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Drought Stress Induces Transgenerational DNA Methylation in Rice

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ABSTRACT

Modification of DNA methylation is one of the important epigenetic mechanism by which plants modulate gene expression when they are exposed to harsh environmental conditions. Often heritable morphological variations are observed in different cultivars which are accompanied by DNA methylation polymorphisms when exposed to abiotic stresses. In this study, an attempt was made to study the transgenerational DNA methylation pattern at the whole genome level in plants subjected to different regimes of moisture *i.e.,* puddled, 100 and 60 per cent field capacities (FC). Based on the methylation sensitive randomly amplified polymorphic DNA analysis (MS-RAPD), hypo methylation was observed in the parental generation under 100 and 60 per cent FC compared to puddled conditions. The offspring of puddled plants grown under puddled, 100 and 60 per cent showed hyper methylation under stress. Offspring from 100 per cent FC plants showed no change in the methylation pattern. Progenies of 60 per cent FC plants showed hypo methylation under stress. The study indicates that drought stress can induce transgenerational hypo methylation in rice.

Keywords: MS-RAPD, Rice, Apo, Drought, Transgeneration, DNA methylation

PLANTS have the ability to protect themselves from adverse environmental conditions by using various strategies. The understanding of how plants sense and respond to various stresses have become an important part of molecular studies (Golldack *et al*., 2011). Among the different environmental stresses, drought stress is considered as the most important stress that affects plant growth and productivity (Farooq *et al*., 2009). Due to the severe effects of drought stress on plants, understanding of different mechanisms under drought tolerance is important which would help to improve crop productivity (Tardieu *et al*., 2018). Several adaptive mechanisms involving genetics have been identified in plants (Kumar *et al*., 2017), but very few studies exist on epigenetic mechanisms.

Epigenetic mechanism involving DNA methylation is being studied in plants and animals (Zemach *et al*., 2010 & Garg *et al*., 2015). DNA methylation is known to occur at three different contexts in plants *i.e.,* CG (Cytosine followed by Guanine residues), CHH and CHG (H is any nucleotide) (Bewick and Schmitz, 2017). Plant development is known to be controlled by DNA methylation under normal conditions and is also known to be linked to environmental stimuli in plants. DNA methylation is known to be modified under drought stress, which further affects gene expression (Tang *et al*., 2014), and is passed on to the next generation (Zheng *et al*., 2017). Studies on transgenerational DNA methylation is gaining importance as it has been identified that past environmental conditions of parents reflect upon the stress adaptation mechanism of the progenies (Zheng *et al*., 2017). DNA methylation is stored as plant stress memory (Goodrich and Tweedie, 2002), and inherited epigenetic marks help plants to be prepared for new stress (Yu *et al*., 2013). Better understanding on these types of mechanisms would be helpful for targeted crop improvement.

Rice is considered as a staple food of nearly three billion people in the world (Carriger and Vallée, 2007). As it is grown in anaerobic lowland conditions, nearly 3000 to 5000 litres of water is required to produce one kilogram of rice (Bouman *et al.,* 2002). A feasible option to address the issue of decreasing freshwater situation for rice is practising cultivation under semi-irrigated, aerobic conditions. Aerobic rice is a production system in which specially developed varieties are grown in well-drained, non-puddled and non-saturated

soils. Due to frequent spells of drought at critical stages of crop growth, it is necessary to ensure the drought adaptation of aerobic rice. Efforts are being made to examine various adaptive mechanisms for targeted crop improvement. Many genetic pathways linked to drought stress adaptation has been identified in rice (Mohanty *et al*., 2016), however, limited information is available on epigenetic regulation in rice. In fact, very few studies have been reported on transgenerational epigenetic mechanism under stress in crops (Wang *et al*., 2010, 2016). Thus, the main objective of the study was to understand the epigenetic mechanism and the inheritance of variation in DNA methylation under drought stress in rice.

Material and Methods

Plant material and experimental design

Two days old seedlings of rice (*Oryza sativa*) genotype, Apo, (aerobic, upland cultivar) were transplanted in polybags in a containment facility under controlled conditions for 15 days. Twenty days old healthy seedlings were transplanted to pots (30 kg capacity) filled with a mixture of garden soil and grown in green house conditions. One set of plants were grown under puddled condition while other sets were maintained at 100 per cent soil field capacity (FC) by gravimetric approaches (Karaba *et al*., 2007). At vegetative stage (30 days after transplanting), a subset of the plants maintained at 100 per cent FC was exposed to drought stress (60 per cent FC). For checking the transgenerational variation, the progenies of the puddled plants were grown in puddled, 100 and 60 per cent FC, similarly the progenies of the 100 and 60 per cent FC plants were subjected to puddled, 100 and 60 per cent FC. For methylation analysis, fourth leaf of every tiller was collected 14 days post stress imposition.

DNA methylation studies and MS-RAPD analysis

The cetyl trimethyl ammonium bromide (CTAB) method was used to extract DNA (Doyle and Doyle, 1987). The methylation sensitive randomly amplified polymorphic DNA (MS-RAPD) analysis was performed as described by Erturk *et al*. (2014). Two enzymes *Hpa*II, which cannot cut CCGG when internal cytosine is methylated and *Msp*I, which cannot cleave CCGG when the external cytosine is methylated, were used for this analysis. Out of 40 primers, ten and five primers that gave reproducible results were selected for analysing the variation in DNA methylation in the parental generation and their progenies, respectively. The PCR program run in a thermal cycler (ProFlex PCR systems by Applied Bio systems) was as follows: 3 min at 95°C, followed by 45 cycles of 95°C (1min), 35°C, (1min) and 72°C (90sec), and a final extension of 10 min at 72°C. The PCR products were seperated on 1.5 per cent (w/v) agarose gel and amplified product was detected by ethidium bromide staining (Sambrook *et al*., 1989). Polymorphism in the MS-RAPD profile was identified as the disappearance of a normal band (hypo methylation) and appearance of a new band (hyper methylation) in 100 and 60 per cent FC in comparison to puddled condition (Erturk *et al*., 2014).

Results and Discussion

Methylation sensitive randomly amplified polymorphic DNA (MS-RAPD) in parental generation

The MS-RAPD analysis was performed in the drought tolerant rice genotype Apo (Muthukumar *et al*., 2017) grown under three different water regimes. When

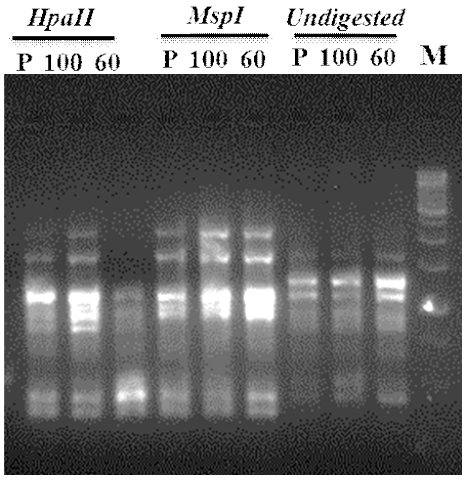

Fig. 1: Representative image of MS-RAPD profile from DNA of rice plants of parental generation challenged with drought stress

P- Puddled, 100- 100 per cent FC, 60- 60 per cent FC

HpaII and *MspI*-Indicates the products generated from *HpaII* and *MspI* digested DNA

DNA from plants grown under puddled and 100 per cent FC were subjected to MS-RAPD analysis using the primer Y18 (GTGGAGTCAG), similar numbers of fragments were observed in *Hpa*II and *Msp*I, which suggest that cytosines were fully methylated (Table 1). Whereas hemi methylation (only internal cytosine) was observed under 60 per cent FC, as reduced number of bands were noticed in the DNA digested with *Hpa*II than *Msp*I (Fig.1, Table 1). Similar trend was observed in the most of the primers used for this analysis. Thus, it can be concluded that methylation is reduced under 60 per cent FC. This suggests that drought stress induces hypo methylation in Apo, which is a drought tolerant genotype. A similar pattern was observed in maize plants exposed to heavy metal such as chromium (Cr) in the form of chromium nitrate (Erturk *et al*., 2014) and also in other crops like cotton (Karaca *et al*., 2019). DNA methylation is known to be reversed when plants are exposed to different stresses (Wang *et al*., 2010). The methylome of a tolerant genotype is known to be more stable than a susceptible genotype (Zheng *et al*., 2013).

TABLE 1

Polymorphic bands obtained from the MS-RAPD analysis of genomic DNA isolated from leaf tissues of rice genotype, Apo grown under different moisture levels in (parent generation)

Primer	Puddled		Total number of bands				Total polymorphic bands			
			100		60		100		60	
	H	M	H	M	H	M	H	M	H	M
P 15	6	6	4	4	6	4	2	2	0	2
R 2	3	2	3	2	1	2	0	0	2	0
R 6	2	5	4	3	1	3	2	2	1	2
R 15	2	2	2	2	1	2	0	0	1	0
R 20	2	1	2	2	1	1	0	1	1	0
T 7	8	4	5	5	2	6	3	1	6	2
X 11	4	5	5	5	4	2	1	0	0	3
Y 16	3	2	2	2	4	2	1	0	1	0
Y 17	1	6	6	6	1	6	5	0	0	0
Y 18	8	8	8	8	6	8	0	0	2	2

H= *HpaII*, M=*MspI,* FC= Soil moisture capacity

TABLE 2

Polymorphic bands obtained from the MS-RAPD analysis using leaf tissues of rice genotype, Apo having puddled parents under different moisture (first generation)

Primer	P to P		Total number of bands				Total polymorphic bands			
			P to 100		P to 60		P to 100		P to 60	
	H	M	H	M	H	M	H	M	H	M
R 2	4	2	4	2	3	2	0	0	1	0
R 6	3	3	2	2	2	3	1	1	1	0
R 15	2	2	2	2	1	1	0	0	1	1
R 9	2	3	4	4	2	3	2	1	0	0
X 11	4	4	3	4	4	4	1	0	0	0

H= HpaII, M=MspI, P= Puddled, 100= 100% FC, 60= 60% FC, FC= Soil moisture capacity

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Transgenerational variation of DNA methylation

Variation in the DNA methylation was analyzed between two generations. When the *Hpa*II and *Msp*I digested products of the offspring of puddled plants were analysed using the random primers, internal cytosine was observed to be methylated under puddled, 100 and 60 per cent FC (Fig. 2, Table 2). The methylation pattern in the progenies from 100 per cent FC did not change when subjected to 100 and 60 per cent FC (Table 3). This indicates that the methylation

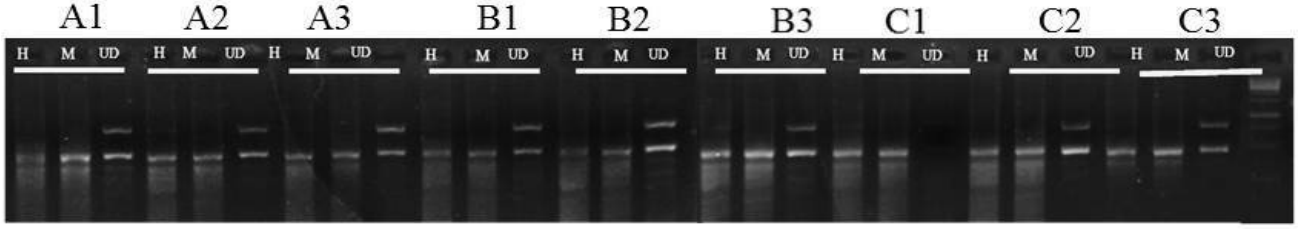

Fig. 2: Representative image of MS-RAPD PCR with primer R2 showing the difference in banding pattern in rice genotypes of the offspring

A1 = Puddled to Puddled, A2 = Puddled to 100% FC, A3 = Puddled to 60% FC, B1 = 100% FC to Puddled, B2 = 100% FC to 100% FC, B3 = 100% FC to 60% FC, C1= 60% FC to Puddled, C2 = 60% FC to 100% FC and C3 = 60% FC to 60% FC. H, M - Indicated products generated by HpaII and MspI digested DNA, UD - Undigested DNA

TABLE 3

Polymorphic bands obtained from the MS-RAPD analysis using leaf tissues of rice genotype, Apo grown from seeds of plants subjected to 100% FC (100% FC parents) under different moisture levels (first generation)

Primer	Total number of bands						Total polymorphic bands			
Apo	100 to P		100 to 100		100 to 60		100 to 100		100 to 60	
	H	M	H	M	H	M	H	M	H	M
R2	2	2	1	1	1	1	1	1	1	1
R6	3	4	3	3	3	3	0	1	0	1
R15	2	2	2	2	2	1	0	0	0	1
R9	4	4	5	5	4	5	1	1	0	1
X11	2	2	2	2	3	3	0	0	1	1

H= HpaII, M=MspI, P= Puddled, 100= 100% FC, 60= 60% FC, FC= Soil moisture capacity

TABLE 4

Polymorphic bands obtained from the MS-RAPD analysis using leaf tissues of rice genotype, Apo plants grown from seeds of plants subjected to 60% FC (60% FC parents) under different moisture levels (first generation)

Primer	Total number of bands						Total polymorphic bands			
Apo	60 to P		60 to 100		6 to 60		60 to 100		6 to 60	
	H	M	H	M	H	M	H	M	H	M
R2	2	2	1	1	1	1	1	1	1	1
R6	4	3	3	4	2	2	1	1	2	1
R15	2	2	1	2	1	2	1	0	0	0
R9	3	3	3	2	1	3	0	1	2	0
X11	3	3	3	3	4	4	0	0	1	1

H= HpaII, M=MspI, P= Puddled, 100= 100% FC, 60= 60% FC, FC= Soil moisture capacity

was maintained under stress conditions in the progenies. However, when the offspring of the plants grown under 60 per cent FC were subjected to 100 and 60 per cent FC a reduction in the band number was observed in *Hpa*II and *Msp*I (Table 4). This suggests that there was a reduction in methylation under stress compared to puddled.

The study indicates that the plants subjected to stress in the previous generation could retain the methylation status in the next generation under stressful condition. This data suggests that the pattern of DNA methylation maybe transgenerational. These results are consistent with earlier studies which suggest that directly induced DNA methylation is transmitted to the next generation faithfully (Zheng *et al*., 2017).

In this study, when plants were exposed to similar water status, there was retention of methylation in the next generation which suggests that drought induced DNA methylation maybe transgenerational. Global methylome analysis along with global transcriptome analysis would give a better clarity on the epigenetic regulation mechanism.

REFERENCES

BEWICK, A. J. AND SCHMITZ, R. J., 2017, Gene body DNA methylation in plants. *Curr. Opin. Plant Biol.,* **36:** 103-110.

BOUMAN, B. A. M., WANG, H., YANG, X., ZHAO, J. AND WANG, C., 2002, Aerobic rice (Han Dao) : a new way of growing rice in water-short areas. In Proceedings of the 12th international soil conservation organization conference. Beijing, China: Tsinghua University Press, **26** : 31.

CARRIGER, S. AND VALLÉE, D., 2007, More crop per drop. *Rice Today*, **6** (2) : 10 - 13.

DOYLE, J. J. AND DOYLE, J. L., 1987, A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem.,* **19** : 11 - 15.

ERTURK, F. A., AGAR, G., ARSLAN, E., NARDEMIR, G. AND SAHIN Z., 2014, Determination of genomic instability and DNA methylation effects of Cr on maize (*Zea mays* L.) using RAPD and CRED-RA analysis. *Acta. Physiol. Plant.,* **36** : 1529 - 1537.

FAROOQ, M., WAHID, A., KOBAYASHI, N., FUJITA, D. AND BASRA, S. M. A., 2009, Plant drought stress: effects, mechanisms and management. In *Sustainable agriculture*, 153 - 188, Springer, Dordrecht.

GARG, R., CHEVALA, V. N., SHANKAR, R. AND JAIN, M., 2015, Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response. *Sci. rep*., **5** : 14922.

GOLLDACK, D., LÜKING, I. AND YANG, O., 2011, Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. *Plant cell rep*., **30** (8) : 1383 - 1391.

GOODRICH. J., S. AND TWEEDIE, 2002, Remembrance of things past : chromatin remodelling in plant development, *Annu. Rev. Cell Dev. Bio.,* **18** : 707 - 746.

KARABA, A., DIXIT, S., GRECO, R., AHARONI, A., TRIJATMIKO, K. R., MARSCH - MARTINEZ, N., KRISHNAN, A., NATARAJA, K. N., UDAYAKUMAR, M. AND PEREIRA, A., 2007, Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. *Proc. Natl. Acad. Sci., USA* ., **104** (39) : 15270 - 15275.

KARACA, M., AYDIN, A. AND INCE, A. G., 2019, Cytosine methylation polymorphisms in cotton using TD-MS-RAPD-PCR. *Mod. Phytomorphol.,* **13** : 13 - 19.

KUMAR, S., BEENA, A. S., AWANA, M. AND SINGH, A., 2017, Physiological, biochemical, epigenetic and molecular analyses of wheat (*Triticum aestivum*) genotypes with contrasting salt tolerance. *Front. Plant Sci.,* **8** : 1151.

MUTHUKUMAR, M, SASIKALA, R, ROBIN, S. AND RAVEENDRAN, M., 2017, Developing improved versions of a popular rice variety (Improved White Ponni) through marker assisted backcross breeding. Green Farming : *Int. J Appl. Agric. Horti. Sci*., **8** (3)**:** 1 - 6.

MOHANTY, B., KITAZUMI, A., CHEUNG, C. M., LAKSHMANAN, M., BENILDO, G., JANG, I. C. AND LEE, D. Y., 2016, Identification of candidate network hubs involved in metabolic adjustments of rice under drought stress by integrating transcriptome data and genome-scale metabolic network. *Plant Sci.*, **242** : 224 - 239.

SAMBROOK, J., FRITSCH, E. F., MANIATIS, T., 1989, Molecular Cloning : A Laboratory Manual. Harbor Laboratory Press, Cold Spring Harbor, New York.

TANG, X. M., TAO, X., WANG, Y., MA, D. W., LI, D., YANG, H. AND MA, X. R., 2014, Analysis of DNA methylation of perennial ryegrass under drought using the methylation-sensitive amplification polymorphism (MSAP) technique. *Mol. Genet. Genomics.*, **289** (6) : 1075 - 1084.

TARDIEU, F., SIMONNEAU, T. AND MULLER, B., 2018, The physiological basis of drought tolerance in crop plants : a scenario-dependent probabilistic approach. *Annu. Rev. Plant Biol*., **69** : 733 - 759.

WANG, W. S., PAN, Y. J., ZHAO, X. Q., DWIVEDI, D., ZHU, L. H., ALI, J., FU, B. Y. AND LI, Z. K., 2010, Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (*Oryza sativa* L.). *J. Exp. Bot.*, **62** (6) : 1951 - 1960.

WANG, W., QIN, Q., SUN, F., WANG, Y., XU, D., LI, Z. AND FU, B., 2016, Genome-wide differences in DNA methylation changes in two contrasting rice genotypes in response to drought conditions. *Front. Plant Sci.,* **7**: 1675.

YU, Y., YANG, X., WANG, H., SHI, F., LIU, Y., LIU, J. AND LIU, B., 2013, Cytosine methylation alteration in natural populations of Leymus chinensis induced by multiple abiotic stresses. *PloS one.,* **8** (2): e55772.

ZEMACH, A., KIM, M. Y., SILVA, P., RODRIGUES, J. A., DOTSON, B., BROOKS, M. D. AND ZILBERMAN, D., 2010, Local DNA hypomethylation activates genes in rice endosperm. *Proc. Natl. Acad. Sci., USA,* **107** (43) : 18729 - 18734.

ZHENG, X., CHEN, L., LI, M., LOU, Q., XIA, H., WANG, P. AND LUO, L., 2013, Transgenerational variations in dna methylation induced by drought stress in two rice varieties with distinguished difference to drought resistance. *Plos One.,* **8** (11) : e80253.

ZHENG, X., CHEN, L., XIA, H., WEI, H., LOU, Q., LI, M., LI, T. AND LUO, L., 2017, Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. *Sci. Rep*., **7**, 39843.

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