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RESEARCH ARTICLE

Yield-enhancing SPIKE allele from the *aus*-subtype indica rice and its allele specific codominant marker

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Abstract. Improving spikelet number without limiting panicle number is an important strategy to increase rice productivity. In this study, a spikelet number enhancing SPIKE-allele was identified from the *aus* subtype *indica* rice, cv. Bhutmuri, which has an identical *japonica* like corresponding sequence including a retrotransposon sequence, usually absent in *indica* genotypes, like IR64. An allele-specific single-tube PCR-based codominant marker targeting an A/G single-nucleotide polymorphism (SNP) at the 3′UTR was identified for easier genotyping. The yield enhancing ability of the Bhutmuri-SPIKE allele carrying RILs and NILs over IR64-SPIKE allele carrying alleles was due to increased number of filled grains/panicle. More than three times higher abundance of SPIKE transcripts was observed in Bhutmuri and NILs carrying this allele compared with IR64 and its allele carrying NILs. Higher rate of photosynthesis at more than 900 $\mu molm^{-2}s^{-1}$ light intensity and more than six small vascular bundles between the two large vascular bundles in the flag leaves of Bhutmuri and its allele carrying NILs were also observed. The identified SPIKE allele and the marker associated with it will be useful for increasing the productivity of rice by marker-assisted breeding.

Keywords. SPIKE allele; rice; marker; photosynthesis rate; yield-enhancement.

Introduction

Improving the yield potentiality of rice is inevitable to meet the future production demand without having to expand the land under cultivation (Tester and Langridge 2010). Moreover, with the global population forecast to rise, and the availability of arable land likely to fall, developing high yielding crop varieties with desired traits is decidedly timely (kersey *et al.* 2020). In India and other South Asian countries, improving the yield potentiality is further challenging as farmers and consumers mostly prefer short duration (<120 days) high-yielding varieties with slender grain. In South Asian countries where multiple cropping is a common practice, planting short-duration varieties allow farmers to prepare for the next crop more easily. As days to heading is positively correlated with number of grain/panicle and seed-weight in rice, it is relatively difficult to develop short duration high-yielding varieties with desired grain qualities. The semi-dwarf high yielding short and medium duration varieties of the green revolution were developed by introducing the *Sd1* gene, which is defective in the 20-oxidase gibberellic acid biosynthetic enzyme, into tall backgrounds (Khush 2001; Spielmeyer *et al.* 2002). Further yield enhancement is being achieved by pyramiding of several yield-enhancing QTLs and genes from diverse sources. Mapping QTLs followed by cloning of genes controlling several yield attributing parameters like Gn1a (Ashikari *et al.* 2005), SPIKE/NAL1 (Fujita *et al.* 2013), APO1 (Terao *et al.* 2010), DEP1 (Huang *et al.* 2009) for grain number, LAX, SPA (Komatsu *et al.* 2003) for panicle number, GW28 (Song *et al.* 2007), GS3 (Fan *et al.* 2006), GS5 (Li *et al.* 2011) for grain shape are allowing breeders to enhance the productivity of rice following the candidate gene based approach.

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SPIKE (Os04g0615000) gene on chromosome 4 of rice enhances yield by improving spikelet number per panicle. The gene is also allelic to the factor responsible for wider leaf breadth (Chen *et al.* 2012) and greater spikelet number (LSCHL4) of temperate *japonica* var. Nipponbare. SPIKE encodes a putative trypsin like serine/cysteine protease, although its molecular function remains unknown (Qi *et al.* 2008; Cho *et al.* 2014). A 30-bp deletion in SPIKE causes a narrow leaf phenotype (Nal1), by affecting polar auxin transport and reducing the number of vascular bundles between the two veins, indicating its role in leaf growth and development (Qi *et al.* 2008). The GREEN FOR PHOTOSYNTHESIS (GPS) locus, which increases the number of mesophyll cells leading to thickened leaves and pleotropic enhancement of photosynthetic rate via improved carboxylation in the *indica* genotype, Takanari, is also a transcript variant of SPIKE (Takai *et al.* 2013). However, the yield enhancing allele of SPIKE is only reported in tropical and temperate *japonica* rice. Its yield-enhancing ability in different *indica* background was explained by high transcript abundance and dissimilarity in three amino acid residues in the *indica* allele. Although the *aus* and aromatic subpopulations of *indica* rice have originated from different pathways, allelic status of SPIKE gene in *aus* and aromatic subpopulations is unknown. Further, the *japonica* allele of SPIKE cannot greatly improve yield when transferred to relatively high yielding long duration *indica* background (Takai *et al.* 2017), forcing breeders to mine for novel alleles of SPIKE within the *indica* subpopulation. Identification of yield enhancing SPIKE allele in *aus* or aromatic subpopulation will be useful in enhancing yield as the allele can be easily transferred to *indica* type high yielding varieties (HYVs) lacking this allele due to easy cross-compatibility.

Although, allele-specific PCR based dominant markers are reported from two single-nucleotide polymorphisms (SNPs) in SPIKE, they require two separate reactions to confirm homozygous or heterozygote status of the locus (Kim *et al.* 2016). Thus, identification of single tube codominant marker associated with yield enhancing SPIKE allele will be more useful in selecting yield-enhancing SPIKE homozygous lines at the early segregating generations.

In this study, a yield enhancing SPIKE allele is identified from an *aus* subtype *indica* landrace, which is identical to the corresponding *japonica* allele. A single tube PCR based codominant marker from an SNP in the 3′UTR is developed, and the yield enhancing ability of the identified allele is confirmed in recombinant inbred line (RIL) and near isogenic line (NIL) populations.

Materials and methods

Plant materials

One-hundred rice genotypes mostly comprising of landraces (*indica*, aromatic and *aus* types) from Bengal were selected (table 1in electronic supplementary material at http://www.ias.ac.in/jgenet/). Two pairs of crosses namely IR64 × Bhutmuri and Vandana × Shatabdi were used to develop RILs and NILs. The RIL population was developed by crossing the parental pairs followed by single seed descent up to F_8 generation. Homozygous lines (n=165, 172) of each parental cross were maintained as RILs population. From F_4 population, heterozygote lines at the SPIKE locus were selected and advanced to F_7 by selecting only heterozygotes. Selected heterozygotes in F_7 were selfed and homozygotes for the SPIKE alleles were selected using a PCR based codominant marker developed in this study. These homozygous lines for alternate alleles of SPIKE were considered as NILs.

PCR based primer from SNP

The whole gene, including a part of 3′ regulatory element from six genotypes was sequenced using 12 overlapping primer pairs (table 3 in supplementary material). The sequence-alignment was made by MULTIALIGN (http://multalin.toulouse.inra.fr) multalin to detect SNPs between the genotypes. Therefore, two SNP (A/G) specific internal primers along with a pair of flanking control primers were designed for PCR based genotyping of SPIKE locus. SNP based allele-specific primer pair (INWL-G and INNL-A) has the final sequence 5′-TCAGAAAAATTGAACCTGCAG TAAGCTTCA-3′ and 5′-ATATGCTTGCCAAAGCCATTC AAGCTCACC-3′. For SNP genotyping, PCR was performed in a 25 μL reaction volume that contained 20 ng of genomic DNA, 2.5 μL 10x buffer, 2 μL 2.5 mM dNTP mix, 10 pmole each control primers, 5 pmole each SNP specific primers and 1 U Taq polymerase (Promega, USA) per reaction under standard reaction conditions, i.e. 94°C for 5 min of first denaturation followed by 35 cycles each denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and polymerization at 72°C for 60 s.

Evaluation of yield and spikelet numbers

Grain yield and spikelet numbers were evaluated during wet season in the university instructional farm where the soil was new alluvial with a neutral pH (6.8) following augmented design. Swarna, IR64 and Satabdi were kept as checks in each block of 10 genotypes. Genotypes and the two RIL populations and NIL-SPIKE population along with their parents were grown under irrigated conditions. The yield of all genotypes was assessed for two consecutive years in 2018 and 2019 in 3 × 6 m^2 plots with three replications in completely randomized block design. Yield was recorded from 2 × 5 m^2 area of the plot leaving half a metre from all sides to reduce border effect. RILs were grown in 4 m rows with three lines for each, following 25 cm spacing in augmented design. Bhutmuri, IR64 and Shatabdi were kept as replicated controls in each block of 20 RILs. Adjusted

means were considered for comparison. Basal fertilizer was applied two days before transplanting at 6 gNm^{-2}, 6 gPm^{-2} and 6 $gK\ m^{-2}$. Two top dressings were made further with 2 gNm^{-2} at the tillering and heading stage. Yield and its component traits were recorded from 10 randomly selected plants from each replication at maturity. Grain yield was recorded at 14% moisture content.

Estimation of photosynthesis rate and leaf related parameters

Mean flag-leaf width of each genotype including the RILs and NILs was measured from five randomly selected hills. For each selected hill, measurements were taken from three flag leaves. Photosynthesis rate (Pn) of the flag leaf was estimated with a portable gas-exchange system (LI-6800; LI-COR, USA) two days after heading under ambient CO_2 concentration (380 $molm^{-2}s^{-1}$) in the leaf chamber of LI-6800. Measurements for 8 s were repeated three times and mean values were calculated. The Pn was estimated for 2 days in succession and an average value was calculated for further analysis. Leaves were exposed to light at a 1300 $\mu molm^{-2}s^{-1}$ photosynthetic active radiation (PAR). Plants were examined from 9:00 am to 11:00 am when Pn was close to the daily maximum (Hirasawa *et al.* 1992). Mean small vascular bundle number (VBN) of each genotype was counted from the transverse section of the widest part of three flag leaves in each hill. Five randomly selected plants were considered for each genotypes including RILs and NILs.

Quantitative real time PCR

Total RNA was extracted from the 25–50 mm young panicle of three independent plants using RNeasy plant mini kit (Qiagen, USA) followed by treating the samples with RNAse free DNase to remove contaminant DNA, if any. First strand cDNA was synthesized with a High Capacity cDNA Reverse Transcription Kit with an oligo $d(T)_{18}$ primer (Invitrogen, USA). Quantitative real-time PCR analysis was performed on a StepOne Plus Real-Time PCR System (Applied Biosystems, USA) using a SYBR green PCR master mix (Invitrogen, USA) as described earlier (Bhattacharyya *et al.* 2003). To confirm the specificity of amplification, melting curve analysis was performed. Significant effect of SPIKE alleles ($P < 0.05$) were tested using Student's *t*-test. Difference in each yield attributing trait in each category across the two years was also tested by ANOVA.

Results

Development of the single-tube allele specific PCR based codominant marker

Since SPIKE gene is allelic to NAL1 (narrow leaf 1), initial screening was done by measuring leaf breadth of 100 genotypes. Flag leaf breadth (FLB) of Asanla, Bhutmuri, Shatabdi and Bidhan Suruchi was more than 2.0 cm, whereas the mean leaf breadth of 100 genotypes was 1.3 ± 0.3 cm (table 1 in electronic supplementary material). Most genotypes had less than 1 cm leaf breadth as in Gobindabhog (0.7 cm) and Vandana (0.8 cm). Based on sequence comparison of SPIKE, Shatabdi and Bhutmuri (MW115951) were found to have identical sequence to that of *japonica* genotype, Daringan (Os04g0615000). Nucleotide polymorphism and their positions in SPIKE genomic sequences of Bhutmuri, Shatabdi, Asanla, Vandana and IR64 along with their FLB are shown in table 1.

Identical SNPs in SPIKE sequence were observed between Koshikari and Bhutmuri, both of which had flag-leaf width more than 2 cm (figure 1 in electronic supplementary material). Bhutmuri possess six small vascular bundles in between two large vascular bundles as compared to four in IR64. An A/G SNP located at the 3′UTR was targeted for the development of single tube allele specific codominant marker for easier genotyping. The SNP specific internal primers combine with one of the external primers to amplify distinct bands based on the presence of A/G SNP in genotypes. External primers (SPIKE-F and SPIKE-R) also produce a common 340 bp band in all genotypes. The

Table 1. Sequence polymorphism in SPIKE genomic sequence among Bhutmuri, IR64 and other four *indica* genotypes along with their FLB.

	Position of SNPs (nucleotide)								
Genotype	7092 Intron2	7169 Intron2	7230 Intron2	7404 Exon3	8622 Exon5	8648 Exon5	**9082 3′UTR**	9126 3′UTR	FLB (cm)
Koshikari (reference)	G	A	T	A	T	A	G	T	>2
Bhutmuri	G	A	T	A	T	A	G	T	2.1
Shatabdi	G	A	T	A	T	A	G	T	2.1
Asanla	T	G	C	G	C	G	A	C	0.7
Vandana	T	G	C	G	C	G	A	C	0.8
IR-64	T	G	C	G	C	G	A	C	1.3

Bold fonts indicate the position of SNP used for codominant primer design.

internal primer INWL-G (internal wide leaf) combines with SPIKE-R to amplify a 230 band in genotypes carrying Bhutmuri SPIKE allele, while INNL-A (internal narrow leaf) combines with SPIKE-F to amplify a 130 bp band in genotypes carrying IR64-SPIKE allele (figure 1a). Thus four primers in a PCR results in three possible outcomes (figure 1b). Amplification of a band of 230 bp indicates a line is homozygous for Bhutmuri-SPIKE allele, while amplification of a band of 130 bp indicates a line is homozygous for the IR64-SPIKE allele. A common band of 340 bp is amplified by all genotypes as PCR-control. Amplification of both 230 bp and 130 bp bands indicates an individual is heterozygous at the SPIKE locus. One hundred genotypes were genotyped using the newly developed single-tube PCR assay with all four primers. PCR products were resolved in 1.5 % agarose gel. Of the 100 genotypes, no genotype carried the same SNP (G) at the 9081 position in the 3′UTR except Bhutmuri, Dular and Shatabdi (table 1 in electronic supplementary material).

Relative quantification of SPIKE

Relative abundance of SPIKE transcripts in young panicle (25 to 50 mm length) of Bhutmuri and Shatabdi were 3–7 folds higher as compared to that of IR64 and Vandana. Although Shatabdi showed higher expression as compared to Bhutmuri, statistically this difference was insignificant at the 5% level of significance. More than 3-fold higher expression was also observed in NIL lines carrying either Bhutmuri or Shatabdi allele (figure 2).

Effect of Bhutmuri-SPIKE on yield and its attributing parameters

Bhutmuri recorded highest yield in both 2018 and 2019 (587 g/m^2 and 607 g/m^2), which was higher than the yield of IR 64 (figure 3). No significant difference was observed between Shatabdi (543 and 530 g/m^2) and Vandana (517 g and 487 g) on the same field. Heterozygote derived NILs carrying either Bhutmuri or Shatabdi SPIKE alleles out yielded the NILs carrying Vandana or IR64 SPIKE allele. Three heterozygote lines were selected

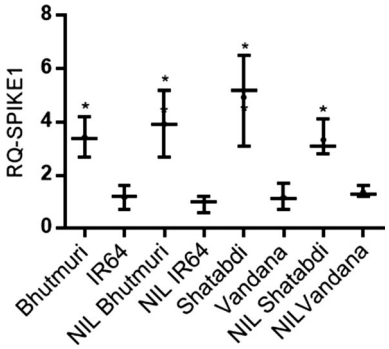

Figure 2. Relative abundance of SPIKE transcript in 25 mm young panicle from the genotypes and NILs with three biological replications. *Difference with its right-hand site values is significant at $P < 0.05$.

in F_4 using the newly designed codominant marker and advanced to F_7 by selecting only heterozygotes for the SPIKE allele in each generation (figure 2 in electronic supplementary material). More than 15% higher yield in NILs carrying Bhutmuri-SPIKE allele than NILs carrying IR64-SPIKE allele in field confirmed the positive effect of Bhutmuri-SPIKE allele on yield. No difference was observed in panicle number per plant among the parental genotypes (figure 3) as well as between NILs, but number of filled grains was greater in NIL-Bhutmuri (S) and NIL-Shat (S) relative to IR64 and Vandana NILs. The extent of increase in yield and filled grains in NIL-Bhutmuri was higher in both the years.

Two RIL population (F_9), developed from the cross, IR64 × Bhutmuri and Vandana × Shatabdi with 165 and 172 homozygous lines respectively were grouped into two using SNP-derived PCR-based codominant marker. Figure 3 in electronic supplementary material describes gel picture showing codominant marker based genotyping of Bhutmuri and IR64. Three lines were found to be heterozygotes and accordingly discarded from association analysis. SNP-based genotyping identified 53 RILs with Bhutmuri-SPIKE allele and 112 with IR64 SPIKE allele. RILs carrying Bhutmuri and IR64 allele had a mean PY 17.9 g ± 6.8 and 14.7 g ± 5.1. Accordingly, RILs carrying Bhutmuri-SPIKE allele also produced significantly higher number of filled grains (126 ± 33 vs 83 ± 27) with wider flag leaf

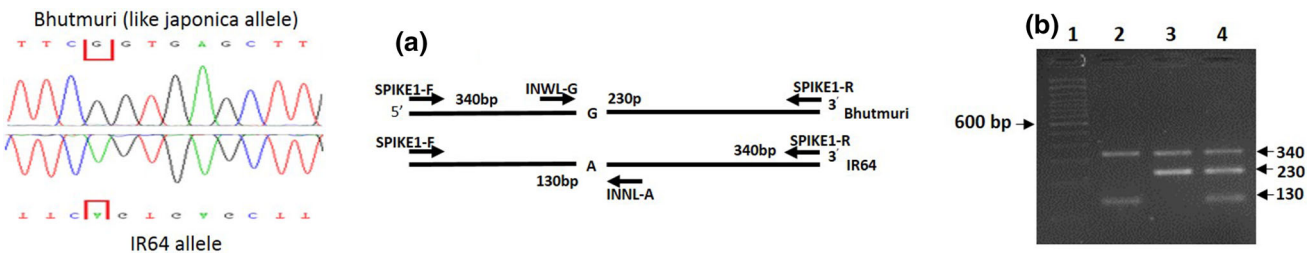

Figure 1. (a) Strategy for PCR based markers from the SNP (A/G) at the 3′UTR and expected band size for SNP (A/G) derived primer pairs. (b) Gel picture of SNP derived PCR based codominant marker; 1, 100-bp ladder; 2, IR64; 3, Bhutmuri; 4, heterozygote; size of fragments is shown by arrow head.

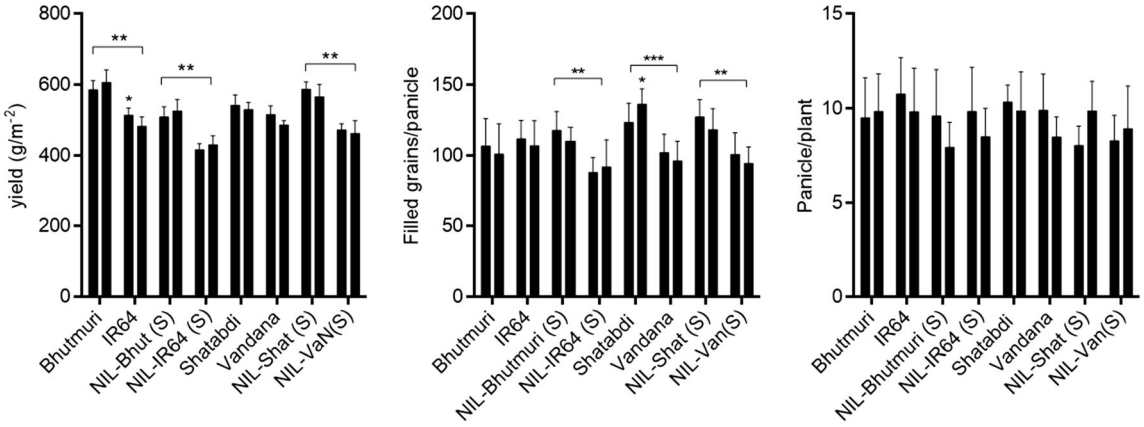

Figure 3. Comparison of filled grains per panicle, chaffy grains per panicle and plant yield (g) between two parents and their NILs from the crosses Bhutmuri × IR64 and Shatabdi × Vandana in successive two years (two closed bars); NILs carrying Bhutmuri-SPIKE-1 allele, NIL-Bhutmuri (S), Vandana allele, NIL-Van(S), Shatabdi-SPIKE-1 allele, NIL-Shat(S), and IR64 allele, NIL-IR64 (S) and their respective parents. *, ** And ***shows $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively by two-way ANOVA analysis.

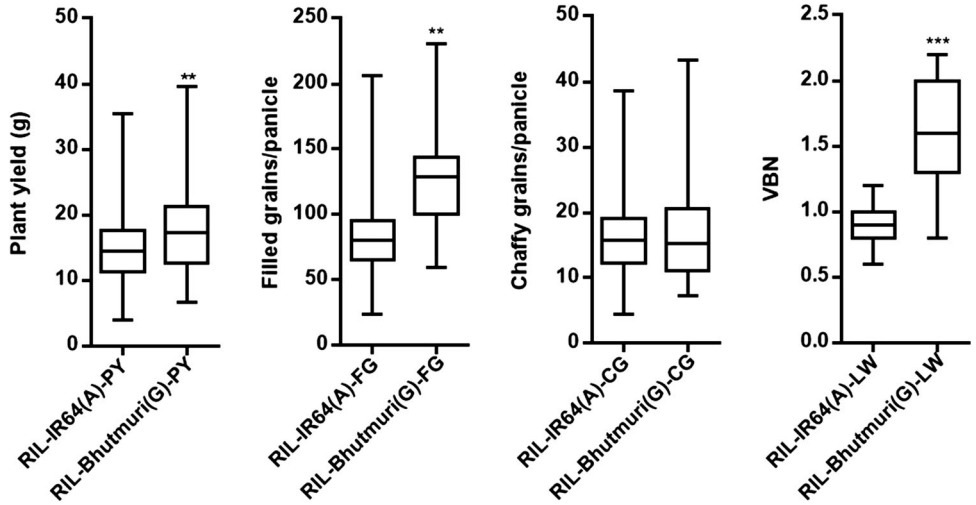

Figure 4. Comparison of plant yield (g), filled grains per panicle, chaffy grains per panicle and flag leaf-width for two groups of RILs from the cross Bhutmuri and IR64; one carrying IR64-SPIKE allele (RIL-IR64(A) and another with Bhutmuri-SPIKE allele, RIL-Bhutmuri(G). ** And ***shows $P < 0.01$ and $P < 0.001$, respectively by single point ANOVA analysis between two groups of RILs.

width, but no difference in chaffy grains was noticed for the studied RILs (figure 4).

Comparison of net photosynthesis rate (P_n) and other physiological parameters

Narrow variation in net photosynthesis (17.7 to 23.3 $\mu molm^{-2}s^{-1}$) was observed among the four parental genotypes. However, the difference between two parental pairs, i.e. Bhutmuri vs IR64 (17.7 ± 3.5 vs 23.3 ± 2.8 $\mu molm^{-2}s^{-1}$) and Shatabdi vs Vandana was significant. NIL-Bhutmuri exhibited high net photosynthesis rate (19.1 ± 1.5) than NIL-IR64 (15.2 ± 2.9), even though IR64 showed higher rate of photosynthesis (figure 5). Shatabdi and its allele carrying NILs exhibited highest photosynthesis rate and VBN (figure 5). We also compared the effect of light intensity on Pn, particularly, for the Bhutmuri-SPIKE carrying NILs. When light intensity was low (<800 $\mu molm^{-2}s^{-1}$), difference in Pn between Bhutmuri and IR64 carrying NILs was negligible, but at optimum PAR (1000 $\mu molm^{-2}s^{-1}$) NIL-Bhutmuri showed clear advantage (19.2 $\mu molm^{-2}s^{-1}$) over IR 64-carrying NILs (15.1 $\mu molm^{-2}s^{-1}$).

However, when VBN was compared, clear superiority was observed both for Bhutmuri and Shatabdi and their allele carrying NILs (figure 5) with wider flag leaves. Both Shatabdi and Bhutmuri contained >6 VBN between two large vascular bundles, and their SPIKE allele carrying RILs and NILs also showed broader leaves with significantly more vascular bundles between two major veins; >6 VBN vs <5 VBN (figure 1 in electronic supplementary material).

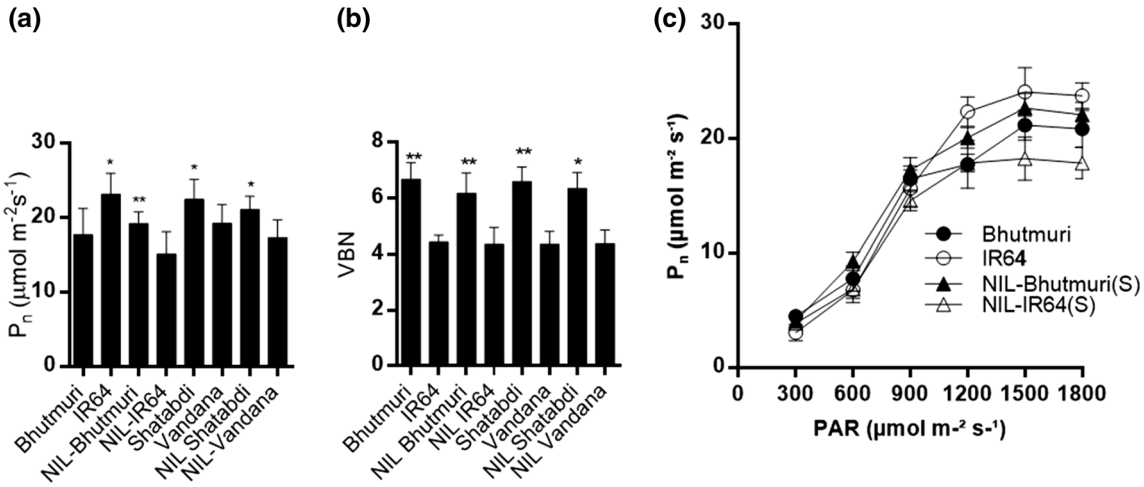

Figure 5. Comparison of (a) net photosymthesis rate (Pn), (b) vascular bundle number between two large vascular bundles among the parents and their NILs; NILs carrying Bhutmuri-SPIKE-1 allele, NIL-Bhutmuri, Vandana allele, NIL-Van, Shatabdi-SPIKE-1 allele, NIL-Shat, and IR64 allele, NIL-IR64 and (c) effect of different light intensity on Pn. * And ** signifies difference is significant at $P < 0.05$, and $P < 0.01$ respectively by unpaired *t*-test.

Discussion

Identification of novel alleles of yield enhancing genes can help to increase the intrinsic productivity of rice *vis-a' -vis* future demands of production without having to expand the land area for cultivation. SPIKE gene on chromosome 4 of rice increases the productivity of rice by improving the grain numbers per panicle without reducing panicle numbers and also by exerting pleotropic effects on leaf and photosynthetic traits. No yield enhancing allele of SPIKE is reported in *indica* subpopulation of rice. In this study, a *japonica* like allele of SPIKE was identified from the *indica* rice Bhutmuri and Shatabdi. Sequence analysis identified Bhutmuri, Dular and Shatabdi, which are *aus* subtype *indica* rice, carry an identical 5895 bp retrotransposon insertion as in the *japonica* genotype, Koshihari and Daringan. Based on sequence analysis, eight SNPs in the SPIKE gene was identified. An A/G SNP at 3′UTR was used to develop a PCR based codominant marker, which clearly distinguished *japonica* type Koshikari or Daringan allele from the *indica* subtypes. Another SNP (T/C) in 3′UTR was unable to distinguish *japonica* from the two *indica* lines, TN1 and Dee-geo-woo gene (Takai *et al.* 2013). As these two *indica* type rice lines were used for the development of several high yielding semi-dwarf cultivars, there are chances of obtaining false positives using the T/C SNP as a marker. Further, the available SNP-derived PCR based marker (Kim *et al.* 2016) targets two SNPs in exons 3 (G/A) and 5 (G/A), which requires two separate reactions for the identification of heterozygote. The identified single-tube allele-specific PCR based marker in this study will be useful for easier discrimination of lines at the early segregating generation with yield enhancing SPIKE allele and heterozygotes. Wide variation in leaf blade width (LBW) of 100 genotypes was present ranging from 0.4 cm to 2.2 cm. Although LBW of five genotypes, Bhutmuri, Asanla, Dular, Shatabdi and Triguna was more than 2 cm, yield enhancing SPIKE allele was present in Bhutmuri, Dular and Shatabdi only, indicating the role of some other genes for the wider leaves of Asanla and Triguna. Sequence analysis confirmed that Asanla and Triguna do not have the similar SNP like Butmuri and Shatabdi in the 3′UTR. *Japonica* type SPIKE allele in Shatabdi might have originated from new plant type (NPT) breeding programme as CR10 14, one of its parents, originated from a tropical *japonica* × *indica* cross. However, Bhutmuri and Dular are *aus* subtype upland landraces with red pericarp, cultivated in the southern part of Bengal. Thus, these genotypes have novelty in terms of the alleles they carry at the SPIKE locus. Panicle size was larger in Shatabdi than in Vandana but equal in Bhutmuri and IR64. The yield enhancing ability of the identified SPIKE allele was confirmed in RIL and NIL populations. Bhutmuri SPIKE allele showed yield advantage over the IR64 allele by producing greater number of grains; both in RILs and NILs. Similarly, RILs carrying the Shatabdi allele produced greater filled grains per panicle without reducing the panicle number in both the years. NIL-Bhutmuri produced greater yield per plant over IR64 mainly by improving spikelet number *vis-à-vis* filled grain number/panicle. Vandana and Bhutmuri matured earlier (115–120 days) than IR 64 (125–130 days). These results indicate that the Bhutmuri SPIKE allele may be useful for developing short duration high yielding rice varieties.

Depending on the SPIKE-allelic structure, various explanations are given for its yield enhancement ability; like, polar auxin transport ability and high transcript-abundance during panicle initiation stage (Takai *et al.* 2013), high photosynthesis rate by improving mesophyll cells between two large vascular bundle without size reduction or lateral extension (Takai *et al.* 2013). One yield enhancing-

QTL surrounding the SPIKE locus was also identified (Marathi *et al.* 2012; Singh *et al.* 2018). Here, high photosynthesis rate and transcript abundance of Bhutmuri-SPIKE in developing young panicles may be the reason behind the yield-enhancing effect of the SPIKE gene. Additionally, wider flag leaves might have played some role in enhancing yield as wider leaves have enhanced translocation ability. Pn enhancement ability of the Shatabdi allele was also observed along with higher vascular bundle number.

In conclusion, this study identified a yield enhancing allele of SPIKE from the *aus* subtype *indica* genotype Bhutmuri with an identical sequence to that of the *japonica* SPIKE allele (Os04g0615000). The SPIKE allele exhibited higher transcript abundance in young panicles and was able to enhance yield in RILs and NILs carrying this allele. The *japonica* like *aus* subtype *indica* allele of SPIKE identified in this study may be used to develop short duration high yielding varieties of rice. This is also the first report of an *aus* subtype landrace carrying a yield enhancing *japonica* like SPIKE allele. The allele-specific single tube codominant marker developed will be useful for marker-assisted introgression of the yield enhancing SPIKE allele into multiple *indica* backgrounds more easily.

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