Identification of Novel QTLs for Spikelet Fertility, Panicle Compactness and Phytohormone Ethylene to Decipher Poor Grain Filling of Basal Spikelets in Dense Panicle Rice

Sudhanshu Sekhar
ICAR - National Rice Research Institute Cuttack India
Jitendra Kumar
ICAR-NRRI Cuttack
Soumya Mohanty
ICAR-NRRI Cuttack
Niharika Mohanty
ICAR-NRRI Cuttack
Rudraksh Shovan Panda
ICAR-NRRI Cuttack
Swagatka Das
ICAR-NRRI Cuttack
Birendra Prasad Shaw
Institute of Life Sciences
Lambodar Behera (lambodarjamujhadi@gmail.com)
ICAR-NRRI Cuttack India

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Abstract

High grain number is positively correlated with grain yield in rice, but it is compromised because of poor filling of basal spikelets in dense panicle bearing numerous spikelets. The phenomenon that turns the basal spikelets of compact panicle sterile in rice is largely unknown. In order to understand the factor(s) that possibly determines such spikelet sterility in compact panicle cultivars, QTLs and candidate genes were identified for spikelet fertility percentage, panicle compactness and ethylene production that significantly influence the grain filling using recombinant inbred lines developed from a cross between *indica* rice cultivars, PDK Shriram (compact, high spikelet number) and Heera (lax, low spikelet number). Novel QTLs, *qSFP1.1*, *qSFP3.1* and *qSFP6.1* for spikelet fertility percentage; *qIGS3.2* and *qIGS4.1* for panicle compactness; and *qETH1.2*, *qETH3.1* and *qETH4.1* for ethylene production were consistently identified in both *kharif* seasons of 2017 and 2018. The comparative expression analysis of candidate genes like *ERF3*, *AP2*-like ethylene-responsive transcription factor, *EREBP*, *GBSS1*, E3 ubiquitin-protein ligase *GW2*, and LRR receptor-like serine/threonine-protein kinase *ERL1* associated with identified QTLs revealed their role in poor grain filling of basal spikelets in dense panicle. These candidate genes thus could be important for improving grain filling in compact-panicle rice cultivars through biotechnological interventions.

Introduction

Rice is a staple crop for about half of human beings all over the world, especially in Asia and Africa. However, since last decade the production of rice has remained more or less static to approximately 450 metric tons, which need to be increased at least 1.5 times by the year 2050 to feed the increasing world population (Alexandratos and Bruinsma 2012). The failure to increase the production has largely been due to failure of the breeding programme to produce high yielding varieties, although rice varieties with large panicle bearing numerous spikelets have been successfully developed, like New plant type (NPT) lines by International Rice Research Institute (IRRI) and ‘super’ rice or ‘super’ hybrid rice in China (Peng et al. 2008; Yang et al. 2010). The failure of the spikelets to undergo proper grain filling to produce well filled grain, particularly of the basal spikelets, has been the major cause of the poor harnessing of the grain yield potential of the rice varieties selected for large or compact panicle bearing numerous spikelets (Sekhar et al. 2015a).

The anthesis in a rice panicle is not synchronous. It is basipetal asynchronous. Anthesis starts from the spikelets borne on the primary branches of the apical region of the panicle and proceeds towards the spikelets located on the basal primary or secondary branches in six to seven days. The asynchronous anthesis is largely responsible for variations in quality and weight of the kernels produced in the apical and basal spikelets with the basal spikelets producing smaller grains compared with that produced by the apical spikelets (Yang et al. 2000; Panda et al. 2015; Panda et al. 2018). The difference in quality of the grain produced by the apical and basal spikelets because of asynchronous anthesis is visible more in the compact panicle bearing numerous spikelets compared with that in the lax panicle bearing fewer (< 250) spikelets (Panda et al. 2018).

Many reasons have been indicated for differential grain filling in the apical and basal spikelets of compact panicle rice. Some of the earlier studies suggested comparatively lesser translocation of assimilates from the leaf sheath to the sink in the basal spikelets than that in the apical spikelets to be the cause poor grain filling in the former than in the latter (Tsukaguchi et al. 1999; Yang et al. 2002). In contrast, it has been seen experimentally that poor grain filling in the basal spikelets of the compact-panicle is a result of sink limitation rather than the limitation of flow of carbohydrate from the source. Studies have shown that the assimilates partitioned into the endosperm cells of the basal spikelets, particularly of those located on the secondary branches of the panicle remain unused during the course of grain development (Umemoto et al. 1994; Panda et al. 2015; Zhang et al. 2014a). Furthermore, spikelet thinning treatment in which sufficient numbers of apical spikelets are removed from the panicle just before heading has shown that the

basal spikelets develop into a well filled grain similar to that of the apical spikelets, suggesting that the basal spikelets of compact panicle are genetically competent to produce well developed mature grain, the quality that could be masked by factors unknown so far (Kato 2004). The research conducted so far has revealed that the poor filling of grain in the basal spikelets compared with apical ones could be low activity of the starch synthesizing enzymes, like sucrose synthase (SUS), starch synthase (SS), granule bound starch synthase (GBSS), UDPase, adenosine diphosphate glucose pyrophosophorylase (AGPase), starch debranching enzymes (DBE) and starch branching enzyme (SBE) (Kato et al. 2007; Ishimaru et al. 2005; Tang et al. 2009; Wang et al. 2008; Panda et al. 2015; Panda et al. 2016; Yang and Zhang 2010; Zhang et al. 2014a), although the evidences are only circumstantial. Besides, the plant hormone ethylene has been indicated to be involved in various regulatory and signalling pathways linked to the grain filling process, and has an inhibitory role in grain filling (Panda et al. 2015; Sekhar et al. 2015b; Panda et al. 2016). The inhibitory role of ethylene in grain filling is basically indicated from the fact that the basal spikelets of compact panicle produces more ethylene than that of the lax- panicle, and ethylene receptors and the downstream signalling components of ethylene are constitutively expressed more in the compact panicle spikelets than that in the lax panicle spikelets (Panda et al. 2015; Sekhar et al. 2015a, b; Panda et al. 2016). Fu and Xue (2010) and Sekhar et al. (2015b) have further proved that expression of RSR1, a rice *AP2/EREBP* family transcription factor (in the ethylene signalling pathway) is negatively correlated with the expression of type I starch synthesis like *GBSS1* genes.

Keeping in view the fact that the normal agricultural practices being followed and continuing breeding programs are no more effective in increasing grain yield of rice, it is highly necessary to understand the reason of trade-off between spikelet numbers and grain filling in compact panicle so as to increase the rice production. Identification of QTLs is one important method through which the phenotypic variation can be linked to the genes involved in such variation, opening the avenue for genetic manipulation for improvement of the trait of interest. Many QTLs linked to yield related traits, such as grain size, grain width, grain weight, etc. have been identified (Wan et al. 2006; Song et al. 2007; Li et al. 2011; Qiu et al. 2012; Kang et al. 2018). QTL, *qTSN4.4/SPIKE/LSCHL4* associated with spikelet number (TSN) (Fujita et al. 2012; 2013; Zhang et al. 2014b*), SCM2/APO1* with panicle architecture (Ookawa et al. 2010; Terao et al. 2010), *GS3* and *Gn1a*, which controls grain weight and length (Fan et al. 2006) and grain number (Ashikari et al. 2005), respectively have been identified. *GIF1* associated with grain filling has been identified, which encodes a cell wall invertase required for carbon partitioning during early grain-filling in rice (Wang et al. 2008). The panicle length determines the number of primary and secondary branches and number of spikelets per panicle (Bai et al. 2016). Huang et al. (2009) reported that many Chinese high yielding rice varieties have the dominant allele at the dense and erect panicle1 *(DEP1*) locus on chromosome 9 with mutation (625bp deletion) that causes truncation of a phosphatidylethanolamine-binding protein domain like protein. The effect of this mutant allele is that there occurs enhance meristematic activity that results in increased number of grains per panicle and reduced length of the inflorescence internode. The elongation of the rachis and primary and secondary branches is on the other hand is under the control of *DEP2* (dense and erect panicle) gene, which when mutated results in compact panicle architecture without significant increase in the spikelet numbers; *dep2* loci has 31bp deletion in exon 6 of *LOC_Os07g42410* (Li et al. 2010). Further, Qiao et al. (2010) reported a third type of dense and erect panicle mutant, *dep3*, which has 408-bp genomic deletion within the third exon and 3'-UTR of the *LOC_Os06g46550* gene. It shows a significant increase in the spikelet number (33.9% more compared to the wild type), but the panicle length, shape and size of spikelets decreases or remains unchanged. *SP1* (SHORT PANICLE 1), *Ghd7*, *Ghd7.1*, and *Ghd8/DTH8* also regulate panicle length, the number of primary and secondary branches (Xue et al. 2008; Yan et al. 2011, 2013). A few other loci like *TAW1* (*TAWAWA1*) and *LRK1* (LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASE 1), *LAX2* (LAX PANICLE2), regulate panicle branching including the number of secondary and tertiary branches (Zha et al. 2009; Tabuchi et al. 2011; Yoshida et al. 2013). *WFP* encodes *OsSPL14* and higher expression of *OsSPL14* promotes grain filling and panicle branching in rice at reproductive stage (Miura et al. 2010). All these studies suggested that the panicle morphology is determined by complex interactions of various gene products, and that grain

yield is highly dependent on the panicle architecture, including the panicle length, the number of primary and secondary branches, compactness and laxness of panicle.

In present study, an attempt was made to identify QTLs and candidate genes associated with spikelet fertility percentage, panicle compactness (inter-grain space) and phytohormone ethylene production using RIL mapping population developed from the cross between two *indica* rice cultivars, PDK Shriram (compact panicle, high spikelet number per panicle) and Heera (lax panicle, low spikelet number per panicle) and further to investigate the possible cause of poor grain filling or sterility in basal spikelets of dense panicle.

Materials And Methods

Plant materials

Two *indica* rice cultivars, PDK Shriram and Heera, contrast for spikelet numbers and panicle compactness were selected for the identification of QTLs associated with spikelet fertility percentage, panicle compactness (inter-grain spacing) and phytohormone ethylene production. PDK Shriram bears compact and heavy panicle with high spikelet numbers (350-360), while Heera bears lax-panicle with less number of spikelets (100-110) (Fig. 1). A mapping population consisting 188, F_{11} recombinant inbred line developed from the cross PDK Shriram and Heera by single-seed descent method.

Phenotypic evaluation of RIL mapping population for spikelet fertility and panicle compactness

All 188 RILs along with parents, PDK Shriram and Heera were planted in the experimental fields of ICAR-National Rice Research Institute, Cuttack, Odisha, India during the *kharif* seasons of 2017 and 2018 following an Alpha Lattice design each with two replications. The 25 days old seedlings were transplanted in the experimental plots. The row-to-row spacing of 20cm and plant-to-plant spacing 15cm were maintained. Each row contained 20 single plants. The fertilizer dose of 80kg N, 40kg P_2O_5 and 40kg K_2O_5 per hectare was applied. The standard agronomic practices and need based plant protection measures were undertaken for normal plant growth. Five plants were selected from the middle of the row from each replication and observations were recorded on number of spikelets per panicle, number of sterile spikelets per panicle, percentage of filled spikelets/grains per panicle, panicle length and length of the primary branches per panicle. The total length (cm) of the primary branches was divided by the total number of spikelets on the panicle to give the inter-grain space. Three panicles per plant for 5 plants with two replications were used for the generation of data on the panicle morphology. Mean data were used for further analysis.

Estimation of ethylene production in the spikelets

Ethylene production in the apical and basal spikelets of the parents PDK Shriram and Heera was measured on the early days of anthesis, such as 0, 3, and 6 days after anthesis (DAA). The apical and basal spikelets sampled were moistened and immediately inserted separately in rubber stopper 10 ml tubes and sealed after 20 min. The sealed tubes with spikelets were incubated in darkness for 5h. After incubation, the headspace gas (1 ml) was drawn with the help of air tight chromatography syringe and injected into the GC column of gas chromatography (Varian CP-3800) fitted with flame ionization detector. The data were represented as the mean ± SD of five observations and expressed as pmol ethylene produced $h^{-1}g^{-1}$frwt.ml^{-1} headspace air (Sekhar et al. 2015b). Ethylene production in all 188 RILs was also measured for 3 days after anthesis of basal spikelets to generate phenological data for ethylene production.

Genotyping of RIL mapping population

Genomic DNA isolation was carried out from the leaf tissues of the parents and all 188 RILs using Cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). The quality of the DNA was checked by electrophoresis on 0.8% agarose gel and the quantity was determined using Nano spectrophotometer. A set of 1200 random SSR markers and 225 SNP markers were used to identify polymorphic SSR and SNP (single nucleotide polymorphic) markers between parents, PDK Shriram and Heera. All the microsatellite markers used in this study were available in the Gramene database (http://www.gramene.org). 118 polymorphic SSR markers (Supplementary Table S1) were used to genotype 188 RILs along with parents. 225 SNP sequences were downloaded from OryzaSNP@MSU databases (http://rice.plantbiology.msu.edu) and used to design assay panels (nine assay panels). These nine assay design panels were used to survey parental polymorphism using the Agena MassArray®SNP genotyping system. Twenty-two polymorphic SNP markers (Supplementary Table S2) identified and were subjected to again design assay panel (one panel). SNP genotyping was performed in 188 RILs and parents using the AgenaMassArray® SNP genotyping system.

Construction of linkage map and identification of QTLs

The genotype data on 188 RILs generated by 118 polymorphic SSR and 22 single nucleotide polymorphic (SNP) markers was used for the construction of linkage map using integrated QTL IciMapping, Version 4.1 software (Meng et al. 2015). LOD score 3 was used to construct the linkage group. The Kosambi mapping function was used to convert the recombination frequency into genetic distances in centi-Morgan (cM) (Kosambi 1944). The inclusive composite interval mapping (ICIM) was used to identify QTLs associated with spikelet fertility percentage, inter-grain space (panicle compactness) and phytohormone ethylene production. The inclusive composite interval mapping (ICIM) software provided more efficient background control via a two-step mapping strategy as compared to composite interval mapping (CIM). This software avoids the possible increase of sampling variance and complicated background marker selection process in CIM (Li et al. 2012). The one-dimensional scanning of the whole genome was carried out with mapping parameters of 1cM, the probability value for entering variables (PIN) of 0.001 with a threshold LOD score of 3 in order to identify significant QTLs (Lander 1995).

In silico analysis for identification of the candidate genes associated with QTLs

The genes located within three important QTL regions were searched using NCBI gene bank (https://www.ncbi.nlm.nih.gov/) and rice genome annotation project (RAP-DB) (https://rapdb.dna.affrc.go.jp/). These QTLs regions are between Affx93259116 and RM12224 on chromosome 1 containing QTL *qETH1.2*, RM588 and RM20045 on chromosome 6 containing three QTLs *qIGS6.1*, *qIGS6.2* and *qETH6.1*, RM20500 and RM20506 on chromosome 6 containing two QTLs *qSFP6.1* and *qIGS6.3*. The position of genes/loci was obtained from Gramene database (http://www.gramene.org). The number genes in QTL regions were listed and candidate genes were selected.

Expression studies of the candidate genes

Apical and basal spikelets were sampled on 6, 9 and 12 days after anthesis in both parents PDK Shriram and Heera, frozen into liquid nitrogen and stored at -80^{0}C until use. Among RILs, one high spikelet number compact panicle line, 166A and one lax panicle low spikelet number line, 14A were also considered for sampling of the spikelets, similar to that of the parents. Total RNA was isolated from the collected samples using TRIZOL reagent (Invitrogen) following reagent user manual. Quality and quantity of the RNA isolated from the individual samples was checked on Agarose gel and Nanodrop spectrophotometer, respectively. Quanti-Tect Reverse Transcription Kit (Qiagen) was used for conversion of total isolated RNA to cDNA following the protocol outlined in the kit's manual. The cDNAs prepared from the individual samples were used for the study of expression of the genes of interest identified in the QTL regions. Expression of a gene was studied by qPCR taking the cDNA as template and SYBR green (Agilent). Primers specific to

the gene of interest were designed using Primer Blast software at the NCBI site. Required amount of SYBR green, cDNA template and the primers for a gene was mixed in a final volume of 20 µl and PCR was run on Bio-Rad CFX Manager Version 3.1 Real Time PCR detection system. Rice actin was used as an internal control. The relative level of templates of the individual gene in the apical and basal spikelets was quantified following Pfaffl (2001), and the result was expressed as fold change in expression in the basal spikelets over apical ones. Seven candidate genes were selected from three QTL regions and used in the expression studies (Supplementary Table S3).

Results

Phenological variation in the parents and RIL mapping population

Total spikelet number per panicle was much higher in PDK Shriram (350-360) than Heera (100-110) (Table 1 and Fig. 1). The inter-grain space (IGS) was much less in PDK Shriram (Mean: 0.268 cm) compared with Heera (Mean: 0.661 cm). In contrast to SFP and IGS, the ethylene production was more in PDK Shriram in basal spikelets (Mean: 0.0319 $pmol.g^{-1}frwt.ml^{-1}air.h^{-1)}$ than in Heera (Mean: 0.0165 $pmol.g^{-1}frwt.ml^{-1}air.h^{-1}$) for 3 day after anthesis (Fig. 2). Furthermore, the production of ethylene was always more in the basal spikelets compared with the apical spikelets in all the days after fertilization in both the cultivars (Fig. 2). The phenological data for parents and two RIL lines 166A and 14A were shown in Table 1 for both the *kharif* seasons of 2017 and 2018. The spikelet fertility among RILs varied from 21.22% to 89.76% with an average of 69.23% in *kharif* 2017, while 45.22% to 97.13% with an average of 73.17% in *kharif* 2018 (Table 2). The IGS in the RIL population varied from 0.28 cm to 0.95 cm with an average of 0.51 cm in *kharif* 2017, while 0.26 cm to 1.03 cm with an average of 0.53 cm in *kharif* 2018. The ethylene production varied from 0.01 $pmol.g^{-1}$ frwt. ml^{-1} air. h^{-1} to 0.201 pmol. g^{-1} frwt. ml^{-1} air. h^{-1} in *kharif* 2017, while 0.01 pmol. g^{-1} frwt. ml^{-1} air. h^{-1} to 0.25 $pmol.g^{-1}frwt.ml^{-1}air.h^{-1}$ with an average of 0.0294 $pmol.g^{-1}$frwt. ml^{-1} air. h^{-1} in *kharif* 2017 and 0.03 $pmol.g^{-1}$frwt. ml^{-1} air. h^{-1} in *kharif* 2018 (Table 2).

It was also observed the difference in the production of ethylene was higher between the basal spikelets of the two cultivars than between the apical spikelets with PDK Shriram showing more production of ethylene than Heera. In addition, the production of ethylene increased greatly in the basal spikelets in PDK Shriram with increase in the days after anthesis from 0 to 3 days, but the increase was not much in Heera (Fig. 2). Table 2 shows the values for skewness and kurtosis for SPF, IGS and ethylene production for the *kharif* seasons of 2017 and 2018. The normal frequency distribution of RILs was observed in SPF and IGS (Fig. 3a, 3b). The ethylene production recorded for 3 DAA in the basal spikelets of RILs, showed highly positive skewness and kurtosis (Table 2).

Correlation analysis between traits

Inter-grain space (IGS) showed significant positive correlation with spikelet fertility percentage (SFP) for the seasons data, *kharif* 2017 (0.313) and *kharif* 2018 (0.325) at the level $p \leq 0.01$ (Table 3). IGS, on the other hand, showed significant negative correlation (-0.128 in *kharif* 2017 and - 0.191 in *kharif* 2018) with ethylene production (ETH) at an early stage after anthesis (3 DAA) in the RIL population at the level $p \leq 0.05$. However, no significant correlation was observed between ETH and SFP in both the *kharif* seasons (Table 3).

Identification of QTLs for spikelet fertility percentage, inter-grain space and ethylene production

A total of 118 (9.83%) out of 1200 SSR markers and 22 (9.78%) out of 225 SNP markers (Supplementary Table S1, S2) were found polymorphic between parents, PDK Shriram and Heera. Seven markers which showed segregation distortion ($P<0.001$) were not used in linkage map construction. The remaining 133 polymorphic markers were used to construct a linkage map. A total of 17 linkage groups were constructed after screening with LOD threshold 3 using the

IciMapping V4.1 software. The linkage map covered a total of genetic distance of 1878.3cM (Table 4) with an average marker interval of 19.17cM. One-dimensional scanning of the whole genome was carried out with mapping parameters of step size 1cM with PIN (probability value for entering variables) value 0.001 and a threshold LOD score of 3 in order to identify significant QTLs associated with spikelet fertility percentage (SFP), inter grain space (IGS) and ethylene production (ETH) in *kharif* seasons of 2017 and 2018 (Fig. 4). A total of 14 QTLs was identified in *kharif* 2017, while 15 QTLs were identified in *kharif* 2018 on five different chromosomes of rice (Table 5). A total of four QTLs (*qSFP1.1*, *qSFP3.1*, *qSFP6.1* and *qSFP8.1*) for spikelet fertility percentage, eight QTLs (*qIGS1.1*, *qIGS3.1*, *qIGS3.2*, *qIGS4.1*, *qIGS4.2*, *qIGS6.1, qIGS6.2* and *qIGS6.3*) for inter-grain space, and seven QTLs (*qETH1.1, qETH1.2, qETH3.1, qETH4.1, qETH4.2, qETH6.1* and *qETH6.2*) for ethylene production were identified. Four QTLs (*qSFP1.1, qSFP3.1, qSFP6.1* and *qSFP8.1*) for spikelet fertility percentage, five QTLs (*qIGS3.2, qIGS4.1, qIGS6.1, qIGS6.2* and *qIGS6.3*) for inter-grain space and five QTLs (*qETH1.1, qETH1.2, qETH3.1, qETH4.1* and *qETH6.1*) for ethylene production were identified in *kharif* 2017, while three QTLs (*qSFP1.1, qSFP3.1* and *qSFP6.1*) for spikelet fertility percentage, seven QTLs (*qIGS1.1, qIGS3.1, qIGS3.2, qIGS4.1, qIGS4.2, qIGS6.1* and *qIGS6.2*) for inter-grain space and five QTLs (*qETH1.2, qETH3.1, qETH4.1, qETH4.1* and *qETH6.2*) for ethylene production were identified in *kharif* 2018 (Table 5). Three QTLs (*qSFP1.1, qSFP3.1* and *qSFP6.1*) for spikelet fertility percentage, four QTLs (*qIGS3.2, qIGS4.1, qIGS6.1* and *qIGS6.2*) for inter-grain space and three QTLs *(qETH1.2, qETH3.1* and *qETH4.1*) for ethylene production were consistently identified in both *kharif* seasons of 2017 and 2018. QTLs explained phenotypic variance, which varied from 5.423% (*qETH4.1* in *kharif* 2017) to 12.653 % (*qIGS6.1* in *kharif* 2017) (Table 5). The LOD score found to vary from 3.045 on chromosome 8 (*qSFP8.1* in *kharif* 2017) to 25.462 on chromosome 6 (*qETH1.1* in *kharif* 2018). The QTLs for spikelet fertility percentage and inter-grain space on 6 (*qSFP6.1, qIGS6.3*) were found to be same region on chromosome 6 between RM20500 and RM20506 in *kharif* 2017, while QTL (*qSFP1.1, qIGS1.1*) found to be same region on chromosome 1 between RM10552 and HVSSR1-31 in *kharif* 2018. Further, *qSFP3.1* and *qIGS3.2* were also identified in the same region between RM14906 and RM16 on chromosome 3 in both *kharif* seasons of 2017 and 2018, while *qIGS6.2* and *qETH6.2* were identified in the same region between RM19480 and RM20045 in *kharif* season of 2018 (Table 5, Fig. 5).

Analysis of candidate genes associated with identified QTLs

In silico search was done in order to identify genes present in the chromosomal regions associated with identified QTLs. NCBI gene bank database (https://www.ncbi.nlm.nih.gov/) and rice genome annotation project (RAP-DB) were used to search genes in QTL regions. 1438, 1929 and 111 genes were identified in QTL regions, Affx93259116-RM12224 (*qETH1.2*), RM588-RM20045 (*qIGS6.1, qIGS6.1* and *qETH6.1*), and RM20500-RM2050 *(qSFP6.1* and *qIGS6.3*), respectively (Supplementary Table S4a). From among these genes, important candidate genes in QTL regions were listed in Supplementary Table S4b, c, d. Six genes were predicted for ethylene production in the QTL region of *qETH1.2* on chromosome 1 (Supplementary Table S4b). On chromosome six, ten genes were predicted in the QTL region of *qIGS6.1, qIGS6.2* and *qETH6.1* for inter-grain space and ethylene production (Supplementary Table S4c), while 5 genes were predicted in QTL region of *qSFP6.1* and *qIGS6.3* associated with spikelet fertility percentage and inter-grain space, respectively (Supplementary Table S4d).

Expression analysis of candidate genes associated with identified QTLs

Comparative expression studies of seven candidate genes associated with three identified QTL regions containing six QTLs (*qETH1.2, qIGS6.1, qIGS6.2* and *qETH6.1, qSFP6.1* and *qIGS6.3*) were carried out at the exponential stage of grain filling at 6 day, 9 day and 12 day after anthesis in both apical and basal spikelets of parents (PDK Shriram and Heera) and two RILs, one lax-panicle low grain (RIL-14A) and one compact-panicle high grain number (RIL-166A). One ethylene responsive gene ethylene responsive transcription factor 8 also named as ethylene responsive factor 3 (*ERF3*) associated with *qETH1.2* on chromosome 1 expression was higher in basal spikelets as compared to apical spikelets

in compact panicle PDK Shriram and RIL-166A, while their expression was not significant between apical and basal spikelets in lax panicle Heera and RIL-14A (Fig. 6a). Further, five candidate genes identified on chromosome 6 in the QTL region *of qETH6.1, qIGS6.1* and *qIGS6.2* were selected for their expression analysis. Two ethylene responsive genes, *AP2*-like ethylene-responsive transcription factor (Fig. 6b) and *EREBP* (Fig. 6c) identified in the region of QTL, *qETR6.1* showed higher expression in basal spikelets of compact panicle cultivar, PDK Shriram and RIL-166A, while no significant difference was found the expression in apical and basal spikelets of lax panicle cultivar, Heera and RIL-14A. The expression pattern of *GBSS1* (Fig. 6d) identified in QTL region of *qIGS6.1* and *qIGS6.2* shows opposite to the expression pattern of the other genes (Fig. 7, 8, 9). The enzyme showed a greater expression in the apical spikelets compared with the basal spikelets in PDK Shriram and RIL-166A on all the days of post anthesis (Fig. 6d). In Heera and RIL-14A, the expression of the enzyme was more or less similar in both apical and basal spikelets (Fig. 6d). E3 ubiquitin-protein ligase (*GW2*)(*LOC107276853*), which was linked to the QTL*, qSFP6.1* and *qIGS6.3* in the marker region RM20500**-**RM20506 on chromosome 6 showed higher expression in the basal spikelets compared with the apical spikelets in the compact panicle cultivar, PDK Shriram and RIL-166A (Fig. 7a). However, the opposite was the case with regard to expression of E3 ubiquitin-protein ligase in the lax panicle Heera and RIL-14A sample (Fig. 7a). The expression pattern of serine carboxypeptidase II-2 (*LOC4340344*), which was also identified on chromosome 6 in the marker region RM19480- RM19496 of QTL *qIGS6.2,* was similar as E3 ubiquitin-protein ligase. The expression of the protein was higher in the basal spikelets compared with the apical ones in PDK Shriram (HGN) and RIL-166A, while the protein had a lower expression in the basal spikelets compared with the apical ones in Heera and RIL-14A on all the days post anthesis, except on 6 DAA (Fig. 7b). The expression pattern of the LRR receptor-like serine/ threonine-protein kinase ERL1 genes, which was also identified in the QTL region of *qIGS6.2* and *qETH6.1* on chromosome 6, was somewhat different from E3 ubiquitin-protein ligase and serine carboxypeptidase II-2. The expression of the protein was although mostly higher in the basal spikelets compared with the apical ones in general in PDK Shriram and RIL-166A, the expression was low in the basal spikelets than in the apical spikelets on 6 DAA in RIL-166A (Fig. 7c). The expression of protein on the other hand was much lower in the basal spikelets than in the apical spikelets in Heera, particularly on 9 and 12 DAA (Fig. 7c). The expression of LRR receptor-like serine/threonine-protein kinase ERL1, however, remained more or less similar in both apical and basal spikelets in RIL-14A (Fig. 7c), in contrast to the expression of E3 ubiquitin-protein ligase (Fig. 7a) and serine carboxypeptidase II-2 (Fig. 7b). Their expression was found upregulated in apical spikelets in Heera as compared to basal spikelets. However, in compact panicle cultivar PDK Shriram and RIL-166A, its expression was more in basal spikelets as compared to apical.

Discussion

Grain filling stage is crucial and very complex in rice life cycle. Several factors regulate the grain filling process determining the final grain yield. In addition, the number of spikelets per panicle plays an important role in determining the grain yield of the crop. Hence, increasing the spikelet number per panicle is essential in order to enhance the yield potential of rice. However, breeding programme to increase the number of spikelets per panicle is accompanied by poor filling of the basal spikelets particularly in secondary branch that limits the grain yield and quality, as is evident from the poor grain filling percentage of PDK Shriram bearing numerous spikelets compared with the high grain filling percentage of Heera bearing fewer spikelets (Table 1). Similar findings have been noted in other studies as well (Mohapatra et al. 1993; Naik and Mohapatra, 1999; Kato et al. 2007; Sekhar et al. 2015a, b; Panda et al. 2015; Panda et al. 2016; Panda et al. 2018). The major factors that have come to knowledge in poor filling of grains on a panicle bearing numerous spikelets is a decrease in inter-grain space (IGS), which was also observed in the present study in PDK Shriram (Table 1), as well as reported by others (Sekhar et al. 2015a, b; Panda et al. 2015; Panda et al. 2016, Panda et al. 2018). In addition, a higher production of ethylene in the basal spikelets of the compact panicle has also been reported to influence grain filling negatively (Yang et al. 2006; Sekhar et al. 2015b; Panda et al. 2015; Panda et al.

2016), similar to that found in PDK Shriram (Fig. 2). However, the mechanistic details of inhibition of the grain filling process in basal spikelets of compact-panicle bearing numerous spikelets are yet to be understood. Nevertheless, in the present study, the identification of QTLs controlling the traits and their associate genes may unmask the cause of inhibition of grain filling in high spikelet number compact panicle cultivars at the genetic level.

The RIL population developed from PDK Shriram and Heera showed normal distribution for two phenotypic traits, SFP and IGS (Fig. 3a, 3b and Table 2) in both *kharif* seasons of 2017 and 2018. No significant skewness or kurtosis was found for these traits, while ethylene (ETH) production showed skewed distribution (George & Mallery 2010). No significant correlation was found between ETH and SFP (Table 3), indicating that ethylene production might not be tightly linked with SFP, in contrast to reports available that high ethylene production in the spikelets is the cause of poor grain filling in basal spikelets of the compact panicle (Sekhar et al. 2015a, b; Panda et al. 2015). Rather, SFP might be linked to the factors other than ETH, like IGS. Support to no possible link of SFP with ETH comes from the fact that in the present case, a lesser SFP was also observed in the RIL population showing low ethylene production (Fig. 3). Nevertheless, the inhibitory role of ethylene may not be totally ruled out, as ethylene-induced signalling component has been found to be inhibitory to granule bound starch synthase (Sekhar et al. 2015b; Fu and Xue 2010). Significant positive correlation between SFP and IGS, is however, certainly visible from the data of the RIL population (Table 3). It means, inter-grain space (IGS) in panicle has an inhibitory role of grain filling that is lower the IGS, higher the poor grain filling particularly seen in basal spikelets. Wang et al. (2008) reported a negative relationship of poor grain filling with IGS. The compact panicle cultivars having lower inter-grain space showed lower grain weight and width, and higher chalky grain percentage and amylose content among grains within a panicle than the lax panicle cultivars.

In our study, a total of seven novel QTLs associated with ethylene production (*qETH1.1, qETH1.2, qETH3.1, qETH4.1, qETH4.2, qETH6.1* and *qETH6.2*) were identified. No QTL has been identified earlier for ethylene production in these QTL regions. A total of four QTLs, *qSFP1.1, qSFP3.1, qSFP6.1* and *qSFP8.1* for spikelet fertility percentage have been identified. Three QTLs, *qSFP1.1, qSFP3.1* and *qSFP6.1* associated with spikelet fertility percentage are found to novel as no QTL has been previously identified in regions between RM10552 and HVSSR1-31 on chromosome 1, RM14906 and RM16 on chromosome 3, and between RM20500 and RM20506 on chromosome 6, respectively. A total of seven QTLs (*qIGS1.1, qIGS3.1, qIGS3.2, qIGS4.1, qIGS4.2, qIGS6.1* and *qIGS6.2*) have been identified for inter-grain spacing. Further, *qIGS3.2* and *qIGS4.1* was novel as no QTL for panicle density or inter-grain space has been detected previously in the associated QTL region. Previously, QTL on chromosome 1 named *sf1* associated with spikelet fertility in the region of 34264553 bp -36658883 bp on chromosome 6, and QTL for spikelet density named *sd6* in the region of 6023974 bp - 9537572 bp have been identified (Lin et al. 1995). These QTLs are present in the marker region of detected QTLs *qIGS6.1* and *qIGS6.2*. A total of four QTLs have been identified for spikelet fertility previously, *qFER-6* in the region of 3416533 bp -3416728 (Suh et al. 2005), *qSPTF6* in the region of 4234080 bp- 5096867 bp (Yan et al. 2003), *S5* in the region of 6283432 bp-9284248 bp (Yan et al. 2000) and *spf6* in the region of 6283432 bp-7177183 bp (Song et al. 2005). The traits, SFP, IGS and ETH may have controlled with expression of certain genes associated with identified QTLs. The important to note was, however, that QTLs, *qSFP1.1, qIGS1.1* and *qSFP6.1, qIGS6.3* were identified in the same regions of chromosome 1 and chromosome 6, respectively ((Table 5 and Fig. 5). Interestingly, these two traits also bears significant correlation between them (Table 3), suggesting that the genes are associated with QTLs for SFP and IGS. Further, *qETH6.2* shared same region with *qIGS6.2* on the chromosome 6, suggesting that the inhibition in grain filling could be linked to production of ethylene or inter-grain space at least some of the genes regulating IGS and ETH share a common region on the chromosome, and the genes associated with these QTLs may have pleiotropic effects on regulation of these traits. The gene, ERF3 (*Os01g0797600*), an ethylene responsive downstream signalling component associated with QTL, *qETH1.2* on chromosome 1 shows higher expression (Fig. 6a) in basal spikelets as compared to apical spikelets in compact panicle cultivar, PDK Shriram and RIL-166A, while its expression was almost same in apical and basal spikelets of lax panicle cultivar, Heera and RIL-14A. Similar results were also found for the

expression of *AP2*-like ethylene-responsive transcription factor (*Os06g0145700*) (Fig. 6b) and *EREBP* transcription factor (*Os06g0194000*) (Fig. 6c) associated with QTL, *qETH6.1* on chromosome 6. The results indicated that due to higher evolution of ethylene in basal spikelets of compact panicle cultivar, the perception of ethylene might be higher that leads to higher expression of downstream ethylene signalling component in basal spikelets (Sekhar et al. 2015b). Further, *AP2* like ethylene-responsive element binding protein family transcription factor, also known as rice starch regulator (RSR1) and it negatively regulates the expression of type I starch synthesis genes (Fu and Xue 2010). Hence, due to higher expression of *AP2* like EREBP family transcription factor in basal spikelets of compact panicle cultivar, there might be inhibition of expression starch synthesis related genes may lead to poor grain filling in inferior spikelets. Further, one of type 1 starch synthesis related gene, granule bound starch synthase 1 (*GBSS1*) was also identified in the QTL region of *qIGS6.1* and *qIGS6.2*. Their spatio-temporal expression analysis of the enzyme reciprocated the grain filling pattern in the lax- as well as the compact-panicle, as the expression of the enzyme was more or less similar in the both the apical and basal spikelets of the lax-panicle cultivar, Heera and RIL-14A, while the expression of the enzyme was down regulated in the basal spikelets compared with the apical ones in compact panicle cultivar, PDK Shriram and RIL-166A indicating the negative regulator of the expression of type I starch synthesis genes by *AP2/EREBP* family transcription factor. So, we propose a model for ethylene-mediated poor grain filling in basal spikelets of dense panicle rice cultivars (Fig. 8). Further, over expression of *AP2/ERF* family transcription factor *OsEATB* (ERF protein associated with tillering and panicle branching was also reported to promote the branching and involve in reduction of panicle length through restrict internode elongation by down regulating a GA biosynthetic gene ent-kaurene synthase A (Qi et al. 2011) that leads to lower inter-grain space and increases compactness having numerous spikelets per panicle. Harrop et al. (2019) also reported that *AP2/EREBP*-like genes were involved in inflorescence branching and architecture in domesticated rice. E3 ubiquitin-protein ligase (*GW2*) has been reported to play very important role in the regulation of weight of spikelets; the loss of function of *GW2* increases grain filling rate and larger spikelets hull (Song et al. 2007). Thus, a greater expression of this gene in the basal spikelets in the compact-panicle cultivar, PDK Shriram and RIL-166A (Fig. 7a) might be one of the possible causes of poor filling of grains in them compared with the apical spikelets. Song et al. (2007) has also reported that *GW2* negatively regulates cell division by targeting its substrate(s) to proteasomes for regulated proteolysis. Furthermore, Choi et al. (2018) reported that *GW2* negatively regulates the seed size by targeting *EXPLA1* for degradation through its E3 ubiquitin ligase activity. In addition, *GW2* regulates the seed size through direct interactions with proteins involved in carbohydrate metabolism by modulating their activity or stability and controlling disulfide bond formation in various proteins during seed development (Lee et al. 2018). Since both the parent PDK Shriram and RIL-166A shows poor grain filling in the basal spikelets, and the same is accompanied by compactness of their panicle, a low IGS in them could be a result of pleiotropic effect of greater expression of E3 ubiquitin-protein ligase in the basal spikelets compared with apical, the possible cause of poor grain filling in the former than in the latter. The relationship between spatial difference in expression of E3 ubiquitin-protein ligase and panicle compactness or panicle architecture, however, certainly needs further detailed investigation.

Serine carboxypeptidase II-2 was another important enzyme that was identified in the QTL region of *qIGS6.2* and *qETH6.1*, and found a greater expression in the basal spikelets compared with apical ones in PDK Shriram and RIL-166A (Fig. 7b). The role of serine carboxypeptidase II-2 has so far not been investigated. However, some other serine carboxypeptidase like *GS5/OsSCP26* and *SCP46* has been reported to be a positive regulator of grain width and weight (Li et al. 2011; Li et al. 2016). Higher expression of LRR receptor-like serine/threonine-protein kinase in the basal spikelets compared to the apical ones in PDK Shriram and RIL-166A (Fig. 7c) could also be the reason of a low IGS and compactness of panicle in them in contrast to Heera and RIL-14A. The possible role of LRR receptor-like serine/threonine-protein kinase in regulating IGS in panicle is reflected from the fact that the enzyme is the largest receptor like protein kinase having a diverse role in plant growth, development including organogenesis, morphogenesis, hormone signalling, and abiotic and biotic stress response in plants (Li and Tax, 2013; Diévart and

Clark, 2003). It has also been reported that LRR receptor-like serine/threonine-protein kinase ERL1 regulates inflorescence architecture and organ shape as well as stomatal patterning, including density and clustering, together with ER and ERL2 (Clark et al. 1997; Cano-Delgado et al. 2004; Shpak et al. 2004; Deyoung et al. 2006; Stenvik et al. 2008; Tsuwamoto et al. 2008; Xu et al. 2008). Thus, the QTLs, *qIGS6.1*, *qIGS6.2* and *qETH6.1* on chromosome 6 that harbour genes encoding proteins like LRR receptor-like serine/threonine-protein kinase remains to be answered how their higher expression in the basal spikelets in comparison with the apical spikelets would change the entire panicle architecture from lax to compact type.

Grain filling in rice is a complex process, particularly keeping in view the differential filling of the grains in the spikelets based on their spatial location in compact-panicles. The differential filling of grains in the compact-panicle in which the basal spikelets remain largely unfilled or poorly filled has, in fact, been a major obstacle in rice breeding program intended to increase rice production. An attempt was made to understand the molecular basis of differential grain filling through identification of QTLs for poor grain filling, inter-grain space and ethylene production in the mapping RIL population of Heera (lax panicle) and PDK Shriram (compact panicle). Five genes associated these traits like *ERF3*, *AP2*-like ethylene-responsive transcription factor (*Os06g0145700*) and EREBP transcription factor (*Os06g0194000*), granular bound starch synthase 1 and E3 ubiquitin-protein ligase stand distinct are involved in regulation of grain filling in basal spikelets of dense panicles, while the role of serine carboxypeptidase II-2, LRR receptor-like serine/threonine-protein kinase in compactness of panicle and grain filling was not known. These genes thus could be important candidate genes in improving the grain filling in compact-panicle rice cultivars showing poor grain filling through biotechnological interventions. The QTLs harbouring these genes may in fact be useful to transfer the grain filling trait into the rice cultivars of interest through molecular breeding approach.

Declarations

Declarations The authors declare that they have no competing interests

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Conflicts of interest The authors declare that they have no Conflicts of interest

Availability of data and material The data that support the findings of this study are available online

Code availability Not applicable

Ethics approval and Consent to participate Not applicable

Authors Contributions SS, BPS and LB designed the work. SM and LB developed RIL population. SS and LB conducted most of the experiments, including RIL mapping population development, phenotyping of RILs, expression analysis, linkage and QTL mapping analysis. JK, NM, RSP and SD conducted genotyping experiment using RIL population. SS drafted the manuscript. BPS and LB corrected the manuscript.

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Supplementary data Supplementary Table S1: List of polymorphic SSR markers used for genotyping of RILs developed from the cross between PDK Shriram and Heera. Supplementary Table S2: List of polymorphic SNP markers used for genotyping of RILs developed from the cross between PDK Shriram and Heera. Supplementary Table S3: List of candidate genes used in qRT-PCR for validation. Supplementary Table S4a: No of genes identified in selected QTL regions. Supplementary Table S4b: List of candidate genes (CG) associated with *qETH1.2* Supplementary Table S4c: List of candidate genes (CG) associated with *qIGS6.1*, *qIGS6.2* and *qETH6.1.* Supplementary Table S4d: List of candidate genes (CG) associated with *qSFP6.1* and *qIGS6.3*

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Tables

Table 1 Morphological feature of panicles of parental cultivars and two RILs selected based on high spikelet number (compact) and low grain number (lax) panicle and their fertility percentage.

Cultivars	Duration of cultivars (days)	Year	Panicle length (cm)	Total grain numbers	Filled grain (%)	Inter-grain space (cm)
HGN	140	2017	21.13 ± 0.54	350 ± 10	69.975	0.266 ± 0.06
		2018	22.14 ± 0.60	344 ± 11	70.235	0.270 ± 0.08
		Mean (2017 & 2018)	21.64 ± 0.58	347 ± 15	70.11	0.268 ± 0.07
Heera	90	2017	20.67 ± 1.13	100 ± 11	85.82	0.621 ± 0.03
		2018	20.96 ± 0.83	98 ± 10	87.41	0.701 ± 0.06
		Mean (2017 & 2018)	20.82 ± 0.48	99 ± 11	86.62	0.661 ± 0.04
RIL-166A	145	2017	21.82 ± 0.38	356 ± 12	59.42	0.286 ± 0.012
		2018	22.58 ± 0.31	361 ± 9	55.69	0.226 ± 0.071
		Mean (2017 & 2018)	22.2 ± 0.35	358 ± 10	57.56	0.256 ± 0.042
RIL-14A	88	2017	20.82 ± 0.51	98 ± 8	86.86	0.630 ± 0.081
		2018	20.11 ± 0.22	95 ± 10	88.49	0.690 ± 0.044
		Mean (2017 & 2018)	20.47 ± 0.36	96 ± 11	87.68	0.67 ± 0.064

Inter-grain space = Total length of primary branch / total number of spikelets per panicle. All values are mean of five replicates.

Table 2 Statistical analysis of spikelets fertility percentage, spikelet number, inter-grain space (panicle compactness) and ethylene production in the RILs derived from PDKV Shriram (HGN) and Heera grown during *kharif* 2017 and *kharif* 2018.

Traits name	Year	RILs					
		Min.	Max.	Average	± SD	Kurtosis	Skewness
Spikelets fertility percentage (SFP)	2017	21.22	89.76	69.23	12.495	0.990	-0.955
	2018	45.28	97.13	73.17	8.605	1.669	-0.902
Inter-grain space (IGS) in cm	2017	0.28	0.95	0.51	0.118	1.002	0.841
	2018	0.26	1.03	0.53	0.154	0.635	0.790
Ethylene (ETH) in $pmol.g^{-1}frwt.\ ml^{-1}$ air. h^{-1}	2017	0.01	0.201	0.0294	0.008	4.205	2.932
	2018	0.01	0.250	0.03	0.0092	4.019	3.305

Table 3 Correlation among spikelet fertility percentage (SFP), inter-grain space (IGS) and ethylene production (ETH) in 188 RILs developed from the cross between PDK Shriram (HGN) and Heera grown during *kharif* seasons of 2017 and 2018.

Pearson Correlation						
	SFP		IGS		ETH	
	2017	2018	2017	2018	2017	2018
SFP	1	1	0.313**	0.325**	0.015	-0.105
IGS	0.313**	0.325**	1	1	-0.128*	-0.191*
ETH	0.015	-0.105	-0.128*	-0.191*	1	1
N	188					
**. Correlation is significant at the 0.01 level (1-tailed).						
*. Correlation is significant at the 0.05 level (1-tailed).						

Table 4 Summary of polymorphic markers obtained in parental polymorphism survey used for genotyping of RILs and construction of linkage map.

Chr#	No of markers used in parental polymorphism survey			No of polymorphic markers obtained in parental polymorphism survey			% of polymorphism	Number of markers used for construction of linkage map	Linkage group	Length for marker interval of linkage group (cM)
	SSR	SNP	Total	SSR	SNP	Total				
1	115	10	125	9	2	11	8.80	10	LG1	243.43
2	102	25	127	11	4	15	11.81	14	LG2 LG3 LG4	114.63
3	120	10	130	27	0	27	20.77	26	LG5	653.5
4	98	26	124	15	7	22	17.74	20	LG6 LG7 LG8 LG9	320.1
5	115	25	140	16	4	20	14.29	20	LG10	256.74
6	80	20	100	8	1	9	9.00	8	LG11	59.04
7	95	25	120	6	1	7	5.83	7	….	0
8	85	22	107	6	1	7	6.54	7	LG12 LG13	65.47
9	116	17	133	5	0	5	3.76	5	….	0
10	90	16	106	1	1	2	1.89	2	….	0
11	92	18	110	9	0	9	8.18	8	LG14 LG15	117.36
12	92	11	103	5	1	6	5.83	6	LG16 LG17	48.03
Total	**1200**	**225**	**1425**	**118**	**22**	**140**	**-**	**133**		**1878.3**

LG- Linkage group

Table 5 QTLs identified for spikelet fertility percentage (SFP), inter-grain space (IGS) and ethylene production (ETH) using RILs derived from PDK Shriram (HGN) and Heera in *kharif* seasons of 2017 and 2018.

Year	Traits Name	QTL Name	LG	Chr#	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add
2017	**SFP**	*qSFP1.1*	1	1	30	RM10552	HVSSR1-31	7.037	10.636	-23.856
		qSFP3.1	5	3	478	RM14906	RM16	4.752	10.23	-23.826
		qSFP6.1	11	6	55	RM20500	RM20506	6.107	9.995	16.229
		qSFP8.1	12	8	9	RM23513	RM264	3.045	7.567	-2.997
	IGS	*qIGS3.2*	5	3	478	RM14906	RM16	10.874	11.464	-0.246
		qIGS4.1	6	4	135	RM16952	RM17063	3.656	11.442	-0.052
		qIGS6.1	11	6	35	RM588	RM19480	6.191	12.653	0.153
		qIGS6.2	11	6	44	RM19480	RM19496	7.983	11.831	0.153
		qIGS6.3	11	6	56	RM20500	RM20506	16.854	9.093	0.246
	ETH	*qETH1.1*	1	1	31	HVSSR1-31	HVSSR1-44	18.530	5.714	-0.045
		qETH1.2	1	1	240	Affx93259116	RM12224	19.556	5.996	-0.045
		qETH3.1	5	3	10	RM14272	RM14292	7.458	6.469	0.035
		qETH4.1	6	4	11	RM16519	RM16569	10.794	5.423	0.048
		qETH6.1	11	6	23	RM19496	RM20045	8.35	6.447	0.043
2018	**SFP**	*qSFP1.1*	1	1	29	RM10552	HVSSR1-31	12.375	9.792	-24.957
		qSFP3.1	5	3	481	RM14906	RM16	7.812	9.528	-24.956
		qSFP6.1	11	6	56	RM20500	RM20506	10.759	10.353	27.996
	IGS	*qIGS1.1*	1	1	28	RM10552	HVSSR1-31	4.343	9.555	-0.157
		qIGS3.1	5	3	475	RM14825	RM14906	5.103	10.487	-0.126
		qIGS3.2	5	3	481	RM14906	RM16	4.094	10.999	-0.135
		qIGS4.1	6	4	132	RM16952	RM17063	5.204	11.47	-0.082
		qIGS4.2	6	4	151	RM17063	RM252	4.451	9.037	-0.080
		qIGS6.1	11	6	35	RM588	RM19480	4.457	5.984	0.131
		qIGS6.2	11	6	43	RM19480	RM19496	4.175	6.35	0.131
	ETH	*qETH1.2*	1	1	239	Affx93259116	RM12224	25.462	5.471	-0.051
		qETH3.1	5	3	8	RM14272	RM14292	14.527	6.873	0.047
		qETH4.1	6	4	10	RM16519	RM16569	21.001	5.445	0.048
		qETH4.2	6	4	17	RM16569	RM16770	9.416	6.561	0.048
		qETH6.2	11	6	43	RM19480	RM19496	14.112	6.846	0.052

LG-Linkage group; Chr# - Chromosome number, PVE-Phenotypic variance

Figures

Figure 1

Parental rice cultivars PDK Shriram (HGN) and Heera showing compact- and lax-panicle type, respectively.

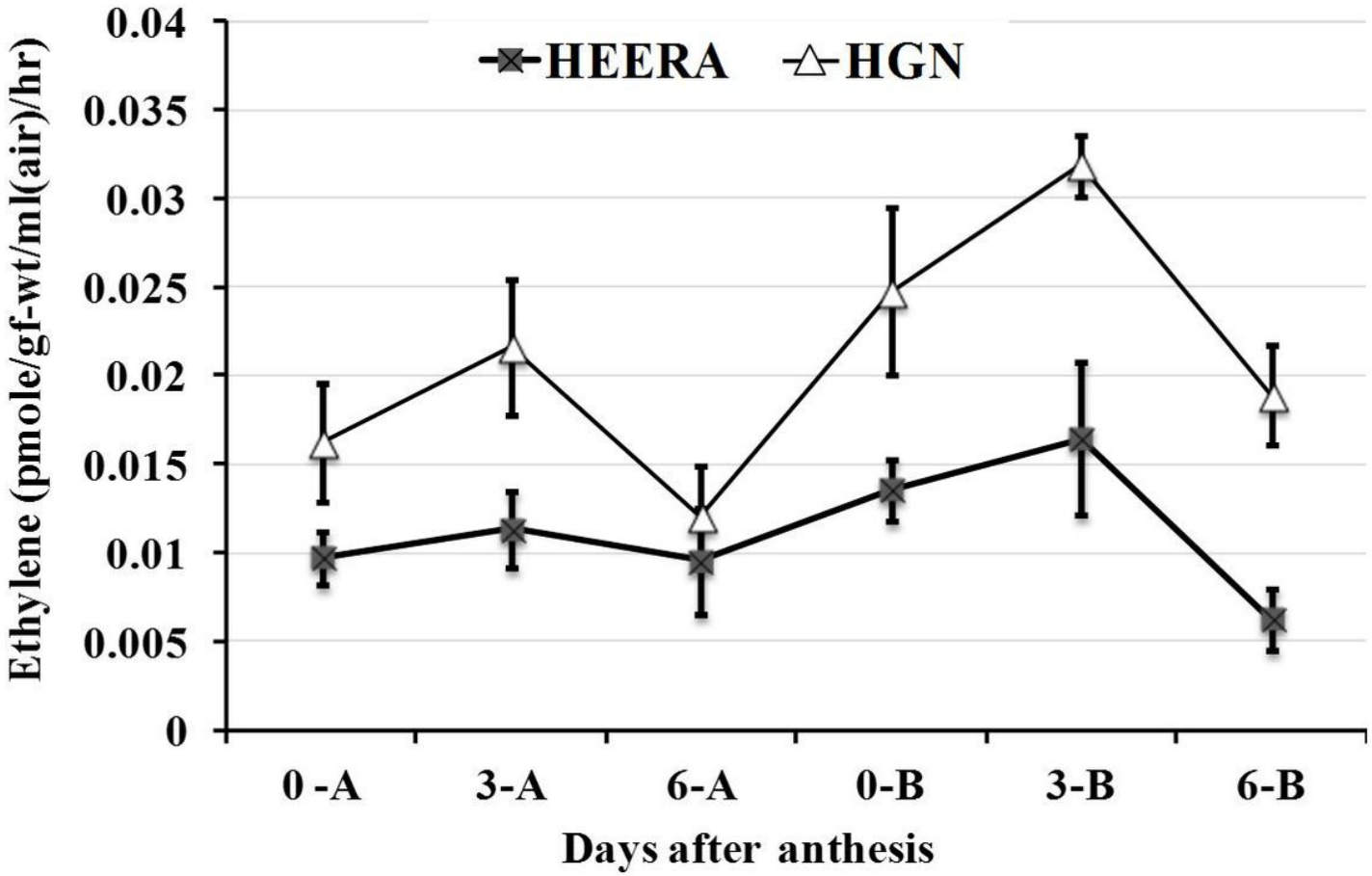

Figure 2

Ethylene production in apical and basal spikelets of compact panicled cultivar PDK Shriram (HGN) and lax-panicled cultivar Heera on different days after anthesis (0, 3 and 6). The data are the mean (± SD) of five observations. There was significant difference ($p \leq 0.05$) for the ethylene production between apical and basal spikelets in HGN. A denotes apical spikelets; B denotes, basal spikelets.

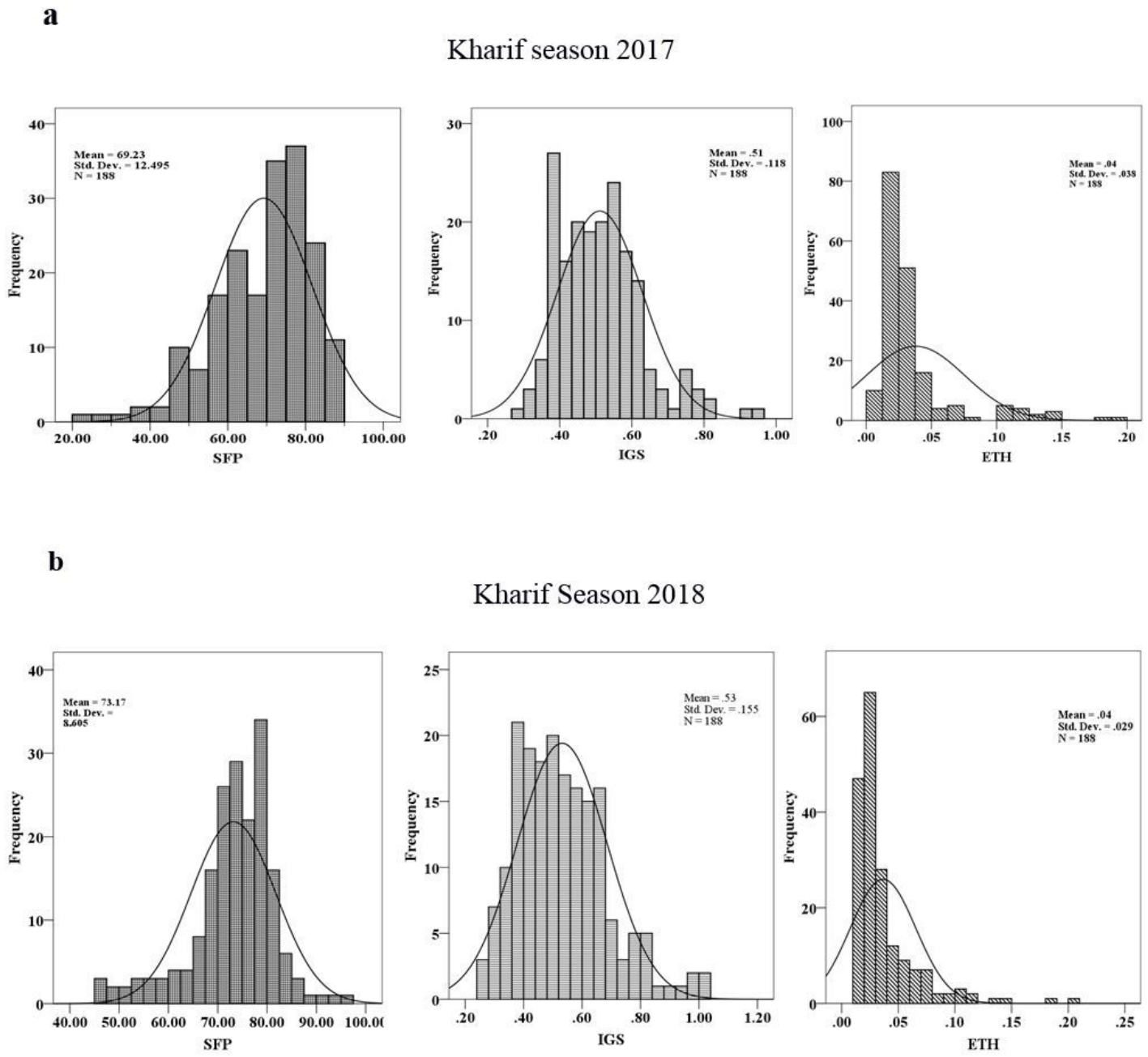

Figure 3

Frequency distribution of RILs for the traits, spikelet fertility percentage (SFP), inter-grain space (cm)(IGS) and ethylene (ETH) production (pmole/gfwt/ml (air)/hr), derived from PDK Shriram (HGN) and Heera. X-axis shows values of traits and Y-axis shows frequency RILs for the traits, SFP, IGS and ETH in kharif 2017 (3A), and in kharif 2018 (3B).

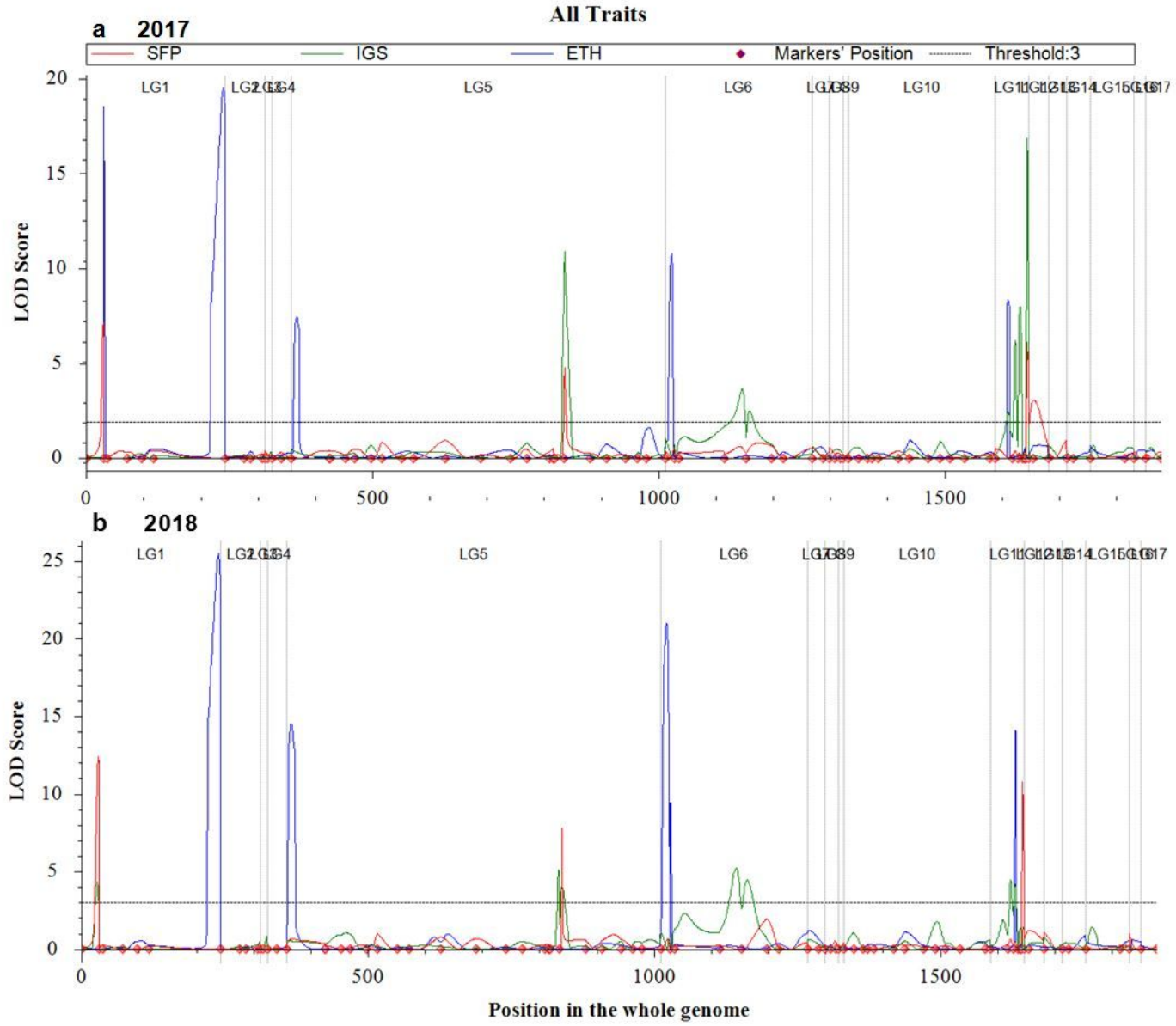

Figure 4

One-dimensional scanning of whole genome for identification of QTLs associated with spikelet fertility percentage (SFP), inter-grain space (IGS) and ethylene production (ETH) in kharif 2017 and kharif 2018, detected at threshold LOD score 3 using QTL ICI mapping V4.1 software. Graph shows identified QTL peaks on linkage groups. Fourteen peaks corresponding to fourteen QTLs were identified in kharif 2017 on chromosome 1 (LG1), chromosome 3 (LG5), chromosome 4 (LG6), chromosome 6 (LG11) and chromosome 8 (LG12). Fifteen peaks corresponding to fifteen QTLs were identified in kharif 2018 on chromosome 1 (LG1), chromosome 3 (LG5), chromosome 4 (LG6) and chromosome 6 (LG11). LG - Linkage group.

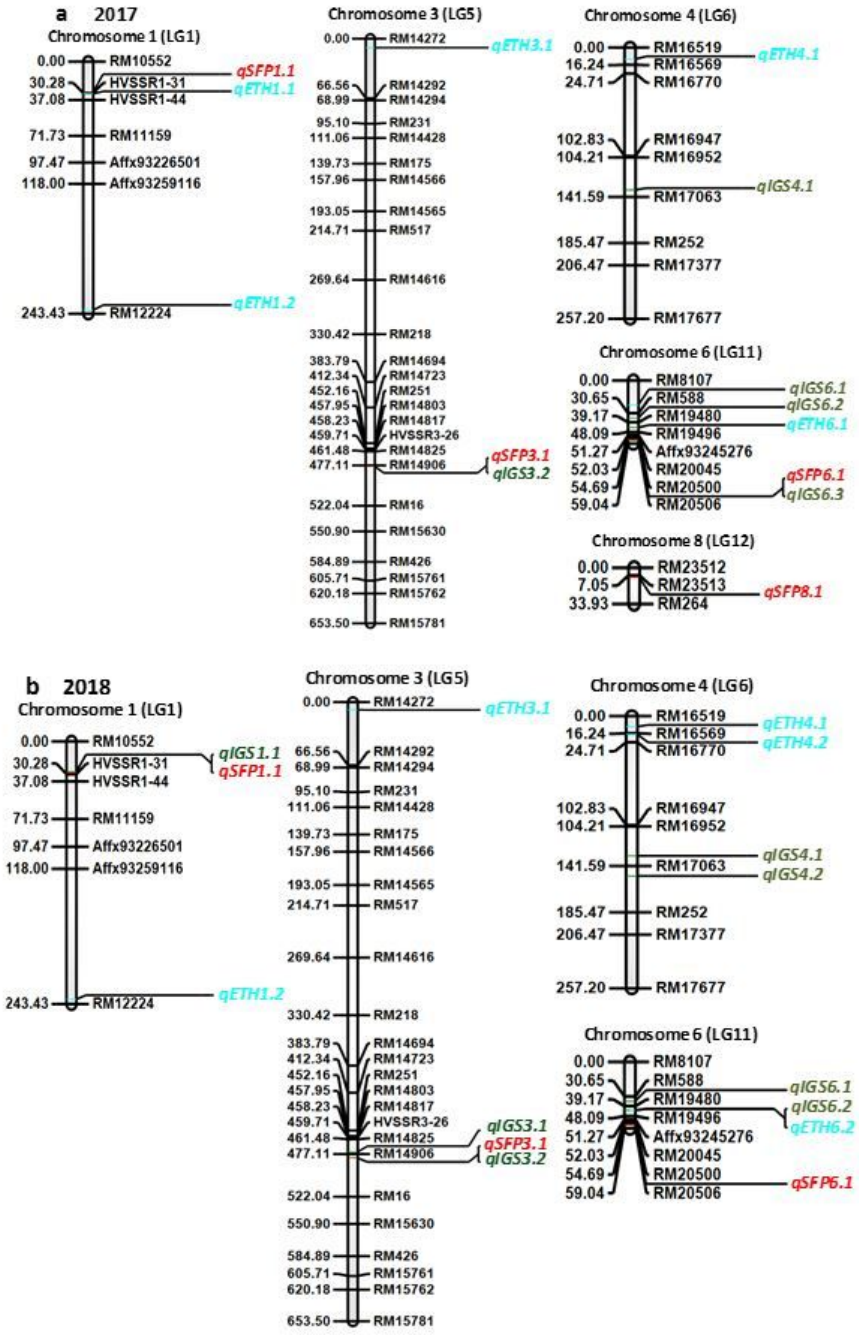

Figure 5

Linkage map showing QTLs associated with spikelet fertility (SFP), inter-grain space (IGS) and ethylene production (ETH). QTL identification was done using integrated QTL IciMapping, Version 4.1 software for (a) kharif 2017, and (b) kharif 2018. Markers and QTLs are represented on right side of linkage group, while values on left side represented linkage distance in cM, LG-Linkage group.

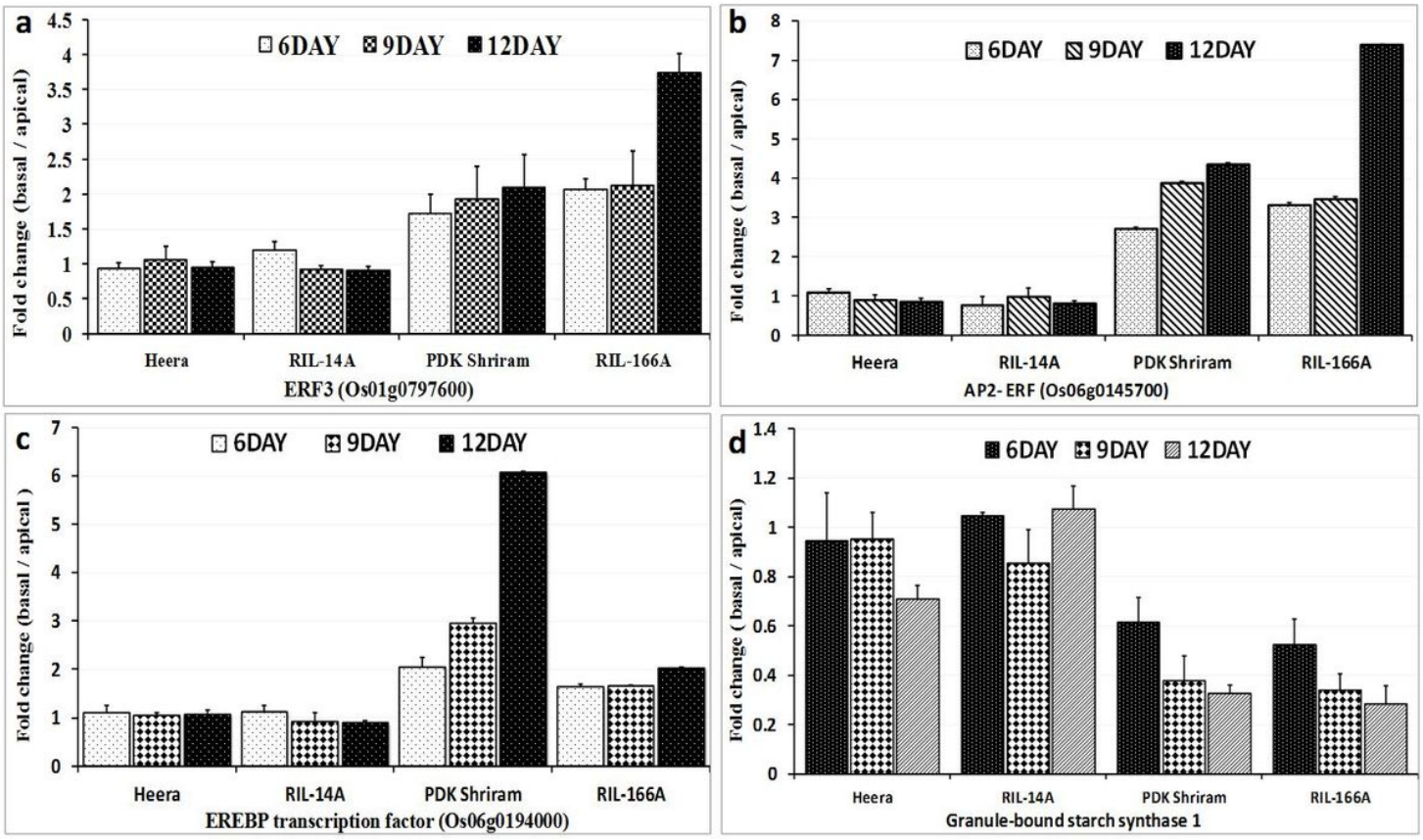

Figure 6

Relative expression level in fold of ethylene-responsive genes and GBSS1. (a) Ethylene responsive transcription factor 8 also named as ethylene responsive factor 3 (ERF3), (b) AP2-like ethylene-responsive transcription factor (AP2-ERF), (c) Ethylene-responsive element binding protein (EREBP) transcription factor, and (d) Granule bound starch synthase 1 (GBSS1) on 6th day, 9th day and 12th day after anthesis in the basal over apical spikelets of compact panicled PDK Shriram (HGN), RIL-166A and lax-panicled Heera, RIL-14A, determined by qRT-PCR. Rice actin was taken as internal reference. Data represent mean (±SD) using three biological replicates.

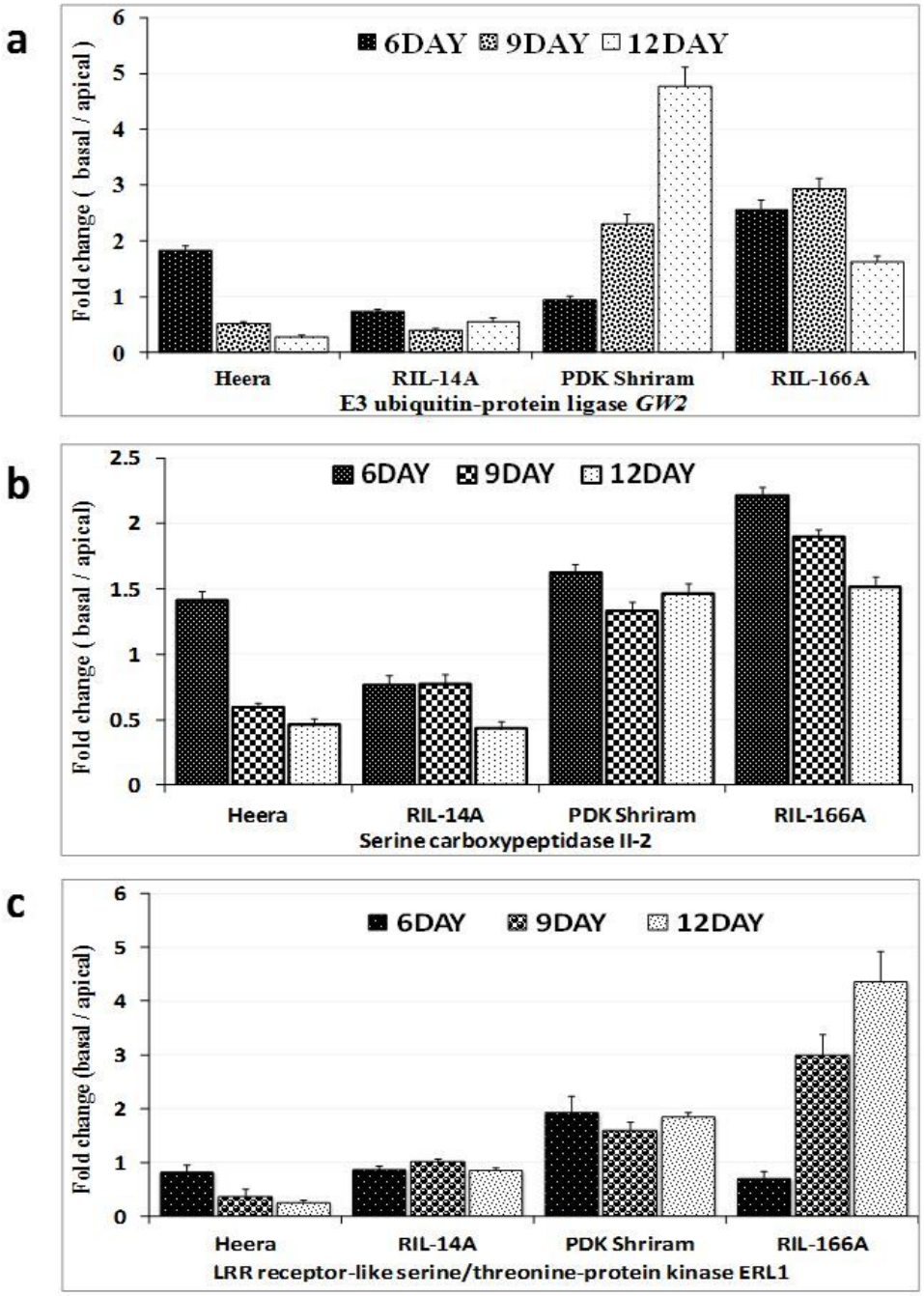

Figure 7

Relative expression level in fold of (a) E3 ubiquitin-protein ligase GW2, (b) serine carboxypeptidaseII-2, and (c) LRR receptor-like serine/ threonine-protein kinase ERL1 on 6th day, 9th day and 12th day after anthesis in the basal over apical spikelets of compact panicled PDK Shriram (HGN), RIL-166A and lax-panicled Heera, RIL-14A, determined by qRT-PCR. Rice actin was taken as internal reference. Data represent mean (±SD) of three biological replicates.

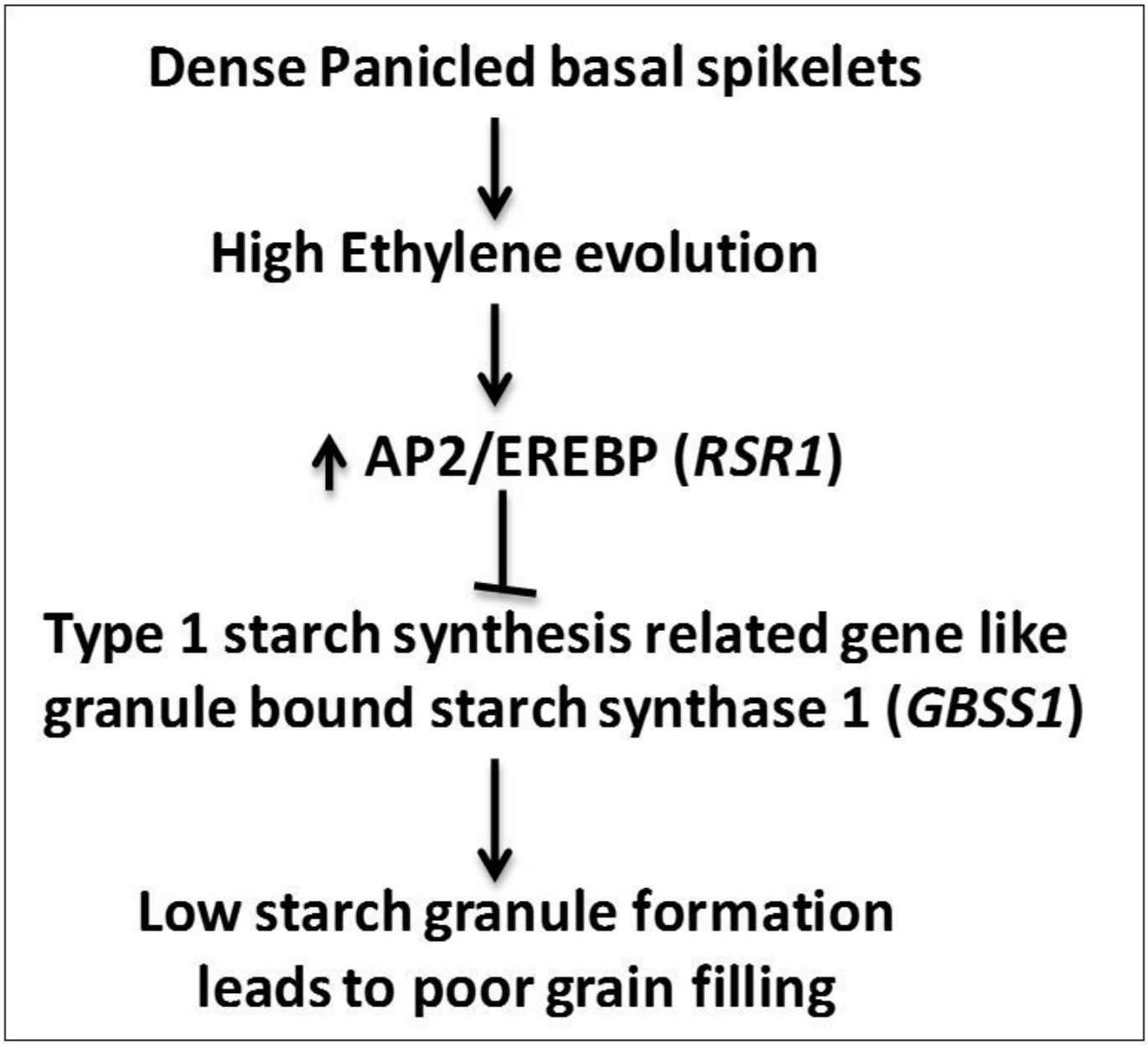

Figure 8

Model for poor grain filling in basal spikelets of dense panicle of rice, regulated by phytohormone ethylene. AP2- EREBP acts as a negative regulator of starch synthesis related genes leads to poor grain filling.