The role of cis-regulatory variation in drought responses in chickpea

Background

Transcriptional regulation facilitates plants to mount appropriate responses to environmental cues and stresses. Expression regulation is controlled by cis-acting elements (e.g., promoters), bound to target alleles and/or trans-acting factors (e.g., transcription factors), impacting both gene alleles. Different evolutionary and selection histories for each of these regulatory elements are thought to accompany interspecific adaptive divergence especially to local environments. In hybrids, maternal and paternal cis-regulatory alleles are exposed to the same trans-acting factors; thus, the relative allele expression provides a measure of relative cis-regulation of the transcriptome (Figure 1). Allele specific expression (ASE) analysis is a powerful tool for studying the effect of such regulatory variation. In combination with differential expression analysis between parents, trans- regulatory elements can simultaneously be identified and trans-regulation quantified for the characterization of evolution of the so-called evolutionary scenarios. Thus, the levels of conserved and compensatory cis-regulatory evolution can also be estimated. Gene and gene-environment interactions can therefore be explored by studying the genetic architecture of gene expression as a key to candidate gene and variants discovery for stress tolerance breeding. Using RNA-seq, we studied the regulatory architecture of gene expression in chickpeas by exploring the transcriptome of F1 hybrids from reciprocal crosses between C. arietinum and C. reticulatum with their parents. We further identified drought-stress driven changes in regulatory divergence between wild and cultivated chickpeas.

Methods

F1 hybrids generated from reciprocal crosses between wild (Cicer arietinum) and cultivated chickpeas (Cicer reticulatum) were grown under unstressed and declining soil moisture conditions along with parents (Figure 2.1), the implemented leaf RNA-seq, variant-aware read alignment, variant calling and filtering followed by genome-wide read-backed phasing resulting in aggregated gene-level haplotypic counts of reads overlapping several SNPs per gene (Figure 2c). Significant cis effects, taken as deviations from 1:1 haplotypic expression ratios in F1s, were determined using binomial and Fisher’s exact tests and expressed as log2-fold changes in the alternative haplotypes over the alternative haplotypes (Figure 2e). The F1 parent 1 parental expression ratio was used to estimate the composite trans effect per locus. We calculated cis-regulatory divergence as the log-transformed ratio of reads mapping to C. arietinum and C. reticulatum haplotypes in the hybrid sample: log2(A/B) (Figure 2d). Differences in expression that were not attributed to the cis-regulatory divergence were attributed to trans-regulation (log2(A/B)trans-effect). Similar analyses were carried out to determine the stress specific variations in hybrids and parents. In addition, we compared allelic expression levels between parents and hybrids to determine possible specific allelic deviations in hybrids while interactions with parental expression differences were used to classify each gene’s expression and regulatory architecture following Fisher’s exact tests.

Results


Discussion and Future activities

Hundreds of genes and tens of pathways show significant allelic inbalance in chickpeas. Differential trans-regulation may predominate expression divergence in the Cicer genus. Interestingly, multiple phototrophic, metabolic, cell reproduction, protein synthesis and turnover pathways among others, are all impacted by cis-regulatory variation impacting extensive wild varied evolutionary and selection strategies for the two species. Genes involved in sugar and carbon metabolism, secondary metabolism and DNA repair were determined to show ASE exclusively in response to drought stress. We are currently characterizing this and more data for the overall expression and regulation architecture, to develop a more complete snapshot of gene and gene by environment cis and trans regulatory impact on interspecific divergence and environmental adaptation.

References