

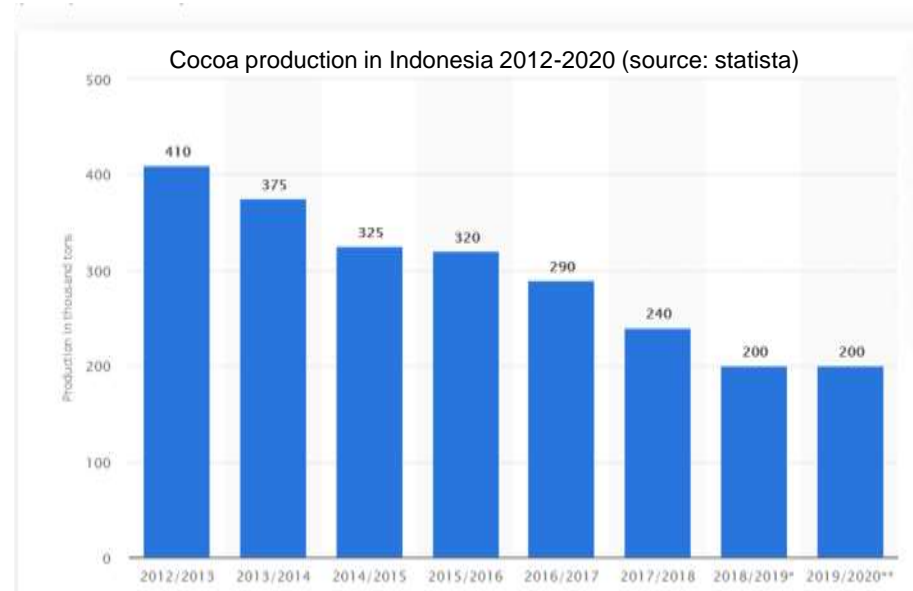
Mapping candidate genes for resistance to Vascular-streak dieback disease of cocoa

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- › Vascular streak dieback (VSD) threatens cacao production in Indonesia and other countries in the Asia/Pacific region
- › Losses ~10%
- › Serious impacts on livelihoods of smallholder farmer families
- › Management is labour-dependent
- › Factor in the decline of cocoa production in Indonesia



- Causal fungus *Ceratobasidium theobromae*
- Basidiospores are released and wind dispersed during proplonged rain
- Basidiospores thought to penetrate flush leaves and colonise xylem
- Spreads through petioles to branches
- Management requires regular pruning
- Susceptibility varies between cocoa genotypes



VSD symptoms

Marginal leaf necrosis



Dark vascular streaks



Enlarged lenticels causing roughening of the bark.



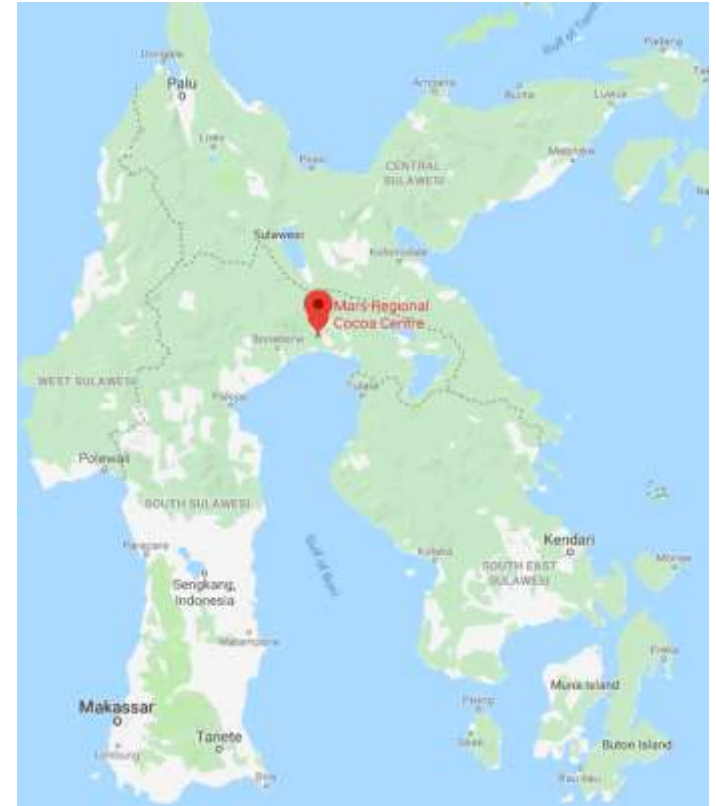
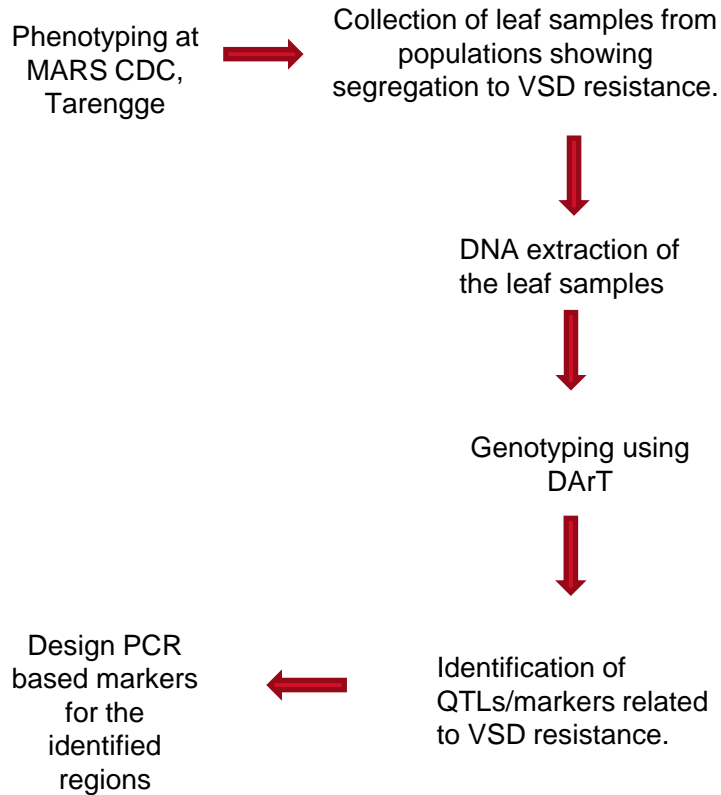
Three blackened
vascular traces at the
leaf scar



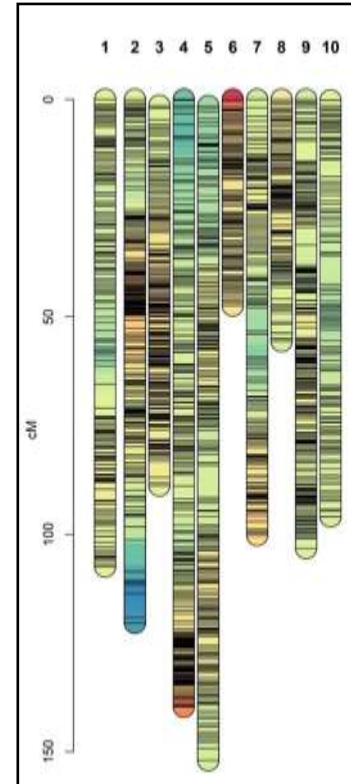
Basidiocarp development on leaf
scars

- To identify QTL associated with VSD resistance in cacao and investigate the genes that may underlie these.
- To develop high throughput PCR based markers associated with VSD resistance for use in marker-assisted selection.



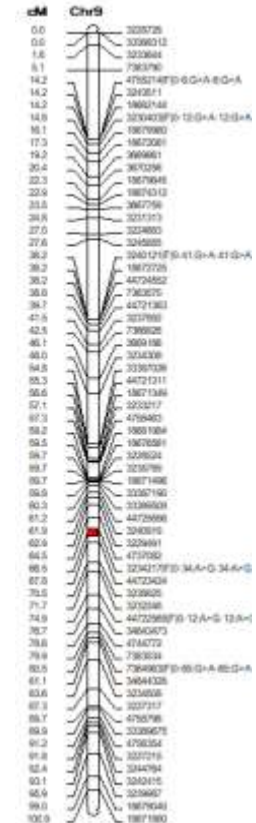
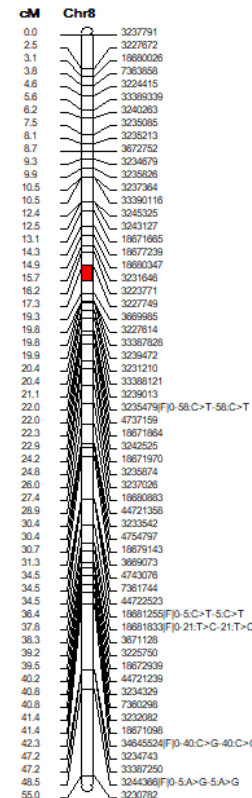


- Phenotypic data for 2017-2019 was kindly provided by MARS CDC, Tarengge, Indonesia.
- › 140 lines derived from a population of S1 (resistant) x CCN51 (susceptible) were genotyped by DArT Pty Ltd.
- › A genetic linkage map was developed using 8,000 DArT markers and 2,000 SNP markers.
- › QTL analysis was performed using R/QTL package in Rstudio 1.4



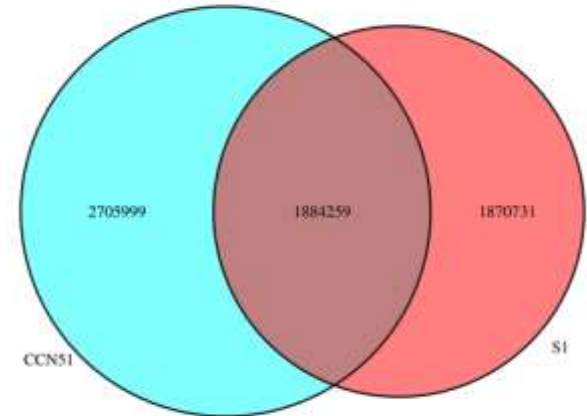
Linkage map of S1 x CCN51

- QTL analysis identified two significant QTLs, one on chromosome 8 and one on chromosome 9
- These QTLs co-located with the QTLs identified by Epaina (2014) for VSD on a different population (KA2-101xK81) in Papua New Guinea
- QTLs were also located in close proximity to QTLs identified for *Phytophthora* pod rot and Frosty Pod disease by Lanaud et al. (2009).



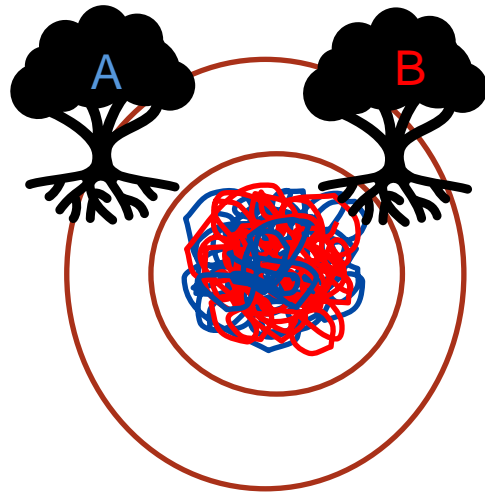
- › PCR based markers flanking the QTL region of chromosome 8 and chromosome 9 were designed
 - › Markers were validated in two populations with the common parent CCN51
 - › Five SSR markers that showed polymorphism for the resistant and susceptible lines were common to both populations
-

- Genome of resistant genotype S1 and susceptible genotype CCN51 was sequenced using paired-end reads
- Genome assembly was aligned to reference cacao genotype Matina 1-6
- DNA resequencing identified polymorphic and common SNV/Indel mutations and structural variants between S1 and CCN51
- Gene specific SNV/Indels or structural variants could be screened for polymorphism between resistant and susceptible lines
- This would enable identification of the cluster of genes associated with VSD resistance

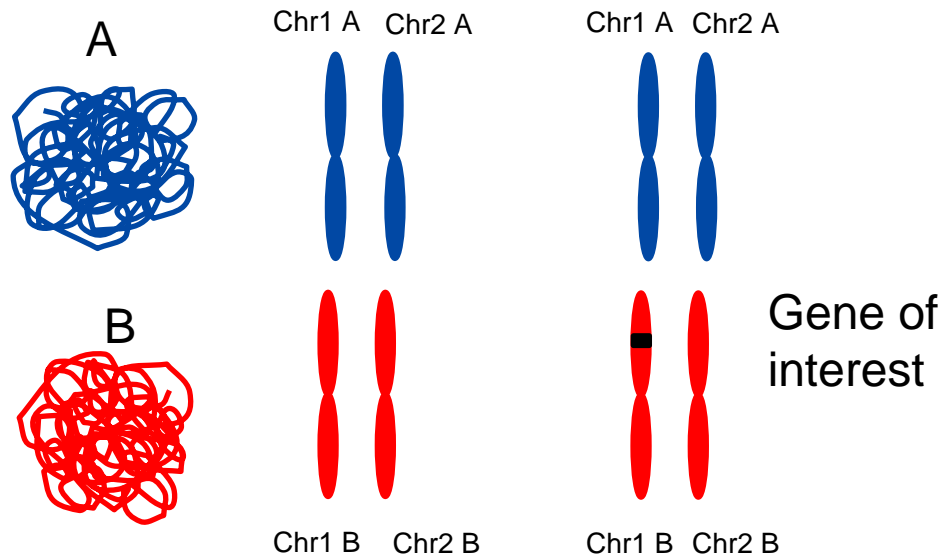


Diploid genome assembly goals

- Until very recently genomes were published as collapsed haploid
- Now possible to separate out the two parental genomes and build chromosome level assemblies

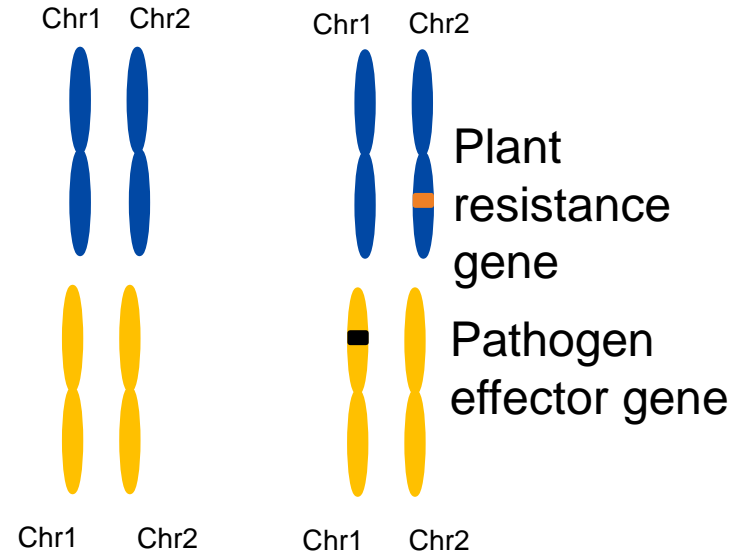
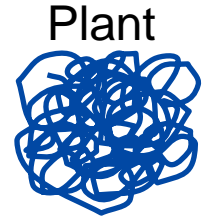
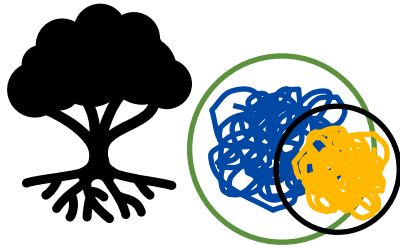


A/B parental DNA in the nucleus



Potential for both plant and pathogen genomes from one sample

Plant infected with a pathogen



MARS Cocoa Research Centre

European Cocoa Association, CAOBISCO and FCC

Australian Centre for International Agricultural Research