Kirkhouse Times

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The Kirkhouse Trust Mobile Laboratory at CRIG
by Dr Jemmy Takrama

Highlights

The Kirkhouse Trust Mobile Laboratory is an ex-RAF Bedford truck converted to a working lab for molecular techniques. It is kept and managed by the Cocoa Research Institute of Ghana (CRIG).

Fig.1 The Mobile Lab visits Central University College


Dr Jemmy Takrama

The Mobile lab treks to Research Stations and Universities across Ghana and is used by both research staff and students. It was initially valued because similar facilities existed in very few universities when it first arrived in the country. The users targeted has evolved over time, and the facility now trains: (i) researchers and technicians in molecular breeding techniques, and (ii) students in the DNA laboratory technology – initially limited to final year and graduate students, but now extended to third year students too. Since the launch of the project, many universities have made strides in building physical facilities for DNA work but are still constrained by limited access to state-of-the-art equipment but mostly by irregular and inadequate supply of molecular biology grade chemicals and reagents. Hence, the KT Mobile Lab continues to be heavily depended upon by the Universities and lately by polytechnics.

The locations of these institutions are widely spread involving journeys of 70,200 and even 500kms from the Ghanaian capital, Accra. The lab has standing room for at most 12 persons, so to enable use by larger student numbers, the equipment in the mobile lab is moved into the main labs of the institutions and the work conducted there. The Mobile lab is fitted with an electricity generator that is used during the frequent periods of power outages.

A brief history of the Mobile Lab

The Trust, realising it would be unable to meet the demands of all the stakeholders in the region, came up with the concept of a mobile lab, and the Co-ordinator at CRIG volunteered to manage the facility. The Mobile Laboratory was inaugurated by the British High Commissioner in Ghana on 22 March 2007. The Mobile Lab was originally planned to move from country to country but it was soon realised crossing national boarders in West Africa was a major obstacle and the laboratory project in Ghana was implemented to test its feasibility. The innovative idea was to provide this facility in the field to plant breeders engaged in cowpea breeding so that the lab could be stationed at the site of the breeding projects for marker assisted breeding.

Science organisations which have used the Mobile Lab for research work and student training:

Cocoa Research Institute of Ghana; Forest Research Institute of Ghana, Plant Genetic Resources Institute and Crops Research Institute of the Council for Scientific and Industrial Research, Kwame Nkrumah University of Science and Technology; the University of Cape Coast; the University of Education in Winneba; the Central University College; the University of Ghana Medical School; the University for Developmental Studies, and the Accra Polytechnic.

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The Kirkhouse Trust Mobile Laboratory at CRIG *continued*

Achievements

Capacity building is the greatest achievement of the Mobile laboratory; currently about 1,000 students benefit from the use of the Mobile Lab per year. As of December 2014, 6,846 students and researchers have used the lab. The lab also offers training avenues to researchers and technicians in the West African sub-region. A lab technician from Cameroon and a Research Scientist from Senegal came to CRIG for training.

Fig.2 Students carry out molecular breeding work in the Mobile Lab

Experiments conducted in the Mobile lab

Electrophoresis of CTAB extracted DNA is run on agarose gels and on horizontal polyacrylamide gels (hPAGE) and visualized on a UV transilluminator. Experiments conducted to train participants on PCR are done using SSR markers together with DNA extracted from various local sources. Cowpea SSRs are supplied by KT; those for cocoa and cashew are provided by CRIG. Lately, experiments on the preparation, purification and activity profiling of Taq polymerase using a cloned Taq plasmid are also conducted during workshops run for students from the Universities and polytechnics. Pre-lab lectures are given by the Coordinator during the workshops. Topics covered include Protein extraction and purification; Plasmids, restriction enzymes and cloning; PCR; and SSRs and SNPs.

Fig.3 Student learning molecular techniques using Mobile Lab equipment

The Kirkhouse Mobile Lab Team

The Mobile lab is managed by the Coordinator, Dr Jemmy Takrama and assisted by a Technician, Mr Bernard Armooh, and a Driver, Mr Kasim Amadu. The Coordinator is a Principal Investigator at CRIG, the Technician and Driver are both funded by the Kirkhouse Trust.

It is gratifying to see the expression of a sense of accomplishment and amazement on the faces of participants after seeing for the first time the floating cotton wool-like strands of DNA in their Eppendorf tubes and bands of DNA and PCR products following electrophoresis on agarose and polyacrylamide gels. Experiments on Taq give participants an overview of bacterial growth dynamics, cloning with plasmids, enzyme induction and purification as well as activity determination using PCR.

Extracts from Testimonials

...we have had the privilege of receiving this essential training for our students for the past three years or so more than 200 students have been exposed to modern techniques of molecular biology ...each student has the opportunity to have a hands-on experience in molecular biology techniques. Rev. Abraham Quarcoo,Head of Department,Science Lab Technology Dept, Accra Polytechnic

We are benefiting immensely from the use of the Kirkhouse Trust equipment, chemicals and reagents and the technical support from the experienced technicians who provide hands-on training to our students. Prof. Richard Akromah, Dean, Faculty of Agriculture, KNUST

The idea of the mobile laboratory has been a laudable one and I think it should be continued and supported to bring molecular biology education to the door steps of Ghanaians especially in areas that are not well endowed with laboratories…. Courage K. S. Saba, Lecturer, University for Developmental Studies (UDS), Tamale

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The WACC Annual Meeting 2015 *by Moussa Diangar*

I am Moussa Diangar from Senegal and I am working on cowpea resistance to *Striga gesnerioides*.

Moussa Diangar

Whenever the topics "cowpea", "breeding", "striga", "markers" or "screening" crop up, there is a keen desire for more knowledge and information. It is an honour for me to share my impressions and feelings regarding my attendance of the Kirkhouse Trust funded West African Cowpea Consortium Annual Meeting. I have attended this meeting for the past 3 years, and I know that it has helped me to acquire plenty of new knowledge.

The WACC researchers meet once a year to present the progress of their projects. They come from different countries and backgrounds in West Africa. The meeting exposes us all to a lot of new knowledge, so it is always a pleasure and a challenge to be part of it. Ghana hosted the meeting this year. Thank you to Dr Francis Kusi from the Savanna Agricultural Research Institute for the hospitality. I have once again benefited from many discussions on cowpea.

As breeders and researchers, we are all striving to breed well adapted cowpea varieties for the farmers to grow. This short sentence implies a broad area of investigation! Being the most widely grown grain legume in Sub-Saharan West and Central Africa, cowpea production is subject to a whole range of constraints to production. One of the most important ones is the parasitic weed *Striga gesnerioides* which attaches itself to the cowpea root via haustoria. In Senegal, the major cowpea growing areas are infested with striga, a situation which depresses cowpea yield. It is thought that race 6 is common in Senegal and so far no varieties resistant to this race have been released.

The meeting also included a journal critique session. This was the most exciting such session that I have ever attended. I really loved it. For those of us who are just starting to build our scientific careers, this kind of training gives a deal of insight into writing our own scientific papers. Also highly interesting was the discussion on seed systems in West Africa chaired by Dr Eugene Terry. It helped me to understand what needs to be done in all participating countries before we can expect major improvements in this sector. Appropriate strategies are being developed in the WACC community, but still many challenges remain to be taken up, especially in terms of the production of quality seed and the provision of financing for producers. We hope that in the near future a collaboration with CORAF/WECARD will generate some sustainable solutions.

Fig.1 Participants of the WACC Annual Meeting in Accra

Another very important point I want to highlight is the potential benefit to us of access to Controlled Environment Growth Chambers. In my country we can only manage two crossing cycles per year and this does slow our progress. The presentation by Prof Southern shows us that it should not be too difficult to increase the rate to 3 per year, thereby greatly improving our breeding efficiency. So we do hope, as so strongly promoted by our colleague Dr Lucky Omoigui from Nigeria, that Kirkhouse Trust will provide us with a workable Controlled Environment Growth Chamber to enable us to maintain a consistent crossing programme throughout the year.

Finally, I would like to use this opportunity to thank our sponsor for the facilities and support provided; it is true that we have come a long way thanks to the generosity of Kirkhouse Trust.

Moussa Diangar is currently studying towards a PhD at the West African Centre for Crop Improvement (WACCI). He is also working on the Kirkhouse Trust funded project at the Institut Sénégalais de Recherches Agricoles (ISRA)

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HA 4, a high yielding photoperiod-insensitive determinate variety of dolichos bean

by Dr M. Byre Gowda

Dr M. Byre Gowda

Various common names given to dolichos lablab (*Lablab purpureus* (L.) Sweet include field bean, hyacinth bean, Indian bean, bonavista bean and Egyptian kidney bean. The species is a member of the family Fabaceae, sub-family Faboideae, tribe Phaseoleae and sub-tribe Phaseolineae. Around 90% of its production in India is concentrated in the southern state of Karnataka. The crop is grown as a source of fresh vegetable (immature pods and grains), dry seed and forage. It provides an important source of protein to the large number of Indians whose diet is vegetarian. It is cultivated either as a pure stand or as an intercrop along with millet, groundnut, maize and sorghum. The area of production in Karnataka is around 67 Kha, generating a dry grain yield of some 73 Kt. Traditional varieties are photoperiod-sensitive and indeterminate, which restricts the timing of the crop's cultivation to the rainy season. Until recently, most production has been used for subsistence, but of late, there has been an upswing in interest for its use as a cash crop. Commercial production is, however, incompatible with the traditional photoperiod-sensitive, indeterminate, non-synchronously flowering phenotype, as these traits lead to heterogeneity in maturity. The University of Agricultural Sciences (UAS) in Bangalore, India has therefore led a concerted research effort to develop photoperiod-insensitive and determinate varieties to allow the crop to be grown in a commercial manner.

Fig.1 HA 4 curved green pods

HA 3 was the first photoperiod-insensitive, determinate variety to be bred at UAS. Its improvement has been focused on combining its favourable plant type with the highly prized pod fragrance expressed by a landrace maintained at UAS. Selection was based on photoperiod-insensitivity, determinacy and the production of highly fragrant, curved and constricted pods. Late generation materials were evaluated both on various research stations and in farmers' fields to identify those associated with high yield potential. The best of these was finally released under the name 'HA 4'.

Salient features of HA 4

HA 4 is both photoperiod-insensitive and determinate, growing to a height of around 1m. Its leaves are trifoliate, broad and ovate, of length 7.5-10 cm. It reaches flowering 40–45 days after sowing. Its 30-45 cm long inflorescences are erect and terminal, comprising several short-stalked white flowers on elongated peduncles. The pods are curved and constricted, and are green when immature; they vary in length and breadth from 6.0 to 8.0 cm and 1.5 to 2.5 cm. Each pod contains an average of four tan-coloured seeds. Green pods can be harvested 65–70 days after sowing; full maturity is reached after 95–105 days. The variety is recommended for cultivation in Karnataka during the rainy, post-rainy or summer seasons. The pod fragrancy trait is expressed throughout the year, which is not the case for traditional varieties. HA 4's yield potential is 1.0-1.2 t/ha dry seed or 4.5-5.0 t/ha green pods.

Fig.2 HA 4 at the flowering stage

Impact of HA 4

Prior to the release of HA 4, dolichos bean production was predominantly carried out as an inter-crop during the rainy season. However, following its release, there has been an increasing level of its production as a sole crop in all the three growing seasons, made possible by the variety's photoperiod-insensitivity, determinacy and retention of pod fragrance. Traditional varieties were only able to produce green pods during the period November-January, but the widespread adoption of HA 4 has extended green pod availability to throughout the year. The variety is currently grown over some 8,000 ha, making a significant contribution to the increase in productivity from 0.25 t/ha before its release, to 0.8-1.2 t/ha after it.

Dr Byre Gowda is an Assistant Professor at the University of Agriculture, Bangalore. He has been supported by the Kirkhouse Trust for 10 years to carry out research on genetic improvement of dolichos. In 2014 he was awarded the Nagamma Dattatreya Rao Desai Award for outstanding adaptive Agricultural Research for Agroclimatic region of Karnataka.

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Visit to the Lab of Prof. Mike Timko at the University of Virginia for Research and Training *by Sory Diallo*

One of my important current research objectives is to identify and validate SSR markers linked to race 2 Striga resistance genes in cowpea. For this purpose I spent six months during 2014 at Mike Timko's laboratory at the University of Virginia (UVA). We exploited populations bred from crosses between cowpea variety IT97K-499-35 (source of resistance) and the Striga race 2 susceptible Malian cultivars Amary-Sho, M'Barawa and CZ06-3-1. The F2 populations segregate in the field for resistance to Striga when tested at a hot spot in Mali. We were able to confirm this by challenging them with a Striga strain collected from Koporo in a pot trial carried out at UVA.

Picture1. Sory Diallo

The first requirement was to establish which SSRs were informative between the parents of each cross, after which the selected SSRs were applied to analyse segregation in the F2 populations. DNA was prepared from leaves using a CTAB-based method. Before using it as a PCR template, an aliquot was run through a 2% agarose TAE gel to enable an estimate to be made of its concentration and to check its integrity. A more precise estimate of the concentrations was then derived by spectrophotometry. I used 10 µL PCRs with a standard programme of 94°C / 4 min, followed by 35 cycles 94°C / 30 s, 56°C / 30 s and 72°C / 30 s, finishing with a final 10 min extension step. The PCR products were electrophoresed through a 2% agarose gel and visualised after ethidium bromide staining.

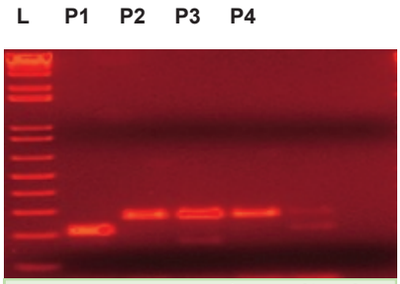

Fig.1 Figure1: SSR CP 333/334 profiles of IT89KD-499-35, M'Barawa, AmarySho, CZ06-3-1. P1 is IT89KD-499-35 allele (200bp), P2 is M'Barawa allele (300bp), P3 is AmarySho allele (300bp), P4 is CZ06-3-1 allele (300bp)

Six of the >300 SSR markers screened (CP 333/334, CP 115/116, CP 743/744, MA62, MA127 and SSR1) were found to be informative between the susceptible and the resistant parents. Of these, four (CP 33/334, CP115/116, CP743/744, MA127) were co-dominant and two (MA62, SSR1) were dominant (see Fig. 1). In the IT89KD-499-35 x M'Barawa population, 25 progeny carried the IT89KD-499-35 CP333/334 allele, 17 the M'Barawa allele and 57 were heterozygous (Fig. 2).

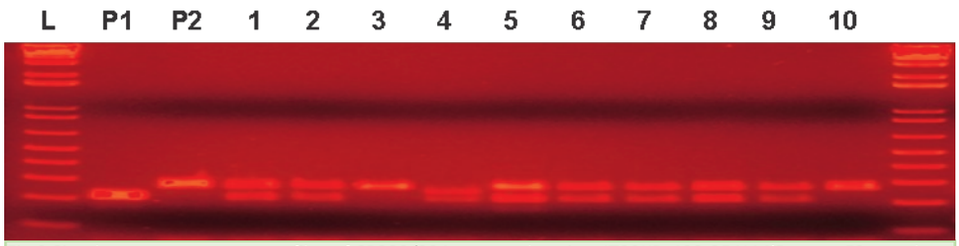

Fig.2 Simple sequence repeat profiles of CP333/334 screened against IT89KD-499-35 X M'Barawa F2 population. L represents the 100 bp DNA molecular size marker, P1 is IT89KD-499-35 allele, P2 is M'Barawa allele, and 1 to 10 are population allele.

Of the six informative SSRs, the one mapping closest to the Striga resistance gene was SSR1 (five recombinants out of 100). Thus we intend to use SSR1 as resistance marker, in the same way that our colleagues in INERA, Burkina Faso are doing.

This training visit was an ideal way for me to acquire the necessary skills to perform marker-assisted selection. I am confident that I am now ready to take on this sort of research in any lab.

Sory Diallo is a PhD student at the University of Ouagadougou, Burkina Faso. He also works at the Institut d'Economie Rurale, Mali, where he is carrying out his research work. His PhD, titled "Marker Assisted Selection for Striga gesneroides resistance in cowpea (Vigna unguiculata (Willt) Vatke)", is funded through a Kirkhouse Trust scholarship. The Trust also funded a trip to the University of Virginia, United States to receive training and carry out molecular work as part of his PhD research.

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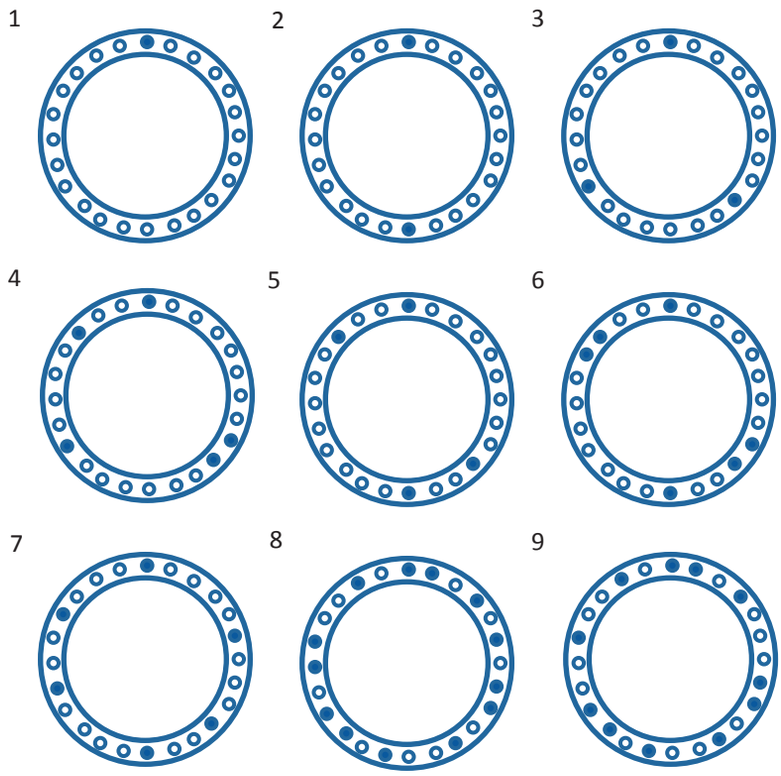
Kirkhouse Times Puzzle No.1

Balancing Centrifuges

Below is a diagram showing the distribution of tubes in a centrifuge. Which of the centrifuges are balanced?

The answer is given under the puzzle.



Answer: Balanced centrifuges: 2, 4, 5, 6, 9

How to Balance a Centrifuge

- For an even number of tubes place each tube directly opposite another tube.
- For an odd number of tubes place three in an equilateral triangle formation, further tubes should be placed directly opposite each other.

Awards and graduations

Francis Kusi, SARI / University of Ghana: Award of Doctor of Philosophy Crop Science (Entomology), *Deployment of the cowpea aphid resistance gene for cowpea improvement in Ghana*.