Abstract

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Title: Characterization of genes involved in pollen development and anther opening in barley

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One of today´s biggest challenges is the achievement of food security around the world. Threats such as climate change make it a necessity to adapt agricultural food production systems for a constantly growing population. Therefore, the performance of important crops such as wheat and barley has to increase. This could be achieved through hybrid lines, which outperform their parents in yield or stress resistance traits. Hybrid seed production requires crossing two different lines and, therefore, efficient methods of emasculation or induction of male sterility in self-pollinating crops such as barley. However, only a handful of methods have practical use today. In order to develop new approaches to control male fertility in barley, a better understanding of the male reproductive organs (anthers) is necessary. To start filling this research gap, this study provides an in-depth description of post-meiotic anther development in barley and characterizes the function of *MSG32* and *MSG36*, two genes required for barley male fertility.

Histological and ultrastructural analysis of male sterile *msg32* mutants revealed post-meiotic pollen degeneration with defects in pollen wall development and tapetum function and degeneration. In contrast, *msg36* mutants are impaired in the separation of specialized septum and stomium cells, which prevents anther opening and proper pollen release. The mutated nuclear genes underlying the male sterile phenotypes of *msg32* and *msg36* were identified by genetic mapping and RNA sequencing, and confirmed with mutant alleles already existing or newly generated with genome editing. *MSG32* and *MSG36* encode a putative mitochondrial aldehyde dehydrogenase and a putative pectin-degrading enzyme, respectively.

Both genes revealed peaks of expression at the stages when the cellular defects occurred in the mutants. Furthermore, preliminary RNA *in situ* hybridisations revealed localized expression within the developing pollen and tapetum cells for *MSG32*, and within the septum and stomium cells for *MSG36*, supporting the observed cellular defects in the corresponding mutants.

A model for the function of MSG32 in barley anther development is presented based on the hypothesis that this enzyme is indispensable to maintain cell homeostasis of pollen or tapetum cells. In contrast, previous mutant studies of *MSG32* orthologues in Arabidopsis and maize suggest that

they either play a minor role or are not essential for pollen and tapetum development in those species. Therefore, the function or metabolic context of mitochondrial aldehyde dehydrogenases seems less conserved between anthers of different species. Based on previous work and its putative function, MSG36 is hypothesized to degrade pectin, an essential component of cell walls, to allow the separation of septum and stomium cells, and, therefore, anther opening and pollen release.

In conclusion, these studies extend the emerging knowledge on barley anther development and future experiments are proposed to test if the identified genetic factors or related pathways could serve as putative targets of male fertility manipulation to improve the current systems of hybrid seed production.