Molecular Responses of Arabidopsis MET1 Cytosine Methyltransferase Mutants to Salinity

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Abstract

In this study, we investigated the morphological and molecular responses of *Arabidopsis met1-7* and *met1-3* null mutants under salinity stress. In this context, global DNA methylation changes of mutants exposed to salt stress were compared and expressions of *DRM2*, *Pol IV* and *Pol V* genes known to be involved in DNA methylation in plants were analyzed. We found that *met1-7* and *met1-3* mutants have a higher rate of hypomethylation than Col-0 under all conditions. According to the results of gene expression analysis, the increase in expression of *DRM2*, *Pol IV* and *Pol V* genes involved in CNN methylation in mutants than Col-0 plant suggests that hypomethylation is directly related to CG regions, but in *met1* mutants, the lack of CG methylation is tried to be compensated by RdDM. In addition, we analyzed the expression of the *TERT* gene as a stress response indicator in order to examine the effect of salt stress on the telomerase enzyme in *met1-7* and *met1-3* mutants. Contrary to expected, we found that there was an increase in the expression of *TERT* gene in the salt stress applied plants. Within the scope of all the data, it is thought that in *met1* mutants RdDM pathway is activated in order to deal with the lack of DNA methylation in CG islands. As a conclusion, it is believed that the morphological and molecular data obtained on the effects of NaCl application to *met1* mutants will help us to understand the epigenetic basis of stress mechanisms.

Introduction

Plants have a major impact on ecosystem balance. They have important roles such as being food sources for animals and humans. However, it has become difficult to provide the required amount of food due to the increasing population and the decrease in agricultural areas as a result of salinity and drought stress. The first step to find solutions to these problems is to understand the relevant pathways very well in model organisms such as *Arabidopsis thaliana*.

After salt exposure molecular mechanisms are activated in plants in order to tolerate stress conditions. Epigenetic modifications, one of these molecular mechanisms, affect the stability of the genome by changing the covalent bonds between DNA and protein without changing the DNA structure. Studies have shown that epigenetic modifications can mediate stress tolerance memory during repeated exposure to certain environmental stresses. Some genetic changes that can occur in plants' reproductive cells throughout their lifetimes can then be passed on to subsequent generations. For this reason, plant systems are a rich resource for the study of epigenetic inheritance (Molinier et al. 2006; Henderson and Jacobsen 2007; Kinoshita and Seki 2014).

DNA methylation, which is one of the most common epigenetic modifications in *A. thaliana*, accounts for about 30% of its genome (Haag and Pikaard 2011; Sahu et al. 2013). Cytosine methylation in plants occurs in three types: CG, CNG as symmetric and CNN as asymmetric (where N represents A, T, or C) (Kong et al. 2018). There are 3 enzymes mainly involved in the methylation of cytosine in plants. The first enzyme is methyltransferase 1 (MET1), which is responsible for methylation of CG sequences and a homologue of the mammalian DNMT1. The second enzyme responsible for methylation of CNG and a small amount of CNN sequence is chromomethylase 3 (CMT3). This plant-specific enzyme is largely deposited in TE's and induces ectopic methylation of protein-coding genes. It is also thought to depend on direct physical binding to H3K9me2 to target chromatin and methylation. The third enzyme, domains rearranged methylase (DRM), which is a

homologue of DNMT3 in mammals, is mainly responsible for maintaining asymmetric CNN methylation with siRNAs and plays a minor role in the maintenance of CNG methylation. Also it is required for *de novo* methylation of cytosines in all sequence contexts (Ashapkin et al. 2002; Henderson and Jacobsen 2007; Johnson et al. 2007; Law and Jacobsen 2010; Wendte et al. 2019; Papareddy et al. 2021). However, plants have developed a unique *de novo* DNA methylation pathway called RNA-directed DNA methylation (Quadrana and Colot 2016; Brocklehurst et al. 2018). Plant specific Pol IV and Pol V derived from Polymerase II (Pol II) play roles in RNA-directed DNA methylation (Chinnusamy and Zhu 2009; Zhang and Zhu 2011). Pol IV is responsible for the biosynthesis of siRNAs, while Pol V is responsible for targeting the siRNAs to loci affected by DNA methylation (Haag and Pikaard 2011).

Telomeres are known to be very sensitive to changing environmental conditions plants are exposed. Studies have shown that telomeres shorten under increasing stress conditions (Von Zglinicki 2002; Chan and Blackburn 2004; Houben et al. 2008). In other words, defects in the expression of *TERT* gene and as well as methylation can cause developmental anomalies (D'Amico-Willman et al. 2021). Moreover, it has been observed that after a certain period of time, when stress conditions disappear, telomeres lengthen again. Hence, the search for *TERT* expression has the potential to be an indicative biomarker.

Studies have been conducted using the *met1-7* and/or *met1-3* mutants, but the molecular changes that occur as a result of salinity stress have not been studied (Jullien et al. 2006; Pontvianne et al. 2013; Rigal et al. 2016; Blevins et al. 2017). Arkan et al. (2018) stated in their study that salt stress causes hypomethylation in plants and this hypomethylation is not only related to the *MET1* gene. Besides this, DRM2-related *de novo* methylation is also affected. Considering these studies, molecular mechanisms must be understood well in order to make plants resistant to the stress conditions they encounter and maintain this resistance for generations. Consequently, it was decided to examine the effect of salt on *met1-7* and *met1-3* mutants to understand the role of the *MET1* gene under salt stress.

In this study, to understand the relationship between the hypomethylation caused by NaCl stress and the *MET1* gene, *A. thaliana* Col-0 plants and null mutants of *met1-7* and *met1-3* were exposed to salt stress. To this extent, methylation level of the genome and the expressions of *DRM2*, *Pol IV* and *Pol V* genes, which are known to be involved in the RdDM mechanism were analyzed. Also the expression of the *TERT* gene was analyzed in order to examine the effects of salt stress on the telomeres of *met1* mutants.

Material And Methods

Plant material and growth conditions

A. thaliana Col-0 (Wild type), *met1-7* and *met1-3* seeds were kindly provided from Dr. Ralf Stracke (Bielefeld University, Center for Biotechnology), Dr. Binglian Zheng (Fudan University, Department of Biochemistry and Molecular Biology) and Dr. Hidetoshi Saze (Plant Epigenetics Unit, Okinawa Institute of Science and Technology), respectively. *met1-7* and *met1-3* lines have been described previously and backgrounds of these two mutants are based on the Col-0 ecotype (Saze et al. 2003; Li et al. 2017).

The Col-0, *met1-7*, and *met1-3* seeds were surface sterilized and placed on Murashige and Skoog basal medium (MS) (Murashige and Skoog 1962) which was supplemented with sucrose (3%, w/v) and pH was adjusted to

5.8. Agar (0.9%, w/v) was added for solidifying and the medium was sterilized by autoclaving at 121°C under 1 atm pressure for 15 minutes. Col-0, *met1-7* and *met1-3* seeds were germinated under fluorescent light in a plant growth chamber [16 hours light / 8 hours dark conditions, 1400 lux (Sanyo, MLR-352H)] at 25°C like as Arkan et al. (2018).

Bioinformatic analysis on MET1 enzyme

Self-fertilization and seeding studies performed in our laboratory gave positive results in *met1-7*, but were inconclusive in *met1-3* similar with Saze et al. (2003). The fact that the two *met1* mutants morphologically different significantly from each other and after observing only *met1-7* mutant seed yields, the regions where the T-DNA insert entered were examined in more detail to investigate the answer that may have caused this difference.

The bacterial Ti-plasmid (T-DNA) inserts disrupt the conserved regions by entering the beginning of the 2nd exon in the *met1-7* mutants and at the beginning of the 7th exon in the *met1-3* mutants (TAIR 2021a, 2021b). The locations of the T-DNA inserts in the mutants are shown in Fig. 1.

The amino acid sequences of the *A. thaliana* MET1 enzyme (NP_199727.1) were obtained from the NCBI site (<https://www.ncbi.nlm.nih.gov/protein/>) and the catalytic sites of the enzyme were obtained using the 'Conserved Domain Database, CDD' program on NCBI (<https://www.ncbi.nlm.nih.gov/cdd/>). The exon sequences disrupt by the T-DNA regions were converted into amino acid sequences with the 'Translated BLAST: blastx' program (<https://blast.ncbi.nlm.nih.gov>) and the projections of the MET1 enzyme on the amino acid sequences were determined by the BLASTp program (<https://blast.ncbi.nlm.nih.gov>). Then, the regions were colored using the ChimeraX (version 1.1) program (<https://www.cgl.ucsf.edu/chimerax/>) for reevaluating the importance of T-DNA insertion sites on enzyme activity (Pettersen et al. 2021).

Stress treatment and morphological analysis

Seven days old *A. thaliana* Col-0, *met1-7* and *met1-3* plants germinated on MS medium were transferred to MS medium containing 0, 100 and 150 mM NaCl for stress application. Col-0, *met1-7* and *met1-3* plants were incubated 7 days under fluorescent light in a plant growth chamber [16 hours light / 8 hours dark conditions, 1400 lux (Sanyo, MLR-352H)] at 25°C.

The number of leaves, leaf surface area and root length of Arabidopsis *met1-7* and *met1-3* plants were determined and later compared morphologically with the control group. Leaf surface areas of *A. thaliana* plants were calculated according to the equation used by Carus and Çatal (2005). After the morphological analysis, the collected plant samples were kept at -80°C until the usage in molecular analysis.

DNA isolation and global DNA methylation (5-mC) level analysis

~100 mg plant sample was harvested from each group treated with NaCl stress and DNA isolation was carried out with a GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific, K0792) according to the manufacturer's protocol. Global methylation changes (%) in genomic DNA were detected by MethyFlash Global DNA Methylation (5-mC) ELISA Easy Kit (EpiGentek, p-1030) according to the manufacturer's protocol. The 5-mC in the DNA of all biological samples were reported as the amount of methylated cytosine relative to the genomic content (percentage) of cytosine (Tellez-Plaza et al. 2014).

RNA isolation and qPCR analysis

For qPCR analysis, total RNA was isolated with Hibrizol (Hibrigen, Turkey) from *A. thaliana* Col-0, *met1-7*, and *met1-3* plants exposed to NaCl stress. Then, the RNAs were checked for their integrity, purity and quantity. Their purities and quantities were checked by NanoDrop 2000 (Thermo Scientific) and their integrities were analyzed by agarose gel electrophoresis (%1 TAE). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific, 4368814) according to the manufacturer's recommended protocol.

qPCR amplification was performed with a Roche LightCycler Nano instrument by using 2X SYBR Green Master mix (Hibrigen, 0220-UB-775). The following PCR protocol was applied: 50°C/30 s, followed by 40 cycles of 94°C/30 s, 59°C/61°C/65°C for 30 s (59°C for *TERT*, 61°C for *Actin*, *Pol IV*, *Pol V* and 65°C for *DRM2* respectively) and 72°C for 30 s. qPCR reactions were carried out with 2 technical replicates for each 3 biological replicates. The fold changes were calculated using $2^{-\Delta\Delta C_t}$ values, and relative expressions were shown as log2 fold changes (Livak and Schmittgen 2001). The primer sequences are given in Table 1. *TERT* gene (AT5G16850) primer pair was designed using the Primer3 program (<http://primer3.ut.ee/>).

Table1 The primer sequences used in gene expression analysis

Gene	Primers	References
DRM2	F: 5'-AACAAAGCTGCCCCACTCG-3'	Naydenov et al., 2015
	R: 5'-ATACGCCACAGTATCAACCTG-3'	
Pol IV	F: 5'-GAAACGCATTCTCCACAGTTAC-3'	Naydenov et al., 2015
	R: 5'-GCGACCCGGATTCTTTTGAC-3'	
Pol V	F: 5'-CATCCGTCTGCGTACCCTG-3'	Naydenov et al., 2015
	R: 5'-TCAACCGTGATGAAGTCAACG-3'	
TERT	F: 5'-AATCTCCCGCCTCTTTCACA-3'	
	R: 5'-GTTTACGCGGCATTACACCT-3'	
Actin 8	F: 5'-GCCAGTGGTCGTACAACCG-3'	Sáenz-Mata et al., 2012
	R: 5'-TCATGAGGTAATCAGTAAGGTCAC-3'	

Statistical analysis

Morphological experiments with *A. thaliana met1-7* and *met1-3* plants, evaluation of the results obtained with the "MethylFlash™ Methylated DNA Quantification" kit, and gene expression analysis were statistically performed using the GraphPad Prism® 7.0 software using two-way ANOVA with post-hoc Tukey's test. Results with $p<0.05$ were considered significant. Morphological experiments were performed on at least 30 plants for each replicate (in total 3 biological replicates), and methylation level analysis as well as gene expression analysis were performed with 3 biological and 2 technical replicates.

Results

Morphological effects of stress application

The morphologies of the *met1-7*, *met1-3* null mutants and the control plants after 7 days exposure to NaCl stress are given in Fig. 2. As a result of the morphological analysis, leaf number for different NaCl concentrations in the control group Col-0 and *met1-7*, *met1-3* null mutants did not change significantly between the groups. But the number of leaves was significantly reduced within all plant groups except in the *met1-3* mutants between the 100 and 150 mM NaCl concentrations.

It was observed that the leaf surface areas decreased significantly between control and 150 mM NaCl concentrations in the Col-0 plants. Also, it was observed that the leaf surface areas of *met1-3* mutants were affected more than the *met1-7* mutants after 150 mM NaCl application.

And finally, it was observed that the root length decreased significantly except between the control group and the 100 mM NaCl application in both mutants. Compared to Col-0, *met1-3* was more sensitive to salt stress than *met1-7* plant and root length was significantly shortened in all groups. The root length was significantly shortened between the control group of *met1-7* and the Col-0, also between each NaCl concentrations applied to *met1-3* and Col-0. Lastly, the root length was decreased between both mutants for each concentration. The graphs of the morphological analysis are given in Fig. 3, and in addition to the graphical data, average values and standard errors are also given in Table 2.

Table2 Determination of the morphological effects of different NaCl concentrations in Col-0, *met1-7* and *met1-3* plants. Each value represents the average of repetitions and the standard error ($\pm$ SH)

Col-0	NaCl (mM)	Leaf Number	Leaf Surface Area (cm ²)	Root Length (cm)
	0	9.047±0.216	0.405±0.032	4.097±0.169
	100	7.195±0.235	0.346±0.029	2.984±0.141
	150	5.787±0.215	0.269±0.020	1.560±0.090
met1-7	NaCl (mM)	Leaf Number	Leaf Surface Area (cm ²)	Root Length (cm)
	0	8.455±0.261	0.409±0.033	2.922±0.152
	100	6.936±0.238	0.328±0.026	2.800±0.124
	150	5.605±0.265	0.292±0.034	2.023±0.120
met1-3	NaCl (mM)	Leaf Number	Leaf Surface Area (cm ²)	Root Length (cm)
	0	8.313±0.362	0.266±0.023	2.124±0.144
	100	6.682±0.297	0.234±0.031	1.600±0.197
	150	5.500±0.336	0.137±0.020	0.640±0.092

T-DNA insertion region in 3D structure of MET1 enzyme

The analysis with ChimeraX Program (version1.1) showed that the region disrupted by the T-DNA insert in the *met1-7* mutant does not coincide with any of the 4 active regions of the MET1 protein (NP_199727.1) consisting of 1534 amino acids whereas the T-DNA insert of the *met1-3* mutant disrupts the active site where S-adenosylmethionine (SAME) binds to the methyltransferase1 protein (Fig. 4).

Global DNA methylation

As a result of the methylation analysis, mutants were found to be less methylated than Col-0 in each NaCl concentration. In control conditions, *met1-3* mutants had less methylation compared to *met1-7*, but there was no significant change in *met1-3* between applied NaCl concentrations, while hypomethylation occurred between control and 100 mM in *met1-7*. In addition, it was observed that between 100 and 150 mM NaCl treatments methylation increment has occurred. These findings could indicate that the treatment with 150 mM NaCl could have activated additional mechanisms in the *met1-7* mutant other than *met1-3*. However, when considering the entirely, mutants were found to be less methylated than Col-0 at each NaCl concentration. The results of methylation analysis are given in Fig. 5, and the percentages of methylation levels and standard errors are given in Table 3.

Table3 Determination of the global DNA methylation percentages and standard errors of Col-0, *met1-7* and *met1-3* plants after different concentrations of NaCl treatment

NaCl (mM)	Col-0	<i>met1-7</i>	<i>met1-3</i>
0	4.726±0.296	4.176±0.146	2.452±0.032
100	3.752±0.068	1.979±0.116	2.700±0.027
150	4.888±0.541	3.936±0.114	2.994±0.281

Expression of *DRM2*, *Pol IV*, *Pol V* and *TERT* genes

As a result of the analysis, expression of the *DRM2* gene changed by 0.4 fold in *A. thaliana* Col-0 plant after 100 mM NaCl application compared to control, and increased by 1.4 and 1.2 fold in *met1-7* and *met1-3*, respectively. And there was no change in Col-0 after 150 mM NaCl application, while it was increased by 1.65 and 1.45 fold in *met1-7* and *met1-3*, respectively. In the expression change of *Pol IV* gene, it was determined that 100 mM NaCl application caused an increase in *met1-7* and *met1-3* groups 1.37 and 2 fold respectively, while 150 mM NaCl application caused an increase of 1.7, 2.73 and 1.95 fold at Col-0, *met1-7* and *met1-3* plants, respectively. The expression of *Pol V* gene after 100 mM NaCl application caused an increase of 1.3, 2.1 and 1.55 fold in Col-0, *met1-7* and *met1-3* groups respectively. After 150 mM NaCl application, the expression of *Pol V* gene in Col-0 plant was changed by 0.7 fold, while in mutants it was increased by 2.85 and 1.8 fold, respectively. Lastly, the expression of *TERT* gene in Col-0 after 100 mM NaCl application changed by 0.6 fold, while in *met1-7* and *met1-3* mutants it was increased by 1.7 and 1.5 fold, respectively. After 150 mM NaCl application, the expression of *TERT* gene in Col-0 plant was changed by 0.7 fold, while in *met1-7* and *met1-3* mutants it was increased by 1.6 and 1.2 fold, respectively. As a result of the two-way ANOVA with post-hoc Tukey test, there was a statistically significant increment of *TERT* gene expression between Col-0 and *met1* mutants at 100 mM NaCl application. Statistical significance was not determined in the expression analysis among other genes. The graphical representation of the gene expression analysis is given in Fig. 6 and relative fold changes are given in Table 4.

Table 4
Relative expression fold change values of *DRM2*, *Pol IV*, *Pol V* and *TERT* genes.

<i>DRM2</i>				<i>Pol IV</i>			<i>Pol V</i>			<i>TERT</i>		
NaCl (mM)	Col-0	met1-7	met1-3	Col-0	met1-7	met1-3	Col-0	met1-7	met1-3	Col-0	met1-7	met1-3
0	1	1	1	1	1	1	1	1	1	1	1	1
100	0.4	1.4	1.2	1	1.37	2	1.3	2.1	1.55	0.6	1.7	1.5
150	0.95	1.65	1.45	1.7	2.73	1.95	0.7	2.85	1.8	0.7	1.6	1.2

Discussion

Salt stress that plants encounter in nature is usually caused by NaCl (Ngara and Ndimba 2014). High NaCl concentrations in soils cause ionic stress by disrupting the ionic balance of Na⁺, Cl⁻, K⁺ and Ca²⁺ together with

osmotic stress. Excessive amounts of Na⁺ ions entering the stem cells are carried to other leaves, tissues and organs and tried to be tolerated. This situation causes morphological changes in plants (Yang and Guo 2018; Liu et al. 2019). Considering all this information, in this study leaf number, leaf surface area and root length of epigenetic mutant plants treated with 100 and 150 mM NaCl during 7 days were examined. Similar to our morphological results, Arıkan et al. (2018) reported that the application of 100 and 150 mM NaCl to *Arabidopsis* Col-0 plants restricted root and shoot growth. Also, Baek et al. (2011) stated that the *met1-3* mutant was hypersensitive to NaCl and they observed a salt-sensitive phenotype in root growth, and this situation could be attributed to the loss of methylation in the putative small RNA target region in the *AtHKT1* promoter. In a previous study, it was stated that after 75 and 150 mM NaCl application, the *met1-3* mutants were sensitive to salt stress, although not at the same degree as the *ddm1* mutants (Yao et al. 2012). Moreover, Huang et al. (2013) stated that the epigenetic mutants (*ros1* and *rdm16ros1*) of *Arabidopsis* plants were adversely affected morphologically with 75, 100 and 125 mM NaCl application at seedling stage. Similar to all this research, we have found that the *met1-3* mutant is more sensitive to salt stress than the *met1-7* mutant, morphologically. In many studies, this morphological sensitivity observed in epigenetic mutants compared to Col-0 plants supports the importance of epigenetic mechanisms in plant development.

In addition to the analysis of mutants' responses to salt stress, seed productivity was also analyzed. In order to obtain homozygous mutant seeds, seeds were tried to be obtained by self-fertilization method. Seeds could be obtained from the *met1-7* mutant, while they could not be obtained from the *met1-3* mutant. This has led us to think that in the *met1-3* plant, unlike *met1-7*, the insertion of the T-DNA insert at the beginning of the 7th exon may cause some defects in the alternative arrangements of RNA transcripts, and this may disrupt the functioning of some pathways involved in gametophyte formation. In previous studies, it was stated that the seeds of the *met1-3* mutant were smaller than normal and the plants in the first generation were sterile (FitzGerald et al. 2008). However, the cause of infertility is not specified. We attribute the reason for this problem, which we encountered with this study, that the T-DNA insert entering the *met1-3* mutant exactly coincides with the active region of the MET1 protein. Bioinformatic analysis showed that the T-DNA insertion in *met1-3* mutant most probably disrupts the active site where S-adenosylmethionine (SAME) binds to the methyltransferase1 protein. It is thought that the disrupted region in the *met1-7* mutant may not cause any impairment in its function, although it disrupts the 3D structure of the protein. It is thought that MET1 protein, which is known to play an active role in embryogenesis, causes infertility due to its inability to function properly.

Stress responses in plants cause changes in methylation in the coding region of genes involved and regulate gene expression (Sudan et al. 2018). For this reason, detecting epigenetic changes in the plant genome shows great importance in the understanding of stress responses of plants. DNA methylation is mostly observed on cytosine bases. The main enzymes responsible for cytosine methylation in plants are MET1, CMT3 and DRM2. The MET1 enzyme, which has a great role in these regulations, is responsible for 80-90% of the cytosine-level methylation in plants (Zangi et al. 2020). In this study, we used the null mutants *met1-7* and *met1-3*, in which the functional MET1 enzyme of *A. thaliana* plant is not synthesized. The methylation changes of these *met1* mutants were investigated and the 5-mC content at the genome level was determined. Likewise, Arıkan et al. (2018), the application of 100 and 150 mM NaCl during 7 days to *A. thaliana* Col-0 plant caused hypomethylation at the genome level. Boyko et al. (2010) reported in their study that unlike Arıkan et al. (2018), they encountered hypermethylation in the progeny of *Arabidopsis* plants exposed to salt stress. Moreover, Zhong et al. (2009) indicated that salt stress caused hypermethylation in some regions of the genome even

though hypomethylation was found overall in the genome as a result of application of 100 and 150 mM NaCl during 5 days to bread wheat ("*Triticum aestivum* L. ").

As with many cellular signaling pathways, molecular responses to salt stress are expected to be influenced by negative regulation as well as positive regulation of the gene expression (Zhu 2000). In parallel with our data, Gao et al. (2020) stated that gene expression of *DMR2* decreased in *Ginkgo biloba* at 150 mM NaCl stress, but increased in chickpea roots. These results suggest that the inactivation of the *MET1* gene has an effect on hypomethylation, and in this case DRM2-related *de novo* methylation plays a more effective role as a compensator in *met1-7* and *met1-3* mutants. Also, expressions of *Pol IV* and *Pol V* genes were examined in order to evaluate the NaCl stress application in terms of RdDM in the absence of functional MET1 enzyme. Similar to our findings, Naydenov et al. (2015) observed that *Pol IV* and *Pol V* expression also increased under heat stress and stated that the expression of these genes could change not only through RdDM but also by acting in other regulatory processes.

DNA methylation has an important role in regulating promoter activity. Many studies have shown that promoter hypomethylation occurs mostly in the CG regions and is also dependent on the methyltransferases MET1 and DRM2. Accordingly, it was concluded that the *TERT* gene could be controlled by the MET1 system. Defects in DNA methylation are known to lead to developmental abnormalities. Zangi et al. (2020) suggested that mutations occurring in methylation systems may affect *TERT* gene expression, leading to developmental anomalies. In their study, they have shown that expression levels of the *TERT* gene increased nearly 14 fold in homozygous *met1* mutant plants compared to wild type. At the same time, *TERT* gene expression increased nearly 2 fold in heterozygous *met1* mutants compared to wild type. They have reached the conclusion that the *TERT* gene is regulated by methyltransferases and may be involved in developmental abnormalities caused by mutation in the MET1 methyltransferase system. Zangi et al. (2020) also suggested that the mutation involved in the MET1 methyltransferase systems decreased the methylation of CG islands in the promoter of the *TERT* gene and consequently increased the expression of the *TERT* gene. It is known that overexpression of telomerase leads to telomere elongation. Therefore, it is thought that overexpression of telomerase in plants with the *met1* mutation may be associated with limited growth and developmental abnormalities in Arabidopsis. It is possible that the effects and deficiencies of the *met1* mutation can be compensated by other methylation systems, thereby improving the phenotype (Zangi et al. 2020). Despite this, Ogrocká et al. (2012) reported that DNA methylation in the putative *TERT* promoter region is not a dominant factor in the regulation of *TERT* transcription. They analysed the telomerase activity in *met1-3* mutant seedlings, they have found no significant change in *TERT* transcription. Whereas, repeated analysis with *met1-3* mutants revealed significantly lower telomerase activity in young leaves, the amount of *TERT* transcript in young leaves of Col-0 samples and 7 day seedlings of *met1-3* mutants was similar. Our result is different from Zangi et al. (2020) but similar to Ogrocká et al. (2012). The expression of *TERT* gene is similar in Col-0 and *met1* mutants not treated with NaCl. However, there is an increase in *TERT* expression compared to Col-0 in NaCl treated mutants. According to these findings, it was concluded that to cope up with NaCl stress, *TERT* expression was upregulated due to the increasing RdDM in the mutants.

In conclusion, hypomethylation occurred at the genome level in mutants and they were left behind morphologically compared to Col-0 plants. It is consistent with the previous reports showing that the *MET1* gene negatively affects the morphological characteristics of plants under NaCl stress. The reason for the

increased expression of *DRM2*, *Pol IV*, *Pol V*, and *TERT* genes after NaCl application to *met1* mutants is thought to support the RdDM pathway to cope with DNA methylation deficiency in CG islands.

In order to make plants resistant to stress, molecular stress mechanisms must be understood. It is thought that the data obtained from this study will contribute to the basic knowledge to understand the effects of salt stress on epigenetic mechanisms.

Declarations

Author contributions

YVY and NTK designed the experiments. YVY and BA performed the experiments. All authors analyzed data, wrote and approved the manuscript. All authors contributed to the final version of the manuscript.

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Financial interests

The authors declare they have no financial interests.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate All authors agreed with participate in this study.

Consent for publication All authors agreed with the publication of this study. The submitted work contains original research that has not been published elsewhere.

Ethical approval This study does not contain any studies with human participants or animals performed by any of the authors.

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Figures

Figure 1

The locations of the T-DNA inserts in the *met1-7* and *met1-3* mutants (TAIR, 2021a and 2021b).

Figure 2

A. thaliana plants exposed to 0, 100 and 150 mM NaCl during seven days. **Row A** belongs to Col-0 plants exposed to different concentrations of NaCl. **Row B** belongs to *met1-7* plants exposed to different concentrations of NaCl. **Row C** belongs to *met1-3* plants exposed to different concentrations of NaCl

Figure 3

Morphological analysis of *A. thaliana* Col-0, *met1-7* and *met1-3* plants. **A** represents the differences between leaf numbers of plants exposed to 0, 100 and 150 mM NaCl. **B** represents the differences between leaf surface areas of plants exposed to 0, 100 and 150 mM NaCl. **C** represents the differences between root lengths of plants in each group individually exposed to 0, 100 and 150 mM NaCl. **D** represents the differences in root lengths of plants exposed to 0, 100 and 150 mM NaCl between Col-0 and mutant groups. **E** represents the differences in root lengths of plants exposed to 0, 100 and 150 mM NaCl between *met1-7* and *met1-3* mutant groups.

Figure 4

Representative image of the 3D structure of the methyltransferase 1 protein. The region shown in red in the image shows the location of the T-DNA insert entering the *met1-7* mutant, and the region shown in blue shows the region where the T-DNA insert enters the *met1-3* mutant and disrupts the protein structure

Figure 5

Graphical representation of the global DNA methylation change in percent (%) of Col-0, *met1-7* and *met1-3* plants after different concentrations of NaCl treatment

Figure 6

Relative gene expression analysis of Col-0, *met1-7* and *met1-3* plants. **A:** *DRM2* gene, **B:** *Pol IV* gene, **C:** *Pol V* gene and **D:** *TERT* gene expression level