ABSTRACT

QUANTITATIVE TRAIT LOCI MAPPING OF DROUGHT RESISTANCE TRAITS IN RICE *(Oryza sativa* L.) LINE ADAPTED TO TARGET POPULATION OF ENVIRONMENT

By

N. MANIKANDA BOOPATHI

Degree : Doctor of Philosophy in Biotechnology

Chairman : Dr. P. Shanmugasundaram
Professor, Department of Biotechnology, CPMB
Tamil Nadu Agricultural University
Coimbatore- 641 003

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Among the abiotic stresses, drought is recognized as a major constraint to rainfed rice production. Genetic improvement of drought resistance addressed through conventional breeding approach has met with limited success. Identifying quantitative trait loci (QTLs) linked to drought resistance will help to develop drought resistant cultivars through marker aided selection strategy. Considerable progress has been made in this direction. However, most QTL mapping studies were made using *indica/japonica* crosses employing restriction fragment length polymorphic markers and the results revealed that most of the drought resistance alleles are contributed by *japonica* ecotypes. These alleles may not be expressed under lowland conditions predominant in Indian subcontinent, since these two ecotypes are grown in entirely different ecosystems. It is desirable to look for genetic variation among rice accessions within *indica* ecotype preferably using rice lines adapted to target population of environment and identify for drought resistance QTLs using simple PCR based markers for effective marker aided selection.

An F_7 recombinant inbred (RI) line population was thus developed from a cross involving IR20/Nootripathu, an *indica* landrace adapted to target population of environment of Tamil Nadu and used for genetic map construction with several PCR based markers such as SSR, ISSR, RAPD, ESTs and SSR derived from ESTs. A total of, 1125 primers were used and 120 were found to be polymorphic between the parents. Among these, 80 markers that segregated in the expected ratio of 1:1 were selected for map construction using MAPMAKER/EXP MS-DOS 3.0. Fifty-six markers were assigned to eleven rice chromosomes covering a total map length of 652 cM.

Two field trials were conducted, one each at managed stress environment (MSE) and target population of environment (TPE) using F_8 RI lines of this population for QTL mapping of drought resistance traits. Significant variation was found among the RI lines for the various physiological and plant production traits under water stress in both the trials. Significant positive correlations between biomass under stress and plant height (r = 0.59**) and tiller numbers (r = 0.61**) and significant negative correlation were found between biomass under stress and leaf rolling (r = -0.42**), leaf drying (r = -0.61**) and canopy temperature (r = -0.37**) were found in MSE. Similar relations were found in TPE as well. Single marker analysis of the 80 markers for the 11 traits identified 54 QTLs, which individually explained 2.1 to 28.7 per cent of the phenotypic variation. There were 15 QTLs for leaf rolling, 23 QTLs for leaf drying, 12 QTLs for drought recovery, 19 QTLs for plant height, 16 QTLs for tiller number, 8 QTLs for biomass under stress, 20 QTLs for canopy temperature, 4 QTLs for relative water content, 4 QTLs for basal root thickness, 3 QTLs for SPAD chlorophyll reading and 1 QTL for days to 50 per cent flowering under stress. Some of the markers were identified for more than one trait whereas

some of the markers were identified as common for the same trait in both the trials. In addition, the markers identified in this study were also previously identified as QTLs linked to various drought resistance component traits in different genetic backgrounds. Thus this study identified consistent simple PCR based markers linked to drought resistance traits using locally adapted *indica* ecotype and may be useful in marker-aided selection for drought resistance improvement in rice.

CHAPTER I

INTRODUCTION

Rice is the principal food crop of the world and is cultivated worldwide over 148 million hectares (Mha) in a broad spectrum of growing environments ranging from upland to lowland, aerobic to deep water and temperate to equatorial conditions. About 27 per cent of world's rice area is under rainfed lowland without assured water supply during critical periods of crop growth (Khush, 1997). Another 12 per cent is under uplands without any surface water accumulation. Since much of the area under rice is rainfed, yields are drastically reduced by the occurrence of drought stress due to insufficient and uneven rainfall (Widawsky and O'Toole, 1990). Of world's rainfed lowland rice area of 41 Mha, 95 per cent is in Asia. In south and southeast Asia, future increases in rice production will rely on rainfed ecosystems (Garrity *et al*., 1986). Nearly 50 per cent of this area is classified as drought-prone and may experience frequent and severe water deficits at any time during the rice growth. Rice environments in India are extremely diverse. Of the 44 Mha of total rice area in India, 33 per cent is rainfed lowland and 15 per cent is upland (http://www.fao.org). By 2025, India must increase its rice production by at least 70 per cent to feed the growing population, which should be achieved with less water and labour from ever shrinking arable land available for rice cultivation. In Tamil Nadu, area under rice cultivation is 2.2 Mha, of which 6.2 per cent is under dry and semi dry conditions. Due to monsoon failure in the past three years (2000-2003), water table is declining at an alarming rate and irrigated rice area is fast decreasing over the years. It is reported that nearly 50 per cent of the districts in Tamil Nadu are drought prone (Ramasamy *et al.,* 2003).

Development of high yielding drought tolerant rice cultivars will considerably improve rainfed rice production. However, little effort has been done to improve the genetic potential of rice for drought resistance because of the low heritability of yield under stress, the inherent variation in the field and there is usually only one experimentally droughted crop per year (Ribaut *et al*., 1997). Alternatively, yield improvements in water-limited environments could be achieved by identifying secondary traits contributing to drought resistance and selecting for those traits in a breeding program. Putative traits contributing to drought resistance in rice have been

reviewed (Nguyen *et al.*, 1997). However, these traits are rarely selected for in crop improvement programs because phenotypic selection for these traits involves complex, difficult and labour-intensive protocols and cost demanding experimental conditions. In addition, these protocols are destructive in nature resulting in loss of breeding materials for further use.

The development of molecular markers has revolutionized the genetic analysis of complex traits such as drought resistance in crop plants. Molecular markers help to track the genetic loci controlling drought resistance without having to measure the phenotype, thus reducing the need for extensive field testing over space and time (Nguyen *et al.,* 1997). The availability of a genetic map saturated with molecular markers helps to map quantitative trait loci (QTLs) linked to drought resistance traits and crop productivity in stressful environments. Once the tightly linked markers have been identified, the QTLs can be selected for in breeding program using marker assisted selection (MAS) much more efficiently than was possible previously. QTLs have been detected for several root-related traits, osmotic adjustment (OA), dehydration tolerance and other shoot-related drought resistance component traits in rice (see Boopathi *et al.,* 2002). Genetic dissection of drought resistance component traits in rice via linkage to molecular markers began only recently (Champoux *et al.,* 1995). The utility of these QTLs depends on the magnitude of phenotypic variation explained and on their consistency over environments. There was consistency in several of the QTLs identified in these experimental populations, despite the presence of QTL X environment interactions.

Although previous analysis indicated the map positions of QTLs associated with drought resistance traits, the effect of those traits on plant production under drought has not yet been established fully. Thus, there is a need to determine whether the QTLs linked to drought resistance traits also affect yield under stress. By comparing the coincidence of QTLs for specific traits and QTLs for plant production under drought, it is possible to test whether a particular constitutive or adaptive response to drought stress is of significance in improving field level drought resistance (Lebreton *et al*., 1995). Such associations would also improve the efficacy of MAS in breeding for drought tolerance in rice. Doubled haploid (DH) lines

derived from CT9993/IR62266 were used by Babu *et al*., (2003) to locate the QTLs linked to rice performance under drought and to genetically dissect the nature of association between drought resistance traits and yield under drought in the field. They have shown co-location of QTLs for root traits and yield under stress. They also showed that root traits had positive correlations with yield and yield components under drought stress.

However, in all these studies, QTLs for drought resistance traits have been studied using populations derived from *indica/japonica* inter-subspecific crosses and majority of the alleles for desirable root traits are contributed by *japonica* ecotypes. However, the *indica* and *japonica* types are grown in diverse ecosystems and the trait that confers drought resistance in one environment may not be useful in another ecosystem (Ingram *et al*., 1994). Further, *japonica* alleles may not be expressed under lowland conditions (Wang *et al*., 1994). Hence, it is desirable to look for genetic variation among rice accessions within *indica* ecotypes and map QTLs using populations derived from *indica* parents. Thus Ali *et al*. (2000) and Kamoshita *et al.,* (2002) used IR58821/IR52561 (*indica/indica*) derived recombinant inbred (RI) lines to map QTLs for root traits. Several QTLs for root traits identified in this population were found to be common across different rice populations. However, identification of the QTLs for plant production under stress and drought resistant traits in rice lines adapted to target population of environments (TPE) will further improve the efficacy of MAS.

Different kinds of molecular markers *viz.,* Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), microsatellites or Single Sequence Repeats (SSR) and isozymes were used in mapping QTLs for drought resistance traits. Most of the identified QTLs were flanked by RFLP and AFLP markers. Though these markers are robust and reliable, it involves tedious, time consuming protocols besides handling hazardous

radioactive chemical. Identification of polymerase chain reaction (PCR) based non-radioactive markers will pave way for routine use of MAS for drought resistance improvement. Thus, the present study was conducted with the objectives:

1. To construct genetic linkage map using a RI line population derived from rice line adapted to the TPE using PCR based markers and

2. To map QTLs linked to drought resistance traits and field performance in rice.

CHAPTER II

REVIEW OF LITERATURE

Rice

Rice (*Oryza sativa* L.) is an intimate part of the culture, food habits and economy of many societies and is one of the most important crops for mankind. It is the basic food of more than 3 billion people and it accounts for 50 to 80 per cent of their daily calorie intake. It is grown on 148 million hectares (Mha) and the annual world production is close to 600 million tons (M t) (IRRI, 2002). To meet the growing demand for food and to sustain food security for people in low- income countries, rice production has to be raised by another 70 per cent over the next three decades. This means raising the rice yield from the current level of 3.7 to 6.3 t/ha by 2020 if these countries can maintain their rice growing area at current levels (Fisher *et al*., 2000). For the irrigated ecosystem, the rice yield will be difficult to rise from the current levels of 5 to 6 t/ha. The potential for increasing yield in the rainfed ecosystem is vast, as the current yield is only about 2.0 t/ha (compared to 5.0 t attainable yields) and nearly 40 per cent of the total rice area is grown under rainfed conditions (Fisher *et al*., 2000) and future increases in rice production will rely on rainfed ecosystems (Garrity *et al*., 1986).

Rice and drought

Rice is a heavy consumer of water, requiring around 5000 liters of water to produce 1 kg of rice and is less efficient in the way it uses water than either wheat or maize. In Asia, where 90 percent of all rice is grown and the vast majority of it is consumed, 72 percent of fresh water resources are used for irrigating rice crops. However, water availability has been shrinking as domestic and industrial demand has increased. In the tropics of south and southeast Asia, only 41 per cent of the rice area is irrigated (IRRI,

2002). Yield loss due to drought is 227 kg/ha (20 per cent of average yield) for upland ecosystem. In a typical year, abiotic stresses decrease rice yields by about 15 per cent in Asia, more than twice the damage caused by biotic stresses (Dey and Upadhaya, 1996). Almost half of the land planted to rice in Asia and almost all of the rice in Africa is rainfed and the yields are seriously limited by water stress. Obviously, drought is the most important abiotic constraint in the upland ecosystem (Evenson, 1996). Hossain (2000) has estimated that the global yield loss due to drought is about 22 M t of unmilled rice worth US $ 2.86 billion annually.

Rice is the main food of 65 per cent of the population in India. It constitutes about 52 per cent of the total food grain production and 55 per cent of total cereal production. Rice environments in India are extremely diverse. During 2002, of the 42 Mha of rice area, 33 per cent are rainfed lowland, 45 per cent irrigated, 15 per cent upland, and 7 per cent flood-prone (www.fao.org). Since the major portion of the area under rice in India is rainfed, production is strongly tied to the distribution of rainfall. In some of the states, erratic rainfall leads to drought during the vegetative period, but later on the crop may be damaged by submergence due to high rainfall. Widawsky and O'Toole (1990) evaluated that the annual drought loss from the 24 Mha of rice of eastern India alone was 3 M t. Drought was responsible for pushing India's 2002-03 (April-March) economic growth down to 4.4 from 5.6 per cent in the previous year. India's food grain production is 184 M t in 2002-03, a decline of 13.2 per cent against record production of 212 M t in 2001-02 (Ramasamy *et al*., 2003).

Improving the yield of rainfed rice can be achieved by selecting directly for yield under stress in breeding program. However, the ability to select for yield is severely hampered by year-to-year variability in rainfall pattern and low heritability of yield under water stress (Blum, 1988; Ludlow and Muchow, 1990). Consequently, it has been suggested that improvements in yield could be achieved more efficiently by

identifying secondary traits that allow a plant to escape, avoid or tolerate water stress and selecting for those traits in a breeding programme (O'Toole, 1982; Blum, 1988; Ludlow and Muchow, 1990).

Mechanisms of drought resistance in rice

In general, rice plant uses less than 5 per cent of the water absorbed through roots from the soil. The rest is lost through transpiration, which helps to maintain leaf energy balance of the crop. The effect of water stress may vary with variety, growth stage of the rice crop and degree and duration of water stress. There may be two kinds of traits *viz*., constitutive and adaptive traits, which confer drought resistance in rice (Kamoshita *et al*., 2002). Constitutive traits are expressed under anaerobic, non-water stressed conditions, do not require water stress for their expression and may demonstrate variation that is subsequently modified by adaptive traits. Adaptive traits can be defined as those, such as osmotic adjustment (OA), which are expressed in response to water deficit. Identifying traits of importance in drought resistance is difficult due to the complexity of climatic variation in precipitation and evapotranspiration, the diversity of the rice hydrological environments, the relationship between soil moisture status and nutrient availability and differential plant interactions with this environment. Traits which are contributing drought resistance in rice have been reviewed by several researchers (Fukai and Cooper, 1995; Nguyen *et al*., 1997; Price and Courtois, 1999; Price *et al*., 2002). All the traits have either positive or negative influence on yield, depending on the existing drought situation (timing, severity and duration) and depending on whether a survival or production mechanism is necessary. The best combination of traits depends, therefore, on the nature of the drought stress. This emphasizes the need for a good characterization of drought occurrence in the target area for breeding programs (Fukai and Cooper, 1995). The problem of adaptation to drought conditions in rice is complex and unique as compared with most other crops. The following are the traits, which are demonstrated for their importance in drought resistance in rice.

Phenology

If a pattern of drought occurrence can be identified, the plant can escape drought by having the most sensitive phenological stages coinciding with the periods of lower risks of drought stress either through manipulation of the plant duration or through manipulation of the cropping calendars. For example, in a terminal stress situation, a common phenomenon in south Asia, breeding for short duration varieties is a simple strategy with proven efficacy. The duration of upland varieties of Bangladesh and eastern India are generally below 95 days, which matches the short monsoon season. The role of plant developmental and phenological factors in affecting crop response to drought stress, such as moderated water-use through reduced leaf area and shorter growth duration have already been discussed (Blum, 1988).

Root system

The possession of deep and thick root system which allows access to water deep in the soil profile is considered crucially important in determining drought resistance (Mambani and Lal, 1983). The trait may less important in rainfed lowland rice, where hardpans may severely restrict root growth. Here, the ability to penetrate a hard layer is considered important and genetic variation in the ability to penetrate a layer of hard wax has been demonstrated (Yu, *et al*., 1995). This trait may also be useful in upland rice where high penetration resistance may limit rooting depth and where soils will harden as they dry. The penetration of roots through uniform hard layers is probably achieved through the possession of large root diameter which resists buckling (Clark *et al*., 1997), but when the impedance is due to a coarse textured sandy or stony horizon, thin roots would penetrate more easily. The investment of carbon in a deep root system may have a yield implication because of loss of carbon allocation to the shoot. The rapid development of deep or thick root systems may, therefore, be of limited value if terminal drought occurs early in the crop cycle, but it is

certainly important for intermittent and later terminal drought situations. It is also important to note that root growth is influenced by the environment. Chemical or physical adverse conditions such as low water potential or high/low soil temperature directly inhibit root growth. Biological factors in the rooting environment such as root feeding nematodes, termites, mites and aphids that can severely reduce root proliferation or rooting depth and thereby affect drought resistance (Audebert *et al*., 2000). The shoot environment can also indirectly influence root growth either *via* carbon supply or signaling process (e.g., light interception, water status, nutrient status). At the genetic level, the response of roots to the environment is poorly understood because roots are intrinsically difficult to study, particularly in the natural environment.

Irrespective of root axial resistance, a few long roots can theoretically sustain reasonable evapotranspirational demand at adequately high leaf water potential (Blum *et al*., 1988). The ability of rice to reach deep soil moisture or to penetrate compacted soil is linked with the capacity to develop a few thick (fibrous) and long root axes (Ingram *et al*., 1994). Thick roots persist longer and produce more and larger branch roots, thereby increasing root length density and water uptake capacity. When drought stress develops, the root/shoot dry matter ratio increases (Hemamalini *et al*., 2000; Kamoshita *et al*., 2002 and 2002a). Sometimes even the absolute size of the root increases. Most certainly root morphology and distribution changes. Drought resistance improvement through breeding program using root traits is limited due to requirement of labour intensive, destructive and expensive phenotyping protocols. Whatever the desirable root ideotype may be, it would be extremely difficult to perform selection based on measuring the root phenotype (Nguyen *et al*., 1997).

Osmotic adjustment

Osmotic adjustment (OA) is increasingly recognized in several crop plants as an effective component of drought resistance, which has a positive direct or indirect effect on plant productivity under drought stress (Ludlow and Muchow, 1990). Generally, when cells are subjected to slow dehydration, compatible solutes are accumulated in the cytosol resulting in the maintenance of cell water content against the reduction in apoplastic water potential. The compatible solutes- various sugars, organic acids, amino acids, sugar alcohols or ions (most commonly K^+)- differ with plant species and genera (Morgan, 1984). The main solutes that are responsible for OA in rice under water deficit conditions were not elucidated. Rice does not accumulate glycine betaine (Ishitani *et al*., 1993) because of a deficiency in choline monooxygenase and betaine aldehyde deydrogenase. Rice accumulates proline but the extent of proline accumulation and its contribution to OA has not been evaluated. The support of leaf turgor by OA in rice was well reflected in delayed leaf rolling when water deficit developed. Results indicate that leaf rolling and leaf death can be delayed by OA in rice (Hsiao *et al*., 1984). However, more data are needed on the contribution of OA to rice performance under different drought stress conditions. Traditional upland cultivars generally tend to excel in root growth and soil moisture extraction capacity while lacking in OA (Nguyen *et al*., 1997). These cultivars usually develop severe leaf dehydration and leaf rolling as soon as soil moisture is depleted. It can be speculated that under upland situations with deep soil moisture there may have been a selective advantage to deep and thick root systems, which served to maintain high leaf water status and dehydration avoidance. Under such conditions, deep roots have evolved in adapted materials. OA did not evolve under such conditions because plants were usually avoiding severe water deficit. The capacity for OA may have evolved where leaf tissue water status was often reduced by water deficit, such as in lowland rice where deep rooting is often deterred by the sub soil compaction. These different modes of response to drought stress require validation and further research to suggest clues to desirable breeding strategies with respect to the different rice environments.

Dehydration tolerance

Dehydration tolerance (the ability of leaves to tolerate desiccation level water stress) assists the plant organs to survive short-term water deficits. The lowest leaf water potential that leaves reach just prior to death (lethal leaf water potential) has been used to determine dehydration tolerance. During terminal stress, dehydration tolerance may allow plants to maintain metabolic activity for longer time and to translocate more stored assimilates to the grain (Fukai and Cooper, 1995). Plants with the ability to adjust osmotically or tolerate dehydration may delay leaf rolling, stomatal closure and maintain leaf expansion with little cost, which should promote resistance particularly in the terminal drought situation. So, if dehydration tolerance of rice is increased by breeding approaches then it could be possible to increase or at least stabilize the yield of rainfed rice. Genotypic variation for dehydration tolerance capacity of rice is large (Lilley and Ludlow, 1996; Babu *et al*., 2001). However, incorporation of this trait in breeding program is hampered by complex experimental protocols requiring heavy investment in creating controlled environment facilities.

Shoot related drought resistance traits

Leaf rolling

Several mechanisms of drought resistance are associated with the shoots of rice. Leaf rolling (drought avoidance) reduces the water loss in addition to reducing the leaf area exposed to heat and light radiation. Varieties differ in their ability to roll leaves under similar water deficit (Turner *et al*., 1986). There is some evidence that enhanced ability to roll leaves confers a yield advantage under drought conditions (Singh and Mackill, 1991). However, most breeders consider the triggering of leaf rolling as an indication of a plant suffering and select against its early manifestation.

Green leaf area

It has been suggested that plants which are able to retain green leaf area are better able to recover after drought and give good yield (Fukai and Cooper, 1995). Leaf drying, often used in field scoring, is the reverse side of the stay green ability and has been shown to be correlated with leaf relative water content. However, it has proved difficult to separate the green leaf retention from the possible underlying mechanisms of drought resistance since the process of drought recovery in terms of mechanisms, importance or genetic variation is poorly understood.

Stomatal closure and canopy temperature

Another mechanism of drought avoidance in the rice shoot is fast stomatal closure which acts to reduce water losses. Varietal differences in the sensitivity of stomatal conductance to leaf water status do exist (Turner *et al*., 1986). The contribution of stomatal conductance to drought performance in the field is yet to be identified. However, a plant with sensitive stomata would only be adapted to a situation of relatively severe drought. But during mild drought, rapid stomatal closure would reduce photosynthesis when there is no need to do so. Canopy temperature can also be used since low canopy temperature may indicate more favorable soil moisture conditions. This characteristic could be valuable in selection, but measuring them requires extremely uniform soils to eliminate any subsoil spatial variation (Richards, 1991).

Cell membrane stability

The cell membrane is one of the main cellular targets common to different stresses (Levitt, 1980). The extent of its damage is commonly used as a measure of tolerance to various stresses in plants such as freezing, heat, drought and salt. Cell membrane stability (CMS) or the reciprocal of cell membrane injury is a physiological index widely used for the evaluation of drought and temperature tolerance. This method was developed for a drought and heat tolerance assay in sorghum and measures the amount of electrolyte leakage from leaf segments. Its reliability as an index of heat stress tolerance is supported in several plant

species by good correlation between CMS and plant performance in the field under high temperature and water stress. The genetic variation in heat tolerance in various crops has been studied using CMS as one of the component traits. Phenotype selection for CMS may not always lead to accurate results for breeding purposes because of its complex nature and its strong interaction with the environment. Thus, the evaluation of this trait should be done in a controlled environmental situation (Levitt, 1980).

Water use efficiency

Connected to stomata and leaf rolling is water use efficiency (WUE, the ratio between carbon gained for water used). Analysis of WUE generally relies on measuring carbon isotope discrimination (Farquhar, 1989). This has been shown to vary between rice varieties (Dingkuhn *et al*., 1991), suggesting that upland varieties need less water for every molecule of carbon fixed. A plant, which is more water use efficient, should be more successful in a drought environment, particularly late in the growing season when transpiration accounts for the majority of total evaporation. WUE can be either positively or negatively related to production under stress, which is largely dependent on the genotype's capacity to sustain transpiration and WUE alone might be questionable as a selection criterion (Blum, 1982). Therefore, WUE can even be a misleading parameter if selection for high WUE is performed under drought stress where genotypic variation in deep soil moisture extraction is possible. It is realized that results from selection for WUE (by carbon isotope discrimination) depend very much on the environmental conditions in which such selection is performed (Hall *et al*., 1994). It also seems that the results from selection for high WUE may be unpredictable. In several crops, the correlations between WUE and dry matter production were inconsistent in experiments conducted over different water regimes and years.

Epicuticular wax

It has been repeatedly shown that total crop dry matter production is linearly and positively related to crop transpiration (Nguyen *et al*., 1997). This relationship is partly derived from the fact that the control of both transpiration and CO_2 exchange is dependant on stomatal acitivity. However, loss of water can also occur through nonstomatal pathways for which no return in CO_2 fixation is expected. Non-stomatal resistance to water loss from leaves can also be considered a drought avoidance mechanism. An important non-stomatal pathway is the leaf cuticle. Research suggests that rice has a low cuticular resistance to water loss compared with other grasses but variation between varieties exists and this may have potential in breeding for improvement in drought resistance (O'Toole and Cruz, 1983). The fact that traditional upland rice cultivars have relatively higher epicuticular wax supports the hypothesis that high epicuticular wax is an important drought resistance attribute in rice. The specific effects of the amount, the composition and the form of cuticular wax in rice were explored (O'Toole, 1982), but the quantification of these factors with respect to rice performance under drought stress is still needed. Further, physiological and biochemical work is required to logically link cuticular resistance and epicuticular wax with drought resistance and for efficient manipulation in breeding program.

Other traits

The value of improving the use of absorbed light, resistance to photoinhibition and capacity for non-photochemical quenching to improve drought resistance of rice has been described (Horton, 2000). In addition, a genetic basis for difference in resistance to photoinhibition in rice has been demonstrated (Jiao and Ji, 2001). These traits are physiologically, biochemically and genetically complex in themselves and interact with each other. Since abscisic acid (ABA) has been shown to be involved in regulating stomatal conductance, OA and root conductivity, interest has been shown in measuring ABA contents in order to establish relationships with drought resistance. Varietal differences in leaf ABA content and sensitivity to applied ABA exist in rice (Dingkhun *et al*., 1991a).

In summary, an utilizable secondary trait in breeding for drought resistance in rice should be 1) genetically associated with grain yield under drought; 2) highly heritable; 3) stable and feasible to measure; 4) not associated with yield loss under ideal growing conditions (Ludlow and Muchow, 1990). However, these traits are rarely selected for in traditional rice improvement programs because phenotypic selection for these traits involves complex, difficult and labour-intensive protocols, the tremendous diversity of environments and water availability and the large genotype X environment interactions which complicates selection (Fukai and Cooper, 1995). Knowledge from physiological studies indicated that the ability of the root systems in exploiting deep soil moisture and the capacity for OA during water stress, are considered as major drought resistance traits in rice. They can also be negatively correlated due to tight genetic linkage of some of the controlling genes as was shown for OA and root morphology (Lilley *et al*., 1996; Zhang *et al*., 2001). Therefore the impact of one trait in isolation may be difficult to establish. One promising approach is to map genetic loci (quantitative trait loci, QTLs) influencing drought resistance traits and crop productivity in stressful environments. Once the tightly linked markers have been identified, they can be used to develop marker assisted selection (MAS) strategy for breeding applications. Molecular markers allow breeders to track the genetic loci controlling drought resistance without measuring the phenotype, thus reducing the need for extensive field testing over space and time (Nguyen *et al*., 1997). High resolution mapping and physical mapping can be followed for isolation of the drought resistance genes by map based cloning techniques. The genes of interest can be used in functional studies and crop improvement through genetic transformation.

Genetic linkage map in rice

Construction of linkage map is essentially the first step in QTL mapping. Such maps allow genetic dissection of QTL, facilitate high-resolution genetic mapping and positional cloning of important genes,

assist in local comparisons of synteny within and across the species and provide an ordered scaffold on which complete physical maps can be assembled (Harushima *et al*., 1998). The concepts for detecting QTL using linked major genes were developed early in 20th century (Sax, 1923). Some studies also demonstrated the feasibility of this type of analysis (Thoday, 1961). However, it was difficult to put this concept into practice with conventional morphological markers due to their limited number, poor genetic relationships *etc*., Recent progress in DNA markers and their linkage maps have provided an efficient tool and methods for mapping individual loci conferring not only monogenic, but also polygenic traits (Tanksley, 1993; Paterson, 1995). Since Paterson *et al.,* (1988) described genetic dissection of several quantitative traits into single Mendelian factors in tomato, many QTLs have been clarified using DNA markers in various crop plants. For rice, the first molecular marker based genetic map was constructed by McCouch *et al.,* (1988) and since then several linkage maps were constructed in rice using different mapping populations including high density Restriction Fragment Length Polymorphism (RFLP) maps (Saito *et al.,* 1991; Causse *et al.,* 1994; Kurata *et al.,* 1994) and Expressed Sequence Tags (ESTs) maps (Harushima *et al.,* 1998; Wu *et al.,* 2002). These maps provide the foundation for molecular genetic analysis of almost any traits of interest and thus have number of advantages over classical genetic maps for genetic research and breeding. However, the RFLP technique could not be used routine in breeding programme since it involves lengthy protocols, use of radioactive chemicals and produce limited number of alleles per locus and so often difficult to find polymorphisms within a subspecies (Akagi *et al.,* 1996). Microsatellite markers (Simple Sequence Repeats, (SSR)), which are highly polymorphic and easily assayed by Polymerase Chain Reaction (PCR) with small samples of genomic DNA, should be a value for breeding programs. In combination with the pool of 500 previously mapped SSR markers (Temnykh *et al.,* 2001), a total of 2740 SSR markers for rice or approximately one SSR in every 157 kb have been recently released (McCouch *et al.,* 2002) for more precise genetic analysis of complex traits in rice. But development of SSR markers requires prior sequence information. Another kind of marker, called as Amplified Fragment Length

Polymorphism (AFLP), has been described (Vos *et al.,* 1995) and it was utilized in the genetic map construction along with previously mapped RFLP markers in rice (Maheswaran *et al.,* 1997). These AFLP markers were combined with RFLP markers. Similarly, Random Amplified Polymorphic DNA (RAPD) markers have been integrated into the RFLP map in rice (Subudhi and Huang, 1997). AFLP and RAPD markers offer several advantages such as high frequency of polymorphism, rapidity, technical simplicity, use of fluorescence, requirement of a few nanograms of DNA, no requirement of prior information of the DNA sequence and feasibility of automation.

QTL mapping of drought resistance traits in rice

The availability of high density linkage maps is valuable as a resource for studies that genetically dissect out the complex traits such as drought resistance. QTL mapping provides a potential tool for conducting physiological and genetical research to understand and improve drought resistance. It eases screening for traits that are difficult to quantify and influenced by environmental stimuli (Hanson *et al*., 1990).

A good progress has been made in identifying molecular markers linked to various drought resistance traits in rice. A review paper has been published, as a part of this study, based on the available literature and it is attached in Annexure I (Boopathi *et al*., 2002). Table 2 summarizes the details of QTLs identified so far, for different drought resistant traits and their flanking markers in different mapping population. The first report on QTLs associated with various root morphological characters have been reported in a CO39/Moroberekan recombinant inbred (RI) line population under green house conditions (Champoux *et al*., 1995). They have also identified QTLs linked to drought avoidance in the field under water deficit stress at three different growth stages using the same mapping population. It is encouraging to note that over 50 per cent of the putative QTLs associated with root characters in the greenhouse study

mapped to the same chromosomal locations as QTLs influencing drought avoidance in the field experiments (McCouch and Doerge, 1995).

Using the same RI lines, Ray *et al*., (1996) mapped QTLs for root penetration ability using wax petrolatum layer. Clustering of QTLs associated with root traits was observed as that of Champoux *et al*., (1995). This suggests that specific regions of the rice genome containing genes that determine root morphology may be clustered in certain chromosomal regions. These regions may contain clusters of genes or genes with pleiotropic effect. Most of the QTLs linked to tiller number are mapped closely to chromosomal regions identified as associated with total root number. These results suggested that molecular marker could play a significant role in studying the relationship of shoot and root related drought resistant traits. This issue can be investigated further in a rice population developed specifically for the purpose of studying these traits.

An analysis was conducted using the subset of this population to identify and map QTLs associated with dehydration tolerance and OA (Lilley *et al*., 1996) and the identified QTLs were compared to root traits and leaf rolling scores measured in the same lines. It is interesting to note that the putative OA locus and two of the dehydration tolerance QTLs on chromosome 8 were close to the regions associated with root morphology. From their results it was suggested that OA and dehydration tolerance is negatively correlated with root morphological characters associated with drought avoidance. High OA and dehydration tolerance is associated with CO39 (*indica*) alleles and extensive root systems were associated with Moroberekan (*japonica*) alleles. It was suggested that to combine high OA with extensive root systems, the linkage between these traits needs to be broken.

It is obvious that QTL detection depends on the cross combination used in the analysis, because detection of QTL is based on allelic differences in QTL between parental lines. Thus, an important question is whether QTL detected in one population are shared with QTL detected in other populations. QTL analysis of the same traits using different cross combinations will be necessary to answer this question (Yano and Sasaki, 1997). Yadav *et al*., (1997) studied doubled haploid (DH) population derived from IR64/Azucena cross and mapped the genes controlling root morphology and distribution. The main QTLs were common between traits, which indicate that there is a possibility to modify several aspects of root morphology simultaneously. The *sd-1* locus on chromosome 1 (Huang *et al*., 1996), which has massive effect on plant height and tillering, was found to show co-location with QTLs governing root system in this study. However, the QTL on chromosome 7 that was associated with effects on maximum root depth did not seem to be linked with a QTL for plant height. This suggests that it may be possible to decrease the height of traditional tall upland rice varieties without diminishing the quality of their root system. By comparing the QTLs identified by Champoux *et al*., (1995), they have identified common QTLs depending on the traits. Development of isogenic lines would help to clarify the proper value of the common QTLs by eliminating the confounding effects of other genomic regions and to fine tune their location.

QTLs controlling drought avoidance mechanisms (such as leaf rolling, leaf drying, relative water content of leaves and relative growth rate under stress) were analyzed in this DH population in three field trials with different drought stress intensities in two sites (Courtois *et al*., 2000). Some of the QTLs were common across the trials and traits. QTLs detected for leaf rolling, leaf drying and relative water content were mapped in the same location as QTLs controlling root morphology in the previous study using the same population (Yadav *et al*., 1997). QTLs identified for leaf rolling in this population located similarly as that of the QTLs for leaf rolling in other population (Champoux *et al*., 1995).

A randomly chosen subset of 56 DH lines derived from this cross were grown in poly-vinyl chloride cylinders to study the root morphology and associated traits under well watered conditions and low-moisture stress at two growth stages during the vegetative phase (Hemamalini *et al*., 2000). In total 15 QTLs were detected from both the growth stages and only three were common between the stages. This reveals that different sets of QTLs 'show up' under different developmental stages within the vegetative stage itself. Further, absence of common QTLs for root traits between two developmental stages and two moisture regimes in this study suggests the existence of parallel genetic pathways operating at different growth stages and moisture regimes.

Using a wax petrolatum layer system simulated to compacted soil layers, root traits were evaluated with a subset of this DH lines (Zheng *et al*., 2000). QTLs for root penetration index, penetrated root thickness, penetrated root number and total root number have been located. Common QTLs linked to root penetration index and basal root thickness were noted across experimental systems (Yadav *et al*., 1997) and genetic background (Ray *et al*., 1996). This suggests that both root penetration ability and root thickness may be controlled by genes, which are closely linked or have pleiotropic effect. No QTLs for maximum penetrated root length were detected by interval mapping, although five RFLP markers were found significantly associated with this trait using single marker analysis. Root length is known to be highly sensitive to environmental variation (Champoux *et al*., 1995) and therefore is more difficult to improve than other root traits such as root thickness.

Another extensively analyzed population for QTLs linked to drought resistance, is Bala/Azucena developed by Price *et al*., (1997). They reported the construction of a linkage map and its use in mapping the QTLs controlling maximum root length at various stages of root development, adventious root thickness and root volume in an F_2 population. QTLs for different days/stages showed different types of genetic

effect. Some QTLs observed in the Bala/Azucena population are evident in the CO39/Moroberekan population (Champoux *et al*., 1995; Ray *et al*., 1996), whilst some are not. The same population was used for mapping two shoot related mechanisms *viz.,* stomatal conductance and leaf rolling along with heading date (Price *et al*., 1997a). This F_2 population was forwarded to F_6 and a more detailed linkage map was constructed to analyze the QTLs for root penetration ability with modified wax petrolatum layer (Price *et al*., 2000). It is interesting to note that some of the QTLs for root penetration ability reported here are close to QTLs for root morphology reported in the F_2 (Price *et al*., 1997). However, the differences in the reported locations of QTLs between this study and Ray *et al*., (1996) are probably due to the different populations studied and to the different methods used for assessing the root penetration phenotype. Comparison of the QTLs identified in this study with previous reports of QTLs for root morphology suggest that alleles which improve root penetration ability may also either make the roots longer or thicker. In another study, QTLs for drought avoidance based on the field trials in the Philippines and West Africa (Price *et al*., 2002a) have been localized. QTLs for leaf rolling and drying, relative water content were mapped for each site and across the site. However, there was relatively poor correlation between traits measured in the two sites suggesting there may be some different genetic components contributing to drought resistance in the different environments. The same experimental materials were used to map QTLs for root morphology and distribution using soil filled chambers exposed to contrasting water deficit regimes (Price *et al*., 2002b). QTLs for the deep root weight, maximum root length, root shoot ratio, number of deep roots and root thickness were identified. Some were revealed only in individual experiment and/or for individual traits, while others were common to different traits or experiments.

A comprehensive analysis of dissecting physiological and morphological traits related to drought resistance and partitioning of drought resistance into components and comparative QTL analysis, would contribute a better understanding of the genetic basis for drought resistance in plants. The parents, CT9993 and IR62266 were studied at morphological and physiological level and shown to differ in root system and

OA (Babu *et al*., 2001). In order to better understand the mechanisms of drought tolerance *via* OA and drought avoidance *via* a deep root system in rice, a molecular dissection of QTLs for both OA and root traits in one genetic background is important. Hence, genomic regions responsible for CMS were studied in the greenhouse in a slowly developed drought stress environment by using rice DH lines derived from CT9993/IR62266 (Tripathy *et al*., 2000). No significant correlation was found between CMS and relative water content, indicating that the variation in CMS was genotypic in nature. They have located nine putative QTLs for CMS and one of the QTL on chromosome 8 mapped on the same locus as the OA mapped by Lilley *et al*., (1996). Moreover, several QTLs involved in root morphology and the drought avoidance in rice have been identified in this region (Champoux *et al*., 1995). The mapping of CMS QTLs in this region suggests that this region might contain genes for different traits responsible for conferring drought resistance in rice. The same DH lines were used to map the QTLs associated with root traits and OA (Zhang *et al*., 2001). Consistent QTLs for drought responses across genetic backgrounds were detected. Comparative mapping identified three conserved regions associated with various physiological responses to drought in several grass species. This result suggests that these regions conferring drought adaptation have been conserved across grass species during genome evolution and might be directly applied across species for the improvement of drought resistance in cereal crops.

Rice develops roots under anaerobic soil conditions with ponded water, prior to exposure to aerobic soil conditions and water stress in rainfed lowlands. Constitutive root system development in anaerobic soil conditions has been reported to have a positive effect on subsequent expression of adaptive root traits and water extraction during water stress (Kamoshita *et al*., 2000). The effects of phenotyping environment on identification of QTLs for constitutive root morphology traits were studied (Kamoshita *et al*., 2002a) using greenhouse experiments and the results emphasized the careful selection of phenotyping environment which relate closely to the target environment where the traits to be expressed and

interpretation of results which otherwise leads to misplacing the QTLs. In spite of large environmental effects, even in well-watered anaerobic conditions, they have identified stable QTLs across the experiments in CT9993/IR62266 DH lines. Physical mapping of the putative QTLs for deep root morphology traits would help to elucidate how rooting depth and deep root mass are genetically controlled at the molecular level. QTLs linked to plant height, number of tillers, total root number, root dry weight, total plant length and root to shoot length ratio were identified in this population under well watered conditions (Kanbar *et al.,* 2002). Some of the alleles governing the root related traits were from IR62266, which indicates that inferior parent can also contribute favorable alleles for root traits.

Solution culture and paper culture, which simulates lowland condition and upland condition, respectively were used to locate QTLs linked to seminal root length in a RI population developed from IR1552/Azucena (Zhang *et al.,* 2001a). The same population was used to find QTLs for seminal root length, adventitious root number, lateral root length, lateral root number and the relative parameters under flooding and upland conditions (Zheng *et al.,* 2003). The comparative study for the QTLs detected in this study and those reported from two other populations with Azucena as a parent has shown that several identical QTLs for root elongation were found across three populations with positive alleles from Azucena. Genomic regions governing OA under water stress have been identified in an advanced backcross inbred population of IR62266/IR60080 (Robin *et al.,* 2003). The QTL locations were in good agreement with previous studies on this trait on rice and other cereals. Single marker analysis of P124/IR64 backcross population evaluated for maximum root length under well-watered and low stress conditions identified four RAPD markers linked to root length (Toorchi *et al.,* 2002).

Drought resistance component traits, described above, can interact with each other in modifying the plant water status. The real test for drought resistance is continuous growth and production under stress. Three traits, which perhaps encapsulate all the drought resistance components, are leaf expansion

(as an indication of plant turgor), biomass production and ultimately grain production under stress. Although previous analysis indicated the map positions of QTLs associated with drought resistance traits and their co-location, the effects of those traits on plant production under drought has not yet been properly established. Thus there is a need to determine whether the QTLs linked to drought resistance traits also affect yield under stress. By comparing the coincidence of QTLs for specific traits and QTLs for plant production under drought, it is possible to test whether a particular constitutive or adaptive response to drought stress is of significance in improving field level drought resistance (Lebreton *et al.,* 1995). Such associations would also improve the efficacy of MAS in breeding for drought tolerance in rice. QTLs associated with grain yield and root morphological traits were mapped in IR64/Azucena DH population under contrasting moisture regimes (Venuprasad *et al.,* 2002). CT9993/IR62266 DH lines were used to identify the QTLs linked to rice performance under drought and to genetically dissect the nature of association between drought resistance traits and yield under drought in the field (Babu *et al*., 2003).

Rice subspecies and habitat

Rice is cultivated in four continents and very large germplasm collections are available offering many possibilities of identifying adaptive traits and tolerance characters towards abiotic stresses. Cultivated rice belongs to the *Oryza sativa* complex, which contains the two cultivated species, *O. sativa* and *O. glaberrima* and several wild species, which are considered as direct ancestors of the cultivated ones. *O. sativa* is cultivated all over the world, whereas *O. glaberrima* is cultivated only in Africa. Within the *O.sativa* species, two major groups of ecogeographic races are distinguished, the *indica* and *japonica* types. They roughly correspond to rice grown in tropical regions of southeast Asia and in more temperate regions of Japan and northern China, respectively. *Indica* and *japonica* varieties cross-hybridize but usually many plants in the progeny are sterile or partially sterile (Khush, 1997). Large and well-known genetic diversity exists in the subspecies level is a valuable resource for both classical and biotechnology assisted breeding.

Most of the populations used in QTL analysis of drought resistance traits were derived from an *indica/japonica* cross, because of the high frequency of polymorphism based on wide variation. Development of a deep and extensive root system is one adaptive strategy of plants for drought avoidance (Ludlow and Muchow, 1990). Upland *japonica* cultivars appear to rely on its deep and extensive root system to achieve its demonstrated capacity for drought avoidance, where as *indica* cultivars have different adaptive strategies including shortening of growth duration and tissue level tolerance. Whether a drought avoidance strategy based almost entirely on a well developed root system in *japonica* background can be combined with tissue level tolerance and/or short growth duration to improve plant performance under water stress in specific environments is a question which is central to drought resistance breeding in cereals (Champoux *et al.,* 1995). The phenomenon of 'return to parental type' after repeated generations of selfing following *indica/japonica* hybridization is familiar to rice breeders and makes it difficult to obtain favourable recombinants through traditional means (Oka 1988). Differential adaptation to edaphic factors, such as soil, water and temperature regimes and genetically controlled sterility barriers, separate these two major sub-species (Oka, 1964). Evaluation of upland *japonica* /lowland *indica* populations under anaerobic lowland conditions may be confounded by the difference in adaptation to lowland conditions (Kamoshita *et al*., 2002). Cross combinations used in breeding programs are mainly same ecotype crosses, such as *japonica/japonica* and *indica/indica*. Therefore, more QTL analysis based on crosses between closely related varieties, especially the *indica/indica* cross, will be necessary for identification of QTL alleles which will be useful in rice breeding (Yano and Sasaki, 1997). Ali *et al*., (2000) analyzed RILs developed from two *indica* parents, IR58821/IR552561, to map QTLs for root traits in two different seasons. They have identified not only common QTLs between two seasons and but also consistent QTLs across genetic backgrounds. The effect of phenotyping environment and genetic background on QTLs identification were examined by using this population (Kamoshita *et al*., 2002). QTLs for shoot biomass, deep root morphology

and root thickness were mapped. Consistent QTLs across the experiments and genetic backgrounds were detected. Results from these studies suggest that some amount of similarity exists between *japonica/indica* crosses and *indica/indica* crosses in the genetic control of root traits.

Marker aided selection and near isogenic lines for drought resistance improvement

QTLs presented in this section associated with different drought resistance mechanisms assessed at different sites, methodologies and seasons confirms the complexity of the genetics of drought resistance in rice. It also illustrates the degree of QTL by year and QTL by site interaction and demonstrates the value of calculating averages for identification of the more stable but small effect QTLs. A significant proportion of the phenotypic variability of several of these putative drought resistance traits is explained by the segregation of relatively few genetic loci, thus leading to the possibility of indirect selection of these complex traits using marker-assisted selection (MAS) strategy (Babu *et al.,* 2003). This information is potentially valuable to breeders and enables researchers to target specific regions in order to produce near isogenic lines (NILs) at some QTLs. These NILs will allow more accurate determination of environmental stable QTLs and understood and further allow for the assessment of the impact of QTLs on yield under drought. They could also aid in the identification of the genes responsible for the QTLs through candidate gene and/or positional cloning approaches (Price *et al*., 2002). Shen *et al.,* (2001) reported improvement of rice root system by MAS of several root QTLs. They have also studied the possible effects of these introgressed segments on other agronomic traits through pleiotrophy or linkage drag. Work is currently underway to transfer the QTL for root morphological traits from Azucena into a popular Indian variety, Kalinga III by MAS (Price *et al.,* 2002a). NILs are being developed for OA with *japonica* background. NILs shall serve as valuable material to test the utility of the introgressed QTL. This will also lead to understand the mechanisms underlying physiological and molecular nature of the QTL and to evaluate the contribution of the QTL to yield in the target environment (Price *et al.,* 2002b).

Target population of environment

To improve the drought resistance of rainfed lowland rice, mapping populations from crosses between parental lines that are equally well adapted to target environments should be evaluated (Kamoshita *et al*., 2002). Focusing on the variation within single ecotype might hasten progress toward drought resistance and the locally well-adapted germplasm will increase the efficiency of breeding. Traditional rice varieties are still being grown in rainfed uplands even though they give low but definite yield. There is a need to develop rice varieties with higher yield but retaining the drought tolerance capacity of traditional accessions. The necessity of QTL identification based on the variation from the crosses between two related varieties belonging to the same subspecies adapted to target population of environment (TPE) has been emphasized by various authors (Ingram *et al*., 1994; Redona and Mackill, 1996; Yano and Sasaki, 1997). Further, upland rice environments vary widely in terms of climate and edaphic factors, making it difficult to use genetic material developed for one location in other locations (Moncada *et al.,* 2001).

Molecular markers

Most of the QTLs linked to drought resistance traits were flanked by mostly RFLP and few Amplified Fragment Length Polymorphisms (AFLP) markers. Though RFLP markers are reliable, it involves tedious, time consuming protocols besides handling hazardous radioactive chemical. Hence, they are not suitable for routine MAS. The RFLP and AFLP markers need to be converted to a simple, rapid and inexpensive polymerase chain reaction (PCR) based markers, like STS, to enhance and economize the breeding programs. This involves extra effort in conversion of this marker besides establishing the polymorphism between the parents as that of original RFLP or AFLP markers. Identification of simple PCR

based non-radioactive markers linked to putative drought resistance component traits will hasten MAS for drought resistance improvement. SSRs, Inter Simple Sequence Repeats (ISSRs) and Randomly Amplified Polymorphic DNAs (RAPDs) are well-established PCR based markers being involved in mapping process.

The candidate gene approach has been applied in plant genetics in the past decade for the characterization and cloning of QTLs (Pflieger *et al*., 2001). Candidate genes are genes involved in the expression of a given trait. They can be identified either from previously sequenced genes of known function or from cDNA libraries constructed specific to different organs, developmental stages or stress responses. Expressed Sequence Tags (ESTs) are partial or single pass sequencing of more or less randomly chosen cDNA clones from libraries at all stages of plant growth and development. They allow fast and affordable gene identification. Development of EST based markers is dependent on extensive sequence data of regions of the genome that are expressed. They are highly reproducible and can be directly associated with functional genes. A number of ESTs specific to drought response are now available in the EST database (dbEST). It will be important to resolve to what extent the allelic variation in these genes affects drought tolerance in rice. Hybridization based RFLP markers have been developed from ESTs and used extensively for the construction of high-density genetic linkage maps in rice (Harushima *et al*., 1998; Wu *et al.,* 2002). The genetic factors underlying constitutive and adaptive morphological traits of roots under different water-supply conditions was investigated using RI lines derived from IR1552/Azucena by exploiting the genetic map constructed with EST clones and cDNA-AFLP clones (Zheng *et al.,* 2003). Two genes for cell expansion, *OsEXP2* and endo-1,4-β-D-glucanase *Ecase* and four cDNA-AFLP clones from root tissues of Azucena were mapped on the intervals carrying the QTLs for seminal as well as lateral root length. Robin *et al.,* (2003) found a candidate gene that was closely linked to QTL for OA. The tight linkage between these candidate genes and the QTLs for root traits and OA may demonstrate a causal relationship. However, further investigation of these genes for stimulated root elongation under water-

limited stress in rice is needed before drawing conclusions on what gene lies beneath the QTL. The candidate genes used in these studies were engaged as radioactive probe as that of RFLP. Development of PCR based EST markers could be useful in QTL mapping and efficient MAS for drought resistance improvement in rice. Further, ESTs allow a computational approach to the development of SSR for which previous development strategies have been expensive (Sreenivasulu *et al*., 2002). Pattern-finding programs can be employed to identify SSRs in the ESTs. Readily available EST sequence information allows the design of primer pairs, which can be used to identify the length polymorphism among the parental lines. Hence, the present study has been conducted to construct genetic linkage map of rice using a RI line population derived from locally adapted germplasm using PCR based markers *viz.,* SSR, ISSR, RAPD and EST derived SSRs and to map QTLs linked to drought resistance traits under field conditions in rice.

Table 1. Representative rice genetic linkage maps using different kinds of molecular markers

Parents	Mapping population	Number and Type of loci mapped	Length of the map (cM)	Reference
IR34583-19-3-3/Bulu Dalam	53 F_2 progenies	135 RFLP	1389	McCouch *et al*., (1988)
Kasalath/FL134		322 RFLP	1836	Saito *et al.,* (1991)
Nipponbare/Kasalath	186 F_2 progenies	1383 EST	1575	Kurata *et al*., (1994)
O. sativa/O. longistaminata	113 BC progenies	726 RFLP	1491	Causse *et al*., (1994)
IR64/ Azucena	135 DH lines	175 RFLP	2005	Huang *et al*., (1997)
IR64/ Azucena	60 DH lines	208 AFLP	-	Maheswaran *et al*., (1997)
IR64/Azucena	60 DH lines	242 RAPD	2900	Subudhi and Huang (1999)
Nipponbare/Kasalath	186 F_2 progenies	2275 EST	1522	Harushima *et al.,* (1998)
IR64/Azucena	96 DH lines	500 SSR	-	Temnykh *et al.,* (2001)
		2240 SSR	-	McCouch *et al.,* (2002)

BC- Back Cross; DH- Dobled Haploid; RFLP- Restriction Fragment Length Polymorphism; EST- Expressed Sequence Tag; AFLP- Amplified Fragment Length Polymorphism; RAPD- Random Amplified Polymorphic DNA; SSR- Simple Sequence Repeat

Table 2. Details of mapping population, linkage map characteristics and QTLs identified for drought resistant traits in rice

Parents	Population§	Number and type of markers used	Linkage map coverage (cM)	Traits	QTLs identified			Maximum phenotypic variance (%)	References
					No. of QTLs	Across trials/ experiments	Across Population		
Co39/Moroberekan	281 F_7 RILs (203)	127 (RFLP)		Root thickness	18	-	-	56	Champoux et al., (1995)
				Root shoot ratio	16	-	-	38	
				Root dry weight per tiller	14	-	-	35	
				Deep root weight	8	-	-	18.5	
				Maximum root depth	4	-	-	-	
				Drought avoidance (leaf rolling)	18	5	-	35	
Co39/Moroberekan	281 F_7 RILs (202)	127 (RFLP)		Number of penetrating roots	4	-	-	8	Ray et al., (1996)
				Total number of roots	19	-	-	19	
				Root penetration index	6	-	-	13	
				Tiller number	10	-	-	14	
Co39/Moroberekan	281 F_7 RILs (52)	127 (RFLP)		Dehydration tolerance	5	-	-	36	Lilley et al., (1996)
				Osmotic adjustment	1	-	-	32	
				Relative water content	2	-	-	35	
IR64/Azucena	135 DH lines (105)	175 (RFLP, RAPD, isozyme)	2005	Total root weight	23	-	3	11.9	Yadav et al., (1997)
				Deep root weight	17	-	-	14.9	
				Deep root weight to shoot ratio	26	-	3	22.3	
				Deep root weight per tiller	20	-	3	19.6	
				Maximum root length	25	-	1	20.9	
				Root thickness	8	-	2	10.4	
`Bala/Azucena	178 F_2 plants (30)	71 (RFLP)	1280	Maximum root length	10	1	4	37.7	Price et al., (1997)
				Root volume	1	-	-	10.2	
				Adventitious root thickness	2	-	2	14.7	
Bala/azucena	178 F_2 plants (178)	71 (RFLP)	1280	Leaf rolling	1	-	-	6.2	Price et al., (1997a)
				Stomatal behaviour	4	-	-	18.4	
				Days to heading	3	2	-	32.5	
IR64/Azucena	135 DH lines (56)	175 (RFLP, RAPD, isozyme)	2005	Plant height	4	2	-	29.2	Hemamalini et al., (2000)
				Number of tillers	11	-	-	25.7	
				Root length	5	-	3	15.4	
				Total root number	10	-	-	25.1	
				Root volume	5	-	-	21.4	
				Root thickness	5	1	2	26.7	
				Root dry weight	2	-	-	20.8	
				Root shoot ratio	1	-	1	12.7	

				Leaf drying (Drought score)	2	-	-	16.1	
				Leaf rolling	1	-	-	11.9	
IR64/Azucena	135 DH lines (105 & 85)	175 (RFLP, RAPD, isozyme)	2005	Leaf rolling	11	4	6	23.3	Courtois et al., (2000)
				Leaf drying	10	1	-	19.4	
				Relative water content	11	1	-	18.5	
				Relative growth rate	10	-	-	16.5	
Bala/Azucena	205 RILs (104)	135 (RFLP, AFLP)	1680	Number of tillers	1	-	-	12.4	Price et al., (2000)
				Number of roots	3	-	1	10.3	
				Number of penetrated roots	7	-	-	16.7	
				Penetrated: total roots (root penetration index)	7	-	2	18.0	
IR1552/Azucena	150 RILs (150)	207 (RFLP, AFLP)	2419	Seminal root length	2	-	-	11.2	Zhang et al., (2001a)
Bala/Azucena	205 RILs (176,118,142& 110)	142 (RFLP, AFLP, SSR)	1779	Leaf rolling	5	1	5	20.4	Price et al., (2002)
				Leaf drying	11	-	8	17.6	
				Relative water content	8	-	7	25.6	
Bala/Azucena	205 RILs (140)	142 (RFLP, AFLP, SSR)	1779	Total dry weight/plant mass	8	2	-	21.3	Price et al., (2002a)
				Root to shoot dry weight ratio	11	2	2	28.0	
				Root mass below 90 cm	6	-	3	16.0	
				Basal root thickness	7	-	-	18.2	
				Root thickness at 90 cm	14	2	8	18.3	
				Maximum root length	6	2	4	17.4	
				Number of root past 100 cm	12	4	-	22.8	
IR64/Azucena	135 DH lines (109)	175 (RFLP, RAPD, isozyme		Penetrated root number	2	-	-	9.0	Zheng et al., (2000)
				Total root number	2	-	-	14.3	
				Root penetration index	4	-	1	13.5	
				Penetrated root thickness	4	-	3	16.4	

IR58821/IR52561	166 RILs (166)	399 (RFLP, AFLP)	2022	Total root number	2	-	-	12.2	Ali et al., (2000)
				Penetrated root number	7	3	-	27.2	
				Root penetration index	6	3	2	26.2	
				Penetrated root thickness	8	5	2	13.9	
				Penetrated root length	5	-	-	12.8	
CT9993/IR62266	154 DH lines (154)	315 (RFLP, AFLP, SSR)	1788	Cell membrane stability	9	-	-	42.1	Tripathy et al., (2000)
CT9993/IR62266	154 DH lines (154)	315 (RFLP, AFLP, SSR)	1788	Osmotic adjustment	5	-	1	12.9	Zhang et al., (2001)
				Root penetration index	4	-	3	11.0	
				Basal root thickness	6	-	4	37.6	
				Penetrated root thickness	11	-	3	31.3	
				Root pulling force	6	-	-	19.9	
				Total root dry weight	5	-	-	20.2	
				Penetrated root dry weight	3	-	-	16.8	
				Penetrated root length	1	-	-	17.0	
CT9993/IR62266	154 DH lines (115 / 100 / 127 / 154)	315 (RFLP, AFLP, SSR)	1788	Shoot biomass	7	1	-	56.8	Kamoshita et al., (2002)
				Deep root mass	7	1	-	35.5	
				Deep root ratio	6	1	-	51.8	
				Deep root per tiller	6	1	-	40.4	
				Maximum Rooting depth	9	1	-	16.8	
				Root thickness 0-10cm	6	2	-	36.4	
				Root thickness 20-25cm	3	1	-	21.8	
IR58821/IR52561	166 RILs (166/164)	399 (RFLP, AFLP)	2022	Shoot biomass	2	-		13.8	Kamoshita et al., (2002a)
				Deep root mass	5	-		21.4	
				Deep root ratio	5	2	5	27.4	
				Deep root per tiller	6	-		21.6	
				Maximum Rooting depth	5	-	1	29.9	
				Root thickness 0-10 cm	6	-	2	15.1	
				Root thickness 20-25 cm	2	-	1	23.2	
CT9993/IR62266	154 DH line (154/40)	315 (RFLP, AFLP, SSR)	1788	Relative water content	2	-	-	58.8	Babu et al., (2003)
				Canopy temperature	1	-	-	47.1	
				Leaf rolling	3	-	-	16.5	
				Leaf drying	3	-	-	20.8	
				Days to heading-stress	3	-	-	9.3	
				Days to heading-control	4	-	-	27.0	
				Plant height-stress	4	1	-	46.8	
				Plant height-control	5	1	-	46.5	
				Grain yield-stress	5	-	-	22.3	
				Biomass-stress	2	-	-	17.2	
				Biomass-control	6	-	-	22.2	

				Grains per panicle-stress	1	-	-	13.6	
				Grains per panicle-control	2	-	-	21.1	
				Harvest index-stress	1	-	-	14.7	
				Harvest index-control	2	-	-	13.0	
				Relative yield	2	-	-	22.0	
CT9993/IR62266	154 DH lines (127)	315 (RFLP, AFLP, SSR)	1788	Plant height	7	-	-	20.0	Kanbar *et al.,* (2002)
				Number of tillers	4	-	-	12.5	
				Total root number	1	-	-	10.1	
				Root dry weight	1	-	-	7.6	
				Total plant length	3	-	-	12.8	
				Root to shoot length ratio	1	-	-	7.8	

Parents	Population§	Number of markers used	Linkage map coverage (cM)	Traits	QTLs identified			Per cent of Maximum phenotypic variance	References
					No. of QTLs	Across trials/ experiments	Across Population		
Co39/Moroberekan	281 F_7 RILs (203)	127 (RFLP)		Root thickness	18	-	-	56	Champoux et al., (1995)
				Root shoot ratio	16	-	-	38	
				Root dry weight per tiller	14	-	-	35	
				Deep root weight	8	-	-	18.5	
				Maximum root depth	4	-	-	-	
				Drought avoidance (leaf rolling)	18	5	-	35	
Co39/Moroberekan	281 F_7 RILs (202)	127 (RFLP)		Number of penetrating roots	4	-	-	8	Ray et al., (1996)
				Total number of roots	19	-	-	19	
				Root penetration index	6	-	-	13	
				Tiller number	10	-	-	14	
Co39/Moroberekan	281 F_7 RILs (52)	127 (RFLP)		Dehydration tolerance	5	-	-	36	Lilley et al., (1996)
				Osmotic adjustment	1	-	-	32	
				Relative water content	2	-	-	35	
IR64/Azucena	135 DH lines (105)	175 (RFLP, RAPD, isozyme)	2005	Total root weight	23	-	3	11.9	Yadav et al., (1997)
				Deep root weight	17	-	-	14.9	
				Deep root weight to shoot ratio	26	-	3	22.3	
				Deep root weight per tiller	20	-	3	19.6	
				Maximum root length	25	-	1	20.9	
				Root thickness	8	-	2	10.4	
`Bala/Azucena	178 F_2 plants (30)	71 (RFLP)	1280	Maximum root length	10	1	4	37.7	Price et al., (1997)
				Root volume	1	-	-	10.2	
				Adventitious root thickness	2	-	2	14.7	

Bala/azucena	178 F_2 plants (178)	71 (RFLP)	1280	Leaf rolling	1	-	-	6.2	Price et al., (1997a)
				Stomatal behaviour	4	-	-	18.4	
				Days to heading	3	2	-	32.5	
IR64/Azucena	135 DH lines (56)	175 (RFLP, RAPD, isozyme)	2005	Plant height	4	2	-	29.2	Hemamalini et al., (2000)
				Number of tillers	11	-	-	25.7	
				Root length	5	-	3	15.4	
				Total root number	10	-	-	25.1	
				Root volume	5	-	-	21.4	
				Root thickness	5	1	2	26.7	
				Root dry weight	2	-	-	20.8	
				Root shoot ratio	1	-	1	12.7	
				Leaf drying (Drought score)	2	-	-	16.1	
				Leaf rolling	1	-	-	11.9	
IR64/Azucena	135 DH lines (105 & 85)	175 (RFLP, RAPD, isozyme)	2005	Leaf rolling	11	4	6	23.3	Courtois et al., (2000)
				Leaf drying	10	1	-	19.4	
				Relative water content	11	1	-	18.5	
				Relative growth rate	10	-	-	16.5	
Bala/Azucena	205 RILs (104)	135 (RFLP, AFLP)	1680	Number of tillers	1	-	-	12.4	Price et al., (2000)
				Number of roots	3	-	1	10.3	
				Number of penetrated roots	7	-	-	16.7	
				Penetrated: total roots (root penetration index)	7	-	2	18.0	
IR1552/Azucena	150 RILs (150)	207 (RFLP, AFLP)	2419	Seminal root length	2	-	-	11.2	Zhang et al., (2001a)
Bala/Azucena	205 RILs (176,118,142& 110)	142 (RFLP, AFLP, SSR)	1779	Leaf rolling	5	1	5	20.4	Price et al., (2002)
				Leaf drying	11	-	8	17.6	
				Relative water content	8	-	7	25.6	
Bala/Azucena	205 RILs (140)	142 (RFLP, AFLP, SSR)	1779	Total dry weight/plant mass	8	2	-	21.3	Price et al., (2002a)
				Root to shoot dry weight ratio	11	2	2	28.0	
				Root mass below 90 cm	6	-	3	16.0	
				Basal root thickness	7	-	-	18.2	
				Root thickness at 90 cm	14	2	8	18.3	
				Maximum root length	6	2	4	17.4	
				Number of root past 100 cm	12	4	-	22.8	
IR64/Azucena	135 DH lines (109)	175 (RFLP, RAPD, isozyme		Penetrated root number	2	-	-	9.0	Zheng et al., (2000)
				Total root number	2	-	-	14.3	
				Root penetration index	4	-	1	13.5	
				Penetrated root thickness	4	-	3	16.4	
IR58821/IR52561	166 RILs (166)	399 (RFLP, AFLP)	2022	Total root number	2	-	-	12.2	Ali et al., (2000)
				Penetrated root number	7	3	-	27.2	
				Root penetration index	6	3	2	26.2	
				Penetrated root thickness	8	5	2	13.9	

				Penetrated root length	5	-	-	12.8	
CT9993/IR62266	154 DH lines (154)	315 (RFLP, AFLP, SSR)	1788	Cell membrane stability	9	-	-	42.1	Tripathy et al., (2000)
CT9993/IR62266	154 DH lines (154)	315 (RFLP, AFLP, SSR)	1788	Osmotic adjustment	5	-	1	12.9	Zhang et al., (2001)
				Root penetration index	4	-	3	11.0	
				Basal root thickness	6	-	4	37.6	
				Penetrated root thickness	11	-	3	31.3	
				Root pulling force	6	-	-	19.9	
				Total root dry weight	5	-	-	20.2	
				Penetrated root dry weight	3	-	-	16.8	
				Penetrated root length	1	-	-	17.0	
CT9993/IR62266	154 DH lines (115 / 100 / 127 / 154)	315 (RFLP, AFLP, SSR)	1788	Shoot biomass	7	1	-	56.8	Kamoshita et al., (2002)
				Deep root mass	7	1	-	35.5	
				Deep root ratio	6	1	-	51.8	
				Deep root per tiller	6	1	-	40.4	
				Maximum Rooting depth	9	1	-	16.8	
				Root thickness 0-10cm	6	2	-	36.4	
				Root thickness 20-25cm	3	1	-	21.8	
IR58821/IR52561	166 RILs (166/164)	399 (RFLP, AFLP)	2022	Shoot biomass	2	-		13.8	Kamohita et al., (2002a)
				Deep root mass	5	-		21.4	
				Deep root ratio	5	2	5	27.4	
				Deep root per tiller	6	-		21.6	
				Maximum Rooting depth	5	-	1	29.9	
				Root thickness 0-10 cm	6	-	2	15.1	
				Root thickness 20-25 cm	2	-	1	23.2	
CT9993/IR62266	154 DH line (154/40)	315 (RFLP, AFLP, SSR)	1788	Relative water content	2	-	-	58.8	Babu et al., (2003)
				Canopy temperature	1	-	-	47.1	
				Leaf rolling	3	-	-	16.5	
				Leaf drying	3	-	-	20.8	
				Days to heading-stress	3	-	-	9.3	
				Days to heading-control	4	-	-	27.0	
				Plant height-stress	4	1	-	46.8	
				Plant height-control	5	1	-	46.5	
				Grain yield-stress	5	-	-	22.3	
				Biomass-stress	2	-	-	17.2	
				Biomass-control	6	-	-	22.2	
				Grains per panicle-stress	1	-	-	13.6	
				Grains per panicle-control	2	-	-	21.1	
				Harvest index-stress	1	-	-	14.7	
				Harvest index-control	2	-	-	13.0	
				Relative yield	2	-	-	22.0	
CT9993/IR62266	154 DH lines	315 (RFLP,	1788	Plant height	7	-	-	20.0	Kanbar *et al.,*

	(127)	AFLP, SSR)		Number of tillers	4	-	-	12.5	(2002)
				Total root number	1	-	-	10.1	
				Root dry weight	1	-	-	7.6	
				Total plant length	3	-	-	12.8	
				Root to shoot length ratio	1	-	-	7.8	
Caiapo/*O.rufipogon*	274 BC_2F_2 (300)	125 (RFLP, SSR)	-	Days to heading	4	4	-	14.0	Moncada et al., (2001)
				Plant height	6	6	2	21.0	
				Panicles per plant	2	-	-	18.3	
				Percentage of sterility	2	1	1	13.0	
				Grains per plant	4	4	2	12.0	
				1000 grain weight	5	4	2	22.0	
				Yield per plant	2	2	2	14.0	
IR1552/Azucena	150 RIL (96)	249 (RFLP, SSR, cDNA-AFLP)		Seminal root length	4	-	4	13.4	Zheng *et al*., (2003)
				Relative seminal root length	2	-	-	13.9	
				Adventitious root number	7	-	3	18.2	
				Relative adventitious root number	1	-	-	15.0	
				Lateral root length	4	-	2	14.4	
				Relative lateral root length	1	-	-	11.9	
				Lateral root number	2	-	-	11.7	
				Relative lateral root number	1	-	-	12.3	
IR62266/IR60080	150 BC_3F_3 (142)	167 (RFLP, SSR, candidate genes)	1370	Osmotic adjustment	19	12	3	25.0	Robin *et al.,* (2003)
P124/IR64	700 BC_1F_2 (69)	35 (RAPD)	-	Maximum root length	4	-	-	23.1	Toorchi *et al*., (2002)
IR64/Azucena	135 DH (90, 84, 56 & 109)	260 (RFLP, SSR, RAPD, isozymes)	2457	Days to flowering	2	-	-	24.6	Venuprasad *et al.,* (2002)
				Plant height	2	1	1	20.0	
				Grain yield	1	1	-	15.7	
				Harvest index	1	1	-	19.7	
				Days to maturity	1	-	-	20.4	
				Root thickness	1	-	-	26.9	
				Root volume	1	-	-	29.1	
				Root dry weight	1	-	-	30.7	
				Maximum root length	1	-	-	12.9	

§ Subset of population used for phenotyping is indicated in parenthesis; DH- Doubled Haploids; RIL- Recombinant Inbred Lines.

Caiapo/*O.rufipogon*	274 BC_2F_2 (300)	125 (RFLP, SSR)	-	Days to heading	4	4	-	14.0	Moncada et al., (2001)
				Plant height	6	6	2	21.0	
				Panicles per plant	2	-	-	18.3	
				Percentage of sterility	2	1	1	13.0	
				Grains per plant	4	4	2	12.0	
				1000 grain weight	5	4	2	22.0	
				Yield per plant	2	2	2	14.0	
IR1552/Azucena	150 RIL (96)	249 (RFLP, SSR, cDNA-AFLP)		Seminal root length	4	-	4	13.4	Zheng *et al*., (2003)
				Relative seminal root length	2	-	-	13.9	
				Adventitious root number	7	-	3	18.2	
				Relative adventitious root number	1	-	-	15.0	
				Lateral root length	4	-	2	14.4	
				Relative lateral root length	1	-	-	11.9	
				Lateral root number	2	-	-	11.7	
				Relative lateral root number	1	-	-	12.3	
IR62266/IR60080	150 BC_3F_3 (142)	167 (RFLP, SSR, candidate genes)	1370	Osmotic adjustment	19	12	3	25.0	Robin *et al.,* (2003)
P124/IR64	700 BC_1F_2 (69)	35 (RAPD)	-	Maximum root length	4	-	-	23.1	Toorchi *et al*., (2002)
IR64/Azucena	135 DH (90, 84, 56 & 109)	260 (RFLP, SSR, RAPD, isozymes)	2457	Days to flowering	2	-	-	24.6	Venuprasad *et al.,* (2002)
				Plant height	2	1	1	20.0	
				Grain yield	1	1	-	15.7	
				Harvest index	1	1	-	19.7	
				Days to maturity	1	-	-	20.4	
				Root thickness	1	-	-	26.9	
				Root volume	1	-	-	29.1	
				Root dry weight	1	-	-	30.7	
				Maximum root length	1	-	-	12.9	

§ Subset of population used for phenotyping is indicated in parenthesis; DH- Doubled Haploids; RIL- Recombinant Inbred Lines; BC-Back Cross progenies; RFLP-Restriction Fragment Length Polymorphism; RAPD- Random Amplified Polymorphic DNA; SSR-Simple Sequence Repeats; cDNA- complimentary DNA; AFLP- Amplified Fragment Length Polymorphism.

Caiapo/*O.rufipogon*	274 BC_2F_2 (300)	125 (RFLP, SSR)	-	Days to heading	4	4	-	14.0	Moncada et al., (2001)
				Plant height	6	6	2	21.0	

				Panicles per plant	2	-	-	18.3	
				Percentage of sterility	2	1	1	13.0	
				Grains per plant	4	4	2	12.0	
				1000 grain weight	5	4	2	22.0	
				Yield per plant	2	2	2	14.0	
IR1552/Azucena	150 RIL (96)	249 (RFLP, SSR, cDNA-AFLP)		Seminal root length	4	-	4	13.4	Zheng *et al*., (2003)
				Relative seminal root length	2	-	-	13.9	
				Adventitious root number	7	-	3	18.2	
				Relative adventitious root number	1	-	-	15.0	
				Lateral root length	4	-	2	14.4	
				Relative lateral root length	1	-	-	11.9	
				Lateral root number	2	-	-	11.7	
				Relative lateral root number	1	-	-	12.3	
IR62266/IR60080	150 BC_3F_3 (142)	167 (RFLP, SSR, candidate genes)	1370	Osmotic adjustment	19	12	3	25.0	Robin *et al.,* (2003)
P124/IR64	700 BC_1F_2 (69)	35 (RAPD)	-	Maximum root length	4	-	-	23.1	Toorchi *et al*., (2002)
IR64/Azucena	135 DH (90, 84, 56 & 109)	260 (RFLP, SSR, RAPD, isozymes)	2457	Days to flowering	2	-	-	24.6	Venuprasad *et al.,* (2002)
				Plant height	2	1	1	20.0	
				Grain yield	1	1	-	15.7	
				Harvest index	1	1	-	19.7	
				Days to maturity	1	-	-	20.4	
				Root thickness	1	-	-	26.9	
				Root volume	1	-	-	29.1	
				Root dry weight	1	-	-	30.7	
				Maximum root length	1	-	-	12.9	

§ Subset of population used for phenotyping is indicated in parenthesis; DH- Doubled Haploids; RIL- Recombinant Inbred Lines; BC-Back Cross progenies; RFLP-Restriction Fragment Length Polymorphism; RAPD- Random Amplified Polymorphic DNA; SSR-Simple Sequence Repeats; cDNA- complimentary DNA; AFLP- Amplified Fragment Length Polymorphism.

Triats*	Population	Number of QTLs identified	Markers identified on Chromosome #												Reference
			1	2	3	4	5	6	7	8	9	10	11	12	
Root thickness **-control**	**CO39/Morobereka n**	**18**	**RG197, RG811**	**RG347**	**RG104A, RZ576, RG910**	**RG214, RG788**	**-**	***waxy***	**CDO533, RG528**	**RZ66, RG136**	**RG533, RZ12**	**RZ892**	**CDO365**	**RZ397**	**Champoux et al., (1995)**
-stress	**IR64/Azucena**	**8**	**RG381, RZ19, RG690, RZ730, RZ801**	**PAL1, RZ58**	**-**	**-**	**RZ649, RZ67**	**-**	**-**	**AMY3DE , RZ66, AC5, RG418B,**	**-**	**-**	**-**	**-**	**Yadav et al., (1997)**
-control	**IR64/Azucena**	**5**	**-**	**RG157,**	**-**	**-**	**RG313,**	**-**	**-**	**AC5,**	**Amy3ABC,**	**-**	**-**	**-**	**Hemamalini et**

				RZ318, Pall, RZ58			RZ556			RG418B	RZ228,				al., (2000)
-stress	Bala/Azucena	7	RM212	-	RG191	C513, RM348	-	e12m37.7	-	-	C506	-	-	G124	Price et al., (2002a)
-stress	CT9993/IR62266	6	-	TGMSP2, ME9_7	EM19_11, RZ474	RG939, RG476	-	-	-	RZ997, EM14_1	ME2_17, C711	-	-	ME10_3, ME6_6	Zhang et al., (2001)
-control	CT9993/IR62266	6	CDO345, ME10-14	R3393, RZ58, EM18-13, ME9-7	-	RG476, RG214	-	-	-	EM14-1, ME211	-	-	ME7-2, EM18-19	-	Kmoshita et al., (2002)
-control	IR58821/IR52561	6	PC32M5, PC31M10	-	R1925, RG1356	-	-	-	-	PC27M15, C1121, PC75M12, PC32M7, PC75M13, G1073	PC32M8, RZ536, PC11M4	-	-	-	Kamoshita et al.,, (2002a)
-control	IR64/Azucena	1	-	RG157, RZ318	-	-	-	-	-	-	-	-	-	-	Venuprasad *et al.*, (2002)
Root thickness at 20-25cm –control	CT9993/IR62266	3	-	ME10-18, C106, EM13-3, RG158	-	RG476, RG214	-	-	-	-	-	-	-	-	Kamoshita et al., (2002)
-control	IR58821/IR52561	2	-	-	-	RZ467, PC184M13, RZ536, PC11M4	-	-	-	-	-	-	-	-	Kamoshita et al., (2002a)
Root thickness at 90cm-stress	Bala/Azucena	14	C86, G393	C601	-	RM348,	-	R2654, e12m36.18	C507,	R2676	G385, G1085	C701	C189	e12m37.13	Price et al., (2002a)
Adventious root thickness-control	Bala/Azucena	2	-	RG171,	-	-	C624	-	-	-	-	-	-	-	Price et al., (1997)
Penetrated root thickness –stress	IR64/Azucena	4	RZ730, RZ801	-	RG104, RG348	RG163, RZ590	-	-	-	-	Amy3ABC, RZ228	-	-	-	Zheng et al., (2000)
-stress	CT9993/IR62266	11	ME6_4, EM18_10, RG957, RG345	ME2_7, EMP2_7, ME9_7, K706	-	RG939, RG476	-	R2549, RG716	RG417, EM17_3	-	RG553, EM14_6, ME9_6, K985	-	-	RG9, ME10_1, ME4_5, ME10_8	Zhang et al., (2001)
-stress	IR58821/IR52561	8	PC15M11, PC3M3, C49, PC11M10	RG256, PC32M10, PC3M11, PC33M8	-	PC33M5, PC38M9,	-	RG123, R2654	RG351, PC11M7,	-	-	RZ892, BCD386	-	-	Ali et al., (2000)
Root pulling force -stress	CT9993/IR62266	6	-	EM13_3, RG158	EM11_9, CDO20, EM13_1, R2170	RG214, RG620	RM164, EM15_5	-	-	-	-	-	ME2_6, RM21	-	Zhang et al., (2001)
Shoot biomass -control	CT9993/IR62266	7	-	R1843, ME2-7, RG437, ME10-18, TGMSP2, ME9-7	-	-	-	R682, EM14-9	-	-	-	-	G257, RM21, C950, R1506	ME108, ME79	Kamoshita et al,., (2002)
-control	IR58821/IR52561	2	-	-	-	RZ536, PC11M4	-	-	-	PC75M13, G1073	-	-	-	-	Kamoshita et al., (2002a)
Biomass-stress	CT9993/IR62266	2	-	-	RZ313, EM17-1	-	-	-	-	-	-	-	-	ME6-12, G2140	Babu et al., (2003)
Biomass-control	CT9993/IR62266	6	-	ME2-7, EMP2-7, EM14-4, RZ386	RG369, EM19-4,	RG620, C107	-	-	-	ME2-1, EM16-6	ME9-3, ME9-6	-	-	-	Babu et al., (2003)
Root/Shoot ratio	CO39/Morobereka	16	CDO920,	RG139	RG104A,	RG214,	-	*waxy*	CDO405	RZ66	RG533,	RZ892	CDO365	RZ397	Champoux et

-control	n		RG140		RZ576, RG910	RG910					RZ12				al., (1995)
-stress	IR64/Azucena	26	RZ19, RG690, RZ730, RZ801	PAL1, RZ58	-	-	RZ390, RG313, RZ556, RG403, RG13	RG424, RG162, RG172, CDO544, AMY2A, RG433, CAT1	RG711, EST9, RZ337B, CDO497, CDO418, RZ978, CDO38, RG351	AMY3DE, RZ66, AC5, RG418B, AMP2	RZ422, AMY3ABC, RZ228, RZ12	-	-	-	Yadav et al., (1997)
-control	IR64/Azucena	1	RG173, Amy1B	-	-	RG449	-	-	-	-	-	-	-	-	Hemamalini et al., (2000)
-stress	Bala/Azucena	11	G393, C86, C949	RG83,	-	RG163	-	e12M36.18	-	G187, R202	G385, G1085	-	-	-	Price et al., (2002a)
-control	CT9993/IR62266	1	CDO345, RZ909	-	-	-	-	-	-	-	-	-	-	-	Kanbar *et al.,* (2002)
Total dry weight-stress	Bala/Azucena	8	C86, C949, RM212	-	G164,	-	-	-	RG650,	e18M43.8,	G1085	C701	C189	-	Price et al., (2002a)
Root dry weight/tiller -control	CO39/Morobereka n	14	CDO920, RG140	RG139, RG437	RG104A, RG910	RG788	-	*waxy*	RG351, RG528	RG136	RG533	-	-	RG181, RG9	Champoux et al., (1995)
-control	IR64/Azucena	1	-	RG171, RG157	-	-	-	-	-	-	-	-	-	-	Hemamalini et al., (2000)
-stress	IR64?Azucena	1	-	-	RG348, RG104	-	-	-	-	-	-	-	-	-	Hemamalini *et al.,* (2000)
-stress	CT9993/IR62266	5	RG109, EM11_11	ME2_7, EMP2_7	-	RG190, EM15_3	-	ME4_11, ME7_5	-	-	-	RG257, ME5_16	-	-	Zhang et al., (2001)
-stress	IR64/Azucena	1	-	-	RM231, RZ329	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
-control	CT9993/IR62266	1	-	-	-	ME6-10, RG449	-	-	-	-	-	-	-	-	Kanbar *et al.,* (2002)
Deep root dry weight/tiller -stress	IR64/Azucena	20	RG381, RZ19, RG690, RZ730, RZ801	RG171, RG157	-	-	-	AMY2A, RG433, CAT1	RG711, EST9, RZ337B, CDO497, CDO418, RZ978, CDO38, RG351	RZ66, AC5, RG418B, AMP2	AMY3ABC, RZ228, RZ12, RG667	-	-	-	Yadav et al., (1997)
Deep root weight -control	CO39/Morobereka n	8	-	RG139, RG437	RG910	RG788	RG351	-	-	RG136	RZ12	-	-	RG181	Champoux et al., (1995)
-stress	IR64/Azucena	17	RG381, RZ19, RG690, RZ730, RZ801	-	-	-	-	AMY2A, RG433, CAT1	RG711, EST9, RZ337B, CDO497, CDO418, RZ978, CDO38, RG351	-	RZ206, RZ422,, AMY3ABC, RZ228, RZ12, RG667	-	-	-	Yadav et al., (1997)
-stress	Bala/Azucena	6	C86, C949	C601,	-	C513	-	-	C451	R2676	G1085	-	-	-	Price et al., (2002a)
-control	CT9993/IR62266	7	RM212, R2417	RG437, ME10-18	CDO20, RG409, RZ474, C746	-	-	-	RG404, CDO38	-	-	-	G257, ME2-6, ME7-2, EM18-19	-	Kamoshita et al., (2002)
-control	IR58821/IR52561	5	-	PC32M10, RG151	PC20M11, PC20M12	PC75M3, RZ536	-	-	-	-	PC33M1, PC32M8	-	PC48M15, PC31M8	-	Kamoshita et al., (2002a)
Deep root ratio -control	CT9993/IR62266	6	C813, RG957,	RG437, ME10-18	-	-	G387, ME5-13	-	-	-	-	-	G257, ME2-6, ME6-7,	-	Kamoshita et al., (2002)

			R1944, RG400										ME7-2		
-control	IR58821/IR52561	5	-	RG256, RG151	PC20M11, PC20M12	PC75M3, PC11M4	-	-	-	-	PC32M8, RZ596	-	PC41M14, PC48M15	-	Kamoshita et al., (2002a)
Penetrated root weight -stress	CT9993/IR62266	3	-	-	-	RG939, RG476	-	-	-	-	R41, ME2_10	-	-	RG9, ME10_1	Zhang et al., (2001)
Maximum root depth -control	CO39/Morobereka n	4	-	RG324, RG139	-	-	-	-	-	-	RZ12	-	CDO365	-	Champoux et al., (1995)
-stress	IR64/Azucena	25	RG381, RZ19, RG690	RG171, RG157, RZ318, PAL1, RZ58	RZ519, PG1, CDO87	-	RZ390, RG313, RZ556, RG403	RG433, CAT1	RG711, EST9, RZ337B, CDO497, CDO418, RZ978, CDO38, RG351	AC5, RG418B, AMP2	RZ422,, AMY3ABC, RZ228, RZ12, RG667	-	-	-	Yadav et al., (1997)
-stress	IR64/Azucena	3	RG381, RZ19	RG171, RG157	-	-	-	-	-	-	RZ206, RZ422	-	-	-	Hemamalini et al., (2000)
-control	IR64/Azucena	2	-	-	-	RG214, RG143, RG163, RZ590	-	-	-	-	-	-	-	-	Hemamalini *et al.,* (2000)
-control	Bala/Azucena	10	RG213	RG171	-	-	RG13	RG213, RZ593, RZ682	-	-	-	G89a	G44, RG2	-	Price et al., (1997)
-stress	Bala/Azucena	6	-	C601	-	RG190	-	-	RG650	RG598,	e12m36.13, G1085	C701	-	-	Price et al., (2002a)
-control	CT9993/IR62266	9	RM212, R2417,	RG437, ME10-18, RZ58, EM13-3, EM18-13, TGMSP2	-	-	C119, C859	-	ME7-1, EM18-4	-	ME47, ME1010, ME59, ME58	-	RM206, RG1109	-	Kamoshita et al., (2002)
-control	IR58821/IR52561	5	PC31M10, PC34M6	-	-	CDO456, PC79M8, RZ467, PC184M13, PC75M3, RZ536, RZ214, C1016	-	-	-	-	-	-	-	-	Kamoshita et al., (2002a)
-control	IR64/Azucena	1	-	-	-	-	-	-	-	-	RZ12, RM201	-	-	-	Venuprasad *et al.,* (2002)
-stress	P124/IR64	4	No chromosomal location. Under stress: OPBC10, OPAK19. Under low moisture condition OPAH13, OPBF02												Toorchi *et al.,* (2002)
Seminal root length-stress	IR1552/Azucena	1	-	-	-	-	-	-	-	RG1	-	-	-	-	Zheng *et al.,* (2001a)
-stress	IR1552/Azucena	3	S1746, RG109B	L16, CDO718	-	-	-	-	-	-	RG570, RG667	-	-	-	Zhang et al., (2003)
-lowland	IR1552/Azucena	1	RG109B, RG690	-	-	-	-	-	-	-	-	-	-	-	Zhang et al., (2001a)
-flooding	IR64/Azucena	2	-	L16, CDO718	-	-	-	-	RG650, CDO497	-	-	-	-	-	Zheng *et al.,* (2003)
Relative seminal root length	IR1552/Azucena	2	S1746, RG109B	-	-	-	-	-	-	-	RG570, RG667	-	-	-	Zheng *et al.,* (2003)
Lateral root length-stress	IR1552/Azucena	2	-	-	RG409, T17	-	-	AAC-CTT10, RZ140	-	-	-	-	-	-	Zheng *et al.,* (2003)

Lateral root length-**flooding**	**IR1552/Azucena**	**2**	**-**	**-**	**RG409, T17**	**-**	**RG313, E1328**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Zheng *et al.*, (2003)**
Relative lateral root length	**IR1552/Azucena**	**1**	**-**	**-**	**-**	**-**	**-**	**AAC-CTT10, RZ140**	**-**	**-**	**-**	**-**	**-**	**-**	**Zheng *et al.*, (2003)**
Total plant length-**control**	**CT9993/IR62266**	**3**	**RG109 ME10-14,**	**RG437, ME10-18**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**ME-7-9, ME10-8**	**Kanbar *et al.*, (2002)**
Deep root per tiller **-control**	**CT9993/IR62266**	**6**	**RM212, R2417**	**RG437, C106**	**-**	**RG476, RG214**	**G387, ME5-15**	**-**	**-**	**-**	**-**	**-**	**C477, EM17-10, ME7-2, EM18-19**	**-**	**Kamoshita et al., (2002)**
-control	**IR58821/IR52561**	**6**	**-**	**PC47M3, PC173M5, PC32M10, RG151**	**-**	**PC11M12, PC28M1, PC73M4, PC180M10**	**-**	**PC48M13, PC32M6**	**PC41M6, CDO385**	**-**	**-**	**-**	**-**	**-**	**Kamoshita et al., (2002a)**
Penetrated root length **-stress**	**CT9993/IR62266**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**G1465, C950**	**-**	**Zhang et al., (2001)**
-stress	**IR58821/IR52561**	**5**	**PC17M5, PC15M10**	**PC33M9, PC79M6**	**RG510, R3226**	**-**	**-**	**-**	**PC41M6, CDO385**	**-**	**-**	**-**	**RG103, PC74M2**	**-**	**Ali et al., (2000)**
Drought avoidance/ leaf rolling **-stress**	**CO39/Morobereka n**	**18**	**RG462**	**RG324, RG139, RG437, RG544**	**RZ394, RG910**	**RG214, RG190**	**-**	**RZ516, RZ192**	**CDO533**	**RG1**	**RG662**	**RZ892, RZ561**	**CDO365, RZ53**	**-**	**Champoux et al., (1995)**
-control	**Bala/Azucena**	**1**	**C949**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Price et al., (1997a)**
-stress	**IR64/Azucena**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**PGMS0.7, CDO59**	**-**	**-**	**-**	**-**	**-**	**Hemamalini et al., (2000)**
-stress	**IR64/Azucena**	**11**	**RZ730, RZ801, RG810, RG331**	**-**	**RZ892, RG100, RZ574, RZ284, RG910, RG418A**	**RG163, RZ590**	**RZ67, RZ70**	**-**	**RG477, PGMSO7, CDO59**	**-**	**RG757, C711, RZ12, RG667**	**-**	**-**	**-**	**Courtois et al., (2000)**
-stress	**Bala/Azucena**	**5**	**C949, RZ14**	**-**	**C136**	**-**	**C624, C43**	**C76**	**G20, R1440, G89**	**-**	**-**	**-**	**-**	**-**	**Price et al., (2002)**
-stress	**CT9993/IR62266**	**3**	**RG109, ME10-14**	**-**	**-**	**-**	**-**	**-**	**-**	**ME6-13, G187**	**-**	**-**	**ME10-16, ME6-2**	**-**	**Babu et al., (2003)**
Number of penetrating roots **-stress**	**CO39/Morobereka n**	**4**	**RG162**	**-**	**RZ393**	**-**	**-**	**RG172**	**-**	**-**	**-**	**-**	**-**	**RG869B**	**Ray et al., (1996)**
-control	**Bala/Azucena**	**7**	**-**	**G45, C601**	**e12m37.4, e12m36.16**	**-**	**C624**	**-**	**-**	**-**	**-**	**e12m37.2**	**C189**	**-**	**Price et al., (2000)**
-stress	**IR64/Azucena**	**2**	**-**	**RZ318, Pall**	**-**	**-**	**-**	**-**	**RZ337B, CDO497**	**-**	**-**	**-**	**-**	**-**	**Zheng et al., (2000)**
-stress	**IR58821/IR52561**	**7**	**BCD134, RZ776**	**PC33M8, PC21M1, RG256, PC32M10, C499, PC11M1, AA7-2b, AA7-2a**	**PC73M13, PC3M5, C746, RZ448**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Ali et al., (2000)**
Number of roots past 100cm **-stress**	**Bala/Azucena**	**12**	**C949, RZ14,**	**e18M43.8, C601**	**RG409, G164,**	**-**	**RG346**	**R2654**	**RG650**	**e18m43.4, R902, e12m36.7**	**G1085**	**-**	**RG2**	**RM247**	**Price et al.,(2002a)**
Total number of roots **–stress**	**CO39/Morobereka n**	**19**	**RG350, RG77, RG811,**	**RG139, RZ103**	**RG745, RZ394**	**RG214, RG476C, RG329,**	**RG13**	***waxy*, CDO475, RZ516,**	**CDO533, RZ272, RG528**	**RG1, RZ66, RG136**	**RG553, RZ404**	**-**	**RG1109, CDO365, RG211,**	**RZ397**	**Ray et al., (1996)**

			RG140			RG163		RZ144, RG162, RG653					RG167, RZ53, RG118		
-control	IR64/Azucena	6	RG472, RG246, W1, RG173	-	-	-	-	pRD10B, RG648	RG477, PGMS0.7, CDO59	-	-	RG134, RZ500	-	-	Hemamalini et al., (2000)
-stress	IR64/Azucena	4	-	RG171, RG157, Pal I, RZ58, RZ123, RG520,	-	RG190, RG908	-	-	-	-	-	-	-	-	Hemamalini *et al.,* (2000)
-control	Bala/Azucena	3	RG173, R117	-	-	-	-	-	-	-	-	e12m37.2	-	-	Price et al., (2000)
-stress	IR64/Azucena	2	RG246, K5	-	-	-	-	-	RG104, RG348	-	-	-	-	-	Zheng et al., (2000)
-stress	IR58821/IR52561	2	-	-	R1925-RG1356	-	-	-	PC75M8, PC32M1	-	-	-	-	-	Ali et al., (2000)
-control	CT9993/IR62266	1	-	-	-	RZ565, EMP3-10	-	-	-	-	-	-	-	-	Kanbar *et al.,* (2002)
Adventitious root number -stress	IR1552/Azucena	3	RG109B, RM315, CDO920, BCD134	AGG-CAG13 G45	-	-	-	-	-	-	-	-	-	-	Zheng *et al.,* (2003)
Adventitious root number -flooding	IR1552/Azucena	4	-	-	RZ399, RZ448, RG104, ACC-GTG2	AAG-CAA4, RG396	-	-	-	-	RM328, RG570	-	-	-	Zheng *et al.,* (2003)
Relative adventitious root number	IR1552/Azucena	1	-	-	RM282, RZ574	-	-	-	-	-	-	-	-	-	Zheng *et al.* (2003)
Lateral root number -stress	IR1552/Azucena	1	-	-	RG191, AAC-CAG5	-	-	-	-	-	-	-	-	-	Zheng *et al.,* (2003)
Lateral root number -flooding	IR1552/Azucena	1	-	-	-	-	-	AAC-CAG7, AAC-CTT10	-	-	-	-	-	-	Zheng *et al.,* (2003)
Relative lateral root number	IR1552/Azucena	1	-	-	-	RM252, AGG-CAG7	-	-	-	-	-	-	-	-	Zheng *et al.,* (2003)
Root penetration index -stress	CO39/Morobereka n	6	RG324, RG73	-	-	RG620, RG476C, RG329	RG360	RG653	-	-	-	-	CDO365, RG118	-	Ray et al., (1996)
-control	Bala/Azucena	7	-	G45, C601	e12m37.4, e12m36.16	-	C624	-	-	-	-	e12m37.2	C189	-	Price et al., (2000)
- stress	IR64/Azucena	4	-	RZ123, RG520	RG104, RG348	-	-	-	CDO418, RZ978	A18A1120, TGMS1.2	-	-	-	-	Zheng et al., (2000)
-stress	CT9993/IR62266	4	-	-	EM19_4, EM13_1	EM14_5, ME2_13, RG939, RG476	-	-	-	-	-	-	-	ME6_12, RG9	Zhang et al., (2001)
-stress	IR58821/IR52561	6	-	RG256, PC32M10, PC33M8, PC21M1, C499, PC11M1, AA7-2b, AA7-2a	PC73M13, PC3M5	-	-	-	-	-	-	RZ892, BCD386	-	-	Ali et al., (2000)
Number of	CO39/Morobereka	10	RG140	RG139,	RG476C,	-	-	*waxy,*	-	RG1,	-	-	CDO365,	RG323,	Ray et al.,

tillers/plant **-stress**	**n**			**RZ103**	**RG329, RZ740, RG788, RG449**			**RZ516**		**RG136**			**RG211, RZ53, RG118**	**RG181, RG9**	**(1996)**
-control	**Bala/Azucena**	**1**	**C949**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Price *et al.*, (2000)**
-control	**IR64/Azucena**	**6**	**RG810, RG331**	**-**	**RZ448, RZ519,**	**RG449, RG788,**	**-**	**-**	**-**	**RZ143, RG20, A10K250, AG8Aro, AC5, RG418B**	**-**	**-**	**-**	**-**	**Hemamalini et al., (2000)**
-stress	**IR64/Azucena**	**5**	**-**	**RG171, RG157**	**RZ329, RG348**	**RG91, RG449**	**-**	**-**	**-**	**RZ617, RG978**	**RZ206, RZ422**	**-**	**-**	**-**	**Hemamalini *et al.*, (2000)**
-control	**Bala/Azucena**	**1**	**C949**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Price et al., (2000)**
-control	**CT9993/IR62266**	**4**	**G359, RG140, R2417, RM212**	**-**	**RG409, RG224**	**ME7-7, EMP3-1c**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Kanbar *et al.*, (2002)**
Dehydration tolerance **-stress**	**CO39/Morobereka n**	**5**	**RG109**	**-**	**RG96**	**-**	**-**	**-**	**CDO533, RG128**	**RG20, RG333**	**-**	**-**	**-**	**-**	**Lilley et al., (1996)**
Osmotic adjustment**-stress**	**CO39/Morobereka n**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**RG1**	**-**	**-**	**-**	**-**	**Lilley et al., (1996)**
-stress	**CT9993/IR62266**	**5**	**ME2-12, RG140**	**RM263, R3393**	**EM17_1, C63**	**-**	**-**	**-**	**-**	**G2132, R1394A**	**EM14-6, ME4-13**	**-**	**-**	**-**	**Zhang et al., (2001)**
-stress	**IR62266/IR60080**	**19**	**RM84, RM220, RM243, RG811, RM265, OSR2, RM259, RM315,**	**OSR9A, RG171**	**RG224, OSR5, CDO1395**	**C335, RG375**	**OSR35, RZ390, RM31, BCD738**	**-**	**RM234, RM11, OSR22, RZ989, CDO38**	**RM25, RG1, CDO116, RM34, RM284, RM210, RM80**	**-**	**C809, R716**	**CSU116**	**-**	**Robin *et al.*, (2003)**
Relative water content **–stress**	**CO39/Morobereka n**	**2**	**-**	**-**	**-**	**-**	**RG182, RG13**	**-**	**-**	**RG1**	**-**	**-**	**-**	**-**	**Lilley et al., (1996)**
-stress	**IR64/Azucena**	**11**	**RG146, RG345, RZ730, RZ801, RG810, RG331**	**-**	**RG104, RG348, RZ403, RG179**	**-**	**RZ649, RZ70**	**PRD10B, RG648, CDO544, RG653**	**-**	**-**	**RG451, RZ404**	**-**	**-**	**RG574, RZ816, SDH1, RG463**	**Courtois et al., (2000)**
-stress	**Bala/Azucena**	**8**	**RZ14, C949**	**-**	**RG191,**	**C734,**	**C43, C624**	**RZ516**	**-**	**G1073**	**R1687**	**G89d**	**-**	**-**	**Price et al., (2002)**
-stress	**CT9993/IR62266**	**2**	**RM212, C813**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**RM215, RG667**	**-**	**-**	**-**	**Babu et al., (2003)**
Total root weight **– stress**	**IR64/Azucena**	**23**	**RG381, RZ19, RG690, RG730, RZ801**	**-**	**-**	**-**	**RG403, RG13, CDO105, RZ649, RZ67, RZ70, RZ225**	**RG424, RG162, RG172, CDO544, RG653, AMY2A, RG433, CAT1**	**RG711, EST9, RZ337B, CDO497, CDO418, RZ978**	**-**	**G103, RZ206, RZ422**	**-**	**-**	**-**	**Yadav et al., (1997)**
Plant height **-control**	**IR64/Azucena**	**4**	**RZ430, RZ801,**	**-**	**RZ448, RZ337A,**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**RG958, CDO344**	**Hemamalini et al., (2000)**

					RZ519,										
-stress	IR64/Azucena	2	RZ430, RZ801,	-	RZ448, RZ337A, RZ519,	-	-	-	-	-	-	-	-	-	Hemamalini *et al.,* (2000)
-stress	CT9993/IR62266	4	RG109, ME10-14,	RG437, ME10-18	-	RG416, RG214	-	-	RG528, RG769	-	-	-	-	-	Babu et al., (2003)
-stress	Caiapo/*O. rufipogan*	6	RZ462, RZ613, RZ513, RM104, RZ801	RG256b, RM207, RM266, RM207	-	RG169, CDO244, RZ740	CDO202, RZ925	-	-	-	-	-	-	-	Moncada et al,. 2001
-stress	IR64/Azucena	1	RZ801, RG810	-	-	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
-control	CT9993/IR62266	6	EM11-11, RG109, ME10-14,	RG437, ME10-18	-	RG476, RG214	-	-	RG404, CDO38	-	-	-	-	-	Babu et al., (2003)
-control	IR64/Azucena	1	RM810, RG348	-	-	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
-control	CT9993/IR62266	7	RG109, ME10-14	ME2-7, EMP2-7	-	-	-	-	-	C1121, ME5-3, ME5-7, EM15-10	ME9-6, K985, RM242	RG257, EMP2-9, ME5-16	-	ME7-9, ME10-8	Kanbar *et al..,* (2002)
Root volume -control	IR64/Azucena	3	-	-	-	-	RG403, RZ556, RZ67, RZ70	-	RZ337A, CDO497	-	-	-	-	-	Hemamalini et al., (2000)
-stress	IR64/Azucena	2	-	RG171, RG157,	RG104, RG348	-	-	-	-	-	-	-	-	-	Hemamalini *et al.,* (2000)
-stress	IR64/Azucena	1	-	-	RM231, RZ329	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
-control	Bala/Azucena	1	-	-	-	-	-	-	-	-	-	-	-	RG181	Price et al., (1997)
Leaf drying /Drought score -stress	IR64/Azucena	2	-	-	-	RG908, RG91	-	-	-	-	-	-	-	RG181, RG958	Hemamalini et al., (2000)
-stress	IR64/Azucena	10	RG146, RG345, RG810, RG331	-	-	RG908, RG91, RG143, RG620	RZ67, RZ70	CDO544, RG653	CDO418, RZ978	-	-	G2155, RG134	RG1094, RG167	SDH1, RG463	Courtois et al., (2000)
-stress	Bala/Azucena	11	R117, R2635, C178	e18m43.8, C601,	R1618,	C513,	RG119		-	C39, G338	G1073	-	-	e12m36.2	Price et al., (2002)
Relative growth rate -stress	IR64/Azucena	10	RG146, RG345, RG810, RG331	RG654, RG256	RG104, RG348, RZ284, PRD10A	-	RZ556, RG229	RG653, AMY2A	RG511, RG477, CDO38, RG351	-	RZ206, RZ422	-	-	AF6, RG457	Courtois et al., (2000)
Stomatal behaviour -control	Bala/Azucena	4	-	-	RG191, RG745	-	-	-	RG351	-	-	-	-	G24	Price et al., (1997a)
Cell membrane stability -stress	CT9993/IR62266	9	CDO345, ME10-14	-	EM11-2, RZ403,	-	-	-	EM17-3, ME2-15	G2132, R1394A, EM18-5, RG598	RZ698, RM219, ME9-6, K985	-	CDO365, ME6-7,	EM19-5, RG901	Tripathy et al., (2000)
Days to Heading -control	Bala/Azucena	3	-	-	C643	-	-	-	-	G1010	-	G1082	-	-	Price et al., (1997a)
-stress	CT9993/IR62266	3	-	-	R2170, RZ672,	-	-	-	-	ME9-1, ME2-1,	RG667, RM201	-	-	-	Babu et a.l., (2003)
-stress	Caiapo/*O. rufipogan*	4	-	RM266, RM207	RG104, RZ329,	-	-	-	RG30, RM125	-	-	-	-	-	Moncada et al., (2001)

					RZ576, RZ22										
Days to heading –control	CT9993/IR62266	4	-	-	RG104, EM11-9, R2170, RZ672	-	-	-	-	G2132, G1073	RM215, RG667	-	-	-	Babu et al., (2003)
-control	IR64/Azucena	1	-	-	RG104, RG348	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
Days to heading -mean	IR64/Azucena	1	-	-	RM231, RZ329	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
Days to maturity -mean	IR64/Azucena	1	-	-	RG104, RG348	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
Canopy temperature -stress	CT9993/IR62266	1	-	ME9-7, K706	-	-	-	-	-	-	-	-	-	-	Babu et al., (2003)
Panicles per plant -stress	Caiapo/*O. rufipogan*	2	-	-	-	-	-	RM3, CDO078	-	-	-	-	RZ537, RZ900	-	Moncada et al., (2001)
Percentage of sterility -stress	Caiapo/*O. rufipogan*	2	-	-	-	-	-	-	-	-	-	CDO98, RM304, RM147, RZ500	-	-	Moncada et al., (2001)
Grain yield -stress	CT9993/IR62266	5	EM18-10, L1087, RG811, ME2-16	-	-	RG476, RG939	-	-	-	-	-	EMP2-9, ME5-16	-	EM14-2, EM19-5	Babu et al., (2003)
-stress	Caiapo/*O. rufipogan*	2	RZ513, RZ613	-	-	-	-	-	-	-	-	-	RZ537, RZ900	-	Moncada et al., (2001)
Grain yield -control	IR64/Azucena	1	-	-	RZ329, RZ892	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
Grains per panicle -stress	CT9993/IR62266	1	-	-	-	RG476, RG939	-	-	-	-	-	-	-	-	Babu et al., (2003)
Grains per plant - stress	Caiapo/*O. rufipogan*	4	RZ513, RZ613	RG256b, RM207	-	-	-	*Waxy,* RZ1002	-	-	-	-	RZ537, RZ900	-	Moncada et al., (2001)
Grains per panicle -control	CT9993/IR62266	2	EM11-11, RG109	-	RG104, EM11-9	-	-	-	-	-	-	-	-	-	Babu et al., (2003)
1000 grains weight -stress	Caiapo/*O. rufipogan*	5	RZ613, RZ513, RG462, RZ613	-	RZ996, RM227	-	-	-	-	-	-	-	RZ537, RZ900, RM254, RM224	-	Moncada et al., (2001)
Harvest index -stress	CT9993/IR62266	1	-	-	-	-	-	-	-	G187, ME2-11	-	-	-	-	Babu et al., (2003)
Harvest index -control	CT9993/IR62266	2	RZ543, RG1028	-	-	-	-	-	-	-	-	-	G257, ME2-6	-	Babu et al., (2003)
Harvest index - (control & mean)	IR64/Azucena	1	-	-	RZ329, RZ892	-	-	-	-	-	-	-	-	-	Venuprasad *et al.,* (2002)
Relative yield	CT9993/IR62266	2	-	EM11-10, EM18-13	-	-	-	-	-	-	-	-	EM18-8, G320	-	Babu et al., (2003)

* stress- QTL identified under water stress conditions; control- QTLs identified under well watered conditions; mean- average over both experiments.

CHAPTER III

MATERIALS AND METHODS

The experiments for the present study were conducted during 2000-2004 in the laboratories of Department of Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India and in the experimental fields of Paddy Breeding Station, Centre for Plant Breeding and Genetics, Wetland Farm, Centre for Soil and Crop Management Studies, TNAU, Coimbatore and Agricultural Research Station, TNAU, Paramakudi. The genetic map construction and QTL mapping were done in Plant Breeding, Genetics and Biochemistry Division, International Rice Research Institute, Manila, Philippines.

Plant materials

In this study, a cross involving IR20/Nootripathu was used to develop a recombinant inbred line (RIL) population for linkage map construction and QTL mapping of drought resistance traits. These two parental lines are well adapted to the rainfed target population of environments (TPE) and differ for a range of root related traits and drought resistance in the field. Nootripathu, an *indica* landrace adapted to rainfed upland, has a deep and thick root system (Babu *et al*., 2001) and has higher drought tolerance. On the other hand, IR20, a lowland *indica* ecotype, has shallow and thin root system (Babu *et al*., 2001) and is drought sensitive. A F_7 RIL population was developed by single seed descent from a cross involving IR20XNootripathu at Paddy Breeding Station, TNAU, Coimbatore, India (Figure 1).

Selection of RI lines

A total of 397 F_7 RI lines were forwarded to F_8 and data on plant height, days to 50% flowering, number of tillers, number of productive tillers, days to maturity and yield were collected. Using random numbers, a subset of 250 RI lines were selected and their frequency distributions for the measured traits were used to confirm their transgressive segregation and normal distribution. These 250 F_7 RI lines were used for genotyping and the same RI lines but at F_8 generation used for phenotypic evaluation.

Isolation of genomic DNA (Gawel and Jarret, 1991)

DNA was isolated from the parents and the 250 RI lines as below and the quantity and quality of the DNA was checked for polymerase chain reaction (PCR).

Materials

a. Leaf samples

2g of fresh leaf samples were collected from parents and 250 RI lines from field.

b. Cetyl Trimethyl Ammonium Bromide (CTAB) Extraction Buffer (100 mL):

CTAB	-	2% W/V
Tris HCl pH 8.0	-	100 mM
Sodium chloride	-	1.4 M
EDTA	-	20 mM

(Tris, NaCl and EDTA were autoclaved and 2% CTAB was added after autoclaving and buffer was preheated every time before it used).

c. Tris EDTA (TE) buffer:

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

This was dissolved and made upto 100 mL, autoclaved and stored at 4°C

d. Ice cold Isopropanol

e. Chloroform : Isoamyl alcohol (24 : 1 V/V)

f. Sodium acetate (3.0 M) pH 5.2 (pH adjusted using glacial acetic acid)

g. Ethanol (100% and 70%)

h. RNAase A -10 mg/mL; RNAase A dissolved in TE and boiled for 15 minutes at 100°C to destroy DNAase and stored at –20°C.

Extraction of genomic DNA

- 2 g of leaf bits were transferred into prechilled mortar, frozen using liquid nitrogen and ground to fine powder.
- The fine powder was allowed to thaw in the presence of 10 mL of pre-heated extraction buffer and incubated for 30-45 minutes at 65°C with occasional mixing.
- Equal volume of Chloroform: Isoamylalcohol mixture (24:1 V/V) was added and mixed by inversion for 1 hour.
- It was centrifuged at 10,000 rpm for 20 minutes at room temperature.
- The clear aqueous phase was transferred to a new sterile tube. Equal volume of ice cold Isopropanol was added and mixed gently by inversion and then kept in the freezer until DNA was precipitated out.
- Using blunt end tips the precipitated DNA was spooled out into an eppendorf tube.
- The spooled DNA was air dried after removing the supernatant by brief spin.
- 500 µl of TE was added to dissolve the DNA and then 10 µl of RNase was added and incubated at 37°C for 30 minutes.
- 500 µl of Chloroform: Isoamylalcohol mixture was added and centrifuged for 10 minutes.
- Aqueous phase was transferred to another eppendorf without disturbing the inner phase.
- 2.5 volume of absolute Alcohol and 1/10 volume of Sodium acetate were added and kept for overnight incubation.
- Then it was centrifuged and the supernatant was discarded. To this 500 µl of 70% and 100% ethanol was used subsequently to wash the DNA using centrifugation.
- Alcohol was discarded and DNA was air dried completely.
- Then the DNA pellet was dissolved in 150-250 µl of TE (depends on the pellet size) and stored at 4°C.

Quantification of DNA

Materials

a. 10X TNE (Tris Sodium EDTA) buffer:

Tris - 100 mM

EDTA - 10 mM

Sodium chloride - 2 mM

This was dissolved, pH adjusted to 7.4, made upto 100 mL, autoclaved and stored at 4°C.

b. Hoechst 33258 dye (1 mg in 1 mL sterile water)

c. Calf thymus standard DNA (1 mg in 1 mL sterile water)

d. Rice genomic DNA

e. Fluorometer (Model DYNA Quant 200, Hoefer, California, USA)

f. Assay Buffer (for high range i.e., >1000ng):

Hoechst dye - 100 µl

10X TNE buffer - 10 mL

Distilled water - 90 mL

Protocol

- The instrument was switched on 15 minutes prior to use for stabilization.
- 2 mL of assay buffer was taken in a clear quartz cuvette and calibrated to read 'O' (blank).
- 2 µl of calf thymus standard DNA was added to the blank and calibrated to 1000 mg per mL at 260 nm.
- 2 µl of unknown DNA sample was added to 2 mL of assay buffer.

- The quantity of DNA present in the sample was read as 'x' ng/µl at 260 nm.

Parental Genotyping

The parents were screened with different types of markers *viz*., Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Random Amplified Polymorphic DNA and PCR based Expressed Sequence Tags (ESTs) and SSRs derived from ESTs in order to construct a genetic linkage map.

Standardization of PCR reactions

1. The reagents and sterile water were divided into aliquots to minimize the number of sampling errors.
2. To avoid cross contamination, via the electrophoresis equipment, the gel combs and casting trays were washed using 3% acetic acid.
3. If there was any doubt about a critical result, the experiment was again repeated until to get an unambiguous result.
4. Various control strategies have been followed to carry out PCR reaction successfully.

Important controls used in the standardization of the PCR reactions were (Newton, 1995):

1. PCR in the absence of exogenously added DNA was used as negative control to check the DNA-free status of reagents and solutions.

2. PCR with positive control DNA was used to check the completeness of PCR mixture (to check the quantity of essential components including $MgCl_2$ in the cocktail mixture).

3. PCR with sufficient quantity of positive control DNA was used to amplify weak but consistent amplicons and to identify sensitivity and efficiency of PCR.

4. Negative and positive controls were used to check for spurious background bands and reaction specificity and to identify PCR parameters (includes annealing temperature and number of cycles) that were suitable for amplifying expected products.

Simple Sequence Repeats

Microsatellites or simple sequence repeats (SSRs) are small repeats of one or few tandemly arranged nucleotides spread throughout eukaryotic genomes. The technical efficiency and multiplex potential of SSRs makes them preferable for high throughput mapping, genetic analysis and marker aided selection. SSR markers are co-dominant, multi-allelic and can be readily used to analyze both *indica* and *japonica* germplasm and facilitates the integration of results from independent studies. In addition, the polymorphic nature of many microsatellites is of particular value when analyzing closely related genotypes, as is often the case in breeding programs working within narrowly adapted gene pools (McCouch *et al*., 2002). Microsatellite DNA markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellites (Weber and May 1989). In general microsatellites have been derived sequence information obtained from DNA libraries and published sequence data (Akagi *et al*., 1996). A total of 627 SSR markers have been used for parental genotyping in this study. The marker name, chromosome number, forward and reverse primer sequence of the each SSR marker is given in Table 3. This includes the 615 microsatellites that are available to the public (the name starts with RM) (Temnykh et al., 2000; McCouch et al., 2002) and twelve newly developed simple sequence repeats (the name starts with abbreviations of the candidate genes with a suffix SSR) based on the sequence data of candidate genes that were used in this study.

***In silica* data mining for SSR markers from EST seqeunce**

On the basis of annotated ESTs, noval molecular markers such as SSR can be developed. The full length gene sequences of the candidate genes (FASTA-formatted) used in this study were analyzed for the presence of SSR motif by using Perlscript, Simple Sequence Repeat Identification Tool (www.gramene.org). Microsatellites were considered to contain motifs that are between one and six nucleotides in size. Thereby the minimum length criteria were defined

as being ten repeat units for mononucleotides, six repeat units for dinucleotides and five repeat units for all higher order repeats (Thiel *et al*., 2003). After identification of the SSR motif, unique sequence of 100 bp flanking the SSR motif on either side was obtained from the full-length sequence.

Primer design

Once the SSR containing candidate genes identified, flanking primers of the each sequence were designed using PRIMER0.5 (www.genome.wi.mit.edu/ftp/pub/software/primer.0.5). To force the selection of flanking primers, the 'target' option was used representing the position of the respective microsatellite enlarged by three positions at each side. Besides, other parameters such as primers of 18-24 bp long, devoid of secondary structure or consecutive tracts of a single nucleotide, GC content of around 50%, melting temperature (Tm) of 60°C and preferably G- or C- rich at the 3' end have also been used. Thus, primers were designed defining loci ranging from 100 to 300 bp. The list of twelve SSR primers developed from ESTs, chromosome number and forward and reverse primers is given in Table 3 (with a suffix SSR).

PCR amplification

The cocktail for PCR amplification of respective SSR fragments was prepared as follows.

Reaction mixture (15µl) contains:

Stock		Aliquot	Final concentration
DNA (50ng)	-	1.0 µl	50.0 ng
dNTPs (2.5mM)	-	0.6 µl	100.0 µM
Forward Primer (20µM)	-	0.15 µl	0.2 µM
Reverse Primer (20µM)	-	0.15 µl	0.2 µM
Taq DNA polymerase (3 units/µl)	-	0.1 µl	0.02units
Buffer (10X)	-	1.5 µl	1X

Sterile distilled water - 11.5 µl

Total - 15.0 µl

The reaction mixture was given a momentary spin for thorough mixing of the cocktail components. Then 0.2mL PCR tubes were loaded in a thermal cycler.

The thermal cycler is programmed as follows

Profile 1: 94°C for 5 min - Initial denaturation

Profile 2: 94°C for 45 sec - Denaturation

Profile 3: 55°C for 45 sec - Annealing

Profile 4: 72°C for 1 min - Extension

Profile 5: 72°C for 5 min - Final extension

Profile 6: 4°C for infinity to hold the sample.

Profile 2, 3 and 4 were programmed to run for 35 cycles.

Electrophoretic analysis

After PCR amplification, products were separated by electrophoresis on metaphor agarose gels and visualized by ethidium bromide staining. Usually, for better resolution and detection of smaller differences in amplified products, polyacrylamide gel and silver staining is preferred. Besides saving cost and time, 3% Metaphor agarose gels can achieve similar efficiency in resolution of amplified products. Initially the parental polymorphism was established in 6% polyacrylamide gel and then it was used in 3% Metaphor agarose gel electrophoresis.

PolyAcrylamide Gel Electrophoresis (PAGE)

Materials

a. 40% Acrylamide (19:1):

Acrylamide 38g

Bisacrylamide 2g

(Dissolved in 50 mL milli Q water and the volume made upto 100 mL, and stored in brown bottle)

b. 5% polyacrylamide denaturating stock solution:

Urea 272.0 g

40% Acrylamide 70.3 mL

Milli Q water 200.0 mL

(Stirred with low heat until urea dissolves, filtered through 0.22μm cellulose acetate filter paper and 28.1 mL of 10X TBE buffer was added and then made up to 500 mL with milli Q water).

c. 10X TBE (Tris Borate EDTA) buffer:

Tris base 107.8 g

Boric acid 55.03 g

EDTA ($Na_2.2H_2O$) 8.19 g

(Dissolved in 800 mL milli Q water filtered through 0.22μm filter paper and made up to 1000 mL and stored at 4°C).

d. 10 % Ammonium persulphate:

Ammonium persulphate 0.1 g

Sterile water 1.0 mL

(It was prepared freshly at every time).

e. Bind silane:

(For Silanizing solution of 500 mL)

Ethanol 497.5 mL

Glacial Acetic Acid 2.5 mL

Bind silane 0.75 µl

f. Fixer:

10 % Acetic Acid

(2 liter has been prepared and kept in dark place in a brown bottle)

g. Staining solution:

Silver nitrate 2 g

Formaldehyde 3 mL

Milli Q water 2 Lt.

(Kept in dark place in a brown bottle. Solution was reused upto six times if there was no contamination).

h. Developing solution:

Sodium carbonate 60 g

Milli Q water 2 Lt.

(Pre chilled at 4°C and immediately prior to use 40 µl of Sodium thiosulphate and 3mL Formaldehyde were added)

i. Mannual sequencing loading buffer:

Formamide 10 mL

Xylene cyanol FF 10 mg

Bromo Phenol blue 10 mg

0.5M EDTA 200 µl

PAGE gel casting

Plate preparation

- The large and small glass plates were soaked in 2% NaOH solution overnight.
- Then the plates were cleaned using tap water and distilled water using scrubber.
- 3 mL of racin or repellent was applied on large plate and cleaned with kim wipes and again cleaned with distilled water and absolute alcohol.
- 3 mL of Bind silane was applied on small plate and cleaned with kim wipes and again cleaned with distilled water.
- Both the plates were again wiped with absolute alcohol.
- Vaseline was applied to both the sides of the spacers.
- The spacers were placed with rubber adapter on either sides of the large plate and the small plate was placed on top of the large plate in such a way that it was seated uniformly on the edges and sides.
- Then the plates were clamped and the edges were sealed with cello tape.

Gel matrix preparation

- 70 mL of 6 % polyacrylamide denaturation solution was taken in a conical flask and 300 µl of 10 % Ammonium persulpate solution and 30 µl of TEMED were added and mixed well.
- The plates were kept in slanting position in such a way that the gel matrix flows freely into the plates and air bubbles comes out freely.

- The solution was poured into the plates with the help of 10 mL syringe. After the matrix spreaded uniformly throughout the plate, the comb was placed and the plates were clamped on the top.

Sample loading and gel running

- The gel was allowed to polymerize for 3 hours.
- After removing the comb, the gel setup was mounted on a Hoefer (USA) electrophoresis apparatus.
- After flushing the wells with running buffer (0.5X TBE), the gel was pre-run for 45 minutes.
- Samples were prepared by adding 5 µl of PCR reaction mix with 2 µl of loading dye and denaturing at 95°C for 5 minutes and snap cooling on ice.
- After flushing the wells again, the denatured DNA samples were loaded onto gel.
- The electrophoresis was resumed and allowed to proceed at 40 watts (constant) till Bromophenol blue reached the bottom of the gel.

Staining of Gel

After careful removal of the small plate with gel from the assembly, the gel with plate has been given the following washing treatments with various solutions.

- Fixer for 15 minutes or till the dye disappears.
- Double distilled water for 5 minutes (twice).
- Staining solution for 15 minutes.
- Double distilled water for 10 seconds.

- Developer for 3 minutes or till band appears.
- Acetic acid for 2-3 minutes.
- Double distilled water for 2 minutes.
- Sodium hydroxide for 10 minutes.
- Double distilled water for 3 minutes.
- Then the gel was carefully air dried and documented.

Metaphor agarose gel electrophoresis

Metaphor agarose (BioWhittaker Molecular Applications, Vallensbaek Strand, Denmark) is a high resolution agarose that can resolve PCR products and small DNA fragments that differ in size by 2%. It has an intermediate melting temperature (75°C) with twice the resolution capabilities of the finest-sieving agarose products. It was suggested by the manufacturer that 3% Metaphor agarose in 1X TBE buffer is sufficient enough to resolve 50-250 bp DNA fragments.

Materials

a. Loading dye:

Glycerol	50% (V/V)
Bromophenol blue	0.5% (W/V)

b. 10X TBE (Tris Borate EDTA) buffer:

Tris base	107.8 g
Boric acid	55.03 g
EDTA ($Na_2.2H_2O$)	8.19 g

(Dissolved in 800 mL milli Q water filtered through 0.22μm filter paper and made up to 1000 mL and stored at 4°C).

The following steps were followed for gel casting.

- A beaker with two to four times the volume of the gel solution was chosen and 1X prechilled TBE buffer was added.
- The solution was stirred with constant speed and the Metaphor agarose powder was sprinkled slowly and mixed well with Teflon coated magnetic stir bar.
- Metaphor agarose was soaked in the buffer for 15 minutes before heating (This reduces the tendency of the agarose solution to foam during heating).
- The solution level was marked in the beaker and then heated in a microwave oven on medium power for 3 minutes.
- The beaker was removed from microwave oven and gently swirled to resuspend any settled powder and gel pieces.
- The solution was reheated on high power for 2 minutes or until all of the particles are dissolved.
- The beaker was removed and gently swirled and sufficient hot distilled water is added to get the initial volume and mixed thoroughly.
- The solution was allowed to cool to 50-60°C after adding Ethidium bromide (1µg/ml) and then casted on a clean template.
- The molten Metaphor agarose was allowed to cool in room temperature for 15 minutes and then placed at 4°C for 15 minutes to obtain optimum resolution and gel handling characteristics.
- 7µl of PCR products and 3µl of agarose gel loading dye was used to load the samples into the well and the gel was run @ 5V/cm of the gel size.

- Then the ethidium bromide stained gel was documented (AlphaImager™ 1200, Alpha Innotech Corporation, California, USA). The gel was reused for four times or until it gives poor quality in documentation.

Inter Simple Sequence Repeats

Inter Simple Sequence Repeats (ISSR) analysis involves the PCR amplification of regions between adjacent, inversely oriented microsatellites using a SSR containing primer (Zeitkiewicz *et al*., 1994). This technique can be undertaken for any species that contains a sufficient number and distribution of SSR motifs and has the advantage that genomic sequence data is not required (Goodwin *et al*., 1997). The primer used in ISSR analysis can be based on any of the SSR motifs (di-, tri-, tetra- or penta-nucleotides) found at microsatellite loci, giving a wide array of possible amplification products and can be anchored to genomic sequences flanking either side of the targeted SSRs. For ISSR analysis to be successful, pairs of SSR must occur within a short distance (in base pairs) that is amplifiable by a PCR reaction, which produces a band that is resolvable on standard polyacrylamide or agarose gels (Zeitkiewicz *et al*., 1994). ISSR primers used in this study and their sequence are given Table 4. A single primer was used in each PCR reaction, which was carried out in a total volume of 15 µl reaction containing the following components.

Stock	Aliquot	Final concentration
DNA 50 ng/µl	1.00 µl	50.00 ng
dNTPs (2.5 mM)	1.20 µl	200.00 µM
Primer (20 µM)	0.50 µl	0.60 µM
10X assay buffer	1.50 µl	1X

Taq polymerase (3 units)	0.18 μl	0.036units
Magnesium Chloride (2mM)	0.20 μl	26.6 μM
Sterile distilled H_20	10.42 μl	
Total	15.00 μl	

The reaction mixture was given a momentary spin for thorough mixing of the cocktail components. Then 0.2mL PCR tubes were loaded in a thermal cycler.

The thermal cycler is programmed as follows

Profile 1: 94°C for 5 min - Initial denaturation

Profile 2: 94°C for 1 min - Denaturation

Profile 3: depends on primer Tm for 1 min - Annealing

Profile 4: 72°C for 2 min - Extension

Profile 5: 72°C for 10 min - Final extension

Profile 6: 4°C for infinity to hold the sample.

Profile 2, 3 and 4 were programmed to run for 39 cycles.

Agarose gel electrophoresis

The PCR products were analyzed using 2% agarose gel electrophoresis in 1X TBE buffer, stained with ethidium bromide and documented.

Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD markers (Williams *et al*., 1990) are PCR-based markers and are generated using arbitrary primers. The Operon (Operon Technologies Inc., California, USA) 10-base primer kits, which have used in this study (Table 5), have a (G+C) content of 60 to 70% and they have no self-

complimentary ends. A single 10bp oligonucleotide primer is used to amplify genomic DNA. DNA amplification product is generated for each genomic region that happens to be flanked by a pair of 10bp sites (in the appropriate orientation), which are within 5000 bp of each other. Amplification products were analyzed by electrophoresis. Genomic DNA from two different individuals often produces different amplification fragment patterns. A particular DNA fragment which is generated for one individual but not for another represents a DNA polymorphism and can be used as a genetic marker. These markers are inherited in a Mendalian fashion (Williams *et al*., 1990) and segregation of these markers among the progeny of a sexual cross can be used to construct a genetic map. The cocktail for the DNA amplification was prepared as follows (slightly modified from Williams *et al*., 1990).

Stock	Aliquot	Final concentration
DNA 50 ng/μl	1.00 μl	50.00 ng
dNTPs (2.5 mM)	1.20 μl	200.00 μM
Primer (20 μM)	0.50 μl	0.60 μM
10X assay buffer	1.50 μl	1X
Taq polymerase (3 units)	0.18 μl	0.036units
Magnesium Chloride (2mM)	0.20 μl	26.6 μM
Sterile distilled H_20	10.42 μl	
Total	15.00 μl	

The reaction mixture was given a momentary spin for thorough mixing of the cocktail components. Then the 0.2 mL PCR tubes were loaded on to a thermal cycler.

The thermal cycler (PTC-100^{TM}, MJ Research Inc, USA) is programmed as follows:

Profile 1: 94°C for 2 min – Initial denaturation

Profile 2: 94°C for 1 min – Denaturation

Profile 3: 37°C for 1 min – Annealing

Profile 4: 72°C for 1 min – Extension

Profile 5: 72°C for 5 min – Final extension

Profile 6: 4°C for infinity to hold the sample

Profile 2, 3 and 4 were programmed to run for 35 cycles.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to separate amplification products.

Materials

a. Loading dye:

Glycerol 50% (V/V)

Bromophenol blue 0.5% (W/V)

b. 10X TBE (Tris Borate EDTA) buffer:

Tris base 107.8 g

Boric acid 55.03 g

EDTA ($Na_2.2H_2O$) 8.19 g

(Dissolved in 800 mL milli Q water filtered through 0.22μm filter paper and made up to 1000 mL and stored at 4°C).

Protocol

- Open ends of the gel casting plate were sealed with cello tape and placed on a perfectly horizontal leveled platform.
- 2% Agarose was added to 1X TBE and boiled till the agarose dissolved completely and then cooled to 50-60°C. Ethidium bromide was used as a staining agent at the final concentration of 1μg/mL.
- Agarose gel was poured into the gel-casting tray; the comb was placed properly and allowed to solidify.
- After solidification of the agarose, the comb and cello tape were removed.
- DNA samples (10μl) were mixed well with 2.5μl of agarose gel loading dye and were loaded into the gel wells. 100 bp ladder (Bangalore Genei Pvt. Ltd., Bangalore**)** was also added in one well as standard markers.
- The gel was run at 5 V/cm for 4-5 hours and bands were visualized and documented in gel documentation system (AlphaImager™ 1200, Alpha Innotech Corporation, California, USA).

Candidate genes/ Expressed Sequence Tags (ESTs)

The candidate gene approach has been applied in plant genetics in the past decade for the characterization and cloning of Mendelian trait loci and quantitative trait loci (QTLs). The idea is to propose a linkage between previously sequenced genes of known function and major QTL and thus giving a biological meaning to markers. The candidate genes may be structural genes or genes involved in the regulation of a metabolic pathway. The working hypothesis assumes that a molecular polymorphism within the candidate genes is related to phenotypic variation (Pflieger *et*

al., 2001). Candidate genes for several genes associated with abiotic stresses have been identified. ESTs, a single pass, partial sequences from cDNA clones, has become an extensively used strategy for candidate gene discovery and mapping in a wide range of organisms. Reddy *et al*., (2002) reported large scale EST development from cDNA libraries constructed from drought stressed leaf and root tissues of an upland *O. sativa* subsp. *indica* cultivar Nagina 22. ESTs were screened against the current GenBank database using the BLAST algorithm and putative functions of the ESTs were assigned after applying a stringency level of *E* value. ESTs were selected from Reddy *et al.,* (2002) and initial parental genomic survey was done with selected ESTs in this study, to resolve to what extent the allelic variation exists in these genes, which affect drought tolerance in rice. The ESTs were selected in such a way that they have shown matches with recognized drought responsive/resistance candidate genes in rice with *E* value of 10^0. Full length sequences of candidate genes were obtained from NCBI data base. Based on the sequence information, the gene specific primers (Forward and Reverse primers) were constructed using PRIMER0.5 software (http://www.genome.wi.mit.edu/ftp/pub/software/primer.0.5). The following global parameters were used to design the primers.

Maximum mispriming : 12

Maximum primer size : 20

Minimum primer size : 18

Maximum melting temperature : 63

Minimum melting temperature : 57

Minimum GC content : 30

Maximum GC content : 80

Maximum N's accepted : 0

Maximum 3' complementarity : 3.0

The list of candidate genes and their size (bp) and chromosome number used in this study are given in Table 6. The primers were synthesized (Microsynth GmbH, Switzerland) and used to screen the parents. The dilution of the primers was done according to the manufacturer's instructions. Initially Gradient PCR was used to standardize the annealing temperature for different primers used in this study. At the annealing temperature range of 55-58°C, all the primers could able to amplify specific genomic regions. However, some of the primers have also amplified non-specific or stutter bands, which may be due to slippage of *Taq* polymerase during amplification processes or due to poor PCR conditions and so those primers were not included in the study. The PCR cocktail used for candidate gene amplification is as follows:

Stock	Aliquot	Final concentration
DNA (50ng)	- 1.0 µl	50.0 ng
dNTPs (2.5mM)	- 0.6 µl	100.0 µM
Forward Primer (20µM)	- 0.15 µl	0.2 µM
Reverse Primer (20µM)	- 0.15 µl	0.2 µM
Taq DNA polymerase (3 units/µl)	- 0.1 µl	0.02units
Buffer (10X)	- 1.5 µl	1X
Sterile distilled water	***- 11.5 µl***	
Total	- 15.0 µl	

The reaction mixture was given a momentary spin for thorough mixing of the cocktail components. Then 0.2mL PCR tubes were loaded in a thermal cycler.

The thermal cycler is programmed as follows

Profile 1: 94°C for 5 min	- Initial denaturation
Profile 2: 94°C for 1 min	- Denaturation
Profile 3: 55°C for 1.5 min	- Annealing

Profile 4: 72°C for 1 min - Extension

Profile 5: 72°C for 5 min - Final extension

Profile 6: 4°C for infinity to hold the sample.

Profile 2, 3 and 4 were programmed to run for 35 cycles.

Agarose gel electrophoresis

The PCR products were run on 2% agarose gel since the product size was more than 500bp and the amplified products were scored for parental polymorphism. A 100bp marker was used to know the approximate fragment size of the PCR product. The non-specific fragments, though it generated repeatedly, were omitted since they lead to false polymorphic bands.

Segregation of polymorphic markers in RI lines

All the polymorphic primers found between IR20/Nootripathu were screened for their segregation in the 250 RI lines. RAPD and ISSR primers which have generated more than 2 polymorphic markers were distinguished by molecular size of the marker. A genotypic scoring was made for all the RI lines based on the banding pattern of IR20 or Nootripathu and data sheet was prepared for MAPMAKER/EXP analysis. All the SSR, ISSR, RAPD and GSP markers were evaluated individually by the χ^2 test for goodness of fit against a 1:1 segregation ratio of IR20 and Nootripathu alleles at a 0.05 probability level. The markers, which have shown extreme distortion from the threshold level were eliminated from the map construction process.

Map construction

Map construction and single marker analysis of this study was done at Plant Breeding, Genetics and Biochemistry Division, International Rice Research Institute, Philippines. The

genetic linkage map was constructed using MAPMAKER/EXP MS-DOS 3.0 (Lander *et al*., 1987; Lincoln *et al*., 1992) software with the Haldane mapping function after all the heterozygote data had been entered as missing data. Initially, by using the SSR data, the linkage groups were determined using "group" command with a minimum LOD score of 8.0 and maximum distance of 50 cM. Different linkage groups belong to same chromosome were grouped again with low LOD score (3.0). The order of the linkage groups was determined using the "compare", "try" and "ripple" commands. Assignment of the linkage groups to the respective chromosomes was based on the rice SSR map developed by Temnykh *et al.,* (2001) and McCouch *et al.,* (2002). Then the "group" command was employed to group the other types of markers and to identify the linkage group to which each RAPD, ISSR and EST markers were belonged. The "order" and "ripple" commands were used to order all the markers on each linkage group. If the linkage groups contained too few markers for the "order" command, the "compare" and "ripple" commands were used. Some markers could not be uniquely placed by the "order" command and were excluded from the map. Similarly, the markers, which had considerably lengthened map when included, have also been excluded. "Make chromosome" and "sequence" commands were used to assign each linkage group to the respective chromosome numbers and "map" and "draw map" were used to construct the linkage maps of the chromosomes.

Field trials

Field trials were conducted under upland conditions in the experimental fields of Tamil Nadu Agricultural University, India at two different locations: Wetland Farm, TNAU, Coimbatore – Managed Stress Environment (MSE; Trial 1) during dry season (February-June, 2003) and Agricultural Research Station, Paramakudi – Target Population of Environment (TPE; Trial 2) during wet season (September-February, 2003-2004) for QTL mapping of drought

resistance traits. The site, soil and drought stress characteristics of the two trials are summarized in Table 7.

Managed Stress Environment: Coimbatore (Trial 1)

A subset of 236 RI lines along with the parents were evaluated in a randomized block design in 2.0 X 0.2 m size plots under irrigated (non-stress) control and water stress treatments in Trial 1. Both the treatments were replicated three times. Hand sowing of seeds (@100 kg/ha) with 20 X 10 cm spacing (between and within rows) was done in dry soil. A buffer channel of 1.0 m wide and 0.75 m deep along the length of the experimental plot divided the control and stress plots. All the plots were surface irrigated to field capacity once a week, except when water stress was imposed by withholding irrigation to stress plots from 60 days after sowing (DAS). NPK fertilizers were applied at the rate of 120:40:40 kg/ha. Insect and weed control measures were applied periodically as required.

Field measurements

In Trial 1, during the initial ten days of the stress period, there were intermittent rains and after that there was a continuous rain free days for 18 days. Changes in soil moisture and penetration resistance were monitored periodically in stress plots with a Thetaprobe and a penetrometer, respectively. Leaf rolling and drying scores were taken at mid day, 15 days after last rainfall during the stress period. Leaf relative water content (RWC) was determined at mid day, 16 days after last rainfall and canopy temperature was taken 17 days after last rainfall. Stress was relieved 18 days after last rainfall and recovery score was made 3 days after rewatering. Following this both control and stress plots were regularly irrigated until harvest. The plants were harvested 120 DAS and total above ground biomass was recorded.

Target Production Environment: Paramakudi (Trial 2)

All the 250 RI lines along with the parents were evaluated in a randomized block design in 2.0 X 0.2 m size plots under irrigated (non-stress) control and water stress treatments in Trial 2. Both the treatments were replicated three times. Hand sowing of seeds (@100 kg/ha) with 20 X 10 cm spacing (between and within rows) was done in dry soil. A 3.5 m width of bund along

the length of the experimental plots divided the control and stress plots. The control plots received tanker irrigation

for three times in one-week interval after panicle initiation while the stress plots were rainfed from sowing to harvest. NPK fertilizers were applied at the rate of 50:25:25 kg/ha. While P and FYM (at the rate of 12.5 tons/ha) were applied in full as basal dose during sowing, N and K were applied in two splits as top dressing. Paddy micronutrient mixture was applied at the rate of 12.5 kg/ha at 39 DAS. Insect and weed control measures were applied periodically as required.

Field measurements

In Trial 2, leaf rolling and leaf drying scores were made at midday, 14 days after cessation of rain along with canopy temperature and chlorophyll reading using SPAD chlrophyll meter (SPAD-502, Minolta Camera Co., USA). Plants were harvested at maturity, 120 DAS. Data on plant height, biomass, number of tillers and days to 50% flowering were recorded.

Leaf rolling and drying

The extent of leaf rolling was determined at mid day using 1 to 7 scale standardized for rice (IRRI, 1996), where smaller number indicates full turgidity and bigger number indicates complete rolling. Similarly, leaf drying score was made using 1 to 7 scale standardized for rice (IRRI, 1996), where smaller number indicates green leaf and bigger number indicates complete drying.

Relative water content (RWC) (Barrs and Weatherly, 1962)

A subset of 129 RI lines was used for RWC measurement in Trial 1. A section of, about 7 cm long, youngest fully expanded leaf was cut into a clean, dry, preweighed and numbered glass vial and capped airtight. While cutting the leaf into the vial, care was taken in such a way that the basal portion of the leaf was towards the bottom of the vial. The leaf samples were brought to the laboratory and the fresh weight (FW) of the leaf was recorded along with the capped-vial using

an electronic analytical balance having a readability of 0.0001g. The leaf was allowed to rehydrate for 4 hr by adding 2 mL of distilled water along the sides of the vial using a pipette. The rehydrated leaf was taken out of the vial using clean forceps, gently blotted to remove external moisture using Kim wipe paper and weighed for turgid weight (TW). Since the detached rice leaves lose water rapidly, care was taken to avoid delay in measuring TW. The water in the vial was poured off and the leaf sample was transferred to the vial and left uncapped. The leaf samples were then oven-dried at 80°C for 24 hr along with the uncapped vial. After oven drying the vial was allowed to stand for ten minutes at room temperature and the dry leaf weight (DW) was recorded. Leaf RWC was calculated as,

$$RWC = \frac{(FW-DW)}{(TW-DW)}$$

Where, FW, TW and DW are fresh, turgid and oven dry weights of the leaf sample, respectively and expressed in percentage.

Canopy temperature

Canopy temperature was measured for 129 RI lines (the same lines used for measuring RWC) in Trial 1 and all the RI lines in Trial 2 with a Telatemp infrared thermometer (Model AG-42, Telatemp Corporation, Fullerton, CA, USA.) with an 8° field of view equipped with a 10.5 – 12.5 µm band pass filter. The infrared thermometer was held so that the sensor viewed only the canopy from a distance of 1 m at an oblique angle of about 20° above the horizontal: this position and distance gave an elliptical canopy target that was 0.42 m long and 0.14 m wide with an area of 0.05 m^2 and prevented the thermometer sensing the soil surface when the leaves were rolled (Garrity and O'Toole, 1995). All canopy temperature measurements were made in a south-facing direction and within 2hr of solar noon, thus minimizing sun angle effects.

Drought recovery score

Visual scoring for drought recovery were made based on 0 to 7 scale, where the smaller number indicates more drought tolerance or greater recovery ability and high score indicates

completely drought susceptible or completely unrecovered. Drought recovery was assessed 3 days after rewatering.

Basal root thickness

Roots were cut from the shoot-root joint in Trial 1 and basal root thickness (mm) was measured at 2 mm from the base of the shoot. Three roots were selected in a single plant and the same was followed for all the rice accessions grown under stress. Root thickness was measured using digital micrometer.

Biomass

The total above ground biomass of the accessions was determined by sampling all plants in the plot in paper covers and properly sun dried. It was weighed in an electronic balance and expressed in g/m^2.

Statistical analysis of the phenotypic data

Analysis of variance was done using the general linear model (GLM) procedure of the SAS program (SAS Institute Inc., 1990) to check the genetic variance among the RI lines for all traits. Since there was no significant difference in between control and water stress treatments, the data on plant height, number of tillers and biomass has been considered as stress data and thus ultimately all the six replications were used for mean and covariance analysis. The data obtained from all the traits were standardized before the statistical analysis. The frequency distributions of all the traits were performed to test the skenewss of the traits towards the parents. From the covariance values, the broad sense heritability or repeatability (*H)* were calculated for each trait by using the following formula,

$$H = \sigma^2_G / (\sigma^2_G + \sigma^2_e/k)$$

Where σ^2_G and σ^2_e were the genetic and residual variances, respectively and 'k' was the number of replications. Phenotypic correlations among the traits within the trial were computed using the mean trait value.

Single marker analysis

Single marker analysis was conducted to test the marker trait linkage by simple ANOVA using the PROC GLM on SAS software (SAS Institute Inc, 1990). All the marker data and the standardized mean traits value for 250 RI lines were used for calculating two marker classes (IR20 and Nootripathu homozygote) and their variances. The significant threshold for identification of a QTL was set at $Pr < 0.02$ for single marker analysis. The R^2 value was used as per cent of variance explained by the marker on the particular trait of test.

Table 3. List of microsatellite markers and sequence information of forward and reverse primers used in this study.

S. No	Primer name	Forward primer	Reverse primer
1	RM1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
2	RM4	TTGACGAGGTCAGCACTGAC	AGGGTGTATCCGACTCATCG
3	RM5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
4	RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
5	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
6	RM14	CCGAGGAGAGGAGTTCGAC	GTGCCAATTTCCTCGAAAAA
7	RM16	CGCTAGGGCAGCATCTAAAA	AACACAGCAGGTACGCGC
8	RM17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
9	RM18	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC
10	RM19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
11	RM20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
12	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
13	RM22	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTTCACTCGTC
14	RM23	CATTGGAGTGGAGGCTGG	GTCAGGCTTCTGCCATTCTC
15	RM24	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
16	RM29	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG
17	RM35	TGGTTAATCGATCGGTCGCC	CGACGGCAGATATACACGG
18	RM36	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTACC
19	RM39	GCCTCTCTCGTCTCCTTCCT	AATTCAAACTGCGGTGGC
20	RM42	ATCCTACCGCTGACCATGAG	TTTGGTCTACGTGGCGTACA
21	RM44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
22	RM50	ACTGTACCGGTCGAAGACG	AAATTCCACGTCAGCCTCC
23	RM55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
24	RM60	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC
25	RM70	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC
26	RM71	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG
27	RM80	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
28	RM81	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
29	RM83	ACTCGATGACAAGTTGAGG	CACCTAGACACGATCGAG
30	RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
31	RM101	GTGAATGGTCAAGTGACTTAGGTC	ACACAACATGTTCCCTCCCATGC
32	RM104	GGAAGAGGAGAGAAAGATGTGTG	TCAACAGACACACCGCCACCGC
33	RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
34	RM107	AGATCGAAGCATCGCGCCCGAG	ACTGCGTCCTCTGGGTTCCCGG
35	RM109	GCCGCCGGAGAGGGAGAGAGAG	CCCCGACGGGATCTCCATCGTC
36	RM124	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC
37	RM127	GTGGGATAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG
38	RM129	TCTCTCCGGAGCCAAGGCGAGG	CGAGCCACGACGCGATGTACCC
39	RM131	TCCTCCCTCCCTTCGCCCACTG	CGATGTTCGCCATGGCTGCTCC
40	RM138	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG
41	RM145	CCGGTAGGCGCCCTGCAGTTTC	CAAGGACCCCATCCTCGGCGTC
42	RM148	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG
43	RM149	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG
44	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
45	RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
46	RM160	AGCTAGCAGCTATAGCTTAGCTGG	TCTCATCGCCATGCGAGGCCTC
47	RM164	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
48	RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC

49	RM170	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC
50	RM176	CGGCTCCCGCTACGACGTCTCC	AGCGATGCGCTGGAAGAGGTGC
51	RM190	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG
52	RM191	CCCATCCTCACCGATCTCTCTAAAC	GTGCGCACGGAGGAGGAAAGGG
53	RM200	CGCTAGGGAATTTGGATTGA	CGATGAGCAGGTATCGATGAGAAG
54	RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
55	RM203	CCTATCCCATTAGCCAAACATTGC	GACGCCAACCTGGAGTTAATTACC
56	RM204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC
57	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
58	RM207	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC
59	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
60	RM209	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
61	RM210	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG
62	RM211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG
63	RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
64	RM213	ATCTGTTTGCAGGGGACAAG	AGGTCTAGACGATGTCGTGA
65	RM214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA
66	RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
67	RM216	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
68	RM217	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
69	RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
70	RM220	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT
71	RM221	ACATGTCAGCATGCCACATC	TGCAAGAATCTGACCCGG
72	RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
73	RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
74	RM226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
75	RM230	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG
76	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
77	RM233	CCAAATGAACCTACATGTTG	GCATTGCAGACAGCTATTGA
78	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
79	RM239	TACAAAATGCTGGGTACCCC	ACATATGGGACCCACCTGTC
80	RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC
81	RM242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
82	RM244	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT
83	RM245	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG
84	RM246	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT
85	RM247	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
86	RM250	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
87	RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
88	RM252	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG
89	RM253	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
90	RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
91	RM258	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC
92	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
93	RM261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC
94	RM262	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC
95	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
96	RM264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
97	RM265	CGAGTTCGTCCAAGTGAGC	CATCCACCATTCCACCAATC
98	RM269	GAAAGCGATCGAACCAGC	GCAAATGCGCCTCGTGTC
99	RM270	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
100	RM276	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
101	RM277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
102	RM278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC

103	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG
104	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
105	RM281	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG
106	RM285	CTGTGGGCCCAATATGTCAC	GGCGGTGACATGGAGAAAG
107	RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
108	RM300	GCTTAAGGACTTCTGCGAACC	CAACAGCGATCCACATCATC
109	RM314	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC
110	RM315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
111	RM318	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC
112	RM320	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC
113	RM321	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG
114	RM322	CAAGCGAAAATCCCAGCAG	GATGAAACTGGCATTGCCTG
115	RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
116	RM327	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC
117	RM328	CATAGTGGAGTATGCAGCTGC	CCTTCTCCCAGTCGTATCTG
118	RM330	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC
119	RM331	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG
120	RM332	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC
121	RM342	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
122	RM343	CCACGAACCCTTTGCATC	GTGATGATGCGTCGGTTG
123	RM344	CAGAGACAATAGTCCCTGCAC	GTAGGAGGAGATGGATGATGG
124	RM345	ATTGGTAGCTCAATGCAAGC	GTGCAACAACCCCACATG
125	RM346	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGGAC
126	RM347	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG
127	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
128	RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
129	RM350	TGATCGTCGCGATTCCCGGC	CCCCACCCTGCGCCTCTCCC
130	RM351	CCATCCTCCACCGCCTCTCG	TGGAGGAAGGAAAGGGGACG
131	RM400	ACACCAGGCTACCCAAACTC	CGGAGAGATCTGACATGTGG
132	RM401	TGGAACAGATAGGGTGTAAGGG	CCGTTCACAACACTATACAAGC
133	RM402	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG
134	RM404	CCAATCATTAACCCCTGAGC	GCCTTCATGCTTCAGAAGAC
135	RM405	TCACACACTGACAGTCTGAC	AATGTGGCACGTGAGGTAAG
136	RM406	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG
137	RM407	GATTGAGGAGACGAGCCATC	CTTTTTCAGATCTGCGCTCC
138	RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
139	RM409	CCGTCTCTTGCTAGGGATTC	GGGGTGTTTTGCTTTCTCTG
140	RM410	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
141	RM411	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
142	RM412	CACTTGAGAAAGTTAGTGCAGC	CCCAAACACACCCAAATAC
143	RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
144	RM414	ATTGCAGTCATGCAGCAGTC	ATATCTCCAATGTGGCAGGG
145	RM415	CTTCGATCCATCATCCATGG	ATTGCTGTACGCAGTTTCGG
146	RM416	GGGAGTTAGGGTTTTGGAGC	TCCAGTTTCACACTGCTTCG
147	RM417	CGGATCCAAGAAACAGCAG	TTCGGTATCCTCCACACCTC
148	RM419	TCTCCTTTGGTATGCGTGTG	GCTGCTGCTCCACTTTTCTC
149	RM420	GGACAGAATGTGAAGACAGTCG	ACTAATCCACCAACGCATCC
150	RM421	AGCTCAGGTGAAACATCCAC	ATCCAGAATCCATTGACCCC
151	RM422	TTCAACCTGCATCCGCTC	CCATCCAAATCAGCAACAGC
152	RM423	AGCACCCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC
153	RM424	TTTGTGGCTCACCAGTTGAG	TGGCGCATTCATGTCATC
154	RM425	CCAACGAAGATTCGAAGCTC	CAGCACCATGAAGTCGCC
155	RM426	ATGAGATGAGTTCAAGGCCC	AACTCTGTACCTCCATCGCC
156	RM427	TCACTAGCTCTGCCCTGACC	TGATGAGAGTTGGTTGCGAG

157	RM428	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTTG
158	RM429	TCCCTCCAGCAATGTCTTTC	CCTTCATCTTGCTTTCCACC
159	RM430	AAACAACGACGTCCCTGATC	GTGCCTCCGTGGTTATGAAC
160	RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
161	RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
162	RM434	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG
163	RM435	ATTACGTGCATGTCTGGCTG	CGTACCTGACCATGCATCTG
164	RM437	ACACCAACCAGATCAGGGAG	TGCTCGTCAATGGTGAGTTC
165	RM438	CTTATCCCCCCGTCTCTCTC	CTCTCTGCCACCGATCCTAC
166	RM439	TCATAACAGTCCACTCCCCC	TGGTACTCCATCATCCCATG
167	RM440	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
168	RM441	ACACCAGAGAGAGAGAGAGAGAG	TCTGCAACGGCTGATAGATG
169	RM442	CTTAAGCCGATGCATGAAGG	ATCCTATCGACGAATGCACC
170	RM443	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG
171	RM444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG
172	RM445	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC
173	RM446	ACAGCGAATACTCCAGACGG	TATCTCCCCCCAAATTCCTC
174	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
175	RM448	TCTGATCTTGATGCAGGCAC	TCTCCCGATTTGGACAGATC
176	RM449	TTGGGAGGTGTTGATAAGGC	ACCACCAGCGTCTCTCTCTC
177	RM450	AAACCACAGTAGTACGCCGG	TCCATCCACATCTCCCTCTC
178	RM451	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC
179	RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
180	RM453	CGCATCTCTCTCCCTTATCG	CTCTCCTCCTCGTTGTCGTC
181	RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
182	RM456A	TTGTAGTCCGGGTCGTAACC	GATAGAATAGGGAGGGGGGG
183	RM456B	TTGTAGTCCGGGTCGTAACC	GATAGAATAGGGAGGGGGGG
184	RM456C	TTGTAGTCCGGGTCGTAACC	GATAGAATAGGGAGGGGGGG
185	RM457	CTCCAGCATGGCCTTTCTAC	ACCTGATGGTCAAAGATGGG
186	RM458	GGTGATCTGCATTGTCAACG	TGCAATGGATCTAGCGACTG
187	RM459	CTGCAATGCTGCATGACC	CACTTTCTCTGCAGCACCAG
188	RM460	TGATCGACAGCGTTCTTGAC	GCCTGGCCCACATAATTAAG
189	RM461	GAGACCGGAGAGACAACTGC	TGATGCGGTTTGACTGCTAC
190	RM463	TTCCCCTCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG
191	RM464	AACGGGCACATTCTGTCTTC	TGGAAGACCTGATCGTTTCC
192	RM465A	GTGCCTCCATCATCATCATC	TAGGACAAGCGAAGAAACCG
193	RM465B	GTGCCTCCATCATCATCATC	TAGGACAAGCGAAGAAACCG
194	RM465C	GTGCCTCCATCATCATCATC	TAGGACAAGCGAAGAAACCG
195	RM466	TCCATCACCACATTCCCC	ACCCTTCTCTCGCTCTCTCC
196	RM467	GGTCTCTCTCTCTCTCTCTCTC	CTCCTGACAATTCAACTGCG
197	RM468	CCCTTCCTTGTTGTGGCTAC	TGATTTCTGAGAGCCAACCC
198	RM469	AGCTGAACAAGCCCTGAAAG	GACTTGGGCAGTGTGACATG
199	RM470	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG
200	RM471	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC
201	RM472	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG
202	RM473A	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
203	RM473B	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
204	RM473C	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
205	RM473D	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
206	RM473E	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
207	RM475	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
208	RM476A	CCGCAGCGATAGAGAGAGAG	TCAAGATGATCCACACGCC
209	RM476B	CCGCAGCGATAGAGAGAGAG	TCAAGATGATCCACACGCC
210	RM477	TCTCGCGGTATAGTTTGTGC	ACCACTACCAGCAGCCTCTG

211	RM479	CCCCTTGCTAGCTTTTGGTC	CCATACCTCTTCTCCTCCCC
212	RM480	GCTCAAGCATTCTGCAGTTG	GCGCTTCTGCTTATTGGAAG
213	RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG
214	RM482	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC
215	RM483	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGTAGCC
216	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
217	RM485	CACACTTTCCAGTCCTCTCC	CATCTTCCTCTCTTCGGCAC
218	RM487	TTTCTCGAACGCAGGAGAAC	GCTAGGAACATCAACCCGAG
219	RM488	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
220	RM489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
221	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
222	RM491	ACATGATGCGTAGCGAGTTG	CTCTCCCTTCCCAATTCCTC
223	RM492	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC
224	RM494	GGGAGGGGATCGAGATAGAC	TTTAACCTTCCTTCCGCTCC
225	RM496	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC
226	RM497	TCCTCTTCACCTATGGGTGG	GCCAGTGCTAGGAGAGTTGG
227	RM498	AATCTGGGCCTGCTCTTTTC	TCCTAGGGTGAAGAAAGGGG
228	RM499	TACCAAACACCAACACTGCG	ACCTGCAGTATCCAAGTGTACG
229	RM502	GCGATCGATGGCTACGAC	ACAACCCAACAAGAAGGACG
230	RM503	CACCTTTCACACACACACAC	GCCCCACTAACAAAACCAAG
231	RM504	TCTATAATGTAGCCCCCCCC	TTTCAGGGGCTTCTACCAAC
232	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
233	RM508	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC
234	RM509	TAGTGAGGGAGTGGAAACGG	ATCGTCCCCACAATCTCATC
235	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
236	RM511	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC
237	RM512	CTGCCTTTCTTACCCCCTTC	AACCCCTCGCTGGATTCTAG
238	RM513	TCTAGTGGCCTCAAAAAGGG	GCAACGAAATCATCCCTAGC
239	RM514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
240	RM515	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
241	RM516	GTTTCCTGCATGCTTGGAAC	ATGTGATTGTATCAGGCTCG
242	RM517	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC
243	RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
244	RM519	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
245	RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
246	RM521	TTCCCTTATTCCTGCTCTCC	GGGATTTGCAGTGAGCTAGC
247	RM523	AAGGCATTGCAGCTAGAAGC	GCACTTGGGAGGTTTGCTAG
248	RM524	TGAAGAGCAGGAACCGTAGG	TCTGATATCGGTTCCTTCGG
249	RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG
250	RM526	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG
251	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
252	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
253	RM529	CCCTCCCTTCTGTAAGCTCC	GAAGAACAATGGGGTTCTGG
254	RM530	GCACTGACCACGACTGTTTG	ACCGTAACCCGGATCTATCC
255	RM531	GAAACATCCCATGTTCCCAC	TCGGTTTTTCAGACTCGGTC
256	RM532	TCTATAATGTAGCCCCCCCC	TTTCAGGGGCTTCTACCAAC
257	RM534	ACAAAACCAAGGGCCTAACC	CTTCGTGCGAGCCATCTC
258	RM535	ACTACATACACGGCCCTTGC	CTACGTGGACACCGTCACAC
259	RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
260	RM537	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC
261	RM538	GGTCGTTGAAGCTTACCAGC	ACAAGCTCTCAAAACTCGCC
262	RM539	GAGCGTCCTTGTTAAAACCG	AGTAGGGTATCACGCATCCG
263	RM540	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC
264	RM541	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG

265	RM542	TGAATCAAGCCCCTCACTAC	CTGCAACGAGTAAGGCAGAG
266	RM544	TGTGAGCCTGAGCAATAACG	GAAGCGTGTGATATCGCATG
267	RM545	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
268	RM546	GAGATGTAGACGTAGACGGCG	GATCATCGTCCTTCCTCTGC
269	RM547	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
270	RM548	TCGGTGAGAAACTGAGAGTACG	AAGGAGGCCATCTCAATGTG
271	RM549	ACGAACTGATCATATCCGCC	CTGTGGTTGATCCCTGAACC
272	RM550	CTGAGCTCTGGTCCGAAGTC	GGTGGTGGAAGAACAGGAAG
273	RM551	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
274	RM552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC
275	RM553	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC
276	RM554	GTTCGTCCGTCTCTCGTCTC	CCCAAAAATCTGTGCCTCTC
277	RM555	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC
278	RM556	ACTCCAAACCTCACTGCACC	TAGCACACTGAACAGCTGGC
279	RM557	GTGGCGAGATCTATGTGGTG	GCTTTGTGTGTGTGTGTGTG
280	RM558A	GAACTCCTCGAACTCGATGC	AGGCATTCAACCTGTTCGAC
281	RM558B	GAACTCCTCGAACTCGATGC	AGGCATTCAACCTGTTCGAC
282	RM559	ACGTACACTTGGCCCTATGC	ATGGGTGTCAGTTTGCTTCC
283	RM561	GAGCTGTTTTGGACTACGGC	GAGTAGCTTTCTCCCACCCC
284	RM562	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
285	RM563	CGACCCTAGGGTTTCTCC	CTCGACGTCGTGGAAAGC
286	RM564	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG
287	RM565	AGTAACGAGCATAGCAGGCG	GCAAAGCCTTCAGGAATCAG
288	RM566	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC
289	RM567	ATCAGGGAAATCCTGAAGGG	GGAAGGAGCAATCACCACTG
290	RM569	GACATTCTCGCTTGCTCCTC	TGTCCCCTCTAAAACCCTCC
291	RM570	GTTCTTCAACTCCCAGTGCG	TGACGATGTGGAAGAGCAAG
292	RM571	GGAGGTGAAAGCGAATCATG	CCTGCTGCTCTTTCATCAGC
293	RM572	CGGTTAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC
294	RM573	CCAGCCTTTGCTCCAAGTAC	TCTTCTTCCCTGGACCACAC
295	RM574	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC
296	RM576	GGACGGCGAGTTCATAAATAG	CTTGATGGGATAAAAGCATCAG
297	RM577	GCTTTCCCTCTAACCCCTCT	GGATGTACCGCTGACATGAA
298	RM579	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT
299	RM581	ACATGCGTGATCAACAATCG	AATTGGATGTGGATGCACG
300	RM582	TCTGTTGCCGATTTGTTCG	AAATGGCTTACCTGCTGTCTC
301	RM584	AGAAAGTGGATCAGGAAGGC	GATCCTGCAGGTAACCACAC
302	RM585	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG
303	RM586	ACCTCGCGTTATTAGGTACCC	GAGATACGCCAACGAGATACC
304	RM587	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC
305	RM588	GTTGCTCTGCCTCACTCTTG	AACGAGCCAACGAAGCAG
306	RM589	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG
307	RM591	CTAGCTAGCTGGCACCAGTG	TGGAGTCCGTGTTGTAGTCG
308	RM592	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA
309	RM593	TCCCGTATGTAACGTGCCA	GACAAGAGAACATCGCTAGG
310	RM594	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC
311	RM595	CCTTGACCCTCCTCTTACTT	TCCTATCAAAATTTGGCAAC
312	RM596	ATCTACACGGACGAATTGCC	AGAAGCTTCAGCCTCTGCAG
313	RM597	CCTGATGCACAACTGCGTAC	TCAGAGAGAGAGAGAGAGAGAG
314	RM598	GAATCGCACACGTGATGAAC	ATGCGACTGATCGGTACTCC
315	RM599	CTGACCGTTGTTGGTCATTG	TTCCCAGAGAACCAAGGATG
316	RM600	AAACGTGTGTTAGCCTGTTAGG	CATATGCTAGTGGTGCTAGCG
317	RM6464	ACACTCTCTCTCCTCGCTGC	CGAGGAGAATACTCGTTCGG

318	RM6515	GCTCGGCTAGTGACGATTTC	GTGGTAGGCGACATAGCTCC
319	RM1254	GTGGTAGGCGACATAGCTCC	CATGCAGATTAGAGGTGGAC
320	RM3652	GCTACAGAAACCAATTCCGG	CGGAGGCGTCTCTAGTCTAC
321	RM6463	AGGCACAGAGCGAAACCG	CCAAGCGGTTCAAGTACGTC
322	RM7466	CGGTCTGCCTAGCTTGTCTC	ACCGAACACGGAAAAGCC
323	RM8110	GAATTATTATGGTGAAGAGTTAGTTGG	TCCATATAGTAGTAGTAATCTACTTACTCCTA
324	RM8077	TGTAAAGTTGTCAAGGGACTACTC	GGGGTATAGTAGACAACATCAAAA
325	RM3235	TAAGTGAGAGCTAGTGATGGCG	CAGCCACTCAAAAACCATCC
326	RM6769	TTGCTGACCTCCGACCAC	CAAGAAGAACGACGAGGAGG
327	RM6630	TCATCAGCAGGTCAGTTTGG	AGATACGCAGGTACACGACG
328	RM8141	GATATTTAAGTCGTAAGAGAACACACAC	GATAATACACCGTACGACATAAAAAATA
329	RM8148	GCCTTCACCGCTTCACCC	GTCTGCAACGCATCGAAGAAA
330	RM8093	TCATGGTTTGTCATGGAATGTTATTATTCA	AACAAATAGCATGCACACATGATACTGATG
331	RM6466	CGAACGAGAACTCCCTCATG	ATTGCACCAAGAGGAGATCG
332	RM6613	GCGTTCATGTGGTCCTGG	TCCTCCTCTGACGCCTTATC
333	RM8268	AAATCGACATTCTCTGTTGC	ATGGCTTACCTGCTGTCTC
334	RM8132	AAACCAATCCCTTATATACTCCC	GAGAGAATTCGAGATCGGTG
335	RM6039	ACGCTGGTGGTGGTTGTG	ACGGTTTGTACGGCTTGATC
336	RM6642	CGTAACGTCCGGATCGATC	TACACGGATTGATCGAGCAG
337	RM8066	TTACTACTGCATATCACGAC	CTTCCAAGCTACTATCAAAC
338	RM8070	AAATGGACTCGCTCCTAAAC	AGGAGCGAATTTTATTGCTACT
339	RM8053	AGACATTGCCGATGATAGG	AAGTACCCCACCGAATAGAG
340	RM8051	CGCGGTTAATGTCATCTGA	CAAGACTGACCCTAAAACCATAC
341	RM1287	GGAAGCATCATGCAATAGCC	GGCCGTAGTTTTGCTACTGC
342	RM3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC
343	RM1321	CTTGCATGACTACACGAGTCG	TATCCTGAGCGAGATCAGGG
344	RM6902	CGGAGAAGACCGAGACGTAG	TCCCGTGTAAAAGTTGGGAC
345	RM1329	GAGCTCAATCGAATCTAGACC	ATTGACATTCCTTTGCTTTG
346	RM6740	GATGGGATAGACAAGCGCAC	CCATCTAGGAGTACTAGTCTTCGC
347	RM8079	TTCACTTTATGATTATTCTTTGCAAAA	TACCAAAATAGAGGAGAACTAGTGATG
348	RM5638	GGCTTCCTCATCGCCATC	CTGAGCAGCATTCCAGTCTG
349	RM7449	TGTGATGGTGATCTAAGCCG	CTCGATCATATGGCTGCAAG
350	RM7056	GAAACGTGTAGCAGTACGCC	ACCAAGCTCTTCATCAACGG
351	RM8129	AGTACAGAACCACACAACACTG	GTCTCGCACGTTCCAATAT
352	RM3366	TGTTTTGCGTATTTATAGGATG	CAAGAAGTACATGGGACCTG
353	RM2574	CTTGGGTTCGAGTAGGATAA	TCCACCAGAATTTGATCAAT
354	RM8260	AATCTAACGTTTGACTATCCATC	TCTACCAGTACTCCCTTCACC
355	RM3143	AGCCTGGATAAGATGGTTCG	CGAGAAGACCCAGTTTCTGC
356	RM3241	GCTACCTTGCCTCCTTCTCC	AACGAAAATCGGGGCGTTAC
357	RM6800	GTCCACGAGATGGACTCCTC	TTCTGAAGGCCAGGCCAG
358	RM5764	CGACGCTGTCTCTTGTTGAG	CATTCGTTTCACCAATGGCC
359	RM6151	TTCGCTTTATGGGCCTAGTC	AGCAGATGGAGAAGCGAATC
360	RM6616	AATCCGATCGTACGAGCAAC	GACAAGGGAAGGAAACCCTC
361	RM3703	GAGAGAGAGGGAAGGGAAGG	GCTCCCCGACATTTAAACTG

362	RM5512	TTCTGGATTACACAGCACGG	TGGGTGGCTTCTTTGTTGAC
363	RM3294	TTACACACACTACGGACGCG	CCTGGTGGTACCTCTCTTAATC
364	RM5664	GCTGACGAAGAGAAAATGGG	GCATTCGTCTCGTCTTTGAG
365	RM2483	TTTTAGAACACAAGGAGTAG	GAAGATAGTACTTCCTCTGTAG
366	RM7006	CTCGTTTATCCTCCCAGTGC	CACTTGTATCCAGAAGCAGG
367	RM6493	GTCTTCGTGTCGGCCATG	CAAGCCAGACCTAGGGTTTG
368	RM2939	CAAGAACTAGACCGGTGTC	CATGGGACCAGCTATTACT
369	RM5459	AGTTGTTGTCACCTCCTGCC	AATAAATAGGAGCCCGGGTC
370	RM5699	ATCGTTTCGCATATGTTT	ATCGGTAAAAGATGAGCC
371	RM1358	GATCGATGCAGCAGCATATG	ACGTGTGGCTGCTTTTGC
372	RM5015	CAAATTGGGTAGATACTCAA	ATGATTAACACGTACGATTC
373	RM1313	TGTGTCTGAAAACCAAGGGG	CGTCCAAGCTGTTCGTTCTC
374	RM5179	ATGAGCTAATGTTTCTAAGC	CAAATTGATTAGTTTGAACC
375	RM7426	TGACATGGATCGATCAGCTC	ATAAGGGTACGGGGAACCAG
376	RM6844	CAGAGCAGGAACAGATGCTG	GTCCAAGAAAGGCACGAGAG
377	RM3630	CAGCTACTGTTCCATGGTGG	GCCATCAACTCCCGGATC
378	RM2634	GATTGAAAATTAGAGTTTGCAC	TGCCGAGATTTAGTCAACTA
379	RM3688	GTTGAATCAAGCTGTGCAGC	AGCTAGGCAAAGCATGCATG
380	RM5363	TCCCTCCCTGGCTTTTTTAC	AGCAACGCGGTGAGAGAC
381	RM6379	AAGTCCACTCATGCTGATGC	TTGATGGCCACCTCTTCTTC
382	RM3515	ACGCTTGTGGTGTTTAATAC	CACTGTGAATACACAGGAAC
383	RM1942	CTGCTCAATGATACAGGA	GGCATCCACTAAATTTAGATA
384	RM6361	ACAAGTTGGAGCTTGGAAGC	ATATGATCGATGGCGACTCC
385	RM1385	ATGACAGGTAAGGTGTGGTG	TGAACATCATCTTCGAATCC
386	RM1367	GCATCGTTCATGTACACTGG	CTGCTACGCTGCTACTCCTAG
387	RM1342	GAAGCAAGAAACCAAAGATG	CTTTCGGTCTCAAGCAATAT
388	RM5305	CCTTCCCTATGCTATGCTGC	GATGGGGAGTAATGGTGTGG
389	RM6465	TTCCCATCGCAACTGACC	ATCTGATGCGCTTCCTTGAG
390	RM6424	AGCGAATCAGGTGACTCCAC	ACACCATCCATCTCCAGTCC
391	RM6290	CAGGAGTGAGTAGCAGAGCG	CATCGGACGGTCACGAAG
392	RM3316	TTCGACGATTCTGTACACGC	CATGATCCCAAATGCATGGG
393	RM6519	CCTTTCCTCACTCTCCTCCC	TCTGGTCGAAGAAGTCGTCC
394	RM8255	AGTGCTCATGTGACACTGC	CATCCATCCATTCTTGCTC
395	RM7307	ACATGCCTCCTCGTAAATGC	ATTTCCATGCAGAGGCAGAG
396	RM5404	GGCCATCCATCTCCTGTATG	GACACACACAGGGTTGGTTG
397	RM3202	TTCACTTCCTATTGGCGGC	TCATCATCAGTCCAGCATCG
398	RM3372	GAGCGACCAAAGAATCCAAG	CCACGGGGAGCTGATGAAG
399	RM7072	CTAATCCTATTGATTTAGGG	AGTCTAGTGTCAACCTTCTC
400	RM3117	GCCATCTCTCTCTCTCTCTC	CCTTAGCTCATCAAGCGAGG
401	RM1278	CCATAGCAATTTAGCCATAT	TCTAATTCTCCCCAACACTA
402	RM3467	ATAATGGCAGGGTTGTCTCG	CTCGGTGAGCCTCCTACAAC
403	RM7565	TGATTCCTGCGACCGATC	CATGCATGCTCTCCTTAGGC
404	RM5477	CACGCATTGTTCGTGATAGG	GCTAATACCGAGCGAGATGG
405	RM1002	GAACCAGACAAGCAAAACGG	AGCATGGGGATTTAGGAACC

406	RM3461	AAAGTCTCCCTGTTGTAGCC	CATGAACGTAAAGCAAACG
407	RM3280	TCTAGCTTCTGGGAAGCAGC	CACCTGTCCTAACATTGCTACG
408	RM5748	CAGTTGGCAATTGTCACGAG	TCGAACATATCCAAGCCTCC
409	RM6080	CAGAGGAAGCAAGGAGATCG	CCATCGGGAGAGAAAGAGAG
410	RM6931	GCTTGTACCTTCTGTTGGCC	CTGTAACTCGTTCTCCACGC
411	RM3180	GGGTCGGATAGCCACACAC	GAGGTAATCTCGCGGAGTTG
412	RM7585	CCTCCTCCCTCGACTACCTC	GGTGTGTCGGTGTGATATGC
413	RM3892	CGAACCAAATCCCACATCTC	ACGACGAATCTACAAACCGG
414	RM8213	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG
415	RM5900	TTCTACGTTTGACCGTCA	TCTAGGAGCGTTTGTAGGAG
416	RM8219	AACCATTGTTGAGCAAATTC	GATAAGCAGGGATTGGAAAG
417	RM5951	TGATCCCAGAACTGAACACG	AAGACGTGTCGTGTGGTGTG
418	RM5424	CACCAGACAGACGCCACAG	CGTATATATCGCATGCACCG
419	RM7313	TATGTGGGCTCGTGGGTC	TCTCATCCGTTTCTTCCACC
420	RM3337	TCCCCAATTATATCCCCTCC	GATTGGGGGAAGAGGAAGAG
421	RM5221	ATTATCTTCTCCTCCATACC	CCTTCTTGAAAATTAAATTG
422	RM1165	CTAGGACGAAGGGGAAGGAC	ACCACCACCATTCCAAACTC
423	RM7051	CTCGATGAGCTTGGCGTC	TTCAGTGTTCATCGCCTCTG
424	RM1136	ATGTCATCCAGAGTCGCCTC	AGGACGTATTCACACACGAC
425	RM1302	TCTTCCCCTGAACGTGAAAG	CCTTCTCTCCCAACATCTCG
426	RM3092	GTTAAGGTGAAATTCATTGG	ACGACCAGACTCCTACTACA
427	RM7187	CAGCGAACGTGGTGTCTTC	CCCACACCAACTTCTCGC
428	RM6148	CTCAGACTTCTGTCCAGGTCG	CGACGTTGTGCACCTCAC
429	RM5030	AGATTTTAGTGGTCCAAACA	ACTCAATTTCAACAATGGTG
430	RM3843	ACCCTACTCCCAACAGTCCC	GGGGTCGTACGCTCATGTC
431	RM3306	CCTTTTACCTTTCATAGCAA	ACAAGAAGATGGTGAGTGAT
432	RM3648	TACCCTTTCTTCCCCAAACC	ACCTCCTCCTCCACTTCTCC
433	RM6057	TCATGTTGTTGCTCCTCCTG	AGGGAGAGAGACAGCAGCAG
434	RM8218	CTCTACCGAATCCATCGTC	CTCTACCGAATCCATCGTC
435	RM6481	TGAGGGCGACGACGAAAG	ATCTCAGTCAGCACAGGCAG
436	RM2799	CTCCGTTTCAGGATATAAGA	AGATGTTAATGAATTCAGGC
437	RM7474	TTTGGTACGGACAGGAAAGG	CGTCCACTCTTCAATCTCCC
438	RM6006	CTCGGCGATGAACAGCTC	AGAAGATCATGAAGCGGTCG
439	RM3216	ATTTTATCCATCTCCAAGTC	GATCTATCGGTTATCCAACT
440	RM2811	AGCCTCCTACCTCTAAACCT	GCGGAGAGAGTAAGAAGTTC
441	RM3536	GGGTGAGTGCGACAGAGATG	CATGTCTCCCCCTCACCCTC
442	RM5633	GTGTAGCTGCTAGGCCGAAC	TTCCTTTCGCTACGTTGGAC
443	RM5688	GCAGTGTCCAACCATCTGTG	ATCTGGTCACCCTTTGCTTG
444	RM3643	AGCATGAGCAGGTGCTAGTG	CGTTGCATGTGTGATGGC
445	RM5749	GTGACCACATCTATATCGCTCG	ATGGCAAGGTTGGATCAGTC
446	RM3397	GGTGAGCTCCACACACACAC	GGGAATGGTTCAACATGAGG
447	RM2565	TGGTGACATTTTTTAGTTGG	ACTCAGTTTTTATTTGCACG
448	RM7313	TATGTGGGCTCGTGGGTC	TCTCATCCGTTTCTTCCACC
449	RM5757	CCTGAGACCATATGCTGCTG	GAGGGAGCATCATTAGCTGG

450	RM3263	CCCCCTCCTTTAATTTGCAC	CTCCTGATCCTCATGGATGG
451	RM6005	CTCCGTTCCCTACTTCCCTC	GGGAGGGAGATATCGGAGTC
452	RM4838	TACCAGTGCAAAACATAGTA	ATTAATGAAATGGAACACAC
453	RM3334	GAAGGCCGAGAGTGAGAATG	GAGAGAGAAAGGGAGAGACTAGC
454	RM6300	CTTTGCTTTCGTGCCTTGTC	CGATGAATCCACTCCTCCTC
455	RM1024	GCATATACCATGGGGATTGG	GGGATTGGGATAATGGTGTG
456	RM5579	CAAATATTGGCAAATAAACT	ATATTGCCTCATGGTAATAA
457	RM6517	TTCTTCCTCCTTTTCCCTCG	ATTGGTCAGATCGAACCTGG
458	RM5874	GAAAAGATCCTGGCTCGTTG	GCATCATCGCCAGAGCTC
459	RM3193	AACGCCTATATTAACGCGCC	CGACGTGGAGGAGAGAAGAG
460	RM2422	AACATGGGAAACACTAATAA	AAGATTTGAACCACAGTAGA
461	RM7293	CCTAGGGGATCCAAGATGTC	GCACGGATCTACATACATGC
462	RM5140	GACGAGGTTGTTTATTAGTG	CTTATTTTCACGTGTACGTT
463	RM4554	GCCGATCATCTAATCTAATC	ACAGAAGCATTATCCGTATC
464	RM1237	CTCCGCGAGCTTTAGAAGAG	CACATACTCTGGCTCTCCCG
465	RM8211	GTTTGGGAAGGAGGAATG	AAGTAGAAACGGCCAACAC
466	RM3575	CCTGGAATGATGATGGAAGG	GTTTTGCTTCCTGGAAGTGC
467	RM8120	AAGATGAGTAAGTTTAATTGACCTGAT	GAAAGCCTATCACTATATATCTAACTAAGC
468	RM3132	GGCCCTCCACTTCTCTCTTC	GGCAAAACCAAGAAGGGAAG
469	RM8075	ACCAAATAAGCCTCTAATGGCA	GTAGCAAACTGATAGTTTTGTCACTAAAG
470	RM1369	AACCTGAGAGTGCCAATTGG	TCCCCTAGTAAAGCGGATTC
471	RM4923	TCCATCTAACAGAGTAAACA	TAGGATAGGGAAGTAGGATA
472	RM3414	TAGGGCAATTGTGCAAGTGG	TTGGGAATTGGGTAGGACAG
473	RM5815	GCCAAGCAGAATCTGAATCC	CTCCAACAAGAGAAAGGGGG
474	RM1163	TCTAGGGTTAGGGTTTCGCC	AGGTCGGTTTCCTTTTGTCC
475	RM4608	CAGGTAATAGTCATACTCCT	GGAAACTAGATTAGCTCATA
476	RM4173	TAGATTTGTCTTGGAAAATA	AACATAACTTTGACTTCTTG
477	RM6779	CACAGCCTCTCACAAGGGAG	AGGACGAGGAGCAGGAGGAG
478	RM8270	TGAATTCTGCAAATCAACATC	ATCAACATCTAATTATCTTTCTTCAC
479	RM3431	ATCCAAATCCAATGGTGC	GCGAAAGGGAACATTCTG
480	RM8240	TGATTGGTGATAATTGGAGAG	ACGAGGTTCTCGAGATGG
481	RM6836	TTGTTGTATACCTCATCGAC	AGGGTAAGACGTTTAACTTG
482	RM4589	GTTTAAACATGGGAGGTGTC	CGAAATTTCTGAAATTTGGA
483	RM6697	GCAAGATCCAGTCGATTTGG	ATAACATGAGCATCTCCCCG
484	RM5100	AAAAGAGTCTCTTCTCTCT	AGGACACATACTGTGTATAA
485	RM6133	TGCGATTGATCTACCGCC	AAAGAAGAAGAGTTGCCGGC
486	RM8010	GAGCCACTGCTATATAAAGC	ACCAAAATCCAAACTTTGTA
487	RM8007	AATAGGATGGATCATGGATA	CATCTCATCAGGAACCTAAC
488	RM6728	GGGTATGTGTCGCTATTTTA	GAAATCTGGAATTTTCCCTA
489	RM3718	AGCGCTCGAGAATTTCTAGG	ATGCTGACGTCACCCCAC
490	RM2256	GTGCTTGCATATAACCTATA	AGATCAACCTTCTTATTCAG
491	RM3449	TCAGGTACACCATGGTGGTG	GTACGATGGTAGTGGGGCAC
492	RM8037	ATTATCGGGTGGAGTTAGAG	AGCGTTTGTAGGAAGTTTTA
493	RM7110	GCCGTCCGTTATAAAACGAG	CCGGTTGAGATGGTGAGC

494	RM6767	ACATTCTTGATCTACGTGGC	AATTATGGTTGCTAGGTTGG
495	RM5380	CCACCCTGTTCATCTCGC	ATGGCGATACCACCACTCTC
496	RM3423	AGCAGGCATATAAAGGTGCC	TGGCCTCAGATTCAGGAAAC
497	RM1019	GTTTGAACAGTAGGACTTGT	AGAACATCTCACACTTCTCT
498	RM1959	CTATTGTACCTGCTCTCATC	ACATCGGTACTGATAATGTT
499	RM6925	TGAGAGGACGCTTGAAGAGG	GCACCTAGTGACTGAAGGTTG
500	RM3702	AAGGCATGCATCTGTGTCTG	CTACTTCTGCTGAGCCAAAG
501	RM6863	GCTGCAGAATTAAGGAGAAC	TGCTCAAAATAATCAGCTCC
502	RM4955	GCATCCAGCAATATAATCAA	CAAGGATTTTGTTAAGTGGG
503	RM5068	GAGGTGTTTATAGAAGTAGG	AATTAGCTTATCTTGTGTTC
504	RM6999	TTATCTGGGATCCATCGAGC	GTGAATTTCCTTGGAGGGAC
505	RM3374	ATGAACTAGTGAACCCCCCC	GTAGCGGTAGCTGCAAAAGC
506	RM6208	TCGAGCAGTACGTGGATCTG	CACACGTACATCTGCAAGGG
507	RM3215	CGGCGTAGCTAAATTTGGAC	ATGGCGAGCAAGGAAGTAAG
508	RM2366	ATTGCCTATATTCATATGGA	GTTATCTGTTACTTCCTTCG
509	RM2910	CAGCTGCTCATATTCATATA	ATAAGGTACTTCATCCGTTA
510	RM7285	GCGGCTATTGTAAGTGTTTG	TATATGAGTGCCACATGACG
511	RM1309	CAGATCACAACCCTGCACTG	ATGGTTGCAACCTCTCCCTC
512	RM5808	AGAAAGAGGAGGGGGAAGTG	CCATCGAGACATCCATCTCC
513	RM1578	TAAAAACCTCTCAAAGCACC	GAAAATTTGAAACATGGGAT
514	RM7580	CTCCAAGAGAGAATGCCAG	GATTTACTAATCTCGCCACG
515	RM1868	ATGGATACTTCCACTTCCAC	CACACATGTGCAAGATGTAC
516	RM4413	AAGCTCTAAACTACTCAAGC	ATGACAATGTGATCTAACAC
517	RM6021	AAGCCCCTCCTCTCCAAGC	GAAGAGCGCCACGATGTC
518	RM1817	TAGTATTCTTTCCTTACAGA	ATTGAAAACTTAACAAATAG
519	RM6444	GGGGGTGGTAAGAAGAGGAG	TAATCCACGATGGACTGCAG
520	RM6475	AGATCAAAGCAACGGCTAGC	GAACAGAGAGGGGACGTGTC
521	RM6567	CGATGTGTCGTCGTCGTC	AGCTCCTCGTGCAGAAGAAG
522	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC
523	RM3025	GGTGGCAAGAAGTTCCTAAT	GATTTCCATACAACCTGTGC
524	RM2214	AACATGTTTGTGAACCGATA	ATAAAAGGAATGCCTTCTTG
525	RM3533	TTCCAACCTGTCAGGGAATC	CATTTCCCTTCCCTCTCCTC
526	RM6543	CGGGCTCCTGAACAGTCTAC	GCAATATCTCATTCTCGGGC
527	RM3164	TCCTCCTGCTAGCTGCCTAG	TCGCCTTCCTTTTCACTCAC
528	RM5535	CGTTCGTGGAGTGGTATGTG	CATACCGAAGTGAGGAACTCG
529	RM3909	CCTCTTTGGACATAATGGGC	GTCCTCCCCACAGAACACAC
530	RM3919	GTGAGTGATCTTCATCAGTG	CGATGGTTATCTGTAAACAG
531	RM6867	AGAGAGCACAATCGGAGTCG	GCAGCAGCAACAAGATGTTC
532	RM5786	AGCAGCTATAGCTTAGCTGG	AGAGACACAGGCAAGTCATC
533	RM3787	CGAAAAACGAGCGAGCAC	GACGCTGGTAAGCAAAGCTC
534	RM6862	GGCAAGATCGTTGGAAGAAC	TTACCTGTCGTTTCCCTTCG
535	RM6643	TGGTGTTATTCCGAGGCTTC	GAGAGAGAGAGGAGATTTTGGG
536	RM1099	CTCGGCGAATCAGAGAAGAC	ATCCTAACGTGCCTATCCCC
537	RM5384	GTACTACTGCTGTTGCTGTG	ATTAAAACCAATGACAGTGC

538	RM6816	GGCTCGCTCTCCATTGATAG	TATAGAGGGCTCACATGGCC
539	RM6294	ATGCTCATCACCAAGGATCC	GCTGCACCATTTGGTCACTG
540	RM2482	CATGTGCTTTCACAGAAAGT	GGCTCAATGACAACTAAACA
541	RM2885	GGCGTCATACATTAAAATAC	GTTTCTATATGCATGTGTCC
542	RM6797	CCTCCTCCATCAGGATCATC	GCTAGGTTGAATGCCCGTAC
543	RM5799	ATCGAACCATCCAGGATGAC	TTGCACAAGAGGCAACACTC
544	RM6196	TACCAAAGGAAGCGGTTCTG	TTCTTAGTTGCCTTCTCGGC
545	RM1896	GGACAGGGTAAAGTGTTAGA	CCTAAGACCTATCAACTCCA
546	RM6854	CTGTGGGAGAAATCTTTGTA	AGAGATGATCAATCCGTCTC
547	RM6771	GCATCAAGCGAATCTTAGCC	TAGTCGCCGATGGATAAACC
548	RM3700	AAATGCCCCATGCACAAC	TTGTCAGATTGTCACCAGGG
549	RM7038	AGGTGGTGAGGGTGAACTTG	TGGGATTAGAGCTTTGGTGG
550	RM6839	CTACTGTTGCAGGCTTGCAG	CAGAGGAGGAGATCGAGAGG
551	RM5122	CTCGCAATTTATACGTAATC	CTCACGAAATAAAATGAGTG
552	RM7424	AGAAGCCCATCTAGCAGCAG	TCAAGCTAGCCACACAGCTG
553	RM7175	ACAGTAAACGTGGTGCCTCC	AGAAGTAGCCTCGAGGACCC
554	RM4405	TGAAGCAATTTGATTTTCAG	GAGCTGGCCTTTATTAACTG
555	RM5102	AATTTTCACCTACATTGTAA	AAGCATAGAAATGTTTGTAT
556	RM6364	GTAGGTGAGGAGGATCTTGT	AATTTCTCGATTCTTCCTTC
557	RM2224	AAAGTTGGTATAGGTGTCAC	GATTATGGTATGTTGCTACA
558	RM2960	TACTCAATTTGAACCATGGA	GATTGTGGGTACATGTGAAC
559	RM2125	TACCTCCTAGCTTTACTTAT	ACTGATCTCTATCTCATTGT
560	RM1236	AGAAAAGTTAATTCCAAAGG	CAAGGAATTCTAGAGGAGTG
561	RM3152	ACAGGTTTGCAGATTACATA	CCCATCTTTAATACCTTCAA
562	RM8201	TCTGTTTATAAGCGCAGCAC	GCCGGCGAGCTACTACTAC
563	RM1859	TCGTAAGAACATGGAGAACC	GGATTTTCTGATAGCGGTAA
564	RM1083	CCTTGATTGCAGCATCCG	TTGAGCCTTTTACGAGACGG
565	RM5304	CAGCCCATCTCTCTCCTCTG	GATAGCAGGAAGAGGCGTTG
566	RM3470	TGATGTGATCTCCTCCTGGC	AGAGCTGCAGAGGAGACAGC
567	RM1873	CTGACAGGACATTAAAAAAC	CCTCATCCTTAATCTCTTTA
568	RM6704	AATCGAATCTGGATATCTTG	CTTCTACCTAGCTACCGAGA
569	RM5373	GGAGATGCTATAGCAGCAGTG	ATTGCTCCTTACCACCTTGC
570	RM6737	CATTGGGGGTGGATAAAGAG	TATCCTCTACTCCCTCGGCC
571	RM6469	ATCATCTCGCCGTACTCCTC	GGAGTCGTCGTCGATGTGG
572	RM3510	TAAGATCGTAAGATCGCGGC	AGGCAGGAAGAGGTGGAGG
573	RM6745	GCGCCTTTAGATGCTACTTG	CAGCTCCATCGTAAGCAAAG
574	RM5841	CCTCTCTCTCTCTCTCCCCC	TGTTATTGGCACGTGGTGTG
575	RM3773	CTGGATGAAAGGATACAACA	CACATTATCTGTCAAGGTCC
576	RM1761	ACGCTTAAAGAACATTTGAT	GCGATTAACTTTTAACCATT
577	RM3717	AGCTCTACCTTTGCTGTCGG	AACTCCCTAGACCCACCTGC
578	RM1240	CCATGAGCTAGTAACTGCAGC	GGATCGCAAAATCTGGCATC
579	RM5927	TGGTCTCGTCTCTCATGTGC	TAGCCCGGAAGTATGATCCC
580	RM1812	CAGCTAGTGAGCTCCTAGTG	GCTAACCCACCAACTTATTC
581	RM1124	CTGGCCACTTAAGTAAGTGC	CGGTTGACCTCGATATAATC

582	RM6544	ACCACTATGCACCCTTCGTC	GAATGCTCTGCTTCGTTTCC
583	RM5128	TAAGTAATGATCATTGGTAA	GTGCCATATATATATGTTGA
584	RM6894	AATCTCCACTGCAGCGATTC	CGAATGGTCAAACGTAGGTG
585	RM4469	AATTTCTCATGTTTTCTTCC	AGTTATTCTAAGGGAGGGAC
586	RM6115	CGCCATAGTCGATGACATTG	GCATCGCAAGCTTATCTTCC
587	RM3428	ATTCATGCTTCCTTTCAGTG	GATTACTGGTTTGCCATTTG
588	RM5558	GCTGACTTCACACTGCGATC	GGCCACTTTCCAAACATCAG
589	RM7303	ACAGGAGGGGAATTGACCAG	CAGTGCTTAGCTGTAAGCTGC
590	RM2110	ATGTGGACAATGATATATGT	CTCCGTTTCATATTATAAGA
591	RM3605	GATGGACGACGAGTAGTGGG	CTCTCCATTTTTCCCCTTCC
592	RM4926	ATTGGACACTTAATTGCAGC	ATTGTTATATACGGGCACCA
593	RM7277	GCTGAACGTTTCAATATGTA	GTTTGTAGGGAGTTTAATGG
594	RM2191	GATAAGCATTTTAGAACACA	ACTAGACCAAGGAATTATTG
595	RM3926	TCATCTCGTAAAATTTCGATTCCGATG	TAATCACCCGGTGCAACGCA
596	RM1880	ACCACTAAATAAGCACATAC	GGCATCATACATTAAAATAC
597	RM7582	TGGAAGGGAAACAACGTACC	AGAAGCTGTAGCCAGATCGC
598	RM6288	CCATGGTGACCGTGGTGAC	CCAGCAGCAGTACATGGCC
599	RM7448	GATTCTGTGTTTCGCTGCTG	TAGCCCGCTGCTCTTCTCTC
600	RM5746	TCGCTACGTCGACTGATTTG	TCGCTACGTCGACTGATTTG
601	RM6973	ATATCATCAGTCGGCAGCAG	CAACTCCAGCTTCGCCAAC
602	RM3103	CAGACAACTTGTAATGTACG	ATGTCATGGGAGATAATTAA
603	RM1036	CTCATTTGTCGATTGCCGTC	ATGGGAGGAGTGATCAAACG
604	RM5746	TCGCTACGTCGACTGATTTG	ATATCATCAGTCGGCAGCAG
605	RM1246	AGCTCGATCCCCTAGCTCTC	TTGGAGAAGGTCACCTGCC
606	RM5341	GCCTGCTGCATTTTCCATAC	GATACATGGACGATGCATGC
607	RM7120	TGCCCAAAATATATGAAACC	TTTTCTTGTTGAATGGGAAC
608	RM3326	CTCATCACCATCGTCACCAC	TCGTCGGGAGAGAGAGAGAG
609	RM4589	GTTTAAACATGGGAGGTGTC	CGAAATTTCTGAAATTTGGA
610	RM6869	GAGCTCCTTGTAGTGACCCG	ATCAGCCTCGCCAGCTTC
611	RM3331	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC
612	RM5990	TAGCCTCCCTCCTCTTCCTC	AGATGGAGGTGGAGGTGGAG
613	RM6947	ATTAAACGTCCACTGCTGGC	GCTAGGTTAGTGGTGCAGGG
614	RM2854	ATGAGAGAGAGAAAGAGAGT	AATGGAGAGAAAAAGTATTA
615	RM6615	GTCGACATGCGGATGCTG	ACCTCCATCTTGGCCTTCTC
616	APSSR	AGAGGAGGAGGAGGAGCTAA	TCTCATGCTCCGCCTCGCGT
617	R40C1SS	TCCCGAAGAGAGCAAGATGT	GATCTTGAAGGTGGGCTGGT
618	SLKESSR	TTCGTCGGAGGAGGGTAGTG	TGGCGCCGTCGCCGTCGCCG
619	STSSR	GGGAACATCCCAGCGTTC	GGAGATTTAAAACCACGGAAAA
620	EN242SS	GAACGGGGCCGCCGCCGCCG	GATTGAAGGCTTTCTGATGT
621	RGPSSR	CACCTCCTCCTCCTCCTCTT	TCTCCAGGAAGTCCAGGTTG
622	OSGRPSS	CGCCGATGCCCGCGGCACAC	TTATCCTTAGCTATCCGTGT
623	EBP89SS	AGGAGAACCACCCGGAAG	CCGACGAAAAAGAAAACGAA
624	RPESSR	ATCAAAAGCTCTCGCTGCTC	ATGATGCCCTCACTCGGAAT
625	CAB2RSS	AATACGCCAAGGTGTTCGTC	CATGTCTACCTCGCTCAGCA

626	MYBSSR	GCCACTGCCGTAGCGCCGCT	CGACCTCAACCTCGAGCTCT
627	ZFPSSR	TCTGCACGTCGTTCGCCAAG	CCACATGCGCCGGCACAGGG

Table 4. List of ISSR markers and their sequences used in this study.

Primer name	Sequence	Primer name	Sequence
ISSR01	ATA TAT ATA TAT ATA TT	ISSR51	GTG TGT GTG TGT GTG TYG
ISSR02	ATA TAT ATA TAT ATA TG	ISSR52	TCT CTC TCT CTC TCT CRA
ISSR03	ATA TAT ATA TAT ATA TC	ISSR53	TCT CTC TCT CTC TCT CRT
ISSR04	TAT ATA TAT ATA TAT AA	ISSR54	TCT CTC TCT CTC TCT CRG
ISSR05	TAT ATA TAT ATA TAT AC	ISSR55	ACA CAC ACA CAC ACA CYT
ISSR06	TAT ATA TAT ATA TAT AG	ISSR56	ACA CAC ACA CAC ACA CYA
ISSR07	AGA GAG AGA GAG AGA GT	ISSR57	ACA CAC ACA CAC ACA CYG
ISSR08	AGA GAG AGA GAG AGA GC	ISSR58	TGT GTG TGT GTG TGT GRT
ISSR09	AGA GAG AGA GAG AGA GG	ISSR59	TGT GTG TGT GTG TGT GRC
ISSR10	GAG AGA GAG AGA GAG AT	ISSR60	TGT GTG TGT GTG TGT GRA
ISSR11	GAG AGA GAG AGA GAG AC	ISSR61	ACC ACC ACC ACC ACC ACC
ISSR12	GAG AGA GAG AGA GAG AA	ISSR62	AGC AGC AGC AGC AGC AGC
ISSR13	CTC TCT CTC TCT CTC TT	ISSR63	AGT AGT AGT AGT AGT AGT
ISSR14	CTC TCT CTC TCT CTC TA	ISSR64	ATG ATG ATG ATG ATG ATG
ISSR15	CTC TCT CTC TCT CTC TG	ISSR65	CCG CCG CCG CCG CCG CCG
ISSR16	CAC ACA CAC ACA CAC AT	ISSR66	CTC CTC CTC CTC CTC CTC
ISSR17	CAC ACA CAC ACA CAC AA	ISSR67	GGC GGC GGC GGC GGC GGC
ISSR18	CAC ACA CAC ACA CAC AG	ISSR68	GAA GAA GAA GAA GAA GAA
ISSR19	GTG TGT GTG TGT GTG TA	ISSR69	GTT GTT GTT GTT GTT GTT
ISSR20	GTG TGT GTG TGT GTG TC	ISSR70	TGC TGC TGC TGC TGC TGC
ISSR21	GTG TGT GTG TGT GTG TT	ISSR71	TAT TAT TAT TAT TAT TAT
ISSR22	TCT CTC TCT CTC TCT CA	ISSR72	GAT AGA TAG ATA GAT A
ISSR23	TCT CTC TCT CTC TCT CC	ISSR73	GAC AGA CAG ACA GAC A
ISSR24	TCT CTC TCT CTC TCT CG	ISSR74	CCC TCC CTC CCT CCC T
ISSR25	ACA CAC ACA CAC ACA CT	ISSR75	CTA GCT AGC TAG CTA G
ISSR26	ACA CAC ACA CAC ACA CC	ISSR76	GAT AGA TAG ACA GAC A
ISSR27	ACA CAC ACA CAC ACA CG	ISSR77	TGC ATG CAT GCA TGC A
ISSR28	TGT GTG TGT GTG TGT GA	ISSR78	GGA TGG ATG GAT GGA T
ISSR29	TGT GTG TGT GTG TGT GC	ISSR79	CTT CAC TTC ACT TCA
ISSR30	TGT GTG TGT GTG TGT GG	ISSR80	GGA GAG GAG AGG AGA
ISSR31	ATA TAT ATA TAT ATA TYA	ISSR81	GGG TGG GGT GGG GTG
ISSR32	ATA TAT ATA TAT ATA TYC	ISSR82	VBV ATA TAT ATA TAT AT
ISSR33	ATA TAT ATA TAT ATA TYG	ISSR83	BVB TAT ATA TAT ATA TA
ISSR34	AGA GAG AGA GAG AGA GYT	ISSR84	HBH AGA GAG AGA GAG AG
ISSR35	AGA GAG AGA GAG AGA GYC	ISSR85	BHB GAG AGA GAG AGA GA
ISSR36	AGA GAG AGA GAG AGA GYA	ISSR86	VDV CTC TCT CTC TCT CT
ISSR37	TAT ATA TAT ATA TAT ART	ISSR87	DVD TCT CTC TCT CTC TC
ISSR38	TAT ATA TAT ATA TAT ARC	ISSR88	BDB CAC ACA CAC ACA CA

ISSR39	TAT ATA TAT ATA TAT ARG	ISSR89	DBD ACA CAC ACA CAC AC
ISSR40	GAG AGA GAG AGA GAG AYT	ISSR90	VHV GTG TGT GTG TGT GT
ISSR41	GAG AGA GAG AGA GAG AYC	ISSR91	HVH TGT GTG TGT GTG TG
ISSR42	GAG AGA GAG AGA GAG AYG	ISSR92	TAG ATC TGA TAT CTG AAT
ISSR43	CTC TCT CTC TCT CTC TRA	ISSR93	NNN NNN NNN NNN NNN
ISSR44	CTC TCT CTC TCT CTC TRC	ISSR94	TGG TAG CTC TTG ATC ANN
ISSR45	CTC TCT CTC TCT CTC TRG	ISSR95	AGA GTT GGT AGC TCT TGA
ISSR46	CAC ACA CAC ACA CAC ART	ISSR96	AGG TCG CGG CCG CNN NNN
ISSR47	CAC ACA CAC ACA CAC ARC	ISSR97	CCG ACT CGA GNN NNN NAT
ISSR48	CAC ACA CAC ACA CAC ARG	ISSR98	GAT CAA GCT TNN NNN NAT
ISSR49	GTG TGT GTG TGT GTG TYA	ISSR99	CAT GGT GTT GGT CAT TGT
ISSR50	GTG TGT GTG TGT GTG TYC	ISSR100	ACT TCC CCA CAG GTT AAC

SINGLE LETTER ABBREVIATIONS FOR MIXED BASE POSITIONS

N = (A,G,C,T); R = (A,G); Y = (C,T); B = (C,G,T) (i.e. not A); D = (A,G,T) (i.e. not C); H = (A,C,T) (i.e. not G); V = (A,C,G)

(i.e. not T); K = (G,T) (Keto in large groove); M = (A,C) (amino in large groove); S = (G,C) (Strong [3 H-bonds]); W = (A,T)

(Weak [2 H-bonds])

Table 5. List of RAPD markers and their sequences used in this study.

S. No	Primer Name	Sequence
1	OPAF1	CCTACACGGT
2	OPAF2	CAGCCGAGAA
3	OPAF3	GAAGGAGGCA
4	OPAF4	TTGCGGCTGA
5	OPAF5	CCCGATCAGA
6	OPAF6	CCGCAGTCTG
7	OPAF7	GGAAAGCGTC
8	OPAF8	CTCTGCCTGA
9	OPAF9	CCCCTCAGAA
10	OPAF10	GGTTGGAGAC
11	OPAF11	ACTGGGCCTC
12	OPAF12	GACGCAGCTT
13	OPAF13	CCGAGGTGAC
14	OPAF14	GGTGCGCACT
15	OPAF15	CACGAACCTC
16	OPAF16	TCCCGGTGAG
17	OPAF17	TGAACCGAGG
18	OPAF18	GTGTCCCTCT
19	OPAF19	GGACAAGCAG
20	OPAF20	CTCCGCACAG
21	OPAG1	CTACGGCTTC
22	OPAG2	CTGAGGTCCT
23	OPAG3	TGCGGGAGTG
24	OPAG4	GGAGCGTACT
25	OPAG5	CCCACTAGAC
26	OPAG6	GGTGGCCAAG
27	OPAG7	CACAGACCTG
28	OPAG8	AAGAGCCCTC
29	OPAG9	CCGAGGGGTT
30	OPAG10	ACTGCCCGAC
31	OPAG11	TTACGGTGGG
32	OPAG12	CTCCCAGGGT
33	OPAG13	GGCTTGGCGA
34	OPAG14	CTCTCGGCGA
35	OPAG15	CCCACACGCA
36	OPAG16	CCTGCGACAG
37	OPAG17	AGCGGAAGTG
38	OPAG18	GTGGGCATAC
39	OPAG19	AGCCTCGGTT
40	OPAG20	TGCGCTCCTC
41	OPBB1	ACACTGGCTG
42	OPBB2	CCCCCGTTAG
43	OPBB3	TCACGTGGCT
44	OPBB4	ACCAGGTCAC
45	OPBB5	GGGCCGAACA
46	OPBB6	CTGAAGCTGG
47	OPBB7	GAAGGCTGGG
48	OPBB8	TCGTCGAAGG
49	OPBB9	AGGCCGGTCA
50	OPBB10	ACTTGCCTGG
51	OPBB11	TGCGGGTTCC

52	OPBB12	TTCGGCCGAC
53	OPBB13	CTTCGGTGTG
54	OPBB14	GTGGGACCTG
55	OPBB15	AAGTGCCCTG
56	OPBB16	TCGGCACCGT
57	OPBB17	ACACCGTGCC
58	OPBB18	CAACCGGTCT
59	OPBB19	TTGCGGACAG
60	OPBB20	CCAGGTGTAG
61	OPR1	TGCGGGTCCT
62	OPR2	CACAGCTGCC
63	OPR3	ACACAGAGGG
64	OPR4	CCCGTAGCAC
65	OPR5	GACCTAGTGG
66	OPR6	GTCTACGGCA
67	OPR7	ACTGGCCTGA
68	OPR8	CCCGTTGCCT
69	OPR9	TGAGCACGAG
70	OPR10	CCATTCCCCA
71	OPR11	GTAGCCGTCT
72	OPR12	ACAGGTGCGT
73	OPR13	GGACGACAAG
74	OPR14	CAGGATTCCC
75	OPR15	GGACAACGAG
76	OPR16	CTCTGCGCGT
77	OPR17	CCGTACGTAG
78	OPR18	GGCTTTGCCA
79	OPR19	CCTCCTCATC
80	OPR20	ACGGCAAGGA
81	OPS1	CTACTGCGCT
82	OPS2	CCTCTGACTG
83	OPS3	CAGAGGTCCC
84	OPS4	CACCCCCTTG
85	OPS5	TTTGGGGCCT
86	OPS6	GATACCTCGG
87	OPS7	TCCGATGCTG
88	OPS8	TTCAGGGTGG
89	OPS9	TCCTGGTCCC
90	OPS10	ACCGTTCCAG
91	OPS11	AGTCGGGTGG
92	OPS12	CTGGGTGAGT
93	OPS13	GTCGTTCCTG
94	OPS14	AAAGGGGTCC
95	OPS15	CAGTTCACGG
96	OPS16	AGGGGGTTCC
97	OPS17	TGGGGACCAC
98	OPS18	CTGGCGAACT
99	OPS19	GAGTCAGCAG
100	OPS20	TCTGGACGGA
101	OPA1	CAGGCCCTTC
102	OPA2	TGCCGAGCTG
103	OPA3	AGTCAGCCAC

104	OPA4	AATCGGGCTG
105	OPA5	AGGGGTCTTG
106	OPA6	GGTCCCTGAC
107	OPA7	GAAACGGGTG
108	OPA8	GTGACGTAGG
109	OPA9	GGGTAACGCC
110	OPA10	GTGATCGCAG
111	OPA11	CAATCGCCGT
112	OPA12	TCGGCGATAG
113	OPA13	CAGCACCCAC
114	OPA14	TCTGTGCTGG
115	OPA15	TTCCGAACCC
116	OPA16	AGCCAGCGAA
117	OPA17	GACCGCTTGT
118	OPA18	AGGTGACCGT
119	OPA19	CAAACGTCGG
120	OPA20	GTTGCGATCC
121	OPB1	GTTTCGCTCC
122	OPB2	TGATCCCTGG
123	OPB3	CATCCCCCCT
124	OPB4	GGACTGGAGT
125	OPB5	TGCGCCCTTC
126	OPB6	TGCTCTGCCC
127	OPB7	GGTGACGCAG
128	OPB8	GTCCACACGG
129	OPB9	TGGGGGACTC
130	OPB10	CTGCTGGGAC
131	OPB11	GTAGACCCGT
132	OPB12	CCTTGACGCA
133	OPB13	TTCCCCCGCT
134	OPB14	TCCGCTCTGG
135	OPB15	GGAGGGTGTT
136	OPB16	TTTGCCCGGA
137	OPB17	AGGGAACGAG
138	OPB18	CCACAGCAGT
139	OPB19	ACCCCCGAAG
140	OPB20	GGACCCTTAC
141	OPC1	TTCGAGCCAG

142	OPC2	GTGAGGCGTC
143	OPC3	GGGGGTCTTT
144	OPC4	CCGCATCTAC
145	OPC5	GATGACCGCC
146	OPC6	GAACGGACTC
147	OPC7	GTCCCGACGA
148	OPC8	TGGACCGGTG
149	OPC9	CTCACCGTCC
150	OPC10	TGTCTGGGTG
151	OPC11	AAAGCTGCGG
152	OPC12	TGTCATCCCC
153	OPC13	AAGCCTCGTC
154	OPC14	TGCGTGCTTG
155	OPC15	GACGGATCAG
156	OPC16	CACACTCCAG
157	OPC17	TTCCCCCCAG
158	OPC18	TGAGTGGGTG
159	OPC19	GTTGCCAGCC
160	OPC20	ACTTCGCCAC
161	OPK1	CATTCGAGCC
162	OPK2	GTCTCCGCAA
163	OPK3	CCAGCTTAGG
164	OPK4	CCGCCCAAAC
165	OPK5	TCTGTCGAGG
166	OPK6	CACCTTTCCC
167	OPK7	AGCGAGCAAG
168	OPK8	GAACACTGGG
169	OPK9	CCCTACCGAC
170	OPK10	GTGCAACGTG
171	OPK11	AATGCCCCAG
172	OPK12	TGGCCCTCAC
173	OPK13	GGTTGTACCC
174	OPK14	CCCGCTACAC
175	OPK15	CTCCTGCCAA
176	OPK16	GAGCGTCGAA
177	OPK17	CCCAGCTGTG
178	OPK18	CCTAGTCGAG
179	OPK19	CACAGGCGGA

180	OPK20	GTGTCGCGAG
181	OPM1	GTTGGTGGCT
182	OPM2	ACAACGCCTC
183	OPM3	GGGGGATGAG
184	OPM4	GGCGGTTGTC
185	OPM5	GGGAACGTGT
186	OPM6	CTGGGCAACT
187	OPM7	CCGTGACTCA
188	OPM8	TCTGTTCCCC
189	OPM9	GTCTTGCGGA
190	OPM10	TCTGGCGCAC
191	OPM11	GTCCACTGTG
192	OPM12	GGGACGTTGG
193	OPM13	GGTGGTCAAG
194	OPM14	AGGGTCGTTC
195	OPM15	GACCTACCAC
196	OPM16	GTAACCAGCC
197	OPM17	TCAGTCCGGG
198	OPM18	CACCATCCGT
199	OPM19	CCTTCAGGCA
200	OPM20	AGGTCTTGGG
201	OPN1	CTCACGTTGG
202	OPN2	ACCAGGGGCA
203	OPN3	GGTACTCCCC
204	OPN4	GACCGACCCA
205	OPN5	ACTGAACGCC
206	OPN6	GAGACGCACA
207	OPN7	CAGCCCAGAG
208	OPN8	ACCTCAGCTC
209	OPN9	TGCCGGCTTG
210	OPN10	ACAACTGGGG
211	OPN11	TCGCCGCAAA
212	OPN12	CACAGACACC
213	OPN13	AGCGTCACTC
214	OPN14	TCGTGCGGGT
215	OPN15	CAGCGACTGT
216	OPN16	AAGCGACCTG
217	OPN17	CATTGGGGAG

218	OPN18	GGTGAGGTCA
219	OPN19	GTCCGTACTG
220	OPN20	GGTGCTCCGT
221	OPAH1	TCCGCAACCA
222	OPAH2	CACTTCCGCT
223	OPAH3	GGTTACTGCC
224	OPAH4	CTCCCCAGAC
225	OPAH5	TTGCAGGCAG
226	OPAH6	GTAAGCCCCT
227	OPAH7	CCCTACGGAG
228	OPAH8	TTCCCGTGCC
229	OPAH9	AGAACCGAGG
230	OPAH10	CCTACGTCAG
231	OPAH11	TCCGCTGAGA
232	OPAH12	TCCAACGGCT
233	OPAH13	TGAGTCCGCA
234	OPAH14	TGTGGCCGAA
235	OPAH15	CTACAGCGAG
236	OPAH16	CAAGGTGGGT
237	OPAH17	CAGTGGGGAG
238	OPAH18	GGGCTAGTCA
239	OPAH19	GGCAGTTCTC
240	OPAH20	GGAAGGTGAG
241	OPAK1	TCTGCTACGG
242	OPAK2	CCATCGGAGG
243	OPAK3	GGTCCTACCA
244	OPAK4	AGGGTCGGTC
245	OPAK5	GATGGCAGTC
246	OPAK6	TCACGTCCCT
247	OPAK7	CTTGGGGGAC
248	OPAK8	CCGAAGGGTG
249	OPAK9	AGGTCGGCGT
250	OPAK10	CAAGCGTCAC
251	OPAK11	CAGTGTGCTC
252	OPAK12	AGTGTAGCCC
253	OPAK13	TCCCACGAGT
254	OPAK14	CTGTCATGCC
255	OPAK15	ACCTGCCGTT

256	OPAK16	CTGCGTGCTC
257	OPAK17	CAGCGGTCAC
258	OPAK18	ACCCGGAAAC
259	OPAK19	TCGCAGCGAG
260	OPAK20	TGATGGCGTC
261	OPE1	ACGGATCCTG
262	OPE2	GAGGATCCCT
263	OPE3	CCTGATCAGG
264	OPE4	GGTGATCAGG
265	OPE5	CCGAATTCCC
266	OPE6	GGGAATTCGG
267	OPE7	CCGATATCCC
268	OPE8	GGGATATCGG
269	OPE9	CCAAGCTTCC
270	OPE10	GGAAGCTTGG
271	OPE11	TTGGTACCCC
272	OPE12	ACGGTACCAG
273	OPE13	GGCTGCAGAA
274	OPE14	TGCTGCAGGT
275	OPE15	CCAGTACTCC
276	OPE16	GGAGTACTGG
277	OPE17	AACCCGGGAA
278	OPE18	TTCCCGGGTT
279	OPE19	CCTCTAGACC
280	OPE20	GGTCTAGAGG
281	OPI1	ACCTGGACAC
282	OPI2	GGAGGAGAGG
283	OPI3	CAGAAGCCCA
284	OPI4	CCGCCTAGTC
285	OPI5	TGTTCCACGG
286	OPI6	AAGGCGGCAG
287	OPI7	CAGCGACAAG
288	OPI8	TTTGCCCGGT
289	OPI9	TGGAGAGCAG
290	OPI10	ACAACGCGAG
291	OPI11	ACATGCCGTG
292	OPI12	AGAGGGCACA
293	OPI13	CTGGGGCTGA

294	OPI14	TGACGGCGGT
295	OPI15	TCATCCGAGG
296	OPI16	TCTCCGCCCT
297	OPI17	GGTGGTGATG
298	OPI18	TGCCCAGCCT
299	OPI19	AATGCGGGAG
300	OPI20	AAAGTGCGGG
301	OPL1	GGCATGACCT
302	OPL2	TGGGCGTCAA
303	OPL3	CCAGCAGCTT
304	OPL4	GACTGCACAC
305	OPL5	ACGCAGGCAC
306	OPL6	GAGGGAAGAG
307	OPL7	AGGCGGGAAC
308	OPL8	AGCAGGTGGA
309	OPL9	TGCGAGAGTC
310	OPL10	TGGGAGATGG
311	OPL11	ACGATGAGCC
312	OPL12	GGGCGGTACT
313	OPL13	ACCGCCTGCT
314	OPL14	GTGACAGGCT
315	OPL15	AAGAGAGGGG
316	OPL16	AGGTTGCAGG
317	OPL17	AGCCTGAGCC
318	OPL18	ACCACCCACC
319	OPL19	GAGTGGTGAC
320	OPL20	TGGTGGACCA

Table 6: List of drought responsive ESTs used in this study, their gene name, size and chromosomal location.

EST ID #	Name of the gene / EST	Chromosome #	Gene size (bp)
GSP1	Chlorophyll a/b binding protein	1	952
GSP2	Ribosomal protein S4	1	936
GSP3	Peroxiredoxin	1	570
GSP4	Root specific Rcc3	2	646
GSP5	Shaggy like kinase etha	2	1709
GSP6	Rab28 protein	3	1772
GSP7	Reversibly glycosylated protein	3	1080
GSP8	Alpha tubulin	3	1602
GSP9	EF-1 alpha	3	1555
GSP10	*OsCDPK7*	4	2092
GSP11	Cytochrome B5	5	750
GSP12	EF-hand Ca^{2+} binding protein	6	588
GSP13	Sucrose synthase	6	2568
GSP14	Aquaporin	7	1314
GSP15	Novel protein	-	1420
GSP16	Thioredoxin H	7	2876
GSP17	Translation initiation factor	7	718
GSP18	Glyoxylase I	8	931
GSP19	Heat shock protein	9	296
GSP20	ABA and stress inducible protein	-	836
GSP21	Early nodulin	-	600
GSP22	Glutathione dependant dehydroascorbate reductase precursor	-	855
GSP23	**Metallothionein like protein**	**-**	**538**
GSP24	Ascorbate peroxidases	7	959
GSP25	Dehydrin	-	824
GSP26	Sucrose transporter	-	7400
GSP27	Alpha 1 tubulin	7	1677
GSP28	Beta expansin	10	1173
GSP29	Calmodulin	-	773
GSP30	Q group of receptor for activated C-kinase	-	1227
GSP31	Small GTP binding protein	2	836

GSP32	rab related GTP binding protein possessing GTPase activity	1	881
GSP33	Metallothionein like protein II	-	540
GSP34	Sucrose 6F phosphate phosphohydrolase	1	1714
GSP35	Succinate dehydrogenase subunit 3	2	2819
GSP36	NADH dehydrogenase	8	1131
GSP37	Beta glucosidase isozyme 2 precursor	-	1733
GSP38	Glutaredoxin	4	529
GSP39	Ca^{2+} sensitive 3'(2'), 5 diphospho nucleoside 3'(2') phoshohydrolase	-	1431
GSP40	Mitochondrial ATP synthase 6KD subunit	-	452
GSP41	H protein subunit Glycine decarboxylase	10	764
GSP42	UDP- Glucoruronic acid decarboxylase	3	1013
GSP43	Heat shock protein 82	9	667
GSP44	En 242 homologe of wound inducible basic protein	-	308
GSP45	ATP/ADP translocator	-	1544
GSP46	Mitochondrial phosphate translocator	-	1520
GSP47	Glycine rich RNA binding protein	-	810
GSP48	Hydroxy praline rice glycoprotein	4	2630
GSP49	Glycine rich protein	-	660
GSP50	26S proteosome regulatory particle triple-A ATPase subunit 2b	-	660
GSP51	26S proteosome regulatory particle non-ATPase subunit 9b	-	1189
GSP52	Ethylene responsive protein	3	1344
GSP53	Reversibly glycosylated polypeptide	3	1366
GSP54	22 KDa protein of photosystemII	1	1069
GSP55	10 KDa phosphoprotein potential component of photosystem II	4	324
GSP56	Ribulose 5 phosphate 3 epimerase	3	993
GSP57	Triosephosphate isomerase	1	4127
GSP58	Triosephosphate isomerase	1	1050
GSP59	Mitochondrial ribulose bisphosphate carboxylase/oxygenase	-	790
GSP60	Small subunit of ribulose 1, 5 bisphosphate carboxylase	-	797
GSP61	Chloroplast ATP synthase beta subunit	12	1521
GSP62	Light harvesting chlorophyll a/b binding protein of PS II	1	1002
GSP63	Light harvesting chlorophyll a/b binding protein	1	1432
GSP64	Chlorophyll a/b binding protein	7	1152
GSP65	PS II D1 protein		360
GSP66	RuBISCO activase small subunit		1641

GSP67	Fructose 1, 6 bisphosphate	1	1518
GSP68	Glyceraldehyde 3 phosphate dehydrogenase	8	1316
GSP69	Elongation factor 1 beta	7	1375
GSP70	21 KD polypeptide	-	772
GSP71	Ubiquitin protein fused to a ribosomal protein	3	610
GSP72	Cyc07	-	1065
GSP73	5S ribosomal RNA	1	1089
GSP74	Alanine:glyoxylate aminotransferase	8	540
GSP75	Aldolase	1	309
GSP76	Vegetative storage protein	5	1100
GSP77	*OsMYB1*	-	1157
GSP78	Zinc finger protein	3	830

Table 7. Site, soil and drought characteristics of field trials.

Particular	Trial 1, Coimbatore	Trial 2, Paramakudi
Elevation (meters above sea level)	427	40
Latitude & Longitude	11^{o}N, 77^{o}E	9^{o} N, 70^{o} E
Soil texture	Clay loam	Black clay
Soil pH	8.4	8.1
Characterization of stress	Severe	Severe
Timing of start of stress (Days after sowing)	60	78
Duration of the stress period (days)	28	51
Rainfall during the stress period (mm)	51	2
Number of continuous rain free days during stress period	18	45
Rainfall during crop period (mm)	306	233
Mean temperature (^{o}C)		
Maximum	36.5	37.8
Minimum	18.0	19.0
Average Relative Humidity (%)	70	86.5

CHATPER IV

EXPERIMENTAL RESULTS

This chapter describes the laboratory and field results obtained from the various experiments conducted to construct a linkage map and to identify the quantitative trait loci (QTL) associated with drought resistance traits under field conditions in rice.

Mapping Population

A recombinant inbred (RI) lines population was developed from a cross involving IR20/Nootripathu which are well adapted to the rainfed target population of environments (TPE) and they differ for a range of root related drought resistance traits. A total of 397 F_7 RI lines were developed by single seed descent and a subset of 250 RI lines was selected randomly from this population. The transgressive segregation of the selected F_7 RI lines for plant stature is shown in Plate 1. These selected RI lines were used for genotyping and phenotyping to identify QTLs linked to drought resistance traits.

Genotyping of parents and RI lines

The genomic DNA was isolated from the parents and RI lines and the concentration of DNA was diluted to 50 ng/µl. The quality and the quantity of DNA in all the samples were checked for their suitability in PCR reactions and the reaction mixture for different molecular markers were standardized. Initially, the parents were genotyped for their polymorphism using simple sequence repeats (SSR), inter simple sequence repeats (ISSRs), random amplified polymorphic DNA (RAPD), candidate genes or expressed sequence tags (EST) and SSRs derived from ESTs. The percentage of polymorphism between the two parents varied with different kinds of markers.

Simple sequence repeats

A total of 627 SSR markers that are distributed throughout the rice chromosomes were selected to screen the two parents. Among these, 82 primers could able to distinguish the two parents, but only 63 primers (10.04 per cent polymorphism) were produced unambiguous polymorphic segregating bands in the RI lines. Rest of the 19 primers produced ambiguous, non-reproducible stutter bands which could not be scored in RI lines. The genotypic score for the 63 polymorphic SSR primers on the RI lines was done to construct the linkage map. A representative SSR primer PCR profile is given in Plate 2. Each polymorphic SSR marker was identified by the rice microsatellite (RM) primer number.

Inter simple sequence repeats

Among the 100 ISSR primers used for parental survey, 28 primers could able to amplify fragments in these two genotypes. Within these 28 primers, 13 had identified as polymorphic primers. But only 10 primers (35.7 per cent polymorphism) have generated 16 informative polymorphic markers in the RI lines. Those primers, which have been used for genotypic scoring of progenies, were ISSR07, ISSR09, ISSR10, ISSR12, ISSR17, ISSR23, ISSR40, ISSR41, ISSR57 and ISSR89. Each polymorphic ISSR marker was identified by the starting letter “I” suffixed with primer number and “S”, followed by molecular weight in bp. For example, if the primer ISSR07 generated a polymorphic fragment of 1383 bp, it was named as “I07S_1383”. A representative ISSR primer PCR profile is given in Plate 3.

Random amplified polymorphic DNA

Totally 320 random decamer operon primers (OP) were used for parental screening and 50 were found to be polymorphic between the parents. Among these, 46 primers (14.37 per cent polymorphism) could able to generate reproducible and unambiguous segregating bands and were used for genotypic

scoring of 61 RAPD markers in the RI lines. The polymorphic random decamer primers are OPA01, OPA02, OPA04, OPA10, OPA11, OPA13, OPA14, OPA19, OPAK19, OPB04, OPB05, OPB06, OPB08, OPB12, OPB18, OPB20, OPBB01, OPBB07, OPC20, OPC04, OPC06, OPI03, OPK09, OPK10, OPK11, OPK12, OPK15, OPK16, OPK20, OPL08, OPL14, OPL19, OPL20, OPM06, OPM10, OPN18, OPP12, OPP16, OPR02, OPR04, OPR20, OPS09, OPS10, OPS11, OPS12 and OPS20. Each polymorphic RAPD marker is identified by the primer name suffixed with the molecular weight in base pairs. For example, if operon primer A01 generated a polymorphic fragment of 961 bp, it was named A01_961. If AK01 generated it, it was named A10K_961, since the marker name is required to be unambiguous and to contain not more than 8 characters for the MAPMAKER program. A representative RAPD primer PCR profile is given in Plate 4.

Expressed sequence tags

Seventy eight EST markers were used to screen the parents and eight were found to be polymorphic between the parents. Ultimately only one primer (GSP23- Metallothionein like protein) produced a segregating band (1.28 per cent polymorphism) in all the RI lines and it was used for mapping effort. Rest of the seven primers produced ambiguous stutter bands and are not pursued further.

SSR derived from ESTs

None of the 12 primers used in this study developed polymorphism between the parents.

As a whole, among the 1125 primers used for parental screening, 120 primers (10.63 per cent) alone generated 141 informative markers and these were used in genetic map construction. Segregating bands were scored as either A (IR20 allele) or B (Nootripathu allele) and ambiguous or improperly amplified and heterozygous bands (progenies having both the parental alleles) were scored as M (missing data).

Segregation of polymorphic markers

All the polymorphic markers (SSRs, ISSRs, RAPDs and ESTs) were evaluated individually by the χ^2 test for goodness of fit against a 1:1 segregation ratio at a 0.01 and 0.05 probability levels. Table 8 shows the χ^2 value calculated for segregation of the individual markers in the RI lines along with the chromosomal location of the SSR markers.

Map construction

Most of the polymorphic markers showed extreme distortion towards IR20 and so clustered as a single group irrespective of their chromosomal location. Those markers have been removed from the mapping process and only 80 markers that are segregating in the expected ratio at the 0.05 probability levels were used. Markers in bold letters in Table 8 are the 61 polymorphic markers that were highly deviating from the expected segregation ratio in the RI lines. Initially the selected 80 polymorphic markers were grouped using high LOD (8.0) with low minimal distance (30 cM) and this gathered the 56 markers into 17 linkage groups and 24 unlinked markers. Then the SSR markers belong to the same chromosome but in different linkage groups were combined together with low LOD (3.0) and optimum distance (50 cM) along with the other type of (ISSR/RAPD/EST) markers. This resulted 11 linkage groups and they were assigned to rice chromosomes except for chromosome 7 since both of the SSR markers belongs to this chromosome was not found to be linked and both of the SSR marker on this chromosome were unlinked to anyone of the RAPD/ISSR/EST markers. In addition to these 11 linkage groups, there were three unlinked groups containing RAPD markers and they were not assigned to any chromosome. Rest of the unlinked markers were tried to group into the established linkage groups and those, which are not linked to those linkage groups or those markers that lengthened the map distance, were left as such. The linkage map was drawn with the Haldane mapping function (Figure 2) with a total map length of 652 cM and compared with the published maps for their relative order and distance.

All the seven SSR markers of chromosome 1 used in the mapping process were grouped as single linkage group with same order as that of the published map (McCouch *et al.,* 2002). This linkage group did not contain RAPD, ISSR or EST marker. Among the seven SSR markers used for chromosome 2, five markers were grouped together and RM3294 and RM5699 remained unlinked with any one of the other groups. This linkage group did not contain RAPD, ISSR or EST marker. The chromosome 3 had three markers, which include two SSR markers, and one ISSR marker, I17S_1882. Only one SSR marker alone was used on chromosome 4 in the mapping process and it was grouped with a RAPD marker, C20_1000 with 15.8 cM distance. Two SSR markers were grouped along with a RAPD marker, K11_425 in chromosome 5. Chromosome 6 had two SSR and two RAPD markers (K16_1756 and S12_1000) as a single linkage group and one SSR marker (RM1369) of this chromosome remained unlinked. None of the markers were assigned to chromosome 7 since the SSR markers (RM10 and RM11) of this chromosome were unlinked in this study. Chromosome 8 had two linkage groups. One had RM152 and a RAPD marker, L19_450 and another had RM210 and two RAPD markers, A14_377 and A04_750. One of the SSR marker (RM230) of this chromosome remained unlinked. All the four SSR markers in the chromosome 9 were grouped as a single linkage group along with a RAPD marker, A10_600. Chromosome 10 had a linkage group containing one SSR marker (RM330), two RAPD markers (B18_850 and B01B_250) and one EST marker (GSP23). The SSR marker RM1859 of this chromosome remained unlinked in this study. Linkage group of chromosome 11 had one SSR marker (RM209) and five RAPD markers (I03_500, A13_1339, A01_961, L14_475 and L20_1113). Chromosome 12 had two linkage groups. One group included a SSR marker (RM101) and a RAPD marker (N18_1399). Another group had a SSR marker (RM17) and an ISSR marker (I40S_1300).

Variation of drought resistance traits under water stress

Two field trials were conducted with RI lines along with the parents in the experimental fields of Tamil Nadu Agricultural University, India for QTL mapping of drought resistance traits in rice (Trial 1: Plate 5). Data on various traits such as leaf rolling, leaf drying, relative water content (RWC), canopy temperature, drought recovery, SPAD chlorophyll reading, plant height, number of tillers, days to 50 per cent flowering and biomass under water stress were recorded. Significant variation in all the investigated traits indicated the presence of genetic variation for drought response between the parents (Plate 6) and among the RI lines studied (Plates 7a, 7b and 8). Transgressive segregation in both directions was observed for most of the traits. The frequency distribution of phenotypes for the traits evaluated in this study approximately fitted a normal distribution as shown in Figure 3 for Trial 1. The mean trait values and broad sense heritability (*H*) of the RI lines along with the parents for two trials are summarized in Table 9. These results are briefly described here below:

Leaf rolling

In Trial 1, the average leaf rolling across the RI lines was 6.0 and it ranged from 3.0 to 7.0. IR20 has higher leaf rolling (6.7) than Nootripathu (4.0) under water stress and in some of the RI lines it was as low as 3.0. The leaf rolling has high *H* (0.73) in Trial 1 whereas it is moderate in Trial 2 (0.57).

Leaf drying

Maximum leaf drying score was noticed in IR20 (7.0) whereas Nootripathu had had a score of 4.3 and minimum leaf drying score was as low as 2.3 in some of the RI lines in Trial 1. The average leaf drying across the RI lines was 5.8. Repeatability of this trait was varied with the environment. Trial 1 had high *H* (0.70) where as Trial 2 had moderate *H* (0.58) for leaf drying.

Stress recovery

Complete recovery was not observed in any of the test materials. However, it ranged from 1.0 to 7.0 in Trial 1 and among the parental lines Nootripathu had better recovery (3.0) than IR20 (6.3). The repeatability of this trait was high (0.91) for Trial 1.

Canopy temperature

The mean canopy temperature observed across the RI lines was 43.0°C in Trail 1 and 32.8°C in Trial 2 and it ranged from 38.4 to 46.6°C in Trial 1 and 27.6 to 40.5°C in Trial 2. IR20 recorded higher canopy temperature than Nootripathu in both the trials. The *H* for this trait was high in both the trials.

Relative water content

In Trial 1, Nootripathu had higher RWC (61.8 per cent) than IR20 (42.3 per cent). The average RWC among the RI lines was 59.12 per cent with the minimum of 34.10 and a maximum of 81.4 per cent. The repeatability of this trait was found to be low (0.10) in this trial.

Basal root thickness

Among the parental lines, thicker roots were observed in Nootripathu (1.09 mm) whereas IR20 had thinner roots (0.7 mm) in Trial 1. The mean root thickness among the RI lines was 0.92 mm with a range of 0.55 to 1.22 mm. The *H* for this trait was moderately high (0.78) in this trial.

Plant height

Nootripathu was taller than IR20 in both trials. The RI lines have different heights ranging from 24.9 to 72.2 cm with a mean of 40.5 cm in Trial 1 and in Trial 2 it ranged from 42.0 to 86.5 cm with a mean of 62.5 cm. The repeatability of this trait was found to be high in Trial 1 and 2.

Number of tillers

IR20 had reduced number of tillers (5.3) than Nootripathu (7.9) under water stress in Trial 1. The average number of tillers among the RI lines was 5.8 and it ranged from 2.4 - 12.2 in Trial 1, whereas in Trial 2 it ranged from 5.3 - 10.3. However, the repeatability of this trait was high in Trial 1 (0.67) and low in Trial 2 (0.30).

Biomass under stress

Among the parental lines, Nootripathu had produced higher biomass (157.3 g/m^2) than IR20 (33.0 g/m^2) in Trial 1. Biomass in the RI lines ranged from as low as 64.6 to as high as 1638.0 g/m^2 in Trial 1 with a mean of 177.6 g/m^2 whereas the mean value in Trial 2 was 292.3 g/m^2. However, *H* value was higher in Trial 1 (0.91) than Trial 2 (0.56).

SPAD chlorophyll reading

Nootripathu had higher chlorophyll content (35.6) than IR20 (38.6) in Trial 2 and in RI lines it ranged from 26.6 to 45.3 with a mean of 36.0. The broad sense heritability for this trait was high (0.61).

Correlation between drought resistance traits and biomass under water stress

The phenotypic correlations among the traits showed that parameters of water stress indicators were significantly correlated with biomass production under stress in both the trials.

The correlation coefficients (r) among various traits under drought stress were presented in Table 10 and 11. Significant positive correlations were found between biomass under stress and plant height (r = 0.59**), tiller number (r = 0.61**) and basal root thickness (r = 0.20**) in Trial 1. Similarly in Trial 2, significant positive correlations were found for biomass under stress and plant height (r = 0.47**). A significant negative correlation was found between water stress indicators *viz.,* leaf rolling (r = -0.42**), leaf drying (r = -0.61**) and canopy temperature (r = -0.37**) with biomass under stress in Trial 1. In Trial 2, a significant negative correlation was observed between leaf rolling (r = -0.13**) and leaf drying (r = -0.11*) with biomass under water stress.

Molecular markers linked to drought resistance traits

Single marker analysis was performed with all the markers used in linkage map construction with the phenotypic data obtained from Trial 1 and 2, since the linkage map constructed was very sparse in most of the chromosomes. The significant threshold for identification of QTL or molecular marker linked to a trait was set as less than 0.02 probability level for single marker analysis. Table 12 and 13 represent the markers identified for different traits at this stringent threshold level, in Trial 1 and 2, respectively. SSR markers *viz.,* RM 212 (chromosome 1), RM263 (chromosome 2), RM5424 (chromosome 4), RM6862 (chromosome 9) and RAPD markers *viz.,* C20_1000 (chromosome 4), S12_1000 (chromosome 6) were found to be linked with same drought resistant traits under water stress conditions in the field across the trials (Figure 4). However, markers have also been identified specific to the particular trial. For example, in Trial 1, RM11 (chromosome 7) was identified as a marker linked to leaf drying and in Trial 2, RM289 on chromosome 5 was found to be linked to plant height.

QTLs linked to leaf rolling

A total of 15 QTLs (12 and 4 QTLs in Trial 1 and 2, respectively) were identified for leaf rolling. Only one marker, RM6862 on chromosome 9, was common between the two trials. None of the RAPD and ISSR markers identified were common between trials. RM1342 had shown highest variation (6.29 per cent) in Trial 1, whereas in Trial 2, L20_1113 had shown highest variation (5.61 per cent) for this trait.

QTLs linked to leaf drying

Twenty-four QTLs were identified for leaf drying. Among these, 16 markers had shown putative linkage with leaf drying in Trial 1 and 11 markers had linked to this trait in Trial 2. Three RAPD markers were common in both the trials. No SSR and ISSR markers were found to be common between the two trials. Highest variation for this trait was shown by the marker RM3515 (6.21 per cent) in Trial 1 and P16_600 (5.85 per cent) in Trial 2.

QTLs linked to drought recovery

Stress recovery score could not be made in Trial 2 because of severe stress due to complete monsoon failure. However, 12 QTLs were identified for this trait in Trial 1. RM5424 had shown highest variation (9.83 per cent) for stress recovery followed by RM1342, which explained 5.88 per cent variation on this trait.

QTLs linked to plant height

Fifteen QTLs in Trial 1 and 11 QTLs in Trial 2 were identified for plant height under water stress. Among them six QTLs were common in both the trials and thus a total of 20 QTLs were found to be linked

with plant height in this study. RM212 has exhibited highest phenotypic variation for this trait in both the trials: 28.69 and 22.9 per cent in Trial 1 and 2, respectively.

QTLs linked to number of tillers

Totally 16 QTLs were identified for tiller number in both the trials *i.e.,* 8 QTLs in Trial 1 and 8 QTLs in Trial 2. None of the markers were common between the trials. In Trial 1, highest phenotypic variation (7.61 per cent) was explained by RM5424 and B06_700 had explained the highest variation of 6.15 per cent in Trial 2.

QTLs linked to biomass under stress

Eight putatively linked QTLs were identified for biomass under water stress in this study. Among these, two QTLs were common for both the trials and four were unique to Trial 1 and two were unique to Trial 2. RM5424 had highest phenotypic variation on this trait both in Trial 1 (4.37 per cent) and Trial 2 (5.27 per cent).

QTLs linked to canopy temperature

Totally 20 QTLs were identified for canopy temperature in both the trials and only one QTL is common across the trials. Trial 1 had 4 QTLs and Trial 2 had 17 QTLs linked to canopy temperature under water stress. The highest phenotypic variation for this trait was given by RM1342 (5.22 per cent) in Trial 1 and RM6836 (11.31 per cent) in Trial 2.

QTLs linked to relative water content

Four QTLs were found to be linked with RWC in Trial 1. Among these, S12_1000 had shown highest phenotypic variation (7.11 per cent) for this trait.

QTLs linked to basal root thickness

Four QTLs were identified for basal root thickness under water stress in Trial 1. Among these C20_1000 exhibited highest phenotypic variation (4.29 per cent) for this trait.

QTLs linked to SPAD chlorophyll reading

Three QTLs were identified for SPAD chlorophyll reading in Trial 2 and RM5424 had shown 7.33 per cent phenotypic variation for this trait.

QTLs linked to days to 50 per cent flowering

Only one QTL, I23S_1761 was found to be linked with days to 50 per cent flowering under water stress in Trial 2 and it explained 7.66 per cent phenotypic variation.

In summary, a total of 54 QTLs were identified for the 11 different traits under water stress condition in two different field trials, which individually explained 2.1 to 28.69 per cent of phenotypic variation. Common QTLs were found to control the same trait in both the trials. Several chromosomal regions linked to more than one trait were identified (Figure 4). It is also noticed that those regions were found to be linked with different traits across genetic backgrounds. Thus consistent QTLs for drought resistance traits and plant production under water stress were detected in this study.

Table . Chromosome wise distribution of polymorphic SSR marker between IR20/Nootripathu

Chr#1	Chr#2	Chr#3	Chr#4	Chr#5	Chr#6	Chr#7	Chr#8	Chr#9	Chr#10	Chr#11	Chr#12
RM5, RM9, RM151, RM212, RM259, RM302, RM306, RM488, RM1287, RM6464, RM8051, RM8077	RM154, RM213, RM263, RM485, RM1342, RM3294, RM3515, RM5699, RM7426	RM232, RM426, RM5477, RM517	RM1136, RM3643, RM5424, RM5749, RM7187, RM8213	RM164, RM289, RM3631	RM170, RM204, RM276, RM314, RM1369, RM6836	RM10, RM11, RM346, RM3423	RM152, RM210, RM230	RM107, RM242, RM278, RM410, RM3164, RM5122, RM6294, RM6862	RM330, RM1859, RM3773, RM6745	RM209	RM4A, RM17, RM19, RM20, RM101

Table 8. Segregation of polymorphic markers in the IR20/Nootripathu RI lines and chromosomal location of SSR markers.

S. No.	Marker	Chromosome Number	Number of IR20 alleles	Number of Nootripathu alleles	χ^2 value
1	**RM6464**	**1**	**248**	**2**	**242.064**
2	RM151	1	105	112	4.552
3	**RM8077**	**1**	**248**	**2**	**242.064**
4	RM8051	1	139	109	3.616
5	RM1287	1	106	120	3.088
6	RM9	1	86	140	13.968
7	**RM306**	**1**	**239**	**6**	**217.256**
8	**RM5**	**1**	**248**	**2**	**242.064**
9	RM488	1	113	121	1.28
10	RM212	1	104	129	3.656
11	RM302	1	116	110	2.448
12	**RM485**	**2**	**116**	**67**	**27.56**
13	RM154	2	124	112	1.36
14	RM3294	2	113	114	2.12
15	RM5699	2	142	89	12.68
16	**RM7426**	**2**	**249**	**1**	**246.016**
17	RM3515	2	143	94	10.28
18	RM1342	2	137	95	8.352
19	RM263	2	95	132	7.592
20	RM213	2	129	114	1.096
21	RM5477	3	110	123	1.832
22	**RM517**	**3**	**248**	**1**	**244.04**
23	RM232	3	108	123	2.344
24	**RM426**	**3**	**248**	**1**	**244.04**
25	**RM8213**	**4**	**247**	**3**	**238.144**
26	**RM5749**	**4**	**245**	**3**	**234.272**
27	**RM3643**	**4**	**111**	**106**	**4.456**
28	RM5424	4	138	102	5.584
29	**RM1136**	**4**	**81**	**167**	**29.6**
30	**RM7187**	**4**	**98**	**82**	**20.624**
31	RM289	5	116	125	0.648
32	RM164	5	110	129	1.928
33	**RM3631**	**5**	**92**	**135**	**9.512**
34	RM1369	6	82	128	14.864
35	**RM204**	**6**	**247**	**3**	**238.144**
36	**RM276**	**6**	**150**	**99**	**10.408**
37	RM314	6	86	134	12.816
38	RM6836	6	123	110	1.832
39	**RM346**	**7**	**248**	**2**	**242.064**
40	RM10	7	131	96	7.016
41	RM11	7	105	129	3.328
42	**RM3423**	**7**	**248**	**2**	**242.064**
43	RM152	8	123	108	2.344
44	RM210	8	138	92	10.064

45	RM230	8	110	110	3.6
46	RM5122	9	117	124	0.52
47	**RM410**	**9**	**118**	**97**	**6.664**
48	RM242	9	105	116	3.848
49	RM3164	9	109	100	7.048
50	**RM278**	**9**	**115**	**123**	**0.832**
51	RM6862	9	125	91	9.248
52	**RM107**	**9**	**118**	**100**	**5.392**
53	**RM6294**	**9**	**247**	**3**	**238.144**
54	RM330	10	140	69	26.888
55	RM1859	10	112	111	2.92
56	**RM6745**	**10**	**247**	**3**	**238.144**
57	**RM3773**	**10**	**248**	**2**	**242.064**
58	RM209	11	132	94	8.08
59	RM20	12	133	101	5.12
60	**RM4A**	**12**	**83**	**155**	**21.312**
61	**RM19**	**12**	**225**	**7**	**191.392**
62	RM101	12	110	113	2.952
63	RM17	12	100	107	7.592
64	BB01_1000		82	167	28.904
65	**BB01_700**		**207**	**40**	**111.592**
66	BB01_250		163	83	25.664
67	**BB07_900**		**32**	**208**	**124.304**
68	C20_1000		120	125	0.2
69	**C04_1000**		**199**	**48**	**91.24**
70	C06_1617		155	95	14.4
71	**C06_1243**		**221**	**29**	**147.456**
72	I03_500		115	133	1.312
73	I03_350		136	112	2.32
74	**K09_2111**		**57**	**191**	**71.84**
75	K11_225		86	162	23.12
76	K12_425		134	106	3.536
77	R20_527		11	224	182.376
78	A01_961		123	126	0.04
79	A02_888		106	139	4.456
80	A04_750		116	132	1.04
81	**A10_800**		**20**	**213**	**150.152**
82	A10_600		88	143	13.544
83	A11_844		177	70	45.832
84	**A13_1645**		**203**	**46**	**98.6**
85	A13_1339		141	108	4.36
86	A14_377		133	114	1.48
87	**A19_750**		**68**	**179**	**49.32**
88	AK19_950		121	104	3.656
89	**AK19_450**		**222**	**4**	**192.4**
90	B01_500		144	103	6.76
91	**B05_700**		**247**	**1**	**242.08**
92	B06_700		147	103	7.744
93	**B08_750**		**160**	**75**	**29.8**
94	**B12_600**		**50**	**182**	**70.992**

95	B18_850		158	87	20.264
96	**B18_675**		**244**	**3**	**232.36**
97	**B20_600**		**155**	**92**	**15.912**
98	**K15_1000**		**86**	**164**	**24.336**
99	K16_1000		78	169	33.16
100	**K16_1756**		**197**	**53**	**82.944**
101	**K16_1573**		**49**	**201**	**92.416**
102	**K20_1381**		**162**	**83**	**25.064**
103	**L08_1000**		**248**	**2**	**242.064**
104	L08_400		108	139	3.88
105	L14_475		125	119	0.288
106	L19_450		116	129	0.776
107	L20_1113		96	154	13.456
108	M06_475		150	88	15.952
109	**M10_530**		**74**	**173**	**39.24**
110	N18_1399		132	107	2.984
111	**N18_500**		**97**	**142**	**8.584**
112	N18_425		141	101	6.656
113	**P12_575**		**144**	**97**	**9.16**
114	P16_600		140	101	6.408
115	**R02_1000**		**230**	**12**	**190.352**
116	**R04_1175**		**38**	**190**	**94.352**
117	**R20_525**		**18**	**201**	**137.8**
118	S09_1537		117	132	0.904
119	S09_1180		118	131	0.68
120	S10_400		107	134	3.24
121	S11_450		115	117	1.312
122	S12_1000		98	121	5.96
123	**S12_300**		**175**	**47**	**68.672**
124	**S20_1075**		**164**	**52**	**54.8**
125	IS07_1383		151	98	11.24
126	**IS07_900**		**227**	**22**	**168.104**
127	**IS07_625**		**205**	**42**	**106.312**
128	IS07_500		171	78	34.6
129	IS09_800		133	111	2.08
130	IS10_1000		138	105	4.552
131	IS12_2023		129	117	0.64
132	IS17_1882		142	106	5.2
133	IS23_1761		102	137	5.384
134	IS40_1300		104	144	6.416
135	**IS40_1118**		**74**	**173**	**39.24**
136	**IS40_700**		**152**	**91**	**15.08**
137	**IS41_1900**		**187**	**54**	**71.08**
138	**IS57_850**		**56**	**180**	**62.288**
139	**IS89_800**		**139**	**96**	**8.296**
140	IS89_900		111	125	1.568
141	GSP23		117	89	10.88

χ^2 Tabulated value at 0.01 probability level = 6.63

0.05 probability level = 3.84

Markers in bold letters were extremely deviated from the expected segregation ratio.

Table 9. Trait mean values for IR20, Nootripathu and RI lines for the two field trials.

Trait	Trial	IR20	Nootripathu	RI lines		SD	H
				Mean	Range		
Leaf rolling	Trial 1	6.7	4.0	5.99	3.0 - 7.0	0.71	0.73
	Trial 2	5.7	5.0	6.57	3.0 - 7.0	0.66	0.57
Leaf drying	Trial 1	7.0	4.3	5.79	2.33 - 7.0	0.67	0.70
	Trial 2	4.3	3.0	4.83	2.33 - 7.0	0.95	0.58
Recovery	Trial 1	6.3	3.0	4.88	1.0 - 7.0	1.15	0.91
Canopy temperature (°C)	Trial 1	43.67	39.7	42.98	39.36 - 46.57	1.35	0.75
	Trial 2	35.47	34.33	32.76	27.56 - 40.5	2.24	0.95
Relative water content (%)	Trial 1	42.3	61.8	59.12	34.10 - 81.40	8.80	0.10
Plant height (cm)	Trial 1	27.45	49.55	40.54	24.88 - 72.18	7.73	0.89
	Trial 2	43.76	56.44	62.45	41.99 - 86.46	10.01	0.87
Number of tillers	Trial 1	5.25	7.92	5.80	2.38 - 12.16	1.43	0.67
	Trial 2	7.9	7.2	7.18	5.28 - 10.27	0.86	0.30
Biomass (g/m^2)	Trial 1	32.88	157.28	177.55	64.63 - 1638	119.09	0.91
	Trial 2	148.33	185.0	292.3	302.23 - 1077.0	128.33	0.56
Basal root thickness (mm)	Trial 1	0.70	1.09	0.92	0.55 - 1.22	0.12	0.78
Days to 50% flowering (days)	Trial 2	-	86.0	82.38	64.74 - 88.09	5.01	0.31
SPAD value	Trial 2	35.57	38.57	35.98	26.6 - 45.33	3.27	0.61

SD- standard deviation
H- Broad sense heritability

Table 10. Correlation coefficients among Canopy temperature (CT), leaf rolling (LR), leaf drying (LD), recovery (REC), relative water content (RWC), plant height (PH), number of tillers (TN), basal root thickness (BRT) and biomass (BM) under drought stress in the field in Trial 1 (Coimbatore, 2003 dry season) in a RI line population of rice.

	CT	LR	LD	REC	RWC	PH	TN	BRT	BM
CT	1.00	0.41**	0.49**	-0.52**	0.001	-0.28**	-0.40**	-0.13	-0.37**
LR		1.00	0.75**	-0.69**	-0.05	-0.27**	-0.57**	-0.22**	-0.42**
LD			1.00	-0.76**	0.04	-0.51**	-0.70**	-0.27**	-0.61**
REC				1.00	0.03	0.45**	0.66**	0.40**	0.50**
RWC					1.00	0.05	0.07	-0.17*	0.06
PH						1.00	0.38**	0.25**	0.59**
TN							1.00	0.25**	0.61**
BRT								1.00	0.20**
BM									1.00

* Significant at 0.05 probability level

** Significant at 0.01 probability level

Table 11. Correlation coefficients among Canopy temperature (CT), leaf rolling (LR), leaf drying (LD), SPAD reading (SPAD), plant height (PH), number of tillers (TN), days to 50 per cent flowering (DF) and biomass (BM) under drought stress in the field in Trial 2 (Paramakudi, 2003-04 wet season) in a RI line population of rice.

	CT	LR	LD	SPAD	PH	TN	DF	BM
CT	1.00	0.15**		-	-0.17**	-0.19**	-	-
			0.10*	0.04			0.02	0.01
LR			0.41**	-0.17**	-0.32**	-0.20**	-0.19**	-0.13**
		1.00						
LD				-	-0.18**	-		-
			1.00	0.06		0.11*	0.01	0.11*
SPAD					-		-	
				1.00	0.07	0.10*	0.01	0.06
PH						-0.34**		0.47**
					1.00		0.10	
TN							-	
						1.00	0.03	0.01
DF								
							1.00	0.002
BM								
								1.00

* Significant at 0.05 probability level
** Significant at 0.01 probability level

Table 12. Marker loci associated with drought resistance traits based on single marker analysis of trait mean obtained from Trial 1.

Marker	Chromosome Number	F	Pr> F	R^2 (%)
1. Markers linked to leaf rolling				
RM154	2	4.9	0.028	2.18
RM3515	2	11.63	0.0008	5.02
RM1342	2	14.38	0.0002	6.29
RM213	2	8.11	0.0048	3.48
RM6862	9	4.87	0.0285	2.38
RM101	12	7.73	0.0059	3.62
B01B_1000		5.01	0.0261	2.12
B06_700		12.02	0.0006	4.92
P16_600		8.13	0.0048	3.52
S10_400		8.55	0.0038	3.69
I10S_1000		4.86	0.0285	2.10
I23S_1761		13.8	0.0003	5.88

2. Markers linked to leaf drying				
RM212	1	5.61	0.0188	2.54
RM154	2	9.2	0.0027	4.01
RM3515	2	14.56	0.0002	6.21
RM1342	2	13.15	0.0004	5.79
RM263	2	5.15	0.0242	2.39
RM213	2	6.51	0.0114	2.81
RM5424	4	10.82	0.0012	4.65
RM11	7	6.55	0.0112	2.92
RM210	8	6.14	0.014	2.80
RM242	9	6.08	0.016	2.30
RM101	12	5.72	0.0177	2.70
C20_1000	4	7.09	0.0083	3.02
A10_600	9	4.97	0.0269	2.26
B06_700		5.22	0.0232	2.20
N18_425		7.79	0.0057	3.36
I23S_1761		9.12	0.028	3.96
3. Markers linked to stress recovery				
RM8051	1	7.59	0.0063	3.19
RM3515	2	11.37	0.0009	4.91
RM1342	2	13.36	0.0003	5.88
RM263	2	6.79	0.0098	3.13
RM5424	4	24.19	0.0001	9.83
RM101	12	8.61	0.0037	4.01
C20_1000	4	10.9	0.0011	4.56
B06_700		5.51	0.0198	2.32
N18_425		6.68	0.0104	2.90
S10_400		8.21	0.0046	3.55
I23S_1761		5.69	0.0179	2.51
I89S_900		5.26	0.0228	2.33
4. Markers linked to plant height				
RM488	1	20.72	< 0.0001	8.74
RM212	1	86.49	< 0.0001	28.69
RM302	1	39.9	0.0001	15.97
RM3515	2	11.12	0.001	4.81
RM1342	2	6.14	0.014	2.79
RM263	2	5.56	0.0192	2.58
RM5424	4	6.79	0.01	2.95
C20_1000	4	7.35	0.0072	3.12
RM210	8	6.06	0.0146	2.77
A04_750	8	7.01	0.0087	2.96
L19_450	8	6.98	0.0088	2.97
RM3164	9	7.45	0.0069	3.74

RM242	9	6.5	0.016	2.8
L14_475	11	7.66	0.0061	3.26
L20_1113	11	8.26	0.0044	3.44
5. Markers linked to tiller number				
RM151	1	5.96	0.015	2.86
RM154	2	6.38	0.0122	2.82
RM5699	2	6.36	0.012	2.87
RM5424	4	18.28	0.0001	7.61
RM101	12	6.98	0.0089	3.28
N18_1399	12	5.44	0.0205	2.40
A19K_950		9.64	0.0022	4.41
N18_425		13.32	0.0003	5.61
6. Markers linked to biomass under stress				
RM212	1	6.59	0.0109	2.97
RM1342	2	5.51	0.0199	2.51
RM5424	4	10.13	0.0017	4.37
C20_1000	4	7.29	0.0075	3.10
RM3164	9	7.64	0.0063	3.83
N18_425		6.59	0.0111	2.84
7. Markers linked to canopy temperature				
RM1342	2	6.39	0.0128	5.22
A14_377	8	5.25	0.0235	3.88
A04_750	8	5.69	0.0186	4.25
I23S_1761		4.94	0.0281	3.86
8. Markers linked to relative water content				
RM314	6	5.11	0.0258	4.32
RM6836	6	5.36	0.0224	4.31
S12_1000	6	8.19	0.0051	7.11
A01_961	11	5.95	0.0161	4.37

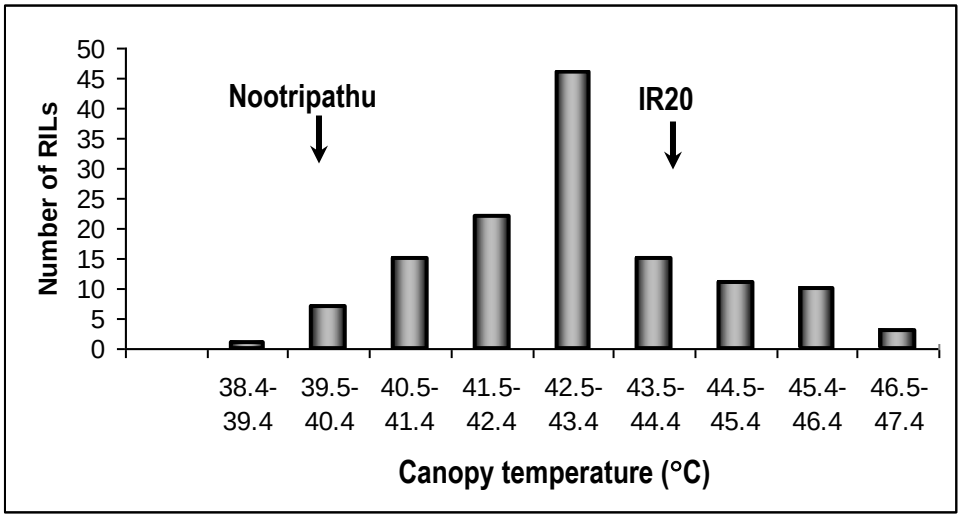
9. Markers linked to basal root thickness				
C20_1000	4	10.23	0.0016	4.29
K16_1000	6	6.3	0.0128	2.68
N18_1399	12	5.94	0.0156	2.62
S10_400		5.14	0.0243	2.25

Table 13. Marker loci associated with drought resistance traits based on single marker analysis of trait mean obtained from Trial 2.

Marker	Chromosome Number	F	Pr> F	R^2 (%)

1. Markers linked to leaf rolling				
RM3164	9	5.02	0.0262	2.5
RM6862	9	5.96	0.0155	2.88
L14_475	11	10.00	0.0018	4.2
L20_1113		13.86	0.0002	5.61
2. Markers linked to leaf drying				
C20_1000	4	11.1	0.001	4.62
RM314	6	8.11	0.0049	3.79
RM6836	6	12.58	0.0005	5.5
L19_450	8	8.38	0.0042	3.52
RM5122	9	5.58	0.019	2.43
RM3164	9	8.09	0.0262	4
A10_600	9	6.55	0.0122	2.96
A13_1339	11	5.18	0.237	2.19
B06_700		12.50	0.0005	5.11
L08_400		8.17	0.0046	3.4
P16_600		13.91	0.0002	5.85
3. Markers linked to plant height				
RM488	1	11.04	0.001	4.78
RM212	1	64.45	<0.0001	22.9
RM302	1	34.81	<0.0001	14.41
RM263	2	6.43	0.0119	2.93
RM289	5	5.32	0.022	2.29
S12_1000	6	4.84	0.0289	2.3
RM152	8	16.13	0.0001	6.89
L19_450	8	7.34	0.0073	3.08
B01B_750	10	5.12	0.0246	2.17
L20_1113	11	7.5	0.006	3.09
B06_700		11.95	0.0006	4.8
4. Markers linked to tiller number				
RM212	1	11.14	0.001	4.86
RM302	1	5.84	0.0165	2.68
RM152	8	5.09	0.025	2.28
L19_450	8	7.01	0.0087	2.94
B06_700		15.41	0.0001	6.15
I07S_700		5.24	0.023	2.19
I10S_1000		5.62	0.0186	2.41
I23S_1761		5.19	0.0237	2.26
5. Markers linked to biomass under stress				
RM212	1	7.48	0.0067	3.32
RM5699	2	5.25	0.0217	2.4
RM5424	4	12.58	0.0005	5.27

RM6836	6	8.1	0.0048	3.59
6. Markers linked to canopy temperature				
RM8051	1	8.17	0.0047	3.42
RM263	2	9.74	0.0021	4.41
RM5424	4	8.81	0.0033	3.77
C20_1000	4	17.88	<0.0001	7.24
K11_225	5	7.32	0.0073	3.07
RM314	6	10.73	0.0012	4.95
RM6836	6	27.54	<0.0001	11.31
S12_1000	6	26.88	<0.0001	11.64
L19_450	8	18.13	<0.0001	7.34
A19K_950		5.22	0.0233	2.38
B06_700		10.20	0.0016	4.19
M06_475		25.84	<0.0001	10.38
P16_600		38.65	<0.0001	14.71
S09_1180		8.6	0.0037	3.58
I09S_800		4.83	0.0289	2.07
I12S_2023		23.79	<0.0001	9.41
I23S_1761		20.74	<0.0001	8.54
7. Markers linked to SPAD reading				
RM5424	4	17.79	<0.0001	7.33
C20_1000	4	9.03	0.0029	3.8
S12_1000	6	5.21	0.0235	2.48
8. Markers linked to days to 50 per cent flowering				
I23S_1761		8.38	0.0046	7.66



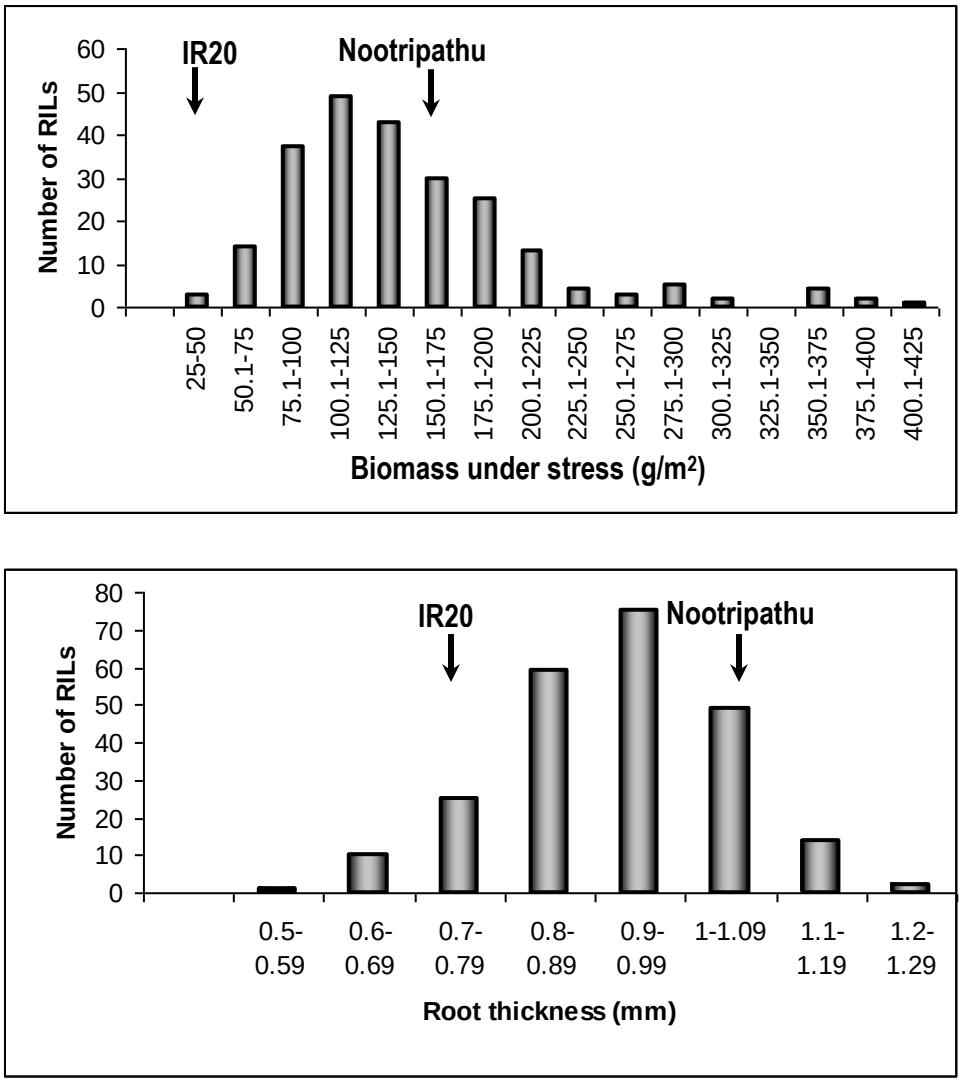


Figure 3. Frequency distribution of phenotypic traits among the F_7 RI lines derived from IR20/Nootripathu

CHAPTER V
DISCUSSION

Food security in Asia is challenged by increasing food demand as a result of increasing population growth and threatened by declining water availability. Rice is the most important staple food in Asia, where it provides 35-80% of total calorie intake. Cultivated rice (*Oryza sativa* L.) is generally a diverse species with broad adaptation to a wide range of growing environments including both tropical and temperate climates and irrigated and rainfed environments (IRRI, 2002). It is grown in more than 148 million hectares (Mha) globally and more than 45 per cent of the world's rice is grown in rainfed ecosystems where water deficit occurs frequently due to uncertain and uneven rainfall distribution patterns and yields are seriously affected by drought. Of world's rainfed lowland rice area of 41 Mha, 95 per cent is in Asia (IRRI, 1995). In south and southeast Asia, future increases in rice production will rely on these rainfed ecosystems (Garrity *et al*., 1986). Of the 44 Mha of total rice area in India, 33 per cent is rainfed lowland and 15 per cent is upland (www.fao.org). In Tamil Nadu, area under rice cultivation is 2.2 Mha, of which 6.2 per cent is under dry and semi dry conditions. Due to monsoon failure in the past three consecutive years, water table is declining at an alarming rate and rice area is fast decreasing over the years, rendering the distinction as rainfed and irrigated rices as obsolete in this State. Thus, drought is by far the leading environmental stress-limiting rice productivity in rainfed and irrigated ecosystems. Developing rice cultivars with inherent capacity to withstand drought stress would help to stabilize rice production especially in rainfed ecosystems. However, progress in genetic improvement of rice for water-limiting environments has been slow and more limited (Evenson and Gollin, 2003), due to lack of knowledge about the mechanism of tolerance, poor understanding of the inheritance of tolerance, low heritability of yield under water stress and lack of efficient techniques for screening breeding materials for drought tolerance (Khush, 2001).

Alternatively, several putative traits contributing to drought resistance in rice have been proposed and selection of these traits in breeding program could lead to yield improvements in water-limited environments (Nguyen *et al*., 1997). However, these traits are rarely selected for in crop improvement programs because phenotypic selection for these traits involves complex,

difficult and labor-intensive protocols and cost demanding experimental conditions. In addition, these protocols are destructive in nature resulting in loss of breeding materials for further use. The advent of molecular markers has revolutionized the genetic analysis of complex traits such as drought resistance in crop plants. Molecular markers help to track the genetic loci controlling drought resistance without having to measure the phenotype, thus reducing the need for extensive field testing over space and time (Nguyen *et al.,* 1997). The availability of a genetic map saturated with molecular markers helps to locate quantitative trait loci (QTLs) linked to drought resistance traits and crop productivity in stressful environments. Once the tightly linked markers have been identified, the QTLs can be selected for in breeding program using marker-assisted selection (MAS) strategy much more efficiently than was possible previously. QTLs have been detected for several root-related traits, osmotic adjustment, dehydration tolerance and other shoot-related drought resistance component traits in rice (see Boopathi *et al.,* 2002). However, all these studies were done using mapping populations derived by crossing *indica* x *japonica* parental lines and majority of the positive alleles for drought resistance traits are contributed by *japonica* parents. *Japonica* alleles may not express in lowland ecosystem, typical of most Indian rainfed lowland rice environment (Wang *et al.,* 1994; Redona and Mackill, 1996; Yano and Sasaki, 1997). Hence, it is desirable to look for genetic variation among rice accessions within *indica* ecotypes (Ingram *et* al., 1994) and map QTLs using populations derived from *indica* rice lines adapted to target population of environments (TPE).

Mapping population

Choosing parents is one of the most important steps in breeding program. The two parents (IR20/Nootripathu) used in this study are adapted to the TPE and they have shown to have difference in drought resistance component traits (Babu *et al.,* 2001). Nootripathu is a locally well-adapted landrace having good root system development and field performance under water stress conditions. Whereas, IR20 is an improved, consumer preferred variety but has shallow root system and is drought sensitive. Use of locally adapted cultivar as a parent ensures the recovery of a high proportion of progenies with adaptation and quality that are acceptable to farmers. Use of improved elite modern variety in crosses with an adapted parent could help in blending of disease and insect resistance and grain qualities. Thus each parent complements the weakness of the other (Atlin, 2003). These two parental lines were used to develop a

recombinant inbred (RI) line population by single seed descent. For many mapping purposes RI lines are superior to F_2 or backcross populations because they constitute a permanent population in which segregation is fixed (Reiter *et al.,* 1992).

Molecular markers and parental polymorphism

So far the QTLs identified for several drought resistance traits were mostly restriction fragment length polymorphism (RFLP) and amplification fragment length polymorphism (AFLP) markers (Boopathi *et al.,* 2002). Though these markers are robust and reliable, they involves tedious, time consuming protocols besides handling hazardous radioactive chemical. Identification of polymerase chain reaction (PCR) based non-radioactive markers will pave way for routine use of MAS for drought resistance improvement. Simple sequence repeats (SSR), inter simple sequence repeats (ISSR), randomly amplified polymorphic DNA (RAPD) are well-established PCR based markers used in mapping efforts (Mohan *et al.,* 1997).

The candidate gene approach has been applied in plant genetics recently for characterization and cloning of QTLs (Pflieger *et al*., 2001). Candidate genes are genes involved in the expression of a given trait. Expressed Sequence Tags (ESTs) are partial or single pass sequencing of more or less randomly chosen cDNA clones from libraries at all stages of plant growth and development. They allow fast and affordable gene identification. Development of EST based markers is dependent on extensive sequence data of regions of the genome that are expressed. They are highly reproducible and can be directly associated with functional genes. A number of ESTs specific to drought response are now available in the EST database (dbEST). It will be important to resolve to what extent the allelic variation in these genes is associated with drought tolerance in rice. The tight linkage between these candidate genes and the QTLs for root traits, water stress indicators or plant production under water stress may demonstrate a causal relationship. The candidate genes used so far were engaged as radioactive probe as that of RFLP. Development of PCR based EST markers could be useful in QTL mapping and efficient MAS for drought resistance improvement in rice. Further, ESTs allow a computational approach to the development

of SSR for which previous development strategies have been expensive (Sreenivasulu *et al*., 2002). Pattern-finding programs can be employed to identify SSRs in the ESTs (www.gramene.org). Readily available EST sequence information allows the design of primer pairs, which can be used to identify the length polymorphism among the parental lines. Hence, the present study was conducted with several PCR based markers *viz.,* SSR, ISSR, RAPD, PCR based ESTs and SSRs derived from ESTs.

In general, the parents exhibited low level of polymorphism since both are *indica* ecotypes. Low level of polymorphism between the parents was also noticed in the intra-sub specific (Ali *et al.,* 2000) and even in inter-sub specific crosses (McCouch *et al.,* 1988; Price and Tomas, 1997) in rice. Generally monomorphic regions are expected in the genome of a population derived from intervarietal crosses or closely related parents. Despite a relatively large number of PCR based markers used in this study for parental polymorphism, it resulted in very low level of polymorphism, which leads to poor genome coverage in linkage map construction and several large gaps on the map. The polymorphic SSR markers found in the chromosome 7 and 11 for SSR markers are extremely low in this study due to monomorphic genetic make-up commonly shared by the closely related *indica* parents. Only 10.6 per cent of the 1128 primers have generated informative polymorphism between the parents and have been used in linkage map construction. ISSR primers have generated high level of polymorphism (35.7 per cent), followed by RAPD primers (14.37 per cent), SSR primers (10.19 per cent) and ESTs (1.48 per cent).

Linkage map construction

Using a minimum LOD score of 3.0 and a maximum distance of 50 cM, 17 linkage groups were obtained with 24 markers unlinked. Fourteen of the linkage groups were assigned to rice chromosomes because they contained at least one SSR marker known to be from that chromosome (Causse *et al.,* 1994). No linkage group was assigned to chromosome 7. Similar types of linkage groups were observed and no linkage map was assigned to chromosome 9 (Price and Tomas 1997). But use of more number of markers to find polymorphism between the parents made it possible to construct the linkage map for chromosome 9 (Price *et al.,* 2000). Thus,

exploitation of more number of the SSR markers in chromosome 7 will lead to construct a linkage map for chromosome 7 in the population of the present study. Eventhough ISSR and RAPD markers generated high level of polymorphism they could not be mapped in this study since the polymorphic SSR markers in the linkage groups did not link to the RAPD and ISSR markers. This may be due to low level of polymorphism in the parents for SSR markers and only those polymorphic SSR markers were used for initial grouping of all the markers. Thus, when more number of SSR markers identified for these linkage groups then these unlinked markers will be useful for mapping.

Two linkage groups were assigned to chromosome 8 and 12. This may be due to the large gap (> 50 cM) found between the polymorphic SSR markers, which could be clearly seen from the published map (Temnykh *et al.,* 2000; McCouch *et al*., 2002). The order and distance of the marker in this study was comparable with the published map except for chromosome 2 where the marker order has been slightly changed. Change of marker order was also observed on chromosome 4 in another population of rice (Price *et al.,* 2000). It was not possible to assign three linkage groups formed by the RAPD markers to the rice chromosomes. However, use of more SSR markers could help to assign these markers as well as unlinked markers to the rice chromosomes and may fill the gaps found in this study. Five RAPD markers were removed from the map because their inclusion significantly lengthened the linkage group. Those markers were S09_1180 on chromosome 1, M06_475, C06_1617 and A11_844 on chromosome 2 and A02_888 on chromosome 8. Thus, large gaps in the linkage map remained, notably the whole of chromosome 7 and upper and lower portions of chromosomes 3, 4, 5, 6, 8, 10 and 12. The unassigned RAPD linkage groups may fit any one of the missing locations. Unassigned linkage groups found in the initial study were placed on the gaps found in the map when more number of markers were used in the later study (Price *et al.,* 2000).

Among 141 polymorphic markers only 80 markers (56.73 per cent) approximately segregated in the expected 1:1 ratio and so they were used in mapping process. Within the markers that are deviating from the expected 1:1 segregation, eight SSR markers (RM6464, RM8213, RM5, RM517, RM426, RM346, RM3423 and RM3773) and one RAPD marker (L08_1000) segregated in a peculiar way. The RI lines were extremely skewed towards IR20 for

these markers and only two RI lines (RIL#179 and RIL#214) had banding pattern as that of Nootripathu for these nine primers. A representative PCR profile of one of these eight SSR primers is shown (Plate 9). Interestingly, these two lines performed well under water stress conditions in the field trials compared to other RI lines (Plate 7a and 7b). In addition, RIL#179 and RIL#214 had 19 and 22 per cent of IR20 alleles, respectively, for the rest of the markers. This shows that these two lines are not a mixture of Nootripathu parental seeds with RI lines. So, it may possible to conclude that these primers might be linked to field performance of the rice lines under water stress. However, further studies on these markers will confirm their linkage to drought resistance.

Variation of drought resistance traits under water stress in field conditions

Significant variation found among the RI lines for investigated traits in both the trials, indicated the presence of genetic variation for drought response between the parents and among the RI lines in this study. Severe drought stress was imposed on the plants as indicated by average leaf rolling and leaf drying scores across the RI lines. Nootripathu had higher plant height, tiller number and biomass than IR20 in both the trials and thicker roots in Trial 1 under water stress conditions in the field. This reveals the superiority of this landrace over IR20 in drought resistance under field conditions.

Significant differences were observed among the RI lines for all traits in both the trials. Transgressive segregation of traits was observed for several traits and they were approximately fitted to the normal distributions. Significant differences for plant phenology and production under stress and for indicators of plant water stress have been reported among a subset of 100 DH rice lines of CT9993/IR62266 (Blum *et al.,* 1999). In the present investigation, significant difference was found in biomass between the two trials. This reveals that large environmental impact on the trait expression in these two trials. The broad sense heritability of this trait in Trial 1 and 2 were 0.91 and 0.56, respectively and this also explained the higher environmental effect on biomass production under water stress.

In Trial 1, both biomass and recovery recorded highest H (0.91) and lowest H was noticed for relative water content (0.10). However, canopy temperature measured on the same RI

lines used for relative water content had a *H* value of 0.75. This explains that there may be different genetic mechanisms involved in control of these traits and they are highly influenced by the environment.

In summary, there was a significant genotypic effect for most traits in both the trials except for relative water content in Trial 1 and days to 50 per cent flowering and number of tillers in Trial 2. It was evident that environment had considerable influence on the expression of these traits. Similar level of low *H* was found earlier for per cent spikelet fertility and days to heading under water stress in CT9993/IR62266 DH line population of rice (Babu *et al*., 2003).

Correlations between water stress indicators and biomass under water stress

Due to increased evaporative demand and severe water stress in Trial 1 and 2, respectively, plant biomass was the only measure of plant production in these trials. Significant positive correlations were observed between biomass under stress and plant height, number of tillers and basal root thickness in Trial 1. The highest correlation coefficient was recorded between biomass and tiller number (r = 0.61**) followed by biomass and plant height (r = 0.59**) under stress. A relatively lower correlation coefficient was observed between biomass and basal root thickness (r = 0.20**) under water stress. Similar correlations were found by Champoux *et al.,* (1995) between root thickness and plant height. Water stress indicators such as leaf rolling, leaf drying and canopy temperature were negatively correlated with basal root thickness and biomass under stress. Leaf relative water content had shown non-significant correlation with biomass under stress.

In Trial 2, significant positive correlation was found between plant height and biomass under stress. Significant negative correlations were found between water stress indicators such as leaf rolling and leaf drying with biomass under stress. Negative correlations of water stress indicators with biomass and positive correlations of plant production traits with biomass were also observed in rice (Babu *et al.,* 2003).

Identifications of putative QTLs linked to drought resistance traits under field condition by single marker analysis

Single marker analysis was performed with the SSR, ISSR, RAPD and EST markers that are used in mapping process i.e., markers, which are segregating approximately in 1:1 ratio. Relatively high statistical threshold ($Pr < 0.02$) was used in this study to declare a marker linked to the particular trait compare to previous studies. This helps to avoid false positives and false negatives. We choose to present this data despite relatively lower significance levels because it includes markers associated with one trait overlapping with putative QTLs for other traits. A low statistical threshold was used earlier to declare QTL for maximum root length using single marker analysis in rice (Toorchi *et al.,* 2002). Similarly, Champoux *et al.,* (1995) used lower threshold because it includes markers associated with one root trait overlapping with other root traits. The R^2 statistic represents the proportion of phenotypic variation explained by each putative QTL. Since the linkage map generated in this study is sparse and did not cover the entire rice chromosomes, single marker analysis was used to detect the QTLs linked to the investigated traits. Rebai *et al.,* (1995) concluded from their study that two-marker mapping provides a relatively small gain (5 per cent) in power over single marker methods when two markers define an interval of width less than 20 cM, but a substantial increase (greater than 30 per cent) in power for intervals upwards of 70 cM, indicating that the gain in power may come from the addition of second marker to the analysis or the addition of information from that marker, rather than the map. Coffman *et al.,* (2003) have also shown that single marker analysis has equal or even more power than two-marker QTL analysis. Price *et al.,* (2000) found that 89 per cent of the QTLs detected by composite interval mapping concurred with those detected by single marker analysis.

In Trial 1, among the 37 SSR markers used for single marker analysis, 20 markers (54.1 per cent) were identified as putatively linked to at least one of the investigated trait. This may be due to low level of polymorphism found between the parents and as a consequence, most of the difference in the genomic content may be linked to their phenotypic difference (Ali *et al.,* 2000). RM1342 on chromosome 2 had shown to have putative linkage with six traits *viz.,* leaf rolling, leaf drying, canopy temperature, drought recovery, plant height and biomass and RM5424 on chromosome 4 had identified to have putative linkage with five traits *viz.,* leaf drying, drought recovery, plant height, number of tillers and biomass under water stress (Table 12). Each of the markers, RM3515 on chromosome 2 and RM101 on chromosome 12 had been found to have

putative linkage with four traits (leaf rolling, leaf drying, drought recovery and plant height). This indicates the chromosomal region of these markers on the rice chromosomes may contain genes that are having linkage or pleiotropic effect on several traits that are involved in drought resistance in rice under field conditions. Most of the markers have shown approximately 3 per cent variance on the traits. However, RM212 had revealed highest variation (28.7 %) for plant height followed by RM5424, which had 9.2 per cent variation for stress recovery and 7.4 per cent variation for tiller number. Relatively low level of phenotypic variation was also noticed in other studies for drought resistance traits under water stress in rice (Champoux *et al.,* 1995; Price *et al.,* 2002a, b). Among the RAPD markers, C20_1000 on chromosome 4 had putative linkage with five traits (leaf rolling, drought recovery, basal root thickness, plant height and biomass) and N18_1399 on chromosome 12 had shown to have linkage with three traits (drought recovery, basal root thickness and number of tillers) and N18_425 had shown to have linkage with four traits (leaf drying, drought recovery, number of tillers and biomass). Among the ISSR markers, I23S_1761 had putative linkage with four traits (leaf rolling, leaf drying, drought recovery and canopy temperature) measured under water stress in the field.

Similarly, in Trial 2, RM212 on chromosome 1, RM5424 on chromosome 4 and RM6836 on chromosome 6 had found to be linked with three traits. Among the RAPD markers, B06_700 and L19_450 on chromosome 8 had QTLs linked to four different traits investigated in this study. The ISSR marker I23S_1761 had linkage with three different traits measured under water stress. This confirms the tight linkage or pleiotropic effect of these markers on the expression of the identified traits. There was also a set common QTLs identified for both the trials, which strengthens the validity of the QTLs over environments.

The EST marker, GSP23 on chromosome 10 did not show linkage with any of the investigated traits in both the trials. This may be due to monomorphic genomic regions shared by the parents (only 1.48 per cent polymorphism was found for EST markers), which reveals that both parents share common functional genes in their genome and the difference exists only on the expression of these genomic regions. Hence, it may be possible to conclude that identification of water stress specific regulatory elements is crucial for elucidation of drought resistance mechanism in these two parents at genetic and molecular level (Wang *et al.*, 2003).

ESTs and SSRs derived from ESTs specific to drought resistance is being used in mapping efforts in rice (Shashidhar *et al.*, 2004; Reddy *et al.*, 2004) and sorghum (Reddy *et al.*, 2004). Mapping ESTs in relation to other molecular markers will take us one step closer to assigning biological meaning to markers.

Colocation of QTLs

If QTLs that were identified in one environment for a given trait, often mapped at the same place as QTLs affecting other traits in the same or another site, they can strengthen the coherence of the overall pattern. In Trial 1, there were two markers (B06_700 and I23S_1761) common for leaf rolling, leaf drying, drought recovery and canopy temperature. Markers RM154, RM3515, RM1342, RM213 and RM101 were common for leaf rolling and leaf drying and RM210 was common for leaf rolling and canopy temperature. Identification of common location for different water stress indicators confirms the strength of the QTLs identified in this study. In defining the strategy to improve the drought resistance of upland rice, one hypothesis was that a deep and thick root system would improve water extraction from the soil and contribute to maintenance of turgor and of relative water content (Courtois *et al.*, 2000). Varieties with such root systems should experience less leaf rolling and leaf drying (Lilley and Fukai, 1994). We would therefore expect to recover at the same position some or all of the QTLs for basal root thickness and water stress indicators such as leaf rolling, leaf drying and recovery. N18_1399 was found to be common for basal root thickness and recovery and S10_400 was identified as a marker linked to leaf rolling, basal root thickness and recovery. For plant production traits, RM212, RM1342, RM3164 had identified as common markers for plant height and biomass production and N18_425 was common for tiller number and biomass production under water stress. RM5424 on chromosome 4 had shown linkage to plant height, tiller number and biomass under stress. Similarly, C20_1000 on chromosome 4 was linked to plant height, biomass and basal root thickness. The correlation study had shown that positive relationships existed between these traits. This reveals that the chromosomal segments of the above said markers had genes that are having pleiotropic effect on controlling these plant production traits under water stress in the field.

In Trial 2, RM314, RM6836, B06_700, C20_1000, L19_450 and P16_600 were shown to have linkage with leaf drying and canopy temperature and RM3164 had identified as maker common to leaf rolling and leaf drying. RM212 was linked to plant height, tiller number and biomass production under water stress. Similar kind of relationship between water stress indicators (e.g., leaf rolling and leaf drying) and relative water content and relative growth rate under water stress was observed in rice and common markers were identified for these traits (Courtois *et al.,* 2000).

QTLs across the trials

Consistency of QTLs across different environments is essential for marker-aided selection to be effective. Analysis of QTLs controlling different traits involved in drought avoidance and plant production under stress had shown common QTLs for several traits in both the trials. RM6862 on chromosome 9 had shown to have linkage with leaf rolling and A10_600 on the same chromosome had linkage with leaf drying in both the trials. RM488, RM212 and RM302 on chromosome 1, RM263 on chromosome 2 and L19_450 on chromosome 8 were identified as markers linked to plant height in both the trials. RM488, RM212 and RM302 were located within 20.7 cM interval on chromosome 1 in this study. In addition, RM212 was also linked to biomass production under water stress in both the trials. RM5424 on chromosome 4 was associated with biomass under stress in both the trials. In addition, this marker had linkage with plant height, number of tillers, leaf rolling, leaf drying, drought recovery, canopy temperature and SPAD chlorophyll reading in either one of the trials. These markers, consistently identified for various traits across the trials may be useful as potentially valuable candidate markers for the transfer of these QTLs into elite background through marker aided selection.

However, there are putative QTLs identified in one environment, which were not identified, in another environment. For example, none of the 16 markers identified for tiller number in both the trials were found to be common between the trials. This indicates the phenomenon of QTL X environment interaction and thus the genetic control of quantitative trait under field conditions is complex. Another reason might be low repeatability found for this trait as shown by the low broad sense heritability in Trial 2, which decrease the ability to detect the

QTLs. Low repeatability and uncommon markers across the trials have also reported for relative growth rate (Courtois *et al.*, 2000) and root morphological traits under different water deficit regimes (Price *et al.*, 2002a) in rice. Further trials with more replications would be required to increase the precision of this trait and thus it will increase the accuracy in identification of QTL for this trait.

QTLs across genetic backgrounds

A comparison of QTLs detected in this present investigation was made with those identified by previous researchers on different populations of rice for different traits. Most of the previous studies used RFLP and AFLP markers and we exploited PCR based markers such as SSR, ISSR, RAPD and ESTs. Only very few studies used SSR markers (Zhang *et al.*, 2001; Price *et al.*, 2002b; Robin *et al.*, 2003) and only those markers were compared with the markers identified in this study. Since inconsistent map distances between markers in different maps hampered precision in the comparison, the comparative results should be considered as indicative.

The QTL region containing RM212 on chromosome 1 is one such example. RM212 was identified to have linkage with leaf drying, plant height, tiller number and biomass under water stress conditions in this study (Figure 5). The marker interval R2417-RM212-C813 on chromosome 1 was associated with deep root mass, deep root ratio and deep root per tiller in CT9993/IR62266 DH population (Komashita *et al.*, 2002a). The region was found to regulate relative water content under field drought condition (RM212-C813) (Babu *et al.*, 2003) and number of tillers (R2417-RM212) in the same population (Kanbar *et al.*, 2003). The chromosomal segment R2417-RM212-C86 on chromosome 1 was also linked to plant mass, basal root thickness and root-shoot ratio in Bala/Azucena RI lines (Price *et al.*, 2002b). The chromosomal segment RM212-RM265 was identified as a QTL for osmotic adjustment in IR62266/IR60080 BC_3F_3 population (Robin *et al.*, 2003). Interestingly, from the published map using SSR markers (Temnykh *et al.*, 2001), it is obvious that RM212 and RZ730 were located within 7.9 cM distance. The major gene controlling the semi-dwarf stature, *sd-1*, was located near RZ730 (Huang *et al.*, 1996). This gene is known to affect many aspects of plant morphology and physiology: plant height, tillering, panicle length, responsiveness to fertilizer,

biomass and harvest index through pleiotropic effects (Xia *et al.*, 1991; Courtois *et al.*,1995) as well as root system development (Yadav *et al.,* 1997). Single marker analysis of RM212 in association with different productive traits revealed that this marker was linked with plant height, total and productive number of tillers, length of internode, stem diameter and width of flag leaf in the RI lines derived from CO43/W1263 (Hemalatha, 2003). Thus, RM212 may be linked to drought resistance traits and plant production under water stress in the field in rice.

Similarly, RM263 on chromosome 2 had shown linkage to leaf drying, recovery, canopy temperature and plant height in this study (Figure 6). The chromosomal segment RM263-R3393 was identified as a QTL for osmotic adjustment in CT9993/IR62266 DH population (Zhang *et al.,* 2001). RM11 identified as a marker linked to leaf drying in this study but could not be mapped on chromosome 7 due to very low level of polymorphism found in the parents for this linkage group. Similarly, RM210 on chromosome 8 was found to be linked to leaf drying, canopy temperature and plant height in this study. The RM11-OSR22 marker interval on chromosome 7 and the genomic segment flanked by RM284-RM210 on the chromosome 8 were linked to osmotic adjustment in IR62266/IR60080 BC_3F_3 population (Robin *et al*., 2003). Fine mapping of these regions with candidate genes may resolve the functional relationship between the QTL and the investigated traits in rice under water stress in the field.

Single marker analysis of RM242 on chromosome 9 revealed that it was linked to leaf drying and plant height under stress in this study (Figure 7). The region on chromosome 9, ME9_6 – RM242, was shown to have linkage with penetrated root thickness in CT9993/IR62266 DH population (Zhang *et al.,* 2001; Ganesh *et al.,* 2004). In the same population, RM242-RG667 had shown to have linkage with days to 50 per cent flowering under water stress condition in the field (Babu *et al.,* 2003) and ME9_6 – RM242 had linkage with plant height (Kanbar *et al.,* 2003). So it is obvious that the region of RM242 on chromosome 9 may contain genes that are having pleiotropic effect on drought resistance traits and plant production under water stress conditions in the field in rice.

Though some degree of probabilistic prediction is possible for the timing and intensity of drought spells, drought stress is still largely unpredictable. We require alleles having an effect

across as many drought situations as possible for effective marker-aided selection program, rather than alleles conferring specific adaptation in a given environment. Consistent QTLs were identified in this study in both the trials and also found to be consistent across genetic backgrounds. The genomic segments, RM212 – RM302 on chromosome 1, RM5424-C20_1000 on chromosome 4 and markers RM263 on chromosome 2, RM210 on chromosome 8 and RM242 on chromosome 9, thus would be reasonable candidates for development of near isogenic lines and further genetic dissection of drought resistance by molecular fine mapping to identify tightly linked markers. Further work is needed to saturate this linkage map using highly polymorphic molecular markers such as single nucleotide polymorphisms (Feltus *et al.,* 2003; Reddy *et al.,* 2004) since very low level of polymorphism was found between the parents in this study. Further research is needed to determine whether QTLs identified in these active segments also affects the grain yield under water stress. Thus the present study had identified putative markers linked to drought resistance traits and may be useful in marker-aided selection for drought resistance improvement of rice for TPE.

Table 2. Details of mapping population, linkage map characteristics and QTLs identified for drought resistant traits in rice

Parents	Population§	Number and type of markers used	Linkage map coverage (cM)	Traits	QTLs identified			Maximum phenotypic variance (%)	References
					No. of QTLs	Across trials/ experiments	Across Population		
Co39/Moroberekan	281 F_7 RILs (203)	127 (RFLP)		Root thickness	18	-	-	56	Champoux et al., (1995)
				Root shoot ratio	16	-	-	38	
				Root dry weight per tiller	14	-	-	35	
				Deep root weight	8	-	-	18.5	
				Maximum root depth	4	-	-	-	
				Drought avoidance (leaf rolling)	18	5	-	35	
Co39/Moroberekan	281 F_7 RILs (202)	127 (RFLP)		Number of penetrating roots	4	-	-	8	Ray et al., (1996)
				Total number of roots	19	-	-	19	
				Root penetration index	6	-	-	13	
				Tiller number	10	-	-	14	
Co39/Moroberekan	281 F_7 RILs (52)	127 (RFLP)		Dehydration tolerance	5	-	-	36	Lilley et al., (1996)
				Osmotic adjustment	1	-	-	32	
				Relative water content	2	-	-	35	
IR64/Azucena	135 DH lines (105)	175 (RFLP, RAPD, isozyme)	2005	Total root weight	23	-	3	11.9	Yadav et al., (1997)
				Deep root weight	17	-	-	14.9	
				Deep root weight to shoot ratio	26	-	3	22.3	
				Deep root weight per tiller	20	-	3	19.6	
				Maximum root length	25	-	1	20.9	
				Root thickness	8	-	2	10.4	
`Bala/Azucena	178 F_2 plants (30)	71 (RFLP)	1280	Maximum root length	10	1	4	37.7	Price et al., (1997)
				Root volume	1	-	-	10.2	
				Adventitious root thickness	2	-	2	14.7	
Bala/azucena	178 F_2 plants (178)	71 (RFLP)	1280	Leaf rolling	1	-	-	6.2	Price et al., (1997a)
				Stomatal behaviour	4	-	-	18.4	
				Days to heading	3	2	-	32.5	
IR64/Azucena	135 DH lines (56)	175 (RFLP, RAPD, isozyme)	2005	Plant height	4	2	-	29.2	Hemamalini et al., (2000)
				Number of tillers	11	-	-	25.7	
				Root length	5	-	3	15.4	
				Total root number	10	-	-	25.1	
				Root volume	5	-	-	21.4	
				Root thickness	5	1	2	26.7	
				Root dry weight	2	-	-	20.8	
				Root shoot ratio	1	-	1	12.7	

				Leaf drying (Drought score)	2	-	-	16.1	
				Leaf rolling	1	-	-	11.9	
IR64/Azucena	135 DH lines (105 & 85)	175 (RFLP, RAPD, isozyme)	2005	Leaf rolling	11	4	6	23.3	Courtois et al., (2000)
				Leaf drying	10	1	-	19.4	
				Relative water content	11	1	-	18.5	
				Relative growth rate	10	-	-	16.5	
Bala/Azucena	205 RILs (104)	135 (RFLP, AFLP)	1680	Number of tillers	1	-	-	12.4	Price et al., (2000)
				Number of roots	3	-	1	10.3	
				Number of penetrated roots	7	-	-	16.7	
				Penetrated: total roots (root penetration index)	7	-	2	18.0	
IR1552/Azucena	150 RILs (150)	207 (RFLP, AFLP)	2419	Seminal root length	2	-	-	11.2	Zhang et al., (2001a)
Bala/Azucena	205 RILs (176,118,142& 110)	142 (RFLP, AFLP, SSR)	1779	Leaf rolling	5	1	5	20.4	Price et al., (2002)
				Leaf drying	11	-	8	17.6	
				Relative water content	8	-	7	25.6	
Bala/Azucena	205 RILs (140)	142 (RFLP, AFLP, SSR)	1779	Total dry weight/plant mass	8	2	-	21.3	Price et al., (2002a)
				Root to shoot dry weight ratio	11	2	2	28.0	
				Root mass below 90 cm	6	-	3	16.0	
				Basal root thickness	7	-	-	18.2	
				Root thickness at 90 cm	14	2	8	18.3	
				Maximum root length	6	2	4	17.4	
				Number of root past 100 cm	12	4	-	22.8	
IR64/Azucena	135 DH lines (109)	175 (RFLP, RAPD, isozyme		Penetrated root number	2	-	-	9.0	Zheng et al., (2000)
				Total root number	2	-	-	14.3	
				Root penetration index	4	-	1	13.5	
				Penetrated root thickness	4	-	3	16.4	

CHAPTER VI

SUMMARY

This chapter outlines the summary of this study conducted with the objectives of linkage map construction and QTL mapping of drought resistance traits in locally adapted rice with a long term goal of developing drought resistant high yielding cultivars for rainfed target production environment through marker aided selection.

- IR20 and Nootripathu were selected as parents for the development of recombinant inbred (RI) line population for mapping quantitative trait loci (QTLs) associated with drought resistance traits in rice under field conditions. Both the parents are well adapted to the target production environment and they differ for a range of root related drought resistance traits.
- A subset of 250 F_7 RI lines were selected randomly from the total population of 397 RI lines for genotyping and phenotypic evaluation under water stress in the field.
- In total, 627 SSR and SSRs derived from ESTs, 100 ISSR, 320 RAPD and 78 ESTs were used for parental polymorphism and respectively revealed 10.04, 35.7, 14.37, 1.28 and 0 per cent polymorphism between the parents. These polymorphic markers were screened for their segregation in the RI lines.
- Eighty polymorphic markers approximately segregated in the expected 1:1 ratio were used to construct a genetic map using MAPMAKER/EXP MS-DOS 3.0.
- Seventeen linkage groups with 56 markers and 24 unlinked markers were formed with a minimum LOD of 8.0 and maximal distance of 30 cM. Fourteen linkage groups were assigned to all the rice chromosomes except chromosome 7 and three linkage groups were left unassigned.

- The linkage map with a total map length of 652 cM was drawn with the Haldane mapping function and compared with the published maps for their relative order and distance between the markers.
- Field trails were conducted, one at managed stress environment (MSE- Coimbatore) and another at rainfed target population of environment (TPE-Paramakudi), using F_8 RI lines for QTL mapping of drought resistance traits under water stress.
- Significant variation was found for water stress indicators such as leaf rolling, leaf drying and canopy temperature and plant production traits such as plant height, number of tillers and biomass under water stress between the parents and among the RI lines.
- Significant positive correlations were found between biomass under stress and plant height, tiller number and basal root thickness in MSE and biomass under stress and plant height in TPE. Negative correlation was found between water stress indicators such as leaf rolling, leaf drying and canopy temperature and biomass under stress.

- Single marker analysis of the 80 polymorphic markers with the phenotypic values for 11 different traits from the two trials revealed association of 54

markers and identified as QTLs linked to drought resistance traits under field conditions.

- The percent of phenotypic variation explained by each marker ranged from 2.1 to 28.7 per cent.
- QTLs that were identified in one environment for a given trait had also been mapped at the same place as QTLs affecting other related traits in the same or another experimental site.

For example, in MSE, there were two markers (B06_700 and I23S_1761) common for leaf rolling, leaf drying, recovery and canopy temperature.

- Analysis of QTLs controlling different traits involved in drought avoidance and plant production under stress has shown that common QTLs for different traits across the trials. For example, RM212 was linked to plant height and biomass under stress in both the trials.
- QTLs identified in this study have also been noticed in other genetic backgrounds for different drought resistance component traits in rice. RM212 on chromosome 1 was identified as QTL associated with leaf drying, plant height, tiller number and biomass under water stress in this study. This marker region was also detected as QTLs for deep root mass, deep root ratio, deep root/tiller, relative water content, number of tillers, plant mass, basal root thickness, root-shoot ratio and osmotic adjustment in different genetic backgrounds and in different environments in rice.

- RM263 on chromosome 2 was linked to leaf drying, plant height and recovery in this study whereas, this marker region was found to be linked to osmotic adjustment in another genetic background.
- RM11 on chromosome 7 was linked to leaf drying and RM210 on chromosome 8 was associated with canopy temperature, leaf drying and plant height in this study. These regions were identified as QTLs for osmotic adjustment in another genetic background.
- RM242 on chromosome 9 was associated with plant height and leaf drying in this study whereas this region was identified as QTL linked to penetrated root thickness, days to heading and plant height in another genetic background.

- Thus this study had identified putative simple PCR based markers linked to drought resistance traits across environments in locally adapted rice genotypes and are consistent across genetic backgrounds and they may be useful in efficient marker aided selection for drought resistance improvement in rice.

Plate 1. Transgressive segregation for plant stature among the recombinant inbred lines of IR20/Nootripathu

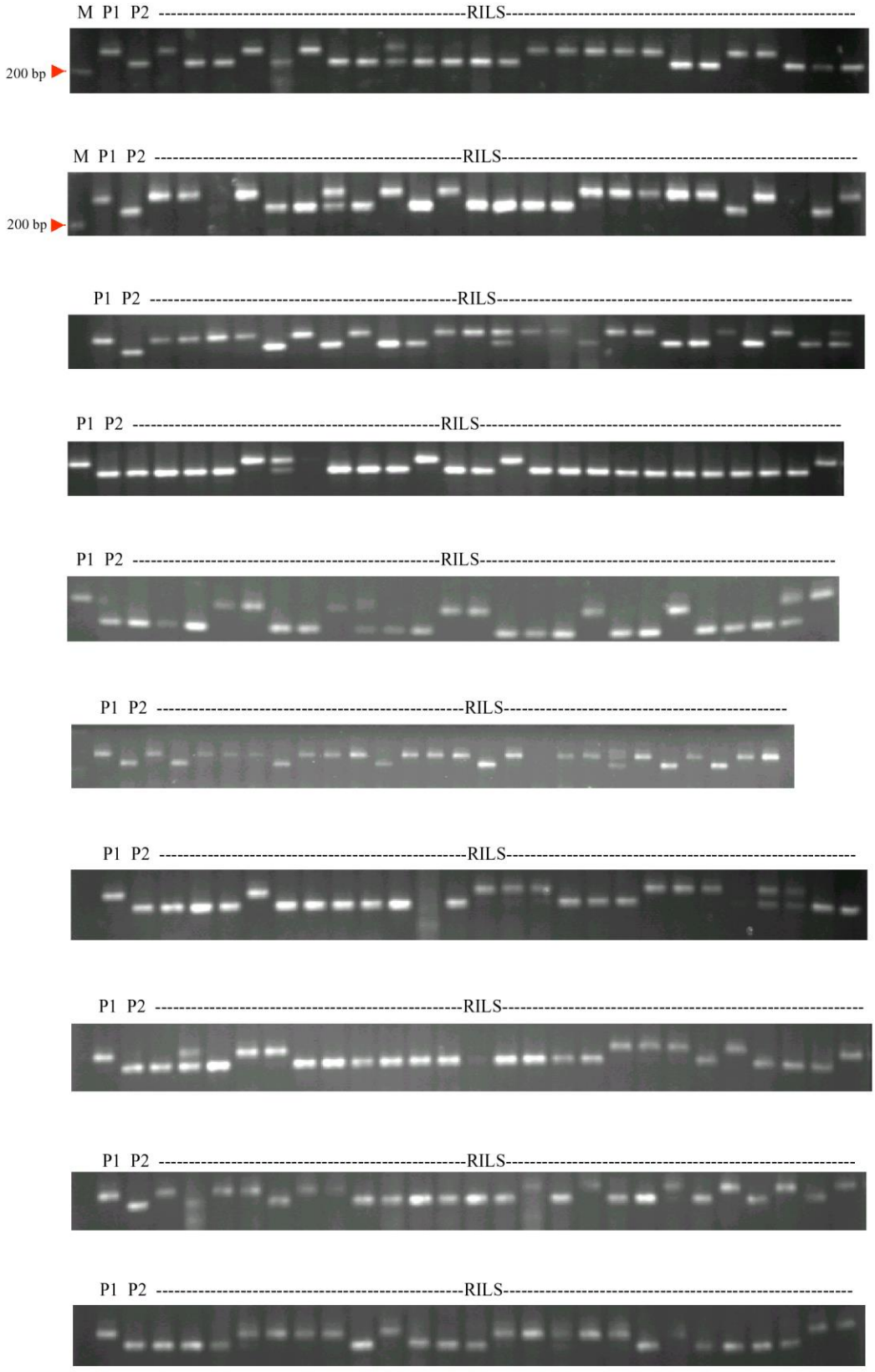


Plate 2 . RM212 marker segregation among the recombinant inbred lines of IR20/Nootripathu

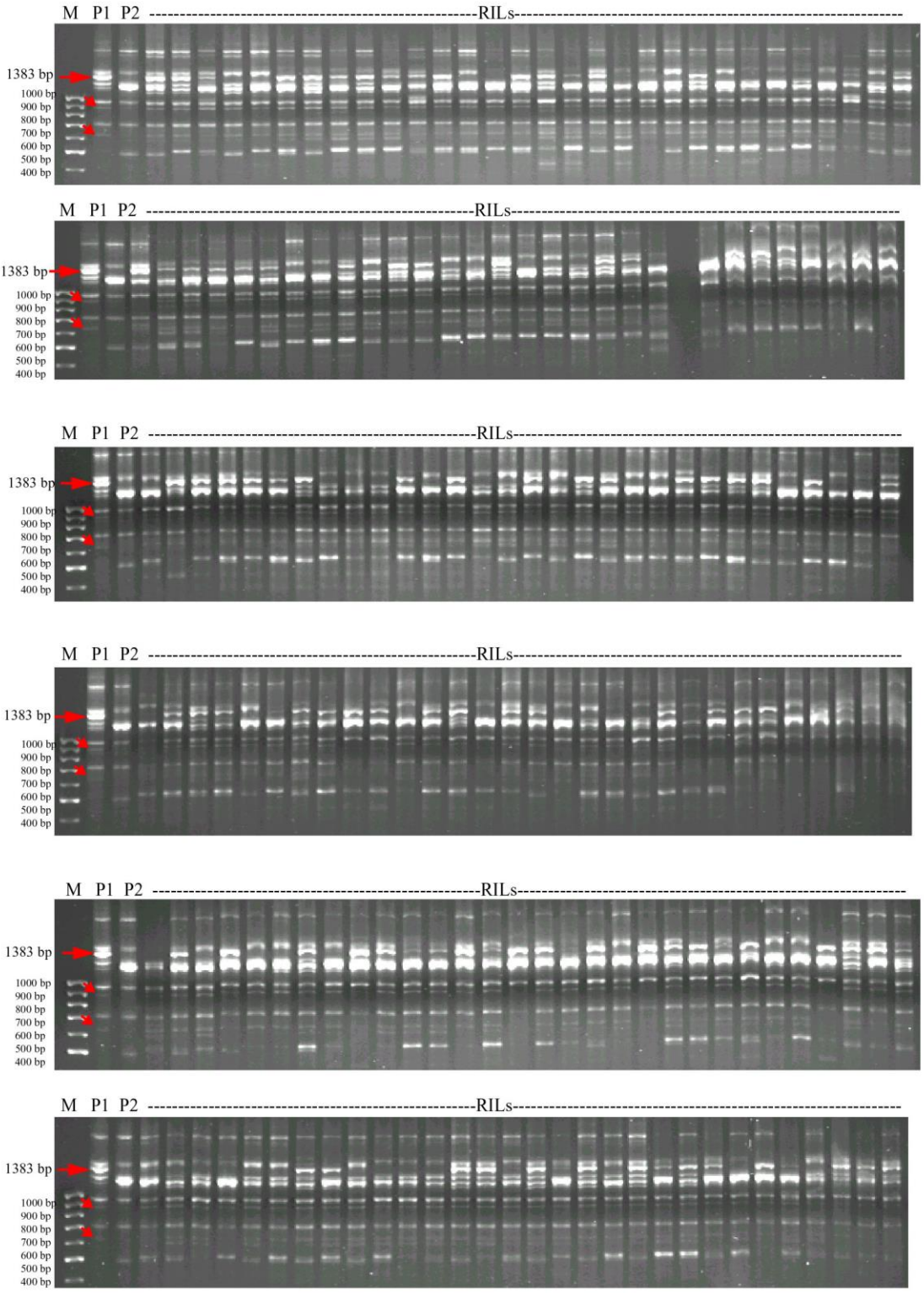


Plate 3. I07S_1383 marker segregation among the recombinant inbred lines of IR20/Nootripathu

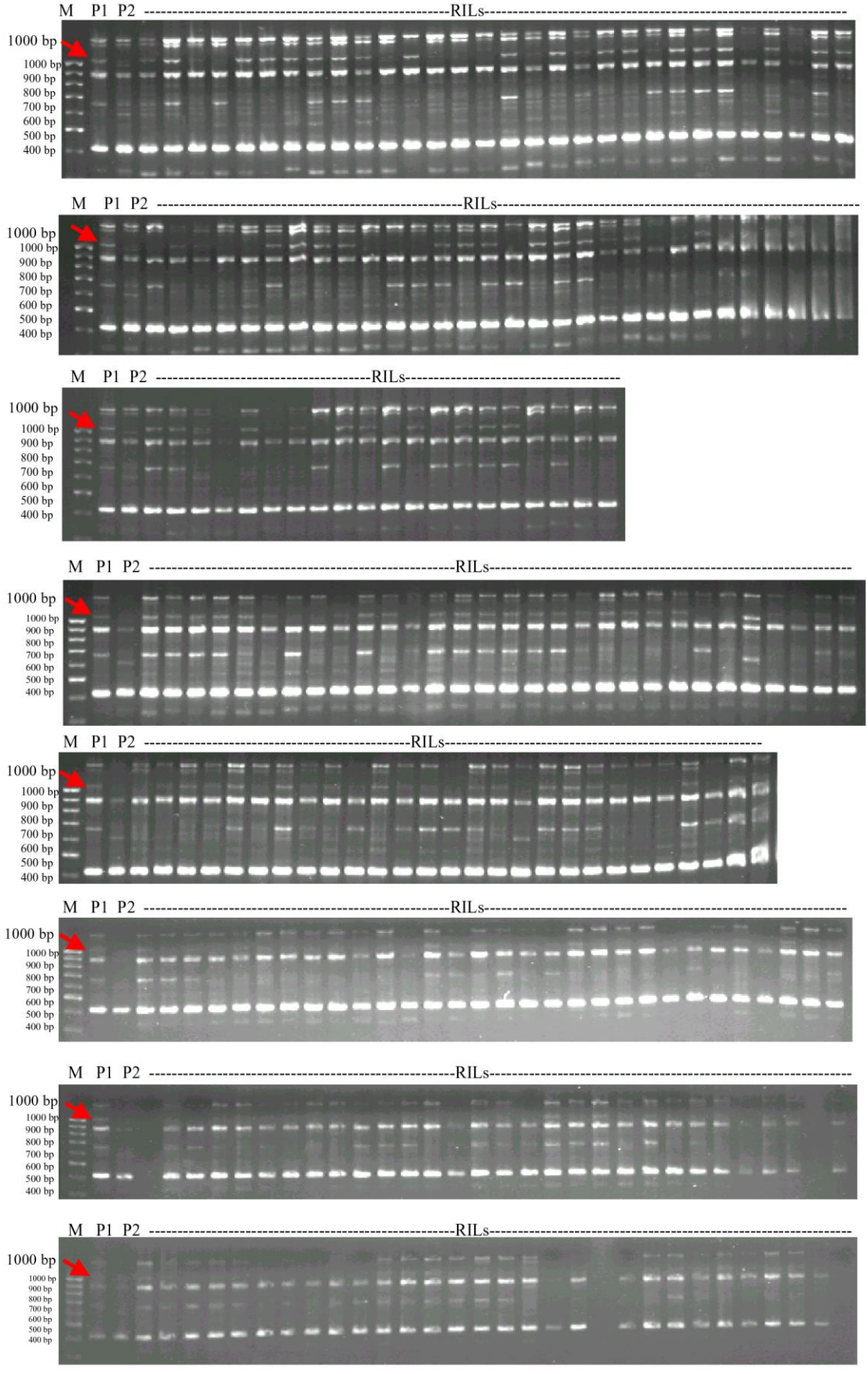


Plate 4. C20_1000 marker segregation among the recombinant inbred lines of IR20/Nootripathu

Plate 5. Field screening of recombinant inbred lines for drought resistance traits

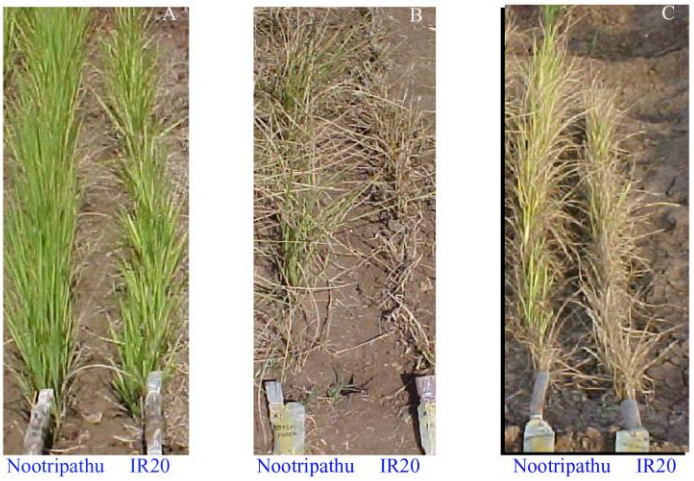


Plate 6. Variation in field drought tolerance between IR20 and Nootripathu
A- Irrigated Control; B- Water Stress; C- Drought Recovery

Plate 7a. Variation in field drought tolerance among the recombinant inbred lines

Plate 7b. Variation in field drought tolerance among the recombinant inbred lines

Plate 8. Variation in drought recovery among the recombinant inbred lines

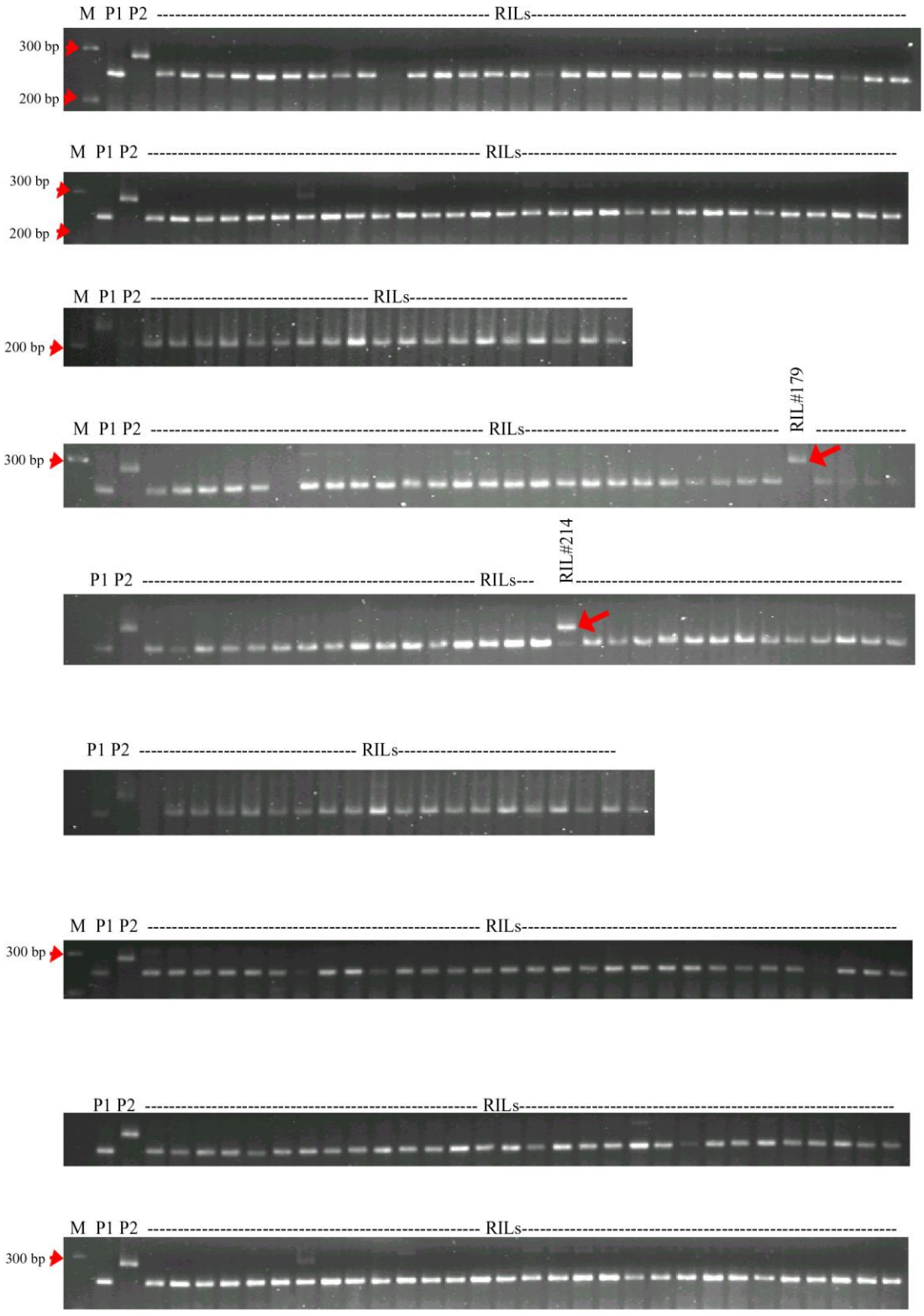


Plate 9. RM8213 marker segregation among the recombinant inbred lines of IR20/Nootripathu