_{1+}\ n_2 - 2)$ degrees of freedom at 5 % and 1 % of probability.

3.3.3 Online tools used in the study

In biotechnology online research tools and databases are of utmost importance and play a major role. In this study online tools and databases has supported to a great extant GRAMENE a grass database is used in the study.

3.3.3.1 GRAMENE

As an information resource, Gramene purpose is to provide added value to data sets available within the public sector, which will facilitate researchers' ability to

understand the grass genomes and take advantage of genomic sequence known in one species for identifying and understanding corresponding genes, pathways and phenotypes in other grass species. In the current study gramene has been explored to extract all essential information of SSR markers, biotic stress marker and candidate gene like sequence homology, product size and primer sequences (Plate 6).

STEP 1 –Open GRAMENE home page www.gramene.org

STEP 2 –Enter GRAMENE home

STEP 3 – collect all available information on marker

Plate 06: Steps involved in extracting marker details from the GRAMENE online database

IV RESULTS AND DISCUSSION

The results obtained from the various experiments conducted to achieve different objectives of the study are presented under the following subheadings:-

4.1 Discern the extent of variation for root morphological characters in Rice and Sorghum

4.1.1 Analysis of variance

4.1.2 Mean performance of selected during field and root experiment genotypes

4.1.3 Correlation studies

4.1.4 Path-coefficient analysis

4.2 Compare sequence variation for root related genetic materials across crops

4.2.1 Screening of genotypes with the selected primers

4.2.2 Sequencing the amplified root related primers

4.3 Establish associations between variations at the sequence with phenotypic data and mine multiple alleles

4.3.1 Identification of variation in the sequence

4.3.2 Associating of nucleotide variation with phenotypic variation

4.1 Discern the extent of variation for root morphological characters in Rice and Sorghum

4.1.1 Field Experiment: *Kharif*-2015 and Summer-2016

4.1.1.1 Analysis of variance

The mean sum of squares due to various sources of variation for nine characters in five genotypes of each rice and sorghum during *kharif*-2015 and summer-2016 is represented in Table 8 and 9 respectively. Highly significant differences among the genotypes were observed for all the characters indicating variability for the yield associated traits except for total number of tillers in rice during *Kharif*-2015 and for harvest index during *Kharif* -2015 and stem girth during summer-2016 in sorghum.

Table 8: Analysis of variance for grain yield and related characters of rice accessions under aerobic condition during *Kharif*-2015 (season-I) and Summer-2016 (season II)

Mean sum of squares	Source of variation	Season I						Season II					
	df	Replication	Genotype	Error	CD @		CV	Replication	Genotype	Error	CD @		CV
		3	4	12	5%	1%		3	4	12	5%	1%	
	PH	68.66	1945.61***	22.75	7.34	10.3	4.47	47.85	1966.17***	31.02	8.58	12.03	5.05
	TNT	4.46	101.40	3.84	3.02	4.23	13.27	6.61	155.31***	3.90	3.04	4.26	12.24
	NPT	5.23	95.09***	3.80	3.00	4.21	14.23	5.41	153.26***	4.47	3.25	4.56	13.70
	PL	19.12	12.97**	1.58	1.93	2.71	5.21	3.91	31.52**	3.65	2.94	4.12	7.33
	DFF	1.43	503.01***	2.78	2.57	3.60	1.66	1.65	381.25***	6.31	3.87	5.42	2.57
	DM	1.68	412.38***	2.12	2.24	3.14	1.14	10.73	749.57***	3.77	2.99	4.19	1.51
	BM	36.26	320.16**	51.37	11.04	15.48	11.44	36.58	352.92**	55.92	11.52	16.15	12.11
	HI	0.219	2.83**	0.462	1.04	1.46	17.50	0.21	5.05***	0.36	0.92	1.30	14.90
	GY/P	8.06	24.13**	3.45	2.86	4.01	11.00	8.24	52.93***	4.39	3.2289	4.52	13.00

**- Significant at the 0.01 level
* - Significant at the 0.05 level

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index, GY: Grain Yield per plant (g)

Table 9: Analysis of variance for grain yield and related characters of sorghum genotypes during *Kharif*-2015 (season-I) and Summer-2016 (season II)

Mean sum of squares	Source of variation	Season I							Season II				
	df	Replication	Genotype	Error	CD @		CV	Replication	Genotype	Error	CD @		CV
		3	4	12	5%	1%		3	4	12	5%	1%	
	PH	53.82	3913.32***	138.84	18.15	25.45	6.71	97.49	3364.67***	214.49	22.56	31.63	8.50
	EHL	0.92	41.11**	6.25	3.85	5.40	9.17	1.41	41.94**	6.76	4.00	5.61	10.30
	SL	51.36	4560.06***	122.97	17.08	23.95	7.47	38.79	4437.45***	134.79	17.88	25.07	7.94
	SW	1.06	94.77***	0.86	1.43	2.01	9.29	0.00	0.006	0.016	0.19	0.27	10.92
	DFF	2.66	180.67***	1.04	1.57	2.20	1.49	3.73	197.87***	1.27	1.73	2.43	1.59
	DM	10.00	593.07***	2.04	2.20	3.08	0.96	3.06	529.12***	3.19	2.75	3.85	1.18
	BM	5.91	649.92***	14.12	5.79	8.11	2.81	8.05	556.80***	23.30	7.43	10.42	3.78
	HI	0.01	1.90	0.02	0.26	0.37	11.16	0.74	4.84*	1.05	1.58	2.21	52.04
	GY/P	4.98	3786.72***	58.86	11.82	16.57	7.88	1001.38	4905.25**	831.76	44.43	62.29	32.45

**- Significant at the 0.01 level
* - Significant at the 0.05 level

PH: Plant height (cm)
EHL: Earhead length
DFF: Days to 50 % flowering
BM: Biomass (gm)
GY: Grain Yield per plant (g)
SL: Shoot length (cm)
SW: Stem girth (cm)
DM: Days to maturity
HI: Harvest index

4.1.1.2 Mean performance of all selected genotypes

The genetic variability parameters such as, minimum, maximum, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (H^2) and genetic advance as per cent mean for quantitative traits and root related traits of rice and sorghum genotypes for *Kharif*-2015 and summer-2016 are presented in Tables 10, 11, 12 and 13 respectively.

4.1.1.2.1 Plant Height in rice and sorghum

Among the selected rice genotypes, range of variation for plant height was from 79.82 cm (ARB6) to 131.5 cm (AM65) with an average value of 106.71 cm. High PCV and GCV of 21.02 per cent and 20.54 per cent respectively, high heritability of 95.5 per cent and high GAM of 41.35 per cent were recorded during *Kharif*-2015.

During summer-2016, range of variation for plant height was from 84.15 cm (ARB6) to 135.10 cm (AM65) with an average value of 110.24 cm. High PCV and GCV of 20.58 per cent and 19.95 per cent respectively, high heritability of 94.0 per cent and high GAM of 39.84 per cent were recorded for this trait.

Among the selected sorghum genotypes, range of variation for plant height was from 152.95 cm (SJH-1) to 230.70 cm (MLHT) with an average value of 175.59 cm. Moderate PCV and GCV of 18.73 per cent and 17.49 per cent respectively, high heritability of 87.2 per cent and high GAM of 33.64 per cent was recorded during *kharif*-2015.

During summer-2016, range of variation for plant height was from 150.25 cm (SJH-1) to 223.10 cm (MLHT) with an average value of 172.16 cm. Moderate PCV and GCV of 18.38 per cent and 16.30 per cent respectively, high heritability of 78.60 per cent and high GAM of 29.76 per cent was recorded for this trait.

4.1.1.2.2 Total Number of tillers in rice

Among the selected rice genotypes during *Kharif*-2015, range of 6.97 (Moroberekan) to 19.92 (AM72) tillers with an average of 14.77 was observed for this

Table 10: Estimates of mean, range and genetic parameters for different traits in rice accessions during *Kharif*-2015 (season-I)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
PH	106.71 ±2.38	79.82	ARB6	131.5	AM65	20.54	21.02	95.5	41.35
TNT	14.77 ± 0.98	6.97	Moroberekan	19.92	AM72	33.42	35.96	86.4	63.99
NPT	13.70 ± 0.97	6.10	Moroberekan	18.65	AM72	34.85	37.65	85.7	66.47
PL	24.14 ± 0.62	22.40	ARB6	26.45	AM65	6.98	8.71	64.2	11.53
DFF	100.40 ± 0.83	84.12	MTU1001	114.37	Moroberekan	11.13	11.26	97.8	22.69
DM	127.15 ± 0.72	111.62	MTU1001	138.12	AM65	7.96	8.04	98.0	16.24
BM	62.60 ± 3.58	51.80	ARB6	74.73	MTU1001	13.09	17.39	56.7	20.30
HI	3.88 ± 0.34	2.80	AM72	5.01	MTU1001	19.84	26.46	56.2	30.65
GY/P	16.90 ± 0.92	15.38	MTU1001	21.22	AM72	13.45	17.37	59.9	21.45

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as per cent of mean

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index
GY: Grain Yield per plant (g)

Table 11: Estimates of mean, range and genetic parameters for different traits in rice accessions during Summer-2016 (season II)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
PH	110.24 ± 2.78	84.15	ARB6	135.10	AM65	19.95	20.58	94.0	39.84
TNT	16.13 ± 0.98	7.15	Moroberekan	23.05	AM72	38.14	40.06	90.7	74.81
NPT	15.44 ± 1.05	6.55	Moroberekan	22.20	AM72	39.50	41.81	89.3	76.87
PL	26.05 ± 0.95	22.21	MTU1001	29.51	AM65	10.13	12.50	65.6	16.90
DFF	97.75 ± 1.25	84.50	MTU1001	111.00	Moroberekan	9.90	10.23	93.7	19.74
DM	127.90 ± 0.97	113.0	MTU1001	143.00	Moroberekan	10.67	10.78	98.0	21.77
BM	61.70 ± 3.73	50.31	ARB6	74.440	MTU1001	13.96	18.49	57.0	21.72
HI	4.03 ± 0.30	2.53	AM72	5.23	MTU1001	26.83	30.69	76.4	48.32
GY/P	16.11 ± 1.04	13.21	Moroberekan	22.40	AM72	21.62	25.23	73.4	38.16

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance**;** $\mathbf{h^2}$: Broad sense heritability; **GAM**: Genetic advance as percent of mean

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index
GY: Grain Yield per plant (g)

Table 12: Estimates of mean, range and genetic parameters for different traits in sorghum genotypes during *Kharif*-2015 (season-I)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
PH	175.59 ± 5.89	152.95	SJH-1	230.70	MLHT	17.49	18.73	87.2	33.64
EHL	27.26 ± 1.25	23.30	MLHT	29.90	Dhanvi	10.82	14.19	58.2	17.02
SL	148.33 ± 5.54	123.40	SJH-1	207.40	MLHT	22.45	23.66	90.0	43.88
SW	10.03 ± 0.46	1.33	MLHT	12.32	Dhanvi	48.28	49.16	96.4	97.66
DFF	68.20 ± 0.51	61.00	CSH-14	77.00	Roagro	9.82	9.93	97.7	20.01
DM	148.40 ± 0.71	129.50	CSH-14	159.00	Roagro	8.19	8.24	98.6	16.75
BM	133.45 ± 1.87	118.25	Roagro	150.50	SJH-1	9.44	9.85	91.8	18.65
HI	1.54± 0.08	1.18	CSH-14	2.77	MLHT	44.39	45.77	94.0	88.69
GY/P	97.31 ± 3.83	45.38	MLHT	124.06	SJH-1	31.36	32.34	94.1	62.67

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; h^2: Broad sense heritability; **GAM**: Genetic advance as percent of mean

PH: Plant height (cm)
EHL: Earhead length
DFF: Days to 50 % flowering
BM: Biomass (gm)
GY: Grain Yield per plant (g)
SL: Shoot length (cm)
SW: Stem width (cm)
DM: Days to maturity
HI: Harvest index

Table 13: Estimates of mean, range and genetic parameters for different traits in sorghum genotypes during Summer-2016 (season II)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
PH	172.16 ±7.32	150.25	SJH-1	223.10	MLHT	16.30	18.38	78.6	29.76
EHL	25.23 ± 1.30	21.35	MLHT	27.90	Dhanvi	11.75	15.63	56.5	18.20
SL	146.15 ± 5.80	123.00	SJH-1	204.60	MLHT	22.44	23.80	88.9	43.57
SW	1.18 ± 0.06	1.15	SJH-1	1.25	MLHT	4.22	10.07	17.6	3.64
DFF	71.00 ± 0.56	63.00	CSH-14	79.50	Roagro	9.87	10.00	97.5	20.08
DM	151.00 ± 0.89	133.25	CSH-14	161.00	Roagro	7.59	7.68	97.6	15.45
BM	127.55 ± 2.41	114.00	Roagro	139.50	SJH-1	9.05	9.81	85.1	17.20
HI	1.97 ± 0.51	1.16	SJH-1	3.71	MLHT	49.34	71.71	47.3	69.93
GY/P	88.86 ± 14.42	31.89	MLHT	121.92	SJH-1	35.91	48.40	55.0	54.88

PCV: Phenotypic coefficient of variance; **GCV:** Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as percent of mean

PH: Plant height (cm)
EHL: Earhead length
DFF: Days to 50 % flowering
BM: Biomass (gm)
GY: Grain Yield per plant (g)
SL: Shoot length (cm)
SW: Stem width (cm)
DM: Days to maturity
HI: Harvest index

trait. High PCV and GCV of 35.96 per cent and 33.42 per cent, high heritability of 86.4 per cent and high GAM of 63.99 per cent were recorded for this trait in these genotypes.

Among the selected rice genotypes during summer-2016, range of 7.15 (Moroberekan) to 23.05 (AM72) tillers with an average of 16.13 was observed for this trait. High PCV and GCV of 40.06 per cent and 38.14 per cent, high heritability of 90.07 per cent and high GAM of 74.81 per cent were recorded for this trait.

4.1.1.2.3 Number of productive tillers in rice

During *Kharif*-2015, an average of 13.70 was observed for this trait with a range of 6.10 (Moroberekan) to 18.65 (AM72) tillers. High PCV of 37.65 per cent, high GCV of 34.85 per cent, high heritability of 85.7 per cent and a high GAM of 66.47 per cent was recorded for this trait in these genotypes.

During summer-2016, an average of 15.44 was observed for this trait with a range of 6.55 (Moroberekan) to 22.02 (AM72) tillers. High PCV of 41.81 per cent, high GCV of 39.50 per cent, high heritability of 89.3 per cent and a high GAM of 76.87 per cent was recorded for this trait in these genotypes.

4.1.1.2.4 Panicle length in rice and Earhead length in sorghum (cm)

Average value for panicle length recorded in these selected rice genotypes during *Kharif*-2015 was 24.14 cm with a range of 22.40 cm (ARB6) to 26.45 cm (AM65). Low PCV of 8.71 per cent, low GCV of 6.98 per cent, high heritability of 64.2 per cent and moderate GAM of 11.53 per cent was recorded for this trait.

Average value for panicle length recorded in these selected rice genotypes during summer-2016 was 26.05 cm with a range of 22.21 cm (MTU1001) to 29.51 cm (AM65). Moderate PCV of 12.50 per cent, moderate GCV of 10.13 per cent, high heritability of 65.6 per cent and moderate GAM of 16.90 per cent was recorded for this trait.

Among the selected sorghum genotypes, range of variation for Earhead length during *Kharif*-2015 was from 26.30 cm (MLHT) to 29.90 cm (Dhanvi) with an average

value of 27.26 cm. Moderate PCV and GCV of 14.19 per cent and 10.82 per cent respectively, moderate heritability of 58.2 per cent and moderate GAM of 17.02 per cent was recorded for this trait.

Among the selected sorghum genotypes, range of variation for Earhead length during summer-2016 was from 21.35 cm (MLHT) to 27.90 cm (Dhanvi) with an average value of 25.23 cm. Moderate PCV and GCV of 15.63 per cent and 11.75 per cent respectively, moderate heritability of 56.5 per cent and moderate GAM of 18.20 per cent was recorded for this trait.

4.1.1.2.5 Shoot length in sorghum (cm)

Among the selected rice genotypes during *kharif*-2015, range of 123.40 cm (SJH-1) to 207.40 cm (MLHT) with an average of 148.33 cm was observed for this trait. High PCV and GCV of 23.66 per cent and 22.45 per cent, high heritability of 90.0 per cent and high GAM of 43.88 per cent were recorded for this trait in these genotypes.

Among the selected rice genotypes during summer-2016, range of 123.00 cm (SJH-1) to 204.60 cm (MLHT) with an average of 146.15 cm was observed for this trait. High PCV and GCV of 23.80 per cent and 22.44 per cent, high heritability of 88.9 per cent and high GAM of 43.57 per cent were recorded for this trait.

4.1.1.2.6 Stem width in sorghum (cm)

During *Kharif*-2015, an average of 10.03 cm was observed for this trait with a range of 1.33 cm (MLHT) to 12.32 cm (Dhanvi). High PCV of 49.16 per cent, high GCV of 48.28 per cent, high heritability of 96.4 per cent and a high GAM of 97.66 per cent was recorded for this trait in these genotypes.

During summer-2016, an average of 1.18 cm was observed for this trait with a range of 1.15 (SJH-1) to 1.25 cm (MLHT). Moderate PCV of 10.07 per cent, low GCV of 4.22 per cent, low heritability of 17.6 per cent and a low GAM of 3.64 per cent was recorded for this trait in these genotypes.

4.1.1.2.7 Days to 50 per cent flowering in rice and sorghum

During *Kharif*-2015, Minimum mean value of 84.12 days (MTU1001) and maximum mean value of 114.37 days (Moroberekan) with an average value of 100.40 days. Moderate PCV and GCV of 11.26 per cent and 11.13 per cent, high heritability of 98.0 and moderate GAM of 16.24 per cent was observed in rice genotypes.

During summer-2016, Minimum mean value of 84.50 days (MTU1001) and maximum mean value of 111.00 days (Moroberekan) with an average value of 97.75 days. Moderate PCV of 10.23 per cent, low GCV of 9.90 per cent, high heritability of 93.7 per cent and moderate GAM of 19.74 per cent was recorded in these genotypes for this trait.

During *Kharif*-2015, among the selected sorghum genotypes minimum mean value of 61.00 days (CSH-1) and maximum mean value of 77.00 days (Roagro) with an average value of 68.20 days. Low PCV and GCV of 9.93 per cent and 9.82 per cent, high heritability of 97.7 and high GAM of 20.01 per cent was observed.

Among the selected sorghum genotypes, Minimum mean value of 63.0 days (CSH-14) and maximum mean value of 79.5 days (Roagro) with an average value 71.00 days of Moderate PCV and GCV of 10.00 per cent and 9.87 per cent, high heritability of 97.5 and high GAM of 20.8 per cent were observed.

4.1.1.2.8 Days to maturity in rice and sorghum

Average value for days to maturity recorded in these selected rice genotypes was 127.15 with a range of 111.62 (MTU1001) to 138.12 (AM65) days was recorded. Low PCV of 8.04 per cent, low GCV of 7.96 per cent, high heritability of 98.0 per cent and moderate GAM of 16.24 per cent was recorded in these genotypes for this trait during *Kharif*-2015.

Average value for days to maturity recorded in these selected rice genotypes was 127.19 with a range of 113.0 (MTU1001) to 143.00 (Moroberekan) days was recorded. Moderate PCV of 10.78 per cent, Moderate GCV of 10.67 per cent, high heritability of

98.0 per cent and high GAM of 21.77 per cent was recorded in these genotypes for this trait during summer-2016.

Average value for days to maturity recorded in these selected sorghum genotypes during *Kharif*-2015 was 148.40 days with a range of 129.50 days (CSH-1) to 159.00 (Roagro) days was recorded. Low PCV of 8.24 per cent, low GCV of 8.19 per cent, high heritability of 98.6 per cent and moderate GAM of 16.75 per cent was recorded in these genotypes for this trait.

Average value for days to maturity recorded in these selected sorghum genotypes during *Kharif*-2015 was 151.00 days with a range of 133.25 (CSH-1) to 161.00 (Roagro) days was recorded. Low PCV of 7.68 per cent, low GCV of 7.59 per cent, high heritability of 97.6 per cent and moderate GAM of 16.45 per cent was recorded in these genotypes for this trait.

4.1.1.2.9 Biomass in rice and sorghum

During *Kharif*-2015, an average of 62.60 g was observed for this trait with a range of 51.80 (ARB6) to 74.73 (MTU1001) g. Moderate PCV and GCV of 17.39 %, and 13.09 %, moderate heritability of 56.7 % and high GAM of 20.30 % was recorded for this trait in rice genotypes.

During summer-2016, an average of 61.70 g was observed for this trait with a range of 50.31 (ARB6) to 74.44 (MTU1001) g. Moderate PCV and GCV of 18.49 % and 13.96 %, moderate heritability of 57.0 % and high GAM of 21.72 % was recorded for this trait in these genotypes.

In sorghum during *Kharif*-2015, an average of 133.45 g was observed for this trait with a range of 118.25 (Roagro) to 150.50 (SJH-1). Low PCV and GCV of 9.85 %, and 9.44 %, high heritability of 91.8 % and moderate GAM of 18.65 % was recorded for this trait in these genotypes.

During summer-2016, an average of 127.55 g was observed for this trait with a range of 114.00 (Roagro) to 139.50 (SJH-1). Low PCV and GCV of 9.81 % and 9.05 %,

high heritability of 85.1 % and high GAM of 17.20 % was recorded for this trait in these genotypes.

4.1.1.2.10 Harvest index in rice and sorghum

An average of 3.88 was observed for this trait in rice with a range of 2.80 (AM72) to 5.01 (MTU1001). High PCV of 26.46 %, moderate GCV of 19.84 %, moderate heritability of 56.2 % and high GAM of 30.65 % was recorded during *Kharif*-2015 for this trait in these genotypes.

An average of 4.03 was observed for this trait with a range of 2.53 (AM72) to 5.23 (MTU1001). High PCV of 30.69 %, high GCV of 26.83 %, high heritability of 76.4 % and high GAM of 48.32 % was recorded during summer-2016 for this trait in these genotypes.

An average of 1.54 was observed for this trait in sorghum with a range of 1.18 (CSH-14) to 2.77 (MLHT). High PCV of 44.39 %, high GCV of 44.77 %, high heritability of 94 % and high GAM of 88.69 % was recorded for this trait in these genotypes during *kharif*-2015.

An average of 1.97 was observed for this trait with a range of 1.16 (SJH-1) to 3.71 (MLHT). High PCV of 71.71 %, high GCV of 48.34 %, moderate heritability of 43.7 % and high GAM of 69.93 % was recorded for this trait in these genotypes.

4.1.1.2.11 Grain yield per plant (g/p) in rice and sorghum

Average value for grain yield per plant recorded during *Kharif*-2015 in these selected rice genotypes was 16.90 g/p with a range of 15.38 g/p (MTU1001) to 21.22 g/p (AM72). Moderate PCV of 17.37 per cent, moderate GCV of 13.45 per cent, moderate heritability of 59.9 per cent and high GAM of 21.45 per cent was recorded for this trait.

Average value for grain yield per plant recorded during summer-2016 in these selected rice genotypes was 16.11 g/p with a range of 13.21 g/p (Moroberekan) to 22.40

g/p (AM72). High PCV of 25.23 per cent, high GCV of 21.62 per cent, high heritability of 73.4 per cent and high GAM of 38.16 per cent was recorded for this trait.

Average value for grain yield per plant recorded during *Kharif*-2015 in these selected sorghum genotypes was 97.31 g/p with a range of 45.38 g/p (MLHT) to 124.07 g/p (SJH-1). High PCV of 32.34 per cent, high GCV of 31.36 per cent, high heritability of 94.1 per cent and high GAM of 62.67 per cent was recorded for this trait.

Average value for grain yield per plant recorded during summer-2016 in these selected sorghum genotypes was 88.86 g/p with a range of 31.89 g/p (MLHT) to 121.92 g/p (SJH-1). High PCV of 48.40 per cent, high GCV of 35.91 per cent, moderate heritability of 55 per cent and high GAM of 54.88 per cent was recorded for this trait.

4.1.2 Root experiment: *Kharif*-2015 and Summer-2016

Roots are the principal plant organ for nutrient and water uptake. Under water stress conditions, a deep root system is considered as an important trait for drought resistance. Since root characters are invisible, information on relationship between plant type and root growth would facilitate the breeder to select for root traits based on above ground characters. Therefore, improving our understanding of the interactions between root function and drought in rice could have a significant impact on global food security and production.

In the present investigation, root morphology analysis was conducted for all five genotype of each rice and sorghum in *Kharif*-2015 and summer-2016, intact roots was obtained by gentle washing at maturity and observations were recorded. Comprehensively, results for root morphology study can be reported under different sections as under:

4.1.2.1 Analysis of variance

The mean sum of squares due to various sources of variation for seven characters in five genotypes of each rice and sorghum during *Kharif*-2015 and summer-2016 is represented in Table 14 and 15 respectively. Highly significant differences among the

Table 14: Analysis of variance for root related characters of rice accessions in PVC pipes during *kharif*-2015 (season-I) and Summer-2016 (season II)

Mean sum of squares	Source of variation	Season I						Season II					
	df	Replication	Genotype	Error	CD @		CV	Replication	Genotype	Error	CD @		CV
		3	4	12	5%	1%		3	4	12	5%	1%	
	RL	123.31	960.16***	83.71	14.09	19.76	15.70	296.45	1876.45**	281.61	25.85	36.24	27.89
	RN	1496.74	906.16	752.90	42.27	59.26	31.35	540.33	2395.20**	412.00	31.27	43.84	27.76
	RV	289.43	1044.42	360.64	29.25	41.01	37.75	1108.18	3846.92*	1050.05	49.92	69.99	54.50
	RFW	144.12	979.17**	132.38	17.72	24.85	18.44	125.17	285.46***	27.69	8.10	11.36	18.86
	RDW	8.58	59.03***	4.44	3.24	4.55	12.91	1.81	46.22***	1.59	1.94	2.72	10.14
	TPL	267.75	3220.64***	92.21	14.79	20.74	7.44	411.51	4794.82***	236.72	23.70	33.23	12.24
	RSLR	0.007	0.068**	0.007	0.13	0.18	15.56	0.01	0.10*	0.02	0.22	0.31	26.43

**- Significant at the 0.01 level

* - Significant at the 0.05 level

RL: Root length (cm) RN: Number of roots
RV: Root volume (cm^3) RFW: Root fresh weight (g)
RDW: Root dry weight (g) TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Table 15: Analysis of variance for root related characters of sorghum genotypes in PVC pipes during *Kharif*-2015 (season-I) and Summer-2016 (season II)

Mean sum of squares	Source of variation	Season I							Season II					
	df	Replication	Genotype	Error	CD @		CV	Replication	Genotype	Error	CD @		CV
		3	4	12	5%	1%		3	4	12	5%	1%	
	RL	803.46	8303.17	486.67	33.98	47.64	29.10	147.25	1160.80	489.00	34.06	47.76	15.62
	RN	2813.38	4098.17	2240.84	72.93	102.24	53.82	20833.40	2159.75	3973.65	97.11	136.15	43.02
	RV	16.31	788.50*	177.90	20.54	28.80	33.13	40760.00	43157.50	19614.16	215.76	302.49	44.60
	RFW	10.58	95.12*	26.62	7.94	11.14	10.06	23.66	160.87***	16.04	6.17	8.65	9.65
	RDW	0.91	21.83	8.14	4.39	6.16	17.01	1.25	25.57**	4.70	3.34	4.68	12.29
	TPL	1886.71	15045.07***	723.67	41.44	58.10	11.65	150.73	5781.25*	1094.98	50.98	71.47	12.23
	RSLR	0.02	0.11	0.01	0.16	0.22	25.26	0.01	0.10*	0.02	0.23	0.32	18.11

**- Significant at the 0.01 level

* - Significant at the 0.05 level

RL: Root length (cm) RN: Number of roots
RV: Root volume (cm^3) RFW: Root fresh weight (g)
RDW: Root dry weight (g) TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

genotypes were observed for all the characters indicating variability for the root related traits except for root number and root volume in rice during *Kharif*-2015. It is noticed that CV is highest for root related traits than the field characters in the present study but it can be applied for root characters.

4.1.2.2 Mean performance of all selected genotypes

The phenotypic variance measures the magnitude of variation arising out of difference in phenotypic values while, the genotypic variance measures the magnitudes of variation due to differences in genotypic values. The absolute values of phenotypic and genotypic variances cannot be used for comparing the magnitude of variability for different characters since the mean and units of measurement of the characters may be different. Hence, the coefficients of variation expressed at phenotypic and genotypic levels have been used to compare the variability observed among different characters. While, genotypic co-efficient of variation indicates the amount of genetic variability present in the character, the heritability estimates aid in determining the relative amount of heritable portion of variation. However, heritability values itself provides no indication of the amount of genetic progress that would result from selecting the best individuals. Ramanujam and Tirumalachar (1967) while studying the genetic variability in red-pepper discussed the limitation of estimating the heritability in broad sense as it included both additive and non-genetic effects. According to them heritability estimates in broad sense would be reliable if accompanied by high genetic advance.

The knowledge about the amount of genetic variability present in a crop species with respect to yield and its attributes and their association, which reflects the nature and degree of relationship between any two measurable characters is of great importance in achieving improvement in that crop. Therefore, in the present investigation, variability parameters *viz.*, range, PCV, GCV, broad sense heritability and genetic advance as per cent of mean as well as correlation coefficients were estimated rice and sorghum genotypes for *Kharif*-2015 and summer-2016 are presented in Tables 16, 17, 18 and 19 respectively.

Table 16: Estimates of mean, range and genetic parameters for different root traits in rice accessions during *Kharif*-2015 (season I)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
RL	58.27 ± 4.57	42.75	ARB6	84.12	MTU1001	25.40	29.86	72.4	44.50
RN	87.52 ± 13.71	63.12	ARB6	102.00	MTU1001	7.07	32.13	4.8	3.20
RV	50.30 ± 9.49	28.25	ARB6	68.75	MTU1001	25.99	45.83	32.2	30.36
RFW	62.36 ± 5.75	39.97	AM65	79.85	MTU1001	23.33	29.74	61.5	37.69
RDW	16.32 ± 1.05	11.88	AM65	21.18	MTU1001	22.63	26.06	75.4	40.49
TPL	128.95 ± 4.80	95.50	ARB6	169.00	MTU1001	21.68	22.93	89.5	42.25
RSLR	0.55 ± 0.04	0.39	AM65	0.72	MTU1001	22.26	27.16	67.2	37.58

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as per cent of mean

RL: Root length (cm) RN: Number of roots
RV: Root volume (cm^3) RFW: Root fresh weight (g)
RDW: Root dry weight (g) TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Table 17: Estimates of mean, range and genetic parameters for different root traits in rice accessions during summer-2016 (season II)

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
RL	60.15 ± 8.39	42.50	ARB6	97.75	MTU1001	33.19	43.36	58.6	52.35
RN	73.10 ± 10.14	48.75	ARB6	113.25	AM72	30.46	41.21	54.6	46.37
RV	59.45 ± 16.20	22.75	ARB6	103.75	MTU1001	44.47	70.35	40.0	57.92
RFW	27.88 ± 2.63	20.15	ARB6	41.66	Moroberekan	28.78	34.41	69.9	49.59
RDW	12.44 ± 0.63	10.42	AM72	18.40	MTU1001	26.83	28.68	87.5	51.71
TPL	125.65 ± 7.69	88.75	ARB6	178.50	MTU1001	26.86	29.52	82.8	50.35
RSLR	0.55 ± 0.07	0.37	AM65	0.79	MTU1001	25.78	36.92	48.7	37.08

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as per cent of mean

RL: Root length (cm) RN: Number of roots
RV: Root volume (cm^3) RFW: Root fresh weight (g)
RDW: Root dry weight (g) TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Table 18: Estimates of mean, range and genetic parameters for different root traits in sorghum genotypes during *Kharif*-2015

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
RL	75.80± 11.03	41.50	SJH-1	152.50	MLHT	58.31	65.17	80.1	107.49
RN	87.95 ± 23.66	44.50	CSH-14	128.25	MLHT	24.50	59.13	17.2	20.91
RV	40.25 ± 6.66	28.25	Roagro	58.75	Dhanvi	30.69	45.17	46.2	42.97
RFW	51.25 ± 2.58	44.25	MLHT	55.50	CSH-14	8.07	12.90	39.1	10.40
RDW	16.77 ± 1.42	15.13	SJH-1	20.80	CSH-14	11.02	20.27	29.6	12.34
TPL	230.85± 13.45	181.00	SJH-1	330.75	MLHT	25.92	28.41	83.2	48.70
RSLR	0.41 ± 0.05	0.26	CSH-14	0.65	MLHT	38.48	46.03	69.9	66.26

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as percent of mean

RL: Root length (cm)
RN: Number of roots
RV: Root volume (cm^3)
RFW: Root fresh weight (g)
RDW: Root dry weight (g)
TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Table 19: Estimates of mean, range and genetic parameters for different root traits in sorghum genotypes during summer-2016

Characters	Mean ± SE	Range				GCV (%)	PCV (%)	h^2 (%)	GAM (%)
		Min	Variety	Max	Variety				
RL	141.55 ± 11.05	112.50	CSH-14	154.25	Roagro	9.15	18.10	25.6	9.53
RN	146.50 ± 31.51	121.75	CSH-14	175.75	Dhanvi	14.53	40.49	12.9	10.74
RV	314.00 ± 70.02	212.50	MLHT	475.00	Dhanvi	24.43	50.85	23.1	24.18
RFW	41.50 ± 2.00	32.00	MLHT	48.50	Roagro	14.50	17.41	69.3	24.86
RDW	17.65 ± 1.08	14.75	SJH-1	20.50	CSH-14	12.94	17.84	52.6	19.32
TPL	270.50 ± 16.54	206.50	CSH-14	303.25	MLHT	12.65	17.60	51.7	18.74
RSLR	0.84 ± 0.07	0.66	MLHT	1.02	SJH-1	16.83	24.72	46.3	23.60

PCV: Phenotypic coefficient of variance; **GCV**: Genotypic coefficient of variance; **h^2**: Broad sense heritability; **GAM**: Genetic advance as percent of mean

RL: Root length (cm)
RN: Number of roots
RV: Root volume (cm^3)
RFW: Root fresh weight (g)
RDW: Root dry weight (g)
TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

4.1.2.2.1 Mean performance

The mean performance of all five elite rice accessions and sorghum genotypes in respect of root traits are briefly presented below:

4.1.2.2.1.1 Root Traits

The mean performance of all rice sorghum genotypes in respect of root traits (RL, RN, RV, RDW, RSWR and RSLR) and Shoot traits (SL and TPL) in PVC pipe experiment for *kharif*-2015 and summer-2016 are presented below.

4.1.2.2.1.1.1 Root length (RL, cm) in rice and sorghum

During *Kharif*-2015 mean RL of the selected rice genotypes was 58.27 cm with a range of 42.75 (ARB6) to 84.12 (MTU1001) cm (Table 20a). The genotypes showed moderate PCV (29.86 %) and GCV (25.40 %). High h^2 (72.4 %) and high GAM 44.50 %.

During summer-2016 mean RL of the selected rice genotypes was 60.75 cm with a range of 42.50 (ARB6) to 97.75 (MTU1001) cm. The genotypes showed high PCV (43.36 %) and GCV (33.19 %), moderate heritability (58.6 %) and high GAM 52.35 %.

During *Kharif*-2015 mean RL of the selected sorghum genotypes was 75.80 cm with a range of 41.50 (SJH-1) to 152.50 (MLHT) cm (Table 20b). The genotypes showed high PCV (65.17 %) and GCV (58.31 %), high h^2 (80.1 %) and high GAM 107.49 %.

During summer-2016 mean RL of the selected sorghum genotypes was 141.55 cm with a range of 112.50 (CSH-14) to 154.25 (Roagro). The genotypes showed moderate PCV (18.10 %) and GCV (9.15 %), moderate heritability (25.6 %) and moderate GAM 9.53 %.

4.1.2.2.1.1.2 RN (Root number) in rice and sorghum

During *Kharif*-2015 mean RN of the selected rice genotypes was 87.52 with a range of 63.12 (ARB6) to 102.00 (MTU1001). The genotypes showed high PCV (32.13 %) and moderate GCV (7.07 %), low heritability (4.8 %) and GAM 3.26 % (Table 20c).

Table 20a: Means of root length (RL) of rice accessions in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root length	
	Season I	**Season II**
ARB6	42.7500	42.5000
Moroberekan	57.1250	55.5000
AM65	51.7500	50.0000
AM72	55.6250	55.0000
MTU1001	84.1250	97.7500
Mean	**58.2750**	**60.1500**
C.V.	15.7011	27.8993
F ratio	11.4690	6.6631
F prob.	0.0005	0.0046
S.E.	4.5749	8.3907

Table 20b: Means of root length (RL) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Length	
	Season I	**Season II**
MLHT	152.5000	146.5000
Roagro	80.5000	154.2500
SJH-1	41.5000	153.0000
CSH-14	44.7500	112.5000
Dhanvi	59.7500	141.5000
Mean	**75.8000**	**141.5500**
C.V.	29.1038	15.6223
F ratio	17.0610	2.3738
F prob.	0.0001	0.1105
S.E.	11.0304	11.0567

During summer-2016 mean RN of the selected rice genotypes was 73.10 with a range of 48.75 (ARB6) to 113.25 (AM72). The genotypes showed high PCV (41.21 %) and GCV (30.46 %), moderate heritability (54.6 %) and high GAM 46.37 %.

During *Kharif*-2015 mean RN of the selected sorghum genotypes was 87.95 with a range of 44.50 (CSH-14) to 128.25 (MLHT). The genotypes showed high PCV (59.13 %) and high GCV (24.50 %), low heritability (17.2 %) and high GAM 20.91 % (Table 20d).

During summer-2016 mean RN of the selected sorghum genotypes was 146.50 with a range of 121.75 (CSH-14) to 175.75 (Dhanvi). The genotypes showed high PCV (40.49 %) and moderate GCV (14.53 %), moderate heritability (12.9 %) and GAM 10.74 %.

4.1.2.2.1.1.3 RV (Root volume, cm^3) in rice and sorghum

During *Kharif*-2015 mean RV of the selected rice genotypes was 50.30 with a range of 28.25 (ARB6) to 68.75 (MTU1001) (Table 20e). The genotypes showed high PCV (45.83 %) and high GCV (25.99 %), moderate heritability (32.2 %) and high GAM 30.36 %.

During summer-2016 mean RV of the selected rice genotypes was 59.45 with a range of 27.75 (ARB6) to 103.75 (MTU1001). The genotypes showed high PCV (70.35 %) and GCV (44.47 %), high heritability (40 %) and high GAM 57.92 %.

During *Kharif*-2015 mean RV of the selected sorghum genotypes was 40.25 with a range of 28.25 (Roagro) to 58.75 (Dhanvi) (Table 20f). The genotypes showed high PCV (45.17 %) and high GCV (30.69 %), moderate heritability (46.2 %) and high GAM 42.97 %.

During summer-2016 mean RV of the selected sorghum genotypes was 314.00 with a range of 212.50 (MLHT) to 475.00 (Dhanvi). The genotypes showed high PCV (50.85 %) and moderate GCV (23.1 %), moderate heritability (24.18 %) and GAM 10.74 %.

Table 20c: Means of root number (RN) of rice accessions in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Number	
	Season I	**Season II**
ARB6	63.1250	48.7500
Moroberekan	94.8750	59.7500
AM65	84.1250	69.7500
AM72	93.5000	113.2500
MTU1001	102.0000	74.0000
Mean	**87.5250**	**73.1000**
C.V.	31.3500	27.7672
F ratio	1.2036	5.8136
F prob.	0.3591	0.0077
S.E.	13.7195	10.1489

Table 20d: Means of root number (RN) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Number	
	Season I	**Season II**
MLHT	128.2500	163.7500
Roagro	69.5000	144.5000
SJH-1	101.2500	126.7500
CSH-14	44.5000	121.7500
Dhanvi	96.2500	175.7500
Mean	**87.9500**	**146.5000**
C.V.	53.8232	43.0286
F ratio	1.8289	0.5435
F prob.	0.1882	0.7071
S.E.	23.6688	31.5185

Table 20e: Means of root volume (RV) of rice accessions in PVC pipe experiment during *Kharif*-2015, Summer-2016

Genotypes	Root Volume	
	Season I	Season II
ARB6	28.2500	22.7500
Moroberekan	62.5000	71.2500
AM65	42.0000	39.5000
AM72	50.0000	60.0000
MTU1001	68.7500	103.7500
Mean	**50.3000**	**59.4500**
C.V.	37.7546	54.5073
F ratio	2.8960	3.6635
F prob.	0.0685	0.0358
S.E.	9.4953	16.2023

Table 20f: Means of root volume (RV) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Volume	
	Season I	Season II
MLHT	29.2500	212.5000
Roagro	28.2500	320.0000
SJH-1	51.7500	330.0000
CSH-14	33.2500	232.5000
Dhanvi	58.7500	475.0000
Mean	**40.2500**	**314.0000**
C.V.	33.1377	44.6021
F ratio	4.4323	2.2003
F prob.	0.0198	0.1304
S.E.	6.6690	70.0253

4.1.2.2.1.1.4 RFW (Root fresh weight, g) in rice and sorghum

During *Kharif*-2015 mean RFW of the selected rice genotypes was 62.36 g with a range of 39.97 (AM65) to 79.85 (MTU1001) g. The genotypes showed high PCV (29.74 %) and GCV (23.33 %), high heritability (61.5 %) and GAM 37.69 % (Table 20g).

During summer-2016 mean RFW of the selected rice genotypes was 27.88 g with a range of 20.15 (ARB6) to 41.66 (Moroberekan) g. The genotypes showed high PCV (34.41 %) and GCV (28.78 %), high heritability (69.9 %) and high GAM 49.59 %.

During *Kharif*-2015 mean RFW of the selected sorghum genotypes was 51.25 g with a range of 44.25 (MLHT) to 55.50 (CSH-14) g (Table 20h). The genotypes showed high PCV (12.90 %) and moderate GCV (8.07 %), high heritability (39.1 %) and moderate GAM 10.4 %

During summer-2016 mean RFW of the selected sorghum genotypes was 41.50 g with a range of 32.00 (MLHT) to 48.50 (Roagro) g. The genotypes showed moderate PCV (17.41 %) and GCV (14.50 %), high heritability (69.3 %) and GAM 24.86 %

4.1.2.2.1.1.5 RDW (Root dry weight, g) in rice and sorghum

During *Kharif*-2015 mean RDW of the selected rice genotypes was 16.32 g with a range of 11.88 (AM65) to 21.18 (MTU1001) g. The genotypes showed high PCV (26.06 %) and GCV (22.63 %), high heritability (75.4 %) and GAM 40.49 % (Table 20i).

During summer-2016 mean RDW of the selected rice genotypes was 12.44 g with a range of 10.42 (AM72) to 18.40 (MTU1001) g. The genotypes showed high PCV (28.68 %) and GCV (26.83 %), high heritability (87.5 %) and high GAM 51.71 %.

During *Kharif*-2015 mean RDW of the selected sorghum genotypes was 16.77 g with a range of 15.15 (CSH-14) to 20.80 (CSH-14) g (Table 20j). The genotypes showed moderate PCV (12.90 %) and GCV (8.07 %), high heritability (39.1 %) and moderate GAM 10.4 %.

Table 20g: Means of root fresh weight (RFW) of rice accessions in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Fresh Weight	
	Season I	**Season II**
ARB6	55.9313	20.1500
Moroberekan	62.2738	41.6600
AM65	39.9750	25.6700
AM72	73.7913	29.4275
MTU1001	79.8550	22.5375
Mean	**62.3653**	**27.8890**
C.V.	18.4492	18.8686
F ratio	7.3964	10.3087
F prob.	0.0030	0.0007
S.E.	5.7529	2.6311

Table 20h: Means of root fresh weight (RFW) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Fresh Weight	
	Season I	**Season II**
MLHT	44.2500	32.0000
Roagro	55.5000	48.5000
SJH-1	48.2500	38.7500
CSH-14	55.2500	44.5000
Dhanvi	53.0000	43.7500
Mean	51.2500	**41.5000**
C.V.	10.0682	9.6511
F ratio	3.5728	10.0286
F prob.	0.0385	0.0008
S.E.	2.5800	2.0026

Table 20i: Means of root dry weight (RDW) of rice accessions in PVC pipe experiment during *Kharif*-2015, and Summer-2016

Genotypes	Root Dry Weight	
	Season I	**Season II**
ARB6	13.7650	10.4475
Moroberekan	15.5150	12.1275
AM65	11.8888	10.8325
AM72	19.2513	10.4275
MTU1001	21.1813	18.4000
Mean	**16.3203**	**12.4470**
C.V.	12.9185	10.1430
F ratio	13.2814	29.0000
F prob.	0.0002	0.0000
S.E.	1.0542	0.6313

Table 20j: Means of root dry weight (RDW) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root Dry Weight	
	Season I	**Season II**
MLHT	15.1550	15.2500
Roagro	16.3275	18.5000
SJH-1	15.1325	14.7500
CSH-14	20.8025	20.5000
Dhanvi	16.4800	19.2500
Mean	**16.7795**	**17.6500**
C.V.	17.0129	12.2939
F ratio	2.6788	5.4319
F prob.	0.0833	0.0099
S.E.	1.4273	1.0849

During summer-2016 mean RDW of the selected sorghum genotypes was 17.65 g with a range of 14.75 (SJH-14) to 20.50 (CSH-14) g. The genotypes showed moderate PCV (17.84 %) and moderate GCV (12.94 %), high heritability (52.6 %) and moderate GAM 19.32 %

4.1.2.2.1.1.6 TPL (Total plant length, cm) in rice and sorghum

During *Kharif*-2015 mean TPL of the selected rice genotypes was 128.95 cm with a range of 95.50 (ARB6) to 169.0 (MTU1001) cm. The genotypes showed high PCV (22.93 %) and GCV (21.68 %), high heritability (89.5 %) and GAM 42.25 %.

During summer-2016 mean TPL of the selected rice genotypes was 125.65 cm with a range of 88.75 (ARB6) to 178.50 (MTU1001) cm. The genotypes showed high PCV (29.52 %) and GCV (26.86 %), high heritability (82.8 %) and high GAM 50.35 %.

During *Kharif*-2015 mean TPL of the selected sorghum genotypes was 230.80 cm with a range of 181.00 (SJH-1) to 330.75 (MLHT) cm. The genotypes showed high PCV (28.41 %) and GCV (25.92 %), high heritability (83.2 %) and GAM 48.70 %.

During summer-2016 mean TPL of the selected sorghum genotypes was 270.50 cm with a range of 206.50 (CSH-14) to 303.25 (MLHT) cm. The genotypes showed moderate PCV (17.60 %) and GCV (12.65 %), moderate heritability (51.7 %) and GAM 18.74 %.

4.1.2.2.1.1.7 RSLR (Root to shoot length ratio) in rice and sorghum

During *Kharif*-2015 mean RSLR of the selected rice genotypes was 0.55 with a range of 0.39 (AM65) to 0.72 (MTU1001). The genotypes showed high PCV (27.16 %) and GCV (22.26 %), high heritability (67.2 %) and GAM 37.58 % (Table 20k).

During summer-2016 mean RSLR of the selected rice genotypes was 0.55 with a range of 0.37 (AM65) to 0.79 (MTU1001). The genotypes showed high PCV (36.92 %) and GCV (25.78 %), moderate heritability (48.70 %) and high GAM 37.08 %.

During *Kharif*-2015 mean RSLR of the selected sorghum genotypes was 0.41 with a range of 0.26 (CSH-14) to 0.65 (MLHT) (Table 20l). The genotypes showed high PCV (46.06 %) and GCV (38.48 %), high heritability (69.9 %) and GAM 66.26 %.

During summer-2016 mean RSLR of the selected sorghum genotypes was 0.84 with a range of 0.66 (MLHT) to 1.02 (SJH-1) cm. The genotypes showed high PCV (24.72 %) and moderate GCV (16.83 %), high heritability (46.3 %) and GAM 23.60 %.

On overall basis, MTU1001, AM72 and Moroberekan in rice, whereas in sorghum Roagro, MLHT and SJH-1 were superior for the root morphological traits *viz*., maximum root length etc.

Many workers have emphasized upon the role of roots in providing drought tolerance, but it was difficult to record root morphological data with accuracy. The root morphological studies in PVC pipes is one of the efficient and simple strategy to explore various root traits, their variability and role in providing tolerance ability by rice plants.

Co-efficient of variation (GCV and PCV): The range in mean values does not reflect the total variance in the material studied. Hence, actual variance has to be estimated for the characters to know the extent of existing variability. The co-efficient of variation (PCV and GCV) which is calculated by considering the respective means have been used for the comparisons. High values of these parameters indicate wider variability and vice versa. In the same context, a narrow difference between the PCV and GCV implies lesser influence of environment on these traits.

Broad sense heritability gives an idea about portion of observed variability attributable to genetic differences. Since the scope of heritability is restricted by their interaction with the environment, they alone provide no information of the genetic advance that would result from selection. Johnson *et al.* (1955) reported that heritability estimates along with genetic gain would be more useful than the former alone in predicting the effectiveness of selecting the best individuals. Therefore, it is essential to consider the predicted genetic advance along with heritability estimate as a tool in the selection programme for better efficiency in the selection. High heritability coupled with

Table 20k: Means of root to shoot length ratio (RSLR) of rice in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root to Shoot Length Ratio	
	Season I	**Season II**
ARB6	0.5371	0.5050
Moroberekan	0.4798	0.4700
AM65	0.3954	0.3725
AM72	0.6388	0.6150
MTU1001	0.7291	0.7900
Mean	**0.5560**	**0.5505**
C.V.	15.5614	26.4376
F ratio	9.1860	4.8039
F prob.	0.0012	0.0151
S.E.	0.0433	0.0728

Table 20l: Means of root to shoot length ratio (RSLR) of sorghum genotypes in PVC pipe experiment during *Kharif*-2015 and Summer-2016

Genotypes	Root to Shoot Length Ratio	
	Season I	**Season II**
MLHT	0.6599	0.6600
Roagro	0.4938	0.9575
SJH-1	0.2710	1.0225
CSH-14	0.2663	0.6900
Dhanvi	0.3676	0.8800
Mean	**0.4117**	**0.8420**
C.V.	25.2694	18.1115
F ratio	10.2766	4.4545
F prob.	0.0008	0.0195
S.E.	0.0520	0.0762

high genetic advance reveals the presence of lesser environmental influence and prevalence of additive gene action in their expression (Panse and Sukathme, 1967).

In selected rice genotypes, the PCV and GCV were highest for all root traits RV, RN, RL, RFW, RDW and RSLR, the PCV and GCV were highest for yield related traits such as TNT, NPT, PH and HI during *Kharif*-2015 and Summer-2016. During *Kharif*-2015 high heritability and GAM observed for RL, RN RSLR and PH, TNT, NPT. During summer-2016 high heritability and GAM observed for RFE, RDW, TPL and PH, TNT, NPT, HI, PL. (Figure 4-7).

The PCV and GCV in sorghum were highest for root traits RL, RN, RV and RSLR. The PCV and GCV were highest for yield related traits such as SL, SW, GY/p, HI, BM and EHL during *Kharif*-2015 and Summer-2016. During *Kharif*-2015 and summer-2016 high heritability and GAM observed for all yield and root traits except EHL, SW and BM (Figure 8-11).

High GCV and PCV for root dry weight, root volume and moderate GCV for shoot height was reported by Latha (1997). High GCV and PCV for root dry weight were reported by Gireesha (1999). Kanbar (2001) obtained high PCV and GCV for root morphological and related traits except shoot height, which was found to possess moderate GCV and PCV. Prabuddha (2003) reported high GCV and PCV values for maximum root length, root volume, root dry weight, number of tillers and shoot dry weight while moderate values for shoot height. Since the genetic co-efficient of variation does not indicate the heritable portion of variability, heritability (broad sense) estimates will be significant to a breeder in predicting the accuracy with which a genotype can be recognized by phenotypic expression.

On the whole, co-efficient of variation indicated considerable amount of variability for most of the traits except DFF, DM, BM, RSLR, RN and PL. The close correspondence between the estimates of GCV and PCV for most of the traits indicated lesser environmental influence on the expression of these traits, which is also reflected by their high heritability values.

Overall results show that high h^2 coupled with moderate GA as per cent of mean was recorded for PL and DFF in rice. Higher h^2 for RDW was reported by Shashidhar *et al.* (1990) and Shahid *et al.* (1994). Hemamalini (1997) obtained higher expected GA as per cent of mean for RV. High h2 with high GA for GY and yield attributes were also reported by Bidhan *et al.* (2001) and Bhandarkar *et al.* (2003). High heritability values for these traits were also reported by Prabuddha (2003).

The present investigation revealed high h^2 coupled with high GA as per cent of mean for most of the characters except RN in rice and RL, RN, RV and RDW in sorghum indicating the presence of considerable variation and additive gene effects. Hence, improvement of these characters could be effective through phenotypic selection.

4.1.3 Correlation Studies

Correlation analysis has been used to determine the type and magnitude of the association between a pair of characters. These associations provide a better understanding of the contribution of one trait in building up the genetic makeup of the other trait of a crop.

4.1.3.1 Correlation coefficient analysis in rice

The estimates of phenotypic correlation coefficient for 16 characters along with grain yield/p for *Kharif*-2015 are presented below (Table 21). For Summer-2016 were presented in Table 22.

4.1.3.2 Association among the traits during *Kharif*-2011(Season I)

RL had highly significant and positive association with RN (0.62), RDW (0.60), TPL (0.90) and RSLR (0.75) at 1 % significance level. Highly significant and positive association of RN was observed with SL (0.48) at 5 % significance level and with TPL (0.62) at 1 % significance level. RV showed positive association with SL (0.49) at 5 % significance level and with TPL (0.74) and RSLR (0.57) at 1 % significance level.

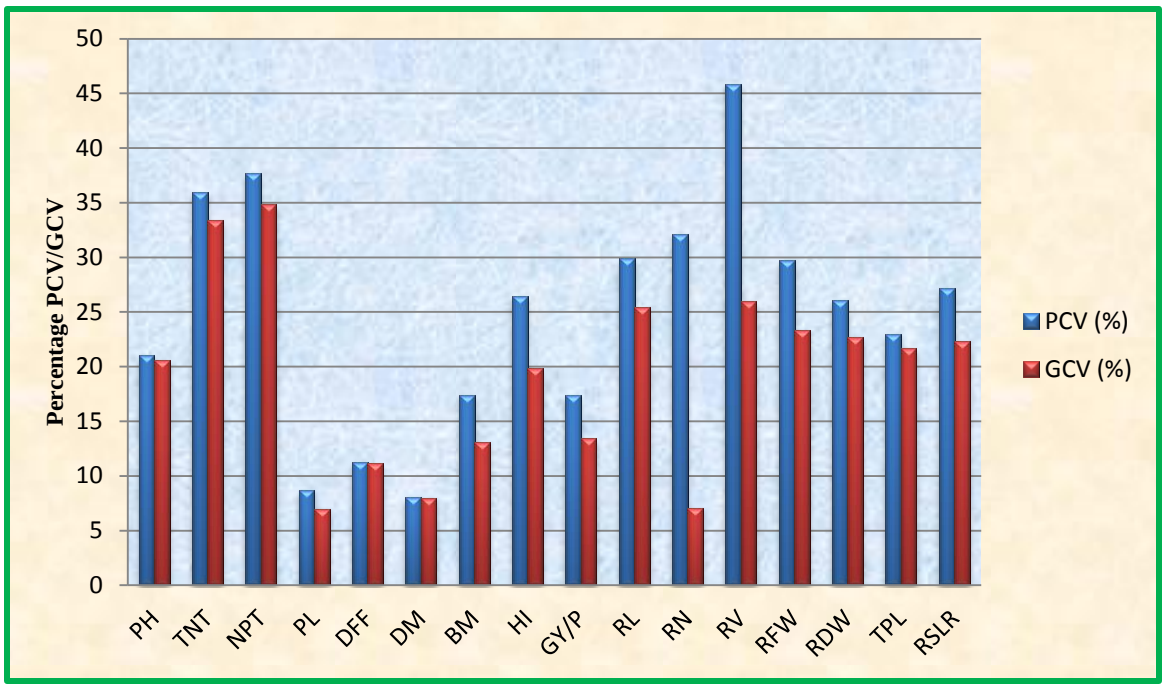


Fig. 04: Genotypic and Phenotypic coefficient of variation of all sixteen characters in selected rice genotype during *kharif*-2015

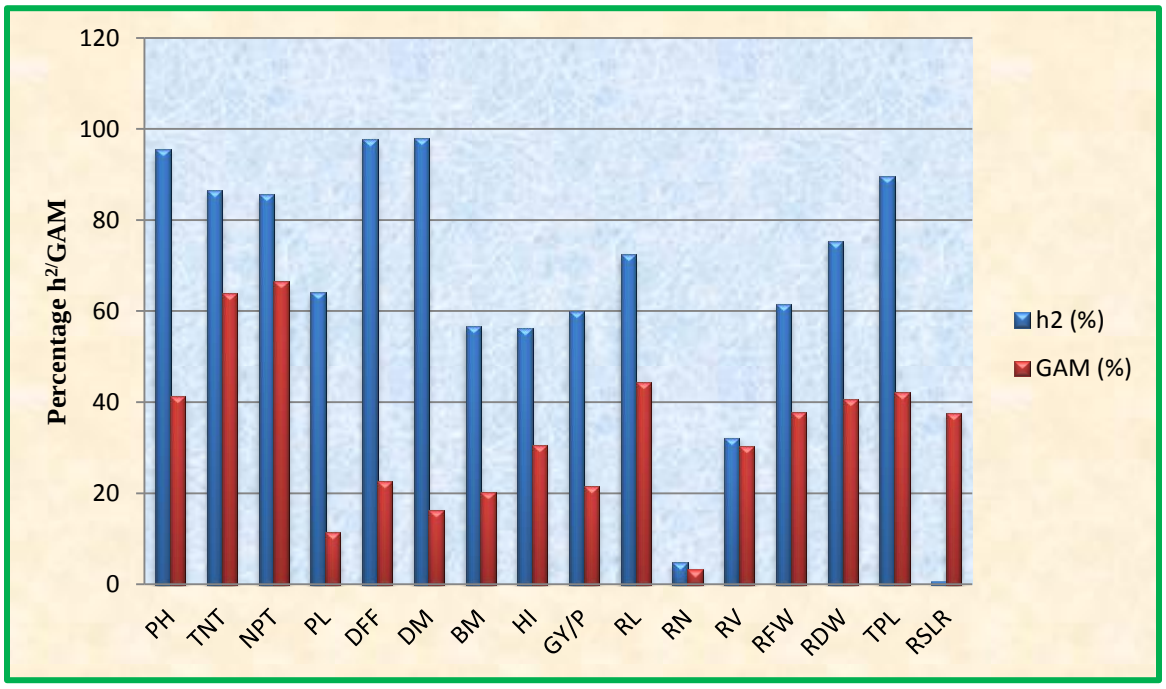


Fig. 05: Broad sense heritability and genetic advance as per cent mean correlation of all sixteen characters in selected rice genotype during *kharif* -2015

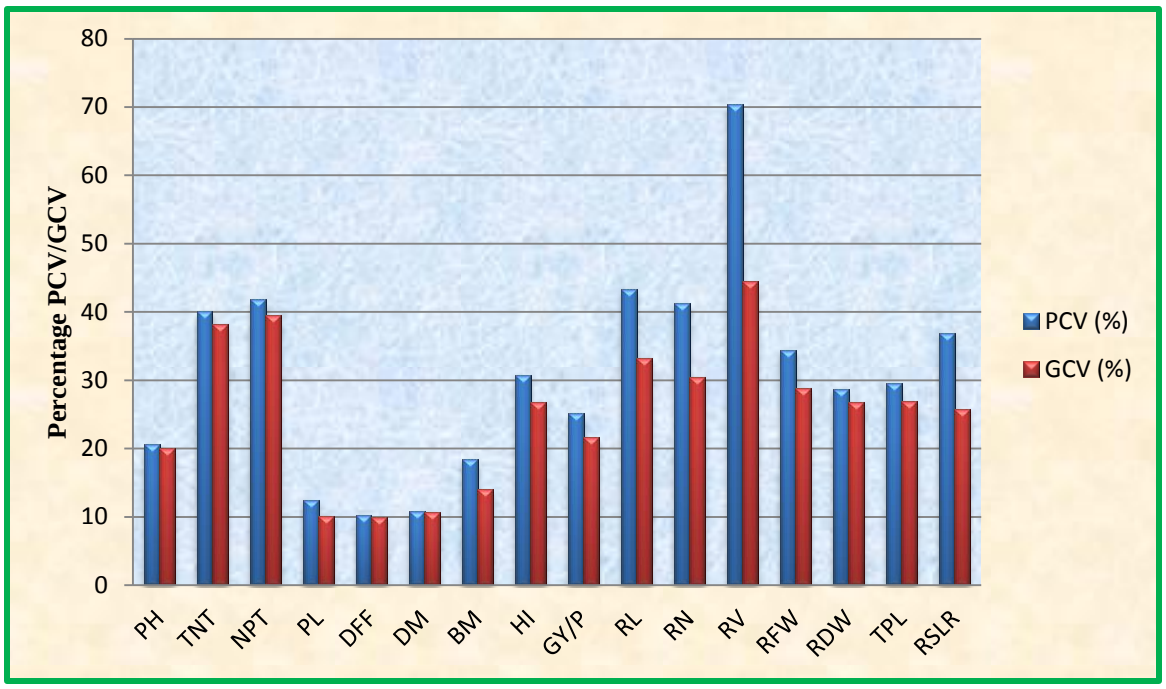


Fig. 06: Genotypic and Phenotypic coefficient of variation of all sixteen characters in selected rice genotype during summer-2016

Fig. 07: Broad sense heritability and genetic advance as per cent mean correlation of all sixteen characters in selected rice genotype during summer-2016

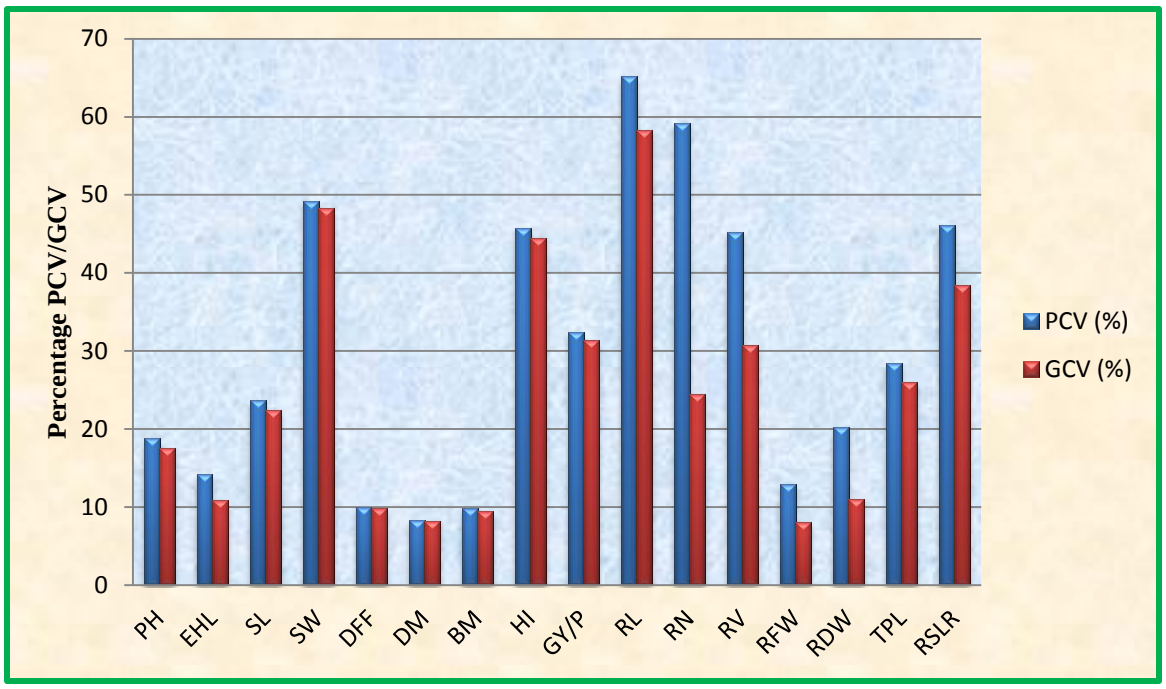


Fig. 08: Genotypic and Phenotypic coefficient of variation of all sixteen characters in selected Sorghum genotype during *kharif* -2015

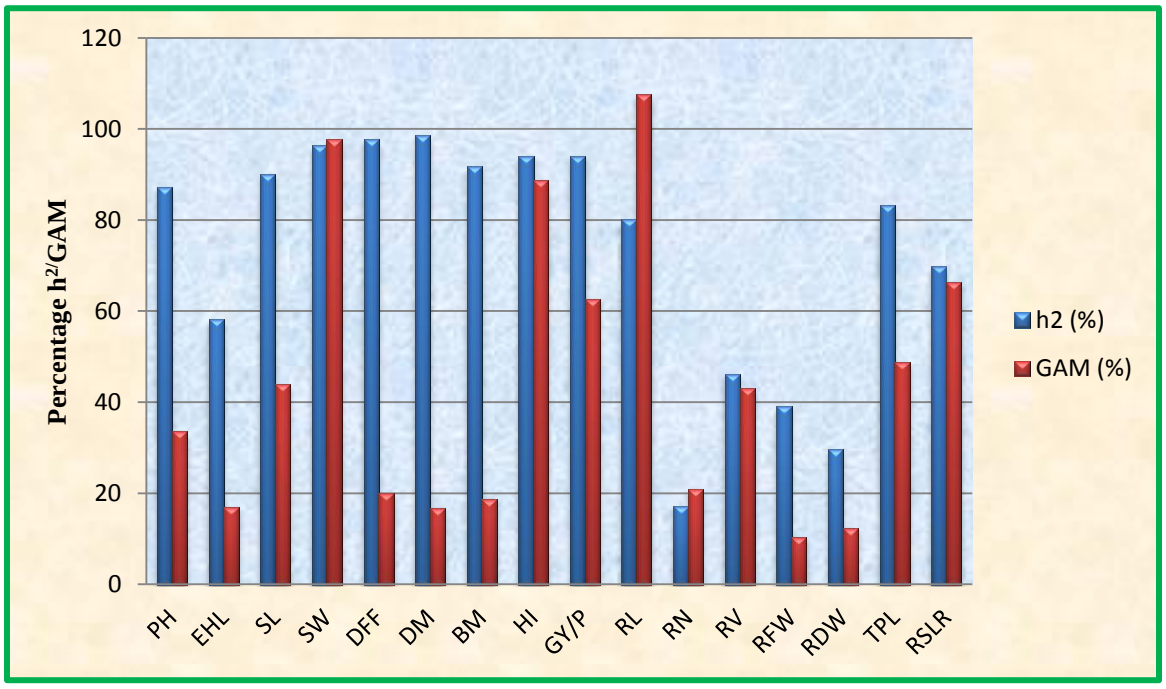
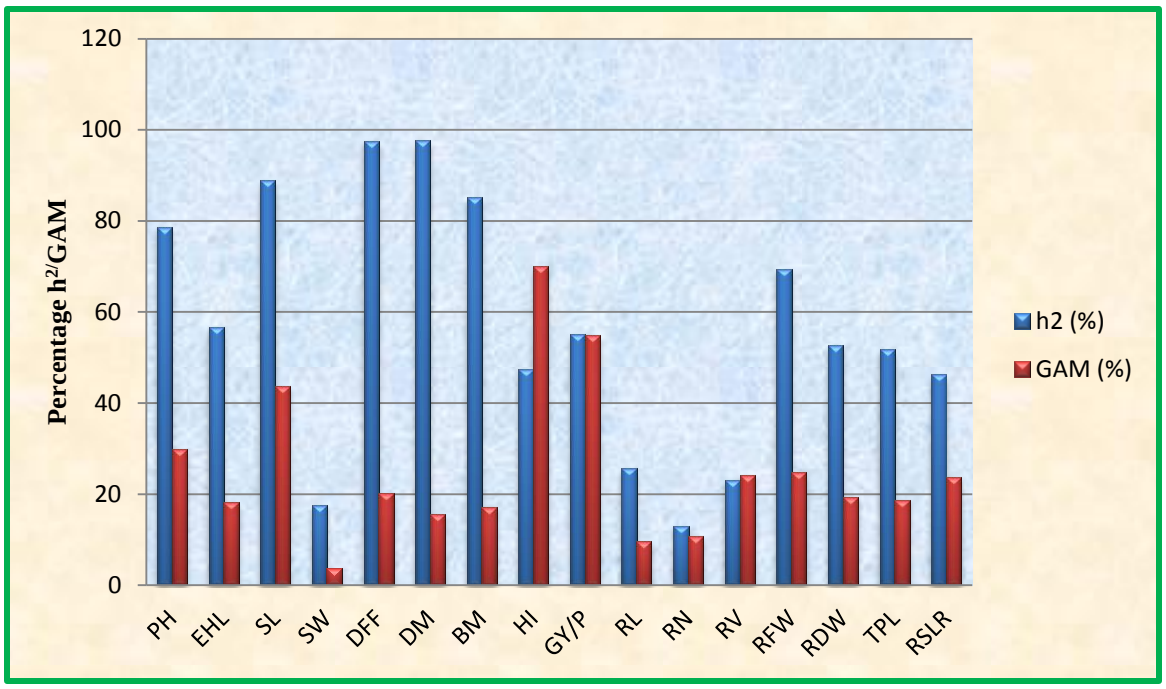


Fig. 09: Broad sense heritability and genetic advance as per cent mean correlation of all sixteen characters in selected Sorghum genotype during *kharif* 2015



Fig, 10: Genotypic and Phenotypic coefficient of variation of all sixteen characters in selected Sorghum genotype during summer-2016

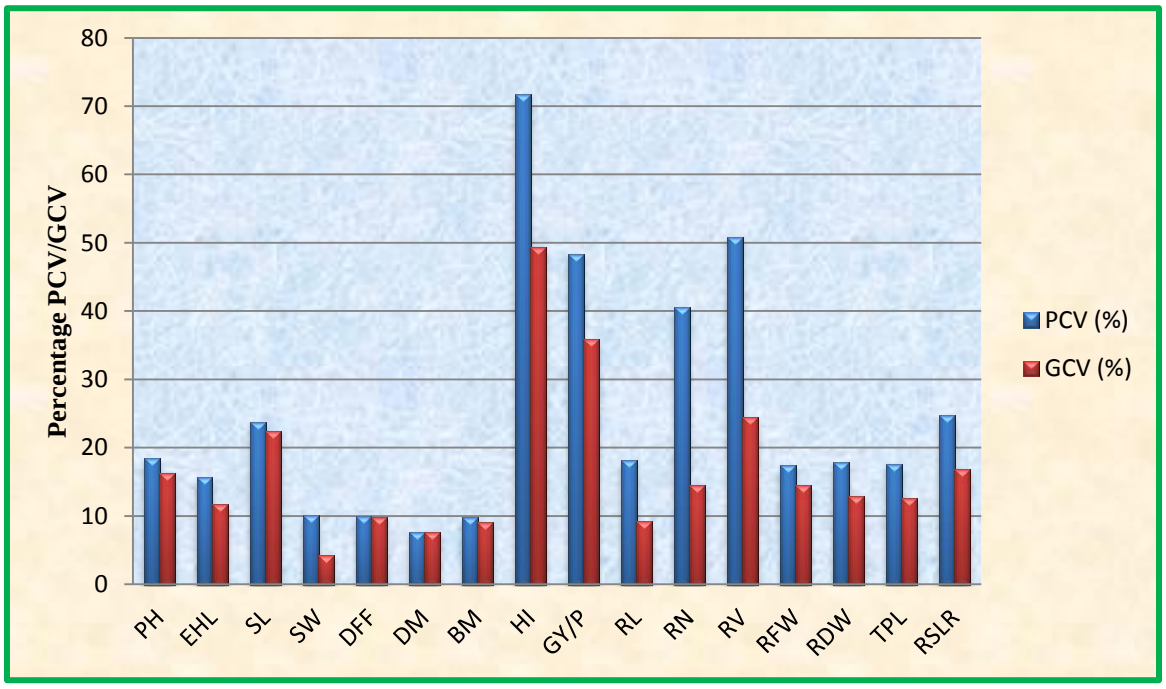


Fig. 11: Broad sense heritability and genetic advance as per cent mean correlation of all sixteen characters in selected Sorghum genotype during summer-2016

Table 21: Phenotypic correlation coefficients among root, shoot and grain yield traits of rice accessions under field and pipe experiment during *Kharif*-2015 (Season I)

	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	RSLR	GY/P
PH	-0.66**	-0.62**	0.36	0.28	0.31	0.55*	0.56*	0.29	0.26	0.30	-0.29	-0.18	0.07	0.21	-0.38	-0.38
TNT	1	0.99***	0.10	-0.50*	-0.22	-0.40	0.46*	-0.12	-0.18	-0.36	0.07	0.15	-0.13	-0.14	0.36	0.36
NPT		1	0.17	-0.45*	-0.14	-0.44*	-0.49*	-0.18	-0.23	-0.40	-0.01	0.07	-0.18	-0.20	0.28	0.36
PL			1	0.33	0.62**	-0.15	-0.26	-0.16	0.19	0.00	-0.32	-0.30	-0.09	-0.15	-0.33	0.27
DFF				1	0.88***	-0.18	-0.17	-0.56	-0.08	-0.14	-0.50	-0.58**	-0.28	-0.48*	-0.72***	0.02
DM					1	-0.35	-0.37	-0.61	-0.10	-0.25	-0.64	-0.64**	-0.39	-057**	-0.76***	0.16
BM						1	0.85	0.73	0.34	0.57	0.39	0.36	0.51*	0.70***	0.30	0.31
HI							1	0.55	0.20	0.37	0.19	0.13	0.26	0.46*	0.11	-0.74***
RL								1	0.62**	0.81	0.63	0.60**	0.60**	0.90***	0.75***	-0.10
RN									1	0.79	0.40	0.31	0.48*	0.62**	0.43	0.07
RV										1	0.61	0.41	0.49*	0.74***	0.57**	0.01
RFW											1	0.72***	0.63**	0.70***	0.80***	0.24
RDW												1	0.75***	0.75***	0.72***	0.32
SL													1	0.88***	0.54*	0.27
TPL														1	0.73***	0.08
RSLR															1	0.21

* Significant at 5%, ** significant at 1 %, ***significant at 0.1%

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (g)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

Table 22: Phenotypic correlation coefficients among root, shoot and grain yield traits of rice accessions under field and pipe experiment during summer-2016 (Season II)

	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	RSLR	GY/P
PH	-0.63**	-0.61**	0.27	-0.11	0.04	0.64**	0.59**	0.31	-0.18	0.27	0.13	0.40	0.13	0.27	-0.15	-0.37
TNT	1	0.99***	0.05	-0.39	-0.47*	-0.44*	-0.72***	-0.13	0.36	-0.24	-61**	-0.31	-0.21	-0.19	0.19	0.66***
NPT		1	0.12	-0.36	-0.43	-0.45*	-0.73***	-0.18	0.33	-0.30	-0.59**	-0.36	-0.26	-0.24	0.13	0.66***
PL			1	0.35	0.25	-0.05	-0.31	-0.47*	0.07	-0.32	0.21	-0.65**	-0.25	-0.44*	-0.58**	0.38
DFF				1	0.87***	-0.24	0.07	-0.61**	-0.37	-0.38	0.51*	-0.57**	-0.28	-0.55*	-0.60**	-0.30
DM					1	-0.27	0.18	-0.54*	-0.61**	-0.41	0.35	-0.47*	-0.55*	-0.63**	-0.62**	-0.54*
BM						1	0.76***	0.67**	-0.10	0.64**	0.07	0.68***	0.56**	0.72***	0.38	-0.24
HI							1	0.45*	-0.51*	0.39	0.06	0.64**	0.30	0.45*	0.15	-0.79
RL								1	0.32	0.92***	-0.12	0.82***	0.46*	0.91***	0.88***	-0.10
RN									1	0.45*	0.12	0.02	0.34	0.38	0.47*	0.67***
RV										1	0.16	0.68***	0.52*	0.88***	0.82***	-0.02
RFW											1	-0.14	0.27	0.03	-0.20	0.00
RDW												1	0.51*	0.81***	0.63**	-0.33
SL													1	0.78***	0.43	0.15
TPL														1	0.82***	-0.00
RSLR															1	0.11

* Significant at 5%, ** significant at 1 %, ***significant at 0.1%

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (g)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

RFW had highly significant and positive association with RDW (0.72), TPL (0.70) and RSLR (0.80) at 1 % significance level and significant and positive association with SL (0.63) at 5 % significance level. RDW had highly significant and positive association with SL (0.75), TPL (0.75) and RSLR (0.72) at 1 % significance level. TPL had highly significant and positive association with RSLR (0.73) at 1 % significance level.

4.1.3.3 Association among the traits during summer-2016 (Season Il)

RL had highly significant and positive association with RV (0.92), RDW (0.82), TPL (0.91) and RSLR (0.88) at 1 % significance level and with SL (0.46) at 5 % significance level. Highly significant and positive association of RN was observed with RV (0.45) and RSLR (0.47) at 5 % significance level.

RV had showed highly significant positive association with SL (0.52) at 5 % significance level and with RDW (0.68), TPL (0.88) and RSLR (0.82) at 1 % signify cance level. RDW had highly significant and positive association with SL (0.51) at 5 % significance level and with TPL (0.81) and RSLR (0.63) at 1 % significance level. TPL had highly significant and positive association with RSLR (0.82) at 1 % significance level

4.1.3.4 Correlation of grain yield with component characters

Grain yield plant-1 (GY/p) did not showed positive association with component traits. Instead it showed a negative association with HI (-0.74) at 1 % significance level during *Kharif*-2015

Grain yield plant-1 (GY/p) had showed highly significant and positive association with TNT (0.66), NPT (0.66) and RN (0.67) at 1 % significance level. And also showed significantly negative correlation with DM (-0.54) at 5 % significance level during summer-2016.

The phenotype of a plant is the result of interaction of a large number of factors. Therefore, the ultimate or final characters can be known through the correlation studies.

The correlation analysis helps in examining the possibility of improving yield through indirect selection of its component traits which are highly correlated.

Correlation among the root traits was found highly significant in both the seasons. Significantly high correlation was found between RL and RN as well as RL and RDW and this trait is always associated with all the root traits in both seasons. Positive and highly significant association of maximum root length and its impact on other root morphological traits and yield component has been extensively reported and emphasized by many workers *viz*. Sheeba *et al*., (2006), Haider *et al*., (2012) and Toorchi *et al.*, (2002, 2006).

From all association studies it was observed that during both season all the root characters having strong association among them. Both RN and RL is always associated with the SL, TPL and it is contributing to RDW also. Whereas RDW has contributed significantly towards SL, TPL and RSRL. In conclusion the root traits were more helpful during season and all the root traits are mutually associated and also contributing towards the traits like SL, TPL and GY/p. This was in accordance with the findings of Latha (1996), Gireesha (1999) and Prabuddha (2003).

4.1.3.5 Correlation coefficient analysis in sorghum

The estimates of phenotypic correlation coefficient for 16 characters along with grain yield/p for *Kharif*-2015 are presented below (Table 23). For Summer-2016 were presented in Table 24.

4.1.3.6 Association among the traits during *Kharif*-2015 (Season I)

Plant height exhibited positive and significant association with shoot length (0.99), harvest index (0.91), total plant length (0.88), root length (0.89) and root to shoot length ratio (0.72) at 1 % significance level.

Positive and highly significant association of plant height with maximum root length, root number and fresh root weight at phenotypic level was also reported by Cui *et al.* (2007) and the present results also indicated the same pattern.

Table 23: Phenotypic correlation coefficients among root, shoot and grain yield traits of sorghum genotypes under field and pipe experiment during *Kharif*-2015 (Season I)

	EHL	SL	SW	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	TPL	RSLR	GY/P
PH	-0.52*	0.99***	-0.91***	0.42	0.29	-0.43	0.91***	0.88***	0.43	-0.29	-0.56**	-0.12	0.89***	0.72***	-0.91***
EHL	1	-0.60**	0.63**	0.05	0.29	0.30	-0.57**	-0.29	0.21	0.39	0.07	-0.23	-0.28	-0.13	0.53**
SL		1	-0.93***	0.39	0.24	-0.44	0.92***	0.86***	0.38	-0.32	-0.53*	-0.09	0.87***	0.69***	-0.91***
SW			1	-0.41	-0.32	0.40	-0.97***	-0.83***	-0.33	-0.36	0.56**	0.23	-0.82***	-0.69***	0.93***
DFF				1	0.84***	-0.77***	0.42	0.65**	0.23	0.38	-0.08	-0.39	0.71***	0.76***	-0.62***
DM					1	-0.45*	0.36	0.53*	0.36	-0.01	-0.18	-0.50*	0.56**	0.64**	-0.49*
BM						1	-0.39	-0.60**	0.03	0.68***	-0.20	0.08	-0.63**	-0.69***	0.62***
HI							1	0.86***	0.38	-0.34	-0.48*	-0.32	0.84***	0.73***	-0.94***
RL								1	0.58**	-0.35	-0.38	-0.45*	0.97***	0.95***	-0.90***
RN									1	0.41	-0.45*	-0.62**	0.53*	0.57**	-0.28
RV										1	-0.16	-0.13	-0.35	-0.35	0.46*
RFW											1	0.08	-0.44	-0.22	0.39
RDW												1	-0.34	-0.54*	0.24
TPL													1	0.92***	-0.91***
RSLR														1	-0.81***

* Significant at 5%, ** significant at 1 %, ***significant at 0.1%

PH: Plant height
EHL: Earhead Length
SL: Shoot Length
SW: Stem Width
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RN: Number of roots
RV: Root volume (cm^3)
RFW: Root fresh weight (g)
RDW: Root dry weight (g)
TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Table 24: Phenotypic correlation coefficients among root, shoot and grain yield traits of sorghum genotypes under field and pipe experiment during summer-2016 (Season II)

	EHL	SL	SW	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	TPL	RSLR	GY/P
PH	-046*	0.98***	0.50*	0.44*	0.29	-0.46*	0.57**	0.02	-0.05	-0.33	-0.68***	-0.26	0.21	-0.62**	-0.61***
EHL	1	-0.58**	0.21	0.06	0.28	0.36	-0.29	0.30	0.17	0.55*	0.25	-0.03	0.12	0.53*	0.33
SL		1	0.41	0.40	0.24	-0.47*	0.60**	-0.02	-0.02	-0.40	-0.67**	-0.26	0.20	-0.64**	-0.63***
SW			1	0.11	0.12	-0.08	0.20	0.16	-0.01	-0.01	-0.35	-0.20	0.19	-0.18	-0.28
DFF				1	0.87***	-0.62**	0.64**	0.40	0.21	-0.01	-0.02	-0.16	0.43	0.00	-0.60***
DM					1	-0.32	0.47*	0.52*	0.32	0.20	-0.09	-0.26	0.59**	0.19	-0.43
BM						1	-0.52	-0.04	0.02	0.38	0.02	-0.03	0.06	0.27	0.54*
HI							1	0.25	0.23	-0.16	-0.32	-0.38	0.39	-0.17	-0.91***
RL								1	-0.05	0.35	-0.18	-0.42	0.84***	0.76***	-0.27
RN									1	0.43	0.10	0.25	0.11	0.00	-0.33
RV										1	0.22	0.34	0.18	0.48*	0.10
RFW											1	0.68***	-0.32	0.27	0.38
RDW												1	-0.55*	-0.17	0.29
TPL													1	0.52*	-0.39
RSLR														1	0.18

* Significant at 5 %, ** significant at 1 %, ***significant at 0.1%

PH: Plant height
EHL: Earhead Length
SL: Shoot Length
SW: Stem Width
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RN: Number of roots
RV: Root volume (cm^3)
RFW: Root fresh weight (g)
RDW: Root dry weight (g)
TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

Positive and highly significant association of stem width with root fresh weight (0.56) at 1 % significance level. And also showed significantly negative correlation with RL (-0.83) and RSLR (-0.69) at 1 % significance level. DFF and DM had highly significant and positive association with RL (0.65 and 0.53), TPL (0.71 and 0.56) and RSLR (0.76 and 0.64) at 1 % significance level.

Positive and highly significant association of biomass root volume (0.68) at 1 % significance level. And also showed significantly negative correlation with RL (-0.60), TPL (0.63) and RSLR (-0.69) at 1 % significance level. HI had highly significant and positive association with RL (0.86), TPL (0.84) and RSLR (0.73) at 1 % significance level and negative correlation with RFW (-0.48) at 5 % significance level.

RL had highly significant and positive association with RN (0.58), TPL (0.97) and RSLR (0.95) at 1 % significance level. It is also observed that RL had negative association with RDW (-0.45) at 5 % significance level.

Highly significant and positive association of RN was observed with TPL (0.53) 5 % significance level and with RSLR (0.57) at 1 % significance level and negative association with RFW (-0.45) at 5 % significance level and RDW (-0.62) at 1 % significance level. RDW had significant negative association with RSLR (-0.54) at 5 % significance level.

4.1.3.7 Association among the traits during summer-2016 (Season II)

Plant height exhibited positive and significant association with shoot length (0.98), harvest index (0.57) at 1 % significance level and with SW and DFF at 15 % significance level. And also showed significantly negative correlation with RFW (-0.68) and RSLR (-0.62) at 1 % significance level.

Positive and highly significant association of earhead length with RV (0.55) and RSLR (0.53) at 5 % significance level. Positive and highly significant association of days to maturity with RL (0.52) at 5 % significance level and TPL (0.59) at 1 % significance level.

RL had highly significant and positive association with TPL (0.84) and RSLR (0.76) at 1 % significance level. Highly significant and positive association of RV was observed with RSLR (0.48) 5 % significance level. RFW had significant and positive association with RDW (0.68) at 1 % significance level.

4.1.3.8 Correlation of grain yield with component characters

Grain yield plant-1 (GY/p) showed positive association with EHL (0.53), SW (0.93) and BM (0.62) at 1 % significance level and with RV (0.46) at 5 % significance level and also it showed a negative association with PH (-0.91), SL (-0.91), DFF (-0.62), HI (-0.94), RL (-0.90), RSLR (-0.81) at 1 % significance level and with DM (-0.49) at 5 % significance level during *Kharif*-2015.

Grain yield plant-1 (GY/p) had showed highly significant and positive association with BM (0.54) at 5 % significance level. And also showed significantly negative correlation with PH (-0.61), SL (-0.63), DFF (-0.60) and HI (-0.91) at 1 % significance level during summer-2016.

Correlation among the root traits was found highly significant in both the seasons. Significantly high correlation was found between RL and RSLR and RDW in both seasons.

From all association studies it was observed that during both season all the root characters having strong association among them. Both RN and RL has is always associated with the SL, TPL and it is contributing to RDW also. Whereas RL has contributed significantly towards SL and TPL, and this trait is always associated with all the root traits.

4.1.4 Path-coefficient analysis

The path-coefficient analysis was carried out to discern direct and indirect effects of yield attributing traits on grain yield and root traits on grain yield. Results are presented for rice in Table 25 and 26. For sorghum results are presented in Table 27 and 28.

During *Kharif*-2015: In rice genotypes, root length had the highest positive direct effect of 2.44 on grain yield per plant whereas root fresh weight had lowest positive direct effect of 0.28. Among the characters under study, total number of tillers had highest negative direct effect of -1.62 on grain yield per plant whereas days to 50 % flowering had lowest negative direct effect of -0.41.

In the selected genotypes, total plant length had the highest positive indirect effect of 2.22 *via.*, root length towards grain yield whereas, root number had lowest positive indirect effect of 0.03 *via*., days to 50 % flowering towards grain yield.

Among the characters under study, number of productive tillers had highest negative indirect effect of -1.60 *via* total number of tillers towards grain yield whereas, number of productive tillers had lowest negative indirect effect of -0.004 via root fresh weight towards grain yield. Similar results for this trait in rice were obtained by Sravan *et al.* (2012).

During summer-2016: In rice genotypes, total number of tillers had the highest positive direct effect of 4.3 on grain yield per plant whereas, biomass had lowest positive direct effect of 0.3. Among the characters under study, number of productive tillers had highest negative direct effect of -4.8 on grain yield per plant whereas, root dry weight had lowest negative direct effect of -0.08.

In the selected genotypes, root fresh weight had the highest positive indirect effect of 2.8 *via* number of productive tillers towards grain yield whereas, root fresh weight had lowest positive indirect effect of 0.01 *via* root dry weight towards grain yield. The similar results were also reported by Mohankumar *et al.* (2011).

Among the characters under study, total number of tillers had highest negative indirect effect of -4.7 *via* number of productive tillers towards grain yield whereas, root number had lowest negative indirect effect of -0.001 *via* root dry weight towards grain yield.

Table 25: Estimates of direct and indirect effects of yield components on grain yield at phenotypic level in selected rice genotypes during *Kharif* -2015

	PH	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	RSLR
PH	**-1.5240**	1.0105	0.9485	-0.5572	-0.4358	-0.4723	-0.8330	-0.8549	-0.4545	-0.4059	-0.4694	0.4433	0.2746	-0.1133	-0.3274	0.5930
TNT	1.0771	**-1.6245**	-1.6095	-0.1703	0.8182	0.3597	0.6555	0.7522	0.2044	0.2937	0.5848	-0.1236	-0.2438	0.2165	0.2347	-0.5951
NPT	-0.5103	0.8124	**0.8200**	0.1458	-0.3733	-0.1178	-0.3653	-0.4080	-0.1522	-0.1932	-0.3326	-0.0132	0.0621	-0.1507	-0.1692	0.2337
PL	0.1335	0.0383	0.0649	**0.3650**	0.1228	0.2271	-0.0583	-0.0980	-0.0620	0.0698	0.0021	-0.1175	-0.1129	-0.0349	-0.0550	-0.1238
DFF	-0.1190	0.2095	0.1894	-0.1399	**-0.4160**	-0.3669	0.0764	0.0739	0.2330	0.0352	0.0586	0.2120	0.2440	0.1197	0.2004	0.3008
DM	0.2676	-0.1912	-0.1240	0.5372	0.7615	**0.8634**	-0.3094	-0.3256	-0.5337	-0.0929	-0.2211	-0.5568	-0.5606	-0.3450	-0.4966	-0.6644
BM	0.6564	-0.4845	-0.5349	-0.1917	-0.2207	-0.4303	**1.2008**	1.0302	0.8787	0.4126	0.6915	0.4783	0.4389	0.6194	0.8447	0.3710
HI	-0.8358	0.6899	0.7413	0.3998	0.2647	0.5618	-1.2783	**-1.4900**	-0.8322	-0.3054	-0.5554	-0.2921	-0.2031	-0.3943	-0.6982	-0.1701
RL	0.7307	-0.3082	-0.4546	-0.4162	-1.3724	-1.5143	1.7928	1.3683	**2.4499**	1.5376	2.0013	1.5612	1.4899	1.4718	2.2202	1.8543
RN	0.0984	-0.0668	-0.0870	0.0706	-0.0313	-0.0398	0.1269	0.0757	0.2318	**0.3694**	0.2946	0.1511	0.1167	0.1782	0.2307	0.1601
RV	-0.3693	0.4317	0.4864	-0.0068	0.1689	0.3071	-0.6906	-0.4470	-0.9796	-0.9564	**-1.1991**	-0.7323	-0.5033	-0.5994	-0.8935	-0.6843
RFW	-0.0837	0.0219	-0.0046	-0.0927	-0.1467	-0.1856	0.1146	0.0564	0.1834	0.1177	0.1758	**0.2878**	0.2084	0.1823	0.2043	0.2310
RDW	-0.0651	0.0542	0.0274	-0.1117	-0.2120	-0.2346	0.1321	0.0493	0.2198	0.1141	0.1517	0.2616	**0.3614**	0.2710	0.2727	0.2603
SL	0.0221	-0.0397	-0.0547	-0.0285	-0.0856	-0.1190	0.1536	0.0788	0.1788	0.1436	0.1488	0.1885	0.2233	**0.2977**	0.2627	0.1627
TPL	-0.2278	0.1532	0.2188	0.1597	0.5108	0.6098	-0.7458	-0.4969	-0.9608	-0.6621	-0.7900	-0.7527	-0.8000	-0.9356	**-1.0602**	-0.7787
RSLR	0.3632	-0.3419	-0.2661	0.3166	0.6749	0.7183	-0.2884	-0.1066	-0.7065	-0.4045	-0.5327	-0.7492	-0.6724	-0.5101	-0.6856	**-0.9334**
GY/P	-0.3862	0.3648	0.3612	0.2798	0.0281	0.1667	-0.3163	-0.7421	-0.1015	0.0733	0.0089	0.2464	0.3232	0.2733	0.0848	0.2169
PARTIAL R^2	0.5885	-0.5926	0.2961	0.1021	-0.0117	0.1440	-0.3798	1.1057	-0.2488	0.0271	-0.0107	0.0709	0.1168	0.0814	-0.0899	-0.2025

RESIDUAL EFFECT= 0.0578

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (g)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

Table 26: Estimates of direct and indirect effects of yield components on grain yield at phenotypic level in selected rice genotypes during summer-2016

	PH	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	RSLR
PH	**-1.1104**	0.7067	0.6815	-0.3042	0.1255	-0.0447	-0.7121	-0.6611	-0.3468	0.2059	-0.3013	-0.1529	-0.4467	-0.1450	-0.3098	0.1698
TNT	-2.7698	**4.3521**	4.3294	0.2364	-1.7236	-2.0599	-1.9406	-3.1335	-0.5867	1.5914	-1.0839	-2.6549	-1.3667	-0.9520	-0.8454	0.8655
NPT	2.9544	-4.7885	**-4.8136**	-0.5864	1.7701	2.0920	2.1984	3.5237	0.8920	-1.5969	1.4501	2.8743	1.7803	1.2624	1.2011	-0.6608
PL	0.3024	0.0599	0.1344	**1.1036**	0.3951	0.2839	-0.0653	-0.3470	-0.5213	0.0827	-0.3587	0.2386	-0.7192	-0.2841	-0.4957	-0.6483
DFF	0.0324	0.1135	0.1054	-0.1026	**-0.2865**	-0.2519	0.0688	-0.0214	0.1749	0.1087	0.1108	-0.1489	0.1639	0.0813	0.1599	0.1730
DM	-0.0230	0.2696	0.2475	-0.1465	-0.5008	**-0.5696**	0.1543	-0.1050	0.3120	0.3520	0.2377	-0.2026	0.2692	0.3142	0.3623	0.3540
BM	0.2352	-0.1635	-0.1675	-0.0217	-0.0881	-0.0994	**0.3667**	0.2812	0.2461	-0.0402	0.2356	0.0268	0.2494	0.2078	0.2675	0.1403
HI	-0.3572	0.4320	0.4392	0.1887	-0.0448	-0.1107	-0.4601	**-0.6000**	-0.2707	0.3114	-0.2362	-0.0410	-0.3876	-0.1813	-0.2728	-0.0912
RL	0.2504	-0.1081	-0.1486	-0.3787	-0.4894	-0.4392	0.5381	0.3617	**0.8017**	0.2643	0.7433	-0.1012	0.6607	0.3749	0.7341	0.7105
RN	0.0258	-0.0510	-0.0462	-0.0104	0.0529	0.0861	0.0153	0.0723	-0.0459	**-0.1394**	-0.0628	-0.0176	-0.0028	-0.0477	-0.0540	-0.0656
RV	-0.3490	0.3203	0.3875	0.4180	0.4972	0.5368	-0.8262	-0.5064	-1.1924	-0.5797	**-1.2862**	-0.2169	-0.8771	-0.6694	-1.1427	-1.0667
RFW	0.0659	-0.2920	-0.2859	0.1035	0.2488	0.1703	0.0350	0.0327	-0.0604	0.0605	0.0807	**0.4787**	-0.0676	0.1336	0.0183	-0.0995
RDW	-0.0356	0.0278	0.0327	0.0576	0.0506	0.0418	-0.0601	-0.0571	-0.0729	-0.0018	-0.0603	0.0125	**-0.0884**	-0.0454	-0.0719	-0.0564
SL	-0.1203	0.2015	0.2415	0.2371	0.2614	0.5081	-0.5218	-0.2783	-0.4307	-0.3150	-0.4794	-0.2570	-0.4735	**-0.9210**	-0.7215	-0.4039
TPL	0.4458	-0.3104	-0.3987	-0.7178	-0.8920	-1.0163	1.1657	0.7264	1.4632	0.6189	1.4197	0.0609	1.2995	1.2519	**1.5980**	1.3143
RSLR	0.0798	-0.1037	-0.0716	0.3065	0.3150	0.3242	-0.1995	-0.0793	-0.4624	-0.2455	-0.4327	0.1084	-0.3331	-0.2288	-0.4291	**-0.5217**
GY/P	-0.3732	0.6661	0.6671	0.3830	-0.3086	-0.5484	-0.2435	-0.7910	-0.1002	0.6774	-0.0236	0.0074	-0.3397	0.1512	-0.0017	0.1131
PARTIAL R^2	0.4144	2.8990	-3.2112	0.4227	0.0884	0.3123	-0.0893	0.4746	-0.0804	-0.0944	0.0304	0.0035	0.0300	-0.1392	-0.0028	-0.0590

RESIDUAL EFFECT= 0.0302

PH: Plant height
TNT: Total number of tillers
NPT: Number of productive tillers
PL: Panicle length
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (g)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

Table 27: Estimates of direct and indirect effects of yield components on grain yield at phenotypic level in sorghum genotypes during *Kharif* -2015

	PH	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	SDW	SG	RSLR
PH	**-6.3844**	3.3805	-6.3564	5.8626	-2.7385	-1.8878	2.7769	-5.8671	-5.6492	-2.8044	1.9139	3.5893	0.8029	-4.7258	-5.7324	0.2191	2.4176	-4.6533
EHL	-1.1172	**2.1099**	-1.2797	1.3324	0.1090	0.6120	0.6355	-1.2184	-0.6283	0.4539	0.8345	0.1606	-0.4931	-0.4157	-0.6032	1.2394	1.0911	-0.2803
SL	2.9981	-1.8263	**3.0113**	-2.8013	1.1935	0.7382	-1.3275	2.7853	2.5962	1.1683	-0.9774	-1.6120	-0.2774	2.1545	2.6290	-0.2918	-1.2404	2.1012
SW	0.3781	-0.2600	0.3830	**-0.4117**	0.1710	0.1347	-0.1648	0.4031	0.3446	0.1398	-0.1497	-0.2334	-0.0986	0.2620	0.3415	-0.0252	-0.1198	0.2864
DFF	-0.0562	-0.0068	-0.0520	0.0544	**-0.1311**	-0.1105	0.1015	-0.0561	-0.0864	-0.0312	0.0504	0.0105	0.0518	-0.0905	-0.0933	-0.0157	0.0580	-0.1006
DM	-0.4285	-0.4204	-0.3553	0.4741	-1.2219	**-1.4492**	0.6664	-0.5248	-0.7787	-0.5334	0.0243	0.2743	0.7386	-0.7332	-0.8157	-0.5280	0.1129	-0.9384
BM	-0.7822	0.5416	-0.7928	0.7199	-1.3929	-0.8269	**1.7983**	-0.7055	-1.0920	0.0581	1.2348	-0.3751	0.1476	-1.0420	-1.1483	0.4298	1.3767	-1.2431
HI	1.5803	-0.9930	1.5906	-1.6833	0.7364	0.6227	-0.6746	**1.7196**	1.4907	0.6612	-0.5980	-0.8347	-0.5509	1.0297	1.4447	-0.0503	-0.4183	1.2608
RL	6.1846	-2.0813	6.0260	-5.8496	4.6049	3.7556	-4.2441	6.0591	**6.9895**	4.0709	-2.4607	-2.6814	-3.1573	4.9710	6.8184	1.3012	-2.0432	6.6929
RN	-0.9979	-0.4887	-0.8814	0.7715	-0.5408	-0.8362	-0.0735	-0.8735	-1.3232	**-2.2719**	-0.9536	1.0237	1.4272	-0.6749	-1.2076	-1.3253	-0.5854	-1.3175
RV	-0.4196	0.5535	-0.4543	0.5090	-0.5380	-0.0234	0.9610	-0.4867	-0.4927	0.5875	**1.3996**	-0.2264	-0.1920	-0.4227	-0.5033	0.6137	0.8140	-0.4968
RFW	0.3201	-0.0433	0.3048	-0.3228	0.0457	0.1078	0.1188	0.2764	0.2184	0.2566	0.0921	**-0.5694**	-0.0492	0.2813	0.2525	0.1680	0.0973	0.1307
RDW	0.1136	0.2111	0.0832	-0.2164	0.3568	0.4604	-0.0741	0.2894	0.4081	0.5675	0.1240	-0.0780	**-0.9034**	0.0196	0.3134	0.4478	0.3528	0.4923
SL	-0.3551	0.0945	-0.3433	0.3053	-0.3311	-0.2427	0.2780	-0.2873	-0.3412	-0.1425	0.1449	0.2370	0.0104	**-0.4798**	-0.4070	0.0357	0.2682	-0.3108
TPL	-2.0911	0.6658	-2.0333	1.9314	-1.6582	-1.3109	1.4871	-1.9566	-2.2719	-1.2379	0.8375	1.0327	0.8080	-1.9758	**-2.3290**	-0.2723	0.9199	-2.1513
SDW	-0.0754	1.2901	-0.2129	0.1342	0.2626	0.8002	0.5250	-0.0642	0.4089	1.2812	0.9630	-0.6481	-1.0886	-0.1634	0.2568	**2.1963**	1.5650	0.6659
SG	1.8173	-2.4818	1.9769	-1.3961	2.1228	0.3740	-3.6742	1.1675	1.4029	-1.2366	-2.7912	0.8199	1.8743	2.6828	1.8956	-3.4197	**-4.7992**	1.2363
RSLR	-1.5960	0.2909	-1.5279	1.5233	-1.6802	-1.4178	1.5136	-1.6054	-2.0968	-1.2698	0.7773	0.5028	1.1933	-1.4184	-2.0227	-0.6639	0.5641	**-2.1897**
GY/P	-0.9114	0.5363	-0.9134	0.9369	-0.6298	-0.4998	0.6292	-0.9452	-0.9011	-0.2827	0.4656	0.3922	0.2437	-0.7414	-0.9105	0.0589	0.4313	-0.8152
PARTIAL R^2	5.8190	1.1316	-2.7504	-0.3857	0.0826	0.7244	1.1315	-1.6254	-6.2984	0.6423	0.6517	-0.2233	-0.2201	0.3557	2.1206	0.1293	-2.0701	1.7850

RESIDUAL EFFECT= 0.00

PH: Plant height
EHL: Earhead Length
SL: Shoot Length
SW: Stem Width
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

Table 28: Estimates of direct and indirect effects of yield components on grain yield at phenotypic level in sorghum genotypes during summer-2016

	PH	TNT	NPT	PL	DFF	DM	BM	HI	RL	RN	RV	RFW	RDW	SL	TPL	SDW	SG	RSLR
PH	**-14.2931**	6.5766	-14.0660	-7.1634	-6.3839	-4.2617	6.6224	-8.2283	-0.3843	0.8032	4.8125	9.7440	3.7977	-4.5374	-3.0215	-0.8285	4.3851	8.8872
EHL	-1.4999	**3.2597**	-1.8994	0.7152	0.2232	0.9429	1.1999	-0.9701	0.9802	0.5551	1.8240	0.8467	-0.1161	-0.2122	0.3961	1.7647	1.4916	1.7374
SL	12.3926	-7.3378	**12.5927**	5.2783	5.1288	3.1253	-5.9259	7.6220	-0.2884	-0.2640	-5.1170	-8.4912	-3.3955	4.4545	2.6080	-0.3317	-4.1871	-8.1652
SW	-0.3833	-0.1678	-0.3206	**-0.7648**	-0.0867	-0.0985	0.0613	-0.1595	-0.1251	0.0084	0.0129	0.2704	0.1555	-0.1275	-0.1465	-0.2918	-0.0410	0.1439
DFF	-0.7752	-0.1188	-0.7069	-0.1968	**-1.7355**	-1.5208	1.0908	-1.1174	-0.7073	-0.3705	0.0245	0.0376	0.2900	-0.5990	-0.7523	-0.3486	0.7229	-0.0144
DM	0.1240	0.1203	0.1032	0.0536	0.3644	**0.4158**	-0.1349	0.1958	0.2197	0.1363	0.0860	-0.0403	-0.1107	0.2070	0.2467	0.1384	-0.0644	0.0818
BM	0.6678	-0.5305	0.6782	0.1155	0.9058	0.4677	**-1.4413**	0.7621	0.0652	-0.0375	-0.5568	-0.0307	0.0539	-0.1984	-0.0879	-0.6192	-1.2591	-0.3945
HI	-0.0185	0.0096	-0.0194	-0.0067	-0.0207	-0.0151	0.0170	**-0.0321**	-0.0082	-0.0074	0.0053	0.0104	0.0122	-0.0133	-0.0126	-0.0032	0.0086	0.0056
RL	0.0358	0.4009	-0.0305	0.2181	0.5433	0.7046	-0.0603	0.3411	**1.3332**	-0.0684	0.4709	-0.2473	-0.5657	0.6494	1.1206	0.4013	-0.0030	1.0178
RN	0.1001	-0.3033	0.0373	0.0197	-0.3802	-0.5835	-0.0464	-0.4119	0.0914	**-1.7807**	-0.7794	-0.1916	-0.4491	-0.4169	-0.2094	-0.1535	-0.2796	-0.0099
RV	-0.0182	0.0302	-0.0219	-0.0009	-0.0008	0.0112	0.0208	-0.0090	0.0191	0.0236	**0.0539**	0.0119	0.0188	-0.0001	0.0102	0.0088	0.0157	0.0264
RFW	0.9784	-0.3728	0.9678	0.5074	0.0311	0.1390	-0.0305	0.4625	0.2662	-0.1544	-0.3171	**-1.4352**	-0.9793	0.5226	0.4675	0.3712	0.2386	-0.3955
RDW	-0.6462	-0.0866	-0.6558	-0.4946	-0.4063	-0.6478	-0.0910	-0.9252	-1.0320	0.6133	0.8491	1.6594	**2.4321**	-1.2972	-1.3603	-1.1419	-0.6993	-0.4200
SL	-0.0341	0.0070	-0.0380	-0.0179	-0.0371	-0.0535	-0.0148	-0.0444	-0.0524	-0.0252	0.0003	0.0392	0.0574	**-0.1075**	-0.0949	-0.0347	-0.0301	-0.0206
TPL	0.3995	0.2296	0.3914	0.3620	0.8193	1.1213	0.1153	0.7440	1.5886	0.2223	0.3564	-0.6156	-1.0571	1.6680	**1.8900**	0.6843	0.3254	1.0014
SDW	0.0720	0.6725	-0.0327	0.4741	0.2495	0.4134	0.5337	0.1254	0.3739	0.1071	0.2032	-0.3213	-0.5832	0.4006	0.4498	**1.2422**	0.8597	0.2367
SG	0.1539	-0.2295	0.1668	-0.0269	0.2089	0.0777	-0.4382	0.1343	0.0011	-0.0788	-0.1460	0.0834	0.1442	-0.1402	-0.0864	-0.3471	**-0.5016**	-0.1074
RSLR	2.1295	-1.8255	2.2207	0.6443	-0.0284	-0.6741	-0.9374	0.5972	-2.6145	-0.0191	-1.6774	-0.9436	0.5914	-0.6563	-1.8147	-0.6525	-0.7334	**-3.4249**
GY/P	-0.6148	0.3338	-0.6332	-0.2839	-0.6052	-0.4362	0.5406	-0.9135	-0.2734	-0.3368	0.1052	0.3861	0.2966	-0.4040	-0.3978	-0.1418	0.2489	0.1860
PARTIAL R^2	8.7872	1.0882	-7.9733	0.2171	1.0504	-0.1814	-0.7791	0.0294	-0.3645	0.5997	0.0057	-0.5542	0.7212	0.0434	-0.7518	-0.1761	-0.1248	-0.6371

RESIDUAL EFFECT= 0.00

PH: Plant height
EHL: Earhead Length
SL: Shoot Length
SW: Stem Width
DFF: Days to 50 % flowering
DM: Days to maturity
BM: Biomass (gm)
HI: Harvest index, GY: Grain Yield per plant (g)

RL: Root length (cm)
RV: Root volume (cm^3)
RDW: Root dry weight (g)
RSLR: Root to shoot length ratio
RN: Number of roots
RFW: Root fresh weight (g)
TPL: Total plant length (cm)

The overall path coefficient analysis in rice revealed that tiller number, root number and number of productive tillers in that order, followed by biomass were major characters having positive direct effect and association with grain yield per plant. Whereas, Root fresh weight, root dry weight followed by root number were major characters having positive indirect effect and association with grain yield per plant

During *Kharif*-2015: In sorghum genotypes, root length had the highest positive direct effect of 6.98 on grain yield per plant whereas root volume had lowest positive direct effect of 1.39. Among the characters under study, plant height had highest negative direct effect of -6.38 on grain yield per plant whereas days to 50 % flowering had lowest negative direct effect of -0.13.

In the selected genotypes, plant height had the highest positive indirect effect of 6.18 *via.*, root length towards grain yield whereas, root dry weight had lowest positive indirect effect of 0.01 *via* shoot length towards grain yield.

Among the characters under study, number of shoot length had highest negative indirect effect of -6.35 *via* plant height towards grain yield whereas, earhead length had lowest negative indirect effect of -0.006 via days to 50 % flowering towards grain yield.

During summer-2016: In sorghum genotypes, shoot length had the highest positive direct effect of 12.59 on grain yield per plant whereas, root volume had lowest positive direct effect of 0.05. Among the characters under study, plant height had highest negative direct effect of -14.29 on grain yield per plant whereas, harvest index had lowest negative direct effect of -0.03.

In the selected genotypes, plant height had the highest positive indirect effect of 12.39 *via* shoot length towards grain yield whereas, earhead length had lowest positive indirect effect of 0.07 *via* shoot length towards grain yield.

Among the characters under study, shoot length had highest negative indirect effect of -14.06 *via* plant height towards grain yield and it had lowest negative indirect effect of -0.0001 *via* root volume towards grain yield.

The relationship between grain yield and its components may be negative or positive but it is the net result of direct effect of that particular trait and indirect effects *via* other traits. Hence, it is necessary to determine the path-coefficients which partition the observed correlation into direct and indirect effects and also reveals the cause and effect relationship between grain yield and their related traits.

The overall path coefficient analysis in sorghum revealed that root length and root volume in that order, followed by shoot length were major characters having positive direct effect and association with grain yield per plant. Whereas, plant height Root dry followed by earhead length were major characters having positive indirect effect and association with grain yield per plant.

EXPERIMENT II

4.2 Compare sequence variation for root related genetic materials across crops

4.2.1 Molecular marker analysis of selected genotypes

4.2.1.1 Root related genes

The list of root related genes obtained by survey along with traits associated is shown in Table 29 and Plate 7.

Out of seven genes four genes were amplified in all rice accessions but could not able to amplify in sorghum. These genes were not able to amplify in some of the genotypes as explained above. The probable reason for the monomorphism could be these genes are present in the genome and their expression levels were controlled by many of the regulatory elements, so by looking into the upstream regions and designing specific primers to these regions seems to be helpful to look into the polymorphism. Even though they are monomorphic bands in rice there could be variation within the amplified products, may be variations at nucleotide level.

4.2.1.2 Screening genotypes with molecular markers

Among the markers, Simple Sequence Repeats (SSRs) are widely used in genetic diversity and parental analysis owing to their co-dominant nature, high reproducibility,

Table 29: The list of root related genes obtained by survey along with traits associated

Sl. No.	Gene	Chr. number	Function	Amplified in	
				Rice	Sorghum
1.	*EXP15*	3	Root Hair Development	400bp	NA
2.	*OsGLU3*	4	Root Elongation	300bp	NA
3.	*OSCSLD*	10	Root Hair Development	250bp	NA
4.	*GLR3.1*	4	Root Elongation	NA	NA
5.	*EXP17*	3	Root Hair Development	500bp	NA
6.	*OsIAA11*	3	Lateral Root Development	NA	NA
7.	*OsIAA13*	3	Lateral Root Development	NA	NA

NA- No Amplification

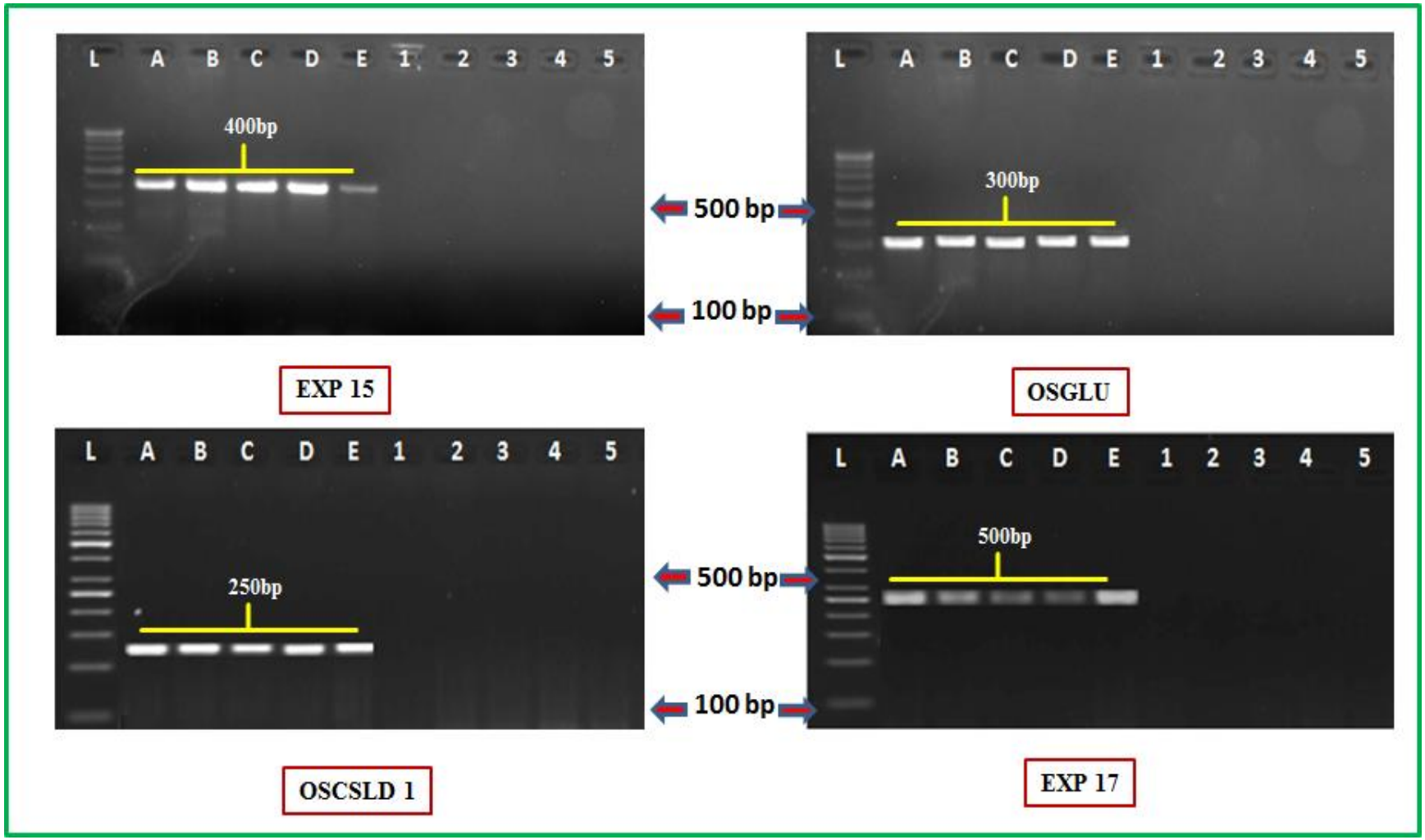


Plate 7: PCR Profile of candidate genes EXP 15, OSGLU, OSCSLD1 and EXP 17

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

abundance in the genome and transferability across species or genera. The development of these markers for a species might be costly and time consuming. Hence, screening existing markers through transferability test from closely related species or family is resource conscious.

Molecular markers that are tightly linked to the gene/QTL are useful in easy identification of desirable characters hence, help in bringing desirable changes in the genotypes. List of amplified markers used in the present investigation with their associated trait is shown in Table 30 and 31. The expected product size of the primers used ranged from 147bp to 450bp, and annealing temperature from 52^0 C to 64.6^0 C which is explained in material and methods section.

Out of the 62 primers only twelve markers namely RM38, RM60, RM149, RM166, RM201, RM212, RM234, RM242, RM318, RM3769, RM5844 and RM6925 were polymorphic in rice. The primers profile is displayed in Plates 8, 9 & 10.

4.2.1.3 Sequencing the amplified root related markers

Amplified products of three primers (out of 62 primers) from ten genotypes were sequenced (Appendix VII-IX). The length of each amplicon is varied within each primer. RM166 had 560bp, RM149 had 350bp and RM5844 had 280bp of amplicon length.

4.2.1.4 Identification of the sequence variations among the genotypes

Ten sequences of all the sequenced primers were subjected to alignment using Clustalw (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The pattern of similarities or dissimilarities, in amplicons and the positions where these occur, was recorded.

All the amplicons were found to have variations at nucleotide level which were less in RM166 and more RM149 amplicon at nucleotide level. Nucleotide variations observed were given in Plates 11-13.

In case of RM166, changes were noticed from 01 positions to 20^{th} position for both rice and sorghum which were mostly deletions. There is no change observed

Table 30: List of SSR markers with associated QTL and Traits.

Sl. No.	Marker	Trait	QTL	Reference
1	RM168	Maximum Root Length	*qMXM-3*	**1**
2	RM194	Tiller number	*nt5.1*	**-**
		Maximum Root Length	*qmrl*	**2**
3	RM201	Deep root thickness	-	**3**
		Maximum Root Length	*qMXN-9*	**4**
4	RM206	Root penetration	-	**3**
		Panicle Number	*qPn11*	**6**
5	RM212	Grain yield	*Yld1.1*	**3**
		Tiller number	-	**3**
		Maximum Root Length	-	**2**
6	RM213	Total Root Number	*Qtrn2.2*	**7**
7	RM215	Maximum Root Length	-	**8**
		Panicle length	*Pl1.1*	**9**
8	RM221	Root Penetration	-	**3**
9	RM229	Root length & PH	*Ph11.1*	**10**
10	RM231	Grain yield	*qGY3.1*	**11**
11	RM234	Total root weight	-	**12**
		Days to heading	*Dth7.2*	**9**
12	RM242	Plant height	*Qph9.1*	**3**
		Maximum Root Length	-	**2**
13	RM248	Grain yield	*qGY7*	**5**
14	RM262	Root thickness	*qRTH-2*	**4**
15	RM318	Deep root weight	-	**3**
16	RM420	Root fresh weight	*Rfw7*	**13**
17	RM3810	Root thickness	*qRTH1*	**4**

1. Yano *et al*., 2008
2. Shashidhar *et al*., 2010
3. Price *et al*., 2002
4. Yano *et al*., 2008
5. Gramene
6. Yuan *et al*., 2011
7. Hemamilini *et al*., 2000
8. Hittalmani *et al*., 2003
9. Mccouch *et al*., 2003
10. Price and tomos, 1997
11. Lanceras *et al*., 2004
12. Yadav *et al*., 1997
13. Qu *et al*., 2008

Table 31: Amplification details of selected SSR markers in rice and sorghum

Sl. No.	Marker	Chr No.	R_O	S_O	Both	Sl. No.	Marker	Chr No.	R_O	S_O	Both	Sl. No.	Marker	Chr No.	R_O	S_O	Both
1	RM212	1	P	M	✔	22	RM48	2	M	M	✔	43	RM331	8	M	X	X
2	RM3810	1	M	X	X	23	RM168	3	M	X	X	44	RM38	8	P	M	✔
3	RM3825	1	M	X	X	24	RM231	3	M	M	✔	45	RM195	8	M	M	✔
4	RM157	1	M	X	X	25	RM60	3	P	M	✔	46	RM506	8	M	X	X
5	RM128	1	M	M	✔	26	RM563	3	M	M	✔	47	RM72	8	M	X	X
6	RM140	1	P	M	✔	27	RM148	3	M	M	✔	48	RM407	8	M	X	X
7	RM543	1	M	X	X	28	RM36	3	M	X	X	49	RM201	9	P	M	✔
8	RM5	1	M	X	X	29	RM185	4	M	X	X	50	RM205	9	M	X	X
9	RM428	1	M	X	X	30	RM252	4	M	X	X	51	RM215	9	M	X	X
10	RM213	2	M	X	X	31	RM518	4	M	X	X	52	RM242	9	P	M	✔
11	RM221	2	M	M	✔	32	RM1153	4	M	M	✔	53	RM296	9	M	X	X
12	RM250	2	M	X	X	33	RM348	4	M	X	X	54	RM316	9	M	X	X
13	RM262	2	M	X	X	34	RM194	5	M	X	X	55	RM328	9	M	X	X
14	RM263	2	M	X	X	35	RM146	5	M	M	✔	56	RM107	9	M	X	X
15	RM315	2	M	X	X	36	RM5844	5	P	M	✔	57	RM2125	10	M	X	X
16	RM 7278	2	M	M	✔	37	RM169	5	M	X	X	58	RM304	10	M	X	X
17	RM211	2	M	M	✔	38	RM170	6	M	X	X	59	RM206	11	M	X	X
18	RM166	2	P	M	✔	39	RM248	7	M	X	X	60	RM229	11	M	X	X
19	RM324	2	M	X	X	40	RM420	7	M	M	✔	61	RM4	11	M	X	X
20	RM318	2	P	M	X	41	RM234	7	P	M	✔	62	RM144	11	M	X	X
21	RM53	2	M	X	X	42	RM223	8	M	X	X						

R_o- Amplified only in Rice; S_o- Amplified only in Sorghum

P-Polymorphic; M-Monomorphic

between 21 to 121 positions in both rice and sorghum. At positions 122 to 130 deletions were found for T; deletions at 129, 133, and 145th positions for T only in rice and changes were also observed at 200 to 270yth position in both rice and sorghum. So there some are conserved domains across the crops. Nucleotide variation is present in Plate 11.

In case of RM149, there has been changes at 09 and 10th positions, in both crops. Deletions at position 30, 45 and 55th. Change of T to A at 100, 101 and 103rd in rice. It is highly variable across the crops, because nucleotide changes for this primer in rice and sorghum are different. More deletions found in both crops from 250 to 350th positions. Nucleotide variations are present in Plate 12.

In case of RM5844, deletions and changes were found at 10 to 40th positions; this region has more variation for rice alone. Only deletions were found in rice from 400 to 480th position. Whereas, deletions and changes were found at 170 to 180th positions in sorghum alone. Nucleotide variation is present in Plate 13.

EXPERIMENT III

4.3 Establish associations between variations at the sequence with phenotypic data and mine multiple alleles

4.3.1 Associating the nucleotide variation with phenotypic variation.

All the fifteen primers which manifested changes in nucleotides for the nine genotypes were selected. To establish associations between variations at the sequence with phenotypic data analysis of scores was done in SPSS (Statistical Product and Service Solutions) package; results revealed that there was no association with any phenotype.

4.3.1.1 Single-Marker Analysis

Single-Marker Analysis was performed using phenotypic and genotypic data (Table 32). RM38, RM60, RM149, RM166, RM201, RM212, RM234, RM242, RM315, RM318, RM324, RM327, RM3769, RM5844 and RM6925 which were polymorphic. They showed statistically significant association with maximum root length, root dry

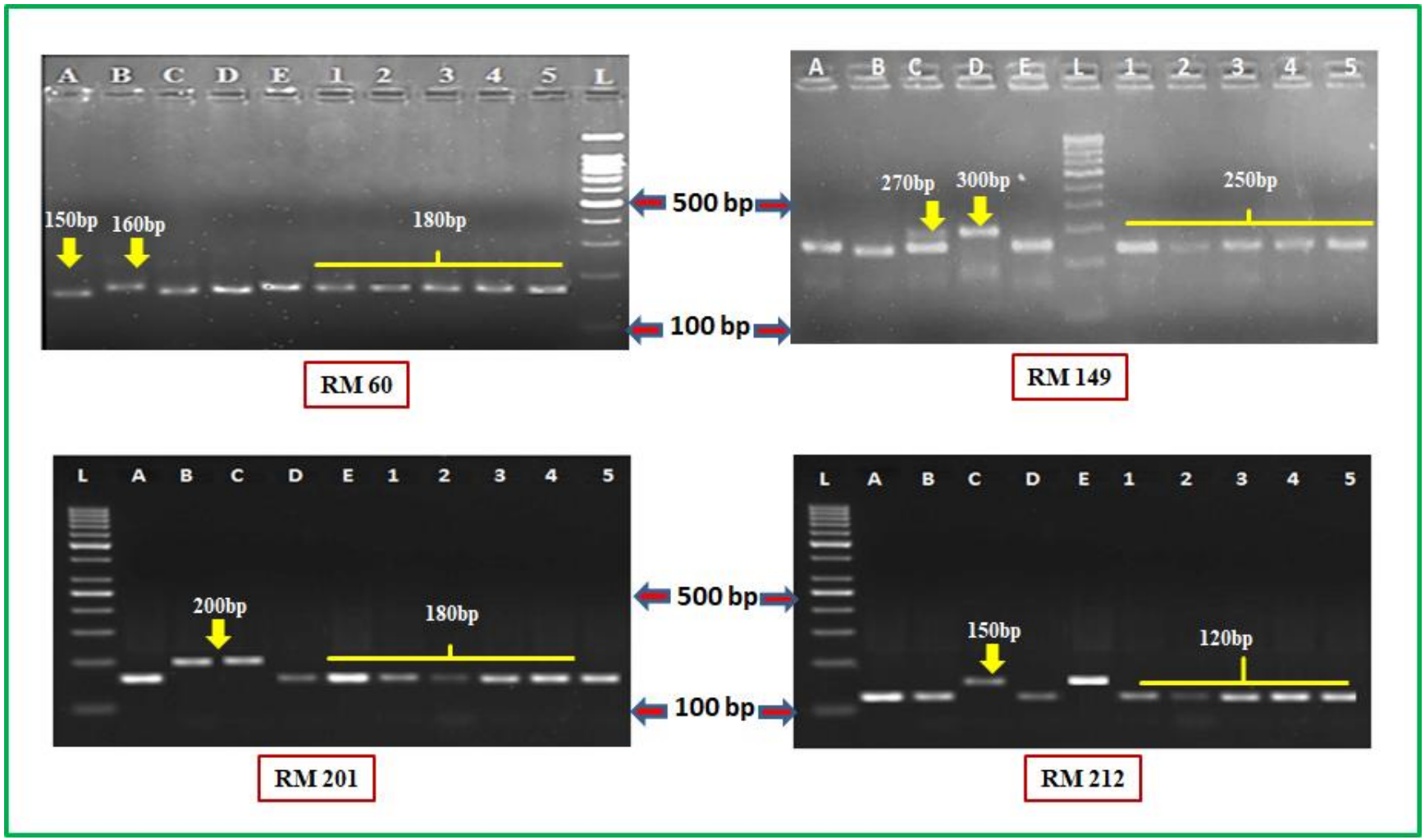


Plate 8: PCR Profile of SSR Primer RM 60, RM 149, RM 201 and RM 212

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

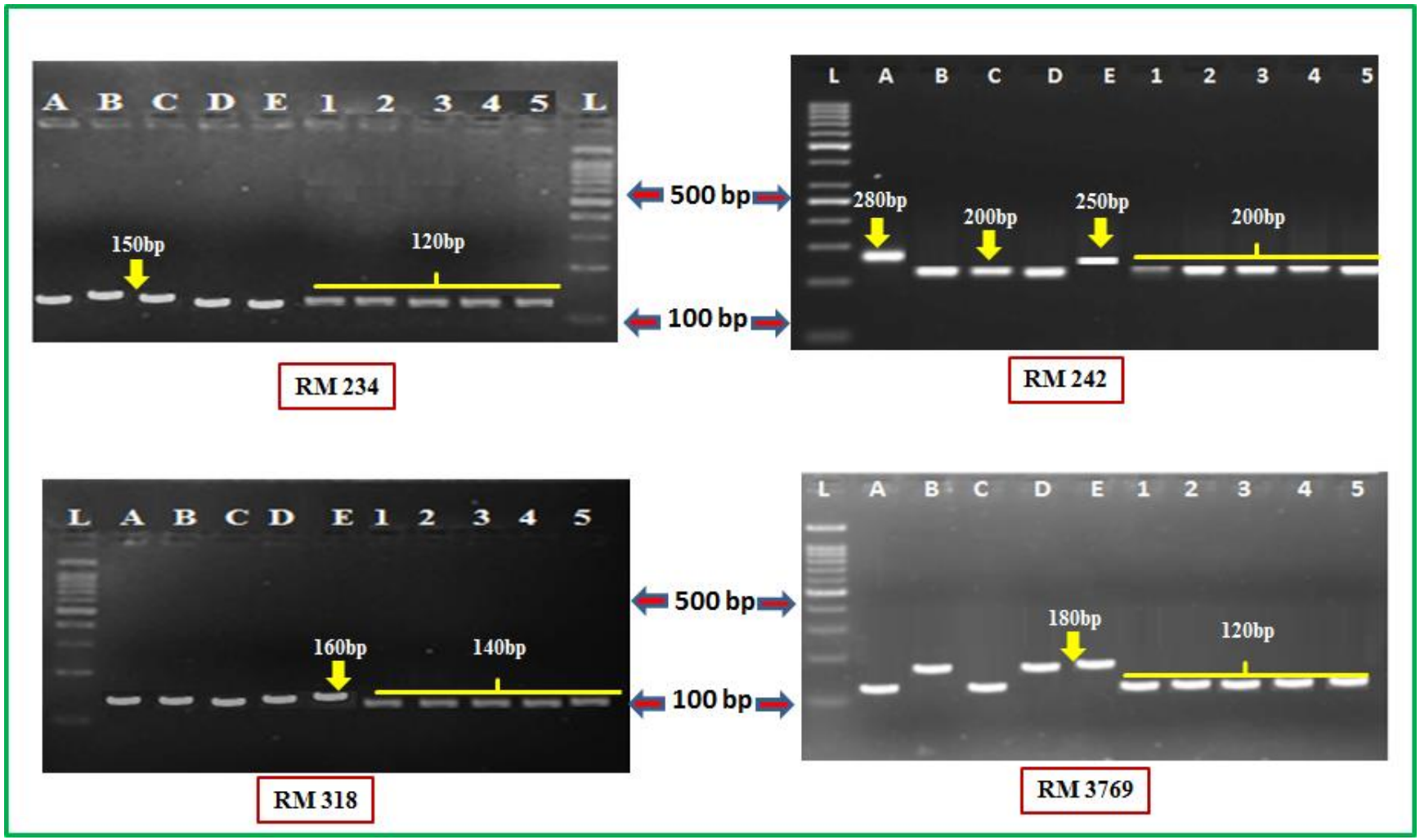


Plate 9: PCR Profile of SSR Primer RM 234, RM 242, RM 318 and RM 3769

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

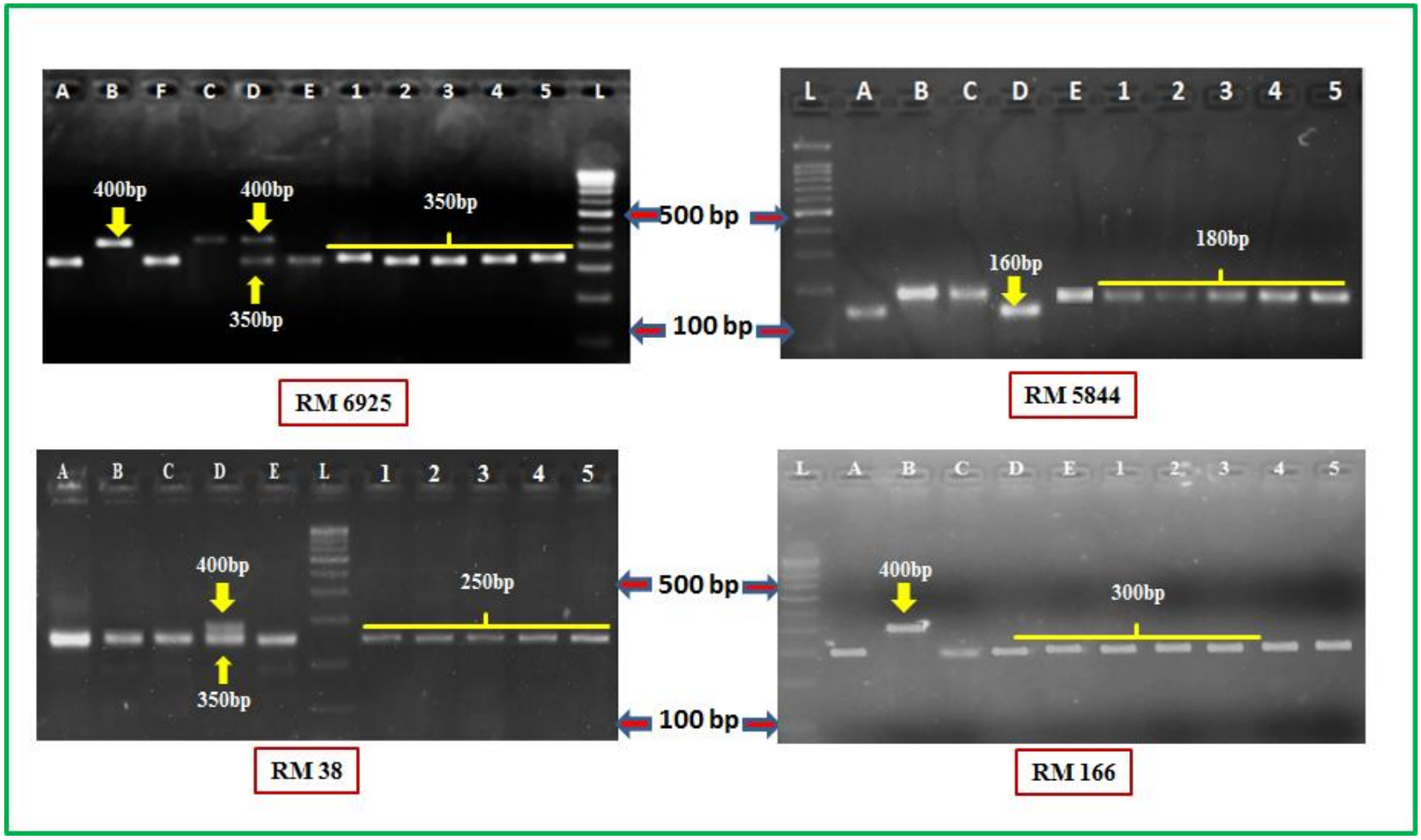


Plate 10: PCR Profile of SSR Primer RM 6925, RM 5844, RM 38 and RM 166

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; F-Azucena;1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

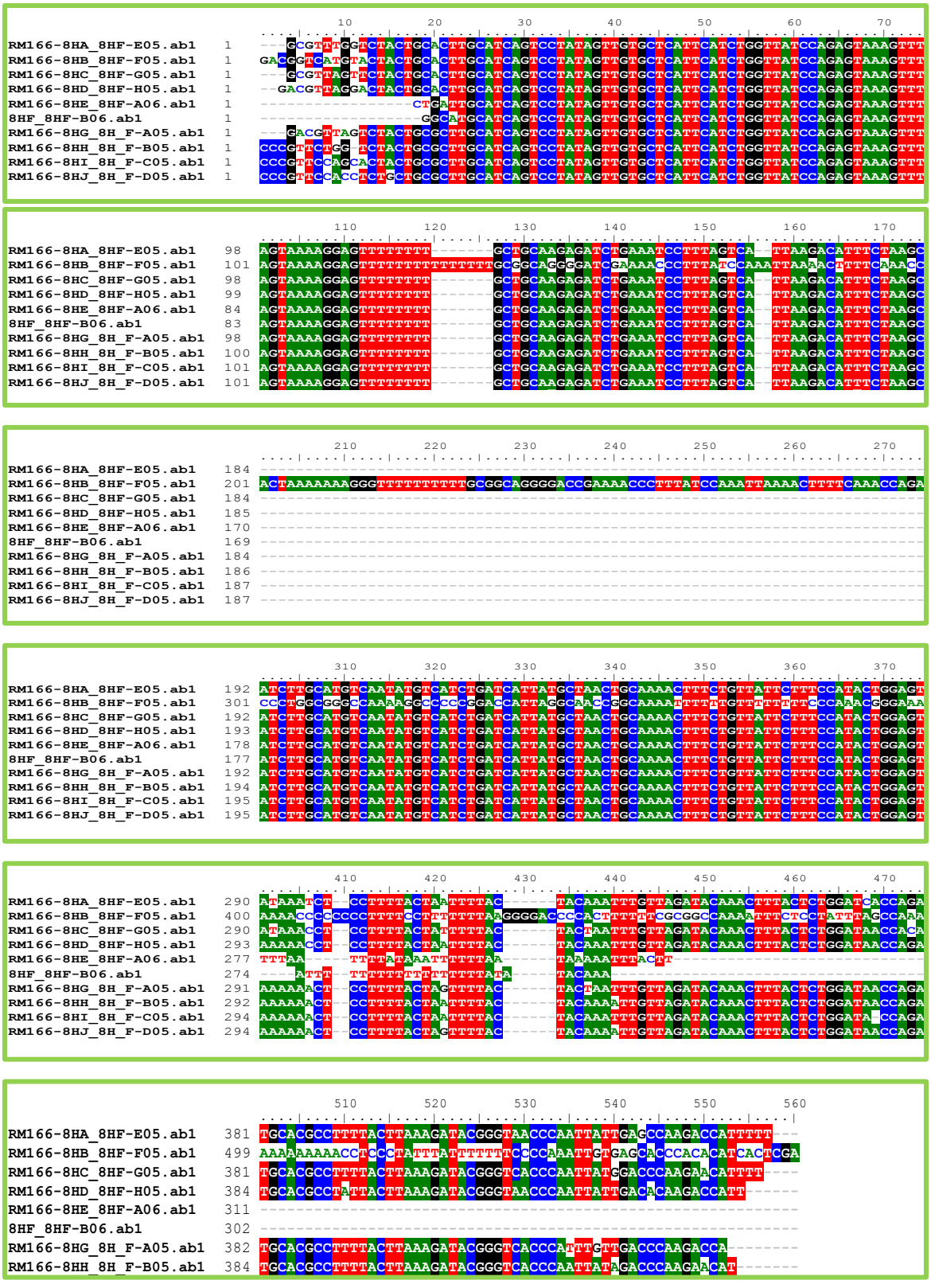


Plate 11: Nucleotide change profile of RM166

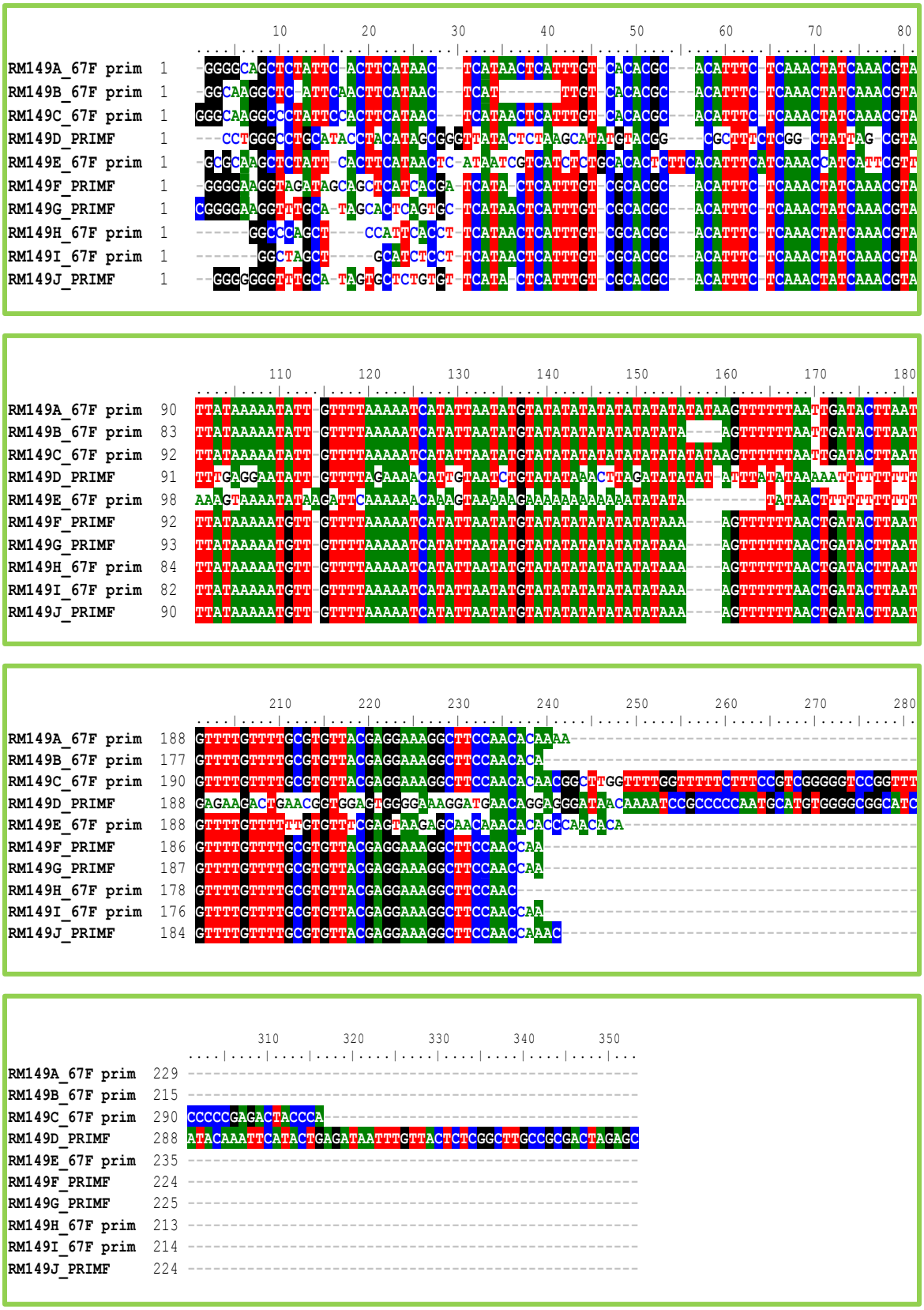


Plate 12: Nucleotide change profile of RM149

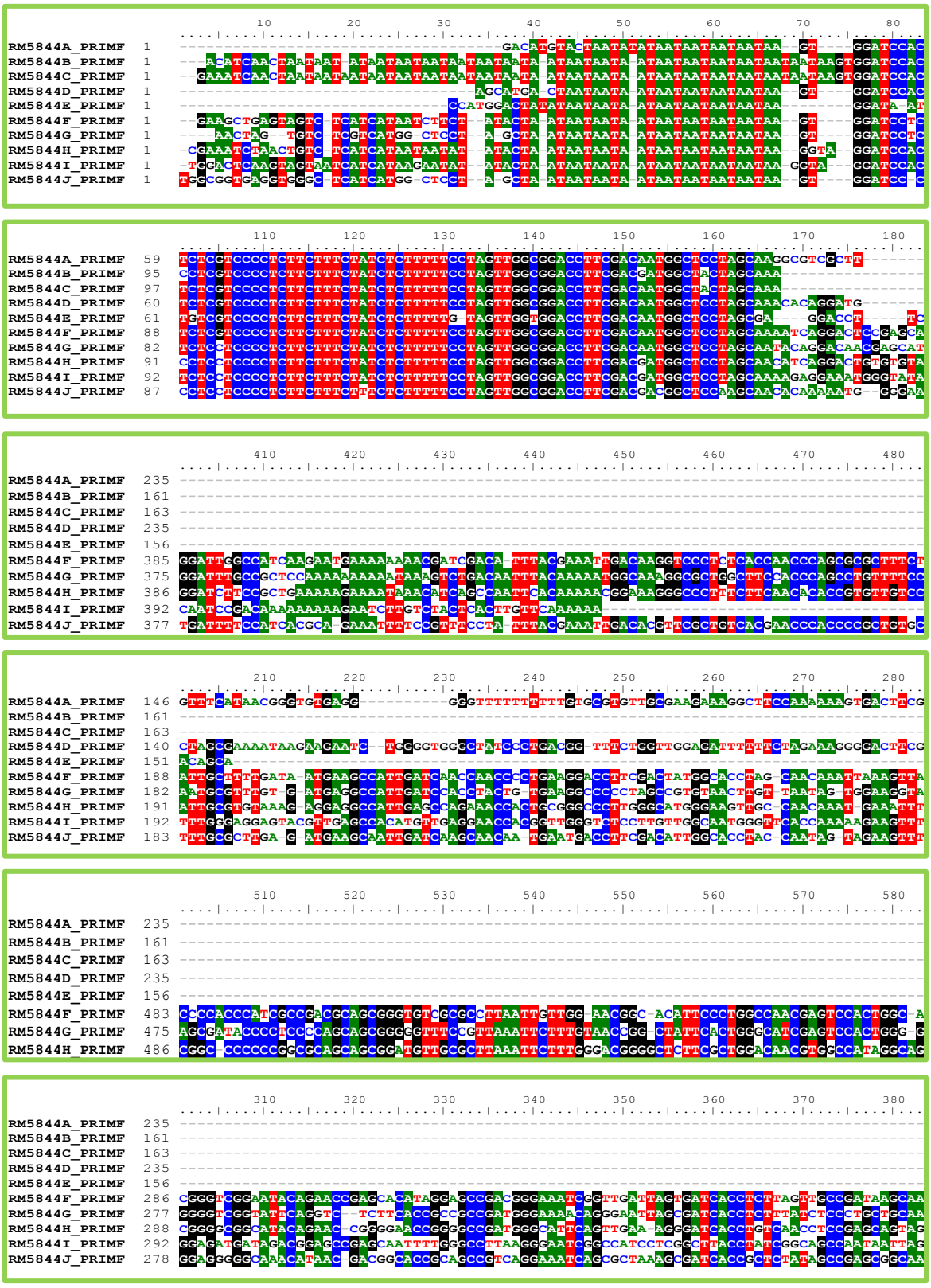


Plate 13: Nucleotide change profile of RM5844

weight and root volume. This proves that the markers are tightly linked to MRL, RV and RDW hence they can be successfully employed to check the root related traits of the concerned genotypes. It can be concluded that RM201, RM242 and RM318 can be used to select for MRL, RV and RDW and related traits in breeding programs. Whereas, RM201 and RM242 markers can be used for MAS in sorghum for root length and root volume traits.

This was in accordance with findings from, Yue *et al*., 2005, a QTL, RM316–RM219 was earlier found to be linked to multiple traits with relatively large effects on root traits in rice. RM29 and RM318 located Xipeng *et al*. (2011) reported similar association of RM29 and RM318 with root traits in rice. Similarly in this study, RM 242, RM 201, and RM318 located on chromosome #9 and #2 were linked with the root volume, total root length, and root dry weight.

Kamoshita *et al.,* 2002 identified the chromosome 9 were also linked with putative QTLs for deep root morphological traits in three different populations. Yue *et al*., 2006 reported that the SSR and RFLP markers on chromosome # 9 were linked with the root length and root mass.

The markers, RM28089, RM511and RM28166 on chromosome 12 were linked with grain yield, plant height reported by Kumar *et al*. (2013). Marker, RM348 on chromosome 4 was associated with deep root dry weight. RM316 was reported to be linked to total root weight in rice (Xipeng *et al*., 2011). Horii *et al*. (2006) and Qu *et al*. (2008) reported RM1287 and RM202 to be linked to root traits in rice. Steele *et al*. (2006) found RM318 linked to deep root dry weight in rice.

4.3.2 Allele mining

Among the sixty two markers used in present study, as expected, most of them showed amplicons in all the genotypes of rice. However, only twenty seven markers showed amplification in sorghum. Of the twenty seven amplified SSR markers, twenty four were amplified in all sorghum genotypes, whereas one marker (RM324) amplified only in two sorghum varieties and two were amplified (RM315 and RM128) in four

Table 32: Single-marker analysis of root related markers

Primer	Root Traits	P	R^2 (%)
RM201	RL	0.0008***	13.65
	RN	0.07	44.6
	RV	0.25	36.6
	RFW	0.22	10.9
	RDW	0.18	26.5
	RSLR	0.28	12.54
RM242	RL	0.003*	22.08
	RN	0.21	10.28
	RV	0.002*	26.35
	RFW	0.34	11.08
	RDW	0.13	42.85
	RSLR	0.21	15.68
RM318	RL	0.09	18.27
	RN	0.14	7.28
	RV	0.18	10.68
	RFW	0.17	5.28
	RDW	0.04*	26.25
	RSLR	0.19	19.65

* Significant at 5 %, ** significant at 1 %, ***significant at 0.001%

RL: Root length (cm) RN: Number of roots
RV: Root volume (cm^3) RFW: Root fresh weight (g)
RDW: Root dry weight (g) TPL: Total plant length (cm)
RSLR: Root to shoot length ratio

sorghum varieties (Plate 14, 15, 16 & 17). This indicates that the rice SSR primers are operating efficiently in sorghum. Other thirty five markers were not amplified in sorghum even after replicating the experiment thrice. Hence, absence of bands is true absence of that particular DNA sequence in the genomic DNA of the respective crop and not due to any kinds of technical error and indicates null allele. It is well known that null allele is also an information hence absence of allele is also unique for that genotype. Unique bands are observed in present study for markers RM166 and RM318 which is shown in Table 33. Therefore from this studied it is identified that, different size of band or no band is an allele across the crops.

Both rice and sorghum are homozygous and homogeneous they are expected to have one band for all primer pairs; surprisingly AM72 manifested two bands for markers RM6925 and RM38.

Table 33: Amplification profile of selected genotypes for SSR and gene specific markers in rice and sorghum

Rice markers	ARB6	Moroberekan	AM65	AM72	MTU1001	MLHT	Roagro	SJH-1	CSH-14	Dhanvi
RM38	250	250	250	**250 + 400**	250	250	250	250	250	250
RM60	150	160	180	180	180	180	180	180	180	180
RM149	250	250	270	300	250	250	250	250	250	250
RM166	300	400	300	300	300	300	300	300	300	300
RM201	180	200	200	180	180	180	180	180	180	180
RM212	120	120	150	120	150	120	120	120	120	120
RM234	120	150	150	120	120	120	120	120	120	120
RM242	280	200	200	200	250	200	200	200	200	200
RM315	120	120	120	120	120	120	0	120	120	120
RM318	140	140	140	140	160	140	140	140	140	140
RM324	500	500	500	500	500	500	0	0	0	500
RM327	180	180	180	180	180	0	0	0	0	0
RM3769	120	150	120	180	180	120	120	120	120	120
RM5844	160	180	180	160	180	180	180	180	180	180
RM6925	350	400	350	**400+350**	350	350	350	350	350	350
RM128	180	180	180	**180**	180	180	180	180	180	0
EXP15	400	400	400	400	400	0	0	0	0	0
OsGLU3	300	300	300	300	300	0	0	0	0	0
OSCSLD	250	250	250	250	250	0	0	0	0	0
EXP17	500	500	500	500	500	0	0	0	0	0

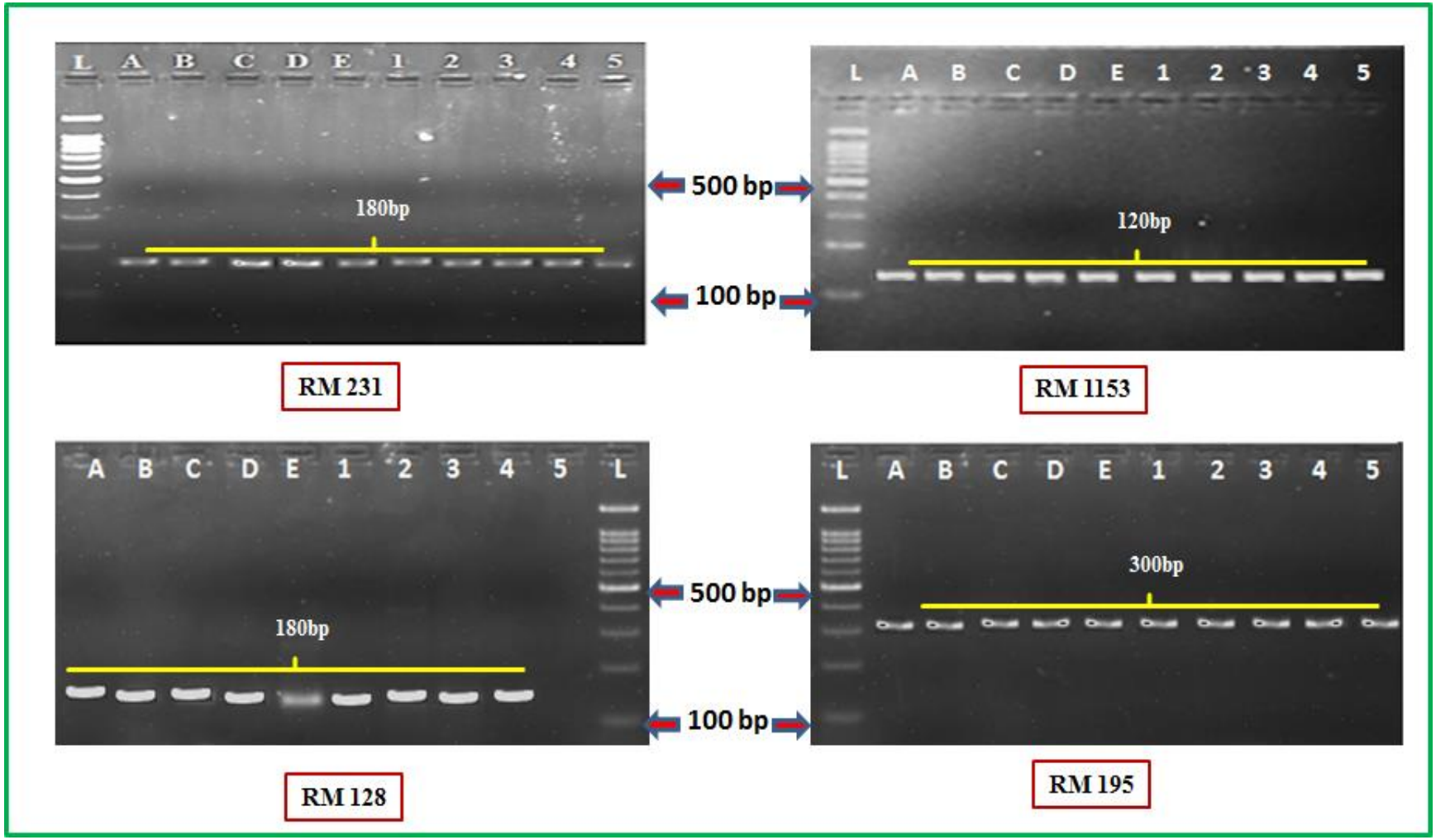


Plate 14: PCR Profile of SSR Primer RM 231, RM 1153, RM 128 and RM 195

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

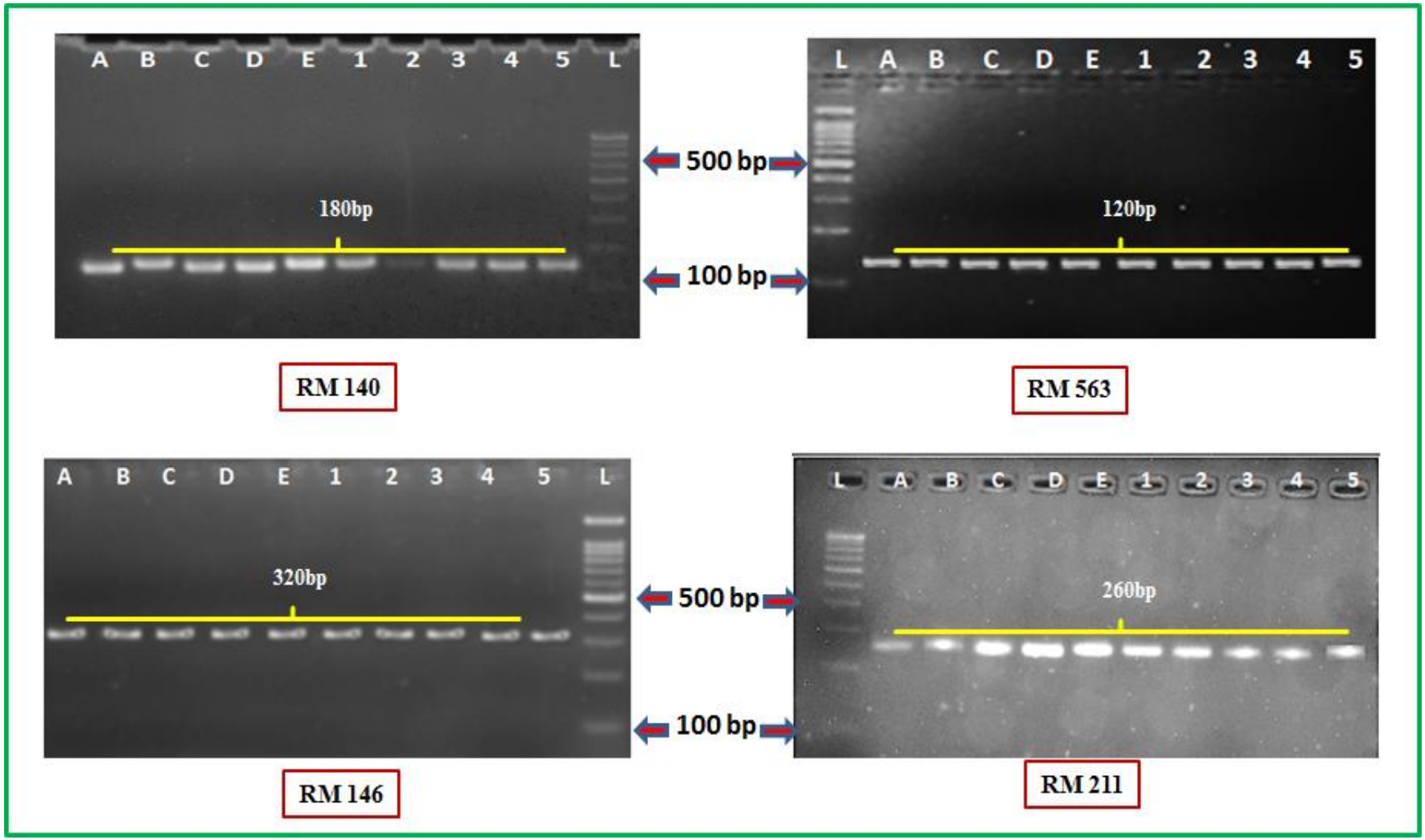


Plate 15: PCR Profile of SSR Primer RM 140, RM 563, RM 146 and RM 211

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

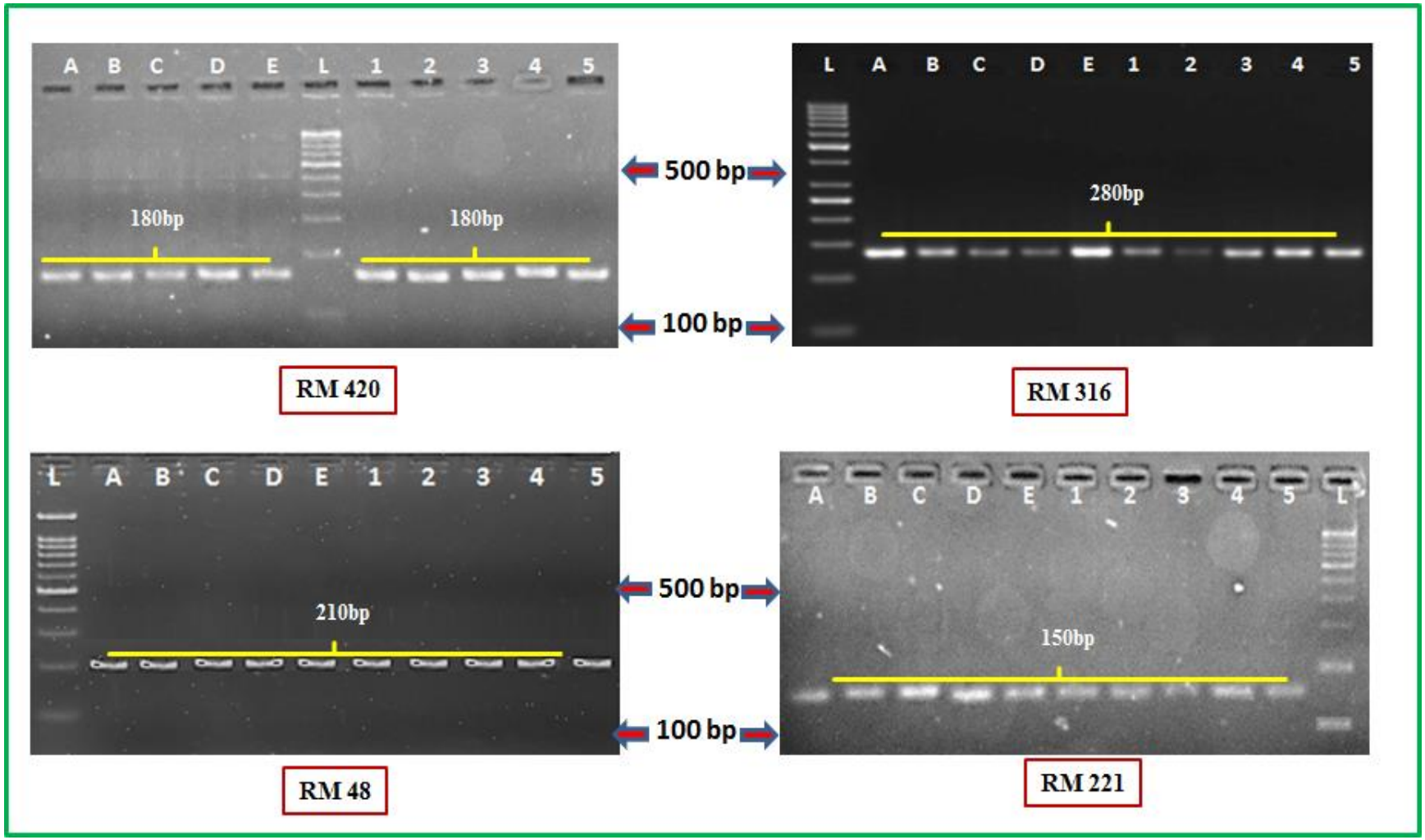


Plate 16: PCR Profile of SSR Primer RM 420, RM 316, RM 48 and RM 221

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

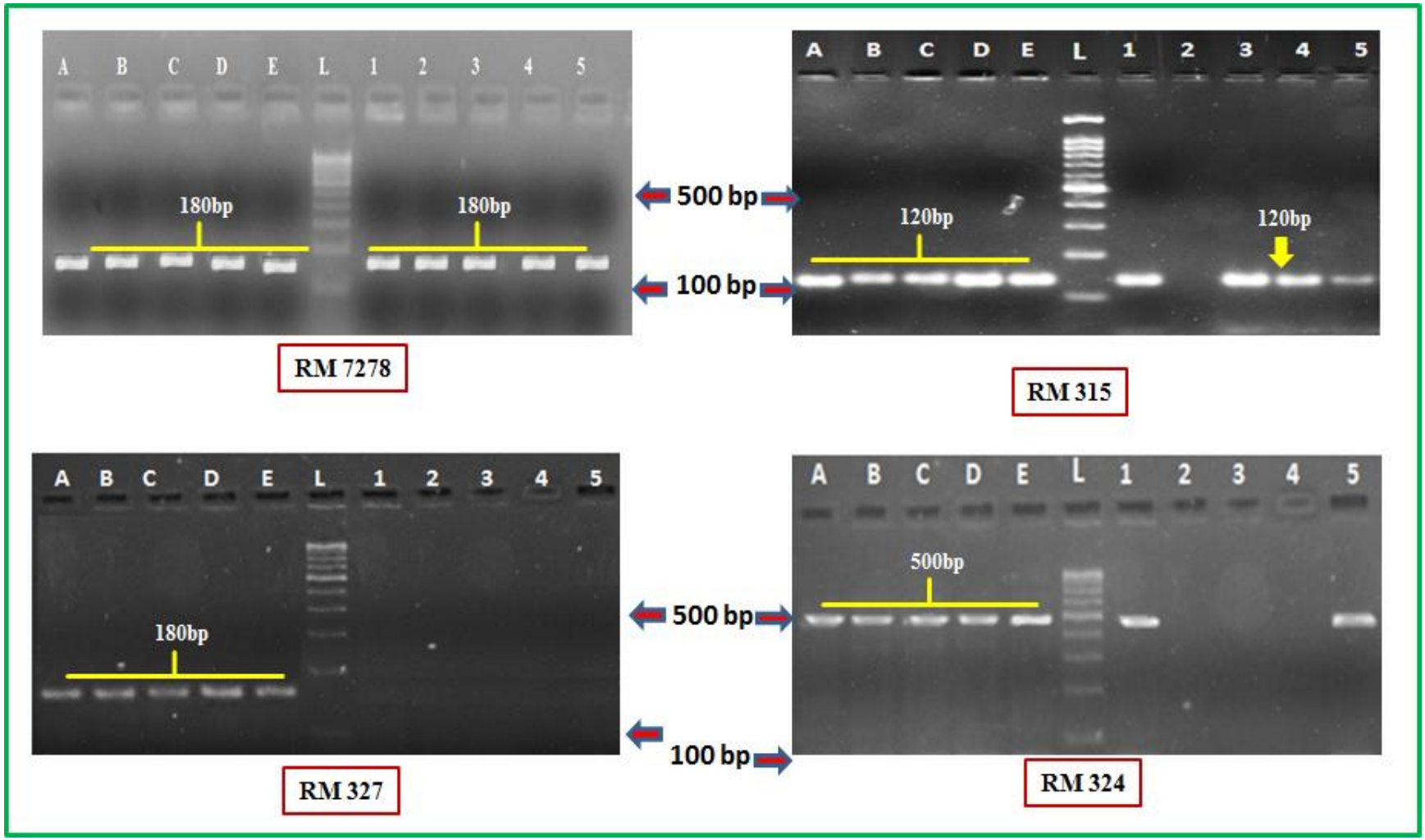


Plate 17: PCR Profile of SSR Primer RM 7278, RM 315, RM 327 and RM 324

Legend: L is 100 bp DNA ladder; A-ARB 6; B-Moroberekan; C-AM 65; D-AM 72; E-MTU1001; 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

V SUMMARY

Rice is one of the most important cereal crops, feeding more than 50 % population of the world. To meet the demand of increasing population, rice production has to be improved continually. As a very important part of rice plant, root system plays multiple roles in rice growth. Almost all of the hot spots about rice research are associated with rice root. Rice is the obvious choice for the first whole genome sequencing of a cereal crop and it is the smallest of the major cereal crop genomes at an estimated 400 to 430 Mb. The next largest genome of an important cereal crop is that of sorghum at 750 to 770 Mb. However, the number of rice SSR markers that have been transferred into sorghum is still limited, and there is a lack of systemic surveys from different rice chromosomes.

The present study was designed to look into the root characters under two different seasons and also look into the stability parameters of these root traits and identification and amplification of rice SSR markers, root gene specific markers in different sorghum genotypes. A novel PVC pipe-root experimental methodology in rice and sorghum was employed to observe the behaviour of the plant. There are no reports in sorghum using PVC pipe-root technique. This is the first attempt to study on root related traits using available rice markers.

Five genotype each from rice and sorghum are used and replicated four times both in field and pipe experiment. This experiment was done both in *Kharif*-2015 and Summer-2016 to study their stability across the seasons. All the root, shoot and yield parameters were recorded at maturity. The findings are summarized below.

5.1 Discern the extent of variation for root morphological characters in Rice and Sorghum

Analysis of variance pointed out significant differences among the selected rice and sorghum genotypes for all the characters indicating presence of sufficient amount of variability for all the characters among the genotypes studied.

5.1.1 Mean performances

In selected rice genotypes, the PCV and GCV were highest for all root traits RV, RN, RL, RFW, RDW and RSLR, the PCV and GCV were highest for yield related traits such as TNT, NPT, PH and HI during *Kharif*-2015 and Summer-2016, suggesting that, these characters are under the influence of genetic control. Hence, these characters can be relied upon and simple selection can be practiced for further improvement. Moderate GCV and PCV values were observed for DFF, BM and HI indicated considerable amount of variability for these characters. High h2 and high GA recorded for all traits indicated the presence of considerable variation and additive gene effects. Hence, improvement of these characters could be effective through phenotypic selection.

The PCV and GCV in sorghum were highest for root traits RL, RN, RV and RSLR. The PCV and GCV were highest for yield related traits such as SL, SW, GY/p, HI, BM and EHL during *Kharif*-2015 and Summer-2016. During *Kharif*-2015 and summer-2016 high heritability and GAM observed for all yield and root traits except EHL, SW and BM.

5.1.2 Association among the traits

The association among root was stronger during both seasons. Both RN and RL has is always associated with the SL, TPL and it is contributing to RDW also. Whereas RDW has contributed significantly towards SL, TPL and RSRL in both rice and sorghum.

5.1.3 Direct and Indirect effect

The overall path coefficient analysis in rice revealed that tiller number, root number and number of productive tillers in that order, followed by biomass were major characters having positive direct effect and association with grain yield per plant. Whereas, Root fresh weight, root dry weight followed by root number were major characters having positive indirect effect and association with grain yield per plant

The overall path coefficient analysis in sorghum revealed that root length and root volume in that order, followed by shoot length were major characters having positive direct effect and association with grain yield per plant. Whereas, plant height Root dry followed by earhead length were major characters having positive indirect effect and association with grain yield per plant.

5.2 Compare sequence variation for root related genetic materials across crops

There are no reports on identification and amplification of rice SSR markers, root gene specific markers in sorghum. From this experiment it was observed that, the rice SSR markers can be a valuable marker source for those plant species for which little molecular marker information is available. This study also suggests that more rice SSR markers derived from those rice chromosome regions showing a very high transferability should be tested in the future with the aim of obtaining more markers in the sorghum genotypes. Trait specific markers from rice when transferred to sorghum could be used directly for MAS.

The sequence variations among the genotypes were observed in both used crops in present study. All the amplicons were found to have variations at nucleotide level which were less in RM166 and more RM149 amplicon at nucleotide level.

5.3 Establish associations between variations at the sequence with phenotypic data and mine multiple alleles

To establish associations between variations at the sequence with phenotypic data analysis of scores was done in SPSS (Statistical Product and Service Solutions) package; results revealed that there was no association with any phenotype. Further move on to check association between marker and trait.

Single-Marker Analysis showed statistically significant association with maximum root length, root dry weight and root volume for rice. This proves that the markers RM201 and RM242 can be used for MAS in sorghum for root length and root volume traits.

Among the sixty two markers used in present study, as expected, most of them showed amplicons in all the genotypes of rice. However, some markers are amplified only in some sorghum genotypes. This indicates that the rice SSR primers are operating efficiently in sorghum.

Unique bands are observed in present study for markers RM166 and RM318 as explained in results by Table 33. This unique allele should be sequenced to compare normal allele to find whether it is associated with any trait or not, if yes it is better to known why it is associated with trait. The work can be carried over for further studies by looking into the below listed future investigations.

FUTURE LINE OF WORK

- Sorghum markers should be used on rice
- Rice markers can be used for MAS in sorghum
- Opportunity of using single marker analysis, if at all these markers can show polymorphism within the sorghum genotypes.
- Novel molecular markers required to be screened and validated for their association with drought tolerant traits.

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APPENDIX I

Meteorological data of the experimental area during 2015 at GKVK, Bengaluru

Year/ Months	Rainfall (mm)			Mean temperature (°C)						Mean Relative Humidity (%)			Mean Sunshine hours (hr day^{-1})			Mean wind speed (km hr^{-1})			Pan evaporation (mm day^{-1})		
				Maximum			Minimum														
	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D
January	1.3	10.0	8.7	27.5	27.4	-0.1	14.0	15.2	1.2	86.0	91.0	5.0	8.9	8.2	-0.7	6.9	6.7	-0.2	5.2	3.8	-1.4
February	9.6	0.0	-9.6	29.8	29.9	0.1	15.3	15.3	0.0	80.7	86.0	5.3	9.5	12.6	3.1	6.8	7.7	0.9	6.3	5.8	-0.5
March	12.9	21.2	8.3	32.6	32.2	-0.4	18.0	18.9	0.9	75.3	85.0	9.7	9.4	8.7	-0.7	6.8	8.0	1.2	7.7	6.7	-1.0
April	47.6	142.1	94.5	33.8	32.1	-1.7	20.5	21.0	0.5	79.3	84.0	4.7	8.9	7.9	-1.0	6.7	6.3	-0.4	7.7	5.8	-1.9
May	99.3	140.2	40.9	33.1	31.4	-1.7	20.5	20.6	0.1	81.5	87.0	5.5	8.3	6.8	-1.5	8.2	6.6	-1.6	7.2	4.3	-2.9
June	76.3	100.0	23.7	29.6	29.1	-0.5	19.6	19.8	0.2	85.8	90.0	4.2	5.8	5.2	-0.6	12.9	10.2	-2.7	6.1	3.7	-2.4
July	101.3	66.0	-35.3	28.2	29.3	1.1	19.1	19.5	0.4	88.1	91.0	2.9	4.4	6.2	1.8	12.3	9.3	-3.0	5.4	3.9	-1.5
August	134.3	71.0	-63.3	27.6	29.3	1.7	18.9	19.6	0.7	89.0	92.0	3.0	4.7	5.7	1.0	10.5	8.3	-2.2	4.9	3.8	-1.1
September	194.9	254.6	59.7	28.1	28.7	0.6	18.8	19.2	0.4	88.9	91.0	2.1	5.8	5.5	-0.3	7.1	6.0	-1.1	5.1	4.0	-1.1
October	167.9	80.8	-87.1	27.8	29.3	1.5	18.3	19.1	0.8	88.0	89.0	1.0	6.0	6.6	0.6	5.6	5.8	0.2	4.7	4.5	-0.2
November	53.5	180.4	126.9	26.7	25.3	-1.4	16.6	17.9	1.3	87.0	94.0	7.0	6.2	3.3	-2.9	6.1	7.2	1.1	4.5	2.8	-1.7
December	11.7	4.2	-7.5	26.2	27.6	1.4	14.5	19.9	5.4	86.7	91.0	4.3	7.3	6.5	-0.8	7.0	6.4	-0.6	4.6	3.4	-1.2
Total/Mean	**910.6**	**1070.5**	**159.9**	29.3	29.3	0.1	17.8	18.8	1.0	84.7	89.3	4.6	7.1	6.9	-0.2	8.1	7.4	-0.7	5.8	4.4	-1.4

Note: N- Normal meteorological data (mean of 1976 – 2014), A - Actual meteorological data, D - Deviation from the normal (A-N)

APPENDIX II

Meteorological data of the experimental area during 2016 at GKVK, Bengaluru

Year/ Months	Rainfall (mm)			Mean temperature (°C)						Mean Relative Humidity (%)			Mean Sunshine hours (hr day^{-1})			Mean wind speed (km hr^{-1})			Pan evaporation (mm day^{-1})		
				Maximum			Minimum														
2016	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A	D
January	1.4	2.4	1	27.5	27.5	0.0	14.0	14.7	0.7	86.0	90.0	4.0	8.8	7.6	-1.2	6.8	6.5	-0.3	5.1	4.2	-0.9
February	9.0	0.0	-9	29.8	31.2	1.4	15.3	16.2	0.9	80.7	85.0	4.3	9.4	9.0	-0.4	6.8	7.1	0.3	6.2	5.2	-1.0
March	16.9	4.2	-12.7	32.6	34.0	1.4	18.0	19.9	1.9	75.3	86.0	10.7	9.3	8.2	-1.1	6.8	6.8	0.0	7.6	6.9	-0.7
April	48.2	1.2	-47	33.8	35.8	2.0	20.5	23.1	2.6	79.3	84.0	4.7	8.9	9.1	0.2	6.7	6.6	-0.1	7.7	8.6	0.9
May	100.7	112.6	11.9	33.1	33.1	0.0	20.5	21.4	0.9	81.5	88.0	6.5	8.2	7.8	-0.4	8.1	7.4	-0.7	7.1	6.3	-0.8
June	78.6	141.4	62.8	29.5	28.4	-1.1	19.6	19.9	0.3	85.8	92.0	6.2	5.7	4.7	-1.0	12.8	9.1	-3.7	6.0	3.0	-3.0
July	101.9	268.2	166.3	28.1	27.6	-0.5	19.1	19.3	0.2	88.1	94.0	5.9	4.3	4.1	-0.2	12.2	9.3	-2.9	5.3	3.4	-1.9
August	130.4	27.2	-103.2	27.6	28.1	0.5	18.9	19.5	0.6	89.0	92.0	3.0	4.7	5.7	1.0	10.4	9.2	-1.2	4.8	4.0	-0.8
September	197.4	44.4	-153	28.0	27.8	-0.2	18.8	19.0	0.2	88.9	92.0	3.1	5.7	3.5	-2.2	7.1	7.0	-0.1	5.0	3.4	-1.6
October	165.5	30.2	-135.3	27.8	29.6	1.8	18.2	18.0	-0.2	88.0	85.0	-3.0	6.0	7.9	1.9	5.5	4.6	-0.9	4.7	4.9	0.2
November	58.5	0.0	-58.5	26.7	29.6	2.9	16.5	16.2	-0.3	84.7	82.0	-2.7	6.2	8.5	2.3	6.1	6.2	0.1	4.5	4.6	0.1
December	11.9	63.5	51.6	26.2	27.2	1.0	14.5	14.7	0.2	84.4	85.0	0.6	7.2	6.9	-0.3	6.9	6.4	-0.5	4.5	3.9	-0.6
Total/Mean	920.4	695.3	-225.1	29.2	30.0	0.8	17.8	18.5	0.7	84.3	87.9	3.6	7.0	6.9	-0.1	8.0	7.2	-0.8	5.7	4.9	-0.8

Note: N- Normal meteorological data (mean of 1976 – 2015), A - Actual meteorological data, D - Deviation from the normal (A-N)

APPENDIX III

Mean performance of rice genotypes in field experiment (Season-I and Season-II)

Genotypes	PH	TNT	NPT	PL	DFF	DM	BM	HI	GY/P
Season I									
1	79.8250	18.4500	17.1500	22.4000	98.1250	123.8750	51.8013	3.4046	15.6148
2	119.9750	6.9750	6.1000	23.6508	114.3750	133.2500	67.8585	4.2824	16.5210
3	131.5000	14.2000	13.8750	26.4583	106.3750	138.1250	61.1990	3.9103	15.7520
4	87.5250	19.9250	18.6500	25.5750	99.0000	128.8750	57.4373	2.8041	21.2280
5	114.7250	14.3250	12.7500	22.6333	84.1250	111.6250	74.7360	5.0166	15.3880
Season I									
1	84.1500	20.9000	20.2500	24.4350	101.7500	137.2500	50.3195	3.5325	14.7955
2	118.0500	7.1500	6.5500	26.7350	111.0000	143.0000	67.2725	5.0750	13.2150
3	135.1000	15.2000	15.0500	29.5150	97.5000	132.2500	60.0200	3.8175	15.7925
4	89.8500	23.0500	22.2000	27.3675	94.0000	114.0000	56.4825	2.5350	22.4075
5	124.0500	14.3500	13.1500	22.2150	84.5000	113.0000	74.4400	5.2300	14.3575

Legend: 1-ARB 6; 2-Moroberekan; 3-AM 65; 4-AM 72; 5-MTU1001

APPENDIX IV

Mean performance of sorghum genotypes in field experiment (Season-I and Season-II)

Genotypes	PH	EHL	SL	SW	DFF	DM	BM	HI	GY/P
Season I									
1	230.7000	23.3000	207.4000	1.3300	73.2500	155.7500	124.2500	2.7775	45.3800
2	164.4000	29.3000	135.1000	12.0775	77.0000	159.0000	118.2500	1.2400	95.8100
3	152.9500	29.5500	123.4000	12.2950	63.5000	143.0000	150.5000	1.2125	124.0600
4	167.4000	24.2500	143.1500	12.1475	61.0000	129.5000	134.2500	1.1875	113.4225
5	162.5000	29.9000	132.6000	12.3265	66.2500	154.7500	140.0000	1.3025	107.9200
Season I									
1	223.1000	21.3500	204.6000	1.2525	76.7500	158.5000	118.2500	3.7175	31.8975
2	161.9500	27.3500	132.0500	1.1550	79.5000	161.0000	114.0000	2.4075	81.0100
3	150.2500	27.5000	123.0000	1.1525	65.7500	145.7500	139.5000	1.1650	121.9225
4	164.8000	22.0500	141.1000	1.1700	63.0000	133.2500	126.5000	1.2175	105.3475
5	160.7000	27.9000	130.0000	1.1850	70.0000	156.5000	139.5000	1.3525	104.1550

Legend: 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

APPENDIX V

Mean performance of root and its associated traits of rice genotypes in PVC pipe experiment during *Kharif*-2015 and summer-2016

Genotypes	RL	RN	RV	RFW	RDW	TPL	RSLR
Season I							
1	42.7500	63.1250	28.2500	55.9313	13.7650	95.5000	0.5371
2	57.1250	94.8750	62.5000	62.2738	15.5150	132.6250	0.4798
3	51.7500	84.1250	42.0000	39.9750	11.8888	109.1250	0.3954
4	55.6250	93.5000	50.0000	73.7913	19.2513	138.5000	0.6388
5	84.1250	102.0000	68.7500	79.8550	21.1813	169.0000	0.7291
Season I							
1	42.5000	48.7500	22.7500	20.1500	10.4475	88.7500	0.5050
2	55.5000	59.7500	71.2500	41.6600	12.1275	127.7500	0.4700
3	50.0000	69.7500	39.5000	25.6700	10.8325	101.2500	0.3725
4	55.0000	113.2500	60.0000	29.4275	10.4275	132.0000	0.6150
5	97.7500	74.0000	103.7500	22.5375	18.4000	178.5000	0.7900

Legend: 1-ARB 6; 2-Moroberekan; 3-AM 65; 4-AM 72; 5-MTU1001

APPENDIX VI

Mean performance of root and its associated traits of sorghum genotypes in pipe experiment during *Kharif*-2015 and summer-2016

Genotypes	RL	RN	RV	RFW	RDW	TPL	RSLR
Season I							
1	152.5000	128.2500	29.2500	44.2500	15.1550	330.7500	0.6599
2	80.5000	69.5000	28.2500	55.5000	16.3275	247.0000	0.4938
3	41.5000	101.2500	51.7500	48.2500	15.1325	181.0000	0.2710
4	44.7500	44.5000	33.2500	55.2500	20.8025	189.7500	0.2663
5	59.7500	96.2500	58.7500	53.0000	16.4800	205.7500	0.3676
Season I							
1	146.5000	163.7500	212.5000	32.0000	15.2500	303.2500	0.6600
2	154.2500	144.5000	320.0000	48.5000	18.5000	273.7500	0.9575
3	153.0000	126.7500	330.0000	38.7500	14.7500	294.7500	1.0225
4	112.5000	121.7500	232.5000	44.5000	20.5000	206.5000	0.6900
5	141.5000	175.7500	475.0000	43.7500	19.2500	274.2500	0.8800

Legend: 1-MLHT, 2-Roagro, 3-SJH-1, 4-CSH-14, 5-Dhanvi

APPENDIX VII

Sequence amplified by RM166

ARB6

GCGTTTGGTCTACTGCACTTGCATCAGTCCTATAGTTGTGCTCATTCATCTGGT
TATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAAGG
AGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCTA
AGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATGTCA
TCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGTA
CCGGTTTAGGATCATGCAGCAAAATAAATCTCCTTTTACTAATTTTACTACAA
ATTTGTTAGATACAAACTTTACTCTGGATCACCAGATGAATGAGCACCCCTTT
GGGACGGATGCACGCCTTTTACTTAAAGATACGGGTAACCCAATTATTGAGC
CAAGACCATTTTT

Moroberekan

GACGGTCATGTACTACTGCACTTGCATCAGTCCTATAGTTGTGCTCATTCATC
TGGTTATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAA
AGGAGTTTTTTTTTTTTTTTGCGGCAGGGGATCGAAAACCCTTTATCCAAATT
AAAACTTTTCAAACCAGGAAAAAAGTAGAAACCCTATAAAAACTAAAAAAA
GGGTTTTTTTTTTGCGGCAGGGGACCGAAAACCCTTTATCCAAATTAAAACTT
TTCAAACCAGAAAAAAAGAAAAAACCCAATTACCCCCCCCTGGCGGGCCAA
AAGGCCCCCGGACCATTAGGCAACCGGCAAAATTTTTTGTTTTTTTTCCCAAA
CGGGAAACCCGGTTTAGGAACGCCCAAAAAAAAAAACCCCCCCCTTTTCCTT
TTTTTAAGGGGACCCCACTTTTTTCGCGGCCAAAATTTCTCCTATTTAGCCAA
AGGATTTCAAATCCCTGGGCAAAAAAAAAAAAAAACCTCCCTATTTATTTTTT
TCCCCAAATTGTGAGCACCCACACATCACTCGA

AM65

GCGTTAGTTCTACTGCACTTGCATCAGTCCTATAGTTGTGCTCATTCATCTGGT
TATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAAGG
AGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCTA
GCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATGTCAT
CTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGTAC
CGGTTTAGGATCATGCAGCAAAATAAACCTCCTTTTACTATTTTTACTACTAA
TTTGTTAGATACAAACTTTACTCTGGATAACCACATGAATGAGCACAACTTTG
GGACGGATGCACGCCTTTTACTTAAAGATACGGGTCACCCAATTATGGACCC
AAGAACATTTT

AM72

GACGTTAGGACTACTGCACTTGCATCAGTCCTATAGTTGTGCTCATTCATCTG
GTTATCCAGAGTAAAGTTTGTATCTACAATTTAGTAGTAAAACTAGTAAAAG
GAGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCT

AAGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATGTC
ATCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGT
ACCGGTTTAGGATCAATGCAGCAAATAAAAACCTCCTTTTACTAATTTTACTA
CAAATTTGTTAGATACAAACTTTACTCTGGATAACCAGATGAATGAGCACAA
CTTTGGGACTGATGCACGCCTATTACTTAAAGATACGGGTAACCCAATTATTG
ACACAAGACCATT

MTU1001

CTGATTGCATCAGTCCTATAGTTGTGCTCATTCATCTGGTTATCCAGAGTAAA
GTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAAGGAGTTTTTTTTGCTG
CAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCTAAGCATGAATAAAG
ATGAATCCTATTATCCCCATCTTGCATGTCAATATGTCATCTGATCATTATGCT
AACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGTACCGGTTTAGGTTCA
TGCAGCAAAATTTAATTTTATAAATTTTTTAATAAAAATTTACTT

MLHT

GGCATGCATCAGTCCTATAGTTGTGCTCATTCATCTGGTTATCCAGAGTAAAG
TTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAAGGAGTTTTTTTTGCTGC
AAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCTAAGCATGAATAAAGT
ATGAATCCTATTATCCCCATCTTGCATGTCAATATGTCATCTGATCATTATGCT
AACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGTACCGGTTTAGGATCA
TGCAGCAAAATTTTTTTTTTTTTTTTTATATACAAA

Roagro

GACGTTAGTCTACTGCGCTTGCATCAGTCCTATAGTTGTGCTCATTCATCTGG
TTATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAAG
GAGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTCT
AAGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATGTC
ATCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAGT
ACCGGTTTAGGATCATGCAGCAAAAAAAAAACTCCTTTTACTAGTTTTACTAC
TAATTTGTTAGATACAAACTTTACTCTGGATAACCAGATGAATGAGCACAACT
TTGGGACTGATGCACGCCTTTTACTTAAAGATACGGGTCACCCATTTGTTGAC
CCAAGACCA

SJH-1

CCCGTTCTGGTCTACTGCGCTTGCATCAGTCCTATAGTTGTGCTCATTCATCTG
GTTATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAAA
GGAGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTTC
TAAGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATGT
CATCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGAG
TACCGGTTTAGGTCATGCAGCAAAAAAAAAACTCCTTTTACTAATTTTACTAC
AAAATTGTTAGATACAAACTTTACTCTGGATAACCAGATGAATGAGCACAAA

CTTTGGGACTGATGCACGCCTTTTACTTAAAGATACGGGTCACCCAATTATAG
ACCCAAGAACAT

CSH-14

CCCGTTCCAGCACTACTGCGCTTGCATCAGTCCTATAGTTGTGCTCATTCATCT
GGTTATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAA
AGGAGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTT
CTAAGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATG
TCATCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGA
GTACCGGTTTAGGATCATGCAGCAAAAAAAAAACTCCTTTTACTAATTTTACT
ACAAATTTGTTAGATACAAACTTTACTCTGGATACCAGATGAATGAGCACAA
CTTTGGGACTGATGCACGCCTTTTACTTAAAGATACGGGTAACCCAATTTTTG
ACCCAAGACCATCC

Dhanvi

CCCGTTCCACCTCTGCTGCGCTTGCATCAGTCCTATAGTTGTGCTCATTCATCT
GGTTATCCAGAGTAAAGTTTGTATCTAACAATTTAGTAGTAAAACTAGTAAA
AGGAGTTTTTTTTGCTGCAAGAGATCTGAAATCCTTTAGTCATTAAGACATTT
CTAAGCATGAATAAAGTATGAATCCTATTATCCCCATCTTGCATGTCAATATG
TCATCTGATCATTATGCTAACTGCAAAACTTTCTGTTATTCTTTCCATACTGGA
GTACCGGTTTAGGATCATGCAGCAAAAAAAAAACTCCTTTTACTAGTTTTACT
ACAAAATTGTTAGATACAAACTTTACTCTGGATAACCAGATGAATGAGCACA
ACTTTGGGACTGATGCAGGCCTGTTACTTAAAGATACGGGTAACCCAATTGTT
GACCCAAGACCA

APPENDIX VIII

Sequence amplified by RM149

ARB6

GGGGCAGCTCTATTCACTTCATAACTCATAACTCATTTGTCACACGCACATTT
CTCAAACTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATATTGTTTT
AAAAATCATATTAATATGTATATATATATATATATATATAAGTTTTTTAATTG
ATACTTAATTAATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAA
GGCTTCCAACACAAAA

Moroberekan

GGCAAGGCTCATTCAACTTCATAACTCATTTGTCACACGCACATTTCTCAAAC
TATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATATTGTTTTAAAAAT
CATATTAATATGTATATATATATATATATATAAGTTTTTTAATTGATACTTAATTA
ATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAAGGCTTCCAACA
CA

AM65

GGGCAAGGCCCTATTCCACTTCATAACTCATAACTCATTTGTCACACGCACAT
TTCTCAAACTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATATTGTT
TTAAAAATCATATTAATATGTATATATATATATATATATATATAAGTTTTTTAATT
GATACTTAATTAATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAA
AGGCTTCCAACACAACGGCTTGGTTTTGGTTTTTCTTTCCGTCGGGGGTCCGG
TTTAGGACCTGCCGCCAAAAACCCCCCGAGACTACCCA

AM72

CCTGGGCCTGCATACCTACATAGCGGGTTATACTCTAAGCATATGTACGGCGC
TTTCTCGGCTATTAGCGTAACTTGGATGTTTGAAGTTTTTGAGGAATATTGTTT
TAGAAAACATTGTAATCTGTATATAAACTTAGATATATATATTTATATAAAAA
TTTTTTTTTGTCAACATATGATACTTTGAGAAGACTGAACGGTGGAGTGGGGA
AAGGATGAACAGGAGGGATAACAAAATCCGCCCCCAATGCATGTGGGGCGG
CATCAAACAAAAAATAGATTTACATACAAATTCATACTGAGATAATTTGTTA
CTCTCGGCTTGCCGCGACTAGAGC

MTU1001

GCGCAAGCTCTATTCACTTCATAACTCATAATCGTCATCTCTGCACACTCTTC
ACATTTCATCAAACCATCATTCGTTATCTTGTATCTTTTAAATTAAAGTAAAA
TATAAGATTCAAAAAACAAAGTAAAAAGAAAAAAAAAAAATATATATATAA
CTTTTTTTTTTTTTATCTTGTCAATCGGGTGTTTTGTTTTTTGTGTTTCGAGTAA
GAGCAACAAACACACCCAACACA

MLHT

GGGGAAGGTAGATAGCAGCTCATCACGATCATACTCATTTGTCGCACGCACA
TTTCTCAAACTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATGTTGT
TTTAAAAATCATATTAATATGTATATATATATATATATAAAAGTTTTTTAACTGA
TACTTAATTAATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAAG
GCTTCCAACCAA

Roagro

CGGGGAAGGTTTGCATAGCACTCAGTGCTCATAACTCATTTGTCGCACGCAC
ATTTCTCAAACTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATGTT
GTTTTAAAAATCATATTAATATGTATATATATATATATATAAAAGTTTTTTAACT
GATACTTAATTAATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAA
AGGCTTCCAACCAA

SJH-1

GGCCCAGCTCCATTCACCTTCATAACTCATTTGTCGCACGCACATTTCTCAAA
CTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATGTTGTTTTAAAAA
TCATATTAATATGTATATATATATATATATAAAAGTTTTTTAACTGATACTTAATT
AATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAAGGCTTCCAAC

CSH-14

GGCTAGCTGCATCTCCTTCATAACTCATTTGTCGCACGCACATTTCTCAAACT
ATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATGTTGTTTTAAAAATC
ATATTAATATGTATATATATATATATATAAAAGTTTTTTAACTGATACTTAATTA
ATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAAGGCTTCCAACC
AA

Dhanvi

GGGGGGGTTTGCATAGTGCTCTGTGTTCATACTCATTTGTCGCACGCACATTT
CTCAAACTATCAAACGTAATTTGTTTCTATGAAGTTTTATAAAAATGTTGTTTT
AAAAATCATATTAATATGTATATATATATATATATAAAAGTTTTTTAACTGATAC
TTAATTAATCGTGTCAATAGGTTGTTTTGTTTTGCGTGTTACGAGGAAAGGCT
TCCAACCAAAC

APPENDIX IX

Sequence amplified by RM5844

ARB6

GACATGTACTAATATATAATAATAATAAGTGGATCCACATGTCATCCCTT
CTCTCTCTCGTCCCCTCTTCTTTCTATCTCTTTTTCCTAGTTGGCGGACCTTCGA
CAATGGCTCCTAGCAAGGCGTCGCTTTTCGTCTAACAGTTTCATAACGGGTGT
GAGGGGGTTTTTTTTTTGTGCGTGTTGCGAAGAAAGGCTTCCAAAAAAGTGA
CTTCGCGTGGGGCGAGCACAAA

Moroberekan

ACATCAACTAATAATATAATAATAATAATAATAATAATAATAATAATAATAA
TAATAATAATAATAAGTGGATCCACATGTCATCCCTTCTCTCCCTCGTCCCCT
CTTCTTTCTATCTCTTTTTCCTAGTTGGCGGACCTTCGACGATGGCTACTAGCA
AA

AM65

GAAATCAACTAATAATAATAATAATAATAATAATAATAATAATAATAATAAT
AATAATAATAATAATAAGTGGATCCACATGTCATCCCTTCTCTCTCTCGTCCC
CTCTTCTTTCTATCTCTTTTTCCTAGTTGGCGG**ACCTTCGACAATGGCTACTA
GCAAA**

AM72

AGCATGACTAATAATAATAATAATAATAATAAGTGGATCCACATGTCATCCC
TTCTCTCTCTCGTCCCCTCTTCTTTCTATCTCTTTTTCCTAGTTGGCGGACCTTC
GACAATGGCTCCTAGCAAACACAGGATGGATCCTAGCGAAAATAAGAAGAA
TCTGGGGTGGGCTATCCCTGACGGTTTCTGGTTGGAGATTTTTTCTAGAAAGG
GGACTTCGCGTGGGTTTTGCACGT

MTU1001

CCATGGACTATATAATAATAATAATAATAATAATAAGGATAATAAGTTATTA
CTTCTAACTGTCGTCCCCTCTTCTTTCTATCTCTTTTTGTAGTTGGTGGACCTTC
GACAATGGCTCCTAGCGAGGACCTTCAACAACGGCTCCTACCAACAGCA

MLHT

GAAGCTGAGTAGTCTCATCATAATCTTCTATACTAATAATAATAATAATAATA
ATAATAAGTGGATCCTCATGTCATCCCTTCTCTCTCTCGTCCCCTCTTCTTTCT
ATCTCTTTTTCCTAGTTGGCGGACCTTCGACAATGGCTCCTAGCAAAATCAGG
ACTCCGAGCAGATCACAACATCATACAATTGCTTTTGATAATGAAGCCATTG
ATCAACCAACCCCTGAAGGACCTTCGACTATGGCACCTAGCAACAAATTAAA
GTTACTCGGCTACTTGACGACCGGGTCGGAATACAGAACCGAGCACATAGGA
GCCGACGGGAAATCGGTTGATTAGTGATCACCTCTTAGTTGCCGATAAGCAA

ATGATGGTCGTAGTGTGGATTGGCCATCAAGAATGAAAAAAAACGATCGACA
TTTACGAAATTGACAAGGTCCCTCTCACCAACCCAGCGCGCTTTCTGATGTTC
CCCAATGAACCCCACCCATCGCCGACGCAGCGGGTGTCGCGCCTTAATTGTT
GGAACGGCACATTCCCTGGCCAACGAGTCCACTGGCAGCTACCACAATGCAG
ATGAGCCCATTCGCTTCCAACATGGTGGGGGGCAAACTGCTCCACAAGTCCA
CAAAAACAAAAGATAGAAATTCAATGACAAAGATCTTCTAG

Roagro

AACTAGTGTCTCGTCATGGCTCCTAGCTAATAATAATAATAATAATAATAATA
AGTGGATCCTCATGTCATCCCTTCTCTCTCTCCTCCCCTCTTCTTTCTATCTCTT
TTTCCTAGTTGGCGGACCTTCGACAATGGCTCCTAGCAATACAGGACAACGA
GCATGACCAATCTATGAAATAAATGCGTTTGTGATGAGGCCATTGATCCACCT
ACTGTGAAGGCCCCCTAGCCGTGTAACTTGTTAATAGTGGAAGGTACATGGC
TCCTTGACGGAGGGGTCGGTATTCAGGTCTCTTCACCGCCGCCGATGGGAAA
ACAGGGAATTAGCGATCACCTCTTTATCTCCCTGCTGCAAATGCAAGGACGT
AGGTTGGATTTGCCGCTCCAAAAAAAAAAATAAAGTCTGACAATTTACAAAAA
TGGCAAAGGCGCTGGCTTCCACCCAGCCTGTTTTCCGATGGCCCTGAAAATA
CAGCGATACCCCTCCCCAGCAGCGGGGGTTTCCGTTAAATTCTTTGTAACCGG
CTATTCACTGGGCATCGAGTCCACTGGGGCCTACTACAAATGCAAGATCAGC
CCATTCCCTGCCGCCAGGGTGGTAGCAAACTGCTCCGCCAGCCACAAAAACA
AAGCAATGAATTCATGACAAGATCTTCGAGCCCTGTTTGAACTTGTGTAATTA
ATGTTACCTAGCGTTACATAGACACTGGGTCGCTAGCATCTTTGTTTCGCTCC
ACCCCCA

SJH-1

CGAAATCTAACTGTCTCATCATAATAATATATACTAATAATAATAATAATAAT
AATAATAAGGTAGGATCCACATGTCATCCCTTCTCCCCCTCCTCCCCTCTTCTT
TCTATCTCTTTTTCCTAGTTGGCGGACCTTCGACGATGGCTCCTAGCAACATC
AGGACTGTGTGTATGTTGTTGTATGAAATAATTGCGTGTAAAGAGGAGGCCA
TTGAGCCAGAAACCACTGCGGGCCCTTGGGCATGGGAAGTTGCCAACAAATG
AAATTTCACGGCTCCTTGACGGCCGGGGCGGCATACAGAACCGGGGAACCGG
GGCCGATGGGCATTCAGTTGAAAGGGATCACCTGTCAACCTCCGAGCAGTAG
ATAAAAGGTGGTAATGTGGATCTTCCGCTGAAAAAGAAAATAAACATCAGCC
AATTCACAAAAACGGAAAGGGCCCTTTCTTCAACACACCGTGTTGTCCGATG
GGGCTAATAGTACCGGCCCCCCCGGCGCAGCAGCGGATGTTGCGCTTAAATT
CTTTGGGACGGGGCTCTTCGCTGGACAACGTGGCCATAGGCAGCCTACTACA
AAGGCAAGACGAGCCCGTTCGCTGCCGTCGGGGTGGGAGGGAACTGCTCCCC
TAGGTCTCCA

CSH-14

TGGACTCAAGTAGTAATCATCATAAGAATATATACTAATAATAATAATAATA
ATAATAATAAGGTAGGATCCACATGTCATCCCTTCTCTCTCTCCTCCCCTCTTC
TTTCTATCTCTTTTTCCTAGTTGGCGGACCTTCGACGATGGCTCCTAGCAAAA

GAGGAAATGGGTATATAGAATTAACCGGGGCATTTGGGAGGAGTACGTTGAG
CCACATGTTGAGGAACCACGGTTGGGTCTCCTTGTTGGCAATGGGTTCACCAA
AAAGAAGTTTCTCCGTCCCTTGATGGGGGAGATGATAGACGGAGCCGAGCAA
TTTTGGGCCTTAAGGGAATCGGCCATCCTCGGCTTACCTATCGGCAGCCAATA
ATTAGACAGAATTTGGTTCTACCAATCCGACAAAAAAAAAGAATCTTGTCTA
CTCACTTGTTCAAAAAA

Dhanvi

TGGCGGTGAGGTGGGCTCATCATGGCTCCTAGCTAATAATAATAATAATAAT
AATAATAAGTGGATCCCATGTCATCCCTTCTCCCCCTCCTCCCCTCTTCTTTCT
TTCTCTTTTTCCTAGTTGGCGGACCTTCGACGACGGCTCCAAGCAACACAAAA
ATGGGGAAGATCACATATCTACATTTGCGCTTGAGATGAAGCAATTGATCAA
GCAACAATGAATGACCTTCGACATTGGCACCTACCAATAGTAGAAGTTTCAT
GGCTACTAGAGGACGGAGGGGGCAAACATAACGACGGCACCGCAGCCGTCA
GGAAATCAGCGCTAAAGCGATCACCGCTCTATAGCCGAGCGGCAAAAACGA
GGAGGTCGTGCTGATTTTCCATCACGCAGAAATTTTCCGTTTCCTATTTACGA
AATTGACACGTTCGCTGTCACGAACCCACCCCGCTGTGCTAGGTTTCCGATGT
ACAGGCACCAACCGACGCCGAACGGGTGTTTCTTTTAATTCTGGGTAACGGA
CTAATCCATTGCCATGGTGTACAATGGCGCCTATCACTAGGGCCAGACTAGTC
ATACA

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Transferability of Rice SSR Markers to Sorghum

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ABSTRACT

In the present investigation, five genotypes each from rice and sorghum were evaluated for marker transferability. Upon PCR amplification, twenty markers distributed evenly on rice chromosomes, were separated on Agarose Gel. It was observed that nine rice primers amplified in sorghum. This showed that rate of transferability (45.0 %) of rice primers among sorghum genotypes. Hence, screening existing markers through transferability test from closely related species or family is resource efficient.

Keywords: Genome, molecular markers, *oryzasativa*, sorghum, transferability

SORGHUM and rice belongs to family Poaceae. *Sorghum bicolor* is a widely grown cereal crop, particularly in Africa, ranking 5th in global cereal production. Sorghum's genome is relatively small (~730 M) and simple (10 chromosomes, diploid) compared to other C_4 crops in the Poaceae subfamily, such as maize and sugarcane (Dillon *et al*., 2007 and Luo *et al*., 2016).

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world. Rice, wheat, and maize together account for about half of the world's food production and rice itself is the principal food of half of the world's population (Joshi *et al*., 2010). Rice is the obvious choice for the first whole genome sequencing of a cereal crop. The rice genome is well mapped and well characterized, and it is the smallest of the major cereal crop genomes at an estimated 400 to 430 Mb. The next largest genome of an important cereal crop isthat of sorghum at 750 to 770 Mb and the wheat genome is nearly 37 times the size of the rice genome at close to16,000 Mb (Chapman *et al*., 2015).

It has been estimated that rice diverged from the common ancestor of sorghum and maize approximately 50 million years ago (Paterson, 2004). Sorghum-rice alignments based on the completely sequenced *S. bicolor* and *O. sativa* genomes, demonstrate high levels of DNA conservation between the two species. In addition, the number and sizes of sorghum gene families are similar to those of Arabidopsis and rice. It has been observed that 39.9 per cent of rice sorghum aligned sequences are conserved at the 70 per cent / 100 bp level and 77.5 per cent of the length of sorghum exon sequences overlap with those of rice (Yu *et al*., 2002; Matsumoto *et al*., 2005; Paterson and Bowers, 2009). However, the number of rice SSR markers that have been transferred into sorghum is still limited, and there is a lack of systemic surveys from different rice chromosomes.

Due to their ubiquitous distribution in genomes, Simple Sequence Repeats (SSR) markers showed a high transferability among common cereal species. The transferability of SSR markers across species orgenera has been reported in several cereal cropssuch as rice, wheat, barley, sorghum, maize and bitter gourd (Pandian *et al.,* 2000; Cordeiro *et al.,* 2001; Gupta *et al.,* 2003; Thiel *et al*., 2003; Wang *et al*., 2005; Zhang *et al*., 2005; Tang *et al*., 2008; Singh *et al*., 2013 and Saxena *et al*., 2015). The transferability of Rice SSR to Bamboo genera has been established by Chen *et al.,* 2010. Thus, developed SSR markers from the model crops could also be transferred to other cropsystem for sustaining beneficial agronomical traits.Therefore, transferable SSR markers from the complete sequenced rice genome would be useful for genetic analyses in sorghum.

Several studies have been conducted on the transferability of SSR markers across species or genera of several cereal crops which belong to grass family such as rice and wheat (Varshney *et al.,* 2005);

rye and triticale (Kuleung *et al*., 2004); barley (Thiel *et al*., 2003); sugar cane (Cordeiro *et al*., 2001; Banumathi *et al*., 2010), sorghum (Savadi *et al*., 2012) major cereal to minor grass (Wang *et al*., 2005) and pearl millet (Yadav *et al*., 2008). There are also reports about transferability of SSRs from cereals such as rice and sugar cane to bamboo species (Sharma *et al*., 2008). Sharma *et al.* (2009) also conducted an experiment on identification and amplification of EST-SSR markers in different bamboo species. However, there has been no report on transferability of rice SSR markers to sorghum. The objective of this study was to perform the transferability test of SSR markers from rice to sorghum and to employ the transferable markers in the genetic diversity analysis of selected sorghum genotypes.

Material and Methods

Plant materials and experimental conditions : The present experiment was conducted during *Kharif*-2015. The experimental material for this investigation consists of five genotypes each from riceand sorghum (Table I). They were grown at field of aerobic rice laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru in Randomized Complete Block Design (RCBD) with three replications. Total genomic DNA was isolated from young leaves of 21 days old plants using cetyltrimethylammonium bromide (CTAB) procedure, as described by Doyle & Doyle (1990).

Table I

List of rice sorghum varieties used in present study

Genotype	
Rice	Sorghum
AM 65	CSH-14
AM 72	DHANVI
ARB 6	MLHT
MOROBEREKAN	ROAGRO
MTU1001	SJH-1

Selection of rice SSR markers : Selection of rice SSR markers information on rice SSR markers was derived from McCouch *et al*. (2002) and the Gramene website (http://www.gramene.org). Total of twenty SSR markers were used to screen for transferability. The details of SSR primers used are presented in Table II.

PCR amplification : Primers developed for rice SSR by McCouch *et al*. (1997) were used for the PCR reactions with the DNA of the five sorghum species. To standardize the PCR conditions, the annealing temperature of each rice SSR marker used with sorghum DNA was the same as that originally used with rice DNA (McCouch *et al*., 2002).

PCR amplification reactions were done in 10 µl reaction mixtures, containing 2 µl of template DNA (50ng/µl), 0.5 µl of each forward and reverse primer (5pmol), 4 µl of PCR master mix and 3 µl ddH_2O. PCR cycling consisted of initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing (according to different SSR primer pairs) and primer elongation at 72 °C for 1 min followed by afinal extension at 72 °C for 10 min. Amplified products were stored at -20 °C until further use. PCR amplification of the markers was carried out in Mastercycler® Nexus Gradient, Eppendorf. Agarose electrophoresis was done with 3 per cent gels to visualize the amplicons and then photographed using Alpha Innotech gel documentation instrument.

Among the markers SSRs are widely used in genetic diversity and parental analysis owing to their co-dominant, high reproducibility, abundance in the genome and transferability across species or genera. The development of these markers for a species might be costly and time consuming. Hence, screening existing markers through transferability test from closely related species or family is resource conscious.

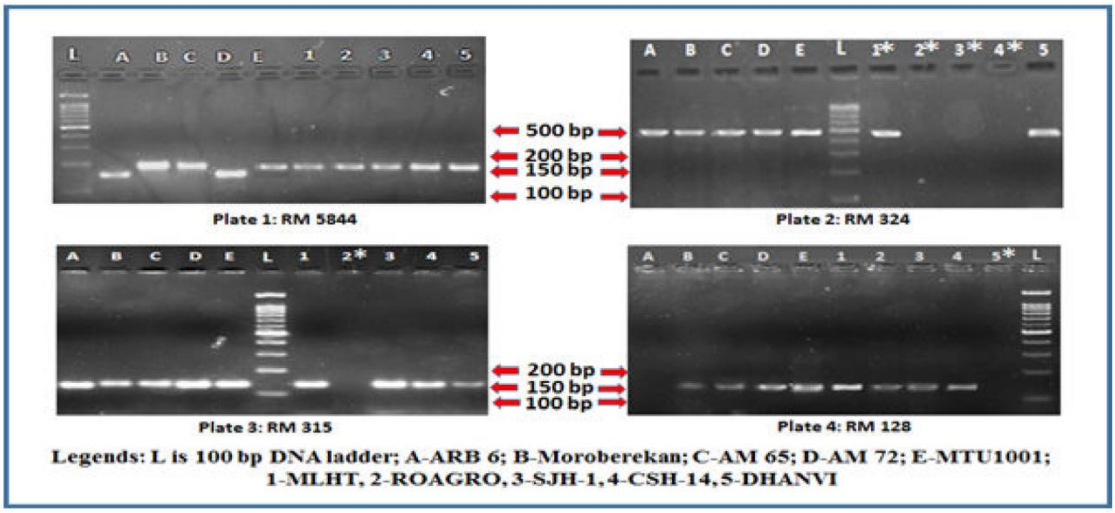
Results and Discussion

Among the twenty markers used in present study, as expected, most of them showed amplicons in all the genotypes of rice. However, only nine primers *viz.*, RM166, RM234, RM315, RM324, RM318, RM128, RM5844, RM140 and RM348 showed amplification in sorghum.

TABLE II

List of selected rice SSR markers tested for their transferability to sorghum

Primer	Chromosome No.	Forward (5' --3')	Reverse (5' --3')	Optimized Annealing Temperature (°C)	Expected Product size (bp)	Repeat motif
RM157	1	CCTCCTCCTCACGAATCCCGCC	GGGCTTCTTCTCCGCCGGCTTC	60	106	(CT)11(TC)10
RM315	1	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	56.6	133	(AT)4(GT)10
RM128	1	AGCTTGGGTGATTTCTTGGAAGCG	ACGACGAGGAGTCGCCGTGCAG	63.9	148	(GAA) 9
RM140	1	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	64.6	261	(CT)12
RM543	1	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC	55	98	(GCG)10
RM211	2	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	60	161	(TC)3A(TC)18
RM166	2	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	60.3	321	(T)12
RM324	2	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	60	175	(CAT)21
RM318	2	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC	60.3	140	(GT)15
RM563	3	CGACCCTAGGGTTTCTCC	CTCGACGTCGTGGAAAGC	60	185	(CCT)6
RM148	3	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG	59.9	129	(TG)12
RM185	4	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC	64.6	197	(AGG)9
RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	68.4	216	(CT)19
RM518	4	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	60	171	(TC)15
RM1153	4	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	60.3	114	(AG)13
RM348	4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	50.4	136	(CAG)7
RM146	5	CTATTATTCCCTAACCCCCATACCCTCC	AGAGCCACTGCCTGCAAGGCCC	60	345	(CT)11(CT)7
RM5844	5	TGACTAACGTGGCATCCATG	GCTAGGAGCCATTGTCGAAG	60	195	(ATA)22
RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	60.3	156	(CT)25
RM195	8	AGAAAGAGAGGCCGTCGGCGGC	GGGCTCACCCCCAAACCTGCAG	63.9	311	(GA)9(CT)8



* Not amplified

Of the nine amplified SSR markers, two were amplified in all sorghum genotypes (RM348 and RM5844) (Plate 1), whereas, RM324 amplified only in two sorghum varieties (Plate 2) and two were amplified (RM315 and RM128) in four sorghum varieties (Plate 3 and 4). This indicates that the rice SSR primers are operating efficiently in sorghum. Other eleven markers were not amplified in sorghum even after replicating the experiment thrice. Hence, absence of bands is true absence of that particular DNA sequence in the genomic DNA of the respective crop and not due to any kinds of technical error and indicates null allele.

TABLE III

Transferability of rice SSR markers in sorghum genotypes

Sorghum Genotypes	Amplified markers (x)	Transferability* (%)
CSH-14	2	10.0
DHANVI	2	10.0
MLHT	2	10.0
ROAGRO	1	5.0
SJH-1	2	10.0
Total	9	45.0

* Transferability, the ratio of amplified markers (x) / tested markers (n)

Among the selected 20 rice SSR markers, nine SSR markers were successfully amplified in sorghum, which is a transferability of 45.0 per cent (Table III). However, transferability rate differed among sorghum genotypes. The transferability rate obtained in this study was higher than the one reported for pearl millet using sorghum, rice and other cereals (Yadav *et al*., 2008). However, the rate is slightly lower than the rice SSR transferability tobamboo that was reported to be 68 per cent (Chen *et al*., 2010). It is possible to improve the transferability rate of markers by using markersthat were developed from expressed sequences (Kuleung *et al*., 2004).

The transferability (45.0%) in this study was lower than that reported for the transfer of apple SSR markers cross-amplified inpear (100%; Yamamoto *et al*., 2001) and for maize SSR markers to Miscanthus (74.5%; Hernandez *et al*., 2001). However, it was higher than that reported for rice SSR markers amplified in Indian bamboo species (44.9%; Sharma *et al*., 2008) and the intrageneric amplification of SSR markers in the grass family (Kuleung *et al*., 2004; Saha *et al*., 2006) and intergeneric amplification of barley (Thiel *et al*., 2003). The transferability of rice SSR markers into sorghum in our study was lower, possibly due to the lack of sequence similarity in sorghum, which are the primer binding sites for SSRs in rice.

The results of study determines that the rice SSR markers can be a valuable marker source for those

plant species for where little molecular marker information is available. This study also suggests that more rice SSR markers derived from those rice chromosome regions showing a very high transferability should be tested in the future with the aim of obtaining more markers in the sorghum genotypes. Trait specific markers from rice when transferred to sorghum could be used directly for MAS.

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Molecular Marker Based Genetic Diversity Analysis in Rice Genotypes (*Oryza sativa* L.) using SSR Markers

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ABSTRACT

Assessment of genetic diversity is important in plant breeding if there is to be improvement by selection. The role of a broad genetic base and systematically characterized germplasm in the crop improvement of cultivated plants has been well recognized. Genetic variability studies are important in selection of parents for hybridization as sound crop improvement depends upon the magnitude of variability in the base population. In the present investigation five rice genotypes were evaluated for genetic diversity. Upon PCR amplification the alleles were separated on Agarose Gel Electrophoresis system. Initial polymorphism detection was conducted using twenty primer pairs distributed on five rice chromosomes. A total of 65 alleles were detected with an average of 3.25 alleles per locus. The polymorphism information content (PIC) reflections of alleles diversity frequency among the varieties, which is ranged from 0.215 to 0.791, with an average of 0.493. RM 260 was found as the best marker for identification of genotypes as revealed by PIC values. The highly informative markers identified in this study could be utilized in further studies for comparative mapping and marker assisted selection for drought tolerance.

Key words: *Genetic Diversity, SSR, Oryza sativa, Molecular markers*

INTRODUCTION

Rice is the staple food and about half of the world population depends on rice for their survival. It is cultivated in 114 a country across the globe, but 90 per cent of world's rice is grown in Asia. India has the largest area under rice among the rice growing countries in the world and ranks second in production after China. Rice is grown in almost all the states of India, but major rice producing states fall in the regions of middle and lower gangetic plains, as well as the coastal lowlands of peninsular India. Being a leading producer of rice, there is a need of more production per unit area to fulfil the needs of ever growing population. In order to meet this, hybridization is a prime consideration for which the diversified genotypic assessment has to be done.

Genetic diversity is a pre-requisite for any crop improvement program as it helps in estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombinations for further selection and introgressing desirable genes from diverse germplasm[12,29].

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Which is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level[31]. Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space[10].

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. The recent development of DNA markers has provided new opportunities for the genetic improvement of rice cultivars[5]. Satellite loci also known as simple sequence repeats (SSRs) are the most commonly used molecular markers because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective[11]. SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies[19].

Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome[23,28]. In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity[16]. These markers can detect a significantly higher degree of polymorphism in rice[19,20] which becomes ideal for studies on genetic diversity and intensive genetic mapping[7]. They have been used for characterizing genetic diversity in several crop species including sorghum[8,27], maize[25], cotton[15] and wheat[24].

In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species[4,18,26]. Rice microsatellites also have a demonstrated utility for gene-tagging and marker-assisted selection[6] and are polymorphic between[1,23] and within rice varieties[21]. These studies showed that SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes. The important advantages of microsatellites are that they are usually single locus and because of the high mutation rate, are often multi-allelic. Microsatellite or simple sequence repeat (SSR) markers are considered to be appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently. The aims of this study was to investigate the genetic diversity among five rice genotypes using SSR markers.

MATERIAL AND METHODS

The present experiment was conducted during *kharif*-2015 (at Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru, India).

Plant materials and experimental conditions

The experimental material for this investigation consists of five rice genotypes, which are grown at field of aerobic rice laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India in Randomized Complete Block Design (RCBD) with three replications. The studied genotypes details are presented in Table 1.

Table 1: Name, origin, type, pedigree and some features of the studied genotypes

Sl. No	Genotype	Origin	Type	Parentage
1	ARB 6	UASB, India	Indica	Bddha X IR 64
2	Moroberekan	Republic of Guinea	Tropical japonica	IR8-24-6 (M307H5)
3	AM 65	UASB, India	Japonica	Azucena X moroberekan
4	AM 72	UASB, India	Japonica	Azucena X moroberekan
5	MTU1001	India	Japonica	Vajram x MTU 7014

Isolation of genomic DNA

Genomic DNA was isolated from young leaves of 21 days old plants using CTAB method as per the protocol described by Doyle and Doyle[9].

DNA quality estimation

The genomic DNA was quantified spectrophotometrically both at 260 nm and 280 nm wavelengths. The absorbance at 260 nm allows the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg of double stranded DNA. A pure sample of DNA shows the ratio of OD 260/280 as 1.8. Ratios less than 1.8 indicate contamination in the isolation either with phenol or with proteins. The values higher than this indicate the presence of RNA in the isolation.

Normalization of the DNA concentration was done to bring all the DNA concentrations to a relatively equal level (25ng/µl) by appropriate dilutions for PCR reaction. Dilution was done with double distilled sterile water.

SSR markers

Total of twenty simple sequence repeat (SSR) markers were used for studying molecular diversity. The details of SSR primers used are presented in Table 2.

Table 2: Details of the microsatellite primers used in present study

Sl. No	Name	Product size (bp)	Forward primer (5'---->3')	Reverse primer (5'--->3')	Repeat motif
1	RM1261	167	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	(AG)16
2	RM319	134	ATCAAGGTACCTAGACCACCAC	TCCTGGTGCAGCTATGTCTG	(GT)10
3	RM212	136	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	(CT)24
4	RM263	199	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	(CT)34
5	RM324	175	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(CAT)21
6	RM279	174	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	(GA)16
7	RM315	133	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	(AT)4(GT)10
8	RM72	166	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	(TAT)5C(ATT)15
9	RM248	102	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(CT)25
10	RM219	202	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	(CT)17
11	RM260	111	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34
13	RM511	130	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC	(GAC)7
14	RM543	98	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC	(GCG)10
15	RM279	174	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	(GA)16
16	RM321	200	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG	(CAT)5
17	RM566	239	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	(AG)15
18	RM1153	114	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	(AG)13
19	RM246	116	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	(CT)20
20	RM525	131	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	(AAG)12

PCR amplification

PCR amplification reactions were done in 10 µl reaction mixtures, containing 2 µl of template DNA, 0.5 µl of each forward and reverse primer, 4 µl of PCR master mix and 3 µl ddH_2O. Thermal cycler was used with the following PCR profile: an initial denaturation step at 94°C for 5 min, followed by 35 cycles

of denaturation at 94°C for 1 min, annealing at 55°C for 30 seconds and primer elongation at 72°C for 1 min and then a final extension at 72°C for 10 min. Amplified products were stored at -20°C until further use.

The SSR amplification products were separated on 2.5 % agarose gel supplemented with ethidium bromide. The TAE 1X was used as a running buffer and 100 bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for 2 hours. Gels were then visualized and photographed using Alpha Innotech gel documentation instrument.

Data analysis

Polymorphic information content (PIC) values were calculated for each SSR locus based on Anderson *et al*.[2]. The amplified bands were scored for each SSR primer pairs based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. These binary data matrix was then utilized to generate genetic similarity data among the different rice genotypes. Both matrices were then analyzed using the NTSYS pc statistical package version 2.2. The data matrices were used to calculate genetic similarity based on Jaccard's similarity coefficients.

RESULTS AND DISCUSSION

Molecular diversity in rice is done by using SSR markers. It is the complimentary sequences of DNA which lie close to the particular gene or QTL. So by annealing the primer we can amplify our target region close to the gene.

Polymorphism level of among rice cultivars was evaluated by calculating allelic number and PIC values for each of the twenty SSR loci evaluated. A total of 65 alleles were detected at the loci of twenty microsatellite markers across five rice genotypes. The results revealed that all the primers showed distinct polymorphisms among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. Among the polymorphic markers, 6 produced two alleles each, 7 produced three alleles each, 4 generated four alleles each, 2 produced five alleles each and only one produced six alleles (table 3).

The number of alleles per locus ranged from 2 (RM319, RM 212, RM 324 *etc*.,) to 6 alleles (RM260) with an average of 3.25 alleles across the twenty loci. The Value is comparable to 1-8 allele per SSR locus with an average number of alleles of 4.58 per locus for various classes of microsatellite[26].

The amplicon size of all five genotypes for each marker alleles varied from 103-130 bp produced by RM260 and 215 – 262 bp produced by RM566. Of the 65 alleles scored all of 65 were found to be polymorphic. Maximum number of polymorphic alleles (6) was obtained with the marker RM260, while the minimum numbers of polymorphic alleles (2) was obtained by using RM319, RM212, RM324, RM315, RM543 and RM321.

PIC value

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability and it is a reflection of allele diversity and frequency among varieties. PIC values ranged from 0.215 to 0.791 with an average of 0.493 (table 3). The highest PIC value 0.791 was obtained for RM260 followed by respectively RM219 (0.74), and RM72 (0.70). PIC value revealed that RM260 was considered as best marker for five test genotypes. The PIC value observed, are comparable to three previous estimates of microsatellite analysis in rice via 0.34-0.88 [30], 0.20-0.90 with an average of 0.56 [13]. Figure 1 showed gel pictures of amplified fragment using primer designed for the SSR marker RM219 and RM263.

Table 3. Number of alleles, highest frequency allele and Polymorphism Information Content (PIC) Values found among five rice germplasms for twenty SSR markers

SL. No	Primer name	Chromosome number	Amplicon size range (bp)	Allele number	Annealing temperature	PIC value
1	RM1261	12	166 - 189	4	50	0.641
2	RM319	1	140 - 144	2	55	0.370
3	RM212	1	122 - 125	2	55	0.325
4	RM263	2	161 - 178	3	55	0.502
5	RM324	2	135 - 154	2	55	0.370
6	RM279	2	150 - 162	3	55	0.407
7	RM315	1	140 - 144	2	55	0.325
8	RM72	8	151 - 184	5	55	0.700
9	RM248	7	118 - 124	3	55	0.502
10	RM219	9	186- 216	5	55	0.740
11	RM260	12	103 - 130	6	55	0.791
12	RM511	12	129 - 135	3	55	0.407
13	RM543	1	114 - 121	2	55	0.215
14	RM279	2	150 - 162	3	55	0.407
15	RM321	9	196 - 200	2	55	0.325
16	RM566	9	215 - 262	4	55	0.641
17	RM1153	4	119 - 140	3	55	0.407
18	RM246	1	114 - 125	3	50	0.580
19	RM525	2	122 - 152	4	55	0.641
20	RM7	3	159 - 172	4	55	0.57

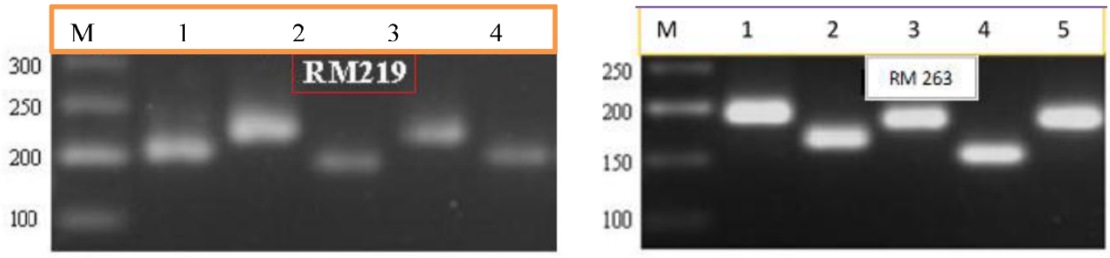

Fig. 1: Agarose gel electrophoresis of PCR amplified fragments for the highest polymorphic SSR markers RM219, and RM263, M is 100 bp DNA ladder; 1-ARB 6; 2-Moroberekan; 3-AM 65; 4-AM 72; 5-MTU1001.

Many studies have been reported on the assessment of genetic diversity in a relatively large set of cultivated germplasm. This include diversity analysis of high yielding cultivars[19], aromatic rice[17], indigenous aromatic rice[14] and even lowland rice[3,32] using various molecular fingerprinting techniques like RFLP, RAPD, SSR, AFLP etc. The informative primers would prove useful in marker-assisted selection, linkage mapping and gene tagging for specialty traits. In the conclusion, SSR markers based molecular fingerprinting could serve as a sound basis in the identification of genetically distant accessions as well as sorting of duplicate germplasm of morphologically close accessions.

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