

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

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Running Title: *Phytophthora palmivora* tolerance in four cacao genetic groups

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 environment 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 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 multibillion dollar chocolate industry. As the plant was dispersed throughout the tropics in the 18th and 19th centuries, originally only a few cultivars were transplanted (a 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, 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; Iwaro et al., 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, Marañón, 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 analysed 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; Peakall and Smouse, 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 to 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; Peakall and Smouse, 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 two days with a 12 hr:12 hr day/night cycle, with daytime light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and darkness at night. After two 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 nine 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 nine 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 two-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 two days with a 12 hr:12 hr day/night cycle with daytime light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and darkness at night. Leaves were photographed 48 hr 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), 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 coordinates 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 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 Marañon 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 to 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 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 placed 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 $p = .0062$ and $.0018$, 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 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.

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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.

Figure Legends

Figure 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.

Figure 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.

Figure 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.

Supplementary Information

Supplementary Table S1. Tree Names, Genotypes, Plant number, Barcode Number assigned for this study, Genetic Group membership based on previous analysis (Motamayor 2008) and GPS coordinates for each tree included in our analysis. Replicate trees in column 1 have clone names appended with a number (1,2,3) indicating position in the field.

Supplementary Table S2. List of 10 reference cacao germplasm groups including 211 individual accessions used in the experiment and their origin.

Supplementary Table 3. List of offtype trees identified in CATIE's IC3 collection. The SNP profiles of these clones did not match a reference SNP set from a progenitor tree at the International Cacao Genebank in Trinidad (ICGT). Tc##s indicate SNP IDs, genotyping data is shown for non-matching loci. The SNP loci used to identify the CATIE NA 246 trees as PA 3 are also listed.

Supplementary Table S4. Results of assignment test performed using STRUCTURE. Accessions row lists 10 cacao genetic groups. Likelihood of membership to each group is displayed as calculated from five runs with 50,000 iterations per run.

Supplementary Table S5. List of genotypes (most susceptible to most tolerant) based on standard least squares analysis of detached leaf phenotyping data. Connected letter report based on pairwise student's t tests, genotypes not connected by the same letter have statistically significant differences in lesion size ($p < 0.05$).

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 (mjpg9@psu.edu). All relevant data is presented in the manuscript and its associated supplemental files.

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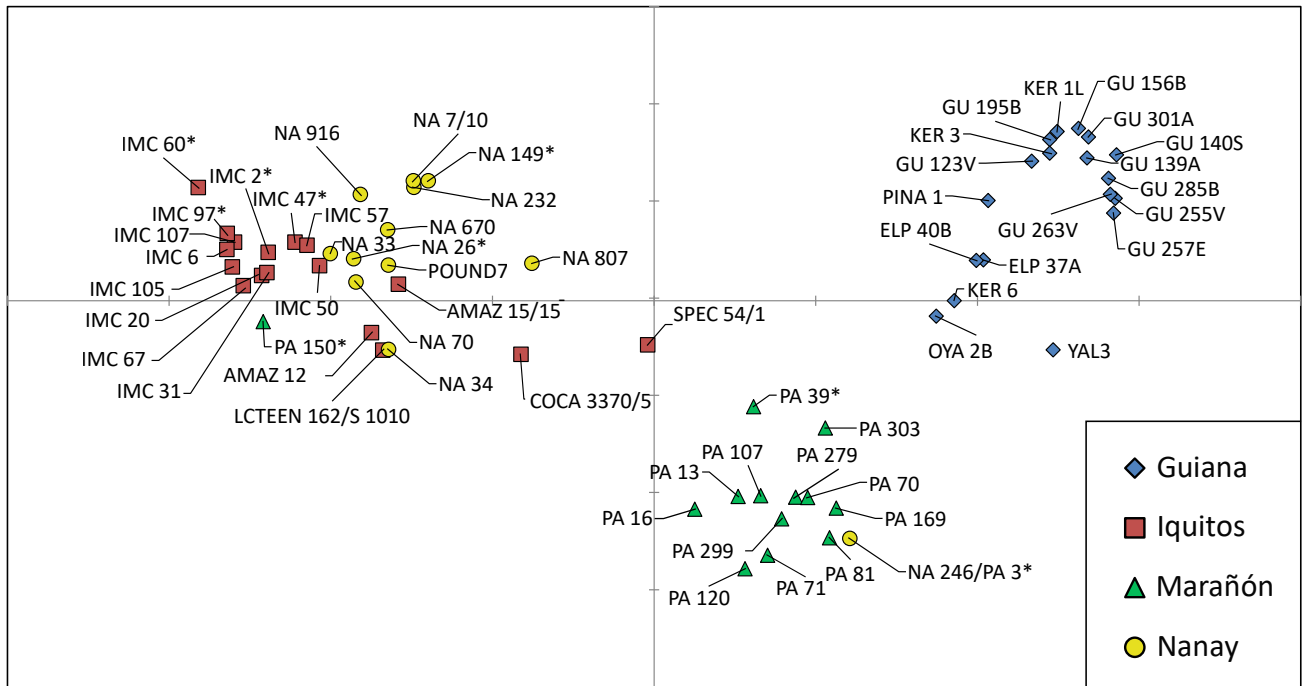
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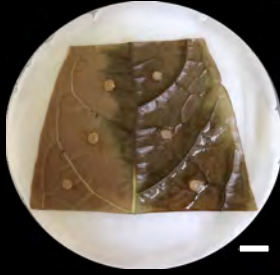
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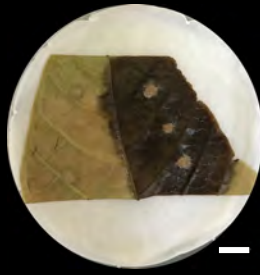
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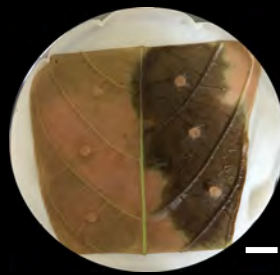
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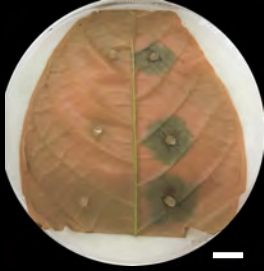
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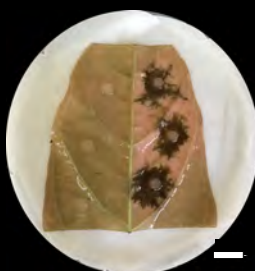
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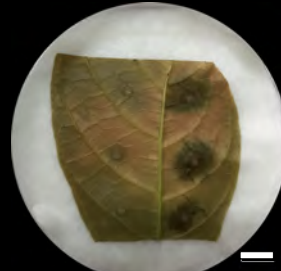
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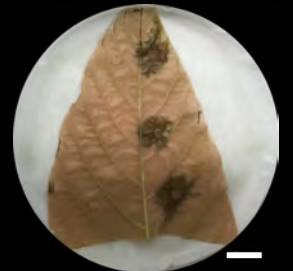
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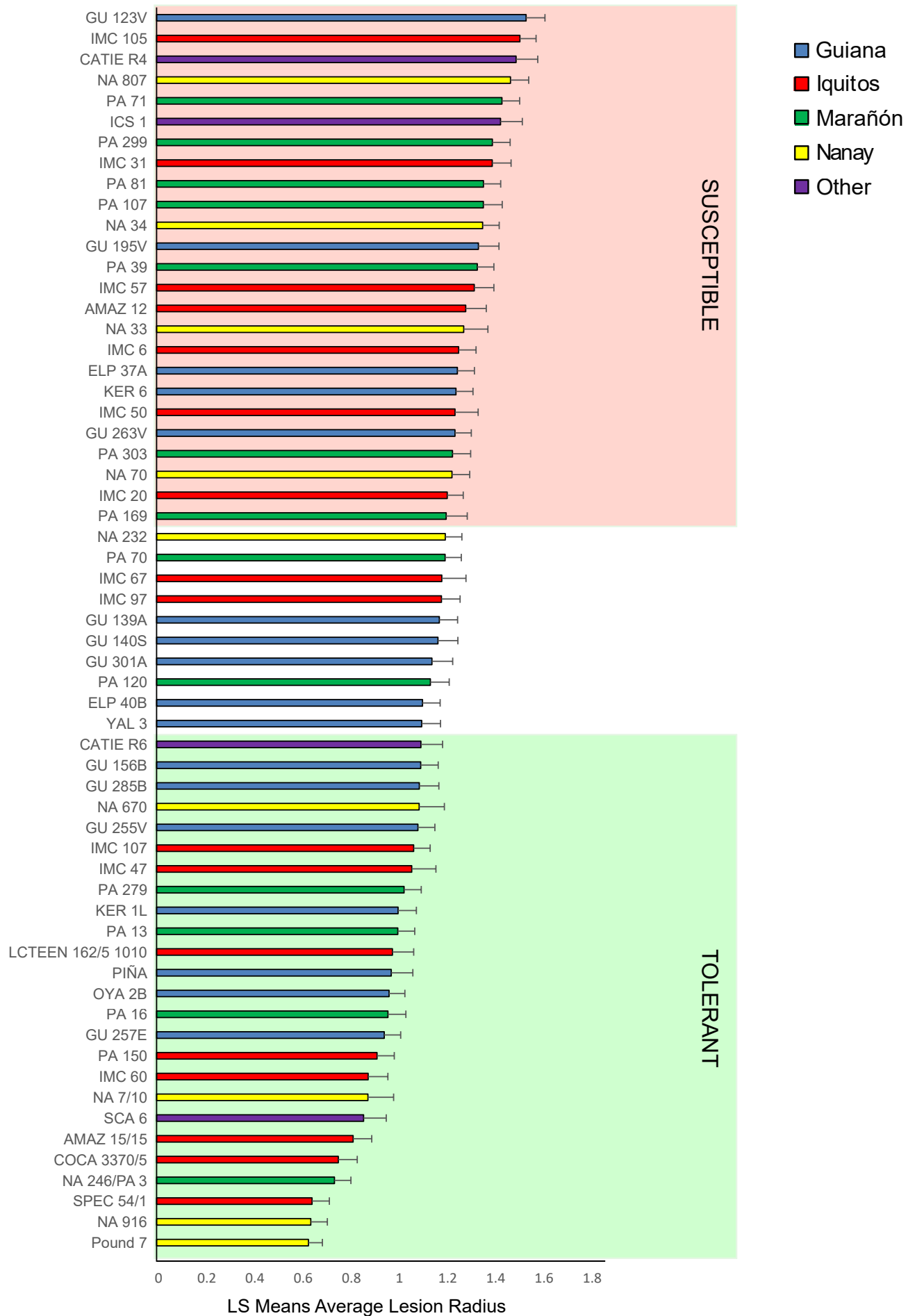


NA 916



POUND 7





Supplemental Table S1. Tree Names, Genotypes, Plant number, Barcode Number assigned for this study, Genetic Group membership based on previous analysis (Motamayor 2008) and GPS coordinates for each tree included in our analysis.

Tree Name	Genotype	Plant Num	Barcode Number	Genetic Group	GPS
Catongo Blanco 1	Catongo Blanco	1	P000181	Amelonado	9.875233333,-83.65606667
Catongo Blanco 2	Catongo Blanco	2	P000182	Amelonado	9.875216667,-83.6561
Catongo Blanco 3	Catongo Blanco	3	P000183	Amelonado	9.8752,-83.6561
Criollo 13 1	Criollo 13	1	P000184	Criollo	9.87605,-83.65683333
Criollo 13 2	Criollo 13	2	P000185	Criollo	9.876033333,-83.65683333
Criollo 13 3	Criollo 13	3	P000186	Criollo	9.876016667,-83.65685
GU-123V 1	GU-123V	1	P000126	Guiana	9.87575,-83.65741667
GU-123V 2	GU-123V	2	P000127	Guiana	9.875733333,-83.6574
GU-123V 3	GU-123V	3	P000128	Guiana	9.87575,-83.65738333
GU-139A 1	GU-139A	1	P000129	Guiana	9.876833333,-83.65718333
GU-139A 2	GU-139A	2	P000130	Guiana	9.876833333,-83.65721667
GU-139A 3	GU-139A	3	P000131	Guiana	9.876816667,-83.65721667
GU-140S 1	GU-140S	1	P000132	Guiana	9.8758,-83.6574
GU-140S 2	GU-140S	2	P000133	Guiana	9.875783333,-83.65745
GU-140S 3	GU-140S	3	P000134	Guiana	9.875783333,-83.65748333
GU-156B 1	GU-156B	1	P000135	Guiana	9.877016667,-83.65671667
GU-156B 2	GU-156B	2	P000136	Guiana	9.877,-83.65675
GU-156B 3	GU-156B	3	P000137	Guiana	9.876983333,-83.65676667
GU-195V 1	GU-195V	1	P000138	Guiana	9.87575,-83.65741667
GU-195V 2	GU-195V	2	P000139	Guiana	9.87575,-83.6574
GU-195V 3	GU-195V	3	P000140	Guiana	9.87575,-83.65738333
GU-255V 1	GU-255V	1	P000141	Guiana	9.875833333,-83.65741667
GU-255V 2	GU-255V	2	P000142	Guiana	9.875816667,-83.65746667
GU-255V 3	GU-255V	3	P000143	Guiana	9.875816667,-83.6575
GU-257E 1	GU-257E	1	P000144	Guiana	9.8757,-83.65738333
GU-257E 2	GU-257E	2	P000145	Guiana	9.8757,-83.65736667
GU-257E 3	GU-257E	3	P000146	Guiana	9.8757,-83.65735
GU-263V 1	GU-263V	1	P000147	Guiana	9.875833333,-83.65745
GU-263V 2	GU-263V	2	P000148	Guiana	9.875816667,-83.65745
GU-263V 3	GU-263V	3	P000149	Guiana	9.8758,-83.65746667

GU-285B 1	GU-285B	1	P000150	Guiana	9.87705,-83.65676667
GU-285B 2	GU-285B	2	P000151	Guiana	9.8770333333,-83.65678333
GU-285B 3	GU-285B	3	P000152	Guiana	9.877016667,-83.6568
GU-301A 1	GU-301A	1	P000153	Guiana	9.8766,-83.6572
GU-301A 2	GU-301A	2	P000154	Guiana	9.8766,-83.65721667
GU-301A 3	GU-301A	3	P000155	Guiana	9.8765833333,-83.65723333
ELP-37A 1	ELP-37A	1	P000157	Guiana	9.8765833333,-83.65715
ELP-37A 2	ELP-37A	2	P000158	Guiana	9.876566667,-83.6572
ELP-37A 3	ELP-37A	3	P000159	Guiana	9.87655,-83.65721667
ELP-40B 1	ELP-40B	1	P000160	Guiana	9.876716667,-83.65728333
ELP-40B 2	ELP-40B	2	P000161	Guiana	9.876716667,-83.65728333
ELP-40B 3	ELP-40B	3	P000162	Guiana	9.876716667,-83.6573
KER-1L 1	KER-1L	1	P000163	Guiana	9.876866667,-83.65723333
KER-1L 2	KER-1L	2	P000164	Guiana	9.87685,-83.65725
KER-1L 3	KER-1L	3	P000165	Guiana	9.87685,-83.65728333
KER-3 1	KER-3	1	P000166	Guiana	9.877616667,-83.65726667
KER-3 2	KER-3	2	P000167	Guiana	9.877616667,-83.65728333
KER-3 3	KER-3	3	P000168	Guiana	9.8776,-83.6573
KER-6 1	KER-6	1	P000169	Guiana	9.87735,-83.65715
KER-6 2	KER-6	2	P000170	Guiana	9.877366667,-83.65716667
KER-6 3	KER-6	3	P000171	Guiana	9.87735,-83.65716667
OYA-2B 1	OYA-2B	1	P000172	Guiana	9.8766,-83.6572
OYA-2B 2	OYA-2B	2	P000173	Guiana	9.8765833333,-83.65721667
OYA-2B 3	OYA-2B	3	P000174	Guiana	9.876566667,-83.65721667
PINA 1	PINA	1	P000175	Guiana	9.877416667,-83.65735
PINA 2	PINA	2	P000176	Guiana	9.8773833333,-83.6574
PINA 3	PINA	3	P000177	Guiana	9.877366667,-83.65741667
YAL-3 1	YAL-3	1	P000178	Guiana	9.87675,-83.65733333
YAL-3 2	YAL-3	2	P000179	Guiana	9.8767333333,-83.65733333
YAL-3 3	YAL-3	3	P000180	Guiana	9.876716667,-83.65736667
UF-712 1	UF-712	1	P000187	Guiana	9.8758833333,-83.65693333
UF-712 2	UF-712	2	P000188	Guiana	9.875866667,-83.65695
UF-712 3	UF-712	3	P000189	Guiana	9.87585,-83.65696667
AMAZ-12 1	AMAZ-12	1	P000001	Iquitos	9.8768,-83.65703333
AMAZ-12 2	AMAZ-12	2	P000002	Iquitos	9.8768,-83.65705

AMAZ-12 3	AMAZ-12	3	P000003	Iquitos	9.876783333,-83.65706667
AMAZ-15/15 1	AMAZ-15/15	1	P000004	Iquitos	9.876733333,-83.65708333
AMAZ-15/15 2	AMAZ-15/15	2	P000005	Iquitos	9.8767,-83.65708333
AMAZ-15/15 3	AMAZ-15/15	3	P000006	Iquitos	9.8767,-83.6571
COCA-3370-5 1	COCA-3370-5	1	P000007	Iquitos	9.875116667,-83.65611667
COCA-3370-5 2	COCA-3370-5	2	P000008	Iquitos	9.875083333,-83.65615
COCA-3370-5 3	COCA-3370-5	3	P000009	Iquitos	9.87505,-83.65618333
IMC-105 1	IMC-105	1	P000010	Iquitos	9.877333333,-83.6573
IMC-105 2	IMC-105	2	P000011	Iquitos	9.877333333,-83.6573
IMC-105 3	IMC-105	3	P000012	Iquitos	9.877333333,-83.65733333
IMC-107 1	IMC-107	1	P000013	Iquitos	9.875366667,-83.65653333
IMC-107 2	IMC-107	2	P000014	Iquitos	9.875366667,-83.65656667
IMC-107 3	IMC-107	3	P000015	Iquitos	9.87535,-83.65658333
IMC-2 1	IMC-2	1	P000016	Iquitos	9.8772,-83.65713333
IMC-2 2	IMC-2	2	P000017	Iquitos	9.877183333,-83.65715
IMC-2 3	IMC-2	3	P000018	Iquitos	9.877183333,-83.65718333
IMC-20 1	IMC-20	1	P000019	Iquitos	9.876883333,-83.65716667
IMC-20 2	IMC-20	2	P000020	Iquitos	9.876883333,-83.65721667
IMC-20 3	IMC-20	3	P000021	Iquitos	9.876883333,-83.65723333
IMC-31 1	IMC-31	1	P000022	Iquitos	9.877283333,-83.65775
IMC-31 2	IMC-31	2	P000023	Iquitos	9.877283333,-83.65776667
IMC-31 3	IMC-31	3	P000024	Iquitos	9.877283333,-83.65778333
IMC-47 2	IMC-47	2	P000025	Iquitos	9.877083333,-83.6573
IMC-47 3	IMC-47	3	P000026	Iquitos	9.877066667,-83.65728333
IMC-50 1	IMC-50	1	P000028	Iquitos	9.877166667,-83.65766667
IMC-50 2	IMC-50	2	P000029	Iquitos	9.87715,-83.65768333
IMC-57 1	IMC-57	1	P000031	Iquitos	9.8769,-83.65676667
IMC-57 2	IMC-57	2	P000032	Iquitos	9.8769,-83.65676667
IMC-57 3	IMC-57	3	P000033	Iquitos	9.876866667,-83.65678333
IMC-6 1	IMC-6	1	P000034	Iquitos	9.87715,-83.65688333
IMC-6 2	IMC-6	2	P000035	Iquitos	9.87715,-83.6569
IMC-6 3	IMC-6	3	P000036	Iquitos	9.877133333,-83.65693333
IMC-60 1	IMC-60	1	P000037	Iquitos	9.87675,-83.6567
IMC-60 2	IMC-60	2	P000038	Iquitos	9.87675,-83.65673333
IMC-60 3	IMC-60	3	P000039	Iquitos	9.87675,-83.65673333

IMC-67 1	IMC-67	1	P000040	Iquitos	9.876916667,-83.65653333
IMC-67 2	IMC-67	2	P000041	Iquitos	9.876916667,-83.65656667
IMC-67 3	IMC-67	3	P000042	Iquitos	9.876916667,-83.65658333
IMC-97 1	IMC-97	1	P000043	Iquitos	9.874966667,-83.65643333
IMC-97 2	IMC-97	2	P000044	Iquitos	9.874983333,-83.65643333
IMC-97 3	IMC-97	3	P000045	Iquitos	9.874983333,-83.65646667
LCTEEN 162/S 1010 1	LCTEEN 162/S 10	1	P000046	Iquitos	9.877416667,-83.65731667
LCTEEN 162/S 1010 2	LCTEEN 162/S 10	2	P000047	Iquitos	9.877416667,-83.65731667
LCTEEN 162/S 1010 3	LCTEEN 162/S 10	3	P000048	Iquitos	9.877416667,-83.65733333
SPEC-54/1 1	SPEC-54/1	1	P000049	Iquitos	9.875166667,-83.65621667
SPEC-54/1 2	SPEC-54/1	2	P000050	Iquitos	9.875166667,-83.65623333
SPEC-54/1 3	SPEC-54/1	3	P000051	Iquitos	9.87515,-83.65625
PA-107 1	PA-107	1	P000087	Maranon	9.87585,-83.65736667
PA-107 2	PA-107	2	P000088	Maranon	9.875833333,-83.65738333
PA-107 3	PA-107	3	P000089	Maranon	9.875833333,-83.6574
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PA-13 1	PA-13	1	P000093	Maranon	9.876216667,-83.65665
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PA-150 3	PA-150	3	P000098	Maranon	9.87625,-83.65708333
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PA-16 2	PA-16	2	P000100	Maranon	9.876666667,-83.65665
PA-16 3	PA-16	3	P000101	Maranon	9.876666667,-83.65666667
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PA-169 2	PA-169	2	P000103	Maranon	9.87585,-83.65715
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PA-303 1	PA-303	1	P000111	Maranon	9.876416667,-83.65661667
PA-303 2	PA-303	2	P000112	Maranon	9.876433333,-83.65661667
PA-303 3	PA-303	3	P000113	Maranon	9.876416667,-83.65661667
PA-39 1	PA-39	1	P000114	Maranon	9.8754,-83.65621667
PA-39 2	PA-39	2	P000115	Maranon	9.875383333,-83.65623333
PA-39 3	PA-39	3	P000116	Maranon	9.875383333,-83.65625
PA-70 1	PA-70	1	P000117	Maranon	9.87595,-83.65748333
PA-70 2	PA-70	2	P000118	Maranon	9.875933333,-83.6575
PA-70 3	PA-70	3	P000119	Maranon	9.8759,-83.6575
PA-71 1	PA-71	1	P000120	Maranon	9.87635,-83.65643333
PA-71 2	PA-71	2	P000121	Maranon	9.876333333,-83.65645
PA-71 3	PA-71	3	P000122	Maranon	9.876316667,-83.65646667
PA-81 1	PA-81	1	P000123	Maranon	9.87575,-83.65711667
PA-81 2	PA-81	2	P000124	Maranon	9.87575,-83.65716667
PA-81 3	PA-81	3	P000125	Maranon	9.875733333,-83.6572
NA-149	NA-149	1	P000052	Nanay	9.877633333,-83.65746667
NA-232 1	NA-232	1	P000053	Nanay	9.877166667,-83.6576
NA-232 2	NA-232	2	P000054	Nanay	9.87715,-83.6576
NA-232 3	NA-232	3	P000055	Nanay	9.877133333,-83.6576
NA-246 1	NA-246	1	P000056	Nanay	9.87715,-83.65698333
NA-246 2	NA-246	2	P000057	Nanay	9.877133333,-83.657
NA-246 3	NA-246	3	P000058	Nanay	9.877116667,-83.65701667
NA-26 1	NA-26	1	P000059	Nanay	9.87695,-83.65766667
NA-26 2	NA-26	2	P000060	Nanay	9.876916667,-83.65766667
NA-26 3	NA-26	3	P000061	Nanay	9.8769,-83.6577
NA-33 1	NA-33	1	P000190	Nanay	9.877016667,-83.6576
NA-33 2	NA-33	2	P000062	Nanay	9.877016667,-83.65761667
NA-33 3	NA-33	3	P000063	Nanay	9.877033333,-83.6576
NA-34 1	NA-34	1	P000064	Nanay	9.876516667,-83.65661667
NA-34 2	NA-34	2	P000065	Nanay	9.8765,-83.65661667
NA-34 3	NA-34	3	P000066	Nanay	9.876466667,-83.65663333
NA-670 1	NA-670	1	P000067	Nanay	9.877283333,-83.6577
NA-670 2	NA-670	2	P000068	Nanay	9.877283333,-83.65773333
NA-670 3	NA-670	3	P000069	Nanay	9.877266667,-83.65776667

NA-70 1	NA-70	1 P000070	Nanay	9.8771,-83.65805
NA-70 3	NA-70	3 P000071	Nanay	9.877116667,-83.65806667
NA-710 1	NA-710	1 P000072	Nanay	9.876933333,-83.6577
NA-710 2	NA-710	2 P000191	Nanay	9.876916667,-83.65771667
NA-710 3	NA-710	3 P000074	Nanay	9.876883333,-83.65775
NA-807 1	NA-807	1 P000075	Nanay	9.877383333,-83.65736667
NA-807 2	NA-807	2 P000076	Nanay	9.877383333,-83.65738333
NA-807 3	NA-807	3 P000077	Nanay	9.87735,-83.6574
NA-916 1	NA-916	1 P000078	Nanay	9.87695,-83.65716667
NA-916 2	NA-916	2 P000079	Nanay	9.876933333,-83.65718333
NA-916 3	NA-916	3 P000080	Nanay	9.876916667,-83.65723333
Pound-7 1	Pound-7	1 P000081	Nanay	9.87545,-83.65688333
Pound-7 2	Pound-7	2 P000082	Nanay	9.87545,-83.6569
Pound-7 3	Pound-7	3 P000083	Nanay	9.875433333,-83.65691667
Pound-7B 1	Pound-7B	1 P000084	Nanay	9.876933333,-83.65725
Pound-7B 2	Pound-7B	2 P000085	Nanay	9.876916667,-83.65725
Pound-7B 3	Pound-7B	3 P000086	Nanay	9.8769,-83.65725

Supplementary Table 2. List of 10 reference cacao germplasm groups including 211 individual accessions used in the experiment and their origin.

Population (group)	Origin	Sample size	Provider
Nacional	Ecuador	20	INIAP, Ecuador; SPCL, USDA
IMC (Iquitos)	Peru	20	ICG,T, Trinidad
Nanay	Peru	20	ICG,T, Trinidad
Parinari (Marañón)	Peru	20	ICG,T , Trinidad
Scavina (Contamana)	Peru	21	ICG,T, Trinidad and; ICT, Peru
Criollo	Puerto Rico Honduras Nicaragua Madagascar Costa Rica	22	SPCL, TARS, USDA; CATIE, Costa Rica
Amelonado	Puerto Rico Belize Madagascar Trinidad	21	SPCL, TARS, USDA; ICG,T , Trinidad
LCT EEN (Curaray)	Ecuador	24	INIAP, Ecuador
Purus	Brazil	22	SPCL, USDA
Guiana	French Guiana	21	CIRAD, France
Total		211	

Supplementary Table 3. List of offtype trees identified in CATIE's IC3 collection. The SNP profiles of these clones did not match a reference SNP set from a progenitor tree at the International Cocoa Genebank in Trinidad (ICGT). Tc##s indicate SNP IDs, genotyping data is shown for non-matching loci.

Accession	Field	Tc14	Tc15	Tc37	Tc46	Tc52	Tc53	Tc56	Tc59	Tc72	Tc95	Tc12	Tc14			
ID	Collection	Collected	Status	Tc25	4	0	2	9	9	4	0	1	3	3	53	58
IMC 2	ICGT,	MARPER	Ref	G G	A C	G T	A A	A A	C C	C T	G G	A A	G G	A A	G G	C G
IMC 2_1	CATIE	ICCC	Offtype	C G	C C	T T	A T	A G	A C	C C	G T	A C	G T	A T	T T	G G
IMC 2_2	CATIE	ICCC	Offtype	C G	C C	T T	A T	A G	A C	C C	G T	A C	G T	A T	T T	G G
IMC 2_3	CATIE	ICCC	Offtype	C G	C C	T T	A T	A G	A C	C C	G T	A C	G T	A T	T T	G G

Accession	Field	Tc15	Tc22	Tc24	Tc37	Tc42	Tc52	Tc53	Tc56	Tc59	Tc89	Tc95	Tc10	Tc10	Tc11	Tc14				
ID	Collection	Collected	Status	Tc25	Tc32	0	6	2	2	9	9	4	0	1	1	3	60	75	65	58
IMC 47	ICGT,	MARPER	Ref	G G	T T	G T	G T	T T	A A	G G	C C	C T	G T	A A	C T	A T	C T	A A	C C	G G
IMC 47_2	CATIE	ICCC	Offtype	C G	A T	T T	C G	C T	A T	A G	A C	C C	G G	A C	T T	A A	C C	A T	C T	C G
IMC 47_3	CATIE	ICCC	Offtype	C G	A T	T T	C G	C T	A T	A G	A C	C C	G G	A C	T T	A A	C C	A T	C T	C G

Accession	Field	Tc14	Tc15	Tc15	Tc56	Tc95	Tc10	Tc12	Tc14		
ID	Collection	Collected	Status	4	0	1	0	3	60	53	14
IMC 60	ICGT,	6B	Ref	C C	G T	C T	G T	A A	C C	T T	T T
IMC 60_1	CATIE	ICCC	Offtype	A C	T T	C C	G G	A T	C T	G G	C T
IMC 60_2	CATIE	ICCC	Offtype	A C	T T	C C	G G	A T	C T	G G	C T
IMC 60_3	CATIE	ICCC	Offtype	A C	T T	C C	G G	A T	C T	G G	C T

Accession	Field	Tc22	Tc37	Tc52	Tc53	Tc64	Tc87	Tc12	Tc14			
ID	Collection	Collected	Status	Tc32	6	2	9	4	5	2	53	58
NA 149	ICGT,	MARPER	Ref	A T	G G	A T	A C	C T	A G	C C	G T	C C
NA 149_1	CATIE	ICCC	Offtype	T T	C C	A A	C C	C C	A A	G G	T T	G G

Accession	Field	Tc14	Tc19	Tc24	Tc37	Tc56	Tc57	Tc61	Tc64	Tc91	Tc92	Tc99	Tc10	Tc12	Tc14	Tc14	Tc14			
ID	Collection	Collected	Status	4	3	2	2	0	7	9	5	7	9	4	62	70	42	58	84	
NA 246	ICGT,	MARPER	Ref	A C	A C	C T	A T	G G	G G	C C	A A	C T	G G	T T	A G	T T	T T	C C	A A	
NA 246_1	CATIE	ICCC	Offtype	A A	A A	C C	A A	C C	A A	G T	C G	T T	A G	T T	C C	C T	A A	C C	C G	G G
NA 246_2	CATIE	ICCC	Offtype	A A	A A	C C	A A	G T	C G	T T	A G	T T	C C	C T	A A	C C	C C	C G	G G	
NA 246_3	CATIE	ICCC	Offtype	A A	A A	C C	A A	G T	C G	T T	A G	T T	C C	C T	A A	C C	C C	C G	G G	

Accession	Field	Tc14	Tc15	Tc19	Tc22	Tc42	Tc52	Tc53	Tc89	Tc91	Tc99	Tc99	Tc10	Tc10	Tc10	Tc11	Tc12	Tc14	Tc14				
ID	Collection	Collected	Status	Tc25	Tc32	4	0	3	6	9	9	4	1	7	4	8	60	62	75	65	53	14	84
NA 26	ICGT,	5B	Ref	C G	A T	C C	T T	A C	G G	A G	A C	C C	T T	C C	T T	A A	C C	G G	A T	C T	G T	C T	A G
NA 26_1	CATIE	ICCC	Offtype	G G	T T	A C	G G	A A	C G	G G	C C	C T	C C	C T	C T	A G	C T	A G	A A	C C	T T	T T	A A
NA 26_2	CATIE	ICCC	Offtype	G G	T T	A C	G G	A A	C G	G G	C C	C T	C C	C T	C T	A G	C T	A G	A A	C C	T T	T T	A A
NA 26_3	CATIE	ICCC	Offtype	G G	T T	A C	G G	A A	C G	G G	C C	C T	C C	C T	C T	A G	C T	A G	A A	C C	T T	T T	A A

Accession	Field	Tc14	Tc15	Tc19	Tc22	Tc23	Tc24	Tc53	Tc56	Tc57	Tc61	Tc87	Tc91	Tc92	Tc99	Tc99	Tc10	Tc12	Tc13	Tc14	Tc14	Tc14	Tc15				
ID	Collection	Collected	Status	Tc25	Tc32	4	1	3	6	0	2	4	0	7	9	2	7	9	4	8	60	53	50	14	42	58	20
PA 150	ICGT,	MARPER	Ref	G G	A A	A C	C C	A A	C C	G G	C C	C T	T T	C G	T T	G G	C T	C C	C C	A G	C C	T T	A C	T T	C C	G G	C T
PA 150_1	CATIE	ICCC	Offtype	C G	T T	C C	T T	A C	C G	A A	C T	C C	G G	G G	C T	C C	C C	C G	C T	A A	C T	G T	C C	C C	T T	C G	C C
PA 150_2	CATIE	ICCC	Offtype	C G	T T	C C	T T	A C	C G	A A	C T	C C	G G	G G	C T	C C	C C	C G	C T	A A	C T	G T	C C	T T	C G	C C	
PA 150_3	CATIE	ICCC	Offtype	C G	T T	C C	T T	A C	C G	A A	C T	C C	G G	G G	C T	C C	C C	C G	C T	A A	C T	G T	C C	C C	T T	C G	C C

Accession	Field	Tc14	Tc15	Tc15	Tc19	Tc24	Tc37	Tc46	Tc53	Tc57	Tc59	Tc61	Tc64	Tc72	Tc87	Tc92	Tc99	Tc99	Tc10	Tc10	Tc11	Tc12	Tc12	Tc13	Tc14	Tc14	Tc15		
ID	Collection	Collected	Status	Tc32	4	0	1	3	2	2	9	4	7	1	9	5	3	2	9	4	8	60	62	65	53	70	50	84	20
PA 39	ICGT,	5A	Ref	A T	A C	G G	C C	A A	C C	A A	A A	C T	C G	A C	T T	A A	G G	G G	C G	C C	A G	C C	A G	C C	T T	C T	A C	A G	C C
PA 39_1	CATIE	ICCC	Offtype	T T	C C	G T	C T	A C	C T	A T	A G	T T	C C	C C	C T	G G	G T	C G	G G	C T	A A	C T	A A	C T	G T	C C	A A	A A	C T
PA 39_2	CATIE	ICCC	Offtype	T T	C C	G T	C T	A C	C T	A T	A G	T T	C C	C C	C T	G G	G T	C G	G G	C T	A A	C T	A A	C T	G T	C C	A A	A A	C T
PA 39_3	CATIE	ICCC	Offtype	T T	C C	G T	C T	A C	C T	A T	A G	T T	C C	C C	C T	G G	G T	C G	G G	C T	A A	C T	A A	C T	G T	C C	A A	A A	C T

Sample	Collection	Collected	Status	Tc42	Tc9	Tc0	Tc3	Tc2	Tc9	Tc9	Tc3	Tc8	Tc7	Tc0	Tc1	Tc4	Tc6	Tc7	Tc6	Tc9	Tc9	Tc10	Tc10	Tc11	Tc12	Tc12	Tc13	Tc14	Tc14	Tc15										
IMC 97_1	CATIE	ICCC	No ref	G G	G T	A T	A C	T T	G G	T T	C T	G G	T T	C C	G G	A A	C T	A A	A G	G T	G G	C C	G G	A G	C C	T T	A A	A T	C C	C C	C C	A T	A A	C C	G G	C C	A A	T T	C C	T T
IMC 97_2	CATIE	ICCC	No ref	G G	G T	A T	A C	T T	G G	T T	C T	G G	G T	G G	A A	C T	A A	A G	G T	G G	C C	G G	A G	C C	T T	A A	A T	C C	C C	C C	A T	A A	C C	G G	C C	A A	T T	C C	T T	
IMC 97_3	CATIE	ICCC	(non-	A A	G G	A A	A A	C C	A G	C C	T T	A A	T T	C C	C C	C C	A G	G G	T T	A A	T T	C G	A A	T T	G G	G G	A A	G G	T T	T T	A A	G G	T T	A A	C G	T T	C C	T T	C C	

Supplementary Table S4. Results of assignment test performed using STRUCTURE. Accessions row lists 10 cacao genetic groups. Likelihood of membership to each group is displayed as calculated from five runs with 50,000 iterations per run.

Accessions	Inferred ancestry									
	Curaray	Purus	Guiana	Nanay	Criollo	Contamana	Iquitos	Amelonado	Nacional	Marañón
AMAZ 12	0.131	0.011	0.003	0.507	0.003	0.057	0.240	0.011	0.029	0.008
AMAZ 15/15	0.004	0.254	0.025	0.015	0.001	0.005	0.635	0.017	0.002	0.042
CATIE R4	0.004	0.003	0.059	0.004	0.001	0.002	0.002	0.047	0.306	0.572
CATIE R6	0.000	0.001	0.001	0.994	0.000	0.000	0.001	0.001	0.000	0.001
CATONGO BLANCO	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.997	0.000	0.001
COCA 3370-5	0.023	0.006	0.020	0.025	0.004	0.134	0.461	0.116	0.049	0.163
CRIOLLO 13	0.005	0.004	0.003	0.003	0.973	0.002	0.003	0.002	0.002	0.003
ELP 37A 1	0.000	0.001	0.605	0.002	0.000	0.000	0.001	0.388	0.000	0.001
ELP 40/B	0.001	0.001	0.668	0.003	0.000	0.001	0.002	0.322	0.000	0.003
GU 123/V	0.001	0.001	0.994	0.001	0.000	0.001	0.001	0.001	0.000	0.001
GU 139/A	0.001	0.000	0.995	0.001	0.000	0.000	0.001	0.001	0.000	0.001
GU 140/S	0.000	0.000	0.996	0.001	0.000	0.000	0.001	0.000	0.000	0.001
GU 156/B	0.001	0.001	0.995	0.001	0.000	0.000	0.001	0.001	0.000	0.001
GU 195/V	0.004	0.003	0.977	0.005	0.002	0.001	0.001	0.005	0.001	0.003
GU 255/V	0.003	0.003	0.925	0.016	0.006	0.003	0.001	0.008	0.014	0.020
GU 257/E	0.000	0.001	0.995	0.001	0.000	0.001	0.001	0.001	0.000	0.001
GU 263/V	0.000	0.000	0.995	0.001	0.000	0.000	0.001	0.001	0.000	0.001
GU 285/B	0.000	0.001	0.995	0.001	0.000	0.000	0.001	0.001	0.000	0.001
GU 301/A	0.000	0.000	0.995	0.001	0.000	0.000	0.001	0.001	0.000	0.001
IMC 105	0.005	0.013	0.001	0.004	0.001	0.001	0.959	0.001	0.012	0.001
IMC 107	0.001	0.001	0.001	0.006	0.000	0.001	0.988	0.001	0.001	0.001
IMC 2	0.003	0.001	0.002	0.004	0.000	0.003	0.979	0.001	0.005	0.001
IMC 20	0.001	0.001	0.001	0.002	0.000	0.001	0.991	0.001	0.001	0.001
IMC 31	0.001	0.001	0.001	0.002	0.000	0.001	0.992	0.001	0.001	0.001
IMC 47	0.006	0.002	0.002	0.002	0.001	0.002	0.884	0.001	0.099	0.001
IMC 50	0.001	0.002	0.002	0.010	0.000	0.001	0.978	0.001	0.003	0.002
IMC 57	0.004	0.003	0.002	0.002	0.001	0.002	0.880	0.001	0.105	0.001
IMC 6	0.001	0.001	0.001	0.003	0.000	0.001	0.991	0.001	0.001	0.001
IMC 60	0.001	0.001	0.001	0.002	0.000	0.001	0.992	0.001	0.001	0.001
IMC 67	0.001	0.001	0.001	0.002	0.000	0.001	0.991	0.001	0.001	0.001

IMC 97 (Tree #1 & #2)	0.000	0.001	0.001	0.020	0.000	0.000	0.974	0.001	0.000	0.001
IMC 97 (Tree #3)	0.001	0.004	0.003	0.004	0.001	0.007	0.006	0.803	0.107	0.064
KER 1/L	0.001	0.001	0.993	0.002	0.000	0.000	0.001	0.001	0.000	0.001
KER 3	0.001	0.001	0.992	0.002	0.000	0.000	0.001	0.001	0.000	0.001
KER 6	0.001	0.007	0.507	0.020	0.000	0.002	0.005	0.441	0.001	0.017
LCTEEN 162/5	0.001	0.015	0.005	0.008	0.001	0.004	0.606	0.028	0.001	0.332
NA 149	0.000	0.001	0.001	0.994	0.000	0.000	0.001	0.001	0.000	0.001
NA 232	0.001	0.001	0.001	0.993	0.000	0.000	0.002	0.001	0.000	0.001
NA 246	0.001	0.001	0.003	0.004	0.000	0.000	0.001	0.070	0.000	0.919
NA 26	0.009	0.004	0.004	0.741	0.006	0.002	0.224	0.001	0.002	0.006
NA 33	0.027	0.004	0.002	0.829	0.001	0.109	0.019	0.003	0.002	0.005
NA 34	0.034	0.048	0.004	0.537	0.004	0.033	0.151	0.061	0.007	0.121
NA 670	0.007	0.043	0.002	0.932	0.002	0.003	0.006	0.001	0.002	0.002
NA 70	0.019	0.003	0.002	0.694	0.001	0.175	0.067	0.022	0.013	0.005
NA 710	0.000	0.001	0.002	0.994	0.000	0.000	0.001	0.001	0.000	0.001
NA 807	0.017	0.179	0.016	0.762	0.003	0.001	0.002	0.002	0.014	0.003
NA 916	0.000	0.001	0.001	0.995	0.000	0.000	0.001	0.001	0.000	0.001
OY 2/B	0.000	0.001	0.423	0.002	0.000	0.001	0.002	0.569	0.000	0.002
PA 107	0.001	0.002	0.051	0.002	0.002	0.016	0.018	0.005	0.011	0.892
PA 120	0.001	0.002	0.003	0.002	0.000	0.001	0.002	0.002	0.001	0.987
PA 13	0.006	0.002	0.023	0.004	0.002	0.007	0.089	0.003	0.042	0.823
PA 150	0.003	0.001	0.001	0.004	0.001	0.347	0.636	0.001	0.003	0.003
PA 16	0.001	0.002	0.004	0.005	0.001	0.002	0.010	0.002	0.001	0.972
PA 169	0.001	0.002	0.053	0.005	0.001	0.001	0.004	0.010	0.001	0.923
PA 279	0.002	0.002	0.039	0.002	0.001	0.001	0.003	0.002	0.001	0.947
PA 299	0.001	0.001	0.004	0.003	0.000	0.001	0.001	0.001	0.001	0.987
PA 303	0.001	0.003	0.111	0.003	0.000	0.001	0.002	0.001	0.001	0.876
PA 39	0.001	0.001	0.001	0.001	0.381	0.001	0.001	0.612	0.000	0.001
PA 71	0.001	0.001	0.002	0.002	0.000	0.001	0.001	0.002	0.002	0.987
PA 81	0.001	0.001	0.002	0.002	0.000	0.002	0.001	0.004	0.002	0.985
PA 70	0.001	0.001	0.011	0.002	0.000	0.002	0.002	0.001	0.003	0.976
PINA	0.001	0.006	0.808	0.172	0.001	0.001	0.002	0.005	0.001	0.004
POUND7	0.009	0.020	0.005	0.912	0.002	0.007	0.028	0.007	0.001	0.010
POUND7B	0.008	0.034	0.004	0.916	0.002	0.010	0.013	0.006	0.001	0.007
SCA 6	0.003	0.003	0.003	0.002	0.004	0.958	0.002	0.004	0.014	0.008

SPEC 54/1	0.001	0.057	0.035	0.371	0.001	0.001	0.065	0.452	0.000	0.017
UF 712	0.012	0.006	0.004	0.004	0.002	0.003	0.007	0.001	0.957	0.004
YAL3	0.001	0.001	0.587	0.001	0.000	0.001	0.001	0.405	0.000	0.003

Supplementary Table S5. List of genotypes (most susceptible to most resistant) based on standard least squares analysis of detached leaf phenotyping data. Connected letter report based on : same letter have statistically significant differences in lesion size ($p < 0.05$).

Genotype	Least Sq Mean	Std Error	Connected Letter Report	Number of Biological Replicates
GU 123V	1.532	0.078380027	A	16
IMC 105	1.506	0.067174295	AB	27
CATIE R4	1.491	0.089931408	ABC	23
NA 807	1.467	0.075659049	ABC	16
PA 71	1.432	0.073466617	ABCD	24
ICS 1	1.426	0.090390574	ABCDE	22
PA 299	1.392	0.07372828	ABCDE	21
IMC 31	1.392	0.078089562	ABCDEF	15
PA 81	1.355	0.071434212	ABCDEFGH	26
PA 107	1.355	0.078252037	ABCDEFGH	13
NA 34	1.352	0.068767856	ABCDEFG	27
GU 195V	1.334	0.085623476	ABCDEFGH	14
PA 39	1.330	0.068733289	BCDEFGHI	23
IMC 57	1.317	0.081354406	ABCDEFGH	16
AMAZ 12	1.282	0.085205849	CDEFGHIJK	15
NA 33	1.274	0.100381718	CDEFGHIJKL	12
IMC 6	1.252	0.072213383	DEFGHIJKL	30
ELP 37A	1.247	0.070600716	DEFGHIJKL	25
KER 6	1.241	0.070636736	DEFGHIJKL	31
IMC 50	1.237	0.095919784	CDEFGHIJKLM	13
GU 263V	1.237	0.068025436	EFGHIJKL	23
PA 303	1.227	0.075222279	EFGHIJKL	17
NA 70	1.225	0.073588581	EFGHIJKLM	18
IMC 20	1.205	0.06639607	EFGHIJKLM	33
PA 169	1.201	0.087358469	EFGHIJKLMNO	12
NA 232	1.197	0.068843632	GHIJKLM	50
PA 70	1.196	0.067169608	FGHIJKLN	29
IMC 67	1.183	0.100285817	EFGHIJKLMNOP	12
IMC 97	1.181	0.077370353	FGHIJKLMNO	16
GU 139A	1.173	0.075603041	GHIJKLMNO	16
GU 140S	1.166	0.082971626	GHIJKLMNOP	15
GU 301A	1.142	0.085961241	HIJKLMNOPQ	12
PA 120	1.135	0.078099902	IJKLMNOPQ	16
ELP 40B	1.103	0.073325924	KLMNOPQR	19
YAL 3	1.100	0.077544225	KLMNOPQR	21
CATIE R6	1.096	0.089882222	JKLMNOPQRST	23
GU 156B	1.095	0.072870057	KLMNOPQR	28
GU 285B	1.089	0.081116252	KLMNOPQRST	23
NA 670	1.089	0.104649764	IJKLMNOPQRST	13
GU 255V	1.083	0.070632849	KLMNOPQRS	29
IMC 107	1.066	0.068517502	LMNOPQRST	24
IMC 47	1.058	0.100407172	KLMNOPQRSTU	12

PA 279	1.026	0.071272083	MOPQRST	26
KER 1L	1.001	0.075932648	NOPQRSTU	19
PA 13	1.000	0.070331827	OPQRSTU	23
STEEN 162/5 101	0.978	0.088224956	OPQRSTUV	10
PIÑA	0.973	0.089364522	OPQRSTUV	13
OYA 2B	0.964	0.065731325	PQRSTU	34
PA 16	0.959	0.074377179	PQRSTUV	19
GU 257E	0.944	0.068083695	QRSTUV	25
PA 150	0.914	0.071634661	RSTUVW	20
IMC 60	0.877	0.081789943	STUVW	16
NA 7/10	0.876	0.106529951	QRSTUVWX	10
SCA 6	0.858	0.093939461	TUVWX	10
AMAZ 15/15	0.815	0.077233243	UVWX	18
COCA 3370/5	0.754	0.078519711	VWXY	13
NA 246	0.738	0.06817577	WXY	26
SPEC 54/1	0.645	0.071789753	XY	21
NA 916	0.639	0.068722166	XY	26
Pound 7	0.630	0.057896559	Y	51