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

Oral Communication Abstract – 6.02

ANNOTATION AND EXPRESSION OF FDM-LIKE GENES IN SEXUAL AND APOMICTIC MODEL SPECIES

BASSO A., BARCACCIA G., GALLA G.

Laboratory of Plant Genetics and Breeding, DAFNAE - University of Padova, Campus of Agripolis, Viale dell'Università 16, 35020 Legnaro (Italy)

Hypericum perforatum, sexual reproduction, aposporous apomixis, RdDM, FDM

Aposporous apomixis is a reproductive strategy that leads to seed production by avoiding female sporogenesis and, eventually, fertilization. In this peculiar reproductive strategy, embryo and endosperm are formed from unreduced gametes developing from somatic cells belonging to the ovule nucellus. Recently gained information on ovule gene expression in the apomictic model species *Hypericum perforatum* L. demonstrated that a few genes involved in the RNA-directed DNA methylation pathway (RdDM) are differentially expressed in ovules collected from apomictic accessions at pre-meiotic stages. In *A. thaliana,* the protein Involved in De Novo 2 (IDN2), together with members of the gene family Factors of DNA Methylation (FDM1, FDM2), acts downstream of the RdDM. In this pathway, IDN2/FDM complex are recruited to the chromatin by the ra-siRNA-Pol V transcript duplex, and then bind the un-methylated DNA to promote DNA methylation of both transposons and protein coding genes. Remarkably, the knock-out of genes involved in the RdDM in sexually reproducing species such as *A. thaliana* and *Z. mays* results in phenotypes mimicking early events of aposporous apomixis. Taken together, these findings let us to hypothesize that RNA-directed DNA methylation might be involved in correct patterning of cell fate determination in the ovule in sexual and apomictic species. This research focuses on genes belonging to the gene family known as Factors of DNA Methylation (FDM1-5) and their closely related IDN2 (Involved in De Novo 2). Our research aim is a better understanding of roles played by these genes in the frame of ovule cell fate determination and gametes formation.

Bioinformatics analyses were performed in order to identify and annotate all gene family members expressed in *H. perforatum* ovules. Gene expression differences between pistils collected from sexual and apomictic accessions were confirmed by qPCR and ISH. Correlated experiments were performed by taking advantage of mutant lines available for *A. thaliana*. IDN2 and FDM1-5 knockout lines were analyzed for alterations in total seed set and plant habits. Mutant lines displayed overlapping phenotypes, including the reduction of seed set. Overall, our phenotypic data are in line with a sporophytic effect resulting in the ovule abortion in *A. thaliana*. GUS reporter lines were adopted to visualize the FDM promoter activity in ovules at different developmental time points. Furthermore, the development of a protocol suitable for whole-mount qPCR assays allowed rapid and reliable quantification of gene expression in micro-dissected ovules. Our results elucidate the role of FDM and IDN2 genes in both sexual and apomicts plants and add new factors affecting the complex events involved in ovule and gametes formation that should be further investigated.