Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2020) **18**, pp. 1648–1650 doi: 10.1111/pbi.13336

Brief Communication

Somatic embryogenesis critical initiation stage-specific ^{m}CHH hypomethylation reveals epigenetic basis underlying embryogenic redifferentiation in cotton

Huihui Guo, Yijie Fan, Haixia Guo, Jianfei Wu, Xiaoman Yu, Junmei Wei, Xin Lian, Li Zhang, Zhongyuan Gou, Yupeng Fan and Fanchang Zeng

State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an, China

Received 4 September 2019;
accepted 7 January 2020.
**Correspondence* (Tel +86-538-8241828; fax +86-538-8242226; email fczeng@sdau.edu.cn)

Keywords: cotton, somatic embryogenesis, embryogenic redifferentiation, single-base resolution DNA methylation, ^{m}CHH hypomethylation, epigenetic marker and molecular basis, lipid pathway.

As a notable illustration of totipotency, somatic embryogenesis (SE) is the developmental reprogramming of somatic cells towards the embryogenesis pathway (Yang and Zhang, 2010). Investigations examining the totipotency process are of great fundamental and practical importance in crop biotechnology. Moreover, high-frequency regeneration of SE has been limited due to the genotype-dependent response. To date, the epigenetic molecular basis underlying embryogenic redifferentiation during SE remains largely unexplored.

Plant embryogenesis is accompanied by changes at chromatin level and reprogramming of gene expression, highlighting the central role of epigenetic regulation (Miguel and Marum, 2011). During SE, DNA methylation is continually changing to satisfy cell requirements (Nic-Can and De-la-Peña, 2014). The methylation of DNA is essential to SE (De-la-Peña *et al.*, 2015; Kumar and Van Staden, 2017). Recently, Ji *et al.* (2019) and Li *et al.* (2019) also reported DNA methylation variations during plant SE.

SE is the concerted process involving multiple cellular pathways controlled by epigenetic and genetic variability (De-la-Peña *et al.*, 2015; Miguel and Marum, 2011). Genome-wide dissection of dynamic methylation modification features is conducive to explaining the complex underlying genotype-dependent SE transdifferentiation at overall level. In this study, a single-base resolution of genome-wide bisulfite sequencing (BS-seq) and transcriptome sequencing was performed to comprehensively analyse the DNA methylation and gene regulatory patterns involved in SE transdifferentiation in two cotton genotypes with distinct embryogenic abilities. Three typical stages of early SE: hypocotyls (HY), nonembryogenic calli (NEC) and primary embryogenic calli (PEC), extending from callus dedifferentiation (NEC-VS-HY) to embryogenic redifferentiation (PEC-VS-NEC) were examined for BS-seq (Figure 1a–c). Two genotypes, Yuzao 1 (YZ) with a high embryogenic ability (>80%) and Lumian 1 (LM) with a very low ability (<10%) (Jin *et al.*, 2006), were selected.

Total methylcytosines (^{m}Cs) were identified at dedifferentiation and embryogenic redifferentiation during early SE in the two genotypes. The percentages of genomic methylation dynamic of ^{m}CG and ^{m}CHG had similar patterns among the samples with the opposite of ^{m}CHH methylation (Figure 1d). The overall ^{m}CG levels accounted to the highest extent followed by ^{m}CHG and then ^{m}CHH (Figure 1e). Notably, ^{m}Cs levels presented different patterns during embryogenic redifferentiation in the two genotypes, continuing to rise in LM but decreased at the PEC stage in YZ (Figure 1e).

The methylcytosine levels of three sequence contexts (^{m}CG, ^{m}CHG and ^{m}CHH) were further overviewed in different genic regions, gene body (exon and intron), 2 kb upstream and downstream of transcription start sites as well. (Figure 1f–h). Results showed that DNA methylation in the three sequence contexts was not evenly distributed among genomic transcriptional elements. Upstream and downstream regions were most highly methylated, particularly for ^{m}CG. Moreover, to assess DNA methylation between developmental specific stages and between genotypes, hierarchical clustering of methylcytosine levels was performed. The results showed that the global pattern of ^{m}CG was more distinguishable between the two genotypes than between the developmental stages (Figure 1f), whereas it was more discernible between the developmental stages at the ^{m}CHH site (Figure 1h). These observations remarkably indicated that methylation levels at the CG site were genotype-specific, whereas differentiation stage-specific at the CHH site during early SE process.

The methylcytosine levels of ^{m}CHH in genome-wide transcriptional elements were further investigated during early SE in LM and YZ (Figure 1i,j). During embryonic redifferentiation, patterns of CHH methylation showed significant differences in the two genotypes. A lower (significantly declined) level of CHH methylation was observed at YZ_PEC (Figure 1j). This result could, to some extent, explain the highly embryogenic redifferentiation ability in YZ, which suggested that CHH hypomethylation marked and distinguished embryonic redifferentiation.

To further investigate SE initiation promoting methylated genes, the differentially methylated genes (DMGs) were identified and significantly enriched in lipid biosynthetic and metabolic processes in YZ embryonic redifferentiation (Figure 1k). Differentially methylated key genes involved in lipid pathway were confirmed to be transcriptionally affected during embryogenic redifferentiation. The results in the highly embryogenic genotype were consistent with and extended our recent report (Guo *et al.*, 2019).

Simultaneously, for association analysis of DNA methylation and expression levels at embryonic redifferentiation during SE transdifferentiation in the two genotypes, a cross-analysis

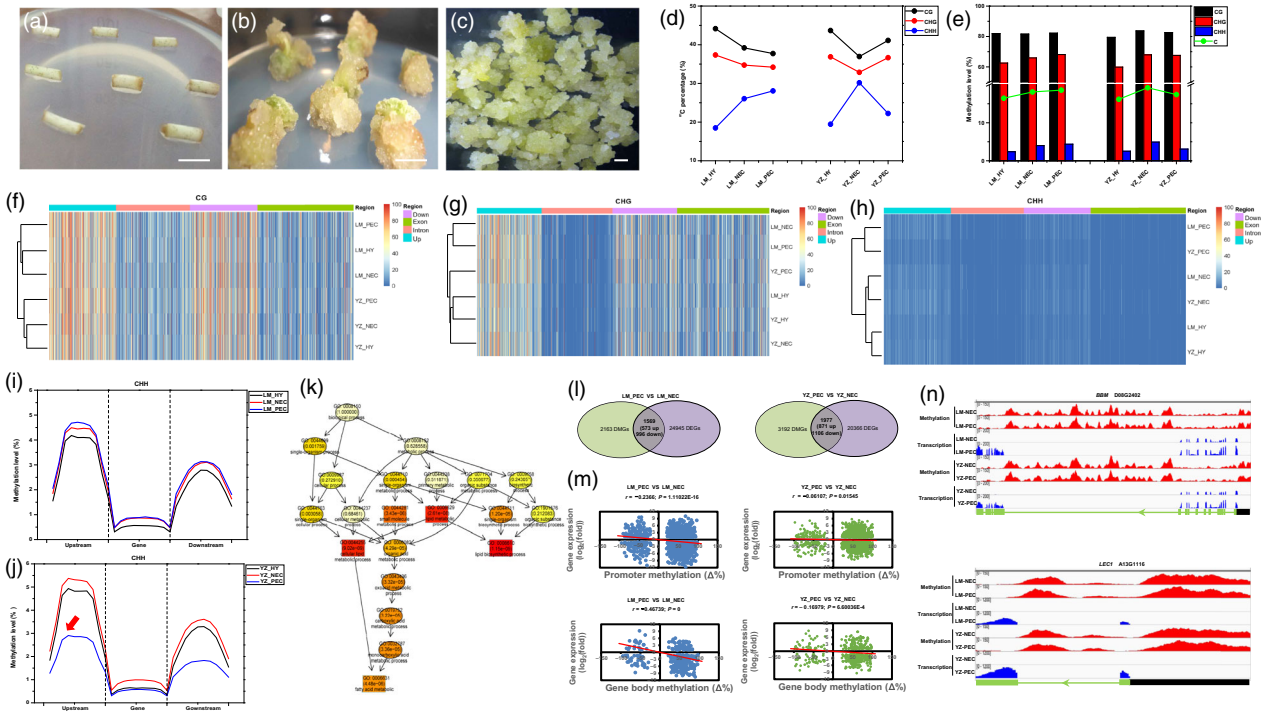

Figure 1 Genome-wide single-base resolution dynamic DNA methylome reveals CHH hypomethylation marked and distinguished the embryogenic redifferentiation. (a–c) Morphology of critical developmental stages during cotton SE. (a) Hypocotyls (HY). (b) Dedifferentiated nonembryogenic calli (NEC). (c) Redifferentiated primary embryogenic calli (PEC). Bar = 1 mm. (d–e) Overall methylcytosines (^{m}CG, ^{m}CHG and ^{m}CHH) during SE transdifferentiation in LM and YZ. (d) Percentage of methylcytosines. (e) Methylation levels of methylcytosines. (f–h) Clustering of methylation levels of ^{m}CG, ^{m}CHG and ^{m}CHH on different transcriptional elements during SE transdifferentiation in LM and YZ. (i) ^{m}CHH methylation levels on different transcriptional elements in LM. (j) ^{m}CHH methylation levels on different transcriptional elements in YZ. (k) Enrichment of differentially methylated genes during embryogenic redifferentiation in YZ (PEC VS NEC). (l–n) Association analysis of DNA methylome and transcriptome during embryogenic redifferentiation in LM and YZ. (l) Codifferential genes with significant variations in both DNA methylation and transcription in two genotypes respectively, combining hyper- and hypomethylated genes at three sequence contexts. (m) Correlation analysis of variations in DNA methylation and transcription on gene-body and promoter regions. (n) Representative genes showing negative correlations between DNA methylation and transcription. *BBM*, *Baby boom*; *LEC1*, *Leafy cotyledon 1*. Tracks of BS-seq and RNA-seq reads were shown for each gene, including the transcribed regions and the upstream regions. Gene structures are shown at the bottom, with light green boxes representing exons, light green lines representing introns, black boxes representing upstream 2 kb regions and arrows indicating transcription direction.

identified 1569 and 1977 genes in two genotypes respectively showing significant variations in both methylation and gene expression (termed codifferential genes) (Figure 1l). Among these genes, 1263/306 and 1606/371 codifferential genes were modified by methylation in their upstream/gene-body regions, respectively. Furthermore, we quantitatively examined the correlations between variations in DNA methylation and variations in gene expression during SE initiation. The results showed that compared with YZ, there was a higher negative correlation of variations in LM in both upstream and gene-body regions (Figure 1m,n), which suggested that transcription variations were more negatively modulated by DNA methylation in LM, the SE recalcitrant genotype in cotton.

For successful achievement of plant SE, genotype-dependent DNA methylation remains crucial. In this study, we reported that CHH demethylation could serve as the critical epigenetic marker and associated with embryonic redifferentiation in the highly embryogenic genotype, while CHH hypermethylation in the recalcitrant genotype, which suggested the negative effect on SE-associated genes during embryonic redifferentiation. However, future research is necessary to explain how DNA methylation is established and to elucidate the molecular mechanisms regulating SE transdifferentiation.

The systematic epigenetic molecular basis underlying cell totipotency and SE transdifferentiation are poorly understood in plants. Especially, the genotype-dependent critical methylation features associated with embryogenic redifferentiation remains largely unexplored. In our study, integrated maps of genome-wide DNA methylomes at single-base resolution and transcriptomes were generated during cotton SE, spanning cell dedifferentiation to embryogenic redifferentiation, in two genotypes with distinct embryogenic abilities. Dynamic DNA methylation variations and their relationships with transcriptional divergence between different genotypes and developmental stages were globally surveyed. Our data revealed that total methylcytosine (^{m}C) levels presented a hypomethylation pattern during embryogenic redifferentiation in the highly embryogenic genotype. DNA methylation (^{m}CG, ^{m}CHG and ^{m}CHH) were significantly distributed on genomic up and downstream transcriptional elements. Significantly, the global pattern of ^{m}CG displayed genotype-specific, and the ^{m}CHH pattern was particularly determined to be differentiation stage-specific during SE process. The

hypomethylated ^{m}CHH notably marked and distinguished embryonic redifferentiation. And differentially methylated genes (DMGs) were significantly enriched in the lipid pathway in embryogenic redifferentiation. Furthermore, systematic association analysis of DNA methylome and transcriptome indicated that gene expression variations were more strongly modulated by DNA methylation in the recalcitrant genotype. Compared with previous significant report of the genome-wide increase in CHH methylation during SE, using one genotype (Ji *et al.*, 2019; Li *et al.*, 2019), our current study characterized CHH hypermethylation in LM with low SE ability, but CHH hypomethylation in YZ with high SE ability during embryogenic redifferentiation process. These results suggested the importance of genotype-dependent methylation modes. The results in this study revealed a comprehensive overview of genotype-dependent dynamic DNA methylation associated with regulated gene expression during cotton SE. Our study provides new insights into the underlying epigenetic molecular basis and critical methylation modes associated with embryogenic competence acquisition during SE transdifferentiation, thereby holding great promise for its advancement in recalcitrant plant species.

Acknowledgements

This work was supported by National Key Research and Development Program (2016YFD0100306; 2018YFD0100303), Taishan Scholar Talent Project from PRC (TSQN20161018) and the National Natural Science Foundation of China (31401428).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

H.H.G. and F.C.Z. conceived and designed the research project. H.H.G., H.X.G., X.M.Y., J.M.W. and Y.P.F. performed cell culture and sampling, H.H.G., Y.J.F. and L.Z. performed all morphological and molecular experiments. H.H.G., J.F.W., X.L, Z.Y.G and L.Z. performed the BS-Seq and RNA-Seq studies and data analysis. H.H.G. and F.C.Z. wrote the article.

References

De-la-Peña, C., Nic-Can, G.I., Galaz-Ávalos, R.M., Avilez-Montalvo, R. and Loyola-Vargas, V.M. (2015) The role of chromatin modifications in somatic embryogenesis in plants. *Front. Plant Sci.* **6**, 635–649.

Guo, H., Guo, H., Zhang, L., Fan, Y., Fan, Y. and Zeng, F. (2019) SELTP assembled battery drives totipotency of somatic plant cell. *Plant Biotechnol. J.* **17**, 1188–1190.

Ji, L., Mathioni, S.M., Johnson, S., Tucker, D., Bewick, A.J., Do Kim, K., Daron, J. *et al*. (2019) Genome-wide reinforcement of DNA methylation occurs during somatic embryogenesis in soybean. *Plant Cell*, **31**, 2315–2331.

Jin, S., Zhang, X., Nie, Y., Guo, X., Liang, S. and Zhu, H. (2006) Identification of a novel elite genotype for in vitro culture and genetic transformation of cotton. *Biol. Plant.* **50**, 519–524.

Kumar, V. and Van Staden, J. (2017) New insights into plant somatic embryogenesis: an epigenetic view. *Acta Physiol. Plant.* **39**, 194–210.

Li, J., Wang, M., Li, Y., Zhang, Q., Lindsey, K., Daniell, H., Jin, S. *et al.* (2019) Multi-omics analyses reveal epigenomics basis for cotton somatic embryogenesis through successive regeneration acclimation process. *Plant Biotechnol. J.* **17**, 435–450.

Miguel, C. and Marum, L. (2011) An epigenetic view of plant cells cultured in vitro: somaclonal variation and beyond. *J. Exp. Bot.* **62**, 3713–3725.

Nic-Can, G.I. and De-la-Peña, C. (2014) Epigenetic advances on somatic embryogenesis of agronomical and important crops. In *Epigenetics in Plants of Agronomic Importance: Fundamentals and Applications* (Alvarez-Venegas, R., ed.), pp. 91–109. Cham: Springer.

Yang, X. and Zhang, X. (2010) Regulation of somatic embryogenesis in higher plants. *Crit. Rev. Plant Sci.* **29**, 36–57.

