III. Final Report—Narrative

a. Activities for Objective 3

Improve common bean productivity for marginal environments in sub-Saharan Africa

Activity 1.

We evaluated a reference collection consisting of 202 genotypes, separated into one Mesoamerican (11 × 11) and one Andean lattice design (9 × 9). The collection was distributed and grown in two sets at the six principal locations for evaluating beans for drought resistance: CIAT–Colombia, SARI–Ethiopia, University of Nairobi–Kenya, DART–Malawi, SELIAN–Tanzania, and CBI–Zimbabwe.

Because of differences in adaptation, different numbers of genotypes were grown in each location and across years while the full set was grown at CIAT–Colombia and the SABRN countries. Additional distributions were made to Zimbabwe and South Africa as part of Godwill Makunde's PhD project (University of the Free State, Republic of South Africa). A description of this collection's genetic structure was published in *Theoretical Applied Genetics (3)*.

Another set of 202 regional and local varieties was established and distributed among locations within the ECABREN and SABRN networks, and evaluated at CIAT–Colombia.

Finally, landraces from Kenya and Ethiopia were evaluated phenotypically and genotypically by Asrat Asfaw as part of his PhD (SARI/Wageningen). The results were published in *Theoretical Applied Genetics (1)*. Coordination with TLII's phenotyping locations was, in many cases, key to the success of this part of *Objective 3*.

Activity 2.

We identified 1,500 SSR loci and evaluated over 1,000 microsatellite markers. Results were published in three articles in *BMC Plant Biology (6)*, *Theoretical Applied Genetics (2)*, and *Genome (4)*, with a fourth submitted to *BMC Genomics (8)*.

The source of these markers varied: EST-SSRs were developed from cDNA, AT-rich microsatellites from enriched libraries, GA- and CA-motif microsatellites from un-enriched libraries, and BAC-end-derived microsatellites from collaboration with the Purdue/USDA sequencing project with a CIAT genotype (G 19833). Over 200 of the markers were genetically mapped and 400 were assessed for polymorphism among the parents and genotypes described above.

Other marker types tested were a legume ortholog GoldenGate OPA Assay of 786 SNPs (with *Objective 5*), 10 ADOC gene markers, and an additional set of 93 SNPs, which were validated by mapping with CEL I nuclease and with SSCP polymorphisms. Results were published in *BMC Genomics (9)* and *Crop Science (10)*, showing the first synteny comparison between common bean and the full-genome sequence of soybean.

Two subtractive and two full-length cDNA libraries were constructed with mRNA from plants either stressed or not stressed by drought. The subtractive libraries were made at CIAT, while the full-length libraries were made at RIKEN (Japan) from two different genotypes representing different genepools for discovering more SNPs and aligning with genomic sequencing. We made 20,000 clones, shipping half to Washington University in St. Louis (MO, USA) for sequencing. The success rate for the full-length clones was very high (96.4%), with an average sequence length of 760 bp and more than 9,000 unigenes found.

Most of the unigenes represented 5′-end sequences of genes that had not previously been sequenced by other EST development efforts. This work builds on sequencing conducted by CIAT/UNAM–Mexico/University of Minnesota, which generated 22,000 ESTs but only 5,000 unigenes. The results validate the utility of full-length cDNA clones, which are unique, compared with shorter, tissue-specific, cDNA libraries. The uni-genes will help us continue analysing ADOC drought gene types ASR, DREB, ERECTA, and SUSY/SPS.

Activity 3.

MAS for biotic resistance, we developed and tested a set of 30 SSR markers for bruchid resistance. This arcelin-based resistance derives from wild beans and, after 200 crosses, was incorporated into Andean beans with commercial seed types, particularly red mottled, cream mottled, and large red. This work is innovative as such resistance had never been deployed in a cultivar, much less in commercial-type lines. Two articles on these new results, published in *Theoretical Applied Genetics (5)* and *BMC Plant Biology (7)*, describe the markers, the complex process of moving the arcelin gene from wild to cultivated backgrounds, and the linkage disequilibrium present at the locus, as compared with other genomic regions.

Resistance to two other biotic stresses was also targeted for pyramiding with each other or with the arcelin gene: bean common mosaic necrosis virus (BCMNV) and common bacterial blight (CBB). Techniques for the rapid assessment of gamete selection populations were developed and perfected for evaluating 4,000 segregants at a time. The techniques proved useful for evaluating double, multiple, triple, and top crosses. Lizzie Kalalokesya (SABRN/University of Zambia) is evaluating three markers for CBB resistance QTLs. Together with Godwill Makunde (CBI), Lizzie was sponsored by TLII and has received laboratory training from CIAT–Colombia and in the Republic of South Africa.

Activity 4.

MAS for abiotic stress resistance, two QTL mapping populations were evaluated in four locations (Awassa–Ethiopia, Thika–Kenya, Chitedze–Malawi, and CIAT–Colombia) over one or more seasons and under drought and no-drought conditions. Root depth and photosynthate mobilisation were the two target traits for the two populations, together with collecting data on yield. This activity formed the theme for the theses by Asrat Asfaw and Felix Waweru (MSc, University of Nairobi). Additional phenotypic data on the best adapted lines and on cross-year adaptation was collected at other locations in each country, including the first QTL evaluation for farmer preference in a bean RIL population!

Activity 5.

We crossed two eastern and southern African varieties (CAL 96/K132 and CAL 143/Napilira) with two drought-tolerant Durango-derived breeding lines (SEA 5 and SEA 15). The idea was to develop new populations for MARS and evaluate drought tolerance in an Andean background. In addition, a North Carolina Design II diallel was created to introgress drought tolerance from five Mesoamerican sources and five Andean sources into five commercial cultivars from southern Africa. The result was the production of over 1,000 CBIB (Crop Breeding Institute Bean) and DAB (Drought Andean Bean) advanced lines in two breeding programmes (CBI–Zimbabwe and CIAT–Colombia, respectively).

Simultaneously, 50 crosses were made with Mexican- or Durango-derived genotypes (e.g. Pinto Villa and SEA lines) with the biotic and abiotic stress sources described above to test their tropical adaptation. Two full advanced backcross populations, for use in TLI–Phase 2, were also developed by crossing two ESA varieties with two Mesoamerican SER lines. These latter are among the most advanced small red beans for drought tolerance.

III. Accomplishments

Objective 3

- Over 1,500 SSR and 800 SNP markers were developed and tested. These represented markers of many different sorts, including groups of drought or orthologous legume genes and genomic or cDNA-based markers and involved collaboration with Objective 5.

- Over 600 genotypes were transferred from CIAT to Eastern and Southern African NARS partners (ECABREN and SABRN network participants) in this Project. Testing was done in collaboration with TLII at improved drought sites throughout the region.

- Emphasis was on pre-breeding for drought tolerance and the use of genetic resources for marker training and for understanding of drought tolerance in common bean (with a reference collection, two sets of regional varieties, and six RIL or Advanced backcross populations developed).

- A set of 10 high-impact documents, covering marker development and use, and germplasm screening, were accepted for publication.

- Six MSc and PhD candidates used TLI-generated populations for their theses and novel research in modern breeding.

IV. Impact

Objective 3

Major impact has been achieved through teamwork among 6 breeding programmes—CBI, CIAT, DART, SARI, SELIAN, and UoN—and a cadre of 6 young scientists trained in modern breeding and use of markers for crop improvement. Impact included:

- Making possible regional evaluations of innovative genetic stocks such as a reference collection (200+ genotypes), diallel-derived drought-tolerant lines (300+ CBIB and DAB lines), and regional varieties (100 + genotypes). Evaluations were carried out at CIAT and across two bean networks in eastern and southern Africa, particularly Ethiopia, Kenya, Malawi, Tanzania, and Zimbabwe. Other crosses and populations with sources of biotic and abiotic resistance were also distributed.

- For drought locations, more than 25 QTLs affecting yield were identified. These should be useful for breeding advanced lines more quickly. Also identified were 30 markers for biotic resistance.

- The value of MAS was shown when, for the first time, commercial-type lines, combining resistance to drought, bruchids, and BCMNV, were made. This was achieved by developing gene-based markers for candidate genes for drought tolerance, using the advanced technology of full-length cDNA cloning. Closely linked markers for arcelin resistance breeding were also identified and deployed, providing bruchid resistance combined with BCMNV resistance. Populations for MARS in Phase 2 were established and will be used to achieve higher levels of drought tolerance in Andean beans.

V. Lessons Learned

Objective 3

- For germplasm evaluation, we learned that climbing beans are less adapted to the construction of a reference collection. We therefore used only bush beans.

- For gene-based marker development, we discovered that many exon sequences have low polymorphism within intra-genepool crosses and therefore we developed genomic SSRs and intron-based markers to complement gene-based markers.

- For breeding activities, we found dwarf lethality in some inter-genepool crosses but overcame this by trying more combinations and using genotypes that did not contain the recessive alleles for this trait.

- The use of large numbers of markers for drought-stress mapping populations overcame limited polymorphism but highlighted the importance of highly polymorphic SSRs (such as AT-rich microsatellites) to track down drought-resistance traits that will be important for TLI–Phase 2.

VI. Challenges

Objective 3

- The depreciation of the dollar was perhaps the biggest challenge, as it changed the available budget in some countries, especially for operations and personnel.

- Coordination of some field locations was a challenge at the beginning, given the political situations in Kenya and Zimbabwe. Fortunately, these were overcome.

- Sequencing full-length genes was more costly than expected because of cost increases at Washington University in St. Louis and because less counterpart funding than expected was received from RIKEN (Japan). Hence, we could sequence only from one end of each gene rather than from both ends. We made up for this by constructing two gene sub-libraries and exceeded our goals in germplasm, pre-breeding, and marker development.

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