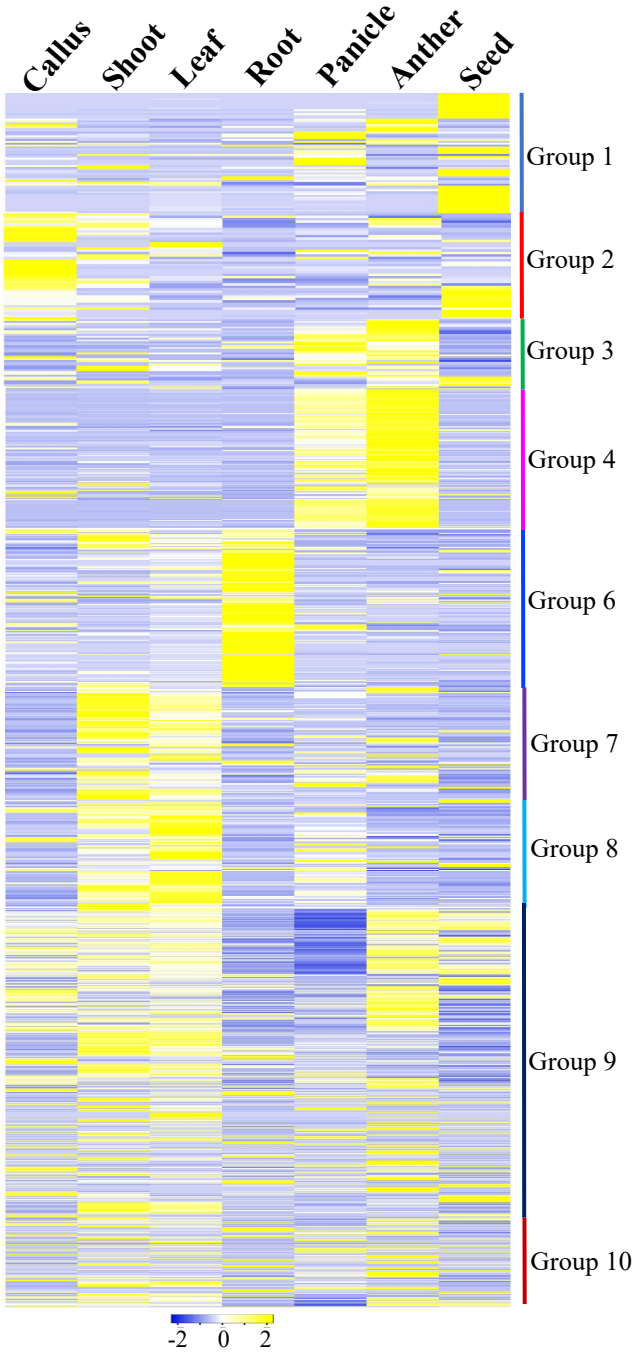


Figure S1. In-silico expression profile of the identified gene sets, belonging to different groups, across different rice tissues as available in the Plant Public RNA-Seq Database (https://plantrnadb.com/). For pollen, there was only one library available, so its data are not included in this heatmap.

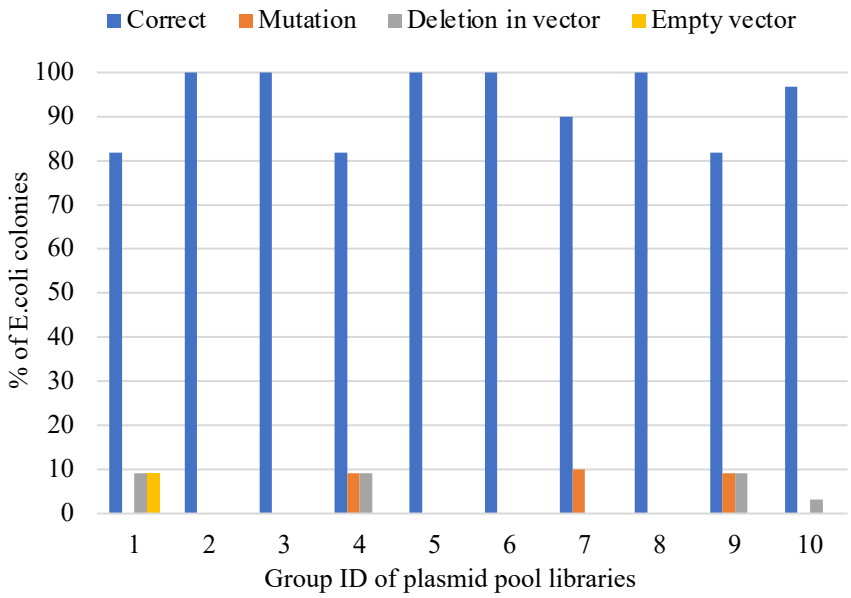


Figure S2. Sanger Sequencing analysis of randomly selected E.coli colonies from the ten plasmid pool libraries. Four different types of reads were obtained: Correct-no any change in sgRNA sequence, Mutation- mutations in sgRNA sequence, Deletion in part of vector sequence, Empty vector- no sgRNA

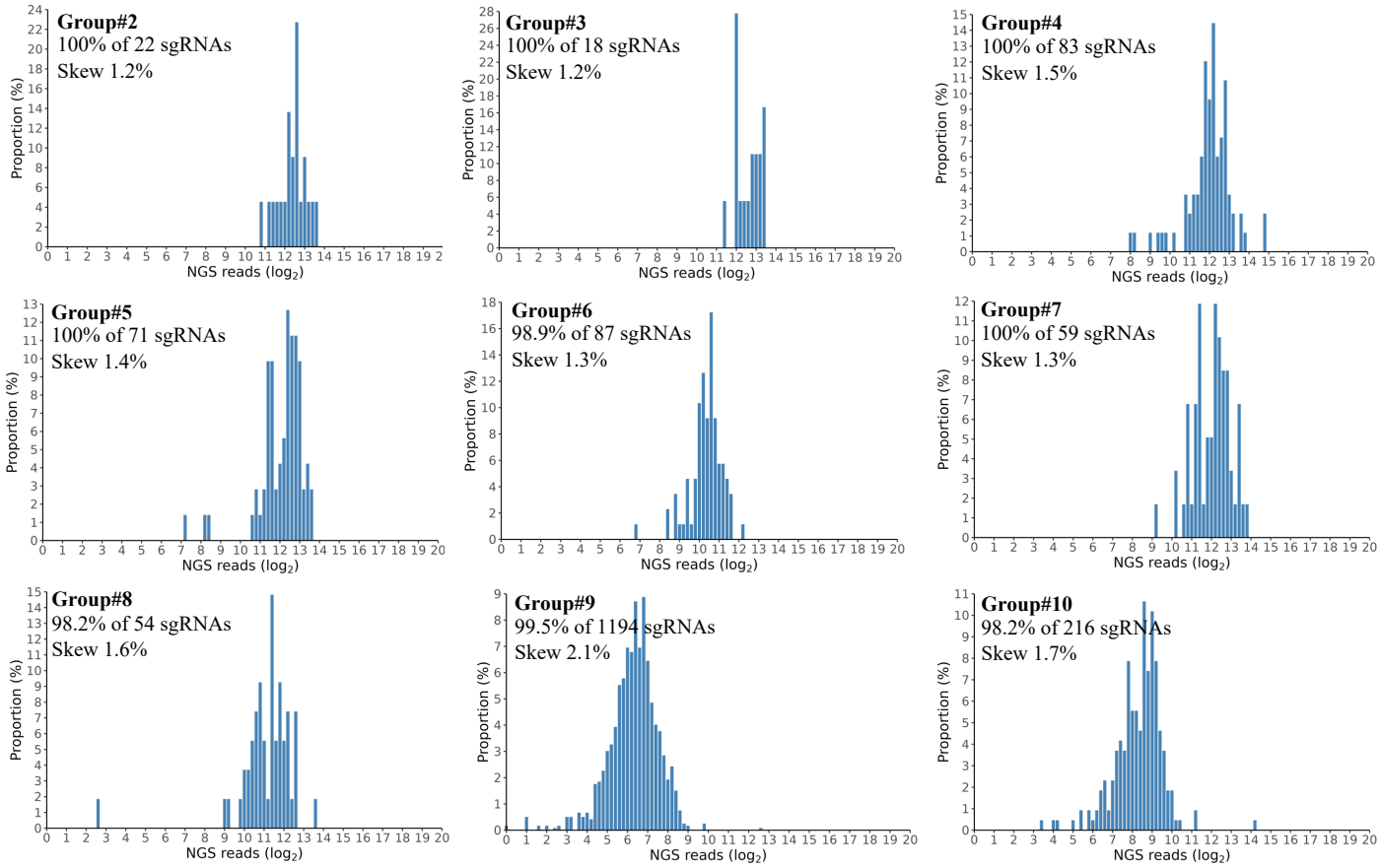


Figure S3. Next Generation Sequencing (NGS) analysis of the developed CRISPR-Cas9 plasmid pool libraries. NGS histograms showing the coverage, skewness and distribution of the sgRNAs in each group's library. The values on the Y-axis shows proportion (%) of the NGS reads, and the values on the X-axis shows log2 values of NGS reads.

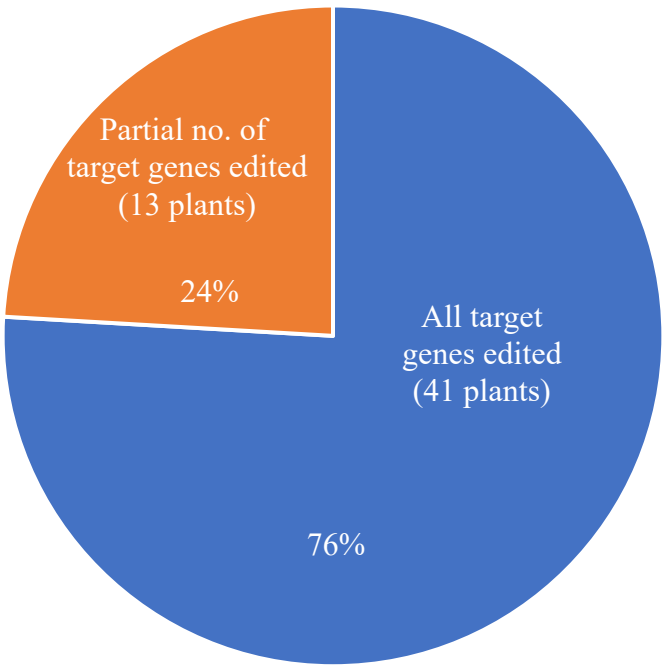


Figure S4. Proportion of different types of plants among all the T0 edited plants. Since sgRNA present in each plant target 2-3 genes, so under the category 'All target genes edited' represent plants in which all the target genes of the sgRNA are edited, while the category 'partial no. of target genes edited' represent the plants in which only partial number of target genes are edited.

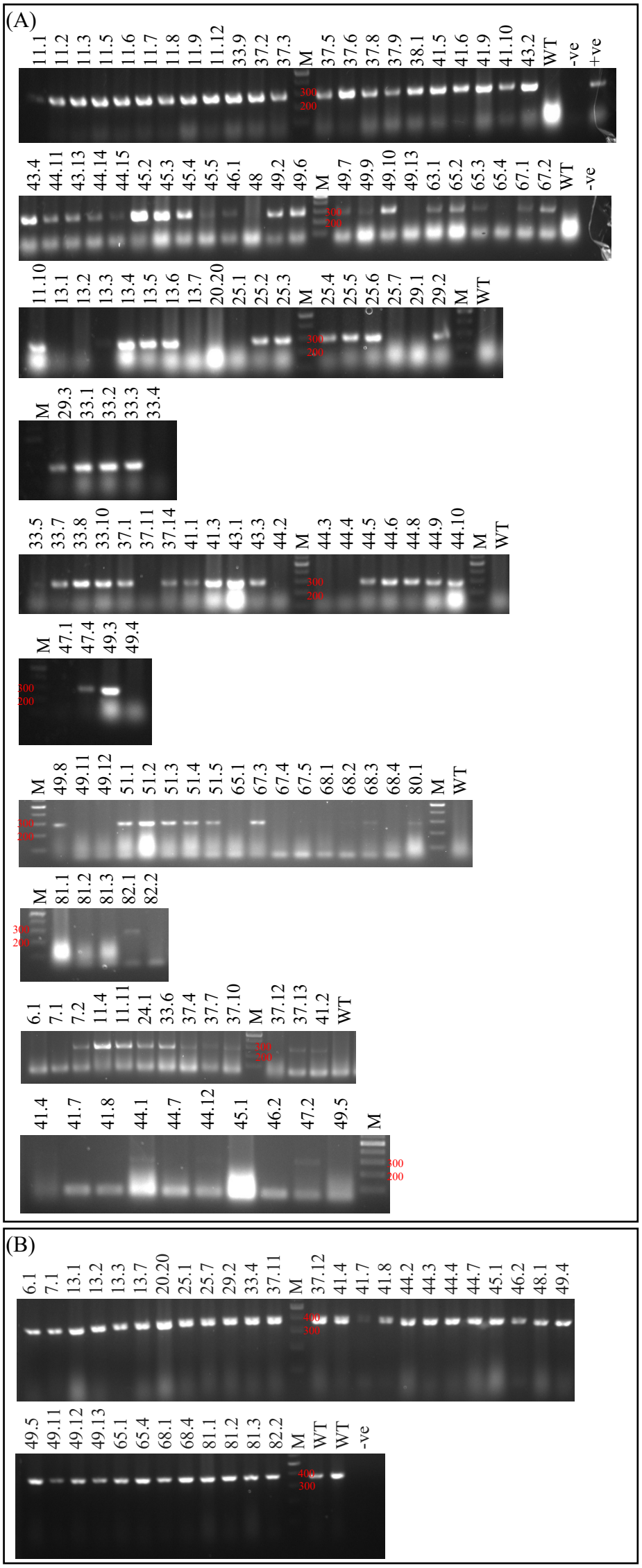


Figure S5. Genotypic analysis of T1 generation plants for transgene presence/absence. (A) DNA Gel images for checking the presence of transgene in T1 plants using primers flanking the gRNA (approach shown in Fig. 2A). Expected band size is 281bp. (B) Plants which didn't give PCR amplification in Figure A were considered as transgene free, and were further checked for gDNA quality by doing PCR by using primers from a native gene, LOC_Os01g14220. Expected band size=375bp. M refers to marker (Thermo Scientific™ O'GeneRuler DNA Ladder Mix, Ready-to-Use 100-10,000 bp), WT=wild type, -ve= water control, +ve= transgenic plant.

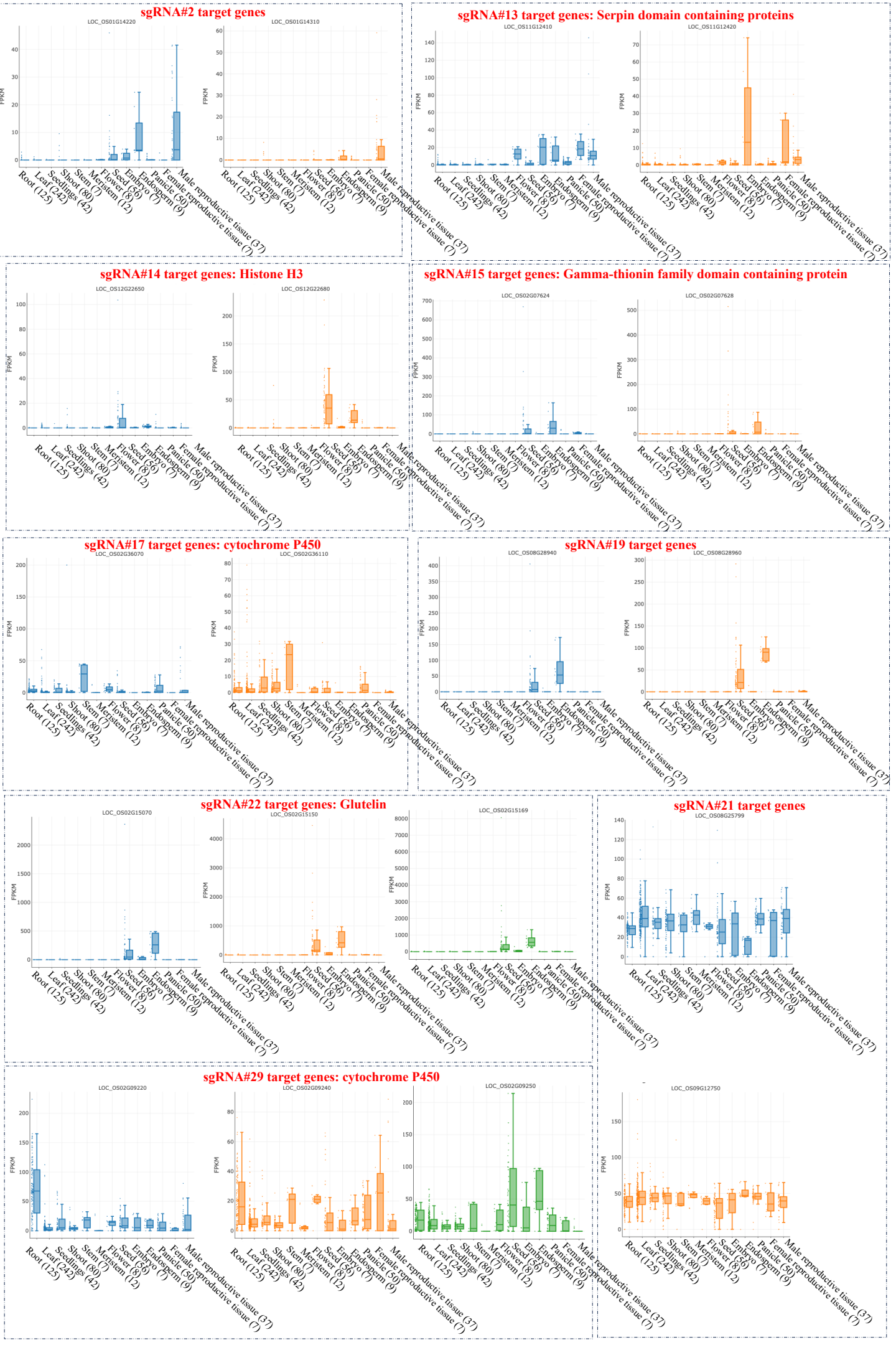
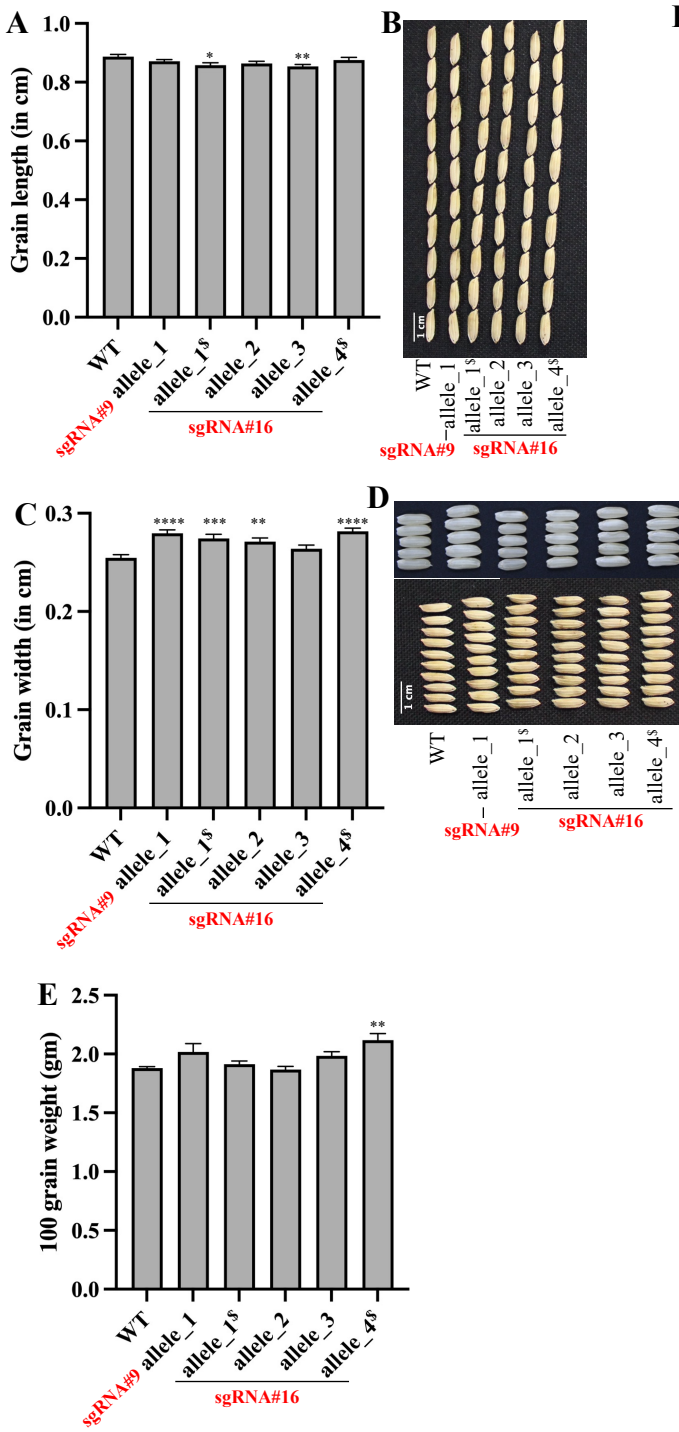


Figure S6. In-silico expression analysis of target genes of sgRNAs identified in T1 population at Plant Public RNA-Seq Database (https://plantrnadb.com/). The numbers in the brackets at the X-axis indicate numbers of RNA-Seq libraries from which the results were derived.



F

gRNA No.		#9		#16	
Target gene		LOC_Os07g11360	LOC_Os07g11380	LOC_Os03g28060	LOC_Os06g39210
Total protein length		163	188	764	763
cut site (aa no.)		119	144	325	232
Mutation types	allele_1	-GGTGTTC, +T:100%	+TT:100%	-	-
	allele_1$	-	-	NA	NA
	allele_2	-	-	-192:58.4%, -207:41.6%	NA
	allele_3	-	-	NA	NA
	allele_4$	-	-	-303:58.9%, -207:41.1%	NA

Figure S7. Analysis of grain length (A, B), grain width (C,D) and 100 grain weight (E) in different genome-edited T1 lines in MTU-1010 background. For sgRNA#9, only one allele was obtained, while for sgRNA#16, multiple alleles were used for phenotyping. **F.** Genotype information for the used genome-edited lines. N>20 was used for both grain length and grain width. For calculating 100 grain weight, only filled grains obtained from all the panicles were mixed and 30 seeds were used as one replicate, and total 3 replicates were used. In Fig (D) along with husked grains, de-husked seeds are also shown. Mean ± SE are plotted here in all the graphs. All the comparisons were made against WT plants. Ordinary one-way Analysis of Variance (ANOVA) was used to calculate p-values, where *=p-value<0.05, **=p-value<0.01, ***=p-value<0.001, ****=p-value<0.0001. $^{\$}$=Transgene free alleles.

A

```
LOC_Os09g24290      MGNDGEKASKLCKEAGGPSLDSNLAPTIVLALTTVPMGWDNRRRKQASTIMNARGRGARN
LOC_Os10g26430      MGNDGEKASKLCKEAGGPSLDSNLAPTIVLALTAVPMGWDNRRRKQASTMMNARGRGARN
                    ********************************:***************:**********

LOC_Os09g24290      GNGPNRGRGGGRAHSDVVVGANTTVLATRRWVGLEIDSVPGNEGQRDIVNYYLRCATGVG
LOC_Os10g26430      GNGRNRGREGGRAHSDVVFGANTTVLATRRWVGLEIDSVPGDEGQRDIVNYYLRRATGVG
                    *** **** *********.**********************:************ *****

LOC_Os09g24290      NGERELAVVGTHHSNRRVTYVVHEPFLQSLKELQVAAVVGVERLMWKSRKDVVHWLNMLI
LOC_Os10g26430      SGERELAVVGTHRSNRRVTYVVHEPFLQSLKELQVAAVVRVERLRWKSRKDVVHWLNMLI
                    .***********:*************************** **** ***************

LOC_Os09g24290      S-----------------------------------------------DVASDEVAICNND
LOC_Os10g26430      SDEMLNPLKLLVAMDSIKQCLITTVESSLRIFAYLAFADTNSVSSITDVTSNEVAICNND
                    *                                              **:*:********

LOC_Os09g24290      GKDAKLANISTTKGSSSSTAGNDSGDFKWLGPESHSKKGKSYKSFWRRGFTFMVHDFVYI
LOC_Os10g26430      GKDAKLANISPTKDSSSSIAGNDSGDFKWLGPESHSKKGKSYKSFWRRGFTFMVHDFVYI
                    **********.**.**** ****************************************

LOC_Os09g24290      LVQHGNKLVAYVEELYEDNHANKMVQIRRFRTLNSTGIQLSPGVNDREILHSDNLQDIGV
LOC_Os10g26430      LVQHGNKLVAYVEELYEDNHANNMVHIRWFRTLNSAGIQLSPSVNDIEILLSDNLQDIGV
                    **********************:**:** ******:******.*** *** *********

LOC_Os09g24290      ECIDGLASVLNEEHFEMFQAIANNTNRQPYLCIRHIDNNSNVKTFDIAQLQGYSEQEIFR
LOC_Os10g26430      ECIDGLASVLNEEHFEKFQAIANNTNRQPYLCIRHIDNNSNVKTFDIAQLQGYSEQEIFR
                    **************** ********************************************

LOC_Os09g24290      IVSGTPPVTVHPDASEGSKNTPRSSARGHHHHRTMENPTASDETNVQATTINVLARNAAP
LOC_Os10g26430      TISGTPPVTGHPDASEGNKNTPRSSARGHHHHQTVENPTAGDETNVQATTINVLACNAAP
                     :******* *******.**************:*:*****.************** ****

LOC_Os09g24290      TESASALINSALEKYLEQYFSHGCLVECLSQDSGIRGCWFIGSVIRRCGDRIKVRYQHLQ
LOC_Os10g26430      TESASGLINSALEKYLEQYFSPGCLVECLSQDSGIRGCWFIGSVIRRRGDRIKVRYQHLQ
                    *****.**************** ************************* ************

LOC_Os09g24290      DPETPRANLEEWLLVTRTANPDTLRIRLSGRTRIRPHNMSERENPSTISVGTVIDGWLYD
LOC_Os10g26430      DPETPRANLEEWLLVTRTANPDMLRIRLSGRTRIRPHNMSERENPSTISVGTVIDGWLYD
                    ********************** ************************************

LOC_Os09g24290      GWWEGIVLKVN---DARRLLAYLPGEKKMVLFRKDQLRHSLEWIDNEWKNFAHREDIARR
LOC_Os10g26430      GWWEGIVLKLVAMSMEFFPLSEFAGEKKMVLFRRDQLRHSLEWIGNEWKNFAHQEDIARR
                    *********:         *: :.*********:**********.*********:******

LOC_Os09g24290      IPSAEDLRIRVITAREVLTREEVMKQLEGLKTNKGGSNSAKPAAEKGSSSSATKKTTPDL
LOC_Os10g26430      IPSAEDLRIRVITAREVPTREEVMRQLEGLQTNKGGSNSAKPAAEKGSSSSATKKTTPDL
                    ***************** ******:*****:*******************************

LOC_Os09g24290      IRHATNDLGSLNFKHVGVPASEEIRTDNKGSQVNLENVLKSDSLKWTERKARGSFGPRM-
LOC_Os10g26430      IRHATNDLGSSNFKHVGVPASEEIRTDNKGSQVNLENVLKLDSLKWTEPLSPRAKWPSRQ
                    ********** ****************************** *******  :  :  *

LOC_Os09g24290      ------------------------------------------------------------
LOC_Os10g26430      LPGTAAPAAAPHAQRSAAGRRRDRGVPLLLLSRRRHRVPTREGRRRRGGFSCGGANDGGG

LOC_Os09g24290      ------------------------------------------------------------
LOC_Os10g26430      DVRADMRREGRRGLQHMEGEGVPREPGGGADSARRRSWGAEGWGGGAGSGAQRKDGGDGG

LOC_Os09g24290      ------------------------------------------------------------
LOC_Os10g26430      AGLAPSPPSSPTWLREAALSPESGGRWAGPAVELGGGDARAAAAEPRGHGNNLRHSGARG

LOC_Os09g24290      -----
LOC_Os10g26430      ERQQE
```

B

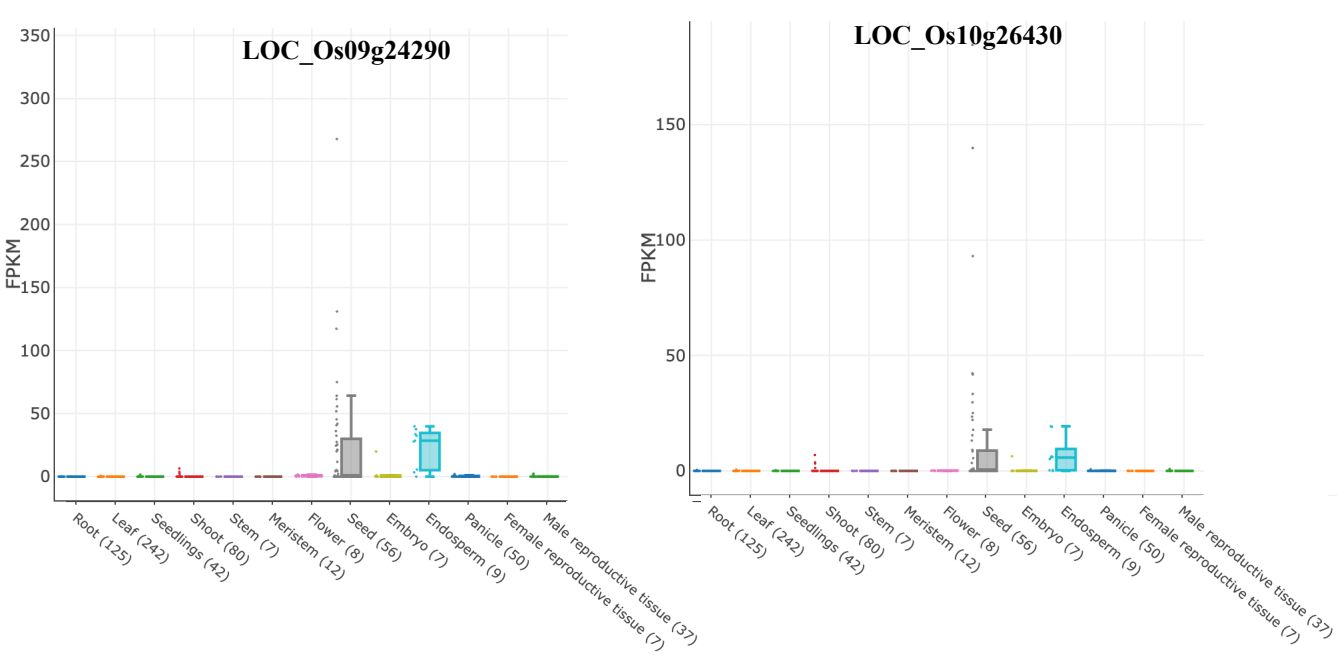


Figure S8. In-silico analysis of LOC_Os09g24290 and **LOC_Os10g26430** ***genes*** **A.** Protein sequence alignment. **B.** In-silico expression analysis at Plant Public RNA-Seq Database (https://plantrnadb.com/). The numbers in the brackets at the X-axis indicate numbers of RNA-Seq libraries from which the results were derived.

A

Allele	LOC_Os09g24290	LOC_Os10g26430
5$	0=78%, +1=12.2%, -2=9.8%	+1=53.1%, 0=46.9%,
6$	0=50%, +1=50%	+1=100%
7	+1=100%	-3=100%
8	+1=56.7%, 0=43.3%,	+1=60.5%, 0=39.5%,
9	+1=100%	+1=56.9%, 0=43.1%,
10	+1=100%	+1=50.4%, 0=49.6%,
11	0=86.7%, +1=13.3%	0=100%
12	+1=71.5%, 0=28.5%,	+3=100%
13$	+2=100%	-1=100%

B

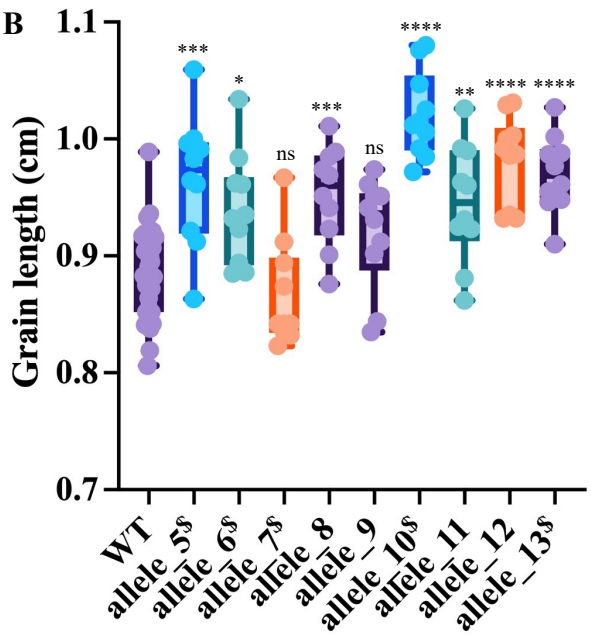


Figure S9. Genotype and phenotype of T1 plants containing gRNA#11, which targets two paralogous genes, LOC_Os09g24290 and LOC_Os10g26430. A. Additional types of alleles identified in the T1 plants **B.** Grain length in the identified different alleles. All the comparisons were made against WT plants. N=20, Ordinary one-way Analysis of Variance (ANOVA) was used to calculate p-values, where ns represents non-significant, * represents p-value<0.05, *** represents p-value<0.001, **** represents p-value<0.0001.