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Heterotic response of genomic regions derived from *Oryza rufipogon* and *O. nivara* in improving grain morphology and quality of indica rice (*Oryza sativa* L.)

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Abstract

Limited backcrossing was followed to introgress useful variability, needed for improving grain morphology and milling traits, from wild *Oryza* species into two indica cultivars PR114 and Pusa44.These alien introgressions lines (ILs), having similar grain quality parameters to the recurrent parent, were used for developing rice hybrids to assess the effect of these introgressions in the heterozygous state and to assess their grain quality parameters, as most of the hybrids commercialized in the tropics have inferior grain quality. These ILs and their recurrent parents, possessing fertility restoration ability for wide abortive cytoplasm, were crossed with CMS line PMS 17A to generate introgressions line hybrids (ILHs).Hybrids developed from recurrent parents were used as a check to compare the performance of ILHs. Based on yield and phenotypic acceptability, six ILHs having enriched genome of *O. rufipogon* and *O. nivara* were selected and analyzed for grain quality traits. All six hybrids observed significant improvement in milled rice recovery (up to 10%), head rice recovery (up to 25%) over ILs and check hybrids.Introgressions were analyzed using polymorphic SSR markers.The majority of *O. rufipogon* and *O. nivara* alleles identified in the study seems to be effective across recipient genotypes and could be used effectively in quality breeding programs.

Key words: Alien introgression lines, heterosis, wild rice, rice quality

Introduction

Wild progenitors and related species constitute an important natural repository of the novel gene(s). During domestication and cultivation from thousands of years, rice-growing farmers may have selected rice lines with better yielding ability with acceptable grain quality. The cross compatible ancestral wild species of rice *viz*., *O. rufipogon* and *O. nivara* have furnished valuable genes for the improvement of productivity (Xiao et al. 1998; Luo et al. 2011; Gaikwad et al. 2014; Qiao et al 2016; Eizenga et al 2017; Qi et al 2018), grain quality and grain morphology related traits (Fasahat et al. 2012; Quio et al. 2016; Eizenga et al. 2017; Qi et al. 2018).

Higher yield and acceptable grain quality are the priority traits for any rice breeding program. Milling quality is measured as brown rice recovery, milled rice recovery and head rice recovery, whereas the appearance is determined by length, breadth, color,and appearance of polished rice. Inheritance of these traits is under polygenic control with epistatic effect (Li et al. 2004), hence their assessment is quite difficult through conventional plant breeding methods. The advent of molecular markers has greatly facilitated the studies of such complex traits. Several quantitative trait loci (QTLs) for milling quality and grain appearance have been reported from cultivated rice (Lee et al. 2005; Kepiro et al. 2008; Bai et al. 2010; Nelson et al. 2012; Zhen et al. 2017) and wild rice (Fasahat et al. 2012; Subudhi et al. 2015; Quio et al. 2016; Furuta et al. 2016; He et al. 2017; Qi et al. 2018).

Efforts are being made in India for increasing area under hybrids from 7 % to 25% of total rice area, however, the pace of hybrid rice adoption in India has

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been slow largely due to inferior grain quality in comparison to pureline varieties. Most of the hybrids commercialized in the tropics are based on a common CMS line (IR58025A). Although these hybrids yielded 15-20% higher than high-yielding popular varieties, their grain quality did not find acceptability by consumers in many parts of India and Bangladesh. Indica/japonica hybrids have shown quite a high heterosis but their grain quality seems to be a major impediment (Virmani et al. 2003).

Hybridization between the cultivated rice genotypes and wild relatives, in general, deteriorated the grain quality characteristics in derived progenies due to linkage drag. Even though, the superiority of Introgression lines (ILs) over recurrent parent have been reported by few researchers. Such sporadic efforts encourage the geneticist to utilize wild relatives in trait improvement, with at least little or no effect on the grain quality indices. Moreover, such directed efforts may also lead to improvement in grain quality traits by harnessing additional alleles that otherwise lost during domestication. Effect of alien introgressions in F_1 hybrid, on the other hand, has not been reported/ studied yet. The present study was, thus, conceived with the hypothesis that alien introgression(s) in homozygous condition may not be able to contribute positively to the phenotype due to the replacement of cultivated genome segment. However, in a heterozygous condition, the recipient complement is conserved, and minor negative effect associated with the alien segment is likely to be masked. Therefore, the present study was planned to explore the effect of novel genes, if any, from wild species in improving milling quality and grain shape traits in hybrids and to locate these responsible segments in improved milling quality of rice hybrids. If so, these heterotic genes/ QTLs can be utilized in addressing the grain quality concerns in otherwise productive hybrids of rice.

Materials and methods

Development of introgression lines and evaluation of hybrids

Backcross breeding program was initiated at Punjab Agricultural University (PAU) Ludhiana, tointrogress useful variability for improving grain yield and quality traits from wild *Oryza* species, into two high yielding *indica* rice varieties *viz*., Pusa44 and PR114. These varieties are widely adapted and restore fertility in wild abortive (WA) cytoplasm from *O. spontanea*. Sixty one accessions (acc.) of different wild species carrying 'AA' genome *viz*., *O. rufipogon* (10), *O. nivara* (29), *O. barthii* (4), one each of *O. longistaminata*, *O. glumaepatula*, *O. meridionalis* and cultivated African rice *O. glaberrima* (25) were used as donors. Two to three backcrosses followed by continuous selfing for five or more generations led to the development of 1780 alien introgression lines (ILs). During backcrossing and selfing, the phenotypic selection was practiced for yield and grain quality attributes.

Out of 1780 ILs, 318 were utilized for the development of CMS based hybrids. Their evaluation has demonstrated that wild species possess the strength to improve the level of heterosis for yield and its component traits (Gaikwad et al. 2014). From this study, further, six high yielding and phenotypically promising hybrids *viz*., ILH299 (PMS17A/IL299), ILH326 (PMS17A/IL326), ILH867 (PMS17A/IL867), ILH901 (PMS17A/IL901), ILH921 (PMS17A/IL921), and ILH951 (PMS17A/IL951) were generated to study the effect of alien introgression on grain morphology and milling quality traits. Parental ILs of these six hybrids were crossed with a cytoplasmic male sterile (CMS) line PMS17A carrying WA cytoplasm to develop test hybrids (ILHs). Among the six ILs used for generating test hybrids, two ILs *viz*., IL299 [Pusa44/ *O. rufipogon* (100219)//2*Pusa44] and IL326 [Pusa44/ *O. rufipogon* (100219)//2*Pusa44] were in Pusa 44 genetic background, whereas, four ILs *viz*., IL867 [PR114/*O. rufipogon*(104433)//2*PR114], IL901 [PR114/*O. rufipogon*(104433)//2*PR114], IL921 [PR114/*O. nivara*(100593)//2*PR114], IL951 [PR114/ *O. nivara* (CR100142A)//2*PR114] were in PR114 genetic background. Recurrent parents *viz*., Pusa44 and PR114 were also crossed with same CMS line to develop check hybrids designated as PMS 17A/ Pusa 44 (CH1) and PMS 17A/PR114 (CH2). Both test and check hybrids had similar genetic background except for the alien introgression segments transferred from wild species.

ILHs, CHs, and recurrent parents were evaluated at Ludhiana, for grain morphology and quality traits in completely randomized design (CRD) in three replications. Observations were recorded on brown rice recovery (BRR), milled rice recovery (MRR), head rice recovery (HRR) and grain length and breadth, further from this length: breadth ratio (L: B ratio) was calculated. Percent brown rice, milled rice and head rice and LB ratio were determined as per the formula are given in SES, IRRI (1996). A composite sample of three replications was taken for determining grain quality. Weighted samples (125g) of clean paddy with a moisture content of 12-14% were dehusked in a Satake Rubber Roll Laboratory Sheller (Satake Engineering Co. Japan), and the shelled rice (brown rice) samples were milled (McGill Miller No.2, USA). The time of polishing was adjusted to obtain a 6 % degree of polish in all the samples. The experiment was repeated in next crop season for confirmation of the results. This was carried out in two experiments. Experiment I was planned to evaluate two test hybrids developed from Pusa44 derived ILs and experiment II was planned to evaluate four test hybrids developed from PR114 derived ILs.In the second year of evaluation, parents of the ILHs (ILs and PMS17B) were also included along with check hybrids and recurrent parents. The statistical analysis was performed using the software SPAR version 2.0 (http://iasri.res.in/spar).

Marker analysis of introgression lines

DNA from these ILs, recurrent and the parent donor lines was isolated from young leaves following CTAB (CetylTrimethyl Ammonium Bromide) method as modified by Saghai- Maroof et al. (1984) and analyzed by using SSR markers (McCouch et al. 2002). Markers spanning the whole genome at approximately 10cM or closer intervals were used to assess parental polymorphism among the donor and the recipient line using 2.5% agarose gel. Out of 200 SSR markers screened, 100 were polymorphic. These polymorphic markers were used for analyzing the introgression and generating graphical genotypes of each introgression line using the software GGT 2.0 (van Berloo 2008).

Results

Performance of introgression line hybrids: Initial evaluation

In earlier study, six ILHs were evaluated for yield and yield component traits and were found to be superior for grain yield along with pollen and spikelet fertility. These ILHs along with their parents *viz*., ILs and PMS17B (maintainer of PMS17A), two CHs were evaluated for quality traits. Analysis of variance revealed that mean square due to genotypes was highly significant for the traits studied, indicating a considerable amount of genetic variation in the genetic material. To assess the effect of alien introgressions segments in homozygous condition, the ILs were compared with their recurrent parent for quality traits (Table 1). Both IL299 and IL326 observed lower BRR, whereas IL326 recorded lower MRR and L:B ratio than Pusa44. These lines recorded a slight increase in HRR and L:B ratio over Pusa44, but this was statistically non-significant. IL867, IL901, IL921 and IL951 recorded lower BRR, MRR and HRR than recurrent parent PR114. IL901 and IL921 had longer grain size and shape, therefore, observed higher L:B ratio over PR114. Initial results indicated that most of these ILs failed to show significant improvement over their recurrent parents for these quality traits. To assess the effect of introgressions in heterozygous background ILHs were evaluated for two consecutive years. The results

Table 1. Performance of six introgression lines evaluated for important quality traits

Line	Generation	BRR	MRR	HRR	LBR
IL299	BC$_2$F$_6$ [Pusa44/*O. rufipogon* (100219)//2*Pusa44]	77.55 ± 0.42^{c}	68.51 ± 0.53	56.98 ± 1.06	2.88 ± 0.10
IL326	BC$_2$F$_6$ [Pusa44/*O. rufipogon* (100219)//2*Pusa44]	75.87 ± 2.05^{c}	66.58 ± 1.17^{d}	58.13 ± 1.72	2.54 ± 0.10
Pusa44	Pureline	79.36 ± 1.07	69.20 ± 0.95	56.69 ± 3.50	2.83 ± 0.05
	LSD (0.01)	1.68	1.68	4.80	0.24
	LSD (0.05)	1.21	1.21	3.45	0.17
IL867	BC$_2$F$_6$ [PR114/*O. rufipogon* (104433)//2*PR114]	77.98 ± 1.70^{d}	57.49 ± 8.90^{c}	49.78 ± 1.26^{c}	3.21 ± 0.04
IL901	BC$_2$F$_6$ [PR114/*O. rufipogon* (104433)//2*PR114]	78.90 ± 0.27	62.88 ± 7.90^{d}	48.14 ± 1.47^{c}	3.56 ± 0.04^{a}
IL921	BC$_2$F$_8$ [PR114/*O. nivara* (100593)//2*PR114]	78.02 ± 0.71^{d}	62.85 ± 7.56^{d}	44.12 ± 0.82^{c}	4.31 ± 0.11^{a}
IL951	BC$_2$F$_8$ [PR114/*O. nivara* (CR100142A)//2*PR114]	77.97 ± 0.72^{d}	62.46 ± 7.56^{d}	56.70 ± 1.03	2.77 ± 0.01^{c}
PR114	Pureline	79.35 ± 0.50	65.99 ± 7.90	57.75 ± 1.33	3.12 ± 0.03
	LSD (0.01)	1.89	4.31	3.36	0.31
	LSD (0.05)	1.35	3.10	2.41	0.23

[a] Significantly higher and [c] lower than the recurrent parent Pusa 44 or PR114 at $P < 0.01$
[b] Significantly higher and [d] lower than the recurrent parent Pusa 44 or PR114 at $P < 0.05$

of ILHs in initial evaluation have been presented in Table 2.

Table 2. Mean values of the ILHs for grain quality traits and extent of heterosis (per cent) over parents check hybrids and recurrent parents in 6 hybrids evaluated in the initial crop season

Traits	Parameters	ILH299^	ILH326^	ILH867˜	ILH901˜	ILH921˜	ILH951˜
BRR	Mean of test hybrid	81.45	81.86	78.89	81.03	80.89	82.07
	Heterosis over CH1	3.61**	4.13**	0.36	3.08*	2.90*	4.40**
	Heterosis over CH2	4.75**	5.28**	1.47	4.22**	4.04**	5.55**
	Heterosis over Pusa44	2.43	2.95*	-0.78	1.92	1.74	3.21*
	Heterosis over PR114	2.42	2.93*	-0.79	1.90	1.73	3.20*
MRR	Mean of test hybrid	72.93	71.06	63.44	66.35	67.05	68.49
	Heterosis over CH1	5.30**	2.59	-8.40**	-4.20**	-3.19*	-1.12
	Heterosis over CH2	20.41**	17.31**	4.73**	9.54**	10.69**	13.07**
	Heterosis over Pusa44	5.74**	3.02*	-8.01**	-3.79*	-2.79	-0.70
	Heterosis over PR114	8.97	6.17	-5.21**	-0.87	0.17	2.33
HRR	Mean of test hybrid	62.41	59.85	58.95	60.67	58.58	65.77
	Heterosis over CH1	6.44**	2.09	0.55	3.48*	-0.09	12.18**
	Heterosis over CH2	10.49**	5.97**	4.37**	7.42**	3.71*	16.45**
	Heterosis over Pusa44	11.04**	6.50**	4.89**	7.95**	4.23**	17.03**
	Heterosis over PR114	9.15**	4.69**	3.11*	6.12**	2.47	15.04**
LBR	Mean of test hybrid	3.42	3.27	3.64	3.68	4.04	3.90
	Heterosis over CH1	10.67**	5.93*	17.90**	19.20**	30.63**	26.21**
	Heterosis over CH2	-0.29	-4.56*	6.21**	7.38**	17.68**	13.70**
	Heterosis over Pusa44	22.58**	17.32**	30.58**	32.01**	44.68**	39.78**
	Heterosis over PR114	10.32**	5.59*	17.52**	18.81**	30.21**	25.80**

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively. CH1: PMS 17A x Pusa44*,* CH2: PMS 17A x PR114
^: ILH developed from Pusa44 derived IL. ˜: ILH developed from PR114 derived IL

All the hybrids gave approximately 80% BRR. Hybrids, ILH299 and ILH326 observed a significant increase in BRR over parents, CH1, and Pusa44. Except for ILH867, the remaining three hybrids in the genetic background of PR114 exhibited slightly higher BRR over CH2. Hybrids ILH299 and ILH326 showed significantly higher MRR over Pusa44 but, none of the hybrids in PR114 background exhibited increase over PR114. All the hybrids showed significant heterosis for HRR over respective CHs and also displayed high LBR than CHs and recurrent parents. Initial evaluation results indicate that ILHs performed better than ILs and recurrent parents. The strategy of using of ILs in hybrid background yielded positive results. These six ILs were then profiled using SSR markers for identification of alien introgressions.

Marker analysis and generation of graphical genotypes

A total of 100 polymorphic SSR markers spanning all 12 chromosomes were used for characterizing each introgression line. Introgression lines *viz*., IL299, IL326, IL867 and IL901 were in BC$_2$F$_6$ generation whereas IL921 and IL951 in BC$_2$F$_8$ generation. Marker profiling of IL299 and IL326 was carried out with 102 and 103 polymorphic markers that covered 1420.8 cM and 1517.2 cM distance within the genome with 7.7 and 5.2% introgressions, respectively from *O. rufipogon* acc. IR100219. IL867 and IL901, which shared a common parentage, were screened by 102 polymorphic markers covering 1574.4 and 1493.5 cM distance within the genome respectively. These ILs

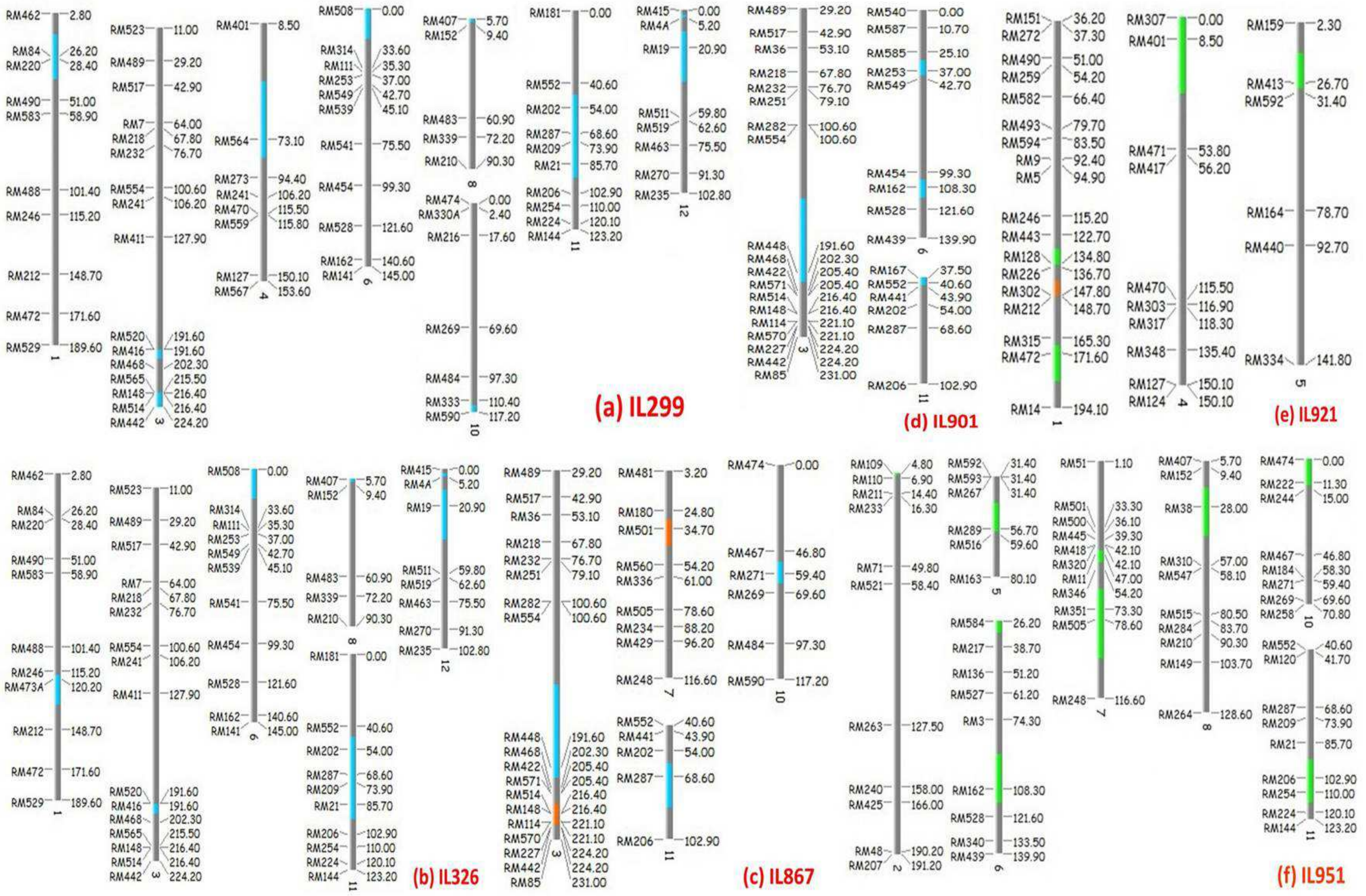

Fig. 1. **Graphical genotypes of six alien introgression lines (a-b) generated after analyzing these with polymorphic SSR markers. Regions in aqua blue are homozygous alien segments from *Oryza rufipogon*; green are homozygous alien segments from *Oryzanivara*and orange are heterozygous alien segments from both the species in respective ILs**

had 3.4 and 2.5% chromosomal segments from *O. rufipogon* acc. IR104433. IL921 and IL951 had introgressions (2.2 and 6.7%) from *O. nivara* acc. IR100593 and CR 100142A respectively. IL 867 and IL921 are having two and one heterozygous segments from respective wild species.Depending upon the introgression pattern of six introgression lines, graphical genotypes for each line were generated (Fig. 1).

Performance of introgression line hybrids: Second evaluation

After molecular marker profiling, six ILs were again involved in the development of ILHs for confirmation of results came out from initial evaluation. In this evaluation, quality traits in six ILHs were compared with CHs, recurrent parents and parents i.e., ILs and PMS17B, which is an isogenic line of PMS17A (Table 3). The results of second-year evaluation were quite confirmatory to the results of an initial evaluation. ILH299 and ILH326 had higher BRR over checks and parents. Similar results were also reflected in PR114 derived hybrids except for ILH867. Most of the hybrids had appreciably higher milling percentage (>62%). For MRR, ILH299 and ILH326 observed significant improvement (3-8%) over parents, CH1 and Pusa44. Three hybrids *viz*., ILH901, ILH951, and ILH921 recorded significant improvement (3-12%) in MRR over parents, CH2 and PR114. All six hybrids had approximately 60% HRR. Hybrids ILH951 observed highest mean value (64.85 %) for this trait. Hybrids ILH299 and ILH326 recorded significant heterosis (7.8 and 25.3%) for HRR over IL299 and IL326 respectively, whereas, ILH901 and ILH921 recorded a significant increase in HRR (23.0 and 30.15 %) over IL901 and IL921 respectively.On the basis of average length and breadth of the kernel, the shape of grain is classified.

Table 3. Mean values of the ILHs for grain quality traits and extent of heterosis (per cent) over parents check hybrids and recurrent parents in 6 hybrids evaluated in the second crop season.

Traits	Parameters	ILH 299^[a]	ILH326^[a]	ILH867~[b]	ILH901~[b]	ILH921~[b]	ILH951~[b]
BRR	Mean of test hybrid	81.1	81.0	78.5	80.2	80.3	80.9
	Heterosis over IL	4.57**	6.8**	0.66	2.92**	1.7*	3.8**
	Heterosis over PMS17B	2.0*	1.9*	-1.3	0.7	0.8	1.7*
	Heterosis over CH 1or CH 2	2.1*	1.9*	0.1	2.2**	2.4**	3.2**
	Heterosis over recurrent parent	2.2*	2.1*	-0.9	1.8*	1.9*	2.0**
MRR	Mean of test hybrid	72.5	71.4	62.0	67.0	67.7	68.3
	Heterosis over IL	5.82**	7.3**	7.9**	6.55**	8.3**	9.3**
	Heterosis over PMS17B	5.3**	3.7*	-2.0	5.7**	6.9**	7.8**
	Heterosis over CH 1or CH 2	3.9*	2.3	2.6	10.8**	12.0**	12.9**
	Heterosis over recurrent parent	4.9**	3.2*	-5.9**	3.5*	3.6*	3.4*
HRR	Mean of test hybrid	61.4	60.3	58.6	59.2	57.4	64.8
	Heterosis over IL	7.8*	25.3**	17.8**	23.0**	30.1**	14.3**
	Heterosis over PMS17B	11.4**	9.4*	8.4**	9.5**	6.2*	19.9**
	Heterosis over CH 1or CH 2	3.4	2.6	3.3	4.3	1.2	14.3**
	Heterosis over recurrent parent	8.3*	6.4	3.5	3.5	1.5	12.3**
LBR	Mean of test hybrid	3.4	3.2	3.6	3.6	3.9	3.8
	Heterosis over IL	19.5**	28.3**	14.0**	1.0	-8.8**	37.4**
	Heterosis over PMS17B	5.6*	0.2	26.1**	24.1**	35.3**	31.4**
	Heterosis over CH 1or CH 2	10.3**	4.7	6.1**	4.4*	13.9**	10.6**
	Heterosis over recurrent parent	21.5**	15.3**	17.5**	15.6**	26.1**	22.4**

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively. CH1: PMS 17A x Pusa44, CH2: PMS 17A x PR114

^: ILH developed from Pusa44 derived IL. ~: ILH developed from PR114 derived IL

[a] ILH 299 & ILH 326 are compared with CH 1; [b] ILH 867 & ILH 901, ILH 921 & ILH 951 are compared with CH 2

Grains having L: B ratio 1 is classified as round, 1.1 to 2.0 and 2.1 to 3.0 is classified as bold and medium respectively while L: B ratio >3 is classified as slender. All the hybrids excelled their recurrent parents for this trait and recorded L: B ratio >3. ILH951 showed the highest magnitude of heterosis (>30%) over IL951 and PMS17B. Except for ILH921 and ILH901 rest, four hybrids had high LBR than respective ILs. Results from two years of evaluation suggest that introgression segments in the homozygous state failed to show improvement in milling quality and grain shape traits in ILs. However, in the heterozygous state, along with the alien segment, the recipient complement was also conserved and it resulted in a significant improvement in hybrids.

Discussion

In the present study, we identified heterotic genomic regions associated with milling traits in rice hybrids developed using alien introgression lines. In these ILs, 38 markers confirmed the presence of 33 alien segments dispersed across the genome. Twenty markers representing 16 alien segments are the exact markers that were associated with QTLs for milling traits and grain shape identified in wild as well as cultivated rice, and twelve markers are in the similar/ adjacent regions where QTLs for grain quality were mapped in earlier studies (Table 4).

Hybrids ILH951 and ILH921 recorded significant improvement for BRR over IL951, IL921 as well as checks. IL951 has *O. nivara* introgressions at RM351, RM505 on CH7 and RM474 on CH10 (Fig. 1). Brown rice QTL *br7* in this region has been mapped in the interval of RM10-RM351 from *O. glaberrima* (Aluko et al. 2004). Similarly, a QTL for BRR from *O. rufipogon* was mapped at marker loci RM474 on CH10 (Septiningsih et al. 2003). IL951 also had introgressions at RM505; the same marker linked to QTL *QBr7* (Table 4) was identified in cultivated rice (Zheng et al. 2007). IL921 carried the *O. nivara* alleles at RM302. RM302 lay in-between the marker interval RM297-RM315, where a QTL for BRR (*br1*) has been reported from *O. glaberrima* (Aluko et al. 2004).

Hybrids ILH299, ILH326, and ILH867 displayed improvement in MRR over respective ILs, which had common introgressed region from *O. rufipogon* marked by RM287 on CH11 (Fig. 1). IL299 and IL326 carried *O. rufipogon* introgressions at RM209, RM21 on CH11 and RM415, RM19 on CH12. Similarly, IL867 had *O. rufipogon* introgression at RM287 on CH11, which is the same location where *O. nivara* alleles have been observed to confer positive effect on MRR (Swamy et al. 2012). Hybrid ILH951 recorded the highest magnitude of heterosis for MRR over IL951, PMS17B and check hybrid. Marker profiling of IL951 revealed the presence of *O. nivara* alleles at RM11, RM505 on CH7, RM289 on CH5 and RM474 and RM38 on CH10 and 8 respectively (Fig. 1). The same markers representing QTLs for MRR *viz*., *mr7* from *O. glaberrima* (Aluko et al. 2004), *mp8.1* from *O. nivara* (Swamy et al. 2012), QTL *MRR* from *O. rufipogon* (Septiningsih et al. 2003) and from cultivated rice QTL *QMr5* and *QMr7* (Zheng et al. 2007) were reported in earlier studies.

In rice breeding, more emphasis is being given to head rice improvement since it is commercially most important and easy to improve. ILH951 recorded significant positive heterosis (>14%) over IL951, PMS17B and check hybrid. IL951 carried *O. nivara* introgression at RM584 on CH6 and RM289 on CH5 (Fig. 1). These introgressions were at same/adjacent positions where QTLs for HRR *viz*., *qHr6*, and *qMHP5* were reported from cultivated rice (Dong et al. 2004; Zheng et al. 2007). Hybrids ILH299 and ILH326 showed improvement in HRR over IL299 and IL326 respectively. IL299 and IL326 had common introgression from *O. rufipogon* at marker loci RM508 (0.0cM) on CH6, and it is in proximity to RM190 (8 cM) which is an important genomic region consisting of QTLs for quality traits (Table 4). Introgression line IL901having genomic segment of *O. rufipogon* has low HRR when compared to its recurrent parent PR114. But, when these genomic segments came in the heterozygous background in ILH901, the HRR get improved when compared to IL901, check hybrid and PR114; these results are as per our hypothesis. IL901 had introgression at the locus RM167 on CH11. The same marker is known to have QTL for HRR (*qhr11.1*) reported by Nelson et al. (2012) in the Western US rice germplasm. It also showed that some genomic segments are conserved between cultivated rice and its wild species; as it is proved that the US weedy rice and germplasm are closely related to *O. rufipogon* and *O. nivara* (Reagon et al. 2010). The results indicated that the effect of wild alleles in heterozygous condition was beneficial, suggesting that, they are associated with more stability of grains during milling.

Medium long to slender rice is preferable in breeding practice with decreasing grain size can lead to a decrease in rice yield. Two traits viz. grain length and grain width decides the grain L:B ratio. Many QTLs

Table 4. Correspondence of reported introgressions from *O. rufipogon* and *O. nivara* with the already reported quantitative trait locus/loci

Traits	ILs	Donor species	Introgression reported on chromosome	The marker associated with introgressed regions in the present study	The marker associated with QTL(s) and the species as reported earlier	Reference
MRR	IL299	*O. rufipogon*	1	RM84, RM220	RM499-RM428 (*mp1.1*) *O. nivara*	Swamy et al. 2012
		4	RM564	RM261-RM241 (*mp4.1*) *O. nivara*		Swamy et al. 2012
	IL299, IL867	*O. rufipogon*	11	**RM209-RM21, RM287**	**RM209-RM21, RM287** (*mp11.1*) *O. nivara*	Swamy et al. 2012
	IL326		12	**RM415-RM19**	**RM415-RM19** (*mp12.1*) *O. nivara*	Swamy et al. 2012
			8	RM407	RM337-RM38 (*mp8.1*) *O. nivara*	Swamy et al. 2012
	IL299	*O. rufipogon*	66	RM508RM584	RM190-RM204 (*QMr6*) *O. sativa*	Zheng et al. 2007
	IL951	*O. nivara*				
	IL299,IL326	*O. rufipogon*	11	RM287	XNpb257 (*qMRP11*) *O. sativa*	Dong et al. 2004
	IL867	*O. rufipogon*	7	RM501	RM125-RM11 (*mr7*) *O. glaberrima*	Aluko et al. 2004
	IL921	*O. nivara*	1	RM302, RM472	RM226-RM104 (*QMr1*) *O. sativa*	Zheng et al. 2007
	IL951	*O. nivara*	7	**RM11**	**RM11** (*mr7*) *O. glaberrima*	Aluko et al. 2004
			10	**RM474**	**RM474** *O. rufipogon*	Septiningsih et al. 2003
			5	**RM289**	**RM289** (*QMr5*) *O. sativa*	Zheng et al. 2007
			7	**RM505**	**RM505** (*QMr7*) *O. sativa*	Zheng et al. 2007
			8	**RM38**	**RM38** (*mp8.1*) *O. nivara*	Swamyet al. 2012
			5	RM289	RM574-RM249 (*mp5.1*), *O. nivara* RM430 (*mp5.2*) *O. nivara*	Swamy et al. 2012
BRR	IL921	*O. nivara*	1	RM302	RM297-RM315 (*br1*) *O. glaberrima*	Aluko et al. 2004
	IL951	*O. nivara*	7	**RM351**	**RM351** (*br7*) *O. glaberrima*	Aluko et al. 2004
			10	**RM474**	**RM474** *O. rufipogon*	Septiningsih et al. 2003
			7/6/5	**RM505**/RM584/RM289	**RM505** (*QBr7*)/RM225 (*QBr6*)/RM509 (*QBr5*)*O.sativa*	Zheng et al. 2007
HRR	IL299	*O. rufipogon*	1	**RM84**	**RM84** (*QHr1*) *O. sativa*	Zheng et al. 2007
	IL299, IL326	*O. rufipogon*	6	RM508	RM190 (*hr6*) *O. glaberrima*	Aluko et al. 2004
					RM190(*hr6*) *O. sativa* ssp. *japonica*	Kepiro et al. 2008
	IL876	*O. rufipogon*	3	**RM570**, RM114	**RM570***O. sativa* ssp. *japonica*	Hao et al. 2009
	IL901	*O. rufipogon*	11	**RM167**	**RM167** (*qhr11.1*) red rice *O. sativa*	Nelson et al. 2012
	IL951	*O. nivara*	56	RM289RM584	C1268 (*qMHP5*) *O. sativa* RM253-RM50 (*QHr6*) *O. sativa*	Dong et al. 2004 Zheng et al. 2007

Table 4. contd....

LBR	IL299, IL326	*O. rufipogon*	12	**RM415-RM19**	**RM415-RM19** (*lwr12.1*) *O. nivara* RM247 (*lwr12.1*) *O. glaberrima* **RM119 (***qGSDLGWDRO12-1*) *O. nivara*	Swamy et al. 2012 Li et al. 2004 Eizenga et al. 2017
	IL299, IL326	*O. rufipogon*	3	**RM514**	**RM514** (*qGW3-2*) *O. rufipogon*	Qiao et al 2016
	IL326	*O. rufipogon*	1	RM473A	RM246 (*lwr1*) *O. glaberrima*	Aluko et al. 2004
	IL901, IL951	*O. nivara*	6	**RM162**	**RM162** (*lwr6*) *O. glaberrima*	Aluko et al. 2004
	IL921	*O. nivara*	1 & 5	**RM302 & RM413**	**RM302** (*lw1*) & **RM413** (*lw5*) *O. rufipogon*	Lee et al. 2005
	IL921	*O. nivara*	1	**RM128**	**RM128** (*qLWR1.2*) *O. rufipogon* **RM128** (*qGL1*) *O. rufipogon*	Qi et al. 2017 Qi et al. 2018
	IL921	*O. nivara*	4	**RM401**	**RM401** (*qGL4*) *O. sativa*	Zhen et al. 2017
	IL921, IL951	*O. nivara*	5	RM413 & RM289	RM13-RM574 (*qLWR5*) *O. sativa*	Bai et al. 2010
	IL951	*O. nivara*	26	**RM109RM584**	**RM109** *O.sativa* **RM584** (*lwr6.1*) *O. nivara*	Jiang et al. 2005 Swamy et al. 2012
	IL951	*O. nivara*	5	**RM289**	**RM289** (*qGL5, aGW5-2*) weedy rice	Subudhi et al. 2015

Markers in bold are common regions associated with grain quality traits in various studies

affected these two traits in previous reports were mapped to similar or in close proximity to genomic regions contributed by two wild species in our study. ILH921 has highest mean value (3.9) for L: B ratio among the hybrids but it failed to show improvement over IL921. IL921 had *O. nivara* introgression at RM302, RM128, and RM472 on CH1; RM413 on CH5 and RM401 on CH4 (Fig. 1). The observed improvement in IL921 is due to introgressions at above marker loci as the same markers were known to have QTLs from *O. rufipogon viz*., *lw1* and *lw2* (Lee et al. 2005); *qLWR 1.2* (Qi et al. 2017) and from cultivated rice (Zhen et al. 2017) (Table 4). The grains of IL921 are very slender and long. This slenderness in the grain is due to introgression from *O. nivara*. IL921 has introgression on CH1 at marker locus RM128 (Fig. 1). Qi et al. (2018) fine mapped a major QTL (*qGL1*) for grain length on CH1 flanked by marker RM128 in *O. rufipogon*. This proves the synteny of genomic segments between two wild species. Also, wild rice seeds have very slender shape and may have many other genes for the grain morphology that have been lost in cultivated rice during the domestication. Though IL921 showed higher L: B ratio, it had lower MRR and HRR over PR114 and ILH921 because extra-long slender grains are prone to breakage. Hybrid ILH299 observed significant improvement over parents and checks. IL299 had introgressions from *O. rufipogon* at RM415 and RM19. The same markers are known to have QTL *lwr12.1* (Swamy et al. 2012) *qSDLGWDRO 12-1* (Eigenga et al 2017) from *O. nivara*. This again proves the synteny between the alleles of these two wild species. IL299 and IL326 had introgression at maker locus RM514 (Fig. 1). The same marker is known to flank a major QTL (*qGW3.2*) for grain width detected in chromosome segment substitution lines developed from *O. rufipogon* as donor parent (Qiao et al. 2016). In recent study, Bhatia et al. (2018) identified two QTLs for grain width (*qgw5.1*) and grain length (*qgl7.1*) on chromosome 5 and 7 respectively in the introgression lines developed by using *O. rufipogon* (Acc. IR 104433) as donor parent. These QTLs showed positive and additive effect contributed by wild species alleles. In our study, the same *O. rufipogon* accession was used as donor parent in the development of IL299 and IL326. Introgression line IL951 carried *O. nivara* chromatin at RM584 and RM162 on CH6 and RM 109 on CH 2 the same markers are known to have QTLs *lwr6.1* from *O. nivara* (Swamy et al. 2012) and *lwr6* from *O. glaberrima* (Aluko et al. 2004) and QTL for LBR from cultivated rice (Jiang et al 2005). From these results, it can be seen that some genomic segments from wild rice *O. nivara* are in common with cultivated African rice *O. glaberrima* though they have a different route of evolution. IL951 also have *O. nivara* segment at RM289 on CH5. The same marker is reported to flank a QTL for grain length and width *viz*., *qGL5* and *qGW5* (Table 4) from weedy rice genotype PSRR-1 (Subudhi et al. 2015).

Markers responsible for the improvement in quality traits through the incorporation of genomic regions from *O. rufipogon* were reported to be governed by QTLs derived from *O. nivara* and vice versa. Regardless of the taxonomical status, these two ecologically distinct taxa are readily distinguished in nature. But due to their evolutionary path, they share some common /conserved genomic regions for yield (Gaikwad et al. 2014) as well as quality traits, as it is also observed in this study. On the whole, the majority of *O. rufipogon* and *O. nivara* alleles that were common with other studies seemed to be effective across recipient genotypes and evaluation sites. SSRs well demonstrate the synteny between genomic regions derived from both the alien species. In this study, ILs did not observe improvement in quality traits over recurrent parents, but their hybrids observed significant improvement over check hybrids and recurrent parents. Alien segments in heterozygous state observed the increased effect for these traits. Our results are in perfect agreement with the findings of Xiao et al. (1998) in rice and Semel et al. (2006) in tomato as they identified QTLs that have a positive effect in heterozygote condition and not in the homozygous condition in the majority. In accordance with the earlier reports, results of the present study also showed the positive comparative link between alien segments with the grain quality traits and their utilization in hybrid development. This has shown a new avenue for the utilization of these loci/QTL in the development of good grain quality rice hybrids in future.

Authors' Contribution

Conceptualization of research (KS, KBG); Designing of the experiments (KS, NSB, TSB, KBG); Contribution of experimental materials (KS, TSB, NS, DB); Execution of field/lab experiments and data collection (KBG, NS, NS,KS); Analysis of the data and interpretation (KBG, NS, NSB, TSB, DB, KS); Preparation of the manuscript (KBG, KS, NS, NSB, TSB).

Declaration

The authors declare no conflict of interest

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