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## Investigating the impact of reduced tetramer formation and DNA binding cooperativity on MADS WT Col-0 complex transcriptional activity

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**Introduction:** Our team uses a combination of genetics and genome wide *in vivo* and *in vitro* techniques, to better understand how dimer and tetramer complexes within the MADS-domain transcription factor family regulate gene expression and flower morphogenesis. SEPALLATA3 (SEP3) and its natural splice variant SEP3<sup>Δ161-174</sup> (SEP3<sup>Δtet</sup>) were used in this study.

**I- SEP3 is A Central Player in Floral Organ Development**

SEP3 is a hub protein that form complexes with four other MADS TFs (AP1, AP3, PI, AG) (Figure 1). Tetramers binds to two CarG boxes cooperatively and triggers specific gene expression (Figure 1). SEP3 is important for the formation of all the flower organs.

In the *sep1sep2sep3* mutant, the 4 whorls are represented by sepals and the flower is indetermined (Pelaz *et al.*, *Nature*, 2000)

Figure 1: The quartet model  
Theissen and Saedler, *Nature*, 2001

**II- *In vivo* evidences that SEP3/AG tetramer regulates flower determinacy**

MADS TFs are comprised of four domains- M, I, K and C (Figure 2). The M domain is highly conserved. Expression of SEP3<sup>Δtet</sup>, impaired in the K domain, restores petals and stamen formation but not the 4<sup>th</sup> whorl neither flower determinacy, in *sep1sep2sep3* mutant (Figure 3). These flower defects are correlated with the impairment of SEP3<sup>Δtet</sup>/AG tetramer formation *in vitro* (Figure 4) and correct binding to *KNUCKLE* (*KNU*) and *CRABS CLAW* (*CRC*) promoters, 2TFs involved in carpel development and flower determinacy (Sun and Ito, *Trends in Plant Sciences*, 2015). Expression of both TFs is miss-regulated in *sep1sep2sep3* plant expressing SEP3<sup>Δtet</sup>, based on qPCR analysis (Hugouvieux *et al.*, *Nucleic Acid Research*, 2018).

Figure 2: MADS-Modular Domain Structure

Figure 3: Complementation analysis of *sep1sep2sep3* flower phenotype with SEP3 and SEP3<sup>Δtet</sup>

**III-RNA seq analysis highlighted few differences in gene expression triggered by SEP3 and SEP3<sup>Δtet</sup>**

MDS plot (Figure 5) shows homogeneity between replicats. While *sep1sep2* flowers showed no clear phenotype (see Figure 2), gene expression is significantly different from WT. As expected, lines expressing SEP3 get closer to *sep1sep2*. SEP3<sup>Δtet</sup> triggers similar pattern of gene expression as SEP3 and only few genes are statistically affected by SEP3<sup>Δtet</sup> (Figure 7). Among them, *CRC* was again identified and was the only one bound by SEP3 in ChIP (Pajoro *et al.*, *Genome Biology* 2014).

Figure 5: Overview of samples homogeneity (Galaxy). RNA-seq analysis was performed on IM and closed buds up to stage 9 (same RNA samples used in II).

Figure 4: EMSA showing that SEP3<sup>Δtet</sup> can form dimer with AG but hardly form tetramers. The DNA belongs to *KNU* promoter and contains 2 CarG-boxes (similar pattern was obtained with *CRC* promoter region). EMSA with other MADS partners (AP3, PI) only showed small impact on tetramer formation with SEP3<sup>Δtet</sup> (Hugouvieux *et al.*, *NAR*, 2018)

Figure 6: Overview of genes up and down between the different genotypes (FDR < 0,05).

**IV-DAP-seq analysis highlighted a genome wide specific binding pattern of SEP3/AG vs SEP3<sup>Δtet</sup>/AG**

SEP3/AG (tetramer) and SEP3<sup>Δtet</sup>/AG (dimer) complexes produced *in vitro* by co-translation were purified by immunoprecipitation (Figure 7) and incubated with DNA. Bound regions were retrieved by immunoprecipitation and sent for sequencing (Genewiz) and further analysed (Figure 8)

**Conclusion and Future experiments**

- 1) Our previous work has demonstrated the requirement of SEP3/AG tetramer formation in determinacy, trough the regulation of *KNU* and *CRC*.
- 2) RNA-seq analysis are being now analysed with less stringency to highlight new potential gene regulation by tetramer vs dimer.
- 3) DAP-seq analysis has identified a specific binding pattern for SEP3/AG tetramer compared to dimers, based on BS spacing that will be shortly tested by EMSA.
- 4) RNA-seq, DAP-seq data will be crossed with ChIP-seq data for SEP3 and AG (Pajoro *et al.*, *Genome Biology*, 2014; Kaufmann *et al.*, *Plos Biol.*, 2009; Ó'Maoiléidigh *et al.*, *The Plant Cell*, 2013) to get list of genes regulated by tetramer vs dimer *in vivo*.
- 5) DAP-seq analyses are being now performed with others MADS combination to unravel MADS specificity between tetramers.
- 6) Plant with enhanced impairment in tetramer formation are as well being generated.

Figure 7: Example of Western blot analysis showing the purification of SEP3/AG tetramers by Immunoprecipitation before incubation with Arabidopsis DNA.

	Input		α-FLAG IP	
	+	-	+	-
SEP3-FLAG	+	-	+	-
AG-Myc	+	+	+	-
α-FLAG				
α-Myc				

Figure 8: Bound regions by SEP3/AG are enriched in 26-36-46bp spaced CarG boxes compared to SEP3<sup>Δtet</sup>/AG bound regions in correlation with the ability of tetramer to bind 2 sites. No difference was found in the binding site sequence between dimer and tetramer. Interestingly in the *KNU* promoter (figure 4) the 2 CarG boxes are separated by 36 bp.