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Title paper: Assessment of the flowering genetic regulatory network in tropical orchids with different lifeforms

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The reproductive transition in angiosperms includes morphological changes when a vegetative shoot apical meristem (VM) forming leaves, becomes an inflorescence meristem (IM) that forms bracts and flowers. This process is controlled in monocots, like *Oryza sativa*, by a Genetic Regulatory Network (GRN) that includes promoters like Heading date 3a (*Hd3a*) (*FLOWERING LOCUS T-FT*), Heading date 1 (*Hd1*) (*CONSTANS-CO*), *FLOWERING LOCUS D* (*OsFD1*) and 14–3–3 proteins that activate floral meristem identity genes. Repressors involved in the maintenance of the vegetative phases include *TERMINAL FLOWER LOCUS 1* (*TFL1*) and *OsMADS55* (*AGAMOUS Like24/SHORT VEGETATIVE PHASE*). Additionally, *FLOWERING LOCUS C* (*FLC*) and *VERNALIZATION 2* (*VRN2*) are important in Poales species that respond to cold. Although flowering mechanisms have been studied in detail in monocot model species, little is known about how the same process occurs in orchids with different habits colonizing different niches. Terrestrial and epiphytic orchids vary in meristem hierarchies and the development of storage organs. We performed a comprehensive analysis of the morpho-anatomical changes from VM to IM in the terrestrial orchid *Epidendrum fimbriatum* with nearly continuous flowering all year long in cloud Andean forests. Using the landmarks for reproductive transition we performed comparative transcriptomic analyses in VM *versus* IM. We used a differential expression gene approach between those stages, and we found 40 DEGs between VM and IM involved in reproductive transition. Furthermore, we used a targeted search to isolate more than 30 orthologs from the canonical flowering GRN in parallel to the DEGs. In addition, we corroborated the results from our RNAseq data with spatio-temporal expression analyses using in situ hybridization and by protein-protein interaction studies using yeast two hybrid experiments. We are currently comparing these results with experiments performed in orchids with seasonal flowering, including *Cattleya trianae*, an orchid with storage organs and epiphytic habit, and *Elleanthus aurantiacus* with terrestrial habit and lacking storage organs. Our results allow us to re-evaluate the flowering GRN in orchids when compared to the model species *O. sativa* and other Poaceae. In general, we have found evidence for: 1) high duplication rates for flowering integrators in orchids but a low percentage of homologs transcriptionally active; 2) the retention of canonical flowering integrators, at the expense of low expression, the loss of key protein interactions and possibly pseudogenization of some homologs; and 3) changes in the transcriptomic profiles in different orchids according to their habits.

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Meristem development
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