Analysis of knock-out lines for the LLP1 gene in Arabidopsis thaliana

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Introduction

• AGP4, also called JAGGER, is an arabinogalactan protein (AGP). AGPs are possible signaling molecules to the pollen tube guidance into the ovule. AGP4 showed to prevent multiple pollen tube growth towards an embryo sac, a mechanism called polytubey.
• An RNAseq analysis of the jagger null mutant vs wt, carried out previously by the SPRED Lab, defined genes with altered expression pattern in the mutant compared to wt; LLP1, legume lectin-like protein 1 gene appeared under-expressed in this study.

Aim

• Study the role of the LLP1 gene during double fertilization and its possible interaction with JAGGER.

Methods

• Genotyped 2 knock-out lines (SALK_36814C and GABI_100H11) of the gene to confirm that they had T-DNA insertion.
• Performed aniline blue to analyze whether there was any change in pollen tube growth.
• Compared the mutant lines seed and silique yield to the wt.

Results

Fig.1 Schematic representation of an A. thaliana pistil and, in more detail, one ovule, showing the different AGPs present along the pollen tube growth pathway (Pereira et al., 2016).

Fig.2 Genotyping of the two knockout lines. A) SALK line genotyping where first we used the left primer (LP) and the right primer (RP). The second we used the border primer (BP), a specific primer for T-DNA and the RP. B) Genotyping of the GABI-kat line that uses the same order of primers as in the SALK line.

Fig.3 Results of the number of seeds and the size of seeds in the two knock-out lines.

Fig.4 Aniline blue staining of A. thaliana pistils after pollen germination, where we can see the ovules and the pollen tubes. From left to right we observe a wt plant, then the llp1 SALK and GABI-kat lines. The asterisks show a normal pollen tube entry into the ovules, only one pollen tube growing to each ovule, no polytubey was observed.

Conclusion

• Most likely the LLP1 gene does not have an interaction in the signaling function to the pollen tube, since only one pollen tube was observed growing into each ovule.
• However, to further understand the interactions between LLP1 and JAGGER during A. thaliana reproduction, we will analyze the llp1 / jagger mutant crosses and lastly evaluate LLP1 expression in the pistil using the LLP1: GFP plants.