RESEARCH ARTICLE

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*

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Abstract

Advanced backcross QTL (AB-QTL) analysis was carried out in two *Oryza nivara*-derived BC_2F_2 populations. For nine traits, we identified 28 QTL in population 1 and 26 QTL in population 2. The two most significant yield-enhancing QTL, *yldp9.1* and *yldp2.1* showed an additive effect of 16 and 7 g per plant in population 1, while *yld2.1* and *yld11.1* showed an additive effect of 11 and 10 g per plant in population 2. At least one *O. nivara*-derived QTL with a phenotypic variance of >15% was detected for seven traits in population 1 and three traits in population 2. The *O. nivara*-derived QTL *ph1.1*, *nt12.1*, *nsp1.1*, *nfg1.1*, *bm11.1, yld2.1* and *yld11.1* were conserved at the same chromosomal locations in both populations. Two major QTL clusters were detected at the marker intervals RM488–RM431 and RM6–RM535 on chromosomes 1 and 2, respectively. The colocation of *O. nivara*-derived yield QTL with yield meta-QTL on chromosomes 1, 2 and 9 indicates their accuracy and consistency. The major-effect QTL reported in this study are useful for marker-assisted breeding and are also suitable for further fine mapping and candidate gene identification.

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Introduction

Rice is one of the most important and widely adapted cereal crops in the world. Its wild relatives are naturally grown in diverse environmental conditions and they harbour a wide range of allelic variations (Tanksley and Nelson 1996; Yano 2001; Quan *et al.* 2012). Further, the *de novo* genetic variations that arise in the progenies of crosses between wild species and elite varieties add to the available genetic diversity (Dong *et al.* 2006). In the past, wild species were largely used to improve disease and pest resistance in rice, but in the last 15 years they have been increasingly used as a source of yield-enhancing QTL (Tanksley and McCouch 1997; Gur and Zamir 2004).

Yield is a complex trait and it needs to be continuously improved. The identification of novel yield-enhancing QTL from wild species using the advanced backcross QTL method is a popular strategy for mapping and simultaneously making available introgression lines that can be evaluated for the presence of QTL and for selecting high-yielding lines (Tanksley and Nelson 1996). Using this strategy, numerous yield-enhancing QTL have been mapped from wild species (Swamy and Sarla 2008).

Wild progenitor species *Oryza rufipogon* and *O. nivara* are easily crossable with cultivated rice and can be readily exploited in breeding programmes without a need for embryo rescue procedures (Lu *et al.* 1998; Niroula *et al.* 2009). *O. rufipogon*, a perennial and diverse wild progenitor of Asian cultivated rice, was most frequently used in yield QTL mapping studies and was found to contribute more favourable alleles than other AA genome wild species. In addition, the *O. rufipogon*-derived QTL are highly congruent and detected on the same chromosomal locations, and this clearly shows the consistency and accuracy of QTL detection (Swamy and Sarla 2011). The other wild progenitor species, *O. nivara*, is also a potential source of yield and yield-related QTL, but still largely needs to be exploited by both conventional and molecular breeding approaches.

O. nivara (Sharma et Shastry) is an annual and a close wild progenitor of Asian cultivated rice. Its accessions have

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abundant genetic diversity (Joshi *et al.* 2000; Sarla *et al.* 2003; Juneja *et al.* 2006) and contributed resistance genes for grassy stunt virus, bacterial leaf blight, blast, brown plant hopper and drought avoidance (Khush 2000; Brar and Khush 2004; Thanh *et al.* 2006; Ali *et al.* 2010), *O. nivara* is also a major source of cytoplasmic male sterility (Hoan *et al.* 1998). Because of their out crossing nature, *O. nivara* accessions are used extensively to study the flow of transgenes (Chen *et al.* 2004). Even though *O. nivara* has contributed significantly to pest and disease resistance in rice, it has rarely been used in yield improvement programmes.

A study was undertaken to exploit the naturally occurring alleles from *O. nivara* by AB-QTL mapping. Two accessions of *O. nivara*, IRGC81848 (Uttar Pradesh) and IRGC81832 (Bihar) from India were used for developing mapping populations in the genetic background of the popular cultivated rice mega variety, Swarna (MTU 7029). These two accessions showed moderate molecular genetic diversity from Swarna and were genetically distinct from *O. nivara* accessions collected from 16 other states (Sarla *et al.* 2003).

The main objectives of this study were to map QTL for yield and yield-related traits, to know the proportion of trait-enhancing alleles that can be obtained from two *O. nivara* accessions and their congruence in the two populations, to compare the colocation of yield QTL with meta-QTL reported for yield, and to identify high-yielding BC_2F_5 Swarna introgression lines.

Materials and methods

Development of mapping populations

The parental material for the experiment included two accessions of *O. nivara* from Uttar Pradesh (IRGC81848) and Bihar (IRGC81832), a popular rainfed lowland cultivated rice variety, Swarna. An advanced backcross strategy as described by Thomson *et al.* (2003) was followed to develop the mapping populations. Crosses were made using Swarna as a female parent and *O. nivara* (IRGC81848 and IRGC81832) as a male parent. The BC_2F_2 populations derived from Swarna × IRGC81848 is designated as population 1 and that derived from Swarna × IRGC81832 is designated as population 2.

Phenotypic evaluation of mapping populations

Two mapping populations consisting of 227 and 245 BC_2F_2 families were grown in two replications in an augmented block design at the Directorate of Rice Research (DRR), Hyderabad, in southern-central India during the wet season (WS) of 2005 and 2006, respectively. Each backcross family and the control (Swarna) consisted of 30 plants planted in three rows of 10 plants each, adopting a uniform spacing of 20 cm between rows and 15 cm between plants. Thirty high-yielding BC_2F_5 lines were evaluated in a 10 m^2 area in two replicates along with the control Swarna during WS2008 and WS2009 at DRR. Standard agronomic practices and need-based plant protection measures were adopted uniformly to raise the crop. Five plants from the middle row were used for taking the following data: days to maturity (DTM): duration in days from seeding to the time when more than 80% of the grains on the panicles were fully ripened; plant height (PH): height from the soil surface to the tip of the primary panicle was measured at the time of harvest; number of tillers per plant (NT): average number of tillers from five plants at the time of harvest; number of productive tillers per plant (NP): average number of panicle-bearing tillers at the time of harvest; number of spikelets per plant (SNP): average number of spikelets from five plants; number of filled grains per panicle (GP): average number of grains from five plants; 1000 grain weight (GW): weight of 1000 randomly selected dried grains; vegetative biomass (BM): average weight of five well-dried plants and grain yield per plant (YDLP): average weight of the dried (14% moisture) and cleaned grains from five plants.

Genotyping of the mapping populations

A set of 250 microsatellite markers uniformly distributed on all 12 chromosomes were screened for polymorphism between Swarna and *O. nivara* accessions, IRGC81848 and IRGC81832. A total of 100 polymorphic microsatellite markers were used to analyse the segregation in population 1, and 75 markers were used in population 2. DNA was extracted from the leaves of two-month-old seedlings using the protocol of Zheng *et al.* (1995). PCR for simple sequence repeat (SSR) primers was performed with 15 μL of final volume containing 45 ng of genomic DNA, 10× buffer, 0.125 mM final concentration of each dNTP, 0.2 μM each of forward and reverse primers, 2% formamide and 1 U of biogene *Taq* DNA polymerase. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, followed by a final extension of 72°C for 5 min. The amplified products were checked for polymorphism or marker segregation on an agarose gel (3%) and recorded for segregating bands. BC_2F_5 lines were genotyped using SSR markers linked to *O. nivara*-derived QTL for yield and related traits.

Linkage map construction and QTL analysis

A linkage map was constructed from the genotypic data of 100 markers in population 1, and 75 markers in population 2 using Join Map 3.0 (Van Ooijen and Voorrips 2001). Linkage groups were assigned to the respective chromosomes based on the rice linkage maps developed by Temnykh *et al.* (2001). Both the maps were integrated to develop a consensus map using Biomercator ver. 2.0 (Arcade *et al.* 2004). Significant thresholds were fixed based on permutation tests at an experiment-wise significance level of 0.05 (Kosambi

1944). Based on 10,000 permutations for each trait, an average LOD value of 3.0 was used for declaring significant QTL. The QTL were identified separately for the two populations by interval mapping (IM) or composite interval mapping (CIM) using QTL Cartographer ver. 2.5 (Wang *et al.* 2011).

Results

Trait analysis

The frequency distribution for yield and yield-related traits is presented in figure 1. The range and number of families showing significant improvement over Swarna are presented in table 1. Transgressive segregants for Swarna were observed for seven traits in both the populations. More than 80 families (35%) showed at least 15% improvement over Swarna for plant height, number of tillers per plant, number of productive tillers per plant, and 1000-grain weight in population 1 and for number of tillers per plant, 1000-grain weight and vegetative biomass in population 2. All the traits followed a near normal distribution except plant height and number of productive tillers per plant in both the populations (figure 1). Correlations between trait pairs were estimated and are presented in table 2. Eighteen correlations in population 1 and 22 correlations in population 2 were found to be significant, and these were observed between days to maturity and number of tillers per plant, number of productive tillers per plant, number of spikelets per plant, and yield per plant; plant height and 1000-grain weight and vegetative biomass; between number of tillers per plant and number of productive tillers per plant; and between number of spikelets per plant and number of filled grains per plant.

Marker analysis

A parental polymorphism survey using 250 microsatellite or SSR markers revealed that 120 (48%) were polymorphic between IRGC81848 and Swarna, and 108 (44%) were

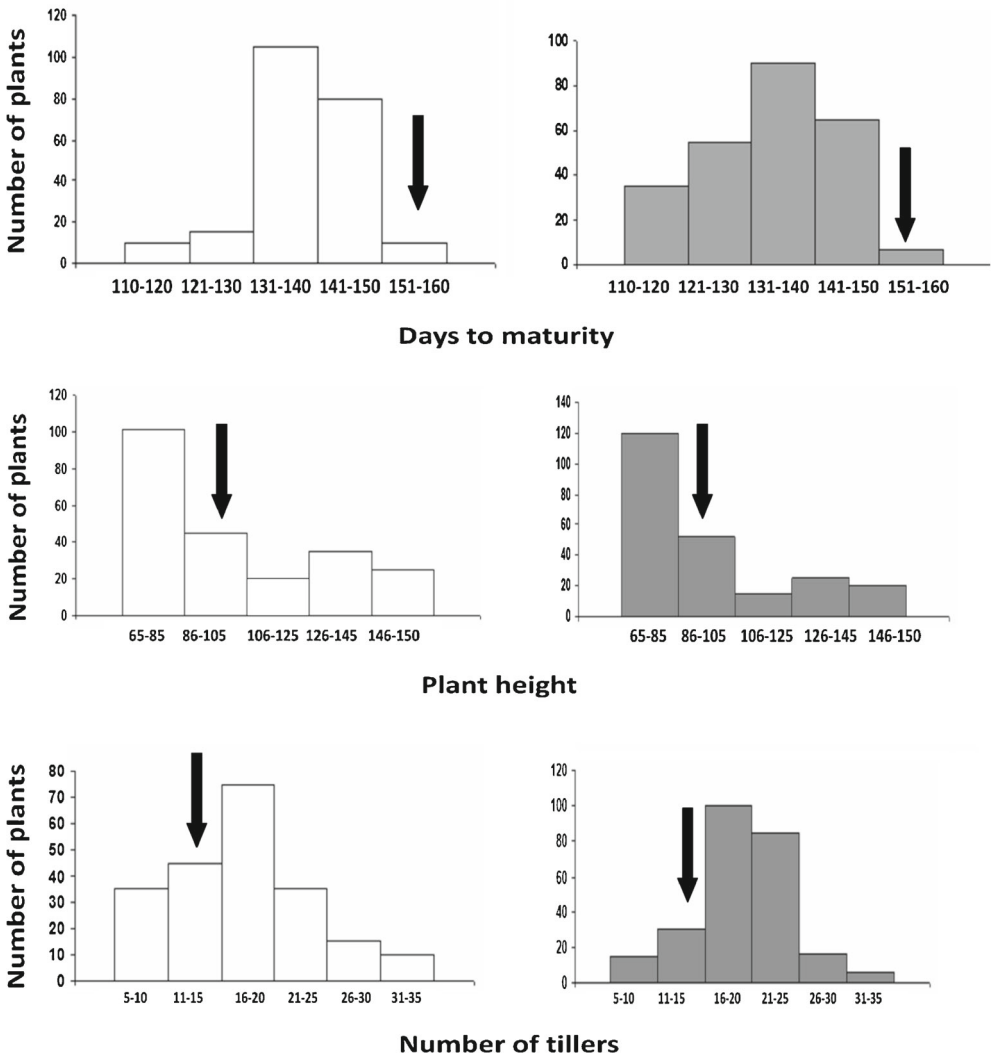

Figure 1 (*continues*)

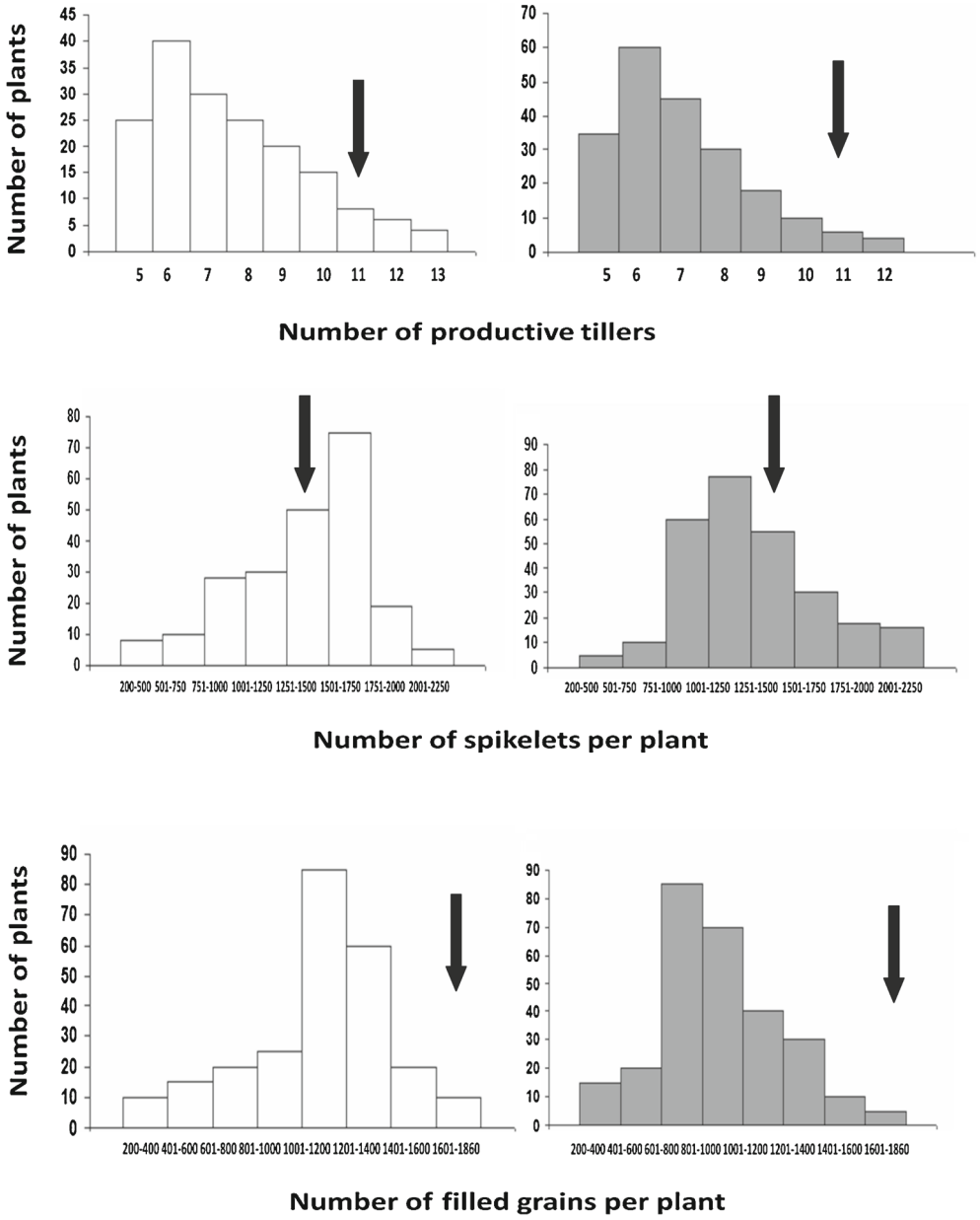

Figure 1 (*contd*)

polymorphic between IRGC81832 and Swarna. One hundred polymorphic SSR markers were used for genotyping 227 BC_2F_2 families in population 1 and 75 SSR markers were used for genotyping 245 families in population 2. The overall mean introgression of *O. nivara* alleles ranged from 2.6 to 38%, with an overall mean introgression of 16.6% in both the populations. Introgression percentage varied from chromosome to chromosome and between the families in both the populations.

QTL analysis

In all, 28 QTL were identified in population 1 and 26 QTL in population 2 (table 3; figure 2). Of these, 22 (78%) and 18 (69%) QTL were derived from *O. nivara* and had a trait-increasing effect. Details on only the significant major QTL identified for each trait are given below.

Days to maturity (DTM): Three QTL were detected in population 1 and two QTL in population 2. All had an increasing effect from Swarna.

Plant height (PH): Three QTL were detected in each population. All were derived from *O. nivara*. The phenotypic variance varied from 5 to 63% in population 1 and from 7 to 46% in population 2. Each of these QTL contributed more than 5% to the total phenotypic variance (PV) and the additive effect varied from 5 to 39 cm.

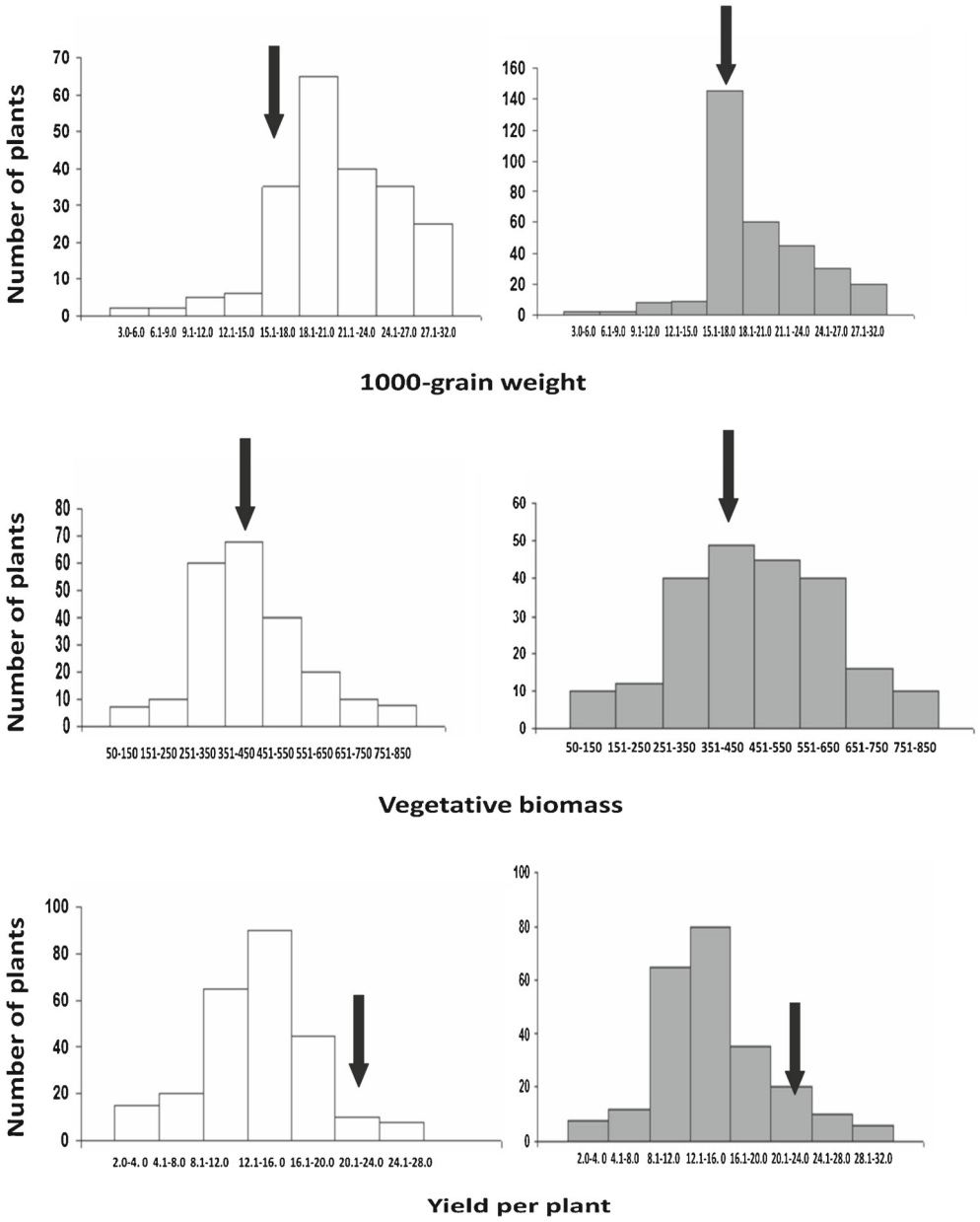

Figure 1. Frequency distribution of nine yield and related traits in two *O. nivara* derived populations. Bars in white indicates population 1; bars in black indicates population 2; arrow indicates Swarna values.

Number of tillers per plant (NT): Two QTL (*nt5.1* and *nt12.1*) were detected in population 1 and four QTL (*nt2.1*, *nt3.1*, *nt11.1* and *nt12.1*) in population 2. All the QTL except *nt2.1* were derived from *O. nivara*. These QTL contributions varied from 6 to 37% to the total PV and also showed a high additive effect.

Number of productive tillers per plant (NPT): Three QTL were detected in each population; all were derived from *O. nivara* except *npt12.2*. Two QTL (*npt6.1* and *npt9.1*) from *O. nivara* contributed 36 and 30%, respectively, to the total PV. These also had a high additive effect on the trait (18 and 30).

Number of spikelets per plant (NSP): Three QTL were detected in each population; all were derived from *O. nivara* except *nsp12.1*. QTL *nsp2.1* was detected at high LOD (6.0). This explained the high PV (15%) with an additive effect of 367 spikelets per plant.

Number of filled grains per plant (NFG): Three QTL were detected in population 1 and two QTL in population 2. All the QTL in population 1 were derived from *O. nivara*. The *nfg2.1* from *O. nivara* was detected at high LOD (6.0) and had PV of 15% with an additive effect of 333 grains per plant. In population 2, *nfg1.1* was derived from *O. nivara* and

Table 1. Mean and range of yield and yield-related traits in two *O. nivara* derived populations.

Trait	Swarna (mean±SE)	*O. nivara* (IRGC81848) (mean±SE)	*O. nivara* (IRGC81832) (mean±SE)	Range P1	Range P2	Number of families showing >15% increase over Swarna P1	Number of families showing >15% increase over Swarna P2
Days to maturity	154±2.8	162±4.3	157±5.2	111–154	115–152	0	0
Plant height (cm)	86±3.2	122±4.2	124±5.3	65–147	64–147	83	70
Number of tillers	12±2.0	62±8.0	58±6.0	5–35	5–32	197	213
Number of productive tillers per plant	11±1.6	52±6.5	48±5.0	5–13	7–12	192	68
Number of spikelet per plant	1666±53	2458±126	2374±183	279–2148	256–2054	58	16
Number of filled grains per plant	1279±38	1538±135	1478±152	201–1857	212–1758	67	47
1000-grain weight (g)	18±0.6	20±1.5	22±1.0	14–32	14–31	202	103
Vegetative biomass (g)	449±23.0	–	–	177–797	50–843	48	126
Yield per plant (g)	21±1.4	–	–	14–31	12–30	15	23

–, Data not available; P1, population 1; P2, population 2.

had a PV of 10% with an additive effect of 274 grains per plant.

Thousand-grain weight (GW): Three QTL were detected for 1000-grain weight in each population. In population 1, all the QTL were derived from Swarna. In population 2, two QTL were derived from *O. nivara*, *gw2.1* contributed 9% to the total PV and added 1 g to grain weight.

Vegetative biomass (BM): Four QTL were detected in population 1, and all were derived from *O. nivara*. Two QTL (*bm2.1* and *bm9.1*) contributed more than 10% to the total PV and also had a high additive effect on the trait (169 and 121 g). Two QTL (*bm5.1* and *bm11.1*) were detected in population 2; both were derived from *O. nivara* and had a PV of 5 and 10%, and an additive effect of 26 and 32 g, respectively.

Yield per plant (YLDP): In population 1, four QTL were detected and all were derived from *O. nivara*. Three QTL (*yldp2.1*, *yldp9.1* and *yldp11.1*), each influenced the trait with more than 10% PV and the additive effects of these QTL were 7.2, 16.6 and 4.1 g, respectively. In population 2, four QTL were detected; all were derived from *O. nivara*. Three QTL (*yldp2.1*, *yldp8.1* and *yldp11.1*) each contributed more than 10% to the total PV and added 6, 12 and 8 g to the yield per plant.

Discussion

Even though wild progenitor species are phenotypically inferior, they harbour many superior alleles which have been left behind during domestication and are often masked by the overall poor genetic background. These superior alleles upon

Table 2. Correlation coefficients for yield and related traits in two *O. nivara* derived populations.

Trait	DTM	PH	NT	NPT	NSP	NFG	GW	BM
PH	−0.065 (−0.231**)							
NT	0.132* (0.252**)	−0.079 (0.264**)						
NPT	0.127* (0.246**)	−0.055 (−0.205*)	0.948** (0.896**)					
NSP	0.147* (0.212**)	0.394** (−0.173**)	0.027 (0.643**)	0.055 (0.725**)				
NFG	0.148* (0.193)	0.394** (−0.105)	0.030 (0.601**)	0.060 (0.694**)	0.998** (0.960**)			
GW	0.109 (−0.221**)	0.180* (0.413**)	−0.035 (−0.115)	−0.042 (−0.065)	0.106 (−0.025)	0.103 (−0.013)		
BM	0.148* (−0.055)	0.453** (0.205**)	0.058 (−0.104)	0.084 (−0.096)	0.953** (−0.024)	0.952** (−0.012)	0.087 (−0.036)	
YDLP	0.161* (0.136*)	0.405** (−0.043)	0.066 (0.305**)	0.083 (0.356**)	0.869** (0.501**)	0.855** (0.513**)	0.096 (0.035)	0.897** (0.205**)

DTM, days to maturity; PH, plant height; NT, number of tillers per plant; NPT, number of productive tillers per plant; NSP, number of spikelets per plant; NFG, number of filled grains per plant; BM, vegetative biomass; GW, 1000-grain weight; YLDP, yield per plant.
Significance levels: $^{*}P < 0.05, 0.126$; $^{**}P < 0.01, 0.166$. Values for population 2 are in parentheses.

Table 3. QTLs identified for yield and yield components traits in two *O. nivara* derived populations.

Trait	Chromosome	Marker interval	Allelic effect	IM			CIM			Population
				LOD	R^2	Additive	LOD	R^2	Additive	
Days to maturity										
dtm1.1	1	RM1–RM490	Swarna	3	4	1.2				P2
dtm2.1	2	RM174–RM243	Swarna	2.9	6	1.1				P2
dtm2.2	2	RM3874–RM106	Swarna	12.8	14	3.8				P1
dtm3.1	3	RM22–RM517	Swarna	3	8	2.8				P1
dtm4.1	4	RM185–RM241	Swarna	16.4	11	3.5	16.5	10	2.4	P1
Plant height										
ph1.1	1	RM488–RM431	*O. nivara*	30	63	−38.9	29.2	56.3	−23.3	P1
ph1.1	1	RM488–RM431	*O. nivara*	6	46	−29.5	31	7	−35.2	P2
ph2.1	2	RM166–RM535	*O. nivara*	16.4	13	−13.1	9.9	12.3	−10.2	P1
ph4.1	4	RM551–RM261	*O. nivara*	3	15	−12.9				P2
ph5.1	5	RM249–RM26	Swarna				6.2	9	5	P2
ph6.1	6	RM314–RM3	*O. nivara*	6.8	5	−8				P1
Number of tillers per plant										
nt2.1	2	RM475–RM263	Swarna	2.7	6	2.4				P2
nt3.1	3	RM55–RM520	*O. nivara*	7.4	37	−10.4	2.7	20	−1.6	P2
nt5.1	5	RM13–RM574	*O. nivara*	3.2	7	−3	3	8	−4.9	P1
nt11.1	11	RM202–RM209	*O. nivara*	3.6	8	−4.6				P2
nt12.1	12	RM415–RM19	*O. nivara*	3	14	−2.5	4.3	19	−6.9	P1
nt12.1	12	RM415–RM19	*O. nivara*	5	12	−1.7	3.2	10	−1.5	P2
Number of productive tillers per plant										
npt1.1	1	RM488–RM128	*O. nivara*	4	13	−2.7	4.7	9	−0.9	P2
npt6.1	6	RM30–RM439	*O. nivara*				16	36	−17.9	P1
npt9.1	9	RM105–RM566	*O. nivara*				12.1	30	−29.9	P1
npt11.1	11	RM206–RM254	*O. nivara*	3.7	6	−6.9				P2
npt12.1	12	RM415–RM19	*O. nivara*	3	6	−2.6				P1
npt12.2	12	RM247–RM519	Swarna	6	12	1.3				P2
Number of spikelets per plant										
nsp1.1	1	RM488–RM431	*O. nivara*				3	5	−160.3	P1
nsp1.1	1	RM488–RM431	*O. nivara*	3.5	10	−274.9	3.1	6	−190.3	P2
nsp2.1	2	RM250–RM166	*O. nivara*	5.9	15	−366.9	6.2	9	−354	P1
nsp3.1	3	RM517–RM7	*O. nivara*	3.3	5	−82.7				P2
nsp9.1	9	RM257–RM288	*O. nivara*	3	6	−34.5				P1
nsp12.1	12	RM341–RM519	Swarna	3	5	33.7				P2
Number of filled grains per plant										
nfg1.1	1	RM488–RM431	*O. nivara*				3	5	−155	P1
nfg1.1	1	RM488–RM431	*O. nivara*	3.5	10	−274.9	3.1	6	−190.3	P2
nfg2.1	2	RM250–RM166	*O. nivara*	5.7	16	−333.3	6	11	−395.6	P1
nfg8.1	8	RM38–RM223	Swarna	3	9	−156				P2
nfg12.1	12	RM341–RM519	*O. nivara*	3	5	−153.7				P1
Thousand-grain weight										
gw1.1	1	RM1–RM490	*O. nivara*	2.6	5	−0.8				P2
gw2.1	2	RM174–RM243	*O. nivara*	3.1	9	−1.1				P1
gw2.2	2	RM324–RM262	Swarna	3	7	3				P2
gw3.1	3	RM22–RM156	Swarna	4.3	12	3.9				P1
gw4.1	4	RM551–RM261	*O. nivara*	2.6	10	−1.9				P2
gw4.2	4	RM348–RM567	Swarna	5.8	13	4.2	5.3	10	3.9	P1
Vegetative biomass										
bm1.1	1	RM9–RM5	*O. nivara*	3	8	−8.1				P1
bm2.1	2	RM6–RM250	*O. nivara*	13.4	25	−168.7				P1
bm5.1	5	RM153–RM413	*O. nivara*	2.6	5	−26.1				P2
bm9.1	9	RM215–RM189	*O. nivara*	3	12	−120.7				P1
bm11.1	11	RM202–RM21	*O. nivara*	2.6	10	−32.8	2.8	12	−41	P1
bm11.1	11	RM209–RM21	*O. nivara*	3.2	6	−78				P2

Table 3 (*contd*)

Trait	Chromosome	Marker interval	Allelic effect	IM			CIM			Population
				LOD	R^2	Additive	LOD	R^2	Additive	
Yield per plant										
yldp2.1	2	RM263–RM535	*O. nivara*	4.1	15	−6.2	2.9	16	−5.3	P2
yldp2.1	2	RM250–RM535	*O. nivara*	8.9	21	−6.2	10.2	20	−7.2	P1
yldp3.1	3	RM55–RM520	*O. nivara*	8.5	9	−3.2	6.1	6	−2.5	P2
yldp8.1	8	RM38–RM223	*O. nivara*	8.8	14	−4.9	5.4	12	−5.2	P2
yldp9.1	9	RM434–RM257	*O. nivara*	15.4	12	−12	12.8	17	−16.6	P1
yldp11.1	11	RM209–RM206	*O. nivara*	3.8	10	−4.1				P1
yldp11.1	11	RM21–RM206	*O. nivara*	5.6	11	−7.4	5.1	10	−8.4	P2
yldp12.1	12	RM341–RM519	*O. nivara*	8.2	6	−3.2				P1

P1, population 1; P2, population 2; IM, interval mapping; CIM, composite interval mapping.

transferring to elite genetic background can further enhance the yield potential of elite rice varieties (Wang *et al.* 2005). In several earlier studies using wild progenitor species as donor parents, superior and favourable alleles have been identified (Xiao *et al.* 1998; Thomson *et al.* 2003; Tian *et al.* 2005; Rahman *et al.* 2008). In this study, in both the *O. nivara* derived populations, transgressive segregants were observed with more than 15% improvement over Swarna. These transgressive segregants are important evidence of the favourable effects of introgressions from wild species.

In general, about half of the QTL mapped from wild species are reported to be favourable (Xiao *et al.* 1998; Septiningsih *et al.* 2003; Thomson *et al.* 2003; Marri *et al.* 2005; Yoon *et al.* 2006; McCouch *et al.* 2007; Swamy and Sarla 2008). The *O. nivara* accessions also contributed trait-enhancing alleles at 78% of the loci in population 1 and at 69% of the loci in population 2 in Swarna genetic background. The results from a parental polymorphism survey also support this hypothesis. *O. nivara* accessions showed 15% less polymorphism with *O. sativa* in comparison with the polymorphism of *O. rufipogon* with *O. sativa* (McCouch *et al.* 2007). The correlations among the traits in both populations were similar, along with the direction of the effect of QTL. Most of the correlated traits were colocated and their QTL were found on the same chromosomal locations, indicating their conservation in the two *O. nivara* accessions.

Several *O. nivara*-derived QTL with a major effect on yield and related traits were identified in both the populations. The major effect yield QTL *yldp9.1* was also found to have pleiotropic effect on several traits. In another *O. rufipogon*-derived population, this chromosomal region has been fine mapped to a 37.4 kb length in which seven traits (plant height, heading date, panicle length, spikelets per panicle, spikelet density, grains per panicle and grain weight) were colocated. Hence, this is a priority locus for map-based cloning, candidate gene identification and allele mining to discover novel, naturally occurring variations for rice improvement (Marri *et al.* 2005; Xie *et al.* 2008).

Two yield QTL (*yldp2.1* and *yld11.1*) were detected in both populations and these were colocated with QTL for number of spikelets per plant, number of filled grains per plant and vegetative biomass, and the trait enhancing alleles were from *O. nivara*. Thus, at this locus, many positive alleles were identified from *O. nivara*. QTL for yield have been reported previously at the same chromosomal region from *O. rufipogon* (Xiao *et al.* 1998; Septiningsih *et al.* 2003; Liu *et al.* 2011). One interesting observation is that QTL for number of spikelets per plant were found to colocate with both *yldp9.1* and *yldp2.1*. If we consider colocation of QTL equivalent to coexpression and compare *yldp9.1* and *yldp2.1*, it is clear that at both loci, the *O. nivara* allele increases the number of spikelets per plant. The other significant grain yield QTL (*yldp3.1*, *yldp8.1* and *yldp12.1*) that increased yield per plant in specific populations is important in QTL pyramiding programmes. All the QTL for number of spikelets per plant were derived from *O. nivara* except *nsp12.1*. The most significant QTL for spikelets per plant (*nsp1.1*) was detected at the same chromosomal location in both populations and also in several *O. rufipogon*-derived populations (Xiao *et al.* 1998; Septiningsih *et al.* 2003; Thomson *et al.* 2003; Marri *et al.* 2005). We noticed that major-effect QTL for number of spikelets per panicle have been frequently reported on chromosomes 1 and 7 (Xiong *et al.* 1999; Tian *et al.* 2005; Li *et al.* 2006; Xing *et al.* 2008). QTL on chromosome 1 was found in both *O. nivara*-derived and *O. sativa*-derived populations, but the one on chromosome 7 was found only in *O. rufipogon*-derived populations. If these QTL from different sources are pooled by pyramiding, the number of spikelets can be improved significantly.

The major-effect QTL *nfg1.1* has also been detected in several earlier mapping studies that involved *O. nivara* and *O. rufipogon* as donor parents (Xiao *et al.* 1998; Septiningsih *et al.* 2003; Marri *et al.* 2005; Wickneswari *et al.* 2012). In most instances, this QTL was colocated with QTL for number of spikelets per plant and it is a worthy locus for marker-assisted breeding to improve the yield of rice varieties, but it has to be ascertained whether this locus has a pleiotropic effect or tight linkage. The numbers of spikelets and grains per plant have a significant influence on yield; up to 18% improvement in yield was observed in several *O. rufipogon*-derived lines by an increased number of spikelets and grains per plant (Xiao *et al.* 1998; Xiong *et al.* 1999). The yield

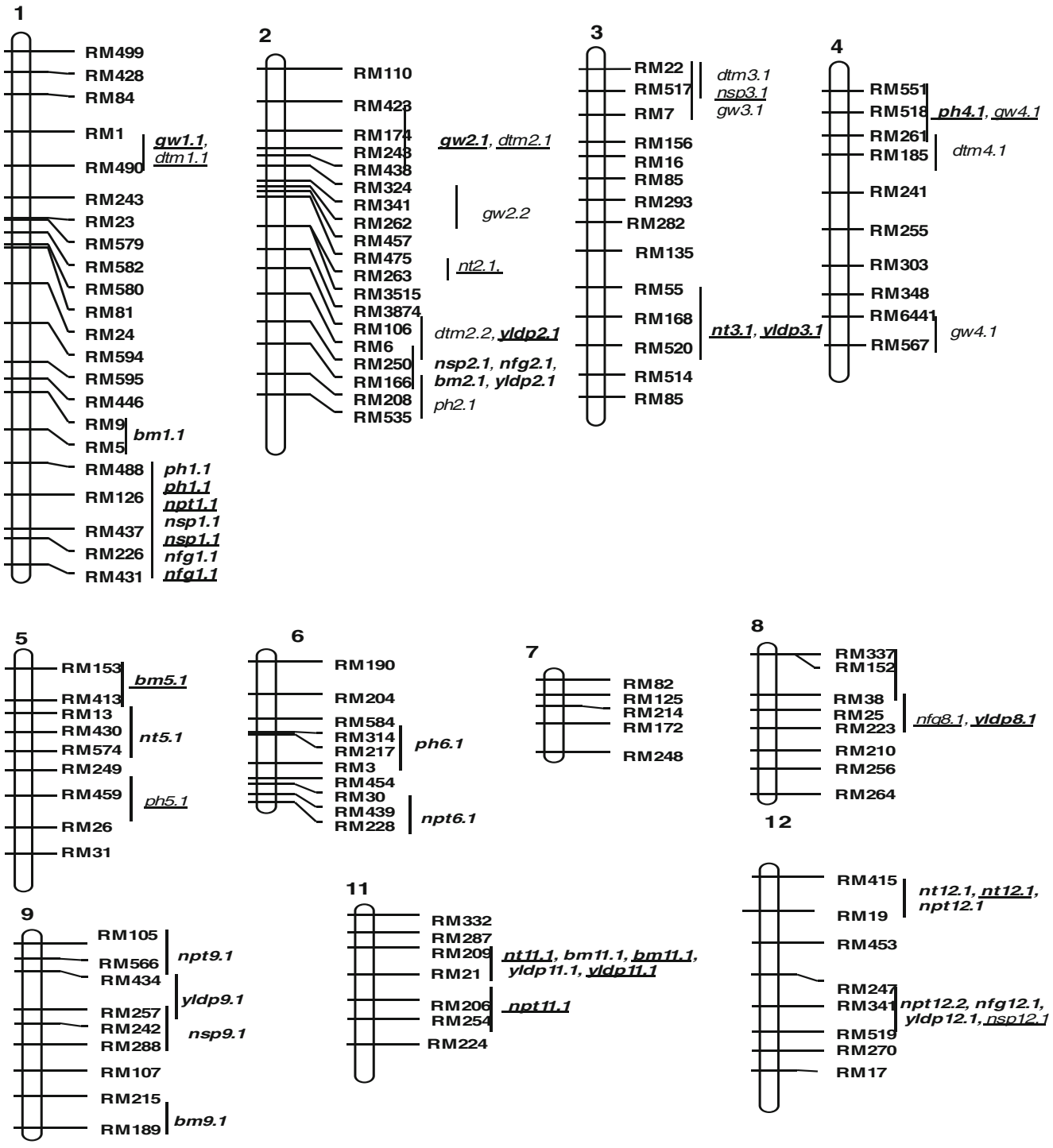

Figure 2. Chromosomal location of QTL for yield and yield-related traits. QTL underlined are from population 2. QTL in bold are derived from *O. nivara*.

advantage in an *O. rufipogon*-derived popular rice variety, Dhanarsi, is also because of its high number of grains per panicle (251 grains) and this variety yielded 6.48 t/ha (Ram *et al.* 2007).

Oryza nivara has high vegetative biomass and all the QTL for vegetative biomass were derived from *O. nivara*. Interestingly, only one QTL (*bm11.1*) was identified at the same locus in both the populations. There are no previous reports of biomass QTL mapped from wild species. The increase in the number of spikelets per plant coupled with higher biomass led to an increase in the number of filled grains in the *O. nivara*-derived progenies.

The major plant height QTL (*ph1.1*) was identified in both the populations. At this chromosomal location, QTL

for plant height have been frequently reported in earlier studies using wild species (Xiao *et al.* 1998; Brondani *et al.* 2001; Septiningsih *et al.* 2003; Thomson *et al.* 2003; Wickneswari *et al.* 2012). It is also interesting to note that this QTL region is near the the *sd1* gene, which is also popularly called the green revolution gene (Cho *et al.* 1994). Detecting new major-effect QTL for plant height is useful in diversifying the genetic base of the green revolution rice varieties, which have the *sd1* gene. Further dissection of this locus provides a basis for comparative mapping and allele mining to detect a new source of plant height genes from distantly related cereal crops.

The *nt5.1* detected in population 1 is congruent with the tiller number QTL reported in *O. rufipogon* (Marri *et al.* 2005; Wickneswari *et al.* 2012), while *nt12.1* was detected in both the populations. We report two new QTL (*npt6.1* and *npt9.1*) from *O. nivara* with a phenotypic variance of >30%. QTL *npt6.1* has been mapped to a region near the monoculm gene (*MOC1*), which is important for tillering in rice (Li *et al.* 2003). Such congruency of QTL/genes for a trait from different populations provide confidence in the accuracy of QTL mapping (Price 2006). It can also help in deciphering the role of candidate genes when alleles derived from different wild species are used in overexpression or loss-of-function studies.

A comparison was made between the yield QTL derived from *O. nivara* with meta-QTL for yield (Swamy and Sarla 2011). The major-effect yield QTL (*yldp2.1* and *yldp11.1*) in both the populations coincided with MQTL2.3 and MQTL11.1, respectively. QTL *yldp9.1* and *yldp12.1* found in population 1 coincided with MQTL9.1 and MQTL12.1, whereas *yldp3.1* and *yldp8.1* identified in population 2 coincided with MQTL3.3 and MQTL8.1, respectively. Thus, a remarkable extent of colocalization was observed in yield QTL from *O. nivara* and *O. rufipogon*.

The results clearly show that yield-enhancing QTL are commonly detected at the same chromosomal locations derived from different wild species but their effect varied across genetic backgrounds (McCouch *et al.* 2007; Swamy and Sarla 2008). It can also be noted that small phenotypic variance values observed in BC_2F_2 populations may increase in subsequent generations of backcrossing as reported for grain weight QTL *gw9.1* (Xie *et al.* 2008). They reported an increase from 12% in BC_2F_2 to 42% in BC_3F_3 and up to 51% in BC_3F_4. The message is that QTL explaining low PV in BC_2F_2 should not be ignored, which may turn out to be ultimately useful for MAS if the PV increases in subsequent generations. The reverse is also true as shown for the effect of root QTL on grain yield in near isogenic lines (NILs) of Azucena in Kalinga III. Here, the NILs with non-target introgressions performed better than NILs with targeted introgression in subsequent generations (Steele *et al.* 2007). These two instances emphasize the fact that most QTL are context-specific and the QTL effect may change as the background genetic milieu changes in each backcross generation.

In BC_2F_5 lines derived from this mapping population, nearly 20% increase in yield was obtained in some lines in small-scale (125 plants in five rows) replicated field trials. The increase was largely associated with the increased spikelets per panicle and number of filled grains per plant. The preponderance of yield QTL-associated markers from *O. nivara* in the 20 high-yielding lines confirms their role in a yield increase (data not shown). If these few high-priority, trait-increasing, major-effect QTL are pooled, it may lead to increased yield even in the presence of extensive phenotypic buffering that often takes place (Mijalski *et al.* 2005). Yet, a yield increase of 15–20% can be easily expected. The evidence for this is the introgression line IET21542 (derived from Swarna × *O. nivara* IRGC 81848), which was successfully evaluated for three years of multi-location testing in the All India Coordinated Rice Improvement Project irrigated medium duration trials and released as DRR Dhan 40 for cultivation in three important rice-growing states of India, namely, West Bengal, Maharashtra, and Tamil Nadu. It gave a mean yield of 5.5 t/ha for over three years and a maximum paddy yield of 10.6 t/ha in the 2011 wet season (DRR AICRIP report on varietal improvement 2012).

Conclusion

In this study, *O. nivara* accessions have contributed trait enhancing alleles for several yield and yield-related traits in the background of popular rice variety Swarna, indicating their potential use in yield improvement programmes. We identified several major effect QTL derived from *O. nivara*, which are reliable for MAS, fine mapping and candidate gene identification. The high yielding BC_2F_5 lines clearly show that the yield and adaptability of rice varieties can be improved considerably using closely-related wild species in crosses with farmer-adapted elite cultivars followed by the advanced backcross method. Several *O. nivara*-derived introgression lines developed in this work will benefit breeding efforts to overcome the adverse effects of climate change and help sustain to global rice production.

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