Phenotyping and molecular marker analysis of selected F_3 segregating lines from aerobic x low land *indica* rice (*Oryza sativa* L.) crosses

By

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CERTIFICATE – I

This is to certify that this thesis entitled, **"Phenotyping and molecular marker analysis of selected F_3 segregating lines from aerobic x low land *indica* rice (*Oryza sativa* L.) crosses",** submitted for the degree of **Master of Science** in the subject of **Molecular Biology and Biotechnology** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Kanika Rani** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course investigation have been fully acknowledged.

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CHAPTER-I

INTRODUCTION

Rice occupies the enviable prime place among the food crops cultivated around the world. It is the staple food of about 3 billion people and its demand is expected to continue to grow as population increases (Carriger and Vallee, 2007). Globally rice is grown over an area of about 165 million ha with an annual production of 750 million tons (FAO, 2015). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's population lives. China and India, which account for more than one-third of global population, produce over half of the world's rice. In India, area under rice cultivation is 43.95 million ha with an annual production of 157.5 million tons (FAO, 2014).Water is a looming crisis due to competition among agricultural, industrial, environmental and domestic users. By 2025 AD, 30% of the human population would be threatened by water scarcity because of the fact that, 70% of water withdrawals is used in irrigated agriculture worldwide (IRRI, 2001).

In Asia, more than 50% of irrigation water is used for rice cultivation. Rice is a heavy consumer of water requiring some 3000 to 5000 L to produce 1 kg of rice (Shen *et al*., 2001). Total water inputs in lowland rice in Asia reportedly vary from 400 mm in heavy clay soils to more than 2000 mm in coarse textured soils with 1300-1500 mm as most average value (Bouman and Tuong, 2001; Tuong *et al*., 2005). A significant portion of the total water requirement for rice production is used for land preparation alone (Bhagat *et al*., 1996). By 2025, it is expected that 2 million ha of Asia's irrigated dry season rice and 13 million ha of its irrigated wet season rice will experience "physical water scarcity," and most of the ~22 million ha of irrigated dry season rice in South and Southeast Asia will suffer "economic water scarcity" (Tuong and Bouman, 2002). In the next 25 years, 15-20 M ha of irrigated rice is projected to suffer from water scarcity particularly wet season irrigated rice in parts of India, China and Pakistan (Carriger and Vallee, 2007).

Of the potential threats to rice productivity, water scarcity and increasing competition for water in irrigated rice systems are perhaps the most pressing in terms of potential impact on overall production levels. Since rice requires more irrigation water than any other grain crops, the increasing water scarcity for agriculture and competition for water from non-agricultural sectors, dictate to an urgent need to improve water productivity in crops such as rice. Water shortage crisis has already reached parts of China as well as central and west parts of India, Pakistan and some parts of Bangladesh. Water-saving irrigation technologies for rice are seen as a key component to deal with water scarcity. In water-short irrigated and rain fed

areas, ways must be sought to reduce water requirements and increase rice productivity. Researchers are developing water-saving technologies, such as alternate wetting and drying, continuous soil saturation (Borell *et al*., 1997), direct dry seeding, ground cover systems (Lin *et al*., 2002) and system of rice intensification (Stoop *et al*., 2002); but it is noteworthy that all these systems use prolonged periods of flooding and hence water losses still remain high.

Aerobic rice is a water-saving rice production system in which potentially high yielding, fertilizer responsive adapted rice varieties are grown in fertile aerobic soils that are non-puddled and have no standing water. The soil is therefore "aerobic" or with oxygen throughout the growing season, as compared to traditional flooded fields, which are "anaerobic". Supplementary irrigation, however, can be supplied in the same way as to any other upland cereal crop (Wang *et al*., 2002; Bouman *et al*., 2005). Hence, shifting gradually from traditional rice production system to growing rice aerobically, especially in water scarce irrigated lowlands, can mitigate occurrence of water related problems. Many countries such as China and Philippines (IRRI) have pioneered in the development of aerobic rice. Growing rice aerobically saves water by: (i) eliminating continuous seepage and percolation (ii) reducing evaporation and (iii) eliminating wetland preparation (Castaneda *et al*., 2002). Aerobic rice shows efficient fertilizer utilization, less incidences of pests/diseases, reduced methane emission, profuse rooting and high tillering, less lodging and high grain and fodder yield, retention of soil structure and quality etc. Thus, aerobic rice cultivation is not only environment-friendly but also saves time, labor, money and water.

Traditionally, rice has been grown under water-limited (non-flooded), aerobic soil conditions in uplands for centuries, but average yields are low (1-2 t /ha), because of adverse environmental conditions (poor soils, little rainfall, weeds etc.), low use of external inputs and low yield potential of upland rice cultivars. Upland varieties have deeper roots than lowland varieties. Plant improvement towards deeper root system would be most effective for better adaptation to occasional or intermittent soil water deficit between irrigation events in rice (Gowda *et al*., 2011). Recently, water efficient 'aerobic rice' varieties have been developed by combining the drought-resistant characteristics of these upland varieties with the high-yielding traits of lowland varieties (Belder *et al*., 2005). In northern China, breeders have produced first-generation temperate aerobic rice varieties with a yield potential of 6 tons per hectare using only 50% of the water used in lowland rice. In the tropics, first-generation aerobic rice varieties are now being produced and initial management recommendations are being developed. In India, the development of aerobic rice varieties was initiated at University of Agricultural Sciences, Bangalore in early 1980s using the available upland paddy and high yielding germplasm and several aerobic rice genotypes were developed using conventional breeding and marker-assisted selection techniques in combination (Girish *et al*., 2006; Toorchi *et al*., 2007).

Molecular markers provide a valuable tool for genetic analysis and plant breeding (Tanksley, 1989). Linkage mapping and marker assisted selection of the target traits can greatly improve the selection efficiency and precision in a rice breeding program. Molecular marker technology can help us not only in the identification of suitable parental lines, diversity and pedigree analyses but also to introgress desirable traits without altering the genetic background. Molecular markers particularly the microsatellite markers (SSRs) have been used to know about the genetic architecture of complex traits in rice based on traditional quantitative trait locus (QTL) & linkage map (Flowers *et al.,* 2000). SSRs are valuable as genetic markers because they detect high level of allelic diversity, are co-dominant, are easily and economically assayed by PCR and are easily automated. The technical efficiency and multiplexing potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies. Over 10,000 microsatellites have already been mapped and developed as molecular markers in rice (McCouch *et al.,* 2002; Temnykh *et al.,* 2001; Wu and Tanksley; 1993).

Root traits are typically complex and controlled by many genes, each with a small identifying genetic variation and QTL for root traits can contribute to our understanding of their role in plant performance under direct seeded conditions. Linkage maps based on molecular markers have been developed for many traits in rice such as drought tolerance (Champoux *et al*., 1995; Price and Courtois, 1999; Henry *et al.,* 2014); drought avoidance by osmotic adjustment (Thanh *et al.,* 2006; Zheng *et al.,* 2006; Uga *et al.,* 2008; Suryapriya *et al.,* 2009) and aroma (Cordeiro *et al.,* 2002). The mapping and tagging of genes with molecular markers is the foundation for marker assisted selection (MAS) in crop plants (Babu *et al*., 2003).

At CCS Haryana Agricultural University, in the Department of Molecular Biology, Biotechnology and Bioinformatics, a breeding program is in progress to transfer the aerobic rice traits from the newly bred aerobic rice varieties (*e.g.* MAS25 and MAS26 developed at University of Agricultural Sciences, Bangalore and MASARB25 developed at IRRI) to some high yielding *indica* and/or important Basmati rice varieties (*e.g.*HKR47, PAU201, Improved Pusa Basmati 1 and Pusa 1121).

Keeping in view the above points, present study was undertaken with the following objectives:

(i) To evaluate selected F_3 segregating lines derived from crosses between aerobic and low-land *indica* rice varieties under aerobic conditions.

(ii) To ascertain the status of molecular markers linked with the aerobic traits in selected F_3 plants.

CHAPTER-II

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is a cereal grain belonging to grass family. Rice is related to other grass plants which produce grain for food such as wheat, oats and barley. Rice plant belongs to the division *Angiospermae*, class *Monocotyledoneae*, order *Glumeflorae*, family *Poaceae* and genus *Oryza.* Originally just two species were cultivated: *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* is native to tropical and subtropical southeastern Asia while *Oryza glaberrima* is native to Africa and less cultivated (Crawford and Shen, 1998). The *Oryza sativa* is the most important with its three subspecies: *indica* (long grain), *japonica* (round grain) and *javanica* (medium grain).

Globally, rice ranks second in only to wheat in terms of area harvested, but in terms of importance as a food crop, rice provides more energy per hectare than other cereal crops. Rice is primarily grown by transplanting of seedling in puddled field which is very cumbersome and labor intensive as it requires 30 man day^{-1} ha^{-1} (Prasad, 2004). Puddling takes up to 30% of total irrigation water application in rice in light textured soils (Aslam *et al.*, 2002). Conventional flooded rice receiving the largest amount of fresh water compared to any other crop is the major contributor to the problem of declining ground water table (0.1 – 1.0 m^{-1}) and increasing energy use (Singh *et al.*, 2002). Asian countries are occupying 89% of the world total paddy area and producing 90% of the total global rice production (FAOSTAT, 2010). In India, rice occupies the largest area among all crops and accounts for as much as 21% of the total cropped area. It has been estimated that world rice production must increase by 30 per cent over the next 20 years to meet projected demands from population increase and economic development.

Increasing scarcity of water for agriculture, caused by increasing urbanization, industrialization, decreasing quality and resources of water has threatened the sustainability of irrigated rice production (Humphreys *et al*., 2005; Peng *et al*., 2009). Drought is one of the main abiotic constraints in rice. Drought is an increasingly important problem limiting rice production in many areas of Asia. Drought naturally affects the rain fed ecosystem because the crop relies strictly on rainfall for its water supply. Yet more rice needs to be produced with less and less water to feed the ever-growing population, this will need judicious water management practices and saving technologies as alternatives to conventional irrigation for rice cultivation.

Aerobic rice culture is one of the most promising alternative water management practices. Aerobic rice is a new type of rice that is aerobic-soil-adapted and input-responsive.

It grows well in non-puddled and non-saturated soils with 70% to 100% of water-holding capacity throughout a growing season. Aerobic rice is specifically developed rice that combines drought tolerance of upland rice with yield potential of lowland rice. Therefore, aerobic rice is considered "improved upland rice" in terms of yield potential and "improved lowland rice" in terms of drought tolerance. 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). While some of these changes can be perceived as positive such as water conservation and decreased methane emissions, there are some negative perceptions also including release of nitrous oxide from the soil, decline in soil organic matter and relatively low yield. The challenge is to develop effective integrated natural resource management interventions which allow profitable rice cultivation with increased soil aeration while maintaining the productivity, environmental protection and sustainability of rice-based ecosystems.

2.1 Effect of water scarcity on rice cultivation

Rice (*Oryza sativa* L.), a model cereal crop with small genome size (389Mb), enriched genetic map, available genome sequence data, large collection of germplasm, is life for more than half of humanity. It is generally grown by transplanting or direct seeding under lowland flooded irrigation system (Bouman and Tuong, 2001; Cantrell and Hettel, 2005). However, irrigated rice is a profligate user of water requiring some 3000 to 5000 L to produce 1 kg of rice (Shen *et al*., 2001). It is common fact that the rice grown under wetland conditions can contribute to the bulk of rice production but consumes huge amount of water and is labor- intensive.

Water is most limited natural resource due to failure of rains and over exploitation of ground water. Increasing scarcity of fresh water threatens sustainability of irrigated rice ecosystem. The situation is further aggravated by drought, global warming, methane emission, adverse climatic changes, over-pumping of ground water causing aquifer resources to decline and the high ‘cost’ of water. It is also reported that the amount of total diverted fresh water used to irrigate rice fields in Asia is about 50%. Seasonal water inputs for puddled transplanted rice vary from 660 to 5,280 mm depending on growing season, climatic conditions, type of soil and hydrological conditions (Tuong and Bouman, 2003).

Transplanting method of rice production has been limited by a number of factors such as water scarcity, high input costs, shortage of skilled labour, suboptimal plant population etc. Transplanting after puddling (a process where soil is compacted to reduce water seepage) has been a major traditional method of rice establishment. But repeated puddling affects soil physical properties adversely by dismantling soil aggregates, reducing permeability in sub-

surface layers, and forming hard-pans at shallow depths which makes land preparation difficult and requires more energy to achieve proper soil tilth for succeeding crops (Gill *et al*., 2014).

Food and water are two of the most important necessities for survival, but, with an increasing demand for food and a looming water crisis, a shortage of both may be on the horizon unless innovative technologies are developed. Water, especially, is fast becoming a precious commodity, as more and more people continue using water for the household, industry and agriculture. More than 75% of the rice production comes from 79 million ha of irrigated lowland. Over 17 million ha of Asia's irrigated rice may experience "physical water scarcity" and 22 million ha may experience "economic water scarcity" by 2025 (Tuong and Bouman, 2003). However, the worsening scarcity of water has threatened the sustainability of the irrigated rice ecosystem. In principle, water is always scarce in the dry season when the lack of rainfall makes cropping impossible without irrigation. Scientists are now taking on the challenging task of developing rice production systems that can cope with water scarcity and that water can be used in other purposes. A reduction of 10% water used in irrigated rice would lead up to a saving of 150,000 million m^3 water that correspond to about 25% of the total fresh water used globally for non-agriculture purposes (Klemm, 1999).

2.2 Direct seeded aerobic rice

In Asia, upland rice is aerobically grown with minimal inputs & it is usually planted as a low yielding subsistence crop in the adverse upland conditions (Lafitte *et al*., 2002). With predictions suggesting that many Asian countries will have severe water problems by 2025, aerobic rice gives hope to farmers who do not have access to enough water to grow flooded lowland rice. The Water-Saving Work Group of the IRRC is committed to further developing this new technology and making it available to farmers in Asia. Aerobic rice is a production system where rice is grown in well-drained, non-puddled, and non-saturated soils. Water requirements can be lowered by reducing water losses due to seepage, percolation and evaporation.

Promising technologies include saturated soil culture and intermittent irrigation during the growing period. However, these technologies still use prolonged periods of flooding, so water losses remain high. A fundamentally different approach is to grow rice like an upland crop, such as wheat, on non-flooded aerobic soils, thereby eliminating continuous seepage and percolation and greatly reducing evaporation. Traditional upland rice has been bred for the unfavorable uplands to give a stable, though low, yield with minimal external inputs. Previous experiments of growing high-yielding lowland rice under aerobic conditions have shown great potential to save water but it has severe yield penalty. A new type of rice is needed to achieve high yields under high-input aerobic conditions.

Aerobic rice is grown in well-drained, non-puddled and non-saturated soils without ponded water (Bouman *et al*., 2007). Economic water use is the main driving force behind aerobic rice cultivation. Irrigation is given at an interval of 5 days up to 25 days; 5-7 days once up to 50 days and during grain filling stage irrigation is provided once in 3 days. Aerobic cultivation saves about 50-60 % of irrigation water. Aerobic rice saved 73% of irrigation water for land preparation and 56% water during the crop growth period. Aerobic rice with micro irrigation practices leads sustainable rice production methodology for immediate future to address water scarcity with more benefits and environmental safety in the scenario of global warming by reduced methane emission (Parthasarathi *et al*., 2012).

From the 1980s to 2002, the area of aerobic rice expanded rapidly to ~ 80,000 ha in the North China Plain (NCP). In the mid-eighties, development of temperate aerobic rice started in northern China and Brazil. In northern China, the grain yields of 8 t ha^{-1} and even higher have been achieved using high-yielding aerobic rice cultivars under appropriate management practices.

Wang *et al*. (2002) reported that advantage of aerobic rice cultivation is as more than 37 m ha of water short irrigated lowlands, 40 m ha of rain fed lowlands and uplands (seasonal RF=600-800 mm) in Asia, which can be rescued from water shortage problems and brought under cultivation. Northern China farmers realized net economic returns of US$ 400-600/ha in fields where water scarcity prohibited the cultivation of lowland rice.

Bouman *et al*. (2005) reported that the highest yield under aerobic conditions was realized in dry season with the approved upland variety APO (5.7 t ha^{-1}) and the lowland hybrid rice Magat (6 t ha^{-1}). On an average, the mean yield of all the varieties was 32% lower under aerobic conditions than under flooded conditions in the dry season and 22% lower in the wet season. Total water input was 1240-1880 mm in flooded fields and 790-1430 mm in aerobic fields. On average, aerobic fields used 190 mm less water in land preparation, and had 250-300 mm less seepage and percolation, 80 mm less evaporation, and 25 mm less transpiration than flooded fields. Without plastic sheets to prevent seepage in flooded fields, the water productivity of rice (with respect to rainfall and irrigation water input) under aerobic conditions was 32-88% higher than under flooded conditions.

Xiaoguang *et al*. (2005) reported that lowland rice variety JD305 (8.8 t ha^{-1}) yielded more than the aerobic rice varieties, HD502 (6.8 t ha^{-1}) and HD297 (5.4 t ha^{-1}), under flooded conditions. But, under aerobic conditions, the aerobic varieties yielded higher than the lowland variety. HD502 produced 3-3.5 t ha^{-1} with 450-500 mm total water input and 5.3-5.7 t ha^{-1} with 650 mm water input. HD297 produced 3-3.5 t ha^{-1} with 450-500 mm total water input and 4.7-5.3 t ha^{-1} with 650 mm water input, reaching values of 0.6-0.8 g grain kg^{-1} water. It showed that the aerobic rice varieties HD502 and HD297 are suitable for water-scarce environments and can stand being periodically flooded.

Changying *et al*. (2008) reported that high-yielding and good-quality aerobic rice varieties adapted to aerobic soil conditions have been released officially and adopted by farmers in North China. The varieties Handao 502 and Handao 297 have been recognized as the most promising varieties reaching a yield level of 3.5 - 5.0 t/ha with 450 - 650 mm water input. Compared with lowland rice, water input in aerobic rice was more than 50% lower and water productivity was 60% higher.

Lampayan *et al*. (2010) reported that aerobic rice yields of up to 4.9 t /ha with 60–150 kg /ha of applied N using Apo variety were attained in wet-season experiments in Central Luzon, Philippines. The rice variety was responsive to applied nitrogen, but risk of lodging increased especially at N rates beyond 90 kg /ha.

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 28.0 q/acre with the highest grain yield of 41.0 q/acre and the lowest grain yield of 18.6 q/acre with a yield advantage of 29.87 per cent over the existing variety (Rasi) in Bangalore Urban district.

Anwar *et al.* (2012) reported that herbicide treatments to aerobic rice fields provided excellent weed control and produced much higher net benefit than weedy or weed-free check. None of the herbicides caused significant phytotoxicity to rice plants. Among the herbicide treatments, sequential application of Cyhalofop-butyl+Bensulfuron at early growth stage followed by Bentazon/MCPA at mid growth stage provided the highest weed control efficiency, productivity and net benefit.

Sunyob *et al.* (2015) conducted glass house and field experiments on five rice varieties: AERON 1, AERON 4, M9, MR211 and MR220-MCL2 against weeds under aerobic rice cultivation. Results revealed that AERON 1 with characteristics of taller plant stature and short growth duration competed better with weed as compared to other varieties.

2.3 Water use efficiency and root-architecture

Yield of rain fed lowland rice, which occupies about 25% of the world's rice areas, has been drastically reduced by drought due to unpredictable, insufficient and uneven rainfall during the growing period (Manickavelu *et al*., 2006). Exploring ways to produce more rice with less water is essential for food security and sustaining environmental health in Asia (Tuong and Bouman, 2002). Improving drought resistance in rice will be necessary for meeting the growing water crisis of the world. To cope with this problem, it requires a deeper understanding of the mechanisms that could facilitate drought resistance (Serraj *et al*., 2011).

Deep roots may provide a key to improve drought resistance in rice because they contribute to water uptake from deeper soil layers during drought. Root traits have been claimed to be critical for increasing yield under soil-related stresses (Lynch, 2007). Adjustment of water uptake to soil-water availability through modifications in physiology,

morphology and anatomy of roots is crucial. Root growth and water uptake ability by the roots are expected to have vital roles in plant water relations (Lafitte *et al*., 2002). For example, root proliferation in the subsoil can alleviate the effects of temporary declines in surface soil moisture (Kato *et al*., 2009).

There are only limited reports on the phenotypic change of root morphology in response to changing soil conditions in rice, possibly due to complexity of this feature and the laborious and time-consuming nature of investigations for root traits (Matsuo *et al*., 2009). Potential to grow deep roots is currently the most accepted target trait and other traits will likely be elucidated as our understanding of plant-soil interactions improves. 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-resistant rice (Gowda *et al*., 2011).

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.

Sathya and Jebaraj (2013) observed genotypic and phenotypic correlations for grain yield and its components. The results revealed that the traits like productive tillers per plant, panicle length, relative water content and dry root weight were significant and showed positive correlation with single plant yield and also among themselves. Therefore, selection for any one of the above characters would bring in simultaneous improvement of other characters and ultimately improving the grain yield.

Raju *et al.* (2014) demonstrated that genotypes that had high roots biomass as well as high CLT (cellular level tolerance) recorded the lowest reduction in spikelet fertility under water limited and aerobic conditions as compared with the low root and low CLT genotypes. This study emphasized the relevance of combining water acquisition and CLT for improving field level tolerance of rice to water limitation.

Wade *et al.* (2015) studied environmental response and genomic regions correlated with rice root growth and yield under drought in the OryzaSNP Panel across Multiple Study Systems. Two genomic regions were identified as hot spots in which root traits and grain yield were co-located; on chromosome 1 (39.7–40.7 Mb) and on chromosome 8 (20.3–21.9 Mb). These regions are good candidates for detailed characterization to contribute to understanding the improvement of rice response to drought.

2.4 Linkage mapping for genes/QTLs for traits promoting adaptation to aerobic conditions

Molecular markers provide a valuable tool for genetic analysis and plant breeding (Tanksley, 1989). Molecular marker technology can help us in identification of suitable parental lines, diversity and pedigree analyses and to introgress desirable traits without altering the genetic background. Recent advances in genome research, particularly in the field of molecular marker technology, have generated opportunity to dissect the variation in quantitative traits in a more meaningful way. Using molecular linkage maps and QTL mapping technology, it is possible to estimate the number of loci controlling genetic variation in a segregating population and to characterize these loci with regard to their map positions on the genome, gene action, phenotypic effects, pleiotropic effect and epistatic interaction between QTL (Parry *et al*., 2005). Molecular markers particularly the microsatellite markers (SSRs) have been used to know about the genetic architecture of complex traits in rice, based on traditional quantitative trait locus (QTL) linkage. SSRs are valuable as genetic markers because they detect high levels of allelic diversity, are co-dominant, are easily and economically assayed by PCR. The technical efficiency and multiplex potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies.

Steele *et al.* (2007) 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 BC_3 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 the new genetic background.

Qu *et al*. (2008) conducted experiments on mapping QTLs of root morphological traits at different growth stages in rice. A mapping population of 120 recombinant inbred lines (RILs) derived from a cross between japonica upland rice 'IRAT109' and paddy rice 'Yuefu' was used for mapping QTLs of developmental root traits. A total of 84 additive-effect QTLs and 86 pairs of epistatic QTLs were detected for the six root traits at five stages. Two main effect QTLs, brt9a and brt9b, were detected at the heading stage and explained 19% and 10% of the total phenotypic variation in basal root thickness (BRT) without any influence from the environment. These QTLs can be used in breeding programs for improving root traits.

Venuprasad *et al*. (2009) performed experiments on Apo/2* $SwarnaF_{4:5}$ population of 490 recombinant inbred lines (RILs) to detect quantitative trait loci (QTL) with large effects

on grain yield under drought stress using bulk-segregant analysis (BSA). It was observed that a few QTL exists in rice which has large effects on grain yield and BSA can detect such QTL alleles relatively cheaply and quickly and aid in rapid screening for large-effect QTL in a large sample of donors.

Vikram *et al*. (2011) studied genetic control of GY (grain yield) under reproductive-stage drought stress (RS) in elite genetic backgrounds in three $F_{3:4}$ mapping populations derived from crosses of N22, a drought-tolerant aus cultivar, with Swarna, IR64, and MTU1010, three high-yielding varieties. Results showed a major QTL for GY under RS, qDTY1.1, was identified on rice chromosome 1 flanked by RM11943 and RM431 in all three populations. 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, under RS. qDTY1.1 also showed a positive effect on GY in non-stress (NS) situations in N22/Swarna, N22/IR64 over both years, and N22/MTU1010 in DS2009.

Dixit *et al.* (2012) (a) studied fine-mapping on four QTLs, qDTY2.1, qDTY2.2, qDTY9.1 and qDTY12.1, for grain yield (GY) under drought using four different backcross-derived populations screened in 16 experiments. They reported that all four of these QTL regions increased GY under stress conditions and did not have any effect on GY under non-stress conditions. qDTY12.1 explained the highest percentage of phenotypic variance for GY under severe upland drought compared with the three other QTLs. However, qDTY2.1, qDTY2.2 and qDTY9.1 have shown a high and consistent additive effect under severe lowland drought stress despite the low phenotypic variance explained by them.

Dixit *et al.* (2012) (b) studied the genetic basis of high grain yield under reproductive-stage drought using an F_3-derived population generated from the cross of upland rice (*Oryza sativa* L.) cultivars Vandana and Way Rarem. They reported that two loci (qDTY2.3 and qDTY3.2) have interaction with qDTY12.1 for GY (grain yield) under severe stress. qDTY2.3 always showed a positive effect on GY under upland and lowland conditions irrespective of stress levels. qDTY3.2 showed a high positive effect on GY under severe upland and lowland stress but showed a negative effect on GY under UNS (upland non-stress) and LMiS (lowland mild stress).

Priyadarshini *et al.* (2013) studied to evaluate the effectiveness of the root QTLs on grain yield and water use efficiency in a set of 116 near isogenic lines (NILs) derived from cross IR64 and Azucena populations which were screened for grain yield and water use efficiency under severe reproductive-stage drought stress. Among NILs with single QTL, the NIL with QTL on chromosome 9 exerted its effect significantly on both grain yield and water use efficiency. Whereas none of two or three QTL combination could not make it significantly, this may be attributed to pleotropic effect of QTLs. However the two roots QTL on chromosome 2 and 7 and three QTL combinations on chromosome 1, 2, and 7 had

comparable water use efficiency as that of Azucena which is a donor of root QTLs. The experimental findings indicate that deeper root may increase grain yield.

Sandhu *et al*. (2013) reported two QTLs (qGY8.1 with an R^2 value of 34.0% and qGY2.1 with an R^2 value of 22.8%) and one QTL (qGY2.2 with an R^2 value of 43.2%) for grain yield under aerobic conditions in the mapping populations MASARB25 x Pusa Basmati 1460 and HKR47 x MAS26, respectively. Co-localization of QTL for yield, root traits, and yield related agronomic traits indicates that the identified QTL may be immediately exploited in marker-assisted-breeding to develop novel varieties with high yield and better aerobic cultivation adaptability.

Henry *et al.* (2014) characterized rice (*Oryza sativa* L.) genotypes with QTLs derived from drought-tolerant traditional variety AdaySel that were introgressed into drought-susceptible high-yielding variety IR64. Of the different combinations of the four QTLs evaluated, genotypes with two QTLs (*qDTY2.2* + *qDTY4.1*) showed the greatest degree of improvement under drought compared with IR64 in terms of yield, canopy temperature, and normalized difference vegetation index (NDVI). Furthermore, *qDTY2.2* and *qDTY4.1* showed a potential for complementarities in that they were each most effective under different severities of drought stress.

CHAPTER-III

MATERIAL AND METHODS

3.1 Material

3.1.1 Plant material

The experimental plant material comprised of seeds harvested from the crosses:

i) MAS25 x HKR47 F_2 plants

ii) MASARB25 x HKR47 F_2 plants

iii) MAS25 x PAU201 F_2 plants

iv) PAU201 x MAS26 F_2 plants

v) MASARB25 x PAU201 F_2 plants

Table 3.1: A brief description of parental rice varieties

Sr. No.	Rice variety/ genotype	Source	Remarks
1.	HKR47	CCSHAU, Hisar	Lowland *indica* rice
2.	MAS25	UAS, Bangalore	Aerobic *indica* rice
3.	MAS26	UAS, Bangalore	Aerobic *indica* rice
4.	MASARB25	IRRI, Manila, Philippines	Aerobic *indica* rice
5.	PAU201	PAU, Ludhiana	Lowland *indica* rice

CCSHAU: CCS Haryana Agricultural University; **UAS:** University of Agricultural Sciences; **IRRI:** International Rice Research Institute and **PAU:** Punjab Agricultural University.

HKR47 and PAU201 are lowland high yielding *indica* rice whereas MAS25 and MAS26 are aerobic rice varieties developed at University of Agricultural Sciences, Bangalore for cultivation under direct-seeded aerobic conditions. MASARB25 (IET-20649) is the drought tolerant rice cultivar developed from IR64/Azucena/xx IR64 crosses at IRRI, Manila, Philippines. MAS26 and MASARB25 have better root length and root biomass, respectively. For the evaluation of root morphology, plants were raised during *Kharif* season of 2014 in pots in the net house of Department of Molecular Biology, Biotechnology and Bioinformatics at CCS Haryana Agricultural University, Hisar whereas raising of plants under field conditions was carried out (during the *Kharif* season of 2014) at Rice Research Station, Kaul (Kaithal). The F_3 plants derived from all the five crosses were evaluated for variation in physio-morphological traits in the field and net house. The F_3 plants were also evaluated for molecular diversity linked to the traits promoting aerobic adaption.

3.1.2 Glassware and Plastic ware

Glassware used was of the borosilicate quality and obtained from Borosil Glass Works Ltd., Mumbai or Corning Glass Company, USA. Disposable Petri dishes were

procured from Tarsons Products Pvt. Ltd., India. Centrifuge tubes were obtained from Fisher Scientific International Co., USA.

3.1.3 Chemicals

Chemicals used for DNA extraction and PCR amplification were of molecular biology grade and were obtained from Sigma Chemicals Co. USA, Life Technologies (India) Pvt. Ltd. and Imperial Bio Medics (Chandigarh, India), Perkin Elmer, Inc., Madison, WI, USA: Applied Biosystems, 850 Lincoln Center Drive, Foster city, CA-94404: GENEI (Bangalore, India). All the other chemicals used were of molecular biology grade or analytical grade and were procured from Sigma Chemicals Co. USA, Promega Inc., USA, Gibco BRL Inc. (Gaitherburg, MD, USA) and E. Merck Ltd. (Worli, Mumbai-400018, India). SSR primers used were obtained from Integrated DNA Technologies, Inc. USA.

3.1.4 Molecular markers

A total of 44 SSR markers widely distributed on 12 rice chromosomes were used in this study. The chromosome location and base sequence of forward and reverse primers of polymorphic SSR markers are given in Table 3.3. The sequence information of these primers is also available at http://www.gramene.org.

3.2 Methods

3.2.1 Phenotypic evaluation

Seeds were grown to generate the respective population(s) and phenotypic evaluation was done in the field and net house.

3.2.1.1 Raising of crop

3.2.1.1.1 Net house experiment

The F_3 populations of the above mentioned crosses were raised during the 2014 kharif season in the net house of the Department of Molecular Biology, Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar.

3.2.1.1.1.1 Layout of the Experiment

F_3 plants were grown using the single seed descent method. All the recommended agronomic practices were followed for raising a good crop.

i) Seeds were grown in the pots (five pots per genotype and one plant per pot) in a net house.
ii) After twenty-one days, Yoshida nutrient solution was added to the pots (500 ml per pot). The composition of Yoshida nutrient solution is given in the Table 3.2.
iii) Each mature plant was harvested separately by cutting from the base of the stem. Sun drying for five days was sufficient to get the grain moisture content of around 14%.
iv) The grains were threshed manually and cleaned removing unfilled grains.

3.2.1.1.2 Field experiment

Seeds derived from selected F_2 plants (a total of 50 F_2 plants from above crosses; 10 F_2 plants from each cross) were sown under aerobic conditions in the field at CCS HAU, Rice

Research Station, Kaul (Kaithal), which falls under semi-tropical regions of North India to raise F_3 populations during the kharif season in 2014.

3.2.1.1.2.1 Layout of the Experiment

The crop was grown using the single seed descent method. All the recommended agronomic practices were followed for raising a good crop. Preparation of the land, seeds and establishment of plants was as per CCS HAU, Rice Research Station Kaul (Kaithal) standard practices.

i) Seeds were grown in rows of 3 m length (3 rows of each selected plant) in the field with plant to plant spacing of 15 cm and row to row spacing of 20 cm.
ii) After one month, weeds were removed manually.
iii) Each mature plant was harvested separately by cutting from the base of the stem. Sun drying for five days was sufficient to get the grain moisture content of around 14%.
iv) The grains were threshed manually and cleaned removing unfilled grains.

Table 3.2: Composition of Yoshida nutrient solution

Sr. No.	Major Salts	g/l	g/250 ml
1.	NH_4NO_3	91.3	22.85
2	K_2SO_4	71.4	17.85
3.	KH_2PO_4	23.1	5.77
	K_2HPO_4	4.3	1.07
4.	$CaCl_2.6H_2O$	175	29.36
5.	$MgSO_4.7H_2O$	324	81
6.	**Minor Salts**		
	$MnCl_2.4H_2O$	1.5	0.37
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.074	0.02
	H_3BO_3	0.930	0.23
	$ZnSO_4.7H_2O$	0.035	0.008
	$CuSO_4.5H_2O$	0.030	0.007
7.	FeEDTA	10.53	2.63

3.2.1.2 Data collection on various physio-morphological traits

The data on various physio-morphological traits was recorded for all the populations, including parent genotypes. The data was subsequently analyzed to determine the variability and correlation coefficient analysis. Data on following physio-morphological traits was recorded:

(i) Plant height (cm)

Height of stem measured from the soil surface until the tip of highest panicle (excluding awn).

(ii) Effective number of tillers per plant

Fully developed tillers bearing panicles of each plant were counted at the time of maturity.

(iii) Panicle length (cm)

Length of panicle (cm) was measured from the base of panicle until the tip of the last grain.

(iv) Number of panicles per plant

Counting of panicles was done during heading stage when panicles started to show exsertion out of boot leaves.

(v) Grain yield per plant (g)

The total weight of cleaned filled grains of each plant was measured in grams.

(vi) Length-breadth ratio

The length and breadth of three seeds from each plant was recorded using digital Vernier Caliper.

(vii) 1000-grain weight (g)

Weight of randomly chosen clean and filled 1000 grains was measured in grams.

(viii) Root length (g)

The root length of fully mature plant was recorded on centimeter scale.

(ix) Fresh and dry root weight (g)

The fresh and dry root weight of fully grown plants was recorded in grams.

(x) Root thickness (cm)

The root thickness of fully grown plants was recorded using digital Vernier Caliper.

3.2.2 Statistical Methods

A. Parameters of variability

(i) Mean: The mean value of each character was worked out by dividing the totals by corresponding number of observations;

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where,

Xi - any observation in i^{th} treatment

n - Total number of observations

(ii) Standard Deviation: The positive square root of mean of squared deviations from arithmetic mean, so called root mean square deviation. The standard deviation is a measure of how widely values are dispersed from the average value (the mean).

STDEV uses the following formula:

$$\sqrt{\frac{\sum (x-\bar{x})^2}{(n-1)}}$$

Where,

x- Sum of all values of the variable

$\bar{x}$- Mean of values

n- Sample size

B. Correlation coefficient analysis:

Data on physio-morphological and root morphological traits was analysed for correlation by using OP-STAT available at website- hau.ernet.in.

3.3 DNA isolation and microsatellite marker analysis

DNA isolation and microsatellite marker analysis were carried out in the Department of Molecular Biology, Biotechnology and Bioinformatics, CCS HAU, Hisar.

3.3.1 Genomic DNA isolation

Genomic DNA was isolated using CTAB method of Saghai-Maroof *et al*. (1984).

i. Leaf samples were taken from 2-3 week old seedlings and cut into small pieces and ground into fine powder using liquid nitrogen in a sterile pestle and mortar.
ii. Approximately five gram of the ground leaf tissue powder was transferred into a 50 ml polypropylene tube. To this, 15 ml of preheated (65ºC) CTAB buffer was added and the samples were thoroughly mixed by inverting the tubes several times.
iii. Incubation was done at 65ºC for 60-90 minutes with regular gentle mixing of samples.
iv. After incubation, samples were cooled to room temperature and 15 ml of Chloroform: Isoamyl alcohol (24:1) mixture was added. Contents were mixed well by inverting the tubes gently for 10-15 minutes.
v. Tubes were then centrifuged at 10,000 rpm for 10 minutes and the upper aqueous layer was transferred into new sterilized 50 ml tubes using a micropipette.
vi. Equal volume of ice-cold isopropanol was subsequently added to precipitate the DNA and the tubes were kept undisturbed for 15 minutes. The DNA was then spooled out and put into 1.5 ml eppendorf centrifuge tubes.
vii. Centrifugation was done at 12,000 rpm for 5 min to pellet down the DNA. The supernatant was discarded and the DNA pellet was washed with 70% ethanol and the DNA pellet was dried overnight at room temperature.
viii. DNA was subsequently dissolved in appropriate volume of T.E. buffer and samples were stored at -20ºC till further use.

Reagents:

CTAB extraction buffer

Tris-HCl (pH 8.0)	1mM
EDTA (pH 8.0)	0.5 M

NaCl	5 M
β-mercaptoethanol	2%
CTAB	2%

T.E. buffer

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

[EDTA - ethylene diamine tetra acetic acid]

3.3.2 RNase treatment

i. RNase solution was prepared by dissolving 10 mg RNase (DNase free RNase-A, Sigma chemical Co. USA, No.R-5503) in 1 ml of (500 µl of 10 mM Tris and 500 µl of 15 mM NaCl) and then boils at 100ºC for 15 minutes.

ii. RNA contamination was removed by adding 1 µl of 10 mg/ml RNase in the dissolved DNA samples. The samples were mixed gently and then incubated at 37ºC for 2-3 hours.

iii. DNA was again extracted by adding equal volume of chloroform: isoamyl alcohol (24:1) mixture. Samples were mixed well and then centrifuged at 10,000 rpm for 10 minutes.

iv. Supernatant was transferred to sterilized eppendorf-tubes. DNA was precipitated by adding 1/10th volume of 5M sodium chloride and 2 volume of chilled absolute alcohol, followed by centrifugation to pellet down the DNA. Pellet was then washed with 70% alcohol, air-dried and finally dissolved in T.E. buffer.

3.3.3 Quality and quantity of genomic DNA

Completely dissolved genomic DNA was checked for its quality and concentration by running DNA samples on 0.8% agarose gels electrophoresis using a standard containing 100 ng/µl genomic λ DNA.

3.3.4 Microsatellite marker analysis

i. A total of 44 SSR markers were screened for polymorphism between the parents (HKR47, MAS25, MAS26, MASARB25 and PAU201). The markers were obtained based on published rice genome maps (IRGSP, 2005). A total of seven, seven, three, five and five molecular markers were used for preparation of DNA fingerprint database of selected F_3 plants derived from the following crosses: MAS25 x PAU201, PAU201 x MAS26, MASARB25 x PAU201, MASARB25 x HKR47 and MAS25 x HKR47 respectively. These primers have been reported to be linked with the traits promoting aerobic adaptation (Shen *et al.*, 2001; Kanagaraj *et al.*, 2010, Table 3.3).

ii. PCR amplifications were performed using PTC – 100^{TM} 96V thermocycler (MJ Research, Inc., Watertown, MA, USA) and Taq DNA polymerase.

iii. The PCR reaction was conducted in a reaction volume of 20 µl containing 1x PCR buffer, 100 µM dNTPs, 0.3 µM of each primer, 1.5 unit Taq DNA polymerase and 50-75 ng template DNA.

iv. PCR amplification was performed with following steps:

Step 1: Initial denaturation at 94ºC for 5 minutes

Step 2: Denaturation at 94ºC for 5 minute

Primer annealing at 55ºC for 1 minute

Extension at 72ºC for 2 minutes

35 cycles of step 2

Step 3: Final extension at 72ºC for 7 minutes before cooling at 4ºC.

v. Amplification products were stored at –20ºC till further use.

3.3.5 Agarose gel electrophoresis

PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels. Gel casting plate was washed, air-dried and its ends were sealed with tape. Agarose was melted in 1X TBE buffer and ethidium bromide (5 µg/ml) was added. Gel solution was then poured into gel casting plate inserted with an appropriate comb to get a 0.4-0.6 cm thick gel. After setting of gel, sealing tapes were removed from both the ends. Gel plate was placed in the electrophoresis chamber and submerged using 1X TBE buffer and comb was removed gently. Samples were prepared by adding 6 X loading dye (Sucrose 4 g, bromophenol blue 0.025 g, xylene cyanol 0.025 g, final volume 10 ml). Samples were loaded in the wells and electrophoresis was carried out at constant voltage (3 v/cm of gel) until dye migrated to other end of the gel. PCR amplified products were viewed under UV light fluorescence using Labnet UV transilluminator.

3.3.6 Data analysis

The presence of band of DNA on agarose gel was taken as one and absence of band was read as zero. The 0/1 matrix was used to calculate similarity genetic distance using ‘simqual’ sub-program of software NTSYS–pc (Rohlf, 1993). Dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc. Principle component analysis (PCA) was done using the ‘PCA’ sub programme of NTSYS-pc.

Table 3.3: A brief description of the microsatellite markers used in the study

Sr. No.	Locus name	Chromosome location	Forward primer	Reverse primer
1	RM17	12	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
2	RM71	2	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG
3	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
4	RM164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
5	RM165	1	CCGAACGCCTAGAAGCGCGTCC	CGGCGAGGTTTGCTAATGGCGG
6	RM175	3	CTTCGGCGCCGTCATCAAGGTG	CGTTGAGCAGCGCGACGTTGAC
7	RM187	11	CCAAGGGAAAGATGCGACAATTG	GTGGACGCTTTATATTATGGG
8	RM200	1	CGCTAGGGAATTTGGATTGA	CGATGAGCAGGTATCGATGAGAAG
9	RM202	11	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
10	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
11	RM208	2	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
12	RM217	6	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
13	RM220	1	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT
14	RM231	3	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
15	RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
16	RM237	1	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
17	RM264	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
18	RM269	10	GAAAGCGATCGAACCAGC	GCAAATGCGCCTCGTGTC
19	RM272	1	AATTGGTAGAGAGGGGAGAG	ACATGCCATTAGAGTCAGGC
20	RM276	6	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
21	RM289	5	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG
22	RM292	1	ACTGCTGTTGCGAAACGC	TGCAGCAAATCAAGCTGGAA

Sr. No.	Locus name	Chromosome location	Forward primer	Reverse primer
23	RM312	1	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
24	RM323	1	CAACGAGCAAATCAGGTCAG	GTTTTGATCCTAAGGCTGCTG
25	RM336	7	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTTCAGG
26	RM341	2	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
27	RM410	9	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
28	RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
29	RM428	1	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTTG
30	RM431	1	TCCTGCGAACTTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
31	RM440	5	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
32	RM475	2	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
33	RM525	2	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG
34	RM545	3	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
35	RM547	8	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
36	RM564	4	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG
37	RM582	1	TCTGTTGCCGATTTGTTCG	AAATGGCTTACCTGCTGTCTC
38	RM6925	8	GAATGAGAGGACGCTTGAAGAGG	GCATTCAGTCCCAGCTTGTATCG
39	RM8020	8	CTGTCCATAGAGGCCTACAAGTATCC	GAATTCGACCCAGATGATAGAAGAGG
40	RM8029	2	CTTCATTTCAGAATGTAAGA	ACCCACATTTATTTAGATGT
41	RM12146	1	AGTATGCCCTGCCCACTACACTAGG	CAGCGAATGGCAAGAGCAACCCTT
42	RM12233	1	CTTGAGTTCGAAGCGAGAAGACG	CACTTGAGCTCGAGACGTAGCC
43	RM16175	3	AGCTTTGGTTTCTTGGCTTTGG	ATTAGCGTTGAACCCAAGTGTGG
44	RM17391	4	ACTTTGCTCTGAACTTGCAGTGG	GCTAGCTGCTATCAGGATTCACG

CHAPTER-IV

RESULTS

In the present investigation, experiments were conducted to evaluate F_3 populations; derived from the fifty selected F_2 plants of the five crosses (MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47 and MAS25 x HKR47) for variation in root morphology, physio-morphological traits and molecular profile for SSR markers linked to traits promoting aerobic adaptation. The results obtained are given below:

4.1.1 Field evaluation of MAS25 x PAU201 F_3population

Enormous variation was observed among 24 MAS25 x PAU201 F_3 plants for plant height (67-120 cm, MAS25–91.5 cm and PAU201–86.7 cm), panicle length (3.67-24.9 cm, MAS25–22.4 cm and PAU201–17.5 cm), number of panicles per plant (1-14, MAS25–11 and PAU201–9.2), number of effective tillers per plant (1-14, MAS25–9.5 and PAU201–7.9), grain yield per plant (1.86-14.4 g, MAS25–11.3 g and PAU201–9.83 g), 1000 grain weight (12.8-26.8 g, MAS25–20.3 g and PAU201–18.9 g) and grain length-breadth ratio (3.12-4.23, MAS25–4.21 and PAU201–3.55) (Table 4.1). Data on physio-morphological traits of the individual F_3 plants is given in Annexure I.

Table 4.1: Range for physio-morphological traits in MAS25 x PAU201 F_3 population grown under aerobic conditions in the field.

Trait	MAS25 Mean (Range)	PAU201 Mean (Range)	MAS25 x PAU201 F_3 population (24 plants)	
			Mean	Range
Plant height (cm)	91.5 ± 0.58 (86-99)	86.7 ± 0.62 (86-88)	80.8 ± 1.48	67-120
Number of panicles per plant	11 ± 0.38 (10-12)	9.2 ± 0.19 (7-11)	6.58 ± 1.28	1-14
Number of effective tillers per plant	9.5 ± 0.75 (8-12)	7.9 ± 0.5 (7-9)	6.56 ± 1.2	1-14
Panicle length (cm)	22.4 ± 0.24 (22.8-24.2)	17.5 ± 0.33 (17-19)	19.1 ± 0.99	3.67-24.9
Grain yield per plant (g)	11.3 ± 0.56 (9.87-12.5)	9.83 ± 0.47 (8.9-11.7)	7.35 ± 1.34	1.86-14.4
1000 grain weight (g)	20.3 ± 0.35 (19.7-21.4)	18.9 ± 0.38 (17.7-20)	20.2 ± 0.78	12.8-26.8
Grain length-breadth ratio	4.21 ± 0.03 (4.2-4.3)	3.55 ± 0.04 (3.2-4.0)	3.87 ± 0.13	3.12-4.23

4.1.2 Net house evaluation of MAS25 x PAU201 F_3 population

Large variation was observed among 22 MAS25 x PAU201 F_3 plants and parental rice genotypes for plant height (56-91 cm, MAS25–68 cm and PAU201–64.3 cm), panicle length (16.9-32.1 cm, MAS25–20.4 cm and PAU201–20.3 cm), number of effective tillers per plant (2-9, MAS25– 4.5 and PAU201–4.2), grain yield per plant (1.77-13 g, MAS25–4.77 g and PAU201 - 2.23 g), 1000 grain weight (13.1-24.6 g, MAS25–21.2 g and PAU201–16.5 g), grain length-breadth ratio (4.26-5.36, MAS25–5.06 and PAU201–4.52), root length (29-54 cm, MAS25–40 cm and PAU201–38.5 cm), root thickness (3.13-24.2 mm, MAS25–12.4 mm and PAU201–6.85 mm), fresh root weight (2.18-24.1 g, MAS25–7.81 g and PAU201–3.35 g) and dry root weight (0.14-8.01 g, MAS25–3.42 g and PAU201–1.1 g) (Table 4.2). Data on various physio-morphological traits of the individual F_3 plants is given in Annexure II.

Table 4.2: Variation for physio-morphological and root morphological traits in MAS25 x PAU201 F_3 population grown in pots in the net house under water-limited conditions

Trait	MAS25 Mean (Range)	PAU201 Mean (Range)	MAS25 x PAU201 F_3 population (22 plants)	
			Mean	Range
Plant height (cm)	68±1.21 (64-71)	64.3±0.86 (62-67)	79.2±1.9	56-91
Number of effective tillers per plant	4.5±0.25 (4-5)	4.2±0.38 (3-5)	5.14±0.31	2-9
Panicle length(cm)	20.4±0.6 (18.8-22.3)	20.3±0.81 (22.5-24.9)	25.3±1.58	16.9-32.1
Grain yield per plant (g)	4.77±0.13 (4.4-5.2)	2.23±0.43 (1.27-3.45)	5.17±1.1	1.77-13
1000 grain weight(g)	21.2±0.41 (20.4-22.6)	16.5±0.81 (14-18.3)	20.7±1.9	13.1-24.6
Grain length-breadth ratio	5.06±0.11 (4.8-5.3)	4.52±0.07 (4.4-4.8)	4.85±0.21	4.26-5.36
Root length (cm)	40±0.7 (38-42)	38.5±0.81 (37-41)	37.6±3.36	29-54
Root thickness (mm)	12.4±1.63 (10.1-18.9)	6.85±0.15 (6.3-7.2)	14.6±2.15	3.13-24.2
Fresh root weight (g)	7.81±0.29 (7.2-8.4)	3.35±0.07 (3.13-3.55)	16±3.04	2.18-24.1
Dry root weight (g)	3.42±0.32 (2.33-4.17)	1.1±0.12 (0.68-1.4)	3.71±0.16	0.14-8.01

4.1.3 Correlation coefficient analysis

Phenotypic correlation coefficient analysis of MAS25 x PAU201 F_3 population, evaluated in field under aerobic condition revealed a positive correlation between grain yield per plant with number of panicles per plant (0.883, p=0.01), number of effective tillers per plant (0.885, p=0.01), panicle length (0.7, p=0.01) and 1000 grain weight (0.619, p=0.01) .

A positive correlation was found between panicle length with number of panicles per plant (0.667, p=0.01), number of effective tillers per plant (0.676, p=0.01) and 1000 grain weight (0.656, p=0.01) (Table 4.3).

Phenotypic correlation coefficient analysis of MAS25 x PAU201 F_3 population, evaluated in net house under aerobic condition revealed a positive correlation between grain yield per plant with dry root weight (0.504, p=0.05), root thickness (0.707, p=0.01), number of effective tillers per plant (0.711, p=0.01), 1000 grain weight (0.837, p=0.01) and grain length-breadth ratio (0.800, p=0.01). A positive correlation was noticed between number of effective tillers per plant with root thickness (0.931, p=0.01), root length (0.604, p=0.01), grain length-breadth ratio (0.698, p=0.01) and dry root weight (0.791, p=0.01).

Root length showed positive correlation with fresh root weight (0.799, p=0.01), root thickness (0.781, p=0.01), plant height (0.820, p=0.01), panicle length (0.813, p=0.01) and 1000 grain weight (0.643, p=0.01). A positive correlation was found between fresh root weight with plant height (0.910, p=0.01), panicle length (0.918, p=0.01) and 1000 grain weight (0.929, p=0.01). Dry root weight showed positive correlation with root thickness (0.609, p=0.01) and grain length-breadth ratio (0.526, p=0.01). A negative correlation was also observed between plant height and root thickness (-0.925, p=0.01) (Table 4.3.1).

4.1.4 Microsatellite marker analysis of MAS25 x PAU201 F_3 plants.

Microsatellite marker data was generated using seven molecular markers for six selected MAS25 x PAU201 F_3 plants.

4.1.5 Variation in allelic profile

Agarose gel displaying allelic polymorphism in MAS25 x PAU201 F_3 plants for SSR marker is shown in (Plate 1). The overall size of PCR amplified products ranged between 100 bp (RM525 and RM528) and 280 bp (RM547) (Table 4.4). At a SSR locus, all the F_3 plants had MAS25 specific allele or PAU201specific allele or alleles specific for both the parents indicating the heterozygous state or absence of both the alleles (Table 4.4). The distribution of PAU201 and MAS25 specific alleles are shown in (Table 4.4.1). Plant number R-17-1, R-17-3 and R-50-2 had the maximum alleles from PAU201 while plant number R-50-2 and R-26-3 had the maximum number of alleles from MAS25.

Table 4.3: Correlation analysis between different morphological traits in MAS25 x PAU201 F_3 population grown in field under aerobic conditions

Trait	Plant height (cm)	Number of effective tillers per plant	Number of panicles per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Plant height (cm)	1						
Number of effective tillers per plant	0.116	1					
Number of panicles per plant	0.164	0.987**	1				
Panicle length (cm)	0.325	0.676**	0.667**	1			
Grain yield per plant (g)	0.257	0.885**	0.883**	0.700**	1		
1000 grain weight(g)	-0.077	0.603**	0.558**	0.656**	0.619**	1	
Grain length-breadth ratio	-0.538**	0.175	0.137	0.065	0.190	0.328	1

Table 4.3.1: Correlation analysis between different morphological traits in MAS25 x PAU201 F_3 population grown under water-limited conditions in net house

Traits	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Root length (cm)	1									
Fresh root weight (g)	0.799**	1								
Dry root weight(g)	-0.156	-0.374	1							
Root thickness (mm)	0.781**	-0.134	0.609**	1						
Plant height (cm)	0.820**	0.910**	-0.353	-0.925**	1					
Number of effective tillers per plant	0.604**	-0.160	0.791**	0.931**	-0.905**	1				
Panicle length (cm)	0.813**	0.918**	-0.237	-0.295	0.984**	-0.282	1			
Grain yield per plant (g)	-0.470	0.160	0.504*	0.707**	-0.121	0.711**	-0.118	1		
1000 grain weight (g)	0.643**	0.929**	-0.455	-0.285	0.875**	0.174	0.883**	0.837**	1	
Grain length-breadth ratio	-0.184	-0.150	0.526**	0.727**	-0.125	0.698**	0.229	0.800**	-0.119	1

Table 4.4: Allelic profile (in bp) of MAS25 x PAU201 F_3 population and parental rice genotypes at seven SSR loci

SSR Marker	Allele size (bp)		Number of plants with		
	MAS25	PAU201	MAS25 Specific allele	PAU201 specific allele	Both PAU201 and MAS25 allele
RM224	180	150	2	3	1
RM231	180	200	3	2	1
RM276	180	140	2	3	1
RM440	210	157	1	1	4
RM525	100	120	4	2	0
RM528	120	100	3	3	0
RM547	260	280	2	2	0

4.1.6 Genetic relationship among MAS25 x PAU201 F_3 population

Similarity coefficient data based on the seven SSR markers was used to calculate the coefficient values among the selected six MAS25 x PAU201 F_3 plants and parental rice genotypes and subjected to NTSYS-pc UPGMA tree cluster analysis. The allelic diversity was used to produce a dendrogram to demonstrate the genetic relationship among six selected F_3 plants and the parental rice varieties (Figure 4.1). All the F_3 plants clustered in two major groups at the similarity coefficient of 0.42. Major group I consisted of MAS25and two F_3 plants while the major group II had PAU201 and four F_3 plants. Genetic relationships among these rice genotypes were also assessed by PCA analysis (NTSYS-pc).

Table 4.4.1: Allelic profile (in bp) of selectedMAS25 x PAU201 F_3 plants and parental genotypes at seven SSR loci

Genotype	RM224	RM231	RM276	RM440	RM525	RM528	RM547
MAS25	180	180	180	210	100	120	260
PAU201	150	200	140	157	120	100	280
R-5-1	-*	180	140	210,157	100	100	-
R-17-3	150	180	140	210,157	100	100	260
R-17-1	150	200	140	210,157	100	120	-
R-50-2	180,150	180,200	140,180	210	100	100	260
R-26-2	-	200	180	157	120	120	-
R-26-3	180	180	180	210,157	120	120	280

*null allele

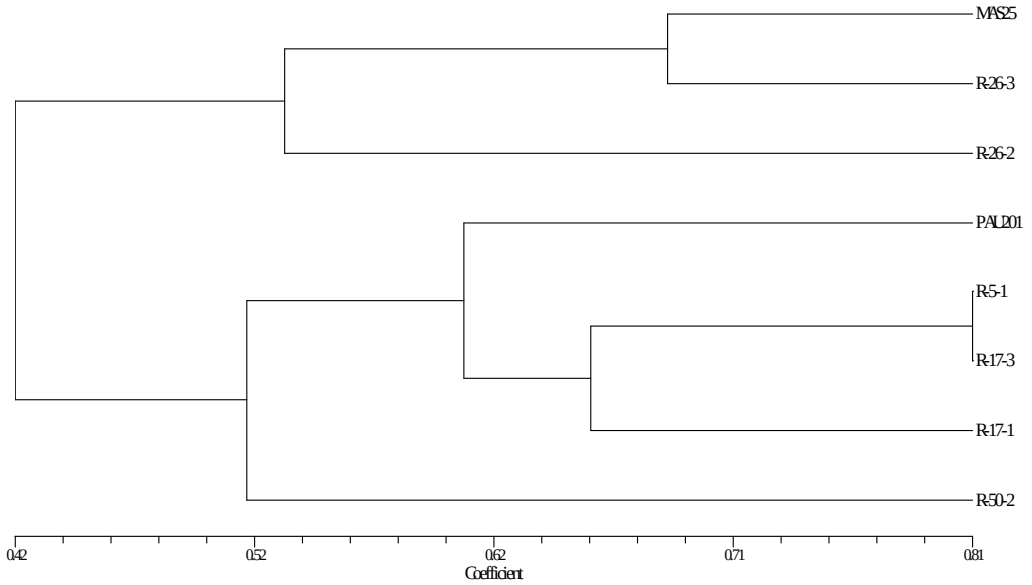

Figure 4.1: Dendrogram (NTSYS-pc) showing genetic relationships among six MAS25 x PAU201 F_3 plants and parental genotypes using allelic data at seven SSR loci

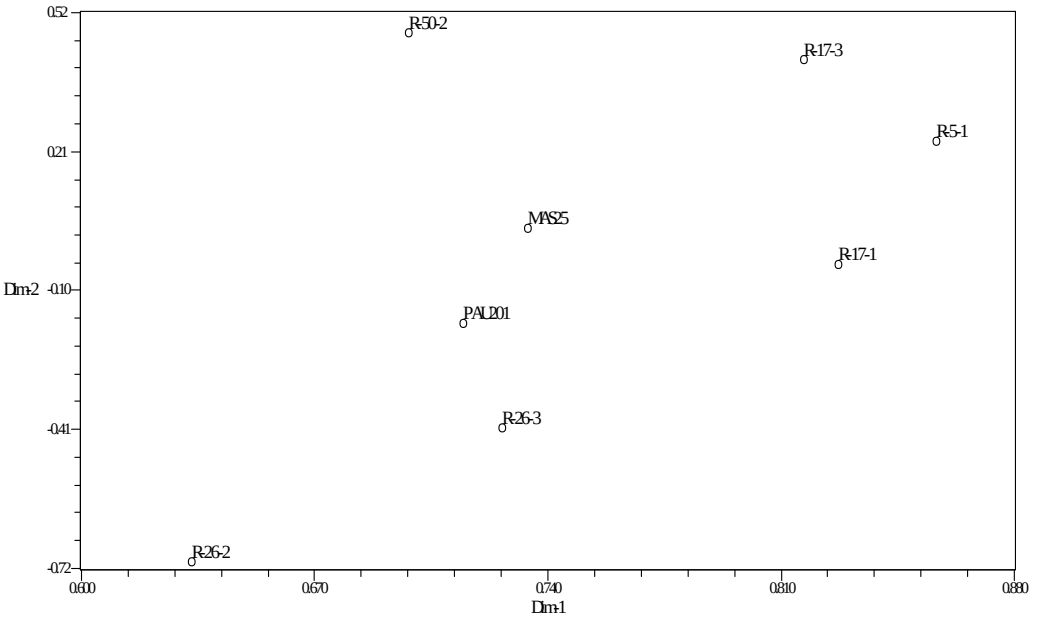

Figure 4.2: Two-dimensional PCA scaling of six MAS25 x PAU201 F_3 plants and parental genotypes using genetic distance matrix data at seven loci

4.1.7 Selection of promising plants

Based on field data, a total of four MAS25 x PAU201 F_3 plants were selected on the basis of plant height, number of effective tillers per plant, grain length-breadth ratio and yield per plant (Table 4.5). Based on net house data, a total of two MAS25 x PAU201 F_3 plants were selected on the basis of root morphology (length & biomass) and grain length-breadth ratio (Table 4.6).

Table 4.5: Data on physio-morphological traits of selected MAS25 x PAU201 F_3 plants

Trait	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MAS25	91.5 ± 0.6	11 ± 0.38	9.5 ± 0.75	22.4 ± 0.24	11.3 ± 0.56	20.3 ± 0.35	4.21 ± 0.03
PAU201	86.7 ± 0.62	9.2 ± 0.62	7.9 ± 0.49	17.5 ± 0.33	9.83 ± 0.47	18.9 ± 0.38	3.55 ± 0.04
R-5-1	83	9	10	22.6	12.9	22.8	3.74
R-17-1	77	14	14	22.4	14.4	26.8	4.23
R-26-2	88	8	8	23.7	11.4	23.3	4.22
R-26-3	78	11	12	21.4	11.2	23.9	4.09

Table 4.6: Data on physio-morphological and root traits of selected MAS25 x PAU201 F_3 plants

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	No. of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 Grain weight (g)	Grain length bredth ratio
MAS25	40 ± 0.7	7.81 ± 0.29	3.42 ± 0.32	12.4 ± 1.63	68 ± 1.21	4.5 ± 0.25	20.4 ± 0.6	4.77 ± 0.13	21.20 ± 0.41	5.06 ± 0.11
PAU201	38.5 ± 0.81	3.35 ± 0.07	1.1 ± 0.12	6.85 ± 0.15	64.3 ± 0.86	4.2 ± 0.38	20.3 ± 0.81	2.23 ± 0.43	16.5 ± 0.81	4.52 ± 0.07
R-17-3	43	26.2	5.08	18.9	88	7	31.4	13	22	4.49
R-50-2	40	24.1	6.7	18.3	80	8	27.5	9.91	24.6	5.11

4.2.1 Field evaluation of MASARB25 x PAU201 F_3 population

Extensive variation was observed among 30 MASARB25 x PAU201 F_3 plants for plant height (60-110 cm, MASARB25–90.6 cm and PAU201–86.7 cm), panicle length (17.1-22.7 cm, MASARB25–23.2 cm and PAU201–17.5cm), number of panicles per plant (3-16, MASARB25–11.8 and PAU201–9.2), number of effective tillers per plant (3-13, MASARB25 – 9.4 and PAU201–7.9), grain yield per plant (3.77-19.3 g, MASARB25 –10.8 g and PAU201–9.83 g), 1000 grain weight per plant (15.8-27.3 g, MASARB25–20.6 g and PAU201–18.9 g) and grain length-breadth ratio (3.51-4.21, MASARB25–4.12 and PAU201–3.55) (Table 4.7). Data on physio-morphological traits of the individual F_3 plants is given in Annexure III.

Table 4.7: Range for physio-morphological traits in MASARB25 x PAU201F_3 population grown under aerobic conditions in the field

Trait	MASARB25 Mean (Range)	PAU201 Mean (Range)	MASARB25 x PAU201 F_3 population (30 plants)	
			Mean	Range
Plant height (cm)	90.6 ± 0.75 (88-92)	86.7 ± 0.62 (86-88)	77.3 ± 1.32	60-110
Number of panicles per plant	11.8 ± 0.58 (11-14)	9.2 ± 0.19 (7-11)	10.5 ± 0.83	3-16
Number of effective tillers per plant	9.4 ± 0.51 (8-11)	7.9 ± 0.5 (7-9)	8.75 ± 0.82	3-13
Panicle length (cm)	23.2 ± 0.15 (22.7-23.6)	17.5 ± 0.33 (17-19)	19.8 ± 0.34	17.1-22.7
Grain yield per plant (g)	10.8 ± 0.46 (6.58-11.4)	9.83 ± 0.47 (8.9-11.7)	8.78 ± 1.11	3.77-19.3
1000 grain weight (g)	20.6 ± 0.37 (19.8-21.9)	18.9 ± 0.38 (17.7-20)	21 ± 0.58	15.8-27.3
Grain length-breadth ratio	4.12 ± 0.04 (4.02-4.21)	3.55 ± 0.04 (3.2-4.0)	3.89 ± 0.1	3.51-4.21

4.2.2 Net house evaluation of MASARB25 x PAU201 F_3 population

Large variation was observed among 21 MASARB25 x PAU201 F_3 plants for plant height (40-95 cm, MASARB25–69.2 cm and PAU201–64.3 cm), panicle length (7.13-25.3 cm, MASARB25–21.3 cm and PAU201–20.3 cm), numbers of effective tillers per plant (1-8, MASARB25 –4.8 and PAU201–4.2), grain yield per plant (0.03-11 g, MASARB25–3.65 g and PAU201–2.23 g), 1000 grain weight per plant (0.3-24.1 g, MASARB25–19 g and PAU201–16.5 g), grain length-breadth ratio (4.26-5.68, MASARB25–5.15 and PAU201–4.52), root length (21-48 cm, MASARB25–41.6 cm and PAU201–38.5 cm), root thickness

(1.86-23.1 mm, MASARB25–15.1 mm and PAU201–6.85 mm), fresh root weight (1.08-24.8 g, MASARB25 – 8.15 g and PAU201–3.35 g) and dry root weight (0.45-8.31 g, MASARB25 – 2.45 g and PAU201–1.1 g) (Table 4.8). Data on various physio-morphological traits of the individual F_3 plants is given in Annexure IV.

Table 4.8: Variation for physio-morphological and root morphological traits in MASARB25 x PAU201 F_3 population grown in pots in the net house under water-limited conditions

Trait	MASARB25 Mean (Range)	PAU201 Mean (Range)	MASARB25 x PAU201 F_3 population (21 plants)	
			Mean	Range
Plant height (cm)	69.2 ± 0.74 (67-71)	64.3 ± 0.86 (62-67)	63.6 ± 1.4	40-95
Number of effective tillers per plant	4.8 ± 0.2 (4-5)	4.2 ± 0.38 (3-5)	3.17 ± 0.96	1-8
Panicle length (cm)	21.3 ± 0.54 (19.8-24.7)	20.3 ± 0.81 (22.5-24.9)	17.8 ± 1.32	7.13-25.3
Grain yield per plant (g)	3.65 ± 0.26 (2.97-4.2)	2.23 ± 0.43 (1.27-3.45)	3.14 ± 1.5	0.03-11
1000 grain weight (g)	19 ± 0.71 (16.7-20.6)	16.5 ± 0.81 (14-18.3)	15.3 ± 1.4	0.3-24.1
Grain length-breadth ratio	5.15 ± 0.04 (5.01-5.26)	4.52 ± 0.07 (4.4-4.8)	4.83 ± 0.17	4.26-5.68
Root length (cm)	41.6 ± 0.51 (40-43)	38.5 ± 0.81 (37-41)	35.4 ± 1.17	21-48
Root thickness (mm)	15.1 ± 0.47 (13.6-16.2)	6.85 ± 0.15 (6.3-7.2)	10.5 ± 1.9	1.86-23.1
Fresh root weight (g)	8.15 ± 0.5 (6.5-9.45)	3.35 ± 0.07 (3.13-3.55)	8.3 ± 2.2	1.08-24.8
Dry root weight (g)	2.45 ± 0.55 (1.64-4.62)	1.1 ± 0.12 (0.68-1.4)	2.28 ± 1.12	0.45-8.31

4.2.3 Correlation coefficient analysis

Phenotypic correlation coefficient analysis of MASARB25 x PAU201 F_3 population, evaluated in field under aerobic condition revealed a positive correlation between grain yield per plant with number of effective tillers per plant (0.694, p=0.01), plant height (0.414, p=0.05) and number of panicles per plant (0.552, p=0.01). A positive correlation was noticed between number of effective tillers per plant with plant height (0.413, p=0.05) (Table 4.9).

Table 4.9: Correlation analysis between different morphological traits in MASARB25 x PAU201 F_3 population grown in field under aerobic conditions

Trait	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Plant height (cm)	1						
Number of panicles per plant	0.159	1					
Number of effective tillers per plant	0.413*	0.718**	1				
Panicle length (cm)	0.110	0.000	0.101	1			
Grain yield per plant (g)	0.414*	0.552**	0.694**	0.327	1		
1000 grain weight (g)	0.101	0.224	0.243	-0.209	0.334	1	
Grain length-breadth ratio	-0.018	0.133	0.153	-0.100	-0.054	0.139	1

Table 4.9.1: Correlation analysis between different morphological traits in MASARB25 x PAU201 F_3 population grown under water-limited conditions in net house

Trait	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Root length (cm)	1									
Fresh root weight (g)	0.432*	1								
Dry root weight (g)	0.236	0.835**	1							
Root thickness (mm)	0.348	0.862**	0.783**	1						
Plant height (cm)	-0.010	-0.173	-0.283	-0.175	1					
Number of effective tillers per plant	0.391	0.635**	0.409*	0.543**	0.175	1				
Panicle length (cm)	0.361	0.401	0.367	0.394	0.470*	0.731**	1			
Grain yield per plant (g)	0.395	0.841**	0.704**	0.673**	-0.076	0.732**	0.438*	1		
1000 grain weight (g)	0.514*	0.482*	0.461*	0.396	-0.052	0.545**	0.489*	0.609**	1	
Grain length-breadth ratio	0.217	0.013	0.125	-0.065	0.259	0.113	0.127	0.048	0.151	1

Net house evaluation revealed a positive correlation between grain yield per plant with fresh root weight (0.841, p=0.01), dry root weight (0.704, p=0.01), root thickness (0.673, p=0.01), number of effective tillers per plant (0.732, p=0.01), panicle length (0.438, p=0.05) and 1000 grain weight (0.609, p=0.01). A positive correlation was found between number of effective tillers per plant with fresh root weight (0.635, p=0.01), dry root weight (0.409, p=0.05), root thickness (0.543, p=0.01), panicle length (0.731, p=0.01) and 1000 grain weight (0.545, p=0.01). 1000 grain weight showed positive correlation with root length (0.514, p=0.05), fresh root weight (0.482, p=0.05), dry root weight (0.461, p=0.05) and panicle length (0.489, p=0.01) (Table 4.9.1).

4.2.4 Microsatellite marker analysis of MASARB25 x PAU201 F_3 plants

Microsatellite marker data was generated using three molecular markers for twelve MASARB25 x PAU201 F_3 plants.

4.2.5 Variation in allelic profile

Agarose gel displaying allelic polymorphism in MASARB25 x PAU201 F_3 plants for SSR marker is shown in (Plate 2). The overall size of PCR amplified products ranged between 110 (RM200) and 220 bp (RM276) (Table 4.10). At a SSR locus, all the F_3 plants had MASARB25 specific allele or PAU201specific allele or absence for both the allele (Table 4.10). The distribution of MASARB25 and PAU201 specific alleles are shown in (Table 4.10.1). Many plants had two alleles from PAU201 while plant number Q-1-1 had the maximum number of alleles from MASARB25.

Table 4.10: Allelic profile (in bp) of MASARB25 x PAU201 F_3 population and parental rice genotypes at three SSR loci

SSR Marker	Allele size (bp)		Number of plants with		
	MASARB25	PAU201	MASARB25 specific allele	PAU201 specific allele	Both MASARB25 and PAU201 allele
RM200	130	110	7	5	-
RM276	220	200	5	6	-
RM453	200	180	5	5	-

4.2.6 Genetic relationship among MASARB25 x PAU201 F_3 population

NTSYS-pc UPGMA tree cluster analysis placed the F_3 plants in two major groups at the similarity coefficient of 0.39. Major group I consisted of PAU201and six F_3 plants while the major group II had MASARB25 and three F_3 plants. Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas 12 F_3 plants were interspersed between the two parental lines with inclination towards PAU201 (Figure 4.3).

Table 4.10.1: Allelic profile (in bp) of selected MASARB25 x PAU201 F_3 plants and parental genotypes at three SSR loci

Plant	RM200	RM276	RM453
MASARB25	130	220	200
PAU201	110	200	180
Q-8-3	130	220	180
Q-8-2	130	200	200
Q-10-1	130	220	180
Q-1-1	130	220	200
Q-25-2	130	200	180
Q-5-2	130	200	180
Q-5-1	130	200	200
Q-17-1	110	200	200
Q-25-3	110	220	200
Q-9-1	110	-*	180
Q-2-1	110	220	-
Q-2-3	110	200	-

*null allele

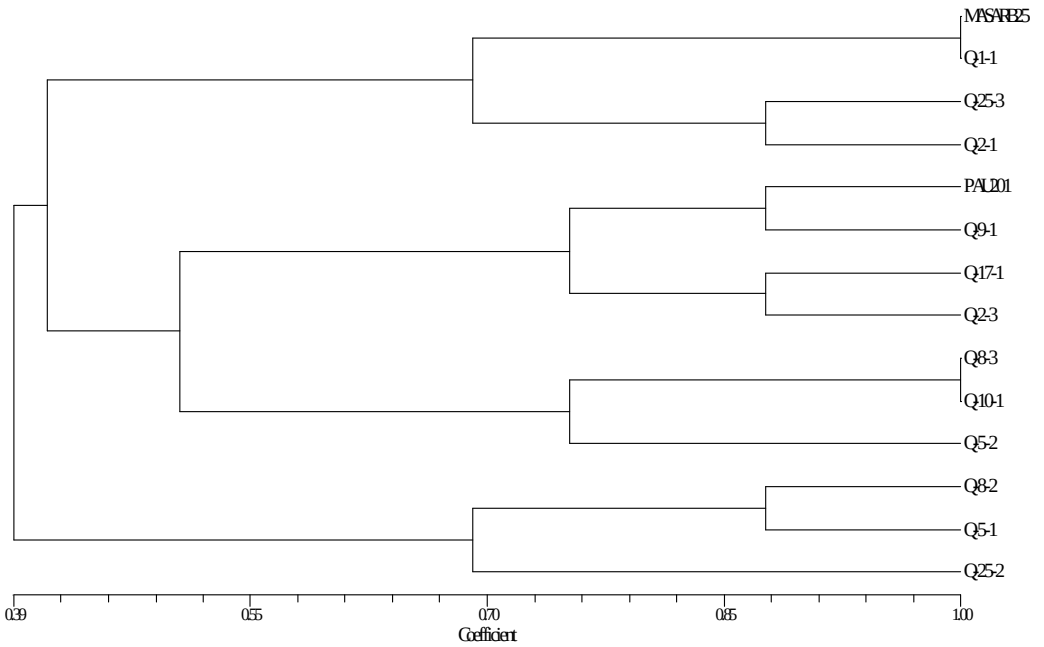

Fig. 4.3: Dendrogram (NTSYS-pc) showing genetic relationships among MASARB25 x PAU201 F_3 plants and parental genotypes based on similarity matrix data obtained using three SSR markers

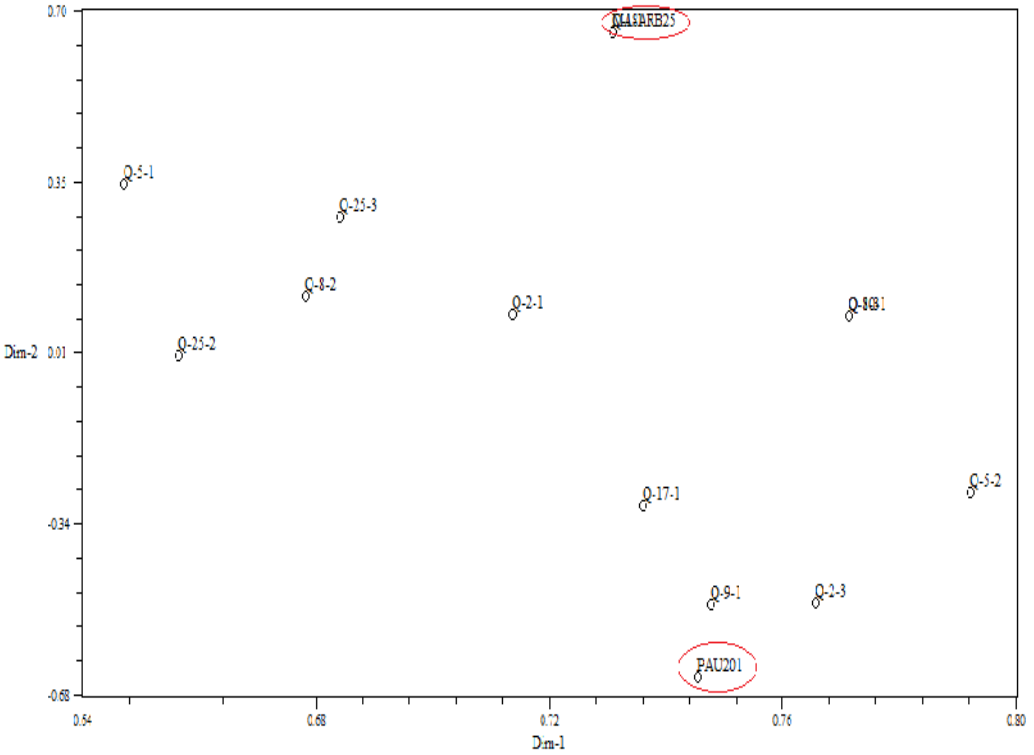

Fig. 4.4: Two-dimensional PCA scaling of MASARB25 x PAU201F_3 plants and parental genotypes using SSR similarity matrix data at three loci

4.2.7 Selection of promising plants

Based on field data, a total of eight MASARB25 x PAU201 F_3 plants were selected on the basis of plant height, number of effective tillers per plant, grain length-breadth ratio and yield per plant (Table 4.11). Based on net house data, a total of four MASARB25 x PAU201 F_3 plants have been selected on the basis of root morphology (length & biomass) and seed length-breadth ratio (Table 4.12).

Table 4.11: Data on physio-morphological traits of selected MASARB25 x PAU201 F_3 plants

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight(g)	Grain length-breadth ratio
PAU201	86.7 ± 0.62	9.2 ± 0.62	7.9 ± 0.49	17.5 ± 0.33	9.83 ± 0.47	18.9 ± 0.38	3.55 ± 0.04
MASARB25	90.6 ± 0.75	11.8 ± 0.58	9.4 ± 0.51	23.2 ± 0.15	10.8 ± 0.46	20.6 ± 0.37	4.12 ± 0.04
Q-1-1	77	12	10	20.5	10.2	18	4.09
Q-2-3	71	9	8	21.1	10.6	23	3.96
Q-5-1	86	15	13	21.1	19.3	24.7	3.82
Q-5-2	110	10	9	19.3	13.7	25.2	3.83
Q-8-3	81	11	11	17.7	10.4	24.6	4.17
Q-9-1	77	15	12	22.7	17.5	19.8	3.68
Q-17-1	76	9	9	20.3	10.5	20.7	3.84
Q-25-1	71	12	10	19.2	10.9	21.4	4.12

Table 4.12: Data on physio-morphological and root traits of selected MASARB25 x PAU201 F_3 plants

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length(cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	41.6 ± 0.51	8.15 ± 0.5	2.45± 0.55	15.1±0.47	69.2± 0.74	4.8± 0.2	21.3± 0.54	3.65± 0.26	19± 0.71	5.15± 0.04
PAU201	38.5 ± 0.81	3.35± 0.07	1.1 ± 0.12	6.85± 0.15	64.3± 0.86	4.2± 0.38	20.3± 0.81	2.23± 0.43	16.5± 0.81	4.52±0.07
Q-2-1	45	10.9	3.34	9.59	64	4	21.1	4.63	19.6	5.68
Q-8-2	44	20.3	3.64	22.7	73	5	24.5	7.56	15.2	4.26
Q-10-1	48	11.7	3.03	13.4	77	4	22	3.09	19.4	5.58
Q-25-3	42	24.8	4.81	23.1	60	8	21.9	3.09	22.5	4.63

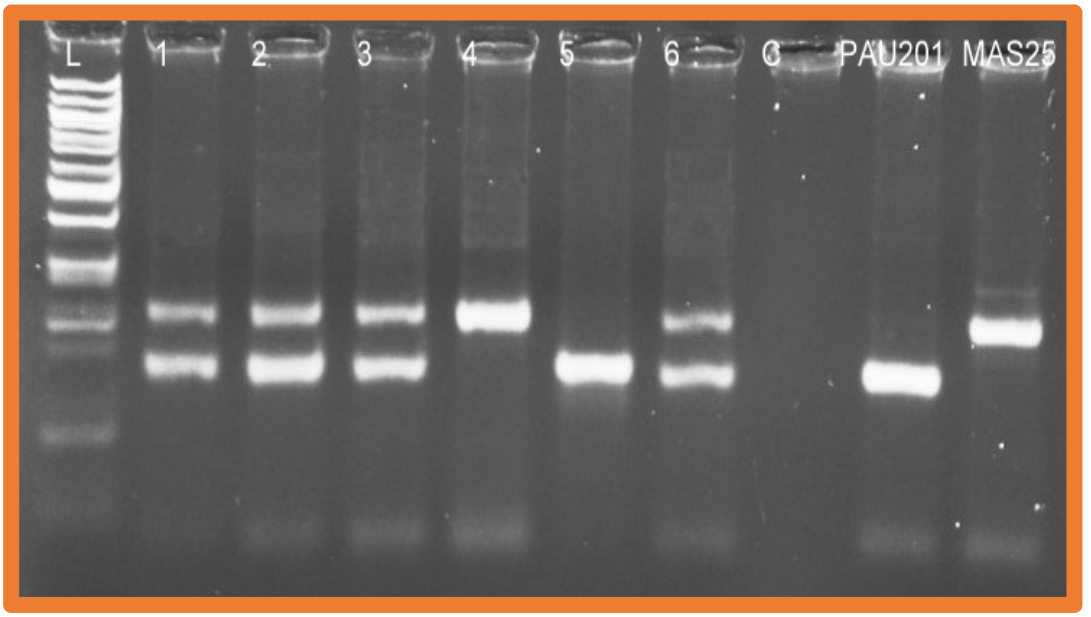

Plate 1- Agarose gel showing allelic polymorphism at RM440 locus among MAS25 x PAU201 F_3 plants

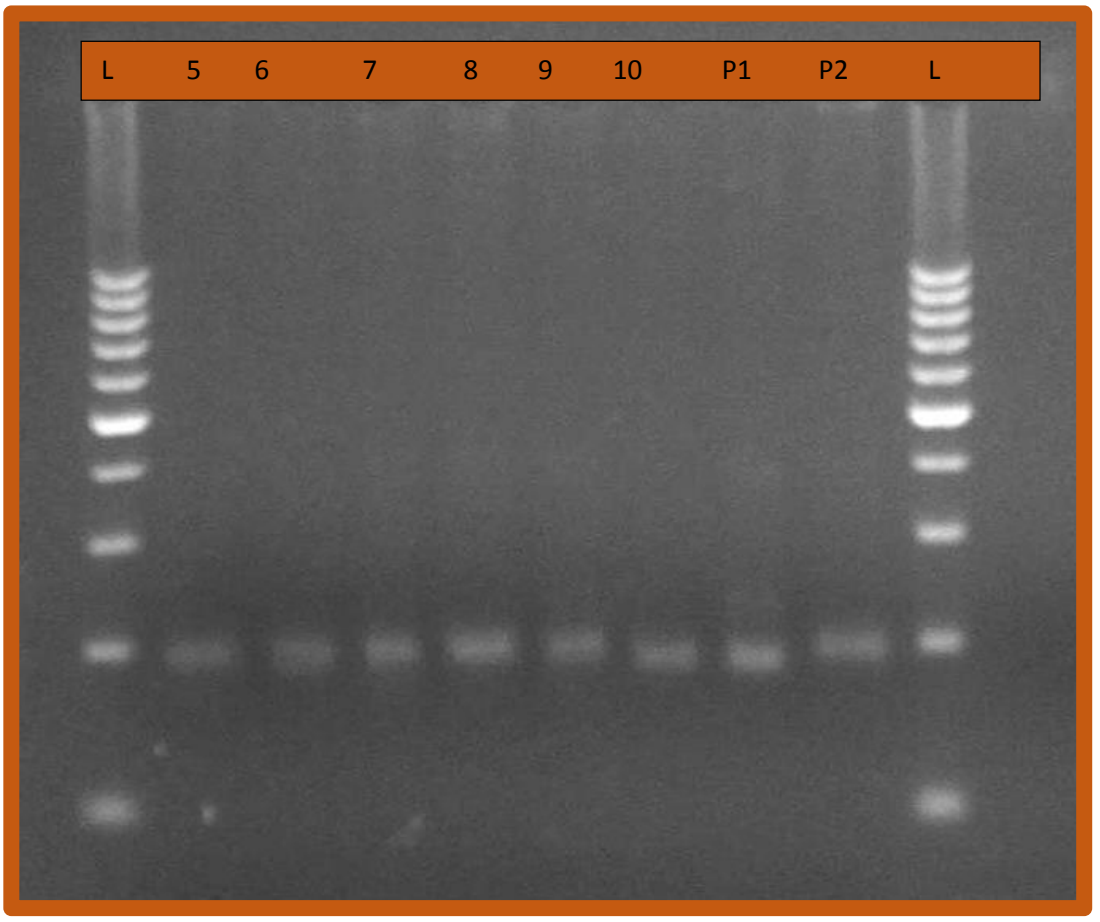

Plate 2- Agarose gel showing allelic polymorphism at RM453 locus among MASARB25 x PAU201 F_3 plants, where L=100 bp ladder, 5-10 F_3 plants, P1=PAU201 and P2=MASARB25, respectively.

4.3.1 Field evaluation of PAU201 x MAS26 F_3 population

Huge variation was observed among 30 PAU201 x MAS26 F_3 plants for plant height (60-108 cm, PAU201–86.7 cm and MAS26–92.1 cm), panicle length (16.9-22.7 cm, PAU201 –17.5cm and MAS26–22.8 cm), number of panicles per plant (4-16, PAU201–9.2, MAS26–11.9), number of effective tillers per plant (4-16, PAU201–7.9 and MAS26–9.6), grain yield per plant (1.39-19.8 g, PAU201–9.83 g and MAS26–11.9 g), 1000 grain weight (12.4-27.8 g, PAU201–18.9 g and MAS26–21 g) and grain length-breadth ratio (3.48-5.18, PAU201–3.55 and MAS26–4.25) (Table 4.13). Data on physio-morphological traits of the individual F_3 plants is given in Annexure V.

Table 4.13: Range for physio-morphological traits in PAU201 x MAS26 F_3 population grown under aerobic conditions

Trait	PAU201 Mean (Range)	MAS26 Mean (Range)	PAU201 x MAS26 F_3 population (30 plants)	
			Mean	Range
Plant height (cm)	86.7 ± 0.62 (86-88)	92.1 ± 0.58 (91-94)	81.6 ± 1.4	60-108
Number of panicles per plant	9.2 ± 0.19 (7-11)	11.9 ± 0.55 (11-14)	9.92 ± 0.96	4.0-16
Number of effective tillers per plant	7.9 ± 0.5 (7-9)	9.6 ± 0.68 (8-12)	9.03 ± 0.9	4.0-16
Panicle length (cm)	17.5 ± 0.33 (17-19)	22.8 ± 0.18 (22.8-25)	20 ± 0.37	16.9-22.7
Grain yield per plant (g)	9.83 ± 0.47 (8.9-11.7)	11.9 ± 0.52 (11.2-12.4)	8.94 ± 1.26	1.39-19.8
1000 grain weight (g)	18.9 ± 0.38 (17.7-20)	21 ± 0.31 (19.2-23)	21 ± 0.76	12.4-27.8
Grain length-breadth ratio	3.55 ± 0.04 (3.2-4.0)	4.25 ± 0.04 (4.15-4.31)	3.86 ± 0.16	3.48-5.18

4.3.2 Net house evaluation of PAU201 x MAS26 F_3 population

PAU201x MAS26 F_3 population (17 plants) showed enormous variation for plant height (35-89 cm, PAU201–64.3 cm, MAS26–68.4 cm), panicle length (6.47-23.9 cm, PAU201–20.3 cm and MAS26–20.7 cm), number of effective tillers per plant (1.0-6.0, PAU201–4.2 and MAS26–4.6), grain length-breadth ratio (2.13-5.59, PAU201–4.52 and MAS26 –4.87), grain yield per plant (0.05-6.28 g, PAU201–2.23 g and MAS26–3.76 g), 1000 grain weight per plant (0.5 -21 g, PAU201–16.5 g and MAS26 –20.8 g), root length (27-47.8 cm, PAU201–38.5 cm and MAS26–40.5 cm), root thickness (5.23-17.8 mm, PAU201–6.85 mm and MAS26–13.5 mm), fresh root weight (2.54-10.8 g, PAU201–3.35 g and MAS26 –7.3 g), dry root weight (0.64-2.94 g, PAU201–1.1 g and MAS26–2.14 g) (Table 4.14). Data of the individual F_3 plant is given in Annexure VI.

Table 4.14: Variation for physio-morphological and root morphological traits in PAU201 x MAS26 F_3 population grown in pots in the net house under water-limited conditions

Trait	PAU201 Mean (Range)	MAS26 Mean (Range)	PAU201 x MAS26 F_3 population (17 plants)	
			Mean	Range
Plant height (cm)	64.3 ± 0.86 (62-67)	68.4 ± 1.29 (66-73)	61.3 ± 1.6	35-89
Number of effective tillers per plant	4.2 ± 0.38 (3-5)	4.6 ± 0.25 (4-5)	3.25 ± 0.7	1-6
Panicle length(cm)	20.3 ± 0.81 (22.5-24.9)	20.7 ± 0.78 (18.4-22.8)	17.3 ± 1.04	6.47-23.9
Grain yield per plant (g)	2.23 ± 0.43 (1.27-3.45)	3.76 ± 0.45 (1.98-4.34)	2.09 ± 1.1	0.05-6.28
1000 grain weight (g)	16.5 ± 0.81 (14-18.3)	20.8 ± 0.38 (19.8-21.9)	12.2 ± 1.54	0.5-21
Grain length-breadth ratio	4.52 ± 0.07 (4.4-4.8)	4.87 ± 0.01 (4.86-4.88)	4.73 ± 0.3	2.13-5.59
Root length(cm)	38.5 ± 0.81 (37-41)	40.5 ± 0.6 (39-42)	35.7 ± 1.08	27-47.8
Fresh root weight(g)	3.35 ± 0.07 (3.13-3.55)	7.3 ± 0.14 (6.9-7.7)	5.67 ± 1.02	2.54-10.8
Dry root weight(g)	1.1 ± 0.12 (0.68-1.4)	2.14 ± 0.65 (0.69-4.13)	1.67 ± 0.53	0.64-2.94
Root thickness (mm)	6.85 ± 0.15 (6.3-7.2)	13.5 ± 0.67 (11.7-15.8)	9.46 ± 1.1	5.23-17.8

4.3.3 Correlation coefficient analysis

Phenotypic correlation coefficient analysis of PAU201 x MAS26 F_3 population evaluated in field revealed a positive correlation between grain yield per plant with number of effective tillers per plant (0.818, p=0.01), number of panicles per plant (0.679, p=0.01), 1000 grain weight (0.479, p=0.01) and grain length-breadth ratio (0.356, p=0.05). A positive correlation was observed between number of effective tillers per plant with 1000 grain weight (0.430, p=0.05) and number of panicles per plant (0.887, p=0.01).Grain length-breadth ratio showed positive correlation with number of effective tillers per plant (0.373, p=0.05) and number of panicles per plant (0.398, p=0.05) (Table 4.15).

Phenotypic correlation coefficient analysis based on net house data revealed a positive correlation between grain yield/plant with root thickness (0.537, p=0.05) and 1000 grain weight (0.483, p=0.05). Fresh root weight revealed a positive correlation with root length (0.705, p=0.01) and root thickness (0.776, p=0.01). A positive correlation was noticed between dry root weight with root thickness (0.666, p=0.01), root length (0.696, p=0.01) and fresh root weight (0.865, p=0.01) (Table 4.15.1). But no significant correlation with yield and root biomass was observed.

Table 4.15: Correlation analysis between different morphological traits in PAU201 x MAS26 F_3 population in field under aerobic conditions

Trait	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight(g)	Grain length-breadth Ratio
Plant height (cm)	1						
Number of panicles per plant	0.068	1					
Number of effective tillers per plant	0.170	0.887**	1				
Panicle length (cm)	0.493**	0.007	0.143	1			
Grain yield per plant (g)	0.346	0.679**	0.818**	0.260	1		
1000 grain weight (g)	-0.030	0.263	0.430*	0.283	0.479**	1	
Grain length-breadth ratio	0.198	0.398*	0.373*	0.279	0.356*	0.192	1

Table 4.15.1: Correlation analysis between different morphological traits in PAU201 x MAS26 F_3 population grown in pots in net house under water-limited conditions

Trait	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height(cm)	No. of effective tiller per plant	Panicle length (cm)	Grain yield per plant (g)	1000 Grain weight (g)	Grain length-breadth ratio
Root length (cm)	1									
Fresh root weight (g)	0.705**	1								
Dry root weight (g)	0.696**	0.865**	1							
Root thickness (mm)	0.626**	0.776**	0.666**	1						
Plant height (cm)	0.081	0.359	0.188	0.292	1					
Number of effective tillers per plant	-0.137	-0.113	-0.037	0.080	0.009	1				
Panicle length (cm)	0.106	0.225	0.302	0.378	0.303	0.779**	1			
Grain yield per plant (g)	0.296	0.331	0.195	0.537*	0.221	0.427	0.414	1		
1000 Grain weight (g)	-0.022	0.041	-0.230	0.164	0.558*	0.066	0.157	0.483*	1	
Grain length-breadth ratio	-0.189	0.039	-0.388	0.107	0.146	0.034	-0.029	0.329	0.504*	1

4.3.4 Microsatellite marker analysis of PAU201 x MAS26 F_3 plants

Microsatellite marker data was generated using seven molecular markers for twelve PAU201 x MAS26 F_3 plants.

4.3.5 Variation in allelic profile

Agarose gel displaying allelic polymorphism in PAU201 x MAS26 F_3 plants for SSR marker is shown in (Plate 4). The overall size of PCR amplified products ranged between 108 bp (RM528) and 200 bp (RM231) (Table 4.16). At a SSR locus, all the F_3 plants had MAS26 specific allele or PAU201specific allele or alleles specific for both the parents indicating the heterozygous state. The distribution of PAU201 and MAS26 specific alleles are shown in (Table 4.16.1).

Table 4.16: Allelic profile (in bp) of PAU201 x MAS26 F_3 population (12 plants) and parental rice genotypes at seven SSR loci.

SSR Marker	Allele size (bp)		Number of plants with		
	PAU201	MAS26	PAU201 specific allele	MAS26 specific allele	Both PAU201 and MAS26 allele
RM224	150	185	6	5	-
RM231	200	180	5	4	1
RM234	180	150	4	2	3
RM276	140	180	4	4	1
RM525	110	120	7	5	-
RM528	108	115	6	5	-
RM547	130	150	3	5	-

4.3.6 Genetic relationship among PAU201 x MAS26 F_3 population

Similarity coefficient data based on the seven SSR markers was used to calculate the coefficient values among the selected twelve PAU201x MAS26 F_3 plants and parental rice genotypes and subjected to NTSYS-pc UPGMA tree cluster analysis (Figure 4.6). All the 12 F_3 plants clustered in two major groups at the similarity coefficient of 0.36. Major group I consisted of PAU201and seven F_3 plants while the major group II had MAS26 and five F_3 plants. Genetic relationships among these rice genotypes were also assessed by PCA analysis (NTSYS-pc). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas eight F_3 plants were interspersed between the two parental lines with inclination towards MAS26 (Figure 4.5).

Table 4.16.1: Allelic profile (in bp) of PAU201 x MAS26 F_3 plants and parental genotypes at seven SSR loci

Genotype	RM224	RM231	RM234	RM276	RM525	RM528	RM547
PAU201	150	200	180	140	110	108	130
MAS26	180	180	150	180	120	115	150
P-48-2	180	180	150	140	110	115	150
P-48-3	180	180	150	140	110	115	150
P-32-3	180	200	150,180	140	110	115	150
P-25-1	-	180,200	150.180	140,180	120	115	150
P-25-3	150	200	180	180	120	108	130
P-23-1	150	180	150,180	180	110	108	150
P-23-2	150	-	-	-	110	108	-
P-12-1	150	200	-	180	110	115	130
P-12-2	180	200	-	180	110	108	-
P-12-3	150	-	180	-	120	108	-
P-14-1	180	180	180	180	120	0	130
P-14-3	180	200	180	140	120	108	-*

*null allele

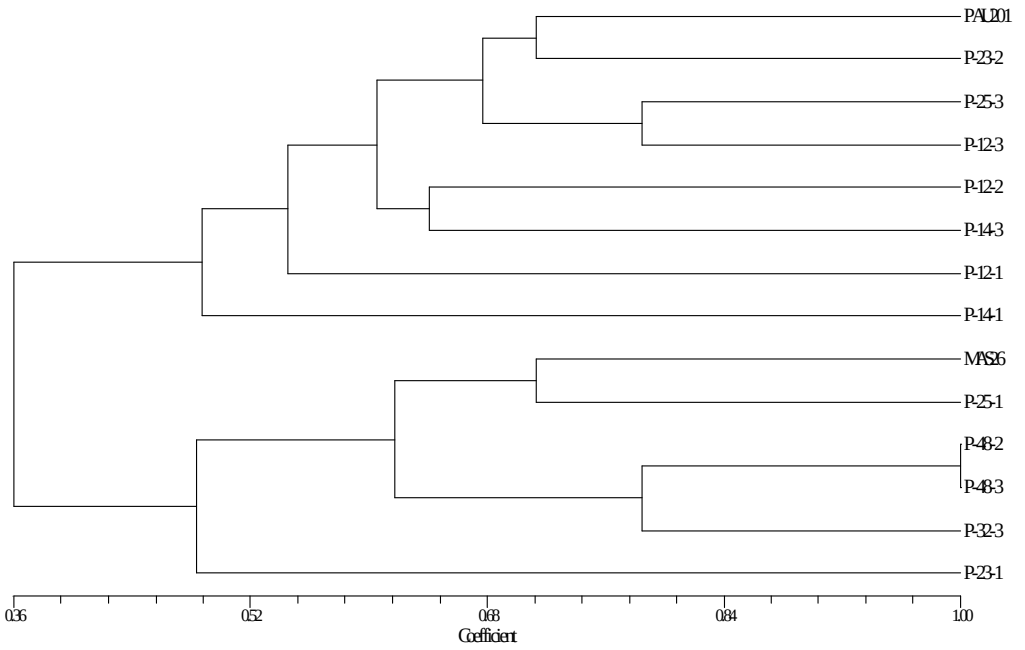

Fig: 4.5: Dendrogram (NTSYSpc) showing genetic relationship among PAU201 x MAS26 F_3 plants and parental genotypes based on similarity matrix data obtained using seven SSR markers

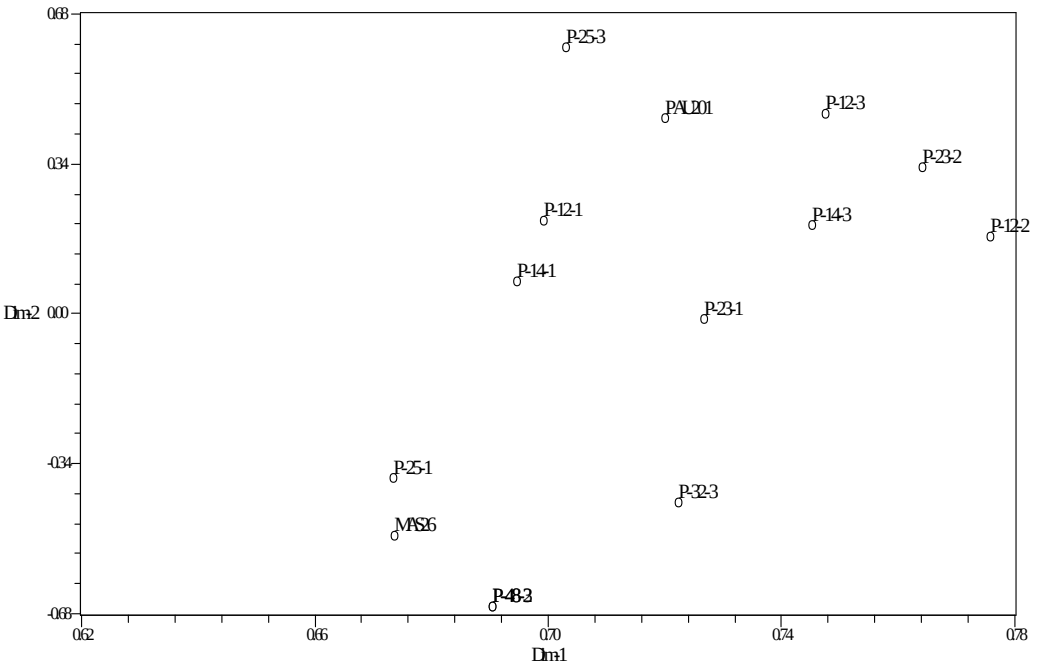

Fig: 4.6: Two-dimensional PCA scaling of PAU201 x MAS26 F_3 plants and parental genotypes using SSR genetic distance matrix data at seven loci

4.3.7 Selection of promising plants

Based on field data, a total of eight PAU201 x MAS26 F_3 plants were selected on the basis of plant height, number of effective tillers per plant, grain length-breadth ratio, grain yield per plant (Table 4.17). Based on net house data, a total of four PAU201 x MAS26 F_3 plants were selected on the basis of root morphology (length & biomass), grain length-breadth ratio and grain yield per plant (Table 4.18).

Table 4.17: Selected PAU201 x MAS26 F_3 plants from field

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g	Grain length-breadth ratio
PAU201	86.7±0.62	9.2±0.19	7.9 ± 0.5	17.5±0.33	9.83±0.47	18.9±0.38	3.55±0.04
MAS26	92.1±0.58	11.9±0.6	9.6±0.68	22.8±0.18	11.9±0.52	21±0.31	4.25±0.04
P-12-1	87	16	16	20.2	19.8	26.8	3.77
P-12-2	75	13	13	20.6	12.5	22.1	3.97
P-12-3	81	11	9	20.8	12.1	20.0	3.82
P-14-1	72	13	13	16.9	12.2	21.9	3.98
P-23-1	92	14	12	20.0	14.31	21.8	3.73
P-23-2	68	11	10	18.0	12.17	20.5	3.92
P-25-1	89	13	12	22.4	12.71	21.1	3.72
P-25-3	91	13	11	21.1	13.92	23.8	5.18

Table 4.18: Selected PAU201 x MAS26 F_3 plants from net house

Genotype	Root length(cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth Ratio
PAU201	38.5± 0.31	3.35± 0.2	1.1± 0.09	6.85± 0.34	64.3± 0.63	4.2± 0.09	20.3± 0.26	2.23± 0.05	16.5± 0.23	4.52±0.12
MAS26	40.5 ± 0.35	7.3 ± 0.37	2.2±0 .11	13.5 ± 0.53	68.4± 0.58	4.6± 0.12	20.7± 0.28	3.76± 0.06	20.8 ±0.34	4.87± 0.14
P-14-3	40.0	7.62	2.31	13.8	62.0	2	14.1	1.17	5.8	4.8
P-32-3	34.0	6.72	1.98	11.8	64.0	6	20.9	6.28	13.1	4.88
P-48-2	47.8	8.04	2.34	14.5	60.0	4	23.9	4.69	10.3	5.04
P-48-3	43.0	10.8	2.62	17.8	89.0	3	22.9	3.41	18.7	4.68

4.4.1 Field evaluation of MASARB25 x HKR47 F_3 population

Enormous variation was observed among 30 MASARB25 x HKR47 F_3 plants for plant height (68-132 cm, MASARB25–90.6 cm and HKR47–85.8 cm), panicle length (11.5-27.7 cm, MASARB25–23.2 cm and HKR47–18.1 cm), number of panicles per plant (2-14, MASARB25–11.8 and HKR47–9.7) , number of effective tillers per plant (2-13, MASARB25 –9.4 and HKR47–8.2), grain yield per plant (2.78-18.2 g, MASARB25–10.8 g and HKR47–9.97 g), 1000 grain weight (12-27.4 g, MASARB25–20.6 g and HKR47–19.8 g) and grain length-breadth ratio (3.6-4.88, MASARB25 – 4.12 and HKR47–3.58) (Table 4.19). Data on physio-morphological traits of the individual F_3 plants is given in Annexure VII.

Table 4.19: Range for physio-morphological traits in MASARB25 x HKR47 F_3 population grown under aerobic conditions in the field

Trait	MASARB25 Mean (Range)	HKR47 Mean (Range)	MASARB25 x HKR47 F_3 population (30 plants)	
			Mean	Range
Plant height (cm)	90.6 ± 0.75 (88-92)	85.8 ± 0.6 (85-87)	80.5 ± 5.54	68-132
Number of panicles per plant	11.8 ± 0.58 (11-14)	9.7 ± 0.93 (8-12)	7.72 ± 1.12	2-14
Number of effective tillers per plant	9.4 ± 0.51 (8-11)	8.2 ± 0.52 (7-9)	7.4 ± 1.09	2-13
Panicle length (cm)	23.2 ± 0.15 (22.7-23.6)	18.1 ± 0.24 (17.6-18.9)	20.3 ± 1.5	11.5-27.7
Grain yield per plant (g)	10.8 ± 0.46 (6.58-11.4)	9.97 ± 0.44 (9.1-11.7)	9.75 ± 1.8	2.78-18.2
1000 grain weight (g)	20.6 ± 0.45 (19.8-21.9)	19.8 ± 0.42 (18.8-20.2)	21.5 ± 2.45	12-27.4
Grain length-breadth ratio	4.12 ± 0.04 (4.02-4.21)	3.58 ± 0.04 (3.34-3.98)	3.99 ± 0.45	3.6-4.88

4.4.2 Net house evaluation of MASARB25 x HKR47 F_3 population

Extensive variation was observed among 17 MASAR25 x HKR47 F_3 plants and parental rice genotypes for plant height (47-77 cm, MASARB25–69.2 cm and HKR47–65 cm), panicle length (10.9-24.4 cm, MASARB25–21.3 cm and HKR47–20.7 cm), number of effective tillers per plant (2-10, MASARB25–4.8 and HKR47–4.2), grain yield per plant (0.87-5.8 g, MASARB25–3.65 g and HKR47–3.33g), 1000 grain weight (9.1-20.2 g, MASARB25 –19 g and HKR47 –18.8 g), grain length-breadth ratio (4.56-5.41, MASARB25 –5.15 and HKR47–4.59), root length (18-48 cm, MASARB25–41.6 cm and HKR47– 39.2 cm), root thickness (4.45-22.6 mm, MAS25–15.1 mm and HKR47–10.4 mm), fresh root weight (1.96-13.5 g, MAS25–8.15 g and HKR47–5.84 g), dry root weight (0.7-5.22 g,

MASARB25 –2.45 g and HKR47–1.62 g) (Table 4.20). Data on physio-morphological traits of the individual F_3 plants is given in Annexure VIII.

Table 4.20: Variation for physio-morphological and root morphological traits in MASARB25 x HKR47 F_3 population grown in pots in the net house under water- limited conditions

Trait	MASARB25 Mean (Range)	HKR47 Mean (Range)	MASARB25 x HKR47 F_3 population (17 plants)	
			Mean	Range
Plant height (cm)	69.2 ± 0.74 (67-71)	65 ± 0.51 (64-67)	64.9 ± 0.84	47-77
Number of effective tillers per plant	4.8 ± 0.2 (4-5)	4.2 ± 0.38 (3-5)	3.99 ± 0.9	2-10
Panicle length(cm)	21.3 ± 0.54 (19.8-24.7)	20.7 ± 0.26 (20.1-21.4)	19.4 ± 0.9	10.9-24.4
Grain yield per plant (g)	3.65 ± 0.26 (2.97-4.2)	3.3 ± 0.18 (2.9-3.7)	2.18 ± 0.96	0.87-5.8
1000 grain weight(g)	19 ± 0.71 (16.7-20.6)	18.8 ± 0.97 (15.6-21.3)	14.2 ± 1.1	9.1-20.2
Grain length-breadth ratio	5.15 ± 0.04 (5.01-5.26)	4.59 ± 0.12 (4.34-4.87)	4.92 ± 0.1	4.56-5.41
Root length (cm)	41.6 ± 0.51 (40-43)	39.2 ± 0.67 (37-41)	36.3 ± 1.26	18-48
Root thickness (mm)	15.1 ± 0.47 (13.6-16.2)	10.4 ± 0.77 (10.2-14.6)	12.2 ± 1.47	4.45-22.6
Fresh root weight (g)	8.15 ± 0.5 (6.5-9.45)	5.84 ± 0.12 (5.5-6.2)	4.97 ± 1.36	1.96-13.5
Dry root weight (g)	2.45 ± 0.55 (1.64-4.62)	1.62 ± 0.29 (1.03-2.67)	1.66 ± 0.9	0.7-5.22

4.4.3 Correlation coefficient analysis

Phenotypic correlation coefficient analysis of MASARB25 x HKR47 F_3 population evaluated in field revealed a positive correlation between grain yield per plant with number of panicles per plant (0.867, p=0.01), grain length-breadth ratio (0.788, p=0.01) and 1000 grain weight (0.731, p=0.05) . A positive correlation was observed between 1000 grain weight with number of panicles per plant (0.472, p=0.05), number of effective tillers per plant(0.432, p=0.05), plant height (0.917, p=0.01) and panicle length (0.878, p=0.01). A positive correlation was noticed between number of effective tillers per plant with plant height (0.527, p=0.01) and panicle length (0.588, p=0.01) (Table 4.21).

A negative correlation was observed between number of panicles per plant with plant height (-0.813, p=0.01) and with panicle length (-0.657, p=0.01). Grain yield per plant

Plate 6: F_3 Plants derived from MASARB 25 x HKR 47 grown in field and net house under aerobic conditions.

Plate 7: Variation among different root parameters in different F_3 plants alongwith MASARB 25 and HKR 47.

showed negative correlation with plant height (-0.774, p=0.01) and panicle length (-0.647, p=0.01) (Table 4.21).

Net house evaluation under aerobic condition revealed a positive correlation between grain yield per plant with dry root weight (0.465, p=0.05), fresh root weight (0.579, p=0.01), number of effective tillers per plant (0.572, p=0.05), 1000 grain weight (0.811, p=0.01) and panicle length (0.528, p=0.05). A positive correlation was found between number of effective tillers per plant with root thickness (0.571, p=0.05), fresh root weight (0.670, p=0.01) and dry root weight (0.681, p=0.01). Root thickness showed positive correlation with fresh root weight (0.877, p=0.01), dry root weight (0.849, p=0.01) and root length (0.554, p=0.05). A positive correlation was found between fresh root weight with plant height (0.910, p=0.01), panicle length (0.918, p=0.01) and 1000 grain weight (0.929, p=0.01). Dry root weight also showed positive correlation with root length (0.488, p=0.05) and fresh root weight (0.951, p=0.01) (Table 4.21.1).

Table 4.21: Correlation analysis between different morphological traits in MASARB25 x HKR47 F_3 population in field under aerobic conditions

Trait	Plant height (cm)	Number of effective tillers per plant	Number of panicles per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Plant height (cm)	1						
Number of effective tillers per plant	0.527**	1					
Number of panicles per plant	-0.813**	-0.180	1				
Panicle length (cm)	0.896**	0.588**	-0.657**	1			
Grain yield per plant (g)	-0.774**	-0.142	0.867**	-0.647**	1		
1000 grain weight(g)	0.917**	0.432*	0.472*	0.878**	0.731*	1	
Grain length-breadth ratio	-0.852**	-0.169	0.854**	-0.803**	0.788**	-0.193	1

Table 4.21.1: Correlation analysis between different morphological traits in MASARB25 x HKR47 F_3 population grown under water-limited conditions in net house

Trait	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
Root length (cm)	1									
Fresh root weight (g)	0.598**	1								
Dry root weight (g)	0.488*	0.951**	1							
Root thickness (mm)	0.554*	0.877**	0.849**	1						
Plant height (cm)	0.240	0.388	0.299	0.322	1					
Number of effective tillers per plant	0.198	0.670**	0.681**	0.571*	-0.042	1				
Panicle length (cm)	0.077	0.265	0.247	0.431	0.296	0.340	1			
Grain yield per plant (g)	0.423	0.579**	0.465*	0.453	0.371	0.572*	0.528*	1		
1000 grain weight (g)	0.380	0.260	0.138	0.255	0.172	0.223	0.572*	0.811**	1	
Grain length-breadth ratio	-0.071	0.238	0.338	0.347	-0.315	0.400	0.293	0.015	-0.119	1

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4.4.4 Microsatellite marker analysis of MASARB25 x HKR47 F_3 plants

Microsatellite marker data was generated using five molecular markers for eighteen selected MASARB25 x HKR47 F_3 plants.

4.4.5 Variation in allelic profile

Agarose gel displaying allelic polymorphism in MASARB25 x HKR47 F_3 plants for SSR marker is shown in (Plate 4). The overall size of PCR amplified products ranged between 110 bp (RM175) and 235 bp (RM200) (Table 4.22). At a SSR locus, all the F_3 plants had MASARB25 specific allele or HKR47specific allele or recombinant allele or null allele (Table 4.22). The distribution of PAU201 and MAS25 specific alleles are shown in (Table 4.22.1). Plant number M-21-3, M-34-3, M-34-1, M-34-2 and M-21-1 had the maximum alleles from MASARB25 while plant number M-21-1, M-22-2, M-13-2, M-14-2 and M-19-2 had the maximum number of alleles from HKR47.

Table 4.22: Allelic profile (in bp) of selected MASARB25 x HKR47 F_3 plants and parental rice genotypes at five SSR loci

SSR Marker	Allele size (bp)		Number of plants with		
	MASARB25	HKR47	MASARB25 Specific allele	HKR47specific allele	Recombinant allele
RM175	250	110	1	5	7*
RM200	205	235	10	7	0
RM217	200	175	5	5	0
RM276	100	200	10	4	3**
RM475	160	180	3	5	3***

*** 120 bp & 190 bp alleles; ** 180 bp alleles; *** 150 bp alleles**

4.4.6 Genetic relationship among MASARB25 x HKR47 F_3 population

Similarity coefficient data based on the five SSR markers was used to calculate the coefficient values among the selected 18 MASARB25 x HKR47 F_3 plants and parental rice genotypes and subjected to NTSYS-pc UPGMA tree cluster analysis. All the F_3 plants clustered in two major groups at the similarity coefficient of 0.54 (Figure 4.2). Major group I consisted of MASARB25 and ten F_3 plants while the major group II had HKR47 and eight F_3 plants. Genetic relationships among these rice genotypes were also assessed by PCA analysis (NTSYS-pc).

Table 4.22.1: Allelic profile (in bp) of selected MASARB25 x HKR47 F_3 plants and parental genotypes at five SSR loci

Genotype	RM175	RM200	RM217	RM276	RM475
MASARB25	250	205	200	100	160
HKR47	110	235	175	200	180
M-21-3	110	205	200	100	180
M-34-3	120	205	200	100	-
M-13-1	120	235	200	100	-
M-34-1	110	205	200	100	150
M-34-2	120	205	200	100	180
M-24-2	120	205	-	-	-
M-39-3	-	-	-	100,180	-
M-19-3	120	235	-	100	-
M-21-1	250	235	175	100	160
M-21-2	110	235	-	100.180	-
M-30-2	-	235	-	100,180	-
M-22-2	-	235	175	200	160
M-13-2	110	235	175	-	160
M-14-2	110	205	-	200	180
M-14-3	-	205	-	200	150
M-19-2	190	205	175	200	180
M-39-1	190	205	175	-*	150
M-39-2	-	205	-	-	180

*null allele

4.4.7 Selection of promising plants

Based on field data, a total of 12 MASARB25 x HKR47 F_3 plants were selected on the basis of plant height, number of effective tillers per plant, grain length-breadth ratio and yield per plant (Table 4.23). Based on net house data, a total of six F_3 plants were selected on the basis of root morphology (length & biomass) and grain length-breadth ratio (Table 4.24).

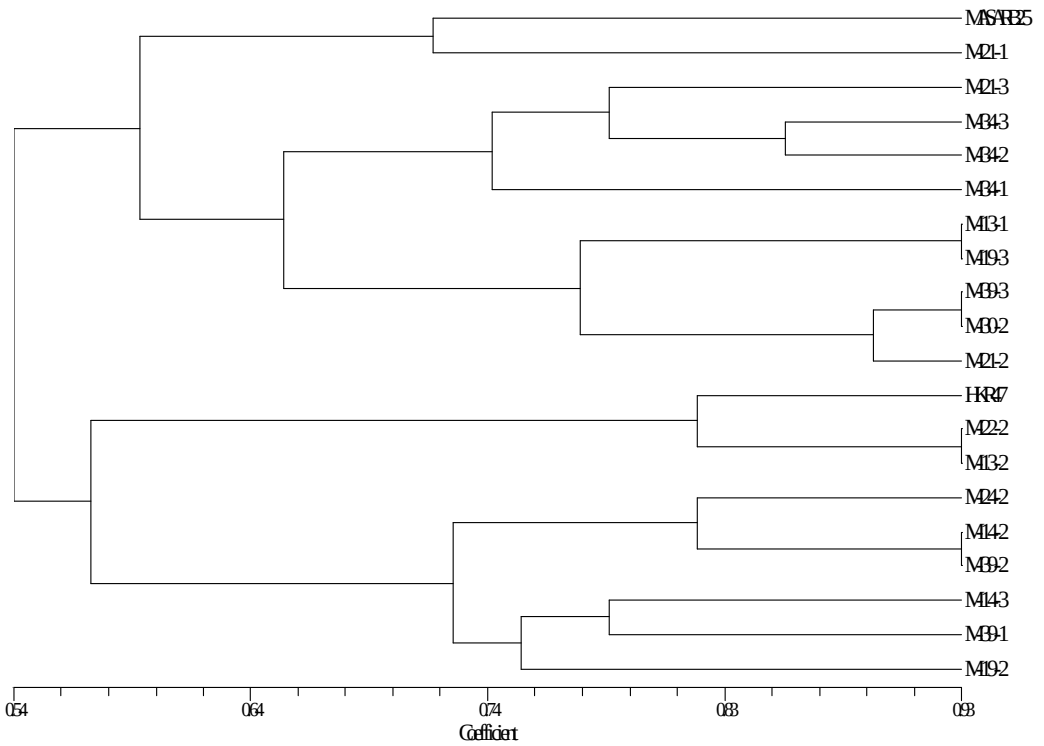

Figure 4.7: Dendrogram (NTSYS-pc) showing genetic relationships among MASARB25 x HKR47 F_3 plants and parental genotypes based on similarity matrix data obtained using five SSR markers

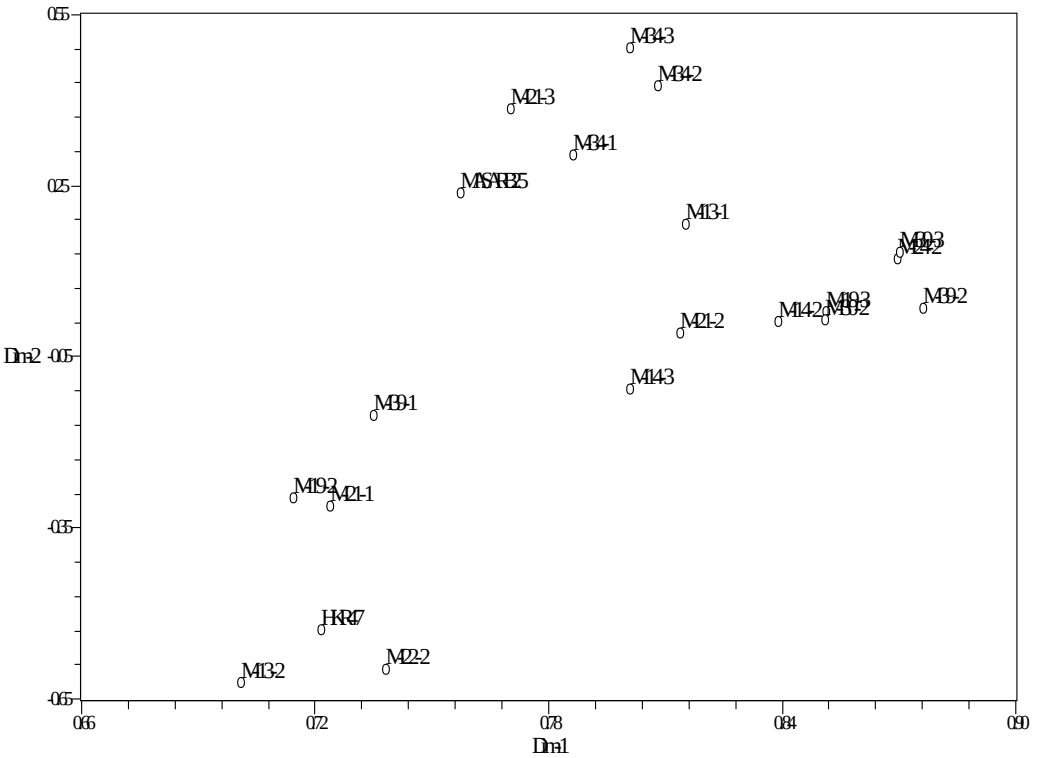

Figure 4.8: Two-dimensional PCA scaling of MASARB25 x HKR47 F_3 plants and parental genotypes using genetic distance matrix data at five loci

Table 4.23: Selected MASARB25 x HKR47 F_3 plants from field

Trait	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	90.6 ± 0.75	11.8 ± 0.58	9.4 ± 0.51	23.2 ± 0.15	10.8 ± 0.46	20.6 ± 0.37	4.12 ± 0.04
HKR47	85.8 ± 0.6	9.7 ± 0.93	8.2 ± 0.52	18.1 ± 0.24	9.97 ± 0.44	19.8 ± 0.42	3.58 ± 0.04
M-13-1	78	14	12	22	17.8	20.1	4.20
M-13-2	75	9	8	18.4	10.6	20.6	3.83
M-14-2	80	12	11	20.6	17.6	19.2	4.25
M-14-3	75	6	6	18.3	12.8	22.9	4.07
M-19-3	81	14	12	21.7	18.2	20.6	3.78
M-21-1	78	13	13	21.2	11.8	20.2	3.85
M-21-3	86	9	9	21.1	10.2	20.8	3.97
M-22-2	78	13	13	19.3	12.2	22.9	4.14
M-24-2	77	8	10	21.1	13.4	20.9	4.06
M-30-2	74	9	8	23.1	12.8	26.7	3.74
M-34-2	84	9	7	24.6	11.0	24.1	4.11
M-39-3	132	4	4	27.7	12.8	20.5	4.08

Table 4.24: Selected MASARB25 x HKR47 F_3 plants from net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length(cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	41.6 ± 0.51	8.15 ± 0.5	2.45 ± 0.55	15.1 ± 0.47	69.2 ± 0.74	4.8 ± 0.2	21.3 ± 0.54	3.65 ± 0.26	19 ± 0.71	5.15 ± 0.04
HKR47	39.2 ± 0.67	5.84 ± 0.12	1.62 ± 0.29	10.4 ± 0.77	65 ± 0.51	4.2 ± 0.38	20.7 ± 0.26	3.3 ± 0.18	18.8 ± 0.97	4.59 ± 0.12
M-34-1	43	10.1	3.0	19.8	77	5	24.4	5.8	20.2	4.90
M-34-3	41.8	7.61	3.45	17.6	71	3	22.8	2.49	17.9	4.83
M-39-2	34.6	4.65	1.32	12.2	61	6	19.9	3.65	19.8	4.81
M-39-1	41	3.08	0.89	8.51	64	4	23.9	3.32	17.6	4.93
M-19-2	43	10.1	3.0	19.8	77	5	24.4	5.8	20.2	4.90
M-21-2	46	13.5	5.22	22.6	65	10	17.5	3.3	10	5.21

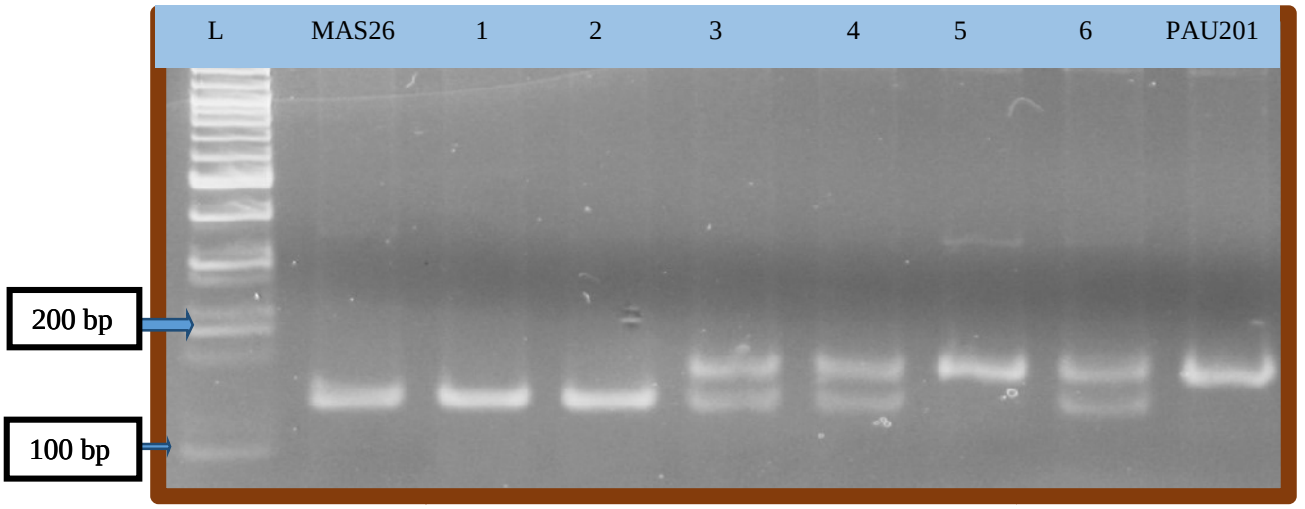

Plate 3- Agarose gel showing allelic polymorphism at RM234 locus among PAU201 x MAS26 F_3 plants

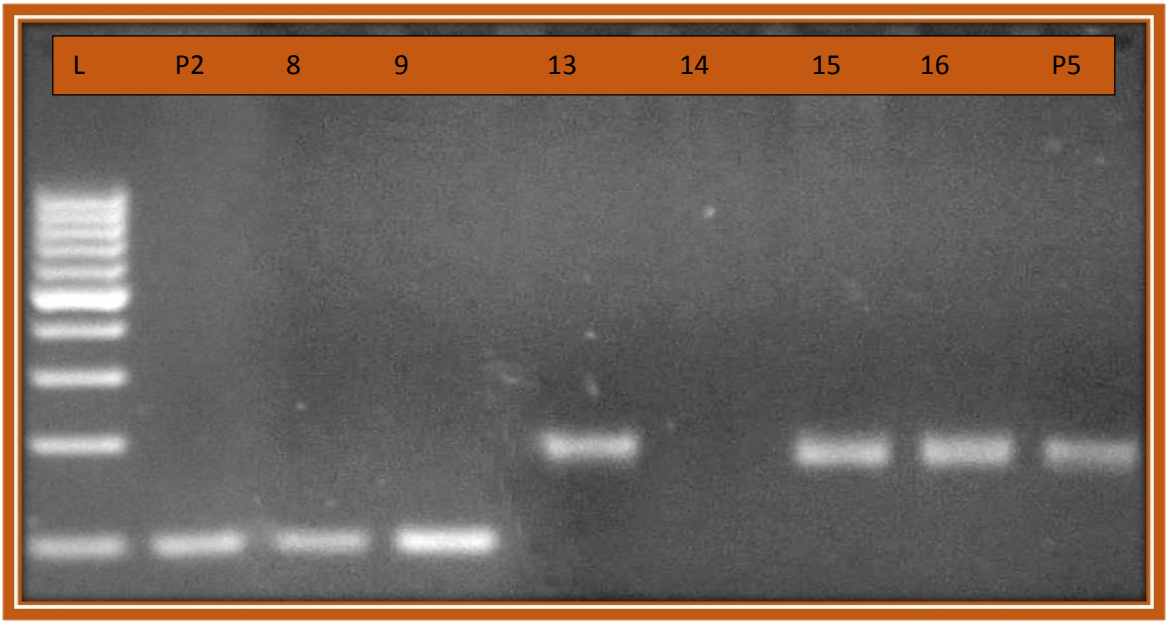

Plate 4- Agarose gel showing allelic polymorphism at RM276 locus among MASARB25 x HKR47 F_3 plants

4.5.1 Field evaluation of MAS25 x HKR47 F_3 population

Enormous variation was observed among 30 MAS25 x HKR47 F_3plants for plant height (64-110 cm, MAS25–91.5 cm and HKR47–85.8 cm), panicle length (15.8-24 cm, MAS25–22.4 cm and HKR47–18.1 cm), number of panicles per plant (4-16, MAS25–11 and HKR47–9.7), number of effective tillers per plant (4-14, MAS25–9.5 and HKR47–8.2), grain yield per plant (2.94-15.8 g, MAS25–11.3 g and HKR47–9.97 g), 1000 grain weight (12.8-23.9 g, MAS25–20.3 g and HKR47–19.8 g) and grain length-breadth ratio (3.43-4.29, MAS25–4.21 and HKR47–3.58) (Table 4.25). Data on physio-morphological traits of the individual F_3 plants is given in Annexure IX.

Table 4.25: Range for physio-morphological traits in MAS25 x HKR47 F_3 population grown under aerobic conditions in the field

Trait	MAS25 Mean (Range)	HKR47 Mean (Range)	MAS25 x HKR47 F_3 population (30 plants)	
			Mean	Range
Plant height (cm)	91.5 ± 0.58 (86-99)	85.8 ± 0.6 (85-87)	83.7 ± 0.9	64-110
Number of panicles per plant	11 ± 0.38 (10-12)	9.7 ± 0.93 (8-12)	8.27 ± 1.1	4.0-16
Number of effective tillers per plant	9.5 ± 0.75 (8-12)	8.2 ± 0.52 (7-9)	7.83 ± 0.89	4-14
Panicle length (cm)	22.4 ± 0.24 (22.8-24.2)	18.1 ± 0.24 (17.6-18.9)	19.9 ± 0.42	15.8-24
Grain yield per plant (g)	11.3 ± 0.56 (9.87-12.5)	9.97 ± 0.44 (9.1-11.7)	7.79 ± 1.1	2.94-15.8
1000 grain weight (g)	20.3 ± 0.35 (19.7-21.4)	19.8 ± 0.42 (18.8-20.2)	20.0 ± 0.66	12.8-23.9
Grain length-breadth ratio	4.21 ± 0.03 (4.2-4.3)	3.58 ± 0.04 (3.34-3.98)	3.91 ± 0.12	3.43-4.29

4.5.2 Net house evaluation of MAS25 x HKR47 F_3 population

Large variation was observed among 12 MAS25 x HKR47 F_3 plants and parental rice genotypes for plant height (50-94 cm, MAS25–68 cm and HKR47–65 cm), panicle length (12.7-22.8 cm, MAS25–20.4 cm and HKR47–20.7 cm), number of effective tillers per plant (2-8, MAS25– 4.5 and HKR47–4.2), grain yield per plant (0.09-7.49 g, MAS25–4.77 g and HKR47–3.33g), 1000 grain weight (0.9-22.2 g, MAS25–21.2 g and HKR47–18.8 g), grain length-breadth ratio (4.3-6.1, MAS25–5.06 and HKR47–4.59), root length (28-45 cm, MAS25–40 cm and HKR47–39.2 cm), root thickness (1.58-26.0 mm, MAS25–12.4 mm and HKR47–10.4 mm), fresh root weight (4.24-23.4 g, MAS25–7.81 g and HKR47–5.84 g), dry root weight (0.53-8.14 g, MAS25–3.42 g and HKR47–1.62 g) (Table 4.26). Data on physio-morphological traits of the individual F_3 plants is given in Annexure X.

Table 4.26: Variation for physio-morphological and root morphological traits in MAS25 x HKR47 F_3 population grown in pots in the net house under water-limited conditions

Trait	MAS25 Mean (Range)	HKR47 Mean (Range)	MAS25 x HKR47 F_3 population (12 plants)	
			Mean	Range
Plant height (cm)	68 ± 1.21 (64-71)	65 ± 0.51 (64-67)	68.5 ± 1.6	50-94
Number of effective tillers per plant	4.5 ± 0.25 (4-5)	4.2 ± 0.38 (3-5)	4.42 ± 0.85	2-8
Panicle length(cm)	20.4 ± 0.6 (18.8-22.3)	20.7 ± 0.26 (20.1-21.4)	19 ± 0.69	12.7-22.8
Grain yield per plant (g)	4.77 ± 0.13 (4.4-5.2)	3.3 ± 0.18 (2.9-3.7)	3.1 ± 1.3	0.09-7.49
1000 grain weight(g)	21.2 ± 0.41 (20.4-22.6)	18.8 ± 0.97 (15.6-21.3)	15.2 ± 1.7	0.9-22.2
Grain length-breadth ratio	5.06 ± 0.11 (4.8-5.3)	4.59 ± 0.12 (4.34-4.87)	5.1 ± 02	4.3-6.1
Root length (cm)	40 ± 0.7 (38-42)	39.2 ± 0.67 (37-41)	37.9 ± 0.76	28-45
Root thickness (mm)	12.4 ± 1.63 (10.1-18.9)	10.4 ± 0.77 (10.2-14.6)	10.3 ± 1.83	1.58-26.0
Fresh root weight (g)	7.81 ± 0.29 (7.2-8.4)	5.84 ± 0.12 (5.5-6.2)	13.3 ± 1.46	4.24-23.4
Dry root weight (g)	3.42 ± 0.32 (2.33-4.17)	1.62 ± 0.29 (1.03-2.67)	3.47 ± 0.98	0.53-8.14

4.5.3 Correlation coefficient analysis

Phenotypic correlation coefficient analysis of MAS25 x HKR47 F_3 population evaluated in field revealed a positive correlation between grain yield per plant with number of panicles per plant (0.815, p=0.01), grain length-breadth ratio (0.470, p=0.01), plant height (0.464, p=0.01) and panicle length (0.638, p=0.01) . A positive correlation was observed between number of effective tillers per plant with number of panicles per plant (0.971, p=0.01), grain yield per plant (0.787, p=0.01), grain length-breadth ratio (0.503, p=0.01) and panicle length (0.409, p=0.05). A positive correlation was noticed between number of panicles per plant with grain length-breadth ratio (0.491, p=0.01) and panicle length (0.456, p=0.01) (Table 4.27).

Net house evaluation under aerobic condition revealed a positive correlation between grain yield per plant with root length (0.685, p=0.05), root thickness (0.650, p=0.05) and 1000 grain weight (0.743, p=0.01). A positive correlation was found between root thickness with fresh root weight (0.653, p=0.05) and dry root weight (0.841, p=0.01). Dry root weight also showed positive correlation with fresh root weight (0.747, p=0.01) (Table 4.27.1).

Table 4.27: Correlation analysis between different morphological traits in MAS25 x HKR47 F_3 population in field under aerobic conditions

Trait	Plant height (cm)	Number of effective tillers per plant	Number of panicles per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight(g)	Grain length-breadth ratio
Plant height (cm)	1						
Number of effective tillers per plant	0.152	1					
Number of panicles per plant	0.187	0.971**	1				
Panicle length (cm)	0.591**	0.409*	0.456**	1			
Grain yield per plant (g)	0.464**	0.787**	0.815**	0.638**	1		
1000 grain weight (g)	0.186	0.243	0.266	-0.015	0.326	1	
Grain length-breadth ratio	0.134	0.503**	0.491**	0.197	0.470**	0.316	1

Table 4.27.1: Correlation analysis between different morphological traits in MAS25 x HKR47 F_3 population grown under water-limited conditions in net house

Traits	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight(g)	Grain length-breadth ratio
Root length (cm)	1									
Fresh root weight (g)	-0.357	1								
Dry root weight (g)	-0.222	0.747**	1							
Root thickness (mm)	-0.111	0.653*	0.841**	1						
Plant height (cm)	0.085	-0.074	0.062	-0.024	1					
Number of effective tillers per plant	-0.376	0.008	0.075	0.079	-0.277	1				
Panicle length (cm)	-0.174	-0.149	0.024	0.135	0.432	0.162	1			
Grain yield per plant (g)	0.685*	0.463	0.473	0.650*	0.396	0.139	0.424	1		
1000 Grain weight (g)	-0.287	-0.182	0.342	0.198	0.028	0.228	0.204	0.743**	1	
Grain length-breadth ratio	-0.112	-0.058	0.058	-0.126	-0.106	0.539*	-0.349	0.170	0.386	1

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4.5.4 Microsatellite marker analysis of MAS25 x HKR47 F_3 plants

Microsatellite marker data was generated using five molecular markers for ten selected MAS25 x HKR47 F_3 plants.

4.5.5 Variation in allelic profile

Agarose gel displaying allelic polymorphism in MAS25 x HKR47 F_3 plants for SSR marker is shown in (Plate 5). The overall size of PCR amplified products ranged between 105 bp (RM441) and 235 bp (RM200). At a SSR locus, all the F_3 plants had MAS25 specific allele or HKR47 specific allele or null allele (Table 4.28). The distribution of HKR47 and MAS25 specific alleles are shown in (Table 4.28.1). Plant number N-24-2 and N-14-3 had the maximum alleles from MAS25 while plant number N-14-2 had the maximum number of alleles from HKR47.

Table 4.28: Allelic profile (in bp) of selectedMAS25 x HKR47 F_3 plants and parental rice genotypes at five SSR loci

SSR Marker	Allele size (bp)		Number of plants with		
	MAS25	HKR47	MAS25 Specific allele	HKR47 specific allele	Both MAS25 and HKR47 alleles
RM200	210	235	3	7	0
RM217	200	180	3	7	0
RM276	200	220	4	2	3
RM441	105	170	5	1	4
RM453	180	200	6	4	0

Table 4.28.1: Allelic profile (in bp) of selectedMAS25 x HKR47 F_3 plants and parental genotypes at five SSR loci

Genotype	RM200	RM217	RM276	RM441	RM453
MAS25	210	200	200	105	180
HKR47	235	180	220	170	200
N-11-2	235	180	200	105,170	180
N-6-1	235	180	200	105	200
N-24-3	235	180	200,220	105	180
N-10-3	235	200	200.220	105	200
N-14-1	210	200	220	170	180
N-24-2	210	200	200,220	105,170	180
N-14-3	210	180	200	105	180
N-40-3	235	180	200	105	200
N-14-2	235	180	220	105,170	200
N-47-3	235	180	-*	105,170	180

*null allele

4.5.6 Genetic relationship among MAS25 x HKR47 F_3 population

NTSYS-pc UPGMA tree cluster analysis placed the F_3 plants in two major groups at the similarity coefficient of 0.35 (Figure 4.2). Major group I consisted of MASARB25 and two F_3 plants while the major group II had HKR47 and eight F_3 plants. Genetic relationships among these rice genotypes were also assessed by PCA analysis (NTSYS-pc).

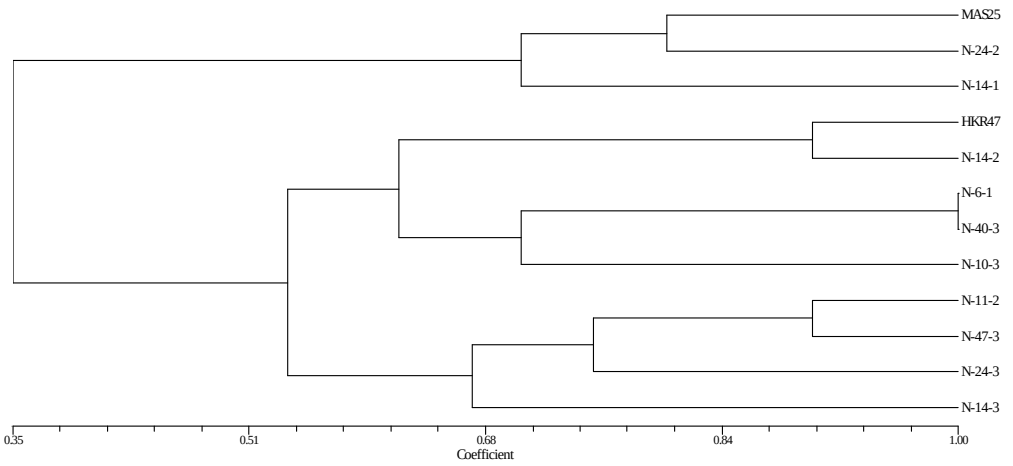

Figure 4.9: Dendrogram (NTSYS-pc) showing genetic relationships among MAS25 x HKR47 F_3 plants and parental genotypes using allelic data at five SSR loci

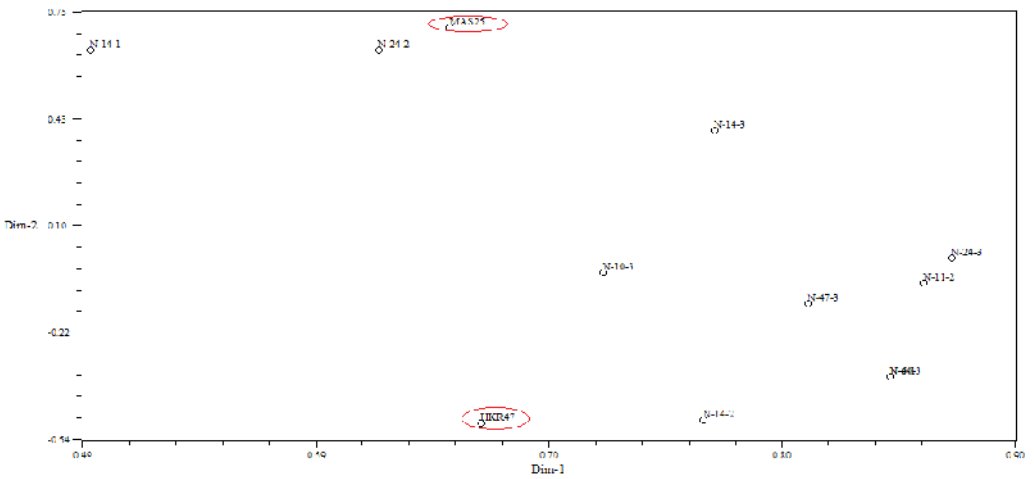

Figure 4.10: Two-dimensional PCA scaling of MAS25 x HKR47 F_3 plants and parental genotypes using genetic distance matrix data at five SSR loci

4.5.7 Selection of promising plants

Based on field data, a total of seven MAS25 x HKR47 F_3 plants were selected on the basis of plant height, number of effective tillers per plant, grain length-breadth ratio and yield per plant (Table 4.29). Based on net house data, three F_3 plants have been selected on the basis of root morphology (length & biomass) and grain length-breadth ratio (Table 4.30).

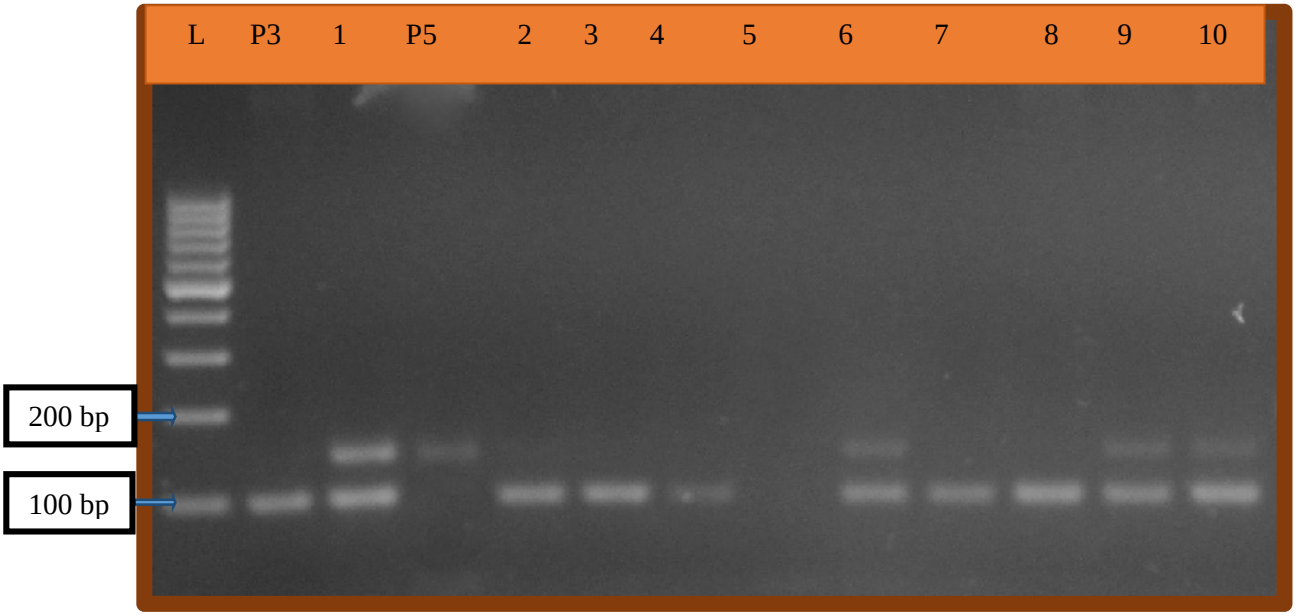

Plate 5- Agarose gel showing allelic polymorphism at RM441 locus among MAS25 x HKR47 F_3 plants, where L=100 bp, P3=MAS25, P5=HKR47 and 1-10 F_3 plants, respectively.

Table 4.29: Selected MAS25 x HKR47 F_3 plants from field

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MAS25	91.5 ± 0.6	11 ± 0.38	9.5 ± 0.75	22.4 ± 0.24	11.3 ± 0.56	20.3 ± 0.35	4.21 ± 0.03
HKR47	85.8 ± 0.6	9.7 ± 0.93	8.2 ± 0.52	18.1 ± 0.24	9.97 ± 0.44	19.8 ± 0.42	3.58 ± 0.04
N-6-1	86	12	14	21.5	11.9	21.9	4.09
N-10-3	85	9	11	19.1	10.5	20.6	4.29
N-11-2	85	9	9	22.0	11.8	19.6	4.19
N-14-3	85	8	11	22.7	10.3	21.2	3.85
N-24-3	85	13	14	19.6	15.8	23.5	4.05
N-40-3	90	11	11	20.5	10.1	22.9	4.23
N-47-3	91	14	16	24.0	15.0	22.1	4.09

Table 4.30: Selected MAS25 x HKR47 F_3 plants from net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 Grain weight (g)	Grain length-breadth ratio
MAS25	40 ± 0.7	7.81± 0.29	3.42± 0.32	12.4± 1.63	68 ± 0.12	4.5 ± 0.25	20.4 ± 0.6	4.77 ±0.13	21.2 ±0.41	5.06 ± 0.11
HKR47	39.2± 0.67	5.84± 0.12	1.62± 0.29	10.4± 0.77	65± 0.51	4.2± 0.38	20.7± 0.26	3.3± 0.18	18.8± 0.97	4.59±0.12
N-10-3	44	12.4	4.42	10.9	76	3	21.20	4.73	22.2	5.1
N-24-3	38	4.24	2.49	3.12	94	4	21.1	7.49	18.9	5.2
N-40-1	33	23.4	8.14	26.0	58	6	20.3	6.12	18.5	5.0

CHAPTER-V

DISCUSSION

Availability of irrigation water is becoming the major factor that limits rice cultivation in both rain fed and irrigated ecosystems (Kumar *et al*., 2011). Growing demand for water from an expanding industrial sector, in addition to increasing residential requirements, has rapidly reduced available water for agriculture (Molden, 2007) thereby increasing the intensity of drought in rain fed ecosystems and aggravating water shortage in irrigated ecosystems. This scenario demands the development and dissemination of ecosystem-specific water-saving technologies that can reduce water requirements of the rice crop without a yield penalty.

Aerobic rice is an emerging agronomical production system that uses 50 % or less water than conventional low-land rice. In aerobic rice cultivation, potentially high yielding, fertilizer responsive adapted rice varieties are grown in fertile aerobic soils that are non-puddled and have no standing water. Supplementary irrigation, however, can be supplied in same way as to any other upland cereal crop (Wang *et al*., 2002 and Bouman *et al.*, 2005).Water uptake in rice depends on root system (Nguyen *et al*., 1997); consequently, studying the root system is one of the most important aspect for understanding mechanism underlying aerobic adaptation and water stress tolerance in rice.

Experiments were carried out to evaluate F_3 populations derived from five crosses (MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47and MAS25 x HKR47) for various physio-morphological traits (plant height, number of panicles per plant, number of effective tillers per plant, panicle length, grain yield per plant, 1000 grain weight and grain length-breadth ratio) and root morphological traits (length, thickness and biomass) during *kharif* season of 2014 and for allelic profile of several microsatellite markers linked with the traits promoting adaptation to aerobic soils. Phenotypic correlation coefficient was calculated to evaluate the association between various traits.

5.1 Field evaluation of F_3 populations

Experiments conducted under aerobic conditions clearly showed that grain yield in aerobic rice varieties (MAS25, MAS26 and MASARB25) were significantly higher as compared to the lowland *indica* (PAU201 and HKR47) rice varieties. Grain yield of MAS25 was 12.6% and 11.4% greater than PAU201 and HKR47 respectively. MASARB25 showed 9.2% and 8% higher yield than PAU201 and HKR47 respectively. Yield of MAS26 was 17.2% greater than PAU201.

Sandhu *et al.* (2012) reported that under aerobic conditions, number of panicles per plant, number of grains per panicle and 1000 grain weight of lowland *indica* rice varieties decreased by 11–28 %, 5–37 % and 5–10 % respectively, whereas the aerobic rice varieties showed an increase of 9–23 %, 5–15 % and 15–25 % respectively. The aerobic rice genotypes had optimum yield under aerobic conditions while in lowland *indica* rice varieties, yield was declined by 22.7 %–27.4 %. Gandhi *et al.* (2011) reported that grain yield obtained by growing MAS 946-1 (aerobic rice variety), under aerobic situation was at par with submerged rice. Babu *et al*. (2011) reported that under aerobic and water-stress conditions, the aerobic varieties MAS25, MAS26, MAS946-1, IR 58025B and SEL 128 showed high yield potential and yield stability. In all the areas, where water is relatively scarcer than land, total rice production can be maximized by growing aerobic rice.

Wide variation was observed for various physio-morphological traits like plant height, number of effective tillers per plant, panicle length, grain yield per plant, 1000 grain weight and grain length-breadth ratio in F_3 populations derived from all the five crosses (MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47and MAS25 x HKR47). This large variation could be due to segregation of genes and QTLs for promoting aerobic adaptation in rice as these traits are quantitative. Similar type of results are observed for cooked kernel elongation (Ahn *et al.*,1993), partial resistance to blast (Wang *et al*., 1994), heterosis (Xiao *et al*., 1995) and for plant height and heading date (Li *et al*., 1995; Xu *et al*., 1995) in rice.

In the present study, grain yield per plant revealed a positive and significant correlation with number of effective tillers per plant, panicle length and 1000 grain weight in MAS25 x PAU201 F_3 population; with plant height and number of effective tillers per plant in MASARB25 x PAU201 F_3 population; with number of effective tillers per plant, 1000 grain weight and grain length-breadth ratio in PAU201 x MAS26 F_3 population; with 1000 grain weight and grain length-breadth ratio in MASARB25 x HKR47 F_3 population and with number of effective tillers per plant, panicle length, plant height and grain length-breadth ratio in MAS25 x HKR47 F_3 population. These results were supported by Ramesha *et al*. (2010), they observed that grain yield was positively correlated with number of effective tillers per plant, panicle length, spikelet fertility and thousand grain weight. Mirza *et al.* (1992) reported positive correlation of number of panicles/m^2 and grain yield with number of tillers/plant. Plant height had registered positive and significant association with grain yield per plant (Akhtar *et al*., 2011; Yadav *et al*., 2011; Seyoum *et al*., 2012). Grain length-breadth ratio, panicle length and number of effective tillers per plant showed significant positive correlation with grain yield per plant (Nagaraju *et al*., 2013). Criterion for a character to be an index of drought tolerance/aerobic adaptation is that it has a positive significant correlation coefficient with grain yield under water stress (Prakash and Anandan, 2012).

5.2 Net house evaluation of F_3 populations

Root traits have been claimed to be critical for increasing yield under soil-related stresses (Lynch, 2007; Serraj *et al*., 2004). 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. A deep penetrating and thickened root system with large xylem vessels has been shown to allow upland rice varieties to extract more water from the soil, resulting in higher yield potential under water-scarce conditions (Nguyen *et al*., 1997). Fukai and Cooper (1995) reported that under low-moisture stress, traits that help the plant to gain access to additional reserves were more important than traits associated with reducing moisture losses. Among the several factors contributing to enhance stress tolerance, root characters are considered to be a vital component of dehydration postponement mechanism since they contribute to regulation of plant growth and extraction of water and nutrients from deeper layers (Thanh *et al*., 1999). Several components of root morphology contributing to drought tolerance have been identified (Ekanayake *et al*., 1985). Maximum root length and root dry weight were good indicators of drought avoidance in upland rice. Plants having deeper root system should colonize a large soil volume and improve the water uptake from the lower layers where water is expected to be available, this would help to maintain a good plant water potential which has a demonstrated positive effect on yield under stress (Mambani and Lal, 1983).

In the present study, F_3 populations derived from all the five crosses showed large variation for physio-morphological and root traits indicating a greater diversity among these populations. The experiments conducted clearly showed that root length, root thickness and root biomass in aerobic rice varieties (MAS25, MAS26 and MASARB25) were significantly higher compared to the low-land *indica* rice varieties (PAU201 and HKR47). The range for root parameters (root length, root thickness, fresh root weight and dry root weight) for F_3 populations of five crosses were given in Tables 4.2, 4.8, 4.14, 4.21 and 4.27 respectively. Under water-limited conditions in net house, some of the F_3 plants of five different crosses showed transgressive segregation for root traits, yield and grain quality. Several F_3 plants had greater root length (14 plants), dry root weight (12 plants), grain yield per plant (12 plants) or grain length-breadth ratio (14 plants) than the respective aerobic rice parents. Transgressive segregation appears through recombination within the chromosomal segment, these reshuffling results in formation of newly derived extreme or transgressive phenotype. Hagiwara *et al*. (2006) and Xu *et al*. (1998) observed similar result for grain characteristics and tiller angle in rice respectively. Genetic variation due to epistasis among loci in heterogeneous environment also leads to transgressive segregation (Fry *et al*., 1998; Leips and Mackay, 2000). Kaushik *et al*. (2003) reported similar type of trangressive

segregation for salt tolerance in F_3 population derived from cross between salt-tolerant CSR10 (*indica*) and salt-sensitive Taraori Basmati (HBC19) varieties of rice.

Grain yield per plant and number of effective tillers per plant showed significant and positive correlation with dry root weight and root thickness in MAS25 x PAU201 and MASARB25 x PAU201 F_3 populations. In PAU201x MAS26 F_3 population, grain yield per plant revealed a positive and significant correlation with root thickness. In MASARB25 x HKR47 F_3 population, grain yield per plant and number of effective tillers per plant both showed significant and positive correlation with fresh root weight and dry root weight. In MAS25 x HKR47 F_3 population, grain yield per plant showed significant and positive correlation with root length and root thickness.

Significant and positive association of these traits indicates that selection based on these traits would ultimately improve grain yield under drought stress situations. A high positive correlation of root traits is a clear indication that thicker and deeper roots facilitate easy uptake of water from deeper layers of soil and help the plants improve their water relationship and thereby yield. Similar results were already reported by Sheeba (2005) for root length; Anbumalarmathi (2005) for dry root weight and Sinha *et al*. (2000) for root thickness.

The above results were also supported by Toorchi *et al*. (2003); they observed significant increases in mean values of root length in the sampling at maturity under low-moisture stress conditions. For relatively large soil water reservoir (deep soil), increase in rooting depth, conductance and root to shoot ratio (by length) results in increased soil water uptake capacity and enhances the grain yield (O'Toole 1982). Positive association between plant height and shoot dry weight, maximum root length, root thickness, root number and root dry weight have been observed (Ekanayake *et al*. 1985; Kanbar and Shashidhar, 2004). The interrelationships between root morphological characters and yield-related traits clearly identified the importance of root thickness and root dry weight.

5.3 Analysis of molecular diversity among F_3 populations

Rice microsatellite (RM) or simple sequence repeat (SSR) markers are robust and co-dominant (i.e., they can detect heterozygous loci), exhibit high allelic variation and are widely distributed throughout the *Oryza* genome (McCouch *et al.*, 1997). A total of 3-7 polymorphic microsatellite markers linked to the traits promoting aerobic adaptation were used to analyze the segregation in the selected F_3 plants derived from the five crosses.

The NTSYS-pc UPGMA tree cluster analysis and 2-D PCA scaling of selected F_3 plants derived from the five crosses clearly showed large variation among two parental genotypes and F_3 plants were invariably interspersed between them as shown in Figures 4.1 to 4.10. Notably in crosses between MASARB25 x HKR47, Q-1-1 was 100% similar while Q-2-1 and Q-25-3 were 88% similar to MASARB25. In MAS25 x HKR47, plant N-24-2 showed

79% resemblance with MAS25. But in some cases, plants were close to other parent too. In MASARB25 x HKR47, plants M-22-2 and M-13-2 were 82% similar to HKR47 and in MASARB25 x PAU201, plant Q-9-1 exhibited 88% similarity with PAU201. Some F_3 plants showed inclination towards PAU201 in PAU201 x MAS26, towards MASARB25 in MASARB25 x HKR47 and towards HKR47 in MAS25 x HKR47 F_3 population, respectively. Such results are expected in F_3 plants derived from two diverse genotypes PAU201 and HKR47 (*indica* rice varieties) and MASARB25, MAS25 and MAS26 (aerobic rice varieties) and has also been observed earlier. Aggarwal *et al*. (2002) characterized basmati and new elite rice genotypes into three major groups by similarity coefficients (UPGMA cluster analysis) and the PCA analysis. Ramadan *et al.* (2015) reported that among seven rice genotypes evaluated for drought, highest similarity coefficient (0.804) was found between Azuciena (*japonica)* and IRAT170 (drought tolerant). Also, high similarity percentage was observed between the moderately drought tolerant variety Giza178 and each of Azuciena (0.533) and IRAT170 (0.508).

Rice includes large root architecture and growth patterns, but the environmental response of root growth among genotypes is just as diverse. Root traits are generally controlled by many genes through quantitative trait loci (QTL). First study to locate genes controlling rice root traits with molecular markers was done by Champoux *et al*., 1995. Since then many QTLs related to root traits have been identified in rice (Gowda *et al*., 2011). Several markers have been identified to be linked with aerobic root traits/drought tolerance in rice (Qu *et al*., 2008). QTLs have been identified in rice populations for basal root thickness (Price *et al*., 2000; Zhang *et al*., 2000; Shen *et al*., 2001; Steele *et al*., 2006; Gomez *et al*., 2009; Kanagaraj *et al*., 2010; Singh *et al*., 2012). The co-location of QTLs for root traits with those of yield under drought stress was reported in rice by Salunkhe *et al*. (2011).

Six MAS25 x PAU201 F_3 plants, 12 MASARB25 x PAU201 F_3 plants, 12 PAU201x MAS26 F_3 plants, 18 MASARB25 x HKR47 F_3 plants and ten MAS25 x HKR47 F_3 plants were selected for analysis of molecular diversity. SSR markers used in this study might be linked with QTLs promoting aerobic adaptation. RM547 on chromosome 8 showed significant association with root length (Bernier *et al*., 2007; Sandhu *et al*., 2013). On chromosome 3, RM475- RM263 marker interval has been found to be related with shape of grain (Rabiei *et al*., 2004; Yoon *et al*., 2006; Kwon *et a*l., 2008) and RM175-RM22 to be related with seedling height (Abdelkhalik *et al*., 2005; Han *et al*., 2007). RM528-RM400 interval with peak marker RM528 on locus $qDTY_{6.1}$ controls grain yield under drought condition in lowland severe stress and lowland non stress ecosystems (Dixit et al., 2014). Xipeng *et al*. (2011) and Hemamalini *et al*. (2000) reported RM525 to be linked to root traits only but not for yield traits in rice. Qu *et al.* (2008) reported that RM525 on chromosome 2 is

linked to basal root thickness, fresh and dry root weight. Courtois *et al.* (2012) noticed that RM234 on chromosome 7 is linked to maximum root length trait.

5.4 Selection of promising plants

Field evaluation of F_3 plants derived from five crosses under aerobic conditions led to identification of 39 plants having higher or comparable grain yield and/or grain length-breadth ratio than the parental genotypes under aerobic conditions. Though root traits were not studied, the better growth and yield productivity in these selected F_3 plants may be due to the presence of aerobic genes/QTLs promoting better adaptation to aerobic conditions. Several of these selected high-yielding F_3plants were in fact close to the low-land rice parent (PAU201and HKR47), indicating large amount of genetic content from these parents in addition to MAS25, MAS26 and MASARB25 genes/QTLs from the aerobic rice parent promoting adaptation to aerobic conditions.

The net house evaluation of F_3 plants also led to the identification of 19 rice genotypes on the basis of root length and root biomass (comparable to respective aerobic rice parent), higher or comparable grain yield and grain length-breadth ratio (comparable to respective lowland *indica* rice parent).

All the selected lines from this study will serve as novel material for the selection of stable direct seeded aerobic varieties requiring less water. Development of these aerobic rice genotypes could be a significant benefit to farmers and save precious water for industrial and domestic purposes.

CHAPTER-VI

SUMMARY AND CONCLUSION

In the present study, experiments were carried out to evaluate F_3 populations derived from five crosses (MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47and MAS25 x HKR47) for various physio-morphological traits (plant height, number of panicles per plant, number of effective tillers per plant, panicle length, grain yield per plant, 1000 grain weight and grain length-breadth ratio) and root morphological traits (length, thickness and biomass) during *kharif* season of 2014 and for allelic profile of several microsatellite markers linked with the traits promoting adaptation to aerobic soils. Phenotypic correlation coefficient was calculated to evaluate the association between various traits. The research work can be summarized and concluded as under:

6.1 Field evaluation

- Enormous variation was observed among MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47 and MAS25 x HKR47F_3 plants for all the physio-morphological traits including number of effective tillers per plant (1-14, 3-13, 4-16, 2-13 and 4-14), grain yield per plant (1.86-14.4, 3.77-19.3, 1.39-19.8, 2.78-18.2 and 2.94-15.8 g) and length-breadth ratio of grain (3.12-4.23, 3.51-4.21, 3.48-5.18, 3.6-4.88 and 3.43-4.29) respectively.
- Grain yield per plant revealed a positive and significant correlation with number of effective tillers per plant, panicle length and 1000 grain weight in MAS25 x PAU201 F_3 population; with plant height and number of effective tillers per plant in MASARB25 x PAU201 F_3 population; with number of effective tillers per plant, 1000 grain weight and grain length-breadth ratio in PAU201 x MAS26 F_3 population; with 1000 grain weight and grain length-breadth ratio in MASARB25 x HKR47 F_3 population and with number of effective tillers per plant, panicle length, plant height and grain length-breadth ratio in MAS25 x HKR47 F_3 population.

6.2 Net house evaluation

- Extensive variation was observed among MAS25 x PAU201, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47 and MAS25 x HKR47 F_3 plants for all the traits including grain yield per plant (1.77-13, 0.03-11, 0.05-6.28, 0.87-5.8 and 0.09-7.49 g), grain length-breadth ratio (4.26-5.36, 4.26-5.68, 2.13-5.59, 4.56-5.41 and 4.3-6.1), root length (29-54, 21-48, 27-47, 18-48 and 28-45 cm), root thickness (3.13-24.2, 1.86-23.1, 5.23-17.8, 4.45-22.6 and 1.58-26.0 mm), fresh root

weight (2.18-24.1, 1.08-24.8, 2.54-10.8, 1.96-13.5 and 4.24-23.4 g) and dry root weight (0.14-8.01, 0.45-8.31, 0.64-2.94, 0.7-5.22 and 0.53-8.14 g) respectively.

- Under water-limited conditions in net house, some of the F_3 plants of five different crosses showed transgressive segregation for root traits, yield and grain quality. Several F_3 plants had greater root length (14 plants), dry root weight (12 plants), grain yield per plant (12 plants) or grain length-breadth ratio (14 plants) than the respective aerobic rice parents.
- Grain yield per plant and number of effective tillers per plant showed significant and positive correlation with dry root weight and root thickness in MAS25 x PAU201 and MASARB25 x PAU201 F_3 populations. In PAU201x MAS26 F_3 population, grain yield per plant revealed a positive and significant correlation with root thickness. In MASARB25 x HKR47 F_3 population, grain yield per plant and number of effective tillers per plant both showed significant and positive correlation with fresh root weight and dry root weight. In MAS25 x HKR47 F_3 population, grain yield per plant showed significant and positive correlation with root length and root thickness.

6.3 Microsatellite marker analysis

- A total of 3-7 polymorphic microsatellite markers linked to the traits promoting aerobic adaptation were used to analyze the segregation in the selected F_3 plants derived from the five crosses. Molecular marker analysis of selected F_3 plants of the five crosses (MAS25 x PAU201-6 plants; MASARB25 x PAU201-12 plants; PAU201x MAS26-12 plants; MASARB25 x HKR47-18 plants and MAS25 x HKR47-10 plants), showed that these plants either had an allele of one of the parent in homozygous state or alleles from both the parents (heterozygous state). In MASARB25 x HKR47 F_3 population, recombinant alleles were also observed.
- The NTSYS-pc UPGMA tree cluster analysis and 2-D PCA scaling of selected F_3 plants derived from the five crosses clearly showed large variation among two parental genotypes and F_3 plants were invariably interspersed between them. Some of the F_3 plants showed inclination towards PAU201 in PAU201 x MAS26, towards MASARB25 in MASARB25 x HKR47 and towards HKR47 in MAS25 x HKR47 F_3 populations, respectively.
- Allelic status of several SSR markers, which have been reported earlier to be linked with QTLs promoting aerobic adaptation, was ascertained for the selected F_3 plants derived from the five crosses.

6.4 Selection of promising plants for future

- Field evaluation of F_3 plants derived from five crosses under aerobic conditions led to identification of 39 plants having higher or comparable grain yield and/or grain

length-breadth ratio than the parental genotypes under aerobic conditions. Though root traits were not studied, the better growth and yield productivity in these selected F_3 plants may be due to the presence of aerobic genes/QTLs promoting better adaptation to aerobic conditions.

- The net house evaluation of F_3 plants also led to the identification of 19 rice plants on the basis of higher or comparable grain yield, root length and root biomass (comparable to respective aerobic rice parent), grain length-breadth ratio (comparable to respective low land *indica* rice parent). These plants shall be further analyzed in order to select stable high yielding aerobic rice lines.

BIBLIOGRAPHY

Abdelkhalik, A.F., Shishido, R., Nomura, K. and Ikehashi, H. (2005). QTL based analysis of heterosis for grain shape traits and seedling characteristics; I: an *indica-japonica* hybrid in rice (*Oryza sativa* L.). *Breeding Science*.**55**: 41- 48.

Aggarwal, R.K., Shenoy, V.V., Ramadevi, J., Rajkumar, R. and Singh, L. (2002). Molecular characterization of some Indian Basmati and other elite rice genotypes using fluorescent-AFLP. *Theoretical and Applied Genetics*.**105:** 680-690.

Ahn, S.N., Bollich, C.N., McClung, A.M. and Tanksley, S.D. (1993). RFLP analysis of genomic regions associated with cooked-kernel elongation in rice. *Theoretical and Applied Genetics.* **87:** 27-32.

Akhtar, N., Nazir, M.F., Rabnawaz, A., Mahmood, T., Safdar, M.E., Asif, M. and Rehman, A. (2011). Estimation of heritability, correlation and path coefficient analysis in fine grain rice. *The Journal of Animal & Plant Sciences*.**21(4):** 660-664.

Anbumalarmathi, J. (2005). Genetic analysis for drought tolerance and yield component traits in rice (*Oryza sativa* L.). Ph. D. Thesis, TNAU, Coimbatore.

Anwar, M.P., Juraimi, A.S., Samedani, B., Puteh, A. and Man, M. (2012). Critical period of weed control in aerobic rice. *The Scientific World Journal*. pp: 1-10.

Aslam, M., Qureshi, A.S. and Horinkova, V.M. (2002). Water saving strategies for irrigated rice. *Journal Drain Water Management*.**6:** 25-36.

Babu, N.N., Hittalmani, S., Shivakumar, N. and Nandini, C. (2011). Effect of drought on yield potential and drought susceptibility index of promising aerobic rice (*Oryza sativa* L.) genotypes. *Electronic Journal of Plant Breeding*.**2(3):** 295-302.

Babu, R.C., Nguyen, B.D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S.K., Palchamy, A., Sadasivam, S., Sarkarung, S., Wade, L.J. and Nguyen, H.T. (2003). Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. *Crop Sciences*.**43:** 1457-1469.

Belder, O., Bouman, B.A.M., Spiertz, J.H.J., Peng, S., Castaneda, A.R. and Visperas, R.M. (2005). Crop performance, nitrogen and water use in flooded and aerobic rice. *Plant and Soil*.**273:** 167-182.

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. and Atlin, G. (2007). A Large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sciences*.**47:** 505–516.

Bhagat, R.M., Bhuiyan, S.I. and Moody, K. (1996). Water, tillage and weed interactions in lowland tropical rice: a review. *Agricultural Water Management*.**31:** 165-184.

Borell, A., Garside, A., and Shu, F. K. (1997). Improving efficiency of water for irrigated rice in a semi-arid tropical environment. *Field Crops Research*.**52:** 231–248.

Bouman, B.A.M. and Tuong, T.P. (2001). Field water management to save water and increase its productivity in irrigated rice. *Agricultural Water Management*.**49(1):** 11-30.

Bouman, B.A.M., Feng, L., Tuong, T.P., Lu, G., Wang, H. and Feng, Y. (2007). Exploring options to grow rice using less water in northern China using a modeling approach II. Quantifying yield, water balance components, and water productivity. *Agricultural Water Management.***88:** 23-33.

Bouman, B.A.M., Peng, S., Castaneda, A.R. and Visperas, R.M. (2005). Yield and water use of irrigated aerobic rice systems. *Agricultural Water Management.***74:** 87-105.

Cantrell, R.P. and Hettel, G.P. (2005). Research strategy for rice in the 21st century. *In*: Rice is life: Scientific perspective for the 21st Century. (Eds.) Toriyama, K., Heong, K.L., Hardy, B. *Proceedings of the world Rice Research Conference*, Tokyo and Tsukuba, Japan.

Carriger, S. and Vallee, D. (2007). More crop per drop: Rice Today. IRRI.**6 (2):** 10-13.

Castaneda, A.R., Bouman B.A.M., Peng, S. and Visperas, R.M. (2002). The potential of aerobic rice to reduce water use in water-scarce irrigated low lands in the tropics. *In*: *Water- wise Rice Production.* (Eds.) Bouman, B.A.M., Hengsdijk, H., Hardy, B., Bindraban, P.S., Tuong, T.P. and Ladha, J.K. IRRI, Los Banos, Philippines. pp.124-129.

Champoux, M.C., Wang, G., Sarkarung, S., Mackill, D.J., O'Toole, J.C., Huang, N. and McCouch, S.R. (1995). Locating genes associated root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical Applications of Genetics.***90:** 969–981.

Changying, X.C., Yang, X., Bouman, B.A.M., Deng, W., Zhang, Q., Yan, W., Zhang, T., Rouzi, A. and Wang, H. (2008). Optimizing yield, water requirements and water productivity of aerobic rice for the North China Plain. *Irrigation Science.***26:** 459–474.

Cordeiro, G.M., Christopher, M.J., Henry, R.J. and Reinke, R.F. (2002). Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. *Molecular Breeding.***9:** 245–250.

Courtois, B., Frowin, J. and Greco, R. (2012). Genetic diversity and population structure in a European collection of rice (*Oryza sativa* L.). *Crop Sciences.***52:** 1663-1675.

Crawford, G. and Shen, C. (1998). The origins of rice agriculture: recent progress in East Asia. *Antiquity.***72**: 858-866.

Dixit, S., Singh, A., Sta Cruz, M.T., Paul, T.M., Amante, M. and Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genetics.***15**:16.

Dixit, S., Swamy, B.P.M., Vikram, P., Ahmed, H.U., Sta Cruz, M.T., Amante, M., Atri, D., Leung, H. and Kumar, A. (2012, a). Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. *Theoretical Applications of Genetics.***125:** 155–169.

Dixit, S., Swamy, B.P.M., Vikram, P., Bernier, J., Sta Cruz, M.T., Amante, M., Atri, D. and Kumar, A. (2012, b). Increased drought tolerance and wider adaptability of qDTY12.1 conferred by its interaction with qDTY2.3 and qDTY3.2. *Molecular Breeding.***30:** 1767–1779.

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

FAOSTAT (2010). Available at: www.faostat.fao.org.

Flowers, T.J., Koyama, M.L., Flowers, S.A., Sudhakar, C., Singh, K.P. and Yeo, A.R. (2000). QTL: their place in engineering tolerance of rice to salinity. *Journal of Experimental Botany.***51**: 99-106.

Food and Agriculture organization (FAO). 2014. Available online at: http://www.fao.org/.

Food and Agriculture organization (FAO). 2015. Available online at: http://www.fao.org/.

Fry, J.D., Nuzhdin, S.V., Pasyukova, E.G. and Mackay, T.F.C. (1998). QTL mapping of genotype-environment interaction for fitness in *Drosophila melanogaster*. *Genetics Research*.**71**:133-141.

Fukai, S. and Cooper, M. (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Research*.**40**: 67–86.

Gandhi, V.R., Rudresh, N.S., Shivamurthy, M. and Hittalmini, S. (2011). Performance and adoption of new aerobic rice variety MAS 946-1 (Sharada) in southern Karnataka. *Karnataka Journal of Agricultural Sciences.* **25(1)**: 5-8.

George, T., Magbanua, R., Garrity, D.P., Tubaria, B.S. and Quiton, J. (2002). Rapid yield loss of rice cropped successively in aerobic soil. *Agronomy Journal*.**94**: 981-989.

Gill, J.S., Walia, S.S. and Gill, R.S. (2014). Direct seeded rice: An alternative rice establishment technique in north-west India – A review. *International Journal of Advanced Research.* **2(3)**: 375-386.

Girish, T.N., Gireesha, T.M., Vaishali, M.G., Hanamareddy, B.G. and Hittalmani, S. (2006). Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza sativa* L.) from an *indica* x *japonica* cross for growth and yield traits under aerobic conditions. *Euphytica*.**152**: 149-161.

Gomez, M.N., Boopathi, M., Kumar, S.S., Ramasubramanian, T., Chengsong, Z., Jeyaprakash, P., Senthil, A. and Babu, R.C. (2009). Molecular mapping and location of QTLs for drought-resistance traits in indica rice (*Oryza sativa* L.) lines adapted to target environments. *Acta Physiologiae Plantarum*.**32**:355-364.

Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E. and Serraj, R. (2011). Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research*.**122**: 1–13.

Gramene database. http://www.gramene.org

Hagiwara, W.E., Onishi, K., Takamure, I. and Sano, Y. (2006). Transgressive segregation due to linked QTLs for grain characteristics of rice. *Euphytica*.**150**: 27-35.

Han, L., Qiao, Y., Zhang, S., Zhang, Y., Cao, G., Kim, J., Lee, K. and Koh, H. (2007). Identification of quantitative trait loci for cold response of seedling vigor traits in rice. *Journal of Genetics and Genomics*.**34**: 239-246.

Hemamalini, G.S., Shashidhar, H.E. and Hittalmani, S. (2000). Molecular marker assisted tagging of root morphological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). *Euphytica*.**112**: 69-78.

Henry, A., Swamy, B.P.M., Dixit, S., Torres, R.D., Batoto, T. C., Manalili, M., Anantha, M.S., Mandal, N.P. and Kumar A. (2014). Physiological mechanisms contributing to the QTL combination effects on improved performance of IR64 rice NILs under drought. *Journal of Experimental Botany.* doi:10.1093/jxb/eru506.

Humphreys, E., Meisner, C., Gupta, R., Timsina, J., Beecher, H.G., Lu, T.Y., Singh, Y., Gill, M.A., Masih, I., Guo, Z.J. and Thompson, J.A., (2005). Water saving in rice–wheat systems. *Plant Production Sciences*.**8**: 242–258.

IRGSP (2005). The map-based sequence of the rice genome. *Nature*.**436**: 793–800.

IRRI [International Rice Research Institute] (2001). Enhancing water productivity in rice-based production systems. *In*: Enhancing water productivity and sustainability of favorable environments project summary reports. IRRI, Los Banos, Philippines. pp. 25-27.

Kanagaraj, P., Prince, K.S.J., Sheeba, J.A., Biji, K.R., Paul, S.B., Senthil, A. and Babu, R.C. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Science.***98**: 836-839.

Kanbar, A. and Shashidhar, H.E. (2004). Correlation and path analysis for root morphological traits in *indica* x *indica* population of rice (*Oryza sativa* L.). *Crop Research*. **27(1)**:94–98.

Kanbar, A., Toorchi, M., and Shashidhar, H. E. (2009). Relationship between root and yield morphological characters in rainfed low land rice (*Oryza sativa* L.). *Cereal Research Communications.* **37(2)**: 261–268.

Kato, Y. and Okami, M. (2011). Root morphology, hydraulic conductivity and plant water relations of high-yielding rice grown under aerobic conditions. *Annals of Botany.***108**: 575–583.

Kato, Y., Okami, M. and Katsura, K. (2009). Yield potential and water use efficiency of aerobic rice (*Oryza sativa* L.) in Japan. *Field Crops Research.***113**: 328–334.

Kaushik, A., Saini, N.S., Jain, R.K. and Singh, R.K. (2003). Genetic and molecular marker analysis of a segregating population derived from a cross between salt-tolerant *indica* variety CSR10 and Taraori Basmati. *Euphytica.***134**: 231-238.

Klemm, 1999. Water saving in rice cultivation. *In*: Assessment and orientation towards the 21st century. *Proceedings of 19th session of the International Rice Commission*, 7-9 September 1998, Cairo, Egypt. Food and Agriculture Organization. pp. 110–117.

Kumar, V. and Ladha, J.K. (2011). Direct seeding of rice: recent developments and future research needs. *In: Advances in Agronomy*, (Eds.). L.S. Donald, USA: Newark, Delaware. **111:** 297–413.

Kwon, S.J., Cho, Y., Kwon, S.W., Oh, C.S., Suh, J.P., Shin, Y.S., Kim, Y.G., Holligan, D., Wessler, S.R., Hwang, H.G. and Ahn, S.N. (2008). QTL mapping of agronomic traits using an RIL population derived from a cross between temperate *japonica* cultivars in rice (*Oryza sativa* L.). *Breeding Science.***58**: 271-279.

Lafitte, H.R., Courtois, B. and Arraudeau, M. (2002). Genetic improvement of rice in aerobic systems: progress from yield to genes. *Field Crops Research.***75**: 171-190.

Lampayan, R.M., Bouman, B.A.M., Dios, D.J.L., Espiritu, A.J., Soriano, J.B., Lactaoen, A.T., Faronilo, J.E., and Thant, K.M. (2010). Yield of aerobic rice in rain fed lowlands of the Philippines as affected by nitrogen management and row spacing. *Field Crops Research.***116**: 165–174.

Leips, J. and Mackay, T.F.C. (2000). Quantitative trait loci for life span in *Drosophila melanogaster* interactions with genetic background and larval density. *Genetics.***155**:1773-1788.

Li, Z., Pinson, S.R.M., Stansel, J.W. and Park, W.D., (1995). Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.).*Theoretical and Applied Genetics.***91:** 374-381.

Lin, S., Dittert, K., Tao, H., Kreye, C., Xu, Y., Shen, Q., Fan, X. and Sattelmacher, B. (2002). *In*: *Proceedings of International Workshop on Water wise Rice Production*, 8-11 April 2002, Los Banos, Philippines, and IRRI. pp. 187-196.

Lynch, J.P. (2007). Roots of the second green revolution. *Australian Journal of Botany.***55**: 493–512.

Mambani, B. and Lal, R. (1983). Response of upland rice cultivars to drought stress. III. Screening varieties by means of variable moisture along a toposequence. *Plant Soil.* **73:** 73-94.

Manickavelu, A., Nadarajan, N., Ganesh, S.K., Gnanamalar, R.P. and Babu, R.C. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regulation.***50**: 121–138.

Matsuo, N., Ozawa, K. and Mochizuki, T. (2009). Genotypic differences in root hydraulic conductance of rice (*Oryza sativa* L.) in response to water regimes. *Plant Soil.***316**: 25–34.

McCouch, S.R., Chen, X. and Panaud, O. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology*. **35(2)**: 89–99.

McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research.***9**: 199–207.

Mirza, M. J., Faiz, F.A. and Majid, A. (1992). Correlation studies and path analysis of plant height, yield and yield components in rice (*Oryza sativa* L.). *Sarhad Journal of Agriculture.* **8(6)**: 647-653.

Molden, D. (2007). Water for food, water for life: a comprehensive assessment of water management in agriculture. International Water Management Institute, London.

Nagaraju, C., Sekhar, M.R., Reddy, K.H. and Sudhakar, P. (2013). Correlation between traits and path analysis coefficient for grain yield and other components in rice (*Oryza sativa* L.) genotypes. *International journal of applied biology and pharmaceutical technology*. **4(3)**: 137-142.

Nguyen, H.T., Babu, R.C. and Blum, A. (1997). Breeding for drought resistance in rice: physiology and molecular genetic considerations. *Crop Science.***37**:1426–1434.

O'Toole, J.C. (1982). Adaption of rice to drought prone environments. *In:* Drought resistance in crops with emphasis on rice. Los Banos, Philippines. pp. 195–213.

Parry, M.A.J., Flexas, J. and Medrano, H. (2005). Prospects for crop production under drought: research priorities and future directions. *Annals of Applied Biology*.**147**:211-226.

Parthasarathi, T., Vanitha, K., Kumar, L. P. and Kalaiyarasi, D. (2012). Aerobic rice-mitigating water stress for the future climate change. *International journal of Agronomy and Plant Production.* **3**(7): 241-254.

Peng, S., Tang, Q. and Zou, Y. (2009). Current status and challenges of rice production in China. *Plant Production Science.***12:** 3–8.

Prakash, M. and Anandan, A. (2012). Molecular characterization, morphophysiological and biochemical evaluation of F_2 and F_3 generation of MAS 946-1 x ADT 43 under aerobic condition. *Asia-Pacific Chemical, Biological & Environmental Engineering Society Procedia.***4**: 22-29.

Prasad R (2004). Recent advances in rice agronomy. *Indian Farming*.**54**: 7-10.

Price, A.H. and Courtios, B. (1999). Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. *Plant Growth Regulators*.**29**: 123-133.

Price, A.H., Steele, K.A., Moore, B.J., Barraclough, P.B. and Clark, L.J. (2000). A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root penetrationability. *Theoretical and Applied Genetics*.**100**: 49–56.

Priyadarshini, S.K., Shivanna, H. and Shailaja, H. (2013). Response of NILs with single and multiple QTLs for root length on grain yield and water use efficiency in rice (*Oryza sativa* L.) under aerobic condition. *International Journal of Agriculture, Environment and Biotechnology.***6**: 403-412.

Qu, Y., Mu, P., Zhang, H., Chen, C. Y., Gao, Y., Tian, Y., Wen, F. and Li, Z. (2008). Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica.***133**: 187–200.

Rabiei, B., Valizadeh, M., Ghareyazie, B., Moghaddam, M. and Ali, A.J. (2004). Identification of QTLs for rice grain size and shape of Iranian cultivars using SSR markers. *Euphytica.***137**: 325–332.

Raju, B.R., Narayanaswamy, B.R., Mohankumar, M.V., Sumanth, K.K., Rajanna, M.P., Mohanraju, B., Udaykumar, M. and Sheshshayee, M.S. (2014). Root traits and cellular level tolerance hold the key in maintaining higher spikelet fertility of rice under water limited conditions. *Functional Plant Biology.***41**: 930–939.

Ramadan, E.A., Elmonghazy, A.M. and El-Mowafi, H.F. (2015). Molecular markers based genetic diversity analysis for drought tolerance in rice (*Oryza sativa* L.) using SSR markers. *Proceedings of International Journal of Scientific Research in Agricultural Sciences.***2**:137-146.

Ramesha, M.S., Hasan, M.J., Kulsum, M.U., Akter, A. and Masuduzzaman, A.S.M. (2010). Genetic variability and character association for agronomic traits in hybrid rice (*Oryza sativa* L.). *Bangladesh Journal of Plant Breeding and Genetics.* **24(1)**: 45-51.

Rohlf, F.J. (1993). NTSYS-pc: numerical taxonomy and multivariate analysis system. Version 1.8. Exeter Software, New York.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. (1984). Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of National Academic Science.***81**: 8014-8019.

Salunkhe, A. S., Poornima, R., Prince, K. S. J., Kanagaraj, P., Sheeba, J. A., Amudha, K., Suji, K. K., Senthil, A. and Babu, R. C. (2011). Fine mapping QTL for drought resistance traits in rice (*Oryza sativa* L.) using bulk segregant analysis. *Molecular Biotechnology.***49:** 90-95.

Sandhu N., Jain, S., Chowdhury, V.K. and Jain, R.K. (2012). Microsatellite diversity among aerobic and lowland *indica* rice genotypes with differential water requirements**.** *Indian Journal of Genetics and Plant Breeding.***72 (4)**:463-467.

Sandhu, N., Jain, S., Kumar, A., Mehla, B.S. and Jain, R.K. (2013). Genetic variation, linkage mapping of QTL and correlation studies for yield, root, and agronomic traits for aerobic adaptation. *BMC Genetics.* **14:** 104-119.

Sathya, R. and Jebaraj, S. (2013). Genetic studies for yield and physiological traits in three line aerobic rice hybrids. *Plant Gene and Trait.* **4(9)**: 48-52.

Serraj, R., Krishnamurthy, L., Kashiwagi, J.W., Kumar, J., Chandra, S. and Crouch, J.H. (2004). Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Research.***88**:115–127.

Serraj, R., McNally, K.L. and Slamet-Loedin, I. (2011). Drought resistance improvement in rice: an integrated genetic and resource management strategy. *Plant Production Science.***14**: 1-14.

Seyoum, M., Alamerew, S. and Bantte, K. (2012). Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice. *Journal of Plant Sciences*. **7 (1)**: 13-22.

Sheeba, A. (2005). Genetic studies on drought tolerance and stability of temperature sensitive genetic male sterility (TGMS) based rice hybrids. Ph.D. Thesis, TNAU, Coimbatore.

Shen, L., Courtois, B., McNally, K.L., Robin, S. and Li, Z. (2001). Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theoretical and Applied Genetics.***103**:75–83.

Singh, A.K., Choudury, B.U., and Bouman, B.A.M. (2002). The effect of rice establishment techniques and water management on crop-water relations. *In:Water-Wise Rice Production*.(Eds.) Bouman, B.A.M., Hengsdijk, H., Hardy, B., Bindraban, P.S., Tuong, T.P. and Ladha, J.K. IRRI, Los Banos, Philippines.

Singh, S., Pradhan, S., Singh, A. and Singh, O. (2012). Marker validation in recombinant inbred lines and random varieties of rice for drought tolerance. *Australian Journal of Crop Science*.**6:** 606-612.

Sinha, P.K., Prasad, K. and Mishra, G.N. (2000). Studies on root characters related to drought resistance and their association in selected upland rice genotypes. *Oryza*.**37**: 29-31.

Steele, K. A., Price, A. H., Shashidhar, H. E. and Witcombe, J. R. (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theoretical and Applied Genetics.***112**: 208–221.

Steele, K.A., Virk, D.S., Kumar, R., Prasad, S.C. and Witcombe, J.R. (2007). Field evaluation of upland rice lines selected for QTLs controlling root traits. *Field Crops Research.***101**: 180-186.

Stoop, W., Uphoff, N. and Kassam, A. (2002). A review of agricultural research issues raised by system of rice intensification (SRI) from Madagascar: opportunities for improving farming systems for resource-poor farmers. *Agriculture Systematics.***71**: 249-274.

Sunyob, N.B., Juraimi, A.S., Hakim, M.A., Man, A., Selamat, A. and Alam, M.A. (2015). Competitive ability of some selected rice varieties against weed under aerobic condition. *International Journal of Agriculture & Biology.***17**: 61–70.

Suryapriya, P., Snehalatha, A., Kayalvili, U., Krishna, A.R., Singh, S. and Ulaganathan, K. (2009). Genome-wide analyses of rice root development QTLs and development of an online resource, Rootbrowse. *Bioinformation*.**3**:279–282.

Tanksley, S.D. (1989). Mapping polygenes. *Annual Review of Genetics.***27**: 205-233.

Temnykh, S., DeClerck, G., Lukashowa, A., Pivovich, L., Cartinhour, S. and McCouch, S. (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon association and genetic marker potential. *Genome Research.***11**:1441-1452.

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

Thanh, P. T., Sripichitt, P., Chanprame, S. and Peyachoknagu, S. (2006). Transfer of drought resistant character from wild rice (*Oryza meridionalis* and *Oryza nivara*) to cultivated rice (*Oryza sativa* L.) by backcrossing and immature embryo culture. *Kasetsart Journal.***40**: 582–594.

Toorchi, M., Shashidhar, H.E. and Hittalmani, S. (2007). Tagging QTLs for maximum root length in rain fed lowland rice by combined selective genotyping and STMs markers. *Journal of Food, Agriculture & Environment.* **5(2)**: 209-210.

Toorchi, M., Shashidhar, H.E., Gireesha, T.M. and Hittalmani, S. (2003). Performance of backcrosses involving transgressant doubled haploid lines in rice under contrasting moisture regimes: yield components and marker heterozygosity. *Crop Science.***43**: 1448–1456.

Toorchi, M., Shashidhar, H.E. and Sridhara, H. (2006). Influence of the root system on grain yield and related traits in rain fed lowland rice (*Oryza sativa* L.). *Pakistan Journal of Biological Sciences*.**9**: 2267–2272.

Tuong, T.P. and Bouman, B.A.M. (2002). Rice production in water-scarce environments. *Proceedings of the Water Productivity Workshop*, Colombo, Sri Lanka. Colombo (Sri Lanka): International Water Management Institute.

Tuong, T.P. and Bouman, B.A.M. (2003). Rice production in water-scarce environments. *In*: Water productivity in agriculture: limits and opportunities for improvement. (Eds.) Kijne, J.W., Barker, R. and Molden, D. UK: CABI Publishing. pp. 53-67.

Tuong, T.P., Bouman, B.A.M. and Mortimer, M. (2005). More rice, less water – integrated approaches for increasing water productivity in irrigated rice-based systems in Asia. *Plant Production Science.***8**: 231-241.

Uga, Y., Okuno, K. and Yano, M. (2008). QTL underlying natural variation in stele and xylem structures of rice root. *Breeding Science*.**58**: 7–14.

Venuprasad, R., Dalid, C.O., Del Valle, M., Zhao, D., Espiritu, M., Sta Cruz, M.T., Amante, M., Kumar, A. and Atlin, G.N. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics.***120**: 177–190.

Vikram, P., Swamy, B.P.M., Dixit, S., Ahmed, H.U., Sta Cruz, M.T., Singh, A.K. and Kumar, A. (2011). qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics.***12**: 89.

Wade, L.J., Bartolome, V., Vasant, V.D., Muthukumar, C., Prabakar, S.M. and Henry, A. (2015). Environmental response and genomic regions correlated with rice root growth and yield under drought in the OryzaSNP Panel across Multiple Study Systems. PLoS ONE. **10(4)**:e0124127.

Wang, G.L., Mackill, D.J., Bonman, J.M., McCouch, S.R., Champoux, M.C. and Nelson, R.J. (1994). RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics.***136**: 1421–1434.

Wang, H., Bouman, B.A.M., Dule, Z., Wang, C. and Moya, P.F. (2002). Aerobic rice in northern China: opportunities and challenges. *In*: Water-wise Rice Production. (Eds) Bouman, B.A.M., Hengsdijk, H., Hardy, B., Bindraban, P.S., Tuong, T.P. and Ladha, J.K. *Proceedings of the International Workshop on Water-wise Rice Production*, 8-11 April, Los Banos, Philippines. Los Banos (Philippines): International Rice Research Institute. pp. 143-154.

Wu, K.S. and Tanksley, S.D. (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular Genetics and Genomics*.**241**:225-235.

Xiao, J., Li, J., Yuan, L. and Tanksley, S.D. (1995). Dominance is major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics.***140**:745-754.

Xiaoguang, Y., Bouman, B.A.M., Huaqi, W., Zhimin, W., Junfang, Z. and Bin, C. (2005). Performance of temperate aerobic rice under different regimes in North China. *Agricultural Water Management.* **74(2)**: 107-122.

Xipeng, D., Li, X. and Xiong, L. (2011). Evaluation of near-isogenic lines for drought resistance QTL and fine mapping of a locus affecting flag leaf width, spikelet number, and root volume in rice. *Theoretical and Applied Genetics.***123:** 815-26.

Xu, J., Constantino, S.V., Magpantay, G., Bennett, J., Sarkarung, S. and Huang, N. (1998). Classification of rice germplasm II. Discrimination of *indica* from *japonica* via analysis of amplicon length polymorphism. *Plant Cell Reports.*17.

Xu, Y.B., Shen, Z.T., Chen, Y. and Zhu, L.H. (1995). Molecular mapping for quantitative trait loci controlling yield component characters using a maximum likelihood method in rice (*Oryza sativa* L.). *Acta Genetica Sinica.***22:**46-52.

Yadav, S.K., Pandey, P., Kumar, B. and Suresh, B.G. (2011). Genetic architecture, interrelationship and selection criteria for yield improvement in rice. *Pakistan Journal of Biological Sciences.* **14(9)**: 540-545.

Yoon, D.B., Kang, K.H., Kim, H.J., Ju, H.G., Kwon, S.J., Suh, J.P., Jeong, O.Y. and Ahn, S.N. (2006). Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa japonica* cultivar Hwaseongbyeo. *Theoretical and Applied Genetics.***112**: 1052–1062.

Zhang, L.Q. (2000). Correlation analysis between grain shape and brown rice shape in hybrid rice. *Fujian Science and Technology of Rice and Wheat.***18**: 4–6.

Zheng, B., Yang, L., Mao, C., Zhang, W. and Wu, P. (2006). QTLs and candidate genes for rice root growth under flooding and upland conditions. *Acta Genetica Sinica*.**33:** 141-151.

ANNEXURE

Annexure I

Data on various physio-morphological traits of F_3 plants derived from MAS25 x PAU201 grown under aerobic conditions in the field

Genotype	Plant height(cm)	Number of effective tillers per plant	Number of panicles per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
PAU201	86.7 ± 0.62	7.9 ± 0.5	9.2 ± 0.19	17.5 ± 0.33	9.83 ± 0.47	18.9 ± 0.38	3.55 ± 0.04
MAS25	91.5 ± 0.58	9.5 ± 0.75	11.0 ± 0.38	22.4 ± 0.24	11.3 ± 0.56	20.3 ± 0.35	4.21 ± 0.03
R-1-1	80	7	8	19.1	6.78	21.3	4.01
R-1-2	76	8	8	21.4	8.8	17.6	4.01
R-1-3	74	7	7	19.6	7.55	19.9	3.48
R-5-1	83	9	10	22.6	12.9	22.8	3.74
R-5-2	81	7	7	21.0	9.34	21.6	4.01
R-5-3	74	3	3	16.1	1.86	12.8	3.84
R-9-1	74	2	2	10.5	1.91	13.7	3.91
R-9-2	67	7	7	18.1	5.73	20.1	3.74
R-9-3	72	6	6	17.2	4.04	20.9	3.94
R-14-1	71	5	5	18.8	7.2	20.4	4.00
R-14-2	77	10	11	20.6	7.25	19.4	3.74
R-14-3	75	4	4	16.8	3.8	19.0	3.97
R-17-1	77	14	14	22.4	14.4	26.8	4.23
R-17-2	74	3	3	20.3	5.29	22.4	3.91
R-17-3	69	9	8	19.6	9.49	24.0	4.01
R-26-1	75	1	1	3.67	3.1	13.9	3.92
R-26-2	88	8	8	23.7	11.4	23.3	4.22
R-26-3	78	11	12	21.4	11.2	23.9	4.09
R-40-1	116	8	9	21.7	9.12	20.0	3.12
R-40-2	101	3	3	15.5	2.2	14.5	3.51
R-40-3	120	6	6	24.9	9.01	20.8	3.57
R-50-1	70	3	3	17.9	3.16	25.5	3.97
R-50-2	79	8	9	23.0	10.7	20.1	4.06
R-50-3	72	4	4	20.1	3.69	21.1	3.95
Range	67-120	1-14	1-14	3.67-24.9	1.86-14.4	12.8-26.8	3.12-4.23
Mean ± S.E.	80.8 ± 1.48	6.56 ± 1.2	6.58 ± 1.28	19.1 ± 0.99	7.35 ± 1.34	20.2 ± 0.78	3.87 ± 0.13

Annexure II

Data on physio-morphological and root morphological traits in MAS25 x PAU201 F_3 plants grown under water-limited conditions in the net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight(g)	Grain length -breadth ratio
MAS25	40 ± 0.7	7.81± 0.29	3.42 ± 0.32	12.4 ± 1.63	68.0 ± 1.21	4.5 ± 0.25	20.4 ± 0.6	4.77 ± 0.13	21.2 ± 0.41	5.06 ± 0.11
PAU201	38.5± 0.81	3.35± 0.07	1.1 ± 0.12	6.85 ± 0.15	64.3± 0.86	4.2 ± 0.38	20.3 ± 0.81	2.23 ± 0.43	16.5 ± 0.81	4.52 ± 0.07
R-1-1	43	26.2	5.08	18.9	88	7	31.4	13.0	22.0	4.49
R-1-2	11	5.67	1.2	5.23	91	4	30.7	2.99	22.1	4.86
R-5-1	17	3.64	1.14	7.2	58	6	22.3	1.83	16.1	5.36
R-9-1	40	24.1	6.7	18.3	80	8	27.5	9.91	24.6	5.11
R-9-2	28	3.8	1.54	4.19	89	5	29.4	2.75	21.9	4.93
R-9-3	25	5.75	1.63	6.78	82	5	29.5	3.58	18.7	4.73
R-9-4	23	8.63	1.81	5.23	88	3	27.8	2.0	23.5	4.68
R-9-5	35	6.09	1.01	6.34	86	5	30.7	2.63	1.2	4.01
R-14-1	35	5.53	1.38	5.43	86	5	24.7	3.6	22.5	5.01
R-14-2	41	8.11	2.51	9.84	90	6	25.7	7.5	22.5	4.69
R-14-5	30	6.98	1.38	5.3	80	3	23.1	3.21	21.6	4.94
R-17-3	29	6.63	1.27	5.49	86	3	26.4	3.44	22.3	4.75
R-17-4	35	2.18	0.14	3.13	83	2	20.9	2.03	21.3	4.75
R-26-1	32	5.8	1.36	7.19	68	5	25.1	1.82	17.9	5.06
R-26-2	33	5.27	3.04	9.85	74	6	25.3	4.37	21.5	5.01
R-26-3	37	3.05	1.13	12.5	81	7	32.1	2.56	21.2	4.96
R-26-5	37.5	5.24	2.48	10.3	84	5	27.6	3.94	21.1	4.86
R-27-4	48	5.84	1.67	4.93	80	5	16.9	4.38	22.4	4.86
R-40-4	41	4.67	1.5	7.49	90	9	22.0	2.56	15.3	4.26
R-45-3	43	15.59	3.98	7.31	64	5	19.5	7.26	13.1	4.65
R-50-1	36.2	9.69	3.55	6.27	56	4	21.5	1.77	17.0	4.35
R-50-5	32	3.2	1.06	5.42	58	5	17.3	2.41	23.5	5.31
Range	29-54	2.18-24.1	0.14-8.01	3.13-24.2	56-91	2-9	16.9-32.1	1.77-13.0	13.1-24.6	4.26-5.36
Mean ± S.E.	37.6 ± 3.36	16 ± 3.04	3.71 ± 0.16	14.6 ± 2.15	79.2 ± 1.9	5.14 ± 0.31	25.3 ± 1.58	5.17 ± 1.1	20.7 ± 1.9	4.85 ± 0.21

Annexure III

Data on various physio-morphological traits of F_3 plants derived from MASARB25 x PAU201 grown under aerobic conditions in the field

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant(g)	1000 grain weight(g)	Grain length-breadth ratio
MASARB25	90.6 ± 0.75	11.8 ± 0.58	9.4 ± 0.51	23.2 ± 0.15	10.8 ± 0.14	20.6 ± 0.45	4.12 ± 0.04
PAU201	86.7 ± 0.62	9.2 ± 0.19	7.9 ± 0.5	17.5 ± 0.33	9.83 ± 0.47	18.9 ± 0.38	3.55 ± 0.04
Q-1-1	77	12	10	20.5	10.2	18.0	4.09
Q-1-2	78	9	6	19.4	6.75	15.8	3.67
Q-1-3	74	8	6	20.9	5.18	15.8	3.97
Q-2-1	70	11	11	19.4	9.19	22.7	3.79
Q-2-2	95	9	8	18.8	6.45	19.9	3.91
Q-2-3	71	9	8	21.1	10.6	23.0	3.96
Q-5-1	86	15	13	21.1	19.3	24.7	3.82
Q-5-2	110	10	9	19.3	13.7	25.2	3.83
Q-5-3	70	10	6	17.8	5.28	22.0	3.76
Q-6-1	60	3	3	19.2	3.77	20.8	3.91
Q-6-2	84	9	8	20.1	6.7	20.1	3.76
Q-6-3	66	8	6	17.7	5.86	23.5	4.08
Q-8-1	71	14	11	18.2	8.7	27.3	3.94
Q-8-2	87	12	10	20.8	9.53	22.4	3.96
Q-8-3	81	11	11	17.7	10.4	24.6	4.17
Q-9-1	77	15	12	22.7	17.5	19.8	3.68
Q-9-2	72	8	5	22.1	7.8	23.8	3.53
Q-9-3	75	9	6	21.1	6.88	20.2	3.51
Q-10-1	110	14	13	19.2	9.68	20.8	3.92
Q-10-2	74	12	7	19.4	6.39	20.4	4.21
Q-10-3	65	16	8	17.1	7.75	21.3	3.99
Q-17-1	76	9	9	20.3	10.5	20.7	3.84
Q-17-2	69	10	10	18.8	7.02	19.3	3.63
Q-17-3	73	9	9	19.6	5.73	21.6	4.04
Q-25-1	71	12	10	19.2	10.9	21.4	4.12
Q-25-2	71	8	7	19.3	7.97	21.7	4.0
Q-25-3	65	13	12	18.7	8.47	20.6	3.9
Q-35-1	74	12	9	21.8	7.66	21.0	4.15
Q-35-2	76	9	8	20.4	7.79	17.3	4.17
Q-35-3	68	11	8	21.3	6.61	16.8	3.61
Range	60-110	3.0-16	3.0-13	17.1-22.7	3.77-19.3	15.8-27.3	3.51-4.21
Mean ± S.E.	77.3 ± 1.32	10.5 ± 0.83	8.75 ± 0.82	19.8 ± 0.34	8.78 ± 1.11	21.0 ± 0.58	3.89 ± 0.10

Annexure IV

Data on physio-morphological and root morphological traits in MASARB25 x PAU201 F_3 plants grown under water-limited conditions in the net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant(g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	41.6 ± 0.51	8.15 ± 0.5	2.45 ± 0.55	15.1 ± 0.47	69.2 ± 0.74	4.8 ± 0.2	21.3 ± 0.54	3.65 ± 0.26	19.0 ± 0.71	5.15 ± 0.04
PAU201	38.5 ± 0.81	3.35 ± 0.07	1.1 ± 0.12	6.85 ± 0.15	64.3 ± 0.86	4.2 ± 0.38	20.3 ± 0.81	2.23 ± 0.43	16.5 ± 0.81	4.52 ± 0.07
Q-1-3	28	5.34	2.45	5.34	74	3	20.5	2.93	17.2	4.37
Q-2-1	36	5.73	1.65	8.56	67	2	18.9	1.01	17.8	4.57
Q-2-3	39.5	3.35	1.07	6.85	55	2	12.0	1.09	10.5	4.52
Q-2-4	40	2.75	0.8	5.34	70	2	22.6	1.25	9.87	4.91
Q-5-1	39	3.4	1.08	9.66	54	2	12.8	2.93	18.4	4.74
Q-5-2	21	2.64	1.22	11.6	69	1	8.6	0.03	0.3	5.0
Q-5-4	29	8.17	1.63	5.8	62	1	9.17	1.24	11.9	5.01
Q-6-1	32.8	6.48	1.83	4.32	60	5	19.0	4.45	17.8	5.36
Q-6-2	35	8.49	2.76	14.0	66	3	17.1	1.56	12.4	5.04
Q-6-4	45	10.9	3.34	9.59	64	4	21.1	4.63	19.6	5.68
Q-8-1	22	1.08	0.45	2.89	55	1	7.13	1.02	8.3	4.49
Q-8-4	41	2.29	0.77	2.26	55	1	7.83	0.98	10.8	4.86
Q-10-2	27	1.85	0.85	1.86	95	4	25.3	2.56	11.4	5.09
Q-10-5	44	20.3	3.64	22.7	73	5	24.5	7.56	15.2	4.26
Q-17-2	48	11.7	3.03	13.4	77	4	22.0	3.09	19.4	5.58
Q-17-5	37	11.6	2.98	12.2	50	2	15.1	1.09	13.6	4.35
Q-25-3	33	19.1	8.31	21.6	40	3	18.9	7.56	19.6	4.98
Q-25-5	35	4.18	1.35	8.79	63	2	14.4	4.01	24.1	4.69
Q-35-1	33	9.43	1.86	14.0	58	5	21.3	2.20	19.4	4.87
Q-35-2	42	24.8	4.81	23.1	60	8	21.9	11.0	22.5	4.63
Q-35-3	30	4.64	1.81	11.0	52	4	21.6	1.15	11.0	4.34
Range	21-48	1.08-24.8	0.45-8.31	1.86-23.1	40-95	1-8	7.13-25.3	0.03-11.0	0.3-24.1	4.26-5.68
Mean ± S.E.	35.4 ± 1.17	8.3 ± 2.2	2.28 ± 1.12	10.5 ± 1.9	63.6 ± 1.4	3.17 ± 0.96	17.8 ± 1.32	3.14 ± 1.5	15.3 ± 1.4	4.83 ± 0.17

Annexure V

Data on physio-morphological traits in PAU201 x MAS26 F_3 plantsgrown under aerobic conditions in the field

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant(g)	1000 grain weight(g)	Grain length-breadth ratio
PAU201	86.7 ± 0.62	9.2 ± 0.19	7.9 ± 0.5	17.5 ± 0.33	9.83 ± 0.47	18.9 ± 0.38	3.55 ± 0.04
MAS26	92.1 ± 0.58	11.9 ± 0.55	9.6 ± 0.68	22.8 ± 0.18	11.9 ± 0.52	21.0 ± 0.31	4.25 ± 0.04
P-6-1	70	5	5	21.7	6.45	23.0	3.55
P-6-2	97	4	4	17.8	4.73	19.4	3.63
P-6-3	104	5	5	21.5	7.7	12.5	3.73
P-12-1	87	16	16	20.2	19.8	26.8	3.77
P-12-2	75	13	13	20.6	12.5	22.1	3.97
P-12-3	81	11	9	20.8	12.1	20.0	3.82
P-14-1	72	13	13	16.9	12.2	21.9	3.98
P-14-2	69	9	9	19.6	7.67	27.8	3.55
P-14-3	81	9	8	21.2	9.75	24.7	3.68
P-23-1	92	14	12	20.0	14.3	21.8	3.73
P-23-2	68	11	10	18.0	12.2	20.5	3.92
P-23-3	72	7	7	19.1	8.92	21.5	3.93
P-24-1	66	9	8	19.5	6.35	27.1	3.94
P-24-2	60	9	7	17.2	1.39	13.9	3.67
P-24-3	77	12	9	18.7	5.9	19.1	3.98
P-25-1	89	13	12	22.4	12.7	21.1	3.72
P-25-2	108	9	9	22.7	8.52	21.3	4.14
P-25-3	91	13	11	21.1	13.9	23.8	5.18
P-31-1	80	5	5	21.1	6.21	22.3	3.84
P-31-2	65	7	7	18.7	5.33	19.3	3.48
P-31-3	73	6	6	20.3	5.33	20.7	3.57
P-32-1	80	9	9	21.0	6.03	19.2	3.77
P-32-2	81	8	7	21.0	4.73	19.1	3.73
P-32-3	107	12	10	19.7	11.4	21.5	3.64
P-45-1	75	12	9	19.5	8.1	21.2	3.83
P-45-2	65	11	6	16.9	3.89	12.4	3.74
P-45-3	100	11	10	21.0	7.58	19.9	3.8
P-48-1	82	12	11	20.4	7.66	22.8	4.1
P-48-2	83	12	10	22.0	9.85	21.3	4.13
P-48-3	81	13	11	20.4	11.2	21.3	4.18
Range	60-108	4.0-16	4.0-16	16.9-22.7	1.39-19.8	12.4-27.8	3.48-5.18
Mean ± S.E.	81.6 ± 1.4	9.92 ± 0.96	9.03 ± 0.9	20.0 ± 0.37	8.94 ± 1.26	21.0 ± 0.76	3.86 ± 0.16

Annexure VI

Data on physio-morphological and root morphological traits in PAU201 x MAS26 F_3 plants grown under water-limited conditions in the net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
PAU201	38.5± 0.81	3.35± 0.07	1.1 ± 0.12	6.85 ± 0.15	64.3 ± 0.86	4.2 ± 0.38	20.3 ± 0.81	2.23 ± 0.43	16.5 ± 0.81	4.52 ± 0.07
MAS26	40.5 ± 0.6	7.3 ± 0.14	2.14 ± 0 .65	13.5 ± 0.67	68.4 ± 1.29	4.6 ± 0.25	20.7 ± 0.78	3.76 ± 0.45	20.8 ± 0.38	4.87 ± 0.01
P-6-1	41	9.6	2.39	9.9	64	3	18.1	1.25	11.8	4.98
P-6-2	36	6.78	1.99	9.68	54	2	13.9	1.11	10.7	5.10
P-6-4	34	6.72	1.98	11.8	64	6	20.9	6.28	13.1	4.88
P-12-4	32	5.71	1.53	8.14	42	3	15.1	2.68	5.89	4.77
P-14-5	30	4.07	1.16	6.04	79	2	13.2	1.18	11.9	4.79
P-23-4	30	3.42	1.01	5.28	70	5	20.2	0.6	11.3	4.64
P-23-5	40	7.62	2.31	13.8	62	2	14.1	1.17	5.8	4.80
P-24-3	47.8	8.04	2.34	14.5	60	4	23.9	4.69	10.3	5.04
P-24-5	44	6.91	1.56	7.92	58	1	6.47	1.09	10.9	4.82
P-31-3	27	2.69	0.90	9.6	50	4	17.9	0.09	6.0	4.52
P-31-5	28	6.35	1.75	8.32	67	3	19.0	1.71	16.4	5.34
P-32-1	42	5.81	2.94	8.09	45	3	17.7	0.05	0.5	2.13
P-32-4	28	3.57	1.31	5.23	68	4	21.3	1.18	11.4	4.75
P-45-4	33	2.62	0.64	7.62	35	4	16.2	1.90	12.1	5.59
P-48-1	36.5	3.84	1.08	9.01	64	2	14.3	3.43	21.0	4.89
P-48-2	27	2.54	0.81	6.7	60	2	12.1	1.82	16.9	4.62
P-48-3	43	10.8	2.62	17.8	89	3	22.9	3.41	18.7	4.68
Range	27-47.8	2.54-10.8	0.64-2.94	5.23-17.8	35-89	1-6	6.47-23.9	0.05-6.28	0.5-21	2.13-5.59
Mean ±S.E.	35.7 ± 1.08	5.67 ± 1.02	1.67 ± 0.53	9.46 ± 1.1	61.3 ± 1.6	3.25 ± 0.7	17.3 ± 1.04	2.09 ± 1.1	12.2 ± 1.54	4.73 ± 0.3

Annexure VII

Data on various physio-morphological traits of F_3 plants derived from MASARB25 x HKR47 grown under aerobic conditions in the field

Genotype	Plant height (cm)	Number of panicles per plant	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	90.6 ± 0.75	11.8 ± 0.58	9.4 ± 0.51	23.2 ± 0.15	10.8 ± 0.14	20.6± 0.45	4.12 ± 0.04
HKR47	85.8 ± 0.6	9.7 ± 0.93	8.2 ± 0.52	18.1 ± 0.24	9.97 ± 0.44	19.75 ± 0.42	3.58 ± 0.04
M-13-1	75	9	8	18.4	10.6	20.6	3.83
M-13-2	78	14	12	22.0	17.8	20.1	4.2
M-13-3	69	8	7	21.3	5.26	18.6	4.88
M-14-1	68	4	4	16.9	4.15	21.7	3.85
M-14-2	80	12	11	20.6	17.6	19.2	4.25
M-14-3	75	6	6	18.3	12.8	22.9	4.07
M-19-1	73	8	8	18.7	7.23	27.4	3.69
M-19-2	72	7	7	22.1	10.5	20.4	3.81
M-19-3	81	14	12	21.7	18.2	20.6	3.78
M-21-1	78	13	13	21.2	11.8	20.2	3.85
M-21-3	86	9	9	21.1	10.2	20.8	3.97
M-21-2	83	8	7	19.3	9.16	19.4	3.6
M-22-1	91	5	5	18.8	4.67	20.6	3.92
M-22-2	78	13	13	19.3	12.2	22.9	4.14
M-22-3	77	12	11	17.2	7.31	20.6	3.94
M-24-1	83	8	8	18.7	7.14	20.0	3.67
M-24-2	77	8	10	21.1	13.4	20.9	4.06
M-24-3	79	7	7	18.1	7.64	23.0	3.62
M-30-1	87	2	2	11.5	2.78	20.2	3.75
M-30-2	74	9	8	23.1	12.8	26.7	3.74
M-30-3	78	5	5	17.7	4.53	12.0	4.06
M-34-1	76	7	7	21.8	10.8	23.0	4.01
M-34-2	84	9	7	24.6	11.0	24.1	4.11
M-34-3	81	4	4	24.3	10.2	22.9	4.18
M-38-1	81	2	2	20.6	4.53	22.7	3.94
M-38-2	76	7	7	18.9	5.97	22.2	4.5
M-38-3	74	7	7	18.9	6.94	22.3	4.1
M-39-1	88	5	5	23.3	10.8	24.0	3.99
M-39-2	82	6	6	22.9	10.7	23.4	4.27
M-39-3	132	4	4	27.7	12.8	20.5	4.08
Range	68-132	2.0-14	2.0-13	11.5-27.7	2.78-18.2	12-27.4	3.6-4.88
Mean ± S.E.	80.5 ± 5.54	7.72 ± 1.12	7.4 ± 1.09	20.3 ± 1.5	9.75 ± 1.8	21.5 ± 2.45	3.99 ± 0.45

Annexure VIII

Data on various physio-morphological and root morphological traits of MASARB25 x HKR47 F_3 plants grown under water-limited conditions in the net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
MASARB25	41.6 ± 0.51	8.15 ± 0.5	2.45 ± 0.55	15.1 ± 0.47	69.2 ± 0.74	4.8 ± 0.2	21.3 ± 0.54	3.65 ± 0.26	19.0 ± 0.71	5.15 ± 0.04
HKR47	39.2 ± 0.67	5.84 ± 0.12	1.62 ± 0.29	10.4 ± 0.77	65.0 ± 0.51	4.2 ± 0.38	20.7 ± 0.26	3.3 ± 0.18	18.8 ± 0.97	4.59 ± 0.12
M-13-1	32.8	3.42	0.91	8.54	64	2	11.7	0.87	11.1	4.75
M-13-4	31	1.96	0.71	4.45	64	2	13.8	1.08	10.2	4.86
M-21-2	31	3.27	1.16	11.5	57	4	21.6	1.2	11.7	5.41
M-21-3	30	3.18	1.09	8.51	65	2	14.5	1.19	11.5	4.96
M-21-5	36	5.25	1.85	19.0	77	3	22.4	0.91	9.1	4.99
M-22-4	18	2.89	0.9	6.37	70	4	20.7	1.04	9.4	4.66
M-24-1	35	2.63	0.97	5.97	65	3	20.3	1.26	12.3	4.94
M-30-1	41.8	7.61	3.45	17.6	71	3	22.8	2.49	17.9	4.83
M-30-3	48	6.32	1.66	17.0	60	3	20.3	1.01	14.5	4.91
M-30-4	24	2.75	1.48	12.1	47	5	20.3	1.34	12.6	5.28
M-34-4	41	3.08	0.89	8.51	64	4	23.9	3.32	17.6	4.93
M-34-5	43	10.1	3.0	19.8	77	5	24.4	5.8	20.2	4.90
M-38-2	45	2.6	0.7	7.4	61	2	10.9	1.04	10.3	4.56
M-38-3	36	4.84	1.39	13.3	63	4	18.5	1.4	13.5	4.83
M-38-4	46	13.5	5.22	22.6	65	10	17.5	3.3	10.0	5.21
M-39-3	36	2.47	0.7	10.2	67	5	22.9	3.53	18.8	4.84
M-39-5	34.6	4.65	1.32	12.2	61	6	19.9	3.65	19.8	4.81
Range	18-48	1.96-13.5	0.7-5.22	4.45-22.6	47-77	2-10	10.9-24.4	0.87-5.8	9.4-20.2	4.56-5.41
Mean ± S.E.	36.3 ± 1.26	4.97 ± 1.36	1.66 ± 0.9	12.2 ± 1.47	64.9 ± 0.84	3.99 ± 0.9	19.4 ± 0.9	2.18 ± 0.96	14.2 ± 1.1	4.92 ± 0.1

Annexure IX

Data on various physio-morphological traits of MAS25 x HKR47 F_3 plants grown under aerobic conditions in the field

Genotype	Plant height (cm)	Number of effective tillers per plant	Number of panicles per plant	Panicle length (cm)	Grain yield per plant (g)	1000 grain weight (g)	Grain length-breadth ratio
HKR47	85.8 ± 0.6	8.2 ± 0.52	9.7 ± 0.93	18.1 ± 0.24	9.97 ± 0.44	19.8 ± 0.42	3.58 ± 0.04
MAS25	91.5 ± 0.58	9.5 ± 0.75	11.0 ± 0.38	22.4 ± 0.24	11.3 ± 0.56	20.3 ±0.35	4.21 ± 0.03
N-6-1	86	12	14	21.5	11.9	21.9	4.09
N-6-2	90	4	4	18.1	6.29	23.8	3.86
N-6-3	77	6	6	19.9	7.54	23.3	3.72
N-10-1	81	5	5	19.3	4.67	19.4	4.0
N-10-2	94	6	6	21.4	7.82	21.0	3.43
N-10-3	85	9	11	19.1	10.5	20.6	4.29
N-11-1	84	6	6	20.9	7.0	20.9	3.64
N-11-2	85	9	9	22.0	11.8	19.6	4.19
N-11-3	90	7	7	19.1	6.52	23.9	4.03
N-14-1	85	9	9	19.3	10.3	22.8	4.09
N-14-2	88	11	13	21.2	9.41	23.4	4.12
N-14-3	85	8	11	22.7	10.3	21.2	3.85
N-20-1	69	6	6	15.8	4.13	21.5	4.29
N-20-2	86	8	8	19.9	8.32	13.5	3.82
N-20-3	86	8	8	21.0	8.44	19.0	4.08
N-24-1	71	7	7	16.1	2.94	19.4	3.76
N-24-2	83	12	12	20.9	9.53	20.0	4.05
N-24-3	85	13	14	19.6	15.8	23.5	4.05
N-40-1	87	7	8	20.7	7.38	18.5	3.99
N-40-2	83	7	7	21.5	6.29	14.6	3.95
N-40-3	90	11	11	20.5	10.1	22.9	4.23
N-47-1	110	5	5	21.7	8.43	18.5	3.95
N-47-2	86	5	5	20.5	5.65	23.0	3.81
N-47-3	91	14	16	24.0	15.0	22.1	4.09
N-48-1	75	7	7	17.9	5.43	17.7	3.6
N-48-2	78	5	5	20.1	4.14	12.8	3.43
N-48-3	64	6	6	18.3	5.91	19.4	3.92
N-49-1	75	8	8	19.3	4.88	15.2	3.84
N-49-2	83	7	7	17.2	3.31	19.5	3.7
N-49-3	80	7	7	17.8	4.13	22.8	3.7
Range	64-110	14-4	4.0-16	15.8-24.0	2.94-15.8	12.8-23.9	3.43-4.29
Mean ± S.E.	83.7 ± 0.9	7.83 ± 0.89	8.27 ± 1.1	19.9 ± 0.42	7.79 ±1.1	20.0 ± 0.66	3.91 ± 0.12

Annexure X

Data on various physio-morphological traits of MAS25 x HKR47 F_3 plants grown under water-limited conditions in the net house

Genotype	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root thickness (mm)	Plant height (cm)	Number of effective tillers per plant	Panicle length (cm)	Grain yield per plant(g)	1000 grain weight (g)	Grain length-breadth ratio
HKR47	39.2 ± 0.67	5.84 ± 0.12	1.62 ± 0.29	10.4 ± 0.77	65.0 ± 0.51	4.2 ± 0.38	20.7 ± 0.26	3.3 ± 0.18	18.8 ± 0.97	4.59 ± 0.12
MAS25	40 ± 0.7	7.81 ± 0.29	3.42 ± 0.32	12.4 ± 1.63	68.0 ± 1.21	4.5 ± 0.25	20.4 ± 0.6	4.77 ± 0.13	21.2 ± 0.41	5.06 ± 0.11
N-10-3	44	12.4	4.42	10.9	76	3	21.2	4.73	22.2	5.1
N-10-5	35	17.2	4.53	12.0	67	2	14.5	2.34	15.5	5.3
N-11-1	45	14.4	3.99	12.3	68	2	12.7	1.1	11.3	4.8
N-11-4	41	9.83	2.62	8.94	62	8	16.6	1.55	15.3	6.1
N-24-3	38	4.24	2.49	3.12	94	4	21.1	7.49	18.9	5.2
N-24-4	30	18.6	4.33	9.97	84	6	20.0	4.63	18.2	5.4
N-40-1	33	23.4	8.14	26.0	58	6	20.3	6.12	18.5	5.0
N-40-2	28	10.7	2.18	5.64	50	6	18.5	2.93	21.2	5.0
N-40-4	41	10.3	2.65	5.89	50	6	18.0	1.31	12.3	5.1
N-47-1	38	11.2	3.03	9.17	72	3	22.7	2.77	16.6	4.6
N-47-2	40	11.9	0.53	1.58	56	3	16.7	0.09	0.9	4.8
N-48-3	39	15.7	2.78	13.6	85	4	22.8	0.12	1.2	4.3
Range	28-45	4.24-23.4	0.53-8.14	1.58-26.0	50-94	2-8	12.7-22.8	0.09-7.49	0.9-22.2	4.3-6.1
Mean ±S.E.	37.9 ± 0.76	13.3 ± 1.46	3.47 ± 0.98	10.3 ± 1.83	68.5 ± 1.6	4.42 ± 0.85	19.0 ± 0.69	3.1 ± 1.3	15.2 ±1.7	5.1 ± 02

ABSTRACT

Title of Thesis	:	**Phenotyping and molecular marker analysis of selected F_3 segregating lines from aerobic x low land *indica* rice (*Oryza sativa* L.) crosses**
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Year of award of degree	:	2015
Major subject	:	Molecular Biology and Biotechnology
Total number of pages in dissertation	:	70+ix+X
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Key words: Aerobic rice, *indica* rice, SSR

Experiments were conducted to evaluate F_3 segregating populations derived from the five crosses (PAU201 **x** MAS25, MASARB25 x PAU201, PAU201 x MAS26, MASARB25 x HKR47 and MAS25 x HKR47) for various physio-morphological and/or root traits and microsatellite markers linked to the traits promoting aerobic adaptation. MASARB25, MAS25 and MAS26 are aerobic while PAU201 and HKR47 are low-land *indica* rice varieties. In all the five populations, wide variation was observed for plant height, panicle length, number of panicles per plant, number of effective tillers per plant, root length, root thickness, fresh and dry root weight, 1000 grain weight, grain length-breadth ratio and grain yield per plant. In these populations, significant positive correlation was observed between yield per plant with plant height, effective no. of tillers per plant, length-breadth ratio, 1000 grain weight, root biomass and/or root length. The NTSYS-pc UPGMA tree cluster analysis and 2-D PCA scaling of selected F_3 plants derived from the five crosses clearly showed large variation among two parental genotypes and F_3 plants were invariably interspersed between them. A number of promising F_3 plants have been selected, which had higher grain yield, root length and biomass greater than MAS25, MAS26 and MASARB25 for further progeny analysis. Most of these selected plants had the desired allele for the markers reported earlier to be linked with the aerobic adaptation traits (RM234 and RM547 for root length on chromosome 7 and 8 respectively; RM525 for root thickness and root biomass on chromosome 2).

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