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IAEA Laboratories Seibersdorf, International Atomic Energy Agency, Vienna, Austria

Contents

The Animal Production and Health Laboratory

EXECUTIVE SUMMARY

The global livestock sector is highly dynamic with an estimated asset value of around US $1.5 trillion. This sector employs at least 1.3 billion people globally and directly supports the livelihood of more than 600 million smallholder farmers in the developing world, particularly in South Asia and Sub-Saharan Africa. Livestock production systems contribute to increased household income and accelerated growth of the rural economy, thereby improving food and nutritional security among poor and marginal people. Livestock also serves as an invaluable insurance to deal with adversity and emergencies of rural poor and this sector is expected to make a significant contribution towards reducing rural poverty. Among the livestock, small ruminants form an important component of rural households worldwide, especially in arid and semi-arid regions. However, using livestock as a tool for poverty alleviation is not without challenges. Animal diseases not only threaten the ability to accumulate livestock assets, they also lower livestock productivity. Zoonotic diseases also endanger the health of livestock-dependent people and around seventy percent of the new diseases that have emerged in humans over recent decades were of animal origin.

With increasing human population and continuous increase in demand for animal protein/products, a further intensifications and expansion of livestock systems are expected. This means increased interaction of human, livestock and wildlife that will potentially lead to increased incidence of zoonotic diseases, like Ebola, influenza, etc. Added to it, globalization and climate change are redistributing pathogens, vectors and hosts with resultant changes to the human and animal disease landscapes. Another major effect of increased intensification of animal production and consequent monoculture is the growing threat to livestock biodiversity. The state of the world's animal genetic resources report shows that approximately one livestock breed has been lost every month during the last decade alone. This is a huge concern for sustainable livestock production and future global food security. To address these issues and to support IAEA and FAO member states (MSs) in overcoming these challenges, activities of the Animal Production and Health Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture are focused on two major areas: (i) control of transboundary animal diseases through the development and transfer of tools related to diagnosis, molecular epidemiology and preventive vaccination and (ii) improving the genetic potential of local livestock breeds to increase productivity and conservation of livestock biodiversity through effective implementation of the global plan of action on animal genetic resources in MSs.

The major focus of transboundary animal diseases in 2014 continued to be those diseases affecting small ruminants. Peste des petits ruminants (PPR) is a highly infectious transboundary animal disease that causes global losses of between US $1.45 billion and $2.1 billion each year. Since its first identification in the early 1940s in Côte d'Ivoire, PPR has steadily expanded its geographical distribution beyond its original endemic region in Western Africa. Indeed, a significant and dramatic geographical expansion of the disease has occurred over the last 15 years resulting in large parts of Central Asia, South Asia and East Asia now being endemic for PPR. The control of PPR is considered an essential element in the fight for

global food security and poverty alleviation and hence a global initiative for its eradication is currently being proposed and expected to kick off in early 2015. APHL has been very active in the development of tools for the control of this disease, in particular specific and rapid diagnostic tests for PPR based on serological methods. Studies on molecular epidemiology of PPR, particularly analysis of samples from Kenya, indicated transboundary movement of lineage III viruses between Eastern African countries. This will have significant implications on surveillance and control of this important disease as it moves southwards in Africa. Further, to better understand the spread and evolution of the disease, APHL initiated in 2014 the development of a simple and cost effective real time PCR-based assay for differentiation of PPR virus lineages, the preliminary results of which are very promising.

Apart from PPR, the other important disease that causes significant economic losses to sheep and goat farmers worldwide is capripox. This is another viral disease on which APHL has a well-recognized expertise. In 2014, APHL embarked on the development of user-friendly methods to differentiate live attenuated capripox vaccines from the pathogenic field isolates. With the increasing incidence of capripox outbreaks in previously vaccinated herds, this test is expected to help in ruling out the vaccine involvement in such outbreaks. Towards APHL's efforts to detect multiple pathogens from samples collected in resource poor settings, the pan-pox detection system and the multi-parametric detection system for respiratory pathogens, developed in 2013, were further successfully validated in 2014 with additional field samples from different regions. These technologies have already attracted significant interest from some MSs and are expected to be transferred to national laboratories in Asia and Africa in 2015 through the VETLAB network under the Peaceful Uses Initiative (PUI) of the IAEA.

In addition to PPR and capripox, two other important diseases were subject of R&D in APHL in 2014: African swine fever (ASF) and animal trypanosomosis. For ASF, a highly contagious swine disease that emerged into East Europe from its well-known endemic area in Africa, the molecular epidemiology study was continued in 2014. ASF Virus (ASFV) isolates from ten African countries (Burkina Faso, Cameroon, Cap Verde, Central African Republic, Chad, Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia, Nigeria and Senegal) were characterized. Further, to facilitate ASF genotyping and to automate the determination of the virus profile, a tool named "ASF CVR profiler" was developed at APHL for the benefit of MSs. This online tool will be made freely available to MS researchers in 2015 and is expected to help them in identifying the type of ASFV circulating in the countries with more efficiency and in less time. With respect to trypanosomosis, in 2013 it was established that low level irradiation doses produce non-virulent but viable and metabolically active Trypanosomes. Comparative genomic analysis of irradiated versus non-irradiated parasites will help to identify genes that are modified by irradiation and will form the basis for the development of a vaccine. To facilitate this, an expression micro-array platform for Trypanosomes was developed by APHL in 2014 and will be made available for the use of the global Trypanosome research community.

Finally, APHL continued DNA marker discovery in goats to identify and validate SNPs (Single Nucleotide Polymorphisms) for testing the association with parasite resistant phenotypes. Gastro-intestinal parasites cause more than US $10 billion loss to the global livestock industry every year and Haemonchus parasite is a major menace to sheep and goat farmers. Considering the growing level of anthelmintic resistance in these worms and differences

among the variants, APHL initiated the development in 2014 of a simple and cost-effective assay to differentiate the three major Haemonchus species infecting ruminants in Asia. The test will help MSs to identify the principal circulating species/variants in their countries and to formulate sustainable strategies for the control of these parasites. The assay was tested successfully and will be validated further for its sensitivity and specificity during 2015. With respect to the implementation of the global plan of action on animal genetic resources to protect livestock biodiversity, APHL supported genetic characterization of Madagascar native cattle and Myanmar goats using DNA based technologies. More than 300 animals belonging to three breeds each of cattle and goat were genotyped and sequenced to assess their genetic variability. In addition, APHL in collaboration with headquarter staff embarked on the development of a "Genetics Laboratory Information and Data Management System (GLIDMaS)". The platform is expected to support animal geneticists in managing large volumes of data intended for animal selection, conservation and genetic improvement. Different modules were created in 2014 and testing and validation of the system will be continued in 2015.

In addition to R&D, another pillar of APHL is promoting capacity building in IAEA and FAO MSs. As part of these activities, APHL staff undertook four technical field support missions in 2014 (Burkina Faso, Cameroon, Democratic Republic of the Congo and Madagascar) to build capacity in MS veterinary diagnostic and animal genetic laboratories. With the objective of promoting the implementation of quality assurance and quality management in counterpart veterinary diagnostic laboratories, APHL also continued to conduct a proficiency test for the diagnosis of PPR. It further conducted three group training courses and hosted five fellows and three interns, all funded by either extra-budgetary funds or by the IAEA Technical Cooperation (TC) Department.

APHL activities carried out in 2014, in particular related to capacity building, benefited from the financial support of the FAO-WHO-OIE Tripartite project "Identify" (USA-funded project), the IAEA-PUI projects (USA and Japan funded PUI projects) and the African Renaissance Fund (South Africa-funded project).

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

The use of irradiation-based technology for the development of vaccines against animal Trypanosomosis

Trypanosomosis is an important parasitic disease in mammals caused by a blood parasite belonging to the Trypanosoma genus. These protozoan parasites are transmitted either mechanically as in the case of *T. evansi*, or through an insect vector in the cases of *T. congolense* and *T. vivax*, both of which are transmitted by Tsetse flies of the Glossina genus. Trypanosomes not only present a public health issue in cases where they cause human disease, but are also a hindrance to the development of livestock resources, with approximately 8.7 million km^2 of the African continent infested by Tsetse flies, putting about 4.6 million cattle at risk annually. Trypanosomosis in livestock is currently controlled by the use of drugs that kill parasites in infected cattle or through the eradication of the Tsetse fly vector. There are currently no viable vaccines available for the prevention of the disease in cattle.

Preliminary experiments at APHL with irradiation of *T. evansi* at a low dose of 200Gy produced viable parasites that are able to replicate *in vitro* under laboratory conditions. Irradiation doses higher than 200Gy resulted in loss of long term viability. This showed that low level irradiation produces non-virulent but viable and metabolically-active parasites that could potentially induce strong pro-inflammatory responses in the host. This observation provides the basis for a vaccine that could present a large repertoire of antigens to the host immune system in a single inoculation. The study progressed with testing three rounds of inoculation using 1 x 106 irradiated parasites in several groups of mice, using different doses of irradiation ranging from 0Gy to 600Gy. It was observed that mice inoculated with viable parasites irradiated at 200Gy did not develop an infection when compared to other groups of mice inoculated with parasites irradiated with lower irradiation doses. Sera collected from immunised mice also showed that parasites irradiated at 200Gy elicit the highest pro-inflammatory response, and the lowest non-inflammatory response when compared to other doses. Pro-inflammatory responses are important during the early phase of a trypanosome infection and are responsible for clearing the first wave of parasites during a natural infection in livestock.

In order to further explore this concept, comparative genomic analysis of irradiated versus non-irradiated parasites is necessary to identify genes that are modified by irradiation and are responsible for the loss of virulence during inoculation. *In vitro* cultures of parasites were used for irradiation with doses ranging from 0Gy to 600Gy, replicating the mouse experiment. RNA was isolated from irradiated parasites to evaluate differential expression of genes across this dose range. The information derived from this experiment will be used for targeted sequencing of associated DNA and to obtain a global view of the effect of irradiation on gene expression across the Trypanosome genome. Top hits will be identified as possible virulence factors that can be developed as potential vaccine targets. As part of this initiative, APHL developed an expression micro-array platform that covers the genomes of three trypanosome species, *T. brucei*, *T. evansi* and *T. congolense* (Table 1). Such a multi-species genomic tool targeting Tsetse as well as non-Tsetse transmitted Trypanosomes is expected to be used by researchers across different laboratories for studies on gene expression in these protozoan parasites. The experiments using microarray to study gene expression in irradiated parasites is currently under progress.

Table 1. Micro-array design for three trypanosome species

Parameter	*T. brucei*	*T. congolense*	*T. evansi*
# probe selection regions	15 180	13 396	17 711
# probe sets on array	4 583	10 362	5 637
total probes on the array	108 905	259 050	140 595
% genes represented on the array	96.68%	93.50%	90.35%

Pestes des petits ruminants

Peste des petits ruminants (PPR) is a highly infectious transboundary animal disease that affects mainly sheep, goat, and small wild ruminants. Sheep and goats contribute considerably to the cash income and nutrition of small farmers in many countries and the disease, which

provokes high fever, rapid emaciation, respiratory collapse and mortality, causes global losses of between US $1.45 billion and $2.1 billion each year. The control of PPR is thus considered an essential element in the fight for global food security and poverty alleviation. Indeed, it is for this reason that PPR is presently being considered as the next animal disease for global eradication. APHL has been very active in the development of tools for the control of this disease, in particular specific and rapid diagnostic tests and the generation of genetic data for a better understanding of the molecular epidemiology of the disease.

Molecular epidemiology of PPR

Characterization of PPR virus (PPRV) from Kenya

In May 2011 in Turkana County of north-western Kenya, tissue samples were collected from goats suspected of having died of PPR disease. The samples were processed and tested by reverse-transcriptase PCR for the presence of PPR viral RNA. The positive samples were sequenced and identified as belonging to PPRV lineage III (Fig. 1). Full genome analysis of one of the positive samples revealed that the virus causing disease in Kenya in 2011 was 95.7% identical to the full genome of a virus isolated in Uganda in 2012 and that a segment of the viral fusion gene was 100% identical to that of a virus circulating in Tanzania in 2013. These data strongly indicate transboundary movement of lineage III viruses between Eastern Africa countries and has significant implications for surveillance and control of this important disease as it moves southwards in Africa.

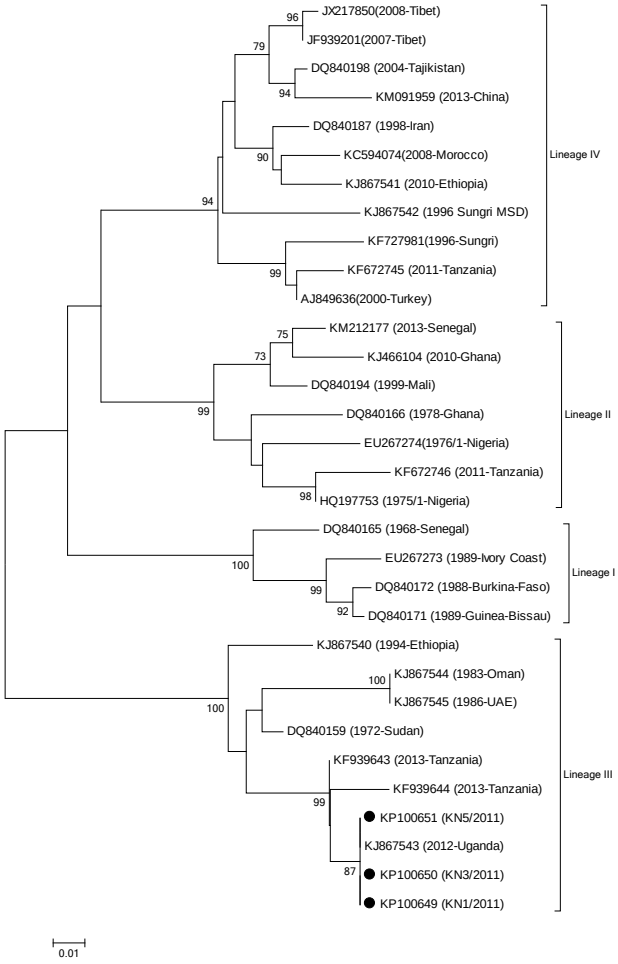

FIG. 1: Phylogenetic analysis of PPRV from Kenya using the NP gene fragment

Full genome sequencing of PPR virus

Four major lineages of PPRV have been reported to circulate among small ruminants across Asia and Africa. Lineage I is mainly circulating in West and Central Africa, lineage II and III in the Middle East and East Africa and lineage IV in Asia and Africa. Full genome sequence information on different PPRV lineages helps in better understanding the evolution and spread of this important disease.

First complete genome sequence of PPRV lineage III

Tissue samples were collected from three goats suspected to have died of PPR in Kenya in 2011 and shipped to the APHL for further characterization. Phylogenetic analysis of the amplicons generated from positive tissue samples revealed that they contained viral RNA from PPRV lineage III. The RNA from one positive lung sample was then selected for full genome sequencing. The organization of the KN5/2011 genome (15,948 bp) was as expected with a 107 nt genome promoter region at the 3' end followed by the transcription units for the N, P, M, F, H and L proteins and the antigenome promoter at the 5' end. The genome has the highest nucleotide sequence identity (87.2%) with the lineage II virus Nigeria 76/1 (EU267274) and the lowest identity (82.5%) with the lineage IV virus Sungri/96 (KF727981). It shares 84.7% nucleotide sequence identity with ICV89 (EU267273), the only full genome sequence of a lineage I PPRV available in NCBI-GenBank. The M is the most conserved of the proteins and has between 96.7 and 97.9% identity with lineage II viruses while the V protein is the least conserved having its highest identity (81.2%) with the lineage II viruses Nigeria 76/1 (EU267274) and Nigeria 75/1 (HQ197753). This is the first available complete genome of a lineage III PPRV and provides important information that, in combination with data on experimental and field infections in animals, will provide a clearer understanding of the genetic influences on host specificity, viral pathogenicity and transmission of PPRV.

Complete genome sequence of a PPRV lineage I isolated in 1969 in West Africa

In March 2013 a lyophilized specimen, dating back to 1969, was shipped by the Laboratoire de Virologie ISRA/LNERV, Dakar, Sénégal to APHL for further characterization. Phylogenetic analysis of the sequence of the amplicon generated revealed that the virus belonged to lineage I. The organization of the PPRV lineage I E32/1969 genome (15,948 bp) was identical to that seen for other PPRVs with a 107 nt genome promoter region at the 3' end followed by the transcription units for the N, P, M, F, H and L proteins and the antigenome promoter at the 5' end. The genome has the highest nucleotide sequence identity (97.1%) with the lineage I virus ICV89 (EU267273) and the lowest identity (89.3%) with the lineage III virus KN5/2011 (KM463083).

Development of real time PCR based assay to differentiate PPRV lineages

Considering the significance of PPRV lineages in establishing the spread and evolution of PPR, there is a need for rapid and specific tools to differentiate different lineages circulating among small ruminant populations. The lineage differentiation of the PPRV is presently carried out by sequence analysis of a fragment of N or F gene sequences. A simple and cost effective real time PCR based assay will help to screen large numbers of samples in a relatively short period of time to better understand the molecular epidemiology of disease. APHL initiated the development of a lineage differentiation assay based on the conserved regions of the PPRV genome and lineage specific sequences. As part of this initiative, targeted sequencing of PPRV across different lineages and countries of origin was conducted to generate information on the conserved regions. This information was combined with full genome sequence data available for selected samples to identify lineage specific variations. These variations are being targeted to design primers and probes and develop the real time PCR based test for genotyping PPRV lineages. The pilot test developed was able to successfully differentiate lineage IV and I from

lineage II and lineage III and efforts are currently underway to develop a single reaction based assay for differentiation of all four lineages.

Development of a diagnostic serological test for PPR

After Rinderpest became the first animal disease eradicated worldwide, PPR, a disease caused by a closely-related Morbillivirus, has become the next target for eradication. There are highly sensitive and specific tests for the detection of PPRV. However, the currently available tests for the serological diagnosis of the disease still present some cross reactivity between Rinderpest and PPR viruses. Since Rinderpest surveillance is expected to continue for some time, it is of great importance to develop an early and rapid disease diagnostic test to specifically detect PPR and avoid cross reaction with Rinderpest. With this in mind, APHL initiated the development of a sensitive and specific PPR serological assay based on luminescence. A nucleic acid vector was constructed to express a fusion protein composed of a fragment of the most abundant PPRV protein, the nucleoprotein, and luciferase, an enzyme that produces light following degradation of a substrate. The fragment of the PPR nucleoprotein serves as a hook to capture PPR antibodies when present in the test serum and retain them on the test plate. Using this procedure, an assay that enables a specific serological diagnosis of PPR has been developed. During 2014, this assay has been used to test and validate several PPR and Rinderpest serum samples (experimental and field) and compared to commercially available ELISA kits, as well as to the Viral Neutralization Test, the gold standard for PPRV detection in serum samples. The preliminary results on sensitivity and specificity of the new assay are promising.

Production of monoclonal antibodies against goat SLAM, the cell surface receptor used to improve efficiency of PPRV isolation

Isolation and propagation of PPR virus in cell culture is important to understand its characteristics, pathogenesis and diagnosis. The use of primary cell cultures and African green monkey kidney (Vero) cell line has several drawbacks, including low efficiency, i.e. the likelihood of isolating the virus is very low and, even if successful, often requires multiple, sequential blind passages and many weeks in culture before any cytopathic effect (CPE) can be observed. In 2009, APHL initiated the development of a recombinant cell line that can express a cell surface receptor, Signaling Lymphocyte Activation Molecule (SLAM), and enhance the efficiency of PPRV propagation in non-lymphoid cells. This newly developed cell line (CHS-20) has been transferred to Member State laboratories upon request to support research on PPR. However, to date, no antibody is available to demonstrate the expression of recombinant SLAM protein. The screening of positive cells is done only by demonstrating the presence of the mRNA by RT-PCR assay. The only commercially available antibodies are from human origin and they failed to detect the expression of the goat SLAM protein. To address this issue, APHL embarked on the production of monoclonal antibodies (mAbs) against goat SLAM to better characterize the production of SLAM protein in CHS-20 cells. Two mAbs specific to goat SLAM were produced and used to characterize CHS-20 cells by immunofluorescence staining (IF) and flow cytometry methods. SLAM protein expression was detected in CHS-20 cells with the mAb A8 (2A6), but not in the parental Flp-CV-1 cells (Fig. 2). Flow cytometry analysis indicated that CHS-20 cells were expressing two levels of SLAM protein (Fig. 3) and mAb A8 (2A6) could be used to sort out the cells expressing high level of SLAM protein.

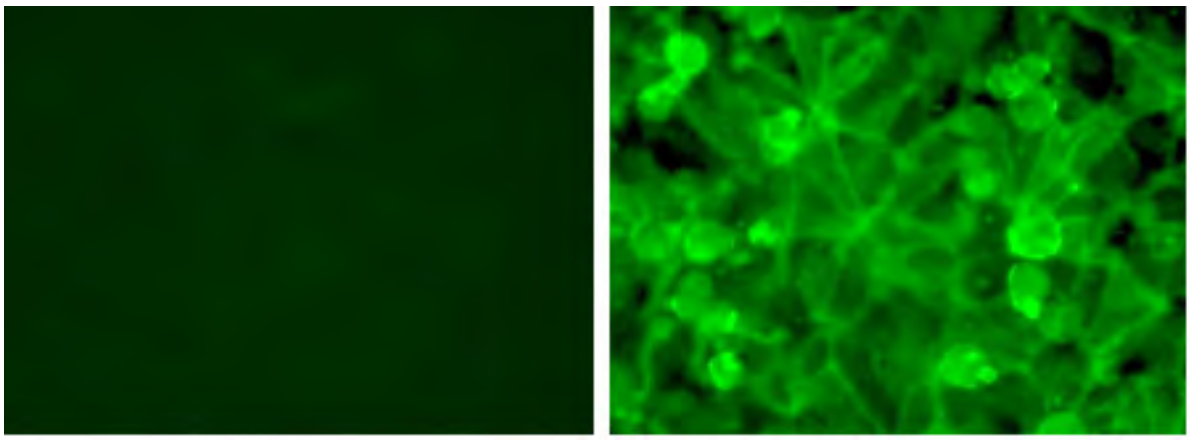

FIG. 2: Detection of the goat SLAM protein expression by IF staining with anti-SLAM mAb A8 (2A6); Flp-CV-1 cells, negative control (left); CHS-20 cells (right)

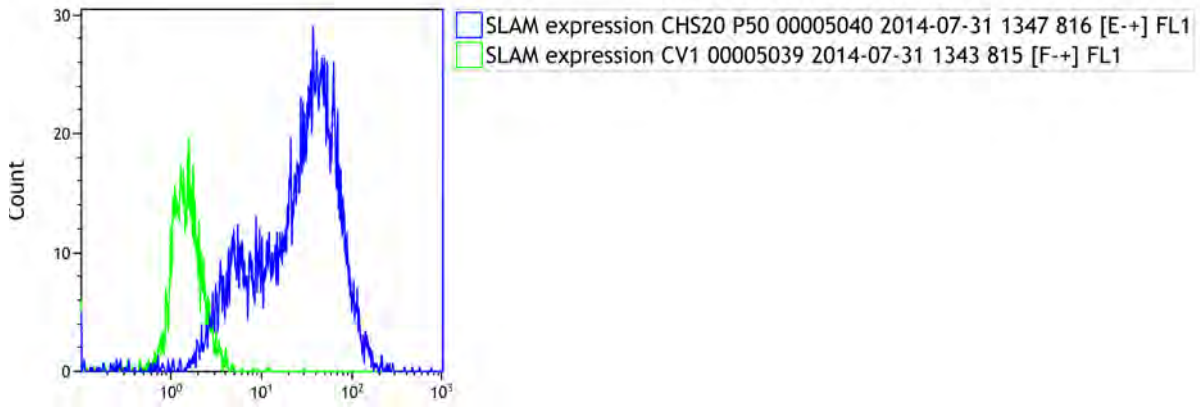

FIG. 3: Detection of the goat SLAM protein expression by fluorescent staining using anti SLAM mAb A8 (2A6)

African swine fever

African swine fever is one of the most devastating transboundary animal diseases for the swine industry, both in Africa and the Balkan region. The Balkan region was first contaminated in 2007 through the shipment of contaminated animal products. With the illegal movement of pork products, illegal disposal of contaminated waste products from areas infected with ASF, and lack of proper biosecurity measures, all areas of the world, will be at risk for incursion of the disease. Even without modern transportation, the current spread of the disease in wild boars puts a threat on moving the virus towards Europe and the West. APHL is currently performing research on African swine fever epidemiology and genomics in collaboration with several Member States (MS), funded through technical cooperation projects, IAEA coordinating research project (CRP-D32031- Early and Rapid Diagnosis and Control of transboundary Animal Diseases — Phase II: African Swine Fever or extra budgetary projects (PUI and ARF).

Due to the lack of information about currently circulating genotypes and subtypes of ASF virus (ASFV) several MS requested APHL to molecularly characterize their local strains between 2012 and 2014. Over 200 ASFV isolates from Burkina Faso, Cameroon, Cap Verde, Central African Republic, Chad, Côte d'Ivoire, Democratic Republic of Congo, Ethiopia, Nigeria

and Senegal were characterized. The results show that all western African, Cameroon and Chad isolates are from the P72 genotype I. In Central African Republic, genotype IX was identified in addition to genotype I. DRC had genotypes I, IX and XIV. Ethiopia had two unassigned genotypes (Fig. 4).

Multiple subtypes (2 to 17) were identified in most of these countries based on the central variable region (CVR) profile of the 9RL gene. The high number of ASFV variants within each of these countries illustrates the importance of using the CVR profile for ASFV isolates typing. In order to automate the determination of the CVR profile of ASFV, a tool named "ASF CVR profiler" was developed at APHL (Fig. 5). This is a web-based application which allows an automated translation of the partial or full 9RL gene nucleotide sequence into its corresponding amino acid sequence, the sparsing of the amino acid sequence into tetramers and the matching of each tetramer with the corresponding 1 letter code. This rapid and user friendly tool will facilitate researchers in member states to perform post-sequencing process quickly and efficiently to type ASFV and better understand molecular epidemiology of the disease.

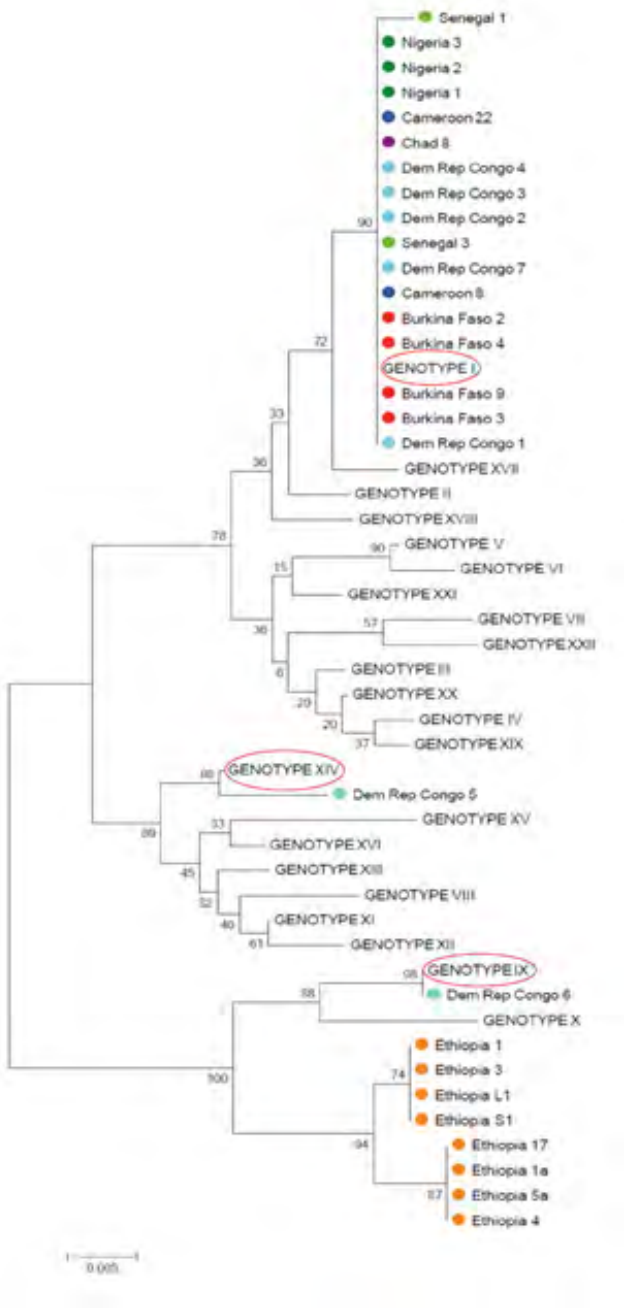

Fig. 4: Phylogenetic analysis of ASFV isolates from Africa

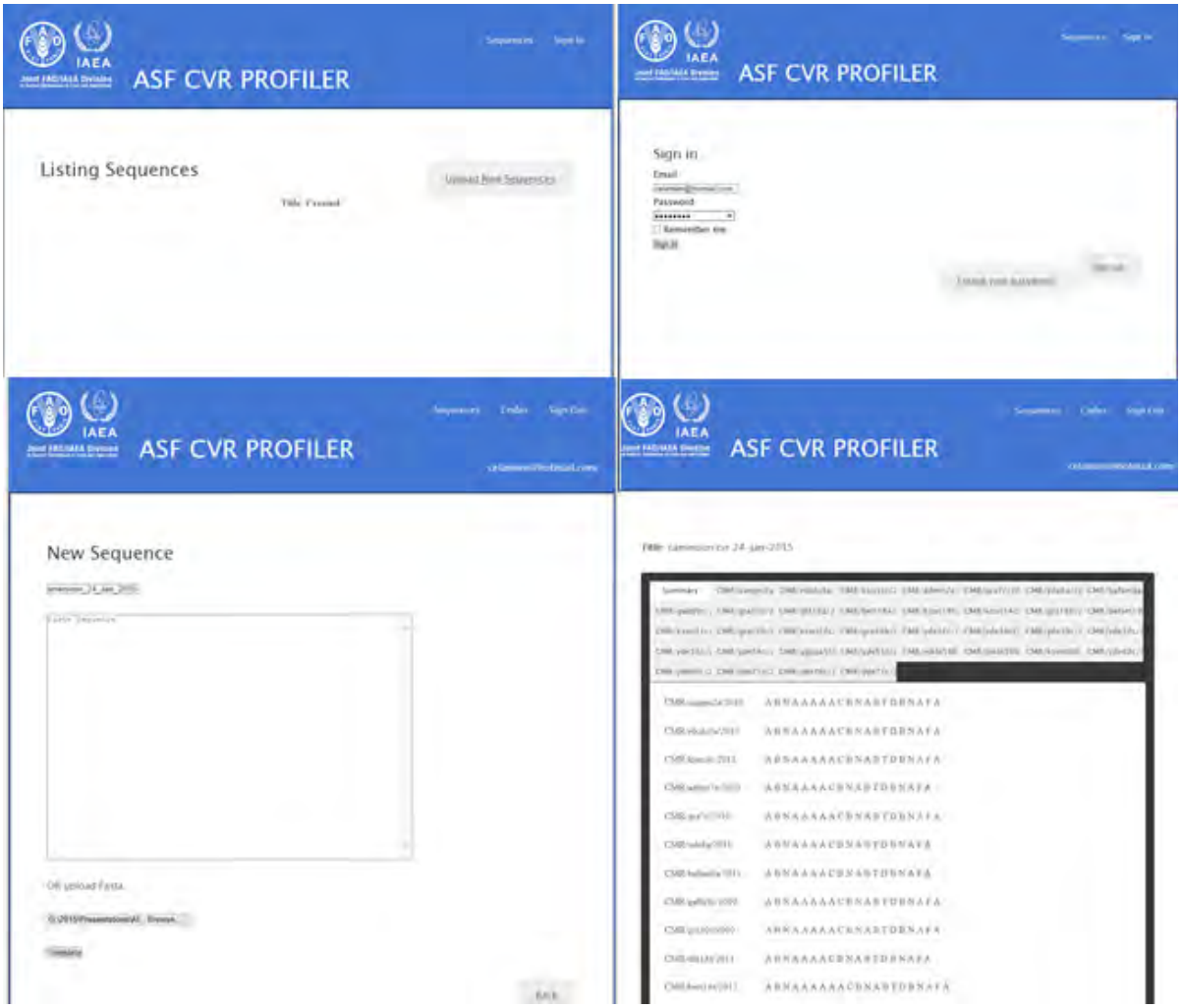

FIG. 5: Screen shots of the ASFV CVR profiler

A better assessment of the molecular epidemiology of ASF at the country and regional level is crucial to understand the dynamics of ASF spread. Having a more complete picture of the molecular epidemiology of different genotypes of ASF will allow MS to produce regional strategies to control the spread of disease within their country and between countries during movement and trade. APHL also initiated the development of an ASFV isolate bank for comparative biological and genomic studies. Currently only 11 full genomes out of 22 known genotypes are present in the public databases. This lack of information about all the genotypes leaves a gap in our understanding of the disease and how to combat it. The increased knowledge of full genome analysis coupled with strain pathogenicity analysis will help in improving ASFV characterization and facilitating the development of a vaccine to control this devastating disease. To this end the APHL has already started to sequence the full genomes of multiple isolates of ASFV and the data are currently being analysed. It is anticipated that the full genome of isolates for all remaining genotypes whose full genomes are not yet available, will be generated in this study.

Capripox Disease

Capripoxviruses are responsible for economically important diseases of ruminants. Sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV), the three members of the genus Capripoxvirus (CaPV) of the *Poxviridae* family affect sheep, goats and cattle, respectively. These three viruses are not strictly host specific and are antigenetically very similar. Routine differentiation tools to allow accurate identification of pathogen is essential to implement better control strategies for capripoxviruses. For the prevention and control of the disease in endemic countries of Asia and Africa, vaccination has been the main tool using a limited number of live attenuated vaccine strains. Live attenuated capripoxvirus vaccines are thought to provide cross protection in both homologous and heterologous hosts, however, adverse reactions following vaccination and vaccination failures have recently gained importance, creating an urgent need for tools to differentiate vaccine strains from pathogenic field isolates.

Genotyping tools for Capripoxvirus

In 2014, APHL has actively promoted the use of capripoxvirus differentiation, by transferring to MS, several tools that were developed in the laboratory between 2009 and 2013. Fourteen countries, all members of the ARF and PUI project networks, have benefited from this technology transfer through field support missions by APHL staff and two training courses organized by APHL in 2014. Furthermore these techniques were adopted by some partners of the APHL for routine use: The National Veterinary Institute and National Animal Health Diagnostic and Investigation Centre, Ethiopia, the high security laboratory of AGES, Austria and the Capripox reference laboratory of the Pirbright Institute, UK.

Additionally, software was developed to handle data generated by the quencher induced fluorescence shut down (QFS) PCR, a quantitative PCR method recently developed at APHL for capripoxvirus genotyping (Fig. 6). This is a Java based desktop application that allows the determination of the quantification cycle (Cq) and melting temperatures (Tm), and the display of both amplification and melting plots. Additional features include the calculation of standard curves and sample concentrations, the determination of mean Cq values and concentration of replicates as well as their standard deviations. This software will be made freely available to MS scientists.

With the increasing incidence of reported capripox outbreaks in previously vaccinated herds in several MS, a proper test to differentiate the vaccine strain from outbreak isolates and to demonstrate or to rule out the vaccine involvement in such outbreaks is highly crucial. In 2014, APHL embarked on the development of user-friendly methods to differentiate live attenuated capripox vaccines from the pathogenic field isolates. This was facilitated by previous work of this laboratory to sequence and compare the full genomes of capripoxvirus. This work has allowed the identification of markers to differentiate the virulent isolates from attenuated strain within each of the SPPV and the LSDV genotypes. A molecular assay was developed to differentiate sheep poxvirus field isolates from vaccine strains (Fig. 7). This assay was validated using outbreak samples collected at several geographical locations as well as sheep pox vaccines produced and used in African countries. This tool will help with diagnostics and surveillance in both enzootic and disease free areas.

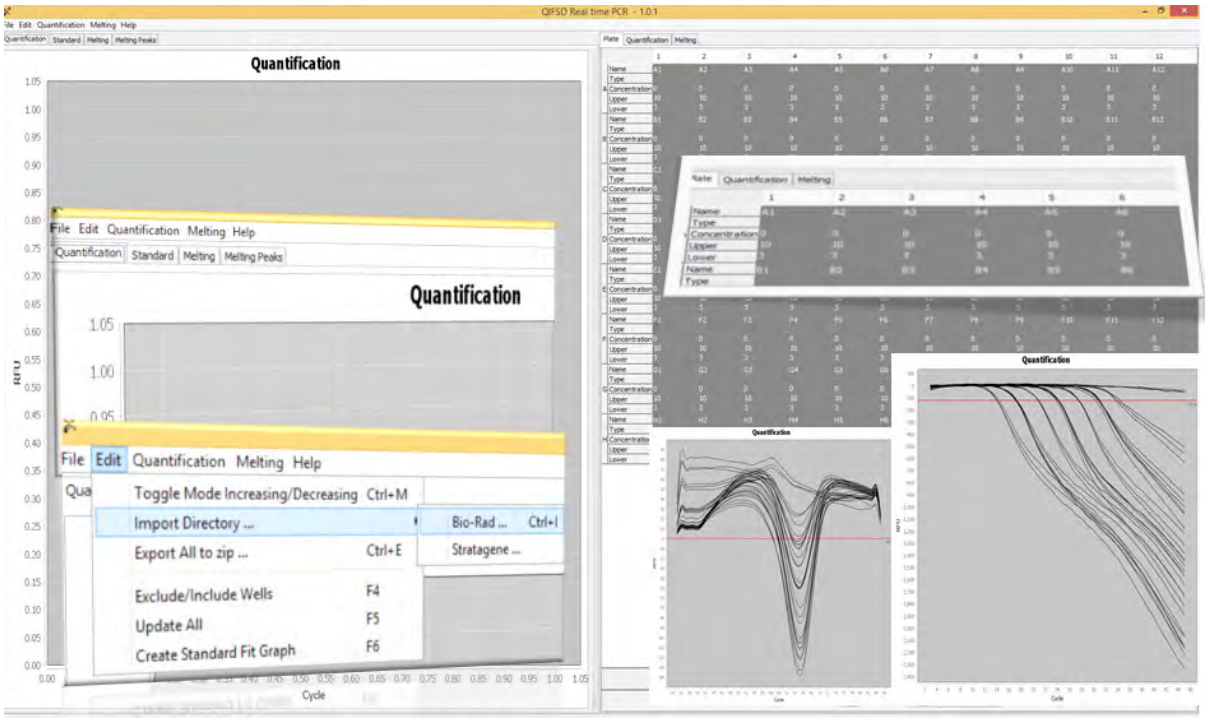

FIG. 6: QFS-PCR software overview

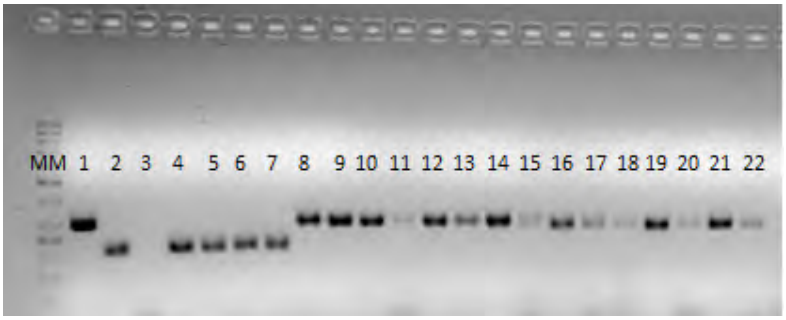

Fig. 7: Classical PCR to differentiate SPPV vaccines strains from SPPV field isolates. MM: 50bp DNA ladder; Lanes 1, 2 and 3: controls (SPPV Field, SPPV vaccine and negative control); Lane 4 – 7: SPPV vaccine strains; Lanes 8-12: capripoxvirus field strains

Molecular epidemiology of Capripox

The tools developed at APHL for the molecular epidemiology of capripoxviruses are now well appreciated and adopted by several MS laboratories. For instance, these tools were recently used to rule out the implication of the vaccine following a LSD outbreak in vaccinated herds in Ethiopia. A manuscript describing this work is currently being processed. This shows that the proper identification of vaccine strains and outbreak isolates is crucial for better vaccine matching and safer use of available vaccines. Additionally, some MS laboratories have requested that APHL characterise their local strains of capripoxviruses. In 2014, on the request of counterparts from Mongolia and Ethiopia, capripoxvirus isolates collected from sheep and goats in Mongolia, and from sheep, goat and cattle in Ethiopia, were molecularly characterised. The results showed that in Mongolia all sheep isolates were SPPV and all goat

isolates were GTPV. In contrast, for Ethiopia, both sheep and goat isolates were found to be GTPVs. All cattle isolates of Ethiopia were identified as LSDVs.

Detection of multiple pathogens in sheep and goats

Identification of causative organism is a significant challenge in the diagnosis of infectious diseases, particularly when clinical symptoms overlap for different pathogens. This becomes even more complex in resource-poor settings where sophisticated testing procedures and highly trained personnel for diagnosis of different diseases are not available. Hence, in 2012, APHL initiated a program for development of multi-parametric pathogen detection assays in specific sample types based on the observed clinical symptoms. A multi-parametric assay is a powerful approach to detect several pathogens in one diagnostic test from various sample types collected based on disease symptoms. This approach optimizes the chance to detect the responsible pathogens for the specific symptoms and enhance animal diseases surveillance and management capacity, especially at the domestic animal/wildlife interface, in Member States.

Pan poxvirus detection method

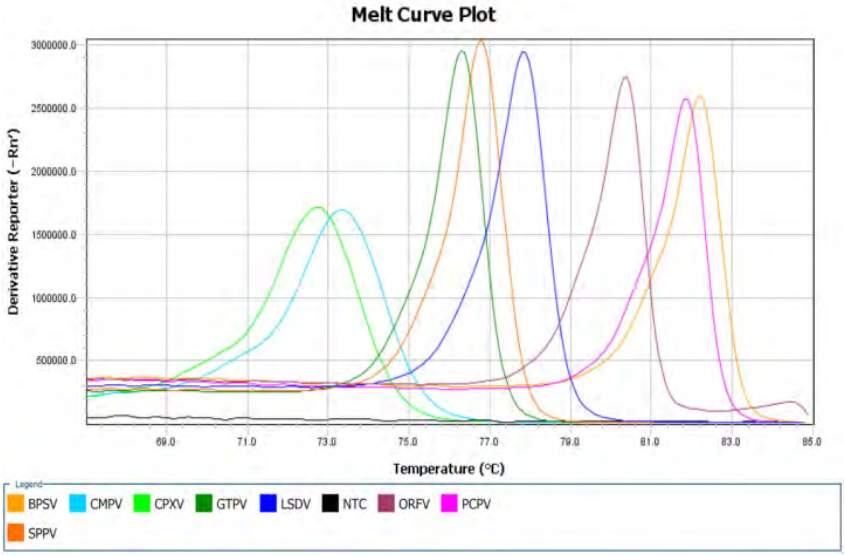

Fig. 8: Multiparameric detection of 8 poxviruses using the QuantStudio 6 Flex Real-Time PCR System (Life Technologies)

A pan-pox detection method designed and evaluated earlier at APHL, was further validated in 2014 using samples collected at various geographical locations. This assay can detect, in a single reaction tube, up to 8 poxviruses affecting ruminants and camels: sheeppox virus (SPPV), goatpox virus (GTPV), lumpy skin disease virus (LSDV), orf virus (ORFV), bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), and camelpox virus (CMPV) and cowpox virus (CPXV) (Fig. 8). The current validation study using samples collected from various outbreaks in sheep, goats, cattle and camels shows the interesting potential of the assay for confirmatory diagnosis. Indeed, sample collected in Ethiopia in sheep suspected of having capripox disease were found to be infected by ORFV, a parapoxvirus. Additionally, the cross-platform compatibility of the assay was assessed in 2 additional qPCR platforms other than the CFX96 Touch™ Real Time PCR Detection System (Bio-Rad): the LightCycler

480 Instrument II (Roche Life Science) and the QuantStudio 6 Flex Real-Time PCR System (Life Technologies). All three platforms were able to perform the assay and deliver similar genotyping results. It is expected that the assay transfer to IAEA and FAO member states will start in 2015.

Taqman multiplex assay for detection of viral and bacterial pathogens

Two multi-parametric assays developed at APHL for the detection of respiratory pathogens were validated for the analytical performance. The two assays were capable of detecting *PPRV, capripoxviruses, parapoxvirus, Mycoplasma capricolum subsp. capripneumoniae (Mccp) and Pasteurella* (Fig. 9). The assays were evaluated for the sensitivity, specificity and repeatability. The field samples obtained from animals showing respiratory symptoms and those mainly suspected for PPR, Capripox and Parapox were screened. The assays efficiently detected the suspected pathogens. All the results were further confirmed either by sequencing or using an existing PCR based assay. The strength of the assay was also illustrated by detection of multiple infections in few of the field samples screened at APHL and at Member state laboratories. Fellows and training participants from different member states were trained at APHL to implement the assay in their respective laboratories.

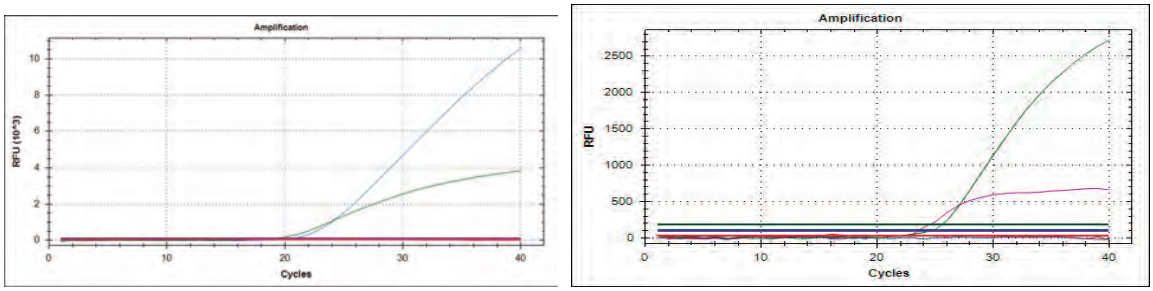

Fig. 9: Taqman multiplex assay detecting left: Pasteurella (Blue), PPRV in a sample (Green) and Capripoxvirus (Purple); and right: PPRV (Green) in another field sample

Liquid array based assay to detect respiratory pathogens

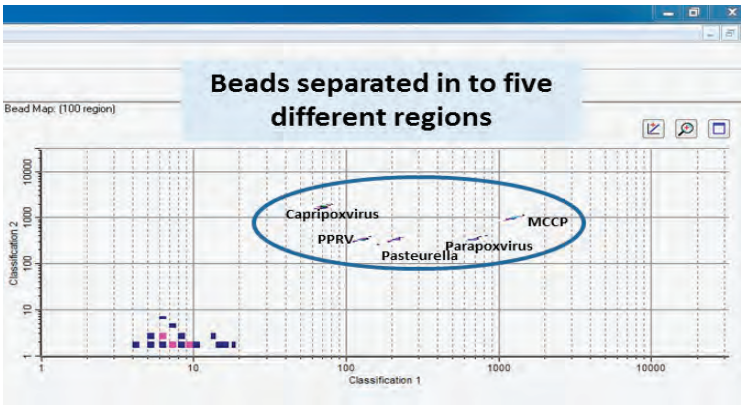

Fig. 10: Detection of five different respiratory pathogens by Luminex based assay in sheep and goats

The nucleic acid based tests are evolving with technology advancements. Technologies such as bead based arrays, microarrays, microfluidic chips, etc. allow rapid detection of multiple pathogens in a single assay with increased throughput. Such technologies can help to

overcome the limitation of number of pathogens targeted in a single assay. A liquid array-based assay targeting more pathogens causing respiratory diseases in ruminants is currently under development. The pilot Luminex assay developed using a combination of fluorescence and bead size coupled to different pathogen specific nucleic acid probes allows detection of five different respiratory pathogens in small ruminants (Fig. 10).

Animal Genetics

Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity

SNP (Single Nucleotide Polymorphism) marker discovery in goats

Parasitic infections in goats cause severe economic loss to the tune of more than $ 10 billion every year across the world. Management of gastro-intestinal parasites has become complicated with increased prevalence of drug resistant parasites. In 2010, IAEA initiated a coordinated research project on DNA marker based genetic improvement of host resistance against parasites as a long term strategy for their effective control. Animal Production and Health Laboratory is involved in the process of discovering novel candidate gene markers and development of genotyping tools for testing of goats under field trial in different Member States. As part of these efforts, targeted re-sequencing of 77 candidate genes (Fig. 11- NCBI GenBank candidate gene symbols) involved in various innate and adaptive immune pathways was conducted to identify new single nucleotide polymorphic (SNP) markers. A total of 187 SNP markers were discovered across the goat genome, of which 170 markers were selected for assay development based on criteria like minor allele frequency and suitability for designing allele specific primers. These markers are being currently validated for testing the samples from indigenous goat breeds under field trial in different Member States.

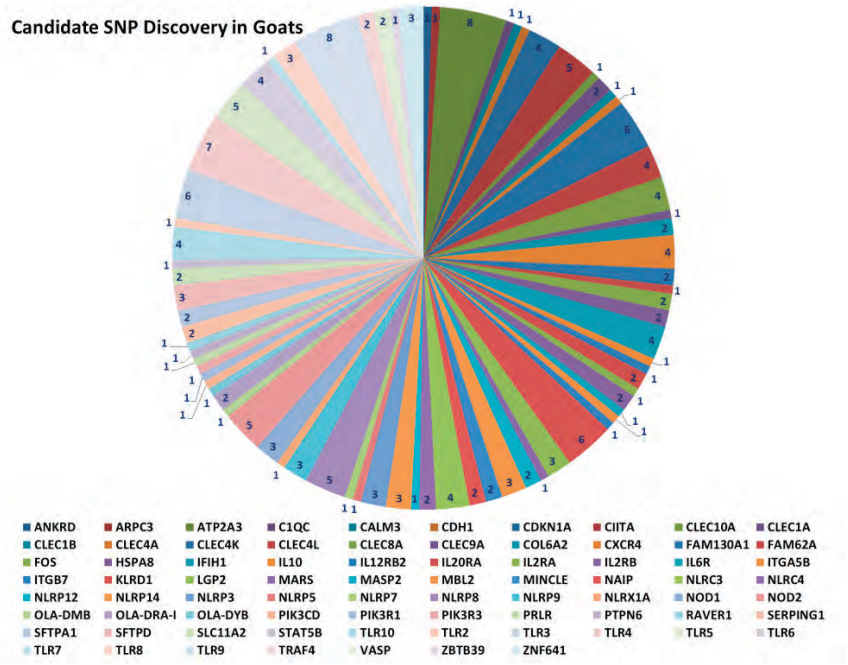

Fig. 11: Distribution of newly discovered goat SNPs across various candidate genes

Development of a real time PCR based assay to differentiate sympatric Haemonchus species infecting ruminants

Haemonchus parasites are trichostrongyloid nematodes and one of the major parasites affecting ruminants (sheep, goat, cattle and camel) around the world. The adult worms suck blood from the abomasum of ruminant hosts and causes anaemia, oedema, diarrhoea and even death. Apart from the loss due to mortality and slow growth, the growing level of anthelmintic resistance in these parasites is a serious challenge. The level of anthelmintic resistance in different species/populations of *Haemonchus* varies greatly. Three different sympatric species of *Haemonchus* are infecting ruminants in Asia viz. *H. contortus, H. placei and H. longistipes. H. contortus* is predominantly a parasite of sheep and goat, *H. placei* infect cattle while *H. longistipes* is more commonly found in camels. The correct identification of different species/variants, as well as knowledge regarding the epidemiology and genetic characterization of the principal circulating species/variants, is essential for the establishment of sustainable control strategies. APHL initiated the development of a real time PCR (polymerase chain reaction) based assay to differentiate the three major Haemonchus species infecting ruminants in Asia. Species specific single nucleotide polymorphic (SNP) variations were utilized to develop the assay. Primers for PCR amplification of ITS2 gene and a snap back probe to detect species specific SNPs were designed. Asymmetric PCR followed by melting curve analysis samples showed three distinct melting temperatures specific to each of the three Haemonchus species (Fig. 12). The assessment of sensitivity and specificity of this assay is currently under progress.

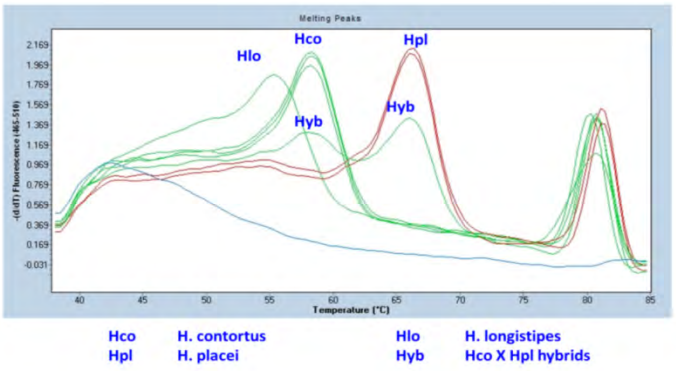

FIG. 12: Melting curve analysis of PCR amplicons showing specific melting peaks for each of the three sympatric Haemonchus species

Genetic Characterization of Indigenous Livestock Breeds

The Joint FAO/IAEA Division is supporting member states especially in Asia and Africa in implementing Global Plan of Action on animal genetic resources (AnGR) to protect, conserve and improve livestock biodiversity through capacity building and training. APHL supported Madagascar, Angola and Myanmar on molecular genetic characterization of indigenous breeds using nuclear and extra-nuclear DNA markers.

DNA marker based breed assignment in Madagascar cattle

Cattle in Madagascar are reared primarily for meat, milk and draught purposes. Madagascar government introduced the policy of crossbreeding local Malagasy zebu cattle to improve productivity and develop dairy type and meat type composites. However, absence of pedigree

and performance records under field conditions resulted in varying exotic blood levels and poor adaptability to local management conditions. Optimal level of exotic blood in Malagasy crossbreds and composites has not been optimized and hence farmers still prefer to rear Malagasy zebu cattle. DNA marker based genetic analysis has potential applications for breed assignment and stabilize exotic inheritance in Madagascar cattle under field conditions. This requires generation of baseline genotype data on Malagasy zebu cattle and new composite breeds. Except for certain physical features and phenotypic characteristics, no information on genetic diversity and structure of local zebu cattle and composites is available. A total of 172 samples collected from three major cattle breeds of Madagascar (Malagasy Zebu, Renitelo and Manjani Boina) were analysed by genotyping 27 microsatellite marker loci and sequencing control region (D-loop) of mitochondrial genome.

Microsatellite based analysis revealed high level of within breed genetic diversity in Madagascar cattle breeds. High genetic differentiation was observed among Malagasy zebu and composite crossbred cattle (9.1%). Phylogeny and principal component analysis based on inter-individual allele sharing distance revealed strong population structure existing within three Madagascar cattle breeds (Fig. 13). Microsatellite genotypes revealed strong potential for breed assignment in Madagascar cattle. With prior population information on allele frequency distribution in three breeds, 100% correct assignment was achieved in Malagasy Zebu and Renitelo breeds, while 93.75% correct assignment was observed in Manjani Boina.

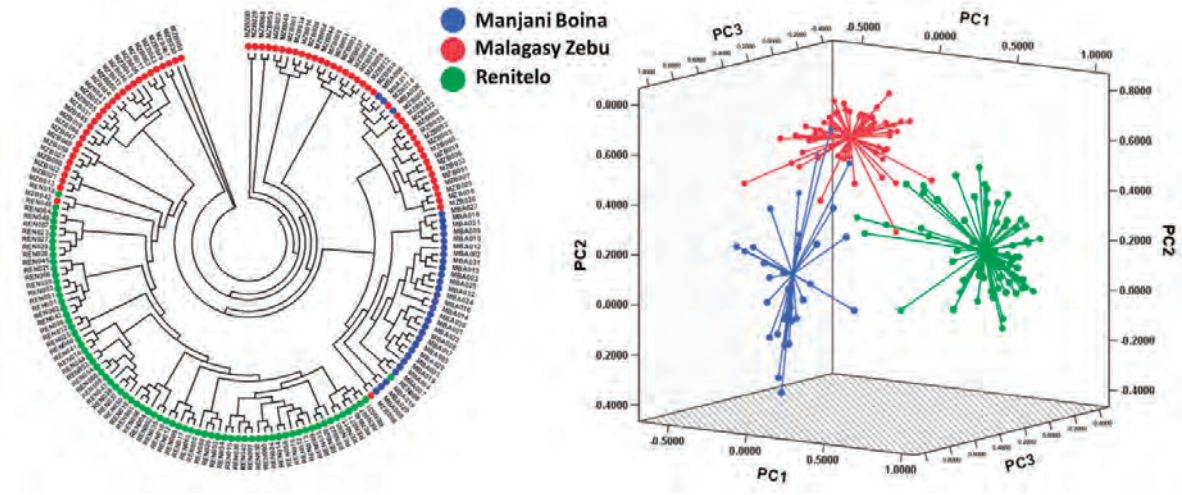

Fig. 13: Phylogeny and genetic structure of Madagascar cattle breeds

Genetic diversity, population structure and phylogeography of Myanmar goats

Myanmar has primarily an agriculture driven economy with a contribution of 42% to the national gross domestic product, of which livestock plays an important role providing employment and livelihood to the rural masses. Goats in Myanmar are mostly reared for meat purpose and are able to adapt well to the prevailing harsh dry conditions. Three major native goat breeds, Jade Ni, Nyaung Oo and Waithar Li are available in Myanmar, however, baseline information on the phenotypic and genetic characteristics on these goat breeds are not available. Such information is required to formulate and implement effective strategies for their genetic improvement and conservation. A total of 147 goats from all three indigenous breeds were genotyped at 27 microsatellite loci. Genetic diversity in terms of allelic

polymorphisms, observed and expected heterozygosities were found to be moderately high. Considerable heterozygosity deficit ranging from 5.5% to 8.2% was observed in Myanmar goat breeds. Most of the genetic variations were found within breeds and only 1.9% of the total observed variation was explained by between breed differences. Genetic structure analyses showed complete admixture of Nyaung Oo and Waithar Li goats indicating high rate of gene flow among these populations (Fig. 2). Population stratification was observed in Jade Ni with a subset of individuals clustering distinctly. Variations in mitochondrial DNA control region revealed 22 distinct haplotypes belonging to two major haplogroups, A and B. Haplogroup A was found to predominate Myanmar goats similar to other goat populations in Asia. Comparative analysis of mtDNA variations indicated possible Chinese origin of the maternal haplotypic lineages of Myanmar goats.

Genetic relationship of domestic sheep breeds with primitive Asian wild Urial sheep

The geographical region extending from Eurasia to North West of Indian sub-continent is home to the wild progenitors of present day domestic sheep. Asian Urial sheep was initially proposed as the first sheep domesticate while later reports suggested them to be more primitive than Asiatic mouflons in the evolutionary scale. In continuation of Joint FAO/IAEA Division's efforts on characterization of small ruminant genetic resources of Asia and as part of the research project implemented by University of Veterinary and Animal Sciences, Pakistan (funded by world wide fund for nature), baseline information on genetic diversity of Urial sheep was generated and compared with domestic sheep breeds around the world.

High levels of genetic diversity in terms of allelic diversity, observed and expected heterozygosity and inter-individual allele sharing distance was observed within the wild Urial sheep (*Ovis vignei punjabiensis*). Comparison of Urial sheep with domestic sheep breeds located near the domestication centers revealed relatively closer relationship of Urial with West Asian and East European sheep than South Asian sheep. No evidence was observed to support the hypothesis of Urial sheep as potential wild ancestor for Asian domestic sheep lineage. To address the question on the taxonomic status of Urial sheep, mitochondrial DNA diversity was assessed to evaluate its evolutionary relationship with closely related taxa. Analysis of mitochondrial DNA clearly established the divide between the lineages of *O. vignei* and *O. orientalis* populations. Also, the genetic differences between *O. vignei bochariensis* and *O. vignei punjabiensis* was significantly higher ($P<0.01$), despite both the populations being classified as sub-species of Urial type sheep. The tests for selective neutrality indicated purifying selection in Punjab Urial sheep while Asiatic Mouflon appears to have experienced a strong population bottleneck in the recent past. Considering the declining population trend and loss in diversity due to possible genetic bottleneck events, there is an urgent need to implement strong conservation measures for *O. vignei punjabiensis* in the region.

Genetics Laboratory Information and Data Management System (GLIDMaS)

Development and transfer of bioinformatics tools to animal genetics laboratories worldwide continues to be an important strategy to support FAO/IAEA Member States in managing their livestock biodiversity and improving productivity of local animal breeds. Animal Production and Health subprogramme initiated the development of a "Genetics Laboratory Information and Data Management System (GLIDMaS)" that will allow users to manage genetic repository, genetic and genomic resources), DNA marker tools, DNA sequence data,

genotype data and laboratory inventory (equipment, reagents and consumables) available in a standard molecular genetics laboratory (Fig. 14). GLIDMaS platform will be a standalone application and will not need special software on user computers. One of the major objectives of developing GLIDMaS is to provide tools for quality assurance and formal accreditation of MS genetic laboratories. The platform is also expected to support animal geneticists in managing large volumes of molecular data intended for animal selection, conservation and genetic improvement. Different modules for manual entry, editing and import of data have been created; testing and validation of the system is currently under progress.

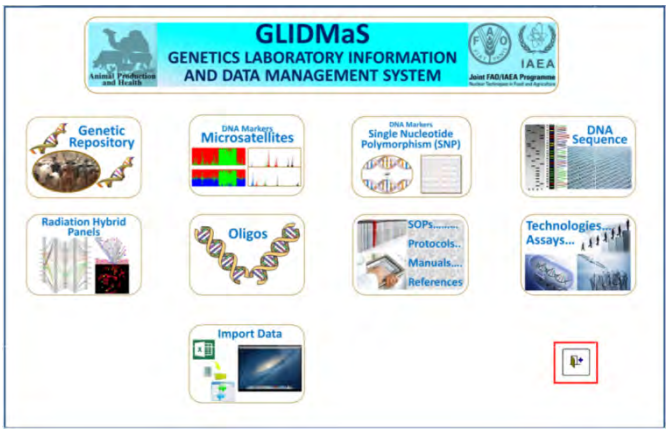

FIG. 14: Genetics Laboratory Information and Data Management System

CAPACITY BUILDING AND SERVICES

Facilitating Access to Sequencing Services for MS Veterinary Laboratories

Nucleic acid sequencing is becoming an integral part of veterinary diagnostics due to its added value for the accurate identification of pathogens. Nowadays, many laboratories rely on service providers to perform their sequencing work. In developing countries where no service provider is available, laboratories must ship their PCR products using a rapid courier to ensure the integrity of the samples for sequencing. A pilot project is being conducted by FAO and IAEA through the Joint FAO/IAEA programme in 10 African countries to facilitate their access to sequencing services (for more information see: http://www.fao.org/3/a-i3752e/i3752e07.pdf). As part of this project, a study was performed at APHL to evaluate the stability of PCR products at room temperature. This work was initiated to simulate the situation where the products are sent by normal courier. The results show that purified PCR products remain stable for up to 30 days when kept at room temperature and can give good quality sequences at this time point. This indicates that most countries could send their purified PCR products for sequencing using a normal courier. This will reduce the cost of transport and allow sustainable use of sequencing services by MS veterinary laboratories after completion of the pilot project.

Technical Field Support Missions to Build Capacity in MS Veterinary Diagnostic and Animal Genetic Laboratories

APHL staffs were actively involved in the transfer of technologies to IAEA MSs, particularly those related to molecular diagnosis and epidemiology for the control of transboundary animal diseases and DNA marker based animal breed characterization to protect livestock biodiversity

and improve animal productivity. Four technical field support missions were undertaken by staff of APHL to install and calibrate critical equipment, demonstrate work flow on disease diagnostic procedures, surveillance and epidemiology and animal genotyping for molecular characterization of breeds in MS veterinary and animal genetic laboratories.

- **Laboratoire National Vétérinaire (LANAVET),** Cameroon. In the framework of the PUI, ARF and Identify, access to DNA sequencing services has been provided to ten Sub-Saharan African countries. This access allows the laboratory to perform sequencing based molecular diagnostic assays. To support these assays and establish a sequencing work flow in the laboratory, a field support mission was carried out at LANAVET, Garoua, Cameroon, from 7 to 12 April 2014. During this mission, the entire sequencing work flow from sample preparation, submission to the sequencing service provider to the data analysis was established. A total of sixteen participants from LANAVET, Cameroon and IRED, N'Djamena, Chad took part in the on-site training in these procedures.

- **Laboratoire National d'Elevage (LNE), Burkina Faso**. A staff member of APHL travelled to Ouagadougou, Burkina Faso from 7 to 11 April 2014, to support the implementation of gene based identification of pathogens. A real time PCR instrument was transferred to Laboratoire National d'Elevage (LNE), Ouagadougou, Burkina Faso to improve its diagnostic platform after initial calibration at APHL. During the mission, the laboratory was assisted in setting up the instrument and workflow, and the laboratory staff was trained on protocol selection procedures and detection of animal diseases by real time PCR. Eleven scientists and technicians from three local institutions in Burkina Faso (Laboratoire National d'Elevage du Burkina, lnstitut de l'Environnement et de Recherches Agricoles and the University of Koudougou) benefited from the training.

- **Central Veterinary Laboratory, Kinshasa, Democratic Republic of Congo**. A staff member from APHL visited this laboratory from the 13 to 17 October 2014. The primary aim of the visit was to transfer and install a real-time PCR instrument in the laboratory under ARF project and to assist in the activation of the sequencing service provided to partners under the Identify project. The real-time PCR instrument was successfully installed and tested. CVL staff members were assisted in the setting up of two real-time protocols for peste des petit ruminants (PPR) and African swine fever (ASF) using the new instrument. In addition, PCR amplicons were generated from positive samples of PPRV, ASFV and rabies virus and prepared for shipment to a sequence provider in Europe.

- **Animal Genetics and Reproduction Laboratory, Centre National de la Recherche Appliquee au Developpement Rural (FOFIFA), Antananarivo, Madagascar** APHL supported to establish a molecular biology laboratory for animal breed characterization and genetic selection at Antananarivo, Madagascar. Various equipment required to perform animal genotyping experiments including DNA extraction, nucleic acid measurement, PCR, gel electrophoresis and documentation provided by IAEA through a Technical Cooperation Project (MAG5020). A field expert mission was made to install the equipment and demonstrate the practical work flow on DNA marker based genetic analysis for genotyping and sequencing. An electronic animal identification system was introduced to FOFIFA staff and field demonstration was made to implement performance recording for milk and meat production in cattle. Five scientific staff and one technician participated in the demonstration cum hands on training.

Proficiency Testing

Proficiency Testing (PT), sometimes referred to as External Quality Assurance or EQA in animal disease diagnosis is an exercise to evaluate the performance of participating laboratories in the routine application of a disease diagnostic test, by testing specimens of undisclosed content. The primary purpose of proficiency testing is to help laboratories detect and take appropriate measures to correct any unacceptably inaccuracy in their produced/ reported results. In 2014, the Animal Production and Health Laboratory (APHL) has continued the organization of its peste des petits ruminants (PPR) PT exercise. This time, on top of the evaluation of PPR diagnosis based on the nucleic acid detection (NAD) test, APHL also introduced the evaluation of PPR diagnosis by serological methods (Sero (ELISAs)). 26 counterpart laboratories were contacted for participation, of which results were received from 14 laboratories for serological evaluation exercise while 12 counterpart laboratories sent back their results on nucleic acid amplification-based evaluation. Below is the list of participating laboratories (Table 2).

Table 2:. List of participating laboratories responding on NAD and/or Sero PPR PT2014

Country	Institute
Bangladesh	SAARC Regional Leading Diagnostic Laboratory-PPR
Cameroon	Laboratoire national vétérinaire (LANAVET)
Chad	Institut de recherche en Elevage pour le Développement (IRED)
Côte d'Ivoire	Laboratoire Central Vétérinaire de Bingerville
DRC	Central Veterinary Laboratory
Ethiopia (NAHDIC)	National Animal Health Diagnostic and Investigation Center (NAHDIC),
Ethiopia (NVI)	National Veterinary Institute (NVI)
Ghana	Accra Veterinary Laboratory
Kenya	Central Veterinary Laboratories Kabete
Mali	Laboratoire central vétérinaire de Bamako
Pakistan	National Institute for Biotechnology & Genetic Engineering (NIBGE)
Senegal	Laboratoire National de l'Elévage et de Recherches Vétérinaires
Sudan	Central Veterinary Research Laboratory Center
Tanzania	Center for Infectious Diseases and Biotechnology
Turkey	Pendik Veterinary Control and Research Institute
Uganda	National Animal Disease Diagnostics and Epidemiology Center,

lab ID	Samples sets	Tested				TRUE		Total of Samples	Sensitivity	Specificity	x/y - Scores - %
		Positive	Negative	False Positive	False Negative	Positives	Negatives				
						8	4	12			
						8	4	12			
Lab 3	Samples set 3					8	4	12			
Lab 5	Samples set 5					8	4	12			
						8	4	12			
Lab 7	**Samples set 7**	6	4	0	2	**8**	**4**	**12**	**75.0%**	**100.0%**	**83.3%**
Lab 8	Samples set 8					8	4	12			
Lab 9	**Samples set 9**	8	4	0	0	**8**	**4**	**12**	**100.0%**	**100.0%**	**100.0%**
Lab 10	**Samples set 10**	8	4	0	0	**8**	**4**	**12**	**100.0%**	**100.0%**	**100.0%**
Lab 11	Samples set 11					8	4	12			
Lab 12	Samples set 12					8	4	12			
Lab 13	Samples set 13					8	4	12			
Lab 14	**Samples set 14**	7	4	0	1	**8**	**4**	**12**	**87.5%**	**100.0%**	**91.7%**
						8	4	12			
Lab 16	Samples set 16					8	4	12			
Lab 17	**Samples set 17**	3	4	0	5	**8**	**4**	**12**	**37.5%**	**100.0%**	**58.3%**
Lab 18	**Samples set 18**	8	3	1	0	**8**	**4**	**12**	**100.0%**	**75.0%**	**91.7%**
						8	4	12			
Lab 20	Samples set 20					8	4	12			
Lab 21	**Samples set 21**	8	4	0	0	**8**	**4**	**12**	**100.0%**	**100.0%**	**100.0%**
Lab 22	Samples set 22					8	4	12			
Lab 23	**Samples set 23**	7	4	0	1	**8**	**4**	**12**	**87.5%**	**100.0%**	**91.7%**
Lab 24	**Samples set 24**	4	3	1	4	**8**	**4**	**12**	**50.0%**	**75.0%**	**58.3%**
Lab 25	**Samples set 25**	8	4	0	0	**8**	**4**	**12**	**100.0%**	**100.0%**	**100.0%**
lab 26	**Samples set 26**	8	4	0	0	**8**	**4**	**12**	**100.0%**	**100.0%**	**100.0%**
Lab 4	**Samples set 27**	7	0	4	1	8	4	12	**87.5%**	**0.0%**	**58.3%**

Legend (color codes) Positive Negative False Positive False Negative

FIG. 15: Summary results of the 2014 PPR NAD proficiency testing

A number of well characterised samples (8 positives and 4 negatives for nucleic acid detection systems and 10 positives and 6 negatives for serological detection systems) were sent as blind, labelled with random number to each participating laboratory and the counterparts were asked to determine the diagnostic status of these samples following the method of their choice. The returned data were collected, analysed and the results including a short report were sent back to each participating laboratory. Each laboratory (participant/counterpart) has received the full coded results of this run (PPR PT2014) where he/she could recognise only his/her own results.

The short accompanying report was to comment the findings and advise where necessary. From the 12 responsive participating laboratories to the NAD evaluation, "unfortunately" only five counterparts have had 100% correct results (Fig. 15); on the other hand, it was a pleasure to see that all 14 responsive participating laboratories that used serological evaluation have demonstrated 100% correctness of their results (Fig. 16). The later shows how well the ELISA technique was transferred/established in these laboratories. The analysis of the NAD evaluation results suggested that the majority of participating laboratories which failed this PT need more training on the implementation of the RT-PCR techniques (s) and/or more stringent implementation and monitoring of quality control and GLP on the laboratory processes. The next run PPR proficiency testing will be organized in 2015.

lab ID	Samples sets	Tested				TRUE		Total of Samples	Sensitivity	Specificity	x/y - Scores - %
		Positive	Negative	False Positive	False Negative	Positives	Negatives				
Lab 1											
Lab 2	Samples set 2										
Lab 3	Samples set 3										
Lab 4	Samples set 4										
Lab 5	**Samples set 5**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 6											
Lab 7	**Samples set 7**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 8	Samples set 8										
Lab 9											
Lab 10	**Samples set 10**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 11	Samples set 11										
Lab 12	**Samples set 12**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 13	**Samples set 13**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 14	**Samples set 14**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 15											
Lab 16											
Lab 17	**Samples set 17**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 18	**Samples set 18**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 19											
Lab 20											
Lab 21	**Samples set 21**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 22											
Lab 23	**Samples set 23**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 24	**Samples set 24**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 25	**Samples set 25**	10	6	0	0	10	6	16	100.0%	100.0%	100%
Lab 26											
Lab 4	Samples set 27	10	6	0	0	10	6	16	100.0%	100.0%	100%

Legend (color codes) Positive Negative False Positive False Negative

FIG. 16. Summary results of the 2014 PPR Sero proficiency testing

Meetings

A technical meeting with directors of veterinary laboratories participating in the ARF-PUI project to strengthen animal disease diagnostic capacities in selected sub-Saharan countries was held at the IAEA's Headquarters in Vienna, Austria, from 4 - 6 February 2014. Nine partner laboratories from eight countries, Botswana, Burkina Faso, Cameroon, Chad, Democratic Republic of Congo, Ethiopia (2 laboratories), Mali, Mozambique and Zambia were represented. The meeting consisted of presentations by the project coordinators explaining the background and aims of the ARF-PUI-project followed by presentation from each laboratory. It was evident from the presentations that the main TADs of concern to the majority of the countries and, therefore of relevance to this project, were of Foot-and-Mouth disease, contagious bovine pleuropneumonia, African Swine Fever, peste des petits ruminants, Trypanosomosis and Rabies. Together with guidance of project coordinators, each partner developed a work plan for 2014/2015 and presented a list of specific requirements (e.g. reagents, equipment and training) necessary to achieve their goals. MS Directors of veterinary laboratories requested field support missions from IAEA to strengthen their capacity in implementing diagnostic procedures for the control of transboundary animal diseases. Participants also stressed the need to take actions for introduction, implementation and maintenance of quality assurance (QAQC) in their different laboratories: organization of proficiency tests of the diseases listed, instrument calibration, expert visit for advice, training courses and workshops.

Training courses

- **A training course** on the Diagnosis of Transboundary Animal Disease: Pathogen Typing Using Molecular Techniques was held from 25 August - 5 September 2014 at the National Veterinary Institute, Debre Zeit, Ethiopia. The specific purpose of this training

was to provide practical knowledge on different techniques used for virus typing and subtyping and general knowledge on the epidemiology and control strategies for peste des petits ruminants (PPR), capripox disease (CaP), Newcastle disease (ND) and highly pathogenic avian influenza (HPAI). The theoretical and practical trainings were delivered by lecturers from the Friedrich-Loeffler-Institut (FLI), Germany, AU-PANVAC, Ethiopia, NVI, Ethiopia and a staff member of the Joint FAO/IAEA Division. Twenty veterinary diagnostic laboratory scientists from 14 Sub-Saharan African countries (Botswana, Burkina Faso, Cameroon, Chad, Côte d'Ivoire, Democratic Republic of Congo, Ethiopia, Kenya, Mali, Mozambique, Namibia, Senegal, United Republic of Tanzania and Zambia), participated in the training.

- **A training course** on the Diagnosis of Transboundary Animal Diseases: Practical Approaches for Introducing New Assays for Routine Use in Veterinary Diagnostics Laboratories was held from 15- 26 September 2014, at IAEA Laboratories, Seibersdorf, Austria. The purpose was to reinforce the participant's knowledge on the set up and routine use of molecular assays for the early detection of transboundary animal diseases (TADs). More specifically, practical steps in introducing new assays for routine use in a laboratory were demonstrated. The theoretical and practical training was delivered by experts from OIE (Paris, France), FAO (Rome, Italy), CODA/CERVA (Brussels, Belgium), and the joint FAO/IAEA Division. Fifteen veterinary diagnostic laboratory scientists from 14 Sub-Saharan African countries (Botswana, Burkina Faso, Cameroon, Chad, Côte d'Ivoire, Democratic Republic of Congo, Ethiopia, Kenya, Mali, Mozambique, Namibia, Senegal, United Republic of Tanzania and Zambia), participated in the training.

- **A regional training Course** on "Genetic characterization of indigenous livestock breeds using DNA markers" was conducted from 11-22 August, 2014 as part of the regional technical cooperation project (RAS5063) on "Improving the reproductive and productive performance of local small ruminants by implementing reliable artificial insemination programs". 14 participants from five countries Iraq, Yemen, Oman, Syria and Jordan participated in the training course. The aim of the course was to enhance knowledge of participants on breed survey and monitoring, animal identification, DNA marker techniques and genomic tools for characterization and improvement of indigenous livestock breeds.

Fellowship and Internship Training

In 2014, the APHL hosted five fellows and three interns in the following areas:

Name	Country	Status	Duration	Topic
Moumouni Sanou	Burkina Faso	Fellow	15th November 2013 to 13th February 2014	Single nucleotide polymorphic (SNP) marker genotyping of candidate genes related to parasite resistance in Djallonke sheep
Norbertin Ralambomanana	Madagascar	Fellow	3rd February 2014 to 30th April 2014	Genetic characterization of Madagascar Zebu cattle using nuclear and extra nuclear DNA markers
Kiala Sebastino	Angola	Fellow	3rd February 2014 to 1st May 2014	Genetic diversity analysis of indigenous livestock breeds using DNA markers
Pann Pwint Phyu	Myanmar	Fellow	25th June 2014 to 21st September 2014	Genetic characterization, population structure and phylogeography of indigenous goat breeds from Myanmar
Jean de Dieu Baziki	AU-PANVAC Ethiopia	Fellow	29th September 2014 to 28th October 2014	Use of molecular assay in vaccine quality control
Timothy Yusufu Woma	Nigeria	Intern	25th November 2013 to 24th June 2014	Molecular diagnosis and epidemiology of peste des petits ruminants viruses from Nigeria
Kadidia Tounkara	Mali	Intern	1st July 2014 to 31st March 2015	Development of molecular diagnostic assay to differentiate PPRV lineages
James Jenkins	South Africa	Intern	14th July 2014 to 23rd September 2014	Development of bioinformatics platforms for molecular diagnostics and molecular epidemiology

PUBLICATIONS

HEIDARIEH, M., HEDAYATI, R.A.D. M., MIRVAGHEFI, A.R., DIALLO, A., MOUSAVI, S., SHEIKHZADEH, N., SHAHBAZFAR, A.A. (2014). Effect of gamma irradiation on inactivation of *Ichthyophthirius multifiliis* trophonts and its efficacy on host response in experimentally immunized rainbow trout (*Oncorhynchus mykiss*). Turkish Journal of Veterinary and Animal Sciences, 38: 388-393.

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DUNDON, W.G., ADOMBI, C., WAQAS, A., OTSYINA, H.R., ARTHUR, C.T., SILBER, R., LOITSCH, A., DIALLO, A. (2014). Full genome sequence of a peste des petits ruminants virus (PPRV) from Ghana. Virus Genes 49(3):497-501. doi: 10.1007/s11262-014-1109-1.

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BOUSSINI, H., LAMIEN, C.E., NACOULMA, O.G., KABORE, A., PODA, G., VILJOEN, G. (2014). Prevalence of Rift Valley fever in domestic ruminants in the central and northern regions of Burkina Faso. Rev Sci Tech., 2014 Dec; 33(3):893-901.

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HUSSAIN, T., PERIASAMY, K., NADEEM, A., BABAR, M.E., PICHLER, R., DIALLO, A. (2014). Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates form domestic ruminants in Pakistan. Veterinary Parasitology, 206: 188-199.

GOYAL, S., DUBEY, P.K., KUMARI, N., NIRANJAN, S.K., KATHIRAVAN, P., MISHRA, B.P., MAHAJAN, R., KATARIA, R.S. (2014). Caprine Toll-like receptor 8 gene sequence characterization reveals close relationships among ruminant species. International Journal of Immunogenetics, 41: 81-89 doi: 10.1111/iji. 12075**.**

JEYAKUMAR, M., THIRUVENKADAN, A.K., SARAVANAN, R., KATHIRAVAN, P., PANNEERSELVAM, S., MALMARUGAN, S. (2014). Microsatellite based genetic characterization of Kanni Adu, Kodi Adu and Salem Black goats of Tamil Nadu. Indian Journal of Animal Sciences, 84(9): 1023-1026.

THIRUVENKADAN, A.K., JAYAKUMAR, V., KATHIRAVAN, P., SARAVANAN, R. (2014). Genetic architecture and bottleneck analyses of Salem Black goat breed based on microsatellite markers. Veterinary World, *7*(9):733-737.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Centre de Coopération Internationale pour la Recherche Agronomique et le Développement (CIRAD), France	PPR and capripox research
The Pirbright Institute, UK	Capripox research
National Animal Health Diagnostic and Investigation Center (NAHDIC), Ethiopia	Capripox research
National Veterinary Institute (NVI), Ethiopia	Capripox research
Pan African Veterinary Vaccine Centre (PANVAC), Ethiopia	Livestock vaccine quality
Laboratoire Central Vétérinaire (LCV), Mali	Capripox and PPR research
Laboratoire Vétérinaire de Kinshasa, DRC	ASF, PPR research
Institute for Veterinary Disease Control, Austrian Agency for Health and Food Security (AGES), Mödling, Austria	Exotic animal diseases research (Capripox, PPR, ASF)
Laboratoire National Vétérinaire (LANAVET), Cameroon	ASF
Special Pathogens Unit of the National Institute for Communicable Diseases, South Africa	Rift Valley Fever
Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV/ISRA), Senegal	Capripox, PPR, ASF
Livestock Breeding and Veterinary Department (LBVD), Myanmar	Animal Genetics
Département Productions Animales,Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso	Animal Genetics
University of Veterinary and Animal Sciences (UVAS), Pakistan	Haemonchus Research
Centre National de la Recherche Appliquee au Developpement Rural (FOFIFA), Madagascar	Animal Genetics
Universita Cattolica del Sacro Cuore,(UNICAT), Italy	Livestock biodiversity research
National Bureau of Animal Genetic Resources (NBAGR), India	Livestock biodiversity research
Instituto de Investigacao Veterinaria (IIV), Angola	Animal Genetics
Department of Population Genetics, Veterinary Medical University (VETMEDUNI), Austria	Animal Genetics
Swiss Institute of Bioinformatics, Switzerland,	E-learning

EXTRA-BUDGETARY SUPPORT

IDENTIFY PROJECT: Support for strengthening animal health laboratory capacities in hot spot regions to combat zoonotic diseases that pose a significant public health threat. Tripartite FAO/OIE/WHO, funded by the United States Agency for International Development (USAID)

AFRICAN RENNAISANCE FUND (ARF): Improvement of veterinary laboratory capacities in South Saharan African countries. Funded by the Department of International Relation and Cooperation of the Republic of South Africa.

PEACEFUL USES INITIATIVE (PUI): The improvement and capacity building of nuclear and nuclear related animal disease diagnostic capacities of veterinary laboratories at the regional level in Africa Funded by the United States Department of State and Japan.

The Food and Environmental Protection Laboratory

EXECUTIVE SUMMARY

The Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides assistance to Member States in implementing food control systems to ensure the safety and quality of the food supply, safeguarding consumer health and helping to facilitate international trade. Technical support is provided for food traceability and contaminant control systems and for authenticity testing to support food safety and combat economic loss through the illegal production and marketing of counterfeit and adulterated products. Activities include applied research, the development, validation, transfer and application of nuclear and related methods such as stable isotope measurements and metabolomics for food traceability, isotope dilution assays for chemical contaminant detection and control, and radiotracer studies for contaminant transfer. The application of these technologies and methods in Member States is supported by the development and provision of technical protocols, advice and guidance, and inputs for the development of international standards.

The FEPL advocates a farm-to-fork approach for effective systems to help ensure food safety, quality and security. Member State laboratories are the chief recipients of the FEPL outputs. However, in order for control systems to be effective and sustainable, they must involve stakeholders at all points along the food supply chain. Our approach, therefore, is to enhance the capabilities of analytical laboratories and to encourage interactions between the laboratories and multiple stakeholders, thereby providing essential feedback and advice to help build the capacity to assess and manage risks and improve agricultural practices.

Research and development achievements in 2014 included the development and evaluation of analytical methods to underpin food traceability systems and for food authentication, with a focus on important commodities in international trade and targets for fraudulent practices such as counterfeiting or adulteration. Complementary methods employing stable isotope analysis and metabolomics were developed to facilitate the authentication of honey, and the effectiveness of combining these and additional methods such as trace element analysis and molecular spectroscopy were investigated using multivariate data analysis. Methodology was developed to modify the existing official method for honey authenticity and overcome problems with that method in authenticating high-value manuka honey. Methods for the detection of adulteration of fruit juices by untargeted and targeted metabolomics, previously investigated in 2013, were further developed and applied to the authentication of Indian fruits and fruit juices with research partners in India. The FEPL commenced work as a research partner in the EU 7th Framework project ‘FoodIntegrity’. The FEPL continued to coordinate and provide technical input to two coordinated research projects on food traceability and authenticity. From these projects, thirty-six new methods for food analysis and protocols were drafted and tested for sample collection and preparation for fifteen food commodities. Three reference materials for stable isotope analysis have been developed and distributed and are being analysed by the project partners. In addition, almost one thousand authentic food samples have been collected, many of which have been analysed for several parameters, in order to populate a database.

Achievements related to the control of residues and contaminants in food included the transfer and application of a method for the estimation of pesticide soil sorption parameters using ^{14}C labelled pesticides to provide data for risk assessment. A multi-residue method for pesticide residues in potatoes was optimised and validated and a method for the detection and confirmation of residues of a range of different classes of veterinary antimicrobials in meat was developed and validated.

Outreach activities included planning and holding the FAO/IAEA International Symposium on Food Safety and Quality: Applications of Nuclear and Related Techniques, which was held in Vienna, Austria, 10-13 November 2014. The symposium had fifty seven speakers, about ninety poster presentations and more than three hundred participants from more than eighty countries. The results of FEPL research were presented at four international conferences, and the FEPL participated in the scientific committees for three major international conferences on food integrity and regulatory analysis for food contaminant control.

Capacity building activities in 2014 included the technical management of twenty national and five regional Technical Cooperation Projects. In addition to the international symposium, three training workshops were held. More than one hundred and thirty personnel were trained through these workshops. The FEPL also hosted three interns. The sustainable, formal network of food safety laboratories in Latin America and the Caribbean, the Red Analitica de Latino America y el Caribe (RALACA), which was initiated and established with FEPL assistance in 2012, was expanded from sixteen laboratories to fifty laboratories in nineteen countries, and is effectively promoting and supporting food safety and environmental sustainability in the region. The FEPL was represented in the Global Food Safety Partnership, providing input to the food safety technical working group and the laboratory capacity working group.

Publications by FEPL staff in 2014 included fourteen papers in conference proceedings/books of abstracts and 3 papers in the peer-reviewed scientific literature.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head
Frew, Russell David	Food Safety Specialist
Maestroni, Britt Marianna	Food Scientist
Jandrić, Zora	Analytical Chemist
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Ochoa, Victoria	Intern
Leithner, Yasmin	Intern
Massinger, Barbara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Food traceability and authenticity

Food quality, including food safety, is a major concern facing the food industry today. Current food labelling and traceability systems cannot guarantee that the food we eat is authentic, of good quality and safe. Globalization in food trade has increased the need for effective food control systems to protect consumers from impure, contaminated and fraudulently presented food. The need for analytical methods to help combat food fraud, to underpin mechanisms for tracing the origin of food commodities, authenticating food products, detecting adulteration and verifying food traceability has grown rapidly in recent years, and will most probably increase in the future with the continuing growth in the complexity of food supply chains. FEPL will expand its activities in this field of work and provide assistance to Member States as and when the need arises.

Discrimination of honey of different floral origins by a combination of various chemical parameters

In recent years, there has been growing interest in verifying the floral origins of honeys, especially in the characterisation of unifloral honeys, which are often more valuable than polyfloral honeys. Certain types of unifloral honey have claimed benefits for human health and are used in the treatment of wounds and diseases because of their healing and antibacterial properties. New Zealand manuka (*Leptospermum scoparium)* honey, for example, has been proven to have non-peroxide antibacterial activity. According to the Codex Alimentarius Standard for Honey and the relevant European Commission Directive, the use of botanical designation is allowed if a honey originates predominantly from the indicated floral source. Adulteration in terms of the dilution or substitution of high value honeys with those of lower value has increased in recent years. Therefore, discrimination of honey by floral origin is of great importance.

Identification of the floral origin of honey is typically achieved by pollen characterisation, complemented by sensory and physico-chemical analysis. However, pollen identification requires a high degree of skill and in some cases gives erroneous results, while the determination of physico-chemical parameters is broad and cannot be uniformly applied to all honey varieties. Therefore, there is an ongoing need to develop reliable, practical methods to discriminate between honeys of different floral origins. The high commercial value of some New Zealand honeys (*L. scoparium* (manuka), *Kunzea ericoides* (kanuka), and *Trifolium spp*. (clover)) has motivated intensive investigation by different research groups, their characterisation being mostly based on targeted analysis of extractable organic components. Most, if not all, of the studies to date have employed a single technique (sensory, microscopic, chromatographic, or spectroscopic) applied to one group of samples, and the methods have very limited applicability.

In FEPL, therefore, a study was carried out to explore the feasibility of combining a number of physico-chemical techniques, applying multivariate analysis to the results of sample analysis to gain additional information and to determine the most appropriate methodology for discrimination of honeys of various floral origins. This approach was applied to four authentic honeys with different floral origins (rata, kamahi, clover and manuka) obtained directly from honey producers in New Zealand. The data processing was performed on the results of chemical analyses performed in FEPL and with partners in the Queen's University of Belfast, UK and Oritain Global Limited, Invermay Agricultural Centre, Mosgiel, New Zealand, using elemental profiling, stable isotope measurements (NZ), metabolomics (ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QToF MS), FEPL), and vibrational spectroscopy (near-infrared (NIR), fourier transform infrared (FT-IR), and Raman spectroscopy) fingerprinting (UK). Orthogonal partial least squares projections to latent structures discriminant analysis (OPLS-DA) was used to determine which technique or combination of techniques provided the best classification and prediction ability.

Using OPLS-DA, the four sample groups were separated using metabolite data with a discrimination rate of 52.3%, while the discrimination rate for trace elements/isotopic data was 24.3%. The data were further analysed with a focus on discrimination of manuka honey, which is of particularly high value. For honey to be called manuka the first floral source should be more than 70% and for the other honeys to be classified as unifloral the primary source should be more than 45%. Only data from manuka and clover samples were analysed, since clover was considered as the most common floral contaminant of the indigenous honeys. The discrimination of manuka and clover honeys is illustrated in FIG 1. Higher contents of secondary pollen had an effect on sample clustering obtained by metabolite data (red circle, ~26% rewarewa; orange circle, ~30% manuka; and black circle, ~30% clover).

The study demonstrated the usefulness of multivariate statistical analysis of the results from multiple chemical techniques for the classification of honey. Variability in metabolite (for all four honeys, prediction value Q2=0.52; manuka and clover, Q2=0.76)) and the trace element/ isotopic (manuka and clover, Q2=0.65) fingerprint were the best discriminators; the other chemical parameters showed promise when combined (manuka and clover, Q2=0.43). For each of the analytical techniques, the most influential variables responsible for separation between honey groups were identified.

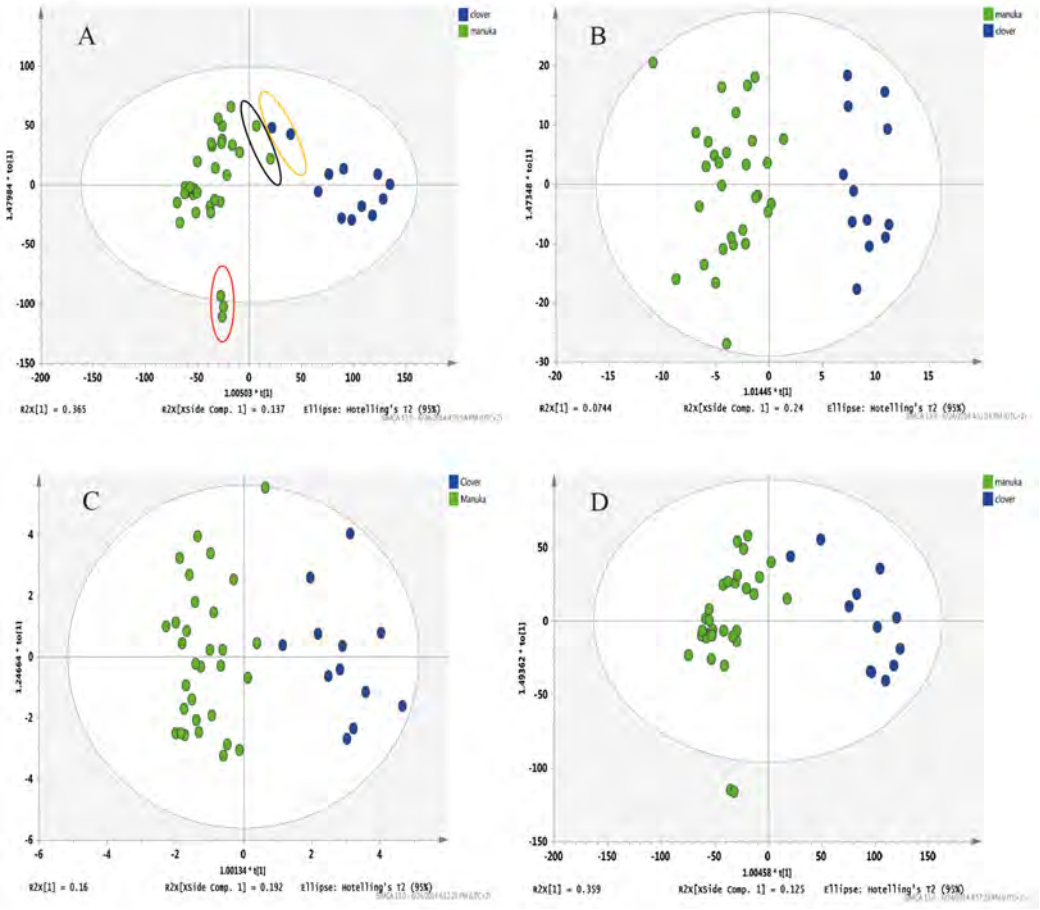


FIG. 1: OPLS-DA scores plots showing clustering of clover and manuka honey obtained by chemical analysis of: (A) metabolic data; (B) spectroscopic data (NIR, FT-IR and RAMAN); (C) stable isotope ratios of carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) combined with concentrations of major, minor and trace elements; and (D) all techniques combined.

To provide increased confidence in these results and in the applicability of the methodology, the range and number of unifloral honey samples needs to be expanded through additional studies, but these results indicate that chemomentric analysis of the results of different analytical techniques can provide a very powerful tool for authenticity testing of food commodites such as honey, that are difficult to analyse by conventional techniques, in Member States.

Stable isotopes applied to authenticating honey

One of the earliest applications of nuclear techniques for food authenticity was the use of carbon isotopes to detect the addition of cheap sugars to honey in the 1970's. The principle is that the cheap sugar (fructose) is derived from corn and has a higher $^{13}C/^{12}C$ that the fructose from honey. This difference is due to the different photosynthetic pathways; corn is a C4 plant and that mechanism does not discriminate against the 13C as much as the C3 pathway used by most honey-producing plants. Consequently the two plant types have quite different carbon isotope ratios (Fig. 2). Thus measurements of the carbon isotope ratios can distinguish between the two sources of fructose. However, within each plant population there is natural variability in isotope ratios. This makes detecting the addition of small amounts of corn syrup difficult. The test was further refined by its developers to use $^{13}C/^{12}C$ measurements on

protein purified from the honey as an internal reference, based on the fact that if the sugar and protein are from the same plant then they should be closely related in isotopic ratio. This refined method was adopted by the Association of Analytical Chemists as an official method (AOAC 998.12) and is part of the Codex Alimentarius standard for testing authenticity of honey. This test is generally reliable. However, some honey, notably New Zealand manuka, has a frequent fail rate. Manuka is a premium honey valued for its non-peroxide antimicrobial activity (NPA). The NPA is thought to be due to high levels of methyl glyoxal (MGO) and it is the manuka honey with high levels of MGO that fail the C4 sugar adulteration test. Work by FEPL indicates that this is partly due to the beekeeping practice of feeding sugar to bees during the winter. However, that does not explain the late season failures, or that the extent of failure increases as manuka honey ages. The MGO levels in manuka increase with age and it has been shown that high MGO is correlated with high apparent C4 sugar content.

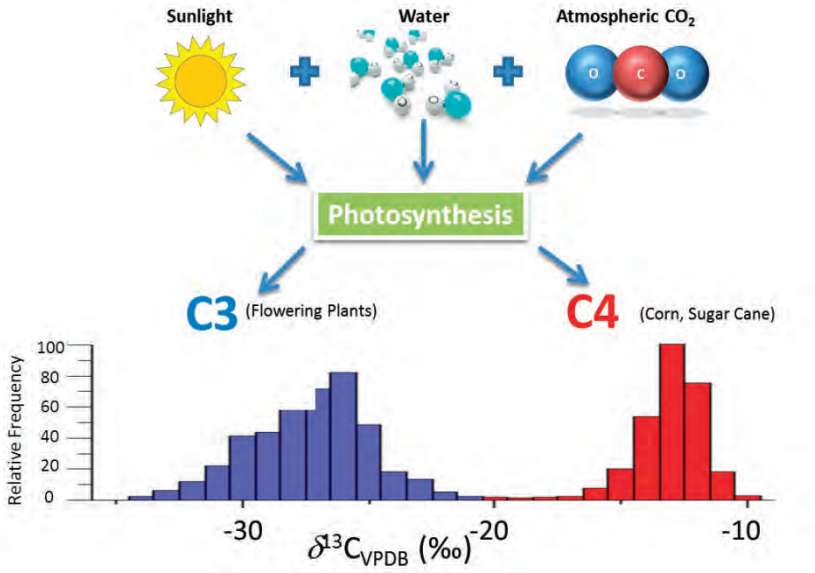


FIG. 2: The distribution of 13C/12C (expressed as d13C values) for C3 and C4 plants. The more complex biochemical reactions involved in eth C3 pathway results in greater discrimination against the 13C hence the more negative values for the 13C/12C when expressed as δ-values.

Current research in this field in FEPL is focused on modifying the AOAC method to overcome these false positives in the C4 sugar adulteration. A method has been developed for the removal of MGO prior to the purification of the protein that is measured as internal standard. It is hoped that the removal of the MGO will eliminate the interference in the isotope test. Tests are now underway to establish the optimum conditions for the removal of MGO and to show that the additional procedure does not affect the isotopic composition of the purified protein. Once those tasks are completed the work will move to the validation stage and involve other laboratories to test the procedure.

A consequence of MGO being responsible for the NPA that gives manuka honey its high value is that it provides motivation for unscrupulous operators to attempt to gain higher prices by doping honey with MGO. Work being conducted in parallel with the modification to the C4 sugar test aims to apply stable isotope measurements to see if different sources of MGO may be distinguished, hence providing a test for adulteration with MGO. So far, methods

have been established for quantifying MGO in honey by GC and HPLC. We are awaiting the availability of GC-IRMS facilities in FEPL to complete this work.

Authentication of Indian citrus fruit/fruit juices by untargeted and targeted metabolomics

Citrus fruits are one of the most important horticultural crops grown in India. India ranks sixth in the production of citrus fruit in the world. Over the last 30 years, the area and production under citrus cultivation has increased at the rate of 11 and 9%, respectively, demonstrating sustainable expansion of the citrus industry. Of the various types of citrus fruits grown in India, mandarin (kinnow, nagpur, coorg, and khasi), sweet orange (mosambi, jaffa, malta, and satgudi) and lime/lemon are of commercial importance. Sweet orange is commercially important for the production of palatable juice.

Their economic value makes citrus fruits a target for misrepresentation and their juices a target for adulteration. This has a negative impact on both industry and consumers, since high quality authentic products have to compete with less expensive adulterated ones. The value of citrus fruit commodities is variable in international trade and consequently there is a risk of finding undeclared mixes on the market, in which cheaper fruit juices (like mandarin) are used to dilute juices stated as 100% orange juice.

The addition of non-*Citrus sinensis* to *Citrus sinensis* juice is not allowed in the European Union countries. Codex Alimentarius guidelines state that *Citrus sinensis* juice may contain up to 10% *Citrus reticulata* juice, while the Food and Drug Administration (FDA) permits the addition of 10% *Citrus reticulata* to pasteurized and canned orange juice, and up to 5% of *Citrus aurantium* to frozen concentrated orange juice. Whilst some of the analytical methods already published are applicable to individual cases, none can meet the testing requirements for authenticity for all of these regulations.

The FEPL, in collaboration with research partners in the University of Delhi, India, undertook a study to explore the feasibility of using untargeted (using ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry, UPLC-QToF MS) and targeted (using high-performance liquid chromatography- mass spectrometry LC-MS/MS) analysis to discriminate authentic and adulterated citrus fruits/fruit juices, as well as to explore the capability of this approach for the verification of the authenticity of specific varieties of Indian citrus fruits or juices prepared from them.

Authentic citrus fruit samples (Kinnow manadarin (*Citrus nobilis* x *Citrus deliciosa*), Jaffa and Mosambi orange (*Citrus sinensis*), and Red blush grapefruit (*Citrus paradisi*)) were obtained from the Indian Agriculture Research Institute and analysed by an untargeted method using ultra performance liquid chromatography-quadrupole-time of flight mass spectrometry to identify characteristic markers that could potentially be used to control citrus fruit authenticity. The most influential markers identified were: hesperidin, neohesperidin, narirutin, naringin, and limonin-17-β-D-glucopyranoside. A targeted liquid chromatography-tandem mass spectrometry method was then optimised for the analysis of these markers. Ratios of limonin glucoside to hesperidin and narirutin, and narirutin to hesperidin have the potential to be used to test for authenticity of Indian citrus fruits/fruit juices and to detect adulteration down to 2% (Fig. 3). In addition, using an untargeted qualitative approach and applying principal component analysis, it was possible to discriminate between authentic and adulterated samples down to 1%.

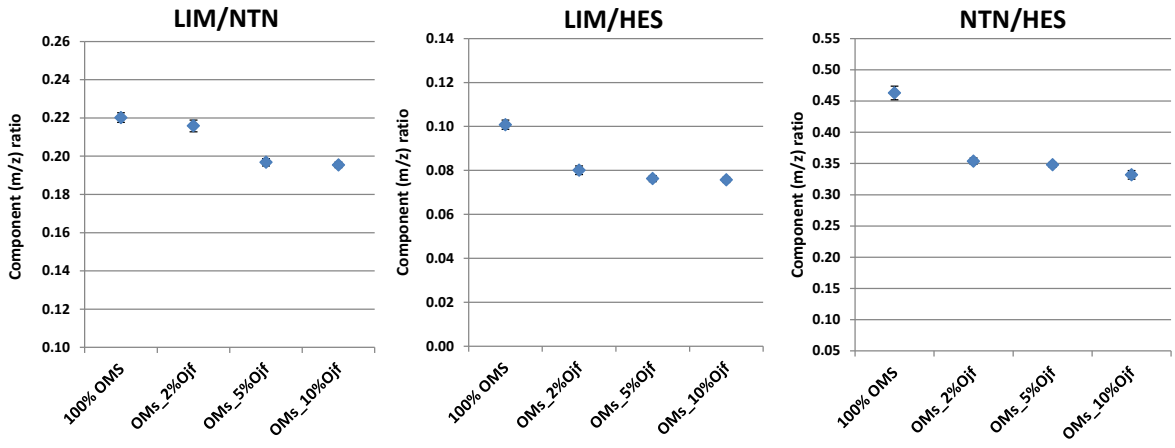


FIG. 3: LIM/NTN, LIM/HES, and NTN/HES ratios in pure Mosambi orange juice (OMs_100%) and Mosambi orange adulterated with Jaffa orange (OJf) at 2, 5, and 10% adulteration level (mean ± SD, n=6).

There is evidence in the scientific literature that, in India and other countries, commercial orange juices, even some of those labelled as 100% pure orange juice, may often have other citrus juices added to increase the flavour and colour. Based on the results from the analysis of the limited number of samples in this study, the use of this methodology could help to improve quality control testing of commercial fruit juices in India and to increase confidence in the quality of Indian citrus juices on the market, as well as to support exports.

EU 7th Framework project 'FoodIntegrity'

The FEPL is a research partner in the multi-national project, 'FoodIntegrity', funded under the EU 7th Framework mechanism, which commenced in 2014.

Providing assurance to consumers and other stakeholders about the safety, authenticity and quality of European food (integrity) is of prime importance in adding value to the European agri-food economy. The integrity of European foods is under constant threat from fraudulently labelled imitations that try to exploit that added value. The FoodIntegrity project will directly address this issue and will be an international focal point for harmonisation and exploitation of research and technology for insuring the integrity of European food. Comprising an inner core of project participants from industry, academia, research institutes, technology providers and a global network of stakeholders, FoodIntegrity will rationalise and harmonise capability to provide a coherent structure and process for assuring the food supply.

In 2014, the FEPL produced outputs under three work packages (WP) within the project: WP1, Food Integrity network; WP2, Knowledge Base; and WP 10, Industrial Integration. Participation in the project fosters integration of the work done in our IAEA coordinated research projects in this field with related work within the EU, and leverages the expertise available in the project consortium to the benefit of our member States.

Coordinated research

The FEPL coordinates and provides technical input to two coordinated research projects (CRP) in the field of food traceability and authenticity. The project 'Implementation of Nuclear Techniques to Improve Food Traceability' commenced in 2011 and is due to end in 2016. The

project has 16 participating laboratories in 15 countries. The second research coordination meeting for this project was held in Lisbon, Portugal from 26-30 May 2014. Progress by most partners was as expected according to the work plans.

To date, the project has resulted in the development or implementation of 36 new methods for food analysis and protocols have been drafted and tested for sample collection and preparation for 15 food commodities. Methodology developed or validated in the CRP has been transferred to 20 Member States through the technical cooperation programme (project RAS5062). Three reference materials for stable isotope analysis have been developed and distributed and are being analysed by the project partners. In addition, almost 1000 authentic food samples have been collected, many of which have been analysed for several parameters, in order to populate a database.

The second project in this field of work, 'Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety' has 15 participating laboratories in 15 countries. Research is proceeding as planned in this project. Results achieved in 2014 include the development and distribution of protocols for the preparation of milk water, casein and whey for isotopic analysis, and for the extraction of fatty acids from milk and bulk stable isotope analysis for C and N isotopes.

Control of Residues and Contaminants in Food

The control of unwanted chemicals in food, such as residues of veterinary drugs or pesticides used in food production, or natural contaminants such as mycotoxins, remains an area of high importance to Member States, as demonstrated by the high number of requests for assistance through the IAEA technical cooperation programme, our sister offices in FAO, and requests directly from Member States to the FEPL. Activities performed in FEPL to underpin capacity building in this area include applied research on analytical methodology to enable Member States to perform targeted risk assessment and the development or adaptation and validation of analytical methods for the detection, quantification and control of residues and contaminants.

Nuclear techniques applied to the generation of input data for first tier risk assessment

As part of an initiative under the 'Red Analitica de Latino America y el Caribe' (RALACA) network, the FEPL contributed to generating field sorption data for chlorpyriphos, to be used as input parameters in first tier risk assessment models. The goal of this study was to use nuclear technology to determine the soil sorption coefficient (Kd) and the soil organic carbon sorption coefficient (Koc) of chlorpyrifos as it behaves in a formulation as well as a pure active ingredient when applied to soil.

Chlorpyrifos is an organophosphate insecticide, used to control foliage and soil-borne insect pests on a variety of food and feed crops. Approximately 10 million pounds of chlorpyriphos formulations are applied annually in agricultural settings. This pesticide is moderately persistent in soils, with a half-life generally between 60 and 120 days, but this can vary from two weeks to over a year depending on the soil type and climatic conditions. The soil sorption coefficient (Koc) is typically between 652 to 30381 L kg^{-1}. The concentration and persistence of chlorpyrifos in water will vary depending on the type of formulation. For example, a large increase in chlorpyrifos concentrations occurs when emulsifiable concentrations and wettable powders are released into water. As the pesticide adheres to sediments and suspended organic

matter, concentrations rapidly decline. The increase in the concentration of insecticide is not as rapid for granules and controlled release formulations in water, but the resulting concentration persists longer. The pesticide is very toxic to fresh water fish, aquatic invertebrates and estuarine and marine organisms.

The pesticide soil sorption test was performed using the OECD batch equilibration method with 14C-chlorpyrifos as a radiotracer according to the procedure described in FIG. 4. A liquid scintillation counter was used to determine the activity of the radiotracer extracts.

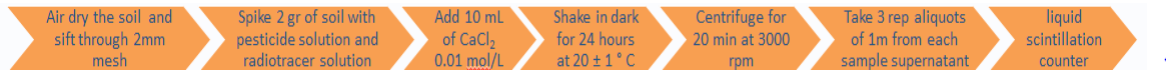


FIG. 4: Schematic of the soil sorption test.

The Freundlich adsorption isotherm is an empirical relation between the concentration of a pesticide adsorbed onto the soil (Cs) in relation to the amount of pesticide in equilibrium in the solution (Ce):

The Freundlich adsorption isotherms for the active ingredient and the commercial formulation are shown in Fig 5.

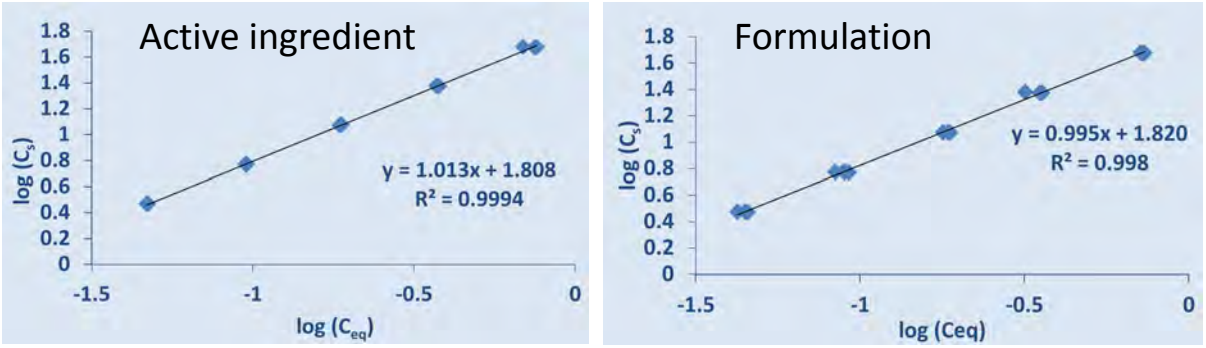


FIG. 5: Freundlich isotherms for chlorpyrifos active ingredient and commercial formulation.

From the isotherms, Kd and Koc for this soil were calculated to be 64.34 and 1641 L kg^{-1} (active ingredient), and 66.08 and 1686 L kg^{-1} (formulation).

Koc is regarded as a more universal parameter since it is related to the hydrophobicity of the pesticide molecule, which applies to a given pesticide in all soils. The Koc values obtained in this study are within the range of data published in the literature for chlorpyriphos (652 to 30381 L Kg^{-1}).

The difference in Koc values between the active ingredient and the formulation of chlorpyrifos is small (2.7%). However, information in the scientific literature indicates that formulation additives could make the pesticide more available in water, for example in the case of run-off. It is, therefore, important to determine the Koc of both the formulation and the active ingredient. The determination of pesticide sorption parameters (Kd and Koc) in local soils is important to calibrate tools such as the Pesticide Impact Rating Index (PIRI) in order to

produce more reliable results for assessing pesticide management practices and environmental changes.

The use of the radiolabelled pesticide is an efficient and a rapid means of generating input data for environmental modelling.

Validation of a gas chromatographic method for several pesticides in potatoes

Potatoes are an important staple food all over the world. To protect the crop from various diseases, farmers apply a range of regulated pesticide formulations which can sometimes leave residues in the crop. To help ensure safe food for consumers it is important to apply end point testing of food products to provide feedback on the effectiveness of pesticide application practices in avoiding harmful levels of these residues, and to ensure that any products containing potentially harmful concentrations of the pesticides do not enter the food chain.

As part of an initiative in the framework of the 'Red Analitica de Latino America y el Caribe' (RALACA) network, the FEPL contributed to the validation of a multi-residue method for 26 pesticides in potato. The method included the pesticides that are most frequently employed in agricultural production of potatoes and was validated according to the Codex Alimentarius Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993). The method performance was characterized in terms of its scope, specificity, accuracy, sensitivity, repeatability and within laboratory reproducibility.

The multi-residue method used QuEChERS clean-up and gas chromatography with mass selective detection (GC-MSD) as summarised in Fig. 6. The processed and homogenized sample was extracted with acidified acetonitrile. A mixture of anhydrous magnesium sulphate, sodium chloride and sodium citrate salts was added for pH adjustment and phase separation. After shaking and centrifugation, an aliquot of the organic phase was cleaned up using primary-secondary amine material (PSA) and anhydrous magnesium sulphate. The extract was then concentrated under a stream of nitrogen and re-dissolved in ethyl acetate. The final extract was filtered and injected for analysis by GC-MSD.

The method was validated for 26 pesticides at 3 spiking levels covering a range of Codex MRLs: 10 µg kg^{-1} as the low spike, 100 µg kg^{-1} as the medium spike and 500 µg kg^{-1} as the high spike.

The average recoveries for all analytes were in compliance with the Codex within laboratory method validation criteria (60-120%), and repeatability and reproducibility values also met Codex requirements for precision (relative standard deviations <20%).

The method is quick and relatively cheap and can be applied to the analysis of potato samples for the determination of several classes of compounds in regulatory laboratories in both developed and developing countries. The validation of the method will be supported and extended in the laboratories in which it is implemented by method performance verification during routine analysis and through the analysis of certified reference materials, participation in proficiency tests and/or other inter-laboratory comparisons.

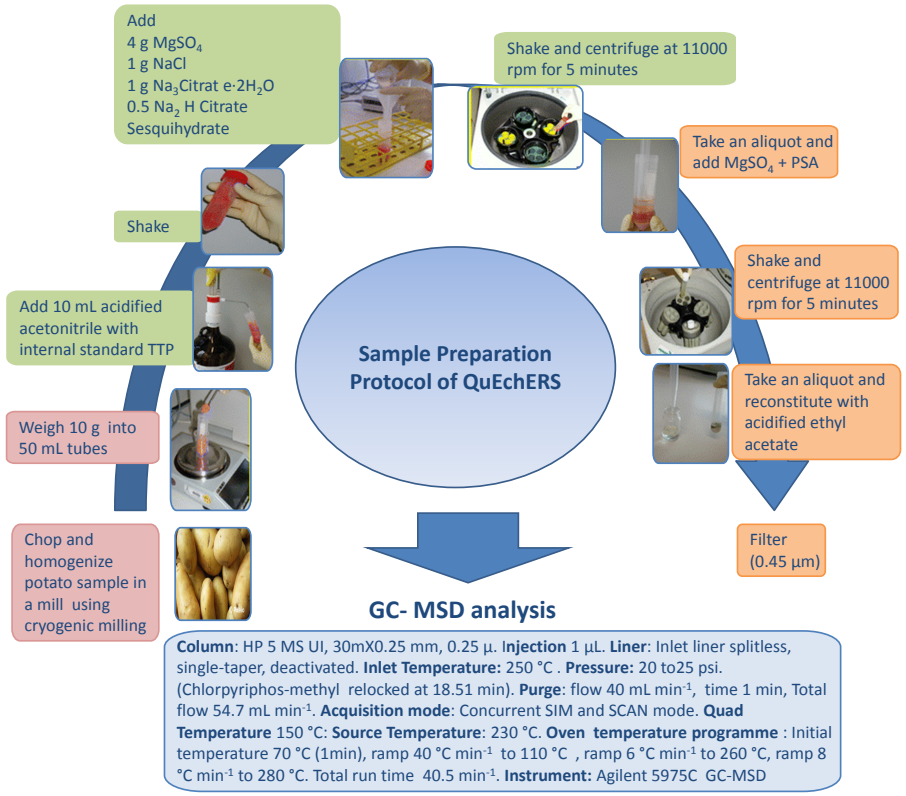


FIG. 6: Schematic of the method.

The use of analyte protectants and matrix-matched calibration in gas chromatographic analysis for pesticide residues in potatoes

In pesticide analysis, it is common for various pesticides to be prone to matrix effects, which can affect the signal from the pesticide and can therefore lead to errors in the calculated concentrations. Since the effective elimination of the sources of the matrix-induced response enhancement is not feasible in practice, some means of compensating for such effects is necessary. One approach commonly applied is the use of matrix-matched calibration standards – that is, calibration standards injected in a matrix similar to the samples being analysed. Another more recent approach is the use of analyte protectants (AP). Analyte protectants minimize pesticide interactions with the active sites in the gas chromatographic system and protect them from degradative interactions. A study was carried out in FEPL to compare and evaluate the use of AP and matrix-matched calibration in a gas chromatographic analytical method for pesticide residues in potatoes.

Homogenized samples were spiked with a mixture of pesticides at 0.5 mg kg^{-1} and analysed by the multi-residue method validated in FEPL and described above. The AP used in the study was a combination of ethylglycol, gulonlactone and sorbitol in ethylacetate: DMSO (80:20).

The effect of AP was tested at different concentrations over the range 0.3 – 2.4 mg mL^{-1} gulonolactone / 3.0 – 24.0 mg mL^{-1} ethylglycol to find an optimal value for compensation of matrix effects. Fig.7 shows the recoveries for selected pesticides when the calibration was prepared in matrix (MM), solvent without AP (SOL), or solvent with increasing amount of AP (SOL+AP25-SOL+AP200). The recovery in solvent at AP levels AP40-AP60 approached the

recovery obtained through matrix matching. However increasing the amount of AP decreased the precision for all pesticides studied. The effect of AP on the recovery values was variable among the pesticides. For most pesticides studied, there was no relationship between the amount of AP and recovery. Azoxystroyn, etho-prophos and pyrimethanil showed a simple linear relationship indicating zero as the optimal concentration of AP in solvent.

In conclusion, under our laboratory conditions, the use of AP for solvent calibration did not improve the results. Matrix-matched calibration by the preparation of calibration standards in blank extracts, which imparted similar matrix-induced enhancement as in the sample extracts, was acceptable and was therefore used as a quantification approach in the multi-residue method for pesticides in potato. This information is important in terms of the transfer of the method to Member State laboratories.

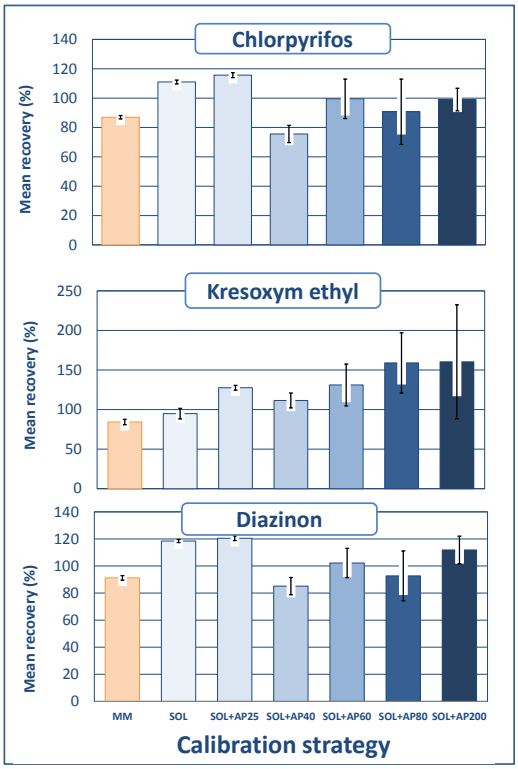


FIG. 7: Mean recovery values and standard deviations for the different calibration strategies in three of the pesticides analysed.

A multi-residue method for the determination of antimicrobials in meat

Antimicrobial agents are often used in animal food production to prevent and/or treat disease and as growth promoters. This can lead to residues in edible tissues with possible detrimental effects on human health including the development of antibiotic-resistant bacteria. Effective control systems require the monitoring of animal derived foods for antimicrobial residues.

A multi-residue method was developed and validated in FEPL for the determination of a range of antibiotic residues in porcine muscle using liquid chromatography - triple quadrupole mass spectrometry (LC-MS/MS). The method was validated for the determination of 29 antibiotics, including representatives of the sulphonamides (SAs), quinolones (QLs) and aminoglycosides (AGs).

The matrix was extracted with EDTA/KH_2PO_4 mixture in acidic conditions (pH 4.0), followed by concentration and clean-up by solid-phase extraction using STRATA columns, concentration by evaporation and reconstitution in water. The extracts were analysed by LC-MS/MS, with gradient elution using a HILIC column and a precursor ion and two transition ions measured for each compound.

The performance of the method was evaluated based on the validation guidelines of European Commission Decision 2002/657/EC. Recoveries, calculated for all compounds using matrix-matched external standard calibration, ranged from 68.3-103% (SAs), 85-101% (QLs) and 75-84% (AGs). Precision was acceptable for all analytes, with repeatability ranging from 5.6-22.5% and within-lab reproducibility between 4.1 and 20.4%. Ion chromatograms for representative of each class compounds are presented in Fig. 8.

The results of the validation demonstrate the suitability of this method for the detection, identification and confirmation of 29 antibiotics in meat. The method is applicable for routine analysis in food control laboratories equipped with LC-MS/MS.

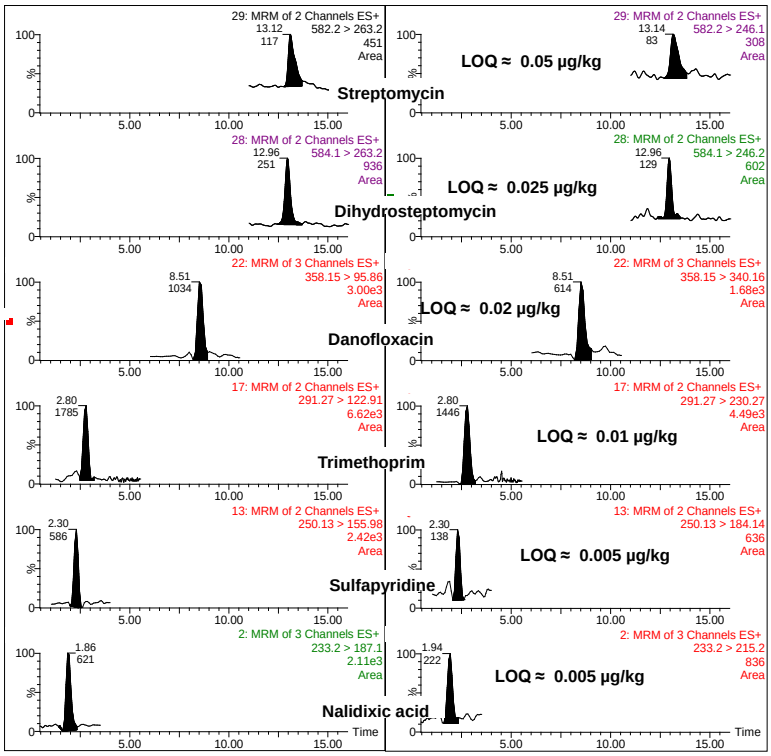


FIG. 8: Ion chromatograms of 2 transitions (quantification and qualifier) of representative SAs, QLs and AGs at the limit of quantification.

Coordinated research

The FEPL participated in the CRP 'Development of Radiometric and Allied Analytical Methods to Strengthen National Residue Control Programs for Antibiotic and Anthelmintic Veterinary Drug Residues', which ended in 2014. Under this CRP, FEPL collaborated with the research team in the Shenzhen Centre for Disease Control and Prevention, Shenzhen, China in the development of a method for the simultaneous determination of 16 aminoglycoside residues in porcine tissues by liquid chromatography tandem mass spectrometry. The method is being implemented in China and a manuscript has been submitted for consideration for publication in a peer-reviewed scientific journal.

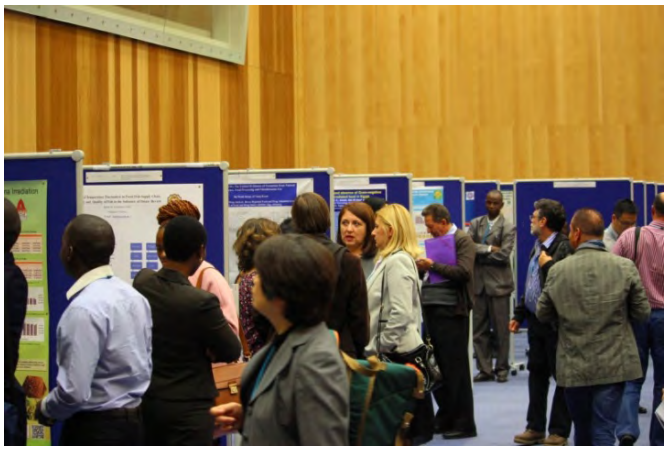
Dissemination of Research Results

The methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training courses and publications in the scientific literature. The online resource developed by the Food and Environmental Subprogramme, 'The Food Contaminant and Residue Information System' (FCRIS, http://nucleus.iaea.org/fcris/) provides a wealth of useful data on food contaminants and residues. FCRIS includes analytical method databases which are continually updated with methods developed in the FEPL, as well as others submitted by laboratories in Member States. The methods databases for veterinary drug residues and for pesticide residues were, developed in response to requests from the Codex Committees on Residues of Veterinary Drugs in Food and on Pesticide Residues.

Conferences

- International Symposium on Food Safety and Quality: Applications of Nuclear and Related Techniques, Vienna, Austria, 10-13 November 2014. This symposium, organised by the Food and Environmental Protection subprogramme, provided a major avenue for dissemination of the research results from FEPL and from a wide range of collaborators and scientists working in related fields. FEPL staff gave one oral and seven poster presentations

(listed in the references section of this report), and there were 57 oral and 90 poster presentations from more than 300 participants. A special edition of the peer-reviewed scientific journal "Food Control" will be dedicated to papers presented at the symposium.



Discussions during a scientific poster session at the FEP symposium

- 248th American Chemical Society National Meeting and Exposition, San Francisco, USA, 10-14 August 2014. The FEPL Head gave an invited oral presentation on the application of stable isotope measurements and metabolomics for traceability and authenticity of milk, fruit juices and honey, in the session 'Authentication and Adulteration of Food', organised by the ACS Division of Agricultural and Food Chemistry.

- 13th International Union of Pure and Applied Chemists (IUPAC) International Congress of Pesticide Chemistry. This congress was held in conjunction with the ACS meeting mentioned above. The FEPL Head participated in a number of sessions of the congress. Several posters were presented in these sessions by counterparts in IAEA technical cooperation projects and other collaborators, including two posters co-authored by staff from the FEPL at Seibersdorf: 'Comparison of estimated KD and KOC for pesticides using pure active ingredient and formulated product in soils from Latin America and Europe using radiometric techniques' and 'Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale'. Both posters focused on technology and methods transferred through training courses run by the FEPL. The methods are being effectively disseminated by the RALACA laboratory network, established under FEPL projects.

- 2nd Food Integrity and Traceability Conference, Belfast, UK, 8-10 April 2014. The conference focused on mechanisms and systems to protect the integrity of the food supply chain. This included the control of threats posed by microbiological and chemical contamination of food, traceability along the food supply chain to facilitate targeted and efficient recall of contaminated products and allow trace-back to contamination sources for mitigation and ongoing improvement of farm-to-fork food safety systems, and guaranteeing the authenticity of food products to combat fraudulent practices and control adulteration for economic, food safety and cultural reasons. The conference had more than 350 participants from more than 30 countries world-wide. The FEPL Head presented a poster entitled 'Discrimination of honey of different floral origins by a combination of various chemical parameters', which was the result of collaborative research between FEPL, Queen's University Belfast, UK, and Otago University, New Zealand. This work represents potential methodology for combating fraud and helping to ensure food safety in Member States in the future. As a member of the Scientific Committee of the conference, Mr Cannavan also chaired a plenary session dedicated to early stage researchers.

The FEPL is also represented through the Laboratory Head in the Scientific and Publishing Committees of the 3rd Saskatoon International Workshop on Validation and Regulatory Analysis, to be held in Calgary, Canada, 16-19 June 2015, and as Chair of the Scientific Committee for the EuroResidue VIII Conference on Residues of Veterinary Drugs in Food, to be held in Egmond aan Zee, The Netherlands, 23-25 May 2016. Preliminary work for both events was ongoing in 2014.

CAPACITY BUILDING

The FEPL provided technical management for twenty national and five regional TCPs in 2014. The expertise available in FEPL and the methods and techniques developed were also used to support technology transfer to Member States through a range of training courses and workshops, both at Seibersdorf and in Member States.

Analytical methods and technology packages were transferred and applied through the TCPs and through training workshops implemented using extra-budgetary funding. The workshops were designed to allow the trainees to train further laboratory personnel in their home institutes, thereby maximising the impact of the training. The methodology transferred provides feedback to food chain stakeholders enabling them to optimise production practices and the use of agrochemicals, improving both food safety and environmental sustainability.

International Symposium on Food Safety and Quality: Applications of Nuclear and Related Techniques

This symposium was held by the Food and Environmental Protection subprogramme in Vienna, Austria, 10-13 November 2014. The Scientific Secretary and Assistant Scientific Secretary, both FEPL staff members, provided the main *input* to the organisation of the symposium, ably assisted by professional and support staff from both the laboratory and headquarters. More than 300 researchers, laboratory analysts, policymakers, regulators, food producers and others participated. More than 80 countries were represented, and more than half of the participants were from developing countries. There were 57 oral and about 90 poster presentations on contemporary and novel applications involving nuclear and related techniques, and the participants discussed different perspectives and future opportunities. The symposium show-cased the areas of work covered by Food and Environmental Subprogramme and also provided a forum for interdisciplinary networking between professionals from different backgrounds, national institutes, academia, industry, and international organizations.

Workshop on Food Control Systems and the Role of the Different Stakeholders in the Food Supply Chain

This workshop was held on 14 November 2014 as a satellite event to the International Symposium on Food Safety and Quality. More than 80 symposium participants registered for the workshop, which covered food control systems, the role of the analytical laboratory and national food safety agencies in the farm-to-fork chain, decision making based on quality input data and risk assessment, risk based management options, and risk communication. The workshop included presentations from invited experts and panel discussions.

Training workshop on the Application of Quality Assurance and Control in Analytical Laboratories to Address Food Safety and Quality

Protection of the integrity of the food supply is of utmost importance in terms of food security, food safety and quality, consumer protection and international trade. Techniques to maintain and assure the quality and safety of food are necessary throughout the food production and supply chain. The need for methods to monitor and verify food safety and quality is evidenced by the ever growing list of food product recalls due to contamination. Emerging issues have highlighted the need for continued refinement, development and innovation to improve measures to ensure food safety and quality. Intensive practical and theoretical training is required to support Member States in meeting these requirements.

To help meet these requirements, the Joint FAO/IAEA Division held a training workshop on "Application of Quality Assurance and Control in Analytical Laboratories to Address Food Safety and Quality" which took place in IAEA Vienna International Centre (10-14 November 2014) and IAEA Laboratories, Seibersdorf, Austria (17-21 November 2014). The workshop was funded under the 'Peaceful Uses Initiative' as part of the project 'Sustainability of Capacity Building Activities to Improve Food Safety and Quality through Nuclear Technology and Networking'.

The workshop was focused on food safety and quality and included protection of the integrity of the food supply chain as a holistic process, involving multiple stakeholders and requiring the application and integration of different analytical methods and processing technologies. It brought together experts in these fields who presented contemporary applications and discussed future perspectives and opportunities, providing a forum for interdisciplinary networking between all stakeholders in the farm to fork food chain.

The first week of the workshop included participation in the International Symposium on Food Safety and Quality, and in the satellite event on Food Control Systems and the Role of the Different Stakeholders in the Food Supply Chain. The second week was held in the Food and Environmental Protection Laboratory at Seibersdorf and focused on intensive training in analytical methodology and techniques. The workshop had 23 participants from 21 countries.

Training Course on the Analysis of Stable Isotope and Trace Element Composition of Food Materials

The capability to certify food origin or authenticity is of significant economic importance to many stakeholders in developing countries. For example, some food products can be marketed using labels (e.g. GI, Geographic Indication) that are based on standards of identity or composition related to a very specific production area. This adds value to such products

in terms of marketability and increased export value. Basmati rice from India and Pakistan, for example, is defined by its cultivar and also by its area of production. Genomic techniques can easily confirm the cultivar of Basmati rice, while isotopic and elemental fingerprinting is essential to determine the geographical origin. Isotopic parameters have recently been added to the PDO (Protected Denomination of Origin) technical specification of certain kinds of cheese and other food commodities are undergoing similar characterisation.

Training on high resolution mass spectrometry in the FEPL

The IAEA project (RAS/5/062) on 'Building Technological Capability for Food Traceability and Food Safety Control Systems Through the Use of Nuclear Analytical Techniques' further strengthens the National Project Teams of each participating Member States to respond to the needs of the industries in the region to enhance food safety through improved traceability systems. Activities and work plans are designed to advance the capability of the region in utilizing nuclear techniques in systems for verifying the origin of food products.

A core component to the project is training in the analysis of the stable isotope and trace element composition of food materials. A training course was held from 3-14 February 2014 in cooperation with the Government of the Philippines through the Philippine Nuclear Research Institute (PNRI). The meeting was attended by 26 participants from Member States as well as several participants from the host country. Participating countries were Bangladesh, Cambodia, China, Indonesia, Iraq, Kuwait, Jordan, Malaysia, Myanmar, Nepal, Oman, Pakistan, Philippines, Singapore, Sri Lanka, Syrian Arab Republic, T.T.U.T.J of T. Palestinian A, Thailand, Vietnam and Yemen.

The objectives for the training were to:

- Promote the use of nuclear and complimentary techniques for food authentication through education and provision of education resources;
- Provide training in analytical techniques (stable isotopes and trace elements) as applied to food analysis;
- Provide training in data handling and interpretation;
- Increase confidence in maintaining and troubleshooting instruments;
- Review, develop and refine individual work plans; and
- Promote networking.

The trainees were from a variety of backgrounds. Only 8 participants were stable isotope analysts and the remainder worked mainly with trace metal analyses. The course was organized to concentrate on the stable isotope techniques in the first week and in the second week covered trace metal analysis and inductively coupled plasma mass spectrometry. The main points of the trainees' assessment of the course were that their knowledge of the subject covered in the course had improved significantly by the end of the course, and they expected that the training would have a significant and immediate impact on their performance in the workplace.

The RALACA Laboratory Network

A sustainable, formal laboratory network for food safety and environmental sustainability, the Red Analitica de Latino America y el Caribe (RALACA), was initiated and established with FEPL assistance in March 2012. Nine laboratories were involved initially, and in 2013-14 the network was expanded to include 50 laboratories in 19 countries. The FEPL maintains input and assists with coordination of the network through membership in the board. In 2014 the scope of activities of the network was broadened to include areas such as risk assessment and environmental biomonitoring, which enables laboratory support of the full 'farm-to-fork' food supply chain. This increased scope also required some restructuring of the management and coordination mechanisms, including the formation of new committees. With FEPL support under the Peaceful Uses Initiative project, 6 of the laboratories have been accredited under ISO/IEC 17025 (2005) and 3 additional labs were in the final stages of accreditation by the end of 2014. The RALACA network is self-sustaining and is already playing an important role in the transfer of technology and methodology developed in the FEPL to the Latin America/Caribbean region, as well as sharing expertise, technology and methodology between participating laboratories and countries. This will greatly enhance the regions ability to pre-empt or react to food safety issues that arise. The RALACA web site address, also established with FEPL assistance, is http://red-ralaca.net.

The Global Food Safety Partnership

The Global Food Safety Partnership (GFSP) is a public private partnership dedicated to food safety capacity building. The main GFSP objective is to support improved food safety systems as demonstrated by enhanced agri-food value chains for economic growth and improved public health outcomes in developing and middle income countries. The GFSP approach is intended to fill a gap whereby food safety initiatives would be better coordinated and accessible to improve impact.

The third annual GFSP meeting and conference, held in Cape Town, South Africa,from 8-12 December 2014 , brought together approximately 150 participants from UN organisations, NGOs, the private sector, and academia. The meeting consisted of three days of working group and bilateral meetings and two days open conference, with roundtable meetings and a food safety regional leadership dialogue event. The FEPL is represented in the Food Safety Technical Working Group, making available the experience of the FEPL in important applications of nuclear and related techniques, web-based resources relevant to food safety (the Food and Environmental Protection Subprogramme's FCRIS database - method databases to support the Codex Committee on Residues of Veterinary Drugs in Food and on Pesticide Residues, e-learning courses on laboratory procedures for food safety, and food irradiation databases), TCPs and CRPs in food safety, traceability and authenticity.

The outputs from the Joint FAO/IAEA Division are directly relevant to the GFSP. The experience gained from implementing IAEA TCPs and CRPs can be used to help make the GFSP projects more effective, and synergy between the Joint Division's projects and those of the GFSP will be to the benefit of recipient countries and institutes, including IAEA TCP counterparts.

Fellowships, Scientific Visitors and Interns

In January 2014, Ms Laura Natalia Fernandez Cedi completed a 4-month period as an intern in the laboratory. During her time in FEPL time, Natalia worked with staff in a number of areas, principally on methodology for the authentication of foods using metabolomics. She also provided support in activities related to the RALACA laboratory network and the set-up of its web site.

In April, Ms Victoria Ochoa commenced an internship in the laboratory, working with FEPL colleagues on the development and validation of a multi-residue method for pesticide residues in potato, and also using her native Spanish language skills in the translation and preparation of technical documents.

In July 2014, Ms Yasmin Leithner joined the laboratory. Yasmin's work during her internship included support in the development and application of analytical methods for food authenticity and traceability by stable isotope analysis, covering on a range of food types.

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JANDRIĆ, Z., FREW, R., MCCOMB, K., HAUGHEY, S, ELLIOTT, C., CANNAVAN, A. (2014). Discrimination of honey of different floral origins by a combination of various chemical parameters. Book of abstracts of the 2nd Food Integrity & Traceability Conference, Belfast, UK, 8-10 April 2014, 57.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Laboratorios Microbioticos s/c/ Ltda, São Paulo, Brazil	Method development for food contaminants; technology transfer to Latin America
Centro de Contaminacion Ambiental (CICA), University of Costa Rica (UCR), Costa Rica	IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB)
Institut für Lebensmittel-, Arzneimittel- und Umwelt-Analytik (ILAU), Germany	Collaborations on research activities linked to CRP D5.20.35 on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale"
Division of Land and Water, Commonwealth Scientific and Industrial Research Organisation (CSIRO),, Australia	
Environmental Chemistry, Ecotoxicology, Pesticides and Radioactivity Department, State General Laboratory, Ministry of Health, Cyprus	
Austrian Agency for Health and Food Safety (AGES), Austria	Collaboration on accelerated capacity building for risk analysis and contaminants in food
Austrian Institute of Technology, Austria	Collaboration on nuclear techniques for research into interactions between environmental/food contamination Collaboration on the use of stable isotope measurements for traceability of foods and animals
Ashtown Food Research Centre, Ireland	Partner laboratory in EU Project "ProSafeBeef"
Institute of Agri-food and Land Use, Queen's University Belfast, UK	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queen's University Belfast, UK	Research activities in isotope-ratio methods for food traceability
Chemistry and the Environment Division, International Union of Pure and Applied Chemistry (IUPAC)	Collaboration on compendium of agrochemicals information
Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA)	Training for Member State scientists and regulators on food safety and quality
Agrolab, México	
Laboratorio Nacional de Insumos Agrícolas, Colombia	

Institution	Topic
Agilent Technologies, PA, USA	Training for Member State scientists in analytical techniques
RIKILT Institute for Food Safety, the Netherlands	Research into causes of food contamination with veterinary drug residues
Institute for Application of Atomic Energy, Department of Agro-Ecological Environment, Chinese Academy of Agricultural Sciences (CAAS), China	Development of methodology for food traceability and residues analysis
Technical University Munich, Germany	Development of radioassay protocols
World Health Organization (WHO), Lyon Office for National Epidemic Preparedness and Response	Global survey of laboratory quality standards
World Organization for Animal Health (OIE)	
World Food Programme	Control of mycotoxins in food stocks
Department for Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, Novi Sad, Serbia	Transfer of natural plant toxins through the environment to food
International Federation for Animal Health (IFAH)	Quality control of trypanocidal drugs in sub-Saharan Africa
GALVmed	
UNODC	
University of Strathclyde, UK	
Manchester Metropolitan University, UK	
Laboratoire de Contrôle des Médicaments Vétérinaires, Dakar, Senegal	
Tanzania Food and Drug Authority, Tanzania	
University of Otago, New Zealand	Collaboration on the use of stable isotope measurements for traceability of foods Development and validation of new certified reference materials for stable isotope analysis Research into new stable isotope techniques for verifying the integrity of honey products
Food and Environmental Research Authority, UK	Collaborations on research activities linked to CRP D5.20.37 on ’Implementation of Nuclear Techniques to Improve Food Traceability’, EU ’FoodIntegrity‘ project

The Insect Pest Control Laboratory

EXECUTIVE SUMMARY

The Insect Pest Control Laboratory (IPCL) is part of the Joint FAO/IAEA Division's food and agriculture programme and develops environmentally friendly methods for area-wide control of insect pests, such as fruit flies, tsetse flies and disease transmitting mosquitoes. It plays a key role in the implementation of the Insect Pest Control subprogramme.

In the plant pest area, work has continued under an FAO/IAEA/USDA agreement entitled: "*Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies*". Emphasis of the work has been on assessing whether cold treatments can be made generic for fruit species and cultivars and populations within fruit flies species. So far it looks promising that cold treatments can be made more generic than they currently are.

The IPCL continued to support the work under the Co-ordinated Research Project on resolving cryptic fruit fly species issues. Field cages experiments were carried out to assess the attraction of female *Anastrepha fraterculus* to pheromones of males from the various morphotypes of this species complex.

With respect to the *Bactrocera dorsalis* complex (*B. dorsalis s.s., B. papayae, B. philippinensis, B. invadens* and *B. carambolae*), genetic and cytogenetic screening of populations of these taxa did not reveal any evidence for the presence of reproductive symbionts, such as *Wolbachia, Rickettsia, Cardinium, Spiroplasma* and *Arsenophonus*. Part of these results contributed to the recent synonymization of *B. papayae, B. philippinensis, B. invadens* to *B. dorsalis s.s.*

A colony of *Drosophila suzukii* commonly known as the spotted-wing drosophila was maintained at the IPCL in 2014. The fly is a major invasive pest in North America and Europe. Work has focussed on development of mass-rearing technology and quality control protocols.

Efforts were initiated to assess the feasibility of cryopreservation of fruit flies. In collaboration with USDA, the IPCL developed the cryopreservation for the Mediterranean fruit fly *Ceratitis capitata.* Two cryopreserved lines of the genetic sexing strain VIENNA 8 were established and quality control analysis after their revival showed that both maintained their key characteristics.

In the area of livestock pests, work continued on the separation of male and female tsetse pupae using near infrared (NIR) technology. A new system was obtained from the USA that uses NIR LEDs. Preliminary tests showed that the system is capable of separating *Glossina palpalis gambiensis* pupae with 95% accuracy at 6-7 days before emergence, at a sort rate of 10-20 pupae per second.

Gamma ray dose rates can be measured using transfer standard dosimeters through services offered by many national standards laboratories. For X-ray systems, no such standard dosimeters have been calibrated and the IPCL, in collaboration with the standards laboratory in Brazil, developed a calibration for alanine EPR dosimeters in the X-ray field of a Rad Source RS2400 machine. Based on this calibration, a dose measurement service will be developed and offered to facilities using X-rays during 2015.

The impact of irradiation on the establishment of endosymbionts in the tsetse fly *Glossina morsitans morsitans* was assessed The results indicate that irradiating 5-7 days old male flies with 110 Gy reduced the replication rate of *Sodalis* and the salivary gland hypertrophy virus, but increased the prevalence of *Wolbachia*.

A series of interspecific crosses were carried out between the tsetse fly subspecies *Glossina morsitans morsitans* (Gmm) and *G. m. centralis* (Gmc) to explore the impact of the endosymbiont *Wolbachia* and other tsetse-associated bacteria on hybrid sterility. The flies were treated with tetracycline to knock down the *Wolbachia* and associated bacteria. This allowed the establishment of a Gmm x Gmc hybrid colony at the IPCL that is now in its 10th generation. The hybrids showed no mating isolation when tested in field cages against Gmm and Gmc flies.

Different molecular tools have been evaluated against laboratory colonies and field collections of tsetse flies to provide quick, robust, easily and massively applicable and cheap markers for species identification. Simple agarose gel electrophoresis of some markers, coupled with some limited sequencing in some cases, show promise as a 'toolkit' for accurate identification of a number of tsetse species.

Work continued on the development of mass-rearing technologies for disease transmitting mosquitoes. As a result of all the experiments, detailed data has been collected over many months on the productivity of *Anopheles arabiensis*, which gives a clear idea of the level of male production which is possible using the techniques that have been developed.

Robust and efficient sex separation methods are not yet available for mosquito species such as *Aedes albopictus*. The IPCL and collaborators (University of Illinois, USA and University of Guangzhou, China) proposed a protocol combining the sterile insect technique (SIT) with the incompatible insect technique (IIT). By applying low irradiation doses females would be completely sterilized while the released males would be fully sterile due to the effects of both *Wolbachia* and irradiation. Even if females were to be accidentally released, these would be sterile and at the same time unable to transmit dengue or to replace the pest population. This combined strategy is currently being considered for the population control of *Ae. albopictus*, a primary dengue vector in mainland China.

In 2014, the IPCL hosted five cost-free experts, eight consultants, thirteen interns, twelve fellows and three scientific visitors. The Plant Pests group delivered 82 shipments of fruit fly eggs, pupae, dead samples or other biological material to 53 institutes in 23 countries. The Livestock Pests group had 49 consignments shipped to 13 institutes in 10 countries. The Vectors of Human Diseases group shipped biological material to 15 institutes in 14 countries, whereas the Genetics and Molecular Biology group had 9 consignments to 7 institutes in 5 countries.

STAFF

Name	Title
Vreysen, Marc	Laboratory Head
Abd Alla, Adly	Molecular Biologist/Virologist
Bourtzis, Kostas	Molecular Biologist/Geneticist
Gilles, Jeremie	Entomologist (Human Health Pests)
Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Livestock Pests)
Yamada, Hanano	Junior Professional Officer (until May 2014)
Targovska, Asya	Senior Laboratory Technician
Wornoayporn, Viwat	Senior Laboratory Technician (until July 2014)
Adun, Henry	Laboratory Technician
Ahmad, Sohel	Laboratory Technician
Almenar, David	Laboratory Technician
Marin, Carmen	Laboratory Technician
Mohammed, Hasim	Laboratory Technician
Schorn, Elisabeth	Laboratory Technician
Soliban, Sharon	Laboratory Technician (until June 2014)
Cancio Martinez, Elena	Laboratory Attendant
Dammalage, Thilakasiri	Laboratory Attendant
Gembinsky, Keke	Laboratory Attendant
Lapiz, Edgardo	Laboratory Attendant
Sto. Tomas, Ulysses	Laboratory Attendant
Massinger, Barbara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Plant Pests

Bactrocera *spp.*, Anastrepha *spp. and* Ceratitis *spp. in culture at the IPCL*

More than 50 populations of *Bactrocera*, *Ceratitis* and *Anastrepha* species are being maintained at the ICPL to conduct studies in collaboration with external research institutions or with the other research groups within the IPCL. Experiments revolved around solving cryptic species issues, post-harvest treatments, cytogenetics, and competitiveness studies of strains artificially infected with the reproductive symbiont *Wolbachia*. In addition, the colonies provided reference biological material for taxonomy training courses.

Phytosanitary treatments under the FAO/IAEA/USDA agreement

Work continued under an FAO/IAEA/USDA agreement entitled: "*Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies*". The rationale of this project is that there are a number of important Tephritid fruit flies that pose a high-level threat of entry into uninfested Member States, but approved quarantine post-harvest treatments are lacking for these species and their hosts. The main thrusts are determination if cold treatments can be made generic for fruit species and cultivars and populations within fruit flies species (Fig. 1). So far it looks promising that cold treatments can be made more generic than they currently are.

One of the key issues is purported differences among populations of *Ceratitis capitata* suggested from the published literature between key countries, such as Australia and Argentina. Field populations of *C. capitata* were obtained from Australia and Argentina, and comparative research has been initiated. This research is internationally crucial because if significant differences truly exist among populations of the same species it will mean that treatments for the International Plant Protection Convention's International Standard on Phytosanitary Measures #28, *Phytosanitary treatments for regulated pests*, will have to be country-specific, jeopardizing the entire effort in developing international treatments.

FIG. 1: (left) Ms Elena Cancio Martinez preparing a cage with fruit flies to infest fruits, and (right) placing infested fruit in a "Thermotron" for cold treatment experiments

The FAO/IAEA Coordinated Research Project on Generic Phytosanitary Irradiation Doses has been finalised and results are being published in a special issue of the Florida Entomologist. Main results are that generic doses can be proposed for the following groups: *Anastrepha* spp. (70 Gy), mealybugs (250 Gy), weevils (150 Gy), Lepidoptera larvae (250 Gy), Lepidoptera pupae (400 Gy), mites (400 Gy), insects except pupae and adult Lepidoptera (300 Gy). These results will greatly enhance application of phytosanitary irradiation.

Pheromone extraction of Anastrepha fraterculus

In collaboration with visiting scientists from Argentina, field cages experiments were carried out to assess and confirm the attraction of female *Anastrepha fraterculus* to pheromones of males and explore whether the various morphotypes/populations that have the same timing of mating activity but exhibit some degree of sexual isolation were as attractive to females of their own morphotype/population than to females from other morphotype/populations. The assessment of female fly responses to artificial leks containing pheromone-calling males was done with morphotypes from Argentina and Brazil. Results are in the process of being analysed.

FIG 2: Ms Maša Brinovec maintaining a colony of Drosophila suzukii

Drosophila suzukii

Drosophila suzukii, commonly known as the spotted-wing drosophila, is a vinegar fly—closely related to *Drosophila melanogaster*. Native to Southeast Asia, *D. suzukii* recently was reported in North America in 2008 and is now widespread all over Canada, USA and Mexico. The pest has also been found in Europe, including Belgium, France, Italy and Spain. This pest is a major concern for the

soft fruit production sector. The IPCL has maintained a colony of this pest as part of a research project to assess the possibility of integrating the sterile insect technique (SIT) into the control of this pest in cultivars that grow in confined conditions (e.g. greenhouses). The first phase of the research project is to develop mass-rearing technology and quality control protocols (e.g. develop larval diets and conduct reproductive sterilization radiation dose response studies and related competitiveness assays) (Fig. 2).

Use of symbiotic bacteria to reduce mass-rearing costs and increase mating success in selected fruit pests in support of SIT application

One of the bacterial species (*Enterobacter* sp.) isolated from the Mediterranean fruit fly by the Genetics and Molecular Biology group was selected for studies to assess the possibility of using bacteria as an alternate source of protein or as a probiotic in the larval and adult diets. First assessments have shown that using *Enterobacter* sp. as a probiotic resulted in an increment of the rearing efficiency from egg to pupae and a reduction of the larval development time. More research is ongoing to verify if similar benefits can be found when the Mediterranean fruit fly colonies are up-scaled to mass-rearing level. In addition, research is ongoing to verify if *Enterobacter* sp. can replace brewer's yeast as a source of protein. This research comprises large scale cultivation/production of the bacteria to get sufficient volume to set up the respective evaluation to verify if the bacteria can provide nourishment to the larvae and adults of various fruit flies.

Livestock Pests

Tsetse pupal sorting

The need for a more effective way to separate the sexes of tsetse flies remains a significant impediment to expanding the use of the SIT for the control of tsetse flies. Currently, adult flies must be chilled to immobilize them and then sort them by hand, a slow and laborious process that is not practical for large colonies. For shipping sterile males from a rearing facility to a distant release site, the best technique currently is to use the difference in emergence date between males and females (most females emerge before males) but the separation is not perfect and it requires that the male pupae, which are on the point of emergence, are chilled to prevent emergence during transport. This both increases the complexity of the shipping arrangements and also impacts on the quality of the shipped males.

FIG 3: Ms Zelda Moran sorting pupae of Glossina palpalis gambiensis *using the new near infrared tsetse pupal sorter*

The ideal system would permit the separation of the sexes in the pupal stage several days before emergence, so that there is time to handle and ship the pupae before emergence without the need of chilling. Several years ago, researchers in the USA showed that a near infrared (NIR) grain sorting machine also detected the presence of insects in the grains and subsequent work showed that NIR can be used to distinguish the sex of tsetse pupae about 5 days before emergence. The IPCL obtained one of these machines for testing, but it proved to be very

slow and unreliable, achieving sorting one day and failing the next for no apparent reason. It was also very expensive and is now no longer available so an alternative system was sought.

A new system was obtained from the USA in 2014. The new system uses NIR LEDs to define the viewing wavelength, which provide much brighter illumination allowing the system to capture the data much faster and making the system more robust, reliable and much cheaper. Preliminary tests show that the system is capable of separating *Glossina palpalis gambiensis* pupae with about 95% accuracy at 6-7 days before emergence, at a sort rate of 10-20 pupae per second. The process of refining the system in cooperation with the manufacturers is continuing (Fig. 3).

IR microscopy

The success of the NIR systems with sorting pupae lead us to ask what character exactly the system detects to produce the sorting. The standard idea from previous work was that the dominant wavelengths in the calibration were in the region of the absorption of hydroxyl bonds, and that therefore the systems were probably detecting carbohydrate reserves. However, in order to get more information we set up an infrared microscopy system, using IR LEDs of various wavelengths from 860 to 1060 nm and a simple microscope eyepiece camera with the IR filter removed. This showed us that the puparium integument, which is dark brown to black in the visible spectrum, is partially transparent in the infrared. Using this system we were able to observe the development of the true pupa inside the puparium. Tests with other cycloraphan diptera (Tephritidae and *Musca domestica*) showed that the puparium in these species is also transparent in the near infrared.

This new information will lead to a better understanding of pupal development in tsetse and hence to better methods for sex separation.

X-ray dosimetry

In the past decade, X-ray sources have been considered as an alternative to isotopic irradiators because of the transport problems and security issues of isotopic irradiators. However, the calibration of X-ray sources is considerably more difficult than that of isotopic sources as X-ray generators produce a complete spectrum of photons from zero to the maximum energy of the machine, whereas isotopic sources produce essentially mono-energetic photons (gamma rays). The absorption of mono-energetic photons is well known, but the absorption of X-rays depends on the energy so in each configuration the initial spectrum will be modified in different ways depending on the absorption material, producing a complex and poorly defined spectrum.

Gamma ray dose rates can be measured using transfer standard dosimeters through services offered by many national standards laboratories. For X-rays, no transfer standard dosimeters have been calibrated in the energy range required, leaving only ion chambers and calorimeters for measuring doses. Calorimeters are impractical at the doses needed for insect sterilization and ion chambers are complex to operate and calibrate. Therefore, in conjunction with the standards laboratory in Brazil we developed a calibration for alanine EPR dosimeters in the X-ray field of a Rad Source RS2400 machine. Alanine is a good dosimeter for use as a transfer standard dosimeter and is frequently used for gamma dosimetry. Based on this calibration,

a dose measurement service will be developed and offered to facilities using X-rays during 2015.

Elimination of salivary gland hypertrophy virus infection from the Glossina pallidipes *colony at the Kality facility in Ethiopia*

As reported previously, some tsetse species carry a virus that in a certain proportion of individuals leads to salivary gland hypertrophy (SGH) and these individuals show reproductive abnormalities. In natural tsetse populations the SGH prevalence is in general low (0.5-5%). In a colony of *Glossina pallidipes* originating from Uganda and maintained at the IPCL, the frequency of SGH ranged from 4 to 10%. However, PCR analysis confirmed that the virus was widely distributed in laboratory colony flies. The virus was also detected in samples of *G. pallidipes* from a colony maintained at the Kality facility in Ethiopia with an SGH prevalence up to 77%. This high prevalence was associated with the poor performance of the colony.

During the last decade research was conducted to develop virus management strategies to control and/or eliminate the virus infection from the *G. pallidipes* colony in the Kality facility in Ethiopia. The management strategy relies on using the combination of a clean feeding system (each fly cage is offered fresh uncontaminated blood) and treatment of the blood meal with the antiviral drug valacyclovir. This management system resulted in the elimination of the salivary gland hypertrophy symptom from the infected colony after two years of implementation. Continuing solely thereafter with the clean feeding system resulted in the elimination of the virus infection as demonstrated with the absence of the virus infection when tested with PCR at three years post implementation.

Breaking the tsetse hybrid sterility barrier by the suppression of tsetse associated bacteria

It is well known that some tsetse subspecies (e.g. *Glossina morsitans morsitans* (Gmm) and *Glossina m. centralis* (Gmc)) can successfully mate with each other but they do not produce any offspring or, if offspring is produced, these are sterile. The tsetse-associated endosymbiont *Wolbachia* causes cytoplasmic incompatibility (CI) that leads to embryonic lethality, which might be the reason behind the sterility of the F_1 resulting from interspecific crosses. To explore the impact of *Wolbachia* or any other tsetse associated bacteria in interspecies hybrid sterility we started a series of interspecies crosses using Gmm and Gmc treated with tetracycline (20µg/ml). The Gmc males that were offered six blood meals that had been treated with tetracycline before mating with untreated Gmm females produced a larger number of F1, F2 and F3 offspring as compared with Gmc males that were only offered three blood meals that were supplemented with tetracycline. Further descendants from the Gmc males (fed six times on tetracycline supplemented blood) that had been crossed with Gmm females (fed with untreated blood) showed good performance in subsequent generations (up to F10). This Gmm X Gmc hybrid colony is now well established at the IPCL.

FIG 4. Ms Erica Ras conducting mating compatibility studies in a field cage with hybrids resulting from the cross Glossina morsitans morsitans *x* G. m. centralis

The mating compatibility of the hybrid flies derived from this colony was tested against Gmm and Gmc flies and the result indicate no mating isolation of these flies (Fig. 4).

Analysis of the impact of irradiation treatment on tsetse microbiota

The SIT has proven to be an effective control tactic for tsetse that is based on the mass-production of the targeted species, the sterilization by irradiation and the sequential release of (ideally only) sterile males. There is however an inherent risk that releases of sterile tsetse males might increase the disease incidence before achieving eradication in endemic areas. Therefore, sterile tsetse males are fed blood with trypanocides before release. Nevertheless, the development of tsetse strains refractory to trypanosome infection would be ideal for SIT programs. One approach currently under consideration is to modify *Sodalis* to produce anti-trypanosome factor(s) in the released sterile males through a paratransgenesis approach. In such cases knowing the impact of irradiation on the establishment of modified *Sodalis* in the flies would be crucial to the successful implementation of this combined approach. Therefore, we investigated the impact of irradiation on the establishment of *Sodalis* in the tsetse fly *G. m. morsitans*. The results indicate that irradiating 5-7 day old male flies with 110 Gy does not increase the mutation rate, as assessed in 15 genes, but it does have a negative impact on the replication rate of *Sodalis* and the salivary gland hypertrophy virus. In contrast the irradiation treatment increased the prevalence of *Wolbachia*. Further investigation to characterize the impact of irradiation on tsetse microbiota is in progress.

Human Disease Vectors

Research in the Human Disease Vectors (HDV) group of the IPCL has expanded from three to four main mosquito vectors of human diseases, i.e. *Anopheles arabiensis* and *An. gambiae*, vectors of malaria, as well as *Aedes albopictus* and now *Ae. aegypti*, both vectors of dengue and chikungunya.

Optimising and standardising mass-rearing of mosquitoes

Efforts to develop standardised rearing protocols for *An. arabiensis* have shown significant progress, using the mass rearing equipment designed and produced at the IPCL as previously reported. A daily larval feeding regime has been developed, which synchronises pupation more successfully and makes it easier to handle the larvae remaining after the initial pupal

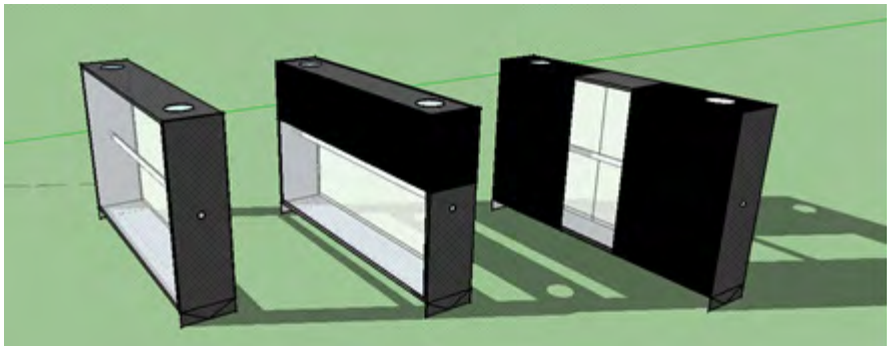

FIG 5: 3D model of the standard mass-rearing cage without the external resting site shrouding (left), the standard resting site configuration used for the experimentation (middle), and the vertical resting site shrouds (right). A low horizontal 'horizon' (middle) was determined to be the optimum for encouraging mating and maximising egg production

collection, hence reducing the workload. The timing of blood feeding and egg collection has been thoroughly investigated, and a new regime that reliably produces high numbers of eggs with the minimum necessary input of manpower has been trialled and seems to be working well. Tests have also been done to determine the optimal number of larvae per tray and adults per cage to maximise production, along with different configurations of 'artificial horizon' to encourage mating within cages (Fig. 5). Through all of these experiments detailed data have been collected over many generations on the productivity of the colony, which gives a clear idea of the level of male production possible using the techniques that have been developed. All of these data and methodological improvements have been used to draft a "Guidelines for standardised mass rearing of *Anopheles* mosquitoes", which will shortly be available to Member States. Similar work is now being undertaken with the two *Aedes* species in preparation of analogous guidelines, including experiments into egg quantification, storage and hatching.

Combining irradiation and Wolbachia *infections for population control of* Aedes albopictus

The mosquito SIT and incompatible insect technique (IIT) applications, which are based on the induction of male sterility through irradiation and *Wolbachia* infection, respectively, highly depend on the availability of a perfect sexing system because the release of females may result in the transmission of human pathogens and may also affect the efficacy of the applications. In addition, for the IIT-based applications, the accidental release of fertile transinfected females may result in population replacement, rather than population suppression. However, robust and efficient sex separation methods are not yet available for mosquito species, including *Ae. albopictus*. The IPCL and collaborators (University of Illinois, USA and University of Guangzhou, China) have therefore investigated whether the SIT can be combined with IIT. By applying low irradiation doses, females could be fully sterilized while both irradiation and *Wolbachia* infection would contribute to the complete sterility of females. In addition, it has been shown that *Wolbachia* transinfection provides some level of protection against dengue in females. Thus, the application of low dose irradiation would sterilize the females while the released males would be fully sterile due to the effects of both *Wolbachia* and irradiation. Even if females were to be accidentally released, these would be sterile and at the same time unable to transmit dengue. This combined strategy has been developed and is being evaluated at the IPCL and is currently being considered for the population control of *Ae. albopictus*, a primary dengue vector in mainland China.

Genetics and Molecular Biology

Cryopreservation of the insect lines

Following recommendations of an external review committee, the Genetics and Molecular Biology (GMB) group initiated efforts to assess the possible cryopreservation of the large number of insect strains maintained in the GMB group in order to preserve their genetic properties and stability and at the same time to potentially reduce the costs of continuous strain maintenance. In collaboration with USDA (Arun Rajamohan), we developed the cryopreservation for the *Ceratitis capitata* (medfly) genetic sexing strain VIENNA 8, which is the strain currently used in almost all medfly mass rearing facilities and medfly SIT operational programmes throughout the world. Two cryopreserved lines were established and quality control (QC) analysis after their revival showed that both lines maintained their key

characteristics, including (a) stability of the temperature sensitive lethal gene (*tsl*) which is responsible for female killing at early embryonic stages at high temperatures, (b) stability of the 'sex dimorphism' character of the strain, as evident from the pupae colour dimorphism (brown for males and white for females) and (c) acceptable recovery rates, as measured by the percentage of egg to adult recovery. It is worth noting that the results of the QC analysis were comparable with the VIENNA-8 strain originally developed and kept in the IPCL for many generations. A cost-benefit analysis is in progress in order to conclude whether this approach is beneficial and economically sustainable compared to continuous strain maintenance.

Evaluation of VIENNA-7 and VIENNA-8 Ceratitis capitata *lines from mass-rearing facilities around the world*

The *Ceratitis capitata* VIENNA-7 and VIENNA-8 lines currently used in mass-rearing facilities for SIT purposes around the world were collected and reared at the IPCL as separate lines to evaluate their properties and stability under the same laboratory conditions. Regarding the main properties of these genetic sexing strains, all lines exhibited the expected *tsl* character and the 'sex dimorphism' stability, although few escapers were observed in some of the lines. Interestingly, although these lines share a common origin, they show substantial differences in recovery rates both at standard and higher temperatures. The possible factors for this phenomenon, which might affect the quality of the lines, are currently under investigation.

Species identification in tsetse and fruit flies

Precise species identification is critical for both SIT applications and implementation of quarantine policies. Limits among species are sometimes unclear, especially in cases of complexes of species. Moreover, there are different factors that can lead to speciation; therefore efforts to resolve species limits must be multi-disciplinary. Research currently ongoing at the IPCL and/or in collaboration with different research groups focuses on developing tools that can contribute to this important area of major significance for SIT application and, in the case of fruit flies, for international trade of agricultural commodities.

Tsetse flies

Different molecular tools are being evaluated against laboratory colonies and field collections to provide quick, robust, easily and massively applicable and cheap (if possible) markers for species identification. Among the available tools, focus has been placed on (a) sequencing of different mitochondrial genes, (b) sequencing or electrophoresis of amplicons of the ITS1 nuclear region, (c) agarose gel electrophoresis or fragment analysis of available microsatellite markers and (d) genotyping of reproductive symbionts present, such as *Wolbachia*. Currently available results indicate that simple agarose gel electrophoresis of some markers, coupled with some limited sequencing in some cases, can deliver a 'toolkit' for accurate identification of a number of tsetse species.

Fruit flies

Much effort is placed on resolving limits of species within species complexes, such as the Bactrocera dorsalis, the Anastrepha fraterculus and the Ceratitis FAR complex by (a) genetic and cytogenetic analysis, (b) symbiont analysis and (c) molecular diagnostics. During 2014, emphasis was given to five economically important members of the B. dorsalis complex (B.

dorsalis s.s., B. papayae, B. philippinensis, B. invadens and B. carambolae) currently maintained at the IPCL. With respect to the genetic and cytogenetic analysis of these taxa, it is known that chromosomal rearrangements are considered very important in the speciation process of Diptera species; however, no diagnostic chromosomal differences were found among them (Fig. 6). With regards to the symbiont analysis, it is known that reproductive symbionts, such as Wolbachia, have been implicated into pre- and post-mating isolation phenomena. Screening populations of B. dorsalis s.s., B. papayae, B. philippinensis, B. invadens and B. carambolae did not reveal any evidence for the presence of reproductive symbionts, such as Wolbachia, Rickettsia, Cardinium, Spiroplasma and Arsenophonus in these taxa. These results together with much additional evidence from collaborators contributed to the recent synonymization of B. papayae, B. philippinensis, B. invadens to B. dorsalis s.s. By employing genetic and cytogenetic, symbiont and molecular diagnostics tools, we are currently expanding this research line to additional members of the B. dorsalis species complex, as well as to the A. fraterculus and the Ceratitis FAR species complexes.

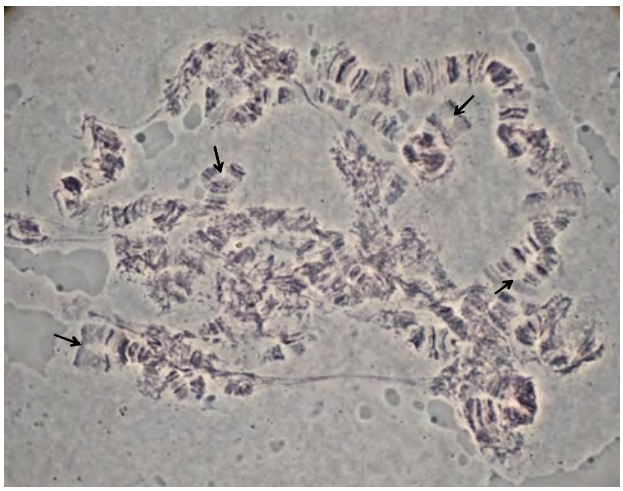

FIG. 6: Cytogenetic analysis of F1 hybrids among two distantly related member of the B. dorsalis *complex. Note the characteristic asynapses of the homogous chromosomes indicated by arrows*

CAPACITY BUILDING AND SERVICES

In 2014, the IPCL hosted five cost-free experts (CFE), eight consultants (C), thirteen interns, twelve fellows and three scientific visitors (SV) (the latter two categories funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
AVGOUSTINOS, Antonios	Greece	C	12 mth	Endosymbionts and their effect on the productivity and competitiveness of insects
KYRITSIS, Georgios	Greece	C	12 mth	
MORAN, Zelda	USA	Intern	6 mth	Rearing of tsetse
RAS, Erica	Netherlands	Intern	9 mth	
KALANTAROW, Inessa	Israel	Intern	3 mth	
HALLMAN, Guy	USA	CFE	2 mth	Post-harvest treatment of fruit flies
DEMIRBAS, Guler Uzel	Turkey	C	8 mth	Developing management strategies for the tsetse virus in support of tsetse eradication projects
MEKI, Irene	Kenya	Intern	9 mth	
INCE, Ikbal Agah	Turkey	C	6 mth	

Name	Country	Status	Duration	Topic
MUTIKA, Gratian	Zimbabwe	CFE	6 mth	Handling protocols for tsetse
UL HAQ, Ihsan	Pakistan	CFE	1 mth	Rearing of fruit flies
BLOUCH, Ammara	Pakistan	Intern	6 mth	
CONTE, Claudia	Argentina	C	6 mth	
YU, Dalin	China	Intern	8 mth	Data management
WEILER, Dorrotya	Slovenia	Intern	2 mth	
CULBERT, Nicole	UK	Intern	1 mth	
ZHANG, Dongjing	China	CFE	6 mth	
ZHENG, Minlin	China	CFE	6 mth	
LEES, Rosemary	UK	C	1 mth	
MAIGA, Hamidou	Burkina Faso	C	8 mth	Developing mass-rearing techniques and the SIT package for disease transmitting mosquitoes
DAMIENS, David	France	C	11 mth	
BIMBILE, Severin	Burkina Faso	Intern	4 mth	
COMBEAU, Robin	France	Intern	4 mth	
NANVUMA, Cynthia	Uganda	Intern	12 mth	
BRUNET, Robin	UK	Intern	1 mth	
IZABAL NOGUEDA, Daniela	Mexico	Intern	4 mth	
ALYAS, Tahani Bashir Abelkareim.	Sudan	Fellow	3 mth	Mosquitoes
WOOD, Oliver	South Africa	Fellow	6 mth	
ALLECK, Malini	Mauritius	Fellow	10 d	
BAKIR, Sevgi	Turkey	Fellow	10 d	
YANG, Jianquan	China	Fellow	1 mth	
SASMITA, Hadian Iman	Indonesia	Fellow	1 mth	
OLANRATMANEE, Phanthip	Thailand	Fellow	1 mth	
OUEDRAOGO SANON, Gisele	Burkina Faso	Fellow	3 mth	Tsetse
MUBVUTA, Lloyd	Zimbabwe	Fellow	10 d	
DESALEGN TAMERE, Tazezew	Ethiopia	Fellow	4 d	
BERIHU GIRMAY, Alem	Ethiopia	Fellow	4 d	
TRAORE, Astan	Mali	Fellow	2.5 mth	
SHERENI, William	Zimbabwe	SV	1 wk	
CHAMISA, Andrew	Zimbabwe	SV	1 wk	
GAZIT, Yoav	Israel	SV	1 wk	Fruit flies

In 2014, the Plant Pests group delivered 82 shipments of fruit fly eggs, pupae, dead samples or other biological material to 53 institutes in 23 countries. The Livestock Pests group had 49 consignments shipped to 13 institutes in ten countries. The Human Disease Vector group shipped biological material to 15 institutes in 14 countries, whereas the Genetics and Molecular Biology group had nine consignments to seven institutes in five countries (Fig. 7).

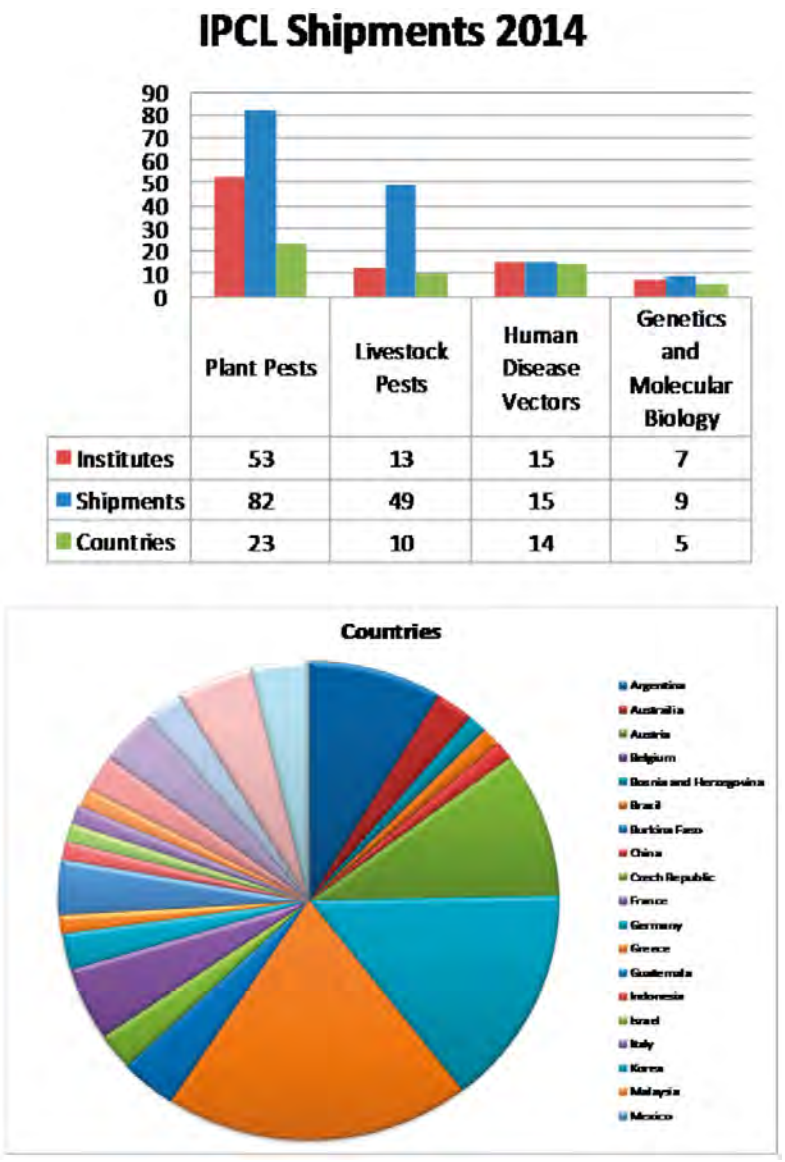

FIG 7: Shipments of the IPCL in 2014 to different countries as a proportion of total shipments (lower); shipments of the different research groups of the IPCL (upper).

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The Plant Breeding and Genetics Laboratory

EXECUTIVE SUMMARY

The Plant Breeding and Genetics Laboratory (PBGL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture focusses on mutation breeding to increase biodiversity for desired traits of crop plants and hence to accelerate the breeding of varieties with higher yield, yield stability, nutrition and improved resistance to environmental stresses such as disease, drought and salinity. It plays a key role in the implementation of the Plant Breeding and Genetics subprogramme and provides assistance to Member States in the fields of mutation induction, mutation screening and mutation discovery.

As regards conventional irradiation techniques for mutation induction it is becoming increasingly important to establish alternatives to gamma irradiation. This is mainly due to restrictions imposed on the transfer of radioactive materials and difficulties in establishing new gamma irradiators in Member States, or refitting old ones. Mutation induction using X-rays is one such possible alternative and PBGL is currently developing transferable and standardized protocols for X-ray irradiation in order to promote the wide use of this technology. Initial results of our comparative radiosensitivity experiments indicate that the biological effect of X-ray irradiation of seeds is proportional to the dose rate.

The use of ion beams is a further possible alternative, increasing evidence suggesting that ion beams not only efficiently induce mutations, but also induce different types of mutations compared to gamma rays or chemical mutagens. The ion beams appear to preferentially induce deletions, the size of these deletions greatly depending on the linear energy transfer (LET). The proper dose range for ion beams has yet to be sufficiently clarified though ion beams are expected to induce mutations in the low dose ranges that do not greatly affect plant survival. Thus, practical research on the optimization of ion beam irradiation was initiated and carried out by PBGL in collaboration with the IAEA's Nuclear Science and Instrumentation Laboratory. So far, similar trends in the radio-sensitivity curves of barley and wheat were observed with ion beam and gamma ray within a specific range of the ion beam current and energy.

After the process of mutation induction, the challenge is to select from large segregating populations those plants that express the new desired character and to further develop these plants into genetically stable lines and finally into new varieties. One way to speed up this process is the use of doubled haploid technology. PBGL has now developed a new protocol on using pollen irradiation for haploid induction in cucurbits (soon to be placed on the PBG website and subsequently to be published).

In 2014 the foundation was laid for the future activities of the PBG subprogramme related to using mutation breeding for developing coffee varieties resistant to the coffee leaf rust disease. Optimum seed irradiation doses were determined as a first step to developing a protocol for mutation breeding in coffee.

Progress was also made in the screening of mutants, be it phenotypically to select for a certain trait, e.g. drought tolerance, or on the DNA level. Plant mutation breeding works

because mutagenesis creates changes in the DNA sequence of plants that affect the plants' characteristics (phenotype) and are stably heritable and passed from generation to generation. The ability to record and characterize those induced DNA changes accurately is a potentially powerful tool for both breeders and applied researchers.

The PBGL has developed a new kit and protocol (see chapter 14 of http://www-naweb.iaea.org/nafa/pbg/public/2014-Laboratory-Manual-Version-2-4.pdf) to assist researchers in their efforts for molecular characterization of induced and natural mutations. This latest kit is designed for room temperature extraction of single-strand-specific nucleases used in enzymatic mismatch based polymorphism enzymes necessary for mutation discovery assays, such as TILLING and Ecotilling, and for the validation of production of doubled haploid plants. All steps are performed at room temperature using a standard microcentrifuge and non-toxic chemicals. Toxic chemicals and complex dialysis are avoided as compared to previous PBGL protocols. The enzyme extraction kit represents the third in a series of kits designed for molecular characterization of plants that we distribute to Member States upon request. The kits are expected to foster the application of molecular tools for crop improvement in countries that do not have advanced molecular laboratories.

Due to its unique position in combining capacities and competencies in many fields of mutation breeding of various crop types, from induction of mutations to screening techniques and mutation discovery at the molecular level, PBGL continues to be a highly sought after location for training. This was reflected also in the dynamic flow of trainees in 2014, with 18 Fellows from 11 Member States, an increase of 64% compared to 2013. In addition, nine Scientific Visitors from seven Member States and nine Interns from seven Members States were hosted at the PBGL in 2014. The PBGL also contributed to group training courses held in other Member States in supplying irradiated standards to demonstrate radiosensitivity testing and low cost kits for DNA extraction and characterization. The PBGL continues to provide an irradiation service to Member States for plant mutation induction; in 2014 it responded to 45 irradiation requests from 26 Member States, showing the demand for this service to be continuously high. It was particularly interesting to note that not only the 'traditional' crop species were being sent for irradiation, but also new crops are included, such as a herb (sage), an ornamental tree (*Catalpa bignonioides*, the Indian bean tree) and a biofuel crop (*Jatropha curcus*).

In 2014 the first Ug99 resistant mutant wheat varieties were released in Kenya, 'Eldo Ngano1' and 'Eldo Mavuno 1'. The PBGL did the initial seed irradiation and was also involved in individual and group training of participants of the related TC project. In recognition of this input PBGL team members received the 2014 IAEA Superior Achievement Award (One-House Award). Collaborations have been established now with the University of Sydney, Australia; the Agriculture and Agri-Food Canada, Manitoba, Canada; and The Genome Analysis Centre and The Sainsbury Laboratory in Norwich in order to identify the mutations that confer the resistance.

The PBGL underwent a change in management during 2014. Brian Forster, who started as Laboratory Head in September 2011, left the Agency in August 2014 to take up new challenges in Indonesia. Since mid-August Stephan Nielen is the Acting Laboratory Head. Mr Nielen has worked three years at PBGL during his first tenure at the IAEA (1997-2004) and returned in 2011 as Technical Officer in the Plant Breeding and Genetics Section.

STAFF

Name	Title
Forster, Brian Peter	Laboratory Head (until 1 August 2014)
Nielen, Stephan	Acting Laboratory Head (from 18 August 2014)
Till, Bradley John	Plant Breeder/Geneticist
Ghanim, Abdelbagi Mukhtar Ali	Plant Breeder
Matijevic, Mirta	Technician
Jankowiak-Cieslak, Joanna Beata	Technician
Berthold, Guenter	Field/Greenhouse Worker
Draganitsch, Andreas	Technician
Bado, Souleymane	Technician
Huynh, Owen Anthony	Technician (until 1 December 2014)
Lorenz, Anne	Implementation Assistant
Mletzko, Joanna Malgozata	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Plant Breeding and Genetics Laboratory (PBGL) continues to provide support to the Plant Breeding and Genetics (PBG) subprogramme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture through its three main activities: 1) research and development (R&D) in the field of plant mutation induction and mutation screening, 2) capacity building (group and individual training) and 3) technical services (primarily irradiation requests). The efforts of the PBGL in these area can be classified into three broad categories: 1) development and adaptation of methods for the rapid and accurate discovery of novel induced mutations, 2) use of discovery technologies to monitor and optimize the mutation induction process and 3) adaptation of basic molecular methodologies so that they are low-cost, reduced in toxicity and suitable for laboratories in developing countries.

Mutation induction

Optimization of ion beam irradiation as an efficient alternative to gamma rays for mutation induction in crop plants

Heavy ion beams provide an alternative option for efficient mutation induction. Ion beams (proton, helium and the heavier charged particles) deposit high energy locally compared to gamma rays and X-rays. The biological effect of ion beams shows a higher rate of growth inhibition, lethality and survival rate.

Research has been jointly initiated with the IAEA's Nuclear Science and Instrumentation Laboratory (NSIL) to optimize mutation induction protocols using the ion beam facility at the Ruder Boskovic Institute in Zagreb, Croatia. An aluminium platform was constructed by NSIL

to mount seeds of different crops on carbon tape for irradiation treatment (Fig. 1, A and B). Radiosensitivity tests of wheat, barley, sesame and tomato seeds were conducted with proton beam and the results were compared to conventional gamma- and X-ray methods. Different combinations of energy, current and exposure times were used to optimize the dose rate for the different crops. Similar trends in radio-sensitivity of barley and wheat were observed between ion beam and gamma ray when the ion beam current and energy specification were between 70–480pA and 7MeV proton, respectively (Fig. 1, C and D). For barley seeds GR_{50} and GR_{30} were 88 Gy and 145 Gy (Gamma rays) and 61.5 pA and 133.7 pA (ion beam), respectively, while for wheat GR_{50} and GR_{30} were 506 Gy and 653 Gy and 265.5 pA and 360 pA, respectively. The optimization process will be expanded to other crops and mutation rates will be scored in subsequent generations. The results will serve as guidelines for effective use of ion beam irradiation and published together with a series of protocols (initially on the PBG website starting in 2015).

Mutation induction using X-ray irradiation

The availability of gamma sources for mutation induction is limited and new installations or refurbishing of old irradiators is becoming difficult due to restrictions imposed on the shipment of radioactive elements. X-ray machines are a good alternative because there are no such restrictions and they have been shown to be efficient in mutation induction. Demand for X-ray irradiation by Member States is expected to increase with the decreasing availability of gamma sources. The PBGL has initiated research to develop protocols for use of X-ray for treatment of seeds and vegetative plant propagules for mutation induction. Established irradiation protocols need to be transferable and standardized, which necessitates the selection of a machine that is stable and can be widely used.

After moisture equilibration of seed samples in a desiccator with 60% glycerol, X-ray mutagenesis was carried out with the in-house RS2400 irradiator (Rad Source Technologies, USA) and the results compared to those of gamma (^{60}Co) rays. In addition, in collaboration with the Arid Land Research Center, Tottori University, Japan, and the University of Ljubljana, Slovenia, the in-house machine was compared with two X-ray machines with rotating-tray platforms from Hitachi and Faxiton, respectively. Representative seed propagated crops were used, including cereals (wheat, barley, sorghum, rice), legumes (mustard, beans, chickpea), oil crops (sesame, sunflower, groundnut), tomato and chilli for the tests using the in-house machine, while a subset of wheat, barley, sorghum, mustard and sesame was tested in the external machines. The irradiated seeds (M1) were planted in both glasshouse and field. Furthermore, *in vitro* material from banana and cassava were treated with the in-house X-ray machine and gamma source. Radiosensitivity was measured as relative reduction in germination and growth rate as compared to non-treated samples. The tested doses were between 0-750 Gy for seeds and 0-60 Gy for *in vitro* materials.

The LD_{50}, LD_{30}, GR_{50} and GR_{30} were determined for each crop and irradiation source. It was noted that despite the similarity in germination and growth rates, a variation exits depending on the sources used. These variations were due to the fact that different sources have different energy levels, thus irradiation is done at different dose rates (measured as Gy/min) and also have different settings (e.g. X-ray machine with or without rotating sample chamber). Therefore, irradiation dosages cannot be easily transferred from one machine to the other without considering the dose rate and the type of machine. It is hence important to

establish proper dosimetry calibration of the radiation facility and to run a radiosensitivity test prior to each mutation breeding experiment in order to determine the optimum dose for mutation induction. Table 1 summarizes the results for two barley varieties irradiated with two X-ray and three gamma sources. The LD_{50} and LD_{30} achieved with the two X-ray machines indicate that the biological effect of X-rays is lower when seeds are irradiated at lower dose rate and vice versa.

Procedures are currently being developed for X-ray irradiation of seed and vegetatively propagated crop plants and will be published in 2015, initially on the PBG website.

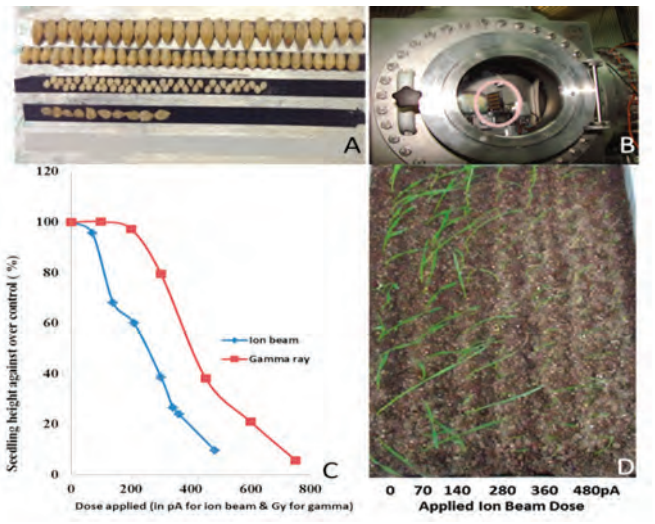


FIG. 1: A) Constructed seed mounting platform with seeds mounted on carbon tape; B) Irradiation chamber with the platform highlighted with circle; C) graph showing comparative response of wheat seeds to different doses of ion beam and gamma ray and D) an image showing radio-sensitivity of wheat seedlings in response to different doses of ion beam irradiation.

Table 1: LD_{50} and LD_{30} of different X-ray and gamma ray irradiators on two barley varieties (Eunova and naked barley) after germination in the field at the PBGL

Irradiators (dose rate)	Eunova		Naked barley	
	LD_{50}	LD_{30}	LD_{50}	LD_{30}
2Gy/min (X_2)	372.7	229.3	321.9	196.6
12Gy/min (X_1)	246.9	136.4	231.6	127.4
8Gy/min (G_1)	265.9	158.1	203.8	93.9
140Gy/min (G_3)	292.0	145.8	234.3	121.3
150Gy/min (G_2)	251.5	144.5	247.8	145.7

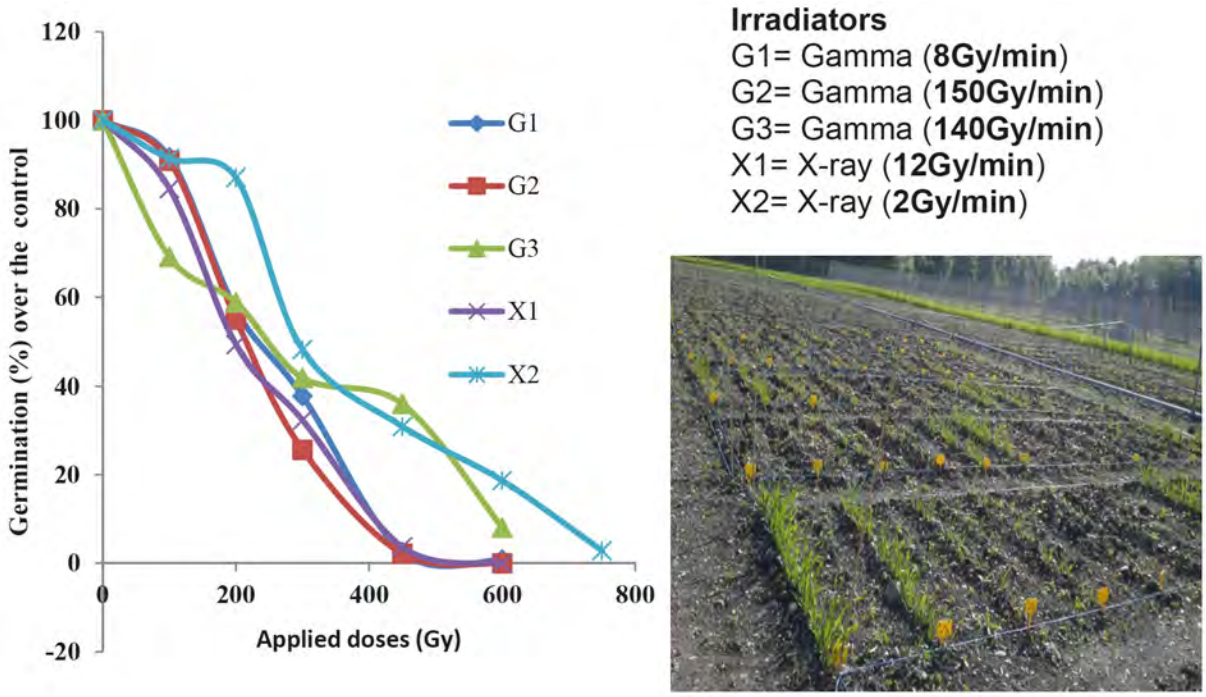


FIG. 2: Radiosensitivity test of barley variety Eunova with two different X-ray (X1 and X2) and three different gamma ray (G1, G2, and G3) irradiators. The photo shows germinating M1 plants in the PBGL experimental field.

Pollen Irradiation for doubled haploid production in cucurbits

Maluszynski *et al.* (2003, Kluwer Academic Publishers, ISBN 1-4020-1544-5) developed protocols for the induction of haploid plants in various plant species using different methods, such as anther/microspore and ovule culture, pollen irradiation and wide hybridization. Haploids are either spontaneously doubled or induced to double through treatment with e.g. colchicine. The doubled haploid technology has been widely used to accelerate breeding programmes, increase efficiency of selection and produce inbred lines for hybrid production. The PBGL is now carrying out adaptive R&D to develop new protocols for doubled haploid production in more crop plants. We have developed a protocol in 2014 (soon to be placed on the PBG website and subsequently to be published) on the use of pollen irradiation for haploid induction in cucurbits. This protocol describes the process from the retrieval of pollen and pollen irradiation to pollinating flowers, collection of fruits, embryo rescue and verification of haploidy by flow cytometry (Fig. 3). Doses of up to 200 Gy were tested in Styrian oil pumpkin, cucumber, and sweet gourd. The number of embryos per 100 seeds and *in vitro* germination of rescued embryos were affected by the dose used for pollen irradiation. An intern from Slovenia and fellows from Bangladesh, Ghana and Sudan assisted in the development and training on this protocol, and now plan to integrate it into the mutation breeding activities in their home countries. The procedures described in this protocol are also being taught at regional training courses organized by the PBG.

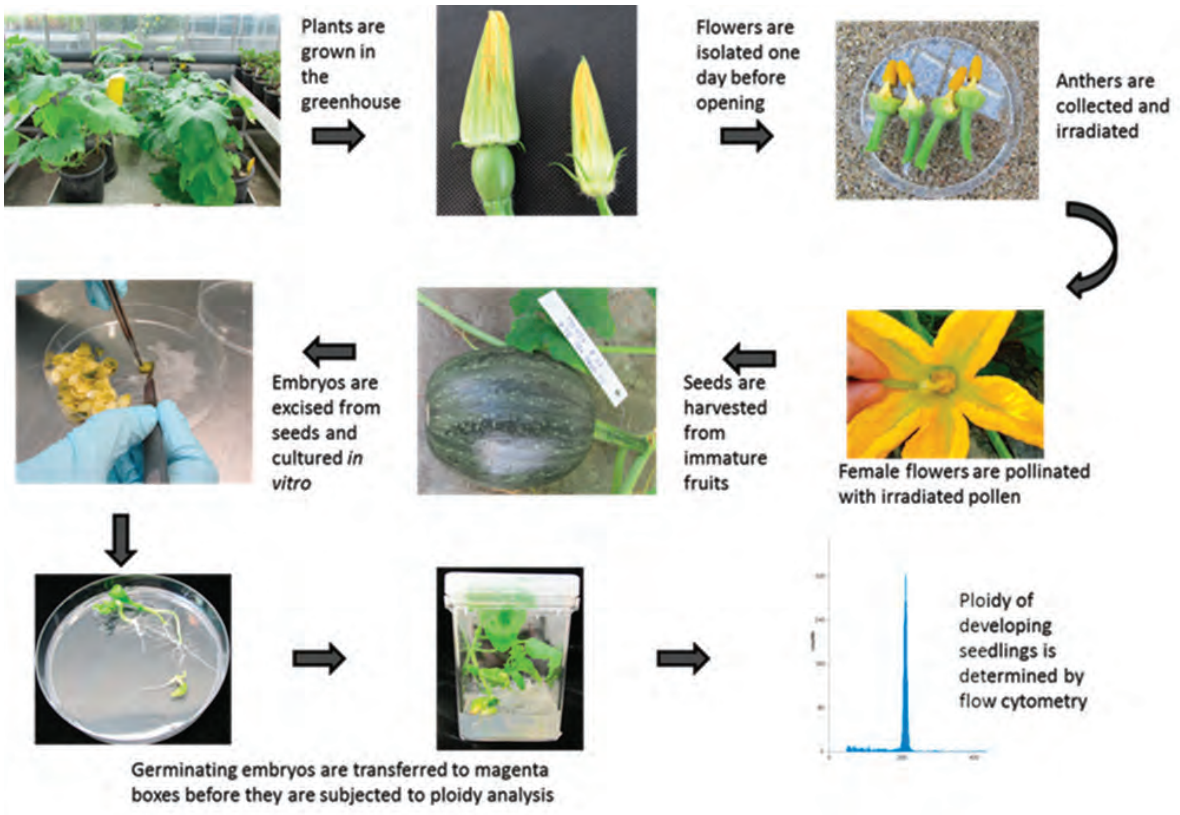


FIG. 3: Production of doubled haploid cucurbits using irradiation

Mutation induction for improvement of Coffee

Coffee is the most traded commodity worldwide after petroleum oil. It is grown in more than 80 countries and is a major source of income. The two main cultivated species are *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta), with 75% and 25% of coffee production, respectively.

Coffee leaf rust disease (*Hemileia vastatrix)*, an endemic disease of coffee, has recently become a major threat to global coffee production and has therefore generated a strong interest in mutation breeding. In addition, poor yield, caffeine content and biennial bearing are other potential targets for coffee improvement. However, while mutation induction has been established for numerous crop plants and trees, it has yet to be explored for coffee improvement. This is rather surprising since important coffee varieties arose through spontaneous mutations, indicating that mutation induction might be a promising technique.

In order to develop protocols for mutation induction in coffee, PBGL performed irradiation tests on seeds of three Arabica coffee varieties - Mundo Novo, Padang and Kona - to assess their radiosensitivity. The in-house gamma source (with a dose rate of 8Gy/min) and the X-ray irradiator RS2400 (Rad Source Technologies, USA) were used. Treatments of 0 to 1000 Gy were applied to investigate the response of seeds to irradiation after moisture equilibration (12-15%). Four replicates (25 seeds) of each variety were evaluated in Petri dishes at 30ºC with a photoperiod of 12 hours and germination rates were recorded (Fig. 4). The LD_{30} and LD_{50} were determined for each variety at 30 days after sowing in order to assess effective

dosages for mutation induction. The results were similar for both types of irradiation and also among the three varieties, with an indication of Padang being slightly more sensitive to radiation (Table 2). This study will now be expanded to vegetative propagules as alternative mutation breeding strategy in consideration of the long bean-to-bean generation time of four years.

Table 2: LD_{30} and LD_{50} values of three coffee varieties irradiated with two sources (Gamma ray and X-ray)

Variety	Gamma ray		X-ray		Relative Biological Effect	
	LD_{30}	LD_{50}	LD_{30}	LD_{50}	LD_{30}	LD_{50}
Mundo Novo	435.3	776.7	379.0	750.1	0.9	1.0
Padang	350.6	721.0	356.5	693.8	1.0	1.0
Kona	395.8	775.3	364.9	724.6	0.9	0.9

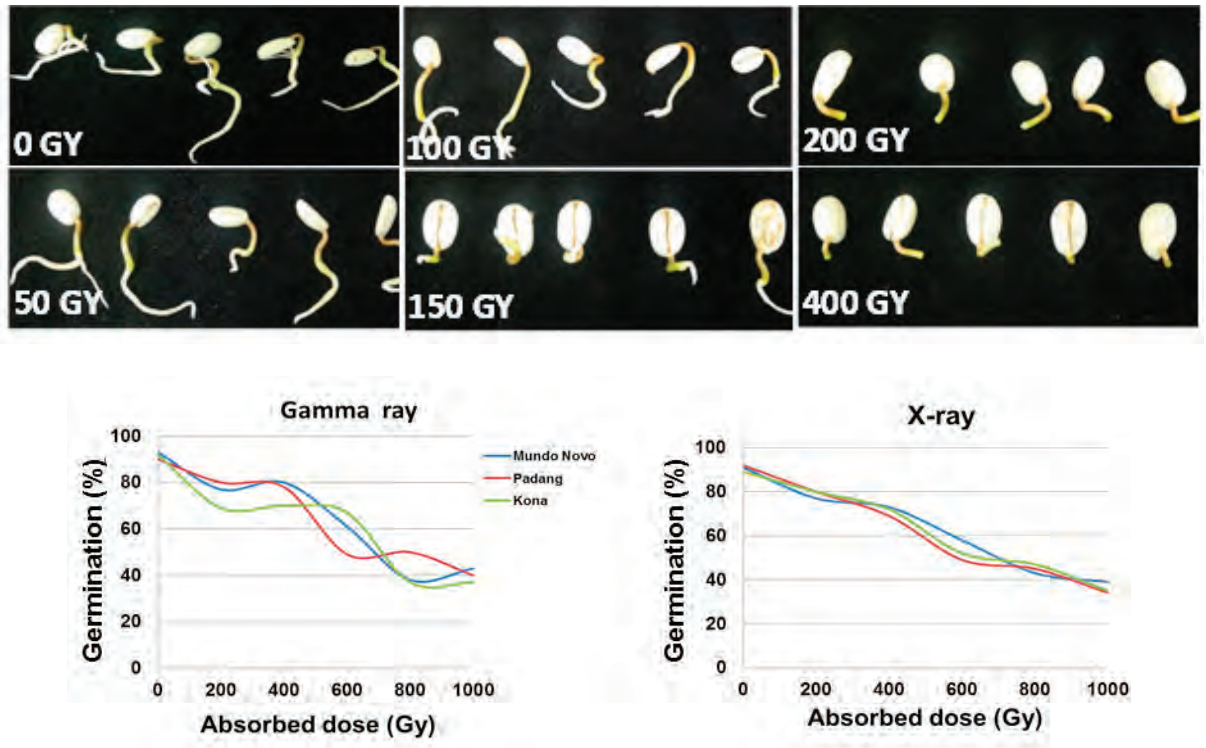


FIG. 4: Germinating M1 seeds treated with 0 to 400Gy of gamma ray (top); graphs comparing effect of different doses of gamma- (bottom left) and X-ray (bottom right) on germination (%).

Mutation Screening and Mutation Discovery

Ideally, mutation screening should be rapid, low-cost and reduce the influence of the environment in what is known as the genotype-environment or GxE interaction. Screening

may involve evaluation of the physical appearance of a plant (its phenotype) or of the changes in the DNA (its genotype).

Pre-field phenotyping of lentil mutants for drought tolerance using polyethylene glycol

Drought is one of the main abiotic stresses that limit plant growth and productivity and challenges global food security. Three varieties and four mutant lines of lentil were used to develop drought screening methods to be used for screening in mutation breeding for drought stress. Four concentrations (0.0%, 10%, 15% and 20%) of polyethylene glycol (PEG-6000) were used to induce plant-water deficit stress in an aerated hydroponic system (Fig. 5). Drought stress was imposed on 14-day old seedlings with frequent changes and replenishment of the solutions every 3-5 days. Plant growth, chlorophyll content, biomass, stay-greenness and harvest index were recorded. Genotypes were classified based on relative performance into drought sensitive, intermediate and tolerant. In the 20% PEG solution BINA 208 had the highest harvest index. As harvest index is directly correlated to stress tolerance BINA 208 was considered the most tolerant genotype. Field evaluation is now planned to validate the PEG screening method in drought prone areas of Bangladesh, the ultimate objective being to develop a technology package for drought screening of crop plants in the laboratory, glasshouse and the field to be used by Member States in their mutation breeding programmes.

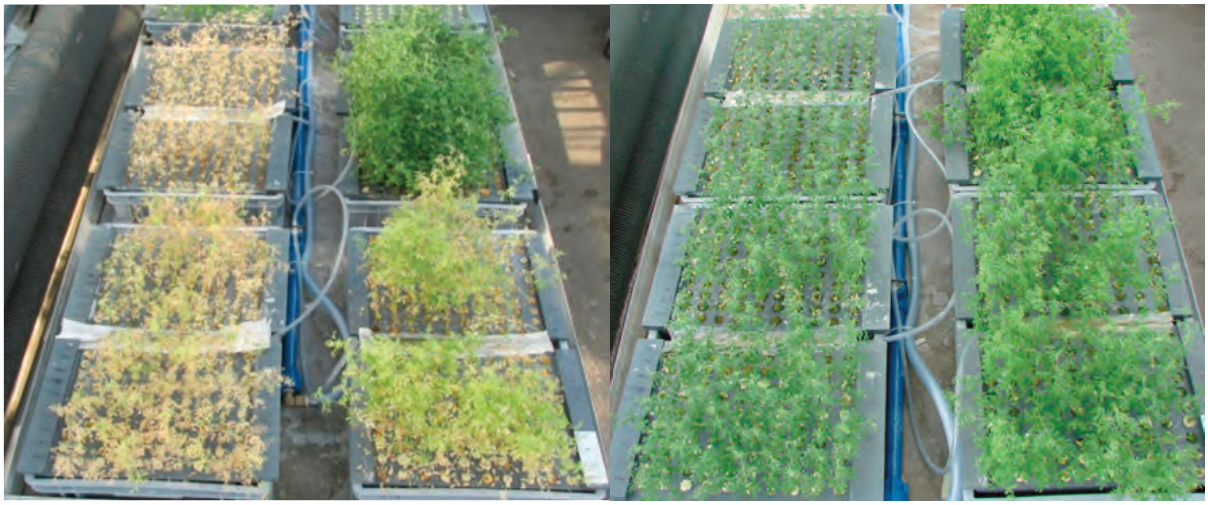


FIG. 5: Lentil genotypes grown in a hydroponic system in Eshida's nutrient solution ten (right) and twenty (left) days after imposition of water stress using PEG-6000 at four treatments (0, 10, 15 and 20%). Note the obvious relative effect on plant biomass and chlorophyll content with increasing concentrations of PEG in the nutrient solution.

Mutation discovery

Natural and induced mutations are effective tools for functional genomics and plant breeding. Methods for rapid discovery and genotyping of mutations are important for a variety of applications including marker development, marker assisted selection, population genetics, and reverse-genetics. Single nucleotide polymorphisms (SNPs) represent the most common type of natural nucleotide variation in plants. Polymorphism discovery techniques commonly used for mutation based reverse genetics (known as TILLING) and the discovery of natural variation (referred to as Ecotilling) use enzymatic mismatch cleavage. This is a powerful approach in that any small mutation (SNP or indel) can be rapidly discovered by treatment of

DNA with a nuclease that cleaves at the site of the polymorphism. Single-strand-specific nucleases have been commonly used in TILLING assays and also for the recovery of natural nucleotide variation (known as Ecotilling) and thousands of nucleotide variants have been discovered. Assay costs can be dramatically reduced through self-extraction of nucleases, but the standard procedure requires large preparatory centrifuges, toxic chemicals and specialized dialysis. We have simplified this procedure so that nuclease can be prepared at room temperature using a bench top microcentrifuge common to most molecular biology laboratories. We have also eliminated the use of toxic chemicals. The whole procedure can be completed in one day. Enzyme for approximately 3000 reactions can be produced from 18 ml of celery juice. The cost of enzyme for one reaction is less than 0.06 euro cents. Enzyme produced by this method is suitable for mutation discovery (Fig. 6). A protocol on "Enzyme extraction for low-cost mutation discovery" is currently being prepared for publication.

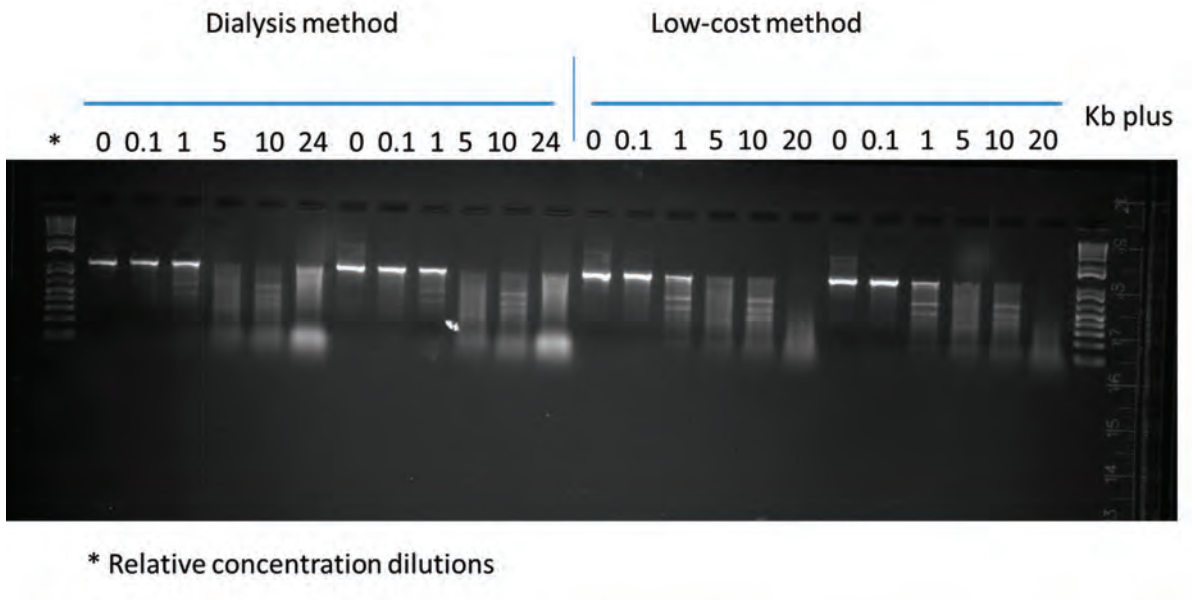


FIG. 6: Enzyme activity tests comparing traditional dialysis based purification (left) and the low-cost method (right). Mutations are easily identified as lower molecular weight cleaved fragments at the 1x concentration.

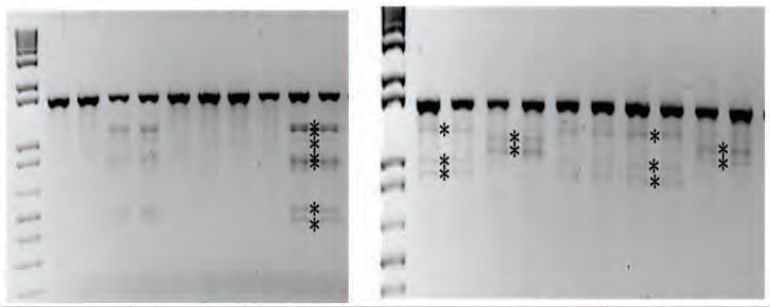
The PBGL has developed a new assay kit based on this protocol (Fig. 7). The kit provides all the materials needed to prepare approximately 3000 reactions worth of enzyme from 18 ml of celery juice. The kit was demonstrated to Member State delegates at the PBG stand during the 2014 IAEA General Conference. The enzyme extraction kit represents the third in a series of kits designed for molecular characterization of plants that the PBGL distributes to researchers upon request.

Molecular methods for screening mutant plants can be especially useful where the application of traditional methods is limited due to difficulties in phenotypic selection or in the method of plant propagation. One such example is the application of mutation breeding to develop citrus resistant to the disease known as greening. Huanglongbing (HLB), or citrus greening, is caused by *Candidatus liberibacter asiaticus* bacteria. The disease affects all citrus growing countries in Asia, except Japan. In the Americas it affects production in Brazil, Florida and parts of Mexico. The bacteria cannot be cultured, thus limiting the ability to do mass

screening on mutant seedlings to select rare mutants with enhanced resistance. Mutation breeding is also complicated by the fact that citrus is grafted and disease resistance alleles may be important in root stock, scion or both. Citrus is an example where reverse-genetics may provide an effective means to breed disease resistance. In this approach mutations in disease resistance genes are targeted and recovered prior to any phenotypic screening. This allows selection of mutations in both root and scion prior to laborious and time-consuming field trials. In 2014 the PBGL began collaborating with the University of Florida to develop the reverse-genetic method known as TILLING for citrus. As a first step we adapted our low-cost mutation assays to evaluate natural genetic variation in breeding material (see Fig. 8) and are now optimizing tissue culture methods for inducing mutations. The presence of putatively deleterious heterozygous polymorphisms in citrus (a phenomenon known as Muller's ratchet) is similar to what the PBGL previously observed in bananas. This is an important finding as phenotypes from otherwise recessive mutations may be observable prior to sexual fertilization or doubled haploidy due to the fact that many loci may be functionally haploid.

FIG. 7: The new enzyme extraction kit from the PBGL comes with a detailed protocol and all materials necessary to prepare 3000 reactions worth of nuclease for mutation discovery from 18mls of starting plant juice extract.

Another example of collaborative research is the optimization of mutation breeding methods for grass pea. Grass pea, *Lathyrus sativus* L., is an important crop of economic significance in many countries of Africa and Asia and serves as a food, feed and fodder. *L. sativus* is resistant to both drought and waterlogging conditions. However, genetic improvements are required for the crop to meet its full potential. For example, humans and animals may develop lathyrism when eating large amounts of the crop due to the



#	View On Sequence	Nucleotide Change	Effect	Restriction Enzyme Differences from REBASE		PSSM Difference	Zygosity
				Gained in Variant	Lost from Reference		
1	G C	A594G	K157E			3.2	Homo
2	G C	A640C	K172T	FnuDII	Bce83I, SmlI	10.1	Hetero
3	G C	T710G	S195R		RsaI, ScaI, TatI		Homo
4	G C	A749G	P208=	BcefI	BccI		Hetero
5	G C	A759G	K212E	Hin4I	MseI		Homo
6	G C	G796A	S224N	Tsp4CI	AluI, CviJI, NspBII, PvuII	-7.1	Homo
7	G C	T857C	I244=	BciVI, Hpy188I	AsuII, TaqI, XmnI		Hetero
8	G C	G896C	M257I	BinI, DpnI, MboI, XhoII	BcefI, SfaNI		Homo
9	G C	G899A	P258=	BccI	BcefI		Homo
10	G C	G1090T	R322M	AflIII, BspLU11I, NlaIII, NspI		2.9	Homo
11	G C	G1224C	V367L	BsmAI, Hpy178III		-10.9	Homo
12	G C	T1289C	N388=	MaeIII			Homo
13	G C	C1415T	D430=				Homo
14	G C	T1563G	F480V		AluI, CviJI, HindIII		Hetero
15	G C	C1731A	Q536K			-2.0	Homo

FIG. 8: Low-cost mutation discovery was adapted for the discovery of natural homozygous and heterozygous polymorphism in different citrus genotypes (top). Protein sequence evaluation (bottom) shows the putative effect of amino acid substitutions.

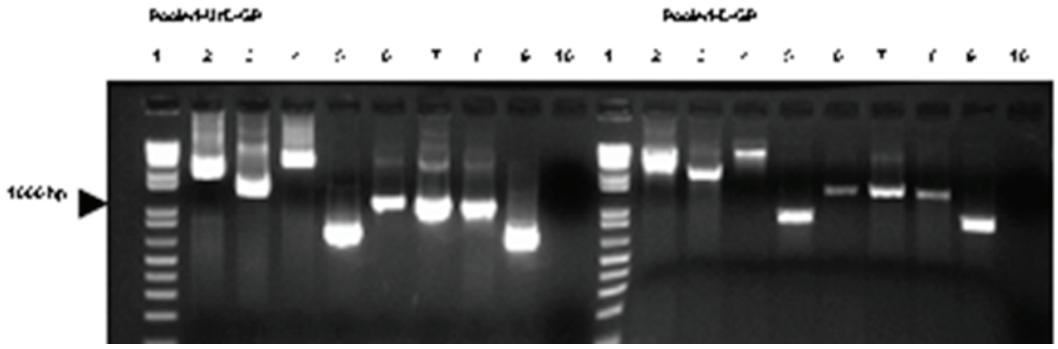


FIG. 9: Agarose gel images of PCR amplification using test primers. Undigested (UnD) and CEL I digested (D) PCR products from wild type pooled grass pea samples visualized on a 1.5% agarose gel.

natural accumulation of the neurotoxin ODAP. The PBGL collaborated with the John Innes Centre, UK, *RevGen*UK and Bench-Bio, India, on grass pea mutation breeding, With the PBGL optimizing gene primer design, low-cost (Fig. 9) and higher throughput mutation discovery methods (Fig. 10) (Sen et al., 2014a). Collaborators are now developing a large mutant population.

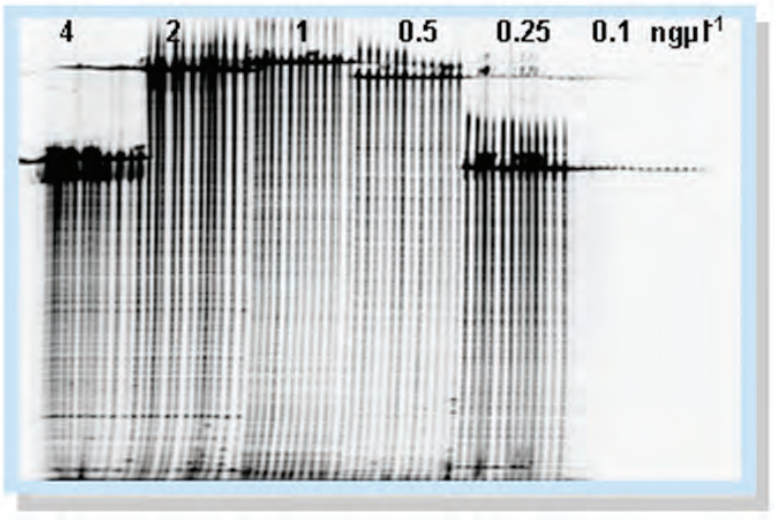


FIG. 10: Higher throughput mutation discovery using fluorescence DNA labelling. Assay optimization was performed by testing different DNA concentrations (labelled on top of image) from pooled genomic DNA samples.

Citrus and grass pea are just two examples of efforts by the PBGL to develop low-cost and non-toxic methods that can be easily adapted to many different crops. By working with research fellows and experts visiting the PBGL we have successfully adapted PBGL protocols for over 20 species (see chapters 2, 13 and 14 of http://www-naweb.iaea.org/nafa/pbg/public/2014-Laboratory-Manual-Version-2-4.pdf). We are currently developing higher throughput mutation discovery methods based on next-generation sequencing. These are especially important for marker development in mutation breeding projects where traditional mapping populations have not been developed.

The technology landscape in the area of plant genomics is rapidly evolving. Of the many tools (both bench and bioinformatics) being developed, only a handful are specifically optimized for discovery of rare induced mutations. Amplicon-based approaches for the recovery of induced point mutations, like those caused by chemical mutagens, are now quite mature and work well across species. Broader spectrum mutagens, such as gamma- and X-ray irradiation, are more challenging. The PBGL is collaborating with the University of California-Davis to develop alternative strategies for the molecular recovery of mutations in gamma irradiated crops. Owing to the fact that plant genomes are quite large, whole genome sequencing approaches remain cost-prohibitive for all but the best funded laboratories. The collaboration, therefore,

seeks to explore methodologies for mutation recovery from reduced representation libraries. For example, the amount of DNA in a plant genome that contains genes encoding proteins (the exome) is relatively constant across different genomes, while the size of the entire genome varies considerably (Fig. 11). The majority of induced mutations important for breeding are potentially in gene sequences. Therefore, large savings can be had by targeting the plant genes (exome) for mutation analysis.

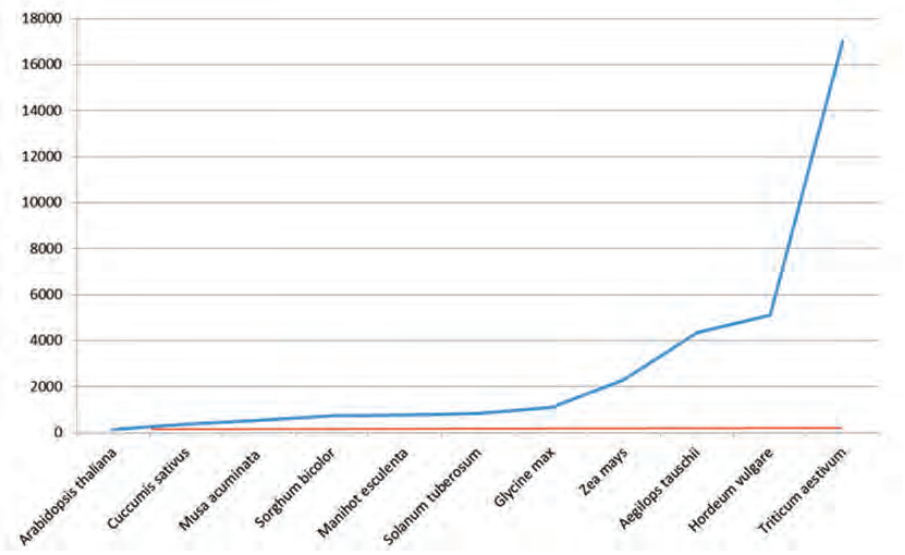


FIG. 11: Sizes of different plant genomes in millions of base pairs (in blue) versus the size of plant exomes (red).

Efforts are ongoing to use exome capture strategies to catalogue gamma induced mutations in both seed and vegetatively propagated crops. Preliminary data is promising with candidate deletions of between 100 and 700 kilobases being recovered (Fig. 12). Interestingly, the same dosage applied to two different genotypes did not give the same result even when standard LD_{50}/GR_{50} dosage optimizations were used to calibrate the treatments. This suggests that more can be done to better optimize gamma irradiation dosages for mutation breeding. We envision a time in the future when sequencing based approaches will be routinely used to calibrate dosages and greatly enhance the efficiency of mutant population development. These tools could then also be used for the development of precise molecular markers for breeding, something that is expensive and time-consuming using traditional methods.

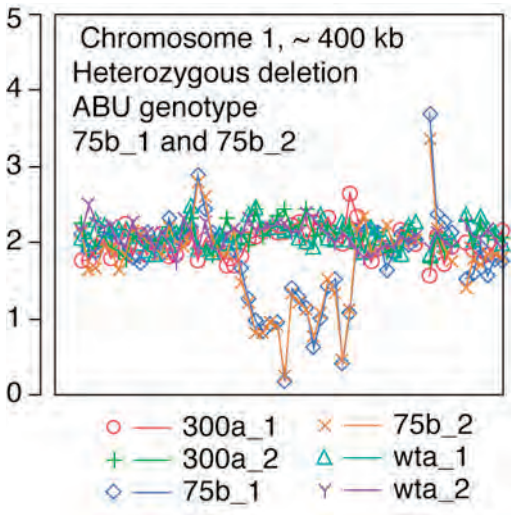


FIG. 12: Recovery of mutations induced by gamma irradiation of seeds of Sorghum bicolor. Data for chromosome 1 is shown. Colours represent different mutant plants. Copy number (left) is set at 2 for a diploid species. Sequence reads below 2 represent regions of genomic deletions. Two sibling mutants have inherited the same deletion of approximately 400,000 base pairs.

CAPACITY BUILDING AND SERVICES

Training courses

Plant Mutation Breeding and Efficiency Enhancing Techniques

A two-week regional training course on *Plant Mutation Breeding and Efficiency Enhancing Techniques* took place at the PBGL, Seibersdorf (10-20 June 2014). Ten participants (Iraq (5), Jordon (3) and Syrian Arab Republic (2)) from the TC project RAS/5/058 on *Supporting Mutation Breeding Approaches to Develop New Crop Varieties Adaptable to Climate Change* attended the training course as did one scientific visitor (Burkina Faso), seven fellows (Algeria, Bangladesh (2), Burundi, Ghana (2) and Sudan) and three interns (Ethiopia, Poland and Slovenia). Lectures, practicals and demonstrations were presented by staff of the PBGL and the PBG Section. The training covered areas of mutation induction and detection, techniques to enhance the efficiency of mutant development and accelerate mutation breeding programmes, such as rapid generation cycling, doubled haploid methods and molecular markers. Participants were also trained in methods of seed irradiation for mutation induction, including measurements of radiosensitivity. The training course focused on wheat and barley, which are key crops for many Member States. Participants practiced procedures in anther and microspore culture of wheat and barley for the induction of haploid plants and *in vitro* culture of immature embryos for rapid generation cycling of selected mutant lines.

Training course participants

Group Training on Chemical Mutagenesis of Barley Seed

An ad hoc training course was organized from 23–27 March 2014 on methods of chemical mutagenesis of seed propagated crops. All steps of the PBGL-developed protocol (soon to be made available online) were taught, from seed preparation to post-mutagenesis decontamination. Participants from four Member States attended (Democratic Republic of Congo, Germany, Nigeria and Turkey) the Group Training on Low-cost DNA Extraction, Purification of Enzymes and Mutation Discovery.

Participants of group training

An *ad hoc* training course was organized from 1-12 September 2014 covering low-cost protocols developed at the PBGL for tissue desiccation, low-cost DNA extraction, bench-top enzyme purification and mutation discovery (available at http://www-naweb.iaea.org/nafa/pbg/public/LowCost_MolecularMethods_PBGL_2013_V1_6.pdf). This training represented the first PBGL training on enzyme purification using the new protocol

Fellows scoring different mutant phenotypes in the barley 'Chromosome walk' demonstration in which mutant lines are arranged according to their genetic location on chromosomes (May 2014).

described earlier in this newsletter. The training was scheduled to coincide with Scientific Visitors from Mongolia and Palestine. Additional participants included research fellows, interns and visiting experts from Bangladesh, China, Ghana, Poland and the United Republic of Tanzania.

In 2014, the PBGL hosted 18 Fellows from 11 Member States, nine Scientific Visitors from seven Member States, nine Interns from seven Members Sates and two cost-free experts from China. Details are given below:

Name	Country	Status	Duration	Topic
Hannachi, Abderrahmane	Algeria	Fellow	3 months	Mutation detection in barley
Dussoruth, Babita	Mauritius	Fellow	3 months	Mutation detection in banana
Randrianarivony, Hery Lalao Lwyset	Madagascar	Fellow	4 month	Mutation induction and detection in rice
Rafiri, Matumelo Alice	Lesotho	Fellow	3 month	Mutation induction in potato
Ntho, Motlatsi James	Lesotho	Fellow	2 month	
El Achouri, Kaoutar	Morocco	Fellow	4 months	
Ndayihanzamaso, Privat	Burundi	Fellow	3 months	Mutation detection
Nunekpeku, Wonder	Ghana	Fellow	6 months	Mutation induction in oil palm
Sapey, Enock	Ghana	Fellow	6 months	
Diamuini, Aime	Democratic Republic of Congo	Fellow	3 months	Mutation induction and detection in cassava
Roy, Snigdha	Bangladesh	Fellow	6 months	Mutation induction and screening for abiotic stress tolerance
Kabir, Alomgir	Bangladesh	Fellow	6 months	
Mustafa, Nada Siddig	Sudan	Fellow	6 months	Mutation induction in wheat and sorghum
Abdessalem, Zeltni	Algeria	Fellow	1 month	Methods in plant mutation breeding
Yona, Neema	The United Republic of Tanzania	Fellow	3 months	Mutation detection in heat stress tolerant rice mutants
Wahiba, Amri Epse Tiliouine,	Algeria	Fellow	3 months	Mutation detection in chickpea
Saraye, Banumaty,	Mauritius	Fellow	2 months	Heat stress screening of tomato mutants

Name	Country	Status	Duration	Topic
Saraye, Banumaty	Mauritius	SV	1 week	Participation in a scientific conference
Gazinski, Johanna	Germany	SV	3 days	Mutation induction in barley seed
Kozak-Stankiewicz, Kamila	Poland	SV	3 weeks	Validation of doubled haploid lines by enzymatic mismatch methods
Nocen, Joanna	Poland	SV	13 days	
Yadamsuren, Myagmarsuren	Mongolia	SV	2 weeks	
Hamdan, Yamen A.S.	Palestine	SV	2 weeks	Mutation induction and detection in crop plants
Nandkangre, Herve	Burkina Faso	SV	2 weeks	
Jouhar, Mohammed	Syria	SV	2 weeks	Development of low cost method for mutant characterization
Kupc, Malgorzata	Poland	SV	2 weeks	Mutation induction and detection in crop plants
Taassob Shirazi, Farzaneh	Islamic Republic of Iran	Intern	12 month	Mutation induction in barley, screening and accelerated breeding
Dada, Keji	Nigeria	Intern	4 months	Mutation induction in coffee
Sen, Ayse	Turkey	Intern	5 months	Mutation detection
Szablinska, Joanna	Poland	Intern	3 month	Mutation detection in rice
Hasanazzaman Rani, Md	Bangladesh	Intern	5 months	Mutation induction in rice, screening and accelerated breeding
Kamruzzaman, Md	Bangladesh	Intern	5 months	
Kosmrlj, Kristina	Slovenia	Intern	3 months	Development of ion beam irradiation
Balachew, Tsegahiwot A.	Ethiopia	Intern	3 months	
Kolloch, Krzysztof	Poland	Intern	2 months	Mutation detection in citrus
Tao, Lan	China	Cost Free Expert	6 months	Mutation induction and identification in rice
Chen, Zhiwei	China	Cost Free Expert	5 months	

Irradiation services provided to Member States in 2014

The PBGL provides an irradiation service to Member States for mutation induction. In 2014 a total of 45 requests for plant irradiation services using gamma rays from 26 Member States were received and handled. These are listed below and included 36 plant species. For each request (unless otherwise stated), we carry out radiosensitivity tests to determine the optimal irradiation dose for mutation induction (some examples are given below). We therefore normally request that Member States send us sufficient seeds for this initial test (usually 100–300 seeds). Once the optimal dose is determined this is applied to the rest of the seed samples and the M1 seeds are returned to the Member State.

Apart from seed samples, which represent the normal case, we also irradiate other plant parts, especially for crops that are predominantly vegetatively propagated. Our service in 2014 included ongoing work on developing mutant populations of potato using a protocol that has been developed at PBGL. This was done for four Member States: Kenya (three potato varieties), Lesotho (four potato varieties), Morocco (one potato variety) and United Kingdom (potato microtuber production collection). The total number of irradiation requests since records began now stands at 1410 at the end of 2014. The trend of applying mutation induction to a wider range of crops continues as can be seen in requests for irradiation of a herb (sage), an ornamental tree (the Indian bean tree, *Catalpa bignonioides*)) and biofuel crops (*Jatropha curcus*).

The number of requests for irradiation is continuously high and more and more Member States make use of the PBGL service. This is thought to be due, in part, to the regulations and restrictions imposed on setting up and refurbishing gamma irradiators; the source of gamma rays are radioactive isotopes such as ^{60}Co and ^{137}Cs. The PBGL is therefore becoming increasingly important as an international centre for mutation induction using gamma irradiation. To offset this dependency and to increase capacity in Member States the PBGL is conducting R&D activities in the application of X-ray and ion beam irradiation for mutation induction.

Request no.	Country	Crop
1366	Oman	Date palm, wheat, barley, banana
1367	Bangladesh	Wheat, groundnut, onion
1368	Bangladesh	Rice
1369	Algeria	Wheat (TA, TD)
1370	UK	*Vica faba*
1371	Germany	*Heliantus anus*
1372	Uzbekistan	Cotton, *Paulownia*
1373	Democratic Republic of Congo	*Jatropha curcas*
1374	Democratic Republic of Congo	Soybean
1375	Albania	*Phaseolus vulgaris*

Request no.	Country	Crop
1376	Lesotho	*Ipomea batotas*
1377	Slovenia	*Catolpa bignonioides*
1378	UK	*Triticum turigidum*
1379	Bangladesh	Lentil
1380	Sudan	Sorghum, cotton and bread wheat
1381	Iraq	Cowpea
1382	Benin	Maize, amaranth
1383	Palestine	Barley, durum wheat
1384	Democratic Republic of Congo	Cassava
1385	Iraq	Sesame
1386	Jordan	Barley
1387	Jordan	Barley
1388	Syria	Wheat, barley
1389	UK	Wheat
1390	Democratic Republic of Congo	Maize
1391	UK	Potato
1392	Italy	Tomato, onion, lettuce
1393	Nigeria	*Digitaria exilis* (Fonio)
1394	UK	Wheat
1395	Sri Lanka	Rice
1396	Albania	Wheat
1397	Sri Lanka	Onion, chili, soybean, mungbean
1398	Oman	Wheat, barley
1399	Senegal	Cowpea
1400	Uzbekistan	*Paulownia*
1401	Slovakia	Pea
1402	Nigeria	Cowpea
1403	UK	Wheat
1404	Jordan	Barley, wheat

Request no.	Country	Crop
1405	Ghana	Sorghum, oil palm
1406	UK	*Hosta*
1407	Kuwait	Barley
1408	Sierra Leone	Rice
1409	Nigeria	Sesame
1410	Kenya	*Brachiaria ruziziensis, Dolichos lablab*

Mutation breeding information sheets

The PBGL continues to produce its very popular 'Mutation breeding information sheets'. These are mostly produced by our fellows and highlight successes in plant mutation breeding in individual countries and provide some background information relating to these countries. In 2014, we revised the layout and six new information sheets were produced (Burundi, Kuwait, Morocco, Qatar, United Kingdom, Viet Nam) bringing the total number of sheets to twenty-five.

New-look Information Sheets for Visitors.

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
International Center for Tropical Agriculture (CIAT), Cali, COLOMBIA	Induction and detection of mutation events in South American cassava lines for enhanced productivity and competitiveness through value addition
International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA	Induction and detection of mutation events in African cassava lines for enhanced productivity and competitiveness through added value
International Rice Research Institute (IRRI), Manila, PHILIPPINES	Induced mutations in rice for tolerance to abiotic stresses (including salinity); protocol development for salt tolerance testing
International Network for the Improvement of Banana and Plantains (INIBAP), Bioversity International, Montpellier, FRANCE	Induced mutations in *Musa* for tolerance to biotic stresses
Austrian Institute of Technology, Health & Environment Department, Tulln, AUSTRIA	Gene expression profiling in drought
University of Natural Resources and Life Sciences, Tulln, AUSTRIA	Methods on marker assisted breeding; NIRS analysis in characterising mutant seed phenotypes; Mutants for barley fodder, quality testing
University of Agriculture, Department of Plant Physiology, Krakow, POLAND	Banana phenotyping for drought tolerance
University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, AUSTRIA	Induced and natural mutation induction in crop plants including under-studied crops
University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, AUSTRIA	Statistical data evaluation
Agri-Science Queensland, Hermitage Research Facility, Warwick, AUSTRALIA	Barley crossing method; barley mutant stocks and mutation breeding
The James Hutton Institute, Invergowrie, Dundee, Scotland, UK	Barley crossing method; barley genetic stocks, genetic markers for low lignin mutants
University of Dundee, Dundee, Scotland, UK	Molecular genetics of lignin mutants for fodder barley
Nordic Genetic Resource Center, Alnarp, SWEDEN	Classic barley mutants, mutant gene descriptions and nomenclature
University of California, Davis Genome Center, Davis, USA	Developing next generation sequencing strategies for discovery of induced mutation events in genomes of vegetatively propagated crops

University of Ljubljana, Ljubljana, SLOVENIA	X-ray irradiation for mutation induction; pollen irradiation for haploid production
Ruder Boskovic Institute, Zagreb, CROATIA	Ion beam irradiation
University of Vienna, Molecular Systems Biology, Vienna, AUSTRIA	Metabolomics of mutant crops
John Innes Centre, Norwich, UK	Reverse-genetics in grass pea
Bench-Bio, Gujarat, INDIA	Reverse-genetics in grass pea
University of Florida, Citrus Research and Education Center, Mature Citrus Biotechnology Facility, Lake Alfred, USA	Citrus reverse-genetics
Gregor Mendel Institute, Vienna, AUSTRIA	Adaptation to climate change
University of Sydney, Sydney, AUSTRALIA	Disease resistance in wheat
Agriculture and Agri-Food Canada, Manitoba, CANADA	Disease resistance in wheat
The Genome Analysis Centre, Norwich, UK	Disease resistance in wheat
The Sainsbury Laboratory, Norwich, UK	Disease resistance in wheat
Stellenbosch University, Stellenbosch, SOUTH AFRICA	Disease resistance in banana
Du Roi Laboratory, Greater Tzaneen Rural, SOUTH AFRICA	Disease resistance in banana

The Soil and Water Management & Crop Nutrition Laboratory

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farming communities to climate change and variability by protecting soil and water resources and optimizing soil, water and nutrient management practices. The SWMCNL also helps Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well as remediating the impact of such events on soil and agricultural water resources.

In 2014, the SWMCNL provided a broad range of services: (i) Develop and validate affordable isotope, nuclear and related conventional techniques for climate-smart agriculture, (ii) Support the improvement of nuclear emergency response in food and agriculture, (iii) Train technical staff and scientists from Member States in the analyses of isotopes and in the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices, (iv) Conduct isotope analyses to projects where analytical facilities are not available, and (v) Provide quality assurance services to Member States.

Research and Development activities at the SWMCNL in 2014 focused on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. This included improvements in the use of Lead-210 as a tracer for long-term erosion events, precise soil sampling devices, the use of Carbon-13 and Nitrogen-15 for assessing soil organic carbon dynamics, and cosmic-ray soil moisture neutron probe for area-wide soil moisture assessment. In addition, new activities were initiated in the field of agricultural water management through the implementation of an on-line soil moisture sensor network for better understanding of the role of crop residues in retaining soil moisture. All these activities are in support of Coordinated Research Projects (CRP) of the SWMCN Subprogramme.

A further major activity of the SWMCNL is its contribution to capacity building in Member States. Besides six individual fellows and interns, one interregional and one group training course were held in the SWMCNL in 2014, with the participation of 42 fellows from 26 countries, each receiving four to six weeks of intensive training in the application of isotopic and nuclear techniques to improve soil and water management and crop nutrition.

A new IAEA publication, "*Guidelines for Using Fallout Radionuclides to Assess Erosion and Effectiveness of Soil Conservation Strategies*" *(IAEA-TECDOC-1741)*, on the use of fallout radionuclides (FRNs) to assess soil erosion magnitude in agricultural lands, was published in 2014. These guidelines provide researchers with step-by-step guidance and up–to-date information on the use of FRNs to assess soil erosion rates of agricultural lands for developing management practices that can minimize land degradation and improve land productivity and environmental sustainability. Information was further released to Member States through

48 publications as book chapters, conference papers and publications in international peer-reviewed journals.

In 2014, 3365 and 140 samples were analysed for stable isotopes and FRNs, respectively, at the SWMCNL. Most analyses were carried out in support of research and development activities in the SWMCNL and focused on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. The SWMCNL also provided isotope analyses to the Plant Breeding and Genetics Laboratory of the Joint FAO/IAEA Division.

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Weltin, Georg	Senior Laboratory Technician
Resch, Christian	Senior Laboratory Technician
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Name	Title
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[1] Retired in June 2014 and returned as a consultant in December 2014; [2] Left in July 2014; [3] April to June 2014; [4] August to October 2014; [5] September to October 2014; [6] Joined in December 2014; [7] Joined in December 2014; [8] April to December 2014; [9] Joined in December 2014.

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management and Crop Nutrition Laboratory (SWMCNL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture plays a key role in the implementation of the Soil and Water Management & Crop Nutrition (SWMCN) Subprogramme.

The SWMCNL assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farmers' communities to climate change and variability by optimizing soil, water and nutrient management practices. These efforts are supported by a new generation of robust and affordable isotope and nuclear techniques that can be used *in-situ* at the plot (on-farm) and area-wide level.

Climate change is a major threat to food security. Changes in weather patterns have brought storms, floods, droughts and extreme temperatures impacting sustainable agricultural production. These have resulted in soil erosion, land degradation, increased greenhouse gas emission and crop failures worldwide. The need to maintain agricultural production in these challenging conditions has never been greater. Consequently, there is an increasing demand from Member States for technical assistance and training in evaluating the impact of climate change and variability on soil and agricultural water resources, as well as for soil and water management packages for climate change mitigation and adaptation.

The SWMCNL also supports Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well as remediating the impact of these events on soil and agricultural water resources.

Based on the experience during recent nuclear emergencies affecting food and agriculture, there is a critical need to effectively improve data collection, management and visualization for timely dissemination and communication to stakeholders in affected areas. Member States have therefore requested urgent technical assistance in improving nuclear emergency preparation and response in food and agriculture.

All activities at the SWMCNL are driven by Member States' demands. The SWMCNL provides a broad range of services:

- Develop and validate isotope and nuclear techniques in support of Co-ordinated Research Projects (CRPs) and Technical Co-operation Projects (TCPs). Ten isotopic and nuclear techniques have been developed or adapted at the SWMCNL over the last 50 years, which are now well established across the world. Currently, six techniques are under development;
- Support the improvement of nuclear emergency response in food and agriculture;
- Train technical staff and scientists from Member States in the analyses of isotopes and the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices (through individual fellowships, group training or training courses);
- Provide isotope analyses to projects where analytical facilities are not available;
- Provide quality assurance services to Member States.

Climate-smart Agriculture

Fallout ^{210}Pb as a soil and sediment tracer in catchment sediment budget investigations

In addition to the *Guidelines for Using Fallout Radionuclides to Assess Erosion and Effectiveness of Soil Conservation Strategies* (IAEA-TECDOC-1741), a review paper (Mabit *et al.*, 2014) was also published in 2014 in Earth-Science Reviews (impact factor 7.1). This paper provided a comprehensive evaluation and discussion of the applications of $^{210}Pb_{ex}$ as a tracer in terrestrial and aquatic environments, with particular emphasis on catchment sediment budget investigations. It summarizes the state-of-the-art, latest knowledge relating to the use of this tracer, the main assumptions, the requirements (including the need for accurate analytical measurements and for parallel validation), and the limitations that must be recognised when using this fallout radionuclide as a soil and sediment tracer. Lessons learned and current and future research needs in the environmental and radiochronological application of $^{210}Pb_{ex}$ are also presented and discussed.

This review is timely as increasing anthropogenic pressures coupled with climate change impacts on natural resources have resulted in a quest for innovative tracing techniques for understanding soil redistribution processes and assessing the environmental status of soil resources. Among the different existing tracers, the fallout component of the radioisotope lead-210, also termed unsupported or excess lead-210 ($^{210}Pb_{ex}$) when referring to its presence in soil or sediment, arguably offers the broadest potential for environmental applications due to its origin and relatively long half-life (22 years). For more than five decades, $^{210}Pb_{ex}$ has been widely used for dating sediments, to investigate sedimentation processes and, since the 1990s, to provide information on the magnitude of soil and sediment redistribution.

Development of the Fine Increment Soil Collector (FISC) for performing precise soil and sediment sampling

Soil and sediment related research for environmental impact assessments requires accurate depth incremental sampling to perform detailed analysis of physical, geochemical and biological properties of soil and exposed sediment profiles. Existing equipment does not allow the collection of soil/sediment increments at millimetre resolution.

In 2014, the SWMCNL developed the Fine Increment Soil Collector (FISC) that allows close control of incremental soil/sediment sampling. It assists in the easy recovery of the material collected by using a simple screw-thread extraction system (Fig. 1). The FISC has been designed specifically to enable standardized scientific investigation of shallow soil/sediment samples. In particular, applications have been developed under two CRPs: D1.20.11 on "*Integrated Isotopic Approaches for an Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion*" and D1.50.15 on "*Response to Nuclear Emergencies Affecting Food and Agriculture*".

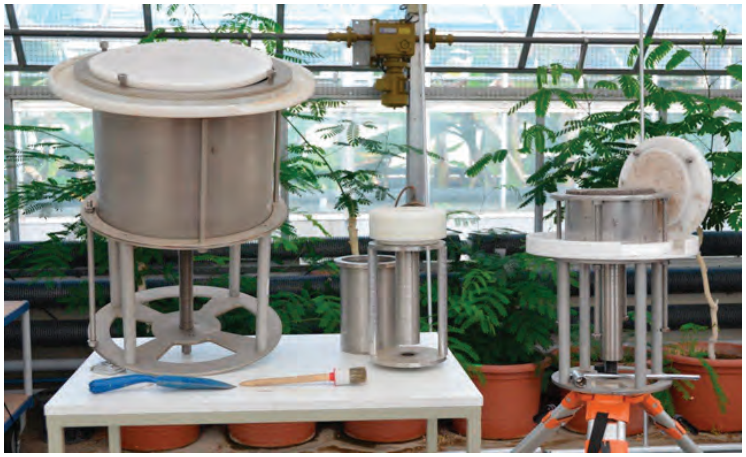


FIG. 1: Different diameters of the Fine Increment Soil Collector (FISC)

The FISC can improve the determination of the depth distributions of fallout radionuclides (FRNs) such as Beryllium-7 [^{7}Be], Caesium-137 [^{137}Cs], Lead-210 [$^{210}Pb_{ex}$], Plutonium-239 and 240 [$^{239+240}$Pu], which are frequently used for soil erosion and sediment transport studies and/or sediment fingerprinting. Such a device offers great potential to investigate radioisotopes depth distributions also associated with recent fallout events, such as that associated with nuclear emergencies. Furthermore, prior to remediation activities – such as topsoil removal – in contaminated soils and sediments (e.g. by heavy metals, pesticides or nuclear power plant accident releases), basic environmental assessments often require the determination of the extent and the depth penetration of the different contaminants; such precision can be provided by using the FISC.

In comparison with existing sampling tools, the FISC has the following advantages and benefits: (i) it permits sampling of soil/sediment at the top of the profile, (ii) it is easy to adjust so as to collect soil/sediment at millimetre resolution, (iii) it is simple to operate by one person, (iv) incremental samples can be performed in the field or at the laboratory, (v) it permits precise evaluation of bulk density at millimetre vertical resolution, and (vi) sample size can be tailored to analytical requirements. Moreover, the FISC is easy to transport and can be easily hand-carried in the field. It involves minimum disruption in the field and it can be constructed at very low cost by a mechanical workshop. Its maintenance is minimal and it can be stored in a limited space.

The FISC has been disseminated through a peer-review publication in the Journal of Soils and Sediments (see Mabit *et al.*, 2014) and a presentation at the European Geosciences Union–General Assembly in 2014. It will be published as a detailed Standard Operating Procedure (SOP) in 2015.

The FISC has attracted the attention and interest in several institutions from Member States (China, Japan, Switzerland and United Arab Emirates)

Development of guidelines on the use of Carbon and Nitrogen isotope techniques to determine C distribution and storage

In order to evaluate the sustainability and efficiency of soil carbon sequestration measures and the impact of different management and environmental factors, information on soil organic matter (SOM) stability and mean residence time (MRT) is required. The MRT is a measure of the average time required to completely renew the SOM in a pool, which can range from a few months to millennia. However, this information on SOM stability and MRT is expensive to determine via radiocarbon dating, precluding a widespread use of stability measurements in soil science. Since 2012, the SWMCNL in collaboration with the KU Leuven (Belgium) tested an alternative method, first developed by Conen *et al.* in 2008 for undisturbed Alpine grassland systems, using Carbon-13 (^{13}C) and Nitrogen-15 (^{15}N) stable isotope ratios in more frequently disturbed agricultural soils. Since only information on carbon and nitrogen concentrations and their stable isotope ratios is required, it is possible to estimate the SOM stability at greatly reduced costs compared to radiocarbon dating. Using four different experimental sites in Austria and Belgium located in various climates and soil types, this research demonstrated the effectiveness of using the C/N ratio and $\delta^{15}N$ signature to determine the stability of mOM (mineral associated organic matter) relative to POM (particulate organic matter) in an intensively managed agro-ecological setting. Combining this approach with $\delta^{13}C$ measurements allowed discrimination between different management (grassland vs cropland) and land use (till vs no till) systems. With increasing depth the stability of mOM relative to POM increases, but less so under tillage compared to no-till practises. Applying this approach to investigate SOM stability in different soil aggregate fractions, it corroborates the aggregate hierarchy theory. The organic matter in the occluded micro-aggregate and silt & clay fractions is less degraded than the SOM in the free micro-aggregate and silt & clay fractions. The stable isotope approach can be particularly useful for soils with a history of burning, and thus containing old charcoal particles that prevent the use of ^{14}C to determine the SOM stability.

In 2014, further validation of the above-mentioned model has been carried out on soil samples collected in Senegal, China and Kenya. These results will be reported in the 2015 laboratory activities report.

This validation is being implemented in the context of the CRP D1.50.12, on "*Soil Quality and Nutrient Management for Sustainable Food Production in Mulch Based Cropping Systems in Sub-Saharan Africa*".

Update on ^{13}C-labelling of plant materials through the use of walk-in growth chambers

In 2013, the SWMCNL installed a pair of walk-in growth chambers, each measuring $12m^3$ in volume. These growth chambers, with controlled temperature, relative humidity and carbon dioxide (CO_2), are being used to label plant materials, in a cost-effective way, with ^{13}C for incubation experiments to better understand soil organic carbon dynamics under a changing climate and to support research activities for improving climate-smart agriculture in Member States.

After an intensive effort of making the growth chamber gas leak-free, installing gas mass flow controllers to ensure continuous ^{13}C labelling and putting in place an automatic drip irrigation system for avoiding the need of entering the chambers during the labelling, the labelling finally began in April 2014. For three months (until June 2014) 36 pots with two to three maize

plants per pot were labelled at a set temperature of 22°C and 50% humidity (Fig. 2). After one month, the temperature was increased to 25°C to stimulate plant growth. Maize plants were harvested at the flowering stage. The results indicated that the harvested maize shoot biomass (1 kg of dry matter) had a maximum ^{13}C enrichment of 340 δ‰, as targeted in the design.

FIG. 2: Walk-in growth chambers with labelled maize plants prior to harvest

Future experiments in 2015 will focus on using various inert growth substrates to minimize CO_2 contributions from soil at the initial stage and so improve the uniformity of the ^{13}C-labelling of the plant material.

This research was also conducted in association with CRP D1.50.12 on "*Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-Based Cropping Systems in Sub-Saharan Africa*".

Method for the purification of inorganic phosphate in soil- and sediment samples prior to analysis of δ^{18O} isotopic abundance in phosphate

Phosphorus has one stable isotope (^{31}P) and several radioisotopes such as ^{32}P and ^{33}P, which have very short half-lives, making it difficult for any long-term study on P dynamics. Due to this constraint and the radioactive nature of ^{32}P and ^{33}P, researchers have started to explore the potential of oxygen isotopes in inorganic P compounds for improving P management. Soils subjected to different farm management practices (e.g. fertiliser or manure applications) show different $\delta^{18}O$-PO_4 signatures, indicating the potential of $\delta^{18}O$ as an isotopic tracer for studying P cycling, tracing P sources and ultimately providing a better understanding of soil P dynamics in agro-ecosystems.

The method for measuring $\delta^{18}O$ isotopic abundance in phosphate involves acid extraction of inorganic phosphate from organic matter in soils and other organic rich materials followed by a series of precipitation steps and resin treatments in order to purify the phosphate and hence eliminating all oxygen sources other than phosphate that could compromise the final result. In the end phosphate is converted into pure silver phosphate (Ag_3PO_4) without isotopic alteration and analysed by Thermal Conversion Elemental Analyser – Isotope Ratio Mass Spectrometer (TC/EA-IRMS).

An SOP has been produced describing this improved method in detail. In this document, the following steps have been described: (1) HCl-extraction, (2) Dissolved organic matter removal, (3) Ammonium phospho-molybdate (APM) precipitation and dissolution, (4) Magnesium ammonium phosphate (MAP, struvite) precipitation and dissolution, (5) Cation removal, (6) Silver phosphate precipitation and (7) Analysis by TC/EA-IRMS. This SOP will be published in 2015, as a joint document including several SOPs (e.g. Fine Increment Soil Collector, Soil Organic Carbon Fractionation methods) produced by the SWMCNL.

This research was conducted in the context of the CRP D1.20.12 on "*Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-Livestock Production Systems*".

Assessing area-wide soil water content using the Cosmic Ray Neutrons Probe

The importance of surface soil water (rooting zone) has become evident with climate change affecting rainfall patterns and crop production. The possible use of the Cosmic Ray Neutron Probe (CRNP) for measuring surface soil water has become increasingly popular. The advantage of the CRNP is that it is a non-invasive technique for measuring soil water content at an area-wide scale (with a footprint of up to 30 ha), in contrast to more conventional techniques that measure mainly at field scale (point) level.

In 2014, further validation of the use of the CRNP for area-wide soil moisture assessment has been carried out at the Petzenkirchen research station, 100 km west of Vienna, as part of the *Doctoral Programme for Water Resource Systems* of the Technical University of Vienna and the Federal Agency for Water Management. This CRNP (CRS 1000/B model), the first in Austria and installed in late autumn 2013, consists of two neutron counters (one tuned for slow, the other for fast neutrons), a data logger and an Iridium modem. The Petzenkirchen research station is located in an undulating agricultural landscape, characterized by heavy Cambisols and Planosols, and winter wheat and barley as main crops in winter and maize and sunflower in summer.

As no other instrument operates at a comparable scale, field validation is inherently difficult due to different crop establishment and agricultural practices within the wide CRNP footprint. It is therefore probably best accomplished by comparing the CRNP readings to an aggregate of point measurements. The preferred method is to compare the probe against the most reliable direct measurements of water content determined from oven-dried soil cores. This was done at different times, with each point in time representing different average water contents. While this procedure is accurate, it has the drawbacks of being labour-intensive and only capable of providing intermittent data. Some researchers have therefore used dense networks of for instance buried electromagnetic probes (Time Domain Reflectometry - TDR; Time Domain Transmission - TDT) for an indirect comparison of soil moisture, which has the advantage of providing continuous data for analysing soil moisture dynamics. The drawback is that the independent measurements are then indirect and their accuracy and bias have to also be measured.

An *in-situ* soil moisture network has been established in Petzenkirchen, consisting of 32 stations of TDT sensors measuring soil water at four depths (0.05, 0.10, 0.20 and 0.50 m) over an area of 66 ha. This TDT network is used to validate the CRNP technique. TDR probes were also installed

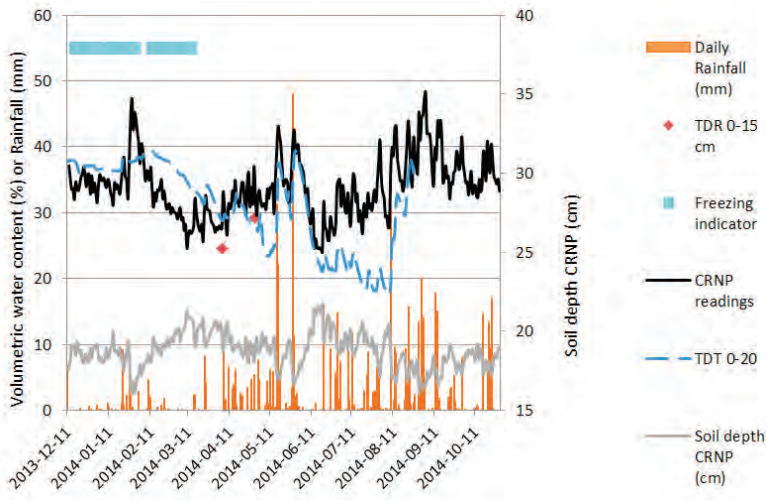


FIG 3: Validation of Cosmic Ray Neutron Probe at Petzenkirchen station

in the upper 15 cm of the soil during two field campaigns in April 2014 to further validate the CRNP. The results showed that CRNP reflects rainfall patterns and snow incidents (Fig. 3). The TDT also showed agreements with CRNP readings especially when no crop was present.

This validation is being implemented under CRP D1.20.13, on *"Landscape Salinity and Water Management for Improving Agricultural Productivity"*.

The measurement of the SWMCNL Cosmic Ray Soil Moisture Neutron Probe can be followed in real-time via the COsmic-ray Soil Moisture Observing System (COSMOS) network on: http://cosmos.hwr.arizona.edu/Probes/StationDat/087/index.php.

Validation of online soil moisture sensors on the role of crop residues in retaining soil moisture

The SWMCNL started in 2014 the validation of the use of online soil moisture sensors to understand the role of crop residues throughout the cropping season on soil moisture availability under mulch-based cropping systems. These activities have been implemented in the SWMCNL long-term trial at Grabenegg, Lower Austria, in cooperation with the Austrian Agency for Health and Food Safety (AGES). This trial, which was implemented in 2011, compares different crop rotations under mulch and no-mulch systems, with initial focus on soil organic carbon sequestration.

FIG. 4: Protecting data network cables at the Grabenegg experimental station

In 2011, a fully equipped weather station was set up at the trial site. This weather station has now been upgraded by adding a soil sensor network with Watermark™ granular matrix sensors for measuring soil matric potential, and Decagon 5TM™ sensors for simultaneous measurement of soil temperature and soil water content.

Sensors are placed at a depth of fifteen centimetres, comparing plots with and without mulch, with three sensor positions on each plot and this replicated on three different plots. Cables are put under ground in protective tubing (Fig. 4). Data generated are remotely accessible and will be used to interpret the effect of retaining plant residues (mulch-based cropping system) on soil water dynamics (content and potential) and soil temperature while rotating crops and practicing minimum tillage farming.

This research is conducted under CRP D1.50.12 on "*Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-Based Cropping Systems in Sub-Saharan Africa*".

Climate change impact and adaptation in fragile environments

In 2014, new activities were initiated by the SWMCNL to develop and/or adapt isotope and nuclear techniques for assessing climate change impact and developing climate change adaptation strategies in fragile agricultural environments, such as mountainous agro-ecosystems.

Consultants Meeting on soil and water conservation for climate change adaptation in agricultural uplands

Under the guidance of the SWMCNL, a consultants meeting was held from 8-12 December 2014 at the IAEA's Headquarters in Vienna to develop a new CRP focusing on soil and water conservation for climate change adaptation in agricultural uplands. Four consultants from Austria, New Zealand, UK and USA attended the meeting.

Upland agro-ecosystems - defined as less-favoured higher altitude environments, including areas with low soil quality and/or limited access to water - will face three major challenges related to food security and climate change in the coming decades: (1) increasing food production on marginal land, (2) optimising soil protection and improving water use efficiency for adapting to climate change, and (3) contributing to climate change mitigation.

The envisaged CRP will aim to (i) identify and test combinations of nuclear and conventional techniques to assess the impacts of changes occurring in upland agro-ecosystems, (ii) distinguish and apportion the impact of climate variability and agricultural management on soil and water resources in uplands and (iii) support adaptive agricultural management for soil and water conservation in uplands to reduce the impacts of climate variability. Nuclear techniques, including FRNs such as ^{137}Cs, ^{210}Pb, ^{7}Be and potentially $^{239+240}$Pu, Compound-Specific Stable Isotope (CSSI) techniques based on the measurement of ^{13}C natural abundance signatures of specific organic compounds (i.e. fatty acids), and Cosmic Ray Soil Moisture Neutron Probe (CRNP) will be used to fulfil these objectives.

The targeted start of the CRP is early 2016.

Exploratory characterisation of benchmark site of Rauris, Austria

Under the newly planned CRP on soil and water conservation for climate change adaptation in agricultural uplands, the SWMCNL will assist in implementing a long-term benchmark site in the *National Park Hohe Tauern* located in the high Austrian Alps, in close collaboration with several Austrian universities (e.g. University of Vienna and University of Graz) and institutions (e.g. National Park Hohe Tauern, Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Federal Agency for Water Management, Austrian Research Centre for Forests), involved in climate change impact assessment research in the Alps. This benchmark site is also part of the interregional Technical Cooperation Project INT/5/153 project on "*Assessing the Impact of Climate Change and its Effects on Soil and Water Resources in Polar and Mountainous Regions (2014–2017)*", and will be later used in TC fellowship training activities implemented by the Austrian partners.

A first activity has been an exploratory soil survey (October 2014), whose focus was the characterisation of four points (under grazing land) in the watershed of the Rauris valley at different altitudes between 900 and 1600 m above sea level, and under different land management (FIG. 5). Over 40 soil samples (each composed of five subsamples) were taken. In addition, two undisturbed cores were also taken. These samples will be analysed for general soil fertility, soil organic carbon stability and quality (^{13}C and ^{15}N signatures), phosphate-^{18}O signatures and fallout radionuclide inventories.

This information will allow the Austrian colleagues to implement further assessments and simulation experiments to better understand the impact of climate change on soil and

agricultural water in the European Alps and hence to help farmers to better adapt best land and water management practices to combat the negative effects of climate change in this region.

FIG. 5: Summer meadows at the benchmark site in the European Alps (National Park Hohe Tauern)

Protocols for assessing the impact of climate change on land-water-ecosystem quality in polar and mountainous regions

An Expert Meeting was held from 10-13 November 2014 at the IAEA Headquarters to define protocols for addressing the drivers of scientific investigations in 13 benchmark sites of the interregional Technical Cooperation Project INT5153 on "*Assessing the Impact of Climate Change and its Effects on Soil and Water Resources in Polar and Mountain Regions*", and to prepare common sampling and analytical procedures.

The following major research questions were targeted to identify specific research protocols:

1. "What is the impact of climate change on soil and soil organic carbon in polar and mountainous regions?", with major emphasis on temperature sensitivity of soil organic carbon, quality, stability along topological sequences in mountain regions and the active layer vs permafrost in polar regions, as a base for the better understanding of positive feedback mechanisms.
2. "What is the impact of climate change on (i) water availability and (ii) soil-sediment redistribution processes in polar and mountainous regions?", with major emphasis for instance on: (soil) water availability monitoring, biomass production due to induced drought or increased water availability, soil-sediment redistribution processes at field-slope-catchment level in mountain (intervened and not-intervened areas) and polar regions.
3. "How is the cryosphere affected by long-term and current climate change?", with major emphasis for instance on: soil temperature in topological sequences at different depths (linked with question 1), sediment cores and dating for paleo-climatic assessment.

The meeting was attended by 14 participants from nine Member States and resulted in detailed protocols. These protocols are currently being tested in polar and mountainous regions worldwide.

Nuclear Emergency Response in Food and Agriculture

Response to nuclear emergencies affecting food and agriculture

This CRP (CRP D1.50.15) aims to develop and assess systems of innovative data collection, management and geo-visualization platforms that can be used for both routine monitoring and emergency response to nuclear and radiological incidents that could affect food and agriculture.

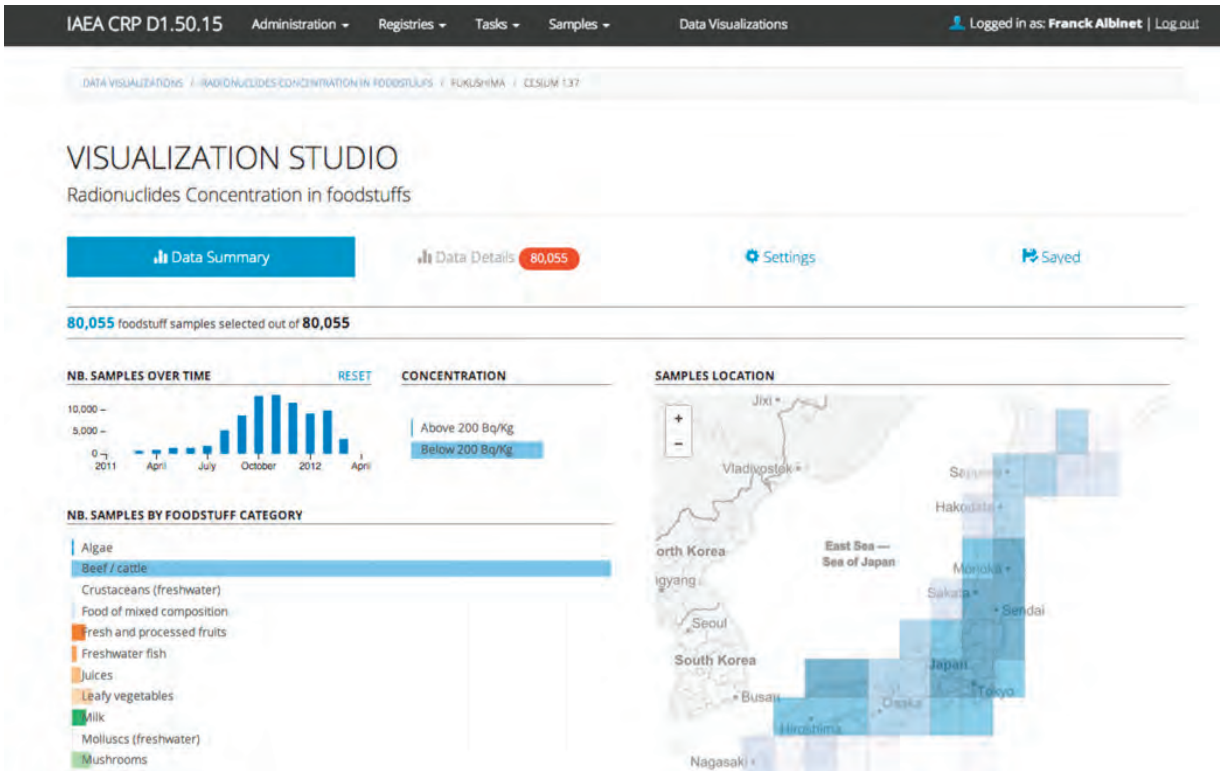


FIG. 6: Prototype of data management and visualization tool for nuclear emergency response in food and agriculture (simulated data are used for this visualization)

In 2014, a first protocol for data collection, management and visualization for the emergency phase (food restriction phase) has been developed by the SWMCNL in close collaboration with CRP participants. The protocol aims to optimize the response time of Member States regarding decision making on food restrictions and food safety communication strategies in case of nuclear or radiological emergencies. In order to make rapid, timely decisions whether food restrictions need to be enforced, simple procedures/protocols for collecting samples, managing minimal-sample attributes and minimal-sample laboratory result attributes and geo-visualization for effective emergency communication are needed.

Based on the above protocol, an information system that links data management and visualization is currently being developed and validated by the CRP participants (Fig. 6). This system will be developed in such a way that it can be linked with existing data exchange platforms (compatibility) of the IAEA, such as the *Unified System for Information Exchange on Incidents and Emergencies* (USIE) and the *International Radiation Monitoring Information System* (IRMIS) managed by the IAEA's *Incident and Emergency Centre* (IEC). The system will allow international organisations to follow up on the nuclear emergency response for food safety for advice purposes on food restrictions when requested at the national and international level. The CRP also includes the formulation of policy guidelines on nuclear emergency response in food and agriculture for competent authorities of IAEA and FAO Member States. Both the proposed information system and the policy guidelines will help Member States to be better prepared for nuclear emergencies affecting food and agriculture.

CAPACITY BUILDING AND SERVICES

Training Courses

Interregional Training Course on the Use of Fallout Radionuclides and Compound-Specific Stable Isotope Techniques for Precision Soil Conservation, 6–31 October 2014, Seibersdorf, Austria

From 6-31 October 2014, the SWMCNL organized an interregional training course on the use of FRNs and CSSI techniques for precision soil conservation. This training was given by scientists and technicians from the SWMCN Subprogramme in close collaboration with leading scientists from Austria, Belgium, Chile, New Zealand and the UK. Twenty-two fellows from 16 Member States, covering Africa, Asia, Europe and Latin America (Brazil, Chile, China, Ivory Coast, Cuba, Madagascar, Mexico, Morocco, Peru, Russian Federation, Tajikistan, Thailand, Tunisia, Uruguay, Venezuela and Vietnam), participated in the training. The main focus was to provide participants with advanced knowledge in using independently and conjointly FRNs and CSSI techniques for investigating soil degradation and assessing soil conservation effectiveness.

FIG. 7: Interregional training course reported by UN Radio

Through this training, the integrated and combined use of FRNs and CSSI techniques has been introduced for the first time on a worldwide scale. This will allow improving the cost-effectiveness of soil conservation at catchment level, and implementing soil erosion control measures where most needed (precision soil conservation). Because of the interregional character of the course, scientists will further strengthen their international networks, very fitting as preparatory activity to the International Year of Soils 2015.

The training was funded by the IAEA Technical Cooperation (TC) Department through national, regional and interregional TC projects. Feedback from participants showed that this advanced interregional training course was highly appreciated. More information on this training course can be found on UN Radio (Fig. 7) at: http://www.unmultimedia.org/radio/english/2014/12/nuclear-science-can-help-contain-soil-erosion-land-degradation/

Training Course on Integrated Nutrient-Water Management at Field and Area-wide Scale, 19 May–27 June 2014, Seibersdorf, Austria

From 19 May-27 June 2014, the SWMCN held a training course on "*Integrated Nutrient-Water Management at Field and Area-Wide Scale*" at Seibersdorf, Austria. The training was given by scientists and technicians from the SWMCN Subprogramme in close collaboration with scientists from the public and private sector, i.e. universities, national and international research for development organizations and companies, based in Austria and abroad (Belgium, Kenya, USA, UK). Twenty fellows from eleven Member States (Bangladesh, Botswana, Benin, Burundi, Iraq, Ivory Coast, Kenya, Oman, Senegal, Yemen and Zimbabwe) with various backgrounds (researchers and technicians mostly in the field of irrigation management) participated in the training.

The main focus of the training course was on: (i) improving nutrient management in rainfed and irrigated agriculture,(ii) monitoring nutrient balances and use efficiency at field scale, (iii) increasing the efficiency of water management in rainfed and irrigated agriculture at field and area-wide scale, (iv) monitoring soil moisture at field and area-wide scale, (v) assessing soil water balance and crop water relations, and (vi) demonstrating the use of the AquaCrop simulation model for improving soil water management and irrigation scheduling.

Besides lectures and laboratory and field work, a field excursion was organized to research stations of the AGES and the Federal Agency for Water Management in Grabenegg and Petzenkirchen (100 km west of Vienna) to learn about on-farm research on nutrient and water management at field and area-wide scales. Mr Dirk Raes from the KU Leuven (Belgium) participated as lecturer on the use of FAO's AquaCrop simulation model on crop water use and irrigation scheduling, which was very much appreciated, as he has been the main developer of the model. The training was funded by the IAEA Technical Cooperation Department through several national TC projects.

A self-assessment at the end of the training activity gave the organizers the opportunity to assess the effectiveness of the training course at an individual level. Feedback from the participants showed that hands-on training with equipment was highly appreciated and led to improved confidence in the handling of instruments and trouble-shooting. This training is useful for Member States as they prepare to meet the challenges of climate change to farming communities and help to develop climate-smart agriculture, particularly on water management practices.

Analytical Services

In 2014, 3365 samples were analysed for stable isotopes and 140 samples were measured for fallout radionuclides, respectively, in the SWMCNL. Most of the analyses that were carried out in support of research and development activities at the SWMCNL focused on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. Analytical support was provided also to the FAO/IAEA's Plant Breeding and Genetics Laboratory, with about 700 samples analysed.

External Quality Assurance: Annual Proficiency Test on ^{15}N and ^{13}C isotopic abundance in plant materials

Worldwide comparisons of stable ^{15}N and ^{13}C isotope measurements provide confidence in the analytical performance of stable isotope laboratories and hence are an invaluable tool for external quality control.

The 2014 Proficiency Test (PT) on ^{15}N and ^{13}C isotopic abundance in plant materials, jointly organized by the University of Wageningen, the Netherlands, has been successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, http://www.wepal.nl) is accredited for the organisation of inter-laboratory studies by the Dutch Accreditation Council.

Every year, one ^{15}N-enriched plant test sample is included in one round of the WEPAL IPE (International Plant-Analytical Exchange) programme. A bulk amount of uniformly ^{15}N-enriched plant material is produced by the SWMCNL and sent to WEPAL for processing. This ^{15}N-enriched material is sent out together with three other non-enriched plant samples. Participants are invited to perform analysis in the WEPAL IPE scheme, including ^{15}N (enriched and/or natural abundance level), total N (N-elementary), Kjeldahl-N, ^{13}C and total C (C-elementary).

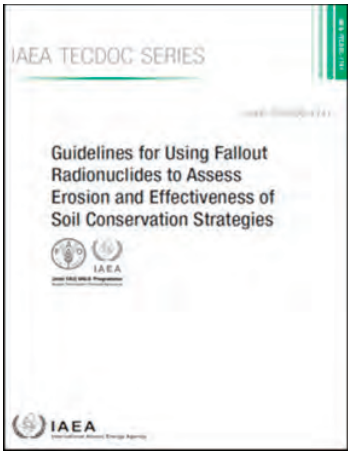
A special evaluation report for IAEA participants on the analytical performance in the stable isotope analysis is issued by the SWMCNL and sent to the participants together with a certificate of participation additional to the regular WEPAL evaluation report. The participation fee for one round per year is covered by the SWMCNL.

Participants registered in the PT scheme were provided with the WEPAL test sample set, IPE 2014.2, consisting of the four test samples, each of 20 g plant material. Ten stable isotope laboratories participated in this PT-round and all ten laboratories reported isotope abundance data: (Africa (1): Morocco; Asia (3): Pakistan and Philippines (2 labs); Europe (4): Belgium, France, Germany and Italy; Latin America (2): Brazil and Chile).

Seven out of ten laboratories participating in the nitrogen analysis reported 15N-data within the control limits for the enriched plant sample (Fig. 8) and seven out of nine participating laboratories in carbon analysis reported ^{13}C isotopic abundance results within the control limits (Fig. 9).

Guidelines, Software and Information

TECDOC 1741[1], Publication of the Guidelines for Using Fallout Radionuclides to Assess Erosion and Effectiveness of Soil Conservation Strategies, 213 p.



The conservation of soil and water resources has become a major concern in ensuring global food production. Soil erosion is a worldwide threat and represents the main mechanism of land degradation in both developed and developing countries. To control soil erosion, there is a need to monitor the impacts

[1] http://www-pub.iaea.org/books/IAEABooks/10501/Guidelines-for-Using-Fallout-Radionuclides-to-Assess-Erosion-and-Effectiveness-of-Soil-Conservation-Strategies

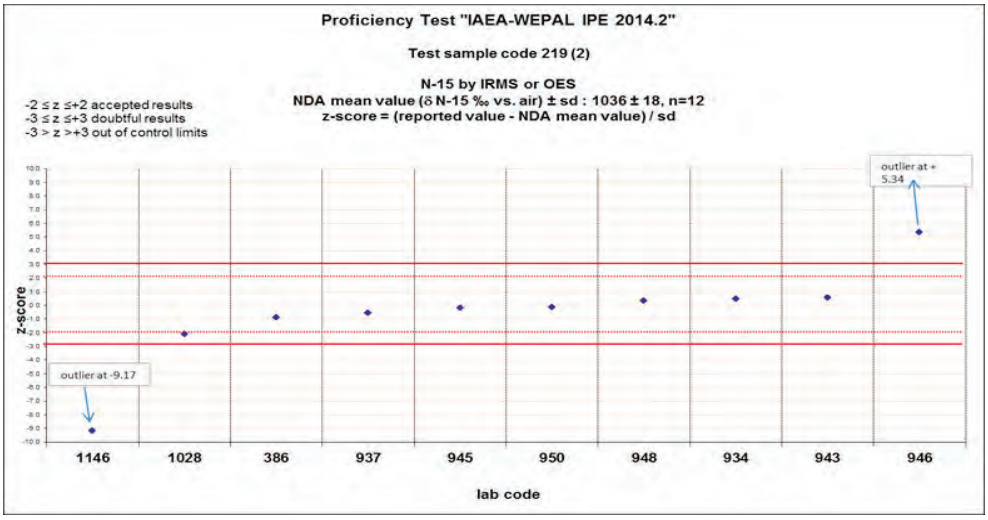


FIG. 8: Z-score evaluation of the ^{15}N analysis

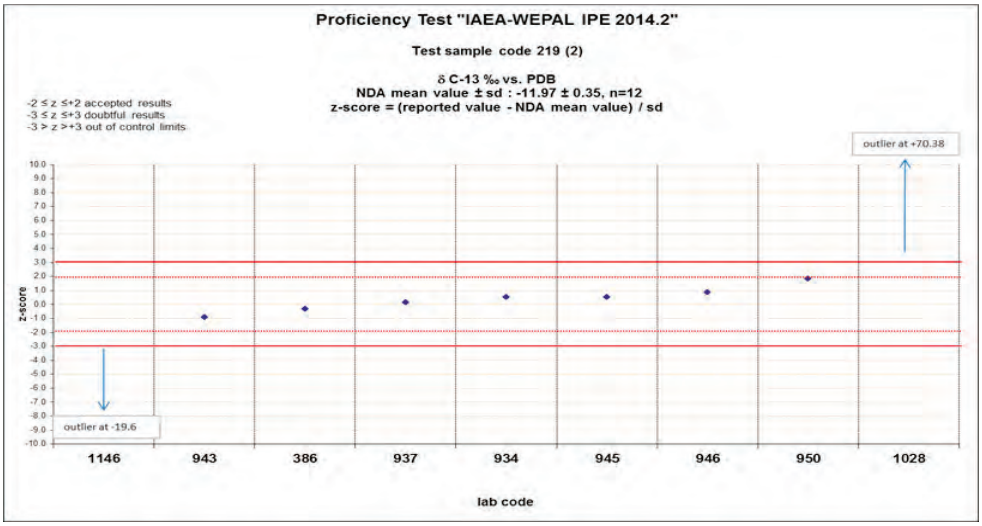


FIG. 9: Z-score evaluation of the ^{13}C analysis

of land use and assess the effectiveness of specific soil conservation technologies. Fallout radionuclides (FRNs) have proven to be a cost effective tool to trace soil redistribution caused by erosion within landscapes, from plot to basin scale, and to complement information provided by conventional erosion measurements and modelling.

The *Guidelines for Using Fallout Radionuclides to Assess Erosion and Effectiveness of Soil Conservation Strategies* is the IAEA's most recent significant contribution in using FRNs to investigate and combat soil erosion.

The purpose of this publication is to provide up-to-date information on the use of FRNs, such as ^{137}Cs, ^{210}Pb and ^{7}Be, to assess soil erosion magnitude in agricultural land. It summarizes the state of the art in the use of these FRNs as tracers, the main assumptions, the requirements and their limitations, which need to be recognized when using FRNs as soil tracers. This publication summarizes the experiences and knowledge gained since the end of the 1990s in the use of FRNs by the IAEA and by scientists from both developed and developing countries involved in IAEA research networks.

This publication delivers comprehensive step-by-step guidance in the application of FRNs for investigating soil erosion and soil redistribution affecting agro-ecosystems to an audience of scientists, technicians and extension workers, undergraduate and graduate students, and staff of nongovernmental organizations involved in agricultural development at the local, national, regional and international levels.

These FRN guidelines are in high demand; by the end of 2014, it had been downloaded 700 times while 710 hard copies had been procured.

CSSIAR v1.0 Software – a new tool to improve soil conservation at catchment level

Under the regional TC Project RLA5064 on *Strengthening Soil and Water Conservation Strategies at the Landscape Level by Using Innovative Radio and Stable Isotope and Related Techniques*, and with the guidance of the *Instituto Tecnológico de Sonora, Mexico, Universidad Austral de Chile, Chile, Universidade Federal Fluminense, Brazil, and the National Institute of Water and Atmospheric Research, New Zealand*, a new software, called CSSIAR, was developed to assess soil erosion apportionment using data obtained from CSSI analysis, a technique that allows assessing soil redistribution in agricultural landscapes and forest plantations, as well as identifying hotspots of soil erosion. This will help researchers and policy makers to enhance and improve soil conservation measures at the catchment level. This technique is based on the measurement of ^{13}C isotope signatures of specific organic compounds in the soil profile (e.g. fatty acids derived from a specific land use).

CSSIAR v1.0 is based on SIAR (Stable Isotope Analysis in R) by Andrew Parnell, but with a more user friendly programme interface, and has been created to assess soil apportionment and to identify hot spots of land degradation. CSSIAR v1.0 enables the analysis of larger sets of data and gives more detailed statistical information (including uncertainty) about the proportion of sediment contribution from different land uses in a catchment. This software runs on R, which is free and can be downloaded on the R website (http://www.r-project.org/). CSSIAR v1.0 is available on the following IAEA website: http://www-naweb.iaea.org/nafa/swmn/models-tool-kits.html.

The software has been presented and tested during the Interregional Training Course on the Use of Fallout Radionuclides (FRNs) and CSSI Techniques for Precision Soil Conservation, held in October 2014 at Seibersdorf, Austria.

Thirteen contributions from the Soil and Water Management & Crop Nutrition Subprogramme at the EGU 2014, Vienna, Austria

The 2014 General Assembly of the European Geosciences Union (EGU), held in Vienna from 28 April-2 May 2014, received a total of 15470 abstracts, with approximately 10% (1480 abstracts) in the Soil System Sciences (SSS) Division for 52 scientific sessions, in addition to 836 abstracts in 28 sessions that were co-organized by the SSS Division. This makes the SSS Division the second largest of the EGU, indicating the importance of soils in the earth system. The EGU is an appropriate platform where the Soil and Water Management & Crop Nutrition Subprogramme can interact with other areas of the Geosciences.

In 2014, the SWMCN Subprogramme participated with thirteen contributions, i.e. ten from the SWMCNL and three from the Section. Eight scientists and technicians from the SWMCN

Subprogramme participated in the EGU 2014 to present the work carried out at the Joint FAO/IAEA Division. The presentations dealt with the use of isotope and nuclear techniques in the field of soil erosion, soil organic carbon dynamics and agricultural water management at landscape level.

Details of the contributions can be found in the Publications section of this Report. More information about the EGU 2014 is available on: http://www.egu2014.eu.

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Arctic and Antarctic Research Institute, Russian Federation	Climate change impact assessment in fragile environments
Austrian Agency for Health and Food Safety (AGES), Grabenegg, Austria	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques Assessment of soil moisture availability under mulch-based cropping systems
Austrian Polar Research Institute, Austria	Climate change impact assessment in fragile environments
Austrian Research Centre for Forests, Austria	Climate change impact assessment in fragile environments
Chengdu Institute of Mountain Hazards and Environments; Chinese Academy of Sciences, China	Climate change impact assessment in fragile environments
Climate / Air Pollution Group, Agroscope, Switzerland	Soil organic carbon dynamics, Soil Organic Carbon-14 dating

Institution	Topic
Centre National de l'Energie, des Sciences et de Techniques Nucléaires (CNESTEN), Morocco	Development of guidelines for using fallout radionuclides to assess erosion and effectiveness of soil conservation strategies
Eidgenössiche Technische Hochschule (ETH), Switzerland	Use of oxygen-18 isotopes in phosphate to trace phosphorous sources and cycling in soils
Environment Agency Austria	Climate change impact assessment in fragile environments
Federal Agency for Water Management, Petzenkirchen, Austria	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
Fujian Agriculture and Forestry University, China	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques
GRID-Arendal, Norway	Climate change impact assessment in fragile environments
Institute of Geography; Russian Academy of Sciences, Russian Federation	Climate change impact assessment in fragile environments
International Institute for Tropical Agriculture (IITA), Nairobi, Kenya	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques
Liverpool John Moore University, UK	Climate change impact assessment in fragile environments
Lomonosov Moscow State University, Russian Federation	Climate change impact assessment in fragile environments
National Institute of Water and Atomospheric Research, New Zealand	Climate change impact assessment in fragile environments
National Park Hohe Tauern, Austria	Climate change impact assessment in fragile environments
Technical University of Vienna, Austria	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
Universidad Austral de Chile, Chile	Development of guidelines for using fallout radionuclides to assess erosion and effectiveness of soil conservation strategies
Universidade do Estado de Rio De Janerio, Brazil	Climate change impact assessment in fragile environments
Universidade Federal Fluminense, Brazil	Climate change impact assessment in fragile environments
University of Basel, Switzerland	Development of guidelines for using fallout radionuclides to assess erosion and effectiveness of soil conservation strategies

Institution	Topic
University of Cologne, Germany	Climate change impact assessment in fragile environments
University of Exeter, UK	Development of guidelines for using fallout radionuclides to assess erosion and effectiveness of soil conservation strategies Climate change impact assessment in fragile environments
University of Ghent, Belgium	Climate change impact assessment in fragile environments
University of Gothenburg, Sweden	Climate change impact assessment in fragile environments
University of Graz, Austria	Climate change impact assessment in fragile environments
University of Idaho, USA	Climate change impact assessment in fragile environments
University of Leuven, Belgium	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques
University of Natural Resources and Life Sciences (BOKU), Vienna, Austria	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques
University of Nebraska-Lincoln, USA	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
University of Plymouth, UK	Review of the potential and limitation of Beryllium-7 as short term radio tracer of soil movement Development of guidelines for using fallout radionuclides to assess erosion and effectiveness of soil conservation strategies
University of Vienna, Austria	Climate change impact assessment in fragile environments
University of Vienna, Isotope Research and Nuclear Physics, Austria	Soil dating using carbon-14
USDA, USA	Climate change impact assessment in fragile environments
Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Austria	Climate change impact assessment in fragile environments

An update on the ReNuAL Project for the FAO/IAEA Agriculture & Biotechnology Laboratories

Breaking Ground on the Future Nuclear Applications Laboratories

On 29 September 2014, IAEA Director General Yukiya Amano was joined in Seibersdorf by representatives of Member States and the Food and Agriculture Organization of the United Nations (FAO), as well as IAEA staff members, to break ground on the Renovation of the Nuclear Applications Laboratories (ReNuAL) project, and to celebrate the 50th anniversary of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. There were over 200 participants, with 48 Member States represented.

Participants join the DG in the ground-breaking

ReNuAL is an initiative to modernize the eight laboratories in Seibersdorf that belong to the IAEA's Department of Nuclear Sciences and Applications. The project calls for the construction of a new Insect Pest Control Laboratory (IPCL) to replace the existing IPCL, and a new Flexible Modular Laboratory (FML) to house three additional laboratories, by the end of 2017.

DG Amano was joined for this event by FAO Deputy Director General and Coordinator for Natural Resources Ms Maria Helena Semedo, and IAEA Board of Governors Chair Ms Marta Ziakova, who each delivered remarks in support of ReNuAL and the achievements of the Joint FAO/IAEA Division.

In his remarks, DG Amano said, "Our symbolic ground-breaking today marks the start of the implementation of the ReNuAL project. I am confident that with the active support of Member States, by 2017, we will have a cluster of modern, well-equipped laboratories here in Seibersdorf that we can all be proud of."

DG Amano and DDG Semedo cut a 50th anniversary cake

Moving from Planning to Construction

Initial rendering of the new Insect Pest Control Laboratory (IPCL)

In July, an architectural and engineering firm was contracted to develop the conceptual designs for the Insect Pest Control Laboratory and the Flexible Modular Laboratory, and to update the master plan for the Seibersdorf site. This plan will guide the development to be carried out in the frame of ReNuAL and other related initiatives on the site.

The conceptual design for the IPCL has been completed, and will be completed for the FML by the end of November. Planning for the latter is more complex as it will house multiple laboratories and is being designed to allow laboratory space to be more easily adapted to different activities and needs, and to be modular to make any future expansion more cost-effective.

The IPCL will house laboratory sub-groups dealing with plant pests, livestock pests, human disease vectors and genetics/microbiology. The FML is designed to house laboratories with similar activities to maximize synergies, for example, through the sharing of equipment and certain types of laboratory space. For this reason, the FML will house the Food and Environmental Protection Laboratory, the Soil and Water Management & Crop Nutrition Laboratory, and the Terrestrial Environment Laboratory.

Initial rendering of the new Flexible Modular Laboratory (FML)

The purpose of the conceptual designs is to provide the basic layout and structure of the new buildings, and in doing so to provide a greater degree of certainty regarding the costs of construction.

Upon completion of the conceptual designs, the detailed designs will be developed. These will build further on the conceptual designs and add greater detail by making more concrete decisions on smaller elements of the two buildings, such as the number, size and type of windows, and the number and type of light fixtures to be used. The design process will be completed with the objective of keeping to ReNuAL's €31 million budget.

The process to procure the required design services began in December 2014, with the contract expected to be awarded in March 2015. This would allow for the completion of the designs in mid-2015, at which point bids for the construction contract could be solicited, with construction to begin in the fourth quarter of 2015. This schedule would ensure completion of the new buildings, as planned, by the end of 2017.

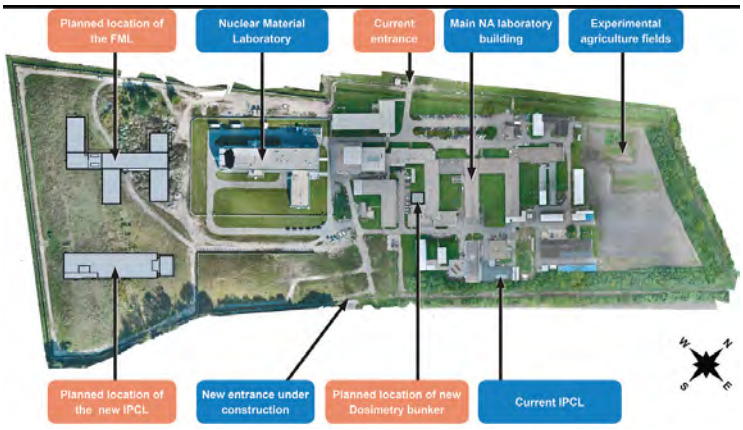
Bids for construction can only be sought, however, if the necessary funds for construction are pledged or available. It is essential, therefore, that these commitments be made by 1 May 2015 to allow ReNuAL to remain on schedule. Delays in receiving these commitments will delay completion of the project, with negative cost implications.

Giving Shape to the Seibersdorf Site

The contract that provided for the development of the conceptual designs of ReNuAL's new buildings also included the creation of a site development plan for the project to identify the land on the Seibersdorf site to be used for the new Insect Pest Control Laboratory (IPCL) and Flexible Modular Laboratory (FML). This plan was completed in October and took into account the identified needs of the other departments in Seibersdorf to ensure that the new buildings to be constructed under ReNuAL would not interfere with any other planned development on the site.

In connection with this, various options for locating the new IPCL and FML on the site were examined, and an architectural and engineering firm was engaged to conduct a cost-benefit analysis to identify the best option. This analysis determined that the IPCL and FML should be built in the greenfield on the southwest of the Seibersdorf site.

Building in the greenfield ensures that construction will not disrupt the ongoing operations of the NA laboratories on the east of the site, and it will facilitate completion of the new buildings by 2017. Additionally, the development of the west of the site that includes the establishment of necessary technical and traffic infrastructure will provide long-term benefits by supporting any future development in this area.



Building Momentum in Resource Mobilization

As was reported to the 59th General Conference in September, ReNuAL had by then raised approximately €860 000 in cash and funding for cost-free experts. These funds and experts have been used to support the initial planning for the project and are supporting the design work that is being carried out.

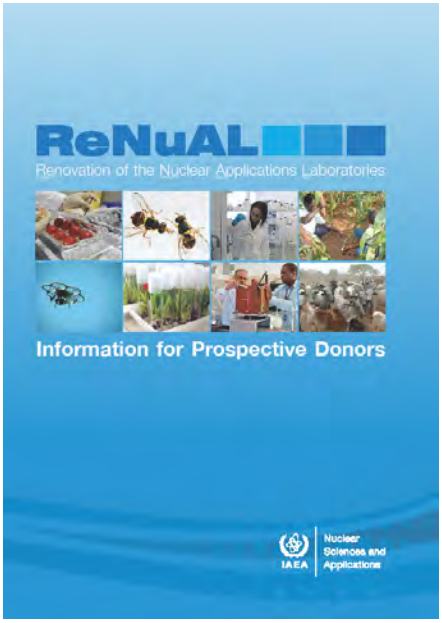
Also, during the General Conference, China announced the in-kind donation of an irradiator that can potentially serve the needs of several laboratories: the Animal Production and Health Laboratory, the Insect Pest Control Laboratory and the Plant Breeding and Genetics Laboratory.

During the November meeting of the Agency's Board of Governors, four Member States announced extrabudgetary contributions to ReNuAL. Germany announced a €1.3 million cash contribution for the procurement of equipment that is urgently needed by the laboratories and that can immediately be used in the existing facilities. Switzerland also announced a cash contribution of €230 000 to fund equipment. Indonesia announced an additional cash contribution of €20 000, to be used as needed to support the project. The United States also provided a €750 000 cash contribution to fund the next stage in designing the Insect Pest Control Laboratory.

Regarding other equipment needs, Agency staff held meetings with manufacturers of linear accelerators to discuss possibilities for establishing partnerships that could include acquisition of an accelerator at reduced or no cost for the new Dosimetry Laboratory bunker to be constructed. The discussions were positive and will continue in the months ahead.

ReNuAL Extrabudgetary Contributions 2014 (€)

Japan	500 000
Kazakhstan	73 600
Rep. Korea	36 500
Russia	103 186
USA	999 112
Other	1 000
Germany	1 334 369
Indonesia	20 500
Switzerland	230 000
Total	**3 298 267**



A donor package describing the elements of ReNuAL in detail as well as their funding requirements was completed in December 2014 and made available to Member States during a Technical Briefing held on 15 December. This package includes the equipment list for ReNuAL, but does not address elements to be implemented or additional equipment to be procured under ReNuAL Plus.

The package will be updated as needed to ensure that potential donors can obtain the most recent information on the project. The package can be obtained in hard copy form by sending a request to nuclearapplications@iaea.org, or electronically through the ReNuAL website at www-naweb.iaea.org/na/renual.

Seeking Biosafety Level 3 Laboratory Capabilities

A number of Member States have expressed support for the establishment by the Agency of biosafety level 3 capabilities that would enable the Animal Production and Health Laboratory to respond to emerging challenges related to transboundary animal diseases. These capabilities are one of the group of project elements now defined as ReNuAL Plus, which was introduced by DG Amano in September to ensure that needs additional to those identified under ReNuAL can be addressed – provided the necessary extrabudgetary resources are available.

The process for licensing and constructing such a facility is complex and can take three to five years. For this reason, the Secretariat has been reviewing various options for obtaining biosafety level 3 capabilities. These include their establishment in Seibersdorf, or possibly at a facility in Mödling belonging to the Austrian Agency for Health and Food Safety (AGES in German). This facility already has biosafety level 3 capabilities and the associated infrastructure that is required, and therefore can potentially support the capabilities sought by the Agency.

AGES facility in Mödling

The IAEA has been granted permission by the Austrian Agency for Health and Food Safety (AGES in German) to use a new biosafety level 3 laboratory under construction at an AGES facility in Mödling beginning 1 July 2015. Access to this facility will provide the IAEA with new capacities that are required to respond to emerging Member State demands in the area of transboundary animal diseases.

The IAEA received this permission following consultations with AGES and Austrian authorities, and after a review of a 2004 Memorandum of Understanding currently in place between the IAEA and AGES. The review found that no changes to the memorandum were required for AGES to allow the IAEA access to its facility in Mödling. Only the memorandum's technical annex will require revision to ensure the IAEA has the necessary flexibility to address diseases and issues of current and emerging importance to Member States.

While this access will help address some of the IAEA's immediate needs for biosafety level 3 capabilities, the IAEA, AGES and Austrian authorities will continue to explore the possibility of an IAEA-owned facility on the AGES campus in Mödling in the months ahead.

A Year of Progress

The ReNuAL project officially began only on 1 January 2014, and from this date rapid and substantial progress was made throughout the year from planning to project implementation and resource mobilization. The project's feasibility study was completed in February, and based on this study the Strategy for the Renovation of the Nuclear Applications Laboratories in Seibersdorf was developed and presented to Member States in June. The addendum to the strategy that established ReNuAL Plus was released in September, and the ground-breaking ceremony was held in Seibersdorf that same month.

This marked the transition from planning to implementation, and significant steps have since been taken. As reported above, the site development plan and conceptual designs, as well as the donor package, were completed in the fourth quarter of 2014.

The efforts made in 2014 helped to raise approximately €3 million for ReNuAL, with over €2 million of this total pledged in the fourth quarter alone. This provides good reason for optimism as the project moves into 2015.

Outreach Activities

Forty-seven delegations visited the NA Laboratories in 2014. These included high-level delegations from Burkina Faso, Burundi, Honduras, Philippines and USA; delegations from the Permanent Missions in Vienna of Belgium, European Union, France, Malaysia, Pakistan, South Africa, State of Kuwait, USA and Viet Nam; 26 National Liaison Officers; diplomats from Armenia, Austria, Bulgaria, Burkina Faso, Croatia, Czech Republic, Denmark, Ecuador, European Union, Finland, France, Islamic Republic of Iran, Italy, Latvia, Libya, Luxembourg, Mexico, Namibia, the Netherlands, New Zealand, Oman, Pakistan, Peru, the Philippines, Portugal, Romania, Russian Federation, San Marino, Serbia, South Africa, Spain, Sudan, Switzerland, UK, USA, Uruguay and Yemen; the CVT Valorisation Sud, France; Eritrean Science and Technology Development Agency; ROSATOM, Russian Federation; Biotechnical Facility of the University of Ljubljana, Slovenia; National Centre for Radiation Service, New Zealand; a scientific delegation from the USA; a group of journalists from Jordan; several IAEA and FAO delegations, including FAO DDG Semedo (on the occasion of the 50th anniversary of the Joint FAO/IAEA Division).

Impressum

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