GENETIC DIVERSITY IN THE ANTHRACNOSE PATHOGEN INFECTING *STYLOSANTHES* IN BRAZIL, INDIA AND CHINA

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Abstract

This work aimed to determine the genetic diversity of *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in Brazil, China and India. A total of 132 isolate originating from *S. seabrana, S. macrocephala, S. capitata, S. scabra,* and *S. guianensis* were used. Four major genetic groups were identified from an analysis of genetic diversity using selection-neutral DNA markers. Group 1 contained 20 isolates and this may represent a genotype that migrated from the center of diversity in Brazil and Colombia to Australia, Thailand and India. Group 2 consisted of 66 Brazilian isolates and group 3 had 19 isolates from Australia, Burundi, Brazil, China, Colombia, Ivory Coast and Peru. The 27 isolates in group 4 were very diverse with >50% dissimilarity between some isolates. Genetic diversity in Brazil and China was more extensive than in the Indian pathogen population.

Introduction

Despite their agronomic suitability, commercial utilization of *Stylosanthes* spp. has been severely curtailed by anthracnose caused by *Colletotrichum gloeosporioides*. The genetic plasticity of *C. gloeosporioides* at its center of diversity in South and Central America (Chakraborty et al., 1997; Kelemu et al., 1997) has caused the demise of at least five of the eight cultivars released (Miles and Lascano, 1997). There is a similar history of cultivar devastation by anthracnose in Australia, China, Thailand, The Philippines and other parts of South East Asia (Guodao et al., 1997).

Durability of anthracnose resistance may be predicted by the propensity of a pathogen population to change and an improved knowledge of pathogen diversity is essential for this. Selection-neutral Random Amplified Polymorphic DNA (RAPD) markers have been used to demonstrate genetic variation in *C. gloeosporioides* population infecting *S. scabra* in Australia (Chakraborty et al., 1999) and *S. guianensis* in Colombia (Kelemu et al., 1999). Scant information is available on the genetic diversity of isolates infecting other *Stylosanthes* spp. in the center of diversity or in any of the countries where these species are increasingly used for animal production. Through an international collaboration between Australia, Brazil, China, Colombia and India, this work examines the genetic diversity in 132 isolates of *C. gloeosporioides* originating from *S. seabrana, S. macrocephala, S. capitata, S. scabra,* and *S. guianensis* from these and other countries.

Material and Methods

Isolates were imported under quarantine permit to the CRC for Tropical Plant Pathology laboratories at the University of Queensland, Australia, for genetic analysis. Single-spore cultures were multiplied on ¼ strength clarified V8 juice broth and mycelia and conidia were ground in an

extraction buffer (250 mM Tris HCl, 200 mM NaCl, 25 mM EDTA and 5% SDS), centrifuged, cleaned with phenol chloroform, followed by a chloroform wash and DNA was precipitated in ethanol. For RAPD analysis DNA from duplicate samples of each isolate was amplified by polymerase chain reaction for 8 arbitrary decanucleotide primers (Operon Technologies Inc. Alameda, CA94501) in a programmable thermocycler (MJ Research, USA). Amplification product (15 μl) was electrophoresed in 1.6% agarose gel containing 0.024% ethidium bromide.

Similarity values were calculated from pooled data using the formula $F = 2m_{xy}/[m_x + m_y]$, where m_{xy} = the number of shared bands and m_x and m_y are the number of bands in each isolate. The Unweighted Pair Group Method with Arithmetic Mean was used for a cluster analysis of the similarity values and the SAS procedure 'TREE' was used to generate a dendrogram (SAS Institute, Cary, NC).

Results and Discussion

A total of 92 reproducible RAPD bands ranging from 380 to 2800 bp were obtained using the 8 primers. Using a dissimilarity value greater than 25% from the cluster analysis, the majority (105) of the isolates could be grouped into three main groups (Fig. 1). The remaining group of 27 isolates from Brazil, China, Colombia, Nigeria and The Philippines are very diverse and many of these are genetically distinct with more than 50% dissimilarity between isolates. Majority of these was isolated from *S. guianensis,* with a smaller number from *S. hamata* and *S. capitata.*

Group 1 contains 20 isolates from *S. capitata, S. fruticosa, S. scabra* and *S. seabrana.* Of these, 3 isolates were from Australia representing the 3 dominant races of Biotype A, 3 were from Colombia, one each from Brazil and Thailand and the remaining 12 were from India. This group may represent the original genotype that migrated from the center of diversity in Brazil and Colombia to become established in Australia, Thailand and India.

Group 2 consists of 66 isolates; all from Brazil and 44 of these are of the same genotype. Over 80% of group 2 isolates originate from the same set of selected accessions of *S. capitata, S. macrocephala* and *S. scabra*. Similar association between *S. guianensis* and *S. scabra* and *C. gloeosporioides* genotypes has been recorded earlier in Australia (Chakraborty et al., 1999) and Colombia (Kelemu et al., 1999).

The 19 isolates in group 3 come from Australia, Burundi, Brazil, China, Colombia, Ivory Coast and Peru. Eleven of these are from *S. guianensis* including an isolate of Biotype B from Australia. It is not known if isolates in this group have similar symptomatology, host range and karyotype to the Australian Biotype B. We know, however, that many group 2 isolates produce discrete lesions similar to the Australian Biotype A on all host species including *S. guianensis* (Perrott and Fernandes, unpublished information). Further investigation is needed to determine the existence of distinct pathogen Biotypes in Brazil.

Due to the small number of isolates studied in this work, it is premature to comment on the genetic diversity of *C. gloeosporioides* population in China and India. However, some practical observations can be made. The genetic structure of the pathogen population in India appears to be relatively simple, with less than 10% overall dissimilarity. This may indicate that the pathogen has established in India following a single introduction. *S. fruticosa* is believed to be indigenous to India and the pathogen population on this host may be more diverse. We have used only 2 isolates from this host and a more extensive collection is warranted. Nevertheless, a lack of extensive genetic diversity does not imply a limited pathogenic variation in India. Evidence from Australia suggests that different pathogen races can arise from the same genetic background (Chakraborty et al., 1999). By comparison, the genetic structure of *C. gloeosporioides* population in China is far more complex. The high level of dissimilarity between some isolates indicates that the pathogen may have been introduced on more than one occasion. Given the relatively short history of *Stylosanthes*

utilization in China, it is unlikely that the pathogen would have evolved to produce such extensive diversity.

The extensive diversity observed in the anthracnose pathogen in this study highlights the need for new *Stylosanthes* cultivars with different resistance to minimize potential anthracnose damage to the limited range of cultivars available in Brazil, China and India.

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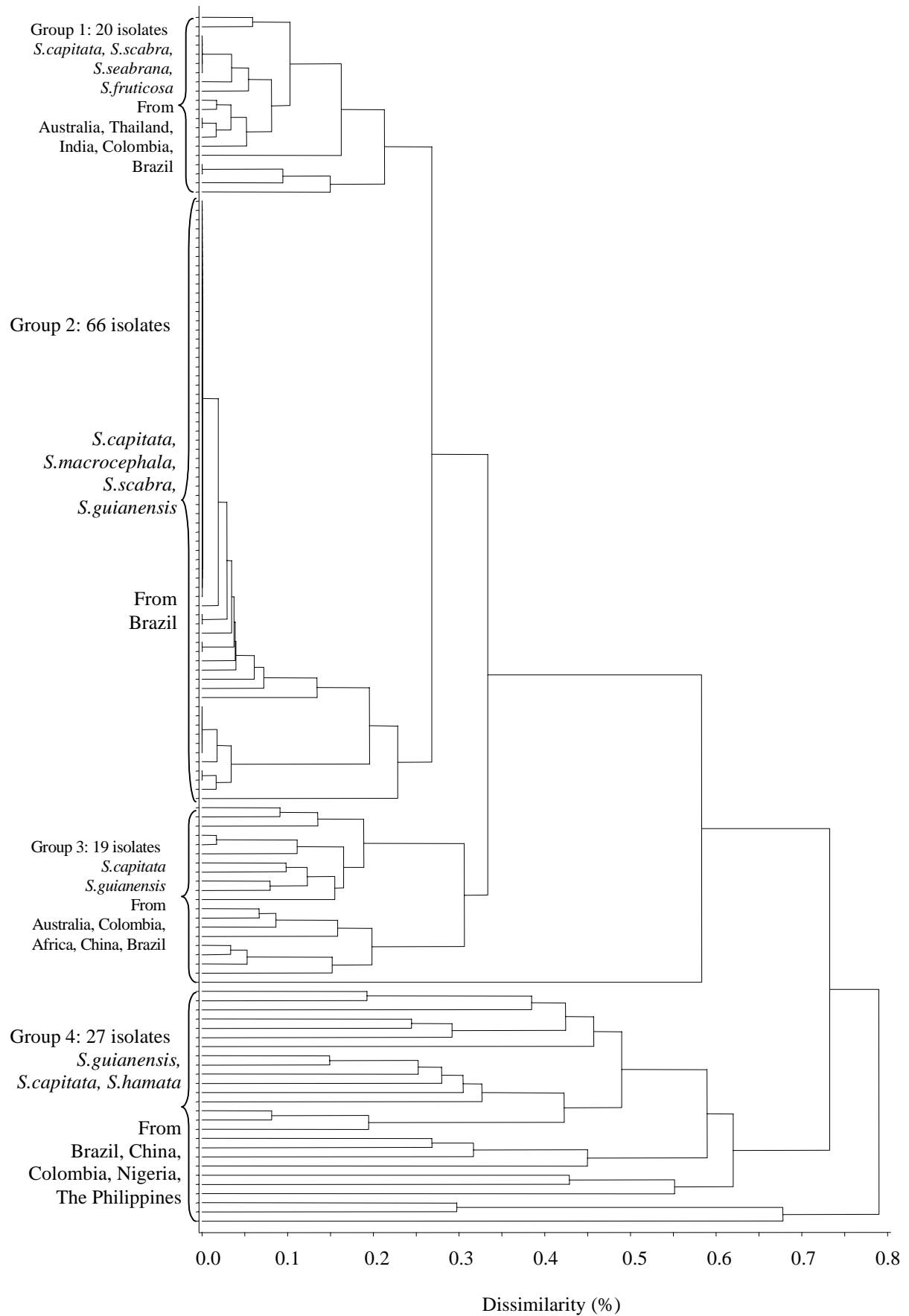
Figure 1 - Genetic dissimilarity between 132 *Colletotrichum gloeosporioides* isolates collected mainly from Australia, China, Colombia, Brazil and India.