International Journal of Multidisciplinary Research and Development
www.allsubjectjournal.com
ISSN Online: 2349-4182 ISSN Print: 2349-5979
Received: 19-06-2023, Accepted: 06-07-2023, Published: 21-07-2023
Volume 10, Issue 7, 2023, Page No. 56-62

The effect of 5-aza-2'deoxycytidine and zebularine on the mutants of *Arabidopsis* gene responsible for DNA methylation

A S Nasirova[1], M Y Hasanova[2], R A Alakbarov[3], A S Orujova[4]

[1] PhD, Scientific Researcher of AR Ministry of Science and Education, Institute of Dendrology, Baku, Azerbaijan

[2] PhD, General Director of AR Ministry of Science and Education, Institute of Dendrology, Baku, Azerbaijan

[3] PhD, Head of the Plant Systematics Laboratory of AR Ministry of Science and Education, Institute of Dendrology, Baku, Azerbaijan

[4] Master in Pharmacognosy, Teacher of Nakhchivan State University, Nakhchivan, Azerbaijan

Abstract

Mutants of *Arabidopsis* gene responsible for DNA methylation show different growth inhibition in the presence of DNA methylation inhibitors. DNA methylation plays an important role in regulating the transposon activation in plants. Therefore, there has been used chemical restriction of DNA methyltransferases as a temporary alternative in order to study the effect of DNA methylation loss in *Arabidopsis thaliana*. To understand the effect of DNA methylation, mutants of *Arabidopsis thaliana* seedlings were treated with either 5-aza-2'-deoxycytidine (5-Aza-2') or Zebularine (Zeb), which is known to be an effective chemical demethylation agent.

In this study, we use phenotype results of DNA methylation of the effects of either Zebularine or 5-Aza-2'-deoxycytidine treatment on mutants of *Arabidopsis thaliana* seedlings. DNA methylation raise was observed in Aza-treated plants than control plants. In this result, there have been revealed that, DNA methylation inhibitors – 5-Aza-2'-deoxycytidine treatment inhibiting DNA methyltransferase activity enhanced on cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 mutants of *Arabidopsis*.

Based on the results of the research, it was found that, growth inhibition of mutants of *A. thaliana* occurs under 5-Aza-2'-deoxycytidine treatment. As a result of the research there have been investigated that, the cmt3 mutants [cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3] showed less growth inhibition than the wild type under 5-Aza-2'-deoxycytidine treatment.

Mutants of *Arabidopsis thaliana* seedlings which treated with Zebularine, showed a slight general growth inhibition on comparison with the mutants of *A. thaliana* seedlings on MS. In contrast to the cmt3 mutants on 5-Aza-2' treatment, there have not been observed any DNA methylation on cmt3 mutants treated with Zebularine.

The result showed that, three mutants: cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 of *Arabidopsis* gene responsible for DNA methylation have been stronger on the comparison with the Col WT in 5-aza-2'-deoxycytidine (5-Aza-2') treatment. When analyzing phenotype results, it has been cleared that, all the MS plate seedlings have been showed well development than 5-Aza-2' treatment.

In this study there have also been measured comparative fresh weights of mutants of *Arabidopsis thaliana* (17 days seedlings). The result showed that: fresh weights of cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 have been almost the same under normal and 5-Aza-2'-deoxycytidine treatment conditions.

Keywords: *Arabidopsis* mutants, 5-aza-2'-deoxycytidine, zebularine

Introduction

DNA methylation is a preserved epigenetic mark that adjusts multiple processes, including gene expression, genome stability and gene imprinting, and consequently disruption of DNA methylation can lead to developmental abnormalities [Zhang *et al*., 2018] [18]. In plants, CG, CHG and CHH sequence context can be found in DNA methylation (where H is A, C or T), and it is extremely enriched over heterochromatic transposable elements (TEs) and repeats, where it plays a distinguished role in silencing their expression at the transcriptional level (Transcriptional Gene Silencing (TGS)). DNA methylation can also initiate TGS when it is present in gene regulatory regions. Moreover, methylation of intronic TEs and repeats has been demonstrated to effect mRNA processing mechanisms such as alternative splicing and alternative polyadenylation [Zhang *et al*., 2018] [18]. In some instances, DNA methylation can also boost gene expression, which has recently been shown to be partially mediated by the DNA methyl-readers SU(VAR)3-9 homologs SUVH1 and SUVH3 [Harris *et al*., 2018 [8]]. Consequently, there are many types of natural and induced epialleles that alter their expression in response to methylation changes and that influence multiple physiological processes [Zhang *et al*., 2018] [18]. DNA methylation, especially in the CG context, has been also presented over gene bodies in many plant species. Its function here is not so well comprehended [Bewick & Schmitz, 2017 [1]], although a recent study noticed a role in suppressing intragenic antisense transcripts [Choi *et al*., 2020 [3]].

DNA methylation at the 5′ position of cytosine contributes to the epigenetic regulation of nuclear gene expression and to genome stability [Robertson *et al*., 2005 [14]; Slotkin *et al*., 2007] [16]. DNA methylation, histone modifications, histone variants and some non-coding RNA (ncRNA) changes of epigenetic changes affect chromatin structure and in turn, the accessibility of genetic information. As a result, DNA methylation is important to many biological processes, and disruption of DNA methylation can lead to developmental abnormalities in plants and mammals [Robertson *et al*., 2005 [14]; Lang *et al*., 2017 [11]; Cortellino, 2011 [5]]. DNA methylation is preserved in plants and mammals, and certain patterns of genomic DNA methylation are vital for development. In both plants and mammals, DNA methylation is catalyzed by conserved DNA methyltransferases using S-adenosyl-l-methionine as the methyl donor, whereas active DNA demethylation involves a base excision repair pathway5–9. An RNAdirected DNA methylation pathway is crucial for de novo methylation in plants but is less important in mammals [Watanabe *et al*, 2011 [17]; Matzke *et al*., 2014 [12]].

DNA methylation, in combination with histone modifications and non-histone proteins, determines chromatin structure and accessibility. Therefore, DNA methylation assists to adjust gene expression, transposon silencing, chromosome interactions.

The chemical inhibition of DNA methyltransferases has been used as a temporary alternative to study the effect of DNA methylation loss in plants [Pecinka and Liu, 2014 [13]]. The most widely used chemical demethylating representatives to inhibit DNA cytosine methylation and reactivated silenced genes consist of 5-azacytidine (5-Aza), 5-aza-2'-deoxycytidine (5-Aza-2') [Jones and Taylor, 1980 [10]], and Zebularine [Driscoll *et al*., 1991 [7]]. These agents serve as nonmethylable cytosine analogues, incorporating into the DNA double helix in the place of cytosine with each cycle of DNA sequencing [Pecinka and Liu, 2014] [13]. Previous studies have shown that, Aza covalently attaches to DNA methyltransferases, creating nucleoprotein adducts, which runs out the number of active DNA methyltransferase enzymes in the cell [Jones and Taylor 1980 [10]; Creusot *et al*. 1982 [6]; Christman *et al*. 1983 [4]; Santi *et al*. 1984] [15]. Nevertheless, 5-Aza has a short half life of only 4 hours. Therefore it needs to be supplied several times in typical experiment. [Champion *et al*, 2010 [2]]. Another DNA methylation inhibitor, Zebularine (Zeb), a more constant alternative to Aza, inhibits DNA methylation in an analogical manner, although it is not thought to develop an irreversible bond with DNA methyltransferases [Champion *et al.*, 2010 [2]]. Zeb was also found to form tight complexes with bacterial methytransferases, resulting in a strong DNA methylation inhibition [Hurd *et al*., 1999 [9]]. Therefore, it is a suitable inhibitor in plant studies. Although Aza and Zeb have been widely utilized in plants, a comprehensive phenotype analysis of either chemical on DNA methylation has been missing. In this study we demonstrate that, Aza and Zeb treatment of mutants of *Arabidopsis thaliana* results in DNA methylation on phenotype. Although some effect of DNA methylation of Aza and Zeb treated plants have been studied, but further experiments is needed to determine responsible gene. This study provides a detailed look into the DNA methylation inhibitors's effect on phenotype.

Plant Materials, Growing condition and Methods

Wild type *Arabidopsis thaliana* seeds of the ecotype "Columbia" (Col-0) (1) and seeds of the mutants of *Arabidopsis thaliana* [cmt3-Ex (2); cmt3-Int (3); cmt3-St (4); cmt2 (5); cmt2/3 (6); ddm1 (7); drm2 (8); met1 (9); LerWT (10); Ler(cmt3) (11)] were obtained and sterilized by an ethanol-based method (as a I group).

After preparation of solid Murashige and Skoog medium plates, each plates have been added 10 ml of 5-Aza-2'-deoxycytidine or Zebularine, (10 ml DNA methylation inhibitor for each round petri dishes) and kept in 4 °C for a day. 10 seeds of mutants of *Arabidopsis thaliana* have been cultivated on one part and 10 seeds of wild ecotype of Col-0 have been cultivated on another half of each petri dish divided into two parts. All seed samples have been grown in the presence of either 5-Aza-2'-deoxycytidine or Zebularine for 7, 9, 11, 14, 16 days (10 ml DNA methylation inhibitor for each round petri dishes). As a control, all seed samples have been grown on Murashige and Skoog (MS) medium plates with no chemical demethylating agent. After two days of stratification period at 4°C, the seeds were transferred to 21°C and allowed to grow under constant light.

In this study there have also been measured comparative fresh weights (17 days seedlings) under normal, 5-Aza-2'-deoxycytidine and Zebularine treatment conditions. In order to calculate of comparative fresh weights 17 days seedlings, seedlings have been measured on scale.

Discussion

Arabidopsis thaliana is the most commonly used model plant species in plant science worldwide. In this study mutants of *Arabidopsis thaliana* [cmt3-Ex (2); cmt3-Int (3); cmt3-St (4); cmt2 (5); cmt2/3 (6); ddm1 (7); drm2 (8); met1 (9); LerWT (10); Ler(cmt3) (11)] and Col WT (1) (as a control) have been studied in the presence of either 5-Aza-2'-deoxycytidine or Zebularine and MS (for control) for 7, 11, 14, 16 days. To evaluate the effect of 5-Aza-2'-deoxycytidine or Zebularine, a sample of each treatment of DNA methylation of mutants of *Arabidopsis* have been taken photographs [Figure 1; 2].

Aza treatment

In 7 days picture's phenotype results there have not been observed any significant differences on seedlings of *Arabidopsis thaliana* mutants [Figure 1].

In 11 days picture's phenotype results: all the MS plate seedlings have been showed well development than 5-Aza-2'-deoxycytidine treated plants. cmt3-Ex, cmt3-Int, cmt3-St have been stronger on the comparison with Col WT on 5-Aza-2' treatment [Figure 1].

In 14 days picture's phenotype results: all the MS plate seedlings have been showed well development than 5-Aza-2'-deoxycytidine treated plants. cmt3-Ex, cmt3-Int, cmt3-St have been completely stronger on the comparison with Col WT on Aza treatment. cmt2/3 have been shown stronger development than Col WT on 5-Aza-2' treatment [Figure 1].

In 16 days picture's phenotype results: all the MS plate seedlings have been showed well development than Aza treatment. cmt3-Ex, cmt3-Int, cmt3-St have been completely stronger on the comparison with Col WT on Aza treatment. cmt2/3 have been showed stronger development than Col WT on Aza treatment [Figure 1].

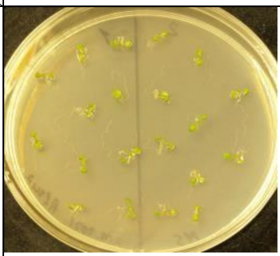
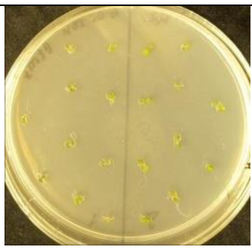
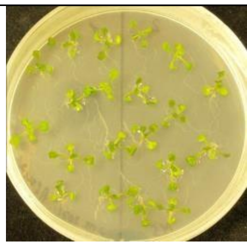
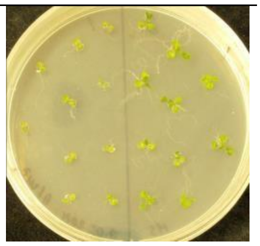
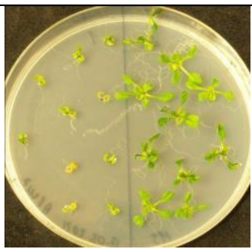
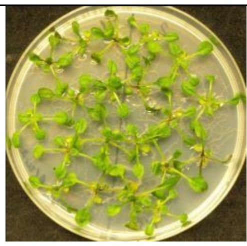
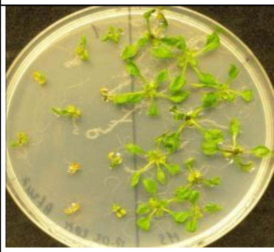
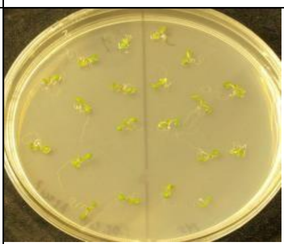
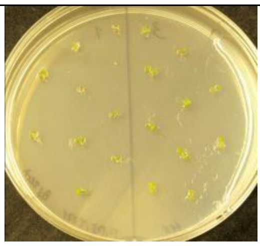
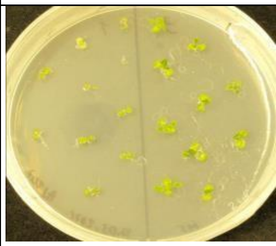
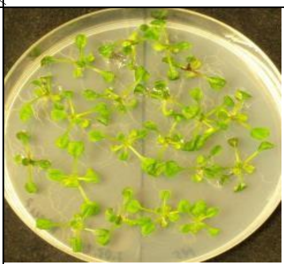
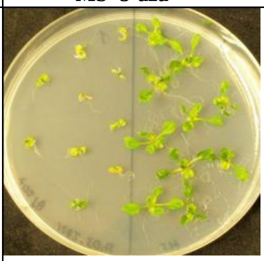
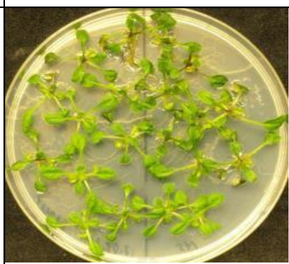
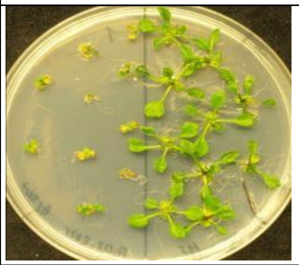
Zebularine treatment

In 7 days picture's phenotype results there have not been observed any significant differences on seedlings of *Arabidopsis thaliana* mutants that treated with Zebularine [Figure 2].

In 11 days picture's phenotype results: all the MS plate seedlings have been showed the same growth with Zebularine treated plants [Figure 2].
In 14 days picture's phenotype results: all the MS plate seedlings have been showed very slight well development than Zebularine treated plants [Figure 2].
In 16 days picture's phenotype results: all the MS plate seedlings have been showed slight well development than Zebularine treatment [Figure 2].

Fresh weight
In this study there have also been measured comparative fresh weights of mutants of *Arabidopsis thaliana* (17 days) under MS conditions as control, and either Zebularine or 5-Aza-2'-deoxycytidine treatment conditions. Results showed that: fresh weights of cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 have been almost the same under normal and 5-Aza-2-deoxycytidine treatment conditions [Table 1; 2]. This is the result of DNA methylation on the mutants of *A. thaliana*. The comparative fresh weight of these 3 mutants has been: cmt3-Ex – 0,421 g; cmt3-Int – 0,396 g, cmt3-St – 0,348 g; cmt2/3 – 0,358 g; on MS; cmt3-Ex – 0,331 g; cmt3-Int – 0,350 g, cmt3-St – 0,369 g; cmt2/3 - 0,317 on 5-Aza-2'-deoxycytidine.

The Effect of 5-AZA-2' deoxycytidine and zebularine on the mutants of Arabidopsis Gene responsible for DNA methylation

Sample / Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+5-aza	Sample / Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+5-aza
			11 days		
Sample / Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+5-aza	Sample / Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+5-aza
14 days			16 days		
Sample / Days	Col WT – cmt3-Int - MS	Col WT – cmt3-Int MS+5-aza	Sample / Days	Col WT – cmt3-Int – MS	Col WT – cmt3-Int MS+5-aza
7 days			11 days		
Sample / Days	Col WT – cmt3-Int – MS	Col WT – cmt3-Int MS+5-aza	Sample / Days	Col WT – cmt3-Int – MS	Col WT – cmt3-Int MS+5-aza
14 days			16 days		
Sample / Days	Col WT – cmt3-St – MS	Col WT – cmt3-St MS+5-aza	Sample / Days	Col WT – cmt3-St – MS	Col WT – cmt3-St MS+5-aza

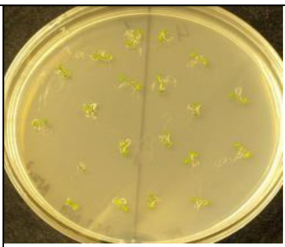
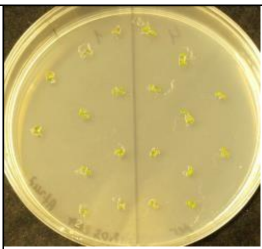
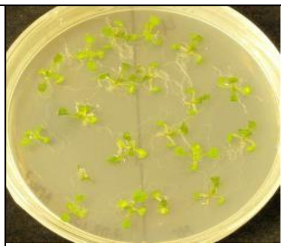
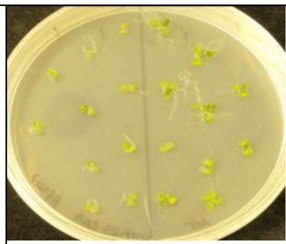
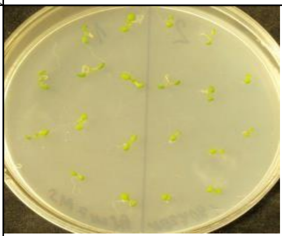
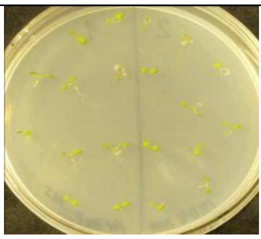
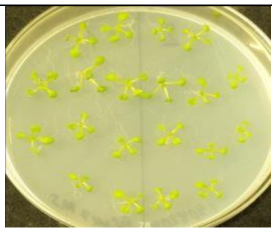
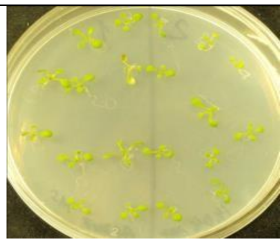
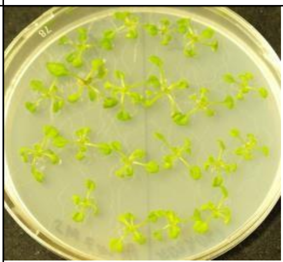
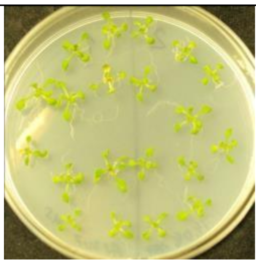
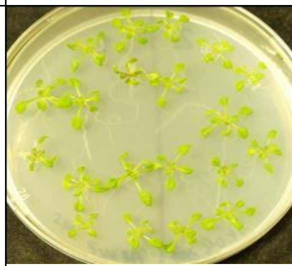
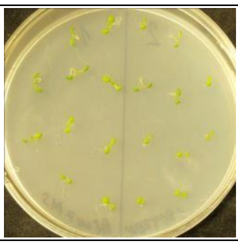
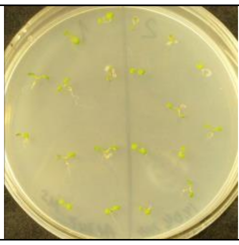
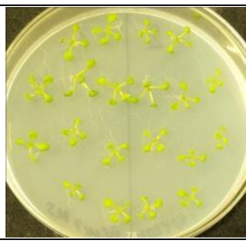
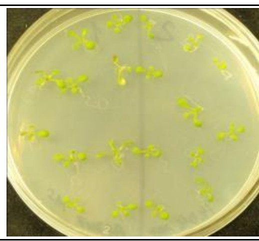
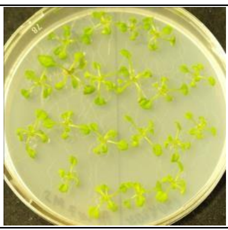
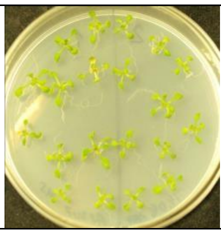
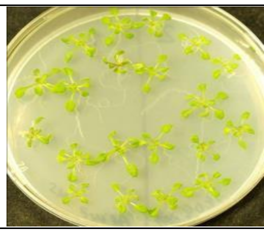
7 days			11 days		
Sample Days	Col WT – cmt3-St - MS	Col WT – cmt3-St MS+5-aza	Sample Days	Col WT – cmt3-St – MS	Col WT – cmt3-St MS+5-aza
Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb	Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb
7 days			11 days		
Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb	Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb
14 days			16 days		
Sample Days	Col WT – cmt3-Int - MS	Col WT – cmt3-Int MS+Zeb	Sample Days	Col WT – cmt3-Int – MS	Col WT – cmt3-Int MS+Zeb

Fig 1: The effect of 5-Aza-2' deoxycytidine on the mutants of *Arabidopsis thaliana*

Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb	Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb
7 days			11 days		
Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb	Sample Days	Col WT – cmt3-Ex – MS	Col WT – cmt3-Ex MS+Zeb
14 days			16 days		
Sample Days	Col WT – cmt3-Int - MS	Col WT – cmt3-Int MS+Zeb	Sample Days	Col WT – cmt3-Int – MS	Col WT – cmt3-Int MS+Zeb

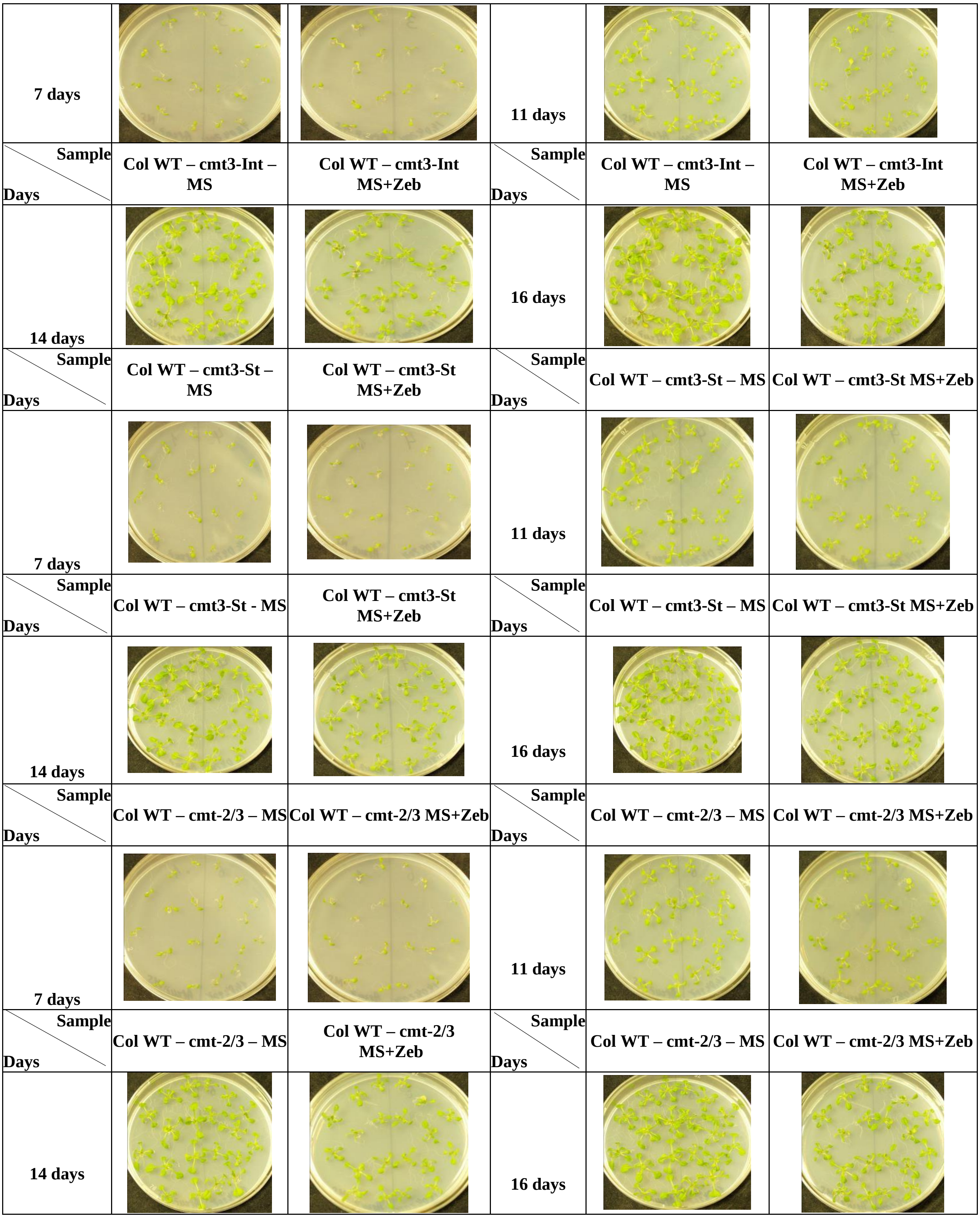


Fig 2: The effect of Zebularine on the mutants of *Arabidopsis thaliana*

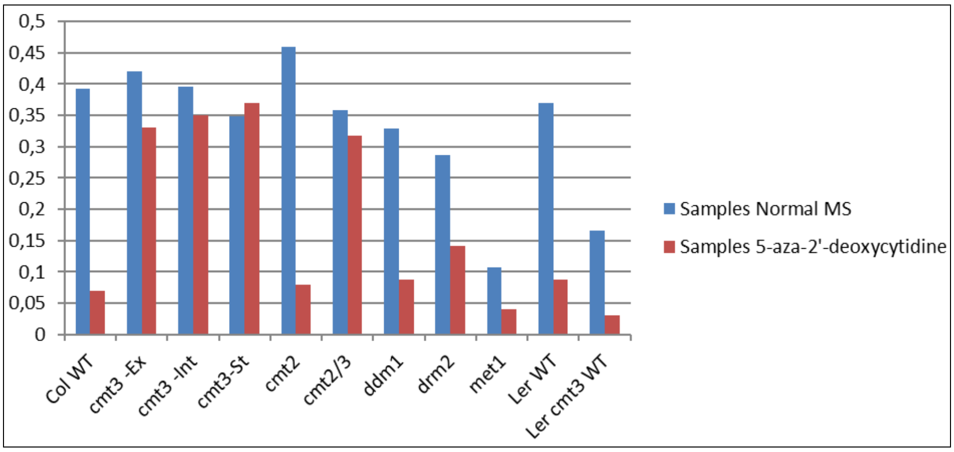


Table 1: Fresh weight comparison on MS and 5-aza-2'-deoxycytidine

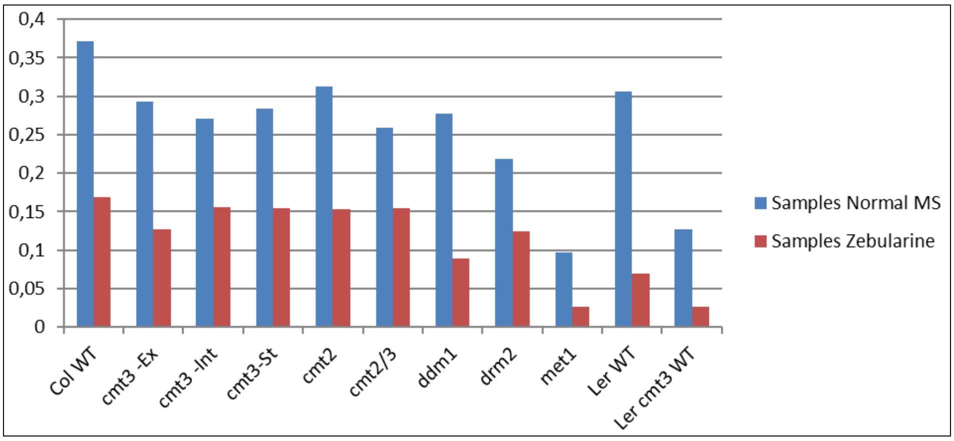


Table 2: Fresh weight comparison on MS and Zebularine

Results

In this study, we use phenotype results of DNA methylation of the effects of either Zebularine or 5-Aza-2'-deoxycytidine treatment on mutants of *Arabidopsis thaliana* seedlings. DNA methylation raise was observed in Aza-treated plants than control plants. In this result, there have been revealed that, DNA methylation inhibitors – 5-Aza-2'-deoxycytidine treatment inhibiting DNA methyltransferase activity enhanced on cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 mutants of *Arabidopsis*.

Based on the results of the research, it was found that, growth inhibition of mutants of *A. thaliana* occurs under 5-Aza-2' treatment. As a result of the research there have been investigated that, the cmt3 mutants [cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3] showed less growth inhibition than the wild type under 5-Aza-2' treatment.

The results under Zebularine treatment showed a different result from those under 5-Aza-2' treatment. So that, although a slight general growth inhibition have been observed on the development of all *Arabidopsis thaliana* mutants treated with Zebularine, but how ever, no DNA methylation observed on cmt3 mutants.

The result showed that, four mutants: cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3 of *Arabidopsis* gene responsible for DNA methylation have been stronger on the comparison with the Col WT in 5-aza-2'-deoxycytidine treatment. When analyzing phenotype results, it has been cleared that, all the MS plate seedlings have been showed well development than Aza treatment. Based on the research results, it was found that DNA methylation in *Arabidopsis thaliana* samples is mainly observed in cmt3 mutants treated with Aza, that the methylation have been observed on *A. thaliana* mutants are: cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3.

Analysis of fresh weights of mutants of *Arabidopsis thaliana* (17 days seedlings) on MS conditions as control, and either Zebularine or 5-Aza-2-deoxycytidine treatment, it has been revealed that, fresh weights of 4 mutants (cmt3-Ex, cmt3-Int, cmt3-St, cmt2/3) of *A. thaliana* seedlings has been almost the same under normal and 5-Aza-2'-deoxycytidine treatment conditions. This is the result of DNA methylation on the mutants of *A. thaliana*.

References

1. Bewick AJ, Schmitz RJ. Gene body DNA methylation in plants. Current Opinion in Plant Biology,2017:36:103–110.
2. Champion C, Guianvarc'h D, Sénamaud-Beaufort C, Jurkowska RZ, Jeltsch A, Ponger L, *et al*. Mechanistic insights on the inhibition of c5 DNA methyltransferases by zebularine. PLoS One,2010:5:e12388.
3. Choi J, Lyons DB, Kim MY, Moore JD, Zilberman D. DNA methylation and histone H1 jointly repress transposable elements and aberrant intragenic transcripts. Molecular Cell,2020:77:310–323. e 7.
4. Christman JK, Mendelsohn N, Herzog D, Schneiderman N. Effect of 5-azacytidine on differentiation and DNA methylation in human promyelocytic leukemia cells (HL-60). Cancer research,1983:43:763-769.

5. Cortellino S, *et al*. Thymine DNA glycosylase is essential for active DNA demethylation by linked deaminationbase excision repair. Cell,2011:146:67–79.
6. Creusot F, Acs G, Christman JK. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'-deoxycytidine. The Journal of biological chemistry,1982:257:2041-2048.
7. Driscoll JS, Marquez VE, Plowman J, Liu PS, Kelley JA, Barchi JJ Jr. Antitumor properties of 2(1H)-pyrimidinone riboside (zebularine) and its fluorinated analogues. J Med Chem,1991:34:3280–4.
8. Harris CJ, Scheibe M, Wongpalee SP, Liu WL, Cornett EM, Vaughan RM, Li XQ, *et al*. A DNA methylation reader complex that enhances gene transcription. Science,2018:362:1182–1186.
9. Hurd PJ, Whitmarsh AJ, Baldwin GS, Kelly SM, Waltho JP, Price NC, *et al*. Mechanism-based inhibition of C5-cytosine DNA methyltransferases by 2-H pyrimidinone. J Mol Biol,1999:286:389–401.
10. Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell,1980:20: 85-93.
11. Lang Z, *et al*. Critical roles of DNA demethylation in the activation of ripening- induced genes and inhibition of ripening- repressed genes in tomato fruit. Proc. Natl Acad. Sci. USA,2017:114:E4511–E4519.
12. Matzke MA, Mosher RA. RNA- directed DNA methylation: an epigenetic pathway of increasing complexity. Nat. Rev. Genet,2014:15:394–408.
13. Pecinka A, Liu CH. Drugs for plant chromosome and chromatin research. Cytogenetic and genome research,2014:143:51-59.
14. Robertson KD. DNA methylation and human disease. Nat. Rev. Genet,2005:6:597–610.
15. Santi DV, Norment A, Garrett CE. Covalent bond formation between a DNA cytosine methyltransferase and DNA containing 5-azacytosine. Proceedings of the National Academy of Sciences of the United States of America,1984:81:6993-699.
16. Slotkin RK, Martienssen R. Transposable elements and the epigenetic regulation of the genome. Nat. Rev. Genet,2007:8:272–285.
17. Watanabe T. *et al*. Role for pi RNAs and noncoding RNA in de novo DNA methylation of the imprinted mouse Rasgrf1 locus. Science,2011:332:848–852.
18. Zhang HM, Lang ZB, Zhu JK. Dynamics and function of DNA methylation in plants. Nature Reviews Molecular Cell Biology,2018:19:489–506.