## SUMMARY

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Functional analysis of *ago1*, *ago2*, and *ago10* mutants

MSc in Agricultural Biotechnology

ARGONAUTEs (AGOs) are the protein group that exists in eukaryotic and procaryotic cells having partly distinct and overlapping functions, specifically in gene silencing pathways together with small RNA (sRNA) as the guide. AGOs – sRNA complexes are important to activate silencing activities. sRNA plays a major role in guiding AGOs into specific targets through sequence complementarity. However, individual ago mutants were extensively studied, and there is still small knowledge revealing the possible overlapping functions among AGO proteins. Particularly, to extend our knowledge about other AGOs than AGO1 taking part in miRNA pathway, AGO2 and AGO10 seem to be appropriate candidates. The objective of this research is to analyze the functional redundancy of AGO1, AGO2, and AGO10 1) through phenotypical analysis of single, double, and triple mutants impaired in the respective genes. 2) To investigate the possible substitution or backup function of AGO2 and AGO10 in the miRNA pathway with the analysis of miR168 and miR159 content in the mutant plants. 3)To investigate the expression of AGO1 mRNA as the target of miR168 in the presence of analyzed mutations, and 4) to visualize the loading efficiency of miR168 into AGO-RISC complex in *ago1-25* and *ago1-25, 2-1, 10-1* mutants.

Single mutants were provided by the research group and originated from the NASC seed bank. Double and triple *ago* mutants were produced by the crossing method of *Arabidopsis thaliana.* F1 progenies of the crossing were tested for their heterozygous nature and self-pollinated. Homozygous plants were selected and left to set seeds. Current work used the next generations/ The seeds were surface sterilized with hypochlorite and germinated *in vitro* on MS medium (Duchefa premix) for one week under continuous light and a constant 21 °C. Seedlings were then kept in peat blocks for further 3 weeks under short day (8 hours light) conditions at 21 °C, planted into pots, and placed on long days (16 hours a day). The phenotypic observation was conducted at the indicated time points (3, 5, 11, and 16 weeks old). Direct PCR was conducted to directly detect the existence of mutant in the plant progeny and total RNA extraction was conducted for further miRNA analysis, including Northern Blotting and FPLC.

Based on the observation, all *ago1* mutation-containing plants displayed the typical serrated, curled leaves with long stems. But the intensity of the distortion of organs differed amongst single, double, and triple

mutants with low fertility and different life spans. Some major alterations on the generative organs can also be observed in all mutants having *ago1.* Moreover, an extreme defect can be seen on triple mutants in their later developmental phase with undefined organs and unstructured symmetrical growth, including inflorescence stem, flower, and silique. We also presume that AGO2 and AGO10 can partially act as substitutions of AGO1 and function as a backup AGO for mir168 and mir159 through miRNA analysis on the mutant plants. The result of semi-quantitative RT-PCR can also explain the likelihood between AGO1 expression levels on some analyzed plant mutants where it is highly controlled by the disturbance of the mir168 population except *ago1-27* mutant. moreover, gel-filtration analysis toward *ago1-25* single and triple mutants revealed the presence of more intense unbound mir168 in the triple mutant having the more severe *ago1-25* mutation. The invisible loaded miR168 in both of the mutants could be a consequence of the suppression of the AGOs and the less active RISCs.

In conclusion, the suppression or lack of the investigated AGO proteins, especially AGO1 could trigger some alterations in the development of plants while AGO2 and AGO10 could act as substitutes for AGO1 function. The elevation of AGO1 mRNA level in mutants can be due to the up-regulation of transcription and/or lowered down-regulation of it through the decreased miR168. Further in-depth analysis is needed to prove the action of AGO2 and AGO10 in other aspects of the miRNA pathway and to reveal the possible role of other AGOs.