Indian Journal of Ecology (2023) 50(3): 811-814
DOI: https://doi.org/10.55362/IJE/2023/3974

Manuscript Number: 3974
NAAS Rating: 5.79

Influence of Induced Mutagenesis on DNA Methylation among Mutants of Groundnut (*Arachis hypogaea* L.)

I.S. Tilak and R.S Bhat

Department of Biotechnology, University of Agricultural Sciences, Dharwad-580 005, India
E-mail: istilak.220@gmail.com

***Abstract*:** Influence of induced mutagenesis on DNA methylation was studied among the two sets of parents and their EMS-derived mutants (first set consisting of TMV 2 and its mutant TMV 2-NLM and the second set consisting of DER and its mutant VL 1). The number of methylated sites increased in the mutants over their parents in both the sets and the rate of increase was more in the first set than in the second set. B genome accumulated a greater number of DNA methylated sites in both sets. Among the various contexts (CPG, CHG and CHH) of DNA methylation, CHG regions showed the highest number of methylated sites in the first set, while the CPG regions showed a greater number of methylated sites in the second set. The first set showed a higher rate of increase in methylated sites in the intronic and while the second set showed a higher rate of increase in methylated sites in the exonic region of mutants compared to parents. Overall, the study indicated the influence of induced mutagenesis on DNA methylation pattern among the mutants.

***Keywords*:** Groundnut, Ethyl methanesulfonate, Mutants, DNA methylation

Groundnut (*Arachis hypogaea* L.) is an important legume food and oilseed crop worldwide, which is a cultivated allotetraploid (2n = 4X = 40). The breeding efforts mainly focus on productivity, disease resistance, insect resistance, oil quality, oleic acid content Genomics-assisted breeding has been successfully employed (Kolekar et al 2017). Varshney et al (2014) with the development of genomic resources including the genome sequence of the cultivated allotetraploid groundnut (Bertioli et al 2019). Enormous phenotypic variability despite limited genetic variability in groundnut suggests the role of epigenetic changes in generating phenotypic diversity (Bhat et al 2020). Epigenetic changes include DNA methylation, histone modification, acetylation, phosphorylation, ubiquitylation and sumoylation (Weinhold, 2006). Epigenetic factors showing transgenerational inheritance have been identified (Miryeganeh and Saze 2020). Importance of these modifications in plant growth and development has been well documented (Kumar and Mohapatra 2021). In plants, genome-wide DNA methylation modifications have been observed due to drought (Sharma et al 2016), hybridization (Liu et al 2015, Zhu et al 2017) induced and spontaneous mutations (Shen et al 2006, Ma et al 2016). Mutagenesis is known to modify the DNA methylation in Arabidopsis (Zilberman et al 2007), rice (Shen et al 2006) and pigeonpea (Junaid et al 2018). However, not much information is available on the influence of mutagenesis on DNA methylation in groundnut. Therefore, an effort was made in this study to compare the DNA methylation pattern between the parent and the mutant genotypes of groundnut.

MATERIAL AND METHODS

Plant material: Two sets of parents and mutants (EMS derived) were used; the first set consisted of TMV 2 and its mutant TMV 2-NLM and the second set consisted of DER and its mutant VL 1. TMV 2 is typical Spanish bunch variety with a wide-elliptic leaflet, while TMV 2-NLM is a Virginia runner with a linear-lanceolate leaflet.

DNA isolation and library construction: DNA of all four genotypes was isolated using Qiagen DNeasy Plant Mini Kit (Cat # 69104). Bisulfite treatment was done using Zymo EZ DNA Methylation-Gold Kit. DNA methylome library was constructed using illumine TruSeq ® DNA Methylation Kit. The quality of the library was checked using Tape Station and Qubit.

Bisulfite sequencing and analysis: DNA sequencing was carried out using Illumina Hiseq 2500 with two technical replicates and without any biological replicates. Raw fastq files were pre-processed using Adapter- Removal v2 (Schubert et al 2016) tool. Using bwa-meth (Pedersen *et al*2014) program, the preprocessed reads were aligned with the *Arachis hypogaea* reference genome downloaded from Peanut- Base (IPGI 2017). The genomic sites showing DNA methylation were identified using Methyl Dackel program. Differential methylation was analyzed using methyl kit (Akalin et al 2012) R package. The DNA methylation pattern was

compared across the genotypes at *q*-value cutoff 0.01 and methylation percentage change cutoff 25 using methyl Kit.

RESULTS AND DISCUSSION

Mapping of the reads: On an average, 281,174,853 bisulfite sequencing reads were generated for each sample. These reads were mapped onto the reference genome of *Arachis hypogaea* with an average mapping rate of 98.78%. Mutant TMV 2 had a marginally higher mapping rate over TMV 2-NLM. Similarly, VL 1 had a marginally higher mapping rate than its parent DER. The number of mapped reads at each DNA methylated site ranged from 1 to 274. On an average, 280, 424, 853 DNA methylation sites were among the four genotypes. Of them, 75, 612, 348 sites showed DNA methylation with 100% reads showing methylation. The number of sites increased to 100, 460, 546 when only 50% reads showing methylation were considered. The B sub-genome exhibited higher DNA methylation sites (184, 183, 182) than the A sub-genome (121, 143, 295) across the genotypes. CHG (where H=A, C or T) region showed the highest methylation sites (121,450,697) followed by CPG (120, 529, 828) and CHH (63, 345, 952) region across all genotypes. These results are in line with the previous reports (Zemach et al 2010, Feng et al 2010) indicating that in plants DNA methylation is found both in CpG and non-CpG (CHG and CHH, where H is A, C or T) contexts, in contrast to mammals where DNA methylation occurs predominantly at CpG dinucleotides.

DNA methylation changes between TMV 2 and TMV 2 - NLM: Changes in DNA methylation between the parent (TMV 2) and the mutant (TMV 2-NLM) were compared in the first set. The number of methylation sites increased in the mutant TMV 2-NLM (25, 44, 52, 659) when compared to its parent TMV 2 (21, 90, 13, 323) when 100% reads showing methylation were considered (Table 1). Likewise, the number of methylated sites increased in the mutant TMV 2-NLM (78, 856, 996) compared to its parent TMV 2 (68, 893, 302). This increase in the methylated sites was more frequent in the B genome than in the A genome, indicating that the B genome is more prone to DNA methylation (Table 2). CHG context showed the highest increase in DNA methylation in the mutant (Table 3). Both genic and non-genic regions showed increased DNA methylation. In genic region, intronic regions (1,687,698 in TMV 2-NLM) showed higher rate of increase in DNA methylation than exonic regions (1,018,559 in TMV 2-NLM). Overall, the number of genes showing DNA methylation increased in the mutant TMV 2-NLM (54,463) over the parent (51,866) (Table 4). Further, the differentially methylated sites between TMV 2 and TMV 2-NLM (Bhat et al 2020) were reported, 37 genes exhibiting differential methylation, of which eight showing differential expression were also reported (Bhat et al 2020).

DNA methylation changes between DER and VL 1; DNA methylation was also compared between the parent (DER) and the mutant (VL 1) in the second set. The number of methylation sites increased in the mutant VL 1

Table 1. Methylation sites and methylated sites among the parents and their mutants in groundnut

Genotypes	Total number of methylation sites	Total number of methylated sites	Frequency of methylated sites	Rate of increase in methylation sites of mutants over its parents (%)	Rate of increase in methylated sites of mutants over its parents (%)
TMV 2	219,013,323	68,893,302	0.31		
TMV 2-NLM	254,452,659	78,856,993	0.31	16.18	14.46
DER	261,792,657	77,745,065	0.30		
VL 1	275,442,003	79,831,117	0.29	5.21	2.68

Table 2. Methylated sites in the A and B genomes of the parents and their mutants in groundnut

Genotypes	Total number of methylated sites		Rate of increase in methylated sites of mutants over its parents (%)	
	A genome	B genome	A genome	B genome
TMV 2	27,365,742	41,527,560		
TMV 2-NLM	31,097,925	47,759,068	14.00	15.01
DER	30,970,192	46,774,873		
VL 1	31,709,436	48,121,681	2.39	2.88
TMV 2	27,365,742	41,527,560		
TMV 2-NLM	31,097,925	47,759,068	14.00	15.01
DER	30,970,192	46,774,873		
VL 1	31,709,436	48,121,681	2.39	2.88

Table 3. Methylated sites among the contexts of parents and their mutants in groundnut

Genotypes	CPG	CHG	CHH	Rate of increase in CPG of mutants over its parents (%)	Rate of increase in CHG of mutants over its parents (%)	Rate of increase in CHH of mutants over its parents (%)
TMV 2	26,820,849	27,768,275	14,304,178			
TMV 2-NLM	30,608,417	30,706,545	17,542,031	14.12	10.58	22.68
DER	30,760,532	31,247,081	15,737,452			
VL 1	32,340,030	31,728,796	15,762,291	5.13	1.54	0.16

Table 4. Methylated sites in exonic and intronic regions among the parents and their mutants in groundnut

Genotypes	Exonic	Intronic	Rate of increase in exonic region of mutants over its parents (%)	Rate of increase in intronic region of mutants over its parents (%)	Genes with methylation
TMV 2	853,885	1,407,484			51,866
TMV 2-NLM	1,018,559	1,687,698	19.29	19.91	54,463
DER	964,170	1,618,932			53,555
VL 1	1,045,365	1,723,338	8.42	6.45	54,876

(27,54,42,003) when compared to its parent DER (26,17,92,657) when 100% reads showing methylation were considered. Similarly, the number of methylated sites increased in the mutant VL 1 (79,831,117) compared to its parent DER (77,745,065) (Table 1). This increase in the methylated sites was more pronounced in the B genome than in the A genome (Table 2). In this set, CPG context showed the highest increase in DNA methylation in the mutant (Table 3). Both genic and non-genic regions showed increased DNA methylation. In genic region, exonic regions (1,045,365 in VL 1) showed a higher rate of increase in DNA methylation than intronic regions (1,723,338 in VL 1). Overall, the number of genes showing DNA methylation increased in the mutant VL 1 (54,876) over the parent (53,555) (Table 4).

DNA methylation changes between TMV 2 versus TMV 2-NLM and DER versus VL 1: Changes in DNA methylation between the parent and the mutant were compared across the two sets. The rate of increase in the total number of methylation sites and the methylated sites in the mutant (over the parent) was more in the first set (~0.31) than in the second set (~0.29) (Table 1). The rate of accumulation of DNA methylated sites in the B genome over the A genome of the mutant was also more in the first set as compared to the second set (Table 2). The rate of increase was highest in CHH context in the first set (Table 3). In contrast, the rate of increase was highest in CPG context in the second set, indicating that the methylation in the contexts (CPG, CHH and CHG) was genotype-specific. Though the genic and non-genic regions showed higher methylated sites in the mutant over the parent in both the sets, intronic regions exhibited a higher rate of increase in methylated sites in the first set, while the exonic regions showed a higher rate of increase in the second set (Table 4). These results are in the line with the results indicating the higher methylated sites at intronic region (Rigal et al 2012) and exonic region (Wang et al 2014) among Arabidopsis mutants.

CONCLUSIONS

The genome-wide DNA methylation analysis among the parents and their EMS-derived mutants revealed that the induced mutagenesis increases the DNA methylation in groundnut. This might contribute to the overall phenotypic variability which can be employed for groundnut improvement.

REFERENCES

Dhawan AK, Vijay Kumar and Shera PS 2013. Ecological Perspectives in Pest Management for Sustainable IPM. *Indian Journal of Ecology* 40(2): 167-77

Akalin A, Kormaksson M, Li S Garrett-Bakelman, Figueroa FE, ME Melnick A and Mason CE 2012. MethylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. *Genome Biology* **13**(10):1- 9.

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SC, Ren L, Farmer AD, Pandey MK and Samoluk S 2019. The genome sequence of segmental allotetraploid peanut (*Arachis hypogaea*). *Nature Genetics* **51**(5): 877- 884.

Bhat RS, Rockey J, Shirasawa K, Tilak IS, Patil MB and Lachagari VR 2020. DNA methylation and expression analyses reveal epialleles for the foliar disease resistance genes in peanut (*Arachis hypogaea* L.). *BMC Research Notes* **13**(1): 1-7.

Feng S, Cokus SJ, Zhang X, Chen PY, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME and Ukomadu C 2010. Conservation and divergence of methylation patterning in plants and animals. *Proceedings of the National Academy of Sciences* **107**(19): 8689- 8694.

IPGI 2017. High-quality genome assembly for peanut (*Arachis hypogaea* L.), cultivar "Tifrunner".

Junaid Kumar H, Rao AR, Patil AN, Singh NK and Gaikwad K 2018. Unraveling the epigenomic interactions between parental inbreds resulting in an altered hybrid methylome in pigeonpea. *DNA Research* **25**(4): 361-373.

Kolekar RM, Sukruth M, Shirasawa K, Nadaf HL, Motagi BN, Lingaraju S, Patil PV and Bhat RS 2017. Marker−assisted backcrossing to develop foliar disease resistant genotypes in TMV 2 variety of peanut (*Arachis hypogaea* L.). *Plant Breed*ing **136**: 948-953.

Kumar S and Mohapatra T 2021. Dynamics of DNA methylation and its functions in plant growth and development. *Frontiers in Plant Science* **12**: 858.

Ma X, Wang Q, Wang Y, Ma J, Wu N, Ni S, Luo T, Zhuang L, Chu C, Cho SW, Tsujimoto H and Qi Z 2016. Chromosome aberrations induced by zebularine in triticale. *Genome* **59**(7): 485-492.

Miryeganeh M and Saze H 2020. Epigenetic inheritance and plant evolution. *Population Ecology* **62**(1): 17-27.

Pedersen BS, Eyring K, De S, Yang IV and Schwartz DA 2014. Fast and accurate alignment of long bisulfite-seq reads. *arXiv preprint arXiv:*1401.1129.

Rigal, M, Kevei Z, Pélissier T and Mathieu O, 2012. DNA methylation in an intron of the IBM1 histone demethylase gene stabilizes chromatin modification patterns. *The EMBO. Journal* **31**(13): 2981-2993.

Schmitt F, Oakeley EJ and Jost JP 1997. Antibiotics induce genome-wide hyper methylation in cultured *Nicotiana tabacum* plants. *Journal of Biological Chemistry* **272**(3): 1534-1540.

Schubert M, Lindgreen S and Orlando L, 2016. Adapter removal v2: Rapid adapter trimming, identification and read merging. *BMC Research Notes* **9**(1): 1-7.

Received 27 November, 2022; Accepted 15 April, 2023

Sharma R, Vishal P, Kaul S and Dhar MK 2016. Epiallelic changes in known stress-responsive genes under extreme drought conditions in *Brassica juncea* (L.) Czern. *Plant Cell Reports* 1-15.

Shen S, Wang Z, Shan X, Wang H, Li L, Lin X, Long L, Weng K, Liu B and Zou G 2006. Alterations in DNA methylation and genome structure in two rice mutant lines induced by high pressure. *Science China Life Sciences* **49**(2): 97-104.

Varshney RK, Pandey MK, Janila P, Nigam SN, Sudini H, Gowda MVC, Sriswathi M, Radhakrishnan T, Manohar S and Nagesh P, 2014. Marker-assisted introgression of a QTL region to improve rust resistance in three elite and popular varieties of peanut (*Arachis hypogaea* L.). *Theoretical and Applied Genetics* **127**(8): 1771-1781.

Wang J, Marowsky NC and Fan C 2014. Divergence of gene body DNA methylation and evolution of plant duplicate genes. *PloS one* **9**(10):110357.

Weinhold B 2006. Epigenetics: The science of change. *Environental Health Perspectives* **114**(3): 160-167.

Zemach A, McDaniel I E, Silva P and Zilberman D 2010. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328**(5980): 916-919.

Zhu W, Hu B, Becker C, Dogan ES, Berendzen KW, Weigel D and Liu C 2017. Altered chromatin compaction and histone methylation drive non-additive gene expression in an interspecific *Arabidopsis* hybrid. *Genome Biology* **18**(1): 157.

Zilberman D, Gehring M, Tran RK, Ballinger T and Henikoff S 2007. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nature Genetics* **39**(1): 61- 69.