

**Figure S1. Schematic diagram of the whole procedure of the CRISPR-Cas and TALEN systems for gene editing in *P. patens* plants.**

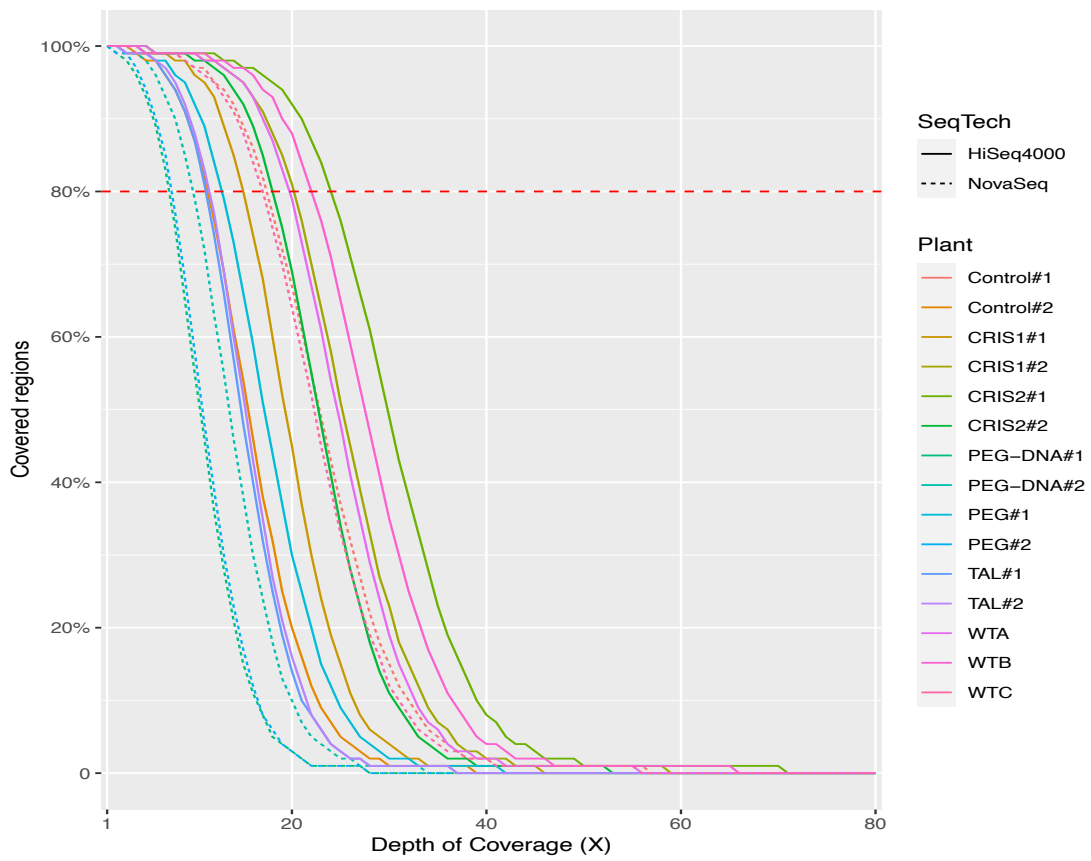
Wild-type plants issued from WT<sub>A</sub>, WT<sub>B</sub> and WT<sub>C</sub> single spores were vegetatively propagated with regular grinding of protonemal tissue for at least 8 weeks to give respectively the WT<sub>A</sub>, WT<sub>B</sub> and WT<sub>C</sub> clones. Wild type protoplasts are issued from 6-day-old protonemal tissue from the WT<sub>A</sub>, WT<sub>B</sub> or WT<sub>C</sub> clones. Non treated or treated (PEG, PEG + mock plasmid, PEG + SDNs plasmids) regenerating plants where vegetatively propagated with regular grinding of protonemal tissue for 8 weeks before DNA extraction. Wild-type, controls and edited plants in red are the ones that have been sequenced. Pictures of this figure are just symbolic and do not represent the actual samples/plants that have been used in the study.

**TALen<sub>402</sub>-TALen<sub>405</sub>**

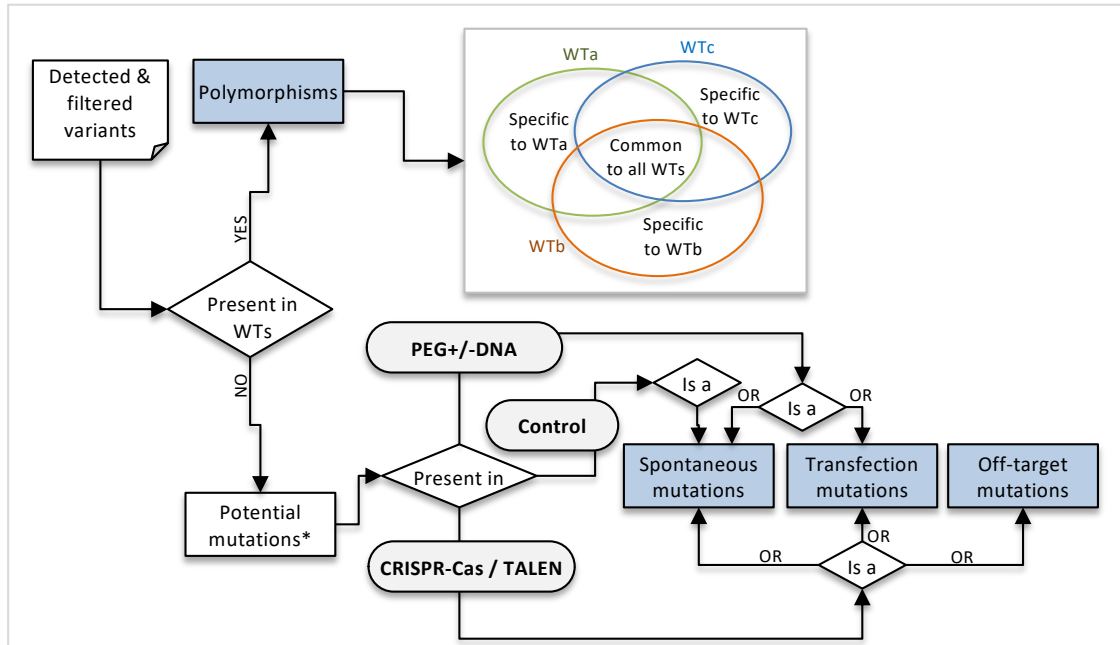
	TALen <sub>402</sub>	TALen <sub>405</sub>
WT	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#1	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#2	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#3	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#4	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#5	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#6	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#7	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#8	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#9	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#10	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#11	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#12	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#13	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	
TAL#14	AGGCCCTGTGATTAGGGAAAGAGTATAGTCTAGAGTATGGTACCGATTGCATTGAGATGCACGTTGG	

**Figure S2. Mutations induced by the TALEN system on the *APT* gene.**

Blue and purple letters plus arrows indicate the sequences targeted by TALen<sub>402</sub> and TALen<sub>405</sub> respectively, in red target sequence for the FOK1 nuclease dimer. Point mutations are in bold capital letters on a yellow background and deletions with dashes. In the frames, the microhomologies potentially involved in Alt-EJ-mediated repair of the TALEN-induced DSBs.



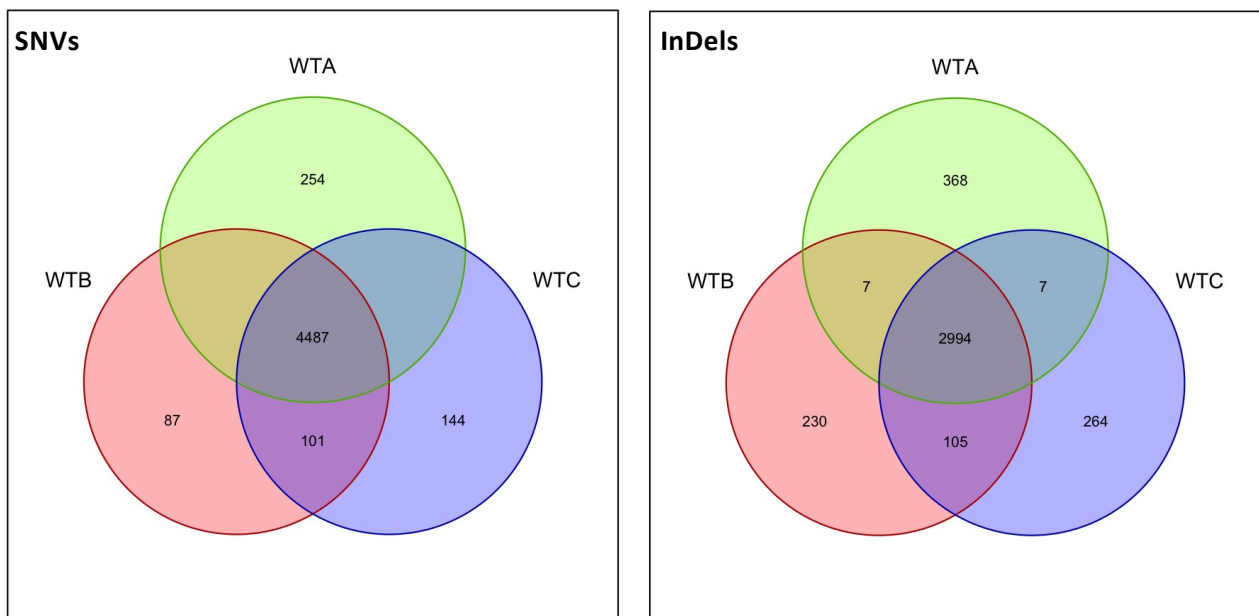
**Figure S3. Depth of coverage.**  
 Mean sequencing read depth for each conditions.



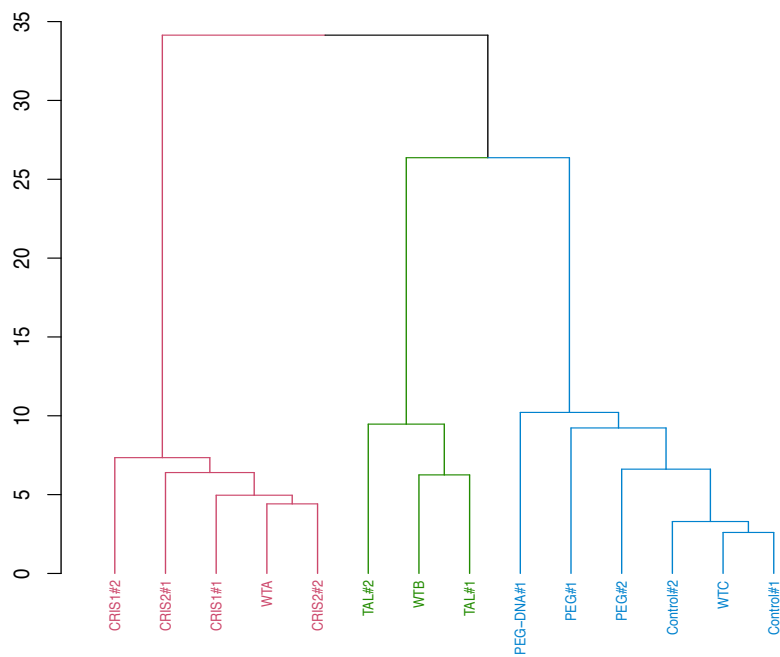
**Figure S4. Classification of variants.**

The variants detected in WTs are classified as polymorphisms. We distinguish the polymorphisms common to the three WTs and polymorphisms specific to each WT line. Variants considered as potential mutations are those detected in treated plants but not in corresponding parents (WT). The spontaneous mutations can occur in control or treated plants, thus present in all treated plants. The mutations due to transfection can occur in plants that have undergone treatment with PEG, thus present in treated plants. The SDN induced mutations (target mutations and off-target mutations) can occur only in plant that have undergone treatment with SDN (CRISPR-Cas or TALEN in our case).

\* The variants present in treated plants and also in parents but at sub-clonal level are considered as chimeric variants and classified as spontaneous mutations



**Figure S5. Polymorphism between the wild types used in this study and the reference genome.** Venn diagram of (a) SNVs and (b) InDels diversity of the wild types sequenced in this study with comparison to the reference genome sequences previously released.



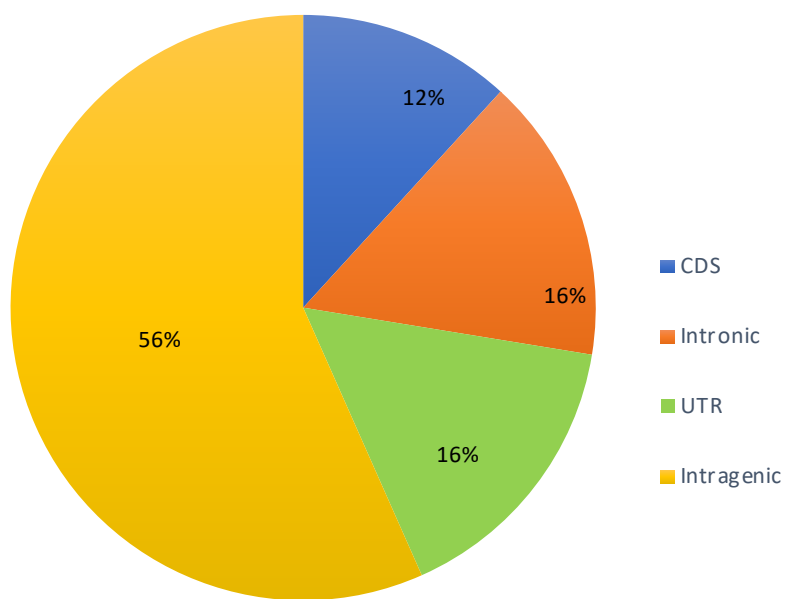
**Figure S6. Sample clustering based on all detected variants.**

Hierarchical clustering of the different samples, based on Euclidian distances between detected variants per sample.

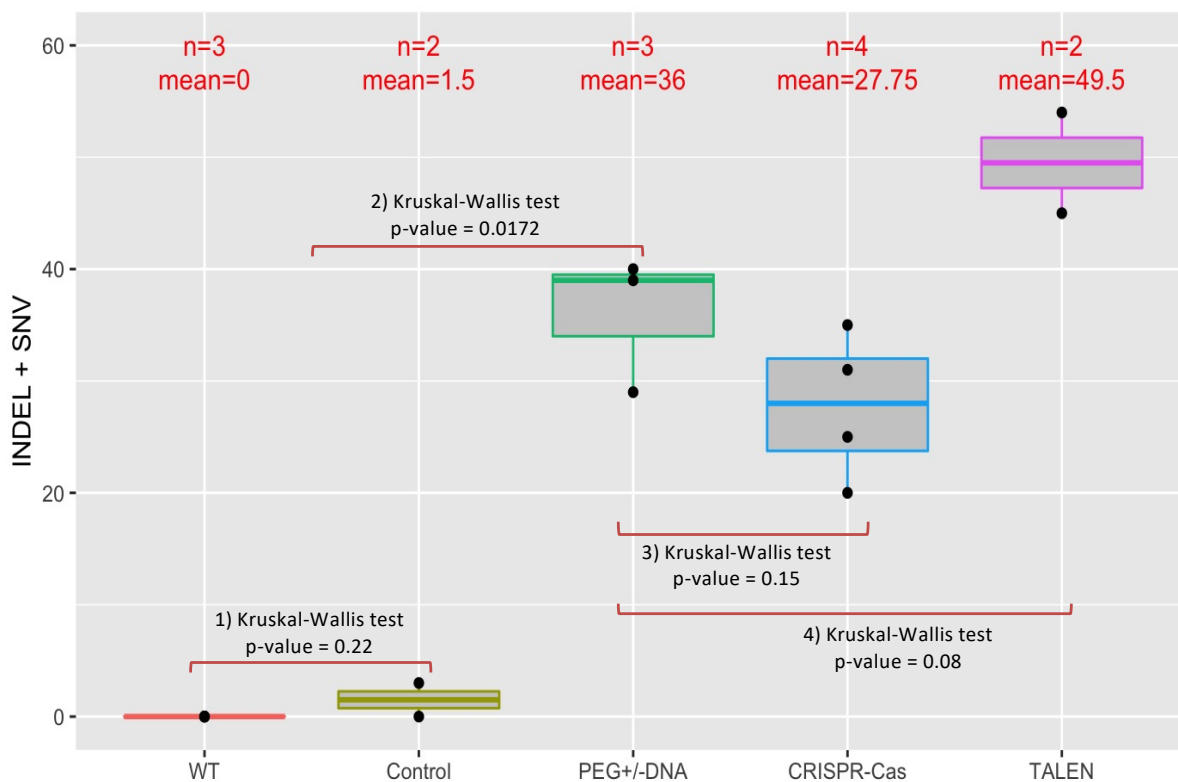
Used functions :

```
dist.mat <- dist(t(var.mat), method = "euclidean")
```

```
hclust(dist.mat, method = "complete")
```



**Figure S7. Distribution of variants normalised by genomic regions sizes.**  
The number of variants for each type of genomic regions has been normalised by the proportion of each of these regions in the genome.



**Figure S8. Boxplot for Kruskal-Wallis test of number of variants for the different treatments.**

- 1) test between WT plants and Control plants.
- 2) test between WT + control plants and PEG+/-DNA treated plants.
- 3) test between PEG+/-DNA treated plants and CRISPR-Cas treated plants.
- 4) test between PEG+/-DNA treated plants and TALEN treated plants.



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**Figure S9. Sequence of the TALEN<sub>402</sub> expression cassette.**

The rice Actin 1 promoter is in blue, the three tandem FLAG epitope tags is in red, the SV40 NLS is in green, in pink the TAL effector repeat array is in pink, the nonspecific DNA cleavage domain of the FokI endonuclease is in orange, the CaMV35S terminator is in black. Codons corresponding to the repeat Variable Diresidue (RVD), specific to A (NI) are highlighted in blue, specific to T (NG) are highlighted in red, specific to G (NS) are highlighted in yellow. Nucleotide sequence recognized is GTGATTAGGGAAGAGTA.

TCGAGGTCATTCATATGCTTGAGAAGAGAGTCGGGATAGTCCAAAATAAAAACAAAGGTAAGATTACCTGGTCAAAAGTGAAAACATCAGTTAAA  
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 ACCAAAATCCAGGGTACCAGCTCGAATTCAGCT

**Figure S10. Sequence of the TALEN<sub>405</sub> expression cassette.**

The rice Actin 1 promoter is in blue, the three tandem FLAG epitope tags is in red, the SV40 NLS is in green, in pink the TAL effector repeat array is in pink, the nonspecific DNA cleavage domain of the FokI endonuclease is in orange, the CaMV35S terminator is in black. Codons corresponding to the repeat Variable Diresidue (RVD), specific to A (NI) are highlighted in blue, specific to T (NG) are highlighted in red, specific to G (NS) are highlighted in yellow, specific to C (HD) are highlighted in green. Nucleotide sequence recognized is ATGGCTAACGTAAGTCT.