Detection of Glucose Accumulation after Pollination in Plant Ovules

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Abstract

The processes underlying the translocation of sugar from leaves to ovules after pollination are not well understood. In this study, a genetically encoded ultra-high sensitive glucose biosensor was used to detect the translocation of sugar molecules from the leaf to the ovules in *Arabidopsis thaliana*. Through an 8-day time series experiment detecting the accumulation of glucose, it was found that around 4 days after pollination (DAP), glucose started to accumulate in the phloem unloading regions, concurrent with endosperm development. Sugar levels then started increasing up to 8 DAP, when seed development was also observed. Overall, an analysis of the glucose accumulation levels in plant ovules after pollination has been conducted.

1. Introduction

A fertilized egg of an organism requires energy to mature and fully grow into an organism. For plants, the plant egg cells, which are ovules, are fertilized with the male counterpart, the pollen, to form a zygote and subsequently grow into a seed. This process that leads to fertilization of the ovules is referred to as pollination.

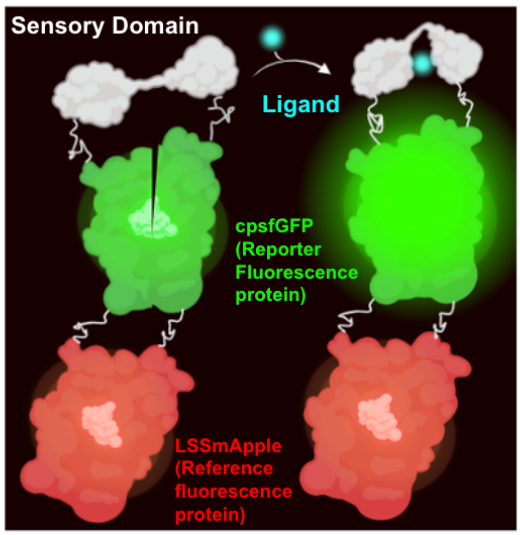
When a plant pollinates and is fertilized, sugar is transported from the leaves where photosynthesis is conducted over to the ovule, or the seed, after pollination and fertilization. Even though the mechanisms behind the developmental stages of the embryo have been studied to analyze the endosperm development to show that sugar content increases after fertilization (Chen et al., 2015), the exact mechanisms behind the transfer of sugars from leaves to ovules are not well understood and require further research.

Current literature suggests there has been research conducted on the transfer of different molecules in plant models, like phytohormones like gibberellin (Ishikawa et al., Unpublished). Sucrose is the main mobile form for long-distance translocation. An ultra-high sensitive glucose biosensor has been developed, which enables the detection of glucose accumulation in the phloem unloading

region of the root following sucrose transport from the shoot to the root (Ishikawa et al., unpublished). However, detecting endogenous sugar changes, such as those occurring before and after fertilization, remains highly challenging due to technical limitations.

To detect such sugar gradients, a genetically encoded biosensor was used in the process. This biosensor is based on the genetically encoded dual fluorophore Matryoshka sensor (Ejike et al., 2023). The biosensor, with a cpsfGFP green-fluorescence protein and an LSSmApple red fluorescence protein, detects sugar to emit fluorescence (Figure 1). The red fluorescent protein functions as a reference channel, enabling normalization of potential artifacts such as sample drift and differences in protein levels among tissues. In contrast, the green fluorescent protein increases its fluorescence intensity when the sensory domain of the sensor binds to endogenous glucose in plant cells. We can infer from this biosensor that change of emission ratio (GFP/LSSmApple) means that sugar level changes in the plant cell.

Figure 1



Structure of a ultra-high sensitive glucose biosensor

Note. From "Root-to-shoot, shoot-to-root translocation and symplasmic unloading of gibberellin detected by new ultra-sensitive GA nano sensor," Ishikawa et al. (2024)

Using this genetically encoded biosensor in the *A. thaliana*, we have analyzed the ovules postpollination using the confocal microscope to see the changes that happen to the ovules in terms of structural and morphological changes using *Arabidopsis thaliana* as the model.

2. Materials and Methods

The time point was from 1 day to 8 days after pollination (DAP), and a sample was taken for each day, making it a total of 8 samples. The trial was conducted 2 times. An unfertilized sample was taken and is referred to as the 0-day sample and used as a control. A total of 17 samples were analyzed in the experiment.

First, we used transgenic, biosensor-expressing *Arabidopsis* plant females to acquire unpollinated, intact pistils and removed the petals to only have the pistil remaining without any anthers or other parts. Then, using the anther of the wild-type Arabidopsis plant, each transgenic sample was pollinated accordingly. Consequently, they were left to grow until the designated time point.

At similar time points, 1 DAP to 8 DAP, the pistil was acquired from the plant. Then, the pistil was dissected to acquire around 10 different ovules in 1 pistil using a scalpel and dissecting tool.

The acquired ovules were consequently transferred over to the slides, and then a pollen tube growth (PTG) buffer was applied onto the samples on the slides so that they would not dry.

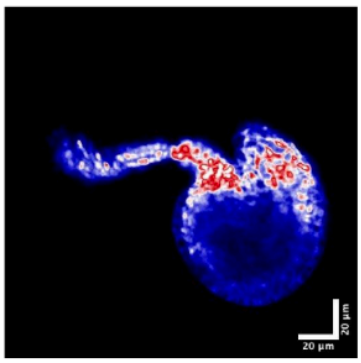
Then, the Leica Stellaris 8 confocal microscope was used to image the ovules. The fluorescent markers were TurboGFP for green and mRFP1 for red with an excitation spectrum of 488 nm. The magnification was 20x zoom and 63x zoom, with the 63x zoom immersed in distilled water.

The emission ratio for the biosensor was detected using both green and red fluorescence channels, and then the emission ratio was calculated to check for the emission ratio levels.

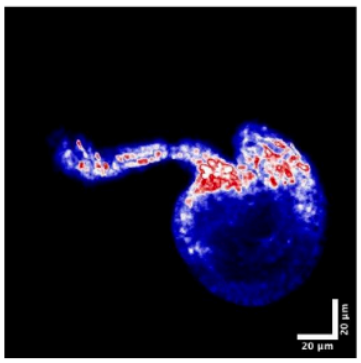
3. Results

We can see that the 0 DAP samples have low levels of emission ratio, as only the chalaza region, which is the region where nutrients enter the ovule, has emission ratio levels. Other regions, like the phloem, have lower levels of emission ratio (Figure 2). Hence, we can hypothesize that the sugar is detected mostly in the chalaza region and some areas near the phloem.

Figure 2

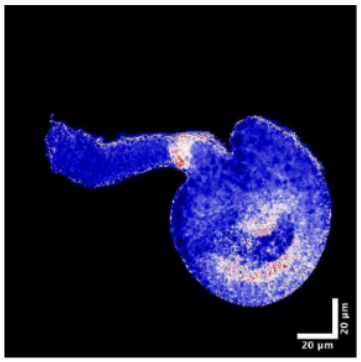


Green Channel



Red Channel

Brightfield

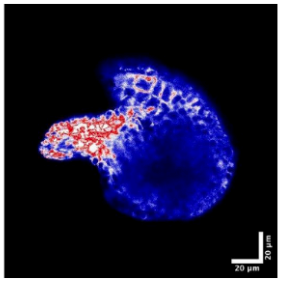
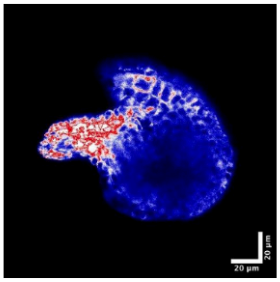
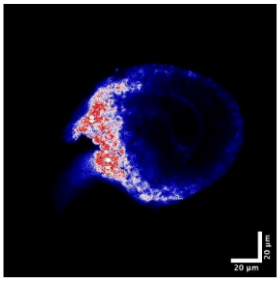
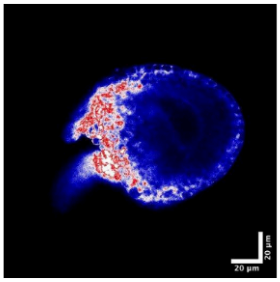
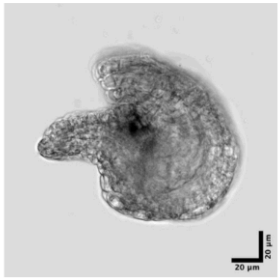
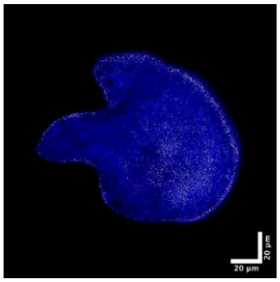
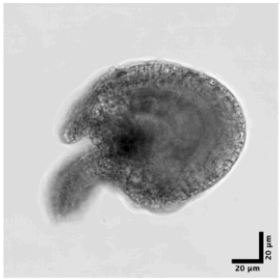
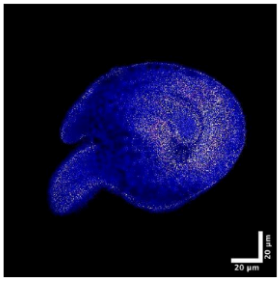


Emission Ratio

Figure 2: Images of 0 DAP ovule (Scale = 20µm)

Starting from the 1 DAP samples, we can see that there is also little to no endosperm development for these samples going up to 3 DAP, showing a similar emission ratio compared to the 0 DAP sample (Figure 3). The ovule structure has not changed, and we can see that there are some levels of sugar in the chalaza region.

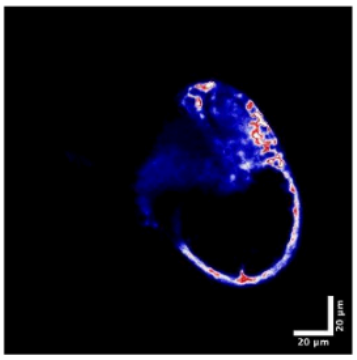
From 3 DAP, we observe a slight change in the ovule's composition, as the number of cells in the central region has relatively decreased compared to 1 and 2 DAP. Additionally, there is a higher level of emission ratio in the central area near the chalaza (Figure 4).

Figure 3		Figure 4	
			
Green Channel	**Red Channel**	**Green Channel**	**Red Channel**
			
Brightfield	**Emission Ratio**	**Brightfield**	**Emission Ratio**
Figure 3: Images of 1 DAP ovule (Scale = 20μm)		Figure 4: Images of 3 DAP ovule (Scale = 20μm)	

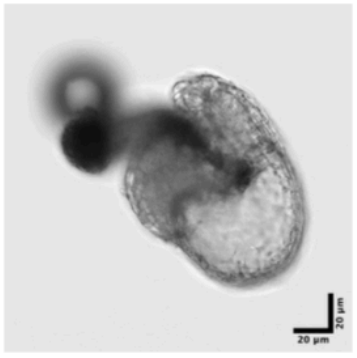
From 4 DAP, we can see that the endosperm has been developing in the ovule, as we can see that there is a vacant region in the ovule with a lower number of cells compared to the 1 DAP to 3 DAP samples. We can see that at this point, as the endosperm develops, nutrients, including sugar and others, will likely be entering the ovule from the leaf (Figure 5).

The results indicated that the endosperm development started around 4 DAP, which suggests that sugar and nutrient molecules have started to move between 3 DAP and 4 DAP time points. Also, there is a higher level of sugar in the phloem unloading region, which is the region where the phloem transports nutrients from the leaves to the ovules and unloads them.

Figure 5



Green Channel | **Red Channel**

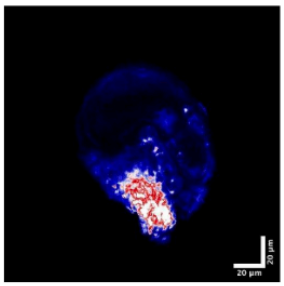
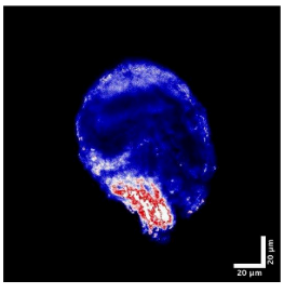
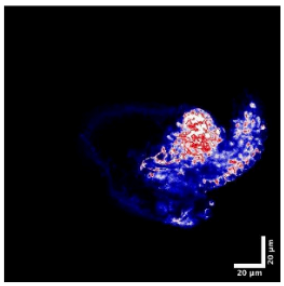
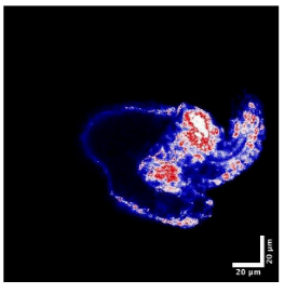
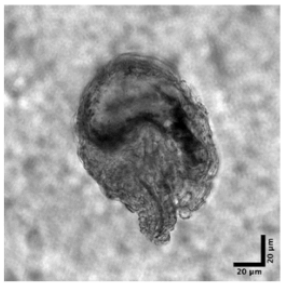
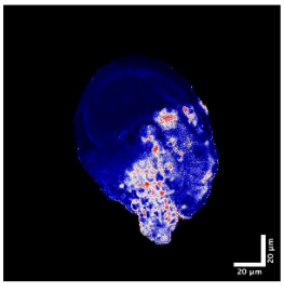
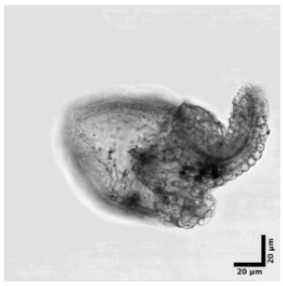
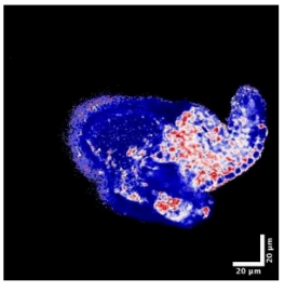


Brightfield | **Emission Ratio**

Figure 5: Images of 4 DAP ovule (Scale = 20µm)

From 5 DAP to 8 DAP, we can observe enlargement of the ovules and hence an increase in the size of the endosperm as the ovule increases in starch content. Also, the sugar detection levels in the phloem unloading regions increase, suggesting that as time passes, sugar accumulation in the phloem unloading region increases (Figure 6).

We can clearly see in the 7 DAP example for the bright field image and the emission ratio image that the phloem unloading region area displays higher levels of emission (Figure 7).

Figure 6		Figure 7	
			
Green Channel	**Red Channel**	**Green Channel**	**Red Channel**
			
Brightfield	**Emission Ratio**	**Brightfield**	**Emission Ratio**
Figure 6: Images of 5 DAP ovule (Scale = 20µm)		Figure 7: Images of 7 DAP ovule (Scale = 20µm)	

4. Discussion

From the results, we can infer that starting from 1 DAP to 3 DAP, there are low levels of detected sugar in the ovule, as we can see low levels of emission ratio in the 1 to 3 DAP ovules. Starting from 4 DAP, we can observe endosperm development and increased levels of sugar detection in the phloem unloading region up to the 8 DAP ovule.

Previous data on total sugar amount in *Arabidopsis* ovules suggests that the starch amount in the ovule increases dramatically from around 3 days after pollination up to around 8 days, and decreases again dramatically. The glucose amount would increase from 3 days up to 6 days, and then decrease again until 8 days, where it starts to increase again. Sucrose starts to increase around 13 days after fertilization (Figure 8, Baud et al., 2002).

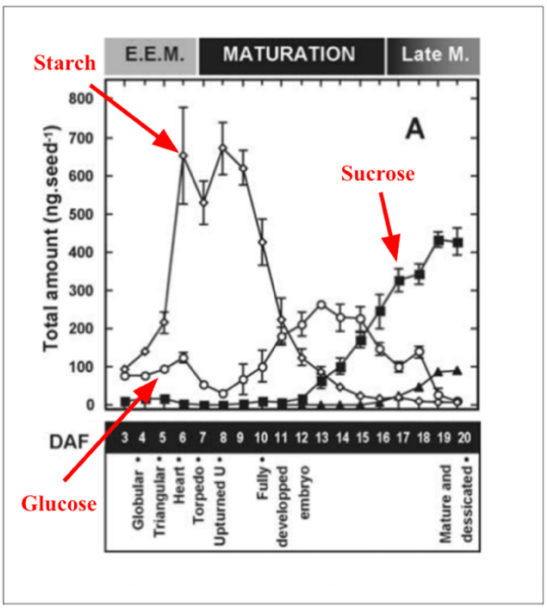


Figure 8 : Graph of total sugar amount in Arabidopsis ovules

Note. From "An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS," Baud et al. (2002)

Using this data and comparing the data that we have from the imaging, we can ask two further questions for further studies. As for the increase of glucose levels, as the levels of glucose start to increase in the graph from 3 days up to 6 days, we could hypothesize that there is an undetermined signalling molecule that signals tissue like leaves, to transport sugar molecules into the ovule to the phloem unloading region during the initial development of the ovules (Figure 9).

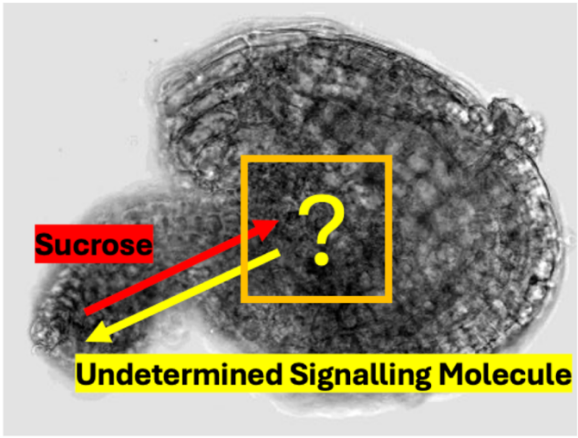


Figure 9 : Mechanism behind "Undetermined Signalling Molecule" that signals transportation of sugar to ovules

Second, for the decrease of starch levels, we see that there is a drastic decrease in starch levels starting from around 8 days after fertilization, while there is an increase in both the sucrose and the glucose. As the embryo would start to grow larger in size, it would likely require high levels of nutrients, including sugar. We could hypothesize that there could potentially be another undetermined signalling molecule from the embryo that signals the breakdown of starch in the endosperm into smaller sugars like glucose (Figure 10).

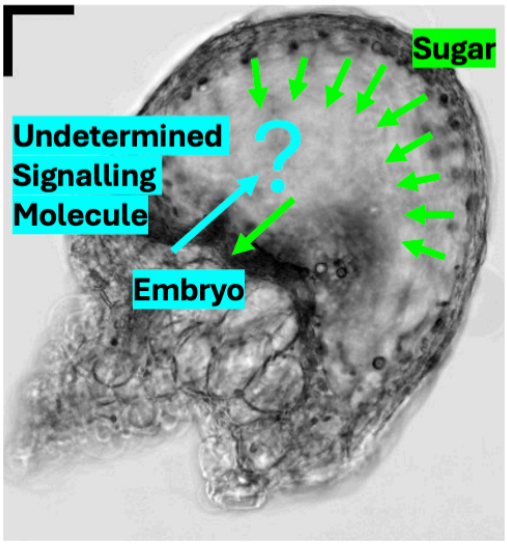


Figure 10 : Mechanism behind "Undetermined Signalling Molecule" that signals breakdown or larger sugar molecules

Further studies to discover or research the mechanisms and the potential molecules related to glucose accumulation or starch breakdown could be conducted. Also, live cell imaging could be conducted on the *Arabidopsis* ovules to check for real-time movement of the sugars from the leaves to the ovules (Desnoyer & Grossniklaus, 2023).

However, some points should be addressed during the experiment, as there could potentially be errors regarding pollination, as the sample could've self-pollinated or the sample wasn't pollinated properly, and this could affect the time point of the results. It was found in some of the samples that they had ovule development that was incongruent with previous literature. Hence, for future experiments, whether pollination has been done correctly should be checked in order to conduct an accurate experiment.

5. Acknowledgements

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Thank you to everyone who helped me throughout the journey. 本当に ありがとうございます!

6. References

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