

Supplementary material

Supplementary Table 1: Cross-efficiency rate and advanced generation duration in biotron breeding system. Data are mean \pm SD ($n = 5-13$). Days to flowering refers to the flowering of ‘Yukinko-mai’ (1st breeding event), F₁, BC₁F₁, BC₂F₁, BC₃F₁, and BC₃F₂ individuals. Data in parentheses in columns 2 and 3 represent the total numbers. †Embryo rescue and tiller removal were not performed so as to obtain the maximum number of true seeds.

Supplementary Table 2: Annotation of SNPs and indels in BC₃F₂ #31-2-4.

Supplementary Figure 1: Schematic representation of speed-breeding technique based on a biotron that controls temperature, daylength, and humidity, tiller removal, and embryo rescue.

Supplementary Figure 2: Frameshift deletion in *Os09g0356200* affects days-to-heading of ‘YNU31-2-4’ plants. (A) Frameshift deletion in *Os09g0356200*; red box indicates indel position (Chr. 9:1144980). (B) Distribution of days-to-heading. Genotypes: Y, ‘Yukinko-mai’ homozygous; H, heterozygous; K, ‘Kaijin’ homozygous. ‘YNU31-2-4’ plants were grown under normal field conditions to assess differences in heading within the population.

Supplementary Figure 3: Agronomic trait assessment of ‘Kaijin’, WT, and ‘YNU31-2-4’ plants under control field condition. (A) Phenotypes of plants after flag leaf emergence (95 days after germination); (B) mature rice seeds and (C) brown rice grains.

Supplementary Figure 4: ‘YNU31-2-4’ maintains higher relative water, chlorophyll, and proline contents at the seedling stage under salt stress. (A) Relative water content, (B) total chlorophyll content, and (C) proline content in shoots of 20-day-old seedlings. Data are mean \pm SD of three independent biological replicates. Bars labeled with the same letter are not statistically different (Duncan’s multiple range test, $P < 0.05$).

Supplementary Figure 5: ‘YNU31-2-4’ plants have an enlarged root system. (A) Root phenotype comparison of ‘Kaijin’, WT, and ‘YNU31-2-4’. Seedlings were cultured in Yoshida’s nutrient solution and photographed at 2 weeks. (B) Root length and (C) root fresh weight of plants grown under stress-free condition for 2 weeks. Data are mean \pm SD of four independent biological replicates, each being a pool of 5 individuals. Bars labeled with the same letter are not statistically different (Duncan’s multiple range test, $P < 0.05$).

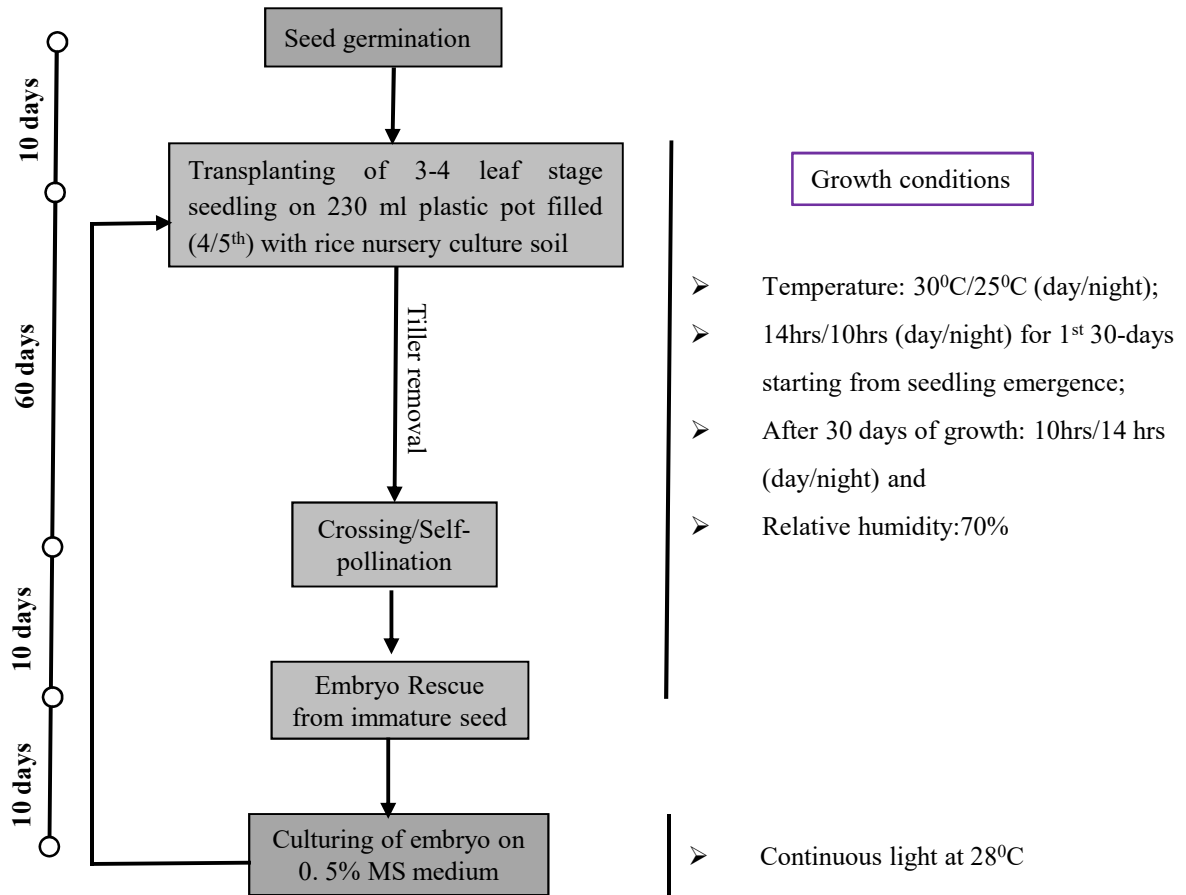
Supplementary Table 1. Cross-efficiency rate and advanced generation duration in biotron breeding system.

Breeding event	No. of spikelets crossed	No. of seed developed	Success rate (%)	Days to flowering	Total duration (days)
‘Kaijin’ × ‘Yukinko- mai’	9.00 ± 3.24 (81)	4.66 ± 2.44 (42)	53.84 ± 25.83	67.0 ± 1.58	77.4 ± 1.14
F ₁ × ‘Yukinko-mai’	8.38 ± 2.33 (67)	5.00 ± 2.45 (40)	57.82 ± 13.37	69.4 ± 1.14	79.4 ± 1.14
BC ₁ F ₁ × ‘Yukinko-mai’	9.15 ± 3.02 (119)	6.15 ± 2.12 (80)	68.85 ± 16.22	70.4 ± 1.67	80.6 ± 1.51
BC ₂ F ₁ × ‘Yukinko-mai’	9.41 ± 2.31 (113)	6.25 ± 2.70 (75)	64.95 ± 14.37	70.0 ± 1.41	80.0 ± 1.41
BC ₃ F ₁ -selfing	–	–	–	71.2 ± 1.30	81.2 ± 1.30
BC ₃ F ₂ -selfing	–	–	–	70.6 ± 1.67	107.8 ± 4.15 [†]

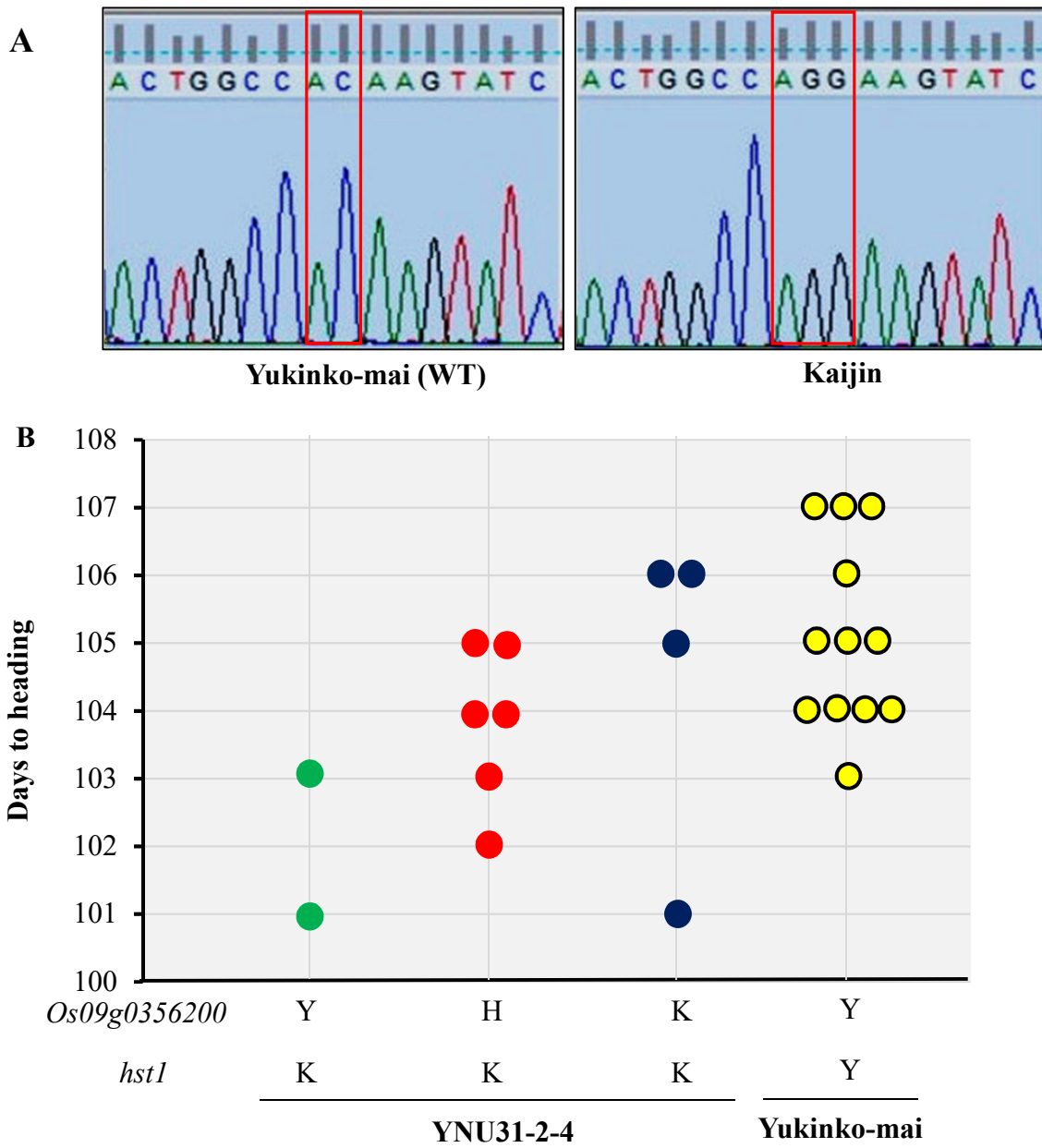
Supplementary Table 2: Annotation of SNPs and Indels in BC3F2 (#31-2-4)

Location/Variant type	Yukinko-mai (Homo)		Kaijin (Homo)		Heterozygous	
	SNPs	Indels	SNPs	Indels	SNPs	Indels
1kb DOWNSTREAM	10,715	3,547	216	113	666	204
3'UTR	2,877	882	86	26	180	51
INTRON_others	11,570	3,902	438	215	1335	325
INTRON_Splice donor	7	7	-	-	2	1
INTRON_Splice acceptor	10	7	-	1	1	1
CDS_Splice region	272	51	18	9	16	5
CDS_Synonymous	2,856	141	75	3	262	2
CDS_Nonsynonymous	2,839	16	70	1	230	-
CDS_Inframe INDEL	-	285	-	10	-	7
CDS_Frameshift	-	277	-	20	-	9
5'UTR	1,629	493	23	12	75	24
1kb UPSTREAM	11,735	3,640	255	111	914	289
INTERGENIC	68,341	16,751	1,807	611	5,984	1,143

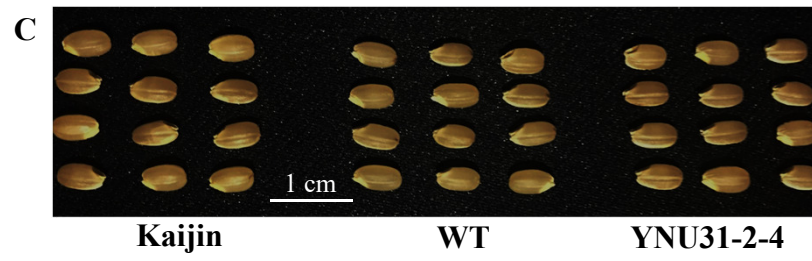
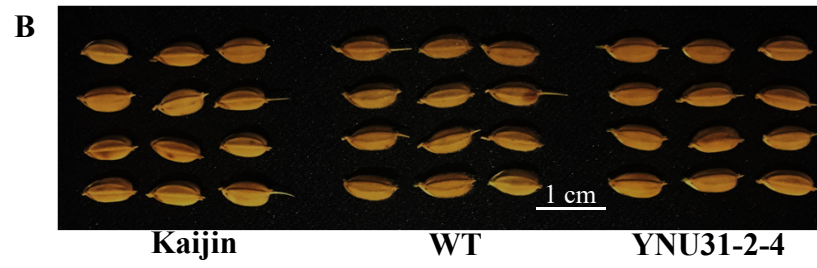
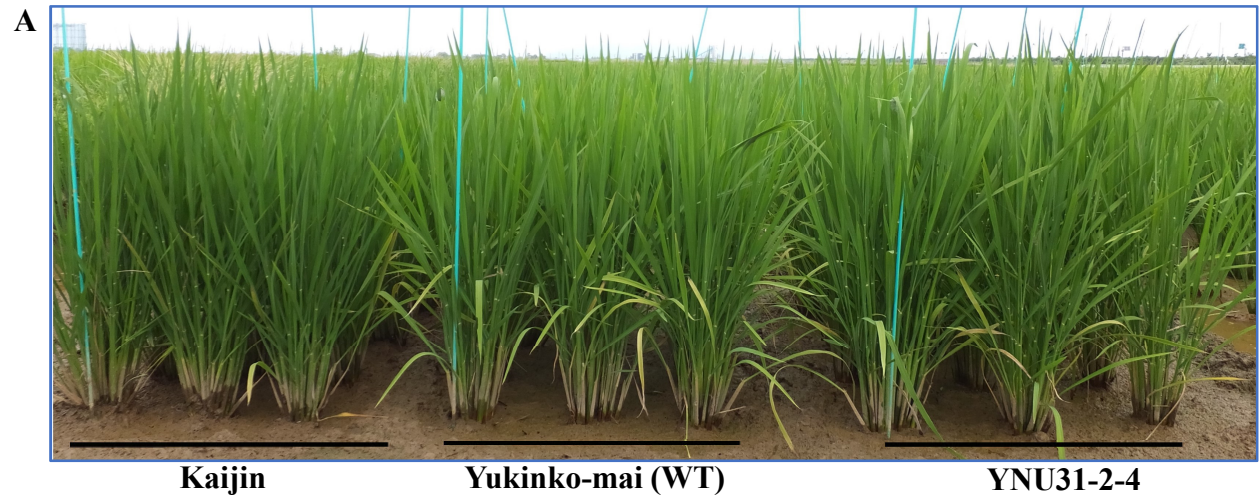
Supplementary Figure 1



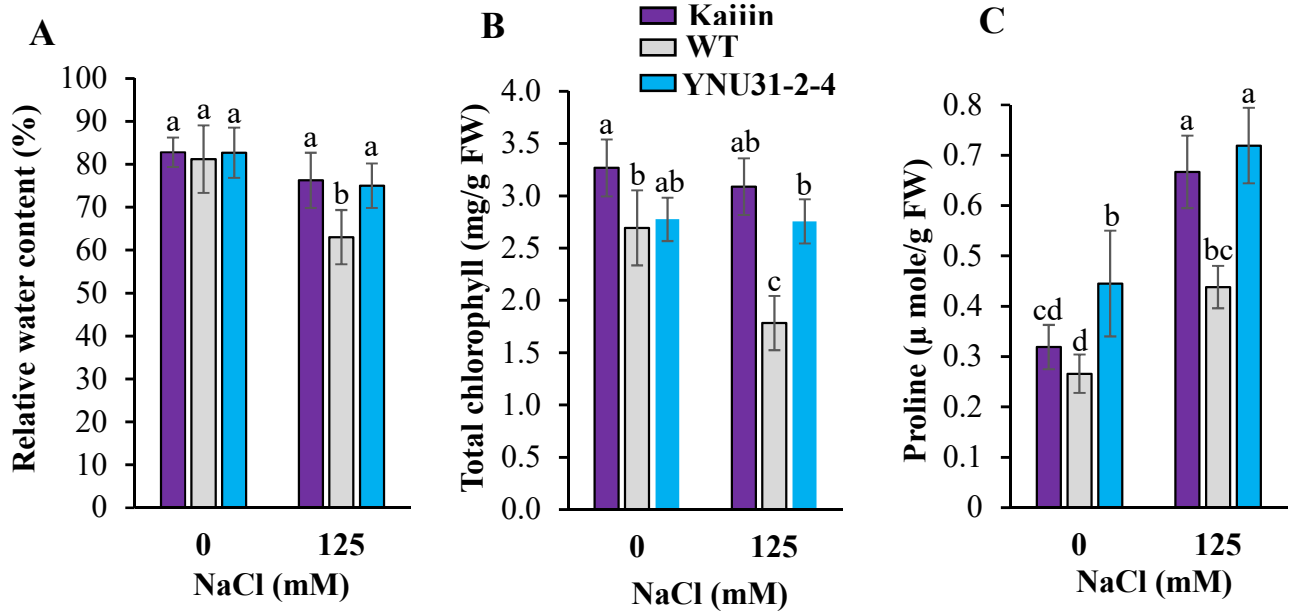
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

