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Highlights

CIAT training

In the last few years two new projects have joined the Kirkhouse Trust (KT)-funded African Bean Consortium (ABC): the Rwandan Agricultural Board (RAB) in Rwanda and the Southern Agricultural Research Institute (SARI) in Ethiopia. Along with the existing ABC projects in Tanzania, Uganda and Kenya, the common goal is to improve common (or French) bean (*Phaseolus vulgaris*) by using a combination of conventional breeding and marker assisted selection.

Figure 1: Theory session at RAB, Rwanda

A major contribution from KT is to establish functional on-site laboratories, to allow the necessary molecular and pathology procedures to be carried out. Just as important as the equipment is the capacity building element, especially for new projects such as those in Ethiopia and Rwanda, as this will ensure that the staff have familiarized themselves with all of the relevant equipment and protocols.

A key provider of training and capacity building has been the International Centre for Tropical Agriculture (CIAT) in Kawanda, Uganda, which was party to much of the training delivered at the outset of the ABC programme. During 2015, two CIAT staff members (Allan Male and Catherine Akam) spent time at SARI (January) and RAB (May); at each location, they oversaw an intensive two week long training course which combined hands-on practical training with theory.

Figure 2: Pathology session at SARI, Ethiopia

In both cases, an important activity was to unpack the equipment which had been donated by KT, and to set up the working laboratory space.

Figure 3: Screenhouse session at SARI

On the molecular side, the ABC projects utilize DNA extraction (from plants and/or fungal/bacterial cultures), PCR amplification and gel electrophoresis. The demonstrations at both RAB and SARI both featured two DNA extraction methods: the "Mahuku method" and the use of FTA cards. On the pathology side, the activities focused on the collection of diseased plant tissues from the field, media preparation, and the isolation and conservation of fungal cultures.

Yayis Rezene, PI, SARI ABC project: 'The training addressed many of the skill gaps in basic molecular biology and pathology which need to be addressed before our technicians and scientists can work effectively in the area of marker assisted selection. The training indeed equipped us with a wide range of knowledge of relevance to marker assisted selection.'

Annuarite Uwera, MSc student, RAB ABC project: The molecular session focused on the practicalities

Figure 4: Collection of samples from the field in Rwanda

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CIAT training *Cont'd...*

of applying markers in our common bean breeding programme: for instance, we learnt how to prepare various reagent solutions, how to extract DNA, how to reconstitute primers, how to set up PCRs, how to pour agarose gels, how to optimize PCR conditions for various primer combinations, how to run gels and how to visualize and interpret the amplicon patterns.

Allan Male

The CIAT trainers: Both Allan and Catherine were involved in the original training for the ABC conducted in 2010. They both work as research assistants at CIAT. Allan has attended several of the ABC annual meetings, where his input during laboratory troubleshooting sessions and project presentations has always been invaluable. Catherine has previously featured in several KT training videos demonstrating pathology techniques.

Catherine Akam

The dolichos bean (Dolichos lablab) improvement programme in Kenya *By Professor Miriam Kinyua*

Figure 5: Farmer participatory harvesting of seed lots

The recent release of the four dolichos varieties ELDO-KT Black 1, ELDO-KT black 2, ELDO-KT cream and ELDO-KT Maridadi represents the culmination of a KT supported research programme spanning eight years. The programme to develop improved Dolichos lablab varieties started with a survey in 2005 of the dolichos bean growing areas in Kenya.

Selections were made based on a varied set of traits. Other germplasm was sourced from the Gene Bank of Kenya collections. Over 200 germplasm entries were screened in five locations in Kenya. A breeding programme was finally initiated based on ten selections, intercrossed as a half-diallel. A set of 20 selections was made in the F4 generation, following a pedigree breeding programme. The number of selections was reduced firstly to ten and finally to six, which were entered into the National Performance Trial (NPT) in 2012.

In September 2014, four of the entries, B1, M1, W7 and G1 were recommended for release by the NPT committee. On February 4th 2015, the National Variety Release Committee announced their release, with the given names ELDO-KT black (1 and 2), ELDO-KT cream and ELDO-KT Maridadi. The varieties are variable with respect to maturity time (early to medium), maturity type (uniform to variable), growth habit (determinate to semi-determinate), and seed colour (black, cream and spotted); these features provide a wide choice to producers to address the diverse demands of the Kenyan consumers. The seed is currently being multiplied by the Freshco Seed Company.

Figure 6: Kephis staff inspecting seed

Figure 7: Farmer participatory organoleptic test experiment in Meru

It is anticipated that the project will have a positive impact on local food security and income generation in the small scale farmer community in Kenya. A number of young scientists have been effectively prepared for a career as plant breeders, and the equipping of the laboratory at Eldoret is fulfilling a need for the modern facilities associated with current breeding technology. We appreciate the sustained contribution from KT of funds needed for screening and NPT testing, for lab equipment and supplies, and for the training of five MSc and three PhD students, as well as support for several other students. Over the years,

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The dolichos bean (Dolichos lablab) improvement programme in Kenya *Cont'd...*

the staff working on the dolichos programme have published seven papers in international peer reviewed journals, as well as delivering five conference papers and contributing to a book describing dolichos breeding (published in 2012).

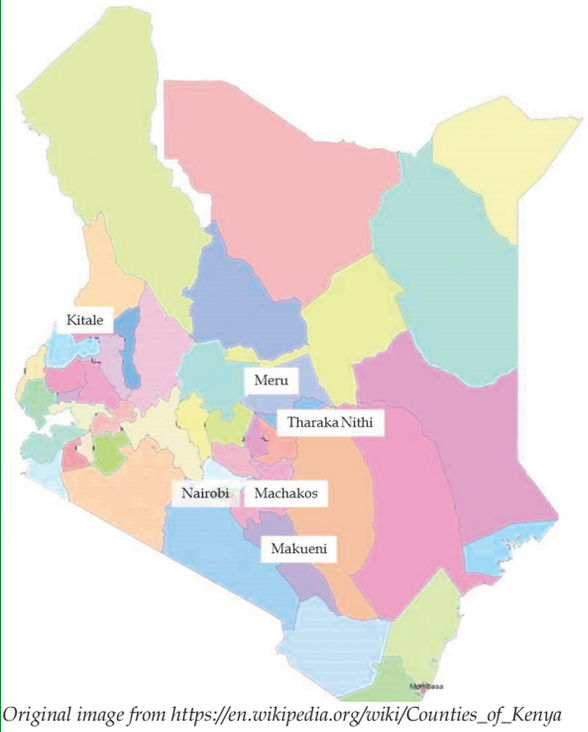
With thanks to KT for their foresight and generosity.

A selection of publications:

- Kinyua, MG and Kipligat, OK. (2012) *Dolichos (Lablab purpureus. L sweet) bean improvement using mutation and biotechnological techniques,* Nairobi: Danste Agencies.
- Kimani, E. Wachira, F. et al. (2012), Molecular Diversity of Kenyan Lablab Bean (Lablab purpureus (L.) Sweet) Accessions Using Amplified Fragment Length Polymorphism Markers. *American Journal of Plant Sciences*, Vol. 3 (3), pp. 313-321.
- Kimani, E. Wachira, F. et al. (2010), Biochemical analysis of cassava quality traits in central rift characterization of volatile compounds of Kenyan lablab bean (Lablab purpureus) accessions and their flavour attributes. *East African Agricultural and Forestry Journal*, Vol. 76 (1&2), pp.220-226.
- Shivachi, A. Kiplagat, K. et al. (2012), Microsatellite analysis of selected Lablab purpureus genotypes in Kenya. *Rwanda Journal* Vol. 28, pp. 39-52.
- Shivachi, A. Kinyua, MG et al. (2012), Cooking time and sensory evaluation of selected Dolichos (Lablab purpureus) genotypes. *African Journal of Food Science and Technology,* Vol. 3(7) pp. 155-159.

According to the Freshco website (www.freshcoseeds.co.ke), Freshco was founded in 2000 with the primary objective of producing, processing and distributing high quality seeds and planting materials to the farming community in Kenya and East and Central Africa (ECA) region at large. The company has its principal offices located in Nairobi. Freshco Seeds is a member of Seed Trade Association of Kenya (STAK).

Captain James Karanga, CEO of Freshco seeds, was kind enough to answer some questions we put to him regarding Dolichos



Original image from https://en.wikipedia.org/wiki/Counties_of_Kenya

Question: What area of Kenya do you cover in terms of providing legume/Dolichos beans to farmers?

Answer: Meru, Makueni, Kitale, Tharaka Nithi and Machakos

Question: Can you tell us your plans for multiplying up the new Dolichos beans? E.g. amount of beans available after 1, 2 and 3 years?

Answer:

Dolichos bean	Year 1(t)	Year 2(t)	Year 3(t)
Basic seed	0.25	0.4	0.7
Certified seed	15	25	40

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The dolichos bean (Dolichos lablab) improvement programme in Kenya *Cont'd...*

Question: How are you advertising the availability of the new Dolichos varieties?

Answer: Freshco Seed Company does the advertisement of Dolichos varieties through setting up demonstration plots for field days and agricultural shows, providing free samples and discounts on prices to farmers.

Question: What do farmers pay for the beans? Are all the varieties the same price?

Answer: The farmers buy dolichos beans at ksh.230 per kilogram of certified seed. The price is the same for all dolichos varieties.

Awards, graduations and publications

Congratulations to Paul Gepts who in July 2015 became Distinguished Professor at UC Davis. Paul also received the Frank N. Meyer Medal for Plant Genetic Resources from the Crop Science Society of America (https://www.crops.org/awards/view/87) which is awarded yearly to an individual who has contributed significantly to the study and conservation of crop genetic resources.

- Arunga, E. et al (2015). Genetic diversity of determinate French beans grown in Kenya based on morpho-agronomic and simple sequence repeat variation. Journal of Plant Breeding and Crop Science Vol. 7 (8), pp. 240-250.
- Akinbo, O. et al. (2015). Introgression of root protein and yield traits from backcross hybrids between cassava and its wild progenitor (Manihot esculenta ssp flabellifolia). Journal of Plant Breeding and Crop Science, 7 (1), 1-8.
- Krishnamurthy, S. L. et al. (2015). Assessment of AFLP marker based genetic diversity in chilli (Capsicum annuum L & C baccatum L.). Indian Journal of Biotechnology, 14, pp. 49-54.
- Ramesh, S . et al. (2015). RAPD marker-based genetic diversity among released finger millet (Eleusine coracana. Gaertn.) cultivars with known pedigree. The Bioscan, 10:2, 741-746 (Supplement on Genetics and Plant Breeding).

The Kirkhouse Trust Training Grant Scheme: This scheme offers partial funding for scientists and students wishing to attend a training course. Further information can be found on the Kirkhouse Trust website.
To apply please email info@kirkhousetrust.org to request an application form.

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Laboratory hints and tips

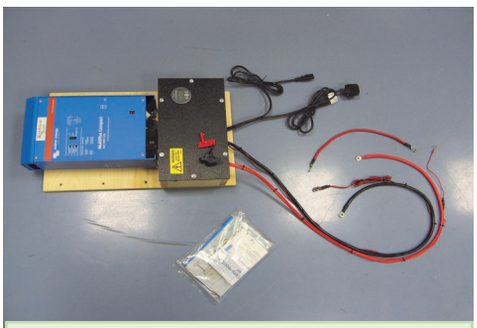

Figure 8: The UPS device

Uninterruptible power supply - UPS: A battery backup device is supplied as part of the molecular equipment supplied by Kirkhouse Trust. Two deep cycle batteries are required and these have to be sourced locally. It is very important that Project staff obtain the batteries and connect the battery back-up device so that it is operational.

A non-fluctuating power supply is essential for equipment such as the PCR machine and centrifuge. If the battery back-up device is not used the smooth running of the lab could be affected as irregular power will damage the circuit board and the equipment will, at some point, fail.

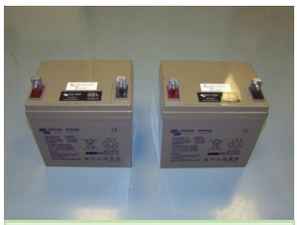

Figure 9: Two deep cycle batteries are required for the UPS

PCR mastermix: The Trust is now supplying the PCR mastermix 'GE Illustra PCR premix beads'. Although this product is very expensive, the advantage of the beads is that they are extremely stable due to a stringent lyophilisation process in their manufacture. This means that unopened this premix is stable indefinitely at ambient and higher temperatures and, therefore, unaffected during delays in shipping.

However, once opened the plate of tubes must be kept in a desiccator with active silica gel. Please note that each tube contains a bead which is made up to 25ul (the Bioneer premix previously supplied was made up to 20ul). As with the Bioneer premix, it is possible to divide the mix into two PCR reactions using the extra PCR tubes supplied without any loss in sensitivity.

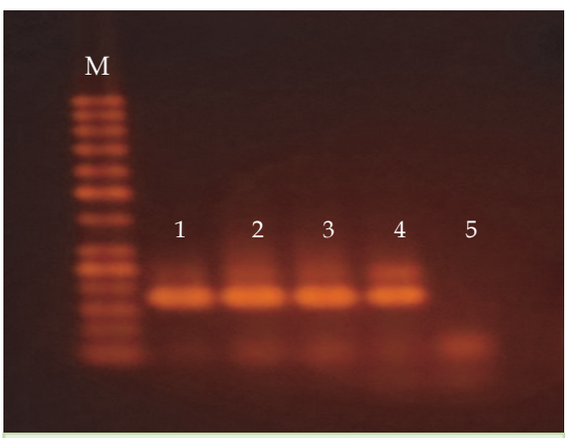

Figure 10:
[1] Sample at ambient temperature,
[2] Sample at ambient temperature **(1 week)**,
[3] Sample at ambient temperature **(2 weeks)**,
[4] Sample at ambient temperature **(5 weeks)**
[5] Blank punch at ambient temperature.

Pipettor Exchange Programme: Kirkhouse Trust has an ongoing annual Pipettor Exchange Programme so that all projects have their pipettes calibrated and repaired if necessary on an annual basis. Regular calibration is important to ensure that the volumes being dispensed are accurate. However, to maintain pipettes in good working order it is important to regularly check them for any damage and to clean them by wiping over with 70% ethanol.

Figure 11: KT pipettor set

To prevent contamination and corrosion pipettes should never be stored on their side with liquid in the tips. Likewise, when handling pipettes should always be held vertically. Where possible, pipettes should be stored vertically so that any liquid that has entered the barrel is prevented from travelling further inside and corroding it.

FTA purification reagent: Kirkhouse Trust is now supplying a substitute for the GE-Whatman branded FTA purification reagent. We have compared the substitute with the branded purification reagent in the KT lab and both were equally effective in the extraction of DNA from the FTA punch. The new product is supplied by Severn Biotech and is TE buffer containing 10% Tween 20, with a final pH of 8.0.

The Kirkhouse Trust website (www.kirkhousetrust.org) has several training videos freely available to watch. The most recent is of the horizontal polyacrylamide gel electrophoresis (hPAGE) system developed in collaboration with Cleaver Scientific (www.cleaverscientific.com) and based on the published work of Izzo et al (2006).

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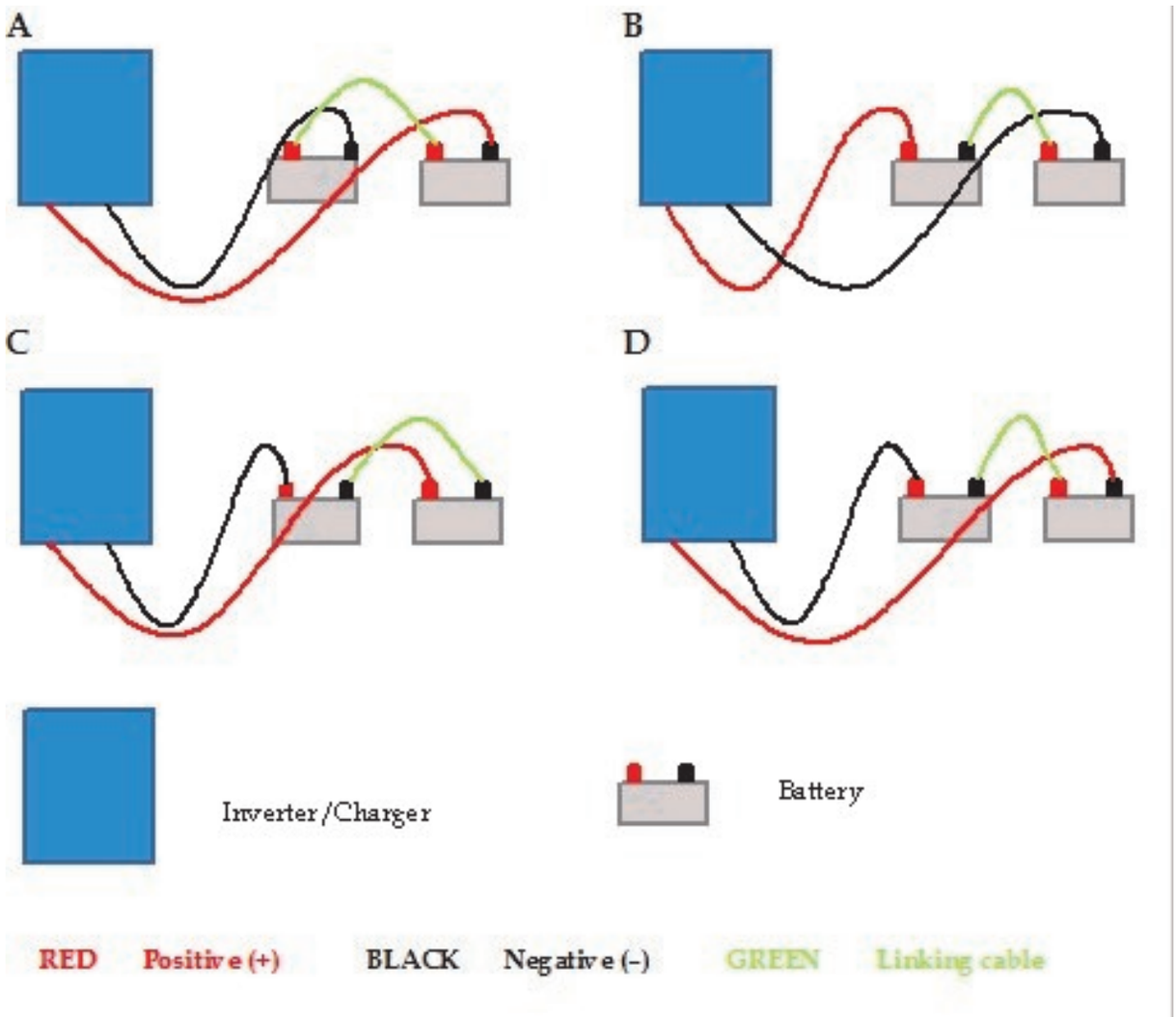
Battery Back-up Puzzle

The following diagrams are of the Battery Back-up systems the Kirkhouse Trust sent to laboratories funded by the Trust. Can you select which of these systems is wired correctly (answer below)

A video showing how to connect the battery back-up system correctly is available on the Kirkhouse Trust website at http://www.kirkhousetrust.org/batterybackup.html and also on YouTube.



Answer: B. The red, positive cable from the inverter charger should be linked to the positive terminal of one battery and the black, negative cable from the inverter charger should be linked to the negative terminal of the second battery. The cable linking the two batteries should be connected to the remaining negative terminal on one battery and positive terminal on the second battery. **Any other system will not work and may damage the equipment**

Congratulations to Pavithravani BV and her husband Sharan on the birth of their daughter on the 27th November 2015.