

Identification of novel strain-specific and environment-dependent minor QTLs linked to fire blight resistance in apples

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Introduction

Fire blight, caused by the gram negative bacterium *Erwinia amylovora* (EA), threatens apple and pear production globally. Identifying novel functional alleles is needed for apple cultivars with enhanced fire blight resistance. The majority of QTLs identified are strain-specific and not effective for multiple pathogen strains, as are often present in orchards. Durable broad-spectrum resistance can be created through combining multiple monogenic and polygenic resistances with complementary action toward different strains.

QTL analysis

- Total shoot length and fire blight lesion length were measured after 6-10 weeks when lesions had ceased extension and a distinct margin was visible between necrotic and healthy stem tissue
- Percentage of shoot length necrosis (PSN) was calculated as necrosis/total shoot length length
- Best linear un-biased predictions (BLUPs) were estimated using PSN for each genotype/year and strain in R software
- The apple genetic map was composed of 578 SSR, SNPlex, HRM, GBS markers (Norelli *et al.* 2017)
- QTL analysis was done using Kruskal-Wallis and Interval mapping in MapQTL 5 software



Mapping population and inoculation

- 169 F1 progeny of GMAL 4593 mapping population, 'Royal Gala' (female) x 'PI 613981' (male)
- Progeny and parents were grafted onto rootstocks in three replications and grown in greenhouse in 2008 in Geneva, NY, and in the field in 2010 and 2011 at Kearneysville, WV
- Inoculation was done using three different bacterial strains (Table 1) by cutting young leaves with scissors dipped in *Ea* inoculum

Strain	Location	Host	Isolator	Trial Year
AFRS273	Unknown	Unknown	J.L. Norelli	2010, 2011
Ea273	New York	M x domestica 'R.I. Greening'	S.V. Beer	2008, 2011
LP101	Washington	Malling 26 apple rootstock	P.L. Pusey	2011

Table 1. *Erwinia amylovora* (Ea) strains used in this study.



Strain-specific novel fire blight QTLs

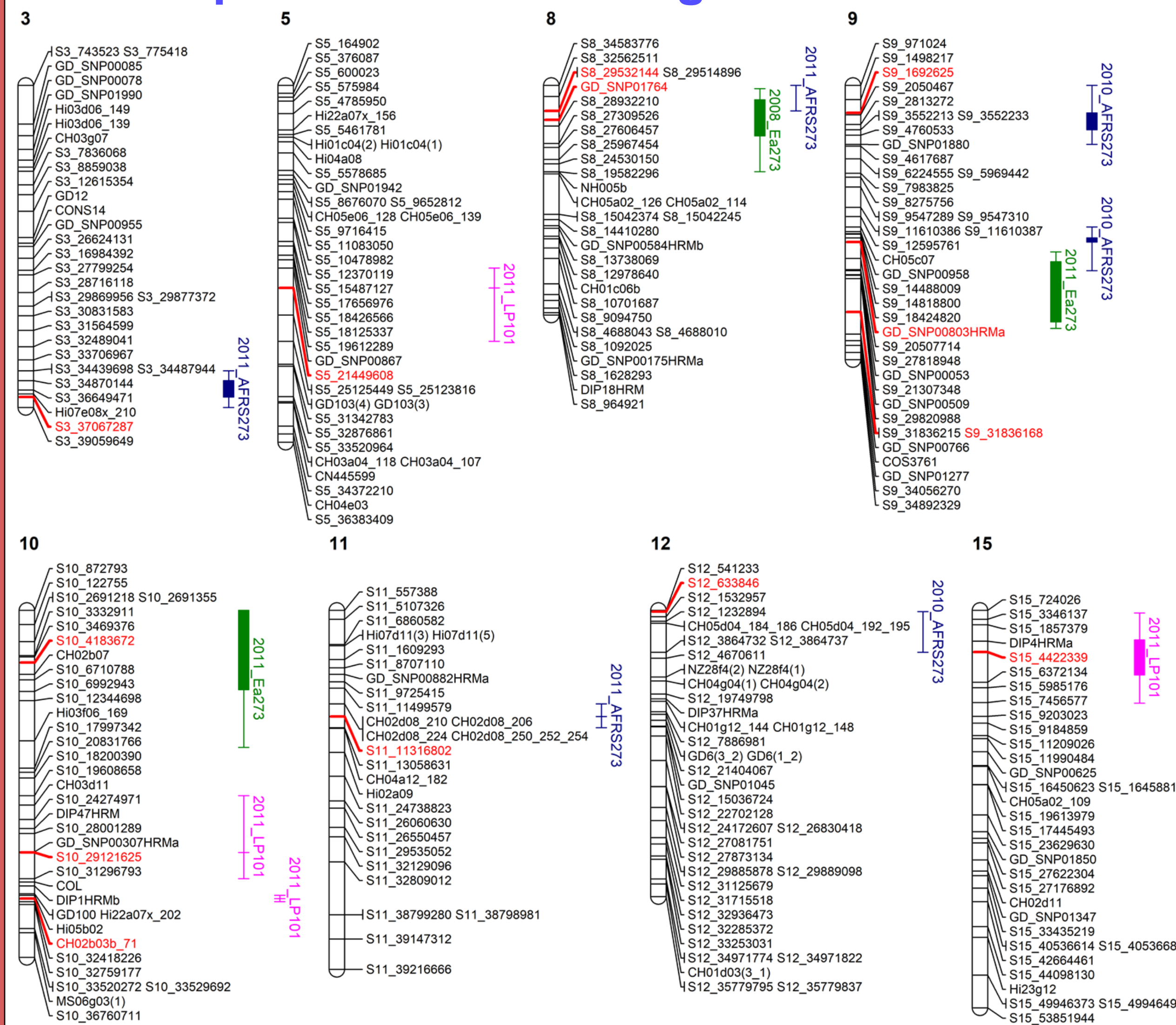


Fig. 3 Fire blight QTLs in GMAL 4593 mapping population for Ea273, AFRS273, LP101 in 2008, 2010, 2011. LOD threshold for a significant QTL in IM was set as 4.5 (95%) and KW was ****:0.005. The QTLs detected with strain Ea273 using IM are represented in green, the lines correspond to the 1- and 2-LOD confidence intervals. The QTLs detected with AFRS273 and LP101 using KW are in blue and pink, respectively; boxes correspond to markers significant at $p=0.005$ and the confidence interval lines for the significant QTL are up to $p=0.1$.

Strain-specific fire blight response

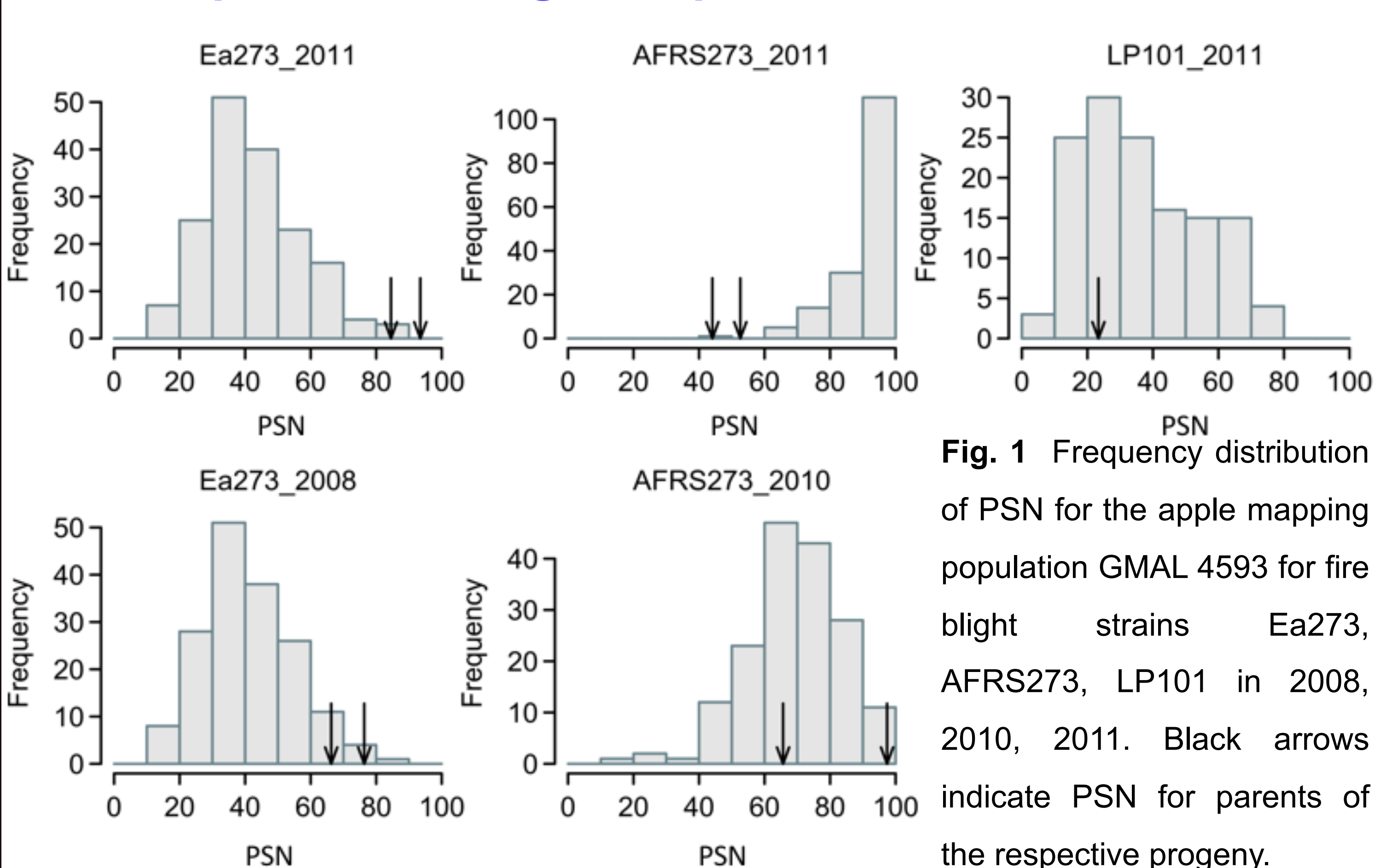
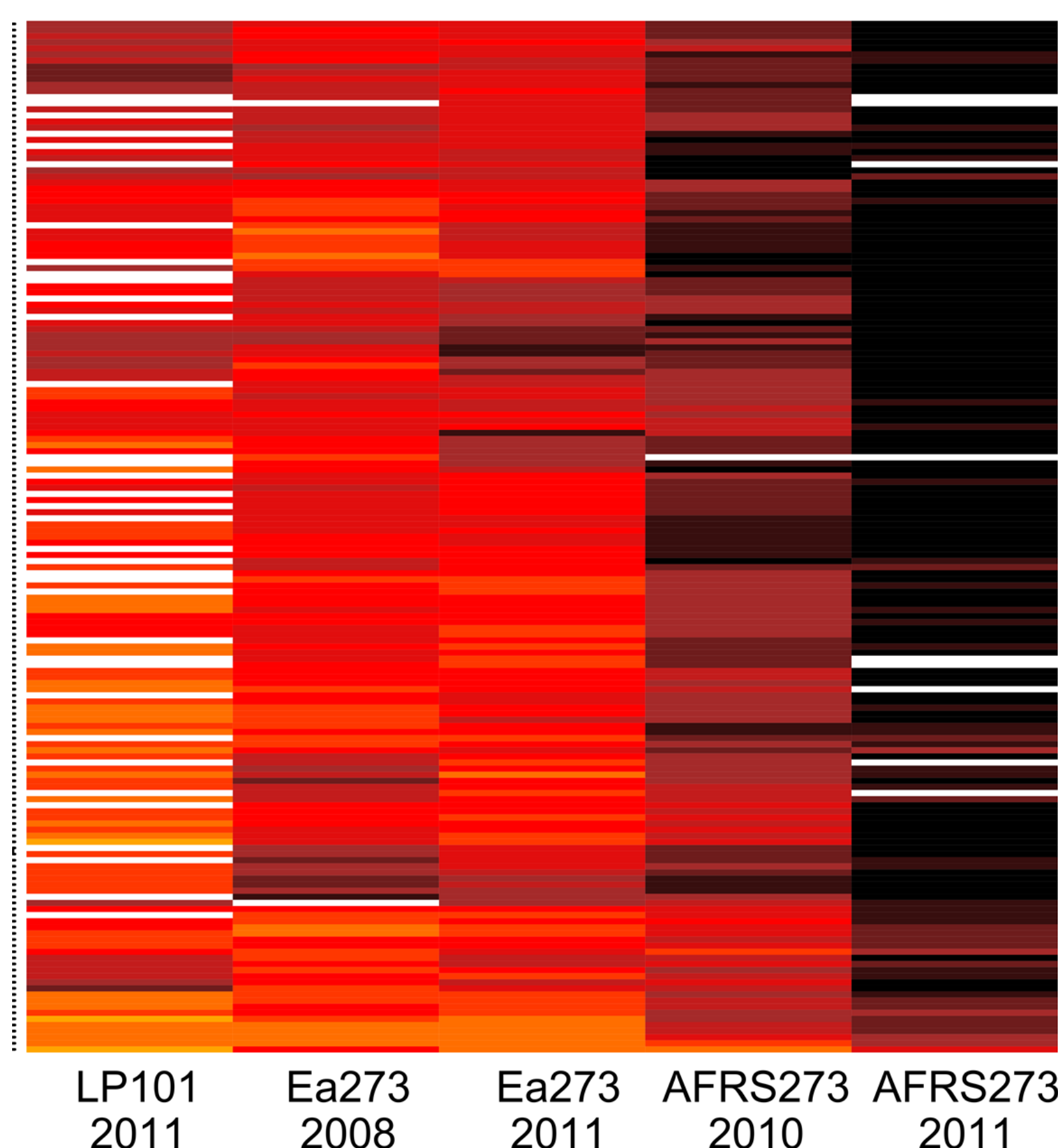
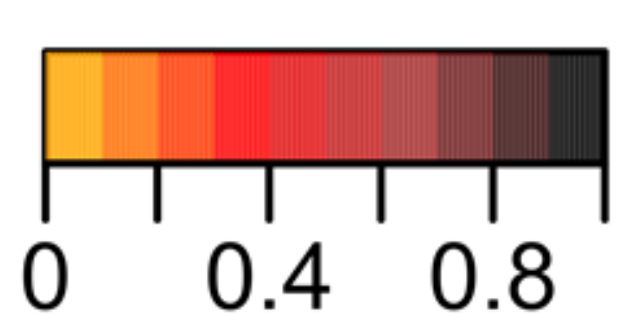


Fig. 1 Frequency distribution of PSN for the apple mapping population GMAL 4593 for fire blight strains Ea273, AFRS273, LP101 in 2008, 2010, 2011. Black arrows indicate PSN for parents of the respective progeny.

Best linear unbiased predictors (BLUPs)

for percentage of shoot necrosis (PSN) of fire blight for GMAL 4593 apple mapping population for three years (2008, 2010, 2011) and three *Erwinia amylovora* strains (Ea273, AFRS273, LP101). Columns represent different datasets and lines represent genotypes. The colors correspond to the percentage from yellow (0%) to black (100%); white corresponds to missing data.



Conclusions

- The progeny of the GMAL 4593 mapping population are segregating for fire blight resistance with varying responses to three *E. amylovora* strains (Fig.1 and 2)
- Progeny plants inoculated with strain Ea273 in 2008 and 2011 had normal distributed PSN. Genotypes inoculated with strain AFRS273 in 2011 skewed towards high PSN, indicating severe fire blight infection, while LP101 infection had more genotypes below 40% necrosis, indicating low to mild severity of fire blight infection compared to other datasets (Fig. 2)
- A total of 13 significant fire blight QTLs were identified (Fig. 3) in eight linkage groups using interval mapping at 95% confidence and Kruskal-Wallis at P -value= 0.005
- The QTLs on LG10 for strain Ea273 in 2011 and strain LP101 in 2011, and on LG15 for strain LP101 could be QTLs previously identified with strain CFBP1430 in cultivar 'Florina' and 'Co-op16 x Co-op17' mapping population, respectively
- The majority are far enough from previously identified fire blight QTLs to assume they are new loci representing novel resistance mechanisms
- Experimental conditions in the greenhouse and field, and between years, and virulence levels of strains might be responsible for strain- and year-specific QTLs

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