Marker discovery and application for the Taro (*Colocasia esculenta)* pathogen *Phytophthora colocasiae*

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Abstract

Taro, a non-graminaceous monocot, is a major staple crop in the Pacific, and is grown widely in the Caribbean, Africa, and Asia. *Phytophthora colocasiae,* causal agent of Taro Leaf Blight (TLB), seriously threatens the sustainability of this important crop. Resources for studying TLB are limited and a next generation sequencing approach was implemented to develop single nucleotide polymorphism (SNP) markers useful for studying natural populations and tracking virulence/pathogenicity in crosses. Sequencing was focused on the 200-400 bases surrounding the SgrA1 restriction enzyme cut site. A total of approximately 6M 55-75bp Illumina reads were generated for two isolates (an A1 and an A2 mating type) recovered from Taro at different locations in Vietnam. Reads were aligned to the *Phytophthora capsici* reference genome and variable sites identified. We will present an overview of the sequence coverage, heterozygosity, total number of putative SNPs and application of a subset of the identified SNPs to natural populations of *P. colocasiae* in Vietnam.

Introduction

Phytophthora colocasiae was first described causing leaf infections on taro in Java in 1900 and is considered to be a host specific and primarily a foliar pathogen. It is heterothallic (obligatory out crossing) and sexual oospores are formed in paired cultures of the A1 and A2 mating types. A survey of *P. colocasiae* on taro on the agriculturally important Hainan Island in South China revealed both mating types were common within single fields and the authors suggest this area may be within the center of diversity/origin for *P. colocasiae*. A more recent survey (2003) of isolates collected from taro in South East Asia and the Pacific using isozymes revealed significant variation in Thailand, Vietnam, Papua New Guinea, the Philippines, and Indonesia and that no isolate genotypes is shared among different countries. This differs from reports for Hawaii and Taiwan where only single mating types (either all A1 or all A2) were recovered from extensive surveys. The apparent low diversity on these islands may be due to recent introductions of virulent strains.

The work presented here supports efforts at the University of Hawaii to develop new resistant lines of Taro. Our goal is to develop a battery of SNP markers useful for characterizing the population dynamics of *P. colocasiae* and for characterizing virulence and pathogenicity in laboratory crosses.

Materials and Methods

We utilized a **R**estriction site **A**ssociated **D**NA sequencing approach known as **RAD** to obtain deep sequence coverage of the 200-400 base pairs surrounding the restriction cut site for the SgrA1 enzyme. Briefly, we extracted high quality DNA from two *P. colocasiae* isolates, an A1 (LT7299) and an A2 (LT7291) mating type recovered from Phú Hồ and Phú Lương (respectively) Vietnam. The DNA was processed by Floragenex, Inc. (Eugene, OR, USA) to cut the DNA, add the appropriate adaptors and to prepare the DNA for paired-end sequencing on an Illumina Genetic Analyzer. Sequences were aligned to the *P. capsici* genome (a close relative) and putative single nucleotide polymorphism (SNP) sites identified using the following criteria: Sequences must be >90% similar to reference and a site must have at least 10X coverage with uniquely aligned reads with an average quality score of 20. If a site has an alternate allele in 35 to 65% of the reads it is scored as heterozygous (**Aa**), if the alternate allele is in >90% of the reads it is scored as homozygous alternate allele (**aa**).

Figure 1: Healthy Taro (**A**),Typical foliar lesion caused by *P. colocasiae* (**B**) and Taro corms in the market in Vietnam (**C**).

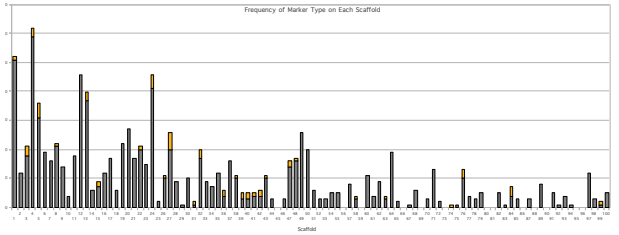
Results and Discussion

A total of 3.7 and 5.5 million reads were obtained from LT7291 and LT7299, respectively. Of these, ≈15% were able to be aligned to the *P. capsici* reference genome at 90% similarity.

When filtered based on the genotyping criteria (described above) a total of 389,269 nts had 10X coverage in both isolates with 1152 nts polymorphic between the two isolates and 24,941 nts fixed for an alternate allele in both isolates compared to P. capsici (Table 1). 821 nts were heterozygous in LT7291 and 776 nts were heterozygous in LT7299 suggesting a heterozygous site every 500bp in these two isolates.

	LT 7291 Aa	LT 7291 aa	LT 7291 AA
LT 7299 Aa	512	104	160
LT 7299 aa	109	24941	37
LT 7299 AA	200	30	

Table 1: Summary of sites useful for characterizing in vitro *P. colocasiae* crosses. Loci with fixed differences (N=67, orange) are useful for identifying oospore progeny. Sites heterozygous in at least one isolate (N=1085, gray) are potentially useful in mapping and for characterizing population dynamics. The remaining sites (N=24,941, white) are sites fixed between *P. colocasiae* and *P. capsici*.



Graph 1: Distribution of polymorphic markers across the largest scaffolds in the *P. capsici* reference genome (100 out of the 917 total scaffolds).Colors correspond to table highlights.

Thus far, our analyses have been intentionally conservative and there are likely many additional markers available for characterization in this set of sequencing data. In the future, we will investigate less stringent mapping criteria (e.g. 80%) and a comparison of contigs directly between the two isolates (no mapping to *P. capsici*). But, for our immediate purposes of (i) characterizing crosses between *P. colocasiae* isolates and (ii) characterizing genotypic and genetic diversity within natural populations, these markers are likely sufficient and valuable. Our immediate next step is to characterize a subset in a cross to determine Mendelian inheritance before application to natural populations.

Literature

Lebot, V., C. Herail, T. Gunua, J. Pardales, M. Prana, M. Thongjiem and N. Viet. 2003. Isozyme and RAPD variation among *Phytophthora colocasiae* isolates from South-east Asia and the Pacific. Plant Pathology. 52: 303-313.