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Bean variety "Kablanketi" story *by Professor Susan Nchimbi Msolla*

Highlights

Sokoine University of Agriculture (SUA) TANZANIA—*Origins:* Kablanketi is believed to have been introduced in Tanzania from Malawi as long ago as 1948 by Tanzanians returning from the gold mines in Johannesburg in South Africa. In those years, men from Southern parts of Tanzania (Mbeya, Rukwa, Ruvuma and Iringa regions) used to go to South Africa to be employed in gold mines. Most of them left their families behind, who remained engaged in agriculture.

When the men came back to Tanzania, they often brought with them money together with other things such clothes, a radio and kitchen utensils, just to mention a few. Since they'd left their wives and children back at home cultivating their fields, sometimes they also brought back seeds of various crops, hoping they would grow well back in their homeland.

According to two elderly men Mr.Mboneshe Mwashuya (almost 98 years old) and Mr. Tuloline Mwalyemba (88 years old) from Ivwanga village in Mbozi district, the seeds brought included those of bean, and Kablanketi was one of the types they brought. This type of bean seed was collected from a town known as Salima in Malawi, since on their way back to southern Tanzania they had to pass through Malawi (Figure 1). Initially, this variety was called *Namawula*, the name of the tree which has a grey mottled bark, resembling the colour of the seed of this variety. Later on, the colonial people called it *Kablanketi* a swahili word meaning blanket because the seeds of this variety also resembled the colour of the blankets which those people carried on their journey back from South Africa.

Figure 1: Map of Malawi and part of southern Tanzania showing Salima town (almost the centre of Malawi) where Kablanketi is believed to come from and Mbeya town in Tanzania where this variety started to be grown in Tanzania

Note: Red Boxes = Salima a town in Malawi and Mbeya town in Tanzania

In the southern parts of Tanzania especially in Mbeya and Rukwa regions, as is also the custom in Malawi, farmers like to grow a mixture of beans and sometimes use and sell them in that form. So when Kablanketi was introduced it was incorporated as part of the composition of the bean mixtures. After being used for some time farmers realized that Kablanketi was a particularly tasty component and that it cooked more quickly than other beans. Farmers then started sorting Kablanketi out of the mixture and selling it separately to traders.

In the mid 1980s, the variety started to become popular in other parts of the country as well, and people were calling it *maharage ya Mbeya,* meaning beans from Mbeya. Some called it *soya* – probably because the seed has a round shape resembling that of the soya bean. Kablanketi became a popular market class competing with yellow types, Calima types (Lyamungo 85 and Lyamungo 90) and red kidney types (Canadian Wonder and Bwana shamba).

'*Kablanketi*, a swahili word meaning blanket'

Traits—Kablanketi is a semi climber with Type II plant type which does not need staking. It flowers

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Bean variety "Kablanketi" story *continued...*

32-35 days after planting and reaches maturity on average 74 days after planting. The most common version of Kablanketi has round seeds and is grey or purple mottled. There are other versions of Kablanketi that differ in seed shape and colour tone. Seed shapes include round and oval (elongated), and the colour of the seed can be grey, purple, brown or mottled (speckled) (Figure 2).

Figure 2: Some types of Kablanketi

Kablanketi is appreciated for its fast cooking and good taste when cooked - both as dry bean or as a fresh bean (when the seeds have changed their colour but are not quite dry). The women food vendors in urban areas like this variety because they do not have to spend a lot of time cooking it and at the same time its preparation is less costly in terms of fuel usage. Despite all these good traits, however, Kablanketi plants are susceptible to most bean diseases, so the variety is low yielding and its seed quality is sometimes poor. The average grain yield of Kablanketi is 500kg/ha.

Improvement of Kablanketi—Because of Kablanketi's fast cooking and other good culinary characteristics, the Bean Breeding Programme at Sokoine University of Agriculture started crossing it with the improved bean variety Rojo, which has good levels of disease resistance. A backcross breeding procedure was undertaken to improve Kablanketi for disease resistance, yield and plant type. Four backcrosses with Kablanketi were made followed by inbreeding, and a Participatory Variety Selection (PVS) exercise was then undertaken. The result of this programme was the release of two new varieties, namely Mshindi and Pesa. Mshindi has the same seed colour as Kablanketi (grey) while Pesa is red. The seed yield of Mshindi and Pesa is 1000-1500kg/ha.

In the current ABC project in Tanzania funded by the Kirkhouse Trust, Mshindi has been used as a recurrent parent in a programme aimed at developing multiple disease resistance bean varieties. This programme specifically deals with angular leaf spot (ALS), common bacterial blight (CBB), bean common mosaic virus (BCMV) and bean common mosaic necrosis virus diseases (BCMNV). The breeding goal is to introgress genes for resistance for these diseases into Mshindi assisted by molecular markers (Marker Assisted Selection-MAS). At present the project has generated some lines that combine the genes for resistance; the current effort is aimed at advancing these selection by inbreeding for further selection using both genotyping and phenotyping.

> 'The breeding goal is to introgress genes for resistance for diseases into Mshindi assisted by molecular markers (Marker Assisted Selection-MAS)'

Expectations from breeding—The expectations from breeding is to produce at least two new bean varieties which carry genes for resistance for each of ALS, CBB, BCMV and BCMNV, while having the seed type and seed quality characteristics of Kablanketi. These varieties are being targeted for both the lowland (450- 1000masl) and mid altitude (1001-1500masl) areas of Tanzania.

Acknowledgements *– My deep appreciation goes to the two farmers Mr.Mboneshe Mwashuya and Mr. Tuloline Mwalyemba who volunteered to provide information about Kablanketi. I also thank Ms. Mary Ndimbo (PhD student at SUA) who assisted in connecting me with the farmers and in collecting information. My sincere appreciation goes to Kirkhouse Trust for giving me the chance to share this information in the Kirkhouse Trust Newsletter and also for continuous financial support for the ABC project activities at SUA.*

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A research journey *by Luseko Amos Chilagane*

Sokoine University of Agriculture (SUA) TANZANIA—My research journey started back in 2008 when I was funded by KT to undertake an MSc in Crop Science at SUA as part of the SUA ABC Project "Use of marker assisted selection (MAS) to improve selection efficiency in breeding for resistance to major diseases of common bean in Tanzania".

Back then I had only limited experience in carrying out research and especially in the use of modern tools like MAS. Through my participation in this project I have now become much more confident in my field of research and was able finish my MSc in 2012 and then to move on to a PhD, carrying on with the project both as a student and as a researcher. Currently I see myself more as a researcher than a student thanks to the knowledge and expertise I have acquired as a result of my participation in the project. As part of the ABC project we hold an annual meeting and training workshop, where we present project updates and progress over the year. Apart from meeting annually we never stop communicating during the rest of the year, raising whatever concerns we have and sharing our experiences with other members of the ABC consortium (Kenya, Rwanda and Uganda) and the project administration, which all helps to ensure that the project goes well.

During the course of my MSc I was offered the chance to go to US as a short term visiting scholar at University of California Davis (USA). This was a great opportunity for me to learn more, since there I was working in the laboratory with other scholars from US and elsewhere. My stay exposed me to what other scholars/scientists are doing, since we all did our research together in the same laboratory, troubleshooting technical problems together and learning from each other's research. In addition, I joined a UCD course "Applied Bioinformatics" which was offered during the time of my visit, and also benefited from a special program for marker discovery training facilitated by Prof Paul Gepts. This training helped me to gain knowledge in bioinformatics which is a key component of molecular breeding. I was able to explore the functionality of the Phaseolusgene database, which is a great tool for marker discovery and the design of alternative molecular markers that can be used in Africa (Page 4 'DNA Marker Analysis ').

Last year I was selected to participate in the "VI International Conference on Legume Genetics and Genomics" (VI ICLGG) held in India; this was my first participation in an international conference. Thanks to the confidence I had built up in myself as a result of taking part in the ABC Project, I felt able to present a poster, which was awarded a prize as the "Best Poster". This made me realize how every bit of what we are doing in the project is important, as I recalled that we had a session of poster presentation during one of our annual meetings in Tanzania, given by Robert Koebner. My poster was based on my MSc research work where I used molecular markers to incorporate three different genes for disease resistance into a particular market class of beans in Tanzania as part of the big project undertaken at SUA.

During my MSc work, I have come up with several ideas which have been incorporated into the current SUA project and will form the basis of my PhD program. This will be to use molecular tools to characterize the causative agent for angular leaf spot, a prominent disease of beans in Tanzania. During my MSc program, I was able to identify some resistant local accessions for this disease, and the idea will be to design markers for these resistances, so that we can readily pyramid them into our popular bean varieties.

I owe my sincerest thanks to Prof Susan Nchimbi–Msolla (Project leader), Prof Cornell Rweyemamu (Head of Department) and the KT team at large for taking part in facilitating this research journey, I hope we will continue working together as we are doing and make a positive impact on bean production in Tanzania.

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DNA Marker Analysis Using the PhaseolusGenes Database *by Tamara Miller*

UC Davis CALIFORNIA— The speed of hybrid selection during plant breeding can be greatly improved by using molecular markers which allow plants to be distinguished by the genes carried from either parent. For instance in a cross between parents resistant and susceptible to Angular Leaf Spot it would be helpful to be able to test the progenies and determine which carry the resistance genes so that only those carrying the resistance gene allele are grown to maturity.

If the parents have different DNA sequences, or polymorphisms, near the resistance gene those differences can be exploited to allow resistant plants to be distinguished from susceptible plants. A DNA marker linked to the resistance locus can be assayed using the Polymerase Chain Reaction so that plants carrying the resistance genes can be identified and carried forward

Finding the DNA differences— The first step in finding polymorphic markers between resistant and susceptible plants is to identify DNA differences between the parental plants at a locus closely linked to the resistance locus. In the case of making crosses between ALS susceptible and resistant plants, the marker associated with the Phg-2 resistance locus (SN02) is the same sequence (monomorphic) between resistant and susceptible plants used in the ABC breeding programs. This makes it impossible to use the marker in a PCR to differentiate between progeny carrying either the resistant or susceptible Phg-2 alleles.

> 'The speed of hybrid selection during plant breeding can be greatly improved using molecular markers '

Since the precise location of Phg-2 in the common bean genome is not known, it is necessary to look for alternative polymorphic markers closely linked to SN02. The rationale for doing this is that since SN02 is located very near to Phg-2, then so will a marker closely linked to SN02 be near Phg-2.

PhaseolusGenes database— The PhaseolusGenes database has sequence and primer data for most published common bean and soybean markers; using this we can search for SN02, find the location of this marker along the G19833 common bean reference genome and locate candidate markers nearby. To use the PhaseolusGenes database to locate candidate markers, first navigate to http://phaseolusgenes.bioinformatics.ucdavis.edu/ and enter the name of the marker to be located in the search box on the upper right-hand part of the screen.

Use the following steps to locate and test markers near SN02 on the common bean sequence map:

- Enter 'SN02' into the search box in the upper right-hand corner of the screen.
- Click on the search result 'SN02'.
- On the page listing published data for SN02, click the link, 'P. vulgaris v.1.0', under 'Blast this sequence' to find sequences in the G19833 reference genome with high similarity to SN02.
- Click 'browser' in the top search result.
- After the genome browser loads showing the top 'track' with the SN02 locus (appears as a black bar at the very top of the browser), click on tracks below it to show SCAR, SSR, SNP or Indel markers surrounding SN02.
- To find sequences of the individual markers and the primers used to amplify them, click on their names.

Figure 1: PhaseolusGenes homepage

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DNA Marker Analysis Using the PhaseolusGenes Database *continued...*

- On the next page click the top most link next to 'Outside link', which leads to the data sheet for that marker.
- Get the published primers from the marker data sheet and use them in a PCR using DNA from resistant and susceptible parents as the templates.
- After PCR, run the amplification products on a 5% horizontal page gel to resolve the fragments, and note whether the DNA from the resistant parent is a significantly different sized product compared to that of the susceptible parents.

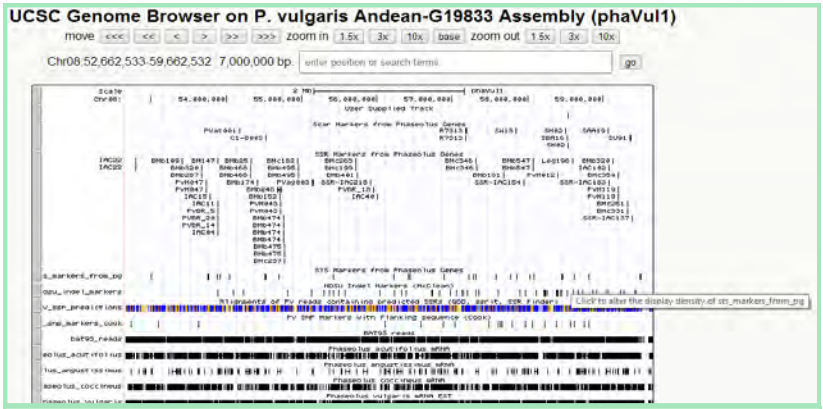
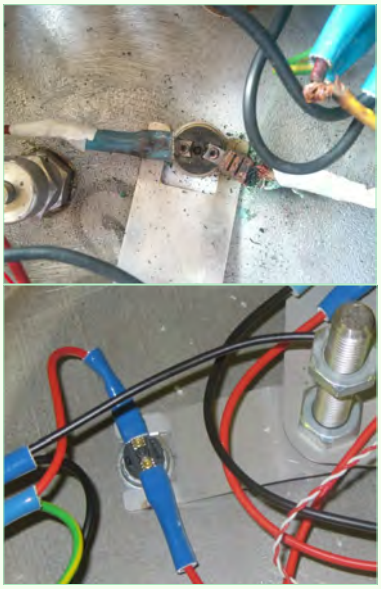


Figure 2: Genome browser showing the SN02 locus

Testing the primers – Published primers used to amplify markers from different parental DNA may not always result in robust amplification because the primer sequences may not match the target sequences with 100% similarity. If PCRs do not produce amplification after the first test, touchdown PCR may be used. Here rather than using multiple tubes each with a different cycling parameter (gradient PCR), a single tube is used and multiple cycles are programmed to run at incrementally lower temperatures. This ensures that the first hybridization/amplification will be between reactants with the greatest complementarity which will out compete any non-specific hybridization which may occur at sub-optimal temperatures.

Once at least one polymorphic marker between parents is found, progenies resulting from crosses between the parents can be screened at the seedling stage and plants with the marker allele corresponding to the resistant parent can be kept while those carrying the allele from the susceptible parent are discarded.

Laboratory hints and tips



Does your Lab have equipment which could be brought back into productive service with a little attention?

Nicholas Olango, a PhD student at NaCRRI Uganda, successfully repaired a Dixons autoclave. This had a corroded element (left, top) and following consultation with the Kirkhouse Trust and the manufacturers, a replacement element was sent out and successfully fitted (left, bottom).

Well done Nicholas, your actions saved £1300 – the price of a replacement – and the potential delays and disruption associated with waiting for new items to be approved, purchased and dispatched.

For PCR, it is encouraging that many of the labs have been dividing the 20µl reaction AccuPower PCR premix into 2 x 10µl reactions. This doubles the number of tests that can be performed without loss of resolution. Additional PCR tubes are supplied by KT in order to do this.

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Awards

- Paul Gepts was selected by the USDA-Agricultural Research Service to give the 2013 edition of the B.Y. Morrison Memorial Lecture series at the annual meeting of the American Society for Horticultural Science in Palm Desert, California .

'The B.Y. Morrison Memorial Lecture series was established in 1968 by the Agricultural Research Service to honour the memory of Benjamin Y. Morrison and to recognize scientists who have made outstanding contributions to horticulture and other environmental sciences, to encourage the use of these sciences, and to stress the urgency of preserving and enhancing natural beauty.'

- Congratulations to Ms. Arpita Srivastava who has received a Shri Dwarika Nath Memorial gold medal Award for the best thesis on vegetable science for the year 2012.

The medal was awarded by the Indian Society of Vegetable Science for best Doctoral thesis work on vegetable crops. The award was established in 1998 to encourage young scientists and recognises talent at the National level. Ms Arpita carried out her molecular research at the KT funded lab at the University of Agriculture, Bangalore, India.

- Dr Mohan Rao, Associate Professor at University of Agricultural Sciences, Bangalore was selected for the ICAR Best Teacher award for the year 2013.

The award is based partly on progress made and credentials in academics during the last five years. Dr Mohan Rao is the Kirkhouse Trust coordinator for KT funded projects at the University and was also the PI for a Kirkhouse Trust project 'Tagging, mapping and validation of QTL's for anthracnose resistance and development of anthracnose tolerant cultivars (hybrids/RIL's) in hot pepper (Capsicum annum L.)'

Introductions—Justine Nakibuule

Justine has a Bachelor's degree in biological sciences of Kyambogo University (Uganda) and currently works at the National Crops Resources Research Institute (NaCRRI) - Namulonge (Uganda) as a Research technician on the beans program.

She is responsible for the: isolation of different pathogens for root rots and ALS currently, pathogen identification and maintenance of clean cultures; field surveys for assessment of disease incidences and severity in common beans and collection of diseased samples for pathogen isolation in the laboratory; participation in molecular activities; maintenance of clean and contaminant free working environment in the laboratory; extending support to special project students and those on internship, and any other duties as may be assigned by her supervisor. Justine attended the ABC annual meeting 2013 *"The ABC meeting was so educative and such an eye opener to many aspects of the work I am handling, and I actually enjoyed every bit of it."*

Recent publications

- Chilagane, L.A. et al (2013). Incorporation of resistance to angular leaf spot and bean common mosaic necrosis virus diseases into adapted common bean (Phaseolus vulgaris L.) genotype in Tanzania. African Journal of Biotechnology. Vol. 12 (27) 4343-4350
- Uzabakiriho, J. D. et al (2013). Co-infection of Tylosema esculentum (Marama bean) seed pods by Alternaria tenuissima and a Phoma spp. African Journal of Biotechnology Vol. 12(1), pp. 32-37.
- Nzungize, J. R. et al (2012). Pythium root rot of common bean: biology and control methods. A review. Biotechnol. Agron. Soc. Environ. 16(3), pp. 405-413 .
- Akinbo, O. et al. (2012). QTL Analysis for Root Protein in a Backcross Family of Cassava Derived from Manihot esculenta ssp flabellifolia. Tropical Plant Biol. 5, pp. 161–172
- Arunga, E.E. et al. (2012). Characterization of Uromyces appendiculatus isolates collected from snap bean growing areas in Kenya. African Journal of Agricultural Research Vol. 7(42), pp. 5685-5691