Detecting CSSLs and yield QTLs with additive, epistatic and QTL × environment interaction effects from *Oryza sativa × O. nivara* IRGC81832 cross

Divya Balakrishnan, Malathi Surapaneni, Venkateswara Rao Yadavalli, Krishnam Raju Addanki, Sukumar Mesapogu, Kavitha Beerelli & Sarla Neelamraju

Chromosome segment substitution lines (CSSLs) are useful tools for precise mapping of quantitative trait loci (QTLs) and the evaluation of gene action and interaction in inter-specific crosses. In this study, a set of 90 back cross lines at BC$_2$F$_8$ generation derived from Swarna × *Oryza nivara* IRGC81832 was evaluated for yield traits under irrigated conditions in wet seasons of 3 consecutive years. We identified a set of 70 chromosome segment substitution lines, using genotyping data from 140 SSR markers covering 94.4% of *O. nivara* genome. Among these, 23 CSSLs were significantly different for 7 traits. 22 QTLs were detected for 11 traits with 6.51 to 46.77% phenotypic variation in 90 BILs. Three pleiotropic genomic regions associated with yield traits were mapped on chromosomes 1, 8 and 11. The marker interval RM206-RM144 at chromosome 11 was recurrently detected for various yield traits. Ten QTLs were identified consistently in the three consecutive years of testing. Seventeen pairs of significant epistatic QTLs (E-QTLs) were detected for days to flowering, days to maturity and plant height. Chromosome segments from *O. nivara* contributed trait enhancing alleles. The significantly improved lines and the stable QTLs identified in this study are valuable resource for gene discovery and yield improvement.

Rice is one of the major crops which grow across wide range of environments and supports majority of the world population mainly located in developing countries of Asia, West Africa, Latin America and the Caribbean. Global rice yields and total rice consumption are projected to rise by 12% and 13% respectively by 2027. However there is a large gap between anticipated production and consumption due to expected population rise and restricted cultivated area expansion in major rice growing countries of Asia. Even though a large number of rice cultivars exist in the world, the most widely grown varieties are few and they harbour a limited genetic diversity due to common parents and origin within *Oryza sativa* species. The crop is exposed to multiple stresses and evolving pests, as range of cultivation is wide and also climate scenario is changing. Therefore, much focus is needed to widen the gene pool of cultivated germplasm to sustain and improve yield potential. Prebreeding and utilization of wild accessions for introgression of novel genes / QTLs / chromosomal regions are gaining more importance in rice improvement.

Chromosome segment substitution lines (CSSLs) are prebreeding genetic stocks which can be utilised in crop breeding and genomics studies for improvement of various traits. Wild species are a reservoir of valuable genes, however the unpredictability of genotypic potential of wild species in a cultivar background, cross incompatibility and sterility in inter specific crosses are common hindrance in prebreeding programs. CSSLs developed using wild donors can overcome these problems as a bridging prebreeding product and can be used for identification, mapping and introgression of novel alleles from the wild. CSSLs can be utilized for faster and easier development of NILs and varieties than using an un-adapted or wild genotype directly. Since a single major introgression is present in each line, its effects are not masked by other components of donor genome and thus linkage drag and negative effect on QTL is limited in an ideal CSSL set.

ICAR-Indian Institute of Rice Research, Hyderabad, India. ☉ e-mail: sarla_neelamraju@yahoo.com
About fifty inter-specific and intra-specific CSSL libraries are reported in rice in the last two decades. Most of the initial CSSLs developed in rice involved indica japonica crosses but the use of wild species as donor has increased since the last two decades. Accessions from wild rice species viz., O. rufipogon, O. nivara, O. glumaepatula, O.barthii, and O. meridionalis were used as donor parent in CSSL development programmes. Furuta et al. developed CSSLs using Oryza rufipogon accessions as donor. Rangel et al. developed CSSL in indica cultivar BG 90–2 using O. glumaepatula. Arbelez et al. developed two populations of interspecific introgression lines (ILs) in cv. Curinga, a tropical japonica upland cultivar using two wild donor species, O. meridionalis and O. rufipogon Griff having 76.73% to 97.6% donor genome respectively. CSSLs are ideal for accurate mapping of gene/ QTL and genetic analysis, largely due to low interaction of genetic backgrounds. Thus, CSSLs have been used to identify chromsome regions affecting any trait, mapping, fine-mapping and cloning genes from novel genomic regions and QTL hotspots. In rice, superiority of introgression lines over recurrent parents has been reported, however the effect of alien introgression using CSSLs and their advantage over RILs/ BILs has not been studied extensively. Ideally if each CSSL has only a segment from the donor it becomes easy to define the QTL position. Usually a primary CSSL population contains more than one donor segment in each line, so the gene and marker frequencies do not fit into mendelian ratios as that of F2 and BC mapping population. Therefore, stepwise regression-based likelihood ratio test (RSTEP-LRT) is used for QTL mapping in CSSLs. Inclusive composite interval mapping (ICIM) was used in QTL mapping and to study the epigenetic interactions due to its unbiased QTL effect estimation.

In our previous study, population was developed from Swarna / O. nivara IRGC81848 for QTL mapping for yield traits. Individually advanced lines were genotyped at BC2F8 and a set of 74 CSSLs were constructed with donor genome coverage of 89%. Another population developed from a cross between Swarna and O.nivara IRGC81832, was evaluated for yield and quality related traits and QTLs were mapped at BC2F8 generation. This population was advanced to BC2F9 and was phenotyped in this study for 3 consecutive wet seasons and genotyped using SSR markers to identify a set of CSSLs representing complete genome of O. nivara as overlapping segments substituting Swarna segments and to detect major QTLs for yield traits.

Methods

The research work was conducted at Indian Institute of Rice Research (IIRR), Hyderabad during the period 2014 to 2017 wet seasons. The area is located at latitude of 11°00’ N longitude of 77°00’ E and an elevation of 426.72 m above mean sea level (MSL).

Plant material. Advanced back cross introgression lines derived from a cross between an elite cultivar Swarna (O. sativa) as a recurrent parent and a wild accession O. nivara IRGC81832 as a donor parent were used in this study at BC2F9 to BC2F10 stage after conducting single panicle selection for consecutive generations from BC2F3 and these NPK or K lines are now designated as NK 1–90.

Phenotypic evaluation. The experiment was conducted following randomised complete block design (RCBD) with three replications at experimental plots in Indian Institute of Rice Research (IIRR), Hyderabad. A set of advanced backcross introgression lines (BC2F9) along with recurrent parent Swarna, were raised during wet seasons of 2014 (E1), 2015 (E2) and 2016 (E3). 25 days old seedlings were transplanted following 15 cm interplant spacing and 20 cm inter row spacing. Package of practices for fertilizer application and pest control measures under irrigated conditions were followed to avoid yield loss. Randomly selected five plants from each replication were evaluated for 4 yield-related traits, viz., days to 50% flowering (DFF), days to maturity (DM), plant height (PH), number of tillers per plant (NT), number of productive tillers per plant (NPT), biomass (BM), single plant weight (PW), grain yield/plant (GY), bulk yield per square metre (BY) and two derived traits; harvest index (HI) and per day productivity (PDP). The observations on yield and morpho-agronomic traits were recorded from the field experiments following Standard Evaluation System (IRRI, 2013).

The phenotypic data was analysed using Statistical Tool for Agricultural Research (STAR v2.0.1, IRRI) for the descriptive statistics (Supplementary Table 1) and ANOVA (Supplementary Table 2). Broad-sense heritability was calculated using the formula: \[ h^2 = \frac{\sigma^2_g}{\sigma^2_g + e^2}, \] where \( \sigma^2_g \) is the genotypic variance and \( e^2 \) is the environmental variance. Levene and Bartlett test for homogeneity of variance was conducted for yearly means and a Shapiro-Wilk test for normality (Supplementary Table 3 and 4) were performed to test the assumptions of the ANOVA (Supplementary Table 5). Significant pairwise comparisons were carried out comparing with control Swarna. Boxplot and histogram of each trait in different seasons were derived using R software. Phenotypic and genotypic correlation among trait averages was computed for Proteins \( > t \) at 0.05, 0.01 and 0.001 in STAR (STAR v2.0, IRRI) using Pearson’s product-moment correlation using replicated phenotypic data across the years. The observed trait data were analyzed with PB tools (Version 1.4, http://www.irri.org/products) by considering blocks as random and entry as fixed effect to obtain LS mean estimates and were then used for QTL analysis.

SSR genotyping. Genomic DNA was extracted from leaf using CTAB method. The extracted DNA was estimated at 260 nm/280 nm and 260 nm/230 nm for quality and quantity using UV spectrophotometer. The estimated DNA quantity was between 94 to 1294 ng/ul with 1.8 to 2.1 OD at 260 nm/280 nm and 260 nm/230 nm. For genotyping, working DNA concentration of 40 ng/ul was made as template. A set of 300 markers including 165 SSRs which are equally distributed throughout rice genome were used to study parental polymorphism and 140 SSR markers, identified as polymorphic were used in screening CSSL population. Genomic DNA was isolated.
from fresh leaf samples of 90 lines following CTAB method. 140 SSR markers distributed across the genome were used to genotype the mapping population. PCR was performed in thermal cycler (G-STORM, USA) with a final reaction volume of 10µl containing 15 ng of genomic DNA, 1X assay buffer, 200 µM dNTPs, 1.5 mM MgCl₂, 10 pmol of forward and reverse primer and 1 unit of Taq DNA polymerase (Thermo Scientific). PCR cycles were programmed as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min and a final extension of 10 min at 72 °C. Amplified products were resolved in 3% agarose gel prepared in 0.5 X TBE buffer and electrophoresis was carried out at 120 V for 2 h. Gels were stained with ethidium bromide and documented using gel documentation system (Alfa imager, USA).

Identification of CSSL and QTL analysis. SSRs were scored as co-dominant markers, and recorded as ‘A’ for band presence female parent Swarna and “B” for presence of male O.nivara parent and “AB” for heterozygous bands. CSSLs were identified using segregation data of 140 polymorphic SSR markers in 90 BILs in the background of Swarna genome using CSSL Finder (http://mapdisto.free.fr/CSSLFinder/). Substituted chromosome segments were estimated on the basis of origin of alleles of flanking markers. A chi-square test for the 1:1 segregation ratio was conducted in the advanced backcross mapping population and no segregation distortion was found. Linkage map was constructed based on genotypic data of 90 BILs using 140 polymorphic SSR markers on all chromosomes. MAP functionality was used for the construction of genetic linkage map involving interfaces of grouping, ordering, and rippling procedures in map construction. Ordering of loci is done by an efficient approximate algorithm of nnTwoOpt in ICIM.

The Kosambi’s mapping function was used to transform recombination frequencies between linked loci into cM distances. QTL mapping was performed by single marker analysis (SMA), inclusive composite interval mapping (ICIM-ADD) of additive and dominant QTL and inclusive composite interval mapping of epistatic QTL (ICIM-EPI) functions implemented in the QTL IciMapping v4.1 (www.isbreeding.net) in a stepwise regression for the adjusted means of each trait. The threshold LOD value was determined by a permutation test involving 10000 runs at a significance level of p = 0.05 and the QTLs in a particular genomic region with LOD values larger than this threshold for each trait were used. The LOD test statistic used was –2ln (L0/L1), where L0/L1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) and the alternative hypothesis (there is a QTL in the interval). The QTLs were deemed to exist only at positions where the LOD score exceeded the corresponding significant threshold. Estimation of position, genetic effects and phenotypic variation percentage of QTLs was done at the significant LOD peak in the region under consideration. The phenotypic variance explained by a single QTL was estimated by the square of the partial correlation coefficient (R²). Estimates of the R² value or the phenotypic variance explained (PVE) and the additive effects of QTL at their peak LOD positions were obtained from the output of QTL analysis. Parent for trait enhancing allele was identified using the sign of the additive effects, negative sign denotes that trait –enhancing allele is from O. nivara.

Mapping of additive and digenic epistasis genes with chromosome segment substitution lines was carried out with QTL IciMapping v4.026 with CSSL (Chromosome Segment Line) option using method ‘Likelihood ratio test’ based on stepwise regression for additive QTL (RSTEP-LRT-ADD) which specifically deals with QTL mapping in collections with multiple introgressions per line32. Parameters setting was ‘Multi collinearity control’ = −1 (equiv- alent to deletion of duplicated markers only) and ‘PIN’ = 0.0001 (PIN: the largest-value for entering variables in stepwise regression of residual phenotype on marker variables). Logarithm of odds (LOD) threshold values for each trait was computed after 1000 permutations using a type I error = 0.05 and reported in corresponding tables as footnotes. The proportion of variance explained by a single QTL was as provided from single marker analysis in RSTEP-LRT-ADD. QTL-by-environment interaction analysis in biparental population was carried out using MET. Epistatic effect QTLs were analysed using ICIM-EPI with a probability value (PIN) of 0.0001 and threshold LOD26.

Results

Phenotyping. The variance components and descriptive statistics of yield related traits are presented in Table 1. The genotypes showed significant variation for BY, PH, DFF, BM and TDM in the BIL population. High broad-sense heritability (h²) of 96.43%, 95.98%, 94.15% and 58.45% was observed for days to flowering, days to maturity, plant height and panicle weight respectively. The variance component associated with genotypes contributed to 92% of total variation. The mean performance of BILs using pooled data for three seasons showed significant increase over Swarna for 7 traits. 31 transgressive BILs that were significantly superior or inferior to the recurrent parent were identified after evaluating the BILs for yield traits over three years from their frequency distribution (Supplementary Fig. 1). Of these BILs, NK61 out-performed Swarna for GY and NK58 and NK83 for BY in all three seasons. 18 BILs with significantly lower DFF than Swarna were identified. For PH and BM, 13 BILs were identified respectively with significantly higher trait values compared to Swarna (Supplementary Table 6). Among 31 lines identified through significant pair wise comparison 21 showed significantly positive values for 7 traits and 16 showed significantly negative values for 3 traits compared to Swarna. Highly significant phenotypic associations were observed among the traits and across years (Table 2). Although all yield traits were positively associated and were significant at P < 0.01, DFF and DM showed negative association with all yield traits except biomass. BY showed strong positive correlation (P < 0.01) with NT, NPT, BM, PW, TDM, HI and PDP. PH was highly significantly correlated with both TDM and BM. GY showed highly significant correlation with BM and BY. PW was significantly correlated with GY, TDM, HI, BY and PDP but negatively with DFF and DM.

CSSL identification. When genotypic data was subjected to CSSL finder, 70 out of 90 lines formed a minimal set of CSSLs with 11.94% of donor and 88.6% of recurrent parent segments (Fig. 1). The minimum donor genome in a CSSL was 2.33% in NK24 and maximum was 26.51% in NK61. NK61 was significantly better than...
| Trait | Parents | Mapping Population | Heritability |
|-------|---------|--------------------|-------------|
| | Swarna (IRGC81832) | Oryza nivara | |
| **Parents** | **Swarna** | **Oryza nivara** (IRGC81832) | **BC1F2** |
| **DFF** | 120 ± 4.3 | 132 ± 7.9 | 112.95 ± 6.36 | 50.5 | -2.01 | 5.2 | 83 | 121 | 38 | 0.82 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.97 |
| **DM** | 134 ± 2.8 | 157 ± 5.2 | 142.27 ± 6.37 | 40.61 | -1.93 | 4.95 | 112.5 | 151 | 38.5 | 0.84 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.96 |
| **PH** | 86 ± 3.2 | 124 ± 5.5 | 84.31 ± 19.13 | 365.83 | 1.23 | 0.64 | 60.44 | 137.22 | 36.78 | 0.84 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.97 |
| **NT** | 12 ± 2.0 | 58 ± 6.0 | 12.05 ± 1.56 | 2.42 | 0.94 | 2.49 | 8.44 | 17.89 | 9.45 | 0.94 | 1.20E-03 | 1.20E-03 | 0.32 |
| **NPT** | 11 ± 4.5 | 48 ± 1.5 | 10.7 ± 1.48 | 2.2 | 0.46 | 0.9 | 8.69 | 15.44 | 8.55 | 0.98 | 5.64E-01 | 5.64E-01 | 0.28 |
| **PW** | 1.83 ± 0.15 | 0.93 ± 0.03 | 2.08 ± 0.46 | 20.21 | 0.3 | 0.66 | 1.11 | 3.15 | 2.04 | 0.96 | 5.32E-02 | 5.32E-02 | 0.80 |
| **GY** | 12 ± 1.4 | 1.80 ± 0.47 | 13.19 ± 3.91 | 15.32 | 0.86 | 1.38 | 5.23 | 27.73 | 22.5 | 0.96 | 2.16E-02 | 2.16E-02 | 0.76 |
| **BM** | 11 ± 1 | 16.00 ± 2.65 | 25.96 ± 6.68 | 44.63 | 0.78 | 0.3 | 13.77 | 44.64 | 30.87 | 0.94 | 1.33E-03 | 1.33E-03 | 0.81 |
| **TDM** | 13 ± 2 | 17.80 ± 2.74 | 39.15 ± 9.43 | 88.89 | 0.74 | 0.25 | 22.27 | 65.95 | 43.68 | 0.95 | 2.17E-03 | 2.17E-03 | 0.79 |
| **HI** | 34.47 ± 6.73 | 10.50 ± 3.02 | 33.37 ± 5.98 | 34.61 | -0.01 | -0.07 | 16.36 | 46.62 | 50.26 | 0.99 | 8.09E-01 | 8.09E-01 | 0.78 |
| **PDP** | 0.09 ± 0.03 | 0.09 ± 0.03 | 0.82 | 1.06 | 2.01 | 0.04 | 0.21 | 0.17 | 0.93 | 1.34E-04 | 1.34E-04 | 0.78 |

Table 1. Variability, descriptive statistics and yield traits means (± standard deviation) of parents and of BC1F2 population. DFF- days to 50% flowering, DM- days to maturity, PH- plant height, NT- number of tillers, NPT- number of productive tillers, PW- panicle weight, GY- yield per plant, BM- Biomass, BY- bulk yield, TDM- total dry matter, HI- harvest index, PDP- per day productivity, G Mean- Genotypic mean, PCV- Phenotypic mean, GCV- Genotypic coefficient of variation, GCV-Genotypic coefficient of variation, h²- Heritability, GA-Genetic advance, GG- Genetic gain.

The Swarna for YLDP and PDP and negatively significant for DFF and DM. NK24 was not significant for any trait. The number of substituted chromosome segments per line ranged from 2 to 16 with an average of 8 in the CSSL set and the number of segments observed per chromosome were 2 to 11 with an average of 6 segments. 70 CSSLs represented 94.4% of O. nivara genome but the donor genome was absent in small regions on chromosomes 5, 6, 9, 11 and 12. Chromosomes 5, 9, 11 and 12 were represented by 3 CSSLs each and chromosome 1 by 14 CSSLs. The line NK24 had two donor segments on chromosomes 1 and 5 while CSSLs NK2, NK11, NK18, NK19 and NK21 had only three segments each on different chromosomes (Supplementary Table 7). NK22 introgression of 120.31 cM showed only six substituted segments. Similarly, NK50 had the lowest size of 5.82 cM O. nivara segment substitutions with 84.88% Swarna alleles and 15.12% of donor alleles.

**Construction of linkage map and QTL mapping.** QTL detection was carried out using composite interval mapping (CIM), mapping of additive and digenic epistasis genes with chromosome segment substitution lines (CSL) and QTL-by-environment interaction analysis (MET) to dissect the genetic basis of yield traits. Consistent detection of QTLs was observed across the analytical methods in same genomic region. Eight chromosomes (chromosome 1, 2, 3, 4, 7, 8, 11 and12) showed significant genomic regions associated with major QTLs for yield traits. LOD scores of QTLs ranged from 2.55 to 22.98 and explained PVE from 6.51% to 46.77% (Table 3). QTLs were detected above threshold LOD values for all the traits except for number of productive tillers after 1000 permutations. 3 QTLs each for days to flowering and days to maturity were detected in chromosome 3, 7 and 12. For plant height 3 QTLs qPH1.1, qPH1.2 and qPH4.1 were mapped. One QTL each was identified for number of tillers (qNT2.1), panicle weight (qPW11.1), grain yield (qGY11.1), biomass (qBM1.1) and harvest index (qHI4.1). Two QTLs, qBY11.1 and qBY11.1 were mapped for bulk yield and qTDM11.1 and qTDM11.1 for total dry matter. For per day productivity (PDP) three QTLs were detected in chromosomes 1, 8 and 11. The QTL on chromosome 1 with the flanking markers RM128 and RM431 was associated with PH, BM and TDM and...
Table 2. Phenotypic and genotypic correlation coefficients among twelve yield related traits for three environments. Significance levels: *P < 0.05, and **P < 0.01. Upper diagonal shows the genotypic correlation of mean data across years and lower diagonal shows phenotypic correlations of year wise and mean data. DFF- days to 50% flowering, DM- days to maturity, PH- plant height, NT- number of tillers, NPT- number of productive tillers, PW- panicle weight, GY- yield per plant, BM- Biomass, BY- bulk yield, TDM- total dry matter, HI- harvest index, PDP- per day productivity.
explained phenotypic variation of 46.77%, 27.11% and 15.17% respectively and the trait-increasing alleles were from *O. nivara*. This was also confirmed by single marker analysis and interval mapping (Supplementary Table 8 and 9). The QTL on chromosome 11 in the region between RM206 and RM144 showed significant effects for grain yield with PVE of 18.92%. The same region harboured QTLs for PW with 15.40%, BY with 15.91% and PDP with 15.46% phenotypic variation. The QTL flanked by RM137 and RM408 on chromosome 8 was associated with PDP and BY. This QTL explained 9.58% of the variation for PDP and 10.85% for BY. A QTL was identified in chromosome 2 with PVE of 14.19% for NT and in chromosome 4 with a PVE of 14.88% for HI. DFF and DM showed common QTL between RM16 and RM55 in chromosome 3, between RM214 and RM11 in chromosome 7 and between RM19 and RM247 in chromosome 12 with PVE ranging from 16.15% to 26.33%. Additive effect showed that trait enhancing alleles for PH, BM, PW, GY, TDM and PDP were contributed from the donor parent *O. nivara* while the alleles for DFF and DM were from Swarna.

**MET QTL mapping.** Three years phenotypic data was subjected to MET functionality to detect QTL by environment interaction in 90 BILs. 31 significant QTLs were detected above threshold LOD MET under 1000 permutations with a probability value (PIN) of 0.0001. METQTLs were identified for the traits on all chromosomes except 6 and 9. The QTL by a single QTL ranged from 3.17% to 45.88% at LOD levels from 4.11 to 58.91 (Table 4). QTLs for DFF and DM at chromosome 7 (RM214-RM11) and chromosome 12 (RM19-RM247), PW at chromosome 11 (RM206-RM144) and PH at chromosome 1 (RM128-RM431) were found to have high LOD (AxE). PW and BY QTL at chromosome 11 (RM206-RM144) and BY QTL at chromosome 8 (RM137-RM408) showed highest QTL by environment phenotypic variance. A significant QTL for panicle weight between RM206 and RM144 in chromosome 11 with a PVE of 16.50% was identified and showed highest additive by environment PVE of 3.41%. Two QTLs on chromosomes 8 and 11 for bulk yield were detected with large additive effects across 3 environments and 14.07% and 9.67% PVE respectively.

Among the QTLs identified from 90 BILs data through ICIM, 10 were detected across 3 environments for DFF, DM, PH, NT or TDM consistently and 4 were detected in at least 2 environments for PDP and GY and with a PVE above 10% (Supplementary Table 10). QTLs that were mapped to the genomic regions located within the
same set of flanking markers were considered as representing the same QTL. The QTL region within RM128 and RM431 on chromosome 1 showed PVE ranging from 38.74 to 44.11% for PH across the environments, and the same region showed QTL for BM also in all the 3 years. Similarly, the locus between RM16 to RM55 at chromosome 3, RM214 to RM11 at chromosome 7 and RM19 to RM247 at chromosome 12 showed major QTLs for DFF and DM consistently over the years. QTL for NT was detected between RM8080 and RM341 at chromosome 2 in all the 3 years, with PVE varying between 11.28 to 15.32%. Similarly, region RM206 to RM144 at chromosome 11 was consistently identified for TDM. QTLs for GY and PDP were detected on chromosome 8 (RM137-RM408) and chromosome 11 (RM206-RM144), in 2 consecutive years.

The locus RM128-RM431 at chromosome 1 showed QTLs for grain yield, harvest index, biomass, total dry matter and plant height by multi environment testing (MET) QTL mapping and also with high PVE and LOD. Another region RM137-RM408 at chromosome 8 had QTLs for total dry matter, bulk yield and per day productivity and QTLs showed additive by environment interaction. Similarly, the region between RM206 and RM144 on chromosome 11 also harboured QTLs for plant height, biomass, total dry matter, bulk yield, grain yield, per day productivity and panicle weight showing high PVE, PVE (additive) and PVE (additive by environment). 21 out of 31 QTLs showed negative additive effects, indicating that the majority of introgressed segments from O.nivara in these regions contributed to trait enhancing alleles.

QTL mapping using CSSL set. A likelihood ratio test based on stepwise regression-based likelihood ratio test (RSTP-LRT) was used to map QTLs in marker defined chromosomal regions of 70 non-idealized CSS lines. This method ensures accuracy of QTL detection using CSSLs. The results of QTL mapping from BILs and CSSL set or a subset representing the whole donor genome of mapping population was compared. Altogether 10 marker loci were identified as significantly linked with trait improvement using 3 environment data (Table 5). Among these RM214, RM55 for DFF and DM; and RM431 for PH were consistently associated with trait increase in three environments and also the average environment. RM206 showed trait increase for BY, PDP and GY in 2015, 2016 and in mean. Similarly RM25 was a significant locus for panicle weight in CSL QTL mapping for two environments. These markers also showed significant trait linkage when analysed through single marker analysis using the complete set of back cross introgression lines. The mean value of the QTL genotype of parents M(QQ) and M(qq) showed that Swarna has higher values of QTL genotype for all traits except DFF and DM.
### Table 3. Additive effect QTLs for yield traits based on phenotyping under three environments E1, E2, and E3 and detected using QTL IciMapping 3.2. LOD threshold values at 10000 permutations: DFF-8.8495, DM-6.0825, PH-9.5269, NT-3.2942, NPT-2.3922, PW-1.6816, GY-5.0034, BM-2.4836, BY-bulk yield, TDM-total dry matter, HI- harvest index, PDP- per day productivity. Chr-chromosome No. Pos-Position cM, LOD-Logarithm of odds ratio, PVE - Phenotypic variance explained, E1-wet season 2014, E2-wet season 2015, E3-wet season 2016.

| QTL | Trait | Chr | Pos | Left Marker | Right Marker | LOD | PVE(%) | Additive effect |
|-----|-------|-----|-----|-------------|--------------|-----|--------|----------------|
| qBM1.1 | BM | 1 | 264 | RM128 | RM431 | 5.95 | 27.11 | -6.11 | 3.55 | 5.17 | 8.49 | 15.09 | 22.73 | 33.26 | -3.94 | -6.35 | -6.84 |
| qPH1.1 | PH | 1 | 265 | RM128 | RM431 | 22.98 | 46.78 | -23.38 | 18.90 | 18.15 | 24.46 | 44.11 | 49.47 | 58.74 | -24.92 | -21.03 | -26.14 |
| qTDM1.1 | TDM | 1 | 264 | RM128 | RM431 | 3.19 | 15.18 | -7.07 | 3.20 | 4.70 | - | 15.96 | 19.99 | -8.13 | -7.86 |
| qPH1.2 | PH | 1 | 289 | RM226 | RM58 | 3.34 | 6.59 | -7.44 | 4.89 | - | 11.96 | - | - | -11.64 | - |
| qPDP1.1 | PDP | 1 | 313 | RM5 | RM212 | 2.55 | 11.69 | -0.02 | - | - | - | - | - | - |
| qNT2.1 | NT | 2 | 107 | RM8080 | RM341 | 4.24 | 14.19 | -2.68 | 3.18 | 3.69 | 5.03 | 15.32 | 13.07 | 11.28 | -2.73 | -2.45 | -2.34 |
| qDF3.1 | DF3 | 3 | 159 | RM16 | RM55 | 6.42 | 18.28 | 5.17 | 7.55 | 4.55 | 6.24 | 16.60 | 16.16 | 18.20 | 4.43 | 5.86 | 5.17 |
| qDM1.1 | DM | 3 | 159 | RM16 | RM55 | 5.75 | 17.50 | 5.02 | 5.59 | 4.51 | 5.75 | 15.00 | 16.07 | 17.50 | 4.13 | 5.84 | 5.02 |
| qPH4.1 | PH | 4 | 53 | RM17499 | RM16742 | 3.71 | 6.51 | 18.43 | - | - | 5.01 | - | 7.29 | - | 21.87 |
| qHI4.1 | HI | 4 | 30 | RM470 | RM348 | 2.78 | 14.88 | -5.18 | - | - | - | - | - | - |
| qDFF1.1 | DFF | 7 | 48 | RM214 | RM11 | 7.50 | 16.35 | 9.54 | 8.50 | 6.07 | 7.40 | 15.82 | 14.23 | 16.32 | 8.38 | 10.80 | 9.55 |
| qDM7.1 | DM | 7 | 48 | RM214 | RM11 | 7.21 | 16.16 | 9.43 | 7.46 | 6.05 | 7.21 | 15.33 | 14.21 | 16.16 | 8.22 | 10.77 | 9.43 |
| qBY1.1 | BY | 8 | 13 | RM1173 | RM408 | 2.79 | 10.85 | -100.29 | - | - | 5.35 | - | 22.89 | - | -176.37 |
| qPDP8.1 | PDP | 8 | 16 | RM1373 | RM408 | 3.63 | 9.58 | -0.03 | 3.90 | 3.83 | - | 11.46 | 16.04 | -0.05 | -0.02 |
| qBY11.1 | BY | 11 | 7 | RM206 | RM144 | 4.80 | 15.92 | -103.27 | - | - | 5.51 | - | 17.43 | - | -125.93 |
| qPDP11.1 | PDP | 11 | 6 | RM206 | RM144 | 5.73 | 15.47 | -0.03 | - | - | - | - | - | - | -0.03 | -0.03 |
| qPW11.1 | PW | 11 | 18 | RM206 | RM144 | 2.68 | 15.41 | -0.32 | - | - | - | - | - | - | - |
| qTDM11.1 | TDM | 11 | 10 | RM206 | RM144 | 4.46 | 15.94 | -8.79 | 3.01 | 4.17 | 3.63 | 10.17 | 12.26 | 14.16 | -10.23 | -9.24 | -8.04 |
| qGY11.1 | GY | 11 | 7 | RM206 | RM144 | 5.17 | 18.93 | -4.05 | - | - | 4.51 | 4.37 | - | 23.30 | 15.98 | -4.13 | -4.23 |
| qDFF12.1 | DFF | 12 | 40 | RM19 | RM247 | 10.16 | 26.30 | 5.85 | 12.72 | 6.68 | 9.90 | 28.76 | 20.53 | 26.28 | 5.57 | 6.16 | 5.85 |
| qDM12.1 | DM | 12 | 40 | RM19 | RM247 | 9.65 | 26.33 | 5.80 | 10.69 | 6.66 | 9.66 | 27.77 | 20.53 | 26.33 | 5.11 | 6.15 | 5.80 |

### Epistatic QTL.
17 significant epistatic QTL were detected from 90 BILs for DFF, DM and PH at threshold LOD > 5 with PVE ranging from 2.44 to 25.85% (Table 6). Other traits did not show any epistasis above LOD 5. At LOD > 3 we found minor interactions between number of loci across chromosomes (Fig. 3). A total of 6 digenic interactions were detected for days to fifty percent flowering. Interestingly, three of them occurred between significant main effect QTLs for DFF and non-significant loci in other chromosomes. A negative digenic interaction involving loci at chromosome 1 and chromosome 5 was detected and both additive effects were from O. nivara. Four of the epistatic interactions for DM were similar in case of DFF involving the main effect QTLs identified through ICIM. However, two different loci RM493-RM1220 on chromosome 1 and RM55-RM85 on chromosome 3 showed epistatic interaction with the same locus RM19-RM247 on chromosome 12 for DFF and DM respectively. An epistatic interaction between chromosome 3 (RM55-RM85) and chromosome 12 (RM19-RM247) explained a high phenotypic variance of 25.85%. Six digenic loci showing epistatic interactions were detected for plant height. Single locus RM16742-RM307 on chromosome 4 showed interactions with loci in chromosomes 1, 3, 5, 8, 9 and 10. It is worthwhile to note that the epistatic interactions had slightly decreased LOD values of already detected main effect QTLs. The total PVE explained by the epistatic QTLs was lower than the PVE of main effect QTLs for plant height.

### Ethics statement.
The authors declare that the experiments comply with the current laws of the country in which they were performed and in compliance with ethical standards.

### Discussion
Swarna is one of the most popular mega varieties in India and other rice growing countries and well-known for its high yield, long duration and stable performance across different ecosystems. Introgessions from wild species O. nivara are reported to improve yield traits in Swarna and a variety DRRDhan 40 has also been released. Improved backcross introgression lines with favourable agronomic traits and high yield stability were also identified through multi environment trials in Swarna background. In this study the advanced back cross introgression lines (BC$_F_{10}$) of Swarna/O. nivara were phenotyped for three years for yield and related traits and transgressive segregation with significantly positive and negative difference with the recurrent parent was observed (Supplementary Fig. 2) indicating the existence of allelic variations for each trait in each parent. Continuous frequency distribution was observed for all traits demonstrating quantitative inheritance of the traits. DFF, DM, NT, NPT, Y and PDP showed kurtosis values above 1 and among these only PDP showed +ve
The skewness. Except these, the absolute values of skewness and kurtosis were less than 1 for all traits, and showed normal distribution indicating the suitability for QTL analysis. In addition, PH, BM, BY, and TDM also showed higher standard deviations suggesting significant effect of environment in their phenotypic expression. Significant pair wise comparison showed 31 lines significantly different than Swarna for atleast one trait and 27 lines showed significant difference for more than one trait. This suggests interspecific combination of two divergent parents results in accumulation of superior alleles with additive and complementary effects which can help to not only broaden the genetic base but create desirable novel variation in cultivar gene pools. Lines NK58 and NK83 showed significantly higher bulk yield, biomass and plant height and are valuable breeding materials for yield improvement. NK61 which showed significantly higher grain yield than Swarna and significantly lower days to flowering and maturity has commercial value as an early, high yielding breeding line. A set of lines which are either positively or negatively significant over Swarna for days to flowering and days to maturity were identified in this biparental population and they are useful in dissecting the genetics of heading date in rice.

**Yield QTL, ICIM, MET, EPI.** Among the 22 major QTLs identified in this study through ICIM, all the QTLs except those for DFF, DM and one QTL for PH showed trait enhancing alleles from *O. nivara*. This indicates that in general introgression of alleles from *O. nivara* into the elite cultivar background had a beneficial effect on improving most of the yield traits. Major QTL for PH at region flanked by RM128 and RM431 on chromosome 1 was the most consistent across all 3 environments with increasing effect from *O. nivara* and explained the highest proportion of PVE (15–33%) and both the flanking markers were previously reported to be associated with plant height. This establishes the robustness of mapping QTLs in our study. Another QTL for plant
height was found between RM226 and RM5 in chromosome 1. Kadambari et al.\textsuperscript{37} reported a major QTL alternate semi-dwarf 1 (asd1) governing plant height on chromosome 1(chr01:2559897–2564321) approximately 36 Mb upstream from asd1. Several factors, such as the number of detected QTL, the degree of linkage of markers with the genes, the range of variation within the population, the population size, genetic background and phenotyping environments influence the magnitude of phenotypic variation explained by a QTL\textsuperscript{38}. The main limitation in using QTLs for marker assisted selection and breeding is the poor consistency of QTL effects across environments and so stable QTLs need to be identified\textsuperscript{39,40}. Dissection of candidate chromosomal regions to elucidate

| Trait | Chr | Marker interval 1 | Chr 2 | Pos2 | Marker interval 2 | LOD | PVE (%) | Add1 | Add2 | Add \(\times\) Add |
|-------|-----|-------------------|-------|------|-------------------|-----|---------|------|------|------------------|
| DFF 3 | 140 | RM16              | 4     | 5    | RM55              | 5.44| 8.86    | 2.70 | 2.25 | –3.97           |
| DFF 1 | 200 | RM237             | 5     | 35   | RM163             | 5.19| 10.08   | –1.55| –2.57| 4.85            |
| DFF 1 | 225 | RM495             | 7     | 35   | RM1132            | 5.02| 10.51   | 5.85 | 6.08 | –7.32           |
| DFF 2 | 65  | RM207             | 35    | RM1132| RM214            | 5.93| 8.89    | 5.01 | 4.51 | –5.54           |
| DFF 4 | 105 | RM307             | 35    | RM1132| RM214            | 5.03| 9.67    | 5.82 | 6.33 | –7.47           |
| DFF 7 | 35  | RM1132            | 11    | 0    | RM206             | 5.50| 10.47   | 5.51 | 3.93 | –5.73           |
| DFF 1 | 85  | RM493             | 12    | 90   | RM19              | 5.53| 14.28   | 3.91 | –2.82| 2.27            |
| DM 3  | 140 | RM16              | 4     | 5    | RM55              | 5.11| 9.70    | 2.59 | 2.26 | –3.90           |
| DM 2  | 65  | RM207             | 7     | 35   | RM1132            | 5.54| 10.96   | 5.03 | 4.49 | –5.45           |
| DM 7  | 35  | RM1132            | 11    | 0    | RM206             | 5.11| 11.74   | 5.47 | 3.87 | –5.59           |
| DM 3  | 185 | RM55              | 12    | 90   | RM19              | 6.92| 25.85   | 2.54 | –4.32| 4.47            |
| PH 1  | 230 | RM495             | 4     | 60   | RM16742           | 6.39| 4.19    | 12.35| 4.24 | –17.96          |
| PH 3  | 160 | RM16              | 4     | 60   | RM16742           | 5.54| 2.72    | 14.88| 3.16 | –15.73          |
| PH 4  | 60  | RM16742           | 5     | 40   | RM163             | 5.59| 2.72    | 2.28 | 16.12| –14.39          |
| PH 6  | 60  | RM16742           | 7     | 90   | RM44              | 6.05| 3.38    | 5.41 | 12.14| –17.83          |
| PH 4  | 60  | RM16742           | 9     | 20   | RM215             | 6.00| 4.39    | 6.13 | 10.74| –19.26          |
| PH 4  | 60  | RM16742           | 10    | 75   | RM228             | 6.34| 2.44    | 3.37 | 14.61| –15.49          |

Table 6. Epistatic QTLs influencing yield traits based on adjusted mean values from three environments E1, E2, E3. DFF- days to 50% flowering, DM- days to maturity, PH- plant height, Chr- Chromosome, Pos- Position in cM. LOD -Logarithm of odds ratio, PVE - Phenotypic variance explained, ADD- Additive effect, E1-wet season 2014, E2-wet season 2015, E3-wet season 2016.
the genetic mechanisms for grain yield, using multi-environment testing is a key approach in crop improvement. Therefore, to detect such chromosomal regions we conducted MET QTL mapping by validating the phenotypic performance in three environments for accurate mapping and effective use of QTL genes.

QTLs for DFF and DM colocalised in three regions on chromosomes 3, 7 and 12 and explained more than 55% cumulative phenotypic variance in these regions for each trait. Thomson et al.41 identified a stable QTL dh7.1 with peak marker RM214 using BC2F2 population. QTL reported as linked to heading date42,43. The region flanked by RM214 – RM11 on chromosome 7 was reported as the same chromosome region as in our study. RM11, the other flanking marker of qDFF7.1 and Qdm7.1, was also reported as linked to heading date42,43. The region flanked by RM214 - RM11 on chromosome 7 was reported as a major QTL Qhd742, Hd44, dh7.151,42, qDTHI–7–145 and hd790 for heading date. Similarly, flanking markers (RM16-RM55) for qDFF3.1 were associated with QTLs dt3.2, dt3.3, dt3.441,42 and RM19 of dth12.1 was reported to be linked to heading date previously41. Interestingly these regions were also reported as linked to several other traits e.g. yield per plant, grain width, number of tillers per plant, spikelet fertility and grain weight46,49,50 (Supplementary Table 11). QTL analysis for heading date conducted over five years located stably expressing QTL on chromosomes 351.

The QTL qPW11.1 flanked by markers RM206 and RM144 was detected for PW on chromosome 11 and explained 15.40% of the phenotypic variation. The regions RM128-RM431 at chromosome 1, RM137-RM408 at chromosome 8 and RM206-RM144 at chromosome 11 showed clusters of significant QTLs for various yield traits with QTL by environment interaction. The chromosome 11 cluster had QTLs for panicle weight, grain yield, bulk yield, total dry matter and per day productivity. RM206 was reported to be significantly associated with panicle length, grain sterility, spikelet setting density, percent sterility, plant height, number of productive tillers, yield per plant, 1000 grain weight, panicle diameter and seed shattering41,43,51. Marathi et al.41 reported that RM144 was linked to panicle length and yield per plant. A major QTL containing genes Pikh/Pi54 was previously detected between RM206 and RM14454 for resistance to neck blast and leaf blast55,56. RM206 is also reported as linked to canopy temperature at reproductive stage in drought57. RM408 was found closely linked to multi-tiller and dwarf mutation on the short arm of chromosome 858.

These traits were also correlated and have contributory effect on each other. Wang et al.38 suggested pleiotropic effects of individual locus or tight linkage between loci in the same region is responsible for significant correlation between traits. The nature and direction of these strong associations suggests that these traits are under similar genetic control, conferred by pleiotropic effects of the underlying genes. It is mainly due to cluster of co-expressed genes serving different functions and selection of these QTLs may help in simultaneous improvement of these traits59. The QTL effect always depends on the background genome as well as direction of correlation among the traits even though the QTLs for correlated traits are colocalised60. Development and phenotyping of segregating population of recombinants of these regions are promising in unravelling genetic mechanisms underlying these regions61.

Grain yield and yield component traits are typically quantitative in nature and are influenced by environment and epistatic interactions62. A total of 10 putative major effect QTLs were detected across three environments and 31 QTLs with significant LOD values were identified for all yield traits. QTL region flanked by RM206 and RM144 at chromosome 11 for panicle weight was detected with significant LOD values and highest PVE additive × environment interaction suggesting it as a stable QTL. Even though, QTLs related to days to flowering, maturity and plant height were consistent with significant LOD levels their phenotypic variance in MET analysis was low indicating major effect of environment and the environment interaction on phenotypic expression of these traits. Hosseini et al.63 detected QTL clusters for rice grain appearance quality traits with main, epistatic and QTL × environment interaction effects using two populations of backcross inbred lines (BILs) and suggested that co-located QTL or genes with pleiotropic effects could control these traits. Li et al.48 carried out QTL × E interaction studies of rice doubled-haploid lines in nine diverse environments for heading date and plant height and found varying effects of QTL both in magnitude and direction due to more pronounced epistatic effects and non
expression of some QTLs, weak expression or their different expression in different environments. Quantitative traits are also influenced by interaction between non allelic genes and QTL × environment interactions and even the minor effect interactions influence trait expression significantly. It is observed that in most of the cases, complete effects of detected QTLs are not expressed in advanced generations or in marker assisted selection. This is mainly due to the fact that generally the major effect of QTLs are determined only by the additive component, however epistatic and environmental interactions are mostly ignored. Simultaneous detection of major effect, epistatic and environmental interaction is required for unbiased determination of QTL effect and breeding value.

A total of 17 pair wise epistatic interaction QTLs were in this study above a threshold LOD of 5 with significant additive × additive (AA) effects and showed interaction loci for either days to flowering or plant height. We found only 17 significant epistatic interactions but a large network of weak epistatic interactions among yield traits. Additive by additive effects of epistatic QTLs were comparatively lower than additive effect of any of the corresponding QTL. This might be mainly due to presence of few individuals with rare loci combinations in the populations, interference from other loci and low epistatic variance from interactions.

Interestingly, all the pair wise interacting loci for days to flowering and maturity involved at least one of the major QTL except an interaction QTL pair on chromosome 1 and 5. 7 pairs of loci showed epistatic interaction for days to 50% flowering (DFD) and 3 of them also showed interaction effects for days to maturity (DM) and the fourth pair of epistatic loci for DM had only one common QTL with DFD. Yang et al. characterized epistatic interaction between heading date genes of different chromosomes in rice. Exploration of epistatic interactions among multiple QTLs improved the understanding on genetic network of flowering and the information is useful for pyramiding of QTLs. Subudhi et al. demonstrated the presence of epistatic interactions and found RM214 linked to Ghd7/qHD7–1 is involved in 5 pairs of epistatic interacting loci among different flowering related QTLs. Similar to our study, Nemato et al. and Zhang et al. also reported the involvement of novel epistatic loci (which are not detected in composite interval mapping) in regulation of flowering. Yadav et al. reported the existence of epistatic interactions between QTLs that influence the expression of a complex trait such as grain yield under stress and non-stress conditions.

The QTL for plant height at chromosome 4 (RM16742-RM307) showed interactions with all the 6 loci for plant height on other chromosomes. However, these 6 interacting epistatic loci for PH did not involve the major QTL for plant height close to semidwarf gene at chromosome 1 suggesting its independent major effect. Zhao et al. reported that digenic epistasis was generally not observed among detected major QTLs; as observed in our study. Thomson et al. showed that DTH4–3(I) (dth4.1) located on chromosome 4 and closely linked with marker RM307 controls days to heading. Zhao et al. mapped a semidwarf gene sd-12 closely linked to RM307. Gaikward et al. also reported that O. nivara allele at RM307 was responsible for increased plant height in introgression lines. In our study this QTL even with a small additive effect from O. nivara showed a complementary effect on other QTLs for PH indicating an important role of epistatic interaction in phenotypic variation.

However, these epistatic loci showed significant interactions only in any one of three environments.

QTLs with main, epistatic and QTEn environment interaction effects were identified previously for salt tolerance, yield traits, grain chalkiness and heading date to dissect the genetic mechanisms of these complex traits. Zhang et al. reported ICIM is an efficient tool to find epistatic QTLs and unbiased by population type or size and the genetic effects from simulation studies using F2 and doubled haploid (DH) populations. Wang et al. reported that epistatic interaction effects depend on the significance and the direction of effects of participating QTL with a consistent trend towards negative epistatic effects as in the current study and these are of much importance when the QTLs/ gene involved are co introgressed or pyramid for crop improvement. Cumulative effects of QTL were significant for flowering duration and pyramiding QTL by marker-assisted selection would be an appropriate strategy to improve the traits showing cumulative or complementary epistasis.

Yield QTL in BC2F2 vs. BC2F2. QTL mapping in this study was based on BILs and CSSLs at BC2F2 generation phenotyped for three years in normal irrigated conditions. The current mapping population was derived through single panicle selection from an earlier generation of BC2F2. We compared the previous study where QTLs were detected with CIM and IM using QTL Cartographer in a population of 245 BC2F2 families and genotyped with 75 SSR markers; to understand the changes in QTL detection and QTL effect after 6 generations of selfing. Interestingly the single significant QTL for GY identified in the present study was also reported in the same region with a common flanking marker RM206 in the previous study. It is noteworthy, that QTL for plant height at chromosome 4, number of tillers at chromosome 2 and bulk yield at chromosome 8 were in the same region in both BC2F2 and BC2F8 generations showing a similar region of genetic control for components of biomass production. Population size and marker density directly affect the accuracy of QTL mapping. However, in this study marker density was higher while population size was lesser than that of BC2F2. The LOD levels for the same QTL were lower in BC2F8 than in BC2F2. A QTL for plant height showed highest LOD in both BC2F2 and BC2F8 with common flanking marker RM128. This region was previously reported for plant height and is close to semi dwarf gene sd1 and QTLs for other yield contributing traits.

Malathi et al. compared BILs of O. nivara IRG58-42 and found only 3 out of 15 QTLs in BC2F2 were reported previously in BC2F2 generation. Similarly, Rangel et al. and Wickneswari et al. identified few common major QTLs across generations. Studies comparing previous QTL mapping with an advanced generation of BILs or back crosses found genetic distance of intervals was reduced resulting in more accurate mapping of QTLs with shorter average marker distances. Since many major epistatic or environmental QTLs are context-dependent and do not appear across generations it is prudent to assess worthiness of a QTL for use in MAS. The advanced backcross generations also help in discovering minor QTLs with significant role in phenotypic expression due to a uniform genetic background which may have been masked in an early generation.
with large number of introgressions. Any QTLs which are stable over backgrounds, generation and genomic context are high priority candidates for fine mapping, cloning, gene discovery and marker assisted breeding to improve the associated trait. Ten QTLs \( qBM1.1 \), \( qDFF3.1 \), \( qDFF7.1 \), \( qDFF12.1 \), \( qDM3.1 \), \( qDM7.1 \), \( qDM12.1 \), \( qPH1.2 \), \( qNT2.1 \) and \( qTDM11.1 \) are considered as stable QTLs in our study.

**CSSLs developed and identification of linked CSS.** CSSL library was identified from this advanced back cross population (BC\(_2\)F\(_4\)) generated from an interspecific cross of \( O. sativa \) and \( O. nivara \) IRGC81832. Three sets of CSSLs were developed and QTLs were reported previously using different accessions of \( O. nivara \) by Malathi et al.\(^\text{29} \), Xin et al.\(^\text{38} \), Furuta et al.\(^\text{37} \), Ma et al.\(^\text{88} \). Subudhi et al.\(^\text{87} \) reported that the increased recurrent parent genome recovery of individual lines of CSSL helps in improving confidence of QTL by reducing the background noise in the mapping population. The presence of more than one chromosome segment substitution and missing segments is mainly because this population was generated after only two back crosses and no genomic or phenotypic selection during selfing. Incomplete genome coverage of wild accession is mainly attributed to inadvertent or deliberate selection in the early generation which may have removed the lines with donor segments contributing to any unfavourable trait in the population. Presence of genetic factors for cross incompatibility, hybrid sterility, lethality and recombination barriers which are well known in wild introgressions also are the probable reasons for missing segments in substitution lines. Chromosome segments in the CSSL genome composition graph showed uneven distribution of many segments on the two sides of the diagonal segments along with heterozygosity and missing segments and it might reduce mapping accuracy.\(^\text{39} \) NK61 with maximum 26.31% alleles from \( O. nivara \) was the only CSSL showing significantly higher grain yield and lower flowering days compared to the parent Swarna. Thus, wild introgressions enhance yield and testing under large scale multi-location trials is required.

CSSLs with uniform genetic background harbouring specific chromosome segments from a donor species are ideal for mapping of QTLs.\(^\text{38,37} \) Nine out of 20 QTL (45%) detected using CSSLs were observed in at least two environments and also based on mean data across environments. In comparison, only 14 out of 45 QTLs (31%) from the larger BIL population were detected in more than one environment indicating usefulness of CSSLs in identifying stable QTLs compared to primary mapping populations. It was demonstrated that CSSLs are more powerful than primary mapping populations like RIL and F\(_2\) to detect QTLs in multi environment testing using a set of inter specific CSSLs and RILs of cabbage (Brassica rapa)\(^\text{37} \). Most of the major QTLs were consistently detected across mapping populations and methods, however variation was observed in number, loci and phenotypic variance of many QTLs. In CSSLs the masking and interaction effects of multiple genes and loci are comparatively lower than in other mapping populations. This leads to more precise detection of observed variation in QTL mapping along with the genome effect of a background genotype.\(^\text{75,76} \) Thus, CSSLs has the advantage of increasing the accuracy of QTL mapping while reducing the population size to be phenotyped, by representing the whole genome of the donor parent in a comparatively smaller subset.

Significant quantitative trait loci (QTLs) for different yield traits were located in 10 chromosomal segments above threshold levels and in at least one environment. These included QTLs for BM, BY, DFF, DM, H1, PDP, PH, PW and GY located in all chromosomes except 5, 6 and 9. The QTL locus near RM431 with large phenotypic variance for plant height and identified in 3 environments was previously identified as associated with sd\(_1\) gene.\(^\text{20,31,82} \) This may be a new allele for semi dwarf locus which can be confirmed by sequencing. RM431 was reported as closely linked flanking marker for a major and consistent QTL \( qDTY1.1 \) in multiple elite genetic backgrounds under both drought stress and non-stress condition\(^\text{81} \) and the same marker was used for transfer of the QTLs for yield under drought into different genetic backgrounds.\(^\text{82} \)

The QTL locus near RM206 showed large phenotypic variance for BY, H1, PDP and GY with trait increase contributed by \( O. nivara \) alleles across two environments. QTL loci RM16-RM55 on chromosome 3 and RM214-RM11 on chromosome 7 for days to flowering and days to maturity showed low environment interaction and were consistently detected across the environments. But interaction between chromosome substitution and environment was not significant in case of regions at chromosome 1 (RM431) for BM, at chromosome 2 for PW, at chromosome 3 for DFF and DM, at chromosome 4 and 12 for PH and at chromosome 8 for GY and PDP indicated by their low LOD (QTL \( x \) E). For enhancing grain yield, \( O. nivara \) allele at RM206 could be explored in breeding for wide adaptation. Wild donor genome segments detected through macro-segment mapping using CSSLs need further fine mapping to understand the genetic mechanisms for phenotypic expression.\(^\text{85} \) Near isogenic lines and secondary mapping populations targeting these chromosome substitution segments are essential to assess the use of QTLs in MAS.

Identification of QTL candidate genes for yield and related traits would help in unravelling genotype to phenotype relationships. In genome wide QTL meta analysis reported by Swamy et al.\(^\text{17} \), PPR genes were associated with 15 out of 23 meta QTLs for yield. PPR genes are known to restore fertility in rice.\(^\text{40} \) In the present study, cytochrome b561 underlies \( gY8.1 \) for yield. Cytochrome b561, a protein family plays an important role in plant growth, development, and prevention of damage to plants from excess light under drought condition.\(^\text{41} \) Recently, the novel MYB-like transcription factor OsMPH1 was identified, where the over expression of OsMPH1 increases plant height and grain yield in rice.\(^\text{42} \) SnRk1 (Sucrose-non fermentation1-related protein kinase1) activity plays multiple essential roles in plant growth and in modulating early seedling development after seed germination and in controlling age-dependent leaf senescence.\(^\text{43} \) MYB-like transcription factor and SnRK1 underlie the stable QTLs for plant height (\( qPH1 \)), biomass (\( qBM1 \)) and total dry matter (\( qTDM1 \)) on chromosome 1 in our study. Lim et al.\(^\text{44} \) identified strong candidate genes underlying \( qTln2 \) for tiller number, which encode receptor-like protein kinase precursor, DUF803 domain containing proteins. A major QTL \( qSSL1b \) for seedling shoot length was delimited to 80.5 kb region and 16 genes were annotated which includes DUF803 domain containing protein, expressed proteins and candidate region for plant height on chromosome 1.\(^\text{45} \) Thus, several candidate genes
including F box protein and DUF domain and zinc finger proteins underlie the major effect QTLs for plant height, yield and biomass.

Among 31 significant lines identified in the BILs, 23 also belonged to the CSSL set. Among these 22 lines for DFF and 4 lines for DM were positively significant and 11 were negatively significant for these two traits. Two lines NK41 and NK70 which were negatively significant for flowering days were positively significant for plant height. Plant height and days to flowering were associated in Swarna/O. nivara introgression lines16,19. In all, 8 lines were positively significant for plant height and 7 of them had a chromosome segment harbouring QTL for plant height at chromosome 1 from O. nivara. Five of the lines positively significant for PH were positively significant for BM also. NK61 showed significantly higher value than Swarna for GY and PDP and significantly lower value for DFF and DM. This line harboured alleles from O. nivara for GY, PW and PDP at chromosome 8 and for GY, BY, HI, PDP at chromosome 11 but Swarna alleles for DFF and is an important line for fine mapping of major QTLs for these traits.

Among the CSSLs, 4 lines were positively significant over Swarna for days to flowering and maturity and harboured Swarna alleles at all the three QTL loci significantly associated with DFF and DM. Conversely, 11 significantly negative lines for flowering days harboured a combination of alleles of O. nivara and Swarna except in case of NK79, NK82 and NK88 where alleles at chromosome 3 for DFF and DM were only from O. nivara. Secondary mapping populations from crosses involving a QTL-CSSL with recurrent parent or among two CSSLs carrying different QTL segments will help in developing near isogenic lines (NILs), fine mapping and studying QTL × QTL interaction16,27,28. The set of lines which show significant phenotypic and corresponding genotypic difference for the particular chromosome can be used to create a secondary mapping population for fine mapping of the specific trait.

Conclusion
A CSSL set of 70 lines of Swarna/ Oryza nivara IRGC 81832 with 94.4% genome coverage of O. nivara was identified. The 23 significantly different CSSLs identified in this study with wide range of variation for 7 yield traits can be further crossed among themselves for trait pyramiding and to study gene interactions in a largely similar genetic background. The chromosomal region between RM206 and RM144 showed a significant major QTL qGY11.1 for GY detected consistently across years and average environment. The same locus was mapped as closely linked to BY, GY and PDP in the derived CSSL population also. This is a high priority candidate region for yield improvement. Similarly ten consistent QTLs qBM1.1, qDFF3.1, qDFF7.1, qDFF12.1, qDM3.1, qDM7.1, qDM12.1, qPH1.2, qNT2.1 and qTDM11.1 for BM, DFF, DM, PH, NT and TDM were observed over the years and across different mapping techniques such as SMA, ICIM, MET and CSL options. CSSLs with marker defined chromosome segments help in identification of QTLs, underlying genes, and superior lines for any trait of interest and in broadening the genetic base of cultivars.

Data availability
All data generated or analysed during this study are included in this published article and its Supplementary information files.

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References
1. OECD-FAO.Agricultural Outlook 2018-2027. https://doi.org/10.1787/19991142(2018).
2. Swamy, B. P. M. & Sarla, N. Yield-enhancing quantitative trait loci (QTLs) from wild species. Biotechnol. Adv. 26, 106–120 (2008).
3. Warnbugu, P. W., Ndjonjodjop, M. N. & Henry, R. J. Role of genomics in promoting the utilization of plant genetic resources in gene banks. Briefings in Functional. Genomics 17, 198–206 (2018).
4. Balakrishnan, D., Surapaneni, M., Mesapogu, S. & Neelamraju, S. Development and use of chromosome segment substitution lines. Rice. 11, 25, https://doi.org/10.1186/s12284-018-0216-3 (2018).
5. Ali, M. L., Sanchez, P. L., Yu, S., Lorieux, M. & Eizenga, G. C. Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from Oryza wild species into cultivated rice (O. sativa L.). Rice. 3, 218–234 (2010).
6. Hao, W., Jin, J., Sun, S.Y., Zhu, M.Z. & Lin, H.X. Construction of chromosome segment substitution lines carrying overlapping chromosome segments of the whole wild rice genome and identification of quantitative trait loci for rice quality. J. of Plant Physiol. and Mol. Biol. 32, 354–62. (in Chinese with an English abstract) (2006).
7. Tian, F. et al. Construction of introgression lines carrying wild rice (Oryza rufipogon Griff) segments in cultivated rice (Oryza sativa L.) background and characterization of introgressed segments associated with yield-related traits. Theor. Appl. Genet. 112, 570–80 (2006).
8. McCouch, S. R. et al. Through the genetic bottleneck: O. rufipogon as a source of trait-enhancing alleles for O. sativa. Euphytica 54, 317–339 (2007).
9. Furuta, T. et al. Development and evaluation of chromosome segment substitution lines (CSSLs) carrying chromosome segments derived from Oryza rufipogon in the genetic background of Oryza sativa L. Breed. Sci. 63, 468–473 (2014).
10. Rangel, P. N., Brondani, R. F. V. & Brondani, C. Agronomic and molecular characterization of introgression lines from the interspecific cross Oryza sativa (BG90-2) × Oryza glumaepatula (RS-16). Genet. Mol. Res. 7, 184–195 (2008).
11. Arbelaez, J. D. et al. Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice, O. meridionalis and O. rufipogon in a common recurrent parent, O. sativa cv Curinga. Mol. Breed. 35, 81 (2015).
12. Jing, L. et al. A heading date QTL, qHD7.2, from wild rice (Oryza rufipogon) delays flowering and shortens panicle length under long-day conditions. Sci. Rep. 8, 2928 (2018).
13. Ujije, K., Toshin, Y., Masahiro, Y. & Ken, I. Genetic factors determining varietal differences in characters affecting yield between two rice (Oryza sativa L.) varieties, Koshihikari and IR64. Genet. Res. Crop Evol. 63, 97–123 (2016).
14. Ishimaru, K. et al. Loss of function of the IAA-glucose hydrolase gene TGW6 increases rice grain weight and increases yield. Nat. Genet. 45, 707–711 (2013).
15. Mulansittil, W. et al. Finding the superior allele of japonica-type for increasing stem lodging resistance in indica rice varieties using chromosome segment substitution lines. Rice. 11, 25, https://doi.org/10.1186/s12284-018-0216-3 (2018).
16. Kaladhari, K., Swamy, B. P. M., Babu, A. P., Reddy, C. S. & Sarla, N. Mapping quantitative trait loci for yield traits in BC2F2 population derived from Swarna x O. nivara cross. *Rice Genet. Newsletter* 24, 34–36 (2008).
17. Swamy, B. P. M., Kaladhari, K., Reshma, M. S., Viraktamath, B. C. & Sarla, N. Molecular mapping of QTLs for yield traits and related traits in Oryza sativa cv Swarna x O. nivara (IRGC81848) backcross population. *Rice Sci.* 18, 178–186 (2011).
18. Swamy, B. P. M., Kaladhari, K., Reddy, A. G., Viraktamath, B. C. & Sarla, N. Mapping and introgression of QTL for yield and related traits in two backcross populations derived from Oryza sativa cv. Swarna and two accessions of O. nivara. *J. Genet.* 93, 643–653 (2014).
19. Gaikwad, K. B. et al. Yield-enhancing heterotic QTL transferred from wild species to cultivated rice Oryza sativa L. *PLoS ONE* 9, e96934 (2014).
20. Malathi, S. et al. Identification of major effect QTLs for agronomic traits and CSSLs in rice from Swarna/ O. nivara derived backcross inbred lines. *Front. Plant. Sci.* 8, 1027 (2017).
21. Haritha, G. et al. Oryza nivara Sharma et Shastry. In: Mondal T., Henry R. (eds) The Wild Oryza Genomes. Compendium of Plant Genomes. Springer, Cham, https://doi.org/10.1007/978-3-319-71997-9_20, 207–238 (2018).
22. Brar D. S. & Khush G. S. Wild relatives of rice: a valuable genetic resource for genomics and breeding research. In: Mondal T., Henry R. (eds) The Wild Oryza Genomes. Compendium of Plant Genomes. Springer, Cham.1-26 pp (2018).
23. Meng, L., Li, H., Zhang, L. & Wang, J. QTL iCIMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal* 3(3), 269–83 (2015).
24. Li, H. H., Ye, G. Y. & Wang, J. A modified algorithm for the improvement of composite interval mapping. *Genetics* 175, 361–374 (2007).
25. Wang, J. CIM. inclusive composite interval mapping of quantitative trait genes. *Acta Agromonica Sinica* 35, 239–245 (2009).
26. Li, H., Ribaut, J.-M., Li, Z. & Wang, J. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. *Theor. Appl. Genet.* 116, 243–260 (2008).
27. Li, H., Heanne, S., Banziger, M., Li, Z. & Wang, J. Statistical properties of QTL linkage mapping in biparental genetic populations. *Heredity* 105, 257–267 (2010).
28. Swamy, B. P. M. et al. QTL analysis for grain quality traits in 2 BC2F2 populations derived from crosses between Oryza sativa cv Swarna and 2 accessions of O. nivara. *J. Heredity* 103, 442–452 (2012).
29. Balkrishnan, D. et al. Genotype x environment interactions of yield traits in backcross introgression lines derived from Oryza sativa cv. Swarna/Oryza nivara. *Front. Plant. Sci.* 7, 1530 (2016).
30. Team, R. D. C. R: A Language and Environment for Statistical Computing. Vienna, Austria (2013).
31. Sarla, N. DRR Dhan40- with yield enhancing QTLs from wild species. *DRR Newsletter* 2 (2014).
32. Paterson, A., Bowers, J. E. & Chapman, B. A. Ancient polyploidization preadinating divergence of the cereals, and its consequences for comparative genomics. *Proc. of Nat. Acad. of Sci. USA* 101, 9903–9908 (2004).
33. Wicknessari, R. et al. Identification and validation of quantitative trait loci for agronomic traits in advanced backcross breeding lines derived from Oryza rufipogon x Oryza sativa cultivar MR219. *Plant Mol. Biol. Rep.* 30, 929–939 (2012).
34. Wang, J. K. et al. QTL mapping of grain length in rice (Oryza sativa L.) using chromosome segment substitution lines. *Genet. Res.* 88, 93–104 (2006).
35. Prasad, G. S. V. et al. Indian rice varieties released around the world. *Curr. Sci.* 80, 1508–1511 (2001).
36. Yang, H. et al. Sequencing consolidates molecular markers with plant breeding practice. *Theor. Appl. Genet.* 128, 779–795 (2015).
37. Kadambari, G. et al. QTL-Seq-based genetic analysis identifies a major genomic region governing dwarfishness in rice (Oryza sativa L.). *Plant Cell Rep.* 4, 677–687 (2018).
38. Wang, J. et al. Identification of QTLs with main, epistatic and QTL environment interaction effects for salt tolerance in rice seedlings under different salinity conditions. *Theor. Appl. Genet.* 125, 807–815 (2012).
39. Xin, D. et al. QTL location and epistatic effect analysis of 100-seed weight using wild soybean (Glycine soja Sieb. &Zucc.) chromosome segment substitution lines. *PloS ONE* 11(3), e0149380 (2016).
40. Bentolila, S., Alfonso, A. A. & Hanson, M. R. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Nat. Acad. Sci. USA* 99, 10887–10892 (2002).
41. Thomson, M. J. et al. Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between O. rufipogon and the O. sativa cultivar Jefferson. *Theor. Appl. Genet.* 107, 479–493 (2003).
42. Lin, Y.-R. et al. Mapping of quantitative trait loci for plant height and heading date in two inter-specific crosses of rice and comparison across Oryza genus. *Bot. Studies* 52, 1–14 (2011).
43. Pinson, S. R. M. et al. Registration of a rice gene mapping population consisting of ‘TeQing’-into-‘Lemon’ (TIL) backcross introgression lines. *J. of Plant. Registr.* 6, 128–135 (2012).
44. Yano, M. & Sasaki, T. Genetic and molecular dissection of quantitative traits in rice. *Genetics* 172, 1287–1300 (2006).
45. You, A. et al. Identification of quantitative trait loci across recombinant inbred lines and testcross populations for traits of agronomic importance in rice. *Genetics* 172, 1287–1300 (2006).
46. Courtois, B. et al. Mapping QTLs associated with drought avoidance in upland rice. *Mol. Breed.* 6, 55–66 (2000).
47. Li, Z. K. et al. QTL × environment interactions in rice. I. Heading date and plant height. *Theor Appl Genet* 108, 141–153 (2003).
48. Tong, H. H. et al. Identification of related QTLs at late developmental stage in rice (Oryza sativa L.) under two nitrogen levels. *Acta Genet. Sin.* 33, 458–467 (2006).
49. Zhang, J., Liu, X., Tong, H., Lu, Y. & Li, J. Association mapping for important agronomic traits in core collection of rice (Oryza sativa L.) with SSR markers. *PloS ONE* 9(10), e111508 (2014).
50. Wei, X. et al. Breeding strategies for optimum heading date using genotypic information in rice. *Mol. Breed.* 25, 287–298 (2010).
51. Marathi, B. et al. QTL analysis of novel genomic regions associated with yield and yield related traits in new plant type based recombinant inbred lines of rice (Oryza sativa L.). *BMC Plant. Biol.* 12, 137 (2012).
52. Lim, J. H., Yang, H. J., Jung, K. H., Yoo, S. C. & Paek, N. C. Quantitative trait locus mapping and candidate gene analysis for plant architecture traits using whole genome re-sequencing in rice. *Mol. Cell. Biol.* 37, 149–160 (2014).
53. Noenphaib, A. et al. QTL mapping for leaf and neck blast resistance in Khoa Dawk Mali IL105 and Jao Hom Nm recombinant inbred lines. *Science Asia* 32, 133–142 (2006).
54. Fjelldstrom, R., Mc-Clung, A. M. & Shank, A. R. SSR markers closely linked to the Pi-2 locus are useful for selection of blast resistance in an array of rice germplasm. *Mol. Breed.* 17(2), 149–157 (2006).
55. Wu, Y. Y. et al. Fine mapping and identification of blast resistance gene Pi-Ihk1 in a broad-spectrum resistant japonica rice land race. *Plant Pathol.* 103, 1162–1168 (2013).
56. Yue, B. et al. Genetic analysis for drought resistance of rice at reproductive stage in field with different types of soil. *Theor. Appl. Genet.* 111, 1127–1136 (2005).
57. Xue, J. J., Wu, S. H., Zhang, H. Y., Xu, P. Z. & Wu, X. J. Genetic analysis and gene mapping of multitalter and dwarf mutant d63 in rice. *Rice Sci.* 13, 179–184, https://doi.org/10.1666/S1672-6308(13)00130-4 (2013).
58. Wang, Y. et al. Construction of chromosome segment substitution lines of Chinese cabbage (Brassica rapa L. ssp. pekinensis) in the background of RcBr (B. rapa L. ssp. dichotoma) and characterization of segments representing the bolting trait. *Mol. Breed.* 38, 35 (2018).
60. Dufey, I. et al. Novel QTLs in an interspecific backcross Oryza sativa x Oryza glaberrima for resistance to iron toxicity in rice. *Euphytica* **204**, 609–625 (2015).

61. Kalladan, R. et al. Identification of quantitative trait loci contributing to yield and seed quality parameters under terminal drought in barley advanced backcross lines. *Mol. Breeding* **32**, 71–90 (2013).

62. Xing, Y. Z. et al. Characterization of the major effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet* **105**, 248–257 (2002).

63. Hosseini, M. et al. Detection of QTLs with main, epistatic and QTL × environment interaction effects for rice grain appearance quality traits using two populations of backcross inbred lines (BILs). *Field Crop Res* **135**, 97–106 (2012).

64. Qi, Z. et al. Identification of quantitative trait loci (QTLs) for seed protein concentration in soybean and analysis for additive effects and epistatic effects of QTLs under multiple environments. *Plant Breed* **133**, 499–507 (2014).

65. Li, S., Wang, J. & Zhang, L. Inclusive composite interval mapping of QTL by environment interactions in biparental populations. *Ecol Evol* **10(7)**, e0132414 (2015).

66. Mackay, T. F. C. The genetic architecture of quantitative traits. *Annu. Rev. Genet.* **35**, 303–339 (2001).

67. Yamamoto, T., Lin, H. X., Sasaki, T. & Yano, M. Identification of heading date quantitative trait locus Hd6 and characterization of its epistatic interactions with Hd2 in rice advanced backcross progeny. *Genetics** **154**, 885–891 (2000).

68. Lin, H. X., Yamamoto, T., Sasaki, T. & Yano, M. Characterization and detection of epistatic interactions of three QTLs, Hd1, Hd2 and Hdl, controlling heading date in rice using nearly isogenic lines. *Theor Appl Genet* **101**, 1021–1028 (2000).

69. Yang, Z. et al. Analysis of epistasis among QTLs on heading date based on single segment substitution lines in rice. *Sci. Rep.* **8**, 3059 (2018).

70. Subudhi, P. K. et al. Genetic interaction involving photoperiod-responsive Hd1 promotes early flowering under long-day conditions in rice. *Sci. Rep.* **8**, 2081 (2018).

71. Nemoto, Y., Nonose, Y., Yano, M. & Iwasa, T. Hd1A CONSTANS ortholog in rice, functions as an Ehd1 repressor through interaction with monocot-specific CCT-domain protein GhD7. *Plant J.* **86**, 221–233 (2016).

72. Zhang, Z. et al. Alternative functions of Hd1 in repressing or promoting heading are determined by GhD7 status under long-day conditions. *Sci. Rep.* **7**, 5388 (2017).

73. Yadav, S. et al. Epistatic interactions of major effect drought QTLs with genetic background loci determine grain yield of rice under drought stress. *Sci. Rep.* **9**, 2616 (2019).

74. Zhao, X., Zhou, L., Ponce, K. & Ye, G. The usefulness of known genes/QTLs for grain quality traits in an indica population of diverse breeding lines tested using association analysis. *Rice* **8**, 29 (2015).

75. Shang, L. G. et al. Partial dominance, over dominance, epistasis and QTL by environment interactions contribute to the heterosis in two upland cotton hybrids. *G3 Genomes Genet.* **6**, 499–507 (2016).

76. Zhao, X. Q. et al. Molecular mapping of two semidwarf genes in an indica rice variety Aitaijin 3 (Oryza sativa L.). *Front. Biol. China* **1**, 93–98 (2006).

77. Qin, M., Zhao, X., Ru, J., Zhang, G. & Ye, G. Bigenic epistasis between QTLs for heading date in rice analyzed using single segment substitution lines. *Field Crops Res.* **178**, 16–25 (2015).

78. Zhang, L., Li, H. & Wang, J. The statistical power of inclusive composite interval mapping in detecting digenic epistasis showing common F2 segregation ratios. *J. Int. Plant Biol.* **54**, 270–279 (2012).

79. Wang, X. et al. QTL epistatic analysis for yield components with single-segment substitution lines in rice. *Plant Breed.** https://doi.org/10.1111/phb.12578** (2018).

80. Stange, M. et al. High-density linkage mapping of yield components and epistatic interactions in maize with doubled haploid lines from four crosses. *Mol. Breeding* **32**, 533–546 (2013).

81. Vikram, P. et al. qDTY(1:1), a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet* **12**, 89(2011).

82. Eizenga, G. C., Prasad, B., Jackson, A. K. & Jia, M. H. Identification of rice sheath blight and blast quantitative trait loci in two different O. sativa x O. nivara advanced backcross populations. *Mol. Breeding* **31**, 889–907 (2013).

83. Li, X. et al. Unraveling the complex trait of harvest index with association mapping in rice (Oryza sativa L.). *PLoS ONE* **7**(1), e29350 (2012).

84. Wickneswari, R. & Bhuiyan, M. A. R. Exploring wild accessions for development of high yielding new rice genotypes. *Malaysian Appl. Biol.* **43**, 89–95 (2014).

85. Qi, Z. et al. Identification of major QTLs and epistatic interactions of seed protein concentration in soybean under multiple environments based on a high-density map. *Mol. Breeding* **36**, 55 (2016).

86. Tanksley, S. D. & Nelson, J. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor Appl Genet* **92**, 191–203 (1996).

87. Furuta, T. et al. Convergent loss of awn in two cultivated rice species Oryza sativa/ Oryza glaberrima is caused by mutations in different loci. *Genes Genomes Genet.* **5**, 2267–2274 (2015).

88. Ma, X. et al. Genomic structure analysis of a set of Oryza nivara introgression lines and identification of yield associated QTLs using whole genome resequencing. *Sci. Rep.* **6**, 274–275 (2016).

89. Yang, H. et al. Chromosome segment detection for seed size and shape traits using an improved population of wild soybean chromosome segment substitution lines. *Physiol. Mol. Biol. Plants* **23**, 877–887 (2019).

90. Takai, T. et al. Genetic mechanisms underlying yield potential in the rice high-yielding cultivar Takanari, based on reciprocal chromosome segment substitution lines. *BMC Plant Biol.* **14**, 295–306 (2014).

91. Ou, X. et al. Examining two sets of introgression lines reveals background-independent and stably expressed QTL that improve grain appearance quality in rice (Oryza sativa L.). *Theor Appl. Genet.* **130**, 951–967 (2017).

92. Singh, R. et al. to variety: harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. *Plant Sci.** **242**, 278–287, pmid:26566845 (2016).

93. Opyro, S. O. & Moriyama, E. N. Mining Cytochrome b561 proteins from plant genomes. *Int. J. Bioinform. Res. Appl.* **6**(2), 209–221 (2010).

94. Cho, Y. H., Kim, G. D. & Yoo, S. D. Giant chloroplast development in ethylene response1-1 is caused by a second mutation in accumulation and replication of chloroplast1 in Arabidopsis. *Mol. Cells* **33**, 99–103 (2012).

95. Swamy, B. P. M. Genome wide mapping of quantitative trait loci (QTLs) for yield and grain quality traits in O. sativa cv Swarna x O. nivara backcross population. Ph.D Thesis, Osmania University, Hyderabad, India (2009).

96. Kanbe, T. et al. Identification of QTLs toward improvement of plant type in rice (Oryza sativa L) using Koshibhikari / Kasalath chromosome substitution lines and backcross progeny F2 population. *Plant Prod. Sci.* **11**, 447–456 (2008).

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**Author contributions**
S.N. and D.B. conceptualized, designed and supervised experiments. M.S., S.M., V.Y., K.A. and K.B. did phenotyping and genotyping. D.B. analysed the data and wrote the manuscript with contributions from SN, all authors read and contributed to the revision of manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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Correspondence and requests for materials should be addressed to S.N.

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