CONSTRUCTION OF EXPRESSION VECTORS FOR SPATIAL AND TEMPORAL EXPRESSION OF MICRORNAS ARGONAUTA9 INTERACTORS IN *ARABIDOPSIS THALIANA*.

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Introduction. Apomixis is a method of asexually reproduction through of seeds, there is currently great interest in achieving induce this type of reproduction into agronomical crops. In 2010 was published the existence of ARGONAUTE9 (AGO9) protein in the model plant *Arabidopsis thaliana*; AGO9 protein controls female gamete formation by restricting the specification of female gametophyte precursors (Olmedo-Monfil *et al*., 2010; Durán-Figueroa y Vielle-Calzada, 2010), the mutant ago9 has a phenotype similar to apospory-apomictic plants. ARGONAUTE proteins bind to small regulatory RNAs, which are key posttranscriptional regulators of eukaryotic gene expression by complementary sequences. Recently, microRNAs (miRNAs) have been associated with the formation of gametes, seeds and embryos. miRNAs interactors of AGO9 are suggested involved in regulation of female gametogenesis and therefore, in apomixis mechanisms. In this work, was carried out the design and construction of expression vectors with the promoter genomic region (RGMP) of miRNAs, RGMP was fused to GUS reporter gene, promoter activity was observed for each gene and deduce miR spatial and temporal expression of miRNA AGO9 interactor.

Methods. 13 RGMP were cloned in the subcloning vector pBS, the RGMP from these clones was used to make the construction with the pBI vector of 5 RGMP::GUS. We used the floral-dip method for Arabidopsis transformation using an *Agrobacterium tumefaciens* cell culture.

Results. The analysis of miRNAs through the activity of its promoter, is the strategy of test to know the spatial and temporal expression in Arabidopsis**.** An analysis of cis elements of the genomic regions of the MIR genes selected, allowing suggest the promoter region corresponding to each miRNAs, for that we designed following primers for RGMP with the restriction sites HindIII and XbaI for each miRNA and selected from the PCR products was accomplished by cloning the vector pBS (Fig.1 A).

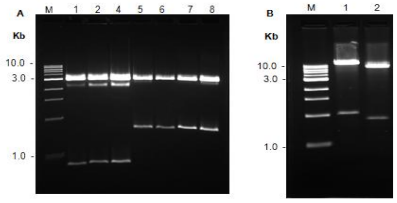

Fig.1 Enzymatic restrictions. It shows the correct insertion of the RGMP of mir158 RGMP (496 bp) and the mir822 (844 bp) into the pBS (A) and pBI vector (B).

Then was conducted uidA fusion gene (beta-glucuronidase, GUS) with the RGMP of miRNAs, the vector used was pBI already containing in their structure the GUS gene (Fig.1 B). Transformed Arabidopsis plants containing the RGMP of miR858 (Fig.2 a-f) and miR157 (Fig.2 g-j). GUS staining showed that miR858 expressed in different tissues of *Arabidopsis thaliana* like leaf, petal and siliques; meanwhile the miR858 expression was observed in anthers.

Fig.2 Analysis of the expression of the RGMP-miRNA of miR858 and miR157 in *Arabidopsis thaliana*.

These results were checked by PCR with primers specific for amplifying the RGMP and the uidA gene (Fig.3).

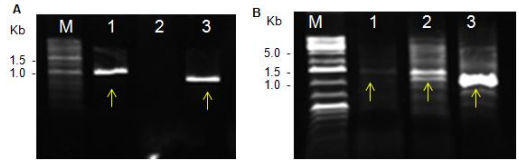

Fig.3 PCR of RGMP::GUS with specific primers in Arabidopsis; (A) RGMP of miR858 and (B) miR157**.**

Conclusions. We generated transgenic plants containing the two RGMP of miRNAs fused to GUS gene expression for to evaluate the spatial and temporal expression in *Arabidopsis thaliana*.

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References.

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