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#### Cowpea flower bud thrips (Megalurothrips sjostedti [Trybom]) by Dr Sobda Gonné

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Cowpea production is constrained by a complex of insects throughout its life cycle and also during seed storage. One of the most devastating of these pests is the cowpea flower bud thrip (Megaluropthrips sjostedti), which can inflict substantial yield losses, reaching 100% in cases of severe infestation. The adults are very



Figure 1: Male (A) and female (B) thrip

small (Figure 1) and highly fecund.

Thrip larvae damage the plant by feeding on its flowers, resulting in at best their distortion and discoloration, and at worst their abortion and consequent yield reduction (Figure 2). The insects

are especially difficult to control because of their wide host range and that thrips populations build up rapidly and their ability to fly in mass helps them to spread and colonise a new population of host plants in a short period. Currently the most effective control measure available is to apply repeated doses of insecticide, but even this strat-

egy is not fully effective as the ability of some of the insects to escape the spray by sheltering within the flower can drive the rapid development of insecticide resistance. The majority of resource-poor

farmers are in any case unable to afford the purchase of both the necessary chemicals and effective spraying equipment. A more sustainable approach would be to deploy genetic resistance against infestation, which may be feasible, since several cowpea accessions have been shown to suffer only limited damage when infested by thrips.

#### Screening cowpea germplasm for thrip resistance

Several KT-funded projects supported within the



Figure 2: Distortion of floral parts (A) and complete flower abortion (B)

West African Cowpea Consortium (WACC) are collaborating to develop a common screening proto-



Figure 3: Flowers infested with thrips (A) and tubes containing chopped cowpea peduncles (B)

col for thrip resistance. The protocol includes both the rearing of insects and the artificial infestation of test plants.

#### **Rearing of thrips**

The first step is to collect naturally infested flowers of a known susceptible cultivar (such as Vita-7) from the field; these are har-

Dr Sobda Gonné is the PI of a Trust funded project at the Institut de Recherche Agricole pour le Developpement (IRAD) in Cameroon. He recently qualified for a PhD at the West African Centre for Crop Improvement (WACCI). The IRAD project continues his work on thrips resistance.

### Cowpea flower bud thrips (Megalurothrips sjostedti [Trybom]) Cont'd...

vested, immersed briefly in 5% household bleach and then rinsed in water to remove any remaining bleach. The peduncles are chopped into pieces small enough to fit into a rearing tube, after a tissue has been pushed into the bottom of the tube to soak up any free moisture. Thrips are brought to the laboratory by collecting field-grown flowers (Figure 3A), which are dissected using a pair of forceps. The adult females within the flowers are transferred into the rearing tubes using an aspirator, and a thin film of honey is added to provide them with sustenance. Finally, the tubes are capped with nylon fabric to prevent the thrips escaping (Figure 3B).

Eggs are typically laid on the peduncle material within 72 hours, at which point the adults are removed and the thrip eggs (attached to the chopped peduncle material) are provided with disinfected cowpea peduncles as a source of food for the

hatching larvae; hatching is expected to be complete within five days of the eggs having been laid. The larvae moult four times (five stages) before reaching adulthood, a process which takes at least six days under normal temperature (26-35°C) and relative humidity (10%) conditions. The entire life cycle lasts 19-21 days.

#### Infestation procedure

Staggered planting of the germplasm to be tested is carried out in order to coincide the formation of buds on the host plants with the production of thrip larvae at the appropriate stage. The procedure is as follows:

- 1. 25-30 nymphs at the fifth larval stage (6-7 days after hatching) are taken from the rearing tubes and placed on the buds of the host plants using either a fine camel hair brush or an aspirator.
- 2. Each infested plant is enclosed in a mesh sleeve (Figure 4) and the infestation is allowed to develop for 20-25 days.
- 3. Damage to the host is scored using the Jackai & Singh 1-9 scale. The scoring involves an assessment of the amount of damage to the flowers, the extent of stipule browning and the degree of peduncle elongation.
- 4. Thrip numbers (adults and nymphs) are counted from two or three flowers per plant, using a stereomicroscope.

## The WACC DNA Fingerprinting and Background Selection project *by Tumie Akintewe*

Over the years, there have been remarkable successes in the cowpea breeding programmes to achieve the WACC's goal which is to develop improved cowpea varieties. Of these are the Aphid-resistant advanced cowpea breeding line- Sarc 1-57-2, and Striga-resistant lines- UAM09 1055-6 (FUAMPEA 1) and UAM09 1051-1 (FUAMPEA 2) developed in Ghana and Nigeria respectively. The development of the aforementioned varieties and many others that are in the pipeline have relied on the use of conventional breeding methods enhanced by Marker Assisted Selection (MAS) whereby molecular markers (such as SSRs and SNPs) which are linked to the gene(s) of interest(s) are carefully selected and used.

The certification and subsequent release of improved cowpea varieties has resulted in the need for a form of quality control to confirm or validate their true identity. This interesting subject of being unable to verify the genuine resistant varieties on sale was raised during the annual WACC meeting in 2012. For this reason, the DNA Fingerprinting and Background Selection project was introduced in 2013 to address the issue through the following aims:

- To develop a (multiplex) PCR-based technique and protocol that would enable breeders "fingerprint" their improved varieties following their release for quality control purposes.
- To create a genome-wide set of markers that would enable the use of Background Selection as a way to recover the recurrent parent's traits in the improved varieties.

To achieve these aims, there was the need to establish a working protocol first. This has been fulfilled by trialling various existing techniques after which a suitable one was selected and optimised.

Tumie Akintewe was a Laboratory Technician at the Kirkhouse Trust where she carried out the fingerprinting and background selection project. Tumie has now moved on to the University of Birmingham and the Trust thanks her for her valuable contribution to the Trust's objectives and wishes her all the best for the future.



Figure 4: Plants enclosed in a mesh sleeve



## The WACC DNA Fingerprinting and Background Selection project Cont'd...

So far, the most promising technique that has been used in the project is the multiplex PCR using standard primers. This method shares very close similarities with the standard multiplex PCR whereby several target loci are amplified with a universal primer pair in a single reaction. However, in the present project several primers which have been designed to amplify specific targets were used. To begin, suitable markers needed to be selected and this selection was based on a number of key features such as:

- Being codominant
- Being polymorphic. The ability to produce sufficient size differences is an important feature that would enable the discrimination of homozygous and heterozygous lines for a particular trait
- Wide distribution across the genome which is an important feature for Background Selection purposes

Selected markers that fulfilled the aforementioned criteria were from on the list of informative SSR markers that are currently used by the WACC projects for assaying Striga and Aphid resistance. Others were selected using an SSR-based linkage map for cowpea published by Mebeaselassie et al (2011). Sequence information of these markers/primers was obtained from the Cowpea Genomics Knowledgebase (CGKB) which is managed by Professor Mike Timko's research team at the University of Virginia (UVA).

To date, several multiplex systems of up to four markers per reaction (Figure 1) have



Figure 1: Multiplex system for fingerprinting improved cowpea varieties using optimised standard PCR conditions. Informative SSRs namely SSR1 (blue arrow), CP171/172 (black arrow), C42-2B (green arrow) and 333/334 (orange arrow) were multiplexed using various cowpea varieties from the UK, Nigeria and Ghana. Amplified samples were run on 6% acrylamide gel at 120V for 4-5hrs. 1x TBE running buffer and peristaltic pump was used. been optimised successfully to enable the Fingerprinting and Background Selection of improved cowpea varieties.

Going forward, SNP markers developed by Dr Erik Ohlson at the UVA, and described in the accompanying article, are now being explored and tested for their incorporation into a multiplex system. Results from an initial trial (Figure 2) look promising. This approach could overcome the size limitation posed by SSR markers, as most of them all within the 200bp region, limiting the number that are suitable for multiplexing. Dr Erik Ohlsen's article on this work can be found on page 6.



**Figure 2**: Initial multiplex trial using SNP markers 2\_15464 (blue arrow), 2\_02911 (green arrow), 2\_03535 (black arrow) and 2\_02471 (orange arrow) on various cowpea varieties from the UK, Nigeria and Ghana. Amplified samples were run on 6% acrylamide gel at 120V for 5hrs. 1x TBE running buffer and peristaltic pump was used.

#### Awards

- Congratulations to **Dr Kelvin Kamfwa** of the University of Zambia, on receiving the "Early career grain legume scientist" award at the Common bean/cowpea international conference in Burkina Faso. This award was presented by the US Feed the Future Innovation Lab for Collaborative research on Grain Legumes. Dr Kamfwa is an ABC PI and will be working on genetic improvement of the common bean in Zambia.
- Congratulations to Dr Clare Mukankusi of CIAT, Uganda for being nominated for the Achievement Award of the Bean Improvement Cooperative 2017 which will be presented at the Bean Improvement Cooperative 2017 Meeting in Michigan State University at the end of October. Dr Mukankusi is a plant breeder and PI at CIAT in Kawenda, Uganda, and has helped The Kirkhouse Trust with training and advice..
- Congratulations to Dr Idah Sithole-Niang of the University of Zimbabwe, one of five scientists to be awarded with the Addgene-Seeding Labs Plasmid grant.



## Genetics of Fusarium wilt resistance in cowpea using both conventional and

#### molecular marker approaches by Catherine C. Danmaigona

Fusarium wilt on cowpea was first reported in Nigeria by Oyekan in 1975. However, little effort has been devoted to breeding for improved varieties. The pathogen can cause up to 100% damage on a susceptible cultivar if the environmental conditions suit its multiplication. Disease symptoms include leaf chlorosis, leaf abscission, plant stunting and wilting; infected plant roots become partially or totally reddish-brown in colour. Once the infection starts, it spreads throughout the plant, leading eventually to the plant's death (Fig. 1). Vascular discolouration can clearly be seen when an infected plant root is cut lengthwise (Fig 2). The potentially devastating effect of Fusarium wilt on cowpea production in Nigeria prompted our research programme to seek sources of genetic resistance, to reveal the mode of inheritance of these resistances and to discover molecular markers linked to each resistance gene. The overall goal was to develop cowpea varieties able to yield a crop even



Fig 1 Symptoms of diseased cowpea plants.



Fig. 3 Spores of Fusarium oxysporum f.sp. tracheiphilum

when the disease is present.

The advice provided by my supervisor and P.I. Dr. Lucky Omoigui has been very instrumental in the success of this pathology work. I was able to complete all the pathology and the laboratory screening in the molecular biology laboratory at UAM (Figs 3 and 4) with guidance from Prof. E.J. Ekefan, a pathologist.

The initial stage of this work involved sampling of diseased plants from the field, isolation of the Fusarium wilt pathogen (Figs 3, 4) and the preparation of spore suspensions to be used as inoculum.

Sixty cowpea accession were screened for resistance using three different methods, namely the "laboratory seed soak method" (Aigbe et al., 2010, Fig. 5), the "screen house seedling root deep method" (Ribeiro et al. 1979, Fig. 6) and field screening (in two locations). The three assays were well correlated with one another, so that in future, any of them can be used to identify Fusarium wilt resistant plants. The laboratory seed soak method proved to be the fastest assay.

The phenotyping revealed a high level of resistance in the three accessions TVu 134, TVu 410 and TVu 109-1, which were all taken forward for crossing with a susceptible parent to study the mode of inheritance of the resistances. The F1, F2 and BC1F1 progenies from the crosses TVu 134 × TVu 984, TVu 410× IT99K-573-2-1 and



TVu 109-1 × TVu 231-2 were phenotyped using both the "laboratory seed soak method" and "the seedling root deep method". The segregation patterns revealed were consistent with each of the resistances being controlled by a single dominant gene.





Fig 2 Diseased cowpea plants showing vascular discolouration symptoms symptomatic of Fusarium wilt



Fig. 4 Single spore of Fusarium oxysporum isolated and growing on PDA medium.

As part of my research, I spent a 6 month training period in molecular biology in Prof. Timko's laboratory at UVa. My task was to test 2,200 SSR primer pairs for informativeness between TVu 134 and TVu 984. Of these, 150 gave a positive result. I then used these primer pairs for a bulk segregant analysis of the TVu 134 × TVu 984 F2 progeny, which resulted in identifying potential marker/trait linkage for three SSR loci. A linkage analysis based on 200 F2 progeny and the three SSR primers showed that marker C13-16 was closely linked to the Fusarium wilt resistance in TVu 134. The outcomes of my research have paved the way for implementing marker-assisted selection for resistance to Fusarium wilt.

The training I received has made me more confident in carrying out molecular biology experiments, especially in the use and application of marker-assisted selection, and this year I successfully defended my master's thesis and was awarded an MSc

Catherine Danmaigona recently qualified for an MSc at the University of Agriculture, Makurdi (UAM), Nigeria, funded by the Trust. Catherine continues this work as a valuable member of the project team at UAM.



Fig. 5. The laboratory seed soak method.

# Genetics of Fusarium wilt resistance in cowpea using both conventional and molecular marker approaches *Cont'd*...

degree. My experience with pathology procedures has given me a secure grounding in molecular crop resistance breeding. During the course of my work I have encountered several other important wilt-causing pathogens, which has brought home



to me that curing the problem of cowpea wilt will require incorporating resistance to these pathogens as well.

This article would be incomplete without an acknowledgement of the contributions and opportunities provided for me. I wish to sincerely thank the Kirkhouse Trust for awarding me a scholarship for my MSc degree in plant breeding. The training

grant gave me an opportunity to be well trained and better equipped in the field of plant breeding using advanced molecular tools. I also wish to express my heartfelt gratitude to Prof. Mike Timko, who has been an inspiration; and to the entire team at UVa, who were all helpful and generous with their support. I finally acknowledge my P.I. Dr. Lucky Omoigui for his unwavering guidance, encouragements and mentorship. Thank you so much for giving me this opportunity.

#### Laboratory Highlights - Dust Hoods by Ann Lonie and Cynthia Sam

Kirkhouse Trust (KT) has collaborated with Cleaver Scientific Ltd to design and build an improved dust hood for KT projects involving pathology work. Following feedback on its original hood the Trust has developed an improved dust hood (Fig 1 and 2) which includes a fan and a HEPA filter, LED light and an access port hole for the microscope cable. The angled front of the dust hood now features three hinges allowing users to either close it completely, use it with a minimal gap or open it up fully when using the microscope. We asked three of our recipients of the new dust hood to share their opinions.



"It is a simple equipment but it is serving a great purpose... Minimum contamination is being experienced as compared to directly working on the bench" *Dr Reuben Otsyula, KALRO* 

"..my opinion after using it is that it is good" *Kelvin Kamfwa*, *University of Zambia* 

"It's simple to use and well designed not require extra space in the lab and the

**Fig. 1.** Pathogen section of a newly set-up KALRO lab in Kenya featuring the new and improved dust hood. **Fig. 2.** New dust hood in use at SARI Ethiopia

lighting and fan system provides good and convenient, contamination free working" Yayis Rezene Tedla, SARI

#### Graduations

- Congratulations to Dr N. Aichatou Coulibaly of Institut National de la Recherche Agronomique du Niger for completing a Ph.D at Université de Ouagadougou, thesis titled: "Développement de varietés de niébé résistantes au Striga gesnerioides (wild) au Niger par la méthode du backcross Assistée par Marqueurs Moléculaires."
- Congratulations to Dr Sory Diallo of Institut d'Economie Rurale, Mali for completing a Ph.D at Université de Ouagadougou, thesis titled : "Developpement de Varieties Elites de Niebe (*Vigna unguiculata* (L.) Walp.) Resistantes au Striga (*Striga gesnerioideS* (Willd.) Vatke) et Identification de Marqueurs SSRs Associes au Gene de Resistance a la Race SG2"
- Congratulations to Dr Sobda Gonné of Institut de Recherche Agricole pour le Développement for completing his Ph.D studies at the West Africa Centre for Crop Improvement, with a thesis titled: "Genetic Studies of Cowpea [*Vigna unguiculata* (L.) Walp] for Resistance to Thrips (*Megalurothrips sjostedti Trybom*) in Cameroon".



## Development of PCR-based codominant SNP markers for cowpea genotyping studies by Dr Erik Ohlsen

Thanks to their abundance, single nucleotide polymorphisms (SNPs) have rapidly become the genetic marker of choice in many genotyping studies. A number of technologies have been developed for their detection, including micro-arrays, genotyping-by-sequencing and the "Kompetitive Allele Specific PCR" (KASP) assay, to name but a few. Most of these genotyping platforms, however, require specialized equipment and expensive reagents, which many breeding labs cannot realistically afford. Even the simpler, PCR-based approach referred to as CAPS (Cleaved Amplified Polymorphic Sequences) is still problematic as it requires the maintenance of a number of costly restriction enzymes. Consequently, simple sequence repeats (SSRs) have remained the predominant marker used for marker assisted selection in most West African cowpea molecular breeding programmes, including those supported by the Kirkhouse Trust.



Several PCR-based SNP genotyping methods have been developed which demand neither expensive reagents nor specialized equipment, but instead rely on reagents and equipment which is available in most molecular breeding labs. A variation of the so-called "allele specific PCR" (AS-PCR) is one of these: it is a relatively easily optimized protocol in which one common primer and two allele specific primers are combined in a single PCR reaction1.. The AS-PCR reaction is designed to convert a known SNP into a codominant, PCR-based marker which can be scored on a gel (Fig. 1). In order to take advantage of the large collection of cowpea SNPs which has been documented, KT has supported the conversion of nearly 200 SNPs from The Cowpea iSelect Consortium Array. The chosen set of SNPs to convert to AS-PCR provides a wide coverage of the genome, and are spaced on average less than 5 cM apart. The intention is to encourage the use by cowpea breeders of these markers within their molecular breeding programmes. They have several advantages over SSRs: their position on the genetic and physical map of cowpea is secure, they are more informative than most SSRs, and they are evenly distributed across the cowpea genome. The AS-PCR products are designed to be readily separated by electrophoresis through a 6% polyacrylamide gel (Fig. 2), so are well suited for marker assisted selection and genetic mapping. The AS-PCR primers should shortly be available to all of the KT-funded cowpea breeding programmes.

<sup>1</sup>Gaudet, M. et al. (2007). Single-reaction for SNP genotyping on agarose gel by allele-specific PCR in black poplar (*Populus nigra L.*). Plant Mol. Biol. Report. 25: 1–9



Figure 1. The allele-specific PCR reaction. One common primer and two allele specific primers are used in a single reaction. The long and short tailed allele specific primers differ in length by 10 nt allowing for the two potential PCR products to be distinguished from each other by PAGE. A destabilizing mismatch is introduced within 5 base pairs of the 3' end of each allele specific primer to increase specificity.



Figure 2. Cowpea lines 524B (P1) and Suvita-2 (P2) and their F1 progeny (524B x Suvita-2) genotyped with four converted allele-specific PCR markers (2\_11056, 2\_54765, 2\_23058, 2\_10954). The amplified PCR products were visualized on a 6% PAGE stained with EtBr

Dr Erik Ohlsen is a Research Associate at the University of Virginia (UVa), U.S.A. He works in the laboratory of Prof. Michael Timko carrying out, amongst other tasks, marker development and training of members of the West African Cowpea Consortium as part of the Kirkhouse Trust funded project at the UVa.