Ecological Genetics and Genomics 11 (2019) 100036

Contents lists available at ScienceDirect

Ecological Genetics and Genomics

journal homepage: www.elsevier.com/locate/egg

Evaluation of yield and seedling vigour related traits of Swarna/ *Oryza nivara* backcross introgression lines under three environment conditions

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ARTICLE INFO

Keywords:
Seedling vigour
Yield
Oryza nivara
G × E interaction
Stability

ABSTRACT

Wild species are valuable resources in prebreeding programmes for broadening the genetic base and to identify novel genetic variants. In this study we used advanced backcross introgression lines (BILs) generated from Swarna/*Oryza nivara* cross. Fifteen BILs were evaluated for three seasons (*kharif 2014, rabi 2015* and *kharif 2015*) for seedling vigour and yield related traits. Among these, seven BILs showed significantly higher yield over Swarna. G × E interaction analysis for yield traits in three environments showed that BILs 166-2-9S, 166-2-3S, 166-23S, 166-9S, 166S and 166-32S were stable with high means for different yield traits. BIL166-9S showed high seedling vigour and thousand grain weight (TGW) and BIL166-30S had high grain number in all three environments. BIL248S (DRR Dhan40) showed high single plant yield (SPY) and spikelet fertility (SPF) in both *kharif* seasons. Trait correlation showed significant positive correlation of panicle length with SPY and TGW. Days to fifty percent flowering (DFF) was significantly associated with thousand grain weight (TGW). Significant correlation was also observed between vigour traits and yield. In addition, seed germination percentage showed significant correlation with plant height and tiller number. Root length was associated with SPY and TGW. Molecular screening to detect the presence of yield related QTLs revealed that high yielding BILs 166-2-9S, 166-9S and 166-21S showed *O. nivara* alleles for two yield enhancing QTLs *qyldp2.1* (RM250-RM535) and *qyldp9.1* (RM434-RM257) reported previously. These stable BILs are useful in rice improvement and to fine map QTL/genes for seedling vigour and yield.

1. Introduction

Enhancement in production of rice (*Oryza sativa* L.), the staple food crop of majority of global population, is essential to meet the increasing consumer demands. Declining availability of resources such as land and water along with drastic climate change hinders increase in production of rice. Improving the yield potential of varieties is a solution to tackle this condition of input and environmental limitations. However, the existing cultivars developed largely from intra specific crosses of *Oryza* spp. have reached a yield plateau so there is a need to use wild and related species to widen the gene pool and increase the yield potential of cultivars. Wild accessions have a significant role in breeding programs as they have many novel genes and were underutilized for crop improvement due to their crossing incompatibility and other undesirable linked traits [26]. Introgression lines are an excellent bridging material for transfer of genes from wild donors to cultivars, as they are more cross compatible and are in an adapted genetic background. They are also useful in detecting novel useful alleles from un-adapted, less-productive wild rice accessions.

Traditional breeding methods have helped in using wild species to improve yield in crops. Exploring novel QTLs from wild x elite advanced backcross introgression lines (BILs) is an effective approach for further improving yield traits of popular rice cultivars [21,26]. *Oryza nivara* is a close wild progenitor of Asian cultivated rice and it belongs to primary gene pool for improving rice [12,19]. Its accessions have abundant genetic diversity and contributed resistance genes for grassy stunt virus, bacterial leaf blight, blast, brown plant hopper and drought stress. *O. nivara* is also a major source of cytoplasmic male sterility and out crossing trait [4]. Even though *O. nivara* has contributed significantly to pest and disease resistance in rice, it has only recently been used in yield improvement programmes [25]. In 2013, DRRDhan40 variety derived from *O. sativa/O. nivara* introgression was released from Indian Institute of Rice Research [23].

Yield is a complex quantitative trait controlled by number of genes, component traits and their interactions with the environment. Grain yield of rice is derived from number of contributing characters like panicles per unit area, number of grains per panicle and weight of thousand grains. Good crop establishment including ability to

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https://doi.org/10.1016/j.egg.2019.100036
Received 10 May 2018; Received in revised form 24 January 2019; Accepted 22 March 2019
Available online 26 March 2019

germinate, fast seedling emergence and seedling growth also affect crop yield. Seedling vigour is a very important indicator for crop establishment and early vegetative growth in rice [30]. It is a major determinant for strong seedling establishment and growth under adverse environmental conditions. It is considered as a main breeding target especially in direct seeded method or dry sowing systems. The required traits are better germination rate, seedling establishment, weed-smothering ability, dry matter accumulation, and resistance to biotic and abiotic stresses for resource saving and cost-effective crop production [5]. The variation in seedling vigour is a result of difference in amylase, ascorbic acid, antioxidant enzymes and other growth hormones. Seedling vigour is measured as length or weight of shoot and root, number of tillers per plant, chlorophyll content, and amylase activity in seeds and oxidation rate in roots [5,6].

The information on stability and adaptability helps in selection of environment specific genotypes as well as for general adaptation [11,18]. AMMI model collectively considers environment (E), genotype (G) and their interaction with each other (G × E) as individual parameters for the evaluation purpose whereas the GGE biplot developed by Ref. [28] evaluates the interaction by considering the genotype (G) and genotype's environmental interaction (GE). [5] reported that component traits of seedling vigour *viz,* germination rate, shoot dry weight and seedling fresh weight showed complex quantitative nature and G × E interactions. In this study, 16 selected BILs derived from Swarna/*Oryza nivara* (BC_2F_8) were evaluated for 17 phenotypic traits related to seedling vigour and yield in three seasons and screened for the presence of reported yield QTLs to identify stable BILs for further crop improvement.

2. Materials and methods

Yield traits of fifteen breeding lines G3-G18 **(Table 1)** derived from an elite BIL 166S (Swarna / *O. nivara*), were studied along with parent Swarna and three other already reported stable BILs (148S (G1), 14S (G2) and 248S (G19-released as DRR Dhan40) (Supplementary Table 1). Among these BILs 166-2-3S, 166-20S and 166-32S were of early duration maturity. BILs166-2-10S, 166-2-7S, 166-2-5S, 166-9S, 166-2-11S, 166-30S, 166-23S, 166-2-2S, 166-23-1S, 166-2-10S, 166-1S and 166S, were of mid-early duration. Field experiments were conducted at Indian Institute of Rice Research Farm, Hyderabad (17° 19′ N and 78° 29′ E) 549 MSL during *kharif* 2014, *rabi* 2015, *kharif* 2015. The soil is alkaline vertisol with pH 7.94. BIL166S was selected in BC_2F_2 and it produced several derivatives in BC_2F_3 which were fixed by selfing up to BC_2F_8 generation by single panicle selection. BIL166S in BC_2F_3 were also advanced upto BC_2F_8.

2.1. Phenotyping in field

At anthesis, DIF (days to initial flowering), DFF (days to 50% flowering) and DHF (from the date of sowing to the anthesis of 80% of spikelets) were recorded. PH (plant height), TN (tiller number) and PTN (productive tiller number) were recorded for 5 plants per replication in each genotype before harvest. Five panicles collected for each replication were measured for panicle topology traits *viz.,* panicle length (PL), number of filled grains (FG), number of unfilled grains (UFG), panicle weight (PW), grain number (GN), thousand grain weight (TGW), spikelet fertility (SPF). After harvest, SPY (g) was measured as an average of five plants yield after drying to 12–14% seed moisture content, following standard evaluation system (SES, IRRI). Harvest Index was measured using formula; HI =(single plant yield/Total dry matter) × 100

Spikelet fertility (SPF %) was calculated using formula.SPF(%) = (Filled grain number/Total grain number) × 100

Panicle weight of each genotype was taken as average weight of five dried panicles at 12% moisture content. Thousand grain weight (g) was measured from 1000 cleaned and filled seeds using seed counter and weighing machine. Vigour related traits were estimated based on germination% in 65 h after germination, length, fresh weight, and dry weight of 14 days old seedlings using petriplate method [6,24] and paper roll test method [30] in controlled lab conditions.

2.2. Germination test

The germination test was performed using petriplate method. Seeds were dried in hot air oven at 50 °C for 3 days to overcome seed dormancy and for uniform germination. 100 well dried seeds from each BIL were placed in a sterilized petriplate lined with Whatman filter paper

Table 1
Mean vigour traits of 166S (Swarna x *O. nivara* elite BIL) derived lines and 4 control lines.

Code	BILs	SL 7DAS	RL 7DAS	SL 14DAS	RL 14DAS	FWT	DWT	RSL	RRL	G% (24 h)	G% (65 h)	SVI (24 h)	SVI (65 h)
G1	148S	4.40	9.13	12.10	14.35	0.12	0.06	63.64	36.36	90.00	98.00	5.40	5.88
G2	14S	3.88	8.34	10.89	18.71	0.11	0.10	64.42	55.42	76.00	98.00	7.60	9.80
G3	166–1	2.15	6.46	8.84	10.81	0.11	0.09	75.68	40.28	28.00	100.00	2.52	9.00
G4	166-2-10	4.08	6.17	8.06	8.72	0.13	0.13	49.44	29.22	10.00	65.00	1.30	8.45
G5	166-2-11	4.03	10.27	11.26	19.92	0.09	0.09	64.25	48.43	35.00	98.00	3.15	8.82
G6	166-2-2	2.78	3.98	7.56	5.95	0.09	0.09	63.29	33.18	51.00	98.00	4.59	8.82
G7	166-2-3	4.60	10.67	11.00	16.02	0.09	0.08	58.20	33.37	90.00	100.00	7.20	8.00
G8	166-2-5	1.68	2.07	8.62	6.40	0.09	0.06	80.57	67.67	11.00	67.00	0.66	4.02
G9	166-2-7	6.30	12.94	9.92	16.86	0.15	0.07	36.49	23.25	20.00	95.00	1.40	6.65
G10	166-2-9	3.73	8.86	11.82	16.43	0.10	0.08	68.49	46.05	19.00	92.00	1.52	7.36
G11	166–20	5.44	12.40	8.30	14.38	0.11	0.05	34.46	13.77	46.00	99.00	2.30	4.95
G13	166-23-1	3.73	9.14	10.07	17.06	0.14	0.08	63.01	46.45	18.00	99.00	1.44	7.92
G14	166–23	4.03	6.95	6.89	11.25	0.08	0.07	41.58	38.20	66.00	98.00	4.62	6.86
G15	166–30	1.90	6.56	8.36	13.23	0.09	0.08	77.27	50.42	27.00	100.00	2.16	8.00
G16	166–32	6.26	14.18	7.65	16.05	0.09	0.03	18.17	11.65	1.00	61.00	0.03	1.83
G17	166–9	4.43	9.69	14.63	20.08	0.12	0.11	69.74	51.74	15.00	95.00	1.65	10.45
G18	166S	1.78	5.78	6.93	10.54	0.09	0.06	74.39	45.20	59.00	100.00	3.54	6.00
G19	248S	3.93	10.26	7.77	18.57	0.08	0.08	49.49	44.75	26.00	94.00	2.08	7.52
G20	Swarna	2.50	8.64	7.81	14.51	0.12	0.09	67.99	40.45	25.00	98.00	2.25	8.82
	Min	1.68	2.07	6.89	5.95	0.08	0.03	18.17	11.65	1.00	61.00	0.03	1.83
	Max	6.30	14.18	14.63	20.08	0.15	0.13	80.57	67.67	90.00	100.00	7.60	10.45
	Average	3.77	8.55	9.39	14.20	0.10	0.08	58.98	39.78	40.62	92.57	3.00	7.21

BILs- Back cross inbred lines, SL 7DAS- Shoot length 7 days after sowing, RL 7DAS- Root length 7 days after sowing, SL 14DAS- Shoot length 14 days after sowing, RL 14DAS- Root length14days after sowing, FWT- Fresh weight, DWT- Dry weight, RSL%- Rate of increase in Shoot length, RRL%- Rate of increase in Root length, G%(24 hrs)- Germination percentage at 24 h, G%(65 hrs)- Germination percentage at 65 h, SVI(24 h)- Seedling vigor index at 24 h, SVI(65 h)- Seedling vigor index at 65 h.

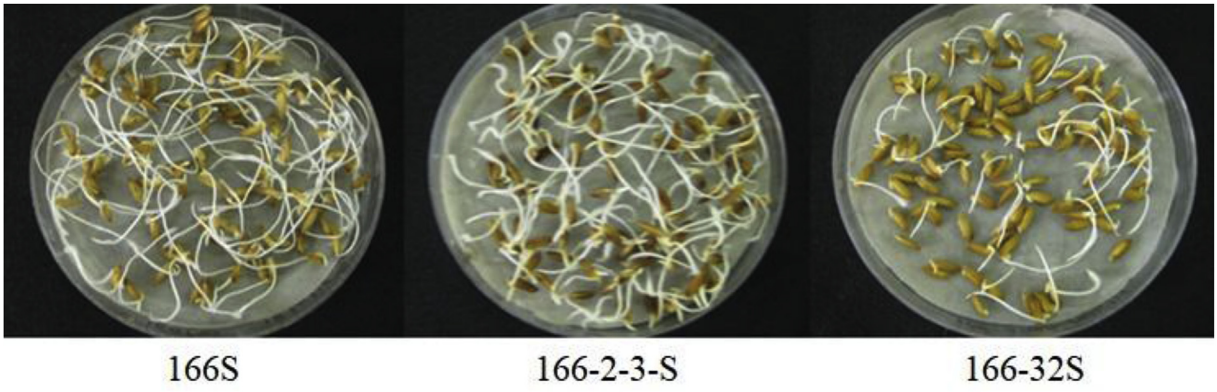

Fig. 1. Higher rate and percentage of germination 65 h after germination in two BILs 166S and 166-2-3S compared to 166-32S.

with sufficient moisture for seed germination. 10 ml distilled water was added and these plates were kept in oven at 32 ± 1 °C and the germinated seeds were counted after 24 h when the embryonal axes emerged [6]. Germination percentage was calculated by counting the number of germinated seeds at the time periods of 24 h and 65 h from the time of sowing maintained in the same temperature (Fig. 1).

2.3. Seedling growth test

Brown germination sheets with water absorbing capacity were used to maintain moisture for seedling growth in Paper-roll test method (Fig. 2). 10 seeds were placed in each paper roll at equal distance (2 cm) and rolled properly after labelling. Paper rolls were arranged vertically in a tray filled with water and the measurements were taken after 7 days and 14 days intervals in two replications each [14]. 20 days after sowing, fresh shoot and root weights were taken by removing the seed pulp attached with seedling (10 seedlings for each) and those seedlings were kept in oven at 62 ± 1 °C up to 5 days and then dry shoot and root weights were measured.

2.4. Seedling vigour index (SVI)

Five seedlings from each replication were selected at random on the 7th and 14th day after germination, and seedling length was measured. The same seedlings were dried at 62 ± 1 °C for 5 days and weighed. The mean seedling length and dry weight were used for estimation of SVI in two different methods using the following formulas.SVL based on seedling length = Mean seedling length(cm)

× Germination(%)

SVW based on seedling dry weight SVW = Mean seedling dry weight(mg)

× Germination(%)

2.5. Statistical analysis

The field experiment was conducted in a randomised complete block design with three replicates. Statistical significance of the parameter means was computed for individual environment, and then a combined analysis of variance was performed, using PB tools (Version 1.4, http://bbi.irri.org/products). Multiple correlations between yield and yield related parameters were estimated. Phenotypic data from BILs and checks were used to conduct stability analysis using AMMI and GGE biplot models. These genotypes were evaluated for high yield that was with high PC1 value and with high stability. Stability of yield traits across three environments was examined by AMMI and it involves both additive and multiplicative components of two way data structure this enables predicting genetic potentiality and environment influence on it [3].

AMMI with biplot origin analyze the yield data for genotypic contribution and G × E interaction (genotype × environment interaction) in three environments. GGE biplot ((genotype) + (genotype × environment)) method explains the genotypic and genotype × environment interaction by AEC (Average environment co ordinate) which is the line passing through the biplot origin and the average environment indicated by a circle which is a sum of PC1 and PC2 values of all environments. PC1 and PC2 were first used to construct a 2 dimensional biplot. These biplots helps to evaluate the genotypes and environments by enabling the visualisation through graphical method.

2.6. Molecular screening

2.6.1. DNA extraction

DNA was extracted from young leaf samples [8] of the recurrent parent Swarna, *O.nivara*, 16 lines of 166s derived BILs, and three other sib-BILs. 400 µL of CTAB buffer was added to the leaf samples for cell

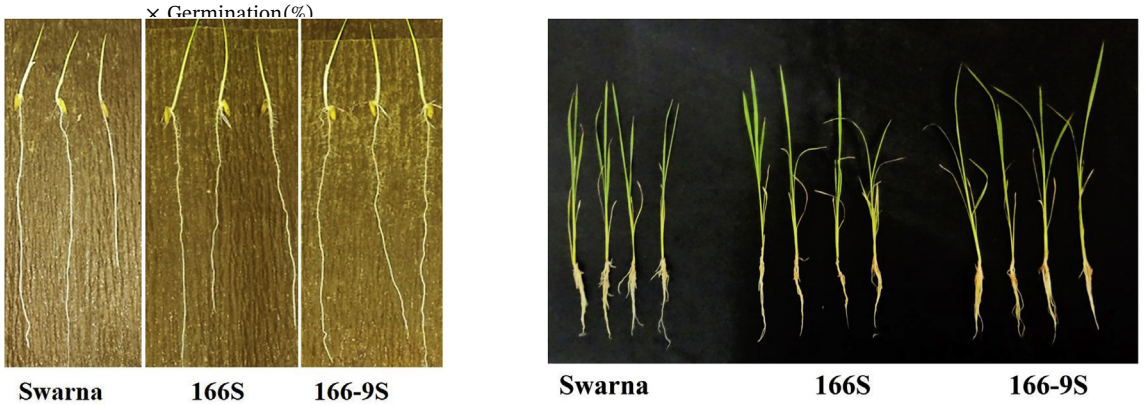

Fig. 2. Seedling growth in paper towel method at 7 days after germination.

Table 2
Variability of 17 yield related traits of 166S (Swarna x *O. nivara* elite BIL) derived lines and 4 control lines in 3 seasons.

	Kharif 2014						Rabi 2015						Kharif 2015					
Variable	Min	Max	Mean	Var	SD	SE	CV	Min	Max	Mean	Var	SD	SE	CV	Min	Max	Mean	Var	SD	SE	CV
DIF	90.00	126.00	106.17	92.88	9.64	2.15	9.08	90.00	121.00	109.00	86.53	9.30	2.08	8.53	96.00	129.00	112.65	120.56	10.98	2.46	9.75
DFF	92.00	129.00	111.22	81.78	9.04	2.02	8.13	94.00	127.00	114.85	77.92	8.83	1.97	7.69	98.00	133.00	118.75	103.49	10.17	2.27	8.57
DHF	96.00	145.00	119.25	136.59	11.69	2.61	9.80	120.00	155.00	143.20	80.06	8.95	2.00	6.25	101.00	139.00	128.40	118.33	10.88	2.43	8.47
SPY	7.98	33.21	19.38	54.87	7.41	1.66	38.22	8.67	36.57	23.15	67.05	8.19	1.83	35.37	3.43	46.27	18.92	119.65	10.94	2.45	57.82
BM	16.02	43.36	29.88	76.82	8.76	1.96	29.33	18.00	59.00	34.49	143.89	12.00	2.68	34.78	14.03	67.43	34.04	212.51	14.58	3.26	42.83
TDM	25.74	76.57	49.26	222.28	14.91	3.33	30.26	30.02	95.57	57.64	337.45	18.37	4.11	31.87	17.83	95.20	52.95	353.63	18.80	4.20	35.51
HI	25.36	49.53	38.71	43.78	6.62	1.48	17.09	24.59	49.17	40.05	52.22	7.23	1.62	18.04	4.84	55.19	35.34	193.16	13.90	3.11	39.32
PH	77.72	164.80	106.45	477.31	21.85	4.89	20.52	22.73	142.67	83.24	1898.33	43.57	9.74	52.34	56.37	153.73	93.74	595.28	24.40	5.46	26.03
TN	6.20	19.40	12.61	12.59	3.55	0.79	28.14	8.67	57.47	25.67	288.35	16.98	3.80	66.16	10.00	42.00	22.81	75.85	8.71	1.95	38.18
PTN	6.20	18.60	12.02	12.64	3.56	0.80	29.57	8.00	43.98	19.87	143.95	12.00	2.68	60.38	10.00	42.00	19.87	58.39	7.64	1.71	38.45
PW	1.73	5.70	3.73	1.29	1.14	0.25	30.49	1.73	5.96	3.93	1.37	1.17	0.26	29.83	1.41	6.21	3.79	2.10	1.45	0.32	38.22
TGW	2.04	27.67	16.84	27.67	5.26	1.18	31.25	9.73	23.57	16.50	11.76	3.43	0.77	20.79	11.00	25.33	16.53	14.98	3.87	0.87	23.42
PL	21.08	27.00	23.69	3.48	1.86	0.42	7.87	20.13	29.80	23.67	5.06	2.25	0.50	9.50	19.53	27.43	23.37	3.67	1.92	0.43	8.20
FG	105.33	297.83	167.05	2253.66	47.47	10.62	28.42	105.33	305.00	175.20	2999.45	54.77	12.25	31.26	80.33	290.67	165.77	2506.84	50.07	11.20	30.20
UFG	5.17	57.67	22.45	203.19	14.25	3.19	63.49	4.67	41.95	23.85	128.03	11.32	2.53	47.44	2.00	73.67	22.96	264.62	16.27	3.64	70.86
GN	118.00	333.67	189.50	2714.64	52.10	11.65	27.49	124.33	334.67	199.05	3321.30	57.63	12.89	28.95	111.67	332.67	188.72	2689.97	51.86	11.60	27.48
SPF	72.82	96.71	88.28	38.79	6.23	1.39	7.06	76.60	96.25	87.75	35.58	5.96	1.33	6.80	52.16	98.89	87.50	98.85	9.94	2.22	11.36

DIF- Days to initial flowering, DFF- Days to fifty percent flowering, DHF- Days to hundred percent flowering, SPY(g)- Single plant yield, BM(g)- Biomass, TDM- Total dry matte(g)r, HI- Harvest index, PH- Plant height (cm), TN- Tiller number, PTN- Productive tiller number, PW- Panicle weight(g), TGW- Thousand grain weight, PL- Panicle length(cm), FG- Filled grains, UFG- Unfilled grains, GN- Grain number, SPF- Spikelet fertility.

lysis and easy grinding, after grinding another 400 µL of CTAB buffer was added. This mixture was kept in water bath up to 60°c for 1 h. After heating the mixture was allowed to cool up to room temperature and then 800 µL of chloroform, isoamyl alcohol (24:1) was added to the 800 µL solution. After mixing vigorously, samples were centrifuged at 12000 rpm for 15 min. It formed two layers; upper aqueous layer (DNA) and bottom organic layer (cell debris). The upper aqueous solution was transferred to another centrifuge tube (1.5 µL) and chilled isopropanol with equal amounts of aqueous solution was added for precipitation of DNA and the mix was kept in −20° for 2 h and was then centrifuged at 10000 rpm for 10 min to separate the precipitate from the solution. After centrifugation the solution was discarded by retaining the DNA precipitate carefully and 70% ethanol was added to the precipitate and centrifuged at 8000 rpm for 5 min to remove impurities. Then the tube was kept for drying to eliminate the ethanol traces and 100 µL TE buffer was then added to DNA pellet and gently tapped to dissolve DNA. DNA quantification was done by using Nano Drop ND-1000 Spectrophotometer (Wilmington, USA).

2.6.2. PCR reaction

The DNA was diluted to 50 ng for 10 µL reaction PCR in 96 well Genaxy PCR plate in G-storm PCR tetrad. DNA quantity was estimated spectro-photometrically using nanodrop. SSR primers were obtained from Integrated DNA Technology (IDT, USA (**Supplementary Fig. 1**). Each reaction mix of 10 µl contained 3 µl genomic DNA (50 ng), 1 µl each of forward and reverse primer (20 mm), 1 µl of 10× buffer (0.1 M Tris, pH 8.3, 0.5 M KCl, 7.5 mM $MgCl_2$, 0.1% gelatin), 0.2 µl of 10 mM dNTPs (from 2.5 mM stock) and 5 units of Taq polymerase (0.1 µl), 3.6 µl sterile distilled water. All the PCR reagents were purchased from Fermentas, India. PCR conditions for SSR–PCR were initial denaturation 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 at 72 °C for 7 min.

2.6.3. SSR genotyping

The amplification products were mixed with loading buffer (40% sucrose and 0.25% bromophenol blue) and resolved on 4.5% agarose gel in 0.5X TBE buffer under room temperature at a constant voltage of 150 V and detected by ethidium bromide staining. The resolved bands were documented using a gel documentation system (Perkin Elmer, Geliance 200 imaging system). The banding patterns obtained were scored in a binary matrix based on the presence (1) or the absence (0) of a particular band. After PCR reaction was completed the samples (7 µL) were loaded in wells of agarose gel (3%), the gel was run at 120 V in agarose gel electrophoresis unit clear bands were obtained. Gel documentation showed the difference in band size. Molecular screening was done using SSR markers flanking previously reported yield QTLs and was graphically presented using GGT ver 2.0 (Supplementary Fig. 1).

3. Results

3.1. Seedling vigour

Wide variation was observed among the BILs when compared with Swarna, BILs 248S, 148S and 14S for germination percentage, shoot and root length, seedling fresh weight and dry weight (**Table 1**). Germination percentage of BILs 166-2-11S, 166-2-2S, 166-23S, 166-20S, 166-1S, 166-30S and 166S was higher than Swarna. There was a wide variation between germination percentage of BILs 166S and 166-32 with 166S (**Fig. 1**). BIL166-2-10S, 166-23-1S, 166-2-2S and 166-2-7S showed higher shoot length (SL) than Swarna at 7th day after germination. BILs 166-23-1S, 166-2-7S, 166-1S, 166-2-5S, 166-30S, 166-20S, 166-2-10S showed higher SL than Swarna and 248S at 14 days. 166-32S showed high root length (RL) at 7 d after germination, 166-9S showed highest RL at 14 d after germination. Likewise, BILs 166-23-1S, 166-2-

7S, 166-2-10S, 166-32S and 166-2-3S had higher RL than Swarna at 14 d after germination.

Fresh weight (FWT) values were higher than Swarna in BIL166-2-7S followed by 166-23-1S, 166-2-10S, and 166-9S. All the BILs showed higher FWT than 248S. BIL166-9S and 166-2-10S had higher dry weight (DWT) than Swarna and 248S. BILs166-1S, 166-2-11S, 166-2-2S, 166-23-1S, 166-2-10S, 166-2-3S and 166-30S showed higher DWT than 248S only. BIL166-2-5S showed higher rate of increase in shoot length (RSL) and rate of increase in root length (RRL) than Swarna and 248S. BILs 166-30S, 166S, 166-9S, 166-2-10S, 166-1S were phenotyped with high RSL% than Swarna and 248S. BILs 166-9S, 166-30S, 166-2-11S, 166-23-1S, 166-2-10S, 166S showed higher RRL% than 248S and Swarna. BIL166-2-3S had higher seedling vigour index at 24 h than Swarna and 248S. BILs 166-23S, 166-2-2S, 166S, 166-2-11S, 166-1S, 166-20S showed higher SVI at 24 h than Swarna and 248S and 166-1S, 166-2-2S, 166-2-11S showed higher SVI at 65 h.

3.2. Yield

Fifteen BILs showed wide diversity among them for yield traits in three seasons (**Table 2**). Days to fifty percent flowering values ranged from 98 days (166-2-3S) to 129 days (166S) in *Kharif* 2014, in *Rabi* 2015 it ranged between 102 days (166-2-3S) to 133 days (166-1S) and in *Kharif* 2015 it was between 94 days (166-2-3S) to 127 days (166-1S). BIL166-9S showed high yield per plant in *Kharif* 2014 and *Rabi* 2015, but in *Kharif* 2015 showed low yield levels. In *Kharif* 2014, yield ranged from 7.98 g (166-2-5S) to 33.21 g (166-9S). In *Rabi* 2015 it was from 3.43 g (166-2-10S) to 34.53 g (166-9S) and in *Kharif* 2015, it ranged from 8.67 g (166-2-5S) to 36.57 g (166-2-10S). 166S showed highest SPY and HI; BILs166-9S, 166-2-3S, 166-30S, 166-2-2S, and 166-23S showed high and maximum mean values for SPF, TGW, GN and PW. 166-20S had highest TN and PTN. Overall, BIL166-9S showed higher values for all the yield traits followed by 166S, 166-1S, 166-2-3S and 166-2-7S.

BILs were compared with their recurrent parent Swarna for yield as they had different grain type, panicle architecture (**Supplementary Fig. 2**). All BILs flowered earlier (2–21days) than Swarna except 166S and 166-1S (**Table 2**). Two BILs 166-2-10S and 166-2-11S showed higher SPY than Swarna. Eight BILs 166S, 166-9S, 166-2-3S, 166-21S, 166-2-7S, 166-1S, 166-32S and 166-20S showed both higher SPY and higher HI than Swarna. 166-20S, 166-2-5S, 166-2-10S, 166-2-2S, 166-21S, 166-32S and 166-2-7S had highest TN and PTN. 166-2-2S, 166-9S, 166-2-3S, 166-2-7S, 166-2-10S, 166-2-5S, 166-23-1S, 166-30S and 166-2-11S showed higher PW and TGW than Swarna. BILs166-2-7S, 166-2-2S and 166-23-1S showed higher GN and SPF. Except 166S and 166-1S (121-125 d for days to initial flowering), all the BILs were earlier duration (1-20 d earlier when compared with Swarna and released variety 248S (DRRDhan40) and 166-2-3S and 166S showed highest HI values 46.80% and 46.98% respectively than Swarna and 248S. BILs also showed different panicle architecture compared with recurrent parent Swarna. GN was comparatively higher in 166-23-1S, 166-2-11S, and 166-30S than 248S. Two BILs 166-9S and 166-2-3S were earlier maturity duration than Swarna and 248S across the three seasons.

3.3. Correlation

Correlation analysis was conducted for yield and vigour traits (**Table 3**). Highly significant positive correlation was observed between yield traits, PW and FG, PL and SPF, FG and GN; DFF with TGW and GN; SPY with BM, HI and SPF; BM with PL and SPF; PH with TGW and PL; TGW with PL and SPF and highly significant negative correlation was observed between HI and PW, PH and TN, TGW and GN. Significant positive correlation was observed between DFF and FG; SPY and PL; PH and SPF; PW and GN and significant negative correlation between TGW and FG.

Highly significant positive correlation was observed between yield

Table 3
Correlation matrix of yield and vigour traits in 166S (Swarna x *O. nivara* elite BIL) derived lines along with control.

	DFF	SPY	BM	HI	PH	TN	PW	TGW	PL	FG	GN	SPF	SL	RL	DWT	RSL	RRL	G (%)	SVI
DFF	1																		
SPY	0.13	1																	
BM	−0.10	0.47***	1																
HI	0.15	0.80***	−0.12	1															
PH	−0.10	0.26	0.34*	−0.01	1														
TN	−0.16	−0.23	0.16	−0.27	−0.67***	1													
PW	0.01	−0.23	0.34*	−0.46***	−0.03	0.33*	1												
TGW	0.49***	0.39*	0.33*	0.26	0.54***	−0.27	−0.23	1											
PL	−0.21	0.44**	0.56***	0.14	0.71***	−0.25	0.15	0.64***	1										
FG	0.43**	0.02	0.08	−0.13	0.18	−0.29	0.46***	−0.40**	0.09	1									
GN	0.46***	−0.12	−0.09	−0.18	0.08	−0.28	0.42**	−0.52***	−0.06	0.97***	1								
SPF	−0.12	0.52***	0.71***	0.14	0.43**	−0.06	0.20	0.48***	0.64***	0.19	−0.05	1							
SL	−0.38*	0.27	0.34*	0.08	0.62***	−0.40*	0.12	0.69***	0.51***	−0.06	−0.15	0.36*	1						
RL	−0.08	0.61***	0.07	0.60***	0.37*	−0.44**	−0.24	0.41**	0.42**	0.17	0.09	0.30*	0.61***	1					
DWT	0.02	0.08	0.40*	−0.16	0.14	−0.11	0.20	−0.02	−0.05	0.04	−0.04	0.32*	0.30*	0.04	1				
RSL	0.17	−0.09	0.19	−0.28	0.52***	−0.35*	0.24	0.17	0.08	0.18	0.12	0.28	0.26	−0.2	0.40*	1			
RRL	0.14	−0.09	0.13	−0.30*	0.45**	−0.31*	0.14	0.15	0.05	0.20	0.18	0.13	0.28	0.01	0.35*	0.83***	1		
G%	0.19	0.26	−0.24	0.42**	0.42**	−0.53***	−0.31*	0.37*	0.09	−0.47***	−0.45**	−0.1	0.21	0.30*	0.12	0.33*	0.15	1	
SVI	0.14	0.22	0.20	0.10	0.30*	−0.37*	−0.30*	0.39*	0.01	−0.43**	−0.43**	−0.07	0.39*	0.23	0.88***	0.50***	0.40	0.55**	1

Significance levels: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. DFF- Days to fifty percent flowering, SPY(g)- Single plant yield, BM(g)- Biomass, HI- Harvest index, PH(cm)- Plant height, TN- Tiller number, PW(g)- Panicle weight, TGW(g)- Thousand grain weight, PL(cm)- Panicle length, FG- Filled grains, GN- Grain number, SPF- Spikelet fertility, SL- Shoot length, RL- Root length, DWT- Dry weight, RSL- Rate of increase in shoot length, RRL- Rate of increase in root length, G%- Germination percentage at 65 h, SVI- Seedling vigor index.

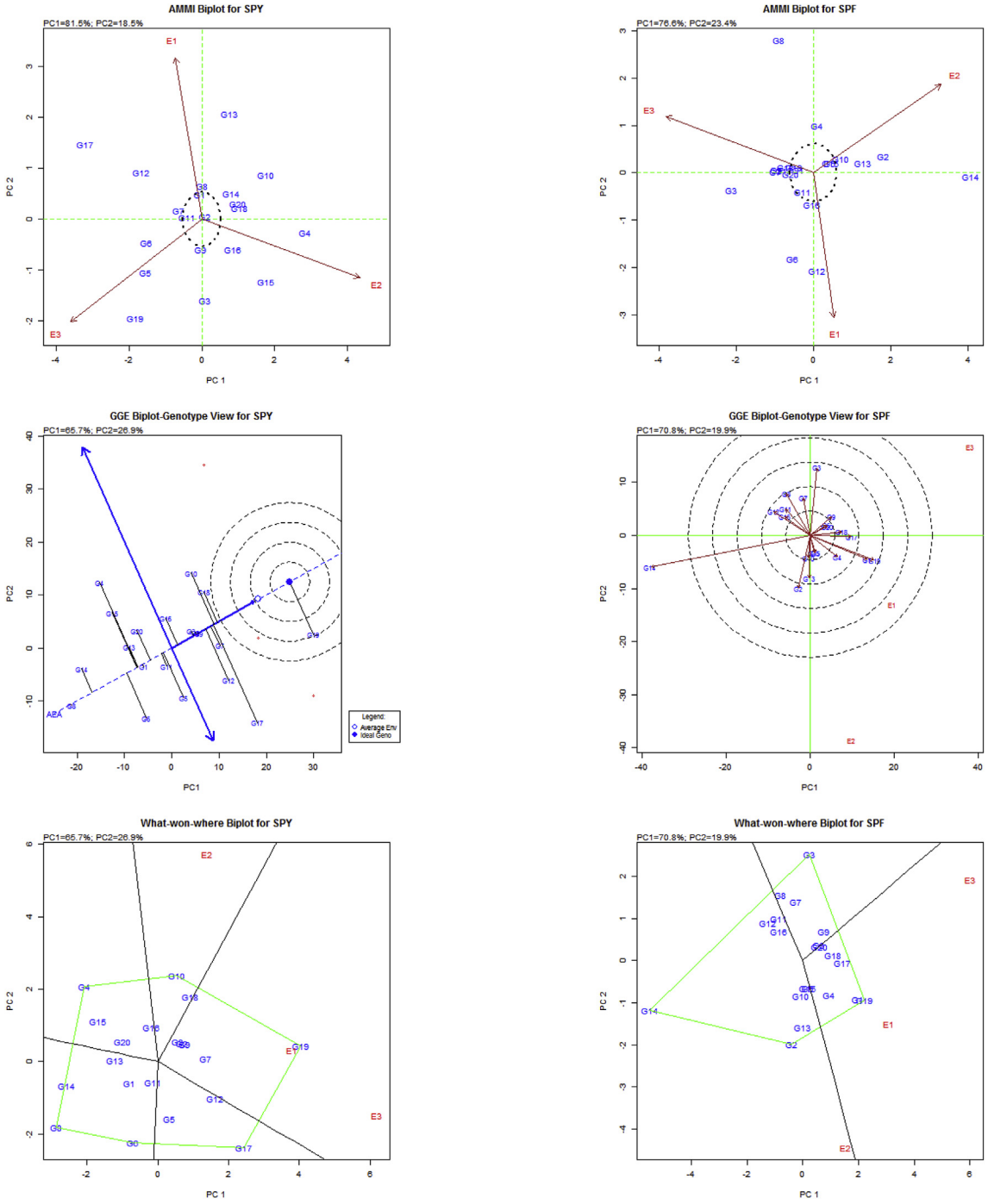

Fig. 3. AMMI and GGE biplot for the primary component of interaction (PC1) or main effect of BILs in different environments for single plant yield and spikelet fertility. Polygon views of the GGE biplot based on symmetrical scaling for 'which-won-where' pattern of BILs in three environments for single plant yield and spikelet fertility.

and vigour traits eg. SPY with RL; HI with RL; TGW with SL; PL with SL; PH with SL and RSL. Highly significant negative correlation was observed between G% with TN and G% with FG. There were significant positive correlations between HI and G%, TGW and RL, PL and RL, PH with RRL and G%. The traits, TN and RL, FG with G% and SVI, GN with G% and SVI showed significant negative correlation. Correlation in vigour traits was highly significant and positive between SL and RL, DWT and SVI, G% and SVI, RSL with RRL and SVI.

3.4. Stability analysis

We employed both AMMI and GGE biplot analysis for stability analysis and what-won-where plots (Fig. 3) of GGE graphs indicated best genotypes for each or all environments. The vertex genotype in each sector shows the sector as its best responsive environment and the response plot explains the specific genotype with high yield in a particular environment and found that for PL, BIL166-2-10S was ideal in E1 and E3 (both *Kharif* seasons) and 148S was ideal in E2. For SPF, 248S was better in E1 and E3 (*Kharif* seasons). In E2 166-23S and 14S were most responsive for SPF. For SPY, 248S was suitable in E1 and E3,

166-2-3S and 166-2-10S in E2 and 166S and 166-32S in E1. For TGW, 148S was best in E1 and E3 and 166S was best in E2. For DFF, BIL166-1S showed high mean values for the three environments. BIL166-30S showed high grain number in three environments and was most responsive in those environments.

Analysis showed significant results for yield and stability by AMMI biplot, average environment co-ordinate method (ranking genotypes based on their mean yield and stability) and genotype ranking based on ideal genotype. In case of DIF, BIL166-1S showed high mean value and it was near zero in biplot origin and showed stable performance across the environments. Other genotypes showing positive genotypic response in mean and stability for DIF were BIL166S, Swarna, 166-2-10S, 166-23-1S, 166-2-2S, 166-23S, 166-21S, 166-30S and 166-9S. For GN, 166-30S was the most stable genotype followed by BILs 166-2-11S, 166-2-2S, 166-23-1S, 166-2-10S, 248S, 166-2-7S, 166-23S and 166-32S. In addition, 166S and 166-2-3S were very close to the origin and were stable with high mean SPY. For HI, BILs 166S, 14S, 166-32S, 166-20S, Swarna were near to the biplot origin and had high mean value and stability. For PW, 166-2-2S was most stable. For TGW 148S was best followed by 166-2-3S. 14Sand 248S showed high yield stability.

3.5. Detection of known yield QTLs from O. nivara in BILs

To detect *O. nivara* derived yield QTLs in BILs, 13 reported linked SSR markers were used. *O. nivara* alleles were present at flanking markers RM9 and RM5 of QTL *bm1.1* in 166-20S and 248S. QTLs *dtm2.7, nsp1.2* were detected in 166-9S and 166-2-9S between RM250 and RM166 and QTL *bm2.2* between RM166 and RM535 on chromosome 2. Interestingly, 166-9S had high SPY and BM. Yield enhancing allele of *O. nivara yldp9.1* between flanking markers RM434 and RM257 was identified in eight BILs which were higher yielding than Swarna. *nt12.1* was present between flanking markers RM415 and RM19 in 166-23-1S, 166-2-5S but showed only one of the flanking marker with *O.nivara* allele for each of the three QTLs *dtm9.3, yldp9.1* and *nt12.1*. In these, *nt12.1* responsible for higher number of tillers was found in 166-2-5S and the trait enhancing allele was from *O. nivara*.

4. Discussion

Wild species are an excellent source to introgress novel genetic variation into cultivars. In this study, among the 15 BILs evaluated for yield traits in three seasons seven BILs 166S, 166-9S, 166-30S, 166-20S, 166-23S, 166-2-5S and 166-2-9S were significantly higher yielding than Swarna. Of these seven BILs, four BILs (166S, 166-2-5,166-9 and 166-30) were part of a set of CSSLs and had 10–14% chromosomal segments from *O. nivara* [21]. BIL166-2-3S was an early line (94 days to initial flowering) among the 16 BILs in all three seasons and can be used as a donor for earliness.166S was previously identified as one of the stable lines when tested in multiple environments [7].

Significant correlations between yield and vigour traits have been reported previously by Ref. [2]; [29] and [1]. Our results showed highly significant positive correlation of SPY and TGW with seedling root length. Previous studies showed association of SPY and TGW with seedling dry weight [2]. [29] reported association of root length with shoot length and dry weight. We observed positive significant association of SVI with DWT, SL with RL, and PL with both TGW and SPY. Highly significant positive correlation was observed between DFF and TGW in our study. There was significant positive association between DWT and SL and also between SVI and DWT. Rapid dry weight accumulation at early stage is an essential component trait in determining seedling vigour irrespective of environment [5,17,20].

Uniformity in germination and faster biomass accumulation during initial seedling establishment is an important phenotypic trait for direct seeded system in rice irrespective of climate conditions for a good crop stand and weed competitiveness [20,27]. BILs 166-2-7S and 166-32S showed high shoot and root length after 7 days. However, at 14 days, another BIL166-9S had highest shoot and root length which was reflected in its highest SPY, TGW and SVI among all BILs. Thus seedling vigour differs depending on when it is measured at 7 d or 14 d. At the early stage of seedling growth, the growth is largely dependent on seed reserve and usually the vigour improves with seed size. Rapid early growth might be due to large seed size which may supply more sugar for seedling growth [6]. High seedling vigour might also be due to high amylase activity or rapid utilization of reducing sugars. Early vigour of seeds can be increased by sowing larger seeds and often the size of the embryo is a major factor determining seedling vigour. BIL166-9S has highest seed weight among all the BILs along with 166-2-3S, which might have contributed to its high vigour. [9] evaluated advance backcross population of M-202/*O. nivara* IRGC100195 for seedling vigour traits of shoot length, root length, coleoptile length, mesocotyl length and identified that *O. nivara* alleles improved seedling vigour by increasing coleoptile and shoot length.

Of all these 15 BILs, 166S and 166-1S were already identified as stable genotypes in the previous study of the same cross using genotype × environment interactions and showed good seedling vigour [1,7]. BIL 166-9S with high vigour and yield (26.54 g/plant), harbouring alleles for spikelet number per panicle from *O. nivara* at RM9 and RM 434 can be directly use to transfer the alleles into modern cultivars [16]. [17] reported that 166S exhibited superior performance under direct seeded condition and conservation agriculture practices. IL166S was reported to be also drought and salinity tolerant. It showed high % seed germination even under 200 mM NaCl, had significantly high proline level and its grain yield was least affected in saline (150 mM) soil in pots [10]. BIL 166S, designated as IET21938 (RPBio4918-166S), was tested in 12 saline, alkaline locations in India and it was better than best check CSR36 for alkaline areas at one location Kumarganj (UP). Otherwise stable lines 166-2S and 166-30S were identified as highly susceptible to heat specially in dry season after evaluating 48 introgression lines for heat tolerance under irrigated field conditions [22]. Rao et al. reported stable photosynthesis rate in 166-1 and 166-2 and 166-2 showed highest water use efficiency in wet season. 166s was reported as salinity tolerant based on their seed germination and seedling growth response under different salinity treatments. The attenuation in yield potential was comparatively low in 166S due to its efficient compartmentalization of Na^+ in leaf tissue [10].

Molecular screening of BILs was carried out for the presence of reported yield related QTL alleles from *O. nivara*. Two BILs 166-2-9S and 166-9S with high BM had *O. nivara* alleles of a QTL reported for biomass production *qbm1.1* at RM5-RM9 chromosomal region. This chromosomal region for the QTL *qbm1.1* was identified in the BC_2F_2 population of the same cross of Swarna/*O. nivara* and the trait enhancing allele was from *O. nivara* [25]. Interestingly the same flanking marker RM9 was reported for seedling vigour related traits such as germination percentage, shoot fresh weight and dry weight [5,20]. Also, 166-2-9S showed *O. nivara* allele for YLDP at earlier reported yield QTL region RM250-RM535 linked to *qyldp2.1* and BIL 166-9S at RM434-RM257 for *qyldp9.1* [25]. BIL166-20S with high tiller number had *O. nivara* alleles at RM9-RM5 and Swarna alleles at loci RM415-RM19 on chromosome 12. QTLs *qnt12.1, qnpt12.1* for TN and QTL *qlwr12.1* for length/breadth ratio, *qmp12.1* for milling percentage were also reported at the same region [25]. RM250 flanking *qyldp2.1* in 166-2-9 was earlier found to be associated with dry matter accumulation in a panel of 629 rice genotypes representing breeding lines and landraces studied under direct seeded aerobic situation. RM19 was associated with germination rate, seedling dry weight and shoot length [5] explaining the association of seedling vigour and yield traits and the common genomic regions contributing to both. QTL *qNPT1.1* was identified at RM5 locus in BC_2F_8 mapping population of Swarna/*O. nivara* IRGC81848 cross and increasing effect was from *O. nivara* [21]. [15] reported QTL *qnt1.1* at RM9 locus in another cross of Swarna/*O. nivara* IRGC81832 BC_2F_2 mapping population and trait enhancing alleles were from *O. nivara*.

166-2-9S had yield enhancing alleles from *O. nivara* at RM250 locus on chromosome 2 and 166-9S with *O. nivara* alleles at RM9 and RM434 position on chromosomes 1 and 9 respectively. 166S has homozygous *O. nivara* alleles at other loci (RM219, RM517, RM6318, RM25, RM4, RM254) linked to yield [13]. Thus these derivatives of 166S are an interesting genetic stock that can be screened for several traits of interest.

5. Conclusion

Our results demonstrate that *O. nivara* introgression lines can help improve seedling vigour and yield related traits. Of fifteen 166S derived lines, 166-30S was the most stable line for grain number and had higher seed germination, shoot and root length than Swarna. Seven BILs performed significantly higher than Swarna for yield traits PTN, PW, SPY, BM, HI, FG and GN. BILs 166S, 166-30S, 166-9S, 166-2-9S with high SPY and BM and 166-20S with high TN are potential donors for rice improvement programs. Significant association was also observed between yield and vigour related traits. Stable lines identified in this study are useful in breeding programmes for direct seeded rice and for mapping and gene discovery for different traits.

Acknowledgements

This research was conducted under project (ABR/CI/ BT/11) on Mapping Quantitative Trait Loci (QTLs) for yield and related traits using backcross inbred lines (BILs) from Elite x Wild crosses of rice (*Oryza sativa* L.) as part of ICAR- National Professor Project (*F.No*: Edn/27/4/NP/2012-HRD) funded by Indian Council of Agricultural Research, New Delhi, India. These lines were developed in Department of Biotechnology (DBT) New Delhi, India funded project BT/AB/FG-2 (Ph-II) 2009 to SN. The authors are grateful to the Director, ICAR- IIRR for providing facilities

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.egg.2019.100036.

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