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Assessing the flowering genetic regulatory network in neotropical orchids

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Abstract:

During the reproductive transition in flowering plants a vegetative apical meristem (SAM) transforms into an inflorescence meristem (IM) that forms bracts and flowers. In grasses, like rice, a Genetic Regulatory Network (GRN) involved in reproductive transitions has been identified. It includes the integration of promoters and repressors from different gene lineages, which have undergone different duplication events at different timepoints in angiosperm diversification. With the objective to understand the evolution and expression of flowering GRN in Orchidaceae, we performed comprehensive phylogenetic analyses of all genes from the flowering GRN and analyzed by RT-PCR the expression of targeted homologs in key developmental stages. Our ML results indicate that *FT/TFL1, FD, FLC/FUL, SOC1* and *AGL24/SVP* gene lineages have been subject to multiple duplications in monocots as well as in Orchidaceae as a result of recorded whole genome duplication events. Conversely, *FLC* genes are lost in Orchidaceae, suggesting major changes in the repression of flowering. Our studies also show active expression of many target genes in *Elleanthus aurantiacus* (Orchidoideae) in the SAM and in IM indicating important functions in the reproductive transition. We describe how the flowering GRN in orchids has significant variations in copy number and expression patterns when compared to the canonical rice flowering GRN

Keywords: AGAMOUS LIKE 24; Flowering; FLOWERING LOCUS T; FLOWERING LOCUS C; FLOWERING LOCUS D; Gene Evolution; Genetic Regulatory Network; Orchidaceae; SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1.

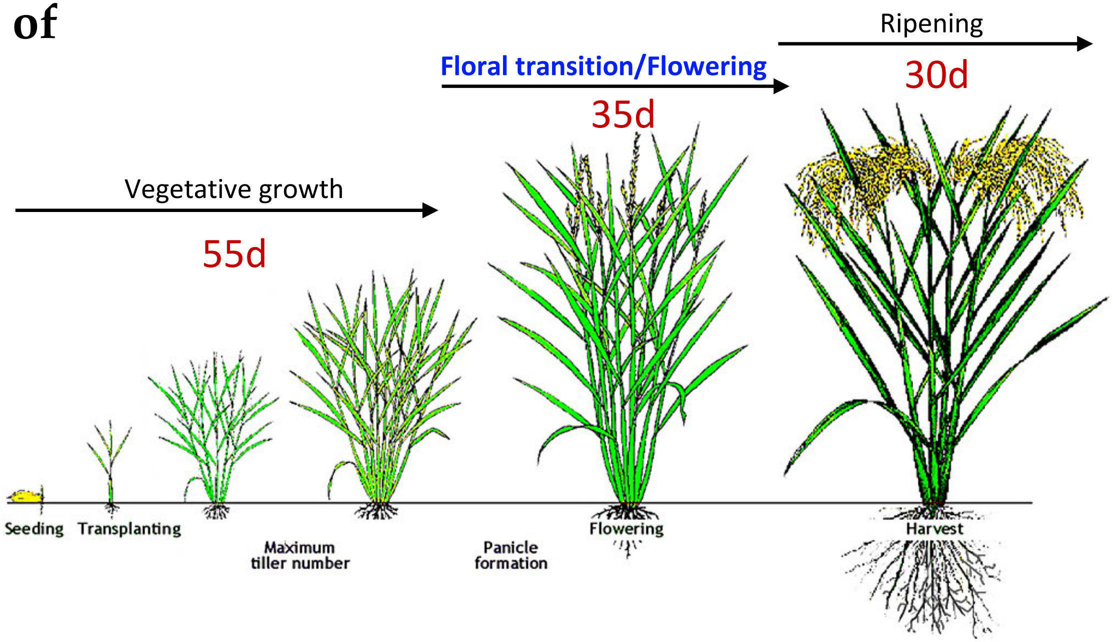
Flowering conditions in Angiosperms: a case of study in Orchids

☀ ❄ 🌡 ⏱

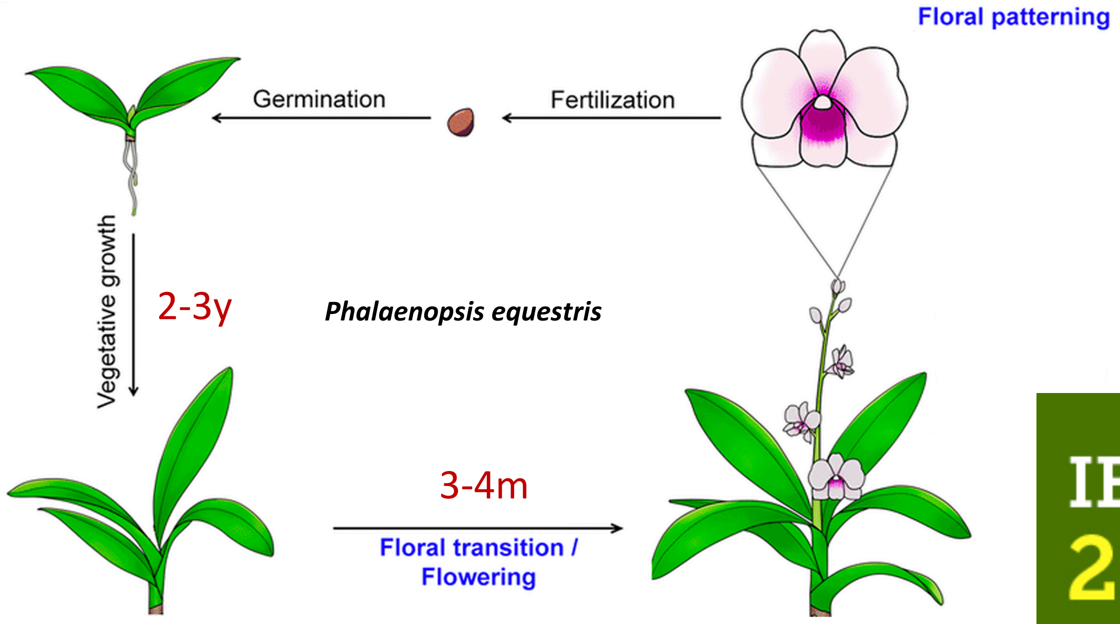
Aging

Hormones

Oryza sativa



🌡 ⏱ Hormones Aging ☁



Teo et al 2019

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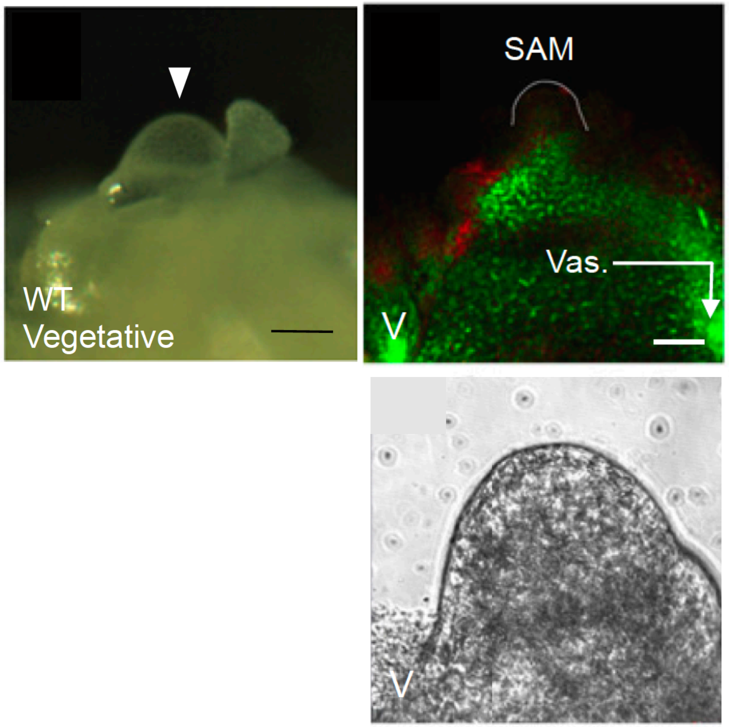
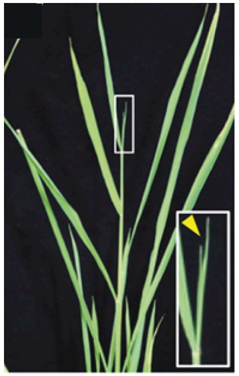
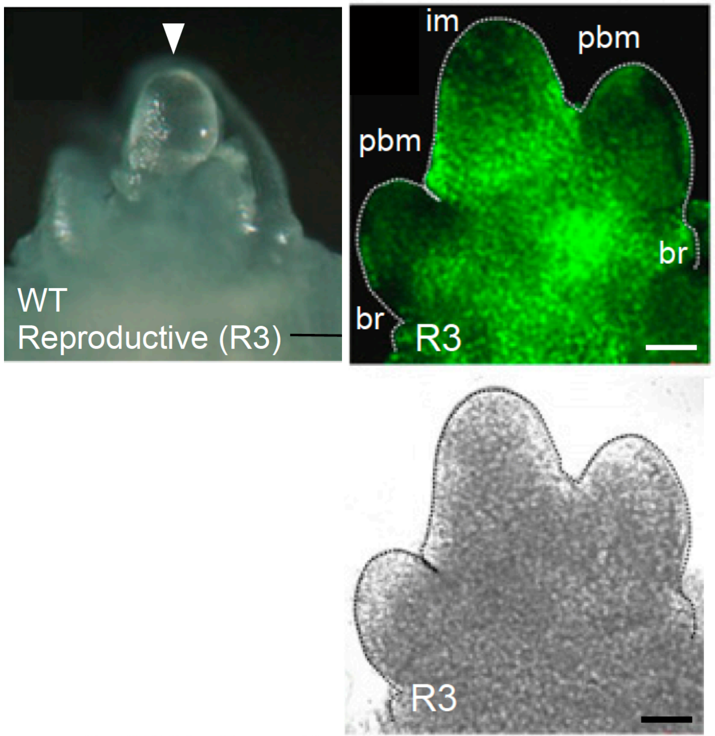
The reproductive transition include changes between meristem identities

Shootㅤ Apical Meristem (SAM)

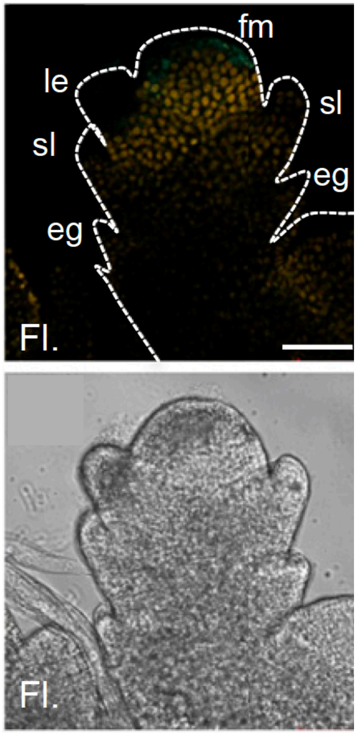




Inflorescence Meristem (IM)

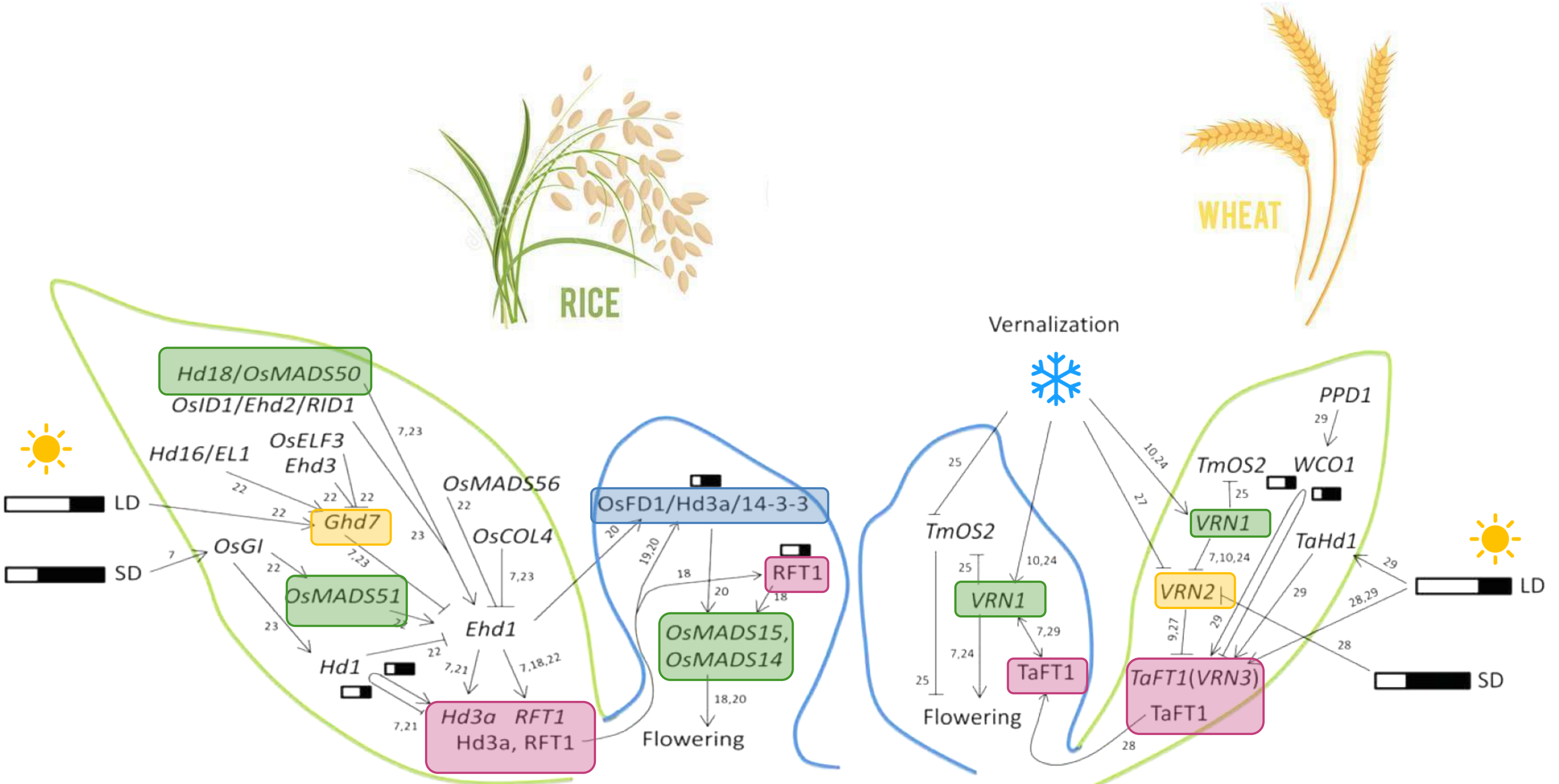


Floral Meristem (FM)



Tamaki et al. 2015, Guo et al. 2018

Summarized Genetic Regulatory Network (GRN) that controls vegetative to reproductive transition in grasses



🟪	🟦	🟩	🟧	⟶	⊣	❄	☀
PEBP	*bZIP*	*MADS-box*	*CO-like*	Positive regulators	Negative regulators	Vernalization	Photoperiod

Wang et al 2017

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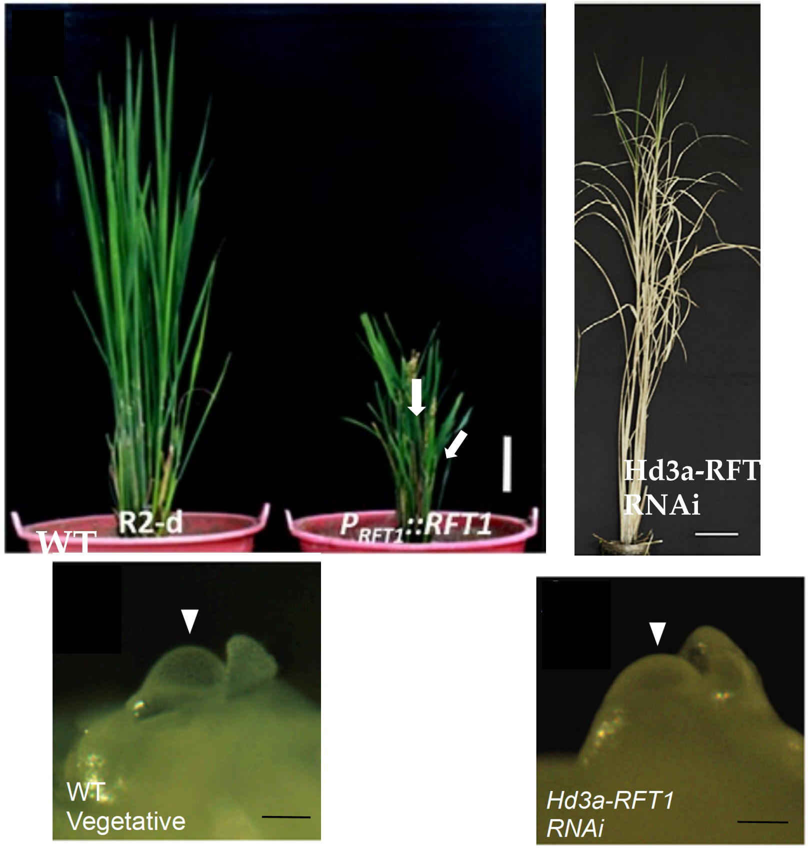
FT and *TFL1* are paralogous and mobile signals with opposite functions in the transition to flowering

FLOWERING LOCUS T (FT)



WT *35s::RFT* *Hd3a-RFTRNAi*

TERMINAL FLOWER 1 GENES (TFL1)

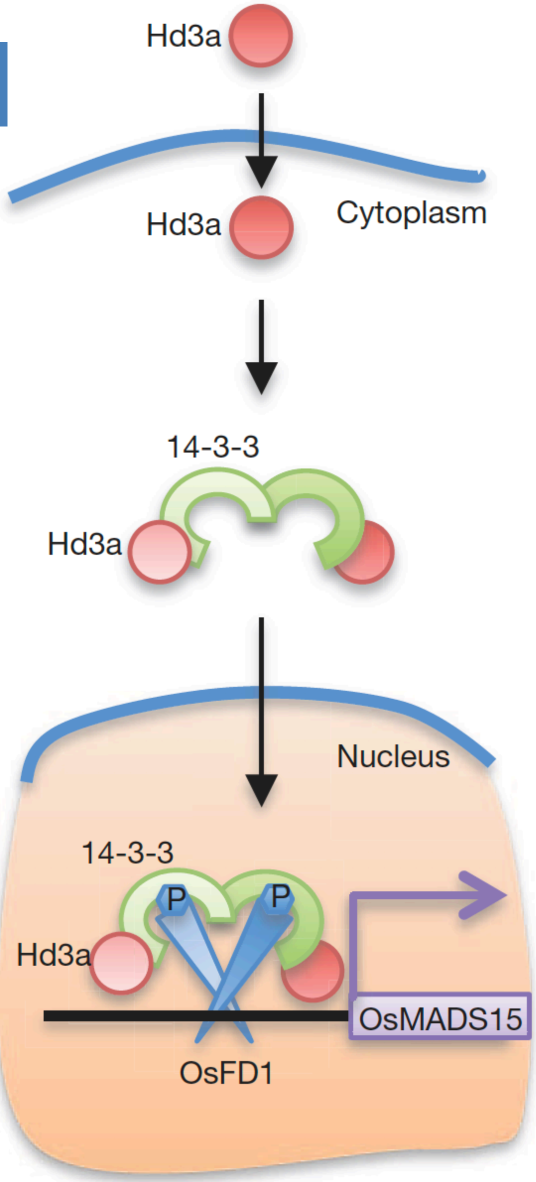
WT *35s::OscCEN1*

Zhang et al. 2005, Komiya et al. 2008, Tamaki et al. 2015, Zhao et al. 2015

FT interact with FD to control flowering through a Florigen Activation Complex (FAC)

FLOWERING LOCUS D GENES (*FD*)

WT *Osfd1*



Taoka et al. 2011, Kaneko-Suzuki et al. 2018 , Cerise et al. 2020

SOC1 together with *AGL24* directly activates Floral Meristem (FM) identity genes *AP1/FUL*

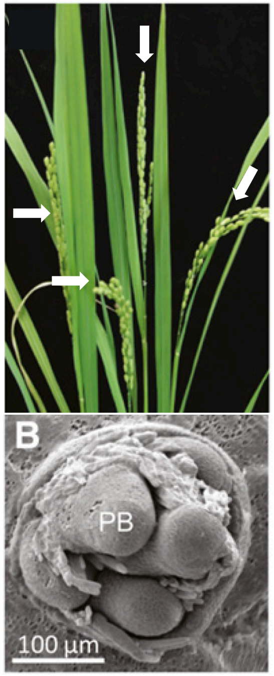
Suppressor Of Overexpression of CONSTANS genes (*SOC1*)

AGAMOUS-Like 24 genes (*AGL24*)

WT *Osmads50/51*

WT *35S::OsMADS50*

APETALA1/FRUITFULL genes (*AP1/FUL*)



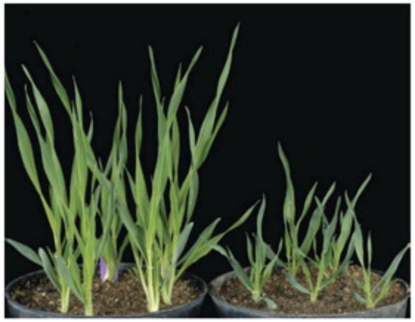
WT

Osmads14/15/18/pap

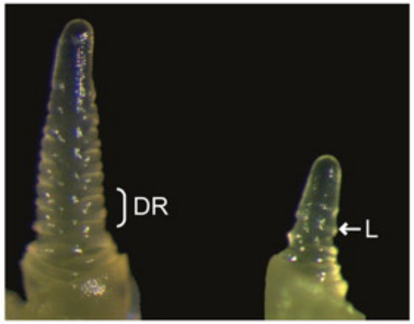
Lee et al. 2008, Kobayashi et al. 2012

Vernalized grasses respond to cold exposure to activate flowering

FLC-like genes

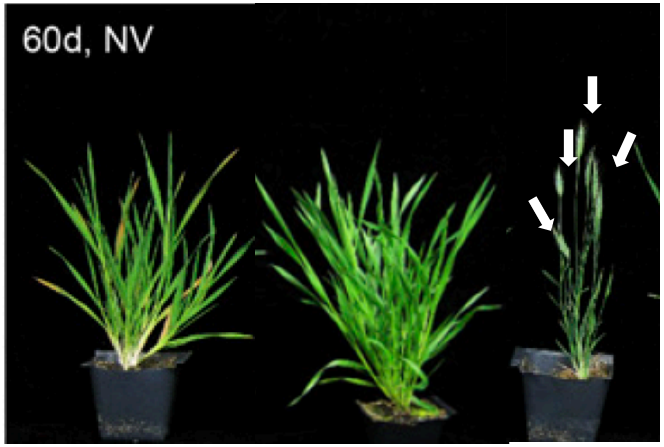


WT *OxHvOS2-20*



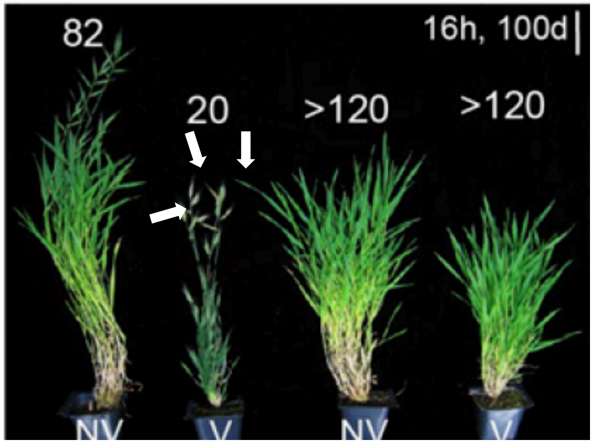
WT *OxHvOS2-20*

VRN2 genes

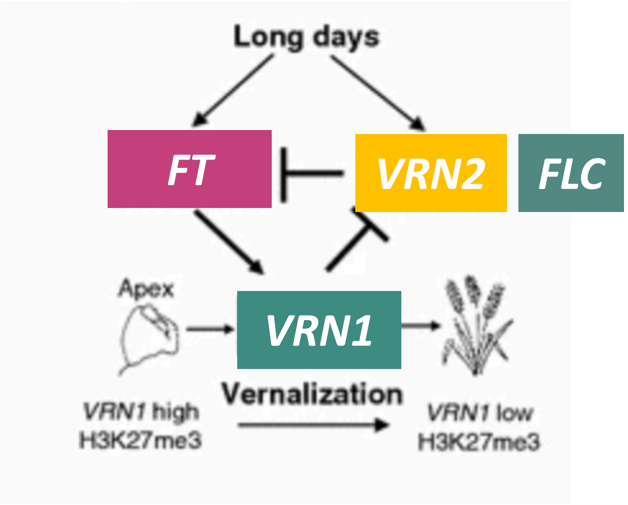


WT *UBI:VRN2* *Bdvrn2*

VRN1 genes



WT *Bdvrn1*



FT ***VRN2*** ***FLC***

VRN1

Distelfeld et al. 2010, Woods et al. 2016, Greenup et al. 2018

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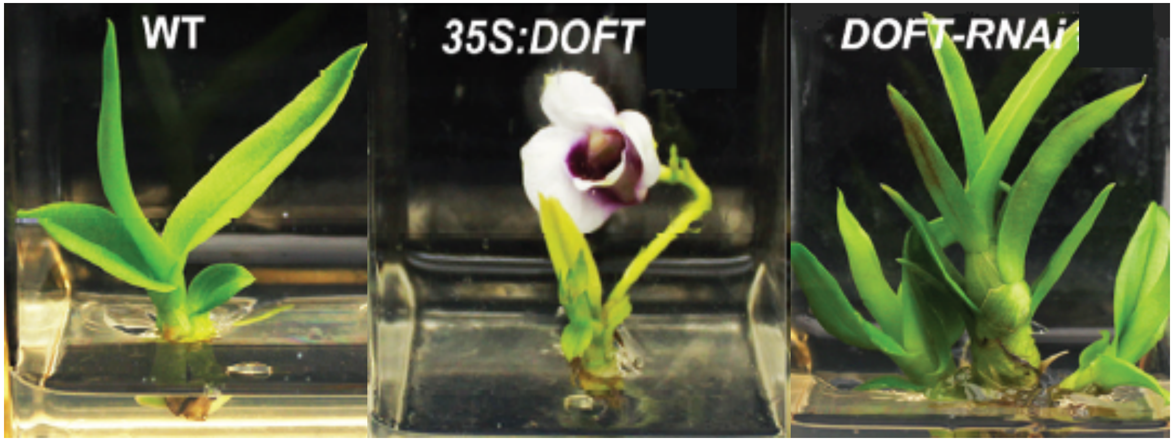
There are only some reports on the expression of *FT/TFL1* and *MADS-box* genes in orchids

Dendrobium Chao Praya Smile

Phalaenopsis equestris

Oncidium Gower Ramsey

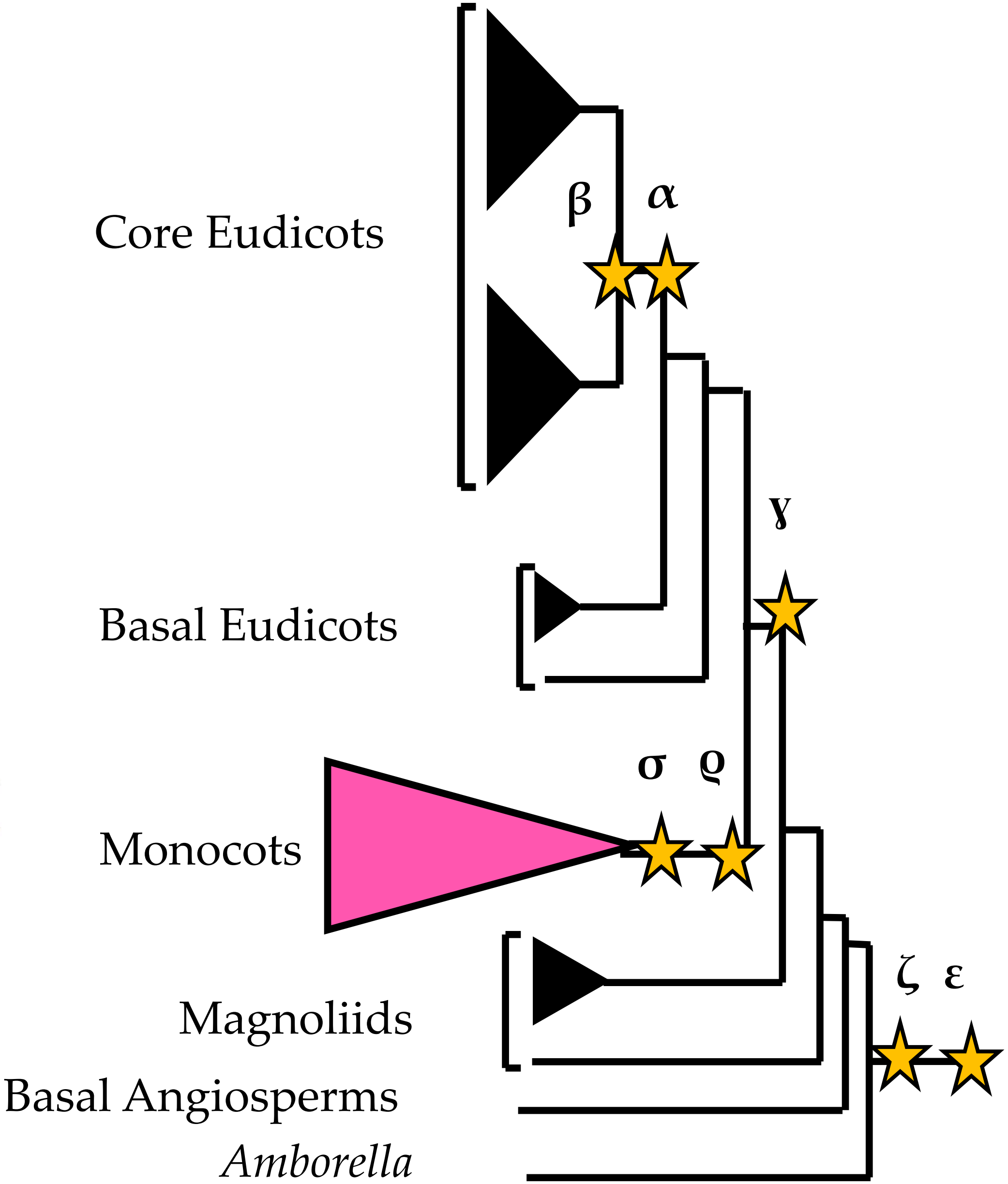
Cymbidium goeringii



FT genes in *Dendrobium* that are involved in the promotion of flowering

Wang et al 2017

Orchid genetic complement is not the same than model species



Wang et al 2017

Results: We have sequenced 13 neotropical orchid mixed transcriptomes

Vanilla aphylla

Gomphichis scaposa

Tolumnia Cherry red *x* Ralph yagj

Oncidium Grower Ramsey

Oncidium Twinkle

Masdevallia coccinea

Masdevallia wendlandiana

Maxillaria aurea

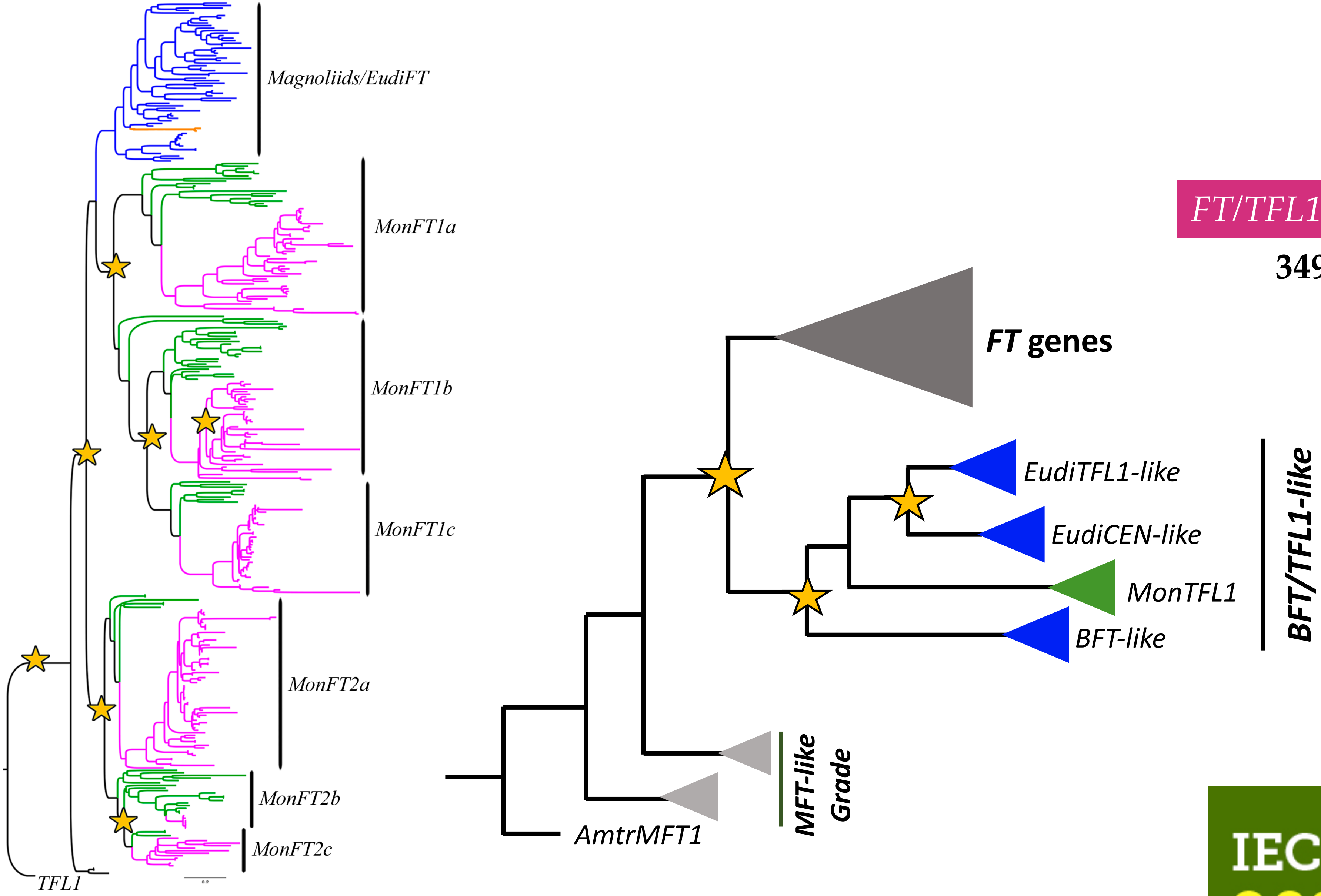
Stelis pusilla

Epidendrum fimbriatum

Miltoniopsis roezlii

Elleanthus aurantiacus

FT genes have undergone a lot of duplications in Angiosperms



FT/TFL1-Like

349 seq

● Eudicots ● Non-Orchids Monocots ● Orchids ● Early divergent angiosperms ☆ Duplication event

Ospina-Zapata et al 2020

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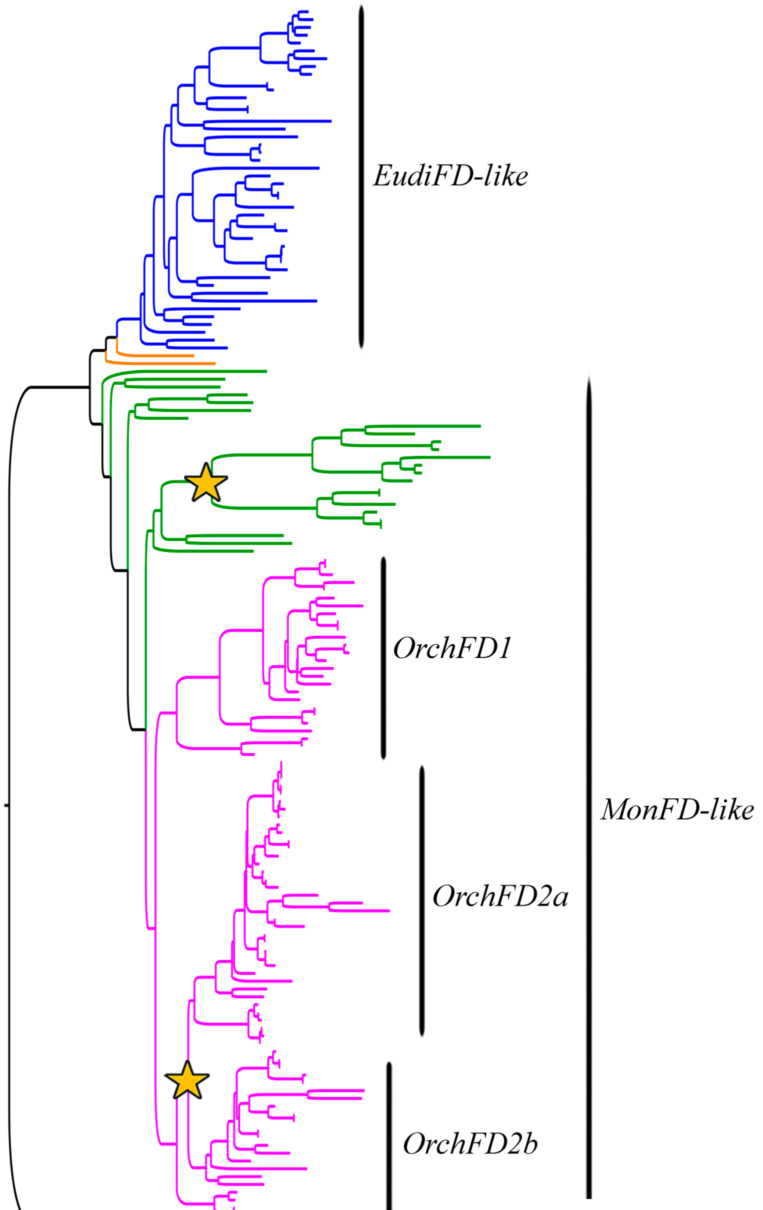
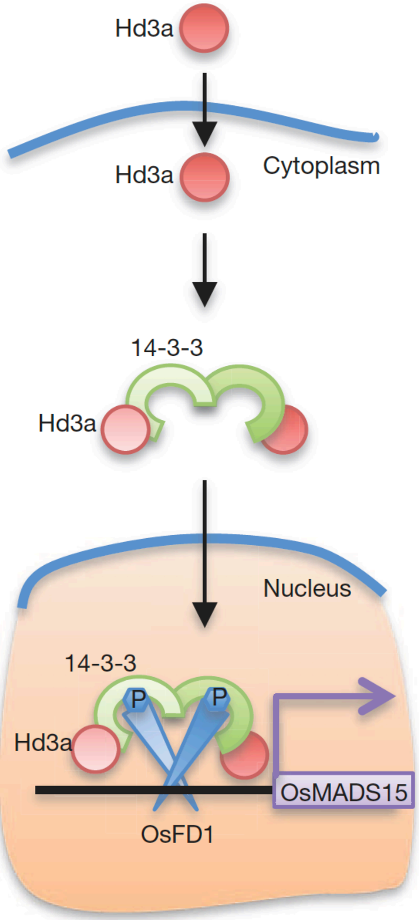
FD genes have undergone at least three duplication events prior to the diversification of the Orchidaceae



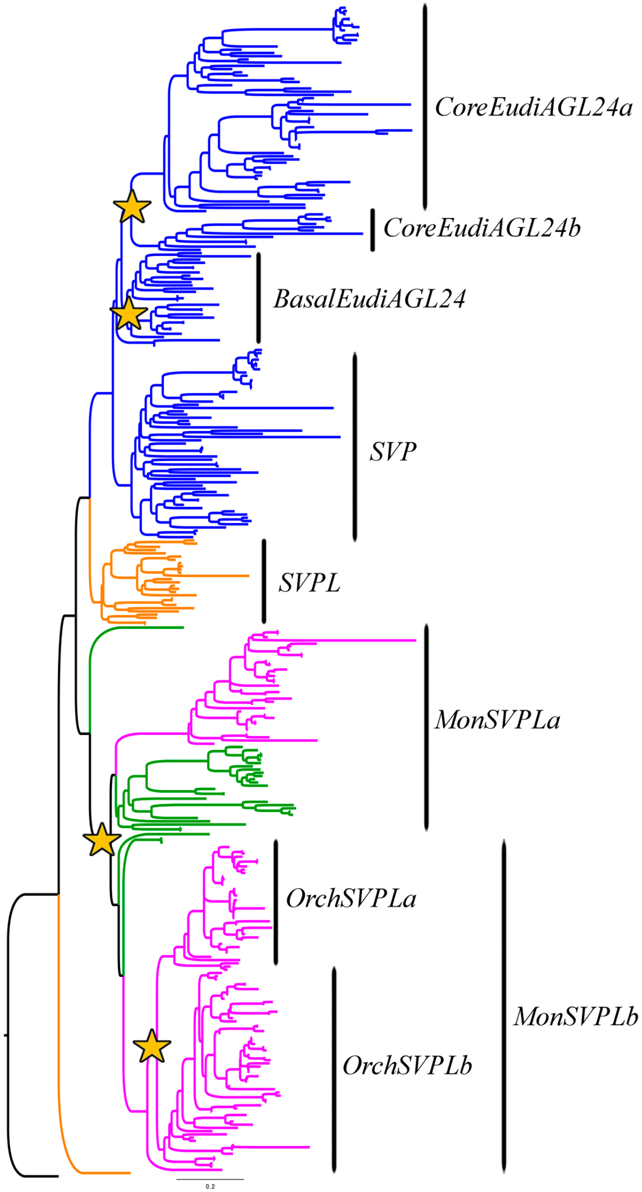


FD-like

156 seq

● Eudicots ● Non-Orchids Monocots ● Orchids ● Early divergent angiosperms ☆ Duplication event

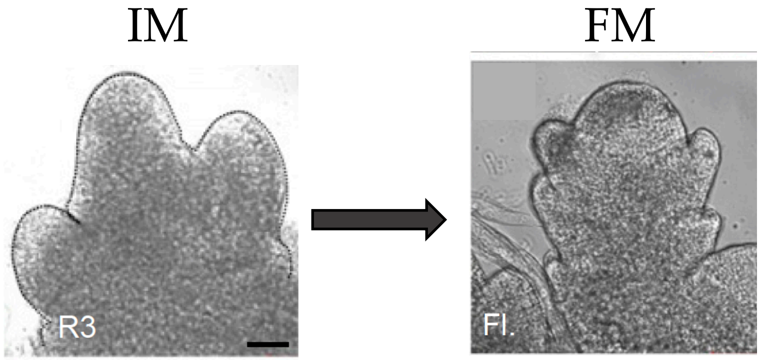
Orchidaceae have at least three copies of *AGL24/SVP* genes



● Eudicots ● Non-Orchids Monocots ● Orchids ● Early divergent angiosperms ☆ Duplication event

AGL24/SVP-like

363 seq



The *AGL24* and *SVP* clades are eudicot specific.

Ramirez-Ramirez et al. in prep

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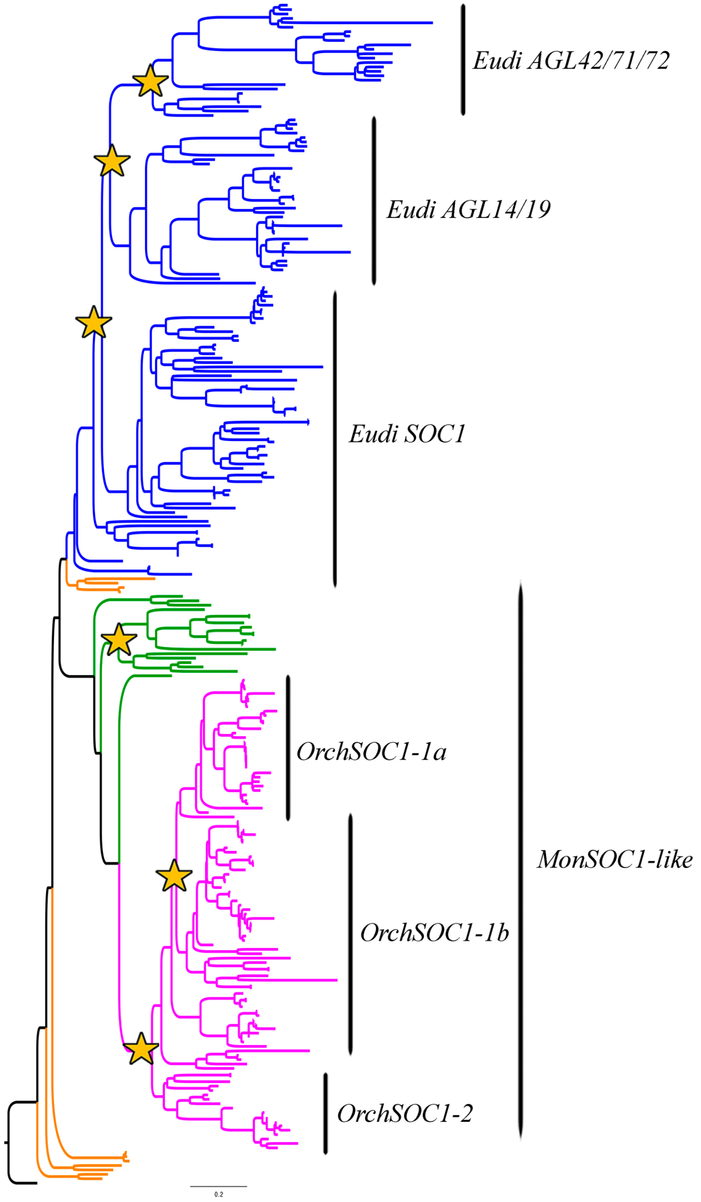
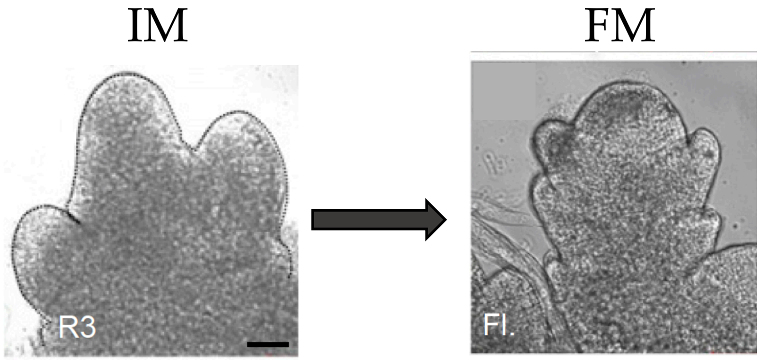
SOC1 genes have three independent duplications prior to the diversification of the Orchidaceae.



● Eudicots ● Non-Orchids Monocots ● Orchids ● Early divergent angiosperms ☆ Duplication event

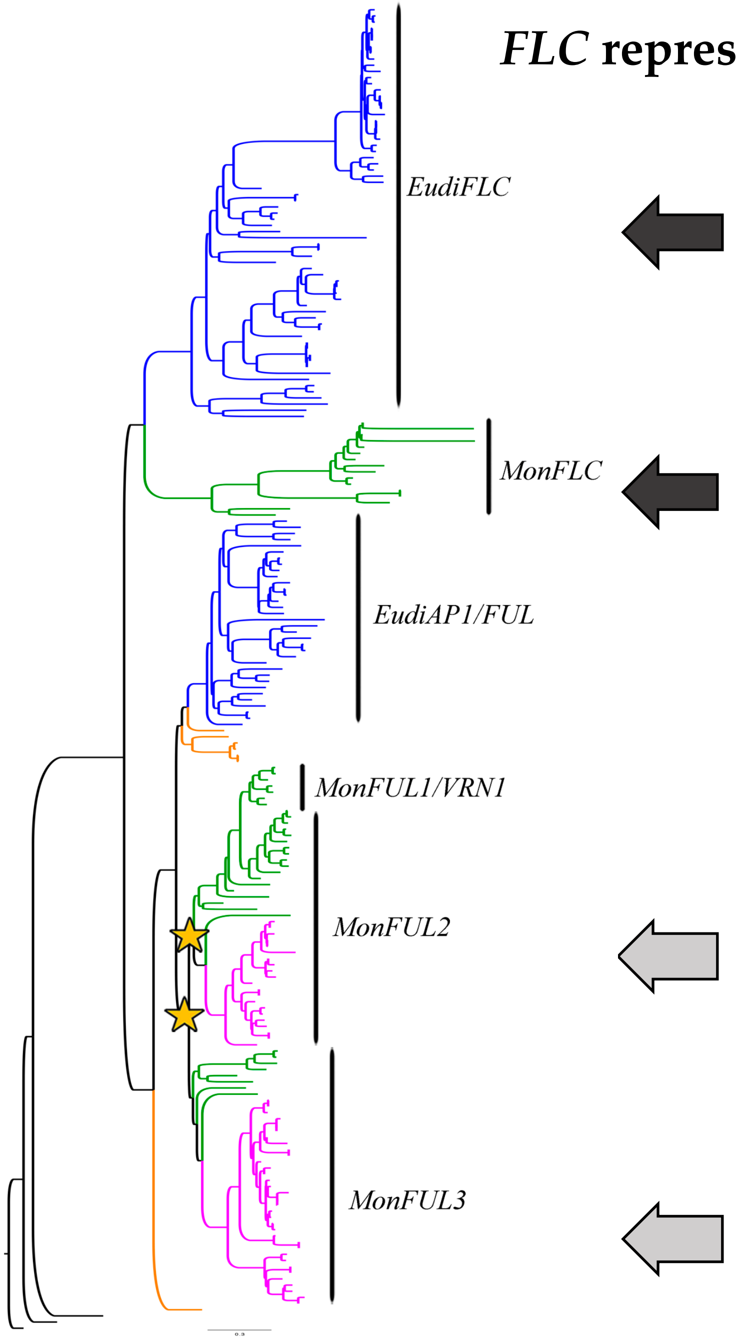
SOC1-like

268 seq



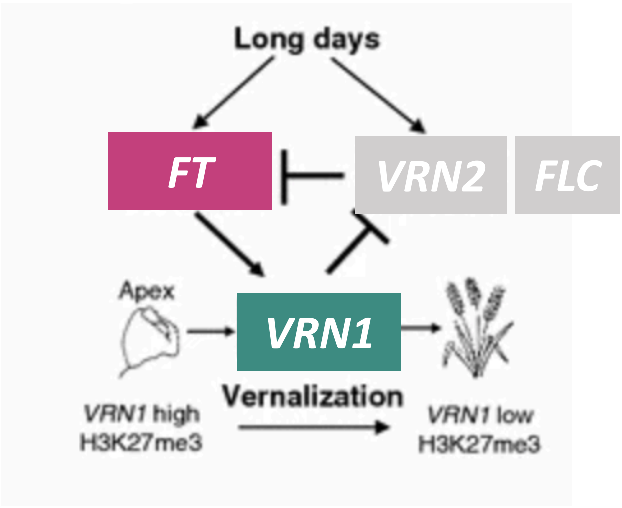
SOC1 genes have at least three duplications prior to the diversification of eudicots.

FLC repressor and *VRN1* promoter genes are lacking in orchids



FLC/FUL

215 seq



FUL homologs are duplicated in both eudicots and monocots

● Eudicots ● Non-Orchids Monocots ● Orchids ● Early divergent angiosperms ☆ Duplication event

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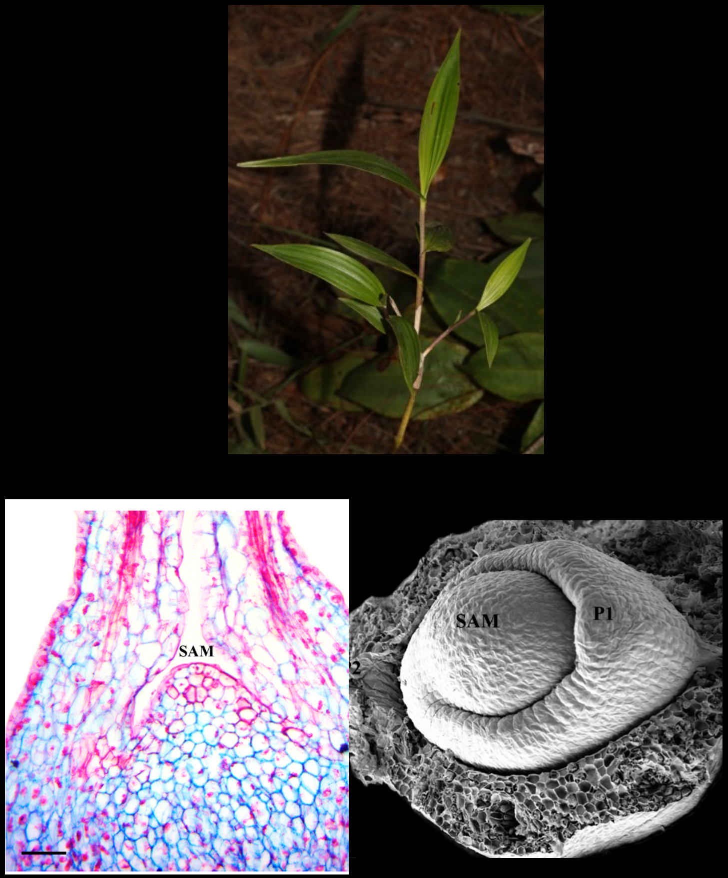
Developmental stages in flowering transition in *Elleanthus aurantiacus*

Shoot Apical Meristem (SAM)



Inflorescence Meristem (IM)

Floral Meristem (FM)

E. aurantiacus plants grow on 1400-2700m altitude and form racemose inflorescences on rainy season two times per year

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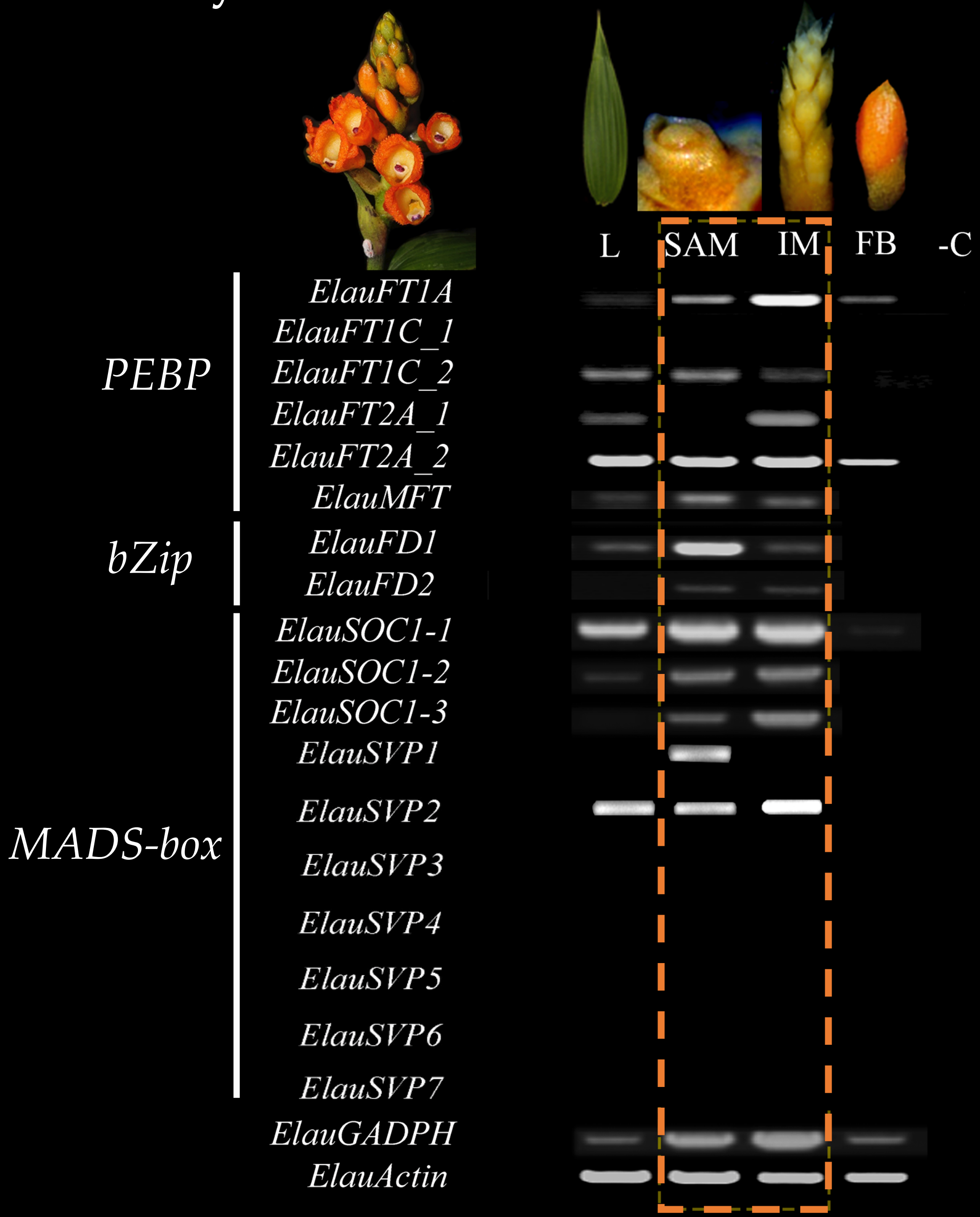
Qualitative expression analysis of GNR in *Elleanthus aurantiacus*

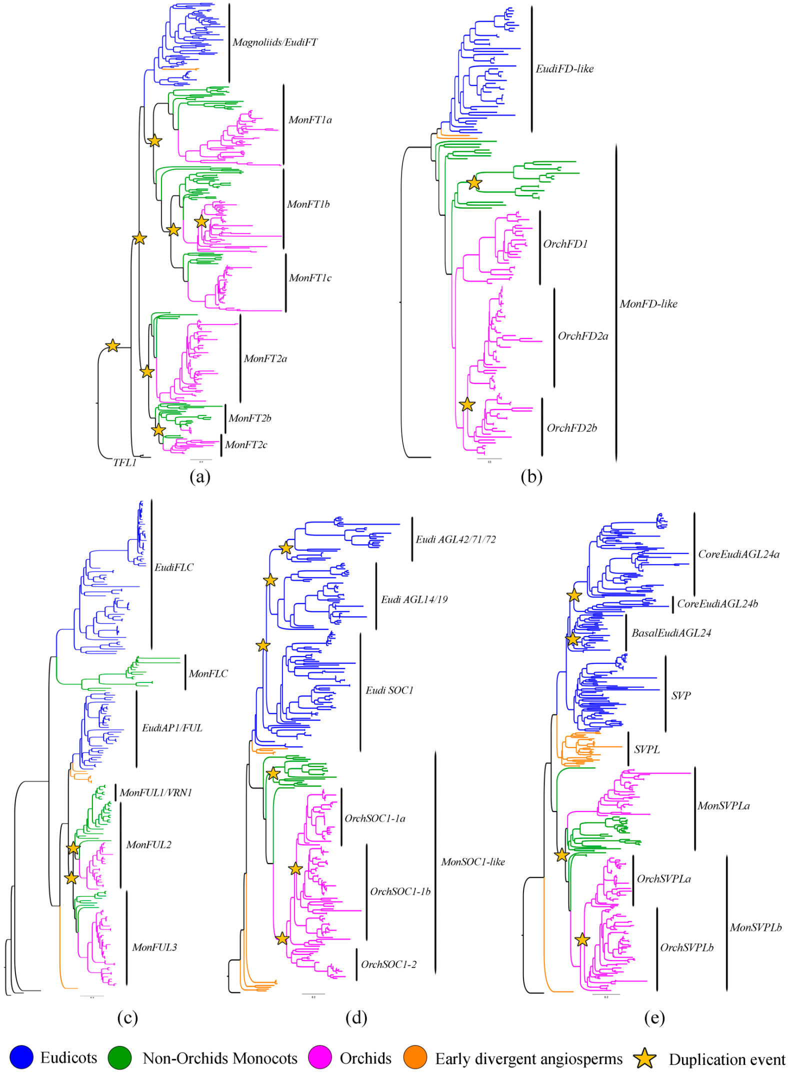
GRN genes are expressed in the SAM and in IM, suggesting important functions in the reproductive transition



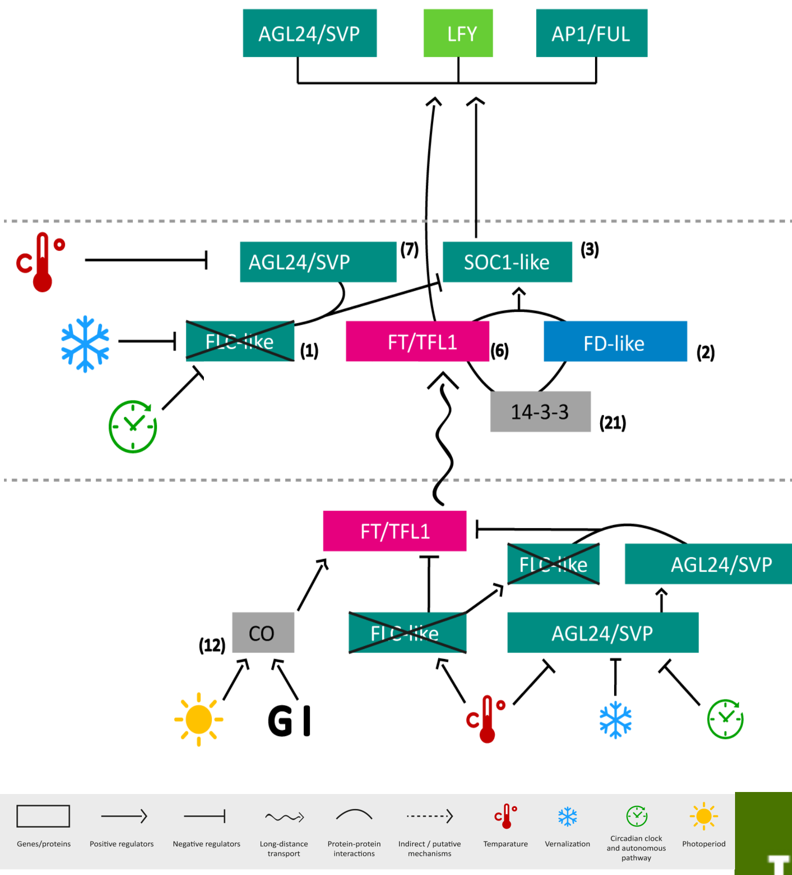
Ospina et al. 2020, Ramirez-Ramirez et al. in prep.

Conclusions

1: GRN in orchids have undergone different evolution pathways in comparison with grass model species, due to independent duplication events



2: GRN has remarkable changes in the increase of gene copy number in orchids with unknown functions



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BOTANICAL SOCIETY OF AMERICA

2019 BSA Graduate Student Research Award


Dr. Natalia Pabon-Mora


Diego Ospina-Zapata


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¿Questions?

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Thank you..