

The identification of bambusa sp

[Science](#), [Biology](#)



The identification of Bamboo using various PCR and Sequencing Techniques

Abstract Often the incorrect bamboo species is sold to unsuspecting customers at shops. This can have a disastrous effect on their garden. Three separate and unknown Bamboo leaf samples were taken and were required to be distinguished genetically from one another. Using ITS-PCR DNA amplification techniques, the ITS region DNA was amplified and used in PCR-RFLP and RAPD PCR in order to determine the genetic identity of each sample. Sequencing was performed, and results allowed us to distinguish between samples (to a certain extent.)

Introduction

Bamboos are a group of woody perennial green plants (Wikipedia et al. 2006) that are found in many parts of the world. There are 91 genera and about 1, 000 species of bamboo (Wikipedia et al. 2006). They are found in diverse climates, from cold mountains to hot tropical regions. Bamboo is a highly desirable plant grown for many reasons in plantations and gardens around the world. Many reasons it is grown are that it is a beautiful ornamental plant with unique properties. Bamboo is also an extremely strong plant that is light; it is used in many building applications for floorboards, and is also often used in furniture making.

There are a number of taller growing species that are effective at blocking out the eyes of pepping toms and nosy neighbors. There are two main forms of bamboo, each form describing the way in which the bamboo itself spreads. These are known as “ clumping” (monopodial) and “ running” (sympodial) forms. (Wikipedia et al. 2006) Clumping bamboo species tend to spread underground slowly. Running bamboo species are highly variable in their tendency to spread; this is related to both the species and the soil and

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climate conditions. Some can send out runners several metres a year, while others can stay in the same general area for long periods.

If neglected, they can be invasive over time and can cause problems by moving into adjacent areas. The reputation of bamboo as being highly invasive is often exaggerated, and situations where it has taken over large areas is often the result of years of untended or neglected plantings. Many invasive bamboo species are often sold, unsuspectingly to people, who plant them without realizing this. The result of this is the complete takeover of ones garden. Some species of bamboo can grow at a tremendous rate, some at over 36inches (90cm) a day, providing it is provided with ideal conditions (OneEarth, 2006).

Plant Biosecurity breaches often occur when bamboo plants are imported with incorrect or false labeling, often in an attempt to bring illegal ornamental species in to the country for indoor use. This ‘ black market’ operation is a serious threat to native species of plants, and, if a threatening sympodial bamboo species is imported and planted in place of a monopodial (which is preferred, as they do not spread), serious damage to native forests and grasslands can occur (NGIA, 2006). Some of the techniques that can be used to identify to a species level are PCR-ITS, RAPD, and PCR-RFLP.

These will be used to identify our unknown samples of bamboo. Aim To identify, to a species level, using nucleotide analysis and sequencing techniques, three unknown samples of bamboo. Materials For DNA extraction
3 Unknown Bamboo Samples (Leaves) Mortar and Pestle Liquid Nitrogen
Quiagen ‘ Dneasy’ DNA Extraction Kit Centrifuge tubes Pipettes and Tips Ice

and Esky Quantification of DNA Well Combs (10uL) Wells UV Transilluminator
Agarose Tris Borate EDTA Ethidium Bromide Loading Dye Centrifuge Tubes
Gel Tank (To run agarose gel electrophoresis) Pipettes and Tips

For ITS based PCR 5uL of extracted DNA 5x Reaction buffer MilliQ (Ultra Pure Water) DNTP's (dATP, dGTP, dCTP, dTTP) PCR Machine MgCl₂ Centrifuge Tubes Pipettes and Tips For RAPD-PCR ITS-PCR DNA product 5x Reaction buffer MilliQ (Ultra Pure Water) MgCl₂ Primers OPM-01 and OPM-17 Wells Well Combs (10uL) UV Transilluminator Agarose Tris Borate EDTA Ethidium Bromide Loading Dye Centrifuge Tubes Gel Tank (To run agarose gel electrophoresis) Pipettes and Tips For ITS-RFLP ITS-PCR DNA product Enzymes Hha1 and Rsa1 Buffer Red (Rsa1) Buffer C (Hha1) MilliQ (Ultra Pure Water)

Wells Well Combs (10uL) UV Transilluminator Agarose Tris Borate EDTA Ethidium Bromide Loading Dye Centrifuge Tubes Gel Tank (To run agarose gel electrophoresis) Pipettes and Tips Methods DNA Extraction and Purification - Quiagen Dneasy Kit ITS-RFLP ITS Region is a particular sequence of DNA which is present in all organisms. It is a region, in between each common sequence, contains DNA that is highly conserved and unique amongst a particular species, and is thus not used to translate into proteins. Enzymes are used to restrict or cut the DNA at certain points.

The location of the cuts depends on nucleotide sequence that the enzyme recognizes. The number of nucleotides in sequence determines size of the restricted piece of DNA in base pairs (BP). ITS-PCR This is done to amplify the ITS region DNA which is highly conserved and unique to each individual

species Primers ITS 1 and ITS 4 are used because the ITS region (18s, 5.8s and 28s regions) are common in all organisms. The region in between the 18s and 28s is the region that is highly conserved and unique to any given species. Added to Master Mix (containing buffer solution) PCR'd ITS Region DNA is amplified out RAPD

RAPD Primers OPM-01 and OPM-17 are added to the ITS-PCR DNA product and where are given a genetic fingerprint of the DNA. HOW, WHEN, WHAT,

WHERE, WHO? What was done? Sufficient detail for repetition by others

Results (facts only) (2) HOW, WHEN, WHAT, WHERE? What was found?

Presentation of results as simply and clearly as possible Figures to present data and concepts clearly and concisely (a picture is worth 1000 words)

Types of figures: photographs, drawings, tables, graphs Numerical data as tables or graphs (graphs preferred) Text to point out trends (not repeat information in figures) Discussion (3) WHY, WHAT, WHO?

What does it mean? Interpretation of results relative to the hypothesis or aim

Comparison with work of others References (6) WHO? List of all references

cited in text <http://www.bonsai-bci.com/species/bamboo.html> Sabrina

Caine Last modified accessed 01/06/06 <http://en.wikipedia.org/wiki/Bamboo>

wikipedia last modified 27/05/06 accessed 01/06/06 [http://www.1earth.com.](http://www.1earth.com.au/collect/wicker_furniture.html)

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1Earth Antiques and Appraisals [http://www.ngia.co.nz/news/507bamboo.](http://www.ngia.co.nz/news/507bamboo.php)

[http://www.ngia.co.nz/news/507bamboo.](http://www.ngia.co.nz/news/507bamboo.php)

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