

**Molecular and physiological studies to identify
traits enhancing rice cultivars adaptation to
aerobic conditions for sustainable rice
production in water short situations**

By

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(2009BS38D)**

*Thesis submitted to the Chaudhary Charan Singh
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HISAR – 125004**

2013

CERTIFICATE – I

This is to certify that this dissertation entitled, **“Molecular and physiological studies to identify traits enhancing rice cultivars adaptation to aerobic conditions for sustainable rice production in water short situations”** submitted for the degree of Doctor of Philosophy in the subject of Molecular Biology and Biotechnology to the Chaudhary Charan Singh Haryana Agricultural University, Hisar is a bonafide research work carried out by **Nitika Sandhu (Admn. No. 2009BS38D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

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

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CERTIFICATE – II

This is to certify that this dissertation entitled, **“Molecular and physiological studies to identify traits enhancing rice cultivars adaptation to aerobic conditions for sustainable rice production in water short situations”**, submitted by **Nitika Sandhu** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**, in the subject of **Molecular Biology and Biotechnology** has been approved by the Student’s Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

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Date:

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ABBREVIATIONS

APS	:	Ammonium per sulphate
bp	:	base pair
CIM	:	Composite interval mapping
cM	:	centi Morgan
CTAB	:	Cetyl trimethylammonium bromide
DAS	:	Days after sowing
DS	:	Dry season
DSR	:	Direct seeded rice
EDTA	:	Ethylene diamine tetra acetic acid
EVV	:	Early vegetative vigour
g	:	grams
LOD	:	Logarithm of odds
LDS	:	Lowland drought stress
LR	:	Likelihood ratio
LRW	:	Lowland re-watered
LWW	:	Lowland well watered
MAB	:	Marker Assisted Backcrossing
MAS	:	Marker Assisted Selection
NS	:	Non stress
NR	:	Number of nodal root
PHT	:	Plant height
PAGE	:	Polyacrylamide gel electrophoresis
PCA	:	Principle component analysis
PCR	:	Polymerase chain reaction
QTLs	:	Quantitative trait loci
RGR	:	Relative growth rate
RHD	:	Root hair density
RHL	:	Root hair length
SSR	:	Simple sequence repeats
RS	:	Reproductive stress
SSS	:	Seedling stage stress
UDS	:	Upland drought Stress
URW	:	Upland re-watered
UWW	:	Upland well watered
UPGMA	:	unweighted pair-group method with arithmetic average
WinQTL	:	Windows QTL
WS	:	Wet season

CHAPTER- I

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal food crops in the world. Rice cultivation is one of the most important agricultural activities on earth, with nearly 90% of it being produced in Asia and supplies more than 50% of calories consumed by world's population. It is an annual grass of family *Poaceae* (*Gramineae*) and belongs to the genus *Oryza*. The genus *Oryza* that has two cultivated species, *O. sativa* (Asian) and *O. glaberrima* (African), and about 21 wild species, is widely distributed in tropical and subtropical regions (Vaughan, 1989). Asia is the biggest rice producer, accounting for 90% of the world's production and consumption of rice. China and India, which account for more than one-third of global population supply over half of the world's rice. Globally rice is grown over an area of about 149 million ha with an annual production of 600 million tones (Bernier *et al.*, 2009).

Food security in Asia is challenged by increasing food demand and threatened by declining water availability. More than 75% of the rice supply comes from 79 million ha of irrigated land. Thus, Asia's present and future food security depends largely on the irrigated rice production system. However, the water-use efficiency of rice is low, and growing rice requires large amounts of water. In Asia, irrigated agriculture accounts for 90% of total diverted freshwater, and more than 50% of this is used for rice cultivation. Until recently, this amount of water has been taken for granted, but now the global "water crises" threatens the sustainability of irrigated rice production.

Increasing scarcity of water has threatened the traditional rice cultivation practices all over the world (Tuong and Bouman, 2003). 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 (Tuong and Bouman, 2003). Irrigation for agriculture consumes 2/3 of the world's fresh water and rice alone consumes more than 50 per cent of the water used for irrigation in Asia.

Overexploitation of groundwater has caused serious problems in many parts of India including Haryana and Punjab; groundwater tables have dropped on average by 0.5 - 1.0 m y^{-1} in states of India (Bouman and Tuong, 2001). 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, 2003).

Decreasing water resources for rice cultivation has prompted research on the development of water efficient 'aerobic rice' varieties by combining the drought-resistant

characteristics of upland varieties with the high-yielding traits of lowland varieties (Belder *et al.*, 2005). Aerobic rice is a production system in which specially developed varieties are grown in well-drained, non-puddled and non-saturated soils. The soil is therefore “aerobic” or with oxygen throughout the growing season, as compared to traditional flooded fields, which are “anaerobic.” Rice is characterized by a shallow root system compared with other cereal crops, having limited water extraction below 60 cm (Fukai and Inthapan, 1988). The form of the rice root system also varies with the cultivation method (Yoshida and Hasegawa, 1982). In upland conditions with direct sowing, the root system generally develops deeper than in transplanted plantings in lowland conditions. Compared with traditional lowland rice cultivation, water inputs in aerobic rice were > 50% lower (only 470-650 mm), water productivities 64-88% higher, gross returns 28-44% lower (345-633 \$ ha⁻¹) and labor use 55% lower. It is hard to say if aerobic rice will replace the traditional rice varieties, but there is no doubt that aerobic rice may find its place in water-short irrigated lowlands, fields on upper slopes or terraces in undulating, rainfed lowlands, favorable uplands etc.

Direct-seeded rice (DSR) is a feasible alternative to conventional puddled transplanted rice with good potential to save water, reduce labour requirement, mitigate green-house gas (GHG) emissions and adapt to climatic risks, and the yields can be comparable with that of transplanted rice if the crop is properly managed (Kumar and Ladha, 2011). Although development of suitable varieties and agronomic packages for promoting the DSR is underway (Pathak *et al.*, 2011), thus far no variety has been developed specifically for direct-seeded conditions that are prone to drought/flooded and nutrient deficient conditions at the seedling stage. Although it is often emphasized that enhancing nutrient-use efficiency is critical across the globe both to sustain yields in low-input agriculture and to reduce fertilizer inputs in high input agriculture, it is not clear if traits that enhance efficiency in low-input systems also confer efficiency in high-input systems (Rose *et al.*, 2012). Therefore, better understanding of root-related traits and how root trait QTL interacts to affect soil resource acquisition across a range of environments will be an important tool in the breeding of direct seeded rice varieties.

Root architectural plasticity, the ability to exhibit morphological and physiological responses to a changing environment, may play an important role in plant adaptation to heterogeneous environments (Bradshaw, 1965; Sultan, 2000; Pigliucci, 2001) and is important in maintaining crop productivity (Wang and Yamauchi, 2006) with low inputs. Such plasticity is especially evident in the root system since it adopts a particular architecture under one condition, but can change architecture in response to change in condition. Root system plasticity has been implicated in causing genotype x environment interactions (MacMillan *et al.*, 2006). Interest has increased in understanding the molecular control of root

development in rice because of the importance of root system establishment and plasticity in plant performance in the field under direct seeded cultivation conditions.

During the vegetative stage, rapid groundcover achieved with early vegetative vigour (Poorter and De Jong, 1999; Shipley, 2006; Dingkuhn *et al.*, 1999) can reduce soil evaporation and accelerate root access to soil water and nutrients (Zhao *et al.*, 2006a). Direct seeded conditions decrease the number of nodal roots per internode and stimulate the elongation of nodal roots (Kondo *et al.*, 1999). Other traits that may be important for direct-seeded rice are root hair growth and lateral root growth. Phosphorus uptake is closely related to root hair length, and plants grown under phosphate-limiting conditions form longer root hairs (Bates and Lynch, 1996; Zhang *et al.*, 2003). Increased lateral root growth increased phosphorus uptake in common bean (Lynch and Van Beem, 1993) and maize (Zhu and Lynch, 2004), nitrogen uptake (Sen *et al.*, 2013) and yield under varying water and nutrient regimes (Hochholdinger and Tuberosa, 2009) in maize, and water uptake under drought in rice (Suralta *et al.*, 2008 and 2010). Interest has increased in understanding the molecular control of root development in rice because of the importance of root system establishment and plasticity in plant performance in the field under direct seeded cultivation conditions.

Historically, genetic diversity and intraspecific classification in Asian rice has been studied using morphological, serological and hybrid fertility parameters in combination (Kato *et al.*, 1928), morphogeographical (Matsuo, 1952; Oka, 1958), isozymatic (Glaszmann, 1987) data and more recently using the DNA markers (Jain *et al.*, 2004; McCouch *et al.*, 2001). 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. 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 (Flowers *et al.*, 2000). SSRs are valuable as genetic markers because they detect high levels of allelic diversity, are co-dominant, are easily and economically assayed by PCR and are easily automated. 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. Rice genome sequence shows the presence of one SSR marker every 157 bp. 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 genetic effect; such traits are typically controlled by quantitative trait loci (QTL; Sharma *et al.*, 2011). 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 important traits in rice such

as drought tolerance (Champoux *et al.*, 1995; Price and Courtois, 1999), phosphorus uptake (Ismail *et al.*, 2007), and aroma (Cordeiro *et al.*, 2002). Molecular markers could be used to tag quantitative trait loci (QTL) and to evaluate their contributions to the phenotype by selecting for favorable alleles at these loci in a marker-assisted selection scheme aiming to accelerate genetic advance. Bulk segregant analysis (BSA) is a cost-effective strategy to identify tightly linked QTL. Several QTL for grain yield under drought were identified in rice using this method (Vikram *et al.*, 2011, Ghimere *et al.*, 2011, Mishra *et al.*, 2013, Yadaw *et al.*, 2013). The concept of QTL detection was first proposed by Sax (1923) and the first known statistical approach was anticipated by Thoday (1961). High resolution linkage mapping would help to elucidate gene functions and gene regulation as well as their expression. The mapping and tagging of the genes with molecular markers is the foundation for marker assisted selection (MAS) in crop plants.

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

OBJECTIVES:

1. To evaluate aerobic × non-aerobic *indica* rice F₂ population for physio-morphological and root traits.
2. To study the genetic structure of an aerobic × non-aerobic F₂ population using markers linked to root trait(s).
3. To study the genetic structure of roots flexibility in an aerobic × non-aerobic mapping population in alternating aerobic to anaerobic situation.
4. To identify large effect QTL(s) contributing to high grain yield under aerobic condition in populations derived from the crosses Kali Aus x MTU1010 and Kali Aus x IR64.
5. To identify QTL(s) for high early vigor and root traits contributing to high yield under seedling stage drought in populations derived from crosses Aus 276 x MTU1010 and Mikhudeb x MTU1010.

Globally, rice ranks second only to wheat in terms of area harvested, but in terms of importance as a food crop, rice provides more energy per hectare than any other cereal crop. Rice (*Oryza sativa* L.) belongs to the genus *Oryza* which includes more than 25 wild species (diploid or tetraploid) either perennial or annual. *Oryza sativa* has two subspecies, *indica* and *japonica*, which are adapted to diverse environmental conditions ranging from upland to lowland and from tropical to temperate zones. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's population lives. Rice is planted on about 154 million hectares annually, which account for ~11% of the world's cultivated land (Khush, 2000). 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.

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 rainfed rice ecosystems because the crop relies strictly on rainfall for its water supply. It also increasingly affects the irrigated rice ecosystem because of undependable irrigation water (IRRI, 1997). Drought is the source of huge yield losses. Widawsky and O'Toole (1996) evaluated at 3.0 million tons the annual drought losses from the 24 million hectares of rice of Eastern India, which represented 22% of all losses from technical constraints. In Asia, irrigated agriculture accounts for 90 percent of the total diverted freshwater used, and more than 50 percent of this is used to irrigate rice. Until recently, this quantity of water was taken for granted, but this situation cannot continue. As the water crisis deepens, the emphasis is on water saving through irrigation systems that are water-efficient. This means developing plants that are high-yielding even when grown under recurrent mild water deficit.

About 70 percent of the water currently withdrawn from all freshwater sources worldwide is used for agriculture. Traditionally rice cultivation is carried out in submerged puddled soils by transplanting. The crop is irrigated regularly with standing water up to 5-6 inches. It takes about 5000 liters of water to produce one kg of rice. The looming global water crisis threatens the sustainability of irrigated rice, which is the Asia's biggest water user. Farmers and irrigation managers have shown keen interest in new technologies that can cope with the decreasing water scarcity. Under this situation many farmers are discouraged from growing rice and switch on to other crops requiring less water. To tackle these problems, research has been directed towards the development of "Aerobic rice" varieties that combine the drought-resistant characteristics of upland varieties (grown on non-flooded sloping islands) with the high-yielding traits of lowland varieties (grown in irrigated, flooded

fields). The International Rice Research Institute (IRRI) used the term “aerobic rice” for the first time which refers to both water-efficient irrigated as well as rainfed rice made much more productive through limited irrigation. It is expected to cause profound changes in water conservation, soil organic matter turnover, nutrient dynamics, carbon sequestration, soil productivity, weed ecology and greenhouse gas emissions. While some of these changes can be perceived as positive (e.g. water conservation and decreased methane emissions), others are perceived as negative (e.g. release of nitrous oxide from the soil and decline in soil organic matter). 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 The need for water efficient rice

As rice is mostly grown under continuously flooded conditions in lowland or irrigated fields, it is the largest water consumer of all the major cereals (Toorchi *et al.*, 2003). However, water shortage is an ever-increasing problem and has become the most serious constraint to rice production and yield stability in many rice-growing areas, particularly in rainfed ecosystems (Nguyen *et al.*, 1997). The reasons for this decline are diverse and location-specific, but include decreasing quality, decreasing resources, and increased competition from urban and industrial users (Postel, 1997). The worsening scarcity of water threatens the sustainability of the irrigated rice ecosystem. 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 approximately 22 million ha of irrigated dry season rice in South and Southeast Asia will suffer “economic water scarcity” (Tuong and Bouman, 2003). Severe water shortages have also led to a recent rapid increase in the area cultivated in upland rice in northern China (Wang *et al.*, 2002). In principle, water is always scarce in the dry season when the lack of rainfall makes cropping impossible without irrigation. The grain production in China has declined about 16% from its peak of 512 million tons in 1998 to 430 million tons in 2003. It is projected that China has to produce 50% more grains with ~20% less lands and ~30% less water to feed its ever growing population in next 20 years. Similarly, drought has been the single greatest factor limiting rice production in approximately 46 million ha of rainfed rice fields of Asia where rice is the main food for subsistence for most poor farmers (Pandey, 2000). Water scarcity, drought, salinity and global warming have seriously threatened rice cultivation in several parts of the world including India.

Rice has relatively few adaptations to water-limited conditions and is extremely sensitive to drought stress as it has the evolutionary particularity of being semiaquatic (Lafitte *et al.*, 2004). The global reduction in rice production due to drought averages 18 M t annually (O’Toole, 2004).

Rice growing environment where water stress is likely to occur, can be grouped in to two major categories, upland and rainfed lowland. Upland areas may have deep soils with extractable soil content, although water stress development is generally more severe than in lowlands areas (Lang *et al.*, 2003). It is not simply the lack of water that lowers yield potential, but also the timing and duration of drought stress related to phenological processes (Jongdee *et al.*, 2002). There are three basic drought patterns affecting rice production: early (occurring during vegetative growth, after establishment but before maximum tillering), intermittent and late (occurring after panicle initiation) drought stresses (Fukai and Cooper, 1995). Yield reductions, as a result of drought stress, are mainly from a reduction in tiller numbers (Jongdee *et al.*, 2006), small leaf area growth and stomatal closure (Boonjung, 1993) and reduced spikelet fertility (Liu *et al.*, 2006).

2.2 Aerobic/Direct seeded rice

Aerobic rice or upland dry direct seeded cultivation is an approach to grow the crop like an irrigated upland crop, such as wheat or maize. Unlike lowland rice, it is grown in unpuddled, non-saturated (i.e., aerobic) soil without flooded water. It is a new concept of reducing water requirement for rice in which rice is grown like an upland crop with high inputs and supplementary irrigations when rainfall is insufficient (Bouman, 2001). It is responsive to high inputs, can be rainfed or irrigated and can tolerate occasional flooding also (Bouman and Tuong, 2001).

It has various advantages, such as low input requirement, crop manageability, yield competitiveness, profitability, early establishment which enhances productivity; resistance to pests and diseases and weed competitiveness (Bouman and Tuong, 2001); promises substantial water savings by minimizing seepage and percolation and also greatly reducing evaporation (Bouman, 2002).

From the 1980s to 2002, the area of upland direct seeded rice expanded rapidly to about 80,000 ha in the North China Plain (NCP) (Wang *et al.*, 2002). In the mid-eighties the development of temperate aerobic rice started in northern China and Brazil. In China, breeders have produced aerobic rice varieties with an estimated yield potential of 6-7 t ha⁻¹ (Wang *et al.*, 2002).

Bouman (2002) studied the performance of aerobic rice varieties under irrigated conditions in northern China. They conducted experiments with aerobic varieties HD502 and HD297 and lowland variety JD305 under aerobic and flooded conditions. The harvest index of both aerobic varieties remained relatively high under aerobic conditions, with lowest values of 0.27-0.28 for HD297 and 0.34-0.35 for HD502 in 2002. The relatively high HI of the aerobic varieties compensated for their relatively short growth duration so that their yields were higher than that of JD305 in all treatments.

Xiaoguang *et al.* (2005) reported that lowland rice variety JD305 (8.8 t ha⁻¹) yielded more than the aerobic/ upland direct seeded rice varieties HD502 (6.8 t ha⁻¹) and HD297 (5.4 t ha⁻¹) under flooded conditions. But, under aerobic conditions, the 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 and more. 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 with that of the lowland variety under flooded conditions, reaching values of 0.6-0.8 g grain kg⁻¹ water. It showed that the HD502 and HD297 are suitable for water-scarce environments, and can stand being periodically flooded.

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 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.

In field experiments near Kaifeng in the North China Plain, Feng *et al.* (2007) obtained relatively low yields of 2.4-3.6 t ha⁻¹ with 750-1,000 mm of total water input. Bouman *et al.* (2007) reported yields of upland direct seeded rice obtained by farmers around Kaifeng of up to 5.5 t ha⁻¹ with sometimes as little as 566 mm of total water input, with only one or two supplementary irrigation applications.

In Brazil, a breeding program to improve plant rice has resulted in aerobic varieties with a yield potential of up to 6 t ha⁻¹ (Pinheiro *et al.*, 2006). The development of tropical aerobic rice is of relatively recent origin.

Using improved upland rice varieties, George *et al.*, (2002) reported upland direct seeded rice yields of 1.5-7.4 t ha⁻¹ in uplands with 2,500 to 4,500 mm of annual rainfall in the Philippines. Yields of 6 t ha⁻¹ and more, however, were realized only incidentally in the first years of cultivation, and most yields were in the 2-3 t ha⁻¹ range.

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

Yang *et al.* (2005) and Bouman *et al.* (2006) reported data on yield, water use, and water productivity of aerobic rice cultivars grown under different irrigation regimes in a field experiment near Beijing in 2001-2002. Rice variety, HD297, showed yields of 3-3.5 t ha⁻¹ with 450-500 mm total water input (rainfall plus irrigation) and 4.7-5.3 t ha⁻¹ with 650 mm water input and more. Using the same variety in field experiments near Kaifeng, Feng *et al.* (2007) reported yields of 2.4-3.6 t ha⁻¹, using 750-1,100 mm total water input. Bouman *et al.* (2007) reported yields of upland direct seeded obtained by farmers around Kaifeng of up to

5.5 t ha⁻¹ with sometimes as little as 566 mm of total water input, with only one or two supplementary irrigation applications.

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

Zhao *et al.* (2006b) reported data on vegetative growth, grain yield, under weed-free (YF) and weedy conditions (YW) and weed suppressive ability (WSA) of aus, *indica*, tropical *japonica* germplasm groups. They found that *aus* cultivars were similar to *indica* types in early growth and WSA but poor in YF. Tropical *japonica* groups and groups derived from *indica/japonica* were inferior to *aus* and *indica* groups in early growth and WSA. *Indica* rice germplasm seemed to be most suitable for breeding high-yielding and weed suppressive upland direct seeded for the tropics.

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

2.3 Seedling vigour

Compared with transplanted rice fields, DSR conditions may be more favorable for the growth of weeds which compete with rice for nutrients, moisture and sun light and can cause large yield losses (Pathak *et al.*, 2011). To improve initial crop establishment and competitiveness of direct-seeded rice, varieties with higher germination and faster seedling emergence with more vigorous seedlings under upland direct seeded conditions must be selected to minimize the risks encountered in direct-seeding (Azhiri-Sigari *et al.*, 2005). During the vegetative stage, rapid groundcover achieved with early vegetative vigour (Poorter and De Jong, 1999; Shipley, 2006; Dingkuhn *et al.*, 1999) can reduce soil evaporation and accelerate root access to soil water and nutrients (Zhao *et al.*, 2006a). Early vegetative vigour can be defined as a high relative growth rate (RGR) during exponential growth before canopy closure (Dingkuhn *et al.*, 1999; Poorter and De Jong, 1999; Shipley, 2006). Early vegetative vigour depends on the assimilate source (light capture and photosynthetic rate) as well as sink constituted by structural growth (leaf appearance rate, potential size, and tiller outgrowth).

Early vegetative vigour may also accelerate depletion of soil water reserves, making less water available for later crop stages (Zhang *et al.*, 2005). However in dry direct seeded environments early vegetative vigour is associated with yield stability (Okami *et al.*, 2011). Differences in early vegetative vigour of rice cultivars affecting crop establishment under direct-seeded conditions have earlier been reported in Asia (Garrity *et al.*, 1992; Caton *et al.*, 2003), Latin America, and Africa (Fischer *et al.*, 1997; Johnson *et al.*, 1998; Dingkuhn *et al.*, 1999).

2.4 Water efficiency, root system and nutrient uptake

Agricultural production in the twenty-first century is predicted to be more limited because of lower availability and increased cost of water and nutrient resources (Lal *et al.*, 2007). Water uptake in rice depends on the root system (Nguyen *et al.*, 1997); consequently, studying the root system is one of the most important parts of understanding tolerance in rice. Drought resistance in rice can be improved through an improved root system. There exists genotypic variation in the ability of roots to penetrate compacted soil layers and simulated compacted soil layers (O'Toole, 1982; Yu *et al.*, 1995). Developing cultivars with high root-penetration ability has been recognized as an important breeding objective for drought resistance improvement in rice (Hanson *et al.*, 1990), and will have great impact to boost rice production and sustain yield stability in rainfed lowland areas. However, incorporation of selection criteria into breeding programs has been difficult due to lack of reliable and efficient screening techniques and the laborious, time-consuming nature of measuring root traits such as root-penetration ability and root thickness (Garrity *et al.*, 1986; Ingram *et al.*, 1994; O'Toole, 1989).

One important aspect for understanding dry direct seeded adaptation is the response of root growth and root development under aerobic conditions, including efficient uptake of water and nutrients. Roots are important for maintaining crop yields, which is vital when plants are grown in soils containing insufficient supplies of water or nutrients (Bengough *et al.*, 2011), and roots are one of the primary sites for stress signal perception that initiates a cascade of gene expression responses to water deficit (Rabello *et al.*, 2008). Previous studies showed that plant growth largely depends on the severity of the stress; mild water deficit leads to growth inhibition of leaves and stems, whereas roots may continue to elongate (Peng *et al.*, 2006). Furthermore, root architecture is a key trait for dissecting the genotypic differences in rice responses to water deficit (Henry *et al.*, 2011). Identifying genetic variation and QTL for root traits can contribute to our understanding of their role in plant performance under direct seeded conditions. Direct seeded conditions decrease the number of nodal roots per internode and stimulate the elongation of nodal roots (Kondo *et al.*, 1999). Other traits that may be important for direct-seeded rice are root hair growth and lateral root growth. Phosphorus uptake is closely related to root hair length, and plants grown under phosphate-limiting conditions form longer root hairs (Bates and Lynch, 1996; Zhang *et al.*, 2003). Increased lateral root growth increased phosphorus uptake in common bean (Lynch and Van

Beem, 1993) and maize (Zhu and Lynch, 2004), nitrogen uptake (Sen *et al.*, 2013) and yield under varying water and nutrient regimes (Hochholdinger and Tuberosa, 2009) in maize, and water uptake under drought in rice (Surlata *et al.*, 2008 and 2010).

Rice displays a complex root structure comprising several root types mostly of postembryonic origin. In rice, earlier work suggests that, under a broad range of conditions, a deep and thick root system is critical for maintaining yield under stress and this indicates that plant root architecture should be a major determinant of abiotic stress tolerance under different field conditions (Mambani and Lal, 1983). Adjustments in the rice root system include various changes in morphology, anatomy, physiology, architecture, and biomass partitioning between roots and shoots. The environmental stimuli that induce a plastic response of the rice root system include not only soil characteristics (mineral nutrients and water status, compactness, and temperature) but also aboveground resources such as radiation. These stimuli affect root system behaviour through their direct role in plant biomass production (passive plasticity in response to carbon assimilate availability) as well as acting as a signal for the regulatory systems involved in root morphogenesis (ontogenetic plasticity). In a number of species, root characteristics such as increased root length density, maximum root length, root thickness, rooting depth, number of nodal root, root : shoot ratio and branching of root systems have all been associated with plant adaptation to water-stress (Sharp and Davies, 1985; Marcum *et al.*, 1995, Huang *et al.*, 1997) under dry direct seeded cultivation condition.

Crop breeding programs that concentrate on root system properties offer a way of improving the cost-effectiveness and sustainability of fertilizer use. However, breeding for improved root performance is a challenging pursuit. Root systems are difficult to access and measure unless grown in artificial substrates - and then they may grow and function quite differently than in field soils (Bengough and Mullins, 1990; Tinker and Nye, 2000; Gregory *et al.*, 2009; Wojciechowski *et al.*, 2009). Furthermore, root system architecture is an aggregate characteristic that results from several distinct and simultaneous processes in the roots (Lynch, 1995) and is influenced by the sufficiency of resources (light, mineral nutrients, water) for shoot activities (Huck and Hillel, 1983; Huck *et al.*, 1983; Husain *et al.*, 1990).

The development of rice varieties with improved root systems for upland dry direct seeded ecosystems, however, has been slow and problematic because of the difficulty in phenotyping of target root traits. Roots are very well affected by G (genetic) x E (soil environment) interaction and thus, affect the consistency of its expression. Root phenotyping can be done in hydroponics, soil-filled pots, field conditions, etc. (Surlata *et al.*, 2013) to empower researchers and breeders to screen for key root traits in their respective breeding programs. Of these drought phenotyping environments, actual field conditions provide the most accurate and relevant results for breeding work. However, field environment is also the most difficult for carrying out root phenotyping since it is more expensive and requires more

careful and sophisticated sampling techniques. It also needs more time for sampling and analysis.

For breeders, component traits like root plasticity directly or indirectly contributing to yield are useful if they are easy to measure and correlated with yield, while having greater genetic diversity than yield itself (Tuberosa *et al.*, 2002). Phenotyping for molecular breeding purposes allows developing molecular probes for marker-based selection. In this context, it is important that markers for component traits of a complex trait have proven physiological complementarities (synergies) while being under distinct genetic control.

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

2.5 Linkage mapping for genes/QTL for root traits, nutrient uptake and yield

Molecular markers provide a valuable tool for genetic analysis and plant breeding (Tanksley, 1989). DNA based molecular markers offers several advantages over traditional phenotypic markers and have found their application in various fields like taxonomy, physiology, genetic engineering etc. Microsatellite markers (SSRs) have been widely used to fingerprint accession, genotype identification and diversity analysis, linkage mapping of genes/QTL and marker-assisted selection (McCouch *et al.*, 2001). 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 the QTL (Parry *et al.*, 2005). During the last 10 years, a large number of quantitative trait loci (QTL) controlling rice root morphological parameters have been detected in several mapping populations by teams interested in improving drought resistance in rice.

Ling *et al.* (2002) and Price *et al.* (1997) reported that the long and thick root system ratio of root weight to shoot weight and root penetration ability of upland rice contributes greatly to the drought tolerance. Several of the QTL identified for root length are consistent across mapping populations (Price and Courtois, 1999; Courtois *et al.*, 2000) and common genomic regions across populations and even across species, have been identified for root thickness, root penetration, and stomatal behavior (Zhang *et al.*, 2001). A particular region (interval RG690-RG730) on chromosome 1 with semi-dwarf genes (*sd1*) has been identified to possess QTL for root depth and thickness and for other drought traits in IR64 × Azucena

DHL population (Courtois *et al.*, 2000). However, Shen *et al.*, (2001) reported that *sd1* region is not pleiotropically associated with the root traits.

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

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

Toorchi *et al.*, (2007) adapted selective genotyping strategy and STMs markers for mapping QTL for maximum root length in rainfed lowland rice. Total of 69 extreme plants were selected from P124 x IR64 mapping population for selective genotyping because of good combination of genetic factors conferring drought tolerance and yielding ability. Forty-two pairs of STMS primer pairs that were earlier found to be polymorphic between the IR64 and Azucena were selected and used in the study. A total of 4 QTL were found for maximum root length under well-watered and low moisture stress and mean conditions.

Liu *et al.* (2008) identified quantitative trait loci (QTL) associated with basal root thickness (BRT) using a 120 recombined inbred lines (RILs) rice population derived from a cross between IRAT109 (a *japonica* upland rice cultivar) and Yuefu (a *japonica* lowland rice cultivar). A major QTL, conferring thicker BRT, designated *brt4*, was selected to study the effects of marker-assisted selection (MAS) using two early segregating populations derived from crosses between IRAT109 and two lowland rice cultivars. This major QTL might be useful for rice drought tolerance breeding.

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

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

Peng *et al.* (2006) compared crop performance of aerobic and flooded rice varieties continuously for eight seasons. The yield difference between aerobic and flooded rice varieties ranged from 8 to 69%, depending on the number of seasons that aerobic rice has been continuously grown, the season (dry or wet), and variety. The difference was attributed more to difference in biomass production than to harvest index.

Bernier *et al.* (2009) measured the effect of qtl_{12.1} on grain yield and associated traits in 21 field trials: ten at IRRI in the Philippines and 11 in the target environment of eastern India under drought conditions. The relative effect of the QTL on grain yield increased with increasing intensity of drought stress, from having no effect under well-watered conditions.

Kanagaraj *et al.* (2010) carried out bulked segregant analysis using 23 recombinant inbred (RI) lines and 1206 rice microsatellite primer pairs. Markers reported to be associated with several drought resistance component traits and will be useful in marker assisted breeding for drought resistance in rice.

A number of QTL associated with nutrient uptake have been detected in rice (Ni *et al.*, 1998; Wissuwa *et al.*, 1998; Ming *et al.*, 2000), maize (Zhu *et al.*, 2005), wheat (Su *et al.*, 2009; Su *et al.*, 2006), common bean (Liao *et al.*, 2004; Yan *et al.*, 2004) and soybean (Li *et al.*, 2005a; Liang *et al.*, 2010). Nutrient-efficient plants produce higher yields per unit of nutrient applied or absorbed compared with other plants grown under similar agroecological conditions (Fageria *et al.*, 2008). Enhancing nutrient-use efficiency is critical across the globe both to sustain yields in low-input agriculture and to reduce fertilizer inputs in high input agriculture (Rose *et al.*, 2012).

Sandhu *et al.* 2011 reported that aerobic rice genotypes possessed deeper and denser root system and performed better compared to lowland *indica* rice varieties and grain yield was positively correlated with the root length and biomass suggesting that root length and biomass might be the key factor for improving grain yield under water-saving conditions.

Sandhu *et al.* (2012) studied polymorphism in a set of 15 rice genotypes comprising of aerobic and Basmati, *indica* and *japonica* rice varieties using 50 SSR markers. Greater divergence was recorded between Basmati and aerobic rice varieties as compared to *indica* and aerobic rice varieties. Two SSR markers (RM212 and RM302) amplified specific alleles in all the five aerobic rice genotypes, which were absent in non-aerobic rice genotypes.

Sandhu *et al.* (2013) reported two QTLs (qGY_{8.1} with an R² value of 34.0% and qGY_{2.1} with an R² value of 22.8%) and one QTL (qGY_{2.2} with an R² value of 43.2%) for grain yield under aerobic conditions in the mapping populations MASARB25 × Pusa Basmati 1460 and HKR47 × 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.

3.1 MATERIALS

3.1.1 Plant material

The experimental plant material comprised of the crosses:

- (i) HKR47 x MAS26 (F_2 and F_3 population)
- (ii) MASARB25 x Pusa Basmati1460 (F_2 and F_3 population)
- (iii) Aus276/3*MTU1010 (BC_2F_4 population)
- (iv) Mikhudeb x MTU1010 (F_1)
- (v) Aus276/3*IR64 (BC_2F_4 population)
- (vi) Kali Aus/2*MTU1010 (BC_1F_4 population)
- (vi) Kali Aus/2*IR64 (BC_1F_4 population)

HKR47 and Pusa Basmati 1460 (PB1460) are the lowland high-yielding rice cultivars unadapted to cultivation in aerobic conditions and MAS26 (developed at the University of Agricultural Sciences, Bangalore), MASARB25 (developed at IRRI, Manila, Philippines) are upland and aerobic adapted genotypes. MAS26 (donor parent) has better root length and MASARB25 (donor parent) has better root biomass. IR64 is a drought-sensitive mega variety widely cultivated in many countries in Asia due to its high-yielding ability, desirable quality traits, and acceptable tolerance for major biotic stresses and MTU1010 is a moderately drought-sensitive rice variety widely grown in India. These two varieties are good improved background parents in breeding for high-yielding and drought-tolerant rice varieties. Kali Aus (donor parent) is a drought-tolerant traditional donor from India. It is a medium-duration line characterized by a distinct purple base and tip during its vegetative growth stage. Aus276 (donor parent) is an early maturing, drought-tolerant traditional donor and Aus pure line originating from Bangladesh.

3.1.2 Chemicals

Chemicals used for preparing Yoshida medium, DNA extraction and PCR amplification were obtained from Sigma Chemicals Co. USA, Life Technologies (India) Pvt. Ltd., 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., Promega Inc., Gibco BRL Inc. (Gaithersburg, MD, USA) and E. Merck Ltd. (Worli, Mumbai-400018, India).

3.1.3. Molecular markers

RM1 - RM600 microsatellite markers were used for assessing the molecular diversity. The rice microsatellite primer pairs were obtained from Sigma Chemicals Co. USA. The original source, repeat motifs, primer sequences and chromosomal position of these markers can be found in the Rice Genes database (<http://www.gramene.Org/micrsat/RMprimers.html>). Specific primers for betaine aldehyde dehydrogenase 2 (*BAD2*) gene (Bradbury *et al.*, 2005) were used for assessing the diversity between Basmati and non-Basmati rice varieties. These primers were got synthesized from Integrated DNA technologies, Inc. USA.

3.2. Methods

Different types of experiments were conducted for evaluation of root and agronomic traits in which different kind of stress was induced at different stages (Table 3.1).

3.2.1 Development of mapping populations

3.2.1.1 Experiment 1: HKR47 × MAS26 and MASARB25 × Pusa Basmati 1460

The F₂:3 populations were developed at CCSHAU, Hisar from crosses involving MAS26 and MASARB25 as male parents and HKR47 and Pusa Basmati 1460 as female parents. True F₁ seeds from the cross were grown to get F₂ seeds. Seeds harvested from HKR47 × MAS26 and MASARB25 × Pusa Basmati 1460 F₁ plants were germinated in petri plates and then a total of 235 and 250 germinated seeds from the two populations, respectively, were raised to maturity in pots (25 cm diameter × 25 cm height) with one plant/pot in a net house in 2010. Seeds obtained from each F₂ plant were evaluated in fields at the Rice Research Station, Kaul, Karnal, India, during 2011 and 2012 under dry direct-seeded aerobic cultivation practices. Five promising F₂ plants from each population were also backcrossed with respective recipient parent.

3.2.1.2 Experiment 2: Aus276/3*MTU1010 and Kali Aus/2*MTU1010

Aus276/3*MTU1010 population consisted of backcross-derived lines (BC₂F₄). Populations were developed at IRRI Philippines, through crossing of Aus276 with IR64 or MTU1010 and the resulting hybrids (F₁) were backcrossed twice to IR64 or MTU1010 to produce BC₂F₁ lines. BC₂F₁ lines were then selfed and bulked to produce BC₂F₂ lines, and this procedure of selfing and bulking was continued until BC₂F₄ lines were produced.

Kali Aus/2*MTU1010 population consisted of backcross-derived lines (BC₁F₄). Populations were developed at IRRI, Philippines through crossing of a drought-resistant parent with a drought-susceptible parent and the resulting hybrid (F₁) was backcrossed to the two susceptible recipient parents to produce BC₁F₁. The BC₁F₁s were then selfed and bulked to produce BC₁F₂ and this procedure of selfing and bulking was continued until BC₁F₄ lines were produced.

Table 3.1 Details of the experiments conducted in 2010-2013 with their corresponding growing environment

Exp	Location	Cultivation condition	Environment code	Stage of water Stress	Date of planting/transplanting	Population
1	CCSHAU, Hisar	Pot house	2010	Seedling	28 July, 2010	HKR47 x MAS26
	CCSHAU, Hisar	Pot house	2011	Seedling	August 5, 2011	HKR47 x MAS26
	CCSHAU, Hisar	Pot house	2012	Seedling	July 30, 2012	HKR47 x MAS26
	CCSHAU, Hisar	Pot house	2010	Seedling	28 July, 2010	MASARB25 x PB1460
	CCSHAU, Hisar	Pot house	2011	Seedling	August 5, 2011	MASARB25 x PB1460
	CCSHAU, Hisar	Pot house	2012	Seedling	July 30, 2012	MASARB25 x PB1460
2	IRRI, Philippines	Lysimeter	2012WSLDS	Seedling	29 August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSLRW	Seedling	29 August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSWW	Seedling	29 August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSUDS	Seedling	17August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSURW	Seedling	17August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSWW	Seedling	17August, 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Lysimeter	2012WSLDS	Seedling	29 August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Lysimeter	2012WSLRW	Seedling	29 August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Lysimeter	2012WSWW	Seedling	29 August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Lysimeter	2012WSUDS	Seedling	17August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Lysimeter	2012WSURW	Seedling	17August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Lysimeter	2012WSWW	Seedling	17August, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DS	Reproductive	21 December, 2013	Kali Aus/2*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DS	Well-watered	21 December, 2013	Kali Aus/2*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DS	Reproductive	21 December, 2013	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DS	Well-watered	21 December, 2013	Aus276/3*MTU1010
	CIRAD, France	Hydroponics	2013	Control (P)	8 July, 2013	Kali Aus/2*MTU1010
	CIRAD, France	Hydroponics	2013	Seedling (P/8)	8 July, 2013	Kali Aus/2*MTU1010
	CIRAD, France	Hydroponics	2013	Control (P)	8 July, 2013	Aus276/3*MTU1010

	CIRAD, France	Hydroponics	2013	Seedling (P/8)	8 July, 2013	Aus276/3*MTU1010
3	IRRI, Philippines	Direct seeded upland	2012DSRS	Reproductive	11 January 2012	Kali Aus/2*IR64
	IRRI, Philippines	Direct seeded upland	2013DSRS	Reproductive	22 January 2013	Kali Aus/2*IR64
	IRRI, Philippines	Direct seeded upland	2012DSNS	Non stress	3 January 2012	Kali Aus/2*IR64
	IRRI, Philippines	Direct seeded upland	2013DSNS	Non stress	18 December 2013	Kali Aus/2*IR64
	IRRI, Philippines	Direct seeded upland	2012DSRS	Reproductive	6 January 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DSRS	Reproductive	22 January 2013	Kali Aus/2*MTU1010
	IRRI, Philippines	Direct seeded upland	2012DSNS	Non stress	3 January 2012	Kali Aus/2*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DSNS	Non stress	20 December 2013	Kali Aus/2*MTU1010
4	IRRI, Philippines	Direct seeded upland	2012UDSNS	Non stress	11 January, 2012	Mikhudeb x MTU1010
	IRRI, Philippines	Direct seeded upland	2012LDSNS	Non stress	18 January, 2012	Mikhudeb x MTU1010
	IRRI, Philippines	Direct seeded upland	2012UDSRS	Reproductive	11 January, 2012	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2013UDSRS	Reproductive	22 January, 2013	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2012UDSNS	Non stress	3 January, 2012	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2013UDSNS	Non stress	20 December, 2013	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2012WSSSS	Seedling	20 June, 2012	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2013DSRSS	Seedling	17 January, 2013	Aus276/3*IR64
	IRRI, Philippines	Direct seeded upland	2012UDSRS	Reproductive	11 January, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2013UDSRS	Reproductive	22 January, 2013	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2012UDSNS	Non stress	3 January, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2013UDSNS	Non stress	20 December, 2013	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2012WSSSS	Seedling	20 June, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Direct seeded upland	2013DSRSS	Seedling	17 January, 2013	Aus276/3*MTU1010
	IRRI, Philippines	Transplanted lowland	2012LDSRS	Reproductive	20 December, 2012/16 January, 2012	Aus276/3*IR64
	IRRI, Philippines	Transplanted lowland	2012LDSNS	Non stress	10 December, 2012/5 January, 2012	Aus276/3*IR64
	IRRI, Philippines	Transplanted lowland	2012LDSRS	Reproductive	20 December, 2012/ 14 January, 2012	Aus276/3*MTU1010
	IRRI, Philippines	Transplanted lowland	2012LDSNS	Non stress	10 December, 2012/ 5 January, 2012	Aus276/3*MTU1010

A total of 10 best and 10 worse yielding lines were selected from each population. In 2012 green house experiment, a set of 40 lines (20 from each population) and 3 checks (Kali Aus, Aus276 and MTU1010) were planted in three main-plot treatments (drought, well-watered and re-watered) under upland and lowland conditions. In 2013 field trial, a set of 40 lines (20 from each population) and 3 checks (Kali Aus, Aus276 and MTU1010) were planted in four replication under well watered and reproductive stress condition. After analysis of 2012 green house experiment data 24 lines were discarded (12 lines from each population) because of second selection criterion. The second criterion of selection was water uptake and dry root biomass. Thus, from each population a total of 8 lines with all possible combination of water uptake and dry root biomass (high water uptake/high DRW, low water uptake/low DRW, high water uptake/low DRW and low water uptake/high DRW) were selected for root measurement, characterization of aboveground response to drought and other agronomic trait evaluation. In 2013 Rhizoscope experiment, CIRAD, France, a set of 40 lines (20 from each population) and 4 checks (Kali Aus, Aus276, MTU1010 and IR64) were planted in four tanks (two replications for control and two replications for stress) each holding 48 plants under control (P) and stress conditions (P/8).

3.2.1.3 Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

Populations used consisted of backcross-derived lines (BC_1F_4 in 2012DS and 2013DS) from crosses of Kali Aus/2*IR64 and Kali Aus/2*MTU1010. BC_1F_4 populations were developed at IRRI, Philippines as mentioned above in experiment 2. Three hundred random lines from each BC_1F_4 population were selected.

3.2.1.4 Experiment 4: Mikhudeb x MTU1010, Aus276/3*MTU1010 and Aus276/3*IR64

The population Mikhudeb x MTU1010 were developed from crosses involving Mikhudeb as male parent and MTU1010 as female parents. True F_1 seeds from the cross were grown to get F_2 seeds. Seeds harvested from F_1 plants were planted under upland and lowland conditions.

Populations used consisted of backcross-derived lines (BC_2F_4) from crosses of Aus276/3*IR64 and Aus276/3*MTU1010. BC_2F_4 populations were developed at IRRI, Philippines as mentioned above in experiment 2. Three hundred lines from each BC_2F_4 population were selected.

3.2.2 Management of mapping populations

3.2.2.1 Experiment 1: HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460

Seeds harvested from HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460 F_1 plants were germinated in petri plates and then a total of 235 and 250 germinated seeds from the two populations, respectively, were raised to maturity in pots (25 cm diameter x 25 cm height) with one plant/pot in a net house in 2010. The pots were irrigated with 1 liter of water for the first 15 days, and then with 1 liter after every third day up to panicle emergence. After

every fifth day, the pots were irrigated with full-strength nutrient solution for the first 21 days, and with half-strength nutrient solution thereafter. Seeds obtained from each F₂ plant were evaluated in fields at the Rice Research Station, Kaul, Karnal, India, during 2011 and 2012 under dry direct-seeded aerobic cultivation practices that involved dry seeding at approximately 2-cm depth in dry-ploughed and harrowed aerobic plots with row spacing of 30 cm (2011) and 25 cm (2012), resulting in a seed rate of approximately 305 seeds m⁻² in 2011 and 365 seeds m⁻² in 2012. The seeds obtained from each selected F₂ plant (20% best and 20% worst, 94 plants in the case of HKR47 × MAS26 and 100 plants in the case of MASARB25 × Pusa Basmati 1460) were grown in 2-m single-row plots with two replications using a seeding density of 2 g per linear meter of row to record observations for the root study, grain yield, agronomic traits, and genotyping. For the yield trial, the plots were randomized by using Crop Stat version 7.2. Aerobic fields were irrigated for about 1 week with a 2-3-cm water layer to facilitate crop establishment; thereafter, the fields were re-irrigated once at a 10-day interval. Nitrogen was applied at 60 kg ha⁻¹ basal after sowing, 60 kg ha⁻¹ 25 days after sowing, and 60 kg ha⁻¹ 55 days after sowing. In addition, 30 kg P ha⁻¹, 40 kg K ha⁻¹, and 5 kg Zn ha⁻¹ were applied as basal and 30 kg P ha⁻¹ at 25 days after sowing. The plots were kept weed-free by manual weeding.

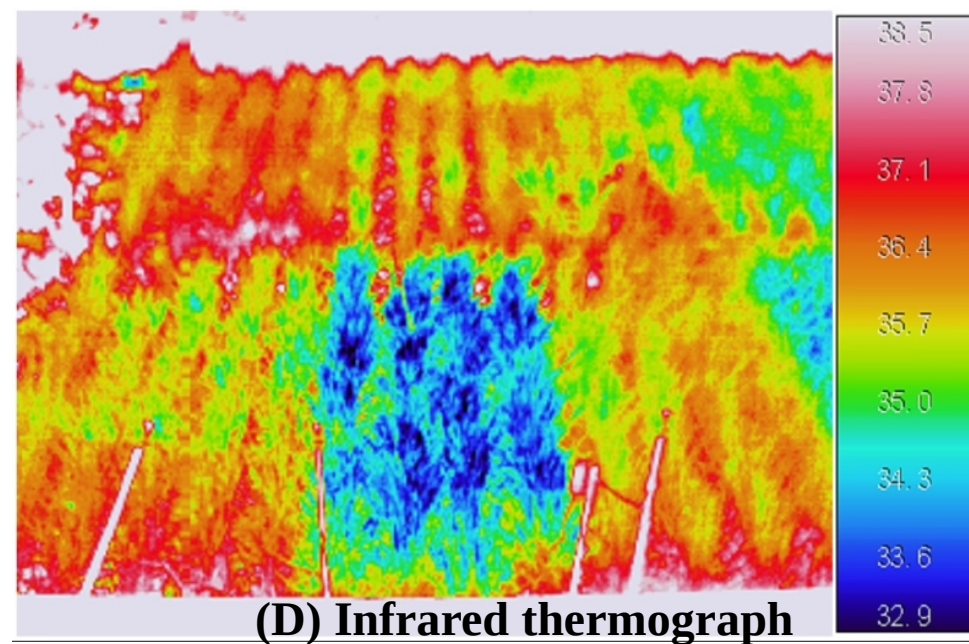
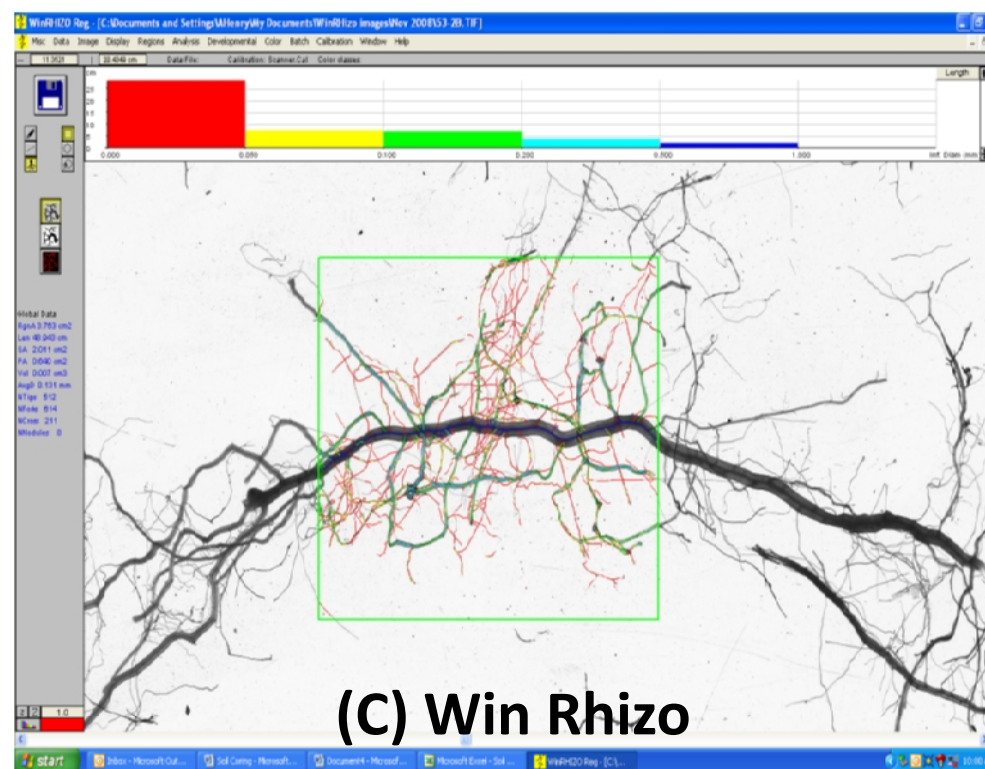
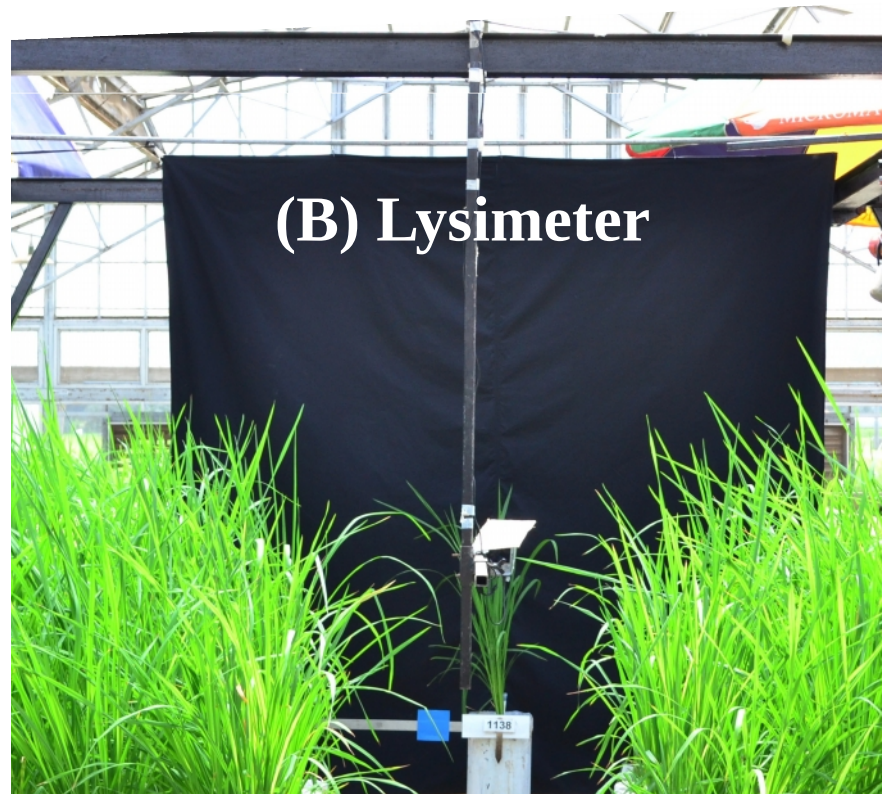
3.2.2.2 Experiment 2: Aus276/3*MTU1010 and Kali Aus/2*MTU1010

3.2.2.2.1. Greenhouse experiment

In the greenhouse, 40 lines (20 from each population), plus the three parents (Kali Aus, Aus276 and MTU1010) were grown in 1.05 m long x 0.19 m diameter PVC pipes lined with plastic bags and filled with soil collected at the IRRI upland farm (Plate 1 Fig. A and B). Plastic bags had holes at the bottom to allow drainage. The experiment was a split-plot design, with water treatments as the main plots and genotypes as sub-plots, and was conducted in 2012, with each line being planted in one replication and three main-plot treatments (well-watered, drought and re-watered) under upland and lowland conditions both. The seeds were pre-germinated in petriplates on August 13, 2012. For the upland condition three seeds per pot per lines were transferred to the pots on August 17, 2012. For the lowland condition the germinated seeds were first transferred to the seedling trays on August 17, 2012 and then three seedlings per pot per line were transplanted in the pots on August 29, 2012. Three seeds were placed in each pot and thinned to one seedling per pot at 20 DAS in upland and 15 DAT in lowland treatment. The PVC pipes were filled with soil and saturated with water for a few days in order for the soil to settle. More soil was then added, in order to bring the soil level about 5 cm below the edge of the pipes. Under both upland and lowland condition, the drought and re-watered treatment were drained 30 DAS. The well-watered pipes received 1000 ml of water twice a week to maintain the soil near field capacity for the whole duration of the experiment. In the drought-stress and re-watered treatment, the

Plate 1

- (A) and (B) Lysimeter study set up in greenhouse
- (C) Analysis of scanned root images for architectural attributes using WinRhizo v. 2007
- (D) Infrared thermograph using the line tool (one line selected per planted row) in Report generator v. 1.7 software (NEC Avio Infrared Technologies Co. Ltd., Tokyo, Japan).



irrigation was the same initially, but was stopped at 30 DAS. For the well watered and drought treatment the plants were sampled at 70 DAS and the re-watered treatment was re-watered once on the same day. For the re-watered treatment the plants were sampled at 85 DAS.

3.2.2.2.2. Field experiment

Field evaluation was conducted under upland conditions at IRRI, Los Baños, Philippines (14° N 121° E, 21 m above sea level) during the dry seasons of 2013. The soil of the IRRI upland farm is a Maahas clay loam. Plots were 4 m long, with inter-row spacing of 0.25 m and a seeding rate of 2 g of seed per linear meter, resulting in a density of approximately 390 seeds m⁻². Basal fertilizer applications equivalent to 40 kg P and 40 kg K ha⁻¹ were applied in the form of single super phosphate and potassium chloride, and 120 kg ha⁻¹ of N in the form of ammonium sulphate was applied in three even splits around 21, 42 and 61 DAS. Both well-watered control trials and drought stress trials were conducted. Irrigation was supplied weekly by basin irrigation for the entire season. The drought stress trial was drained 40 DAS.

3.2.2.3 Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

Reproductive-stage drought stress (RS) and nonstress (NS) trials were established in alpha lattice design with two replications under upland situation. Seeds were dry-direct-seeded in the soil using the seed density same as above population. Basal applications equivalent to 40 kg P and 40 kg K ha⁻¹ were given in the form of single superphosphate and potassium chloride; 120 kg ha⁻¹ of N in the form of ammonium sulfate was applied in three even splits around 21, 42, and 61 d after seeding. Weeds were controlled through application of pre- and post-emergence herbicides supplemented by hand weeding. Pests and diseases were monitored regularly and controlled through application of pesticides. The NS experiments were managed like irrigated lowland rice trials, where no water stress was employed. In both years, the NS trial was sprinkler-irrigated twice weekly and the RS trials were sprinkler-irrigated twice weekly during establishment and early vegetative growth; irrigation frequency in the latter was reduced at 56 and 40 d after sowing in 2012 and 2013, respectively. Plots were re-irrigated periodically when soil water tension fell below -50 kPa at 30-cm soil depth. At this soil water potential, most lines wilted and exhibited leaf drying.

3.2.2.4 Experiment 4: Mikhudeb x MTU1010, Aus276/3*MTU1010 and Aus276/3*IR64

For Mikhudeb x MTU1010, seeds were dry-direct-seeded in the upland soil using the seed density same as above population. In case of lowland seeds were sown in a raised-bed nurser.

In case of Aus276/3*MTU1010 and Aus276/3*IR64, two types of experiments were conducted: those for evaluation of DSR traits in which seedling stage stress was induced, and those for evaluation of agronomic traits in which reproductive stage stress was induced (Table

1). The field experiments on DSR traits were carried out in an automated rainout shelter under direct seeded conditions in the 2012WS and 2013DS. The field experiment for the study of agronomic traits was carried out under lowland and upland conditions in 2012DS and 2013DS, and a well-watered control treatment (non-stress) was included each season. All 300 BC₂F₄ lines were included in each experiment. The field used for the seedling stage stress experiment was in an automatic rain out shelter (ROS) that had been fallow five years before the experiment was conducted. Soil characteristics included pH 7.7 (0.01 M CaCl₂); 16.0 meq 100g⁻¹ Ca; 7.8 meq 100g⁻¹ Mg; 1.09 meq 100g⁻¹ K; 20 µg g⁻¹ P; 0.101 % Kj N; 39% clay; 21% sand; and 36% silt). A basal fertilizer was incorporated into the soil at a rate of 45-45-45 kg ha⁻¹ at 2 DAS. No topdressing was applied in order to maintain low-nutrient conditions. Seeding was conducted at approximately a 2-cm depth at 2 g per linear meter in dry-ploughed plots randomized in an augmented design (CropStat version 7.2) with one replication of each population and three checks (Aus276, MTU1010 and IR64) repeated ten times. Single-row plots (3 m) were planted with row spacing of 30 cm. The central one meter of each plot was thinned to an interplant spacing of about 5 cm and maintained undisturbed to record observations for grain yield and yield attributing traits, and the remaining two meters of each row were used for destructive sampling. Irrigation was stopped once all plots had emerged, and stress was initiated by closing the rainout shelter at 9 DAS until the experiments were re-watered (31 DAS) and kept well-watered until maturity. Trials for agronomic traits under lowland and direct-seeded upland conditions (Table 1) were planted in an alpha lattice design with 2 m row with two replications and three checks (Aus276 and MTU1010 or IR64) repeated ten times in each replication. For all lowland trials, seeds were sown in a raised-bed nursery and 26-day-old seedlings were transplanted to the main field with each hill containing one seedling. For direct-seeded upland trials, the seed density was same as above population. The non-stress treatments in the lowland trials were maintained flooded and the direct-seeded upland trials were sprinkler-irrigated twice weekly. Stress treatments were irrigated during establishment and early vegetative growth, but irrigation was stopped in the reproductive-stage drought stress treatments at 30 d after transplanting (DAT)/51 DAS in the 2012 lowland trial and at 56 and 40 DAS in 2012 and 2013, respectively, in the direct seeded upland trials and. Plots were re-irrigated periodically when most lines were wilted and exhibited leaf drying.

3.2.3 Characterization of mapping populations

3.2.3.1 Experiment 1 (HKR47 x MAS26 and MASARB25 × Pusa Basmati 1460)

The data on root morphological traits-root length (RL, cm), root thickness (RT), root number (RN), root volume (RV), fresh and dry root weight (FRW and DRW in g), and fresh and dry shoot weight (FSW and DSW in g) from six plants from each line at maturity were recorded and analyzed. For the measurement of root traits, plants were removed from the soil.

Soil sections that contained roots were removed by digging a hole and put into a sieve and gently washed with a hose until only the roots remained. The roots and shoots were then separated by cutting from the top soil line. For fresh root weight, the roots were blotted gently with a soft paper towel to remove any free surface moisture. Then, the roots and shoots were weighed immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data). Root volume was measured by the actual volume displacement analysis, which measures the volume of water displaced when roots are submerged in a vessel of water. Volume displacement analysis has the advantage of providing a fast measure of new root production. In addition, its non-destructive nature permits repeated measures over time. The thickness of the root crown was measured using a vernier calliper. Root numbers were counted manually. Root length was measured using a centimeter scale. For dry root weight, the roots and shoots were dried in an oven set to low heat (100°F) for 3 days, and then cooled in a dry environment. Once cooled, they were weighed on a scale.

At physiological maturity or 80-85% of the panicles turned into golden yellow and the panicles at the base were already at hard dough stage, data were recorded on agronomic traits, plant height in cm (PH), effective number of tillers per plant (TN), panicle length in cm (PL), number of panicles per plant (P/P), number of grains per panicle (S/P), 1,000-grain weight (1,000 GW), length/breadth ratio (L/B), and grain yield in Kg ha^{-1} (GY). Grain yield per plot was recorded after harvesting, threshing, and drying to moisture content adjusted to 14% and converted to kg ha^{-1} .

3.2.3.2 Experiment 2: Aus276/3*MTU1010 and Kali Aus/2*MTU1010

3.2.3.2.1. Greenhouse experiment

The water consumption by plants was monitored by weighing pipes the pots 32, 39, 46, 53, 60 and 60 DAS. Water consumption was calculated as the difference of the initial pot weight and the pot weight at different times. For the well watered treatment the pots were replenished with the same amount of water lost by the plant following each weighing schedule. For sampling the plants were cut at the stem base and oven-dried to measure above-ground biomass production. Plastic bags were then pulled out of the pipes. The maximum rooting depth was measured.

Soil was then cut into four sections (0–20 cm depth, 20–40 cm depth, 40–60 cm depth and below 60 cm depth) (Plate 2 Fig. A). Within every soil section, roots were carefully washed to remove all soil particles and stored in 70% alcohol until it was possible to measure them. The root samples were scanned (Epson V700, California, USA) and the scanned images were analyzed for architectural attributes using WinRhizo v. 2007 d (Régent instruments, Québec, Canada) (Plate 1 Fig. C). A pixel threshold of 200 was set for the analysis. The diameter class of <0.05 mm, 0.05-0.1 mm, 0.1-0.2 mm, 0.2-0.5 mm, 0.5-1 mm and >1mm

were used. Analysis of fine root production was conducted for both S-type laterals (<0.05 mm diameter) and total lateral root growth (S and L-type; <0.2 mm diameter) according to Yamauchi *et al.* (1987a).

3.2.3.2.2. Field experiment

3.2.3.2.2.1. Characterization of above ground response to drought

Mercury manometer-type tensiometer (15 and 30 cm soil depth) and PVC tubes (4 cm diameter, 1 m long) for frequency domain reflectometry readings were installed at three locations in the drought treatment field, as soon as the soil had solidified after draining. Soil water potential based on the height of the mercury column, volumetric soil moisture at 10 cm increments to a depth of 70 cm (Diviner 2000, Sentek Sensor Technologies, Stepney SA, Australia), and water table depth were monitored in the selected plots up to three times weekly thereafter. Tubes installed within the plot of specific genotypes were located at the mid-point between rows and hills, ~30 cm from the edge of the plot. Stomatal conductance (gs) was measured three times weekly using a Li-1600 steady-state porometer (Li-Cor Biosciences, USA). Measurements were taken on the abaxial (lower) side of the last fully elongated leaf. Canopy temperature was measured three times weekly in the drought treatment using IR gun. Thermal images were acquired at the mid-day (between 11:00 AM and 3:00 PM) with six plots per image (three in front and three behind) using an NEC TH7800 Infrared camera (NEC Avio Infrared Technologies Co. Ltd., Tokyo, Japan) from a 3.5-m-tall ladder positioned 10 cm in front of the plots. Canopy temperature was determined using the line tool (one line selected per planted row) in Report generator v. 1.7 software (NEC Avio Infrared Technologies Co. Ltd., Tokyo, Japan) (Plate 1 Fig. D). Time to flowering was recorded when the 50% of the hills in a plot were flowering.

At harvesting, three plants per plot were randomly measured to estimate plant height. Above-ground biomass was sampled by cutting 1m length of the first row of the plot at ground level at maturity. This material was oven-dried, weighed and subsequently used to derive the traits biomass yield and harvest index. Panicle number and harvest index were calculated as per Bernier *et al.* (2007). Biomass yield was obtained by dividing grain yield by harvest index.

3.2.3.2.2.2. Root measurements

Soil samples for root measurement were acquired with a 20-cm x 20 cm monolith sampler in selected plot in both drought and well watered treatment to a depth of 60 cm at 82 DAS (Plate 2 Fig. B). Monoliths were centered over one planted hill, and cores were sampled at the mid-point between rows and hills. The samples were separated into depth increments of 15 cm of sampled soil. Three subreplicate samples per plot were collected.

Roots were either washed from the soil immediately after sampling or stored at -4 °C until washing (within 2 weeks). Root samples were washed by repeatedly mixing the soil with water in a container, and pouring the root water suspension over a 1-mm plastic sieve

Plate 2

- (A) Root measurement from Lysimeter study in greenhouse
- (B) Root measurement with a 20-cm x 20 cm monolith sampler in Field
- (C) Root washing for further scanning of root images for architectural attributes
- (D) Set up of Rhizoscope facility for root phenotyping at CIRAD, France.

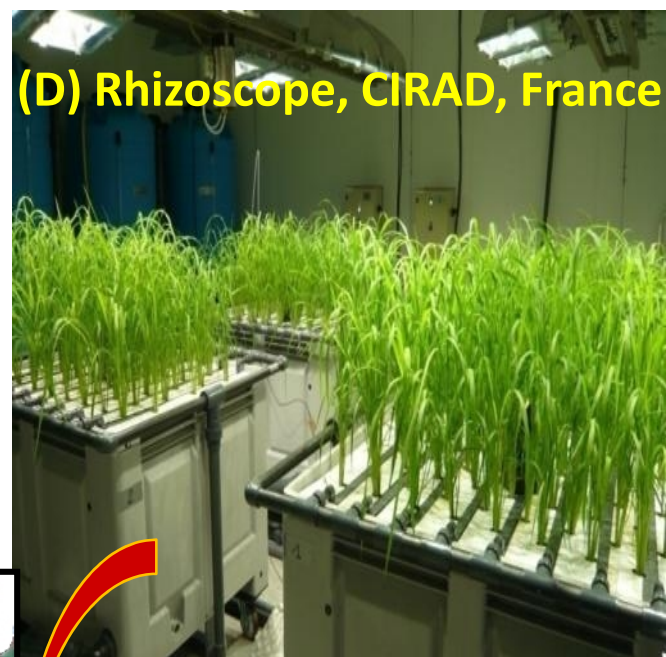


(A) Root sampling in Screenhouse

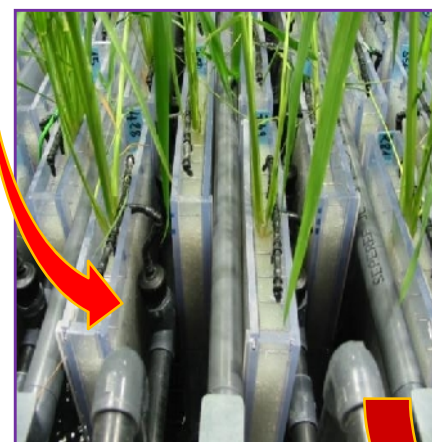


(B) Root sampling in Field

(C) Root Washing



(D) Rhizoscope, CIRAD, France



(Plate 2 Fig. C). All samples were stored in 70% alcohol until scanning. Root samples were scanned and analyzed as green house experiment.

3.2.3.2.3. Rhizoscope study

3.2.3.2.3.1. Characterization of Rhizoscope study

Rhizoscope is a new high-throughput screening system for phenotyping rice root traits. The Rhizoscope is based on the use of 2-D “nail board rhizoboxes,” made up of plexiglass (50 cm x 20 cm x 2 cm), filled with transparent soda-glass-beads (SGB) to simulate soil conditions, and bathed with a re-circulating nutrient solution. SGB (5-mm-diameter) composed of 75% SiO₂, 14% Na₂O + K₂O, 10% CaO, and 31% AlO₃ were used as substrate. SGB test the ability of the root system to grow through a medium with uniform “mechanical impedance” (MI); thus, it can be considered an analog of uniform soil (Plate 2 Fig. D). SGB advantages include easy manipulation, low cost, suitability for axenic culture, recyclability, chemical inertness, and availability in multiple sizes. Beads are homogeneously arranged in the rhizoboxes without external pressure. Consequently, the substrate is not completely rigid and can be displaced by roots exerting a pressure equivalent to MI. The system makes it possible to monitor root growth without removing plants from the boxes. Multiple nondestructive measurements can be made as root distribution and architecture are kept “intact” after SGB removal, a very important asset as traits can be recorded using high-quality digital images. The images were then evaluated by Image-Pro Plus 5.1 (Copyright Media Cybernetics, MD, USA) analytical software. We explored the suitability of the rhizobox for evaluating root plasticity in response to P deficiency, a major constraint to rice productivity. Plants were grown for 21 days and received a modified Yoshida nutrient solution under control (P) and stress (P/8) conditions. pH and solution temperature was maintained at –5.5 and 25 °C, respectively. The data were recorded on shoot biomass, number of tiller, root cone angle, root diameter, maximum root depth, number of roots below 30 cm depth, number of roots below 20 cm depth, total dry root biomass, root/shoot ratio, root biomass (0-20 cm, 20-30 cm and below 30 cm).

3.2.3.2.3.2. Anatomical characterization of Rhizoscope study

For anatomical study three 2 cm long root sections were collected from each plant from root apex. The agar blocks were prepared using 2% agarose. The agar blocks were then cut with Thermo Scientific Microm Microtome HM 650 V (Microm International GmbH, part of Thermo Fisher Scientific, Germany) (thickness: 60µm, frequency 70 Hz, amplitude 0.7 mm). The pictures were taken by microscope Leica DM4500 (Leica Microsystems, Inc., France) using velocity 3D image Analysis Software (Copyright PerkinElmer Inc, Massachusetts, USA). The images were then analyzed by using Image-Pro Plus 5.1 (Copyright Media Cybernetics, MD, USA) and the following anatomical traits were

measured: metaxylem vessel number, cortical diameter, root diameter, stele diameter and cortical cell layer number.

3.2.3.3 Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

Days to flowering (DTF) was recorded when 50% of the plants in the plot had exerted their panicles. Plant height (PH) was measured as the mean height of five random plants for each entry measured from the base of the plant to the tip of the panicle during maturity stage. Grain yield (GY) was determined at physiological maturity or when 80–85% of the panicles turned into golden yellow and the panicles at the base were already at the hard dough stage; the harvested grains were threshed, dried to 14% moisture content, and weighed for yield computation (kg ha^{-1}).

3.2.3.4 Experiment 4: Aus276/3*MTU1010 and Aus276/3*IR64

3.2.3.4.1 Emergence and early vegetative vigour

Seedling emergence (first and full emergence) was recorded in 2013DSSSS only. Seedlings were sampled three times, at 15, 22 and 30 DAS, in 2012WSSSS and 2013DSSSS. Three plants per plot were removed from the soil by digging a hole with trowel (10 cm depth), put into a sieve and the roots gently washed. The number of nodal roots and adventitious roots per plant were counted manually. The roots and shoots were then separated and shoots were dried in an oven at 60°C for 3 days.

In 2012WSSSS and 2013DSSSS, early vegetative vigour was calculated in terms of relative growth rate (determined by biomass accumulation). RGR (relative growth rate) was calculated as:

$$\frac{[(\ln (\text{dry shoot weight at sampling 2}) - \ln (\text{dry shoot weight at sampling 1}))]}{(\text{date of sampling 2} - \text{date of sampling 1})}.$$

In 2013UDSRS, early vegetative vigour was recorded on 1-9 scale at 30 DAS according to the IRRI Standard Evaluation System for rice (IRRI, 1996).

3.2.3.4.2 Characterization of root hair length and density

In 2012WSSSS and 2013DSSSS, the roots from the third sampling (30 DAS) were stored in 70% ethanol. Root hairs on the basal 2-3 cm of nodal roots were visually evaluated as described by Vieira *et al.* (2007) by two to three researchers, each observing different roots, after staining with 0.05% trypan blue and using a rating scale of 1-5 to rank the root hair length and density (Table 3.2). Lateral roots were visually evaluated using a rating scale of 0-3 (Table 3.2) in 2013DSSSS.

3.2.3.4.3 Nutrient analysis at seedling stage and reproductive stage

In 2012WSSSS and 2013DSSSS, a total of 100 lines from the both populations covering a range of ratings for root hair length and density were selected. The shoots from three plants per plot at 22 DAS from both populations were sampled and dried at 70°C. Shoot samples were ground and ashed at 600°C for 16 h. The ash was dissolved in 0.1 N HCl, and

phosphorus concentration was then determined using a colorimetric assay by spectrophotometry (Murphy and Riley, 1962). At 95 DAS, the same above mentioned 100 lines from the Aus276/3*IR64 population were chosen for nutrient analysis in 2013DSSSS. Shoots from three plants per plot were dried at 70°C, ground and analyzed by inductively coupled optical-emission spectrometry at the IRRI Analytical Service Laboratory. Nutrient uptake was calculated as concentration x biomass.

Table 3.2 Root hair length, density and lateral root rating scale

Root hair rating	Category	Root hair density	Root hair length
1	no root hair	0	0
2	short and sparse	1	1
3	short and dense	2	1
4	long and sparse	1	2
5	long and dense	2	2
Lateral root rating			
0	none or almost none	-	-
1	sparse	-	-
2	dense	-	-
3	dense and branching	-	-

3.2.3.4.4 Nematode study

During the root hair rating, we observed that the densest root hairs were found on parts of the nodal root that were swollen, which a preliminary evaluation suggested might be associated with nematode infection. Therefore, at 95 DAS, the above mentioned 100 lines from the Aus276/3*IR64 population were chosen for quantifying nematode infection in 2013DSSSS. The roots of the three plants per plot (20 cm depth, sampled by shovel) were used for nematode assays. The nematode galls per plant were visually evaluated using a rating scale of 0 - 5 (Hussey and Janssen, 2002).

3.2.3.4.5 Spatial mapping of soil and plant parameters

A map of apparent soil EC to a depth of 0.75 cm was done between 2012WS and 2013DS when there was no standing crop (EM38, Geonics, Inc; Leica GPS) according to Klassen *et al.* (In preparation). GPS points were also acquired at each sampling point at 95 DAS in 2013DSSSS, when shoot and root samples for nematode gall rating were collected. Spatial heat maps for nematode gall rating, root hair length, density, phosphorus uptake, dry shoot weight and GY (based on the position of each plot) were prepared. Spatial data were modelled in R (version 3.0.1, package: raster) using the thin plate spline regression function (*Tps*) and converted to a raster for plotting the heat maps using the predict function (*predict*).

3.2.3.4.6 Characterization of agronomic traits

Days to flowering (DTF) was recorded when 50% of plants in the plot exerted their panicles in 2012UDSNS/RS, 2013UDSNS/RS, 2012LDSNS/RS, 2012WSSSS and 2013DSSSS. Plant height (PH) was measured as the mean height of the five random plants for each entry measured from the base of the plant to the tip of the panicle during maturity stage. Above-ground biomass was sampled by cutting a 0.5 m length of the first row of the plot at ground level at maturity. This material was oven-dried, weighed and subsequently used to determine biomass and harvest index (HI; grain yield/total aboveground biomass). Grain yield (GY) was harvested at physiological maturity or 80-85% of the panicles turned into golden yellow and the panicles at the base were already at hard dough stage; harvested grains were threshed, dried to 14% moisture content, and weighed to determine grain yield (kg ha⁻¹).

3.2.4. Statistical analysis

Statistical analysis of the data on individual characters was performed using SAS v9.1 (SAS Institute, Inc., 2002-2003). Line means were estimated using the REML option of the SAS MIXED procedure taking lines as fixed and replicates and blocks within replicates as random. The phenotypic r(P) correlation coefficients for all possible pairs of characters were calculated from the variance and covariance was estimated:

$$r(P) = \sigma_{xy}(P) / [\sigma^2_x(P) \times \sigma^2_y(P)]$$

Where

$\sigma_{xy}(P)$ = Phenotypic covariance between characters x and y

$\sigma^2_x(P)$ = Phenotypic variance of character x

$\sigma^2_y(P)$ = Phenotypic variance of character y

The phenotypic correlation coefficients were tested against a standardized tabulated significant value of r with (n-2) degree of freedom as per the procedure of Fisher and Yates, (1963).

Broad-sense heritability (H) was estimated as: $H = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_E}{r}}$

where σ^2_G is the genotypic variance, σ^2_E is the error variance, and r is the number of replications.

3.2.5. Genotyping of mapping populations

All DNA marker work for HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460 populations was conducted in the Department of Molecular Biology and Biotechnology, CCSHAU, Hisar, India. Molecular work for Kali Aus/2*IR64 and Kali Aus/2*MTU1010 populations was carried out at the Molecular Markers Application Laboratory (MMAL) of IRRI's Plant Breeding, Genetics, and Biotechnology Division. For all six fresh leaves from each line were collected in bulk and genomic DNA was isolated from 1-month-old plants using the CTAB method (Saghai-Marooof *et al.*, 1984). DNA quantity was estimated by

ethidium bromide staining (CCSHAU, Hisar) or SYBR Safe™ staining (IRRI) on 1% agarose gels using a standard containing 100 ng/μl genomic DNA. Amplification of simple sequence repeat (SSR) markers using polymerase chain reaction (PCR) was carried out in experiment 1 and 3 with a 15-μl reaction mixture that contained 3 μl DNA templates, 1.5 μl 10x PCR buffer, 2.0 μl of MgCl₂, 0.5 μl each of forward and reverse primers, 0.5 μl of 1 mM dNTP, and 0.5 μl of Taq DNA polymerase. A drop of mineral oil was added to each well to prevent the mixture from evaporating and the plate was covered with a PCR sealing film. Finally, the addition of 10x loading buffer to the PCR product prior to loading (1.3 μl of 10x loading buffer for every 10 μl PCR products) was done.

The PCR profile for SSR described by Thompson *et al.* (2006) was used: initial denaturation at 94 °C for 5 min; then 35 cycles of denaturation at 94 °C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and final extension at 72°C for 5 min and storage at 10 °C forever. PCR products were resolved using high-resolution 4% (CCSHAU, Hisar), 6 and 8% polyacrylamide gel (IRRI) electrophoresis (PAGE) as described by Sambrook *et al.*, (1989). The gel was run in 1x TBE at 95 volts for 1 to 3 h, depending on the product size of the SSR marker. Gels were stained with SYBR Safe™ DNA gel stain and were viewed after 20 min in experiment 3. Following electrophoresis in experiment 1, bands were visualized via silver staining protocols (Promega Inc., Madison, WI, USA). Two plates were separated and the notched plate with gel was treated with fixing solution (10% acetic acid) for 20 min, followed by 3 washings of two min each with distilled water. Plate was then treated with staining solution (0.1% w/v silver nitrate, 0.15 %, v/v formaldehyde) for 30 min, washed with distilled water for 10 seconds and treated with developing solution (3% w/v sodium carbonate, 0.15% v/v formaldehyde, and 0.2% w/v sodium thiosulfate) till bands appeared. The gel was finally fixed for 5 min with fixing solution (10% glacial acetic acid), washed with distilled water and dried overnight. Permanent record of banding pattern was taken by digital camera (Nikon Coolpix-850). The DNA samples were diluted with 1x TE into an equal concentration of 5 ng/μL and were submitted to LGC Genomics Ltd. UK for genotyping I experiment 4.

3.2.5.1. Experiment 1: HKR47 x MAS26 and MASARB25 × Pusa Basmati 1460

A total of 300 mapped SSR and aroma gene-specific *BAD2* markers (for which basmati serves as a parent) were screened for polymorphism between the parents. The markers were obtained based on published rice genome maps (IRGSP 2005) and their physical position (Mb) on the indica genome (www.gramene.org) was used as a reference. A total of 91 and 112 markers showed polymorphism and were run on 94 and 100 plants for HKR47 x MAS26 and MASARB25 × Pusa Basmati 1460 populations, respectively.

3.2.5.2. Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

A polymorphism survey was carried out among the parents IR64, MTU1010, and Kali Aus using 600 SSR markers (Temnykh *et al.*, 2001, McCouch *et al.*, 2002, IRGSP

2005). The marker order was taken from published rice genetic maps (Temnykh *et al.*, 2001). From each population, 4% of the highest and 4% of the lowest yielding lines were selected based on GY data from the stress trials of 2012DS. The DNAs of the highest yielding lines were mixed in equal quantity to prepare the high bulk and the DNAs of the lowest yielding lines were mixed to prepare the low bulk for each population. For BSA, 134 and 109 polymorphic SSR markers for Kali Aus/2*IR64 and Kali Aus/2*MTU1010, respectively, were used to cover the entire rice genome and to identify markers showing a significant banding pattern for high and low bulks in Kali Aus/2*IR64 and Kali Aus/2*MTU1010 populations, respectively. Polymorphic markers where tolerant parent allele bands correspond to the high bulk and susceptible parent allele bands correspond to the low bulk under RS were used to genotype the whole population. Single-marker regression analysis was carried out to identify significant markers associated with GY under RS using Qgene (Nelson, 1997). After getting significant markers, additional polymorphic markers on both sides of the significant markers were run on the whole population to determine the QTL flanks.

3.2.5.3. Experiment 4: Aus276/3*MTU1010 and Aus276/3*IR64

The DNA samples were diluted with 1x TE into an equal concentration of 5 ng/μL and were submitted to LGC Genomics Ltd. UK for genotyping. A polymorphism survey was carried out by LGC Genomics Ltd. UK among the parents IR64, MTU1010, and Aus276 using 1894 SNP markers. A total of 150 polymorphic SNP markers distributed on all 12 chromosomes at equal 10 cM for each population were selected for whole-population genotyping. The whole-population genotyping was carried out by LGC Genomics Ltd. UK.

3.2.6. Genetic analysis

Genetic similarities between the cultivars were measured by the similarity coefficient based on the proportion of shared electromorphs using the ‘Simqual’ subprogram of the NTSYS-PC (Version 2.02 Exeter Software, Setauket, NY, USA) software package (Rohlf *et al.*, 1993). The resultant distance matrix data were used for two-dimensional scaling of rice genotypes by principal component analysis (PCA). Linkage analysis was performed using the Map Manager/QTX computer program (Manly *et al.*, 2001) using the Kosambi function and linkage evaluation of $P = 0.001$. The ripple command was used to verify the marker order. QTL analysis was performed using Windows QTL Cartographer version 2.5 (Wang *et al.*, 2011). For interval mapping (IM) analysis, an LOD threshold score of 2.5 was selected. The proportion of the total phenotypic variation explained by each QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the QTL to the total sum of squares). To more accurately determine QTL positions, composite interval mapping (CIM) was performed with default parameters (permutation time 300, significance level 0.05, model 6; standard model, method 3; forward and backward method, walk speed 2 cM, etc.). QTL analysis results were confirmed with Q Gene and QTL Network (Yang *et al.*, 2007) software.

Experiment 1: Two aerobic × nonaerobic populations were studied for root traits, grain yield, and yield-related agronomic traits to identify QTL conferring a potential yield advantage under aerobic conditions; identify QTL for yield-related agronomic traits under aerobic conditions; identify root traits having a positive correlation with grain yield under aerobic conditions; identify QTL for root-related traits with a positive correlation with grain yield under aerobic conditions; and identify QTL for yield, yield-related agronomic traits, and root traits that co-localize with the aim to introgress identified QTL in HKR47 and Pusa Basmati 1460 rice varieties using marker-assisted breeding to develop rice varieties better adapted to aerobic conditions.

Experiment 2: A set of 40 lines from Aus276/3*MTU1010 and Kali Aus/2*MTU1010 population was used to identify genotypes with high levels of root growth plasticity (in terms of deep root growth) in multiple conditions (upland dry direct seeded, lowland, induced by drought or low P) under multiple environment (lysimeter, field and rhizoscope) and to address the question: are genotypes with the most root growth plasticity the highest yielding and the least root growth plasticity the lowest yielding?

Experiment 3: Experiment was conducted to identify QTL for GY under RS that show a large and consistent effect. Bulk segregant analysis, followed by whole genotyping with selected markers, was used to identify significant markers putatively linked with high GY under drought.

Experiment 4: In this experiment, traits hypothesized to be important for direct-seeded conditions were characterized, including seedling emergence, early vegetative vigour, nutrient uptake, and root morphology [nodal root number (NR), root hair length (RHL) and root hair density (RHD)], and were analyzed in relation to grain yield (GY) under direct seeded, seedling-stage drought conditions. To study the genetic control of GY under seedling and reproductive-stage drought stress, two BC₂F₄ mapping populations derived from crosses of Aus276, a drought-tolerant aus variety, with MTU1010 and IR64, high-yielding *indica* mega-varieties, with the aim to identify QTL that showed a large and consistent effect for GY, early vegetative vigour, nutrient uptake, root morphology, and other agronomic traits.

4.1 Experiment 1

4.1.1 Phenotypic analysis of parents and populations

MAS26 and MASARB25, the aerobic genotypes, outyielded the lowland parents, HKR47 and Pusa Basmati 1460, in both years under dry direct-seeded aerobic conditions. Root length, root volume, root thickness, root number, and root biomass of the tolerant parent were found to be higher than those of the susceptible parent. Mean values of RL, RV, RT,

RN, FRW, DRW, FSW, and DSW for the two populations are presented in Table 4.1 (Plate 3, Fig. A). Mean and range values of PH, TN, PL, P/P, S/P, 1,000 GW, and GY on two populations under dry direct seeded aerobic conditions in 2011 and 2012 are presented in Table 4.2.

4.1.2 Correlation analysis

In both mapping populations, grain yield was found to be positively correlated with root length, root number, root volume, and root biomass, suggesting that these traits might be the key factor for improving grain yield under aerobic conditions (Table 4.3).

Phenotypic correlation coefficient analysis of MASARB25 $\times$ Pusa Basmati 1460 showed that GY is positively and significantly correlated with the effective number of tillers (0.442, $p = 0.01$), panicle length (0.411, $p = 0.01$), panicles plant⁻¹ (0.427, $p = 0.01$), seeds panicle⁻¹ (0.377, $p = 0.01$), and length/breadth ratio (0.689, $p = 0.01$). In this population, root number is positively and significantly correlated with panicles plant⁻¹ (0.365, $p = 0.01$), panicle length (0.324, $p = 0.01$), seeds panicle⁻¹ (0.367, $p = 0.01$), GY (0.285, $p = 0.05$), dry root weight (0.575, $p = 0.01$), fresh root weight (0.630, $p = 0.01$), and root length (0.602, $p = 0.01$). Root thickness has been found to be positively and significantly correlated with fresh root weight (0.544, $p = 0.01$), root length (0.352, $p = 0.01$), root number (0.770, $p = 0.01$), dry root weight (0.625, $p = 0.01$), panicles plant⁻¹ (0.335, $p = 0.01$), and GY (0.278, $p = 0.05$). Root volume is positively and significantly correlated with panicle length (0.358, $p = 0.01$), dry root weight (0.575, $p = 0.01$), fresh root weight (0.621, $p = 0.01$), root thickness (0.587, $p = 0.01$), root number (0.740, $p = 0.01$), and root length (0.700, $p = 0.01$). Dry root weight is positively and significantly correlated with GY (0.330, $p = 0.01$) and seeds panicle⁻¹ with panicle length (0.604, $p = 0.01$). Root length was found to be negatively correlated with plant height (Table 4.3).

Phenotypic correlation coefficient analysis of HKR47 $\times$ MAS26 showed that GY is positively and significantly correlated with panicle length (0.512, $p = 0.01$), panicles plant⁻¹ (0.294, $p = 0.05$), seeds panicle⁻¹ (0.476, $p = 0.01$), and length/breadth ratio (0.725, $p = 0.01$). In this population, seeds panicle⁻¹ is positively and significantly correlated with panicle length (0.694, $p = 0.01$) and panicles plant⁻¹ (0.529, $p = 0.01$). Root number is positively and significantly correlated with GY (0.386, $p = 0.05$) and fresh root weight (0.482, $p = 0.01$). Root thickness has been found to be positively and significantly correlated with fresh root weight (0.626, $p = 0.01$), root number (0.746, $p = 0.01$), and GY (0.408, $p = 0.01$). Root volume is positively and significantly correlated with fresh root weight (0.492, $p = 0.01$), root thickness (0.728, $p = 0.01$), and GY (0.455, $p = 0.01$). Root length is positively and significantly correlated with GY (0.317, $p = 0.05$) and seeds panicle⁻¹ (0.355, $p = 0.01$). Fresh root weight is positively and significantly correlated with panicles plant⁻¹ (0.545, $p = 0.01$), GY (0.346, $p = 0.01$), and effective number of tillers (0.539, $p = 0.01$) (Table 4.3).

Table 4.1 Mean and range for various root traits in two populations under pot-house conditions

Trait	HKR47	MAS26	MASARB25	Pusa Basmati 1460	HKR47 x MAS26		MASARB25 x Pusa Basmati 1460	
					Mean	Range	Mean	Range
RL	26±0.784	39.45±0.958	32.43±0.975	21.89±1.156	27.54	17-49	29.46	12-48
RN	92±1.172	97±1.059	110.0±2.045	92.0±1.685	83.3	34-220	98	35-222
RV	7.3±0.497	8.9±0.677	15.0±0.779	12.0±0.931	8.0	4-18	10.1	3-22
RT	2.4±0.884	2.9±0.679	3.72±0.553	2.98±0.478	2.85	1.8-4.4	3.27	2.1-5.6
FRW	2.64±0.948	3.34±0.869	4.88±0.585	3.79±0.664	3.08	1.30-6.39	4.04	1.34-11.13
FSW	23.36±1.558	26.18±1.037	28.77±1.156	23.39±1.473	22.27	15.32-46.44	25.88	16.14-56.34
DRW	1.22±0.447	0.89±0.375	1.56±0.661	1.13±0.449	0.92	0.24-2.94	1.22	0.22-3.62
DSW	10.18±0.836	11.99±0.991	12.74±0.868	10.89±0.921	9.18	3.42-19.72	12.13	4.34-35.26

RL: Root length (cm), RN: Root number, RV: Root volume (ml), RT: Root thickness (mm), FRW: Fresh root weight (g), FSW: Fresh shoot weight (g), DRW: Dry root weight (g), DSW: Dry shoot weight (g)

Table 4.2 Mean and range for various agronomic traits in two populations under direct-seeded aerobic field conditions

Trait	HKR47		MAS26		MASARB25		Pusa Basmati 1460		HKR47 x MAS26				MASARB25 x Pusa Basmati 1460			
									Mean		Range		Mean		Range	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
PH	82	86.3	71	78.0	75	82	80	103	75.9	81.9	63-92	71-101	70.6	81.2	50-85	66-97
TN	18	15	21	18	19	21	15	12	13	16	3-30	8-28	13.88	16.22	9-28	11-24
P/P	16	13	20	15	16	19	13	11	12	14	3-26	5-25	11	15	7-24	6-21
S/P	74	80	88	91	75	80	82	89	58	65	48-109	40-100	62	55	43-107	32-99
GY	820	747	972	932	908	852	788	717	760	834	302-1450	358-1222	702	878	401-1598	323-1410
1,000 GW	27.5	22.4	29.4	23.4	29.4	35.9	28.3	8.8	30.7	23.3	22.7-59.7	30.0-51.1	33.8	39.9	25.3-54.0	20.2-64.2
PL	20.8	21.7	17.4	19.5	18.4	20.9	20.9	22.5	19.1	21.3	14.4-25.5	18.8-27.5	19.0	17.7	14.8-23.1	15.5-28.6
L/B	4.15	3.89	4.34	4.24	4.66	4.27	5.23	4.91	4.20	3.29	3.89-4.73	3.31-4.44	5.09	4.99	4.30-5.84	4.77-6.12

PH: Plant height (cm), *TN*: Effective number of tillers plant⁻¹, *P/P*: No. of panicles plant⁻¹, *S/P*: Seeds panicle⁻¹, *GY*: Grain yield (Kg ha⁻¹), *1,000 GW*: 1,000-grain weight (g),

PL: Panicle length (cm), *L/B*: Length/Breadth ratio

Table 4.3 Phenotypic correlation coefficients between agronomic and root traits in MASARB25 x Pusa Basmati and HKR47 x MAS26-derived populations

		L/B	P/P	PH	PL	S/P	TN	GY	DRW	DSW	FRW	FSW	RL	RN	RT	RV
MASARB25 x Pusa Basmati 1460	L/B	1														
	P/P	0.404*	1													
	PH	0.277*	0.461**	1												
	PL	0.354**	0.420**	0.397**	1											
	S/P	0.202	0.494**	0.265*	0.604**	1										
	TN	0.415**	0.975**	0.485**	0.426**	0.476**	1									
	GY	0.689**	0.427**	0.297*	0.411**	0.377**	0.442**	1								
	DRW	0.301*	0.222	0.235	0.488**	0.241*	0.210	0.330**	1							
	DSW	0.063	0.352**	0.125	0.266*	0.310**	0.311**	0.246*	0.648**	1						
	FRW	0.306*	0.305*	0.130	0.451**	0.336**	0.297*	0.379**	0.782**	0.612**	1					
	FSW	0.152	0.313**	0.090	0.245*	0.335**	0.276*	0.340**	0.651**	0.918**	0.663**	1				
	RL	0.146	0.000	-0.078	0.258*	0.245*	0.013	0.235*	0.281*	0.392**	0.397**	0.417**	1			
	RN	0.106	0.365**	0.101	0.324**	0.367**	0.337**	0.285*	0.575**	0.867**	0.630**	0.857**	0.602**	1		
HKR47 x MAS26	RT	0.130	0.335**	0.134	0.337**	0.349**	0.281*	0.278*	0.625**	0.775**	0.544**	0.745**	0.352**	0.770**	1	
	RV	0.172	0.279*	0.241*	0.358**	0.295*	0.261*	0.265*	0.575**	0.606**	0.621**	0.586**	0.700**	0.741**	0.587**	1
	L/B	1														
	P/P	0.105	1													
	PH	0.044	0.492**	1												
	PL	0.259	0.457**	0.431**	1											
	S/P	0.212	0.529**	0.422**	0.694**	1										
	TN	0.113	0.997**	0.480**	0.460**	0.532**	1									
	GY	0.725**	0.294*	0.330*	0.512**	0.476**	0.296*	1								
	DRW	0.101	0.468**	0.404**	0.462**	0.557**	0.466**	0.352**	1							
	DSW	0.187	0.505**	0.205	0.530**	0.552**	0.507**	0.377**	0.569**	1						
	FRW	0.126	0.545**	0.419**	0.514**	0.573**	0.539**	0.346**	0.834**	0.699**	1					
	FSW	0.155	0.452**	0.200	0.384**	0.429**	0.453**	0.221	0.494**	0.878**	0.681**	1				
	RL	0.239	0.297*	-0.059	0.324*	0.355**	0.289	0.317*	0.285*	0.662**	0.353**	0.521**	1			
	RN	0.270*	0.697**	0.384**	0.371**	0.417**	0.695**	0.386**	0.449**	0.517**	0.482**	0.419**	0.413**	1		
	RT	0.225	0.640**	0.239	0.482**	0.554**	0.628**	0.408**	0.485**	0.718**	0.626**	0.551**	0.475**	0.746**	1	
	RV	0.322*	0.636**	0.296*	0.441**	0.468**	0.63**	0.455**	0.442**	0.614**	0.492**	0.531**	0.533**	0.810**	0.728**	1

L/B: Length/breadth ratio, P/P: No. of panicles/plant, PH: Plant height, PL: Panicle length, TN: Tiller number, GY: Grain yield, DRW: Dry root weight, DSW: Dry shoot weight, FRW: Fresh root weight, FSW: Fresh shoot weight, RL: Root length, RN: Root number, RT: Root thickness, RV: Root volume, *Correlation is significant at 0.05 level, ** Correlation is significant at 0.01 level

4.1.3. Genotypic analysis of mapping populations

Polymorphism of HKR47 with MAS26 and of MASARB25 with Pusa Basmati 1460 was 31.7% and 37.3%, respectively. A total of 226 and 184 alleles were identified in the HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460 populations by using 300 SSR markers (Plate 3, Fig. B, C, and D), respectively. MASARB25 x Pusa Basmati 1460-derived mapping populations were also evaluated for aroma using aroma gene-specific *BAD2* markers. A total of 13 lines were found to be homozygous and 24 lines were found to be heterozygous for aroma.

Similarity coefficient data based on the proportion of shared alleles using 91 and 112 SSR markers were used to calculate the coefficient values among the selected HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460 lines, respectively, through UPGMA tree cluster analysis. Allelic diversity was used to produce a dendrogram (cluster tree analysis, NTSYS-PC) to demonstrate the genetic relationship among selected plants and the parental rice varieties. All 94 and 100 plants from the two populations, HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460, respectively, clustered in two major groups at the similarity coefficients of 0.520 and 0.540, respectively. Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two-dimensional PCA scaling showed that the parental genotypes were quite distinct, whereas the mapping population genotypes were interspersed between the two parental lines (Figures 4.1 and 4.2).

Several QTL associated with root traits and agronomically important traits in the two mapping populations were detected in the MASARB25 x Pusa Basmati 1460 (Table 4.4) and HKR47 x MAS26 (Table 4.5) populations. A total of 35 QTL associated with 14 traits were mapped on chromosomes 1, 2, 5, 6, 8, 9, and 11 in MASARB25 x Pusa Basmati 1460-derived population (Table 4, Figure 4.3). A total of 14 QTL associated with nine traits were mapped on chromosomes 1, 2, 8, 9, 10, 11, and 12 in HKR47 x MAS26-derived population (Table 4.5, Figure 4.4).

QTL $qRL_{8.1}$ with a peak at RM544 on chromosome 8 with an R^2 value of 25.1% and $qRL_{8.2}$ with a peak at RM547 on chromosome 8 with an R^2 value of 38.8% and QTL $qRL_{8.2}$ with a peak at RM547 on chromosome 8 with an R^2 value of 19.8% and $qRL_{9.1}$ with a peak at RM524 on chromosome 9 with an R^2 value of 31.6% were identified associated with root length in the mapping populations MASARB25 x Pusa Basmati 1460 and HKR47 x MAS26, respectively. $qRL_{8.2}$ was identified in both populations.

In addition, QTL $qGY_{8.1}$ with RM339 on chromosome 8 being the peak marker with an R^2 value of 34.0% and $qGY_{2.1}$ on chromosome 2 with RM7562 being the peak marker with an R^2 value of 22.8% (Figure 4.5) and QTL $qGY_{2.2}$ on chromosome 2 with RM526 being the peak marker with an R^2 value of 43.2% (Figure 4.6) were identified for grain yield under aerobic conditions in the mapping populations MASARB25 x Pusa Basmati 1460 and HKR47 x MAS26, respectively.

Plate 3

- (A) Screening of F_2 population for root traits
- (B) Agarose Gel Electrophoretic pattern showing allelic polymorphism among HKR47 x MAS26 derived F_2 at RM205 locus
- (C) Agarose Gel Electrophoretic pattern showing allelic polymorphism among MASARB25 x Pusa Basmati 1460 derived F_2 at RM256 locus
- (D) A silver stained gel showing allelic polymorphism among MASARB25 x Pusa Basmati 1460 and HKR47 x MAS26 derived F_2 at RM333, RM526 and RM 258 locus

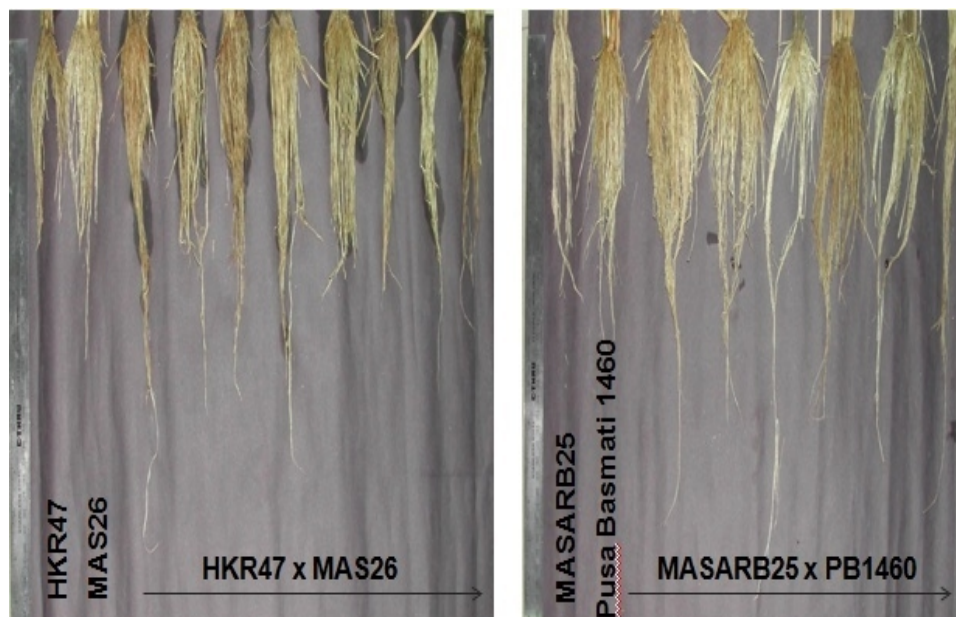
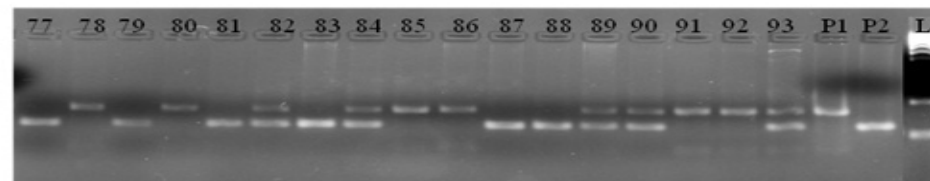
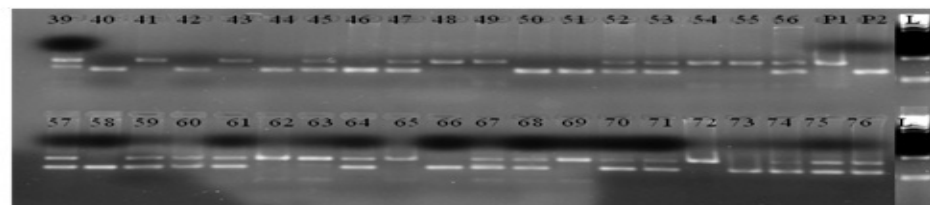
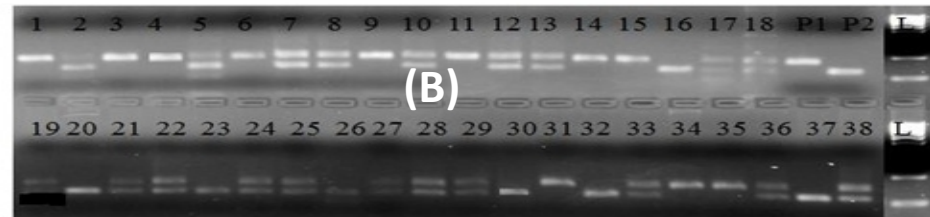
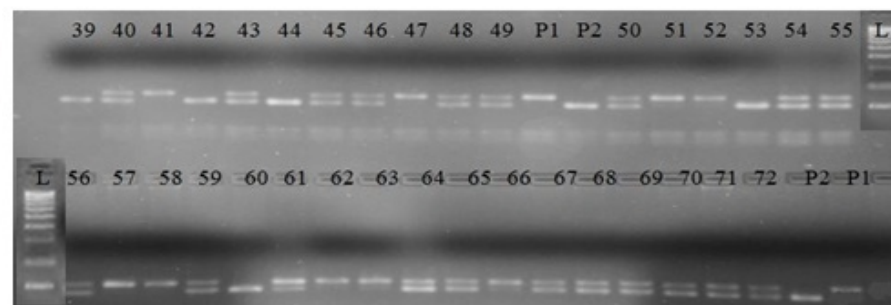
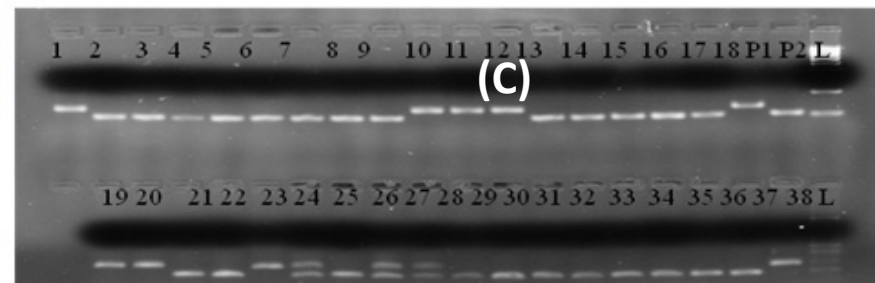


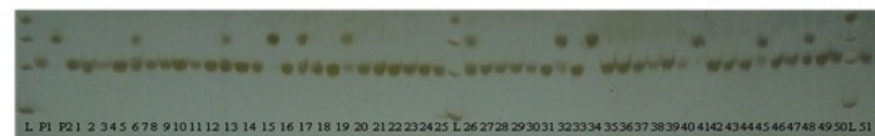
Fig (A): Screening of F₂ Populations



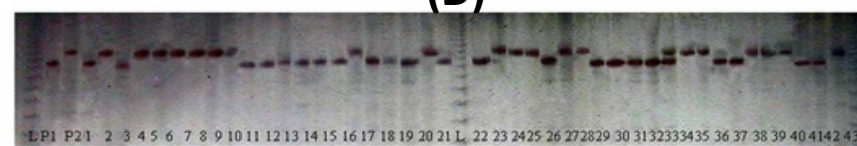
Agarose gel showing allelic polymorphism among parental rice varieties and selected F₂ plants at RM205 locus. Lane L-100 bp ladder, P1-MAS26, P2-HKR47, 1-93: F₂ plants



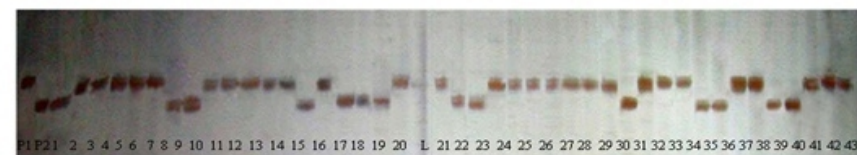
Agarose gel showing allelic polymorphism among parental rice varieties and selected F₂ plants at RM256 locus. Lane L-100 bp ladder, P1-MASARB25, P2-Pusa Basmati 1460, 1-72: F₂ plants



A silver stained gel showing allelic polymorphism among parental rice varieties and selected F₂ plants at RM333 locus. Lane L-20 bp ladder, P1-MASARB25, P2-Pusa Basmati 1460, 1-52: F₂ plants



A silver stained gel showing allelic polymorphism among parental rice varieties and selected F₂ plants at RM526 locus. Lane L-20 bp ladder, P1-HKR47 P2- MAS26, 1-43: F₂ plants



A silver stained gel showing allelic polymorphism among parental rice varieties and selected F₂ plants at RM258 locus. Lane L-20 bp ladder, P1-MASARB25, P2-Pusa Basmati 1460, 1-43: F₂ plants

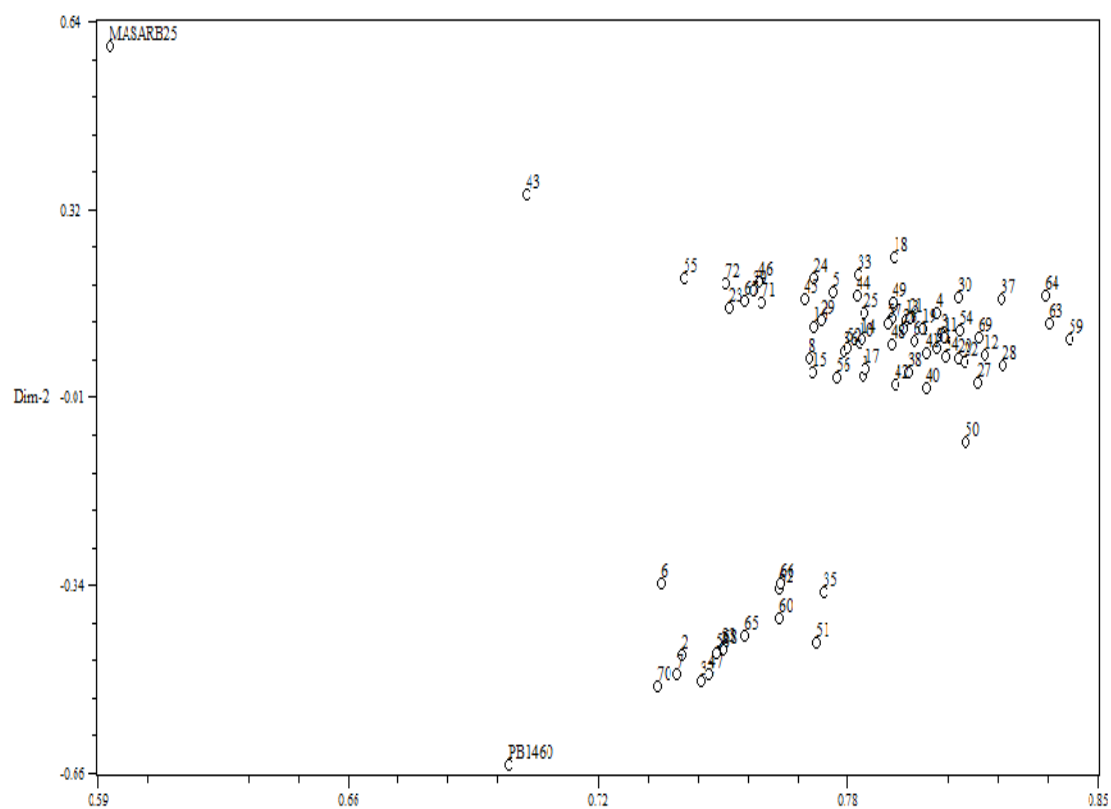


Figure 4.1 Two-dimensional PCA scaling of MASARB25 x Pusa Basmati 1460 based on SSR diversity data

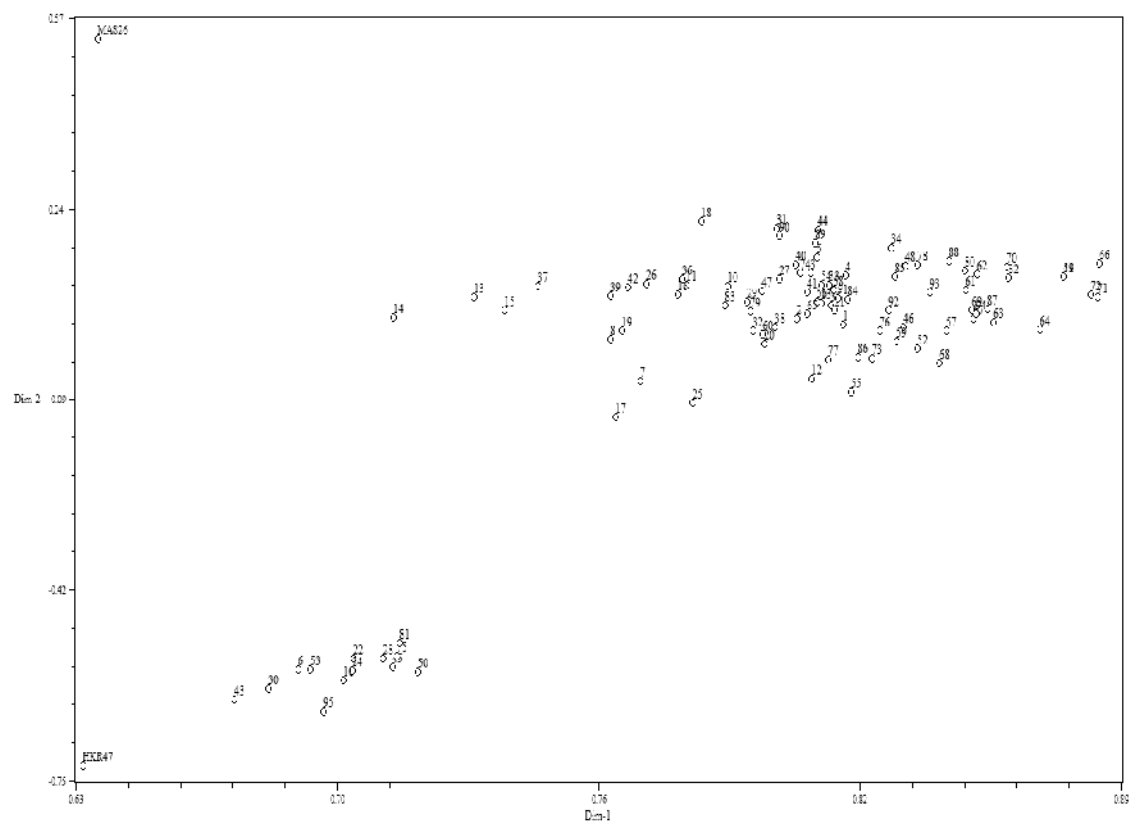


Figure 4.2 Two-dimensional PCA scaling of HKR47 x MAS26 based on SSR diversity data

Table 4.4 QTL associated with agronomic and aerobic root traits of MASARB25 x Pusa Basmati 1460 population

Trait	Name	Chr. no.	Position (cM)	Flanking markers	Flanking markers (cM)	LR	LOD	Additive effect	R ²	DPE ^a
Root length	qRL _{8.1}	8	36.5	RM408-RM544	0.6-38.5	21.51	4.6	-3.244	25.1	P
	qRL _{8.2}	8	58.3	RM547-RM331	58.1-65.0	33.35	7.2	4.066	38.8	M
Root volume	qRV _{2.1}	2	0.8	RM485-RM7033	0.0-7.9	16.8	3.7	2.492	19.7	M
	qRV _{6.1}	6	20.3	RM587-RM204	10.7-25.1	11.7	2.5	-2.349	18.1	P
Root thickness	qRT _{5.1}	5	6.7	RM122-RM574	6.4-41.0	15.04	3.3	0.375	22.3	M
	qRT _{6.1}	6	26.0	RM204-RM225	25.1-26.2	14.40	3.1	-0.33	17.4	P
Fresh root weight	qFRW _{2.1}	2	2.5	RM485-RM7033	0.0-7.9	17.27	3.8	1.194	28.9	M
	qFRW _{2.2}	2	60.0	RM174-RM300	47.5-66.0	12.11	2.6	-1.049	24.6	P
	qFRW _{6.1}	6	3.4	RM589-RM587	3.2-10.7	11.71	2.5	-0.901	17.2	P
Dry root weight	qDRW _{2.2}	2	48.1	RM174-RM300	47.5-66.0	12.5	2.7	-0.298	18.4	P
	qDRW _{8.2}	8	123.0	RM458-RM3331	122.9-123.2	14.1	3.1	-0.294	18.1	P
Fresh shoot weight	qFSW _{2.1}	2	6.1	RM485-RM7033	0.0-7.9	13.8	3.0	5.204	20.6	M
	qFSW _{6.2}	6	28.5	RM225-RM314	26.2-33.6	22.6	4.9	-6.020	31.4	P
	qFSW _{6.1}	6	21.1	RM587-RM204	10.7-25.1	18.1	3.9	-5.730	30.5	P
Dry shoot weight	qDSW _{2.1}	2	5.8	RM485-RM7033	0.0-7.9	11.9	2.6	3.260	23.1	M
	qDSW _{6.1}	6	25.1	RM204	25.1	18.7	4.1	-3.510	29.4	P
Plant height	qPH _{2.1}	2	2.2	RM485-RM7033	0.0-7.9	14.5	3.0	14.45	24.3	M
	qPH _{6.1}	6	56.2	RM6836-RM527	54.1-61.2	12.3	2.7	12.34	18.9	M
	qPH _{9.1}	9	56.1	RM524-RM410	13.2-64.4	21.49	4.7	-8.330	67.7	P
	qPH _{11.2}	11	99.8	RM21-RM206	85.7-102.9	17.81	3.9	-4.580	21.0	P
Panicle length	qPL _{2.1}	2	4.4	RM485-RM7033	0.0-7.9	20.92	4.5	2.030	28.6	M
	qPL _{11.1}	11	39.0	RM4-RM441	0.0-43.9	15.68	3.4	-1.820	26.4	P
	qPL _{11.2}	11	48.1	RM441-RM21	43.9-85.7	16.59	3.6	-1.860	28.0	P
	qPL _{8.1}	8	112.8	RM230-RM433	112.2-116.0	25.24	5.4	1.260	16.5	M
	qPL _{9.1}	9	56.2	RM524-RM410	13.2-64.4	17.15	3.7	-2.050	64.2	P
Tiller number	qTN _{2.1}	2	152.7	RM1092-RM318	146.6-152.8	11.97	2.6	-0.979	14.5	P
	qTN _{1.1}	1	86.9	RM10916-RM246	67.4-115.2	13.77	3.0	-2.340	20.3	P
	qTN _{8.2}	8	58.3	RM547-RM331	58.1-65.0	20.32	4.4	-3.361	47.9	P
Panicles/plant	qNPP _{2.1}	2	153.0	RM318-RM6	152.8-154.7	12.05	2.6	-0.979	14.5	P
	qNPP _{8.2}	8	58.4	RM547-RM331	58.1-65.0	1.64	6.4	-0.947	13.7	P
Seeds/panicle	qNSP _{11.1}	11	37.5	RM4-RM441	0.0-43.9	14.85	3.2	-10.840	38.9	P
	qNSP _{11.2}	11	47.8	RM441-RM21	43.9-85.7	14.02	3.0	-10.060	34.7	P
L/B	qLBR _{11.1}	11	55.1	RM441-RM21	43.9-85.7	14.53	3.2	-0.277	39.1	P
Grain yield	qGY _{8.1}	8	72.2	RM339	72.2	21.54	4.7	-199.20	34.0	P
	qGY _{2.1}	2	10.2	RM7562-RM279	8.6-13.8	13.74	3.0	147.25	22.8	M

^aDirection of phenotypic effect; *M* and *P* indicate MASARB25 and Pusa Basmati 1460 alleles, respectively. Additive effect is the effect of substituting a MASARB25 allele for a Pusa Basmati 1460 allele; Its positive value indicates that MASARB25 has the positive allele; *LR*: Likelihood ratio, *LOD*: log₁₀ of an odds ratio, *R*²: percent phenotypic variance

Table 4.5 QTL associated with agronomic and aerobic root traits of HKR47 x MAS26 population

Trait	Name	Chr. no.	Position (cM)	Flanking markers	Flanking markers (cM)	LR	LOD	Additive effect	R ²	DPE ^a
Root length	qRL _{8.2}	8	57.8	RM310-RM547	57.0-58.1	13.58	2.9	1.980	19.8	M
	qRL _{9.1}	9	34.1	RM524-RM257	13.2-66.1	11.87	2.6	-2.800	31.6	H
Root thickness	qRT _{1.1}	1	107.9	RM488-RM237	101.4-112.9	20.43	4.4	0.406	38.4	M
Dry root weight	qDRW _{8.1}	8	14.6	RM152-RM310	9.4-57.0	21.26	4.6	-0.439	44.7	H
Plant height	qPH _{8.1}	8	101.5	RM256	101.5	16.00	3.5	-4.424	28.2	H
	qPH _{11.1}	11	11.9	RM4-RM202	5.0-6.2	16.10	3.5	-5.682	42.6	H
Panicle length	qPL _{12.1}	12	47.4	RM28048	47.4	13.40	2.9	1.952	36.8	M
	qPL _{10.1}	10	70.8	RM258	70.8	11.78	2.6	1.200	21.6	M
Tiller number	qTN _{8.3}	8	123.2	RM3331	123.2	16.60	3.6	-1.730	26.4	H
	qTN _{8.1}	8	15.7	RM152-RM310	9.4-57.0	11.90	2.6	-3.124	29.3	H
Panicles/plant	qNPP _{8.3}	8	123.2	RM3331	123.2	13.82	3.0	-1.340	20.2	H
	qNPP _{8.1}	8	16.2	RM152-RM310	9.4-57.0	13.51	2.9	-2.790	31.6	H
Seeds/panicle	qNSP _{8.1}	8	123.2	RM3331	123.2	11.46	2.5	-186.300	15.3	H
Grain yield	qGY _{2.2}	2	118.5	RM475-RM526	92.5-136.3	12.56	2.7	145.100	43.2	M

^aDirection of phenotypic effect; *H* and *M* indicate HKR47 and MAS26 alleles, respectively. Additive effect is the effect of substituting a MAS26 allele for an HKR47 allele; its positive value indicates that MAS26 has the positive allele; *LR*: Likelihood ratio, *LOD*: log₁₀ of an odds ratio, *R*²: percent phenotypic variance

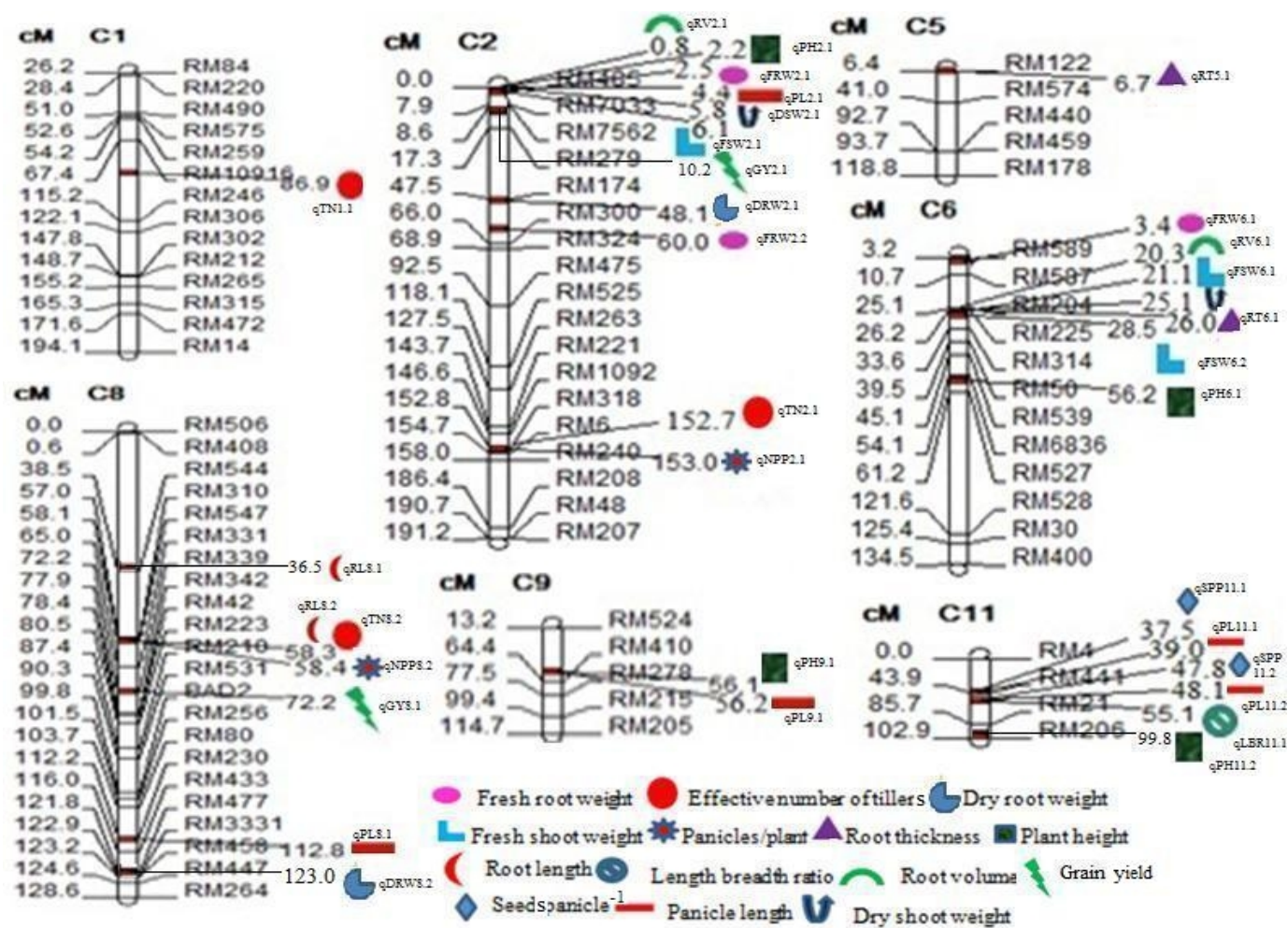


Figure 4.3: QTL associated with agronomic and aerobic root traits of MASARB25 x Pusa Basmati 1460 population

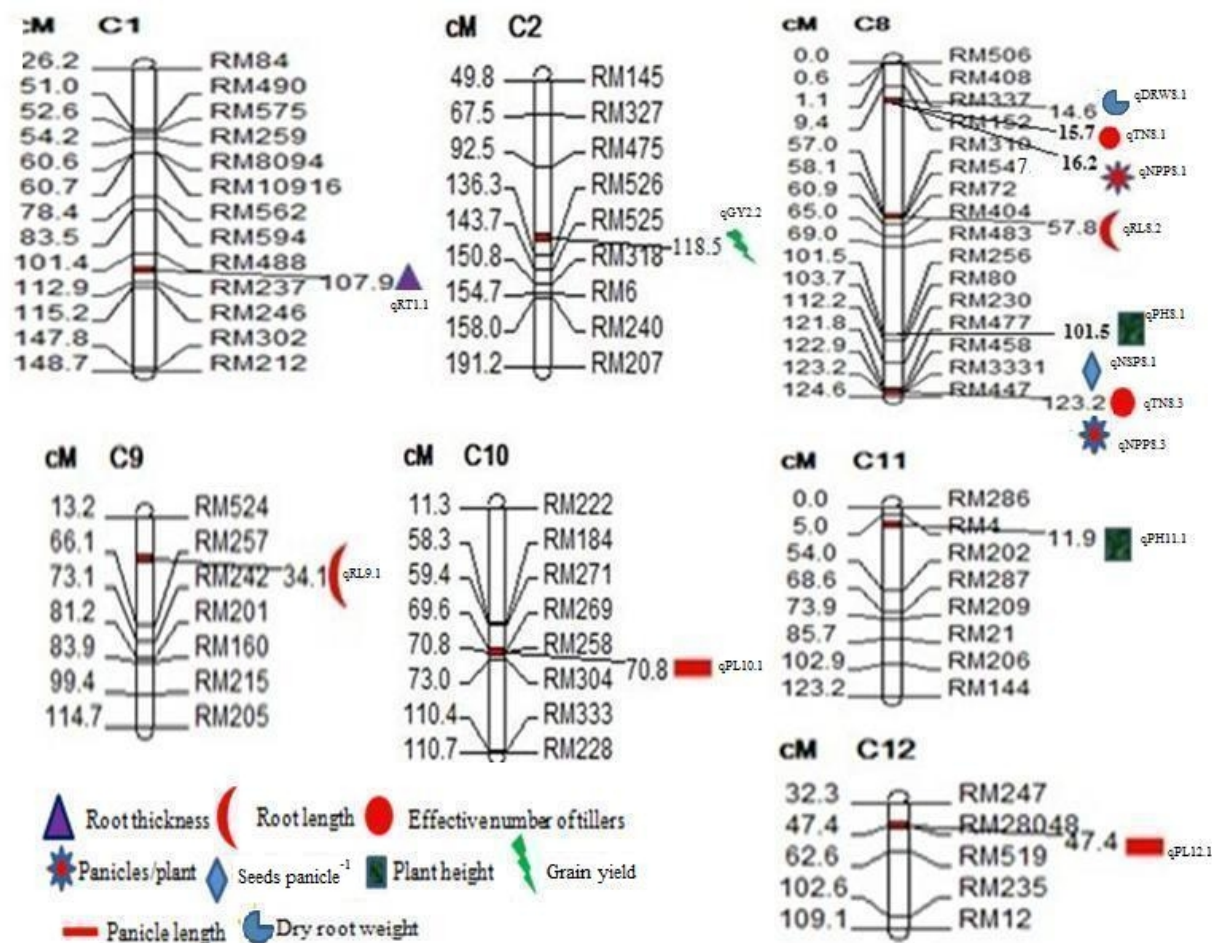


Figure 4.4 QTL associated with agronomic and aerobic root traits of HKR47 x MAS26 population

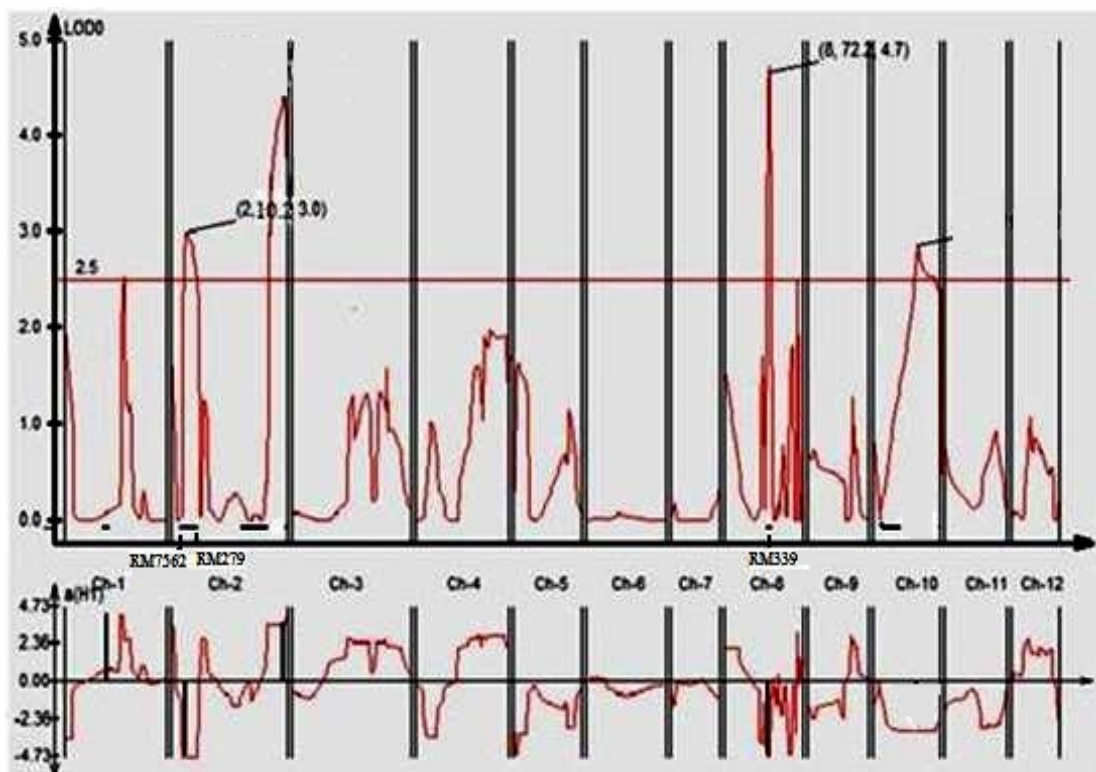


Figure 4.5 QTL likelihood curves of LOD scores for grain yield showing significant regions within qGY_{2.1} and qGY_{8.1} under aerobic conditions in MASARB25 x Pusa Basmati 1460 population

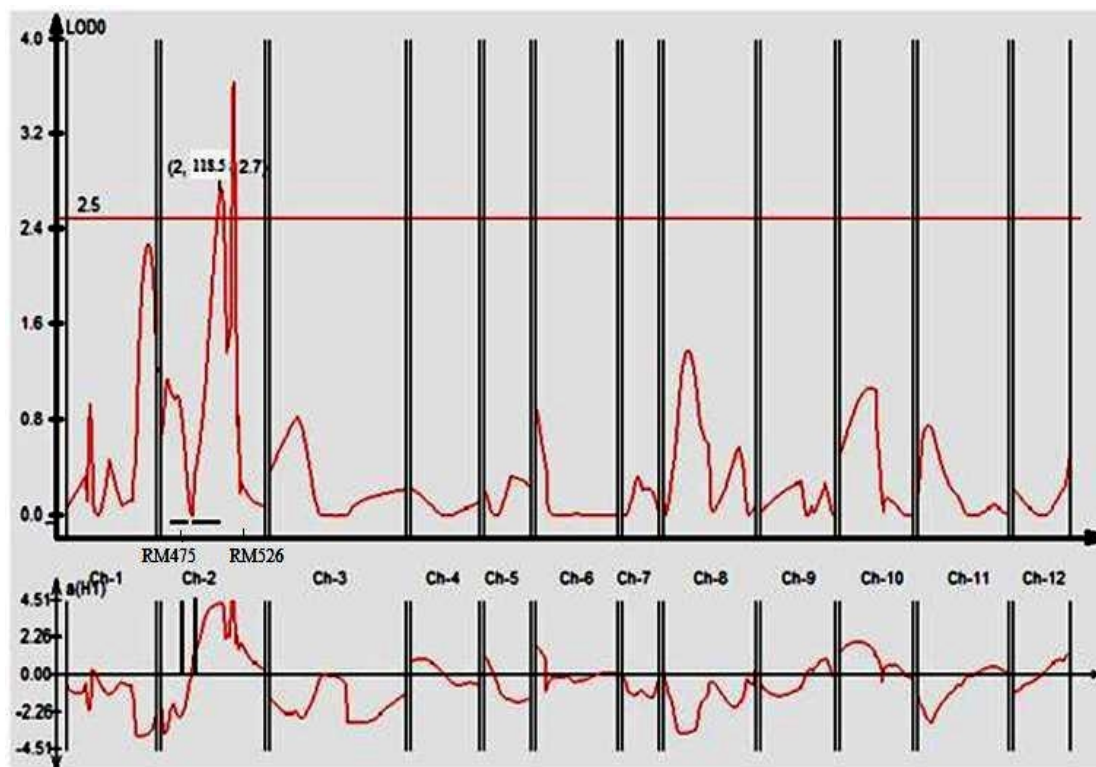


Figure 4.6 QTL likelihood curves of LOD scores for grain yield showing significant regions within qGY_{2.2} under aerobic conditions in HKR47 x MAS26 population

For the MASARB25 × Pusa Basmati 1460 population on chromosome 2 in a region of 0-10.2 cM, seven QTL were reported to be significantly associated with seven different root traits and agronomically important traits (RV, FRW, FSW, DSW, PH, PL, and GY) (Figure 4.7). These coexisting chromosomal regions governing root and yield traits provide a unique opportunity for breeders to introgress such regions together as a unit into lowland varieties through MAS/MAB and to develop cultivars possessing higher yield and increased adaptation to aerobic conditions.

In HKR47 × MAS26-derived mapping population, RM256 on chromosome 8, RM28048 on chromosome 12 and RM258 on chromosome 10, and RM3331 on chromosome 8 showed significant association with plant height, panicle length, and number of tillers per plant, respectively (Table 4.5). RM339 on chromosome 8 and RM204 on chromosome 6 showed significant association with grain yield and dry shoot weight in MASARB25 × Pusa Basmati 1460-derived mapping population. In this study, three QTL for root thickness (chromosomes 1, 5, and 6), two for root volume (chromosomes 2 and 6), three for fresh root weight (chromosomes 2 and 6), three for dry root weight (chromosomes 2 and 8), three for fresh shoot weight (chromosomes 2 and 6), two for dry shoot weight (chromosomes 2 and 6), six for plant height (chromosomes 2, 6, 8, 9, and 11), seven for panicle length (chromosomes 2, 8, 9, 10, 11, and 12), five for tiller number (chromosomes 1, 2, and 8), four for number of panicles per plant (chromosomes 2 and 8), three for number of seeds per panicle (chromosomes 8 and 11), one for length/breadth ratio (chromosome 11), and three for grain yield (chromosomes 2 and 8) were identified (Tables 4.4 and 4.5). $qGY_{2.1}$ and $qGY_{8.1}$, individually and combined, and $qGY_{2.2}$ exhibited a grain yield improvement of 24%, 28%, 37%, and 36%, respectively, in 2011 and 18%, 25%, 39%, and 24%, respectively, in 2012 under aerobic conditions (Table 4.6).

Adjacent to the QTL $qGY_{8.1}$ within a region of 14.4 cM, other QTL ($qTN_{8.2}$, $qNPP_{8.2}$, and $qRL_{8.2}$) has been reported (Figure 4.8). A 25.1-cM segment between RM589 and RM314 on chromosome 6 affecting different root (RV, RT, and FRW) and shoot (FSW and DSW) traits under aerobic conditions in the case of MASARB25 × Pusa Basmati 1460 mapping population (Figure 4.9).

A number of lines with higher yield under aerobic conditions, higher root length and dry biomass, GY, and length/breadth ratio, and with Pusa Basmati 1460-specific alleles in homozygous or heterozygous condition at the *BAD2* locus (Table 4.7). These lines will serve as novel materials for the selection of stable aerobic Basmati rice varieties.

Table 4.6 Yield improvement of lines possessing QTL (+ QTL) for grain yield under aerobic conditions over lines not possessing QTL (– QTL) for the two populations

Trial	QTL	+QTL	–QTL	PB1460/HKR47	% Yield improvement
MASARB25 x Pusa Basmati 1460					
2011	qGY _{2.1}	1255.3	1014.9	788	24
	LSD _{0.05}	163.4	131.6	126.6	
	qGY _{8.1}	1352.3	1057.0	788	28
	LSD _{0.05}	170.2	126.2	126.6	
	qGY _{2.1} and qGY _{8.1}	1500.3	1094.8	788	37
	LSD _{0.05}	195.4	141.0	126.6	
2012	qGY _{2.1}	1087.2	922.9	717	18
	LSD _{0.05}	134.6	112.2	104.2	
	qGY _{8.1}	1237	987.2	717	25
	LSD _{0.05}	151.2	124.2	104.2	
	qGY _{2.1} and qGY _{8.1}	1317	949.5	717	39
	LSD _{0.05}	173.8	136.8	104.2	
Combined over 2011 and 2012	qGY _{2.1}	1171.3	968.9	753	20.8
	LSD _{0.05}	149.0	122.0	115.4	
	qGY _{8.1}	1294.6	1022.1	753	26.6
	LSD _{0.05}	160.7	125.2	115.4	
	qGY _{2.1} and qGY _{8.1}	1408.7	1022.2	753	37.8
	LSD _{0.05}	184.6	138.9	115.4	
HKR47 x MAS26					
2011	qGY _{2.2}	1958	1436	820	36
	LSD _{0.05}	215.4	166.8	113.8	
2012	qGY _{2.2}	1617	1302	747	24
	LSD _{0.05}	183.6	144.8	101.2	
Combined over 2011 and 2012	qGY _{2.2}	1788	1369	784	30.6
	LSD _{0.05}	197.0	151.8	107.4	

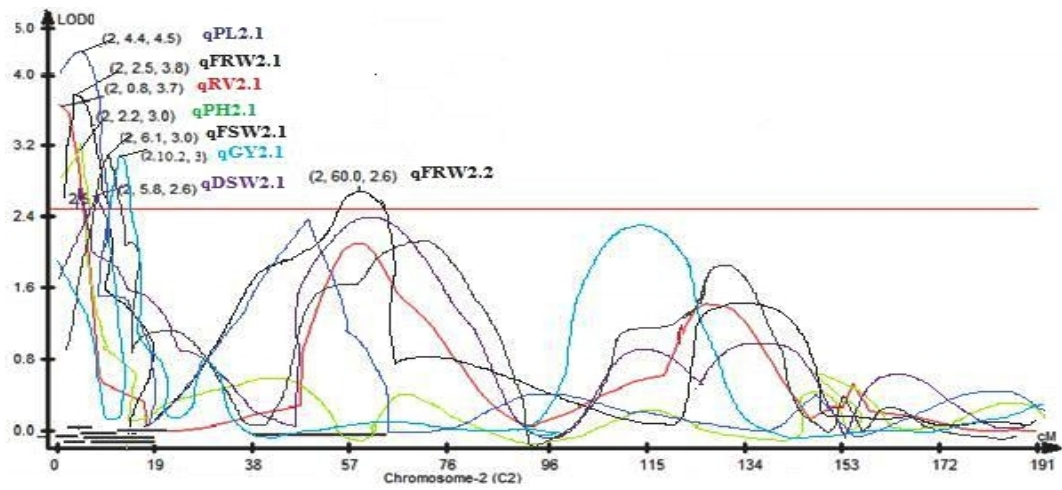


Figure 4.7 QTL likelihood curves of LOD score of a segment of 10.2 cM on chromosome 2 in MASARB25 x Pusa Basmati 1460 population

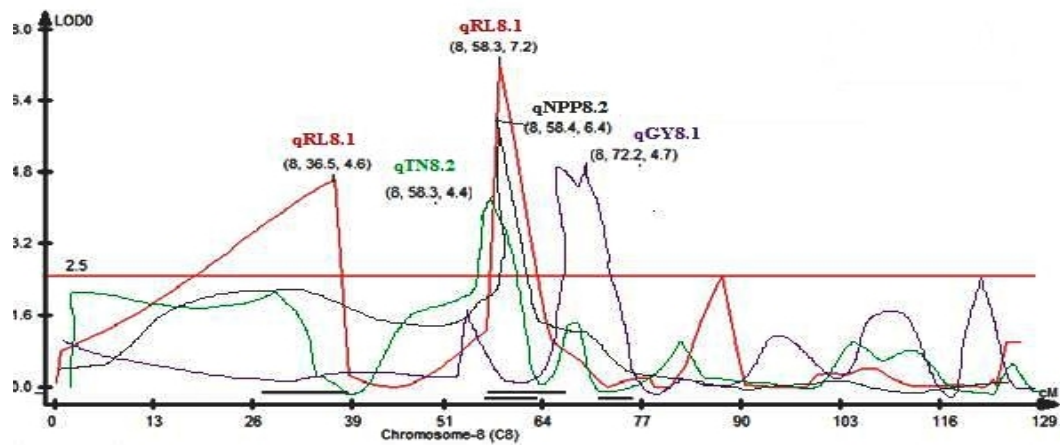


Figure 4.8 QTL likelihood curves of LOD score of coexisting qtl on chromosome 8 in MASARB25 x Pusa Basmati 1460 population

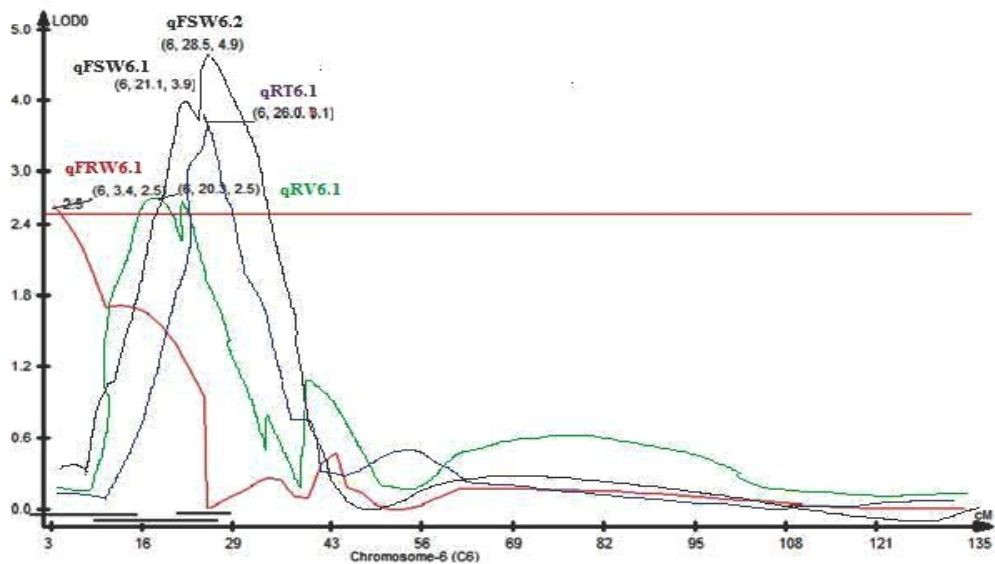


Figure 4.9 QTL likelihood curves of LOD score of coexisting qtl on chromosome 6 in MASARB25 x Pusa Basmati 1460 population

Table 4.7: Values of mean yield and mean root traits of selected promising lines under aerobic conditions

Population	Pusa Basmati 1460	MASARB25	MASARB25 x Pusa Basmati 1460	HKR47	MAS26	HKR47 x MAS26
No. of promising lines selected	-	-	42	-	-	52
GY (kg ha ⁻¹)	717	852	1889.3	747	932	2346.2
LSD _{0.05}	104.2	133.3	234.1	101.2	156.7	198.2
RL	20.22	27.22	29.93	20.00	29.45	33.42
LSD _{0.05}	0.996	1.175	1.112	1.332	1.545	1.443
RV	13.0	16.0	18.8	7.1	8.2	8.8
LSD _{0.05}	0.621	0.545	0.775	0.399	0.452	0.576
RN	88	102	109	85	92	97
LSD _{0.05}	1.994	2.223	2.899	1.132	1.459	1.886
RT	2.5	3.2	3.6	2.0	2.6	3.1
LSD _{0.05}	0.445	0.776	0.356	0.778	0.597	0.662
FRW	3.45	4.67	5.02	2.42	3.12	4.01
LSD _{0.05}	0.532	0.445	0.668	0.448	0.439	0.665
DRW	1.02	1.34	1.57	1.12	0.77	1.34
LSD _{0.05}	0.554	0.454	0.379	0.229	0.236	0.332
Aroma	++	--	++ , +-	--	--	--

GY: Grain yield, RL: Root length, RN: Root number, RV: Root volume, RT: Root thickness, FRW: Fresh root weight, DRW: Dry root weight, ++ Aromatic (homozygous), +- : Aromatic (heterozygous), -- : Non-aromatic (homozygous)

4.2 Experiment 2

4.2.1 Lysimeter and Field study

A 1204-pot lysimeter study was conducted to study the water uptake rates, shoot growth dynamics and root growth plasticity of Kali Aus/2*MTU1010 and Aus276/3*MTU1010 lines contrasting for yield. Variation was observed among the genotypes for the water uptake rates (Figure 4.10), total water uptake/total root biomass (TWU/TRB) (Figure 4.11 A, B, C, D) and DRW/DSW (Figure 4.11 E, F, G, H).

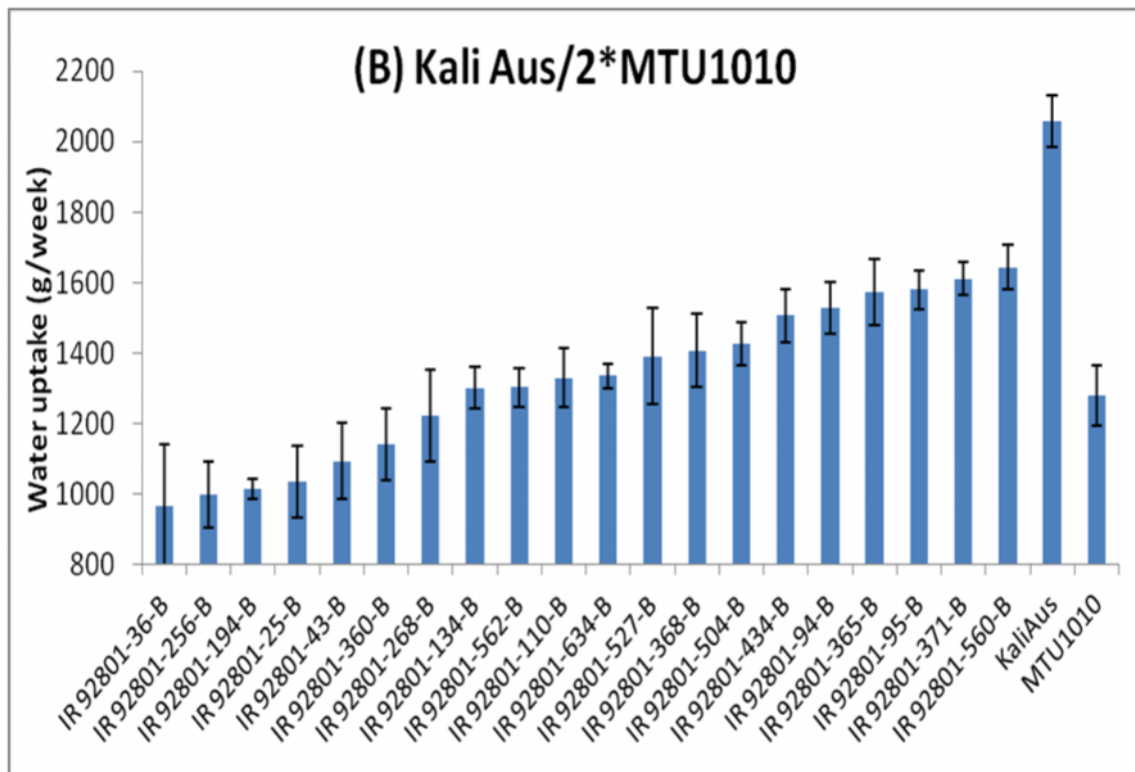
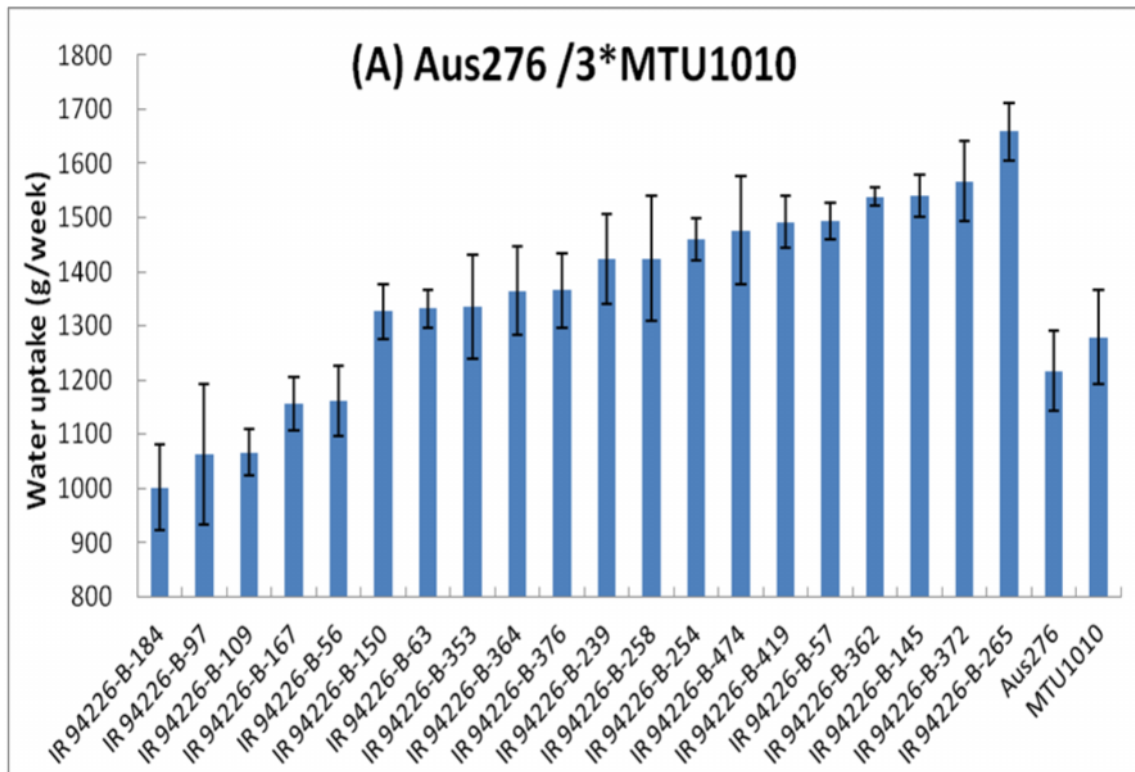


Figure 4.10 Variation for water uptake (g/week) among genotypes in Aus276/3*MTU1010 (A) and Kali Aus/2*MTU1010 (B) populations under UDS conditions

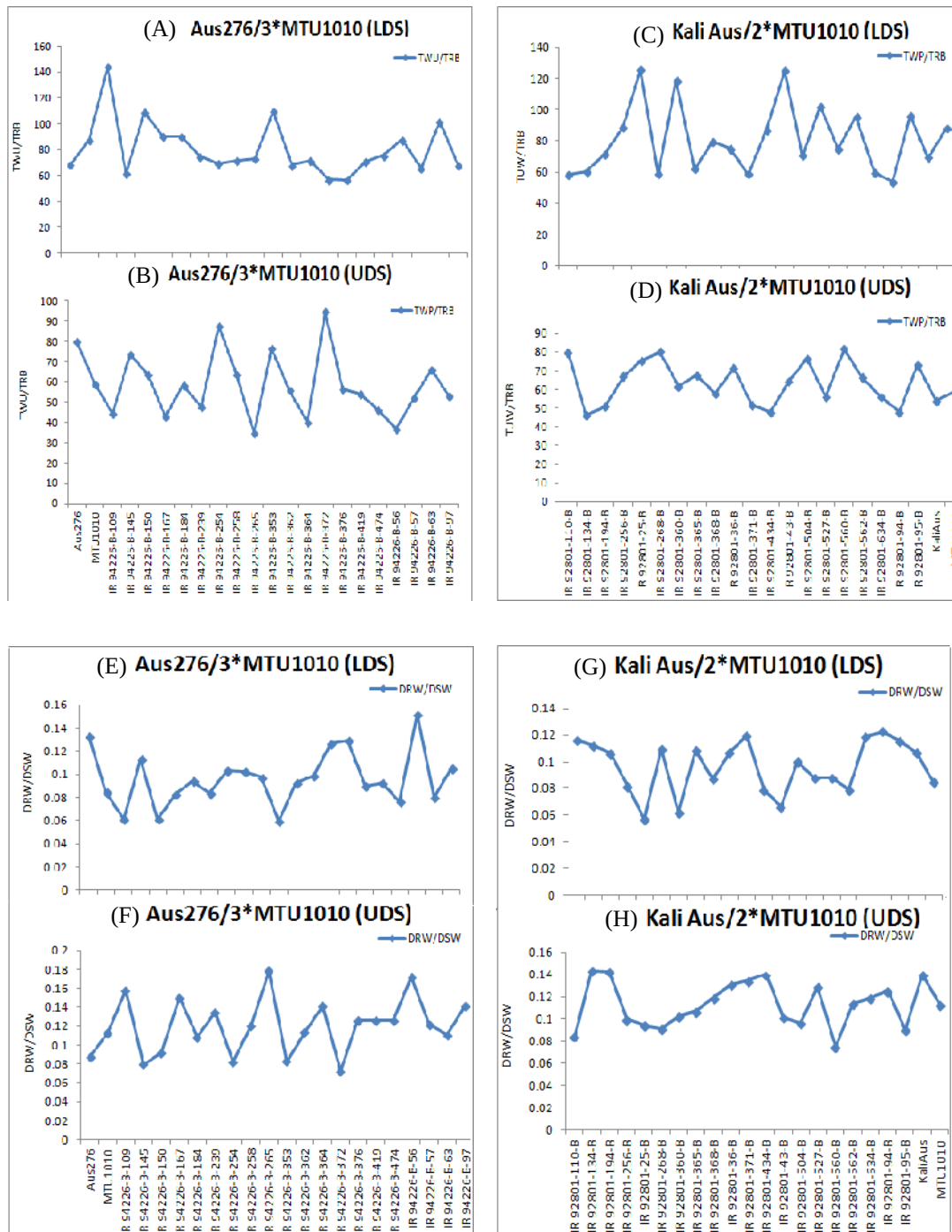


Figure 4.11 Variation for total water uptake/total root biomass (TWU/TRB) in Aus276/3*MTU1010 (A, B), Kali Aus/2*MTU1010 (C, D) populations and dry root weight/dry shoot weight (DRW/DSW) in Aus276/3*MTU1010 (E, F), Kali Aus/2*MTU1010 (G, H) populations

We calculated plasticity in term of root length, dry root weight, elongation of lateral root, branching of lateral root and we are exploring them by analyzing the data in different ways and at different depth (0-15, 15-30, 30-45 and 45-60 cm), we want to figure out which one showed the best differences among genotypes under different conditions (lysimeter, field and hydroponics) and treatments (LWW, LDS, LRW, UWW, UDS and URW).

The genotypes that were showing the consistent plasticity across different treatment was shown in Figure 4.12. On the basis of water uptake and dry root weight a total of eight genotypes from each population were selected for field studies (Table 4.8).

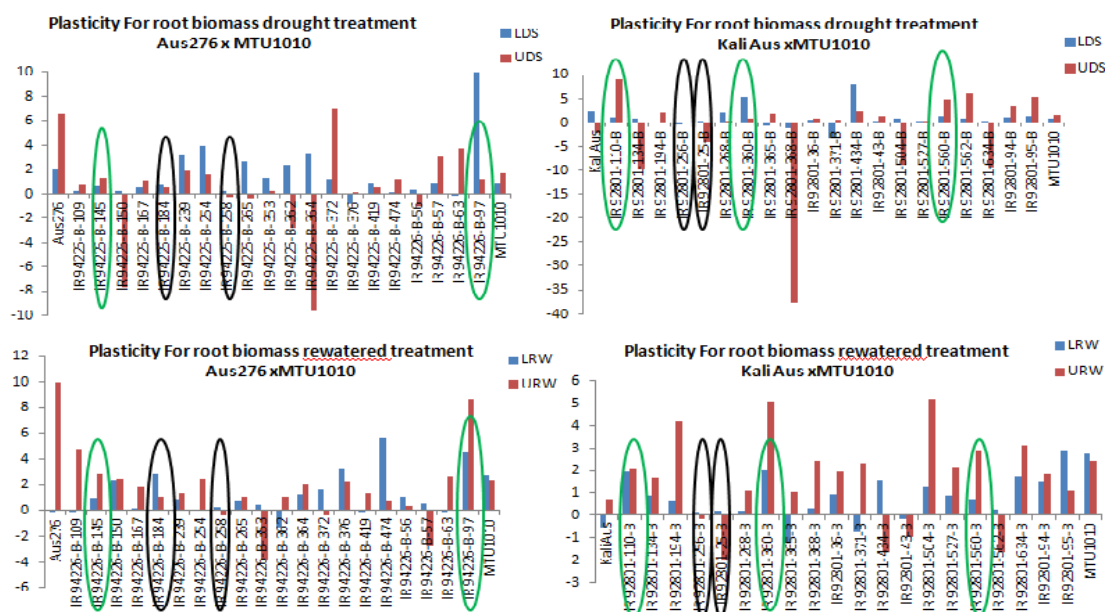


Figure 4.12 Variation in plasticity for root biomass for Aus276/3*MTU1010 and Kali Aus/2*MTU1010 populations (green color: genotypes that were able to maintain their plasticity across different treatment, black outlines: genotypes that were not able to maintain their plasticity across different treatment)

Data on stomatal conductance, canopy temperature, soil water potential and plasticity in term of root length, dry root weight, elongation of lateral root and branching of lateral root were recorded. Variation in plasticity for deep root length, deep lateral root and deep root weight under field conditions is shown Figure 4.13. The data of root plasticity was explored in different ways at different depth. Good correlation between root plasticity in lysimeter and field at a depth of 45-60 cm in field and 40-60 cm in lysimeter is shown in (Figure 4.14). Good correlation was observed between root growth in lysimeter and field (Figure 4.15). Plasticity in % lateral root growth was observed more due to elongation than branching of lateral root in Aus276/3*MTU1010 (Figure 4.16 (A)) and Kali Aus/2*IR64 (Figure 4.16 (B)) populations.

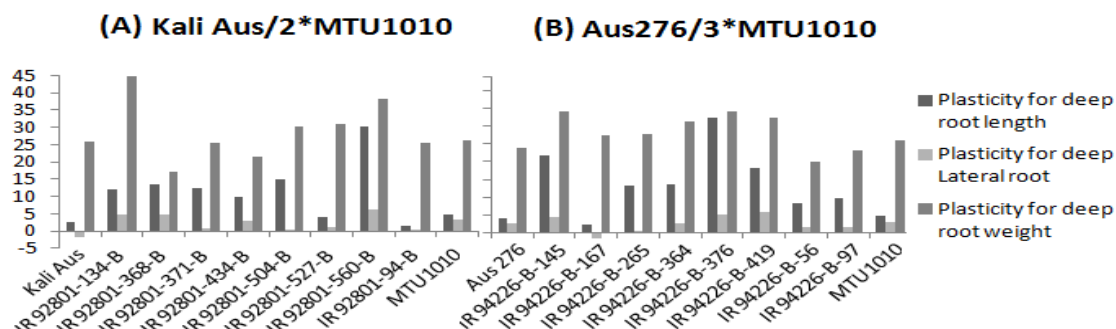


Figure 4.13 Variation in plasticity for deep root length, deep lateral root and deep root weight under field conditions in Kali Aus/2*MTU1010 (A) and Aus276/3*MTU1010 (B) populations

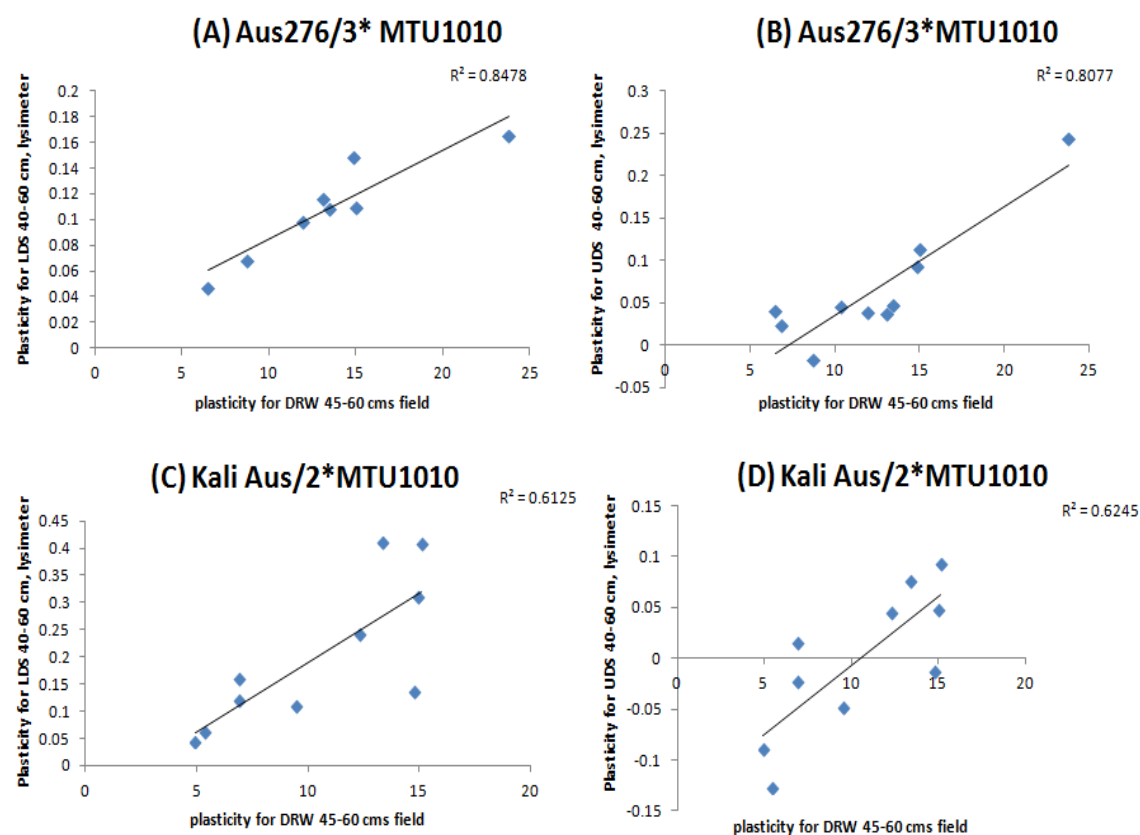


Figure 4.14 Correlation between root plasticity at a depth of 45-60 cm in field and 40-60 cm in lysimeter

Table 4.8 List of selected sixteen genotypes from Lysimeter study for root sampling and soil moisture content measurement in field

S. No.	Population	Genotype	Trait
1	Aus276 x MTU1010	IR 94226-B-56	High water uptake & High DRW
2	Aus276 x MTU1010	IR 94226-B-419	High water uptake & High DRW
3	Aus276 x MTU1010	IR 94226-B-97	High water uptake & High DRW
4	Aus276 x MTU1010	IR 94226-B-376	Low water uptake & Low DRW
5	Aus276 x MTU1010	IR 94226-B-167	Low water uptake & Low DRW
6	Aus276 x MTU1010	IR 94226-B-145	Low water uptake & Low DRW
7	Aus276 x MTU1010	IR 94226-B-364	High water uptake & Low DRW
8	Aus276 x MTU1010	IR 94226-B-265	Low water uptake & High DRW
9	Kali Aus x MTU1010	IR 92801-434-B	High water uptake & High DRW
10	Kali Aus x MTU1010	IR 92801-134-B	High water uptake & High DRW
11	Kali Aus x MTU1010	IR 92801-94-B	High water uptake & High DRW
12	Kali Aus x MTU1010	IR 92801-527-B	Low water uptake & Low DRW
13	Kali Aus x MTU1010	IR 92801-368-B	Low water uptake & Low DRW
14	Kali Aus x MTU1010	IR 92801-504-B	Low water uptake & Low DRW
15	Kali Aus x MTU1010	IR 92801-560-B	High water uptake & Low DRW
16	Kali Aus x MTU1010	IR 92801-371-B	Low water uptake & High DRW

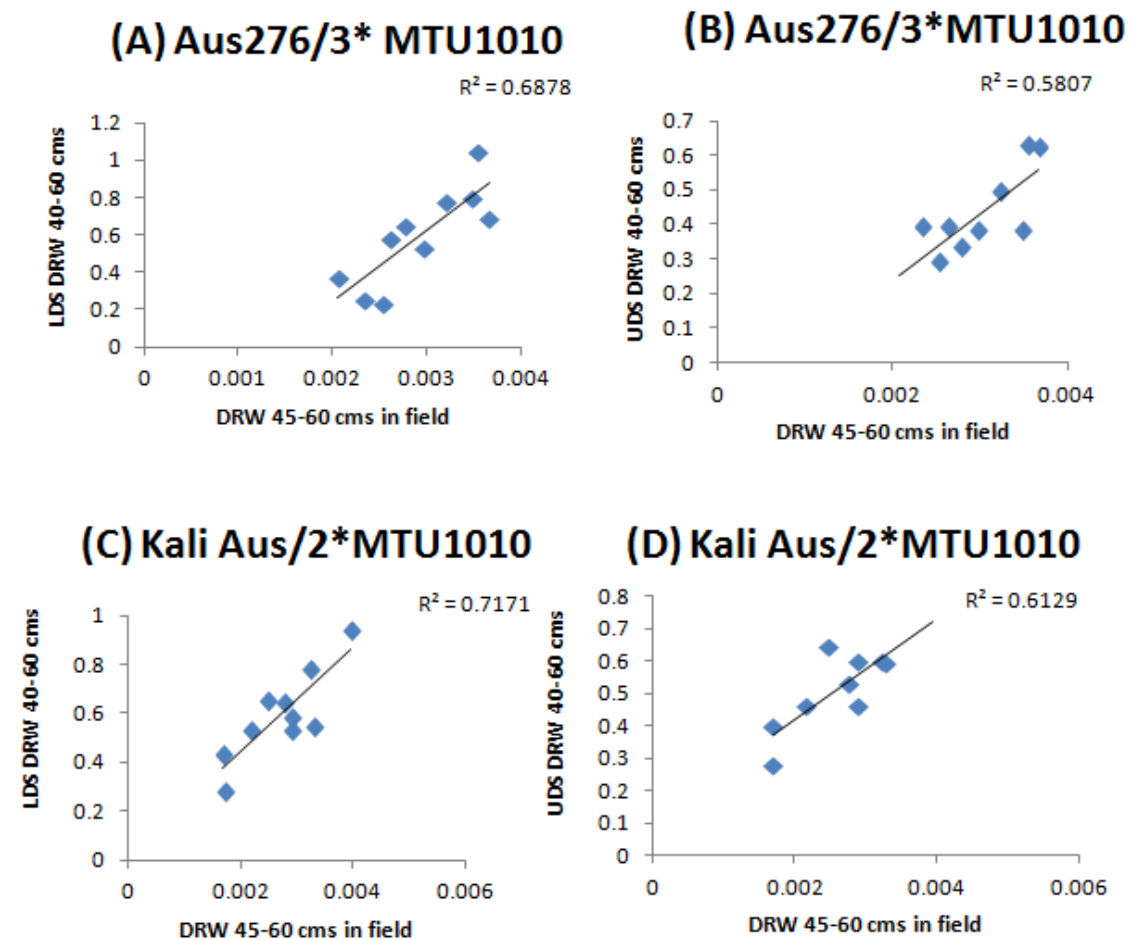


Figure 4.15 Correlation between root growth (dry root weight) at a depth of 45-60 cm in field and 40-60 cm in lysimeter

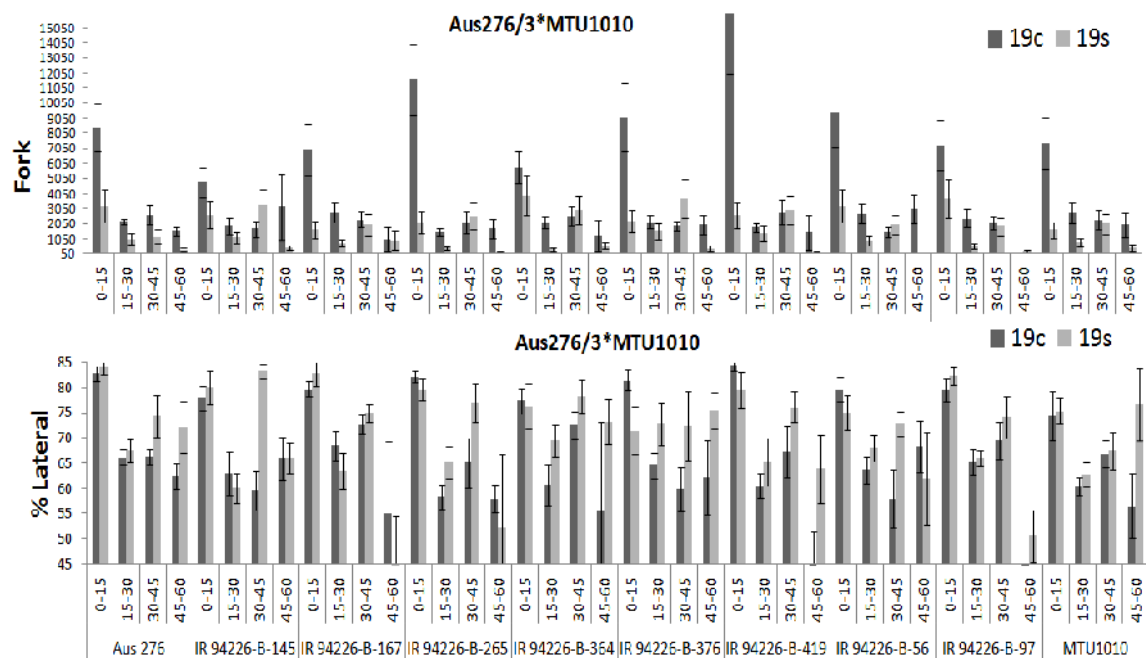


Figure 4.16(A) Plasticity in fork and % lateral root growth in Aus276/3*MTU1010 population at different depth (0-15, 15-30, 30-45 and 45-60 cm)

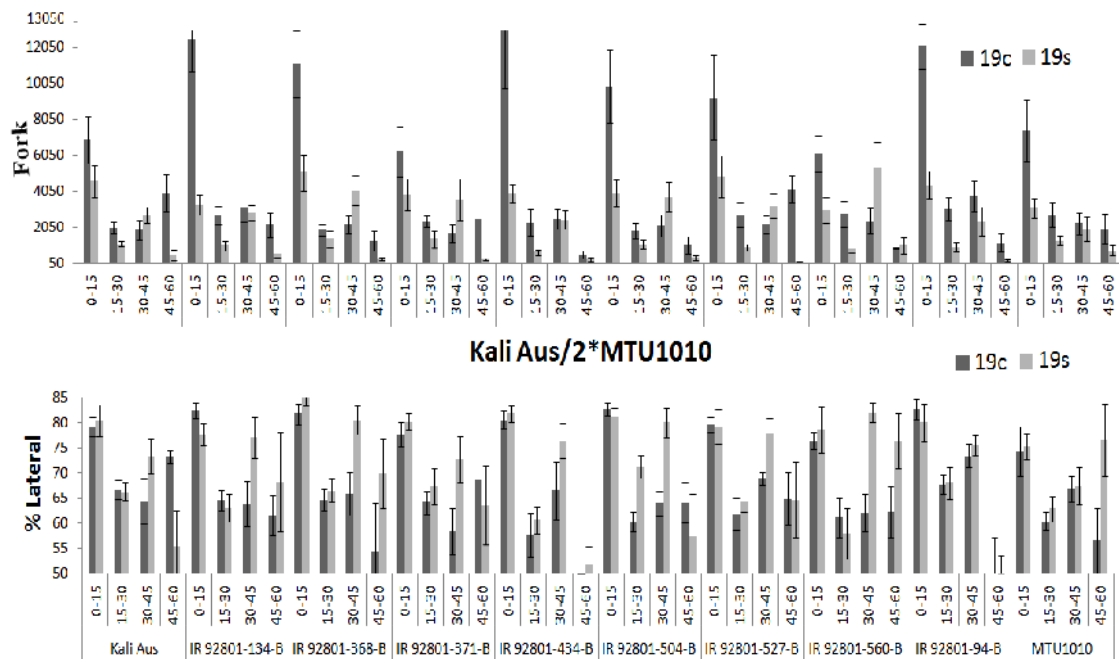
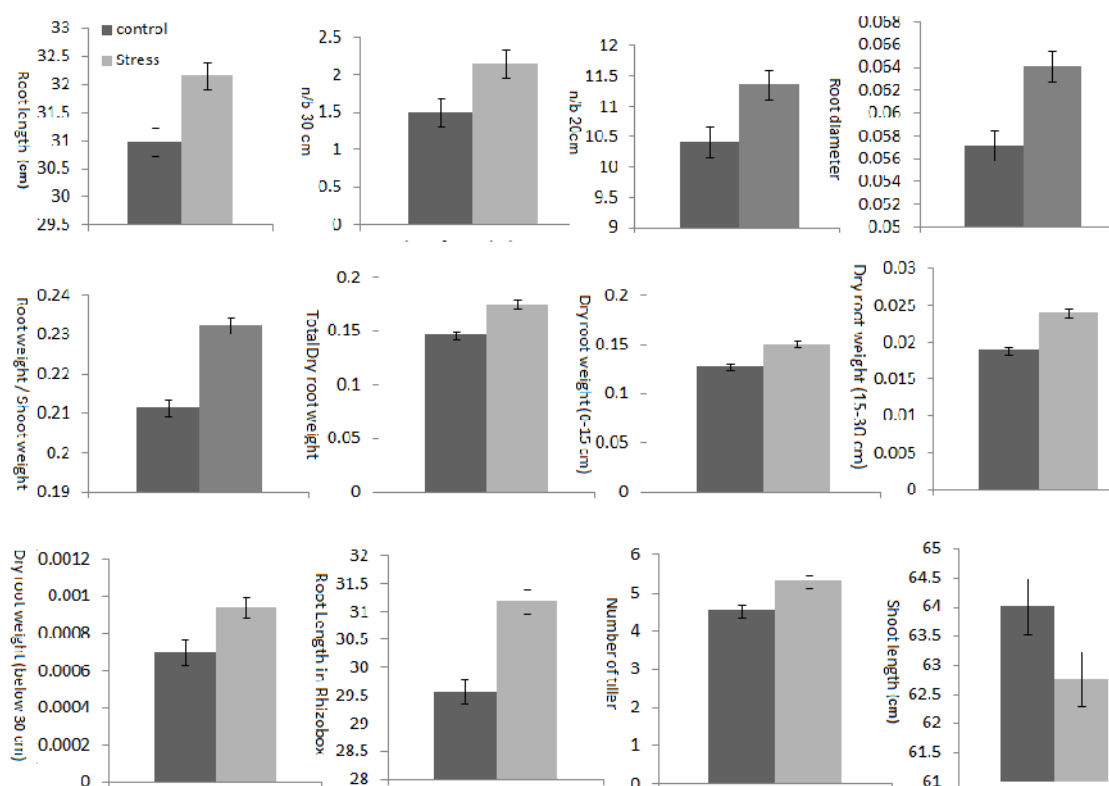


Figure 4.16(B) Plasticity in fork and % lateral root growth in Kali Aus/2*MTU1010 population at different depth (0-15, 15-30, 30-45 and 45-60 cm)

4.2.2 Rhizoscope study

The performance of Kali Aus/2*MTU1010 and Aus276/3*MTU1010 lines were also observed under nutrient deficient conditions (P/8) at CIRAD, France. Rhizoscope is a new high-throughput screening system for phenotyping rice root traits. Results of ANOVA revealed a significant difference among the treatment and the genotypes and treatment had an effect on genotypes for the root morphological and anatomical traits (Table 4.9 and 4.10). Root length were found to be positively and significantly correlated to the dry root weight at different depth, dry shoot weight and number of roots below 20 or 30 cm. Tiller number was reported to be positively and significantly correlated to the number of roots below 20/30 cm, root length and dry root weight at different depth (Table 4.11). Number of metaxylem was reported to be significantly correlated with the root and stele diameter and stele area (Table 4.12). The root length, number of roots below 20/30 cm, root diameter, root/shoot weight and dry root weight at different depth were found to be increased under stress (P/8) conditions as compared to the control (P) conditions (Figure 4.17).



n/b 30 cm: number of roots below 30 cm, n/b 20 cm: number of roots below 20 cm

Figure 4.17 Graphical representation of various root and shoot traits under normal (P) and stress (P/8) conditions

Under the phosphorus deficit conditions the roots were trying to increase the surface area (Plate 4). Anatomical characterization was shown in Plate 5. The root diameter, number of cortical cell layer and metaxylem number were found to be varied among the genotypes under the stress conditions (Plate 5). PCA analysis showed that the root traits like root plasticity, number of metaxylem and number of cortical cell layer were lying towards the positive F_2 axis (Figure 4.18 and 4.19). Therefore the genotypes lying on the positive side of F_2 axis have better root plasticity. On the basis of lysimeter and field study conducted at IRRI and rhizoscope study conducted at CIRAD, France, a total of six genotypes were selected from each population for further evaluations (Table 4.13).

Table 4.9 Mean and variance analysis results of root morphological traits under control and stress (P/8) conditions

	DRW 0-15 cm	DRW 15- 30 cm	DRW b/30 cm	DSW	R/S	RL	n/b 30 cm	n/b 20 cm	TDRW	Root Angle	Root diameter	Tiller number
Control mean	0.1272	0.0186	0.000947	0.7096	0.2165	30.98	1.50	10.42	0.146	77.30	4.537	4.54
Stress mean	0.1504	0.0240	0.00064	0.7750	0.2274	32.15	2.15	11.37	0.175	77.53	5.311	5.31
treatment	11.54**	13.21**	3.26*	2.04	40.56***	11.63**	5.93**	7.24**	12.98**	0.01	15.77***	10.66**
replication	0.07	8.79*	0.39	3.03	10.71	0.13	4.75	3.68	0.61	0.27	3.33	5.31
genotype	1.56	4.93***	3.01**	1.49	7.49***	3.76**	2.77 **	4.80***	1.81	1.06	2.10 *	1.55
treat*geno	1.35	3.75**	2.13*	0.98	3.08***	3.09**	1.24	2.76**	1.48	0.69	2.01	0.64
treat*rep*geno	0.94	2.83***	1.74	0.56	3.46**	2.74***	1.02	1.81**	0.99	0.92	1.03	0.43
Error	0.0024	0.0021	0.00012	0.0949	0.005	5.335	0.125	1.667	0.029	10.02	0.014	0.54
CV	33.18	46.91	162.27	41.52	10.03	7.32	98.74	21.86	33.57	24.76	19.31	32.38

Factors influencing these traits are expressed as F-values

*P<0.05; **P<0.01; ***P<0.001

DRW: dry root weight, DSW: dry shoot weight, R/S: root/shoot dry weight, RL: root length, n/b 30 cm: number of roots below 30 cm, n/b 20 cm: number of roots below 20 cm, TDRW: total dry root weight.



Plate 4 Difference in root architecture under control (P) and stress (P/8) conditions in 2-D “nail board rhizoboxes at CIRAD, France

Plate 5

Anatomical characterization of 2 cm long root sections collected from each plant root apex

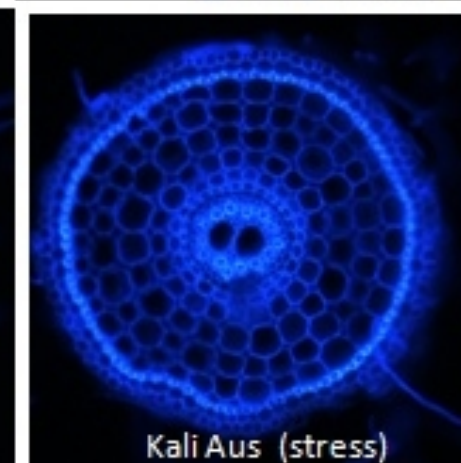
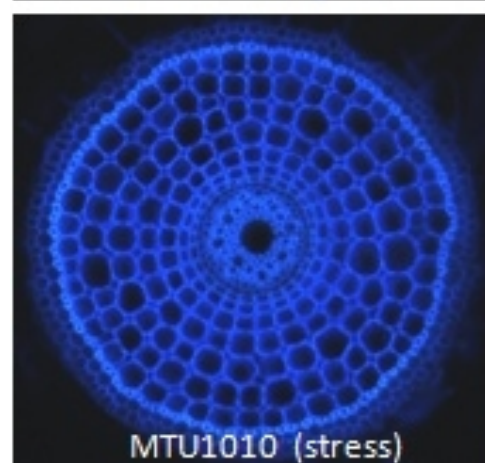
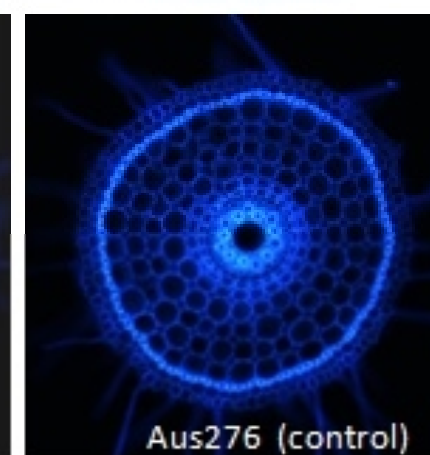
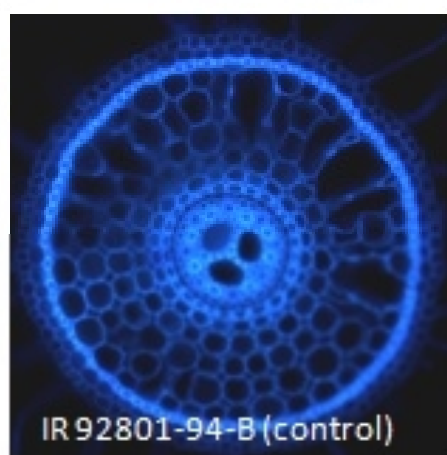
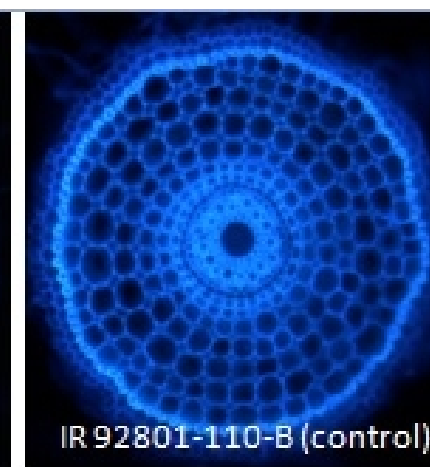
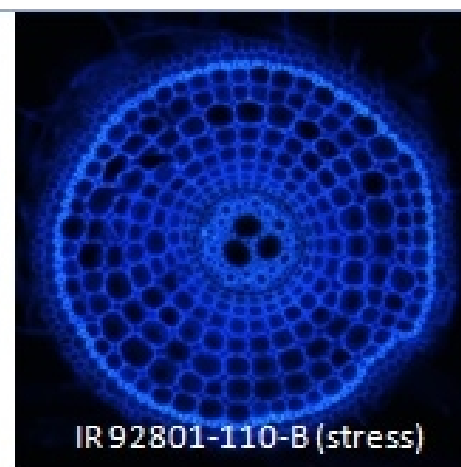
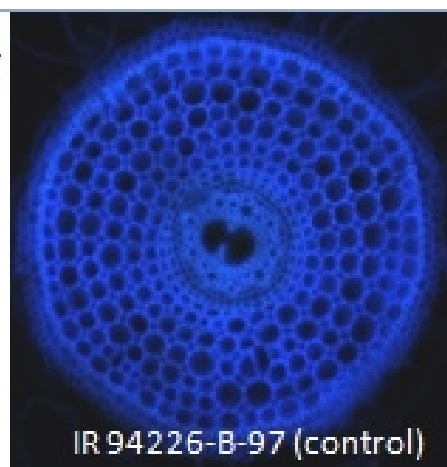
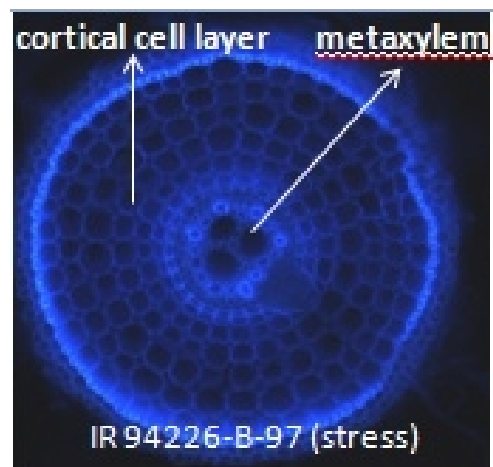


Table 4.10 Mean and variance analysis results of root anatomical traits under control and stress (P/8) conditions

	Cortical Diameter	number of cell layer in cortex	Root area	Root Diameter	Stele Area	Stele diameter	no. of metaxylem
Control mean	907.36	6.395	881490	1057.3	55078	277.44	1.9848
Stress mean	898.82	6.542	835841	1041.1	53127	264.92	1.8106
treatment	0.03	0.16	0.51	0.09	0.08	0.63	0.59
genotype	0.81	1.77	0.99*	0.67	0.43*	2.07*	1.03*
treatment*genotype	0.44	1.23*	0.64*	0.46	0.32	2.22	0.58
Error	34.72	0.1212	45123	37.989	4734.4	11.18	0.1603
CV	25.90	12.66	35.40	24.39	58.95	27.77	56.92

Factors influencing these traits are expressed as F-values

*P<0.05; **P<0.01; ***P<0.001

Table 4.11 Correlation analysis between different morphological traits under stress (P/8) condition

	DRW0-15 cm	DRW15-30 cm	DRW b/30 cm	DSW	R/S	RL	n/b 30 cm	n/b 20 cm	SL	TDRW	Root angle	Root diameter	tiller
DRW0	1												
DRW15	0.4823***	1											
DRW b/30	0.5163***	0.4192**	1										
DSW	0.9191***	0.4581***	0.4633***	1									
R/S	-0.0756	0.4388***	0.0766	-0.325***	1								
RL	0.6805***	0.5078***	0.5732***	0.6511***	-0.0245	1							
n/b 30 cm	0.6718**	0.5190**	0.7287***	0.6363***	0.0464***	0.751***	1						
n/b 20 cm	0.7620***	0.4903***	0.4143***	0.7623***	-0.146**	0.709***	0.6463**	1					
SL	0.5123***	0.2443***	0.2566***	0.6222***	-0.418***	0.444***	0.2989***	0.4878**	1				
TDRW	0.9646***	0.6959**	0.5625**	0.8907***	0.0702	0.714***	0.7131***	0.7718***	0.4931	1			
Root angle	0.2819***	0.0632	0.0894	0.2446**	-0.0422	0.169**	0.1741**	0.2265**	0.1557**	0.249***	1		
Root diameter	0.5333***	0.2996***	0.3734***	0.5361***	-0.0550	0.514***	0.4966***	0.4476***	0.252***	0.529***	0.0676	1	
tiller	0.7645***	0.4058***	0.3646***	0.8140***	-0.238***	0.561***	0.5913**	0.6477***	0.3658**	0.748***	0.1397	0.4845**	1

*P<0.05; **P<0.01; ***P<0.001

DRW: dry root weight, DSW: dry shoot weight, R/S: root/shoot dry weight, RL: root length, n/b 30 cm: number of roots below 30 cm, n/b 20 cm: number of roots below 20 cm, SL: shoot length, TDRW: total dry root weight.

Table 4.12 Correlation analysis between different anatomical traits under stress (P/8) condition

	Cortical Diameter	No. of cell layer in cortex	Root area	Root Diameter	Stele Area	Stele diameter	no. of metaxylem
Cortical Diameter	1						
No. of cell layer in cortex	0.4916***	1					
Root area	0.7853***	0.5742**	1				
Root Diameter	0.8853**	0.6299*	0.9082***	1			
Stele Area	0.6863	0.5499	0.8750**	0.7873***	1		
Stele diameter	0.1309	0.5134**	0.5391**	0.5499***	0.5719***	1	
no. of metaxylem	0.3854*	0.4858***	0.407**	0.4428**	0.5630**	0.3792**	1

*P<0.05; **P<0.01; ***P<0.001

Table 4.13 List of selected genotypes on the basis of comparison of results from Lysimeter, Field and Rhizoscope

Population	Genotype	Yield	Trait
Kali Aus/2*MTU1010	IR 92801-134-B	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 92801-504-B	High	LWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 92801-560-B	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 92801-94-B	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 92801-110-B	low	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 92801-360-B	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
Aus276/3* MTU1010	IR 94226-B-145	low	LWU & High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 94226-B-364	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 94226-B-419	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 94226-B-56	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 94226-B-167	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity
	IR 94226-B-97	High	HWU, High gS and low canopy temp, good correlation in field and lysimeter in root growth and plasticity

HWU: high water uptake, LWU: low water uptake, gS: stomatal conductance,

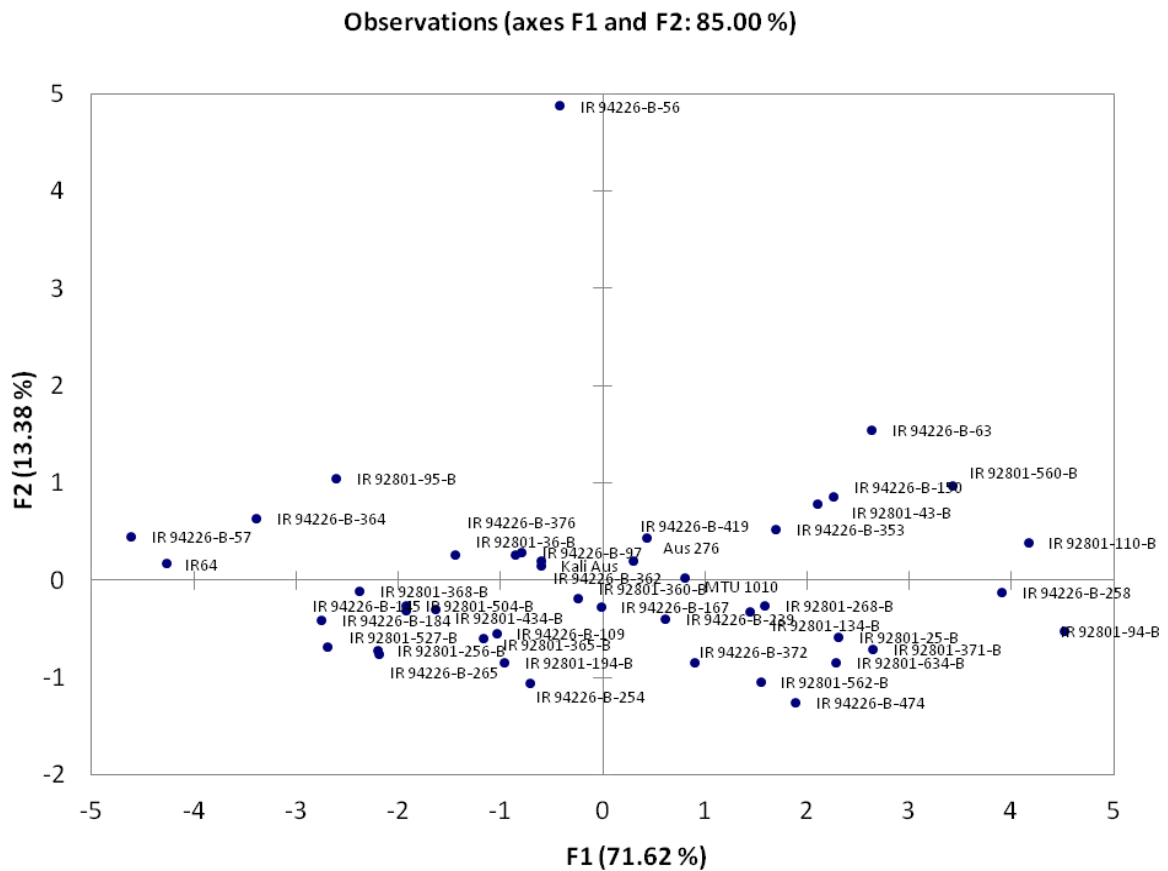
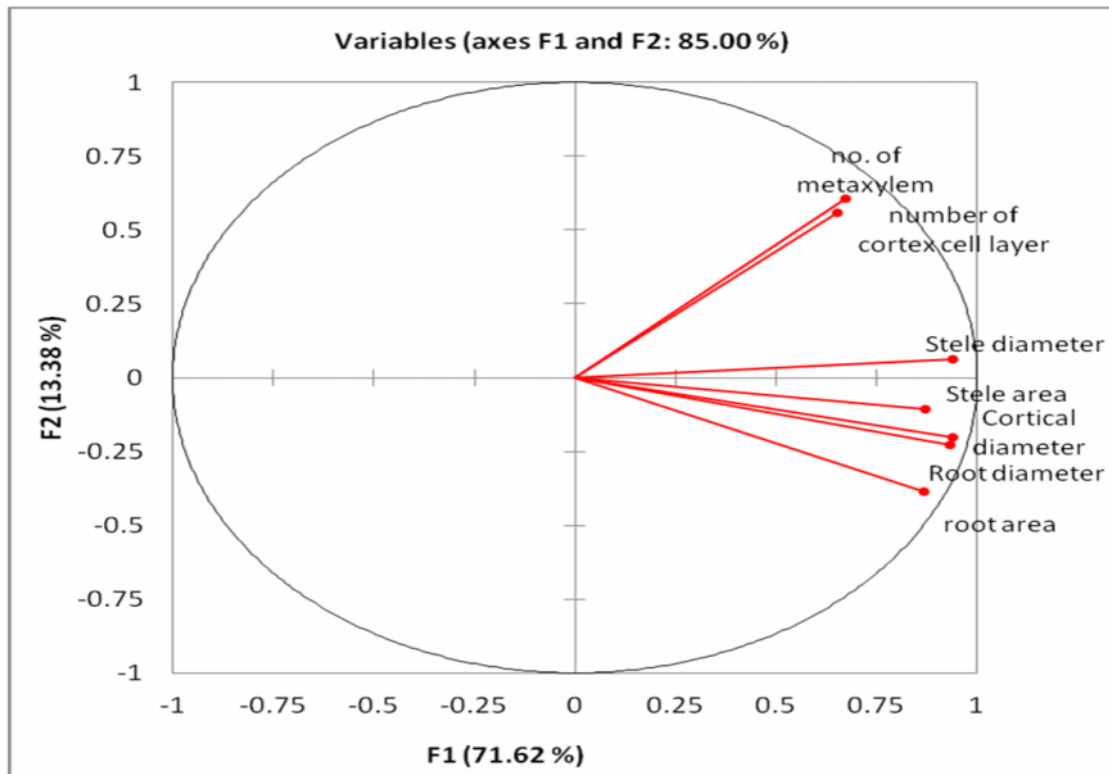


Figure 4.19 PCA for root anatomical traits and genotypes under stress (P/8) condition

4.3 Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

4.3.1 Phenotypic performance of the populations

The mean GY of the population during 2012DS under stress was 664 kg ha⁻¹, whereas GY during 2013DS was 638 kg ha⁻¹, indicating the severity of drought stress in these experiments (Table 4.14). Kali Aus, the parent donor of drought tolerance, consistently outyielded the recipient parent MTU1010 in the 2012 and 2013 RS trials, except for the NS trial under which MTU1010 showed a 20% yield advantage over Kali Aus (Table 4.14). A higher yield reduction of MTU1010 was observed under stress conditions as compared with Kali Aus: it showed a 47% and 42% yield advantage over MTU1010 in the 2012 and 2013 stress trials, respectively (Table 4.14). The best performing line yielded 1654 kg ha⁻¹, outyielding the tolerant parent by 47%, while the least performing line yielded only 118 kg ha⁻¹ (Table 4.14). The Kali Aus/2*IR64 mapping population showed an overall mean GY of 722 kg ha⁻¹ and 582 kg ha⁻¹ during the 2012DS and 2013DS stress trials, respectively. As compared with NS yield, 50% GY reduction was incurred during the 2012DSRS trial, while only 39% GY reduction was observed in the 2013DS RS trial. Under RS condition, Kali Aus consistently outperformed IR64, with a yield advantage of 45% in the 2012DS. Under RS, GY ranged from 123 kg ha⁻¹ to 1890 kg ha⁻¹ during 2012DS and from 156 kg ha⁻¹ to 2863 kg ha⁻¹ during 2013DS. On the other hand, GY ranged from 253 kg ha⁻¹ to 3070 kg ha⁻¹ under NS condition (Table 4.14). Comparing with the mean GY of the NS trial in the 2012 DS (2156 kg ha⁻¹), 70% and 69% GY reduction was noted in 2012DS and 2013DS RS trials, respectively (Table 4.15). Transgressive segregants for GY under RS conditions were also observed (Table 4.16).

The response of genotypes in terms of agronomic parameters differed significantly under both RS and NS conditions. The mean PH of Kali Aus/2*MTU1010 mapping population was 85 cm in 2012DS; in 2013, the mean PH was 61 cm under RS and 93 cm under NS conditions, respectively (Table 4.14). For the Kali Aus/2*IR64 mapping population, the recorded mean PH was 76, 100, and 107 cm during 2012 DS, 2013DS under RS, and 2012DSNS, respectively. DTF was significantly affected by drought stress as reflected by a flowering delay of as many as 22 and 18 days (2012DS) and 28 and 21 days (2013DS) for Kali Aus/2*MTU1010 and Kali Aus/2*IR64 populations, respectively.

4.3.1.1 Correlation and broad-sense heritability of traits under drought-stress condition

For the Kali Aus/2*MTU1010 population, GY was negatively correlated to DTF in both RS years (2012DS and 2013DS) and NS condition (Table 4.17). Similarly, a negative correlation between GY and DTF in all treatments for the Kali Aus/2*IR64 population was observed (Table 4.17). PH was positively correlated to GY in both RS and NS conditions in the Kali Aus/2*IR64 population and Kali Aus/2*MTU1010 population (Table 4.17).

Heritability of GY for Kali Aus/2*MTU1010 population was moderate (0.47) in the 2013DSRS trial, while it measured 0.34 and 0.45 during RS and NS conditions of 2012DS, respectively (Table 4.18).

Table 4.14 Descriptive trait statistics for parents (Kali Aus, IR64, and MTU1010) and mapping populations subjected to stress and non-stress conditions

Population	Trait		Stress, 2012	Stress, 2013	Nonstress, 2012
Kali Aus/2*MTU1010	GY	Kali Aus	874	727	2658
		MTU1010	465	419	3119
		Population mean	664	638	2156
		Highest line	1654	2650	3385
		Lowest line	118	159	166
		LSD _{0.05}	472	381	1193
	PH	Kali Aus	74	55	91
		MTU1010	80	62	89
		Population mean	85	61	93
		Highest line	111	82	128
		Lowest line	62	43	70
		LSD _{0.05}	23	13.4	22
	DTF	Kali Aus	84	85	74
		MTU1010	92	95	78
		Population mean	86	102	77
		Highest line	110	121	88
		Lowest line	77	82	69
		LSD _{0.05}	9	15.6	5
Kali Aus/2*IR64	GY	Kali Aus	910	759	2731
		IR64	495	335	2432
		Population mean	722	582	2454
		Highest line	1890	2063	3070
		Lowest line	123	188	253
		LSD _{0.05}	557	312	1117
	PHT	Kali Aus	68	101	90
		IR64	61	85	84
		Population mean	76	100	107
		Highest line	106	87	122
		Lowest line	50	36	73
		LSD _{0.05}	22.5	9.4	24
	DTF	Kali Aus	87	85	76
		IR64	92	93	80
		Population mean	79	67	79
		Highest line	104	116	95
		Lowest line	80	75	68
		LSD _{0.05}	11.2	15.9	8

GY: grain yield (Kg h⁻¹), PH: plant height (cm), DTF: days to flowering (d).

Table 4.15 Comparison of overall mean values of grain yield, plant height, and days to flowering under stress (2012DS and 2013DS) and nonstress (2012DS) conditions

Water condition	Grain yield (kg ha ⁻¹)	RYR (%)	Plant height (cm)	HR (cm)	DTF	FD
Kali Aus/2*MTU1010						
2012DSNS	2156	-	93	-	77	-
2013DSRS	638	70	61	34	102	25
2012DSRS	664	69	85	9	86	9
Kali Aus/2*IR64						
2012DSNS	1454	-	107	-	79	-
2013DSRS	582	60	100	7	67	-12
2012DSRS	722	50	76	29	79	0

RYR: relative yield reduction, HR: height reduction, DTF: days to flowering, FD: days of flowering delayed

Table 4.16 Values of mean yield, mean DTF, and mean PH of selected promising genotypes for the two populations

Population	Year	MTU1010	Kali Aus/2* MTU1010	IR64	Kali Aus/2*IR64
Promising lines selected (no.)			10		10
GY (kg ha ⁻¹)	2012DSRS	465	925	495	947
	2013DSRS	419	1084	335	1023
	2012DSNS	3119	3230	2432	2315
	2013DSNS	2733	3009	2178	2418
Best line	2012DSRS	465	1654	495	1890
	2013DSRS	419	2650	355	2063
	2012DSNS	3115	3385	2432	3070
	2013DSNS	2733	4237	2178	3268
PH	2012DSRS	80	90	61	78
	2013DSRS	62	68	85	87
	2012DSNS	89	101	84	90
	2013DSNS	92	96	90	95
Best line	2012DSRS	80	109	61	92
	2013DSRS	62	78	85	86
	2012DSNS	89	112	84	110
	2013DSNS	92	107	90	103
DTF	2012DSRS	92	83	92	88
	2013DSRS	95	94	93	86
	2012DSNS	78	75	80	77
	2013DSNS	82	80	85	83
Best line	2012DSRS	92	79	92	82
	2013DSRS	95	91	93	78
	2012DSNS	78	69	80	67
	2013DSNS	82	72	85	74

GY: grain yield (Kg ha⁻¹), PH: plant height (cm), DTF: days to flowering (d). Best line – line combining high yield potential and good yield under drought.

Table 4.17 Correlation analysis between grain yield and agronomic traits of mapping populations derived from Kali Aus/2*IR64 under drought stress and nonstress conditions

Population	Grain yield	Days to flowering	Plant height
Kali Aus/2*MTU1010	2012DSRS	-0.40**	0.37*
	2013DSRS	-0.25*	0.68**
	2012DSNS	-0.59**	0.51**
Kali Aus/2*IR64	2012DSRS	-0.50**	0.16
	2013DSRS	-0.37*	0.54**
	2012DSNS	-0.22	0.62**

*Significance at 5%; **significance at 1%.

Table 4.18 Broad-sense heritability (*H*) of grain yield (GY), days to flowering (DTF), and plant height (PH) under stress, combined stress, and nonstress conditions for Kali Aus/2*IR64 and Kali Aus/2*MTU1010 mapping populations

Population	Traits	<i>H</i>			
		2012DSRS	2013DSRS	Combined stress	2012DSNS
Kali Aus/2*MTU1010	DTF	0.52	0.36	0.44	0.58
	PH	0.24	0.59	0.42	0.32
	GY	0.34	0.47	0.39	0.45
Kali Aus/2*IR64	DTF	0.22	0.50	0.36	0.61
	PH	0.12	0.39	0.26	0.18
	GY	0.24	0.17	0.21	0.51

For the Kali Aus/2*IR64 population, *H* for GY under drought stress was fairly low, measuring only 0.24 and 0.17 during 2012DS and 2013DS, respectively, but relatively high (0.51%) under NS condition (2012DS; Table 4.18).

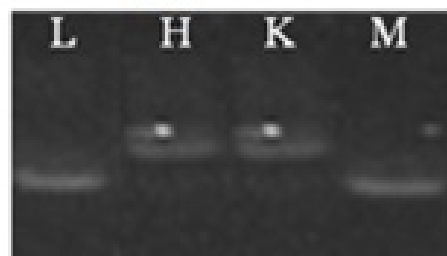
4.3.2 Bulk segregant analysis

In the Kali Aus/2*MTU1010 mapping population, seven out of the 109 markers used were found significant in BSA. These markers were RM259 (29.18 cM) and RM315 (143.9 cM) on chromosome 1, RM211 (7.92 cM) and RM263 (101.4 cM) on chromosome 2, RM471 (73.8 cM) on chromosome 4, RM253 (21.27 cM) on chromosome 6, and RM234 (99.9 cM) on chromosome 7. These markers showed high-bulk bands and low-bulk bands similar to those of Kali Aus (tolerant, donor) and MTU1010 (sensitive, recipient), respectively (Plate 6). In the Kali Aus/2*IR64 mapping population, eight markers showed differential banding patterns in high and low bulks, which corresponded to those of the tolerant (Kali Aus) and sensitive (IR64) parents (Plate 6). The markers identified were RM259 on chromosome 1, RM573 and RM341 on chromosome 2, RM545 on chromosome 3, RM274 on chromosome 5, RM434 and RM105 on chromosome 9, and RM28089 on chromosome 12.

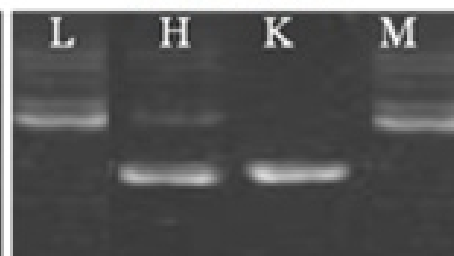
Plate 6

Simple Sequence Repeat (SSR) markers showing significant banding patterns between tail bulks (High – H, and Low – L), parents (Kali Aus – K, MTU1010 – M), (A-G) and (K - Kali Aus/donor parent; I – IR64/sensitive parent) (H-O)

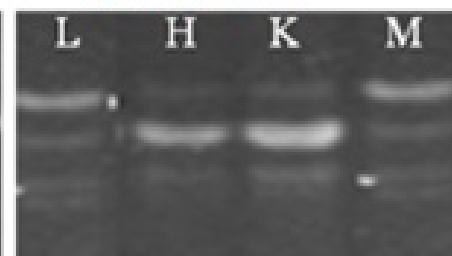
(A) RM259



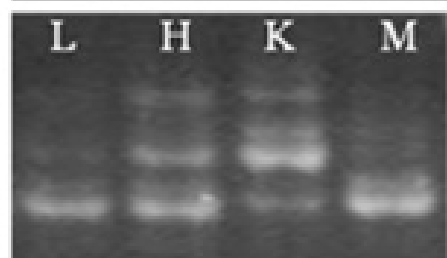
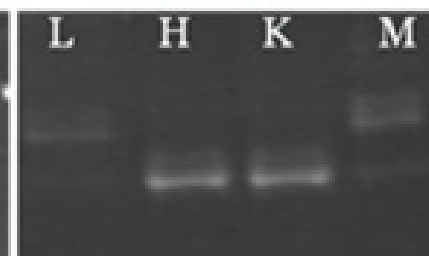
(B) RM315



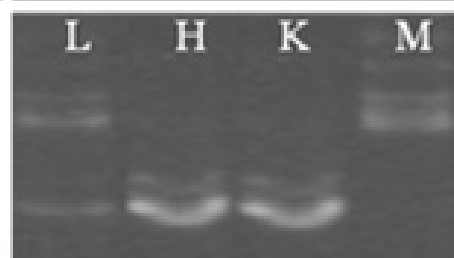
(C) RM211



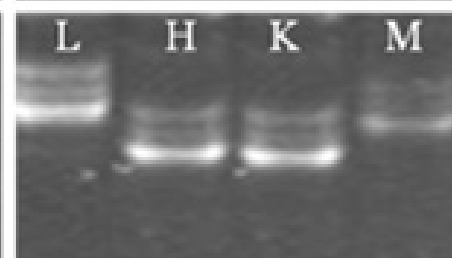
(D) RM263



(E) RM471



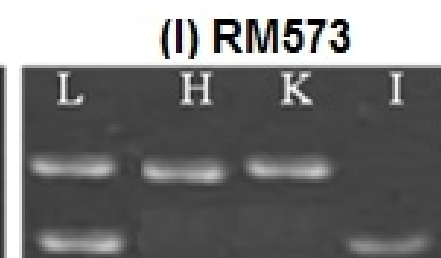
(F) RM253



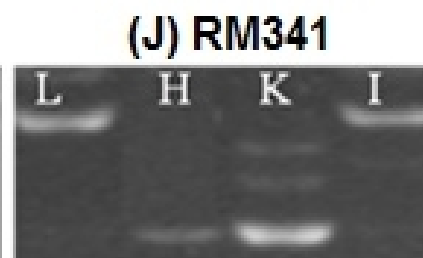
(G) RM234



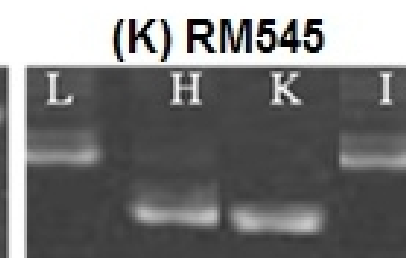
(H) RM259



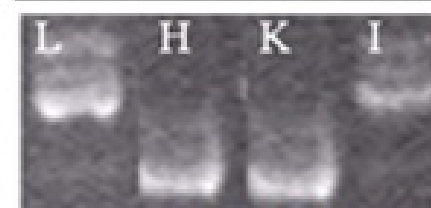
(I) RM573



(J) RM341



(K) RM545



(L) RM274



(M) RM434



(N) RM105



(O) RM28089

4.3.3. QTL analysis

4.3.3.1. GY QTL in the Kali Aus/2*MTU1010 population

QTL with consistent effect for GY were mapped using composite interval mapping (CIM) located on chromosomes 1 and 2. These QTL for GY labeled as qDTY_{1.1} and qDTY_{2.2} are positioned at 50.2 and 72.9 cM flanked by markers RM259–RM315 and RM211–RM263, respectively (Figure 4.20, Table 4.19).

Table 4.19 QTL identified under upland drought-stress conditions in two populations

Trait	QTL	Year	Flanking markers	Position (cM)	AE (%)	F value	R ² (%)
Kali Aus/2*MTU1010							
GY	qDTY _{1.1}	2012DSRS	<u>RM259</u> –RM315	49.2	17	9.0	6.3%
		2013DSRS		50.2	19	8.2	4.5%
		Combined stress		50.2	19	11.6	7.1%
	qDTY _{2.2}	2012DSRS	<u>RM211</u> –RM263	70.2	21	9.3	6.2%
		2013DSRS		72.9	30	10.9	6.0%
		Combined stress		70.9	24	8.6	5.8%
DTF	qDTF _{2.2}	2012DSRS	<u>RM211</u> –RM263	67.9	-3.8	8.4	4.5%
		2013DSRS		73.9	-4.0	7.7	4.0%
		Combined stress		72.9	-3.7	12.3	7.8%
PH	qPH _{1.1}	2012DSRS	RM315– <u>RM431</u>	144.0	2.0	7.5	4.6%
		2013DSRS		145.0	4.2	16.8	8.3%
		Combined stress		144.0	2.5	17.1	9.1%
Kali Aus/2*IR64							
GY	qDTY _{1.1}	2012DSRS	RM488– <u>RM315</u>	109.2	14	9.0	6.5%
		2013DSRS		108.2	18	7.0	7.7%
		Combined stress		110.2	15	8.9	5.3%
	qDTY _{2.3}	2012DSRS	RM263– <u>RM573</u>	104.2	10	7.7	5.0%
		2013DSRS		107.4	11	5.2	4.8%
		Combined stress		106.4	11	9.7	7.4%
		2012DSNS	RM263– <u>RM573</u>	110.4	8.3	8.0	4.3%
		2013DSNS		112.6	12	5.4	4.0%
		Combined non-stress		108.8	7	7.8	6.3%
		Epistatic QTL	2012DSRS	RM315– <u>RM431</u>	144.0, 53.6	-35	3.8, 4.4
	2013DSRS		<u>RM324</u> –RM263	144.0, 60.6	-46	4.0, 4.8	6.2%
DTF	Epistatic QTL	2012DSRS	RM154– <u>RM324</u>	24.3, 101.4	1.28	5.7, 4.3	6.7%
		2013DSRS	RM263– <u>RM573</u>	14.3, 101.4	2.64	4.4, 3.9	5.4%
		2012DSNS	<u>RM259</u> –RM306	32.2, 128.2	2.02	4.4, 3.3	7.4%
		2013DSNS	RM450– <u>RM250</u>	30.2, 124.4	2.21	4.0, 5.7	6.5%

DTF: days to flowering; PH: plant height; GY: grain yield; AE: additive effect, additive effect depicted as % of mean trial. Underline markers are the markers closest to peak.

The additive effect of qDTY_{1.1} on grain yield represented 17 and 19% of the trial mean for 2012DS and 2013DS, respectively. The qDTY_{2.2} exerted a positive additive effect of 21 and 30% of the trial mean for 2012DS and 2013DS, respectively under RS. The combined analysis showed a higher additive effect (19 and 24% of the trial mean) compared with both RS years, indicating a valid consistency for effect of QTL on GY under RS. The increase in GY for RS can be attributed to the Kali Aus allele, the tolerant parent, as reflected by the GY improvement of lines with QTL in contrast to lines without the QTL. The consistent effect of QTL for GY under the respective RS years and in combined RS over 2 years is supported by the significant F static values ($P < 0.01$). At qDTY_{1.1}, Kali Aus homozygotes significantly outyielded the MTU1010 homozygotes under RS condition for RM315 and RM259, individually and combined, with 22, 15, and 32%, respectively, in 2012DS and 15, 12, and 33% GY advantage in 2013DS (Table 4.20). Similarly, Kali Aus homozygotes for qDTY_{2.2} significantly outyielded the MTU1010 homozygotes under RS condition for RM211 and RM263, individually and combined, with 14, 9, and 20%, respectively, in 2012 DS and 13, 15, and 20% GY advantage in 2013DS. qDTY_{1.1} and qDTY_{2.2}, combined, exhibited a GY improvement of 26 and 33% in 2012DS and 2013DS, respectively (Table 4.20).

4.3.3.2. GY QTL in the Kali Aus/2*IR64 mapping population

Significant and consistent-effect QTL qDTY_{1.1} and qDTY_{2.3} for GY were identified at chromosomes 1 and 2 flanked by markers RM488–RM315 and RM263–RM573 positioned at 109.4 cM and 104.4 cM (Table 4.19, Figure 4.21) with additive effect of 14 and 10% of trial mean under RS condition in 2012 DS, respectively; 18 and 11% under RS condition, respectively, in 2013DS; and 15 and 11% of trial mean in combined stress, respectively. qDTY_{1.1} contributed 16, 12, and 19% increase in GY under 2012DSRS condition; 18, 22, and 27% under 2013DSRS condition, for RM315 and RM488, individually and combined, respectively, which can be attributed to the Kali Aus allele, the drought-tolerant parent (Table 4.20). For qDTY_{2.3}, the homozygotes for RM573 and RM263 exhibited a GY improvement of 15, 6, and 18% for 2012DSRS and 24, 28, and 35% under 2013DSRS condition, individually and combined, respectively. qDTY_{1.1} and qDTY_{2.3}, combined, exhibited a GY improvement of 19 and 31% in 2012DSRS and 2013DSRS, respectively (Table 4.20).

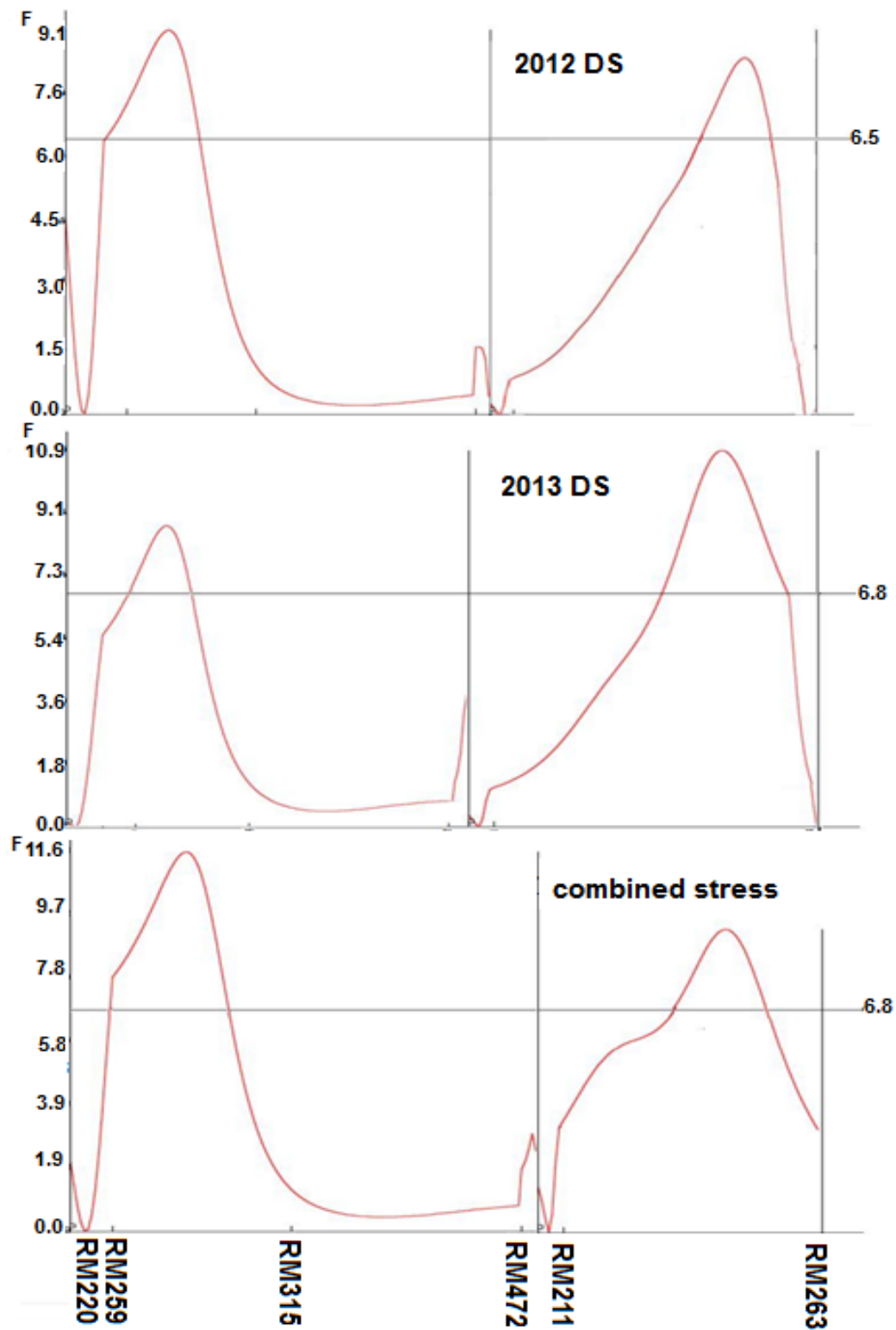


Figure 4.20 *F*-static curve indicating consistent QTL for grain yield (qDTY_{1.1} and qDTY_{2.2}) of Kali Aus/2*MTU1010 mapping population under stress 2012, stress 2013, and combined stress conditions located on chromosome 1 and 2. Genetic distance (cM) between two markers is exhibited on X-axis while horizontal line corresponds for critical *F*-value ($P < 0.01$)

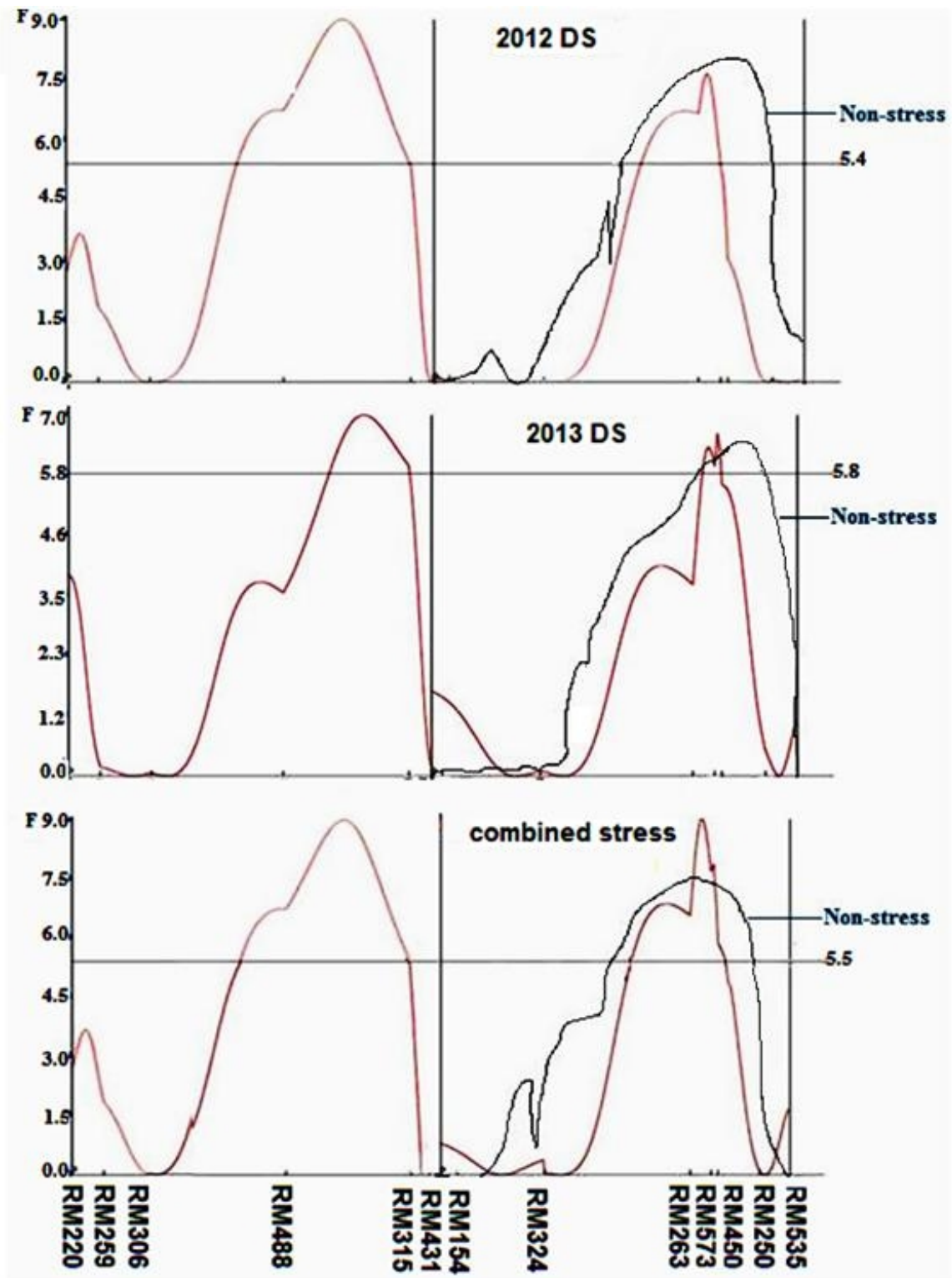


Figure 4.21: *F*-static curve indicating consistent QTL for grain yield ($qDTY_{1.1}$ and $qDTY_{2.3}$) of Kali Aus/2*IR64 mapping population under 2012DSRS, 2013DSRS, and combined stress conditions (Red line) and under 2012DSNS, 2013DSNS, and combined non-stress conditions (Black line) located on chromosome 1 and 2. Genetic distance (cM) between two markers is exhibited on X-axis while horizontal line corresponds for critical *F*-value ($P < 0.01$)

Table 4.20 Yield improvement of genotypes possessing the donor alleles (+) at the QTL for grain yield under drought stress over lines not possessing the donor alleles (-) for the two populations

Kali Aus/2*MTU1010						
QTL	Trial	Markers	QTL (+)	QTL (-)	MTU1010/IR64	Yield improvement (%)
qDTY _{1.1}	2012DSRS	RM315	568	441	465	22
		RM259	535	458	465	15
		RM315 + RM259	614	348	465	32
	2013DSRS	RM315	483	406	419	15
		RM259	468	438	419	12
		RM315 + RM259	555	392	419	33
qDTY _{2.2}	2012DSRS	RM211	528	428	465	14
		RM263	505	440	465	9
		RM211 + RM263	559	351	465	20
	2013DSRS	RM211	473	451	419	13
		RM263	482	446	419	15
		RM211 + RM263	504	419	419	20
qDTY _{1.1} + qDTY _{2.2}	2012DSRS	RM315 + RM259 + RM211+ RM263	586	350	465	26
qDTY _{1.1} + qDTY _{2.2}	2013DSRS	RM315 + RM259 + RM211 + RM263	557	406	419	33
Kali Aus/2*IR64						
qDTY _{1.1}	2012DSRS	RM315	576	377	495	16
		RM488	554	418	495	12
		RM315 + RM488	590	332	495	19
	2013DSRS	RM315	395	350	335	18
		RM488	409	343	335	22
		RM315 + RM488	426	366	335	27
qDTY _{2.3}	2012DSRS	RM573	570	418	495	15
		RM263	526	440	495	6
		RM573 + RM263	586	320	495	18
	2013DSRS	RM573	418	362	335	24
		RM263	429	357	335	28
		RM573 + RM263	452	322	335	35
qDTY _{1.1} + qDTY _{2.3}	2012DSRS	RM315 + RM488 + RM573 + RM263	588	326	495	19
qDTY _{1.1} + qDTY _{2.3}	2013DSRS	RM315 + RM488 + RM573 + RM263	439	344	335	31

4.3.3.3. QTL for DTF and PH

A significant QTL, qDTF_{2.2}, for DTF was mapped in Kali Aus/2*MTU1010 mapping population flanked by markers RM211–RM263 positioned at 67.9 cM in 2012DSRS and at 73.9 cM in 2013DSRS. The QTL for DTF co-located with qDTY_{2.2}.

A QTL for PH was mapped in Kali Aus/2*MTU1010 under both environments in both seasons. It is located near marker RM315 and flanked by markers RM315–RM431 on chromosome 1 (Table 4.19).

In addition to the major-effect, single-locus QTL (qDTY_{1.1} and qDTY_{2.3}) for GY in the Kali Aus/2*IR64 mapping population, epistatic interaction was observed for GY on chromosome 1 at marker interval RM315–RM431 (qDTY_{1.1}) and chromosome 2 at RM324–RM263 (qDTY_{2.1}; identified previously by Venuprasad *et al.*, 2009) in 2012DSRS and 2013DSRS with an additive effect of 35 and 46% of the population mean. Between the detected major loci epistatic interaction for DTF between genomic locations on chromosome 2 at marker intervals RM154–RM324 (qDTY_{2.1}; identified previously by Venuprasad *et al.*, 2009) and RM263–RM573 (qDTY_{2.3}) positioned at 4.3 cM and 101.4 cM, respectively, in 2012DS, 2013DSRS and on chromosome 1 at marker intervals RM259–RM306 and RM450–RM250 (qDTY_{2.3}) positioned at 32.2 cM and 128.2 cM, respectively, in 2012DS, and 2013DSRS were identified (Table 4.19).

4.4 Experiment 4: Mikhudeb/MTU1010, Aus276/3*MTU1010 and Aus276/3*IR64

4.4.1. Mikhudeb/MTU1010

Mikhudeb, drought tolerant donor was crossed with MTU1010, drought susceptible parent. The seeds obtained from the cross Mikhudeb x MTU1010 was germinated under upland dry direct seeded and lowland conditions both. The germination % was 2% and 4% respectively. This was not enough to carry out the QTL study under wide range of environment and conditions. Therefore, it could not be included for the further studies.

4.4.2. Aus276/3*MTU1010 and Aus276/3*IR64

4.4.2. 1. Identification of QTL for seedling-stage and agronomic traits for direct-seeded rice

A wide range of phenotypic responses was observed among the BC₂F₄ lines in the seedling-stage stress trials for DSR traits and in the reproductive-stage stress trials for agronomic traits (Table 4.21 and 4.22). A total of 26 major QTL with consistent effects associated with 21 traits were mapped using composite interval mapping (CIM) on chromosomes 1, 4, 5, 6, 8, 9 and 10 in the Aus276/3*IR64 population (Figure 4.22, Table 4.23), and 21 QTL associated with 13 traits with were mapped on chromosomes 1, 2, 4, 6, 8, 9, 10 and 11 in the Aus276/3*MTU1010 population (Figure 4.23, Table 4.24). Most QTL identified in both populations were contributed by Aus276. The consistent effect of

QTL under different cultivation conditions over 2 years is supported by the significant F static values ($P \leq 0.01$).

For agronomic traits, grain yield QTL $qGY_{10.1}$, $qGY_{6.1}$ and $qGY_{1.1}$ were identified to be stable in both populations over both years and in multiple conditions (Figure 4.22 and 4.23; Tables 4.23 and 4.24). In most cases, the increase in GY can be attributed to the donor parent Aus276 (Tables 4.23 and 4.24). However, in some cases a negative value of additive effect for the grain yield QTL can be attributed to the recipient parent (MTU1010/IR64), as reflected by the GY improvement in lines with and without the grain yield QTL (Tables 4.25 and 4.26). For days to flowering, $qDTF_{6.1}$ and $qDTF_{10.1}$ were identified, of which $qDTF_{10.1}$ co-located with $qGY_{10.1}$ in both years in multiple conditions in the Aus276/3*IR64 population (Table 4.23).

Table 4.21 Descriptive trait statistics for parents (Aus276, MTU1010 and IR64) and mapping populations subjected to seedling stage stress under direct seeded upland conditions

Trait	Population	2012WSSS	2013DSSS
Seed weight	Aus276	2.579± 0.26	2.72± 0.21
	MTU1010	1.849± 0.18	1.43± 0.32
	Population mean	2.034± 0.015	2.11± 0.009
	Aus276	2.34± 0.24	2.12±0.25
	IR64	1.98± 0.13	1.66±0.14
	Population mean	2.07± 0.014	2.05±0.014
Dry shoot weight (15 DAS)	Aus276	0.058± 0.019	0.146± 0.039
	MTU1010	0.062± 0.012	0.150± 0.091
	Population mean	0.061± 0.001	0.139± 0.002
	Aus276	0.071 ± 0.025	0.17±0.049
	IR64	0.067± 0.021	0.14±0.09
	Population mean	0.076±0.0014	0.15± 0.003
Dry shoot weight (22 DAS)	Aus276	0.182± 0.025	0.272±0.032
	MTU1010	0.188± 0.063	0.230±0.020
	Population mean	0.198± 0.004	0.234±0.004
	Aus276	0.17±0.069	0.33±0.08
	IR64	0.19± 0.072	0.28± 0.02
	Population mean	0.20± 0.004	0.27±0.005
Dry shoot weight (30 DAS)	Aus276	0.414± 0.17	0.465± 0.10
	MTU1010	0.355± 0.11	0.375± 0.14
	Population mean	0.401± 0.01	0.380±0.006
	Aus276	0.48± 0.018	0.49± 0.016
	IR64	0.44± 0.019	0.45± 0.016
	Population mean	0.51± 0.012	0.46± 0.009
First Emergence (Days to first emergence)	Aus276	-	3.0±0.41

	MTU1010	-	4.0± 0.56
	Population mean	-	3.6± 0.023
	Aus276	-	3.0±0.49
	IR64	-	4.1± 0.21
	Population mean	-	3.3± 0.03
Full emergence (Days to full emergence)	Aus276	-	7.0±0.80
	MTU1010	-	8.0±0.12
	Population mean	-	7.4±0.046
	Aus276	-	6.9±1.15
	IR64	-	7.8±1.01
	Population mean	-	6.5±0.07
RGR ₁₂	Aus276	0.167±0.05	0.0874±0.079
	MTU1010	0.152±0.01	0.0645± 0.038
	Population mean	0.196±0.003	0.0724± 0.002
	Aus276	0.126±0.35	0.096± 0.04
	IR64	0.151± 0.22	0.097± 0.01
	Population mean	0.166 ± 0.02	0.081± 0.002
RGR ₂₃	Aus276	0.116± 0.053	0.0763± 0.042
	MTU1010	0.092±0.034	0.0706± 0.019
	Population mean	0.137±0.003	0.0721± 0.002
	Aus276	0.178±0.50	0.058± 0.05
	IR64	0.160±0.44	0.068± 0.04
	Population mean	0.209± 0.03	0.073±0.003
RGR ₁₃	Aus276	0.283± 0.063	0.164± 0.048
	MTU1010	0.241± 0.033	0.135± 0.080
	Population mean	0.260± 0.004	0.144± 0.003
	Aus276	0.279±0.07	0.154± 0.050
	IR64	0.220±0.04	0.166± 0.011
	Population mean	0.246±0.004	0.175± 0.003
Nodal root number (15 DAS)	Aus276	10.4±1.16	7.8±2.29
	MTU1010	11.9±1.12	9.2±1.73
	Population mean	10.7±0.182	8.4±0.132
	Aus276	14.0±2.43	9.6±2.05
	IR64	8.0±2.31	9.0±1.11
	Population mean	10.4±0.14	10.52±0.12
Nodal root number (22 DAS)	Aus276	13.4±2.73	10.1±1.45
	MTU1010	10.1±1.18	9.3±1.60
	Population mean	12.6± 0.158	12.8±0.084
	Aus276	20.4± 3.01	13.0± 4.7
	IR64	13.0 ± 2.9	16.3± 2.22
	Population mean	13.1±0.17	18.4±0.27
Nodal root number (30 DAS)	Aus276	17.2±2.01	15±2.83
	MTU1010	15.4±1.19	17.6±1.28

	Population mean	23.9± 0.231	22.7±0.221
	Aus276	25.6± 3.97	22.0± 2.14
	IR64	20.7± 0.23	23.3± 4.9
	Population mean	18.0± 2.81	28.3± 0.29
Root hair length	Aus276	1.408± 0.32	1.467±0.43
	MTU1010	1.161± 0.30	1.133±0.76
	Population mean	1.243± 0.018	1.349±0.025
	Aus276	1.54±0.32	1.30± 0.35
	IR64	1.02±0.24	1.11± 0.11
	Population mean	1.30±0.019	1.27± 0.02
Root hair density	Aus276	1.446± 0.24	1.43±0.43
	MTU1010	1.322± 0.11	1.267±0.48
	Population mean	1.157± 0.014	1.32±0.025
	Aus276	1.36±0.29	1.39± 0.30
	IR64	1.09±0.11	1.16± 0.14
	Population mean	1.24±0.017	1.24± 0.018
Lateral root rating	Aus276	-	2.5±0.14
	MTU1010	-	1.8±0.33
	Population mean	-	2.2±0.021
	Aus276	-	2.1±0.22
	IR64	-	1.7±0.81
	Population mean		2.6±0.047
Biomass	Aus276	55.34±9.09	45.56±3.97
	MTU1010	59.23±13.06	36.64±5.73
	Population mean	54.67±1.33	31.29±4.85
	Aus276	45.41±12.43	43.08± 9.56
	IR64	54.57± 8.26	26.88± 4.11
	Population mean	61.69±1.41	37.81± 5.46
Harvest index	Aus276	0.290± 0.064	0.170±0.072
	MTU1010	0.262± 0.021	0.139±0.016
	Population mean	0.274± 0.004	0.137±0.004
	Aus276	0.243±0.061	0.186±0.07
	IR64	0.296±0.065	0.102±0.02
	Population mean	0.232± 0.004	0.128±0.004
Grain yield	Aus276	561±88.83	680±198.25
	MTU1010	472±59.68	545±187.75
	Population mean	523± 92.58	770±201.23
	Aus276	861±100.01	962±211.02
	IR64	684±101.12	867± 119.7
	Population mean	766±80.59	886±121.74

Table 4.22 Descriptive trait statistics for parents (Aus276, IR64, and MTU1010) and mapping populations subjected to reproductive stress and non-stress treatments in lowland and direct-seeded upland conditions

Population	Trait		2012UDSRS	2013UDSRS	2012UDSNS	2012LDSRS	2012LDSNS
Aus276/MTU1010	GY	Aus276	1201	1098	2942	1305	3622
		MTU1010	643	522	3388	1172	3432
		Population mean	889	770	1662	1550	4244
		Highest line	1679	1267	3900	2846	6848
		Lowest line	129	122	265	588	1848
		LSD _{0.05}	504	683	1420	894	1542
	PH	Aus276	92	69	95	83	96
		MTU1010	73	66	102	86	100
		Population mean	77	64	103	106	122
		Highest line	112	84	134	136	154
		Lowest line	48	41	70	70	81
		LSD _{0.05}	22	17	26	21	22
	DTF	Aus276	75	93	78	80	80
		MTU1010	85	91	71	82	81
		Population mean	92	98	80	83	78
		Highest line	109	112	92	87	84
		Lowest line	72	80	67	71	72
		LSD _{0.05}	12	12	9	17	4
	EVV	Aus276	-	3.6	-	-	-
		MTU1010	-	4.7	-	-	-
		Population mean	-	4.9	-	-	-
		Highest line	-	2.4	-	-	-
		Lowest line	-	8.6	-	-	-
		LSD _{0.05}	-	2.4	-	-	-
	HI	Aus276	-	0.0098	-	-	-
		MTU1010	-	0.0069	-	-	-
		Population mean	-	0.0051	-	-	-
		Highest line	-	0.0302	-	-	-
		Lowest line	-	0.0014	-	-	-
		LSD _{0.05}	-	0.0107	-	-	-
Aus276/IR64	GY	Aus276	1696	961	2557	3346	4085
		IR64	593	492	1663	2368	3211
		Population mean	1073	717	1167	2563	4586
		Highest line	2152	1470	2770	5992	7378
		Lowest line	258	118	175	733	2182
		LSD _{0.05}	377	591	1173	1813	1636
	PHT	Aus276	87	70	96	104	98
		IR64	59	55	84	73	104
		Population mean	75	65	106	100	135
		Highest line	112	83	136	126	166
		Lowest line	50	42	70	65	74
		LSD _{0.05}	21	17	26	17	23
	DTF	Aus276	81	88	75	81	80
		IR64	95	98	85	84	80
		Population mean	93	96	83	82	77

		Highest line	108	111	113	97	82
		Lowest line	72	88	71	71	68
		LSD _{0.05}	11	10	9	11	4
	EVV	Aus276	-	4.2	-	-	-
		MTU1010	-	5.6	-	-	-
		Population mean	-	5.0	-	-	-
		Highest line	-	1.5	-	-	-
		Lowest line	-	8.9	-	-	-
		LSD _{0.05}	-	2.6	-	-	-
	HI	Aus276	-	0.0076	-	-	-
		MTU1010	-	0.0030	-	-	-
		Population mean	-	0.0048	-	-	-
		Highest line	-	0.0220	-	-	-
		Lowest line	-	0.0010	-	-	-
		LSD _{0.05}	-	0.013	-	-	-

Table 4.23 QTL identified under different cultivation conditions in the Aus276/3*IR64 population

Trait	Chr	Year	Flanking markers	Marker closest to Peak	Position	Additive effect	Additive effect %	R ² (%)
GY	qGY _{6.1}	2012UDSRS	id6010515-id6015531	id6015531	107.5	-15.44	18.61%	10.1%
		2013UDSRS			104.5	-22.78	35.77%	11.2%
	qGY _{8.1}	2012UDSRS	id8003773-ud8001270	id8003773	56.3	-71.82	36.58%	10.4%
		2013UDSRS				-52.33	26.62%	8.2%
	qGY _{9.2}	2012UDSRS	id9004128-id9005890	id9005890	58.0	96.85	31.29%	7.4%
		2013UDSRS				42.39	44.44%	8.4%
	qGY _{9.1}	2012WSSSS	id9003600-id9004128	id9003600	49.6	30.09	15.71%	6.1%
		2013DSSSS				10.77	32.86%	6.1%
	qGY _{10.1}	2012UDSNS	id10005369-id10006378	id10006378	52.3	164.63	14.25%	7.0%
		2013UDSNS			58.7	183.31	8.0%	4.3%
		2012LDSNS			57.7	-289.61	6.28%	8.1%
		2012LDSRS			58.7	-361.22	13.32%	12.6%
	qGY _{1.1}	2013UDSNS	K_id1024836-id1026726	id1026726	162.3	-542.09	23.65%	7.6%
		2012LDSNS				-526.39	11.42%	8.1%
DTF	qDTF _{6.1}	2012LDSNS	ud6000218-id6004089	id6004089	26.4	-1.72	2.21%	12.6%
		2012LDSRS				-2.78	3.29%	12.2%
	qDTF _{10.1}	2012UDSNS	id10003870-id10005369	id10005369	45.7	-1.47	1.77%	7.6%
		2013UDSNS				-1.50	1.83%	8.5%
		2012LDSNS			49.7	-1.65	2.12%	14.4%
		2012LDSRS				-1.83	2.17%	9.5%
	qDTF _{4.1}	2013UDSNS	id4001205-id4002844	id4002844	13.2	-3.47	5.7%	4.9%
PHT	qPHT _{1.1}	2012UDSNS	K_id1024836-id1026726	id1026726	162.3	-6.51	6.06%	8.4%
		2013UDSNS				-4.28	3.8%	12.0%
		2012UDSNS	K_id1021259-id1024167	id1024167	144.2	-4.22	3.68%	12.2%
		2013UDSNS				-4.70	4.18%	14.9%
		2012LDSNS				-3.62	2.62%	11.5%
		2012LDSRS				-4.87	4.92%	17.2%
		2012UDSRS	K_id1021259-id1024167	id1024167	142.2	-3.07	4.12%	7.7%
		2012UDSRS				-3.07	4.12%	7.7%

	qPHT _{4.1}	2013UDSRS			142.2	-2.91	4.54%	9.6%
		2013UDSNS	id4002844-id4004639	id4004639	20.2	1.95	1.73%	6.2%
		2013UDSRS	id4011542-id4012189	id4011542	121.6	-1.87	2.92%	6.8%
	qPHT _{10.1}	2012LDSNS	id10005369-id10006378	id10005369	52.3	-8.24	5.97%	12.8%
		2012LDSRS				-4.73	4.78%	12.5%
SW	qSW _{5.1}	2012UDSRS	id5004150-id5006615	id5006615	52.6	-0.11	5.4%	7.3%
		2013UDSRS				-0.12	5.6%	7.1%
NR	qNR _{4.1}	2012WSSSS	id4001205-id4002844	id4001205	14.2	1.10	5.23%	7.1%
		2013DSSSS			13.2	1.28	5.41%	6.6%
EVV Epistatic QTL	qEVV _{9.2}	2013UDSRS	K_id1021259-id1024167	id1024167	144.2	0.54	10.32%	7.7%
	qEVV _{9.1}	2012WSSSS	id9003600-id9004128	id9003600	49.6	0.025	10.57%	5.8%
		2013DSSSS				0.034	21.93%	6.5%
	1 9	2013DSSSS	K_id1024836-id1026726, id9003600- id9004128	id1026726, id9003600	158.3, 49.6	0.032	20.62%	6.3%
RHL	qRHL _{8.1}	2012WSSSS	id8002306-id8003773	id8003773	46.2	-0.086	6.43%	7.1%
		2013DSSSS			43.3	-0.097	7.43%	6.8%
RHD	qRHD _{8.1}	2012WSSSS	id8000536-id8000845	id8000845	15.4	0.0617	5.1%	6.6%
		2013DSSSS				0.0627	5.2%	6.5%
	qRHD _{5.1}	2012WSSSS	id5004150-id5006615	id5006615	51.6	0.0885	7.3%	6.3%
		2013DSSSS				0.0984	8.1	6.6%
HI	qHI _{6.1}	2012WSSSS	ud6000218-id6004089	id6004089	30.4	0.024	11.60%	8.9%
		2013DSSSS			27.7	0.025	13.39%	6.2%
	qHI _{10.1}	2013UDSNS	id10005369-id10006378	id10005369	56.3	0.018	8.6%	7.3%
		2012WSSSS	id10006378-id10006890	id10006890	76.8	0.019	9.23%	7.7%
		2013DSSSS			71.3	0.027	17.87%	6.1%
Biomass	qBio _{6.1}	2012WSSSS	id6006288-id6010515	id6010515	59.6	-11.12	15.92%	8.1%
		2013DSSSS			61.6	-18.88	19.91%	4.6%
	qBio _{10.1}	2012WSSSS	id10003870-id10005369	id10005369	45.7	-6.58	10.37%	6.3%
		2013DSSSS			47.7	-8.78	9.14%	7.8%
GY Epistatic QTL	6 8	2012UDSRS	id6010515-id601553 id8003773-ud8001270	id6015531 id8003773	107.5 56.3	52.98	43.86%	4.6%
	6 9		id6010515-id6015531 id9004128-id9005890	id6015531 id9005890	107.5 58.0	-70.46	48.87%	4.2%
	6	2013UDSRS	id6010515-id601553	id6015531	104.5	44.32	47.23%	5.1%

	8		id8003773-ud8001270	id8003773	56.3			
	6		id6010515-id6015531	id6015531	104.5	-39.55	44.14%	6.5%
	9		id9004128-id9005890	id9005890	58.0			
	4	2012WSSSS	id4008719-id4009390	id4008719	84.0	-2.89	14.69%	7.0%
	8		id8002306-id8003773	id8003773	52.2			
Seedling emergence	8	2013DSSSS	id8000845-id8001326	id8001326	25.3	-0.325	4.35%	7.2%
Epistatic QTL	9		id9006773-id9007315	id9007315	86.1			
RHD	5	2013DSSSS	id5004150-id5006615	id5006615	51.6	0.177	14.74%	9.4%
Epistatic QTL	7		id7005557-ud7002101	ud7002101	112.6			
DTFNS ,	4	2013UDSNS	id4004639-id4005212	id4004639	39.0	-1.20	1.45%	7.3%
Epistatic QTL	7		id7005557ud7002101	ud7002101	108.6			
Al conc	qAl _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-602.57	54.43%	3.0%
Cu conc	qCu _{8,1}	2013DSSSS	id8000845-id8001326	id8000845	29.3	0.5114	10.36%	11.7%
Fe conc	qFe _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-643.67	38.26%	13.3%
	Epistatic 4 & 6	2013DSSSS	id4005570-id400683	id4005570	51.0	-326.03	70.03%	11.7%
			id6006288-id6010515	id6010515	68.6			
N conc	qN _{5,1}	2013DSSSS	id5004150-id5006615	id5006615	51.6	-0.1180	12.46%	20.7%
Mn conc	Epistatic 4 & 6	2013DSSSS	id4005570-id4006835	id4005570	51.0	-8.6689	18.49%	11.9%
			id6006288-id6010515	id6010515	69.6			
P conc	qP _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-0.0615	23.3%	12.3%
Al uptake	qAl _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-1622.17	43.76%	11.5%
Fe Uptake	qFe _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-4481.39	78.49%	11.7%
	Epistatic 4 & 6	2013DSSSS	id4005570-id400683	id4005570	51.0	-8371.9	64.64%	11.7%
			id6006288-id6010515	id6010515	68.6			
Mn uptake	Epistatic 4 & 6	2013DSSSS	id4005570-id4006835	id4005570	51.0	-265.76	20.96%	16.9%
			id6006288-id6010515	id6010515	69.6			
P uptake	qP _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-3.0610	42.51%	12.3%
Zn uptake	qZn _{4,1}	2013DSSSS	id4002844-id4004639	id4004639	20.2	113.96	15.77%	12.0%
Nematode GR	qGR _{5,2}	2013DSSSS	id5006615-id5007304	id5006615	63.3	-0.9310	28.8%	13.7%

GY: Grain Yield, DTF: Days to flowering, PHT: Plant height, SB: Shoot biomass, SW: Seed weight, NR: Nodal root number, RHL: Root hair length, RHD: Root hair density, EVV: Early vegetative vigor, HI: Harvest index, Al: Aluminum, Cu: Copper, Fe: Iron, N: Nitrogen, Mn: Manganese, P: Phosphorus, Zn: Zinc, GR: Gall rating, conc: Concentration

Table 4.24 QTL identified under different cultivation conditions in the Aus276/3*MTU1010 population

Trait	Chr	Year	Flanking markers	Marker closest to Peak	Position	Additive effect	Additive effect %	R ²
GY	qGY _{1.2}	2012UDSNS	id1000001-K_id1000955	id1000001	0.1	-231.40	11.7%	6.9%
		2013UDSNS				-234.77	8.3%	7.1%
		2012UDSRS				-44.09	20.6%	7.3%
		2013UDSRS				-15.41	21.8%	6.8%
	qGY _{1.1}	2012LDSNS	K_id1024973-id1026726	id1026726	167.7	-307.35	7.1%	9.3%
	qGY _{6.2}	2012UDSRS	id6006288-id6013772	id6006288	60.6	-58.30	26.8%	7.9%
		2013UDSRS				-48.82	20.2%	6.2%
	qGY _{6.1}	2012WSSSS	id6013772-id6015665	id6015665	105.1	-7.41	14.0 %	7.2%
		2013DSSSS				-8.89	17.1%	6.8%
		2012LDSRS	id6015665-ud6001296		108.3	142.99	8.7%	6.3%
	qGY _{10.1}	2012UDSRS	id10005474-id10006378	id10006378	61.2	-64.15	30.0%	8.9%
		2013UDSRS			60.0	-32.33	43.7%	10.0%
	qGY _{11.1}	2012LDSRS	id11001466-id11002436	id11002436	24.3	225.54	13.8%	8.0%
	qGY _{11.2}	2012UDSNS	id11009201-id11010385	id11010385	108.1	-61.63	8.7%	7.3%
		2013UDSNS				-37.39	6.8%	7.6%
DTF	qDTF _{1.1}	2012UDSNS	K_id1021259-id1024167	id1024167	145.2	4.4252	5.9%	6.9%
		2013UDSNS	id1024167-K_id1024973		149.8	3.3731	4.5%	6.6%
		2012LDSNS	K_id1021259-id1024167		143.2	0.7457	9.6%	7.5%
PHT	qPHT _{1.1}	2012UDSNS	id1024167-K_id1024973	id1024167	151.8	-4.0507	3.9%	8.2%
		2013UDSNS				-4.6728	4.1%	8.9%
		2012UDSRS				-2.5295	3.8%	6.6%
		2013UDSRS				-3.7866	5.9%	9.4%
		2012LDSNS				-6.3468	5.2%	9.9%
		2013LDSRS				-4.5307	4.3%	8.3%
	qPHT _{1.2}	2013LDSRS	id1005271-id1006691	id100527	42.5	-7.4538	7.0%	8.2%

NR	qNR _{5.1}	2012WSSSS	id5000759-id5001182	id5001182	10.3	0.9077	5.2%	6.7%
		2013DSSSS			12.3	0.9391	6.1%	6.6%
EVV	qEVV _{9.1}	2012WSSSS	ud9000737-id9002704	ud9000737	49.4	0.0189	8.9%	6.4%
		2013DSSSS				0.0136	9.3%	6.8%
RHL	qRHL _{1.1}	2012WSSSS	id1000001-K_id1000955	id1000001	0.1	0.0813	6.5%	6.6%
		2013DSSSS				0.0945	7.4%	6.9%
RHD	qRHD _{1.1}	2012WSSSS	id1005271-id1006691	id1005271	44.5	0.1194	9.8%	6.3%
		2013DSSSS				0.1331	8.2%	6.7%
Seedling emergence	qDTE _{9.1}	2013DSSSS	ud9000737-id9002704	ud9000737	40.4	0.1578	4.2%	6.6%
Biomass	qBio _{11.1}	2012WSSSS	id11001466-id11002436	id11002436	25.3	-11.30	20.7%	7.9%
		2013DSSSS				-10.55	18.2%	7.3%
HI	qHI _{11.1}	2013UDSNS	id11001466-id11002436	id11002436	28.1	-6.13	10.2%	10.0%
	qHI _{11.2}	2013UDSRS	id6006288- id6013772	id6013772	85.5	-9.22	13.3%	8.2%
P conc (veg stage)	qPC _{6.1}	2012WSSSS	id6006288-id6013772	id6013772	95.6	-0.2125	17.4%	14.4%
		2013DSSSS			88.6	-0.3157	27.5%	13.5%
P uptake (veg stage)	qPU _{4.1}	2012WSSSS	id4008522-id4009900	id4008522	80.9	-0.4256	14.1%	16.1%
		2013DSSSS			81.9	-0.4211	13.8%	16.4%
Epistatic qtl	qPU _{1.1}	2012WSSSS	id1005271-id1006691	id1005271	45.5	-1.1654	38.6%	16.7%
	qPU _{6.1}	2013DSSSS	id6006288-id6013772	id6006288	59.6	-1.1597	38.1%	17.4%
DTF (epistatic qtl)	qDTF _{4.1}	2012UDSRS	id4006835-id4008522	id4006835	66.6	2.0344	2.2%	5.7%
	qDTF _{6.1}		id6013772-id6015665	id6013772	104.1			3.0%
Dry shoot weight (epistatic qtl)	qDSW _{2.1}	2012WSSSS	id2007378-id2007507	id2007507	72.6	0.0172	28.1%	10.8%
	qDSW _{10.1}		id10006378-id10006740	id10006740	67.8			

GY: Grain Yield, DTF: Days to flowering, PHT: Plant height, HI: Harvest index, SW: Seed weight, NR: Nodal root number, RHL: Root hair length, RHD: Root hair density, EVV: Early vegetative vigor, P: Phosphorus, Veg stage: Vegetative stage

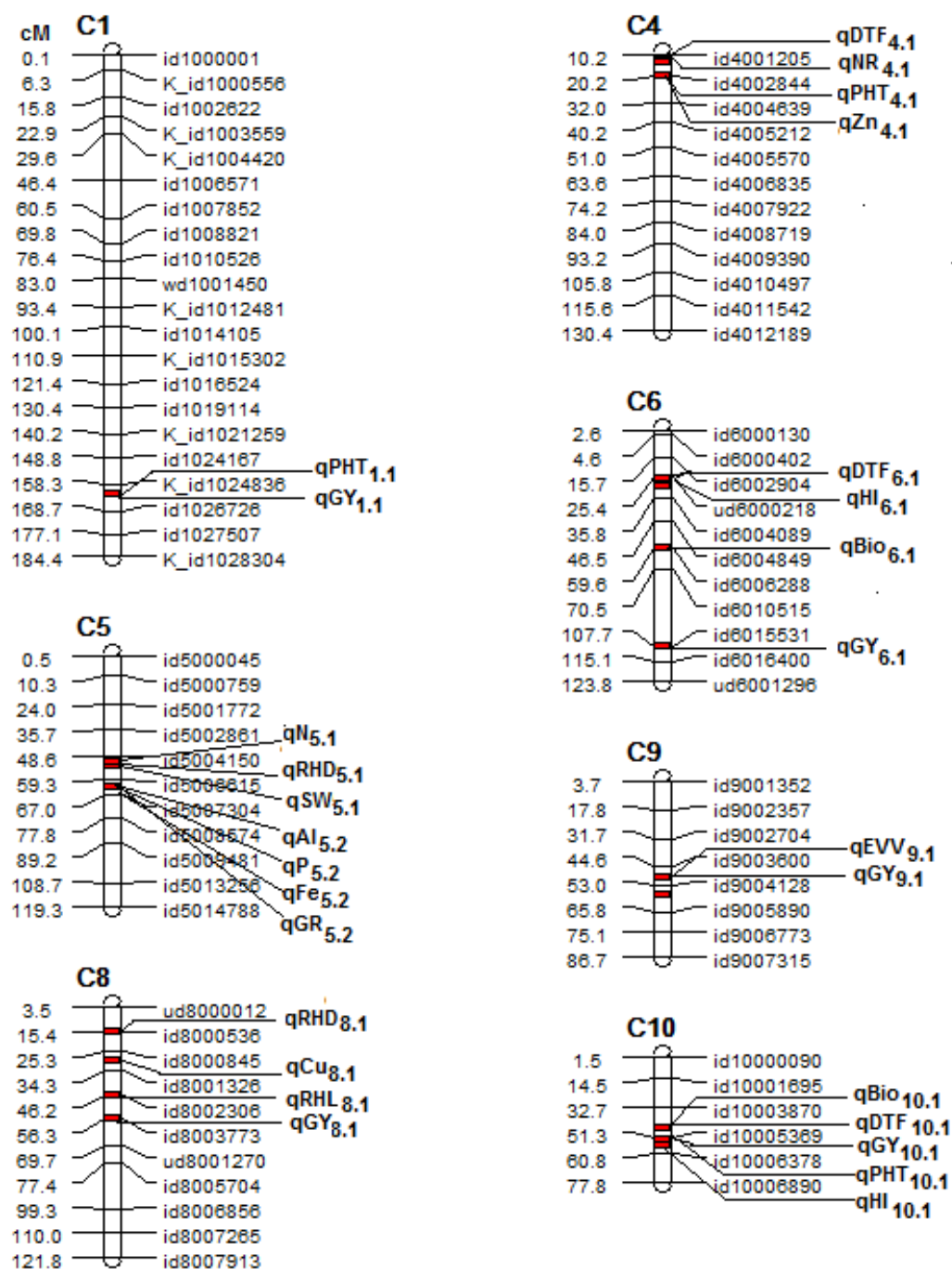


Figure 4.22 Chromosome map of QTL detected across 16 studies under seedling stage stress, reproductive stage stress, and well-watered conditions in lowland and direct-seeded upland trials in the Aus276/3*IR64 population

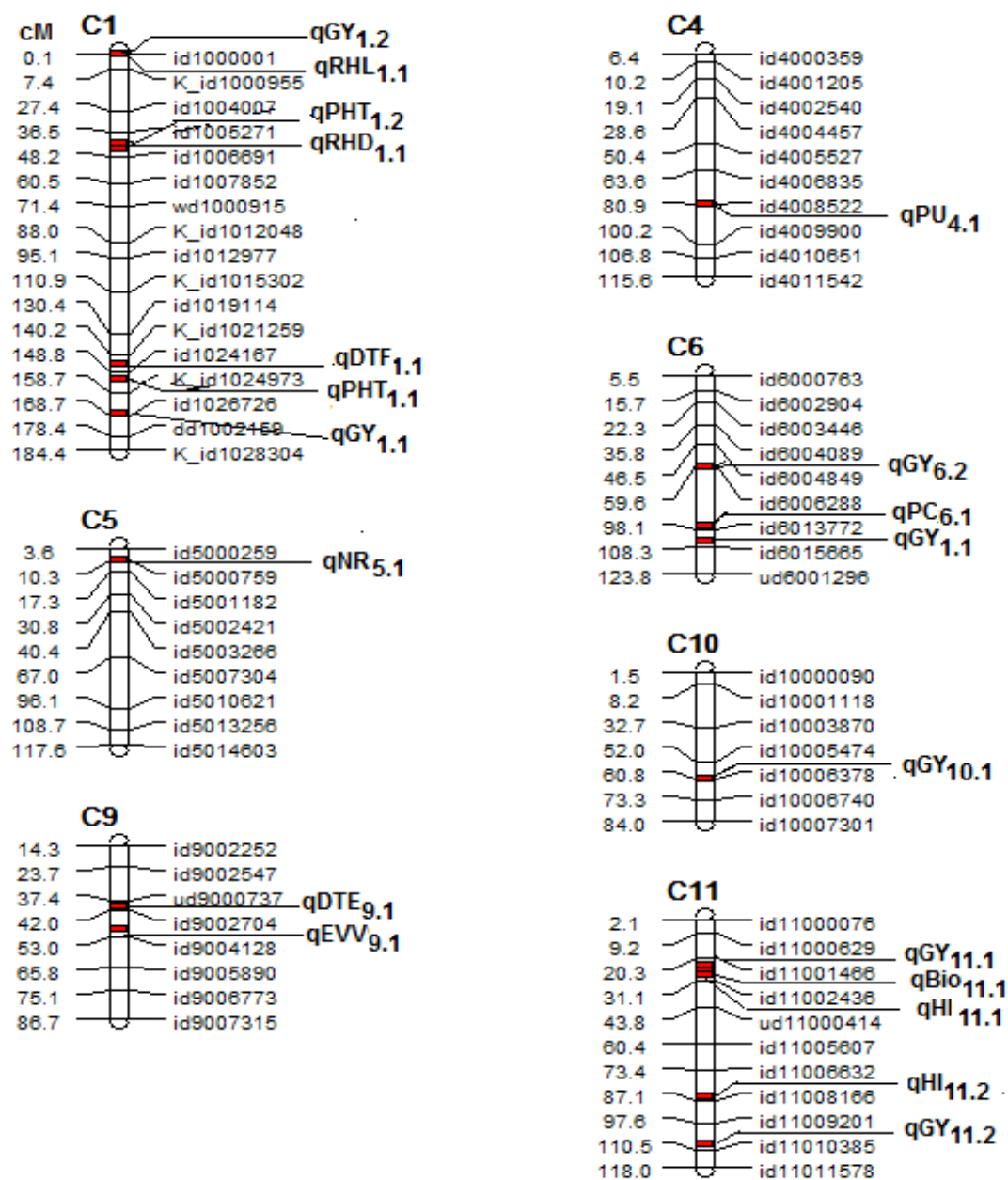


Figure 4.23 Chromosome map of QTL detected across 16 studies under seedling stage stress, reproductive stage stress, and well-watered conditions in lowland and direct-seeded upland trials in the Aus276/3*MTU1010 population

Table 4.25 Percentage improvement of genotypes possessing QTL (QTL +) under different situation over lines not possessing QTL (QTL -) for the Aus276/3*IR64 population

Aus276/3*IR64							
Trait	QTL	Experiment	Markers	QTL (+)	QTL (-)	IR64	Increase (%)
GY	qGY _{6.1}	2012UDSRS	id6010515	1195.64	1059.44	593	12.9%
			id6015531	1159.59	1002.23	593	15.7%
			id6010515+ id6015531	1197.14	1012.29	593	18.7%
		2013UDSRS	id6010515	821.56	741.48	492	10.8%
			id6015531	809.17	709.33	492	14.1%
			id6010515+ id6015531	839.32	713.80	492	17.6%
	qGY _{8.1}	2012UDSRS	id8003773	1268.48	1017.11	593	24.7%
			ud8001270	1230.14	1036.28	593	18.7%
			id8003773+ud8001270	1457.83	1154.09	593	26.3%
		2013UDSRS	id8003773	795.77	738.81	492	7.7%
			ud8001270	764.77	684.31	492	11.8%
			id8003773+ud8001270	852.50	712.84	492	19.6%
	qGY _{9.2}	2012UDSRS	id9004128	1169.25	1005.00	593	16.3%
			id9005890	1189.01	1095.09	593	8.6%
			id9004128+id9005890	1215.66	1017.34	593	19.5%
		2013UDSRS	id9004128	785.45	717.54	492	9.4%
			id9005890	814.51	758.47	492	7.4%
			id9004128+id9005890	850.61	766.65	492	11.0%
		2012WSSSS	id9003600	644.34	547.39	533	17.7%
			id9004128	561.54	453.44	533	23.8%
			id9003600+id9004128	711.32	533.23	533	33.4%
		2013DSSSS	id9003600	870.34	752.85	727	15.6%
			id9004128	756.57	650.85	727	16.3%
			id9003600+id9004128	925.33	749.13	727	23.5%
	qGY _{9.1}	2012UDSRS	id10003870	1756.12	1555.57	1663	12.9%
			id10005369	1683.05	1592.10	1663	5.7%
			id10003870+id10005369	1808.55	1579.09	1663	14.5%
		2013UDSRS	id10003870	2521.26	2343.66	1926	7.6%
			id10005369	2250.08	2131.14	1926	5.5%
			id10003870+id10005369	2429.04	2177.25	1926	11.6%
		2012LDSNS	id10003870	4498.38	4265.36	3211	5.4%
			id10005369	4573.57	4268.18	3211	7.1%
			id10003870+id10005369	4990.27	4487.97	3211	11.2%
		2012LDSRS	id10003870	2761.69	2465.60	2368	12.1%
			id10005369	2827.73	2422.45	2368	16.7%
			id10003870+id10005369	3241.93	2703.48	2368	19.9%
	qGY _{10.1}	2013UDSRS	K_id1024836	2441.99	2217.57	1926	10.1%
			id1026726	2332.11	2212.51	1926	5.4%
			K_id1024836+id1026726	2709.14	2402.37	1926	12.8%
		2012LDSNS	K_id1024836	4601.77	4367.01	3211	5.3%
			id1026726	4864.22	4295.79	3211	13.2%
			K_id1024836+id1026726	4980.83	4178.59	3211	19.2%
RHL	qRHL _{8.1}	2012WSSSS	id8002306	1.391	1.110	1.02	25.3%
			id8003773	1.410	1.198	1.02	17.5%
			id8002306+id8003773	1.580	1.090	1.02	44.9%
		2013DSSSS	id8002306	1.301	1.010	1.11	28.8%
			id8003773	1.376	1.145	1.11	20.2%
RHD	qRHD _{6.1}	2012WSSSS	id5004150	1.39	1.10	1.09	26.4%
			id5006615	1.30	1.09	1.09	19.3%
			id5004150+id5006615	1.46	1.06	1.09	37.7%
		2013DSSSS	id5004150	1.23	1.07	1.17	14.9%
			id5006615	1.24	1.04	1.17	18.2%
			id5004150+id5006615	1.27	1.02	1.17	24.5%
	qRHD _{8.1}	2012WSSSS	id8000536	1.25	1.09	1.09	22.5%

		2013DSSSS	id8000845	1.27	1.01	1.09	25.7%		
			id8000536+id8000845	1.33	1.05	1.09	26.7%		
			id8000536	1.38	1.17	1.17	17.9%		
			id8000845	1.34	1.11	1.17	20.7%		
			id8000536+id8000845	1.39	1.12	1.17	24.1%		
NR	qNR _{4,1}	2012WSSSS	id4001205	24.86	21.63	20.7	15.1%		
			id4002844	22.71	19.52	20.7	16.3%		
			id4001205+id4002844	26.61	22.13	20.7	20.2%		
		2013DSSSS	id4001205	23.93	21.21	23.3	12.8%		
			id4002844	23.52	21.77	23.3	8.1%		
EVV	qEVV _{9,1}	2012WSSSS	id4001205+id4002844	24.59	20.95	23.3	17.4%		
			id9003600	0.233	0.203	0.220	14.8%		
			id9004128	0.264	0.245	0.220	7.8%		
		2013DSSSS	id9003600+id9004128	0.284	0.246	0.220	15.5%		
			id9003600	0.172	0.155	0.166	10.9%		
P uptake (reproductive stage)	qP _{5,2}	2013DSSSS	id9004128	0.169	0.152	0.166	11.1%		
			id9003600+id9004128	0.192	0.161	0.166	19.2%		
			id5006615	8.15	6.77	6.18	20.4%		
		P conc (seedling stage)	2012WSSSS	id500730	7.80	6.42	6.18	21.5%	
				id5006615+id500730	9.21	7.44	6.18	23.8%	
id5006615	3.15			2.66	1.73	18.4%			
P conc (reproductive stage)	2013DSSSS		id500730	2.95	2.35	1.73	25.5%		
			id5006615+id500730	3.23	2.54	1.73	27.1%		
		id5006615	2.50	1.86	1.36	34.4%			
	Al uptake	qAl _{5,2}	2013DSSSS	id500730	2.40	1.92	1.36	25.0%	
				id5006615+id500730	2.80	1.99	1.36	40.7%	
id5006615				0.282	0.241	0.250	17.1%		
Al conc			qFe _{5,2}	2013DSSSS	id500730	0.270	0.232	0.250	16.4%
					id5006615+id500730	0.303	0.245	0.250	23.7%
	id5006615	7034.31			6262.56	5762.1	12.3%		
	Fe uptake	qN _{5,1}		2013DSSSS	id5007304	5845.47	4851.22	5762.1	20.5%
					id5006615+id500730	6351.48	5185.66	5762.1	22.5%
id5006615			532.90		460.00	233	15.7%		
Fe conc			qZn _{4,1}	2013DSSSS	id5007304	447.11	395.79	233	13.2%
					id5006615+id500730	772.22	638.00	233	21.1%
	id5006615	7163.13			6265.88	5673.1	14.3%		
	N uptake	qCu _{8,1}		2013DSSSS	id5007304	5753.01	5175.33	5673.1	11.2%
					id5006615+id500730	6461.20	5386.93	5673.1	19.9%
id5006615			516.90		467.00	229.4	10.5%		
N conc			qGR _{5,2}	2013DSSSS	id5007304	433.94	379.95	229.4	14.2%
					id5006615+id500730	736.89	633.00	229.4	16.3%
	id5006615	24.60			21.08	23.80	16.7%		
	Zn uptake	qN _{5,1}		2013DSSSS	id5007304	27.00	23.68	23.80	14.0%
					id5006615+id500730	21.30	17.47	23.80	21.9%
id5006615			0.916		0.746	0.832	22.8%		
Cu conc			qZn _{4,1}	2013DSSSS	id5007304	0.852	0.733	0.832	16.2%
					id5006615+id500730	0.887	0.685	0.832	29.5%
	id4002844	754.17			676.23	670.18	11.5%		
	Nematode gall rating	qGR _{5,2}		2013DSSSS	id4004639	820.13	708.24	670.18	15.8%
					id4002844+id4004639	829.70	715.47	670.18	16.0%
id8000845			4.614		3.992	4.51	15.6%		

Table 4.26 Percentage improvement of genotypes possessing QTL (QTL +) under different conditions over lines not possessing QTL (QTL -) for the Aus276/3*MTU1010 population

Aus276 x MTU1010							
Trait	QTL	Trial	Markers	QTL (+)	QTL (-)	MTU1010	Improvement (%)
GY	qGY _{1.1}	2012UDSNS	id1000001	3680	3285	3388	12.0%
			K_id1000955	3831	3509	3388	9.2%
			id100000+K_id1000955	3849	3339	3388	15.3%
		2013UDSNS	id1000001	4203	3866	4048	8.7%
			K_id1000955	4208	3922	4048	7.3%
			id100000+K_id1000955	4316	3724	4048	15.9%
		2012UDSRS	id1000001	806	711	643	13.4%
			K_id1000955	766	691	643	10.9%
			id100000+K_id1000955	928	775	643	19.5%
		2013UDSRS	id1000001	693	594	522	16.6%
			K_id1000955	651	585	522	11.3%
			id100000+K_id1000955	790	667	522	18.3%
	qGY _{1.2}	2012LDSNS	K_id1024973	4428	4037	3432	9.7%
			id1026726	4472	4098	3432	9.1%
			K_id1024973+id1026726	4582	4025	3432	13.8%
	qGY _{6.1}	2012UDSRS	id6006288	715	626	643	14.4%
			id6013772	747	644	643	16.1%
			id6006288+id6013772	857	694	643	23.6%
		2013UDSRS	id6006288	666	595	522	12.1%
			id6013772	807	721	522	12.0%
			id6006288+id6013772	846	714	522	18.5%
	qGY _{6.2}	2012WSSSS	id6013772	541	473	472	14.3%
			id6015665	471	418	472	12.7%
			id6013772+id6015665	538	458	472	17.5%
		2013DSSSS	id6013772	594	525	545	13.1%
			id6015665	564	512	545	10.2%
			id6013772+id6015665	683	577	545	18.3%
	qGY _{10.1}	2012UDSRS	id10005474	709	641	643	10.6%
			id10006378	722	632	643	14.3%
			id10005474+id10006378	882	740	643	19.2%
		2013UDSRS	id10005474	594	510	522	16.5%
			id10006378	513	458	522	11.9%
			id10005474+id10006378	740	608	522	21.7%
	qGY _{11.1}	2012LDSRS	id11001466	1533	1401	1172	9.4%
			id11002436	1903	1776	1172	7.1%
			id11001466+id11002436	2136	1827	1172	16.9%
EVV	qEVV _{9.1}	2012WSSSS	ud9000737	0.218	0.202	0.092	8.1%
			id9002704	0.208	0.197	0.092	5.7%
			ud9000737+id9002704	0.222	0.198	0.092	11.8%
		2013DSSSS	ud9000737	0.152	0.136	0.0706	11.7%
			ud9000737+id9002704	0.160	0.139	0.0706	15.7%
Seedling emergence	qDTE _{9.1}	2013DSSSS	ud9000737	3.71	3.83	4.0	3.2%
			id9002704	3.71	3.83	4.0	3.0%
			ud9000737+id9002704	3.43	3.79	4.0	9.5%
NR	qNR _{5.1}	2012WSSSS	id5000759	26.7	26	24	2.7%
			id5001182	27.4	25.7	24	6.8%
			id5000759+id5001182	28.1	24.6	24	14.2%
		2013DSSSS	id5000759	25.5	24.6	23	3.9%
			id5001182	25.1	24.2	23	3.9%
			id5000759+id5001182	26.8	24.0	23	11.7%
RHL	qRHL _{1.1}	2012WSSSS	id1000001	1.33	1.28	1.16	4.1%
			K_id1000955	1.57	1.40	1.16	12.3%
			id1000001+K_id1000955	1.71	1.46	1.16	17.3%
		2013DSSSS	id1000001	1.34	1.25	1.13	6.9%
			K_id1000955	1.50	1.36	1.13	10.1%
			id1000001+K_id1000955	1.70	1.49	1.13	13.9%
RHD	qRHD _{1.1}	2012WSSSS	id1005271	1.33	1.26	1.32	5.6%
			id1006691	1.40	1.25	1.32	12.4%
			id1005271+id1006691	1.50	1.26	1.32	19.2%
		2013DSSSS	id1005271	1.28	1.19	1.27	7.4%
			id1006691	1.22	1.17	1.27	4.3%
			id1005271+id1006691	1.29	1.16	1.27	11.4%

GY: Grain yield, EVV: Early vegetative vigor, NR: Number of nodal root, RHL: Root hair length, RHD: Root hair density

For plant height, qPHT_{1.1} was mapped in both populations in both years in multiple conditions, (Tables 4.23 and 4.24) and qPHT_{10.1} co-located with qGY_{10.1} and qDTF_{10.1} on chromosome 10 under lowland stress and non-stress conditions in 2012 (Table 4.23). A number of QTL were also detected for traits hypothesized to be beneficial for direct-seeded conditions. The root hair density QTL qRHD_{5.1} was detected and co-located with qSW_{5.1} for seed weight in the Aus276/3*IR64 population (Table 4.23). The root hair length QTL qRHL_{1.1} co-located with qGY_{1.2} for grain yield, which is near to qRHD_{1.1} for root hair density in the Aus276/3*MTU1010 population (Figure 4.24, Table 4.24). qEVV_{9.1} for early vegetative vigour was mapped in both populations in multiple seasons under direct seeded upland conditions and co-located with the qGY_{9.1} for grain yield (Figure 4.27). The majority of the QTL identified for nutrient uptake and nutrient concentration were on chromosome 5 in the Aus276/3*IR64 population and this was also the region in which qGR_{5.2} for nematode gall rating was detected (Figure 4.25). For leaf phosphorus concentration, qPC_{6.1} located near to qGY_{6.2} for grain yield under direct seeded upland conditions in the Aus276/3*MTU1010 population.

The percentage increase of lines possessing a QTL compared with lines not possessing the QTL were highest for root hair length (37.5-44.9%) in the Aus276/3*IR64 population and for grain yield (ranging up to 27%) in the Aus276/3*MTU1010 population. In addition to the major-effect, single-locus QTL for GY in the Aus276/3*IR64 mapping population, major epistatic QTL for GY showing interaction between genomic locations on chromosomes 6 and 8 and between chromosomes 6 and 9 were identified (Table 4.23, Figure 4.26). Another epistatic QTL showing interaction between genomic locations on chromosome 1 and 6 under direct seeded upland conditions was identified for phosphorus uptake (Table 4.24) which co-located with qRHD_{1.1} and qGY_{6.1} in the Aus276/3*MTU1010 population.

Characterization of traits for direct-seeded rice

The donor parent Aus276 showed higher seedling emergence rates, early vegetative vigour, nutrient uptake, nodal root number, root hair length, and root hair density than the recipient parents IR64 and MTU1010, and this was reflected by higher mean values for these traits in the BC₂F₄ populations (Table 4.21). In general, the QTL for various traits showed a stronger phenotypic effect in the background of IR64 than in the background of MTU1010.

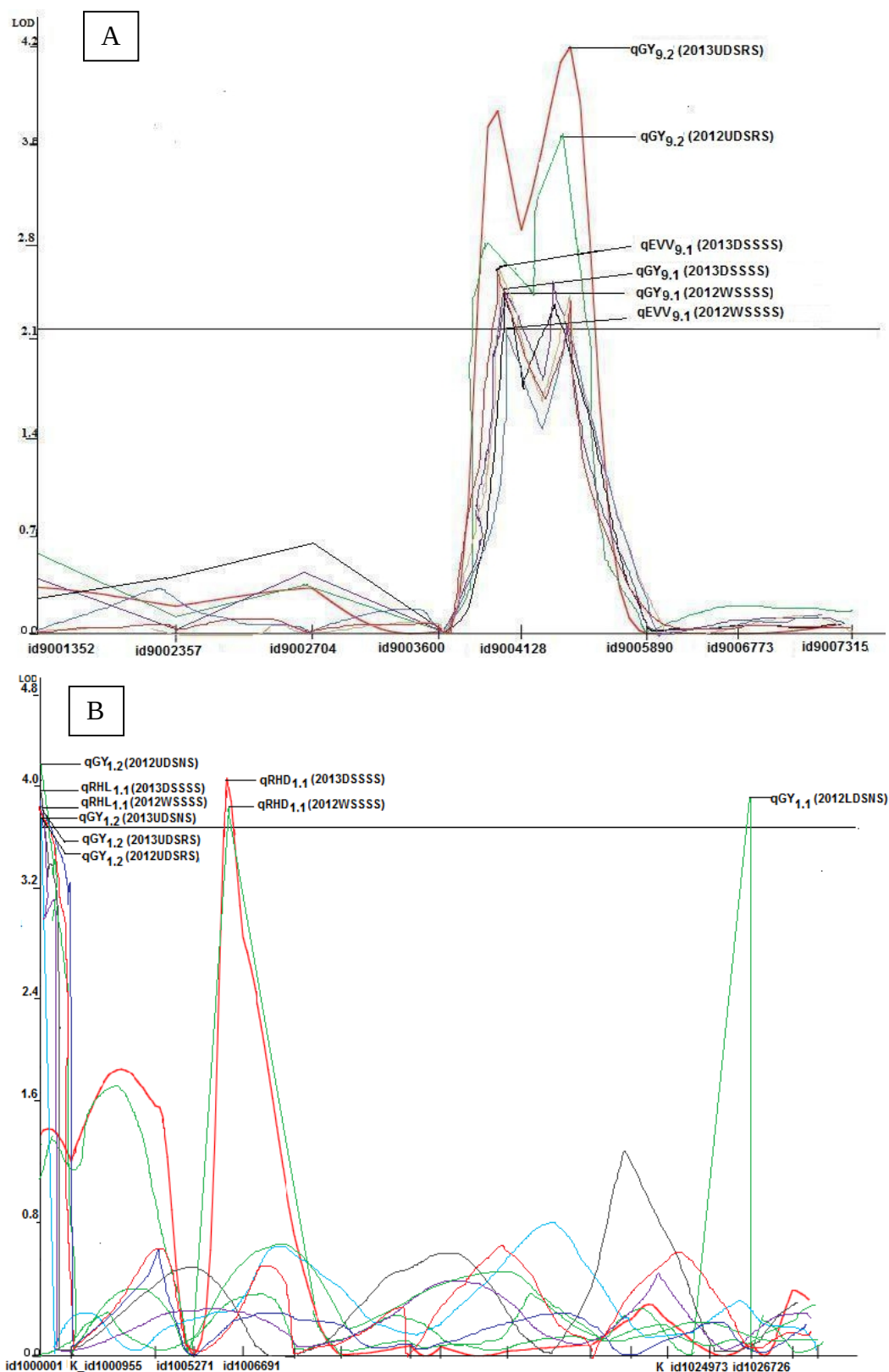


Figure 4.24 QTL likelihood curves of LOD score of coexisting QTL. Regions shown are where QTL for traits hypothesized to be beneficial for direct-seeded conditions co-located with QTL for grain yield; (A) early vegetative vigour in the in Aus276/3*IR64 population on chromosome 9, and (B) root hair length in the Aus276/3*MTU1010 population on chromosome 1

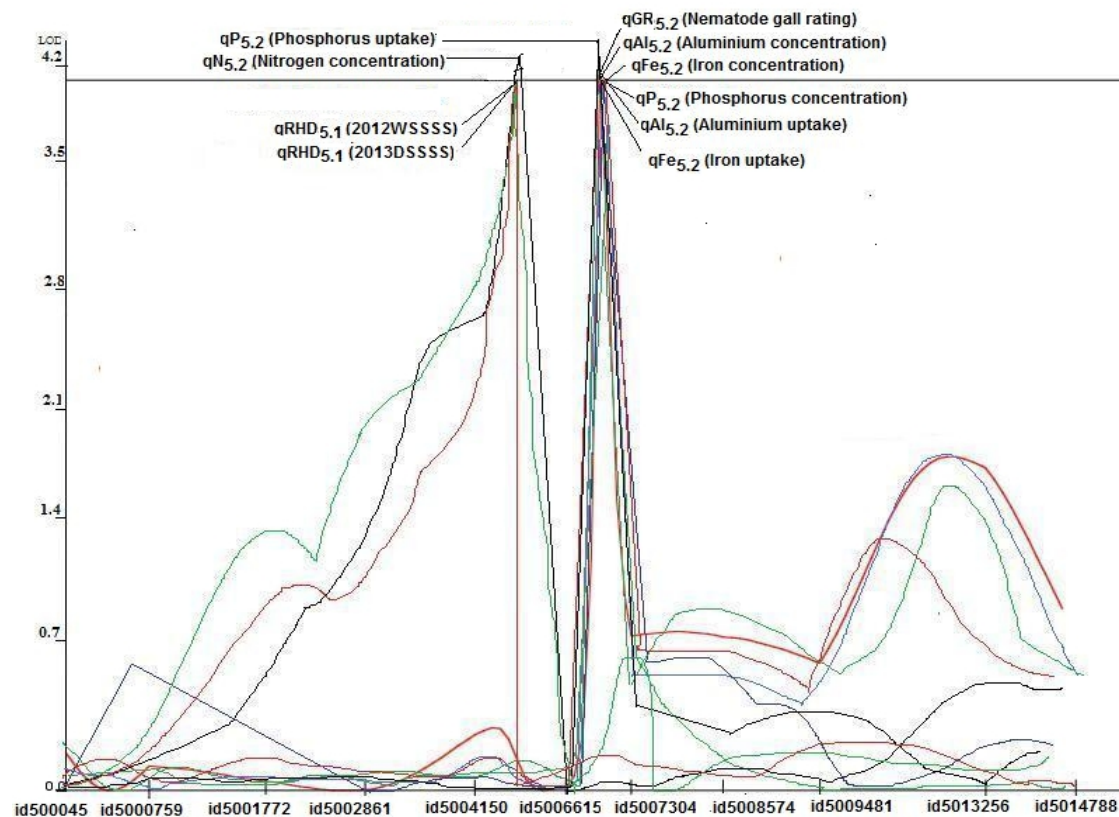


Figure 4.25 QTL likelihood curves of LOD score of coexisting QTL for (Al, Fe, N and P uptake/concentration, root hair density and nematode gall rating) on chromosome 5 in the Aus276/3*IR64 population

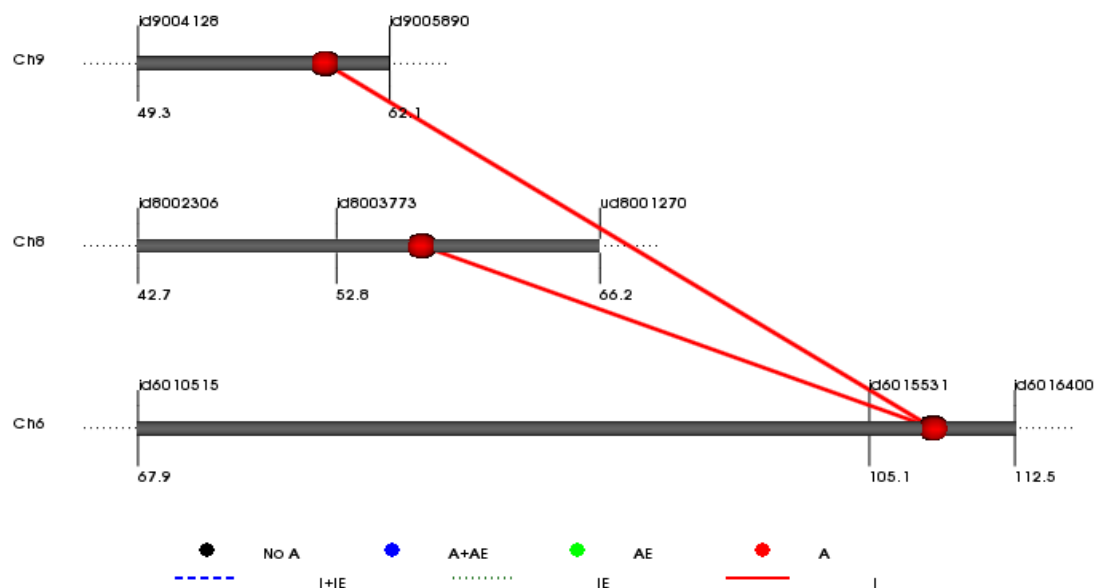


Figure 4.26 Major and epistatic QTL for GY showing interaction between genomic locations on chromosome 6, 8 and 9 in the Aus276/3*IR64 population (A: Additive effect, I: Epistatic main effect, IE: Epistasis x Environment interaction, AE: Additive x Environment interaction)

Root hairs were rated from field grown roots (Plate 7, Fig. A) and showed a range of densities, lengths, and overall morphologies; unlike agar-grown or paper rollup-grown root hairs, the root hairs from soil-grown roots were not always completely straight. In some samples, denser root hairs were observed at a swollen portion of the root (Plate 7, Fig. F), and preliminary evaluation indicated an interaction in the swollen portion with dense root hairs and nematode infection. The interaction of dense root hair growth, nematode gall rating, and nutrient uptake was confirmed by the co-location of QTL for these traits in the Aus276/3*IR64 population (Figure 4.25).

In the Aus276/3*IR64 population, root hair density was positively correlated with GY and for lateral root growth was correlated with GY and RHD (Table 4.27). In the Aus276/3*MTU1010 population, early vegetative vigour (relative growth rate) was correlated with GY, and nodal root number was correlated with early vegetative vigour, seedling emergence and GY (Table 4.27). GY was negatively correlated with DTF in multiple environments (Table 4.28). Heritability of GY was moderate in the upland trial while it was relatively high in lowland trial (Table 4.29). No significant positive correlation was found between phosphorus uptake and root hair length and density. To investigate the correlation between traits and the potential effects of spatial variation, a soil EC map and heat maps of the traits were generated; each showed distinct spatial variation patterns across the experimental field (Plate 7, Fig. B).

From each population, BC₂F₄ lines were selected in which the most stable grain yield QTL were present, as well as QTL for early vegetative vigour, nodal root number, RHD, and RLD. Of those, six lines were identified that showed the most stable and high yield in both years and in multiple conditions (Figure 4.27). Compared to the parents (IR64 and MTU1010), many of these selected lines (but not all) showed higher nodal root numbers and high dry shoot biomass related to early vegetative vigour throughout seedling stage (Figure 4.28). Likewise, many but not all of the selected lines showed longer and denser root hairs and higher lateral root rating than the parents (Figure 4.29).

Table 4.27 Correlation analyses between grain yield, root traits and early vegetative vigour of mapping populations derived from the Aus276/3*IR64 and Aus276/3*MTU1010 populations under RS and SSS upland direct seeded conditions

Aus276/3*IR64													
	GY (UDSRS)		GY (SSS)		# NR		EVV		RHL		RHD		LR
	2012DS	2013DS	2012WS	2013DS	2012WS	2013DS	2012WS	2013DS	2012WS	2013DS	2012WS	2013DS	2013DS
GY (UDSRS)	1	1											
GY (SSS)	0.1664*	0.4357*	1	1									
# NR	0.0806	0.0096	0.0843	0.0063	1	1							
EVV	0.0614	0.0316	0.0096	0.0277	0.1108	0.0195	1	1					
RHL	0.0562	0.0019	0.0522	0.0222	0.0603	0.0011	0.0019	0.0207	1	1			
RHD	0.0586	0.0623	0.1278*	0.1117*	0.0265	0.0739	-0.0014	-0.0163	-0.1222	-0.0669	1	1	
LR	-	-0.0190	-	0.1089*	-	-0.1125	-	-0.1095	-	-0.0217	-	0.1062*	1
Seedling Emergence	-	0.0343	-	0.0793	-	0.0117	-	0.1199*	-	0.0619	-	0.0198	-0.1469*
Aus276/3*MTU1010													
GY (UDSRS)	1	1											-
GY (SSS)	0.0359	0.0264	1	1									-
# NR	0.0387	0.0682	0.1159	0.1097*	1	1							-
EVV	0.0360	0.1827**	0.0053	0.1238*	0.2272**	0.0630*	1	1					-
RHL	-0.0645	-0.0365	-0.0741	-0.0041	0.0173	0.0747	0.056	0.1013	1	1			-
RHD	0.0233	0.0762	0.0275	0.0212	0.0313	-0.0399	0.089	0.1333*	0.1347*	0.172**	1	1	-
Seedling Emergence	-	0.0159	-	0.0926	-	0.1959**	-		-	0.0710	-	0.0324	-

GY: Grain yield, NR: Nodal root number, EVV: Early vegetative vigour, RHL: Root hair length, RHD: Root hair density, LR: Lateral root

*P<0.05, **P<0.01

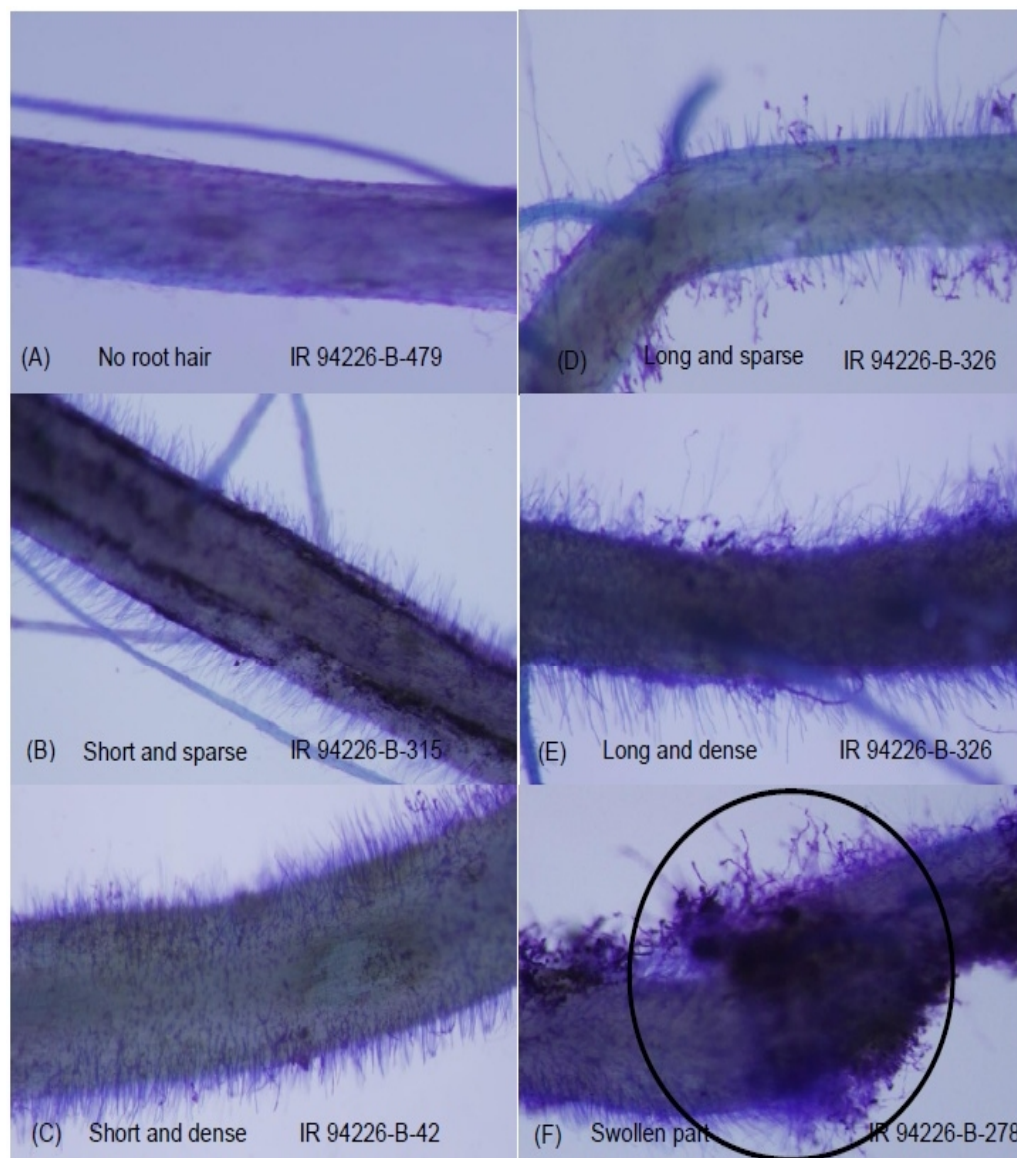


Fig (i): Variation in root hair length and density among lines under dry direct seeded upland field conditions (A - E) Root hair rating (scale 1-5), (F) swollen root with dense root hair.

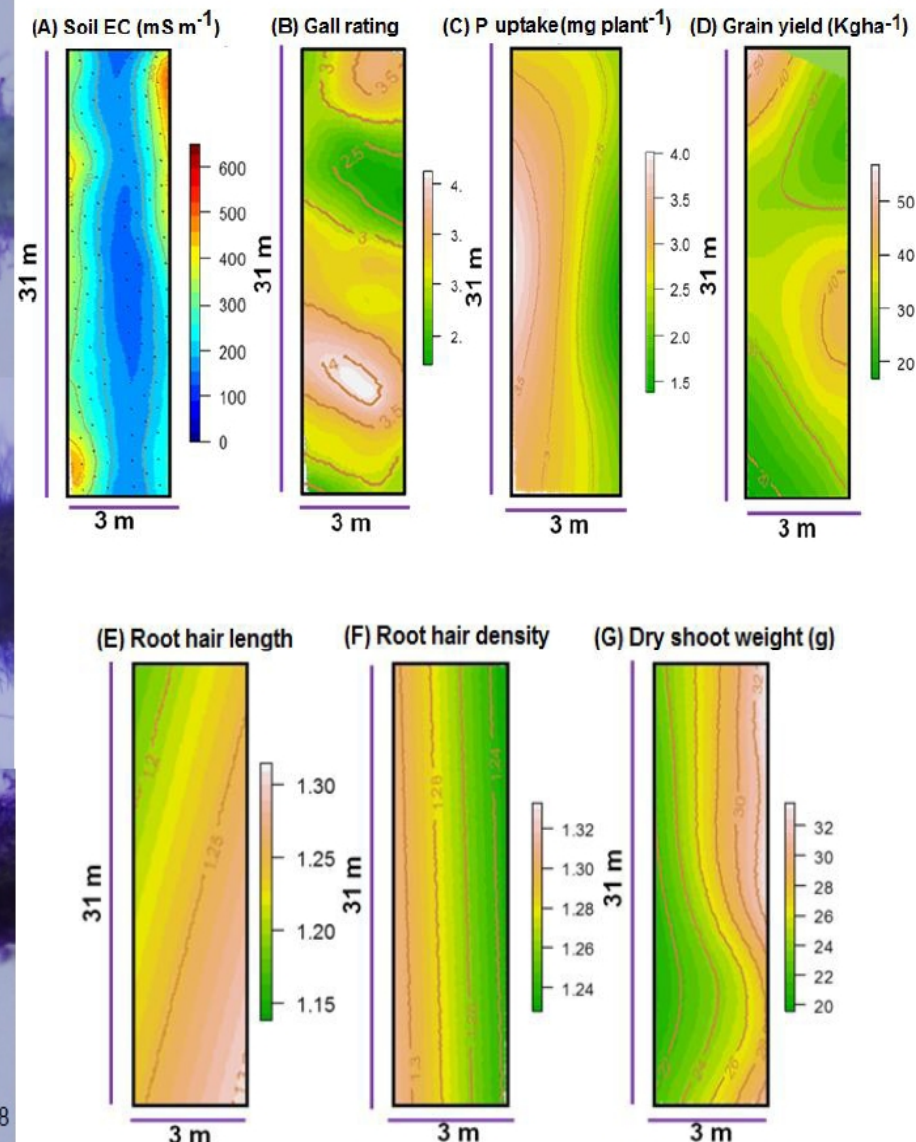


Fig (ii): (A) Soil EC map and (B-G) spatial heat maps for GR (95 DAS), PU (22 DAS), GY (130 DAS), RHL, RHD(30 DAS), DSW (95 DAS), in the 2013DSSSS trial in the Aus276/3*IR64 population using R (version 3.0.1, package: raster)

Table 4.28 Correlation analysis between grain yield and agronomic traits of mapping populations derived from Aus276/3*MTU1010 and Aus276/3*IR64 under reproductive stress and non-stress conditions

Population	Grain yield	Days to flowering	Plant height
Aus276/3*MTU1010	2012UDSRS	-0.32**	0.41*
	2013UDSRS	-0.14*	-0.22**
	2012UDSNS	-0.47*	0.02
	2012LDSRS	-0.30*	0.19*
	2012LDSNS	-0.24**	0.50**
Aus276/3*IR64	2012UDSRS	-0.45**	0.22**
	2013UDSRS	-0.65*	0.26*
	2012UDSNS	-0.61	0.35**
	2012LDSRS	0.15*	0.06
	2012LDSNS	-0.01	0.52**

*Significance at 5%; **significance at 1%.

Table 4.29 Broad-sense heritability (H) of grain yield (GY), days to flowering (DTF), and plant height (PH) under stress, combined stress, and nonstress conditions for Aus276/3*MTU1010 and Aus276/3*IR64 mapping populations

Population	Traits	<i>H</i>					
		2012UDSRS	2013UDSRS	2012UDSRS + 2013UDSRS	2012UDSNS	2012LDSRS	2012LDSNS
Aus276/3*MTU1010	DTF	0.17	0.38	0.28	0.50	0.42	0.71
	PH	0.22	0.53	0.37	0.45	0.73	0.81
	GY	0.42	0.34	0.38	0.50	0.38	0.64
Aus276/3*IR64	DTF	0.26	0.43	0.34	0.49	0.56	0.85
	PH	0.29	0.50	0.39	0.48	0.78	0.79
	GY	0.33	0.46	0.39	0.57	0.26	0.67

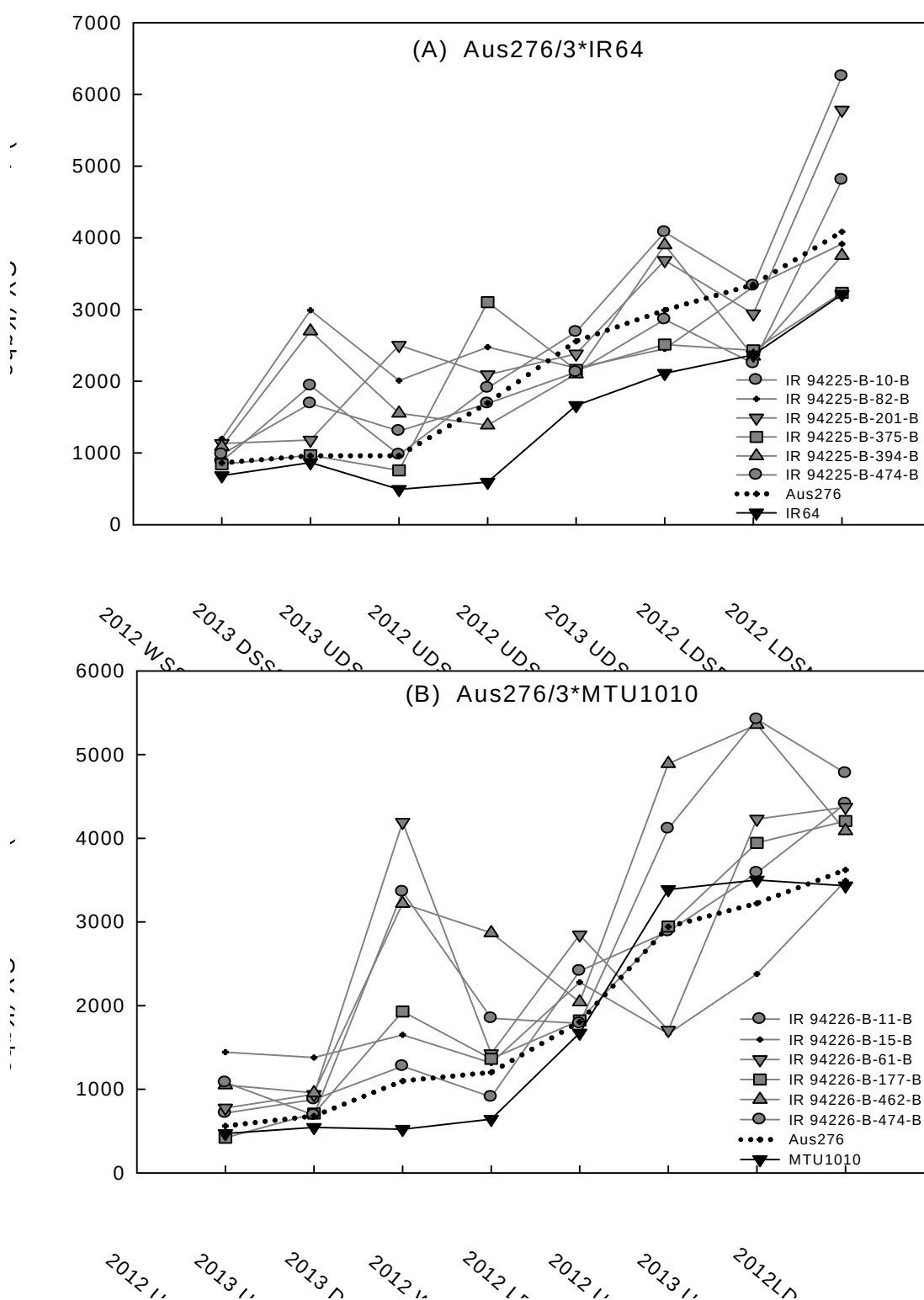


Figure 4.27 Response of six selected lines in terms of grain yield (GY) under different cultivation conditions (A) Aus276/3*IR64 (B) Aus276/3*MTU1010 population. In experiment 2012WSSSS and 2013DSSSS, each line was grown in one replicate in an augmented design. In experiment 2012UDSRS, 2013UDSRS, 2012LDSRS and 2012LDSNS, each line was grown in two replicate in an alpha lattice design

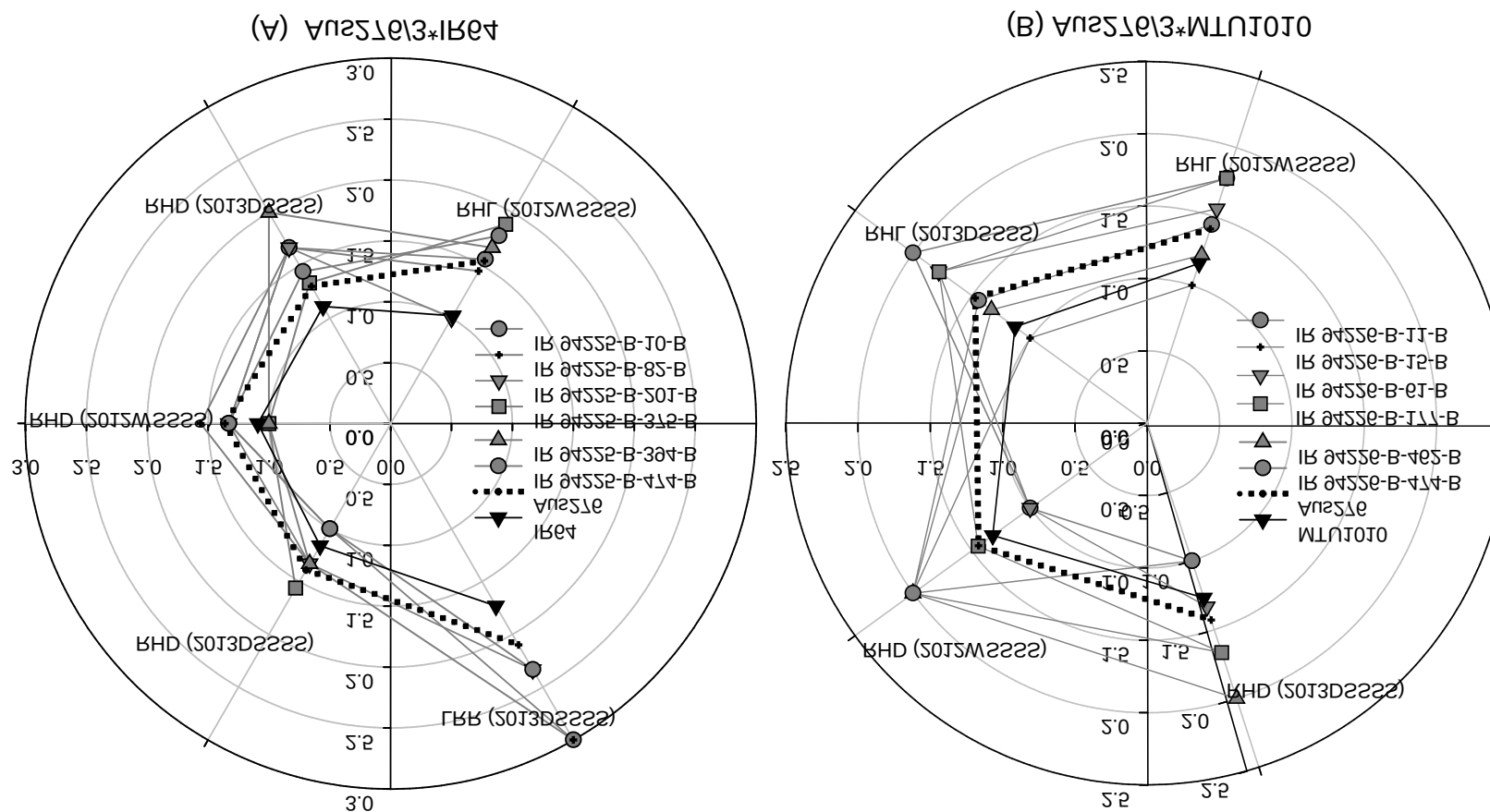


Figure 4.29 Response of six selected lines in terms of root hair length (RHL), root hair density (RHD) and LRR (lateral root rating) under dry direct seeded upland conditions in the Aus276/3*IR64 (A) and Aus276/3*MTU1010 (B) populations. Each line was grown in one replicate in an augmented design

Water shortage is an ever-increasing problem and has become the most serious constraint to rice production and yield stability in many rice-growing areas, particularly in rainfed ecosystems (Nguyen *et al.*, 1997). Agricultural production in the twenty-first century is predicted to be more limited because of lower availability and increased cost of water and nutrient resources (Lal, 2007). Water uptake in rice depends on the root system (Nguyen *et al.*, 1997); consequently, studying the root system is one of the most important parts of understanding tolerance in rice. Roots are the principal plant organ for nutrient and water uptake. Therefore, improving our understanding of the interactions between root architecture/plasticity and water scarcity in rice could have a significant impact on global food security. The identification of QTL linked to root traits and yield under aerobic conditions in target populations of an environment is critical. Marker-assisted selection using consistent-effect QTL is an efficient approach for developing appropriate aerobic rice varieties. Trait selection is another important concern in molecular breeding for aerobic/direct seeded traits. As in the case of drought, QTL for a number of physiological and morphological traits have been identified but they have limited implications in breeding drought-tolerant and water efficient direct seeded rice varieties.

In the present investigation experiments were conducted to: identify QTL conferring a potential yield advantage under aerobic/ direct seeded conditions; identify root traits having a positive correlation with grain yield under aerobic/direct seeded conditions; identify genotypes with high levels of root growth plasticity (in terms of deep root growth) in multiple conditions (upland, lowland, induced by drought or low P) and to identify a positive correlation of root plasticity with grain yield under dry direct seeded cultivation conditions; BSA approach was applied to identify QTL showing consistent effects in the background of two popular high-yielding varieties, IR64 and MTU1010, under upland reproductive-stage drought stress; identify QTL with large and consistent effects for traits thought to be beneficial for direct-seeded rice: seedling emergence, early vegetative vigour, root morphology, nutrient uptake, drought tolerance, and grain yield. We hypothesized that the some of the seedling-stage traits investigated might have a strong enough effect on plant growth to be correlated with grain yield at harvest.

The root system is considered as one important component for solving the problem of water scarcity. The improvement of upland rice through a deeper root system is considered to be a promising way to increase water uptake, reduce lower canopy temperature, increase stomatal conductance, and ultimately increase grain yield under water-stress conditions. A

deeper and thickened root system having large xylem vessels has been shown to allow upland rice varieties to extract more water from the soil, resulting in a higher yield potential under water-scarce conditions (Nguyen *et al.*, 1997). Such root traits are highly likely to have a positive effect on increasing the adaptation of rice genotypes to aerobic conditions. Growth of the rice root, in terms of total dry matter, maximum root depth, root length density, root number, and root volume, increases until flowering stage and then decreases sharply to maturity (Yoshida and Hasegawa, 1982).

5.1 Experiment 1: HKR47 x MAS26 and MASARB25 x Pusa Basmati 1460

Correlation analysis was carried out to identify how root morphological characters influence the grain yield and yield morphological traits under aerobic conditions. Our study identified a significant and positive correlation between some of the root traits (root number, root volume, root thickness, root hair density, lateral root and nodal root number and dry root weight) with yield and early vegetative vigour under dry direct seeded conditions indicate the role of improved water and nutrient uptake at the seedling stage in improving rice yield for direct-seeded conditions. This shows that a well developed root system will help the plant in maintaining high plant water status which ultimately leads to increase in yield potential under water-deficit conditions.

Correlated characters are of interest for three main reasons: in connection with the genetic causes of correlation through the pleiotropic action of genes, to know how selection for one character will cause a simultaneous change in other characters, and to determine the relationship between character and fitness. In our study, on chromosome 2 in a region of 0-10.2 cM, seven QTL were reported to be significantly associated with seven different root traits and agronomically important traits (RV, FRW, FSW, DSW, PH, PL, and GY) in the case of MASARB25 × Pusa Basmati 1460 population. Two QTL, qGY_{2.1} in the 10-cM region between RM7562 and RM279 on chromosome 2 in MASARB25 × Pusa Basmati 1460 population and qGY_{2.2} in the 118.5-cM region between RM475 and RM526 on chromosome 2 in HKR47 × MAS26, for grain yield under aerobic conditions were identified. qGY_{2.1} and qGY_{8.1} in MASARB25 × Pusa Basmati 1460 showed a stable high over two different years and combined over two years individually, and an increased stable effect over two different years and combined over two years. Similarly, in HKR47 × MAS26, qGY_{2.2} showed a stable high effect over individual years and combined over two years. These QTL were found to be adjacent to the earlier reported QTL qDTY_{2.2} and qDTY_{2.3} (Bernier *et al.*, 2007) for grain yield under drought stress. Although the effect of the two identified QTL needs to be validated in different genetic backgrounds, under the present scenario successful introgression of the identified QTL following marker-assisted backcrossing can be used to improve the popular varieties Pusa Basmati 1460 and HKR47. Similarly, QTL for root length within the qGY_{2.1} region (MacMillan *et al.*, 2006) and for root thickness adjacent to qGY_{2.2} (Champoux

et al., 1995) have been reported. Coexisting chromosomal regions/loci governing different traits provide a unique opportunity for breeders to introgress such regions together as a unit into high-yielding lowland varieties through MAS/MAB and to develop cultivars possessing increased adaptation to aerobic conditions.

Two QTL, $qRL_{8.2}$ in both mapping populations and $qRL_{8.1}$ in MASARB25 $\times$ Pusa Basmati 1460, for root length have been reported in our investigation. Qu *et al.*, (2008) reported QTL for fresh root weight and root number coinciding with $qRL_{8.2}$ linked to marker RM331. Studies also compiled numerous earlier reports of QTL for root length (Uga *et al.*, 2010), root thickness, and root number (Li *et al.*, 2005a) adjacent to or coinciding with $qRL_{9.1}$. In HKR47 $\times$ MAS26 mapping population, $qRT_{1.1}$ was reported for root thickness with an LOD of 4.4 and R^2 value of 38.4%. These QTL may confer a grain yield advantage under direct-seeded conditions and this is supported by the earlier reported large-effect consistent QTL ($qDTY_{1.1}$) for grain yield under drought (Ghimire *et al.*, 2012) in the region of $qRT_{1.1}$. Under dry direct-seeded conditions, the QTL peak ($qGY_{8.1}$) for grain yield was seen in MASARB25 $\times$ Pusa Basmati 1460 at RM339 with an R^2 value of 34%. At the same position and linked to the same marker (RM339), $qDTY_{8.1}$ in Basmati334/Swarna was reported by Vikram *et al.*, (2012) for grain yield under drought. Hanamaratti, (2007) reported RM339 on chromosome 8 associated with relative yield and drought susceptibility index in IR64 $\times$ Binam-derived NILs under drought stress. Adjacent to the QTL $qGY_{8.1}$ within a region of 14.4 cM, other QTL ($qTN_{8.2}$, $qNPP_{8.2}$, and $qRL_{8.2}$) has been reported.

In a segment of 21.7 cM on chromosome 8 (101.5- 123.2 cM), QTL for DRW, TN, NPP, PL, NSP, PH, and GY were also reported in our study. The effect of these regions on a number of traits that is likely to impart improved aerobic adaptation. GY strongly suggests the presence of more than one gene within these QTL affecting a wide range of traits. These genes conferring a GY advantage under aerobic adaptation may have undergone strong natural selection to stay together in the course of evolution. These sub-QTL with a discernible phenotypic effect on GY may affect the same/different physiological traits in response to different severities of stress, leading to a GY advantage. QTL $qPL_{12.1}$ (RM28048) identified in our study in HKR47 $\times$ MAS26 mapping population showed an effect on a yield-attributing trait (i.e., panicle length) under dry direct-seeded conditions. Bernier *et al.* (2007, 2009) reported QTL $qtl_{12.1}$ linked to the same marker. Bernier *et al.* (2009) and Dixit *et al.* (2012) showed a consistent large effect on grain yield under drought over a wide range of environments in the Philippines and eastern India. This confirms that this QTL is effective not only at IRRI, where it was initially detected, but also in at least a part of the target environment of eastern and northern India, where improved drought resistance in upland rice cultivars is an important breeding objective. This is also supported by the study of Bernier *et al.* (2007) that mentioned that this locus is associated with a low QTL $\times$ environment

interaction under severely stressed conditions, which is one of the two major requirements for the use of a QTL in MAS. The QTL for biomass, harvest index, days to flower, plant height, flowering delay, drought response index, and panicle number under stress were mapped in the same region. *qtl_{12.1}* also influences water uptake under upland stress and increases harvest index (Bernier *et al.*, 2007). In our study, we reported a 25.1-cM segment between RM589 and RM314 on chromosome 6 affecting different root (RV, RT, and FRW) and shoot (FSW and DSW) traits under aerobic conditions in the case of MASARB25 × Pusa Basmati 1460 mapping population. In this region, a large-effect QTL (*qDTY_{6.1}*) associated with grain yield in aerobic environments was identified in a total of 20 hydrological environments over a period of five seasons and in five populations in three genetic backgrounds using bulk-segregant analysis (Venuprasad *et al.*, 2009). Co-localization of *qDTY_{6.1}* with a region identified to govern several root and shoot traits is an implication that this is an important region for improving yield under aerobic conditions.

5.2 Experiment 2: Aus276/3*MTU1010 and Kali Aus/2*MTU1010

Root architectural plasticity, the ability to exhibit morphological and physiological responses to a changing environment, may play an important role in plant adaptation to heterogeneous environments (Sultan, 2000; Pigliucci 2001) and is important in maintaining crop productivity (Wang and Yamauchi, 2006) with low inputs. Such plasticity is especially evident in the root system since it adopts a particular architecture under one condition, but can change architecture in response to change in condition. Spatial localization of roots is an important root architecture strategy for efficient resource acquisition when resources are heterogeneous in time and space. Dry root biomass, water uptake and root plasticity at a depth of 40-60 cm in lysimeter and 45-60 cm under field condition showed good positive correlation. An established correlation between root system size and resistance to water stresses was reported by Price *et al.* (2002), Tuberosa *et al.*, (2002). Plasticity in % lateral root growth is more due to elongation than branching. Consistently high root growth plasticity was observed across different treatment and environments. Some plants respond to water deficit in term of increase in root length, root biomass and lateral root production. In our study root growth was measured at different depth in different treatment. More consistent results in different treatment were obtained in term of dry root biomass at a depth of 40-60 cm under lysimeter and 45-60 cm under field conditions. While the field is more realistic, it also includes many potentially confounding environmental factors. Consistency in results under lysimeter and field conditions indicates that results from a controlled environment were confirmed in the field validates our greenhouse methods, and also indicates that the architectural traits we examined are robust across environments. Variation was observed among the genotypes for plasticity for root length, root biomass, lateral root elongation and branching of lateral roots under phosphorus deficit conditions. Root system plasticity has

been implicated in causing genotype x environment interactions (MacMillan *et al.*, 2006). One particular variety or species will try to allocate more of its dry matter to growth of deep roots whereas another may give more priority to producing an elongated but shallow root system (Lynch, 1995). The genotypes with the most root growth plasticity were found to be the high yielding genotypes. Under the phosphorus deficiency enhanced lateral root development resulted in increase in surface area; as a result of plastic response, which may leads to increase in nutrient uptake that ultimately leads to increase in productivity.

Increased lateral root growth increased phosphorus uptake in common bean (Lynch and Van Beem, 1993) and maize (Zhu and Lynch, 2004), nitrogen uptake (Sen *et al.*, 2013) and yield under varying water and nutrient regimes (Hochholdinger and Tuberosa, 2009) in maize, and water uptake under drought in rice (Suralta *et al.*, 2008 and 2010). Under the phosphorus deficit conditions root diameter, number of cortical cell layer and number of metaxylem were found to be higher as compared to the control conditions. Plasticity was most pronounced under low water and phosphorus levels. A number of QTL associated with nutrient uptake have been detected in rice (Ni *et al.*, 1998; Wissuwa *et al.*, 1998; Ming *et al.*, 2000), maize (Zhu *et al.*, 2005), wheat (Su *et al.*, 2009; Su *et al.*, 2006), common bean (Liao *et al.*, 2004; Yan *et al.*, 2004) and soybean (Li *et al.*, 2005b; Liang *et al.*, 2010). Nutrient-efficient plants produce higher yields per unit of nutrient applied or absorbed compared with other plants grown under similar agroecological conditions (Fageria *et al.*, 2008). Although it is often emphasized that enhancing nutrient-use efficiency is critical across the globe both to sustain yields in low-input agriculture and to reduce fertilizer inputs in high input agriculture, it is not clear if traits that enhance efficiency in low-input systems also confer efficiency in high-input systems (Rose *et al.*, 2012). Therefore, better understanding of root-related traits and root plasticity and how root plasticity QTL interacts to affect soil resource acquisition across a range of environments will be an important tool in the breeding of rice varieties.

5.3. Experiment 3: Kali Aus/2*IR64 and Kali Aus/2*MTU1010

BSA is indeed a cost-effective scheme of identifying QTL alleles as considerable savings in time and inputs can be achieved in genotyping efforts, thus allowing resources to be allocated to more precise identification of QTL with large effects (Vikram *et al.*, 2012). In the case of drought, QTL $\times$ genetic background interaction has been reported to be a major factor limiting the use of QTL for MAB in rice (Bernier *et al.*, 2007). In our study, three QTL (qDTY_{1.1}, qDTY_{2.2}, and qDTY_{2.3}) were identified using one common donor parent (Kali Aus) in the backgrounds of mega varieties IR64 and MTU1010. qDTY_{1.1} showed consistent effect against both improved genetic backgrounds and in both seasons. The additive effect of qDTY_{1.1} on GY represented around 17 and 19% of the trial mean for 2012 DS and 2013 DS,

respectively, in the Kali Aus/2*MTU1010 population and 14 and 18% of the trial mean for 2012 DS and 2013 DS, respectively, in the Kali Aus/2*IR64 population. It is noteworthy to mention that $qDTY_{1.1}$ had a significant effect on GY under both RS and NS conditions in N22/Swarna, N22/IR64, and N22/MTU1010 backgrounds in two seasons (Vikram *et al.*, 2011). $qDTY_{1.1}$ also showed a significant effect on PH under both environments in Kali Aus/2*MTU1010 populations in both seasons in our study. This is similar to the effect on PH under RS and NS in N22/Swarna, N22/IR64, and N22/MTU1010 backgrounds in two seasons in the study of Vikram *et al.* (2011). QTL for GY under RS has also been reported by Kumar *et al.* (2007) and Ghimire *et al.* (2012) in this region. The allele that increased GY under RS was contributed by a comparatively susceptible parent in a study conducted by Kumar *et al.* (2007). However, the positive allele in our study was contributed by the tolerant parent, Kali Aus, similar to what Vikram *et al.* (2011) and Ghimire *et al.* (2012) found in their studies: the tolerant allele was contributed by tolerant parent N22 and Dhagaddeshi, respectively, at this locus. Apart from this, the region has shown a rich diversity of QTL related to various drought-tolerance traits such as root-related traits (Gomez *et al.*, 2010), PH (Bernier *et al.*, 2007), root dry weight (Zhang *et al.*, 2001), grains per panicle (Babu *et al.*, 2003), relative water content under drought (Kanbar *et al.*, 2003), biomass, basal root thickness, and osmotic adjustment (Robin *et al.*, 2003). Two different QTL, $qDTY_{2.2}$ and $qDTY_{2.3}$, were mapped in Kali Aus/2*MTU1010 and Kali Aus/2*IR64, respectively, despite a common donor parent being used to develop both populations. This could possibly be due to the presence of interaction between the QTL and the genetic background of the recipient parent or epistatic interaction (Holland, 2001, Li *et al.*, 2000). The $qDTY_{2.2}$ exerted a positive additive effect around 21 and 30% of the trial mean for 2012 DS and 2013 DS, respectively, under RS and $qDTY_{2.3}$ showed additive effect of 10 and 11% of the trial mean for 2012 DS and 2013 DS, respectively. Combined analysis showed an additive effect of 24 and 11% of the trial mean for $qDTY_{2.2}$ and $qDTY_{2.3}$, respectively, indicating a valid consistency with respect to the effect of QTL on GY under RS. Several QTL have been previously reported to have a significant effect on GY under RS, which proved to be highly specific to the background parent (MacCaferri *et al.*, 2008). Nonetheless, it is still possible that the effect of these QTL on GY could be validated in other genetic backgrounds. The mapped QTL can be introgressed into the background of drought.

In our study, the genotypes with the most root growth plasticity were found to be the high yielding genotypes and have low canopy temperature, higher dry root biomass, high water uptake and high stomatal conductance. It may be hypothesized that under the stress conditions root traits plays an important role in water uptake, low canopy temperature leads to decrease in the loss of water and such plastic root responses resulted in increase stomatal

conductance and photosynthesis, eventually increased dry matter production, leads to increase in productivity.

5.4. Experiment 4: Aus276/3*MTU1010 and Aus276/3*IR64

Direct relationships between seedling-stage traits and grain yield were established in terms of QTL co-location and phenotypic correlation. The co-location of QTL for grain yield with root hair length on chromosome 9 and early vegetative vigour on chromosome 1, as well as the concentration of QTL for nutrient uptake and concentration and root hair density on chromosome 5 implies that these could be key genetic regions for improving rice for direct seeded conditions. The significant and positive correlations between some root traits (root hair density, lateral root and nodal root number) and early vegetative vigour and yield under dry direct seeded conditions indicate the role of improved water and nutrient uptake at the seedling stage in improving rice yield for direct-seeded conditions.

Interestingly, QTL for root hair length and GY and for root hair density and phosphorus uptake were found to be co-located with each other in both populations. Compared with flooded paddy soils, reduced nutrient uptake, especially of nitrogen and phosphorus, under dry direct seeded conditions has been the most important factor for lower yield in direct seeded of rice systems (Alam, 1999). The characterization of root-related traits and QTL associated with these root traits (Sandhu *et al.*, 2013) that increase nutrient uptake under direct seeded conditions can help develop direct seeded rice varieties with high yield potential. The co-location of QTL for seedling emergence, EVV, RHL, DTF, PH, biomass and HI with a region identified to govern GY is an implication that these QTL for adaptation to direct seeded conditions may have undergone strong natural selection to stay together over the course of evolution. These regions likely accumulated in aus cultivars during evolution and are coming from aus backgrounds. Drought tolerance donors like Aus276 have been categorized in the aus cluster (a pool of traditional varieties commonly used as donors for abiotic stress tolerance traits into cultivated varieties) by genome-wide SNP-based diversity analysis (McNally *et al.*, 2009) and may have originally been cultivated in dry-direct seeded conditions.

QTL for phosphorus uptake in rice have been reported on chromosomes 2, 6, 10, and 12 (Wissuwa *et al.*, 2001). Although QTL for root hair density and phosphorus uptake co-located in this study on chromosome 5, no direct significant correlation between phosphorus uptake at seedling stage or reproductive stage and root hair length and density were observed. It may be hypothesized that there was a strong G x E interaction between these traits that obscured their direct correlation. However, maps of soil EC and spatial heat maps for nematode gall rating, P uptake, GY, root hair length, density and dry shoot weight (based on the position of each plot) were not similar for the different traits measured, highlighting the genetic variation for these traits. The root hairs tend to be sparse in P sufficient plants

(Gahoonia and Nielsen, 1997). So it may be possible that under low input conditions some of lines became P deficient first, and then formed more root hairs. It is also likely that the complex nature of root growth accounts for the lack of direct relationship between root hair growth and phosphorus uptake observed here. Root architecture encompasses many interacting traits and components within a trait. Root hair growth was only evaluated here at the basal end of the nodal roots and may differ in different parts of the root zone. Rice lateral roots fall into several different classes (Yamauchi *et al.*, 1987a, b) and be highly affected by fluctuating conditions throughout a growth season (Suralta *et al.*, 2008 and 2010), and were only evaluated here by an overall rating at the basal end of the nodal roots. The number of nodal roots varied and originated from varying numbers of nodes; although this trait was not quantified in our study, it may be important for soil resource acquisition and should be further investigated (Lynch and Brown, 2012).

The co-location of QTL on chromosome 5 in the Aus276/3*IR64 population indicates an interaction between nematode infection, root hair growth, and nutrient uptake. It may be possible that nematode infection lead to a change in root hair density that increased the ability to take up nutrients under dry direct seeded conditions. A positive effect of root-knot nematodes on root hair growth, root exudation and nutrient uptake has been reported in barley (Haase *et al.*, 2007). However, nematode infection is a serious concern for rice growing in aerobic soils including dry-direct seeded conditions (Pankaj *et al.*, 2012). Therefore, the interaction of nematode infection with root hair density observed here should be further characterized to identify any potential tradeoffs in selecting for traits for direct seeded rice.

Use of cultivars with early vegetative vigour facilitating good crop establishment and better competitive ability with weeds is an important strategy for enhancing and stabilizing the yield of direct seeded upland rice. Though several high yielding cultivars have been released for cultivation in upland conditions, few have been widely adopted by farmers due to a lack of either high seedling vigor or preferred grain traits (Sujay, 2007). In the present study, qEVV_{9.1} for early vegetative vigour and qGY_{9.1} for grain yield co-located in both populations under dry direct seeded conditions. Earlier reports of QTL for root length (Uga *et al.*, 2010; Sandhu *et al.*, 2013), root thickness, and root number (Li *et al.*, 2005b) were located near to or coinciding with qEVV_{9.1} and qGY_{9.1} in the present study. Although co-location of physiological traits and grain yield have been previously reported (Gautami *et al.*, 2012; Venuprasad *et al.*, 2002; Hanamaratti 2007), it is not often that seedling stage traits will be related to yield due to differences in the time of their occurrence and fluctuations in environmental conditions between those time points. A better understanding of the nature of association among seedling traits, namely early vegetative vigor, nodal root number, RHL, RHD and GY is expected to provide a new dimension in planning selection strategies for rice adapted to a range of conditions.

A wide range of conditions (upland, lowland, seedling stage stress and low-input soil, reproductive stage stress and non stress) was used to evaluate the populations because after direct seeded any of these conditions could be experienced by the farmers and also in order to confirm that whether the loci is associated with a low level of QTL x environment interaction, which is one of the two major requirements for use of a QTL in MAS. Large-effect QTL for grain yield under drought have been identified (Bernier *et al.*, 2007; Venuprasad *et al.*, 2009) and their successful introgression has established a yield advantage under drought (Swamy *et al.*, 2013). For developing drought tolerant and direct seeded rice with high yield potential, three major QTL with a large effect on grain yield across a wide range of environment and background have been identified in our study; these can also be incorporated into future QTL introgression work to improve rice yield under drought. Several of the QTL identified for GY in the present study (qGY_{1.1}, qGY_{6.1}, qGY_{8.1}, qGY_{9.1} and qGY_{10.1}) were previously reported to be consistent across different mapping populations and conditions (Ghimire *et al.*, 2012; Vikram *et al.*, 2012; Sandhu *et al.*, 2013; Dixit *et al.*, 2012). The occurrence of different QTL despite a common donor parent being used to develop both populations (Aus276/3*IR64 and Aus276/3*MTU1010) may possibly be due to the presence of interaction between the QTL and the genetic background of the recipient parent or epistatic interaction (Holland 2001; Li *et al.*, 2000). In general, more phenotypic variation was observed in Aus276/3*IR64 population as compared to the Aus276/3*MTU1010 population.

In addition to the effects of QTL at single loci, it has been hypothesized that epistasis is one component of the genetic basis of quantitative traits, and a large portion of the main-effect QTL in rice are involved in epistatic interactions (Xing *et al.*, 2002). In the present study, we observed epistatic interactions between several QTL showing effects on different traits. The positive interaction between the genomic regions for root traits and nutrient uptake ultimately that these interacting loci should also be considered when introgressing QTL to develop rice for dry direct seeded conditions.

The magnitude and direction of influence of co-located loci on the different phenotypes will markedly affect the utility of such loci in selection for simultaneous improvement of these traits (Hittalmani *et al.*, 2003). Coexisting chromosomal regions/loci governing different traits provide a unique opportunity for breeders to introgress such regions together as a unit into high-yielding lowland varieties through MAS/MAB and to develop cultivars possessing increased adaptation to dry direct seeded conditions. The identified QTL for root traits for higher nutrient uptake could be further used to increase the yield under direct seeded condition. All of the selected lines from this study will serve as novel materials for the selection of stable direct seeded rice varieties. Development of these direct seeded varieties could be of significant benefit to farmers in the region who depend on upland rice for food security. The challenges ahead are the effective use of these roots and GY QTL and

their combinations in breeding for direct seeded rice varieties, fine mapping of QTL to facilitate precise introgression without undesirable linkages, and understanding the physiological and molecular mechanisms associated with these major-effect QTL under direct seeded conditions. MAB program using the selected lines is currently underway.

In the present investigation experiments were conducted to: identify QTL conferring a potential yield advantage under aerobic/ direct seeded conditions; identify root traits having a positive correlation with grain yield under aerobic/direct seeded conditions; identify genotypes with high levels of root growth plasticity (in terms of deep root growth) in multiple conditions (upland, lowland, induced by drought or low P) and to identify a positive correlation of root plasticity with grain yield under dry direct seeded cultivation conditions; BSA approach was applied to identify QTL showing consistent effects in the background of two popular high-yielding varieties, IR64 and MTU1010, under upland reproductive-stage drought stress; identify QTL with large and consistent effects for traits thought to be beneficial for direct-seeded rice: seedling emergence, early vegetative vigour, root morphology, nutrient uptake, drought tolerance, and grain yield. We hypothesized that the some of the seedling-stage traits investigated might have a strong enough effect on plant growth to be correlated with grain yield at harvest.

1. Our study identified a total of 35 QTL associated with 14 traits on chromosomes 1, 2, 5, 6, 8, 9, and 11 in MASARB25 × Pusa Basmati 1460 population and 14 QTL associated with nine traits on chromosomes 1, 2, 8, 9, 10, 11, and 12 in HKR47 × MAS26-derived population. These identified QTL included three large-effect stable QTL for increased yield under aerobic conditions and QTL for several root-related traits likely to increase water and nutrient uptake under aerobic conditions.
2. A number of plants were identified with higher root length and dry biomass, yield per plant, length-breadth ratio, and with Pusa Basmati 1460 specific alleles in homozygous or heterozygous condition at the *BAD2* locus; these lines shall serve as novel materials for the selection of stable aerobic Basmati rice varieties.
3. A mechanism associated with higher yield of promising lines under dry direct-seeded conditions, indicating relocation of resources during grain filling, is suggested by coexisting QTL for root and yield-attributing traits.
4. A good correlation was observed between root growth in lysimeter, field and rhizoscope study.
5. A good correlation was reported between water uptake in lysimeter and leaf water status in field.
6. A good correlation was found between root plasticity in lysimeter, field and rhizoscope study.

7. Plasticity in % lateral root growth was observed to be more due to elongation than branching of lateral root.
8. Consistently high plasticity was reported across different treatment and environment. Consistency in results under lysimeter, field and rhizoscope conditions indicates that results from a controlled environment were confirmed in the field validates our greenhouse methods, and also indicates that the architectural traits we examined are robust across environments.
9. On the basis of lysimeter and field study conducted at IRRI and rhizoscope study conducted at CIRAD, France, a total of six genotypes were selected from each population for further evaluations.
10. The study identified three large and consistent-effect QTL, $qDTY_{1.1}$, $qDTY_{2.2}$, and $qDTY_{2.3}$ on GY under reproductive-stage drought stress in Kali Aus/2*MTU1010 and Kali Aus/2*IR64 populations, these identified QTL for GY under drought can be used to improve the GY of mega varieties MTU1010 and IR64, respectively, over different degrees of severity of drought stress through MAB and provide farmers with improved varieties that effectively combine high yield potential with good yield under drought.
11. QTL $DTY_{2.3}$ has shown an effect across both lowland and upland ecosystems in the Kali Aus/2*IR64 population and QTL $DTY_{1.1}$ were identified in both populations in the upland ecosystem.
12. A total of 28 QTL associated with 22 traits and 20 QTL associated with 12 traits were mapped in Aus276/3*IR64 and Aus276/3*MTU1010 population, respectively.
13. The QTL $qGY_{6.1}$, $qGY_{10.1}$, $qGY_{1.1}$ and $qEVV_{9.1}$ were found to be effective in both Aus276/3*IR64 and Aus276/3*MTU1010 populations under wide range of conditions; $qEVV_{9.1}$ was found to be co-located to the $qDTE_{9.1}$ and $qGY_{9.1}$. The QTL for nutrient uptake were located on chromosome 5, co-located with $qGR_{5.2}$ and $qRHD5.1$.
14. The positive interaction between the genomic regions for root traits and nutrient uptake ultimately that these interacting loci should also be considered when introgressing QTL to develop rice for dry direct seeded conditions.
15. The identified QTL for root traits for higher nutrient uptake could be further used to increase the yield under direct seeded condition.
16. Co-localization of QTL for yield, early vegetative vigour and root traits indicates that the identified QTL may be immediately exploited in marker-assisted-breeding to develop novel high-yielding rice varieties for direct seeded conditions.

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ANNEXURE-I

Composition of Yoshida nutrient solution

Sr. No.	Major Salts	g/l	g/250 ml
1.	NH ₄ NO ₃	91.3	22.85
2	K ₂ SO ₄	71.4	17.85
3.	KH ₂ PO ₄	23.1	5.77
	K ₂ HPO ₄	4.3	1.07
4.	CaCl ₂ .6H ₂ O	175	29.36
5.	MgSO ₄ .7H ₂ O	324	81
6.	Minor Salts		
	MnCl ₂ .4H ₂ O	1.5	0.37
	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.074	0.02
	H ₃ BO ₃	0.930	0.23
	ZnSO ₄ .7H ₂ O	0.035	0.008
	CuSO ₄ .5H ₂ O	0.030	0.007
7.	FeEDTA	10.53	2.63
8.	FeCl ₃ .6H ₂ O	7.7	1.925
	Citric acid Monohydrate	11.9	2.975

ANNEXURE-II

Composition of solution used in Rhizoscope study at CIRAD, France

Solution	Salt	Molecular weight	Stock solution (g/L)	Concentration stock solution (mm)	Final concentration	Amount to 1 Litre
NH ₄ + Mg	(NH ₄) ₂ SO ₄	132.14	33	250	0.5 Mm	2 mL
(solution 1)	MgSO ₄ .7H ₂ O	246.49	197.2	800	1.6 mM	
Ca + K	Ca(NO ₃) ₂ .4H ₂ O	236.15	141.7	600	1.2 mM	2 mL
(solution 2)	KNO ₃	101.11	35.4L	350	0.7 mM	
Oligo-elements (solution 3)	MnSO ₄ .H ₂ O	169.02	1.7	10	10 µM	1 mL
	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	1235.6	0.2	0.16	0.16 µM	
	ZnSO ₄ .7H ₂ O	287.54	0.2	0.7	0.7 µM	
	CuSO ₄ .5H ₂ O	249.68	0.2	0.8	0.8 µM	
	H ₃ BO ₃	61.83	1.4	22.6	22.6 µM	
FeEDTA (solution 4)	FeSO ₄	278	5.56	20	100 µM	5 mL
	Na ₂ EDTA	372.24	7.44	20	100 µM	
KH ₂ PO ₄ (control) (P)	KH ₂ PO ₄	136.09	27.2	200	0.4 mM	2 mL
KH ₂ PO ₄ (stress) (P/8)	KH ₂ PO ₄	17.01	3.4	25	0.05 mM	0.25 mL

ANNEXURE III

DNA fingerprint database of HKR47 x MAS26 derived lines using SSR markers

Plant	RM204	RM234	RM237	RM475	RM169	RM447	RM458	RM440	RM310	RM240	RM544	RM314	RM251	RM477
HKR47	106	156	134	208	191	118	189	211	98	145	248	106	147	221
MAS26	178	162	137	200	167	105	180	209	116	136	252	108	150	219
A1(1)	106/178	156/162	137	208/200	191/167	118/105	180	211/209	116	145	252	106/108	147/150	219
A2	178	162	137	208/200	191/167	118	189	211/209	98/116	145	248/252	108	147/150	221
A3	178	156/162	137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
A4	106/178	156/162	134/137	208/200	191/167	118/105	189/180	211/209	116	145/136	252	106/108	147	221/219
A5	106/178	156	137	208/200	191	118	189/180	211/209	98/116	136	248/252	106/108	147	221/219
A5(1)	106	162	134/137	208	191	118	189/180	209	116	136	252	106	150	221/219
A6	106	162	134	208/200	191	118	180	211/209	98/116	145/136	248/252	106	147/150	219
A11	178	156	137	208/200	191/167	105	180	211/209	98	145/136	248	108	147	219
B1	178	156/162	134/137	208/200	167	118/105	189/180	211/209	98/116	145	248/252	106/108	150	221/219
B1(1)	106/178	162	134	200	191	118	189	209	98/116	136	248/252	106/108	147/150	221
B2(1)	106	162	137	200	167	105	180	211	98/116	145/136	248/252	106	147/150	219
B6	106/178	156	134	208	167	118	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
B10	106	156/162	134	208	167	118/105	180	211/209	116	136	252	106	150	219
C1	178	162	137	200	191/167	118	180	211/209	116	145	252	108	147	219
C1(1)	106	162	137	200	167	118/105	189/180	211	116	136	252	106	147	221/219
C2	178	162	137	200	191	118/105	189	211/209	98	145	248	108	150	221/219
D2	106	156/162	137	200	191	118	189	211/209	116	145	252	106	150	221
D3	106/178	162	137	200	191/167	105	189/180	209	98/116	136	248/252	108	147	221/219

D4	106/178	156/162	137	200	167	118	189	209	98	136	248	106/108	147/150	221
E1	106/178	156/162	137	200	191/167	118/105	189/180	211/209	98/116	145	248/252	108	147	221/219
E2	106	156/162	137	200	191/167	118/105	189/180	211/209	116	145	252	106/108	147/150	221/219
E2(1)	106	162	134	208	191/167	118	189	211/209	98/116	145/136	248/252	106/108	147/150	221
E3	178	156/162	134	208	191/167	105	189/180	209	98/116	145/136	248/252	106/108	147/150	221/219
E4	106/178	156/162	134	208	191/167	105	180	209	98/116	145/136	248/252	106/108	150	219
E5	106/178	156/162	134	208	191/167	118	189/180	211/209	98/116	145/136	248/252	106/108	150	221/219
E6	178	156/162	134	200	167	118/105	189/180	209	116	145	252	108	150	221/219
E6(1)	106/178	162	137	200	167	105	180	211/209	98	145/136	248	106/108	147/150	219
E6(2)	106/178	162	137	200	191/167	118/105	189/180	211/209	98	145/136	248	108	147	221/219
E7	106	156	137	200	167	118/105	189/180	209	116	145/136	252	106	147	221/219
E7(1)	178	156/162	137	200	167	118/105	189	211/209	116	145/136	252	106/108	147	221
E8	106/178	162	137	200	167	118/105	189/180	211/209	116	136	252	106	150	221/219
E9	178	156/162	137	200	191/167	118/105	189/180	211/209	98	145	248	106/108	150	221/219
E10	178	156/162	137	200	191/167	118/105	189/180	209	98/116	145	248/252	106/108	147	221/219
E11	106/178	162	137	208/200	191/167	105	180	211/209	98/116	145	248/252	106/108	150	219
E12	106/178	156/162	137	208/200	167	118/105	189/180	211/209	116	145	252	108	147	221/219
E13	106/178	156	137	208/200	167	105	189/180	209	116	145	252	106/108	147/150	221/219
E16	106	162	137	208/200	191/167	118/105	189/180	209	98	136	248	106	147/150	221/219
E18	106/178	156/162	137	200	191/167	118/105	189	211/209	98	145/136	248	106/108	147/150	221
E19	178	162	137	208	167	105	180	211/209	98	145/136	248	108	147/150	219
E19(1)	106/178	162	137	208	191/167	105	180	211/209	98	145/136	248	106/108	147	219
E20	106/178	162	134	208/200	167	118	189	211/209	98/116	145/136	248/252	106/108	147/150	221

Ea	106/178	162	134	200	191	105	180	211/209	116	145/136	252	106/108	150	219
AK1	106/178	156/162	134	208	191	118/105	189/180	211/209	98	145	248	106/108	147/150	221/219
AK2	106/178	162	134	200	191/167	118/105	189/180	209	98/116	145/136	248/252	106/108	147/150	221/219
AK3	106/178	156/162	134	208	191/167	118/105	180	211/209	98	145/136	248	108	147/150	219
AK4	106/178	156/162	134/137	208	191/167	118	189/180	211/209	116	145/136	252	106	147	221/219
AK5	106	156/162	137	208	167	118/105	189/180	209	98	145/136	248	106	150	221/219
AK6	106/178	156/162	134/137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
AK7	106/178	162	137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	150	221/219
AK8	106/178	156	134	208/200	191	118	189/180	211/209	98/116	145/136	248/252	106/108	150	221/219
AK9	106/178	162	137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	150	221/219
AK10	106/178	156	134	208/200	191	118	189/180	211/209	98/116	145/136	248/252	106/108	150	221/219
AK11	106/178	162	137	200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	108	147/150	221/219
AK12	106/178	156/162	134/137	200	191/167	118	189	209	98	145/136	248	108	147/150	221
AK13	106/178	156	134	200	191/167	118/105	189/180	211/209	98/116	145	248/252	106/108	147/150	221/219
AK14	106/178	162	137	208/200	191/167	118/105	189/180	209	98/116	145	248/252	106/108	147/150	221/219
AK15	106	156	134	208/200	191/167	118/105	180	211/209	98/116	145/136	248/252	106/108	150	219
AK16	106	162	137	200	167	118/105	189/180	211/209	116	145/136	252	106/108	150	221/219
AK17	106/178	162	137	208/200	191/167	118/105	189/180	211	98	145/136	248	106/108	147	221/219
AK18	106/178	156	134	208/200	191/167	118/105	189/180	211/209	116	145/136	252	106	150	221/219
AK19	106/178	156/162	134/137	208	191/167	118/105	189/180	211/209	98	145/136	248	106/108	147/150	221/219
AK20	106/178	162	137	208	191/167	118/105	180	211/209	98/116	145/136	248/252	106/108	147/150	221
AK21	106	162	137	200	191/167	118/105	189/180	211	98/116	145/136	248/252	106/108	147/150	221/219
AK22	106/178	156/162	137	200	191	118	189	211/209	98	145/136	248	106/108	147/150	221

AK23	106/178	156/162	137	208	191	118/105	189	209	98	145/136	248	106	147/150	221
AK24	106/178	162	137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
AK25	106/178	156/162	137	208	191	118/105	189/180	211/209	98/116	145	248/252	106/108	147/150	221/219
AK26	106/178	156/162	134/137	208	191/167	118/105	189/180	211	98/116	145	248/252	106/108	147/150	221/219
AK27	106/178	156	134/137	208/200	191/167	105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
AK28	106/178	156/162	137	208/200	191/167	118/105	189/180	211/209	98	145/136	248	106/108	147/150	221/219
AK29	178	156/162	137	208/200	191/167	118/105	189/180	211/209	98/116	145	248/252	106/108	147/150	221/219
AK30	106/178	162	137	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
AK31	106/178	162	134	208/200	191/167	118/105	189/180	211/209	98/116	145/136	248/252	108	147/150	221/219
AK32	106/178	156/162	137	200	167	105	189	211/209	116	145/136	252	106/108	147/150	219
AK33	106/178	156/162	134/137	208/200	191/167	105	180	211/209	98/116	145/136	248/252	106/108	147	219
AK34	178	156	134/137	208/200	191	105	189/180	211/209	98/116	145	248/252	106/108	147	221/219
AK35	106	156	134/137	208	191/167	118	189	211/209	98/116	145	248/252	106/108	150	221
AK36	106/178	162	137	208/200	191/167	105	189/180	209	98/116	145	248/252	106/108	147/150	221/219
AK37	106/178	162	137	200	191/167	118	189	211/209	98/116	145	248/252	108	147/150	221
AK38	106/178	156/162	137	208/200	191/167	118/105	189/180	211/209	116	145/136	252	106/108	147/150	221/219
AK39	106/178	156/162	134/137	208/200	191	118/105	189/180	209	98/116	145	248/252	106/108	147/150	221/219
AK40	106/178	156/162	137	208/200	191	118/105	189/180	211/209	98	145	248	108	147/150	221/219
BK1	106	156/162	137	200	191	118/105	189	209	116	145/136	252	106/108	147/150	221
BK2	106/178	156/162	134/137	200	191	118/105	180	211/209	98/116	145	248/252	106/108	147/150	219
BK3	106/178	162	137	208/200	191/167	118/105	180	211/209	116	145/136	252	106/108	147/150	219
BK4	178	156/162	134	208/200	191/167	118	189	211/209	98/116	145/136	248/252	106	147/150	221
BK5	106/178	156/162	134/137	208/200	191/167	105	180	211/209	98/116	145/136	248/252	106/108	147	219

BK6	106/178	156/162	137	200	191/167	105	180	211/209	98/116	145/136	248/252	106/108	150	219
BK7	106/178	156/162	134/137	208/200	191	118/105	189/180	209	116	145/136	252	106/108	150	221/219
CK3	106/178	162	137	200	167	105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
CK4	106/178	162	137	208/200	191	118/105	189/180	211/209	98/116	145	248/252	108	147/150	221/219
DK2	106/178	162	137	208/200	191/167	118/105	180	211	98/116	145	248/252	106/108	147/150	219
EK1	106/178	156/162	137	208/200	191/167	105	189/180	211	98/116	145	248/252	108	150	221/219
EK2	106/178	156/162	137	208/200	191	118/105	189/180	211/209	98/116	145/136	248/252	106/108	147/150	221/219
EK3	106/178	162	137	200	191	105	180	211/209	98/116	145/136	248/252	106/108	147/150	219
PLANT	RM5412	RM258	RM317	RM287	RM318	RM333	RM3331	RM416	RM247	RM201	RM205	RM152	RM202	RM228
HKR47	106	152	190	108	154	180	100	114	138	156	125	151	172	150
MAS26	103	150	192	118	156	176	129	112	135	144	120	142	189	110
A1(1)	103	150/152	192	108/118	154	176	129	112	135/138	156/144	125/120	151	172	150
A2	106/103	150	192	108/118	154	176	100	112	135/138	156/144	120	151	172/189	110
A3	103	152	190	118	154	176	129	112	135	156	120	142	172/189	150
A4	103	152	192	118	154	176	129	112	135	144	125	142	189	110
A5	106/103	150/152	192	108/118	156	176	129	112	135	144	125	142	172/189	150
A5(1)	106/103	150/152	190	108	156	180/176	129	112	135/138	156/144	125/120	142	172	150
A6	106/103	150	192	108/118	154/156	176	129	112	135/138	156/144	125	151	172	110
A11	106/103	150/152	192	108/118	154/156	176	129	114	135	144	125	151	189	150
B1	103	150/152	192	108/118	154	176	129	112	135/138	156/144	120	151	172	150
B1(1)	103	150/152	190	108/118	156	180	129	112	135/138	144	125/120	142	172	110/150
B2(1)	106/103	150/152	192	118	154	176	129	112	135	156/144	125	142	172/189	150
B6	106/103	150/152	192	118	154	180	129	112	135/138	156/144	125/120	142	189	150

B10	106/103	150/152	192	108	156	176	100	112	135	144	125	142	172	110
C1	106/103	152	190	108	156	180	100	112	135	144	125	142	172	150
C1(1)	103	150/152	192	118	156	176	129	112	135/138	156/144	125/120	151	189	150
C2	103	150/152	192	108	154	180	129	114/112	135/138	144	125	142	172/189	150
D2	106/103	150/152	190	108/118	154	180	129	112	135/138	156/144	125/120	151	172/189	150
D3	103	150	192	108	156	180/176	100	112	135	144	125/120	142	172/189	150
D4	103	150/152	190	118	156	176	129	112	135/138	156/144	125	151/142	189	110
E1	103	150	190	108	154	176	129	112	135/138	156	120	151/142	172/189	150
E2	103	152	190	118	154	176	129	112	135/138	156/144	125/120	151/142	172/189	150
E2(1)	103	152	192	108/118	154/156	176	129	112	135/138	144	125	142	172/189	150
E3	106	150/152	190	108	154/156	176	129	112	135/138	144	125/120	142	172	110
E4	103	152	192	108/118	154	180/176	129	114/112	135/138	156/144	120	142	172	110/150
E5	106/103	150	192	108	154	180	129	114	135	156	120	142	172	110/150
E6	103	150	192	108/118	154	176	129	114	135/138	144	125/120	142	172/189	110/150
E6(1)	106/103	152	190	108/118	154	180	129	112	135/138	144	125/120	142	172/189	150
E6(2)	106/103	150	192	108/118	156	176	100	112	135	156/144	125/120	142	172/189	150
E7	106/103	150	192	108/118	154	180	129	112	135	156/144	120	142	172/189	110
E7(1)	103	152	192	118	154/156	176	129	112	135	156/144	125	151/142	189	150
E8	106/103	150/152	192	118	154/156	180/176	100	114/112	135	156/144	120	151/142	172/189	110/150
E9	106/103	152	192	108	154	180/176	129	112	135	156/144	125/120	142	172/189	150
E10	106/103	150/152	190	108/118	154	176	100	112	135	156/144	125/120	151/142	172/189	150
E11	103	150/152	192	108/118	154	176	129	112	135/138	156/144	120	142	172/189	150
E12	103	150/152	192	108/118	154	176	129	114	135/138	156/144	125	142	172/189	150

E13	103	150/152	192	108	154	180	129	112	135	156/144	120	142	172/189	150
E16	106/103	150/152	190/192	108	156	176	129	114	135	156	120	142	172	110/150
E18	106/103	150/152	190/192	108	154/156	180/176	129	114/112	135	144	125	142	172	110/150
E19	103	150/152	192	108/118	154/156	180	129	114/112	135	144	125/120	142	189	150
E19(1)	103	150/152	192	108/118	154/156	180	129	112	135/138	156/144	120	142	172	150
E20	106/103	150	192	108/118	154	176	100	112	135/138	144	125	151/142	172/189	150
Ea	106/103	150	190	108/118	154/156	180	100	114	135	156/144	125/120	151/142	172	110
AK1	106/103	150/152	192	108	154	176	129	112	135	156/144	120	142	172	150
AK2	103	150/152	192	108/118	156	176	129	112	135	156/144	120	142	172/189	150
AK3	103	150/152	192	108/118	154	176	129	112	135	156/144	120	142	189	110/150
AK4	106/103	152	192	108/118	154	180	129	112	135	156/144	120	151/142	172/189	110/150
AK5	103	150/152	190/192	108	154	180	129	112	135	144	125	142	172	110/150
AK6	103	152	192	118	154	176	129	112	135	144	125	142	172	110/150
AK7	106/103	150/152	192	108/118	156	176	129	112	135/138	156/144	125/120	142	172	110/150
AK8	106/103	150/152	192	108/118	154/156	180/176	129	112	135	156/144	125/120	142	172	110/150
AK9	106/103	150/152	192	108/118	156	176	129	112	135/138	156/144	125/120	142	172	110/150
AK10	106/103	150/152	192	108/118	154/156	180/176	129	112	135	156/144	125/120	142	172	110/150
AK11	106/103	150	192	108	154	180/176	129	114/112	135	156/144	125	142	172/189	110/150
AK12	103	150	192	108/118	154/156	176	129	112	135/138	156/144	120	151/142	172	150
AK13	106/103	152	190/192	108	154	180/176	129	114/112	135/138	156/144	125	151	172	150
AK14	103	150	192	108/118	154	180	129	114/112	135/138	156	125/120	151/142	172	110/150
AK15	106/103	150/152	192	108/118	154/156	180	129	114/112	135/138	156/144	125/120	151/142	172	150
AK16	106/103	152	192	108/118	154/156	176	100	114/112	138	156/144	120	142	172	150

AK17	103	150/152	192	108/118	154	176	129	112	135/138	156/144	125/120	151	172	150
AK18	106/103	150	190	108/118	154/156	180/176	100	112	135/138	144	125	151/142	172/189	150
AK19	106/103	150	192	108/118	154	180/176	129	114	135	156/144	120	142	172/189	110/150
AK20	106/103	150/152	192	108/118	156	180/176	129	112	138	156/144	120	142	172/189	110/150
AK21	106/103	150/152	190	108/118	154/156	180/176	129	112	135/138	156/144	125/120	142	172	110/150
AK22	106/103	150/152	190	108	156	176	129	112	135/138	156/144	125/120	142	172	150
AK23	106/103	150/152	190	108/118	154/156	180/176	129	112	135/138	144	120	142	172	110
AK24	106/103	150/152	192	108/118	154	176	129	114/112	135/138	156/144	125/120	151/142	172/189	110/150
AK25	106/103	150	190	108	154	180/176	129	114/112	135/138	144	125/120	142	172/189	110/150
AK26	106	150/152	190	108/118	154	176	129	114/112	135/138	156/144	125/120	151	172/189	150
AK27	103	150/152	190/192	108/118	154/156	180/176	129	112	135/138	156	125/120	142	172	150
AK28	106/103	150/152	192	108/118	154/156	180/176	100	112	135/138	156/144	125/120	142	172/189	110/150
AK29	106/103	152	192	108/118	154	180/176	129	112	135	156/144	125/120	151/142	172/189	110/150
AK30	103	150/152	192	108/118	154/156	176	129	112	135/138	156/144	125/120	151/142	172	110/150
AK31	103	150/152	190	108	154	176	129	112	135/138	156/144	120	151	172	110/150
AK32	106	150	192	108/118	154	180/176	129	112	135	156/144	125	142	172	110/150
AK33	106/103	150/152	190	108/118	154/156	176	129	114	138	156	125/120	142	172/189	150
AK34	106/103	150/152	192	118	154	180/176	129	114/112	135	156/144	125/120	151	172/189	150
AK35	103	152	192	108/118	154	180/176	129	114	135	156/144	125/120	151/142	189	150
AK36	103	152	192	108	154	180/176	129	114/112	135	156/144	125/120	151	189	110
AK37	106/103	150/152	192	108/118	154/156	180/176	129	114	135	156/144	120	151	172/189	110
AK38	103	150/152	192	108/118	154	180	100/129	114	135	156/144	120	142	172/189	110/150
AK39	103	150/152	192	118	154	180/176	129	112	135	156/144	120	151/142	172/189	110

AK40	106/103	150	192	108/118	154/156	180/176	129	112	135	156/144	125/120	151/142	172/189	110/150
BK1	106/103	150	192	118	154	180/176	129	114	138	156/144	125	151/142	172/189	110/150
BK2	106/103	152	192	118	156	180/176	129	112	135	156/144	125/120	151/142	172/189	110
BK3	106/103	150/152	192	108	154	180/176	100	112	138	144	125/120	142	172/189	110/150
BK4	103	152	190	108/118	154	180/176	100	112	135	156/144	125/120	142	172	110/150
BK5	103	152	192	108/118	154	180/176	129	112	135/138	156/144	125/120	142	172/189	110
BK6	106/103	150/152	192	108/118	154	180/176	129	112	135/138	156/144	120	142	172/189	110/150
BK7	103	150/152	192	118	154	180/176	100	112	135/138	156/144	125/120	142	189	110/150
CK3	106	150/152	192	118	154	176	129	112	135/138	144	125	151/142	189	110
CK4	106/103	150/152	190	108/118	154	176	129	112	135/138	144	125	142	172/189	110
DK2	106/103	150/152	190	118	154/156	176	129	114/112	135/138	156/144	125/120	151/142	189	110
EK1	106/103	152	192	118	154/156	180/176	129	112	135/138	156/144	120	151/142	172/189	110
EK2	106/103	152	192	108/118	154	180/176	129	114	135/138	156/144	120	142	172/189	110
EK3	106/103	152	190	118	154	180/176	129	114	135/138	156/144	120	142	189	110
PLANT	RM4	RM6	RM12	RM218	RM72	RM222	RM212	RM525	RM351	RM8094	RM209	RM587	RM528	RM539
HKR47	155	180	252	128	152	222	136	110	134	209	127	202	248	281
MAS26	152	182	250	148	158	218	122	107	142	207	150	217	252	278
A1(1)	155	180	252	128/148	152	222	136/112	110	134/142	209/207	127/150	202/217	248/252	281/278
A2	155	180	252	128/148	152	218	136/112	110	134/142	207	127/150	217	248/252	281/278
A3	155/152	180/182	252	128/148	152	222/218	122	110/107	134/142	207	150	202/217	248	281/278
A4	155/152	180/182	252/250	128/148	152/158	222/218	136	107	134/142	209/207	150	202/217	248	281/278
A5	152	182	252	148	152/158	222/218	136	107	142	207	150	202/217	248/252	281/278
A5(1)	152	182	252/250	148	152/158	222/218	136/112	107	134/142	207	127	202	248/252	281/278

A6	152	180/182	252	128/148	152/158	222/218	122	110/107	134/142	209	127	202	248/252	281
A11	155	180/182	252/250	128	158	222/218	136	110/107	134/142	207	127/150	217	248	281/278
B1	155/152	180	250	148	152/158	222/218	136/112	110	134/142	207	150	217	252	281
B1(1)	155	182	252/250	148	152/158	218	136/112	107	134/142	207	150	202/217	248	281
B2(1)	155/152	180/182	252/250	148	152/158	218	136	110	142	209	150	202	248/252	281/278
B6	155/152	180/182	252	128	152/158	218	136/112	110	134	207	150	202/217	248	281/278
B10	155	182	252	148	152	218	136/112	107	142	207	150	202	248/252	281/278
C1	155/152	182	250	128	152	218	122	107	134	209/207	127/150	217	252	281/278
C1(1)	152	182	250	128	152	222	136/112	107	134	207	127	202/217	252	281
C2	155	180	250	148	158	222/218	122	110/107	134	207	150	217	252	281/278
D2	155/152	180	250	148	152/158	222/218	136	110	134/142	207	127	202	252	281
D3	155/152	182	250	128	152/158	218	136/112	107	134/142	209/207	150	217	248	281/278
D4	155/152	182	250	128/148	158	218	136	107	134	209/207	127	202/217	248	281/278
E1	155/152	180	250	128/148	152	222	136/112	110	142	209/207	150	202/217	248	281/278
E2	155/152	180	250	148	152	218	122	110	134/142	209	150	202/217	248/252	281
E2(1)	155/152	180/182	250	128/148	152/158	218	136/112	110/107	134/142	209/207	127/150	202	248/252	281/278
E3	155/152	180/182	250	128/148	152/158	222/218	136/112	110/107	134/142	209/207	127/150	217	248/252	278
E4	155	180/182	252	148	152/158	218	136/112	110/107	134/142	209/207	150	202	252	281/278
E5	155	180/182	252	148	152/158	222	136/112	110/107	134	207	150	202	252	281
E6	152	180	250	148	152	218	136/112	110	134	207	150	217	248/252	281/278
E6(1)	152	180/182	250	128/148	158	222	136/112	110/107	142	207	150	202/217	248/252	281/278
E6(2)	152	180/182	252	128	158	222	122	110/107	134/142	207	127/150	202/217	248/252	278
E7	155/152	180/182	250	128	152	218	136/112	110/107	134/142	207	127/150	202	248	281

E7(1)	155/152	180/182	250	128/148	152	218	136/112	110/107	134/142	207	150	217	248	281
E8	155/152	182	250	128/148	152	218	136	110/107	134/142	207	150	202/217	252	281/278
E9	155/152	180	250	128/148	158	218	136	110	134	207	127/150	217	248	281/278
E10	155/152	180	250	128/148	152/158	218	136/112	107	142	209/207	150	217	252	281/278
E11	155/152	180	250	148	158	218	122	110	134/142	209/207	127/150	202/217	248/252	281/278
E12	152	180	250	128/148	152	222/218	136/112	110/107	134/142	209/207	150	202/217	248/252	281/278
E13	152	180	250	128/148	152	222	136	110	134/142	209	127/150	202/217	248/252	281/278
E16	152	182	252	128/148	158	222	136	107	134/142	207	150	202	248	281/278
E18	152	180/182	250	128/148	158	218	136/112	110/107	142	207	127	202/217	248/252	281
E19	155/152	180/182	250	128/148	158	222/218	122	110/107	134/142	209	127/150	217	248/252	278
E19(1)	155/152	180/182	250	128	158	218	136/112	110/107	134	209	150	202	252	281/278
E20	155/152	180/182	250	128/148	152/158	218	122	110/107	134/142	207	127/150	202/217	248/252	281/278
Ea	152	180/182	250	148	152	218	122	110/107	142	207	127/150	202/217	248/252	281/278
AK1	152	180	250	128/148	158	218	136	110	134/142	207	127	202/217	248	281/278
AK2	155/152	180/182	250	128/148	152	222/218	136/112	107	142	207	150	202/217	248/252	278
AK3	152	180/182	252	128/148	158	218	136/112	110/107	134/142	207	127/150	217	248	281/278
AK4	155/152	180/182	252/250	128	152	218	136/112	110/107	134/142	207	127/150	202/217	252	281/278
AK5	155/152	180/182	252/250	148	152	218	136/112	110/107	134/142	207	150	202	248/252	281
AK6	155	180/182	252/250	128/148	158	218	136	110	142	209/207	127/150	202/217	248/252	281/278
AK7	155	180/182	252	128/148	152/158	218	136/112	110	134/142	207	127/150	202/217	248/252	281/278
AK8	152155	180/182	252/250	128/148	152/158	222/218	136	107	142	209/207	127/150	202	248	281
AK9	155	180/182	252	128/148	152/158	218	136/112	110	134/142	207	127/150	202/217	248/252	281/278
AK10	152155	180/182	252/250	128/148	152/158	222/218	136	107	142	209/207	127/150	202	248	281

AK11	155/152	180/182	252/250	128	152/158	222/218	136	110/107	134/142	207	127	202/217	248/252	281/278
AK12	152	180/182	252/250	128/148	152/158	218	136/112	110/107	134/142	209/207	127/150	217	248/252	281/278
AK13	155/152	180	252/250	128/148	152	218	122	110/107	142	207	127/150	202	248	281
AK14	155/152	180	252/250	128/148	152/158	222/218	136	110/107	134/142	207	150	202	248/252	281/278
AK15	155/152	180/182	252/250	128/148	152/158	218	136	110	142	207	150	202/217	248/252	281/278
AK16	155/152	180/182	252/250	148	152	218	136/112	110/107	134/142	207	127/150	202/217	248/252	281/278
AK17	155/152	180/182	252/250	128	158	218	136/112	110	142	209/207	127/150	202/217	248/252	281/278
AK18	155/152	180/182	252/250	128/148	152/158	218	136	110/107	134	209	150	202/217	248/252	281
AK19	155/152	180/182	252/250	128/148	158	222/218	136/112	107	134/142	209	150	202/217	248/252	281/278
AK20	155/152	180/182	252/250	128/148	152/158	218	122	110	142	207	150	202/217	248	281/278
AK21	155	180/182	252	128/148	152/158	218	136/112	110/107	134/142	209/207	127/150	202/217	252	281/278
AK22	155/152	180/182	252/250	128/148	152/158	222/218	122	110/107	134/142	209/207	127/150	202/217	248/252	281/278
AK23	155/152	180	250	148	152/158	222/218	136/112	107	142	209	150	202	248	281/278
AK24	155/152	180/182	252/250	128/148	152/158	222	136/112	110/107	142	209/207	127/150	202/217	248/252	281/278
AK25	155/152	180	252/250	128/148	152/158	218	136/112	110	142	207	127/150	202/217	248/252	281
AK26	155/152	180	252/250	128/148	152	218	136/112	110	134/142	209/207	127/150	202/217	252	281/278
AK27	155/152	180/182	252/250	128/148	152	222/218	136/112	110	134/142	207	150	202/217	252	281/278
AK28	152	180/182	252/250	128/148	158	218	136/112	110	142	207	127/150	202/217	252	281/278
AK29	155/152	180	252	128/148	152	218	136/112	110/107	134/142	209/207	127/150	217	248/252	281/278
AK30	155	180/182	252/250	128/148	152/158	218	136/112	110/107	134/142	209/207	127/150	202	252	281
AK31	155	180/182	252	128/148	152/158	218	136/112	110	142	209	127/150	202/217	252	281/278
AK32	152	180/182	252/250	128/148	152/158	218	136/112	110	142	207	127/150	202	248/252	281/278
AK33	152	180/182	252/250	128/148	152/158	222/218	136	110/107	134	209/207	127/150	202/217	248/252	278

AK34	152	180	252/250	128/148	152/158	222	136	110/107	134/142	207	127/150	217	248/252	281/278
AK35	155/152	180	252/250	148	152/158	222/218	122	110	134/142	209/207	127/150	202	248/252	281/278
AK36	155/152	180	252	128/148	152/158	218	136/112	107	142	209/207	127/150	202/217	252	281/278
AK37	152	180	252/250	128/148	158	222/218	136/112	110	142	209/207	127/150	202	252	281/278
AK38	155	180/182	252/250	128/148	152/158	222/218	136/112	110	134/142	209/207	150	202/217	248/252	281/278
AK39	155/152	180/182	252/250	128/148	152/158	222/218	136/112	110/107	134/142	209/207	150	202	252	281/278
AK40	152	180	252/250	128/148	158	222/218	136/112	110/107	134/142	207	150	202/217	252	281/278
BK1	155/152	180/182	252	128/148	152/158	218	136/112	110/107	134/142	207	127/150	202	252	281/278
BK2	155/152	180	252/250	128/148	158	222	122	110/107	134	207	150	202	248	281/278
BK3	152	180/182	252/250	128/148	152	218	136/112	110/107	142	209/207	127/150	217	248	281/278
BK4	152	180/182	252/250	148	152/158	222/218	136/112	110/107	134/142	209/207	150	202/217	248/252	281/278
BK5	155/152	180/182	252/250	128/148	152/158	218	136	110/107	134/142	207	150	202/217	248/252	281/278
BK6	152	180/182	252/250	128/148	158	218	136/112	107	134/142	207	150	202/217	248/252	281/278
BK7	155/152	180/182	250	128/148	152/158	222	136/112	110/107	134/142	207	127/150	202/217	252	281/278
CK3	155/152	180/182	252/250	148	158	222/218	136/112	110/107	134/142	207	127/150	217	248/252	278
CK4	152	180	252	128/148	152	222/218	136/112	110	134/142	207	127/150	202/217	248/252	281/278
DK2	155/152	180	252/250	148	152/158	218	136/112	110	134	207	127/150	202/217	248/252	281/278
EK1	152	180	252	128/148	152/158	222/218	122	110/107	134/142	209/207	150	202/217	248/252	281/278
EK2	152	180/182	252	128/148	152/158	218	136/112	110/107	134/142	209/207	127/150	202/217	252	281/278
EK3	152	180/182	252/250	128/148	152/158	218	136	110	134/142	207	150	202/217	252	281/278

PLANT	RM206	RM524	RM527	RM594	RM517	RM28048	RM488	RM304	RM337	RM302	RM207	RM248	RM225	RM144	RM242
HKR47	125	207	238	300	260	110	215	160	192	190	139	94	125	230	232
MAS26	146	198	240	308	288	97	195	136	158	168	118	104	138	220	194
A1(1)	146	207/198	238/240	300	260/288	110	215	160/136	158	168/190	139/118	104	125/138	220	194
A2	146	198	238/240	300/308	260/288	110	215/195	136	158	168/190	139	104	138	220	194
A3	125/146	207/198	238/240	300/308	288	110/97	195	160	158	190	139/118	104	138	220	232
A4	146	207/198	238/240	300	260/288	110/97	215/195	160	158	168	139	104	138	220	194
A5	125/146	207/198	238/240	300/308	260/288	97	215	160/136	158	168	118	94/104	125/138	220	194
A5(1)	125/146	207	238/240	300/308	260/288	97	215/195	136	158	168	139/118	104	125	230/220	194
A6	125/146	207/198	238	308	260/288	110	195	136	192	168	139/118	94/104	125	230/220	194
A11	125/146	207	238/240	300/308	260	97	215	160/136	192	168	118	104	138	220	194
B1	146	207/198	238	300/308	288	97	215	160/136	158,	168	118	104	138	230/220	194
B1(1)	125/146	198	238/240	300/308	288	97	215/195	160/136	158	168	118	94/104	125/138	220	194
B2(1)	125/146	198	238	300	288	97	215	136	158	168/190	139/118	104	125	230	194
B6	125/146	207/198	240	300/308	260	97	215	160/136	158	168	139	104	125/138	230	194
B10	125/146	207	238/240	308	260/288	97	195	136	158	168	118	104	125	220	194
C1	125	207/198	240	308	260/288	110	195	160	158	168	118	104	138	220	194
C1(1)	125/146	207/198	238/240	300	260	97	215	136	192	168	118	94/104	138	220	194
C2	125/146	198	238/240	300	288	97	195	160	158	168	139	94/104	125/138	220	194
D2	125/146	207/198	238	300	260/288	110	215/195	160/136	192	168	139	104	125/138	230	194
D3	125	207/198	238	300/308	260/288	97	215/195	160	158	168	139/118	104	125/138	220	194
D4	125	198	238	300	260/288	110	215	160/136	158	168	139/118	94/104	125/138	230	194
E1	125/146	207/198	240	300/308	260/288	110	215	136	144	168	139	94/104	125/138	230/220	232
E2	146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	158	168/190	139	94	125/138	230/220	194

E2(1)	146	198	238/240	300	260/288	110/97	215/195	160/136	158	168	139	104	125	220	194
E3	125	198	238/240	300/308	260/288	97	215/195	160/136	158	168	118	94/104	138	220	194
E4	125/146	198	238/240	300/308	288	110/97	195	160/136	158	168	118	94/104	125/138	230	232/194
E5	125/146	198	238	300/308	288	110	215/195	160	158	168	139/118	94/104	125	230/220	232
E6	125/146	207/198	238/240	300/308	260/288	110/97	215/195	136	158	168	139/118	94/104	138	230	194
E6(1)	125/146	198	238/240	300	260	97	215/195	160	158	168	139	104	125/138	220	194
E6(2)	125/146	198	240	300/308	260	110/97	215	160	158	168	139/118	104	125/138	220	232/194
E7	125	207/198	238	300	260	110/97	215	136	158	168	139/118	94/104	125	230/220	232/194
E7(1)	146	207	238/240	300/308	260/288	110/97	195	160	158	190	139	94/104	138	220	232/194
E8	125/146	207/198	238/240	308	288	110/97	195	160/136	158	168	139	94/104	138	220	232/194
E9	125	207/198	238/240	300	288	97	195	160	158	168	139/118	94/104	138	230	232/194
E10	125/146	207/198	238/240	300/308	260	110	215/195	160/136	158	168	139	94/104	138	220	232
E11	125/146	207/198	238/240	300/308	288	110/97	195	160/136	158	168/190	139	104	125/138	220	232
E12	125/146	207/198	238/240	300	260	110	195	136	158	168	139/118	104	125/138	220	232/194
E13	146	198	238	300	260/288	110/97	215/195	160/136	158	190	139	94	125/138	220	232
E16	146	207/198	238/240	300/308	288	97	215	136	158	168	118	94	125	220	232
E18	125/146	207/198	238	300/308	260/288	97	215/195	136	192	168	139	94	125/138	230/220	194
E19	125/146	207	240	300/308	260/288	97	215/195	160/136	158	168	139	94	138	230	194
E19(1)	146	207/198	238/240	300/308	260/288	97	215/195	160/136	158	168	139	94/104	125/138	220	232/194
E20	125/146	198	238/240	300	260	97	215/195	136	158	168	118	94	125/138	220	232/194
Ea	125/146	207/198	240	300/308	260/288	110	195	160/136	158	168	118	94	125/138	230	194
AK1	125/146	207	240	300/308	260/288	97	215	160	158	168	139	104	125/138	230/220	232/194
AK2	125	207/198	240	300/308	260	97	195	160/136	158	168	118	104	125/138	230/220	232/194
AK3	125/146	198	238/240	300/308	260/288	97	215	136	158	168	139	104	138	230	232/194
AK4	125	207/198	238/240	300/308	260/288	97	215/195	160/136	158	168/190	139	104	125/138	230/220	232/194

AK5	125/146	198	238	300/308	288	97	215/195	160	192/158	168	118	104	125	220	232/194
AK6	146	198	238/240	300/308	260/288	97	215/195	160/136	158	168	139	94/104	125/138	230/220	232/194
AK7	125	207/198	238/240	300/308	288	97	215/195	160/136	158	168	139/118	94/104	125/138	230/220	232/194
AK8	125/146	207/198	238/240	300	260/288	97	215	160/136	158	168	139	94/104	125/138	230/220	232/194
AK9	125	207/198	238/240	300/308	288	97	215/195	160/136	158	168	139/118	94/104	125/138	230/220	232/194
AK10	125/146	207/198	238/240	300	260/288	97	215	160/136	158	168	139	94/104	125/138	230/220	232/194
AK11	125	198	238/240	300/308	260/288	110	195	136	158	168	139	94/104	125/138	230/220	232/194
AK12	125/146	207/198	238/240	300/308	260/288	97	215/195	136	158	168	118	94	138	230	232/194
AK13	125/146	198	238	300/308	260/288	97	215/195	160/136	192	168/190	139	104	125	220	232/194
AK14	125/146	198	238	300/308	260/288	97	215/195	136	158	168	118	104	125	220	232
AK15	125/146	207/198	238/240	300	260	110/97	215/195	160/136	158	168	118	104	125/138	230/220	232/194
AK16	125	207/198	240	300/308	260	110/97	195	160	144	168	139	104	125/138	230/220	232/194
AK17	125/146	207/198	238/240	300/308	260	110/97	215/195	160/136	144	168	139/118	94/104	125/138	230	232/194
AK18	146	207/198	238	300/308	260/288	110/97	215/195	136	158	168	139/118	94/104	125/138	220	194
AK19	125/146	198	238/240	300/308	260/288	110/97	215	136	192/158	168	139/118	94/104	125/138	230/220	232/194
AK20	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	168	139/118	94/104	125	220	232/194
AK21	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	168	139/118	94	125	230/220	232/194
AK22	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	158	168	139/118	94/104	125/138	220	232/194
AK23	125/146	198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	168	139	104	125/138	220	232
AK24	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	190	139/118	104	125/138	220	232/194
AK25	125/146	207/198	238/240	300	260/288	97	215	160/136	192/158	168/190	139/118	104	125/138	220	232/194
AK26	125	207/198	238/240	300	260/288	110/97	215/195	160/136	192/158	190	139	104	125/138	230/220	232/194
AK27	125/146	207/198	238/240	300/308	260/288	97	215	160/136	192/158	190	139/118	104	125/138	220	232
AK28	125/146	207/198	238/240	300/308	260/288	110/97	215	160/136	158	168/190	139/118	94	138	230/220	232/194
AK29	125/146	207/198	238/240	300	260/288	110/97	215	160/136	192/158	168/190	139/118	94/104	138	220	232/194

AK30	125/146	198	238/240	300/308	260/288	110/97	215/195	160	192/158	168/190	139/118	94/104	125/138	230/220	232/194
AK31	125/146	198	238/240	300/308	260	97	215	136	192/158	168/190	118	104	125/138	230/220	232/194
AK32	125/146	198	238/240	300/308	260	110/97	195	136	158	168/190	139/118	104	125/138	230	232/194
AK33	125/146	198	240	300/308	288	110/97	195	160/136	192/158	168	118	94/104	125/138	220	232/194
AK34	125/146	207/198	238/240	300	260/288	97	195	160/136	158	168/190	118	94/104	125	220	232/194
AK35	125/146	198	240	300	288	97	195	160/136	192/158/200	168/190	139	104	125/138	220	232/194
AK36	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	168/190	139/118	94/104	125/138	230/220	232/194
AK37	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160/136	158R	168/190	118	94/104	125/138	220	232/194
AK38	125/146	207/198	238/240	300	260	110/97	215/195	160/136	192/158	190	118	94/104	125/138	220	232/194
AK39	125/146	198	238/240	300/308	260/288	110/97	215/195	160/136	158	168/190	139	94/104	125	220	232/194
AK40	125/146	198	238/240	300/308	260/288	110/97	215/195	160/136	192/158	168	118	94/104	125/138	220	232/194
BK1	125	207/198	238/240	300/308	260/288	110	195	160/136	192	168	118	104	125	230	232/194
BK2	125/146	207/198	238/240	300/308	260/288	97	215/195	160/136	158	168/190	139	94/104	125/138	220	232/194
BK3	125/146	198	238/240	300/308	260	97	215/195	160/136	192/158	168/190	139/118	104	125/138	220	232/194
BK4	125	207/198	238/240	300/308	288	110	215	160	192/158	168	118	104	138	230	232/194
BK5	125/146	207/198	238/240	300/308	260/288	110/97	215/195	160	192/158	168/190	139/118	104	125/138	220	232/194
BK6	125	207	238/240	300/308	288	97	195	160/136	192/158	168	139/118	104	125/138	230	232/194
BK7	125/146	198	238/240	308	288	97	195	160/136	158	168/190	118	104	125/138	220	232/194
CK3	125/146	207/198	240	300/308	260/288	110	215	160/136	192/158	168	139	104	138	220	194
CK4	125/146	198	238/240	300/308	260/288	110	195	160	192/158	168	139	104	125/138	230/220	232/194
DK2	146	207/198	238/240	300/308	288	97	215/195	160/136	192/158	168/190	118	104	125/138	230	232/194
EK1	125/146	207/198	238/240	300/308	288	97	215	160/136	158	168/190	139	94/104	125/138	220	232/194
EK2	125	207/198	238/240	300/308	288	110	215/195	160/136	192/158	168	139/118	104	125/138	230/220	232/194
EK3	125/146	198	238	300/308		97	215	160/136	192/158	168	139	94/104	125/138	230/220	232/194

PLANT	RM21	RM26	RM483	RM286	RM257	RM562	RM256	RM259	RM575	RM490	RM230	RM241	RM505	RM408	RM518	RM478
HKR47	160	105	309	112	152	248	107	162	201	107	257	145	197	120	167	201
MAS26	141	102	325	118	150	250	108	160	204	93	255	140	220	128	165	203
A1(1)	160/141	105/102	309	112/118	152/150	248/250	108/107	160/162	201/204	107	257/255	140	197/220	128/120	165	201/203
A2	160/141	105/102	309/325	112/118	152/150	248/250	107	160/162	201/204	107/93	255	140	197/220	128	165	201/203
A3	141	105/102	309/325	118	152	248/250	108/107	160	204	93	257/255	145	197/220	128/120	167/165	203
A4	141	105/102	309/325	112	152/150	248	108/107	160/162	201/204	107/93	255	140	197/220	128/120	167/165	201/203
A5	141	105/102	309/325	112/118	150	248/250	107	160	201/204	107/93	257/255	140	197/220	128	167/165	201/203
A5(1)	160	102	309/325	118	152/150	248/250	108	160/162	201	107/93	257/255	145	197	128/120	167/165	203
A6	160	105/102	309/325	112/118	152/150	250	108	160/162	204	107/93	255	140	197	120	167	201/203
A11	160/141	105/102	309	112/118	152/150	248/250	108/107	160	204	93	257/255	140	197	120	167	201/203
B1	141	105/102	309/325	112	152/150	248/250	108	160	201/204	107/93	257/255	140	220	120	165	201/203
B1(1)	141	102	309/325	118	152/150	248/250	107	160	201/204	107/93	257/255	145	220	128/120	167	201/203
B2(1)	141	105	309/325	112	150	248	108/107	162	201/204	107	255	140	220	128/120	167/165	203
B6	141	105/102	309/325	112	152/150	248/250	107	160/162	201/204	107	255	140	197	128/120	167/165	201/203
B10	141	105/102	309/325	118	152/150	250	107	160	201	107	257/255	140	220	128/120	167/165	201/203
C1	160/141	105/102	309/325	112	152	250	108	160/162	201	107	255	145	197	128	165	201/203
C1(1)	160	105	309/325	118	152	248	107	160/162	201/204	107/93	257/255	140	197	128	165	201/203
C2	141	105/102	309/325	118	152	250	108/107	160/162	201/204	107	257/255	140	197	128/120	165	201/203
D2	160	105/102	309/325	112	152	248/250	108/107	162	201	93	257/255	145	220	120	167	201
D3	141	102	309/325	118	150	248/250	108/107	160	204	107	257/255	140	197/220	128/120	167	203
D4	160	102	309/325	112	150	248/250	108/107	160	201	107/93	255	145	197	128/120	167/165	201
E1	141	105/102	309/325	112/118	150	248	108/107	160	201/204	107/93	255	145	197/220	128/120	167	203
E2	141	105/102	309/325	112/118	152/150	250	108/107	160/162	204	93	257/255	145	197/220	128/120	165	201/203

E2(1)	160/141	105/102	309/325	112/118	150	250	107	160	204	107/93	255	140	197/220	128	165	201/203
E3	160/141	102	309/325	112	150	248	107	160/162	201/204	107/93	255	145	197/220	128/120	167	201/203
E4	160/141	102	309/325	112/118	152/150	248/250	108/107	160	201/204	107	255	140	197/220	128/120	167/165	201/203
E5	141	105/102	309	112/118	152/150	248/250	108/107	160/162	201/204	107/93	255	140	197	128/120	165	201/203
E6	141	102	309/325	118	152/150	248/250	107	160/162	201/204	107/93	257	140	197	128/120	167/165	201/203
E6(1)	141	105/102	309/325	118	152/150	248/250	108	160/162	201/204	107/93	257/255	145	220	128/120	167	201/203
E6(2)	160/141	105/102	309/325	118	152	248	108/107	160/162	204	107/93	257/255	140	220	128	167/165	201
E7	160/141	102	309/325	112/118	150	248/250	108/107	160	204	107	257/255	140	197/220	128/120	167	203
E7(1)	141	105/102	309/325	112	150	248	108/107	162	201	93	257/255	140	197/220	128/120	167/165	201/203
E8	141	105/102	309/325	112	152/150	248/250	108/107	160	201/204	107/93	257/255	140	197/220	128	167/165	201/203
E9	160/141	105/102	309/325	112	152	250	108/107	160/162	201/204	93	255	140	197	128	167/165	203
E10	141	102	309/325	112/118	150	248	108/107	160	204	107/93	255	145	220	128/120	165	201
E11	160/141	105/102	309/325	112/118	152	248/250	108/107	160/162	204	107/93	255	140	220	128/120	167	203
E12	141	105/102	309/325	118	152	248/250	107	160/162	201/204	107	255	140	197/220	128/120	167	203
E13	160/141	102	309/325	118	150	248/250	108	160	201/204	107/93	255	140	197/220	128	165	203
E16	141	102	309/325	112/118	150	248/250	108/107	160	201/204	93	255	145/140	197/220	128	165	201
E18	160	105/102	309/325	112/118	150	248/250	108/107	160/162	201	107/93	255	145/140	220	120	167	203
E19	160/141	105/102	309/325	112	150	248/250	107	162	201/204	107	257	140	220	128	167/165	203
E19(1)	141	105/102	309/325	112/118	150	248/250	107	160	201/204	107/93	255	140	220	128	165	203
E20	160/141	105/102	309/325	112/118	152	248	108/107	160	204	93	257/255	140	220	120	167	203
Ea	160/141	105/102	309/325	118	150	248/250	108	160	201/204	107/93	257/255	145	220	128/120	165	201/203
AK1	160	105/102	309/325	118	152	248/250	108	162	201	107	255	140	197/220	128/120	167	203
AK2	141	102	309/325	112/118	150	248/250	108/107	160/162	201/204	107	257/255	140	220	128/120	167	201/203
AK3	160/141	105/102	309/325	112	152	248/250	108/107	160/162	201/204	107/93	257/255	140	220	128/120	167/165	201/203
AK4	160/141	105/102	309	118	152	248/250	107	160/162	201/204	107/93	257/255	140	197/220	128/120	167/165	201/203

AK5	141	102	309/325	112/118	152	250	108	160	204	107/93	257/255	145/140	197/220	128/120	167/165	201/203
AK6	160/141	105/102	309	112/118	152	248/250	108/107	160	201/204	107/93	257/255	140	220	128	167	201/203
AK7	160/141	105/102	309/325	112/118	150	248/250	108/107	162	201/204	107/93	257/255	140	197/220	128/120	165	201/203
AK8	160/141	105/102	309/325	112	150	248/250	108/107	160	201	107/93	255	140	197/220	128/120	167	201/203
AK9	160/141	105/102	309/325	112/118	150	248/250	108/107	162	201/204	107/93	257/255	140	197/220	128/120	165	201/203
AK10	160/141	105/102	309/325	112	150	248/250	108/107	160	201	107/93	255	140	197/220	128/120	167	201/203
AK11	160	105/102	309/325	112/118	152	248	107	162	201/204	107	255	140	197/220	128	165	201/203
AK12	160/141	102	309/325	112/118	152	248/250	108/107	160	204	93	255	140	197/220	128/120	167	201/203
AK13	160/141	105/102	309/325	112/118	150	248/250	108/107	160/162	201/204	107/93	255	140	197/220	120	165	201/203
AK14	141	105	309	112/118	150	248/250	108	160/162	201/204	107/93	257/255	145/140	220	128/120	165	201/203
AK15	141	105/102	309	112/118	152	248	108/107	162	201/204	107/93	257/255	140	197/220	128/120	165	201
AK16	160/141	105/102	309/325	112/118	152/150	250	108/107	160/162	201/204	107/93	255	140	197/220	128/120	167/165	203
AK17	160/141	105	309/325	112	152/150	248/250	107	160/162	201/204	107/93	257/255	140	197/220	128/120	167	201/203
AK18	141	105/102	309/325	112/118	152/150	248/250	108/107	160/162	204	93	255	145	197	128/120	167/165	201/203
AK19	141	105/102	309/325	112/118	150	248/250	108/107	160/162	201/204	107/93	257/255	140	197/220	128/120	167/165	201
AK20	141	105/102	309	112/118	150	248/250	108/107	160/162	201/204	107/93	255	140	197/220	128/120	167/165	203
AK21	160/141	105	309	112/118	152	248/250	108/107	160/162	201/204	107/93	257/255	145	197/220	128/120	167/165	203
AK22	160/141	105/102	309/325	112/118	152	250	108/107	160/162	204	107/93	257/255	145	197/220	128/120	167/165	203
AK23	141	102	309/325	112/118	150	248/250	108/107	160/162	201/204	107/93	255	145	220	128/120	167/165	203
AK24	160/141	105/102	309/325	112/118	152/150	248/250	108/107	160	201/204	107/93	255	140	220	128/120	167/165	203
AK25	160/141	105/102	309/325	112/118	152/150	248/250	108/107	160	201	107/93	257/255	145	220	128/120	167/165	201
AK26	160/141	105	309/325	112/118	152/150	250	108/107	160/162	201/204	107/93	255	145	197/220	128/120	167/165	201/203
AK27	141	105/102	309/325	112/118	150	248/250	108/107	160/162	201/204	107/93	257/255	145/140	197/220	128/120	165	201/203
AK28	160/141	105/102	309/325	112/118	150	248	108/107	160	204	107/93	257/255	140	197/220	128	167/165	201/203
AK29	160/141	105/102	309/325	112/118	150	248/250	108/107	160/162	201	107/93	257/255	140	197/220	128/120	167/165	201/203

AK30	160/141	105/102	309/325	112/118	152	248/250	107	160/162	201/204	107/93	257/255	140	197/220	128/120	165	201/203
AK31	160/141	105/102	309/325	118	152	248/250	107	160/162	201	107/93	257/255	145	197/220	128/120	165	201/203
AK32	160/141	105/102	309/325	118	150	250	107	160/162	204	107/93	255	140	197/220	120	167/165	203
AK33	160/141	105/102	309/325	112/118	150	250	108/107	160/162	204	107/93	255	145	197/220	120	167/165	201/203
AK34	160/141	105/102	309/325	112	150	248	108/107	160	201/204	107	255	140	197/220	128	165	201/203
AK35	160/141	105/102	309/325	112	152	248	108/107	160/162	201/204	107/93	257/255	140	197/220	128/120	167	203
AK36	160/141	102	309/325	112/118	150	250	108/107	160	201/204	107/93	257/255	140	220	128/120	167	201/203
AK37	160/141	105/102	309	118	152	248	108/107	162	204	107/93	257/255	140	197	128	167	201/203
AK38	141	105/102	309/325	112/118	150	248/250	108/107	160	204	107/93	257/255	140	197/220	120	167/165	203
AK39	141	102	309/325	118	150	248/250	108/107	160	204	107/93	255	140	197	128	167/165	201/203
AK40	141	105/102	309/325	118	152	248/250	108/107	160	201	107/93	257	140	220	128/120	167/165	201/203
BK1	160/141	102	309/325	112	152	248/250	108	160/162	204	107/93	257/255	140	220	128	167/165	203
BK2	141	105/102	309	118	152	248/250	108/107	160	204	107/93	257/255	140	197/220	128	167	201
BK3	160/141	105/102	309	118	150	248/250	108/107	160	204	107/93	255	140	197/220	120	167/165	201
BK4	141	105/102	309/325	112/118	152/150	248/250	108	160	201/204	107/93	257	145	197/220	128/120	167/165	201/203
BK5	141	105/102	309/325	112	152	248/250	108/107	162	204	107	257/255	140	197/220	120	167/165	203
BK6	141	105/102	309/325	112	152/150	250	108/107	160/162	201/204	107/93	257/255	140	197/220	128	167/165	201/203
BK7	160/141	102	309/325	118	152	250	108/107	160/162	204	107/93	257	140	220	128/120	165	203
CK3	160/141	105/102	309/325	118	152	250	108/107	160/162	204	107/93	255	140	197/220	128/120	167	203
CK4	160/141	105/102	309/325	118	150	250	108/107	160/162	201	93	257/255	145	197/220	128/120	167	203
DK2	160/141	105	309/325	118	152	248	108/107	162	201/204	107/93	255	145	197/220	128/120	165	201/203
EK1	141	105	309	112/118	152/150	248	108/107	160/162	201/204	107/93	255	140	197/220	128/120	167	201/203
EK2	160/141	105/102	309/325	118	152/150	248/250	108/107	160/162	204	107/93	255	140	197/220	120	165	201
EK3	141	105/102	309	118	152	248/250	108/107	160/162	204	107/93	257/255	145	197/220	128	167	201/203

PLANT	RM160	RM506	RM10916	RM6836	RM184	RM271	RM519	RM269	RM235	RM145	RM327	RM215	RM246	RM404	RM84	RM80	RM162	RM526
HKR47	90	115	294	240	190	110	140	200	124	190	208	152	115	238	126	165	220	240
MAS26	130	117	296	248	220	100	141	203	106	220	210	154	118	236	113	173	215	220
A1(1)	90/130	115/117	296	248	190	110	141	203	124	220	208/210	152/154	118	238	126	173/165	220/215	240
A2	90/130	115/117	294/296	240/248	190/220	110	140/141	200	124	220	208/210	152	118	236/238	126/113	165	220/215	240
A3	90	115/117	296	240/248	190	110	140/141	203/200	124/106	220	208	154	118	236/238	113	173/165	220	240/220
A4	130	115/117	296	240/248	190/220	110	140	203/200	124/106	220	208/210	152/154	115/118	236/238	126/113	173/165	220	220
A5	130	117	294	248	190	110/100	140/141	203/200	124	220	208/210	154	118	236/238	126/113	165	220/215	220
A5(1)	90/130	115/117	294	248	190/220	110	141	203	124	190	210	152	115/118	236/238	126/113	173	220/215	220
A6	90/130	115/117	294	240	190/220	110	140	203	124/106	190/220	208/210	152	115	236/238	126/113	173	220/215	240/220
A11	130	115/117	294/296	248	190	110	141	203/200	124/106	190/220	208	154	118	238	113	173/165	220	240/220
B1	90/130	115/117	296	240	190/220	110/100	141	203/200	124/106	220	208/210	152	115/118	236/238	126/113	173	215	240
B1(1)	130	115/117	296	248	220	110/100	140/141	203	124/106	190/220	208/210	152/154	115	236/238	126/113	165	220	220
B2(1)	90/130	115/117	294	240/248	220	110/100	141	203/200	124/106	190/220	210	152/154	118	236/238	126	173/165	220/215	240
B6	90/130	115/117	294	248	190	110/100	140/141	203/200	124/106	190/220	208	152/154	115	236/238	126	165	220	240
B10	130	117	294	240	190	110/100	140/141	200	124/106	190/220	210	152	115	236/238	126	165	220/215	220
C1	90/130	117	294	248	190	110/100	140/141	203/200	106	190	208	154	118	236/238	126	173	215	220
C1(1)	130	115	296	240/248	190/220	110/100	140/141	203/200	124	190/220	208/210	154	118	236/238	126/113	165	215	220
C2	130	115/117	294	240/248	190/220	110/100	140/141	203/200	124	220	208/210	154	118	236/238	126	173/165	215	240/220
D2	90	115	296	240/248	190/220	100	141	203/200	106	190/220	208/210	154	118	236/238	113	173/165	215	240
D3	130	115/117	296	248	190/220	100	140/141	203	124/106	190/220	208/210	154	118	236/238	126	173/165	220	220
D4	90/130	115/117	294	240/248	190/220	100	140/141	203	124/106	220	210	152/154	118	236/238	126/113	173/165	220	220
E1	90	117	296	248	190	110	141	203	124/106	190/220	208/210	152	118	236/238	126/113	173/165	220	240
E2	90/130	115/117	296	240/248	190/220	110	140/141	200	124	190/220	208	152/154	118	236/238	113	173/165	220/215	240
E2(1)	130	117	294	240/248	190/220	100	140/141	203/200	124	190/220	208	152/154	115	236/238	126/113	165	220/215	240/220
E3	130	115/117	296	248	190/220	110/100	140/141	200	106	190/220	208/210	152/154	115	236/238	126/113	165	220/215	240/220
E4	90/130	117	294	240/248	190/220	110/100	140/141	203	124	190	208/210	152/154	115	236/238	126	173/165	215	240/220

E5	90	115/117	294	240	190	110	140/141	200	124	220	208/210	152	115	238	126/113	173/165	215	240/220
E6	130	115/117	294	240	190/220	100	140/141	203	124	220	210	154	115	236/238	126/113	165	220/215	240
E6(1)	90	115/117	296	240/248	190	100	140/141	200	124	220	208/210	154	118	236/238	126/113	173	220/215	240/220
E6(2)	90/130	117	296	248	190	100	140/141	200	124	220	208	152/154	118	236/238	126/113	173/165	220/215	240/220
E7	90/130	115/117	296	240	190/220	100	140	203/200	106	190/220	210	152/154	118	236/238	126	173/165	220	240/220
E7(1)	90/130	115/117	294	240	190/220	110	141	203	124/106	190/220	210	152/154	118	236/238	113	173/165	220	240/220
E8	90	117	296	240/248	190	110	141	203/200	124/106	190/220	210	154	118	236/238	126/113	173/165	215	240/220
E9	90/130	117	294/296	240/248	190/220	110	140/141	200	124	190	208	152	118	236/238	113	173/165	220	240
E10	90/130	115/117	294/296	240/248	190/220	110	140/141	203/200	124	220	208	152/154	118	236/238	126/113	173/165	215	220
E11	90	115/117	296	240/248	190/220	110	140/141	203/200	124/106	190	210	152	118	236/238	126/113	173/165	220/215	240
E12	90/130	115	296	240/248	190/220	100	140	203/200	124/106	190	210	152/154	118	236/238	126	165	220/215	240/220
E13	90/130	117	294/296	240/248	190/220	100	140/141	203	124/106	220	208/210	152/154	118	238	126/113	173	220/215	240
E16	90	117	296	240/248	190/220	100	140/141	203	106	190/220	208/210	152	118	236/238	113	173/165	220	220
E18	90/130	115	294/296	240	220	100	140/141	203/200	106	220	208/210	152/154	118	236/238	126/113	173/165	220/215	240/220
E19	130	117	294	248	190/220	110	140/141	203/200	106	190/220	208	152/154	118	236/238	126	165	220/215	240/220
E19(1)	90/130	117	294	240/248	190/220	110	140/141	203/200	106	220	208/210	152	118	236/238	126/113	165	215	240/220
E20	90/130	117	296	240/248	190/220	110	140/141	203	106	220	208/210	152/154	115	236/238	113	173/165	220/215	240/220
Ea	130	117	294/296	248	190/220	100	140/141	203/200	124	220	208	152/154	115	236/238	126/113	173	220/215	240/220
AK1	90/130	115/117	294/296	240/248	190/220	100	140/141	203/200	106	190/220	210	152	115	236/238	126	173	220	240
AK2	90/130	115/117	296	248	190/220	110	141	203/200	106	190/220	208	152/154	115	236/238	126	173/165	220/215	220
AK3	90/130	115/117	294	248	190/220	100	140	203/200	106	220	210	152	115	236/238	126/113	173/165	220	240/220
AK4	90/130	115/117	294	240/248	220	110	141	200	124	190/220	208	154	115/118	238	126/113	165	215	240/220
AK5	90/130	115/117	294	240	190/220	110	141	203/200	106	190/220	208/210	154	118	236/238	126/113	173	220/215	240/220
AK6	90/130	117	296	240/248	190/220	100	140	203/200	106	220	210	154	115/118	238	126/113	173/165	220/215	240
AK7	90/130	115/117	294	240/248	190/220	100	140	203	124	220	208/210	152	118	236/238	126/113	173/165	220/215	240
AK8	90/130	115	294	240/248	190/220	110	141	203	106	190/220	208/210	152	115	236/238	126/113	173/165	220	220
AK9	90/130	115/117	294	240/248	190/220	100	140	203	124	220	208/210	152	118	236/238	126/113	173/165	220/215	240

AK10	90/130	115	294	240/248	190/220	110	141	203	106	190/220	208/210	152	115	236/238	126/113	173/165	220	220
AK11	90/130	117	296	240/248	220	100	140	203/200	124/106	190/220	208/210	154	118	236/238	126	165	220/215	240/220
AK12	90	117	294	240/248	190/220	110	141	203/200	106	190/220	208	152/154	115/118	236/238	113	173/165	220/215	240/220
AK13	90/130	115	296	240	190/220	110	141	200	124/106	190/220	210	152/154	115	236/238	126/113	173/165	220	240/220
AK14	130	115	296	240	190/220	100	140	203	106	190/220	208/210	152	118	238	126/113	173	220/215	240/220
AK15	90/130	115/117	296	240/248	220	110/100	140/141	203	124/106	220	210	152/154	115	236/238	126/113	173/165	220/215	240
AK16	130	115	296	240	190	110/100	140/141	203	124/106	190/220	208/210	152/154	118	236/238	126/113	173/165	220/215	240/220
AK17	130	115/117	294	248	220	110	141	200	124/106	190/220	208/210	154	118	236/238	126/113	165	220/215	240
AK18	130	115/117	294/296	240/248	190/220	100	140	200	124/106	190	210	154	115	236/238	113	173/165	220/215	240/220
AK19	130	115/117	294/296	248	220	110/100	140/141	203	124/106	190/220	208/210	154	115/118	236/238	126/113	173/165	220/215	220
AK20	90/130	115/117	296	240/248	220	110/100	140/141	203	124	190/220	208/210	152	118	238	126/113	173/165	220	240
AK21	130	115/117	296	240	190/220	110/100	140/141	203/200	106	190/220	210	152/154	118	238	126/113	173/165	215	240/220
AK22	90/130	115/117	294	240/248	190/220	110/100	140/141	200	106	190/220	208/210	152	118	236/238	126/113	173/165	220/215	240/220
AK23	90/130	115/117	294	240/248	190/220	110/100	140/141	200	124/106	220	210	152	118	236/238	126/113	173/165	220	220
AK24	90/130	115/117	296	240/248	220	100	140	200	124/106	190/220	208	152/154	118	236/238	126/113	173/165	220/215	240/220
AK25	90/130	115/117	296	240/248	220	100	140	203/200	106	220	208	152/154	118	236/238	126/113	173/165	220/215	240
AK26	130	115/117	296	240/248	190/220	100	140	203/200	106	190/220	208	152/154	115/118	236/238	126/113	173/165	215	240
AK27	90	115/117	296	240/248	220	110	140/141	203/200	106	190/220	208/210	152	115/118	236/238	126/113	173/165	215	240
AK28	90/130	115/117	294/296	240/248	190/220	110/100	140/141	203/200	106	220	210	152/154	118	236/238	126/113	173/165	215	240
AK29	90/130	115/117	294/296	248	190/220	110/100	140/141	200	124/106	190/220	208/210	152/154	118	236/238	126/113	173/165	220/215	240/220
AK30	90/130	115/117	294/296	240/248	190/220	110/100	140/141	203/200	124/106	220	208/210	152	118	236/238	126/113	165	215	240/220
AK31	90/130	115/117	296	240	190/220	110	140/141	203/200	124/106	190/220	208/210	152	115	236/238	126/113	165	215	240
AK32	90/130	115/117	294	248	220	110	140	203	124/106	190/220	208/210	152	118	236/238	126/113	165	220/215	240
AK33	90/130	115/117	294	248	190/220	110/100	140/141	203	124/106	190/220	210	152	115/118	236/238	126/113	173/165	220/215	240/220
AK34	130	115/117	294/296	240/248	190/220	110/100	140/141	203/200	124/106	220	208/210	154	115/118	236/238	126	173/165	220/215	240/220
AK35	90/130	115/117	296	240/248	190/220	110/100	141	200	124/106	190/220	208/210	152	115/118	236/238	126/113	173/165	220/215	240
AK36	90/130	115/117	296	248	190/220	110/100	140/141	203	124	190/220	208/210	152	118	236/238	126/113	173/165	215	220

AK37	90/130	117	296	248	220	110/100	141	203	124/106	190/220	208	152	118	238	126/113	173/165	215	240
AK38	90/130	115/117	294	248	190/220	100	140/141	203	106	220	208/210	152	118	236/238	126/113	173/165	220/215	240
AK39	90/130	115/117	296	240	190	100	140/141	203	124	190/220	208/210	152	115/118	236/238	126/113	173/165	215	240/220
AK40	90/130	115/117	296	240/248	190/220	110/100	140/141	200	124/106	190/220	208/210	152	118	236/238	126/113	173/165	215	240/220
BK1	90/130	115/117	296	240/248	220	100	140/141	203	124/106	220	208	154	118	236/238	126/113	173	215	240/220
BK2	90/130	115/117	296	240/248	190	110	140/141	200	124/106	190/220	208/210	154	115/118	238	126/113	173/165	220	240/220
BK3	90/130	115/117	294/296	240/248	190/220	110	140/141	203/200	124	220	208/210	152/154	118	238	126/113	173/165	220	240/220
BK4	90/130	115/117	294/296	240/248	190/220	110	140/141	203/200	124/106	190/220	208	154	115	236/238	126/113	173	220/215	240/220
BK5	90/130	115/117	296	240/248	190/220	110	140	203/200	124/106	190/220	208	152	115/118	236/238	126	173/165	220/215	240/220
BK6	90/130	115/117	294	240/248	190/220	110/100	140	203/200	124	190/220	208/210	152	118	236/238	126/113	173/165	220/215	220
BK7	90/130	117	296	240/248	190/220	110	140/141	203/200	124/106	190/220	210	152	115/118	236/238	126/113	173/165	215	240/220
CK3	130	117	296	248	190/220	110	140/141	203/200	124/106	190	210	154	118	236/238	126/113	173/165	220/215	240/220
CK4	90/130	117	294	240/248	220	100	140/141	200	124/106	190/220	210	154	118	236/238	113	173/165	220/215	240
DK2	90/130	115/117	296	240/248	220	110/100	140/141	203/200	124/106	190/220	208	152	118	236/238	126/113	173/165	220/215	240
EK1	90/130	115/117	296	240/248	220	110	140/141	203/200	124/106	190/220	208/210	152	118	238	126/113	173/165	220/215	240/220
EK2	90/130	115/117	294	240	190	110/100	140	203/200	124/106	220	208/210	152	118	236/238	126/113	173/165	215	240/220
EK3	90/130	115/117	296	N	190	110/100	141	203/200	124/106	190/220	208/210	152	118	238	126/113	173/165	215	240

ANNEXURE IV

DNA fingerprinting database of MASARB25 x Pusa Basmati 1460 derived population using SSR markers

Plant	RM302	RM6836	RM524	RM490	RM185	RM208	RM265	RM273	RM6	RM11	RM491	RM280	RM215
MASARB25	190	248	198	93	210	180/173	110	211	182	123	381	161	154
PB1460	126	240	205	107	212	173	106	207	173	140	278	158	152
F1	126	248	205	93	212	180/173	110/106	211	173	123/140	278	161/158	154/152
F2	126	248	198/205	93/107	210	180/173	110/106	211	182/173	123/140	278	161/158	154
F3	126	248/240	198/205	93	210/212	180/173	110/106	211	182/173	140	278	161	154/152
F4	126	240	198/205	107	210	180/173	110/106	211	182/173	123/140	278	161/158	154/152
F6	126	248/240	198/205	93	210	180/173	106	211/207	182/173	123/140	278	161/158	154
F7	190	248/240	198/205	93/107	210/212	180/173	110/106	211	182/173	123/140	381	161	154
F8	190	248/240	198/205	93/107	212	180/173	106	207	182/173	123/140	381	158	154
F9	190	248/240	198	107	210/212	180/173	110/106	211	182/173	123/140	278	158	154
F10	190/126	240	198	93	212	180/173	110/106	211	182/173	123/140	381	158	154
F11	190	248/240	198/205	93/107	210	173	106	211/207	182/173	123	381	161/158	154
F12	126	240/248	198	93/107	210	180/173	110/106	211/207	173	123/140	278	161/158	154/152
F13	126	240	198/205	107	210/212	180/173	106	211/207	173	123/140	278	161	154/152
F15	126	240	198	93/107	210/212	180/173	110/106	211/207	182/173	140	381	158	154/152
F16	190/126	248/240	198	93/107	210	173	110/106	211/207	182/173	123/140	381	161/158	154
F17	126	240	198	107	212	180/173	110/106	207	182	140	278	158	154
F18	190/126	248/240	198	93/107	210/212	180/173	110/106	211/207	182	140	278	161	152
F19	126	240	198	93/107	210/212	180/173	106	207	173	140	278	161	152
F20	126	240	198	93	210/212	180/173	110/106	211/207	173	123/140	381	161	154

F21	126	240	198/205	107	210/212	173	110/106	211/207	182/173	123/140	381	158	154
F22	126	248	198/205	93	210/212	180/173	106	207	182/173	123/140	278	161	154
F23	126	248	205	93/107	210/212	180/173	110/106	211/207	182/173	123/140	278	161	154/152
F24	126	240	198	93/107	210	180/173	110/106	211/207	182	140	278	158	152
F26	126	240	198/205	93	210	173	110/106	207	182	140	278	161/158	154/152
F27	190	248	198/205	93/107	210/212	180/173	110/106	207	173	140	278	158	154
F28	126	240	198	93/107	212	180/173	106	211	173	123/140	278	161/158	154/152
F29	126	240	198/205	93/107	210	180/173	110/106	211	182	140	381	161/158	154/152
F30	126	240	198/205	107	210/212	180/173	110/106	207	173	123/140	278	161	154/152
F31	126	248	198	93/107	210/212	173	110/106	211/207	173	123/140	278	161/158	154
F32	190	240	198/205	93/107	210	180/173	110/106	211/207	173	123	381	161/158	154/152
F33	126	248/240	198	93	210/212	180/173	106	211/207	173	123/140	381	161/158	154
F34	190/126	240	205	93	210/212	180/173	110	211/207	173	123/140	278	158	154
F35	126	240	198/205	93	212	173	110/106	211/207	173	140	278	161	154
G1	126	248	198/205	93/107	210	173	110/106	211/207	173	123/140	381	161/158	154
G2	126	240	198/205	107	210	173	110	211	173	140	278	161/158	154
G3	190/126	248/240	198/205	107	210	180/173	110	211/207	173	140	278	161	154
G5	126	248	198	107	210/212	180/173	110	211	182	123	278	161	154
G6	126	248	198	93/107	210/212	180/173	110	211/207	182/173	123/140	278	161/158	154
G7	190/126	248/240	198	93/107	210/212	173	110	211/207	182/173	123/140	278	158	154/152
G8	126	248	198/205	93	210	180/173	110	211	182	123	278	161/158	154/152
G9	126	248/240	198/205	107	210/212	180/173	106	207	182/173	123	278	161/158	154/152
G10	190/126	248/240	198/205	93	210/212	180/173	106	211/207	182/173	123/140	381/278	161	154

G14	126	240	198205	107	212	180/173	106	211/207	173	123/140	278	161/158	154/152
G16	190	248/240	198	93/107	210	180/173	110/106	211	182	140	381	161	154/152
G17	126	248	198/205	107	210	180/173	110/106	211	173	140	278	161	154
G18	126	248/240	198/205	93/107	210/212	180/173	110/106	207	173	123/140	381	161/158	154
G19	126	240	198/205	93/107	212	180/173	110/106	207	182	140	278	161	154
G20	126	240	198/205	93	212	180/173	110/106	211/207	173	140	278	161/158	154
G21	190/126	240248	198/205	93	210	180/173	106	211/207	173	123	278	158	154
G22	190	240	198/205	107	210	180/173	106	207	182	123	278	158	154/152
G23	126	240	205	107	210	180/173	106	211/207	173	123/140	278	161/158	154/152
G23(1)	126	240248	198/205	93	210	180/173	106	211/207	173	123/140	381	161/158	154
G24	190/126	248	198/205	93	212	180/173	106	211/207	173	123/140	381	161	154
G25	190/126	248/240	198	107	210	180/173	110/106	211/207	173	123/140	381	161/158	154
G26	126	248/240	198/205	93/107	210/212	173	110/106	211/207	182/173	123/140	278	161/158	152
Ga(1)	126	248	198/205	93/107	210	180/173	110/106	211/207	182	123	278	161	154
Gb	190/126	240	198	93	210/212	180/173	110/106	211	173	140	381	161	154
FK1	190/126	248/240	198/205	93/107	210/212	180/173	106	211/207	182/173	123/140	278	158	154/152
FK2	126	248/240	198/205	93/107	210	173	106	211/207	182/173	140	278	161/158	154
FK3	126	248/240	198/205	93/107	210	180/173	110/106	211	173	140	278	161	154
FK4	126	248/240	198	93	210	180/173	110/106	211/207	182/173	123/140	278	161/158	154
FK5	126	248/240	198/205	93/107	212	180/173	110/106	211/207	182	123/140	278	161	154/152
FK6	126	240	198/205	93	210	180/173	110/106	211/207	182	140	381	158	154/152
FK7	126	248/240	198/205	93/107	210	173	110/106	211/207	182/173	140	278	161	154/152
FK8	126	248/240	198/205	93/107	210/212	180/173	110/106	211/207	182/173	123/140	278	161/158	154/152

FK9	190/126	240	198	93107	212	180/173	106110	207	173	140	278	161/158	154/152
FK10	190/126	248/240	198	93	210	180/173	106	211/207	173	140	278	161/158	154/152
FK11	190/126	248/240	198	93	210	180/173	110/106	211/207	182/173	123/140	278	161/158	154/152
GK1	126	248/240	205	93/107	210	180/173	110/106	211/207	182/173	123/140	381	161	154
GK2	126	248/240	198/205	93/107	210	180/173	110/106	211/207	182	140	278	161/158	154
GK4	126	248/240	198	107	212	180/173	110/106	211/207	173	140	278	158	154
GK5	190	248/240	205	107	210/212	173	106	211/207	182/173	123	278	161	154/152
GK6	126	248	205	93	212	173	106	211	173	140	381	161/158	154

Plant	RM178	RM174	RM84	RM122	RM222	RM220	RM212	RM210	RM21	RM4	RM12	RM221	RM50	RM42
MASARB25	108	208	126	237	218	127	112	160	150	152	252	210	202	161
PB1460	122	204	113	235	210	124	116	142	135	145	249	202	199	166
F1	122	208/204	126/113	235	218/210	124	112	142	150/135	152/145	252/249	210/202	202	161/166
F2	122	208/204	126/113	237/235	218/210	127/124	116	142	150/135	152/145	252/249	210	202	161/166
F3	122	208	113	237/235	210	127/124	112	160	150	152	252/249	210/202	202	161/166
F4	108/122	208/204	126/113	237/235	210/218	127/124	116	160	150	152/145	249	210	202	161/166
F6	108/122	208	126/113	235	218/210	127	116	160	150	152/145	252/249	210	202	161
F7	122	208/204	126/113	237/235	218/210	124	112	142	150	152/145	252	210/202	202	161
F8	122	208	126/113	237/235	218/210	124	112	160	150	145	249	210	202	161
F9	122	208/204	126/113	237/235	218/210	127/124	112	160/142	150	145	252/249	210	202	161
F10	122	204	126/113	237/235	218/210	124	116	160/142	150	152	249	210	202	166
F11	122	204	126/113	237/235	218/210	124	112/116	160	150/135	145	252/249	210	202	166
F12	122	208	126/113	237/235	210	127	112/116	160/142	150	152/145	252/249	210	202	166

F13	108/122	208	126/113	237/235	218/210	124	116	160/142	150	152/145	249	210	202	166
F15	108/122	208	113	235	218/210	124	112/116	160/142	150	145	252/249	210	202	166
F16	108/122	208/204	126/113	235	218/210	124	112/116	142	150	145	252/249	210	202	161
F17	108/122	204	126	237/235	218/210	127/124	116	142	150	145	252/249	210	202	161
F18	108/122	208/204	126/113	237/235	218/210	124	112/116	160/142	150	145	252/249	210	202	161
F19	108/122	204	126/113	235	218/210	124	112/116	160	150	152/145	249	210	202	161
F20	108/122	208	126	237/235	218/210	124	112	160/142	150	152/145	252	210	202	166
F21	108/122	208/204	126	237/235	218/210	124	112	142	150	152/145	252	210	202	166
F22	108/122	204	126/113	237/235	218/210	127	116	160/142	150	152/145	249	210	202	166
F23	108	204	113	237/235	218/210	127/124	116	160/142	150/135	152/145	252/249	210	202/199	166
F24	108	208/204	113	235	218/210	127/124	112/116	160/142	150/135	152/145	252/249	210/202	202	166
F26	108	208/204	126/113	237/235	218/210	127/124	112	160	150/135	152	252/249	210	202	166
F27	108/122	204	126/113	237/235	218/210	127/124	112/116	160/142	150	152/145	252/249	210/202	202	161
F28	108/122	208	126	237/235	210	127	116	160	150	145	252	210	202	161
F29	108/122	204	126/113	237/235	218/210	124	112/116	160	150	152	252	210	202	161
F30	108/122	204	126/113	235	218/210	127/124	112/116	142	150	152/145	249	210/202	202	161
F31	122	204	126/113	235	218/210	124	116	160/142	150	152/145	249	210/202	202	161/166
F32	108	208/204	126	237	218/210	124	112	160/142	150/135	152	249	210	202	161/166
F33	108	208/204	126/113	237	218/210	124	116	160/142	150/135	152	252/249	210	202	161/166
F34	108	204	126/113	237	218/210	127	116	160/142	150	152	252/249	210	202	161/166
F35	108/122	208/204	113	235	218/210	127	116	160	150	152	252/249	210	202	166
G1	108/122	208/204	126	235	218/210	127/124	116	160/142	150/135	152/145	252/249	210/202	202	161/166
G2	108/122	208/204	113	237/235	210	124	116	142	150	145	252	210	202	161/166

G3	108/122	208/204	113	235	218/210	124	112	160/142	150	145	252/249	210/202	202	161/166
G5	108/122	204	113	235	218/210	124	112/116	142	150	152/145	252/249	210/202	202	166
G6	122	208	126/113	235	218/210	127	112/116	160	150	152/145	252/249	210/202	202	166
G7	122	208	113	235	210	127/124	112/116	142	150/135	152/145	252/249	210/202	202	161
G8	108/122	208	113	235	210	124	112/116	142	150	152/145	249	210	202	161
G9	108	204	113	237	210	127	116	160/142	150	152/145	249	210	202	161
G10	108/122	204	113	237	210	124	116	160/142	150	152/145	252	210/202	202	166
G14	108	208/204	126	235	210	124	112/116	142	150	152	252	210/202	202	161/166
G16	108	208	113	237	218/210	127	112/116	160/142	150/135	145	252	210	202	161/166
G17	108/122	208/204	126	235	218/210	127/124	116	160/142	150	145	252	210/202	202	161/166
G18	108/122	208/204	126	235	218/210	127	116	160/142	150	145	252	210	202	166
G19	122	208	126	237/235	218/210	127	116	142	150	145	252	210/202	202	166
G20	108	204	126	237/235	218/210	127	112/116	160/142	150	145	252/249	210/202	202	161/166
G21	108/122	208/204	126/113	237/235	210	127	112	142	150/135	152	252/249	210/202	202	161/166
G22	108/122	208/204	126/113	235	218/210	127/124	112/116	160	150	152	252	210	202/199	161/166
G23	122	208/204	126	235	218	124	112/116	160/142	150	145	249	210/202	202/199	161/166
G23(1)	108	208/204	126	235	218	127/124	112/116	160	150	145	252	210/202	202/199	166
G24	108/122	204	113	235	218	127	112/116	160/142	150	145	249	210/202	202	166
G25	108/122	204	113	237	210	124	112/116	160/142	150	145	249	210	202	166
G26	108/122	208/204	113	237	210	127/124	116	160/142	150	152	249	210/202	202/199	161
Ga(1)	122	208/204	126	235	218/210	127/124	116	160	150	152	252	210	202	161
Gb	108/122	208/204	126	235	218	127	116	160/142	150	145	249	210/202	202/199	161
FK1	108	208	126	235	210	127/124	112/116	160/142	150	145	252	210/202	202	161/166

FK2	108/122	208	126	235	210	127/124	116	160/142	150	145	252	210	202	161/166
FK3	108	204	126/113	235	210	127/124	116	160/142	150	145	249	210/202	202	161/166
FK4	108/122	208/204	126/113	235	210	127	112/116	160/142	150/135	152	252/249	210/202	202	161/166
FK5	108/122	208/204	126/113	237/235	210	127/124	112/116	160/142	150/135	145	252/249	210/202	202	166
FK6	108/122	208	126/113	235	218/210	127/124	116	160/142	150/135	152/145	252	210	202/199	161/166
FK7	108/122	208	126/113	235	218/210	127/124	112/116	160/142	150	145	252	210/202	202	161/166
FK8	108/122	208/204	126/113	237	210	127/124	112/116	142	150	145	252	210/202	202/199	161
FK9	108/122	208/204	126	235	210	127/124	116	142	150	145	252	210/202	202	161/166
FK10	108	204	126/113	237	210	127/124	116	142	150	152/145	252	210	202	161/166
FK11	108	208	126	235	218/210	127	112	160	150	152/145	252	210/202	202/199	161
GK1	108/122	208/204	126/113	235	218/210	127/124	112/116	160/142	150	152	252	210	202/199	161
GK2	108/122	208/204	126/113	235	218	127/124	116	160/142	150/135	152	252	210/202	202	161
GK4	108/122	204	126	235	218	127/124	116	160	150	145	252	210	202	161
GK5	122	208	126/113	237/235	218/210	127	112	142	150	145	249	210	202/199	166
GK6	122	208/204	126/113	235	218	127/124	116	142	150/135	152	252	210/202	202/199	161

Plant	RM14	RM48	RM331	RM314	RM318	RM416	RM447	RM307	RM263	RM246	RM433	RM458	RM418	RM342
MASARB25	191	199	176	108	156	112	105	124	165	118	222	189	283	141
PB1460	178	204	158	106	138	114	118	126	158	114	224	178	277	145
F1	178	199/204	176	108	138	114	118	126	158	118/114	224	178	283	141
F2	191/178	199/204	158	108	156	114	105/118	124/126	165	118/114	224	178	277	141
F3	191/178	199/204	176	106	156	114	105/118	124/126	165/158	118/114	224	189	283	145
F4	178	204	176	108	156/138	114	105	124/126	165	118/114	222	189	283/277	145

F6	191/178	204	176	108	156/138	112	105/118	124/126	165	118/114	222	178	283/277	141
F7	191/178	204	158	106	156	114	105	124/126	165	114	222	178	283/277	141
F8	178	204	158	106	156	112	105	124/126	165/158	118/114	224	189	283/277	145
F9	191/178	199/204	176	106	138	114	105	126	165	118/114	224	178	283/277	141
F10	191/178	204	176	106	156/138	112	105	126	165/158	114	224	189	283/277	145
F11	191/178	204	176	108	156/138	112	105/118	124/126	165	118/114	224	189	277	145
F12	191/178	199/204	176	106	156	112	105	124/126	158	118/114	224	189	277	141/145
F13	178	204	176	106	138	114	105	124/126	158	114	224	189/178	283/277	141/145
F15	191/178	204	176	108	138	114	118	124/126	165/158	118/114	224	189/178	283	141
F16	191/178	204	158	106	156/138	114	118	124/126	165/158	118/114	224	178	283	141
F17	178	204	158	106	156	112	105	124	165	118/114	224	178	283	145
F18	191	199/204	158	108	156	112	105	124/126	165/158	114	222	189	283/277	145
F19	191/178	204	158	106	138	112	105	124	158	118	222	189	283/277	141/145
F20	191	204	176	108	138	112	105	124/126	165/158	118	224	189/178	277	141/145
F21	191/178	199/204	176	106	138	112	105	124/126	165/158	118	224	189/178	277	141/145
F22	191/178	199/204	176	108	156/138	114	105	124	165	118	224	189/178	277	141/145
F23	191/178	204	176	108	156/138	112	105	124/126	158	118	224	189/178	277	141/145
F24	178	199/204	176	106	138	114	118	124/126	165/158	114	224	189/178	283/277	141/145
F26	191	204	176	106	156	114	118	124	158	118	224	178	283	141
F27	191	199/204	176	108	156	114	105	124	165/158	118	222	189	283	145
F28	191/178	204	176	108	156/138	112	118	126	165/158	118/114	224	189/178	277	141/145
F29	191	204	176	106	138	114	118	126	165	114	224	189	277	145
F30	191	199/204	176	106	156	114	105	124	158	118/114	224	189	277	145

F31	191/178	199/204	176	106	138	112	118	124/126	158	118/114	222	178	277	141
F32	191	199/204	176	106	138	114	105/118	124/126	165/158	118/114	222	178	277	141
F33	178	204	176	106	156	112	105	124/126	165/158	114	224	189/178	283/277	141/145
F34	178	204	176	106	156/138	114	118	124/126	165/158	114	224	189/178	277	141/145
F35	178	204	176	106	138	112	105	124/126	165/158	118	224	189/178	277	141/145
G1	191	204	176	108	138	112	105	124/126	158	118	224	178	277	141
G2	191/178	199/204	176	108	156	114	105/118	126	165/158	118	224	189	283/277	145
G3	178	199/204	176	108	138	112	105/118	124/126	165/158	118	224	189	283	145
G5	178	204	176	108	156	114	118	126	165	114	224	189/178	283	141/145
G6	178	204	176	108	138	114	105/118	124/126	165/158	118	224	189	283/277	145
G7	191/178	204	176	106	156	114	118	124/126	158	118	222	178	283/277	141
G8	191/178	199/204	176	106	156	114	105	126	165/158	118	222	189/178	277	141/145
G9	191/178	199/204	158	106	138	112	105	124	165/158	118	224	189	277	145
G10	191/178	204	158	108	138	114	105	124/126	158	118/114	222	178	283/277	141
G14	191	199/204	176	108	138	114	118	124/126	165/158	118/114	224	189/178	283/277	141/145
G16	191	204	176	106	138	112	105	126	165	118/114	222	189	283	145
G17	191	199/204	176	108	138	114	118	124	165	118	224	189/178	283/277	141/145
G18	191/178	199/204	176	106	138	114	118	124	158	118	222	178	283/277	141
G19	191	204	176	108	156	114	105	126	165	118/114	222	189/178	277	141/145
G20	191	204	176	108	138	112	105	124	158	118/114	222	178	283/277	141
G21	178	204	176	108	138	114	105	126	158	114	224	189	283/277	145
G22	178	199/204	176	106	156	114	105/118	124	165	118	224	189/178	283/277	141/145
G23	178	199/204	176	106	138	114	105	126	165/158	118	224	189/178	277	141/145

G23(1)	178	204	176	108	156/138	112	105	124	165/158	114	224	178	277	141
G24	191/178	204	176	106	138	114	105/118	124	158	118	222	189	283/277	145
G25	191	204	176	108	156/138	114	105	124	165/158	118	224	189	277	145
G26	191	199/204	176	108	156/138	114	105	124	165/158	118/114	224	178	283/277	141
Ga(1)	191	204	158	108	156	114	105/118	124	165	118	222	178	283/277	141
Gb	191/178	199/204	176	106	138	112	105/118	126	165/158	114	224	189	283/277	145
FK1	178	199/204	176	106	156	114	105/118	126	165	118	222	178	277	141
FK2	191/178	199/204	176	106	156/138	112	105	126	165/158	118	224	178	283/277	141
FK3	191/178	199/204	176	106	138	112	105/118	124	165/158	118/114	224	189/178	283/277	141/145
FK4	191/178	204	176	106	138	112	118	124	165/158	118	224	178	283/277	141
FK5	191/178	204	176	108	156	112	118	124	165/158	118	224	178	283/277	141
FK6	191	204	176	108	156	112	118	124	165	118	224	189	283/277	145
FK7	191/178	199/204	176	108	156/138	114	105/118	124	165/158	118/114	222	189/178	283	141/145
FK8	191/178	199/204	176	108	156/138	114	105	124	165/158	118	222	189/178	283	141/145
FK9	178	204	176	108	156/138	114	105	124/126	165/158	118	224	189	277	145
FK10	191/178	199/204	176	108	156/138	112	105/118	124	158	118/114	224	189/178	283	141/145
FK11	191/178	204	158	108	156/138	112	105/118	124/126	165/158	118/114	224	178	277	141
GK1	191	204	176	106	156/138	114	105/118	124	165/158	114	224	189/178	283/277	141/145
GK2	191/178	199/204	176	106	156/138	114	105	124	165/158	118/114	224	189/178	283	141/145
GK4	191	204	176	106	138	114	118	124	165/158	118/114	224	178	277	141
GK5	191	204	176	108	156	114	105	124	165/158	114	224	189	283	141
GK6	178	204	176	108	156	112	105	124	165	114	224	189	283	145

Plant	RM459	RM351	RM441	RM422	RM472	RM485	RM258	RM300	RM225	RM248	RM223	RM528	RM511	RM475
MASARB25	67	142	230	385	270	357	150	131	138	94	153	248	138	208
PB1460	63	134	240	358	268	347	134	163	125	104	150	278	143	200
F1	67	134	240	385/358	270	347	134	131/163	138/125	94/104	153	248/278	143	200
F2	67/63	142/134	240/230	385/358	268	347	134	131/163	138/125	94	153/150	248/278	138	200/208
F3	67/63	142/134	240/230	358	268	357/347	150/134	131	138/125	94	153	248/278	143/138	200/208
F4	67/63	134	240/230	385/358	270/268	347	134	131/163	138/125	104	153	248/278	143	200/208
F6	67/63	142	240	385/358	270/268	347	134	131/163	138/125	104	153/150	248	143/138	208
F7	67	142/134	240/230	358	270	357	150/134	131/163	138/125	104	153/150	248/278	138	200/208
F8	63	142/134	240/230	385/358	268	357/347	150/134	131/163	138/125	94/104	153/150	248	138	200/208
F9	63	142/134	230	385/358	270	357	150/134	131/163	125	94/104	153/150	278	138	200/208
F10	63	142/134	240	385/358	270/268	357	150/134	131/163	125	94/104	153	248/278	138	200/208
F11	67	134	240/230	385/358	268	357/347	134	131	138/125	94	150	248/278	138	200/208
F12	63	134	240	358	270/268	357	134	163	138	94	153	278	143/138	208
F13	67/63	134	240	385/358	268	357	150/134	131/163	138	94	153	248/278	138	200
F15	67	134	230	358	270/268	357/347	150/134	131	138	104	153	248/278	138	200
F16	67/63	142	240	385/358	270/268	357	134	131/163	138	94/104	153/150	278	138	200/208
F17	67/63	142	230	385/358	270/268	357	134	131	125	104	153	278	143/138	200/208
F18	67	142	240	385/358	270/268	357	134	163	138/125	104	153	248/278	138	200/208
F19	67/63	142	240	385/358	270/268	347	134	131/163	125	94	150	248/278	143/138	200
F20	67/63	134	240	385/358	270/268	357/347	134	163	138	94	153	248/278	143/138	200/208
F21	67	134	240	385/358	270	357	134	131/163	138	94	150	248/278	138	208
F22	67	134	240	358	268	357	134	131/163	138/125	104	153	248/278	143/138	200/208

F23	67	142/134	240	385	270/268	357	150/134	131/163	125	104	153/150	248	143/138	208
F24	67	142/134	240/230	358	268	357/347	134	131/163	138/125	104	153	248	143/138	200
F26	67	134	230	385/358	270/268	357	134	131	125	94/104	153/150	248	143/138	208
F27	63	134	230	385/358	270	357	150/134	131/163	138/125	94	153	278	143/138	208
F28	67	142/134	240	385	270/268	357/347	134	131	138	94	153/150	248/278	138	208
F29	67/63	134	240/230	385/358	270/268	357/347	134	131/163	138/125	94	153	248	143/138	200/208
F30	67/63	134	240/230	385	270/268	357	134	131	138/125	94/104	153	278	143/138	200/208
F31	67/63	142/134	240/230	385/358	270/268	347	134	131/163	138/125	94	153/150	248/278	143	200/208
F32	63	134	240	385/358	270	357/347	134	131/163	138/125	94/104	153/150	278	138	200
F33	67	134	240/230	385/358	270	357/347	150/134	131/163	138/125	94/104	153	278	138	208
F34	67	142/134	240	385	268	357	150/134	131/163	125	94/104	150	248/278	143/138	200
F35	63	134	230	385	268	357/347	134	131	138/125	94/104	153	248	143/138	200
G1	67/63	134	240	358	270/268	357	134	131/163	138	94	153	248	143/138	208
G2	67/63	134	240	385	268	357	150/134	131/163	138	94	153/150	278	143/138	208
G3	67	134	230	385	270/268	357	134	131/163	138	94	153	278	143/138	200/208
G5	67	142/134	240	385	268	357/347	150/134	131	138/125	94	153	248/278	138	200/208
G6	63	142/134	240	385	268	357/347	134	131	138	94	153	248/278	143	200/208
G7	63	134	230	358	268	357/347	134	131	125	94	153	248/278	143/138	200/208
G8	67	142/134	240	385	270/268	347	134	131	125	94	153/150	248	143	200
G9	67	142/134	240	385/358	270/268	347	134	131	125	94	153/150	248/278	143/138	200/208
G10	63	142/134	240/230	385/358	270/268	357	134	131	138	104	153	248/278	143/138	200
G14	63	134	240/230	385	268	357	134	131/163	138	94	153	248/278	143/138	200/208
G16	67	134	240/230	385/358	270	357/347	150/134	163	125	94	153	248	138	200

G17	67	134	240/230	385/358	268	357/347	134	163	138	94/104	153	278	138	208
G18	67	134	230	385	268	357/347	134	163	138	94/104	153	248/278	143/138	208
G19	63	134	240/230	385/358	270	357	134	131	138/125	94/104	153	248/278	138	208
G20	63	142/134	240	385/358	268	357/347	134	163	138/125	94/104	153/150	248	143	200
G21	67/63	142/134	240/230	358	270/268	357	134	163	125	94/104	153/150	278	143/138	200
G22	67/63	142/134	240/230	385	270	357/347	134	163	125	94	153/150	248/278	143/138	200
G23	67/63	142/134	240	385/358	270/268	357	134	131/163	138/125	104	153	248/278	138	200/208
G23(1)	67	142/134	240/230	358	270/268	357/347	134	131/163	138/125	94	153/150	278	143/138	200
G24	67/63	142/134	240/230	385	270/268	357	150/134	131/163	138/125	94	153	248/278	138	200
G25	63	134	240	385	270/268	357	134	131/163	138/125	94/104	153	278	138	200
G26	67	134	230	385/358	268	357/347	134	131/163	138	104	153	248/278	143/138	200
Ga(1)	67/63	142/134	240/230	385/358	270	347	134	131/163	138	94/104	150	248	143	208
Gb	67/63	142/134	240/230	385/358	270/268	347	134	163	138/125	94	150	278	138	208
FK1	67	142/134	240	385	268	357/347	134	163	138/125	94/104	153/150	248	143/138	208
FK2	63	142/134	230	385	270/268	357	150/134	131	138/125	104	153/150	248/278	143/138	200/208
FK3	67/63	142/134	240	385	270/268	357	150/134	131	138/125	104	150	248/278	143/138	200/208
FK4	67/63	142	230	385/358	268	357/347	150/134	131	125	94	153/150	248/278	143/138	200/208
FK5	67/63	142	230	385	270/268	347	134	163	138/125	94	153/150	248	143/138	200
FK6	63	134	230	358	268	357	150/134	163	138/125	94/104	153	278	143/138	200
FK7	67/63	134	230	385/358	270	357/347	150/134	131/163	138/125	94	153	248/278	143/138	200
FK8	67	142/134	230	385/358	270	357/347	150/134	131/163	138/125	94	153	248/278	143/138	200
FK9	67	134	240	385	270	357	134	131/163	138	94	153/150	248/278	143	200
FK10	67	142/134	230	385/358	270	357/347	150/134	131/163	138	94	153/150	248/278	143/138	200

FK11	67	142/134	240	385/358	270	357/347	150/134	131	138	94/104	153/150	248/278	143	200/208
GK1	63	142	240	385	268	347	150/134	131/163	138/125	94/104	153/150	248	143/138	208
GK2	67	142/134	240	358	270	357	134	131/163	138/125	104	153/150	248/278	138	200
GK4	67	142/134	230	385	270/268	357	134	131/163	138/125	104	153	248	143/138	208
GK5	67	142	230	358	270	357	134	131/163	138	94	153	248/278	143	200/208
GK6	67	142	230	385/358	270/268	357	150/134	131/163	138	94	153	248/278	143	200/208

Plant	RM264	RM539	RM574	RM3331	RM589	RM5412	RM1092	RM520	RM440	RM28048	RM6314	RM587	RM547	RM518
MASARB25	178	278	160	129	204	103	192	259	211	97	169	217	230	165
PB1460	151	281	155	133	192	106	195	247	150	104	173	202	256	159
F1	178/151	278	155	129/133	204/192	106	195	259/247	211/150	97	169	217/202	230	165/159
F2	151	278	160/155	129	204/192	106	195/192	259/247	211/150	97	169	217/202	256	165/159
F3	178/151	278	155	133	204/192	103	195/192	247	211/150	97/104	169/173	217/202	230/256	165/159
F4	178	278/281	160/155	129/133	204	103	195	247	211/150	97/104	169	217	230/256	165/159
F6	178/151	278/281	160/155	129/133	204/192	103	195/192	259/247	211	97/104	169	202	230/256	165
F7	151	278/281	160	129	204	103	192	247	211	97/104	169	202	256	165
F8	178/151	281	160	129	204/192	103	195/192	259/247	211	104	169	217	256	165
F9	178/151	281	160/155	133	204/192	103	195/192	259/247	211/150	97	169/173	202	230/256	165/159
F10	178	281	160/155	129/133	204	106	192	259/247	211	97/104	169/173	217	230	165/159
F11	178/151	281	160/155	129/133	204	103	195	259/247	211/150	104	169	217/202	230/256	165
F12	178/151	278	160/155	129/133	192	106	195/192	259/247	211	97	173	202	230	165/159
F13	178/151	281	160	129/133	204	103	195	247	211	97/104	169/173	217	230	165
F15	178/151	278/281	155	129	204	103/106	195/192	259/247	211	97/104	169/173	217	230/256	165/159

F16	151	278/281	155	129	204	106	195	259/247	211	97/104	169	202	256	165
F17	151	278/281	160/155	129/133	192	106	195/192	259	211/150	104	169/173	202	256	165/159
F18	178	278/281	160	129/133	204	103/106	195	259/247	211	97	169/173	202	230/256	165/159
F19	178	278	160/155	129/133	204	103	195	259/247	211	97/104	169/173	202	256	165
F20	178/151	278	160/155	129	204	103/106	195/192	259/247	211	97	169/173	217/202	230	165/159
F21	178/151	278/281	160/155	129	204/192	103/106	195/192	259/247	211/150	97	169/173	202	230	165/159
F22	178/151	278/281	160/155	133	204/192	106	195/192	247	211/150	104	173	217	230	165/159
F23	178/151	278/281	160/155	133	204/192	103	195/192	259/247	211	97	169/173	217/202	230/256	165
F24	178/151	278/281	155	129/133	204	103/106	195/192	247	211	104	169/173	217/202	230/256	165/159
F26	151	278/281	160/155	129	204/192	103/106	192	247	211/150	97/104	169	202	230	165/159
F27	178/151	278/281	160	133	204/192	103/106	195/192	259/247	211/150	97/104	169/173	217	230	165/159
F28	178/151	281	155	129	204/192	103/106	195	259	211	97	173	202	230/256	165/159
F29	178/151	278	160/155	133	204/192	103/106	192	247	211	97/104	169	217/202	230	165/159
F30	178/151	278/281	160/155	129/133	204/192	106	195/192	259/247	211	97/104	173	217/202	230	165/159
F31	151	281	155	129/133	192	103	195/192	259	211	97/104	169/173	217/202	230/256	165
F32	151	278/281	160/155	133	204	106	195/192	247	211	97/104	169	217/202	230	165/159
F33	178/151	278/281	160	133	192	103	195/192	259	211	97/104	169/173	217/202	230	165/159
F34	178/151	278/281	160/155	133	204	103	195/192	259/247	211/150	97/104	169/173	217/202	230/256	165/159
F35	178/151	281	160	129/133	204/192	103	195/192	259	211/150	97/104	169/173	217/202	230/256	165/159
G1	151	278	155	129/133	204/192	103	195/192	259	211/150	97	169	217/202	230	165
G2	178	281	160/155	129	204/192	103	195	259/247	211/150	97	169	217/202	230	165/159
G3	178	281	160/155	129	204/192	103	195	259/247	211/150	97	169	217/202	256	165/159
G5	178/151	278	155	129/133	204/192	103	195/192	247	211	97	169/173	217/202	256	165/159

G6	178	278/281	160/155	129/133	204/192	103	195/192	259/247	211	97/104	169/173	217/202	230/256	165/159
G7	151	278/281	155	129/133	204/192	103	195/192	259/247	211	97	169	217/202	230/256	165/159
G8	178/151	278	160/155	133	204/192	103/106	192	247	211	97/104	169	217/202	230/256	165/159
G9	178	281	160/155	129/133	204/192	103	195/192	259	211	104	169/173	217/202	256	165
G10	178/151	278/281	160	129	204/192	103/106	195	259/247	211	104	169/173	217	256	165/159
G14	178/151	278/281	155	129/133	204	103/106	192	259/247	211	97	169/173	217	230/256	165/159
G16	178	278/281	160	129	204	103	195	259	211	97	169	202	230/256	165
G17	151	278	155	129	204	103/106	195	259/247	211	97	169/173	217	230	165/159
G18	151	278	155	129/133	192	103	195/192	259/247	211	97	169/173	202	230	165
G19	151	278/281	160	129	204/192	103	192	259/247	211/150	97	173	217/202	230	159
G20	151	278/281	160	129/133	204	103	195	247	211/150	104	173	217/202	256	165/159
G21	178	278/281	160	129/133	204/192	103	195/192	247	211/150	97	169	217/202	230	165/159
G22	178/151	278	155	133	204/192	103	195/192	259/247	211	97	169	202	230	165/159
G23	178	281	160/155	129	204/192	103	195	259/247	211	104	169/173	217/202	230/256	165/159
G23(1)	178	278	160/155	129/133	204/192	103	195/192	259	211	97/104	169/173	217/202	230/256	165/159
G24	178/151	281	160/155	129	204	103	195/192	247	211/150	97	169	217/202	230/256	165/159
G25	178	278/281	160/155	129/133	204/192	106	195/192	247	211	104	169/173	217/202	230	165/159
G26	178/151	278/281	160/155	129/133	204/192	103	195/192	259/247	211	97	169	217/202	256	165/159
Ga(1)	151	278/281	160	133	204	106	195/192	259/247	211	97/104	169	217	256	165
Gb	178/151	281	160/155	129	204/192	103	195/192	259	211	97/104	169/173	217/202	230/256	159
FK1	178/151	278/281	160/155	129/133	192	103	195/192	259/247	211	97/104	169	217/202	230/256	165/159
FK2	178/151	278/281	160/155	129/133	204/192	106	195	259/247	211/150	97/104	169/173	217/202	230/256	165/159
FK3	178/151	278/281	160/155	129/133	204/192	103	195/192	259/247	211/150	97/104	169	217	230/256	165/159

FK4	178/151	278/281	155	129/133	192	103	195/192	259/247	211	97/104	169/173	217/202	230/256	165/159
FK5	178	278/281	155	129/133	192	103	195/192	259/247	211	104	169/173	217/202	230/256	165/159
FK6	151	278/281	155	129/133	204	103/106	192	247	211/150	97/104	169/173	217	230/256	165/159
FK7	178/151	278/281	160/155	129	204/192	103/106	195	259/247	211	97/104	169/173	202	230/256	165/159
FK8	178/151	278/281	160/155	129/133	192	103	195/192	259/247	211	97/104	169/173	202	230/256	165/159
FK9	178	281	160/155	129	204	103/106	195	259	211/150	97	173	217	230	165/159
FK10	178/151	278/281	160/155	129/133	204	103/106	195/192	259/247	211	97/104	169	217	230/256	165
FK11	151	278	160/155	133	204/192	103	192	259	211	104	169	217	256	165/159
GK1	178/151	278/281	160/155	129/133	204/192	103	195	259/247	211	97/104	169/173	217/202	230	165
GK2	178/151	278/281	160/155	129	204/192	106	192	259	211	97	169	217/202	230/256	165/159
GK4	151	278	155	129	204/192	103	195	259/247	211/150	97	169/173	217/202	230	165
GK5	178/151	278	155	133	204/192	103	192	259/247	211	97/104	169/173	217/202	230	165
GK6	178/151	278	155	129	204	106	195/192	259	211/150	97/104	169	217	230	165

Plant	RM575	RM278	RM279	RM10916	RM7033	RM333	RM544	RM2	RM240	RM235	RM247	RM205	RM477	RM506
MASARB25	204	148	170	296	142	165	252	144	145	118	138	120	220	123
PB1460	201	144	174	294	145	183	248	153	140	106	175	165	218	117
F1	204/201	148	170	296	142	183/165	252	144/153	140	118	138	165	220	123
F2	204/201	148	170/174	296	145/142	165	252/248	144/153	145	118	138	120	220	123
F3	201	144	170	296/294	142	183/165	248	144/153	140	118/106	138	120	218	117
F4	204	148	170	296/294	145/142	183	248	144/153	140	118	138	120	218	117
F6	204	148/144	170	296/294	142	183/165	248	153	145	118	138	120	218	123/117
F7	204/201	144	170	296/294	142	183	252/248	144	145	118/106	138	120	220	117

F8	204/201	144	170/174	296/294	145/142	183	252/248	144	145/140	118	138	120	220	117
F9	204/201	148/144	170/174	296/294	145/142	183	252/248	144	145/140	118/106	175/138	165	218	123
F10	204/201	148/144	170	296/294	142	183	248	144	145/140	118	138	120/165	220	123/117
F11	204/201	144	170/174	296	145/142	183/165	252/248	144	145	118	138	120/165	220	123/117
F12	204/201	148/144	170/174	296/294	145/142	183/165	252/248	144	140	118/106	175/138	120/165	218	123/117
F13	204/201	144	170	296/294	142	183	252	153	140	118	175/138	120	218	123/117
F15	201	144	170/174	296	145/142	165	248	144/153	145/140	118	138	120	218	123/117
F16	201	144	170	296/294	142	165	248	144	145/140	118	138	120	218	117
F17	204	148/144	170	296/294	142	183	248	144	140	118	138	120	220	123/117
F18	201	144	174	296/294	145	183	248	144	140	118	138	120	220	123
F19	204	144	174	296/294	145	183	248	144	140	118	138	120	220	123/117
F20	201	144	170	296/294	142	165	252	144	145/140	118	138	165	220	123/117
F21	204	148/144	174	296/294	145	183/165	252	144/153	145	118	138	120/165	218	117
F22	204/201	148/144	170/174	296/294	145/142	183	248	144/153	145/140	118	138	120/165	220	123/117
F23	204	144	170/174	296/294	145/142	165	248	144	145/140	118	138	120	218	117
F24	204/201	148/144	170/174	296/294	145/142	165	248	144/153	145	118	138	120	218	123/117
F26	201	144	170/174	296	145/142	183/165	252	144/153	145	118	138	165	220/218	123/117
F27	204	144	170	296/294	142	183/165	252	144/153	140	118	138	120	218	123/117
F28	204/201	148	170/174	296	145/142	165	248	144	145	118	138	120	220/218	123/117
F29	204/201	144	170/174	296/294	145/142	183/165	248	144	145	118	138	120	218	117
F30	204/201	144	170	296/294	142	183/165	252	144	140	118/106	138	165	218	117
F31	201	144	170	296/294	145/142	183	248	144/153	140	118	138	120	218	123/117
F32	204/201	144	170	296/294	142	183/165	252	144/153	145/140	118/106	138	120	220	123

F33	204/201	144	170/174	296/294	145/142	183/165	252	153	145/140	118	138	120	220	123/117
F34	204	144	170/174	296/294	145/142	183/165	252/248	144/153	145/140	118	138	120/165	220	123
F35	201	148/144	170/174	296/294	145/142	183/165	248	153	145/140	118	175/138	165	220	123/117
G1	204/201	144	170	296/294	142	183/165	252	144	140	118	138	120	218	117
G2	204/201	144	170/174	296/294	145/142	183	248	144	140	118	138	120	220	117
G3	204/201	144	170/174	296/294	145/142	183/165	248	144	145	118	175/138	120	220	123/117
G5	201	148	174	296/294	145	183	248	144	145	118	138	120	220	123/117
G6	204/201	148/144	170	296	142	183/165	252	144	145/140	118	138	120	218	123/117
G7	204/201	148	174	296	145	183/165	252	144/153	145/140	118	138	120	218	117
G8	201	144	170	296/294	142	183/165	252	144/153	145	118	138	120	218	123
G9	201	144	170/174	296/294	145/142	183	248	144/153	145/140	118/106	175/138	120	218	123/117
G10	204/201	148/144	170/174	296/294	145/142	183	248	144/153	145/140	118	138	120	220	123/117
G14	201	148/144	174	296/294	145	165	248	144	140	118/106	138	165	220	117
G16	204	148	170	296/294	142	165	248	144	145	118	138	120	218	117
G17	204/201	148/144	170/174	296	145/142	183	248	144	140	118	138	120	220	117
G18	204/201	144	170/174	296/294	145/142	165	252	144/153	145	118	138	165	220	123/117
G19	204/201	144	174	296/294	145/142	183	252	144/153	145/140	118	138	120/165	220	123/117
G20	204	144	174	296/294	145/142	183	248	144/153	140	118	175/138	120	220	123/117
G21	204	144	170	296/294	145/142	183	252	144/153	140	118	138	120	218	123/117
G22	204/201	144	170	296	145/142	183/165	248	144	145/140	118	138	120	220	123
G23	201	148/144	170/174	296/294	145/142	183	248	144/153	140	118/106	175/138	120/165	220	123
G23(1)	204/201	144	170/174	296/294	145/142	183	248	144/153	145/140	118	175/138	165	220	117
G24	204	144	170	296/294	142	165	248	153	140	118/106	175/138	165	218	123

G25	204/201	144	170/174	296/294	145/142	183	252	144	145/140	118	138	120/165	220/218	123/117
G26	204/201	144	170	296/294	142	183	248	144	145	118/106	138	120/165	218	123
Ga(1)	204	144	170/174	296/294	145/142	165	248	144	145/140	118	175/138	120	220/218	123/117
Gb	204/201	148/144	174	296/294	145/142	183	252	144	140	118/106	138	120	220/218	117
FK1	204/201	144	170	296/294	142	183/165	248	144	145/140	118	138	120	220/218	123/117
FK2	204/201	144	170	296/294	142	183/165	248	144	140	118	138	120	218	123/117
FK3	204/201	144	170/174	296/294	145/142	183	248	144	140	118	138	120	220	123/117
FK4	204	144	170/174	296	145/142	183/165	252/248	144	140	118	138	120	220	123/117
FK5	204	144	170	296	145/142	183	248	144/153	145/140	118	138	120	220	123
FK6	204	144	170/174	296/294	145/142	183	252/248	144/153	145/140	118	138	120/165	220	123/117
FK7	201	148/144	170	296/294	145/142	183/165	252	144/153	145/140	118	138	120/165	218	123/117
FK8	204/201	148/144	170/174	296/294	145/142	183	252	144	140	118	138	120	220	123/117
FK9	204/201	148/144	170	296/294	142	183	252/248	144/153	140	118	138	165	218	123/117
FK10	204/201	144	170/174	296/294	145/142	165	248	144	140	118	138	120/165	220	123/117
FK11	204/201	148	170	296/294	142	183	252/248	144/153	140	118/106	175	120	218	123
GK1	204/201	144	174	296/294	145/142	165	252/248	144/153	140	118	138	120	218	123
GK2	204/201	144	170/174	296/294	145/142	183	252/248	144/153	140	118	138	165	218	123/117
GK4	204/201	144	170/174	296/294	145/142	183	248	144/153	140	118/106	138	120	218	123/117
GK5	204/201	148	170	296/294	145/142	165	248	144	140	118	138	120	218	123/117
GK6	204/201	148	170	296/294	145/142	165	248	144	140	118	138	120	218	123

Plant	RM531	RM527	RM525	RM204	RM207	RM410	RM206	RM228	RM234	RM310	RM260	RM336	RM335	RM339
MASARB25	108	240	107	178	117	209	164	150	162	98	122	194	150	150
PB1460	115	238	137	131	118	183	124	112	135	116	64	164	129	176
F1	115/108	240/238	137	178/131	118	183	164/124	112	135	98	64	164	150/129	176
F2	115	240	107	178/131	117	209	124	150	162/135	116	122	194/164	150/129	176
F3	115/108	240/238	137	178/131	118	209	164	150	162	116	122	194/164	150/129	176
F4	115/108	240/238	137	178	117	209/183	164	150	162/135	116	64	194/164	150	176
F6	115	240	107	178	117	209	124	112	135	116	122/64	164	150/129	176
F7	115/108	238	107	178	118	209/183	164	112	162/135	116	122	194/164	150/129	176
F8	115	240/238	137	178/131	118	209/183	164/124	150/112	162/135	116	122	194/164	150/129	176
F9	115/108	238	137	131	117	209/183	164	112	162	98	122	194/164	150	150
F10	108	238	107/137	178/131	118	209	164/124	112	162/135	98	122	194/164	129	176
F11	115	240/238	107/137	178	118	183	164/124	150/112	162	116	122	194/164	150/129	150
F12	108	238	137	178/131	118	209	164	150/112	162/135	116	122	194/164	150	176
F13	108	240/238	137	178	117	209/183	124	112	162/135	116	122/64	164	150/129	176
F15	108	240/238	137	178	118	209/183	164/124	150	162/135	98	122/64	194	129	150
F16	115/108	240/238	107/137	178	118	209/183	164	150	162/135	116	122	194	150	176
F17	115/108	240/238	107	178/131	118/117	209	124	150/112	162/135	116	122	194	150/129	150
F18	115	240	137	178/131	118/117	209	124	112	162/135	116	122	194/164	150/129	150
F19	115	240	137	178/131	118/117	209/183	164	150/112	162/135	116	122/64	194	150/129	176
F20	108	240/238	137	178	118	209/183	124	150	162/135	98	122/64	194	150/129	150/176
F21	115/108	240/238	107	178/131	118/117	209	164/124	150/112	162/135	116	122/64	194	150/129	176

F22	108	240/238	137	178/131	118/117	209	164/124	112	162/135	116	122/64	194	129	150
F23	108	240/238	107	131	117	209/183	124	150	162/135	116	64	164	150	176
F24	108	240/238	107	178/131	118/117	209	164	112	162	116	122	194/164	150/129	150/176
F26	108	240	107	131	118/117	209	124	150/112	135	98	122/64	194	150/129	176
F27	108	238	107/137	178/131	118	183	164/124	150	162	98	122/64	194	150/129	150
F28	115/108	240	137	178	118	209	164	150/112	135	116	122	164	129	176
F29	115	240/238	107	131	118/117	183	124	150/112	135	116	122/64	194/164	129	176
F30	108	238	137	178/131	118	183	164	150/112	162/135	116	122/64	194/164	129	176
F31	108	240/238	137	178/131	118	209/183	164/124	112	162/135	116	122/64	194/164	150	150/176
F32	108	240/238	137	178/131	118	209/183	124	112	135	116	122	164	150	176
F33	108	240/238	137	178/131	118	209/183	164/124	150/112	135	98	122/64	194/164	150/129	176
F34	108	238	137	178/131	118	209/183	164	150	135	116	122	164	150/129	176
F35	115	240	107/137	178/131	118	209	164/124	112	162/135	116	122	194	150/129	150
G1	108	238	137	178/131	117	209	124	150/112	135	98	122/64	164	150	176
G2	108	238	107/137	178/131	118	209	124	150/112	162/135	116	122/64	194/164	150	176
G3	108	240/238	137	178/131	118	183	164	112	162/135	116	122	194	150/129	176
G5	115	240	107	178/131	117	183	164	150	162/135	116	122	194/164	150/129	176
G6	108	240/238	107/137	178/131	118	209	164	150/112	135	116	64	194/164	150/129	176
G7	115/108	240/238	137	178/131	118	209/183	164/124	150/112	162/135	116	64	194	129	176
G8	108	240	107	178/131	118	209	164	150/112	135	116	64	164	150/129	176
G9	108	238	107/137	178/131	118	209/183	164	150/112	135	116	122	164	150/129	176
G10	115	240/238	137	178	118	183	164/124	150/112	162/135	116	122	194/164	150/129	176
G14	115/108	238	107/137	178/131	117	209/183	124	150	162/135	98	64	194/164	150/129	150

G16	108	240/238	107	178	118/117	209/183	152	150/112	162	98	122	194	150	176
G17	108	240	137	178	117	209	164	150/112	162/135	116	122	194/164	150/129	176
G18	108	240/238	137	178/131	118	183	152	150	162	98	122	194/164	150	176
G19	108	238	107	178	117	209	164	150/112	162/135	98	122	194/164	150/129	150
G20	108	240/238	137	178	118	183	124	150/112	135	116	122/64	164	150/129	176
G21	108	240	107/137	178/131	118	209/183	124	150	162	116	122/64	164	150/129	176
G22	108	240	107	131	118	183	164/124	150/112	162	116	122/64	194/164	150/129	176
G23	115	238	137	178/131	118/117	183	124	112	135	116	122	164	150	176
G23(1)	115/108	240	137	178/131	118/117	209	164/124	150/112	162/135	116	64	164	150/129	176
G24	115/108	238	137	178/131	118/117	183	124	150	162/135	116	122	194/164	150/129	176
G25	108	240/238	107/137	178/131	118/117	209/183	164/124	150/112	135	98	122	164	150	176
G26	108	240/238	107/137	178	117	183	164/124	150/112	162/135	98	64	194/164	150	176
Ga(1)	115	240	107/137	178	118/117	209/183	124	150	162/135	116	64	194/164	150/129	176
Gb	115	238	137	178/131	118/117	209/183	164/124	150/112	162/135	116	122/64	194/164	129	176
FK1	115/108	240/238	107	178/131	118/117	209	124	112	162/135	116	64	164	150/129	150
FK2	115/108	240/238	137	178/131	118/117	209	124	150	162	116	122	194	150	150
FK3	115/108	240/238	137	178/131	118/117	209/183	164/124	150	135	116	64	194/164	150	176
FK4	115	240/238	107/137	131	117	209/183	164/124	150/112	162	116	64	194	150	176
FK5	115	240/238	107/137	178/131	117	209/183	164/124	150	162/135	116	64	194/164	150/129	176
FK6	115	240/238	107	178/131	118/117	209/183	164	150/112	162/135	98	122/64	194/164	150	150
FK7	115	240/238	137	178/131	118/117	183	164/124	150/112	162/135	116	122/64	194/164	150	176
FK8	115/108	238	137	178/131	117	209/183	164	150/112	162/135	116	64	194/164	150	176
FK9	115/108	238	137	178/131	118/117	209/183	164/124	150/112	135	98	122	194/164	150	150

FK10	108	240/238	107/137	178	118/117	209	164	150/112	162/135	98	122/64	194	150	176
FK11	115/108	240	107/137	178	117	183	164/124	150/112	135	116	122/64	164	150	176
GK1	115	240/238	107/137	178/131	118/117	209	164/124	150	162/135	98	122/64	194	150	176
GK2	115	240/238	107/137	178/131	118	183	164/124	150/112	162	116	122	194	150/129	176
GK4	115/108	240	107/137	178/131	118	183	164	150/112	135	116	64	194/164	150/129	150
GK5	115	240/238	107/137	178	117	209/183	124	150	135	98	64	194	150	176
GK6	108	240/238	107/137	178/131	118/117	209/183	164	150	135	116	64	164	150	176

Plant	BAD2	RM241	RM270	RM306	RM474	RM7562	RM259	RM315	RM30	RM80	RM256	RM324	RM230	RM400	RM408
MASARB25	355/585	140	117	183	238	190	180	160	80	145	107	135	255	198	120
PB1460	257/585	142	114	175	226	181	160	139	106	173	135	138	254	186	126
F1	355/585	142	117/114	183/175	238	190	180160	160	106/80	145/173	135/107	135/138	254	198/186	120
F2	257/585	142	117	183/175	238/226	190	180160	139	106/80	173	135	135/138	254	198/186	120
F3	257/355/585	142	117	183/175	238	190	160	139	106/80	145/173	135/107	135	254	198/186	126
F4	257/355/585	140	117/114	183	238/226	190/181	180	160/139	106/80	145/173	135/107	135/138	255	198/186	126
F6	257/585	142	114	183/175	238/226	190/181	180	160/139	80	173	135	135/138	255	198	126/120
F7	257/585	142/140	117	183/175	238/226	190/181	180160	160	106/80	145/173	135/107	135/138	255	198/186	126
F8	257/585	142/140	117	183/175	238	190	180160	139	80	173	135	135/138	254	198	126
F9	257/585	142	114	175	238	190/181	180160	160	106	145/173	135/107	135/138	254	186	120
F10	355/585	142	117	183	238	190/181	180160	160/139	106/80	145	107	135/138	254	198/186	126/120
F11	257/355/585	142	117/114	183	238	190/181	180160	139	106/80	173	135	135	254	198/186	126/120
F12	257/585	142/140	117/114	183/175	238/226	190/181	180160	160/139	106	145	107	138	254	186	126/120
F13	257/585	142/140	117/114	183	238/226	190	180160	139	106/80	145	107	135/138	254	198/186	126/120
F15	355/585	142/140	117	183	238/226	190	160	160/139	106/80	145	107	135	254	198/186	126/120

F16	257/585	142	117/114	183	238/226	190	160	160/139	106	145/173	135/107	135/138	254	186	126
F17	257/355/585	142/140	117/114	183/175	238/226	190	180	160/139	106	145/173	135/107	135	254	186	126/120
F18	257/585	142/140	117/114	183/175	238	190/181	160	160/139	106/80	173	135	138	255	198/186	120
F19	257/355/585	142/140	117	183/175	238	190/181	180	160/139	106/80	173	135	135/138	255	198/186	126/120
F20	257/585	142/140	117	183/175	238/226	190/181	160	160/139	106/80	145	107	138	254	198/186	126/120
F21	257/585	142/140	114	175	238/226	190/181	180	160	106/80	145/173	135/107	135/138	254	198/186	126
F22	355/585	142/140	114	175	226	190/181	180160	139	106/80	145	107	135/138	254	198/186	126/120
F23	257/355/585	142	117/114	183/175	238/226	181	180	160/139	80	145	107	135/138	254	198	126
F24	355/585	142	117/114	183/175	238	181	180160	139	80	145	107	135/138	254	198	126/120
F26	355/585	142/140	117/114	175	238	181	160	160/139	80	145	107	135	254	198	126/120
F27	355/585	142/140	117/114	175	238/226	190	180	160	106	145	107	135/138	255	186	126/120
F28	257/355/585	142/140	117	183/175	238/226	190	180160	160/139	106/80	145/173	135/107	135	254	198/186	126/120
F29	355/585	142/140	117/114	183/175	238	190	180160	160/139	80	173	135	135/138	254	198	126
F30	355/585	142/140	117/114	175	226	190/181	180160	160/139	106	145	107	135	254	186	126
F31	257/355/585	142/140	117/114	175	238/226	190	160	160/139	106/80	145	107	135/138	255	198/186	126/120
F32	257/355/585	142/140	114	183/175	238/226	190/181	180160	160	106	145	107	135/138	255	186	120
F33	355/585	142	117/114	175	238/226	190/181	180160	160	106	145	107	135/138	254	186	126/120
F34	257/585	142	114	183/175	238/226	190/181	180	139	106/80	145	107	135/138	254	198/186	120
F35	257/355/585	142/140	114	183/175	238/226	190/181	160	139	80	173	135	135	254	198	126/120
G1	355/585	142/140	114	183/175	238/226	190/181	180160	160/139	80	145	107	135/138	254	198	126
G2	257/355/585	142	117	183/175	238/226	190	180160	139	106	145	107	135/138	254	186	126
G3	257/355/585	142	117	183/175	238/226	190	180160	160/139	106	145	107	135/138	254	186	126/120
G5	257/355/585	142	117/114	175	238/226	190/181	160	139	106/80	173	135	135	254	198/186	126/120
G6	257/355/585	142	117	175	238/226	190/181	180160	139	106/80	145	107	135	254	198/186	126/120
G7	257/355/585	142/140	117/114	175	238/226	190/181	180160	139	106/80	145/173	135/107	135	255	198/186	126

G8	257/355/585	142	114	183/175	238	190/181	160	160/139	80	145	107	135	255	198	120
G9	257/355/585	142/140	117/114	183/175	238/226	181	160	160/139	106/80	145	107	135	254	198/186	126/120
G10	355/585	142/140	117	183/175	238	190/181	180/160	160/139	106/80	173	135	135	255	198/186	126/120
G14	355/585	142	117	175	238/226	190/181	160	139	106/80	145/173	135/107	135/138	254	198/186	126
G16	355/585	142/140	117	183/175	238	190/181	180	160	80	145	107	138	255	198	126
G17	355/585	142	117	175	238	190/181	180/160	139	106	145	107	138	254	186	126
G18	355/585	142	117	175	238/226	190/181	180/160	139	106/80	145	107	138	255	198/186	126/120
G19	355/585	142/140	117	183/175	238	190	180/160	160	106/80	145	107	135	255	198/186	126/120
G20	257/355/585	142/140	117/114	175	238/226	190/181	180	139	80	145	107	138	255	198	126/120
G21	257/355/585	142	117/114	175	238/226	190	180	160/139	106	145	107	138	254	186	126/120
G22	257/355/585	142/140	117	183/175	238/226	190/181	180/160	160	106/80	145	107	138	254	198/186	120
G23	257/355/585	140	117	183/175	238/226	190/181	160	160/139	106/80	173	135	135/138	254	198/186	120
G23(1)	257/355/585	142/140	117/114	183/175	226	190/181	180/160	160/139	106	145/173	135/107	135/138	254	186	126
G24	355/585	142/140	117	183/175	238	190/181	180	160/139	106/80	145/173	135/107	135/138	255	198/186	120
G25	355/585	140	114	175	238	190/181	180/160	160/139	106	145	107	135/138	254	186	126/120
G26	257/355/585	140	117/114	183/175	238/226	190/181	180/160	139	106/80	145	107	135/138	254	198/186	120
Ga(1)	257/355/585	140	114	183	238/226	190	180	160	80	173	135	135/138	255	198	126/120
Gb	257/585	142/140	117	183	238/226	181	180/160	160/139	106	173	135	138	254	186	126
FK1	257/585	140	117/114	183/175	238	190	180/160	139	80	145/173	135/107	138	255	198	126/120
FK2	257/355/585	140	117/114	183/175	226	190/181	180/160	160/139	106/80	145/173	135/107	135	254	198/186	126/120
FK3	355/585	140	117/114	183/175	238/226	190/181	180/160	160/139	106/80	145/173	135/107	135	254	198/186	126/120
FK4	355/585	142/140	117/114	175	238/226	190/181	180	139	106/80	173	135	135	254	198/186	126/120
FK5	355/585	142/140	117	183/175	238/226	190/181	180	160/139	80	173	135	138	254	198	120
FK6	355/585	142/140	114	183	238/226	190/181	180	139	106	173	135	138	254	186	126/120
FK7	257/355/585	142/140	117/114	183	238/226	190/181	160	160	106/80	173	135	135/138	255	198/186	126/120

FK8	257/355/585	142/140	117/114	183	238/226	190/181	180160	160	106/80	145/173	135/107	135/138	255	198/186	126/120
FK9	257/355/585	142	117/114	183/175	238	190	180160	160	106/80	145/173	135/107	135/138	254	198/186	126/120
FK10	257/355/585	142	117/114	175	238	190/181	180160	160	106/80	145	107	135/138	254	198/186	126/120
FK11	257/585	140	117/114	183/175	238	190/181	180160	160	106/80	145/173	135/107	135	254	198/186	120
GK1	257/355/585	142	117/114	183/175	238	181	180160	139	80	173	135	135/138	254	198	120
GK2	257/355/585	142	117	183/175	238	181	180160	160	106/80	173	135	135/138	254	198/186	126/120
GK4	355/585	142/140	114	183	238	190	180160	160/139	80	145/173	135/107	135/138	254	198	126/120
GK5	355/585	142/140	117	183	238/226	190	180160	160	106/80	173	135	135/138	254	198/186	126/120
GK6	355/585	142/140	117	183	238	190	180160	160/139	106/80	145	107	135/138	254	198/186	120

ANNEXURE V

List of SNP used for whole population genotyping in Aus276/3*IR64 and Aus276/3*MTU1010 population

Aus276/IR64					Aus276/MTU1010			
Chr	SubjectID	IR64	Aus276	Map position (cM)	SubjectID	MTU1010	Aus276	Map position (cM)
1	id1000001	T:T	C:C	0.1	id1000001	T:T	C:C	0.1
	K_id1000556	G:G	A:A	6.3	K_id1000955	G:A	A:A	7.4
	id1002622	G:G	A:A	15.8	K_id1002899	A:A	G:G	17.7
	K_id1003559	C:C	T:T	22.9	id1004007	C:C	T:T	27.4
	K_id1004420	A:A	T:T	29.6	id1005271	C:T	C:C	36.5
	id1006571	A:A	C:C	46.4	id1006691	T:T	C:C	48.2
	id1007852	G:G	A:A	60.5	id1007852	G:G	A:A	60.5
	id1008821	G:G	C:C	69.8	wd1000915	A:A	G:G	71.4
	id1010526	A:A	T:T	76.4	K_id1011200	G:G	T:T	78.8
	wd1001450	A:A	T:T	83	K_id1012048	C:C	T:T	88
	K_id1012481	T:T	C:C	93.4	id1012977	T:T	C:C	95.1
	id1014105	T:T	C:C	100.1	K_id1015302	A:A	C:C	110.9
	K_id1015302	A:A	C:C	110.9	id1016524	T:T	C:C	121.4
	id1016524	T:T	C:C	121.4	id1019114	A:A	C:C	130.4
	id1019114	A:A	C:C	130.4	K_id1021259	A:A	G:G	140.2
	K_id1021259	A:A	G:G	140.2	id1024167	T:T	A:A	148.8
	id1024167	T:T	A:A	148.8	K_id1024973	A:A	G:G	158.7
	K_id1024836	G:G	C:C	158.3	id1026726	T:T	A:A	168.7
	id1026726	T:T	A:A	168.7	dd1002159	T:T	C:C	178.4
	id1027507	A:A	G:G	177.1	-	-	-	-
	K_id1028304	C:C	T:T	184.4	-	-	-	-
	K_id2000235	T:T	C:C	2.3	-	-	-	-
	K_id2001102	G:G	A:A	7.8	-	-	-	-
2	id2001842	A:A	T:T	16.3	K_id1028304	C:C	T:T	184.4
	id2002865	A:A	G:G	26.8	id2000972	T:T	C:C	6.8
	K_id2003646	C:C	T:T	35.3	id2001301	G:G	A:A	11.7
	id2004534	T:T	G:G	44.4	id2001842	A:A	T:T	16.3
	K_id2004970	G:G	C:C	51.3	K_id2002811	C:T	T:T	26.7
	id2006996	A:A	G:G	58.7	K_id2003393	T:T	C:C	33.8
	ud2001031	C:C	G:G	68.3	id2004534	T:G	G:G	44.4
	id2007518	A:A	C:C	78.6	id2005554	A:G	A:A	54.8
	id2008866	G:G	A:A	89.7	id2007378	G:A	A:A	66.6
	K_id2010102	A:A	G:G	97.1	id2007507	A:A	C:C	77.6
	K_id2011302	T:T	C:C	106.8	id2008415	A:C	C:C	83.3
	K_id2012408	G:G	A:A	123	id2009808	G:G	A:A	95.1
	id2013434	C:C	A:A	130.2	K_id2011302	T:T	C:C	106.8
	id2014684	G:G	C:C	142.9	K_id2012408	G:G	A:A	123
	id2015856	A:A	C:C	153.6	id2013434	C:C	A:A	130.2
	K_id2016481	A:A	G:G	161.2	id2014452	T:G	G:G	141.1

	-	-	-	-	K_id2015767	G:G	A:A	153.1
3	id3000197	A:A	G:G	2.5	K_id2016481	A:A	G:G	161.2
	id3001110	A:A	G:G	11.2	id3000019	C:C	T:T	0.9
	id3002912	A:A	C:C	25.5	id3000757	A:A	G:G	6.6
	id3004086	C:C	G:G	38	K_id3001137	A:A	C:C	12.8
	K_id3005216	A:A	G:G	48.6	id3002912	A:C	C:C	25.5
	K_id3006493	T:T	C:T	59.6	K_id3003697	C:C	T:T	32.5
	K_id3007703	C:C	T:T	73	id3004859	A:A	G:G	44.6
	K_id3008333	G:G	A:A	84.3	id3005956	G:G	A:A	53.8
	wd3001849	G:G	A:A	94.7	K_id3006721	A:A	G:G	62.5
	id3011085	C:C	T:T	110.1	K_id3007703	C:C	T:T	73
	id3011383	A:A	C:C	120.6	K_id3008333	G:G	A:A	84.3
	id3013713	G:G	A:A	129.7	wd3001849	G:G	A:A	94.7
	id3014449	A:A	G:G	138.3	id3011085	C:C	T:T	110.1
	id3015833	A:A	G:G	147.8	id3011383	A:A	C:C	120.6
	id3017403	T:T	C:C	158.9	K_id3013480	T:T	C:C	129
	id3018422	C:C	T:T	165.2	id3014158	T:T	G:G	136.3
	-	-	-	-	id3015703	A:A	G:G	146.9
4	id4001205	C:C	T:T	10.2	id3017403	T:T	C:C	158.9
	id4002844	T:T	C:C	20.2	id4000359	G:G	A:A	6.4
	id4004639	A:A	G:G	32	id4001205	C:C	T:T	10.2
	id4005212	T:T	C:C	40.2	id4002540	A:G	G:G	19.1
	id4005570	G:G	C:C	51	id4004457	T:T	G:G	28.6
	id4006835	T:T	G:G	63.6	id4005072	A:A	G:G	38.2
	id4007922	T:T	A:A	74.2	id4005527	G:G	T:T	50.4
	id4008719	A:A	G:G	84	id4006835	T:T	G:G	63.6
	id4009390	T:T	C:C	93.2	id4007771	A:A	G:G	72.2
	id4010497	A:A	C:C	105.8	id4008522	A:A	T:T	80.9
	id4011542	A:A	G:G	115.6	id4009900	T:T	C:C	100.2
	id4012189	C:C	A:A	130.4	id4010651	C:C	T:T	106.8
5	id5000045	T:T	C:C	0.5	id4011542	A:A	G:G	115.6
	id5000759	T:T	C:C	10.3	id5000259	C:C	T:T	3.6
	id5001772	G:G	C:C	24	id5000759	T:T	C:C	10.3
	id5002861	A:A	G:G	35.7	id5001182	G:A	G:G	17.3
	id5004150	T:T	G:G	48.6	id5002421	G:G	C:C	30.8
	id5006615	T:T	C:C	59.3	id5003266	T:T	G:G	40.4
	id5007304	G:G	A:A	67	wd5000922	A:A	G:G	51.4
	id5008574	G:G	A:A	77.8	id5006615	T:T	C:C	59.3
	id5009481	T:T	G:G	89.2	id5007304	G:G	A:A	67
	id5011044	C:C	T:T	99.4	id5008593	T:T	A:A	77.8
	id5013256	A:A	T:T	108.7	id5010621	A:A	G:G	96.1
	id5014788	C:C	G:G	119.3	id5013256	A:A	T:T	108.7
6	id6000130	A:A	C:C	2.6	id5014603	A:A	G:G	117.6
	id6000402	G:G	A:A	4.6	id6000763	G:G	C:C	5.5
	id6002904	A:A	G:G	15.7	id6002904	A:A	G:G	15.7
	ud6000218	G:G	C:C	25.4	id6003446	C:T	T:T	22.3

	id6004089	T:T	C:C	35.8	id6004089	T:T	C:C	35.8
	id6004849	C:C	T:T	46.5	id6004849	C:C	T:T	46.5
	id6006288	A:A	G:G	59.6	id6006288	A:A	G:G	59.6
	id6010515	G:G	A:A	70.5	id6010185	A:A	G:G	68.4
	id6011854	A:A	G:A	84.1	id6011280	A:A	C:C	77.7
	id6013772	T:T	C:C	98.1	id6012365	T:T	C:C	87.8
	id6015531	A:A	G:G	107.7	id6013772	T:T	C:C	98.1
	id6016400	G:G	A:A	115.1	id6015665	A:A	C:C	108.3
	ud6001296	A:A	G:G	123.8	id6016361	T:T	C:C	114.5
7	ud7000154	T:T	A:A	8.8	ud6001296	A:A	G:G	123.8
	ud7000203	T:T	G:G	17.1	id7000519	A:A	C:C	28.2
	id7000519	A:A	C:C	28.2	id7000598	C:C	T:T	37.3
	id7000661	T:T	C:C	40.6	id7000871	G:G	A:A	43.1
	wd7001838	A:A	C:C	50.2	wd7000903	C:C	T:T	49.4
	wd7002642	G:G	A:A	62.2	id7002571	T:T	C:C	55.3
	id7003593	T:T	C:C	76.8	wd7002642	G:G	A:A	62.2
	id7004560	A:A	C:C	87.4	id7003271	C:C	G:G	73.6
	id7005143	T:T	C:C	99.7	id7003853	G:G	A:A	80.7
	id7005557	C:C	T:T	108.6	id7005036	A:A	T:T	97.9
	ud7002101	T:T	C:C	115.2	-	-	-	-
8	ud8000012	G:G	A:A	3.5	id7005667	A:G	G:G	115.7
	id8000536	T:T	G:G	15.4	ud8000012	G:G	A:A	3.5
	id8000845	A:A	G:G	25.3	id8000337	C:T	T:T	13.2
	id8001326	C:C	T:T	34.3	ud8000186	G:G	C:C	22.8
	id8002306	T:T	C:C	46.2	id8002216	C:C	T:T	45.2
	id8003773	A:A	T:T	56.3	id8003773	A:A	T:T	56.3
	ud8001270	C:C	A:A	69.7	wd8003431	G:G	A:A	63.8
	id8005704	T:T	G:G	77.4	id8005704	T:T	G:G	77.4
	id8006856	G:G	A:A	99.3	id8006856	G:G	A:A	99.3
	id8007265	T:T	G:G	110	id8007144	C:C	A:A	108.6
	id8007913	A:A	T:T	121.8	-	-	-	-
9	id9001352	G:G	A:A	3.7	id8007562	A:A	C:C	117.1
	id9002357	A:A	G:G	17.8	id9002252	C:C	A:A	14.3
	id9002704	A:A	C:C	31.7	id9002547	A:G	A:A	23.7
	id9003600	T:T	A:A	44.6	ud9000737	C:T	C:C	37.4
	id9004128	A:C	A:A	53	id9002704	A:A	C:C	42
	id9005890	A:A	G:G	65.8	id9004128	C:C	A:A	53
	id9006773	T:T	C:C	75.1	id9005890	A:A	G:G	65.8
	id9007315	T:T	G:G	86.7	id9006773	T:T	C:C	75.1
10	id10000090	A:A	G:G	1.5	id9007315	T:T	G:G	86.7
	id10001695	C:C	T:T	14.5	id10000090	A:A	G:G	1.5
	id10003104	A:A	G:G	23.1	id10001118	G:G	A:A	8.2
	id10003870	A:A	G:G	32.7	id10001695	C:C	T:T	14.5
	id10004564	A:A	G:G	42.4	id10003104	A:A	G:G	23.1
	id10005369	C:C	A:A	51.3	id10003870	A:A	G:G	32.7
	id10006378	T:T	C:C	60.8	id10005474	T:C	C:C	52

	id10006890	T:T	A:A	77.8	id10006378	T:T	C:C	60.8
	-	-	-	-	id10006740	A:A	C:C	73.3
11	id11000076	G:G	T:T	2.1	id10007301	G:G	A:A	84
	id11000855	G:G	C:C	14.6	id11000076	G:G	T:T	2.1
	id11002304	C:C	A:A	29.6	id11000629	C:C	A:A	9.2
	ud11000372	T:T	C:C	39.4	id11001466	A:G	A:A	20.3
	id11003441	C:C	T:T	49.5	id11002436	T:T	C:C	31.1
	id11005456	T:T	C:C	57.9	ud11000414	A:A	T:T	43.8
	id11006360	C:C	T:T	69.8	id11005607	T:T	C:C	60.4
	id11007488	T:T	A:A	80.5	id11006632	C:C	T:T	73.4
	id11009027	C:C	G:G	92.7	id11008166	C:C	T:T	87.1
	id11009865	G:G	T:T	102.4	id11009201	A:A	T:T	97.6
	id11010544	G:G	C:C	113.1	id11010385	C:C	T:T	110.5
12	id12000558	T:T	C:C	4.4	id11011578	A:A	C:C	118
	id12001164	A:A	G:G	20.1	id12000605	A:A	G:G	4.6
	id12002396	A:A	G:G	33.9	id12001164	A:A	G:G	20.1
	id12003141	T:T	G:G	42.4	id12002396	A:A	G:G	33.9
	id12005437	C:C	T:T	50.1	id12002797	A:A	G:G	38
	id12006327	A:A	T:T	58.9	id12004974	A:A	T:T	47.3
	id12007245	A:A	G:G	69.2	id12006231	T:T	A:A	58.3
	id12007741	T:T	G:G	78.6	id12007245	A:G	G:G	69.2
	id12008557	A:A	G:G	88.5	id12007741	T:T	G:G	78.6
	-	-	-	-	id12008557	A:A	G:G	88.5
	-	-	-	-	id12009192	G:G	A:A	95.3
	-	-	-	-	id12010012	T:T	C:C	103.5

ABSTRACT

Title of Thesis : **Molecular and physiological studies to identify traits enhancing rice cultivars adaptation to aerobic conditions for sustainable rice production in water short situations**

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Title of Degree : Doctor of Philosophy

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In the present investigation experiments were conducted to: identify QTL conferring a potential yield advantage under direct seeded conditions; identify root traits having a positive correlation with grain yield under direct seeded conditions; identify genotypes with high levels of root growth plasticity in multiple conditions (upland, lowland, induced by drought or low P) and to identify a positive correlation of root plasticity with grain yield; BSA approach was applied to identify QTL showing consistent effects in the background of two popular high-yielding varieties, IR64 and MTU1010, under upland reproductive-stage drought stress; identify QTL with large and consistent effects for traits thought to be beneficial for direct-seeded rice: seedling emergence, early vegetative vigour, root morphology, nutrient uptake, and grain yield. We hypothesized that the some of the seedling-stage traits investigated might have a strong enough effect on plant growth to be correlated with grain yield at harvest. Our study identified a total of 35 QTL associated with 14 traits on chromosomes 1, 2, 5, 6, 8, 9, and 11 in MASARB25 × Pusa Basmati 1460 population and 14 QTL associated with nine traits on chromosomes 1, 2, 8, 9, 10, 11, and 12 in HKR47 × MAS26-derived population. These identified QTL included three large-effect stable QTL for increased yield under aerobic conditions and QTL for several root-related traits likely to increase water and nutrient uptake under aerobic conditions. A number of plants were identified with higher root length and dry biomass, yield per plant, length-breadth ratio, and with Pusa Basmati 1460 specific alleles in homozygous or heterozygous condition at the *BAD2* locus; these lines shall serve as novel materials for the selection of stable aerobic Basmati rice varieties. A good correlation was observed between root growth/plasticity in lysimeter, field and rhizoscope study, between water uptake in lysimeter and leaf water status in field. Consistently high plasticity was observed across different treatment and environment. Three large and consistent-effect QTL, *qDTY_{1.1}*, *qDTY_{2.2}*, and *qDTY_{2.3}* for GY were identified under reproductive-stage drought stress in Kali Aus/2*MTU1010 and Kali Aus/2*IR64 populations. A total of 28 QTL associated with 22 traits and 20 QTL associated with 12 traits were mapped in Aus276/3*IR64 and Aus276/3*MTU1010 population, respectively. The QTL *qGY_{6.1}*, *qGY_{10.1}*, *qGY_{1.1}* and *qEVV_{9.1}* were found to be effective in both Aus276/3*IR64 and Aus276/3*MTU1010 populations under wide range of conditions; the QTL for nutrient uptake were located on chromosome 5, co-located with *qGR_{5.2}* and *qRHD_{5.1}*. The positive interaction between the genomic regions for root traits and nutrient uptake depicted that these interacting loci should also be considered when introgressing QTL to develop rice for dry direct seeded conditions. Co-localization of QTL for yield, EVV, and root traits indicates that the identified QTL may be immediately exploited in MAB to develop novel high-yielding direct seeded rice varieties.

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List of publications:

- **Nitika Sandhu**, Sunita Jain, K. R. Battan and R. K. Jain. 2011. Aerobic rice genotypes displayed greater adaptation to water-limited cultivation and tolerance to polyethyleneglycol-6000 induced stress. *Physiol. Mol. Biol. Plants*, **18 (1)**: 33-43.
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- Novel insights into the plasticity of rice root adaptive development under dry direct seeded cultivation condition. (*In preparation*).

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