Title

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Journal

The plant genome, 16(1)

ISSN

1940-3372

Authors

Jiménez, Nicolás P
Feldmann, Mitchell J
Famula, Randi A
et al.

Publication Date

2023-03-01

DOI

10.1002/tpg2.20275

Received: 29 July 2022 | Accepted: 19 September 2022

DOI: 10.1002/tpg2.20275

The Plant Genome

ORIGINAL ARTICLE

Harnessing underutilized gene bank diversity and genomic prediction of cross usefulness to enhance resistance to *Phytophthora cactorum* in strawberry

Nicolás P. Jiménez[#] | **Mitchell J. Feldmann**[#] | **Randi A. Famula** | **Dominique D. A. Pincot** | **Marta Bjornson** | **Glenn S. Cole** | **Steven J. Knapp**

Dep. of Plant Sciences, Univ. of California, One Shields Ave, Davis, CA 95616, USA

Correspondence
Steven J. Knapp, Dep. of Plant Sciences, Univ. of California, One Shields Ave, Davis, CA 95616, USA.
Email: sjknapp@ucdavis.edu

Assigned to Associate Editor Natalia de Leon.

[#]Nicolás P. Jiménez and Mitchell J. Feldmann contributed equally.

Funding information
California Strawberry Commission; National Institute of Food and Agriculture, Grant/Award Number: 2017-51181-26833

Abstract

The development of strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) cultivars resistant to Phytophthora crown rot (PhCR), a devastating disease caused by the soil-borne pathogen *Phytophthora cactorum* (Lebert & Cohn) J. Schröt., has been challenging partly because the resistance phenotypes are quantitative and only moderately heritable. To develop deeper insights into the genetics of resistance and build the foundation for applying genomic selection, a genetically diverse training population was screened for resistance to California isolates of the pathogen. Here we show that genetic gains in breeding for resistance to PhCR have been negligible (3% of the cultivars tested were highly resistant and none surpassed early 20th century cultivars). Narrow-sense genomic heritability for PhCR resistance ranged from 0.41 to 0.75 among training population individuals. Using multivariate genome-wide association studies (GWAS), we identified a large-effect locus (predicted to be RPc2) that explained 43.6–51.6% of the genetic variance, was necessary but not sufficient for resistance, and was associated with calcium channel and other candidate genes with known plant defense functions. The addition of underutilized gene bank resources to our training population doubled additive genetic variance, increased the accuracy of genomic selection, and enabled the discovery of individuals carrying favorable alleles that are either rare or not present in modern cultivars. The incorporation of an RPc2-associated single-nucleotide polymorphism (SNP) as a fixed effect increased genomic prediction accuracy from 0.40 to 0.55. Finally, we show that parent selection using genomic-estimated breeding values, genetic variances, and cross usefulness holds promise for enhancing resistance to PhCR in strawberry.

Abbreviations: AUDPS, area under the disease progression stairs; CNGC, cyclic-nucleotide-gated calcium channel; EMM, estimated marginal mean; G-BLUP, genomic best linear unbiased prediction; GEBV, genomic-estimated breeding value; GVE, genetic variance explained; GWAS, genome-wide association studies; LMM, linear mixed model; MV-GWAS, multivariate genome-wide association studies; PhCR, Phytophthora crown rot; QTL, quantitative trait loci; REML, restricted maximum likelihood; RKHS, reproducing kernel Hilbert space; SNP, single-nucleotide polymorphism; UCD, University of California–Davis.

1 | INTRODUCTION

Strawberry (*Fragaria ×ananassa* Duchesne ex Rozier) plant health and production are adversely affected by a broad spectrum of diseases caused by soil-borne pathogens, including Phytophthora cactorum (Lebert & Cohn) J. Schröt (Erwin & Ribeiro, 1996; Paulus, 1990). This pathogen has a wide host range, a wide geographic distribution, and causes Phytophthora crown rot (PhCR) of strawberry, a disease that was not reported on strawberry until the middle of the 20th century in Germany (Deutschmann, 1954) and thrives under warm and wet growing conditions (Erwin & Ribeiro, 1996; Wilcox, 1989). Phytophthora cactorum produces zoospores from oospores that can persist in soil or infected plant material for many years (Verdecchia et al., 2021), a factor that limits the utility of crop rotation for reducing the incidence of the disease (Rosskopf et al., 2005; Schneider et al., 2003). Soil fumigation with methyl bromide, an ozone-depleting gaseous chemical introduced in the 1960s to control *P. cactorum* and other soil-borne pathogens, greatly decreased the incidence of the diseases they cause, increased yields, decreased production risk, and enabled a phenomenal expansion of strawberry production (Rosskopf et al., 2005; Schneider et al., 2003; Yagi et al., 1993).

The application of methyl bromide as a soil fumigant was banned by a global treaty established in 2005 to protect the ozone layer (https://www.epa.gov/ods-phaseout/methyl-bromide). The soil fumigants that replaced methyl bromide appear to be less effective for controlling *P. cactorum* and other soil-borne pathogens (Duniway, 2002; Pincot et al., 2022; Rosskopf et al., 2005; Schneider et al., 2003). Pincot et al. (2020) postulated that the widespread adoption of soil fumigation with methyl bromide decreased both natural selection pressure and the imperative of breeding for resistance to Fusarium wilt, Verticillium wilt, and other diseases caused by soil-borne pathogens in strawberry. They showed that a significant percentage of the cultivars worldwide are susceptible to one or both of these wilt diseases and speculated that the frequency of susceptible cultivars increased from the 1960s onward because they could be commercially produced without disease-associated yield losses in methyl-bromide-reliant production systems (Pincot et al., 2020; Pincot et al., 2022).

The apparent scarcity of PhCR resistant cultivars reported in earlier studies (Eikemo et al., 2000, 2003; Mangandi et al., 2017; Shaw et al., 2006) suggests that, similar to the history of breeding for resistance to Verticillium wilt and Fusarium wilt in strawberry (Pincot et al., 2020, 2022), breeding for resistance to PhCR has either not been systematic or widespread, not produced significant genetic gains, not substantially increased the frequency of resistant cultivars, or a combination thereof. This was difficult to assess from previous reports because of the diversity of screening protocols, phenotyping methods, pathogen isolates, experiment designs, environments, population composition, and outcomes, in addition to a dearth of common resistant and susceptible checks across studies (Denoyes-Rothan et al., 2004; Eikemo et al., 2003; Mangandi et al., 2017; Parikka, 1998; Pitrat & Risser, 1977; Seemüller, 1977; Shaw et al., 2006, 2008; van Rijbroek et al., 1997).

Core Ideas

- The strongest sources of resistance were heirloom cultivars developed before the advent of soil fumigation.
- The addition of exotic genetic resources to an elite training population doubled genetic variation.
- Although resistance is genetically complex, a single locus accounted for 44–52% of the genetic variance.
- Genetic gains can be accelerated by genomic prediction of breeding values and cross usefulness.
- A global effort is needed to improve resistance to PhCR, which appears to be inadequate among modern cultivars.

The genetic variation previously uncovered for resistance to PhCR in strawberry has been quantitative with broad-sense heritability estimates in the 0.40–0.66 range and narrow-sense heritability estimates in the 0.26–0.39 range; hence, a significant fraction of the phenotypic variation previously observed for resistance to PhCR has been nongenetic (Denoyes-Rothan et al., 2004; Mangandi et al., 2017; Nellist et al., 2019; Shaw et al., 2006). Although several sources of resistance to this disease have been reported, a high percentage of the previously tested cultivars and other genetic resources worldwide appear to be susceptible, gene-for-gene resistance has not been discovered, and breeding for resistance to this pathogen has been challenging (Mangandi et al., 2017; Marin et al., 2022). Here we explore the feasibility of increasing resistance to PhCR through the application of genome-informed breeding approaches, particularly genomic selection and parent selection using genomic-estimated breeding values, genetic variances, and usefulness criteria (Allier et al., 2019; Goddard et al., 2010; Heffner et al., 2009; Lehermeier et al., 2017).

Historically, breeding for PhCR resistance has depended on phenotypic selection, and more recently on phenotypic selection combined with marker-assisted selection targeting RPc2, a large-effect quantitative trait locus (QTL) identified by Mangandi et al. (2017). Genomic selection could perhaps complement phenotypic selection, accelerate genetic gains, and increase the frequency of highly PhCR-resistant cultivars in strawberry (Bernardo & Thompson, 2016; Goddard & Hayes, 2007; Habier et al., 2007; Heffner et al., 2009; Meuwissen et al., 2001; Poland & Rutkoski, 2016). RPc2 was discovered by QTL mapping and explained 13.7–25.3% of

the phenotypic variation in a University of Florida population (Mangandi et al., 2017).

Earlier studies of the genetics of resistance to PhCR (Denoyes-Rothan et al., 2004; Eikemo et al., 2003; Mangandi et al., 2017; Nellist et al., 2019) predated the development of octoploid reference genomes (Edger et al., 2019; Hardigan et al., 2021a), genotyping platforms populated with single-nucleotide polymorphisms (SNPs) physically anchored to the octoploid genome, and genome-wide alignment of next-generation DNA sequences to the octoploid genome (Hardigan et al., 2020). These advances have enabled the straightforward application of octoploid genome-informed genome-wide association study (GWAS) approaches and high-resolution subgenome specific genetic mapping of DNA variants (Hardigan et al., 2020; Pincot et al., 2020, 2022).

Our working hypothesis was that the PhCR resistance breeding problem cannot be fully or effectively solved by targeting individual QTL (Bernardo, 2008) but rather by applying genomic selection with or without the inclusion of RPc2 or other large-effect QTL as fixed effects, an approach that frequently improves genomic prediction accuracy (Bernardo, 2014; Rice & Lipka, 2019; Rutkoski et al., 2014). Our study included a multivariate GWAS search for large-effect loci in a genetically diverse genomic selection training population (George & Cavanagh, 2015; Segura et al., 2012; Tibbs Cortes et al., 2021; Zhang et al., 2010). Lastly, we undertook this study to shed light on historic genetic gains, the impact of past breeding on genetic variation for resistance to PhCR, and the prevalence of resistance to *P. cactorum* among elite and exotic genetic resources in addition to assessing the feasibility of enhancing resistance to PhCR through genome-informed prediction of breeding values, genetic variances, and usefulness criteria (Crossa et al., 2017; Heslot et al., 2012; Labroo et al., 2021; Lehermeier et al., 2017; Mohammadi et al., 2015).

2 | MATERIALS & METHODS

2.1 | Plant material

Collectively, 435 *F.* × *ananassa*, 18 *F. chiloensis*, and 22 *F. virginiana* individuals (asexually propagated genetic resources) were phenotyped for resistance to PhCR in our study. The origin, identification numbers, taxonomy, and other passport data for these individuals are documented in Supplemental File S1. The *F.* × *ananassa* individuals included 64 cultivars developed at University of California–Davis (UCD), 282 UCD hybrids (offspring from crosses between noninbred parents), 75 non-UCD cultivars, and 14 non-UCD hybrids. Thirty-eight individuals were phenotyped in 1 yr, whereas 437 individuals were phenotyped in both years of our studies. The latter were used as the "training" population for genomic prediction of breeding values and genetic variances. The training population included 321 UCD and 116 non-UCD individuals. Of the latter, 40 were wild ecotypes. The *F. chiloensis* and *F. virginiana* ecotypes were originally collected from habitats across their natural ranges in North and South America (Staudt, 1989, 1999). Other than a single *F. virginiana subsp. platypetala* ecotype (15X001P001) collected near the Trout Creek Campground, CA (41.5° N, −121.9° W), the non-UCD genetic resources were originally acquired as single mother plants from the USDA National Plant Germplasm System National Clonal Germplasm Repository, Corvallis, OR (https://www.ars-grin.gov/). These individuals were multiplied from stolons in Winters, CA, and maintained in the UCD Strawberry Germplasm Collection. To produce bare-root clones ("daughter" plants) for replicated testing, bare-root "mother" plants were harvested in January, temporarily stored in the dark at −3.5 °C, and transplanted to a high-elevation (1,294 m asl) nursery in April 2017 and 2018 (Cedar Point Nursery, Dorris, CA). Clones (bare-root plants) of each individual were harvested in mid-October of each year and stored in the dark at 3.5 °C for 2–3 wk before pathogen inoculation and planting.

2.2 | Pathogen isolates and artificial inoculation protocols

Five isolates of *P. cactorum* (Ph9, Ph10, Ph23, Ph24, and Ph25) cultured from infected plants in coastal California were acquired from Dr. Kelly Ivors (California Polytechnic State University, San Luis Obispo, CA). The *P. cactorum* spore samples we used for artificial inoculation of bare-root plants were produced by California Seed and Plant Labs (https://csplabs.com/, Pleasant Grove, CA) from a mixture of these isolates. Samples were prepared by releasing zoospores in water and creating 1×10^4 spores ml^{-1} spore solutions the day of planting. The roots of each bare-root plant (four per individual per year) were submerged in 40 ml of inoculum solution for ∼5 min before being transplanted to the field. One month after transplanting, 11 g of *P. cactorum*-infected oats were spread around the base of every plant in the field. This inoculum was produced by California Seed and Plant Labs using the protocol described by Ivors (2015). The field was periodically sprinkler irrigated over the following week to promote the spread of spores and infection.

2.3 | Field experiments

Our study populations were phenotyped for resistance to PhCR in field experiments at the UCD Plant Pathology

Research Farm, Davis, CA, in both years of our study (the soil is classified as a Yolo loam [fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents]). Field preparation and agronomic practices were identical to those previously described for Verticillum wilt resistance screening studies done side-by-side with the present study (Pincot et al., 2020). Our planting sites were preplant flat-fumigated by TriCal (https://trical.com/) with a chloropicrin-based fumigant (Pic-Clor 60, Cardinal Professional Products; 560 kg ha^{-1}) and sealed with a totally impermeable film tarp for 1 week post fumigation. Once the tarps were removed, fields were prepared for planting by creating 15.3-cm-high raised beds with 76.2 cm of spacing between beds center-to-center and installing drip irrigation before covering the bed with black plastic mulch. Subsurface drip irrigation was applied as needed to maintain adequate soil moisture throughout the growing season. Fertilization was done via injection through the drip system with 10–34–0 solution in fall and early spring and 32–0–0 solution in late spring and summer of each year. Approximately 198 kg ha^{-1} of nitrogen was applied over the 2017–2018 and 2018–2019 growing seasons.

The study populations were planted on 23 Oct. 2017 and 10 Oct. 2018. The roots of each bare-root plant were submerged in the *P. cactorum* inoculum suspension (1×10^4 spores ml^{-1}) for 5 min before transplanting into a single row in the middle of each planting bed. The between-plant spacing within rows was 30.5 cm. The individuals were arranged in 22 by 22 square lattice experiment designs with 22 individuals per incomplete block × 22 incomplete blocks (484 individuals) and four single-plant replications per individual arranged in complete blocks (replications) (Hinkelmann & Kempthorne, 2007). The R package agricolae (De Mendiburu & Simon, 2015) was used to assign individuals to incomplete blocks and randomize individuals within incomplete blocks.

2.4 | Disease resistance phenotyping

The training population was visually scored for resistance to *P. cactorum* on eight different dates each year using an ordinal disease rating scale from the onset of symptoms until the resistance scores of check cultivars plateaued. We used the time-series progression of symptoms, phenotypes of check cultivars, and shapes of the phenotypic distributions to guide our phenotyping schedule. The 437 individuals selected for inclusion in the training population were phenotyped both years. We used a combination of stunting, wilting, chlorosis, and die back to score plants on a 1 to 5 ordinal scale, where 1 = symptomless; 2 = mild stunting and wilting of outer leaves; 3 = stunted growth, wilting of outer leaves, and mild chlorosis; 4 = wilting and chlorosis throughout the plant canopy; and 5 = complete die back. The entire population was phenotyped once every 10–14 d from 13 April to 28 June in 2018 and from 1 April to 18 July in 2019. We collected 24,600 data points and calculated the area under the disease progression stairs (AUDPS) (Simko & Piepho, 2012) from the eight time points within each year using the R package agricolae (De Mendiburu & Simon, 2015) (Supplemental File S2).

2.5 | DNA isolation and SNP genotyping

DNA was extracted from 0.2 g of dried young leaf tissue with the E-Z 96 Plant DNA Kit (Omega Bio-Tek) according to manufacturer's instructions. To increase DNA quality and yield, Proteinase K was added to the lysis buffer to a final concentration of 0.2 mg ml^{-1} and lysis incubation was extended to 45 min at 65 °C. Training population individuals were genotyped using these DNA samples with an Axiom™ Strawberry 50K SNP array (Hardigan et al., 2020) by ThemoFisher Scientific Axiom Genotyping Services (Palo Alto, CA; https://www.thermofisher.com/). The raw genotypic data files returned by ThermoFisher Scientific were analyzed using the Axiom Analysis Suite Software v4.0.3.3 (https://www.thermofisher.com/us/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/axiom-analysis-suite.html). The SNP calls for the 50K loci were filtered to identify and only include markers with well separated, codominant genotypic clusters and identify and eliminate SNPs with minor allele frequencies <0.05. The filtering process yielded 40,334 SNPs (Supplemental File S3).

The physical addresses of the Axiom SNPs in the octoploid genome were originally ascertained by Hardigan et al. (2020) by aligning DNA sequences for array probes to the 'Camarosa' V1 reference (FaCA1; https://phytozome-next.jgi.doe.gov/info/Fxananassa_v1_0_a1) described by (Edger et al., 2019). They have since been aligned to the haplotype-phased 'Royal Royce' V1 reference (FaRR1; https://phytozome-next.jgi.doe.gov/info/FxananassaRoyalRoyce_v1_0) described by Hardigan et al. (2021a). The 'Royal Royce' physical addresses and chromosome nomenclature described by Hardigan et al. (2020) were used for GWAS analyses in the present study. We have provided a database with physical addresses for both genomes for cross-referencing purposes (Supplemental File S4).

2.6 | Statistical analyses

The resistance scores observed at the eighth time point and AUDPS variable were analyzed using linear mixed model (LMM) functions in the R package lme4::lmer() (https://cran.r-project.org/web/packages/lme4/index.html) (Bates et al., 2015). The raw phenotypic data were initially analyzed using LMMs for square lattice experiment designs with a completely random-effects model. Because the square

lattice was found to be no more efficient than a randomized complete blocks experiment design, statistics are reported for analyses of LMMs for the latter only (Hinkelmann & Kempthorne, 2007). Estimated marginal means (EMMs) for training population individuals were estimated using the R package emmeans (Lenth, 2021) (https://cran.r-project.org/web/packages/emmeans/index.html). The LMM for individual year analyses was as follows:

$$y_{ij} = b_i + G_j + e_{ij} \quad (1)$$

where y_{ij} is the observed phenotype for the jth genotype (individual) in the ith block, b_i is the random effect of the ith complete block, G_j is the random effect of the jth individual, e_{ij} is the ijth residual effect, $i = 1,2,\ldots,4$, $j = 1,2,\ldots,n$, and n is the number of individuals. The LMM for the across-years analysis was as follows:

$$y_{ijk} = b_i + G_j + Y_k + GY_{jk} + e_{ijk} \quad (2)$$

where y_{ijk} is the observed phenotype for the jth genotype (individual) in the ith complete block in the kth year, Y_k is random effect of the kth year, GY_{jk} is the random effect of the interaction between the jth genotype and kth year, e_{ijk} is the ijkth residual effect, and k indexes years.

The variance components in these analyses were estimated using the restricted maximum likelihood (REML) method (Bates et al., 2015). The broad-sense heritability on a clone-mean basis was estimated by $\hat{H}^2 = \hat{\sigma}^2_G/\hat{\sigma}^2_{\bar{P}}$, where $\hat{\sigma}^2_G$ is a REML estimate of the among individuals (genotypes) variance component, $\hat{\sigma}^2_{G\times Y}$ is a REML estimate of the genotype × year interaction variance component, and $\hat{\sigma}_e$ is a REML estimate of the residual variance component, $\hat{\sigma}^2_{\bar{P}} = \hat{\sigma}^2_G + \hat{\sigma}^2_{G\times Y}/y + \hat{\sigma}^2_e/ry$ is a REML estimate of the phenotypic variance on a clone-mean basis, y is the number of years, and r is the harmonic mean of the number of replications per individual ($r = 2.23$ in 2018, 3.60 in 2019, and 5.72 across years). The harmonic mean number of replications was less than four (the number of replicates per individual originally planted) because of the loss of plants to factors other than disease. Narrow-sense genomic heritability was estimated by $\hat{h}^2 = \hat{\sigma}^2_A/\hat{\sigma}^2_{\bar{P}}$, where $\hat{\sigma}^2_A$ is a REML estimate of the genomic additive genetic variance (Endelman, 2011; Mathew et al., 2018). The coefficient of additive genetic variance was estimated by $\mathrm{CV}_A = 100\hat{\sigma}_A/\hat{\mu}$, where $\hat{\mu}$ is population mean.

2.7 | Multivariate GWAS

The EMMs for individuals for each year were analyzed as separate dependent variables in a multivariate GWAS (MV-GWAS) using GEMMA v0.98.1 (Zhou & Stephens, 2012, 2014). The independent variables for these analyses were the genotypes for 39,195 Axiom array SNP markers with alleles coded 0, 1, and 2 (Hardigan et al., 2020). The physical positions of these SNPs in the 'Royal Royce' reference genome are shown in Supplemental File S5. The genomic relationship matrix (**K**) was estimated from the coded SNP marker genotypes as described by Pincot et al. (2020). The **K** matrix was used in MV-GWAS analyses to correct for genetic relationships among individuals (Zhou & Stephens, 2012, 2014). We used a 5% false discovery rate-corrected significance threshold to test the null hypothesis of no SNP effect (Benjamini & Hochberg, 1995). The single most significant SNPs in each cluster of one or more tightly linked SNPs were used as independent variables in multi-locus genetic models to estimate the percentage of the genetic variance explained ($\mathrm{GVE} = \hat{\sigma}^2_M/\hat{\sigma}^2_G$) and the percentage of the phenotypic variance explained ($\mathrm{PVE} = \hat{\sigma}^2_M/\hat{\sigma}^2_{\bar{P}}$) by each locus individually and collectively corrected for other loci in the genetic model, where $\hat{\sigma}^2_M$ is a biased-corrected average semivariance REML estimate of the GVE for one or more SNP marker loci, $\hat{\sigma}^2_G$ is the genetic variance among clonally replicated individuals, and $\hat{\sigma}^2_{\bar{P}}$ is the phenotypic variance on a clone-mean basis (Feldmann et al., 2021). The genotype (individual) effect (G_j) was partitioned into the effects of SNP marker loci and individuals nested in SNP marker loci (the residual genetic variance not explained by SNP marker loci). The initial GWAS identified two significant SNPs for resistance score and eight significant SNPs for AUDPS, which were further analyzed. The SNP marker loci included in multi-locus genetic models were AX-184879834, AX-184292487, AX-184338462, AX-184055612, AX-184127382, AX-184211829, AX-184673648, and AX-184109190 for resistance score and AX-184211684 and AX-184109190 for AUDPS. The REML estimates of the variance components for these loci were used to calculate average semivariance estimates of GVEs and PVEs for each locus (Feldmann et al., 2021). The SNP with the largest effect (AX-184109190) was shared between both traits. We subsequently repeated MV-GWAS analyses of both traits with AX-184109190 as a covariate, which eliminated the other statistically significant signals; hence, GVEs, PVEs, and other statistics were subsequently estimated for AX-184109190 alone. The additive and dominance effects of AX-184109190 were estimated by $\hat{a} = \hat{\mu}_{AA} - \hat{\mu}_{GG}$ and $\hat{d} = (\hat{\mu}_{AG} - \hat{\mu}_{AA} + \hat{\mu}_{GG})/2$, respectively, where $\hat{\mu}_{AA}$, $\hat{\mu}_{AG}$, and $\hat{\mu}_{GG}$ are the EMMs for individuals with AA, AG, and GG genotypes for the AX-184109190 SNP locus (Falconer & Mackay, 1996; Walsh, 2001). The degree of dominance of the AX-184109190 locus was estimated by $|\hat{d}/\hat{a}|$ (Falconer & Mackay, 1996; Walsh, 2001).

2.8 | Genomic prediction of breeding values

The statistical methods used to estimate genetic parameters and genomic-estimated breeding values (GEBVs) in the

present study were previously described by Pincot et al. (2020) in a parallel study of the genetics of resistance to Verticillum wilt (Pincot et al., 2020). Narrow-sense genomic heritability (h^2) was estimated as described by Mathew et al. (2018). The GEBVs for resistance score and AUDPS were estimated using three whole-genome regression methods implemented in the R package BGLR (Pérez & de los Campos, 2014) (https://cran.r-project.org/web/packages/BGLR/index.html) with and without the inclusion of the RPc2-associated SNP marker AX-184109190 as a fixed effect; specifically, genomic best linear unbiased prediction (G-BLUP), the Bayesian Lasso, and reproducing kernel Hilbert spaces (de Los Campos et al., 2010, 2013; Gianola & Van Kaam, 2008; Habier et al., 2013). Across-year EMMs for training population individuals were analyzed in BGLR by assuming a normal distribution for both traits. We used Monte Carlo cross-validation with 1,000 iterations to estimate the accuracy of genomic predictions of GEBVs by randomly sampling 80% of the original individuals without replacement and predicting GEBVs for the other 20% of the individuals. The estimates from individual year and across-year analyses were used to predict GEBVs for individual years and across years. Genomic prediction accuracy was estimated from estimates of the Pearson's correlation coefficient between EMMs and GEBVs (Dekkers, 2007; Dekkers et al., 2021; Van den Berg et al., 2019).

2.9 | Genomic prediction of genetic variances and cross usefulness criteria

We used the approach described by Mohammadi et al. (2015) to estimate genomic-estimated genetic variances for simulated segregating populations (200 individuals per population) arising from 190,532 crosses (a factorial mating design with reciprocals) among 437 individuals in the training population (prospective parents). The across-year EMMs for resistance score, Axiom SNP array genotypes, and a reference genetic map developed for the cultivar 'Camarosa' (Hardigan et al., 2020) were used as input in the R package PopVar to estimate population means ($\hat{\sigma}^2_{G\times E}/\hat{\sigma}^2_{\bar{P}}$) and genetic variances ($\hat{\sigma}^2_G$) for each of the 190,532 simulated populations (Tiede & Neyhart, 2021) (https://cran.r-project.org/web/packages/PopVar/index.html). To compare estimates of these genetic parameters for different subsets of prospective parents under phenotypic and genomic selection scenarios, we selected the 32 most resistant individuals in the training population using EMMs (phenotypic selection) and GEBVs (genomic selection). The EMM cutoff was 2.0, whereas the GEBV cutoff was 2.6. Fifteen of the selected (resistant) parents were common to both subsets, whereas 17 were unique to each subset. The cross usefulness criterion (U) was estimated by $\hat{\mu} + \hat{\sigma}^2_G$ (Lehermeier et al., 2017).

3 | RESULTS & DISCUSSION

3.1 | Heritability of resistance to PhCR

The variation we observed among training population individuals for PhCR resistance score and AUDPS—a time-series metric that estimates the progression of disease symptoms (Simko & Piepho, 2012)—was continuous and approximately normally distributed with phenotypes spanning the entire range in both years of our study (Figure 1). The additive genetic correlation between resistance score and AUDPS was $\hat{r}_A = 0.82$ ($p \leq 2.2 \times 10^{-16}$). The phenotypic variation was noisier in 2017–2018 than in 2018–2019—additive genetic variance and narrow- and broad-sense heritability estimates were lower and the progression of symptoms over time was more erratic in 2017–2018 than in 2018–2019 (Figure 1; Table 1). The inclusion of a small, albeit highly diverse, collection of exotic individuals in the training population doubled or tripled genetic variation for resistance score and AUDPS within and between years. The between-year rank correlations were moderately positive and identical for both traits ($\hat{r} = 0.39, p \leq 2.2 \times 10^{-16}$), and individual × year interactions were nonsignificant—REML estimates of $\hat{\sigma}^2_{G\times E}/\hat{\sigma}^2_{\bar{P}}$ were 0.05 for resistance score and 0.09 for AUDPS (Figure 1; Table 1). Hereafter, we report across-year statistics unless noted otherwise. The EMMs for training population individuals within and across years are documented in Supplemental File S1.

3.2 | Prospective donors of favorable alleles for enhancing resistance to PhCR

We found that 93.2% of the genetic resources (clonally propagated individuals preserved in public germplasm collections) in our study population were moderately to highly susceptible to PhCR (Figure 2; Supplemental File S1). This conclusion was reached by screening artificially inoculated plants (stolon-propagated clones) of cultivars and other hybrid individuals from the UCD population ($n = 346$), a geographically and historically diverse collection of non-UCD heirloom and modern cultivars ($n = 89$), and a phylogenetically and geographically diverse collection of 18 *F. chiloensis* and 22 *F. virginiana* ecotypes. From previous analyses of biodiversity (Hardigan et al., 2020, 2021b; Pincot et al., 2021), these individuals were predicted to broadly sample global diversity and included several check cultivars previously reported to be resistant or susceptible to PhCR (Bell et al., 1997; Denoyes-Rothan et al., 2004; Eikemo et al., 2003; Nellist et al., 2019; Parikka, 1998; Pérez-Jiménez et al., 2012; Pitrat & Risser, 1977; Schafleitner et al., 2013; Seemüller, 1977; van Rijbroek et al., 1997). We compiled a database of previously reported PhCR resistance phenotypes for reference and comparison (Supplemental File S6). As shown in that database, the PhCR

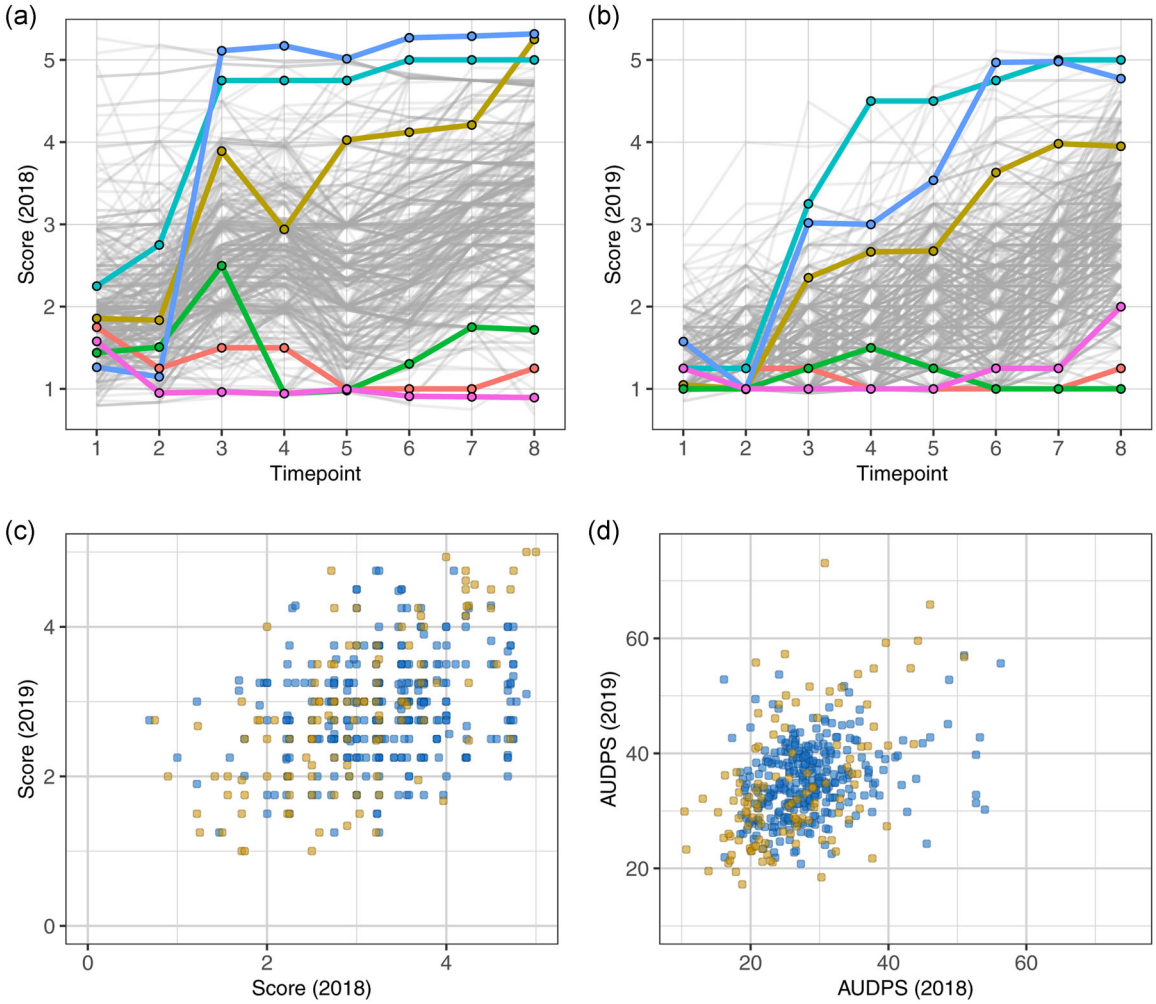

FIGURE 1 Phenotypic variation for resistance to Phytophthora crown rot in strawberry. Estimated marginal means (EMMs) were estimated for 475 individuals from three to four single-plant replicates (clones) per individual per year and eight time points per year in 2017–2018 and 2018–2019 field experiments in Davis, CA (24,600 phenotypic observations are displayed). (a, b) Estimated marginal means for resistance score are shown for each timepoint in both years. The bold colored lines highlight resistant and susceptible individuals: blue = 'Strawberry Mountain' (PI616601), gold = 'Tamella' (PI551411), teal = 'Sitka D' × 'Red Rich' (PI551472), green = 'Cyclone' (PI551412), red = 'Senga Sengana' (PI264680), and pink = 'N2' (PI616675). (c, d) Phytophthora crown rot resistance score and area under the disease pressure stairs (AUDPS) EMMs for University of California–Davis (UCD) individuals (blue) and non-UCD individuals (gold) in the training population are shown for both years. The between-year phenotypic correlations were $r = 0.39$ ($p \leq 0.001$) for both resistance score and AUDPS

resistance classifications for the resistant check 'Senga Sengana' (PI264680; developed in 1954) and the susceptible check 'Tamella' (PI551411; developed in 1964) have been highly consistent across studies and environments.

Of the 475 individuals screened for resistance to PhCR in the present study (Supplemental File S1), 1.3% lacked symptoms and were classified as highly resistant (had across-year EMMs in the $1.0 \leq \bar{y} \leq 1.5$ range), 5.5% had mild symptoms and were classified as resistant ($1.5 < \bar{y} \leq 2.0$), and 93.2% developed moderate to severe symptoms and were classified as moderately to highly susceptible ($2.0 < \bar{y} \leq 5.0$; Supplemental File S1). The most resistant individual in our study was 'Senga Sengana' ($\bar{y} = 1.25$), an heirloom cultivar widely reported to be highly resistant (Denoyes-Rothan et al., 2004; Eikemo et al., 2003; Parikka, 1998; Pitrat & Risser, 1977; Seemüller, 1977; van Rijbroek et al., 1997) (Supplemental File S6). The other individuals in the highly resistant group were 'Cyclone' ($\bar{y} = 1.30$; developed in 1950), 'Addie' ($\bar{y} = 1.38$; developed in 1982), 'MD683' ($\bar{y} = 1.38$; developed in 1955), 12C071P602 ($\bar{y} = 1.47$; developed in 2012), and 'Massey' ($\bar{y} = 1.50$; 1934; Supplemental File S1). Thirty-two individuals had symptom scores in the resistant to highly resistant range ($1.0 \leq \bar{y} \leq 2.0$), of which 20 were cultivars developed 40 to 99 yr before present (Supplemental File S1). Approximately 80% of these cultivars were developed before methyl bromide fumigation was introduced and widely

TABLE 1 REML estimates of narrow-sense genomic heritability ($\hat{h}^2$), broad-sense heritability on a clone-mean basis ($\hat{H}^2$), the proportion of the phenotypic variance explained by the genotype × year interaction variance ($\hat{\sigma}^2_{G\times E}/\hat{\sigma}^2_P$), additive standard deviation ($\hat{\sigma}_A$), coefficient of additive genetic variance ($CV_A = 100\hat{\sigma}_A/\hat{\mu}$) for resistance score and area under the disease pressure stairs (AUDPS) among $n = 437$ training population individuals phenotyped for resistance to Phytophthora cactorum in 2017–2018 and 2018–2019 field experiments in Davis, CA, where $\hat{\mu}$ is the population mean

Trait	Population	Year	$\hat{H}^2$	$\hat{\sigma}^2_{G\times E}/\hat{\sigma}^2_P$	$\hat{h}^2$	$\hat{\sigma}_A$	CV_A
Score	Training	2017–2018	0.49	0.05	0.41	0.21	0.16
		2018–2019	0.59		0.41	0.26	0.19
		Combined	0.67		0.57	0.26	0.18
	UCD	2017–2018	0.22	0.03	0.16	0.06	0.08
		2018–2019	0.44		0.29	0.14	0.14
		Combined	0.51		0.38	0.11	0.12
	Non-UCD	2017–2018	0.75	0.06	0.75	0.66	0.27
		2018–2019	0.80		0.56	0.56	0.28
		Combined	0.81		0.75	0.60	0.27
AUDPS	Training	2017–2018	0.55	0.09	0.37	12.52	0.14
		2018–2019	0.74		0.56	37.16	0.18
		Combined	0.71		0.61	24.82	0.20
	UCD	2017–2018	0.37	0.07	0.36	8.01	0.11
		2018–2019	0.57		0.38	15.26	0.12
		Combined	0.59		0.40	9.27	0.12
	Non-UCD	2017–2018	0.69	0.12	0.52	30.56	0.21
		2018–2019	0.86		0.63	78.79	0.27
		Combined	0.77		0.77	61.56	0.31

Note. Statistics are shown for the training population as a whole and for University of California–Davis (UCD) ($n = 321$) and non-UCD ($n = 116$) subsets of individuals in the training population.

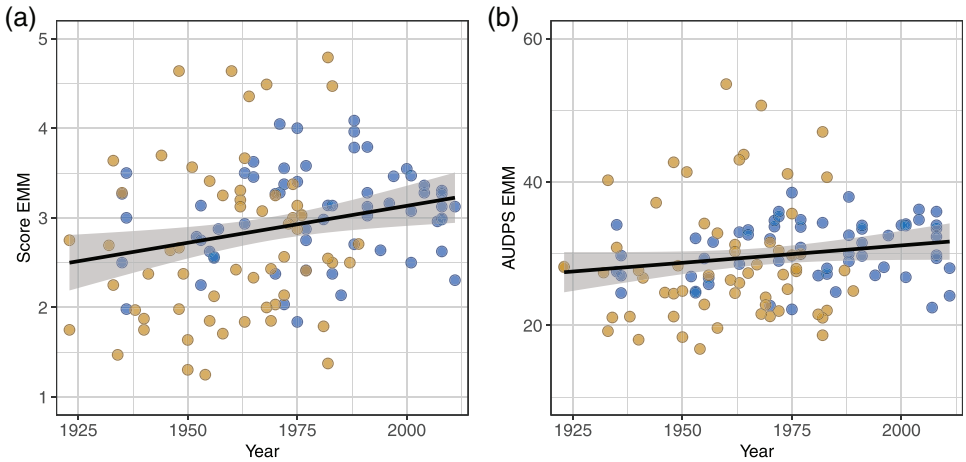

FIGURE 2 Estimated-marginal means for Phytophthora crown rot (PhCR) resistance score and area under the disease pressure stairs (AUDPS) plotted against the year or origin for 60 University of California–Davis (UCD) (blue points) and 62 non-UCD (gold points) cultivars released since 1923. Slopes for linear regressions (solid black lines) with 95% confidence intervals (gray bands) were significant for resistance score ($R^2 = 0.050$; $p \leq 0.01$) and nonsignificant for AUDPS ($R^2 = 0.017$; $p = 0.08$)

adopted in the 1960s (Wilhelm & Paulus, 1980; Wilhelm et al., 1961).

Of the 64 UCD cultivars screened for resistance to PhCR in the present study (Supplemental File S1), only three were found to be resistant: the heirloom cultivars 'Mrak' ($\bar{y} = 1.84$; developed in 1975), 'Tahoe' ($\bar{y} = 1.98$; developed in 1936), and 'Douglas' ($\bar{y} = 2.03$; developed in 1972) (Supplemental File S1). The three modern UCD cultivars reported by Shaw et al. (2006) to be PhCR resistant ('Camino Real', 'Albion', and 'Aromas') were found to be moderately to highly susceptible in our study (Supplemental File S1). The across-year EMMs for these cultivars were $\bar{y} = 2.64$ ('Camino Real'; developed in 1994), $\bar{y} = 3.46$ ('Albion'; developed in 1997), $\bar{y} = 3.79$ ('Aromas'; developed in 1991). Two modern UCD cultivars reported by Shaw et al. (2006) to be PhCR susceptible were found to be susceptible: 'Ventana' ($\bar{y} = 3.16$; developed in 1996) and 'Diamante' ($\bar{y} = 3.02$; developed in 1991). Of the other 282 UCD individuals screened for resistance to PhCR, which represent a broad cross-section of diversity in the circa 2015 UCD population, only 2.5% were classified as resistant (Supplemental File S1). As discussed below, these findings have profound implications for improving resistance to PhCR in the historically and commercially important UCD population and other elite populations that have undergone intense selection and experienced population bottlenecks (Hardigan et al., 2021b).

Finally, we found that PhCR resistance was rare among ecotypes of the octoploid progenitors of cultivated strawberry (Supplemental File S1). Of the 40 *F. chiloensis* and *F. virginiana* ecotypes screened for resistance to PhCR in the present study, only one was found to be resistant ($\bar{y} = 1.56$), whereas the other 39 were found to be moderately to highly susceptible ($2.2 \leq \bar{y} \leq 5.0$). The resistant ecotype was 'N2' (PI616675), an *F. virginiana subsp. virginiana* individual collected from a riparian habitat along the Sainte-Anne River, a tributary of the Saint-Lawrence River in Quebec, Canada (49.1° N, −66.5° W).

3.3 | Genetic gains in breeding for resistance to PhCR have been negligible over the last century

Using the assemblage of 122 cultivars developed since 1923 as a barometer (Figure 1; Supplemental File S1), genetic gains in breeding for resistance to PhCR appear to have declined over the last century (Figure 2). On the ordinal disease symptom rating scale we used, resistance to PhCR decreased as resistance score increased; hence, slopes from linear regressions of resistance score or AUDPS on cultivar year of origin were positive, albeit with weak coefficients of determination because phenotypic variation spanned the entire range over several decades (Figure 2).

On closer inspection, we found that the PhCR resistance phenotypes of cultivars developed since 1980 have drifted toward the population mean ($\bar{y} = 3.1$), with the range dropping by approximately one unit on the ordinal disease rating scale in both the upper (more susceptible) and lower (more resistant) tails of the phenotypic distribution (Figure 2a; Supplemental File S1). The pattern for AUDPS was even more pronounced, with UCD cultivars falling in a narrower range than non-UCD cultivars (Figure 2b). The narrowing of these phenotypic ranges coincided with a population bottleneck in the UCD breeding program (Hardigan et al., 2021b; Pincot et al., 2021) that appears to have fortuitously decreased genetic variation for resistance to PhCR (Table 1; Figure 1). The AUDPS range increased in both directions (toward more susceptible and more resistant) among non-UCD individuals in the training population, which is precisely what one would expect in a random sample of individuals from a gene bank collection.

Although we sampled a broad cross-section of heirloom and modern cultivars and common ancestors of modern cultivars to build the training population (Pincot et al., 2021), a shortcoming of our study was that plants of non-UCD cultivars developed later than 1989 were either unavailable or could not be acquired for testing from public or proprietary breeding programs in North America and Europe. Every UCD cultivar developed and released between 1935 and 2019, however, was tested (Supplemental File S1). The year of origin for the 75 non-UCD cultivars tested in our study ranged from 1854 to 1989 (the median year of origin was 1965); hence, our insights into PhCR resistance were constrained by the spectrum of non-UCD cultivars tested, which were biased toward older off-patent cultivars (Figure 2; Supplemental File S1). The frequency of PhCR-resistant cultivars worldwide could obviously be greater than what we are reporting here. Although that seems improbable (Mangandi et al., 2017; Marin et al., 2022; Nellist et al., 2019), thorough sampling and screening of non-UCD modern cultivars is needed to address this question (Figure 2; Supplemental File S1).

Our data show that a high frequency of unfavorable alleles persist in the broad cross-section of cultivars and elite and exotic genetic resources sampled and, importantly, that the apparent wellspring of favorable alleles found in gene banks have either not been discovered and utilized or have simply been left behind over the last century of breeding (Figures 1 and 2). We suspect that the increase in the susceptibility of cultivars over time was caused by the widespread reliance on methyl bromide and other soil fumigants to suppress soil-borne pathogens in many parts of the world (Rosskopf et al., 2005; Schneider et al., 2003), a consequent decrease in natural selection pressure, inattention to breeding for resistance to PhCR and other soil-borne pathogens, and absence of initiatives to pyramid favorable alleles from multiple sources of resistance, most of which are exotic, while simultaneously

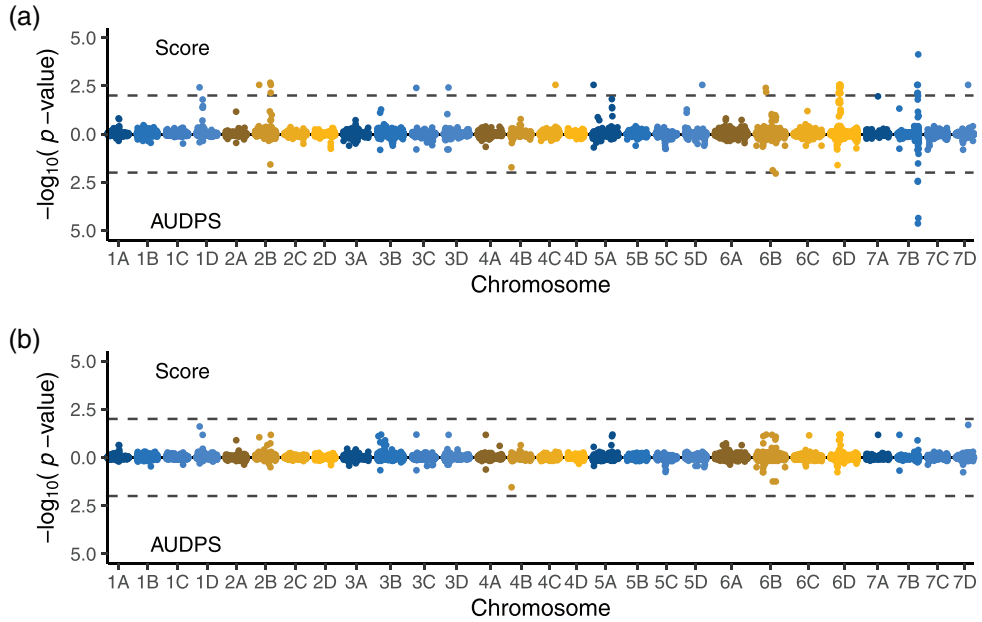

FIGURE 3 Genome-wide search for loci affecting resistance to Phytophthora crown rot in a population of 321 University of California–Davis (UCD) and 116 non-UCD individuals genotyped with an Axiom 50K single-nucleotide polymorphism (SNP) array. Estimated marginal means (EMMs) for resistance score and area under the disease pressure stairs (AUDPS) were estimated from multiple replicates within each year of the study (2017–2018 and 2018–2019). The within-year EMMs were analyzed as separate dependent variables using multivariate genome-wide associations studies. False discovery rate (FDR)-adjusted p values are shown (the horizontal dashed lines depict 0.01 p-value thresholds). The SNP physical positions were ascertained in the haplotype-phased 'Royal Royce' reference genome (FaRR1). (a) Manhattan plot for the initial genome-wide search without covariates. (b) Manhattan plot for a genome-wide search using the RPc2-associated SNP AX-184109190 as a covariate

preserving genetic gains for other horticulturally important traits. The latter poses a significant long-term technical challenge because of the genetic complexity of resistance to this pathogen (as discussed below) and exoticness of most of the promising donors of favorable alleles for improving resistance to PhCR (Table 1; Supplemental File S1). The findings reported here for PhCR resistance are consistent with our findings for Verticillium wilt, another disease caused by a soil-borne pathogen (*Verticillium dahliae* Klebahn) where the genetics of resistance is quantitative and complex, and where genetic gains in breeding for resistance appear to have declined over the last century (Pincot et al., 2020).

3.4 | Multivariate GWAS uncovered the segregation of a large-effect locus

Our initial GWAS search identified nine statistically significant signals for resistance score and two statistically significant signals for AUDPS in the training population (2; Figure 3a). Only one was significant for both traits, a SNP (AX-184109190) associated with a QTL on chromosome 7B that was predicted to be RPc2, a large-effect locus originally discovered by Mangandi et al. (2017) in Florida populations and screening environments. The RPc2-associated SNP AX-184109190 explained 28.7–39.7% of the genetic variance for resistance score and 30.7–55.6% of the genetic variance for AUDPS (2). These percentages were estimated using bias-corrected methods and multi-locus genetic models (Feldmann et al., 2021). Several of the GWAS signals observed for resistance score were weak and hypothesized be false positives. When AX-184109190 was used as a covariate in a multivariate GWAS (George & Cavanagh, 2015; Segura et al., 2012; Tibbs Cortes et al., 2021; Zhang et al., 2010), the other eight statistically significant signals decreased or disappeared altogether, and none exceeded the statistical significance threshold (Figure 3b). Hence, we only found statistical support for a single large-effect QTL predicted to be RPc2 and concluded that RPc2 was the only locus that merited modeling as a fixed effect in our genomic prediction study and the only locus that clearly warrants direct targeting by marker-assisted selection.

3.5 | The dominant RPc2 allele is necessary but not sufficient for resistance to PhCR

The SNP most strongly associated with the RPc2 locus was AX-184109190, an A/G variant (Figure 3; Table 2). We used AX-184109190 as a predictor of RPc2-associated phenotypes in the training population. The favorable SNP allele (A) was completely dominant ($|\hat{d}/\hat{a}| = 1.00$)

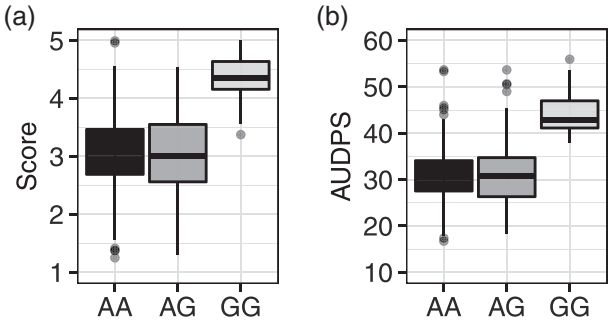

FIGURE 4 Estimated marginal means for Phytophthora crown rot resistance score and area under the disease pressure stairs (AUDPS) among 437 training population individuals segregating for the RPc2-associated A/G single-nucleotide polymorphism (SNP) marker AX-184109190, where A is the favorable and G is the unfavorable allele. The minimum (phenotype for the most resistant individual), median, Q1–Q3 interquartile range, and maximum (phenotype for the most susceptible individual) are plotted for each AX-184109190 SNP genotype for both traits

for resistance score, nearly completely dominant for AUDPS ($|\hat{d}/\hat{a}| = 0.91$), and highly frequent among UCD ($f_A = 0.92$) and non-UCD ($f_A = 0.67$) individuals, where f_A is the frequency of the A allele. The additive effect of the AX-184109190 locus was $\hat{a} = -0.63$ ($p = 1.76 \times 10^{-8}$) for resistance score and $\hat{a} = -6.83$ ($p = 2.23 \times 10^{-11}$) for AUDPS. Similarly, the dominance deviation of the AX-184109190 locus was $\hat{d} = 0.63$ ($p = 9.33 \times 10^{-7}$) for resistance score and $\hat{d} = 6.24$ ($p = 8.42 \times 10^{-8}$) for AUDPS. Hence, the favorable allele increased resistance (decreased the ordinal resistance score) and slowed the progression of disease from the onset of symptoms to the point where symptoms plateaued, which spanned 10–15 wk across years.

We found that 100% of the UCD individuals in the training population ($n = 321$) were either homozygous (A/A) or heterozygous (A/G) for the favorable SNP allele (Figure 4; Supplemental Table S1). The frequency of the favorable allele (f_A) was 0.92 among UCD and 0.67 among non-UCD individuals (Supplemental Table S1). The high frequency of the favorable allele in the UCD population was not expected because resistance score EMMs spanned the entire range from highly resistant ($\bar{y} = 1.41$) to highly susceptible ($\bar{y} = 4.54$) among A/A homozygotes and heterozygotes. This result suggests that the favorable RPc2 allele is necessary but not sufficient for resistance, which was consistent with our finding that AX-184109190 explained 43.6% of the genetic variance for resistance score and 51.6% of the genetic variance for AUDPS across years (Table 2). The wide range of resistance phenotypes observed within AX-184109190 genotypic classes (Figure 4) can be attributed to several factors: nongenetic variation, genetic variation (effects of other QTL), incomplete penetrance of RPc2 alleles, and historic recombination between the SNP and the causal gene underlying RPc2 (MacLeod et al., 2014). One caveat here is that the causal mutation underlying RPc2 is yet unknown and thus almost certainly not in complete linkage disequilibrium with AX-184109190. Even though AX-184109190 appears to be in strong linkage disequilibrium with the QTL, a certain percentage of resistant individuals (RPc2/RPc2 and RPc2/rpc2) carry the G allele and vice versa for susceptible individuals (rpc2/rpc2).

The high-density SNP arrays we developed for strawberry have facilitated octoploid genome-informed GWAS and the discovery of agriculturally important loci (Hardigan et al., 2020; Petrasch et al., 2021; Pincot et al., 2022); however, they only capture and interrogate a fraction of the nucleotide variants found throughout the genome (Hardigan et al., 2021b). SNP-array genotyping has been incredibly powerful and important in octoploid strawberry genetic studies and facilitated the accumulation and integration of genotypic data across populations and studies with minimal genotyping errors or missing data; however, the discoveries and inferences enabled by SNP array genotypes are limited by linkage disequilibrium between the assayed variants and causal mutations (Druet et al., 2014). With the emergence of high-quality, haplotype-phased genomes, strawberry is positioned to pivot toward using whole-genome sequence-facilitated approaches for genomic prediction. Hardigan et al. (2020, 2021b) showed that homologous and homoeologous DNA variation can be disentangled in the octoploid using next-generation sequencing and whole-genome shotgun genotyping-by-sequencing, for example, >80% of short DNA sequences (150 bp) can be unambiguously physically mapped (aligned) in octoploid reference genomes. Hence, using whole-genome sequence data for GWAS and genomic prediction is feasible and should accelerate the discovery of causal variants and improve the accuracy of genomic prediction across populations in octoploid strawberry (Druet et al., 2014; Iheshiulor et al., 2016; Meuwissen et al., 2021; Raymond et al., 2018).

3.6 | Several immunity-related genes are associated with RPc2

We used historic recombination (GWAS) in the training population to narrow the location of RPc2 down to a short DNA segment on chromosome 7B and searched the octoploid genome for annotated genes with predicted or demonstrated plant defense functions that were in strong linkage disequilibrium with RPc2 (Figure 3). Using a $p = 0.001$ statistical significance threshold, AX-184109190 and 10 additional 50K array SNPs were found to be strongly associated with PhCR resistance phenotypes. These SNPs spanned 21.73–22.99 Mb

in the 'Royal Royce' reference genome (https://phytozome-next.jgi.doe.gov/info/FxananassaRoyalRoyce_v1_0). This 1.26-Mb segment harbors 233 annotated genes (Supplemental File S7), of which 18 have immunity-related annotations: seven with homology to intracellular leucine-rich repeat (LRR)-type immune receptors (Bonardi & Dangl, 2012), one with homology to an immunity-associated WRKY transcription factor (Pandey & Somssich, 2009), two with homology to membrane-localized immune receptors (Boutrot & Zipfel, 2017), one with homology to a Ca^{2+}–dependent protein kinase, and seven with homology to cyclic-nucleotide-gated calcium channels (CNGCs; Seybold et al., 2014). Loss-of-function CNGC DEFENSE, NO DEATH1 and 2 mutations have been shown to disrupt broad-spectrum disease resistance and inhibit hypersensitive response cell death in *Arabidopsis* (Clough et al., 2000; Jurkowski et al., 2004). The presence of several CNGCs in close physical proximity to RPc2 makes them intriguing candidates for the causal gene. The gene most proximal to AX-184109190 is homologous to WAK1, another intriguing candidate for RPc2. The WAK1 domain encodes a wall-associated receptor kinase galacturonan-binding protein, senses the presence of cell wall fragments produced during fungal or oomycete attack, and activates plant immune responses in *Arabidopsis* (Brutus et al., 2010).

3.7 | Genomic prediction accuracy was strongly affected by population composition and RPc2 allele frequency

Our genomic prediction analyses were informed by population-specific differences in additive genetic variance and heritability and the discovery that RPc2 was segregating in the training population (Table 1; Figure 3). Genomic-estimated breeding values (GEBVs) were separately estimated for UCD and non-UCD individuals and the complete training population with and without the RPc2-associated SNP AX-184109190 as a fixed effect using three whole-genome regression methods: G-BLUP, reproducing kernel Hilbert space, and the Bayesian Lasso (Table 3). Substantive differences in prediction accuracy were not observed among methods; hence, findings and conclusions are henceforth only presented for G-BLUP unless otherwise noted.

We went into this study without knowing if the RPc2 locus would segregate or have a significant effect in the training population (Table 2; Figure 3). As shown earlier, the favorable RPc2-associated SNP allele was nearly completely fixed (noninformative) in the UCD population; hence, the inclusion of the RPc2-associated SNP marker as a fixed effect decreased the accuracy of genomic predictions among UCD individuals for resistance score. Conversely, including AX-184109190 as a fixed effect increased the accuracy of genomic predictions among non-UCD and training population individuals as a whole because the exotic (non-UCD) individuals segregated for RPc2 (Table 2; Supplemental Table S1). These findings suggest that a fraction of the increase in additive genetic variation associated with the inclusion of exotic (non-UCD) individuals in the training population (Table 1) was caused by the introduction of RPc2 alleles (we are allowing here for the presence of multiple favorable and unfavorable alleles). What this shows, in addition, is that genetic variation associated with loci other than RPc2 was insufficient to drive significant genetic gains for resistance to PhCR in the UCD population without the introduction of novel favorable alleles from non-UCD sources. The prediction accuracy was only in the 0.19 to 0.24 range for resistance score and 0.28 to 0.32 for AUDPS among UCD individuals in the training population (Table 3).

Several noteworthy differences were observed in the EMM × GEBV distributions and genomic selection accuracy estimates between UCD and non-UCD individuals in the training population (3; Figure 5). First, breeding values for UCD individuals were more substantially shrunk toward the population mean than breeding values for non-UCD individuals (Figure 5). The GEBVs for UCD individuals clustered in a vertically narrow band centered on the population mean (3.1), whereas GEBVs for non-UCD individuals were more diffuse, spanned a much wider range (1.9–4.3), and substantially increased prediction accuracy. The prediction accuracy estimates from cross-validations were 0.22 for G-BLUP without the RPc2-associated SNP as a fixed effect among UCD individuals and 0.67 for G-BLUP with the RPc2-associated SNP as a fixed effect among non-UCD individuals. Even those UCD individuals with EMMs in the resistant range (1.0–2.0) had GEBV estimates close to the population mean (3.1) and were consequently predicted to be moderately susceptible (Figure 4). Excluding one or two outliers in the lower and upper tails of the distribution, the GEBV range among UCD individuals (2.6–3.6) was exceptionally narrow (Figure 5).

Second, the phenotypic and breeding value distributions were thinned tailed (Figure 5). Using GEBVs as a selection metric, 19 of the 20 most resistant individuals from the lower tail of the distribution were non-UCD *F.* × *ananassa* cultivars and genetic resources (Figure 5; Supplemental File S1). The GEBV range for those individuals was narrow (1.9–2.5). The genomic-estimated breeding value for the most resistant UCD individual (the cultivar 'Tufts') was 2.2 (Supplemental File S1). Using the phenotypic mean as a selection metric, the two most resistant UCD individuals were 'Mrak' ($\hat{y} = 1.84$) and 'Tahoe' ($\hat{y} = 1.98$); however, the GEBV estimates for both of those cultivars were 3.1 and thus identical to the population mean.

Third, 10 of the 12 most susceptible individuals from the thin upper tail of the GEBV distribution were from the non-UCD population, disconnected from other individuals, and

TABLE 2 Multivariate GWAS statistics for SNP loci associated with Phytophthora crown rot (PhCR) resistance phenotypes observed in a population ($n = 437$) genotyped with a 50K AxiomTM SNP array

						2017–2018		2018–2019		Across years	
Trait	**SNP Marker**	**Chr[a]**	**Postion (bp)[b]**	**MAF[c]**	***p* value[d]**	**PVE (%)[e]**	**GVE (%)[f]**	**PVE (%)**	**GVE (%)**	**PVE (%)**	**GVE (%)**
Multiple loci											
Score	AX-184879834	1D	4,028,756	0.08	$2.07e^{-6}$	3.7	7	0.1	0.1	0.4	0.5
	AX-184292487	2B	21,279,975	0.13	$1.19e^{-7}$	6	11.3	3.7	5.5	4.9	6.4
	AX-184055612	3C	6,126,473	0.21	$2.75e^{-6}$	1.5	2.9	2.9	4.3	2.9	4
	AX-184338462	3D	4,982,835	0.15	$2.21e^{-6}$	1.1	2.1	1.6	2.4	1.9	2.4
	AX-184127382	4C	22,046,504	0.16	$6.82e^{-7}$	0	0	1.5	2.3	0.7	1
	AX-184211829	6B	12,105,164	0.2	$2.47e^{-6}$	1.7	3.2	4.1	6.1	3.8	5
	AX-184211684	6B	25,289,143	0.09	$4.31e^{-4}$	0	0	0.1	0.1	0	0
	AX-184109190	7B	22,332,550	0.14	$4.60e^{-7}$	15.3	29	26.7	39.9	29	39.9
	AX-184673648	7D	16,344,212	0.14	$8.84e^{-7}$	1.8	3.5	0.6	0.9	1.1	1.5
AUDPS	AX-184211684	6B	25,289,143	0.1	$1.24e^{-6}$	4.1	7.1	0.5	0.6	1.2	1.5
	AX-184109190	7B	22,332,550	0.14	$6.34e^{-7}$	17.7	30.7	44.9	55.6	42.6	50.9
Single locus											
Score	AX-184109190	7B	22,332,550	0.14	$4.60e^{-7}$	22.2	38.5	29	43.2	33.6	43.6
AUDPS	AX-184109190	7B	22,332,550	0.14	$6.34e^{-7}$	18.1	30.9	45.4	56.2	43.4	51.6

Note. Artificially inoculated plants (clones) of each individual were phenotyped in Davis, CA field studies in 2017–2018 and 2018—2019. GWAS statistics are shown for resistance score and area under the disease pressure stairs (AUDPS) within and across years. The across years statistics were estimated using the phenotypes for individual years as separate independent variables in MV-GWAS. Statisitcs for multilocus MV-GWAS analyses were estimated using the full complements of SNP marker loci shown for each trait.

[a]Chromosone number.

[b]SNP physical positions were ascertained in the FaRR1 haplotype-phased 'Royal Royce' reference genome.

[c]Minor allele frequency (MAF).

[d]FDR-corrected *p* value.

[e]Percentage of the phenotypic variance on a clone-mean basis (PVE) explained by a locus.

[f]Percentage of the genetic variance (GVE) explained by a locus.

TABLE 3 Accuracy of genomic prediction of Phytophthora crown rot resistance score and area under the disease pressure stairs (AUDPS) breeding values in a training population of 321 University of California–Davis (UCD) and 116 non-UCD individuals phenotyped in 2017–2018 and 2018–2019 field experiments in Davis, CA

		G-BLUP		RKHS		BL	
Trait	**Population**	**GEBV**	**GEBV + RPc2**	**GEBV**	**GEBV + RPc2**	**GEBV**	**GEBV + RPc2**
Score	Training	0.40 ± 0.10	0.50 ± 0.08	0.41 ± 0.10	0.50 ± 0.08	0.39 ± 0.10	0.49 ± 0.08
	UCD	0.22 ± 0.10	0.19 ± 0.16	0.24 ± 0.10	0.20 ± 0.17	0.23 ± 0.10	0.19 ± 0.16
	Non-UCD	0.55 ± 0.14	0.67 ± 0.11	0.55 ± 0.14	0.67 ± 0.11	0.55 ± 0.14	0.65 ± 0.12
AUDPS	Training	0.33 ± 0.10	0.49 ± 0.08	0.34 ± 0.09	0.49 ± 0.08	0.32 ± 0.10	0.48 ± 0.08
	UCD	0.31 ± 0.10	0.30 ± 0.15	0.32 ± 0.09	0.31 ± 0.15	0.28 ± 0.10	0.30 ± 0.15
	Non-UCD	0.36 ± 0.17	0.59 ± 0.13	0.37 ± 0.15	0.60 ± 0.13	0.35 ± 0.17	0.59 ± 0.13

Note. Across-year genomic-estimated breeding value (GEBV) means ± 1 SD were estimated from 1,000 iterations of 80/20 cross validation using genomic best linear unbiased prediction (G-BLUP), reproducing kernel Hilbert space (RKHS), and Bayesian Lasso (BL) whole-genome regression methods applied with and without incorporating the RPc2-associated single-nucleotide polymorphism AX-184109190 as a fixed effect (GEBV and GEBV + RPc2, respectively).

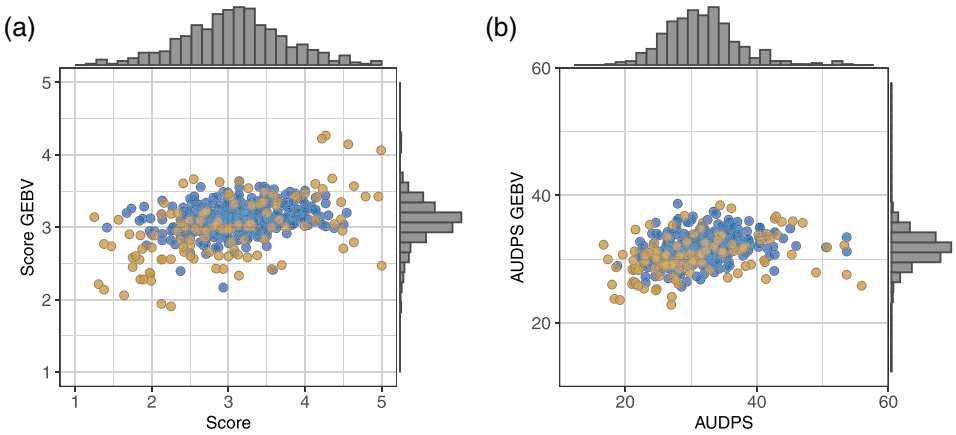

FIGURE 5 Phenotypic and genomic-estimated breeding value (GEBV) distributions for Phytophthora crown rot resistance score and area under the disease pressure stairs (AUDPS) among 437 individuals phenotyped in 2017–2018 and 2018–2019 field experiments in Davis, CA. Across-year phenotypes (estimated marginal means) are shown on the *x* axis. The GEBV means estimated from 1,000 iterations of 80/20 cross-validation using genomic best linear unbiased prediction are shown on the *y* axis. University of California–Davis (UCD) and non-UCD individuals are shown in blue and gold points, respectively

had GEBVs in the 3.57–4.27 range (Figure 5; Supplemental File S1). Seven of the 12 were *F. chiloensis* ecotypes and one was an *F. virginiana* ecotype. These findings further highlight the prediction that unfavorable alleles appear to be substantially more common in the exotic genetic resources we sampled than in the UCD population, which has a lower frequency of highly susceptible individuals. The *F.* × *ananassa* individuals from the highly susceptible tail of the GEBV distribution were the cultivars 'Tamella' (3.67) and Jersey Belle (3.57) and the UCD hybrid 94C016P001 (3.64). 'Tamella' has been widely reported to be highly susceptible (Supplemental File S6) and was clearly susceptible in our study (Figure 2; Supplemental File S1). Hence, the inclusion of exotic genetic resources in the training population widened the phenotypic and breeding value ranges in both directions, which increased genetic variation and genomic prediction accuracy (Tables 1 and 3; Figure 5).

3.8 | Revisiting the selection of prospective donors of favorable alleles for enhancing resistance to PhCR

Among the more compelling story lines to emerge from our study were the scarcity of highly resistant individuals and consequential differences in genetic variation between UCD and non-UCD individuals in the training population, particularly for resistance score in 2017–2018 (Table 1). The inclusion of a small, albeit highly diverse, collection of exotic individuals (n = 116) in the training population doubled or tripled genetic variation for resistance score and AUDPS. Nevertheless, our estimates of the additive coefficient of genetic variation (CV_A) or "evolvability" for the complete training population (0.14–0.20) and elite UCD individuals only (0.08–0.14) highlight the challenge of breeding for resistance to PhCR in strawberry (Table 1).

Several of the PhCR-resistant heirloom cultivars identified in strawberry have roots in early 20th century breeding for resistance to red stele or Lanarkshire disease in Scotland where that disease was initially discovered, heavy clay and cool wet soils are common, and the causal pathogen *P. fragariae* thrives (Adams et al., 2020; Wardlaw, 1927). Weaving back through the breeding history (Eikemo et al., 2000, 2003; Pincot et al., 2021; Van de Weg, 1997), we discovered that many of the cultivars found to be resistant to PhCR have common ancestors previously shown to be resistant to red stele, most notably 'Frith' and 'MD-683' (Supplemental File S1). Frith was a parent or more distant ancestor of several red-stele-resistant cultivars that were found to be resistant to PhCR in the present study, for example, 'Climax' (2.38), 'Red Gauntlet' (2.64), and others with 'Auchincruive' ancestry (Supplemental File S1). 'Fairfax' (1.75) and 'Scotland BK-4', a descendant of 'Frith', are parents of 'MD-683' (1.38), a highly resistant parent found in the ancestry of several cultivars shown to be PhCR resistant, for example, 'Addie' (1.38), 'Stelemaster' (1.64), 'Delite' (1.71), 'Tribute' (1.79), and 'MDUS-5097' (1.89) (Pincot et al., 2021). Several other descendants of 'Fairfax' were found to be resistant to PhCR in the present study, notably 'Empire' (1.75), 'Tribute' (1.79), 'Bounty' (1.84), 'Hood' (1.85), 'Jewel' (1.85), 'Fairland' (1.97), 'Cavalier' (1.98), and 'Red Giant' (2.00) (Supplemental File S1). The interconnections here are intriguing because the development of the cultivars resistant to *P. fragariae* in the early 20th century predated the emergence of *P. cactorum*-caused strawberry diseases in the middle of the century (Deutschmann, 1954; Wardlaw, 1927). The correlation seems more than coincidental; however, the genetic mechanisms underlying resistance to these pathogens are different: gene-for-gene resistance to red stele has been rigorously documented and shown to be widespread (Van de Weg, 1997), whereas resistance to PhCR appears to be quantitative albeit strongly affected by the segregation of the large-effect QTL RPc2 (Table 1; Figure 4) (Mangandi et al., 2017). One or more 'black-box' QTL underlying quantitative resistance to both pathogens could be shared but undiscoverable or simply not yet discovered. Moreover, favorable QTL alleles underlying quantitative resistance to red stele could be masked by the dominance of race-specific resistance genes (Van de Weg, 1997).

The breeding value estimates for cultivars trended upward (toward greater susceptibility) from 1960 onward without a single cultivar released after 1960 falling in the resistant range (1.0, 2.0) for either EMMs or GEBVs (Figure 2). The eight most highly resistant cultivars ($1.25 \leq \bar{y} \leq 1.75$) in our study, with one exception ('Addie', a cultivar developed in 1981), originated between 1923 and 1958 (Supplemental File S1). Among UCD individuals in the training population, the cultivars 'Tufts' ($\bar{y} = 2.93$; GEBV = 2.17; 1963), 'Capitola' ($\bar{y} = 2.38$; GEBV = 2.40; 1983), and 'Santana' (GEBV = 3.58; 1977), and the hybrid 65C065P601 ($\bar{y} = 2.71$; GEBV = 2.63; 1965) had the lowest GEBVs and were consequently predicted to be the most resistant (Supplemental File S1). Using GEBVs as a selection metric, the three most resistant individuals in our study were the non-UCD cultivars 'Sparkle' ($\bar{y} = 2.25$; GEBV = 1.91), 'Earlimiss' ($\bar{y} = 2.13$; GEBV = 1.94), and 'Stelemaster' ($\bar{y} = 1.64$; GEBV = 2.06). Conversely, using phenotypic EMMs as a selection metric, the three most resistant individuals in our study were the non-UCD cultivars 'Senga Sengana' ($\bar{y} = 1.25$; GEBV = 3.14), 'Cyclone' ($\bar{y} = 1.30$; GEBV = 2.21), and 'Addie' ($\bar{y} = 1.38$; GEBV = 2.77). Although the divergence between observed phenotypes and GEBVs was pronounced for some of the individuals documented here (e.g., 'Senga Sengana'), the degree of shrinkage observed toward the population mean was expected. 'Senga Sengana' and 'Cyclone' are especially illustrative examples of validated resistant genetic resources where the divergence between phenotypes and GEBVs markedly differed. The outcome for these two cultivars clearly highlights the merits of selecting prospective donors of favorable alleles using phenotypic means and GEBVs when applying selection to individuals in the training population vs. the more challenging problem of applying selection to nontraining population individuals that have not been phenotyped (Müller et al., 2015). Selection on GEBV alone here would exclude 'Senga Sengana', the most important benchmark of resistance identified in the present and previous studies (Supplemental Files S1 and S6). This sort of hedging seems prudent when selecting parents or prospective donors of favorable alleles because of the uncertainty associated with both phenotypic and breeding value estimates (Tables 1 and 3).

The phenotypic and breeding value differences observed between UCD and non-UCD individuals in the training population (Figures 1 and 5; Supplemental File S1) were aligned with insights gained from previous genome-wide studies of nucleotide diversity. Using diverse genetic resources, Hardigan et al. (2021b) showed that strong directional selection, breeding bottlenecks, and selective sweeps had progressively decreased genetic variation in the UCD population. The decrease was hypothesized to have been driven by significant genetic gains for agriculturally important traits (e.g., fruit yield, size, and firmness) over nearly 70 yr of selection in the UCD population combined with the fixation and loss of alleles through random genetic drift and hitchhiking in selective sweeps (Hardigan et al., 2021b). Our data suggests that resistance to PhCR and Verticillium wilt has declined over the last half century in the UCD population through 2012, the oldest generation analyzed in our studies (Figure 1) (Pincot et al., 2020). The non-UCD individuals phenotyped in the present study appear to harbor favorable alleles (lower tail of the GEBV distribution) and unfavorable alleles (upper tail of the GEBV distribution) that are not present in the UCD

population and are rare among genetic resources preserved in gene banks (Figure 5; Supplemental File S1).

3.9 | Prospects for improving parent selection through cross usefulness prediction

Although the gene bank diversity we sampled (primarily non-UCD heirloom cultivars and octoploid ecotypes) substantially increased genetic variation, novel favorable alleles appear to be scarce and therefore highly dispersed (Figure 1). Their introduction and accumulation in elite populations will require several generations of hybridization and recombination, which could be accelerated by applying genomic selection (Crossa et al., 2010; Labroo et al., 2021; Poland & Rutkoski, 2016). Our evolvability (CV_A) estimates (Table 1) predict that segregating populations developed with parents chosen at random from the training population have a low probability of "producing phenotypic variation that is both heritable and adaptive" (Payne & Wagner, 2019; Pigliucci, 2008). This conclusion is consistent with our estimates of historic genetic gains for resistance to PhCR (Figure 5) and estimates of narrow-sense heritability in elite California and Florida populations (Table 1) (Mangandi et al., 2017). Although parents would obviously not be randomly chosen in practice, the low frequency of outstanding parents for PhCR resistance (individuals that have accumulated favorable alleles for multiple QTL underlying resistance to PhCR) drastically limits the choice of prospective parents, a preponderance of which have been selected for traits other than PhCR resistance; for example, out of 95,266 possible crosses among 437 individuals (excluding reciprocal crosses) in the training population, only 15, or 0.0157%, would be between pairs of prospective parents predicted to be highly resistant (using phenotypic means as the selection criteria). The percentage predicted from GEBV estimates was virtually identical.

To take the parent selection problem one step further, additive genetic variances and usefulness criteria (Allier et al., 2019; Lehermeier et al., 2017) were estimated for phenotypic and genomic selection schemes by simulating segregating populations (full-sib families of 200 individuals each) for all possible crosses (190,532 including reciprocals) among 437 prospective parents (individuals) in the training population (Figures 6 and 7). The latter were split into resistant (R) and susceptible (S) groups using across-year phenotypic EMM and GEBV estimates as selection criteria (Supplemental File S1). The truncation selection cutoffs were EMM < 2.0 for phenotypic selection and GEBV < 2.6 for genomic selection. There were 32 selected parents in both resistant groups (the selected fraction was 32/437 = 0.073) with an intersection of 15 parents between resistant groups (49 different parents were selected between methods). These analyses yielded classic isosceles triangle-shaped $\hat{\mu} \times \hat{\sigma}^2_A$ distributions similar to the those observed for many agriculturally important traits under directional selection in other domesticated plants (Lado et al., 2017; Mohammadi et al., 2015). As predicted, crosses between highly resistant parents ($R \times R$) or highly susceptible parents ($S \times S$) had the smallest predicted genetic variances, whereas crosses between highly resistant and highly susceptible parents ($R \times S$) had the largest predicted genetic variances (Figure 6). Naturally, as selection intensity increased among individuals with EMM < 2.0, the genetic variance range (altitude of the triangle) and maximum (apex of the triangle) decreased, where EMM here was the predicted population mean estimated from 200 individuals per simulated full-sib family.

The subsets of crosses shown in the $\hat{\mu} \times \hat{\sigma}^2_A$ distribution were chosen to study the outcomes of different phenotypic or genomic selection scenarios where resistant UCD and non-UCD individuals were selected and crossed to every individual in the training population or subsets of resistant individuals. The breeding scenarios we explored produced several insights. First, broadly speaking, crosses between resistant non-UCD parents and all other parents unlocked more genetic variation and were predicted to be more resistant (had lower population means) than crosses between UCD parents and all other parents (Figure 6a,b). This was fully expected and predicted by the differences observed in the whole-genome regression shrinkage of breeding values discussed earlier (Figure 5). Second, the genetic parameter estimates for crosses with resistant UCD parents (blue points) were shifted upward (increased genetic variance) and leftward (decreased population mean) for genomic selection (Figure 6b) compared with phenotypic selection (Figure 6a). This pattern suggests that genomic selection on cross usefulness should produce greater gains than phenotypic selection (Figures 6 and 7). Third, we observed a distinct band of prospective crosses to the right of the primary distribution that traced to the least resistant parents and most exotic genetic resources in the training population, for example, *F. chiloensis* and *F. virginiana* ecotypes. These crosses were predicted to unleash the most genetic variation for resistance to PhCR albeit with lower population means (Figure 6a,b). There were a small number of crosses with non-UCD resistant parents near the apex in the right-shifted exotic band that warrant further exploration and inclusion in a long-term breeding strategy. These had maximum genetic variance with population means that were only slightly >2.0.

Shifting to the lower (more resistant) tail of the $\hat{\mu} \times \hat{\sigma}^2_A$ and $\hat{\mu} \times U$ distributions and focusing only on crosses between resistant parents, we found that genomic and phenotypic selection scenarios identified different complements of crosses (Figures 6c,d and 7b,c). Moreover, the complements of UCD × UCD crosses (blue points) and UCD

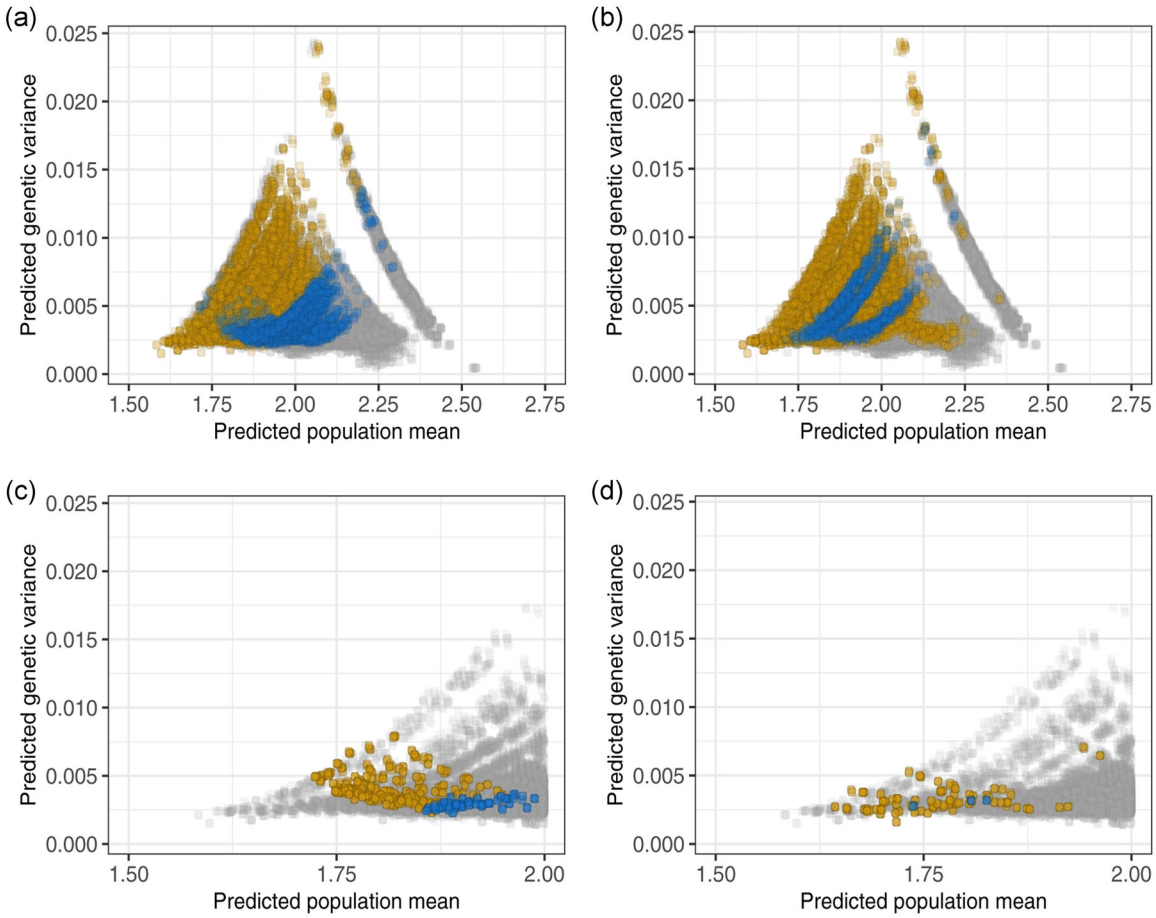

FIGURE 6 Genomic-estimated genetic variances and population estimated marginal means (EMMs) for Phytophthora crown rot resistance score are shown for 190,532 simulated segregating populations ($n = 20$ full-sib individuals per population) developed from crosses (with reciprocals) among 437 individuals (prospective parents) in the training population. The prospective parents were classified as resistant or susceptible using resistance score EMMs or genomic-estimated breeding values (GEBVs) as a selection criteria to model the outcomes of phenotypic and genomic selection, respectively. The truncation selection cutoffs were EMM < 2.0 for phenotypic selection and GEBV < 2.6 for genomic selection. There were 32 parents in the resistant groups (32/437 = 0.073) for phenotypic and genomic selection with an intersection of 15 parents between resistant groups. (a) and (c) show estimates for phenotypic selection, whereas (b) and (d) show estimates for genomic selection. (a) and (b) show statistics for all possible crosses between resistant non-University of California–Davis (UCD) parents and all other training population individuals (gold points) and all possible crosses between resistant UCD parents and all other training population individuals (blue points). (c) and (d) display the lower tails of the EMM distribution (population EMMs < 2.0) and highlight crosses between resistant parents. The gold points identify UCD × non-UCD crosses, whereas the blue points identify UCD × UCD crosses. The grey points identify non-UCD × non-UCD crosses

× non-UCD crosses (gold points) differed between genomic and phenotypic selection (Figures 6c,d and 7b,c). The gray points identify non-UCD × non-UCD crosses. The UCD × non-UCD crosses depict possible outcomes for the exact breeding scenario that motivated our study, the identification and introduction of novel favorable alleles from highly resistant sources (found to be heirloom cultivars) into modern UCD cultivars. We observed the predicted dichotomy between UCD × UCD and UCD × non-UCD crosses for the phenotypic selection scenario with lower genetic variances and population means for the former (Figure 6c). The pattern was quite different for the genomic selection scenario where only three UCD × UCD crosses (0.003% of the crosses simulated) were found in the tail and the UCD × non-UCD crosses were shifted leftward toward greater resistance (had lower population means and lower genetic variances) compared with the pattern observed for phenotypic selection (Figure 6c,d).

These analyses clearly identified the most promising crosses for pyramiding favorable alleles from different sources and introducing novel favorable alleles from non-UCD sources into elite UCD sources. The triangular $\hat{\mu} \times \hat{\sigma}^2_A$ and ellipsoidal $\hat{\mu} \times U$ distributions predict that useful genetic variation can be unlocked by crosses among the resistant parents identified in our study, both exotic and elite (Figures 6 and 7). However, the altitudes and apices of the $\hat{\mu} \times \hat{\sigma}^2_A$ distributions (Figure 6c,d) clearly show that the small fraction of crosses between pairs of highly resistant parents unlock

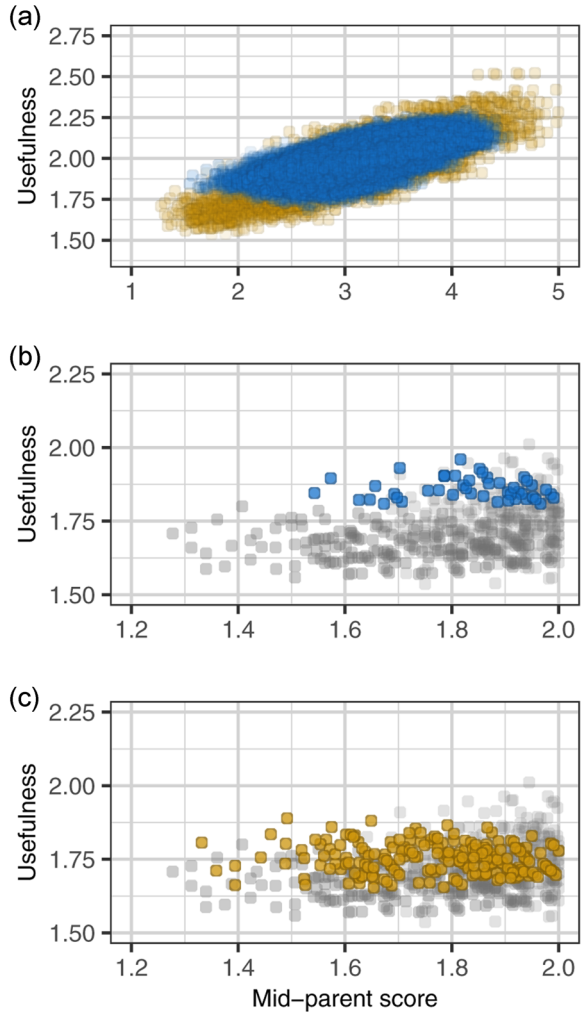

FIGURE 7 Cross usefulness criteria and population estimated marginal means (EMMs) for Phytophthora crown rot resistance score are shown for 190,532 simulated segregating populations ($n = 200$ full-sib individuals per population) developed from crosses (with reciprocals) among 437 individuals (prospective parents) in the training population. (a) Statistics are shown for all possible University of California–Davis (UCD) × UCD crosses (blue points) and all possible non-UCD × non-UCD crosses (gold points). (b) Statistics are shown for the lower tail of the EMM distribution (population EMM < 2.0). Crosses between resistant UCD parents only (UCD × UCD crosses) are shown in blue, whereas other crosses between resistant parents (UCD × non-UCD and non-UCD × non-UCD crosses) are shown in gray. (c) Statistics are shown for the lower tail of the EMM distribution (population EMM < 2.0). Crosses between resistant UCD and non-UCD parents (UCD × non-UCD crosses) are shown in gold, whereas other crosses between resistant parents (UCD × UCD and non-UCD × non-UCD crosses) are shown in gray

three- to fivefold less genetic variance than crosses with maximum genetic variance at the apex of the entire distribution of 190,532 crosses (Figure 6a,b). The latter, of course, have intermediate population means. These results suggest that the resistant individuals either share a preponderance of favorable alleles in common, as would be expected from shared ancestry (Hardigan et al., 2020; Pincot et al., 2021), that the cumulative effects of independent QTL combined in thousands of cross combinations are not additive, or both. The prediction accuracy could, in addition, have been partly caused by population structure rather than linkage disequilibrium between SNP markers and QTL (Daetwyler et al., 2012), which would further explain the patterns we observed.

4 | CONCLUSIONS

Our study highlighted one of the most pressing challenges ahead for strawberry breeders: stacking resistance to *P. cactorum* and other soil-borne pathogens without eroding genetic gains for yield and other agriculturally important traits that have enabled the phenomenal growth of the strawberry industry since the 1950s. The difficulty of that challenge was illustrated by separately analyzing UCD (90% modern era elite) individuals in the training population—100% of those individuals were predicted to be homozygous or heterozygous for the favorable RPc2 allele. Our analysis of the UCD population mimicked a real-world situation where marker-assisted selection could be applied to a population to fix the favorable RPc2 allele thereby removing the segregation of RPc2 from the equation and leaving selection to operate on the "black-box" residual quantitative genetic variation. The specific elite population we targeted (UCD) had significantly less additive genetic variance and appeared to be devoid of many if not most of the favorable alleles for PhCR resistance found in highly resistant heirloom cultivars. We concluded that those alleles had simply been left behind because of the germplasm conservation, breeding priority, and selection decisions made since the inception of the UCD breeding program in the 1920s. Those decisions profoundly affected the spectrum and frequencies of alleles preserved among elite individuals that passed through breeding bottlenecks and were preserved in the 2015 rendition of the UCD population we inherited and broadly sampled to assemble the training population for the present study. Our findings suggest that an influx of novel favorable alleles from exotic genetic resources into our elite, albeit bottlenecked population and perhaps many others, is necessary to replicate the highly resistant phenotypes of heirloom cultivars.

We undertook this study without any knowledge of the strength or spectrum of resistance to *P. cactorum* in the UCD population that has been the source of commercially important and groundbreaking cultivars for nearly a century. When paired with insights gained from earlier population genomic and forward genetic studies, the genome-informed approaches applied here further pulled back the shroud of mystery that has long surrounded the UCD population and genetic resources worldwide for that matter, in addition to

enabling data-driven decisions to unlock genetic variation for resistance to PhCR and other diseases stowed away in phenotypically anonymous strawberry gene bank collections. We concluded that the genetic complexity of resistance to *P. cactorum*, although important, has perhaps been less of a factor in the scarcity of highly resistant modern cultivars and negative genetic gains than widespread inattention to breeding for resistance. This conclusion was reached because a preponderance of the highly resistant cultivars we discovered were developed in the early 20th century decades before methyl bromide fumigation emerged and modern genome-informed breeding approaches were invented. The latter have come somewhat late to strawberry breeding and cultivar development. Although the highly resistant heirloom cultivars documented in the present study are still the benchmark for resistance today, the application of increasingly powerful predictive approaches should enable breeders to replicate the PhCR resistance of early 20th century heirloom cultivars in high-yielding modern cultivars.

AUTHOR CONTRIBUTIONS

Nicolás P. Jiménez: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing-review & editing. Mitchell J. Feldmann: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing-review & editing. Randi A. Famula: Data curation; Methodology; Project administration. Dominique D. A. Pincot: Data curation; Formal analysis; Investigation; Methodology; Resources; Writing-review & editing. Marta Bjornson: Formal analysis; Investigation; Writing-review & editing. Glenn S Cole: Conceptualization; Data curation; Investigation; Resources; Supervision; Writing-review & editing. Steven J. Knapp: Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Supervision; Visualization; Writing-original draft; Writing-review & editing.

ACKNOWLEDGMENTS

This research would not have been possible without the support of Bryan Pellisier and Alexa Sommers-Miller at the UCD Armstrong Plant Pathology Research Farm, Davis, CA, and Bruce Campopiano, Omar Gonzalez, and Eduardo Garcia at the UCD Department of Plant Sciences, Wolfskill Experimental Orchard, Winters, CA. Dr. Kelly Ivors (Driscoll's, Watsonville, CA) kindly shared inoculum of the *P. cactorum* isolates used in our studies.

This research was supported by grants awarded to Steven J. Knapp from the USDA (https://doi.org/10.13039/100000199) National Institute of Food and Agriculture (NIFA) Specialty Crops Research Initiative (#2017-51181-26833), California Strawberry Commission (https://doi.org/10.13039/100006760), and the University of California–Davis (https://doi.org/10.13039/100007707). The graduate studies of Nicolás P. Jiménez were supported by a Fulbright Fellowship (#56160010). The postdoctoral research of Mitchell J. Feldmann and graduate studies of Nicolás P. Jiménez were supported by the aforementioned USDA and CSC grants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data associated with this study are publicly available in a Dryad repository (https://doi.org/10.25338/B86D3M). Supplemental Table S1 displays genotype and allele frequencies for the RPc2-associated SNP marker AX-184109190 among UCD and non-UCD individuals in the training population. Phytophthora crown rot resistance phenotypes (estimated marginal means for resistance score and AUDPS), AX-184109190 SNP marker genotypes, and associated passport data for individuals screened for resistance to PhCR in the present study are stored in Supplemental File S1. The phenotypic data for 475 individuals collected across eight timepoints/year are stored in Supplemental File S2. The genotypic data for 40,334 SNP marker loci × $n = 437$ individuals are stored in Supplemental File S3. The chromosome IDs and physical addresses for 50K Axiom array SNPs in the 'Camarosa' (FaCA1) and 'Royal Royce' (FaRR1) genomes are documented and cross-referenced in Supplemental File S4 using the chromosome nomenclature described by (Hardigan et al., 2020). The GWAS statistics are stored in Supplemental File S5. Supplemental File S6 documents the PhCR resistance phenotypes observed in previous studies (cited in our paper). The physical addresses for annotated genes and 50K Axiom array SNPs found in the 21.73–22.99 Mb window on chromosome 7B harboring the RPc2 locus are shown in Supplemental File S7. The data rows for candidate genes are highlighted in blue.

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How to cite this article: Jiménez, N. P., Feldmann, M. J., Famula, R. A., Pincot, D. D. A., Bjornson, M., Cole, G. S., & Knapp, S. J. (2022). Harnessing underutilized gene bank diversity and genomic prediction of cross usefulness to enhance resistance to *Phytophthora cactorum* in strawberry. *The Plant Genome*, 1–23. https://doi.org/10.1002/tpg2.20275