Abstract

Bamboo serves as an important food source for pandas and has many medicinal and commercial applications. The Memphis Zoo grows a large bamboo farm located in Shelby Farms to feed their two pandas and the pandas in Toronto Zoo, Canada. We studied the genetic variation present in one of the bamboo species, *Phyllostachys bissetti*, from Shelby Farms using Simple Sequence Repeat (SSR) markers. The study provides valuable information for the Memphis Zoo and establishes the methodology for identifying clonal diversity and genetic variation in other bamboo species in natural stands located in China.

Introduction

Worldwide, there are over 1,400 species of bamboo. This member of the grass family, Poaceae, is found on every continent except Antarctica and Europe (Yasmin et al. 2014). Bamboo can be classified into two categories of the subfamily Bambusoideae. These two categories are woody bamboo or herbaceous bamboo. Woody bamboo is the most prevalent of the two and is a staple food source for pandas.

Bamboo reproduces both sexually by flowering and asexually through clonal growth via rhizomes. Most species flower every 60 to 130 years, and it is unknown when or why flowering occurs (Bamboo Botanicals 2014). For most species of bamboo, the entire stand dies after it flowers. There have been recordings of this phenomena occurring in Sichuan, China where 80-90% of its natural stand of *Sinorhizobium fangianum* flowered, leading to a loss of food and habitat for many animals (Taylor 1998). Understanding the genetic variation of a species is necessary to determine the fitness of individuals within a population and the likelihood of extinction (Mandell 2010). It is our goal to develop a method to understand the clonal variation within one species of bamboo and apply it to other species in the future.

Methods

Bamboo leaf samples, from Shelby Farms, were systematically obtained from each row within a stand of bamboo. A leaf was collected from one stalk every eleven feet on both sides of a row. Each leaf was labeled with a number and letter corresponding to the stalk location (see Figure 1). The culms from which the sample was collected was marked with field tape. The plant tissue was placed in a 2.0 ml tube containing three metal pellets and was ground in a SPEX Mixer mill. An Omega DNA Extraction kit was used to extract the DNA of the leaf. Two steps of micro-satellites from Eurofin Genomics were added to the DNA master mix along with one of three simple sequence repeat (SSR) primers, NEB, FAM, or VIC. A polymerase chain reaction (PCR) was performed, and the DNA fragments were visualized using gel electrophoresis. The amplified DNA products were sent to the University of Tennessee’s Molecular research center for fragment analysis. The fragments were analyzed and scored using the 2.6.3 version of Geno Mark computer software.

Results

Based on fragment analysis, it was determined that all three individuals are clones. At each locus, individuals 1, 2, and 3 either exhibited the same heterozygous or homozygous genotype with the exception of a few DNA failures which are indicated by the white box. Individuals with the same color box contain the same genotype for that locus. Figure 1 illustrates the bamboo stand displaying each individual's location and color-coded genotype. Below each picture is the name of the genetic marker used to identify the genotype. At least six out of ten markers have been successful.

Discussion

One main objective of this experiment was to determine a method that successfully extracts and amplifies DNA from bamboo tissue. Based on PCR and fragment analysis results, our protocol was effective. Establishing a methodology to understand the genetic makeup of a population is important for future research of natural stands of bamboo all over the world. If we can measure the genetic variation of individuals within a small population, we could apply this same methodology toward large natural stands in the wild. This protocol could be applied further to understand flowering patterns. We could determine which individuals would most likely flower at the same time based on genetic similarities and environmental factors. Conservation efforts could then be taken in a situation where mass flowering of one species might occur. It is our assumption based on fragment analysis that all individuals within one stand are clones. However, we cannot conclude this until the entire stand is analyzed in the same manner.

References


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