# Genome-Wide Association Mapping Uncovers a Dominant Gene Conferring Resistance to Fusarium Wilt in Strawberry

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## SUMMARY

Fusarium wilt of the cultivated strawberry (*Fragaria x ananassa)* is a soilborne disease caused by *Fusarium oxysporum* f.sp. *fragariae*. Fusarium wilt resistance was evaluated for a panel of 566 accessions from the University of California's strawberry germplasm collection. A genome-wide association study identified 13 SNPs in a 2.3 megabase interval that were significantly associated with resistance. Two selfed mapping populations were constructed and evaluated, and resistance co-localized with these SNPs in a 3:1 phenotypic ratio. This resistance appears to be conferred by a dominantly acting R gene (henceforth labelled as *FoR2C-1)* on linkage group 2C and appears throughout the history of the germplasm evaluated.

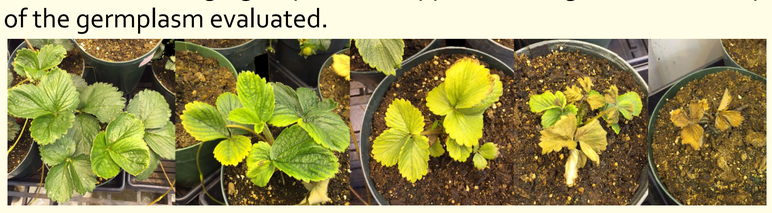

**Figure 1:** General Progression of Fusarium wilt on strawberry plants in a growth chamber. Symptom development (from left to right): a healthy plant; development of chlorosis on younger tissue; development of chlorosis in most tissues; die-back of older tissue; plant death.

## INTRODUCTION

The cultivated allo-octoploid strawberry (*Fragaria x ananassa*) is a valuable crop that is affected by several soil-borne and above-ground pathogens. *Fusarium oxysporum* f. sp. *fragariae* (*FOF*), a host-specific, vascular wilt pathogen, has now been documented on every continent that produces strawberry. In California, which produces ~90% of the United States' strawberries, FOF has been of great concern since its detection in 2006, which coincides with the phase-out of the fumigant Methyl Bromide

While *FOF*-resistant cultivars currently exist, little is known about the genetics underlying Fusarium wilt resistance. Studies into the genetic architecture of *FOF* have yielded results that are diametrically opposed on whether resistance is qualitative or quantitative[1,2] . To gain insights into the genetics of resistance to Fusarium wilt, we completed genome wide association and genetic mapping studies. The former guided the latter, and both were designed to uncover resistance genes if present in strawberry.

The 566 accession panel analyzed in this analysis is representative of the history of the University of California's strawberry breeding program. Historically and commercially important accessions from 1935 to 2012 were included.

## METHODS

**Phenotyping:**

- Replicated trials in 2016 and 2017.
- 566 (2016) and 559 (2017) accessions evaluated.
- 4 replicates per accession planted in an α-lattice, each inoculated with *FOF* by root-dipping into a $5x10^6$ spore solution.
- Ratings based on an established 1 – 5 scale (1 = healthy, 5 = dead) [3], with six timepoints per trial.

**Genome Wide Association Study (GWAS):**

- All accessions genotyped using the 35k Affymetrix® Axiom® Istraw Array[4], resulting in genotypic data for 38,506 SNPs.
- Only good quality, codominant extracted and utilized, resulting in 14,408 markers.
- Markers aligned against the diploid *F. vesca* (v4.0) reference genome [5].
- GWAS performed with 'GENESIS' R package [6], with stratification correction utilizing principal components.

**Genetic Mapping of Two S1 Mapping Populations:**

- 'Portola' and 'Fronteras' identified as heterozygous in region of significant SNPs based on genetic data and selfed.
- Non-replicated progeny planted in an augmented block design alongside the 2017 GWAS panel.
- 93 random progeny from each population genotyped.
- Segregating SNPs mapped using JoinMap 4.1 [7a].
- QTL Mapping performed using MapQTL 6 [7b].

## GWAS RESULTS

Thirteen SNPs exceeded the corrected significance threshold in both years. One additional SNP was significant in 2016 but dropped out in 2017. These SNPs were localized to the telomeric region of chromosome 2 of the *F. vesca* reference genome **(Figure 1a,c).** No significant SNPs were detected elsewhere in the genome at the significance threshold utilized.

Nine of the thirteen SNPs appeared to have a dominant mode of action with the presence of at least one copy of an allele conferring significant resistance **(Figure 1b,d).** These SNPs had mean additive-dominance ratios of 1.00 and 0.86 in 2016 and 2017 respectively. Trait repeatability across years was 0.96. Phenotypic correlation across years was 0.84.

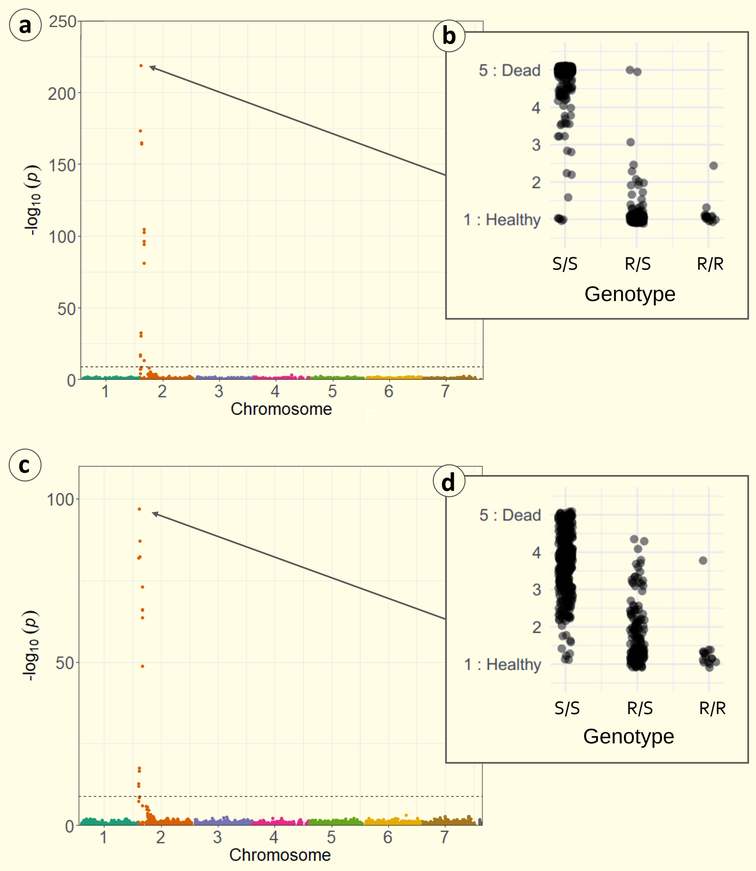


**Figure 2:** SNP-trait associations for Fusarium wilt resistance in strawberry. **a)** Manhattan plot utilizing 2016 phenotypic data, with **b)** inset showing genotype-phenotype plot for the most significant SNP. **c)** Manhattan plot utilizing 2017 phenotypic data, with **d)** inset showing genotype-phenotype plot for the most significant SNP. The most significant SNP was the same in both years. The x-axis of the Manhattan plots indicates genomic location per the diploid *F. vesca* (v4.0) reference genome. Significant SNPs appear above the corrected significance threshold (black horizontal line; $p = 1.2 \times 10^{-9}$ ).

## VALIDATION BY GENETIC MAPPING

Segregation for Fusarium Wilt resistance was observed in a 3.0:1.0 and 2.8:1.2 ratio in the 'Fronteras' and 'Portola' S1 populations respectively. Neither population deviated significantly from the 3:1 segregation ratio expected for a dominant acting Mendelian gene ($\chi^2$ p-values were respectively 0.81 and 0.23 for the 'Fronteras' and 'Portola' populations.)

Genetic mapping indicated a high LOD of ~30 on a single linkage group in both populations **(Figure 3).** Comparison with the University of Florida's UF_14.95 genetic map defined this linkage group as chromosome 2 of subgenome C in both mapping populations [8, using the nomenclature of [9]]. Significant SNPs from the genome-wide association study were associated with the LOD peak in both mapping populations and showed dominance-additive ratios of 0.83 to 1.07.

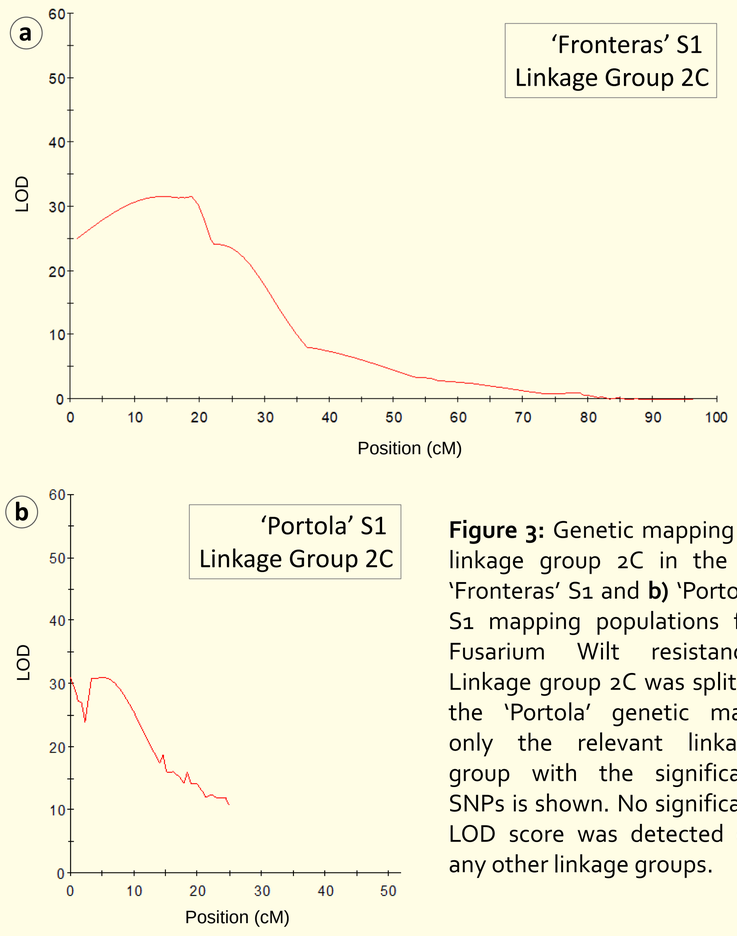


**Figure 3:** Genetic mapping of linkage group 2C in the **a)** 'Fronteras' S1 and **b)** 'Portola' S1 mapping populations for Fusarium Wilt resistance. Linkage group 2C was split in the 'Portola' genetic map; only the relevant linkage group with the significant SNPs is shown. No significant LOD score was detected on any other linkage groups.

## CONCLUSIONS

Based on the results of both the association and genetic mapping experiments, we believe that Fusarium wilt resistance in the University of California's germplasm is governed primarily by a dominant R-gene (henceforth referred to as *FoR2C-1*.) Accessions possessing a resistant haplotype at the SNPs linked to *FoR2C-1* were present throughout the history represented by the panel (1935-2012), with the earliest example being the cultivar 'Shasta' (1935) **(Figure 4).** The majority of resistant individuals were heterozygous for the resistance associated alleles, and, overall, the heterozygous resistant and homozygous susceptible haplotypes were observed in 93.8% of the accessions evaluated. Significant SNPs will be aligned to an upcoming octoploid genome reference, and bulk segregant analysis of the two S1 populations and a subset of the GWAS panel is currently underway. Ideally, this will provide sequence data that further informs development of DNA markers used to screen selections for Fusarium Wilt resistance. Potential novel resistances from older, unique accessions are also being investigated.

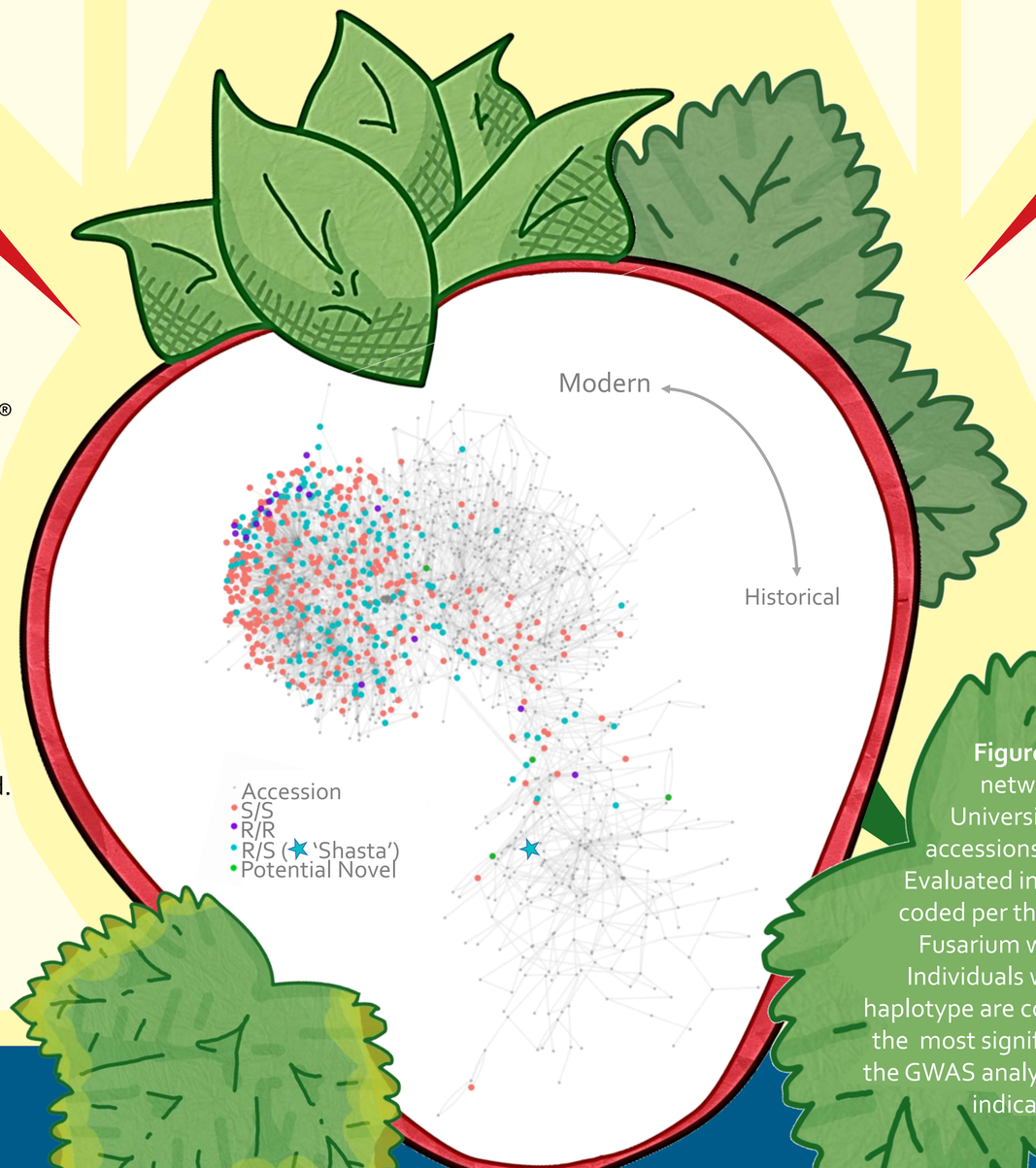


**Figure 4:** Pedigree network showing University of California accessions and ascendants. Evaluated individuals are color-coded per their haplotype at the Fusarium wilt locus *FoR2C-1*. Individuals with a recombinant haplotype are coded per their status at the most significant SNP detected in the GWAS analyses. Cultivar 'Shasta' is indicated by a star.

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[1] Mori T.et al. 2005. J. Jpn. Soc. Hort. Sci., 74, pp.57-59.
[2] Paynter M.L. et al. 2014. J. Am. Soc. Hort. Sci., 139(2), pp.178-184.
[3] Gordon T.R. et al. 2016. Int. J. Fruit Sci., 16(S1), pp.160-168
[4] Affymetrix Inc., Santa Clara, CA, USA.
[5] Edger P.P. et al. 2017. Gigascience. doi: 10.1093/gigascience/gix124.
[6] Conomos M.P. et al. 2017. R Package available at: http://bit.ly/2BUlV3Z
[7] Van Ooijen J.W. (a) 2006. (b) 2009. Kyazma V.B., Wageningen, Netherlands.
[8] Mangandi J. et al. 2017. G3, 7(6), pp.1707-1719.
[9] Van Dijk T. et al. 2014. BMC Plant Bio., 14(1), pp.55.