Identification of marker associated yield and drought tolerance QTLs in rice (*Oryza sativa* L.)

Thesis submitted to

Acharya N. G. Ranga Agricultural University

For the award of the degree of

Doctor of Philosophy

By

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Certificate

Mrs. Akkareddy Srividhya has satisfactorily prosecuted the course of research and that the thesis entitled “**Identification of marker associated yield and drought tolerance QTLs in rice (*Oryza sativa* L.)”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part of thereof has not been previously submitted by her for degree of any university

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Declaration

I hereby declare that the thesis entitled “**Identification of marker associated yield and drought tolerance QTLs in rice (*Oryza sativa* L.)”** embodies the original research work carried out by me at Biotechnology Unit, Agricultural Research Institute, Aharya N.G.Ranga Agril. University, Hyderabad under the guidance of K.Hariprasad Reddy. This thesis has not been submitted for any other degree or diploma to any university or institute. Any assistance received is suitably acknowledged and original works referred to have been cited.

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ABSTRACT

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Progressive improvement of yield is critical for sustained self-sufficiency in rice, India's major staple. Along with yield enhancement, what is considered equally important to raise the productivity level is containment of yield destabilizing factors, especially major abiotic stress like drought, being the most devastating environmental stress for crop production. Rice is one of the very few crop species endowed with rich genetic diversity. In spite of such a huge variability today's cultivars represents not more than 15% of the total variability available in the landraces and wild/weedy relatives.

In all, 45 and 68 QTLs were identified for yield and drought tolerance, respectively in 140 RILs in F_6, developed from the cross of the landrace INRC10192 with IR64. Markers, namely RM1, RM471, RM262, RM427, RM331 and RM404 for yield and its components and RM1, RM225, RM5897, RM427, RM481, RM486, RM218 and RM404 were identified as potential markers for use in crop breeding through MAS. A highly significant locus (*qspy8.1*) for grain yield under moisture stress was identified on chromosome 8 at the marker interval of RM38-RM331 with high phenotypic variation (45.45%). Despite its inferiority for traits of agronomic significance alleles from the landrace had beneficial effect on yield components like NT, NPT, SF, BM, SPY and HI. Moreover, for many of the production and phenology related traits of drought tolerance *viz*., NPT, PSF, PSF, SGW, BM, SPY, SL, RL, SDW, RDW and RS, also had favorable allele effect from the landrace. Stable QTLs offer new opportunities for exploiting them for progressive yield improvement.

ABSTRACT

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Introduction

Rice is endowed with a rich genetic variability. In spite of such a great diversity, the modern rice cultivars have narrow genetic base for most of the agronomically important traits and abiotic stresses like drought. To sustain the demand of an ever increasing population, new avenues have to be explored to increase the yield level of rice. Wild progenitor species and traditional varieties, proven as potential donor sources for complex traits such as yield and drought tolerance would help to realize the dream of sustained food security. The traditional tall varieties and landraces, available in thousands, have hardly been screened for their genetic potential for yield before using them as a parent in breeding for yield enhancement. Most of the traits of economic importance such as yield and its components and traits related to drought tolerance are governed by a number of genes with small effect popularly called polygenic which are differed in expression with environments and difficult to measure phenotypically. However, recent advances in molecular biology have equipped scientists with a wide choice of marker-assisted techniques to identify both quantitative and qualitative traits. Among them the marker-associated quantitative trait loci (QTLs) relating to quantitatively inherited traits like yield and drought tolerant related traits has become handy to plant breeders to identify germplasm/segregants with positive yield genes. Investigations using high-density molecular markers have shown several of the land races and traditional cultivars of rice to represent a rich reservoir of genes directly or indirectly promoting grain yield.

Keeping the foregoing in view, the proposed study on rice was undertaken with the following specific objectives:

1. Mapping marker associated QTLs relating to yield and its major components
2. Tagging the component traits of drought tolerance to molecular marker associated QTLs

Material and methods

A mapping population of 140 RILs in F_6 was developed from the cross of the landrace INRC10192 with the semidwarf high yielding variety IR64 and was evaluated under field conditions using randomized block design at wet land farm of Biotechnology Unit, Hyderabad and RARS, Maruteru, West Godavari District during *kharif* 2004 with two doses of nitrogen fertilizer for yield and its components, while for drought tolerance related components a field experiment was conducted during *rabi* 2006 at BTU, Hyderabad. Six randomly selected plants in each line were phenotyped as well as genotyped with polymorphic rice microsatellite markers (RMs).

For mapping QTLs, two parental varieties were screened using 412 rice microsatellite markers. Of these, 133 showed polymorphism (32.28%). The polymorphic markers were distributed on all the 12 chromosomes and were used for screening the mapping population. A molecular linkage map was constructed employing 63 microsatellite markers spanning a total map length of 1978.9cM using the kosambi mapping function resulting in an average of one marker every 31.41cM, using MAPMAKER/EXP v 1.0 and MapDisto v 1.7 softwares. QTL mapping was carried out employing interval mapping (IM) and composite interval mapping (CIM) methods of the

QTL Cartographer v 2.5. Chi square analysis revealed that 33.33% of the markers showed segregation distortion.

Results

The phenotypic values were found normally distributed with transgressive segregants for all the 12 and 23 traits measured for yield and drought tolerance, respectively. The major findings from QTL mapping are presented according to the objectives here under.

1. **Mapping marker associated QTLs relating to yield and its components**

   A total of 45 QTLs was identified for 12 traits. These QTLs were found to be distributed on all the chromosomes except 6, 11 and 12. Of the 45 QTLs, 18 were identified as “stable” and 25 were novel identified for the first time. The donor parent contributed favorable alleles for 62.22% of the QTLs. 26 QTLs explained more than 30% phenotypic variance. Six markers, namely RM1, RM471, RM262, RM427, RM331 and RM404 were identified as “potential” to use in crop breeding through marker assisted selection (MAS). Interestingly, a single region on chromosome 8 at the marker interval of RM404-RM547 was found to be controlling eight important yield components *viz*., plant height, number of tillers, number of productive tillers, chaffy grains, spikelet number, biomass and harvest index.

2. **Tagging the component traits of drought tolerance to molecular marker associated QTLs**

   A total of 68 QTLs were identified for drought related traits and distributed on all the rice chromosomes except 11 and 12. In all only five QTLs *viz*., *qph5.1*, *qph7.1*, *qph8.1*, *qscg8.2* and *qrs8.1* were identified as “stable” and most of them were novel. Out of 68 QTLs 33 explained more than 30% of the phenotypic variation. The markers RM1, RM225, RM5897, RM427, RM481, RM486, RM218 and RM404 were identified as “potential” and are likely to be useful in practical plant breeding by MAS.

A highly significant locus (*qspy8.1*) for grain yield under stress was identified on chromosome 8 at the marker interval of RM38-RM331. This QTL explained high phenotypic variance (45.45%). The same locus also had a significant effect on as many as 8 traits under severe stress, including plant height, number of productive tillers, chaffy grains and spikelet fertility on secondary branches, biomass, root dry weight and root shoot ratio. Thus the effect of this locus in this population seems to be large enough to support fine mapping and positional cloning of genes, besides the potential use in MAS to improve drought tolerance. Seven candidate genes *viz*., *Os08g07700.1, Os08g07760.1, Os08g08060.1, Os08g08110.1, Os08g08140.1, Os08g10070.1,* and *Os08g16910.1* associated with drought tolerance were identified in this interval. Cloning and functional analysis of these genes may shed precise insights into the molecular and physiological basis of drought tolerance in rice.

Some genomic regions showed multi-trait clusters. For instance, the genomic region between the markers RM404 and RM547 on chromosome 8 accounts for eight yield related traits, while in the marker interval of RM1-RM495 on chromosome 1 accounts for spikelet number and single plant yield and between RM331 and RM404 on chromosome 8 accounts for three yield components with the former reflecting the multigenic control of a trait, and the later two instances either pleiotropism or complex locus with tightly linked non-allelic genes.

Conclusions

The promising QTLs relating to major yield related traits could be used as potential candidates for future improvement by pyramiding as well as fine mapping and positional cloning by gene transfer technology. Stable yield related QTLs offer new opportunities for exploiting them for progressive yield improvement through two approaches *viz*., recombination breeding using concerned QTLs as selectable markers and recombinant DNA technology by transferring selectively cloned QTLs of promise into good agronomic bases. In addition, the novel QTLs identified are good candidates for fine mapping and positional cloning studies, while, the QTLs that are mapped to regions consistent with other studies can be useful for marker assisted transfer of these QTLs. Pyramiding of promising and harmonious QTLs into one cultivar from stress responsive 'productivity' (chromosomes 2 and 8) and 'phenology traits related' (Chromosome 1) chromosomes could help breach yield barriers.

Detection of favorable QTLs for yield components like number of tillers, number of productive tillers, percent spikelet fertility, biomass, single plant yield and harvest index. Moreover, for many of the production and phenology related traits of drought tolerance *viz*., number of productive tillers, spikelet fertility on primary and secondary branches, grain weight on secondary branches, biomass, single plant yield, shoot length, root length, shoot dry weight, root dry weight and root shoot ratio confirms that the traditional tall landraces as the one chosen for the study posses hitherto unexploited yield and drought tolerant genes and utilization of them as potential donors in breeding for yield enhancement would be rewarding.

Contents

S.No.	Title	Page No.
1	Introduction	1
2	Review of Literature	9
3	Materials and Methods	27
4	Results	45
5	Discussion	85
6	Summary and Conclusions	138
7	References	144
8	Supporting Information	160

List of Figures

S.No.	Title	Page No.
Figure 1	Development of mapping population and QTL mapping	29
Figure 2	The mapping population of 140 RILs along with the parents	30
Figure 3	Rice field plots under moisture stress condition	32
Figure 4	Leaf rolling of RILs along with parents under the stress condition	33
Figure 5	Root and shoot pictures of parents (INRC10192 and IR64) and check (Azucena and MTU1061)	37
Figure 6	Polymorphism between IR64/INRC10192 employing rice microsatellite markers	42
Figure 7	Segregation pattern of RM17 locus in recombinant inbred population of cross between IR64 and INRC10192	42
Figure 8	Phenotypic distributions of yield and its components of 140 RILs evaluated at Hyderabad	48
Figure 9	Phenotypic distributions of yield and its components of 140 RILs evaluated at Maruteru	49
Figure 10	Soil moisture percentage measured at two depths of soil in drought experiment under field conditions	50
Figure 11	10 Phenotypic distributions of drought related traits in 140 RILs under field condition	54-55
Figure 12	Phenotypic distributions of drought related traits in 140 RILs under greenhouse condition	58
Figure 13	Distribution of QTLs for yield and its components in the molecular linkage map of IR64/INRC10192	69
Figure 14	QTL cartographer LOD peak for plant height and biomass	71
Figure 15	Distribution of QTLs for drought related traits in the molecular linkage map of IR64/INRC10192	78

List of Tables

S.No.	Title	Page No.
Table 1	Previous reports on major quantitative trait loci (QTLs) for yield and its components	12-14
Table 2	Previous reports on drought related quantitative trait loci (QTLs)	18-20
Table 3	Genes underlying the major QTLs in rice	22
Table 4	Description of yield and its components and their codes	31
Table 5	Description of yield and its components and drought tolerance related traits measured under field conditions	35
Table 6	Description of the drought tolerance related traits measured under greenhouse conditions	38
Table 7	Test of significance of the parents for yield and its components measured under two levels of N at two locations	45
Table 8	Performance of the parents, IR64 and INRC10192 and 140 RILs for yield and its components under two N-levels across two locations	46
Table 9	Test of significance of the parents for yield and its components and drought related traits measured under two water regimes in field condition	51
Table 10	Performance of the parents, IR64 and INRC10192 and 140 RILs for yield and its components and drought related traits under field conditions	52
Table 11	Test of significance of the parents for shoot and root traits and of their relative parameters measured in control and stress treatments under drought stress controlled greenhouse conditions stress	56
Table 12	Performance of the parents, IR64 and INRC10192 and 140 RILs for shoot and root traits and of their relative parameters in control and stress treatments under green house conditions	57
Table 13	Correlation coefficients among yield and its components under two N-levels at two locations	59

Contd..

Table 14	Correlation coefficients among yield and drought related traits under field conditions	61
Table 15	Correlation coefficients among shoot and root traits in Control (C), Stress (S) treatments and Relative parameters (R) in 140 RIL population under greenhouse conditions	63
Table 16	QTLs identified employing interval mapping and composite interval mapping	66
Table 17	QTLs for yield and its components identified in 140 RILs of the cross between IR64 and INRC10192 under two N levels at two locations	67-68
Table 18	QTLs for grain yield and its components and secondary traits detected in 140 RILs of the cross IR64 and INRC10192 under field and greenhouse conditions	76-77
Table 19	Markers associated with gamete abortive and/or sterility genes	93
Table 20	QTLs identified for yield and its components across N-levels and Locations	94
Table 21	Comparison of quantitative trait locus (QTL) reported across the *Oryza* genus	101
Table 22	Candidate genes in the region RM38-RM331 on chromosome 8	115
Table 23	Main effect QTLs detected for drought tolerance	118
Table 24	QTL clusters identified for yield and its components	124
Table 25	QTL clusters identified for drought tolerance traits under field and greenhouse conditions	127
Table 26	Potential QTLs with nearest marker identified in the present study for marker assisted selection (MAS) related to yield and yield components	130
Table 27	Potential QTLs with nearest marker detected under field and greenhouse experiments in the present study for marker assisted selection (MAS) of drought tolerance related traits	131
Table 28	Promising QTLs identified for yield and its components	133
Table 29	Promising QTLs identified for drought tolerance	134

Introduction

1 Introduction

Rice is the world's second most widely grown cereal crop after wheat, and is the staple food for more than one half of the world's population. Accounting for 23 percent of the world's supply of calories (Ashikari *et al.*, 2005), it is one of the most versatile crops grown under a wide range of agro-ecological conditions ranging from irrigated to rainfed lowland, upland, deep water and tidal conditions. The total area under rice is around 145 million hectares with the production of about 645 million tons (2007-2008), of which 90% is produced and consumed in Asia. India is the largest rice growing (44 million hectares in 2007-2008) and the second largest producing (143.5 million tons in 2007-2008) country, but productivity wise standing eighth (3.2 tons per hectare in 2007-2008) (www.usda.gov, www.fao.org and www.oryza.com). Accounting for 43 per cent of the country's food grain production, rice provides over 70 per cent of the dietary energy in the daily intake of food in the traditional rice growing-consuming regions.

With population growing still at a higher rate, rice demand is estimated to be around 200 million tons (130 million tons of milled rice) by 2025. Such a high target has to be achieved only by yield increase from 3.2 to 4.5 t of paddy/ ha. With practically no scope for expansion of net area under rice and limited opportunities for bringing more area under irrigated crop, lack of high yielding varieties to cater to the needs of the vast rainfed ecosystems and plateauing/declining of productivity growth in the irrigated ecology, achieving of the projected yield target would be a challenging task (Siddiq, 1999). This calls for development of varieties with higher yield potential combining tolerance to biotic and abiotic stresses. Given the underexploited opportunities and unexploited potential, the task is not unachievable. Of the immediately exploitable opportunities, consolidation of sizeable yield potential still remaining untapped in the currently available high yielding varieties in the irrigated ecology is the foremost. A recent study (E.A.Siddiq's personal communication) reveals the gap between achievable and actual yields to range between 35 to 65% in the major rice growing states, with the exception of Punjab and Tamilnadu. Narrowing of the gap even by 30% would help add almost 30 million tons by diagnosing and correcting the yield depressing factors. Maximization of yield levels of relatively favorable rainfed shallow lowland ecology could be yet another potential opportunity. Such strategies, no doubt, would greatly help meet the demand projections until 2030. The real challenge would be in coping with the colossal demands of the decades beyond 2030 and that can be achieved by finding new yield thresholds.

Since the advent of the plant type based high yielding varietal technology since the mid sixties there has been research efforts to further raise the genetic yield level. Thanks to the Chinese scientists, who could successfully develop and demonstrate the commercial feasibility of hybrid technology by late seventies. The cytoplasmic male sterility based hybrid technology with yield advantage of about 15% proved a landmark in the history of rice breeding. The technology though found very wide adoption and helped raise the production-productivity level in

China, it is yet to make its impact elsewhere including in India. Hoping that it would prove a potential technology, if the problems constraining its wide adaptation are addressed, the rice scientists are in search of technologies beyond hybrids for progressively raising the ceiling to genetic yield. Of the various strategies being contemplated, discovery and use of still not exploited yield genes in wild/weedy relatives is considered promising (Tanksley and McCouch, 1997; Moncada *et al*., 2001).

Rice is one of the very few crop species endowed with rich genetic diversity. It comprises 23 species grouped under seven genomes *viz*., AA, BB, CC, BBCC, CCDD, EE and FF (Chang, 1976). More than one hundred thousand landraces and improved varieties of the cultivar species along with a few thousand accessions of wild/weedy species available in the gene banks world over largely constitute the diversity. However, it still remains unknown as to how much of useful genetic diversity exists, especially for complex traits like yield and adaptability to abiotic stresses in the least used primitive landraces and wild/weedy progenitor species of *Oryza sativa*. In fact, the germplasm breeders depend on today, represents not more than 15% of the total variability available and the remaining over 85% of the genetic variation remain untapped in the landraces and wild/weedy relatives (Wang *et al*., 1992). The importance of the genepool representing such a large variability is not, however, known beyond finding it as a source of a few rare and valuable major genes like insect pest and disease resistance. But for stray reports, hardly there are reports based on any systematic study to the effect that so neglected germplasm as well could be a source of variability for quantitatively inherited traits like yield. Thus, the greatest challenge to rice breeders is in mining and judiciously exploiting such variability towards raising the genetic yield level.

Along with yield enhancement, what is considered equally important to raise the productivity level is containment of yield destabilizing factors, especially major abiotic stresses like moisture stress and salinity. Over 55% of the rice area is rainfed, while 14% is affected by inland and coastal salinity. Drought and salinity depress yield by about 15-50% and 10-20%, respectively.

Drought being the most devastating environmental stress, efforts have been to improve the crop productivity under water-limiting conditions. In the course of origin and further evolution of rice, natural selection has been in favor of retaining traits of survival, while breeding activity has been towards selectively improving the traits of agronomic value such as higher yield, duration of choice, plant type etc. More than 80 year long history of breeding for tolerances to drought has led us nowhere near development of ideal genotypes capable of resisting the stress, despite availability of reliable sources of tolerance to the stress in the germplasm. In depth study of the phenomenon, however, has enabled us in understanding the physiological responses of plants and not so much the genetic basis of moisture stress.

Like yield and its components, the traits that confer drought tolerance are controlled by many genes, and are referred to as quantitative traits.

Understanding the genetic basis of quantitative traits is difficult as observed phenotype is not exact of the genotype (Falconer and Mackay 1996). Expression of genes that control quantitative traits is greatly influenced by environment and consequently, improvement of such traits by traditional breeding and phenotype based selection methods are time consuming and cumbersome and the gains are harder to realize. Breeders usually overcome this problem by multi-environmental evaluation of the traits, in replicated trials, to quantify the effect of environment. Because of problems encountered while trying to improve quantitative traits by conventional means, breeders and geneticists consider molecular markers as the reliable means to detect chromosomal regions harboring the genes that govern such quantitative traits *per se* and their indices/components.

Morphological and genetic markers were the first generation markers used for selection of quantitative traits. About 80 years ago, Sax (1923) reported the association of quantitatively inherited seed size with simply inherited genetic markers that governed seed coat pigmentation and pattern in common bean (*Phaseolus vulgaris* L.). Subsequently, simply inherited and linked morphological mutations were used as markers for selection of polygenic traits. However, because of paucity of such markers and effect of them on quantitative traits often being larger than that of the linked QTLs, breeding of quantitatively inherited traits has been difficult (Tanksley *et al*., 1998). Later, isozyme markers were used to identify QTLs in maize (Stuber and Edwards 1986). Isozymes have also been used for genetic analysis and to study linkage relationships in rice (Pham *et al* 1990). Number of isozyme markers was too inadequate to be of use for extensive study of quantitative traits.

Falconer and Mackay (1996) observed that if DNA markers linked to genes governing quantitative traits could be found, indirect selection for such markers might improve the efficiency of breeding-selection process for the associated quantitative traits. This strategy is highly reliable as molecular markers are unaffected by environment and not stage or tissue specific. Over the last two decades DNA markers have been extensively used in a variety of crops to determine the number of genes controlling the traits of quantitative inheritance and for tagging them (Tanksley *et al*., 1992; Marri *et al*., 2005).

The DNA markers are of two broad categories, *viz*. restriction enzyme based markers and DNA amplification or PCR based markers. Of the several PCR-based markers employed in genetic mapping studies, microsatellite markers, also known as simple sequence repeats (SSR), are genetically more informative, crop and trait specific, co-dominant and robust. Available in abundance in eukaryote genomes SSR marker analysis requiring only a small DNA template, is technically simple and amenable to high throughput assay (McCouch *et al*., 1997). Over 18,600 SSR markers based high-density linkage map is available today in rice (Gramene V28; www.gramene.org; September 2008). Such molecular maps are powerful tools for elucidating genetic basis of several agriculturally important traits, many of which are quantitatively inherited. The marker associated QTL approach enables detection of genes that are otherwise difficult to identify and by dissecting the QTLs genetic architecture of such traits could be elucidated. In the past, understanding of the molecular basis of polygenic traits had been difficult in the absence of whole genome sequence information. Today, annotated whole genome sequence of rice is available; using which designing of suitable molecular marker(s) for analyzing QTLs is possible. Availability of high density marker linkage maps annotated genome sequence and powerful biometric methods have enabled in-depth study and use of trait-specific QTLs for directed improvement of rice.

Reports on the discovery of marker associated yield QTLs in landraces (Hariprasad, 2003) and wild/weedy species (Xiao *et al*., 1998; Brondani *et al*., 2002; Marri *et al*., 2005 and Lafitte *et al*., 2006), with suggested potential to raise the genetic yield level of rice by about 15-20 percent, have aroused interest among rice breeders to look for more such genes in wild /weedy accessions in the primary and secondary genepools.

Most of the past efforts to combine tolerance to drought in high yield background have been a futile exercise, largely because of its genetically complex nature and lack of precise screening or selection protocols. Molecular marker associated QTL approach has been found quite handy in the recent years. Potential markers and gene clusters have been identified for the various morpho-physiological indices of tolerance to moisture stress in rice (Lilley *et al*., 1996;

Tripathy *et al.*, 2000; Price *et al.*, 2002a; Babu *et al.*, 2003; Lafitte *et al.*, 2006, Bernier *et al.*, 2007; Liu *et al.*, 2008 and Zhao *et al.*, 2008).

Like in yield, genes conferring tolerance to abiotic stresses like drought and salinity remain still uncovered in genotypes other than those known to be tolerant. Recent reports suggest that such sequences could be found in the mapping populations of cross combinations that do not involve proven donor parents, for such traits (Ali *et al.*, 2002, Vijayakumar *et al.*, 2002, Adnan Kanbar *et al.*, 2004, and Ali *et al.*, 2006). Genes discovered from such hidden sources could be new and novel, there is need to identify them as well for adding further strength to marker assisted breeding for tolerance to abiotic stresses like moisture stress.

Keeping in view the need and urgency to find new yield thresholds and defend so achieved gains against yield depressing abiotic stresses like moisture stress on one hand and molecular marker technology available to find new and novel QTLs/ gene clusters relating to these breeding objectives remaining hidden in the still not exploited rice genepools on the other, the present study was undertaken with the following specific objectives:

- Identification of stable molecular marker associated yield QTLs of promise in primitive cultivars.
- Identification of rare recombinants for drought tolerance in yield oriented mapping population and
- Tagging the component traits of drought tolerance to molecular marker associated QTLs.

Review of Literature

2 Review of Literature

Introgression of unique genes from wild/weedy relatives and primitive cultivars enriches genetic variability required for progressively improving crop plants. Rice is endowed with the richest germplasm comprising as many as 23 wild species grouped into seven distinct genomes. Ironically not even 2% of the variability available in the wild genepools has been utilized in the improvement of the crop. Efforts have been although to find potential gene sources not found in the cultivar germplasm and use by devising innovative breeding approaches to overcome crossability barriers. International Rice Research Institute (IRRI) has been successful in transferring many pest resistance genes of value from the wild/weedy species into cultivars. West Africa Rice Development Association (WARDA) and the International Center for Tropical Agriculture (CIAT) have as well been as successful in introgressing useful genes from various wild relatives into elite breeding lines of the Asian cultivars.

Aside Mendelian genes, many QTLs for traits that contribute to complexly inherited quantitative traits like yield and drought tolerance have been reported in the mapping populations involving wild/ weedy species as donors as listed in Table 1 and Table 2. Some of the major findings relating to yield and tolerance to moisture stress are briefly reviewed below.

Yield and its components

International status: In the first ever report on the use of wild species for introgression of quantitative characters, Xiao *et al.* (1996a) had identified in a Malaysian accession of *O.*

rufipogon two yield QTLs, *viz*., *yld1.1* and *yld2.1*, each of which capable of increasing yield by about 18% over the hybrid V64, one of the most popular heterotic hybrids in China. In another study by the same group (Xiao *et al*., 1996b) identified as many as 37 QTLs of promise for 13 yield and yield related traits in the mapping population of 194 recombinant inbred lines in F_8 of an inter sub-specific cross between two elite homozygous lines *viz*., 9024 and LH 422,. The number of QTLs detected for each of the traits ranged between one and six explaining phenotypic variance of 5.0 to 73.7%.

Lu *et al.* (1996) by comparative mapping of QTLs for six agronomic traits across three diverse environments in a doubled–haploid (DH) population of *indica/japonica* cross identified a total of 22 QTLs for heading date, plant height, number of spikelets per panicle, number of grains per panicle, 1000-grain weight and percentage of seed set. Only seven QTLs were found significant in all the three environments, while seven were in two environments and eight in a single environment. QTLs for spikelet number per panicle and grains per panicle were common across environments, while traits like heading date and plant height were more sensitive to environment indicating a trait dependency of QTL-by-environment (Q x E) interaction.

Moncada *et al.* (2001) have reported 25 QTLs introgressed from the accession of *O. rufipogon* from Malaysia into Caiapo, an upland *O.sativa* subsp *japonica* rice variety from Brazil. Two of them were for yield, thirteen for major yield components, four for maturity and six for plant height.

Brondani *et al.* (2002) have also reported introgression of yield-related traits from the wild species, *O.glumaepetula* into cultivated rice through advanced backcross method. They too identified two QTLs each for yield and plant height, four for number of tillers, five for panicle length, 7 for spikelet fertility and six for grain weight.

Yoon *et al.* (2006) for the first time used the distantly related wild species *O.grandiglumis* (2n=48; CCDD) as the donor parent and developed BC_5F_3 lines in the background of Hwaseongbyeo (*japonica*), and identified a total of 39 QTLs for 13 traits. Phenotypic variation associated with each QTL ranged from 4.2% to 30.5% and for 18 (46.2%) of the QTLs *O. grandiglumis alleles* contributed the desirable effect.

National status: Exploitation of wild species (*O. rufipogon*) and primitive cultivars of rice for introgression of yield enhancing QTLs was started first time In India at the Directorate of Rice Research (DRR), Hyderabad, under the National Professor's project entitled "**Enhancement of yield through innovative genetic approaches in rice**".

Hariprasad (2003) detected as many as nine hitherto unexploited QTLs for grain yield and its components *viz.*, harvest index, biomass per plant, grain number/ panicle, per cent spikelet fertility and test grain weight in the F_2 population of the cross involving the primitive cultivar *viz.*, INRC10192 and the high yielding variety IR64 using SSR, ISSR and RAPD markers. The findings confirmed that traditional tall landraces could as well be potential donors in breeding for yield enhancement.

Marri *et al.* (2005) identified 39 QTLs for 13 yield and its components employing advanced backcross method in BC_2F_2 test cross progeny of the cross IR58025A/ *O.rufipogon* (IC22015)// IR58025B/// IR58025B//// KMR3 using single marker analysis with 81 SSRs. Of the 39 QTLs, 30 corresponded with the QTLs reported earlier while remaining nine were novel and reported for the first time. They also reported that in spite of its inferiority for most of the yield related traits, *O. rufipogon* alleles contributed positively to 74% of the QTLs.

Cheema *et al.* (2004) studied 12 BC_2F_5 population of the cross *O. rufipogon* (IRGC 105491) X IR64, raised in a replicated yield trail at two locations, (Ludhiana and Kapurtala in Punjab) for phenotyping of yield related traits. Genotyping of the population was done by microsatellite marker analysis to identify introgressed chromosome segments. SSRs showing frequent introgression were RM3, RM243, RM208, RM251, RM254, and RM561 present respectively on chromosomes 1,2,5,6 and 11. The introgressed lines showed significant increase in grain yield (50%) and yield components *viz.*, tillers/plant (29%) and grains/panicle (32%).

Anand *et al.* (2004) from study of RIL population in F_6 of the cross Pusa 1121 (elite basmati quality rice)/ Pusa 1342 (New plant type high yielding line) have reported QTLs of promise for grain number per panicle, 1000 grain weight, percent filled grains and number of productive tillers per plant.

Table 1. Previous reports on major quantitative trait loci (QTLs) for yield and its components in rice

Trait /QTL	Chr	Marker Interval/Marker	LOD	PVE*	Population	Parents	Reference
Panicle length							
pl1.1	1	RZ730	'-'	14.2	300 BC_2	V20A*	Xiao *et al.,*1998
pl8.1	8	RM210	'-'	6.9	300 BC_2	V20A*	Xiao *et al.,*1998
pl2.1	2	RM250 – RM208	10.9	19.28	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
pl6	6	RM539–RM121	'-'	8.2	BC_5F_3@	*O.grandiglumis***	Yoon *et al*., 2006
pl3.1	3	RM218-E25M48.237P2	7.82	13.65	164 RIL	Milyang(i/j) /Gihobyeo	Cho *et al.,*2007
Panicle no.							
ppl2.1	2	RG256		3.44	300 BC_2	V20A*	Xiao *et al.,*1998
ppl6.1	6	'-'	'-'	'-'	274 BC_2F_2	*O.rufipogon* /Caiapo	Moncada *et al*., 2001
np2.1	2	RM262-RM183	2.5	6.8	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
np2.2	2	RM324-RM262	3.23	10.81	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
qPn3b (N 1)	3	RM293-RM143	2.75	7.94	RIL F_{13}	Lemont/Teqing	Hua *et al*., 2006
qPn3a (N 0)	3	OSR31-RM55	3.45	5.63	RIL F_{13}	Lemont/Teqing	Hua *et al*., 2006
pn11	11	RM224 - RM144	'-'	5.6	BC_5F_3@	*O.grandiglumis***	Yoon *et al*., 2006
ppp1.1	1	RG140-RM243	5.24	16.97	164 RIL	Milyang(i/j) /Gihobyeo	Cho *et al.,*2007
Tiller no.							
nt2.1	2	RM262-RM183	2.5	6.8	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
Grain weight							
gw8.1	8	RZ323	'-'	3.14	300 BC_2	V20A*	Xiao *et al.,*1998
qgw3.1	3	JL107-JL109, RZ452	'-'	17	258BC_2F_2	*O.rufipogon/O.sativa*	Li *et al.,* (2004)
gw2.1	2	RM250-RM208	3.25	10.4	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
gw9.2	9	RM242-RM205	3.21	13.95	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
tgw8.1	8	RM52-RG885	5.88	15.1	164 RIL	Milyang(i/j) /Gihobyeo	Cho *et al.,*2007
tgw2	2	RM290–RM550	'-'	13.4	BC_5F_3@	*O.grandiglumis***	Yoon *et al*., 2006

Contd..

Spikelets/panicle							
spp9.1	9	RG386	‘-’	3.56	300 BC_2	V20A*	Xiao *et al.*,1998
spp6	6	RM539–RM121	‘-’	6.3	BC_5F_3@	*O.grandiglumis***	Yoon *et al.*, 2006
spp1.3	1	RM35-RG811	7.13	16.67	164 RIL	Milyang(i/j) /Gihobyeo	Cho *et al.*,2007
Spikelet no./plant							
spl1.1	1	RM25	‘-’	4.43	300 BC_2	V20A*	Xiao *et al.*,1998
sn2.1	2	RM250-RM208	4.2	19.13	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al.,* 2005
Fertile grains							
gpp8.1	8	RM25	‘-’	4.43	300 BC_2	V20A*	Xiao *et al*.,1998
gn2.1	2	RM250-RM208	3.32	16.65	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
gn5.1	5	RM194-RM169	2.98	6.12	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
gn8	8	R202-C10122	‘-’	‘-’	98 BC_1 F_8	*Nipponbare$*	Ishimaru *et al*., 2005
Plant height							
ph1.1	1	RZ730	‘-’	44.77	300 BC_2	V20A*	Xiao *et al*.,1998
ph4.1	4	Y1065	‘-’	8.49	300 BC_2	V20A*	Xiao *et al*.,1998
ph1.1	1	RM220-RM272	5.32	17.48	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
ph9.1	9	RM257-RM242	3.7	5.63	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
qPh2b (N1)	2	RM250-RM248	3.4	13.89	RIL F_{13}	*Lemont/Teqing*	Hua *et al*., 2006
qPh7a(N0)	7	RM18-RM478	3.19	7.35	RIL F_{13}	*Lemont/Teqing*	Hua *et al*., 2006
cl4.1	4	RM335–RM518	‘-’	6	BC_5F_3@	*O.grandiglumis***	Yoon *et al*., 2006
cl1.1	1	RZ317-RZ14	40.6	9.61	164 RIL	*Milyang(i/j) /Gihobyeo*	Cho *et al*.,2007
cl8.1	8	RG20-RG885	4.49	4.58	164 RIL	*Milyang(i/j) /Gihobyeo*	Cho *et al*.,2007
Semi dwarf							
Sd1	3	XNpb363	‘-’	‘-’	‘-’	‘-’	Ashikari *et al.,* 1999
Harvest index							
hi2.1	2	RM183-RM263	3.12	5.83	251 BC_2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
qtl8.1	8	RM3459-RM149	3.7	10	436 F_3	*Vandana/Way Rarem (i)*	Bernier *et al*., 2007
hi7b	7	RM420-RM248	6.88	23.1	166 F_8 RIls	*Dasanbyeo/TR22183*	Cho *et al*., 2007a

Contd..

Spikelet fertility							
pss8.1	8	RM25	'-'	14.61	300 BC2	V20A*	Xiao *et al*.,1998
sf1.1	1	RM212-RM	3.26	5.2	251 BC2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
sf3.1	3	RM251-RM36	4.35	6.7	251 BC2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
rg5	5	R2558	2.02	19.8	98 BC1 F8	*Nipponbare$*	Ishimaru *et al*., 2005
Yield per plant							
yld5.1	5	RG435	'-'	3	300 BC2	V20A*	Xiao *et al*.,1998
gy2.1	2	RM262-RM263	35.3	50.47	251 BC2	*O.rufipogon/O.sativa*	Marri *et al*., 2005
qYd6	6	RM30-RM439	2.71	8.13	RIL F13	*Lemont/Teqing*	Hua *et al*., 2006
yld1.1	1	RM5-RG462	3.66	8.41	164 RIL	*Milyang(i/j) /Gihobyeo*	Cho *et al*.,2007
yld7.1	7	RG477A-RM2	4.65	9.93	164 RIL	*Milyang(i/j) /Gihobyeo*	Cho *et al*.,2007
yld8.1	8	RM25-E13M59.152P2	2.81	15.94	164 RIL	*Milyang(i/j) /Gihobyeo*	Cho *et al*.,2007
gy9	9	RM242-RM215	4.1	12.9	166 F8 RIls	*Dasanbyeo/TR22183*	Cho *et al*., 2007a
Biomass							
qtl4.1	4	RM6487-RM471	3.8	3	436 F3	*Vandana/Way Rarem (i)*	Bernier *et al*., 2007

'-' means no symbol/ data for related QTLs in the published paper; N1= N-fertilizer- 150kg/hm^2; N0 = without N-fertilizer PVE*- Phenotypic variance explained

V20A*- V20A/*O.rufipogon*//V20B///V20B////Ce64; *O.grandiglumis***- *O.grandiglumis*/ japonica cv. Hwaseongbyeo; *Nipponbare$- Nipponbare/Kasalath// Nipponbare*

i-indica;j-japonica; @ BC5F3 /150 F3

Drought tolerance related traits

In 1998, a large-scale backcross breeding programme was initiated at IRRI involving as many as 195 traditional varieties from different rice growing countries as donors and three elite genotypes comprising two high yielding varieties *viz*., IR64 and Teqing, and a new plant type breeding line IR68552-55-3-2, derived from *indica*/ tropical *japonica* cross, as the recurrent parents. The BC_2F_2 population was then screened for tolerance to abiotic stresses such as drought and salinity. Forty-six QTLs for drought tolerance were detected by linkage and linkage disequilibrium in 38 introgression lines selected under moisture stress condition in the lowland and upland environments in the BC_2F_2 population of the cross involving no known donor as the parent as in IR64/ Type3. Most of the breeding populations involving traditional varieties not known for its tolerance to the stress, similarly resulted in tolerant progeny. Genes from these traditional varieties (TVs) when placed in elite backgrounds showed previously unexpressed phenotypes like tolerance to abiotic stresses (Vijaykumar *et al*., 2002, Ali *et al*., 2002 and 2006). Similar findings were reported by Tanksley and coworkers when they showed that some genes from *O. rufipogon* contributed to enhanced yields when placed in hybrid rice backgrounds. Dr. ZhiKang Li of IRRI who masterminded this strategy of advanced backcrossing programme refers to these newly expressed genes as “hidden alleles”.

several QTLs have been reported for traits that are putatively associated with drought tolerance such as root morphology (Champoux *et al*., 1995), osmotic adjustment

(Robin *et al*., 2003), leaf membrane stability (Tripathy *et al*., 2000), and visual symptoms of leaf rolling and drying (Courtois *et al*., 2000).

In recent years increasingly more attention is given to mapping of QTLs for grain yield and its components under managed stress environments (Zhang *et al*., 1999; Lafitte *et al*., 2002; Venuprasad *et al*., 2002; Babu *et al*., 2003; Lanceras *et al*., 2004; Lafitte *et al*., 2004). Lafitte *et al.* (2002) have reported mapping of QTLs for grain yield and its components in lowland and six upland experiments using a population of doubled haploids derived from the cross IR64/Azucena.

Babu *et al.* (2003) have reported five QTLs related to grain yield over two different trials conducted in southern India under upland drought stress in yet another doubled–haploid (DH) population of 154 lines derived from the cross of CT9993-5-10-1-M, an upland *japonica* ecotype possessing a deep and thick root system and low osmotic adjustment (OA) with IR62266-42-6-2, an *indica* ecotype with a shallow root system and high OA. The QTLs with largest effects explained approximately 20 and 28% of the genetic variation for yield in the stress trails at vegetative and reproductive phases, respectively. But none of the yield QTLs observed was consistent across trials. In all 47 QTLs were identified by them (Babu *et al*., 2003) for various plant water stress indicators, phenology, and production traits under control and water stress treatments, and a region was identified on chromosome 4 that harbored major QTLs for plant height, grain yield and number of grains per panicle under drought stress.

Lafitte *et al.* (2004), in the study of the population derived from the cross Azucena/Bala raised under drip-irrigation system enabling to precisely withhold water from the beginning of panicle emergence until 50% flowering failed to identify QTLs responsible for increased yield under the stress. Lanceras *et al.* (2004), in their study of mapping population derived from the cross CT9993/IR62266 under five different water stress treatments in a single transplanted trial under line-source irrigation, identified four significant QTLs for grain yield under the stress. One of them was detected consistently in three of the five water treatments and one was detected twice. The largest-effect QTL identified in this trial explained about 30% of the genetic variance for yield under severe stress. None of these QTLs corresponded to those previously identified by Babu *et al.* (2003) in the same population.

Yue *et al.* (2005) have reported QTLs affecting yield, biomass, and harvest index under drought stress in pot experiments in two consecutive years. Of them the QTL identified on chromosome 9 between markers RM316 and RM219 was consistent and stress-specific but of relatively small effect, explaining only 14 to 25% of the total phenotypic variation (Yue *et al*., 2005).

Bernier *et al.* (2007) have identified a large effect QTL on chromosome 12 (*qtl12.1*) for grain yield under drought stress at reproductive stage, in a population of 436 random F_3-derived lines from the cross between two upland rice cultivars Vandana and Way Rarem. The QTL is localized to a 10.2 cM region between the SSR markers RM28048 and RM511. Under stress conditions, the locus also was found associated with increased harvest index, biomass yield, and plant height while reduced number of days to flowering. It represented 47% of the average yield under the stress and explaining 51% of the genetic variance.

Price *et al.* (2002) have identified 6 QTLs for root weight and root length, 11 for root to shoot ratio, 12 for number of roots and 14 for root thickness in 140 RILs of the cross of Bala/Azucena. In all, 24 regions were identified to contain the QTLs relating to root traits. Seven QTLs mapped to chromosomes 1, 2,4,7,9 (two QTLs) and 11, were considered by them to be particularly noteworthy.

Adnan Kanbar *et al.* (2004) in their study of BC_1F_3 population of the cross P124 (DH) X IR64 have identified seven putative RAPD makers to show highly significant association with maximum root length (MRL), total root number, root volume, and root dry weight.

Recently, Cui *et al.* (2008) in an experiment of recombinant inbred population derived from Zhenshan 97 x Minghui 63 raised in Yoshida's nutrient solution characterized the response of the seedlings to water deficit imposed by PEG-6000 and identified 26 and 34 QTLs for six seedling characters *viz*., plant height, shoot fresh weight, root fresh weight, root number, maximum root length, and root shoot ratio under both normal and water-deficit conditions respectively.

Table 2. Previous reports on drought related quantitative trait loci (QTLs) in rice

Trait /QTL	Chr	Marker interval	LOD	PVE*	Population	Parents	Reference
Plant height							
‘-’	3	RZ448 & RZ519	1.92	16.8	DH	IR64/Azucena	Hemamalini *et al.,* 2000
phs1.1	1	RG109–ME10_14	14.27	27.8	154 DH	CT/IR*	Babu et. al 2003
qph8.2	8	ME6_13-EMP18_5	7.66	21.2	154 DH	CT/IR*	Lanceras *et al.,* 2004
‘-’	4	RM451 and RM317	2.72	5.46	F_{10}	ZS97B/IRAT109	Liu *et al.,*2005
qtl1.1	1	RM212-RM431	5.1	9	436 F_3	V**	Bernier *et al.,* 2007
Root-shoot ratio							
‘-’	1	R2417-C393, RM212-C86	5.5,3.3	8.9,14.7	F_6	Bala/Azucena	Price *et al.,* 2002
‘-’	2,3,6,7,11	RM208, RM231	‘-’	‘-’	‘-’	‘-’	Li *et al.,* 2005
Panicle no.							
qpn2.1	2	ME2_4-R3128	2.84	11.2	154 DH	CT/IR*	Lanceras *et al.,*2004
qpn4.1	4	ME6_10-EMP2-2	5.37	17.9	154 DH	CT/IR*	Lanceras *et al.,*2004
qtl6.1	6	RM587-RM314	4	5	436 F_3	V**	Bernier *et al.,*2007
qtl12.1	12	RM7195-RM28166	12.9	9	436 F_3	V**	Bernier *et al.,*2007
Panicle length							
qPL-4a	4	RM470-RM451	10.02	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,*2008
qPL-8	8	RM149-RM477	7.04	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,*2008
qPL-9	9	RM553-RM160	4.72	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,*2008
Primary branch no.							
qPBN-4b	4	RM317-RM255	8.32	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
Secondary branch no.							
qSBN-3	3	RM282-RM411	4.02	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
qSBN-4	4	RM255-RM349	5.35	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
qSBN-8	8	RM149-RM477	3.83	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
Grain no./panicle							
qGNP-4	4	RM317-RM255	5.91	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
qGNP-8	8	RM342-RM515	6.21	‘-’	187 F_{10} RI	ZS97B/IRAT109	Liu *et al.,* 2008
gpps4.1	4	RG214–RG620	3.58	13.6			

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Total spikelet no.							
qtsn9.1,9.2,9.3,&9.4	9	K936-C313	3.90-4.73	5.7-17.4	154 DH	CT/IR*	Lanceras *et al.,* 2004
Spikelet fertility							
qSf4	4	RM252-RM303	13.12	11.1	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008
qSf10b	10	RM258-RM228	9.45	7.8	55 ILs	Teqing/Lemont	
Spikelet sterility							
qpss5.1	5	EM19_4-CDO202	3.15	10	154 DH	CT/IR*	Lanceras *et al.,* 2004
qpss8.1	8	RM256-RM210	3.39	11.2	154 DH	CT/IR*	Lanceras *et al.,* 2004
Grain weight							
qGw3	3	RM85-RM148	13.12	'-'	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008
qGw12	12	RM235-RM17	14.76	'-'	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008
Relative water content							
rwc1.1	1	RM212–C813	3.59	12.1	154 DH	CT/IR*	Kebede *et. al* .,2001/ Babu *et. al.,* 2003
Relative yield							
ry2.1	2	EM11_10–EM18_13	4.26	22	154 DH	CT/IR*	Babu *et. al.,* 2003
ry11.1	11	EM18_8–G320	2.94	9.4	154 DH	CT/IR*	Babu *et. al.,* 2003
Biomass/ plant							
bms12.1	12	ME6_12–G2140	5.17	17.2	154DH	CT/IR*	Babu *et. al.,* 2003
qby9.2	9	RM201-RM215	3.56	11.6	154 DH	CT/IR*	Lanceras *et al.,* 2004
bms3.1	3	RZ313–EM17_1	1 4.43	15.5	F_{10}	ZS97B/IRAT109	Liu *et al.,* 2005
'-'	4	RM451 and RM317	2.28	4.9	F_{10}	ZS97B/IRAT109	Liu *et al.,* 2005
qtl6.1	6	RM587-RM314	4.8	2	436 F_3	V**	Bernier *et al.,* 2007
Grain yield							
gys1.1	1	EM18_10–L1087	4.41	11.7	154 DH	CT/IR*	Babu *et. al.,* 2003
qgy4.2	4	EMP2_2-RZ565	3.1	9.4	154 DH	CT/IR*	Lanceras *et al.,* 2004
qtl12.1	12	RM28048-RM511	34	33	436 F_3	V**	Bernier *et al.,* 2007
qGy5	5	RM509-RM163	10.2	5.8	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008
qGy9	9	RM242-RM278	11	4.1			
Chlorophyll content							
qCc2c	2	RM341-RM263	3.69	9.66	RIL F_{13}	Lemont/Teqing	Hua *et al.,* 2006
Gc gene	1	RM462-RM6464	'-'	'-'	170 BC_6F_2	Chongqing 2/ Zhenshan 97B	Wang *et al.,* 2006
qCc3	3	RM36-RM282	10.88	11.9	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008

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Shoot dry matter weight							
qSdw8	8	RM38-RM25	2.4	3.8	RIL F_{13}	Lemont/Teqing	Hua *et al.,* 2006
‘-’	11	G320&G257	3.69,3.57	14.8,15.4	DH	CT9993/IR62266	Kumar *et al.,* 2003
Specific leaf weight							
qSlw10	10	RM258-RM228	9.76	8.2	55 ILs	Teqing/Lemont	Zhao *et al.,* 2008
Shoot length at 28 DAS							
QTL-1.4	1	C86	5	10.5	205 F_6 RILs	Bala/Azucena	MacMillan *et al.,* 2006
QTL-4.2	4	RG163	6.9	12.2	205 F_6 RILs	Bala/Azucena	MacMillan *et al.,* 2006
Root dry weight							
QTL-2.3	2	RM6	3.4	7.8	205 F_6 RILs	Bala/Azucena	MacMillan *et al.,* 2006
QTL-9	9	RM242	4.1	9.7	205 F_6 RILs	Bala/Azucena	MacMillan *et al.,* 2006
Seminal root length							
‘-’	8	RG1	2.53	11.2	150 RILs	IR1552/Azucena	Zhang *et al.,* 2001
‘-’	1	RG109B-RG690	3.37	10	150 RILs	IR1552/Azucena	Zhang *et al.,* 2001
‘-’	1	S1746/RG109B	2.5	11.3	96 RILs	IR1552/Azucena	Zheng *et al.,* 2003
‘-’	2	L16/CDO718	2.58	11.6	96 RILs	IR1552/Azucena	Zheng *et al.,* 2003
‘-’	7	RG650/CDO497	2.46	11.5	96 RILs	IR1552/Azucena	Zheng *et al.,* 2003
‘-’	9	RG570/RG667	2.99	13.4	96 RILs	IR1552/Azucena	Zheng *et al.,* 2003
Maximum root length							
‘-’	9	RM215,RM278	‘-’	2.5,21.7	69 BC_1F_2	P124/IR64	Toorchi *et al.,* 2002
‘-’	10	RM222	‘-’	22.6	69 BC_1F_2	P124/IR64	Toorchi *et al.,* 2002
‘-’	2	RM6	‘-’	1.1	69 BC_1F_2	P124/IR64	Toorchi *et al.,* 2002
QTL-10	10	C701	3.5	8	205 F_6 RILs	Bala/Azucena	MacMillan *et al.,* 2006
No. of tillers							
‘-’	1	RZ810 & RG331	2.1	15.8	DH	IR64/Azucena	Hemamalini *et al.,* 2000
Harvest index							
qhi1.1	1	CDO345-RZ909	2.75	7.8	154 DH	CT/IR*	Lanceras *et al.,* 2004
qtl3.1,3.2,3.37 *3.4*	3	RG104-RM81	2.86-6.27	17.2-24.8	154 DH	CT/IR*	Lanceras *et al.,* 2004
qtl12.1	12	RM7195-RM28166	22	26	436 F_3	V**	Bernier *et al.,* 2007

V**-Vandana/Way Rarem (Indica); **CT/IR* -** CT9993-5-10-1-M/IR62266-42-6-2; ‘-’ means no symbol/ data for related QTLs in the published paper

Map-based cloning of genes related to yield and its components

As of September 2008, 8646 QTLs have been identified and mapped in rice for different traits (Gramene V28; www.gramene.org). Of these, however, only 32 genes (0.36% of the total QTLs) have been cloned. They include QTLs relating to yield and its components as listed in Table3, biotic stresses (blast and blight each of 6 genes) and abiotic stresses (submergence tolerance *sub1A* gene and salinity tolerance gene, *SKC1*). Some of the reports relating to yield and its components are briefly discussed as under.

Ashikari *et al.* (2005) have identified a gene underlying the major QTL (*Gn1*) for grain number on the short arm of the chromosome 1. It has been found to include two loci *i.e.*, *Gn1a* and *Gn1b,* encoding cytokinin oxidase/ dehydrogenase (*OsCKX2*), an enzyme that degrades phytohormone cytokinin. They also reported reduced expression of *OsCKX2* to cause cytokinin accumulation in inflorescence meristems and to increase subsequently the number of grains resulting in enhanced grain yield. Pyramiding of the gene loci for grain number and plant height into the same rice background resulted in 26% more grain number per panicle and 18% reduction in plant height.

He *et al.* (2006) have fine mapped an yield improving QTL *GY2-1* on chromosome 2 and identified a gene cluster encoding leucine rich repeat receptor kinase (LRR) from the cross involving Dongxiong, the wild rice, *Oryza rufipogon* Griff. and Guichao2, an *indica* cultivar.

Table 3 Genes underlying the major QTLs in rice

S.No.	*Gene*	Chr.	Trait affected	Functional mutation	Molecular function	References
1.	*Waxy*	6	Amylose content	SNP in intron affecting mRNA splicing	Granule-bound starch synthase	Wang *et al*., 1995
2.	*MOC1*	6	Tillering	Deletion causing protein truncation	GRAS family nuclear transcription factor	Li *et al*., 2003
3.	*sd1*	1	Plant height	Deletion causing protein truncation	Gibberellic acid-20-Oxidase	Monna *et al*., 2002; Sasaki *et al*., 2002; Speilmeyer *et al*. 2002
4.	*Hd6*	3	Flowering time/heading date	SNP causing an amino-acid substitution	α subunit of protein kinase CK2 (CK2 α)	Takahashi *et al*., 2001
5.	*Hd1*	3	Flowering time/heading date	43-bp deletion in the first exon and a 433-bp insertion in the intron	homolog of ArabidopsisFlowering Time Gene *CONSTANS* (*CO*)	Yano *et al*., 2001
6.	*Hd3a*	3	Flowering time/heading date	Unknown	Ortholog of the *Arabidopsis FLOWERING LOCUS T(FT)* Gene	Kojima *et al*., 2002
7.	*[BAD2]b*	8	Fragrance	Deletion causing protein truncation	Betaine aldehyde dehydrogenase	Bradbury *et al*., 2005
8.	*[Gn1a]b*	1	Grain number	Several possible mutations	Cytokinin oxidase/dehydrogenase	Ashikari *et al*., 2005
9.	*Sh4*	4	Grain shattering	SNP causing an amino-acid substitution	Myb3 transcriptional regulator	Li *et al*., 2006a
10.	*qSH1*	1	Grain shattering	SNP in regulatory region	BEL1-type homeobox transcriptional regulator	Konishi *et al*., 2006
11.	*Rc*	7	Grain pericarp color	Deletion causing protein truncation	Basic helix–loop–helix transcriptional regulator	Sweenay *et al*., 2006
12.	*Rd*	1	Grain pigment synthesis	SNP causing protein truncation	Dihydroflavonol-4-reductase (DFR)	Furukawa *et al*., 2006
13.	*GS3*	3	Grain size/shape	SNP causing protein truncation	Cellular signaling protein with a VWFC module	Fan *et al*., 2006
14.	*[GW2]b*	2	Grain weight/width	Deletion causing protein truncation	RING-type protein with E3 ubiquitin ligase activity	Song *et al*., 2007
15.	*Ghd7*	7	Heading date, plant height and yield	Deletion causing protein truncation	CCT domain protein,	Xue *et al*., 2008
16.	*GIF1*	4	Grain filling	Deletion causing protein truncation	cell-wall invertase	Wang *et al*., 2008a
17.	*PROG1*	7	Plant architecture (Tiller angle and number)	An amino acid substitution in the PROG1 protein	zinc-finger nuclear transcription factor	Jin *et al*., 2008
18.	*PROG1*	7	Prostrate growth of ancestral wild rice to the erect growth of *Oryza sativa*	15 SNPs and 6 indels causing protein truncation	Cys2-His2 zinc-finger protein	Tan *et al*., 2008
19.	*qSW5*	5	Grain size	Deletion causing protein truncation	Hypothetical protein	Shomura *et al*., 2008

A QTL for grain size/ weight on chromosome 3 *i.e*., *GS3* was dissected into a gene, that encodes a putative phosphatidyl ethanolamine-binding protein-like domain (PEPB), a transmembrane region, a putative TNFR (tumor necrosis factor receptor) /NGFR (nerve growth factor receptor) family cystein rich domain and a VWFC (von Willebrand factor type C) module. Comparative sequence analysis identified a nonsense mutation in the second exon of the putative *GS3* gene in all long-grain varieties. This mutation causing a 178 amino acid truncation in the C-terminal region of the predicted protein has led to the conclusion that *GS3* might function as a negative regulator for grain size (Fan *et al.,* 2006).

Recently, Ikeda *et al.* (2007) have reported an *aberrant panicle organization-1 (APO-1)* gene encoding an F-box protein on chromosome 6. The mutant strain of this gene results in reduced panicle length and thereby accommodates less number of spikelets per panicle.

Song *et al.* (2007) have identified a major QTL for grain width *i.e*., *GW2* on chromosome 2, which encodes a previously unknown RING type protein with E3 ubiquitin ligase activity and it is known to function in the degradation by the ubiquitin-proteasome pathway. Further, loss of *GW2* function increased cell number resulting in larger or wider spikelet hull while accelerating grain milk filling rate, which consequently enhances grain width, weight and yield.

Most recently, Wang *et al.* (2008) identified *GIF1* (GRAIN INCOMPLETE FILLING-1) gene that encodes a cell wall invertase required for carbon partitioning during early grain filling phase. Over expression of *GIF1* has been found to increase grain production. Further, study by them of nucleotide diversity in *GIF1* loci between cultivated and wild rice (*O. rufipogon*) has revealed one nucleotide deletion at the 4th exon to generate premature stop codon-TAA. This is reasoned for low grain weight in wild species.

Apart from, the *sd1* gene for plant height (Monna *et al.,*2002; Sasaki *et al.,*2002; Speilmeyer *et al.,* 2002); for tillering, MONOCULM 1 (*MOC 1*) (Li *et al*., 2003); *Hd1, Hd3a* and *Hd6* for heading date (Kojima *et al*., 2002; Takahashi *et al*., 2001; Yano, 2001); and recently, *Ghd7* (Xue *et al*., 2008) gene that appears to control rice yield as well as plant height and flowering time have also been isolated by map based cloning.

Interestingly till today, not even a single gene has been cloned for drought related traits in rice. A more extensive survey of tolerant rice germplasm, currently underway at IRRI, expecting to identify lines carrying major genes for enhanced yield under moisture stress.

Materials and Methods

3 Materials and Methods

To develop mapping population for identification of stable QTLs of promise for yield and drought tolerance related traits, INRC10192, a landrace from an area of rice diversity and IR64, a popular semi-dwarf high yielding variety (HYV), were chosen as parents.

Selection of parents

Landrace: The following two approaches were used for choosing the prospective donor parent among a set of 40 landraces (Hariprasad, 2003).

Genetic diversity: Fourteen (AG) and (GA) repeat based anchored ISSR primers were used to study the genetic diversity of the traditional tall statured landraces *vis-a-vis* IR64. With similarity coefficient ranging from 0.70 to 0.86 with respect to IR64, the landrace INRC10192 was found to be sufficiently distant for qualifying as the donor parent.

Propping: Relative potential of the landraces to respond to high levels of nitrogenous fertilizer (N) application (normal N= 100kg/ha^{-1} and high N-level= 150kg/ha^{-1}) under propped condition as compared to unpropped was used. The rationale of choosing this approach lies in the fact that lodging masked the inherent potential of a genotype to fertilizer response and if that could be prevented, identification of a genotype with high yield potential should not be difficult. This strategy has enabled to identify three landraces *viz*., INRC10062, INRC10066 and INRC10192 as promising. Of them, INRC10192, by virtue of its highest grain yield per plant as well as highest number of productive tillers per plant and high biomass as well as its genetic distance with respect to IR64, qualified as the choice for using as the donor parent.

Semidwarf variety: The widely grown semi-dwarf high yielding variety IR64 was chosen as the other parent. The choice was because of its popularity with farmers for its high yield potential, acceptable grain quality and resistance to many biotic stresses. Significantly, it has been the agronomic base in a wide range of studies including conventional and molecular breeding for resistance to biotic and abiotic stresses in crosses with appropriate donor parents as well as in investigations to understand the molecular basis of complex traits.

Development of mapping population

The population in F_4 generation of the cross involving the popular high yielding variety, IR64 and the landrace, INRC10192 from Assam Rice Collection, made available from the National Professor Project, Directorate of Rice Research (DRR), Hyderabad, formed the basic material to develop mapping population. It was advanced during *kharif* and *rabi* seasons of 2004 to F_5 and F_6, respectively, employing single seed descent (SSD) method at the wet-land farm of the Biotechnology Unit (BTU), Agricultural Research Institute (ARI), Acharya N.G. Ranga Agricultural university, Hyderabad. In all 140 recombinant inbred lines (RILs) in the F_6 generation formed the mapping population (Fig. 1).

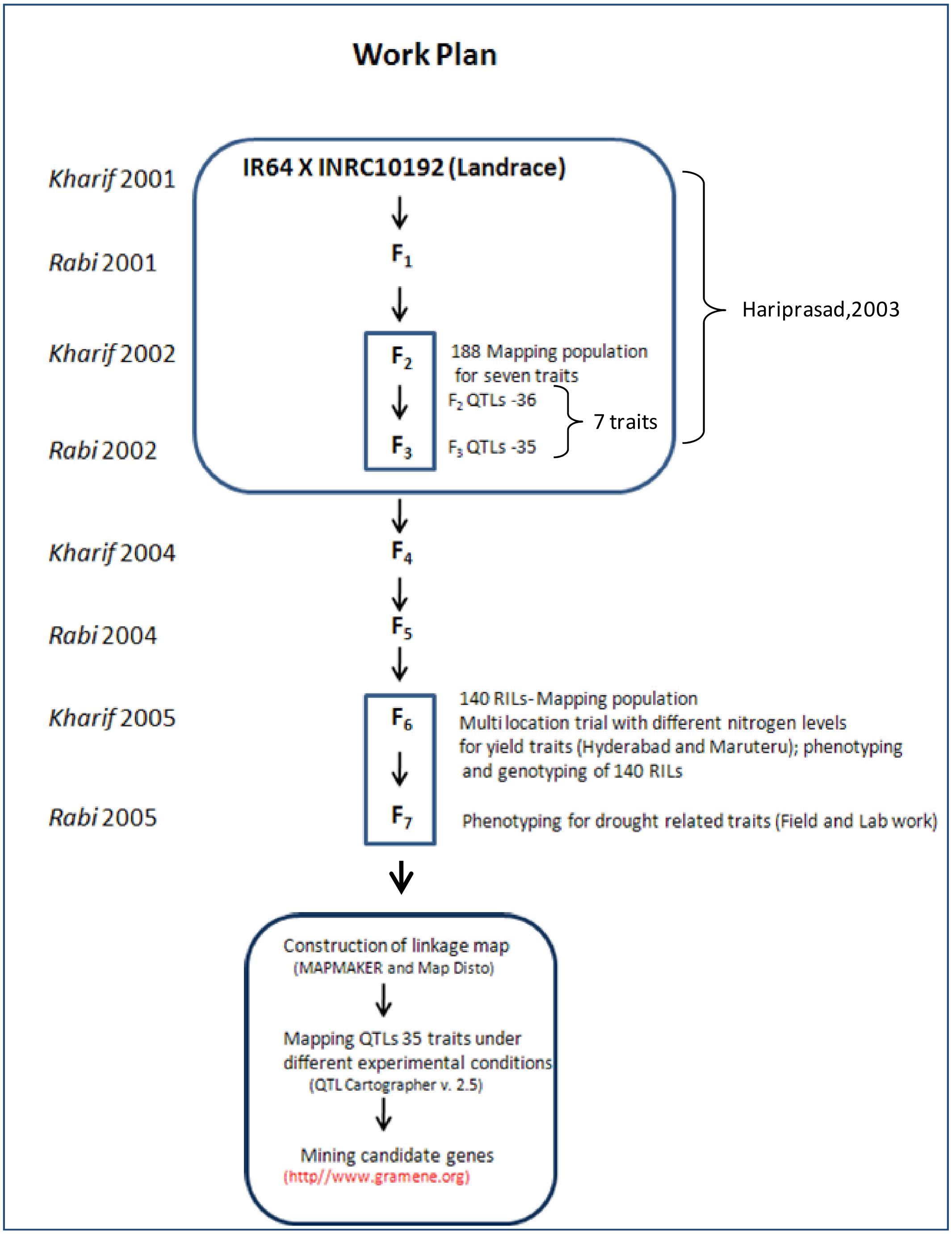


Fig. 1 Development of mapping population and QTL mapping

Phenotyping

Yield and its component traits

The recombinant inbred lines (RILs) were evaluated under field conditions at two locations *viz*., BTU, ARI, Hyderabad and the Regional Agricultural Research Station (RARS), Maruteru, West Godavari District in *kharif*, 2005 at two levels of nitrogen (N) (N1= 100 kg and N2= 150 kg/ha) each with two replications, along with parents as checks (Fig. 2). The field planting followed a split-plot design with two N levels as main plot treatment and plant materials as sub-plot treatment. Nitrogen fertilizer was applied

Fig. 2 The mapping population of 140 RILs along with the parents

in three split doses, one as basal and two by top dressing at 44th and 66th day after sowing. Each of the RILs and the parents consisted of 24 plants planted in 2 rows of 12 plants each adopting a uniform spacing of 20 x 15 cm^2. Six plants in the middle of each of these lines were tagged and leaf samples were collected for DNA isolation. Phenotypic data was recorded on yield and its component traits on these tagged plants as detailed in Table 4.

Table 4 Description of yield and its components and their codes

Trait code	Trait name (units)	Description
PH (N1H/N1M)/(N2H/N2M)	Plant height (cm)	Length of the tallest tiller from soil surface to the tip of the panicle
NT (N1H/N1M)/(N2H/N2M)	Number of tillers/plant (no)	Total number of tillers including productive and non productive ones
NPT (N1H/N1M)/(N2H/N2M)	Number of productive tillers / plant (no)	Tillers bearing the panicles with seed set exceeding 15% only
PL (N1H/N1M)/(N2H/N2M)	Panicle length (cm)	Length from neck to tip of the panicle (excluding awns)
FG (N1H/N1M)/(N2H/N2M)	Number of Fertile grains / panicle (no)	Number of filled spikelets per panicle
CG (N1H/N1M)/(N2H/N2M)	Number of chaffy grains / panicle (no)	Number of sterile spikelets per panicle
SN (N1H/N1M)/(N2H/N2M)	Number of spikelets / panicle (no)	Number of spikelets including chaffy and fertile ones
SF (N1H/N1M)/(N2H/N2M)	Spikelet fertility (%)	Ratio of filled spikelets to the total number of filled and chaffy spikelets per panicle
GW (N1H/N1M)/(N2H/N2M)	1000-grain weight (g)	Weight of 1000 filled spikelets
BM (N1H/N1M)/(N2H/N2M)	Biomass / plant (g)	Total dry weight of plant including straw, filled and chaffy grains
SPY (N1H/N1M)/(N2H/N2M)	Single plant yield (g)	Weight of filled grains per plant
HI (N1H/N1M)/(N2H/N2M)	Harvest index (%)	Ratio of single plant yield to biomass, expressed in percentage

N1H: Nitrogen level 1 at Hyderabad; N1M: Nitrogen level 1 at Maruteru
N2H: Nitrogen level 2 at Hyderabad; N2M: N treatment 2 at Maruteru

Drought response under field conditions

The same set of 140RILs in F_7 generation along with their parents were evaluated under water stress and fully irrigated conditions in a replicated experiment with randomized block design, at the experimental farm of the BTU, ARI, Hyderabad, during *rabi*, 2005 (Fig.3). The rice varieties IR20 and Moroberekan were used as the drought susceptible

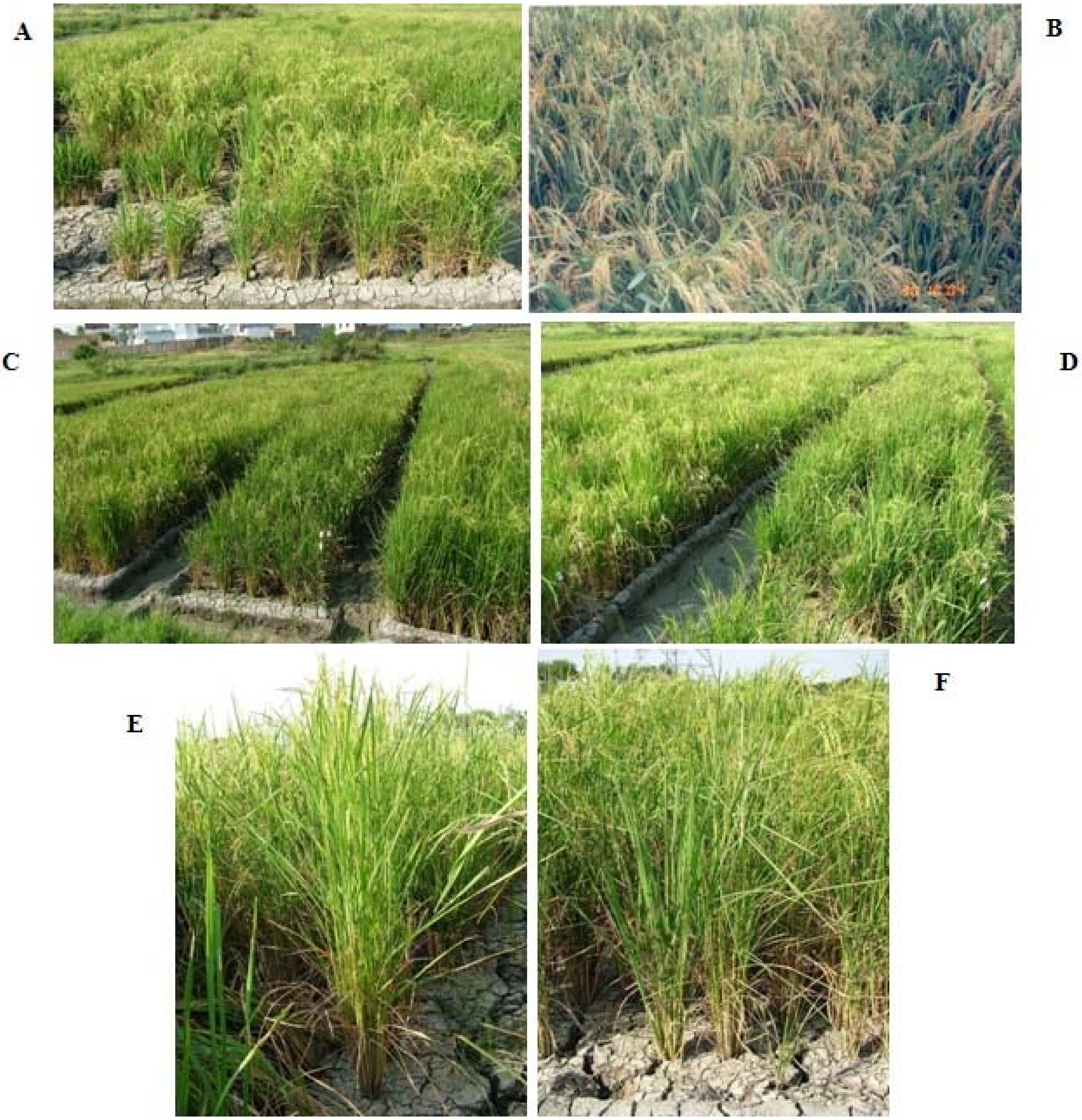


Fig. 3 Rice field plots under moisture stress condition (A, C, D, E and F). Homozygosity of RILs (B)

and drought tolerant checks respectively. The two treatments were separated by 2 m space. Seeds of the RILs were sown in seedbed and 21-day old seedlings were transplanted in two-row plots (24 plants per plot) at a spacing of 20 x 15 cm^2. The crop was managed following standard practices; with a basal dose of 30 kg /ha each of N, P, and K, and two additional N applications of 30 kg/ ha at 44th and 66th day after sowing. Weeds in both the irrigated and water stressed fields were controlled by a combination of chemical and manual methods. Insect pests, particularly stem-borer were controlled chemically. The crop received irrigation for the initial 82 days of the season to ensure normal growth. When panicles were visible on three or more hills, irrigation was completely stopped for the next 21 days for stress plots *i.e.*, until severe leaf rolling was observed in the drought sensitive check and experimental lines (Fig. 4) (Babu *et al*., 2003; Liu *et al*., 2005 and Zou *et al*., 2005).

INRC10192 **RILs** **INRC10192** **IR64**

Fig. 4 Leaf rolling of RILs along with parents under the stress condition

Later, both control and stress plots were regularly irrigated until maturity. Moisture content at 0-15 cm, 15-30 cm below the soil surface was determined in stressed plots, at various times over 15 days from the day of the last irrigation, using gravimetric analysis. Six plants in the middle of each of these lines were randomly chosen for recording observations on the selected drought tolerance related traits. Standard procedures were followed for recording the observations as detailed in Table 5.

Table 5 Description of yield and its components and drought tolerance related traits measured under field conditions

Trait code	Trait name (Units)	Description
PH (C/S)	Plant height (cm)	Length of the tallest tiller from soil surface to the tip of the panicle
NPT (C/S)	Number of productive tillers / plant (no)	Tillers containing panicles with seed set exceeding 15% only
PL (C/S)	Panicle length (cm)	Length from neck to tip of the panicle (excluding awns)
PFG (C/S)	No. of fertile grains on primary branches/ panicle (no)	Number of filled spikelets on primary branches per panicle
PCG (C/S)	No. of chaffy grains on primary branches/ panicle (no)	Number of sterile spikelets on primary branches per panicle
SFG (C/S)	No. of fertile grains on secondary branches/ panicle (no)	Number of filled spikelets on secondary branches per panicle
SCG (C/S)	No. of chaffy grains on secondary branches/ panicle (no)	Number of sterile spikelets on secondary branches per panicle
PSN (C/S)	No.of spikelets on primary branches / panicle (no)	Number of spikelets including chaffy and fertile ones on primary branches
SSN (C/S)	No. of spikelets on secondary branches / panicle (no)	Number of spikelets including chaffy and fertile ones on secondary branches
PSF (C/S)	Spikelet fertility on primary branches (%)	Ratio of filled spikelets to the total number of filled and chaffy spikelets on primary branches per panicle, expressed in percentage
SSF (C/S)	Spikelet fertility on secondary branches (%)	Ratio of filled spikelets to the total number of filled and chaffy spikelets on secondary branches per panicle, expressed in percentage
PGW (C/S)	1000-grain weight on primary branches (g)	Weight of 1000 filled spikelets on primary branches
SGW (C/S)	1000-grain weight on secondary branches (g)	Weight of 1000 filled spikelets on secondary branches
BM (C/S)	Biological yield/plant (g)	Total dry weight of plant including straw, filled grains and chaffy grains
SPY (C/S)	Single plant yield (g)	Weight of filled grains per plant
HI (C/S)	Harvest index (%)	Ratio of single plant yield to biomass, expressed in percentage
RWC (S)	Relative water content of leaves (%)	RWC determined at midday, 15 days after withholding irrigation. Five 2 × 2 cm^2 long portions from the mid section of the penultimate fully expanded leaf blade were cut avoiding major veins and put in a pre-weighed glass vial to obtain the fresh weight (FW). The leaf tissue in the glass vial was re-hydrated with distilled water for 4 hours in the dark. After equilibration, the leaf samples were removed, blotted gently between filter papers, and weighed to get the turgid weight (TW). The re-hydrated leaf samples were oven dried at 70^{o}C to obtain the dry weight (DW). RWC (%) was calculated for the samples collected on the same morning using the formula as follows (Sanguineti *et al.,* 1999 and Robin *et al.,* 2003). RWC = [(FW – DW)/(TW – DW)] × 100
CC (S)	Chlorophyll content (SPAD units)	In the stressed field, CC were measured at midday on the middle parts of three fully expanded flag leaves on the main culms of three middle plants in each line at the flowering time using a SPAD-502 chlorophyll meter (Spectrum Technologies Inc.). CC was presented as the SPAD meter reading/ SPAD units (Hua *et al*., 2006).
SPY (R)	Relative yield (ratio)	Ratio of the parameters under stress to the control, and used to asses drought tolerance (Babu *et al.,* 2003 and Zheng, *et al.,* 2003).
BM (R)	Relative biomass (ratio)	

S- Stress=Drought treated; C-Control= Irrigated; R= Relative parameter

Drought response under greenhouse conditions

Approximately 150 seeds each of 140 RILs in F_7 generation along with parents and IR20 and Moroberekan as drought susceptible and drought tolerant checks respectively, were sown on moistened filter paper in petri dishes and kept in an incubator maintained at 30^{o}C for 48h. The germinated seeds were removed from the incubator and kept under room temperature (27°C ± 1°C) for three days. The seedlings were allowed then to grow in Yoshida's nutrient solution (Yoshida *et al*., 1976) under greenhouse conditions for three days. The seedlings of each of the lines were evenly distributed in four petri dishes, two of which for water deficit (stress) treatment and two for control. Water deficit was created by using half-strength Yoshida's nutrient solution containing 15% PEG-6000 (W/W) with osmotic potential (OP) of -2.36 to -2.95 bars at 25-30˚C, while the control was continued with normal nutrient solution (Yoshida *et al.,* 1976; (Supporting Information 1 and SI Table2). Five days later, OP level of the stress treatment was increased to -4.04/-4.91 bars by replacing 15% with 20% PEG 6000 to the nutrient solution and maintained for two weeks. Though this is considered to be the critical concentration for early seedling stage screening of rice for drought tolerance (Blum *et al*., 1989 and Gloria *et al*., 2002), the seedlings were subjected to still higher stress by ranging further the OP level to -6.15/-7.35 bars (PEG 6000 at 25% of nutrient solution) for four days. The nutrient solution was replenished once a week in control; while for keeping the water potential stable, the solution containing PEG was changed on alternate days in the stress treatment. The pH of both the treatments was adjusted to 5.0 by adding 1 mol/l HCl or NaOH solution every 24 hours.

Response to the treatment was observed 29 days after sowing (DAS). Immediately after removing from nutrient solution, the seedlings were thoroughly washed with distilled water and blotted with tissue paper to remove excess water before observing for root and shoot length (Fig.5).

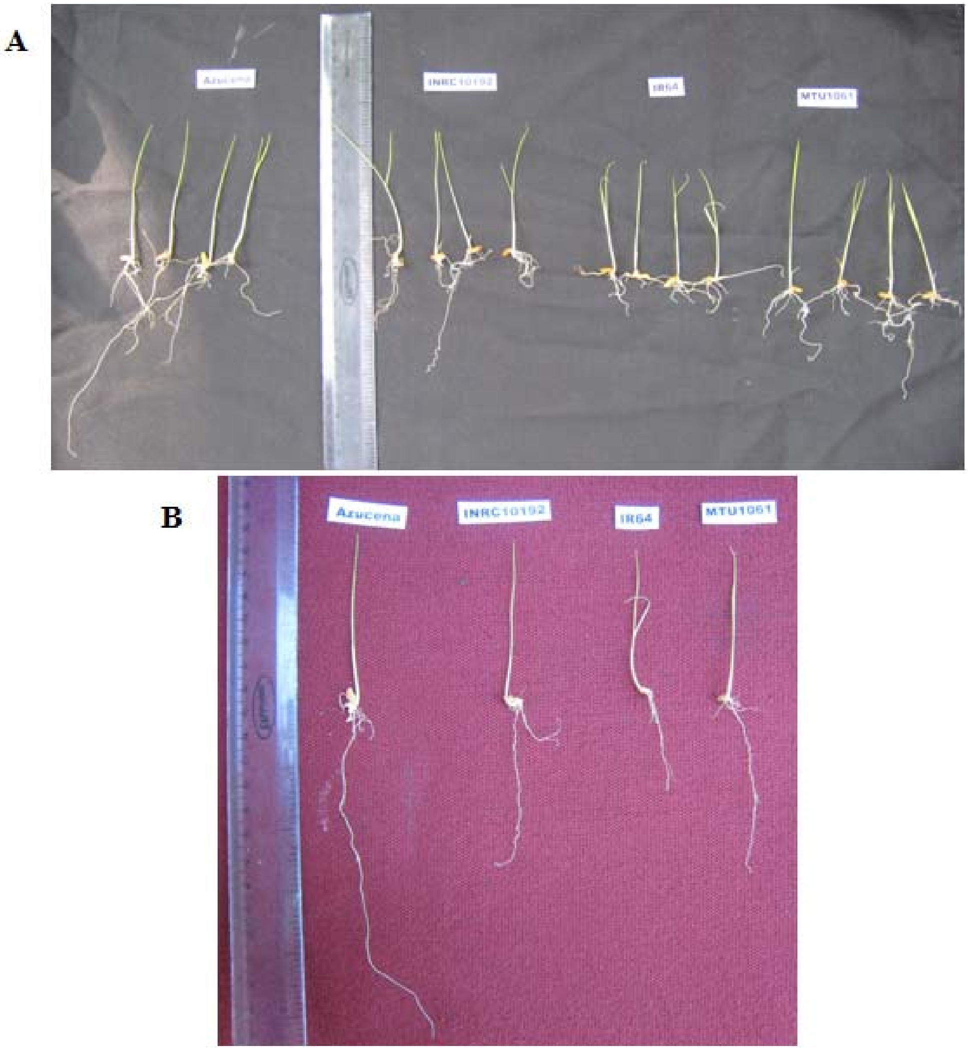


Fig. 5 Root and shoot pictures of parents (INRC10192 and IR64) and control (Azucena and MTU1061)

The same root and shoot samples were used for measuring root and shoot dry weights, after drying them at 70˚C for 48h (Gregorio *et al.,* 2002 and Ali *et al.,* 2006). For study of secondary traits related to drought tolerance, observations were made on six seedlings per replication and averaged following standard procedures as detailed in Table 6.

Table 6 Description of the drought tolerance related traits measured under greenhouse conditions

Trait code	Trait full Name and (Units)	Description
SL (C/S)	Shoot length (cm)	Length from node/collar region to the tip of the shoot
RL (C/S)	Root length (cm)	Length of the seminal root (Seminal root is the longest root at early seedling stage)
SDW (C/S)	Shoot dry weight (mg)	Weight of shoot after oven-drying at 80^{o}C for 24 h.
RDW (C/S)	Root dry weight (mg)	Weight of roots after oven-drying at 80^{o}C for 24 h.
RS (C/S)	Root shoot weight ratio	Ratio of root dry weight to the shoot dry weight.
SL (R)	Relative shoot length	Ratio of the parameter under stress to the control
RL (R)	Relative root length	
SDW (R)	Relative shoot dry weight	
RDW (R)	Relative root dry weight	
RS (R)	Relative root shoot weight ratio	

C- Control: Normal half- strength Yoshida's nutrient solution
S- Stress: PEG-6000 contained Half- strength Yoshida's nutrient solution (Yoshida *et al.,* 1976)

Phenotypic data analysis

Test of significance among parents, and among RILs, transgressive segregants, test for normal distribution (skewness) and correlations among character pairs were computed at $p < 0.05$ and $p < 0.01$ in Microsoft-Excel (2007) using trait averages.

DNA extraction and quantification

DNA was extracted from two month old leaf tissue from all the 140 RILs as well as the parents using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Dellaporta *et al.* (1983) described as under:

Steps followed:

- Approximately 1 g of fresh leaf tissue was ground to fine powder in liquid nitrogen using a pestle and mortar.
- The powder was quickly added with 20ml of pre-warmed (65^{0}C) 2x extraction buffer (2% CTAB, 200 mm Tris–HCl p^H 8.0, 20 mM EDTA; 1.4M NaCl; 1% PVP and distilled water) in a capped polypropylene or oakridge tubes. The clumps were suspended with a spatula, and incubated for 1hr with frequent stirring in a water bath at 65^{o}C.
- 20ml of Chloroform: Iso-amyl alcohol (24:1) mixture was added and kept shaking for 10 min.
- It was centrifuged at 13000 rpm for 15 min and supernatant was collected into a new polypropylene tube. This step was repeated till a clear supernatant was obtained.
- DNA was precipitated by adding 0.7 volume of ice-cold 100% Iso-propanol followed by gentle mixing.
- It was then incubated for 1 hr at -20^{0}C followed by centrifugation at 13000 rpm for 15 min at 4^{0}C. The resultant supernatant was discarded and the DNA pellet was air dried. The pellet was washed with 5 ml of 70% ethanol and spun again at 13000 rpm for 5 min at 4 ^{0}C and was air dried again.
- The DNA pellet was dissolved in 200µl of TE buffer (10mM Tri-HCl, 1mM EDTA p^H 8.0) overnight at room temperature followed by incubation for 1hr at 65^{0}C.

- The DNA was transferred from polypropylene tube to Eppendorf tube using wide-mouthed glass pipette or half cut tip (to avoid DNA shearing), and 5μl RNAase (20mg/ml) (pre-boiled) was added to the DNA solution and incubated at 37^0C for an hour.
- The DNA was re-precipitated by adding 0.1volume of 5M ammonium acetate and two volumes of 100% ethanol followed by gentle mixing and then stored at -20^0C for an hour.
- Samples were centrifuged at 13000rpm for 10 min and the supernatant was drained out.
- 100μl of 70% ethanol was added to the DNA pellet and centrifuged at 13000 rpm for 10min. Supernatant was drained carefully and the DNA pellet was air dried over night.
- The DNA was dissolved in 200μl of TE buffer (10mM Tri-HCl, 1mM EDTA p^H 8.0) at 65^0C for one hour.

The quality of the DNA was checked by loading 5μl of the DNA on a 0.8% agarose gel along with the marker Lambda Hind III digest (MBI Fermentas, Canada) and stained with ethidium bromide. Quantification of the DNA was done based on spectrophotometric measurement of UV absorbance at 260nm. An aliquot of the DNA sample was diluted in TE buffer in a ratio of 1:100 in a microcuvette. Optical density (OD) was determined at 260, 280 and 320nm against TE buffer blank. The DNA concentration was calculated using the formula that 1 OD corresponds to 50μg/ml of DNA. The ratio of OD260 to OD280 provides information on the purity of the DNA sample, where pure DNA preparations show OD260 to OD280 ratio between 1.8 and 2.0.

PCR amplification

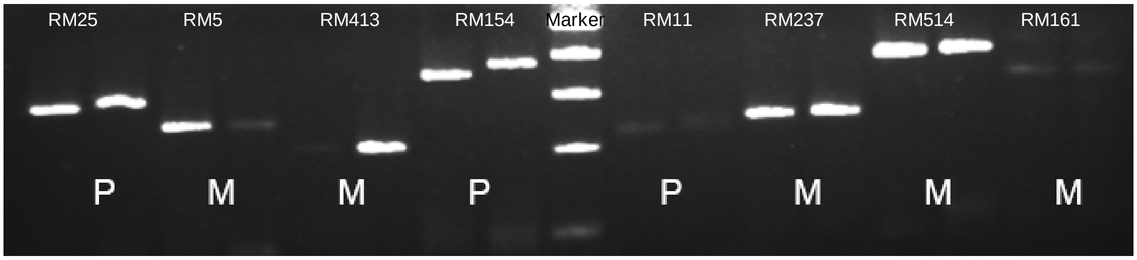
PCR amplification was performed in a 10μl volume containing 10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 0.5 unit of Taq polymerase (Sigma, USA and Genei, Bangalore), 50μM of dNTPs, and 0.1µM of each of forward and reverse primers with 10ng of genomic DNA on Applied biosystems 9700 (ABI 9700) with a Ramp speed of 9700 (Applied Biosystems, USA).Thermal cycling conditions for PCR reaction were: after an initial denaturation at 94^0C for 10 min, the PCR mix was cycled 35 times at 94^0C (denaturation), 55^0C (annealing) and 72^0C (extension) for 45s, 45s, and 60s, respectively (ABI 9700, Applied Biosystems, USA). This was followed by a final extension step at 72^0C for 10min.

Agarose gel electrophoresis

PCR samples were mixed with bromo-phenol blue (0.25% bromo phenol blue and 40% (w/v) sucrose mixed in water) and run on a 3% agarose gel (Sigma) containing ethidium bromide (10mg/ml) along with the marker 50bp ladder (MBI Fermentas, Canada) at 5.3V/cm (Bio-Rad PowerPac 300) for an hour in 0.5x Tris-Acetic acid-EDTA (TAE) buffer (242g Tris base, 57.1 ml Acetic acid 100ml 0.5M EDTA mixed and made up the volume to 1 litre with double distilled water and pH adjusted to 8.5). The resolved PCR bands were documented using Bio-Rad Molecular Imager Gel Doc XR System.

Parental polymorphism and screening of mapping population

A set of 412 microsatellites or SSR (simple sequence repeat) markers (www.gramene.org) spanning all the 12 rice chromosomes were screened between the parents, IR64 and INRC10192 (Fig.6). Of these markers, 133 (32.28%) that showed polymorphism between the parents were used for screening the 140 RILs (Fig.7). The scoring of the population was done as the band corresponding to IR64 (parent A) type or INRC 10192 (parent B) type and given the codes as A or B for RILs and then marker data was used for construction of linkage map. The χ2 goodness of fit against 1:1 segregation ratio was estimated using MapDisto v.1.3 software (Lorieux, 2006).



First lane-INRC10192 and Second lane-IR64; Marker-50bp ladder;P-Polymorphic;M-Monomorphic

Fig. 6 Polymorphism between IR64/INRC10192 employing rice microsatellite markers

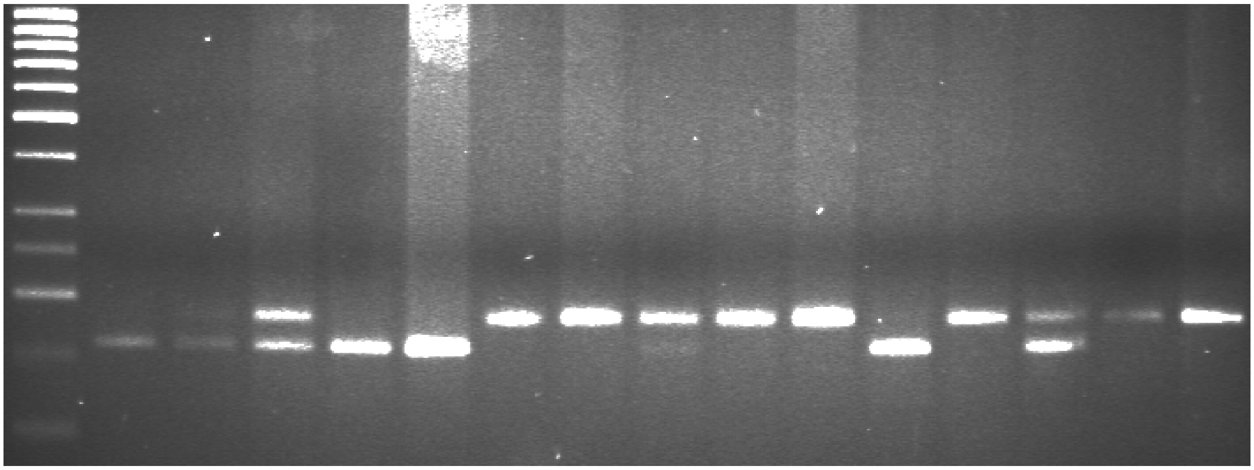


Fig. 7 Segregation pattern of RM17 locus in recombinant inbred population of cross between IR64 and INRC10192

Linkage map construction and QTL detection

Linkage map was constructed using the MAPMAKER version 3.0 (Lincoln *et al.*, 1993) following Kosambi Mapping Function (Kosambi, 1944). Linkage groups were determined using 'group' command with LOD (Logarithm of odds ratio) score of 3 and recombination fraction of 0.4. Order of the markers for each group was determined using 'order' and 'ripple' commands. Assignment of linkage groups to the respective chromosomes was done based on the rice maps developed at Cornell University (McCouch *et al*., 2002).

Each of the scored traits (MAPMAKER result file) along with phenotypic means was subjected to QTL mapping. QTLs were detected by interval mapping [(IM), Lander and Botstein 1989] and composite interval mapping (CIM) procedure of Windows QTL Cartographer v.2.5 software (Wang *et al*., 2006). Composite interval mapping (Zeng, 1994) was conducted using the default settings (e.g., Model 6, five cofactors selected automatically by forward regression with a 10-cM window) (http://statgen.ncsu.edu/qtlcart/cartographer.html). Permutation tests (Churchill and Doerge, 1994) were used to determine chromosome-wise significance thresholds (α=0.05) for QTL detection using QTL Cartographer with 1000 shuffles of the trait data. Quantitative trait loci peaks with significant likelihood ratio test statistics under H3/H0 were recorded.

These two hypotheses are:

H0: $a = 0, d = 0$

H3: $a \neq 0, d \neq 0$

Where a = additive effect and d = dominance effect.

Conversion factor: LOD = 0.217 x LR (likelihood ratio).

QTL Nomenclature

Nomenclature for QTLs was as described by McCouch *et al.* (1997), where a two or three letter abbreviation is followed by the number of the chromosome on which the QTL is found and a terminal suffix, separated by a period, providing a unique identifier to distinguish multiple QTL on a single chromosome.

Mining candidate genes

Candidate genes underlying the potential QTLs were detected using the genome position of markers flanking the QTL (http://orygenesdb.cirad.fr). The genome position of markers was determined using the Nipponbare (*japonica* rice) sequence based rice map from the gramene web site (www.gramene.org).

Results

4 Results

Performance of parents and RILs

Yield and its component traits at Hyderabad and Maruteru locations

The parents IR64 and INRC10192 showed significant differences for all the traits except panicle length (PL) and biomass (BM) in N1; and spikelet number (SN) and BM in N2 in both the N levels at Hyderabad, while at Maruteru they differed significantly for plant height (PH), fertile grains (FG), SN, BM, single plant yield (SPY) and harvest index (HI) in N1 and N2 levels, whereas for number of tillers (NT), number of productive tillers (NPT) and chaffy grains (CG) significant differences were observed between parents in N2 level only (Table7).

Table 7 Test of significance of the parents for yield and its components measured under two levels of N at two locations

	Hyderabad				Maruteru			
	IR64	INRC10192	IR64/INRC10192		IR64	INRC10192	IR64/INRC10192	
Trait	N1/N2	N1/N2	N1/N1	N2/N2	N1/N2	N1/N2	N1/N1	N2/N2
PH	**0.02**	**0.05**	**S**	**S**	**0.003**	**0.00016**	**S**	**S**
NT	0.07	0.57	**0.03**	**0.02**	0.48	0.47	0.3	**0.004**
NPT	**0.03**	0.48	**0.05**	**0.001**	0.33	0.08	0.2	**0.003**
PL	0.4	0.34	0.08	**0.02**	0.09	**0.04**	0.74	0.7
FG	**0.0005**	**0.00023**	**S**	**S**	0.06	0.61	**0.0005**	**0.007**
CG	0.32	0.67	**0.001**	**0.008**	0.3	0.69	0.88	**0.01**
SN	**0.03**	0.81	**0.01**	0.09	0.08	0.66	**0.0004**	**0.01**
SF	**0.03**	0.085	**0.006**	**0.002**	**0.007**	0.68	0.1	0.6
GW	0.6	0.68	**0.01**	**0.01**	0.71	0.89	0.86	0.9
BM	**0.014**	0.32	0.8	0.38	0.13	**0.01**	**0.0014**	**0.0001**
SPY	**0.017**	0.06	**0.038**	**0.013**	**0.0002**	**0.04**	**0.00048**	**0.006**
HI	**0.00086**	**0.05**	**S**	**S**	**0.004**	0.26	**S**	**0.001**

S= *P* >0.00001; Note: Values in bold are significant at corresponding probability levels

Table 8 Performance of the parents, IR64 and INRC10192 and 140 RILs for yield and its components under two N-levels across two locations

		Location-I Hyderabad							Location-II Maruteru						
Trait	N-level	IR64 (n=10)	INRC10192 (n=10)	RILs (n=140)	SD	Range	TS(%)	Skew	IR64 (n=10)	INRC10192 (n=10)	RILs (n=140)	SD	Range	TS (%)	Skew
PH	N1	66.5	136	110.75	22.05	69.88-145.67	13.67	-0.09	64.5	121.25	103.41	21.69	66.25-144.5	28.15	-0.12
	N2	71	143	112.47	21.69	75.00-157.75	2.90	-0.05	82.25	151.75	105.6	20.96	67.25-159.5	17.99	-0.09
NT	N1	31.5	19.25	12.1	2.55	7.00-28.00	98.56	2.30	23	17.75	14.5	3.47	8.25-29.75	88.15	0.96
	N2	35	22.5	12.72	3.02	7.25-34.00	99.28	2.89	25.5	19.75	14.62	3.96	8.25-33.00	94.96	1.75
NPT	N1	21.5	15.25	10.42	1.96	5.00-21.00	98.56	0.78	14.75	9	10.97	2.91	5.50-22.25	36.30	0.99
	N2	24	16.67	10.93	2.66	6.88-28.50	98.56	3.24	18.5	13	11.29	3.32	5.75-29.00	79.86	1.89
PL	N1	20.25	23.5	23.67	2.18	19.25-29.67	57.25	0.13	23	23.25	23.81	1.91	19.37-28.38	96.40	0.23
	N2	21.2	25.5	23.63	1.97	18.13-30.60	26.81	-0.04	24.85	25.25	23.9	1.67	18.4-30.25	87.41	0.25
FG	N1	64	102	111.27	23.76	43.25-188.38	64.75	0.44	82.25	136	100.31	22.7	35-167.75	30.37	0.14
	N2	72	113	112.38	27.32	63.25-205.08	43.88	0.95	102.5	140.25	104.69	23.39	44.5-161.75	56.12	0.25
CG	N1	15.5	36.5	22.36	12.31	6.13-74.25	46.76	1.44	5.25	5.5	25.42	16.46	5.00-123.50	99.28	2.90
	N2	13	33.5	22.36	12.94	3.00-69.80	47.10	1.26	4.3	6.25	24.14	13.06	7.25-86.00	100.00	1.66
SN	N1	79.5	139	133.65	24.66	78.75-227.00	33.09	0.64	88	141.6	124.44	26.08	69.5-229.50	29.63	0.47
	N2	85	146.5	134.42	29.5	80.38-215.00	32.37	0.71	106.75	146.25	129.99	28.09	80.5-213.75	45.32	0.94
SF	N1	80.58	73.4	83.17	8.17	37.48-94.51	81.29	-1.85	93.65	95.21	80.89	9.25	20.31-95.47	98.56	-2.40
	N2	84.5	77.06	83.33	8.44	47.43-97.80	70.50	-0.83	96.64	95.65	80.64	9.23	42.70-94.39	100.00	-1.43
GW	N1	21.75	22.48	22.7	3.77	9.65-46.52	89.86	1.67	22.1	22.29	21.95	2.61	16.09-31.84	91.79	0.75
	N2	21.8	22.35	23.26	4.13	14.40-40.93	93.48	1.73	22.45	22.36	22.03	2.44	17.57-36.76	98.56	1.62
BM	N1	77.5	75.67	54.2	12.51	34.00-136.00	98.55	1.56	59	73	50.4	10.58	32.75-95.25	86.03	1.25
	N2	79	81.5	56.57	14.22	35.79-110.00	99.27	2.27	62.5	78.3	54.04	11.56	36.00-105.00	87.05	1.40
SPY	N1	21.6	14.2	23.14	6.54	7.28-53.52	58.99	1.23	23.3	18.6	15.45	3.79	5.95-26.45	81.29	0.11
	N2	27.75	16.37	27.9	7.8	5.46-71.28	46.76	1.44	28.2	22.4	16.8	4.37	7.21-32.46	97.04	0.91
HI	N1	27.87	18.77	37.64	6.06	7.68-50.04	95.52	-1.72	39.27	25.47	27.68	5.34	13.50-40.51	34.78	-0.01
	N2	35.13	20.09	47.48	6.39	19.28-61.29	98.35	-0.67	45.18	28.61	36	22.54	12.17-66.02	33.83	0.28

For trait names and N-levels refer table 4. RILs= recombinant inbred lines. SD=standard deviation. Range= minimum-maximum. TS= transgressive segregation. Skew= skewness.

The parent IR64 had significant differences for PH, NPT, FG, SN, spikelet fertility (SF), BM, SPY and HI across N levels at Hyderabad (Table 7) and at Maruteru it exhibited highly significant differences for PH, FG, SN, SF, SPY and HI. In contrast, INRC10192 showed significant differences for PH, FG, SF and HI at Hyderabad, while at Maruteru it had significant differences for PH, NPT, PL, BM, SPY and HI.

Mean phenotypic values of RILs in N2 level were relatively higher than those in N1 level at Hyderabad, especially for BM, SPY and HI (Table 8). Under N2, BM and grain yield were 2.37g (4.37%) and 4.76g (20.57%) respectively higher than in N1 level. Additionally, mean of HI in N1 was 37.64%, as against 47.48% in N2, an increase of 9.84%. In the case of PH and FG the increased rate under N2 level over N1 level was 1.55% and 1.00%, respectively. In contrast, smaller differences between N-levels were observed for the remaining traits. On the other hand at Maruteru HI (8.32%), SPY (1.35g), BM (3.64g), SN (5.55), FG (4.38) and PH (2.19cm) showed higher performance in N2 than in N1 level, the increase being 30.06%, 8.47%, 7.22%, 4.46%, 4.37% and 2.12%, respectively.

Transgressive segregation was observed in both the directions for most of the traits ranging from 2.90% for PH to 99.28% for NT at Hyderabad and 17.99% for PH to 100% for CG and SF at Maruteru.

Most of the traits in RILs, showednormal distribution except, NT under both the N levels, NPT and BM under N2 at Hyderabad; while CG under N2 and SF under N1 at Maruteru showed near normal distribution (Table 8; Figs 8 and 9). The trait PH showed bimodal distribution under both the levels of N at both the locations.

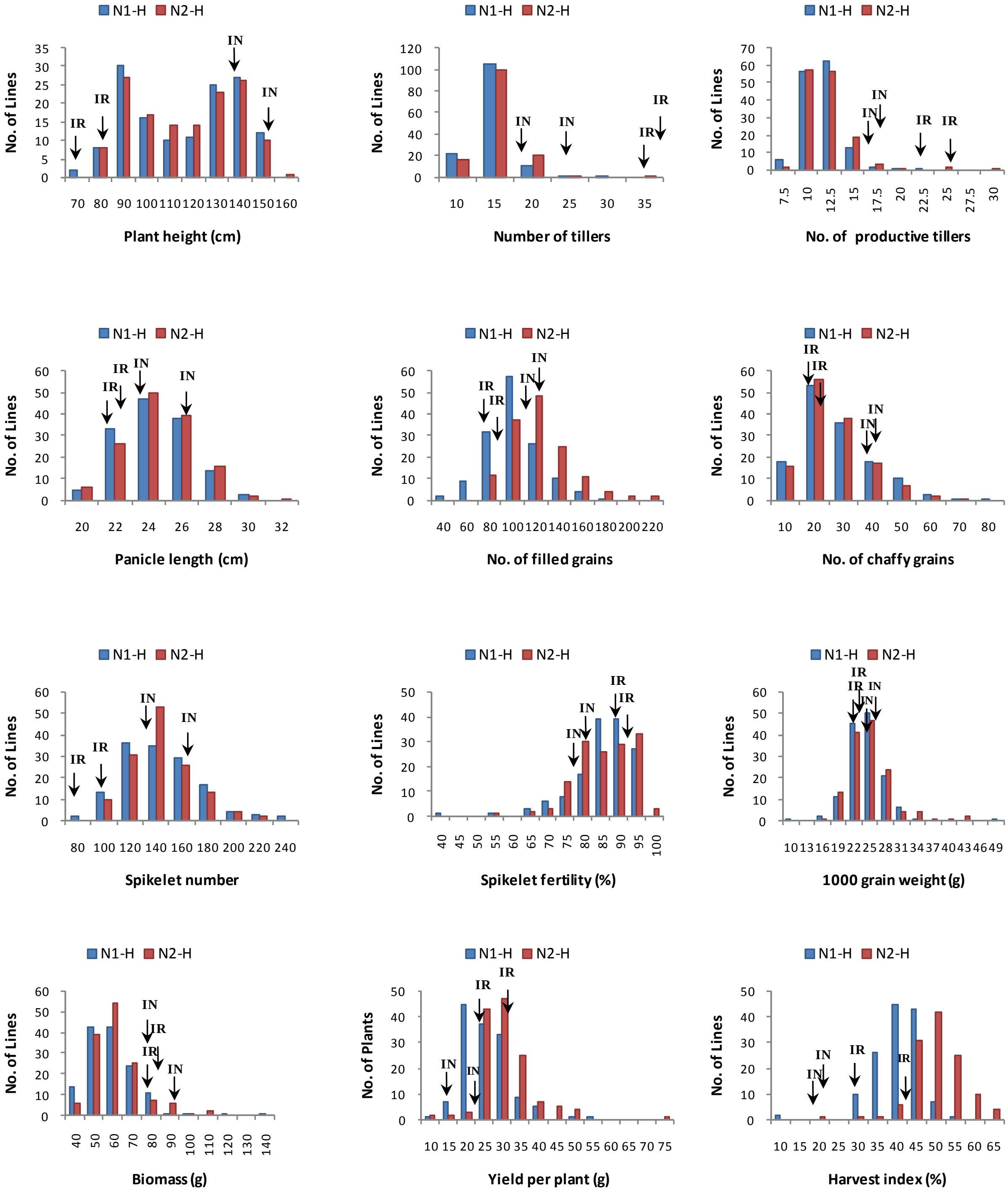


Fig. 8 Phenotypic distributions of yield and its components of 140 RILs evaluated at Hyderabad; IN-INRC10192 and IR-IR64

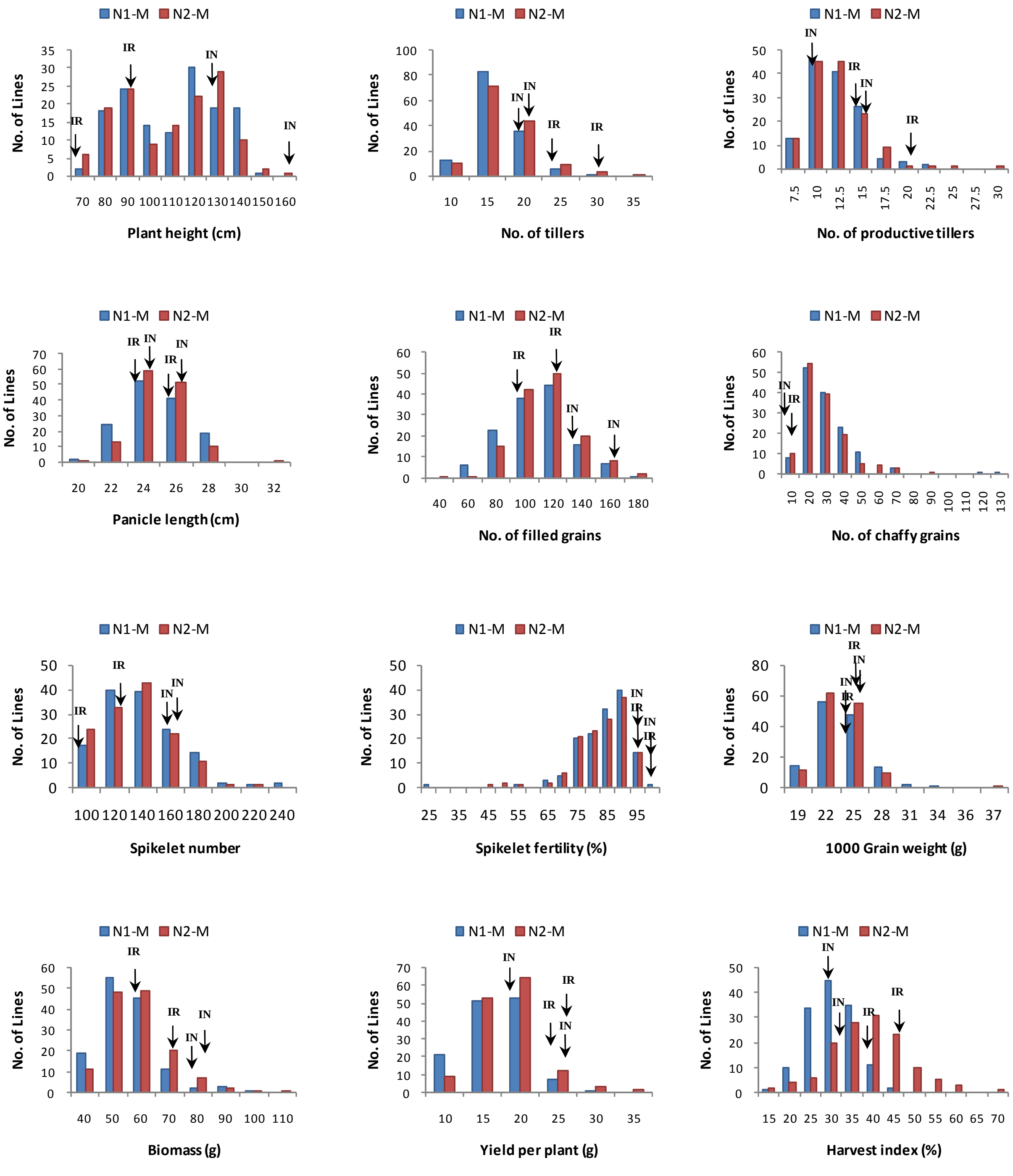


Fig. 9 Phenotypic distributions of yield and its components of 140 RILs evaluated at Maruteru ; IN-INRC10192 and IR-IR64

Response to moisture stress under field conditions

During the first 15 days of the stress, available soil moisture was 72 and 49% of field capacity respectively in 15cm and 30cm of soil layers (Fig 10). Symptoms of severe leaf rolling (Fig. 4) and leaf drying were observed in most of the lines, especially under prolonged stress period (21 days) at and around reproductive stage. Under stress free control conditions, both the parents differed significantly in respect of PH, NPT, BM, SPY and HI, while under stress, the differences between them were much higher for nearly all the traits except NT and NPT (Table 9). The parent IR64 showed significant differences for PH, SFG, SCG, SSN, SSF, SGW, BM, SPY and HI between the two water supply conditions, while INRC10192 differed significantly only in respect of PH and SSF. RWC and CC between parents, under stress conditions.

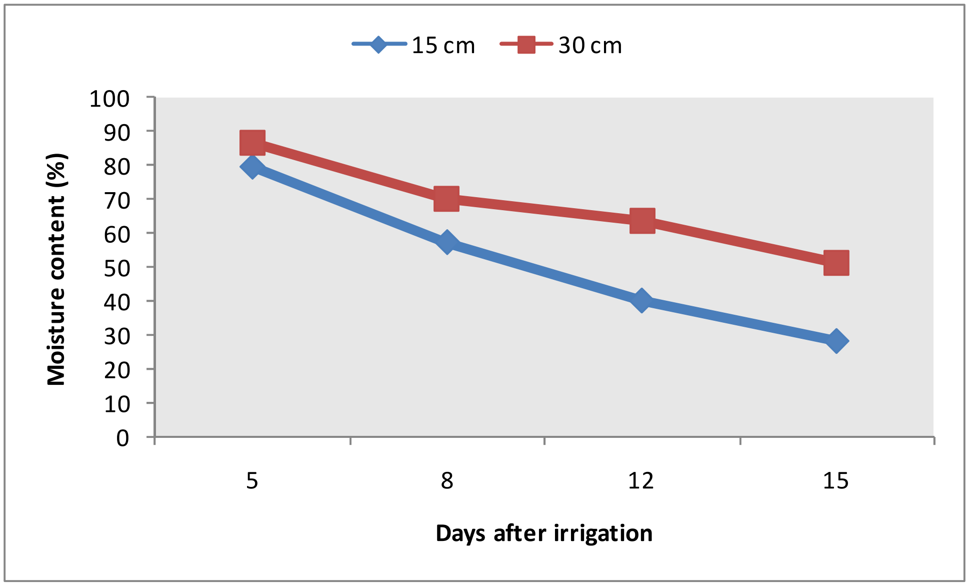


Fig. 10 Soil moisture percentages measured at two depths of soil in drought experiment under field conditions

Table 9 Test of significance of the parents for yield and its components and drought related traits measured under two water regimes in field condition

	IR64	INRC10192	IR64/INRC10192	IR64/INRC10192
Trait	Control/Stress	Control/Stress	Control	Stress
PH	**0.01**	**0.013**	**0.0015**	**0.00073**
NPT	0.18	0.46	**0.017**	0.16
PL	0.57	0.76	0.12	**0.014**
PFG	0.19	0.16	0.21	0.09
PCG	0.13	0.49	0.7	0.21
PSN	0.33	0.31	0.19	**0.001**
PSF	0.13	0.35	0.54	0.45
PGW	0.31	0.55	0.49	0.67
SFG	**0.02**	0.56	0.41	**0.039**
SCG	**0.004**	0.17	0.55	**0.005**
SSN	**0.012**	0.25	0.32	0.07
SSF	**0.0028**	**0.02**	0.9	**0.008**
SGW	**0.04**	0.09	0.46	**0.05**
BM	**0.01**	0.14	**0.019**	**0.0066**
SPY	**0.008**	0.06	**0.016**	**0.0072**
HI	**0.005**	0.27	**0.03**	**0.015**
RWC[a]				**0.05**
CC[a]				**0.02**
SPY (R)				**0.0036**
BM (R)				0.85

[a] =measured under stress. Note: Values in bold are significant at corresponding probability levels

The two parents differed more significantly in grain yield and HI under the stress as compared to in control plots (Table 10), with INRC10192 producing higher SPY (12.3g) than IR64 (3.01g). The RILs on the other hand segregated tremendously for the trait, the range being from 0.02g to 39.35g. The mean yield reduction due to the stress, as compared to the control was 65% and 28% in IR64 and INRC10192 respectively. On an average the RILs showed 43% relative yield under stress is compared to the control. The HI of IR64, decreased dramatically

Table 10 Performance of the parents, IR64 and INRC10192 and 140 RILs for yield and its components and drought related traits under field conditions

	Control							Stress						
Trait	IR64 (n=10)	INRC10192 (n=10)	RILs (n=140)	SD	Range	TS (%)	Skew	IR64 (n=10)	INRC10192 (n=10)	RILs (n=140)	SD	Range	T S (%)	Skew
PH	72	118	96.71	19.37	51-124.5	33.90	-0.09	61.5	113.2	87.27	18.24	48-121	14.5	-0.37
NPT	16.5	10	17.6	5.73	5-33.5	63.25	0.28	11	8	13.48	4.99	3.00-29	80.15	0.53
PL	22	25	21.25	2.27	15.65-29	75.21	0.59	21.3	24.8	20.26	2.14	15-25.5	70.08	-0.15
PFG	41	29	29.14	6.42	8.5-47	55.56	-0.10	29	22	19.51	8.74	2.00-43	75.21	0.01
PCG	2	3	6.83	3.4	1.5-18.5	89.74	0.87	9	4.5	13.17	9.11	1.0-44	73.23	0.92
PSN	43	32	35.71	6.71	17-58	41.88	0.23	38	26.5	29.41	7.09	5.0-50	44.09	0.43
PSF	95	91	81.5	9.31	29.59-95.97	86.32	-1.62	76	83	60.35	29.24	0-96.55	83.76	-0.48
PGW	21.6	21.3	18.68	2.54	13.19-30.52	97.44	0.80	21.2	21.1	17.19	3.41	5-30.33	99.15	-0.04
SFG	67	57	55.91	21.16	19-135.5	81.20	0.70	41.5	49	34.04	17.65	2.0-88	83.48	0.34
SCG	9	8	19.09	9.57	4.5-51	98.29	1.03	26.5	11	32.88	16.63	6.0-88.5	64.29	0.93
SSN	76	65	74.55	22.57	33.5-156.5	75.21	0.62	44	52	59.8	17.71	23-113	81.1	0.54
SSF	88	87.6	74.15	12.48	28.36-93.91	97.46	-0.95	40.21	66	48.33	26.52	0-100	66.09	-0.17
SGW	21.2	20.8	17.55	2.49	12.13-25.81	99.15	0.40	13.43	20.1	16	3.24	6.5-23.49	22.61	-0.39
BM	60.5	73	53.02	14.63	24-106	86.84	0.88	32	58	46.75	12.5	27-82.5	29.51	0.69
SPY	26	20.1	15.23	6.02	4.15-29.47	87.29	0.46	3.01	12.3	6.53	7.02	0.02-39.35	59.54	1.74
HI	42.9	28	28.41	7.62	11.3-61.79	47.01	0.71	18.02	20.21	14.79	12.99	0.06-60.52	86.07	0.82
RWC								59	66	64.62	12.74	26-94	77.42	-0.28
CC								26.92	32.92	30.5	2.36	24.81-36.4	27.14	0.03
SPY (R)								0.35	0.72	0.43	0.7	0.0015-3.53	83.78	2.17
BM (R)								0.83	0.84	1.07	0.42	0.39-2.38	99.01	1.14

For trait names refer Table 5. Control= well-irrigated. Stress= drought at flowering stage. RILs= recombinant inbred population. SD=standard deviation. Range= minimum-maximum. TS= transgressive segregation. Skew= skewness.

from 42.9% in control to 18.02% under the drought stress, while the HI of INRC10192 decreased from 28% to 20.21%. The mean HI of RILs in the control plot was 28.41%, as against 14.79% under the stress treatment. In respect of biomass there was 17% and 16% reduction under the stress in IR64 and INRC10192, respectively. The HI was relatively lower in IR64 (42%) than in INRC10192 (72.18%). Relative BM of RILs under drought ranged between 0.39 and 2.38 times that of the control, with the mean of 1.07.

Mean plant height of the RILs was less by 3.8 cm under the stress, as compared to that in the control plot. The parents INRC10192 and IR64 had height reduction of 4.8cm and 10.5cm under stress when compared to the control. Mean leaf RWC of the RILs declined to 64.62% under the stress. However, in one of the RI lines it was as low as 26%. INRC10192 had higher RWC and SPAD values (66% and 32.92) as compared to IR64 (59% and 26.92), with RILs showing mean SPAD value of 30.5units.

In the control, transgressive segregants in the RILs ranged from 33.90% for PH to 99.15% for SSW, while under the stress it was between 14.5% for PH and 99.15% for PGW. All the traits showed continuous variation both under control and stress conditions with skewness ranging from -1.62 (PSF in the control) to 1.74 (SPY in the stress) and well fitted normal distribution. However, relative yield had a skewness of 2.17, which approximately fitted normal distribution (Table 10, Fig.11). In 13% of the lines panicles were completely sterile.

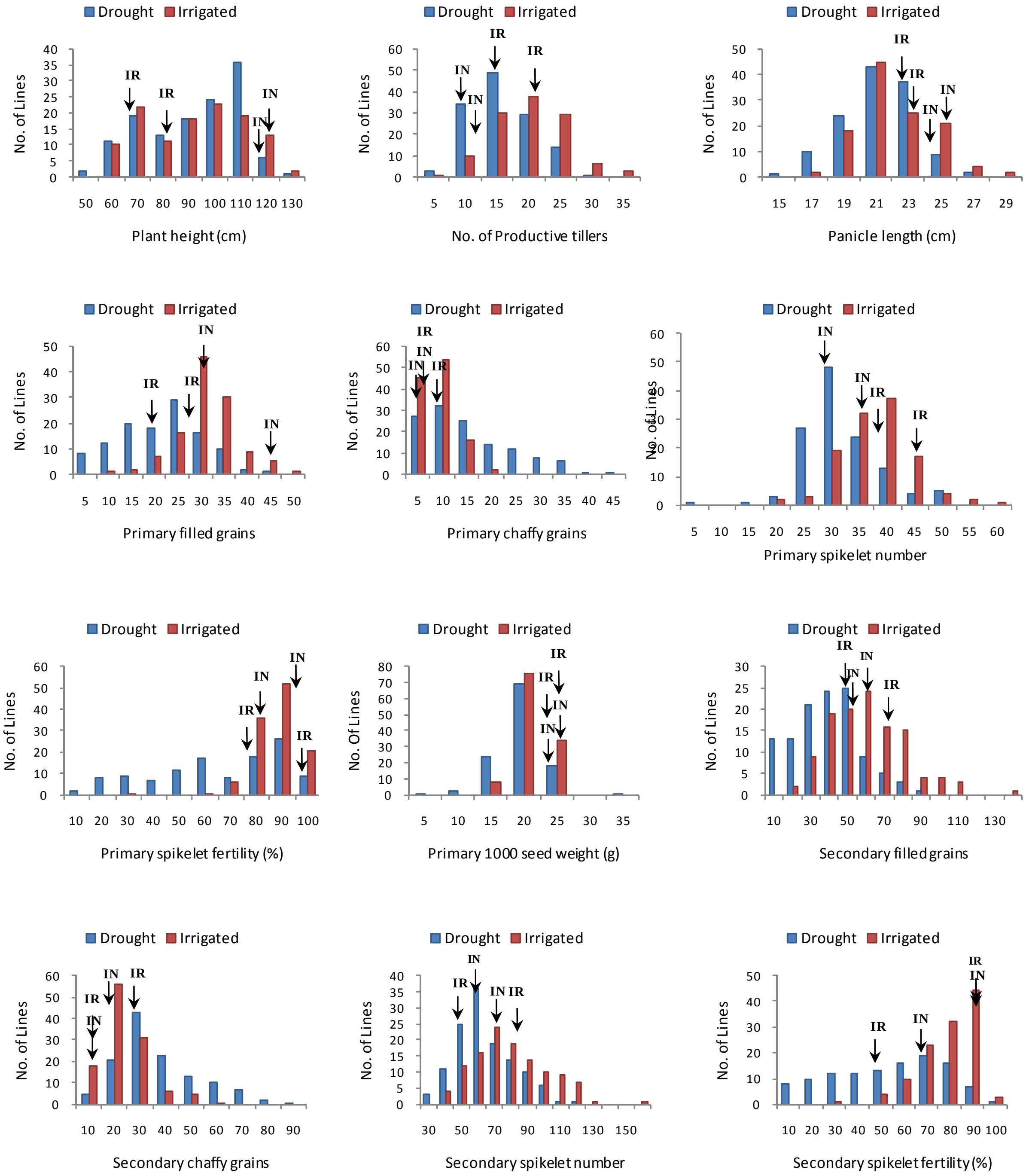


Fig. 11 Phenotypic distributions of drought related traits in 140 RILs under field condition; IN-INRC10192 and IR-IR64

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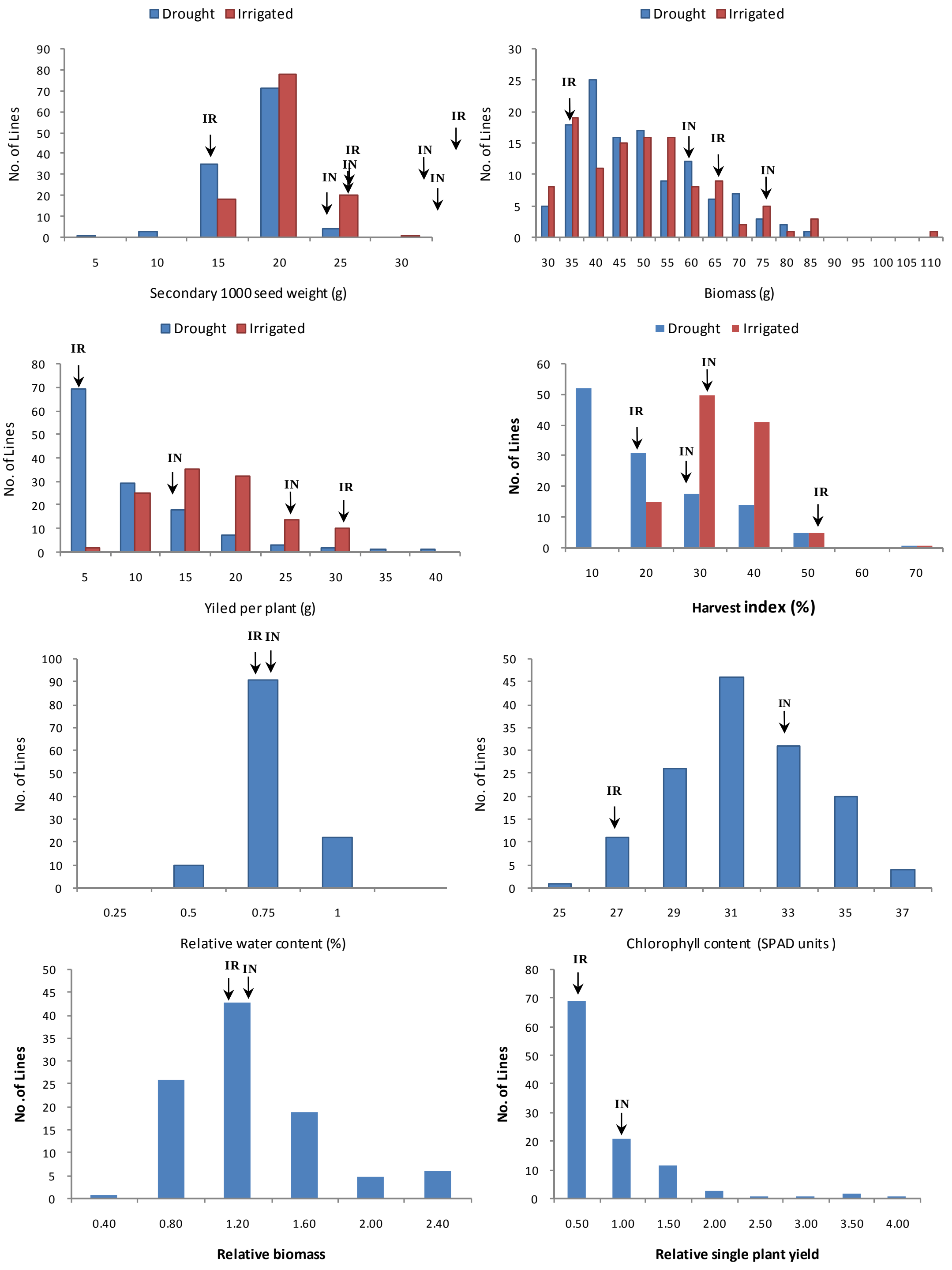

Fig. 11 Phenotypic distributions of drought related traits in 140 RILs under field condition; IN-INRC10192 and IR-IR64

Response to Drought under greenhouse condition

Under both control and stress conditions, the two parents, IR64 and INRC10192 manifested significant differences in all the traits studied, except root dry weight (RDW) in the control (Table 11). On the other hand, significant differences in RDW and root shoot weight ratio (RS) were observed between the two treatments in the case of both the parents. In respect of shoot dry weight (SDW) and root length (RL), INRC10192 only showed significant differences under the stress. Relative parameters measured under stress revealed significant difference between parents for RL (R), RDW (R) and RS (R).

Table 11 Test of significance of the parents for shoot and root traits and of their relative parameters measured in control and stress treatments under drought stress controlled greenhouse conditions

	IR64	INRC10192	IR64/INRC10192	IR64/INRC10192	Rel. par.
Trait	Control/Stress	Control/Stress	Control	Stress	Stress
SL	0.19	0.55	**0.017**	**0.0072**	0.37
MRL	0.73	**0.023**	**0.0095**	**0.0045**	**0.032**
SDW	0.06	**0.03**	**0.04**	**0.007**	0.22
RDW	**0.05**	**0.01**	0.2	**0.01**	**0.02**
RS	0.09	**0.008**	**0.04**	**0.001**	**0.007**

Rel. par. = relative parameter. Note: Values in bold are significant at corresponding probability levels

INRC10192 showed higher mean values than IR64 for all the traits studied (Table 12). Study of RILs under control revealed transgressive segregants to range from 48.54% (MRL) to 93.33% (RS), while under stress to range from 49.49% (RDW) to 99.87% (RS). The relative parameters showed the range to be between 69.47% (RDW) and 92.22% (RS). All the traits showed continuous variation fitting well

Table 12 Performance of the parents, IR64 and INRC10192 and 140 RILs for shoot and root traits and of their relative parameters in control and stress treatments under greenhouse conditions

	Trait	IR64 (n=10)	INRC10192 (n=10)	RILs (n=140)	SD	Range	Ts (%)	Skew
Control (half-strength Yoshida medium)	SL	12.600	15.950	11.513	3.441	3.349-21.8	71.15	0.64
	MRL	6.625	8.750	7.425	1.957	1.887-12	48.54	-0.09
	SDW	0.013	0.016	8.25	3.329	1.5-18.25	90.48	0.36
	RDW	0.010	0.014	10.75	2.861	2.753-25	53.33	0.93
	RS	0.792	0.857	1.291	0.631	0.629-4.142	93.33	1.55
Stress (PEG-6000 + half-strength Yoshida medium)	SL	11.020	15.260	9.328	3.102	2.875-16	74.26	-0.18
	MRL	6.118	9.933	6.075	1.881	3.8-12.7	49.50	-0.16
	SDW	0.010	0.015	7.875	3.241	2.5-15.75	70.21	0.52
	RDW	0.008	0.017	15	7.984	2.925-49.25	49.49	1.45
	RS	0.835	1.082	1.805	1.576	0.213-7.556	97.87	1.19
Relative parameters	SL (R)	0.875	0.957	0.775	0.235	0.16-1.531	84.38	0.1
	MRL (R)	0.923	1.135	0.791	0.294	0.263-1.74	80.21	0.58
	SDW (R)	0.784	0.968	1.01	0.334	0.328-2.05	75.56	0.41
	RDW (R)	0.827	1.222	1.291	0.872	0.229-5.794	69.47	2.22
	RS (R)	1.054	1.262	1.53	0.939	0.138-6.259	92.22	1.95

For trait names refer Table 6. RILs= recombinant inbred population. SD=standard deviation. Range= minimum-maximum. Ts= transgressive segregation. Skew= skewness.

normal distribution except relative RDW (Table 12; Fig. 12). As many as 18 RI lines with very poor growth slowly died.

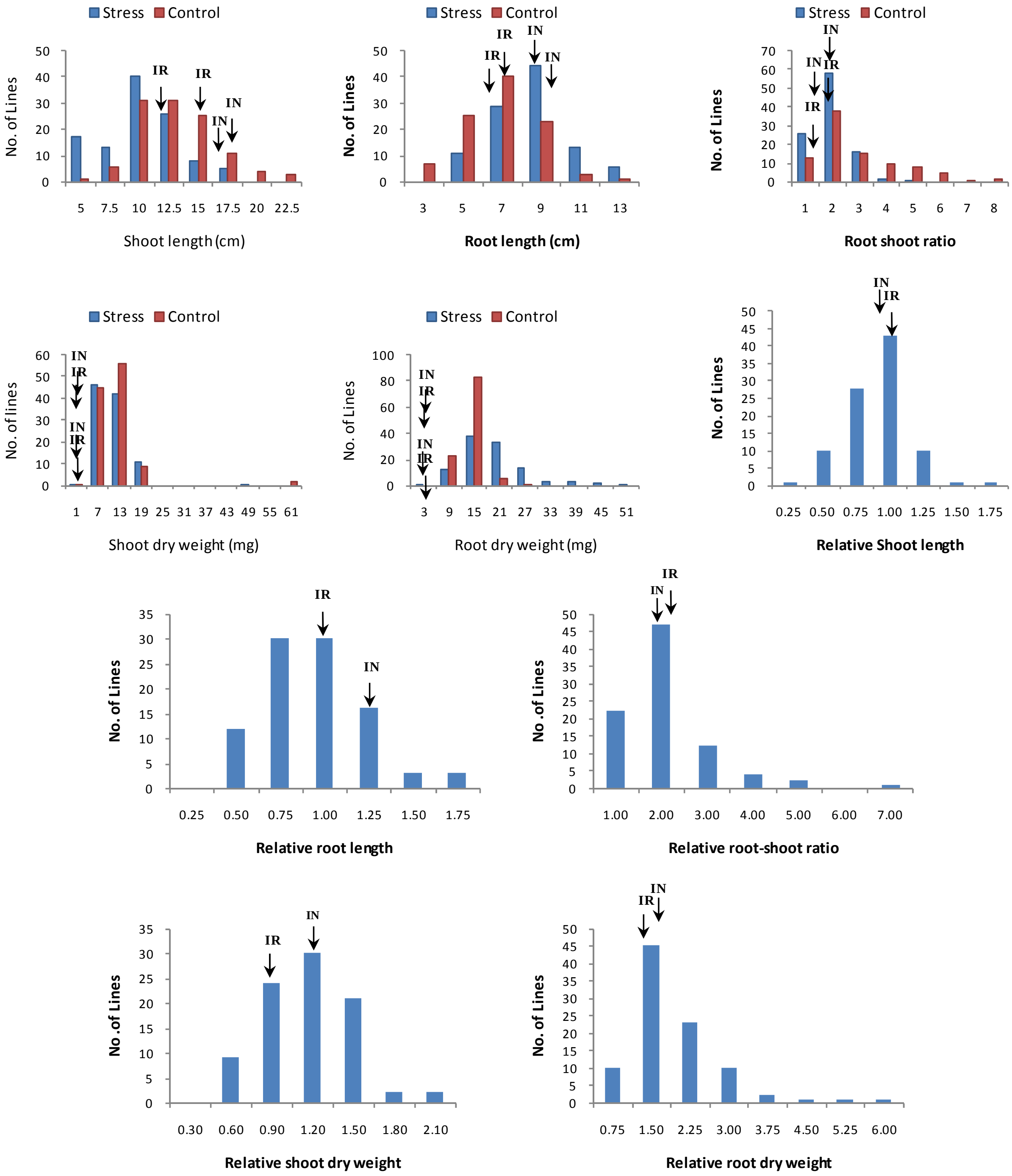

Fig. 12 Phenotypic distributions of drought related traits in 140 RILs under greenhouse condition; IN-INRC10192 and IR-IR64

Trait Correlations

Yield and its component traits

The nature and strength of relationship among the components of yield and traits with yield *per se* was studied of material raised at Hyderabad and Maruteru locations. The data relating to Hyderabad (Table 13), revealed single plant yield (SPY) to show highly significant positive correlations with PH (0.373** and 0.292**), NPT (0.461** and 0.351**), FG (0.554** and 0.444**), SN (0.446** and 0.299**), SF (0.316** and 0.385) BM (0.638** and 0.61788) and HI (0.588 and 0.348**), respectively at N1 and N2 levels. Equally strong positive correlation was observed between yield and panicle length (0.350**) but at N1 level only.

Table 13 Correlation coefficients among yield and its components under two N-levels at two locations

Trait	Component traits with yield in location I		Component traits with yield in location II		Location I	Location II	Location I with Location II	
	N1	N2	N1	N2	N1 with N2	N1 with N2	N1 vs N1	N2 vs N2
PH	**0.373****	**0.292****	0.191	0.142	**0.876****	**0.574****	**0.739****	**0.641****
NT	0.121	0.128	**0.235***	**0.445****	**0.568****	**0.327****	**0.232***	0.163
PT	**0.461****	**0.351****	**0.289****	**0.515****	**0.376****	**0.330****	0.158	0.058
PL	**0.350****	0.148	**0.242***	**0.263****	**0.606****	**0.451****	**0.475****	**0.246***
FG	**0.554****	**0.444****	**0.372****	**0.355****	**0.434****	**0.200***	**0.197***	0.120
CG	-0.158	-0.188	-0.086	-0.072	**0.530****	**0.242***	**0.207***	0.062
SN	**0.446****	**0.299****	**0.255****	**0.282****	**0.446****	**0.343****	**0.313****	0.113
SF	**0.316****	**0.385****	**0.217***	**0.236***	**0.536****	0.114	0.177	0.076
SW	0.138	0.007	**0.237***	0.127	**0.428****	**0.280****	**0.445****	**0.263****
BM	**0.638****	**0.617****	**0.457****	**0.509****	**0.633****	**0.202***	0.135	-0.182
SPY	1.000	1.000	1.000	1.000	**0.442****	0.110	0.037	-0.178
HI	**0.588****	**0.348****	**0.626****	**0.379****	**0.229***	0.022	0.018	-0.051

* = p<0.05; **= p<0.01. Location I : Hyderabad; Location II :Maruteru

At Maruteru, NT (0.235* and 0.445**), NPT (0.289** and 0.515**), PL (0.242* and 0.263**), FG (0.372** and 0.355**), SN (0.252* and 0.282**), SF (0.217* and 0.236*), BM (0.457** and 0.509**) and HI (0.626** and 0.379**) showed positive significant correlations with SPY under both N1 and N2 conditions, respectively. SW however showed positive significant correlation with SPY only at N1 level.

The mean yield and of all the components of it varied significantly with the level of N at Hyderabad (Table 13), while at Maruteru, except SF, SPY and HI; all the traits were significantly different across N-levels. N-levels across locations showed significant effects for PH, PL, CG and SW (N1 with N1), while N2 with N2 had significant effects for PH, PL, CG, SW and SN.

In general, associations among yield components were well correlated at both the locations confirming what was expected of (SI Tables 3 and 4).

Drought response under field conditions

Drought related traits were found to be poorly correlated when control means were correlated with that of stress, however, traits like PH, PL, PSW and SSW showed positive significant associations the values being respectively 0.420**, 0.201*, 0.274** and 0.327**. The correlations among traits were in general well correlated under normal (control) as well as stress conditions. (Table 14).

Correlations of component traits with grain yield under control and stress conditions were significantly positive in respect of PSF (0.220* and 0.469**), PSW

(0.228* and 0.226*), SFG (0.487** and 0.406**), SSN (0.390** and 0.211*), SSF (0.370** and 0.519**), BM (0.817** and 0.360**) and HI (0.509** and 0.835**). Apart from these traits the trait SSW (0.219*) was significantly and positively correlated with yield under stress; while, PSN (0.251*) was similarly correlated with yield under control condition. Further, correlations of SPY with PCG (-0.447**) and SCG (-0.386**) were highly significant but negative under stress condition. Relative yield under stress had significant positive correlation with yield under stress (0.244*), however, it showed negative but not significant correlation with SPY (-0.015) under control (Table 14).

Table 14 Correlation coefficients among yield and drought related traits under field conditions

Trait	Control with stress	Component traits with yield in control	Component traits with yield in stress
PH	**0.420****	0.147	-0.092
PT	0.154	**0.602****	**0.333****
PL	**0.201***	0.189	0.078
PFG	-0.065	**0.304****	**0.385****
PCG	0.088	-0.086	**-0.447****
PSN	0.055	**0.251***	0.133
PSF	-0.088	**0.220***	**0.469****
PSW	**0.274****	**0.228***	**0.226***
SFG	-0.154	**0.487****	**0.406****
SCG	0.118	-0.161	**-0.386****
SSN	0.044	**0.390****	**0.211***
SSF	-0.031	**0.370****	**0.519****
SSW	**0.327****	0.163	**0.219***
BM	0.118	**0.817****	**0.360****
SPY	-0.007	1.000	1.000
HI	0.166	**0.509****	**0.835****
RWC (D)[a]		0.054	0.063
CC (D)		0.104	0.066
SPY (R)[b]		**-0.015**	**0.244***
BM (R)		-0.029	0.026

* = p<0.05; **= p<0.01.

As expected, most of the yield components were positively correlated among themselves in both control and stress conditions (SI Table 5).

Drought response – under greenhouse conditions

Root length (RL) under control had significant positive correlation with shoot length (0.219*) and shoot dry weight (0.215*) (Table 15), while under stress it had highly significant positive correlation with both SL (0.658**) and SDW (0.566**). In contrast, MRL showed significant negative correlation with RDW (-0.286**) and RS (-0.643**) under stress. Root dry weight under control, had strong significant positive association with SL (0.346**) and SDW (0.451**), but under stress, it had significant negative association with SL (-0.195*), MRL (-0.286**) and SDW (-0.228*).

As expected, R S had strong significant negative association with SL (-0.485** and -0.660**) and SDW (-0.745** and -0.706**) under both control and stress conditions, respectively. Under stress, still RS exhibited significant negative and positive association with MRL (-0.643**) and RDW (0.711**), respectively.

Identical traits showed positive significant association, when correlated between control means and stress means for all traits *viz*., SL, MRL, SDW, and RS (0.553**, 0.248*, 0.597** and 0.578**), expect RDW which showed negative correlation (-0.078).

Table 15 Correlation coefficients among shoot and root traits in Control (C), Stress (S) treatments and Relative parameters (R) in 140 RI population under greenhouse conditions

Trait	SL (C)	RL (C)	SDW (C)	RDW (C)	RS (C)	SL (S)	RL (A)	SDW (S)	RDW (S)	RS (S)	SL (R)	RL (R)	SDW (R)	RDW (R)	RS (R)
SL (C)	1.000														
RL (C)	**0.219***	1.000													
SDW (C)	**0.684****	**0.215***	1.000												
RDW (C)	**0.346****	0.013	**0.451****	1.000											
RS (C)	**-0.485****	-0.162	**-0.745****	0.130	1.000										
SL (S)	**0.553****	0.081	**0.525****	0.087	**-0.526****	1.000									
RL (S)	**0.426****	**0.248***	**0.545****	0.169	**-0.491****	**0.658****	1.000								
SDW (S)	**0.456****	0.136	**0.597****	**0.204***	**-0.515****	**0.609****	**0.566****	1.000							
RDW (S)	-0.192	-0.092	**-0.288****	-0.078	**0.226***	**-0.195***	**-0.286****	**-0.228***	1.000						
RS (S)	**-0.335****	-0.158	**-0.576****	-0.119	**0.578****	**-0.660****	**-0.643****	**-0.706****	**0.711****	1.000					
SL (R)	**-0.209***	-0.013	0.030	-0.161	**-0.200***	**0.671****	**0.418****	**0.263****	-0.077	**-0.446****	1.000				
RL (R)	**0.279****	**-0.434****	**0.347****	0.154	**-0.337****	**0.531****	**0.726****	**0.410****	**-0.197***	**-0.472****	**0.404****	1.000			
SDW (R)	**-0.292****	-0.060	**-0.445****	-0.176	**0.331****	0.123	0.068	**0.393****	0.088	-0.169	**0.404****	0.092	1.000		
RDW (R)	**-0.247***	-0.067	**-0.351****	**-0.362****	0.098	-0.172	**-0.285****	**-0.213***	**0.927****	**0.640****	-0.003	**-0.223***	0.154	1.000	
RS (R)	-0.094	-0.041	-0.156	**-0.252***	-0.043	**-0.344****	**-0.379****	**-0.467****	**0.739****	**0.750****	**-0.303****	**-0.294****	**-0.403****	**0.768****	1.000

* = p<0.05; **= p<0.01.

Relative parameters measured under stress were negatively correlated with all traits under control (SL= -0.209*, MRL= -0.43**, SDW= -0.445**, RDW= -0.362** and RS= -0.043); in contrast, to traits measured under stress, showing significant positive correlation (SL= 0.671**, MRL= 0.726**, SDW= 0.393**, RDW= 0.927** and R/S= 0.750**).

Parental polymorphism

A total of 412 microsatellite markers was used for screening of the parents IR64 and INRC10192. Of these, 361 markers had given clearly scorable amplification products, while 21 did not amplify (null alleles) either IR64 or INRC10192, 18 could not be scored on agarose gel and 12 exhibited heterozygosity in either IR64 or INRC10192. In all 133 (32.28%) markers distributed on all the 12 chromosomes were polymorphic between the parents, indicating the possibilities of constructing a linkage map of IR64/INRC10192.

Construction of linkage map

Segregation of marker loci: Of 133 polymorphic markers 92 were chosen for screening the mapping population. Excluding 29 markers that were found to be unlinked and finally, 63 markers were used for construction of genetic linkage map. Of these, 21 markers showed segregation distortion to varied degrees on all the chromosomes, except on chromosome 5 (SI Table 6). The segregation distortion of all the markers, except RM1, RM154, RM589, RM5752 and RM25 were skewed towards the parent IR64.

Map length and marker interval: The linkage map covered 1978.9cM employing Kosambi mapping function, resulting in an average marker interval of 31.41cM (SI Fig, 1).

QTL mapping

Phenotypic and genotypic data relating to 140 RILs were subjected to QTL mapping employing QTL Cartographer 2.5. Many QTLs were identified using interval mapping (IM) and composite interval mapping (CIM) methods with 2.5 as LOD threshold for detecting a QTL.

A total of 73 QTLs, 38 by CIM and 35 by IM methods governing yield and its components was detected under two N levels across two locations (SI Tables 7 and 8). Twenty-eight QTLs were common in both the methods, while, 7 were exclusively detected by IM, and 10 by CIM. The 72 QTLs included 16 and 18 QTLs detected at Hyderabad and 22 and 17 at Maruteru under N1 and N2 levels, respectively (Table 16). These QTLs were mapped to 25 regions spread over all the chromosomes except chromosomes 6, 11 and 12, and most of them were on chromosomes 1, 2, 4, 7, and 8 (Fig 13).

In the case of drought related traits, 70 QTLs were detected by IM (34) and CIM (36). Fourteen QTLs were common for both the methods (SI Tables 9 and

10). Under control and stress conditions in field and greenhouse experiments, number of QTLs identified was 16, 30 and 5, 19; respectively (Table 16). These QTLs were mapped to 23 regions over all the chromosomes except chromosomes 11 and 12and most of them were on chromosomes 1,2,7,8 and 9 (Fig. 14).

Table 16 QTLs identified employing interval mapping and composite interval mapping

Experiment	Location/environment	Treatment	QTLs detected	Total	Grand Total
Yield and its components	Hyderabad	N1H	16		
		N2H	18	34	73
	Maruteru	N1M	22		
		N2M	17	39	
Drought tolerance parameters	Field	Control	16		
		Stress	30	46	70
	Greenhouse	Control	5		
		Stress	19	24	

For codes refer Tables 4, 5 & 6

Yield related QTLs identified at Hyderabad and Maruteru locations

A total of 46 QTLs was detected for 12 traits under two N-levels across two locations (Table 17, Fig. 13) Eighteen of them were identified in one or both the N-levels at one or both the locations, while the remaining 28 QTLs were detected in any one N-level and one location. The QTLs were categorized as (i) QTLs with “major/main effect” identified over one or both the N-levels in one or both the locations, and (ii) QTLs with “minor effect” that were detected under one N-level and one location only.

Table 17 QTLs for yield and its components identified in 140 RILs of the cross between IR64 and INRC10192 under two N levels at two locations

				Location I-Hyderabad						Location II-Maruteru						
			N level	N1			N2			N1			N2			
Trait	QTL	Chr.	Marker interval	LOD	PVE	a0	LOD	PVE	a0	LOD	PVE	a0	LOD	PVE	a0	Al.ef.
PH	*qph2.1**	2	RM154-RM318	6.04	62.02	19.14				3.29	59.87	16.66	8.71	79.36	19.36	IR64
	qph2.2	2	RM106-RM262										4.35	59.02	-17.87	INRC
	*qph2.3**	2^b	RM262-RM5897							4.51	72.12	-17.92	3.76	59.33	-17.94	INRC
	qph3.1	3	RM545-RM168										5.5	59.52	18.21	IR64
	qph3.2	3	RM570-RM517				2.67	57.42	-17.14							INRC
	*qph4.1**	4	RM471-RM252	5.17	61.61	19.09				3.08	71.34	18.07				IR64
	*qph5.1**	5	RM233-RM18602	9.64	62.12	-19.12	7.35	63.88	-18.61	5.85	62.77	-17.18	10.2	67.35	-18.71	INRC
	qph7.1	7^a	RM481-RM427				2.58	16.93	9.07							IR64
	*qph7.2**	7	RM427-RM248	14.74	72.6	19.8	9.48	64.91	18.52	8.94	65.37	17.47	10.87	65.32	18.78	IR64
	qph7.3	7	RM248-RM22171	3.1	17.07	9.26										IR64
	qph7.4	7^b	RM22171-RM5752							4.21	70.42	17.63				IR64
	*qph8.1**	8	RM38-RM331	6.87	62.08	-19.02				4.5	61.02	-16.81	10.61	79.65	-19.4	INRC
	*qph8.2**	8	RM404-RM547	4.48	61.83	19.06	4.76	77.31	19.21							IR64
	qph8.3	8	RM408-RM25										4.05	61.43	18.3	IR64
	*qph9.1**	9^b	RM219-RM434							4.76	71.44	-17.74	10.83	79.69	-19.42	INRC
NT	*qnt1.1*	1^a	RM495-RM243	2.86	12.37	-1.11										INRC
	*qnt8.1**	8^b	RM331-RM404	4.62	39.98	-5.53				4.24	36.53	-6.81				INRC
	*qnt8.2**	8	RM404-RM547	3.84	30.26	-4.99	3.65	31.15	-5.78	6.13	39.3	-6.77				INRC
NPT	*qnpt8.1**	8	RM331-RM404							3.61	25.61	-7				INRC
	*qnpt8.2**	8	RM404-RM547				4.9	29.78	-3.02	2.88	29.56	-6.18				INRC
PL	*qpl7.1*	7	RM481-RM427										2.51	12.77	0.61	IR64
FG	*qfg7.1*	7	RM481-RM427										2.51	14.38	9.03	IR64
	qfg10.1	10^b	RM271-RM591										3	13.63	-8.82	INRC
	qfg10.2	10^b	RM591-RM304										2.91	9.67	-7.44	INRC

Table 17 *continued from previous page.*

Table continues in the following page.

				Location I-Hderabad						Location II-Maruteru						
			N level	N1			N2			N1			N2			
Trait	QTL	Chr	Marker interval	LOD	PVE	a0	LOD	PVE	a0	LOD	PVE	a0	LOD	PVE	a0	Al.ef.
CG	*qcg4.1**	4	RM518-RM471	5.32	50.62	-11.86	3.99	44.08	-10.94							INRC
	*qcg4.2**	4	RM471-RM252	4.42	49.52	-11.6	2.72	34.34	-10.59							INRC
	qcg8.1	8^{a}	RM38-RM331							2.72	34.34	-10.59				INRC
	qcg8.2	8	RM331-RM404							7.71	35.99	-34.99				INRC
	*qcg8.3**	8	RM404-RM547				3.99	44.08	-10.94	8.79	39.04	-30.68	5.29	49.62	-14.43	INRC
SN	*qsn1.1*	1	RM1-RM495				3.13	10.72	-9							INRC
	qsn8.1	8^{a}	RM404-RM547							2.57	43.42	-24.36				INRC
SF	*qsf1.1*	1^{a}	RM243-RM488							2.55	9.81	2.96				IR64
	*qsf4.1**	4	RM518-RM471	3.98	13.62	4.57	3.92	35.45	6.67							IR64
	*qsf4.2**	4	RM471-RM252	3.68	12.64	4.44	3.45	13.06	4.88							IR64
	*qsf8.1**	8	RM404-RM547				2.9	32.41	8.54	4.11	31.07	30.48	4.37	39.67	14.71	IR64
GW	*qgw8.1*	8	RM38-RM331				2.74	44.4	-5.74							INRC
BM	*qbm8.1**	8	RM404-RM547				4.12	44.02	-13.05	4.65	31.85	-15.97	2.7	42.56	-12.6	INRC
SPY	*qspy1.1*	1^{a}	RM1-RM495										2.56	9.47	1.55	IR64
	qspy2.1	2^{a}	RM262-RM5897	2.5	7.84	-1.85										INRC
	qspy9.1	9	RM434-RM242							3.2	10.67	-1.24				INRC
HI	*qhi2.1*	2^{a}	RM154-RM318							3.47	48.69	-3.76				INRC
	qhi2.2	2^{a}	RM318-RM263							2.93	46.97	-3.69				INRC
	qhi3.1	3^{a}	RM168-RM570							2.58	23.84	2.63				IR64
	qhi7.1	7	RM154-RM319				4.06	14.19	-2.59							INRC
	qhi7.2	7	RM22171-RM5752				4.11	14.07	-2.58							INRC
	qhi8.1	8	RM38-RM331	2.68	21.34	6.54										IR64

Location I- Hyderabad; Location II- Maruteru. N1 – N2 refer Nitrogen level 1 (100kg/ha^{-1}) and Nitrogen level 2 (150kg/ha^{-1}) respectively. For trait names refer table4. Chr, refers to Chromosome. LOD, refers to Maximum likely hood ratio of odds i.e LOD score for the QTL. PVE, Phenotypic variance explained by each QTL; a0, Additive effect. Al.ef., Allele effect caused by parents towards QTL (+ = IR64; - = . INRC=INRC10192); [a]Significant only in composite interval mapping.; [b]Significant only in simple interval mapping. *Stable QTLs expressed across N treatment/environment. Individual QTL are designated with italicized abbreviation of the trait and the chromosome number. When more than one QTL affecting a trait is identified on the same chromosome, they are distinguished by decimal numbers.

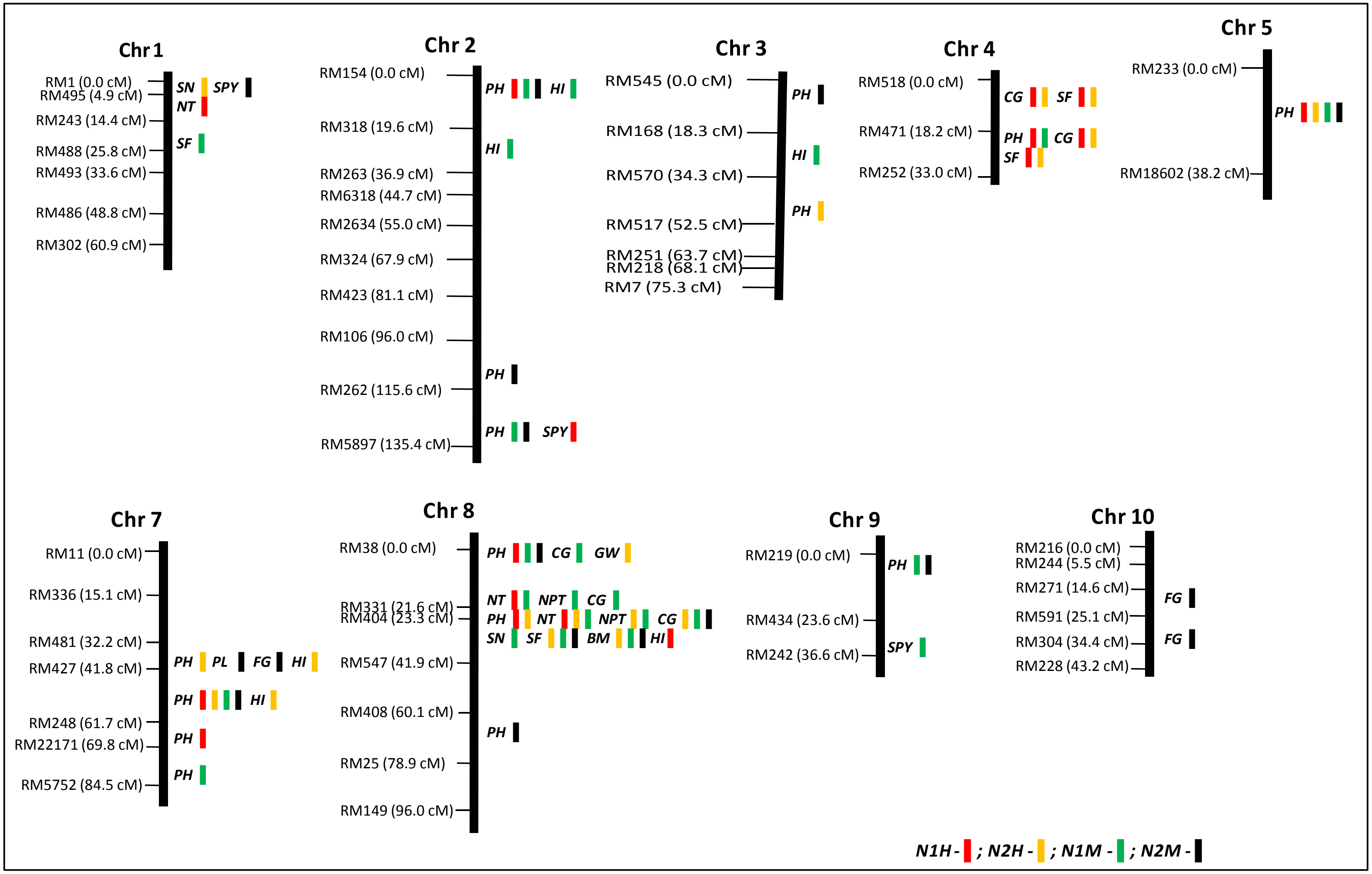

Fig. 13 Distribution of QTLs for yield and its components in the molecular linkage map of IR64/INRC10192. QTLs are indicated right side of the linkage map. Names of the markers represented left side of the linkage map. Numbers in parenthesis are relative genetic distances from one end of the chromosomes in centi morgans (cM).

***Plant height (PH)*:** A total of 15 QTLs distributed over seven chromosomes showed significant association with PH. Of these five QTLs *viz*., *qph2.1*, *qph4.1*, *qph5.1*, *qph7.2* and *qph8.1* were identified at both the locations, with major effect. Out of these two QTLs *viz*., *qph5.1* and *qph7.2* were detected in both N-levels and across both the locations with high LOD score range of 5.85-14.74 (Fig 14), having relatively high phenotypic variation ranging from 62.12 to 72.6%. The IR64 alleles contributed to the QTLs, *qph2.1*, *qph4.1* and *qph7.2*, while INRC10192 alleles to *qph5.1* and *qph8.1* for increased plant height.

Three QTLs *viz*., detected *qph8.2* at Hyderabad; and *qph2.3* and *qph9.1* detected at Maruteru, were found to be of major effect under both the N-levels. The LOD score of them ranged between 3.76 and 10.83 with the phenotypic variance ranging from 59.33 to 79.69%. The allele effect for *qph2.3* and *qph9.1* was from INRC10192, while that for *qph8.2* from IR64.

The remaining seven QTLs; *qph2.2*, *qph3.1*, *qph3.2*, *qph7.1*, *qph7.3*, *qph7.4*, and *qph8.3* were identified with minor effect in one N-level and one location only. These QTLs showed LOD score ranging from 2.58 to 5.5 explaining 16.93 to 70.42% phenotypic variation. Except *qph2.2* and *qph3.2,* for the remaining five QTLs, INRC10192 contributed increased allele effect.

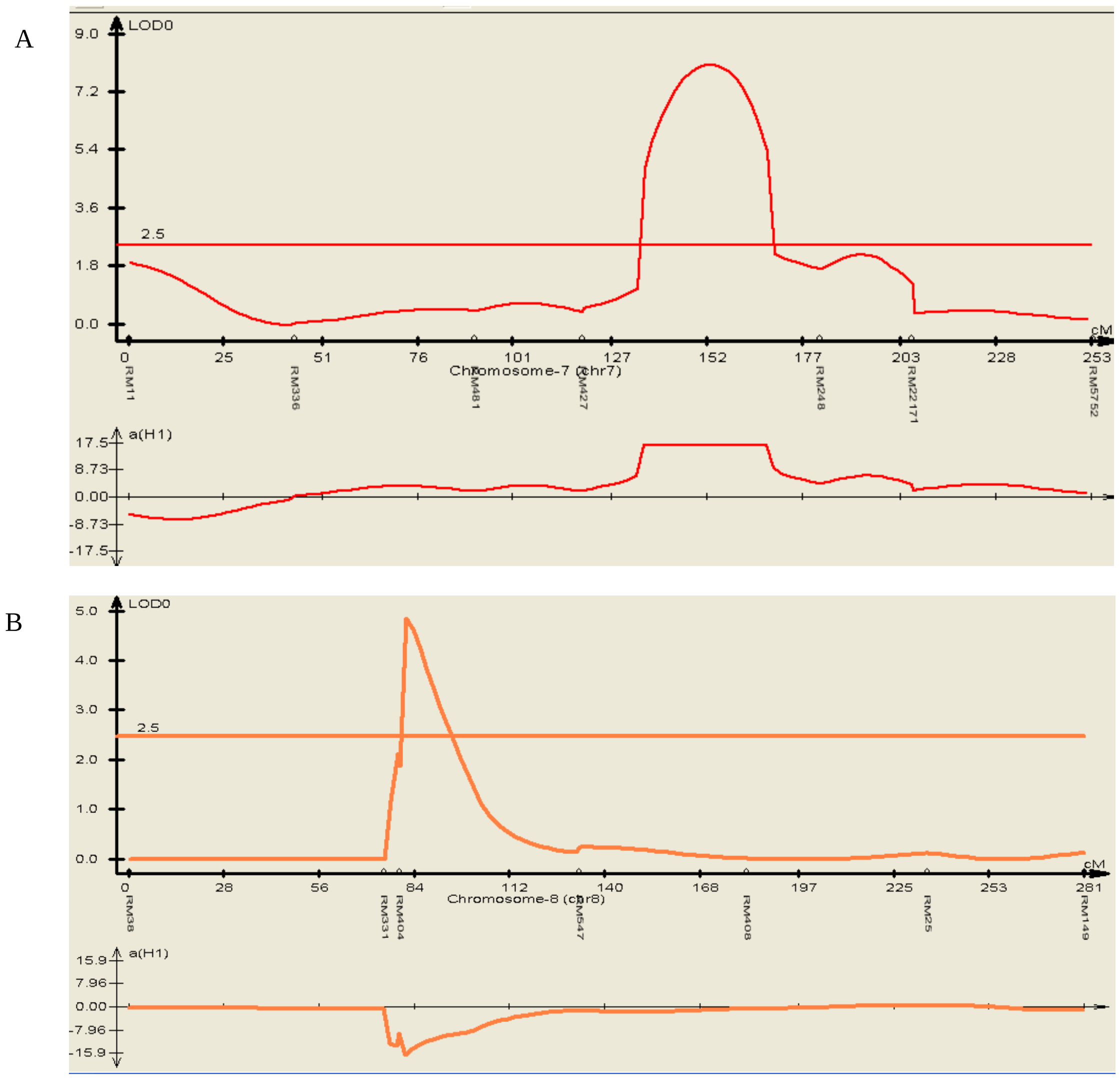

Fig. 14 QTL cartographer LOD peak for plant height (A) and Biomass (B)

Number of tillers (NT): Three QTLs mapped on two chromosomes were found associated with NT. Two QTLs, *qnt8.1* and *qnt8.2* were identified with main effect at both the locations under N-level 1 and *qnt8.2* still in N-level 2 at Maruteru. These QTLs showed LOD values ranging from 3.65 to 6.13 and had high phenotypic variance, ranged between 31.15 and 39.98%. Another QTL *qnt1.1* was identified on chromosome 1 with minor effect under N-level 1 at Hyderabad with LOD score of 2.86 and phenotypic variance explained by this QTL was 12.37%. The favorable allele effect of all three QTLs was from INRC10192.

Number of productive tillers (NPT): Two QTLs on chromosome 8 were significantly associated with NPT. One QTL, *qnpt8.2* mapped between the markers RM404 and RM547 with main effect and the other one *qnpt8.1* mapped between the markers RM331-RM404 were detected as QTL with miner effect. The main effect QTL, *qnpt8.2* was identified in N-level 2 of Hyderabad and N-level 1 of Maruteru with LOD score and phenotypic variance of 2.88, 29.78% and 4.9, 29.56%, respectively; while the minor effect QTL was identified under treatment N1 of Maruteru with LOD score of 3.61 and phenotypic variation of 25.61%. The allele effect of both the QTLs was from INRC10192.

Panicle length (PL): Only one QTL *qpl7.1* was identified for PL between the markers RM481 and RM427 in N-level 2 of Maruteru with minor effect with the LOD score of 2.51. Phenotypic variance explained by this QTL was 12.77%. The allele effect was contributed by IR64.

Fertile grains (FG): Three QTLs *viz*., *qfg7.1, qfg10.1* and *qfg10.2* were identified on chromosomes 7 and 10 with minor effect in N-level 2 of Maruteru between markers RM427-RM248 and RM271-RM304, respectively. These QTLs had LOD score values ranging from 2.51 to 3 and phenotypic variance explained by individual QTL ranged from 9.67 to 14.38%. The increased allele effect of all the QTLs came from IR64.

Chaffy grains (CG): Five QTLs on two chromosomes were associated with CG. The QTL *qcg8.3* was detected at both the locations with main effect. It showed LOD score to range between 3.99 and 8.79 and phenotypic variance explained by the QTL ranging from 39.04 to 49.62%. Two QTLs, *qcg4.1* and *qcg4.2* were detected with main effect under both the N-levels of Hyderabad. They had LOD score and phenotypic variance range of 2.72-5.32, and 34.34-50.62%, respectively. The remaining two QTLs *qcg8.1* and *qcg8.3* were detected only under N-level 1 of Maruteru with minor effect, with LOD score of 2.72 and 7.71 and phenotypic variances explained individually by these QTLs were 34.34% and 35.99%, respectively. INRC10192 contributed the allele effect for all the five QTLs.

Spikelet number per panicle (SN): Two QTLs on chromosome 1 and 8 were identified for SN. The QTL *qsn1.1* with LOD score value of 3.13 was identified in N-level 2 of Hyderabad, while *qsn8.1* (LOD score value-2.57), was identified in N-level 1 of Maruteru with marker interval of RM1-RM495 and RM404-RM547, respectively and both the QTLs had clear minor effect. The phenotypic variation explained by *qsn1.1* was 10.72% and of *qsn8.1* was 43.42%. For both the QTLs the favorable allele effect was from INRC10192.

Spikelet fertility (SF): Four QTLs on three chromosomes were identified for SF. The QTL *qsf1.1* was detected with minor effect in N-level 1 of Maruteru between markers RM243 and RM488. Two QTLs, *qsf4.1* and *qsf4.2* on chromosome 4 were detected in both N-levels (N1 and N2) of Hyderabad, while the QTL *qsf8.1* was found at both the locations as main effect QTL in marker interval of RM404-RM547. These QTLs had LOD score values ranging from 2.55 to 4.37 and phenotypic variance explained by individual QTL ranged from 9.81 to 39.67%. The increased allele effect of all the QTLs came from IR64.

***Grain weight (GW)*:** Only one QTL *qgw8.1* was identified between markers RM38 and RM331 on chromosome 8 with minor effect in N-level 2 of Hyderabad. It had the LOD score value of 2.74 and phenotypic variance explained by it was 44.4%. The increased allele effect was associated with INRC10192.

***Biomass (BM)*:** Single QTL, *qbm8.1* on chromosome 8 was found associated with BM with main effect. This was identified in N-level 2 of Hyderabad (LOD = 4.12, PVE= 44.02%; Fig 14); N-level 1 (LOD=4.65 and PVE= 31.85%) and N-level 2 (LOD= 2.7 and PVE= 42.56%) of Maruteru. This had high LOD and PVE under N2 of Hyderabad and N1 of Maruteru. The allele effect of *qbm8.1* was contributed by INRC10192.

***Single plant yield (SPY)*:** Two QTLs on two different chromosomes were associated with SPY. But none was detected with main effect. One QTL *qspy1.1* was identified on chromosome 1 between markers RM1and RM495 with LOD score of 2.56 in N-level 2 of Maruteru. Phenotypic variance explained by this QTL was 9.47%

and the increased allele effect was contributed by IR64, while the other QTL *qspy2.1* identified on chromosome 2 was between RM262 and RM5897 with minor effect. The LOD score of this QTL was 2.5 and phenotypic variance explained by it was 7.84%. The effect of *qspy2.1* came from INRC10192.

***Harvest index (HI)*:** In all six minor effect QTLs were identified for HI on four chromosomes. The QTLs *qhi2.1*, *qhi2.2* and *qhi3.1* were identified under N-level 1 of Maruteru and *qhi7.1* and *qhi7.2* in N-level 2 of Hyderabad, while *qhi8.1* in N-level 1 of Hyderabad. The LOD score value and phenotypic variance explained by individual QTL ranged from 2.58 to 4.11 and from 14.07 to 48.69%, respectively. Two QTLs *qhi7.1* (LOD= 4.06) and *qhi7.2* (LOD= 4.11) had high LOD scores with phenotypic variance of 14.19% and 14.07%, respectively. In contrast, *qhi2.1* and *qhi2.2* had high phenotypic variance of 48.69% and 49.97%, respectively. All the QTLs, except *qhi8.1* and *qhi3.1*, showed favorable allele effect from INRC10192.

Drought response

A total of 68 QTLs were detected for the 23 traits across control and stress treatments under field and greenhouse conditions (Table 18 and Fig. 15). Six QTLs were identified over two treatments, while the remaining 48 QTLs were detected either in control or stress treatment. The QTLs have been categorized as (i) those identified over the two treatments, QTLs with “major/main effect” and (ii) those detected in either of the treatment QTLs with “minor effect”.

Table 18 QTLs for grain yield and its components and secondary traits detected in 140 RILs of the cross IR64 and INRC10192 under field and greenhouse conditions

				Stress				Control			
Trait	QTL	Chr	Marker interval	LOD	PVE	a0	Al.ef.	LOD	PVE	a0	Al.ef.
Field condition											
PH	*qph1.1*	1^b	RM493-RM486	4.15	71.25	16.1	IR64				
	*qph5.1**	5	RM233-RM18602	5.05	59.62	-15.04	INRC	3.81	70.29	16.51	IR64
	*qph7.1**	7	RM427-RM248	5.57	59.05	14.85	IR64	3.92	70.8	16.69	IR64
	qph8.1	8	RM38-RM331	6.33	65.55	-15.19	INRC				
	*qph8.2**	8	RM404-RM547	5.05	59.51	15.14	IR64	2.76	69.15	16.8	IR64
	qph9.1	9^b	RM219-RM434					3.16	69.79	16.48	IR64
NPT	*qnpt6.1*	6^b	RM204-RM225	2.98	10.48	1.63	IR64				
	qnpt8.1	8^b	RM38-RM331	2.63	54.75	-4.29	INRC				
PCG	*qpcg1.1*	1^a	RM243-RM488					2.78	19.3	-1.52	INRC
	qpcg1.2	1^b	RM493-RM486	2.57	62.44	-8.18	INRC				
	qpcg7.1	7^a	RM336-RM481	2.75	39.28	5.76	IR64				
	qpcg8.1	8	RM404-RM547	2.83	51.81	-7.99	INRC				
PSF	*qpsf2.1*	2	RM262-RM5897	3.89	61.73	-20.3	INRC				
	qpsf7.1	7^b	RM336-RM481	3.76	68.4	-21.12	INRC				
	qpsf8.1	8^b	RM331-RM404					3.47	29.04	19.45	IR64
	qpsf9.1	9^b	RM219-RM434	3.25	69.97	-21.19	INRC				
SCG	*qscg1.1*	1^a	RM243-RM488	3.12	13.86	-6.28	INRC				
	qscg1.2	1^a	RM488-RM493	3.63	19.54	-7.52	INRC				
	qscg4.1	4^a	RM471-RM252	2.73	8.42	5.12	IR64				
	qscg7.1	7^a	RM481-RM427					2.76	9.73	3.23	IR64
	qscg7.2	7^a	RM427-RM248					2.8	9.62	3.22	IR64
	qscg8.1	8^b	RM38-RM331	3.95	61.67	-16.35	INRC				
	*qscg8.2**	8^b	RM404-RM547	3.36	61.35	-16.3	INRC	2.9	40.42	-8.38	INRC
	qscg10.1	10^a	RM271-RM591					3.86	13.1	3.72	IR64
	qscg10.2	10^a	RM591-RM304					3.95	12.84	3.69	IR64
	qssf2.2	2^a	RM423-RM106	2.58	58.92	-18.42	INRC				
	qssf2.3	2^b	RM262-RM5897	2.92	67.82	-19.47	INRC				
	qssf5.1	5	RM233-RM18602	2.97	57.75	-18.27	INRC				
	qssf8.1	8^a	RM38-RM331	3.27	58.05	18.47	IR64				
	qssf8.2	8^b	RM404-RM547					2.85	54.35	11.32	IR64
	qssf9.1	9	RM219-RM434	3.37	57.84	-18.23	INRC				

Table continues in the following page.

Table 18 *continued from previous page.*

				Stress				Control			
Trait	QTL	Chr	Marker interval	LOD	PVE	a0	Al.ef.	LOD	PVE	a0	Al.ef.
SGW	*qsgw2.1*	2^a	RM262-RM5897	2.63	8.33	-0.95	INRC				
BM	*qbm7.1*	7^a	RM481-RM427	2.78	9.85	4.17	IR64				
	qbm7.2	7^a	RM427-RM248	2.78	9.49	4.1	IR64				
	qbm8.1	8^b	RM38-RM331	3.32	62.94	-10.82	INRC				
	qbm10.1	10^a	RM304-RM228	3.2	14.46	4.85	IR64				
SPY	*qspy1.1*	1^b	RM493-RM486					3.82	15.87	4.43	IR64
	qspy1.2	1^b	RM486-RM302					3.83	14.47	4.29	IR64
	qspy8.1	8	RM38-RM331	4.56 (R)	45.95	-91.69	INRC				
HI	*qhi1.1*	1	RM493-RM486					5.17	17.53	4.73	IR64
	qhi1.2	1	RM486-RM302					5.23	17	4.71	IR64
Greenhouse condition											
SL	*qsl1.1*	1	RM1-RM495					4.24	18.4	-1.58	INRC
RL	*qrl3.1*	3^b	RM251-RM218					2.52	11.74	-0.72	INRC
	qrl3.2	3^b	RM218-RM7					2.52	11.51	-0.71	INRC
SDW	*qsdw1.1*	1	RM1-RM495					3.48	13.69	-1.38	INRC
	qsdw2.1	2^a	RM106-RM262	2.63	53.86	-2.42	INRC				
	qsdw2.2	2^a	RM262-RM5897	2.51	53.16	-2.4	INRC				
	qsdw9.1	9^a	RM219-RM434	3.03	53.42	2.39	IR64				
RDW	_qrdw1.1*_	1	RM1-RM495	6.33	32.94	5.26	IR64	5.73 (R)	29.29	53.08	IR64
	qrdw1.2	1^b	RM495-RM243	2.74	17.96	3.49	IR64				
	qrdw7.1	7^a	RM336-RM481					3.89 (R)	13.62	-34.6	INRC
	qrdw8.1	8^b	RM38-RM331	2.91	47.71	-7.87	INRC				
	qrdw8.2*	8^b	RM404-RM547	5.37	51.21	-9.84	INRC	4.16 (R)	28.07	-86.94	INRC
RS	*qrs1.1*	1	RM1-RM495	3.71	25.61	90.77	IR64				
	qrs1.2*	1	RM493-RM486	3.54	47.19	-131.62	INRC	3.32 (R)	19.54	-59.46	INRC
	qrs1.3	1	RM486-RM302					3.72 (R)	24.88	-63.67	INRC
	qrs2.1	2b	RM154-RM319	3.29	62.97	136.93	IR64				
	qrs7.1	7a	RM22171-RM5752					2.71 (R)	23.77	47.79	IR64
	qrs8.1*	8	RM38-RM331	4.92	44.34	-143.33	INRC	3.27	47.64	-55.13	INRC
	qrs8.2*	8	RM404-RM547	5.26	43.89	-148.55	INRC	3.17 (R)	34.64	-86.07	INRC

For trait names refer tables 5 and 6. Chr, refers to Chromosome number. LOD, refers to Maximum likely hood ratio of odds i.e LOD score for the QTL. PVE, Phenotypic variance explained by each QTL. a0, Additive effect. Al.ef., Allele effect caused by parents towards QTL. ^{a}significant only in composite interval mapping. ^{b}significant only in simple interval mapping. *Repeated/Stable QTL across control /stress treatments. Control= irrigated under field or normal half- strength Yoshida's nutrient solution under green house experiment. Stress = drought treated or PEG 6000 contained half- strength Yoshida's nutrient solution under field and green house experiments respectively. (R)= relative parameter.

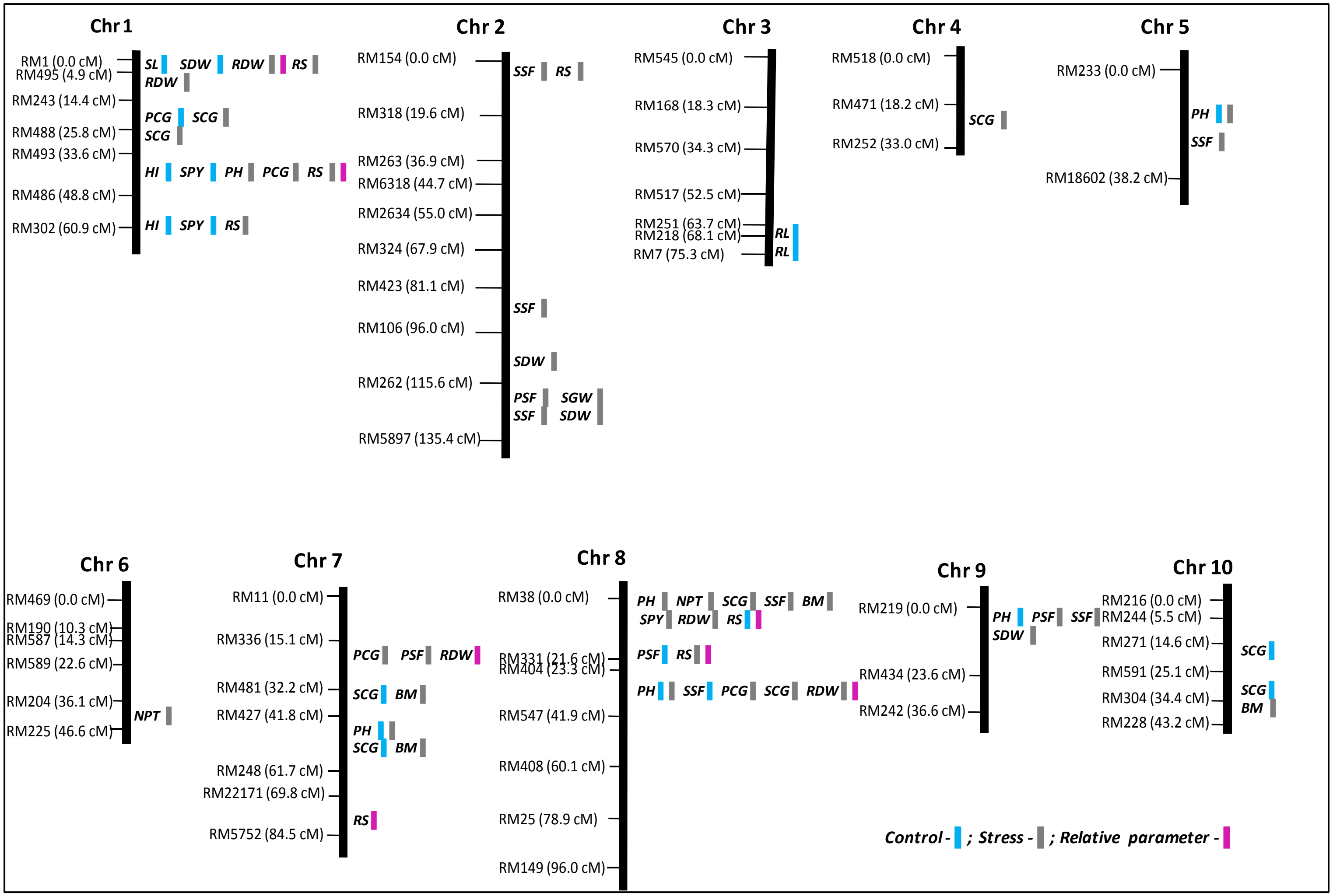

Fig.15 Distribution of QTLs for drought related traits in the molecular linkage map of IR64/INRC10192. QTLs are indicated right side of the linkage map. Names of the markers represented left side of the linkage map. Numbers in parenthesis are relative genetic distances from one end of the chromosomes in centi morgans (cM).

Drought response under field conditions

Plant height (PH): A total of six QTLs on five different chromosomes were identified for PH. Out of which, three *viz*., *qph5.1*, *qph7.1* and *qph8.2* were identified with main effect in both the treatments. These QTLs collectively had LOD scores ranging from 2.76 to 5.57 and phenotypic variation explained by individual QTL ranged between 59.05 and 70.80%. Two QTLs, *qph1.1* and *qph8.1* were identified only under the stress condition, while *qph9.1* was identified in control condition. These had LOD score ranging from 3.16 to 6.33 explaining 65.55 - 71.25% phenotypic variation. For all the QTLs, except *qph5.1*and *qph8.1*under stress, the allele effect was contributed by IR64.

Number of productive tillers (NPT): For NPT, two QTLs were identified under stress treatment. One QTL *qnpt6.1*, located on chromosome 6, explained 10.48% of phenotypic variation with a LOD score of 2.98. The other QTL, *qnpt8.1* detected on chromosome 8, had a LOD score of 2.63 and explained 54.75% of phenotypic variation. The allele effect of *qnpt6.1* and *qnpt8.1* was contributed by IR64 and INRC10192, respectively.

Chaffy grains on primary branches (PCG): Four QTLs namely *qpcg1.1*, *qpcg1.2*, *qpcg7.1* and *qpcg8.1* were identified for PCG on three different chromosomes. Out of these QTLs *qpcg1.2*, *qpcg7.1* and *qpcg8.1* were identified under the stress condition with their LOD score ranging from 2.57 to 2.83, and phenotypic variance explained by individual QTL ranged from 39.28 to 62.44%, while one QTL *qpcg1.1* was identified under control condition on chromosome 1 in the marker interval of RM243-RM488 with LOD score of 2.78 and phenotypic variance explained by this QTL was 19.3%. The allele effect of all the QTLs, except *qpcg7.1*, was contributed by INRC10192.

***Spikelet fertility on primary branches (PSF)*:** Four QTLs on chromosomes 2, 7, 8 and 9 were associated with PSF. Out of them and three QTLs *qpsf2.1*, *qpsf7.1* and *qpsf9.1* were identified under the stress with their LOD score range between 3.25 and 3.89 and phenotypic variance explained by individual QTL ranging from 61.73 to 69.97%. The remaining one QTL *qpsf8.1* was identified under control treatment with LOD score value of 3.47 and phenotypic variance explained by it was 29.04%. The allele effect for all the three chromosomal regions under the stress condition, by INRC10192, while under control condition it was from IR64.

***Chaffy grains on secondary branches (SCG)*:** For SCG, nine QTLs were identified on five chromosomes. Under the stress condition, *qscg1.1*, *qscg1.2*, *qscg4.1*, *qscg8.1* and *qscg8.2* were identified on chromosomes 1, 4, and 8, respectively. While *qscg7.1*, *qscg7.2*, *qscg8.2*, *qscg10.1* and *qscg10.2* on chromosomes 7, 8 and 10 respectively under control condition. The QTL *qscg8.2* between the markers RM404 and RM547 was detected in both the treatments. The LOD score and phenotypic variance explained by individual QTL under the stress condition ranged from 2.73 to 3.95 and from 8.42 to 61.67%, respectively; while under control condition the values ranged from 2.76 to 3.95 and from 9.62 to 40.42%, respectively. The allele contribution of the QTLs on chromosome 1 and 8 were from INRC10192, while for the remaining QTLs it was from IR64.

Spikelet fertility on secondary branches (SSF): A total of seven QTLs identified on four chromosomes for SSF revealed to be of minor effect. Out of them, six namely *qssf2.1, qssf2.2, qssf2.3, qssf5.1, qssf8.1* and *qssf9.1* were identified under the stress condition. LOD score of these QTLs ranged from 2.58 to 3.37 and phenotypic variance explained by individual QTL ranged between 56.64 to 67.82%. All the QTLs, except *qssf8.1,* which had positive allele effect coming from IR64, had positive allele effect contributed by INRC10192. One QTL *qssf8.2* was identified under control condition with LOD score value of 2.85 and phenotypic variance explained by it was 54.35%. The increased allele effect (11.32%) was contributed by IR64.

Grain weight on secondary branches (SGW): A single QTL designated as *qsgw2.1*, was identified on chromosome 2 under the stress condition. This was located in the marker interval of RM262-RM5897 had a LOD score of 2.63. It explained phenotypic variance of 8.33% and INRC10192 alleles contributed favorable effect at this locus.

Biomass (BM): For BM, four QTLs were detected under the stress conditions. All these QTLs were localized in different chromosomal locations, except for two QTLs on chromosome 7 designated as *qbm7.1* and *qbm7.2,* which were found on the same chromosomal regions flanked by markers RM481-RM427 and RM427-RM248, respectively. Other two QTLs, *qbm8.1* and *qbm10.1* were located respectively on chromosomes 8 and 10. The LOD scores of these QTLs were 2.78-3.20 with phenotypic variation explained by individual QTL being 9.49, 62.94%. IR64 alleles contributed to higher BM for three QTLs *viz*., *qbm7.1*, *qbm7.2* and *qbm10.1*, while for the QTL *qbm8.1* the allelic contribution was from INRC10192.

Single plant yield (SPY): Three QTLs located on chromosomes 1 and 8 were identified for SPY and designated as *qspy1.1*, *qspy1.2* and *qspy8.1* flanked by the markers RM493-RM486, RM486-RM302 and RM38-RM331, respectively. All the three QTLs were of minor effect. Two QTLs, *qspy1.1* and *qspy1.2* were identified under control condition, while *qspy8.1* under the stress condition. IR64 alleles of the QTLs on chromosome 1 increased SPY in irrigated condition, while INRC10192 alleles of the QTL, *qspy8.1* increased SPY under severe drought stress. The LOD score and phenotypic variation explained by *qspy8.1,* for the trait under drought was 4.56 and 45.97%, respectively; whereas under control conditions the QTLs, *qspy1.1* and *qspy1.2* had LOD and phenotypic variance of 3.82 and 3.83; 15.87% and 14.47%, respectively.

Harvest index (HI): Two QTLs *viz*., *qhi1.1* and *qhi1.2* for HI were mapped to chromosome 1 under control conditions. The LOD score and phenotypic variance of *qhi1.1* and *qhi1.2* were 5.17, 17.53% and 5.23, 17%, respectively. The alleles of IR64 contributed for increased HI at these QTLs.

Drought response under Greenhouse condition

***Shoot length (SL)*:** A single QTL designated as *qsl1.1* on chromosome 1 was identified for the trait under control condition. It was located in the marker interval of RM1-RM495 with a LOD score of 4.24, and explained 18.4% of phenotypic variance. The allele effect was contributed by INRC10192 (Table 18 Fig. 15).

***Root length (RL)*:** Two QTLs of minor effect influencing RL on chromosome 3 were identified only under control condition. They had same LOD value of 2.52 and phenotypic variance explained by the QTLs, *qrl3.1* and *qrl3.2* was 11.74% and11.51%, respectively. The increased allele effect of these QTLs was contributed by INRC10192.

***Shoot dry weight (SDW)*:** For SDW, in all four QTLs were identified, of which three *viz*., *qsdw2.1*, *qsdw2.2* and *qsdw9.1* were identified on chromosomes 2 and 9 under stress condition. The LOD score and phenotypic variance explained by individual QTL ranged from 2.51 to 3.03, and 53.16 to 53.86%, respectively. One QTL, *qsdw1.1* was identified on chromosome 1 in the marker interval of RM1-RM495 with a LOD score of 3.48 and phenotypic variance of 13.69%. INRC10192 had allele effect for *qsdw1.1*, *qsdw2.1* and *qsdw2.2*, while IR64 contributed at *qsdw9.1* locus.

***Root dry weight (RDW)*:** A total of five putative QTLs for RDW were identified on three chromosomes under stress condition and for relative parameter measured under stress condition. Four QTLs, *qrdw1.1*, *qrdw1.2*, *qrdw8.1* and *qrdw8.2* mapped to chromosomes 1 and 8, explained phenotypic variation ranging from 17.96 to 51.21% and LOD score in the range of 2.74-6.33. Three QTLs *qrdw1.1*, *qrdw7.1* and *qrdw8.2* were identified for relative root dry weight with LOD score of 3.89-5.79 and phenotypic variation explaining 13.62-29.29%. Of these, two QTLs *viz*., *qrdw1.1* and *qrdw8.2*, were identified simultaneously with main effect for RDW under stress and for relative root dry weight measured under stress. IR64 contributed positive allele effect for *qrdw1.1* and *qrdw1.2;* while, for the remaining three QTLs, INRC10192 contributed favorable allele effect.

***Root to shoot dry weight ratio (RS)*:** A total of seven QTLs were found associated with RS. Of them, five namely *qrs1.1*, *qrs1.2*, *qrs2.1*, *qrs8.1* and *qrs8.2* were detected under the stress condition, while *qrs1.2* and *qrs8.2* were found as well for relative R S measured under the stress conditions. Further *qrs1.3* and *qrs7.1* were also found for relative R S under the stress. Besides under the stress, the QTL *qrs8.1* was as well detected under control condition with main effect. All these QTLs collectively showed LOD score in the range of 2.71- 5.76 and explained phenotypic variance ranging from 19.54 to 62.97%. INRC10192 alleles contributed the favorable effect at all the loci except *qrs1.1*, *qrs2.1* and *qrs7.1*.

Discussion

5 DISCUSSION

Despite enviable record of production advance made possible through development and extensive adoption of high yielding varieties of rice during the last four decades culminating is self-sufficiency in food and rice, the declining productivity-production growth trends since last 10 years have pushed the country to a food insecure position. To sustain the growing need, according to economists, rice production has to grow at around 1.8% annually to meet the demand projection of 130 million tons of milled rice by 2025. With limited scope for horizontal growth, the target has to be achieved only through steady increase in productivity level. Hybrid technology with yield advantage of about 15% if widely adopted as happened in China would enable along with HYVs achieve targets of at least two decades from now. The challenge is in developing technologies capable of breaching yield levels of HYVs and hybrids. Of various strategies being contemplated towards this goal, finding new and still not exploited yield related variability from wild/weedy species and primitive landraces and defending so gained yield advantages by guarding against stresses are important. The fact that not even 15% of the variability available in the germplasm and less than 2.0% of the variability available in wild/weedy gene pool has only been utilized in the crop improvement has necessitated going back to such gene pools in search of still not exploited genetic variability. This strategy is on the belief that in the course of origin, domestication and further improvement of rice cultivars not all the available variability had been captured and thus the prospects of identifying and using such variability remaining hidden in the putative ancestor species and cultivars. The proof of the concept has already been demonstrated in rice by identifying yield influencing gene sequences by QTL approach (Xiao *et al*., 1998; Marri *et al*., 2005).

Consolidation of the potential yield is one immediate means to maximize the yield level. In rice, gap between the achievable and actual farmer level yields of HYVs has been estimated to range between 35-65%. Major factors that contribute to the yield gap are biotic and abiotic stresses. Moisture stress is the major yield depressing abiotic factor. Insulation of HYVs with desired level of tolerance to moisture stress would ensure harvest the maximum of the achievable yield like yield. All efforts to develop using known sources of tolerance has been only partially successful. As in the case of yield, hidden variability might be available for such stresses, given the reported influences of finding tolerance to drought and salinity in the progeny of crosses involving parents not-known to be tolerant by QTL approach (Li *et al*., 2005; Ali *et al*., 2006).

Keeping the foregoing in view the present investigation was undertaken with the objective of identifying stable and still not known yield and drought tolerance genes in the advanced generations of the cross between the high yielding variety IR64 and the low yielding primitive landrace INRC10192 not-known to be tolerant to moisture stress by QTL approach. Findings from the following two separate experiments in this regard conducted are discussed as under:

A. Identification of stable yield enhancing QTLs
B. Search for hidden drought response QTLs

A. Identification of stable yield enhancing QTLs

In breeding for quantitatively inherited traits, reliability and success of selection would depend on minimizing the environmental influence in character expression. Conventionally it is achieved by raising and studying experimental material over locations, seasons and growing environments. In the present investigation, accordingly mapping population were screened for phenotypic and genotyping for yield and its components was carried out under two levels of N-fertilizer *i.e.*, ordinary N-level (N1) and high N-level (N2) and at two locations.

Choice of prospective donor (landrace) parent for new QTLs

From a set of 40 landraces, INRC10192 (landrace) was identified as prospective donor parent based on the genetic divergence *vis-a-vis* a HYV and relative response to higher doses of N fertilizer under propped conditions (Hariprasad, 2003). The propping which protects the plants from lodging under high N levels enables to assess inherent genetic yield potential of tall landraces. The findings have indicated the chosen landrace to be genetically distant enough from modern varieties like Jaya and IR64 and likelihood of fertilizer responsiveness of the landrace suggested the prospects of INRC10192 carrying new variability that could be made use of for progressive yield improvement.

Choice of mapping population and phenotyping

Mapping population comprising 140 RILs in F_7 generation developed from the inter-*indica* combination involving the landrace INRC10192 and IR64 has been raised at two locations under two levels of N in the present study. For exploiting QTL approach in crop improvement stability of QTLs are important as polygenic traits governed by QTLs are influenced by environment. Stable QTLs can be identified only by evaluating the RILs over diverse environments. In precisely mapping quantitatively inherited traits, choice of mapping population is important. Advanced generations especially recombinant inbred lines (RILs) and near isogenic lines (NILs) represent highly homozygous lines. Theoretically each line is nearly homozygous at every locus and hence, the variation in the RIL population can be immortalized, by simple selfing for mapping of different traits by different people and at different times (Li *et al.*, 2005).

Phenotyping

Reduced significance of difference between parents in N2 for characters like chaffy grains and spikelet number per plant at Hyderabad, and for fertile grains, spikelet number, percentage spikelet fertility, single plant yield and harvest index at Maruteru, as compared to in N1 level, has revealed the landrace (INRC10192) to show higher response to N fertilization than to IR64. Phenotypic values of RILs for most of the traits had wider range in N2 level as compared to the range in N1 level. Grain yields of RILs are lower in Maruteru than Hyderabad with mean yield reduction of the population being 19.62% and 19.71%, respectively under N1 and N2 levels.

Transgressive segregants were observed for all the traits in positive and negative directions, indicating that neither of the parents carried all positive or all negative alleles. Occurrence of such transgressive segregants is possibly due to accumulation of complementary alleles from the parents at multiple loci in certain RILs (Tanksley, 1993) and G x G interactions (epistasis) (Lanceras *et al.*, 2004). Yield and all its components studied displayed continuous variation in the RIL population at both the locations, suggesting all of them to be of polygenic inheritance (Figs 8 and 9). As expected distribution of percentage chaffy grains and percentage spikelet fertility skewed towards the lowest and highest values respectively. However, plant height though a quantitative trait showed bimodal distribution at both the locations, in conformity with many of earlier reports on the pattern of segregation in crosses involving tall and dwarf (*DGWG*) parents. The pattern reflecting monogenic Mendelian inheritance could be attributed to the role of a major gene like *sd1* in association with more genes tightly linked as a complex locus.

Correlations between trait pairs was by and large on the expected nature across locations, though the strength was less or more in respect of some trait pairs at Hyderabad and Maruteru, suggesting that expression of some genetic components might vary with the location/growing environment.

Construction of molecular linkage map

The tremendous segregation and normal distribution observed in respect of most of the traits in the RILs was indicative that the mapping population had sufficient amount of genetic variation for mapping trait specific QTLs. For mapping QTLs, a genetic framework map was constructed employing 140 RILs and 63 markers. Parental polymorphism of 32.28% observed in the present study; suggest a wide diversity between the landrace, INRC10192 and IR64. The linkage map (LOD-score ≥3.0) places 63 markers on all the 12 linkage groups of the rice genome, spanning a total map length of 1978.9cM with average distance of 31.41cM between markers using the kosambi mapping function (SI Fig 1).

This intra-sub specific (*indica/indica*) map (1978.9cM) is longer than some of the notable maps constructed using inter-sub specific populations, such as 1576 cM (Redona and Mackill, 1998), 1820 cM (Xiong *et al*., 1999) and 1239 cM (Bress-Patry *et al*., 2001) and shorter than 2027 cM (Lu *et al*., 1997), 3058 cM (Maheswaran *et al*., 1997), 1984 cM (Cho *et al*., 1998) and 2344.4 cM (Hariprasad, 2003). Higher average genetic distance between markers in the present study could be attributed to two reasons *viz.,* (i) higher homology between DNA strands in the intra-sub specific (*indica/indica*) population as compared to those in the inter-sub specific populations given the observations of Causse *et al*. (1994), who had reported about 25% reduction in genetic distances in an inter-specific population as compared to that in an inter-sub specific population and (ii) stretching effect of markers on chromosomes contributing to increased map length. Due to stretching effect, total map length has been reported to have increased in rice (Maheswaran *et al*., 1997), sorghum (Biovin *et al*., 1998) and barley (Becker *et al*., 1995). Employment of small population size and more number of segregation distorted markers (33.33%) could be other probable reasons for the stretching effect (Subudhi and Huang, 1999).

The linear order and genetic distances between microsatellite markers reported in the present genetic map are not in total agreement with that of the high-density linkage map of Temnykh *et al* (2001). Such discrepancies is most likely to occur on account of different parental strains involved, number of markers, differences in the size and type of mapping populations employed and levels of polymorphism. Nevertheless, for exploratory mapping, the resolution and genome coverage of the present linkage map may be adequate at least for some of the chromosomes to detect QTLs for major yield related traits.

Marker segregation

Significant distorted segregation - deviation of genotypic frequencies from expected Mendelian ratio (1:1 in RILs), was observed for 21 (33.33%) marker loci. Of these, 16 marker loci were skewed towards IR64 alleles and five loci towards the INRC10192 alleles, resulting in an allele frequency of 58.73% (IR64) to 41.27% (INRC10192) (SI Table 6). The genetic basis of such skewed segregation may be due to either selective abortion of male or female gametes, or selective gametic mating

(Xu *et al*., 1997).The segregation distortion was seen on all the chromosomes, except chromosome 5, suggesting that the distortion was random and not confined to any specific part of the rice genome as opined by Xu *et al*. (1995). Further, these results are in agreement with earlier reports of Amarawathi *et al*. (2008), wherein segregation distortion observed on as many as eight chromosomes.

Segregation distortion is a common feature of inter-specific (Xiong *et al.,* 1999; Moncada *et al*., 2001; Brondany *et al*., 2002; Septiningsih *et al*., 2003) as well as inter-subspecific crosses in rice (Lu *et al.,* 1996; Xu *et al.,* 1995; Mc Couch *et al.,* 1988). Comparison of the present results with earlier reports reveal that the percentage of skewed markers was lower compared to the reports of Moncada *et al.,* 2001 (37.6%) and Thompson *et al.,* 2003 (42.5%) and higher than that of Septiningsih *et al*., 2003 (21.4%), Marri *et al*., 2005 (28.5%) and Yoon *et al*., 2006 (10.2%). This suggests that percentage polymorphism is relative and depends on parental combinations.

Most of the skewed loci found in the present study are located on the same or nearby regions as those reported by Aluko *et al.* (2004); Marri *et al*. (2005) and Yoon *et al*. (2006). The skewness towards landrace may be due to reduced recombination and linkage drag in some regions of distantly related parents (Causse *et al*., 1994, Grandillo *et al*., 1996 and Marri *et al*., 2005). The segregation distortion of the markers RM1, RM218, RM251, RM427, RM17 and RM235 appears presumably to be due to their proximity to the gamete abortive genes (Table 19), whereas RM471 is close to the sterility gene *s-c-2*. In contrast, two markers RM204 and RM427 that shows no segregation distortion are nearer to both the types of genes. Further, the segregation distortion of RM324, RM25, RM331, RM404 and RM271 might be due to their close presence to centromere region.

Table 19 Markers associated with gamete abortive and/or sterility genes

Marker	Chr.	Gamete abortive/ Sterility gene	Reference
RM1	1	ga-9	Kinoshita (1991, 1993)
RM218	3	ga-2	Nakagahra (1972)
RM251	3	ga-3	Nakagahra (1986)
RM204	6	ga-4, s-1	Kinoshita (1991, 1993)
RM471	7	s-c-2	Kinoshita (1991, 1993)
RM427	7	ga-11, s-7	Kinoshita (1991, 1993)
RM17 and RM235	12	ga-13	Rha et al. (1995)

QTL mapping

In all 46 QTLs for yield and its components have been identified and localized on the genetic map under two N-levels and at two locations. The LOD and phenotypic variation explained by each of the QTLs range from 2.5 (*qspy2.1*) to 14.74 (*qph7.2*) and from 7.84% (*qspy2.1*) to 77.69% (*qph9.1*), respectively (Table 17 & Fig 13).

Two QTLs *qph5.1* and *qph7.2* have been identified under both normal and high N levels at both the locations with high LOD score (5.85 to 14.74) and high phenotypic variance (62.12% to 72.6%). They would, therefore, tend themselves for possible exploitation using linked markers with high confidence in breeding programme. Of these two, the QTL, *qph7.2* mapped between the markers RM427-RM248 has also been identified and designated as *qPh7b* in the same region for plant height by Hua *et al*. (2006) with largest additive effect under low nitrogen stress, could be the region having genes that respond to N-fertilization. Recently, Onishi *et al*. (2007) have identified a cluster of six QTLs responsible for plant architecture, which include culm length (*qCL7*) and panicle characters like panicle length (*qPL7*) on the short arm of chromosome 7 in the genetic background of inter-specific cross involving *japonica* cultivar and *O. rufipogon*, the wild relative. Interestingly the QTL cluster for plant height (*qph7.2**) (Table 20 & Fig 13) detected in the present study has also been found to occupy the same genomic region reported by Onishi *et al*. (2007). In addition to this, seven out of 15 QTLs relative to plant height identified in this study have been mapped to the short arm of chromosome 7.

Table 20 QTLs identified for yield and its components across N-levels and Locations

N-level & Location	**QTLs identified**								
N1H	*qph7.3*	*qnt1.1*	*qhi2.1*	*qhi8.1*	*qspy2.1*				
N2H	*qph3.2*	*qph7.1*	*qsn1.1*	*qhi7.1*	*qhi7.2*	*qgw8.1*			
N1M	*qph7.4*	*qnpt8.1*	*qcg8.1*	*qcg8.2*	*qsn8.1*	*qsf1.1*	*qhi2.2*	*qhi3.1*	*qspy9.1*
N2M	*qph2.2*	*qph3.1*	*qph8.3*	*qpl7.1*	*qfg7.1*	*qfg10.1*	*qfg10.2*	*qspy1.1*	
N1H,N2H	*qph8.2**	*qcg4.1**	*qcg4.2**	*qsf4.1**	*qsf4.2**				
N1M,N2M	*qph2.3**	*qph9.1**							
N1H,N1M	*qph4.1**	*qnt8.1**							
N2H,N1M	*qnpt8.2**								
N1H,N2H,N1M	*qnt8.2**								
N1H,N1M,N2M	*qph2.1**	*qph8.1**	*qsf8.1**						
N2H,N1M,N2M	*qcg8.3**	*qbm8.1**							
N1H,N2H,N1M,N2M	*qph5.1**	*qph7.2**							

*, stable QTLs; N1= N-level1; N2= N-level 2; H= Hyderabad; M= Maruteru

Very recently, Tan *et al*., (2008) and Jin *et al*. (2008) have independently identified a semi-dominant gene, *PROG 1* (PROSTRATE GROWTH 1) on chromosome 7 between the markers RM427-RM481, that encodes a single Cys_2-His_2 zinc-finger protein, which is defective in *O. sativa*, leading to erect growth, higher grain number and grain yield. Interestingly, in the present study *qph7.1* and *qph7.2* have been found to be associated with these markers. Moreover the QTL, *qph7.2* has been detected across N-levels and locations in the study of yield as well as under stress and control conditions of drought experiment (SI Fig 1). The present study overall tends support to the general conclusion of Tanksley (1993) that a substantial proportion of QTLs affecting a trait particularly those having major effects can be identified in different genetic populations and under different environments.

Tian *et al.* (2006) fine mapped a QTL for grain number per panicle (*gpa7*) to 35kb region that contains five predicted genes. While fine mapping, they found five panicle related traits (length of panicle, primary branches per panicle, secondary branches per panicle and ratio of grains on primary and secondary branches) to be associated with the same marker interval region of RM481-RM427. Interestingly, Onishi *et al*. (2007) have also detected a cluster of six QTLs in this region for plant height, panicle length, primary and secondary branches, grain number on primary and secondary branches. In the present study also each a QTL for panicle length, (*qpl7.1*), filled grains (*qfg7.1*) and harvest index (*qhi*7.1) have been detected exactly in the same region. Possibly the HI QTL might have resulted from higher ratio of secondary branches and ratio of grains on secondary branches per panicle.

Recently, Ashikari *et al.* (2005) have identified a gene underlying the major QTL controlling grain number/panicle on chromosome 1 *i.e*., *Gn1a* encoding cytokinin oxidase/dehydrogenase (*OsCKX2*), an enzyme that degrades phytohormone cytokinin and concluded that reduced expression of *OsCKX2* caused cytokinin accumulation in inflorescence meristems and increased the number of reproductive organs resulting in enhanced grain yield. Pyramiding of the genes for grain number and plant height into the same rice background has been reported by them to result in 26% more grain number per panicle and 18% shorter in plant height. The QTL detected in the present study namely *qsn1.1* and *Gn1a* are not the same suggesting that *qsn1.1* seemed to harbor other candidate gene(s) that control the grain number through mechanism(s) that remain to be elucidated.

A grain weight QTL (*qgw8.1*) has been identified on chromosome 8 at the marker interval of RM38-RM331 in the present study, the grain weight QTL *gw8.1* fine mapped to about 306.4kb (~1.2cM) by Xie *et al*. (2006) is nearer (~9.5cM) to the grain weight QTL detected in the present study. Thus, it may be concluded that the QTL identified in the current study is same as identified by Xie *et al*. (2006). Further, they have reported this QTL to contribute an increase of 9% over NIL (in *japonica* background) and 19.3% more grains than the *japonica* parent

(Hwaseongbyeo). Besides, they report that the increase in grain yield was due to variation in grain shape and specifically grain length.

The QTL, *qspy9.1* identified on chromosome 9 in the present study for single plant yield is likely to be the same locus as the one reported by earlier workers (Hittalmani *et al*., 2003 and Thompson *et al*., 2003). Cho *et al*. (2003) have reported a grain weight QTL (*qgw9.1*) in the same region. Recently, this region has been fine mapped by Xie *et al*. (2008) and dissected into 370.4kb region containing seven predicted genes. They have identified the seven QTLs relating to 1000-grain weight, spikelets per panicle, grains per panicle, panicle length, spikelet density, heading date and plant height to be in a cluster ($p<0.0001$) in this region, suggesting that a single pleiotropic gene might be acting as a major regulator of this QTL cluster. Yield trials with NILs ($BC_3F_{4;}$ Hwaseongbyeo (*japonica*)/ *O.rufipogon*) have revealed the lines containing a homozygous *O.rufipogon* introgression in this region to out-yield NILs containing Hwaseongbyeo DNA by 14.2-17.7% and out-yield Hwaseongbyeo parent by 16.2-23.7%.

Recently, He *et al.* (2006) have fine mapped an yield improving QTL *GY2-1* on chromosome 2, which is 102.9 kb away from the *qspy2.1* identified in the present study and concluded that it had a haplotype of leucine rich repeat receptor kinase gene cluster, which showed an extensive allelic variation between parents, Dongxiong, the wild species, *Oryza rufipogon* Griff. and Guichao2 (*Oryza sativa ssp indica*). Seven QTLs for SPY have been reported by Hariprasad (2003) using the same mapping population in F_2. Of them, only two QTLs *qspy1.1* and *qspy2.1* coincide with what have been detected in the present study. The striking difference indicates the complex nature of yield and G × E interaction for grain yield.

Stable QTLs of promise

QTLs, which are consistently detected over a range of environments are considered to be "Stable QTLs", and are the preferred target loci in crop improvement. According to Wan *et al.* (2005), QTLs with major effects are more likely to behave as stable QTLs across multiple environments. These QTLs, apart from their suitability for improvement of the traits concerned, also serve as potential candidates for fine-mapping. The stable QTLs also facilitate development of near-isogenic lines and advanced breeding lines. Further, several QTLs, each with different environment specificity, can be introgressed into a single genotype to develop phenotypes performing stably over a range of environments. In conventional plant breeding, selections are made in target environment and testing is done in multiple diverse environments. This exercise is cumbersome and time consuming. However, use of stable marker associated QTL based selection can accelerate the pace of breeding-selection process in rice breeding for polygenic traits like yield. Thus QTLs in general particularly those with main effect would be of great value.

Out of the 46 QTLs detected, 18 have been identified as "stable" and 11 of them *viz*., q*ph2.1, qph4.1, qph5.1, qph7.2, qph8.1, qnt8.1, qnt8.2, qnpt8.2, qcg8.3, qsf8.1, and qbm8.1* have been identified consistently at both the locations irrespective of N-level (Table 20). Using such QTLs would ensure wide suitability of the trait concerned over regions akin to Deccan plateau (Hyderabad) and coastal Andhra (Maruteru) under wide range of fertilizer management. The remaining seven stable QTLs (q*ph2.2, qph8.2, qph9.1, qcg4.1, qcg4.2, qsf4.1 and qsf4.2*) have been identified across N-levels in one and the same location, indicating as well to be stable but with relatively narrow adaption confined to one location. These QTLs might be useful for MAS targeting to that specific location. For instance, the QTLs q*ph8.2* and q*sf4.2* can be of value in Hyderabad region, while q*ph2.2* and q*ph9.1* in Maruteru region.

The remaining 28 QTLs are regarded as of minor effect as they have been identified in either of the environments only. According to Yan *et al*. (1998), QTLs detected in one environment but not in another could be considered to be an indication to the level of G × E interaction. However, it has also been shown that QTLs that are stable and consistently detected across environments may still have significant G × E effects.

It will not be out of context to discuss the utility of QTLs of small effect. Those QTLs explaining low percentage phenotypic variance, but consistently detected could as well be of value, considering the fact that larger genes with small effect characteristic to quantitative traits govern yield and major components of it. In harmonious combinations such QTLs would as well help step up genetic yield level, though not in large measure. In the course of present investigation such QTLs (qfg7.1, qpl7.1, qspy2.1 etc) could as well be found for yield and yield related traits.

Comparison of QTLs across the genus *Oryza*

In rice (*Oryza sativa* L.), around 8646 QTLs have so far been identified (V.28, www.gramene.org; September 2008). Comparison of QTL positions detected in the present study with earlier reports across populations and environments allows researchers to develop testable hypotheses about the behavior of genetic factors underlying the putative QTLs.

When comparing QTL results across the genus *Oryza*, 20 of the 46 QTLs identified in the current study have been mapped to similar locations on the chromosomes as reported by earlier workers (Table 21), indicating that genes underlying such QTLs have wide adaptability to different genetic back grounds (populations) and environments. The remaining 26 QTLs appear to be novel and have been identified for the first time. These findings encourage us the prospects of identifying more and more novel and new variability for yield enhancements in new cross combinations especially involving wild/primitive cultivars. The novel QTLs could be good candidates for fine mapping and positional cloning studies, while the QTLs that are mapped to the region consistent with other studies can be right away applied in marker assisted transfer of them into widely adopted high yielding varieties.

Table 21 Comparison of quantitative trait locus (QTL) reported across the *Oryza* genus

S.No.	QTL	Chr	Marker/Interval	QTLs in Previous studies shared common regions
1	*qsn1.1*	1	RM1-RM495	*sp1*(Xiong *et al*., 1999); *SPKNB* (Brondani *et al*., 2002 and Zhuang *et al*., 2002), gn1.1 Septiningsih *et al*. (2003a); spp1.2 (Cho *et al*., 2007)
2	*qspy1.1*	1	RM1-RM495	*yld1.1* (Xiao *et al*., 1998 and Marri *et al*., 2005); GRYLD (Li *et al*., 1997) Xiong *et al*. (1999); *yldp1.1* (Brondani *et al*., 2002), *yldp1.2* (Septiningsih *et al*., 2003), (Zhuang *et al*., 2002); yld1.1 (Cho *et al*., 2007)
3	*qnt1.1*	1	RM495-243	tp1, *NT* (Li *et al*., 2000, 2006); ppp1.1 (Cho *et al*., 2007); *NP-1* (Zhuang *et al*., 2002)
4	*qspy2.1*	2	RM262-RM5897	*yldp2.1* (Brondani *et al*., 2002); *yldp2.1* (Marri *et al*., 2005); *yd2* (Yoon *et al*., 2006)
5	*qhi2.1*	2	RM318-RM263	*hi2.1* (Marri *et al*., 2005)
6	*qph3.1*	3	RM545-RM168	*qPH-3-2* (Li *et al*., 2006)
7	*qph3.2*	3	RM570-RM517	*qPH-3-3* (Li *et al*., 2006)
8	*qph4.1*	4	RM471-RM252	*PTHT* (Li *et al*., 2006)
9	*qsf4.1*	4	RM471-RM252	sf4 (Zhao *et al*., 2008)
10	*qph7.1*	7	RM336-RM481	*qPh7b* (Hua *et al*., 2006)
11	*qph7.2*	7	RM481- RM427	*qPh7b* (Hua *et al*., 2006); qCL7 (Onishi *et al*., 2007)
12	*qpl7.1*	7	RM427-RM248	qpl7 (Onishi *et al*., 2007),pl7 (Tian *et al*., 2006)
13	*qfg7.1*	7	RM427-RM248	qfg7 (Onishi *et al*., 2007), gpa7 (Tian *et al*., 2006)
14	*qhi7.1*	7	RM427-RM248	hi7b (Cho *et al*., 2007)
15	*qbm7.1*	7	RM427-RM248	sy7b (Cho *et al*., 2007)
16	*qph8.3*	8	RM408-RM25	Qph8 (Mei *et al*., 2005); ht8 (Aluko *et al*., 2006); qPH-8 (Li *et al*., 2006)
17	*qph8.2*	8	RM404-RM547	QPh8a (Zhang *et al*., 2006); ph (Zhuang *et al*., 1997; Xiong *et al*., 1999)
18	*qsn8.1*	8	RM404-RM547	QSpp8, QGpp8 (Zhang *et al*., 2006); spp (Lin *et al*., 1996; Xiao *et al*., 1996; Zhuang *et al*., 1997; Xiong *et al*., 1999)
19	*qgw8.1*	8	RM38 - RM331	*gw8.1* (Xie *et al*., 2006)
20	*qspy9.1*	9	RM242-RM205	yld9.1 (Thomson *et al*., 2003 and Marri *et al*., 2005); qYLD9-1 (Hittalmani *et al*., 2003); gy9 (Cho *et al*., 2007); gy9.1 (Zhao *et al*., 2008); yld9.1 (Cho *et al*., 2007)

B. Search for hidden variability for drought tolerance

Drought is the most devastating among the abiotic stresses. Despite large number of QTLs reported to affect response to drought in rice, there have been only a few attempts to introgress drought-tolerance QTLs into high yielding but drought susceptible genotypes (Courtois *et al*., 2003; Price, 2002). Large chromosomal regions bearing putative QTLs associated with root length in a population derived from the cross between the deep-rooted upland cultivar Azucena and shallow-rooted lowland cultivar IR64 have been introgressed into IR64 background. But majority of the lines carrying the desired introgressions have been found to fail to have deeper roots than IR64 (Shen *et al*., 2001). The reasons for lack of the expected effect of the introgressed segments on root length and yield may be that the target QTLs were responsible for relatively a small proportion of the total phenotypic variation in the mapping experiment (5.6–17.7%) or the introgressed region was large, and therefore desirable genes within it could have been lost because of recombination during backcrossing. Azucena root-related QTLs have also been introduced into the *indica* cultivar Kalinga III, but only one of the five target QTLs had the expected effect on root length. Such introgressions have however, not been reported to consistently improve grain yield under stress (Steele *et al*., 2006).

So far most reported drought-related QTL mapping experiments in rice have employed only five populations (Price *et al*., 2002). Many of the upland parents used in these populations (e.g., CT9993-5-10-1-M and Azucena) are not considered to be highly drought-tolerant in terms of grain yield under severe drought conditions. Many traditional and improved cultivars from drought-prone areas have some tolerance to the stress at reproductive-stage. But they have rarely been used as parents in mapping studies. Extensive survey for drought tolerant rice germplasm, currently underway at IRRI, and use of them in the development of mapping population is expected to result in the identification of lines carrying major QTLs/genes improving yield under such stress conditions (Li *et al*., 2005 and Bernier *et al*., 2007).

Keeping foregoing, the present study was initiated with the objective of detecting hidden drought response QTLs in mapping populations in crosses involving primitive landraces, not known to possess drought tolerance as one of the parents, under two water regimes in field and controlled greenhouse conditions. The findings therefrom are discussed as under:

I. Drought response under field conditions

Performance of parents and RILs

The two parents differed significantly in PH, NPT, BM, SPY and HI under normal conditions, while under the stress, differences between them increased to highly significant level nearly in all the traits. Whereas IR64 showed significant differences for PH, SFG, SCG, SSN, SSF, SGW, BM, SPY and HI between the stress and control conditions INRC10192 differed significantly only in PH and SSF, indicating that the former is relatively more susceptible to moisture stress than the latter.

For most of the traits mean values as expected, decreased under stress as compared to control. For instance, IR64 produced higher yield than INRC10192 under saturated condition. But under stress, the grain yield of it decreased dramatically while INRC10192 showed lower decline. In respect of number of panicles, IR64 produced higher number than INRC10192 under both the treatments. However, reduction in NPT under stress was drastic in IR64 (33.34%) as compared to the landrace (20%).

In the RILs, average yield reduction was as high as 57% under stress as compared to control. According to Pantuwan *et al*. (2002) and Bernier *et al*. (2007); yield reduction of more than 50% under stress is necessary to remove the effect of inherent yield potential and facilitating thereby to clearly identify lines that are truly drought resistant. Further, in 13% of the lines, panicles were completely sterile due to severe and long (21days) stress period coinciding with flowering stage.

The RILs showed around 48% lower average harvest index and 7% higher average biomass in drought stressed plots than in irrigated. Studies by Babu *et al*. (2003) reveal that onset of drought stress at flowering stage was the main cause for reduction of HI and accumulation of BM. Under non-stress condition, population performance for PSF, SSF, SPY and HI was 27%, 35%, 57% and 48%, respectively higher than in stress conditions suggesting them to be the highly stress vulnerable characters. However, the population means for PH, SPY, SGW and SSF were higher than IR64 mean under stress, indicating that the population tends to show their drought tolerance than IR64, which is known to be moderately tolerant to drought. The lines, which showed good grain filling (SSF and SGW) on secondary–branches under stress conditions, appear relatively more drought tolerant because the emergence of secondary-branches and grain filling on them coincided with severe stress period unlike, primary branches that emerged earlier and possibly escaped the stress.

Under stress condition, large variation in the performance of traits was observed among the RILs. Transgressive segregation for SPY in both directions indicated both the parents to contribute tolerance alleles under severe drought stress. In all 34 lines yielded less than drought susceptible check IR20 (1.83g/plant), while 9 lines yielded more than the drought tolerant check Moroberekan (18.6g/plant) (SI Table 11). All the traits under both the treatments showed normal distribution, indicating their quantitative nature. As expected, under stress, in respect of percent spikelet fertility, harvest index and single plant yield the distribution was skewed towards the lowest values, while chaffy grains skewed towards highest values.

Trait Correlations

Drought tolerance of the RILs can be assessed by several parameters, namely grain yield (or biomass) under drought, yield under drought as percentage of yield in control (relative- yield), and drought susceptibility index (Fisher and Maurer, 1978). Relative grain yield (or biomass) has been used as the index of drought tolerance in the present study (Ribaut *et al*., 1997; Blum *et al*., 1999; Babu *et al*., 2003). Relative yield [SPY (R)] under stress, though not significant, is negatively correlated with actual yield in the control among the RILs, indicating that the RILs performing best under control conditions showed marked yield reduction under drought as evident from the performance of PFG, PSF, SFG and SSF (Table 14). On the other hand, SPY (R) under stress has been found to be positively and significantly correlated with yield under drought. Thus, absolute yield of the RILs under stress represents quite well their relative drought tolerance. Similar results have been reported in rice by Blum *et al*. (1999) and Babu *et al*. (2003).

Very strong correlations were observed between grain yield and harvest index under stress and decreased correlation with BM as compared to control, indicating yield differences under the stress to be largely due to differences in the inherent capacity of plants to maintain seed set rather than to accumulate biomass. The findings are in agreement with those of Lanceras *et al*. (2004) who have reported decreased and increased correlations of SPY with BM and HI, respectively. They have further concluded that the strength of correlation between HI and GY was directly proportional to the severity of the stress. Nevertheless, it looks that improvement of HI might be the means to improve yield level under stress as well as non-stress condition, correlation of them with grain yield being high enough.

Significant negative correlation of grain yield with PCG and SCG only under stress is due to delay in flowering and reduced panicle elongation rate as opined by Lafitte *et al*. (2004). Further, Boonjung and Fukai (1996) and Jongdee *et al*. (2002) attribute high yield reduction (40%) to increased spikelet sterility when drought occurred during grain filling phase.

Phenotypic correlation between NPT and SPY was lower under drought stress as compared to that under control condition. Moreover, spikelet number on primary-branches showed significant positive correlation with SPY only under control conditions, suggesting that panicle number and spikelet number are not the major factors in grain yield reduction under drought stress. These findings are in conformity with those of Lanceras *et al*. (2004), who also observed decreased correlations of SPY with NPT and SN, when stress level was severe.

Positive significant correlation (0.219*) observed between SPY and SSW was only under stress, indicating that higher grain yield under drought stress could be realized by selecting for SSW directly within the stress environment rather than indirectly under control conditions. Neither RWC nor CC was found correlated with grain yield under both the treatments, indicating greater genetic independence of them as observed as well by Babu *et al*. (2003).

II. Drought response under greenhouse conditions

Plants have different adaptive mechanisms to minimize the adverse effects of stress, which involve different root and shoot characteristics. Study of root related traits is however difficult, time-consuming and usually imprecise under field conditions. In the present study therefore, Yoshida medium containing PEG 6000 was used to impose water deficit stress for understanding the response of root and shoot traits at seedlings stage.

Performance of parents and RILs

Both the parents have shown response to moisture stress induced by PEG with the response of IR64 being significantly low in respect of SL and RL (Table 11) under water deficit stress even though it is known to be moderately tolerant to drought conditions and INRC10192 showing significant difference for RL only under stress. This is in agreement with Cui *et al*. (2008), who have reported water deficit to inhibit shoot growth (Plant height and Shoot fresh weight) while promoting root growth (Maximum root length, Root fresh weight, Root number and Root Shoot ratio) in a hydroponic experiment with a RI population.

The RI population in the present study, as expected, showed decrease in SL and SDW; and increase in RL, RDW and RS under stress conditions in comparison to the response under control condition. The relative parameters of measured traits showed wide variation, ranging far from 1 (1= similar performance under control and stress conditions) in two directions (Table 12). For instance, relative RL ranged from 0.26 to 1.74 while relative RDW from 0.23 to 5.79, suggesting either inhibition or enhancement of root growth by water deficit. Varied levels of response observed among the lines to water deficit could be attributed to their genotype. Only three lines showed higher root length than Moroberekan (10.8 cm; SI Table 11) under stress. Transgressive segregants were observed in both the directions among the measured traits in the RILs (Table 12 & Fig 12).

Trait Correlations

Significant correlations were observed among most of the trait pairs studied, suggesting that the parameters of shoot and root morphology measured in this population to be inter-related. SL, RL, and SDW were positively and significantly correlated with each other under both control and stress conditions. Therefore, indirect selection of one by the other trait is possible in this population. The results are in accordance with the earlier findings by Yadav *et al*. (1995), Gireesha *et al*. (2000) and Mane *et al*. (2003).

Whereas under control condition, RL was positively correlated with RDW under the stress it showed negative correlation with RDW. These results are in agreement with those of Asch *et al*. (2005). On the basis of the quantification of the effects of different levels of drought stress on dry matter partitioning and root development in rice, they concluded that assimilate partitioning between root and shoot, which was not affected under low moisture stress conditions, but did affect under severe stress by drastically decreasing the partitioning to the root.

SL and SDW showed significant positive association with many other traits. Thus, selection based on these two traits could greatly increase the selection efficiency for other traits as well as to result in improved drought tolerance. Since the root trait RL has been found to be significantly positively correlated with SL and SDW under both control and stress conditions selection based on the shoot traits is too easy, because of the difficulty in monitoring root development as compared to shoot, as opined by Mane *et al*. (2003) and Zheng *et al*. (2003).

In the course of the present investigation relative parameters have been used as drought tolerance index as was used by Zheng *et al*. (2003). The relative parameters for different indices under stress showed significant negative associations (though not significant with RS) with their respective characters under control, indicating that the lines well performed under control do not perform so under stress and they have showed marked reduction in their performance under stress. On the contrary the relative parameters measured under stress had strong positive and significant correlation with traits measured under stress. Therefore, absolute performance of RILs under stress represents quite well their relative drought tolerance.

QTL Mapping

In all 68 QTLs were identified for various drought tolerance related traits, which were located throughout the genome except on chromosomes 11 and 12 (Fig 15).

Of them, 46 QTLs relating to yield and its components under control (30) and stress (16) conditions of field experiment have been identified, whereas, 22 QTLs relating to shoot and root related traits under control (8 QTLs) and stress (14 QTLs), detected under greenhouse conditions. In relating the QTLs to tolerance to drought emphasis was given to those that were detected under stress condition for yield *per se*, relative parameters and response of seedling traits under greenhouse conditions. The findings are discussed as under:

Detection of yield related drought tolerance QTLs

Single plant yield and related components

Two QTLs each for HI and SPY collectively explaining 30.34% of phenotypic variance were detected on chromosome 1 in the marker interval RM493-RM302 under control. Allelic contribution at both the QTL locations for increased yield potential might have been derived from IR64. The QTL, *qSPY8.1* on chromosome 8 flanked by the markers RM38 and RM331 was identified only under stress conditions. Allelic contribution from INRC10192 have increased grain yield at this QTL. Thus, this QTL has possibly contributed to the maintenance of SPY under stress condition through the control of complex biochemical and physiological processes associated with drought tolerance. The phenotypic variance explained by this QTL was very high (45.95%) suggesting transgressive segregants for SPY detected under severe stress condition would be of value.

QTLs contributing to increased drought tolerance through ability to maintain HI and BM in rice have been reported earlier by Babu *et al*. (2003) and Lanceras *et al*. (2004). Interestingly, in the present study the biomass QTL *qbm8.1* detected for drought tolerance has been found to be located in the same marker interval (RM38-RM331) where drought specific yield related QTL *qspy8.1.* This result shows that close mapping of these two QTLs might be responsible for the high positive correlation of SPY with BM observed in the present study. The favorable allele effects for SPY and BM have been contributed by INRC10192.

Spikelet fertility (SF) is not a factor contributing to SPY under well-watered conditions. It is however a factor under drought stress. QTLs for SCG and SSF linked with the QTL for SPY under stress indicating that genotypes with high SSF may have low SCG, which is evident from the significant negative correlations of PCG and SCG with yield (Table 14) and between these two characters under drought (SI Table 4). Sheoran and Saini (1996); and Saini and Lalonde (1998) have reported that changes in carbohydrate levels and enzyme activities associated with inhibition of starch accumulation in pollen are potential causes of spikelet sterility (SS), thereby rendering to chaffy grains. SS has also been found to be affected by slower rate of panicle exertion due to water stress (O'Toole and Namuco, 1983; Lanceras *et al*., 2004). In the present study, on chromosome 7 a QTL (*qscg7.1*) for SCG was identified under control conditions in the region between R481-RM427. This is likely to be the same region as earlier reported for QTLs relating to secondary branch number and ratio of grains on secondary branch number by Tian *et al.* (2006) and Onishi *et al*. (2007).

Under severe stress, the location of a QTL for PH (RM38-RM331) has been found to be the same as that of the QTLs for BM, SSF and NPT. This is in agreement with Lanceras *et al*. (2004), who have also identified QTLs in the same position for PH, BY, percent spikelet sterility (PSS) and panicle number (PN) on chromosome 8 under severe stress. The QTL, *qph7.1* identified under both the water regimes in the marker interval of RM427-RM248. Is the same region, Yue *et al*. (2008) reported a QTL for relative plant height (*qRPH7.1*) under stress. One more QTL (*qph9.1*) detected in the present study on chromosome 9 has also been reported by Yue *et al*. (2008) as *qRPH9.1.*

Interestingly, near the plant height QTL *qph1.1* the semi-dwarfing gene *sd1* has been located. The effect of *sd-1* is to reduce the amount of gibberellic acid in the plant through a defect in GA-20-oxidase (Spielmeyer *et al*., 2002). This QTL is the same as identified under both stress and non-stress conditions by Bernier *et al.* (2007) as, *qtl1.1.*

Two QTLs for spikelet fertility on primary and secondary-branches (*qpsf9.1* and *qssf9.1*) have been identified on chromosome 9 in the marker interval RM219-RM434. Interestingly, Lanceras *et al*. (2004) have identified a QTL (*qpss9.1*) for percent spikelet sterility on the same position of chromosome 9. Zhang *et al*. (2001) have reported a QTL for osmotic adjustment (OA) designated as *os9.1*, in this region indicating that fertility may be maintained through maintenance of OA under stress. Thus, physiological mechanisms for maintaining spikelet fertility via OA under drought stress need to be well investigated.

As of now, many QTLs have been reported for primary and secondary branch number. For instance, Liu *et al*. (2006) identified six QTLs for primary branch number (PBN) on chromosomes 1, 4, 6, 7, 9, and 10, and one QTL for secondary branch number (SBN) on chromosome 4 under control conditions. From drought tolerance angle, one QTL for PBN on chromosome 4 and three QTLs for SBN on chromosomes 3, 4, and 8 have been identified under stress conditions. For identifying tolerance QTLs for flowering stage, spikelet fertility is the ideal character. To our knowledge, this is the first report on detection of drought tolerance QTLs for grain related traits on primary and secondary-branches.

Candidate genes at QTL region(s)

Identification of candidate gene(s) underlying major QTLs, stable QTLs and QTL clusters is an important objective of QTL mapping studies. In the present study, it is noticeable that a region on chromosome 8 (RM38-RM331) has been found to host QTLs for SPY together with eight other yield related traits *viz*., PH, NPT, SCG, SSF, BM, SPY, RDW, RS under stress. Li *et al*. (2005) have been identified as many as 46 QTLs for drought tolerance by linkage and linkage disequilibrium in 38 introgression lines selected under the lowland and upland drought from the IR64/Type3 BC_2F_2 population. Interestingly, this is one of the regions identified as drought tolerant.

Moreover, the QTL, *qspy8.1* present in this region explained quite high phenotypic variance (45.95%) suggesting involvement of a major gene controlling this trait under stress. QTLs having high phenotypic effects can be efficiently targeted for fine mapping and map based cloning of genes of promise.

Seven candidate genes associated with drought tolerance have been identified in this marker interval, RM38-RM331 based on previous reports (http://orygenesdb.cirad.fr) (Table 22). For instance, vacuolar protein sorting is predicted to act as AVP 1 H^+- pump (*Arabidopsis* Vacuolar Protein) in *Arabidopsis* (Park *et al*., 2005). As the transgenic *Arabidopsis* plants over expressing the vacuolar H^+-pyrophosphatase (AVP 1) are more resistant to high concentrations of NaCl as well as moisture stress than the isogenic wild-type strains. The phenotypes of the *AVP1* transgenic plants suggest that increasing of the vacuolar proton gradient results in increased solute accumulation (Na^+ and K^+) and water retention.

Table 22 Candidate genes in the region RM38-RM331 on chromosome 8

S.No.	Gene	Function	References
1	Os08g07700.1	Ethylene-responsive transcription factor 4, putative, expressed	Fujimoto *et al*., 2000; Wang *et al* .,2005
2	Os08g07760.1	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	Wang *et al* .,2005
3	Os08g08060.1	Vacuolar protein sorting 18, putative, expressed	Gaxiola *et al*.,2001; Park *et al*.,2005
4	Os08g08110.1	Diacylglycerol kinase, putative, expressed	Arisz *et al*., 2003; Wang *et al* .,2005
5	Os08g08140.1	Receptor-like kinase, putative, expressed	Xiong *et al*.,2002; Wang *et al* .,2005
6	Os08g10070.1	Serine/threonine-protein kinase receptor precursor, putative, expressed	Wang *et al* .,2005; Xiong and Zhu,2001
7	Os08g16910.1	Mannitol dehydrogenase, putative, expressed	Abebe *et al*.,2003

According to Ali *et al.* (2006) 38% of the drought tolerance regions coincide with those of salt tolerance on chromosomes 1, 2, 6, 8, 10 and 11. Also linkage disequilibrium analysis results reveal that many of the drought tolerance QTLs completely shared the salt tolerance QTLs. Recently, Diedhiou *et al*. (2008) have proved SNF-I type serine/ threonine protein kinase, SAPK4 to regulate stress responsive gene expression under salt stress, including vacuolar H^+- pump, the Na^+/K^+ antiporter etc. and thereby conferring salt tolerance in rice. Based on these reports the locus Os08g10070.1 appears to be another important candidate gene for drought tolerance in this region. In addition, according to Abebe *et al.* (2003) Mannitol dehydrogenase (*mltD*) gene causes accumulation of mannitol, which leads in turn to increased osmotolerance. Thus this gene is also a potent candidate found in the present study. Cloning of these genes may enable gaining precise further insights into the molecular and physiological basis of abiotic stresses like drought in rice.

Comparison of QTLs identified under two water regimes in field and greenhouse experiments:

Nearly all the QTLs reported in this study for drought tolerance are new and not reported previously in other mapping populations. For the 18 traits investigated under field conditions a total of 46 QTLs were mapped under both well watered and water deficit conditions (Table 18). QTLs, which were detected under both control and stress conditions with main effect have been classified as stable QTLs.

Of the 46 QTLs, only 4 QTLs (*qph5.1, qph7.1, qph8.1* and *qscg8.2*) were detected on the same genomic regions in both conditions (Table 23).

Under greenhouse conditions for five traits and of their relative parameters, a total of 22 QTLs have been detected under control and stress conditions. Out of them only 5 have been identified as stable (Table 23), while the remaining have been detected either under stress (10 QTLs) or control (7 QTLs) conditions only. Among these five stable QTLs also only one QTL *viz*., *qrs8.1* has been identified under control as well as stress conditions, while the remaining four QTLs *viz*., *qrdw1.1*, *qrdw8.2, qrs1.2* and *qrs8.2* for traits measured under stress and of their relative parameters measured under the stress.

This lower coincidence of QTLs across two water regimes has as well been reported by Kamoshita *et al*. (2002a and 2002b). They could find only 2 stable QTLs among 31 QTLs detected for 7 traits in two experiments with different sowing dates under anaerobic conditions, suggesting a large effect of the phenotyping environment as defined by temperature and solar radiation on QTL identification for root traits. Further, Zhang *et al*. (2001) showed that QTLs and epistatic loci for seminal root length detected in solution culture were different from those detected in paper culture and revealed a different genetic system responsible for seminal root growth under different water supply conditions.

No QTL was detected for some of the traits like fertile grains, test grain weight on primary and secondary branches and root length under stress conditions,

Table 23 Main effect QTLs detected for drought tolerance

				Stress				Control			
Trait	QTL	Chr	Marker interval	LOD	PVE	a0	Al.ef.	LOD	PVE	a0	Al.ef.
Field exp.											
PH	*qph5.1**	5	RM233-RM18602	5.05	59.62	-15.04	INRC10192	3.81	70.29	16.51	IR64
	*qph7.1**	7	RM427-RM248	5.57	59.05	14.85	IR64	3.92	70.8	16.69	IR64
	*qph8.1**	8	RM404-RM547	5.05	59.51	15.14	IR64	2.76	69.15	16.8	IR64
SCG	*qscg8.2**	8	RM404-RM547	3.36	61.35	-16.3	INRC10192	2.9	40.42	-8.38	INRC10192
Green house exp.											
RDW	*qrdw1.1**	1	RM1-RM495	6.33	32.94	5.26	IR64	5.73 (R)	29.29	53.08	IR64
	*qrdw8.2**	8	RM404-RM547	5.37	51.21	-9.84	INRC10192	4.16 (R)	28.07	-86.94	INRC10192
	*qrs8.1**	8	RM38-RM331	4.92	44.34	-143.33	INRC10192	3.27	47.64	-55.13	INRC10192
	*qrs1.2**	1	RM493-RM486	3.54	47.19	-131.62	INRC10192	3.32 (R)	19.54	-59.46	INRC10192
	*qrs8.2**	8	RM404-RM547	5.26	43.89	-148.55	INRC10192	3.17 (R)	34.64	-86.07	INRC10192

although segregation for these traits was obvious in the RILs. Previous studies by Yu *et al*. (1997); Li *et al*. (1997 and 2001); Xing *et al*. (2002) and Lanceras *et al*. (2004) have found epistasis and G x E interactions to play major role in determining yield and its components and most of the phenology traits, especially under drought conditions to have no main effects at single-locus level. This finding probably explains why no QTL could be detected for such traits. In addition, failure to detect a QTL for any given trait or bias in QTL mapping may be attributed to any one or many of the following reasons:

(i) Two parental inbreds may have identical alleles at a linked QTL, which may thus escape detection,

(ii) Parents may have different alleles at given QTL, having equivalent expression for the trait,

(iii) Type and size of mapping populations,

(iv) Type and number of molecular markers used in the construction of genetic map,

(v) Statistical threshold for detecting putative loci,

(vi) Heritability of target traits (Yano and Sasaki, 1997)

(vii) Low level of polymorphism between parents, and

(viii) Properties of the genome such as variation in recombination rates and gene density among different regions of the genome.

Genetic basis for responses to water stress

Out of the 68 drought tolerance related QTLs identified under both field and greenhouse conditions only 5 QTLs *viz*., *qph5.1*, *qph7.1*, *qph8.1*, *qscg8.2* and *qrs8.1* (7.35%) (Table 23) have been found to be stable across two water regimes. In other words, 63 QTLs (92.65%) were water supply specific. Similar results have been reported earlier by Li *et al*. (2005) and Cui *et al*. (2008). Very low percentage of common (stable) QTLs detected across water supply conditions is in agreement with the significant responses of RILs to water deficit strongly suggesting QTL detection to depend on specific environment and water deficit to induce or inhibit some new genes to express simultaneously.

Among the 13 regions identified for QTL clusters under field condition, 10 have been found to influence related traits under both the water supply conditions. High percentage of common regions under both water supply conditions is due to high correlation of traits between well-watered and water deficit conditions. In addition, three regions (RM423–RM5897 on chromosome 2, RM336–RM481 on chromosome 7 and RM38–RM331 on chromosome 8) have been detected to have effects on related traits only under water deficit conditions. Therefore, the three water supply-specific regions may be closely associated with the responses of lines to water deficit. Notably, this also suggests that water deficit promoted the expression of QTLs located in these regions.

It is assumed that inductive expression of new genes would permit adaptation to stresses. Several studies have documented that gene expression is induced by stresses (Bray, 1997; Kathiresan *et al*., 2006; Rabbani *et al*., 2003). For instance, Rabbani *et al*. (2003) have found 62 genes to be induced by drought in rice. Salekdeh *et al*. (2002) have shown concentrations of several leaf proteins to increase significantly during drought and to decline on re-watering. Thus, QTLs detected only under water-deficit conditions may be in response of plants to stress. So identified QTLs may be useful for marker-assisted improvement of drought tolerance in rice.

Comparison of QTLs detected for morphological indices and performance under drought stress

By comparing the coincidence of QTLs for phenotypic traits and for yield under drought stress, it is possible to test more precisely whether a particular trait is of significance in improving drought tolerance (Lebreton *et al*., 1995). This can be done simply by looking for coincidence of QTLs for two traits.

By comparing locations of QTLs linked to root traits and plant production traits under drought stress, the genomic region RM38-RM331 on chromosome 8 has been found to be of value in breeding for drought resistance in rice. This region has as well been found to co-localize QTLs for root traits (RDW and R S) with as many as six production traits *viz*., PH, NPT, SCG, SSF, BM, and SPY under stress

(Table 25). Some of the recent reports reveal overlap between root QTLs and yield performance (Babu *et al*., 2003; Lafitte *et al*., 2004).

Leaf sheath and stem reserves in rice are important sources of carbohydrate and nitrogen for grain filling, especially at times when transient photosynthesis is inhibited by drought and other abiotic/ biotic stresses like heat or leaf disease. The efficiency of stem reserves in overcoming the effect of drought during grain filling is also dependant on the amount of reserves like Non-structural carbohydrates (NSC) available in the stem before flowering. In general, under non-stress conditions, the NSC accounts for 30% of the carbohydrates only, while under stress the contribution of it rises to more than 50% (Lanceras *et al*., 2004). Interestingly in the present study, SDW, which is the indirect measure for NSC reserves, has been found to be associated three times with SSF two times with PSF and once with SGW in the linked region on chromosome 2 between the markers RM423 and RM5897 (Fig 15) and on chromosome 9 between the markers RM219 and RM434. Thus, selection for such secondary traits can help as well to improve yield under moisture stress conditions.

Further RDW has been found to coincide with PCG and PSF on chromosome 7 at RM336-RM481 region and once with PH, SSF, PCG and SCG on chromosome 8 at RM404-RM547 region. In addition to RDW; root-shoot ratio has also been found to coincide in the marker interval RM404-RM547 on chromosome 8. Further, R S is found to coincide thrice with HI, PCG, PH and SPY on chromosome

1 at RM493-RM302 and once with SSF on chromosome 2 at RM154-RM318. Kamoshita *et al*. (2000b) and Babu *et al*. (2003) have reported that, a QTL for relative yield (*ry2.1*) overlapped with the QTLs for shoot biomass, root depth and root thickness in a DH population on chromosome 2. In other reports, Kamoshita *et al*. (2002a) and Babu *et al*. (2003) have identified a QTL (*Rwc1.1*) for relative water content on chromosome 1, mapped to the same region linked to deep root mass and deep root ratio and deep roots per tiller. In contrast, Price *et al.* (2002) have reported poor co-location of phenology and production traits. The results of the present study on the other hand show considerable amount of co-location of phenology and production traits in the RILs.

Multi-trait clusters

Yield related QTL clusters

In the present study, most of the QTLs detected have been found to cluster in different genomic regions across the rice genome. In all, 12 marker intervals on chromosomes 1, 2, 4, 5, 7, 8, and 9 have been found to harbor multiple QTLs affecting the same or different traits related to yield (Table 24). The number of QTLs in each such cluster is ranging from 2 to 18. It is interesting to note that some of the QTLs for important yield related traits are clustered together. For instance, on chromosome 1 two QTLs for SN and SPY have been found to co-locate in the marker interval of RM1-RM495 suggesting that these two traits can be exploited simultaneously in breeding for yield enhancement employing MAS.

Table 24 QTL clusters identified for yield and its components

S.No.	Marker interval	Chr	Traits	Total No. of QTLs
1	RM1-RM495	1	SN, SPY	2
2	RM154-RM318	2	PH (3), HI	4
3	RM262-RM5897	2	PH (2), SPY	3
4	RM518-RM471	4	CG (2), SF(2)	4
5	RM471-RM252	4	PH (2), CG (2), SF (2)	6
6	RM233-RM18602	5	PH (4)	4
7	RM481-RM427	7	PH, PL, FG, HI	4
8	RM427-RM248	7	PH (4), HI	5
9	RM38-RM331	8	PH (3), CG, GW	5
10	RM331-RM404	8	NT (2), NPT, CG	4
11	RM404-RM547	8	PH (2), NT (3), NPT (2), CG (3), SN, SF (3), BM (3), HI	18
12	RM219-RM434	9	PH (2)	2

-Values in parenthesis indicates no. of QTLs for the respective traits

The linked region RM481-RM248 on chromosome 7 has been found to contain five QTLs for PH, one QTL each for PL, FG and two for HI. Considering the reports by Tian *et al*. (2006) and Onishi *et al.* (2007) on panicle traits and grain number; and Jin *et al.* (2008) and Tan *et al.* (2008) on plant height (*PROG 1* gene in this region) in *O.rufipogon* genome, it is presumed that this region might have played a strong role in the domestication of rice contributing for traits of plant architecture.

It is of interest that at the marker interval of RM404-RM547 on chromosome 8 a cluster of 18 QTLs had been found to repeatedly occur across N-levels and locations, for 8 yield components *viz*., PH, NT, NPT, CG, SN, SF, BM and HI. Further characterization of this region by fine mapping and identification of genes underlying will throw more light on whether the same set of genes regulated differentially or an entirely different set of genes govern these phenotypes.

Similarly, the region between the markers RM331 and RM404 associated with QTLs for chaffy grains, number of tillers and number of productive tillers would as well if this region is involved in tillering efficiency as number of tillers is determined by number of productive tillers.

One more region at map interval of RM38-RM331on chromosome 8 harbors QTLs for PH, CG, and GW. For all of these QTLs INRC10192 has contributed favorable alleles. Recently, Zhang *et al*. (2006) have reported a QTL cluster in this very region for four traits *viz*., spikelets per panicle, grains per panicle, heading date and plant height. In addition, in this region, positive and negative QTLs have been found to be linked together (GW and PH). Association of positive and negative QTLs at the same chromosomal regions has earlier been reported in studies involving wild/weedy species, where positive QTLs for grain weight and panicle length as a cluster has been reported to be linked with negative QTLs for plant height (Septiningsih *et al*., 2003 and Marri *et al*., 2005). In view of the instances of association of positive and negative QTLs at one and the same chromosomal regions, a careful selection strategy is warranted so as to avoid negative characteristics interesting with the targeted crop improvement programmes.

In the course of the present investigation, three regions have been identified as N-specific regions. These include the region on chromosome 1 (RM1-RM495) containing one QTL each for SN and SPY under N2-level. The region (RM481-RM427) on chromosome 7 harboring QTLs for PH, PL, FG and HI under N2-

level and the region on chromosome 8 (RM331-RM404) containing QTLs for NT, NPT, and CG under N-level. These fertilizer responsive regions may be of great value in evolving new plant type based future higher N responsive varieties.

Drought related QTL clusters

In all, 13 intervals distributed over chromosomes 1, 2, 5, 7, 8, and 9 have been found to harbor multiple QTLs affecting the same or different traits related to drought tolerance (Table 25). The number of QTLs in each of the clusters ranges from 2 to 9.

The region RM233-RM18602, on chromosome 5 three QTLs have been found to get clustered, which include one QTL each for SSF under stress, and PH under both the treatments. In the same region on chromosome 5, a QTL for relative spikelet fertility under stress has been identified earlier by Yue *et al*. (2006).

Further, one QTL for plant height, *qph7.2* has been identified under both the water regimes. It is likely to be the same QTL as the one recently reported by Yue *et al*. (2008) as relative plant height (qRPH7.1) QTL under stress.

The region on chromosome 8 flanked by the markers RM38-RM331 contain QTLs for SPY overlapped with several other QTLs related to yield components *viz*., PH, NPT, SCG, SSF and BM under stress. INRC10192 has contributed the favorable alleles for all the QTLs except SSF, in this region. The co-localization of NPT, SSF and BM reveals that high NPT and high SSF contributed to

Table 25 QTL clusters identified for drought tolerance traits under field and greenhouse conditions

S.No.	Marker interval	Chr	Traits	Total No. of QTLs
1	RM1-RM495	1	SL, SDW, RDW (2), RS	5
2	RM243-RM488	1	PCG, SCG	2
3	RM493-RM486	1	PH, PCG, SPY, HI, RS (2)	6
4	RM486-RM302	1	SPY, HI, RS	3
5	RM154-RM318	2	SSF, RS	2
6	RM262-RM5897	2	PSF, SSF, SGW, SDW	4
7	RM233-RM18602	5	PH (2), SSF	3
8	RM336-RM481	7	PCG, PSF, RDW	3
9	RM481-RM427	7	SCG, BM	2
10	RM427-RM248	7	PH (2), SCG, BM	4
11	RM38-RM331	8	PH, NPT, SCG, SSF, BM, SPY, RDW, RS (2)	9
12	RM404-RM547	8	PH (2), PCG, SCG, SSF, RDW (2), RS (2)	9
13	RM219-RM434	9	PH, PSF, SSF, SDW	4

-Values in parenthesis indicates no. of QTLs for the respective traits
-For codes of the traits refer Table 5 and Table 6

high biomass and consequently high yield under stress. The finding is supported by the significant positive correlation seen between SPY with NPT, SSF, PH and BM (Table 14 & SI Table 4). The findings imply molecular mechanisms underlying drought tolerance to overcome the drastic yield reduction that occurs under severe drought stress by ensuring high NPT, SSF and BM. Thus, this region could be a good target for stepping up the yield level of rice under drought conditions.

One QTL for PH under control and one QTL each for PSF and SSF under stress condition have also been found to cluster on chromosome 9 between the marker interval of RM219-RM434. Interestingly, Tripathy *et al*. (2000) and Lanceras *et al*. (2004) report co-location of the QTLs for cell membrane stability and spikelet sterility in this very region.

Two QTLs for shoot dry weight under stress conditions have been located at the interval of RM106-RM5897 on chromosome 2. In a study by Cui *et al*. (2008) the region near RM262 on the same chromosome has been detected to harbor QTLs for shoot fresh weight. However, Xu *et al*. (2001) have detected a QTL for root weight in the same region.

The region RM1-RM243, on chromosome 1 has been found to harbor five QTLs for shoot and root related traits not only in the present study but also in many earlier studies. For instance, Li *et al.* (2005) have reported location of QTLs for root thickness, root number and root dry weight, while length of mesocotyl (F_2) by Redona and Mackill (1996) in the same region. Further, Xue *et al*. (2004) and Price *et al*. (2000) have reported QTLs for PH and root number, respectively in the same region (RM1-RM243). Cui *et al.* (2008) have identified a QTL cluster in the same region for plant height and shoot fresh weight under well watered conditions. Interestingly, in the present study also under well-watered conditions QTLs for shoot length and shoot dry weight have been mapped to the same region. Thus, this region appears to be a good candidate for breeding drought tolerance through MAS as well as for fine mapping and positional cloning of the genes underlying.

Several earlier studies have demonstrated QTLs for correlated traits often to map to the same chromosome regions (Paterson *et al.,* 1991; Lin *et al.,* 1996; Xiao *et al.,* 1996a and Tan *et al.,* 1999). For instance, QTLs related to highly correlated traits like SN and SPY have been found to be located on the same genomic region of RM1-RM495 on chromosome 1. However, this trend is not seen in respect of other correlated traits like PL, FG and SPY. Classical quantitative genetics assumes that trait correlations can be attributed to either pleiotropy or to tight linkage between causative genes. If pleiotropism is the major reason, coincidence of both the location of QTL for related traits as well as the directions of their genetic effects can be expected. If tight physical linkage of the genes concerned is the major reason, the directions of the genetic effect of QTL for different traits may be different although the coincidence of the locations of QTL can still be expected (Wan *et al* 2005). In the present study, for instance, one QTL region RM1-RM495 on chromosome 1 harbors two QTLs for SN and SPY and exhibits similar increasing effects from INRC10192 indicating pleiotropism rather than close linkage between these QTLs. However, effects of QTLs in the region RM404-RM547 on chromosome 8 are in different directions suggesting involvement of tightly linked genes as the cause for the correlation of these traits.

Major findings from the yield and drought response related experiments

Potential markers for marker assisted selection (MAS)

It is difficult and time consuming for breeders to improve quantitative traits through conventional breeding relying excessively on phenotype based selection. Molecular markers, tightly linked to these target traits, could be of help in exercising precision in and the breeding goal selection relatively in a shorter period of time. Prerequisite for identification of markers for MAS is the tight association of marker(s) with the target QTL. According to Wan *et al.* (2006) distance between the marker and the QTL should be as low as possible (< 2.6cM) to avoid linkage drag during introgression of the QTL(s) of interest. Xie *et al.* (2006) have identified eight functional markers, which spans around 0.4cM from region the grain weight QTL (q*gw-9.1*) and used the same for marker assisted introgression of the trait. Further, the same group introgressed another grain weight QTL, *qgw8.1* on chromosome 8 using 9 SSR markers (1.2cM). In the present study, 7 tightly linked markers for eight different traits have been identified for yield and its components (Table 26), whereas for drought tolerance, 7 markers for 6 traits have been identified under

Table 26 Potential QTLs with nearest marker identified in the present study for marker assisted selection (MAS) related to yield and yield components

Trait	N-level & Location	Chr	Marker	Marker-QTL distance	LOD	PVE	Allele effect
NT	N1 L I	1	RM495	2.04	2.86	12.37	INRC10192
	N1 L I, L II	8	RM331	2.01	4.24-4.62	36.53-39.98	INRC10192
	N1 L I, L II & N2 L I	8	RM404	2.06-2.49	3.65-6.13	30.26-39.98	INRC10192
NPT	N1, L II	8	RM331	2.01	3.61	25.61	INRC10192
	N2 L I & N1 LII	8	RM404	2.06-2.49	2.49-4.90	25.61-29.78	INRC10192
CG	N2 L I,LII & N1 LII	8	RM404	0.49	3.57-8.79	35.99-49.62	INRC10192
SN	N2, L I	1	RM1	2.01	3.13	10.72	INRC10192
SF	N1 &N2, L I	4	RM471	0	3.45-3.98	12.64-35.45	IR64
BM	N2 L I & N1 LII	8	RM404	2.06	2.70-4.65	31.85-44.02	INRC10192
SPY	N2, L II	1	RM1	2.01	2.56	9.47	IR64
	N1 L I	2	RM262	0.04	2.5	7.84	INRC10192
HI	N2, L I	7	RM427	0	4.11	14.07	INRC10192
	N1 L I	8	RM404	2.06	2.68	21.34	IR64

For trait names, N-level and Location names refer table 4 stress based on their distance with the concerned QTLs (Table 27). Of these markers, RM331 and RM404 for yield and its components, while RM1, RM486 and RM404 for drought tolerance, seems to be of great value. Apart from being reasonably tightly linked, they contribute higher phenotypic variance (Table 26 and 27) for the traits, with which they are linked. The markers which are so tightly linked could be employed effectively in marker assisted breeding for improvement of yield and tolerance to drought.

Table 27 Potential QTLs with nearest marker detected under field and greenhouse experiments in the present study for marker assisted selection (MAS) of drought tolerance related traits.

Trait	Chr	Marker	Marker-QTL distance	LOD	PVE	Allele effect
Stress						
NPT	6b	RM225	0.13	2.98	10.48	IR64
SCG	4a	RM252	0.02	2.73	8.42	IR64
SGW	2a	RM5897	0.06	2.63	8.33	INRC10192
BM	7a	RM427	0	2.78	9.49	IR64
RDW	1	RM1	2.01	6.33	32.94	IR64
	7a	RM481	0	3.89	13.62	INRC10192
RS	1	RM1	4.01	3.71	25.61	IR64
	8	RM404	8.06	5.26	43.89	INRC10192
	1	RM486	2.43	3.32	19.54	INRC10192
Control						
PSF	8b	RM331 & RM404	2.01 & 2.49	3.47	29.04	IR64
SCG	7a	RM427	0	2.8	9.62	IR64
	10a	RM591	0.03	3.95	12.84	IR64
SPY	1^b	RM486	0	3.83	14.47	IR64
HI	1	RM486	0	5.23	17	IR64
SL	1	RM1	2.01	4.24	18.4	INRC10192
RL	3b	RM218	0.01	2.52	11.51	INRC10192
SDW	1	RM1-RM495	0.01	3.48	13.69	INRC10192

For trait names refer table 5 and 6

Promising QTLs for marker assisted selection (MAS)

If a QTL explains large phenotypic variation (>30%) and is significantly different from other QTLs (e.g. one having much larger LOD score) there is reason to assume that it could be a major gene rather than a QTL. Those QTLs can be regarded as "promising QTLs or major QTLs". Out of the 46 QTLs detected, 26 individually account for more than 30% of the phenotypic variation for yield and its components (Table 28). Similarly, 23 of 46 QTLs have been identified for drought tolerance under field conditions with large phenotypic variation for different production traits. They could be the most rewarding QTLs for use in marker-assisted selection for specific yield traits under stress conditions. Further, in the greenhouse experiment, 10 promising QTLs have been identified for shoot and root related traits (Table 29). It is possible to detect more number of novel and major QTLs by employing RI populations derived from crosses involving primitive landraces as donor parents.

Table 28 Promising QTLs identified for yield and its components

S.No.	Trait	QTL	Chr.	Marker interval	LOD	PVE	a0	Allele effect
1	PH	*qph2.1**	2	RM154-RM318	3.29-8.71	59.87-79.36	19.14-19.36	IR64
2		*qph2.2**	2^b	RM262-RM5897	3.76-4.51	59.33-72.12	17.92-17.94	INRC10192
3		*qph2.3*	2	RM106-RM262	4.35	59.02	17.87	INRC10192
4		*qph3.1*	3	RM570-RM517	2.67	57.42	17.14	INRC10192
5		*qph3.2*	3	RM545-RM168	5.5	59.52	18.21	IR64
6		*qph4.1**	4	RM471-RM252	3.08-5.17	61.61-71.34	18.07-19.09	IR64
7		*qph5.1**	5	RM233-RM18602	5.85-10.2	62.12-67.35	17.18-19.12	INRC10192
8		*qph7.1**	7	RM427-RM248	8.94-14.74	64.91-72.6	17.4719.8	IR64
9		*qph7.4*	7^b	RM22171-RM5752	4.21	70.42	17.63	IR64
10		*qph8.1**	8	RM38-RM331	4.5-10.61	61.02-62.08	19.02-19.4	INRC10192
11		*qph8.2**	8	RM404-RM547	4.48-4.76	61.83-77.31	19.06-19.21	IR64
12		*qph8.3*	8	RM408-RM25	4.05	61.43	18.3	IR64
13		*qph9.1**	9^b	RM219-RM434	4.76-10.83	71.44-79.69	17.74-19.42	INRC10192
14	NT	*qnt8.1**	8^b	RM331-RM404	4.24-4.62	39.98	5.53-6.81	INRC10192
15		*qnt8.2**	8	RM404-RM547	3.65-6.13	30.26-39.3	4.99-6.77	INRC10192
16	NPT	*qnpt8.2**	8	RM404-RM547	2.88-4.9	29.56-29.78	3.02-6.18	INRC10192
17	CG	*qcg4.1**	4	RM518-RM471	3.99-5.32	44.08-50.62	10.94-11.86	INRC10192
18		*qcg4.2**	4	RM471-RM252	2.72-4.42	34.34-49.52	10.59-11.6	INRC10192
19		*qcg8.1*	8^a	RM38-RM331	2.72	34.34	10.59	INRC10192
20		*qcg8.2*	8	RM331-RM404	7.71	35.99	34.99	INRC10192
21		*qcg8.3**	8	RM404-RM547	3.99-8.79	39.04-49.62	10.94-14.43	INRC10192
22	SN	*qsn8.1*	8^a	RM404-RM547	2.57	43.42	24.36	INRC10192
23	SF	*qsf8.1**	8	RM404-RM547	2.9-4.37	31.07-39.67	8.54-14.71	IR64
24	GW	*qgw8.1*	8	RM38-RM331	2.74	44.4	5.74	INRC10192
25	BM	*qbm8.1**	8	RM404-RM547	4.12	44.02	13.05	INRC10192
26	HI	*qhi2.2*	2^a	RM318-RM263	2.93	46.97	3.69	INRC10192

C = control; R = relative parameter; *= stable QTLs

Table 29 Promising QTLs identified for drought tolerance

S.No.	Trait	QTL	Chr	Marker interval	LOD	PVE	a0	Allele effect
Field conditions								
1	PH	*qph1.1*	1^b	RM493-RM486	4.15	71.25	16.1	IR64
2		*qph5.1**	5	RM233-RM18602	5.05	59.62	15.04	INRC10192
					3.81	70.29 (C)	16.51	IR64
3		*qph7.1**	7	RM427-RM248	5.57	59.05	14.85	IR64
					3.92	70.8 (C)	16.69	IR64
4		*qph8.1*	8	RM38-RM331	6.33	65.55	15.19	INRC10192
5		*qph8.2**	8	RM404-RM547	5.05	59.51	15.14	IR64
					2.76	69.15 (C)	16.8	IR64
6		*qph9.1*	9^b	RM219-RM434	3.16	69.79 (C)	16.48	IR64
7	NPT	*qnpt8.1*	8^b	RM38-RM331	2.63	54.75	4.29	INRC10192
8	PCG	*qpcg1.1*	1^b	RM493-RM486	2.57	62.44	8.18	INRC10192
9		*qpcg7.1*	7^a	RM336-RM481	2.75	39.28	5.76	IR64
10		*qpcg8.1*	8	RM404-RM547	2.83	51.81	7.99	INRC10192
11	PSF	*qpsf2.1*	2	RM262-RM5897	3.89	61.73	20.3	INRC10192
12		*qpsf7.1*	7^b	RM336-RM481	3.76	68.4	21.12	INRC10192
13		*qpsf9.1*	9^b	RM219-RM434	3.25	69.97	21.19	INRC10192
14	SCG	*qscg8.1*	8^b	RM38-RM331	3.95	61.67	16.35	INRC10192
15		*qscg8.2**	8^b	RM404-RM547	3.36	61.35	16.3	INRC10192
					2.9	40.42 (C)	8.38	INRC10192
16	SSF	*qssf2.2*	2^a	RM423-RM106	2.58	58.92	18.42	INRC10192
17		*qssf2.3*	2^b	RM262-RM5897	2.92	67.82	19.47	INRC10192
18		*qssf5.1*	5	RM233-RM18602	2.97	57.75	18.27	INRC10192
19		*qssf8.1*	8^a	RM38-RM331	3.27	58.05	18.47	IR64
20		*qssf8.2*	8^b	RM404-RM547	2.85	54.35 (C)	11.32	IR64
21		*qssf9.1*	9	RM219-RM434	3.37	57.84	18.23	INRC10192
22	BM	*qbm8.1*	8^b	RM38-RM331	3.32	62.94	10.82	INRC10192
23	SPY	*qspy8.1*	8	RM38-RM331	4.56	45.95	91.69	INRC10192
Greenhouse conditions								
1	SDW	*qsdw2.1*	2^a	RM106-RM262	2.63	53.86	2.42	INRC10192
2		*qsdw2.2*	2^a	RM262-RM5897	2.51	53.16	2.4	INRC10192
3		*qsdw9.1*	9^a	RM219-RM434	3.03	53.42	2.39	IR64
4	RDW	*qrdw1.1**	1	RM1-RM495	6.33	32.94	5.26	IR64
5		*qrdw8.1*	8^b	RM38-RM331	2.91	47.71	7.87	INRC10192
6		*qrdw8.2**	8^b	RM404-RM547	5.37	51.21	9.84	INRC10192
7	R/S	*qrs1.2**	1	RM493-RM486	3.54	47.19	131.62	INRC10192
8		*qrs2.1*	2b	RM154-RM319	3.29	62.97	136.93	IR64
9		*qrs8.1**	8	RM38-RM331	4.92	44.34	143.33	INRC10192
					3.27	47.64 (C)	55.13	INRC10192
10		*qrs8.2**	8	RM404-RM547	5.26	43.89	148.55	INRC10192
					3.17 (R)	34.64	86.07	INRC10192

C = control; R = relative parameter; *= stable QTLs

Landrace derived QTLs for yield and drought tolerance

The landrace INRC10192 alleles have beneficial effect on 62.22% (Table 17) of the QTLs obtained for yield and its components as against the previous reports of alleles from wild species to have beneficial effect in 35-58% of the QTLs (Xiao *et al*., 1998; Moncada *et al*., 2001; Septiningsih *et al*., 2003 and Thompson *et al*., 2003).

For the traits related to drought tolerance majority of the favorable alleles have come from the landrace INRC10192 (65.86%) (Table 18). McCouch and Doerge (1995) have identified more than 50% of the QTLs for root morphology in Co39/Moroberekan RI population and all the alleles that had positive effect were from Moroberekan, the *japonica* parent.

It is interesting to report here high percentage of transgressive segregants of value observed for nearly all the traits, especially for NPT, SF and SNP, in the mapping population involving the landrace INRC10192 which is not known for superior traits. Recovery, however of useful transgressive segregants in such a population indicates that alleles of the primitive cultivars like INRC10192 might be interacting with those of high yield background like IR64 can result in valuable segregants.

Landrace derived QTLs of promise for MAS

In keeping with the objective of the present investigation *viz*., discovering new QTLs of promise, it is important to identify QTLs with enhancing favorable alleles from the landrace for exploitation in the crop improvement programme. The present study has yielded many QTLs of promise for improvement of yield and its components (Tables 26 & 28) *viz*., number of productive tillers per plant, percent spikelet fertility, biomass, single plant yield and harvest index, for which the trait enhancing favorable alleles have been drawn from the donor parent INRC10192.

In case of drought tolerance, (Tables 27 & 29) many valuable QTLs have been detected using indices like relative parameters, yield *per se* and seedling traits under the stress conditions. Though agronomically unattractive, the results suggest that the landrace INRC10192 had contributed favorable alleles for enhancing the level of tolerance to drought through various trait related QTLs. These results are in agreement with Brondani *et al*. (2002), Li *et al*. (2006) and Mc Couch *et al*. (2007) where they have detected QTLs with trait value enhancing alleles drawn from phenotypically inferior parental success in rice.

Based on the results of the present study as well as of the previous reports four important chromosomes have been identified to carry yield and drought resistance enhancing QTLs/ genes. Chromosomes 1 and 7 control "**plant architecture**", as they harbor QTLs/ genes for plant height (*sd1*, *PROG 1,*) and panicle characters, while chromosomes 2 and 8 may be termed as "**stress responsive**" chromosomes as they carry many important QTLs for tolerance to moisture stress. Chromosome 8 harboring QTLs for as many as 8 important yield components, most of which are from parent landrace may be termed also as "**productivity chromosome**". The QTLs of promise detected on these chromosomes can be used for pyramiding into varieties of with progressively new yield thresholds combining tolerance to moisture stress, which is the major yield depressing factor in rice across ecologies.

Summary and Conclusions

6 Summary and Conclusions

Progressive improvement of yield is critical for sustained self-sufficiency in rice, India's major staple. Following the success story of hybrid technology, of the two strategies being contemplated for raising progressively the ceiling to genetic yield *viz*. tailoring of new plant type and exploiting still uncovered yield genes in the primary gene pool, the later is gaining importance. Along with yield enhancement, what is considered equally important to raise the productivity level is containment of yield destabilizing factors, especially major abiotic stresses like moisture stress and salinity. Drought being the most devastating environmental stress, efforts have been althrough to improve the crop productivity under water-limiting conditions. More than 80 year long history of breeding for tolerances to drought has led us nowhere near development of ideal genotypes capable of resisting the stress, despite availability of reliable sources of tolerance to the stress in the germplasm.

With increasing awareness that wild/weedy species and landraces of the Asian rice cultivar represented over 80% of the total variability and recent reports demonstrating presence of potential yield enhancing and drought tolerant QTLs in such a genepool (Xiao *et al*., 1998 and Li *et al*., 2006), the present study was undertaken with the objective of identifying till unknown and now unexploited genetic variability for yield and its stability in the primitive cultivars using marker associated QTL tagging technique. The study was carried out in two parts *viz*. (a) mapping marker associated with QTLs relating to yield and its major components and (b) tagging the component traits of drought tolerance to molecular marker associated QTLs. Major research findings are summarized as under.

For mapping QTLs, a mapping population of 140 RILs in F_6 was developed from the cross of the landrace INRC10192 with the semi-dwarf high yielding variety IR64. These two parental varieties were screened for parental polymorphism using 412 rice microsatellite markers (RMs). Of these 133 showed polymorphism (32.28%). The polymorphic markers were distributed on all the 12 chromosomes and were used for screening the mapping population.

A molecular linkage map was constructed employing 63 microsatellite markers spanning a total map length of 1978.9cM using the kosambi mapping function, resulting in an average of one marker every 31.41cM, using MAPMAKER/EXP v 3.0 and Map Disto v 1.7 softwares. QTL mapping was carried out employing interval mapping (IM) and composite interval mapping (CIM) methods of the QTL Cartographer v 2.5 with the LOD threshold of 2.5. The following are the major findings from the study.

- In all, 45 and 68 QTLs were identified for yield and drought tolerance, respectively. These QTLs were found distributed on all the chromosomes except 11 and 12. Trait-enhancing alleles were found to be contributed largely by the landrace INRC10192 for most of the yield (62.22%) and drought tolerance (65.86%) components.

- Of the 45 yield related QTLs 18 were stable; and 25 novel identified for the first time in the present study. In respect of drought tolerance only five QTLs *viz*., *qph5.1*, *qph7.1*, *qph8.1*, *qscg8.2* and *qrs8.1* were identified as stable and most of them were new and novel.
- In the yield related study, 26 of the 45 QTLs individually accounted for more than 30% of the phenotypic variation, while for drought tolerance 33 of 68 explained more than 30% of the phenotypic variation. The promising QTLs relating to major yield related traits could be used as potential candidates for future improvement by pyramiding by MAS or by fine-mapping and positional cloning for possible gene transfer through transformation technology.
- Six markers, namely RM1, RM471, RM262, RM427, RM331 and RM404 for yield and its components were identified as potential ones for use in crop breeding through MAS and for drought tolerance RM1, RM225, RM5897, RM427, RM481, RM486, RM218 and RM404 were identified as potential markers. These markers are likely to be very useful in practical plant breeding by MAS.
- Most traits were found to be controlled by more than one QTL located on different chromosomes. Also, genomic regions (QTLs) showing more than one trait was not uncommon. For instance, the genomic region between the markers RM404 and RM547 on chromosome 8 accounts for eight yield related traits. The genomic region between the markers RM1 and RM495 on chromosome 1 accounts for spikelet number and single plant yield. Further the

region between RM331 and RM404 on chromosome 8 accounts for three yield components with the former reflecting the multigenic control of a trait. The latter two instances indicating either pleiotropism or complex locus with tightly linked non-allelic genes.

- A highly significant locus (*qspy8.1*) for grain yield under stress was identified on chromosome 8 at the marker interval of RM38-RM331. This QTL explained high phenotypic variation (45.45%). The same locus also had a significant effect on a wide range of traits under severe stress, including plant height, number of productive tillers, chaffy grains and spikelet fertility on secondary branches, biomass, root dry weight and root shoot ratio. Thus, the effect of this locus in this population seems to be large enough to support fine-mapping and positional cloning of genes, besides the potential use in MAS to improve drought tolerance. Seven candidate genes *viz*., *Os08g07700.1* (Ethylene-responsive transcription factor 4)*, Os08g07760.1* (BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor)*, Os08g08060.1* (Vacuolar protein sorting 18)*, Os08g08110.1* (Diacylglycerol kinase)*, Os08g08140.1* (Receptor-like kinase)*, Os08g10070.1* (Serine/threonine-protein kinase receptor precursor)*,* and *Os08g16910.1* (Mannitol dehydrogenase) associated with drought tolerance for this interval were identified. Cloning and functional analysis of these genes may throw precise insights into the molecular and physiological basis of drought tolerance in rice.

- Stable yield related QTLs offer new opportunities for exploiting them for progressive yield improvement through two approaches *viz*., recombination breeding using concerned QTLs as selectable markers and recombinant DNA technology by transferring selectively cloned QTLs of promise into good agronomic bases.
- In addition, the novel QTLs identified are good candidates for fine-mapping and positional cloning studies, while, the QTLs that are mapped to regions consistent with other studies can be useful for marker assisted transfer of these QTLs.
- Pyramiding promising and harmonious QTLs, into one cultivar from ‘stress responsive’ (Chromosome 2) ‘productivity trait dense’ (Chromosomes 8) and ‘phenology traits related’ (Chromosome 1) chromosomes could help breach yield barriers.
- Despite its inferiority for traits of agronomic significance alleles from the landrace had beneficial effect on yield components like number of tillers, number of productive tillers, percent spikelet fertility, biomass, single plant yield and harvest index. Moreover, for many of the production and phenology related traits of drought tolerance *viz*., number of productive tillers, spikelet fertility on primary and secondary branches, grain weight on secondary branches, biomass, single plant yield, shoot length, root length, shoot dry weight, root dry weight and root shoot ratio, also had favorable allele effect from the landrace. Thus, the study brought out the fact that primitive

landraces do possess potential new allelic variation for grain yield and its major components as well as for drought tolerance.

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Supporting information

Supporting Information 1: Yoshida medium (Yoshida *et al.*, 1976)

Preparation of stock solutions

Element	Reagent (AR grade)	Preparation (g/ 10 liters of distilled water)	
N	NH_4NO_3	914	
P	$NaH_2PO_4.2H_2O$	403	
K	K_2SO_4	714	
Ca	$CaCl_2$	886	
Mg	$MgSO_4.7H_2O$	3240	
Mn	$MnCl_2.4H_2O$	15.0	*
Mo	$(NH_4)_6.MO_7O_{24}.4H_2O$	0.74	
B	H_3BO_3	0.35	
Zn	$ZnSO_4.7H_2O$	0.31	
Cu	$CuSO_4.5H_2O$	77.0	
Fe	$FeCl_3.6H_2O$	119	
	Citric acid (monohydrate)		

Note: Store in glass or polyethylene carboys.

*Dissolve separately combine with 500 ml of 9.34 concentrated H_2SO_4. Make up to 10 liter volume with distilled water.

Preparation of culture solution

Reagents:

1. Sodium hydroxide (1N): Dissolve 40g NaOH in 1 liter of demineralized water.
2. Hydrochloric acid (1N): Put 83ml of concentrated HCl in a 1-liter graduated beaker and make up to volume with demineralized water.
3. Mixed indicator: Dissolve 0.3g of bromocresol green and 0.2g of methyl red in 400 ml of 90% ethanol. This indicator is red below Ph 5.0, purple at pH 5.0 to 5.1 and blue above pH5.1.

Composition of culture solution

Element	Milliliters of stock solution per four liters of culture solution	Concentration of element in nutrient solution (ppm)
N	5	40
P	5	10
K	5	40
Ca	5	40
Mg	5	40
Mn	5	0.5
Mo		0.05
B		0.2
Zn		0.01
Cu		0.01
Fe		2

Procedure:

For every 4 liters of culture solution to be prepared, add 5 ml of each of the stock solution (as set out in ST 2.2) to 1 liter of water in a plastic bucket. Prepare a few extra liters of solution in case of spillage. Stir the solution after adding each reagent to avoid any precipitation. Then, using mixed indicator, adjust the pH of the solution to 5.0 by adding 1N NaOH/ HCl. Stir the solution continuously while adding. Add 12 liters of demineralized water to this four liters to get Half-strength nutrient solution.

SI Table 2: PEG-6000 concentration at different temperatures and its Osmotic potential in bars

Conc (g/kg H_2O)	T (oC)	OP (bars)	Conc (g/kg H_2O)	T (oC)	OP (bars)
100	25	-1.48	100	35	-1.13
110	25	-1.74	110	35	-1.34
120	25	-2.01	120	35	-1.57
130	25	-2.31	130	35	-1.82
140	25	-2.62	140	35	-2.08
150	25	-2.95	150	35	-2.36
160	25	-3.30	160	35	-2.66
170	25	-3.68	170	35	-2.98
180	25	-4.07	180	35	-3.31
190	25	-4.48	190	35	-3.67
200	25	-4.91	200	35	-4.04
210	25	-5.36	210	35	-4.42
220	25	-5.82	220	35	-4.83
230	25	-6.31	230	35	-5.25
240	25	-6.82	240	35	-5.69
250	25	-7.35	250	35	-6.15
260	25	-7.89	260	35	-6.63
270	25	-8.46	270	35	-7.12
280	25	-9.04	280	35	-7.64
290	25	-9.65	290	35	-8.17
300	25	-10.27	300	35	-8.71
310	25	-10.91	310	35	-9.28
320	25	-11.58	320	35	-9.86
330	25	-12.26	330	35	-10.46
340	25	-12.96	340	35	-11.08
350	25	-13.68	350	35	-11.72
360	25	-14.42	360	35	-12.37
370	25	-15.18	370	35	-13.04
380	25	-15.96	380	35	-13.73
390	25	-16.76	390	35	-14.44
400	25	-17.57	400	35	-15.16
410	25	-18.41	410	35	-15.91
420	25	-19.27	420	35	-16.67
430	25	-20.14	430	35	-17.44
440	25	-21.04	440	35	-18.24
450	25	-21.95	450	35	-19.05
460	25	-22.89	460	35	-19.88
470	25	-23.84	470	35	-20.73
480	25	-24.81	480	35	-21.60
490	25	-25.81	490	35	-22.48
500	25	-26.82	500	35	-23.39
510	25	-27.85	510	35	-24.31
520	25	-28.90	520	35	-25.24
530	25	-29.97	530	35	-26.20
540	25	-31.06	540	35	-27.17
550	25	-32.17	550	35	-28.16
560	25	-33.30	560	35	-29.17
570	25	-34.44	570	35	-30.20

SI Table 3 Correlation coefficients among yield and its components under two N-levels at Hyderabad

N2 level

N1 level

Trait	PH	NT	PT	PL	FG	CG	SN	SF	SW	BM	TY	HI
PH	**0.876****	-0.221*	-0.170	0.636**	0.441**	-0.145	0.344**	0.289**	0.181	0.462**	0.292**	-0.354**
NT	-0.072	**0.568****	0.916**	-0.271**	-0.261**	0.077	-0.214*	-0.169	-0.232*	0.449**	0.128	0.403**
PT	0.028	0.780**	**0.376****	-0.224*	-0.221*	0.037	-0.194	-0.120	-0.261**	0.563**	0.351**	0.574**
PL	0.694**	-0.235**	-0.115	**0.606****	0.567**	0.132	0.606**	0.075	0.104	0.371**	0.148	-0.304**
FG	0.482**	-0.130	0.062	0.669**	**0.434****	-0.175	0.870**	0.467**	0.009	0.333**	0.444**	0.015
CG	-0.103	0.068	-0.021	0.127	-0.050	**0.530****	0.330**	-0.916**	-0.168	0.119	-0.188	-0.264**
SN	0.407**	-0.085	0.049	0.673**	0.905**	0.377**	**0.446****	-0.010	-0.070	0.377**	0.299**	-0.122
SF	0.246*	-0.140	0.020	0.137	0.370**	-0.855**	-0.024	**0.536****	0.139	-0.023	0.385**	0.258**
SW	0.208*	-0.289**	-0.304**	0.237*	0.004	-0.119	-0.046	0.134	**0.428****	-0.077	0.007	-0.280**
BM	0.463**	0.530**	0.587**	0.314**	0.387**	0.016	0.370**	0.052	0.026	**0.633****	0.617**	-0.022
TY	0.373**	0.121	0.461**	0.350**	0.554**	-0.158	0.446**	0.316**	0.138	0.638**	**0.442****	0.569**
HI	-0.066	-0.248*	0.109	0.050	0.297**	-0.298**	0.135	0.435**	0.128	-0.109	0.588**	**0.229***

* = p<0.05; **= p<0.01. Note: Data along diagonal (bold) represent the correlation coefficients among twelve traits under two N-levels. Data above the diagonal correspond to correlations under N2 level; Data below the diagonal correspond to correlation under N1 level

SI Table 4 Correlation coefficients among yield and its components under two N-levels at Maruteru

N2 level

	Trait	PH	NT	PT	PL	FG	CG	SN	SF	SW	BM	TY	HI
N1 level	PH	**0.574****	-0.412**	-0.361**	0.629**	0.436**	0.163	0.473**	-0.006	0.236*	0.329**	0.142	-0.039
	NT	-0.351**	**0.327****	0.843**	-0.162	-0.111	-0.040	-0.120	0.013	-0.216*	0.374**	0.445**	0.103
	PT	-0.358**	0.852**	**0.330****	-0.199*	-0.081	-0.061	-0.104	0.044	-0.118	0.474**	0.515**	0.060
	PL	0.612**	-0.187	-0.173	**0.451****	0.550**	0.109	0.549**	0.115	0.161	0.234*	0.263**	0.084
	FG	0.408**	-0.246*	-0.236**	0.547**	**0.200***	-0.062	0.866**	0.466**	0.164	0.147	0.355**	0.189
	CG	0.114	0.089	0.105	0.345**	0.017	**0.242***	0.446**	-0.877**	-0.087	0.234*	-0.072	-0.206*
	SN	0.396**	-0.145	-0.127	0.641**	0.813**	0.594**	**0.343****	-0.021	0.104	0.249*	0.282**	0.065
	SF	0.009	-0.143	-0.151	-0.153	0.320**	-0.901**	-0.267**	**0.114**	0.135	-0.163	0.236*	0.285**
	SW	0.241*	-0.140	-0.185	0.187	0.103	-0.061	0.054	0.043	**0.280****	0.046	0.127	-0.013
	BM	0.312**	0.524**	0.528**	0.280**	0.163	0.259**	0.287**	-0.193	0.029	**0.202***	0.509**	-0.066
	TY	0.191	0.235*	0.289**	0.242*	0.372**	-0.086	0.252*	0.217**	0.237*	0.457**	**0.110**	0.379**
	HI	-0.141	-0.025	0.050	0.056	0.123	-0.086	0.049	0.172	0.140	-0.207*	0.626**	**0.062**

* = p<0.05; **= p<0.01. Note: Data along diagonal (bold) represent the correlation coefficients among twelve traits under two N-levels. Data above the diagonal correspond to correlations under N2 level; Data below the diagonal correspond to correlation under N1 level

SI Table 5 Correlation coefficients among yield and drought related traits under field conditions of 140 RILs developed of the cross IR64/INRC10192

Stress

Control

Trait	RWC	CC	PH	PT	PL	PFG	PCG	PGN	PSF	PSW	SFG	SCG	SGN	SSF	SSW	BM	TY	HI	RTY	RBM
RWC	-	0.047	-0.078	0.034	-0.089	0.098	-0.035	-0.008	0.034	-0.186	0.156	-0.098	-0.003	0.051	-0.167	0.085	0.063	0.065	0.075	0.013
CC	0.047	-	-0.065	0.062	-0.119	-0.164	0.01	-0.072	-0.048	0.044	-0.204*	-0.052	-0.147	-0.008	-0.051	-0.038	0.012	0.066	0.148	0.205*
PH	-0.121	0.105	0.420**	-0.128	0.485**	-0.057	0.063	-0.007	-0.034	0.072	0.067	0.190	0.187	0.013	0.285**	0.399**	-0.092	-0.064	-0.052	0.016
PT	0.12	0.133	-0.171	0.154	-0.085	0.234*	-0.115	0.095	0.260**	0.122	0.195*	-0.116	0.076	0.244*	0.064	0.558**	0.333**	0.129	0.145	0.021
PL	-0.035	0.002	0.607**	-0.207*	0.201*	0.028	0.173	0.273**	-0.042	0.324**	0.168	0.306**	0.400**	0.002	0.430**	0.353**	0.078	0.000	0.028	-0.098
PFG	0.078	0.067	-0.003	-0.057	0.211*	-0.065	-0.519**	0.572**	0.735**	0.317**	0.718**	-0.471**	0.297**	0.700**	0.357**	0.144	0.385**	0.398**	0.02	-0.073
PCG	-0.223*	0.201*	0.059	-0.133	0.131	-0.165	0.088	0.235*	-0.884**	-0.322**	-0.438**	0.625**	-0.104	-0.736**	-0.297**	0.004	-0.447**	-0.500**	-0.078	-0.024
PGN	-0.035	0.161	0.047	-0.116	0.277**	0.871**	0.322**	0.055	0.106	0.075	0.332**	0.082	0.402**	0.211*	0.152	0.087	0.133	0.132	0.032	-0.068
PSF	0.244*	-0.096	-0.066	0.079	-0.009	0.538**	-0.835**	0.073	-0.088	0.419**	0.612**	-0.572**	0.230*	0.855**	0.374**	0.023	0.469**	0.570**	0.056	0.014
PSW	-0.04	0.035	0.269**	-0.115	0.221*	0.226*	0.05	0.249*	0.066	0.274**	0.259**	-0.193	0.185	0.331**	0.709**	0.136	0.226*	0.233*	0.061	-0.016
SFG	0.054	0.095	0.357**	0.091	0.541**	0.455**	-0.164	0.364**	0.312**	0.042	-0.154	-0.369**	0.601**	0.724**	0.211*	0.181	0.409**	0.317**	-0.036	-0.157
SCG	-0.161	-0.013	0.320**	-0.265**	0.428**	-0.08	0.418**	0.136	-0.338**	0.064	-0.047	0.118	0.344**	-0.702**	-0.131	0.032	-0.386**	-0.405**	-0.117	-0.06
SGN	-0.02	0.094	0.479**	-0.008	0.686**	0.371**	0.026	0.381**	0.131	0.064	0.908**	0.362**	0.044	0.202*	0.142	0.188	0.211*	0.17	0.011	-0.115
SSF	0.106	0.016	-0.096	0.143	-0.044	0.421**	-0.399**	0.197*	0.503**	0.052	0.566**	-0.755**	0.209*	-0.031	0.308**	0.115	0.519**	0.538**	0.089	0.009
SSW	-0.025	0.004	0.151	-0.161	0.181	0.226*	0.085	0.263**	0.012	0.728**	-0.107	0.085	-0.073	-0.085	0.327**	0.231*	0.219*	0.221*	0.047	-0.151
BM	0.045	0.240*	0.447**	0.600**	0.351**	0.18	0.027	0.199*	0.057	0.223*	0.434**	0.055	0.432**	0.104	0.173	0.118	0.360**	0.082	0.176	0.004
TY	0.054	0.104	0.147	0.602**	0.189	0.304**	-0.086	0.251*	0.220*	0.228*	0.487**	-0.161	0.390**	0.370**	0.163	0.817**	-0.007	0.835**	0.244*	0.026
HI	0.134	-0.043	-0.185	0.134	-0.016	0.431**	-0.178	0.326**	0.300**	0.171	0.285**	-0.302**	0.118	0.438**	0.241*	0.195*	0.509**	0.166	0.306**	0.109
RTY	0.075	0.148	-0.206*	-0.094	-0.062	0.159	0.123	0.209*	-0.009	0.035	0.006	-0.039	-0.024	0.131	0.129	-0.112	-0.015	0.336**	-	0.449*
RBM	0.013	0.205*	-0.105	0.045	-0.033	0.175	0.079	0.189	0.057	-0.085	0.084	0.083	0.085	0.106	-0.049	-0.049	-0.029	0.099	0.449**	-

* = p<0.05; **= p<0.01. Note: Data along diagonal (bold) represent the correlation coefficients among twenty traits under control and stress conditions. Data above the diagonal correspond to correlations under stress; Data below the diagonal correspond to correlations under control conditions.

SI Table 6 Chi square values of microsatellite markers that showing segregation distortion among 140 RILs of the cross IR64/INRC10192

S. No.	Marker	Chr.	$\chi^2_{1:1}$	*p*	Significance	Skewness
1	RM1	1	29.77	0.00000	*****	INRC10192
2	RM486	1	67.93	0.00000	*****	IR64
3	RM154	2	25.79	0.00000	*****	INRC10192
4	RM324	2	5.05	0.02459	*	IR64
5	RM218	3	13.45	0.00025	***	IR64
6	RM251	3	7.26	0.00706	**	IR64
7	RM471	4	76.17	0.00000	*****	IR64
8	RM589	6	4.84	0.02773	*	INRC10192
9	RM204	6	4.23	0.03967	*	IR64
10	RM427	7	10.61	0.00112	**	IR64
11	RM5752	7	8.13	0.00436	**	INRC10192
12	RM25	8	12.83	0.00034	***	INRC10192
13	RM404	8	105.53	0.00000	*****	IR64
14	RM331	8	98.57	0.00000	*****	IR64
15	RM434	9	20.16	0.00001	*****	IR64
16	RM244	10	9.31	0.00228	**	IR64
17	RM271	10	15.13	0.00010	***	IR64
18	RM304	10	4.45	0.03500	*	IR64
19	RM229	11	4.30	0.03815	*	IR64
20	RM17	12	41.14	0.00000	*****	IR64
21	RM235	12	6.22	0.01262	*	IR64

p=probability

SI Table 7. QTLs detected for yield and its components under two N levels at Hyderabad employing composite interval and interval mapping (CIM and IM)

						CIM						IM				
Trait	N-Level	QTL	Chr	Marker Intrval	Position cM	RFM	LFM	LOD	PVE	a0	Position cM	RFM	LFM	LOD	PVE	a0
PH	N1	qph2.1*	2	RM154-RM318	30.01	30.01	25.09	6.04	62.02	19.14	28.01	28.01	27.09	11.03	82.02	20.01
		qph4.1*	4	RM471-RM252	72.28	19.98	20.02	5.17	61.61	19.09	72.28	19.98	20.02	9.13	81.34	20.01
		qph5.1*	5	RM233-RM18602	36.01	36.01	69.79	9.64	62.12	-19.12	36.01	36.01	69.79	14.77	81.96	-20.00
		qph7.2*	7	RM427-RM248	153.37	33.97	28.43	14.74	72.60	19.80	151.37	31.97	30.43	17.54	81.77	19.98
		qph7.3	7	RM248-RM22171	189.78	7.98	16.12	3.10	17.07	9.26	189.78	7.98	16.12	2.70	18.15	9.39
		qph8.1*	8	RM38-RM331	30.01	30.01	44.89	6.87	62.08	-19.02	30.01	30.01	44.89	11.82	81.88	-19.98
		qph8.2*	8	RM404-RM547	101.46	22.06	31.04	4.48	61.83	19.06	103.46	24.06	29.04	9.51	81.62	20.02
	N2	qph3.2	3	RM570-RM517	128.4	26	26	2.67	57.42	-17.14	128.4	26	26	5.36	76.05	-18.95
		qph5.1*	5	RM233-RM18602	34.01	34.01	71.79	7.35	63.88	-18.61	34.01	34.01	71.79	10.89	78.03	-19.19
		qph7.2*	7	RM427-RM248	147.37	27.97	34.43	9.48	64.91	18.52	147.37	27.97	34.43	12.71	78.28	19.22
		qph7.1	7	RM481-RM427	106.96	15.96	12.44	2.58	16.93	9.07						
		qph8.2*	8	RM404-RM547							99.46	20.06	33.04	4.76	77.31	19.21
NT	N1	qnt1.1	1	RM495-RM243	16.24	2.04	24.56	2.86	12.37	-1.11						
		qnt8.1*	8	RM331-RM404							76.91	2.01	2.49	4.62	39.98	-5.53
		qnt8.2*	8	RM404-RM547	81.46	2.06	51.04	3.84	30.26	-4.99	81.46	2.06	51.04	5.59	40.00	-5.50
	N2	qnt8.2*	8	RM404-RM547	81.46	2.06	51.04	3.65	31.15	-5.78						
NPT	N2	qnpt8.2*	8	RM404-RM547	81.46	2.06	51.04	4.90	29.78	-3.02	81.46	2.06	51.04	10.10	51.98	-6.68
CG	N1	qcg4.1*	4	RM518-RM471	44.01	44.01	8.29	5.32	50.62	-11.86	42.01	42.01	10.29	6.63	58.15	-12.11
		qcg4.2*	4	RM471-RM252	60.28	7.98	32.02	4.42	49.52	-11.60	60.28	7.98	32.02	5.68	56.60	-12.12
	N2	qcg4.1*	4	RM518-RM471	44.01	44.01	8.29	3.99	44.08	-10.94	44.01	44.01	8.29	4.90	52.05	-11.66
		qcg4.2*	4	RM471-RM252	56.28	3.98	36.02	2.72	34.34	-10.59	58.28	5.98	34.02	2.99	46.52	-11.63
		qcg8.3*	8	RM404-RM547	83.46	4.06	49.04	3.57	36.56	-17.74	85.46	6.06	47.04	3.04	43.21	-13.14
SN	N2	qsn1.1	1	RM1-RM495	2.01	2.01	12.19	3.13	10.72	-9.00	2.01	2.01	12.19	2.56	9.80	-8.58
SF	N1	qsf4.1*	4	RM518-RM471	52.01	52.01	0.29	3.98	13.62	4.57	52.01	52.01	0.29	2.85	11.68	4.26
		qsf4.2*	4	RM471-RM252	52.28	0	40	3.68	12.64	4.44	52.28	-0.02	40.02	2.85	11.24	4.24
SF	N2	qsf4.1*	4	RM518-RM471	46.01	46.01	6.29	3.92	35.45	6.67	46.01	46.01	6.29	3.92	42.87	7.69
		qsf4.2*	4	RM471-RM252	52.28	0	40	3.45	13.06	4.88	56.28	3.98	36.02	2.99	33.78	7.04
		qsf8.1*	8	RM404-RM547	83.46	4.06	49.04	2.90	32.41	8.54	83.46	4.06	49.04	3.23	38.59	9.77
GW	N2	qgw8.1	8	RM38-RM331	70.01	70.01	4.89	2.74	44.40	-5.74	68.01	68.01	6.89	2.94	53.21	-5.93
BM	N2	qbm8.1*	8	RM404-RM547	87.46	8.06	45.04	4.12	44.02	-13.05	85.46	6.06	47.04	5.39	53.19	-15.21
SPY	N1	qspy2.1	2	RM262-RM5897	322.04	0.04	56.06	2.50	7.84	-1.85						
HI	N1	qhi8.1	8	RM404-RM547	81.46	2.06	51.04	2.68	21.34	6.54	81.46	2.06	51.04	4.14	34.76	14.71
	N2	qhi7.1	7	RM481-RM427	118.96	27.96	0.44	4.06	14.19	-2.59	118.96	27.96	0.44	3.66	14.13	-2.50
		qhi7.2	7	RM427-RM248	119.37	0	62.4	4.11	14.07	-2.58	119.37	-0.03	62.43	3.69	13.92	-2.49

*= QTLs identified both in Hyderabad and Maruteru regions.

SI Table 8. QTLs detected for yield and its components under two N levels at Maruteru employing composite interval and interval mapping (CIM and IM)

						CIM						IM				
Trait	N-Level	QTL	Chr	Marker Interval	Position	RFM	LFM	LOD	PVE	a0	Position	RFM	LFM	LOD	PVE	a0
PH	N1	qph2.1*	2	RM154-RM318	28.01	28.01	27.09	3.29	59.87	16.66	42.01	42.01	13.09	3.53	69.08	17.42
		qph2.3*	2	RM262-RM5897							483.43	161.43	105.33	4.51	72.12	-17.92
		qph4.1*	4	RM471-RM252							103.72	51.42	11.42	3.08	71.34	18.07
		qph5.1*	5	RM233-RM18602	38.01	38.01	67.79	5.85	62.77	-17.18	48.01	48.01	57.79	7.22	73.24	-18.05
		qph7.2*	7	RM427-RM248	153.37	33.97	28.43	8.94	65.37	17.47	213.74	94.34	31.94	7.78	72.66	17.96
		qph7.4	7	RM22171-RM5752							313.63	107.73	60.43	4.21	70.42	17.63
		qph8.1*	8	RM38-RM331	30.01	30.01	44.89	4.50	61.02	-16.81	86.01	86.01	11.11	4.88	73.02	18.22
		qph9.1*	9	RM219-RM434							46.01	46.01	26.19	4.76	71.44	-17.74
	N2	qph2.1*	2	RM154-RM318							42.01	42.01	13.09	8.71	79.36	19.36
		qph2.2	2	RM106-RM262	296.15	28.05	25.85	4.35	59.02	-17.87	408.83	140.73	86.83	9.88	79.57	-19.50
		qph2.3*	2	RM262-RM5897	348.04	26.04	30.06	3.76	59.33	-17.94	481.43	159.43	103.33	9.07	79.48	-19.50
		qph3.1	3	RM545-RM168	30.01	30.01	26.29	5.50	59.52	18.21	44.01	44.01	12.29	11.27	79.68	19.49
		qph5.1*	5	RM233-RM18602	28.01	28.01	77.79	10.20	67.35	-18.71	38.01	38.01	67.79	13.39	79.55	-19.42
		qph7.2*	7	RM427-RM248	145.37	25.97	36.43	10.87	65.32	18.78	199.74	80.34	17.94	14.29	79.73	19.47
		qph8.1*	8	RM38-RM331							46.01	46.01	28.89	10.61	79.65	-19.40
		qph8.3	8	RM408-RM25	209.77	27.97	25.03	4.05	61.43	18.30	309.84	128.04	75.04	9.27	79.37	19.38
		qph9.1*	9	RM219-RM434							56.01	56.01	16.19	10.83	79.69	-19.42
NT	N1	qnt8.1*	8	RM331-RM404	76.91	2.01	2.49	4.24	36.53	-6.81	119.6	44.7	40.2	4.15	42.57	-7.44
		qnt8.2*	8	RM404-RM547	81.46	2.06	51.04	6.13	39.30	-6.77	126.35	46.95	6.15	6.18	46.91	-6.52
NPT	N1	qnpt8.1*	8	RM331-RM404	76.91	2.01	2.49	3.61	25.61	-7.00	121.6	46.7	42.2	4.98	33.78	-7.42
		qnpt8.2*	8	RM404-RM547	81.46	2.06	51.04	2.88	29.56	-6.18	124.35	44.95	8.15	4.81	39.61	-6.47
PL	N2	qpl7.1	7	RM481-RM427	112.96	21.96	6.44	2.51	12.77	0.61	155.66	64.66	36.26	2.52	14.72	0.65
FG	N2	qfg7.1	7	RM481-RM427							157.66	66.66	38.26	2.51	14.38	9.03
		qfg10.1	10	RM271-RM591							86.67	45.07	14.37	3.00	13.63	-8.82
		qfg10.2	10	RM591-RM304							90.28	17.98	7.62	2.91	9.67	-7.44

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CG	N1	qcg8.1	8	RM38-RM331	70.01	70.01	4.89	2.65	39.17	-20.51						
		qcg8.2	8	RM331-RM404	78.91	4.01	0.49	7.71	35.99	-34.99	121.6	46.7	42.2	9.27	44.88	-39.19
		qcg8.3*	8	RM404-RM547	81.46	2.06	51.04	8.79	39.04	-30.68	124.35	44.95	8.15	10.57	50.64	-32.31
	N2	qcg8.3*	8	RM404-RM547	87.46	8.06	45.04	5.29	49.62	-14.43	128.35	48.95	4.15	5.48	53.25	-15.72
SN	N1	qsn8.1	8	RM404-RM547	89.46	10.06	43.04	2.57	43.42	-24.36						
SF	N1	qsf1.1	1	RM243-RM488	46.8	6	25.4	2.55	9.81	2.96						
		qsf8.1*	8	RM404-RM547							124.35	44.95	8.15	4.11	31.07	30.48
	N2	qsf8.1*	8	RM404-RM547							124.35	44.95	8.15	4.37	39.67	14.71
BM	N1	qbm8.1*	8	RM404-RM547	81.46	2.06	51.04	4.65	31.85	-15.97	126.35	46.95	6.15	5.71	45.51	-14.58
	N2	qbm8.1*	8	RM404-RM547							128.35	48.95	4.15	2.70	42.56	-12.60
SPY	N2	qspy1.1	1	RM1-RM495	2.01	2.01	12.19	2.56	9.47	1.55						
HI	N1	qhi2.1	2	RM154-RM318	36.01	36.01	19.09	3.47	48.69	-3.76						
		qhi2.2	2	RM318-RM263	71.08	15.98	30.22	2.93	46.97	-3.69						
		qhi3.1	3	RM168-RM570	84.3	28	18.1	2.58	23.84	2.63						

*= QTLs identified both in Hyderabad and Maruteru regions.

SI Table 9 QTLs detected for drought related traits under field conditions employing composite interval and interval mapping (CIM and IM)

				CIM						IM					
Treatment & Trait	QTL	Chr	Marker Intrval	Position cM	RFM	LFM	LOD	PVE	a0	Position cM	RFM	LFM	LOD	PVE	a0
Stress															
PH	qph1.1	1	RM493-RM486							115.47	21.97	20.43	4.15	71.25	16.10
	qph5.1*	5	RM233-RM18602	36.01	36.01	69.79	5.05	59.62	-15.04	38.01	38.01	67.79	5.79	72.89	-15.79
	qph7.1*	7	RM427-RM248	151.37	31.97	30.43	5.57	59.05	14.85	149.37	29.97	32.43	6.27	71.94	15.70
	qph8.1	8	RM38-RM331	22.01	22.01	52.89	6.33	65.55	-15.19	24.01	24.01	50.89	5.16	72.03	-15.65
	qph8.2*	8	RM404-RM547	103.46	24.06	29.04	5.05	59.51	15.14	105.46	26.06	27.04	6.05	72.80	16.05
NPT	qnpt6.1	6	RM204-RM225							128.77	27.97	0.13	2.98	10.48	1.63
	qnpt8.1	8	RM38-RM331							60.01	60.01	14.89	2.63	54.75	-4.29
PCG	qpcg1.2	1	RM493-RM486							119.47	25.97	16.43	2.57	62.44	-8.18
	qpcg7.1	7	RM336-RM481	65.77	21.97	25.23	2.75	39.28	5.76						
	qpcg8.1	8	RM404-RM547	93.46	14.06	39.04	2.83	51.81	-7.99	93.46	14.06	39.04	5.09	64.16	-8.65
PSF	qpsf2.1	2	RM262-RM5897	356.04	34.04	22.06	3.89	61.73	-20.30	356.04	34.04	22.06	4.38	70.62	-21.47
	qpsf7.1	7	RM336-RM481							67.77	23.97	23.23	3.76	68.40	-21.12
	qpsf9.1	9	RM219-RM434							34.01	34.01	38.19	3.25	69.97	-21.19
SCG	qscg1.1	1	RM243-RM488	66.8	26	5.4	3.12	13.86	-6.28						
	qscg1.2	1	RM488-RM493	80.19	7.99	13.31	3.63	19.54	-7.52						
	qscg4.1	4	RM471-RM252	92.28	39.38	0.02	2.73	8.42	5.12						
SCG	qscg8.1	8	RM38-RM331							62.01	62.01	12.89	3.95	61.67	-16.35
	qscg8.2*	8	RM404-RM547							91.46	12.06	41.04	3.36	61.35	-16.30
SSF	qssf2.1	2	RM154-RM318	28.01	28.01	27.09	2.73	56.64	-17.90						
	qssf2.2	2	RM423-RM106	245.47	21.97	22.63	2.58	58.92	-18.42						
	qssf2.3	2	RM262-RM5897							356.04	34.04	22.06	2.92	67.82	-19.47
	qssf5.1	5	RM233-RM18602	58.01	58.01	47.79	2.97	57.75	-18.27	48.01	48.01	57.79	2.74	68.07	19.53
	qssf8.1	8	RM38-RM331	44.01	44.01	30.89	3.27	58.05	18.47						
	qssf9.1	9	RM219-RM434	38.01	38.01	34.19	3.37	57.84	-18.23	40.01	40.01	32.19	2.70	69.37	-19.65
SGW	qsgw2.1	2	RM262-RM5897	378.04	56.04	0.06	2.63	8.33	-0.95						

Table continuous in the next page

Table continued from previous page

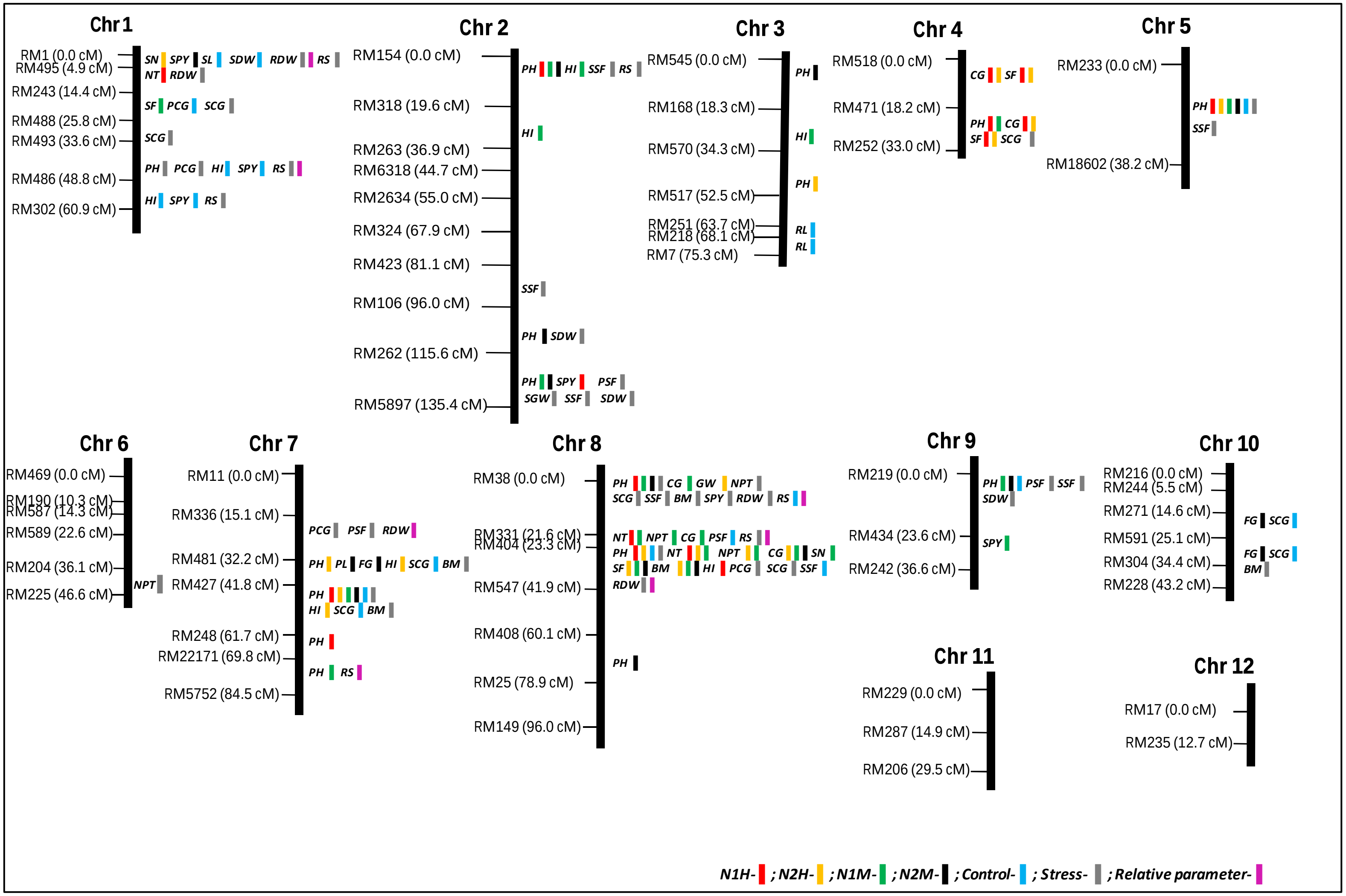
BM	qbm7.1	7	RM481-RM427	118.96	27.96	0.44	2.78	9.85	4.17						
	qbm7.2	7	RM427-RM248	119.37	0	62.4	2.78	9.49	4.10						
	qbm8.1	8	RM38-RM331							50.01	50.01	24.89	3.32	62.94	-10.82
	qbm10.1	10	RM304-RM228	115.86	17.96	5.24	3.20	14.46	4.85						
SPY	qspy8.1	8	RM38-RM331	68.01	68.01	6.89	4.56	45.95	-91.69	70.01	70.01	4.89	6.19	59.88	-108.96
Control															
PH	qph5.1*	5	RM233-RM18602							62.01	62.01	43.79	3.81	70.29	16.51
	qph7.1*	7	RM427-RM248							149.37	29.97	32.43	3.92	70.80	16.69
	qph8.2*	8	RM404-RM547							103.46	24.06	29.04	2.76	69.15	16.80
	qph9.1	9	RM219-RM434							42.01	42.01	30.19	3.16	69.79	16.48
PCG	qpcg1.1	1	RM243-RM488	50.8	10	21.4	2.78	19.30	-1.52						
PSF	qpsf8.1	8	RM331-RM404							76.91	2.01	2.49	3.47	29.04	19.45
SCG	qscg7.1	7	RM481-RM427	118.96	27.96	0.44	2.76	9.73	3.23						
	qscg7.2	7	RM427-RM248	119.37	0	62.4	2.80	9.62	3.22						
	qscg8.2*	8	RM404-RM547	89.46	10.06	43.04	2.90	40.42	-8.38	89.46	10.06	43.04	3.54	52.38	-9.29
	qscg10.1	10	RM271-RM591	71.61	30.01	0.69	3.86	13.10	3.72						
	qscg10.2	10	RM591-RM304	72.33	0.03	25.57	3.95	12.84	3.69						
SSF	qssf8.2	8	RM404-RM547							91.46	12.06	41.04	2.85	54.35	11.32
SPY	qspy1.1	1	RM493-RM486							135.47	41.97	0.43	3.82	15.87	4.43
	qspy1.2	1	RM486-RM302							135.87	0	35.8	3.83	14.47	4.29
HI	qhi1.1	1	RM493-RM486	135.47	41.97	0.43	5.17	17.53	4.73	135.47	41.97	0.43	3.82	15.87	4.40
	qhi1.2	1	RM486-RM302	135.87	0	35.8	5.23	17.00	4.71	135.87	0	35.8	3.83	14.47	4.29

*=QTLs identified under both control and stress treatments.

SI Table 10. QTLs detected for drought related traits under greenhouse conditions employing composite interval and interval mapping (CIM and IM)

					CIM						IM				
Trait	QTL	Chr	Marker Interval	Position cM	RFM	LFM	LOD	PVE	a0	Position cM	RFM	LFM	LOD		a0
Control															
SL	qsl1.1	1	RM1-RM495	2.01	2.01	12.19	4.24	18.40	-1.58	4.01	4.01	10.19	3.31	18.75	-1.56
RL	qrl3.1	3b	RM251-RM218							196.42	12.02	0.18	2.52	11.74	-0.72
	qrl3.2	3b	RM218-RM7							196.61	0.01	20.09	2.52	11.51	-0.71
SDW	qsdw1.1	1	RM1-RM495	0.01	0.01	14.19	3.48	13.69	-1.38	0.01	0.01	14.19	2.86	12.78	-1.30
RS	qrs8.1*	8	RM38-RM331	64.01	64.01	10.89	3.27	47.64	-55.13	66.01	66.01	8.59	2.67	47.73	-58.42
Stress															
SDW	qsdw2.1	2	RM106-RM262	296.15	28.05	25.85	2.63	53.86	-2.42						
	qsdw2.2	2	RM262-RM5897	350.04	28.04	28.06	2.51	53.16	-2.40						
	qsdw9.1	9	RM219-RM434	28.01	28.01	44.19	3.03	53.42	2.39						
RDW	qrdw1.1*	1	RM1-RM495	2.01	2.01	12.19	6.33	32.94	5.26	4.01	4.01	10.19	4.47	29.88	4.88
	qrdw1.2	1b	RM495-RM243							20.24	6.04	20.56	2.74	17.96	3.49
	qrdw8.1	8b	RM38-RM331							68.01	68.01	6.89	2.91	47.71	-7.87
	qrdw8.2*	8b	RM404-RM547							83.46	4.06	49.04	5.37	51.21	-9.84
RS	qrs1.1	1	RM1-RM495	4.01	4.01	10.19	3.71	25.61	90.77	2.01	2.01	12.19	2.67	18.09	75.09
	qrs1.2*	1	RM493-RM486	125.47	31.97	10.43	3.54	47.19	-131.62	125.47	31.97	10.43	3.42	60.89	-146.79
	qrs2.1	2b	RM154-RM318							20.01	20.01	35.09	3.29	62.97	136.93
	qrs8.1*	8	RM38-RM331	64.01	64.01	10.89	4.92	44.34	-143.32	62.01	62.01	12.59	4.60	63.46	-154.49
	qrs8.2*	8	RM404-RM547	87.46	8.06	45.04	5.26	43.89	-148.55	89.46	10.06	43.04	4.75	61.88	-159.49
Rel.Par.															
RDW	qrdw1.1*	1	RM1-RM495	2.01	2.01	12.19	5.73	29.29	53.08	4.01	4.01	10.19	3.98	30.58	53.43
	qrdw7.1	7	RM336-RM481	43.77	0	47.2	3.89	13.62	-34.60						
	qrdw8.2*	8	RM404-RM547	83.46	4.06	49.04	4.16	28.07	-86.94	83.46	4.06	49.04	5.52	50.26	-140.72
RS	qrs1.2*	1	RM493-RM486	133.47	39.97	2.43	3.32	19.54	-59.46	135.47	41.97	0.43	2.53	13.30	-53.32
	qrs1.3	1	RM486-RM302	139.87	3.97	31.83	3.72	24.88	-63.67	137.87	1.97	33.83	2.76	21.38	-62.16
	qrs7.1	7	RM22171-RM5752	241.93	36.03	11.27	2.71	23.77	47.79						
	qrs8.2*	8	RM404-RM547	87.46	8.06	45.04	3.17	34.64	-86.07	85.46	6.06	47.04	5.25	54.51	-109.07

*=QTLs identified under both control and stress treatments. Rel.Par.= relative parameter



SI Fig. 1 Distribution of QTLs for yield and drought related traits in the molecular linkage map of IR64/INRC10192. QTLs are indicated right side of the linkage map. Names of the markers represented left side of the linkage map. Numbers in parenthesis are relative genetic distances from one end of the chromosomes in centi morgans (cM).

List of Abbreviations

List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
bp	Base pairs
cM	Centi morgan
CTAB	Cetyl-trimethyl-ammonium bromide
DAS	Days after sowing
DH	Double haploid
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethyl diamino tetra acetic acid
g	Gram
ha	Hectare
HCl	Hydro chloric acid
hr	Hour
ISSR	Inter-simple sequence repeat
kb	kilo base pairs
LOD	Logarithm of Odds Ratio/ Limit of detection
M	Molar
mM	Milli molar
MAS	Marker assisted selection
min	Minute
mg	Milli gram
mL	Milli litre
mM	Milli molar
MT	Metric tones
PCR	Polymerase chain reaction
PEG	Poly ethylene glycol
PVE	Phenotypic Variance Explained by each QTL
QTL	Quantitative Trait Loci
r	Correlation coefficient
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RM	Rice microsatellites
rpm	revolutions per minute
SD	Standard deviation
SPAD	Special parameters analysis division
SSR	Simple Sequence Repeat
Taq	Thermostable DNA polymerase from *Thermus aquaticus*
TE	Tris-EDTA
UV	Ultra violet
w/v (weight/volume)	Percent concentration of a solute in a solution, by weight per unit volume. For example, 20% w/v = 20 g of solute in 100ml of solution.
^{o}C	Degree centigrade
µL	Micro litre
µM	Micro molar
%	Per cent
i.e.,	Which is to say in other words
viz.,	namely