Quantitative resistance differences between and within natural populations of *Solanum chilense* against the oomycete pathogen *Phytophthora infestans*

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Running title: Quantitative resistance in a wild tomato species

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Abstract

The wild tomato species *Solanum chilense* is divided in geographically and genetically distinct populations that show signs of defense gene selection and differential phenotypes when challenged with several phytopathogens, including the oomycete causal agent of late blight *Phytophthora infestans.* To better understand the phenotypic diversity of this disease resistance in *S. chilense* and to assess the effect of plant genotype vs. pathogen isolate, respectively, we evaluated infection frequency in a systematic approach and with large sample sizes. We studied 85 genetically distinct individuals representing nine geographically separated populations of *S. chilense.* This showed that differences in quantitative resistance properties can be observed between but also within populations at the level of individual plants. Data also did not reveal clear indications for complete immunity in any of the genotypes. We further evaluated the resistance of a subset of the plants against *P. infestans* isolates with diverse virulence properties. This confirmed that the relative differences in resistance phenotypes between individuals were mainly determined by the plant genotype under consideration with modest effects of pathogen isolate used in the study. Thus, our report suggest that quantitative resistance against *P. infestans* in natural populations of a wild tomato species *S. chilense* is likely not the result of specific adaptations of hosts to the pathogen but of basal defence responses that depend on the host genotype and are pathogen isolate-unspecific.

Keywords: *Solanum chilense, Phytophthora infestans*, quantitative resistance, natural populations.

Introduction

Plant immunity can be observed in a qualitative manner, in which plants are either completely resistance or show clear disease symptoms, or in a quantitative way, in which certain plants of the same species are more resistant and others are less resistant. Such quantitative resistance was formally introduced as horizontal resistance in the field of phytopathology by Vanderplank in 1963 and he further extended his concept in 1968. By his definition horizontal resistance is a product of multiple underlying genes, that all have minor effects on the amount of resistance observed. This may explain, why quantitative resistance is observed to be pathogen-genotype independent in many cases. This in contrast to vertical resistance or also called qualitative resistance depends on a single major resistance (*R*) gene and is pathogen genotype dependent. Thus, plants with quantitative or horizontal resistance show a range of intermediate infection phenotypes but no complete resistance. Today the theories and concepts proposed by Vanderplank are well integrated in plant pathology and in use by plant disease epidemiologist in the field (Coutinho *et al.*, 2020).

For natural populations, knowledge on quantitative resistances is limited, when compared to agriculture. However, a number of species has been reported to show quantitative resistance in their natural populations against several pathogens as highlighted in a review by Laine *et al.* (2011). Burdon (1980) was one of the first to show in detail the differences in resistance to the fungal pathogen *Cymadothea trifolii* and *Pseudopeziza trifolii* in a population of white clover, *Trifolium repens,* with a significant difference observed among the least and most resistant genotypes. Another notable example is a study on two populations of *Senecio vulgaris*. The species was shown to have up to 10 different resistance phenotypes against the powdery mildew fungus *Erysiphe fischeri* within an area of 1 m^2 of the population (Bevan *et al.*, 1993). Some recent reports also highlight interesting aspects underlying the quantitative resistance observed. In the case of oak, *Quercus robur*, quantitative resistance to two exotic pathogens *Erysiphe alphitoides* and *Phytophthora cinnamomi* is shown to be present in naive populations, that had not previously been exposed to the pathogens and the resistance is suggested to be delivered by genes encoding proteins with extracellular signaling functions (Bartholomé *et al.*, 2020). A study on six natural genotypes of bittersweet, *Solanum dulcamara*, showed differences in tolerance and resistance against *P. infestans* and interestingly highlights a mechanism of overcompensation in

some genotypes with increase in shoot length and number of flowers in plants with higher disease levels (Masini *et al.*, 2019). Genetic variation in resistance can also be seen in plant-insect interactions. 16 genotypes of wild strawberry, *Fragaria vesca,* show high variation in tolerance to the herbivore *Spodoptera littoralis* but no effect of prior treatment with the important herbivore defence related phytohormone jasmonic acid were observed (Wang *et al.*, 2020). Ultimately, these studies highlight host genotype specific differences in resistance to biotic stress in wild plant species and show the potential of natural populations to be a valuable resource to explore and exploit quantitative resistance for evolutionary and economical aspects.

Here, we investigate phenotypic variation in resistance of the wild tomato species *Solanum chilense*. *S. chilense's* natural habitat is southern Peru and northern Chile. The demography of this species can be described by four clearly defined genotype groups based on four geographical regions: One northern, one central and two southern groups (Böndel *et al.*, 2015). The regions are geographically separated from each other and thus all have their own specific climate. As a result, the populations growing in these four regions are genetically different and abiotic stress adaptations have been reported for example for genes involved in cold- and drought-stress responses (Fischer *et al.*, 2013; Nosenko *et al.*, 2016; Böndel *et al.*, 2018). An advantage of working with *S. chilense* is that it is perennial. Hence, each plant represents a unique genotype from a distinct population but can be kept in the greenhouse and regularly cut back to maintain identical genetic material for years and comparable experiments.

The species has also been established as a genetic model for studies on plant-pathogen interactions. Some accessions of the species possess resistance genes against the fungal pathogen *Verticillium dahlia* or to tomato mottle virus (Tabaeizadeh *et al.*, 1999; Griffiths & Scott 2001). Genes of the *Pto* resistance pathway, conferring resistance to *Pseudomonas sp.,* are shown to be under balancing selection (Rose *et al.*, 2011). Stam and coworkers previously showed that also *R* genes of the nucleotide-binding site, leucine-rich repeat receptor (NLR) gene family show signatures of adaptation through the species. Using targeted resequencing of these important receptor genes they showed that it depends on the population whether *NLRs* are under positive or balancing selection and that only a few *NLRs* show consistent positive or balancing selection signatures through the species. This led to the conclusion that indeed *S. chilense* is a highly variable host species, but that some adaptations, also in pathogen defence-associated genes, depend on or even drive colonization of new habitats (Stam *et al.*, 2019a). Most recently, we

showed that genetic diversity in the species also has immediate effect on the observed resistance phenotypes of the host populations. Two specific *Cladosporium fulvum* resistance gene dependent responses show presence-absence variations between and within populations of the central geographical group. Interestingly, in this study we also found complete loss of major *R* gene mediated resistance responses in the southern part of the species range, which we hypothesize to be related to the extreme environments and probably absence of the pathogen in these regions (Kahlon *et al.*, 2020).

The presence and absence of these *R* genes results in qualitative differences in resistance. However, as mentioned above, many interactions in nature are of quantitative nature. Populations from geographically distinct locations in the *S. chilense* species range show such quantitative variation in the resistance against three common filamentous pathogens of tomatoes, *P. infestans*, *Alternaria solani* and a *Fusarium* species (Stam *et al.*, 2017). The resistance properties against these three pathogens do not follow geographic patterns, but instead show a mosaic of resistance quantities, which led to the conclusion that local pathogen pressures and separation of individual niches likely differ even within geographical regions.

P. infestans was choice of pathogen for this study. It is an oomycete belonging to the Peronosporaceae family and has large economical relevance (Kamoun *et al.*, 2015). It was the cause of the Irish potato famine and it causes significant losses as late blight pathogen on tomatoes and other *Solanaceae* species (Kaushal *et al.*, 2020). It was one of the first oomycetes to have its genome sequenced (Haas, 2009), making it one of the best studied oomycetes at the genomic level. *P. infestans* contains more than 500 genes coding for so-called effectors and for many of these effectors it has been shown that they are essential for infection. It is also well known that the effector repertoire varies between isolates, with complete presence-absence variations as well as point mutations occurring in important effector genes (Thilliez *et al.*, 2019). *P. infestans* occurs globally, with large variation between field populations: some being highly diverse, some being clonal, some following sexual and some asexual reproduction mode (Fry *et al.*, 2015). The origin of *P. infestans* has been either in the Andes (Gómez-Alpizar *et al.*, 2007, Martin *et al*., 2014) or in the Toluca Valley in Mexico (Goss *et al.*, 2014). *P. infestans* has likely been present in areas home to wild tomato species for a long time. A recent publication confirms that *P. infestans* can regularly be detected on wild potato and several tomato populations, growing in close proximity to cultivated potato fields in the Peruvian Andes (Lindqvist-Kreuze

et al., 2020). Where cultivated potato and tomato crops are decimated when infected with these *P. infestans* isolates, these wild plants show often limited infection symptoms. Infection lesions appear to be smaller and do not spread over the whole plant (Lindqvist-Kreuze *et al.*, 2020), suggesting a high level of quantitative resistance to be present in the wild plant populations.

To evaluate above hypothesised presence of quantitative resistance from former mentioned studies, in this study, we looked in detail at the phenotypic variation observed in natural populations of *S. chilense,* tested the quantitative resistance against various isolates of *P. infestans* and elucidated the role of plant genotype and the pathogen isolate in determining quantitative resistance.

Materials and methods

Plants material used and maintained

Seeds of nine accessions/populations of *S. chilense* (LA1958, LA1963, LA2747, LA2932, LA3111, LA3786, LA4107, LA4117A and LA4330.) were obtained from the C. M. Rick Tomato Genetics Resource Center of the University of California, Davis (TGRC UC-Davis, http://tgrc.ucdavis.edu/). Each population represents a random collection of seeds from a wild population and is propagated by TGRC in a way to maintain maximum genetic diversity. Thus, each of the populations used is genetically diverse. Each individual plant within a population is genetically unique and can be considered a unique genotype. For each population 9-10 plants were grown in controlled greenhouse conditions (16h light and 18-20^{0}C temperature). Mature plants of at least one-year-old were used in the study and were maintained throughout the study by bi-weekly cutting. The plants are perennial and can be maintained for a longer time and providing the benefit of allowing repeated testing of the same plants.

Pathogen used and maintained

P. infestans isolates EC1, 88096, 3928 and T-30.4 were obtained from Wageningen University, Pi100, Pi078, Pi054, were obtained from INRAE Avignon. All the isolates were maintained on Rye A Agar (Stam, 2016a) and were sub-cultured on Rye B Agar (Stam, 2016b) before experiments and incubated 9 days in the dark at 16^{0}C and 1 day in the light at room temperature. The sporangia were scratched from the plates after flooding with water, with the help of a pipette

tip and stored at 4^0C for 1-2 hours prior to infection assays. The final concentration used for the infection assays and pathogen phenotype assays were 3000 sporangia/ml.

Detached leaf infection assays with Pi100 isolate

The infection assays were performed on 85 plants representing nine populations of *S. chilense* using a drop inoculation method with Pi100 spores of *P. infestans* on the detached leaf as described in Stam *et al.* (2017). Leaves on our glass house plants differed in age and size. Therefore, the assays were performed in three biological replicates, each several weeks apart. For each biological replicate seven-eight randomly selected leaves were harvested per plant from each of the mother plants and mother plants were cut back between experiments. Surface sterilization of leaves with 70% ethanol was performed by dipping the leaves in the solution briefly, rinsing with water and gently dabbing with a dry tissue to remove the excessive water. The leaves, with the abaxial site facing upwards were then placed in plastic boxes (50x32.5x6.5cm), containing bedding of two layers of wet tissue paper. Drop inoculation was performed on the individual leaflets of each leaf and boxes were kept in the dark at 18-20^0C. Seven days post-inoculation, the infection frequency was recorded per individual leaf by calculating the ratio of the number of leaflets showing mycelial growth divided by the total number of leaflets inoculated.

Phenotyping different *P. infestans* isolates on culture medium and plants

To investigate the growth phenotype of these isolates, drop inoculations of the same sporangia solution used for the infections were done on Rye B Agar culture medium and incubated at 18-20^0C in the dark. Phenotyping was performed with up to five technical replicates and three biological replicates per strain. Each biological replicate was performed at least 10 days apart, with freshly propagated starter cultures. The diameter of the mycelial growth (in cm) was taken 10 days post-inoculation.

Additional infection assays were performed on the central population LA 3111 (nine plants) using the same drop inoculation method with 3000 sporangia/ml of seven isolates of *P. infestans* (Pi100, Pi078, Pi054, EC1, 88096, 3928 and T-30.4) on the detached leaves. The infection frequency was obtained from individual plants, using the same methodology as described above.

Data representation and Statistical analysis

Figures were made using the R package {ggplot2}, analysis of variance were performed using the function aov() in R software, version 3.4.4, (R core Team, 2020), with post hoc Tukey Test function TukeyHSD(), from the package {stats} and p-value was considered significant when lower than 0.05. Generalised linear Mixed Models (GLMM) were generated using glmer() from the package {lme4} (Bartes *et al.*, 2015), as shown in Stam *et al.* (2017) and we used the binomial variable (y) which consisted of number of successful and non-successful infection events per leaf, experimental date was used as a random effect in the both models.

Results

Quantitative resistance differences between populations are independent of the pathogen isolate

In previous study on *S. chilense* after inoculation with the *P. infestans* isolate - called EC1 originating from South America, phenotypic differences were observed between the populations (Stam *et al.*, 2017). We wanted to know whether populations of *S. chilense* possess similar quantitative resistance differences upon inoculation with a very aggressive *P. infestans* isolate - Pi100, originating from *Solanum lycopersicum* in Europe. Every infection event has a certain amount of stochasticity, as spore germination rates are not uniform, even under optimal conditions (Minogue and Fry, 1981). To reduce the variance caused by this effect, we performed a minimum of 1588 drop inoculations per population. This number accumulates from a total of three independent experiments with inoculation of all individual leaflets of each seven to eight leaves from nine to ten individual plants per population.

We observed differences in quantitative resistance in the different populations upon infection with *P. infestans* isolate Pi100 (Figure 1). In our study, all plants have been infected. ANOVA with post hoc Tukey honest significant difference tests, show significant differences between populations in 23 out of 36 pairwise comparisons (Table S1). We calculated the Pearson's correlation coefficient between the overlapping population's mean score of infection frequency from data EC1 (Stam *et al.*, 2017) and our new data Pi100. This coefficient of 0.724162 indicates a positive correlation between the two data sets. Due to a limited sample size (n=6 overlapping populations) we cannot reasonably judge the significance of this correlation. We further had up

to six non overlapping plants per population between the experiments accounting for additional variance within our samples. However these results suggest that the variation in the infection frequency is the result of genetic differences in the host plants.

Individual plant genotypes appear to drive resistance against single *P. infestans* isolate

Next, we wanted to know whether the observed variance in the infection frequencies (Figure 1) is intrinsic to the assays or to the population as whole, or whether significant differences between the individual plants in the population contribute to the large variance.

To evaluate the infection frequency within the population, we separated the data of the drop inoculation on all detached leaves (Figure 1) into infection frequency for each of the individual plants (Figure 2). We observed high variance in the successful infection frequency in individual plants. In the majority of cases (68% of the plants) this variance was smaller than the variance observed for the population as a whole (Table 1 and S2). This indicates that the differences in successful infection of *P. infestans* can at least partly be attributed to genetic differences within the host populations, however other factors beyond our control cannot be disregarded in this outcome (i.e. physiological differences between individual leaf explants from perennial plants).

To test whether the observed differences in infection frequency for each of the plants are statistically significant, we performed an ANOVA, with post hoc Tukey honest significant difference test for all plant-plant comparisons within a population (Table S3). For each population, 40 pairwise comparisons were evaluated (amounting to 360 comparisons), out of which 68 showed to be significantly different at $p<0.05$. The number of significantly different comparisons varies between the populations. Most frequent significant differences were found within the populations LA1963 (18) followed by LA3111 (8), LA3786 (8), LA2932 (8), LA4107 (7), LA4117A (7), LA4330 (7), and LA1958 (5), whereas LA 2747 showed no significant difference in the plants' pairwise comparison. This confirmed that the plant genotypes within populations showed different resistance phenotypes against a single isolate of *P. infestans*.

Growth of different isolates of *P. infestans* on culture medium is significantly different

To elaborate on our first indication that the differences in the plant's resistance are similar irrespective of the isolate of *P. infestans*, we aimed at infection our plants with several genetically diverse isolates. To first judge general differences of those seven isolates apart from

the host plant, we evaluated their growth rate *in vitro*. We used *P. infestans* isolates with known differences in effector diversity, originating from different geographical locations and different hosts. To measure phenotypic growth differences, we performed drop inoculation of sporangia solution (3000/ml) of the *Phytophthora* isolates on the culture medium Rye B Agar B (Figure 3) and measured the radial outgrowth. The outgrowth of the isolates was significantly different for 66.67% of the pairwise comparisons confirming growth differences of these isolates on the culture medium (14 pairwise comparisons out of 21 pairwise comparisons - ANOVA, with post hoc Tukey honest significant difference test, Table S4).

Infection with seven different isolates of *P. infestans* supports host genotype dependency

Knowing that the selected isolates are genetically and phenotypically different, we further evaluated infection frequency of the seven isolates of *P. infestans* on the detached leaves from the nine individual plants of the central population LA3111.We observed that the plant showing the highest resistance (i.e. the lowest infection frequency), upon inoculation with Pi100 (Plant 11) is always the most resistant irrespective of the *P. infestans* isolate used. Similarly, the plant showing the lowest resistance (i.e. the highest infection frequency) to Pi100 (Plant 10) is always the least resistant irrespective of the isolate used (Figure 4).

To test if we observe an effect of the pathogen isolate on the infection frequency on plant genotypes, we performed ANOVA, with post hoc Tukey honest significant difference tests and found 10.05% of the pairwise comparisons to be significantly different (Table S5). To ensure that these observed differences in infection frequency do not correspond to a general difference in growth rate of the *P. infestans* isolates, we compared the mean growth of *P. infestans* isolates on the plates with the mean of infection frequency of these isolates on the plants. We saw no correlation upon evaluation with a Pearson's correlation test (coefficient of -0.064, n=7).

Finally upon testing the effect of the plant genotype and the pathogen isolate on the infection frequency we found both significant but independent (multi-way ANOVA) (Table 2). This supports that no clear effect exists of the interaction between specific plant genotypes and specific isolates, as would be expected for qualitative, race-specific resistance, but rather independent effects on infection frequency. We performed a GLMM model using the inoculation date as random effect and as expected, the additive model (Plant + Pathogen) provides the best

fit (AIC and BIC criteria), while the plant genotype explains most of the differences in infection frequency (Table 3).

Discussion

S. chilense is known for its resistance properties against various pathogens (Tabaeizadeh *et al.*, 1999; Griffiths & Scott, 2001; Ji *et al.*, 2007; Verlaan *et al.*, 2013). It has also been studied extensively in relation to habitat adaptation (Xia *et al.*, 2010; Fischer *et al.*, 2013; Nosenko *et al.*, 2016; Böndel *et al.*, 2018) and as a model for the evolution of pathogen defence mechanisms in plants (Stam *et al.*, 2019a, Stam *et al.*, 2019b, Kahlon *et al.*, 2020). Previously, *S. chilense* populations from different geographical regions were shown to express different levels of resistance against three filamentous tomato pathogens (Stam *et al.*, 2017). In this report, we further characterize the resistance in this species against the oomycete pathogen *P. infestans* as quantitative resistance which is largely determined by host genotypes.

In our previous work, we found significant differences in resistance properties of the different *S. chilense* populations but also had some very important remaining questions (Stam *et al.*, 2017). We observed a large amount of variation in the infection success rate for each of the populations – in some populations ranging from 0/10 infections on one leaf to 10/10 on another leaf. This left the question of whether infection frequency is generally variable and spore germination of *P. infestans* on *S. chilense* is strongly affected by stochastic effects. Using a different *P. infestans* isolate as in our previous study and studying a very high number of infection events, we again observed a high amount of variance within the populations, but also significant differences between the populations (Figure 1) that correlated with our previous results (Stam *et al.*, 2017). The variance was reduced when looking into individual plants per population (Figure 2), which suggests that the resistance outcome is at least in part dependent on the plant genotype under consideration. One possible explanation for the observed variance between plants is the age and morphology of the used leaves, as older, thicker leaves might be harder to penetrate by the pathogen. We minimized this effect by regularly cutting back our plants and randomly selecting leaves for infection, but it remains possible that the selected leaves from some plants were less uniform than for other plants, because *S chilense* is known to be a morphologically diverse species, with large variation between and within populations (Raduski and Igić, 2020) and many

317 quantitative resistance phenomena have been described to be age and physiology-dependent
318 (Niks *et al.*, 2015). We also observed high variability of infection frequency on individual leaves
319 from the same population or even from the same plant. This may have limited the number of
320 statistical significant differences between our genotypes. However, due to the high number of
321 repetitions, we consider these results robust and valuable. They reflect not just a single
322 developmental stage or specific leaf level as considered in most model plant experiments but
323 rather the spectrum of pathogen resistance responses in each given plant. That within individual
324 plants the variance observed was still high indicates other effects as well. These might include
325 stochastic effects affecting spore germination rates. Such variation in spore germination rate is
326 commonly observed with *P. infestans,* also under conductive laboratory conditions (Minogue
327 and Fry, 1981) and infections of wild and cultivated tomato with the pathogen *A. solani* also
328 often resulted in no visible lesions on some of the leaves of generally susceptible accessions
329 (Chaerani *et al*., 2005).
330 Another limitation of our previous study was that we tested only a single isolate of *P. infestans*.
331 It is well-known that *P. infestans* strains are genetically diverse, with complete presence-absence
332 polymorphisms of certain key virulence effector genes (Thilliez*et al.*, 2019). Also, different
333 isolates of *P. infestans* have been shown to be tremendously different in their aggressiveness on a
334 single host (Cooke *et al.*, 2012). Testing a single isolate prevented drawing general conclusions
335 on the observed resistance in *S. chilense*, as outcomes could have been isolate specific.
336 To address this, we looked into the infection frequency of the central population upon
337 inoculation with seven different isolates of *P. infestans*. The infection frequency for most
338 resistant and least resistant plant showed similar outcome regardless of the *P. infestans* isolate
339 used (Figure 4). Looking into the growth phenotype of these isolates on culture medium (Figure
340 3) and correlating it to infection frequency on plant it becomes evident that there is no correlation
341 in infection frequency on plants and radial growth on plates. We further confirmed the larger role
342 of plant genotype over pathogen isolate with GLMM. Multiway ANOVA showed no role of
343 interactions between certain plant genotype with certain pathogen isolate in resistance outcome.
344 Indicating to that the observed quantitative resistance in our study is not the result of mutual
345 adaptation. Thus, the differences observed in our infection assays are more clearly defined by the
346 plant genotype than the pathogen isolate although we observed influence of pathogen isolate
347 (Table 2&3). This does not need to depend on isolate germination and growth speed on plate, but

348 can be related to various other factors that contribute to the general aggressiveness of individual
349 isolates (Pariaud *et al.*, 2009).
350 Even though we used 7 different isolates, from different hosts, we used only one Latin American
351 isolate from a clonal lineage that is spreading throughout Peru (Lindvist-Kreuze *et al.*, 2020).
352 Therefore, there might still be a possibility that adapted pathogens (e.g. collected directly from *S.*
353 *chilense*) would show different results. As shown by Thrall and Burdon, (2003) in more resistant
354 flax populations more virulent rust was observed. This mechanism is also shown in resistant
355 plants of *Plantago lanceolata* and powdery mildew *Podosphaera plantaginis* (Laine, 2005).
356 However, field observations by Lindqvist-Kreuze *et al.*, 2020 and our own observations from
357 field visits in Peru and Chile suggest that in nature, *S. chilense* is rarely visibly infected with *P.*
358 *infestans* and the pathogen was to our knowledge never successfully isolated from plants from
359 southern populations. Thus we expect relatively little influence of specific local adaptations and
360 hypothesize that our results represent general defence differences that are potentially functional
361 against a wide array of pathogens. The absence of strong signatures of local adaptation to a
362 pathogen is also shown in case of 12 species of wild potatoes against 13 populations of the
363 nematode *Globodera pallia.* Similar as in our study host genotype was the deciding factor in
364 resistance rather than the pathogen isolate under consideration (Gautier *et al.*, 2020). Such basal
365 resistances have been shown to be prevalent in the plant kingdom and are expected to be based
366 on more conserved, or core, defence mechanisms, like the recognition of microbe-/pathogen-
367 associated molecular patterns (M/PAMPS). The presence of basal resistance properties, which is
368 sometimes also described as exapted resistance, (e.g. resistance that is present in a plant, without
369 recent adaptation to the pathogen: Newcombe, 1988), has been studied in the context of invasive
370 *Phytophthora* species on several hosts. In case of Norway spruce, *Picea abies,* and Nordmann
371 fir, *Abies nordmanniana,* in Swedish Christmas tree farms, which currently are not under threat
372 by *Phytophthora,* it has been shown that after inoculation with different isolates of *Phytophthora*
373 sp. (isolated from water and soil samples from the same region), all the seedlings of these plants
374 showed a basal level of resistance under laboratory conditions (Pettersson *et al.*, 2018). A study
375 on 72 trees of black alder, *Alnus glutinosa* from 12 different sites (Redondo *et al.,* 2020), showed
376 that all the plants possess a basal level of resistance against *Phytophthora uniformis* before
377 invasion by the pathogen, but also that seedlings from pathogen-invaded locations were more
378 resistant than seedlings from uninvaded locations. Exapted resistance is also shown in the case of

379 powdery mildew, *Erysiphe alphitoides,* and *Phytophthora cinnamomi* in European oak (*Quercus*
380 *rubur*) where multiple genes were shown to be responsible for the variation in resistance between
381 the trees of naive, not previously infected, populations (Bartholomé *et al.*, 2020).
382 The molecular mechanisms underlying the observed phenotypes in our study remain thus far
383 unknown. We did not observe any host genotype-pathogen isolate combination that led to
384 complete resistance or showed hypersensitive reactions, suggesting that no major *R* genes against
385 the used isolates are active in those populations. It has been shown that the perception of
386 conserved M/PAMPs can differ within and between wild tomato species (Roberts *et al.*, 2019).
387 The mechanisms of resistance can also vary within a species as in case of natural accessions of
388 *Arabidopsis thaliana* where few accessions showed resistant against the bacterial pathogen
389 *Pseudomonas syringae* pv. tomato (*Pst*) DC3000. Resistance mechanisms, such as elevated
390 phytohormone levels or the presence of leaf surface barriers, were shown to differ between the
391 accessions (Velásquez *et al.*, 2017). Differences in defence activation within the species are also
392 shown against a generalist herbivore African cotton leafworm, *Spodoptera littoralis,* on the host
393 plant Arugula, *Eruca sativa*, where the activated defence mechanism was associated with
394 geographical origin of the population (Organ *et al.*, 2016).
395 In a study with a panel of six domesticated and six wild tomato accessions, infection severity of
396 97 *Botrytis cinerea* isolates was shown to be determined by complexity of both host and
397 pathogen genotype and a few pathogen genes associated with host preferences (Soltis *et al.*,
398 2019). Similar multigenic effects has been indicated in a genome-wide association mapping
399 analysis of 88 genotypes of quinoa, *Chenopodium quinoa,* showing large variation in disease
400 traits upon inoculation with one isolate of the downy mildew pathogen *Peronospora variabilis*
401 (Colque-Little *et al.*, 2021). With the recent availability of a reference genome sequence for *S.*
402 *chilense* (Stam et al. 2019b), studies to identify the multiple genes underlying the observed
403 differences in quantitative resistance will be the next step.
404 Our findings show an example of quantitative resistance in *S. chilense,* a plant species where we
405 earlier reported qualitative resistance against another pathogen (Kahlon *et al*., 2020). We show
406 that this quantitative variation exists between and within the natural populations of the species
407 and is mainly driven by the plant genotype. The resistance can potentially be defined as exapted
408 resistance as it appears to protect against non-local, un-adapted pathogen isolates. Overall we
409 show that the plants of the wild tomato species *S. chilense* have their own unique resistance

properties and that the species is amenable for detailed studies on quantitative resistance in the lab. In future studies it would also be interesting to look into structural and molecular factors underlying these quantitative differences and possibly associate this with genetic differences.

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576 **Tables and Figures**

577 **Table 1: Variance observed in infection frequency of overall populations and number of**
578 **plants with high and low variance as compared to overall population**

Population	Variance in infection frequency	Number of plants with higher variance than overall population	Number of plants with lower variance than overall population	Number of plants tested
LA3786	0.0815094	4	6	10
LA1963	0.044541	2	8	10
LA1958	0.047965	3	6	9
LA2747	0.0615848	4	5	9
LA3111	0.0605912	3	6	9
LA2932	0.0441113	3	7	10
LA4107	0.0828558	4	5	9
LA4330	0.0375442	3	7	10
LA4117A	0.0367892	2	7	9

579

580 **Table 2: Multiway ANOVA test outcome to evaluate effect of interaction of plant genotype**
581 **of central population LA3111 and *P. infestans* isolate on infection frequency.**

Component	*Df*	*F*-value	*p*-value
Plant	8	21.971	<2e-16
Isolate	6	22.794	<2e-16
Plant:Isolate	48	1.179	0.19

582 Table 3: GLMM showing plant genotype and pathogen isolate effect on infection frequency
583 observed in the central population LA3111 upon inoculation with 7 isolates of *P. infestans*. The
584 lower AIC and BIC predicts a better fitting model. To construct the models we used the binomial
585 variable (y) which is infection outcome, random effect considered in all models was date of the
586 infection.

Model	*AIC*	*BIC*
y~ Plant	4881.3	4934.0
y~ Isolate	4981.0	5023.1
y~ Plant+Pathogen	4548.5	4632.8

587

Figure 1: Infection frequencies in different populations upon inoculation with *P. infestans* isolate Pi100.

The box plots show the median of the infection frequency of a leaf which is the ratio of infected leaflets over total inoculated leaflets. Each population consisted of 9-10 plants. The assay was performed on three separate dates, each time with seven to eight leaves for each individual plant. Each data point indicates the infection frequency of an individual leaf obtained from inoculations of up to 14 leaflets per leaf. The Y-axis shows infection frequency ranging from 0 (no infected leaflets on a leaf) to 1 (all leaflets show infection). The colors represent the geographic regions of the population.

Figure 2: Evaluation of infection frequency in individual plants from different populations. Each individual facet shows different populations (as tested in Figure 1) each box plot shows a single plant tested for infection phenotype. Experiments were performed on three separate dates (with an exception for 1 plant only on two dates), each time with 8 leaves per plant (with an exception in 2 plants with 7 leaves per plant). Y-axis represents infection frequency and X-axis represents different plants; color shows the geographical region of the population (as in Figure 1).

Figure 3: Growth of different isolates of *P. infestans* on culture medium.

The radial plot shows the outgrowth of mycelia (in cm) of different isolates of *P. infestans* 10 days post drop inoculation on Rye B agar medium. Different colors indicate different isolates of *P. infestans*.

Figure 4: Infection frequency of 7 different isolates of *P. infestans* on plants within population LA3111.

Each facet shows an individual plant of the population LA3111, the box plot represents the infection fraction of plants when inoculated with seven isolates of *P. infestans* depicted in different colors. Y-axis represents infection frequency (0-1 as in Figures 1 and 2) and X-axis represents different *P. infestans* isolates.

Data Accessibility Statement

Raw Data and R scripts are available as supplements

Author contributions

Conceptualization: RS, PSK and RH; Investigation: PSK; Data interpretation and evaluation PSK, MV, RH and RS; Writing: PSK and RS. All authors critically reviewed and approved the manuscript.

Conflicts of interests

The authors declare that no competing interests exist.

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