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RESEARCH ARTICLE

Genome-wide in silico analysis of long intergenic non-coding RNAs from rice peduncles at the heading stage

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Abstract Long intergenic non-coding RNAs (lincRNAs) belong to the category of long non-coding RNAs (lncRNAs), originated from intergenic regions, which do not code for proteins. LincRNAs perform prominent role in regulation of gene expression during plant development and stress response by directly interacting with DNA, RNA, or proteins, or triggering production of small RNA regulatory molecules. Here, we identified 2973 lincRNAs and investigated their expression dynamics during peduncle elongation in two Indian rice cultivars, Pokkali and Swarna, at the time of heading. Differential expression analysis revealed common and cultivar-specific expression patterns, which we utilized to infer the lincRNA candidates with potential involvement in peduncle elongation and panicle exsertion. Their putative targets were identified using in silico prediction methods followed by pathway mapping and literature-survey based functional analysis. Further, to infer the mechanism of action, we identified the lincRNAs which potentially act as miRNA precursors or target mimics.

Keywords Long non-coding RNAs (lncRNAs) · Long intergenic non-coding RNAs (lincRNAs) · Rice · Peduncles · Plant height · Panicle

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Introduction

Long non-coding RNAs (lncRNAs) are the transcripts which are longer than 200 nucleotides (nt), but do not exhibit any detectable coding potential (Budak et al. 2020; Caixia et al. 2020). They are ubiquitous in plant genomes and have been associated with diverse aspects of plant growth and development as well as stress response (Ding et al. 2012; Song et al. 2013; Song and Zhang 2017; Wang et al. 2018b). Some of the agronomically important traits regulated by lncRNAs include male fertility (Yu and Zhu 2019), nutrient metabolism (Franco-Zorrilla et al. 2007), flowering (Swiezewski et al. 2009), fruit ripening (Wang et al. 2018a), fruit development (Vlasova et al. 2016), etc. For this, they employ multitude of mechanisms associated with epigenetic modifications, transcriptional regulation, post-transcriptional RNA processing, regulation of protein synthesis and post-translational modifications (Zhang et al., 2020). Epigenetic modifications triggered by lncRNAs include histone modifications, DNA methylation and chromatin remodelling while at transcriptional level, lncRNAs can activate or repress gene expression by directly binding to DNA or regulatory proteins. For example, *COLDAIR* (*COLD ASSISTED INTRONIC NON-CODING RNA*) lncRNA regulates the expression of its target, *FLOWERING LOCUS C* (*FLC*), by mediating RNA–DNA interactions in *Arabidopsis* (Heo and Sung 2011), whereas, *LDMAR* (*LONGDAY-SPECIFIC MALE-FERTILITY-ASSOCIATED*) lncRNA of rice, involved in Photoperiod-Sensitive Male Sterility (PSMS), initiates

silencing of its target gene by regulating DNA methylation (Ding et al. 2012). Additionally, they can interfere with gene expression by acting as target mimics of miRNAs (Caixia et al. 2020). In fact, many lncRNAs also serve as precursors for generating miRNAs and secondary siRNAs. In addition, lncRNAs have also been shown to regulate RNA processing, mRNA stability, protein synthesis, localization and post-translational modifications. Contrary to earlier belief, some lncRNAs have also been shown to encode micropeptides with diverse biological functions (Zhang et al. 2020).

Based on the site of origin, lncRNAs have been classified into several subtypes. These include long intergenic non-coding RNAs (lincRNAs), intronic long non-coding RNAs, and natural antisense transcripts (NATs), which originate from intergenic, intronic, and antisense transcript regions, respectively (Mattick and Rinn 2015). In rice, lincRNAs have been identified from roots (Bazin and Bailey-Serres 2015), shoots (Xu et al. 2016), reproductive tissues (Komiya et al. 2014), and stress-treated samples (Xu et al. 2016; Shin et al. 2018). However, no lincRNAs have been reported from stem internodes of rice, so far.

In rice, the uppermost internode just below the panicle is called peduncle. Peduncle elongation at the time of heading is crucial for emergence of panicle from the leaf sheath also known as panicle exsertion and, completion of anthesis failing which leads to panicle enclosure (Bardenas 1965; Yin et al. 2007). Poor panicle exsertion causes significant impediment to optimum grain yield, as up to 40% spikelets may remain unfertilized due to panicle enclosure in leaf sheath (Devi et al. 2011). Earlier, we reported transcriptomic analysis of rice peduncles in two Indian rice cultivars, Swarna and Pokkali, exhibiting contrasting phenotypic characters with respect to peduncle length, plant height, grain yield, and cell wall composition (Kandpal et al. 2020). In this study, we leveraged that data to identify lincRNAs with potential involvement in regulating these traits in rice. Since, the data has been generated from two different genotypes of rice, we could also decipher the conservation in lincRNAs in both the genotypes.

Materials and methods

Plant material and RNA-sequencing

Earlier, we had generated RNA sequencing data from two genotypes, Swarna and Pokkali, of rice (Kandpal et al. 2020). Details of plant material collection and RNA sequencing experiment were provided (Kandpal et al. 2020) and the raw data was submitted to NCBI-GEO (GSE157727). Briefly, two genotypes of *Oryza sativa* ssp. *Indica*, Pokkali and Swarna, were grown in field conditions and RNA sequencing was performed from three biological replicates each of elongating and non-elongating peduncles collected at the heading stage using Illumina HiSeq platform. Elongating peduncles were collected 2–4 days before heading while non-elongating peduncles were sampled about 2–4 days after heading. After pre-processing of raw reads, merged assembly was used to extract the sequences of all the transcripts using gffread (Pertea and Pertea 2020).

Identification and expression analysis of long intergenic non-coding RNAs (lincRNAs)

The pipeline used for identification of lincRNAs is illustrated in Fig. 1. The longest transcript for each locus was extracted from the merged assembly and subjected to length filtering using a perl script. The Getorf function of EMBOSS package (Rice et al. 2000) was used to identify sequences containing open reading frames (ORFs). Transcripts with ORF length > 300 nt were removed. Coding potential of all transcripts was assessed using coding potential calculator (CPC2) with a score threshold of < 0.5 (Kang et al. 2017). BLASTX with E-value cut-off ≤ 0.001 was used to filter the sequences that aligned with SwissProt database (Magrane and Consortium 2011). Further, Hmmscan with E-value cut-off ≤ 0.001 was performed to check the presence of Pfam (Finn et al. 2014) domains in the remaining sequences. The class code (u) filter was applied to extract the lncRNAs originating from the intergenic regions. Only lincRNAs with FPKM > 0.1 in all three replicates of at least one sample were used for further analysis. Cuffdiff was used to determine the differentially expressed lincRNAs in elongating (EPs) and non-elongating peduncles (NPs) with fold change ≥ 2 and FDR ≤ 0.05 taken as threshold (Trapnell et al. 2012). Data outputs in the form of pie charts and heatmaps were generated using R.

Target gene prediction and functional analysis

LincRNAs may exert their function via *cis* or *trans* regulation of protein-coding genes. *Cis* regulation can be predicted based on the positional association of lincRNAs with protein-coding genes. In this study, genes within 20 kb upstream or downstream of lincRNAs were predicted as the *cis* targets (Deng et al. 2018; Li et al. 2018; Pang et al. 2019). For *trans* regulation, BLASTn (Chen et al. 2015) was used to extract the coding sequences complementary to lincRNAs using sequence identity $\geq 95\%$ and E-value cut-off ≤ 0.01. Pathway enrichment analysis of target genes was performed using MapMan with FDR ≤ 0.05 (Thimm et al. 2004; https://mapman.gabipd.org/) and data was plotted using ggplot package in R.

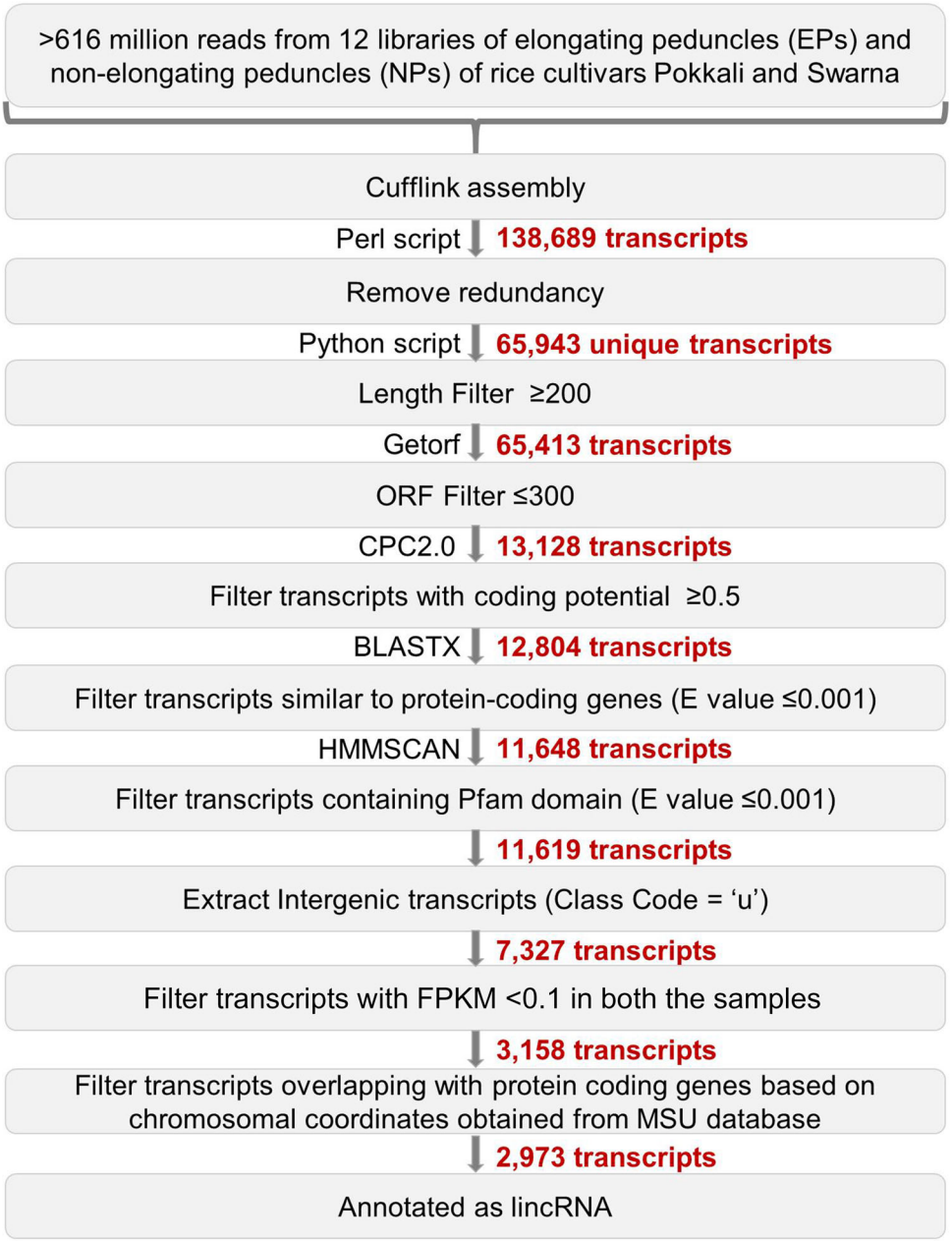

Fig. 1 Workflow used for identification of lincRNAs. Transcripts obtained from sequencing of all the 12 libraries from elongating (EP) and non-elongating peduncles (NP) from rice cultivars, Pokkali and Swarna, were analysed to predict 2973 lincRNAs

Analysis of lincRNA, miRNA, and mRNA interaction network

The information about miRNA precursors was downloaded from miRBase (Griffiths-Jones et al. 2007; http://www.mirbase.org/). Potential miRNA precursors were identified using BLASTn with sequence identity ≥ 95% and E-value cut-off ≤ 0.01. PsRobot was used with default parameters to identify lincRNAs that mimic miRNAs and therefore, may act as decoys (Wu et al. 2012). The predicted lincRNA-miRNA-mRNA interaction networks were visualized using Cytoscape (http://www.cytoscape.org; Smoot et al. 2011).

Identification of conserved lincRNAs in rice

In order to determine if the lincRNAs identified in this study are conserved across other genotypes of rice or have been identified earlier from other rice cultivars in other tissue types, we blast searched (BLASTn) 2973 lincRNAs identified in our study against RiceLncPedia database (Zhang et al. 2020) using e-value cut-off ≤ 1E-5 and percent identity ≥ 90%. Further, we also checked if the lincRNAs, differentially expressed in rice peduncles, are co-located with any of the QTLs listed in RiceLncPedia (http://3dgenome.hzau.edu.cn/RiceLncPedia#/).

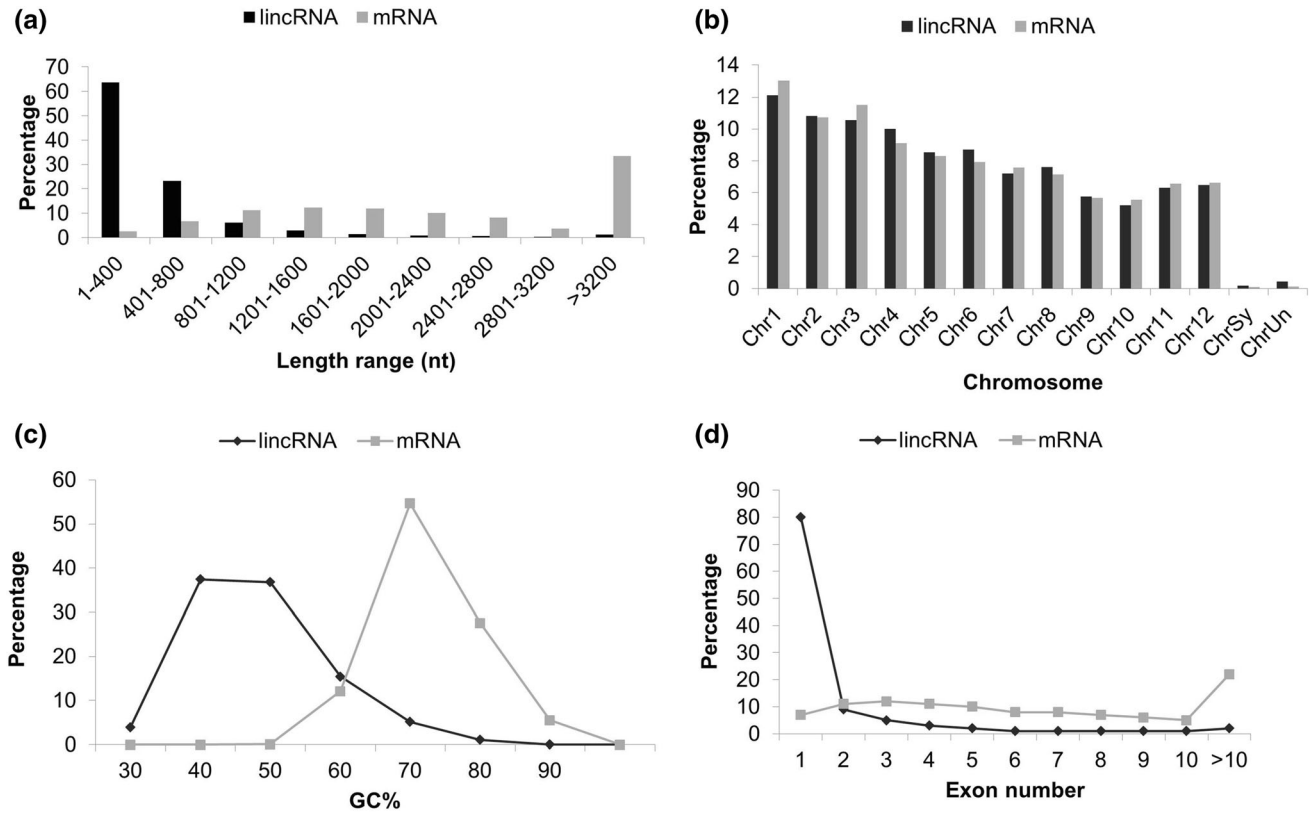

Fig. 2 Comparison of characteristics of lincRNAs and mRNAs. **a** Comparison of length distribution of lincRNAs and mRNAs; **b** Chromosome-wise distribution of lincRNAs and mRNAs; **c** Distribution of percent GC content in lincRNAs and mRNAs; **d** Distribution of number of exons in lincRNAs and mRNAs

Results and discussion

Genome-wide identification and characterization of lincRNAs

We had earlier carried out detailed analysis of gene expression of elongating (EP) and non-elongating peduncles (NP) in two rice cultivars, Pokkali and Swarna at the time of heading (Kandpal et al. 2020). However, a complete understanding of transcriptional dynamics warrants investigation of non-coding transcripts as well. Here, we identified and characterized lincRNAs, a class of lncRNAs which originate from the intergenic regions, expressed in rice peduncles by leveraging the transcriptomic data generated from rice peduncles (Kandpal et al. 2020).

For identification of lincRNAs, we aligned > 616 million clean reads, obtained from RNA seq data to rice genome (MSU Rice Genome Annotation Project Release 7 (http://rice.plantbiology.msu.edu/) and obtained 138,689 genome-aligned transcripts corresponding to 65,943 unique transcripts after removing redundancy (Fig. 1). Further, we filtered these unique transcripts on the basis of length to obtain 65,413 transcripts of ≥ 200 nt. After removing transcripts with ORFs > 300 nt, a set of 13,128 transcripts was obtained. Thereafter, we used CPC2.0 to remove transcripts with discernible coding potential further reducing this number to 12,804 transcripts. These were screened for sequence homology with coding proteins and the presence of Pfam domains. Finally, out of 11,619 transcripts annotated as lncRNAs, 7327 mapped to intergenic regions. Those overlapping with protein-coding genes and with FPKM < 0.1 were further filtered resulting in a set of 2973 expressed lincRNAs (Fig. 1; Table S1).

To obtain a comparative account of structural attributes of lincRNAs and mRNAs, we compared transcript length, chromosomal distribution, GC content and exon numbers of lincRNAs and mRNAs expressed in rice peduncles. The comparison of length showed that the mean length of mRNAs (1733 nt) is much longer than that of lincRNAs (665 nt) with 98.3% of lincRNAs falling in the range of 200–3000 nt (Fig. 2a). The smaller mean length of lincRNAs compared to mRNAs has also been reported previously in rice (Liu et al. 2019). The chromosomal distribution revealed that lincRNAs are distributed on all the chromosomes with the maximum number of lincRNAs localized on the chromosome 1. The overall chromosomal distribution of lincRNAs was similar to that of mRNAs (Fig. 2b). The GC content of lincRNAs varied from 20.72

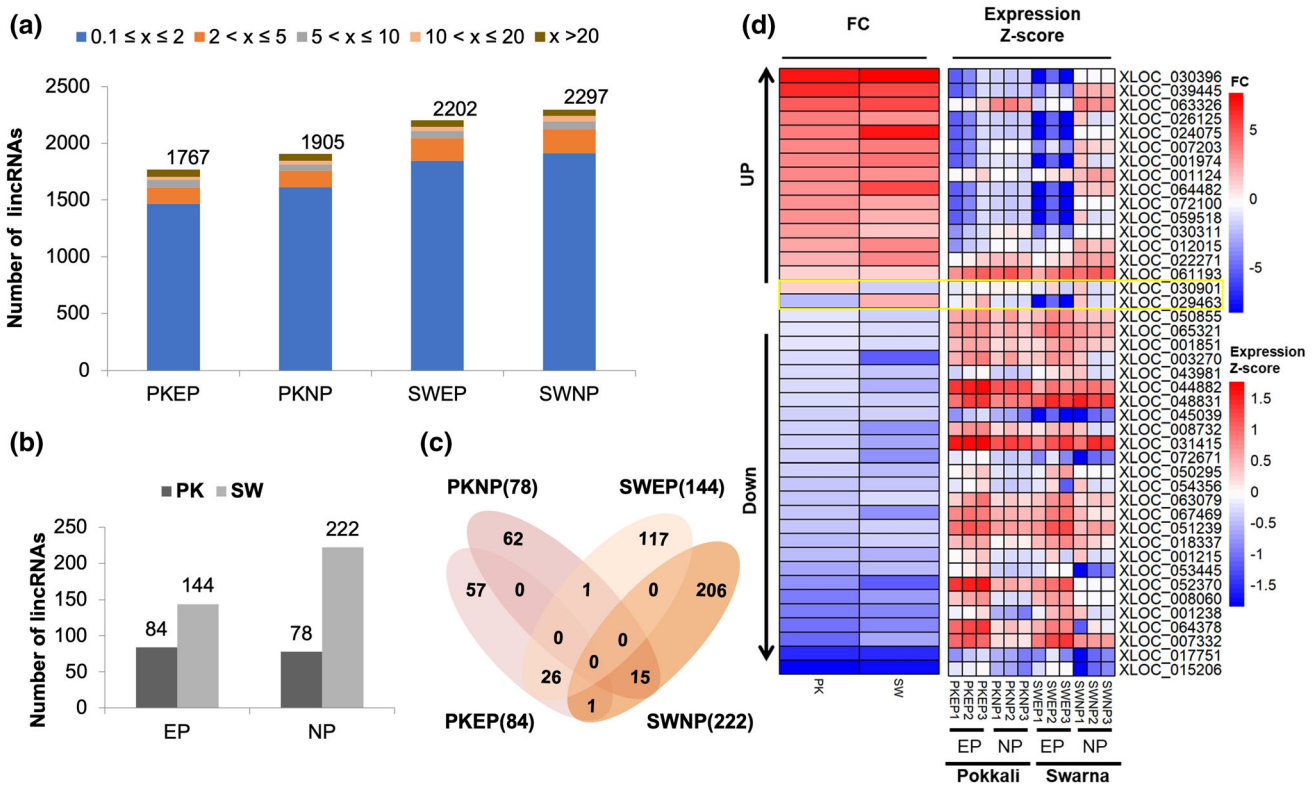

Fig. 3 Expression analysis of lincRNAs. **a** Distribution of lincRNAs in different classes based on their expression level. The X-axis indicates different samples (PKEP—Pokkali elongating peduncles, PKNP—Pokkali non-elongating peduncles, SWEP—Swarna elongating peduncles, SWNP—Swarna non-elongating peduncles) and the Y-axis shows the number of lincRNAs in different classes based on the range of FPKM values. Total number of expressed lincRNAs in each stage is indicated at the top of each bar; **b** The bar graph shows the number of differentially expressed lincRNA (FC $\geq$ 2 and *p*-value $\leq$ 0.05). EP represents lincRNAs with higher expression in elongating peduncles compared to NP while NP represents the number of lincRNA showing higher expression in non-elongating peduncles post heading; **c** Venn diagrams showing overlap between differentially expressed lincRNAs in each sample. Total number of differentially expressed lincRNAs is given in the brackets; **d** Heatmaps show the expression pattern of lincRNAs differentially expressed (FC $\geq$ 2 and *p*-value $\leq$ 0.05) in non-elongating peduncles (NPs) of both the cultivars compared to elongating peduncles (EP). Both fold changes (FC) expression z-scores are plotted separately. Two lincRNAs showing contrasting patterns in Pokkali and Swarna are highlighted by yellow rectangle. The color legend for each heatmap (FC and expression z-score) is provided separately (Color figure online)

to 81.26% with an average GC content of 43.37%, while mRNAs exhibit an average GC content of 60.22% (Fig. 2c). Similar observation was previously reported in rice (Xu et al. 2016). A comparison of number of exons showed that the lincRNAs possessed an average of only 1.6 exons compared to an average of 7.3 exons per mRNA in rice. In fact, 85.5% of lincRNAs identified in our study had only one exon in their transcripts (Fig. 2d). This is also in agreement with the earlier report in rice indicating that these features are characteristic of rice lincRNAs (Xu et al. 2016).

Differential expression analysis of lincRNAs in two rice cultivars

The analysis of expression values obtained from cufflinks revealed that out of 2973 lincRNAs identified here, the highest proportion i.e. 1467, 1613, 1844 and 1913 in Pokkali EP (PKEP), Pokkali NP (PKNP), Swarna EP (SWEP) and Swarna NP (SWNP), respectively had FPKM values lower than 2. Similarly, 142, 145, 199 and 211 lincRNAs had FPKM values between 2 to 5 in PKEP, PKNP, SWEP, and SWNP, respectively. A total of 67, 55, 67, and 69 lincRNAs in PKEP, PKNP, SWEP, and SWNP, respectively showed FPKM range between 5 to 10 while 26, 33, 34, and 49 lincRNAs, respectively had FPKM ranging from 10 to 20. Only 65, 59, 58, and 55 lincRNAs expressing in PKEP, PKNP, SWEP, and SWNP, respectively had FPKM > 20 (Fig. 3a; Table S2).

Further, we identified differentially expressed (DE) lincRNAs with fold change $\geq$ 2.0 at FDR $\leq$ 0.05. In a comparison between NPs versus EPs, 162 and 366 lincRNAs were differentially expressed in Pokkali and Swarna, respectively (Fig. 3a, b, Table S2). Out of 162 DE lincRNAs in Pokkali, 84 were upregulated in EPs while 78 exhibited higher expression in NPs (Fig. 3b). Similarly in

Swarna, 144 and 222 lincRNAs exhibited higher expression in EPs and NPs, respectively (Fig. 3b).

A total of 43 differentially expressed lincRNAs were shared between Pokkali and Swarna indicating conserved functions (Table S3). Conversely, 119 and 323 lincRNAs were differentially expressed exclusively in Pokkali and Swarna, respectively (Fig. 3c, Table S3 and S4). Interestingly, of the 43 lincRNAs shared between Pokkali and Swarna, 41 exhibited the same expression trend in two genotypes with 26 having higher expression in EPs while 15 were upregulated in NPs. Only two lincRNAs (XLOC_029463 and XLOC_030901) showed contrasting expression patterns. XLOC_030901 was upregulated in EPs of Swarna while it was upregulated in NPs of Pokkali. On the other hand, XLOC_029463 showed a higher expression in NPs of Swarna but was upregulated in EPs of Pokkali indicating cultivar-specific modulation (Fig. 3d).

Prediction of putative target genes and functional analysis of lincRNAs

LincRNAs exert their effects via *cis*- or *trans*-regulation of target genes (Kornienko et al. 2013). *Cis*-regulation implies regulation of protein-coding genes in proximal or overlapping regions, while trans-regulation means the regulation of genes which are located on other chromosomes, or further away on the same chromosome (Ramakrishnaiah et al. 2020). In order to determine their putative functions, we carried out identification of both *cis* and *trans* targets of the DE lincRNAs. The *cis*-targets were predicted based on the positions of the lincRNAs while the *trans*-targets were predicted using BLASTn (Deforges et al. 2019). A total of 308 lincRNA-target gene pairs were predicted for 43 common DE lincRNAs. Among these, 260 were *cis* regulatory and 48 were *trans* regulatory (Table S3). In Pokkali, 866 lincRNA-target gene pairs were identified for 119 DE lincRNAs, of which 739 were *cis*-regulated while 127 were predicted as *trans*-regulated (Table S4). Similarly in Swarna, 2202 lincRNA-gene pairs were predicted corresponding to 323 DE lincRNAs (Table S5). Among these, 1957 genes were predicted as *cis*-regulated and 245 as *trans*-regulated. Corroborating with the earlier findings in rice, the number of predicted *cis* targets was much higher than *trans* targets (Liu et al. 2019). This could likely be due to localization of lincRNAs in genic-rich regions as they will have more genes in their proximity.

Further analysis of the DE lincRNAs (1) Common to both genotypes, (2) Exclusively DE in Pokkali, and (3) Exclusively in Swarna, was carried out separately. This analysis not only helped us delineate rice lincRNAs which may be required for panicle exsertion and peduncle elongation, but also revealed genotype-specific differences in lincRNA repertoire of rice peduncles.

Analysis of lincRNAs differentially expressed in both the genotypes

The 43 common DE lincRNAs, differentially expressed in both genotypes, were predicted to target 299 unique genes (Table S3). Out of these 299 target genes, 86 were differentially expressed in elongating stems of rice (Kandpal et al. 2020). The enrichment analysis of 299 target genes using MapMan pathways revealed eight enriched pathways including photorespiration, nitrate transport, glycolysis, cell wall modification, transport of peptides and oligopeptides, and lipid metabolism (Fig. 4; Table S6).

Also, 20 of the target genes have already been functionally characterized with key roles in regulating internode elongation, heading date, panicle architecture and overall yield (Table 1 and S3). For example, XLOC_007332, upregulated in EPs of both the genotypes, is predicted to target *OsMFT1* which is involved in determining the number of spikelets per panicle. *OsMFT1* showed differential expression in Swarna, with higher expression in NPs compared to EPs (Fig. 5) and its loss of function leads to delay in heading and decrease in number of spikelets per panicle (Song et al. 2018). Another lincRNA, XLOC_048831, was also upregulated in EPs and predicted to target *OsSAMS1* which impacts the fertility and grain size in rice (Fig. 5, Table 1) (Li et al. 2011; Chen et al. 2013). Similarly, XLOC_051239, exhibiting higher expression in EPs of both Swarna and Pokkali was predicted to target *OsCOLE1* (*CONTINUOUS VASCULAR RING-LIKE 1*) (Fig. 5, Table 1). *OsCOLE1* is involved in auxin transport and homoeostasis, and its overexpression led to internode elongation and increased auxin content (Liu et al. 2016a). Given the known function of auxins in stem elongation by promoting the cell growth (Dilworth et al. 2017), downregulation of XLOC_051239 after heading in both the genotypes might be associated with regulation of auxin levels. Conversely, XLOC_052370 showed higher expression in EPs, targets *OsSK2*, which was upregulated in developing panicles at heading stage, and has been implicated in defence response, and panicle development (Kasai et al. 2005). XLOC_030311, upregulated in NPs of both the cultivars, was predicted to target *OsCKT1,* which exhibited higher expression before heading (Fig. 5, Table 1). It is also well-known that cytokinins are involved in post-heading grain filling (Yang et al. 2002; Zhang et al. 2009). Ding et al. (2017) reported involvement of *OsCKT1* in regulation of secondary metabolism, sucrose and starch metabolism, chlorophyll synthesis, and photosynthesis.

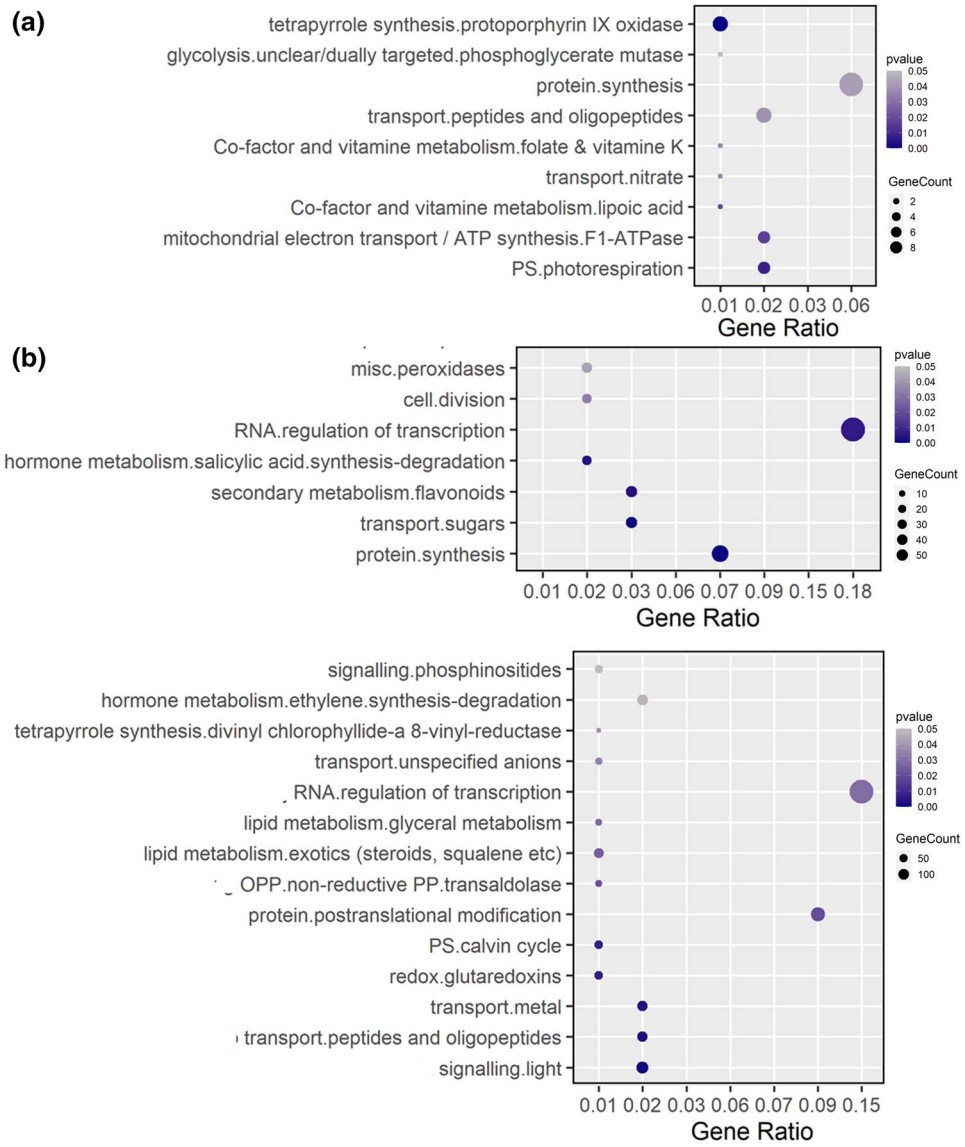

Fig. 4 Results of enrichment analysis of MapMan pathway analysis with target genes of differentially expressed lincRNAs. **a** The dot plot shows the enriched pathways among 299 target genes of 43 lincRNAs differentially expressed in both the cultivars; **b** Dot plot showing the enriched pathways among 806 target genes of 119 lincRNAs differentially expressed only in Pokkali; **c** Dot plot showing enriched pathways among 2052 target genes of 323 lincRNAs differentially only in Swarna

LincRNAs differentially expressed only in Pokkali

Target analysis of the 119 lincRNAs, DE in Pokkali, identified 806 unique genes associated with synthesis of tetrapyrroles, protein synthesis, sugar transport, hormone metabolism, regulation of transcription, cell division, peroxidase activity and secondary metabolism (Fig. 4, Table S7).

Among Pokkali-exclusive DE lincRNAs, 30 were predicted to target 39 genes with previously characterized functions (Table 1). At least 11 of them have demonstrated roles in regulating plant height and architecture. For example, XLOC_007226 is predicted to target *dwarf and gladius leaf 1 (OsDGL1)*, which regulates cell division and elongation in rice by affecting microtubule organization (Fig. 5, Table 1) (Komorison et al. 2005). *Dgl1* mutant exhibits reduced plant height (Komorison et al. 2005). Similarly, XLOC_031298 targets *OsDof12*, which regulates plant architecture in rice (Wu et al. 2015). Upon overexpression, it leads to decreased plant height and smaller panicles (Fig. 5, Table 1). XLOC_017661, upregulated exclusively in Pokkali NPs, targets *OsXTR1* which is involved in brassinosteroid signalling and cell elongation (Fig. 5, Table 1) (Duan et al. 2006). Similarly, XLOC_032318 targets SUMO protease encoding gene, *OsFUG1*, an important regulator of plant height. Knockout of *OsFUG1* led to dwarf phenotype along with defects in panicle architecture, fertility, and seed weight (Fig. 5, Table 1) (Rosa et al. 2018).

Besides, there were several other genes which regulate panicle elongation and other yield traits (Table 1). For example, XLOC_060278 targets *dense and erect panicle 2*

Table 1 List of lincRNAs shortlisted based on the functions of target genes implicated in the regulation of crucial agricultural traits

S. no.	LincRNA	Target gene ID	Target gene name	Traits affected	References
LincRNAs differentially expressed in both Pokkali and Swarna					
1	XLOC_007332	LOC_Os06g30370	*OsMFT1*	Heading time and architecture of the panicle	Song et al. (2018)
2	XLOC_030311	LOC_Os02g50480	*OsCKT1|OHK5|OsHk6*	Secondary metabolism, sucrose and starch metabolism, chlorophyll synthesis, and photosynthesis	Ding et al. (2017)
3	XLOC_048831	LOC_Os05g04510	*OsSAMS1*	Fertility, plant height, grain filling, seed germination and flowering time	Chen et al. (2013)
4	XLOC_051239	LOC_Os05g45280	*OsCOLE1*	Intracellular auxin transport	Liu et al. (2016a, b)
5	XLOC_052370	LOC_Os06g12150	*OsSK2*	Panicle development and defense response	Kasai et al. (2005)
LincRNAs differentially expressed in Pokkali only					
6	XLOC_005413	LOC_Os01g15830	*OsPOX1|ddOs319*	Shows flower preferential cold induced expression	Kim et al. (2012)
7	XLOC_007226	LOC_Os01g49000	*DGL1|OsKTN60*	Cell division, cell elongation, plant height	Komorisono et al. (2005)
8	XLOC_017661	LOC_Os11g33270	*OsXTR1|XTH2*	Cell elongation by regulating brassinosteroid signalling	Duan et al. (2006)
9	XLOC_026351	LOC_Os02g45770	*OsMADS6|MFO1|AFG1*	Endosperm nutrient accumulation	Zhang et al. (2010)
10	XLOC_028753	LOC_Os02g26160	*OsLecRK-S.7|OsLecRK5|OsDAF1*	Callose biosynthesis during pollen development	Wang et al. (2020)
11	XLOC_031298	LOC_Os03g07360	*OsDof12|OsCDF1*	Flowering and plant architecture	Wu et al. (2015)
12	XLOC_032318	LOC_Os03g22400	*OsFUG1*	Panicle architecture, seed weight, fertility and internode elongation	Rosa et al. (2018)
13	XLOC_051818	LOC_Os06g03610	*RUPO*	K + homeostasis required for growth and integrity of pollen tubes	Liu et al. (2016b)
14	XLOC_053898	LOC_Os06g39390	*OsAt10*	Target for improving grass quality for fuel and animal feed	Li et al. (2018)
15	XLOC_060278	LOC_Os07g42410	*DEP2|EP2|SRS1*	Seed size and panicle architecture	Abe et al. (2010)
16	XLOC_062736	LOC_Os04g44150	*OsGA2ox6*	Plant height by regulating GA biosynthesis	Yu and Zhu (2019)
17	XLOC_054408	LOC_Os06g46900	*OsHSA32*	Thermotolerance of rice seeds	Lin et al. (2014)
18	XLOC_033699	LOC_Os03g48170	*OsFLO6*	Starch synthesis and compound granule formation in rice endosperm	Peng et al. (2014)
19	XLOC_045209	LOC_Os04g55560	*OsSHAT1*	Seed shattering in rice	Zhou et al. (2012)
20	XLOC_060282	LOC_Os07g42490	*OsSUS3*	Involved in carbon allocation during grain filling	Hirose et al. (2008)
21	XLOC_060278	LOC_Os07g42410	*OsEP2*	Plant height by regulating GA biosynthesis	Abe et al. (2010)
LincRNAs differentially expressed in Swarna only					
22	XLOC_000741	LOC_Os01g13030	*OsIAA3*	Auxin signal transduction	Jain et al. (2006)
23	XLOC_002964	LOC_Os01g52050	*D61|OsBRI1*	Organ development through controlling cell elongation and division	Nakamura et al. (2006)
24	XLOC_003937	LOC_Os01g66100	*sd1|GA20ox2*	GA biosynthesis and plant height	Li et al. (2010)
25	XLOC_004190	LOC_Os01g70440	*RTS*	Pollen development	Luo et al. (2006)
26	XLOC_004936	LOC_Os01g08320	*OsIAA1*	Plant morphogenesis and cross-talk of auxin and brassinosteroid signaling pathways	Song et al. (2009)
27	XLOC_005638	LOC_Os01g19694	*OSH6*	Bract differentiation	Park et al. (2007)
28	XLOC_008051	LOC_Os01g61940	*OsABCG3*	Pollen development	Chang et al. (2018)
29	XLOC_010934	LOC_Os10g41590	*OsHUB2|FRRP1*	Anther development, flower timing and yield potential	Cao et al. (2015) and Du et al. (2016)
30	XLOC_013193				
31	XLOC_017662	LOC_Os11g33270	*OsXTR1|XTH2*	Cell elongation by regulating brassinosteroid signalling	Duan et al. (2006)

Table 1 (continued)

S. no.	LincRNA	Target gene ID	Target gene name	Traits affected	References
32	XLOC_026650	LOC_Os02g49880	*Ghd2|OsK*	Grain number, plant height, heading date, and abiotic stress tolerance	Liu et al. (2016a, b)
33	XLOC_026761	LOC_Os02g51320	*PGL2|OsBUL1| OsbHLH173*	Grain weight and grain length	Heang and Sassa (2012)
34	XLOC_029653	LOC_Os02g41550	*OsCRY2|CRY2*	Flowering time	Hirose et al. (2008)
35	XLOC_035272	LOC_Os03g10620	*HTD2|D88|D14*	Cell growth, organ development and plant architecture	Gao et al. (2009)
36	XLOC_044076	LOC_Os04g38950	*TDD1|OASB1*	Auxin biosynthesis and basic pattern formation	Sazuka et al. (2009)
37	XLOC_046079	LOC_Os05g09520	*GSE5*	Grain size	Duan et al. (2017)
38	XLOC_050341	LOC_Os05g30750	*OsFTIP7*	Auxin mediated anther dehiscence	Song et al. (2018)
39	XLOC_051064	LOC_Os05g41760	*MFS1*	Spikelet meristem determinacy and floral organ identity	Ren et al. (2013)
40	XLOC_064691	LOC_Os08g20580	*OsCTZFP8*	Cold stress tolerance	Jin et al. (2018)
41	XLOC_072402	LOC_Os06g15620	*OsGSR1|GW6|OsGASR7*	Grain size	Shi et al. (2020)
42	XLOC_035706	LOC_Os03g51030	*PHYA|OsPhyA*	Internode elongation	Iwamoto et al. (2011)
43	XLOC_043092				
44	XLOC_031307	LOC_Os03g07530	*OsFBK12*	Grain size and number	Chen et al. (2013)

(dep2), which regulates cell proliferation during panicle elongation (Fig. 5, Table 1) (Li et al. 2010). XLOC_062736 targets *OsGA2ox6* which positively regulates paclobutrazol, an inhibitor of GA biosynthesis (Yu and Zhu 2019).

We also identified several DE lincRNAs in this subset, which target genes involved in pollen development, seed development and therefore, determine overall yield of the plant (Table 1). Among these, XLOC_054408 and XLOC_033699 were downregulated before heading and predicted to target *OsHSA32* and *OsFLO6*, respectively (Fig. 5). OsFLO6 (FLOURY ENDOSPERM6*)* is involved in starch synthesis by interacting with Isoamylase1 (ISA1). *OsFLO6* mutants exhibit reduced starch content and altered physicochemical properties (Peng et al. 2014). Further, XLOC_045209, XLOC_060282 and XLOC_060278, upregulated after heading, target *OsSHAT1*, *OsSUS3,* and *OsEP2,* respectively. *OsSHAT1* is essential for seed shattering (Zhou et al. 2012), *OsSUS3* (*Sucrose Synthase 3*) has an important role in carbon allocation during grain filling (Hirose et al. 2008), whereas, *OsEP2* is involved in regulation of panicle length and size of the grains (Fig. 5, Table 1) (Abe et al. 2010). Furthermore, of the 806 unique target genes of the Pokkali-specific DE lincRNAs, 119 exhibited differential expression in rice peduncles (Table S4).

LincRNAs differentially expressed only in Swarna

A total of 323 lincRNAs, DE exclusively in Swarna, were predicted to target 2052 unique target genes. These genes belong to 14 enriched pathway categories related to transport of metal, peptides and oligopeptides, signalling, photosynthesis, regulation of transcription, photosynthesis, hormonal signalling, etc. (Fig. 4, Table S8).

Among these, XLOC_003937 targets *SD1* which encodes gibberellin 20-oxidase 2 (GA20ox2), also referred as 'the green revolution' gene (Spielmeyer et al. 2002). Mutants in this gene exhibit dwarf phenotype widely utilized during green revolution in Asia in 1960s for enhancing the lodging resistance and other yield-related traits (Khush 2001). It is a major regulator of vegetative as well as reproductive development through regulation of GA metabolism and signalling (Huang et al. 2010). Another lincRNA, XLOC_026650, upregulated in Swarna EPs, is predicted to target *Ghd2* which in turn has been shown to regulate grain number, plant height, heading date, as well as abiotic stress tolerance in rice (Fig. 5, Table 1) (Liu et al. 2016b). Interestingly, both XLOC_035706 and

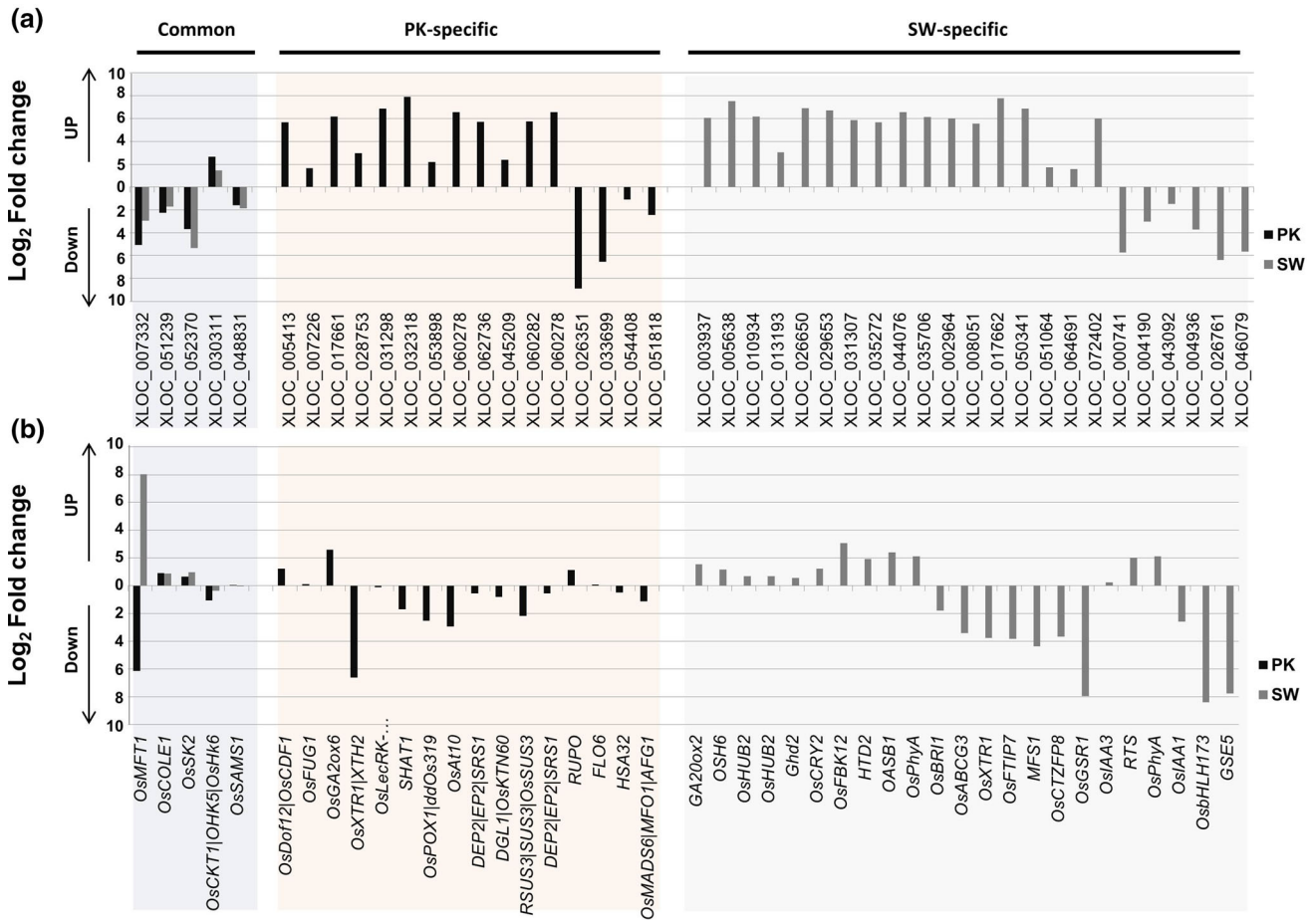

Fig. 5 Relationship between expression patterns of shortlisted lincRNAs and their predicted targets. The bar graphs depict the relationship between shortlisted lincRNAs and their predicted targets with previously characterized functions associated with peduncle elongation. **a** Log_2 fold change in the expression of lincRNAs **b** Log_2 fold change in the expression of respective targets

XLOC_043092, are predicted target the phytochrome gene *OsPHYA*, which plays a role in regulating internode elongation as well as heading date (Fig. 5, Table 1) (Iwamoto et al. 2011; Lee et al. 2016).

Similarly, XLOC_044076 targets *OsTDD1* which catalyses Trp biosynthesis pathway functioning upstream of Trp-dependent IAA biosynthesis (Sazuka et al. 2009). XLOC_004936 was predicted to target *OsIAA1,* a member of Aux/IAA gene family (Thakur et al. 2001). XLOC_031307 was predicted to target an F-box protein coding gene *OsFBK12*. Chen et al. (2013) have reported the role of *OsFBK12* in the regulation of seed size as well as grain number in rice. *OsFBK12* exhibits three-folds increase in NPs in comparison to EPs corroborating with its role in seed development (Kandpal et al. 2020). Further, of the 2052 total putative target genes among these DE lincRNAs, 509 exhibited differential expression during peduncle elongation (Kandpal et al. 2020) (Table S5).

Differentially expressed lincRNAs as potential miRNA precursors

LincRNAs may also act as precursors for miRNA biogenesis (Yoon et al. 2014). Therefore, to identify lincRNAs with potential to act as miRNA precursors, we aligned DE lincRNAs in our study to mature miRNA sequences of rice obtained from miRBase. A total of 22 DE lincRNAs were identified as putative precursors of miRNAs belonging to 23 miRNA families (Fig. 6, Table S9, S10, S11).

Among the common DE lincRNAs, seven (XLOC_045039, XLOC_001238, XLOC_050855, XLOC_051239, XLOC_030901, XLOC_059518, XLOC_063326) were predicted as precursors for 19 miRNAs belonging to eight (osa-miR171, osa-miR408, osa-miR1425, osa-miR1850, osa-miR1857, osa-miR11339, osa-miR11336, osa-miR11343) different miRNA families (Fig. 6, Table S9). Interestingly, some lincRNAs were predicted as precursors for more than two miRNA families. For example, XLOC_045039 was predicted as precursor for osa-miR171 as well as osa-miR11336;

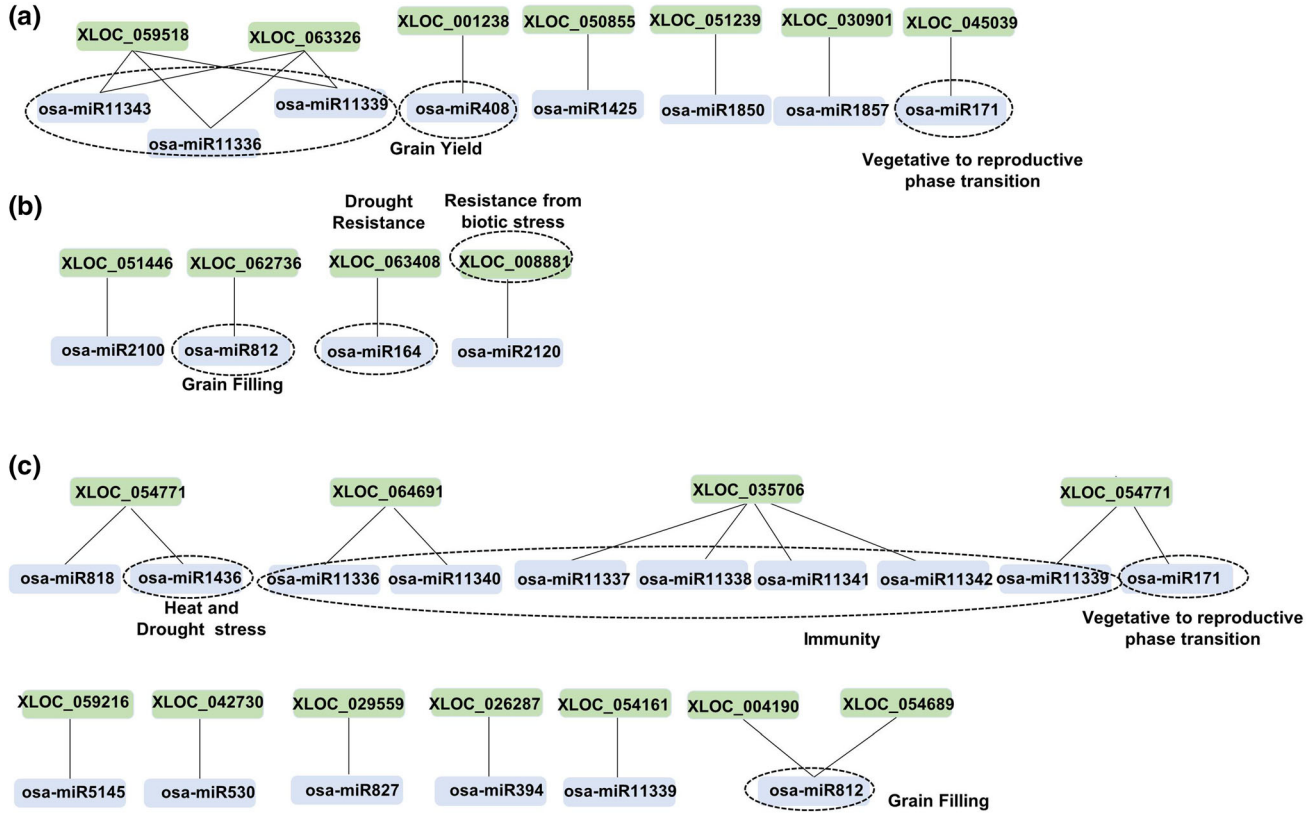

Fig. 6 Identification of lincRNAs as miRNA precursors. Network representation of lincRNAs and miRNAs based on prediction of lincRNAs as putative miRNA precursors for **a** Seven lincRNAs differentially expressed in both the cultivars; **b** Four lincRNAs differentially expressed exclusively in Pokkali and, **c** Eleven lincRNAs differentially expressed exclusively in Swarna. The circles represent miRNAs for which the functions have been characterized, as reported in previous studies

XLOC_059518 was predicted as precursor of osa-miR11336, osa-miR11339, osa-miR11343; while XLOC_063326 may act as precursor of osa-miR11336 and osa-miR11339.

Insights into the previously characterized functions of miRNAs further proffered interesting lincRNA candidates from our study. For example, XLOC_045039, DE in both Pokkali and Swarna, is predicted as precursor of osa-miR171c that has been demonstrated to play critical role in determination of heading date in rice by regulating GRAS transcription factors (Fan et al. 2015). Another lincRNA, XLOC_001238, was predicted as precursor of OsmiR408 which positively regulates grain yield in rice by promoting panicle branching, grain size, and grain number (Zhang et al. 2017).

Among the lincRNAs DE only in Pokkali, four (XLOC_063408, XLOC_008881, XLOC_051446, and XLOC_062736) were predicted as precursors for 15 miRNAs from four miRNA families (osa-miR164, osa-miR2120, osa-miR2100, osa-miR812) (Fig. 6, Table S10). In Pokkali, XLOC_063408, which was upregulated after heading, is predicted as precursor of osa-miR164. Osa-miR164 has been recently shown to target *OsCUC1* gene in rice, which along with *OsCUC3,* regulates meristem boundary specification. Perturbation of their expression adversely impacts plant architecture and male sterility (Wang et al. 2020).

Among the lincRNAs DE only in Swarna, eleven (XLOC_064691, XLOC_009692, XLOC_035706, XLOC_054161, XLOC_054771, XLOC_026287, XLOC_059216, XLOC_042730, XLOC_004190, XLOC_054689, XLOC_029559) were predicted as precursors for 33 miRNAs from 15 miRNA families (osa-miR11336, osa-miR11337, osa-miR11338, osa-miR11339, osa-miR11340, osa-miR11341, osa-miR11342, osa-miR1436, osa-miR171, osa-miR394, osa-miR5145, osa-miR530, osa-miR812, osa-miR818, osa-miR827). Four of the lincRNAs seem to act as precursors for more than one miRNA (Fig. 5, Table S14). XLOC_054161 also acts as precursor for osa-miR171c in Swarna.

Notably, XLOC_062736, a putative precursor of osa-miR812, was upregulated in Pokkali after heading, while in Swarna, three lincRNAs (XLOC_004190, XLOC_054689, and XLOC_068034) were predicted as precursors of osa-miR812. Among these, XLOC_054689 was upregulated after heading while other two were downregulated. Yi et al.

(2013) implicated involvement of osa-miR812 in grain filling.

LincRNAs mimic the miRNA targets

It is well documented that one of the mechanisms with which the lincRNAs regulate gene expression is by mimicking the miRNA targets (Franco-Zorrilla et al. 2007). LncRNAs, referred as endogenous target mimics (eTMs), act as decoys to miRNA targets and thereby, inhibit their negative regulation (Borah et al. 2018). The RNAs mimicking miRNA targets always contain a motif sequence complimentary to the corresponding miRNA. This pairing is not continuous and usually comprises of a bulge in the middle of the miRNA binding site. This bulge stops the cleavage of miRNA target mimics. Thus, these target mimics reduce the activity of miRNA (Wu et al. 2013). We identified the lincRNAs, which possibly act as target mimics using the tool psRobot_mimic. A total of nine DE lincRNAs were predicted to act as target mimics of nine miRNA families (Fig. 6). The common DE lincRNA XLOC_030901 was predicted to be a target mimic of osa-miR2871 (Table S12). A high throughput sequencing study by Barrera-Figueroa et al. (2012) has previously linked osa-miR2871 expression to drought, cold and salt stress conditions.

Pokkali-specific DE lincRNAs, XLOC_002353 and XLOC_053841 upregulated in the EPs, were predicted as target mimics of osa-miR414 and osa-miR171, respectively (Fig. 7, Table S13). Osa-miR414 plays a role in salinity stress response, while osa-miR171 is crucial for vegetative to reproductive phase transition (Macovei and Tuteja 2012; Fan et al. 2015).

In Swarna, six DE lincRNAs XLOC_014510, XLOC_043376, XLOC_066715, XLOC_020903, XLOC_017662 and XLOC_009692 were predicted as target mimics of osa-miR2922, osa-miR2275, osa-miR5501, osa-miR2925, osa-miR2919, and osa-miR2873, respectively (Fig. 7, Table S14). Among these, osa-miR2275 triggers 24 nt phasiRNAs, and is essential for pollen development in rice (Li et al. 2017).

Conservation of rice lincRNAs

In order to identify the conserved lincRNAs, we first determined the conservation of lincRNAs between Swarna and Pokkali. Among the 2973 lincRNAs identified here, 2399 (80.7%) were common to both Swarna and Pokkali, 98 (3.3%) were only detected in Pokkali and 476 (16%) were only detected in Swarna indicating cultivar-specific expression of lincRNAs (Fig. 8a). Further, the lincRNAs identified in this study were compared with the rice lincRNAs from the RiceLncPedia database (Zhang et al. 2020). This database comprises of 6925 non-redundant rice lncRNA transcripts. Using BLASTn analysis, with the e value cut-off of $\leq$ 1E-5, 158 (5.31%) lincRNAs out of 2973 total lincRNAs mapped to the 158 transcripts from RiceLncPedia database (Fig. 8b). This indicated that 5.31% of the lincRNAs, identified in this study, are conserved in rice genotypes, while remaining 94.68% are novel. The conserved lincRNAs may be responsible for crucial functions in development as well as stress tolerance in species-agnostic manner (Zhang et al. 2014; Wang et al. 2015). Out of 158 conserved lincRNAs identified in the present study, 34 were DE, of which 7 were DE in both Pokkali and Swarna, 7 only in Pokkali and 20 only in Swarna (Table S15). Furthermore, since RiceLncpedia database harbours information of lncRNAs overlapping with known QTLs based on chromosomal locations, as deciphered using Q-TARO (QTL Annotation Rice Online) database (Yonemaru et al. 2010) and GWAS SNP tags from Rice SNP-Seek database (Alexandrov et al. 2015), we utilized this information to determine if conserved rice lincRNAs identified in our study overlap with QTLs associated with the important agronomic traits like plant height, panicle and flower development, flowering time, and seed development. Of the 158 conserved lincRNAs, 18 exhibited differential expression and were associated with four characters pertaining to 'dwarf', 'flowering', 'panicle/flower' and 'seed' (Table S16). These lincRNAs co-localized with previously known QTLs (Fig. 9) for heading (*hd8*), plant height (*Sn2a, qPH2-6-1, qPH-2, ph6*), panicle development (*An10, FON3, gp1a, gp6, gp7a, gw7, qLVBSL6, qLVBSL6-1, qPES-3, qPTD-2, qSPN-6, sp2(t), yd7a*), and seed development (gw-6, gy12, qGY6, qGY6-1, QKw5, qTGWT-10, qWIJ-2,yld2.1).

Conclusions

By analysing the transcriptional dynamics of lincRNAs in the developing peduncles of rice using two phenotypically contrasting cultivars Pokkali and Swarna, we identified a total of 2973 lincRNAs of which 2399 were detected in both cultivars. Out these, 162 and 366 lincRNAs were differentially expressed in elongating peduncles at the time of heading of Pokkali and Swarna, respectively. The functional aspects of lincRNA-mediated regulation of panicle elongation and panicle exsertion were explored using the analysis of target genes. The target genes showed enrichment of several biological pathways such as photosynthesis, protein synthesis, regulation of transcription, hormone metabolism, signalling, cell division, cell wall modification, etc. A total of 44 lincRNAs were shortlisted as crucial candidates based on the literature survey (Table 1), while 18 conserved lincRNAs were shortlisted

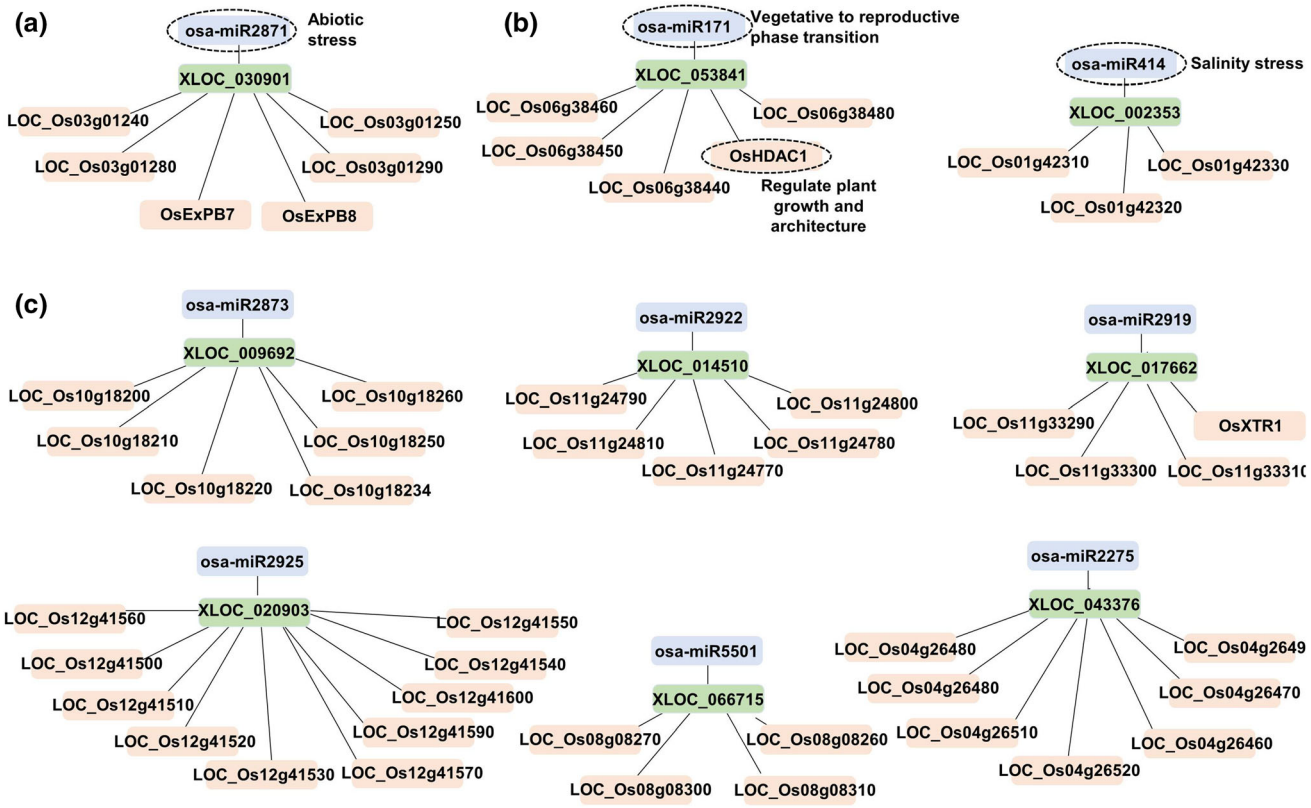

Fig. 7 Identification of lincRNAs acting as target mimics. Networks showing lincRNAs, miRNAs putatively triggered by them, and the genes for which the lincRNAs were predicted as target mimics **a** One lincRNA differentially expressed in both the cultivars; **b** Two lincRNAs differentially expressed in Pokkali and, **c** 6 lincRNAs differentially expressed in Swarna. The circles represent miRNAs for which the functions have been characterized, as reported in previous studies

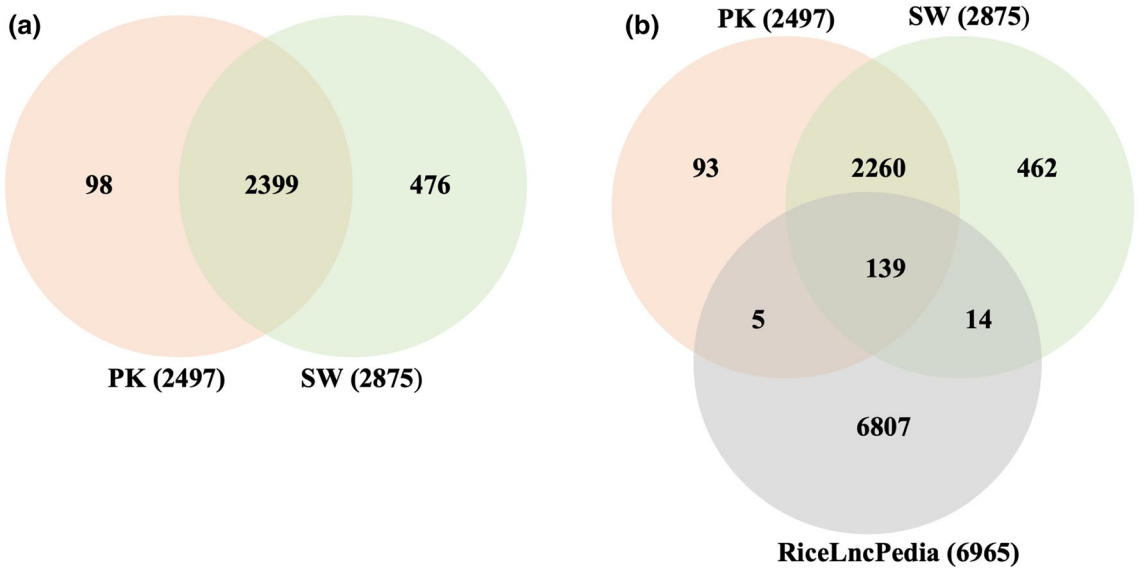

Fig. 8 Identification of conserved lincRNAs. Venn diagram showing lincRNAs conserved in the two rice cultivars used in this study, Pokkali (PK) and Swarna (SW); **b** Venn diagram showing conserved lincRNAs detected based on mapping with lncRNAs in the RiceLncPedia database (Zhang et al. 2020)

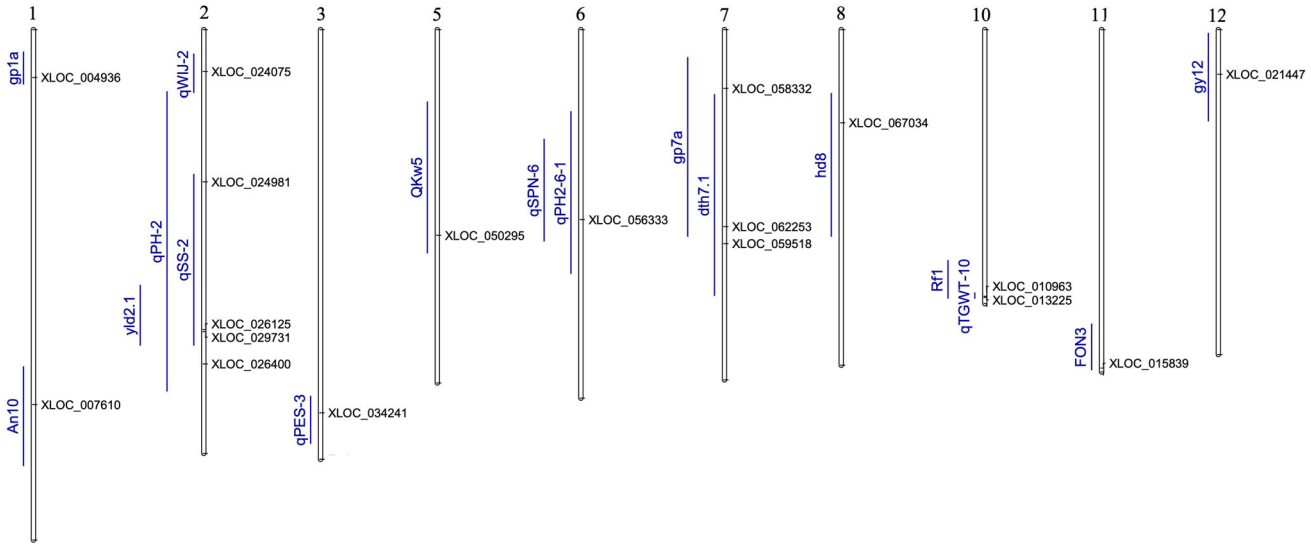

Fig. 9 Chromosomal mapping of lincRNAs co-localized with QTLs. LincRNAs colocalized with the QTLs associated with 'dwarf', 'flowering', 'panicle/flower' and 'seed' traits were mapped on rice chromosomes. The chromosome numbers on which shortlisted lncRNAs/QTLs mapped are given on the top

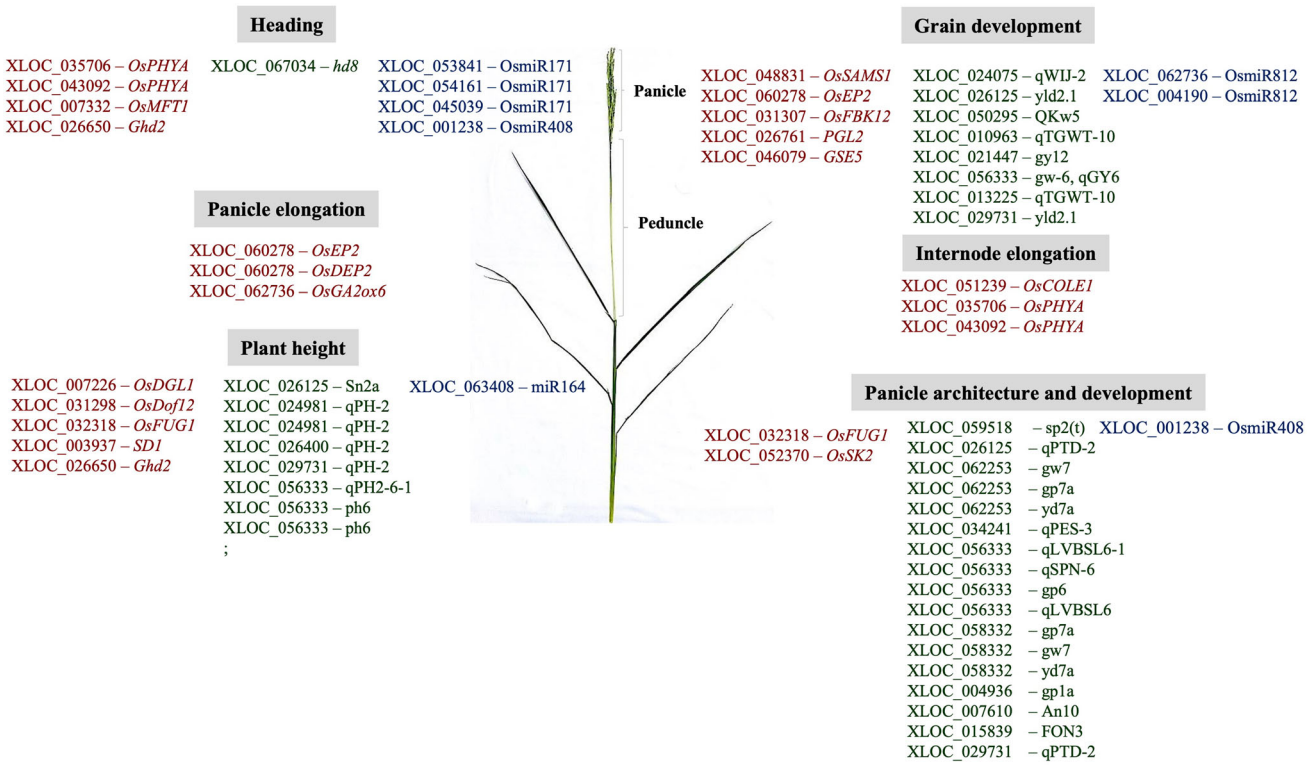

Fig. 10 Rice lincRNA candidates relevant for peduncle elongation and panicle exsertion. Summary of the most crucial rice lincRNA candidates identified in this study with putative roles in determining plant height, panicle elongation, heading, panicle architecture, development, internode elongation and grain development. These were shortlisted based on the target gene analysis, identification of putative miRNA precursors, and mapping with the RiceLncPedia (Zhang et al. 2020). The lincRNAs and target gene pairs are shown in red, lincRNAs and QTL regions are shown in green while lincRNAs and miRNAs are shown in blue (Color figure online)

based on co-localization with previously known QTLs for seed, flowering, panicle, and plant height-related characters (Table S16). The comparative profiling of 44 shortlisted lincRNAs and their predicted targets revealed similar number of lincRNA-target gene pairs showing negative and positive correlation in their expression patterns (Fig. 5). A summary of the most important lincRNAs and their target genes/QTLs/miRNA pairs is presented in Fig. 10 awaiting experimental validation.

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Authors' contributions RS conceived and designed the study. MK performed all the experiments. MK and ND carried out data analysis. MK, ND, and RS drafted the manuscript. All authors read and approved the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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