

Figure S1. The proportion of different mutation rates at each guide RNA site of the *OsIRO3* gene. The gene structure of *OsIRO3* and target positions of guide RNAs (gRNA) are shown (pink). The coding sequences (black boxes), introns (grey boxes), and untranslated regions (lines) of *OsIRO3* are depicted with length (base pairs) provided. The corresponding proportion of different mutations achieved at gRNA 1 and gRNA 2 of the *OsIRO3* gene are calculated from 42 recovered T0 rice plants following transformation.

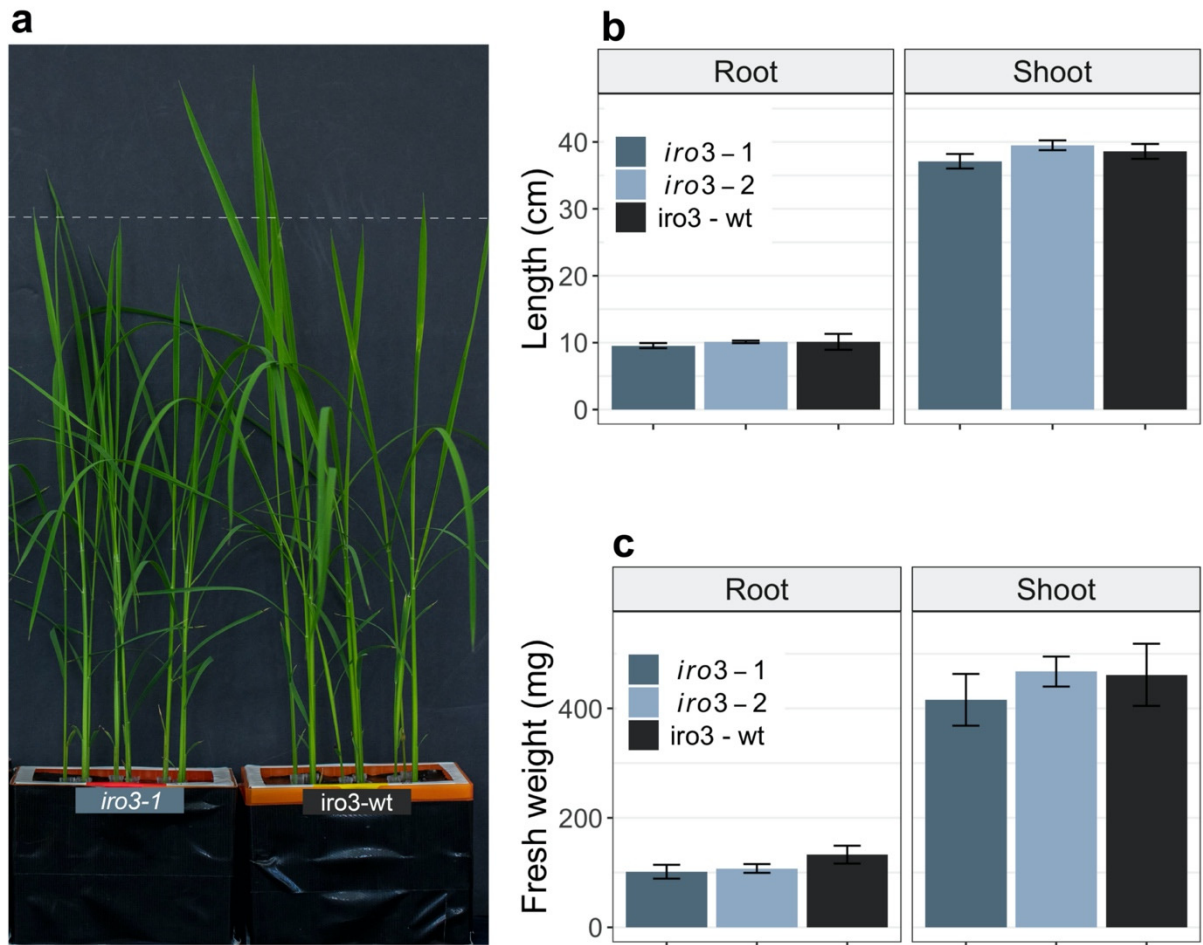


Figure S2. Phenotypic assessment of *iro3-1*, *iro3-2* and *iro3-wt* plants grown under hydroponic alkalinity stress conditions. **(a)** Representative *iro3-1* (navy blue) and *iro3-wt* (black) plants following 7 days of alkalinity stress and a 5 day recovery period. A dashed line is presented at a height of 40 cm. **(b,c)** Length (cm) and fresh weight (mg) of *iro3-1*, *iro3-2* and *iro3-wt* roots and shoots under hydroponic alkalinity stress conditions. Error bars indicate SEM of three biological replicates ($n = 3$) where each biological replicate is comprised of three representative plants.

Table S1. Indicators of alkalinity stress in *iro3-1*, *iro3-2* and *iro3-wt* plants.

Genotype	Alkalinity Tolerance Score	Proportion of dead leaves	Total no. of dead plants
<i>iro3-1</i>	7.00	0.47	10
<i>iro3-2</i>	7.67	0.63	11
<i>iro3-wt</i>	7.00	0.47	8

Table S2. Primers used for quantitative RT-PCR analysis.

Gene	Forward primer (5' – 3')	Reverse primer (3' – 5')	PCR product length (bp)	Annealing temperature (C°)
<i>OsIRO3</i>	TCCATGGGCCTATCTCTGAC	TGCCTATCCGCTTCTAGCAT	139	60
<i>OsIRO2</i>	TGGTTCAGGTCAGCTTGTTG	GTCCTTCACTTCTCTGAAGATGG	166	60
<i>OsYSL15</i>	GGAACAAGATGAACAAGAAGGAG	CGAGAGCAAGGATAGAAGAAGG	108	59
<i>OsIRT1</i>	AGGTCGGTGCTCGTCTTCT	TGTCCCTGTACACCCTGGTC	85	60
<i>OsNAS1</i>	AACTCCGTCATCGTCGCC	GCAAACCTTTCTGCCTTCTCA	165	61
<i>OsNAS2</i>	GTCATCGTCGCTCGCAAG	GAACTCTTCCGCCTTCTGG	152	61
<i>OsNAAT1</i>	GTCCTCACAAGCCCGAAG	TTCATTCCCAGCACACTCC	148	59
<i>OsNAS3</i>	GGTGATCAACTCCGTCATCA	CTACGAGGAGGGCAGCTTCT	187	60
<i>OsACT1</i>	CTATGTTCCCTGGCATTGCT	ACATCTGCTGGAATGTGCTG	156	60
<i>OsELF1</i>	GAAAGCAGCAGAAGAACGGG	TCCTCAAGCTTTGCCATGTCT	126	60
<i>OsP2</i>	CTCCTTCTGGCCCTGTCTCT	CCCCCTAGAATGTTCCCTGA	121	61
<i>OsEF-1a</i>	CTGCTGCAACAAGATGGATG	CAGTCAAGGTTGGTGGACCT	180	60
<i>*OsIRO3</i>	<i>*GCGAGCTGGGTAATATGCTAGA</i>	<i>*GGGCTCAATGACTGTTGGTT</i>	825	60

The asterisks indicate that these primers were used to detect the mRNA *OsIRO3* transcript using reverse transcription-PCR analysis.