

FloralNET, a gene network modeling the transcriptional program of floral development in *Arabidopsis thaliana*

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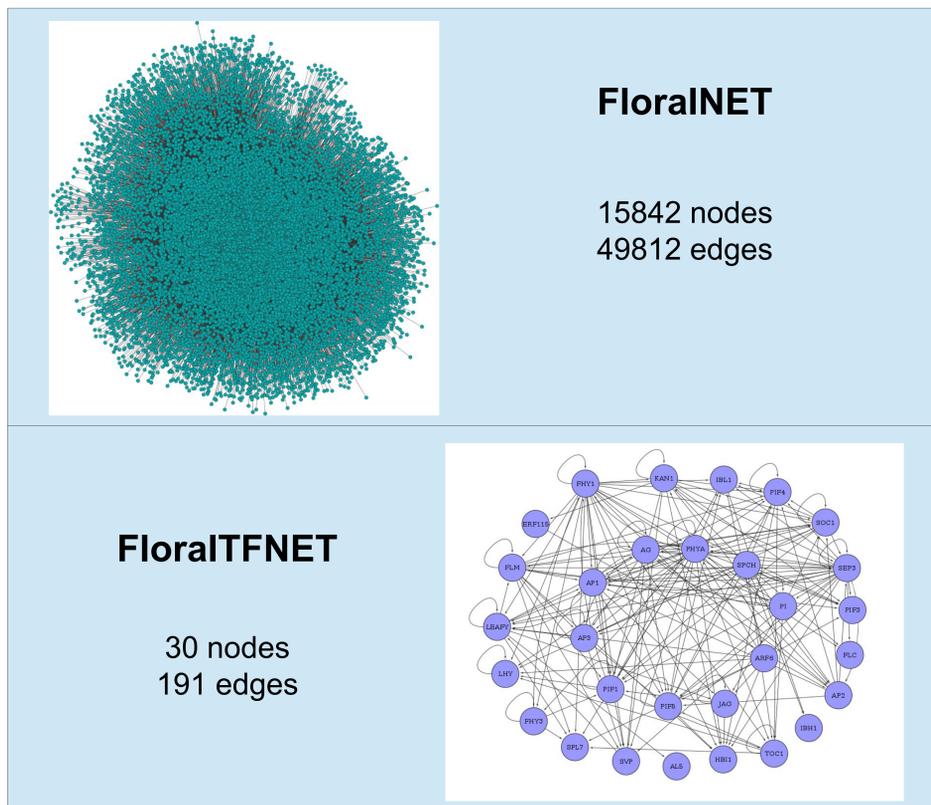
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Introduction

Plants go through a great variety of physiological and morphological changes that are part of their developmental programs. In this work we have analyzed flower development in the model plant *Arabidopsis thaliana* from a molecular systems biology approach. In this sense, we have analyzed all *cis* elements (cistrome) bound by several transcription factors involved in the floral transition. The most direct way to identify the cistrome is chromatin immunoprecipitation followed by high throughput sequencing or ChIP-seq. In this work, we have collected a massive amount of ChIP-seq data from key transcription factors involved in the floral development that has been integrated to produce a model, the transcriptional network called FloralNET. Computational and mathematical analysis of this network have unveiled some properties of the floral development and generate several hypothesis. Finally, using transient expression experiments in *Nicotiana benthamiana*, these hypothesis have been experimentally validated. This validation produces new data that could be integrated again, starting a new refined cycle.

Identification of target genes and construction of the transcriptional networks.

Altogether, data of thirty transcription factors available in the GEO database have been analyzed. The target genes of these transcription factors have been assigned according to the criterion of the nearest downstream gene. We have built a transcriptional network integrating all transcription factor ChIP-seq data. This network, called FloralNET, has 15842 nodes and 49812 edges, representing 58.5% of the *Arabidopsis* genome. From FloralNET we have extracted the network constituted only by the analyzed transcription factors and the interactions between them. We called this network FloralTFNet, constituting the transcriptional regulatory core of FloralNET.

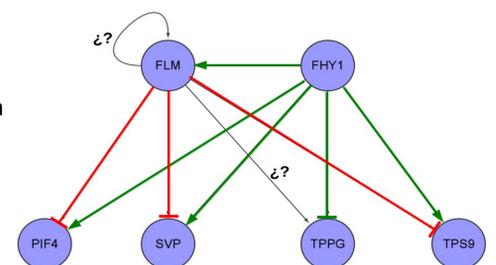


Network Motifs identification

In order to find non random regulatory structures in FloralNET, we performed a network motif identification. A network motif is defined as a subgraph that appears a significantly higher number of times in transcriptional networks than in random graphs. The main motifs of FloralNET are auto-regulation, double feedback and double feedback with output. According with previous studies they are typical motifs in developmental networks.

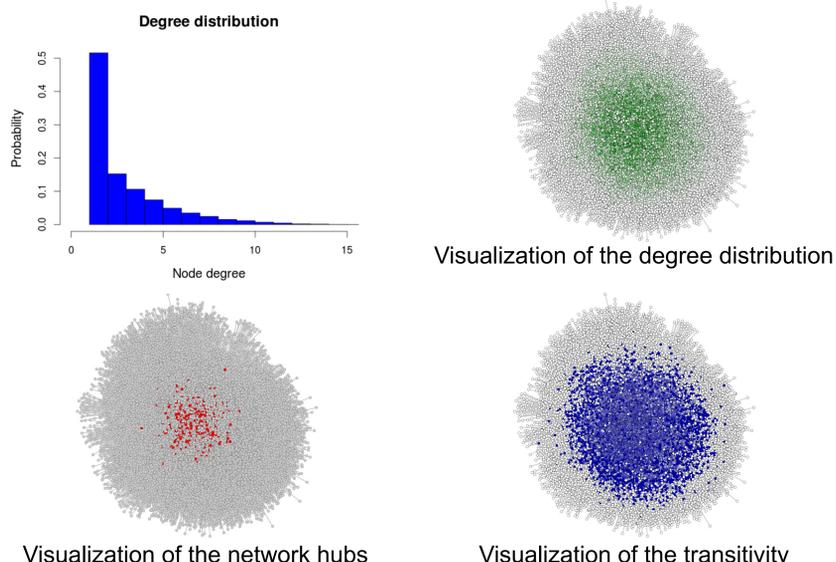
Network Motif	Number of genes	Appearances in FloralNET	Appearances in random networks (mean ± sd)	p-value
Auto-regulation	1	17	6.36 ± 2.21	< 10 ⁻⁵
Double feedback	2	14	0.86 ± 1.07	< 10 ⁻⁵
Double feedback with output	3	73	15.08 ± 4.06	< 10 ⁻⁵

Particularly relevant motif found in FloralNET



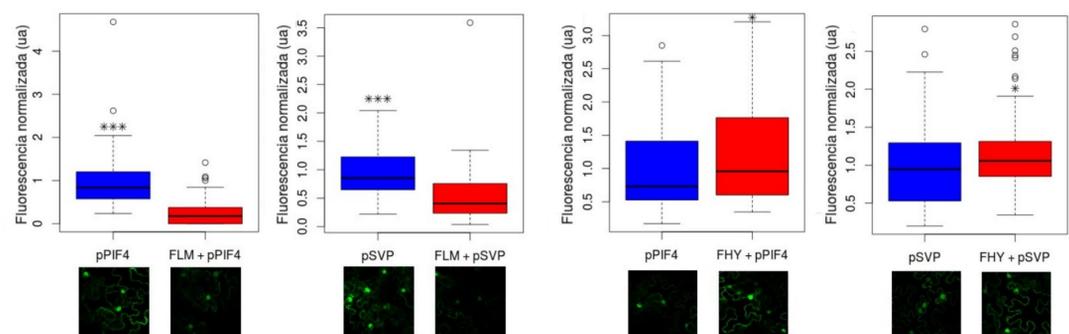
Network topology

We have checked that FloralNET is a scale-free network since the network degree distribution follows a negative exponential distribution, obtaining a p-value of 0.95 using the Kolmogorov-Smirnov test. In scale-free networks most nodes are lowly connected whereas there exist only a few nodes called hubs that are highly connected. To analyze the clustering coefficient of FloralNET we calculated the transitivity of each node and represented it in the network. FloralNET presents a high clustering coefficient with a p-value < 10⁻⁵. This supports the small-world character of FloralNET.



Experimental Validation

According to the motif network described in FloralNET, we have decided to check the transcriptional regulation of *FLM* and *FHY* on a set of target genes. Specifically, we checked if the fluorescence of the Green Fluorescent Protein fused to the promoters of the target genes vary if the putative regulator gene of this promoter is coinfiltrated. Our experimental data confirmed the hypothesis made over the net.



Discussion

The topological properties of FloralNET, scale-free and small-world network, suggest that the transcriptional program that governs the flowering process exhibit relevant properties such as robustness against random mutations, fragility against targeted mutations, high connectivity and fast information propagation. We have found relevant motifs that could have relevant functions in the information processing carried out by the network. One instance of these network motifs has been experimentally validated.

Funding

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