Conserved Genes and Universal Primers in Bananas and Plantain

Genome sequencing of rice, Arabidopsis and other species has given the sequences of genes, and most genes have been assigned functions. Now we need to identify the homologous genes in other species to characterize gene diversity and new functional alleles.

Despite the importance of bananas as a subsistence and cash crop throughput the tropics and sub-tropics, there has been only limited genomic research.

Objectives:

- To develop PCR primers to identify conserved orthologous genes in various species
- To exploit the primers to assess gene diversity and classify accessions
- To identify and characterize genes related to abiotic and biotic stress from banana and plantains (*Musa*)

- The work was carried out in conjunction with training programmes

Published Conserved Orthologue Set (COS) Markers

Fulton, Hoeven, Eannetta & Tanksley (2002, Plant Cell 14:1457) published nine primer pairs designed in tomato and *Arabidopsis* to isolate putatative orthologous genes.

- In banana species:
  - Three gave no amplification
  - Two gave weak bands or smears
  - One amplified retroelement sequences
  - COS1263 amplified 600bp transmembrane protein
  - COS1006 amplified a 220bp fragment
  - COS1358 (cellulose synthase) amplified unreliably and needed to be optimized

COS1263 Amplification in 13 out of 20 *Musa* accessions

Even across large taxonomic distances and using non-optimized primers, about a third of genes are amplified.

Conserved Primer Development

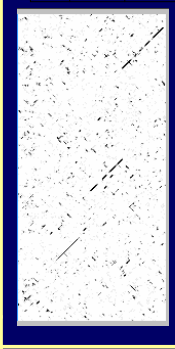
A targeted gene and informatic approach has been used to identify primers to amplify genes in the flavonol pathway and genes which have been related to abiotic stress responses. Where available, EST sequences from *Musa* have been included in the analysis, and both predicted protein and DNA sequences used for alignment. Primer design was more difficult for proteins rich in amino acids encoded by 6 codons (eg leucine, serine) or with AT-rich codons (eg lysine, tyrosine).

Example: optimised forward primer for cellulose synthase

```
hordeum_vulgareBQ468780     AATGATGAATCAGCTACAGTTGAGCTTTGCTGGAGGAGACCGTCAGGGCT 496
secale_cerealeBQ160068      AATGATGAATCACCTACAGTTGAGCTTTGCTGGAGGAGACCGTCAGGACT 417
hordeum_vulgareAV935566     AATGATGAATCAGCTACAGTTGAGCTTTGCTGGAGGAGACCGTCAGGGCT 385
wheatCD240064.1             AATGATGAATCAGCTACAGTTGAGCTTTGCTGGAGGAGACCGTCAGGACT 258
Banana 600138291            GAAGTTGTATCAGCAACAGTTGAACTTTGTAGTAGAAGGCCATCAGGGCT 412
CERES36434A. thaliana       GATATCGAATCTGCTACAGTTGAGCTTTGCTGCAAACCACCATCAGGGCT 230
                             *   * ***  * ********* *****  * *      ** ***** **
```

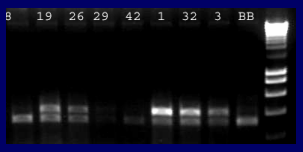
Example: Primers for flavonol pathway genes

No	Name	Primer sequences	Annealing Temp	Size	Gene
1	MYC	TCATCACCGGAGATGCCACGG-F TCCTCGACGACCACCACACG-R	62	~448	Anthocyanin regulatory R-S protein
2	CHS	TCTCCGACGCCTTCAGCACG-F AACATGGAGCCGGAGCCTCGCG-R	56	~501	Chalcone synthase
3	DFR	TGTCTCAAGCCATCAGGGCCT-F GATGTTCCGAGCGCGATACCC-R	56	~380	Dihydroflavonol-4-reductase (Dihydrokaempferol 4- reductase).
4	ACR	AGCAGTCCTACAGCCTCTGCA-F ACGAGTCTGCCGGAGCTTGCC-R	62	~204	Anthocyanin reductase



Dotplot of Musa EST (x) vs Rice BAC (y) showing three exons and three introns. Comparison of ESTs and heterologous BACs shows locations of exons and introns and hence design of intron-spanning primers which are often polymorphic.

Polymorphisms in chalcone synthase products



Conserved Primer Notes

- PCR primers are relatively straightforward to design to isolate genes from genomic DNA in Musa based on cereal and Arabidopsis sequences.
- Design requires typically >60% homology over 100 bp at DNA level, or >30% similarity over 20 aa at protein level. This is less homology than is required for homologue identification by Southern or colony hybridization.
- Use of low annealing temperature or degeneracies in products were equally successful.
- Product polymorphisms and background smears made cycle-sequencing un-usable, so products needed to be cloned.

Major contributors to this work, supported in part or whole by the Generation Challenge Programme include: Ashalatha Nair, Kerala, India; Azhar Mohamad, Malaysia; Dhairyasheel Desai, Pune, India; Ojay Benedict, Uyo, Nigeria; Suresh Kumar, India; Zul Mohamed, Malaysia; Emmanuel Otwe, Ghana; Luis Rodriguez-Zapata, Mexico and the laboratory of Pat Heslop-Harrison and Trude Schwarzacher, Leicester UK

Generation
Cultivating Plant Diversity for the Resource-Poor

University of Leicester

IPGRI