Discovery and characterization of disease resistance loci using a unique gene copy number variant population

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http://comailab.genomecenter.ucdavis.edu/index.php/Poplar

1. Identification of genomic regions that control response to two damaging poplar diseases, leaf spot and leaf rust. In field trials in two US locations and in controlled infection experiments, genome-wide scans will identify poplar loci underlying disease susceptibility.

2. Characterization cell functions governing the response of poplar to leaf pathogens. We will integrate gene expression studies with developmental and microbial responses to identify mechanisms that contribute to disease resistance.

3. Identification of genes that regulate these responses and whose manipulation could result in sustainable, long-term tolerance to the target pathogens. Candidate genes will be evaluated based on their molecular and cellular properties. Individual candidate genes will be tested using transgenic approaches and genome editing.

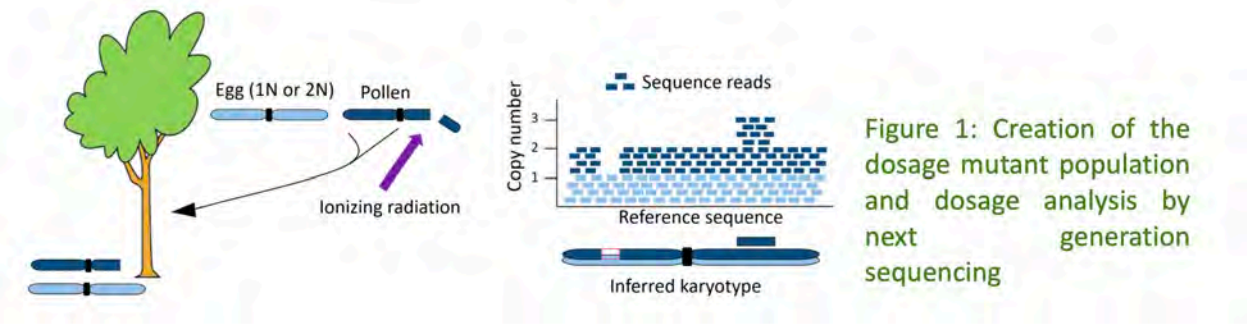

Figure 1: Creation of the dosage mutant population and dosage analysis by next generation sequencing

Pathogenic fungi that colonize poplar leaves and stems reduce yield and can cause failure of industrial bioenergy plantations. Despite extensive study of poplar pathosystems, the genetic control of poplar resistance to pathogens is still poorly understood, underscoring the need for new approaches. We developed a unique functional genomics resource based on gene dosage variation in an elite biomass poplar hybrid (1). We pollinated *Populus deltoides* with gamma irradiated *P. nigra* pollen to produce ~ 800 F1 seedlings. These contain large-scale deletions and insertions that together tile each chromosome multiple times. This resource, developed through previous funding from USDA-DOE Plant Feedstock Genomics for Bioenergy Program, has been used to define loci affecting phenology and biomass (2), and, more recently, leaf shape. Under

natural infection in the field, as well as under controlled inoculations in the greenhouse, we observed a wide variation in disease resistance within our population and were able to identify dosage QTLs influencing resistance of poplar to some of its most important fungal diseases: leaf rust and leaf spot (Melampsora sp., Septoria sp. and *Sphaerulina musiva*). Next, time-course analysis of gene expression during progression of disease symptoms will be performed for selected genotypes and used to develop predictive models of transcriptional networks underlying disease susceptibility. A final set of experiments will aim to identify candidate genes for functional analysis by manipulation using CRISPR-Cas9. Such dosage-sensitive candidate genes with significant effects on disease resistance phenotypes could be exploited in breeding programs through the selection of germplasm with naturally-occurring allelic variation or indels/copy number variation covering resistance loci.

References

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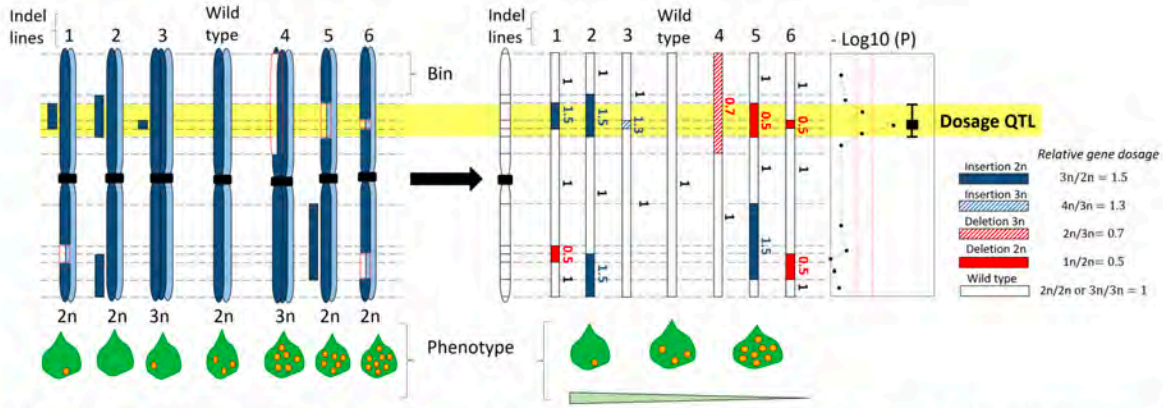

Figure 3: Left: chromosomal bins are defined by the breakpoints of the indels tiled to each chromosome. Right: Genotypes within each bin are assigned a relative dosage value reflecting both the background ploidy level and indel type. Correlations among relative dosage value and phenotypes can then be calculated.

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