

Table S1 Summary of genome sequencing data from *ar21*. SNV, single nucleotide variant.

Reference	Reads	Length	Signatures	Homozygous SNVs (100% probability)	Amino acid change	Pre-mature stop mutation
Chr1	9,243,149	30,427,671	38,207	153	24	1
Chr2	8,702,008	19,698,289	37,966	132	15	0
Chr3	10,041,831	23,459,830	39,822	192	24	2
Chr4	7,056,948	18,585,056	25,737	198	33	2
Chr5	8,997,444	26,975,502	35,195	204	36	4
Mitochondrion	1,446,760	366,924	835	1	0	0
Plastid	9,426,669	154,478	194	19	1	0
Total	54,914,809	119,667,750	177,956	899	133	9

Table S2 Summary of premature stop mutations detected in *ar21*. SNV, single nucleotide variant.

Chromosome	Reference position	Type	Reference	Allele	Count	Coverage	Frequency	Coding region change	Amino acid change	AGI	Annotation
Chr1	1296800	SNV	G	A	36	36	100	1252C>T	Gln418*	AT1G04650	unknown protein
Chr3	4758985	SNV	C	T	29	29	100	3340C>T	Gln1114*	AT3G14270	phosphatidylinositol-4-phosphate 5-kinase family protein
Chr3	8833717	SNV	C	T	31	31	100	1767G>A	Trp589*	AT3G24340	chromatin remodeling 40
Chr4	13634918	SNV	G	A	17	17	100	1795C>T	Gln599*	AT4G27220	NB-ARC domain-containing disease resistance protein
Chr4	16355664	SNV	G	A	55	55	100	326G>A	Trp109*	AT4G34150	Calcium-dependent lipid-binding (CaLB domain) family protein
Chr5	2147613	SNV	C	T	41	41	100	1936C>T	Gln646*	AT5G06930	protein_coding
Chr5	4695796	SNV	C	T	26	26	100	1095G>A	Trp365*	AT5G14570	ATNRT2.7_NRT2.7__high affinity nitrate transporter 2.7
Chr5	23441536	SNV	C	T	50	50	100	697C>T	Gln233*	AT5G57870	eIFiso4G1__MIF4G domain-containing protein / MA3 domain-containing protein
Chr5	24758915	SNV	C	T	30	30	100	409C>T	Arg137*	AT5G61570	Protein kinase superfamily protein

Table S3 Genotyping with the *ar21* x Col F2 population. Genomic DNA was extracted from 107 lines individually. PCR products were sequenced to identify the point mutation in *At5g57870*. T, mutation; C, wild type; H, heterozygous.

Sample#	SNV	Sample#	SNV	Sample#	SNV	Sample#	SNV	Sample#	SNV
1	T	31	C	60	H	93	H	122	H
2	C	32	H	61	H	94	C	124	C
3	C	33	T	62	H	95	C	125	T
4	T	34	H	63	C	96	C	126	H
5	H	35	H	65	T	97	H	127	H
7	C	36	C	66	T	98	H	128	T
9	H	37	H	67	C	100	H	129	C
10	H	39	T	69	H	101	C		
11	H	40	H	70	H	102	C		
12	H	41	H	72	H	103	H		
13	H	42	T	73	T	104	H		
14	C	43	T	74	H	105	C		
15	H	44	T	75	T	107	T		
16	C	45	H	76	H	108	H		
17	C	46	H	77	H	109	T		
18	C	47	H	78	T	110	H		
19	H	50	C	79	H	111	T		
20	T	51	H	81	H	112	C		
21	H	52	H	82	H	114	H		
23	H	53	H	83	H	115	H		
25	H	54	H	85	H	116	T		
26	T	55	C	86	H	117	T		
28	C	56	T	88	H	118	C		
29	C	57	H	89	H	119	H		
30	H	59	H	92	C	121	T		

Table S4 Primer pairs used in this study.

Name	Sequence(5' to 3')	Comments
BarF	AACCCACGTCATGCCAGTTC	BAR gene
BarR	CCATCGTCAACCACTACATC	BAR gene
JV65/66F	TTTCTTTACGGTTTCTAACTTTT	Mapping
JV65/66R	TCATCTTCCCTTTAATTCTGATGA	Mapping
MTH12F	GTAAAATTTTCTATTGCA	Mapping
MTH12R	ATGTCCTCCTGTTCTGTCCA	Mapping
qTUB3F1	ACCATTGCGAGTTCCCGAGT	qRT-PCR
qTUB3R1	AGCGCACCGATAGACGAGAT	qRT-PCR
qFAD3F	TCAAACCCTTTCTTACCACA	qRT-PCR
qFAD3R	TTGGTCCATAGCAACAACCA	qRT-PCR
I4G1-qRTF	AGAGTCCAACGGGAAACTGC	qRT-PCR
I4G1-qRTR	CTTGCGGATTGAACGGGAAC	qRT-PCR
accD-qRTF	CTACTACCGGTGGAGTGACA	qRT-PCR
accD-qRTR	TGTGAACCTTCAGGCACGGCT	qRT-PCR
ACC1-qRTF	CCTAGGTATGCGGTGCATAC	qRT-PCR
ACC1-qRTR	TCGGGCCACCCAGTTGCATG	qRT-PCR
I4g1-RTF	AGCTCCTGTCCTCGTCAAAG	RT-PCR and sanger sequencing
I4g1-RTR	GTCTAAGCTCCTCCCTCAAC	RT-PCR and sanger sequencing
i4g2-LP	AATGCAACAACAAGGTGAACC	T-DNA screening
i4g2-RP	AAGAAGCTCGTACTTCTCCGG	T-DNA screening
i4g1-LP	TCAAGGGCAAACATATCATCC	T-DNA screening
i4g1-RP	TTTTGACTTCACGTTTCCGTC	T-DNA screening
Attb1-gi4g1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTCGTAATGGATCATGCATC	Plasmid construction
Attb2-gi4g1	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTTAGCTCAGAATCAGATAG	Plasmid construction