Kirkhouse Times

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The Work of SARI in Ghana

Highlights

i) Improving an elite Ghanaian cowpea cultivar - *Francis Kusi*

Savanna Agricultural Research Institute (SARI) have identified a monogenic source of resistance to the cowpea aphid, *Aphis craccivora* in the advanced breeders' line SARC1-57-2, and in showing that the SSR marker CP 171/172 is linked to the resistance locus (Fig. 1). The presence of the resistance gene seems to reduce aphids' fecundity when they are forced to feed on resistant plants (Kusi et al., 2010). Efforts are currently being made to use this gene to improve the field resistance of existing cowpea cultivars in Ghana through marker assisted backcrossing, and we have now reached the BC4 generation.

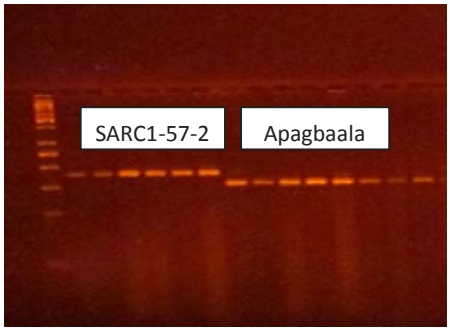

Fig.1 CP I71/172 polymorphism between the aphid resistant parent (SARC1-57-2) and the susceptible parent (Apagbaala)

Polymorphism test

To deploy the aphid resistance gene using marker assisted backcrossing, we first had to be sure that the CP 171/172 marker could distinguish between resistant donor (SARC1-57-2) and the four elite recipient varieties which we were aiming to improve: Padi Tuya (SARC3-122-2), Zaayura (SARC4-75), Songotra (IT97K-499-35) and Bawutawuta (IT95K-193-2). There was a satisfactory polymorphism between Zaayura and SARC 1-57-2 (Fig. 2), but the profiles of the other three varieties were not different from that of the resistantce donor. Thus we were only able to go ahead with the marker-assisted backcrossing using Zaayura.

Fig.2 Polymorphism test between SARC1-57-2 and 4 elite varieties of CSIR-SARI

Subsequently a backcrossing breeding programme was initiated with Zaayura. The F1 hybrid between SARC1-57-2 and Zaayura was backcrossed to Zaayura (the recurrent parent) to generate 20 BC1F1 individuals. These were genotyped using CP171/172 to select those which were heterozygous (Fig. 3), as these are plants predicted to carry one copy each of the SARC1-57-2 and Zaayura alleles at the aphid resistance gene, while the homozygous ones are predicted to carry two copies of the (susceptible) Zaayura allele. The selections were grown in a screen house to confirm their reaction to the aphid. Three resistant plants were selected for the next round of backcrossing to generate BC2F1 plants. The same cycle of crossing, identification of heterozygous lines using CP 171/172 and screenhouse confirmation of resistance was followed till BC4F1 plants had been obtained.

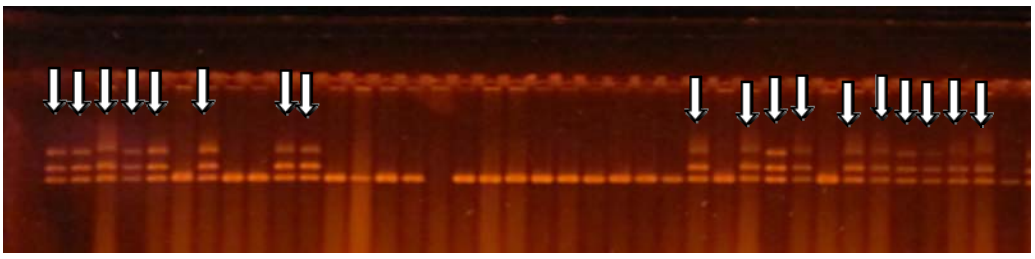

Fig.3 Genotyping and selection of heterozygous individuals (Note the SARC1-57-2 allele now shows two bands)

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Background selection

Currently, we are collaborating with University of California, Riverside (UCR) to identify a set of SNP markers which will be used to recover the genetic background of Zaayura as efficiently as possible. A further aim of the SNP work is to identify marker(s) which are closer to the aphid resistance gene than is the CP 171/172 locus, which is known lie about 10-15 cM away. The following protocol is being followed to achieve these objectives.

1. Send UCR leaf material of SARC1-57-2 and Zaayura in zip-lock bags containing desiccant packs
2. About 100-150 seeds of BC_4F_1 plants will be tested rigorously for aphid resistance (no prior CP 171/172 genotyping), and leaf samples will be collected before the aphid infestation.
3. We are assuming that about half of the plants (50-70 or so individuals) will manifest the resistance. The DNA from these will be genotyped with a large number of SNPs; any SNPs which are shared between all (or most) of the plants will then be regarded as candidates for a better marker for the resistance.
4. Once this information is to hand, then the few closely linked SNPs identified, along with the genome-wide set of informative SNPs derived from the parental screen, can be applied to a further 100 of the BC_4F_1 plants with a view to selecting individuals which carry the Zaayura allele at most of the SNP loci to recover the background.
5. The final selection(s) (in the BC_4F_2 generation) will be challenged by aphids just to be sure that they are still resistant. Choosing homozygous resistant types will mean that the resistance has become genetically fixed - so will not segregate any more in the BC_4F_3 and subsequent generations.

Note that this protocol requires the use of material that has not been genotyped using CP 171/172, because we are trying to find a marker closer to the resistance gene than the CP171/172 locus – ideally therefore, the selections will carry the resistance gene and not contain CP171/172.

Fig.3 SARC-57-2 seed which are black-eyed

Fig.4 Zaayura seed, which are brown-eyed and larger

A Research Scientist with CSIR-Savanna Agricultural Research Institute (CSIR-SARI), Francis Kusi holds an MPhil in Entomology and is currently in his last year of a PhD in Crop Science (Entomology) at University of Ghana, Legon. As an Entomologist, his special interest is in host plant resistance, combining molecular breeding and entomological tools in developing resistant plants. He is currently deploying cowpea aphid resistant gene to improve the field resistance of susceptible cowpea varieties in Ghana, using marker assisted backcrossing methodology. Releasing cowpea varieties that are resistant to cowpea aphid is his priority in the near future and he hopes to identify sources of resistance to other major insect pests of cowpea.

Recent publications by Kirkhouse Trust funded students and scientists

- Okii, D. et al (2014). Morphological diversity of tropical common bean germplasm. African Crop Science Journal, Vol. 22, No. 1, pp. 59 – 67.
- Okii, D et al. (2014). Application of Bioinformatics in Crop Improvement: Annotating the Putative Soybean Rust resistance gene Rpp3 for Enhancing Marker Assisted Selection. J Proteomics Bioinform 7:1.

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The Work of SARI in Ghana *continued* ...

ii) First experiences of the new KT funded lab *by Frederick Awuku*

I am Frederick Justice Awuku (pictured right), a BSc holder from the University of Ghana in chemistry. Aged 25 and from the Eastern region of Ghana. I am currently working at the CSIR-Savannah Agricultural Research Institute located in the Northern Region of Ghana as a senior technical officer. I am the laboratory manager at the biotechnology laboratory funded by the Kirkhouse Trust and also a new member of the Kirkhouse Trust funded project at SARI.

As a chemistry major, working in a biotechnology laboratory for the first time was very challenging. At the beginning, I did make some mistakes in the operation of the equipment, with some laboratory practices and even the use of some of the chemicals. However, it was not long before Francis Kusi organized a training session in which I took part, and this turned my performance around and gave me more insight into the activities of the laboratory. He also organized for me to have some field exposure. All of this helped me gain more knowledge on the project and my activity as a whole.

The cowpea consortium meeting at Togo last year, in which I was recommended to take part, gave me the opportunity to interact with different researchers and to learn more on the broader scope of the Kirkhouse funded projects in West Africa. At this meeting I was able to interact with other technicians, discussing various laboratory practices and also learning from their experiences at their laboratories. This meeting actually had a great impact on me, as it was my first time.

The biotechnology laboratory at SARI has been of great importance to the institute. The equipment needed for the basic molecular work is available, thanks to the Trust. Management of the lab so far has been smooth. I attach great concern to the life span of the equipment so it can last long to benefit the institute. The laboratory currently is used fully for the Kirkhouse funded project and also other researchers whose work has some molecular aspects, making their work less tedious and less time consuming. All work in the laboratory is carried out by me, especially with the equipment.

I must say that the laboratory has been of great importance to SARI and other Researchers in the Northern Region. Thanks to the Kirkhouse Trust.

Clearing Consignments from the Kirkhouse Trust through local Customs

Customs duties exemptions are usually available for consignments of equipment and consumables provided by charities such as the Kirkhouse Trust. This is because they are usually classed as gift or donation for agricultural research. Several of the PIs have worked hard and successfully secured such exemptions**. Failure to do this usually results in long delays in the dispatch process because KT will not send consignments before customs clearing arrangements have been fully explored.**

KT's experience is that the rules are constantly changing and all PIs need to find ways to keep themselves and the Trust updated about the current rules and legislation. Do raise this issue in meetings within your institution and search out administrative contacts who may be able to assist you with complex procedures**. If you have any questions or need help, please do contact Janice Henderson (janice.henderson@kirkhousetrust.org)**

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WACC Annual Meeting *by Deborah Ayeni and Macsamuel Ugbaa*

From Deborah: Every year, I eagerly look forward to the West African Cowpea Consortium (WACC) meeting in anticipation of new things to learn and have never been disappointed. Discussions and training anchored by experts range from improvements in horizontal Polyacrylamide Gel Electrophoresis to new software for data analysis, data mining, advances in molecular tools, and designing a realistic breeding program based on established facts.

Fig. 1 Group photo of meeting paticipants. Deborah is in the front row, centre-left.

The 2013 meeting was an exceptional event. The journal critique session was one new feature that made the meeting unique and I still expect more of it. I also had to do a detailed study of a paper assigned to me for the critique for proper understanding before analysing it. The discussion on SNPs and its utility in breeding programmes was really impactful. Prof. Mike Timko taught extensively on cowpea genespace sequence reads (GSR); emphasising the available information on databases which can be mined for advancements in applying molecular tools to cowpea breeding; fast tracking the release of improved cowpea varieties.

Another important component of the meeting was the presence of experts who are active in plant breeding programmes and with records of good success. In 2013, Prof. Anna Maria Benko Iseppon gave us good insight into the breeding programs in Brazil and their recorded successes with biofortification incorporated. Their targets are similar to that of West Africa. These include: resistance or tolerance to drought, diseases, pests, good adaptability and above all higher yield. It is interesting to learn of how well they have exploited the cowpea genome for genetic improvement via molecular tools including bioinformatics. The knowledge acquired during the session, especially on biofortification, is making a meaningful contribution to on-going research work here in Nigeria.

The Trust's approach to training West African scientists has been unique. The training has been able to bridge the wide gap between the western world and Africa in terms of technology applications. The efficiency of this technology transfer approach is evident in the ability of West African breeders to independently apply molecular tools to crop breeding and consequentially, releasing improved crop varieties. This was not achieved at once. It came through series of trainings under patient and optimistic instructors.

Fig. 2 Macsamuel (second left) in discussion with other participants at the meeting

Overall, this is a meeting that adds value to every attendee. At WACC annual meeting, everyone has something to learn and contribute. *(Deborah Ayeni, Laboratory Assistant and MSc student at University of Agriculture, Makurdi, Nigeria)*

From Macsamuel: One of the high points of WACC Annual meetings for me has always been the training sessions. No doubt, these sessions are appreciated by all WACC members as it has been of great benefit to me due to the invaluable knowledge we gain.

2013's meeting was memorable due to enthusiasm, friendliness and humour of Bere Tchabana who facilitated the hosting in Togo. I enjoyed Prof. Benko Iseppon's presentation of work on cowpea by the Brazilian Cowpea Genome Consortium.

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WACC Annual Meeting *continued*

The introduction of Journal critique by Dr Robert Koebner was challenging as a first time experience. However, it was an interesting and educative event. It provoked a mind set of looking at scientific writing with an objective and critical mind. The session on the use of the new horizontal Polyacrylamide Gel Electrophoresis 3 system was very beneficial.

Overall, the meeting was very heartwarming and the impact shows the Trust is seriously considering future research direction of the consortium with regards to current technology and further training of students. It is always a joy to learn, meet and network with new people in the consortium, and to see how the Trust effort has achieved capacity building, technology transfer and facilitated research on cowpea in the west African sub-region. *(Macsmuel Ugbaa, Research Fellow and MSc Student at University of Agriculture, Makurdi, Nigeria)*

Laboratory hints and tips

Gel estimation of DNA concentration

A PCR sample (4 µl) was run on a 6% gel (hPAGE) next to 4 µl, 2 µl and 1 µl samples of a 50 bp ladder. The was gel post-stained with ethidium bromide. (*Fig. 1*)

To estimate the concentration of the unknown PCR sample, visually compare the intensity of the band with the 50 bp DNA ladder bands.

For example, for the 4 µl ladder lane the intensity of the unknown sample is < 200 bp but > 150 bp. Therefore, the unknown PCR sample can be estimated as follows:

The 200 bp and the 150 bp bands have a concentration of 67 and 30 ng/5 µl respectively. Since 4 µl of ladder was loaded onto the gel, the concentrations of these bands can be calculated to be about 53 ng and 24 ng/4 µl respectively. As the intensity of the unknown sample is closer to that of the 53 ng/4 µl band **it is estimated that the concentration of the unknown PCR sample is 44 ng/4 µl or 11 ng/µl.**

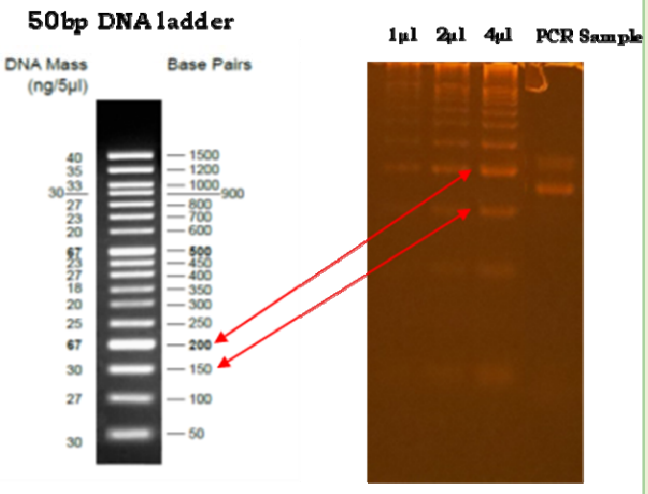

Fig. 1 PCR sample run on 6% gel to estimate concentration

Reconstitution of Oligos

Oligonucleotides supplied by KT are lyophilised. The oligo pellet may become dislodged in transit and end up in the lid of the tubes. Before opening the tube, it is very important spin down every oligonucleotide in the centrifuge.

Dried DNA is usually very easy to resuspend in aqueous solution but some oligos need more time to go into solution than others. Resuspend in TE buffer pH 8.0 (1X).

To reconstitute, use the nanomole quantity for each specific oligo shown on the datasheet. For example, to make a 100 µM concentration stock solution: Take the number of nmoles in the tube and multiply that by 10. This will be the number of µL buffer to add to get a 100 µM solution.

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Identification of Germplasm and DNA Markers linked to Dry Root Rot Resistance in Chickpea *by Dr Sidramappa Talekar*

The title of my PhD thesis was "Identification of Resistant Source(s) and DNA Markers Linked to Genomic Regions Conferring Dry Root Rot Resistance in Chickpea (*Cicer arietinum L.*)"

I set out to screen a set of 520 chickpea accessions to identify sources of resistance to dry root rot, to unravel the genetics of resistance, to identify DNA markers linked to genes conferring resistance and to study the morphological diversity represented in a panel of 529 entries. The resistance screen was a laboratory-based test. It revealed a high level of resistance in three entries, namely PG 06102, BG 2094 and IC 552137. From the pattern of segregation in the F3 generation of the cross L 550 (susceptible) x PG 06102, it was possible to conclude that the resistance in PG 06102 was under monogenic control, with resistance being dominant over susceptibility. A screen of 381 SSR primer pairs realized 52 which were informative between L 550 and PG 06102. A bulk segregant analysis[1] approach was then applied to the DNAs of L 550 x PG 06102 F2 progeny, and this came up with potential marker/trait linkage for four SSR primer pairs. A linkage analysis based on 129 F2 individuals and these four SSR markers showed that two markers (ICCM 0299 and ICCM 0120b) co-segregated with resistance to dry root rot. In the morphological divergence study, I found that the number of days to 50% flowering and seed yield per plant each contributed about 25% of the total diversity present. The four dry root rot resistant selections clustered into different clades[2], showing that they were unlikely to be closely related to one another; this was encouraging as it demonstrated that they could each be used as donors of dry root rot resistance in a non-redundant manner.

Fig.1 Susceptible variety. The tap root is dark, shows signs of rotting and is devoid of most of its lateral and finer roots

Fig.2 Resistant variety

1. Bulk segregant analysis will be covered in a future edition of the Kirkhouse Times

2. A clade is a group consisting of an ancestor and all its descendants, a single "branch" on the "tree of life". *Wikipedia.*

I currently work as an Assistant Professor at the University of Agricultural Sciences, Raichur, Karnataka, India. I pursued my doctoral degree in Genetics and Plant Breeding from UAS, Bangalore, qualifying in 2013, during which time I was a recipient of the Kirkhouse Trust fellowship for doctoral research. My PhD supervisor was Dr Viswanatha, K.P.

I am hoping to be given the opportunity to work as a Postdoctoral fellow. I am looking for a permanent placement at Agriculture Universities and Research Institutes in India and abroad. I would like to continue my career in the research field so that I can contribute something novel to the farming community, maybe in the form of development of new varieties, hybrids, technology which will help to enhance the food grain production. My experience gained from my doctoral research will be of great help to me in this endeavour. I would like to proceed in academic line as well.

♦ *The Kirkhouse Trust would like to thank all those who contributed to this newsletter.*