Tree Genetics & Genomes (2020) 16:1
https://doi.org/10.1007/s11295-019-1396-8

ORIGINAL ARTICLE

Widely distributed variation in tolerance to *Phytophthora palmivora* in four genetic groups of cacao

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Received: 15 May 2019 / Revised: 22 October 2019 / Accepted: 23 October 2019

Abstract

The tropical tree *Theobroma cacao* is the source of chocolate, and its seeds are a major export from many producing countries in Central and South America, Africa, and Asia. Every year, 30–40% of pre-harvest yield is lost due to disease damage. Host plant resistance is the most efficient and environmentally friendly approach for disease management. Historically, cacao germplasm resources have been underutilized in efforts to introduce novel sources of disease tolerance into breeding programs. Maintenance of cacao germplasm also relies on clonally propagated live collections, as cacao seeds do not exhibit dormancy and cannot be stored for more than a few weeks. In this study, we use a 90 SNP array to verify genetic identity of a set of clones in the International Cocoa Collection at CATIE, Costa Rica, and assign the clones into known genetic groups. We also used a detached leaf inoculation technique to measure the susceptibility of 60 genotypes to *Phytophthora palmivora,* a major cacao pathogen with global importance. We identified 24 genotypes with disease tolerance statistically similar to a standard tolerant variety (SCA6) and another 24 which performed similarly to a standard susceptible variety (ICS1). Our results indicate that each of the four included genetic groups show variability for quantitative resistance to *P. palmivora.* These results provide a foundation for future genomic and transcriptomic analysis of disease tolerance and susceptibility in the field at CATIE and provide guidelines for breeders searching for novel sources of tolerance that can be introduced into breeding programs.

Keywords *Theobroma cacao* · *Phytophthora* · Resistance · Genetics

Introduction

Cacao, *Theobroma cacao L.*, is endemic to the Amazon rainforest and is the source of cocoa (Wood and Lass 2008). Exploration of the New World by Europeans led to the species becoming a global cash crop, which is today an important in many developing countries and is the centerpiece of a multi-billion dollar chocolate industry. As the plant was dispersed throughout the tropics in the eighteenth and nineteenth centuries, originally only a few cultivars were transplanted (a

Communicated by A. M. Dandekar

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11295-019-1396-8) contains supplementary material, which is available to authorized users.

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Published online: 05 December 2019

history of cacao germplasm utilization is presented in (Zhang and Motilal 2016)). When new viral, fungal, and oomycete pests arose and threatened production, expeditions for discovery of new germplasm and breeding efforts were undertaken to identify, develop, and distribute more robust varieties. However, lack of genetic diversity still limits development of superior varieties. The total genetic diversity in collections and seed gardens in Cameroon, Ghana, and Nigeria can be traced back to about ten main progenitors which were introduced more than 70 years ago. Genomic analysis of cacao also shows a strong signature of a bottleneck in genetic diversity resulting from domestication (Cornejo et al. 2018). In South America, renewed effort is being used to collect wild accessions and identify useful germplasm. At the same time, thorough characterization of cacao germplasm in collections is essential for reliable discovery of novel sources of resistance/tolerance to biotic and abiotic stresses tolerance which can be introduced into breeding programs (Bailey and Meinhardt 2016).

Until roughly the past decade, cacao genetic material was classified broadly using three groups: Criollo, plants from central America considered to produce fine-flavor cocoa; Amelonado, plants from the Amazon which were more widely used in chocolate production and which were initially transplanted onto African farms; and Trinitario, naturally occurring hybrids of Criollo and Amelonado (Motamayor et al. 2003; Zhang and Motilal 2016). As SSR and SNP markers were applied to studying cacao genetics, the understanding of the genetic structure was refined to comprise 10 distinct populations or genetic groups (Motamayor et al. 2008; Zhang et al. 2009) and additional populations were subsequently added as more collections were made (Zhang et al. 2012). Two annotated cacao genome sequences are now publicly available (Argout et al. 2011; Motamayor et al. 2013; Argout et al. 2017), one from the Criollo group and one from the Amelonado group. An additional 200 cacao genomes have been sequenced, a set that includes individuals representing all genetic groups (Cornejo et al. 2018). Availability of these genomes and genome-scale technologies enables powerful new strategies for functional genomics. A high-density SNP array was used to perform genome-wide association mapping to identify loci and particular genes linked to quantitative resistance to frosty pod rot and witches' broom, caused by fungi *Moniliophthora roreri* and *M. perniciosa*, respectively, and black pod rot, caused by oomycete *Phytophthora* spp. (Romero Navarro et al. 2017; McElroy et al. 2018). Application of these techniques to underutilized cacao genetic material will aid in the discovery of novel sources of disease tolerance and continue to enhance the ability to breed for improved plant health and for other traits of economic interest.

Disease prevalence imposes significant losses on the world's cocoa supply, with 30–40% of the crop destroyed before harvest annually (Bailey and Meinhardt 2016). While different regions are host to different pathogens, the oomycete *Phytophthora* spp. are a global threat. Strains of *P. palmivora* are present throughout cacao's area of cultivation and these alone account for 20–30% of the crop's losses (Flood et al. 2004). *Phytophthora megakarya* is a particularly aggressive species which is currently only present in West Africa (Bailey et al. 2016). *Phytophthora capsici, P. tropicalis, P. heveae* and *P. citrophthora* strains are present in Central and South America (Bowers et al. 2001). Reports of disease tolerance traits and field performance in specific cacao genotypes can be quite variable, which likely reflects both different pathogen strains in different locations and pathogen effects being environmentally dependent (Turnbull and Hadley 2013).

To date, no gene-for-gene mechanism has been described that imparts qualitative disease resistance in cacao for any of its pathogens. Therefore, "resistant" lines used in breeding programs and QTL mapping populations likely exhibit polygenic, quantitative resistance, also termed tolerance. Measurements of the spectrum of disease tolerance to susceptibility in cacao rely on measurements of response to artificial inoculation of leaves (e.g., (Nyassé et al. 1995; Paulin et al. 2008; Thevenin et al. 2012; Lachenaud et al. 2015)) or pods (e.g., Iwaro et al. 2005) and evaluation of disease prevalence in the field (e.g., Pokou et al. 2014). These metrics are generally highly correlated, (Nyassé et al. 1995; Efombagn et al. 2011; Nyadanu et al. 2012), supporting the use of leaf-based assays as a proxy for pod tolerance and field performance.

Application of these screening strategies has advanced the identification of cacao genotypes with disease tolerance (Tahi et al. 2000; Iwaro et al. 2003, 2005; Thevenin et al. 2012; Barreto et al. 2015; Ling et al. 2017). However, there is still a lack of consensus in repeatably and reliably identifying sources of resistance, partially due to mislabeling of germplasm (Surujdeo-Maharaj et al. 2016). Moreover, the genetic basis of differences in quantitative resistance, within and between cacao populations, has yet to be understood. To develop a new scientific approach for utilization of untapped wild cacao germplasm for disease resistance, we propose to use genomics and bioinformatic analyses to identify candidate loci for black pod resistance. This approach will use genomic and RNA-Seq analyses in accessions with varying pathogen resistance to reveal polymorphism in coding genes and transcriptional responses to pathogen inoculation. As the first step of this project, our objective in the present study is to generate reliable phenotypic data of black pod resistance in different wild cacao populations, using standardized phenotyping method. We initiated a thorough curation of the response of diverse genotypes in the CATIE cacao collection to a particular strain of *P. palmivora*, creating a dataset that will act as a reference for future transcriptomic analysis of the defense response in trees at the CATIE collection. In this study, we used SNP genotyping to verify the identity of 187 trees in CATIE collection, which represent 60 cacao accessions in four genetic

groups: Guiana, Iquitos, Marañón, and Nanay. We used a detached leaf phenotyping assay (protocol described in (Fister et al. 2016)) to measure susceptibility of 60 clones, and we distinguished tolerant and susceptible genotypes belonging to each genetic group. Improved understanding of novel sources of disease resistance described here will be a useful resource for cacao breeders and improve genetic diversity within the crop.

Materials and Methods

Genotype selections in the CATIE collection and tree tagging

Leaf samples were collected from trees at the La Montaña field site at the CATIE field station in Turrialba, Costa Rica. Plants used were tagged with barcodes and GPS coordinates were taken for each tree (Supplemental Table S1). We evaluated a subset of the cacao germplasm maintained at IC3 focused primarily on four genetic groups (Guiana, Iquitos, Marañon, and Nanay). Clone names generally are comprised of an alphabetic prefix indicating where clones were collected and a numeral to differentiate from other accessions within the population. We also included four genotypes of interest outside of these genetic groups: Scavina 6 (SCA6), a known source of broad-spectrum disease tolerance (Thevenin et al. 2012; Royaert et al. 2016), Imperial College Selection 1 (ICS1), a Trinitario hybrid known to be highly disease susceptible, and CATIE R4 and R6, two recently developed hybrid genotypes with strong tolerance of the true fungal disease *Moniliophthora roreri*, but which are susceptible and moderately tolerant, respectively, to *Phytophthora* pod rot (Phillips et al. 2009). The majority of genotypes were represented by three clonally propagated trees, some with two, and NA 149 with only one.

SNP genotyping and identity verification of cacao clones

Mature (stage E) leaves (Mejia et al. 2012; Fister et al. 2016) were collected into plastic, resealable bags with desiccating silica gel beads and transported to the USDA-ARS in Beltsville, Maryland for genotyping analysis. DNA was extracted as previously described, and genotyping was performed using a set of 90 SNPs with a 96.96 Dynamic Array™ IFC (Fluidigm, San Francisco, CA). These SNPs were selected from a larger set of 1560 SNPs developed from EST sequences (Argout et al. 2008), and have been used previously to analyze cacao genetic ancestry (Ji et al. 2013; Fang et al. 2014; Lukman et al. 2014; Lindo et al. 2018).

Genotypic data was analyzed to validate identity and determine genetic membership of the sampled trees. Firstly, the clonality (or intra-clone mislabelling) among the multiple individual trees was verified using pairwise multi-locus matching, as implemented in the computer program GenAlex 6.503 (Peakall and Smouse 2006, 2012). Samples with SNP profiles fully matched at all genotyped SNP loci were declared the same genotype. For a subset of cacao accessions, reference genotype data is available, which was generated from the cacao trees maintained in Marper Farm, Trinidad. These trees have been used by cacao researchers as the original trees for most of the Upper Amazon Forastero germplasm (Bartley 2005; Zhang and Motilal 2016). Genotype data of trees sampled at CATIE were compared with these reference data, in all cases where it was available. The genetic integrity of the experimental accessions was also assessed by checking their population memberships. An assignment test for the experimental clones was performed using model-based Bayesian cluster analysis software STRUCTURE v2.3.4 (Pritchard et al. 2000). The analysis included SNP sets representing 10 distinctive cacao germplasm groups, which served as reference material (Motamayor et al. 2008). The full list of cacao accessions representing these 10 groups is presented in Supplemental Table S2. To ensure that the assignment tests were not affected by the sample size of the tested accessions, the sample size of each of the 10 germplasm groups was brought up to 200 using the SIMULATION procedure implemented in the computer program ONCOR (Kalinowski et al. 2007). The simulated populations were then analyzed together with the selected clones from CATIE. An admixed model was selected and the number of clusters (K value) was set to 10. Five independent runs were assessed for each K. All runs were carried out using 50,000 iterations after a burn-in period of 50,000. From the five independent runs, the highest Ln Pr (X|K) value was chosen and presented as bar plots for this experiment.

The genetic relationships among the accessions were analyzed using principal coordinates analysis (PCoA) in GenAlEx v 6.503 (Peakall and Smouse 2006, 2012). One individual per genotype, typically the individual with the least or no missing data, was used in the PCoA. To perform the PCoA, the 90 SNPs were first used to generate a genetic distance matrix using the "codominant-genotypic" setting for distance calculation. The PCoA was subsequently performed using the "covariance-standardized" method.

Phytophthora palmivora growth conditions

Phytophthora cultures of isolate C-14 were prepared as described in (Mejia et al. 2012). Briefly, agar plugs from a mature culture were transferred to new 20% V8 medium. Inoculated plates were kept at 26 °C for 2 days with a 12 h:12 h day/night cycle, with daytime light intensity of 60 μmol m^{-2} s^{-1} and darkness at night. After 2 days of growth, cultures were inspected to ensure typical colony

morphology and that no bacterial or fungal contamination appeared.

Leaf sampling

Leaves were sampled at CATIE over the course of 9 months, March through November, of 2017. We collected leaves between 7:00 and 9:00 AM. Stage C leaves, which are expanded and bronze to light green in color, but still supple, were collected by cutting the petiole and transferring the bag to a plastic bag containing a damp paper towel, which maintained humidity within the bag as previously described (Fister et al. 2016). Leaves were inspected for damage or symptoms of disease, and only healthy, undamaged leaves were collected. Leaves collected from any replicate trees were treated as the same genotype, except in cases where offtypes were identified, but the individual tree the leaf was selected from was also tracked to monitor tree-to-tree effects. After returning to the lab, leaves were washed with tap water, 2–3 cm at the tip and base of each leaf were removed with a scalpel, and the remaining middle section of each leaf was transferred to a petri dish containing a wet paper towel and filter paper (Fister et al. 2016) to prepare for inoculation. At the end of the season, each genotype was sampled a minimum of 10 times.

Inoculation of leaves

Over the 9-month sampling period, a total of 1250 leaf inoculations were performed. Leaves were inoculated as described in (Fister et al. 2016). In short, three agar plugs containing mycelia from 2-day-old *P. palmivora* cultures were transferred to the left, abaxial side of a leaf. To ensure virulence of the inoculum, plugs are always selected from the outer edge of the expanding mycelial growth. Any *Phytophthora* cultures exhibiting abnormal growth are discarded. Three agar plugs without mycelia were transferred to the right abaxial side of the leaf to control for ability of the media alone to produce a lesion. After transferring the agar plugs, leaves were misted with ~ 2 ml of water from an atomizer, and the plates were sealed with parafilm. Plates containing leaves were transferred to an incubator at kept at 26 °C for 2 days with a 12 h:12 h day/night cycle with daytime light intensity of 60 μmol m^{-2} s^{-1} and darkness at night. Leaves were photographed 48 h after inoculation, and photos were used to quantify lesion size. Lesions were defined as brown to black necrotic areas centered around agar plugs carrying mycelia. Lesions sizes were calculated by measuring four radii from the center of the lesion to its perimeter, and the four radii were averaged to calculate an average radius for each lesion. The average radius per lesion was calculated for each of the lesions on each leaf section, and this per-leaf average lesion radius was considered a biological replicate. Over the sampling period, each genotype was sampled at least ten times and on at least three dates. Genotypes that could not be sampled to this depth were excluded from subsequent analysis (NA149 and KER 3).

Statistical analysis

Statistical analysis was performed using SAS (JMP Pro 13, SAS Institute Inc., Cary, NC, 1989–2007). A mixed linear model analysis (JMP®, Version *13*. SAS Institute Inc., Cary, NC, 1989–2018) was conducted, treating genotype as a fixed effect, with likelihood ratio tests for random effects, which included date of inoculation and tree. To generate a connected letter analysis of statistical differences, a Fisher's LSD test was applied.

Results and discussion

Assessment of genetic integrity and population membership

Samples collected from trees at IC3 were genotyped using a previously described set of 90 SNPs (Cosme et al. 2016), and pairwise multilocus matching of the SNP profiles within each clone revealed a high rate of consistency, based on the 90 SNP markers. No intra-clone mislabelling was found among the experimental materials except one case (IMC 97, Tree 3). From each confirmed clone, one tree with the least missing loci was retained for subsequent analysis.

For a subset of trees, we were able to compare SNP data from CATIE to that from reference trees of the same genotypes held at the International Cocoa Genebank in Trinidad. We identified five of these as off-type, where all replicate clones of genotypes in the IC3 collection did not match the corresponding reference SNP profile (Supplemental Table S3), likely due to mislabeling in the collection. By comparing to reference SNP data, we were able to determine that one of these mislabeled genotypes, called NA 246 at CATIE, is likely a clone of the PA 3 genotype. The retained samples with unique SNP profiles were used to perform a principal coordinate analysis of the four populations of interest (Fig. 1). The first three principal coordinates explain 28.3%, 12.2%, and 8.7% of variation, respectively. The first two appear to correlate with longitude and latitude: genotypes in the Nanay and Iquitos groups have been collected from Loreto in northern Peru, the Guiana group was collected in French Guiana, and the Marañon group includes individuals from western Brazil (Motamayor et al. 2008). This observation is consistent with a spatial map of the ancestral lineages (Motamayor et al. 2008), and is consistent with a recent analysis of 200 cacao genomes which described the Western Amazon as the likely center of origin, and differentiation of most genetic groups according to latitudinal geographic distance (Cornejo et al. 2018). The PCoA also highlights two cases (PA 150 and NA 246/PA 3) where mislabeled trees

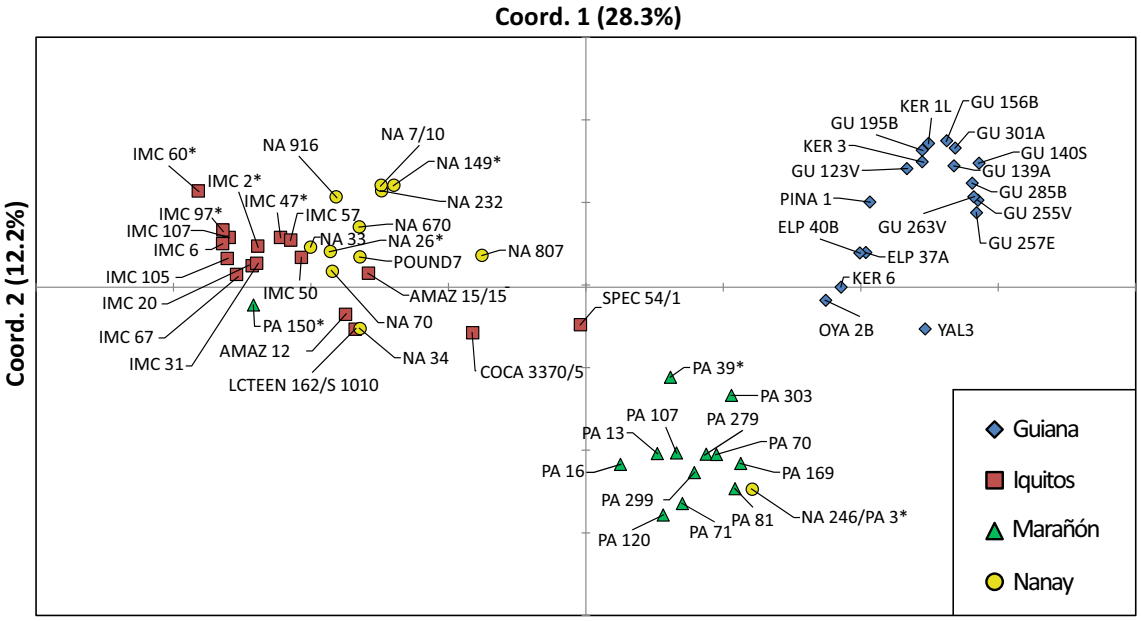


Fig. 1 Principal coordinates analysis of genotypes in populations of interest. Coord. 1 and 2 represent the first two principal coordinates identified by the analysis; percentages indicate proportion of variation explained by the coordinates. Genotypes are color-coded according to previously described genetic group membership (Motamayor et al. 2008). Genotypes marked with an asterisk were found to be offtype by comparing to a reference SNP profile

cluster with incorrect genetic groups. The PA 3 genotype does belong to the Marañon genetic group, resolving this inconsistency.

The result of assignment test for genetic group membership was consistent with the observation in PCoA (Supplemental Table S4). Generally, our analysis confirmed previous genetic group assignments (Motamayor et al. 2008). The assignment test also supported our identification of mislabeled trees: the results suggest that NA 246 belongs to the Marañón group, PA 150 belongs to the Iquitos group, and PA 39 belongs to the Amelonado group. Several accessions were found having a relatively low membership in the previously reported genetic groups, with their Q-value ranged from 0.452 to 0.635 (Supplemental Table 4). These accessions included AMAZ 12, LCTEEN 162/5 1010, COCA 3370/5, OYA 2B, and SPEC 54/1, all of which had a 'border line' status in the assignment test. This result was compatible with the assignment result based on 90 SSR markers (Motamayor et al. 2008), where low memberships were reported for the same accessions although their exact Q-value differed slightly from the present study. We also found one case where genetic group membership differed substantially compared with the description in Motamayor et al. 2008. This is AMAZ-15/15, previously described as an Iquitos clone with a Q-value of 0.82 but

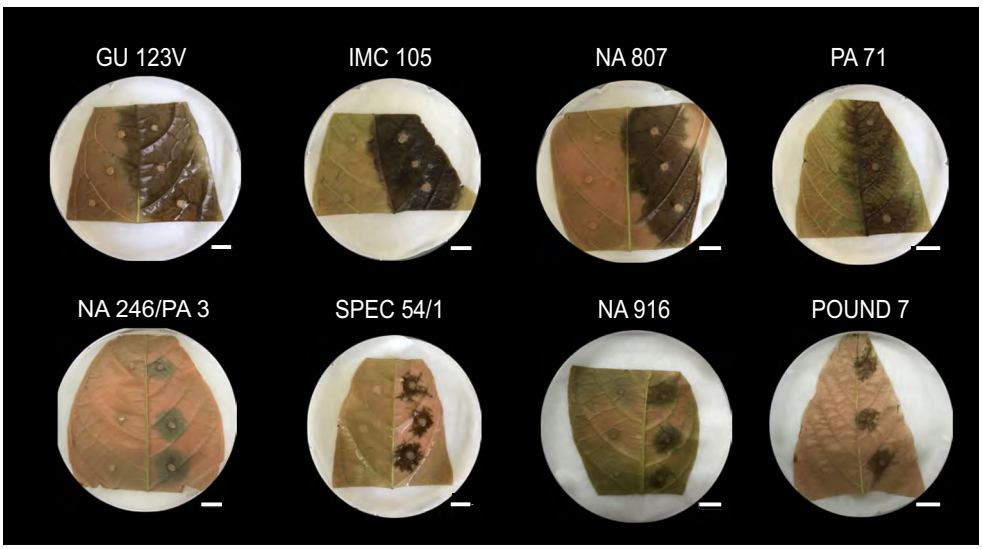
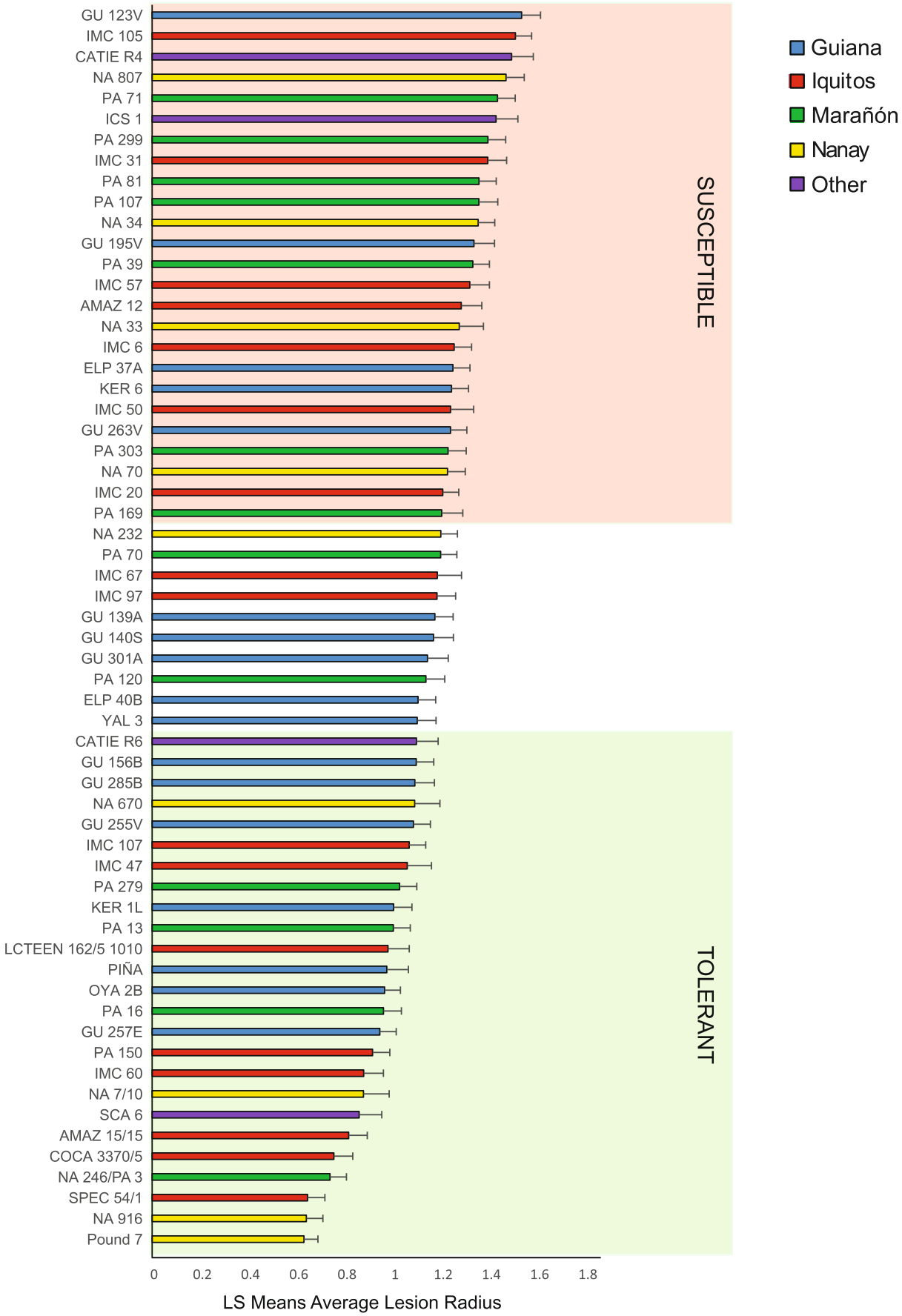


Fig. 2 Representative photographs of inoculated leaves from the four most susceptible and four most tolerant genotypes. Right side of leaves inoculated with V8 media containing *P. palmivora* mycelia, left sides mock inoculated with sterile V8 agar. White bars represent 1 cm. The top row includes photographs of the four most susceptible varieties, and the bottom row includes the four most tolerant


GU 123V
IMC 105
CATIE R4
NA 807
PA 71
ICS 1
PA 299
IMC 31
PA 81
PA 107
NA 34
GU 195V
PA 39
IMC 57
AMAZ 12
NA 33
IMC 6
ELP 37A
KER 6
IMC 50
GU 263V
PA 303
NA 70
IMC 20
PA 169
NA 232
PA 70
IMC 67
IMC 97
GU 139A
GU 140S
GU 301A
PA 120
ELP 40B
YAL 3
CATIE R6
GU 156B
GU 285B
NA 670
GU 255V
IMC 107
IMC 47
PA 279
KER 1L
PA 13
LCTEEN 162/5 1010
PIÑA
OYA 2B
PA 16
GU 257E
PA 150
IMC 60
NA 7/10
SCA 6
AMAZ 15/15
COCA 3370/5
NA 246/PA 3
SPEC 54/1
NA 916
Pound 7
SUSCEPTIBLE
TOLERANT
Guiana
Iquitos
Marañón
Nanay
Other
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
LS Means Average Lesion Radius

◀ **Fig. 3** Summary of detached leaf bioassay measurement. Bars represent least squares means calculated from a standard least squares model. Error bars represent standard error. Bars are color coded according to genetic group. The 'Other' class includes three hybrid genotypes (CATIE R4, CATIE R6, ICS 1) and SCA 6, which belongs to the Contamana genetic group (Motamayor et al. 2008). Genotypes not statistically different from ICS 1 are shaded in red; those not statistically different from, or more tolerant than, SCA 6 are shaded in green

a Q-value of 0.635 was found by our assignment test. These changes in genetic group assignment may be the result of different markers being used in our analysis and in the Motamayor et al.'s work, which was based on 106 SSR markers. Deeper genotyping or sequencing may more conclusively resolve ambiguities in assignment.

Analysis of defense response

Leaves of plants from the four populations of interest were inoculated with *P. palmivora* mycelia using a detached leaf pathogen bioassay (Fister et al. 2016) in order to measure their disease tolerance (Fig. 2). Each of the populations harbored phenotypic variation in tolerance, with multiple clones from each placing into the tolerant and susceptible classes. We included four special interest varieties in our phenotyping program: SCA 6, ICS 1, CATIE R4, and CATIE R6. We grouped the genotypes we phenotyped as tolerant if their lesion sizes were not statistically different from SCA 6, a control tolerant genotype, and as susceptible if their lesion sizes were not statistically different from ICS 1, a model susceptible genotype (Fig. 3, Supplemental Table S5). Pound 7, the most tolerant genotype, had statistically smaller lesion size than SCA 6. Notably all four genetic groups were represented in both of these classes, albeit the Guiana group's most tolerant clone was 11th overall. In a linear mixed model analysis, genotype was highly significant ($p < 0.0001$, df = 59). The random effects of date and tree (Wald $p = 0.0062$ and 0.0018, respectively) also were statistically significant, reflecting environmental effects on response to pathogens. A Fisher's least significant difference test was applied to identify pairwise differences ($p < 0.05$) with a correction for multiple hypothesis testing, and these results were used to generate a connected letters report (Supplemental Table S5), which were used to create the disease susceptibility and tolerance classes.

Cacao is host to several major fungal and oomycete pathogens, which collectively account for a roughly 30% loss in productivity annually (Bailey and Meinhardt 2016). *Phytophthora* diseases of cacao are typically associated with infection of pods, but can also cause foliar disease, particularly in young plants. Moreover, results from leaf-based *Phytophthora* infection bioassays are highly correlated with results from fruit susceptibility assays (Iwaro et al. 1997). The wide range of phenotypes we identified within genetic groups is similar to findings from previously reported screens of Phytophthora infection responses in pod tissue (Iwaro et al. 2003).

Trees in the Guiana genetic group have been extensively evaluated for their response to inoculation with three *Phytophthora* species (Paulin et al. 2008; Thevenin et al. 2012; Lachenaud et al. 2015). One of these reports (Thevenin et al. 2012) focused on response of the clones to a different *P. palmivora* isolate (GY-27), and classified 68 clones as tolerant and another 68 as susceptible using a visual score of infection development (Nyassé et al. 1995). The 17 Guiana clones included in our analysis were also included in theirs. In a few cases the results were in agreement: both datasets identified GU 156B, GU 257E, GU 285B, and KER 1L as tolerant or moderately tolerant. However, their study identified GU 123V as tolerant, which was the most susceptible clone overall in our analysis, and they placed PINA and OYA 2B among the susceptible clones, which we found to be tolerant. Ultimately this result highlights that strain specificity and environmental factors may have a significant role in a plant's tolerance of a particular pathogen species.

Qualitative disease resistance in plants is typically associated with a gene-for-gene interaction between pathogen effector proteins and plant resistance genes (reviewed in (Kushalappa et al. 2016)). No gene-for-gene interaction has been described in cacao, although a hypersensitive-response (HR)–like reaction was recently reported after inoculation of cacao genotype SCA 6 pods with *P. palmivora* zoospores (isolate Gh-ER1349)(Ali et al. 2016). While identification of identification of genes contributing qualitative resistance to cacao diseases would be of significant value to breeding programs, better understanding of sources of quantitative resistance in underutilized cacao populations could also significantly advance breeding efforts. Our results do suggest that genetic variation underlying quantitative resistance is present in each of the cacao genetic groups we phenotyped (Fig. 3). Further work is required to identify specific polymorphisms contributing to the range of phenotypes we observed.

Conclusions

Through this work we determined relative sensitivity/tolerance of *Phytophthora palmivora* of cacao clones in the International Cocoa Collection at CATIE, a collection representing the global genetic diversity of both wild and cultivated cacao. We identified 24 tolerant and 24 susceptible clones, with both classes including genotypes from four of the main genetic groups of the species. Each genetic group surveyed appears to harbor substantial genetic variation yielding a wide range in responses to this pathogen strain. Cacao breeding programs have relied on a very small set of clones for introduction of disease tolerance into new cultivars. The genotypes surveyed here, which belong to under-studied and

under-utilized genetic groups, could offer breeders new selections to include as disease-tolerant parents. Further genomic analyses of these accessions may provide genetic markers that are broadly useful for targeted breeding to increase *Phytophthora* resistance in cacao. Phenotyping the same set of trees via inoculation with other *P. palmivora* strains, other *Phytophthora* species, or cacao's true fungal pathogens would also offer unique insight into race- or species-specificity of each genotype's defense response.

Acknowledgments Thank you to Lena Sheaffer and Melanie Perryman for assistance in project management and transportation of samples. Thank you to Wilbert Phillips-Mora for advice on the use of the IC3 collection. This work was supported by The Pennsylvania State University College of Agricultural Sciences, the Huck Institutes of the Life Sciences, the Penn State Endowed Program in Molecular Biology of Cacao, NSF Plant Genome Research Award 1546863 and by the USDA National Institute of Food and Agriculture, Federal Appropriations under Project PEN04569 and Accession number 1003147.

Author Contributions AF collected leaf material, performed inoculations, analyzed genotyping and phenotyping data, and wrote the manuscript. ML performed the majority of inoculations and contributed to writing and editing the manuscript. DZ performed genotyping analysis and the genetic group assignment test, and contributed to writing and editing the manuscript. JM, PT, and CdP contributed to experimental design and planning, and editing of the manuscript. SM and MJG played primary roles project planning and management and contributed to drafting and editing the manuscript.

Data Archiving Statement All relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes by contacting the corresponding author (mjg9@psu.edu). All relevant data is presented in the manuscript and its associated supplemental files.

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