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Impact on Arabidopsis growth and stress resistance of depleting the Maf1 repressor of RNA polymerase III

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ABSTRACT

Maf1 is a transcription factor that is conserved in sequence and structure between yeasts, animals and plants. Its principal molecular function is also well conserved, being to bind and repress RNA polymerase (pol) III, thereby inhibiting synthesis of tRNAs and other noncoding RNAs. Restrictions on tRNA production and hence protein synthesis can provide a mechanism to preserve resources under conditions that are suboptimal for growth. Accordingly, Maf1 is found in some organisms to influence growth and/or stress survival. Because of their sessile nature, plants are especially vulnerable to environmental changes and molecular adaptations that enhance growth under benign circumstances can increase sensitivity to external challenges. We tested if Maf1 depletion in the model plant Arabidopsis affects growth, pathogen resistance and tolerance of drought or soil salinity, a common physiological challenge that imposes both osmotic and ionic stress. We find that disruption of the Maf1 gene or RNAi-mediated depletion of its transcript is well-tolerated and confers a modest growth advantage without compromising resistance to common biotic and abiotic challenges.

1. Introduction

Maf1 is a highly-conserved transcription factor found in yeasts, animals and plants (Ciesla and Boguta, 2008; Graczyk et al., 2018), with 36% amino acid identity between the human and Arabidopsis proteins. It was first identified in a budding yeast screen for mutations that affect tRNA suppressors and was shown to interact with pol III, the enzyme that synthesizes tRNA (Boguta et al., 1997; Pluta et al., 2001). Further genetic analyses established that *S. cerevisiae* Maf1 represses pol III-mediated transcription when yeast encounter adverse growth conditions, such as nutrient deprivation (Boguta, 2013; Upadhya et al., 2002). This may be important for metabolic economy, as pol III synthesizes highly abundant transcripts that account for ~20% of total RNA content (Willis, 2018). Maf1 represses transcription by binding directly to pol III, sealing off its active site and blocking its recruitment to promoters (Andrade et al., 2020; Vannini et al., 2010; Vorlander et al., 2020).

The ability of Maf1 to repress transcription can be switched off under conditions that favour growth through direct phosphorylation by several kinases, including TOR (Moir and Willis, 2013; Willis and Moir, 2007, 2018). Control of Maf1 by TOR is highly conserved through evolution, having been demonstrated in yeast (Wei et al., 2009), mammals (Kantidakis et al., 2010; Michels et al., 2010; Shor et al., 2010) and plants (Ahn et al., 2019; Andrade et al., 2020; Soprano et al., 2017). Despite a high degree of conservation at the molecular level, the organismal effects of Maf1 disruption vary between species and experimental systems (Willis, 2018). For example, homozygous knockout of the *Maf1* gene in mice results in animals that are viable and fertile, but are slightly smaller and significantly leaner than wild-type; this phenotype has been ascribed to metabolic inefficiency involving degradation of surplus tRNA that is produced due to release of pol III from Maf1-mediated restraint (Bonhoure et al., 2015). In contrast, depletion of Maf1 in Drosophila larvae expressing transgenic RNAi constructs results in elevated tRNA expression that promotes growth and development, resulting in significantly larger final body size (Rideout et al., 2012). Whereas larger flies may be undesirable, enhanced growth of plants is potentially advantageous, if it comes without fitness costs. As a first step to explore this possibility, we used *Arabidopsis thaliana* as a well-characterized model to investigate the impact of Maf1 depletion *in planta*. We find modest enhancement of average growth within popula-

Abbreviations: IM, insertion mutant; pol, RNA polymerase; RNAi, RNA interference; rRNA, ribosomal RNA; RT-qPCR, quantitative reverse transcription polymerase reaction; TOR, target of rapamycin; tRNA, transfer RNA

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Table 1

Gene	Primer sequences (Fwd/Rev)	Annealing temp (°C)	Cycle number	% agarose gel
Actin	TCAGCCGTTTTGAATCTCCG CATCCCAACCATGACACCAT	58	30	1.5
Maf1	CGCCTTTTCCTTCCACCATC CAGCGCTGAAATCGTAGTCC	58	32	1.5
RN7SL	GCTTGTAACCCATGTGGGGA GCTAAACCGTGATCCGTCCA	58	30	1.5
$tRNA^{Pro}$	TCAAAAGGTGTTTGGTCTAGTG ATTGAAGCATAGGGGTGTTC	58	36	2.5
$tRNA^{Thr}$	GAAAAGCCTCCGTAGCATAG GAACTGAAAGCCCCCGG	58	36	2.5
$tRNA^{iMet}$	ACAAAGCAGAGTGGCGGA CATAGCAGAGCCAGGTTTC	58	36	2.5
$tRNA^{Tyr}$	ATCAATCCGACCTTAGCTCA AGATTATCCGACCTGCCG	58	36	2.5
$tRNA^{Trp}$	ATCCGTGGCGCAATGGTAG CGTGAATCGAACACGCAACC	58	36	2.5
5S rRNA	GGATGCGATCATACCAGCACTAA ATCCTAGTACTACTCTCGCCCAA	58	24	1.5

tions when Maf1 levels are reduced by RNAi and when the Maf1 gene is disrupted by insertional mutagenesis. As growth restraint can provide protection against stress, we considered the possibility that plants relieved of Maf1 might be more vulnerable to environmental challenges, even if they thrive under optimal conditions. Indeed, depletion of Maf1 in sweet orange plants (*Citrus sinensis*) was found to increase susceptibility to attack by *Xanthomonas citri*, an economically important bacterial pathogen that devastates crops through canker (Soprano et al., 2018; Soprano et al., 2013). In contrast, we found no evidence under laboratory conditions that Maf1 depletion influences infection of *A. thaliana* by *Botrytis cinerea*, a broad range plant pathogen of substantial economic impact. Clearly, the result might be different in field conditions and with different pathogens. We also tested the effects of two major abiotic stresses that challenge plants, drought and high salinity. In neither case was evidence found that Maf1-depleted plants are more vulnerable to stress. Thus, the restraint of pol III transcription by Maf1 appears not to be essential for organismal protection of growing *Arabidopsis* against key biotic and abiotic stresses encountered in the natural environment. Furthermore, a modest growth advantage can be obtained.

2. Materials and methods

2.1. Plant lines

For RNAi, a 400 bp inverted repeat fragment of *MAF1* cDNA (At5g13240) was designed to generate a hairpin transcript, which is processed into siRNAs by DICER-like endonuclease; it was cloned into pFG5C5941 and transcribed constitutively from the cauliflower mosaic virus 35S promoter. The construct also contained a Basta-resistance plant selection marker. It was introduced into wild-type *Arabidopsis thaliana* (Columbia ecotype) via *Agrobacterium tumefaciens* by floral dipping. Multiple transgenic lines were obtained by screening for Basta resistance. A line having a 3:1 Basta resistance segregation in the T2 generation was found and, in the T3 generation, homozygous lines were obtained with100% resistance. Maf1 knockdown was confirmed by RT-qPCR.

T-DNA insertion mutant (IM) lines SALK_054632 (IM1) and SALK_027781 (IM2) were obtained from the SALK Institute Genomic Analysis Laboratory (http://signal.salk.edu/). (O'Malley et al., 2015).

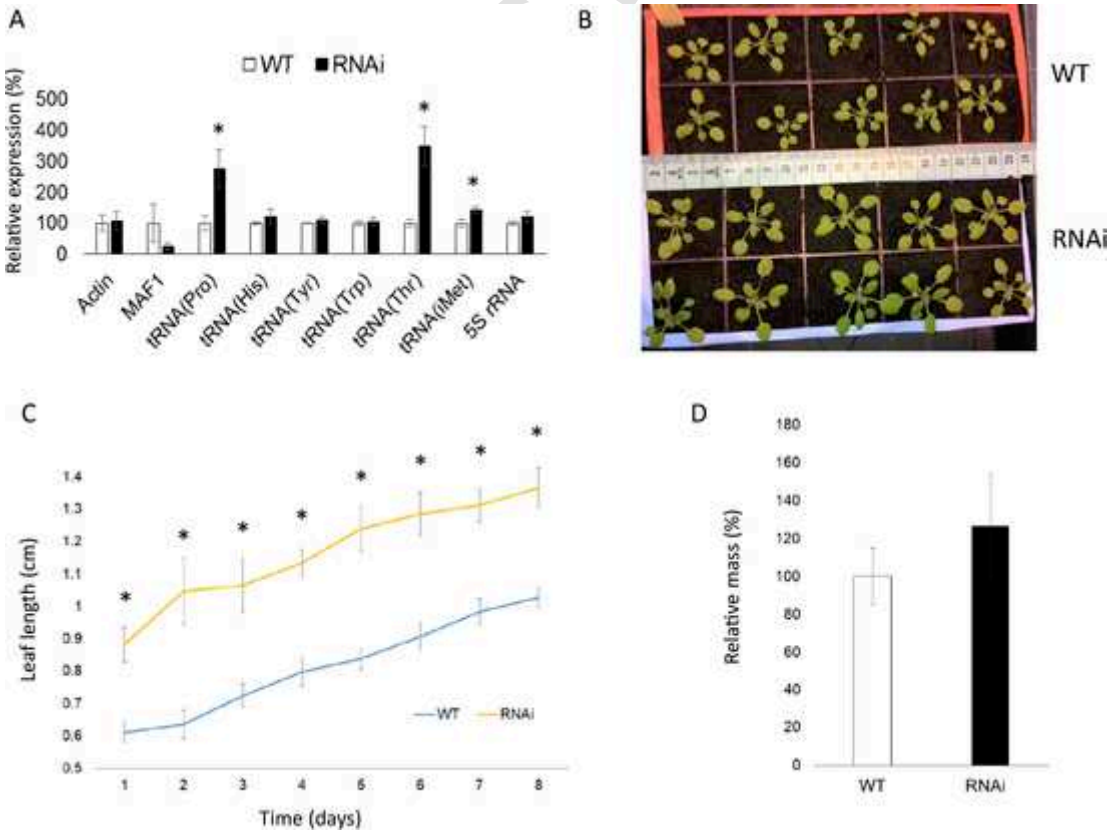

Fig. 1. Effects of Maf1 RNAi on expression of pol III products and growth of plants under optimal conditions. (**A**) Relative expression of indicated genes in aerial parts of wild-type (100%) and RNAi plants. Error bars indicate standard error of the mean; asterisks $P < 0.05$. Data are averaged from three biological replicates. (**B**) Representative image of wild-type and RNAi plants. (**C**) Lengths of > 100 leaves of 10 wild-type and 10 RNAi plants over 8 consecutive days. Error bars standard error. Asterisks $P < 0.00001$. (**D**) Relative mass of aerial parts of wild-type (100%) and RNAi plants.

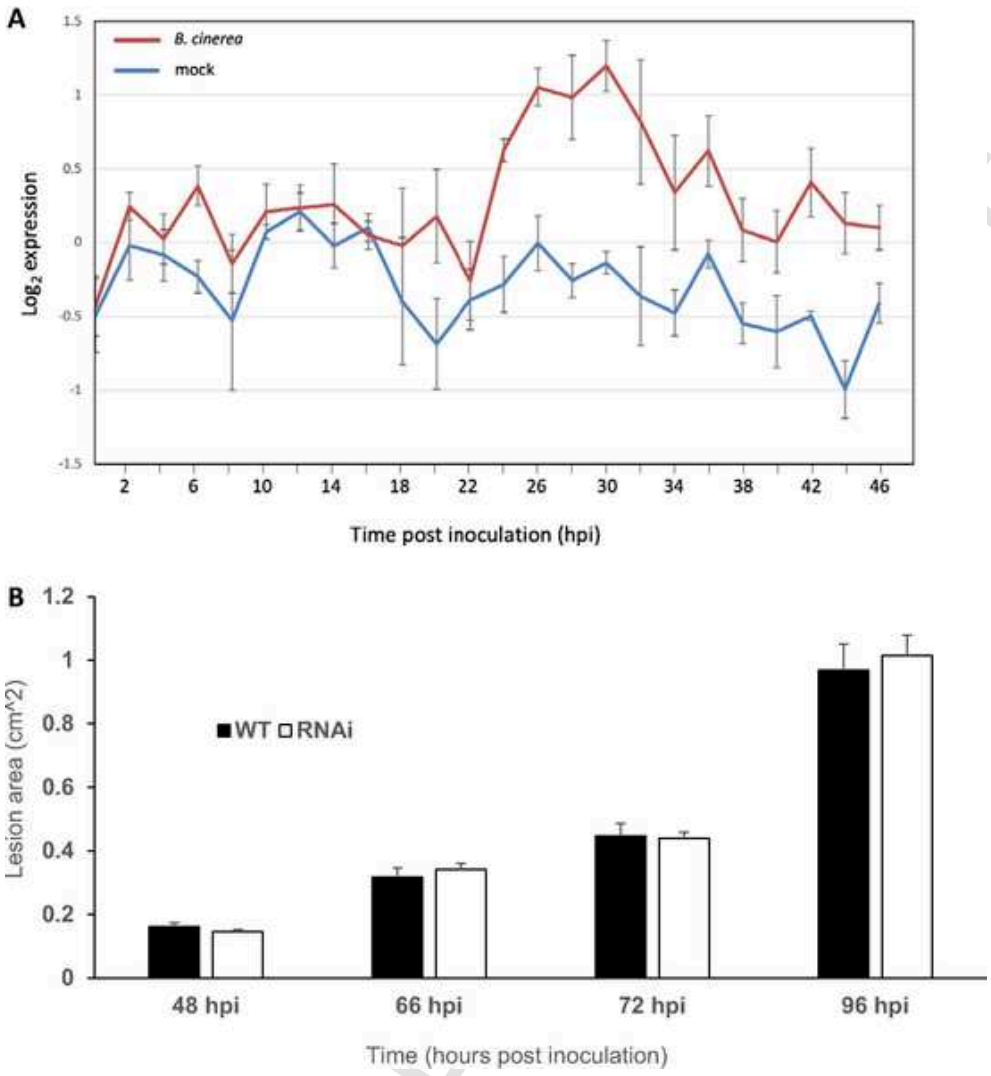

Fig. 2. Although Maf1 is induced by Botrytis infection, its depletion by RNAi does not affect short-term growth of fungal lesions. (**A**) Relative expression level of Maf1 mRNA in Arabidopsis leaves over 48 h following mock-inoculation or inoculation with Botrytis. Data are the normalised mean of 4 biological replicates with Type 1 error indicated. (**B**) Area of fungal lesions in Botrytis-inoculated leaves from wild-type and Maf1 RNAi plants over 4 days following inoculation. Data are from 9 biological replicates with bars indicating standard error of the mean.

Sites of T-DNA insertion and the region targeted in RNAi lines are detailed in Supplementary Fig. S1.

2.2. Growth and phenotypic analysis

All plants (WT, RNAi and T-DNA insertion mutants) were grown in identical greenhouse conditions. Supplementary lighting was switched on at 5.00 and off at 21.00. Plants were watered twice a day, apart from during drought trials. Photographs were taken daily of plants throughout the trial periods, next to a known standard. Leaf/petiole lengths were measured using imagej. Researchers were blinded to plants' genotypes, until all analyses were complete.

Salt stress trials: ten control and ten salt-treatment plants were grown for each genotype (WT and the two T-DNA insertion mutant lines). All plants received identical treatment until day 22 post-germination, when analysis began. For the first four days of analysis, all plants received 200 ml water, every other day. From the fifth day, trays containing control and salt-treatment plants were irrigated with 200 ml water, or 200 ml 75 mM NaCl, respectively, every other day. Plants were harvested on salt-treatment day eight (day twelve of analysis); plant stems were cut at the air–soil interface and aerial mass was measured before RNA extraction.

For severe salt-stress trials, groups of ten WT and ten RNAi plants were irrigated with 200 ml water, 75 mM NaCl, or 150 mM NaCl, every other day. Plants were harvested on day sixteen, as above.

Drought stress trials: eight control and eight drought-treatment plants were grown for each genotype (WT, RNAi, and the two T-DNA insertion mutant lines) in jiffy pots. All plants received identical treatment until day 22 post-germination, when analysis began. Drought treatment was carried out as described (Harb and Pereira, 2011). Briefly, plants growing in jiffy pots were weighed every day, and water content maintained at 30% field capacity (2 g H_2O/g dry soil), to inflict mild drought stress. Well-watered controls were maintained at 100% field capacity. Plants were maintained in a state of drought stress/control conditions for ten days, before being harvested as above.

2.3. RNA extraction and RT-PCR

Leaves were removed from plants and immediately frozen in liquid nitrogen. Leaves were powdered using pestle and mortar, and stored in trizol reagent. RNA was extracted using Invitrogen phasemaker tubes, according to the manufacturer's protocol. RNA concentration was measured using a nanodrop 2000c machine. Thermofisher superscript IV

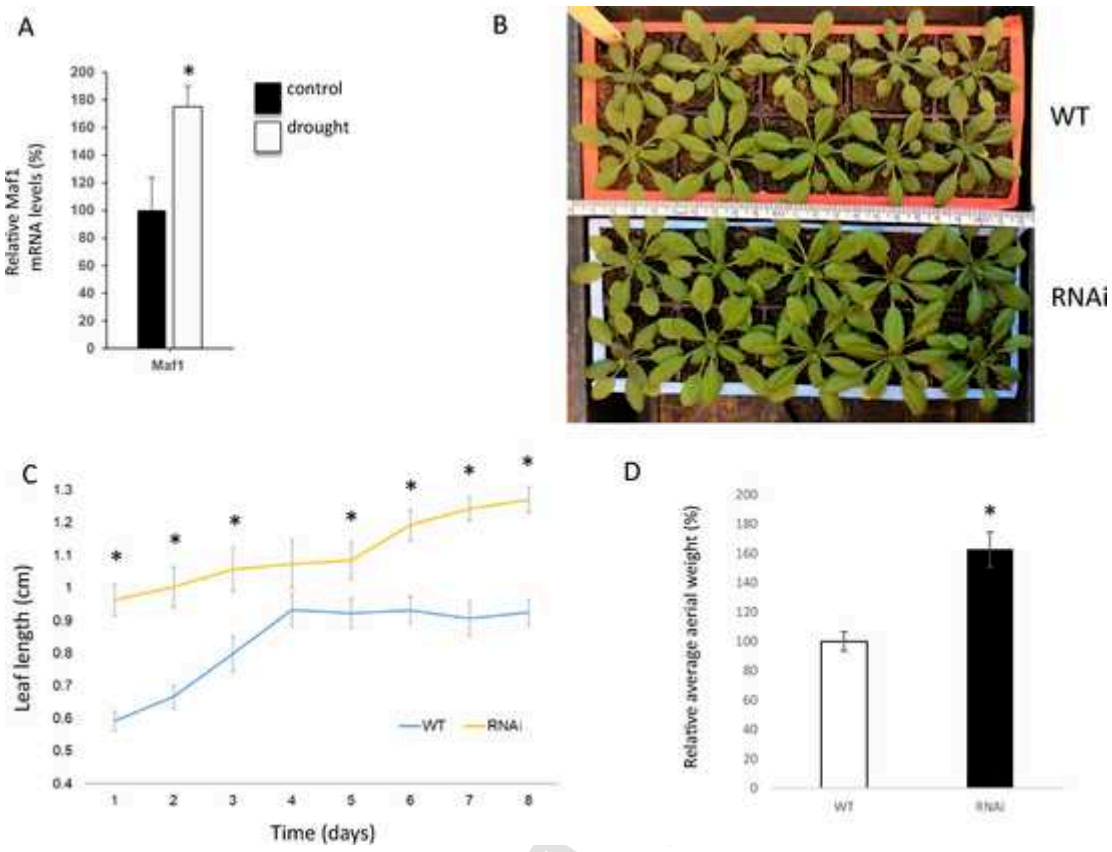

Fig. 3. Response of Maf1 mRNA and effects of Maf1 RNAi on plant growth during drought. (**A**) Relative expression of Maf1 mRNA (normalized to actin mRNA) in aerial parts of wild-type plants maintained with standard (100%) or with low water for 9 days. Error bars indicate standard error of the mean; asterisk $P < 0.01$. Data are averaged from three biological replicates. (**B**) Representative image of wild-type and RNAi plants. (**C**) Lengths of leaves of wild-type and RNAi plants over 8 consecutive days without water. $P < 0.00001$ at days 1,2, 7 and 8; day 3, $P = 0.0015$; day 4, $P = 0.066$; day 5, $P = 0.0198$; day 6, $P = 0.00007$. (**D**) Relative mass of aerial parts of ten wild-type (100%) or RNAi plants after 10 days without water ($P < 0.01$).

was used for reverse transcription, according to the manufacturer's protocol. cDNA was generated from three plants/genotype/condition.

PCRBIO Ultra Polymerase was used for semi-quantitative PCR, according to manufacturer's protocol. Primer sequences and amplification conditions are listed in Table 1. PCR products were stained with 3ul NEB 6X loading dye, and run on 1.5% or 2.5% agarose gels, depending on product size. RT-PCR bands were quantified using ImageJ.

Downstream primers for tRNA amplification were designed to recognise sequence in the primary transcript (pre-tRNA) that is removed during 3′ maturation to generate the mature product. As a consequence, the assays monitored pre-tRNA levels, rather than mature, processed tRNA. This approach was chosen to provide a read-out of transcriptional activity in response to Maf1 depletion.

2.4. Botrytis cinerea infection

Leaves from WT or RNAi plants were mock-inoculated or inoculated with spores of *Botrytis cinerea* strain pepper, as previously (Windram et al., 2012). Lesion area was determined using ImageJ from photographs taken after 48, 66, 72 and 96 hrs. Mean lesion areas were compared using a Student's two-tailed *t*-test. Expression profile of Maf1 mRNA was extracted from processed normalized microarray data GSE39598 (Windram et al., 2012).

2.5. Data processing and statistical analysis

RT-PCR gels were imaged by transilluminator and product intensity was measured using imagej, as described (Antiabong et al., 2016). Expression of each transcript was then normalized to actin expression from the same sample.

All graphs (plant growth and RT-PCR data) were generated in excel, and all statistical analysis (standard error and ANOVA,) performed in Rstudio. One-way ANOVA tests were used in each case to assess the significance of differences in size, mass or gene expression between the different genotypes. Two-way ANOVA tests were used to assess the significance of differences in size, mass or gene expression between the different genotypes in different conditions. All error bars represent standard error of the mean. Researchers were blinded to plants' genotypes until all analyses were completed.

3. Results

3.1. Maf1 RNAi increases expression of a subset of tRNAs and growth of leaves under benign conditions

Expression of Maf1 mRNA was depleted ~ 2-fold using a stably-expressed RNAi constuct. Since Maf1 binds and represses pol III, its depletion is expected to result in elevated pol III transcriptional output. To assess this, RT-PCR was used to detect short-lived, unprocessed primary transcripts (pre-tRNAs), which reflect ongoing transcription more accurately than levels of stable, highly-processed mature tRNAs. Primary transcripts from $tRNA^{Thr}$, $tRNA^{Pro}$ and initiator $tRNA_i^{Met}$ genes were consistently elevated in the RNAi plants, but several other pre-tRNAs showed little or no response (Fig. 1a). We also found minimal change in other pol III transcripts examined, including 5S rRNA. Thus, Maf1 depletion results in selective derepression of a subset of pol III target genes. It is unclear why many pol III-transcribed genes are unresponsive

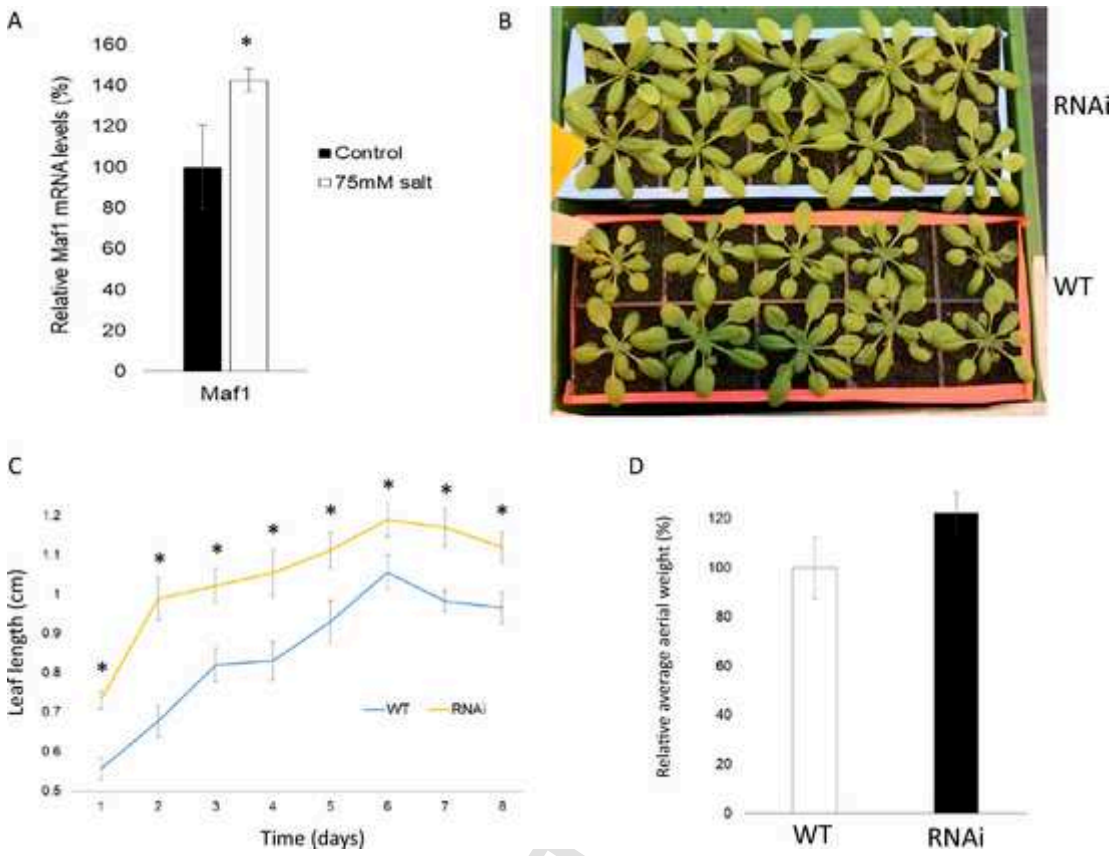

Fig. 4. Response of Maf1 mRNA and effects of Maf1 RNAi on plant growth during salt stress. (**A**) Relative expression of Maf1 mRNA (normalized to actin mRNA) in aerial parts of wild-type plants maintained for 8 days with tap water (100%) or with tap water supplemented with 75 mM NaCl. P = 0.036. Data are averaged from three biological replicates. (**B**) Representative image of wild-type and RNAi plants. (**C**) Lengths of leaves of wild-type and RNAi plants over 8 consecutive days exposure to 75 mM NaCl. P < 0.00001 at days 1 and 2; day 3, P = 0.00064; day 4, P = 0.0037; day 5, P = 0.00789; day 6, P = 0.01882; day 7, P = 0.000031; day 8, P = 0.00339. (**D**) Relative mass of aerial parts of ten wild-type (100%) or RNAi plants after 8 days exposure to 75 mM NaCl (P = 0.0485).

to Maf1 depletion, but similar selectivity has been reported in other organisms (Orioli et al., 2016; Turowski and Tollervey, 2016; Turowski et al., 2016).

Despite substantial variation between individual plants, the Maf1 RNAi plants on average displayed enhanced leaf growth relative to wild-type plants of the same age grown in parallel (Fig. 1b). This difference was most apparent in measurements of leaf length (Fig. 1c), which demonstrate significantly longer leaves in Maf1 RNAi lines relative to wild-type (p = 2e-06). As a consequence, aerial mass is also generally greater in RNAi plants (Fig. 1d), although this shows more variability (p > 0.05).

3.2. Significant depletion of Maf1 has minimal impact on sensitivity of *Arabidopsis* to the pathogen Botrytis cinerea

Sweet orange plants in which Maf1 was partially depleted by RNAi showed dramatic increases in both the number and the size of hyperplastic canker lesions following infection by the bacterial pathogen *Xanthomonas citri* (Soprano et al., 2013). We therefore considered whether Maf1 depletion might affect sensitivity of *Arabidopsis* to biotic stress induced by pathogen attack. We used *Botrytis cinerea*, a necrotrophic fungus with broad host range and high economic impact. Gene expression profiling of RNA extracted from *Arabidopsis* leaves revealed robust induction of Maf1 mRNA one day after *Botrytis* inoculation, raising the possibility that Maf1 might contribute to a defence response (Fig. 2a). However, Maf1 depletion by RNAi had no effect on *Botrytis* infection or lesion development under standard test conditions (Fig. 2b). Although these experiments provide no evidence that Maf1 influences the response of *Arabidopsis* leaves to attack by a specific fungal species, we cannot exclude effects under field conditions nor a role in defense against other pathogens.

3.3. Maf1 RNAi plants tolerate abiotic stresses

A principal function of Maf1 in yeast is to inhibit tRNA production under conditions of stress. A similar function in *Arabidopsis* is suggested by the induction of Maf1 expression in response to water deprivation, a major physiological stress in plants (Fig. 3a). We therefore tested if Maf1 depletion might compromise the ability of plants to withstand stressful conditions. However, the RNAi lines showed no apparent disadvantage during 10 days without water (Fig. 3b-d), maintaining longer leaves (p < 1e-06) and attaining greater aerial mass (p < 0.001).

As soil salinity can be one of the most important environmental stresses for plants, we also investigated the influence of Maf1 under high salt conditions. Addition of 75 mM NaCl to the water used for irrigation led to a ~ 40% increase in expression of Maf1 mRNA, relative to actin mRNA (Fig. 4a). As for plants grown under optimal or drought conditions, Maf1 depletion by RNAi allowed significantly enhanced leaf growth in the presence of 75 mM NaCl (Fig. 4b; p = 0.0068). As a consequence, the leaves of RNAi plants were longer (Fig. 4c) and heavier (Fig. 4d) than those of wild-type after 8 days growth in 75 mM NaCl (p < 0.01). This size difference was maintained during 16 days exposure to 75 mM NaCl, but was lost when the salt concentration was doubled. Nevertheless, the RNAi plants were not significantly more sensitive than wild-type to 150 mM NaCl (Fig. 5), showing that Maf1 depletion has not weakened tolerance of these harsh conditions.

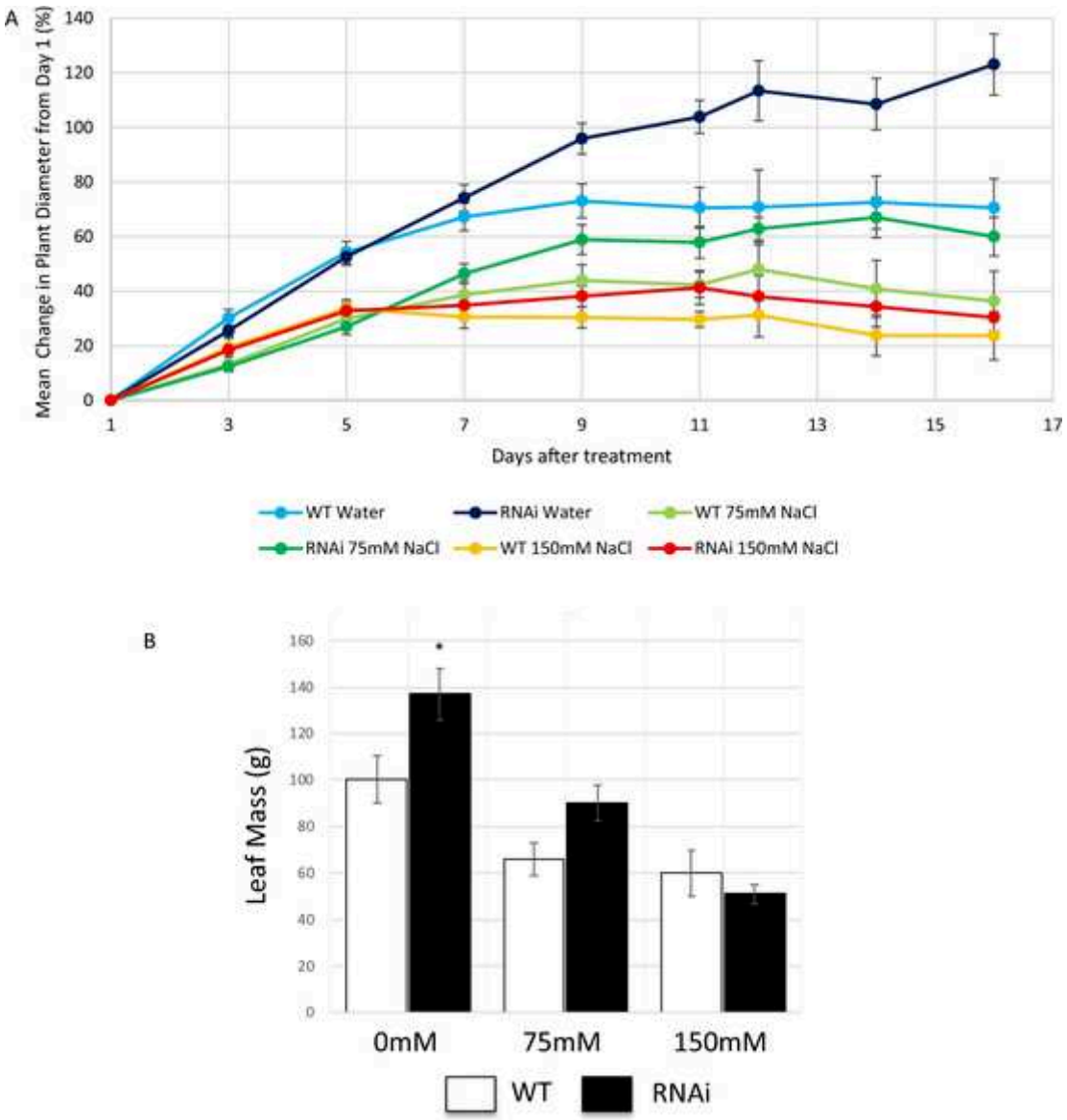

Fig. 5. Effects of Maf1 RNAi on plant growth under prolonged salt stress. (**A**) Mean change in relative diameter of wild-type and RNAi plants maintained for 16 days with tap water alone or supplemented with 75 mM NaCl or 150 mM NaCl, as indicated. (**B**) Relative mass of aerial parts of ten wild-type and RNAi plants after 14 days growth in 0 mM, 75 mM or 150 mM NaCl, as indicated ($P < 0.05$).

3.4. Maf1 mutant lines recapitulate properties of Maf1 RNAi lines

If the phenotype of these RNAi plants reflects depletion of Maf1, rather than off-target effects, it should be reproduced by disruption of the Maf1 gene. To test this, lines were obtained from the Arabidopsis Biological Research Center with T-DNA insertions in the Maf1 locus that reduce its expression by > 5-fold (Supplementary Fig. S2). As with Maf1 RNAi plants, the insertion mutant lines expressed elevated levels of $tRNA^{Thr}$, $tRNA^{Pro}$ and $tRNA_i^{Met}$, relative to wild-type plants grown and harvested in parallel (Fig. 6a). Furthermore, both mutant lines had significantly longer leaves (Fig. 6b and 6c; p = 1.58e-04) and greater aerial weight (Fig. 6d; p = 0.009) than wild-type. Maf1 disruption also enhanced leaf growth under conditions of water deprivation (Fig. 7a and 7b; p = 0.028), although differences in aerial weights were less consistent at the time of harvesting (Fig. 7c; p = 0.15, 0.026 and 0.48 for RNAi, IM1 and IM2, respectively, compared to wildtype). Under 75 mM salt stress, the Maf1 mutants again grew significantly larger than wild-type, in terms of leaf length (Fig. 8a and 8b ; p = 4.44e-09) and aerial mass (Fig. 8c; p = 0.04 for IM1 and 0.03 for IM2). We conclude that enhanced leaf growth under benign and stressful conditions is a recurrent feature of plant lines in which Maf1 expression is compromised by RNAi or insertional mutagenesis.

4. Discussion

Our data support previous studies demonstrating that transcription by pol III in plants is restrained by Maf1, a regulator that is well-conserved through evolution (Ahn et al., 2019; Soprano et al., 2018; Soprano et al., 2013; Soprano et al., 2017). In addition, we found that Maf1 depletion can enhance growth of *Arabidopsis* cultured in soil under greenhouse conditions. Substantial variation between individual plants can obscure this effect on size, but its significance becomes apparent when populations are considered.

Our results contrast with those of Ahn et al., who found that *Arabidopsis* lines carrying T-DNA insertions in the Maf1 gene showed no significant growth differences in liquid culture, despite elevated expression of 5S rRNA and several tRNAs (Ahn et al., 2019). A key experimental difference between those previous studies and our own is that the

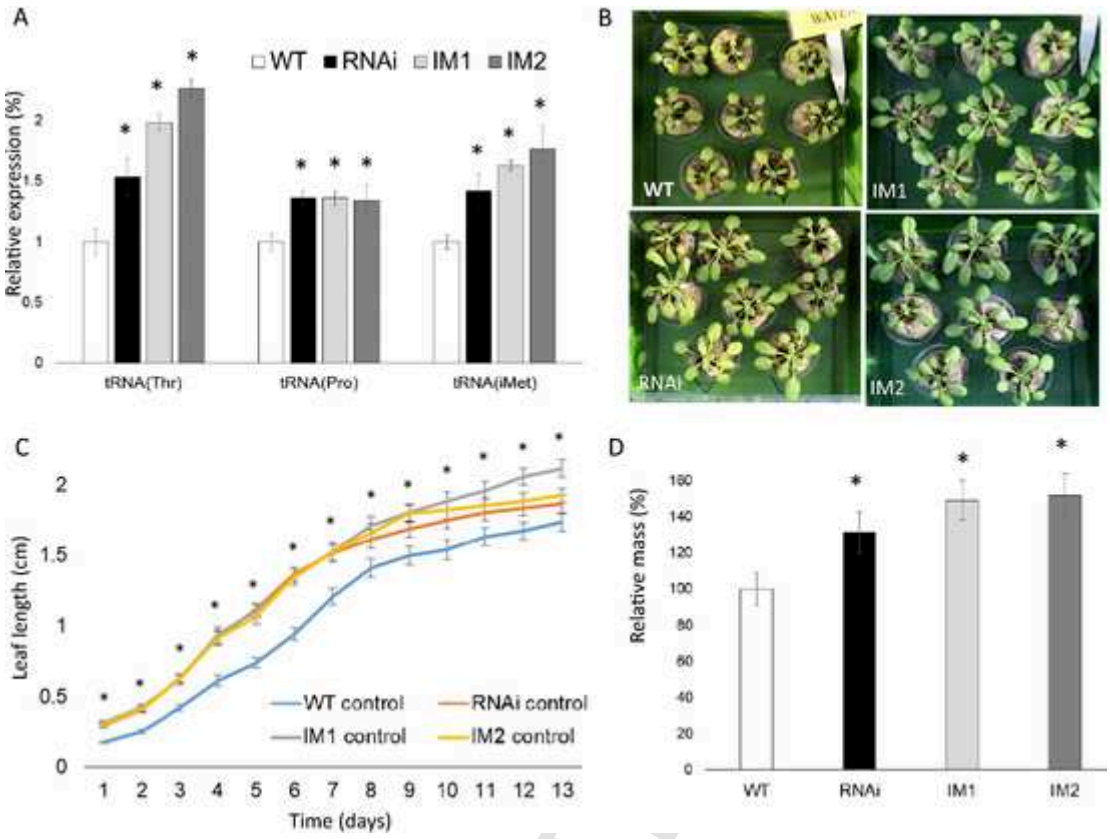

Fig. 6. Effects of Maf1 RNAi and insertion mutations on expression of tRNAs and growth of leaves. (**A**) Relative expression of indicated tRNAs in aerial parts of wild-type, two insertion mutant (IM) and RNAi lines cultivated under benign conditions. Asterisks: $tRNA^{Thr}$, P = 2.74e-08; $tRNA^{Pro}$, P = 0.03; $tRNA_i^{Met}$, P = 1.59e-06. (**B**) Representative images of young wild-type, insertion mutant (IM) and RNAi plants under benign conditions. (**C**) Lengths of leaves of of wild-type, IM and RNAi plants over 13 consecutive days. Asterisks P < 0.005. (**D**) Relative mass of aerial parts of wild-type (100%), IM and RNAi plants. P = 0.009.

former were conducted using seedlings grown in liquid media, whereas all our experiments used older plants grown in soil. It remains to be determined how these cultivation conditions might influence the physiological effects of Maf1, but soil growth represents a more natural environment.

An effect of Maf1 on plant growth is consistent with its direct control by TOR (Ahn et al., 2019; Andrade et al., 2020; Soprano et al., 2017), as in yeast and mammals (Kantidakis et al., 2010; Michels et al., 2010; Shor et al., 2010; Wei et al., 2009), despite major differences in this signaling pathway (Soprano et al., 2018). In *Arabidopsis,* dephosphorylation of Maf1 follows exposure to TOR inhibitors or silencing of the TOR gene and this is accompanied by reduced tRNA expression (Ahn et al., 2019). Four residues in human Maf1 are targeted by TOR to control pol III (Hsu et al., 2011; Kantidakis et al., 2010; Michels et al., 2010; Shor et al., 2010) and these sites are conserved in *Arabidopsis* and *Citrus* Maf1, despite overall conservation of only 38% with the human protein; this conservation does not reflect structural constraints, as the residues cluster in a variable region (Soprano et al., 2017). At least one of these sites in citrus Maf1 can be phosphorylated by recombinant human TOR in vitro (Soprano et al., 2017). The physiological significance of this is indicated by findings that cell hypertrophy and hyperplasia associated with canker development in citrus leaves can be suppressed by a TOR inhibitor, but the affect is attenuated by RNAi of Maf1 (Soprano et al., 2017). *Arabidopsis* TOR responds to photosynthesis-driven glucose availability to activate comprehensive growth programmes (Xiong et al., 2013). As in all systems analysed, TOR then controls protein synthesis, which underlies cell growth (Dobrenei et al., 2016; Wullschleger et al., 2006). Inactivation of TOR in *Arabidopsis* reduces protein production and content (Deprost et al., 2007). Amongst genes induced by TOR, the largest category encodes components of the translational machinery, such as ribosomal proteins (Xiong et al., 2013). Induction of tRNA by switching off its repressor Maf1 might complement this protein synthetic programme,

As in other systems, plant TOR promotes growth when sufficient nutrients are available and environmental circumstances are optimal, impacting overall yield (Dobrenei et al., 2016). Adverse conditions, such as low temperature, trigger TOR inactivation through the stress-induced kinase SnRK2, which responds to the phytohormone abscisic acid (Rosenberger and Chen, 2018). This mechanism allows plants to balance their resources between growth and defense. As Maf1 is an effector of TOR, we had expected that Maf1 RNAi or mutant lines might lose resilience under adversity, being less able to conserve energy by reducing tRNA production. Indeed, Maf1 is required in budding yeast for pol III tRNA repression in response to every stress tested and its absence results in reduced fitness and stress sensitivity (Upadhya et al., 2002). However, phenotypes observed upon Maf1 knockout vary markedly between organisms, despite the conserved molecular function of pol III repression (Willis, 2018). For example, deletion of the Maf1 gene increases stress tolerance in worms (Cai and Wei, 2016). Contrary to expectations, we found that Maf1 depletion did not compromise the ability of *Arabidopsis* to withstand two environmental stress conditions that are of key importance to plants – water deprivation and elevated salt. Future work might investigate if redundant defense mechanisms exist that mitigate loss of Maf1 under these circumstances. Such protection may prove insufficient under harsher challenge. Thus, Maf1 mutant *Arabidopsis* display reduced tolerance to stress induced by hydrogen peroxide or the DNA-damaging agents cisplatin and methyl methanesulfonate (Ahn et al., 2019); the physiological significance of this is unclear.

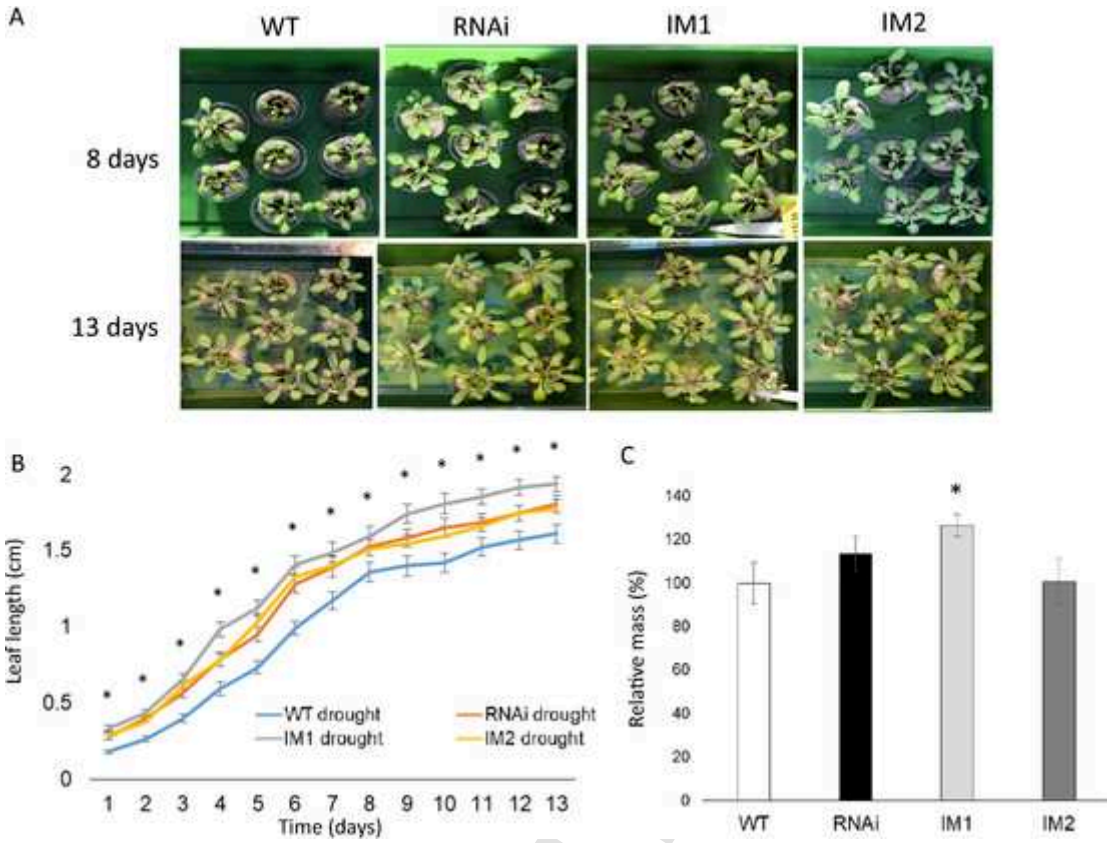

Fig. 7. Effects of Maf1 RNAi and insertion mutations on leaf growth during drought. (**A**) Representative images of wild-type, insertion mutant (IM) and RNAi plants after 8 and 13 days without being watered. (**B**) Lengths of leaves of of wild-type, IM and RNAi plants over 13 consecutive days without water. Asterisks $P < 0.05$. (**C**) Relative mass of aerial parts of wild-type (100%), IM and RNAi plants after 13 days without water ($P = 0.15$ for RNAi, $P = 0.0263$ for IM1 and $P = 0.48$ for IM2).

Strong evidence exists that Maf1 depletion can increase vulnerability of sweet orange plants to a natural biotic stress (Soprano et al., 2018). Thus, Maf1 RNAi resulted in dramatic increases in the size and number of hyperplastic canker lesions following infection of *Citrus sinensis* with the bacterial pathogen *Xanthomonas citri* (Soprano et al., 2013). Indeed, the first example of Maf1 identified in plants was isolated in a screen for targets of TAL virulence factors (Soprano et al., 2013). *Xanthomonas* TAL was shown to bind citrus Maf1 directly and antagonize its ability to repress tRNA expression (de Souza et al., 2012; Soprano et al., 2013). Furthermore, the hypertrophic and hyperplastic growth observed in canker lesions correlates with decreased Maf1 levels (Soprano et al., 2013), consistent with the model that Maf1 can influence growth in plants. In contrast to this protective role against canker, we found no evidence that Maf1 depletion affects sensitivity of *Arabidopsis* under laboratory conditions to attack by the necrotrophic fungus *Botrytis cinerea*. However, considering the wide variety of molecular mechanisms employed by plant pathogens, it seems likely that other situations will be found in which Maf1 is targeted, given its high level of functional conservation and relevance to growth.

Plant breeding to improve yield has in many cases reduced the resistance of modern crops to abiotic stress (Koziol et al., 2012). In contrast, Maf1 depletion allows enhanced growth that is not accompanied by increased sensitivity to drought or salinity, two key challenges to crop productivity. It may be worth considering if manipulation of Maf1, through targeted breeding or genetic engineering, might confer advantages to plant species of nutritional and/or economic value. Consideration of such an approach, however, would need to address the strong possibility that growth advantages might come at the price of increased sensitivity to environmental stresses, such as pathogenic attack.

CRediT authorship contribution statement

Joseph Blayney: Investigation, Validation, Formal analysis. **James Geary:** Investigation, Validation, Formal analysis. **Ruby Chrisp:** Investigation, Formal analysis. **Joseph Violet:** Investigation, Formal analysis. **Liam Barratt:** Investigation, Formal analysis. **Laçin Tavukçu:** Investigation, Formal analysis. **Katherine Paine:** Investigation, Formal analysis. **Fabián E. Vaistij:** Investigation, Formal analysis, Resources. **Ian A. Graham:** Conceptualization. **Katherine J. Denby:** Resources, Conceptualization. **Robert J. White:** Conceptualization, Supervision, Project administration, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

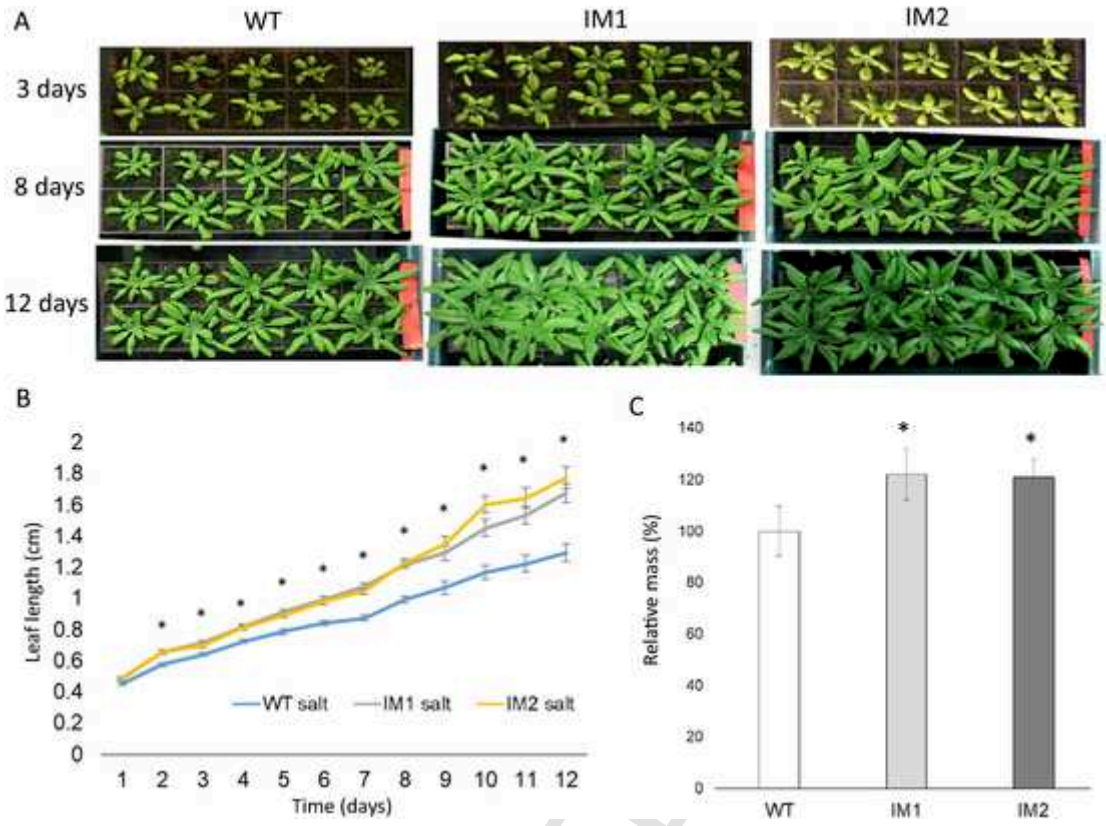

Fig. 8. Effects of Maf1 insertion mutations on leaf growth during salt stress. (**A**) Representative images of wild-type (WT) and insertion mutant (IM) plants after growth for 3, 8 and 12 days in the presence of 75 mM NaCl. (**B**) Lengths of leaves of WT and IM plants over 12 consecutive days exposure to 75 mM NaCl. Asterisks $P < 0.005$. (**C**) Relative mass of aerial parts of wild-type (100%) and IM plants after 12 consecutive days exposure to 75 mM NaCl ($P = 0.04$ for IM1 and $P = 0.03$ for IM2).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.146130.

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