ISSN 1479-2621

Plant Genetic Resources; 1–12
doi:10.1017/S1479262118000187

Swarna × *Oryza nivara* introgression lines: a resource for seedling vigour traits in rice

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

ICAR-National Professor Project, ICAR-Indian Institute of Rice Research, Hyderabad, India

Received 16 March 2018; Accepted 6 July 2018

Abstract

Seedling vigour is an important indicator of crop establishment, subsequent crop growth and yield. Initial seedling vigour is most vital in case of water-limited conditions and in environments where the crop is exposed to different stresses at the early growth stage. Wild and weedy species are well known for their vigour and survival in adverse environmental conditions. Seedling vigour traits of backcross introgression lines (BILs) derived from Swarna × *Oryza nivara* IRGC81848(S) (accession from Uttar Pradesh, India) and IRGC81832 (K) (accession from Bihar, India) were studied in wet (*Kharif*) and dry (*Rabi*) seasons. Seedling vigour was estimated in terms of plant height and tiller number at 30 and 60 d after transplanting under field conditions. In both the seasons, 148S showed highest seedling vigour for plant height. The highest number of tillers were produced by 7K in *Kharif* and 248S in *Rabi* season. 75S showed the highest percentage increase in tiller number consistently. High yielding BILs 166S, 14S and 148S showed higher seedling vigour indices compared with checks Tulasi and Sahbhagidhan. Seedling vigour was also evaluated using paper roll method and shoot length, root length and dry weight were used to estimate vigour index. Season-wise association studies were conducted to determine the relative contribution of seedling vigour to yield traits. Seedling vigour was significantly correlated with yield traits. Markers RM217 and RM253 on chromosome 6 differentiated lines with high seedling vigour from those with low seedling vigour and have the potential for use in marker-assisted breeding.

Keywords: backcross introgression lines, seedling vigour index, SSRs, yield, wild

Introduction

Major shifts in rice cropping system from irrigated to aerobic conditions and also from transplanting to direct sowing are essential in the climate change scenario. Rice cultivation is slowly shifting from irrigated to rainfed and it has been reported that 50% of rainfed rice area of India is under direct seeded rice (DSR) (Anandan *et al.*, 2016). Seedling vigour is a vital trait for crop establishment, better survival, weed competition and nutrient use efficiency and also for normal growth under conditions of limited inputs and abiotic stresses. Quick germination and growth of seedling with high capacity to take nutrients have been shown to be important traits for DSR in rainfed lowlands where soil moisture and nutrients are often limited (Yamane *et al.*, 2018). Even though considerable genetic variation in seedling vigour exists in *Oryza* species it is difficult to enhance vigour traits in genotypes as the inheritance is quantitative and mostly dependent on environment and crop duration (Li and Rutger, 1980; Redona and Mackill, 1996a). Understanding the genetic and molecular basis of traits related to seedling vigour is essential for crop improvement as it determines not only initial establishment but subsequent crop growth and yield.

*Corresponding author. E-mail: sarla_neelamraju@yahoo.com

Wild species are important donors in rice breeding programme as they have many beneficial alleles for rice improvement (Tanksley and McCouch, 1997; Wang *et al.*, 2002; Sarla *et al.*, 2003; Gur and Zamir, 2004; Swamy and Sarla, 2008; Swamy *et al.*, 2011; Eizenga *et al.*, 2013) which were eliminated during domestication. The wild progenitor species *Oryza nivara* is easily crossable with cultivated rice and can be readily exploited in breeding programmes without embryo rescue procedures (Niroula and Bimb, 2009) and has been used in inter-specific crosses as a source of new alleles (Wickneswari *et al.*, 2012; Haritha *et al.*, 2018). *O. nivara* accessions have abundant genetic diversity (Joshi *et al.*, 2000; Sarla *et al.*, 2003; Juneja *et al.*, 2006); however, this species has not been used extensively in breeding programs for seedling vigour traits. In our previous studies *O. nivara* accessions were used as donors to develop backcross introgression lines (BILs) and Chromosome segment substitution lines (CSSLs) libraries in the background of popular Indian rice varieties (Kaladhar *et al.*, 2008; Swamy *et al.*, 2014; Malathi *et al.*, 2017). The introgression lines derived from these crosses have been extensively characterized using molecular markers and field evaluated for yield and other agronomically important traits (Thalapati *et al.*, 2014; Divya *et al.*, 2016; Haritha *et al.*, 2016; Malathi *et al.*, 2017; Prasanth *et al.*, 2017). Previous reports indicated that wild species are an excellent source to introgress novel genetic variation for vigour into cultivars (Rangel *et al.*, 2006; Borjas *et al.*, 2016; Eizenga *et al.*, 2016). Though wild genotypes are known for their high vigour and establishment compared with cultivars, introgression lines derived from them have rarely been evaluated for seedling vigour. This study was undertaken to identify the BILs showing early seedling vigour and to determine if seedling vigour correlates with yield traits and their association with selected molecular markers.

Materials and methods

Plant material

Twenty-three rice genotypes including 14 high yielding BILs derived from BC_2F_6 of Swarna/*O. nivara* (14S, 14-3S, 148S, 166S, 166-1S, 166-2S, 248S, 65S, 70S, 75S, 24K, 250K, 3-1K, 7K) and nine high yielding cultivars of different maturity durations (IR64, Jaya, MTU1010, MTU1081, NLR34449, Sahbhagidhan, Swarna, Tellahamsa and Tulasi) were studied for various seedling vigour related traits (online Supplementary Table S1). S indicates lines originated from BC_2F_2 of the cross Swarna/*O. nivara* IRGC81848, an accession from Uttar Pradesh, India and K lines are from BC_2F_2 of Swarna/*O. nivara* IRGC81832, an accession from Bihar, India. These are the lines selected from the respective mapping population.

Assessment of seedling vigour-related traits

Seedling vigour is measured using different parameters such as germination rate (GR), coleoptile length, shoot-root length, shoot-root weight, plant height and tiller number (Redona and Mackill, 1996a, b; Cui *et al.*, 2002; Sun *et al.*, 2007; Wang *et al.*, 2010). Screening of these BILs was conducted at laboratory conditions during *Kharif* 2013 along with checks. Seedling-vigour related traits such as seed germination percent (GP), seedling shoot length (SL), seedling root length (RL) and dry matter weight were determined by using the paper-roll test method (Zhang *et al.*, 2005b) and petridish method (Subudhi *et al.*, 2015) with two replications. For the study, 70 well-filled grains of each genotype were first treated at 50 °C for 5 d to eliminate residual dormancy, surface-sterilized with 0.6% sodium hypochlorite solution for 15 min, rinsed three times with sterile distilled water and 2 sets containing 25 seeds of each genotype were kept for germination by soaking in sterile distilled water at 30 °C. Germinated seeds were counted at 7 d after incubation. Seed GP was calculated as the percentage of seeds germinated. Seed with both coleoptiles and radical protrusion ≥ the length of the seed itself was considered germinated.

Ten seeds for each replication were then placed equidistantly in sterile distilled water saturated blotter paper mounted on polythene film, each piece of paper is 50 cm long and 30 cm wide. These were rolled up carefully with their two distal edges tied with rubber bands to prevent seed removal; these seed-loaded paper rolls were vertically held in sealed polythene bags to avoid water evaporation and incubated at normal room temperature. At 7 d and 14 d after incubation 10 seedlings for each genotype were sampled to measure their SL and RL. Then the residual seed grains attached to the seedlings were removed and the seedlings were dried in an oven at 80 °C for 3 d. Dry matter weight of seedlings was measured and expressed as mg/10 seedlings. Root to shoot ratio was derived from dividing root dry weight by shoot dry weight (Cairns *et al.*, 2009). Seedling Vigour Index-1(SVI1) was calculated with the help of data recorded on seedling growth at 7 d and 14 d after sowing and germination percentage (GP) according to Kharb *et al.* (1994) using the formula:

$$\text{Seedling Vigour Index 1} = \frac{\text{Seedling length} \times \text{Germination percentage}}{100}$$

Seedling Vigour Index-2 (SVI2) was estimated using seedling dry weight at 14 d after sowing by the formula adopted by Kharb *et al.* (1994)

$$\text{Seedling Vigour Index 2} = \frac{\text{Seedling dry weight} \times \text{Germination percentage}}{100}$$

Field assessment of seedling vigour-related traits

Twenty-five-days-old seedlings were transplanted in the main field (one seedling/hill) during wet season (Kharif) 2013 and dry season (Rabi) 2014 under irrigated conditions at the experimental farm of Indian Institute of Rice Research (IIRR), Hyderabad following a randomized complete block design, with three replications with five rows per replication, 21 plants per row and a spacing of 15 cm between plants within each row and 20 cm between rows. The cultural practices, fertilizer application and plant protection measures were carried out as per standard package of practices during crop growth. Seedling vigour was obtained both for *Kharif* and *Rabi* season in terms of plant height and tiller number from the data taken at 30 d after transplanting and 60 d after transplanting and just before harvest. Observations on crop growth parameters and yield contributing traits were recorded at both pre-harvest and post-harvest stages (IRRI, 2013). At harvest, five plants in the middle of each replication were selected and yield-related traits including days to heading (DH), days to fifty percent flowering (DFF), plant height (PH), tiller number (TN), panicle number per plant (NPT), single plant yield (SPY), bulk yield (BY) and harvest index (HI) were evaluated. The average trait measurements from three replicates in both years were used for data analysis.

Genotyping

Young leaves were collected 20 d after transplantation and genomic DNA was isolated using CTAB method (Doyle and Doyle, 1987). Purity and concentration of DNA were measured using Nano Drop ND-1000 Spectrophotometer (Wilmington, USA). 30 SSR markers linked to reported genes/QTLs (Quantitative Trait Loci) for seedling vigour traits (Zhang *et al.*, 2005a, b; Lu *et al.*, 2007) viz., qsv^{-1}, qsv^{-5}, qsv^{-6} and qsv^{-8} were used to survey the selected 14 BILs compared with the recurrent parent. Polymerase chain reactions (PCR) were carried out in Thermal cycler (Veriti PCR, Applied Biosystems, USA) with the total reaction volume of 10 µl containing 25 ng of genomic DNA, 1× assay buffer, 200 µM of dNTPs, 1.5 mM $MgCl_2$, 10 pmol of forward and reverse primer and 1 unit of *Taq DNA polymerase* (Thermo Scientific). PCR cycles were programmed as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 45 s, 55 °C for 30 s, 72 °C for 45 s and a final extension of 10 min at 72 °C. Amplified products were resolved on 3% agarose gels prepared in 0.5× TBE buffer and electrophoresis was conducted at 120 V for 2 h. Gels were stained with ethidium bromide and documented using gel documentation system (Alfa imager, USA).

Statistical analysis

The field experiments were conducted in a completely randomized block design with three replications. Laboratory screening was conducted using three replications. Statistical analysis was carried out using PB Tools statistical software (version 1.4, http://bbi.irri.org/products) for test of significance. Multiple correlations between vigour and yield traits and for seasonal data and mean were also calculated.

Results

Phenotypic variation for seedling vigour-related traits

Kharif-*2013*

A wide range of variation was found among the set of BILs and cultivars for key traits related to seedling vigour and yield (Table 1). Among all, 148S was the earliest in days to flowering (97 d), days to maturation (120 d) and 7K was late in flowering (138 d) and days to maturation (168 d). 148S flowered 30 d earlier than parent Swarna. Among the nine cultivars, Tellahamsa was early in flowering (100 d) and early in days to maturation (125 d) (online Supplementary Table S2). 148S had the highest vigour for plant height throughout the growth stage (Fig. 1). It also had high SVI1 and high vigour for SL and total dry weight (TDW). Among other BILs, 7K showed high seeding vigour for RL and TDW and 75S showed high RL/SL ratio and vigour for tiller number. 166-1S had the highest RL among BILs and also highest SVI1. 14-3S had least SVI1 and SVI2 (online Supplementary Table S3). Considering yield traits, BY was high in 166S but SPY and HI was high in 14S. 14-3S showed high tiller number before harvest but it had the least SPY, biomass (BM) and HI. Among all the checks, Sahbhagidhan showed high vigour in terms of plant height and RL while Tulasi had high vigour in terms of tiller number and both showed high seedling vigour indices and comparatively higher yield levels.

Rabi-*2014*

In Rabi season also, 148S had high vigour for PH and showed early flowering (99 d) and maturity (122 d). 7K was late in flowering (133 d) and maturity (163 d) among all the BILs. In addition, 248S and 3-1K also

Table 1. Variability of 11 yield-related traits and seven vigour-related traits in Swarna × *O. nivara* BILs

Yield-related traits										Vigour-related traits				
Kharif (wet season) 2013					Rabi (dry season) 2014					Kharif (wet season) 2013				
Variable	Min	Max	Mean	Var	Variable	Min	Max	Mean	Var	Variable	Min	Max	Mean	Var
DFF	97.00	138.33	119.13	11.92	DFF	99.00	133.00	120.39	10.26	GP	48.00	100.00	91.83	14.70
DM	120.00	168.33	146.96	13.84	DM	122.00	163.00	148.22	11.70	SL	7.22	12.75	9.68	1.49
PH	75.33	149.40	99.14	15.43	PH	68.13	136.47	88.18	14.67	RL	11.07	22.10	18.35	2.24
TN	6.40	14.00	9.26	1.72	TN	9.53	22.92	14.89	3.40	R:SDW	0.25	0.86	0.47	0.13
PTN	5.67	13.20	8.35	1.71	PTN	9.33	21.78	14.33	3.15	TDW	0.02	0.10	0.06	0.02
%PH	0.14	0.92	0.52	0.20	%PH	0.28	1.39	0.57	0.21	SVI1	13.75	31.40	25.70	4.89
%TN	0.01	0.31	0.14	0.11	%TN	0.05	3.49	0.75	0.78	SVI2	1.12	10.40	5.67	2.06
BM	12.12	21.42	17.23	2.88	BM	15.86	41.77	24.23	6.49					
SPY	5.35	19.78	12.28	3.28	SPY	7.95	25.66	19.12	4.06					
BY	0.47	2.16	1.43	0.40	BY	0.30	1.81	1.27	0.40					
HI	27.83	56.32	40.76	8.37	HI	32.35	58.04	43.03	6.84					

BM, biomass; BY, bulk yield; DFF, days to fifty percent flowering; DM, days to maturity; GP, germination percentage; HI, harvest index; Min, minimum; Max, maximum; PH, plant height at maturity; PTN, productive tiller number at maturity; RL, root length; R:SDW, root shoot dry weight ratio; %PH, Percent increase in plant height; %TN, Percent increase in tiller number; SPY, single plant yield; SL, shoot length; SVI1, seedling vigour index-2; SVI2, seedling vigour index-2; TN, tiller number at maturity; TDW, total dry weight; Var, variance.

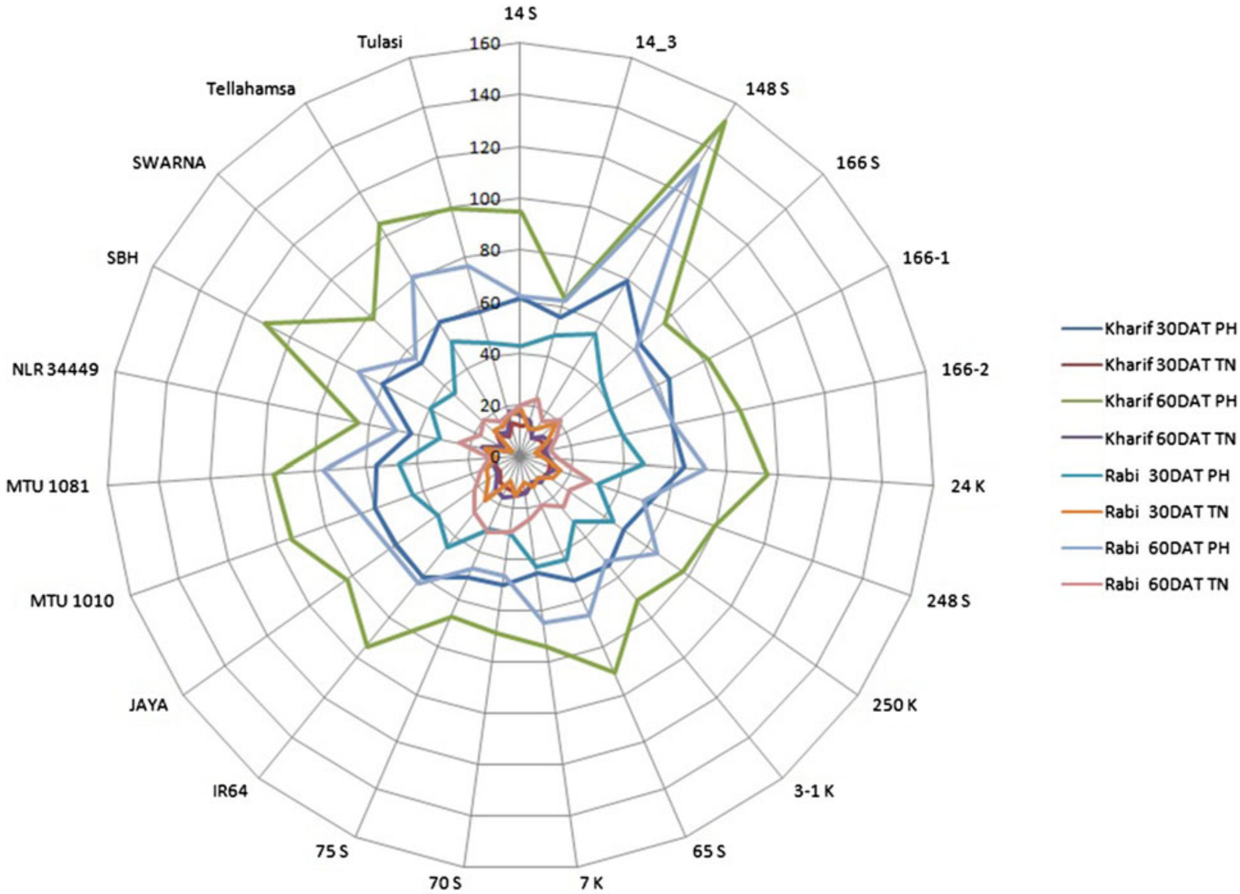

Fig. 1. Variation in plant height and tiller number in seedlings of popular varieties and Swarna ILs at 30 and 60 d after transplanting in field conditions.

showed high vigour for plant height. 75S showed the highest vigour for tiller number at 60 d after transplanting followed by 3-1K, 7K and 14-3S (online Supplementary Table S4). 166S showed high SPY and high tiller number at 30 d after transplanting. Among all the BILs, 166-2S had the least vigour for tiller number. 14S showed high BY, HI and 7K had high BM among all the BILs. 14-3S showed the least SPY, BY and BM. Among checks, Sahbhagidhan showed high SPY and HI during *rabi* season and high SVI2 in laboratory conditions.

In case of TN at 30 d, 7K (Kharif-2013) and 166S (Rabi-2014) showed the highest number; at 60 d, 75S showed high TN in both seasons but at harvest 14-3S (Kharif -2013) and 70S (Rabi-2014) exhibited high TN. High productive tiller number at maturity (PTN) was observed in 14-3S during kharif 2013 and 70S in Rabi 2014. Similarly, phenotypic variation was observed for traits SPY, BY and BM between two seasons. 14S showed high SPY in Kharif 2013, while 166S in Rabi 2014. Likewise, 166S showed highest BY in Kharif 2013 and 14S in Rabi 2014.

Determining seedling vigour using paper-roll method

The results obtained from the paper-roll method for seedling vigour traits revealed that 148S showed good vigour in terms of SL at 7 and 14 d after sowing (Fig. 2) and shoot dry weight (SDW) and SVI1 at 14 d after sowing but it showed very less growth rate in SL at seedling stage among all the BILs and checks. 7K showed high vigour in RL at 7 and 14 d after sowing and low percentage of root elongation. 3-1K showed a high growth rate in RL and lowest RL at 7 d. 166S showed high growth rate in SL, high SVI2 and less vigour in SL and SVI1 at 7 d and RL at 14 d after sowing. 166-2S had highest RDW among the BILs. 14-3S showed less vigour in 14SL, SDW, 14SVI1, SVI2 among all the BILs. High root-shoot dry weight was observed in 75S, 65S, 248S, 166S and 166-2S. Highest TDW was found in 148S and 166S. At 7th and 14th d seedling stage, 148S showed the highest RL and SL among all BILs. However, the tremendous increase was observed from 7 to 14 d in SL in 166S and in RL in 3-1K and 248S. Among all the

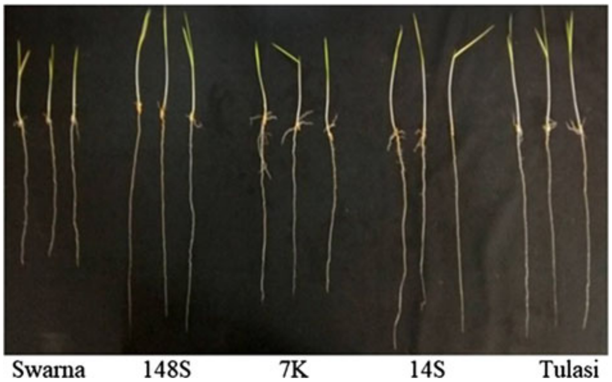

Fig. 2. Variation in seedling vigour among three ILs, Tulasi and Swarna at 14 d after germination using paper roll method.

checks, high SDW, RDW, SVI1 at 7d, SVI2 at 14 d and less growth rate in RL and 14SVI1 was observed in Tulasi. MTU1081 showed good vigour in RL at 7 d and less vigour in terms of SL, RL, RDW, SVI1 and SVI2 at 14 d after sowing. MTU1010 showed high vigour in SL at 14 d and Sahbhagidhan in seedling RL at 14 d after sowing. Tellahamsa showed a high growth rate in seedling RL and SL (online Supplementary Table S4).

Correlation analysis

Correlation analysis (Table 2) revealed that germination% (GP) exhibited significant positive correlation with SPY, BY, HI and both seedling vigour indices. SL was positively correlated with root-shoot dry weight, TDW, SVI1 and SVI2. However, RL and root to shoot ratio were negatively associated with all other vigour parameters. SVI1 and SVI2 were positively correlated with germination%, SL and TDW. DFF and DM were positively correlated with BM but negatively with HI, percent increase in plant height (%PH) and seedling vigour indices in both the seasons. PH showed highly significant positive correlation with % PH and TDW but negative correlation with TN and PTN in both seasons. TN showed highly significant positive correlation with PTN in both seasons. SPY showed significantly positive correlation with HI and SVI2 in both seasons. BY showed significant positive association with GP, TDW and SVI2. Similarly, HI showed significant positive association with GP, SL, R:S DW, TDW, SVI1, SVI2, SPY and BY but showed a negative association with DFF, DM and BM.

Genotyping using vigour-related SSR markers

The lines were screened using 27 previously reported SSR markers for vigour related traits and distributed on all chromosomes except 4, 7 and 10 (online Supplementary Fig. S1). A total of 377 alleles were detected, of which Swarna homozygous alleles accounted for 51%, *O. nivara* homozygous alleles 37% and heterozygous alleles 38%. Considering the *O. nivara* alleles for vigour traits, 14S showed the highest percentage of *O. nivara* alleles followed by 65S. It may be noted that 14S is reported to have 72.6% of recurrent parent genome of Swarna using random genome-wide markers (Divya *et al.*, 2016). Swarna alleles were higher in the most vigourous line 148S but 11% of its alleles were heterozygous. Highest percentage (27.5%) of heterozygous alleles were observed in another high yielding line 166S and its derived line 166-2S. 148S showed more allelic variation for the previously reported markers linked to GP, SL and seedling vigour compared with that seen in other BILs (Table 3). 7K and 14-3S showed *O. nivara* alleles for dry weight-related QTLs. Heterozygous loci for GP and SL were observed in 250K.

Discussion

Seedling vigour is the indicator of ability of plants to accumulate dry matter, tolerance to water limitation and weed competition at an early stage especially in direct and dry sowing conditions (Quarrie *et al.*, 1999; Balasubramanian and Hill, 2002; Zhao *et al.*, 2006; Mahender *et al.*, 2015; Yamane *et al.*, 2018). Genotypic variation for seedling vigour was identified among rice cultivars (Krishnasamy and Seshu, 1989; Redona and Mackill, 1996a, b; Yamauchi and Winn, 1996; Huang *et al.*, 2017). There is enormous genetic diversity for crop growth and vigour in the available rice germplasm especially in landraces, wild and weedy species which can survive in more adverse environmental conditions (Yamauchi *et al.*, 1993; Redona and Mackill, 1996a, b; Namuco *et al.*, 2009).

In this study, variability in vigour traits was observed among BILs at two growth stages and in two seasons in field conditions. Invariably, 148S showed high seedling vigour in both seasons, but other BILs and cultivars showed seasonal variation for both vigour and yield traits. We previously reported that 148S is stable for days to flowering and days to maturity (DM) and 14S and 166S are stable for grain yield compared with Swarna (Divya *et al.*, 2016). 148S showed good vigour in both paper-roll test method and in field conditions. 7K showed good vigour for RL at 7 and 14 d after sowing in paper-roll method and for TN at 30 d in field conditions in Kharif-2013. There are several studies on genotypic variability (Cruz and Milach, 2004; Sharifi, 2010; Zhou and Stephens, 2012; Anandan *et al.*, 2016), mapping of QTLs (Redona and Mackill, 1996b; Cui *et al.*, 2002; Fujino *et al.*, 2004; Xu *et al.*, 2004; Zhang *et al.*, 2005a, b; Kanbar *et al.*, 2006; Lu *et al.*, 2007; Zhou *et al.*, 2007; Rebolledo *et al.*, 2015; Subudhi *et al.*, 2015) and marker-assisted gene pyramiding

Table 2. Correlation matrix of yield and vigour-related traits of Swarna × *O. nivara* BILs using mean phenotypic data of two seasons Kharif (wet season) 2013 and Rabi (dry season) 2014

Season		GP	SL	RL	R:SDW	TDW	SVI1	SVI2	DFF	DM	PH	TN	PTN	%PH	%TN	BM	SPY	BY	HI
Kharif	GP	1																	
Kharif	SL	0.15	1																
Kharif	RL	−0.23	0.29	1															
Kharif	R:SDW	−0.06	−0.51**	0.02	1														
Kharif	TDW	0.24	0.72**	0.11	−0.35*	1													
Kharif	SVI1	0.82**	0.52**	0.30	−0.18	0.43*	1												
Kharif	SVI2	0.62**	0.62**	−0.05	−0.35*	0.88**	0.68**	1											
Kharif	DFF	−0.32	−0.32	−0.14	0.29	−0.21	−0.47*	−0.35*	1										
Rabi	DFF	−0.54**	−0.42*	−0.15	0.20	−0.35*	−0.69**	−0.54**	1										
Kharif	DM	−0.28	−0.44*	−0.14	0.29	−0.29	−0.47	−0.42	0.93	1									
Rabi	DM	−0.46**	−0.49**	−0.14	0.20	−0.39*	−0.64**	−0.56**	0.96	1									
Kharif	PH	0.10	0.58**	0.14	−0.32	0.35*	0.30	0.27	−0.22	−0.21	1								
Rabi	PH	0.25	0.60**	0.01	−0.33	0.36*	0.39*	0.35*	−0.24	−0.25	1								
Kharif	TN	−0.04	−0.37*	−0.18	0.30	−0.35*	−0.20	−0.32	0.26	0.16	−0.41*	1							
Rabi	TN	−0.41*	−0.06	0.13	−0.05	−0.12	−0.29	−0.14	−0.08	−0.15	−0.42*	1							
Kharif	PTN	0.07	−0.35*	−0.24	0.28	−0.37*	−0.12	−0.28	0.23	0.12	−0.37*	0.98**	1						
Rabi	PTN	−0.39*	−0.06	0.14	−0.03	−0.15	−0.25	−0.15	−0.18	−0.23	−0.37*	0.98**	1						
Kharif	%PH	0.31	0.46*	0.19	−0.17	0.33	0.48**	0.33	−0.46*	−0.44*	0.69**	−0.08	−0.05	1					
Rabi	%PH	0.21	0.46*	0.49**	−0.19	0.26	0.50**	0.25	−0.50**	−0.43	0.54**	−0.11	−0.03	1					
Kharif	%TN	−0.25	−0.28	0.28	0.37*	−0.32	−0.17	−0.41	0.11	0.10	−0.24	0.20	0.15	0.09	1				
Rabi	%TN	0.24	−0.17	−0.18	0.31	−0.14	0.10	0.01	−0.02	−0.06	−0.37*	−0.20	−0.21	−0.48**	1				
Kharif	BM	−0.28	0.16	0.06	−0.06	0.15	−0.24	−0.06	0.59**	0.57**	0.37*	0.14	0.09	0.15	−0.08	1			
Rabi	BM	0.04	−0.24	−0.19	0.24	0.05	−0.16	−0.01	0.61**	0.60**	−0.07	−0.34	−0.42*	−0.29	−0.02	1			
Kharif	SPY	0.31	0.18	−0.12	−0.08	0.34	0.21	0.39*	0.10	0.16	−0.12	0.04	0.04	0.02	0.08	0.27	1		
Rabi	SPY	0.50**	0.41	0.18	−0.22	0.68**	0.61**	0.79**	−0.61**	−0.54**	0.07	−0.04	0.00	0.34	0.12	−0.14	1		
Kharif	BY	0.54**	0.29	−0.26	−0.15	0.43*	0.42*	0.56**	−0.17	−0.18	−0.03	−0.05	0.01	0.01	−0.50**	−0.21	0.28	1	
Rabi	BY	0.54**	0.35*	0.23	−0.02	0.67**	0.63**	0.76**	−0.45*	−0.42*	0.11	−0.17	−0.17	0.25	0.04	0.04	0.83**	1	
Kharif	HI	0.47*	0.03	−0.04	0.01	0.16	0.40*	0.36*	−0.43*	−0.35*	−0.40*	−0.12	−0.09	−0.10	0.17	−0.59**	0.60**	0.38*	1
Rabi	HI	0.39*	0.36*	0.23	−0.25	0.51**	0.54**	0.62**	−0.74**	−0.68**	0.01	0.07	0.14	0.43*	0.13	−0.52**	0.89**	0.67**	1

Significance levels: *$P > 0.05$, and **$P > 0.01$.
BM, biomass; BY, bulk yield; DFF, days to fifty percent flowering; DM, days to maturity; GP, germination percentage; HI, harvest index; PH, plant height at maturity; PTN, productive tiller number at maturity; %PH, Percent increase in plant height; RL, root length; R:SDW, root to shoot dry weight ratio; %TN, Percent increase in tiller number; SPY, single plant yield; SL, shoot length; SVI1, seedling vigour index-1; SVI2, seedling vigour index-2; TN, tiller number at maturity; TDW, total dry weight.

Table 3. Genotypes with *O. nivara* alleles for markers linked to seedling vigour-related traits

Phenotyping			Genotyping with markers linked to QTLs reported for seedling vigour					
Trait	Best genotypes identified		QTL	References	Marker Interval	Chr	Traits associated	Genotypes with *O. nivara* alleles
	BILs	Cultivars						
SL	148S	MTU1010	*qSV-1*	Zhang *et al.* (2005a, b)	RM212-RM104-RZ14	1	SL, GR	250K
			qSEV-3-4	Lu *et al.* (2007)	RM148-RM85	3	SL, GR, SV	148S, 14-3S
				Zhang *et al.* (2005a, b)	RM204-RM217	6	SL	148S, 14-3S
				Zhang *et al.* (2005a, b)	RM242-RM278	9	SL	148S, 250K
RL	7K	MTU1081, Sahbhagidhan		Xu *et al.* (2004)	RM318-RM166	2	RS, SDW	–
SVI	148S, 166S	Tulasi	*qSEV-2-1*	Lu *et al.* (2007)	RM279-RM27	2	SV	250K, 14-3S
			qSEV-3-4	Lu *et al.* (2007)	RM148-RM85	3	SL, GR, SV	148S, 14-3S
			qSEV-3-2	Lu *et al.* (2007)	RM251-RM282	3	SV	148S
			qSEV-5-2	Lu *et al.* (2007)	RM163-RM161	5	SV	148S
				Zhang *et al.* (2005a, b)	RM30-RM340	6	SV	148S
			qSEV-8	Lu *et al.* (2007)	RM223-RM210	8	GR, SEV	148S
SDW	148S	Tulasi		Xu *et al.* (2004)	RM318-RM166	2	SDW, RS	–
			qSDW2	Xu *et al.* (2004)	RM240-RM213	2	SDW, RDW, TDW	7K, 14-3S
				Cui *et al.* (2002)	RM44-RM210	8	SDW, RDW	–
RDW	166-2S	Tulasi	*qRDW2*	Xu *et al.* (2004)	RM240-RM213	2	SDW, RDW, TDW	7K, 14-3S
				Zhang *et al.* (2005a, b)	RM44-RM210	8	RDW, R:S,	–
				Zhang *et al.* (2005a, b)	RM284	12	RDW, LDW, R:S	–
TDW	148S, 166S	Tulasi	*qTDW2*	Xu *et al.* (2004)	RM240-RM213	2	SDW, RDW, TDW	–
			qSEV-3-4	Lu *et al.* (2007)	RM148-RM85	3	SL, GR, SV	148S, 14-3S
GR	166S, 3-1K,	NLR34449, Tellahamsa	*qSV-1*	Zhang *et al.* (2005a, b)	RM212-RM104-RZ14	1	GR, SL	250K
				Zhang *et al.* (2005a, b)	RM263 -RM221	2	GR	–
			qSV-6	Zhang *et al.* (2005a, b)	RM217-RM253	6	GR,	–
			qSEV-8	Lu *et al.* (2007)	RM223-RM210	8	GR, SEV	148S
			qSV-8	Zhang *et al.* (2005a, b)	RM230-RM264	8	GR, SL	250K, 14-3S
				Lu *et al.* (2007)	RM44-RM210	8	SDW, RGR, RDW	–
				Zhang *et al.* (2005a, b)	RM229-RM21	11	GR	–
				Zhang *et al.* (2005a, b)	RM19-RM247	12	GR	250K

GR, growth rate; RDW, root dry weight; RGR, root growth rate; RL, root length; R:S, Root shoot ratio; SDW, shoot dry weight; SEV, seedling early vigour; SL, shoot length; SV, seedling vigour; SVI, seedling vigour index; TDW, total dry weight.

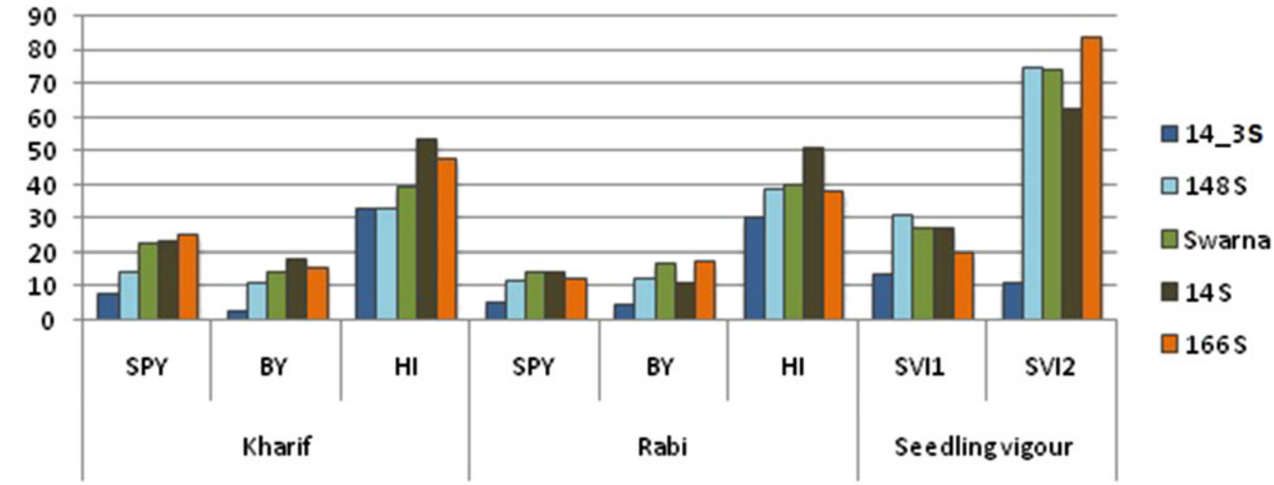

Fig. 3. Seasonal yield and seedling vigour index of four BILs compared with parent Swarna.

(Redona and Mackill, 1996b; Cui *et al.*, 2002; Fujino *et al.*, 2004) related to seedling vigour in rice. QTL hotspots for early vigour in dry DSR were identified on chromosomes 3 and 5 recently (Singh *et al.*, 2017). Attention has been paid to introgression of beneficial alleles from wild rice into elite breeding lines (Multani *et al.*, 1994; Brar and Khush, 1997) through BIL approach (Monforte and Tanksley, 2000; Li *et al.*, 2004). BILs can be developed through a series of backcrosses, containing wild segments in a cultivated genetic background (Tian *et al.*, 2006a, b; Tan *et al.*, 2007; Barone *et al.*, 2009). These BIL populations can accelerate molecular breeding and improve the traits of agronomic importance without much linkage drag from wild species.

Previous studies focused mainly on the traits associated with seedling vigour, including RL, SL and dry weight which contribute to final yield and yield components (Regan *et al.*, 1992; Redona and Mackill, 1996a, b). Variability in seedling vigour depends on several component traits, environment interactions, stress at various crop stages and also post-harvest drying and storage conditions of seed (Bewley *et al.*, 2013; Guan *et al.*, 2013). Laboratory screening procedures have been usually followed to measure seedling vigour in rice in most of the studies (Redona and Mackill, 1996a, b; Zhang *et al.*, 2005a, b). We used paper roll method in laboratory conditions to estimate seedling vigour. The rate of germination and rate of root and shoot growth determines seedling vigour. Both of them indicate nutrient uptake and carbohydrate accumulation in growth and development (Krishnasamy and Seshu, 1989; Redona and Mackill, 1996a, b; Cui *et al.*, 2002). It was observed that 148S attained maximum vigour within 7 d of germination, but other genotypes attained maximum vigour at 14 d. The vigorous phenotype among these BILs was contributed either by plant height, tiller number, BM, root parameters or their combinations. 148S has longer nodal root system with fine lateral roots. Nodal roots form the basic framework of the root system and fine lateral roots form the primary mechanism for absorbing water and nutrients (Gu *et al.*, 2017). The studies using petri plate and paper roll method showed the similar ranking of genotypes for vigour as ranking based on field studies indicating that environmental conditions had little effect in selecting the most vigorous genotypes.

Association studies among yield and vigour traits using data from two seasons showed that DFF had a significant positive correlation with BM and negative correlation with HI, PH and SVI in both the seasons. PH was significantly correlated with SL in a positive direction but with tiller number and root/shoot dry weight ratio in a negative direction in both seasons. SPY exhibited a significantly positive correlation with HI in both seasons. This study revealed significant correlations among the seedling vigour traits in both seasons. Zhang *et al.* (2005a, b) reported that SL is the best predictor of seedling vigour and a phenotypic indicator of plant vigour in rice. In our study, SL and RL were significantly correlated with shoot dry weight and root dry weight but in the negative direction. However, TDW showed a highly positive correlation with SL and seedling vigour indices in both seasons. Cui *et al.* (2002) reported a positive correlation of maximum RL with RDW and SDW. They also reported strong correlation between amylase activity in germinated seed with seedling dry weight. Vigour is associated with amylase activity and breakdown of starch in the endosperm (Williams and Peterson, 1973; Sasahara *et al.*, 1986). SPY and BY showed correlation with SVI2 based on dry weight, while HI showed association with SVI1 that is based on seedling length in all the genotypes (Fig. 3). Singh *et al.* (2017)

reported strong correlations between early vigour and grain yield in Swarna/Moroborekan BC_3F_4 mapping population grown in field and glasshouse conditions.

Molecular screening of BILs with 27 SSRs revealed 37% homozygous *O. nivara* introgressions and 38% of heterozygous alleles. 14S had the highest percentage (12%) of *O. nivara* introgression among BILs. Most of the high yielding BILs were identified to harbour QTL regions for vigour traits which were earlier reported. QTLs *qSV-6* for GR, SL and RL were identified between marker interval RM217 and RM253 (Zhang *et al.*, 2005a, b) and QTL *qSEV-8* was identified for seedling vigour related traits between RM223 and RM210 (Lu *et al.*, 2007). In this study, we found 14S, 148S, 3-1K and 250K have the complete QTL at chromosomal regions between RM217 and RM253 on chromosome 6 from *O. nivara* and 14S, 14-3S, 65S, 70S, 75S, 166S, 166-1S and 7K have the QTL region for seedling vigour from *O. nivara* at the chromosomal region between RM223 and RM210. Singh *et al.* (2017) reported early vigour QTLs in BC_3F_4 lines derived from Swarna × Moroberekan using SNP genotyping and one QTL *qEV6.1* for early vigour was identified on chromosome6.

Similarly, QTL region RM19-RM247 on chromosome 12 was reported to be associated with GR (Zhang *et al.*, 2005a, b). 250K was found to have *O. nivara* alleles for the QTL at this chromosomal region. Thus, it is evident that BILs derived from *O. nivara* have potential alleles for seedling vigour and related traits. 14S and 166S, with higher seedling vigour were also found to be stable BILs for yield and 148S for earliness (Divya *et al.*, 2016). Introgression lines can be shared with researchers under a Material Transfer Agreement (http://www.icar-iirr.org/). These BILs can be utilized to introgress seedling vigour traits into elite breeding lines for DSR.

Acknowledgements

This research was conducted in project (*ABR/CI/BT/11*) on Mapping Quantitative Trait Loci (QTLs) for yield and related traits using backcross inbred lines (BILs from Elite × 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 the 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.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262118000187.

References

Anandan A, Anumalla M, Pradhan SK and Ali J (2016) Population structure, diversity and trait association analysis in rice (*Oryza sativa* L.) germplasm for early seedling vigour (ESV) using trait linked SSR markers. *PLoS ONE* 11: e0152406.

Balasubramanian V and Hill JE (2002) Direct seeding of rice in Asia: emerging issues and strategic research needs for the 21st century. In: Pandey S and International Rice Research Institute (eds) *Direct Seeding: Research Strategies and Opportunities*. Los Banos, Philippines: IRRI, pp. 15–39.

Barone A, Matteo A, Carputo D and Frusciante L (2009) High-throughput genomics enhances tomato breeding efficiency. *Current Genomics* 10: 1–9.

Bewley JD, Bradford KJ, Hilhorst HWM and Nonogaki H (2013) *Seeds: Physiology of Development, Germination and Dormancy*. New York: Springer, 392p.

Borjas AH, Teresa BDL and Prasanta KS (2016) Genetic analysis of germinating ability and seedling vigour under cold stress in US weedy rice. *Euphytica* 208: 251–264.

Brar DS and Khush GS (1997) Alien introgression in rice. *Plant Molecular Biology* 35: 35–47.

Cairns JE, Namuco OS, Torres R, Simborio FA, Courtois B, Aquino GA and Johnson DE (2009) Investigating early vigour in upland rice (*Oryza sativa* L.): Part II. Identification of QTLs controlling early vigour under greenhouse and field conditions. *Field Crops Research* 113: 207–217.

Cruz RP and Milach SCK (2004) Cold tolerance at the germination stage of rice: methods of evaluation and characterization of genotypes. *Agricultural Sciences* 61: 1–8.

Cui KH, Peng SB, Xing YZ, Xu CG, Yu SB and Zhang Q (2002) Molecular dissection of seedling vigour and associated physiological traits in rice. *Theoretical and Applied Genetics* 105: 745–753.

Divya B, D Subrahmanyam, J Badri, AK Raju, YV Rao, Kavitha B, Sukumar M, Malathi S, Revathi P, G. Padmavathi, Babu VR and Sarla N (2016) Genotype × environment interactions of yield traits in backcross introgression lines derived from *Oryza sativa* cv. Swarna/*Oryza nivara*. *Frontiers in Plant Science* 7: 1530.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Eizenga GC, Prasad B, Jackson AK and Jia MH (2013) Identification of rice sheath blight and blast quantitative trait loci in two different *O. sativa*/*O. nivara* advanced backcross populations. *Molecular Breeding* 31: 889–907.

Eizenga GC, Neves PCF, Bryant RJ, Agrama HA and Mackill DJ (2016) Evaluation of a M-202 × *Oryza nivara* advanced backcross mapping population for seedling vigour, yield components and quality. *Euphytica* 208: 157–171.

Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y, Takeuchi Y, Ando T, Lin SY and Yano M (2004) Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 108: 794–799.

Gu D, Zhen F, Hannaway DB, Zhu Y, Liu L and Cao W (2017) Quantitative classification of rice (*Oryza sativa* L.) root length and diameter using image analysis. *PloS ONE* 12: e0169968.

Guan Q, Lu X, Zeng H, Zhang Y and Zhu J (2013) Heat stress induction of miR398 triggers a regulatory loop that is critical for thermotolerance in Arabidopsis. *The Plant Journal* 74: 840–851.

Gur A and Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. *PLoS Biology* 2: e245.

Haritha G, Sudhakar T, Chandra D, Ram T, Divya B and Sarla N (2016) Informative ISSR markers help identify genetically distinct accessions of *Oryza rufipogon* in yield improvement. *Rice Science* 23: 225–2241.

Haritha G, Malathi S, Divya B, Swamy BPM, Mangrauthia SK and Sarla N (2018) *Oryza nivara* Sharma et Shastry. In: Mondal T and Henry R (eds) *The Wild Oryza Genomes. Compendium of Plant Genomes*. Cham: Springer, pp. 207–258. DOI: 10.1007/978-3-319-71997-9_20.

Huang M, Zhang R, Chen J, Cao F, Jiang L and Zou Y (2017) Morphological and physiological traits of seeds and seedlings in two rice cultivars with contrasting early vigour. *Plant Production Science* 20, 95–101.

IRRI (2013). *Standard Evaluation System (SES) for Rice*, 5th edn. Philippines: IRRI.

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK and Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics* 100: 1311–1320.

Juneja S, Das A, Joshi SV, Sharma S, Yogesh VY, Patra BC, Bharaj TS, Sidhu JS and Singh K (2006) *Oryza nivara* (Sharma et Shastry) the progenitor of *O. sativa* (l.) subspecies *indica* harbors rich genetic diversity as measured by SSR markers. *Current Science* 91: 1079–1085.

Kaladhar K, Swamy BPM, Babu AP, Reddy CS and Sarla N (2008) Mapping quantitative trait loci for yield traits in BC_2F_2 population derived from Swarna × *O. nivara* cross. *Rice Genetics Newsletter* 24: 34–36.

Kanbar A, Janamatt M, Sudheer E, Vinod MS and Shashidhar HE (2006) Mapping QTLs underlying seedling vigour traits in rice (*Oryza sativa* L.). *Current Science* 90: 1–10.

Kharb RPS, Lather BPS and Deswal DP (1994) Prediction of field emergence through heritability and genetic advance of vigour parameters. *Seed Science and Technology* 22: 461–466.

Krishnasamy V and Seshu DV (1989) Seed germination rate and associated characters in rice. *Crop Science* 29: 904–908.

Li CC and Rutger JN (1980) Inheritance of cool temperature seedling vigour in rice and its relationship with other agronomic characteristics. *Crop Science* 20: 295–298.

Li J, Thomson M and McCouch SR (2004) Fine mapping of a grain weight Quantitative Trait Locus in the pericentromeric region of rice chromosome 3. *Genetics* 168: 2187-2195.

Lu XL, Niu AL, Cai HY, Zhao Y, Liu JW, Zhu YG and Zhang ZH (2007) Genetic dissection of seedling and early vigour in a recombinant inbred line population of rice. *Plant Science* 172: 212–220.

Mahender A, Anandan A and Pradhan SK (2015) Early seedling vigour, an imperitive trait for direct-seeded rice; an overview on physio-morphological parameters and molecular markers. *Planta* 241, 1027–1050.

Malathi S, Divya B, Sukumar M, Raju A K, Rao YV, Tripura Venkata VGN and Sarla N (2017) Identification of major effect QTLs for agronomic traits and CSSLs in rice from Swarna/*O nivara* derived backcross inbred lines. *Frontiers in Plant Science* 8: 1027.

Monforte AJ and Tanksley SD (2000) Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: a tool for gene mapping and gene discovery. *Genome* 43: 803–813.

Multani DS, Jena KK, Brar DS, Delos Reyes BG, Angeles ER and Khush GS (1994) Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin.to cultivated rice *O. sativa* l. *Theoretical and Applied Genetics* 88: 102–1109.

Namuco OS, Cairns JE and Johnson DE (2009) Investigating early vigour in upland rice (*Oryza sativa* L.). Part I. Seedling growth and grain yield in competition with weeds. *Field Crops Research* 113: 197–206.

Niroula RK and Bimb HP (2009) Overview of wheat × maize system of crosses for dihaploid induction in wheat. *World Applied Science* 7: 1037–1045.

Prasanth VV, Babu MS, Basava RK, Tripura Venkata VGN, Mangrauthia SK, Voleti SR and Neelamraju S (2017) Trait and marker associations in *Oryza nivara* and *O. rufipogon* derived rice lines under two different heat stress conditions. *Frontiers in Plant Science* 8: 1819.

Quarrie SA, Strojanovic J and Pekic S (1999) Improving drought resistance in small grained cereals: a case study, progress and prospects. *Plant Growth Regulators* 29: 1–21.

Rangel PHN, de Morais OP, Brondani C, Rangel PN and Brondani RPV (2006) Selection of rice genotypes with greater seedling vigour under controlled conditions. *Crop Breeding and Applied Biotechnology* 6: 65–72.

Rebolledo MC, Dingkuhn M, Courtois B, Gibon Y, Clément-Vidal A, Cruz DF, Duitama J, Lorieux M and Luquet D (2015) Phenotypic and genetic dissection of component traits for early vigour in rice using plant growth modelling, sugar content analyses and association mapping. *Journal of Experimental Botany* 66: 5555–5566.

Redona ED and Mackill DJ (1996a) Genetic variation for seedling vigour traits in rice. *Crop Science* 36: 285–290.

Redona ED and Mackill DJ (1996b) Mapping quantitative trait loci for seedling vigour in rice using RFLPs. *Theoretical and Applied Genetics* 92: 395–402.

Regan KL, Siddique KHM, Turner NC and Whan BR (1992) Potential for increasing early vigour and total biomass in spring wheat. II. Characteristics associated with early vigour. *Australian Journal of Agricultural Research* 43: 541–553.

Sarla N, Bobba S and Siddiq EA (2003) ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. *Current Science* 84: 683–690.

Sasahara T, Ikarashi H and Kambayashi M (1986) Genetic variations in embryo and endosperm weights, seedling growth parameters and á-amylase activity of the germinated grains in rice (*Oryza sativa* L.). *Japanese Journal of Breeding* 36: 248–261.

Sharifi P (2010) Evaluation on sixty-eight rice germplasms in cold tolerance at germination stage. *Rice Science* 17: 77–81.

Singh UM, Yadav S, Dixit S, Ramayya PJ, Devi MN, Raman KA and Kumar A (2017) QTL hotspots for early vigour and related traits under dry direct-seeded system in rice (*Oryza sativa* L.). *Frontiers in Plant Science* 8: 286.

Subudhi PK, Leon TD, Singh PK, Parco A, Cohn MA and Sasaki T (2015) A chromosome segment substitution library of weedy rice for genetic dissection of complex agronomic and domestication traits. *PLoS ONE* 10: e0130650.

Sun Q, Wang JH and Sun BQ (2007) Advances on seed vigour physiological and genetic mechanisms. *Agricultural Science in China* 6: 1060–1066.

Swamy BPM and Sarla N (2008) Yield-enhancing quantitative trait loci (QTLs) from wild species. *Biotechnology Advances* 26: 106–120.

Swamy BPM, Kaladhar K, Ramesha MS, Viraktamath BC and Sarla N (2011) Molecular mapping of QTLs for yield and related traits in *Oryza sativa* cv Swarna × *O. nivara* (IRGC81848) backcross population. *Rice Science* 18, 178–186.

Swamy BPM, Kaladhar K, Reddy AG, Viraktamath BC and Sarla N (2014) 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*. *Journal of Genetics* 93: 643–653.

Tan LB, Liu FX, Xue W, Wang GJ, Ye S, Zhu ZF, Fu YC, Wang XK and Sun CQ (2007) Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci. *Journal of Integrative Plant Biology* 49: 871–8884.

Tanksley SD and McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277: 1063–1066.

Thalapati S, Guttikonda H, Nannapaneni ND, Babu AP, Reddy CS, Swamy BPM, Batchu AK, Basava RK, Viraktamath BC and Sarla N (2014) Heterosis and combining ability in rice as influenced by introgressions from wild species *Oryza rufipogon* including *qyld* 2.1 sub-QTL into the restorer line KMR3. *Euphytica* 202: 81–95.

Tian F, Li DJ, Fu Q, Zhu ZF, Fu YC, Wang XK and Sun CQ (2006a) Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*O. sativa* l.) background and characterization of introgressed segments associated with yield-related traits. *Theoretical and Applied Genetics* 112: 570–5580.

Tian F, Zhu ZF, Zhang BS, Tan LB, Fu YC, Wang XK and Sun CQ (2006b) Fine mapping of a quantitative trait locus for grain number per panicle from wild rice (*Oryza rufipogon* Griff.). *Theoretical and Applied Genetics* 113: 619–6629.

Wang L, Wang YJ and Porter R (2002) Structures and physicochemical properties of six wild rice starches. *Journal of Agriculture and Food Chemistry* 50: 2695–2699.

Wang ZF, Wang JF, Bao YM, Wang FH and Zhang HS (2010) Quantitative trait loci analysis for rice seed vigour during the germination stage. *Journal of Zhejiang University, Science B* 11: 958–964.

Wickneswari R, Bhuiyan MAR, Kalluvettankuzhy KS, Lim LS, Thomson MJ, Md. Kairudin NMK and Abdullah MZ (2012) Identification and validation of quantitative trait loci for agronomic traits in advanced backcross breeding lines derived from *Oryza rufipogon* × *Oryza sativa* cultivar MR219. *Plant Molecular Biology Reporter* 30: 929–939.

Williams JF and Peterson ML (1973) Relations between α-amylase activity and growth of rice seedlings. *Crop Science* 13: 612–615.

Xu CG, Li XQ, Xue Y, Huang YW, Gao J and Xing YZ (2004) Comparison of quantitative trait loci controlling seedling vigour characteristics at two seedling stages in rice recombinant inbred lines. *Theoretical and Applied Genetics* 109: 640–647.

Yamane K, Garcia R, Imayoshi K, Mabesa-Telosa RC, Banayo NPM, Vergara G, Yamauchi A, Sta. Cruz P and Kato Y, 2018. Seed vigour contributes to yield improvement in dry direct-seeded rainfed lowland rice. *Annals of Applied Biology* 172: 100–110.

Yamauchi M and Winn T (1996) Rice seed vigour and seedling establishment in anaerobic soil. *Crop Science* 36: 680–686.

Yamauchi M, Aguilar AM, Vaughan DA and Seshu DV (1993) Rice germplasm suitable for direct sowing under flooded soil surface. *Euphytica* 67: 177–184.

Zhang ZH, Su L, Li W, Chen W and Zhu YG (2005a) A major QTL conferring cold tolerance at the early seedling stage using recombinant inbred lines of rice (*Oryza sativa* L.). *Plant Science* 168: 527–534.

Zhang ZH, Yu SB, Yu T, Huang Z and Zhu YG (2005b) Mapping quantitative trait loci (QTLs) for seedling-vigour using recombinant inbred lines of rice (*Oryza sativa* L.). *Field Crops Research* 91: 161–170.

Zhao D, Atlin GN, Bastiaans L and Spiertz JL (2006) Developing selection protocols for weed competitiveness in aerobic rice. *Field Crops Research* 97: 272–285.

Zhou L, Wang JK, Yi Q, Wang YZ, Zhu YG and Zhang ZH (2007) Quantitative trait loci for seedling vigour in rice under field conditions. *Field Crops Research* 100: 294–301.

Zhou X and Stephens M (2012) Genome-wide efficient mixed model analysis for association studies. *Nature Genetics* 44: 821–824.