Research article

# Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*

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## Abstract

**Background:** Cultivated rice (*Oryza sativa* L.) is endowed with a rich genetic variability. In spite of such a great diversity, the modern rice cultivars have narrow genetic base for most of the agronomically important traits. To sustain the demand of an ever increasing population, new avenues have to be explored to increase the yield of rice. Wild progenitor species present potential donor sources for complex traits such as yield and would help to realize the dream of sustained food security.

**Results:** Advanced backcross method was used to introgress and map new quantitative trait loci (QTLs) relating to yield and its components from an Indian accession of Oryza *rufipogon*. An interspecific BC$_2$ testcross progeny (IR58025A/*O. rufipogon*//IR580325B///IR58025B////KMR3) was evaluated for 13 agronomic traits pertaining to yield and its components. Transgressive segregants were obtained for all the traits. Thirty nine QTLs were identified using interval mapping and composite interval mapping. In spite of it's inferiority for most of the traits studied, *O. rufipogon* alleles contributed positively to 74% of the QTLs. Thirty QTLs had corresponding occurrences with the QTLs reported earlier, indicating that these QTLs are stable across genetic backgrounds. Nine QTLs are novel and reported for the first time.

**Conclusion:** The study confirms that the progenitor species constitute a prominent source of still unfolded variability for traits of complex inheritance like yield. With the availability of the complete genome sequence of rice and the developments in the field of genomics, it is now possible to identify the genes underlying the QTLs. The identification of the genes constituting QTLs would help us to understand the molecular mechanisms behind the action of QTLs.

## Background

The modern day cultivars of rice, in spite 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 Indian rice varieties for their ancestry revealed 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 (E A Siddiq, personal communication). Rice is endowed with very rich genetic diversity. Wild/weedy species along with very large number of

primitive cultivars and landraces constitute an important reservoir of useful genes. The size of additional variability they can provide would be of great value to the ongoing crop improvement endeavor. Large genetic variability still remains untapped in the wild relatives and primitive cultivars of rice [1]. Considering the large hidden variability and very rare and agronomically important genes they possibly possess, utilization of the wild species is critical to future crop improvement [2]. Utilization of these exotic species as donors in interspecific crosses is one of the strategies to harness their hidden potential and broaden the genetic diversity of the existing gene pool. Over the last decade, wild species in rice have been successfully utilized for introgression of diverse traits such cytoplasmic male sterility (cms) [3-6], abiotic and biotic stress [7-11], yield and its components [12-18] and grain quality [19-21] into the cultivars. A great deal of work in the recent past, on the wild species of rice, concentrated on the utilization of these species for quantitative traits such as yield and its components long with grain quality. In the first ever report on the use of wild species for introgression of quantitative characters, two yield QTLs, *yld1.1 and yld 2.1*, each of which is capable of increasing yield by about 18% have been identified in a Malaysian accession of *O. rufipogon* [12,13]. This was a precursor to many studies resulting in the identification of numerous QTLs pertaining to yield and grain quality [12-21]. Keeping in view the unlimited potential of wild/weedy species of rice for yield genes as evident from the foregoing research, the present study reports the identification and mapping of molecular marker-associated yield QTLs in an Indian accession of *O. rufipogon* (IC 22015). An interspecific testcross population, derived using an advanced backcross QTL strategy (AB-QTL) [22], between *O. rufipogon* and IR 58025A, a widely used cms line in India, was used to map QTLs related to yield and it's components. The AB-QTL method has been successfully employed earlier in tomato and rice to transfer positive alleles from phenotypically inferior wild and weedy species into elite cultivars [23-25,14-19]. In addition to identifying potential novel QTLs for yield and it's components, the results from the current study will provide additional data for comparison with QTLs that are previously documented in rice. Comparisons across different genetic backgrounds will provide information about the conservation of QTLs and help us to understand the interactions of QTL alleles across multiple backgrounds and environments.

## Results

### Trait analysis and field performance

The phenotypic analysis of the 251 testcross families showed that the frequency Distribution of all traits approximately fit normal distribution (Figure 1). As expected in an interspecific cross, character wise frequency distribution of testcross families showed transgressive seg-

regants for all the traits. For a depiction of variation in tiller number and panicle length in the testcross families, see additional file 1. The average grain yield of the test-cross 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 g with an average of 19.5 g. Thirteen testcross families outperformed the hybrid check, 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 1). Of the 251 testcross families studied in all, 75 showed at least 20% increase over KRH2 for three or more yield components.

### Trait correlations

The trait correlations confirmed to the expected results. Significant positive correlations (P < 0.01) included SPY (Single plant yield) × SNP (Spikelet number per plant) (0.552), SPY × GNP (Grain number per plant) (0.581) and HI (Harvest index) × SPY (0.539) whereas, the significant negatively correlated traits included PH (Plant height) × SF (Spikelet fertility) (-0.362), GW (Grain weight) × NT (Number of tillers) (-0.255), GW × NP (Number of panicles) (-0.284) and HI × PH (-0.298). Interestingly GW had no significant effect on PY (Plot yield), but showed negative correlation with NP. For detailed character pair correlations among the traits see additional file 2.

### Marker polymorphism

Two hundred and ten microsatellite markers were used to screen the parents for identifying polymorphic markers. Eighty markers (38%) detected polymorphism. The polymorphism is lower compared to earlier studies involving *O. rufipogon*, where the polymorphism ranged from 60–90% [13,15,17]. Polymorphism is a measure of genetic diversity and varies with the parental combinations used. Earlier studies using a Malaysian accession of *O. rufipogon* (IRGC 105491) have indicated varying frequencies of SSR polymorphism with *indica* (~60%) [13,17] and *japonica* (90%) [15] recurrent parents. The lower percentage polymorphism may be due to a higher degree of genetic similarity between *O. rufipogon* and *O. sativa* used in this study compared to those used earlier.

### Marker segregation

The expected genotypic ratio in the $BC_2$ population would be 3:1 for homozygous IR 58025A : heterozygous IR 58025A/*O. rufipogon* (87.5% IR 58025A alleles to 12.5 *O. rufipogon* alleles). Out of the 80 marker loci, 28.75% (23 markers) were skewed towards one or the other parent resulting in an allele frequency of 83.26% IR 58025A alleles to 16.74% *O. rufipogon* alleles (Table 2). While 12.5% (10 markers) were skewed towards *O. sativa* parent, 16.25% (13 markers) were skewed ($X_2$ > 6.6, p < 0.01) towards *O. rufipogon*.. The skewed markers were distrib-

**Table 1: Mean phenotypic traits for 13 yield components across 251 testcross families as compared to IR 58025A, IC22015 (wild) and KRH2**

| Trait | IR 58025A[a] | IC 22015[b] | KRH2[c] | 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 | 152* | 35 | 117 | 30 – 185 | 101 |
| Grain number/plant | 1064* | 700 | 1187 | 322 – 2310 | 63 |
| Spikelet fertility (%) | 0 | 15–20 | 68 | 42 – 91 | 42 |
| 1000 grain weight (g) | 20 | 11.5 | 22.5 | 17.5 – 31.3 | 1 |
| Yield / plant (g) | 16* | 9 | 19 | 7.5 – 36 | 39 |
| Harvest index | 32* | - | 45 | 27 – 56 | 7 |
| Yield (t/ha) | 4.2 | - | 7 | 3.9 – 9.45 | 13 |

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

a – *O. sativa* parent, b – *O. rufipogon* parent, c- hybrid check

* for the traits where IR 58025A has no corresponding value, the value from IR 58025B, an isogenic line of IR 58025A is used.

uted on chromosomes 1, 2, 3, 5 and 8 with most of the markers on chromosome 2.

### QTL analysis

A total of 39 QTLs were identified using composite interval mapping (CIM) and interval mapping (IM). CIM analysis detected fewer QTLs (25 QTLs) than IM (31 QTLs). While 17 QTLs (43.58%) were detected by both the methods, IM identified 14 QTLs (35.89%) exclusively and 8 QTLs (20.51%) were only detected by CIM (Table 3). Single marker analysis identified a total of 45 QTLs for the 13 traits studied [See additional file 3]. Forty two out of the 45 QTLs identified by single marker analysis were either identified by CIM or IM, so these will not be discussed separately. Three QTLs, *sf1.1*, *spp1.1* and *hi1.1* were only identified by SMA. The variation in the number of QTLs detected by different methods has been previously reported for interspecific crosses involving *O. rufipogon* [15,17]. The 39 QTLs were distributed on chromosomes 1, 2, 3, 5, 8 and 9 (Figure 2).

### Interaction among QTLs

A two-way test to detect epistatic interactions between marker loci was performed using the EPISTAT software [26]. The analysis identified a total of 15 interactions consisting of 20 markers spread across 8 different chromosomes (Table 4). These markers did not contribute to the phenotype singly but had a significant effect on the phenotype in combination with another marker indicating strong G × G interactions. This may be one of the reasons for the transgressive segregants obtained.

## Discussion

### Marker segregation

The allele frequency in a BC$_2$ population without selection would be 87.5% IR 58025A alleles to 12.5% *O. rufipogon* alleles. Twenty three markers (28.75%) were skewed towards one or the other parent resulting in an allele frequency of 83.26% IR 58025A alleles to 16.74% *O. rufipogon* alleles. Ten marker loci (12.5%) were skewed towards *O. sativa* parent, whereas, 13 markers (16.25%) had over representation of *O. rufipogon* alleles. Skewness of markers towards one of the parents has been documented for interspecific as well as intersubspecifc crosses in rice [14-19], [27-29]. A comparison of the results with earlier studies involving *O. rufipogon* revealed that the percentage of skewed markers was lower compared to that reported by Moncada *et al* [15] (37.6%) and Thompson *et al* [18] (42.5%) and higher compared to Septiningsih *et al* (21.4%) [17]. All the three previous studies used same accession of *O. rufipogon* (IRGC 105491)) but different recurrent parents. This suggests that the polymorphism percentage is relative and depends on parental combination. Skewness towards the elite parent could have been due to the intensity of selection imposed in the BC1 generation, while, skewness towards *O. rufipogon* may be due to reduced recombination and linkage drag in some regions of an interspecific population [30,31]. While, segregation distortion of RM251 and RM7 on chromosome 3 may be due to their proximity to the gamete abortive gene, *ga3* [32], the deviation from the mendelian ratio of RM249 and RM44 might be due to the presence of these markers close to the centromeres of chromosome 5 and 8
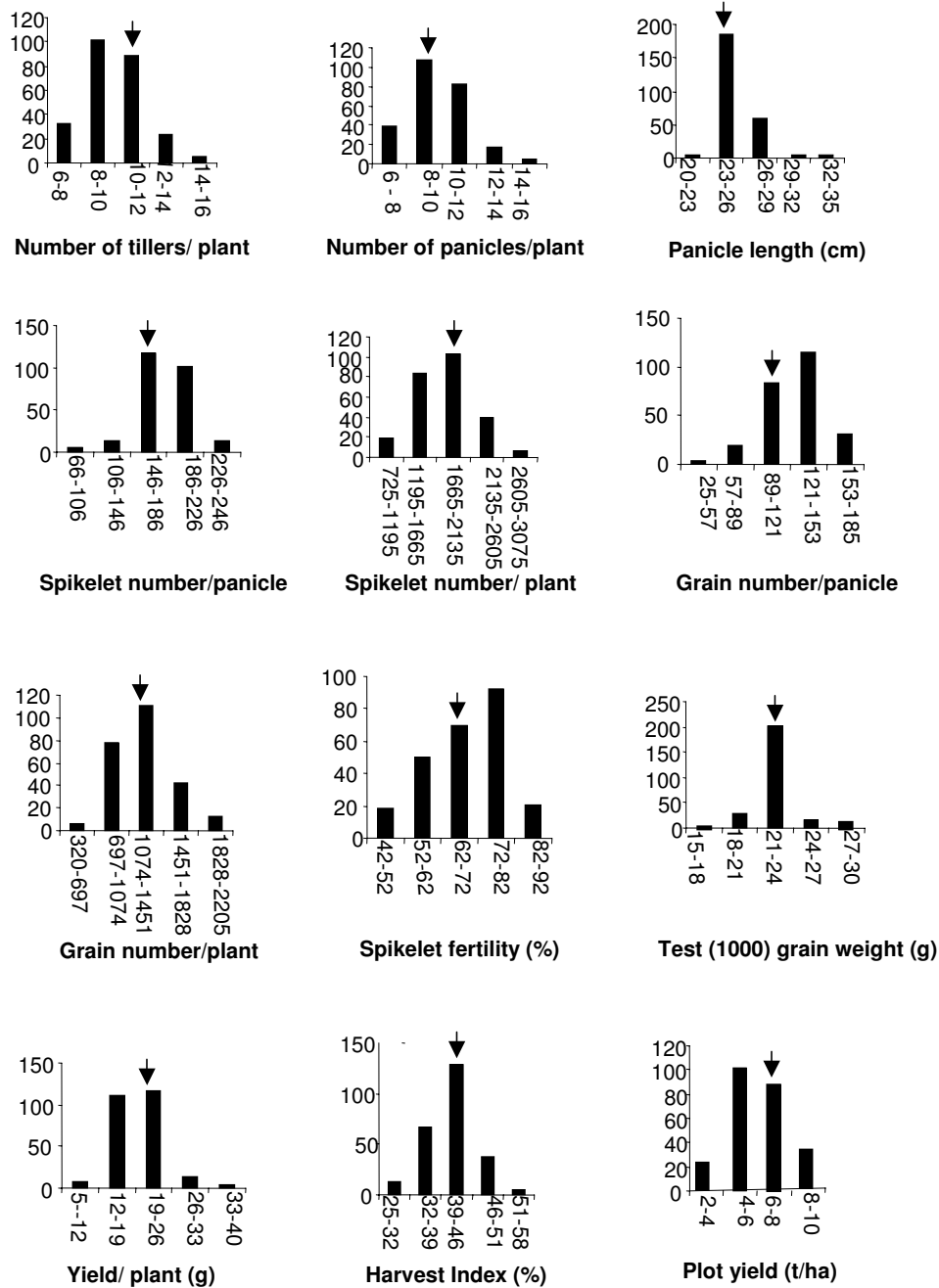
**Figure 1**
Frequency distribution of the 251 testcross families for yield and its components. Arrow indicates the value of the hybrid check, KRH2. Y-axis: Number of individuals.

respectively. Some of the chromosomal regions may have been distorted due to the selection imposed in BC1. The segregation distortion, towards IR 58025A, of marker loci RM84 on chromosome 1, RM207 on chromosome 2 and RM22 on chromosome 3, which exist in close proximity

to dwarfing genes *d18, d29* and *d50* respectively [32] may be due to the selection for semi-dwarf plant height in BC1 generation. The distortion of RM13, RM169 and RM262 (markers associated with QTLs for number of tillers) towards *O. rufipogon*, may be due to the selection imposed

**Table 2: Chi square values of the markers showing segregation distortion in the test cross progeny**

| Marker | Chromosome | $X^2$ | Chr. Position[a] | Skewness[b] |
|---|---|---|---|---|
| RM84 | 1 | 24.88** | 0.0 | IR 58025A |
| RM243 | 1 | 8.28* | 98.1 | IC 22015 |
| RM212 | 1 | 19.47** | 209.8 | IR 58025A |
| RM14 | 1 | 11.92** | 268.4 | IR 58025A |
| RM262 | 2 | 42.54** | 98.6 | IC 22015 |
| RM207 | 2 | 10.64* | 227.5 | IR 58025A |
| RM211 | 2 | 47.40** | 0.0 | IC 22015 |
| RM53 | 2 | 19.47** | 23.2 | IC 22015 |
| RM8 | 2 | 28.83** | 42.3 | IC 22015 |
| RM240 | 2 | 24.88** | 182.3 | IC 22015 |
| RM183 | 2 | 17.81** | 102.4 | IC 22015 |
| RM55 | 3 | 8.28* | 193.7 | IC 22015 |
| RM60 | 3 | 23.00** | 0.0 | IC 22015 |
| RM7 | 3 | 17.81** | 91.6 | IR 58025A |
| RM251 | 3 | 30.95** | 108.6 | IC 22015 |
| RM203 | 3 | 11.92** | 173.1 | IR 58025A |
| RM22 | 3 | 14.72** | 27.5 | IR 58025A |
| RM13 | 5 | 14.23** | 24.8 | IC 22015 |
| RM169 | 5 | 28.85** | 62.4 | IC 22015 |
| RM249 | 5 | 21.19** | 71.1 | IR 58025A |
| RM164 | 5 | 8.28* | 87.0 | IR 58025A |
| RM230 | 8 | 35.37** | 144.2 | IC 22015 |
| RM44 | 8 | 10.64* | 47.4 | IR 58025A |

[a]Location of the marker on the chromosome in centiMorgans
[b]Skewed marker segregation towards the *O. sativa* (IR 58025A) or *O. rufipogon* (IC 22015) parent
*Significant at p < 0.01
**Significant at p < 0.001

on BC1 for high tiller number, a trait that was superior in *O. rufipogon*. RM13 was also shown to exhibit segregation distortion towards *O. rufipogon* in an earlier study [17].

***Trait correlations***
The present study confirms that major components follow significant positive relationship with yield. Most of the trait correlations confirm with those reported earlier for studies involving *O. rufipogon*. Grain weight was negatively associated with both spikelet number per panicle and grain number per panicle [13,17,18]. In the present study, the correlation between grain weight and yield was non significant as also reported earlier for an IR64/*O. rufipogon* cross [17], However other studies on *O. rufipogon* report a positive correlation between yield and grain weight [13,18]. There is no correlation detected in the present study between panicle number and yield, however, a positive correlation between these two traits was reported in an IR64/*O. rufipogon* derived cross [17].

**O. rufipogon** *derived QTLs for yield improvement*
*Oryza rufipogon* alleles had a beneficial effect on 74% of the QTLs obtained for yield and yield components in the present study. This is a higher percentage than docu-

mented for interspecific crosses in rice. In previous studies involving *O. rufipogon*, alleles from wild species had beneficial effect in 35–58% of the QTLs [13,15,17,18]. The higher percentage reported here might indicate the presence of a larger number of favorable alleles in this accession of *O. rufipogon* compared to the one used in the previous studies. Alternatively, IR 58025A might have inferior alleles at many of the loci compared to the *O. rufipogon* alleles or the alleles introgressed from the wild species may interact better with the IR 58025A background compared to the *O. sativa* accessions used earlier. The intensive selection in the BC1 for higher tiller number, a superior trait in *O. rufipogon*, may be another reason for the increased contribution of wild alleles.

The *O. rufipogon* alleles contributed to an increase in panicle length (*pl2.1*, *pl5.1*, *pl9.1*), number of tillers (*nt2.1*, *nt5.1*), number of panicles (*np2.1*, *np2.2*), spikelet number per plant (*snp2.1*, *snp5.1*, *snp5.2*), grain number per panicle (*gn5.1*), grain number per plant (*gnp2.1*, *gnp2.2*, *gnp5.1*), grain weight (*gw2.1*, *gw9.1*), yield per plant (*yldp2.1*, *yldp2.2*, *yldp9.1*), harvest index (*hi2.1*) and plot yield (*yld1.1*, *yld2.1*, *yld8.1*, *yld8.2*, *yld8.3*, *yld8.4*, *yld8.5*). The *O. rufipogon* alleles also resulted in an increase

**Table 3: QTLs related to yield and yield components detected in an IR58025A × *O. rufipogon* (IC 22015) population**

| Trait | Chromosome | Marker Interval | Allele effect | CIM | | | IM | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | LOD | $R^2$ | Additive effect | LOD | $R^2$ | Additive effect |
| **Plant Height** | | | | | | | | | |
| *ph1.1* | 1 | RM220 – RM272 | IC 22015 | 5.32 | 17.48 | -17.33 | 5.46 | 21.12 | -19.09 |
| *ph1.2* | 1 | RM272 – RM259 | IC 22015 | 4.2 | 6.82 | -2.45 | 3.82 | 5.87 | -2.03 |
| *ph9.1* | 9 | RM257 – RM242 | IR 58025A | 3.7 | 5.63 | 9.53 | 4.21 | 7.408 | 10.83 |
| **Number of tillers per plant** | | | | | | | | | |
| *nt2.1* | 2 | RM262 – RM183 | IC 22015 | | | | 2.99 | 11.11 | -1.28 |
| *nt5.1* | 5 | RM169 – RM249 | IC 22015 | 2.53 | 5.9 | -1.06 | | | |
| **Number of panicles per plant** | | | | | | | | | |
| *np2.1* | 2 | RM262 – RM183 | IC 22015 | 2.5 | 6.8 | -1.023 | | | |
| *np2.2* | 2 | RM324 – RM262 | IC 22015 | | | | 3.23 | 10.81 | -1.28 |
| **Panicle length** | | | | | | | | | |
| *pl2.1* | 2 | RM250 – RM208 | IC 22015 | 10.9 | 19.28 | -9.53 | 10.76 | 20.85 | -9.16 |
| *pl5.1* | 5 | RM249 – RM164 | IC 22015 | 6.61 | 18.93 | -9.04 | 6.66 | 20.85 | -9.16 |
| *pl9.1* | 9 | RM242 – RM205 | IC 22015 | 8.11 | 17.3 | -9.24 | 9.48 | 20.85 | -9.16 |
| **Spikelet number per panicle** | | | | | | | | | |
| *sn2.1* | 2 | RM250 – RM208 | IR 58025A | 4.20 | 19.13 | 103.53 | 4.57 | 19.71 | 106.56 |
| **Spikelet number per plant** | | | | | | | | | |
| *snp2.1* | 2 | RM262 – RM183 | IC 22015 | | | | 3.13 | 12.27 | -321.31 |
| *snp5.1* | 5 | RM194 – RM249 | IC 22015 | 3.98 | 11.79 | -413.25 | | | |
| *snp5.2* | 5 | RM169 – RM249 | IC 22015 | | | | 2.52 | 10.29 | -386.74 |
| *snp8.1* | 8 | RM44 – RM350 | IR 58025A | 3.11 | 6 | 383.53 | | | |
| *snp8.2* | 8 | RM44 – RM223 | IR 58025A | - | - | - | 2.83 | 6.1 | 381.67 |
| **Grain number per panicle** | | | | | | | | | |
| *gn2.1* | 2 | RM250 – RM208 | IR 58025A | | | | 3.32 | 16.65 | 72.52 |
| *gn5.1* | 5 | RM194 – RM169 | IC 22015 | 2.98 | 6.12 | -5.67 | 3.45 | 7.65 | -6.82 |
| **Grain number per plant** | | | | | | | | | |
| *gnp2.1* | 2 | RM262 – RM183 | IC 22015 | 2.79 | 5.42 | -171.64 | 3.68 | 12.5 | -256.41 |
| *gnp2.2* | 2 | RM183 – RM263 | IC 22015 | | | | 3.31 | 6.5 | -186.41 |
| *gnp3.1* | 3 | RM16 – RM203 | IR 58025A | 3.14 | 9.8 | 248.31 | 2.71 | 9.98 | 241.4 |
| *gnp5.1* | 5 | RM194 – RM249 | IC 22015 | 5.73 | 12 | -285.18 | 3.35 | 6.5 | -193.2 |
| **Spikelet fertility** | | | | | | | | | |
| *sf1.1* | 1 | RM212 – RM315 | IR 58025A | 3.26 | 5.2 | 2.45 | | | |
| *sf3.1* | 3 | RM251 – RM36 | IR 58025A | 4.35 | 6.7 | 1.45 | 3.72 | 5.78 | 1.27 |
| **Grain weight** | | | | | | | | | |
| *gw2.1* | 2 | RM250 – RM208 | IC 22015 | 3.25 | 10.4 | -5.21 | | | |
| *gw2.2* | 2 | RM324 – RM262 | IR 58025A | | | | 2.91 | 7.16 | 82.41 |
| *gw2.3* | 2 | RM262 – RM183 | IR 58025A | | | | 3.17 | 10.8 | 0.98 |
| *gw9.2* | 9 | RM242 – RM205 | IC 22015 | 3.21 | 13.95 | -4.73 | | | |
| **Yield per plant** | | | | | | | | | |
| *yldp2.1* | 2 | RM262 – RM183 | IC 22015 | 4.38 | 12.21 | -3.54 | 4.35 | 14.2 | -3.79 |
| *yldp2.2* | 2 | RM183 – RM263 | IC 22015 | | | | 3.59 | 7.05 | -2.70 |
| *yldp9.1* | 9 | RM242 – RM205 | IC 22015 | | | | 4.18 | 23.2 | -13.84 |
| **Harvest index** | | | | | | | | | |
| *hi2.1* | 2 | RM183 – RM263 | IC 22015 | 3.12 | 5.83 | -2.85 | 2.76 | 5.64 | -2.8 |
| **Plot yield** | | | | | | | | | |
| *yld1.1* | 1 | RM243 – RM81A | IC 22015 | 4.23 | 6.98 | -3.98 | 3.87 | 5.86 | -3.09 |
| *yld2.1* | 2 | RM262 – RM263 | IC 22015 | 31.92 | 38.46 | -216.04 | 35.33 | 50.47 | -238.51 |
| *yld8.1* | 8 | RM350 – RM210 | IC 22015 | 3.86 | 4.67 | -67.45 | | | |
| *yld8.2* | 8 | RM210 – RM256 | IC 22015 | 3.35 | 3.98 | -62.92 | 4 | 10.88 | -103.54 |
| *yld8.3* | 8 | RM38 – RM25 | IC 22015 | | | | 4.56 | 7.99 | -89.13 |
| *yld8.4* | 8 | RM223 – RM210 | IC 22015 | | | | 7.02 | 20.24 | -138.96 |
| *yld8.5* | 8 | RM256 – RM230 | IC 22015 | | | | 6.42 | 15.35 | -134.53 |
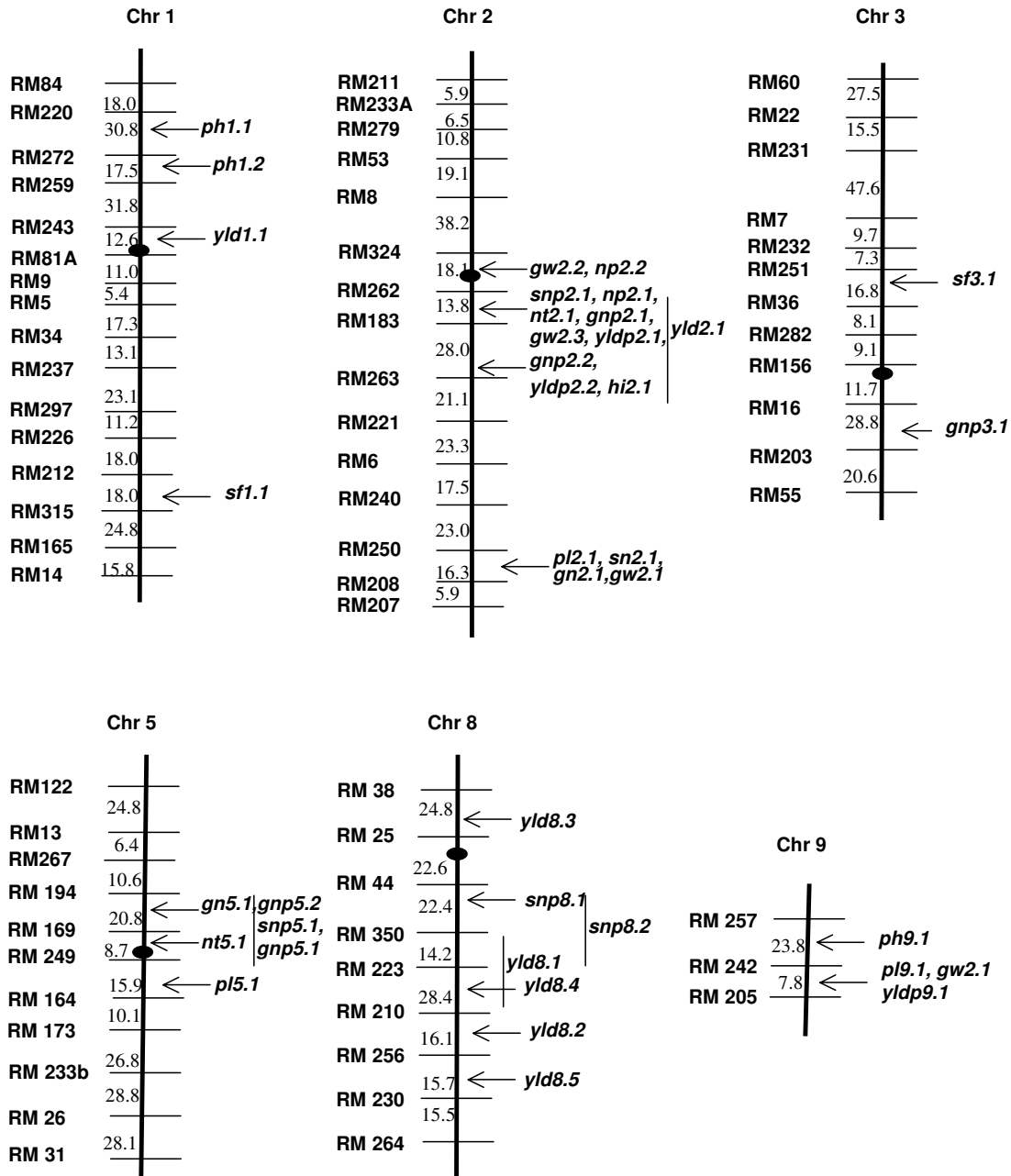
#### Figure 2
Distribution of QTLs on the molecular linkage maps constructed based on BC₂ testcross population of IR 58025A (*O. sativa*) × IC 22015 (*O. rufipogon*) **ph**: Plant height, **nt**: Number of tillers per plant, **np**: Number of panicles per plant, **pl**: Panicle length, **sn**: Spikelet number per panicle, **snp**: spikelet number per plant, **gn**: Grain number per panicle, **gnp**: Grain number per plant, **sf**: Spikelet fertility, **gw**: Grain weight, **yldp**: Yield per plant, **hi**: Harvest index, **yld**: Plot yield.

in plant height (*ph1.1, ph1.2*), however, they did not enhance spikelet fertility and spikelet number per panicle. It is interesting because, the population had transgressive

segregants for both the traits, in spite of the inferiority of *O. rufipogon* for these traits. The alleles in IR 58025A may be dominant at this loci compared to the alleles from *O.*

**Table 4: Significant two-way interactions between marker loci determined using EPISTAT program**

| Trait | Marker 1 | | Marker 2 | | MC-test# |
|---|---|---|---|---|---|
| | Name | Chromosome | Name | Chromosome | |
| NT | RM282 | 3 | RM210 | 8 | 0.002 |
| PL | RM243 | 1 | RM8 | 2 | 0.001 |
| SN | RM22 | 3 | RM169 | 5 | 0.001 |
| SNP | RM282 | 3 | RM210 | 8 | 0.001 |
| GN | RM211 | 2 | RM263 | 2 | 0.000 |
| GNP | RM262 | 2 | RM169 | 5 | 0.002 |
| | RM169 | 5 | RM214 | 7 | 0.000 |
| SF | RM36 | 3 | RM340 | 6 | 0.000 |
| HI | RM5 | 1 | RM240 | 2 | 0.001 |
| | RM272 | 1 | RM211 | 2 | 0.002 |
| | RM272 | 1 | RM50 | 6 | 0.000 |
| | RM60 | 3 | RM257 | 9 | 0.005 |
| PY | RM263 | 2 | RM183 | 2 | 0.000 |
| | RM263 | 2 | RM264 | 8 | 0.077 |
| | RM183 | 2 | RM38 | 8 | 0.073 |

#Monte Carlo simulation using EPISTAT program to evaluate significance of interactions

*rufipogon*. However, despite its inferiority for the trait, the alleles from the wild species had beneficial effect on grain weight indicating that the alleles contributing to grain weight might interact positively with the genetic background of IR 58025A.

### Interaction among QTLs
An analysis to identify the potential epistatic interactions between marker loci, using EPISTAT software [26], identified 20 markers resulting in 15 two-way interactions (Table 4). All these markers had no effect on the trait singly but resulted in an enhanced effect when combined with another marker. The resulting G × G interactions between these markers may be one of the reasons for the appearance of transgressive segregants in the population. Several chromosomal regions were associated with more than one trait, indicating linkage or pleiotropic effects. For example, the QTLs *gnp2.2* and *yldp2.2*, associated with an increase in grain number per plant and yield per plant respectively were located in the same region on chromosome 2. Similarly, the region associated with *nt2.1* which controlled an increase in number of tillers was linked to *np2.1*, *gnp2.1*, *yldp2.1*, *hi2.1* and *yld2.1* controlling an increase in number of panicles, grain number per plant, yield per plant, harvest index and plot yield respectively. The *O. rufipogon* alleles had beneficial effect on all these traits. However, the same region is associated with a negative QTL from *O. rufipogon*, *gw2.3*, resulting in decreased seed weight. At a different chromosomal region, *O. rufipogon* allele associated with a QTL *gw2.1*, leading to an increase in grain weight is linked to two negative QTLs, *sn2.1* and *gn2.1*, which result in decrease in spikelet

number per panicle and grain number per panicle. The reverse is true for the region associated with another QTL for grain weight, *gw2.2*. This negative QTL from *O. rufipogon* is linked with two QTLs corresponding to grain number per plant, *gnp2.1* and yield per plant, *yldp2.1*, where the *O. rufipogon* alleles had positive effect. It is very interesting that the same chromosomal region associated with a positive QTL for grain weight coincides with negative QTLs for spikelet number and grain number and vice versa. As grain weight is negatively correlated with spikelet number and grain number, it is tempting to speculate that the same QTL might contribute to both the phenotypes. Further characterization of this region by fine mapping and identification of genes underlying it will throw more light on whether the same set of genes, regulated differentially, or an entirely different set of genes govern these phenotypes. The association of positive and negative QTLs to the same chromosomal regions was earlier reported for studies involving *O. rufipogon* where the positive traits for grain weight and panicle length together and panicle length alone were linked with negative QTLs for plant height and broken rice respectively [17,19]. *In lieu* with the association of the positive and negative QTLs to same chromosomal regions, a careful selection will be needed to avoid negative characteristics in the crop improvement process.

### Comparison with QTLs from other wild species
A comparison of the QTLs obtained with the other wild species including *O. rufipogon*, *O. glaberrima* and *O. glumaepatula* revealed that 27 out of 39 QTLs obtained in this study had congruent occurrences with QTLs reported

**Table 5: Comparison of QTLs with other studies involving wild rice species**

| Chr./marker | QTLs in this study | QTLs identified in other wild species | | | | | | Ref |
|---|---|---|---|---|---|---|---|---|
| **Chromosome 1** | | | | | | | | |
| RM220 – RM272 | *ph1.1* | *spp1* | *gyp1* | *fgp1* | *gypa1* | | | 16 |
| | | *sp1* | *al1* | | | | | 14 |
| RM272 – RM259 | *ph1.2* | *dth1.1* | | | | | | 18 |
| RM243 – RM81A | *yld1.1* | *yld1.1* | | | | | | 13 |
| | | *BR* | | | | | | 19 |
| RM212 – RM315 | *sf1.1* | *BR* | | | | | | 19 |
| | | *ph1.1* | *pl1.1* | | | | | 17 |
| | | *pss1.1* | *pth1.2* | *ph1.2* | *pl1.1* | | | 18 |
| | | | | | | | | |
| **Chromosome 2** | | | | | | | | |
| RM250 – RM208 | *pl2.1,sn2.1,gn2.1,gw2.1* | *yld2.1* | *gpp2.1* | | | | | 17 |
| | | *gl2.1* | *gw2.1* | | | | | 18 |
| | | *ph2.1* | *gpl2.1* | | | | | 15 |
| | | *ppl2.1* | *gpl2.1* | *yld2.1* | | | | 13 |
| RM262 – RM183 | *nt2.1,np2.1,yld2.1,gw2.3* | *dtf2* | | | | | | 16 |
| *gnp2.1,yldp2.1, snp2.1* | *pl2.1* | | | | | | | 13 |
| RM262 – RM263 | *gnp2.1,yldp2.1,hi2.2* | *dtf2* | *spp2* | *fgp2* | | | | 16 |
| | | *pl2.1* | | | | | | 13 |
| | | *al2* | | | | | | 14 |
| | | *HR* | *CR* | *BR* | | | | 19 |
| | | | | | | | | |
| **Chromosome 3** | | | | | | | | |
| RM36 – RM251 | *sf3.1* | *CR* | | | | | | 19 |
| | | *dth3.2* | *sh3.2* | | | | | 18 |
| | | *kl3.1* | | | | | | 20 |
| | | *amy3* | | | | | | 21 |
| | | | | | | | | |
| **Chromosome 5** | | | | | | | | |
| RM194 – RM249 | *gn2.1,snp5.1,gnp5.1* | *gw5.1* | | | | | | 13 |
| **Chromosome 8** | | | | | | | | |
| RM44 – RM350 | *snp8.1* | *tnr8* | *dnr8* | *plh8* | | | | 16 |
| | | *sh8.1* | | | | | | 18 |
| | | *cp8.1* | | | | | | 20 |
| RM350 – RM210 | *yld8.1* | *GD* | | | | | | 19 |
| | | *gpp8.1* | *ph8.1* | | | | | 18 |
| | | *ph8.1* | *pl8.1* | *gpl8.1* | *yld8.1* | | | 13 |
| | | | | | | | | |
| **Chromosome 9** | | | | | | | | |
| RM257 – RM242 | *ph9.1* | *gw9.1* | | | | | | 13 |
| RM242 – RM205 | *pl9.1,gw9.1* | *pnl* | | | | | | 14 |
| | | *pl9.1* | *spp9.1* | | | | | 13 |
| | | *dyg* | | | | | | 19 |
| | | *pl9.1* | | | | | | 17 |
| | | *gw9.1* | *gpp9.1* | *spp9.1* | *pl9.1* | *yld9.1* | *tt9.1* | 18 |

earlier (Table 5). The QTLs that overlap with other studies fall into two categories: i) QTLs that share similar map position and mapped to same trait and ii) QTLs that share a similar map position, but mapped to a different trait. The QTL for panicle length, *pl2.1*, is mapped to the same region under same name in a study involving *O. rufipogon*/ Jefferson [18], while it is associated with yield components like grains/panicle and yield in a cross involving V20A/*O. rufipogon* [13]. However, the *O. rufipogon* alleles contributed to positive effect in both the cases. In case of *pl9.1*, QTLs under same name and associated with same chromosomal regions were reported previously in three separate studies involving *O. rufipogon* (IRGC 105491) [13,17,18]. However, the same region is associated with a negative QTL for panicle length (pnl) in a study involving another accession (P 16) of *O. rufipogon* [14]. This indi-

**Table 6: Comparison of QTLs across *Oryza* species**

| QTL | QTLs reported in earlier studies |
| --- | --- |
| *ph1.1* | PTHT [33,34], *ph1* [35], *qPH-1* [36], *ph1-1* [37] |
| *ph1.2* | PTHT [33,34,36], *ph1* [35], *ph1-1* (10), *ph1* [38] |
| *ph9.1* | *ph9* [37,38] |
| *np2.1* | *tns2* [35] |
| *np2.2* | PNNB [35,40,57] |
| *pl2.1* | PNL [53], *pl2.1* [18] |
| *pl5.1* | PNL [40], QPl5 [53] |
| *pl9.1* | *qPl9b* [53], *pl9.1* [13], *pl9.1* [17], *pl9.1* [18] |
| *sn2.1* | SPKNB [53] |
| *snp2.1* | *tns5* [35] |
| *snp5.1* | SPKNB [34,40,53] |
| *snp8.1* | SPKNB [40], *tns5* [35] |
| *snp8.2* | SPKNB [40], *tns5* [35] |
| *gn2.1* | *gpp2.1* [17] |
| *gn5.1* | FGRNB [41] |
| *gnp2.1* | FGNRB [39, 55] *nfg* [35] |
| *gnp2.2* | *fgp2* [16] |
| *gnp3.1* | *gp3* [41] |
| *gnp5.1* | *qNFGP-5-1* [56] |
| *sf1.1* | SF [51], *pss1.1* [18] |
| *sf3.1* | SF [51], *S3b* [52], SF [11] |
| *gw2.1* | GW [39], *gw2.1* [41] |
| *gw2.2* | GW [39], *tgwt* [35], GW [54] |
| *gw2.3* | *kw2-2* [55] |
| *gw9.1* | *gw9.1* [18] |
| *yldp2.2* | GRYLDPPL [35,39,40] |
| *yld1.1* | GRYLD [39], *yld1.1* [13] |
| *yld8.1* | GRYLD [39], *yd8* [41], *yld8.1* [13] |
| *yld8.3* | GRYLD [40] |
| *yld8.4* | GRYLD [35,39], *yd8* [41] |

cates an accession based variation in the alleles at this locus, with alleles derived from accessions IRGC 105491 and IC 22015 superior to the alleles from *O. rufipogon* accession, P 16. The alleles from *O. rufipogon* increased yield at *yld1.1*, *yld2.1*, *yldp2.1*, *yldp2.2* and *yld8.1*. While, QTLs with same names as *yld1.1* and *yld8.1* were reported in similar regions in a cross V20A/*O. rufipogon*, the beneficial effect of *yld2.1*, *yldp2.1* and *yldp2.2* coincided with an increase in panicle length [13]. The position of *yld8.1* overlaps with another yield component, grains per panicle, *gpp8.1*, in a cross involving Jefferson/*O. rufipogon* [18]. The QTLs for grain weight, *gw2.1* and *gw9.1* shared same names and had orthologous regions with the QTLs identified in V20A/*O. rufipogon* cross [13]. While, *O. rufipogon* had beneficial effect on grain weight in the present study, they had a negative effect in the earlier study, suggesting that the alleles at this locus might be superior to those reported earlier. However, a negative QTL from grain weight in this study, *gw2.3*, is associated with panicle length, *pl2.1*, in case of [13] where the *O. rufipogon* alleles had beneficial effect.

## Comparison of QTLs across Oryza species

The present study identified a total of 39 QTLs. Thirty QTLs have corresponding occurrences with QTLs reported earlier, while, 9 QTLs (*nt2.1*, *nt5.1*, *snp5.1*, *hi2.1*, *yldp2.1*, *yldp9.1*, *yld2.1*, *yld8.1*, *yld8.5*) are novel and reported for the first time. The results are on the expected lines, as new parental combinations especially involving exotic species are likely to unfold novel variability. Of the three QTLs detected for plant height, the *O. rufipogon* alleles increased plant height at two loci while another QTL decreased plant height. All the three QTLs have been reported previously (Table 6). The similarity of the regions associated with QTLs for plant height with other studies involving *O. rufipogon*, *indica* and *japonica* cultivars indicates that the location of alleles for plant height are conserved across different genetic and environmental backgrounds. The two QTLs for tiller number, *nt2.1* and *nt5.1*, identified in this study are novel and have no correspondences with QTLs reported earlier for this trait. This indicates that these may be a potentially new set of alleles specific for this accession of *O. rufipogon*. All the QTLs for number of panicles have been reported earlier (Table 6).

All the three QTLs for panicle length, *pl2.1*, *pl5.1* and *pl9.1*, were trait enhancing and overlapped with the regions identified earlier for the same trait (Table 6). This is in agreement with earlier studies where *O. rufipogon* alleles had a positive effect [13,16-18]. The large number of studies implicating a similar region as *pl9.1* indicate that this region has a similar predictable effect on the phenotype irrespective of the genetic background. Two QTLs were identified for grain number per panicle (*gn2.1*, *gn5.1*) and one for spikelet number per panicle (*sn2.1*) in the present study. The alleles from *O. rufipogon* had a negative effect on the QTLs on chromosome 2, whereas, they enhanced the number of grains per panicle on chromosome 5. A similar negative effect of *O. rufipogon* alleles had been reported for grain number per panicle, on chromosome 2, in a cross involving IR64/*O. rufipogon* [17]. The negative effect across *O. rufipogon* accessions indicates the superiority of the *O. sativa* alleles at this locus.

Five QTLs were identified for spikelet number per plant and 4 QTLs for grain number per plant. All the four QTLs for grain number per plant were reported earlier, while only four of the five QTLs for spikelet number per plant are documented (Table 6). The *O. rufipogon* alleles had negative effect on *snp8.1*, *snp8.2* and *gnp3.1* whereas, they had beneficial effect on all the other QTLs. The QTL for grain number per plant, *gnp2.2*, coincided with a QTL reported for the same trait (fgp) in a cross involving *O. glumaepatula* [16]. While, the *O. rufipogon* alleles had a beneficial effect in the present study, the *O. glumaepatula* alleles had a negative effect, indicating that this accession

of *O. rufipogon* has a novel set of alleles at this locus that are superior to *O. glumaepatula*. The QTL *snp5.2* is novel and is reported for the first time. Again, this indicates the possibility of the presence of novel alleles in this accession of *O. rufipogon*. Two QTLs, *sf1.1* and *sf3.1*, both conferring negative effect, were identified for spikelet fertility. Both the QTLs have been reported earlier and they also are in agreement with earlier study indicating the negative effect of the *O. rufipogon* alleles on this trait [18]. All the four QTLs for grain weight have been reported earlier indicating that the allele set may be common across most of the genetic backgrounds. The *O. rufipogon* alleles contribute to positive effect for two of these QTLs (*gw2.1*, *gw9.1*), while the other two derive negative effect from the wild alleles. The beneficial effect at *gw2.1* and *gw9.1* is in contrast to what has been previously reported for this trait in a Jefferson/*O. rufipogon* cross, where the *O. rufipogon* alleles have a deprecating effect on both these QTLs [18]. This indicates that alleles at these loci may be superior in this accession of *O. rufipogon* or the same set of alleles might perform better in the IR 58025A background compared to the Jefferson background or the G × E interactions might be at play.

Six of the eight QTLs identified for yield have been reported earlier [35,39-41] suggesting that the QTLs for yield are conserved across different genetic backgrounds. Two QTLs, *yld2*.1 and *yld8.5* are reported for the first time. The *O. rufipogon* alleles had beneficial effect on all the eight QTLs. Of the two QTLs identified for yield per plant, the *O. rufipogon* alleles were responsible for increase in yield in both the cases. While, *yldp2.1* is novel and reported for the first time, *yldp2.2* coincides with the regions reported earlier for this trait (Table 6). The QTL for harvest index, *hi2.1*, is novel and is reported for the first time in this study.

## Conclusion
The study while confirming the view that the progenitor species constitute the largest source of still unfolded variability for traits of complex inheritance like yield and its components has helped identify additional novel variability for yield improvement. The novel QTLs identified are good candidates for fine mapping and positional cloning studies, while, the QTLs that are mapped to regions consistent with other studies can be useful for marker-assisted transfer of these QTLs. The availability of the complete rice genome sequence and rapid advances being made in the area of genomics will help dissect and characterize yield related QTLs further. 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 dependent world is to truly attain and sustain food security.

## Methods
### Choice of parents
IR 58025A, a commercial cms line developed by Directorate of Rice Research (DRR), India, http://www.drrindia.org/ was used as a recurrent parent. IR 58025A grows to a height of 80 cm and is characterized by having long grain type and early maturity along with good milling and eating qualities. The *O. rufipogon* accession, IC22015, collected from Kerala, India, and maintained at DRR was used as a donor parent.

### Development of mapping population
An advanced backcross strategy as described in [13] was followed to develop the mapping population. A single plant of *O. rufipogon* (IC 22015) was used as a male parent and crossed to IR 58025A to generate $F_1$ plants. Fourteen $F_1$ plants, whose hybrid nature was confirmed with microsatellite markers were backcrossed to IR 58025B (an isogenic line of IR 58025A) used as male to produce $BC_1$. Fifty $BC_1$ plants, looking morphologically like IR 58025A were backcrossed to IR 58025B to produce $BC_2$. 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 testcross families. The 251 $BC_2$ testcross families constituted the mapping population. Simultaneously, under similar conditions, a cross was made between IR 58025A and KMR3 to obtain the hybrid, KRH2, to be used as the check.

### Phenotypic evaluation of mapping population
The 251 testcross families, two parents and checks *viz.*, KRH2, Jaya and IR64 were grown under irrigated conditions at DRR in an augmented block design in two replications with checks 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 20 cm × 20 cm. Six plants in the middle of each of these families were evaluated for the following yield related traits: *Plant height (PH)* – length of the tallest tiller (cm) from soil surface to the tip of the panicle. *Tiller number per plant (NT)* – Total number of tillers per plant. *Panicles per plant (NP)* – Panicles with seed set exceeding 15%. *Panicle length (PL)* – length (cm) from neck to tip of the panicle. *Spikelet number per panicle (SN)* – number of spikelets including empty and filled ones averaged over five randomly chosen panicles in each plant. *Spikelet number per plant (SNP)* – total number of spikelets including empty and filled ones in each plant computed as average number of spikelets per panicle × number of productive tillers. *Grain number per panicle (GN)* – number of filled spikelets per panicle averaged over five randomly chosen panicles in each plant. *Grain number per plant (GNP)* – number of filled spikelets in a plant computed as average number of filled spikelets per panicle × number of productive tillers per plant. *Spikelet fertility (SF)* – ratio of

filled spikelets to the total number of filled and unfilled spikelets per panicle, expressed in percentage. *Grain weight (GW) – w*eight (g) of 1000 filled spikelets, averaged over six samples taken from the bulk-harvested grain from each plant. *Harvest index (HI)* – ratio of filled grains to biomass (filled grains, unfilled grains and straw of the plant) in terms of weight (g) expressed in percentage. *Grain yield per plant (Yldp)* – weight (g) of filled grains per plant. *Grain yield (Yld)* – weight (g/kg) of filled grains harvested from each testcross family (40 plants) extrapolated to tonnes per hectare.

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

### DNA extraction
DNA was extracted from two months old leaf tissue using the protocol of Dellaporta [42].

### Parental polymorphism and linkage map construction
A set of 210 randomly selected microsatellite markers (Donated by Rockefeller Foundation to EAS) spanning all the 12 chromosomes were screened among the *O. sativa* and *O. rufipogon* parents. A total of 80 polymorphic microsatellite markers separated by an average distance of 15.37 cM were used to analyze the 251 testcross progeny. Linkage maps were constructed using the Mapmaker version 3.3 [43] following Kosambi Function [44]. Linkage groups were determined using 'group' command with an LOD score of 3.0 and a recombination fraction of 0.5. Order of the markers for each group was determined using 'order' and 'ripple' commands. Assignment of linkage groups to the respective chromosomes was done based on the rice maps developed at Cornell University [18,45].

### QTL analysis
QTLs were analyzed using single marker analysis (SMA), interval mapping (IM) and composite interval mapping (CIM). Single marker analysis wasperformed 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. QTL analysis by interval mapping (IM) and Composite interval mapping (CIM) [46] was done using QTL Cartographer 3.0 [47]. The significant threshold value for identification of a QTL (both for IM and CIM) was determined based on permutation tests at a significance level p

$< 0.01$ [48]. Based on 1000 permutations for each trait, the threshold for IM and CIM corresponded to minimum LOD score value of 2.5. The proportion of phenotypic variance (R2) and additive effect were determined for each trait. The deviations from the expected mendelian ratio was calculated using MapDisto software [49] and the digenic interactions between marker loci were determined using EPISTAT software [26]. The QTL nomenclature followed was as reported in [50].

### Note
The material used in this study can be obtained from Prof E.A. Siddiq, Honorary Professor, Center for DNA Fingerprinting and Diagnostics, Nacharam, Hyderabad, 500 076, India. The raw data used for analysis can be obtained from Dr. M. Pradeep Reddy, Department of Biology, McMaster University, Hamilton, ON, Canada.

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## Authors' contributions
**MPR** participated in the design, carried out the field studies and marker studies, analyzed the data and darafted the manuscript. **NS** participated in the coordination of the study and helped in the design of the study, analysis of data and drafting the manuscript. **VLNR** carried out the field studies, marker studies and helped in analysis of data. **EAS** conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

## Additional material

### Additional File 1
*Transgressive Segregants*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2156-6-33-S1.ppt]

### Additional File 2
*Correlation Data*
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### Additional File 3
*Single Marker QTLs*
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## References

1. Wang ZY, Second G, Tanksley SD: **Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs.** *Theor Appl Genet* 1992, **83**:565-581.
2. Tanksley SD, McCouch SR: **Seed banks and molecular maps: Unlocking genetic potential from the wild.** *Science* 1997, **277**:1063-1066.
3. Lin SC, Yuan GP: **Hybrid rice breeding in China.** *Innovative approaches to rice improvement, IRRI and Manila, Philippines* 1990:35-51.
4. Dalmacio RD, Brar DS, Ishii T, Sitch TA, Virmani SS, Khush GS: **Identification and transfer of a new cytoplasmic male sterility source from *Oryza perennis* into indica rice (*O. sativa*).** *Euphytica* 1995, **82**:221-225.
5. Dalmacio RD, Brar DS, Virmani SS, Khush GS: **Male sterile line in rice (*Oryza sativa*) developed with *O. glumaepatula* cytoplasm.** *International Rice Research Notes* 1996, **21(1)**:22-23.
6. Hoan NT, Sarma NP, Siddiq EA: **Identification and characterization of new sources of cytoplasmic male sterility in rice.** *Plant Breeding* 1997, **116**:547-551.
7. Jena KK, Khush GS: **Identification of genes from *Oryza officinalis* Wall ex Watt to cultivated rice, *Oryza sativa* L.** *Theor Appl Genet* 1990, **80**:737-745.
8. Khush GS: **Origin, dispersal, cultivaton and variation of rice.** *Plant Mol Biol* 1997, **35**:25-34.
9. Khush GS, Bacalangco E, Ogawa T: **A new gene for resistance to bacterial blight from *O. longistaminata*.** *Rice Gen Newslett* 1990, **7**:121-122.
10. Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT: **Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.).** *Theor Appl Genet* 2003, **106**:583-593.
11. Albar L, Ndjiondjop MN, Esshak Z, Berger A, Pinel A, Jones M, Fargette D, Ghesquiere A: **Fine genetic mapping of a gene required for rice yellow mottle virus cell-to-cell movement.** *Theor Appl Genet* 2003, **107**:371-378.
12. Xiao J, Grandillo S, Ahn SA, McCouch SR, Tanksley SD, Li J, Yuan L: **Genes from wild rice improve yield.** *Nature* 1996, **384**:223-224.
13. Xiao J, Li J, Grandillo S, Ahn SN, Yuan L, Tanksley SD, McCouch SR: **Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*.** *Genetics* 1998, **150**:899-909.
14. Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q: **Identification of genetic factors controlling domestication-related traits of rice using an F$_2$ population of a cross between *Oryza sativa* and *O. rufipogon*.** *Theor Appl Genet* 1999, **98**:243-251.
15. Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H Jr, Guimaraes E, Tohme J, McCouch SR: **Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC2F2 population evaluated in an upland environment.** *Theor Appl Genet* 2001, **102**:41-52.
16. Brondani C, Rangel PHN, Brondani RPV, Ferreira ME: **QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatelite makers.** *Theor Appl Genet* 2002, **104**:1192-1203.
17. Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR: **Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*.** *Theor Appl Genet* 2003, **107**:1419-1432.
18. Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobo KB, Xu Y, Martínez R, McCouch SR: **Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson.** *Theor Appl Genet* 2003, **107**:479-493.
19. Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR: **Identification of quantitative trait loci for grain quality in an advance backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*.** *Theor Appl Genet* 2003, **107**:1433-1411.
20. Li J, Xiao J, Grandillo S, Jiang L, Wan Y, Deng Q, Yuan L, McCouch SR: **QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice.** *Genome* 2004, **47**:697-704.
21. Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH: **QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*.** *Theor Appl Genet* 2004, **109**:630-639.
22. Tanksley SD, Nelson JC: **Advanced backcross QTL analysis: a method for simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines.** *Theor Appl Genet* 1996, **92**:191-203.
23. Bernacchi D, Beck BT, Eshed Y, Lopez Y, Petiard Y, Uhlig J, Zamir D, Tanksly SD: **Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicum hirsutum*.** *Theor Appl Genet* 1998, **97**:381-397.
24. Fulton TM, Grandillo S, Beck BT, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD: **Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicum parviflorum* cross.** *Theor Appl Genet* 2000, **100**:1025-1042.
25. Tanskley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T: **Advanced backcross QTL analysis in a cross between elite processing line of tomato and its wild relative *L. pimpinellifolium*.** *Theor Appl Genet* 1996, **92**:213-224.
26. Chase K, Adler FR, Lark KG: **EPISTAT: A computer program for identifying and testing between pairs of quantitative trait loci.** *Theor Appl Genet* 1997, **94**:724-730.
27. Lu C, Shen L, Tau Z, Xu Y, He P, Chen Y, Zhu L: **Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled haploid population.** *Theor Appl Genet* 1996, **93**:1211-1217.
28. Xu Y, Shen Z, Chen Y, Zhu L: **Molecular mapping of quantitative trait loci controlling yield component characters using a maximum likelihood method in rice (*Oryza sativa* L.).** *Chinese J Genet* 1995, **22**:37-42.
29. McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD: **Molecular mapping of rice chromosome.** *Theor Appl Genet* 1988, **76**:815-829.
30. Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Xu K, Xian J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD: **Saturated molecular map of rice genome based on an interspecific backcross population.** *Genetics* 1994, **138**:1251-1274.
31. Grandillo S, Tanksley SD: **QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*.** *Theor Appl Genet* 1996, **92**:935-951.
32. Kinoshita T: **Linkage mapping using mutant genes in rice.** *Rice Genet Newslett* 1998, **15**:13.
33. Huang N, Courtois B, Khush GS, Lin HX, Wang GL, Wu P, Zheng KG: **Association of quantitative trait loci for plant height with major dwarfing genes in rice.** *Heredity* 1996, **77**:130-137.
34. Xu Y, Shen Z, Xu J, Zhu H, Chen Y, Zhu L: **Interval mapping of quantitative trait loci by molecular markers in rice (*Oryza sativa* L.).** *Science in China* 1995, **38(4)**:422-428.
35. Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N, Zheng KL: **Analysis of QTL × environment interaction for yield components and plant height in rice.** *Theor Appl Genet* 1997, **95**:799-808.
36. He P, Li JZ, Zheng XW, Shen LS, Lu CF, Chen Y, Zhu LH: **Comparison of molecular linkage maps and agronomic trait loci between DH and RIL populations derived from the same rice cross.** *Crop Sci* 2001, **41**:1240-1246.
37. Cao G, Zhu J, He C, Gao Y, Yan J, Wu P: **Impact of epistasis and QTL × environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.).** *Theor Appl Genet* 2001, **103**:153-160.
38. Li ZK, Yu SB, Lafitte HR, Huang N, Courtois B, Hittalmani S, Vijayakumar CH, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS, Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT: **QTL × environment interactions in rice. I. heading date and plant height.** *Theor Appl Genet* 2003, **108**:141-153.

39.  Li ZK, Pinson SR, Park WD, Paterson AH, Stansel JW: **Epistasis for three grain yield components in rice (Oryza sativa L.).** *Genetics* 1997, **145:**453-465.
40.  Xiao JH, Li JM, Yuan LP, Tanksley SD: **Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross.** *Theor Appl Genet* 1996, **92:**230-244.
41.  Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Sagai Maroof MA: **Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid.** *Proc Natl Acad Sci* 1997, **94:**9226-9231.
42.  Dellaporta SC, Wood J, Hicks TB: **A plant DNA mini preparation: version II.** *Plant Mol Bio Rep* 1983, **1:**19-21.
43.  Lander ES, Green P, Abrahmson J, Barloe A, Daly MJ, Lincoln SE, Newburg L: **MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations.** *Genomics* 1987, **1:**174-181.
44.  Kosambi DD: **The estimation of map distance from recombination values.** *Ann Eugen* 1944, **12:**172-175.
45.  Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S: **Computational and experimental analysis of microsatellites in rice (Oryza sativa L.): frequency, length variation, transposon associations, and genetic marker potential.** *Genome Research* 2001, **11:**1441-52.
46.  Zeng ZB: **Precision mapping of quantitative trait loci.** *Genetics* 1994, **136:**1457-1468.
47.  Basten CJ, Weir BS, Zeng ZB: **ZMAP- A QTL Cartographer reference manual and tutorial for QTL mapping.** *Department of Statistics, North Carolina State University, Raleigh* .
48.  Churchill GA, Deorge RW: **Empirical threshold value for quantitative trait mapping.** *Genetics* 1994, **138:**963-971.
49.  Lorieux M, Ndjiondjop MN, Ghesquiere A: **A first interspecific O. sativa × O. glaberrima microsatellite genetic linkage map.** *Theor Appl Genet* 2000, **100:**593-601.
50.  McCouch SR, Cho YG, Yano M, Blinstrub M: **Report on QTL nomenclature.** *Rice Genet Newsl* 1997, **14:**11-13.
51.  Redona ED, Mackill DJ: **Quantitative trait locus analysis for rice panicle and grain characteristics.** *Theor Appl Genet* 1998, **96:**957-963.
52.  He YQ, Yang J, Xu CG, Zhang ZG, Zhang Q: **Genetic bases of instability of male sterility and fertility reversibility in photoperiod-sensitive genic male-sterile rice.** *Theor Appl Genet* 1999, **99:**683-693.
53.  Mei HW, Luo LJ, Ying CS, Wang YP, Yu XQ, Guo LB, Paterson AH, Li ZK: **Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two testcross populations.** *Theor Appl Genet* 2003, **107:**89-101.
54.  Yoshida S, Ikegami M, Kuze J, Sawada K, Hashimoto Z, Ishii T, Nakamura C, Kamijima O: **QTL analysis for plant and grain characters of sake brewing rice using a doubled haploid population.** *Breeding Science* 2002, **52:**309-317.
55.  Gao YM, Zhu J, Song YS, He CX, Shi CH, Xing YZ: **Analysis of digenic epistatic effects and QE interaction effects QTL controlling grain weight in rice.** *J Zhejiang Univ Sci* 2004, **5:**371-377.
56.  Zhuang JY, Fan YY, Rao ZM, Wu JL, Xia YW, Zheng KL: **Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice.** *Theor Appl Genet* 2002, **105:**1137-1145.
57.  Kobayashi S, Fukuta Y, Sato T, Osaki M, Khush GS: **Molecular marker dissection of rice (Oryza sativa L.) plant architecture under temperate and tropical climates.** *Theor Appl Genet* 2003, **107:**1350-1356.