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Evolution and development of flowers, fruits and inflorescences of Phalaenopsis and other orchid species

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Summaries

SUMMARY

The enormous number of orchid species encompasses a huge variation in flower and fruit shape. This diversity has evolved over the past 100 million years as an adaptation to a wide variety of pollinators (mainly insects and birds) and seed dispersers (animals, wind). Few studies have been conducted on the evolution and development of orchid flowers, fruits and inflorescences. This thesis focuses on increasing knowledge of the evolutionary development of three orchid floral organs, fruit dehiscence zones, resupination, and inflorescence orientation and lignification.

Transcriptomics has enhanced our understanding of how the genome is expressed in plants and continues to provide new insights into genomes and gene expression and regulation. Gene expression measurement on a genome-wide scale has become an integral part of many orchid studies. In **chapter 2**, I reviewed the brief history of transcriptomics analysis. I explain step-by-step RNA preparation, library preparation, sequencing and bioinformatic analysis. I also discuss innovative approaches to standard RNA isolation as potential sample preparation methods for obtaining tissue-specific RNA. Possible pipelines for assembling transcriptomics data using de novo assembly, reference-guided assembly, or combined approaches are explained, together with possible applications.

Although many evolutionary and developmental studies have been carried out on orchid floral organs, these numbers are insignificant when compared to the number of species and high morphological diversity in this plant family. Using advanced transcriptomic and micro-morphological analyses, in **chapter 3**, I studied the evolution and development of callus, stelidia and mentum. The callus is situated on the labellum and provides a hold-fast to pollinators. The stelidia are lateral outgrowths of the gynostemium that position the head of a pollinator below the anther and above the stigma. And a mentum is situated at the junction of gynostemium and labellum. It functions as a hinge, pushing pollinators landing on a flower against the anther and stigma to stimulate pollinia removal or deposition. For this chapter, two different species were studied: *Phalaenopsis equestris* and *P. pulcherrima*. These two orchid species have very differently shaped flowers: *P. equestris* has a large callus, small stelidia and no mentum; *P. pulcherrima* has a small callus, large stelidia and a pronounced mentum. Ontogenetic observations showed that the callus is the earliest structure to emerge, and the mentum the last. Gene expression analysis revealed that expression of MYB *DIV-2* gene copies corresponds with the development of a small callus of *P. pulcherrima*, and short stelidia and a gynostemium without a mentum of *P. equestris*. The expression of *AP3-4/AGL6-2* gene copies found in the callus of both species, and a vascular bundle beneath the callus, confirm a staminodial origin of this floral

organ. Higher expression of *AP3* clade 2 and 4 and *AGL6* clade 2 and 3 gene copies and vascular bundles in the stelidia of both species confirm the staminodial origin of these particular structures. Vascular bundles from the sepals, petals and stamens were found in the mentum of *P. pulcherrima*, suggesting a mixed sepaloïd-petaloid-staminodial origin. This research revealed evolutionary developmental mechanisms driving the adaptation of *Phalaenopsis* flowers to different pollinators.

Few studies on the evolution and development of orchid fruits have been carried out because of the relatively long life cycle and complex genomes. I studied the evolution and development of fruit dehiscence zone (DZ) formation in **chapter 4** using the emergent orchid model *Erycina pusilla*, which has lipidified dehiscence zones. I applied combined molecular and morphological analyses of 1 week after pollination (WAP) and 3 WAP *E. pusilla* fruits. I also carried out Ancestral Character Reconstruction (ASR) analyses across the five orchid subfamilies. Erect dehiscent fruits with non-lignified DZs and a short ripening period were found to be ancestral. Lignified DZs in orchid fruits evolved multiple times from non-lignified DZs, indicating that lignin metabolic pathways may be derived from those producing lipids. Higher expression of the lipid biosynthesis genes *EpKCS6*, *EpPDH-E1*, and *EpMAH1* was detected in 3 WAP fruit valves of *E. pusilla*. These genes may be involved in the lipidation of DZs since lignified DZs-related genes, such as *IND/HEC3* and *ALC/SPT*, are lowly expressed in the same tissue. The existing genetic models for fruit dehiscence in *Arabidopsis thaliana* requires adjustment for orchids, which is not surprising given the anatomical and macro-morphological differences.

To ensure successful growth, pollination and seed dispersal, plant organs such as roots, leaves, inflorescences, flowers, and fruits resupinate. Resupination, a 180 degree twist of structures during plant development, brings what would be the basal part of an organ to the top, and the apical part to the bottom. Study of resupination in orchids so far focused on flowers. In **chapter 5**, I studied resupination of *E. pusilla* roots by weekly scans of seedling and mature plants using 3D CT scans, incorporated in a time-lapse video with a new bioinformatic pipeline. Roots of *E. pusilla* twisted and resupinated multiple times from early development onwards, also in opposing directions. The most optimal time slots for harvesting tissue for transcriptome analyses of roots of *E. pusilla* were found to be 11 to 12 and 22 to 24 weeks after germination. Using 2D photographs, the movement and resupination of *P. equestris* and *E. pusilla* fruits was captured. Orchid fruit resupination had an independent direction and degree of torsion from initial to final position. Optimal harvesting time to capture genes related to fruit resupination seems to be 9 WAP for *E. pusilla* and 20 WAP for *P. equestris*.

The last orchid trait that I studied for this dissertation was inflorescence orientation. In *Phalaenopsis*, inflorescence orientation and lignification are important traits to focus on for the production of new cultivars. **Chapter 6** presents a study of these traits of different *Phalaenopsis* species and horticultural hybrids. Phylogenetic patterns were also investigated to explore further developing a horticultural hybrid with a sufficiently lignified inflorescence stem that does not require additional support. In the six species and 17 horticultural hybrids studied, the orientation of the inflorescence varied from erect, sub-erect, arching, to pendant. A significant difference in the degree of lignification between the different *Phalaenopsis* species and hybrids was found, not only between peduncle and rachis, but also among the different inflorescence positions analyzed. Topologically, the degree of lignification corresponds with the age of the tissues: the older the tissue, the more lignified it becomes. An erect orientation positively correlates with a higher degree of lignification in the inflorescence stem. Combined ASR and heritability analysis showed that the degree of lignification is a heritable character and that closely related *Phalaenopsis* species or hybrids have a more similar degree of lignification compared to more distantly related ones. We recommend using *P. viridis* and *P.* “Purple Gem” as parental lines for breeding new cultivars with erect and sturdy inflorescences that do not need artificial support of the inflorescences anymore during their development.