Supplementary Diagram 1. Flow diagram of analytical pipeline for this study integrating SEM and residual GWAS approaches.

Integrating growth physiology and calcium status to reveal the genetic basis of lettuce tipburn via GWAS

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Running title: Lettuce tipburn resistance

Data compilation

Phenotyping

DS, PB, Ca, TB

Genotyping

tGBS

Population structure

PCAs, K matrix

Statistical modeling

Response Surface Model (RSM)

Estimate predicted traits (pTB and pCa) from DS and PB

Residual trait calculation

Calculate rTB, rcTB and rCa from DS, PB, (Ca), and PCAs

Structural Equation Modeling (SEM)

Calculate causal relationship DS, PB, Ca → TB

Genetic mapping & bioinformatics

GWAS

Mapping QTLs for DS, PB, Ca, TB, rTB, rcTB, rCa

QTL classification

Compare QTLs to identify direct and mediated TB loci

Bioinformatics

Identify and annotate candidate genes

Legend

DS: developmental score, PB: plant biomass, TB: tipburn incidence, Ca: bulk leaf calcium content, tGBS: tunable genotyping-by-sequencing, PCAs: three principal components, K matrix: kinship matrix, pTB & pCa: traits predicted from growth covariates (DS & PB), rTB : residual TB adjusted for DS, PB, & PCAs, rcTB: residual TB adjusted for DS, PB, Ca, & PCAs, rCa: residual Ca adjusted for DS, PB, & PCAs.