A genetic screen for suppressors of a hypomorphic allele of Arabidopsis *ARGONAUTE1*

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Supplementary Information





Figure S1 Schematic representation of the structure of the AGO1 gene and the AGO1 protein, with indication of the nature of the *ago1-52* mutation and its impact on the mRNA and protein products of the mutated gene. Boxes and lines between boxes represent exons and introns, respectively. White boxes represent untranslated 5' and 3' regions. The PAZ and PIWI domains of the AGO1 protein are shown in blue. Lower case letters are used for intron sequences. Red letters indicate mutated nucleotides or amino acids. The asterisk indicates a stop codon¹⁷.



Figure S2 Double mutants representative of the phenotypic classes defined in this work. (a-d) Depigmented plants: (a) P5 25.1, (b) P6 9.2, (c) P6 9.3 and (d) P6 23.3. (e-h) Severe Ago1-52 mutant phenotype: (e) P2 3.4, (f) P3 39.1, (g) P3 40.3 and (h) P5 26.1. (i-l) Weak Ago1-52 mutant phenotype: (i) P6 3.2, (j) P6 29.1, (k) P8 6.2 and (l) P8 35.3. (m-p) Compact rosette: (m) P4 48.1, (n) P6 3.6, (o) P6 5.1 and (p) P6 8.1. (q-t) Synergistic phenotypes: (q) P1 6.3, (r) P1 10.13, (s) P1 15.11 and (t) P1 23.4. (u-x) Reticulate leaves: (u) P6 65.1, (v) P8 7.1, (w) P8 16.1 and (x) P8 21.1. (y-ab) Other phenotypes: (y) P3 3.5, (z) P3 28.1, (aa) P3 36.4 and (ab) P6 2.4. Pictures were taken at 21 das. Scale bars: 1 mm.

Phenotypic class	M ₂ double	Expressivity in the M ₃ generation			
	mutants	constant	reduced	variable	null
Severe Ago1-52 phenotype	42	11	4	18	9
Weak Ago1-52 phenotype	93	21	6	39	27
Compact rosette	25	3	2	7	13
Depigmented plants	34	8	4	14	8
Reticulate leaves	48	45	0	2	1
Other phenotypes	60	4	5	24	27
Total	302	92	21	104	85

Table S1 Expressivity of the mutant phenotypes in M₃ families, classified by phenotypic class

Values indicate the number of individuals of each type.

	Phenotypic classes in the F ₂						
mas ago1-52	WT ^a	Putative mas	Ago1-52	Putative	Lethal		Other
line backcrossed		single mutant ^b		double mutant ^c	seeds ^d	seedlings ^e	phenotypes
P1 5.33 (<i>mas1</i>) ^h	122	0	16	27			
P2 11.1 (<i>mas2</i>) ⁱ	121	0	8	14			
P4 22.2 ^j	107	0	3	12	15		11 ^f
P5 11.1 ^k	113	0	28	3	3		17 ^f
P7 13.1 (<i>mas7</i>) ¹	160	26	13	27	62	22	
P7 23.1 ^m	284	14	13	77			18 ^g
P7 26.1 (<i>mas4</i>) ⁿ	125	20	31	22	20	18	9 ^g
P8 14.1 (<i>mas3</i>)°	308	0	49	101			
P8 25.1 (<i>mas5</i>) ^p	136	13	14	24		54	

Table S2 Phenotypic segregation in the F_2 progeny of several mas ago1-52 × Ler backcrosses

Values indicate the number of plants from each phenotypic class. ^aPhenotypically wild type class, including *MAS/MAS;AGO1/-* plants. If the *mas* mutation was recessive or dominant but had no morphological phenotype on its own, the *MAS/mas;AGO1/-* plants would also be included in this class. The *mas/mas;AGO1/-* plants would also be included in this class if the *mas* mutation is recessive but has no phenotype on its own. ^bPlants that share some phenotypes with the putative double mutants but not with *ago1-52*. ^cPlants that show a partially suppressed Ago1-52 phenotype. ^dSeeds exhibiting no or abortive germination. ^eSeedlings in which only the cotyledons and the first pair of leaves expand. ^fPlants with mutant phenotypes different among themselves and from that of *ago1-52*. ^gPlants with a phenotype other than that observed in M₂ and M₃. ^{h-p}See Figure ^h3d, ⁱ3e, ^j3f, ^k3g, ^l3j, ^m3l, ⁿ3n, ^o3q and ^p3r.

Marker	Chromosome - Physical	osome - Physical <u>Chromosomes studied</u>			Recombination frequency $(r\pm S_r)^a$			
	map position (bp)	MAS1	MAS2	MAS3	MAS1	MAS2	MAS3	
nga59	1 - 2,768,000	116	84	76	35.34±4.44	50.00±5.46	32.89±5.39	
JV18/19	1 - 5,160,595	116	84	54	24.14±3.97	31.71±5.14	38.89±6.63	
AthZFPG	1 - 8,727,056	104	84	50	16.35±3.63	21.43±4.48	38.00±6.86	
SNP10026	1 - 11,562,061	100	74	74	11.00±3.13	20.27±4.67	36.49±5.60	
F1L21	1 - 15,993,202	116	84	74	6.03±2.21	8.33±3.02	27.03±5.16	
nga128	1 - 20,695,113	116	84	76	15.52±3.36	8.33±3.02	31.58±5.33	
SNP10490	1 - 24,344,448	116	84	76	23.28±3.92	20.24±4.38	30.26±5.27	
nga111	1 - 27,418,736	116	84	76	34.48±4.41	42.86±5.40	25.00±4.97	
nga1145	2 - 682,624	116	84	74	30.17±4.26	47.62±5.45	48.65±5.81	
SNP8895	2 - 7,860,251	116	84	74	12.93±3.12	46.43±5.44	51.35±5.81	
nga1126	2 - 11,670,000	110			3.64±1.78			
nga168	2 - 16,240,385	116	84	74	18.10±3.58	44.05±5.42	64.86±5.55	
nga126	3 - 3,713,432	112	76	74	48.21±4.72	55.26±5.70	47.30±5.80	
MYF24	3 - 6,466,772	98	50	74	43.88±5.00	56.00±7.02	43.24±5.76	
AthGAPab	3 - 9,796,450	116	84	76	46.55±4.63	58.33±5.38	46.05±5.72	
T32N15	3 - 16,986,906	108	84	74	41.67±4.74	47.62±5.45	50.00±5.81	
F28P10	3 - 21,398,175	96	82	62	51.04±5.10	47.56±5.52	50.00±6.35	
nga6	3 - 23,040,009	114	84	74	42.11±4.62	44.05±5.42	48.65±5.81	
nga1111	4 - 5,074,681	116	84	76	40.52±4.56	14.29±3.82	11.84±3.71	
AthF28J12.3	4 - 9,166,451	112	84	76	40.18±4.63	30.95±5.04	6.58±2.84	
nga1139	4 - 15,408,641	116	84	76	43.10±4.60	42.86±5.40	18.42±4.45	
AthCTR1	5 - 979,763	104			49.04±4.90			
nga151	5 - 4,669,932	106	76	76	48.11±4.85	46.05±5.72	43.42±5.69	
nga76	5 - 10,375,531	104		74	50.96±4.90		39.19±5.67	
AthPHYC	5 - 13,721,807	104	84	56	46.15±4.89	50.00±5.46	46.43±6.66	
MNF13	5 - 17,908,612	116	84	52	49.14±4.64	50.00±5.46	40.38±6.80	
MNB8	5 - 23,120,770	114	84	76	51.75±4.68	52.38±5.45	42.11±5.66	

Table S3 Low-resolution mapping of MAS1, MAS2 and MAS3

Genotyping results obtained for mapping populations of F_2 plants derived from crosses involving the wild type Col-0 and a *mas ago1-52* double mutant with a Ler genetic background. r: recombination frequency. *S_r*: Standard error of recombination frequency. Physical map positions of the markers were obtained from the Munich Information Center for Protein Sequences (MIPS) Arabidopsis database. Data corresponding to r < 30% are highlighted in bold and were considered unequivocal evidence of linkage. The linkage found for chromosome 1 corresponds to the *ago1-52* mutation, whose physical map position is 17,885,633 bp.

84 84

106

114

74

74

53.77±4.84 50.00±5.46 54.05±5.79

53.51±4.67 48.81±5.45 56.76±5.76

MUA2

K8K14

5 - 23,956,127

5 - 28,518,213