

Unravelling the *Brassica napus* epigenetic network with an integrated multi-omics approach

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Background:

Brassica napus (canola) is an allotetraploid (AACC, 2n= 38) crop derived from a natural hybridization event between *B. rapa* and *B. oleracea* and it's one of the major oilseed crops worldwide. With only 12.5% of the genome predicted to contain coding sequences and 37.5% composed of repetitive elements, a significant proportion of the 1.1Gb genome is yet to be deciphered. Among these unannotated sequences, cis-regulatory elements (CREs) are known to affect gene expression and modulate agronomically relevant traits in several crops. A major challenge hindering CREs annotation is their highly dynamic activity/state, often times tissue-, condition-, and/or growth stage- dependent.

Objective:

Generating a comprehensive representation of *B. napus* regulatory architecture and chromatin organization by integrating multi -omics approaches, to further elucidate the role of CREs in fine tuning crop traits.

Methods:

To identify *B. napus* CREs, we evaluated five tissues of the 'Express 617' canola accession/ cultivar (seedlings, roots, leaves, floral bud bundles and immature siliques) with three approaches - WGBS, ATACseq and mRNAseq. DNA methylation profiles of the three most diverse tissues (roots, leaves and siliques) were obtained via whole genome bisulphite sequencing (WGBS). Unmethylated regions (UMRs) are expected to capture majority of CREs regardless of their active or inactive state and to be relatively stable across vegetative tissues. Active CREs were detected with ATACseq (Assay for Transposase-Accessible Chromatin), a method that utilizes a hyperactive Tn5 transposase to cut and tag open chromatin regions (OCRs), known to correlate with active CREs presence. Finally, mRNAseq data was generated for all tissues investigated to assess the expression of active CREs target genes and further confirm their regulatory function.

Results:

Several OCRs /UMRs were found conserved amongst all samples and confirmed to be proximal to known essential genes. In contrast, thousands of OCRs identified were unique to each sample type, confirming the highly tissue-specific active state of CREs. Combining chromatin accessibility information with DNA methylation confirmed a significant overlap between OCRs and UMRs, while also identifying CREs inaccessible across all investigated tissues, that have the potential to become accessible (and active) under specific growth conditions or stresses.

Conclusions:

Further elucidation of this regulatory map of canola is a key step towards understanding the role of CREs and the potential impact of cis-regulatory variation on crop traits.