NOVEL ORGAN-SPECIFIC GENETIC FACTORS FOR

QUANTITATIVE RESISTANCE TO LATE BLIGHT IN POTATO

1 **Deissy Katherine Juyo Rojas[1] orcid.org/0000-0001-6220-2554, Johana Carolina Soto Sedano[2]**

2 **orcid.org/0000-0001-7890-4403, Agim Ballvora[3] orcid.org/0000-0003-0949-8311, Jens Léon[3]**

3 **orcid.org/0000-0002-6514-3112, Teresa Mosquera Vásquez[1] orcid.org/0000-0003-4854-1079 ***

4

5 1. Universidad Nacional de Colombia, sede Bogotá, Facultad de Ciencias Agrarias, Carrera 30

6   No. 45 - 03, Building 500 Bogotá, Colombia.

7 2. Universidad Nacional de Colombia, sede Bogotá, Facultad de Ciencias, Carrera 30 No. 45 - 03,

8   Building 421 Bogotá, Colombia.

9 3. University of Bonn, Institute of Crop Science and Resource Conservation (INRES)

10   Katzenburgweg 5, D-53115 Bonn, Germany.

11 *** Correspondence:**

12 Corresponding Author

13 tmosquerav@unal.edu.co   telephone number 571- 3165000 ext. 19101

14 **Keywords: leaves resistance, stem resistance, Group Phureja, Phytophthora infestans, candidate**

15 **resistance gene.**

16 **Abstract**

17 Potato, *Solanum tuberosum*, is one of the highest consumed food in the world, being the basis of the

18 diet of millions of people. The main limiting and destructive disease of potato is late blight, caused by

19 *Phytophtora infestans*. Here, we present a multi-environmental analysis of the response to *P. infestans*

20   using an association panel of 150 accessions of *S. tuberosum* Group Phureja, evaluated in two localities

21   in Colombia. Disease resistance data were merged with a genotyping matrix of 83,862 SNPs obtained

22   by 2b-restriction site–associated DNA and Genotyping by sequencing approaches into a Genome-wide

23   association study. We are reporting 16 organ-specific QTL conferring resistance to late blight. These

24   QTL explain from 13.7% to 50.9% of the phenotypic variance. Six and ten QTL were detected for

25   resistance response in leaves and stem, respectively. *In silico* analysis revealed 15 candidate genes for

26   resistance to late blight. Four of them have no functional genome annotation, while eleven candidate

27   genes code for diverse proteins, including one leucine-rich repeat kinase.

28

29   **1. Introduction**

30   Potato (*Solanum tuberosum*) is the most important non-cereal crop and plays a basic role for the food

31   security and nutrition [1]. Also, potatoes are an important crop for farmers and consumers because of

32   its ability to efficiently transform inputs into a high-quality food with an excellence consumer

33   acceptance.

34   The predominately cultivated potato species worldwide is *S. tuberosum* L. (2n=4x=48), this species

35   contains two groups: Chilotanum (tetraploid potato) and Andigenum [2]. The Group Andigenum has

36   a subset of diploid potatoes (2n=2x=24), the Group Phureja [3]. Phureja has been used worldwide as a

37   genetic source and has contributed to understand the genetic basis of several agronomic traits of potato.

38   It is worth to notice that the current potato reference genome sequence is obtained from DM1-3516

39   R44 a Phureja genotype [4].

40   Potato, as any other vegetative multiplied crop, is affected by several pests and diseases and the most

41   devastating disease affecting this crop is late blight caused by the oomycete *Phytophthora infestans*

42   [5–8]. Late blight has been reported in the main regions where potato is grown, including more than

43   130 countries distributed over Asia, Africa, Oceania, Europe, South, Central and North America [9].

44 Thereby, great concern exists worldwide for possible emergence of new *P. infestans* strains as well as

45 for the number of countries affected by the disease that could be increasing. Although chemical control

46 of late blight is possible, the best alternative is through the use of resistant varieties. In potato, both,

47 pathotype-specific (vertical resistance) and race non-specific resistance (field, horizontal, polygenic or

48 partial resistance) have been reported [6,7,10–12].

49 The potato breeding programs worldwide are focused to obtain late blight resistant cultivars though

50 conventional breeding [13–15]. Despite these programs have successfully developed partial resistant

51 cultivars [16], several challenges remain to be faced. If the selection is based on vertical resistance

52 exists the risk of its breakdown caused by an eventual emergence of new *P. infestans* races [11], but if

53 the selection is based on horizontal resistance, this is highly dependent on the environmental

54 conditions, as well as on the pathogen strains. Thus, the cultivar resistance could change depending on

55 the geographic region [17]. To face these challenges, it is important the detection of quantitative trait

56 loci (QTL) that govern late blight resistance in both tetraploid and diploid potatoes, through methods

57 based on genetic variants capture by DNA molecular markers such as Genome-wide association studies

58 (GWAS) and on multi-environmental phenotyping. This will reduce the time required in the process,

59 and also will contribute to a better understanding of the genetic basis of potato - *P. infestans* interaction.

60 In potato, several successful association mapping studies have been achieved for traits such as diseases

61 resistance [12,18–20]. For diploid potatoes, the search for sources of resistance to late blight through

62 GWAS has been relatively scarce. Two candidate genes and three SNPs associated with quantitative

63 resistance to late blight in the Group Phureja were identified using 371 SNP distributed along the

64 genome [18,21].

65 In the polygenic resistance one of the major limitations is the characterization of the plant-pathogen

66 interaction due to many influencing factors, including the biology, genetics and evolution of the

3

67   pathogen in addition to the environmental conditions [22]. To facilitate the understanding of polygenic

68   resistance, it has been proposed to subdivide the behavior of the disease in the different affected organs

69   of the plant [23].

70   Studies indicate that differences in organ-specific resistances are resulting in an increase of

71   transcription levels, although there is also the possibility that organs develop hypersensibility response

72   (HR) [23–25].

73   The aim of the presented study is to identify QTL for resistance to late blight in landrace accessions *S.*

74   *tuberosum* Group Phureja. We employed a GWAS using phenotypic data from the characterization of

75   disease resistance conducted in two localities and genotyping data obtained from GBS and 2b-RAD

76   approaches. Here, we report 16 organ-specific QTL for resistance to late blight in *S. tuberosum* Group

77   Phureja. The analysis of DNA sequence corresponding to the QTL reveals 15 candidate defense genes

78   that both, contribute to a better understanding of the genetic control of late blight resistance in diploid

79   potato, and also could be used in the future in potato breeding programs.

80

81   **2.   Materials and Methods**

82

83   **2.1.   Plant material**

84   The association panel consists of 150 accessions of *S. tuberosum* Group Phureja from the Working

85   Collection of the Potato Breeding Program at Universidad Nacional de Colombia. From them, 109 are

86   landrace accessions belonging to the Colombian Core Collection (CCC). Five are Colombian

87   commercial genotypes, denoted as commercial cultivars, Criolla Colombia (CrCol), Criolla Guaneña

88   (CrGN), Criolla Latina (CrLSE1), Criolla Paisa (CrPSE1), and Criolla Galeras (CrGL). Four are

89   diploid accessions from the *IPK Germplasm Bank of Germany* (Leibniz-Institut für Pflanzengenetik

4

90   und Kulturpflanzenforschung), which correspond to materials collected in Colombia, Peru and Bolivia

91   by the International Potato Center (CIP). Three accessions are from a segregating F1 population

92   derived from a cross between the susceptible parental 48A.3 and the resistant parental 2A4, that exhibit

93   different levels of resistance to *P. infestans*, and 20 are a new set of landrace accessions (CA) that were

94   collected in Nariño province, located at south of Colombia, in 2013. The cultivars ICA Unica and

95   Diacol Capiro were the resistant and the susceptible controls to late blight, respectively.

96   Each accession was multiplied vegetatively, late blight-free, using seed potato tubers at the

97   experimental station, Centro Agropecuario Marengo of Universidad Nacional de Colombia, with an

98   altitude of 2,516 meters above sea level (masl), annual average temperature of 13.7 °C, and cumulated

99   rainfall of 669.9 mm/year. The production of seeds was carried out during three consecutive crop cycles

100   under the Colombia traditional conditions of potato crop [26].

101

102   **2.2.**   **Genotyping approaches**

103

104   GBS libraries were prepared and analyzed at the Génome Québec Innovation Centre from McGill

105   University, while the 2b-RAD was conducted at the Agriculture and Agri-Food Canada (AAFC). The

106   *Pst*I/*Msp*I enzymes were used for GBS digestion and the selective amplification was performed

107   according to [27]. The *Alf*I enzyme was used for 2b-RAD approach. The DNA fragments generated

108   were sequenced using the MiSeq system from Illumina®.

109   DNA sequencing raw data from GBS and 2b-RAD were analyzed for SNP polymorphisms detection,

110   according to the NGSTools Eclipse Plugin (NGSEP) [28]. The SNP calling was performed by

5

111   alignments to the current potato reference genome from *S. tuberosum* Group Phureja clone DM1-3

112   (v4.03) [29] , in order to obtain an annotated SNP matrix.

113   The filters applied to the annotated SNP matrix allowed to select those SNPs with quality score higher

114   than 40 (-q 40), 0.05 Minor Allele Frequency (MAF), minimum distance of 5 bp between variants, and

115   SNPs located in non-repetitive regions according to the RepeatMasker annotated repeats [29]. Then,

116   the SNP matrix was filtered using SAS® software (SAS institute Inc.). The criteria such as a MAF

117   higher than 0.05 and less than 10% of distorted molecular markers were allowed and those SNPs with

118   Mendelian segregation of 1:1 were selected.

119

120

121

122   **2.3.   Evaluation of potato response to late blight disease**

123

124   The evaluation of the response of the association panel to late blight was carried out in two localities

125   during different crop cycles, starting in May 2010 until July 2014. The combination of localities and

126   the crop cycles conformed seven environments, with specific conditions, that were coded ENV1 to

127   ENV7 (Table 1). The localities were, La Unión in Antioquia, (5°58′22″N 75°21′40″O) and

128   Subachoque, in Cundinamarca (4° 55′ 41″ N, 74° 10′ 25″ W). These localities were selected as

129   important potato production regions in Colombia, and have a high natural inoculum pressure of *P.*

130   *infestans*. The experimental design used for the phenotypic evaluation consisted of an incomplete block

131   design with three biological replicates (plant clones) per genotype, distributed in blocks of 12

132   experimental units of three tubers. The controls for resistance and susceptibility were the cultivars Ica

6

133   Unica and Diacol Capiro, respectively [30]. The plants were evaluated for their response to *P. infestans*

134   under natural infection conditions and inoculum pressure of RG57 (EC-1 clonal lineage), mating type

135   A1, which is the predominant pathogen presented in both localities [31].

136

137   **Table 1. Description of each environment for the phenotyping evaluation for resistance to *Phytophthora infestans* in**

138   **Group Phureja.** It is shown the name of location, the crop season, mean temperatures, mean relative humidity (RH), mean

139   precipitation and code.

Location	Crop season	Temp. (C°)[a]	RH (%)[a]	Mean precipitation (mm/crop season) [a]	Code
La Unión (Antioquia)	May 2010 – August 2010	15.1	86.6	16.5	ENV1*
La Unión (Antioquia)	November 2010 – February 2011	14.2	88.6	8.5	ENV2*
La Unión (Antioquia)	February 2013 - May 2013	14.8	89.1	9.4	ENV3
La Unión (Antioquia)	March 2014 - July 2014	13.6	93.4	4.4	ENV4
Subachoque (Cundinamarca)	May 2010 – August 2010	14.4	86.3	4.4	ENV5*
Subachoque (Cundinamarca)	December 2010- April 2011	13.3	85.7	2.6	ENV6*
Subachoque (Cundinamarca)	November 2013 - February 2014	13.1	80.0	2.6	ENV7

140   [a] Average of environmental parameters (IDEAM www.ideam.gov.co).

141   * Data from [18]

142

7

143   The severity of late blight was estimated and scored according to the Percentage of Direct Visual

144   Estimation of the disease (PDVE) [32] in leaves and stems following the concepts presented by Bock

145   et al., [34] and the procedure described by Álvarez et al., [18]. The evaluations were conducted by

146   expert raters in late blight, who made the estimations along the experiments. The diseased leaves and

147   stems were assessed independently per each plant and for each organ. The severity was estimated as

148   the percentage of the affected area for leaves and stems through a visual direct estimation. For stems,

149   the PDVE was averaged over the number of stems of each genotype. The evaluation started 40 days

150   post sowing (dps) and were performed weekly until the susceptible control was completely affected

151   (PDVE = 100%). Disease progress in time for each plant was analyzed by estimating the Area Under

152   Disease Progress Curve (AUDPC) and the Relative Area Under Disease Progress Curve (rAUDPC) to

153   the total area [33,35]. The rAUDPC value for each genotype per environment was calculated by using

154   the PROCMEAN procedure of SAS®.

155   In order to evaluate the resistance response of the Phureja genotypes to late blight infection, previous

156   data of the resistance in leaves in the population were taken into account. These data correspond to the

157   phenotypic evaluation of late blight response of 104 diploid accessions of the Group Phureja (CCC),

158   in the same two localities from May 2010 to February 2011 (ENV1, ENV2, ENV5, and ENV6) [18]

159   (Table 1) and these evaluations were integrated for the analysis with new evaluations for the same plant

160   material conducted from February 2013 until July 2014 (ENV3, ENV4, and ENV7).

161

162   **2.4.   Analysis of genetic stability**

163

164   The GGE-biplot criteria were centered by two (centered G + GE), without scaling and singular value

165   partitioning (SVP). GGE-biplot analysis was performed using the R package GEA-R [36].

166

167

2.5.   Statistical analysis

169

170   The rAUDPC values were tested for variance components of genotype, genotype by environment, and

171   experimental error through an ANOVA (SI 1). All analyses were performed using the SAS® software.

172   AUDPC data for the trials of late blight resistance evaluation of the CCC population were included for

173   best linear unbiased predictors (BLUPs) estimation and association analysis. These data correspond to

174   the phenotypic evaluation of late blight disease response of 104 diploid accessions of the Group Phureja

175   CCC, at La Unión from May to August 2010, from May to August 2010 at Subachoque, from

176   November 2010 to February 2011 at La Unión, and from December 2010 to April 2011 at Subachoque

177   [18] (Table 1).

178

2.6.   Genome-wide association study for late blight resistance

180

181   GWAS was performed for marker main and marker by locality interaction effects simultaneously using

182   a liner mixed model in PROC MIXED procedure in SAS® program (SAS version 9.2) following the

183   model:

$$Y_{ijk} = \mu + M_i + T_j + M_i * T_j + e_{ijk}$$

185   Where Y_{ijk} is the phenotypic value; μ is the general mean; M_i is the random effect of i-th marker

186   genotype; T_j is the locality effect fixed of j-th locality; $M_i * T_j$ is the interaction random effect of i-th

187   marker with j-th local; and e_{ijk} is the residual. To determine traits of interest in the genome wide

188   detection analysis, a log-of-odds (LOD) threshold with P-value ≤ 0.00001, and 1000 permutations was

189   determined. The QTL model comprises an iterative multiloci procedure. Therefore, the most

190   informative SNP (QTL) was set as a fixed factor during each calculation iteration step. All remaining

191   markers were again incorporated in the next iteration round and re-analyzed. The starting point of each

192   calculation round was determined by the result of the previous iteration. P-values of significant markers

193   were corrected using Probability of False Discovery Rate (PFDR) of lower than 0.05, implemented in

194   the SAS procedure PROC MULTTEST according to Benjamini and Yekutieli [37]. This procedure

195   was repeated until no marker could be detected, which led to a reduction in significant markers and

196   thereby a reduced number of false-positive QTL. A confidence interval of 1000 bp was chosen on both

197   sides of the most significant SNP and designated as putative QTL. SNPs were combined to one joint

198   QTL depending on their estimated (significant) P-value from the first iteration of the multi loci

199   procedure. Therefore, the size of the genetic interval was dependent on the significance value of

200   flanking SNPs.

201   The phylogenetic relationships of the 150 potato genotypes were determined based on the allele

202   frequency of each SNP, the genotypes were assigned to an eventual population, using a Bayesian

203   modeling in the software STRUCTURE v.2.3 [38]. The population structure matrix was used as the

204   matrix Q in the association model.

205

206   **2.7.   QTL mapping and candidate gene proposed**

207

208   The DNA sequences harboring SNPs identified as statistically associated with the phenotypic data of

209   the response to late blight were explored through BLAST (Basic Local Alignment Search Tool)

210   analysis to the current potato reference genome (v4.04). These sequences were searched also in the

211   potato consensus QTL map [39] in order to find co-localizations of SNPs with previous reported QTL.

212

213   **3. Results**

214

215   **3.1.   GBS and 2b-RAD genotyping in *Solanum tuberosum* Group Phureja**

216

217   Plant genome-wide genotyping for single nucleotide polymorphism (SNP) detection was performed

218   by GBS [40] and 2b-RAD [41] approaches, using Illumina Hi-seq (Illumina, Inc.), which produced

219   both in total 349,965,885 reads. Using GBS were obtained 302,965,885 reads of 90 bp, while with 2b-

220   RAD 46,502,388 reads of 36 bp. On average, 88% of the reads, were aligned to unique positions in

221   the potato reference genome (v4.04) [29]. Based NGSEP analyses a total of 87,656 SNPs were

222   identified. From them, 85,755 SNPs were obtained from GBS and 1,901 from 2b-RAD. Finally, the

223   subsequent filtering criteria reduced the matrix to 83,862 SNPs. These SNPs are distributed across of

224   the 12 potato chromosomes with an average of 6,988 for each chromosome. The higher densities of

225   SNPs were found in chromosomes I, III, and IV with 10,427; 8,237 and 8,160 SNPs, respectively

226   (Figure 1). From the resulting matrix of 83,862 SNPs, 60% (49,948) corresponds to transitions while

227   40% (33,914) are transversions, with a ratio of 1.47 (SI 2).

228

229   **Figure 1. Number of SNPs mapped for each chromosome in the potato genome.** Numbers on the

230   right of bars show the total SNPs identified through GBS and 2b-RAD located in each chromosome as

231   indicated by roman letters on y-axis.

11

232

233

234 **3.2.   Evaluation of late blight response in an association panel of Group Phureja**

235

236 Both AUDPC and rAUDPC values for the genotypes for each locality and season showed a continuous

237 distribution. The AUDPC values observed among the genotypes were the highest in the ENV6 (ranged

238 from 1,400 to 13,325), followed by ENV5 (from 503.5 to 11,964.2) and then, by ENV2 (from 1,416.6

239 to 10,227.7). In the Table 2, it is shown the number of evaluated genotypes, the AUDPC and rAUDPC

240 ranges.

241

242 **Table 2. Distribution of the AUDPC and rAUDPC values in the Group Phureja association panel.** Number of

243 genotypes evaluated, range of AUDPC and rAUDPC values.

Location/code	Evaluated genotypes	AUDPC range	rAUDPC range
ENV1	112	437.5 – 12,835	0 – 3.67
ENV2	112	1416.6 – 10227.7	0.28 – 2.08
ENV3	111	0 – 1456.6	0 – 0.46
ENV4	114	0 – 834.1	0 – 0.23
ENV5	112	503.5 – 11964.2	0.07 – 1.89
ENV6	112	1,400 – 13,325	0.25 – 2.37
ENV7	137	4.16 – 1713.4	0 – 0.40

244

12

245 **3.3.   Stability analysis**

246

247   With the purpose of evaluate the performance of Phureja genotypes under different environments; a

248   genotype x environment analysis was conducted through a GGE-biplot analysis of Multi-Environment

249   Trials (MET). The rAUDPC values of each genotype in each plant organ (leaves and stems) were

250   analyzed independently. The GGE-Biplot analysis shows that the two principal components explained

251   77.5% and 91.1% of the total variance caused by G_{plant} + $G_{pathogen}$ + E for Phureja resistance to late

252   blight in leaves and stem, respectively (Table 3 and Figure 2). The leaves analysis showed the two

253   mega-environments conformation, MET 1 (ENV1, ENV2, ENV5, and ENV6) and MET 2 (ENV3,

254   ENV4 and ENV7). But for stem analysis no mega-environments were detected. The rAUDPC values

255   of the genotypes were able to discriminate among seven environments tested. The behavior of

256   genotypes for both leaves and stem fall into two sectors of the graphic. For leaves resistance, the

257   genotypes behavior seems to be quite similar in MET 1, where, the genotypes exhibit more

258   susceptibility in the environment ENV1. In MET 2, the environments ENV3 and ENV4 show also the

259   most susceptible genotypes (Figure 2).

260

261   **Figure 2. Which Won Where/What graphic of GGE-Biplot analysis for leaves and stem**

262   **resistance evaluation.** (a) GGE-Biplot analysis for phenotype evaluation against *P. infestans* in leaves.

263   (b) GGE-Biplot analysis for phenotype evaluation against *P. infestans* in stem. ENV codes are shown

264   in table 1.

265

13

266 **Table 3. Summary of genome associations for resistance to late blight by location.** Plant organ, localization, genome

267 position, QTL for late blight resistance, its significance and gene annotation were the QTL resides.

Plant organ	Location	Genome position	QTL name	P-value	R^2	Gen annotation (Arabidopsis and Tomato)
Leaves	Subachoque (Cundinamarca)	Chr 1 Pos 37547438	RPiLSub1	6,2616E-08	18.8%	Protein Glycoside hydrolase, catalytic domain
		Chr 3 Pos 54281967	RPiLSub3	9,0818E-09	25.1%	Chloroplast protein with Zinc finger domain
		Chr 6 Pos 40366150	RPiLSub6	2,5174E-06	16.3%	Integral membrane transporter family protein
	La Unión (Antioquia)	Chr 3 Pos 3162604	RPiLLU3a	9,15844E-11	28.5%	Ubiquitin receptor protein associated with PEX2 and PEX12.
		Chr 3 Pos 54281967	RPiLLU3b	4,49269E-09	24.3%	Chloroplast protein with Zinc finger domain
		Chr 11 Pos 7171775	RPiLLU11	8,99280E-06	13.7%	Receptor-like protein kinase
Stem	Subachoque (Cundinamarca)	Chr4 Pos 9090691	RPiSSub4	2,04E-06	17.3%	Leucine-rich repeat protein kinase family protein
		Chr 6 Pos 11035921	RPiSSub6	1,14E-09	25.8%	SIT4 phosphatase-associated family protein
		Chr 7 Pos 53724116	RPiSSub7	1,07E-09	25.8%	Actin filament-based movement. Endocytosis
		Chr 10 Pos 51118650	RPiSSub10	1,02E-07	20.8%	DHHC-type zinc finger family protein
		Chr 12 Pos 57373550	RPiSSub12	1,27E-08	23.2%	No functional annotation
	La Unión (Antioquia)	Chr 5 Pos 45069210	RPiSLU5	3,00E-08	22.2%	Ser/Thr protein kinase, BLUS1
		Chr 10 Pos 4646061	RPiSLU10	3,53E-11	27.7%	No functional annotation

14

		Chr 12 Pos 3076623	RPiSLU12a	1,60E-14	33.3%	Serine/threonine-protein kinase. Plasma membrane
		Chr 12 Pos 38707075	RPiSLU12b	7,64E-23	50.9%	No functional annotation
		Chr 12 Pos 56839213	RPiSLU12c	6,32E-18	38.4%	No functional annotation

268

269

270 On the other hand, the genotypes CCC003 (32) and CCC101 (103) were identified as extreme resistant

271 and susceptible to late blight, respectively evaluated in leaves, while the genotypes CCC086 (91) and

272 CC133 (131) were identified in stem as extreme resistant and susceptible, respectively. The numbers

273 in the parentheses are the running ones as shown in the Figure 2. According to the rAUDPC, 44

274 genotypes are resistant in the stem evaluation. For leaves, five genotypes CCC003 (32), CCC051 (68),

275 CCC005 (34), CCC115 (114), and the control Unica (233) were the most resistant.

276

277 **3.4.   Stable QTL identified for late blight resistance in leaves and stem**

278

279 In total 16 organ-specific QTL distributed in nine of the 12 chromosomes were identified (Table 3 and

280 Figure 3). The QTL detected explain from 13.7% to 50.9% of the phenotypic variance (late blight

281 resistance) (Table 3). Six QTL were detected through the leaves evaluation, three under locality

282 Subachoque and the other three for the locality La Unión. Two of these QTL were considered as stable

283 as they were detected in the same organ (leaves) in the two localities, RPiLSub3 and RPiLLU3b for

284 Subachoque and La Union, respectively. The QTL RPiLSub3 explains 25.1% while the QTL

285 RPiLLU3b explains the 24.3% of the resistance to *P. infestans* (Table 3). On the other hand, ten QTL

15

286   were detected for late blight stem resistance. Five QTL were detected in Subachoque locality and the

287   other five in La Unión. The QTL coded as RPiSLU12b explains the highest phenotypic variance of

288   50.9% identified through the stem evaluation.

289

290   **Figure 3. SNPs mapped in the potato chromosomes of Group Phureja.** Genomic distances are

291   given in Mbp according to the PGSC V4.03 pseudomolecules [29]. In red are presented the QTL for

292   late blight resistance detected in the phenotypic response for leaves, while in blue are presented those

293   detected for stems. In black are presented the markers that limit each chromosome in the potato

294   genome. Diagram plotted using MapChart software [41].

295

296   **3.5.   QTL and candidate genes for organ-specific resistance to late blight**

297

298   The DNA genome sequences harboring associated SNPs to late blight resistance were analyzed *in*

299   *silico* using BLAST against the current potato reference genome (v4.04) and to the potato consensus

300   QTL map [29]. Those genes which co-localized with the SNPs associated to late bight resistance were

301   considered as candidate genes for late bight resistance. In total, 15 candidate genes were identified, ten

302   for stem resistance and five for leaves resistance. Among the total of candidate genes, those

303   corresponding to the QTL RPiSSub12, RPiSLU10, RPiSLU12b, and RPiSLU12c have not been

304   previously annotated, while the other 11 candidate genes, have known functional annotation in the

305   current potato genome, with diverse protein domains (Table 3). Interestingly, the QTL RPiSSub4,

306   which explains 17.3% of the resistance to late blight in stem, co-localized with a gene coding for a

307   leucine-rich repeat containing protein kinase, a class of genes shown to be involved in plant immunity.

16

308

4. Discussion

310

311   We identified 16 organ-specific QTL for resistance to *P. infestans* and 15 candidate genes that might

312   have a putative function in the defense response of potato against this pathogen. For that an association

313   panel of 150 accessions from *S. tuberosum* Group Phureja was evaluated under seven environments

314   conditions and genotyped through GBS and 2b-RAD. The locations chosen for the phenotypic

315   evaluation to late blight organ-specific disease response (La Unión-Antioquia and Subachoque-

316   Cundinamarca) are important potato production regions in Colombia with high natural pathogen

317   pressure. Moreover, these are geographic regions where the late blight disease response in the Group

318   Phureja has been evaluated before and the *P. infestans* populations in these regions have been explored

319   [43]. Nevertheless, it will be interesting to explore the actual genetic diversity of the *P. infestans*

320   populations in these two locations, in order to elucidate connections between this variability and the

321   variability found both in the defense response of the evaluated genotypes and also in the identified

322   QTL. Furthermore, this will let directing the potato breeding programs toward the development of

323   resistant adapted materials for these important potato production regions.

324   In the genetic stability analysis, the addition of the phenotypic data (ENV1, ENV2, ENV5, and ENV6)

325   shows a major variation of the resistance behavior in the genotypes and revealed the presence of Meta

326   Environments. In the phenotypic evaluation of stems, the genotypes behavior was less dissimilar with

327   a clear resistance trend. On the other hand, in the leaves phenotypic evaluation, the MET 1 (Figure 3)

328   grouped the phenotypic data, which were obtained when the climate phenomenon called La Niña

329   affected Colombia, and in consequence those crop cycles presented higher relative humidity than MET

330   2. While in the MET 2 those environments considered as normal years with an average relative

17

331 humidity were grouped. Therefore, as previously reported, the environments with higher relative

332 humidity seem to the most favorable for the late blight disease development [5,44].

333 In this study 16 organ-specific QTL conferring resistance in potato Group Phureja against *P. infestans*

334 were identified, explaining high levels of phenotypic variance or late blight resistance (13.7% to

335 50.9%) thus these regions could be considered as QTL with major effects [45]. Ten of these QTL were

336 detected through the stem evaluation while the other six QTL were detected through the leaves

337 evaluation. Despite that it is known that the response in a plant to a pathogen can be organ specific

338 [24], according to our understanding this is the first organ-specific late blight resistance QTL detection

339 study through the phenotyping in leaves and stem from *S. tuberosum* Group Phureja.

340 The organ specific analyses have been considered as a useful way to dissect and understand the plant

341 pathogen polygenic resistance. Detection of organ-specific pathogen resistance QTL has already been

342 published in several crops such as maize (*Zea mays*) [46], tetraploid potato [23–25] and wheat [47].

343 The resistance responses in different plant organs have been associated with the ability and speed of

344 defense related metabolites production, as well as how these metabolites can be transported to other

345 plant organs and then increase the plant pathogen basal defense. Dannan et al., [23] report that one of

346 the advantages of the high level of resistance to late blight exhibited by the potato stem compared to

347 the leaves, is the ability of the stem to recover the plant leaves and thus could have a significant

348 potential in controlling late blight epidemics in the field.

349 Several resistance QTL to late blight have been reported in *S. tuberosum* Group Phureja [4,48,49],

350 however, none of those QTL were identified in this study. This could be explained by the high effect

351 of the environments where the phenotypic evaluations were performed. Also, the plant materials

352 employed, their genetic backgrounds, and the phenotyping restricted to specific plant organ could have

353 an important influence in the QTL detection. Nevertheless, we are reporting a stable QTL, which

18

354   receives its name according to the locality in which it was detected, RPiLSub3 and RPiLLU3b, in

355   Subachoque and La Unión conditions respectively. In Subachoque this QTL explains 25.1% while in

356   La Unión explains the 24.3% of the resistance to *P. infestans*. The phenotypic variance explained by a

357   QTL could change depending on the environment where the phenotypic data were collected and also

358   by the evaluated organ. This variability has been reported in QTL for maize [50], barley [51], and

359   *Brassica rapa* [52].

360   The genome regions harboring the SNPs associated with resistance to late blight harbor genes coding

361   for proteins carrying diverse domains and were established as candidate genes for resistance to late

362   blight. We propose 15 candidate genes, four of them have not functional annotation so far.

363   Interestingly, two of these anonymous QTL, are those explaining the highest percentage of phenotypic

364   variances, RPiSLU12c and RPiSLU12b, which explain 38.4% and 50.9% of the resistance to late

365   blight, respectively. These genomic regions have a big potential to be explored in the near future for

366   functional validation and establish their annotation.

367   Both qualitative and quantitative resistance have been described in the *S. tuberosum - P. infestans*

368   interaction [6,18,46,53]. Thus, it is not surprising that within the set of candidate genes was found a

369   gene coding for a leucine-rich repeat protein kinase family, a protein typically related to qualitative

370   resistance, co-localizing with the RPiSSub4 QTL. This finding reinforces the thesis that the qualitative

371   resistance governed by the Avr-R interactions and the quantitative resistance governed by QTL can act

372   simultaneously. On the other hand, the fact that the majority of the identified candidate genes do not

373   belong to classical protein domains of resistance genes, goes accordingly to previous findings where it

374   has been shown that the proteins carrying the classical resistance domains could be just a few of the

375   whole set of proteins that governs the quantitative resistance [22].

19

376   Here four candidate genes coding for protein kinases were identified co-localizing with the QTL

377   RPiLLU11, RPiSSub4, RPiSLU5, and RPiSLU12a. The role of kinases has broadly described in plant

378   resistance [54–55]. Their roles have been described as a receptor molecule and also involved in the

379   defense signaling pathway [58]. In *S. tuberosum* some protein kinases have been reported such as

380   calcium-dependent protein kinases (CDPKs) decode calcium (Ca^{2+}) signals and activate different

381   signaling pathways involved in hormone signaling and both abiotic and biotic stress responses [59]. In

382   this study, the authors identified different organ-specific expression levels among the genes *CDPKS*.

383   The higher expression of these genes was in roots, stolons, and microtubers compared with the level

384   in leaves and stems (i.e. StCDPK7). Also, the potato CDPKs can be sorted into two classes, those

385   which are ubiquitously expressed in different tissues and those that have specific expression pattern

386   which are approximately one-third of the CDPK family [59].

387   The results presented here demonstrate the great potential of the Group Phureja as an alternative source

388   of resistance to potato late blight. In fact, the diploid nature of this group facilitates its use in molecular

389   breeding programs, whose aim is the transferring of resistance into tetraploid potatoes gene pool. This

390   article shows that the responses of potato plants to *P. infestans* infection are organ-specific. These

391   differential responses can have an important impact on the behavior of the genotype and therefore on

392   its production. Therefore, these results open the door to continue exploring this type of resistance, so

393   in the near future we can develop strategies that integrate the specific responses in the potato

394   improvement and production programs.

395   It is known that the dynamics with which effectors and *R* genes interact in resistant potato genotypes

396   versus susceptible genotypes differ in the level of transcription, as well as in the speed with which it is

397   induced in tuber [25] and in leaves [60]. Now, our results open the way for studies of expression

398   demonstrating the above in potato leaves of the Group Phureja and for the first time in potato stem.

20

399   The identified QTL in this research are an advance in the discovery of new organ-specific genetic

400   factors involved in the resistance response of *S. tuberosum* Group Phureja to *P. infestans* infection.

401   Also, the genomic regions associated and the candidate genes proposed are resistance sources to be

402   functionally validated in the near future. The environment specific QTL here described hides

403   interesting molecular mechanisms to be explored to a better understanding the genotype-environment

404   interactions in the potato - *P. infestans*interaction. Finally, the results could be integrated into potato

405   genetic breeding programs focused on marker assisted selection, as well as, for the development

406   of local adapted new cultivars or by combining or pyramiding these QTL to develop environment

407   independent resistant cultivars.

408

409   **5. Conflict of Interest Statement**

410   The authors declare that the research was conducted in the absence of any commercial or financial

411   relationships that could be construed as a potential conflict of interest. The authors declare no conflict

412   of interest.

413

414   **6. Author's contributions**

415   DJ: Designed and established the experiments, took the data, analyzed the results, prepared the draft

416   of manuscript. JS: Revised and analyzed the results, prepared the draft of manuscript. AB: Contributed

417   with conceptualization of the original idea, revised the results, analyzed the results, revised the draft of

418   manuscript. JL: Revised and analyzed the results, revised the draft of manuscript. TM: Conceptualized

419   the original idea, supervised the whole research, designed the experiments, revised the results and

420   analyses and revised the manuscript.

421

7. Funding

428

429

8. Acknowledgments

437

9. Supporting information captions

439   **SI 1.** a. Analysis of variance for genotype, environment and genotype x environment among the

440   association panel for a. leaves phenotyping. b. stem phenotyping ($p < 0.001$).

441   **SI 2.** Transitions and transversion SNPs from the genotyping matrix of 83,862 SNPs.

442   **SI 3.** rAUDPC values by genotype for leaves and stem resistance evaluations.

443   **SI 4.** GBS and 2b-RAD genotyping data.

444

445   **Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of

446   any commercial or financial relationships that could be construed as a potential conflict of interest.

447

448   **10. Data Availability Statement**

449   The genotyping data supporting the conclusions of this manuscript is available in SI 4.

450

451   **11. References**

452   1.   FAOSTAT. FAOSTAT. In: FAOSTAT [Internet]. 2018 [cited 19 Oct 2018]. Available:
453   http://www.fao.org/faostat/en/#home

454   2.   Spooner DM, Núñez J, Trujillo G, Herrera M del R, Guzmán F, Ghislain M. Extensive simple
455   sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool
456   structure and classification. PNAS. 2007;104: 19398–19403. doi:10.1073/pnas.0709796104

457   3.   Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM. Genetic analysis of the
458   cultivated potato Solanum tuberosum L. Phureja Group using RAPDs and nuclear SSRs. Theor
459   Appl Genet. 2006;113: 1515–1527. doi:10.1007/s00122-006-0399-7

460   4.   The Potato Genome Sequencing Consortium. Genome sequence and analysis of the tuber crop
461   potato. Nature. 2011;475: 189–195. doi:10.1038/nature10158

462   5.   Nowicki M, Foolad MR, Nowakowska M, Kozik EU. Potato and Tomato Late Blight Caused by
463   Phytophthora infestans: An Overview of Pathology and Resistance Breeding. Plant Disease.
464   2011;96: 4–17. doi:10.1094/PDIS-05-11-0458

465   6.   Mosquera T, Alvarez MF, Jiménez-Gómez JM, Muktar MS, Paulo MJ, Steinemann S, et al.
466   Targeted and Untargeted Approaches Unravel Novel Candidate Genes and Diagnostic SNPs for
467   Quantitative Resistance of the Potato (Solanum tuberosum L.) to Phytophthora infestans
468   Causing the Late Blight Disease. Yin T, editor. PLOS ONE. 2016;11: e0156254.
469   doi:10.1371/journal.pone.0156254

470   7.   Ballvora A, Ercolano MR, Weiss J, Meksem K, Bormann CA, Oberhagemann P, et al. The R1
471   gene for potato resistance to late blight (Phytophthora infestans) belongs to the leucine
472   zipper/NBS/LRR class of plant resistance genes. Plant J. 2002;30: 361–371.

473   8.   Leonards-Schippers C, Gieffers W, Schäfer-Pregl R, Ritter E, Knapp SJ, Salamini F, et al.
474   Quantitative resistance to Phytophthora infestans in potato: a case study for QTL mapping in an
475   allogamous plant species. Genetics. 1994;137: 67–77.

476   9.   Centre for Agriculture and Biosciences International. CABI.org [Internet]. 2018 [cited 19 Oct
477   2018]. Available: https://www.cabi.org/

478   10. Ballvora A, Jöcker A, Viehöver P, Ishihara H, Paal J, Meksem K, et al. Comparative sequence
479   analysis of Solanum and Arabidopsis in a hot spot for pathogen resistance on potato
480   chromosome V reveals a patchwork of conserved and rapidly evolving genome segments. BMC
481   Genomics. 2007;8: 112. doi:10.1186/1471-2164-8-112

482   11. Fry WE, Birch PRJ, Judelson HS, Grünwald NJ, Danies G, Everts KL, et al. Five Reasons to
483   Consider Phytophthora infestans a Reemerging Pathogen. Phytopathology. 2015;105: 966–981.
484   doi:10.1094/PHYTO-01-15-0005-FI

485   12. Pajerowska-Mukhtar K, Stich B, Achenbach U, Ballvora A, Lübeck J, Strahwald J, et al. Single
486   Nucleotide Polymorphisms in the Allene Oxide Synthase 2 Gene Are Associated With Field
487   Resistance to Late Blight in Populations of Tetraploid Potato Cultivars. Genetics. 2009;181:
488   1115–1127. doi:10.1534/genetics.108.094268

489   13. Gebhardt C, Valkonen JPT. Organization of Genes Controlling Disease Resistance in the Potato
490   Genome. Annual Review of Phytopathology. 2001;39: 79–102.
491   doi:10.1146/annurev.phyto.39.1.79

492   14. Haverkort AJ, Struik PC, Visser RGF, Jacobsen E. Applied Biotechnology to Combat Late
493   Blight in Potato Caused by Phytophthora Infestans. Potato Research. 2009;52: 249–264.
494   doi:10.1007/s11540-009-9136-3

495   15. Jansky SH, Spooner DM. The Evolution of Potato Breeding. Plant Breeding Reviews. Wiley-
496   Blackwell; 2018. pp. 169–214. doi:10.1002/9781119414735.ch4

497   16. Torres L, Cuesta X, Monteros C, Rivadeneira J. Variedades. In: International Potato Center
498   [Internet]. 2011 [cited 2 Mar 2014]. Available: http://cipotato.org/region-
499   quito/informacion/inventario-de-tecnologias/variedades/

500   17. Barquero M, Goméz L, Brenes A. Resistencia al tizón tardío (Phytophthora infestans) en clones
501   promisorios de papa en Costa Rica. Agronomía Costarricense. 2005;29: 31–45.

502   18. Álvarez MF, Angarita M, Delgado MC, García C, Jiménez-Gomez J, Gebhardt C, et al.
503   Identification of Novel Associations of Candidate Genes with Resistance to Late Blight in
504   Solanum tuberosum Group Phureja. Front Plant Sci. 2017;8: 1040. doi:10.3389/fpls.2017.01040

505   19. Gebhardt C, Ballvora A, Walkemeier B, Oberhagemann P, Schüler K. Assessing genetic
506   potential in germplasm collections of crop plants by marker-trait association: a case study for
507   potatoes with quantitative variation of resistance to late blight and maturity type. Molecular
508   Breeding. 2004;13: 93–102. doi:10.1023/B:MOLB.0000012878.89855.df

509   20. Simko I. One potato, two potato: haplotype association mapping in autotetraploids. Trends Plant
510   Sci. 2004;9: 441–448. doi:10.1016/j.tplants.2004.07.003

511   21. Visker MHPW, Keizer LCP, Van Eck HJ, Jacobsen E, Colon LT, Struik PC. Can the QTL for
512   late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor
513   Appl Genet. 2003;106: 317–325. doi:10.1007/s00122-002-1021-2

514   22. Corwin JA, Kliebenstein DJ. Quantitative Resistance: More Than Just Perception of a Pathogen.
515   Plant Cell. 2017;29: 655–665. doi:10.1105/tpc.16.00915

516   23. Danan S, Chauvin J-E, Caromel B, Moal J-D, Pellé R, Lefebvre V. Major-effect QTLs for stem
517   and foliage resistance to late blight in the wild potato relatives Solanum sparsipilum and S.
518   spegazzinii are mapped to chromosome X. Theor Appl Genet. 2009;119: 705–719.
519   doi:10.1007/s00122-009-1081-7

520   24. Gao L, Bradeen JM. Contrasting Potato Foliage and Tuber Defense Mechanisms against the
521   Late Blight Pathogen Phytophthora infestans. Wong S-M, editor. PLOS ONE. 2016;11:
522   e0159969. doi:10.1371/journal.pone.0159969

523   25. Gao L, Tu Z, Millett BP, Bradeen JM. Insights into organ-specific pathogen defense responses
524   in plants: RNA-seq analysis of potato tuber-Phytophthora infestans interactions. BMC
525   Genomics. 2013;14: 340. doi:10.1186/1471-2164-14-340

526   26. Alarcón J, Torrado E, Nuñez P, Forigua V. Manejo fitosanitario del cultivo de la papa
527   (Solanum tuberosum subsp. andigena y S. phureja). Medidas para la temporada invernal.
528   Produmedios; 2011.

529   27. Sonah H, Bastien M, Iquira E, Tardivel A, Légaré G, Boyle B, et al. An Improved Genotyping
530   by Sequencing (GBS) Approach Offering Increased Versatility and Efficiency of SNP
531   Discovery and Genotyping. PLoS ONE. 2013;8: e54603. doi:10.1371/journal.pone.0054603

532   28. Duitama J, Quintero JC, Cruz DF, Quintero C, Hubmann G, Foulquié-Moreno MR, et al. An
533   integrated framework for discovery and genotyping of genomic variants from high-throughput
534   sequencing experiments. Nucleic Acids Res. 2014;42: e44. doi:10.1093/nar/gkt1381

535   29. Potato Genome Sequencing Consortium, Xu X, Pan S, Cheng S, Zhang B, Mu D, et al. Genome
536   sequence and analysis of the tuber crop potato. Nature. 2011;475: 189–195.
537   doi:10.1038/nature10158

538   30. Ñustez C. Variedades colombianas de papa. Grupo imágenes; 2011.

539   31. Vargas AM, Ocampo LMQ, Céspedes MC, Carreño N, González A, Rojas A, et al.
540   Characterization of Phytophthora infestans Populations in Colombia: First Report of the A2
541   Mating Type. Phytopathology. 2008;99: 82–88. doi:10.1094/PHYTO-99-1-0082

542   32. Nita M, Ellis MA, Madden LV. Reliability and accuracy of visual estimation of Phomopsis leaf
543   blight of strawberry. Phytopathology. 2003; 93.8: 995-1005. doi:10.1094/PHYTO.2003.93.8.995.

544   33. Forbes G, Perez W, Andrade-Piedra J. Field assessment of resistance in potato to Phytophthora
545   infestans: International cooperators guide. 2014. doi:10.4160/9789290604402

546   34. Bock CH, Poole GH, Parker PE, Gottwald TR. Plant Disease Severity Estimated Visually, by
547   Digital Photography and Image Analysis, and by Hyperspectral Imaging. Critical Reviews in
548   Plant Sciences. 2010;29: 59–107. doi:10.1080/07352681003617285

549   35. Campbell CL, Madden LV. Introduction to Plant Disease Epidemiology. 1 edition. New York:
550   Wiley-Interscience; 1990.

551   36. Pacheco Á, Vargas M, Alvarado G, Rodríguez F, Crossa J, Burgueño J. GEA-R (Genotype x
552   Environment Analysis with R for Windows) Version 4.1 [Internet]. 2018. Available:
553   https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10203

554   37. Benjamini Y, Yekutieli D. False Discovery Rate–Adjusted Multiple Confidence Intervals for
555   Selected Parameters. Journal of the American Statistical Association. 2005;100: 71–81.
556   doi:10.1198/016214504000001907

557   38. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus
558   genotype data. Genetics. 2000;155: 945–959.

559   39. Danan S, Veyrieras J-B, Lefebvre V. Construction of a potato consensus map and QTL meta-
560   analysis offer new insights into the genetic architecture of late blight resistance and plant
561   maturity traits. BMC Plant Biology. 2011;11: 16. doi:10.1186/1471-2229-11-16

562   40. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. A Robust, Simple
563   Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PLoS ONE. 2011;6:
564   e19379. doi:10.1371/journal.pone.0019379

565   41. Wang S, Meyer E, McKay JK, Matz MV. 2b-RAD: a simple and flexible method for genome-
566   wide genotyping. Nat Methods. 2012;9: 808–810. doi:10.1038/nmeth.2023

567   42. Voorrips RE. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J
568   Hered. 2002;93: 77–78. doi:10.1093/jhered/93.1.77

569   43. Jaramillo S. Monografía sobre Phytophthora infestans (Mont) de Bary. Facultad de Ciencias
570   Agropecuarias. Universidad Nacional de Colombia. 2003.

571   44. Fry B, Myers K, Mayton H, Small I, Danies G. LATE BLIGHT UPDATE 2015. : 4.

572   45. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative
573   trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic
574   concepts. Euphytica. 2005;142: 169–196. doi:10.1007/s10681-005-1681-5

575 46. Balmer D, de Papajewski DV, Planchamp C, Glauser G, Mauch-Mani B. Induced resistance in
576   maize is based on organ-specific defence responses. The Plant Journal. 2013;74: 213–225.
577   doi:10.1111/tpj.12114

578 47. Golkari S, Gilbert J, Prashar S, Procunier JD. Microarray analysis of Fusarium graminearum-
579   induced wheat genes: identification of organ-specific and differentially expressed genes. Plant
580   Biotechnology Journal. 2007;5: 38–49. doi:10.1111/j.1467-7652.2006.00213.x

581 48. Costanzo S, Simko I, Christ BJ, Haynes KG. QTL analysis of late blight resistance in a diploid
582   potato family of Solanum phureja × S. stenotomum. Theor Appl Genet. 2005;111: 609–617.
583   doi:10.1007/s00122-005-2053-1

584 49. Simko I, Costanzo S, Ramanjulu V, Christ BJ, Haynes KG. Mapping polygenes for tuber
585   resistance to late blight in a diploid Solanum phureja × S. stenotomum hybrid population. Plant
586   Breeding. 2006;125: 385–389. doi:10.1111/j.1439-0523.2006.01232.x

587 50. Asaro A, Ziegler G, Ziyomo C, Hoekenga OA, Dilkes BP, Baxter I. The Interaction of
588   Genotype and Environment Determines Variation in the Maize Kernel Ionome. G3 (Bethesda).
589   2016;6: 4175–4183. doi:10.1534/g3.116.034827

590 51. Tétard-Jones C, Kertesz MA, Preziosi RF. Quantitative trait loci mapping of phenotypic
591   plasticity and genotype-environment interactions in plant and insect performance. Philos Trans
592   R Soc Lond, B, Biol Sci. 2011;366: 1368–1379. doi:10.1098/rstb.2010.0356

593 52. Dechaine JM, Brock MT, Iniguez-Luy FL, Weinig C. Quantitative trait loci × environment
594   interactions for plant morphology vary over ontogeny in Brassica rapa. New Phytol. 2014;201:
595   657–669. doi:10.1111/nph.12520

596 53. Ghislain M, Trognitz B, Herrera M del R, Solis J, Casallo G, Vásquez C, et al. Genetic loci
597   associated with field resistance to late blight in offspring of Solanum phureja and S.tuberosum
598   grown under short-day conditions. Theor Appl Genet. 2001;103: 433–442. doi:10.1007/s00122-
599   001-0545-1

600 54. Huard-Chauveau C, Perchepied L, Debieu M, Rivas S, Kroj T, Kars I, et al. An atypical kinase
601   under balancing selection confers broad-spectrum disease resistance in Arabidopsis. PLoS
602   Genet. 2013;9: e1003766. doi:10.1371/journal.pgen.1003766

603 55. Kohorn BD. Cell wall-associated kinases and pectin perception. J Exp Bot. 2016;67: 489–494.
604   doi:10.1093/jxb/erv467

605 56. Roux F, Noël L, Rivas S, Roby D. ZRK atypical kinases: emerging signaling components of
606   plant immunity. New Phytologist. 2014;203: 713–716. doi:10.1111/nph.12841

607 57. Vásquez AX, Soto Sedano JC, López Carrascal CE, Vásquez AX, Soto Sedano JC, López
608   Carrascal CE. Unraveling the molecules hidden in the gray shadows of quantitative disease
609   resistance to pathogens. Acta Biológica Colombiana. 2018;23: 5–16.
610   doi:10.15446/abc.v23n1.66487

611 58. Tena G, Boudsocq M, Sheen J. Protein kinase signaling networks in plant innate immunity.
612   Curr Opin Plant Biol. 2011;14: 519–529. doi:10.1016/j.pbi.2011.05.006

613   59. Fantino E, Segretin ME, Santin F, Mirkin FG, Ulloa RM. Analysis of the potato calcium-
614   dependent protein kinase family and characterization of StCDPK7, a member induced upon
615   infection with Phytophthora infestans. Plant Cell Rep. 2017;36: 1137–1157.
616   doi:10.1007/s00299-017-2144-x

617   60. Bradeen JM, Iorizzo M, Mollov DS, Raasch J, Kramer LC, Millett BP, et al. Higher Copy
618   Numbers of the Potato RB Transgene Correspond to Enhanced Transcript and Late Blight
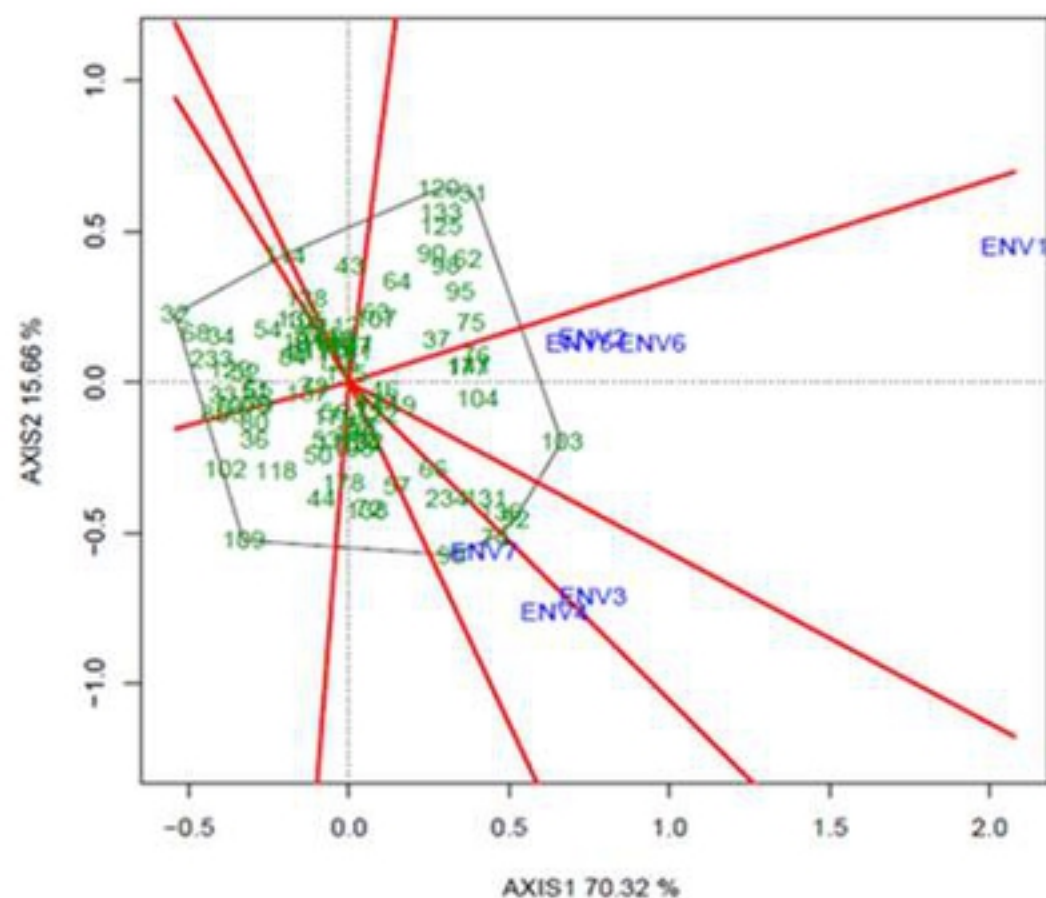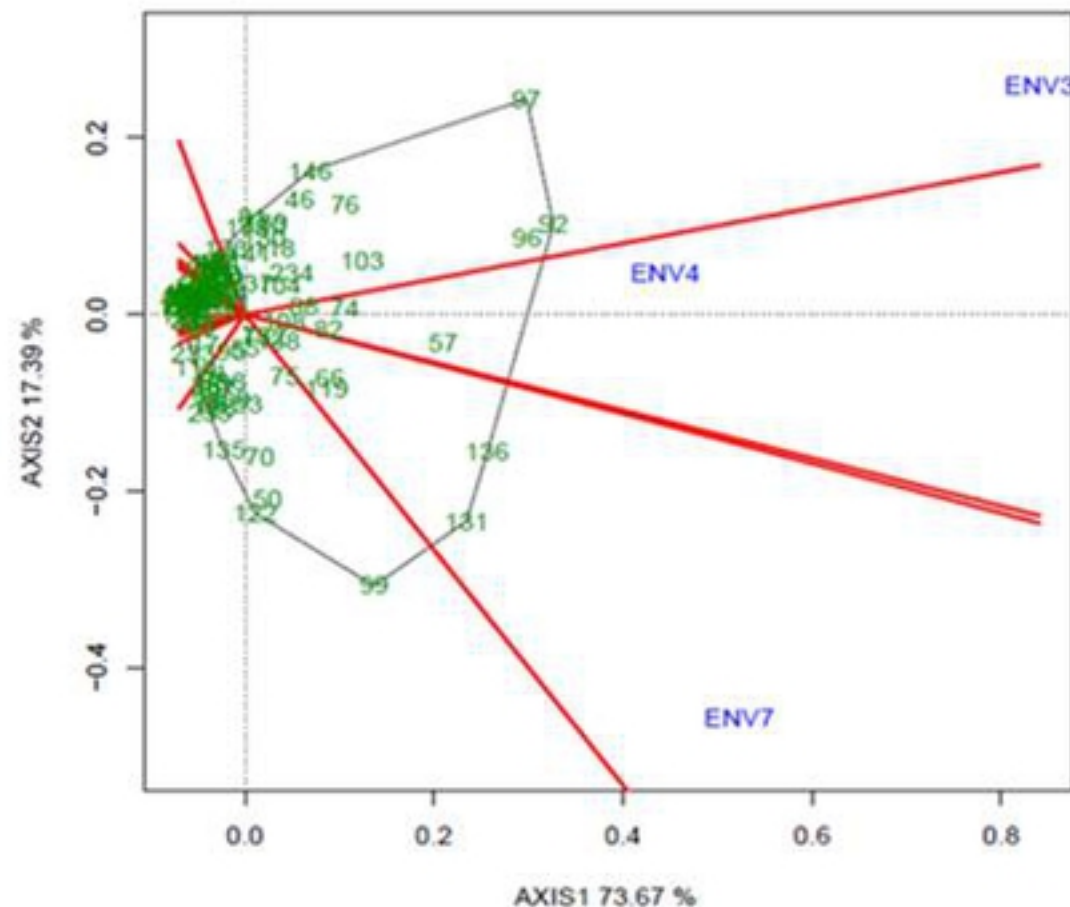619   Resistance Levels. MPMI. 2009;22: 437–446. doi:10.1094/MPMI-22-4-0437

620
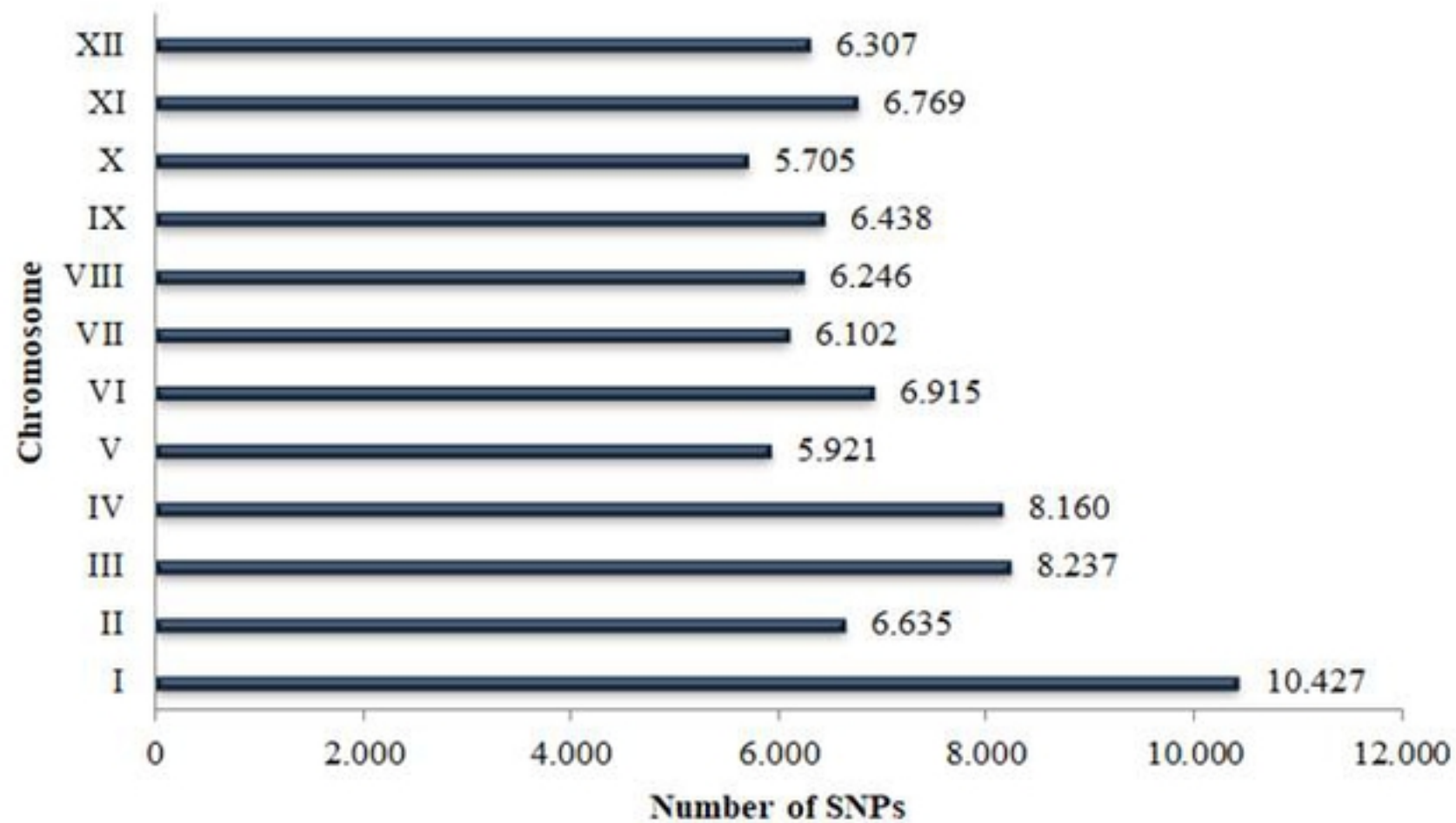
Figure 1. Number of SNPs mapped for each chromosome in the potato genome. Numbers on the right of bars show the total SNPs identified through GBS and 2b-RAD located in each chromosome as indicated by roman letters on y-axis.

Figure 1

Figure 2. Which Won Where/What graphic of GGE-Biplot analysis for foliage and stem resistance evaluation. (a) GGE-Biplot analysis for phenotype evaluation against *P. infestans* in foliage. (b) GGE-Biplot analysis for phenotype evaluation against *P. infestans* in stem. ENV codes are shown in table 1.
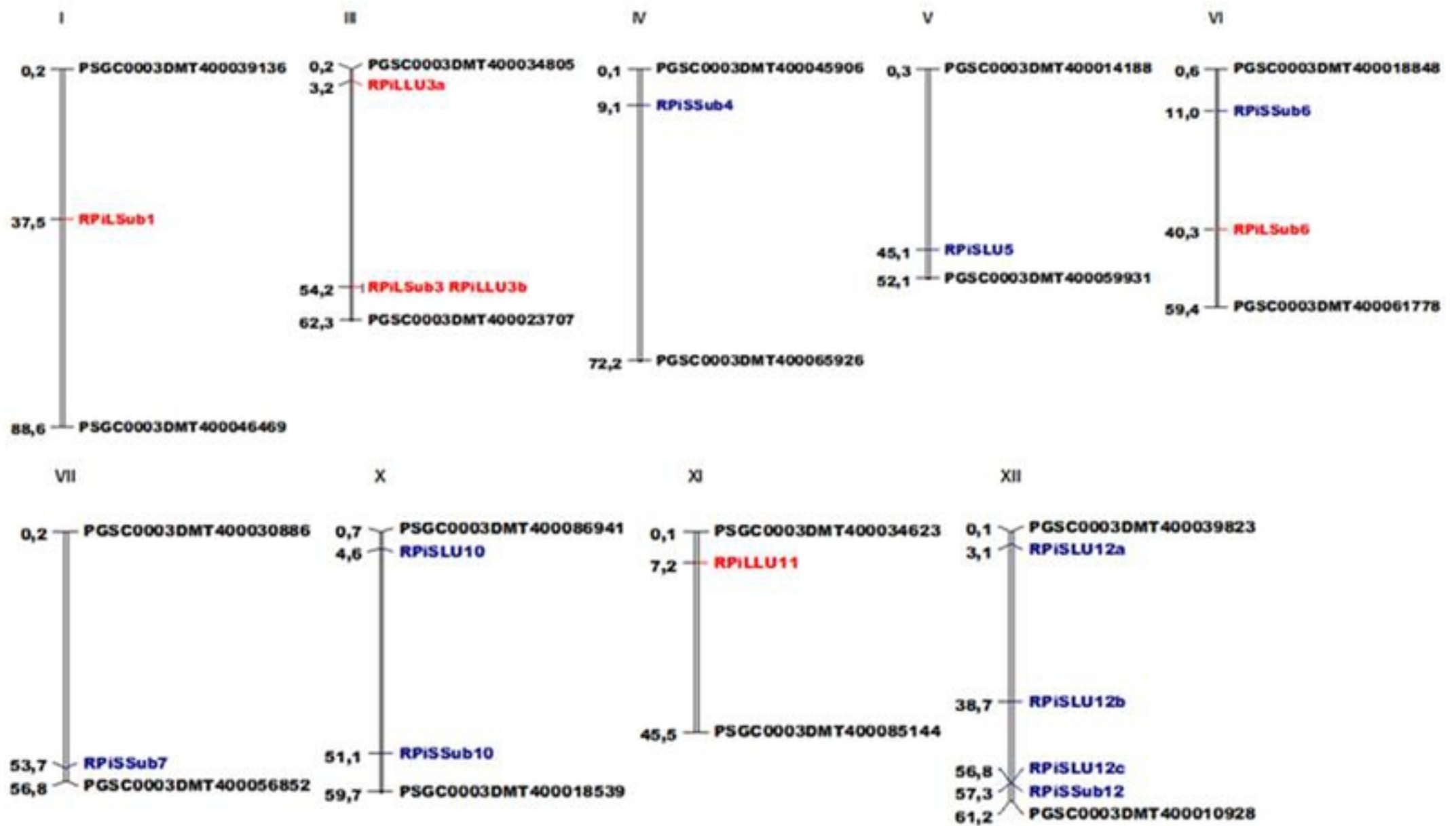
Figure 2

Figure 3. SNPs mapped in the potato chromosomes of Group Phureja. Genomic distances are given in Mbp according to the PGSC V4.03 pseudomolecules (Xu et al., 2011). In red are presented the QTL for late blight resistance detected in the phenotypic response for foliage, while in blue are presented those detected for stems. In black are presented the markers that limit each chromosome in the potato genome. Diagram plotted using MapChart software (Voorrips. 2002).

Figure 3