

1 **Bioinformatic pipeline for phenotype-genotype association analyses with limited**
2 **genotypic data: *Phytophthora infestans* and its resistance to mefenoxam case study.**

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8 **Introduction**

9 A vast number of plant pathogenic organisms that range from virus to parasitic
10 plants can be a threat to food security since they are difficult to ~~control, and~~control and can
11 be highly devastating. Not only is this because their populations are variable in time, space,
12 and genotype but also, because these are constantly evolving, which represents a challenge
13 when it comes to implementing disease control strategies (Strange & Scott, 2005). Usually,
14 disease may then be minimized by the reduction of the pathogen's inoculum, inhibition of
15 its virulence mechanisms, and promotion of genetic diversity in the crop. Combining these
16 strategies can lead to a robust pest management control plan.

17 Genomics represent a valuable approach to gain insight into virulence and
18 pathogenicity of plant pathogens, as well as plant resistance, and defense mechanisms.

19 Exploring the genomic contents of pathogens and plants has allowed the discovery of genes
20 required for disease development (that can be used as control targets) (Cooke et al., 2012;
21 Haas et al., 2009; Raffaele et al., 2010; Vleeshouwers et al., 2008) or resistance, used in
22 breeding programs (Vossen et al., 2013). Furthermore, large scale screening of either
23 pathogen or plant populations can facilitate marker-assisted breeding plans (Singh et al.,
24 2013), or genomic selection (Spindel et al., 2016). Particularly, genome wide association
25 studies provide a powerful tool frequently used to detect genetic markers in diverse
26 collections of organisms, based on historic recombination events and linkage disequilibrium
27 across ~~de-the~~ genome. For example, in germplasm collections these studies seek to identify
28 regions associated with desirable agronomical traits such as yield or disease resistance
29 (Begum et al., 2015; Brown et al., 2015; Gao et al., 2016; Kollers et al., 2013; Schneider et
30 al., 2016; Spindel et al., 2016; Thakur et al., 2015). There are few association studies focused
31 in plant pathogen populations. For the most part, these studies aimed to discover variants
32 responsible for fungicide resistance (Mohd-Assaad et al., 2016; Wessels et al., 2016),
33 virulence (Dalman et al., 2013; Gao et al., 2016) and host specialization (Hartmann et al.,
34 2017) in fungal pathogens.

35 Here, we aimed to create a framework to facilitate the analysis of phenotypic and
36 genotypic information collected from oomycete microorganisms. We have used
37 *Phytophthora infestans* as a model organism to illustrate the potential of a custom pipeline

38 for phenotype-genotype association analyses with limited genotypic data. To this end, we
39 tested the sensitivity of *P. infestans* to mefenoxam during *in vitro* assays and attempted to
40 find significant associations between the observed phenotypes and the genotypes of 12
41 microsatellite markers by combining bioinformatic tools currently available.

42 **Materials and Methods**

43 ***Phytophthora infestans* strain collection**

44 *Culturing*

45 A total of 110 strains collected from 11 locations in Cundinamarca (Bogota, Bojaca, Choconta,
46 Cogua, El Rosal, Mosuera, Sesquile, Subachoque, Tausa, Villapinzon, and Zipaquira) (Table
47 S1) were retrieved from the *Phytophthora* collection in Universidad de Los Andes natural
48 museum and used during phenotyping and genotyping experiments. The strains were routinely
49 cultured in potato dextrose agar (PDA) (Oxoid, Waltham, MA, USA) and clarified V8 agar (2%
50 bacteriological agar, 10% clarified V8, 0.1% CaCO₃), and incubated at 18°C until further use.

51 *Genotyping*

52 The 110 *P. infestans* strains were previously genotyped (Chaves et al, 2018, *in preparation*)
53 using a reported set of 12 polymorphic simple sequence repeat (SSR) markers (Table S2)
54 (Lees et al., 2006; Li et al., 2013). PCR conditions were as follows: 95 °C for 5 min followed
55 by 30 cycles of 95 °C for 30 s, 58 °C for 90 s, 72 °C for 20 s, and a final extension at 60 °C for
56 30 min. PCR products were analyzed on an ABI 3730xl capillary system with POP-7

57 Polymer (ABI, PN 4335615). PCR amplicons were compared with a set of size standards and
58 alleles were scored accordingly (Goss et al., 2014; Lees et al., 2006; Li et al., 2013) using the
59 GeneMapper 5 software (Thermo Fisher, Waltham, MA, USA). One reference isolate from
60 *P. infestans* US-8 (isolate US040009) and US-17 (isolate US970001) clonal lineage was
61 included in all analyses (Danies et al., 2014).

62 **Mefenoxam sensitivity assays**

63 To test mefenoxam sensitivity among the *P. infestans* strains these were cultured on V8 agar
64 plates without mefenoxam and on plates supplemented with 1 µg mL⁻¹ or with 500 µg mL⁻¹ of
65 the fungicide . V8 plates were inoculated by placing a 5 mm plug of actively growing mycelia
66 in the center of each plate, and then incubated at 18°C until the mycelial growth of each strain
67 reached 80% on the non-supplemented plates (1-2 weeks). These bioassays were conducted
68 twice over time and two technical replicates were set up per strain to ensure reproducibility of
69 the results.

70 Mycelial growth and sporulation capacity were assessed for each strain. Mycelial growth was
71 recorded with a ChemiDoc XRS+ (BioRad, Hercules, CA, USA) and calculated using the
72 ImageJ2 software (Rueden et al., 2017; Schindelin et al., 2015). Sporangia production was
73 obtained by collecting sporangia from the plates using a sterile rod, placing it in 1mL of distilled
74 sterile water, counting the sporangia with a hemocytometer and calculating the concentration
75 according to

$$76 \quad \text{sporangia concentration} = \frac{\text{sporangia count}}{4} * 10^4 * \text{dilution}$$

77 The sporulation capacity (sporangia cm⁻²) was then calculated by dividing the number of
78 sporangia mL⁻¹ over the mycelial growth area. Both growth and sporulation measurements were
79 used to calculate inhibition values based on the control (non-supplemented V8 plates).

80 Furthermore, a log-logistic model was used to fit a four parameter dose-response curve for each
81 strain in each bioassay using the DRM function implemented in the R package 'dre' (Ritz &
82 Strebig, 2016). This model is based on the formula:

$$83 \quad f(x) = c + \frac{d - c}{1 + \exp(b(\log(x) - \log(e)))}$$

84 where the parameter e corresponds to the concentration at which the inhibition is exactly
85 halfway between the upper limit, d , and the lower limit, c , inhibition boundaries. The parameter
86 e determines the fungicide ED50 for a given isolate. The parameter b denotes the relative slope
87 around e (Mohd-Assaad et al., 2016; Ritz & Strebig, 2016). The EC50 values were obtained
88 from the model using the ED function implemented in the same R package and transformed
89 using the Log10 function.

90 **Pipeline for phenotype-genotype association**

91 *Phenotypic data analysis*

92 For each strain, best linear unbiased estimator (BLUEs) values were estimated over the EC₅₀
93 across bioassays using the following mixed linear model,

$$94 \quad \log_{10}(EC_{50}) \sim genotype_i + location_j + bioassay_k + Error_{ijk}$$

95 where the genotype is the identity of the strain (fixed effect, 129 levels), location is the sampling
96 site (random effect, 11 levels), and the bioassay is each biological replicate (random effect, 2
97 levels). The variance components were estimated using the restricted maximum likelihood

98 method (REML) (Oehlert, 2012) from the LMER function implemented in the R package
99 ‘lme4’ (Bates et al., 2018). Given that not every bioassay was complete, the BLUE value did
100 not equal the arithmetic mean. Additionally, significance of differences in mefenoxam
101 sensitivity among locations was determined with the T.TEST function in the R package ‘stats’
102 (p.adjust.method=bonferroni).

103

104 *Genotypic data analysis*

105 To determine the effect of covariates during the association analysis, population structure was
106 inferred from microsatellite data from *P. infestans* strains. Considering that most populations
107 of this plant pathogen are clonal or partially clonal, the principal components were obtained
108 with R package ‘poppr’(Kamvar et al., 2015; Kamvar et al., 2014) and used in the MLM
109 association model (parameter Q). Moreover, to estimate relatedness among individuals, we
110 calculated a kinship matrix using the VanRaden method provided in GAPIT (Lipka et al., 2012;
111 VanRaden, 2008). To this end, microsatellite data were converted into a numeric data format,
112 where ‘1’ indicated heterozygous, ‘0’ dominant homozygous, and ‘2’ recessive homozygous
113 genotypes.

114

115 *Phenotype-genotype associations*

116 The effect of each marker associated to mefenoxam sensitivity was tested using a linear mixed-
117 effects model (MLM) with the LMER function included in the R package ‘lme4’ with the
118 BLUE $\log_{10} EC_{50}$ values as the response variable

119

$$BLUE \sim M + Q + K + E$$

120 where M denotes each microsatellite (fixed effect), Q the population structure (fixed effect,
121 covariate), K the kinship or co-ancestry matrix (random effect) and E the residual effects. The
122 null hypothesis in this model is $M = 0$, there is no effect of the genotype on the phenotype
123 (Zhang et al., 2010). Linkage disequilibrium was estimated among loci according to the \bar{r}_d
124 statistic. This linkage measurement is independent of the number of loci sampled (Agapow &
125 Burt, 2001). Associations were considered significant above the bonferroni threshold set to p
126 $>1 \times 10^{-3}$.

127 **Results**

128 **Variation in mefenoxam sensitivity in a collection of *Phytophthora infestans***

129 *Phytophthora infestans* strains sampled from 11 locations across Cundinamarca were tested
130 for mefenoxam sensitivity and the corresponding EC_{50} and BLUE values were calculated for
131 each strain. The distribution of the $\log_{10} EC_{50}$ and BLUE values are presented in figure 1. In
132 both cases, positive values represented strains with some level of resistance to mefenoxam.
133 Significant differences ($p < 0.05$, table S3) in $\log_{10} EC_{50}$ were detected among locations, where
134 the most resistance strains were found in Cogua, while El Rosal, Bojaca y Mosquera
135 displayed highly sensitive strains (figure 2).

136

137 **Genetic analyses showed little population structure and geographic differentiation**

138 According to microsatellite data, principal component (PC) and kinship analyses were
139 performed with the aim to identify genetic relations among the *P. infestans* strains. The PC

140 analysis showed that most strains clustered in one large group that included all geographical
141 locations; therefore, no evidence for population structure was detected. However, four
142 strains did not group within the cluster and corresponded to mefenoxam susceptible strains.
143 The two principal components accounted for 33.81% of the total variance (figure 3A).
144 Furthermore, kinship relations were detected among the strains, according to the matrix
145 distance (figure 3B) and according to the clone correction where total number of individuals
146 (n=110) was reduced to only 7 clones. These results were further used to correct the MLM
147 model implemented during the association analysis.

148 **Four microsatellite loci appear to be associated with mefenoxam resistance**

149 Associations were calculated for the BLUEs values. A total of four individual significant ($p > 10^{-3}$)
150 associations between microsatellite-loci and BLUEs were detected using a mixed linear
151 model corrected by population structure and kinship matrix (figure 5). The microsatellite-trait
152 associations were detected in markers D13, G11, SSR2, and SSR3_Pi02, which also appear to
153 have the highest linkage disequilibrium (Figure 4).

154
155 Given that microsatellites are multiallelic markers, the allelic effect of these four markers on
156 the phenotype was investigated. A positive allelic effect was understood as increased
157 mefenoxam resistance, while a negative allelic effect as sensitivity (figure 6). It was found that
158 in most cases, alleles identified as 0 had a positive effect, while '1' a negative, only in one

159 [case allele '2' had a positive effect on mefenoxam resistance, however no significant](#)
160 [differences were detected \(p>0.05\).](#)

161 Discussion

162 ~~Here~~ ~~In this study~~ we have relied on currently available tools to implement a
163 comprehensive pipeline to ~~study~~ ~~assess~~ potential fungicide resistance-microsatellite
164 associations in natural field populations of *Phytophthora infestans*. Association studies rely
165 on statistical estimations rather than predictions due to the large number of factors
166 interacting or affecting a phenotype whether it is a disease or a desirable agronomical trait
167 (Balding, 2006). To find meaningful associations it is mandatory to accurately phenotype
168 and genotype several individuals using informative markers. We determined phenotype
169 variability in mefenoxam resistance through the calculation of a BLUE value and then
170 associated it with 12 microsatellite markers.

171
172 Population structure can generate spurious genotype- phenotype associations (Balding,
173 2006), and therefore we corrected our association model using a *Q* or population structure
174 covariate. We did not detect population structure among the strains when grouped by their
175 origin (sampling location) according to microsatellite data. However, it was observed that
176 all the strains that did not cluster were highly susceptible to mefenoxam, indicating that
177 this might be a factor determining population structure rather than the sampling location.

178 Fungicide driven population structure has been reported for other plant pathogens such as
179 *Botrytis cinerea* (Wessels et al., 2016), and *Phytophthora capsici* (Quesada-Ocampo et al.,
180 2011). A better understanding of the population dynamics would benefit downstream
181 marker-trait associations analysis by reducing false positive discoveries (Bartoli & Roux,
182 2017). Moreover, high clonality was observed among the strains indicating lack of sexual
183 reproduction and strong kinship relations among individuals. This was expected, given the
184 differences in frequency of recombination and selection across microorganisms, the
185 population structures are usually likely to range from purely clonal to nearly panmictic
186 (Power et al., 2016).

187

188 Although we detected four significant associations using microsatellites, the number of
189 genotyped individual and markers challenges our results and represents a limitation for the
190 implemented statistical models. First, the limited number of individuals reduced statistical
191 power failing to detect differences in allelic frequencies among individuals (Genissel et al.,
192 2017). Furthermore, the microsatellite markers were not evenly distributed across the
193 genome, making the LD calculation inaccurate, and displayed in some cases more than three
194 alleles. To deal with the later, we ~~implanted~~implemented a numerical approach, however
195 the allelic effects might be hindered by this transformation and each allele should be treated
196 as a separate marker (Kollers et al., 2013).

197

198

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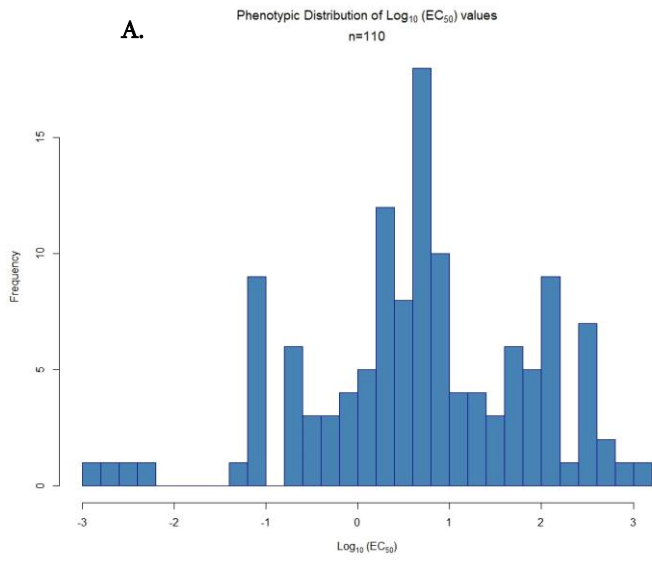
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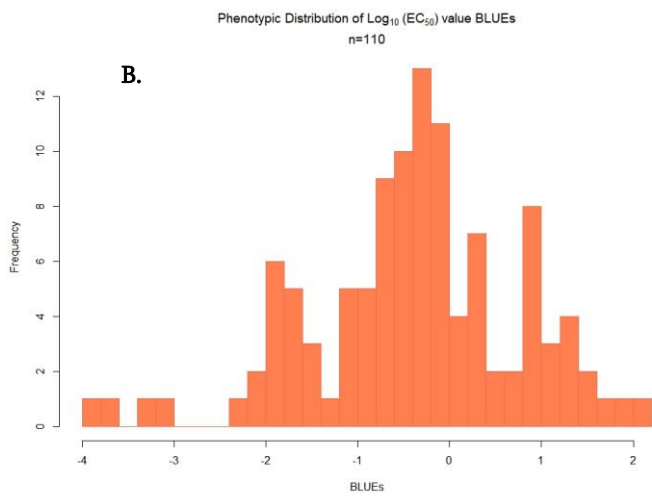
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323

324 **Figures**

325 **Figure 1.**

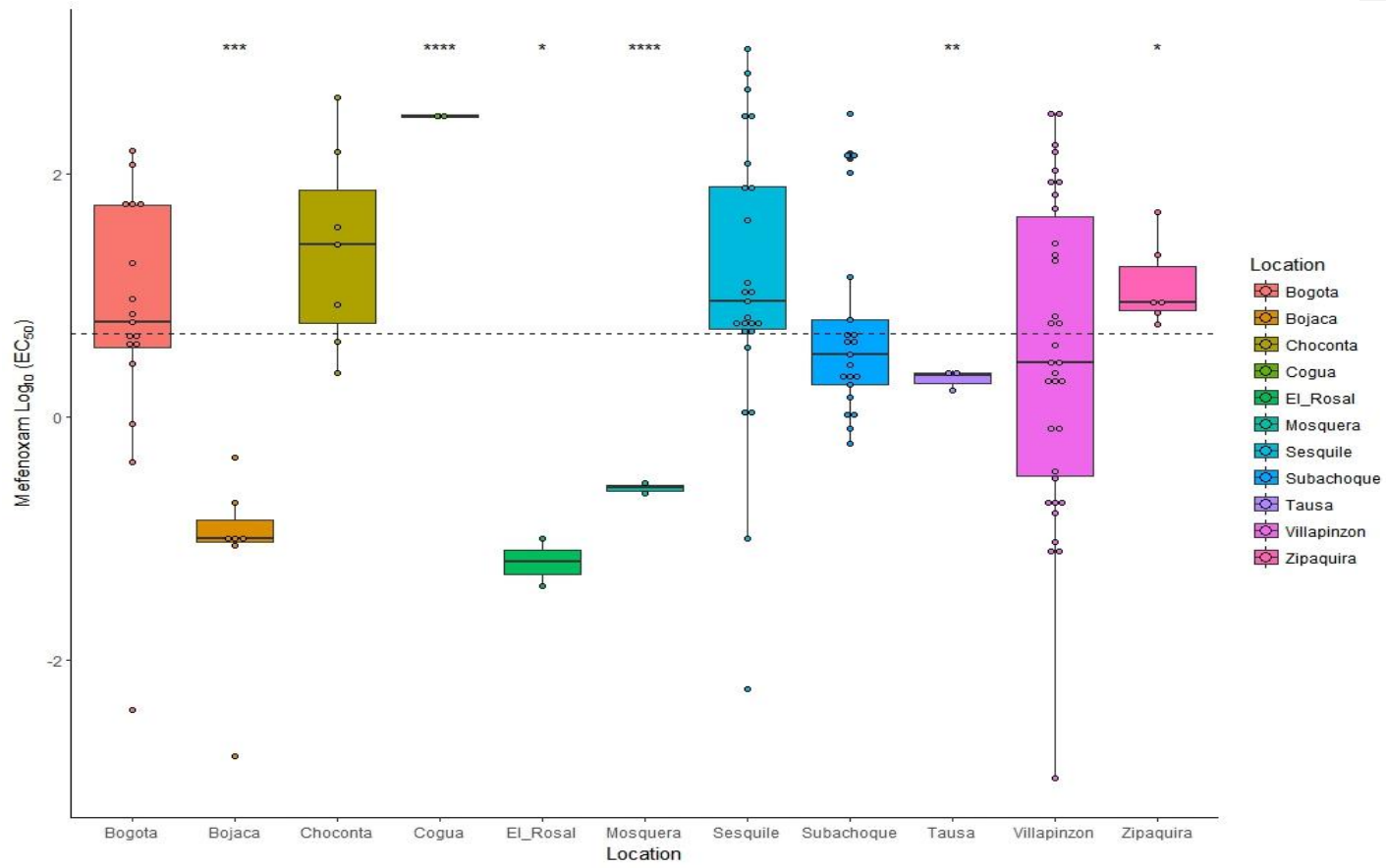


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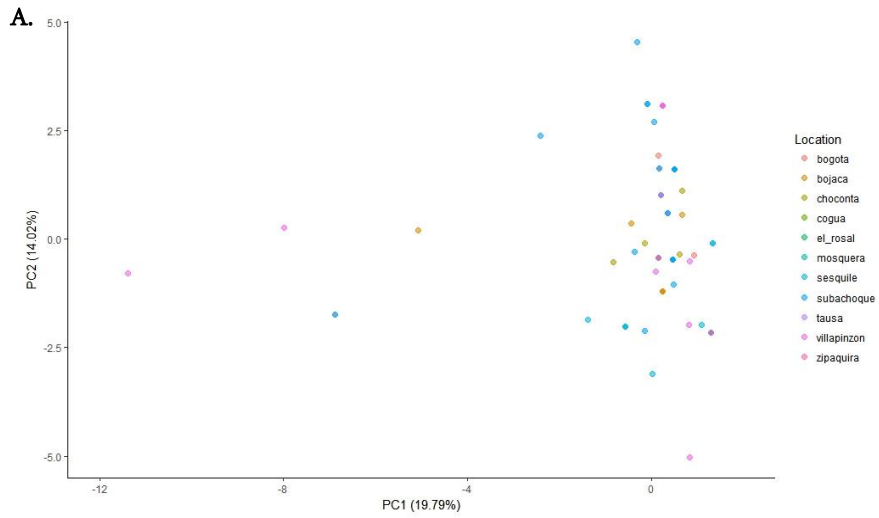
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328 **Figure 2.**



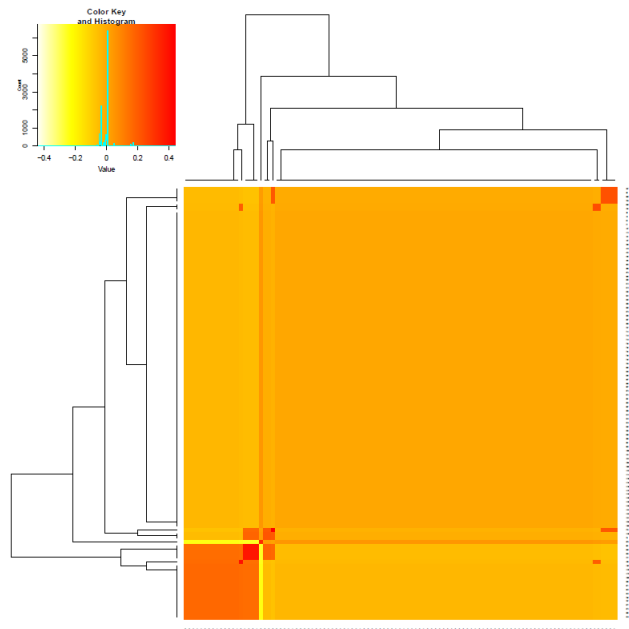
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330 **Figure 3.**



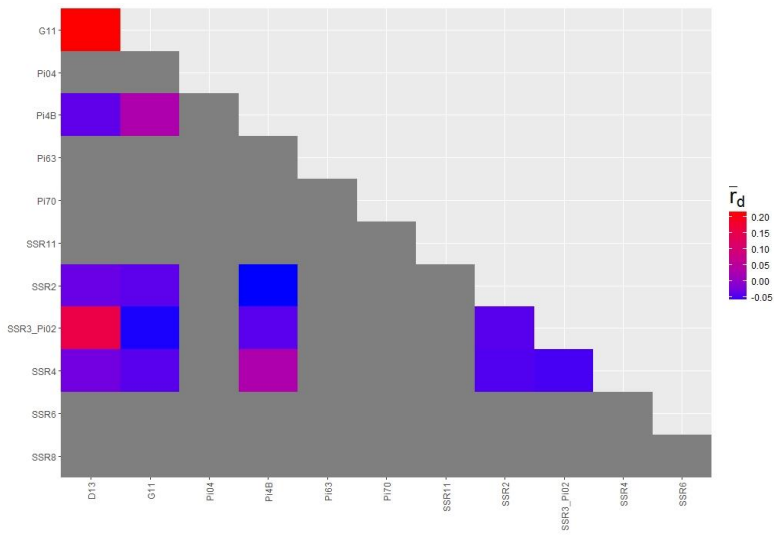
Con formato: Sangría: Izquierda: 0 cm, Sangría francesa: 1,27 cm

331



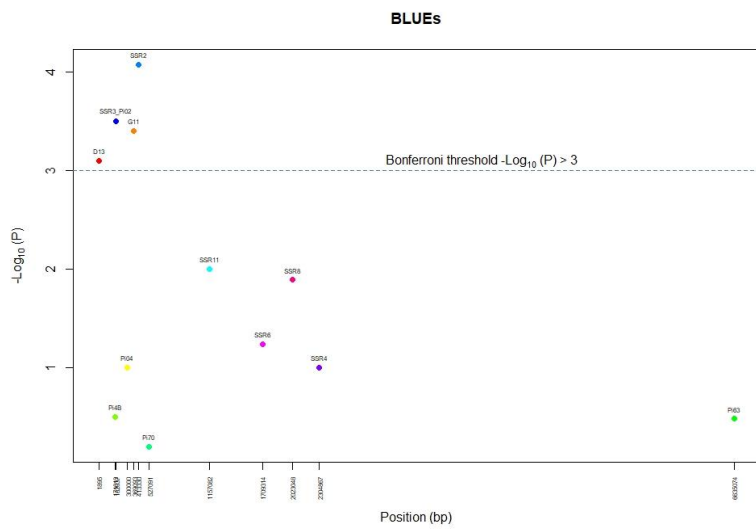
332

333 **Figure 4.**



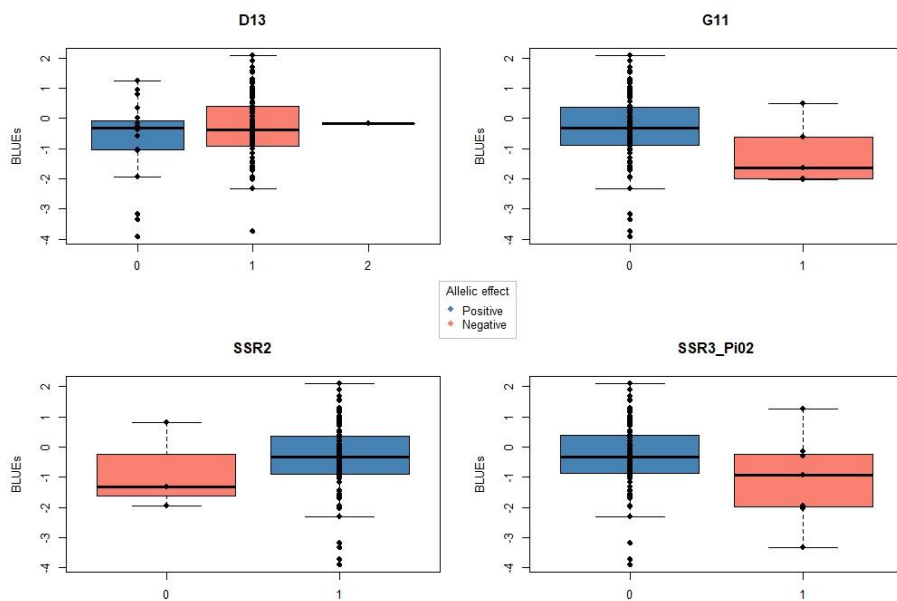
334

335 **Figure 5.**



336

337 **Figure 6.**



338

339 **Figure Legends**

340 **Figure 1. Phenotypic distribution of EC₅₀ (A) and BLUEs (B) values in 110 strains of**
 341 ***Phytophthora infestans*.** The BLUEs of the EC₅₀ were based on two independent mefenoxam
 342 susceptibility assay. A low BLUE or EC₅₀ value indicates susceptibility.

343 **Figure 2. Mefenoxam sensitivity and geographic differentiation among *Phytophthora***
 344 ***infestans* strains.** The boxplots show EC₅₀ of mefenoxam treatment across locations. The
 345 dotted line indicates the median EC₅₀ value

346 **Figure 3. Population structure and kinship relationships among *Phytophthora infestans***

347 **strains. (A)** The Principal component analysis of genetic differentiation among strains is
348 based on 12 microsatellite markers. 34% of the variance is explained by the first two
349 components. **(B)** Heatmap and dendrogram of a kinship matrix estimated based on 12
350 microsatellites among 110 *P. infestans* isolates from 11 locations in Cundinamarca.

351 Figure 4. Linkage disequilibrium represented as \bar{r}_d among microsatellite loci. The color key
352 represents the strength of the disequilibrium in a pair of markers.

353 **Figure 5. Manhattan plot of microsatellite-BLUE associations for mefenoxam sensitivity.** The
354 plot represents the individual significant marker-trait associations sorted according to their
355 genomic location (*Phytophthora infestans* T30-4 reference strain). The dotted line
356 indicates the threshold Bonferroni correction.

357 **Figure 6. Allelic effects of the four most significant loci associated with mefenoxam**
358 **resistance.** The boxplots show the differences in fungicide resistance between strains carrying
359 the most associated allele.

360 **Supplementary Tables**361 **Table S1.** *Phytophthora infestans* strains used in this study.

SampleID	Species	Locality (Country/State/Locality)	Host cultivar	Host cultivar Sensitivity	Original host	Year
BJDC_18	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
BJDC_28	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
BJDC_62	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
BJSR_77	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Superior	Susceptible	<i>S.tuberosum</i>	2016
BJSR_79	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
BJDC_95	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
BJDC_97	<i>P. infestans</i>	Colombia/Cundinamarca/Bojacá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
RODC_161	<i>P. infestans</i>	Colombia/Cundinamarca/El Rosal	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
RODC_167	<i>P. infestans</i>	Colombia/Cundinamarca/El Rosal	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
SUPP_337	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
SUPP_339	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_418	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_422	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_430	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_434	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_439	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
CHPP_454	<i>P. infestans</i>	Colombia/Cundinamarca/Chocontá	Parda Pastusa	Susceptible	<i>S.tuberosum</i>	2016
VPICA_518	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	ICAUnica	Susceptible	<i>S.tuberosum</i>	2016
107	<i>P. infestans</i>	Colombia/Cundinamarca/Mosquera	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
112	<i>P. infestans</i>	Colombia/Cundinamarca/Mosquera	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
552	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
559	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016

572	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
600	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
606	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Diacol Capiro	Moderately resistant	<i>S.tuberosum</i>	2016
612	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Diacol Capiro	Moderately resistant	<i>S.tuberosum</i>	2016
613	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Diacol Capiro	Moderately resistant	<i>S.tuberosum</i>	2016
626	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
644	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
646	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
656	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
658	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
665	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
672	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
673	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
677	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
695	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
696	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
697	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
699	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
707	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
714	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
715	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
719	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
724	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
729	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
730	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
734	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
738	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
746	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	Superior	Susceptible	<i>S.tuberosum</i>	2016
753	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	ICAUnica	Moderately resistant	<i>S.tuberosum</i>	2016
763	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	ICAUnica	Moderately resistant	<i>S.tuberosum</i>	2016

767	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	ICAUnica	Moderately resistant	<i>S.tuberosum</i>	2016
770	<i>P. infestans</i>	Colombia/Cundinamarca/Villapinzón	ICAUnica	Moderately resistant	<i>S.tuberosum</i>	2016
777	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
785	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
800	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
816	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Tuquerreña	Susceptible	<i>S.tuberosum</i>	2016
825	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
830	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
833	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
835	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
843	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
848	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
857	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
861	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
866	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
867	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
871	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
872	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
874	<i>P. infestans</i>	Colombia/Cundinamarca/Bogotá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
881	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
892	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
897	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
908	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
915	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
916	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
919	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
925	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
926	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
936	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016
938	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Superior	Susceptible	<i>S.tuberosum</i>	2016

969	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
976	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Superior	Susceptible	<i>S.tuberosum</i>	2016
981	<i>P. infestans</i>	Colombia/Cundinamarca/Sesquilé	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
992	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Paola	Resistant	<i>S.tuberosum</i>	2016
1005	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
1008	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
1011	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Superior	Susceptible	<i>S.tuberosum</i>	2016
1015	<i>P. infestans</i>	Colombia/Cundinamarca/Zipaquirá	Superior	Susceptible	<i>S.tuberosum</i>	2016
1084	<i>P. infestans</i>	Colombia/Cundinamarca/Tausa	Superior	Susceptible	<i>S.tuberosum</i>	2016
1086	<i>P. infestans</i>	Colombia/Cundinamarca/Tausa	Superior	Susceptible	<i>S.tuberosum</i>	2016
1102	<i>P. infestans</i>	Colombia/Cundinamarca/Tausa	Pastusa Suprema	Susceptible	<i>S.tuberosum</i>	2016
1114	<i>P. infestans</i>	Colombia/Cundinamarca/Cogua	superior	Resistant	<i>S.tuberosum</i>	2016
1130	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Ocarina	Susceptible	<i>S.tuberosum</i>	2016
1134	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Ocarina	Susceptible	<i>S.tuberosum</i>	2016
1136	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Ocarina	Susceptible	<i>S.tuberosum</i>	2016
1137	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Ocarina	Susceptible	<i>S.tuberosum</i>	2016
1146	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Milagros	Moderately resistant	<i>S.tuberosum</i>	2016
1156	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Paola	Resistant	<i>S.tuberosum</i>	2016
1158	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
1159	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
1160	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
1161	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
1162	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Dorada	Susceptible	<i>S.tuberosum</i>	2016
1178	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
1193	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Diacol Capiro	Susceptible	<i>S.tuberosum</i>	2016
1215	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
1228	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Criolla Colombia	Susceptible	<i>S.tuberosum</i>	2016
1251	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016
1290	<i>P. infestans</i>	Colombia/Cundinamarca/Subachoque	Pastusa Suprema	Resistant	<i>S.tuberosum</i>	2016

363 **Table S2.** *Phytophthora infestans* microsatellite marker information.

SSR locus	Product size range (bp)	Position according to <i>P.infestans</i> T30-4 (bp)	Primer Sequence	Reference
PiG11	130-206	369093	FwdNED-TGCTATTTATCAAGCGTGGG Rev-GTTTCAATCTGCAGCCGTAAGA	(Li et al, 2013)
Pi02	255-275	182037	FwdNED-ACTTGCAGAACTACCGCCC Rev-GTTTGACCACTTCTCGGTTT	(Li et al, 2013)
PinfSSR11	325-360	1157082	FwdNED-TTAAGCCACGACATGAGCTG Rev-GTTTAGACAATTGTTTTGTGGTCGC	(Li et al, 2013)
D13	100-210	1895	FwdFAM-TGCCCCCTGCTCACTC Rev-GCTCGAATTCATTTTACAGACTTG	(Li et al, 2013)
PinfSSR8	250-275	2023048	FwdFAM-AATCTGATCGCAACTGAGGG Rev-GTTTACAAGATACACGTCGCTCC	(Li et al, 2013)
PinfSSR4	280-305	2304867	FwdFAM-TCTTGTTTCGAGTATGCGACG Rev-GTTTCACTTCGGGAGAAAGGCTTC	(Li et al, 2013)
Pi04	160-175	300000	FwdVIC -AGCGGCTTACCGATGG Rev-GTTTCAGCGGCTGTTTCGAC	(Li et al, 2013)
Pi70	185-205	527091	FwdVIC-ATGAAAATACGTCAATGCTCG Rev-CGTTGGATATTTCTATTTCTTCG	(Li et al, 2013)
PinfSSR6	230-250	1709314	Fwd-GTTTTGGTGGGGCTGAAGTTTT RevVIC - TCGCCACAAGATTTATTCCG	(Li et al, 2013)
Pi63	265-280	6635074	FwdVIC-ATGACGAAGATGAAAGTGAGG Rev-CGTATTTTCTGTTTATCTAACACC	(Li et al, 2013)
PinfSSR2	165-180	413330	FwdPET-CGACTTCTACATCAACCGGC Rev-GTTTGCTTGGACTGCGTCTTTAGC	(Li et al, 2013)
Pi4B	200-295	171013	FwdPET - AAAATAAAGCCTTTGGTTCA Rev-GCAAGCGAGGTTTGTAGATT	(Li et al, 2013)

364 **Table S3. Pairwise t-test of mean Log₁₀ EC₅₀ values among sampling locations.** Significant (p-value <0.05) values are shown in
 365 bold

	Bogota	Bojaca	Choconta	Cogua	El_Rosal	Mosquera	Sesquile	Subachoque	Tausa	Villapinzon
Bojaca	0,005362	-	-	-	-	-	-	-	-	-
Choconta	1	0,001193	-	-	-	-	-	-	-	-
Cogua	1	0,002502	1	-	-	-	-	-	-	-
El_Rosal	0,733789	1	0,164008	0,041119	-	-	-	-	-	-
Mosquera	1	1	1	0,253576	1	-	-	-	-	-
Sesquile	1	0,000145	1	1	0,202212	1	-	-	-	-
Subachoque	1	0,00566	1	1	0,842868	1	1	-	-	-
Tausa	1	1	1	1	1	1	1	1	-	-
Villapinzon	1	0,015389	1	0,695805	1	1	1	1	1	-
Zipaquira	1	0,015132	1	1	0,522818	1	1	1	1	1

366