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Laboratories

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THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

According to the World Bank, two-thirds of poor people live in rural areas. Most of them keep livestock, which should place this activity in the centre of policies for poverty alleviation, in particular in South Asia and Sub-Saharan Africa where nearly 73% of the world's poor people are currently living (Staal et al. 2009; ILRI report to DFID, July 2012; Robinson et al., 2011). For these people, there is a chance to improve their daily livelihoods. The world food economy is being increasingly driven by a shift in diet and food consumption patterns towards livestock products in developing countries due primarily to urbanization, a rise in incomes and population growth which will provide new opportunities. Meeting the increase in demand for food of animal origin will place enormous pressure on the global food system, the environment, and the sustainable increase in livestock production, mainly poultry, sheep and goats as those animal species have a central role in the world's poorer household, i.e. small farmer's households (ILRI report to DFID, July 2012). Indeed, it is not surprising to find that the distribution densities of sheep and goats correlate to that of the poor and the developing world. Small ruminants not only provide meat for domestic consumption, they also have a high reproduction rate that allows them to be easily sold for cash allowing the purchase of staple foods and other commodities required for the household. In addition, sheep and particularly goats (often referred to as the "cattle of the poor"), provide milk to poor small farmers who cannot afford to buy cattle. Meeting the increase in demand for food of animal origin will place an enormous pressure on the global food system, the environment, the sustainable increase in livestock production, in particular on animal species that are important for the poor This perspective calls for an important and over-riding improvement of the productivities of those species by controlling major animal diseases and improving the genetic potential of local breeds. To address this challenge and help the IAEA and FAO Member States, major activities of the Animal Production and Health Laboratory (APHL) of the IAEA are focused mainly on small ruminants: identification of genetic markers for disease resistance in local breeds to be exploited in breeding strategies, development of tools for the control of major sheep and goats infectious diseases through the use of molecular, nuclear and nuclear-related technologies.

In the animal genetics area, APHL is involved in research and development (R&D) to identify markers for desirable traits and their use in breeding strategies for the improvement of livestock productivities and for breed preservation. In 2013, those activities enabled the discovery of more than 180 SNP (Single Nucleotide Polymorphism) markers across candidate genes involved in pathogen recognition and immune pathways in sheep. Based on those results, genotyping assays were developed for identifying genes or families of genes that are involved in animal resistance/tolerance to gastro-intestinal parasites infestation. Those assays are currently under field testing to ascertain the association of the targeted markers with host resistance against gastro-intestinal parasites. One parasite that is causing enormous losses in sheep and goats is the *Haemonchus* species. Considering the varying levels of drug resistance among *Haemonchus* parasites, APHL made new initiatives to evaluate their genetic diversity and molecular epidemiology. Parasite samples from Pakistan were analysed to assess

distribution of different species/variants of Haemonchus parasites and the frequency of alleles associated with drug resistance.

Amongst the threatening diseases of sheep and goats, peste des petits ruminants (PPR) is the most important and is certainly the main constraint to intensive small ruminant farming where PPR is endemic: Africa, the Near East, the Middle East and large parts of Asia. Due to its high economic incidence, its high morbidity and mortality rates of up to 50-80% in some cases, its potential to spread quickly and over long distances in the field, PPR is classified in the list of animal diseases to be notified to the World Organization for Animal Health (OIE). Since 2007, the geographical distribution PPR has been expanding steadily in Africa and Asia. This expansion is due to intensification of livestock trade but also by the use of new diagnostic tools that are highly sensitive and highly specific. The research and development activities that have been conducted in APHL since 2001 to develop some of those tools have contributed to the improvement of this knowledge on PPR epidemiology. This was again the case in 2013. Indeed in that year, gene sequencing of samples collected in Nigeria showed a change in the distribution of the causal agent, the PPR virus. Stains of this virus are grouped into 4 lineages. The results of samples analysis carried out at APHL showed that the lineage II that was present in Nigeria until the end of the 1990's is being replaced by virus strains of another group, the lineage IV. However, in Benin, a neighbouring country, no change is noted in the virus strain genotype according to the result that was obtained by comparing the gene sequence of a virus isolated in 1969 with that of 2010.

Considering the threat that PPR is causing to food security in many developing countries, there is a growing interest from international organizations and donors to support programmes for early diagnosis, control and eventually eradication of PPR as part of efforts for poverty alleviation and food security. In 2013, experts were meeting regularly to develop a strategy to control and eradicate PPR. Scientists from the Animal Production and Health Subprogramme, because of the work done in APHL on PPR, took part of those meetings. For that strategy, it is advised to combine PPR control, even global eradication, with at least another small ruminant disease. The sheep and goat disease that is the most indicated disease to be targeted in the control programme along with PPR is capripox. This suggestion is made based on the fact that, apart from some exceptions, the global distribution of PPR is similar to that of capripox. This is another viral disease on which APHL has a well-recognized expertise. In 2013, we developed an easy test to perform for the identification and genotyping of capripoxviruses. Another test, a pan-pox virus test, designed for detecting many ruminant poxviruses, including capripoxviruses, was also developed. We also developed a disease diagnosis test for the detection of 4 major pathogens causing respiratory disease symptoms in small ruminants: PPRV, capripoxvirus, the *Mycoplasma capricolum subsp. capripneumoniae* (Mccp) and the bacteria *Pasteurella*. Such a test will constitute an important tool for disease surveillance during the PPR control/eradication programmes.

In addition to PPR and capripox, two other important diseases were subject of R&D in APHL in 2013: trypanosomosis and African swine fever (ASF). After having determined the efficient irradiation dose obtained to create a non-replicative but metabolically active trypanosomes according to *in vitro* studies, *in vivo* tests using mice were carried out in 2013. Results showed that mice inoculated with trypanosomes irradiated at a dose 200 Gy are fully protected against

a homologous challenge. Although those mice did not resist to heterologous challenge, measurement of different immune response parameters in serum of immunized mice indicates that irradiating parasites at levels between 140 Gy and 200 Gy for immunisation potentiates inflammatory immune responses that are known to be important for protection during the early phase of infection. This R&D on trypanosomosis is part of a current IAEA Coordinated Research (CRP) that aims at developing irradiated vaccines for the control of infectious transboundary livestock diseases (CRP D32029).

For ASF, a highly contagious swine disease that emerged into east Europe from its well-known stronghold endemic area in Africa, a molecular epidemiology study was continued in 2013 by analysing samples that were collected in the Democratic Republic of Congo (DRC) between 2003 and 2012 from suspected African swine fever cases. The results indicated the circulation of several strains of ASFV genotype I and IX.

Finally, for animal genetics, APHL supported Myanmar and Zambia to characterize their native cattle breeds using DNA markers. More than 300 animals belonging to six breeds of cattle were genotyped and sequenced to assess their genetic variability. In addition, the global genetic (DNA) repository of indigenous livestock breeds has been significantly strengthened with more than 900 new DNA samples, a valuable reference material for collaborative animal genetic research.

In addition to R&D, another pillar of APHL is promoting capacity building in IAEA and FAO Member States. As part of those activities, APHL, in link with its partners, FAO Animal Health Service in Rome (Italy) and Swiss Institute of Bioinformatics in Geneva (Switzerland), update the bioinformatics eLearning tool that was developed in 2012 for animal pathogen molecular epidemiology studies. With the objective of promoting the implementation of quality assurance and quality management in counterpart veterinary diagnostic laboratories, it conducted a proficiency test for the diagnosis of PPR. It conducted four group training courses and hosted six fellows, all funded by either extra-budgetary funds of by the IAEA Technical Cooperation (TC) Department, an indication of the strong implication of APHL activities with those of TC.

APHL activities that were carried out in 2013, in particular capacity building activities, benefited from the financial of the FAO-WHO-OIE Tripartite project “Identify” (USA-funded Project), the IAEA-PUI programme projects (USA and Japan Funded PUI projects), the African Renaissance Fund (South Africa- funded project).

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

Irradiation-based technology in the development of vaccine for the control of trypanosomosis

Trypanosomosis is an important parasitic disease in mammals. The causal agents, blood parasites of the *Trypanosoma* species, can be transmitted mechanically, as in the case of *Trypanosoma evansi* which affects different animal species, or through a biological vector like the tsetse fly. The *Trypanosoma* species that is transmitted by the tsetse fly is not only a major public health issue that can be highly pathogenic to humans, but is also a big hindrance to the development of livestock resources with about 8.7 million km^2 of the African continent infested by tsetse flies. The disease puts 4.6 million cattle at risk annually. Trypanosomosis is currently controlled through the use of drugs to kill either the insect vector or the infecting parasites themselves. In 2011, APHL started a research programme to explore the possibilities of developing a potential anti-trypanosomal vaccine through the use of irradiation technology. The preliminary experiments are focused on *Trypanosoma evansi* (*T. evansi*), a trypanosome species that is widely distributed in Africa, Asia and Latin America. The results obtained in 2011 and 2012 showed that irradiating 1 x 10^5 parasites using 250 Gy produces parasites that do not replicate but are still able to synthesize proteins, i.e. are metabolically active, for up to

3 days *in vitro* before dying. The study was continued in 2013 by *in vivo* testing in mice using parasites that had been irradiated using different doses ranging from 100 Gy to 600 Gy. Before mouse inoculation, the viability of the irradiated parasites was analysed *in vitro* by microscopic observation and fluorescence using a vital staining dye, the carboxyfluorescein diacetate succinimidyl ester (CFSE). The mice were inoculated with the irradiated trypanosomes three times over two week intervals, subsequently challenged with homologous virulent parasites and finally exposed to heterologous virulent parasites. The results of the experiment are summarized in figure 1. They revealed that irradiation levels at 200 Gy and below lead to a rebound in division (Figure 1, column 3). This trend was also observed using microscopy (Figure 1, column 2). However, mice that were inoculated with parasites irradiated at a dose of 200 Gy did not develop an infection as was the case in mice inoculated with parasites treated with higher doses of irradiation. The possibility of infection was seen to increase with a reduction of irradiation dose used. Mice immunized with parasites irradiated at 140 Gy had a 67% chance of survival and this decreased to 0% when parasites irradiated at 100 Gy were used for immunization (FIG 1, column 4).

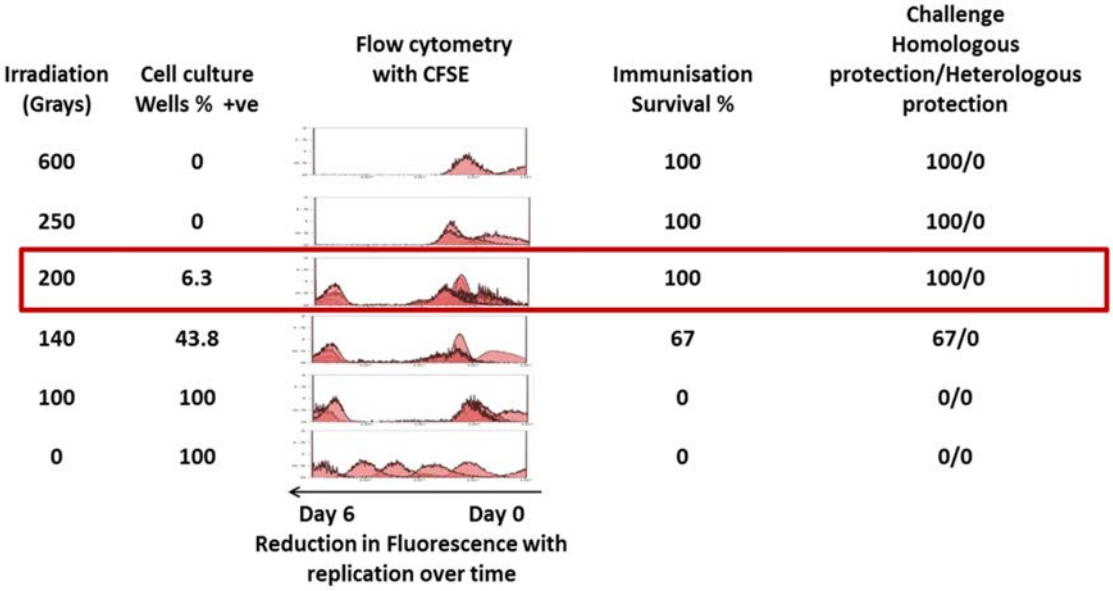

FIG. 1: Gamma irradiation of T. evansi: In vitro and in vivo parasite dynamics of irradiated trypanosomes from 0Gy to 600 Gy.

All groups of mice that survived three rounds of immunization subsequently also survived homologous challenge, though this can be explained by the similarity of the parasite surface glycoprotein used for both immunization and infection (Figure 1, column 5). None of the mice immunized using parasites irradiated at different doses survived heterologous infection, although this experiment is still being undertaken (Figure 1, column 5).

During the period of immunization experiments, serum was regularly collected from all the animals in order to measure different parameters of the immune response and in particular cytokine concentrations, proteins that are involved in host inflammatory immune responses. Mice immunized with parasites irradiated between 140 Gy and 200 Gy displayed particularly higher levels of IL-6, a cytokine in the group that mediates inflammatory responses, when

compared to mice immunized with parasites irradiated at levels higher than 200 Gy (Figure 2). Inflammatory responses are important during the early phase of a trypanosome infection and are responsible for clearing the first wave of parasites. Inducing this type of response using immunization would help in preventing disease. This observation indicates that lower levels of irradiation are able to induce a stronger pro-inflammatory response that is seen early during trypanosome infections.

In comparison, mice immunized with parasites irradiated between 140 Gy and 200 Gy displayed depressed levels of IL-10, a cytokine that belongs to the group that mediates non-inflammatory responses that appear later during a trypanosome infection (Figure 2). Non-inflammatory responses are not protective during the early phase of an infection and only appear later when the host is required to reduce inflammatory responses that can lead to pathology if sustained over a long period of time. Taken together, this indicates that irradiating parasites at levels between 140 Gy and 200 Gy for immunization potentiates inflammatory immune responses that are known to be important for protection during the early phase of infection. Further studies on the *in vivo* dynamics of using irradiated parasites for immunization are required to understand immune related changes in the host and whether it would be possible to exploit these observations to develop a functional irradiated vaccine for trypanosomosis.

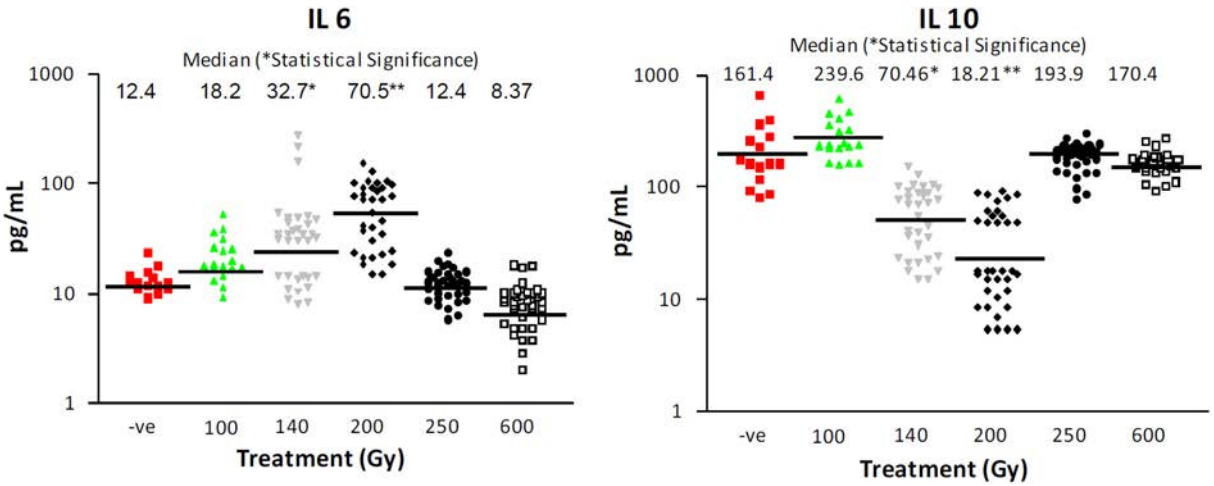

FIG. 2: Quantification of IL6 in mouse sera during immunization. Interleukin levels before infection in sera from mice immunized with parasites irradiated from 100 to 600 Gy. Results from IL-6 are displayed.

Peste des petits ruminants

Peste des petits ruminants is a highly contagious infectious disease of domestic and wild ruminants. It constitutes a major constraint on the increase of small animal production in countries where it is endemic: Africa, the Near East, the Middle East and large parts of Asia. It is so economically important that it is one of the priority diseases of the FAO and is on the list of animal diseases of which outbreaks have to be notified to the OIE (the Word Organisation for Animal Health). As of 2002-2003, the IAEA, at its laboratory in Seibersdorf, 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 PPRV from Nigeria

In July 2013, 140 samples from 79 animals (goats and sheep) suspected of being infected with PPRV and collected from throughout Nigeria (Fig. 3) from 2009 to 2013 were provided by the National Veterinary Research Institute (NVRI), Vom, Nigeria, to the APHL for (Fig. 4) for diagnosis. Thirty–three samples were positive and an analysis of the sequence of the N gene revealed that there are viruses from two distinct lineages of PPRV (lineage IV and II) presently circulating in the country. Both the lineage II viruses (n=7) and the lineage IV viruses (n=26) grouped into two clades [IIA (n=3), IIB (n=4), IVA (n=10) and IVB (n=16)] highlighting further diversity between the viruses circulating in the country (Fig. 4).

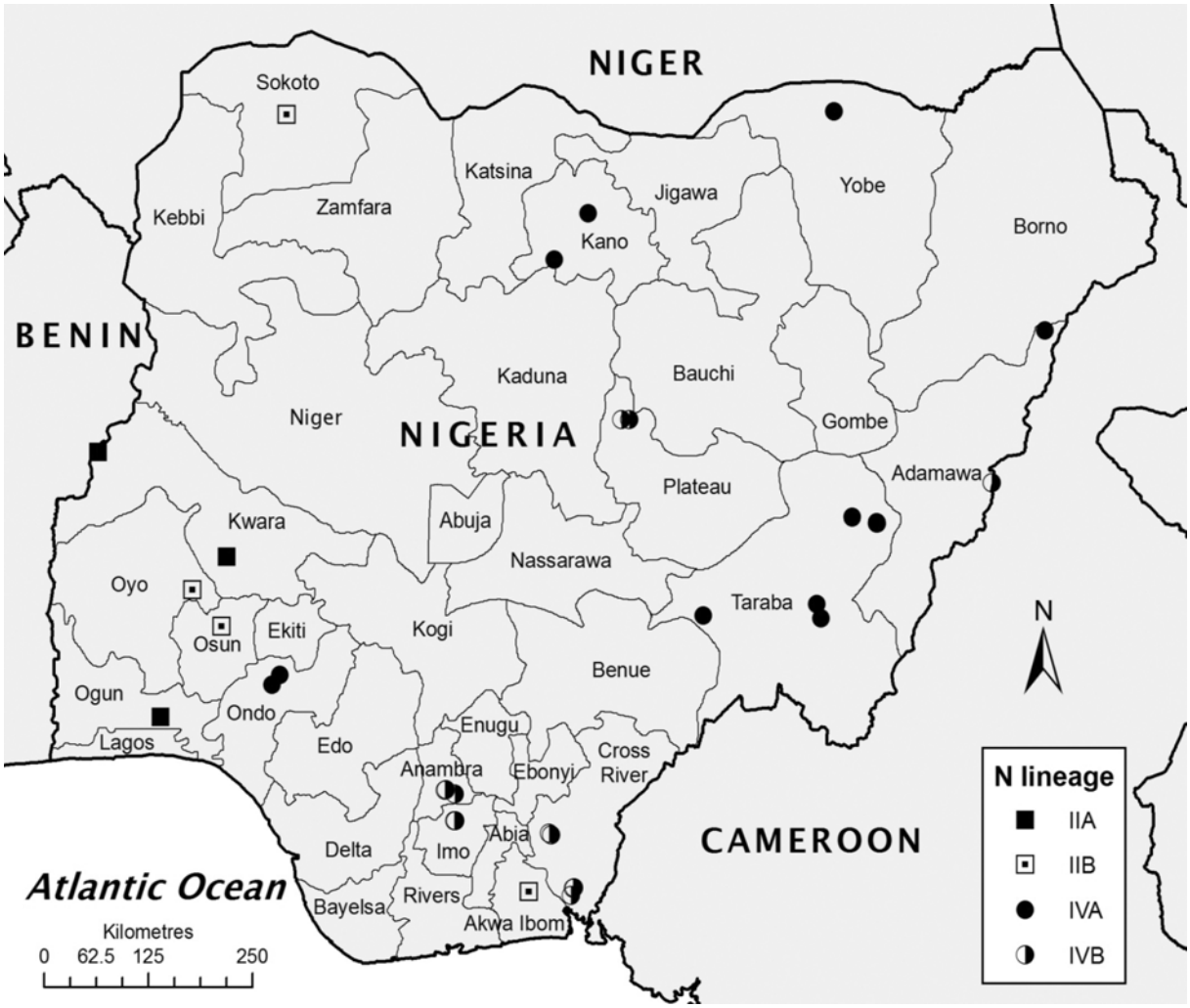

FIG. 3: The distribution of PPRV lineage II (circles) and IV (squares) in different states of Nigeria. The map shows the location of villages where positive PPR samples were collected. Adamawa state (samples were from Gulak and Njobli); Taraba state (samples were from Jalingo, Wukari, Kassa, Maihula and Garbabi); Plateau state (samples were Angwa kurma in Jos); Yobe state (samples were Yusufari); Kano state (samples were from Dogongora and Kano Municipal); Ondo state (samples were from Akure and Idanre); Kwara state (samples were from Baruten); Akwa Ibom state (samples were from Uyo); Imo state (samples were from Eziama-Obaire and Iho); Anambra state (samples were from Adazi-Ani, Eziora, Umuchi and Amada); Oyo state (samples were from Bodija); Sokoto state ((samples were from Sokoto Municipal abattoir); Osun state (samples were from Iregba); Ogun state (samples were from Ijebu-ode); Cross Rivers satate (samples were from Biase, Ikot-Omini and Ibogo).

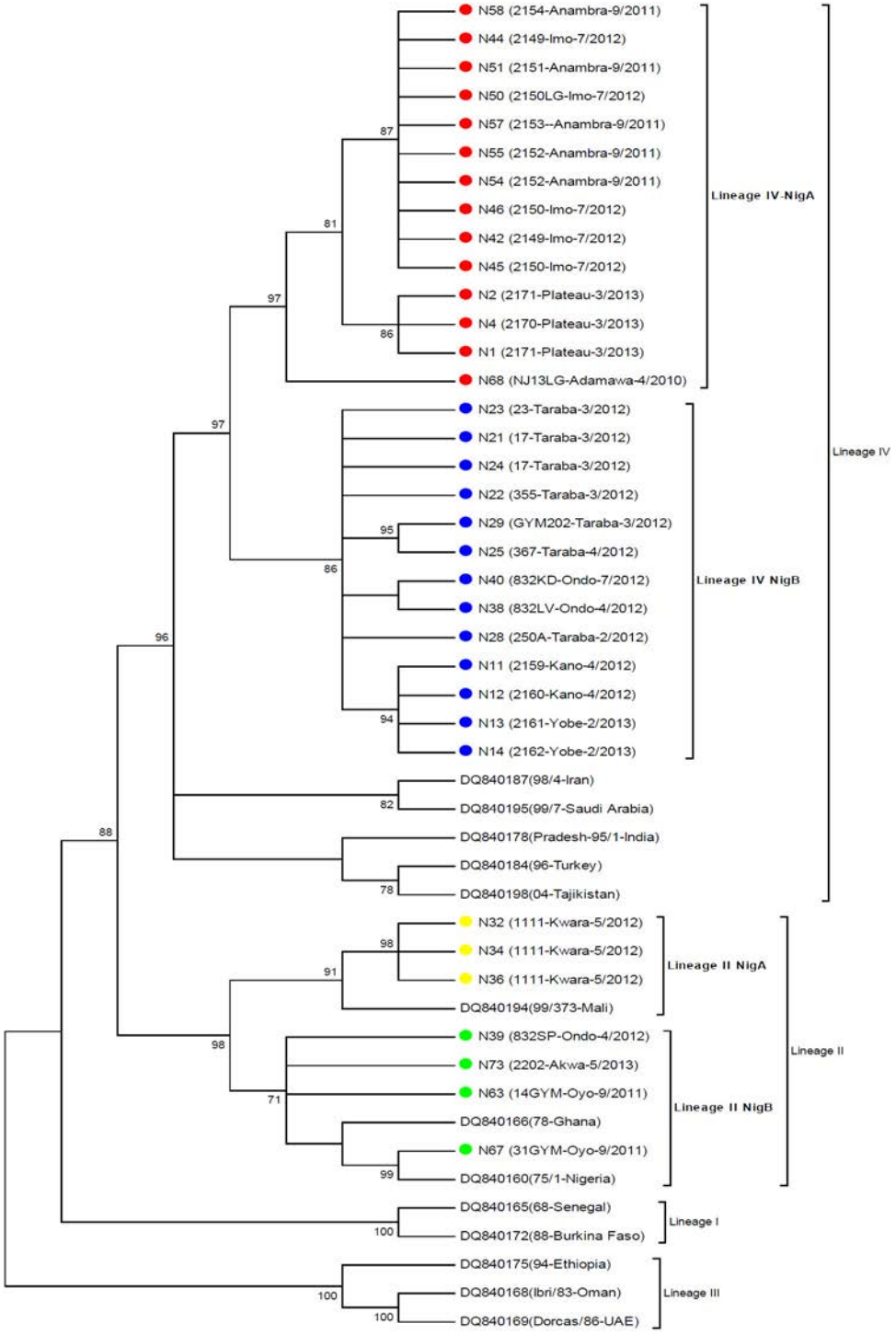

FIG. 4: Phylogenetic analysis of PPRV. Neighbour-joining unrooted cladogram showing the relationship between the PPRV N gene sequences from this study (indicated by black circles, ISO 3166 country code and state code, year of sample collection and sample laboratory number) with unique published sequences obtained from GenBank (indicated by accession number, ISO 3166 country code, year of isolation and name of isolate). The numbers at the nodes are bootstrap values obtained from 1000.

Temporal and spatial analysis of PPRV for the Democratic Republic of Congo (DRC)

In a previous report the identification of possible temporal and spatial changes to PPRV circulating in the DRC from 2009 to 2012 was reported. To add to this study, in July 2013 more samples from different areas of the country were sent by the Laboratoire Vétérinaire de Kinshasa (DRC) to APHL for sequencing. The phylogenetic tree generated using the NP gene fragment (Fig. 5) clearly confirms our previous findings that all isolates were of lineage IV and there was little genetic variation between the isolates over time. There is no evidence of other lineages circulating in the country which is of interest given that lineage III viruses have been identified in Uganda, neighbouring country.

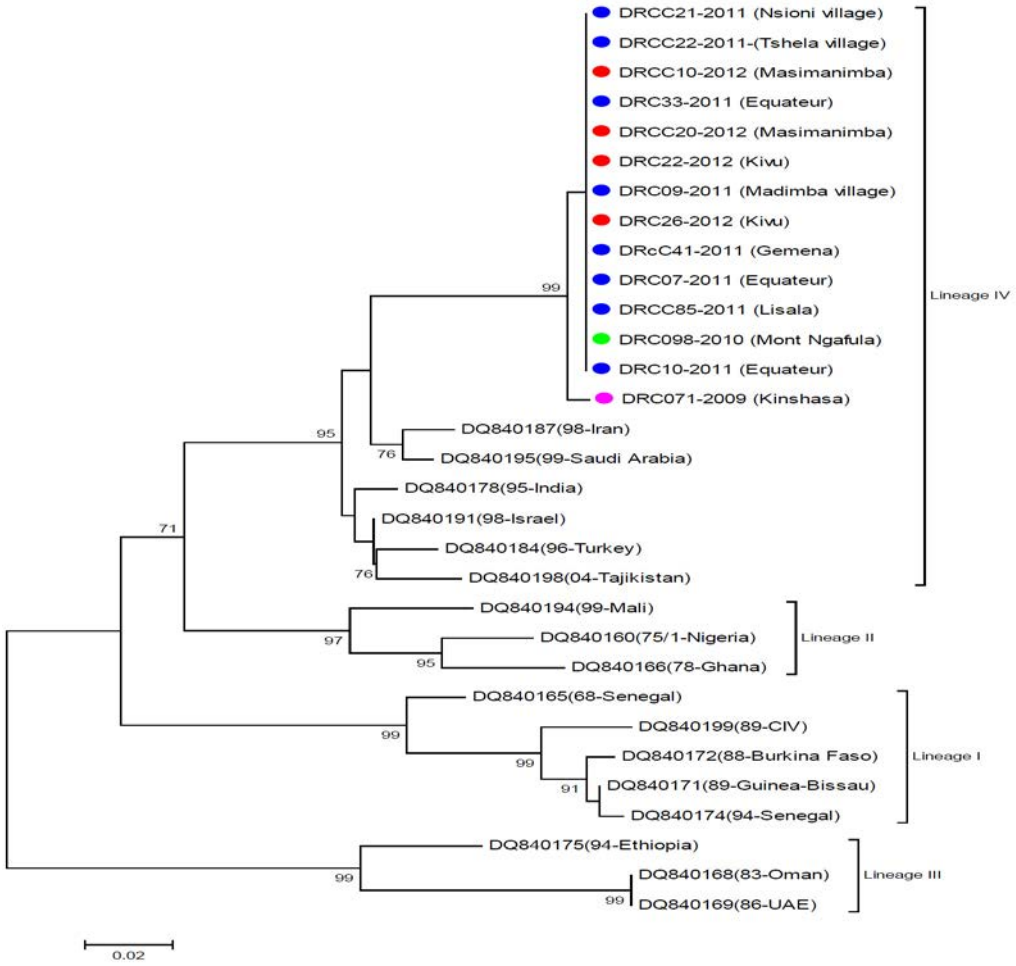

FIG. 5: Phylogenetic analysis of PPRV from DRC using the NP gene fragment

Full genome sequencing of PPRV

A number of full genome sequences of PPRV have been generated. Specific details of the completed genomes are provided below.

- Comparison of the genomes of two peste des petits ruminants viruses isolated 42 years apart in Benin. The full genome coding region of two PPRV from Benin, collected in 1969 and 2011 respectively has been sequenced. This has provided us with a unique opportunity to determine changes in the genomes over a 42-year time

period. Phylogenetic analysis revealed that both viruses clustered within the lineage II. Comparison of the genomes revealed a 95.3% identity at a nucleotide level between each other. At the protein level an analysis of specific amino residues of known or putative function did not identify any significant changes over this period.

- Full genome sequencing of a PPRV isolated in Ghana in 2010.The full genome of a PPRV collected in Ghana on 14/01/2010 has been sequenced. The virus belongs to lineage II and the genome is 97% identical at the nucleotide level to genome of the PPRV isolated from Nigeria in 1976. An analysis of specific amino residues of known or putative function has not identified any significant changes.

Development of a diagnostic test for peste des petits ruminants

Rinderpest was declared to be eradicated worldwide in 2011. A closely related disease, the peste des petits ruminants (PPR), has now become the next target for programmed eradication. As for rinderpest, a highly efficient attenuated vaccine exists that provides a strong and lifelong protective immunity in inoculated animals. There are also highly sensitive and specific tests for the detection of the causal pathogen, the PPR virus. However, the current tests available for serological diagnosis of the disease, although based on the use of monoclonal antibodies specific to each virus, rinderpest virus and PPRV, still present some cross reactions. Since rinderpest surveillance will continue for many years to come, it is of great importance to develop an early and rapid PPR test for the specific serological diagnosis of PPR to avoid confusion with rinderpest. For a year now, work is being carried out at APHL to develop a sensitive and specific PPR serological assay based on luminescence. A nucleic acid vector has been constructed by molecular-based technology to produce in cells a fusion protein composed of a fragment of the most abundant PPRV protein, the nucleoprotein, and the luciferase, an enzyme that will produce light following degradation of a substrate. The fragment of the PPR nucleoprotein serves as a hook to capture the PPR antibodies when present in the test serum and which are then retained on the test plate. Using this procedure, an assay that enables a specific serological diagnosis of PPR has been developed. It has been tested with several PPR experimental samples and compared to a commercially available PPR ELISA kit. The results between the two tests agree 100% for the detection of PPR positive and negative samples. An interesting feature of this assay is the fact that it uses 1µl of sample per reaction compared to the usual 25 to 40µl of commercially available kits. This is especially relevant when sampling and testing for wildlife samples which are precious as they are very expensive to collect.

Production of monoclonal antibodies (mAbs) against the goat signaling lymphocytic activation molecule (SLAM), the cell surface protein used by PPRV as a receptor to enter into the cell host

In 2009 a monkey cell line (CHS20) expressing the goat SLAM protein was developed in APHL; these cells are highly sensitive in isolating PPRV *in vitro*. Since then this cell line has been distributed to Member States upon request. It appears now that for studies that aim at unravelling the pathogenesis of PPR, anti-SLAM antibodies is needed. Such antibodies are not available yet. Therefore work started a year ago to develop and produce anti-goat SLAM monoclonal antibodies (mAb). With this purpose in mind, a recombinant goat SLAM protein was produced and used to immunize mice. Spleen cells from those animals were fused with mice myeloma cells to produce hybrid cells, the hybridomas. Upon screening 3 clones of those

cells (1-H12, 4-A1 and 2-A6) producing antibodies recognizing the recombinant goat SLAM were obtained (see Fig. 6). Those mAbs will be used for the identification and quantification of cells producing the natural goat SLAM and to study the susceptibility of cells to PPRV infection.

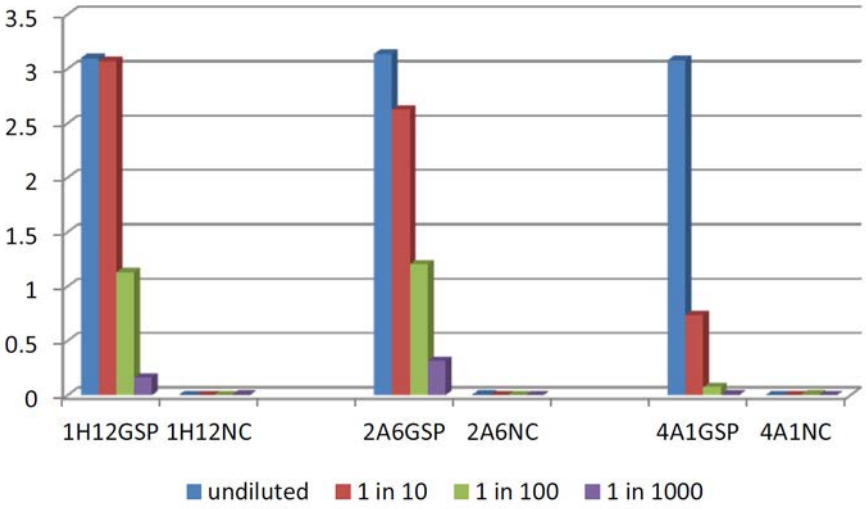

FIG. 6: Titration result by i-ELISA of the 3 hybridoma clones to measure the level of produced anti-goat SLAM antibody. GSP: positive control (purified goat SLAM protein at 1:400), NC: negative control (E. coli culture lysate at 1:400)

African swine fever

African swine fever, a deadly viral infectious disease of pigs is currently on continual expansion in east Europe, mainly through wild boars. Today, no vaccine or treatment is available to control the disease.

In 2013, APHL received 140 swine samples that were collected in the Democratic Republic of Congo (DRC) between 2003 and 2012 from suspected African swine fever cases. Those samples were screened by real time polymerase chain reaction nucleic acid amplification assay (real time PCR). Positive samples were further characterized to determine their genotype by sequencing the appropriate African swine fever virus (ASFV) protein P72 gene, the protein gene classically used by all laboratories to genotype ASF. For the purpose of fine-tuning the genotyping for a better identification of the virus strain, it was previously demonstrated that additional genes should be analysed: the protein p54 gene and the central variable region (CVR) of the gene called 9RL open reading frame. The results showed that ASFV strains circulating in DRC during that period belong to at least 3 different p72 genotypes (Fig. 7). Twentyfour isolates fall into two distinct sub-clusters of the ASVF genotype I. Ten isolates were located in the ASFV genotype IX and one isolate in the ASFV genotype XIV.

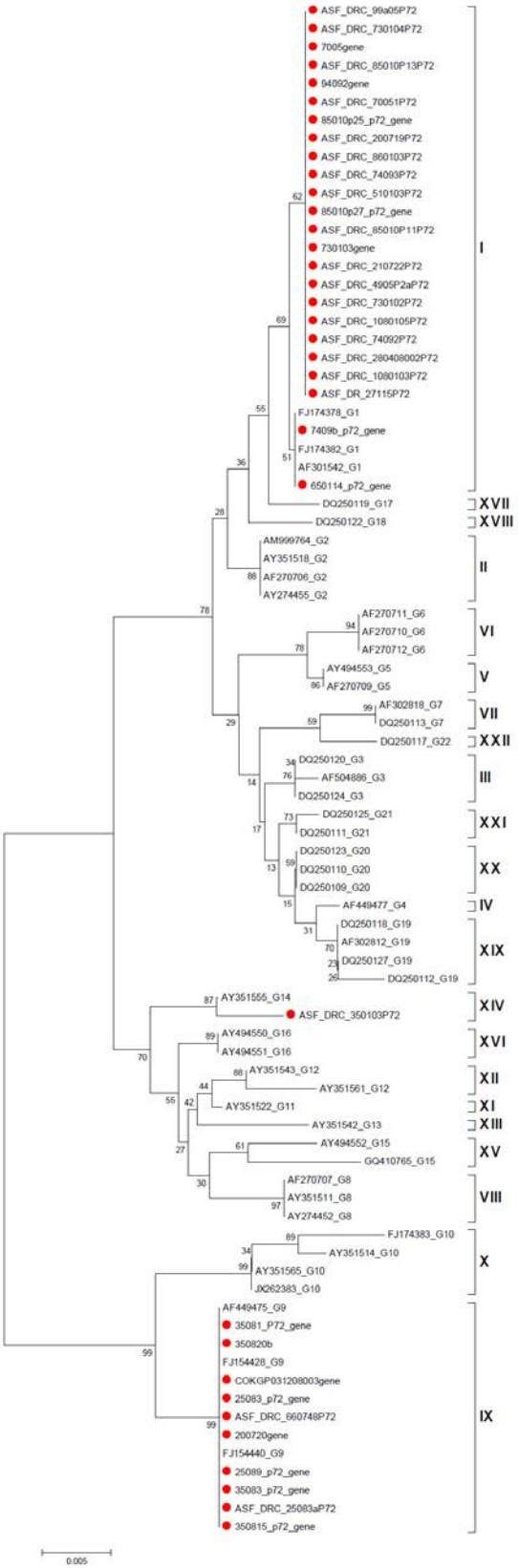

FIG. 7: Neighbour joining tree showing the 22 ASFV p72 genotypes labelled (I-XXII). The tree was constructed using the test of phylogeny, bootstrap method with 1000 replications in MEGA 5. The DRC samples are labelled with a red circle. Of the isolates in the tree, the DRC samples characterized in this study represented genotypes I, IX, and XIV.

The analysis of the P54 gene confirmed the ASFV isolates distribution among the 3 ASFV p72-based genotypes: genotype I, IX and XIV.

The analysis of the CVR profiles within the p72-based genotype I and the genotype IX showed that more subgroups of isolates can be defined within each of these genotypes. This indicated the circulation of several strains of ASFV genotype I (9 different strains) and ASFV genotype IX (2 different strains) in the country.

Capripox disease

Sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV), the three members of the genus capripoxvirus (CaPV) of the *Poxviridae* family are responsible for causing capripox disease in sheep, goats and cattle, respectively. These three viruses are not strictly host specific and are antigenetically very similar. This creates an urgent need for routine differentiation tools to allow an accurate identification of the pathogen to implement better control strategies.

Capripoxvirus genotyping tools

In 2012, APHL designed and evaluated a method for capripoxvirus genotyping based on unlabelled probes. In 2013, the cross platform compatibility of this method was further tested by performing the assay and evaluating its performance on five different real time PCR platforms. Indeed, because various real time PCR platforms are available in Member State laboratories, the cross-platform compatibility study was needed to assess and address the eventual need of setting additional assay parameters for each of these platforms. All the platforms that were tested, apart from one, were found suitable for the assay. The result of this work was disseminated through a publication in the international refereed journal PlosOne in 2013 and transferred to two Member State laboratories in Ethiopia (Fig. 8 as an example of result in testing different viruses). Further efforts to transfer this assay into additional laboratories will take place in 2014. It is expected that this method will facilitate the implementation of capripoxvirus epidemiological studies in veterinary diagnostic and research laboratories with moderate resources.

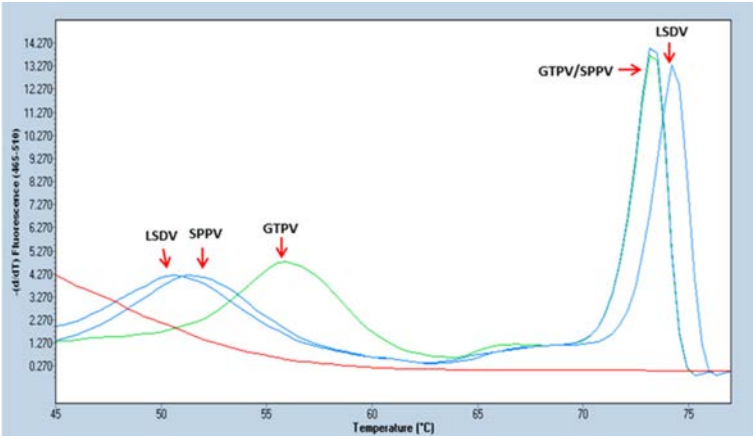

FIG. 8: Snapback primer genotyping of CaPVs using the Roche LightCycler. The melting curves analysis show two melting peaks for each of the CaPV three genotypes (GTPV, SPPV and LSDV) corresponding to the snapback stem melting peak at lower temperature

Molecular epidemiology of capripox

The APHL is recognized by many Member State veterinary laboratories, including the OIE reference laboratories, for the good quality of the tools it has developed for capripoxvirus genotyping and molecular epidemiological analysis of the diseases. Regularly, the laboratory is requested by those Member State laboratories to characterize their local strains of capripoxviruses. At the end 2012 and early 2013, counterparts from Senegal and Sudan requested the APHL to characterise field and vaccine strains of sheeppox virus and lumpy skin disease virus.

Samples that were received from the counterpart in Senegal were collected in sheep and cattle in that country and in Mauritania, a neighbouring country. Upon testing, 3 samples were characterized as belonging to the LSDV group of the capripoxviruses. One sample collected in 1985 in Mauritania was characterized as a SPPV. The sheeppox virus vaccine strain produced in Senegal was characterized as a SPPV.

For Sudan, 4 field samples collected in cattle, and sequential passages of a cattle strain which leaded to the strain attenuation were analysed. The results confirmed both strains to belong to the LSDV group of the capripoxvirus genus.

Multiple pathogen detection

Pan-poxvirus detection method

The APHL initiated a program for multi-parametric pathogen detection assays in specific sample types based on the observed clinical symptoms. Among this category of assays, a pan-pox detection method designed in 2012, was further fine-tuned and evaluated in 2013. This assay was shown to be able, in a single reaction tube, to detect up to 8 poxviruses affecting ruminants and camels: sheeppox virus (SPPV), goatpox virus (GTPV), lumpy disease virus (LSDV), orf virus (OV), bovine popular stomatitis virus (BPSV), pseudocowpox virus (PCPV), and camelpox virus (CMPV) and cowpox virus (CPV). Indeed, the assay can simultaneously differentiate the 3 genus they belong to: capripoxvirus genus (SPPV, GTPV and LSDV), parapoxvirus (OV, BPSV and PCPV) and orthopoxvirus (CMPV and CPV), and further discriminate between the individual species within each genus (Fig. 9).

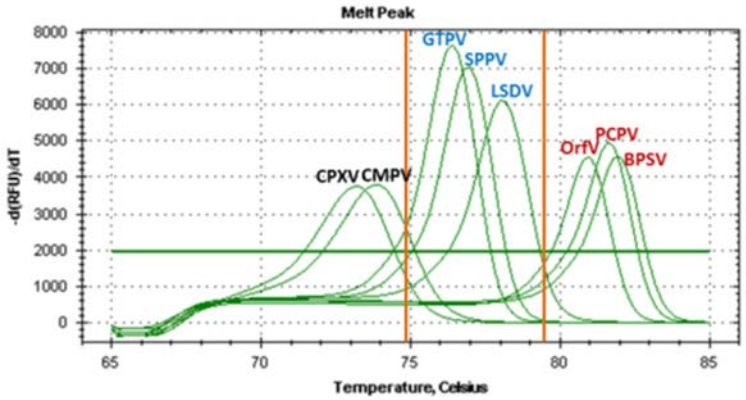

FIG. 9: Multiparameric detection of 8 poxviruses based on their melting peaks.

This type of assay can help in detecting pathogens from non-expected hosts. For instance, the current assay was used to identify pseudocowpox virus in samples from camelpox suspicions. In 2014, the final validation of this assay will be undertaken, before its transfer to IAEA and FAO Member States.

Detection of respiratory pathogens in sheep and goats

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, in certain cases, from an unexpected host and enhancing animal diseases surveillance and management capacity, especially at the domestic animal/wildlife interface, in Member States. In 2013, APHL developed a nucleic acid amplification-based Taqman assay, for the detection of respiratory pathogens highly prevalent in sheep and goats: e.g. PPRV, capripoxvirus, parapoxvirus, *Mycoplasma capricolum subsp. capripneumoniae* (Mccp) and *Pasteurella* (Fig.10). This assay, in a single run, utilizes low sample volumes, increases the rapidity and will improve surveillance of small ruminants respiratory diseases and disease control programmes in Member States.

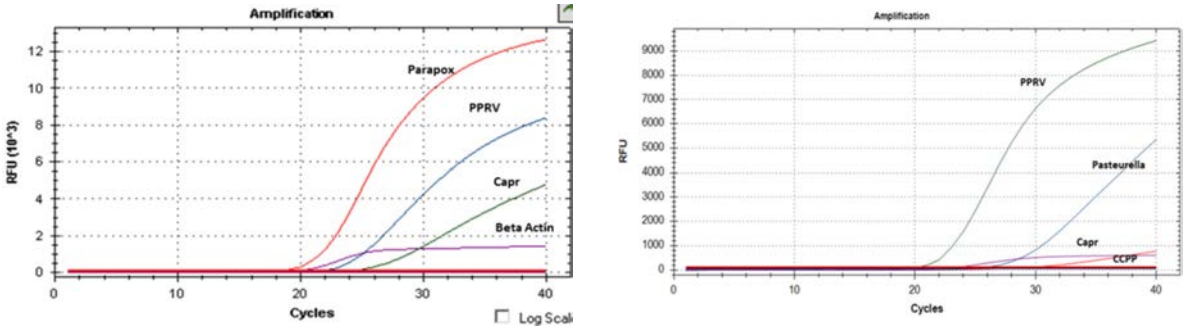

FIG. 10: Taqman assay depicting the detection of five different pathogens in two panels – Panel 1: PPRV (Fam- Blue), capripox (Hex-Green) and parapoxvirus (Texasred-Red) with an internal control beta-actin (Cy5-Indigo) and panel 2: MCCP (Texasred-Red), Pasteurella *(Fam-Blue), capripoxvirus (Cy5-indigo) and PPRV (Hex-Green)*

Animal Genetics

Genetic variation in the control of resistance to infectious diseases in small ruminants for improving animal productivity

Field testing of DNA markers for parasite resistance in sheep

Haemonchus parasite is a gastro-intestinal worm of ruminants. In sheep and goats, it is responsible for anaemia, bottle jaw, and death, mainly during summer months in warm, humid climates. It is one of the major parasites affecting ruminants all over the world. This parasite causes serious production and economic losses to farmers around the world. Breeding sheep and goat for enhanced host resistance would be a long term and sustainable strategy in managing and controlling these parasites. In continuation of its efforts in identifying DNA markers for parasite resistance in sheep, APHL developed genotyping assays for 194 novel DNA (SNPs-Single Nucleotide Polymorphisms) markers identified in several candidate genes related to immune pathways, pattern recognition receptors, major histocompatibility complex,

etc. Of these, 181 DNA marker assays were validated and are currently being tested in around 3000 phenotyped recorded animals which are part of the field trial in six countries. The genotyping of first set of 1536 animals at 181 SNP loci has been completed. The process of sequencing candidate genes for SNP discovery to develop DNA marker set in goats is under progress.

Molecular epidemiology and characterization of Haemonchus *variants*

Ruminant livestock in tropical countries including sheep, goat, cattle and camel are infected by different sympatric species of *Haemonchus* parasites like *Haemonchus contortus, Haemonchus placei, Haemonchus longistipes,* etc. These parasites affect sheep and goats around the world by causing death of young ones and reduction in growth rate and result in low meat production. Apart from this, the growing level of drug resistance in these parasites is becoming a huge challenge in controlling them. The level of anthelminthic (drug) resistance in different species/variants of *Haemonchus* varies. Hence, 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 targeted sequencing of parasite genome to characterize the genetic diversity of *Haemonchus* variants. Three different regions of *Haemonchus* genome viz. nuclear internal transcribed spacer 2 (ITS2), mitochondrial cytochrome oxidase subunit 1 (COI) and mitochondrial nicotinamide dehydrogenase subunit4 (ND4) were optimized for epidemiological screening and genetic diversity analysis. ITS2 sequencing revealed *H. placei* as the predominant parasite infecting cattle while *H. contortus* as the predominant parasite infecting sheep and goat. ITS2 sequences also revealed 12 distinct genotypes of *Haemonchus,* seven among *H. contortus* isolates, one genotype among *H. placei* and four genotypes among the hybrids. High genetic variability was observed in Pakistani *Haemonchus* isolates at ND4 and COI gene loci. Intra-population diversity parameters were higher in *H. contortus* isolates than *H. placei*. Phylogenetic analysis of ND4 and COI sequences did not reveal clustering of haplotypes originating from a particular host indicating high rate of gene flow among *Haemonchus* parasites infecting sheep, goat and cattle in Pakistan. ND4 and COI haplotypes from Pakistan were compared to sequences of *Haemonchus* isolates from 11 countries to elucidate the population structure. *H. contortus* isolates from Pakistan, China, Malaysia and Italy clustered together while the isolates from Yemen and Malaysia were found to be genetically distinct. With respect to *H. placei*, isolates from Pakistan were found to be genetically differentiated from isolates of other countries. (FIG: 11). The tests for selective neutrality did not reveal significant deviations in Pakistani *Haemonchus* populations while significant deviation was observed in Brazilian and Chinese *H. contortus* populations indicating selective forces in operation. Median Joining (MJ) network of ND4 haplotypes network revealed Yemenese *H. contortus* being closer to *H. placei* cluster. β-tubulin isotype 1 genotyping revealed 7.86% frequency of allele associated with benzimidazole resistance at F200Y locus in Pakistani *Haemonchus* isolates. All the resistant alleles were observed only in *H. contortus* isolates while none of the *H. placei* isolates possessed resistant allele. Thus anthelminth resistance in nematodes of cattle, thus appears to develop more slowly than in nematodes infecting small ruminants. These findings provide important information on the genetic variability of *Haemonchus* parasites circulating in Pakistan and will help in formulating effective strategies for their control.

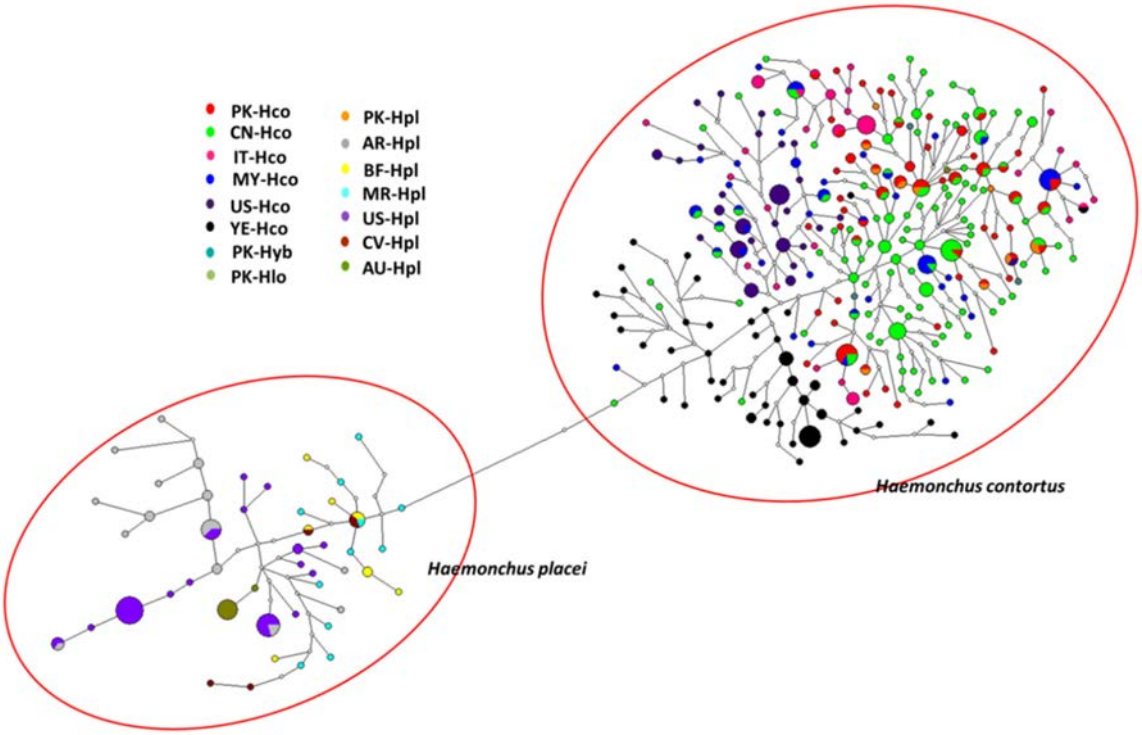

FIG. 12: Median Joining NETWORK of ND4 haplotypes derived from Haemonchus isolates across various countries (Hco-H. contortus; Hpl-H. placei; Hlo-H. longistipes; Hyb-Hco x Hpl hybrid; PK-Pakistan; CN-China; IT-Italy; MY-Malaysia; US-United States; YE-Yemen; CV-Cape Verde; MR-Mauritania; BF-Burkina Faso; AU-Australia; AR-Argentina)

Genetic characterization of indigenous native cattle from Myanmar and Zambia

The livestock biodiversity and existing pool of genetic resources are fundamental to food security. Responding to the rapid loss of livestock biodiversity, international community, through their Interlaken declaration (2007) agreed to adopt the Global Plan of Action (GPA) on animal genetic resources. GPA includes 23 strategic priorities for action grouped into four major priority areas: characterization and monitoring; sustainable use and development; conservation; and policies, institutions and capacity building. IAEA through its National and Regional Technical Cooperation projects support Member States to implement GPA through capacity building and technical support for genetic characterization of livestock using molecular DNA technologies. In continuation of agency's support in implementing FAO's Global Plan of Action on animal genetic resources (AnGR), APHL supported genetic characterization of native cattle from Myanmar and Zambia. Further, to evaluate the genetic structure and phylogeography of global sheep breeds, genotype and sequence data were generated on more than 400 indigenous animals.

Characterization of Myanmar native cattle

Most of the indigenous cattle from Myanmar are draught type breeds which can work for long hours in agricultural fields and pull carts with heavy loads. Much required information on genetic potential of these animals especially genetic variability, level of inbreeding, physical and phenotypic characteristics, etc. were lacking. A total of 162 samples collected from three major cattle breeds of Myanmar (Pyar Zein, Shwe Ni and Shwe Ni Gyi) were analyzed by sequencing control region (D-loop) of mitochondrial genome and genotyping 27 microsatellite

marker loci. Microsatellite genotypes revealed moderate level of within breed genetic diversity and relatively low level of inbreeding in Myanmar cattle breeds. Although morphologically similar, genetic analysis showed Shwe Ni and Shwe Ni Gyi breeds quite distinct from each other (Fig 12). This finding will have significant implication on decisions related to breeding management of these cattle breeds. Mitochondrial DNA sequencing revealed three maternal lineages in Myanmar cattle, two *Bos indicus* lineages and one *Bos taurus* lineage. mtDNA haplotypes of Myanmar cattle were closely related to Vietnamese as well as Indian zebu cattle.

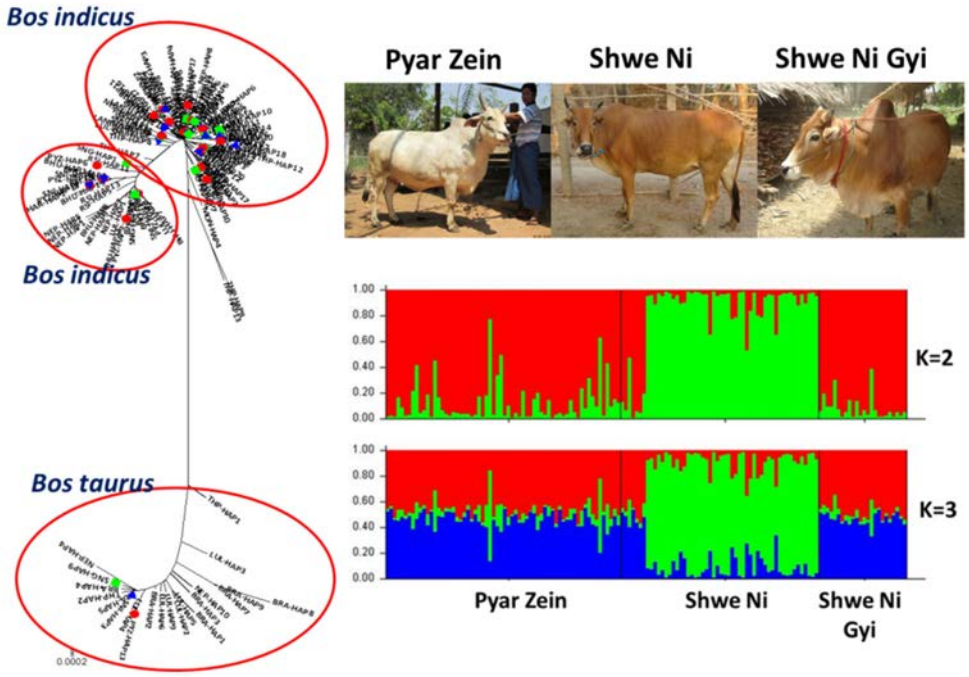

FIG. 13: Phylogeny and genetic structure of Myanmar native cattle

Characterization of Zambian native cattle

Cattle in Zambia are reared primarily for meat as well as for milk. Smallholder farmers are the major keepers of livestock including cattle owning 70-80% of total population. Crossbreeding of local Zambian cattle is practiced in rural as well as peri-urban areas to improve productivity which resulted in replacement of local breeds by exotic or graded animals. Most local livestock populations in Zambia including cattle are not yet classified into discrete breeds. Considering the strong and urgent need to evaluate and characterize indigenous cattle, three populations viz. Angoni, Barotse and Tonga were analysed using microsatellite and mitochondrial DNA markers. High level of within breed genetic diversity was observed in all the three Zambian cattle breeds, with low levels of estimated inbreeding. Genetic structure analysis revealed complete admixture of all three Zambian native cattle breeds although the centroid of Angoni was marginally distinct from Barotse and Tonga.

Global diversity analysis of indigenous sheep breeds

The coordinated research project entitled "Gene based technologies for livestock breeding-Phase I: Characterization of small ruminant genetic resources of Asia" and implemented from 2005 to 2010 generated data on about 35 sheep breeds/populations from seven countries

in Asia. In order to assess the genetic structure of indigenous sheep breeds across different geographical regions, APHL initiated efforts on global analysis of microsatellite genotypes and mitochondrial DNA sequences by merging data from different projects including the above IAEA-CRP, the European Union supported ECONOGENE consortium, the NordGen funded North European sheep diversity project and national projects from different countries. However, data of indigenous sheep breeds from certain regions were still not available including that of Middle East Asia, Latin America and India. Hence, sequencing and genotyping of about 380 samples collected from these regions were started to supplement information on global diversity analysis. As part of this effort, 10 indigenous sheep breeds were genotyped at 19 FAO recommended microsatellite loci. Mitochondrial DNA sequence data were generated in more than 400 animals representing various indigenous breeds from Asia. Analysis of sequence and genotype data and global diversity analysis of indigenous sheep breeds are currently under progress.

Global Reference Genetic Repository of livestock breeds

The genetic repository of livestock breeds at APHL is constantly strengthened by addition of new DNA samples. More than 900 DNA samples from various livestock breeds were added to the repository during the last year.

CAPACITY BUILDING

eLearning

For most of the pathogens causing transboundary animal diseases (TADs), a very specific identification and characterization is needed to:

- Select the better matching vaccine
- Understand the entry and circulation routes for defining control strategies
- Understand the epidemiology and geographical distribution

These can be achieved by applying appropriate molecular and bioinformatics analysis.

Since 2011, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, together with the Animal Health Service (FAO, Rome) and the Swiss Institute of Bioinformatics, Switzerland, are working to develop e-learning applied bioinformatics to promote the genetic and phylogenetic analysis of animal pathogens. The objective of this work is to prepare FAO and IAEA MS veterinary scientists from diagnostic and research laboratories to be self-sufficient in pathogen genetic data analysis for the better management of TADs.

In 2012, the first module on “The phylogenetics of animal pathogens: basic principles and applications” (*http://viralzone.expasy.org/e_learning*) was launched. This was followed by the development and the release of two new modules in 2013. The first one covered multiple sequence alignments (MSA) and the second focused on sequence similarity search. In addition, a chapter combining exercises on both sequence similarity search and multiple

sequence alignments was developed by the SIB to complement the MSA and Blast modules. The exercises are focused on the comparison of MSA modules and the comparison of hemagglutinin sequences of influenza viruses. The characterization of genes with unknown function and protein homology inference are covered for the BLAST module (*http://viralzone.expasy.org/e_learning*).

Proficiency testing

Ring test 2012: PPR nucleic acid amplification based diagnosis

As part of its activities to promote the implementation of quality assurance in MS veterinary diagnostic laboratories, the Animal Production and Health Laboratory (APHL) has been conducting an annual inter-laboratory proficiency test (PT) on the nucleic acid amplification-based assay for peste des petits ruminants (PPR) diagnosis using the reverse transcription polymerase chain amplification (RT-PCR) technique. In 2013, 17 laboratories participated in the 2013 PT. (Table 1).

Country	Institute
Côte d'Ivoire	Laboratoire Central Vétérinaire de Bingerville
Turkey	Viral Diagnostic Laboratory, Pendik Veterinary Control and Research Institute
China	Institute of Animal Science and Veterinary Medicine, Chinese Academy of
Mali	Laboratoire central vétérinaire de Bamako
Pakistan	National Institute for Biotechnology & Genetic Engineering (NIBGE)
Bangladesh	Department of Pathology, Bangladesh Agricultural University
Burkina Faso	Laboratoire National D' Elevage
Sudan	Animal Resources Research Corporation (ARRC); Central Veterinary Research
Nigeria	Federal Ministry of Agriculture and Water Resources; National Veterinary
Cameroon	Laboratoire national vétérinaire (LANAVET), Diagnostique et santé animale
Kenya	Central Veterinary Laboratories Kabete
Tanzania	Center for Infectious Diseases and Biotechnology, Tanzania Veterinary
Ghana	Accra Veterinary Laboratory
Benin	Laboratoire de Diagnostic Vétérinaire et de Sérosurveillance
Ethiopia	National Animal Health Diagnostic and Investigation Center (NAHDIC),
Uganda	National Animal Disease Diagnostics and Epidemiology Center,
DRC	Central Veterinary Laboratory

Table 1: List of participation laboratories

A number of well characterized samples (six positives and four negatives), were sent as blind and labelled with random numbers to each laboratory. The participants were asked to determine the diagnostic status of the samples using any RT-PCR method(s) of their choice with as many repetitions as desired but were only required to supply a single definitive result for each sample. The data was collected, analysed and the results which included a short report were sent back to all the participating laboratories. Each laboratory (participant/counterpart) received the full coded results where he/she could recognize only his/her own results (Table 2). The short accompanying report was to comment on the findings and advise where necessary. Three participating laboratories did not send back data. From the 14 responsive participating laboratories, only three counterparts had 100% correct results (Table 1). The analysis suggested that the majority of participating laboratories failed the test as they did over the previous year. Laboratories that did not pass the test are being assisted in the implementation of the RT-PCR along with the stringent implementation and monitoring of quality control and GLP. The next PPR proficiency test will be organized in the 3rd quarter of 2014.

Animal Production and Heath Laboratory (APHL)
PPR Ring Test 2013
Nucleic Acid Amplification based PPR Diagnosis

Results

Lab. Nr.	Positive	Negative	False Positive	False Negative	Total number of Positives	Total number of Negatives	Total number of Samples	x/y - Scores - %		Remarks
Lab 1	4	3	1	2	6	4	10	7/10	70	
Lab 2	0	3	1	6	6	4	10	3/10	30	
Lab 3	6	4	0	0	6	4	10	10/10	100	100%
Lab 4	6	4	0	0	6	4	10	10/10	100	100%
Lab 5	5	4	0	1	6	4	10	9/10	90	
Lab 7	0	4	0	6	6	4	10	4/10	40	All Neg
Lab 8	5	4	0	1	6	4	10	9/10	90	
Lab 10	4	0	4	2	6	4	10	4/10	40	
Lab 12	6	4	0	0	6	4	10	10/10	100	100%
Lab 13	6	0	4	0	6	4	10	6/10	60	All Pos
Lab 14	3	1	3	3	6	4	10	4/10	40	
Lab 15	6	0	4	0	6	4	10	6/10	60	All Pos
Lab 16	4	3	1	2	6	4	10	7/10	70	
Lab 17	2	4	0	4	6	4	10	6/10	60	
Legend		Positive	False Positive	Negative	False Negative	100% Correctness	All Positive	All Negative		

Table 2: Summary results of the 2013 PPR ring test

Meetings

A threeday workshop on the prevention and control of PPR in the Southern African Development Community (SADC) region was held in Dar es Salaam, Tanzania from the 10 to 12 June, 2013. The workshop was jointly organized by FAO, OIE and IAEA and discussed the preparation of SADC countries in disease diagnosis and the control and management of PPR. Fourteen SADC countries were represented with a total of 28 participants as follows; Botswana (4), DR Congo (2), Kenya (1), Lesotho (2), Madagascar (1), Malawi (2), Mozambique (2), Nambia (2) Zimbabwe (1), Zambia (1), Seychelles (1), South Africa (2), Swaziland (1), Tanzania (6). Experts from FAO, OIE and IAEA as well as specialists on specific topics such as disease surveillance, diagnostics and the control and involvement of wildlife from CIRAD, France and The Royal Veterinary College, UK also participated. The SADC Secretariat and other organisations such as PANVAC-Ethiopia, and USDA-APHIS, South Africa, were also present. The participants discussed approaches required to halt the rapid spread of PPRV in countries that are already affected and at-risk regions with a special focus on the threatened SADC-member countries. A key component of the Workshop was the introduction and discussion of the work of FAO and OIE to develop a Global PPR Control Strategy. The objectives of this work, the elements of the Control Strategy and the time plan for its establishment were presented and discussed in small working groups.

The overall output of the meeting included:

- Review of the PPR situation in Africa and exchange on the risks of introduction in currently PPR-free SADC countries
- Discussion of PPR control strategies and review of the current SADC regional strategy to foster a more comprehensive control of PPR
- Preparation of SADC members for the identification, early detection and management of PPR,

- Enhancement of SADC member's knowledge and practical application of surveillance and epidemiological data on PPR
- Published Proceedings of the Meeting (IAEA/OIE/FAO) can be accessed using the following webpage link: *http://www.rr-africa.oie.int/docspdf/en/2013/PPR/Proceedings.pdf*

Training courses

Training course on peste des petits ruminants (PPR) and ***Mycoplasma capricolum subsp. capripneumoniae*** **(MCCP) diagnosis (13-21, June)** for laboratory diagnosticians was held at the Tanzania Veterinary Laboratory Agency (TVLA), Temeke, Dar es Salaam. Eleven SADC countries were represented with a total of 15 participants as follows; Angola (1), DR Congo (1), Botswana (1), Lesotho (1), Malawi (1), Mozambique (1), Zimbabwe (1), Zambia (1), South Africa (1), Swaziland (1), Tanzania (5). The principle aim of the course was to improve knowledge on the application of early detection and surveillance tools to diagnose PPR and MCCP. This included improving the national and regional surveillance programmes for the two diseases, improving the information on the molecular epidemiology of PPR and MCCP, increasing the efficiency disease management in a quality assured manner, increasing the knowledge on Laboratory biosafety and good laboratory practice in handling the pathogens that cause zoonotic diseases and foster networking between laboratories involved in transboundary animal disease diagnosis in the targeted region.

The seven day course consisted of lectures and benchwork on the early detection, surveillance and epidemiology of PPR and MCCP using nuclear-based or related molecular techniques in addition to antigen capture and serological techniques. The course work was provided by two experts each from CIRAD, France and APHL and was designed to provide essential theoretical and practical information on important aspects of diagnosis of PPRV and MCCP.

The main topics that the programme covered were:

- Cell culture for PPRV isolation,
- Competitive ELISA for PPRV diagnosis,
- Immunocapture for PPRV diagnosis,
- Conventional RT-PCR for PPRV diagnosis,
- Real-Time PCR for PPRV diagnosis,
- Conventional PCR for MCCP diagnosis,
- Competitive ELISA for MCCP,
- Molecular epidemiology of PPRV

The overall feedback for the course was positive with the participants feeling that the training would help them in the performance of their work on returning to their home laboratories.

Avian influenza H7N9: Given the emergence of the new H7N9 avian influenza virus in China in March 2013, the joint FAO-IAEA Division's Animal Production and Health Laboratory in collaboration with the Technical Cooperation Department (TC) conducted two training courses on the serological and molecular detection of H7N9 in order to contribute to the early

detection of this virus and early reaction capabilities in Member States. Both training courses were organized as part of the IAEA's regional TC project 'Supporting Coordinated Control of Transboundary Animal Diseases with Socioeconomic Impact and that Affect Human Health.' The first course was held at the Seibersdorf laboratories from 19 to 30 August 2013 with participants from Albania, Armenia, Azerbaijan, Bulgaria, Bosnia and Herzegovina, Croatia, Georgia, Hungary, Latvia, Former Yugoslav Republic of Macedonia, Montenegro, Serbia and Turkey and the second from 9 to 19 September, 2013 with participants from the Bangladesh, Indonesia, Iran, Iraq, Jordan, Kuwait, Malaysia, Oman Pakistan, Philippines, Sri Lanka, Syria, T.T.U.T.J of T. Palestinian A, Vietnam, Yemen, Myanmar. With the assistance of avian influenza experts from China, FAO, Germany, Italy, the United Kingdom, and the World Organization for Animal Health (OIE), the course enhanced technical, risk assessment and epidemiological knowledge on avian influenza H7N9 through lectures on epidemiology, risk assessment, identification of the virus sub-types involved, sampling and submission procedures (including shipment of pathological samples to the FAO/OIE reference laboratories). The course also offered practical training in current rapid techniques for disease diagnosis, in particular, the use of nuclear-based or related techniques for the identification and characterization of the pathogen(s). In addition, the course included genome sequencing, molecular epidemiological and computer based analysis of H7N9 viruses. Specifically, the on-site computer teaching facilities were used for the analysis of avian influenza genomes using web-based and freeware analysis software

Regional Training Course on "Advanced molecular genetic tools for characterization and improvement of indigenous small ruminants" was conducted at APHL, Seibersdorf from 17th-21st June, 2013. Thirteen participants from four ARASIA countries Iraq, Jordan, Yemen and Oman participated in the training course. The purpose of the training course was to enhance knowledge and capacity of participants in applying advanced molecular genetic tools for evaluation, characterization and genetic improvement of indigenous sheep and goats. With the assistance of experts from Italy and Austria, the participants were given hands on practical training on various DNA based techniques used for animal genotyping, sequencing and breed characterization. The course also offered training on analysis of genetic data using various open source software.

Fellows

In 2013, the APHL hosted four fellows and two interns in the following areas:

Name	Country	Status	Duration	Topic
Curé Georges TSHILENGE MBUYI	Democratic republic of Congo	Fellow	02 September 2013 to 29 November 2013	Molecular epidemiology of ASFV isolates from DRC
Kassedo Nina Benedicte TOILY	Cote d'Ivoire	Fellow	5 November 2012 to 3 March 2013	Laboratory methods for the diagnosis of transboundary animal diseases"
Ms. Ei Thandar	Myanmar	Fellow	15th July, 2013 to 11th October, 2013	Genetic characterization and phylogeography of indigenous Zebu cattle from Myanmar
Ms. Ireen Mbeule	Zambia	Fellow	2nd September, 2013 to 31st October, 2013	Molecular genetic characterization of Zambian native Zebu cattle using DNA markers
Mr. Tanveer Hussain	Pakistan	Intern	1st November, 2013 to 31st December, 2013)	Molecular Epidemiology and genetic characterization of Haemonchus variants infecting domestic ruminants in Pakistan
Mr Timothy Yusufu Woma	Nigeria	Intern	19th July-27th September 2013	Molecular epidemiology analysis of PPRV samples from Nigeria

PUBLICATIONS

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BOUSSINI H., LAMIEN C.E., NACOULMA O.G. OUEDRAOGO A. Rift Valley Fever in Camels in Northern Burkina Faso. Bull. Anim. Hlth. Prod. Afr., (2013), 61, 467-472

PERIASAMY K., PICHLER R., POLI M., CRISTEL S., CETRÁ B., et al. (2014) Candidate Gene Approach for Parasite Resistance in Sheep – Variation in Immune Pathway Genes and Association with Fecal Egg Count. PLoS ONE 9(2): e88337. doi:10.1371/journal.pone.0088337.

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 (1) 81-89 doi: 10.1111/iji. 12075.

DUBEY P. K., GOYAL S., KATHIRAVAN P., MISHRA B.P., GAHLAWAT S. K. KATARIA R.S. (2013). Sequence characterization of river buffalo Toll-like receptor (TLR) genes 1-10 reveals distinct relationship with cattle and sheep. International Journal of Immunogenetics, 40 (2): 140-148. doi: 10.1111/j.1744-313X.2012.01135

KAMISSOKO B., SIDIBE C.A.K., NIANG M., SAMAKE K., TRAORE A., DIAKITE A., SANGARE O., DIALLO A. LIBEAU G. Prévalence sérologique de la peste des petits ruminants (PPR) dans les troupeaux ovins et caprins au Mali. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 2013, 66 : 5-10

LIBEAU G., DIALLO A., PARIDA S. Evolutionary genetics underlying the spread of peste des petits ruminants virus. Animal Frontiers (accepted for publication)

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	RVF
Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV/ISRA), Senegal	Capripox, PPR, ASF
Livestock Breeding and Veterinary Department (LBVD), Myanmar	Animal Genetics
University of Veterinary and Animal Sciences (UVAS), Pakistan	Haemonchus Research
National Institute for Scientific and Industrial Research (NISIR), Zambia	Animal Genetics
Universita Cattolica del Sacro Cuore,(UNICAT), Italy	Livestock biodiversity research
Department of Population Genetics, Veterinary Medical University (VETMEDUNI), Austria	Animal genetics
Swiss Institute of Bioinformatics, Switzerland,	E-learning

EXTRABUDGETARY SUPPORT

In addition to IAEA regular budget and TC projects, activities APHL activities were supported by the following projects:

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 provides services and assistance to FAO and IAEA Member States in their efforts to ensure the safety and quality of the food supply, safeguarding the health of consumers and helping to facilitate international trade. The laboratory helps Member States to implement effective food traceability and contaminant control systems to support food safety, and authenticity testing to combat economic loss through the illegal production and marketing of counterfeit and adulterated products. Assistance is provided through the development, technology transfer and application of nuclear and related technologies 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 holistic, farm-to-fork approach for effective systems to help ensure food safety, quality and security. Member State laboratories are the chief direct recipients of the FEPL outputs and activities. 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 their multiple stakeholders, thereby providing essential feedback and advice to help build and consolidate their capacity to assess and manage risks and also inform and improve agricultural and food production practices.

Research and development achievements in 2013 included the development of new and innovative methods for food traceability and authentication, focusing mainly on honey, fruit juices and dairy products, which are important commodities in international trade and frequent targets for fraudulent practices such as counterfeiting or adulteration. Methodologies established to facilitate the authentication of honey included the development of a technique for analysing light isotopes in honey proteins and also the application of metabolomics and multivariate data analysis as a method to provide chemical fingerprints that allow discrimination between honeys of different floral origin. An untargeted metabolomics technique for the detection of fruit juice adulteration was refined and further developed to provide a more simple method to target a number of chemical markers in juice samples that can be more readily transferred to analytical laboratories. In addition, a number of candidates certified reference materials for light stable isotope analysis were characterised in collaboration with other laboratories, and quality control materials for light isotope analysis of rice and milk samples were produced and distributed to counterpart laboratories. The development of these reference materials underpins the successful application of analytical techniques in laboratories and they are required for the further application and development of these methods. Also as regards dairy products, a new Coordinated Research Project was initiated on accessible technologies for the verification of origin of dairy products as an example control system to enhance global trade and food safety.

Research activities concerning nuclear related technologies for food authenticity are now beginning to stimulate applications for assistance in Member States through technical cooperation projects for implementation in the 2016 to 2017 biennium, with at least four national concepts directly related to authenticity issues, reflecting a growing demand in this area.

Research achievements related to the control of residues and contaminants in food include the development of multi-residue analytical methods for the determination and control of residues of veterinary antimicrobials in meat, and for various pesticide profiles in a number of commodities. Work also continued in an inter-agency project that aims to establish capacity for the quality control of veterinary trypanocidal drugs in sub-Saharan Africa. Analysts from reference laboratories in Senegal and Tanzania were trained in methods developed for this purpose and the methods have been successfully applied in their reference laboratories. There remains a high demand from our Member States for assistance and services related to the control of residues and contaminants in food, with more than 29 national and regional concepts being submitted for future support as new technical cooperation projects for the biennium 2016 to 2017. This level of interest also indicates that our technical expertise in this area is highly regarded and can continue to help ensure food safety.

Outreach activities included the presentation of the results of FEPL research at five international conferences, and the FEPL was involved directly in the scientific committee for a major international conference on food integrity.

Capacity building activities in 2013 included the technical management of eighteen national and seven regional Technical Cooperation Projects. The expertise available in the FEPL and the methods and techniques developed were also used to support technology transfer to Member States. An inter-regional training workshop was held at Seibersdorf and two regional workshops were held in Member States with extra-budgetary funding. The FEPL hosted group fellowship training for four scientists from Zimbabwe, a training course for five scientists from Tanzania and Senegal, and one internship. Two food traceability training courses were held in Southeast Asia. In total, more than one hundred and thirty developing country scientists were trained in various aspects of food safety control. 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 forty nine laboratories in nineteen countries, and is effectively promoting and supporting food safety and environmental sustainability in the region.

Publications by FEPL staff included nine papers in the peer-reviewed scientific literature, eleven papers in conference proceedings/books of abstracts, and one book chapter. Theses references are cited in full under the Publications at the end of this section.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head
Frew, Russell David	Food Safety Specialist
Maestroni, Britt Marianna	Food Scientist
Jandrić, Zora	Analytical Chemist
Rathor, Mohammad Nasir	Laboratory Technician
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Wimberger, Tamara	Team Assistant
Massinger, Barbara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Food traceability and authenticity

Mechanisms to facilitate the effective traceability and authentication of food commodities have become mandatory with respect to food trade and are essential components of farm-to-fork food control systems. The globalization and the increasing complexity of food trade provide both opportunities and risks for producers, manufacturers and traders. With appropriate systems to check, control and reward efforts to ensure the integrity of the food supply chain there are increasing opportunities for those involved in the provision of safe, high quality food. There are also risks, including risks associated with inadvertent or deliberate fraudulent mislabeling of products that can cause damage to the brand image, reduce consumer demand and affect the reputation of the producer country. The consequences can affect commodity prices for genuine, correctly labelled products or even result in loss of market access. Food fraud and questionable provenance also raise issues related to food safety, and it is imperative that a traceability system can rapidly and robustly identify affected products and the origin of the issue, enabling the product to be removed from the supply chain and the risk to be mitigated. Conventional traceability systems (e.g. labelling, radiofrequency tagging) are very good for passing information and tracking the packaging along a supply chain. However, all such systems are vulnerable to fraud and analytical methods to confirm origin or food content have a role to play in ensuring confidence in the safety and quality of food.

The FEPL undertakes research to support Member States in food control. The FEPL is helping to develop systems for the verification of origin and authenticity of foodstuffs using nuclear and related techniques. These techniques utilize chemical measurements of the food itself to verify its authenticity. The parameters measured are inherent properties of the food therefore extremely difficult to counterfeit.

The two categories of analysis currently used are stable isotope measurements and metabolomics. The stable isotopes ratios of the bio-elements (H, C, N, O and S) vary according to different environmental drivers. Measurements of these isotope ratios provide a 'fingerprint' that is unique to the origin and history of that product. The advantage of using isotope systems such as H and O is that their relationship to environmental drivers is well understood, enabling predictive models to be developed. Current work shows that the H-isotope composition of milk can be predicted using global rainfall isotope databases. The approach of using models ground-truthed with authentic samples greatly reduces the cost, a significant barrier to entry for producers wanting to implement origin systems.

A metabolomics approach can be applied to identify biomarkers that can be used to authenticate food products or detect adulteration. For example, untargeted metabolite fingerprinting using UPLC-QToF MS with multivariate data analysis has been applied in the FEPL to identify potential biomarkers for the rapid detection of the adulteration of fruit juices with cheaper alternatives. A simpler, targeted metabolomics method was then optimized and adulteration could be detected at 1%. Untargeted metabolomics and chemometrics were also applied to discriminate between various honeys classified as unifloral in origin.

The results of these studies indicate the potential for the development of effective control systems to improve confidence in the food supply chain using a combination of complementary analytical techniques and data processing.

The research and development activities in FEPL were carried out in collaboration with counterpart laboratories in two international research projects coordinated by the laboratory; "Implementation of Nuclear Techniques to Improve Food Traceability" and "Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety".

Authenticity assessment of honey products

The health benefits of honey have long been recognized. Some honey types (specific botanical and/or geographical origin) have nutraceutical qualities or superior organoleptic properties and thus can command a premium price over other types. Therefore, honey is one of the food commodities most commonly suspected of being subject to mislabeling fraud, and this is an issue of international scale in terms of authenticity and quality control.

An untargeted metabolomics method was developed for the classification of honey of various floral and geographical origins using ultra-performance liquid chromatography-quadrupole time

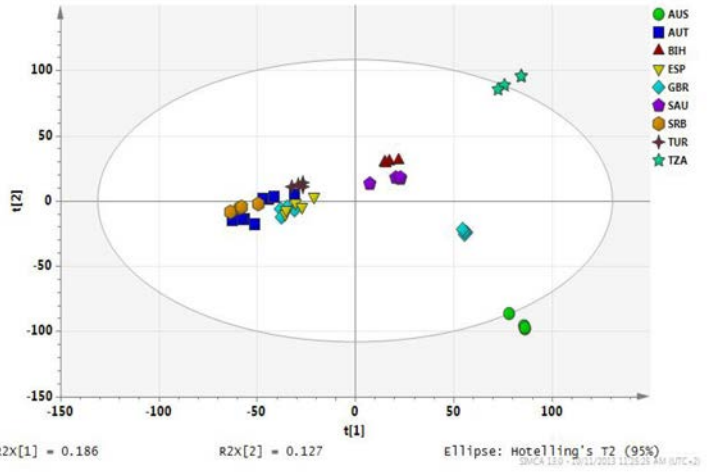


FIG. 1: Discrimination of some polyfloral honeys obtained from: AUS, AUT, BIH, ESP, FRA, GBR, NZL, SAU, SRB, TUR, and TZA.

of flight mass spectrometry (UPLC-QToF MS) to support Member States that trade honey. Evaluation of data by multivariate data analysis allowed the discrimination of New Zealand and Australian honeys from honeys originating in other countries, until now a feat too difficult for analytical techniques.

Research also demonstrated that it was also possible to differentiate between some unifloral honeys of various geographical origins (FIG. 1). Reliable classification was obtained for honeys from Australia, Tanzania (miombo), Great Britain (moor), Bosnia and Herzigovina (Salvia officinalis), and Saudi Arabia (sidr), while honeys from Austria (acacia, sunflower), Spain (thyme), Great Britain (borage), Serbia (acacia), and Turkey (pine) were grouped together. The clusters of acacia honey from Austria and Serbia overlapped.

The results demonstrate the potential of this methodology for the differentiation and classification of honeys traded between countries. Work is ongoing in FEPL to further elaborate and validate the methodology. The application of such methods may represent an effective means to combat fraud, protect the economic interests of Member States trading honey, and ensure that consumers are getting the products with nutritional and medicinal qualities that they are paying for.

Detection of honey adulterated with rice syrup

Current work in the FEPL includes applying stable isotope techniques to the verification of the origin and authenticity of foodstuffs. In particular, we are working on honey as an example food for the development of the analytical protocols. One aspect is to try and counter the fraudulent practice of adulterating honey with rice syrup. Such rice syrup adulteration cannot be detected using the conventional carbon isotope test (AOAC) so we are attempting to develop test that uses hydrogen isotopes as an internal standard. The techniques applied to date include the purification of honey protein and analysis of the deuterium/hydrogen ratio, δ^2H, in the protein, the determination of non-exchangeable δ^2H in sugars (H-exchange, nitration), and measurement of C, H and N isotope ratios in authentic honey samples collected from international sources.

The elaboration of the analytical methodology was completed in 2013 and samples are now being acquired from around the world for analysis.

Differentiating citrus fruit/fruit juices by chemometrics and mass spectrometry

As a continuation of the work started in 2012 on developing methodology for authenticating fruit juices, the potential of coupling chemometric classification methods and mass spectrometry (UPLC-QToF MS) data for the traceability of distinguishing citrus fruit/fruit juices was investigated. Amongst the various supervised recognition methods, discriminant analysis (PLS-DA) and modelling (SIMCA) were employed. The mass spectrometry data processed through PLS-DA demonstrated high classification accuracy for data obtained in negative (goodness of fit, R2, and predictability, Q2, greater than 0.925 and 0.899, respectively) and positive (R2 and Q2 greater than 0.843 and 0.965, respectively) ionization modes for the discrimination of citrus fruits/fruit juices. SIMCA confirmed these results and showed that the category models for the class can be sensitive and highly specific. The applicability of the

models was tested with an external data set of fruit juices adulterated with other fruit juices and water (1% and 5%, respectively). Using the SIMCA algorithm, adulterated samples were easily distinguished from authentic samples showing the possibility of applying this method as a rapid screening technique to trace or confirm the origin of citrus fruit/fruit juices and detect fraud. The results demonstrate that metabolomics has potential as a screening tool for the detection of food fraud by adulteration, and could represent a new strategy in food forensics to enable a rapid response in the global fruit juice market to help regulators to stay one step ahead of fraudsters.

Development of reference materials

A significant issue in applying stable isotope techniques to foodstuffs as a traceability tool is the need for comprehensive authentic data and large databases of analytical data derived from samples of authentic-origin. To ensure the quality of the data in the database it is essential that appropriate reference materials are used i.e. that have a food matrix. At present there are very few food-matrix reference materials certified for their stable isotope ratios and available for laboratory use. An initial survey of a number of currently available IAEA trace element and radionuclide reference materials in 2012 identified some materials that could potentially be used as quality control materials for stable isotope analysis. A joint FEPL project with the FAO/IAEA Soil and Water Management and Crop Nutrition Laboratory, the IAEA Terrestrial Environment Laboratory, and the University of Otago in New Zealand continued with this work in 2013, revealing that many of those materials are suitable for development as reference materials for stable isotope analyses. There is a very useful range of isotope ratio and elemental composition among these materials meaning they may be suitable for a great many applications. These data were presented at the 2013 European Geophysical Union conference in April 2013.

Work is now underway to verify the homogeneity and stability of the selected materials. Once that is completed those that are deemed suitable will be sent to participant laboratories for intensive analysis to verify the isotopic composition and subsequent certification.

In support of the two coordinated research projects and a regional technical cooperation project in the field of food traceability/authenticity, quality assurance materials for the analysis of stable isotopes in rice and in milk were developed and distributed to our counterpart laboratories.

Control of Residues and Contaminants in Food

A number of analytical methods and protocols were developed and validated in the FEPL or in collaboration with counterpart laboratories, and transferred to laboratories in Member States through training at Seibersdorf, in Member States or through coordinated research projects. Protocols were developed for screening and quantitative methods for use in Member States as components of analytical method packages to allow the cost-effective implementation of contaminant control strategies. These included protocols for the detection and enumeration of pesticide residues in a number of commodities, tailored for the needs of specific countries or regions, and methods for the detection and quantification of antibiotics (quinolones and aminoglycosides) in animal-derived foods. The dissemination of these methods was facilitated

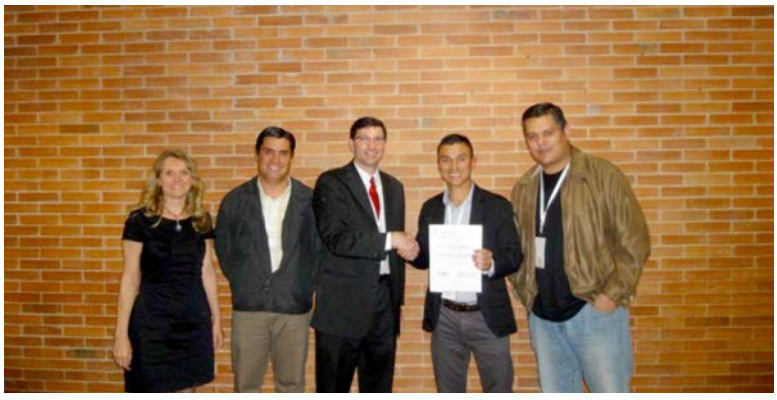
to a large extent by the RALACA network. The information provided by the analytical results can be used to inform and improve agricultural and food production practices and optimize the use of chemical applications to support both food safety and environmental sustainability.

Dissemination of research results

The analytical 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.

FEPL staff participated in, and presented research and development results at, several international conferences and seminars in 2013, including:

- Presentation of an invited talk and a poster at the European Geophysical Union Conference, Vienna, Austria, 7-12 April 2013. Mr. Russell Frew presented a talk on the coordinated research project "Implementation of Nuclear techniques to Improve Food Traceability" and a poster on the development of new reference materials for light isotopes in foodstuffs. The conference had more than 11,000 delegates from approximately 95 countries.
- An oral presentation and several posters at the 4th Latin American Pesticide Residue Workshop (LAPRW) - Food and the Environment, 26-29 May 2013. Ms. Britt Maestroni gave a talk entitled ¨Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale¨, which presented the work of the Red Analitica de Latino America y El Caribe (RALACA) network of laboratories, established with Joint FAO/IAEA Division support. In addition, the representatives of Argentina, Brazil, Chile, Costa Rica, Panama and the Joint FAO/IAEA Division presented the following posters: Integrated Assessment of Environmental Pollution by Pesticides in the upper Valley of Rio Neuquén, Patagonia, Argentina; Evaluation of environmental impacts caused by the application of insecticides on orange crop; Determination of pesticides adsorption isotherms in Chilean



Mr S. Lehotay presenting the CICA/RALACA team with the best poster award

soils using isotopic techniques; Implementation of Good Agricultural Practices (GAP) in the production of fruits in the Machuca-Jesus María watershed, Costa Rica based on analytical laboratory results; Optimization of a method using direct LC- MS/MS injection for determination of pesticides in surface water; Nuclear techniques applied to the generation of input data for environmental modelling; and a poster presented by Mr. Greivin Perez from Costa Rica on ¨Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at the microcatchment La Mula, Guanacaste Costa Rica¨, which was awarded the prize for the best poster of the conference. Mr. Perez, a former trainee in the Food and Environmental Protection Laboratory at Seibersdorf, gave a five minutes presentation about the team's work and accepted the award on behalf of the Costa Rica/RALACA team.

- An invited oral presentation and a poster at the Annual Meeting of the Association of Official Analytical Chemists International (AOAC), Chicago, USA, 25-28 August 2013. AOAC INTERNATIONAL is a globally recognized, independent, not-for-profit association with over 3,000 members worldwide and fourteen international sections representing four continents and more than ninety countries. Major topics covered in the 2013 Annual Meeting and Exposition included food authenticity and the control of residues and contaminants in food. The FEPL Head, Mr. Andrew Cannavan, gave an invited lecture on the possible uptake of antibiotics by plants and their recycling in the food chain and consequent issues with respect to food safety and the development of antibiotic resistance in human pathogens. The data presented was based on work performed using radiotracer techniques and mass spectrometric analytical methods at Seibersdorf and in IAEA CRPs to provide data and information for risk management with respect to the presence of antibiotic residues and other chemical contaminants in the food supply chain. A poster was also presented on work done in the FEPL on novel and transferable methodology for the assessment of fruit juice authenticity.
- Presentation of a poster at the 6th International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 5-8 November 2013. The symposium had approximately 900 registered participants from more than 60 countries world-wide. A poster was presented on "Classification of New Zealand Honey by Mass Spectrometry and Chemometrics". Several posters were also presented by project counterparts and former trainees at Seibersdorf on methodology developed in collaboration with the FEPL. These included posters on the validation of a GC-SIM-MS method for the determination of dithiocarbamate fungicide residues in fruits and vegetables by the Centro de Investigación en Contaminación Ambiental (CICA) in San José, Costa Rica (an IAEA Collaborating Centre) and on the evaluation of the behaviour of different dispersive solid phases in the analysis of pesticide residues in tomatoes consumed in Uruguay by TC counterparts in the Laboratorio de Bromatologia in Montevideo, Uruguay.
- A poster presentation at the 2nd International Food Safety Conference (IFSAC2013), Kuala Lumpur, Malaysia, 2-3 December 2013. The theme of the conference was "Food safety; a critical dimension of food security in emerging economies". The conference was attended by more than 200 participants from more than 15 countries. Ms. Zora Jandrić presented a poster on applied research done in the FEPL on the classification of honey of various floral and geographical origins using ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry and

multivariate data analysis. The aim of this upstream research was to provide analytical methodology for food safety and traceability to support IAEA Member States in ensuring sustainable food systems.

- In 2013, FEPL was also involved in planning the scientific programme of The 2nd International Conference on Food Integrity and Traceability, Queen's University Belfast, UK, 8-10 April 2014, through the Laboratory Head's inclusion in the scientific committee.

CAPACITY BUILDING

The FEPL provided technical management for eighteen national and seven regional technical cooperation projects in 2013. The expertise available in FEPL and the methods and techniques developed were also used to support technology transfer to laboratories and institutes in Member States through various train-the-trainers activities, at Seibersdorf and in Member States. The FEPL provided group fellowship training for four scientists from Zimbabwe on food contaminant control and training for 5 scientists from Tanzania and Senegal on the quality control of trypanocidal drugs. Two interns were also hosted in the FEPL in 2013.

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

Sustainability of capacity building activities to improve food safety and quality through nuclear technology and networking

The FEP Subprogramme commenced this project funded by the USA under the Peaceful Uses Initiative in 2012. The objective of the project is to facilitate greater access for Member States to the peaceful uses of nuclear technology. In 2013, the second year of this 3-year project, one training workshop was held at Seibersdorf and two regional workshops (Latin America and Africa) were held in Member States of this 3-year project. As of December 2013, eight training courses or workshops for scientists, technicians, regulators and policy makers had been held since the project started in mid-2012, with approximately 160 individuals from developing countries participating in training activities directly related to various aspects of food safety, quality and control.

The FAO/IAEA interregional training workshop on "Integrated analytical techniques to control contaminants in food" was held at Seibersdorf, from 25 February to 8 March 2013. The workshop had 21 participants from 15 different developing countries (Argentina, Belize, Brazil, Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, Guatemala, Lebanon, Nicaragua, Panama, Paraguay, Peru, Uruguay) and 12 external lecturers, as well as the FEPL staff. The objective was to present a range of nuclear and related technologies for the control

of contaminants in food in an integrated way, with a special focus on the control of pesticide residues in food.

An FAO/IAEA/ICA regional workshop on "Effective Monitoring of Food Contaminants – Sampling, Method Validation and Quality Control" was held in Bogotá, Colombia, 20-24 May 2013. The workshop was held at the analytical laboratory of the Instituto Colombiano Agropecuario (ICA) with the participation of 30 representatives from 11 countries in Latin America and the Caribbean (Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Paraguay, Peru, and Uruguay).

A regional training meeting on "Quality Assurance/Control of Analytical Methods for Food Contaminants and Traceability" was held in Botswana National Veterinary Lab (BNVL), Gaborone, Botswana October 28 - 1 November 2013. The meeting had 40 participants from 20 countries in Africa (Algeria, Benin, Botswana, Cameroon, Egypt, Ethiopia, Ghana, Kenya, Mauritius, Morocco, Namibia, Nigeria, Senegal, Seychelles, South Africa, Tanzania, Tunisia, Uganda, Zambia and Zimbabwe).

Food traceability training

Two TC-funded training courses under the project "Building technological capacity for food traceability and food safety control systems through the use of nuclear analytical techniques" (RAS5062) were implemented by FEPL staff in 2013. A course was held in Universiti Sains, Penang, Malaysia, from 28 January to 8 February, with 38 participants from 14 countries in Southeast Asia. The trainees gained theoretical knowledge and practical skills in sample collection, sample processing and analysis of trace elements and stable isotopes in food and beverages for the purpose of determining the point of origin of the food. Training was also provided by Mr. Russell Frew to staff of the Chinese Academy of Agricultural Sciences, Beijing, 3-10 November 2013. Mr. Frew trained staff of the Institute if Quality Standards and Testing Technology for Agro-Products (IQSTAP) in setting up and operating a newly installed isotope ratio mass spectrometer, carbon and nitrogen analysis and principles of hydrogen isotope ratio analysis for natural products.

Trypanocide QC training

Training course on trypanocide QC

A training course was held in the FEPL in January 2013 as part of an international project involving FAO, IFAH, GALVmed, IAEA and Manchester Metropolitan University (MMU). The objective of the project is to help to control the quality of drugs used to control tsetse-transmitted African animal trypanosomosis - there is a well-recognized problem of counterfeit or poor quality drugs on the market. In January 2013, Ms. Siya Augustine Assey and Mr. Gerald Sambu Kulwa from Tanzania Food and Drugs Authority (TFDA) laboratory, Dar

Es Salaam and Messrs. Gilbert Akoda, Assiongbon Teko-Agbo and Elhadji Niang from the Laboratoire de Contrôle des Médicaments Vétérinaires (LACOMEV), Dakar, Senegal were trained in FEPL on the chromatographic methods for the quality control of the trypanocidal formulations. The training was funded by GALVmed and implemented by Dr. Oliver Sutcliffe (MMU) and FEPL staff. The initial implementation of the methods is now under way in the TFDA and LACOMEV.

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 the network was expanded to include 49 laboratories in 19 countries. 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 experience gained in setting up RALACA is unique. The concept will be exported to other continents and regions to create awareness of the importance of working in a network of peers. RALACA offers the opportunity to work in a multidisciplinary context, where challenges are shared amongst dedicated committees and addressed collectively using expertise from multiple disciplines and experts. The network is gaining international recognition; the work of RALACA was presented at the 4th Latin American Pesticide Residue Workshop that took place in Bogotá, Colombia, from 26th to 29th May 2013.

The Global Food safety Partnership

The Global Food Safety Partnership is an innovative public-private partnership facilitated by the World Bank, dedicated to improving the safety of food worldwide, focusing on middle income and developing countries. The mission of the GFSP is to improve food safety through capacity building in low and medium income countries in order to improve public health, facilitate trade, accelerate economic growth, and alleviate rural poverty.

The activities of the GFSP are facilitated through Advisory Working Groups (WGs) consisting of experts from public and private sectors and service providers, which provide technical input and expertise in the design and delivery of the GFSP's work program. The Head of the FEPL is a member of the Food Safety Technical Working Group (FSTWG). A meeting of the FSTWG was hosted by UNIDO in Vienna, from 10−11 October 2013, at which the terms of reference for the group were discussed and agreed and work was commenced on elaborating the mechanisms through which the WG would act. These mechanisms were further elaborated and finalized at the second Annual Conference in Singapore, 9-13 December 2013.

It is expected that participation in the GFSP will enable identification of synergies with FAO/IAEA projects to the greater benefit of project counterparts and Member States in the field of food safety.

Fellowships, Scientific Visitors and Interns

Ms Laura Natalia Fernandez Cedi joined the Food and Environmental Protection Laboratory as an intern in September 2013. Natalia brought the benefits of her qualifications in food engineering and previous UN experience as an intern in UNIDO to the FEPL. During her internship she worked on food traceability and authenticity methodology using a metabolomics approach and contributed to the support provided by FEPL to the RALACA Laboratory Network.

Fellows from Zimbabwe training in FEPL

Mr. Tungamirayi C. Mhande, Mr. Bamusi Saidi, Mr. Prosper Jambra, and Ms. Joan Burumu, from the Central Veterinary Laboratory in Zimbabwe, commenced a twoweek group fellowship training in FEPL on 4th February 2013. The Fellows were trained in the field of contaminants and residues in animal products for human consumption, focusing on the quantitative determination of pesticide residues by gas chromatography-mass spectrometry (one week) and veterinary drug residues by liquid chromatography-tandem mass spectrometry (one week). The training also covered topics such as the principles of analytical method development, laboratory quality assurance and quality control, and method validation according to internationally accepted guidelines, focusing on SANCO/12495/2011 and European Commission Decision 2002/657 EC.

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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”

THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

The Insect Pest Control Laboratory (IPCL) continued to focus on three insect groups (different species of tsetse flies, fruit flies and mosquitoes) for the research and development part of our work.

Work continued on developing management strategies of the tsetse salivary gland hypertrophy virus (SGHV) that can impede productivity of certain tsetse colonies such as *Glossina pallidipes*. One method that was further explored was based on the use of oligopeptides to impede the entry of the virus in the midgut cells and hence, to block its transmission. Preliminary trials used an oligopeptide that is homologous to a region of the ODV-e66 protein of baculoviruses. The virus load in *G. pallidipes* fed with oligopeptide-supplemented blood meals showed a reduction by at least two orders of magnitude compared to the controls. Repeating this experiment on a larger colony scale failed to produce the same results, which was most likely related to inadequate solubility of the oligopeptide and suboptimal distribution of the oligopeptide in the supplemented blood.

The IPCL initiated efforts to assess the relation between the prevalence of the SGHV and the endosymbiont *Wolbachia*. The results indicate that a high virus titre was only observed in the flies which had low *Wolbachia* titre and *vice versa*.

Some IPCL staff were part of the International Glossina Genome Initiative (IGGI) that sequenced and annotated the genome of the tsetse fly *Glossina morsitans morsitans.* Genome analysis predicted the presence of 12,308 protein coding genes as well as multiple insertions of *Wolbachia* genome sequences. The results were published in Science and in PLOS Neglected Tropical Diseases.

Research in support of the CRP on resolution of cryptic species complexes of Tephritid pests to overcome constraints to SIT application and international trade focused on *Bactrocera invadens* (Kenya origin) and *Bactrocera dorsalis* (China and Pakistan origin). Pre-zygotic mating compatibility studies combined with F_1 post-zygotic compatibility and F_2 hybrid viability and fertility studies support the hypothesis that *B. dorsalis* and *B. invadens* represent the same biological species; an outcome that poses significant implications for pest management and international trade for sub-Saharan Africa.

Experiments were carried out that aimed at the development of practical methods to enhance the mating behaviour of male *Bactrocera carambolae* through exposure to methyl eugenol (ME) prior to release in the field. ME-feeding enhances male *B. carambolae* mating competitiveness but currently used and available equipment in fruit fly emergence and holding facilities do not allow ME-feeding before release of the adult flies. It was found that exposing males to ME through aromatherapy increased their mating success over untreated males similar to ME feeding. Mating success of ME-aromatherapy-treated males was similar to that of ME-fed males and both treatment groups were significantly better at mating with virgin females

than untreated males. The data of this work clearly indicate that ME-aromatherapy could be a practical approach to be applied before release for improving sterile male *B. carambolae* mating competitiveness, thereby increasing the effectiveness of the sterile insect technique (SIT) against this pest.

Further progress can be reported under the Joint USDA/IAEA 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 Tephritid fruit flies that pose a high-level threat of entry into uninfested Member States, but approved post-harvest quarantine treatments are lacking for several important fruit flies species and their hosts. Work was carried out with hot water treatments, cold treatments and methyl bromide fumigations to help Member States to export their horticultural products.

Comparative quality control analysis of *Wolbachia*-infected and *Wolbachia*-free Mediterranean fruit fly *Ceratitis capitata* lines was initiated. The impact of *Wolbachia* on developmental and demographic parameters (fecundity, egg hatch, egg to pupae recovery, egg to adult recovery, sex ratio, and lifespan) and mating competitiveness was investigated.

Research was conducted towards the detection, isolation and characterization of gut-associated symbionts and to assess their impact on nutrition, digestion, reproduction, mating behaviour and pest status in fruit flies. Both culture-dependent and culture-independent approaches were considered in order to characterize the structure of the symbiotic communities for each targeted species.

Male mating competitiveness is a crucial parameter in many genetic control programmes including the sterile insect technique. The competitiveness of males of the mosquito species *Anopheles gambiae* was evaluated in semi-field cages (1.7m x 1.7m x 1.7m) that were located in a climate controlled insect greenhouse using varying ratios of irradiated to untreated males. Egg hatch was reduced to 64% with a 1:1 irradiated:untreated male ratio, which was higher than the expected 50%. Increasing the irradiated:untreated male ratio to 5:1 did not increase the sterility proportionally to the increase in number of males released, but nevertheless reduced egg hatch to 35% (induced sterility of 65%).

Studies were likewise carried out in field cages to assess the effect of genetic manipulation to develop a genetic sexing strain (based on a dieldrin resistant mutation), dieldrin treatment (to eliminate the females from the production line), and irradiation (with 75 Gy) on the mating competitiveness of *Anopheles arabiensis* males. Whereas genetic manipulation had no effect on males' competitiveness, the irradiation treatment reduced their competitiveness by half. The irradiated males that had been additionally treated with dieldrin as eggs appeared to be more competitive than those that had only been irradiated. This suggests a possible radio protectant effect of dieldrin on the *An. arabiensis* germinal cells.

In 2013, the IPCL hosted eleven cost-free experts, five consultants, five interns, and fifteen fellows (the latter funded by the IAEA's Department of Technical Cooperation).

In 2013, the IPCL carried out 22 shipments of tsetse pupae to six research institutes in Germany, Italy, Switzerland, and the UK. In addition, 200 tsetse DNA samples and 300 dissected tsetse pupae in preservative solution were shipped and fortnightly shipments of tsetse pupae from Slovakia in support of the tsetse project in Senegal were irradiated. It also carried out 22 shipments of different fruit fly species to 10 institutions in Australia, Czech Republic, China, Columbia, Croatia, Greece, Israel, Italy, Mexico, and Spain, and 13 shipments of preserved fly samples to 8 institutions in Australia, Greece, Italy, Japan, Mexico, Malaysia, Pakistan, and USA. In addition, the IPCL shipped 144 DNA samples and 58 materials (diet components, equipment) to 41 institutions in 15 countries. A newly constructed VIENNA 8 *Ceratitis capitata* strain was shipped to Spain and Australia for mas-rearing purposes. The IPCL supplied four egg shipments of *An. arabiensis* to Germany and Sweden and transferred mosquito mass rearing materials / technologies to 11 institutions in Brazil, China, France (La Reunion), Italy, Madagascar, Mauritius, Seychelles, South Africa, Sri Lanka and Sudan.

STAFF

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Caceres, **Carlos**	Entomologist (Plant Pests)
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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Livestock Pests

Salivary gland hypertrophy virus

As reported previously, some tsetse species carry a virus (Figure 1) 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 Insect Pest Control Laboratory (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 a SGH prevalence up to 77%. This high prevalence was associated with the poor performance of the colony.

Development of strategies to impede Glossinavirus entry and transmission using oligopeptide bioassays

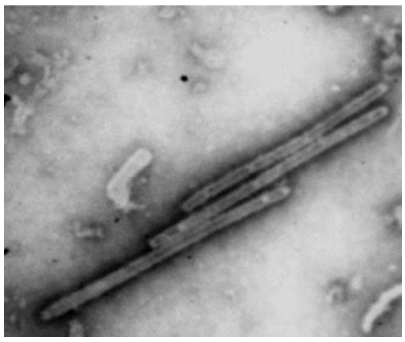

FIG. 1: An electron microscopy photo of the SGHV

For successful infection of a tsetse fly, the Glossinavirus must attach and eventually traverse the fly gut epithelium to find its way into the salivary glands. The virus must, therefore, possess peptide sequences recognized by receptors in the flies' midgut. By screening a phage display library, it was possible to identify oligopeptides that would bind to the mid- and/or hindguts of tsetse flies. Therefore, preliminary trials were initiated to evaluate the potential of using an oligopeptide that contains 12 amino acids to impede the attachment of Glossinavirus in the mid gut of *G. pallidipes*. This short oligopeptide is homologous to a region of the ODV-e66 protein of baculoviruses. The virus load in *G. pallidipes* fed with oligopeptide-supplemented blood meals showed a reduction by at least 2 orders of magnitude compared to the controls.

The excellent results obtained with the oligopeptide in the small scale experiments encouraged implementation of this strategy to impede virus infection at colony scale. Three *G. pallidipes* colonies were established (i) negative control colony, (ii) positive control colony (colony

that was fed virus-contaminated blood), and (iii) flies fed on virus-contaminated blood but supplemented with the oligopeptide (300 ng/ml). The flies were maintained for 6 months and the virus load was estimated by qPCR. The results however indicated no significant differences among treatments in contrast to the results obtained in the small scale experiment. This unexpected result is most likely related to inadequate solubility of the oligopeptide and suboptimal distribution of the oligopeptide in the supplemented blood. Further work is ongoing to improve the solubility of the oligopeptide.

Analysis of the SGHV virus host range

In an attempt to better understand the biology and transmission of SGHV, experiments were carried out with other tsetse species. The SGHV obtained from hypertrophied salivary glands of *G. pallidipes* males was injected into newly emerged adults from *G. brevipalpis, G. palpalis gambiensis, G. morsitans morsitans, G. m. centralis* and *G. pallidipes*. The virus titre was estimated in the injected flies at different times post-infection and the development of SGH was evaluated in F_1 progeny. The virus prevalence significantly increased after injection in *G. p. gambiensis, G. m. morsitans, G. m. centralis* and *G. pallidipes*, but no increase in the virus titre was observed in *G. brevipalpis*. Injection of the virus into adult females of *G. pallidipes* leads to the development of SGH in the F_1 progeny, but no SGH was observed in the F_1 progeny of injected females from *G. p. gambiensis, G. m. morsitans, G. m. centralis* and *G. brevipalpis*. This result indicates that a barrier prevents virus transmission from infected mother to progeny in these species.

Prevalence of Wolbachia *and its correlation with virus prevalence in East Africa*

The prevalence of the endosymbiont *Wolbachia* was estimated in tsetse fly samples collected in East Africa to assess the correlation between the prevalence of the SGHV and *Wolbachia*. A total of 5034 flies were tested for the detection of both SGHV and *Wolbachia* and only 142 flies were found to be infected by both. The results indicate that a high virus titre was only observed in the flies which had low *Wolbachia* titre. In the flies with high *Wolbachia* titre only low virus titre was detected. This result was observed in *G. morsitans* and *G. austeni*, two species which have a high natural prevalence of *Wolbachia* and these data indicates a negative impact of *Wolbachia* on the prevalence of SGHV.

The genome sequence of the tsetse fly Glossina morsitans morsitans *and its associated symbiont* Wolbachia*: extensive horizontal transfer of* Wolbachia *sequences in the tsetse fly chromosomes*

In the frame of the International Glossina Genome Initiative (IGGI), the genome of *Glossina morsitans morsitans* (366 Mb) was sequenced and annotated. Genome analysis predicted the presence of 12,308 protein coding genes as well as multiple insertions of *Wolbachia* genome sequences. In parallel, we sequenced the genome of the cytoplasmic *Wolbachia* endosymbiont (cytWol) associated with *G. m. morsitans*. By *in silico* and molecular and cytogenetic analysis, we characterized the multiple insertions of *Wolbachia* (chrWol) in the host genome. Taken together, these data provide a wealth of information about the biology of tsetse as well as the biological and evolutionary significance of *Wolbachia* infections in this species of tsetse fly. In

addition, they provide the ground for innovative research and potential development of novel approaches for the control of tsetse populations and trypanosomosis.

A manuscript reporting on the genome of *G. m. morsitans* was published in the journal Science while another manuscript describing the genome of the *Wolbachia* strain infecting *G. m. morsitans* and the detailed characterization of the insertions of *Wolbachia* genome sequences into the host chromosomes was published in the journal PLOS Neglected Tropical Diseases.

Plant Pests

Resolution of cryptic species complexes of Tephritid pests to overcome constraints to SIT application and international trade

Anastrepha fraterculus *complex*

In collaboration with visiting scientists from Argentina, field cage experiments were carried out to assess the attraction of female *Anastrepha fraterculus* to the pheromones of males and to 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 morphotypes/populations than to females from other morphotypes/populations. The assessment of female fly responses to artificial leks containing pheromone-calling males was done with morphotypes from Tucumán (Argentina), Parnamirim, Piracicaba, and Vacaria (Brazil). Preliminary results show that females visited more frequently leks that had males than leks with no males and

Tucumán females discriminated between Tucumán males and those from Vacaria. This was not evident for Vacaria or Piracicaba females.

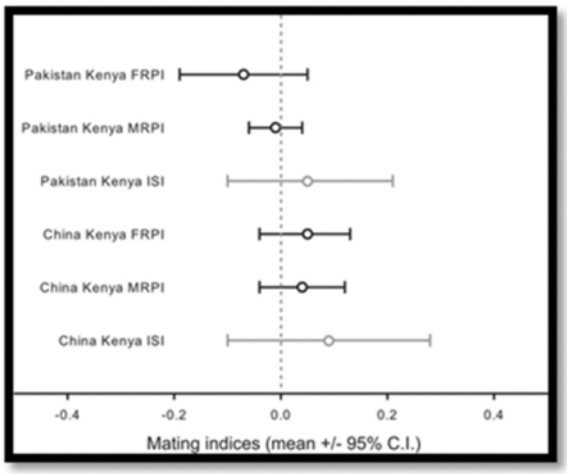

FIG. 2: Indices of sexual isolation (ISI; gray), male relative performance indices (MRPI; black), and female relative performance indices (FRPI; black) for two field cage mating compatibility tests between B. dorsalis *from China and Pakistan against* B. invadens *from Kenya. Confidence intervals crossing zero denote either a) statistically random mating (for ISI) or b) equal participation by the sexes of either test species (MRPI and FRPI).*

Bactrocera dorsalis *complex*

The IPCL, in collaboration with scientists from China and Australia, continued working towards resolving species status among morphologically cryptic pest taxa of the *Bactrocera dorsalis* complex. Mating compatibility tests were carried out in field cages with host trees between populations of the invasive fruit fly *Bactrocera invadens* that originated from Kenya and the Oriental fruit fly *Bactrocera dorsalis* that originated from Pakistan and China. The invasive fruit fly that occurs predominantly in Africa is morphologically and genetically extremely similar to the Oriental fruit fly, but the specific relationship between these two species had remained unclear. Comparative pre-zygotic mating compatibility tests between *B. dorsalis* and *B. invadens* were carried out using standardized field cage mating tests. These tests were followed by an assessment of F_1 post-zygotic compatibility and F_2 hybrid viability and fertility. Results showed that *B. invadens* mated randomly with *B. dorsalis* from both localities (Figure 2) and there were generally high levels of hybrid viability, survival, and fertility of F_1 hybrids. Similarly, the data of the F_2 crosses showed high viability and survival. The fact that *B. dorsalis* and *B. invadens* mated randomly under standard field cage conditions brings us to conclude that, regardless of their specific identities, males of either *B. dorsalis* or *B. invadens* are equally suitable for use against females of either taxon for the purposes of future management approaches such as the SIT. Furthermore, the pre-zygotic compatibility between the two populations endorses the hypothesis that these taxonomic species represent the same biological entity. The confirmation that *B. dorsalis* and *B. invadens* represent the same biological species poses significant implications for pest management and international trade for sub-Saharan Africa.

Aromatherapy for Bactrocera carambolae

Experiments were carried out with the aim to develop practical methods to enhance mating competitiveness of sterile males of some *Bactrocera* species through exposure to methyl eugenol (ME) prior to release in the field.

FIG. 3: Mean mating percentage (±SE) of Bactrocera carambolae males that were either fed methyl eugenol (ME) or treated by ME-aromatherapy or untreated. Tests were carried out in field cages. Mean male mating success followed by different letters are significantly different from each other.

Methyl eugenol (1,2-dimethoxy- 4 -(2-propenyl) benzene) is a natural compound occurring in a variety of plant species. It is a powerful attractant for males of many tropical tephritid fruit fly species of the genera *Bactrocera* and *Dacus* and one of the most commonly used male lures. This behavioural attractiveness has been exploited since the 1950s for fruit fly population monitoring and as part of more environment-friendly lure-and-kill approaches called male annihilation technique (MAT) for controlling fruit flies of economic importance.

ME-feeding is known to enhance male *B. carambolae* mating competitiveness 3 days after feeding which can increase significantly the effectiveness of the SIT. However, the currently used and available equipment in fruit fly emergence and release facilities is not suitable for ME-feeding before release of the adult flies. ME application through aromatherapy could however be a practical alternative for use in such facilities. This study was therefore

carried out to assess the effects of ME-aromatherapy on the mating competitiveness of male *B. carambolae* as compared to ME-feeding. The mating success of the males increased 3 days after exposure to ME-aromatherapy over untreated males similar to ME-feeding 3 days after exposure (Figure 3). Mating success of ME-aromatherapy-treated males was similar to that of ME-fed males and both treatment groups were significantly better at mating with virgin females than untreated males. The data of this work clearly indicate that ME-aromatherapy could be a practical approach for improving male *B. carambolae* mating competitiveness and therefore increasing the effectiveness of the SIT against this pest.

Development of phytosanitary and regulatory treatments for exotic Tephritid fruit flies

Work continued under the Joint USDA/IAEA 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 Tephritid fruit flies that pose a high-level threat of entry into uninfested member states, but approved quarantine post-harvest treatments are lacking for several important fruit flies species and their hosts. Work was carried out with hot water treatments, cold treatments and methyl bromide fumigations.

Hot water immersion treatment

To assess relative tolerance of hot water treatments among different tropical fruit fly species available at the IPCL, plastic bags filled with 450g standard *Bactrocera* carrot-based rearing diet were used as "artificial fruits" to standardize the testing procedure (Figure 4). Testing was conducted for *Bactrocera cucurbitae, B. zonata, B. correcta, B. dorsalis, B. invadens, B. carambolae, B. tryoni, B. philippinensis, B. papayae, Anastrepha fraterculus*, and *Ceratitis capitata* comparing these bioassay containers to the heating of mango. The standard hot water treatment procedures according to the UDSA treatment manual, including a minimum temperature of 46°C and constant water circulation, was used as reference. Data are being analysed for publication.

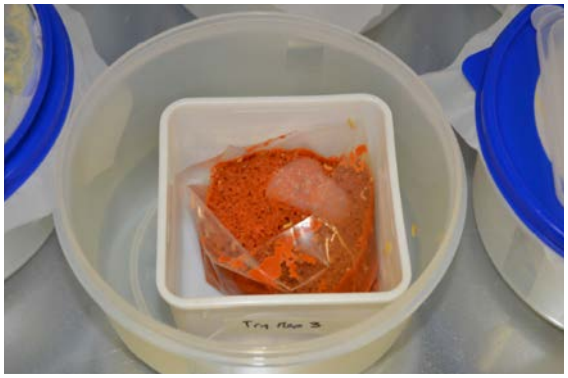

FIG. 4: Artificial fruit used in the hot water immersion treatments

Methyl bromide fumigations

Treatment of late instar larvae to assess relative tolerances to methyl bromide fumigation was completed for various *Bactrocera, Anastrepha* and *Ceratitis* species. *In vitro* fumigations at 15.5°C and at 5°C with a range of doses from 0 - 60 g/m3 and for a period of 2 hours were conducted. Data is being analysed for publication

Cold treatment

Verification of the most tolerant developmental stage to cold treatment for *Anastrepha ludens* on grapefruit was carried out. Efforts were undertaken to improve the infestation

techniques that would increase the rate of larval infestation per fruit. A protocol for testing relative tolerances to 1.5°C of different *Bactrocera* species (eggs and third instars larvae) was developed. "Artificial fruits" were used in a similar manner as the hot water treatment study. The experiments are expected to be completed in the second semester of 2014.

Pests of Human Health

Research in the mosquito group of the IPCL continued to focus on three main pests of human diseases, i.e. *Anopheles arabiensis* and *An. gambiae*, vectors of malaria, and *Aedes albopictus*, a vector of dengue and Chikungunya.

Spiking blood with ivermectin kills female Anopheles gambiae

The elimination of blood-sucking, and potentially disease transmitting female mosquitoes from the release material is of highest priority for mosquito SIT programmes. For those species where genetic sexing strains are not available this can be achieved using behavioural or biological differences of the sexes. For *Anopheles arabiensis* a method was developed that kills all female mosquitoes by offering blood meals that contain 7.5 ppm ivermectin. This method was now tested for *An. gambiae* and the data show that all females could be eliminated on day 4 post-emergence (similarly to the *An. arabiensis* laboratory colony) regardless of the blood source. More females were killed on days 1 and 2 when fresh blood was offered as compared to thawed frozen blood (FIG 5).

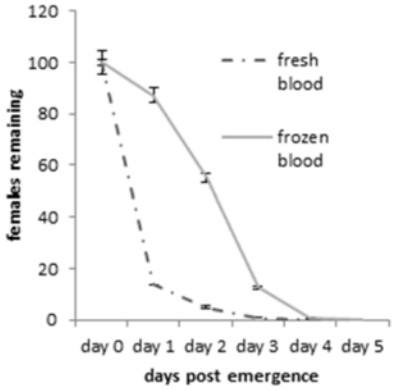

FIG. 5: Elimination of female Anopheles gambiae that were offered a blood meal spiked with ivermectin.

Assessing competitiveness in mosquitoes

Effect of blood feeding on competitiveness

Male mating competitiveness is a crucial parameter in many genetic control programmes including the sterile insect technique. Classical steps in mosquito competitiveness experiments include the separation of the sexes, emergence of the adults, resting period to allow sexual maturation, mating period, blood feeding and egg laying. Female mosquitoes that were offered a blood meal before the mating period also showed a significant increase in egg production. In addition, the female age, when offered the blood meal, appears to influence egg production.

Mating competitiveness of male Anopheles gambiae mosquitoes

The competitiveness of male *An. gambiae* was evaluated in semi-field cages (1.7m x 1.7m x 1.7m) in a climate controlled insect greenhouse. Varying ratios of irradiated males to untreated males to virgin females were tested: 100:0:100 (as control of irradiated males), 0:100:100 (as control of untreated males), 100:100:100, 300:100:100 and 500:100:100.

As previously reported in other mosquito species including anophelines, irradiation of pupae reduced mating competitiveness to some degree. With a 1:1 ratio of irradiated to untreated males, the hatch rate of 64.0 % was higher than the expected 50%. Increasing the ratio to 5:1 did not increase the sterility proportionally to the increase in number of males released, but nevertheless reduced egg hatch to 35% (induced sterility of 65%).

Effect of genetic manipulation, dieldrin treatment, and irradiation on Anopheles arabiensis males mating competitiveness

Before their release in an SIT programme, *An. arabiensis* males have to undergo several treatments. From the original strain, a genetic sexing strain (ANO IPCL1) based on a dieldrin resistant mutation was developed that involves a complex translocation. With the ANO IPCL1 strain, a dieldrin treatment at the egg stage allows for the complete elimination of female mosquitoes from the production line. The absence of female larvae and pupae reduces costs, space and labour requirements. Finally, after the larval development, the male pupae are sterilized by irradiation with a dose of 75 Gy. The impact of genetic manipulation, dieldrin treatment, and irradiation with 75 Gy on the competitiveness of the males was studied.

Three experiments were carried out to assess: (1) the competitiveness of ANO IPCL1 males compared to those of the wild-type strain (*An. arabiensis* Dongola), (2) the competitiveness of irradiated ANO IPCL1 males versus *An. arabiensis* Dongola males, and (3) the competitiveness of dieldrin treated, irradiated ANO IPCL1 males versus *An. arabiensis* Dongola males in a semi-field setting.

The experiments were carried out in semi-field cages (1.7m x 1.7m x 1.7m) that were located in a climate controlled insect greenhouse. Varying ratios of treated:untreated males:virgin females were used. No effect of genetic manipulation on male competitiveness was observed with a ratio of 1:1. Irradiated ANO IPCL1 males were approximately half as competitive as wild-type males when evaluated in this given scenario. The irradiated ANO IPCL1 males that had been additionally treated with dieldrin at egg stage appeared to be more competitive than those that had only been irradiated. This suggests a radio protectant effect of dieldrin on the *An. arabiensis* germinal cells as a possible explanation. According to the current results, somatic damage induced by irradiation could be diminished slightly by the presence of dieldrin residues, which are known to be retained by the insect to adulthood. The data indicate that a ratio of 10:1 of irradiated: fertile males was needed to reduce the cage population's fertility by 81%.

Genetics and Molecular Biology

Comparative quality control analysis of Wolbachia-infected and Wolbachia-free Mediterranean fruit fly lines

The impact of *Wolbachia* on developmental and demographic parameters (fecundity, egg hatch, egg to pupae recovery, egg to adult recovery, sex ratio, lifespan) and mating competitiveness was investigated in the Mediterranean fruit fly *Ceratitis capitata*. Five lines were included in the comparative analysis: the uninfected lines Benakeio and Vienna 8 GSS and the infected lines 88.6 (wCer-2 infected Benakeio line), S.10.3 (wCer-4 infected Benakeio

line) and 56S2 (wCer-2 infected Vienna 8 GSS). All *Wolbachia*-infected Mediterranean fruit fly lines showed significantly reduced egg hatch, egg-to-pupae and egg-to-adult recovery rates as compared to uninfected Mediterranean fruit fly lines. *Wolbachia* had no effect on egg hatch in aged females and on the sex ratio. No difference was observed in the competitive ability of Vienna 8 GSS (uninfected) and 56S2 (wCer2-infected Vienna 8 GSS) males against wild-type males for wild-type females. The other strains seem to be less competitive than the genetic sexing strains with remarkably low competitive ability observed for the 88.6 (wCer2-infected Benakeio) and Benakeio (uninfected) lines.

The gut microflora of fruit flies

Research was conducted towards the detection, isolation and characterization of gut-associated symbionts and to assess their impact on nutrition, digestion, reproduction, mating behaviour and pest status. Both culture-dependent and culture-independent approaches were considered in order to characterize the structure of the symbiotic communities for each targeted species.

Culture-independent approaches based on next generation sequencing techniques (16S rRNA gene pyrosequencing) were used to determine the structure of the gut-associated microbiota in *Ceratitis capitata* (Vienna 8 GSS), *Bactrocera oleae*, *Anastrepha ludens*, *A. grandis* and different morphotypes of *A. fraterculus*. As for the culture-dependent approaches, teneral males and females or of different age, unfed or fed were used for this experimental work.

A total of 122 samples were analyzed by next generation sequencing using pyrosequence analysis. The samples included: larvae, teneral flies, and 5 and 15 days-old adults (males and females). The sequence results indicated that each sample had on average around 21,475 sequences. Data analysis with "Quantitative Insights Into Microbial Ecology" software package indicated that more than 90% of the bacteria present in the gut of fruit flies were Proteobacteria, with 2.9% Firmicutes and 3.7% Actinobacteria. The Proteobacteria were mainly from Gamaproteobacteria (89.9%) and Alphaproteobacteria (1.1%).

Isolation and use of an Enterobacter sp. isolate as probiotic for the development of cost-effective and improved Mediterranean fruit fly mass-rearing and SIT applications

During the last few years, a number of studies have focused on the characterization of the gut symbionts of the Mediterranean fruit fly, presenting evidence for their significance on the host's biology and fitness. In this project, gut bacterial species from the Vienna 8 GSS were isolated and their impact on host life history traits assessed. The *Enterobacter* sp.-based probiotic treatments of Mediterranean fruit fly Vienna 8 larvae had increased egg to pupae recovery, increased adult emergence, shorter egg to pupae developmental time, reduced duration of the pupal stage, and shorter egg to adult developmental time. The use of live bacteria was much more effective than autoclaved bacteria as probiotic, but there was no effect on the sex ratio and on competitiveness of the flies as tested in field cages.

CAPACITY BUILDING

In 2013, the IPCL hosted eleven cost-free experts (CFE), five consultants (C), five interns, and fifteen fellows (the latter funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
AVGOUSTINOS, **Antonios**	Greece	C	11 mth	Endosymbionts and their effect of the productivity and competitiveness of insects
KYRITSIS, **Georgios**	Greece	C	7 mth	
BIAN, **Guowu**	China	CFE	6 wk	Mating compatibility studies with members of the *Bactrocera dorsalis* and *Anastrepha fraterculus* complexes to disentangle their taxonomic status and thereby remove constraints for SIT application and to facilitate international trade
DEVESCOVI, **Francisco**	Argentina	CFE	1 mth	
BRIZOVA, **Radka**	Czech Republic	CFE	1 wk	
UL HAQ, **Ihsan**	Pakistan	CFE	12 mth	
WALSE, **Spencer**	USA	CFE	1 wk	Post-harvest treatment of fruit flies
FONTENOT, **Emily**	USA	CFE	11 mth	
MYERS, **Scott**	USA	CFE	1 wk	
DEMIRBAS, Guler **Uzel**	Turkey	Intern	9 mth	Developing management strategies for the tsetse virus in support of the tsetse eradication projects
BOUCIAS, **Drion**	USA	CFE	1 wk	
MUTIKA, **Gratian**	Zimbabwe	CFE	12 mth	Handling protocols for tsetse
BLOUCH, Ammara	Pakistan	Intern	4 mth	Rearing of fruit flies
YU, **Dalin**	China	Intern	4 mth	Data management
ZHENG, **Minlin**	China	CFE	2 mth	Developing mass-rearing techniques and the SIT package for disease transmitting mosquitoes
ZHANG, **Dongjing**	China	CFE	6 mth	
DAMIENS, **David**	France	C	12 mth	
BALESTRINO, **Fabrizio**	Italy	C	12 mth	
NANVUMA, **Cynthia**	Uganda	Intern	1 mth	
RAJAMOHAN, **Arun**	USA	C	2 wk	Cryopreservation of Fruit flies
MONIB, **Mohamed**	Egypt	Intern	10 mth	Genetics of fruit flies

Name	Country	Status	Duration	Topic
Fathelrhman, **Omnia**	Sudan	Fellow	3 mth	Mosquitoes
Ouedraogo Sanon, **Gisele**	Burkina Faso	Fellow	9 mth	Tsetse
Wang, **Bo**	China	Fellow	6 mth	Fruit flies
Chundydyal, **Sangeeta**	Mauritius	Fellow	1 mth	Mosquitoes
Iyaloo, Diana **Pillay**	Mauritius	Fellow	1 mth	Mosquitoes
Dharmasiri, **Aluth Gedra**	Sri Lanka	Fellow	1 mth	Mosquitoes
Dewagamage, **Isuru Chamara**	Sri Lanka	Fellow	1.5 mth	Mosquitoes
Berihu, **Alem**	Ethiopia	Fellow	3 d	Tsetse
Desalegn, **Tazezew**	Ethiopia	Fellow	3 d	Tsetse
Munhenga **givemore**	South Africa	Fellow	3 mth	Mosquitoes
Traore, **Astan**	Mali	Fellow	3.5 mth	Tsetse
Yang, **Jianquan**	China	Fellow	3 mth	Mosquitoes
Daba, **Habtamu**	Ethiopia	Fellow	3 wk	Tsetse
Woinue, **Johannes**	Ethiopia	Fellow	1 wk	Tsetse
Chisinjila, **Benjamin**	Tanzania	Fellow	1 wk	Tsetse

SERVICES

In 2013, the IPCL carried out 22 shipments of tsetse pupae to six research institutes in Germany, Italy, Switzerland, and the UK. In addition, 200 tsetse DNA samples and 300 dissected tsetse pupae in preservative solution were shipped and fortnightly shipments of tsetse pupae from Slovakia in support of the tsetse project in Senegal were irradiated.

It also carried out 22 shipments of different fruit fly species to 10 institutions in Australia, Czech Republic, China, Columbia, Croatia, Greece, Israel, Italy, Mexico, and Spain, and 13 shipments of preserved fly samples to 8 institutions in Australia, Greece, Italy, Japan, Mexico, Malaysia, Pakistan, and USA. In addition, the IPCL shipped 144 DNA samples and 58 materials (diet components, equipment) to 41 institutions in 15 countries. A newly constructed VIENNA 8 *Ceratitis capitata* strain was shipped to Spain and Australia for mas-rearing purposes.

The IPCL supplied four egg shipments of *An. arabiensis* to Germany and Sweden and transferred mosquito mass rearing materials / technologies to 11 institutions in Brazil, China,

France (La Reunion), Italy, Madagascar, Mauritius, Seychelles, South Africa, Sri Lanka and Sudan.

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EXTRABUDGETARY SUPPORT

In 2013, the IPCL received extrabudgetary resources from the USA under the USDA/APHIS agreement “Development of phytosanitary and regulatory treatments for exotic tephritid fruit flies” (Euro 98,021).

THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

Yield continues to be the number one goal of plant breeders, especially those in developing countries. Attaining high yields is becoming increasingly problematic due to the effects of climate change. Dramatic changes of climate such as temperature extremes, drought, flooding and high winds have obvious and direct impacts on crop failure. Subtle changes in climate can promote the development of pests and diseases, for example small increases in humidity and rainfall are thought to be responsible for severe losses in coffee production due to coffee leaf rust disease. This is particularly problematic in developing countries, for example recent failures of wheat crops in Ethiopia, Kenya and Uganda, not only leaves these countries without a harvest but pushes up commodity prices on world markets. Global economies mean that crop failures in one part of the world affect everyone. Food security can be enhanced by developing more resilient crops and this can be achieved effectively (cheaply and quickly) using plant mutation breeding. The Plant Breeding and Genetics Laboratory (PBGL) focuses on mutation breeding to increase biodiversity for desired traits in crop plants and to accelerate the breeding of mutant varieties with higher yield, yield stability, quality and improved resistance to environmental stresses such as pests, diseases, drought and salinity.

In 2013 the PBGL has been engaged in three programmes of work: 1) Crop improvement for yield and enhanced adaptability to climate change; 2) Integrated and efficient mutation technologies for crop breeding and genetics; and 3) Integrated soil-water-plant approaches to enhance food production and biomass productivity. The work is implemented through research and development (R&D in mutation induction, mutation detection and mutant line development), capacity building (individual and group training) and service provision (irradiation). These laboratory activities have direct links with ongoing IAEA Technical Cooperation (TC) projects and Coordinated Research Projects (CRPs).

Recent advances in our R&D work include the development of *in vitro* mutation induction methods for potato, especially for Kenya, Lesotho and Morocco who provided fellows for training in these methods. The PBGL also carried out the first investigations aimed at developing mutation induction methods for coffee, involving the irradiation treatments of seed for Arabica varieties and irradiation of cuttings for Robusta varieties from Nigeria, again this involved fellowship training. In addition the PBGL has been developing biotechnologies aimed at speeding up the breeding of mutant lines in a range of crops, so that they may be released as new varieties for farmers as soon as possible.

In 2013, research and development activities in the area of mutation screening largely focused on the development and validation of low-cost and non-toxic methods that can be easily adapted for most laboratories in developing countries. Protocols produced by the PBGL were used to develop a curriculum for training courses. Low-cost methods for plant tissue collection, non-toxic DNA extraction and mutation discovery were incorporated into three Seibersdorf training courses in 2013. Kits for low cost methods have also been developed that

are being sent to Member States to facilitate the up-take and utilization of these user-friendly methods.

With respect to capacity building, in 2013 the PBGL hosted 26 individual trainees from 20 countries and organized three training courses for a total of 73 trainees from 30 countries. In addition staff of the PBGL were involved in the organization and training of two inter-regional training courses (Jordan and Qatar) involving 37 trainees from 9 countries.

The PBGL provides an irradiation service for plant materials to Member States. This mainly involves gamma irradiation for mutation induction, but in 2013 requests were also received for X-ray and Ion Beam irradiation treatments. There were a record number of irradiation service requests: 105 for over 50 plant species from 34 countries.

As Technical Officers staff of the PBGL visited and assessed projects in Botswana, Eritrea, Indonesia and Mongolia where they gave technical advice for project implementation in plant mutation breeding methods. Professional staff were also involved in reviewing proposals for Technical Cooperation (TC) funding from Albania, Colombia, DR Congo, Ghana, Indonesia, Lesotho, Mauritius, Pakistan, Thailand and Viet Nam. Practical support to TC projects included the delivery of: low cost kits developed at the PBGL for DNA extraction and Mutation discovery to Botswana, India, Iran, Mauritius and Sudan ; and standard mutant stocks were delivered to Jordan, Oman, Poland, Qatar, Sudan and Yemen for training purposes in radio-sensitivity testing. In 2013 the PBGL, as a partner in the CRP: "Enhancing the efficiency of induced mutagenesis through an integrated biotechnology pipeline" developed low cost methods in DNA characterization for mutation screening and mutant line development and delivered methods to 21 Member States (Algeria, Bangladesh, Bulgaria, Botswana, China, Colombia, DR Congo, Egypt, India, Indonesia, Iraq, Iran, Lebanon, Mauritius, Nigeria, Oman, Saudi Arabia, South Africa, Sudan, Syria, Tunisia, Uganda and Yemen). In addition the R&D work at the PBGL supported activities in the CRP: "Integrated utilization of cereal mutant varieties in crop/livestock production systems" by providing technical advice in screening for mutant traits and simple methods in assessing nutritional quality, these were supplied to all counterparts (Austria, China, Indonesia, Kuwait, Macedonia, Malaysia, Mongolia and Peru). Relevant mutant germplasm developed at the PBGL was also disseminated to plant breeders in these countries and to other interested countries who expressed interest (Iran and Iceland.

Three methods in plant mutation breeding were published in peer reviewed journals. Other publications included a research paper on quality mutants in sorghum and seven presentations in plant mutation breeding in international conference proceedings. Protocols were developed for 1) Low-cost TILLING, 2) Salt tolerance screening and 3) X-ray mutation induction; and Methods were developed in 1) Low-cost DNA characterization of mutant plants, 2) Barley crossing and 3) Molecular characterization of mutant germplasm – a manual, these have been uploaded onto our web-site: *http://www-naweb.iaea.org/nafa/Pbg/public/manuals-pbg.html.* Other outreach information provided to Member States included: Newsletters (July 2013 and January 2014), the book "Plant mutation breeding and biotechnology" and Information Sheets on PBGL interactions with 17 countries.

Along with other FAO/IAEA Agriculture & Biotechnology Laboratories at Seibersdorf the PBGL has be active in supporting the ReNuAL project: **Re**novation of the FAO's and IAEA's **Nu**clear Sciences and **A**pplications **L**aboratories in Seibersdorf, which aims to ensure that all Seibersdorf laboratories including the PBGL are fit-for-purpose and appropriately positioned to meet the evolving needs and demands of Member States with adequate infrastructure in place for the next 20-25 years.

STAFF

Name	Title
Forster, **Brian Peter**	Laboratory Head
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
Seballos, **Gilbert**	Laboratory attendant
Hofinger, **Bernhard**	Technician
Lorenz, **Anne**	Implementation Assistant
Mletzko, **Joanna Malgozata**	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The R&D activities of the PBGL focus on enhancing the efficiency of plant mutation breeding, and target and develop user-friendly methods for plant breeders in developing countries. The three main areas of work: 1) mutation induction; 2) mutation detection and 3) mutant line development.

Mutation induction

The mandate of the PBGL is to use nuclear techniques to improve crop plants. This is done by inducing mutations through irradiation treatment with gamma rays or X-rays. This is a proven and successful method as over 3000 mutant varieties have been produced in over 200 crop species world-wide. Seed is an ideal material for irradiation as it can be produced in large numbers, this is important as the desired mutation is normally a rare event occurring in one in a thousand or less individuals. Seed are also easily shipped to and from a remote irradiation

facility (see Services below). Crops that do not produce seed or are not propagated from seed, i.e. vegetatively-propagated crops such as banana, cassava and potato require different methods for mutation induction. One major success in 2013 has been the development of *in vitro* methods in potato.

In vitro mutation induction in potato

Potato (*Solanum tuberosum*) is an important vegetable and staple food worldwide. It is a tetraploid outbreeding species which maintains a high degree of genetic variation (heterozygosity). Problems facing potato production are yield loss due to diseases (blights, scabs and rotters) and pests such as potato cyst nematode and Colorado beetle. In developing countries many traditional varieties suffer from poor yield and have small tubers, in addition their market value is impaired by undesirable traits such as sunken eyes. Mutation breeding aims to improve such traits in these favoured local varieties. In 2013 the PBGL received requests for both potato mutation induction and training in methods for mutation induction in potato from Kenya, Lesotho and Morocco. Because of this demand the PBGL set out a series of experiments aimed at optimizing methods for *in vitro* mutation induction in potato. The starting materials were tubers provided by Fellows from Kenya, Lesotho and Morocco, these were sent to the PBGL through the Standard Material Transfer Agreement to be planted prior to training.

Tubers were sprouted and plants grown up in the greenhouse to provide shoot materials, which were used to initiate *in vitro* shoot cultures. Sterile, disease and pest free shoot cultures were established and these were multiplied rapidly, thus large numbers of plantlets were established. These cultures were used in radio-sensitivity tests to determine the optimal gamma dose treatment for mutation induction. Treatments included:

- irradiation of *in vitro* nodal cuttings (without leaf) followed by *in vitro* shoot propagation to dissolve chimeras;
- irradiation of *in vitro* nodal cuttings (without leaf) followed by *in vitro* shoot propagation to dissolve chimeras, followed by *in vitro* micro-tuber production;
- irradiation of *in vitro* nodal cuttings (with leaf) followed by *in vitro* micro-tuber production;
- mutation induction by irradiation of *in vivo* micro-tubers.

These methods are illustrated below:

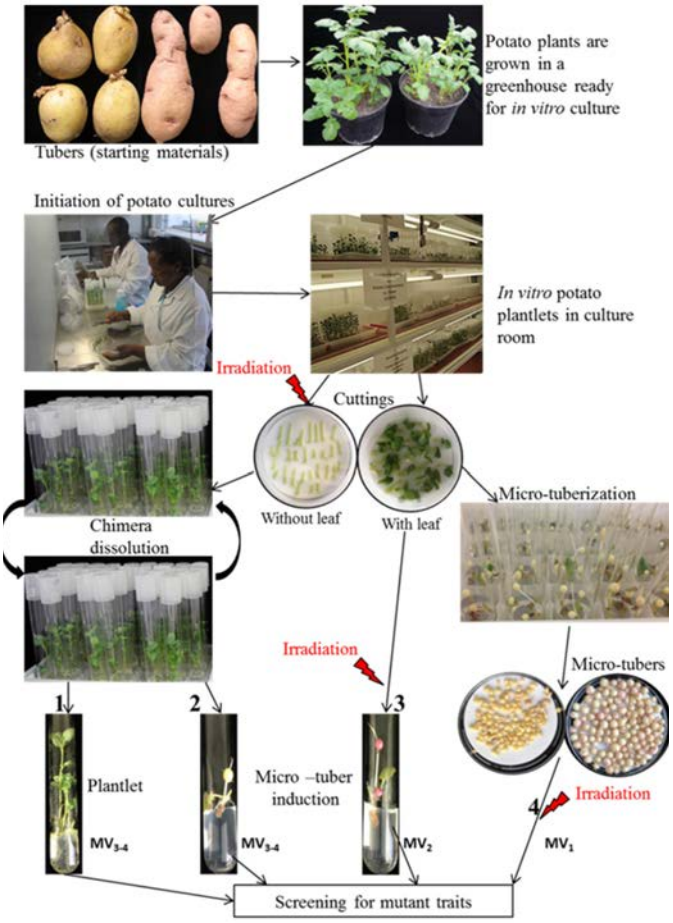


Table: Comparison of methods in potato mutation induction

Method	Time line	Mode of chimera dissolution	Materials targeted for irradiation treatment	Output -deliverable planting materials Mutant vegetative generation produced	Minimum quantity recommended	Planting materials and shipment
1	6-12 months	Rounds of *in vitro* sub-culture	*In vitro* leafless nodal cuttings	*In vitro* plantlets MV_{3-4}	1000	*In vitro* plantlets, requires specialised shipment
2	6-12 months	Rounds of *in vitro* sub-culture	*In vitro* leafless nodal cuttings	Micro-tubers MV_{3-4}	1000	Micro-tubers, easily transported

Method	Time line	Mode of chimera dissolution	Materials targeted for irradiation treatment	Output -deliverable planting materials Mutant vegetative generation produced	Minimum quantity recommended	Planting materials and shipment
3	4-6 months	Limited to micro-tuber production	*In vitro* nodal cuttings with leaf	Micro-tubers MV_2	1000	Micro-tubers, easily transported
4	4-6 months	None	Micro-tubers	Micro-tubers MV_1	1000*	Micro-tubers, easily transported

* A micro-tuber is capable of having several eyes (growing points) and therefore several different mutant genotypes can be produced from one MV_1 micro-tuber, which can be exploited to increase the population size in the next generation and thereby increase the frequency of desired mutant plants.

The PBGL continues to evaluate the various options with a view of establishing recommendations and protocols for *in vitro* mutation induction in potato for use by Member States. Currently Member States provide conventional tubers as starting materials, but there is now a practical option for Member States to produce micro-tubers themselves and send these on for mutation induction. The choice of the various options depends on the facilities (mutation induction and tissues culture) available in the Member State. Mutant populations (several hundred micro-tubers) are to be shipped to Kenya, Lesotho and Morocco in 2014.

Mutation screening

A range of molecular methods can be employed for the characterization of natural and induced nucleotide variation in crop plants. These facilitate a better understanding of gene function and allow a reduction in the time to breed new mutant varieties. Molecular biology, however, can be difficult to master. While efficient, many protocols rely on expensive pre-made kits, or rely on the use of toxic organic chemicals, which is problematic for many Member States, particularly for developing countries. To address this, the PBGL has been developing rapid, accurate and low-cost methods for mutation discovery suitable for developing countries. Such methods have broad applications in breeding from marker assisted selection to reverse-genetics.

Low cost DNA methods

In 2013 PBGL staff focused their efforts on developing protocols for the first steps required for the molecular characterization of mutant plants: proper collection and storage of plant tissue and the extraction of high quality genomic DNA. For collection and storage, a common method is to isolate leaf or root tissues and to flash freeze in liquid nitrogen, followed by prolonged storage at -80°C. While collection of tissues in liquid nitrogen and -80°C storage may be highly suitable for most plant species, it can be impractical in some developing countries. This is because liquid nitrogen can be expensive and hard to procure, and -80

freezers are expensive and require uninterruptable electricity. To avoid these issues, R&D activities of the PBGL have involved adapting methods for collection and storage of plant materials that do not rely on the availability of liquid nitrogen or the availability of constant electricity supplies. The PBGL has been successful in developing an easy-to-use approach utilizing storage of tissues in silica gel at room temperature.

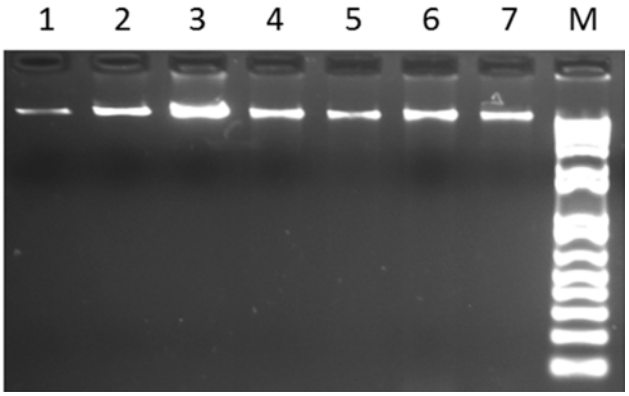
The extraction of high quality genomic DNA can also be problematic. Commercial kits are quick and easy, but can cost over 3 Euros per sample. Many traditional home-made methods utilize toxic organic phase separation that requires special ventilation and proper procedures for disposal of toxic compounds. To address this, the PBGL has developed a non-toxic DNA extraction protocol that provides high quality DNA at about 1/10th the cost of commercial kits, and this has been supplied to Member States.

Leaf tissue dried using silica gel (left) is easily ground using metal beads and a standard vortex mixer found in most laboratories.

DNA extraction methods were combined with previously developed PBGL protocols for low-cost mutation discovery for a training course curriculum. This was used in the three training courses held at PBGL in 2013 (see below) and taught to visitors and fellows in the laboratory. Over 50 scientists were trained from 21 countries in these methods. All students were successful in producing good quality genomic DNA, even though some had never performed such bench experiments before. By the close of 2013 the protocol for this method had been distributed to researchers in 30 Member States (62 individuals): Algeria (2), Bangladesh (4), Botswana (2), Bulgaria (1), China (1), Democratic Republic of the Congo (1), Egypt (1), Eritrea (2), India (1), Indonesia (6), Iran, Islamic Republic of (2), Iraq (6), Jordan (3), Kenya (2), Lebanon (3), Lesotho (1), Madagascar (1), Mauritius (1), Mongolia (2), Morocco (1), Nigeria (1), Oman (1), Saudi Arabia (4), South Africa (1), Sudan (3), Syrian Arab Republic (3), Tunisia (1), Turkey (1), Uganda (1) and Yemen (3).

To support Member States further with this method, supplementary kits containing buffers and materials for DNA extraction plus detailed instructions have been prepared by the PBGL: and are distributed to Member States upon request.

Students at the November training course learning low-cost methods developed at the PBGL.



Genomic DNA samples prepared by Ms. Sasanti Wisiarsih and Mr. Wijaya Murti Indriatama of Indonesia at the FAO/IAEA 2013 training course on "Plant Mutation Breeding: Mutation Induction, Mutation Detection, and Pre-Breeding", held at the PBGL, 3-14 June 2013. Lanes 1-3 represent lambda DNA concentration standards of 3, 10 and 30 ng/µL respectively. Lanes 4-7 represent DNA samples prepared from silica gel dried leaf tissue thus demonstrating the effectiveness of the method.

The PBGL is currently working with counterparts to further validate and expand the utility of these low-cost methods. Through the Technical Cooperation Project MAR5020, the PBGL has been working with counterparts in Mauritius to adapt methods for woody and non-woody crop species. The methods can also be utilized to facilitate the validation and proper use of standard breeding methods. For example, the PBGL has been working on methods to validate the production of doubled haploid (DH) plants. Doubled haploidy is a powerful

breeding approach because all mutations in DH plants are homozygous (genetically pure) and all nucleotide variation is fixed. This means that recessive traits are immediately observable and are true breeding in DH plants. The process of making DHs can be inefficient and it is important that putatively DHs are confirmed homozygous and not heterozygous contaminants. As part of CRP D2.40.12, and in collaboration with CRP agreement holder Dr Jochen Kumlehn's group from IPK Gatersleben, Germany, the PBGL set out to adapt an enzymatic mismatch cleavage screening approach for the validation of DH plants. Assays clearly showed that while F_1 material is heterozygous in target genes, putative DHs are not. The parental origin of genic regions can also be determined easily. This work was published in November 2013 in the journal Plant Methods (see publications list below). In a direct comparison with a standard simple sequence repeat (SSR) molecular marker screening approach, enzymatic mismatch cleavage was successful for 42% of gene targets compared to only 9% success with SSRs.

The success of enzymatic mismatch cleavage for DH screening in barley suggested that the method could be important for other crops. This is because barley has well developed and sophisticated marker information that other species lack. In December 2013 the PBGL began a collaboration with Likyelesh Gugsa of Ethiopia and Ayşe Sen of Turkey, to adapt the DH screening method for the neglected African grain crop Tef. This crop is especially challenging as it is an autotetraploid and its genome has yet to be sequenced. Previous extensive efforts using molecular markers done in another laboratory proved to be unsuccessful. Although the work just began in 2013, the PBGL has had success in developing gene-specific primers suitable for loss-of-heterozygosity screening. This work included an *ad-hoc* 1 week training course for Drs Gugsa and Sen at the PBGL.

Dr. Gugsa (right) being shown how to use low-cost methods for the validation of the production of doubled haploid plant.

Genotypic mutation detection

Although great advances have been made in recent years in terms of technologies to evaluate genome sequences, accurate discovery of rare induced mutations in large plant genomes remains challenging. Unlike natural populations where alleles are fixed, early generations of mutant populations have many new and varied nucleotide variations that segregate. Two plants from two different lines showing the same trait are likely to have completely different mutations that are responsible for the observed phenotype. Furthermore, many mutant varieties registered in the IAEA database have only been self-propagated and not outcrossed. This means that a traditional approach for marker development and gene cloning cannot be applied easily before the development of mapping populations (this typically takes 3-5 years). In recent years, Member State requests to PBGL for methods in discovering the causal gene mutation for their improved trait have been on the rise. In 2013, the PBGL began efforts to evaluate new technologies for the rapid cloning of mutant alleles. As test material, the PBGL choose advanced mutant wheat lines from the TC project INT/5/150 that are resistant to the black stem rust disease, Ug99. The first step taken was to test suitable methods for proper genotypic validation to ensure that mutant and parental material are genetically related. With the assistance of Professor Hermann Buerstmayr of BOKU in Tulln, Austria, a pilot experiment was carried out to evaluate this using a genotyping by sequencing approach. This method evaluated 7000 nucleotide polymorphisms distributed across the three genomes of wheat. Results suggest that this resolution is sufficient to distinguish even closely related Kenyan wheat varieties.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		KENA.1	KENA.2	KENA.3	KENA.4	KENA.5	KENA.6	KENA.7	KENA.8	KENB.1	KENB.2	KENB.3	KENB.4	KENB.5	KENB.6	KENB.7	KENB.8
2	KENA.1	1	0.4368654	0.4360765	0.4371947	0.438616	0.435666	0.4953242	0.4428017	0.4311754	0.4676031	0.429414	0.4303903	0.4320839	0.4769112	0.988665	0.4354873
3	KENA.2	0.4368654	1	0.9868386	0.9850089	0.9848593	0.9862406	0.4062364	0.4145801	0.4294298	0.4515273	0.4266353	0.427819	0.4277397	0.4049424	0.4342142	0.9867042
4	KENA.3	0.4360765	0.9868386	1	0.9872283	0.9872012	0.9868258	0.4080173	0.4121232	0.4269533	0.4518881	0.4246647	0.4255191	0.4261155	0.4063561	0.434688	0.9880853
5	KENA.4	0.4371947	0.9850089	0.9872283	1	0.986494	0.9863201	0.4066375	0.4149015	0.4288693	0.452021	0.4270426	0.4283858	0.4283428	0.4050403	0.4361453	0.9863756
6	KENA.5	0.438616	0.9848593	0.9872012	0.986494	1	0.9862698	0.4075531	0.4133656	0.4280853	0.4480942	0.4257382	0.4268808	0.4273057	0.4060294	0.436508	0.9869058
7	KENA.6	0.435666	0.9862406	0.9868258	0.9863201	0.9862698	1	0.405093	0.4130206	0.4277413	0.450196	0.4255978	0.4265589	0.4270246	0.4056182	0.4342093	0.9876551
8	KENA.7	0.4953242	0.4062364	0.4080173	0.4066375	0.4075531	0.405093	1	0.3973478	0.4013141	0.4262884	0.3994121	0.39869	0.4002213	0.8644268	0.4948088	0.4078747
9	KENA.8	0.4428017	0.4145801	0.4121232	0.4149015	0.4133656	0.4130206	0.3973478	1	0.9538876	0.4553959	0.9524498	0.9533608	0.9544857	0.40394	0.4421753	0.4124158
10	KENB.1	0.4311754	0.4294298	0.4269533	0.4288633	0.4280853	0.4277413	0.4013141	0.9538876	1	0.4547152	0.3841782	0.3870587	0.3860949	0.4087738	0.4312053	0.4267308
11	KENB.2	0.4676031	0.4515273	0.4518881	0.452021	0.4480942	0.450196	0.4262884	0.4553959	0.4547152	1	0.4522191	0.4536391	0.4541405	0.4281984	0.4640185	0.4499591
12	KENB.3	0.429414	0.4266353	0.4246647	0.4270426	0.4257382	0.4255978	0.3994121	0.9524498	0.9841782	0.4522191	1	0.985386	0.985119	0.4047567	0.4293344	0.4248808
13	KENB.4	0.4303903	0.427819	0.4255191	0.4283858	0.4268808	0.4265589	0.39869	0.9533608	0.9870587	0.4536391	0.985386	1	0.9856808	0.4052301	0.4310705	0.4253821
14	KENB.5	0.4320839	0.4277397	0.4261155	0.4283428	0.4273057	0.4270246	0.4002213	0.9544857	0.9860949	0.4541405	0.985119	0.9856808	1	0.4059024	0.4336914	0.4257959
15	KENB.6	0.4769112	0.4049424	0.4063561	0.4050403	0.4060294	0.4056182	0.8644268	0.40394	0.4087738	0.4281984	0.4047567	0.4052301	0.4059024	1	0.4765725	0.405974
16	KENB.7	0.988665	0.4342142	0.434688	0.4361453	0.436508	0.4342093	0.4948088	0.4421753	0.4312053	0.4640185	0.4293344	0.4310705	0.4336914	0.4765725	1	0.4343426
17	KENB.8	0.4354873	0.9867042	0.9880853	0.9863756	0.9869058	0.9876551	0.4078747	0.4124158	0.4267308	0.4499591	0.4248808	0.4253821	0.4257959	0.405974	0.4343426	1
18																	
19																	

A pilot project in comparative genotyping by sequencing, data from different Kenyan wheat lines shows the method is suitable to differentiate genotypically related plants (green boxes).

The next phase of this project, scheduled for 2014, is to collect and evaluate more germplasm followed by testing of different methods of mutation discovery to try and define regions of the wheat genome that are mutated in disease resistant lines.

Mutant line development

Mutation induction takes a few minutes/hours in an irradiator, mutation screening takes a few months/years (but is getting faster with the increasing use of high-throughput systems), the main delay in releasing a mutant variety is the time taken from selecting a desired mutant individual to developing it into an elite line that can be entered into national tests for official certification and release to growers. The breeding method is one factor in this delay and can take 10-15 years to deliver a successful variety. Techniques are available that can speed up the time from mutation induction to mutant variety release. Advances in high-throughput

phenotyping and genotyping, plant propagation and tissue culture provide several opportunities to make efficiency gains and accelerate the delivery of mutant varieties. The PBGL is carrying out R&D to integrate methods in rapid generation cycling, doubled haploidy and marker-assisted selection to speed up the breeding of mutant varieties. Encouraging results were made in shortening crop generation times of major cereal crops (spring wheat, spring barley and sorghum) through the management of plant propagation using small pots, sparse watering and exposure to continuous light in a glasshouse.

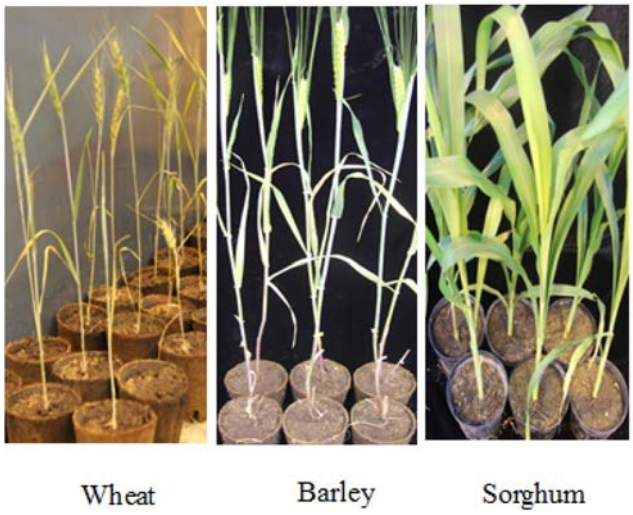
Rapid generation cycling

Ten wheat and 7 sorghum varieties from Kenya and Sudan were used in these trials together with barley mutant stocks from the Bowman mutant backcross series. At the seed milk-ripe stage (15-20 days after pollination) young embryos were rescued and cultured, they immediately germinate thus saving the time taken to ripen and avoiding any seed dormancy issues. Embryo culture in combination with managed plant growth resulted in an average generation time of 48 days in wheat, 40 days in barley and 60 days in sorghum. These time-saving techniques enable the production of 6-8 generations a year. This is enough to reach sufficient genetic uniformity (homozygosity) to advance a mutant line to pre-release official evaluation trails.

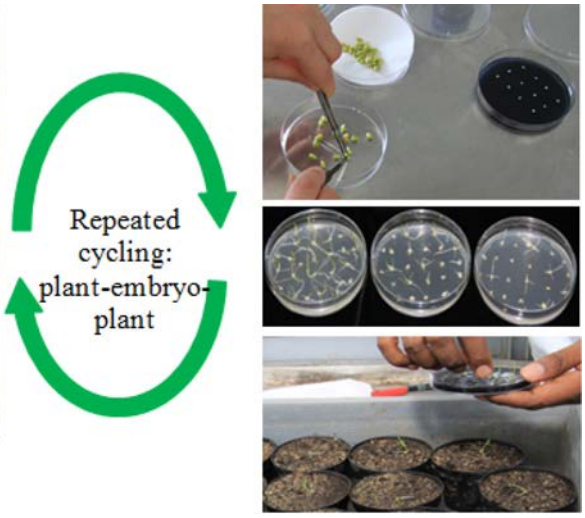
Table 1: Comparing normal and accelerated generation times for wheat, barley and sorghum

Crop	Normal generation time	Generations per year using normal conditions	Accelerated generation time [1)]	Generations per year using rapid generation cycling
Wheat	120-150 days	1-2	45 days	7
Barley	120-150-days	1-2	40 days	8
Sorghum	150-210 days	1-2	60 days	6

[1)] These data are averages for 5-10 varieties from each crop species with the aid of embryo rescue.



Shortening the generation time by growing plants in small pots, watering sparingly and providing continuous lighting



Excision and culture of immature embryos (top) allows precocious germination (middle) and early sowing (bottom) of the next generation

CAPACITY BUILDING

Training courses organized and held at the PBGL

A joint regional training course on: Plant Mutation Breeding, Mutation induction, Mutation detection and Pre-breeding was held at the PBGL (3-14, June). This was organized primarily for participants from Bangladesh and Indonesia involved in the TC projects BGD/5/028 (Assessing crop mutant varieties in saline and drought prone areas using nuclear techniques) and INS/5/039 (Enhancing food crop production using mutation, improved soil and water management and climate change adaptation), respectively. There were 7 participants from Bangladesh and 6 from Indonesia. The training course was open for other applicants from TC projects and included 5 participants from Austria, Botswana (2), Ethiopia and Palestine. The main aim of the course was to provide practical training in methods used in plant mutation breeding. The two week course included lectures, demonstrations, protocols and hands on experience, with practicals in the laboratory, greenhouse and field. The course was designed and taught by staff from the entire Plant Breeding and Genetics Section.

The main topics covered were:

- Capacity building in plant mutation breeding
- Successes in plant mutation breeding
- R&D work at the PBGL
- Methods in plant mutation induction – nuclear techniques
- Irradiation of materials supplied by the trainees/Member State
- Radio-sensitivity testing
- Biological mechanisms of mutation induction
- History of plant mutation breeding

- Tissue culture methods in plant mutation breeding
- Classic mutant genetic stocks
- Phenotypic screening methods for mutant traits
- Genotypic detection of mutation events
- Synergistic biotechnologies in plant mutation breeding
- Accelerated breeding methods for mutant traits

Participants of the June 2013 training course

A regional training course on: Mutation induction techniques and supportive breeding biotechnologies for wheat and barley was held at the PBGL 1-5 July for participants of the TC project RAS/5058 (Supporting mutation breeding approaches to develop new crop varieties adaptable to climate change. Drought, disease, heat and salinity are major constraints affecting sustainable agricultural productivity in ARASIA States. Most cultivated areas depend on rainfall and this can lead to drought. Irrigation helps, but often can raise the incidence of disease. This situation requires an integrated approach to develop technology packages of mutant lines (resistance to diseases, salinity and heat stress, and sustainable high yield under variable climatic conditions) with proper water utilization practices. In this context, mutation induction is a valuable tool in developing drought and salinity tolerant and disease resistant mutant lines of wheat and barley. In order to address these agricultural constraints ARASIA States began mutation a breeding programme under the RAS/5/048 project in 2007 with the assistance of the International Atomic Energy Agency (IAEA). This training course represents on capacity building component of the TC project.

There were 17 trainees from 8 Member States (Iraq, Jordan, Lebanon, Oman, Saudi Arabia, Syrian Arab Republic, and Yemen). The course was also open to fellows at the PBGL at that time. The course was designed and taught by staff of the Plant Breeding and Genetics Section . The main topics of the training course were:

- Capacity building in plant mutation breeding
- Nuclear techniques in plant mutation induction
- Irradiation of materials supplied by the trainees/Member State
- Radio-sensitivity testing
- Biological mechanisms of mutation induction
- History of plant mutation breeding
- Classic mutant genetic stocks
- Phenotypic screening methods for mutant traits

- Genotypic detection of mutation events
- Other enabling biotechnologies in plant mutation breeding
- Accelerated breeding methods for mutant traits

Trainees learning DNA techniques for mutation discovery

A training course on: Breeding techniques for mutant traits with special reference to Ug99 has held at the PBGL 25-29th November for participants of the TC project INT5/150 (Responding to the transboundary threat of wheat black stem rust, Ug99). Ug99, a form of black stem rust, is a devastating disease of wheat first discovered in Uganda in 1999. The disease has spread rapidly and in 2009 the PBGL was instrumental in providing mutant (M1) populations for participants of IN5/150 from which resistant mutants were selected and advanced for future testing to attain varietal status and release to farmers. This success led to additional support from TC in developing this training course that was requested by INT5/150 participants. The purpose of the training course was to provide basic knowledge and skills in accelerated breeding of mutant traits with special reference to Ug99, especially the future development of screening methods for desired mutants that will aid the breeding process. The course was aimed at plant breeders in Member States embarking on mutation breeding for Ug99 resistance in wheat and provided an opportunity for the exchange of germplasm (resistant mutant lines).

There were 16 trainees from 15 Member States 16 (Algeria, Bulgaria, China, Egypt, India, Iran, Iraq, Jordan, Kenya, Lebanon, Saudi Arabia, South Africa, Sudan, Syrian Arab Republic, Tunisia and Uganda). The course was also open to fellows at the PBGL at the time. The course was devised and taught by staff from the Plant Breeding and Genetics Section and in addition a special seminar on the genetics and screening of rust resistance and future goals was led by Prof Hermann Buerstmayr from BOKU, Tulln, Austria. Topics covered by the course included:

- Milestones in the development of Ug99 resistant wheat mutants
- Pre-breeding for effective use of plant genetic resources
- Irradiation of trainee's material
- Radio-sensitivity testing
- Accelerated techniques in plant breeding for mutant traits

- Seminar on current advances in genetics and breeding for disease resistance
- Exchange of seed of mutant lines resistant to Ug99

Presentation of milestones made in screening for Ug99 resistant wheat mutants in Kenya

FELLOWS

In 2013, the PBGL hosted 11 Fellows from 9 Member States, four Interns from 3 Member States, 13 Scientific Visitors from 11 Member States and 1 consultant. Details are given below:

Beshir, **Mayada**	Sudan	Fellow	4 months	Mutation induction and detection using molecular techniques
Ego, **Amos**	Kenya	Fellow	8 months	Induced mutation, molecular screening and validation of Ug99 resistant wheat mutant lines
Elsiddig, **Mohammed**	Sudan	Fellow	4 months	Induced mutations, sorghum mutation screening and associated biotechnologies
Islam, **Md. Rafiqul**	Bangladesh	Fellow	6 months	Mutation induction in vegetable crops and haploid production using irradiated pollen
Rafiri, **Matumelo Rafiri**	Lesotho	Fellow	6 months	Mutation induction in potato, sweet potato and amaranth
Randrianarivony, **Hery Lalao Lwyset**	Madagascar	Fellow	4 months	Mutation induction and detection in rice
Hannachi, **Abderrahmane**	Algeria	Fellow	3 months	Mutation induction and detection in barley
El Achouri, **Kaoutar**	Morocco	Fellow	4 months	*In vitro* mutation induction in potato and mutation detection
Tungalag, **Munkhbat**	Mongolia	Fellow	2 months	Mutation induction and genotypic mutation detection methods in wheat
Purevjav, **Sukhbaatar**	Mongolia	Fellow	2 months	Mutation induction and detection in forage crops
Dussoruth, **Babita**	Mauritius	Fellow	4 months	Mutation induction and detection in banana
Taassob-Shirazi, **Farzaneh**	Iran	Intern (cost-free)	12 months	Rapid introgression of mutant genes for fodder quality in barley
Sen, **Ayşe**	Turkey	Intern (cost-free)	8 months	Molecular characterisation of mutant plants
Kamruzzaman, Mohamed	Bangladesh	Intern (cost-free)	1 month	Methods in plant mutation breeding
Hasanuzzaman, **Rani**	Bangladesh	Intern (cost-free)	1 month	Methods in plant mutation breeding
Tao Lan	China	Consultant	6 months	Mutation induction and detection in rice

Jouhar, **Mohammad**	Syria	Scientific visitor	1 week	Molecular screening of mutations
Yousuf, **Dheyaa**	Iraq	Scientific visitor	1 week	Methods in plant mutation breeding
Mamoori, **Jalal N**	Iraq	Scientific visitor	1 week	Methods in plant mutation breeding
Akel, **Wessam**	Syria	Scientific visitor	1 week	Methods in plant mutation breeding
Tshilenge, **Lukanda**	DR Congo	Scientific visitor	1 week	Methods in plant mutation breeding
Brunner, **Cecilia**	Austria	Scientific visitor	1 week	Mutation induction and screening
Rabealaina, **Brunhilde B**	Madagascar	Scientific visitor	2 weeks	Methods in plant mutation breeding
Zinga, **Innocent**	Central African Republic	Scientific visitor	2 weeks	Methods in plant mutation breeding
Chentouf, **Mouad**	Morocco	Scientific visitor	2 weeks	Mutation induction and screening
Madhu, **Shamduth**	Mauritius	Scientific visitor	1 week	Mutation induction and screening for disease resistance
Simon, **Sibu**	Austria	Scientific visitor	1 week	Methods in plant mutation breeding
Sochacka, **Anna**	Poland	Scientific visitor	1 week	Methods in plant mutation breeding
Gugsa, **Likyelesh**	Ethiopia	Scientific visitor	2 weeks	Plant mutation breeding, genetic markers, doubled haploid screening in tef

* Extra TC funding was sourced to hold this additional training course for INT/5/150 members, this was considered important and urgent due to Member States needs in fighting the threat of the Ug99 disease of wheat.

SERVICES

The PBGL provides an irradiation service to Member States for mutation induction. This is normally of seed samples for gamma ray treatment. However, other plant parts can be irradiated, including *in vitro* cultures and the PBGL also offers irradiation treatments using X-rays for which protocols have been developed. In 2013 the PBGL received its first request for Ion Beam irradiation. Ion Beam is a new tool in plant mutation breeding and in 2013 the PBGL began R&D work in collaboration with the Nuclear Science and Instrumentation Laboratory at the IAEA Laboratories (Seibersdorf, Austria) and the Laboratory for Ion Beam Interactions (Zagreb, Croatia). The aims are to develop protocols for practical use and to

investigate the spectrum of mutants produced in comparison with standard gamma irradiation and X-ray treatments.

The total number of irradiation requests received in 2013 was 105 from 34 Member States, this was a record number of requests for irradiation services, and brought the total number of requests to 1365 since records began (in 1977). Previous highs were 59 requests in 1997 and 64 in 2009. Samples from over 40 crop species were irradiated; in addition irradiation requests were received for several perennial crops and many ornamental plants. The PBGL is performing more irradiation requests for more Member States on a wider range of plant species than ever (see table below).

Requests for mutation induction have been increasing in recent years, 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 Cobalt 60 and Cesium 137. The PBGL is 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.

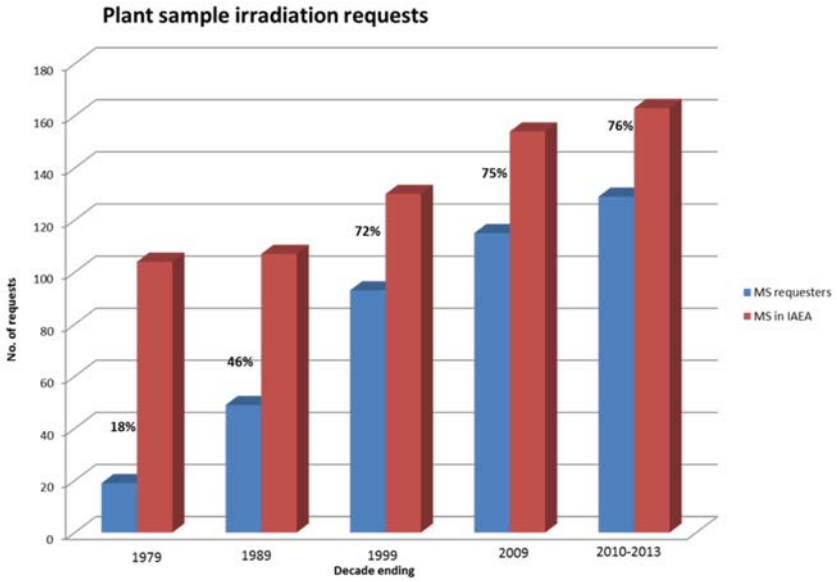
Irradiation services provided to Member States by the PBGL in 2013

Request number	Member State	Plant species
1309	Madagascar	Bambara groundnut
1310	Indonesia	Rice
1311	Turkey	Wheat, chickpea
1312	Germany	Ornamental plants
1313	Turkey	Pepper, tomato, eggplant
1314	UK	Barley
1315	Austria	Barley
1316	Kenya	Potato
1317	Tanzania	Rice
1318	Germany	Ornamental plants
1319	Mongolia	Wheat
1320	Poland	Sugar beet
1321	Netherlands	Ornamental plants
1322	Kenya	Chickpea, finger millet, sorghum, wheat
1323	Botswana	Maize, groundnut, bean, Bambara groundnut
1324	Indonesia	Rice, cotton, soybean, orchid
1325	Palestine	Durum wheat

Request number	Member State	Plant species
1326	Bangladesh	Rice, jute, rapeseed, mustard, groundnut, sesame, mung bean, chickpea, lentil, blackgram, grass-pea, tomato, soybean
1327	UK	Wheat
1328	Nigeria	Hibiscus
1329	Jordan	Barley
1330	Yemen	Wheat
1331	Iraq	Soybean, sesame, maize, wheat, kidney bean
1332	Saudi Arabia	Wheat
1333	Senegal	Rice, sesame, *Vigna spp.*
1334	Netherlands	Ornamentals
1335	Sudan	Wheat, sorghum
1336	Turkey	Pepper
1337	USA	Sugar beet
1338	Macedonia	Barley
1339	Qatar	Barley
1340	Madagascar	Rice
1341	Madagascar	Rice, cassava
1342	Oman	Wheat, barley
1343	Palestine	Durum wheat
1344	Zimbabwe	*Vigna unguculata, Vigna subteranea,* groundnut
1345	UK	Hosta
1346	China	Wheat
1347	Bulgaria	Barley
1348	Iraq	Wheat, tomato
1349	Tunisia	Bread wheat
1350	Sudan	Bread wheat
1351	Jordan	Bread wheat
1352	Kenya	Bread wheat
1353	Uganda	Bread wheat
1354	UK	Bread wheat
1355	UK	Bread wheat

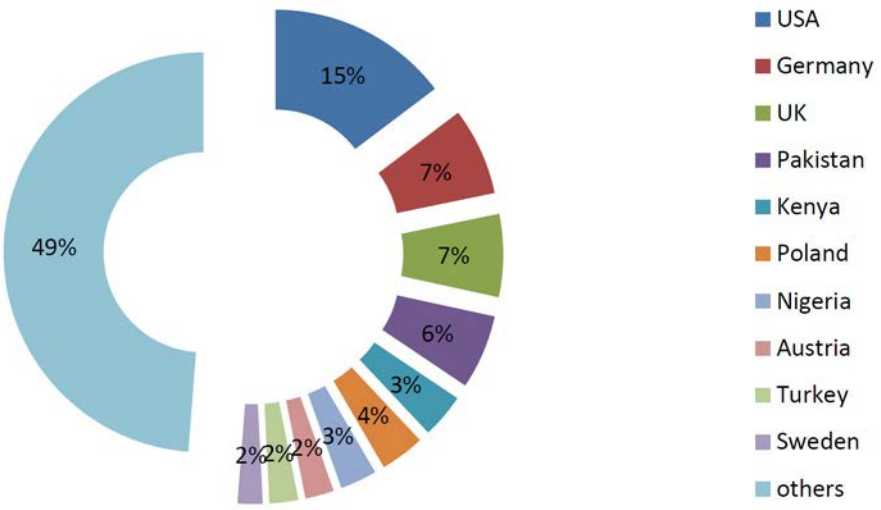
Request number	Member State	Plant species
1356	UK	*Perovskia atriplicifolia*
1357	Benin	*Vitellaria paradoxsa*
1358	Madagascar	Rice
1359	Algeria	Barley
1360	Morocco	Potato
1361	Lesotho	Potato, sweet potato, amaranth
1362	Mongolia	wheat, barley
1363	Mongolia	*Medicago falcate, Melilotus dentotus, Stipa sibirica, Agropyron christatum, Elymus daturicus*
1364	Bangladesh	chilli pepper, cucumber, tomato, eggplant, okra, rice
1365	Nigeria	Robusta and Arabica coffee

The graph below represents a cumulative histogram of plant irradiation requests carried out by the PBGL along with the respective number of IAEA Member States (MSs) for each decade. In the 1970s the PBGL served 18% of MSs. To date, at the end of 2013 (just four years into the current decade) the PBGL has performed plant sample irradiation requests for 76% of MSs.



Graph showing the number of irradiation requests per decade, and as a percentage of IAEA Member States. Note the right hand column shows data up to the end of 2013 and is expected to increase significantly by the end of the current decade (the number of Member States is also increasing).

The pie-chart below shows Member States plant irradiation service requests as a percentage of all requests received since 1977 until the end of 2013, the top ten requesting countries are indicated (others account for 2% or less of total requests).



Member State information sheets developed and distributed in 2013

The PBGL produces 'Information Sheets for Visitors', these describe the importance of agriculture and the problems facing crop production in an individual country, the major crops, interactions with the FAO/IAEA Joint Division, training opportunities and successes in plant mutation breeding. The information is usually compiled by fellows to the PBGL for their home country. In 2013 17 'Information Sheets for Visitors' were compiled for: Afghanistan, Bangladesh, Burkina Faso, China, D.R. Congo, Indonesia, Kenya, Lesotho, Madagascar, Mongolia, Nigeria, Palestine, Peru, Sudan, Thailand, Turkey and USA.

Country information sheets are popular with visitors

Visitors to the PBGL

There were 66 delegation visits to PBGL in 2013, from (in chronological order):

Bangladesh, Angola, Benin, Botswana, Egypt, Kenya, Libya, Mauritius, Niger, South Africa, Seychelles, Uganda, Zimbabwe, IAEA-TC-NLOs,, Germany, Japan, Panama, Myanmar, Croatia, EU, Austria, Belgium, Bulgaria, Cyprus, Czech Rep., Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Netherlands, UK, Croatia, FAO, USA, SAGNA, Indonesia, TCPC, Korea, Palau, South Africa, Philippines, Egypt, GC delegates, Nepal, SAGNE, Russia, USA, TC-NLOs, Korea, Hungary.

PUBLICATIONS

Book chapters and abstracts

ALI, A.M., ELAMEIN, H.M., TAHIR, I.S.A., BAUM, M., FORSTER, B.P. Doubled haploid production in spring wheats of hot irrigated environments. Plant Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, February 18-20, 2013, Vienna, Austria. Medimond - Monduzzi Editore international Proceedings Division, Pianoro, Italy, 2013, p. 49-52. ISBN 978-88-7587-682-1.

BADO, S., KOZAK, K., SEKANDER, H., ALHAJAJ, N., GHANIM, A., FORSTER, B.P., LAIMER, M.. 2013. Resurgence of X-rays in mutation breeding. Plant Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, February 18-20, 2013, Vienna, Austria. Medimond - Monduzzi Editore international Proceedings Division, Pianoro, Italy, 2013, p. 13-16. ISBN 978-88-7587-682-1.

COOPER, J.L., HENIKOFF, S., COMAI, L., TILL, B.J., TILLING and Ecotilling for Rice, *Methods in Molecular Biology: Rice Protocols*, Springer (2012) 39–56.

HUYNH, O.A., HOFINGER, B.J., BESHIR, M.M., JANKOWICZ-CIESLAK, J., GUO, H., FORSTER, B.P., TILL, B.J. Do-it-yourself molecular biology for plant breeding: low-cost tools for developing countries. Plant Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, February 18-20, 2013, Vienna, Austria. Medimond - Monduzzi Editore international Proceedings Division, Pianoro, Italy, 2013, p. 33-36. ISBN 978-88-7587-682-1.

JANKOWICZ-CIESLAK, J., KOZAK-STANKIEWICZ, K., SEBALLOS, G., RAZAFINIRINA, L., RABEFIRAISANA, J., RAKOTOARISOA, N.V., FORSTER, B.P., VOLLMANN, J., TILL, B.J. Application of soft X-ray and near-infrared reflectance spectroscopy for rapid phenotyping of mutant rice seed. Plant Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, February 18-20, 2013, Vienna, Austria. Medimond - Monduzzi Editore international Proceedings Division, Pianoro, Italy, 2013, p. 37-40. ISBN 978-88-7587-682-1.

LAGODA, P.J.L. 2.1 Use of Tissue Culture and Mutation Induction to Improve Banana Production for Smallholders in Sri Lanka. In: Chapter 2: Case Studies in the Crop Sector of Biotechnologies at Work for Smallholders: Case Studies from Developing Countries in Crops, Livestock and Fish (Edited by J. Ruane, J.D. Dargie, C. Mba, P. Boettcher, H.P.S. Makkar, D.M. Bartley and A. Sonnino), Food and Agriculture Organization of the United Nations (FAO); ISBN 978-5-107877-8 (2013) pp. 8–17.

LEE, L.S., TILL, B.J., HILL, H., HUYNH, O.A., JANKOWICZ-CIESLAK, J. Mutation and Mutation Screening. Chapter 8 in: Cereal Genomics, Methods and Protocols Series: Methods in Molecular Biology, Vol. 1099. Eds: Robert J. Henry, Agnelo Furtado. Humana Press, ISBN 978-1-62703-714-3 (2014).

MAGHULY, F., JANKOWICZ-CIESLAK, J., TILL, B.J., LAIMER, M. The use of ECO-TILLING for the genetic improvement of Jatropha curcas. In: Sujatha M., Bahadur B. and Carels N., Compendium of Bioenergy Crops: *Jatropha curcas*. 2; Science Publishers, (USA), ISBN: 978-1461449140 (2013).

MATIJEVIC, M., BADO, S., LAGODA, P.J.L., FORSTER, B.P. 2013. Impact of induced mutations in plant breeding. Plant Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, February 18-20, 2013, Vienna, Austria. Medimond - Monduzzi Editore international Proceedings Division, Pianoro, Italy, 2013, p. 45-47. ISBN 978-88-7587-682-1.

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Peer-reviewed publications

CZYCZYŁO-MYSZA, I., MARCIŃSKA, I., JANKOWICZ-CIEŚLAK, J., DUBERT, F. The effect of ionizing radiation on vernalization, growth and development of winter wheat. Acta biologica Cracoviensia. Series botanica. 01/2013; 55(1):1-6. DOI:DOI: 10.2478/abcsb-2013-0004 pp.1-6 (2013).

HOFINGER, B.J., HUYNH, O.A. JANKOWICZ-CIESLAK, J. MÜLLER, A., OTTO, I., KUMLEHN, J., TILL, B.J. Validation of doubled haploid plants by enzymatic mismatch cleavage. Plant Methods, 9:43 (2013).

JANDRIĆ, Z., RATHOR, M.N., CHHEM-KIETH, S., ADU-GYAMFI, J., MAYR, L., RESCH, C., BADO, S., ŠVARC-GAJIĆ, J., CANNAVAN, A. 2013 Uptake of 14C-atropine and/or its transformation products from soil by wheat (*Triticum aestivum* var Kronjet) and their translocation to shoots. Journal of Environmental Science and Health, Part B. Vol. 48, Iss. 12: 1034-1042.

JANKOWICZ-CIESLAK, J., HUYNH, O.A., DUSSORUTH, B., SARAYE, B., TILL, B.J., Low cost mutation discovery methods suitable for developing countries, ScienceMED 3(3) (2012) 245–249.

MEHLO, L., MBAMBO, Z., BADO, S., LIN, J., MOAGI, S.M., BUTHELEZI, S., STOYCHEV, S., CHIKWAMBA, R. Induced protein polymorphisms and nutritional quality of gamma irradiation mutants of sorghum. Mutation Research 749 (2013) 66–72. dx.doi.org/10.1016/j.mrfmmm.2013.05.002.

Conference abstracts

ALI, A.M., ELAMEIN, H.M., TAHIR, I.S.A., BAUM, M.. FORSTER, B.P. Doubled haploid production in spring wheats of hot irrigated environments. Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

AMOS, E.K., KINYUA, M.G., KIPLAGAT, O.K., ALI, A.M. BADO, S., JANKOWICZ-CIESLAK, J., HUYNH, O.A., HENRY, I., COMAI, L., LAGODA, P.J.L., FORSTER, B.P., TILL, B.J. Induced mutations for increased disease resistance: an example of resistance to Ug99 in spring wheat. Book of Abstracts: International Cereal Biotechnology and Breeding Budapest, Hungary, 5–7 November 2013.

BADO, S., KOZAK, K., SEKANDER, H., ALHAJAJ, N., GHANIM, A., FORSTER, B.P., LAIMER, M. 2013. Resurgence of X rays in mutation breeding. Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

JANKOWICZ-CIESLAK, J., KOZAK-STANKIEWICZ, K., RAZAFINIRINA, L., RABEFIRAISANA, J., RAKOTOARISOA, N.V., FORSTER, B.P., TILL, B.J., VOLLMANN, J. Application of Near-Infrared Reflectance Spectroscopy for rapid phenotyping of mutant seed germplasm. International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February.

HOFINGER, B., HUYNH, O.A., JANKOWICZ-CIESLAK, J., HUANG, B., COMAI, L., MUELLER, A., OTTO, I, KUMLEHN, J., FORSTER, B.P., TILL, B.J., Choose your throughput: Recovery of induced and natural nucleotide variation in plants, Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

HUYNH, O.A., HOFINGER, B., JANKOWICZ-CIESLAK, J., GUO, H., BESHIR, M., FORSTER, B.P., TILL, B.J. Do-it-yourself molecular biology for plant breeding: low-cost tools for developing countries. Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

MATIJEVIC, M., BADO, S., LAGODA, P.J.L., FORSTER, B.P. Impact of induced mutations in plant breeding. Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

TAASSOB-SHIRAZI, F., FORSTER, B.P., HUANG, B., GRAUSGRUBER, H., FRANCKOWIAK, J. Rapid introgression of mutant traits for fodder quality in barley. Book of Abstracts: International Conference on Plant Genetics and Breeding Technologies, Vienna, Austria, 18–20 February 2013.

TAASSOB-SHIRAZI, F., HUANG, B., GRAUSGRUBER, H., FRANCKOWIAK, J.D., FORSTER, B.P. Introgression of mutant alleles in barley. Book of Abstracts: Plant and Animal Genome Conference, San Diego 12–16 January 2013.

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, Glenn Gregorio), 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 and development and deployment of genomics tolls for the crop
Austrian Institute of Technology, Health & Environment Department, (Dr Silvia Fluch, Dr Kornel Burg), Tulln, AUSTRIA	Gene expression profiling in drought
University of Natural Resources and Life Sciences, (Profs. Hermann Buerstmayr; Hans Vollmann and Heinrich Grausgruber), 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, (Dr Marcin Rapacz) Krakow, POLAND	Banana phenotyping for drought tolerance
University of Natural Resources and Life Sciences, Department of Biotechnology, (Prof Margit Laimer, Dr Fatemeh Maghuly) Vienna, AUSTRIA	Induced and natural mutation induction in crop plants including under-studied crops
University of Natural Resources and Life Sciences, Department of Biotechnology, (Dr Theresa Scharl), Vienna, AUSTRIA	Statistical data evaluation
Agri-Science Queensland, (Prof. Jerome Franckowiak), Hermitage Research Facility, 604 Yangan Road, Warwick, QLD 4370, AUSTRALIA	Barley crossing method; barley mutant stocks and mutation breeding
The James Hutton Institute (Dr. William Thomas), Invergowrie, Dundee, DD2 5DA, Scotland, UK	Barley crossing method; barley genetic stocks, genetic markers for low lignin mutants

University of Dundee (Prof. Clare Halpin), Dundee, Scotland, UK	Molecular genetics of lignin mutants for fodder barley
Nordic Genetic Resource Center (Dr. Udda Lundqvist), P.O. Box 41, SE-230 53 Alnarp, SWEDEN	Classic barley mutants, mutant gene descriptions and nomenclature
University of California Davis Genome Center (Prof. Luca Comai and Dr. Isabelle Henry), USA	Developing next generation sequencing strategies for discovery of induced mutation events in genomes of vegetatively propagated crops
University of Ljubljana (Dr Borut Bohanec), Ljubljana, SLOVENIA	X-ray irradiation for mutation induction; pollen irradiation for haploid production
Ruder Boskovic Institute (Dr Milko Jaksic), Zagreb, CROATIA	Ion Beam irradiation

EXTRABUDGETARY SUPPORT

In addition to the IAEA regular budget, ongoing TC projects and CRPs, the activities of the PBGL were supported by:

- A cost-free expert, Mr Lan Tao, working on: "Mutation induction and identification in rice", funded and supported by the Fujian Agriculture and Forestry University, Fujian China and the Chinese Government, respectively.
- Funding from the Bangladesh Institute of Nuclear Agriculture (BINA) to support two cost-free interns in the application of nuclear techniques in plant mutation breeding.
- Funding from the Government of Turkey to support a cost-free intern on: "Molecular characterisation of mutant plants".

THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition (SWMCN) Laboratory is part of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It 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. The SWMCN Laboratory also helps Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well remediating the impact of these events on soil and agricultural water resources.

In 2013, the SWMCN Laboratory provided a broad range of following services: (i) Develop and validate robust and 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 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 SWMCN Laboratory in 2013 focused on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. This included improvements in the use of Beryllium-7 as a tracer for short-term erosion events, the use of cost-effective and rapid in-situ fallout radionuclide measurements for assessing soil erosion and the use of Oxygen-18 isotopes in phosphate to trace phosphorus sources and cycling in soils and ultimately provide a better understanding of soil phosphorus dynamics in agro-ecosystems. In addition, new activities were initiated in the field of area-wide agricultural water management through the testing of the innovative and promising cosmic-ray soil moisture neutron probe. All these different activities have made important progress, and proved to be essential in the implementation of Coordinated Research Projects of the SWMCN Subprogramme.

A second major component of the SWMCN Laboratory is the contribution to capacity building in Member States. In the frame of two group training courses, it hosted forty-one fellows from twenty-three countries, each receiving about four to six weeks, intensive training in the application of isotopic and nuclear techniques to improve soil and water management and crop nutrition. The SWMCN Laboratory also conducted one regional training course of one month duration for twenty-six scientists and technicians from twenty-one countries across the Asia and Pacific region on Soil and Water Management in Agriculture to Support Crop Production in Asia and the Pacific.

One protocol on the use of compound-specific stable isotope techniques for improving precision soil conservation strategies and one protocol on the use of stable isotope probing

for improved soil fertility management were prepared and released on-line in 2013, in the frame of Coordinated Research Projects. Information was released to Member States through 24 publications: 7 publications in international peer-reviewed journals, and 11 in the FAO/IAEA proceedings of the International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria.

In 2013; 4538 and 170 samples were analysed for stable isotopes and fallout radionuclides respectively at the SWMCN Laboratory. Most analyses were carried out for supporting Research and Development activities in the SWMCN Laboratory focused on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture.

In the frame of the planned laboratory modernization, refurbishment and modernization of existing greenhouse facilities were completed in 2013. Walk-in growth chambers have been installed and first experiments have been implemented to label plant materials with carbon-13 isotopes as tracer for soil organic carbon dynamics studies.

STAFF

Name	Title
Dercon, Gerd	Laboratory Head
Adu-Gyamfi, Joseph[1]	Soil Scientist/Plant Nutritionist
Mabit, Lionel[2]	Soil Scientist
Wahbi, Ammar[3]	Technical Officer
Mayr, Leo	Senior Laboratory Technician
Arrillaga, José Luis[4]	Senior Laboratory Technician
Aigner, Martina	Senior Laboratory Technician (50%)
Heiling, Maria	Senior Laboratory Technician (50%)
Weltin, Georg[5]	Senior Laboratory Technician
Toloza, Arsenio	Laboratory Technician
Resch, Christian	Laboratory Technician
Jagoditsch, Norbert	Technical Attendant
Augustin, Franz[6]	Technical Attendant
Mao, Yanling[7]	Consultant
Gonsalves, Basil[8]	Consultant
Mletzko, Joanna Malgorzata[9]	Team Assistant

[1] *Left the SWMCNL in March 2013;* [2] *Joined the SWMCNL in September 2013,* [3] *Joined the SWMCNL in October 2013,* [4] *Left the SWMCNL in February 2013;* [5] *Joined the SWMCNL in July 2013;* [6] *Left the SWMCNL in October 2013;* [7] *Joined the SWMCNL in July 2013;* [8] *Left the SWMCNL in November 2013;* [9] *Joined the SWMCNL in August 2013.*

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management and Crop Nutrition Laboratory (SWMCN Laboratory) 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.

Climate-smart Agriculture

The SWMCN Laboratory 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. Therefore, 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. Recent SWMCN Laboratory success stories included timely response to Member States' requests for more robust and affordable nuclear applications in soil, water and nutrient management, including conservation of land and agricultural water resources.

Nuclear emergency response in food and agriculture

The SWMCN Laboratory also supports Member States to be better prepared in responding in 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 the work of the SWMCN Laboratory is driven by Member States' demands. The SWMCN Laboratory provides a broad range of following services:

- Develop and validate isotope and nuclear techniques for supporting Co-ordinated Research Projects (CRPs) and Technical Co-operation Projects (TCPs). Ten isotopic and nuclear techniques have been developed or adapted at the SWMCN Laboratory 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.

Review and discussion on the key assumptions and challenges surrounding the use of Beryllium-7 (7Be) as a soil and sediment tracer

The assumptions and challenges surrounding the use of ^{7}Be to investigate soil and sediment redistribution in river basins have recently been reviewed (Taylor et al., 2013; see 2013 SWMCN Laboratory publication list) to support the Coordinated Research Project 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. This paper analyses the assumptions made in the context of hillslope erosion studies and additional implications for the use of ^{7}Be as a tracer at a catchment-scale.

A key assumption in hillslope erosion studies is that ^{7}Be fallout is spatially uniform for a typical field or location. It is also important to assume that rainfall received prior to a study event is non-eroding to maintain a uniform inventory and enable estimates of soil redistribution to be attributed to a particular event. This requirement is well recognised by researchers in this field and these conditions have been met in studies shown in the literature. Little attention, however, has been given to the effects of other factors (e.g. atmospheric processes affecting the rainfall field across a site, topographic factors including the influence of vegetation cover), which could influence the uniformity of fallout and therefore the spatial variability of the ^{7}Be inventory.

Assumptions of spatially uniform fallout at the microscale have not been adequately supported by previous research. Studies demonstrated for example the variability in raindrop size distribution across short distances (i.e. 250 m). These factors are, however, likely to translate into minimal gradients in ^{7}Be inventories and it is more likely that factors affecting the direct transfer of ^{7}Be to soil, such as rain shadowing (by e.g. vegetation and topography) and interception by vegetation, will have a greater influence on spatial uniformity. These factors could present a fundamental challenge to the application of ^{7}Be as a hillslope soil erosion tracer unless suitable studies are undertaken to demonstrate otherwise. Plant interception and potential uptake are likely to contribute to significant heterogeneity.

The second key assumption is rapid sorption of the tracer to soil particles. Rapid sorption of ^{7}Be to soil particles upon fallout is assumed to be at shallow soil depth distributions and laboratory batch studies have been reported on this sorption. Applications of ^{7}Be as a tracer have overlooked the potential for high rates of infiltration through preferential flow pathways to increase sorption time, thus, influencing depth distributions, which has implications for erosion modelling using current conversion models. Furthermore, there is potential for ^{7}Be to

be transported in the dissolved phase in overland flow and this remains a key area for research to determine the influence of this upon redistribution estimates.

As a tracer at the catchment scale, ^{7}Be offers a unique opportunity to provide an indication of recent sedimentation and the transport of surface material, which could make a significant contribution to catchment management schemes. Successful use at this scale does, however, rest upon support for the third assumption of irreversible sorption to soil particles in a range of environments and such support is currently lacking. Knowledge of ^{7}Be behaviour with changing physicochemical parameters in fluvial environments is conflicting and there is evidence to suggest that ^{7}Be may be mobilized under reducing, saline or low pH conditions. Impact upon sorption behaviour is likely to be highly site specific and it is a priority that future laboratory studies are coupled with in situ monitoring of parameters to determine the likelihood for increased tracer mobility under representative conditions and timescales.

A detailed appraisal of current knowledge surrounding each assumption is provided together with discussion regarding the potential influence upon tracer estimates and recommendations for further research. Further information can be found in the recently published article of Taylor et al (2013).

Preliminary investigations to assess the usefulness of Beryllium-7 (^{7}Be) as radiotracer in soil covered by vegetation

Different factors may affect the extent of radionuclides' interception by plants and therewith their inventories in soil covered areas. In particular, there is interest in assessing the impact of the vegetation factor for different soil coverage conditions, when using ^{7}Be as radiotracer of soil redistribution in cropped farmland.

Common beans at the early growing stage were selected to perform this experimental study in the SWMCN Laboratory in close collaboration with the Terrestrial Environmental Laboratory, as these plants are known to provide a large foliar surface in a relatively short time (Figure 1). ^{7}Be activity concentration was determined using high resolution gamma-ray spectrometry. A relatively high ^{7}Be interception factor (normalized to the leaf area index-LAI) of 0.62 (LAI value 0.85) was measured after 0.4 mm precipitation. After a second 7.2 mm rainfall, the interception factor had a value of 0.37, for a 3.0 LAI value. Wash-off experiments using deionized water conducted at several hours and at 10 days after the wet foliar interception showed that the removable ^{7}Be fraction was limited to only 35% of the initial concentration deposited on leaves. ^{7}Be incremental depth profiles confirmed that the radionuclide reached only the upper 20 mm of the soil, independently of precipitation amount or soil coverage, having a maximum in the

FIG. 1: Common bean plants for ^{7}Be gamma measurements

first 2.5 mm layer. Moreover, ^{7}Be was not found in plant roots, thus excluding its direct uptake from soil.

Our results suggest that ^{7}Be foliar interception by bean plants is likely to affect the radionuclide inventories and their spatial uniformity in covered soil. Reliable results on short-term erosion using ^{7}Be can be obtained in cropped farmland with limited cover, but only when taking into account the interception factor. The impact of the interception factor is highly dependent on the rainfall intensity and duration, crop species and growing stage of the plants. Further investigations into these variables are required.

This research has been conducted in the context of the Coordinated Research Project (CRP) 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"*.

Landscape Erosion Assessment using the ^{137}Cs-based Method through Laboratory and in-situ Gamma Spectrometry

The objective of this research is to compare in-situ ^{137}Cs measurements using an on-site lanthanum bromide ($LaBr_3$ (Ce)) scintillation detector with those from a conventional laboratory based HPGe detector for assessing soil erosion. We aimed to establish (1) the strength of the relationship between in-situ and laboratory based measurements, and (2) develop improved tools for landscape based soil erosion assessments in our continuing research.

In a pilot study, using stratified soil sampling, five soil cores (to a depth of 1 m with 5 cm increments) were collected from the experimental research station of the Austrian Agency for Health and Food Safety (AGES) located in Grabenegg, Austria. Three soil cores were sampled in the study site (F1, F2 and F3, with F1 being situated at the top of the field slope, followed by F2 and F3 further down, Figure 2). Two soil cores (REF1 and REF2) were collected from two different reference sites (REF1 situated in an undisturbed orchard field and REF2 in an undisturbed pasture; Figure 2). After pre-treatment, the soil samples were analysed, using the HPGe coaxial detector in the SWMCN Laboratory.

FIG. 2: Location of the soil cores sampled

The gamma measurements performed at the SWMCN Laboratory confirmed the undisturbed status of the two reference sites (i.e. exponential decrease in ^{137}Cs content with depth). The ^{137}Cs areal activity of soil cores collected from F1, F2,

F3 were established at 2134±465 Bq m^{-2}, 1835±356 Bq m^{-2} and 2553±340 Bq m^{-2}, respectively, and the reference sites at 3221±444 Bq m^{-2} and 3946±527 Bq m^{-2} for REF1 and REF2, respectively. These results highlight the lower ^{137}Cs inventories of F1, F2 and F3 soil samples as compared to the reference sites, confirming the erosion processes affecting the site under investigation.

Prior to the collection of soil cores (F1, F2, F3, REF1 and REF2), in-situ measurements using a lanthanum bromide scintillator were performed at the same sites. The detector was placed 2 cm above the ground and each measurement was conducted for 900 seconds. Across the study site, clear variation on ^{137}Cs levels was recorded. To convert these measurements to areal activity (i.e. Bq m^{-2}), an efficiency calibration was performed using In Situ Object Counting System (ISOCS), a Canberra software package system. Using ISOCS, a circular plane model with a diameter of 300 cm and a depth of 20 cm was pre-set, and the soil matrix dirt1 (i.e. H 2.2%, O 57.5%, Al 8.5%, Si 26.2%, Fe 5.6%, density 1.6 g cm^{-3}) was selected. The ISOCS incremental mass activity was calibrated using laboratory measurements carried out for the soil samples collected from the field. Preliminary results are presented in FIG 3.

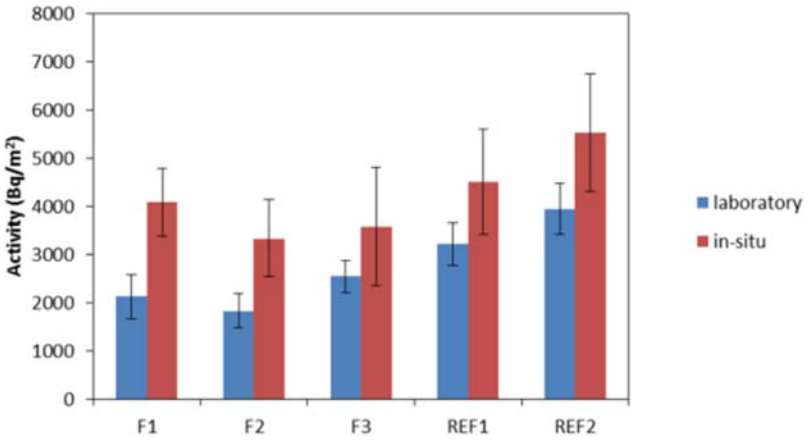


FIG. 3: Comparison of laboratory versus in-situ activity using the circular plane model (Error bars are reported at 2σ) 4

A significant positive correlation (i.e. R^2=0.85; $p < 0.001$) has been established between the ^{137}Cs areal activities obtained with our in-situ and laboratory based measurements (FIG 4).

More research and development are required to validate this innovative cost effective in-situ technique and will be the focus of future investigations conducted by the SWMCN Laboratory. Possible future activities include increasing the height of the LaBr detector above the ground, performing longer counting times, and experimentally comparing results against an in-situ HPGe detector.

This research has been conducted in the context of the Coordinated Research Project (CRP) 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"*.

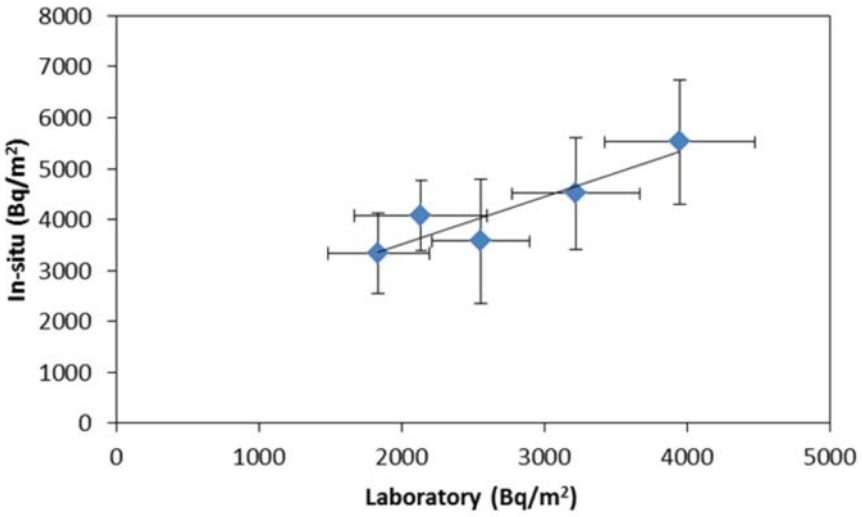


FIG. 4: Laboratory versus in-situ (137Cs), using circular plane model (Error bars are reported at 2σ)

Assessment of soil organic matter stability in agricultural land using isotopic techniques

The common method to determine stability and age of soil organic carbon (SOM) is the ^{14}C radio-carbon technique, which is very expensive and therefore limited in use. Conen et al. (2008) developed a model to estimate the SOM stability based on the isotopic discrimination of ^{15}N natural abundance by soil micro-organisms, and the decreasing C/N ratio during organic matter decomposition. This model has been developed for permanent grasslands in the Swiss Alps under steady-state conditions. The objective of our study was to validate whether this model could be used or adapted, in combination with ^{13}C isotope signatures of SOM, to predict the relative age and stability of SOM fractions in more disturbed agricultural ecosystems, providing support in using this low-cost and more accessible technique in Member States.

In 2013, the investigation was conducted on samples from a long-term experimental trial under different agricultural management practices (e.g. fertilizer and green manure *(Tithonia diversifolia)* application) in Kenya. The results show that the similar trends were found in Humic Nitisols of Kenya as in Luvisol (Figure 5), Cambisol and Chernozem soils from Austria and Belgium, which were tested in 2012. Particulate organic carbon (POM) has a higher C/N ratio and a lower $\delta^{15}N$ signature as compared to the mineral associated soil organic matter fraction (mOM). The POM in top soil (<15 cm) has a lower C/N ratio than in deep soil. The C/N ratio and $\delta^{15}N$ of POM was influenced by agricultural management. It has been estimated that the mOM fraction has a 50 to 2060 times longer turnover rate than POM; the relative age of the SOM rose with increasing soil depth. The combination of the above results with $\delta^{13}C$ data of the soil (to one meter depth) led to a more comprehensive understanding of the processes underlying SOM dynamics. Green manure application decreased significantly the bulk $\delta^{13}C$ signature of the SOM (including POM) in the topsoil, suggesting the importance of the green manure in the build-up of soil organic carbon.

The preliminary results of this research seem to indicate that the model, developed for grasslands, can be used to determine the stability of SOM in agricultural ecosystems. The C/N

ratio and $\delta^{15}N$ signature of the POM and mOM fraction follow the expected model pattern. In addition, the combination of above information with the isotopic $\delta^{13}C$ signature can further enhance the understanding of the processes driving SOM dynamics.

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

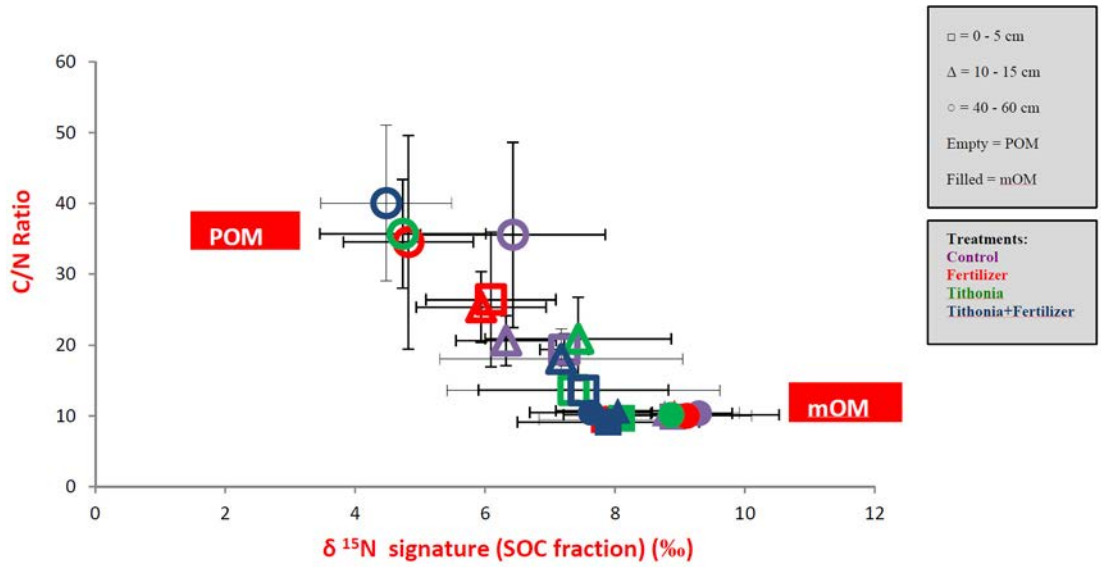


FIG. 5: C/N ratio versus $\delta^{15}N$ signature of different soil organic carbon fractions under different agricultural systems at different depths (Humic Nitisols at Embu, Kenya) (mean values, bars represent standard error, n=3)

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

In 2013, the SWMCN Laboratory installed a pair of walk-in growth chambers with an effective volume of about 12 m^3 each (Figure 6). These growth chambers with temperature, relative humidity and carbon dioxide (CO_2) control, are being used currently in the labelling of plant materials for incubation experiments to better understand soil organic carbon dynamics under a changing climate.

FIG: 6. Walk-in growth chambers with adapted CO_2 regulation and automatic irrigation system

In the first phase, the growth chambers were sealed for minimizing CO_2 losses and more important losses of ^{13}C labelled CO_2. Leakage rates were measured by filling the chambers with elevated levels of CO_2 (about 3000 ppm) and monitoring the decline of the CO_2 concentration over time. Leakage rates were calculated from

the decay constant of the exponential decay curve of the CO_2 concentration (Figure 7). The originally delivered growth chambers were found to have a leakage rate of about 25% per day. The feed through of the CO_2 supply and the cooling tubes could be identified as the major source of leaking. Sealing these leaks with silicone reduced the leakage rate to less than 5% per day.

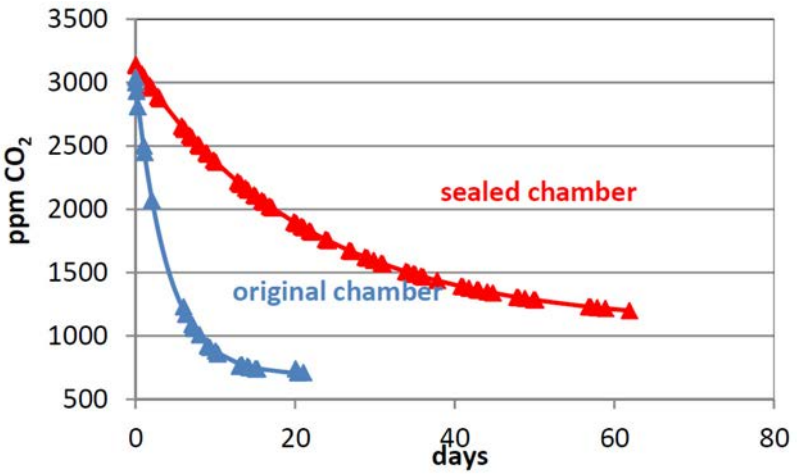


FIG. 7: CO_2 decline in sealed growth chamber

The CO_2 gas inlet was modified to introduce a mixture of (a) pure CO_2 at natural abundance level and (b) CO_2 with 99 atom% ^{13}C (Sigma Aldrich) into the chamber for a period of 15 seconds after a decrease of 10 ppm of the CO_2 level is monitored. The flow rates of the two gases (200 ml/min and 2 ml/min) are controlled by mass-flow-controllers assuring that the ^{13}C enrichment of about 350 δ‰ is constant over the whole growing period (continuous labelling).

In addition, automatic drip irrigation systems were placed in the growth chambers in combination with complementary air humidity controls, avoiding the need of entering into the growth chambers during the ^{13}C-labelling of plant materials.

This research has been conducted in the context of the Coordinated Research Project (CRP) D1.50.12 on *"Soil Quality and Nutrient Management for Sustainable Food Production in Mulch Based Cropping Systems in Sub-Saharan Africa"*.

Testing a new tracer for studying Phosphorus dynamics: Screening of different natural P fertilizers for oxygen-18 isotope 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 receiving different farm management practices (e.g. fertiliser or manure applications) show different $\delta^{18}O\text{-}PO_4$ signatures, indicating the potential as isotopic tracer for studying P cycling, tracing P sources and ultimately providing a better understanding of soil P dynamics in agro-ecosystems.

To contribute to the establishment and the wider application of this new tracer in phosphorus research, the SWMCN Laboratory in Seibersdorf, Austria has adopted this new technology from ETH, Switzerland, and started to screen a variety of seventeen rock phosphates from

different phosphate rocks in fourteen different Member States product in order to estimate the range and variation of ^{18}O isotope abundances.

The method involves acid extraction of inorganic phosphate from the phosphate rock samples followed by a series of precipitation steps and resin treatments in order to purify the phosphate, eliminating all oxygen sources other than phosphate that could compromise the final result (Figure 8). In the end phosphate is converted to pure silver phosphate (Ag_3PO_4) without isotopic alteration and analysed by TC/EA-IRMS (FIG 9).

FIG. 8: Filtration of the HCl-extract of different phosphate rock samples

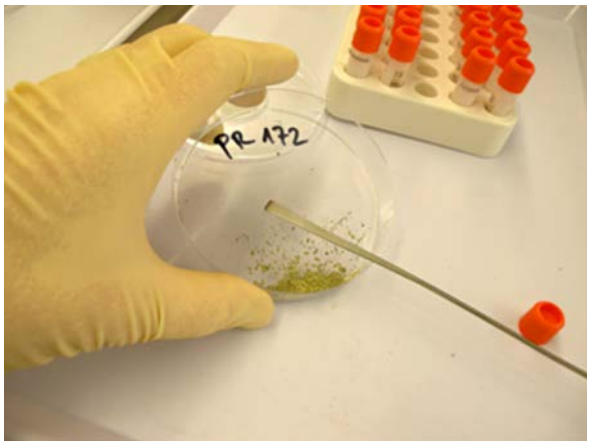


FIG. 9: Transfer of Ag3PO4 crystals to vials prior to TC/EA IRMS analysis

O-18 abundances are ranging from 5 to 22 $\delta^{18}O\text{-}PO_4$ ‰ with an average uncertainty of ± 0.45 ´$^{18}O\text{-}PO_4$ ‰ depending on their geological origin, (e.g. igneous vs. marine sediment) thus confirming previous results in literature (Figure 10). The animal bone sample was within the usual range of O-18 values (17.7 $\delta^{18}O\text{-}PO_4$ ‰).

In 2014, this new analytical technique will be further used to analyse other P sources, in particular from animal origin (e.g. pig or cow manure). This additional screening is the prerequisite for developing and adapting protocols for the use of this technique to improve land management. First surveys will be made in Austrian catchment to screen the feasibility for P tracing from uplands to lowlands through erosion and sediment transport.

This research has been conducted in the context of the Coordinated Research Project (CRP) D1.20.12 on *"Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-livestock Production Systems"*.

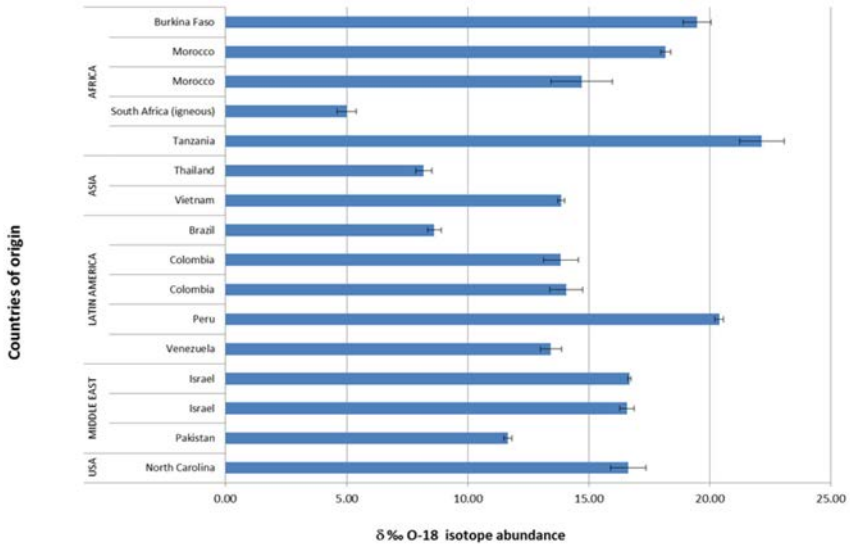


FIG. 10: $\delta^{18}O$-PO_4 ‰ isotope abundance values of the phosphate ions in different rock phosphates sorted by region (Error bars represent combined preparative and analytical errors)

Accessing Area Wide Soil Water Content using Cosmic Ray Neutrons

As one of the first in Europe, the SWMCN Laboratory acquired in 2013 a cosmic ray soil moisture neutron probe (CRS 1000/B model) to test its suitability for measuring area wide soil water content. While the cosmic ray soil moisture neutron probe has been shown to be able to measure soil water content in dryland systems, its suitability and usefulness for agricultural water management has not been tested fully. The technique is a non-invasive intermediate-scale soil water monitoring system that has a water footprint of an area up to 40 ha.

Besides the large area covered by a single device, the measurement is not affected by variations in soil temperature, salinity, bulk texture and density, of remote data accessibility and requires no more than one calibration of the system.

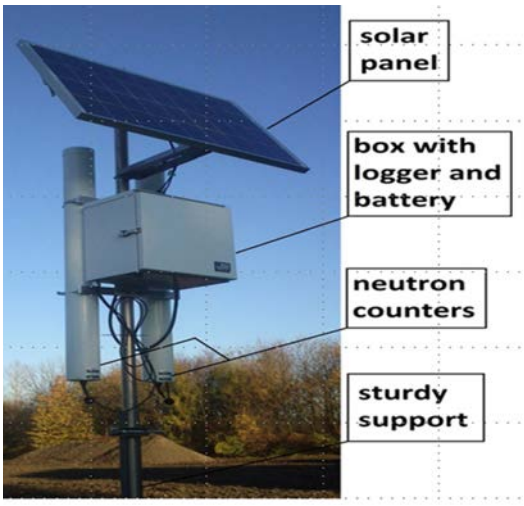


FIG. 11: Cosmic Ray Soil Moisture Neutron Probe

In close collaboration with the Vienna University of Technology and the Austrian Federal Agency for Water Management, the cosmic ray soil moisture neutron probe is currently being validated in a field site near Petzenkirchen in Lower Austria, 100 km west of Vienna (FIG 11).

At this field site, nearly 40 soil water sensors covering an area of 60 ha have recently been installed, besides other conventional devices already operating in the same site, making this location ideal for testing the state of the art device and cross-referencing data obtained from cosmic ray neutrons with measurements by conventional techniques (FIG 12).

FIG. 12: Comparing Cosmic Ray Neutron Probe data (purple) in situ soil moisture sensor network (red) in Petzenkirchen, Austria

This validation is being implemented in the context of Coordinated Research Project (CRP) D1.20.13, on *"Landscape Salinity and Water Management for Improving Agricultural Productivity"*.

The measurement of the SWMCN Laboratory Cosmic Ray Soil Moisture Neutron Probe can be followed in real-time on: *http://cosmos.hwr.arizona.edu/Probes/StationDat/087/index.php.*

Response to Nuclear Emergencies Affecting Food and Agriculture

At the end of 2013, the SWMCN Laboratory started a set of new activities in the frame of the new CRP D1.50.15 on Response to Nuclear Emergencies Affecting Food and Agriculture. This CRP aims to develop and assess systems of innovative data collection, management and geo-visualization platforms that can be used in both routine monitoring and also for emergency response to nuclear and radiological incidents that could affect food and agriculture. The SWMCN Laboratory assists in compiling Standard Operating Protocols (SOPs) for actions required in case of a nuclear emergency affecting food and agriculture, as well as sampling analytical SOPs for activity measurements. The objectives of the CRP are:

- To identify sampling and analytical strategies in nuclear emergencies affecting food and agriculture
- To determine how online geo-visualization tools can influence emergency response strategies, approaches to learning from nuclear accidents, and end-users ability to generate future short-term and long-term scenarios about the impact of nuclear accidents on food and agriculture
- To ensure that systems use common or standardized protocols that can be shared across different software platforms

- To produce low-cost computer-based platforms that are robust and can be used both routinely to monitor every-day sampling as well as in nuclear emergency situations
- To produce decision support tools that will help rapid analysis of the situation in radionuclide contamination in food stuffs.

The first RCM was held from 16 to 20 December 2013 in Vienna, under the joint coordination of the SWMCN Laboratory and Section. Four research contract holders (from China, Morocco, Russian Federation and Ukraine), two technical contract holders from France and Macedonia and three agreement holders from Japan (two) and India attended the RCM.

So far a detailed draft of the first protocol for data collection, management and visualization for the emergency phase (food restriction phase) has been developed by the technical contract holders in close collaboration with colleagues in the SWMCN Laboratory. 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 decide rapidly whether food restrictions need to be enforced, simple procedures/ protocols for collecting samples, managing minimal sample's attributes and minimal sample's laboratory result attributes and geo-visualisation for effective emergency communication are needed. A first information system is currently being developed in such a way that it can be linked with existing data exchange platforms (compatibility) of the IAEA (Figure 13). The system should allow international organizations to follow up the nuclear emergency response for food safety (for advice purposes on food restrictions when requested at national and international level).

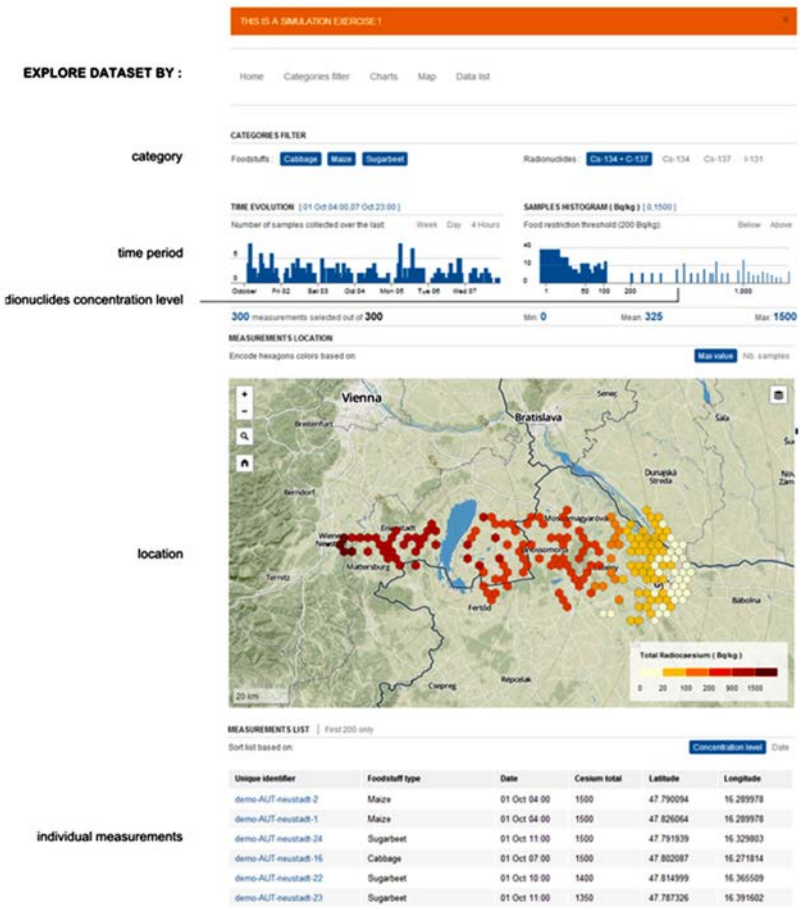


FIG. 13: First prototype of data visualization tool for nuclear emergency response in food and agriculture (simulated data are used for this visualization)

CAPACITY BUILDING

Soil Organic Carbon Dynamics and Management: The Use of Innovative Isotope and Conventional Techniques

From 15 April to 14 May 2013, the SWMCN Laboratory organized a training course on Soil Organic Carbon Dynamics and Management: The Use of Innovative Isotope and Conventional Techniques. Eighteen fellows from ten countries (Algeria, Benin, Cote d'Ivoire, Iraq, Madagascar, Mozambique, Oman, Palestine, Senegal and Zimbabwe) participated in the training.

The main focus of the training was to: (i) understand the importance of soil organic carbon for soil quality and climate change, (ii) soil sampling and sample processing techniques for estimating soil organic carbon, (iii) the use of isotopic and conventional techniques for assessing soil organic carbon dynamics, and (iv) modelling soil organic carbon dynamics. The training was funded by IAEA Technical Cooperation Department through national TC projects. Besides lectures and practical laboratory demonstrations, a technical visit to a long term field experiment run by the IAEA in collaboration with the Austrian Agency for Health and Food

Safety (AGES) at Grabenegg (ca. 120 km from Vienna) was organized to give the possibility of hands-on training in soil sampling (FIG 14).

Feedback from the participants showed that such intensive training, focussing on one topic from theory to application, will improve research capacity significantly. In addition, it was stated that the course helped the participants to better understand the use of modelling to assess soil carbon sequestration. Knowledge gained during the training will also be used to change teaching practices and will help support research and development. The training was participatory with a great deal of interaction between trainers and trainees.

FIG. 14: Fellowship training on soil sampling techniques to monitor soil organic carbon sequestration

Agricultural Water Management: The Use of Isotope, Nuclear and Conventional Techniques

From 24 June to 4 August 2013, the training Course on Agricultural Water Management: The Use of Isotope, Nuclear and Conventional Techniques was held at the SWMCN Laboratory. Twenty-two fellows from 16 Member States (Algeria, Bangladesh, Indonesia, Iraq, Ivory Coast, Madagascar, Malaysia, Mali, Mozambique, Oman, Palestinian Territories, Seychelles, Senegal, Tanzania, Zambia and Zimbabwe) with various backgrounds (students, trainers, researchers and irrigation managers) participated in the training.

The main focus of the training was to: (i) discuss the principles of water management in rainfed and irrigated agriculture, (ii) demonstrate monitoring techniques for estimating soil moisture at plot level, (iii) show the use of isotope, nuclear and conventional techniques for estimating soil water balance and assessing crop water relations, and (iv) model crop water requirements and irrigation scheduling using AquaCrop and related software. The training was funded by the IAEA Technical Cooperation Department through national TC projects.

Besides lectures and laboratory demonstrations, a guided tour to the Marchfeldkanal Water Distribution System close to Vienna was organized to give a practical example of large-scale irrigation management. In addition, experts from companies such as LI-COR Biosciences, Pessl Instruments and Eijkelkamp Agrisearch Equipment demonstrated equipment used to assess crop water use and monitor soil water quality and quantity. This partnership with companies improved the impact of the training course, as it gave the fellows an excellent insight into the latest technologies available for improving agricultural water management.

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. Visits to field sites, where the application of knowledge gained in theory can be experienced, were programme highlights. This training is useful for Member States as they prepare to meet the challenges that climate change will bring to farming communities and develop climate-smart agriculture, particularly on water management practices.

More information about this training course can be found on the UN Radio: *http://www.unmultimedia.org/radio/english/2013/09/tanzania-benefits-from-un-agencies-workshop-onwater/index.html.*

Regional Training Course on agricultural soil and water management to support crop production in Asia and the Pacific

From 7 October to 5 November 2013, the Soil and Water Management & Crop Nutrition Subprogramme brought together twenty-six scientists and technicians from twenty-one countries (i.e. Afghanistan, Indonesia, Iran, Iraq, Jordan, Laos, Malaysia, Mongolia, Myanmar, Nepal, Oman, Pakistan, Palestine, the Philippines, Qatar, Sri Lanka, Syria, Thailand, Vietnam and Yemen) across the Asia and Pacific region for a training programme on soil and water management in agriculture.

They spent one month at the IAEA's Seibersdorf Laboratory, working with the IAEA experts, to gain hands-on experience in the application of nuclear techniques to improve farming practices. Over the course of four weeks, the fellows received a comprehensive overview of soil and water management in agriculture, including the use of nuclear techniques for water use efficiency, fertilizer use efficiency and soil salinity. The training specifically focused on nutrient cycling and soil organic matter dynamics, soil erosion and conservation agriculture practices, direct measurements of soil water content, crop transpiration and soil evaporation, salinization of soil and water and managing salt affected soils and saline waters for crop production.

Feedback from the participants showed a very positive response, as most fellows were not previously aware of the use of any nuclear techniques in soil-water management before, and they learnt and benefited a lot from this training. One of the fellows from Thailand elaborated that though the training lasted for only one month, they learnt a lot from the IAEA experts as well as from group discussion and working together. The training also facilitated a discussion on the development of potential new regional TC projects.

More information about this training course can be found on the following link: *http://www.iaea.org/technicalcooperation/Home/Highlights-Archive/Archive-2013/11122013-TCAP_Group_Fellowship.html.*

The video URL is:
http://www.youtube.com/watch?feature=player_embedded&v=cOSItWQgy5A

ANALYTICAL SERVICES

In 2013, 4538 and 170 samples were analysed for stable isotopes and fallout radionuclides respectively in the SWMCN Laboratory. Most analyses were carried out for supporting Research and Development activities at the SWMCNL focused on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture.

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

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

The annual Proficiency Test (PT) on ^{15}N and ^{13}C isotopic abundance in plant materials, jointly organized by the University of Wageningen, The Netherlands, and funded by the IAEA Soil Water Management and Crop Nutrition Laboratory (SWMCNL) has been successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, *http://www.wepal.nl*) is accredited for the organization of Interlaboratory Studies by the Dutch Accreditation Council. In total twelve stable isotope laboratories participated in this PT-round.

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 SWMCN Laboratory and sent to WEPAL for processing. This ^{15}N-enriched material is sent out together with 3 other, non-enriched plant samples. Participants are invited to perform analysis 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 isotope analysis is issued and sent to the participants together with a certificate of participation additionally to the regular WEPAL evaluation report. The participation fee for one round per year is covered by the IAEA.

Participants registered in the PT scheme were provided with the WEPAL test sample set IPE 2013.2 consisting of the four test samples of 20 g plant material each. In total twelve laboratories reported isotope abundance data [i.e. Africa (1): Morocco; Asia (3): Malaysia, Pakistan, Philippines; Europe (5): Belgium, France, Germany, Italy and Turkey; Latin America (3): Argentina, Brazil and Chile.]

Eight out of twelve (67 %) laboratories participating in the nitrogen analysis reported ^{15}N-data within the control limits for the enriched plant sample (Figure 15) and eight out of nine (89%) participating laboratories in carbon analysis reported ^{13}C isotopic abundance results within the control limits for this test sample (FIG 16).

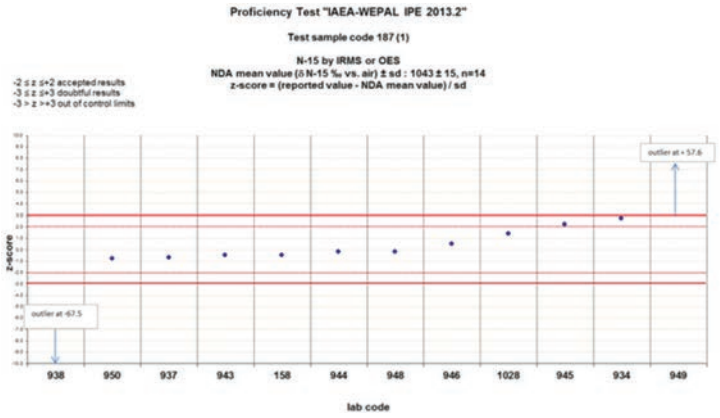


FIG. 15: Z-score evaluation of the ^{15}N analysis

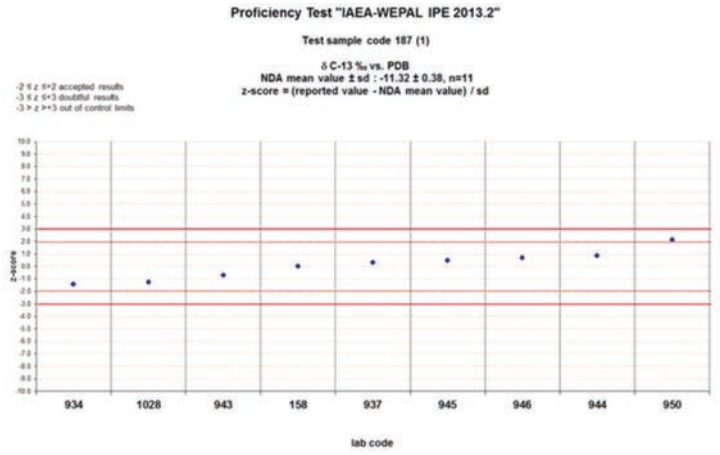
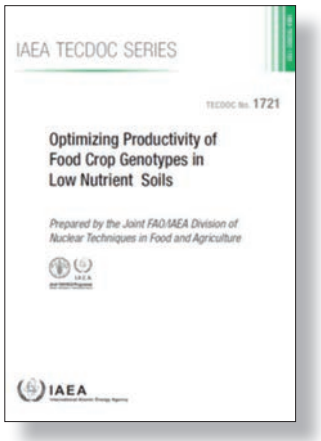


FIG. 16: Z-score evaluation of the 13C analysis

PROTOCOL, GUIDELINES AND INFORMATION RELEASED IN 2013

TECDOC 1721, Selection and evaluation of food crop genotypes tolerant to low nitrogen and phosphorus soils through the use of isotopic and nuclear technique, pp 327.

www-pub.iaea.org/MTCD/Publications/PDF/TE-1721_web.pdf



Global climate change and variability are likely to exacerbate plant abiotic stress in the coming decades by increasing water stress and by accelerating soil fertility degradation. To respond to this set of challenges, there is a need to develop agricultural systems with significantly greater productivity and resilience, while at the same time with more efficient use of limited nutrient resources.

This publication summarizes the output from a FAO/IAEA coordinated research project (CRP) on optimizing productivity of food crop genotypes in low nutrient environments over a five year period across a wide range of geographical areas and environments. It demonstrates that root traits are vital for nitrogen (N) and phosphorus

(P) acquisition from low nutrient soils. The CRP participants created a database on cereals and legumes with better root characteristics and greater productivity in low input agriculture. The use of N-15 and P-32 as tracers was valuable to understanding the physiological explanations for superior genotype performance in low N and P soils.

Research protocol on stable isotope probing to elucidate the role of soil microorganisms in nutrient cycling and soil quality

http://www-naweb.iaea.org/nafa/swmn/SIP-Protocol.pdf

Stable isotope probing is a technique that is used to identify the microorganisms in environmental samples that use a particular growth substrate. The method relies on the incorporation of a substrate that is highly enriched in a stable isotope, such as ^{13}C, ^{15}N or ^{18}O and allows identification of active microorganisms by the selective recovery and analysis of isotope-enriched cellular components. DNA and rRNA are the most informative taxonomic biomarkers and ^{13}C-labelled molecules can be purified from non-labeled nucleic acid by density-gradient centrifugation. The future holds great promise for SIP, particularly when combined with other emerging technologies such as metagenomics.

The protocol is divided into five separate sections: 1. An introduction to centrifugation and stable isotope probing, 2. Experimental design of stable isotope probing experiments, 3. A detailed experimental protocol for stable isotope probing experiments 4. A discussion of possible applications of stable isotope probing to nutrient cycling and soil quality is given, and 5. A list of published SIP studies is given in the Reference section. Stable Isotope Probing sometimes refers to the study of incorporation of stable isotopes into any biomolecule including RNA, DNA, phospholipid fatty acids, and proteins. This protocol is limited to the application of stable isotope probing to the study of DNA and RNA.

Research protocol on the use of compound-specific stable isotope analysis for precision soil conservation strategy development

www-naweb.iaea.org

This protocol has been prepared in order to standardise sampling, sample processing, analysis, and calculations for the use of a forensic technique using compound-specific stable isotopes (CSSI) to identify and apportion soil sources from land use.

This technique, linked with Fallout Radionuclide (FRN) techniques, will enable quantitative assessment of source-specific rates of soil erosion and sediment mass transport. The preparation of these protocols has been carried out in the frame of the FAO/IAEA Coordinated Research Project (CRP) D1.20.11 “Integrated Isotopic Approaches for an Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion”.

Report of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to the General Assembly in the frame of levels and effects of radiation exposure to the nuclear accident after the 2011 Great East-Japan Earthquake and Tsunami

www.unscear.org/unscear/en/publications/2013_1.html

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture contributed to this report through the FAO/IAEA Foodstuff Database. Since March 2011, a database has been compiled on radionuclide concentrations in foodstuffs due to the Fukushima-Daiichi NPP accident under the guidance of FAO/IAEA Joint Division of Nuclear Techniques in Food and Agriculture and in collaboration with the Japanese authorities, including the Ministry of Agriculture, Forestry and Fisheries (MAFF).

The database includes data over 500 types of foodstuffs sampled in all 47 prefectures in Japan. These data were provided through the FAO/WHO International Food Safety Authorities Network (INFOSAN) based on information published and provided by the Japanese Ministry of Health, Labour and Welfare (MHLW) and compiled by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

The database has been used in the UNSCEAR assessment of exposure and dose assessment for the public and environment. For the UNSCEAR assessment, approximately 126000 records on food monitoring were compiled, representing the period of March 2011 until March 2012 (only samples collected from 15 March 2011 through 15 March 2012 were included). In September 2012 these data were made available to the respective working groups of UNSCEAR for the assessment of ingested doses by the population of Japan due to the Fukushima nuclear accident through a relational database prepared in Microsoft Access format (hereinafter referred to as the Fukushima Foodstuff Database).

UNSCEAR 2013 REPORT Vol. I

SOURCES, EFFECTS AND RISKS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation
UNSCEAR 2013 Report to the General Assembly, with scientific annexes

Volume I: Report to the General Assembly, Scientific Annex A

CONTENTS:

Report to the General Assembly [1]
Includes a summary of the materials and conclusions contained in the scientific annex

Scientific Annex:

- Annex A: Levels and effects of radiation exposure to the nuclear accident after the 2011 great east-Japan earthquake and tsunami
  - Attachments of data and methodologies used for the assessment will be available in due course

Available for purchase soon

Japan: Follow-up IAEA International Mission on Remediation of Large Contaminated Areas Off-site the Fukushima Daiichi NPP, 14–21 October 2013, Japan

http://www.iaea.org/newscenter/focus/fukushima//final_report151111.pdf

The incident at TEPCO's Fukushima Daiichi Nuclear Power Plant (NPP) led to the radioactive contamination of large areas in and around Fukushima. The Government of Japan formulated a programme for the remediation of these areas and as part of this remediation programme a series of activities aimed at improving the living conditions of the people affected by the accident have been initiated.

In response to a request made by the Government of Japan, the IAEA organized a fact finding mission to provide assistance to manage the remediation of contaminated areas, to review remediation related strategies, plans and works, and to share findings and lessons learned with the international community. The mission was carried out from 7 to 15 October 2011. The final report of this mission is available on the above mentioned IAEA webpage.

Since then, various remediation activities have been put in place through the joint efforts of the Government of Japan and the local municipalities. However, challenges still remain, for example in remediating the highly contaminated forest areas, implementing radiation protection measures, and securing temporary sites for the establishment of interim storage facilities.

In response to these challenges, the Government of Japan requested the IAEA to carry out a follow-up mission on remediation of large contaminated off-site areas with the main purpose of evaluating the progress of on-going remediation works and providing advice to address remediation challenges.

The mission had the following three main objectives: (a) To provide assistance to Japan in assessing the progress made with the remediation of the Special Decontamination Area and Intensive Contamination Survey Areas; (b) To review remediation strategies, plans and works, initiated as a result of advice provided by the previous IAEA mission on remediation of large contaminated off-site areas; and (c) To share its findings and lessons learned with the international community.

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
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 Assessing soil erosion at landscape level by means of the fallout radionuclides method via *in-situ* gamma spectrometry
Climate / Air Pollution Group, Agroscope, Switzerland	Thermal stability of soil and soil fractions
Eidgenössiche Technische Hochschule (ETH), Switzerland	Use of oxygen-18 isotopes in phosphate to trace phosphorous sources and cycling in soils
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
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
National Institute of Water and Atmospheric Research, New Zealand	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
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 protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
University of Basel, Switzerland	Innovative preliminary test of plutonium isotopes (i.e. $^{239+240}$Pu) to assess soil erosion in mountain grasslands
University of Birmingham, UK	Assessing soil erosion at landscape level by means of the fallout radionuclides method via *in-situ* gamma spectrometry.
University of Ghent, Belgium	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies

University of Hohenheim, Germany	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
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 Plymouth, UK	Review of the potential and limitation of Beryllium-7 as short term radio tracer of soil movement
University of Vienna, Isotope Research and Nuclear Physics, Austria	Soil dating using carbon-14