8th International Conference of the Brazilian Association for Bioinformatics and Computational Biology
OCTOBER 14th to 17th
X-meeting 2012
Unicamp Convention Center | Campinas - São Paulo

Building a transcriptome molecular marker platform for diagnosis of fungal plant pathogens

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Background: Banana, one of the most important crops worldwide is threatened by a destructive disease caused by Fusarium oxysporum f. sp. cubense (Foc). Foc is a soilborne pathogen that produces chlamydospores, enabling the fungus to persist for up to 30 years in soil in the absence of the host. Four races attacking banana cultivars are recognized: race 1 (R1), race 2 (R2), subtropical race 4 (ST4) and tropical race 4 (TR4). R1 and R2 are distributed worldwide causing significant losses in local varieties, ST4 affects Cavendish in the subtropics and TR4, which affects almost all the cultivated bananas, is still restricted to South-East Asia. Despite the importance of this pathogen, genomic data is still scarce and only for TR4 a molecular diagnostic is available. Results: By using an Expressed Sequence Tag (EST) library from all four races of Foc, we developed a molecular marker platform based on single sequence repeats (SSR). The search criteria were restricted to SSR length ranging from dinucleotides (at least 8 repeats) to decanucleotides (at least 4 repeats), rejecting all monomeric sequences. Furthermore, analyses focused on the identification of regions with designable reverse and forward primers enabling amplicons ranging from 300bp to 4,000bp to facilitate validation by wet-lab experiments. This approach resulted in 4,822 SSRs, from which 4,459 were applicable for PCR experiments. The four races had 10,216 transcripts in common resulting in 2,675 SSRs. Those race-specific transcripts are 101, 166, 47 and 78 - with 8, 26, 6 and 11 repeats – for R1, R2, ST4 and TR4, respectively. Interestingly, the longest SSR (112 bp, TCGTTCGT, 14 repeats) was unique for the R1 and R2 VCGs. Conclusions: This SSR platform opens new windows for elucidating the Foc genetic evolution and the development of molecular diagnostics for enhanced disease management and the implementation of quarantine measures.

Keywords: molecular marker, Fusarium oxysporum, fungal pathogen
Concentration area: Genomics Evolution

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