Proceedings of the LXII SIGA Annual Congress
Verona, Italy – 25/28 September, 2018
ISBN **978-88-904570-8-1**

Oral Communication Abstract – 6.05

MCSeEd, A METHOD FOR DETECTING CHANGES IN DNA METHYLATION AND CALLING SNPs WITH OR WITHOUT A REFERENCE GENOME

MARCONI G.*, CAPOMACCIO S.**, COMINO C.***, CERRUTI E.***, ACQUADRO A.***, PORTIS E.***, PORCEDDU A.****, ALBERTINI E.*

*) Department Agricultural, Food and Environmental Sciences, University of Perugia, Perugia (Italy)
**) Department of Veterinary Medicine, University of Perugia, Perugia (Italy)
***) Department of Agricultural, Forest and Food Sciences, Plant Genetics and Breeding, University of Torino, Grugliasco (Italy)
****) Department of Agricultural, University of Sassari, Sassari (Italy)

Zea mais, DNA methylation, SNP calling, stress, development

DNA methylation is a heritable epigenetic modification that is closely associated with gene expression and chromatin structure, and it is critical in the developmental processes of animals, plants, and fungi. In fact, plants can employ DNA methylation to enable relatively rapid adaptation to new environmental conditions as well as to respond to biotic and abiotic stresses. Several investigations have now demonstrated that cytosine methylation plays important role in regulating the response to various stresses. Traditionally, two main approaches have been used for investigating the genome-wide extent of cytosine methylation: digestion with methylation-sensitive restriction enzymes coupled with electrophoresis (i.e. MSAP - Methylation-sensitive amplification polymorphism) and bisulfite conversion followed by NGS (i.e. BS-seq). Both these approaches have some constrains. BS-seq requires a reference genome for the alignment of converted and unconverted sequences while MSAP does not, but at the cost of providing only a small picture of the overall methylation status. Recently, to overcome this limitation, some strategies involving methylation-sensitive restriction enzymes combined with high-throughput sequencing were developed.

Here we propose MCSeEd (Methylation Context Sensitive Enzyme ddRAD) a new method that provides both DNA methylation polymorphisms in symmetric and asymmetric contexts and SNPs between samples, with or without a reference genome. To achieve this, highly multiplexed libraries were generated with a simultaneous double restriction-ligation employing an enzyme sensitive to methylation and an enzyme insensitive to methylation. In particular, we separately employed *Aci*I, *Pst*I and *EcoT22*I in combination with *Mse*I to infer the CG, CHG and CHH contexts methylation state. Moreover, for the best of our knowledge the use of *Dpn*II enzyme allowed us to study, for the first time, the large-scale analysis of changes in Adenine methylation.

To test its effectiveness and reliability, MCSeEd was applied in maize, an important crop with a complex genome, comparing plants grown under normal irrigation (WW) and plants grown under drought stress (DS) of a commercial hybrid and to a comparison between B73 tissues (shoots and roots) collected at 5 days after germination.

Our results, demonstrating the reliability of MCSeEd either in depicting methylation state or calling SNPs, will be reported and discussed.