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Involvement of Cytosine DNA methylation in different developmental stages of *Aeluropus littoralis*

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Abstract

DNA methylation as epigenetic mark plays a key role in normal differential and developmental processes as well as in dynamic gene regulation at the genomic level. To assess DNA methylation pattern in different developmental stages of *Aeluropus littoralis,* methylation sensitive amplified polymorphism (MSAP) was used. Methylation and demethylation status at the CCGG recognition site were tracked by two sets of cytosine methylation-sensitive enzymes (*Msp*I and *Hpa*II), which were classified into three types. The percentage of total bands per type I (non methylation), type II (CpG methylation) and type III (CpCpG methylation) fragments were 75.7, 19.4 and 4.9, respectively. The most frequent methylation events (19.4%) were observed in type II fragment in which full methylation pattern occurred. Out of 480 bands, 33 bands showed methylation alterations between differential developmental stages in all three types of detectable methylation levels. In this study, polymorphic bands had two main directions associated with methylation or demethylation patterns in which methylation level increased during plant development. The methylation and demethylation events at CG sites could be related to developmental stage-specific gene regulation.

Additional keywords: Epigenetic, DNA methylation, *Aeluropus littoralis*, Halophyte, Developmental stages.

Introduction

Genetic and epigenetic information are essential determinants of structural and functional states of organisms (Loidl 2004). Epigenetic regulation governs expression of the genome by processes often associated with chromatin structure. The regulation may be coupled to histone variants, histone post-translational modifications, and DNA methylation, which are involved in a broad spectrum of biological behaviors (Chinnusamy and Zhu 2009). Methylation is a universal DNA modification

(Hendrich and Tweedie 2003) which is raised from the conversion of cytosine into 5-methylcytosine at nuclear DNA. The direct addition of a methyl group can provide docking sites for proteins to alter the chromatin state or affect the covalent modification of resident histones (Alis *et al*. 2007). In plants, cytosine methylation is shared among CpG, CpHpG, and CpHpH contexts, where H stands for adenine, cytosine, or thymine (Feng *et al*. 2010; Chinnusamy and Zhu 2009). The highly frequent occurrence of the genome methylation can be considered on the basis of *Arabidopsis* genome methylation which is placed around 24% of CG dinucleotides in the collection of methylated contexts (Cokus *et al*. 2008).

Methylation plays a crucial role in the regulation of gene expression, genome plasticity and gene silencing (Choi and Sano 2007; Loidl 2004). Transcriptional and post-transcriptional gene silencing are linked with hypermethylation of promoter sequences and transcribed or coding sequences, respectively (Paszkowski and Whitham 2001). It seems that resetting of epigenetic status across development has a significant role in gene expression patterns. In comparison with animals, much less is known about epigenetic modifications in plants (Zhang *et al.* 2010). The epigenetic status in mammals is systematically reprogrammed during developmental stages, featuring erasure and reestablishment of epigenetic marks, and spatially and temporally redefined cytosine methylation patterns (Reik *et al.* 2001).

DNA methylation profiling has become an important technology in many areas of epigenetic researches. The approaches principally investigate methylation patterns either across the entire genome or at specific areas of interest like promoters and other regulatory elements (Rauch and Pfeifer 2010). In general, two techniques are used to assess methylation: (i) the sequencing after modification of genomic DNA with sodium bisulfite and (ii) PCR amplification after digestion of genomic DNA with methylation-sensitive endonuclease (s) (Fuso *et al.* 2006; Tollefsbol 2004). Amplified fragment length polymerphism (AFLP) technique (Vos *et al.* 1995) was modified by Substitution of *Mse* I with two isoschizomer enzymes *Hpa* II and *Msp*I, which has been entitled to methylation sensitive amplification polymorphism (MSAP). In the MSAP, the genome-wide DNA methylation profiling can be investigated based on CCGG site (Xu *et al.* 2000). This technique has been successfully used for plant epigenome studies in a number of plant species viz. maize (Zhao *et al.* 2007, Lu *et al.* 2008; Tan 2010), rice (Sha *et al.* 2005), pepper (Portis *et al.* 2004), hops (Peredo *et al.* 2008), *Brassica napus* L. (Labra *et al.* 2004), *Brassica oleracea* (Salmon *et al.* 2008), and Azalea (Meijon *et al.* 2009).

Halophytic plants are very important genetic resources to study the relationships among genome, epigenome and abiotic stresses. The poaceae halophyte *A. littoralis* as a perennial, rhizomatous monocotyledonous is a diploid (2n-=2X-=14) plant with a relatively small haploid genome of 349Mb and C4 photosynthesis (Barhoumi *et al*. 2007; Wang 2004). Thus, *A. littoralis* has the

potential to become a precious genetic resource for understanding the molecular mechanisms of stress-responses in important monocot crops (Zouari *et al.* 2007; Ben Saad *et al.* 2010; Hashemi et al. 2013). On the other hand, seedling development and plant establishment under natural environmental conditions are among the major concerns in the field of crop research. Therefore, understanding the pattern and extent of cytosine methylation during plant growth and development could provide valuable information about chromatin status and its regulatory role in biological process. The present study employed methylation-sensitive amplified polymorphism technique to analyze genome-wide DNA methylation pattern during *A.littoralis* seedling development.

Materials and methods

Plant materials and growth conditions

The surface-sterilized *Aeluropus littoralis* seeds were transferred on 1/2MS medium (Murashige and Skoog 1962) supplemented with 3% sucrose and 0.7% agar in glass plates. The cultures were incubated at 25 ± 2^{o}C with 16h light/8h dark photoperiod at 100 µmol m^{-2} s^{-1} photon flux density using cool-white fluorescent light. After four days, seedlings were sampled at three sequential growth stages including: the last stage of coleoptile elongation, the complete expansion of seed leaf, and the appearance of the third leaf. At each sampling time, 150 seedlings were harvested in three replications and were immediately placed in liquid nitrogen.

DNA isolation and quantification

One hundred mg of each sample was powdered and genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN, Germany). Quantification and qualification of isolated DNA were determined by agarose electrophoresis and spectrophotometer.

Methylation sensitive amplified polymorphism (MSAP)

MSAP analysis was carried out according to Vos *et al.* (1995) with some modifications. The isoschizomers of *Hpa*II and *Msp*I (Roche Applied Science, Germany) were employed as 'frequent-cutter' enzymes instead of *Mse* I. Both *Hpa*II and *Msp*I recognize the same tetra-nucleotide sequence (5'-CCGG-3'), but they display different sensitivity to DNA methylation (Xu *et al.* 2000; Fang and Chao 2007; Peng and Zhang 2009). Genomic DNA was digested overnight using the *Msp*I/*EcoR*I and *Hpa*II/*EcoR*I enzyme combinations and then linked to the adapters. Sequences of the adapters and primers used in this study are listed in Table 1. Pre-amplification was performed in a 20µl reaction mixture, containing 50ng ligated DNA, 250µM of each dNTPs, 1x Taq buffer PCR (10mM Tric-HCl; 50mM KCl, pH 8.8; 0.08% Nonidet P40), 2mM $MgCl_2$, 1U of *Taq* polymerase (Fermentas, Lithuania) and 0.25µM of each non-selective primer pairs (Alpha DNA, Canada) using a MJ Mini thermal cycler (Bio-Rad, USA). The PCR program consisted of 20 cycles: 94^{o}C for 1min, 55^{o}C for 1min, 72^{o}C for 2min. The pre-amplification product was diluted 20-fold and used as a template for selective amplification.

The PCR program consisted of 12 cycles at 94°C for 30s, 65°C for 30s, and 72°C for 60s with annealing temperature decreasing 0.7°C per cycle; followed by 25 cycles at 94°C for 30s, 56°C for 30s and 72°C for 60s and final elongation at 72°C for 10min. Selective amplified products were separated by a 6% denaturing PAGE using Sequi-Gen GT Cell system (Bio-Rad, USA) and visualized by silver staining. The gels were dried at room temperature for 12h and were scanned with an Imaging Densitometer model GS-800 (Bio-Rad, USA).

Table 1. The list of primer and linker sequences.

Sequence ID	Sequence	Sequence ID	Sequence
*EcoR*I linker 1	CTC GTA GAC TGC GTA CC	HM Linker 1	GAT CAT GAG TCC TGC T
*EcoR*I linker 2	AAT TGG TAC GCA GTC TAC	HM Linker 2	CGA GCA GGA CTC ATG A
*EcoR*I (A)	GAC TGC GTA CCA ATT CA	HM	ATC ATG AGT CCT GCT CGG
*EcoR*I (N)	GAC TGC GTA CCA ATT CN	HM (TCAA)	ATC ATG AGT CCT GCT CGG AA
HM (TCAC)	ATC ATG AGT CCT GCT CGG TCA C	*EcoR*I (AGG)	GAC TGC GTA CCA ATT CAG G
*EcoR*I (AAC)	GAC TGC GTA CCA ATT CAA C	*EcoR*I (GGA)	GAC TGC GTA CCA ATT CGG A
*EcoR*I (AAG)	GAC TGC GTA CCA ATT CAA G	*EcoR*I (AAA)	GAC TGC GTA CCA ATT CAA A
*EcoR*I (ACA)	GAC TGC GTA CCA ATT CAC A	*EcoR*I (AGT)	GAC TGC GTA CCA ATT CAG T
*EcoR*I (ACC)	GAC TGC GTA CCA ATT CAC C	*EcoR*I (ACT)	GAC TGC GTA CCA ATT CAC T
*EcoR*I (ACG)	GAC TGC GTA CCA ATT CAC G	*EcoR*I (AGC)	GAC TGC GTA CCA ATT CAG C

Data analysis

Differences between *EcoR*I/*Msp*I and *EcoR*I/*Hpa*II patterns were considered as epigenetic changes. Each pair of isoschizomers was classified into three types for the analysis of variation in methylation status (Table 2). In type I, *Hpa*II and *Msp*I recognition sites were not methylated and showed the same band patterns. In type II, internal methylation occurred in mCpG site at both strands which could be detected by *Msp*I (full-methylated sequence) while in Type III, methylation happened in mCmCpG or mCpG sites at one strand (hemi-methylated sequence) and was capable of being visualized with *Hpa*II enzyme (Peredo *et al.* 2008).

Results

As shown in Table 3, differential plant development was completely evident during the sampling time increments (four days). It seems that plant seedlings at the second developmental stage (DS2) showed a sharp increase in length and fresh weight where they increased up to 3- and 6-fold in comparison with the first sampling time (DS1), respectively. The DS2 seems to follow growth rather than to follow organ development while the final developmental stage (DS3) tends to generate more plant organs.

Table 2. Schematic presentation of cytosine methylation patterns in MSAP analysis (Salmon *et al.* 2008).

Description	Band type	*Hpa*II	*Msp*I	Enzyme restriction site			
Non-methylated	I	+	+	C	C	G	G
				G	G	C	C
CpG methylation	II	-	+	C	**mC**	G	G
				G	G	**mC**	C
CpCpG methylation	III	+	-	**mC**	**mC**	G	G
				G	G	C	C
		+	-	C	**mC**	G	G
				G	G	C	C
Hypermethylation	IV	-	-	**mC**	**mC**	G	G
				G	G	**mC**	**mC**

The assessment of DNA methylation involvement in regulation of plant growth and development was established on the basis of MSAP profiling technique where different primer combinations were tested. Primer pairs of HM and *EcoR*I (A) used for pre-selective amplification. The 13 primer combinations for *EcoR*I (ANN) and HM (TCAW) were used as selective primers. PCR products between 100 and 800 base pairs with high frequent occurrence in different reactions were considered while fragments with weak intensity and low reproducibility were not evaluated for analysis.

Table 3. Plant characteristics at three different developmental stages.

Developmental stage	Plant age (days after sowing)	Number of leaves	Plant length (mm)		Plant fresh weight (µg)	
			Shoot	Root	Shoot	Root
DS1	5	0	2.8	7.4	75	27
DS2	9	1	7.1	22.2	487	178
DS3	13	2	14.3	27.9	1650	632

Out of the 13 primer combinations, 11 primer pairs produced polymorphic bands at different stages of seedling development (Table 4) while two combinations detected no variation in methylation sites. In total, 480 reproducible amplification products were observed in CCGG sites with an average of 37 bands per each enzymatic combination. The highest and the lowest numbers of bands were 26 and 51 for *EcoR*I (CTA) /HM (TCAC) and *EcoR*I (AAG) /HM (TCAA) primer combinations, respectively. Epigenetic marks as methylation and demethylation at the CCGG recognition site were tracked by the differences between *EcoR*I /*Msp*I and *EcoR*I /*Hpa*II patterns. The percentage of total bands in type I, II and III were 75.7, 19.4 and 4.9, respectively (Table 4). The most frequent methylation events (19.4%) were observed in type II fragment in which full methylation pattern occurred in CCGG recognition site.

Table 4. Data of primer combinations were used in methylation sensitive amplification polymorphism. M: monomorphic bands; P: polymorphic bands.

	Type of bands	Type I		Type II		Type III	
	Number of bands	M	P	M	P	M	P
Primer combination	*EcoR*I (AAA) / HM (TCAC)	24	2	1	1	-	-
	*EcoR*I (AGT) / HM (TCAA)	32	1	4	-	-	-
	*EcoR*I (AAC) / HM (TCAA)	29	3	8	1	1	1
	*EcoR*I (CTA) / HM (TCAC)	20	-	6	-	-	-
	*EcoR*I (AAG) / HM (TCAA)	39	2	9	-	1	-
	*EcoR*I (ACA) / HM (TCAA)	26	3	6	-	2	-
	*EcoR*I (ACC) / HM (TCAA)	27	-	7	-	2	-
	*EcoR*I (AAC) / HM (TCAC)	18	1	6	1	4	-
	*EcoR*I (ACG) / HM (TCAA)	21	-	6	1	8	-
	*EcoR*I (ACT) / HM (TCAA)	26	3	9	2	1	-
	*EcoR*I (AGC) / HM (TCAA)	18	3	8	2	1	1
	*EcoR*I (AAA) / HM (TCAA)	26	2	4	-	1	-
	*EcoR*I (AGG) / HM (TCAA)	30	2	4	1	-	-
	Total	336	22	78	9	21	2
	Percent	72	4.7	16.6	1.9	4.4	0.4

Out of 480 bands, 33 bands showed methylation alterations among differential developmental stages which belonged to all three types of methylation. The number of polymorphic bands (NPB) produced by each primer combination ranged from one (*EcoR*I (AGT) /HM (TCAA)) to seven (*EcoR*I (ACA) /HM (TCAA); *EcoR*I (ACT) /HM (TCAA); *EcoR*I (AGG) / HM (TCAA)) (Table 4).

Twenty one percent of total detected methylation (21%) was identical among different developmental stage. The level of polymorphism for the three types of fragments decreased from type I to III which were 4.7 %, 1.9% and 0.4 % in type I, II, and III, respectively. In Figure. 1, a sample of DNA methylation pattern in primer combination of *EcoR*I ACT /HM TCAA is presented. Different developmental stages were compared using polymorphism frequency of 33 enzymatic recognition sites. *Msp*I recognized 27, 27, and 8 and *Hpa*II restricted 21, 19, and 7 sites at different developmental stages of DS1, DS2, and DS3, respectively. It appears that the variation of DNA cytosine methylation followed an increasing trend along with seedling differentiation (Figure. 2) owing to increase in polymorphism amount. The lowest level of methylation was observed in the developmental stage 1 (DS1) with the cumulative polymorphism percent value equal to 63 and 81 for *Msp*I and *Hpa*II sites. Consequently, the polymorphism values were reduced in DS2 (57 and 81) and DS3 (21 and 24).

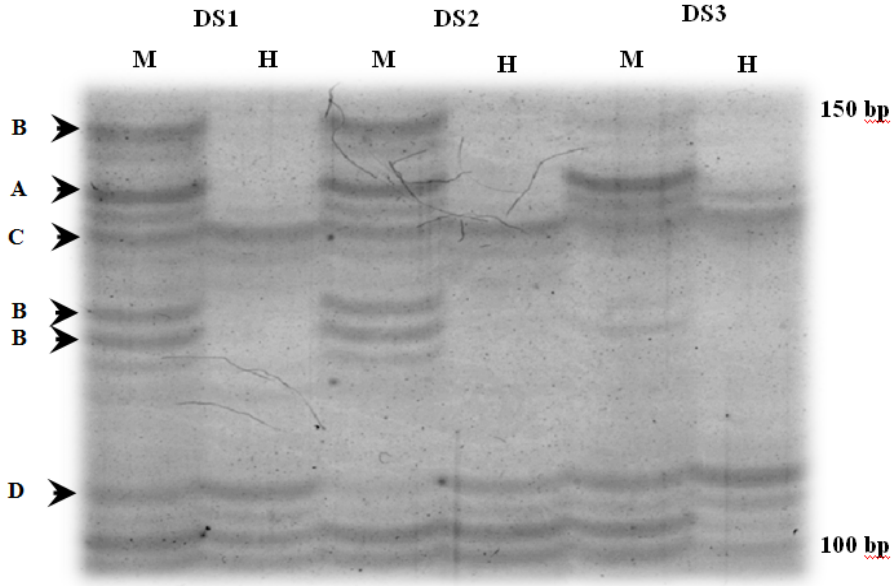

Figure. 1. MSAP profile of Aeluropus littoralis across different developmental stages (DS1, DS2 and DS3) detected by *EcoR*I (ACT) /HM (TCAA) primer combination. Each sample digested by *EcoR*I+Hpa II (H) or *EcoR*I+*Msp*I (M). The arrows indicate different types of bands. The arrow represents those digested by *EcoR*I+*Msp*I but not by *EcoR*I+HpaII as type II fragments (CpG methylation). In the B arrow indicated polymorphism in type II fragment that *Msp*I site disappear in DS3. The C and D arrow showed types I fragment that in D arrow, methylation in *Msp*I site were detected.

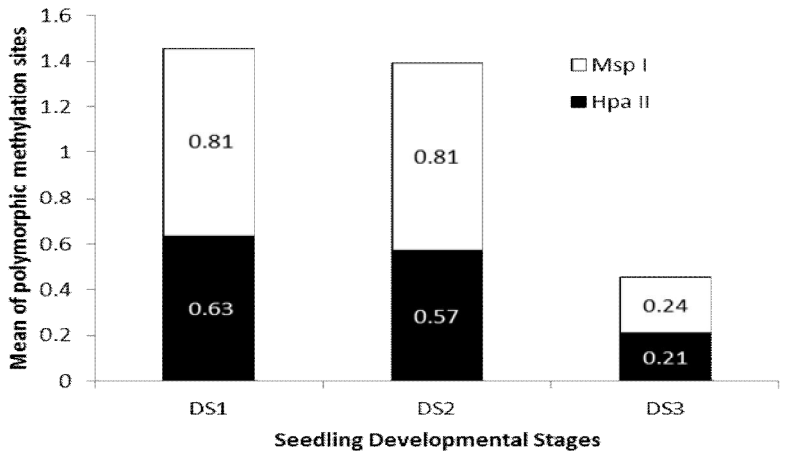

Figure. 2. The mean of 33 polymorphic methylation sites across three developmental stages (DS1, DS2 and DS3) detected by MSAP.

Discussion

AFLP-based MSAP technique is a robust method to make a global overview of methylation pattern that is able to amplify a large number of methylated and demethylated fragments simultaneo-

usly with no need for sequence information. It should be noted that MSAP can only analyze two states of methylation at CCGG sites including full methylation of both the external and internal cytosine and hemi-methylation of the internal cytosine (Zhao *et al.* 2007) and therefore the methylation events could be underestimated. Besides, the isochimeric restriction enzymes, *Msp*I and *Hpa*II, theoretically generate four distinct patterns of which type IV as a hypermethylation status cannot be practically detectable.

Plant growth cycle runs across a series of spatial and temporal developmental events so that successful performance of each phase is a precursor for the proper possibility of other successive immediate events. Seed-seed cycle is a major concept in economic productivity of crop plants in different environmental conditions. Seed germination and early seedling development are the critical points in the growth cycle and plant establishment on the basis of wide reprogramming of cell differentiation. DNA cytosine methylation constructs an integral part of the epigenetic controlling network to tune tissue-, organ- and developmental stage-specific gene expression across plant development. Regulation of cytosine methylation across the plant development is an essential dimension for gene expression remodeling and consequent differentiation.

In plants, the occurrence of different types of DNA methylation is dependent on its own maintenance regulation pathway and mode of inheritance (Salmon *et al.* 2008). The present results indicated that methylation rate increased along with progression in plant development. Total occurrence of methylation in the internal cytosines on both strands (type II) was always more frequent than hemi-methylation on the external cytosine (type III) with 18.5% and 4.8 %, respectively. Despite the different values for the methylation types, the percentage of polymorphic fragments in each type were similar and estimated 11.5% and 9.5%, respectively. In contrast, type I fragments displayed higher levels of methylation alternation compared to types II and III fragments.

In this study, polymorphic bands had two main directions associated with methylation or demethylation patterns. Out of 22 bands in type I, methylation value was higher than demethylation (18 versus 3 bands) and was mainly limited to DS3 stage while no changes were observed in other stages. Demethylation status occurs in both *Msp*I and *Hpa*II recognition sites. Of 18 methylation fragments, 15 bands showed hyper-methylation while the remaining devoted to CpG (one band) and CpCpG (two bands) sites. Similarly, in type II, 7 methylation sites were detected in DS3 and two demethylation events were identified only in DS1 and DS2. Most likely, these methylation and demethylation play main role for switching on or off the key genes in developmental processes. As shown in Figure. 2, *Msp*I and *Hpa*II are strikingly able to detect methylation patterns, which are linked to the developmental stages. In other word, different developmental stages may be explained using differential sensitivity of genome recognition sites to *Msp*I and

Hpa II enzymes. These methylation and demethylation events at CG sites are probably related to developmental stage-specific gene regulation. Methylation in specific regions of genes or in their vicinity can inhibit the expression of these genes, while demethylation of genes has been shown to result in reactivation (Grunau *et al.* 2001). The evidence indicates that, an increase or a decrease in methylation level is temporary and will alter accordingly along with the growth process and developmental stage-dependent manners (Lu *et al.* 2008; Zhang *et al.* 2010). CG full methylated sites (as recognized by *Msp*I) are established during cell divisions by methyltransferase 1 (MET1), whereas CG hypomethylated sites (as recognized by *Hpa* II) are maintained by chromodomain-containing methyltransferase (CMT3) and domains rearranged methyltransferases (DRMs) (Salmon 2008). It seems that dynamic interaction between the methylation of a genome and the activity of the enzymes, DNA methyltransferases and demethylases, determine the final genome-wide pattern of DNA methylation (Rival *et al.* 2008).

Based on the data, along with the progression in plant development and the increase in specialized cell populations, the methylation statuses of genome altered. It means that cells probably turn on (or off) specific of genes by DNA methylation mechanism during differentiation and development. At a given developmental stage, plant response to the environmental variations will strikingly depend on accessible parts of genome and its gene contents. It may partly explain why plant response to the environment alterations can be related to the developmental stage at that condition. The results clearly demonstrated that the MSAP is a highly efficient technique for genome-wide DNA methylation analysis in the *Aeluropus littoralis*.

References

Allis, C.D., Jenuwein, T. and Reinberg, D. 2007. Overview and Concepts, In: Allis, C.D., Jenuwein, T., Reinberg, D., Caparros, M.L. (eds.) Epigenetic, Cold Spring Harbor Laboratory Press pp, 23-40.

Barhoumi, Z., Djebali, W., Chaibi, W., Abdelly, C. and Smaoui, A. 2007. Salt impact on photosynthesis and leaf ultrastructure of *Aeluropus littoralis*. *J Plant Res*, 120: 529-537.

Ben-Saad, R., Zouari, N., Ben-Ramdhan, W., Azaza, J., Meynard, D., Guiderdoni, E. and Hassairi, A. 2010. Improved drought and salt stress tolerance in transgenic tobacco overexpressing a novel A20/AN1 zinc-finger ''AlSAP'' gene isolated from the halophyte grass *Aeluropus littoralis*. *Plant Mol Biol*, 72: 171-190.

Chinnusamy, V. and Zhu, J.K. 2009. RNA-directed DNA methylation and demethylation in plants. *Sci China Ser C-Life Sci*, 52: 331-343.

Choi, C.S. and Sano, H. 2007. Abiotic-stress induces demethylation and transcriptional activation of a gene encoding a glycerophosphodiesterase-like protein in tobacco plants. *Mol Genet Genomics*, 277: 589-600.

Cokus, S.J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C.D., Pradhan, S., Nelson, S.F., Pellegrini, M. and Jacobsen, S.E. 2008. Shotgun bisulphite sequencing of the Arabidopsis

genome reveals DNA methylation patterning. *Nature*, 452: 215-219.

Fang, J. and Chao, C.T. 2007. Methylation-sensitive amplification polymorphism in date palms (*Phoenix dactylifera* L.) and their off shoots. *Plant Biol*, 9: 526-533.

Feng, S., Cokus, S.J., Zhangd, X., Chen, P.Y., Bostick, M., Golle, M.G., Hetzel, J., Jaine, J., Straussf, S.H., Halperne, M.E., Ukomadug, C., Sadlerh, K.C., Pradhani, S., Pellegrini, M. and Jacobsen, S.E. 2010. Conservation and divergence of methylation patterning in plants and animals. *PNAS,* 107: 8689-8694.

Fuso, A., Scarpa, S., Grandoni, F., Strom, R. and Lucarelli, M. 2006. A reassessment of semi quantitative analytical procedures for DNA methylation: Comparison of bisulfite and *Hpa* II polymerase chain reaction based methods. *Anal Biochem,* 350: 24-31.

Grunau, C., Renault, E., Rosenthal, A. and Roizes, G. 2001. MethDB-a public database for DNA methylation data. *Nucleic Acid Res,* 29: 270-274.

Hashemi, S.H., Nematzadeh, Gh., Askari, H. and Ghasemi, Y. 2013. Pattern of DNA cytosine methylation in *Aeluropus littoralis* during temperature stress. *J Plant Mol Breed*, 1: 16-24.

Hendrich, B. and Tweedie, S. (2003) The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends Genet*, 19: 269-277.

Labra, M., Grassi, F., Imazio, S., Fabio, T.D., Citterio, S., Sgorbati, S. and Agradi, E. 2004. Genetic and DNA-methylation changes induced by potassium dichromate in *Brassica napus* L. *Chemosphere,* 54: 1049-1058.

Loidl, P. 2004. A plant dialect of the histone language. *Trends Plant Sci,* 9: 85-90.

Lu, Y., Rong, T. and Cao, M. 2008. Analysis of DNA methylation in different maize tissues. *J Genet Genomics,* 35: 41-48.

Meijon, M., Valledor, L., Santamarıa, E., Testillano, P.S., Risueno, M.C., Rodrıguez, R., Feito, I. and Canal, M.J. 2009. Epigenetic characterization of the vegetative floral stages of azalea buds: Dynamics of DNA methylation and histone H4 acetylation. *J Plant Physiol,* 166: 1624-1636.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant,* 15: 473-497.

Paszkowski, J. and Whitham, S.A. 2001. Gene silencing and DNA methylation processes. *Curr Opin Plant Biol,* 4: 123-129.

Peng, H. and Zhang, J. 2009. Plant genomic DNA methylation in response to stresses: Potential applications and challenges in plant breeding. *Prog Nat Sci,* 19: 1037-1045.

Peredo, E.L., Arroyo-Garcia, R., Reed, B.M. and Revilla, M.A. 2008. Genetic and epigenetic stability of cryopreserved and cold-stored hops (*Humulus lupulus* L.). *Cryobiology,* 57: 234-241.

Portis, E., Acquadro, A., Comino, C. and Lanteri, S. 2004. Analysis of DNA methylation during germination of pepper (*Capsicum annuum* L.) seeds using methylation-sensitive amplification polymorphism (MSAP). *Plant Sci,* 166: 169-178.

Rauch, T.A. and Pfeifer, G.P. 2010. DNA methylation profiling using the methylated-CpG island recovery assay (MIRA). *Methods,* 52: 213-217.

Reik, W., Dean, W. and Walter, J. 2001. Epigenetic reprogramming in mammalian development. *Science,* 293: 1089-1093.

Rival, A., Jaligot, E., Beule, T. and Finnegan, E.J. 2008. Isolation and expression analysis of genes encoding MET, CMT, and DRM methyltransferases in oil palm (*Elaeis guineensis* Jacq.) in relation to the

'mantled' somaclonal variation. *J Exp Bot,* 59: 3271-3281.

Salmon, A., Clotault, J., Jenczewski, E., Chable, V.R. and Manzanares-Dauleux, M.J. 2008. *Brassica oleracea* displays a high level of DNA methylation polymorphism. *Plant Sci,* 174: 61-70.

Sha, A.H., Lin, X.H., Huang, J.B. and Zhang, D.P. 2005. Analysis of DNA methylation related to rice adult plant resistance to bacterial blight based on methylation-sensitive AFLP (MSAP) analysis. *Mol Gen Genomics,* 273: 484-490.

Tan, M. 2010. Analysis of DNA methylation of maize in response to osmotic and salt stress based on methylation-sensitive amplified polymorphism. *Plant Physiol Biochem,* 48: 21-26.

Tollefsbol, T.O. 2004. Methods of epigenetic analysis. *Methods Mol Biol,* 287: 1-8.

Vos, P., Hogers, R., Bleeker, M., Reijand, M., Van-de-Lee, T., Hornes, M., Fritjers, A., Pot, J., Paleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res,* 23: 4407-4414.

Wang, R.Z. 2004. Plant functional types and their ecological responses to salinization in saline grasslands, Northeastern China. *Photosynthetica,* 42: 511-519.

Xu, M.L., Li, X.Q. and Korban, S.S. 2000. AFLP-based detection of DNA methylation. *Plant Mol Biol Rep*, 18: 361-368.

Zhang, M., Kimatu, J.N., Xu, K. and Liu, B. 2010. DNA cytosine methylation in plant development. *J Genet Genomics*, 37: 1-12.

Zhao, X., Chai, Y. and Liu, B. 2007. Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. *Plant Sci,* 172: 930-938.

Zouari, N., Ben-Saad, R., Legavre, T., Azaza, J., Sabau, X., Jaoua, M., Masmoudi, K. and Hassairi, A. 2007. Identification and sequencing of ESTs from the halophyte grass *Aeluropus littoralis*. *Gene*, 404: 61-69.

بررسی متیلاسیون سیتوزین DNA در مراحل نموی گیاه *Aeluropus littoralis*

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چکیده:

متیلاسیون DNA یکی از راه‌های اپی‌ژنتیکی تنظیم دینامیکی بیان ژن در سطح ژنوم بوده که از نقش کلیدی در مراحل مختلف نمو و تمایز برخوردار می‌باشد. به منظور ارزیابی جایگاه متیلاسیون DNA در تحلیل واکنش گیاه شورپسند *Aeluropus littoralis* به شرایط مختلف نموی، از چندشکلی ناشی از متیلاسیون (MSAP) استفاده گردید. میزان تغییرات اپی‌ژنتیکی بصورت متیلاسیون و دمتیلاسیون در جایگاه CCGG با استفاده از مقایسه الگوی باندی ناشی از آنزیم‌های *Hpa*II و *Msp*I بررسی گردید. باندهای تیپ I (عدم متیلاسیون)، تیپ II (متیلاسیون در جایگاه CpG)، تیپ III (متیلاسیون در جایگاه CpCpG) به ترتیب ۷۵/۷ درصد، ۱۹/۴ درصد، ۴/۹ درصد از کل این باندها را به خود اختصاص دادند. بیشترین فراوانی متیلاسیون (۱۹/۴٪) در باندهای تیپ II بوده که در نوکلوتید سیتوزین در هر دو رشته توالی پالیندرومی متیله گردید. از ۴۸۰ باند مشاهده شده، ۳۳ باند در مراحل مختلف نموی از الگوی متیلاسیون متفاوتی برخوردار بودند. تنظیمات اپی‌ژنتیکی در این تحقیق دوجهته و بصورت تغییرات متیلاسیون و غیرمتیلاسیون بوده که در مجموع روند افزایشی در میزان متیلاسیون در طی فرایند نمو گیاه مشاهده گردید. تغییرات متیلاسیون و دمتیلاسیون مشاهده شده در جایگاه CG، می‌تواند در تنظیم بیان ژن وابسته به مراحل مختلف نموی نقش داشته باشد.

کلمات کلیدی: آلوروپوس لیتولاریس، اپی‌ژنتیک، متیلاسیون DNA، مراحل نموی، هالوفیت.