Identification of Yield related Quantitative Trait Loci (QTLs) from progenitor wild species of rice, Oryza rufipogon

A thesis submitted for the degree of
DOCTOR OF PHILOSOPHY

by

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CERTIFICATE

This is to certify that **M. Pradeep Reddy** has carried out the research work embodied in the present thesis entitled ***"Identification of Yield related Quantitative Trait Loci (QTLs) from progenitor wild species of rice, Oryza rufipogon"*** for the degree of **Doctor of Philosophy** under my supervision in the Department of Plant Sciences, School of Life Sciences, University of Hyderabad.

This work has not been submitted for the award of any degree or diploma of any other University or Institute.

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DECLARATION

I hereby declare that the work presented in this thesis entitled ***Identification of Yield related Quantitative Trait Loci (QTLs) from progenitor wild species of rice, Oryza rufipogon"*** has been carried out by me under the supervision of **Prof. E.A. Siddiq,** Honorary Professor, in the Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad- 500 046 and Directorate of Rice Research, Hyderabad. This work has not been submitted for any degree or diploma of any other University or Institute. All the assistance and help received during the course of the investigation have been duly acknowledged.

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1. INTRODUCTION

Rice is the staple food for more than one half of the world's population and is cultivated worldwide under diverse agroclimatic conditions. Over 90 percent of the world's rice is produced and consumed in Asia. Most of the consumers, whose staple is rice l ive in less developed countries. The United Nations' recent publications indicate that the world population would increase by 35% from 5.69 billion in 1995 to 7.67 billion by 2020. The increase will be more than 95% in the developing countries, whose share of the global population is projected to be around 84% in 2020. Over the period absolute population increase would be highest in Asia, where rice has to meet largely its food needs (Pinstrup-Anderson *et al,* 1996). Thanks to the plant type based high yield ing varieties' introduction and extensive adoption of them since mid 1960s enabled many countries in the continent including india attain and remain till now self sufficient in food and rice. To sustain the current level of sufficiency, rice production has to grow by more than 40% by 2020. On the basis of modest income growth of 5 percent and the current consumption pattern, demand projection of milled rice for india has been estimated at 125 million tonnes by 2020 which amounts to an additional 40 million tonnes. 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 as yet to cater to the needs of the vast rainfed ecosystem and plateauing of produ ctivity growth in high productivity areas, achieving of the projected target would be a challenging task (Siddiq, 1999). Consolidation of sizeable yield potential still remaining untapped in the currently available high yielding varieties and maximization of

yield levels of relatively favourable rainfed ecologies would help sustain the growth for a couple of decades maximum. Such opportunities cannot sustain the population growing indefinitely warranting need for technologies with new yield thresholds. Four major strategies are being contemplated world over to raise the ceiling to genetic yield. They are (i) exploitation of hybrid vigour (ii) tailoring of new plant type with more efficient physio -morphological frame (iii) unlocking and use of hitherto untapped yield genes hidden in the land races and wild species of rice and (iv) engineering of biosynthetic pathway of starch biosynthesis. While exploitation of hybrid vigor and development of new plant type varieties are now distinct possibilities for commercialization, given the progress made in China, IRRI and IARI (Khush, 1995). Sustenance of these strategies would depend on availability and rational deployment of new variability for yield related traits.

Traits of economic importance such as yield and its components are of complex inheritance governed by a number of genes with small effect. The yield breakthrough achieved through plant type based varieties in the sixties has been by exploiting a simply inherited non -lodging dwarf trait accessed from a single source Dee-Geo-Wugen. Since then keeping high yields as the common objective, breeding research has been to improve accessory characteristics like resistance to biotic stresses, improved cooking quality etc., rather than for more focused research to raise the genetic yield level. On so generated material representing a very narrow genetic variation breeders continue to depend on for progressive yield improvement. It is the notion among breeders that the improved cultivar gene pool now in use possessed the required level of

variability (allelic variation) for achieving higher yield thresholds that continues to dissuade them from going in search of additional yield genes from the least used primitive land races and wild/weedy progenitor species of *O.sativa*. In fact, the germplasm breeders depend on today represents not more than 15% of the total variability available and over 85% of the genetic variation remains untapped in the wild/weedy relatives (Wang *et al,* 1992). The importance of the wild/weedy gene pool representing such a large variability is not however know beyond finding it as a source of a few rare and valuable major genes. But for stray reports, hardly there is any systematic study to the effect that wild germplasm as well could be a source of varia bility for quantitatively inherited traits like yield. Characteristic to quantitative traits, yield is conditioned by several genes having predominantly either trait-enhancing "positive" or trait-depressing 'negative" alleles. Desirable positive alleles pr esent at very low frequencies in the wild/weedy species are invariably masked by the effects of the deleterious negative alleles occurring at high frequencies. As a result the phenotype of the wild/weedy species looks agronomically inferior leading to the wrong notion among breeders that wild/weedy species component of the germplasm is of low breeding value for yield enhancement. It is increasingly evident that largely due to lack of techniques, potential variability for yield available in the wild germplasm could not be assessed and utilized for long.

Rapid advances in cellular and molecular biology during the last two decades have provided, among several, two innovative tools for genetic manipulation of plant genomes *viz.,* recombinant DNA technology for mo ving

genes across sexual barriers and molecular marker technology for achieving precision in selection. While genetic transformation is dependent on availability of candidate genes, tissue specific promoters and efficient transformation protocols, marker-assisted selection depends on availability of high -density map. The advent of DNA based markers such as Restriction Fragment Length Polymorphism (Helentjaris, 1987), Random Amplified Polymorphic DNA (Williams *et al,* 1990), Simple Sequence Repeats or Microsa tellites (Jeffereys *et* al, 1985a), Amplified Fragment Length Polymorphism (Zabeau and Vos, 1993) and Inter Simple Sequence Repeats (Zeitkiewicz, 1994) have led to the development of high -density molecular maps for plant species. The maps have enabled mapping and tagging of several traits of economic significance. Using Restriction Fragment Length Polymorphism (RFLP) markers, the first ever genome map in plants was reported in maize (Helentjaris *et al,* 1986; Stuber *et al,* 1987) closely followed by tomato (Tanksley and Hewitt, 1988), rice (McCouch *et al,* 1988) and *Arabidopsis* (Cheng *et al,* 1988; Nam *et al,* 1989). Mapping of microsatellite markers in plants was initiated by Zhao and Kochert (1992) in rice using tri-nucleotide repeat $(GGC)_n$, followed by mapping of di-nucleotide repeats by Panaud *et al* (1995). The first report on gene tagging was in tomato (Paterson ef *al,* 1988) followed by a series of reports on successful linking of characters of economic importance with molecular markers (Schachermayr *et al,* 1994). These pioneering efforts have led to better understanding of the genetics of quantitative variations and served as models for subsequent work to exploit the tool in breeding for complexly inherited traits

like yield etc. Like Mendelian traits polygeni c traits have also been mapped using marker association in a variety of crop plants including rice (Paterson *et al* 1988). In rice, using highly saturated molecular map several gene blocks governing genetically complex polygenic traits like yield and its co mponents could be tagged (Xiao *et al,* 1996b, c; Hayes *et al,* 1993).

The range of genetic variation for a trait is often believed to be much greater in exotic (wild) germplasm than among the cultivated types as the latter are usually derived from a small nu mber of ancestral species and have been selected over centuries (Vaughan, 1994). Rice is one such crop endowed with rich genetic diversity but least exploited. The genus *Oryza* comprises 21 wild and 2 cultivated species representing nine different genomes. *Oryza rufipogon* and *O. nivara* closely related to and progenitors of the world over cultivated Asian cultivar *O.sativa* and *O.barthii* and *O. longistaminata* corresponding wild relatives of the African cultivar *O.glaberrima* sharing the AA genome along with cultivars account for the maximum variability. Utilization of these wild relatives, especially *O. rufipogon* and *O. nivara* is critical to future improvement of Asian cultivated rice considering their compatibility with the latter and the fact that sizeable allelic variation of genes related to traits of complex inheritance still remains in them uncaptured in the course of origin and domestication of cultivars (Tanksley and McCouch, 1997). As experienced in other crop species the genetic potential of the progeni tor species growing sympatrically with *O. sativa* has been evident so far from a few valuable Mendelian genes accessed from them and utilized in the improvement of cultivated rice. Whereas *O. rufipogon*

provided the widely used WA (wild abortive) source of c ytoplasmic male sterility for hybrid rice production (Yuan, 1997), 0. *nivara* proved a valuable source for the rare genes for resistance to grassy stunt virus, sheath blight, bacterial leaf blight and abiotic stresses such as acidic soils (Vaughan, 1994; Br ar and Khush, 1997; Khush *et al,* 1999; Dalmacio *et al,* 1996). As against the foregoing reports on the utility of the wild species limited to simply inherited traits, the recent investigations using high -density molecular markers have shown wild species of rice to possess a rich reservoir of genes that directly or indirectly influence grain yield (Xiao *et al,* 1998). The pioneering report has aroused interest all over to search for new allelic variations of yield genes in rice and explore the possibility of using them (Xu, 1997).

During the last five years as many as 70 yield related unique QTLs have been identified in rice by various workers (Zhang and Yu, 2001). Significantly the two unique yield influencing QTLs *yld1.1* and *yld2.1* identified in an accessi on of *O.rufipogon* by Xiao *et al* (1998) have been exploited by conventional breeding by Chinese breeders to raise further the yield ceiling (Yuan, 2001). Keeping in view the unlimited potential of wild/weedy species of rice for yield genes as evident from the foregoing and the high density molecular map now available to identify and tag new yield related QTLs, the present study has been undertaken with the following two specific objectives:

(i) Identification of prospective *Oryza rufipogon* accession for use a s donor having unexploited genetic potential for yield.

(ii) Mapping of unique molecular marker -associated yield QTLs identified in the wild species.

The salient findings threrefrom have been presented in this thesis

2. REVIEW OF LITERATURE

2.1 Molecular Markers and Their Application in Plant Breeding

Sustained global ability to provide adequate quantity of food, feed and fiber from domesticated plant species has been largely through continuous and concerted crop improvement efforts by both farmers and plant breeders spanning many millennia. Progressive yield enhancement employing various breeding/selection approaches has been the major means of production growth althrough as extensively reviewed by Stuber *et al* (1999). The breeding efforts have been on the strength of germplasm in te rms of genetic variability. During the first half of the last century, studies on genetic variability were largely based on gross morphological, anatomical and behavioral features. Subsequently, between 1950s and 1980s, it was possible to look at more subt le variation in the structure of polypeptides. Since 1980s, it has become possible to explore and understand the extent of variation at the level of DNA itself. No matter at what level one is studying, variability- from subtle variations in DNA to an obser vable change in phenotype- distinct differences can be used as genetic markers to identify traits of interest and map to specific chromosomal regions. Since 1990's classical parameters are being complimented by molecular markers involving macromolecules li ke proteins and DNA. Advances in marker technology have not only revolutionized the methods of genetic analysis but also helped greatly to accelerate breeding programme for improvement of traits of complex inheritance.

Though isozymes have been initiall y exploited as molecular markers for variability analysis and construction of framework genome maps, their

availability in small numbers and stage dependant expression proved a serious limitation to their use as markers for assessment of breeding material. DNA markers that obviate these limitations are extensively used in crop breeding programmes. DNA markers are ubiquitous and innumerable, discrete and nondeleterious, inherited in Mendelian fashion, unaffected by environment, and are free of epistatic inte ractions and pleiotropic effects. (Beckman and Soller, 1986; Tanksley *et a*/, 1989). Today, various types of DNA markers are employed for mapping and tagging of agronomically important traits and marker assisted selection techniques so developed are provin g handy in screening segregating populations with precision in breeding programmes. They, once mapped enable dissection of complex traits into component genetic units more precisely (Hayes *et al*, 1993), thus providing breeders with new tools to manage complex traits more efficiently. Basic information and potential of various types of DNA markers such as Restriction Fragment Length Polymorphism (RFLP), and PCR-based markers like microsatellites, minisatellites, RAPDs and their utility are described below in more detail.

2.1.1 Restriction Fragment Length Polymorphism: RFLP is as a result of differences in the nucleotide sequence in the DNA of two different genotypes. Such differences due to either mutations or sequence rearrangements that might have occurred during the course of evolution are detected as variation (polymorphism) in the length of restriction fragments. RFLPs are co -dominant markers and they are very reliable in linkage analysis and selection process. They can be used to study the nature of link ed trait i.e., hetero or homozygous

state, where such information is highly desirable, especially for recessive traits. Single copy genomic DNAs or cDNAs have been used as molecular markers to construct RFLP maps of crop plants including rice (Mc Couch *et al,* 1988; Saito *et al,* 1991), maize (Helentjaris, 1987), lettuce (Landry *et al,* 1987), soyabean (Keim *et al,* 1990), tomato (Yang and Tanksley, 1989), potato (Gebhardt *et al,* 1989) and wheat (Cho *et al,* 1989). Genetic maps based on such markers have many applications in plant breeding. Dense genetic maps developed using these markers have been found useful in resolution of some of the long pending questions from classical genetic studies such as intergenomic relationships between maize and sorghum (Hulbert *et al,* 1990) and between tomato and potato (Bonierbale *et al,* 1988). Inspite of its usefulness, RFLP marker technology is less used being time consuming and expensive.

2.1.2 **PCR-based** markers: The polymerase chain reaction (PCR), an *in vitro* method for enzymatic synthesis of specific DNA sequence, is yet another significant step forward in the field of molecular biology (Mullis and Faloona, 1987). It uses two **oligonucleotide** primers of about 10 -20 nucleotides in length that specifically anneal to opposite s trands flanking the region to be synthesized. Several cycles of DNA denaturation, primer annealing and extension of annealed primers by DNA polymerase, produce an exponential amplification of a specific DNA sequence. There are different kinds of PCR based markers, which are useful in genome mapping and gene tagging.

2.1.2a Random Amplified Polymorphic DNA: Generation of RAPDs, which are dominant markers, involves use of single short but random oligonucleotides.

When the random primers are mixed with genomic DNA, mixture of deoxy ribonucleotide and thermostable DNA polymerase and are subjected to PCR, several DNA fragments are amplified simultaneously. The DNA amplification with random primers exposes polymorphisms that are distributed throughout the genome (Williams *et al,* 1990). RAPD markers in genome mapping and gene tagging have been exploited by many (Martin *et al*, 1991; Paran *et al,* 1991; Michelmore *et al,* 1991; Reiter *et al,* 1992). Lack of repeatability, stability and accurate detection of heterozygotes are some of the limitations of RAPDs, which can however, be overcome by converting them into codominant RFLP markers for stable performance.

2.1.2b Variable Number Tandem Repeats: Variable Number Tandem Repeats (VNTRs) include microsatellites, minisatell ities and hyper variable regions. Microsatellites are arrays of tandemly repeated DNA sequences, which are dispersed throughout the genome (Jeffreys *et al,* 1985a) and are also referred to as " Sequence Tagged Microsatellite Sites (STMS)". Microsatellites consist of around 10-50 copies of motifs ranging from 1 to 5 bp that occur in perfect tandem repetition, as in perfect repeats or in imperfect repeats i.e., together with another repeat type. The repeat number of microsatellites has been demonstrated to be highly variable in animals and inherited in a co -dominant manner (Litt and Luty, 1989; Johansson *et al,* 1992). Presence of microsatellites has been documented in many plants, which include *Arabidopsis* (Bell and Ecker, 1994), barley (Saghai-Maroof *et al,* 1994), *Brassica* (Langercrantz *et al,* 1993), maize (Condit and Hubbel, 1991; Senior and Heun, 1993), rice (Wu and

Tanksley, 1993), soybean (Akkaya *et al,* 1992; Morgante and Olivieri, 1993) and wheat (Roder *et al,* 1995). Microsatellite molecular marker linka ge maps developed in crops such as barley, wheat, rice, soyabean and *Arabidopsis* are gaining increasing importance in recent years.

Minisatellites comprise a class of variable number of tandem repeat (VNTR) loci, in which the repeated sequences are short (<65 bp) and frequently GC rich (Jeffreys *et al,* 1985a,b; Nakamura *et al,* 1987). Highly polymorphic and dispersed, minisatellites present in large numbers appear to be an ubiquitous feature of eukaryotic genomes (Ryskova *et al,* 1988). Minisatellites have be en widely used in humans and various animal species to yield DNA 'fingerprints', which are unique to each individual. A 15bp repeat motif in the protein III gene of bacteriophage M 13 appears to be extremely useful in detecting polymorphism (Vassart *et al,* 1987) and serves as a universal marker for DNA fingerprinting (Ryskova *et al,* 1988). It has been used by them for the first time in plants to identify polymorphic regions in barley. Subsequently it found its use in detection of minisatellite-like sequences in a few gymnosperms and angiosperms (Rogsted *et al,* 1988; Zimmerman *et al,* 1989). Jeffrey's 33.6 and 33.5 minisatellite probes have been used for fingerprinting Asian and African rices (Dallas, 1988), while a human minisatellite probe, pV 47 has been sh own to be polymorphic in *indica*, *japonica* and wild rices (Ramakrishna *et al,* 1995).

2.1.2c Inter Simple Sequence Repeats: Inter-simple sequence repeat analysis involves polymerase chain reaction (PCR) derived amplification of regions between adjacent, inversely oriented microsatellites using a single simple

sequence repeat (SSR) containing primer (Zietkiewicz *et al,* 1994). This technique can be applied to any species that contains sufficient number and distribution of SSR motifs. Its another advantage is th at genomic sequence data is not required (Gupta *et al,* 1994; Godwin *et al,* 1997). The primer used in ISSR analysis can be based on any of the SSR motifs (di -, tri-, tetra-, or penta-nucleotides) found at microsatellite loci, giving a wide array of possible amplification products, and can be anchored to genomic sequences flanking either side of the targeted simple sequence repeats. For ISSR analysis to be successful, pairs of simple sequence repeats must occur within a short distance (in base- pairs) that is amplifiable by a PCR reaction, which produces a band that is resolvable on standard polyacryiamide or agarose gel (Zietkiewicz *et al,* 1994). The potential supply of ISSR markers depends on variety and frequency of microsatellites, which vary with species and SSR motifs, that are targeted (Morgante and Olivieri, 1993; Depeiges *et al,* 1995).

As the ISSR technique amplifies a large number of DNA fragments per reaction, representing multiple loci across the genome, it is an ideal method for fingerprinting rice varieties and a useful alternative to single -locus or hybridization-based methods (Zietkiewicz *et al,* 1994; Godwin *et al*, 1997). The ISSR method has proven its usefulness, especially in the *Gramineae* family for analysis of near isogenic lines (Akagi *et al,* 1996) and varieties (Paterson *et al,* 1997; Swathi *et al,* 2000) of rice, inbred lines of corn (Kantety *et al,* 1995), populations of finger millet (Salimath *et al,* 1995) and accessions of sorghum (Yang *et al,* 1996).

2.1.2d Amplified Fragment Length Polymorphism: Production of amplified fragment length polymorphisms (AFLPs) is based on Selective Restriction Fragment Amplification of DNA fragments (SRFA) (Zabeau and Vos, 1993). SRFA involves three major steps viz., i) cutting genomic DNA with restriction enzyme(s) ii) ligating double stranded adapters to the restriction fragments and iii) amplifying selective restriction fragments using universal primers. SFRA may be performed with a single enzyme, but the best results are achieved when two different enzymes, a rare cutter and a frequent cutter are used. AFLP is a powerful, reliable, stable and rapid assay with potential application in genome mapping, DNA fingerprinting and marker -assisted breeding (Vos *et al*, 1995). This PCR-based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amounts of DNA. The reproducibility of AFLPs is ensured by using restriction site specific adapters and adapter -specific primers with variable number of selective nucleotides under stringent amplification conditions. (Vos *et* a/, 1995). Because of their various advantages, AFLPs **are** currently considered as the molecular markers of choice within the genome mapping community.

Apart from development of new type s of markers, various technological simplifications have been made to resolve the problems associated with molecular marker technology. The recent PCR based approach, gel free visualization of PCR products and automation at various steps are boons to the molecular marker approaches adopted for genome mapping.

2.2 Genetic diversity and prediction of heterosis

Heterosis may be defined as superior performance in growth, vigour, adaptability and other morpho-physiological traits of F_1 population of two diverse parents. Genetic basis of heterosis not precisely understood and attributed is the combined effect of inter-allelic (epistatic) and/or intra-allelic (dominance) interactions in the heterozygous F_1s. Cross combinations involving genetically diverse parents are likely to give more heterotic hybrids than those that are genetically related. Breeders' interest always lies in choosing parents that will yield heterotic combinations for heterosis as well as recombination breeding. Several methods such as *per se* performance of parents and extent of genetic diversity as determined through multivariate analysis using morphological and agronomic traits have been the criteria for choosing parents in commerical hybrid breeding.

With recent developments in the field of molecular biology, molecular markers such as RFLP, RAPD, AFLP and microsatellites have come handy for breeders to assess extent of variability available in the germplasm of a crop plant and identify diverse ones for use in breeding programmes. A lot of studies have been carried out in crop plants to relate heterosis for a given trait with genetic distance of the parents. Correlation between isozyme diversity and combining ability for grain yield estimated in single cross hybrids of maize was found to be low and non-significant (Stuber *et al*, 1999). Probably, low number of isozyme loci representing only a fraction of the genome and fewer parental lines used might have contributed to the non-significant relationship. Later

studies using a large number of loci and cross combinations too revealed no consistent relationship with some combinations showing high correlations between molecular marker distances of the parents and hybrid performance *per se* (Lee *et al,* 1989; Smith *et al,* 1990), while others showing low correlations (Godshalk *et al,* 1990; Dudley *et al,* 1991).

In oilseed rape, study based on RFLP markers although revealed the genetic distance (GD) estimates to be significantly correlated with heterosis for seed yield (Diers *et al,* 1996), general combining ability (GCA) showed stronger relationship. The foregoing reports on the nature and strength of relationships between GD and yield vigour suggest that GD alone cannot vouch for the level of heterosis. Extensive studies carried out in rice to understand the relationship between molecular marker based diversity and hybrid performance, have also revealed variable results. Whereas Khush and Aquino (1994) have shown the level of heterosis expressed in hybrid rice to be directly prop ortional to genetic distance between the respective two parents, Zhang *et al* (1994; 1995) report high correlations between specific heterozygosity and heterosis of yield and biomass in a diallel cross of eight elite *indica* lines widely used in hybrid rice production in China. Saghai Maroof *et al* (1997) also reported subsequently very high correlation between yield heterosis and general heterozygosity from a study of diallel cross involving eight long -grain varieties from southern United States. Results of Xiao *et al* (1996a), who studied relationships between marker distance and hybrid performance in a diallel of intra - and inter-subspecific crosses, indicate correlations to vary with the parental choice with crosses

involving *japonica* showing high, intermediate in those involving *indica* and insignificant in *indica x japonica* crosses. Similarly, results of Zhao *et al* (1999) also indicate that correlations of marker heterozygosity with hybrid performance differed considerably between the two subspecies with con tributions involving *japonica* being higher than those involving *indica* varieties. Interestingly hardly any correlation could be detected in inter-subspecific crosses. In a recent study using STMS and RFLP markers Joshi *et al* (2001), have shown existence of significant correlation between genetic distance of the parents and hybrid performance in rice.

However, in a study on inter-subspecific hybrids of rice it has been shown congruently that parental genotypic divergence had relatively low impact on heterosis for major yield components, *i.e.* panicle number per plant and 1000-grain weight, while great bearing on fertility parameters *i.e.* filled grains per plant and percentage seed set. Thus fertility parameters appeared to be the most sensitive and were influenced maximum by extent of genetic divergence. The findings further showed parents of moderate divergence to express high heterosis at inter-subspecific level (Li *et al,* 1998). Notwithstanding all the foregoing reports on the relationship of lev el of heterosis with genetic diversity of parental source or extent of heterozygosity, none of the latter alone can be a good indicator for predicting heterosis as concluded by Liu and Wu (1998).

2.3 Utilization of wild germplasm as sources of agronomicall y useful traits

The modern day cultivars of rice, inspite of all their high yielding potential and other desirable features are handicapped with narrow genetic base for most

of the agronomically important traits including the dwarf habit, which is the major yield enhancing trait. Recent study of high yielding varieties for their ancestry reveal that hardly 5 to 6 accessions accounted for more than 90% of their genetic constitution confirming that the cultivar gene pool being depended on now for improvement represent hardly 15% of the total genetic variability available in rice germplasm. It amounts to the fact that a large genetic variability still remains untapped in the wild relatives and primitive cultivars of rice (Wang *et al,* 1992). Thus, there is an urgent need to assess the extent of exploitable variability available in the wild/weedy species of the genus *Oryza.*

Rice is endowed with very rich genetic diversity. Wild/weedy species along with very large number of primitive cultivars and land races constitute an important reservoir of useful genes. The genus *Oryza* has 23 species including 2 cultivated species. *O.sativa* of Asia and *O.glaberrima* of Africa (2n=24) belong to AA genome. The wild species are either diploids (2n=24) or tetraploids (2n =48) representing AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ genomes. Morishima and Oka (1981) based on their study of 42 morphological variables clustered the *Oryza* species into three complexes *viz.,* sativa, officinalis and other distantly related species complex. On the basis of their relative sexual compatibility *O.rufipogon*, *O.nivara, O.glumaepatula*, *O.meredionalis. O.breviligulata, O.longistaminata* alongwith the two cultivar species constitute the primary gene pool. They share the AA genome and gene t ransfer among them can be accomplished through conventional hybridization and selection procedures. Species *O.officinalis, O.latifolia, O.alta, O.minuta, O.punctata,*

O.australiensis etc., belonging to the *officinalis* complex constitute the secondary gene pool. They represent CC, BBCC, CCDD, EE genomes. Crosses between species of sativa complex and those of officinalis complex can be accomplished through embryo rescue technique only. Since there is limited homology between the A genome of *O.sativa* and CC, BBCC, CCDD and EE genome species of the Officinalis complex gene transfer is rare and limited. Species *O.meyeriana*, *O.ridleyii* and *O.schlechteri* which belong to GG, HHJJ genomes constitute the tertiary gene pool (Khush, 1997).

Considering the large hidden variability and very rare and agronomically important genes they posssibly possess utilization of the wild species is critical to future improvement of rice. The size of additional variability they can provide, especially in the context of shrink ing gene pool currently depended on would be of great value to the ongoing crop improvement endeavour. Low crossability and limited recombination, however, limit transfer of desired genes from wild species to cultivars (Brar and Khush, 1986; Khush and Brar , 1992). Recent advances in cellular and molecular biology have made exploitation of distantly related species of *Oryza* in rice improvement possible. The first instance of successful use of wild species for rice improvement is the development of the drough t tolerant variety wherein tolerance was transferred from *O.perennis* (Srinivasan *et al,* 1941; Rajagopalan, 1957; 1958). It was much later that introgression of a gene for grassy stunt virus resistance accessed from *O.nivara* was accomplished (Khush, 1997). This achievement prompted breeders at IRRI to intensify the search for more and more resistance genes against major pests

and their biotypes/races. Identification and transfer of a dominant bacterial blight (BB) resistance gene *(Xa21)* from *O.longistaminata* by backcrossing to IR24 is one significant instance (Khush *et al,* 1990). This gene has a very wide spectrum of resistance to more than 30 races of BB. Significantly it was again the closely related A genome species *O.sativa f. spontanea* that provided the wild abortive (WA) source of CMS, now in worldwide use for commercial hybrid rice seed production (Lin and Yuan, 1980). Recently CMS sources alternate to WA have been found in *O.perennis* (Dalmacio *et al,* 1995), *O.glumaepatula* (Dalmacio *et al,* 1995) *O.nivara* and *O.rufipogon* (Hoan *et al,* 1997). Jena and Khush (1990) have been successful in producing several introgressed rice lines with alien resistance genes against different pests. Whereas the introgressed lines derived from the cross *O.sativa x O.minuta* (BBCC) showed resistance to race 6 of BB and race P06-6 of blast (Amante-Bordeos *et al,* 1992), introgressed lines from crosses involving wild species of CCDD and EE genomes resulted in resistance to BPH, WBPH and BB (Multani *et al,* 1994).

Recently, the wild species have been found to be potential sources of variability for quantitative traits such as yield and its components. In the first ever report on the use of wild species for introgression of quantitative characters, Xiao *et al* (1996c) have identified in a Malaysian accession of *O.rufipogon* two yield QTLs, *Yld1.1 and Yld 2.1* each of which is capable of increasing yield by about 18%. In a study involving the same accession of the wild species, an advanced backcross breeding strategy was used t o identify QTLs associated with eight other agronomic traits. The QTLs introgressed from the accession of

O.rufipogon included 2 for yield, 13 for major yield components, 4 for maturity and 6 for plant height (Moncada *et al,* 2001). In a more recent report on introgression of yield -related traits from wild species, *O. glumaepatula* was used to transfer QTLs for yield and its components into cultivated rice (Brondani *et al,* 2002). Most of the experiments for transfer of QTLs from wild species have used advanced backcross method.

2.4 QTL mapping for yield and its components

Most traits of agronomic importance such as yield and its components, nutritional quality and abiotic stress tolerance are quantitatively inherited (Allard, 1960; Hallauer and Miranda, 1988). Characters, whose phenotypic variation is continuous and determined by segregation of multiple loci have often been referred to as quantitative and the inheritance as polygenic (Thoday, 1961). A chromosomal region linked to or associated with a marker gene, which affects a quantitative trait is defined as Quantitative Trait Locus (QTL) (Geldermann, 1975). Although numerous quantitative studies have revealed the relative importance of genes with different actions on quantitative traits, methodology itself does not allow resolution of continuous trait variation into individual underlying mendelian factors. Advances in DNA markers and molecular linkage maps have stimulated a new area of molecular quantitative genetics through mapping of QTLs (Zhikang Li, 2002). One of the most important applications of DNA markers and molecular linkage maps is dissection of genetic variation of quantitative traits into individual mendelian factors through QTL mapping analyses. QTL mapping can thus be defined as the marker-facilitated genetic

dissection of variation of complex phenotypes through appropriate experimental design and statistical analysis of segregating material. In QTL mapping, genes controlling variation of quantitative traits in segregating populations are resolved into individual mendelian factors by determining the number, location, gene effect and action of loci involved and their interactions with other loci and environment. In the past decade, many rice QTL mapping studies have been carried out and QTLs affecting a wide range of phenotypes have been identified. A review of the studies on QTLs relating to yield and its components is as under:

The first ever QTL analysis of yield components associated with heterosis in rice was done by Xiao *et al* (1995). Based on a recombinant inbred line (RIL) population and derived testcross lines, their study identified QTLs associated with nine components of yield in addition to plant height, days to heading and days to maturity. Nineteen QTLs for heading time were mapped on all the 12 chromosomes using doubled haploid (DH) population of an *indica/japonica* cross. Methods of composite interval mapping and multiple trait composite interval mapping were used to map QTLs (Zhou *et al,* 2001). In a study of QTL analysis for yield and yield components of an F_2 population based on data from replicated field trials using vegetative shoots of ratooned plants, 20 QTLs in all for yield, tillers/plant, grains/panicle and grain weight distributed on seven of the 12 chromosomes were detected. Eight of these were detected in both the years of the trial, while the remaining 12 were detected in only one year. It was also shown that these QTLs were highly concentrated in a few chromosomal regions or QTL hot-spots. This indicated that vegetative ratooned F_2 populations have

considerable utility in mapping QTLs, especially if dominant types of gene actions are of concern (Li *et al,* 2000). Redona and Mackill (1998) have identified QTLs controlling six panicle and grain characteristi cs using composite interval mapping in an F_2 population of the cross tropical *japonica x indica*. Two QTLs were identified for panicle size, four for spikelet fertility and seven, four, three and two for length, breadth, shape and weight of grains respectiv ely. Scan-analysis revealed existence of two QTLs with large effects *viz., Hd-1* and *Hd-2,* one in the middle of chromosome 6 and other at the end of chromosome 7, respectively. A secondary scan-analysis carried out by removing phenotypic effects of *Hd-1* and *Hd-2* identified another three QTLs *viz., Hd-3, Hd-4* and *Hd-5* located respectively on chromosomes 6, 7 and 8. The five QTLs explained 84% of the total phenotypic variation in the F_2 population (Yano *et al,* 1997). In a study of 194 recombinant inbred lines (F_8) of an inter sub-specific cross between two elite homozygous lines 9024 and LH422, QTLs for 13 quantitative traits including grain yield were identified. A total of 37 significant QTLs were identified for 13 traits with the number of QTLs detected for each of the traits ranging form one to six and percentage of phenotypic variance explained by each QTL ranged from 5.0 to 73.7% (Xiao *et al,* 1996b).

Comparative mapping of QTLs for six agronomic traits across three diverse environments in a DH p opulation 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 significant in all the thre e environments, while another

seven were in two environments and eight could only be detected in a single environment. QTLs for spikelets 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 (Lu *et al,* 1996). In a study of an F_2 population of *indica x indica* cross, 14 QTLs were identified for yield and its related characters. Three QTLs each were identified for grain weight per plant and 1000 -grain weight, two each for number of panicles per plant and grain number per panicle and one each for spikelet fertility and spikelet number per panicle. One QTL was identified for number of first branc hes per main panicle (Lin *et al,* 1996). Li *et al* (1995) have identified three QTLs *QHd3a, QHd8a* and *QHd9a* for heading date collectively explaining a phenotypic variance of 76.5% and four QTLs for plant height in the F_4 population of a cross involving two s emidwarf varieties *viz.,* 'Lemont' and Teqing'.

In the first ever report on utilization of wild species in rice for improvement of yield and its component characters Xiao *et al* (1998) had identified 68 significant QTLs in the *O.rufipogon* accession from Malaysia. Fifty percent of these had beneficial alleles derived from the phenotypically inferior *O.rufipogon* parent. Using an advanced backcross approach for population development and single marker analysis for identification of QTLs, two QTLs were identifie d on chromosomes 1 and 2 that resulted respectively in 18 and 17% increase in grain yield. In their study on an advanced backcross population derived from an interspecific cross between Caiapo, an upland *O.sativa* subsp *japonica* rice

variety from Brazil, and an accession of *Oryza rufipogon* from Malaysia, Moncada *et al* (2001) have found that 56% of the trait enhancing QTLs identified in this study were derived from *O.rufipogon*. They detected two QTLs each for yield, panicles per plant, and percent spikelet st erility, four for grains per plant, five for 1000 grain weight, four for maturity and six QTLs for plant height. Three methods *viz.,* single point analysis, Interval mapping and composite interval mapping were used for QTL mapping and a comparison of the th ree methods revealed that all of them identified similar QTLs about 80% of the time.

3. MATERIAL AND METHODS

The present study was envisaged to identify and map Quantitative Trait Loci (QTLs) related to yield and its components introgressed from the progenitor species of cultivated rice, *Oryza rufipogon.* The material used and methods employed are detailed as under

3.1: Identification of prospective accession of *O.rufipogon* for use as donor parent for introgression of yield QTLs

3.1.1 Material: The experimental material comprised 25 accessions of *O.rufipogon,* five accessions of *O.nivara,* three cultivars *viz.,* IR64, Jaya and Mahsuri, the restorer line (KMR3) and cytoplasmic male sterile line (IR 58025A) of commercial hybrid KRH2 (Table 1). Fourteen accessions of *O.rufipogon* were obtained from the Genetic Resource Center at the International Rice Research Institute, the Philippines **and** the rest from the germplasm collection maintained under the National Professor (ICAR) project at the Directorate of Rice Research (DRR), Hyderabad, India. The 29 F_1 plants derived from the cross IR 58025A x IC 22015 *(O.rufipogon)* **and** the advanced backcross generations *viz.,* BC_1 (50 plants), BC_2 (251 plants) and 251 testcross families also comprised the experimental material.

3.1.2 Methodology

3.1.2.1 Genetic diversity analysis: Analysis of genetic diversity was done using 14 $(AG)_n$ and $(GA)_n$ based inter simple sequence repeat (ISSR) primers.

3.1.2.1a Extraction of genomic DNA and quantification: DNA was extracted from two month old leaf tissue f rom all the 30 accessions of the wild species as well as the cultivars using the protocol of Dellaporta *et al* (1983), briefly described below.

Table 1: Origin and source of wild and cultivar accessions of rice used in this study

S.No.	Sample No.	Accession No./Variety	Species	Origin	Source
1	R1	IRGC 100189	*O.rufipogon*	Malaysia	IRRI
2	R2	IRGC 100204	*O.rufipogon*	India	IRRI
3	R3	IRGC 100916	*O.rufipogon*	China	IRRI
4	R4	IRGC 101193	*O.rufipogon*	Taiwan	IRRI
5	R5	IRGC 103827	*O.rufipogon*	Bangladesh	IRRI
6	R6	IRGC 103844	*O.rufipogon*	Bangladesh	IRRI
7	R7	IRGC 104311	*O.rufipogon*	Thailand	IRRI
8	R8	IRGC 104409	*O.rufipogon*	Thailand	IRRI
9	R9	IRGC 104433	*O.rufipogon*	Thailand	IRRI
10	R10	IRGC 104479	*O.rufipogon*	Thailand	IRRI
11	R11	IRGC 105308	*O.rufipogon*	India	IRRI
12	R12	IRGC 105325	*O.rufipogon*	India	IRRI
13	R13	IRGC 105400	*O.rufipogon*	China	IRRI
14	R14	IRGC 105569	*O.rufipogon*	Cambodia	IRRI
15	R15	IC 87973	*O.rufipogon*	India	DRR
16	R16	IC 87998	*O.rufipogon*	India	DRR
17	R17	IC 87983	*O.rufipogon*	India	DRR
18	R18	IC 87969	*O.rufipogon*	India	DRR
19	R19	IC 22011	*O.rufipogon*	India	DRR
20	R20	IC 22015	*O.rufipogon*	India	DRR
21	R21	IC 22017	*O.rufipogon*	India	DRR
22	R22	IC 22017-3	*O.rufipogon*	India	DRR
23	R23	IC 22017-5	*O.rufipogon*	India	DRR
24	R24		*O.rufipogon*	Vietnam	DRR
25	R25		*O.rufipogon*	Vietnam	DRR
26	N1	IC 21001	*O.nivara*	India	DRR
27	N2	IC 21009	*O.nivara*	India	DRR
28	N3	IC 21021	*O.nivara*	India	DRR
29	N4	IC 21022	*O.nivara*	India	DRR
30	N5	IC 21023	*O.nivara*	India	DRR
31	S1	IR 64	*O.sativa*	India	DRR
32	S2	JAYA	*O.sativa*	India	DRR
33	S3	MAHSURI	*O.sativa*	India	DRR
34	S4	IR 58025 A	*O.sativa*	India	DRR
35	S5	KMR3	*O.sativa*	India	DRR

R:*O.rufipogon*, **N: *O.nivara*, S: *O.sativa***

Four to five grams of leaf tissue collected fresh from each of the test accessions was ground to fine powder in liquid nitrogen. The powder was added into 15ml of pre warmed (65 °C) DNA extraction buffer in a capped polypropylene tube and clumps were suspended with a glass rod. The mix was kept at 65 °C for 20 minutes with intermittent sh aking. 5ml of 5M potassium acetate was added, thoroughly mixed and incubated at room temperature for 5 minutes with continuous shaking. Equal volume of chloroform:isoamyl alcohol (24:1) was added and incubated at room temperature for 15 minutes with regula r shaking. The mixture was centrifuged for 20 minutes at 3000rpm and supernatant was collected into a new centrifuge tube. DNA was precipitated by adding double the volume of ice-cold isopropanol and incubating the mix for I hour. The DNA was spooled using a sterile glass rod, washed in 70% ethanol and dissolved in 500ul of TE buffer (10mM Tris-HCl, 1mM EDTA). The aliquot was transferred to a microcentrifuge tube and RNase was added to a final concentration of 10ug/ml followed by incubation at 37^{0}C for 1 hour. Following incubation, equal volume of Phenol:chloroform:isoamyl alcohol (25:24:1) was added, thoroughly mixed and centrifuged at 10,000rpm for 15 minutes. The supernatant was collected into a fresh microcentrifuge tube and $1/5^{th}$ volume of 5M ammonium a cetate and two volumes of ice-cold ethanol were added to precipitate DNA. The DNA pellet was washed in 70% ethanol, dried thoroughly and resuspended in 200ul of TE buffer. The quality of the DNA was checked by taking 5ul of the DNA and checking it on a 0.8% agarose gel stained with ethedium 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 was determined at 260, 280 and 320nm against TE buffer blank. The DNA concentration was calculated using the formula that 1OD corresponds to 50ug/ml of DNA. The ratio of OD260 to OD280 provides information on the purity of the DNA sample, where pure DNA p reparations show OD260 to OD280 ratio between 1.8 and 2.0.

3.1.2.1b PCR amplification with ISSR primers: Fourteen $(AG)_n$ and $(GA)_n$ based dinucleotide anchored primers (Table 2) from a set of 100 anchored microsatellite primers (UBC set 9) obtained from the University of British Colombia, Canada were used in a PCR reaction to study the genetic diversity of the wild accessions. A single primer was used in each PCR reaction/experiment, which had a final volume of 25 µl. Each reaction mix of 25 µl contained 3 µl of genomic DNA (30 ng), 3.3 µl of primer (0.2 µM concentration), 2.5 µl of 10x buffer (0.1 M Tris pH 8.3; 0.5M KCl; 7.5 mM $MgCl_2$; 0.1 % gelatin), 1.0 µl of 200 µM dNTPs and 1.0 unit of Taq polymerase. 0.5 µl of formamide and 1.2 µl of spermidine (1mM) were added to increase the stringency of the reaction. PCR amplifications were performed in 96 -well plates on a PE 9700 (Perkin Elmer) Thermal cycler under the following conditions: A hot start of 94 °C for 5 minutes: followed by 44 cycles of denaturing at 94 °C for 1 minute, annealing for 45 seconds, extension at 72 °C for 2 minutes, and final extension at 72 ^{0}C for 5 minutes. The annealing temperature (determined separately for each primer using the formula ((2 x A or T + 4 x G or C) -5)) was adjusted according to the

Tm of the primer used in the reaction and ranged from 45 to 49 °C (Table 2).

Table 2: Details of polymorphism revealed by ISSR primers

S.No	UBC Primer No.	Primer sequence	Annealing temp. (°C)*	No. of bands	No. of polymorphic bands	Percentage polymorphism	Mol.wt.range (bp)
1	807	$(AG)_8$ T	45	09	07	77.7	450-1330
2	808	$(AG)_8$ C	47	13	12	92.3	560-1900
3	809	$(AG)_8$ G	47	10	07	70.0	560-1350
4	810	$(GA)_8$ T	45	14	12	85.7	450-1350
5	811	$(GA)_8$ C	47	08	08	100.0	560-1330
6	812	$(GA)_8$ A	45	10	08	80.0	560-1350
7	834	$(AG)_8$ YT	47	12	12	100.0	400-1300
8	835	$(AG)_8$ YC	49	11	10	90.9	450-1330
9	836	$(AG)_8$ YA	47	12	11	91.6	400-1300
10	840	$(GA)_8$ YT	47	12	10	83.3	500-1300
11	841	$(GA)_8$ YC	48	07	06	85.7	450-1200
12	842	$(GA)_8$ YG	49	11	09	81.8	300-1300
13	884	HBH $(AG)_7$	45	08	06	75.0	500-1100
14	885	BHB $(GA)_7$	47	12	11	91.6	450-1400

B= C ,G, T (not A); Y= Pyrimidine; H= A, T, C (not G)

* For calculating, Tm (°C) = [(A+T)2 + (G+C)4 - 5]; Y=T; B=C; H=T

Following amplification, the products were checked for polymorphism on an agarose gel.

3.1.2.1c Agarose gel electrophoresis: The amplified products were mixed with

bromophenol blue loading buffer (40% sucrose and 0.25% bromo phe nol blue) and resolved on 1.8% agarose gel in 0.5X TBE buffer under room temperature at a constant voltage of 120 V along with a marker (phi X 174 Hae III digest). Gels were stained with ethedium bromide and photographed on Image master VDS Gel scanning system (Pharmacia biotech).

3.1.2.1d Gel scoring and data analysis: Each amplification product was considered as an ISSR marker. Consistency of the bands was checked by repeating the reaction twice and the reproducible bands were scored in all the samples for each of the 14 primers separately. Bands were recorded as present (1) or absent (0). For each band scored, '1' was given to the individuals, which had the presence of that band and '0' to those, which did not have the band of interest. All the numerical and taxonomical analyses were conducted using the NTSYS-pc software version 2.0 from Exetersoftware, NY (Rohlf, 1993). Similarity matching (SM) similarity coefficient values for pairwise comparison between accessions were calculated and a similarity coeffi cient matrix was constructed using the SIMQUAL subroutine. This matrix was subjected to unweighted pairwise group method using arithmetic average analysis (UPGMA) to generate a dendrogram using the SAHN subroutine and Tree plot of NTSYSpc.

3.1.2.2 Hybrid vigour in F_1 plants derived from crosses between IR 58025A and 30 wild accessions: Thirty crosses were made in *Kharif,* 1999 using IR 58025A as female parent and each of the 30 accessions of the wild species as male parent. The F_1's from the crosses were grown under field conditions in *Rabi,* 2000 to evaluate their vegetative vigour. The F_1's showing high vegetative

vigour were chosen for backcrossing. Two characters *viz.,* leaf area and plant height were studied to identify the most vigorous F_1 plants. Observations on the two characters were made two times, i.e. on 40th day and 60th day after transplanting. The average over all the F_1 plants in a cross was taken as the value for that cross. The value obtained 40 days after planting was subtracted from the value obtained 60 days after planting to obtain the values of difference in leaf area (DLA) and difference in plant height (DPH) and the corresponding growth rate was calculated for each of the crosses. The DLA and DPH values were plotted against genetic distance in each of the wild species as compared to IR 58025A. Based on the graph, F_1 plants were chosen for backcrossing.

3.2 Development of mapping population

The F_1 plants of the cross combination selected based on genetic diversity of parents and F_1 vigour were backcrossed to IR 58025B, an isogenic maintainer line of IR 58025A during *Rabi,* 2000 to produce BC_1 (Fig. 1). IR 58025B was used as the male parent. The BC_1 was raised in the subsequent season, *Kharif,* 2000 and 50 BC_1 plants, looking morphologically like IR 58025A were again backcrossed to IR 58025B using it as male parent to produce BC_2. BC_2 plants were raised during *Rabi,* 2001. Out of a population of 3000 BC_2 plants obtained, 251 male sterile plants were randomly selected and testcrossed to KMR3, the restorer of IR 58025A to produce 251 testcrosses. Simultaneously, under similar conditions, a cross was made between IR 58025A and KMR3 to obtain the hybrid KRH2 to be used as the check. Before effecting crosses the 251 plants were evaluated for four yield related characters *viz.,* panicle length,

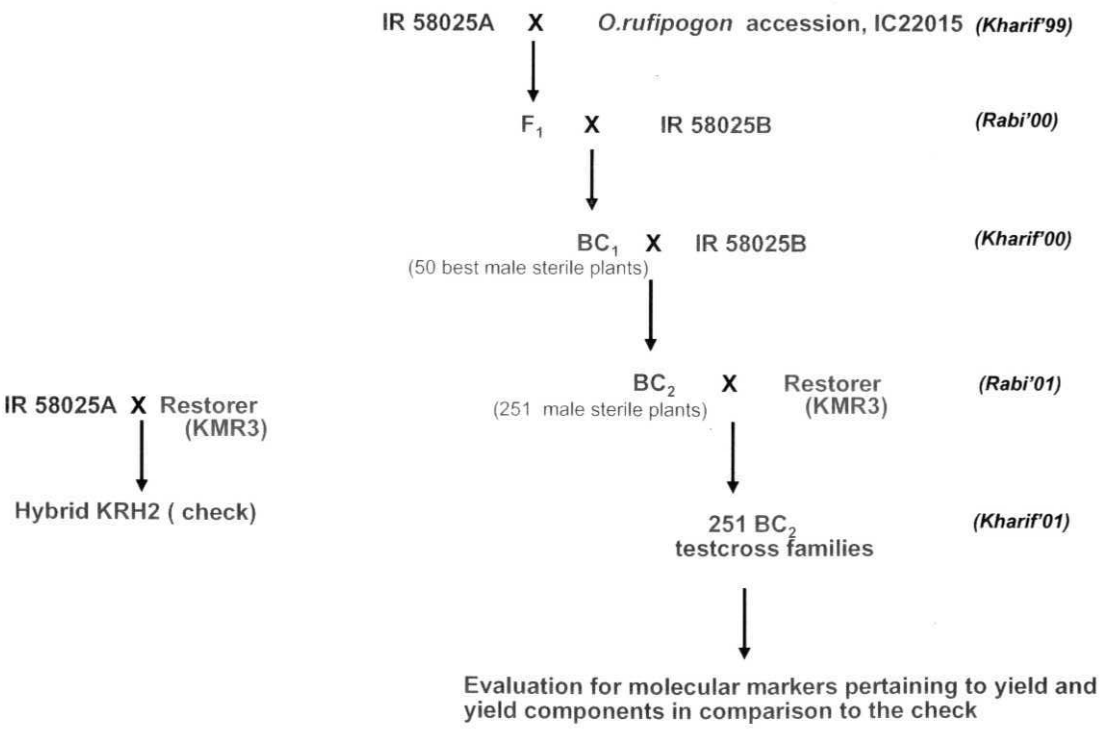


Fig 1: A schematic representation of the workplan for development and study of mapping population

number of panicles per plant, number of tillers per plant and spikelet number per panicle to check, if the randomly selected plants followed normal distribution. The testcrosses along with the hybrid check KRH2 we re grown during *Kharif,* 2001 in the DRR farm to evaluate for yield and major components of it in comparison to the check. The 251 BC_2 testcross families constituted the mapping population.

3.3 Phenotypic evaluation of the mapping population

The 251 testcross families were grown in an augmented design in two replications with checks KRH2, Jaya and IR64 repeated after every 10 families. Each of the testcrosses and the check consisted of 40 plants planted in 4 rows of 10 plants each adopting a uniform spacing of 20cm X 20cm (Fig. 2). Six plants in the middle of each of these families were marked for evaluation of yield related traits. Average of the six plants for each of the traits was taken as the representative value for that family. So computed values of ea ch of the families were compared to the corresponding check KRH2 following it. Thirteen characters were evaluated in all, following standard procedure given as under:

Plant height: Length of the tallest tiller measured in cm from soil surface to the tip of the panicle (awns included).

Tiller number per plant: Total number of tillers including productive and non productive ones.

Panicles per plant: Panicles with seed set exceeding 15% only.

Panicle length: Length in cm from neck to tip of the panicle (exclud ing awns).

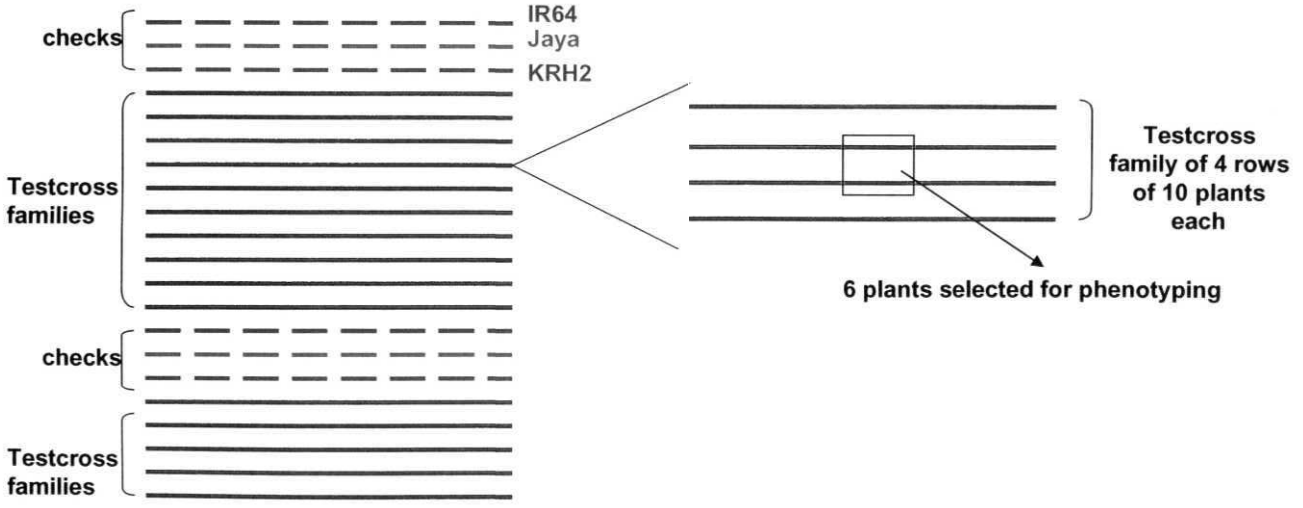


Fig 2: Diagrammatic representation of the Augmented Block Design adopted for raising the mapping population

Spikelet number per panicle: Number of spikelets including empty and filled ones averaged over five randomly chosen panicles in each plant.

Spikelet number per plant: Total number of spikelets including empty and filled ones in each plant computed as average number of spikelets per panicle x number of productive tillers.

Grain number per panicle: Number of filled spikelets per panicle averaged over five randomly chosen panicles in each plant.

Grain number per plant: Number of filled spikelets in a plant computed as average number of filled spikelets per panicle x number of productive tillers per plant.

Spikelet fertility: Proportion of filled spikelets to total number of filled and unfilled spikelets per panicle expressed in percentage.

Test grain weight: Weight in gms of 1000 filled spikelets, averaged over six samples taken from the bulk harvested grain from each plant.

Harvest index: Ratio of filled grains to biomass (filled grains, unfilled grains and straw of the plant) in terms of weight in g ms expressed in percentage.

Grain yield per plant: Weight in gms of filled grains per plant.

Grain yield: Weight in gms/kg of filled grains harvested from each testcross family (40 plants) extrapolated to tonnes per hectare.

Trait correlations: Correlations between character pairs was computed at $p < 0.05$ and $p < 0.01$ in Excel using trait averages.

3.4 Parental polymorphism

DNA of the three parents, IR 58025A, KMR3 and *O.rufipogon* (IC 22015) was extracted using the protocol described in 3.1.2 .1a and analysed for polymorphism using ISSR primers and Rice Microsatellite (RM) primers. A total of 284 RM primers and 75 ISSR primers were used to survey the parents for polymorphism. The RM primers used for parental survey were chosen based on their distribution in the genome and earlier reports. Each microsatellite amplification reaction of 25ul consisted of 2.5ul of 10X reaction buffer, 1 ul of 200uM dNTP, 0.2ul of the forward and reverse primer, 0.5ul formamide and 1 unit of the enzyme Taq polymerase. Each reaction had 30ng of their respective genomic DNA. The amplification was done using PE 9700 thermal cycler as follows: Hot start of 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 45 sec and extension at 72°C for 2 min and a final extension step of 72 °C for 2 min. Following amplification the samples were stored at 4°C for short periods of time and at -20°C for long durations. Amplification using the ISSR primers was done based on the protocol described under section 3.1.2.1b. The amplified products were checked on ethedium bromide stained agarose gels (2.5%) and polymorphic primers were noted. A primer was considered polymorphic, if it amplified a unique band in IC 22015 (wild species) absent both in IR 580 25A and KMR3

3.5 Genotypic evaluation of mapping population

DNA of the mapping population was extracted as described under section 3.1.2.1a and 81 polymorphic microsatellite markers were screened on the mapping population to study th e segregation pattern of them. The population was scored for the presence and absence of the *O.rufipogon* alleles.

3.6 Linkage map construction and QTL mapping

3.6.1 Linkage map construction: Microsatellite linkage maps were constructed for the chromosomes based on the marker segregation data generated using the 81 polymorphic RM primers. Map construction was done using the Mapmaker version 3.3 (Lander *et al,* 1987) following Kosambi Function (Kosambi, 1944). Linkage groups were determined using 'group' comm and with an LOD score of 3.0 and a recombination fraction of 0.5. Order of the markers for each group was determined using command 'first order'. Ungrouped/unlinked markers were assigned to the respective linkage groups using 'try' command. Assignment of linkage groups to the respective chromosomes was done based on the rice maps developed at Cornell University (Temnykh *et al,* 2000).

3.6.2 Single marker analysis: Nomenclature for QTLs was followed as described by McCouch *et al* (1997). The association betwee n phenotype and marker genotype was investigated using single marker analysis and interval mapping. Single marker analysis was done on BC_2 testcross data by regression of field performance on marker genotypes using standard analysis of variance (ANOVA) procedure at a statistical threshold of $p<0.01$ and assuming regular segregation of wild and cultivated alleles in the testcross families. The proportion

of observed phenotypic variance attributable to a particular QTL was estimated as the difference between the mean of the segregants having the *O.rufipogon* allele and the mean of the segregants that did not have the *O.rufipogon* allele. The phenotypic variance over the check KRH2 was also calculated in a similar manner.

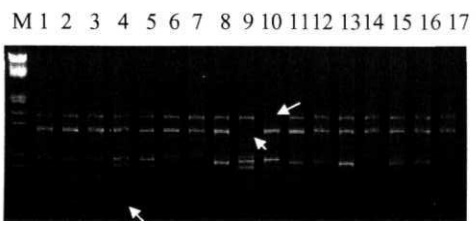
3.6.3 Interval mapping: For analyzing marker-QTL association for each trait and determining the precise location of the putative QTLs, interval mapping of the MAPMAKER/QTL program version 1.1 (Lincoln *et al,* 1992) was conducted. An LOD score of 2.0 was used as the threshold for detecting the locat ion of QTLs. LOD peaks for significant QTLs were used to position the QTL on the linkage map. Gene action (additive effect free of dominance) and percent variation attributable to individual QTLs were estimated at peaks.

4. RESULTS

4.1 Selection of donor parent

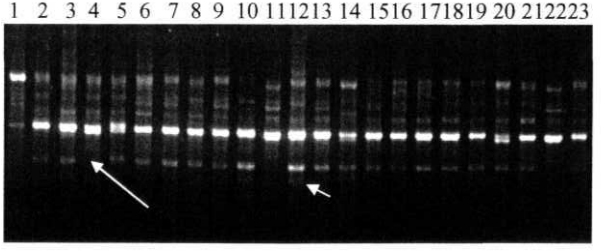
4.1.1 Primers vs. polymorphism: Fourteen ISSR primers in all were found to amplify 7 to 14 bands each ranging from 300 to 1900 bp in the 35 accessions. Of 149 bands scored, 129 (86%) were polymorphic (Table 2). The polymorphism ranged from 70 to 100%. Seven of the primers based on $(AG)_8$ together showed about the same percentage polymorphism (86%) as the other seven primers based on $(GA)_8$ taken together. Among the $(AG)_8$ primers, those with a dinucleotide 3' anchor generated higher polymorphism than those with a single nucleotide 3' or 5' anchor. Among the $(GA)_8$ primers on the other hand, 5' anchored single nucleotide ones showed the highest polymorphism while the dinucleotide anchored primers the least. The two primers, which amplified 100% ploymorphic bands were $(GA)_8C$ (UBC 811) and $(AG)_8YT$ (UBC 834) (Fig. 3). The least polymorphism was generated by $(AG)_8G$ and $(GA)_8A$ i.e., those, which had the same nucleotide as 3' anchor as the nucleotide at 3' end of the repeat motif.

4.1.2 Diversity analysis and correlation of genetic profiles: Genetic diversity analysis based on the 14 ISSR primers revealed highest diversity among the *O.rufipogon* accessions. The extent of diversity in terms of genetic distance ranged from 0.211 to 0.375 *vis a vis* IR 58025A. The accession R24 from Vietnam was the closest to the cultivar IR 58025A, whereas the accession R15 from India the most farthest. Unlike in *O.rufipogon,* the extent of diversity was comparatively narrow among *O.nivara* accessions studied, the range being from 0.2283 to 0.2923. The accession N5 was the closest while N1 the distant from IR



ISSR Primer 811

(a) M – phi x 174 Hae III digest marker
1 – 17: accessions of *O.rufipogon*



ISSR Primer 841

(b) 1 – 23: accessions of *O.rufipogon*

Fig 3: A representative plate of ISSR- PCR products showing polymorphism across accessions

58025A. As compared to *O.rufipogon* and *O.nivara* accessions diversity in *O.sativa* cultivars was still narrower, the range being from 0.13 to 0.18 compared to IR 58025A. Based on their rel ative genetic distance from IR 58025A all the test accessions were grouped into five clusters (Table 3). Cluster-1, which comprised all the test cultivars had a genetic distance ranging from 0.15 to 0.20 and cluster - 2 with diversity in the range of 0.21 to 0.25 included all the *O.nivara* accessions along with a few of the *O.rufipogon* accessions. The remaining *O.rufipogon* accessions were distributed into last three clusters. Those comprising the cluster - 5 with genetic distance in the range of 0.36 - 0.40 included three Indian accessions *viz.,* IC87973, IC87998 and IC87983.

Table 3: Genetic distance of a representative set of *O.rufipogon, O.nivara* and *O.sativa* accessions in comparison with IR 58025A

Genetic Distance	Accessions*
0.15-0.20	IR 64, Jaya, Mahsuri, KMR3
0.21 – 0.25	IRGC101193, IC 21009, IC 21021, IC 21022, IC 21023, IC 22011
0.26 - 0.30	IRGC 100204, IRGC 103827, IRGC 103844, IRGC 104409, IRGC 104433, IRGC 105325, IRGC 105400, ***IC 22015***, \C 21001
0.31 -0.35	IRGC 105569, IC 87969, IC 22017-3, IC 22017-5
0.36 – 0.40	IC 87973, IC 87998, IC 87983

*** Accession details given in Table 1**

A dendrogram generated by cluster analysis (UPGMA method) based on the 149 bands generated by the 14 primers (Fig. 4) revealed that three accessions of *O.rufipogon viz.,* R5 and R6 from Bangladesh and R13 from China did not cluster with any other accessions and each remained separate.

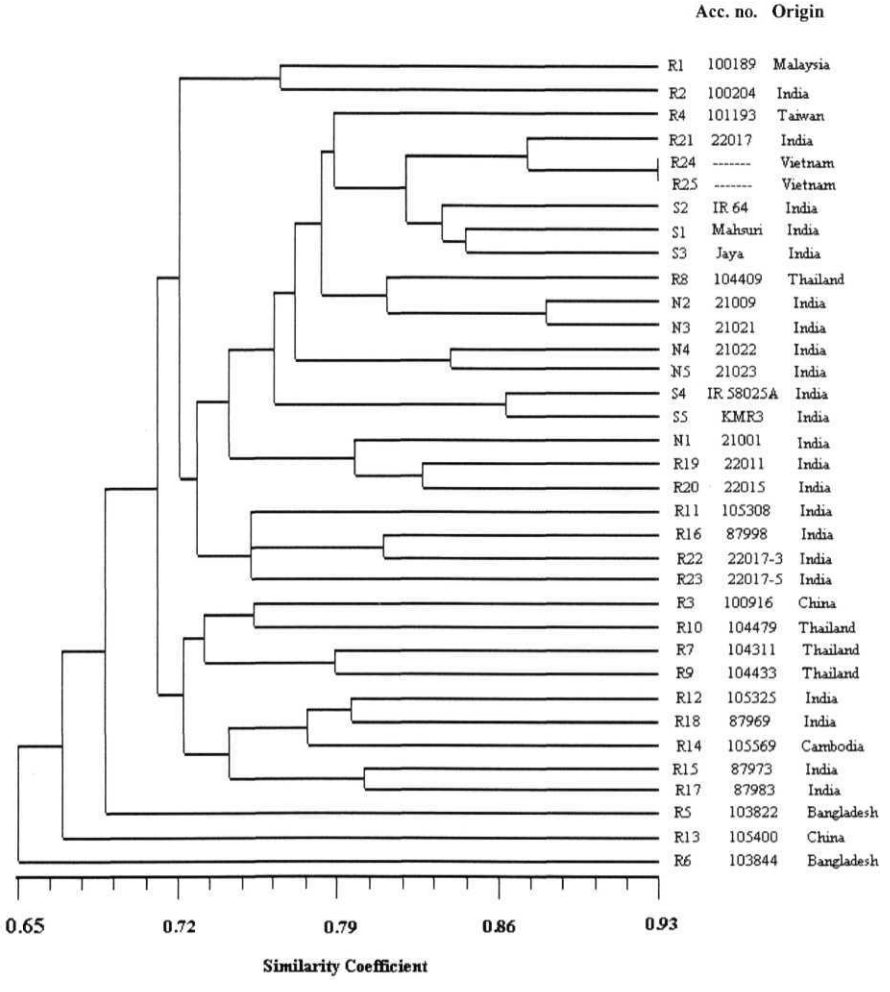


Fig 4: Dendrogram comparing 25 accessions of *Oryza mfipogon* with 5 each of *O. nivara* and *O. saliva* based on the banding profiles generated using 14 ISSR primers. (Abbreviations as in table 1)

The remaining 32 accessions were separable into two major clusters. Cluster 1 consisted of the five accessions one each of *O.sativa* and *O.nivara* and 13 accessions of *O.rufipogon.* Cluster 2 consisted of the remaining nine accessions of *O.rufipogon.* The *O.rufipogon* accessions in cluster 1 were largely from India, Vietnam, Malaysia and Taiwan with one exception from Thailand while cluster 2 consisted of *O.rufipogon* accessions largely from Thailand, Cambodia, India and China. It was of interest that three accessions of Thailand as a group and two accessions from Bangladesh remained outside the two major clusters. Equally, it was interesting that not all accessions from a given geographical area grouped together. For instance, of the 12 *O.rufipogon* accessions from India, eight were in cluster 1 and four in cluster 2. Likewise, of the four accessions from Thailand one was in cluster 1 and thre e in cluster 2.

4.1.3 Hybrid performance: Thirty interspecific hybrids obtained from crosses of IR 58025A with the 30 wild accessions were evaluated for a set of morphological/growth features, which included plant height and leaf area. Difference between **v alues** of 60 days after planting (60 DAP) and 40 days after planting (40 DAP) was taken as the index of growth rate (Table 4). Plant height ranged between 26.6 and 60.16cm at 40 DAP and between 48.55 and 88.66cm at 60 DAP. On both the days the hybrid involv ing *O.rufipogon* accession R24 measured the tallest while the accession R23 the shortest. Growth in terms of difference in plant height (DPH) during 40 DAP and 60 DAP ranged from 18.50 to 41.81 cm with the cross involving N2 showing the least DPH accountin g to a growth rate of 42.18% and that with R20 having the highest DPH value

corresponding to growth rate of 102%. The leaf area at 40 DAP ranged from 11.21 (in the cross with R7) to 32.03cm^2 (in the cross with N1), while at 60 DAP it ranged from 18.24 (in cross with R22) to 46.52cm^2 (in cross with R2).

Table 4: Growth rate of the F_1s in the crosses involving IR 58025A and wild accessions.

Wild Acc.	Genetic distance*	PH 40DAP	PH 60DAP	DPH	Growth rate(%)	LA 40DAP	LA 60DAP	DLA	Growth rate(%)
R24	0.2116	60.16	**88.66**	28.50	47.37	29.83	37.42	7.59	25.44
R4	0.2159	**44.85**	76.85	32.00	71.34	18.43	27.84	9.41	51.05
R25	0.2205	**42.86**	72.82	29.96	69.90	19.78	28.65	8.87	44.84
N5	0.2283	45.32	68.32	23.00	50.75	20.36	29.62	9.26	45.48
N2	0.2361	43.86	62.36	18.50	42.17	22.32	31.32	9.00	40.32
R21	0.2394	46.42	76.50	30.08	64.79	23.12	32.79	9.67	41.82
N3	0.2463	46.66	68.25	21.59	46.27	26.11	37.69	11.58	44.35
R19	0.2500	50.83	84.82	33.99	66.86	28.73	45.86	17.13	59.62
N4	0.2517	44.96	77.86	32.90	73.17	23.27	34.57	11.30	48.56
R6	0.2698	34.78	67.82	33.04	94.99	13.68	22.83	9.15	66.88
R8	0.2715	29.92	60.12	30.20	100.93	12.53	20.37	7.84	62.56
R12	0.2888	37.64	58.27	20.63	54.80	16.55	28.30	11.75	70.99
R2	0.2968	36.80	67.20	30.40	82.60	31.53	46.52	14.99	47.54
N1	0.2923	51.96	85.47	33.51	64.49	32.03	46.15	14.12	44.08
R5	0.2932	45.00	75.30	30.30	67.33	26.53	40.32	13.79	51.97
R20	0.2977	40.63	82.44	41.81	102.90	24.19	46.10	21.91	90.57
R13	0.3015	36.52	70.30	33.78	92.49	14.35	26.54	12.19	84.94
R9	0.3055	34.80	69.56	34.76	99.88	16.71	30.50	13.79	82.52
R7	0.3061	28.27	48.68	20.41	72.19	11.21	20.27	9.06	80.82
R23	0.3070	26.66	48.55	21.89	82.10	12.42	21.63	9.21	74.15
R22	0.3088	30.21	50.50	20.29	67.16	14.81	18.24	3.43	23.16
R18	0.3125	32.20	53.10	20.90	64.90	15.62	24.21	8.59	54.99
R14	0.3129	43.23	63.86	20.63	47.72	23.42	28.20	4.78	20.40
R11	0.3280	40.79	69.33	28.54	69.96	21.72	35.10	13.38	61.60
R3	0.3281	42.32	66.24	23.92	56.52	21.93	35.21	13.28	60.55
R10	0.3484	42.3	67.34	25.04	59.19	22.36	35.78	13.42	60.01
R1	0.3538	42.22	71.55	29.33	69.46	20.24	34.25	14.01	69.21
R17	0.3611	45.65	73.90	28.25	61.88	18.75	32.46	13.71	73.12
R16	0.3750	36.75	61.55	24.80	67.48	15.15	28.70	13.55	89.43
R15	0.3750	38.63	62.34	23.71	61.37	16.1	30.52	14.42	89.56

* Genetic distance *vis a vis* IR 58025 A
PH- Plant height, LA- Leaf area, DAP- Days after planting
DPH- Difference in plant height (60DAP - 40DAP), DLA- Difference in leaf area (60DAP - 40DAP)

Growth in terms of difference in leaf area (DLA) ranged from 3.43 to 21.91 cm ^{2}. The hybrid involving R22 had the lowest DLA corresponding to a growth rate of 23.16% whereas that involving R20 the highest accounting for a growth rate of 90.51% (Fig. 5).

Based on the genetic diversity data and hybrid vigour the *O.rufipogon* accession R20 (IC 22015) was chosen as the donor parent for developing mapping population for identification and study of yield related quantitative trait loci (QTLs). A representative photograph of the *O.rufipogon* accession (IC22015), IR 58025A and the F_1 derived from the cross is shown as Fig.6.

4.2 Distribution of randomly selected BC_2 plants for yield components

The randomly selected 251 BC_2 plants evaluated for four yield components followed a normal distribution with transgressive segregants at both ends. Number of tillers per plant ranged from 8 to 65 while number of panicles per plant from 8 to 60, panicle length from 17 to 36.5 cm and spikelet number per panicle from 69 to **410** (Fig. 7 and 8).

4.3 Phenotypic evaluation of mapping population

4.3.1 Morphological characters: The 251 testcross families developed as detailed under the Material and Methods were grown in an augmented block design with three checks viz., KRH2, IR64 and Jaya repeated once after every 10 testcross families. Each entry consisted of four rows of 1 0 plants each. Six plants randomly chosen from the center of each of the testcross families were used for morphological observations. Average grain yield of the testcross

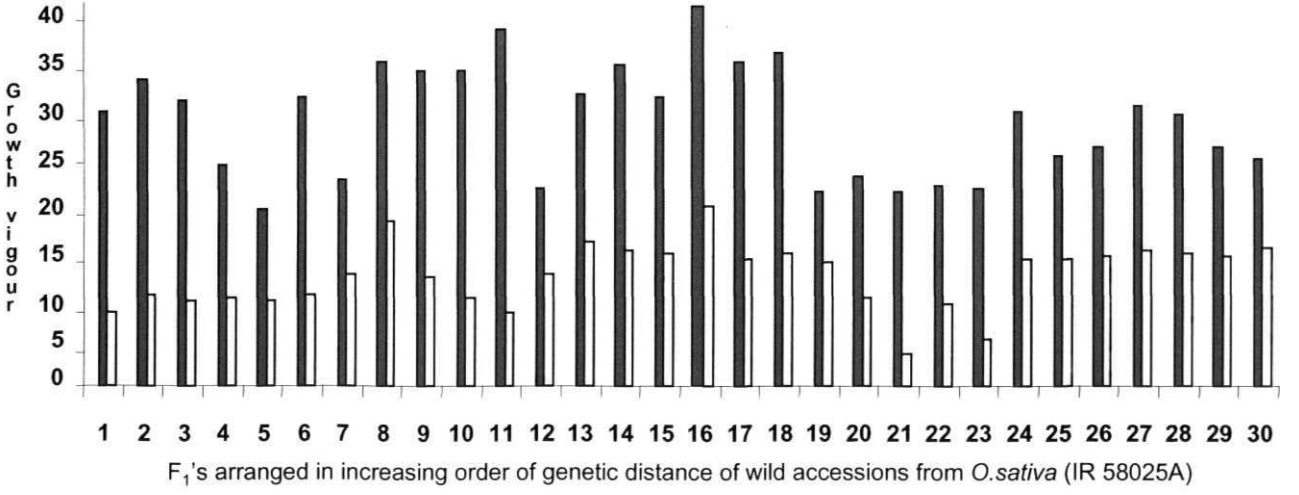
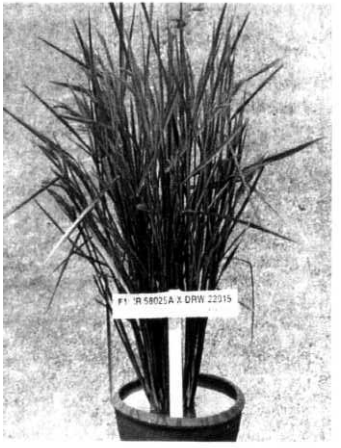
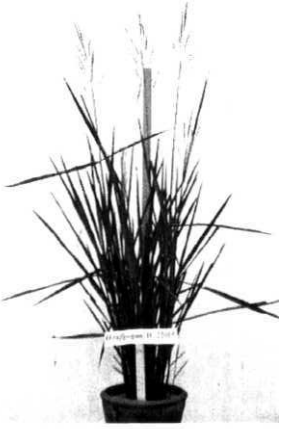


Fig 5: Relative growth of F_1's of IR 58025A with 30 different wild accessions

IR 58025A, *O.sativa*



F_1: IR 58025A x IC 22015



IC 22015, *O.rufipogon*

Fig. 6: A representative plate showing the wild (IC 22015) and cultivar (IR 58025A) parents alongwith the F_1 obtained from the cross

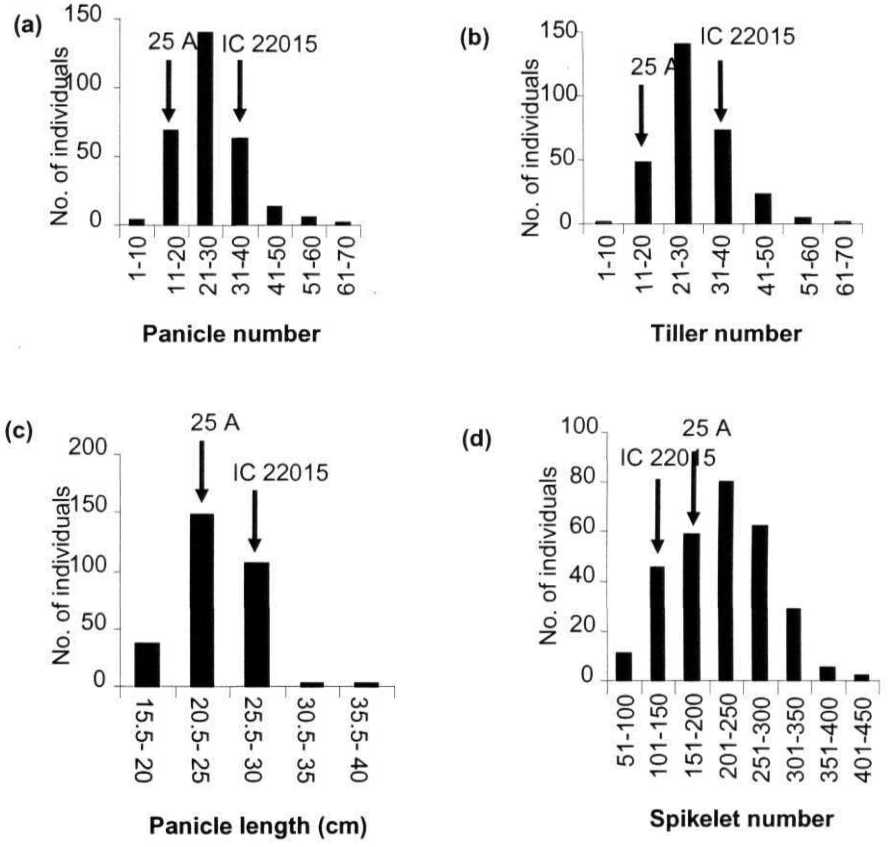
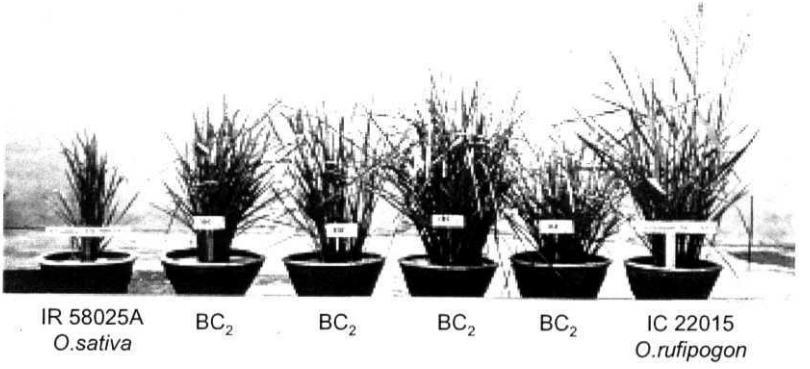


Fig. 7: Frequency distribution of 4 traits in 251 randomly selected BC_2 plants

(a)



(b)

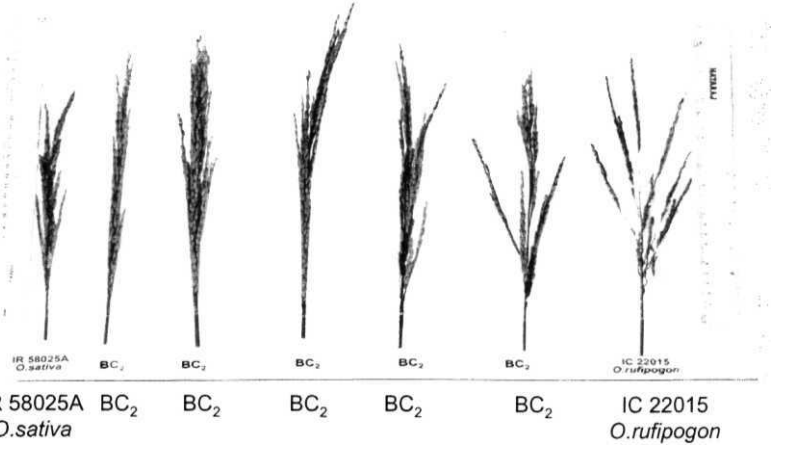


Fig. 8: A representative plate depicting the segregants for plant type (a) and panicle length (b) in the 251 randomly selected BC_2 pants.

families was 6.08 t/ha with the range varying from 3.90 to 9.45 t/ha, while yield per plant ranged from 7.5 to 36.0 gm with an average of 19.5 gm (Table 5). The range for

Table 5: Phenotypic traits for 13 yield components across **251** testcross families as compared to **IR** 58025A, **IC22015** (wild) and KRH2

Trait	**IR** 58025A	**IC** 22015	KRH2	Range in Testcross families	No. of families showing > 20% increase over KRH2
Plant height (cm)	80	119	118	93-177	26
Number of tillers	9	32	11.2	7-16	18
Number of panicles	7	28	10	6-14	20
Panicle length (cm)	24	29	23.5	20.5 – 34.5	1
Spikelet number / panicle	175	150	167	67-265	88
Spikelet number / plant	1350	3500	1880	737-3074	74
Grain number / panicle	-	35	117	30-185	101
Grain number / plant	-	700	1187	322-2310	63
Spikelet fertility (%)	0	15-20	68	42-91	42
1000 grain weight (gm)	20	11.5	22.5	17.5-31.3	1
Yield / plant (gm)	-	9	19	7.5-36	39
Harvest index	-		45	27-56	7
Yield (t/ha)	-	-	7	3.9-9.45	13

cm: Centimeters, gm: Grams, t/ha : Tonnes / hectare

plant height in the testcross families was betwee n 93 and 177cm with an average of 130 cm. Whereas number of tillers per plant ranged from 7 to 16 with an average of 9.98, number of panicles per plant was between 6 and 14 with an average of 9.76. Average panicle length was 25.11cm with range being betwee n

20.5 and 34.5 cm. Spikelet number per panicle and spikelet number per plant had an average of 181.37 and 1772.32 with a range of 67 to 265 and 737 to 3074 respectively. Average grain number per panicle and grain number per plant was 125.28 and 1219.92 re spectively while average percentage spikelet fertility was 68.81, the range being between 42 and 91. Test (1000) grain weight varied from 17.5 to 31.3 gm with an average of 22.37 gm while mean harvest index (HI) was 41.50 with range being between 27 and 56 . Among the yield related traits, number of tillers per plant, number of panicles per plant, spikelet number per plant, test grain weight, harvest index and grain yield *per se* showed decrease in their mean values as compared to the commercial hybrid KRH2, while spikelet number per panicle, grain number per panicle and percentage spikelet fertility showed higher mean value than that of KRH2.

4.3.2 Heterosis over KRH2: Character wise frequency distribution of testcross families showed families expressing valu es higher and lower than corresponding values in KRH2 (Fig. 9). Thirteen testcross families were found to out perform KRH2 by more than 20% for plot yield and as many as 39 families showed more than 20% increase in yield per plant as compared to KRH2 (Table 5). Number of families, which showed at least 20% increase over KRH2 for other yield related traits varied with the character. It was 18 for number of tillers/ plant, 20 for number of panicles/ plant, one for panicle length, 74 for spikelet number/ plant , 63 for grain number/ plant, 42 for spikelet fertility, one for test (1000) grain weight and seven for harvest index. It was noteworthy that 88 families showed more than 20% increase in respect of spikelet number/ panicle and 101 families

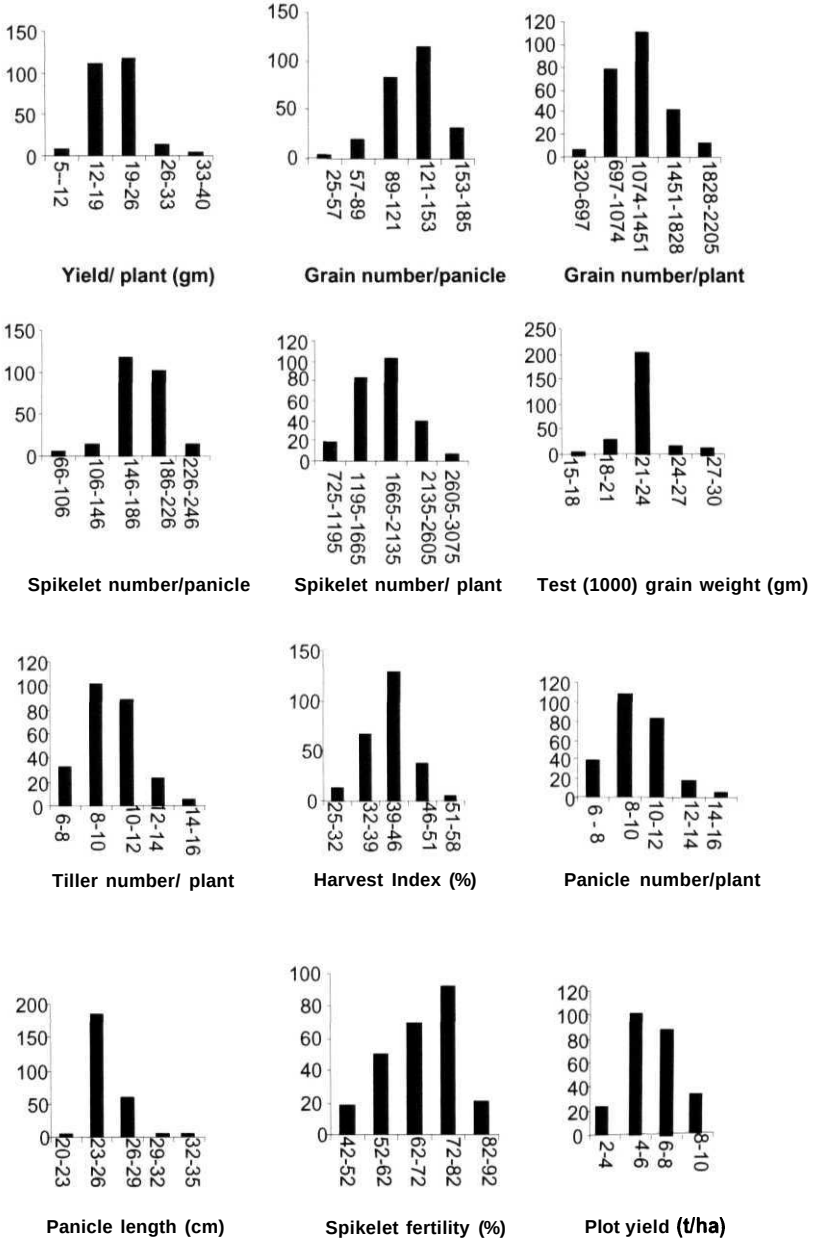


Fig.9: Frequency distribution of the 251 testcross families for yield and its components

for grain number/ panicle. Of the 251 testcross families studied in all, 75 showed at least 20% increase over KRH2 for three or more yield components.

4.4 Trait correlations

Character pair correlations among the traits at $p<0.05$ and $p<0.01$ are summarized in Table 6. The highest correlation (0.982) was found between total number of tillers per plant and number of panicles per plant closely followed by association between total spikelet number per plant and filled grain number per plant (0.823). Plant height was found to follow a negative but significant correlation with percentage spikelet fertility (-0.362) and harvest index (-0.298), while significant positive correlation with number of panicles (0.213), panicle length (0.236) and spikelet number per plant (0.249). Both number of tillers and number of panicles per plant showed positive correlation with spikelet number per panicle and filled grain number per panicle. Panicle length had significant positive correlation with all the traits except number of tillers, number of panicles, percentage spikelet fertility and harvest index. Interestingly yield showed no significant correlation with panicle length. While spikelet number per panicle followed significant correlation with spikelet number per plant, grain number per panicle, grain number per plant and panicle length, grain number per panicle showed significant correlation with spikelet number per plant, spikelet number per panicle, spikelet fertility and panicle length. Harvest index had a strong correlation with grain yield per plant, while it showed a negative correlation with plant height. Yield per plant was found to be strongly correlated with spikelet number per plant, grain number per plant and harvest index, its association with

Table 6: Nature and strength of relationship among the yield components in BC_2 testcross families

	PH	NT	NP	PL	SN	SNP	GN	GNP	SF	GW	SPY	HI	PY
PH	1.000												
NT	0.178	1.000											
NP	0.213	**0.982**	1.000										
PL	0.236	-0.059	-0.052	1.000									
SN	0.147	-0.096	-0.104	0.460	1.000								
SNP	0.249	**0.686**	**0.692**	0.281	**0.637**	1.000							
GN	-0.137	-0.162	-0.176	0.357	**0.716**	0.391	1.000						
GNP	0.016	**0.542**	**0.544**	0.265	**0.537**	**0.823**	**0.719**	1.000					
SF	-0.362	-0.150	-0.167	0.040	-0.002	-0.105	**0.684**	0.461	1.000				
GW	-0.130	-0.255	-0.284	0.151	0.206	-0.059	0.293	0.062	0.260	1.000			
SPY	0.180	0.405	0.434	0.175	0.303	**0.552**	0.318	**0.581**	0.140	0.189	1.000		
HI	-0.298	-0.131	-0.087	0.040	0.145	0.037	0.323	0.218	0.311	0.261	**0.539**	1.000	
PY	-0.070	-0.027	-0.010	0.108	0.144	0.108	0.206	0.181	0.150	0.023	0.213	0.239	1.000

$p < 0.01 = 0.163$ $P < 0.05 = 0.125$

PH-Plant height NT- No. of tillers/plant NP- No. of panicles/plant PL- Panicle length SN- spikelet number./plant
SNP- Spikelet number /plant GN – Grain number/panicle GNP- Grain number/plant SF- Percentage spikelet fertility
GW- test grain weight SPY- Yield per plant HI- Harvest index PY- Plot yield

plot yield was comparatively less strong. Plot yield had no significant correlation with any of the components except harvest index (0.239) and yield per plant (0.213). Interestingly grain weight had no significant effect on yield, but showed negative correlation with number of panicles.

4.5 Parental polymorphism

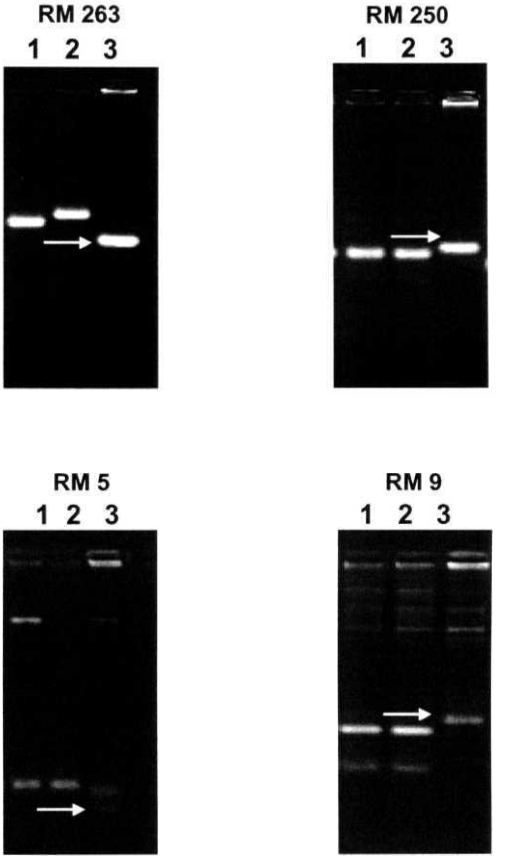
Unique alleles present in the *O.rufipogon* accession and absent in the other two parents *viz.,* IR 58025A and KMR3 were scored as polymorphic. Out of a total of 284 microsatellite primers 107 detected polymorphism between the three parents leading to a polymorphism percentage of 37.6. Thirty three of the total 75 ISSR primers resulted in polymorphism leading to a polymorphism of 44% among the three parents as shown in Table 7. A representative photograph of the microsatellite is shown i n (Fig. 10).

Table 7: Polymorphism as detailed using ISSR and SSR primers in the parents IR 58025A, KMR3 and IC 22015 (*O.rufipogon)*

	ISSR	SSR
No. of primers used	**75**	284
No. of polymorphic primers	**33**	107
Percentage polymorphism	**44**	37.6

4.6 Segregation of molecular markers in BC_2

In an unselected BC_2 population, the expected segregation would be in the ratio of 75% homozygotes (IR 58025A/IR 58025B) to 25% heterozygotes (IR 58025A/*O.rufipogon*), resulting in an allele frequency of 87.5% IR 58025A : 12.5% *O.rufipogon.* The ratio in this population, however, was 83.26% IR 58025A : 16.74% *O.rufipogon,* due to skewed allele frequencies at 29 out of 74



1: IR 58025B
2: KMR3
3: IC 22015 *(O.rufipogon)*
→ Indicates polymorphic band

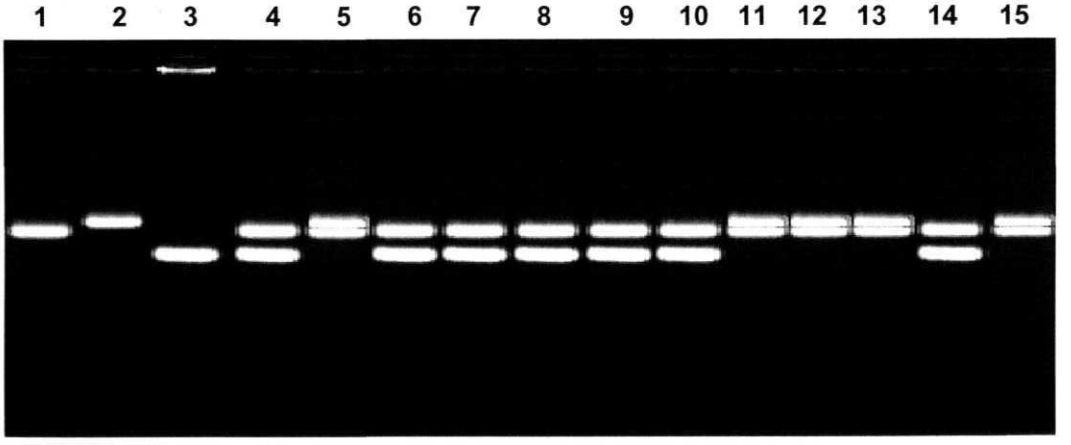
Fig. 10: A representative plate showing parental polymorphism

(39.19%) marker loci. Twenty five percent of these loci were skewed towards IR 58025A and 14% towards *O.rufipogon.* Seven markers *viz.,* RM297, RM242, RM208, RM11, RM2, RM232 and RM205 did not show any segregation indicating segregation distortion in favour of IR 58025A at these loci. A representative photograph of segregating population is shown in Fig. 11.

4.7 Linkage map construction and QTL mapping

4.7.1 Linkage map construction: A genetic linkage map constructed at a minimum LOD threshold of 3.0 based on 81 microsatellite primers resulted in 64 primers forming five linkage groups. The remaining 19 primers did not form any linkage group. The five linkage groups have been assigned to their respective chromosomes based on the rice chromosome map of Temnykh *et al* (2000). The five linkage groups mapped to chromosomes 1, 2, 3, 5 and 8. Chromosome 1 had 17 markers with a total map distance of 424.6CM, while chromosomes 2 and 3 had a map distance of 470.9CM and 481.3CM respectively with 15 markers. Ten markers were spread over chromosome 5 with a total distance of 244.2CM between them, while chromosome 8 had eight markers with a total map distance of 237.7CM (Fig. 12).

4.7.2 Single marker analysis for QTL identification: Single marker analysis using 81 polymorphic microsatellite primers identified a total of 68 QTLs for the 13 traits studied (Table 8). Thirty four (50%) of these QTLs had positive effect on the traits, while the rest negative effect. The details of the QTLs are presented below traitwise.



1: IR 58025A
2: KMR3
3: IC 22015 (*O.rufipogon)*
15: KRH2 (commercial hybrid)
4, 6-10,14 are segregants with allele from the wild species *O.rufipogon* (IC 22015)
5, 11-13 are segregants of KRH2 type, without the introgressed fragment from the wild species (IC 22015)

Fig 11: Plate showing segregation in the mapping population using RM263

Table 8: Details of QTLs identified by single marker analysis

Marker	Chr	QTL	F-crit	Fobs	VC	RC	allele effect	KRH2	Diff.
Plant height									
RM324	2	*ph2.1s*	12.11	0.0006	131.14	120.36	10.78	118	13.14
RM7	3	*ph3.1s*	15.42	0.0001	130.42	108.23	22.19	118	12.42
RM122	5	*ph5.1s*	7.796	0.0056	130.24	115.57	14.67	118	12.24
RM249	5	*ph5.2s*	8.448	0.004	130.11	111.6	18.51	118	12.11
RM173	5	*ph5.3s*	6.999	0.0087	130.78	122.6	8.18	118	12.78
RM248	7	*ph7.1s*	9.25	0.0026	130.59	118.82	11.77	118	12.59
RM210	8	*ph8.1s*	13.64	0.0003	127.58	138.05	-10.5	118	9.58
RM242	9	*ph9.1s*	18.86	2E-05	131.49	118.06	13.43	118	13.49
RM229	11	*ph11.1s*	13.17	0.0003	130.25	107.35	22.9	118	12.25
RM203		*ph.1s*	9.396	0.0024	130.35	115.44	14.91	118	12.35
Grain number per panicle									
RM81A	1	*gpp1.1s*	7.291	0.0074	126.74	113.07	13.67	117	9.74
RM210	8	*gpp8.1s*	9.542	0.0022	127.4	113.41	13.99	117	10.4
Grain number per plant									
RM183	2	*gpl2.1s*	11.62	0.0008	1174.2	1335.9	-162	1187	-12.81
RM16	3	*gpl3.1s*	9.155	0.0027	1259.7	1127	132.7	1187	72.7
RM169	5	*gpl5.1s*	11.8	0.0007	1175.6	1342.1	-166	1187	-11.37
RM223	8	*gpl8.1s*	11.23	0.0009	1235.4	979.06	256.3	1187	48.37
RM219	9	*gpl9.1s*	11.23	0.0009	1235.4	979.66	255.7	1187	48.37
Test (1000) grain weight									
RM84	1	*gw1.1s*	11.54	0.0008	22.36	19.28	3.08	21.4	0.98
RM81A	1	*gw1.2s*	8.322	0.0043	22.45	21.3	1.15	21.4	1.07
RM223B	8	*gw8.1s*	8.268	0.0044	22.44	21.24	1.2	21.4	1.06
RM229	11	*gw11.1s*	10.17	0.0016	22.37	19.85	2.52	21.4	0.99
Number of panicles per plant									
RM84	1	*np1.1s*	9.735	0.002	9.79	7.44	2.35	10	-0.21
RM34	1	*np1.2s*	6.958	0.0089	9.87	9.03	0.84	10	-0.13
RM262	2	*np2.1s*	8.821	0.0033	9.53	10.31	-0.78	10	-0.47
RM214	7	*np7.1s*	8.266	0.0044	9.54	10.29	-0.75	10	-0.46
RM223	8	*np8.1s*	11.34	0.0009	9.85	8.42	1.43	10	-0.15
RM44	8	*np8.2s*	8.272	0.0044	9.82	8.42	1.4	10	-0.18
RM203		*np.1s*	8.479	0.0039	9.82	8.35	1.47	10	-0.18
Number of tillers per plant									
RM84	1	*nt1.1s*	10.63	0.0013	10.01	7.58	2.43	11.2	-1.19
RM262	2	*nt2.1s*	7.679	0.006	9.76	10.48	-0.72	11.2	-1.44
RM214	7	*nt7.1s*	7.161	0.0079	9.77	10.46	-0.69	11.2	-1.43
RM223	8	*nt8.1s*	11.4	0.0009	10.06	8.65	1.41	11.2	-1.14
RM44	8	*nt8.2s*	8.181	0.0046	10.03	8.65	1.38	11.2	-1.17
RM203		*nt.1s*	8.222	0.0045	10.03	8.6	1.43	11.2	-1.17

VC is the heterozygote (25A/*O.rufipogon*), RC is the heterozygote (25A/KMR3)
Allele effect is the difference of the phenotypic means between VC and RC
Diff. Is the percentage increase or decrease in comparison to hybrid KRH2

Table 8: continued....

Panicle length									
RM84	1	*pl1.1s*	11.38	0.0009	25.1	21.8*	3.3	23.5	1.6
RM164	5	*pl5.1s*	8.419	0.004	25.14	23.4	1.74	23.5	1.64
RM228L	10	*pl10.1s*	10.51	0.0014	25.12	22.64	2.48	23.5	1.62
RM229	11	*pl11.1s*	12.8	0.0004	25.12	22.08	3.04	23.5	1.62
RM203		*pl. 1s*	8.467	0.0039	25.13	23.22	1.91	23.5	1.63
Spikelet fertility									
RM272	1	*sf11s*	11.3	0.0009	69.65	62.99	6.66	68,4	1.3
RM297	1	*sf1.2s*	9.995	0.0018	68.75	42.35	26.4	68.4	0.4
RM210	8	*sf8.1s*	17.94	3E-05	70.02	62.08	7.94	68.4	1.67
Spikelet number per panicle									
RM297	1	*spp1.1s*	11.6	0.0008	181.28	102.58	78.7	168	13.55
RM223	8	*spp8.1s*	8.312	0.0043	182.4	160.4	22	168	14.67
Spikelet number per plant									
RM183	2	*spl2.1s*	7.283	0.0074	1772.9	1890.5	-118	1880	-107.1
RM223	8	*spl8.1s*	17.63	4E-05	1797.9	1386.1	411.8	1880	-82.11
RM203		*spl. 1s*	7.274	0.0075	1783	1464.5	318.4	1880	-97.05
Yield per plant									
RM84	1	*yldp1.1s*	7.877	0.0054	19.56	14.2	5.36	19	0.56
RM207	2	*yldp2.1s*	7.47	0.0067	19.63	16.27	3.36	19	0.63
RM183	2	*yldp2.2s*	12.88	0.0004	18.83	21.21	-2.38	19	-0.17
RM223	8	*yldp8.1s*	10.64	0.0013	19.71	16.22	3.49	19	0.71
RM203		*yldp.1s*	9.71	0.002	19.65	15.71	3.94	19	0.65
Harvest index									
RM84	1	*hi1.1s*	10.98	0.0011	41.56	33.16	8.4	45	-3.44
RM297	1	*hit2s*	17.54	4E-05	41.5	23.5	18	45	-3.5
RM26	5	*hid. 1s*	6.978	0.0088	41.83	39.15	2.68	45	-3.17
RM340	6	*hi6.1s*	10.57	0.0013	41.67	36.36	5.31	45	-3.33
RM210	8	*hi8.1s*	11.69	0.0007	41.99	38.6	3.39	45	-3.01
RM223	8	*hi8.2s*	9.596	0.0022	41.71	37.27	4.44	45	-3.29
RM260	11	*hi11.1s*	7.502	0.0066	41.81	38.87	2.94	45	-3.19
Plot yield									
RM263	2	*yld2.1s*	151	2E-27	764.7	544.1	220.6	700	64.7
RM183	2	*yld2.2s*	130.4	1E-24	763.7	549.15	214.6	700	63.7
RM223	8	*yld8.1s*	13.27	0.0003	613.91	481.4	132.5	700	-86.09
RM38	8	*yld8.2s*	17.72	4E-05	658.2	572.8	85.4	700	-41.8
RM350	8	*yld8.3s*	18.91	2E-05	655.6	569.2	86.4	700	-44.4
RM210	8	*yld8.4s*	11.1	0.001	644.05	576.89	67.16	700	-55.95
RM256	8	*yld8.5s*	7.39	0.007	642.7	584.92	57.78	700	-57.3
RM230	8	*yld8.6s*	20.79	8E-06	680.06	580.03	100	700	-19.94
RM264	8	*yld8.7s*	11.46	0.0008	672	587	85	700	-28

Plant height: **Ten QTLs were identified for plant height, of which nine had positive influence resulting in increased height ranging from 8.18 to 22.90 cm, which amounted to 8.12 to 11.43% increase over KRH2.**

Number of tillers per plant: **Six QTLs showed significant effect on this trait. The *O.rufipogon* alleles had a positive effect at *nt1.1s*, *nt8.1s*, *nt8.2s* and *nt.1s* explaining a phenotypic variance of 32.05, 16.98, 15.95 and 16.62% respectively.**

Number of panicles per plant: **Seven QTLs significantly affecting the trait were identified. The *O.rufipogon* alleles increased the number of panicles in respect of five of the QTLs ranging from 0.84 to 2.35 and explaining a phenotypic variance of 9.30 to 31.58% respectively.**

Panicle length: **Five genomic regions were found to be associated with this trait. In respect of all the five QTLs, the *O.rufipogon* alleles increased panicle length by 1.74 to 3.3 cm, which corresponded to 6.89 to 6.98% increase over KRH2.**

Spikelet number per panicle: **Two QTLs significantly influenced spikelet number per panicle effecting increase by 22 and 78.7, which accounted to 8.75 and 8.08% increase respectively over KRH2.**

Spikelet number per plant: **Three QTLs were found to be associated with spikelet number per plant. The *O.rufipogon* alleles had a positive effect in two cases by increasing spikelet number per plant by 411.84 and 318.41, which explained a phenotypic variance of 29.71 and 21.74% respectively.**

Grain number per panicle: **Two QTLs were associated with this trait. *O.rufipogon* alleles effected grain number increase by 13.67 and 13.99, which corresponded to 9.74 and 10.4% increase over KRH2.**

Grain number per plant: **Five QTLs significantly influenced the trait. The *O.rufipogon* alleles increased the number of grains per plant at *gpl9.1s*, *gpl8.1s* and *gpl3.1s* corresponding to a 4.07, 4.07 and 6.12% increase over KRH2, respectively.**

Spikelet ***fertility:*** **Three QTLs were found to be related to percentage spikelet fertility. *O.rufipogon* alleles had a positive effect in all the three cases increasing spikelet fertility by 6.66, 7.94 and 26.4% corresponding to a 1.3, 1.67 and 24 % increase over KRH2.**

Test (1000) grain weight: **Four QTLs had significant effect on test grain weight. The magnitude of the phenotypic effect of these individual QTLs ranged from 1.15 to 3.08 gm, which corresponded to 4 to 5% increase over KRH2.**

Harvest index: **Seven QTLs significantly affected the trait. The *O.rufipogon* alleles increased the harvest index from 2.68 to 18.00 explaining a phenotypic variance of 20.61 to 76.5%**

Yield per plant: **Six QTLs were found to influence significantly yield per plant. The *O.rufipogon* alleles effected yield increases at *yldp1.1s, yldp8.1s, yldp2.1s* and *yldp. 1s* by 5.36, 3.49, 3.36, and 3.94 gm corresponding to a 2.95, 3.32, 4.84 and 3.42% increase over KRH2, respectively.**

Plot Yield: **Nine QTLs had significant effect on plot yiel d. Two QTLs *viz., yld2.1s* and *yld2.2s* mapped to chromosome 2 had a positive effect on yield. Influence of**

O.rufipogon alleles was seen in the increase of yield by 2.14 and 2.20 tons/ha, which amounted to an increase of about 9.2% over KRH2.

4.7.3 Interval mapping of QTLs: Interval mapping with a minimum LOD threshold of 2.0 identified a total of 41 QTLs for all the 13 traits studied over two locations. Out of a total of 41 QTLs, 15 were common at both the locations (Table 9). The QTLs identified are presen ted trait-wise below:

Table 9: Details of Yield and yield components related QTLs identified by interval mapping at two locations

QTL	Location	Marker	Peak LOD score	Phenotypic variance (%)
Plant height				
ph1.1	1	RM220 – RM272	5.50	25.4
	2	RM220 – RM272	7.18	36.8
ph1.2	1	RM210 – RM81A	10.02	38.2
	2	RM210-RM81A	9.20	40.0
ph1.3	2	RM272-RM259	7.29	37.8
ph2.1	2	RM250 – RM208	2.92	18.3
p*l72.2*	1	RM221-RM6	2.50	13.2
ph8.1	1	RM44-RM350	2.06	33.6
	2	RM44-RM350	3.63	41.9
ph8.2	1	RM44-RM350	8.71	36.5
	2	RM44 – RM350	7.60	39.2
ph8.3	2	RM350-RM210	7.50	41.2
Number of tillers				
nt2.1	1	RM262 – RM183	3.63	27.7
	2	RM262-RM183	3.87	37.6
Number of panicles				
np2.1	1	RM262-RM221	3.78	25.5
	2	RM262-RM221	3.34	53.1
np2.2	*2*	RM262-RM183	4.28	41.5
Panicle length				
pl1.1	1	RM220-RM272	2.30	9.8
pl2.1	1	RM250-RM208	10.77	20.9
Spikelet number per panicle				
spp2.1	1	RM250-RM208	4.55	19.9
Spikelet number per plant				
spl2.1	1	RM262-RM183	3.29	22.6
	2	RM262-RM183	3.99	34.1
spl5.1	1	RM169-RM249	2.60	13.9

Table 9: continued.

Grain number per panicle				
gpp1.1	1	RM210 – RM81A	3.38	16.8
gpp2.1	1	RM250 - RM208	3.32	13.3
gpp3.1	1	RM232 – RM36	3.90	21.5
gpp8.1	1	RM210-RM256	2.15	14.5
Grain number per plant				
gpl2.1	1	RM262 – RM183	3.64	18.4
	2	RM262 – RM183	5.56	32
gpl3.1	1	RM16-RM203	2.92	11.8
gpl5.1	1	RM169 – RM249	4.73	27.8
gpl8.1	2	RM230 – RM264	2.36	9.8
Spikelet fertility				
sf2.1	1	RM262 - RM221	4.23	21.2
sf3.1	1	RM232 – RM36	3.12	40.0
	2	RM232 – RM36	8.34	44.9
sf8.1	1	RM210-RM256	**3.99**	16.2
Test (1000) grain weight				
gw1.1	1	RM210-RM81A	2.02	4.2
gw2.1	1	RM262 – RM221	3.22	8.8
gw5.1	2	RM173-RM233b	3.39	6.6
Harvest index				
hit 1	1	RM210 – RM81A	3.02	11.5
	2	RM210 – RM81A	4.32	**13.82**
hi2.1	2	RM183 – RM263	2.76	5.8
hi8.1	1	RM210 – RM256	2.72	14.2
	2	RM210-RM256	4.87	16.7
Yield per plant				
yldp1.1	2	RM237 - RM297	**2.59**	13.6
yldp2.1	2	RM262 – RM183	5.30	30.0
yldp3.1	1	RM16-RM203	4.32	10.2
yldp8.1	2	RM230 - RM264	2.47	11.3
Plot yield				
yld2.1	1	RM183 – RM263	35.1	50.8
	2	RM183-RM263	32.7	48.28
yld8.1	1	RM38 – RM25	3.56	8.0
	2	RM38 - RM25	4.36	7.80
yld8.2	1	RM350-RM210	4.76	8.40
	2	RM350-RM210	4.78	8.74
yld8.3	1	RM230 - RM264	6.89	20.9
	2	RM230 - RM264	5.58	18.39

Plant height: Eight QTLs relating to this trait were identified. Four QTLs *viz., ph1.1, ph1.2*, *ph8.1* and *ph8.2* were common at both the locations, while the rest were unique either to location 1 or 2. The peak LOD score ranged from 2.05 to 10.02, while percentage of phenotypic variance explained by individual QTLs varied from 13.2 to 41.2%.

Number of tillers per plant: Only one QTL, *nt2.1* was identified to affect number of tillers per plant. The QTL was identified at both the locations but the peak LOD score and percentage phenotypic var iance explained differed at both the locations. The QTL was identified with a peak LOD score of 3.63 explaining 27.7% of phenotypic variance at location 1 while the peak LOD was 3.87 at location 2 accounting for a phenotypic variance of 37.6%.

Number of panicles per plant: Two QTLs *np2.1* and *np2.2* were found related to number of panicles per plant. While *np2.1* occurred at both the locations, *np2.2* was identified at location 2 only. The LOD score ranged from 3.34 to 4.28, while the QTLs contributed to a phen otypic variance of 25.5 and 41.5% respectively.

Panicle length: Two QTLs, *pl1.1* and *pl2.1* were identified at location 1. No corresponding occurrence was found at location 2. The LOD score was 2.30 and 10.77 for *pl1.1* and *pl2.1* respectively explaining a phe notypic variance of 9.8 and 20.9%.

Spikelet number per panicle: Only one QTL *spp2.1* relating to the trait was identified at location 1. It had a peak LOD score of 4.55 and explained 19.9% of phenotypic variance.

Spikelet number per plant: Two QTLs, one each on chromosome 2 and chromosome 5 relating to the trait were identified. The QTL *spl2.1* was found at both the locations, while *spl5.1* at location 1 only.

Grain number per panicle: Four QTLs, one each on chromosomes 1, 2, 3 and 8 were identified only at location 1. The LOD score of the QTLs ranged from 2.15 to 3.90, while the phenotypic variance due to these QTLs ranged from 13.3 to 21.5% with the QTL on chromosome 3, *gpp3.1* contributing the maximum.

Grain number per plant: Four QTLs influencing the trait were identified. The QTL *gpl2.1* on chromosome 2 occurred at both the locations, while the QTLs *gpl3.1* and *gpl5.1* were restricted to location 1 and the QTL *gpl8.1* to location 2. The QTLs had a peak LOD ranging from 2.36 to 5.56 with each QTL explaining a phenotypic variance from 9.8 to 32.0%.

Test (1000) grain weight: Three QTLs unique either to location 1 or location 2 were identified. While the QTLs *gw2.1* and *gw1.1* were identified at location 1 *gw5.1* was identified at location 2. The QTLs explained a pheno typic variance of 4.2 –8.8% with peak LOD score ranging from 2.02 to 3.39.

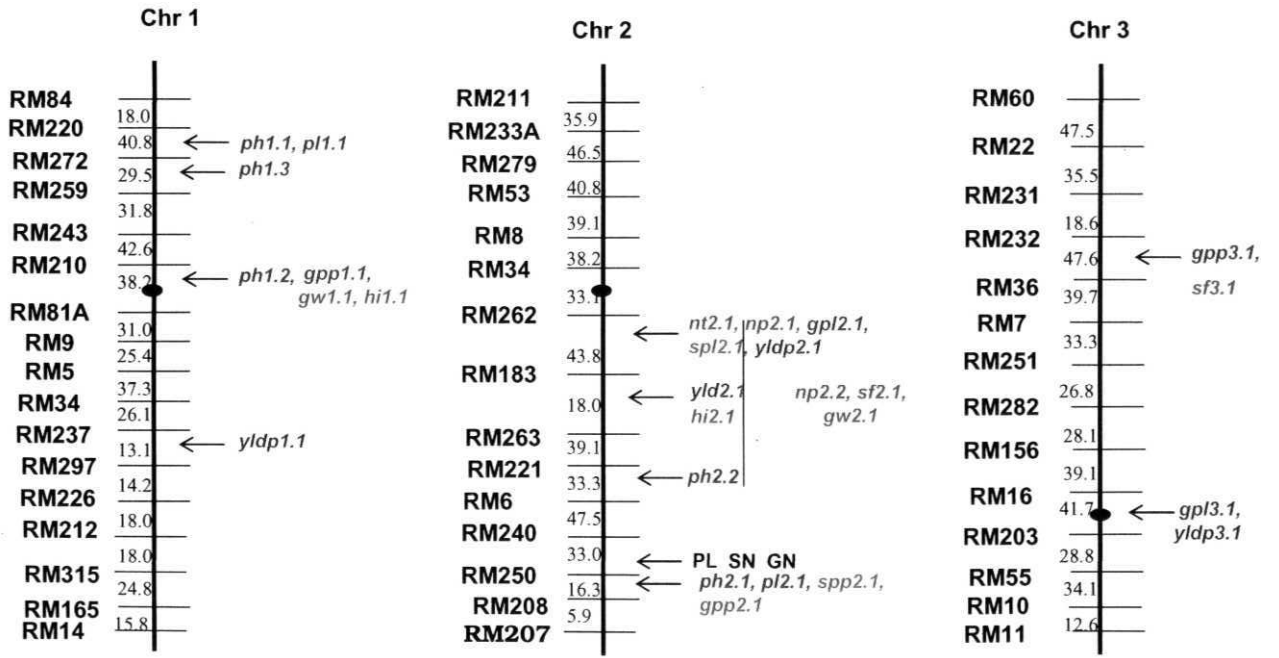
Spikelet fertility: Two QTLs specific to location 1 and one QTL common to both the locations were found to be related to spikelet fertility. They had a peak LOD score ranging from 3.12 to 8.34 with phenotypic variance explaining 16.2 to 44.9%.

Harvest index: Three QTLs relating to the trait were identified and of these, *hit 1* and *hid. 1* were common to both the locations, while *hi2.1* was identified only at location 2.

Yield per plant: **Four QTLs influencing the trait were identified. While the QTL *yldp3.1* was identified at location 1, the other three occurred at location 2.The peak LOD score values ranged from 2.59 to 5.30 and phenotypic variance explained by each QTL was from 10.2 to 3 0.0%. The QTL *yldp2.1* explained the highest phenotypic variance.**

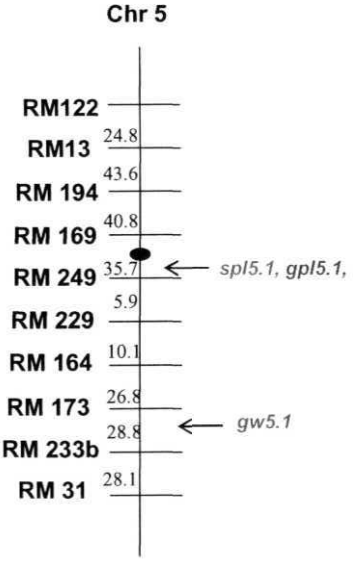
Plot yield: **Three QTLs on chromosome 8 and one on chromosome 2 affecting plot yield were identified. The QTL *yld2.1* was found to explain a phenotypic variance of 50.8%, while those on chromos ome 8 a phenotypic variance of 8 to 20.9% with peak LOD score values ranging from 4.36 to 6.89.**

QTLs identified by interval mapping are only shown.



ph: Plant height; pl: Panicle length; gpp: Grain number per panicle; gw: Test grain weight; HI: Harvest index; nt: Number of tillers per plant; yld: Plot yield; sf: Percentage:spikelet fertility; yldp: Yield per plant; np: Number of panicles per plant. spl: Spikelet number per panicle; spp: Spikelet number per plant; gpl:Grain number per plant

Fig 12: Distribution of QTL's on the molecular linkage maps constructed based on BC_2 testcross populations of IR 58025A / IC 22015



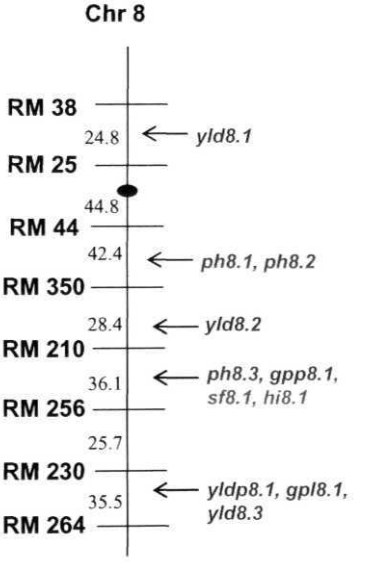


Fig. 12: continued....

5. DISCUSSION

Rice accounts for 27-80% of dietary energy in the average daily intake in the predominantly rice eating Asian countries. Its improvement for enhanced productivity on a sustainable basis is critical for many countries in the region including India to remain food secure. Historically, progressive yield increase in crop plants in general and rice in particular has been through genetic intervention rather than through any other single factor. Among the factors that constrain the envisaged improvement 'limiting genetic variability' is the foremost. In a crop endowed with rich genetic diversity, the limitation is on account of lack of research effort to unfold the variability rather than lack of variability. It is estimated that not even 15% of the available variability has been exploited for its improvement till now. In the long history of its origin, domestication and subsequent improvement only a small fraction of the variability could so far be captured in the cultivars. In other words sizeable allelic variations of genes related to traits of complex inheritance like yield still remain uncovered in the allied wild/weedy species and primitive cultivars. Considered as the "feedstock" for crop improvement, wild germplasm has hardly been used beyond as gene source for very few simply inherited traits of economic significance. The belief that wild species on account of their non-thrifty appearance, might not be the potential donor sources of allelic variation for improvement of quantitatively inherited traits like yield, has so far dissuaded rice breeders all along from exploiting them.

The pioneering attempt to explore and utilize wild species for improvement of quantitative traits like yield was of Frey (1983), who

demonstrated through classical genetic approach in oats (*Avena sativa)* that wild species could also be potential source of new variability for improvement of quantitatively inherited yield. In rice, long before this report, Srinivasan *et al* (1941) and Rajagopalan (1957, 1958) had successfully used wild species like *O.perennis* and *O.sativa f. spontanea* for improvement of yield with tolerance to moisture stress. Recent advancement in the field of molecular biology has provided a variety of sophisticated tools to identify precisely and objectively use the still not exploited yield genes. The most fundamental of these tools is DNA marker technology, which enables building a complete genetic map, which inturn reveals all information about the regions of the genome. By knowing the map position of a gene- no matter it is a simply inherited mendelian trait or polygenically governed quantitative trait, it is possible to trace and select with certainty the same in breeding using closely linked molecular markers. Use of DNA markers started with RFLP markers in tomato and maize (Weller, 1986; Stuber *et al,* 1987; Edwards *et al,* 1987; Tanksley and Hewitt, 1988). These pioneering efforts have led to better understanding of quanti tative traits and make use of the marker technology for crop improvement. In particular, it is the marker technology that helped know the richness of variability in wild germplasm for metric traits as well and prove its utility in overcoming the very much feared problem of linkage drag in interspecific crosses. Demonstration recently through molecular marker analysis that wild allies too carry valuable yield -influencing gene blocks (QTLs) (Xiao *et al,* 1996c; 1998) and increasing conviction that DNA markers would help selectively harness the desired genes of wild species

in yield improvement programme, the present investigation was undertaken not only to further confirm that the wild species, though inferior in their phenotypic appearance could be potential s ource of variability for yield traits but also to find out yield related new QTLs for use in yield enhancement research. The salient findings there from are discussed as under:

5.1 Selection of prospective donor parent

Selection of donor parent is the cruc ial step for mapping and tagging a trait. Parents of contrasting characters are chosen for mapping simply inherited Mendelian traits. In case of improving traits of complex inheritance like yield, breeders largely depend on yield potential *per se* or components of yield that would complement for deciding on the choice of parents. Very few breeders rely on statistically determined genetic diversity for choosing the parents. Relativeness in terms of genetic distance computed using molecular markers as variables through a reliable criteria is seldom used for selection of parents. Those that are distantly placed are considered genetically more diverse and chosen as parents for realizing better recombinants in pedigree breeding and heterotic combinations in hybri d breeding. Studies carried out in maize and rice to relate genetic distance of parents with hybrid performance/heterosis have revealed variable results, some suggesting very strong correlation while others not so strong association.

In the present study a two-pronged strategy *viz.,* genetic diversity based on marker (alleleic) variation and hybrid performance *per se* in terms of growth vigour has been used to select the donor parent. Genetic diversity analysis of

the wild accessions using 14 ISSR primers ha s revealed the accessions R17 and R18 to be relatively more divergent compared to others *vis a vis* the cultivar IR 58025A. The F_1's of the two divergent wild accessions with IR 58025A were not, however, as vigorous as expected. Contrarily, cross combinati ons involving relatively less divergent accessions and the cultivar expressed higher vigour than those involving highly divergent parents. It appears that those wild accessions that are genetically less diverse *vis a vis* cultivars as well could be the right parental choice for looking for yield related new QTLs. Accordingly taking into consideration genetic diversity from IR 58025A (*O.sativa)* as well as hybrid performance *per se,* the *O.rufipogon* accession IC 22015 was chosen as the probable donor parent wit h new genetic variability for yield.

Relationship between marker heterozygosity and hybrid performance is complex owing to germplasm diversity and complex genetic basis of heterosis. Zhang *et al* (1995) report that relationship between molecular marker hetrozygosity and heterosis was variable, depending on the genetic diversity of rice germplasm used. Xiao *et al* (1996b) have observed that random markers like RAPDs and SSRs were useful for predicting yield potential and heterosis of intra-subspecific hybrids, but not of inter-subspecific hybrids. It is generally accepted that association of marker -based genetic distance with hybrid performance depended on the type of crop, the germplasm being studied (Melchinger *et al,* 1992) and on the type of marker being us ed for analysis (Parsons *et al,* 1997). It seems necessary to employ specific markers for those segments that significantly affect expression of heterosis for grain yield. Their

findings suggest that genotypic differences might be useful for preliminary selection of parents with desirable loci and enhanced yield vigour in their hybrid combinations and that it might not accurately predict actual performance of the hybrids. In otherwords, the findings suggest that number of unique and new alleles found in a do nor line need not be a guarantee to high yield heterosis in hybrids involving it and that unique alleles need not necessarily be favorable yield promoting QTLs (Parsons *et al,* 1997).

Keeping the foregoing pitfalls in view, choice of the donor parent *viz., the* wild *O.rufipogon* accession has been done employing together two different approaches *viz.,* genetic distance and hybrid vigour *per se*.The approach is very much same as that followed by Xiao *et al* (1996c).

5.2 Choice of population

Most QTL studies utilize populations, in which alleles of both the parents occur at a relatively high frequency (Shen *et al,* 2001; Liao *et al,* 2001; Li *et al,* 2000; Redonna and Mackill, 1998). While such populations are well suited for conventional QTL mapping, they have seri ous drawbacks when employed to detect and transfer useful QTLs from unadapted germplasm into elite breeding lines. Undesirable QTL alleles in such sources occur at high frequency and epistatic interactions offer difficulty in detecting them statistically (Tanksley and Nelson, 1996). A possible solution to the above problem would be delaying of QTL analysis until the population reaches relatively an advanced generation. Researchers have demonstrated that advanced backcross populations such as BC_2, BC_3 etc., could be used to increase the probability of successful selections

in crosses involving wild germplasm (Xiao *et al,* 1998; Moncada *et at,* 2001).

In this study we adopted advanced backcross QTL analysis method, wherein QTLs were transferred into the cytopla smic male sterile line IR 58025A and BC_2 plants were produced by repeatedly backcrossing with its isogenic maintainer line IR 58025B and practicing negative selection. Male sterile plants in BC2 were crossed to the inbred restorer line KMR3 to produce BC_2 testcross families that served as mapping population. The transfer could also have been done into any other inbred line. But as this work was undertaken largely with the objective of confirming the earlier reports on the value of wild species as the parental sources for unique variability for quantitative traits such as yield, the cytoplasmic male sterile line was used as the recepient parent.

Aside using a cms line as the recepient parent, the method followed for development of mapping population is justif ied as well for its adopting an advanced backcross generation (BC 2) as the mapping population, which ensures, sure transfer of yield enhancing QTLs from wild/weedy relatives into cultivars. Also, this approach while resulting in genotypes increasingly resembling the elite parent with less unfavorable alleles from the wild/weedy parent allows phenotypic selection to further reduce the frequency of deleterious alleles. Further, BC_2 population will have a considerable fraction of the genome of wild donor parent unlike in still more advanced BC 3 or BC_4 which would have more of the recurrent parent alleles and hence have too low statistical power to detect most QTLs (Tanksley and Nelson, 1996).

5.3 Segregation of molecular markers

Against the expected allele frequency of 87.5% (IR 58025A) : 12.5% *(O.rufipogon),* a ratio of 83.26% (IR 58025A) : 16.74% (*O.rufipogon)* has been observed in the population, which may be explained as due to skewed segregation observed at 39.19% of the markers studied. Twenty five percent of these markers show skewness towards IR 58025A while 14% towards *O.rufipogon.* Skewness towards the elite parent can be explained by intensity of selection imposed in the BC_1 generation giving preference to *O.sativa* alleles in the population. Similarly distorted segregation has been reported earlier by Moncada *et al* (2001) in the interspecific cross of *O.sativa x O.rufipogon.* Skewness in segregation towards one or the other parent has been reported earlier in doubled haploid (DH) population (Lu *et al,* 1996) as well as recombinant inbred populations (Xu *et al,* 1995; McCouch *et al,* 1988). Skewness towards *O.rufipogon* may be as a result of reduced recombination between distantly related parental lines as suggested by Causse *et al* (1994) and Grandillo and Tanksley (1996). Seven of the markers used in the present study did not show any segregation and all the alleles were of IR58025A type. Such distortions from the expected Mendelian ratios arise, when one or more classes of gametes are either lethal or the zygo tes derived from them are lethal, so that the normal meiotic segregation pattern is not observed. It is important to know the chromosomal regions associated with such segregation distortion. If a gene that causes segregation distortion is segregating in a population, then

markers close to it would also tend to exhibit distorted ratios. It was interesting to note that three of the eight markers, which did not segregate were located close to gamete abortive *(ga)* locus reported earlier. In conformity with such reports markers RM2 and RM214 near the centromere of chromosome 7 do also not segregate. This in turn is in conformity with earlier reports (He *et al,* 2001). Two new segregation distortion regions have also been identified in the course of the present investigation *viz.,* RM208 on the long arm of chromosme 2 and RM122 at the distant end of the short arm of chromosome 5. These regions might carry some new genes responsible for gamete abortion.

5.4 Character pair associations

Yield is a trait dependant on its major direct components. The components, being independent traits, may be positive or negative in the relationship among themselves. Such a general trend would again depend on various factors like composition of the population and factors interfering wit h the random mating etc. Significantly the present study, besides confirming that major components follow significant positive relationship with yield, has brought out two important facts that the plot yield and yield per plant need not be related though both are strongly related to harvest index and plot yield depends on the number of grains per unit area that in turn is determined by number of productive panicles per unit area and number of grains per panicle. Test grain weight is an important component of yield. But its nature of association with yield would depend on grain number per panicle. Though yield per plant on the expected lines shows positive relationship with most of the major yield components,

selection on that basis would not guarantee yield in plot level. The yield potential of a genotype is its ability to perform in a population.

The present findings on the nature of relationship, therefore, either agree with some reports or deviate from those of others. For instance, the results agree with Brondani *et al* (2001) in respect of character pair correlation between number of panicles (NP) and number of tillers (NT), Yield per plant (Yldp) and spikelet number per panicle (SPP), grain number per panicle (GPP) and SPP, grain number per plant (GPL) and Yldp, which follow a positive association, while panicle length (PL) and NT, PL and NP, test (1000) grain weight (GW) and NT, which have a negative correlation. While the present findings do not agree with those of Brondani *et al* (2001) on a positive correlation of PH with NP and NT, negative correlation obtained between NP and PL in the present study agrees with the results of Yamamoto *et al* (2001), who obtained similar results in a population of inter-subspecific *(indica / japonica)* cross. The results very well agree with that of Moncada *et al* (2001), for all the traits studied but for the correlation of PH with GPP and GW While our results are largely in conformity with those of Xiao *et al* (1998), whose study like ours was on the population of *O.sativa / O.rufipogon* cross, they differ at some points. For instance, as against the negative correlation between PH and NP reported by Xiao *et al* (1998), ours shows positive correlation. Whereas PH has no significant correlation with GPL, SF (spikelet fertility) and GW, findings of Xiao *et al* (1998) indicate positive relationship. Such differences in the nature and strength of character pair associations are bound to be there in interspecific crosses, largely an account of

compatibility-dependant distorted segregation. What is important in studies aimed at finding desirable genes for complexly inherited yield is that both yield per plant and yield per plot along with the components that show consistently positive relationship with both be taken into consideration .

5.5 Heterosis over KRH2

The impact of transfer of unique yield QTLs from the wild species is evident from some or the other testcross families outperforming the commercial KRH2 in one or the other yield related traits. For instance, 39 families outyielde d KRH2 on a per plant basis while 8 and 101 families for spikelets per panicle and filled grains per panicle respectively. On plot basis, 24 families yielded 8 t/ha of which 9 families gave more than 9 t/ha as against 7 t/ha of KRH2.

5.6 Identification and study of yield related QTLs

DNA marker associated QTLs reveal many details which broadly include (a) location of them in the genome (b) number of them governing a trait and their relative contribution to the trait expression (c) influence of one QTL on ma ny traits (pleiotropic) (d) transgressive segregation (e) sensitivity to environment influence etc,. To precisely study the foregoing, high density map is a prerequisite and using powerful statistical methods likelihood intervals for the location of QTLs can be determined (Lander and Botstein, 1989). Based on fine mapping of QTLs, they could be mapped to individual likelihood intervals measuring 0.1% of the genome. This would mean a QTL to be equivalent to 10 to 100 genes, assuming crop plants of average ge nome size consist of 10,000 to 100, 000 genes. Keeping in view the foregoing knowledge base on QTLs, the

results of the present effort on QTL identification have been discussed. Interval mapping and single marker analysis together enabled identification in all of 102 QTLs relating to 13 yield and its major components (Tables 8, 9). Forty one QTLs were mapped to five chromosomes *viz.,* 1, 2, 3, 5 and 8 using interval mapping from the data obtained from two different test locations. Thirty seven percent (15) of the QTLs are common at both the locations and the rest being restricted to either of the locations. A genotype performing better in one environment may not do so in other environment due to strong G x E interaction. The differential performance traceable to relative genetic adaptability to environment is not unusual. It could be interpreted as due to differences in the sensitivity of QTLs governing the trait. Paterson *et al* (1990) have demonstrated the phenomenon in tomato. Of 29 QTLs mapped in three dif ferent environments, only 4 (14%) expressed in all the three environments while 10 (34%) and 15 (52%) respectively in two and one environments. As for their deployment in applied breeding that doing well in all environments could be of value for situations requiring general adaptability. Those which are specific to a given location could be deployed in two ways *viz.,* pyramiding QTLs with different specificities into a single genotype and exploiting the potential QTLs specific to one environment as such to take advantage of specific adaptability. In the present study what have been identified as specific to one or two locations be studied more critically, their stability, before use in the above suggested lines. Single marker analysis has helped identify 61 QTLs distributed over 10 chromosomes. On the five chromosomes analyzed for interval mapping, single

marker analysis has identified 51 QTLs, of which 16 (30%) are in agreement with the results of interval mapping whereas others are not. One QTL on chr -6, three QTLs on chr-7, two on chr-9, one on chr-10 and three QTLs on chr-11 constituted the remaining 10 QTLs. No QTL could be identified on chr. 4 and chr. 12.

One of the major objectives of the present study has been to identify yield related QTLs of promise and place emphasis on study of those reported for the first time. Relating to yield and its components so far as many as 125 QTLs have been reported by various workers as reviewed recently by Zhang and Yu (2001). In the course of the present investigatio n 85 QTLs directly relating to yield and its major components have been identified and of these 56 are being reported for the first time (Table 10). Of 17 QTLs relating to plant height 7 are new and 10 of them are in conformity with the earlier reports (Li *et al* 1995). Out of the 17 QTLs relating to plant height QTL *ph8.1* on chromosome 8 has been identified by both single marker as well as interval mapping. Of the six QTLs identified for number of tillers per plant, *nt2.1* is commonly identified in both typ es of mapping. While *nt1.1s* and *nt8.2s* are newly identified QTLs. The QTLs *nt2.1s, nt7.1s and nt8.1s* are same as earlier reported ones (Lin *et al,* 1996; Yu *et al,* 1997; Li *et al,* 2000; Brondani *et al,* 2001). Except one QTL namely *np8.1s* (Brondani *et al,* 2001), of the eight relating to number of panicles per plant are reported for the first time. Four of the six QTLs identified for panicle length are novel, two QTLs *pl1.1* and *pl2.1* match with those of the earlier results (Xiao *et al,* 1995; 1996c). All the three QTLs for spikelet number per panicle, four for spikelet number per

plant, nine for grain number per panicle, six for spikelet fertility and nine for harvest index are novel QTLs identified for the first time. While two of the six QTLs for grain number per plant are identical to those reported earlier by Yu *et al* (1997), and four of the seven QTLs relating to grain weight are novel.

Table 10: QTLs relating to yield and its components identified using interval mapping and single marker analysis in backcross population involving the ***O.rufipogon*** accession **(IC22015)**

S.No	Trait	Total QTLs identified (IM & SMA)*	Chromosome number	Novel QTLs from this study	Most promising
1	Plot Yield	13	2,8	4	*yld2.1,yld8.3*
2	Yield/plant	8	1,2,8	6	*yldp2.1*
3	Tiller no./plant	6	1,2,7,8	2	*nt2.1*
4	Panicle no./plant	8	1,2,7,8	1	*np2.2*
5	Panicle length	6	1,2,5	4	*pl2.1*
6	Spikelet no./panicle	3	1, 2, 8	3	*spp2.1, spp8.1s*
7	Spikelet no./plant	4	2,5	4	*spl2.1*
8	Grain no./panicle	9	1, 2, 3, 8	9	*gpp3.1*
9	Grain no./plant	6	2, 3, 5, 8	4	*gpl5.1*
10	Spikelet fertility	6	2,3,8	6	*sf3.1*
11	Test grain weight	7	1,2,5	4	---
12	Harvest index	9	1,2,8	9	*hi8.1*
13	Plant height	17	1,2,3,5,7,8,9	7	*ph1.2, ph8.2*
	Total QTLs	102		63	
	Yield related QTLs	85		56	

* IM: Interval mapping, SMA: Single marker analysis

As for yield per plant, of eight QTLs located on chromosomes 1, 2, 3 and 8, six are new while the remaining two *viz., yldp1.1s* and *yldp3.1* are same as reported earlier by Brondani *et al* (2001). Of 13 QTLs identified per p lot yield on chromosomes 2 and 8, *yld2.1* and *yld2.2* on chromosome 2 match with the

earlier reports of Lin *et al* (1996) and Lu *et al* (1996), while the remaining 11 QTLs on chromosome 8 are novel and reported for the first time. The QTL *yld8.1s* has been uniquely identified by single marker analysis, all the remaining QTLs have been identified through both interval mapping and single marker analysis.

The distribution of the yield related QTLs over as many as six chromosomes (1, 2, 3, 5, 7 and 8) suggest chro mosome 2, 8 and 1 to be critical carrying nearly all the yield related QTLs, the least being chromosome 7. The findings broadly agree with earlier reports as for the relative importance of the chromosomes. Another finding of interest is that except total s pikelet number per panicle, all others are controlled by more than one QTLs suggesting that they could be different genes/gene blocks (non allelic) contributing to the expression of a common trait. Considering the fact that each QTL could consist of 10 -100 genes, though a QTL may largely inherit as a complex locus of several genes with small effect, it is separable. The constitution and distribution of the QTLs could explain very well dominance and epistasis as the genetic basis of heterosis.

Interestingly some of the genomic regions account for more than one QTL. For instance the QTL flanked by RM183 and RM263 on chromosome 2 has as many as five QTLs including yield (Yld, NP, GN, SF and GW). Similarly the locus between RM210 and RM81A on chromosome 1 has f our QTLs (PH, GN, GW, HI) followed by the one between RM240 and RM250 on chromosome 2 with three QTLs (PL, SN, GN) and between RM210 and RM256 on

chromosome 1 which also has three QTLs (PH, SF, HI). The genetic basis of such a phenomenon could be explained as due to either tightly linked more than one gene blocks (QTLs) each governing a complex trait or pleiotropic effect of one and the same QTL. Unless we have highly saturated map of genetic markers, the reason behind this phenomenon cannot be resolved.

The study while confirming the view that the progenitor species and primitive cultivars constitute the largest source of still unfolded variability for traits of complex inheritance like yield and its components (Xiao *et al*, 1998) has helped identify additional novel variability for yield improvement. In particular the QTLs such as *yld8.3, spl2.1, gpl2.1, sf3.1* and *np2.1* relating to plot yield, spikelet number per plant, grain number per plant, percentage spikelet fertility, and number of panicles per plant respectively are not only new and reported for the first time but also quite stable as understood from their accounting for high percentage phenotypic variance, high LOD score and consistent occurrence at both the test environments. Whereas, the QTL *yldp2.1* relating to yield per plant, even though occurs at only location is of much promise due to it's accounting for high LOD score and high percentage phenotypic variance. Significantly identification of QTLs affecting harvest index is of particular value, the trait being the route to grain yield either independently or along with biomass. Stable QTLs influencing the components of yield and harvest index such as grain number per panicle, number of ear bearing tillers per plant, test grain weight and percentage spikelet fertility identified for the first time would provide yet another strong base for breeding for genetic yield enhancement. Near isogenic lines for the yield

related QTLs can be developed by validation and subsequent transfer to elite cultivars by both conventional recombination breeding using the QTLs as the selectable markers and gene transfer technology by isolating, cloning and splicing of the QTLs of interest into desired agronomic bases (Paterson, 1998).

The applied value of the yield related QTLs such as *yld1.1* and *yld2.1* discovered by Xiao *et* a/ (1998) is evident incidentally from the recent repots from China that their exploitation by conventional recombination breeding could help enhance the yield level of new plant type varieties by about 30 percent (Yuan, 2001). Rapid advances being made in the area of genomics should help dissect and characterize further both individual as well as multiple yield related QTLs towards selective splicing of them for further raising the genetic yield level of the currently available highest yielding varieties and hybrids in rice. Considering the potential of yield influencing new QTLs, more research is warranted to unearth and use more and more novel yield related gene blocks hidden in closely related wild/weedy species and primitive cultivars, if the rice dependant world is to truly attain and sustain food security.

6. SUMMARY

It is generally believed that the least used primitive landraces and wild/weedy progenitor species of *O.sativa* represent a large still unexploited variability in terms of valuable major genes as well as the genes with small effect governing quantitative traits of agronomic value. In fact, the germplasm breeders depend on today for rice improvement represents not more than 15% of the total variability. The unattractive weed-like appearance of the wild germplasm continues to dissuade the breeders from utilizing them as sources of variability for yield enhancement. With the recent advances in the field of molecular biology a wide array of DNA marker techniques like RFLP, RAPD, AFLP, SSRs, ISSR etc., now it has become possible to the gene blocks popularly known by quantitative trait loci (QTLs) influencing yield and its components. The present study was undertaken with the sole objective of confirming the potential progenitor wild species as the potential source for yield genes and identifying new allelic variations of genes (QTLs) governing yield related traits. The study consisted of two parts namely, identification of prospective donor parent likely to possess the new yield genes, and identification of new marker associated QTLs related to yield and it's components. The following are the salient findings and conclusions:

Material and Methodology:

- A representative set of 25 *Oryza rufipogon* and 5 *O.nivara* accessions assembled from various sources along with five improved varieties of *O.sativa* including the widely used CMS line IR 58025A, its maintainer IR 58025B and restorer (KMR3) formed the experimental material. The 29 F_1

plants derived from the cross IR 58025A x IC 22015 *(O.rufipogon)* and the advanced backcross generations *viz.,* BC_1 (50 plants), BC_2 (251 plants) and 251 testcross families also comprised the experimental material.

- As for identification of donor parent, two approaches *viz.,* genetic diversity as understood at molecular level and the relative hybrid vigour in terms of growth vigour was used.
- The study helped identify the *O.rufipogon* accession, IC 22015 to be the parental choice for the study by the virtue of its hybrid vigour, compatibility and genetic distance from IR 58025A.
- The donor parent was used in a crossing programme for developing mapping population using Advanced Backcross Method. The accession of *O.rufipogon,* IC 22015 used as the male parent was crossed with IR 58025A to generate F_1. The F_1 plants were backcrossed to IR 58025B, the isogenic maintainer (B) line of IR 58025A to generate BC_1. Fifty best BC1 plants were selected and backcrossed again with IR 58025B to produce BC2. Two hundred and fifty one randomly chosen BC_2 plants were crossed to KMR3, the restorer line of the commercial hybrid KRH2 to generate 251 testcross families, which formed the mapping population. The mapping population was grown at two locations to assess stability of QTLs.
- Two types of markers *viz.,* SSRs and ISSRs were used for screening the parents, IR 58025A, KMR3 and IC 22015 (*O.rufipogon).*

- Interval mapping and single marker analysis were used to detect QTLs

Major findings:

- Comparison of 252 BC_2 testcross families with KRH2 revealed 39, 8 and 101 families to give 20% higher grain yield, spikelets per panicle and filled grains per panicle respectively. On plot basis 24 families yielded 8 t/ha and 9 families more than 9 t/ha as against 7 t/ha of KRH2.
- Out of 75 ISSR primers tested, 33 were polymorphic amounting to percentge polymorphism of 44, while107 of the 284 microsatellite primers tested were polymorphic amounting to 37.7% polymorphism.
- The polymorphic microsatellite markers were screened on the mapping population for their segregation pattern. The markers formed five linkage groups corresponding to chromosomes 1, 2, 3, 5 and 8.
- In all 85 QTLs have been identified for yield and its components by usinf interval mapping as well as single marker analysis and of these 63 are novel and reported for the first time. The yield related QTLs have been found to be on three genomic regions. As many as eight genomic regions mapped 2-5 yield related QTLs.
- The QTLs were location specific in their expression. QTLs occurring in both the environments (15) as well in 0 ne or the other environment (26) could be identified.
- Of the two QTLs identified on chromosome 2 in this study one is in agreement with what has been earlier demonstrated by the Cornell group.

- **QTLs such as *yld8.3, yldp2.1, spl2.1, gpl2.1, sf3.1* and *np2.1* relating to plot yield, yield per plant, spikelet number per plant, grain number per plant, percentage spikelet fertility and number of panicles per plant respectively are novel and reported for the first time. Also they were quite stable as understood from their accounting for high percentage phenotypic variance, high LOD score and consistent occurrence at both the test environments.**

Conclusions:

- **The findings have helped unfold hitherto unknown variability for yield and most of the yield components.**
- **While confirming that the wild and weedy species constituted the potential variability for yield and its components, the discovery of new and stable yield related QTLs offer opportunities for exploiting them for progressive yield improvement through two approach es *viz.,* conventional recombinant breeding using the QTLs as selectable markers and recombinant technology for selective transformation of good agronomic bases with QTLs of interest.**

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