

Stress response-enriched EST resources target species:

Cowpea

Generation Challenge Program

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Sarah Hearne

Richard Bishop

Chris Town

Morag Ferguson

Jean Hanson



Cowpea

- ▶ Grown on at least 12.5 M Ha worldwide
- ▶ Staple for C 200 million Africans : protein-rich nutritional supplement to cereal grains and stover for improved human and livestock nutrition.
- ▶ Genome size only slightly larger than that of the model legume *Medicago truncatula* (613×10^6 bp)
- ▶ Well-studied conventional genetics



Cowpea

But

- ▶ Poorly characterised at the genomic level
- ▶ Current limitations to utilisation of contemporary technologies include lack of molecular tools such as SSRs, SNPs and COS markers
- ▶ Impacted on SP1 genotyping, SP3 QTL mapping



Objective

Develop drought stress-response enriched EST resources

A comprehensive annotated EST collection would provide an invaluable tool facilitating;

- The development of new molecular markers
- The identification of putative COS markers
- The development of oligos for microarray and real time PCR



Cowpea



- ▶ **Tvu7778** (drought susceptible)
- ▶ **Tvu11986** (type I drought tolerance: maintenance of all leaves)
- ▶ **Dan IIa** (type II drought tolerance: maintenance of meristematic activity in shoots and growth of young leaves by loss of unifoliate leaves)
- ▶ **12008 D** (a fodder type, unknown response to drought)

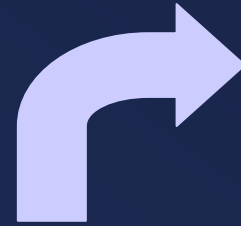
1. Mai-Kpdomi, Y., Singh B.B., Myers, O., Yopp, JR., J.H., Gibson, P.J., Terao, T. 1999 Indian Journal of Genetics, **59** (2) pp. 309-316
2. Maia-Kodomi, Y., Singh, B. B., Terao, T., Myers, O., Yopp, JR., J. H., and Gibson P. J. 1999. Indian Journal of Genetics, **59** (2) pp. 317-323
3. Singh, B.B., Mai-Kodomi, Y. and Terao, T. 1999. Indian Journal of Genetics, **59** (2) pp. 211-220.



Cowpea



Re-watered



Drought



Irrigated



Tissues

Materials harvested from 3 treatments (see poster for time point details)

- Leaves
- Roots
- Shoot including meristems

Extract RNA from each separately then pool in 1:1:1 quantity ratios on a genotype basis (as soon as I leave this meeting!)



Library construction

Normalized libraries

Normalization to be performed by Evrogen (Moscow) (Oct)

Ligation mixes sent to TIGR – prepare libraries and sequence
(Nov, Dec)

Annotation and preparation of unigene set (Dec.....)

Utilization of sequence information:

Marker development: SSRs, SNPs and COS

Oligos for microarray



Modifications.....

Don't use subtractive libraries : more funds for sequencing- could generate as many as 16,000 unique sequences (4000 uniques per library)

Use only one normalized library : larger unigene set, risk loosing rare amplification, how much information lost; value of information gained?

