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THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

According to estimates of the United Nations Department of Economic and Social Affairs/Population Division (UN-DESA, 2015), the human population will reach 9.7 billion in 2050, with the highest growth rate in developing countries. To secure food and alleviate poverty, livestock is a key agricultural sector as the growing world population, particularly in developing countries, increasingly demands animal protein. In these countries, the livestock sector directly supports the livelihoods of 600 million poor smallholder farmers[1]. Sustainability and productivity of the livestock sector in countries with limited resources is challenged by two major issues: the effective exploitation of animal genetic resources to maximize production and adaptability of local breeds to locally available environmental conditions and nutrients, and the preservation and improvement of animal health. Healthy animals produce higher quality food, reduce the needs for therapeutic drugs and thus decrease the burden of antimicrobial resistance and environmental pollution, ensure the maintenance of local and international trade and the generation of incomes, and contribute to preserve public health. A review published in 2012 identified 13 diseases transmitted from animals to humans (zoonoses) important for poor people and responsible for 2.2 million human deaths and 2.4 billion cases of illness every year (DFID, 2012). Nine of these diseases also have a high impact on livestock.

Animal health and exploitation of animal genetic resources were the main drivers of the research and development (R&D) activities conducted in the Animal Production and Health Laboratory (APHL) in 2016.

For an effective control of zoonoses and transboundary animal diseases (TADs), early detection and rapid confirmation of the identity of the pathogen are essential. To sustain the livestock sector and trade in Member States, implementation of efficient early warning systems and surveillance of the animal population are vital. The application of nuclear derived and related techniques in veterinary laboratories can provide rapid tools for serological surveillance of large animal populations and for the sensitive and specific detection of pathogens. R&D activities in 2016 at the APHL led to the development and validation of rapid tests for the detection of multiple pathogens in a single reaction. The tests targeted some of the most important TADs in small ruminants, including peste des petits ruminants (PPR), capripoxvirus and parapoxvirus diseases, and in swine, such as African swine fever (ASF), classical swine fever (CSF), Salmonella diseases and erysipelas. Capripox viruses are responsible for severe diseases in ruminants (e.g. lumpy skin disease in cattle and sheep-pox and goat-pox in small ruminants) and in 2016 they emerged for the first time in several countries in Europe. To monitor the presence or absence of circulation of capripox viruses in large numbers of animals, a novel assay to detect antibodies against these viruses was developed at the APHL. The ultimate purpose is the cost-effective screening of susceptible animal populations. Therefore, such assays represent useful tools for Member State laboratories and international organizations in their battle to sustainably control and/or eradicate animal diseases. Vaccination is also an extremely valid strategy to prevent or control the spread of infectious diseases. On some occasions, vaccinated animals may still harbour and spread the infectious agents. It is therefore important for disease control and eradication purposes to be able to differentiate infected from vaccinated animals (the 'DIVA' strategy). Cost-effective laboratory protocols for capripox viruses to reveal their genetic fingerprint were developed by the APHL. Their application enables laboratories to exactly identify the virus isolate and thus discriminate between field and vaccine strains. Genetic sequencing of viruses was also applied to investigate the molecular epidemiology and infer the origins of important

[1] webarchive.nationalarchives.gov.uk/20130128103201/http://www.dfid.gov.uk/research/mapping-climate.pdf

TADs, such as ASF in pigs and Newcastle disease in poultry. The investigations were conducted in collaboration with Member State laboratories in Ethiopia and Mozambique.

As mentioned earlier, vaccines are important tools in fighting infectious diseases. To be effective, they should guarantee high quality standards, contain the proper antigen at the right concentration, and be prepared, stored and administered properly. Above all, they should provoke in the animal a protective and possibly long lasting immune response and be safe (i.e. not causing the disease). Unfortunately, such vaccines are often not available for the important animal diseases. In 2016, APHL conducted research to explore the mechanisms of attenuation of *Trypanosoma evansi*, a parasite responsible for a cattle disease endemic in large parts of sub-Saharan Africa.

Developing and transferring immunological laboratory tests enabling the *in vitro* evaluation of candidate vaccine and their quality were other important activities in the APHL. *In vitro* assays to measure interferon gamma release and evaluate the cell mediated immune response in cattle were developed at the APHL during 2016.

Several new R&D activities on animal genetics were undertaken by the APHL. The laboratory in Seibersdorf initiated the construction of radiation hybrid panels for mapping camel genome as part of the newly launched coordinated research project (CRP) on 'Application of nuclear and genomic tools to enable for the selection of animals with enhanced productivity traits'. Significant achievements were made with the harvesting and screening of more than 270 clones for final selection into the camel radiation hybrid panel. Under this new CRP, APHL also initiated the development of a low cost DNA marker panel for estimating admixture levels in cattle. The marker panel is expected to help estimating the level of exotic inheritance in crossbred cattle. Regarding the implementation of the Global Plan of Action for Animal Genetic Resources to protect livestock biodiversity, APHL supported Burkina Faso and Niger on molecular genetic characterization of local cattle breeds. Additionally, and in collaboration with our headquarters colleagues, we completed the development and validation of the 'Genetics Laboratory Information and Data Management System (GLIDMaS)'. The system is currently under field testing and is expected to be transferred to genetic laboratories in interested Member States in 2017.

In addition to R&D, APHL was involved in capacity building and technology transfer activities in IAEA and FAO Member States. Two workshops and five training courses on TADs were organized in Vienna and Seibersdorf. A regional training course on 'Genetics of Parasite Resistance in Sheep: Sampling, Data Collection, Management and Analyses' was implemented in Montevideo, Uruguay. APHL hosted 15 fellows/interns for trainings on TADs, immunology, animal genetic resource characterization and DNA barcoding for animal and food traceability. APHL staff undertook eight technical field support missions in Member State laboratories and institutions (Belize, Botswana, Bulgaria, Burkina, Ethiopia, Lao PDR, Nepal and Sudan) to support activities related to animal health and animal production. In September 2016, APHL organized a workshop on 'Community-Based Livestock Breeding Programs in Tropical Environments', in collaboration with the University of Natural Resources and Life Sciences (BOKU, Vienna), the International Livestock Research Institute (ILRI) and the International Centre for Agricultural Research in the Dry Areas (ICARDA).

Technical data and scientific information were shared with Member States and scientific communities through 25 publications in refereed high impact scientific journals.

APHL activities that were carried out in 2016, in particular capacity building and technology transfer activities, also benefited from the financial support of the IAEA Peaceful Uses Initiative (USA and Japan) and the African Renaissance Fund (South Africa).

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[1] left in January 2016; [2] left in February 2016; [3] left in August 2016; [4] stayed from February to August 2016; [5] left in March 2016

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

Use radiation to identify virulent genes in Trypanosoma evansi

Studies that elucidate the impact of low dose irradiation on parasite metabolism and cellular processes were performed using probe based expression arrays. An expression micro-array platform that covers the genomes of three trypanosome species, *T. brucei*, *T. evansi* and *T. congolense* previously developed by APHL was used for this study. Time delay experiments after irradiation with doses of 100 Gy, 140 Gy and 200 Gy were performed at 1h, 6h and 20h to track the post irradiation recovery of parasites. Parasites irradiated using 100 Gy and analysed 20 hours post exposure show 68 genes with known function up-regulated and 18 genes down-regulated. This is in contrast to the up-regulation of 21 genes and the down-regulation of 267 genes when using a dose of 200 Gy. Genes that are consistently down-regulated when using both doses (100 Gy and 200 Gy) include metallopeptidases, carboxypeptidases, kinases and members of the ubiquitin family. Further pathway analysis of the data derived from these experiments reveal that the biological processes significantly associated with low dose irradiation are gene expression, translation and biosynthesis, whereas those associated with higher doses include cellular transport and localisation. Associated functions for low dose irradiation include ribosomal and structural activity. A summary of pathways affected by different irradiation doses is shown in Fig. 1. In conclusion, low dose irradiation experiments affect the expression of important trypanosomal proteases that irradiated parasites mitigate for by directing biological pathways towards increased translation and ribosomal activity. However, when exposed to doses above the 200 Gy thresholds, parasites struggle to survive by maintaining nutrient transport processes that ultimately cannot prevent death.

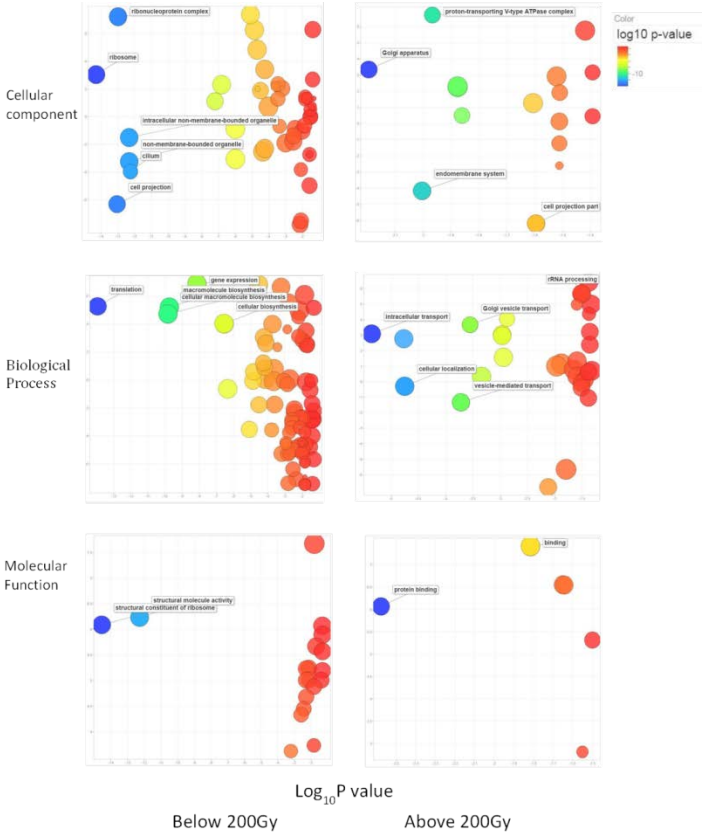

FIG. 1: Gene ontology analysis of irradiated parasites at doses above and below 200 Gy. GO terms with associated p values were analysed using REVIGO software (Supek et al., 2011: dx.doi.org/10.1371/journal.pone.0021800). Output was assembled into three categories: cellular component, biological process and molecular function. Significance values for different pathways were distributed from higher significance in blue to lower significance in red

Further experiments were carried out to evaluate the effect of irradiation in trypanosomes using flow cytometry and further characterize their cellular level activity. Green fluorescent protein (GFP) knock-in parasites were used to show that parasites irradiated with 200 Gy are still able to express GFP (Fig. 2). This indicated that irradiation with 200 Gy, which has been established as the dose that will not cause infection in the host, still allows the parasite to replicate at a lower rate, demonstrating that certain virulent factors are supressed during irradiation.

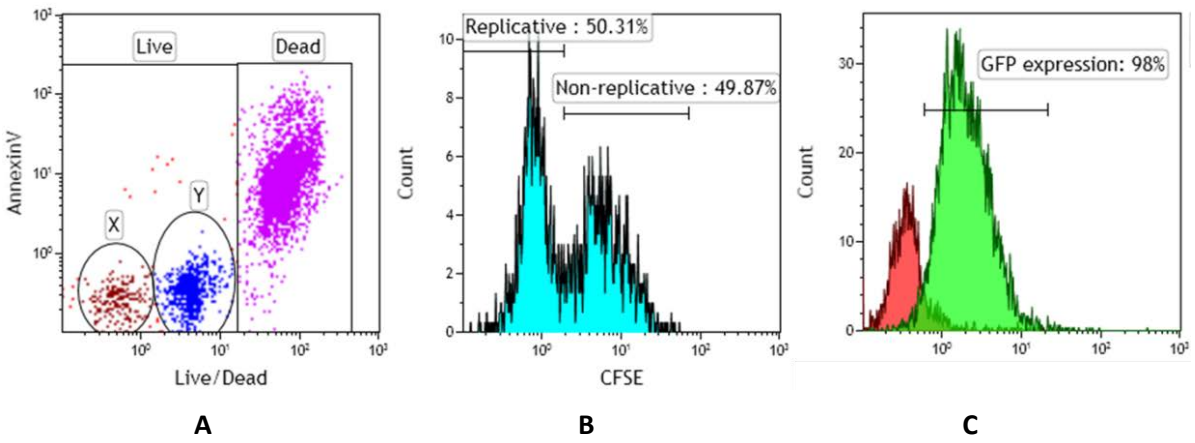

FIG. 2: Functional and phenotypic characteristics of Trypansoma evasi *following irradiation with 200 Gy and cultured for 24 hours. A) Two populations of live and dead parasites can be differentiated based on the amine reactive dye and annexin V binding. The live population is further divided into two populations (X and Y) based on the amine reactive dye. B) Parasites were pulsed with CFSE dye, the live cell population is shown based on the CFSE expression. C) GFP knock-in parasites (green) and wild type parasites (red) are shown after gating on the live cell population following irradiation using 200 Gy.*

APHL plans to use this information to identify trypanosome phenotypes that will lead to a better immune response and that will enhance immunity when used as an irradiated vaccine candidate.

To supplement data generated from irradiation studies, gene knock out clones were characterized *in vivo* using mice as an infection model. Gene knock out parasite clones targeting a prolyl oligopeptidase-like (*Δpop-like*) serine peptidase in *T. evansi* were generated and used to study the dynamics of infection in mice. Analysis using mouse spleen cells in an *in vitro* 24-hour incubation assay reveal that IL10 producing CD3 positive cells display significantly lower values when incubated with *Δpop-like* parasites compared to wild type clones (Fig. 3). These results suggest that prolyl oligopeptidase-like peptidase may play a role in immune responses during *T. evansi* infections by affecting interleukin concentrations in the host.

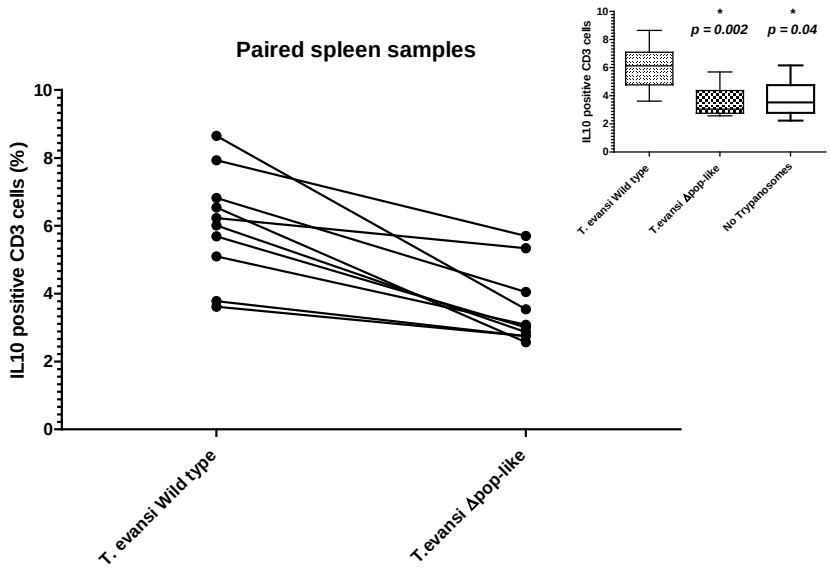

FIG. 3: Flow cytometry analysis of naïve mouse spleen cells. Percentages of IL-10 producing CD3+ T lymphocytes when incubated with wild type parasites compared to Δpop-like clones in paired experiments. Inset; Wilcoxon matched-pairs signed rank test reveals a significant difference between cells incubated with wild-type parasites when compared to spleen cells incubated with Δpop-like clones at a p value of 0.002.

Peste des petits ruminants

Peste des petits ruminants (PPR) is a highly contagious viral disease of wild and domestic small ruminants. The disease can cause serious clinical signs in sheep and goats, often leading to death in

80–100% of the most severe cases. After it was first identified in Cote d'Ivoire in 1942, it has spread to more than 70 countries in Asia, Africa and Middle East with an estimated annual loss ranging from US $1.45 billion to $2.1 billion. In 2016, the infection was diagnosed for the first time in Mongolia, where it is causing severe losses not only in livestock but also in wild ruminants such as the saiga antelope. In 2015, the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) took a lead in developing and implementing a global strategy for the control and eradication of PPR. The APHL has been playing an active role in the development of tools for the control of this disease, in particular specific and rapid diagnostic tests and the generation of genetic data to better understand the molecular epidemiology of the disease and the evolution of the PPR virus (PPRV), both important to prevent the transboundary spread of this economically important disease.

Rapid diagnostic tests based on multiplex PCR reaction to detect important virus pathogens in sheep and goats

RNA viruses such as PPRV often undergo genomic variations creating a challenge in their detection by PCR, and especially by real time PCR, when new variants arise. Such variants may be undetected by a diagnostic test if the mutations happen within the binding site of the PCR primers or probes. This often leads to false negative results, potentially allowing the disease to further spread in the affected area. One solution to increase the chance of detecting all variants is to target multiple genes of the same pathogen. APHL is working in collaboration with Member State laboratories from Cameroun, Ethiopia and Nepal to develop a multi-target multiplex assay for detection of PPRV with high sensitivity and specificity. At present, the method has been validated with very good results on 150 archived samples from different areas of the world.

Preparation of thermostable, lyophilised RNA from PPRV

A standard operating procedure (SOP) was developed to generate thermostable lyophilised RNA isolated from cells infected with PPRV. The ultimate aims were to make available to Member State laboratories a standardized positive control – the thermostable virus RNA - for the molecular detection of PPRV and to facilitate easier storage and cheaper shipment of such diagnostic reference reagents to laboratories worldwide. The SOP describes the addition of a thermostabilizing sugar (trehalose) directly to the purified RNA that is then lyophilised. The positive control was sent at ambient temperature to several Member State laboratories and successfully tested. The reagent is now available on demand from APHL.

African swine fever

Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia

African swine fever (ASF) is a contagious viral disease of pigs transmitted by ticks or through contact between infected and susceptible animals. In domesticated pigs, it leads to acute disease with high mortality and survivors are chronically infected serving as reservoir for further transmission. Wild boars are the natural reservoir in Africa. Endemic in large parts of sub-Saharan Africa, the infection has spread in the last 10 years to the Northern Caucasus and keeps expanding primarily to the West and North, also threatening Europe. The disease creates severe economic hardship for pig farmers and, due to the lack of an effective vaccine, culling and quarantine measures are the only tools to control the disease. As pig production in the affected areas is in many cases a small scale business, farmers often lack the means and education to fend off the disease. To date, a number of issues regarding the epidemiology and pathogenesis of ASF virus (ASFV) are not well understood. The early diagnosis and response, including molecular epidemiology, are very essential for the control of ASF to control and trace the movement of the virus.

Since 2012, APHL has been working on ASFV, supporting affected Member States through the transfer of rapid detection methods and assistance in their efforts to genetically characterize local virus isolates. In 2016, ASFV isolates were genetically characterized upon the request of the

following countries: Burkina Faso, Cameroon, Cap Verde, Central African Republic (RCA), Chad, Cote d'Ivoire, DRC, Ethiopia, Mali, Mozambique, Nigeria, Senegal, Tanzania and Zambia. A major outcome of this research activity was the discovery of the new ASFV genotype 23 in Ethiopia. This work was done in collaboration with Member State laboratories and two ASFV reference laboratories in Spain: the European Union Reference Laboratory for ASF (Centro de Investigacion en Sanidad Animal, INIA, Madrid) and the OIE Reference Laboratory for ASF (VISAVET Health Surveillance Centre, Universidad Complutense Madrid). All the Ethiopian isolates characterized clustered in the same genotype 23.

Detection of multiple pathogens in swine by a real time PCR single tube reaction

African swine fever (ASF), classical swine fever (CSF), salmonellosis and erysipelas are haemorrhagic diseases of swine, sharing common clinical sign such as haemorrhages. Since several of these diseases also share common geographical locations, there is an urgent need for proper differential diagnosis. APHL is currently involved in developing a multiplex real time PCR based assay for detection of viral (ASFV and CSFV) and bacterial (*Salmonella choleraesuis*, *Erysipelothrix rhusiopathiae*) pathogens causing the haemorrhagic diseases in swine. The test was developed in collaboration with the VETLAB Networks in Asia and Africa (supported by the Peaceful Uses Initiative (PUI) and the African Renaissance Fund (ARF)) in the framework of CRP D32031 and validated using 200 clinical samples from Cameroon, Cote d'Ivoire, Ethiopia, Mali, Mongolia, Mozambique, Nigeria and Tanzania. The test showed very good performance and an extensive validation on a larger number of samples from other areas of the world is planned.

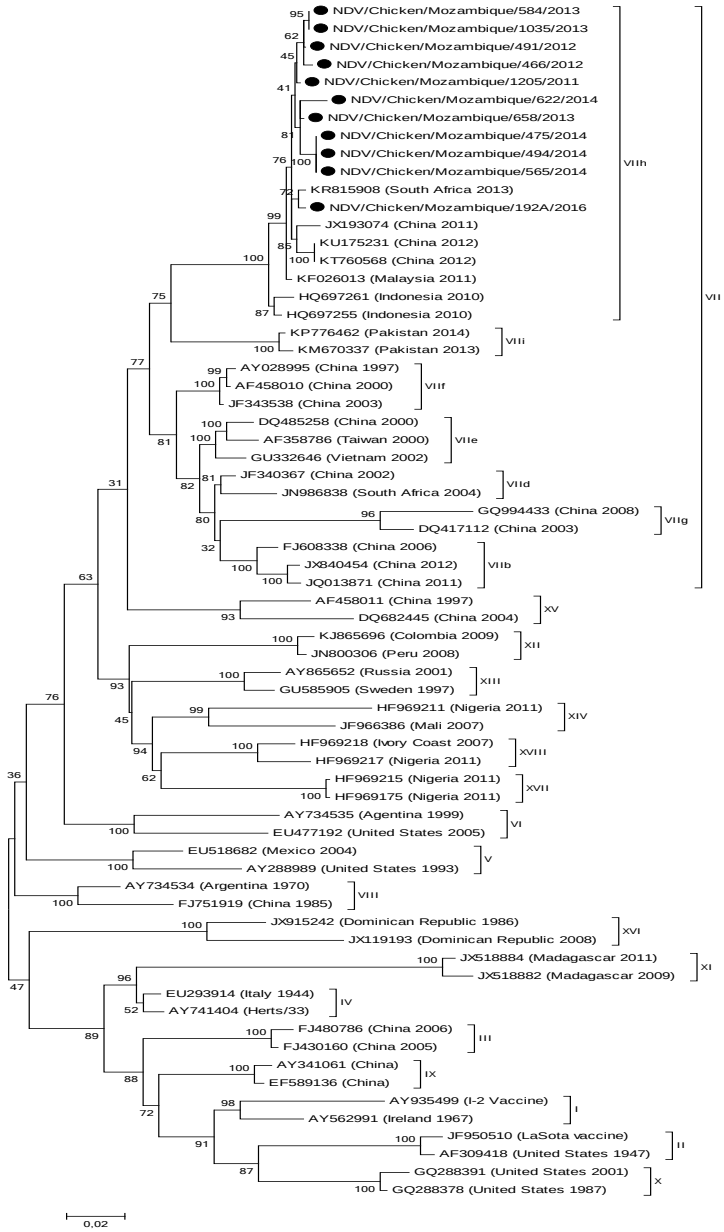

FIG. 4: ML analysis using the MEGA6 software of the full F gene nucleotide sequence (1662 bp) from eleven NDV positive samples from Mozambique (filled circles). The numbers indicate the bootstrap values calculated from 500 bootstrap replicates

Newcastle disease

Molecular epidemiology of Newcastle disease viruses in Mozambique

A phylogenetic analysis of the partial sequence of the fusion (F) protein gene from thirteen isolates of Newcastle disease viruses (NDV) collected in commercial poultry in Mozambique between 2012 and 2014 was performed in collaboration with the Central Veterinary Laboratory, Maputo, Mozambique. The analysis

revealed that the virus clustered within genotype VII and showed high similarity with NDV isolates from south-east Asia (Fig. 4). This is the first report of a genotype VII virus in the country and has important implications for Newcastle disease management and control in Mozambique.

Capripox disease

New diagnostic serology-based tests for capripoxviruses

APHL initiated the development of a capripox serological assay based on luminescence (Fig. 5). A nucleic acid vector was constructed to express a fusion protein composed of a fragment of a capripox protein and luciferase, an enzyme that produces light following degradation of a substrate. The capripox protein fragment serves as a hook to capture the capripox antibodies when present in the test serum. The complex is then retained on a test plate. Using this procedure, an assay that enables a specific serological diagnosis of capripox is developed. During 2016, initial assay development steps were taken. Capripox experimental sera will be used to validate this assay.

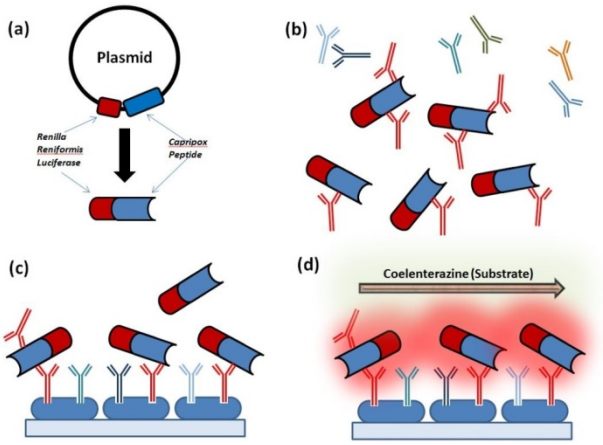

FIG. 5: Design of Capripox-LIPS Assay. (a) A modified circular DNA is made and cells generate a fusion protein (combining capripox and luciferase enzyme proteins). (b) The fusion protein is mixed with serum containing capripox antibodies (positive serum). (c) Special beads capture the antibodies and fusion protein complex. (d) Substrate is added. The enzyme produces light, which is then read by a luminometer

Genotyping of capripox viruses

Capripox viruses (CaPV) are responsible for economically important diseases of ruminants caused by sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV) affecting sheep, goat and cattle, respectively. The infections caused by CaPV are endemic in several countries in Africa and Asia. Recently, outbreaks of LSDV have also been reported in areas of Europe never affected before and SPPV is also expanding its area of distribution. These three viruses are not strictly host specific and are antigenically very similar. Therefore, the availability of rapid differentiation tools to veterinary laboratories can facilitate the implementation of more efficient detection methods and control measures. In endemic areas, capripox diseases are controlled using vaccination usually with live attenuated vaccine. In newly affected European countries, vaccination using live attenuated vaccines has been adopted by a few countries. However, the appearance of pock lesions in vaccinated animal has been reported, creating the need for differentiating vaccine strains from field isolates in order to determine the exact cause of the symptoms. In 2015, two amplification procedures for sequencing the complete RPO30 and GPCR genes of CaPV field isolates and vaccine strains were further validated and refined to improve the assay's sensitivity and facilitate the adoption of the testing procedures by other laboratories. Furthermore, the methods were used in 2016 on novel Ethiopian LSDV and GTPV isolates and on LSDV isolates and vaccine used in the Balkan region of Europe. Notably, it was revealed that the GPCR gene harbours a 12 nucleotides difference in both European and African LSDV field isolates, which can be used to differentiate them from some vaccines strains, such as those related to the Neethling and the KS O-240 type vaccine strains. As an additional advantage, this molecular DIVA for LSDV can also be used to partially sequence the GPCR gene using one pair of primers ((CpGPCR-OL3F -5'-CACAATTATATTTCCAAATAATCCAA -3' and CpGPCR-OL3R -5'-TGTACATGTGTAATTTTAATGTTCGTA3') instead of the three pairs needed for the full GPCR gene. These strategies were discussed and SOPs were developed and shared with the participants from Eastern Europe and Asia at two training courses on CaPV organised in August 2016.

In vitro *assays to evaluate the immune response to pathogens and vaccines in cattle*

IFN-gamma production and release from whole lysed blood is a more accurate measure of cell mediated immunity

Interferon (IFN)-gamma production and release from activated T lymphocytes is an excellent indicator of cell mediated immunity (CMI) and has been a gold standard for a long time. CMI indeed is a very important factor of protective immunity in infectious diseases, especially those caused by intra-cellular pathogens. Moreover, IFN-gamma production by clonally expanded CD4 and CD8 T cells is a good indicator of CMI induced by vaccinations against many intracellular and extracellular pathogens. Traditionally, peripheral blood mononuclear cells (PBMC) are isolated and IFN-gamma production is assayed within this cell population. Whole blood assays to measure IFN-gamma release are easy to perform and provide a better response in detecting bovine tuberculosis. Commercially already kits are available for this purpose. However, in other situations, such as where there is a need to measure IFN-gamma production in CD4 and CD8 cell subsets using flow cytometry or where incubation over several days with an antigen is required, the use of PBMC is preferred. However, experiments done at APHL have shown that, when isolating PBMC, the ratio of isolated PBMC over total cell counts are variable with each experiment. Moreover, signalling derived from non-PBMC compartments are lost when PBMC are assayed exclusively. Therefore, a more accurate protocol to measure IFN-gamma production was indicated and experiments were done to assess the suitability of whole lysed blood.

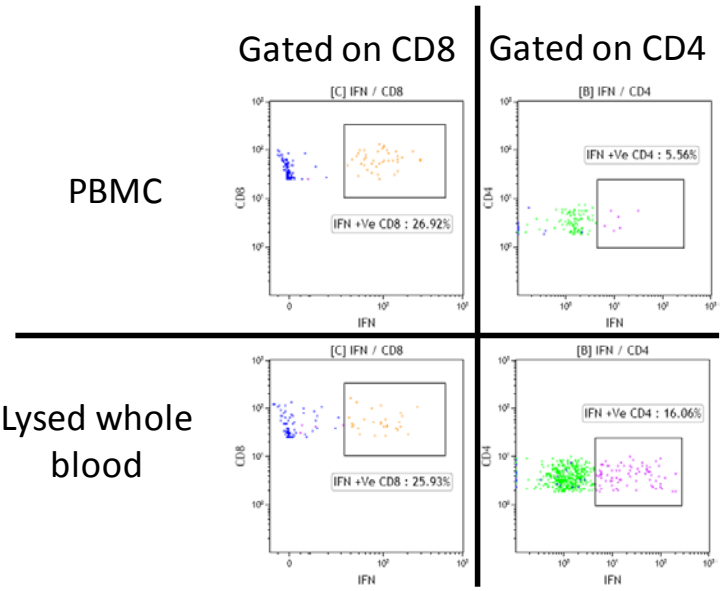

FIG. 6: Expression of IFN-gamma by CD8 and CD4 lymphocytes in response stimulation by PMA and ionomycin. Sheep PBMC or whole lysed blood was cultured for 48 hours and PMA and ionomycin with a golgi transporter inhibitor was added during last 4 hours of the culture. Cells were harvested and stained with live dead marker, preceded by surface staining with CD4 and CD8 antibodies. Next cells were fixed, permeabilized and was stained with an antibody against IFN-gamma. Next samples were analysed though a flow cytometer. Dot plots representing gated live CD8 (left) or CD4 (right) lymphocytes for the IFN-gamma positive cells

PBMC and whole lysed blood were cultured and stimulated with a cocktail of phorbol 12-myristate 13-acetate (PMA) and ionomycin. The IFN-gamma production and release was measured both by flow cytometry and ELISA. The ELISA results showed that total IFN-gamma in the supernatants of the PBMC culture was significantly higher than that of the whole lysed blood culture. However, the per-lymphocyte production of IFN-gamma was higher in whole lysed blood in comparison to PBMC. Further investigation by flow cytometry (Fig. 6) showed that CD4+ lymphocytes produce significantly high IFN-gamma in the whole lysed blood compared to PBMC. Therefore, IFN-gamma production and release from whole lysed blood is a more accurate measure of cell mediated immunity compared to traditional PBMC assays and is relatively easy to perform in less-resourced laboratories. This protocol has been transferred to Sudan where it was performed and proved to be successful.

Simulating *in-vivo* vaccine research in a culture dish

APHL previously introduced a comprehensive *in-vitro* experimental system to assess the vaccine antigens that would help to narrow down candidate vaccines that would be tested in animals. This

concept was based on *in-vitro* priming of T lymphocytes by dendritic cells (DC) to a specific antigen/pathogen. We now optimized this system using two known vaccine antigens (diphtheria and tetanus toxoids). Next, we used the optimized DC based system to assess CD40 ligand (CD40L) as an adjuvant to be used in the irradiated Trypanosome vaccine that is being developed at APHL. For a long time it was believed that immune evasion through changing of the variable surface antigen (VSG) coat by trypanosome parasites would hinder the discovery of an effective vaccine against this parasite and indeed there is no vaccine to this day. However, recent reports show that VSG change alone is not the main cause of immune evasion and that CD40 ligation could overcome the immune suppression induced by trypanosomes. Hence, potency of CD40 ligation as an adjuvant was investigated in a whole cell irradiated trypanosome vaccine using DC based *in vitro* assay. The cell proliferation marker, Ki-67, was used to measure priming in both CD4+ and CD8+ T lymphocytes. Our previous experience had shown that irradiation of T. evansi with a dose of 200 Gy (*T. evansi*-200 Gy) will not cause disease in mice but that it will remain metabolically active and replication competent *in-vitro*. Therefore, we used *T. evansi*-200 Gy as the primary vaccine candidate, with or without CD40 ligation. DCs were pulsed with i) no antigens, ii) *T. evansi*-200 Gy, iii) CD40 ligation (CD40 mAb) or iv) *T. evansi*-200 Gy plus CD40 mAb (*T. evansi*-200 Gy+CD40L). Then DCs were co-cultured with naïve lymphocytes and re-stimulated twice, and the expression of Ki-67 by lymphocytes was measured.

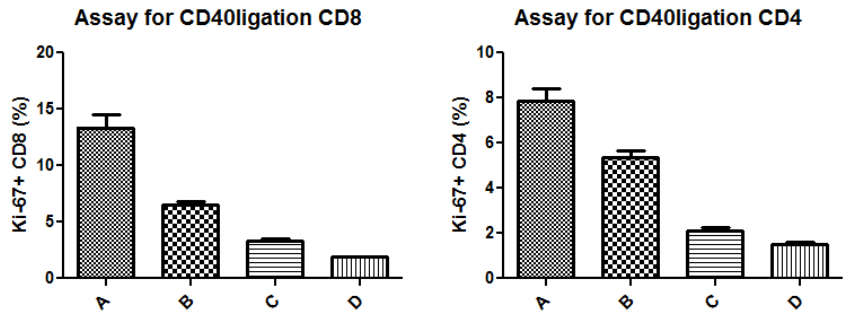

FIG. 7: Expression of Ki-67 by CD8 and CD4 lymphocytes upon re-stimulation with antigen pulsed DCs. Monocyte derived DCs were pulsed with T. evansi-*200 Gy+CD40L (A), CD40 mAb (B),* T. evansi-*200 Gy (C) or no antigens (D) and co-cultured with naïve lymphocytes for 14 days. DCs pulsed as above were added into culture twice for re-stimulation. After final re-stimulation, expression of Ki-67 by lymphocytes was measured. Left panel shows mean (± SEM) percentage of Ki-67+ cells in the total CD8+ cell population. Right panel shows mean (± SEM) percentage of Ki-67+ cells in the total CD4+ cell population*

As shown in Fig. 7, the Ki-67 expression was highest by CD8 and by CD4 lymphocyte with DCs pulsed with *T. evansi*-200 Gy+CD40L (A) whereas it continued to decline with CD40 mAb (B), *T. evansi*-200 Gy (C) and no antigens (D), indicating that CD40 ligation increases the priming of both CD4 and CD8 lymphocytes. These results together indicate the possibility of using DC based *in-vitro* assay to evaluate vaccine candidates and the ability of CD40L to improve the irradiated trypanosome vaccine.

Animal Genetics

Construction of radiation hybrid panels to map camel genome

Advances in genomics have enabled the development of DNA chips (microarrays) that could be used to evaluate and breed genetically superior livestock for increased productivity. Development of DNA microarrays for animal evaluation requires sequencing and mapping of the concerned genomes. A genome map can be developed either by conventional methods or radioisotopic techniques. Conventional methods of mapping, such as genetic linkage mapping, are based on natural recombination events and require pedigreed animals in successive generations. In case of livestock, this not only involve huge costs and a long time (due to long generation intervals), but also result in maps with low resolution. Application of radioisotope techniques help to overcome these limitations by mimicking the genetic recombination events and speeding up the process of genome mapping for faster development of genetic tools to increase livestock productivity. Radiation hybrid panels and genomic tools are not yet available for several important livestock species, including camels.

Considering the significance of camel improvement for pastoralist communities in Africa and Asia, APHL initiated the construction of radiation hybrid panels for the mapping of the camel genome as part of the newly launched CRP on 'Application of nuclear and genomic tools to enable for the selection of animals with enhanced productivity traits'.

^{60}Co was successfully used to irradiate two normal diploid fibroblast cultures derived from male and female dromedary camels, respectively. Irradiated camel fibroblasts were fused with A23 hamster cells to establish two radiation hybrid panels of different resolutions (15 000 rad and 5000 rad). A total of 487 (238 15 000 rad panel and 249 5000 rad panel) camel-hamster hybrid cells were collected, of which 279 (93 15 000 rad panel; 186 5000 rad panel) have been expanded and harvested for subsequent screening with a set of 44 markers. Preliminary analysis revealed a retention frequency of the camel genome ranging between 10% and 90% across different clones. Further screening of hybrid cells is currently underway. The final set of radiation hybrid panels selected after screening will be transferred to the International Camel Genome Consortium and interested Member States for further characterization and mapping. The high resolution radiation hybrid map is expected to help Member States in developing and implementing genomic tools for breeding and improvement of camel productivity.

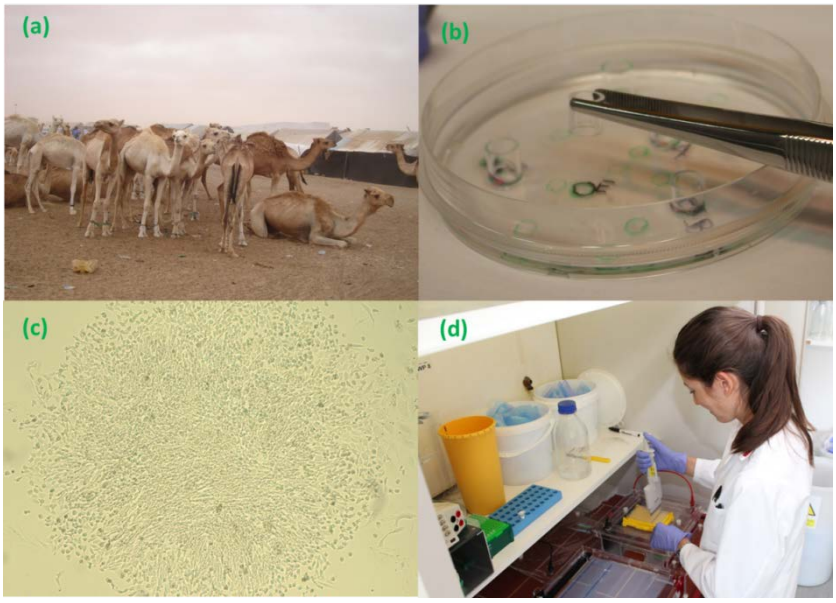

FIG. 8: Construction of radiation hybrid panels for camels (a) A herd of camels (b) Picking up of live hybrid cells (c) A colony of radiation hybrid cells for genome mapping (d) Screening of radiation hybrid cells. A colony of radiation hybrid cells for genome mapping (bottom right)

Estimating levels of admixture in crossbred dairy cattle

Dairy production in the tropics, particularly in South Asia and Sub-Saharan Africa is essentially characterized by smallholder systems with an average of 2-5 animals per household. Genetic improvement of dairy cattle in these smallholder systems has been mainly through crossbreeding. The crossbreds have been found to perform optimally with 50% or 62.5% level of exotic inheritance in certain institutional farms. However, such stabilization of crossbreds with desired genetic makeup did not happen at field level because of lack of infrastructure for animal identification, performance recording, etc. This often resulted in indiscriminate crossbreeding and the production of crossbred cattle with widely varying levels of exotic inheritance. These animals currently face several problems related to adaptability, reproduction and productivity. To address this issue and to assist Member States in stabilizing the crossbreds, APHL initiated the development of a low cost DNA marker panel for estimating admixture levels in cattle. A total of 198 samples collected from indigenous and crossbred cattle in Bangladesh and Myanmar were utilized for genotyping with microsatellite and genome-wide single nucleotide polymorphic (SNP) markers. All the samples were genotyped for 27 FAO recommended microsatellite markers and 60 000 SNP markers available in a ovine-caprine-bovine array from Affymetrix. Preliminary analysis of microsatellite genotypes included parentage testing and evaluation of admixture levels in indigenous and crossbred cattle. Analysis of SNP genotypes derived from crossbred cattle with varying levels of exotic inheritance is currently under progress.

Genetic characterization of indigenous cattle breeds from Burkina Faso and Niger

In continuation of the Joint FAO/IAEA Division's support towards implementing the Global Plan of Action for Animal Genetic Resources (GPA-AnGR), APHL supported the genetic characterization of native cattle breeds from Burkina Faso and Niger during 2016. Most of the indigenous cattle from Burkina Faso and Niger are compact and low milk producers, except for a few moderately producing cattle breeds like Azawak and Goudali. These animals survive on naturally available grasses (e.g. Pennicetum) and crop residues and are well adapted to tropical environments. However, much required information on their genetic potential, such as genetic variability, level of inbreeding, and physical and phenotypic characteristics, are lacking. A total of 286 samples collected from three major cattle breeds in Burkina Faso and seven cattle populations in Niger were analysed by sequencing the control region (D-loop) of the mitochondrial genome. All animals were also genotyped at 27 microsatellite marker loci grouped in six multiplex panels. Basic diversity indices were established for each of these ten breeds/populations and genotype data were utilized to assess phylogeny and genetic structure. Genetic analysis revealed strong sub-population structures among the Bororo cattle reared in different regions of Niger and Burkina Faso, while the Gourounsi breed was genetically distinct from other cattle in the region (Fig. 9). The results of the genetic diversity and structure analysis are expected to facilitate formulation of effective strategies for the conservation and genetic improvement of native Burkina and Niger cattle.

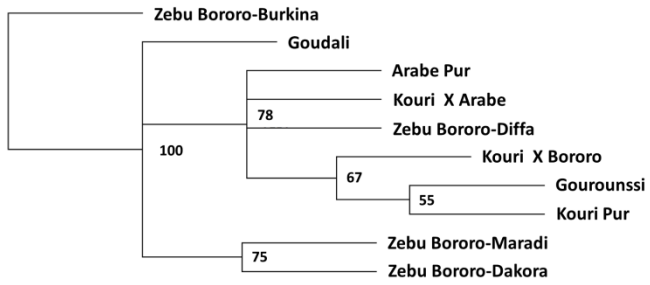

FIG. 9: Phylogeny of native Burkina and Niger cattle breeds

Genetics Laboratory Information and Data Management System (GLIDMaS)

APHL continued its efforts in the development and validation of the Genetics Laboratory Information and Data Management System (GLIDMaS) to support Member States in managing their livestock biodiversity and improving productivity of local animal breeds. New modules and facilities on Genetic Repository, Radiation Hybrid Panels and Data Archives were created. All modules in the GLIDMaS platform were finalized and validated. Field testing, initiated at the end of 2016, will be continued in 2017. Transfer of GLIDMaS to animal genetic laboratories in Member States is expected to begin by the end of 2017.

CAPACITY BUILDING

Emergency response to the re-emergence of highly pathogenic avian influenza virus (H5 subtype) in Africa and Europe

The highly pathogenic avian influenza (HPAI) virus belonging to the H5 subtype poses serious threats to the poultry industry and, in limited resource countries, to the livelihood of rural communities that rely significantly on poultry to secure food or generate income. Some strains of this virus also cause public health concerns as they are capable of crossing the species barriers and infect not only birds and humans but also other animals. In 2016, HPAI viruses belonging to the H5N1, H5N8 and H5N5 subtypes emerged in East Asia and reached several countries in Europe and some in Africa, similar to that of the H5N1 virus in 2005-2008. Upon request of Member States, immediate action was taken to strengthen the preparedness of veterinary services to control the disease. These included the preparation and shipment of emergency diagnostic kits (i.e. boxes containing all the reagents and consumables necessary to address the immediate diagnostic needs of veterinary laboratories) to Cameroon and Bulgaria, assistance to national veterinary laboratories in affected or at-risk

countries, and the organization of a workshop in Vienna with 83 participants from Europe, Asia and Africa to apprise participants on recent advances in the detection of avian influenza as well as on the current epidemiological situation ().

Emergency response to enhance Member State capacities in the diagnosis and control of lumpy skin disease (LSD)

In 2016, the introduction and spread in the European territory of the lumpy skin disease (LSD), an infectious viral disease affecting cattle, caused an extremely serious animal health emergency and challenged the preparedness and capacity of many the veterinary services. In fact, LSD in Europe was considered an 'exotic' disease and the majority of veterinary services and laboratories were inexperienced and unprepared for the detection and control of this virus. Upon requests from Member States in Europe, an emergency action plan was implemented, targeting recently affected countries and countries at high risk of infection. Taking advantage of the VETLAB network and the network of expertise involving OIE, FAO reference laboratories and research institutions in Member States, IAEA in partnership with FAO mobilised resources and implemented the actions briefly described below.

Field support missions. Immediately after the emergence of the first infections in Europe, field support missions were undertaken to evaluate the immediate diagnostic needs of the national veterinary laboratory in Bulgaria and Serbia. Missions focused on laboratory capacity, harmonization of laboratory SOP and efficiency of laboratory testing throughput.

Provision of reagents and laboratory supplies. To address deficiencies in laboratory reagents and supplies to handle the large numbers of suspected samples, diagnostic toolkits, reagents and consumables, along with guidance and SOP for LSD detection and differential diagnosis (e.g. for genotyping LSDV, SPPV and GTPV) were provided to eleven countries in Europe and Central Asia. In addition, to assist Member States in the implementation and validation of the assays in their own laboratories, standard material (viral DNA positive controls) was supplied. This will assist countries to implement rapid detection and early warning systems for LSD.

Training courses. Two training course were organized in August 2016 on the rapid detection and genotyping of capripox viruses, including LSDV, SPPV and GTPV. Thirty-six participants from 22 countries participated and familiarized themselves with the epidemiological and clinical aspects of capripox infections. The participants also took part in intensive practical sessions where they conducted laboratory tests at the bench.

Workshop. To be better prepared to face and control this emerging disease in Europe and Central Asia, a workshop on Advanced Diagnosis and Control of Emerging Transboundary Animal Diseases, with Emphasis on Lumpy Skin Disease was organized in Vienna in November 2016. International experts on LSD and related capripox virus infections presented up-to-date information on the disease, its epidemiology and tools and strategies for its control. Eighty-three participants from national veterinary laboratories, veterinary epidemiologists and officials from the national veterinary services in Europe, Asia and Africa attended the one-week workshop.

Proficiency test for peste des petits ruminants

As in previous years, APHL organized a proficiency test (PT) for PPR diagnosis. The aim was to evaluate, in a qualitative manner, the ability of participating laboratories to determine the presence of antibodies against, or RNA from, PPRV using serological and molecular techniques in a panel of samples prepared and provided by APHL. The test panel consisted of a total of 21 gamma-irradiated sample vials; 10 for nucleic acid and 11 for antibody detection. Twenty-seven laboratories in 24 countries (17 Africa, 6 Asia, 1 Europe) participated and were each allocated a unique identification code.

Seventy-seven percent of participants obtained a perfect score with regard to the serology panel, 19% obtained a score of 80% and 4% obtained a score of 50%.

On the molecular panel, 73% obtained a perfect score, 13% scored 90%, 5% scored 80% and 9% scored 50%.

A final report was sent to participating laboratories. Furthermore, the APHL offers to discuss, in a confidential and individual manner, ways to improve diagnostic capabilities with laboratories that have scored less than 100%.APHL also encourages all members of its laboratory networks to participate in future proficiency tests as useful exercises towards determining the ability of a laboratory to identify PPRV.

Technical field support missions to build capacity in veterinary diagnostic laboratories

APHL has been actively involved in the transfer of technologies to Member States. Seven technical field support missions were undertaken to install and calibrate critical equipment and to demonstrate work flow on disease diagnostic procedures, surveillance and epidemiology of transboundary animal diseases. Some missions also focused on building national capacities in the diagnosis of avian influenza and lumpy skin disease.

Belize

An expert from APHL travelled to Belize from 11-15 July to enhance the capacity of the newly built PCR laboratory by providing hands on practical and theoretical training on nuclear and nuclear related molecular diagnostic techniques for the diagnosis of animal and zoonotic diseases, with a focus on Newcastle disease and avian influenza, as well as to assist with quality assurance and quality management for future ISO 17025 accreditation. Technologies included molecular diagnostic techniques for avian influenza, Newcastle disease and multiplex RT-PCR assay for swine haemorrhagic diseases (classical swine fever, AFS, Salmonella and erysipelas), four separate swine diseases causing similar clinical symptoms.

Botswana

APHL staff travelled to Gaborone, Botswana, to support the activities of the technical cooperation project (TCP) BOT5015 'Establishing District Laboratories that use Nuclear and Molecular Techniques for Early and Rapid Diagnosis of Endemic and Transboundary Animal Diseases' (27 June to 01 July 2016). The mission was carried in conjunction with the project to strengthen animal disease diagnostic capacities in selected Sub-Saharan African countries, supported by the ARF and the PUI. The Botswana National Veterinary Laboratory (BNVL) received the molecular diagnostic platform under the VETLAB Network and a new real-time PCR platform was installed and real-time PCR technology transferred, focusing on multi-targets detection as an additional tool for rapid and accurate diagnosis of transboundary animal diseases (TADs). Laboratory staff was trained on assays for the detection of ASF, PPR viruses, CaPV genotyping and multi-parametric assays for small ruminants respiratory diseases and pox-like disease in ruminants. The molecular diagnostic procedures used at BNVL were reviewed and new procedures were added, comprising assays including internal and exogenous controls, assays targeting deep pathogen identification, multiple pathogens detection methods and sequencing-based identification of pathogens.

Bulgaria

A mission was organized in response to an urgent request by Bulgaria, following the detection of LSD in cattle, to assist the national veterinary laboratory in assessing its needs in terms of LSD virus detection and surveillance. Laboratory PCR-procedures for the rapid detection of LSD virus were evaluated and optimized and the gel-based PCR for sequencing was updated and optimized. Laboratory results, obtained on samples submitted to the laboratory in Sofia and processed, were discussed, enabling laboratory staff to interpret the results and to provide robust feedback to

farmers and decision makers. Furthermore, urgent needs of the laboratory in terms of reagents, lab ware and equipment, were discussed and prioritized.

Burkina Faso

The mission was carried out through TCP BKF5017 to support national efforts to put in place a sustainable native cattle breeding program for Burkina Faso utilizing artificial insemination services and modern animal genetics tools. A field visit was made to smallholder cattle farms to identify the breeding requirements of farmers and assess their willingness to adopt artificial insemination for dairy cattle breeding. It was agreed that the artificial insemination centre will focus on improving the production of frozen semen from Azawak bulls. Practical training on genotyping workflow and analysis of molecular genetic data was provided to 13 participants from Burkina Faso, Niger and Mali, including hands-on training on bioinformatics software for analysis of DNA marker data to assess genetic diversity and population structure. This training has helped the ongoing national efforts to complete the genetic characterization of native livestock breeds in Burkina Faso and Niger.

Lao PDR

Livestock production in Lao PDR, both bovine and caprine, plays a significant role in the socioeconomic development of the country. One of the main constraints, however, is the occurrence of disease outbreaks, as TADs continue to threaten animal health in the country. Through TCP LAO5003, and in collaboration with the VETLAB Network, an APHL staff visited the National Animal Health Laboratory (NAHL) of the Ministry of Agriculture and Forestry, Vientiane, Lao PDR on 18-22 April 2016, to train staff on the diagnosing of PPRV, CSF, NDV, HPAI, ASF, capripox, MCCP, Pasteurella, erysipelas and Salmonella using real-time and conventional molecular diagnostic protocols.

Nepal

A field support mission was performed from 25-29 July 2016 to the Central Veterinary Laboratory in Kathmandu to transfer real-time PCR based multiplex assays for the detection of TADs, sequence analysis and genetic characterization of targeted pathogens and to determine gaps in executing these tests in a routine setting. Five staff of CVL was trained on the multiplex assays for detection of pathogens causing respiratory diseases in small ruminants and haemorrhagic diseases in swine, and on multi-target assays for PPRV detection. During the mission, CVL staff used the multiplex assays to screen outbreak samples and confirmed the presence of PPRV. Staff was also trained on different assays on two different real-time PCRs, its data analysis and trouble shooting.

Ethiopia

In October a field mission in Ethiopia was organized aiming at reviewing the current status of the main national veterinary laboratories, namely the National Animal Health and Disease Investigation Centre (NAHDIC) and the National Veterinary Institute (NVI). Through the support of the Joint FAO/IAEA Division, these laboratories achieved important results in the most recent period, including national (NVI) and international (NAHDIC) accreditation for several laboratory tests. The priorities and main needs of these laboratories in the areas of animal health, infectious disease diagnosis, and vaccine development and production were assessed.

During the mission, potential synergies and areas of collaboration to better support research and development in animal health in Ethiopia were discussed with the National Institute for Control and Eradication of Tsetse and Trypanosomosis (NICETT) and the African Union Pan African Veterinary Vaccine Centre (AU-PANVAC).

Sudan

An expert mission was carried out on December 9-16, 2016 to provide technical expertise to assist the Veterinary Research Institute, Animal Resources Research Corporation of Sudan to develop an irradiated vaccine against Brucellosis in sheep. The current vaccine against Brucellosis is sub-optimal

and an irradiated vaccine would be a major breakthrough not only to Sudan but to all concerned Member States. As part of the mission, a new cell culture facility was set up at the Central Laboratory of the Ministry of Higher Education and Scientific Research in Khartoum. Several protocols to measure cell mediated immune responses that had been developed at APHL were transferred. A two-day workshop on flow cytometry, attended by 19 participants from three national research institutes, was conducted specially targeting vaccine trials.

Meetings

TROPENTAG, workshop on Community-Based Livestock Breeding Programs in Tropical Environment

The TROPENTAG 2016 was organized by the BOKU and the Council for Tropical and Subtropical Agricultural Research on 18-21 September 2016 in Vienna, Austria. As part of the TROPENTAG conference, a workshop on 'Community-Based Livestock Breeding Programs in Tropical Environments' was jointly organized on 19th September, 2016 by the Joint FAO/IAEA Division (APHL), BOKU, the International Livestock Research Institute (ILRI) and the International Centre for Agricultural Research in the Dry Areas (ICARDA). Forty-one participants from Europe and Africa attended the workshop. Presentations were made by the IAEA, BOKU, ILRI and ICARDA covering challenges, potentials and opportunities for community-based approaches in animal breeding in low-input systems, including conventional and genomic breeding tools.

Technical meeting with directors of Asian and African veterinary laboratories participating to the VETLAB Network project

A joint technical meeting of the VETLAB network, with directors of veterinary laboratories in Africa and Asia that are supported by the ARF and the PUI to Strengthen Animal Disease Diagnostic Capacities, was held at the IAEA Headquarters in Vienna, Austria, from 16-18 August 2016. This was the third technical meeting for the African and the second for the Asian laboratory directors. Nineteen participants from 18 countries (Bangladesh, Botswana, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Ethiopia (2), Kenya, Lao PDR, Mongolia, Mozambique, Myanmar, Namibia, Nepal, Senegal, United Republic of Tanzania and Zambia) participated and provided updates on progress, achievements and challenges during the past year.

The meeting was held in parallel with the first Research Coordination Meeting of CRP D32032 on 'Early Detection of Transboundary Animal Diseases to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network (VETLAB Network)' to facilitate interaction between laboratory directors and CRP participants and to obtain a critical assessment of the CRP work plan. The VETLAB partners highlighted the anticipated important contributions of CRP D32032 and specifically apprized the support of IAEA, ARF and PUI. They particularly stressed the importance of the project in bringing several veterinary laboratories from Asia and Africa together to share experiences and expertise and noted their support for the proposed objectives and work plan of the CRP.

Training courses

A training course on 'Transboundary Animal Diseases Diagnoses: Early Detection and Characterization' was held from 5-16 December 2016 at the APHL. The purpose was to promote the application of advanced rapid and differential diagnoses of multiple pathogens in the targeted African and Asian veterinary laboratories and to strengthen the capacity of participant countries to detect, conduct surveillance and perform epidemiological studies on the major viral and bacterial pathogens of a transboundary nature that are associated with respiratory problems in small ruminants. The training consisted of lectures on the principles, and practical sessions on the applications, of molecular and serological diagnostics, differential diagnostics and molecular epidemiology for PPRV, capripoxvirus and other ruminant pathogens. Nineteen VETLAB Network veterinary diagnostic laboratory scientists from 14 Sub-Saharan African and four Asian countries

(Botswana, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Ethiopia (2), Kenya, Lao PDR, Mali, Mongolia, Mozambique, Myanmar, Namibia, Nepal, Senegal, United Republic of Tanzania and Zambia) were trained by APHL and CIRAD (Montpellier, France) experts.

Figure 10: lecturers and participants to training courses organized by APHL and held at Seibersdorf laboratories in 2016.

Two regional training courses on 'Diagnosis of Lumpy Skin Disease' were held at the APHL on 15-19 August and 22-26 August 2016 as part of TCP RER9137. The purpose was to strengthen knowledge on lumpy skin disease and other capripox diseases and to disseminate updated molecular techniques for the detection and differentiation of capripox viruses. In the past two years, several European countries have experienced, for the first time, lumpy skin disease outbreaks. These include Bulgaria, Greece, FYR of Macedonia, Montenegro and Serbia. Many countries are not prepared for this exotic disease and has thus requested the technical support of the Joint FAO/IAEA Division. The two training courses were conducted as part of this support. They consisted of lectures on the diagnosis, epidemiology and control of lumpy skin and other capripox diseases as well as practical sessions on the applications of molecular diagnostics and differentiation of capripoxviruses. They also included practical classes in bio-informatics for analysis of genetic viral sequences, genetic clustering and data sharing in publicly available online genetic databases, such as the NCBI-Genbank. Thirty-seven participants from 23 European Member States attended the courses.

A regional training course on 'Genetics of Parasite Resistance in Sheep: Sampling, Data Collection, Management and Analyses' was held on 5–9 December, 2016, in Canelones, Uruguay. A total of 29 participants from 11 countries (Argentina, Bolivia, Brazil, Costa Rica, Cuba, Dominican Republic, Mexico, Paraguay, Peru, Uruguay and Venezuela) attended the course, which included lectures and practical training on animal identification using an ear tag system that simultaneously allows collection of samples for genetic analysis. Various phenotyping procedures related to parasite resistance characteristics in sheep, including estimation of faecal egg count, packed cell volume and FAMACHA scoring (a method to assess the anaemic level and parasite burden in animals) were covered. The GLIDMaS was also introduced and participants were trained on various modules for managing information on samples collected to assess parasite resistance in sheep. The course also covered practical procedures on extraction and quantification of DNA for sheep genotyping using various molecular markers. Course participants were each provided with an 'Animal Identification Toolkit' to enable sampling and phenotypic data recording in at least 500 sheep. This training will assist ongoing national efforts in Latin America to implement appropriate sheep breeding program as one strategy for the control of gastro-intestinal nematode parasites.

Fellowship and internship training

In 2016, the APHL hosted thirteen fellows in the following areas:

Name	Country	Status	Duration	Topic
AL RUBAYE, Hadeel Hussein, Abdulameer	Iraq	Fellow	1 month	DNA barcoding for rapid and accurate species identification
ATIM, Stella A.	Uganda	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
BHATTACHARJEE, Jayonta	Bangladesh	Fellow	3 months	Genomic analysis of cattle for improvement of milk productivity
EL RAHMAN ALAWAD, Mihad Fath	Sudan	Fellow	2 months	Flow cytometry and ELISA based technologies
GOREISH, Ibtisam Amin Sidahmed	Sudan	Fellow	2 weeks	Laboratory techniques for evaluation of the cellular immune response
MAHADEVAN, Anis Nadia Faisol	Malaysia	Fellow	3 months	DNA barcoding for rapid and accurate species identification
MAKALO, Mabusetsa J.	Lesotho	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques and DNA barcoding
NIYOKWISHIMIRA, Alfred M.	Burundi	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
O'BRIAN, Kabunda	Zambia	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
OUSSAMA, Dehhani	Morocco	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
REDA, Ederar	Morocco	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
SANOU, Moumouni	Burkina Faso	Fellow	2 months	Molecular genetic characterization of native cattle breeds using nuclear and extra-nuclear DNA markers
TRAORE, Amadou	Burkina Faso	Fellow	2 weeks	Analysis of animal genetic data

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EXTRA-BUDGETARY SUPPORT

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 by Japan.

THE FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

EXECUTIVE SUMMARY

The Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides assistance to Member States in implementing food control systems to ensure the safety and quality of the food supply, safeguarding consumer health and helping to facilitate international trade. Technical support is provided for food provenance and authenticity determination and for contaminant control systems. This support underpins food safety and traceability systems and combats economic loss through the illegal production and marketing of counterfeit and adulterated products. Activities include applied research, the development, validation, transfer and application of nuclear and related methods such as stable isotope measurements and metabolomics for food authentication, isotope dilution assays for chemical contaminant detection and control, and radiotracer techniques to study 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, training both in the FEPL and in Member States, and providing input for the development of international standards.

Research and development achievements in 2016 included the development and evaluation of a number of analytical methods to underpin food traceability systems, for food authentication, or to control residues and contaminants in food. The focus was on important commodities in international trade and targets for fraudulent practices such as counterfeiting or adulteration. Methodology was developed, in a preliminary study in collaboration with counterpart scientists in Sri Lanka, for the discrimination of high-value Sri-Lankan teas on the basis of their regional origin within the country and their cultivar, using untargeted metabolomics. Methodology was also developed for testing the authenticity of the high-value product, edible bird's nest, using metabolomics, mid-infrared spectrometry and stable isotope analysis. The methods were transferred to trainees from Malaysia for further validation and application. In collaboration with the Joint Division's Animal Production and Health laboratory, a DNA barcode technique was adapted and successfully applied to identify Nile tilapia and Nile perch, the two main commercial fish species from Lake Victoria. Research was initiated on the application of rapid, cost-effective, field-deployable screening methods for food authenticity. Studies included the mid-infrared spectroscopy method mentioned above for edible bird's nest and the application of a hand-held near-infrared spectrometer for testing the authenticity of extra-virgin olive oil. A comparative study was carried out on efficient methods for the optimisation and ruggedness testing of analytical methods, using a multi-residue pesticide method developed in the FEPL. The results will be posted on the website of the Red Analitica de Latinoamérica y el Caribe (RALACA) food safety laboratory network to assist Member State laboratories in method development and validation.

The FEPL continued to work as a research partner in the EU 7th Framework integrated project 'FoodIntegrity', and commenced work as a partner in the EU Horizon 2020 project 'Authent-Net'. The FEPL coordinated and provided technical input to two coordinated research projects (CRPs) on food traceability and authenticity, one of which concluded in 2016 with successful outputs and outcomes. The projects involved thirty countries. A new CRP focusing on the development of food authentication and quality testing using affordable, field-deployable methods was developed and approved. The FEPL also continued to support the CRP 'Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products', which commenced in 2015 and includes 15 laboratories in 14 countries.

The results of FEPL research were presented at six international conferences, and the FEPL was represented in the scientific committees for two major international conferences on food safety. The FEPL also gave presentations at 'European Development Days' (EDDs) - Europe's leading forum on

development and international cooperation - with approximately 200 visitors to the exhibition stand, and at Austria's 'Long night of research', which had more than 1000 visitors. Advice to Member States on research and capacity building policy included the FEPL's representation on the Food Authenticity Methodology Working Group (AMWG) of the UK's Department for Environment, Food and Rural Affairs (Defra); participation in the International Food Safety Authority Network (INFOSAN) meeting 'New science for food safety: supporting food chain transparency for improved health'; and provision of expertise on the implementation of technical requirements for food safety to the competent authorities in Bulgaria.

Capacity building activities in 2016 included the technical management of twenty national and five regional Technical Cooperation Projects (TCPs). Five training workshops or events were organised by FEPL and held in Member States or at Seibersdorf, and one webinar on analytical methodology was broadcasted. More than one hundred and eighty scientists, analytical chemists, laboratory personnel and food inspectors from more than fifty countries participated in these events. The FEPL hosted three interns, three fellows, two scientific visitors and one visiting scientist during 2016. The FEPL continued to provide technical backstopping and advice to the RALACA network of food safety laboratories in Latin America and the Caribbean, andwas represented in the Global Food Safety Partnership, providing input to the food safety technical working group and the laboratory capacity working group.

Publications by FEPL staff in 2016 included a special issue of the Elsevier journal 'Food Control', comprising 29 peer-reviewed research papers on 'Food safety and quality: applications of nuclear and related techniques', five papers in peer-reviewed scientific journals, twelve conference papers, abstracts or reports, and one book chapter.

STAFF

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Food traceability and authenticity

Food quality and safety are major concerns for both the food industry and consumers. Recurrent food authenticity and safety crises can endanger public health and provoke loss of public confidence in the integrity of the food supply. Globalization in the food trade has increased the need for effective food control systems to protect consumers from contaminated and fraudulently presented food. Food crime — intentional mislabelling or adulteration of food commodities on an organized and large scale for financial gain — has become a major criminal activity that can result in substantial economic losses and damage the reputation of entire commodity sectors, leading to barriers to international trade. Although food fraud is driven by financial gain, there is often a food safety risk, since the adulterant or counterfeit product will not have undergone the same controls as a genuine product and its constituents may be unknown or unfit for human consumption. Examples include the addition of melamine to milk powder to increase apparent protein content, which caused many thousands of cases of illness and several infant deaths due to the toxicity of melamine; the adulteration of high quality extra virgin olive oil with cheaper oils such as groundnut oil, which are undeclared on the label and may cause serious allergic reactions; the spraying of table olives with copper-sulphate solution to enhance their appearance; the use of denatured alcohol in counterfeit white-spirit drinks leading to toxic concentrations of methanol; and even counterfeit rice made from potato starch and industrial synthetic resin.

The need for analytical methods to underpin mechanisms for food authentication and traceability has grown rapidly, and is likely to increase in the future with the increasing complexity of food supply chains and advances in food processing and technology. Nuclear techniques provide essential tools for food authenticity testing.

The measurement of the ratios of naturally occurring stable isotopes of the bio-elements (hydrogen, carbon, nitrogen, oxygen and sulphur) in food can often provide information on their geographical origin or production technique through linkages to the ratios of the isotopes found in the environment or in the production process. Heavy element (e.g. strontium and lead) stable isotopes can also provide information related to the geology of the area of origin or industrial activity, and this ‘signature’ is transferred through soils to plants and animals. Because stable isotopes are intrinsic characteristics of the atoms in the food, their distribution and ratios are difficult to manipulate for fraudulent ends.

Elemental profiling of food provides important information on its safety with regard to the concentration of potentially toxic elements and can provide information that links food to its place of production through soil chemistry. The multi-element composition of animal tissues reflects, to some extent, that of the vegetation that they eat. For example, alkaline metals, especially rubidium and caesium, being easily mobilized in the soil and easily transported into plants, are good indicators of geographical identity.

Metabolomic fingerprinting, the analysis of metabolites that are the result of cellular or molecular processes in an organism, is also used for authenticity testing. Metabolomics can be either targeted, focusing on groups of related metabolites to provide direct functional information for modelling, or untargeted, detecting patterns in the metabolome that can differentiate between sample sets and can be used to build models for classification of unknown samples. An example is spin-generated fingerprint profiling using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy to screen fruit juices and wines.

These techniques, in combination with others such as vibrational spectroscopy, DNA analysis and microbial fingerprinting, are proving invaluable for authenticating foodstuffs.

Although various promising food testing methods have been developed using a variety of analytical techniques, the implementation of effective controls has been hampered to date by factors such as the lack of harmonization of approaches; absence of accessible and sufficiently comprehensive databases of authentic product characteristics and data; absence of suitable food-matrix reference materials; lack of awareness in industry and regulatory bodies of the possibilities for food testing; and the cost of putting the methods in place.

However, recent developments in analytical instrumentation are making the required analytical techniques more accessible. Various categories of instruments that were previously used only in the laboratory are becoming available in more affordable bench-top, portable or hand-held versions, which may be able to provide a screening capability. These include bench-top NMR instruments, portable and hand-held X-ray fluorescence (XRF) and near-infrared spectrometers; some infrared spectrometers are currently available as palm-held instruments costing only a few hundred dollars (for example, the 'SCIO'), connectable to spectral libraries via smartphones or tablets, with the next generation of such instruments integrated into smartphones. Other bench-top or portable techniques that have potential applications for authenticity testing include ion-mobility spectrometry and isotope ratio measurement by laser ablation molecular isotope spectrometry (LAMIS). Research in the FEPL is increasingly focused on these accessible techniques as first-tier or screening options, with more detailed information available from the high-end techniques when required.

The successful application of all such techniques requires research on their application for different food commodities, the development of extensive databases of measurement results from authentic foodstuffs, and robust statistical analysis and modelling.

A non-targeted approach for the discrimination of teas

Tea (*Camellia sinensis* L.) is one of the most popularly consumed beverages worldwide. It has been used as a natural medicine for thousands of years, containing many compounds purportedly beneficial to health. The two most popular types are green (favoured in Asia) and black tea (favoured in western countries). The different growing seasons, geographical regions, processing, and fermentation methods create many varieties of tea, some of which have premium value compared to the others. The expansion of the consumer market, which has increased demand for 'manufactured' food as well as transported 'pure' food such as tea, has motivated adulteration simply because of the opportunity for increased profit. The adulteration of tea has become a common problem. Mixing exhausted tea-leaves with leaves of some other plants (e.g. elder, hawthorn, sloe); addition of the dust from the tea leaves and sand; chemical enhancement of green tea (with Prussian blue and sulphate of lime or gypsum); and simply re-dried and resold tea-leaves, are some of the main examples of tea adulteration. To help address these issues, the FEPL applied an untargeted metabolomics approach that it had previously developed for some other commodities (e.g. honey, fruit juices) to investigate the possibility of distinguishing teas from different origins, and detecting products that had been adulterated.

Tea samples were obtained from the market (black tea from China, India, Nepal and Sri Lanka and green tea from Japan and Kenya, oolong from Taiwan, and rooibos from South Africa), infused in water and analysed by ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC-QToF MS).

Using an untargeted metabolite profiling approach and multivariate statistical data analysis, reliable discrimination was obtained between various tea types (black, green, oolong and rooibos), as well as between black and green teas produced in different countries (Fig. 1A). Some of the metabolites that contribute to discrimination of the sample groups were tentatively identified using a loadings plot (Fig. 1B) and database search.

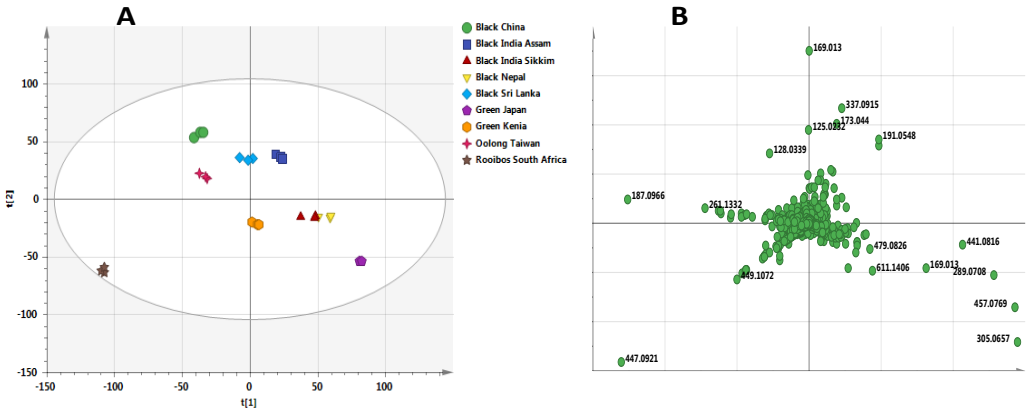


FIG. 1: Principal component analysis performed on tea samples: (A) PCA-X plot of various tea samples; (B) loadings plot

The ability of this methodology to differentiate between different tea varieties and types suggests possible applications of untargeted metabolomics for authentication testing of tea samples. The methodology was, therefore, further developed in FEPL to investigate, in collaboration with CRP and TCP counterparts in Sri Lanka, the possibility of distinguishing Sri Lankan teas from different geographical origins, as well as differentiating between treated and untreated black tea samples. Tea is Sri Lanka's second most important export product, with a value of US $1.5 billion. About 30% of production is in large plantations, with 70% being accounted for by approximately 400 000 smallholders. The selection of specific cultivars, the humidity, cool temperatures, and the rainfall patterns of the country's central highlands provide a climate that favours the production of high-quality tea, which is well known as 'Ceylon tea'. Ceylon tea reportedly contains many compounds beneficial to health. Because of its widespread consumption, the quality control and safety of Ceylon tea are extremely important. Its popularity and value make Ceylon tea a common target for fraud.

Authentic tea samples were obtained directly from four production sites in Sri Lanka; green (Talawakelle, Hanatana, Ratnapura, and Passara) and black (Talawakelle), and analysed by UPLC-QToF MS with multivariate data analysis.

The qualitative models generated using unsupervised principal component analysis (PCA) allowed differentiation between samples of the same cultivar grown in four different regions in Sri Lanka. Examples are given in Fig. 2A/B for cultivars TRI 2025 and 4052.

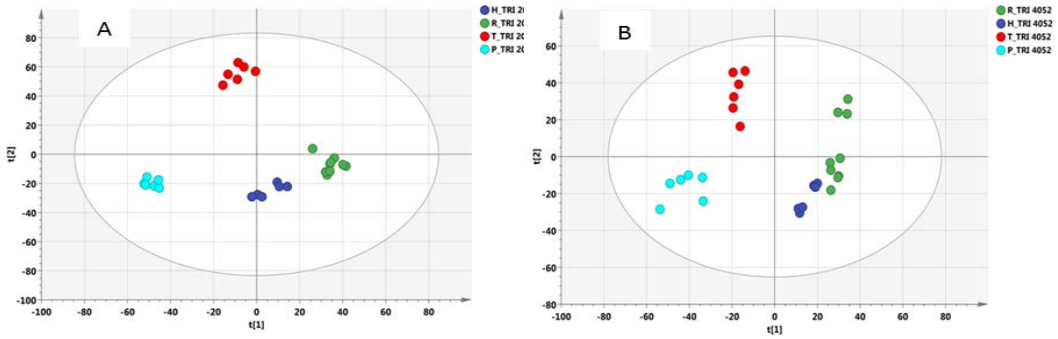


FIG. 2: Principal component analysis performed on two tea cultivars grown in Talawakelle, Hanatana, Ratnapura, Passara: (A) TRI 2025; (B) TRI 4052

Reliable differentiation was also obtained between various cultivars grown in the same region. As an example, Fig. 3A/B shows the PCA models generated for five cultivars grown in the Hanatana and Ratnapura regions.

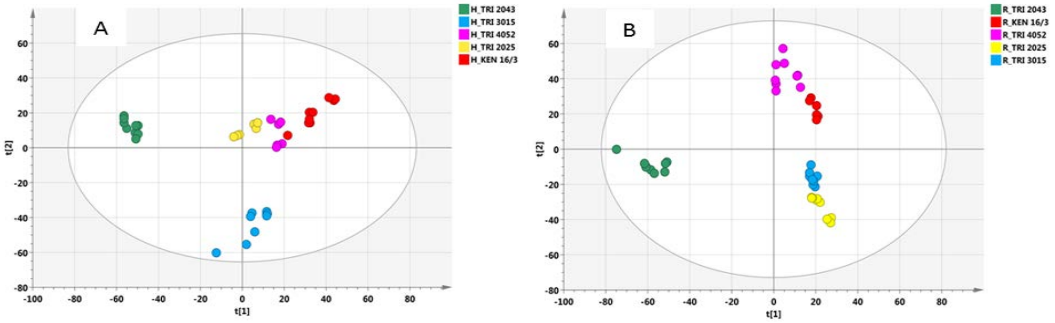


FIG. 3: Principal component analysis performed on various tea cultivars (TRI 2015, 2043, 4052, 3015 and EN 16/3) grown in: (A) Hanatana; (B) Ratnapura

Black tea accounts for about 95% of local consumption in Sri Lanka. Black tea is a fully fermented tea that can be chemically treated to enhance the flavour. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to validate the observed differentiation between treated and untreated black teas (Fig. 4A). An S plot was generated and used to help identify the metabolites contributing significantly to the differentiation (Fig. 4B).

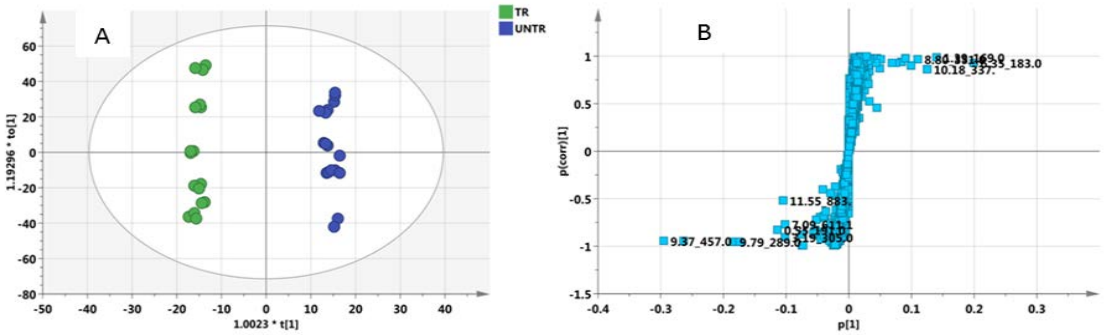


FIG. 4: (A) OPLS-DA scores plot of treated and untreated black teas; (B) S-plot for marker identification

The next stage of this work will be to attempt to identify specific chemical markers that would enable the differentiation of tea varieties and their points of origin using a cheaper, more convenient targeted analytical method. As reported previously, both untargeted and targeted metabolomics are included in the suite of methods, with other techniques such as stable isotope analysis, spectroscopic and trace element profiling, that are being developed to support authenticity testing and food traceability systems.

Authentication of edible birds nest

Edible bird's nest (EBN) is a widely used health food in South East Asia. There is an increasing market for EBN because of its alleged beneficial health effects, such as anti-ageing, growth promotion and immune system-enhancement properties. The economic motivation for adulteration is significant as EBNs rank amongst the world's most expensive animal products for food and medicinal uses. Trade in EBN increased dramatically from approximately US $170 million in 1989 to US $380 million in 2004 and it is expected to grow further with rising demands in East Asian countries. For example, in Thailand the price of white EBN reached 65 000 Baht (US $2170) per kilogram and had a total export value of approximately 126 million Baht (US $4.2 million) per annum in 2007. It has been reported that adulterants such as karaya gum, agar, white jelly fungus, egg-white and isinglass have been added to EBN, and there is a possibility that there are many other adulterants that have not yet been detected. There is a need, therefore, for development of analytical methods for EBN authentication. In FEPL, method development was combined with training of TC Fellows from Malaysia, which has a strong interest in EBN quality and authenticity.

Authentic processed EBN samples of different grades from the swiftlet species *Aerodramus fuciphagus* were obtained directly from processing houses from four different regions (Kelantan, Perak, Sarawak, and Selangor) in Malaysia. Two untargeted approaches were investigated as discriminatory techniques; untargeted metabolomics by high resolution mass spectrometry, and screening using mid-infrared spectroscopy. Both techniques require data processing by means of various chemometric tools.

The objective of the untargeted study using high resolution mass spectrometry was to investigate the potential of differentiating between samples of authentic EBN from different regions within Malaysia. Untargeted metabolite profiling was performed using UPLC-QToF MS with multivariate data analysis. Clear separation between EBN samples from different regions was achieved using OPLS-DA (Fig. 5A). In order to obtain relevant information regarding the metabolic differences between EBN samples, a set of statistically meaningful markers was selected from a loadings plot (Fig. 5B). The method shows promise as a tool that could be harnessed by regulatory bodies to combat fraud involving EBN. With comprehensive characterisation of a wide range of authentic EBN samples and the development of prediction models using the data produced, the metabolomics approach could provide a tool to help to identify the origins of EBN samples and verify their authenticity on a routine basis.

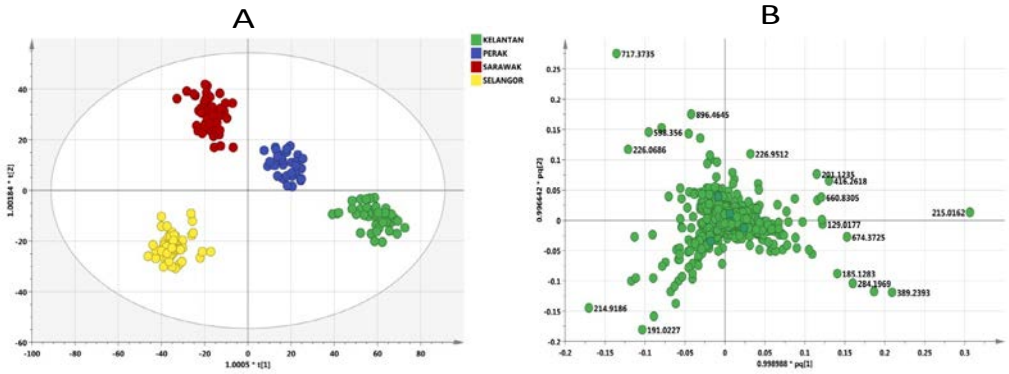


FIG. 5: (A) OPLS-DA scores plot constructed for the classification of EBN according to production region; (B) loading plot

Another approach, which may be applied either as an alternative or a complementary method depending on the information required, is to conduct untargeted quick, relatively cheap, and often non-destructive spectroscopic measurements with subsequent data processing by chemometrics. An example of such a rapid screening method is the use of mid-infrared (MIR) spectrometry combined with a micro-diamond attenuated total reflectance (ATR) measurement adaptor that permits the EBN sample to be measured without preparation of a potassium bromide disk. The MIR measurements are rapid, simple and also need minimal sampling preparation. IR spectroscopy measures the covalent chemical bonds, creating a molecular 'fingerprint' of the chemicals present. This fingerprint can be used to identify and quantify chemicals present in a sample. The IR spectrum region 4000 – 450 cm^{-1} in particular, is able to identify a large number of components and the absorption bands are sensitive to the physical and chemical states of individual constituents.

The samples were dried by lyophilisation for 24 hours, then ground to fine powder form and stored in airtight containers prior to analysis. Adulterant mixtures were prepared gravimetrically at concentrations of 1, 5 and 10% m/m by combining appropriate quantities of adulterants with an authentic EBN sample. Approximately 20 mg of the powdered EBN samples were placed on the ATR crystal and the powder compressed until a transmission of 70% was obtained. Spectra were gathered in transmittance mode between 4000 and 450 cm^{-1} at a resolution of 1 cm^{-1}.

The infrared transmission spectra (%) obtained from authentic Malaysian EBN and for the common structural adulterants karaya gum; agar; and porcine, bovine and fish gelatin, all at 10% m/m in EBN are shown in Fig. 6.

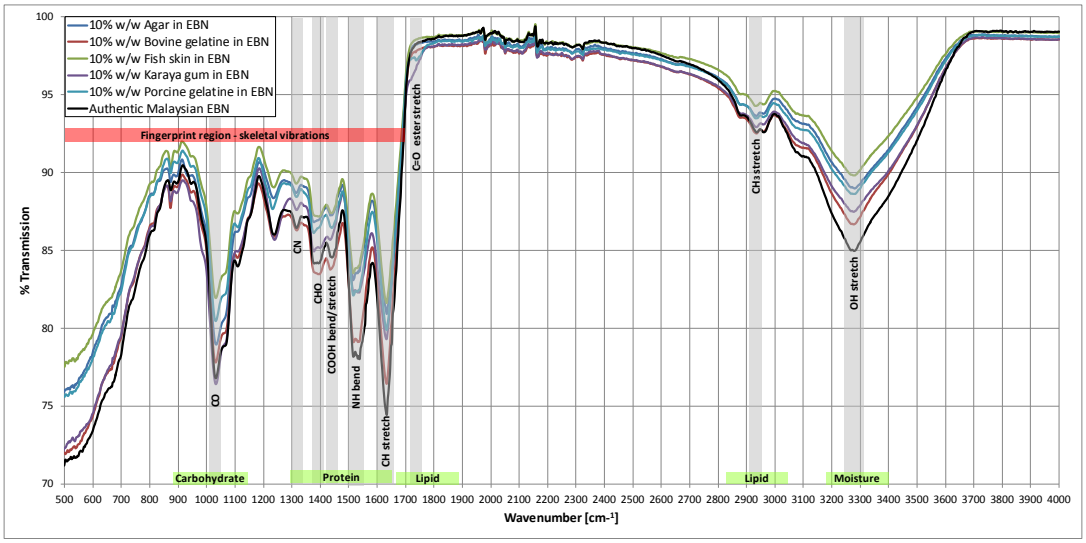


FIG. 6: The infrared % transmission spectra (4000 to 500 cm^{-1}) obtained from an authentic Malaysian EBN using mid-infrared spectroscopy with attenuated total reflectance micro-diamond sampling adapter. The figure also shows the spectra obtained for common structural adulterants at 10% (w/w) in EBN and the absorption bands for a range of functional groups

The data were analysed using data driven soft independent modelling of class analogy (DD-SIMCA), with 91 authentic EBN samples (the target class) used as the 'training samples' to develop a one-class target classification model. The objective was to build a model using authentic EBN samples from a variety of regions in Malaysia, as representative of Malaysian EBN, against which adulterated or counterfeit samples could be tested. The quality of the authentic Malaysian EBN acceptance area was estimated by testing with data from 12 other authentic EBN samples that were not included in the 91 training or target set samples. The cross-validation sample results are plotted in the acceptance area generated from the 91 EBN samples of the training set in Fig. 7A. Nine of the 12 test samples fell within the authentic EBN acceptance area (α = 0.05). Based on this cross-validation test set, the sensitivity was 75%, i.e. the type 1 error rate (of wrong rejections) of the untargeted screening method was 25%. With α = 0.01 for the acceptance area the sensitivity was 100%, i.e. the type 1 error rate was 0% with no wrong rejections of the 12 authentic EBN test set (Fig. 7B).

The model was tested with 18 EBN negative control samples deliberately adulterated in the laboratory, against the target set of 91 authentic EBN samples at the 95% confidence interval.

The overall specificity was 50% with the majority of the EBNs

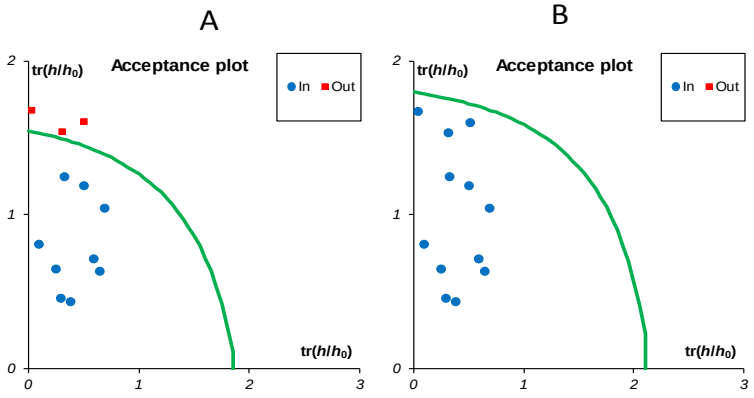


FIG. 7: (A) Application of DD-SIMCA to the analysis of 12 randomly selected 'test samples'. The quality of the authentic EBN acceptance area was estimated by testing with data from 12 other authentic EBN samples that were not included in the 91 training set (or target class) samples. With α = 0.05 the sensitivity was 75%, i.e. the type 1 error rate (of wrong rejections) of the untargeted screening method was 25%. (B) With α = 0.01 the sensitivity was 100%, i.e. the type 1 error rate was 0% with no wrong rejections of the authentic EBN test set

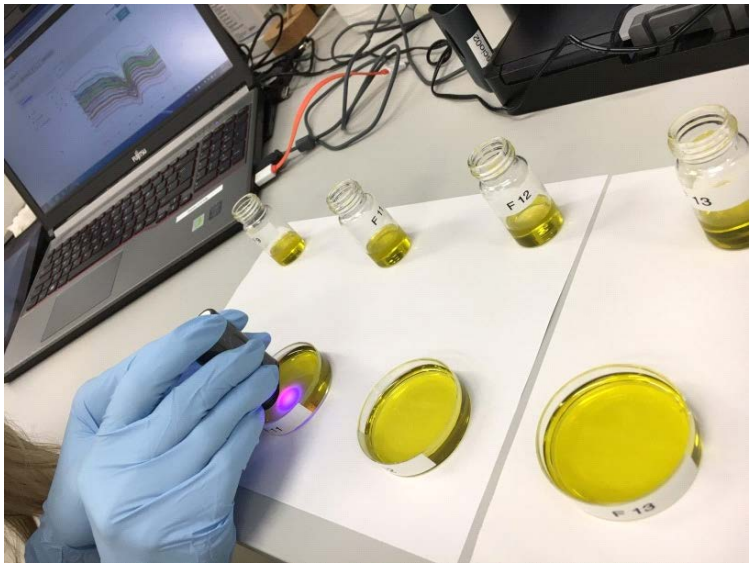
adulterated at the 5 and 10% w/w level identified as 'aliens' and falling outside the 95% confidence boundary shown as a green boundary line in Fig. 7A. This included the common adulterant karaya gum and three other adulterants: agar, porcine gelatin and 'apparent protein' enhancer melamine. It is expected that the specificity will further increase with the analysis of more samples. DD-SIMCA analysis of the six pure adulterant materials gave a specificity of 100%.

Due to the limited number of samples available and incomplete information available for some of the samples, these results can be considered only as preliminary. Nevertheless, the application of IR-ATR spectroscopy, combined with DD-SIMCA data processing, for the authentication of EBN produced in Malaysia has been successfully demonstrated. This technique is accessible, non-destructive, fast and direct, requiring no significant sample preparation. The method combines good sensitivity with acceptable specificity. Development of reliable non-targeted screening methods based on DD-SIMCA and one target class classification are extremely important for identifying and preventing evolving fraudulent trade of adulterated EBN and also to reduce the possibility of unintended side-effects and health risks posed by addition of ingredients unfit for human consumption. With the rapidly growing demand for EBN for both culinary use and traditional medicine the requirement for screening methods is clear. Further work is required to validate the approach for a wider range of adulterants, including flavour enhancers and preservatives.

Detection of Extra Virgin Olive Oil (EVOO) adulteration by SCIO

As an example of the application of relatively cheap and accessible instrumentation for food authenticity testing, a study was initiated to investigate the use of the SCIO hand-held infra-red detector for screening olive oil. Extra virgin olive oil (EVOO) is an increasingly popular food worldwide and is of higher value than other oils. Adulteration of EVOO with lower quality olive oils, or other lower cost edible oils, has been reported. The most common adulterants include hazelnut oil, sunflower oil, soybean oil, corn oil, rapeseed oil, and olive pomace oil.

Using the SCIO, a classification model was built for three types of edible oils (EVOO, rapeseed, and sunflower oil). Fig. 8A shows spectral differences between the evaluated oil types. These differences were significant enough to be able to develop a classification method for the three different oil types with a 100% correct classification rate (Fig. 8B).



The hand-held 'SCIO' infra-red detector being used to develop a screening test for authentic extra-virgin olive oil

Admixtures of EVOO and rapeseed were prepared (up to 50 % adulteration level) and tested. Using the classification models developed, the admixtures of EVOO/rapeseed or sunflower oil at ratios 80/20 were classified as unknown, while the admixtures at a ratio of 90/10 was classified as EVOO. This demonstrates, as proof of principle, that this instrument is capable of detecting adulteration of EVOO with rapeseed or sunflower oil at 20% and higher using the model developed, and with further work and development of spectral libraries and models, could be useful as a rapid, portable tool for protecting the food supply chain from counterfeit and adulterated products.

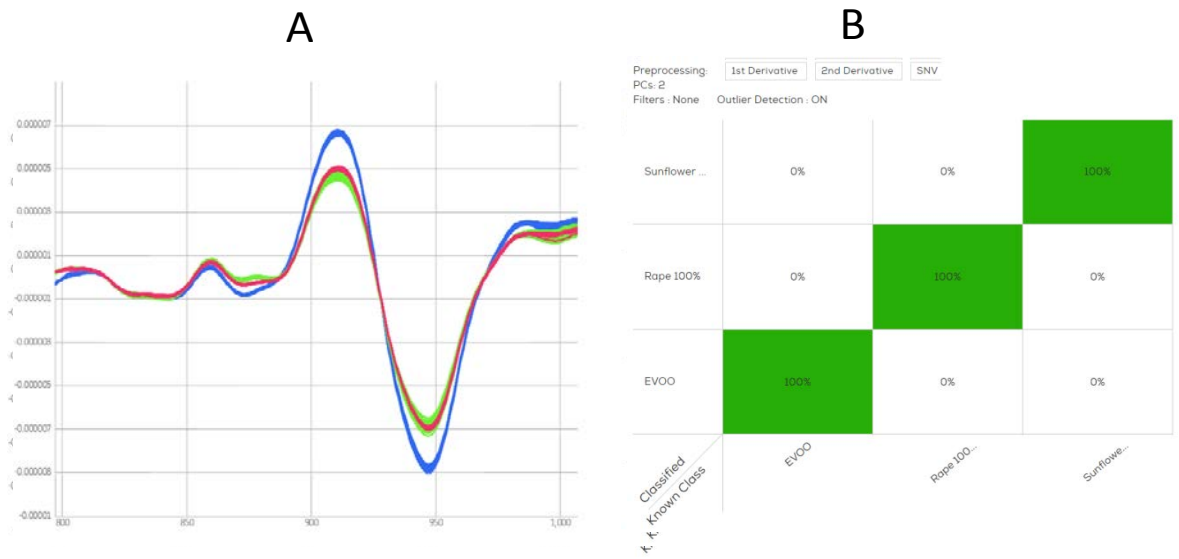


FIG. 8: (A) Spectral differences between EVOO (blue), rapeseed (red), and sunflower (green) oil; (B) classification model

DNA barcode for genetic traceability of Nile perch and Nile tilapia

Another important technique applied in food authenticity testing is DNA barcoding. The application of this technique was investigated for the identification of two important fish species from Lake Victoria, which is Africa's single most important source of inland fishery production. It makes a significant contribution to employment by providing jobs to over 3 million people and attracts investments of around 9% of total exports. In addition, the fish caught in Lake Victoria are a rich source of animal protein for consumption by the local population, providing around 60% of their total animal protein intake.

The two main commercial species of fish from Lake Victoria are Nile perch (*Lates niloticus*), which was introduced to Lake Victoria in the 1950s and 1960s and is exported mainly to Europe, the US and the Middle East, and Nile tilapia (*Oreochromis niloticus*), which was also introduced to the lake in the 1950s and 1960s, contributing to food security as well as income and employment. These species are therefore important commodities to the region, and are a study focus of a collaborating research group in Uganda involved in a CRP coordinated by FEPL.

As well as their food value, the fish species have been widely studied from an evolutionary point of view. The identification of species, or the confirmation of their claimed identity, is a fundamental requirement to ensure high quality standards not only for food export, but also for food traceability, safety and security.

In a collaborative study with the FAO/IAEA Animal Production and Health Laboratory, the applicability of DNA barcoding was investigated for the identification and traceability of sea- and fresh water food, with the genetic identification of species of Nile tilapia and Nile perch as a model. The DNA barcode is based on the extraction of DNA from samples and the amplification of a short mitochondrial DNA fragment, COI (cytochrome c oxidase), which is conserved at the species level. The relevant segment of the COI mitochondrial gene is amplified by single locus polymerase chain reaction (PCR) technique and then sequenced by bioinformatics software, in order to screen the unknown result against a reference sequence available in a public database, such as the 'Barcode of Life Database' (BOLD, www.boldsystem.org). The database permits a species assignment to be made against one of the species in the reference library.

For this study, mitochondrial DNA was extracted from 55 fish samples (26 Nile perch and 29 Nile tilapia samples) collected from three different Ugandan regions of Lake Victoria. In order to optimize

the PCR method, DNA was also extracted from two other different fish samples, one from Italy and one from a Viennese market. The COI gene was amplified using universal primers and the amplicons were analysed using gel electrophoresis to establish that the primers worked well in these samples. The DNA positive fragments for COI were then sequenced and the DNA results obtained were aligned using bioinformatics software. The consensus sequence created was compared with DNA barcode sequences already present in the BOLD database that identifies the fish DNA sequences and provides information about the species from which the sequences came. The results showed that all the sequences of Nile tilapia were matched to *Oreochromis niloticus* and all the sequences of Nile perch were matched to *Lates niloticus* except one sample that was mislabelled as perch but identified as a tilapia by the BOLD System.

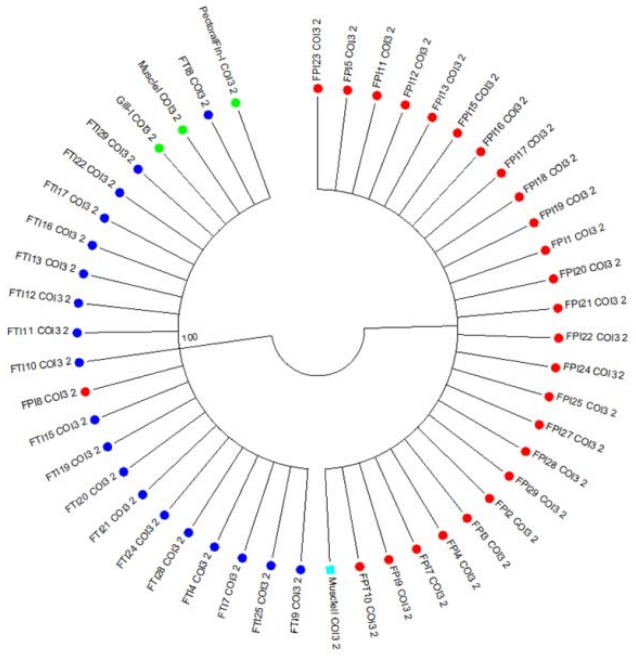


FIG. 9: Phylogenetic tree shows the relation among the two different species of fish

Starting from the information obtained through BOLD, a phylogenetic tree was created to show the evolutionary relationship among the tilapia species (in blue) and the perch species (in red) connected through a central branch (Fig. 9). In the Nile tilapia species, the mislabelled sample of Nile perch can be clearly seen (in red).

The research concluded that DNA barcoding can be used to reliably authenticate Nile perch and talapia fish species from Lake Victoria. This method can also be considered as a molecular tool for the implementation of genetic traceability based on taxonomic identification of other species.

This study exemplifies a key advantage of the FAO/IAEA Agriculture & Biotechnology Laboratories; the ability to collaborate between laboratories to harness the power of multi-disciplinary tools and approaches to solve Member State problems.

Control of Residues and Contaminants in Food

The control of unwanted chemicals in food, such as residues of pesticides or veterinary drugs used in food production, or natural contaminants such as mycotoxins, remains an area of high importance to Member States. Activities performed in FEPL to underpin capacity building in this area include applied research on analytical methodology to enable Member States to perform targeted risk assessment, the development or adaptation and validation of analytical methods for the detection, quantification and control of residues and contaminants, and the development of approaches and protocols to assist in the successful implementation of the methodology in Member State laboratories.

Optimisation and ruggedness testing of an analytical method for pesticide residues in potato

As part of an initiative under the 'Red Analitica de Latino America y el Caribe' (RALACA) network the FEPL validated a multi-residue method for pesticides in potato. One of the parameters to be assessed was the intra-laboratory robustness or ruggedness. The objective of this work was to

implement a worked example for RALACA laboratories to test for the ruggedness of an analytical method.

There is currently no harmonisation in the definitions of the terms robustness and ruggedness. A review of current international guidelines shows that the terms are either used as synonyms or as complementary terms. According to the 'Proposed draft guidance on performance criteria for methods of analysis for the determination of pesticides residues' by Codex Alimentarius (2015), the ruggedness of an analytical method is the resistance to change in the results produced by an analytical method when minor deviations are made from the experimental conditions described in the procedure. The best demonstration of the ruggedness of a method is monitoring its performance on an ongoing basis as part of the analytical quality control applied in the laboratory, to ensure that its performance remains within the parameters established during method validation, with the natural variations that can occur day-to-day, such as different analysts, different batches of reagents, slight variations in working practices, temperature etc. However, an initial demonstration of the ruggedness is often performed as one aspect of the method validation, to give confidence that the method should perform under normal variations in conditions encountered in its routine application. This initial ruggedness testing is typically performed using either multiple replicate analyses, or a fractional factorial design such as that described by Youden and Steiner in the AOAC Statistical Manual, which minimises the number of analyses, time and effort required to detect influences on the measurement results.

In this study the goal was to test the ruggedness of the analytical method for pesticides in potato by assessing the degree of intra-laboratory reproducibility of the method under small, deliberately introduced variations in the conditions of the test. Fig. 10 summarizes the analytical method, with the factors (marked 'X') that were chosen to be varied for the test.

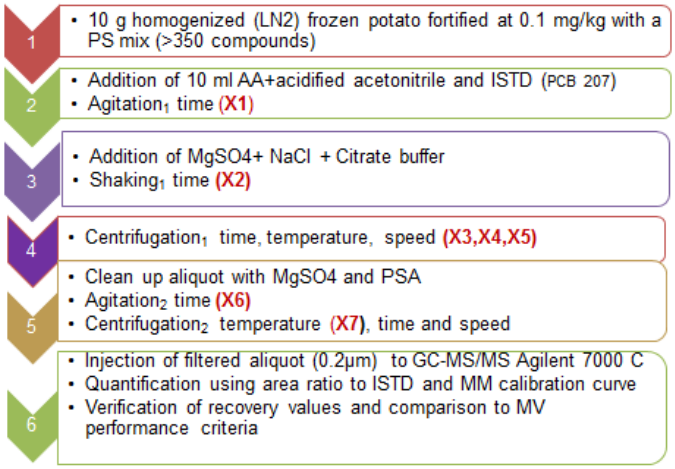


FIG. 10: The method used and the X factors

Among the possible statistical experimental designs that are available, the Plackett-Burman design (PBD), which is based on the Youden-Steiner approach, and the definitive screening design (DSD) were chosen for the study because they are relatively cheap to implement and give substantial information to the analytical chemist on the sources of variability of an analytical procedure. In general the PBD can identify main effects and some two-factor interactions, and was used to study seven factors using eight experimental runs. The DSD can estimate main effects, some two-factor interactions, and also some quadratic effects, and was used to study the same seven factors using 34 experimental runs. Knowledge of the type of effect caused by a variation in conditions is very important in controlling the analytical procedure. Linear effects are easier to take into account or compensate for in the method. Quadratic effects are problematic as one cannot know in which direction the change caused by the factor will be and therefore it will be difficult to account for.

Both designs are best applied early in the development of a method in order to identify and optimise conditions for critical points that must be controlled to ensure the desired method performance. For this purpose, the DSD provides more information on the critical steps in the method, though it does require more time and effort. Optimisation of the method in this way, and identification of the

critical points that must be highlighted for special attention by the analyst in the standard operating procedure or method protocol, enhances the ruggedness of the method before the validation experiments are performed. The method's performance characteristics established during validation can, therefore, be confidently considered as benchmark parameters and can be realistically applied during ongoing quality control of the method. However, the statistical designs may also be applied to methods already developed, or being adapted, to confirm their ruggedness, as was done in this study. The analysis of the results using both designs showed that the method being evaluated was rugged.

A worked example, based on the results of this work, to assist RALACA laboratories in testing for the ruggedness of an analytical method will be posted on the RALACA website (www.red-ralaca.net). This study was carried out with collaborators from LVA (Austria), University of Antwerp (Belgium), University of Leuwen (The Netherlands), Universidad de la Republica (Uruguay) and Agilent Technologies.

Coordinated research

In 2016, the FEPL coordinated and provided technical input to two coordinated research projects (CRPs) in the fields of food authenticity and traceability. In addition, a new CRP focusing on field-deployable analytical methods for food authenticity testing was formulated for commencement in 2017. The FEPL also provided technical backstopping for a CRP focusing on the control of chemical contaminants in foods produced through aquaculture.

The CRP 'Implementation of Nuclear Techniques to Improve Food Traceability' commenced in 2011 and was completed with the final research coordination meeting in November 2016. The project had 16 participating laboratories in 15 countries.

The aim of this CRP was to develop systems based on nuclear technologies that contribute to food safety and traceability by verifying the origin and authenticity of foods and natural commodities. The CRP achieved the first stage of this by demonstrating the applicability of the techniques to a wide range of foodstuffs. The protocols and databases developed are foundational to the future development of food control systems and reducing barriers to international trade.

The CRP successfully demonstrated the feasibility of using stable isotope analysis (SIA) to establish the geographical origins of several important food products produced in developing Member States. The CRP was also an important vehicle for raising awareness of stable isotope and related nuclear techniques such as neutron activation analysis for characterising the elemental profiles of foods to determine their provenance and authenticity. The project has generated a significant number of food authenticity and traceability datasets for the first time, has enhanced Member State capabilities in stable isotope and trace element (SITE) analysis and generated several new methods, SOPs, and training opportunities. Furthermore, the project has facilitated investment by several of the Member States in these capabilities, and helped secure new funding for projects and equipment and facilitate new scientific collaborations and involvement in national and international food authenticity projects and networks. All of these achievements have raised awareness and allowed project members to interact with food industry stakeholders and regulators within their respective Member States helping to bring nuclear techniques into implementation and improving food traceability systems.

The second CRP in this field of work, 'Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety' has 15 participating laboratories in 15 countries. Research is proceeding as planned. The 2nd research coordination meeting was held in Rabat, Morocco, in October 2016. The CRP has initiated useful collaborations between laboratories in different Member States and it provides information with potential regulatory impact, e.g. the detection of milk whose chemical parameters do not comply with that from the claimed origin may be an indication of fraud.

The focus of the next phase of the project is to ensure sufficient sampling, consistency of methods and data quality between participants so that the ultimate goal of generating a sustainable database and maps of the spatial variability in dairy isotopic parameters can be achieved. It is hoped that the IAEA will be able to host and maintain the milk powder database from this CRP to ensure its sustainability and legacy in a similar way to the 'Water Isotope System for Data Analysis, Visualization, and Electronic Retrieval (WISER)' maintained by the IAEA Water Resources Programme.

In 2016, a new CRP project was developed through internal discussions and a consultants' meeting, and approved for commencement of funding in 2017. The project will be coordinated jointly by FEPL and the IAEA Nuclear Science and Instrumentation Laboratory, and will identify and select appropriate analytical techniques and develop protocols to assess the authenticity, safety and quality of food in a field-deployable context. Milk powder and vegetable oils will be used as exemplar commodities to establish methods and guidance for 'front-line' screening to detect economically motivated adulteration of food.

The FEPL also participates in the CRP 'Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products', which commenced in 2015. This project includes 15 laboratories in 14 countries, and the FEPL.

Through coordinated research, this project aims at strengthening Member State analytical laboratories and national chemical residue monitoring programmes, thus contributing to the improvement of food safety, better aquaculture production and management practices as well as enhancement of trade in aquaculture products. New analytical methods will be developed, including improved environmentally friendly sample preparation techniques, validated and transferred amongst Member States laboratories. The FEPL is involved in developing methods and validation protocols for transfer and in-house validation in partner laboratories, and the provision of advice and guidance on method development and validation.

EU Projects

The FEPL is a research partner in two EU-funded projects; the multi-national Integrated Project, 'FoodIntegrity', funded under the EU 7th Framework mechanism, and the Horizon 2020 project 'Authent-Net'.

FoodIntegrity

The integrity of European foods is under constant threat from fraudulently labelled imitations that try to exploit their added value. The European Framework 7 Integrated Project 'FoodIntegrity' started in 2014. Its goal is to provide assurance to consumers and other stakeholders about the safety, authenticity and quality of European food (its integrity), which is of prime importance in adding value to the European agri-food economy. The FEPL is an active participant in the FoodIntegrity project. In July 2016 the project organised a workshop entitled 'Geographic origin and authenticity of food products: from tools to legislation' at the University of Lisbon in Portugal. The aim of this workshop was to present recent developments in analytical tools, as well as applications and quality control systems that can be applied to the authentication of a range of food products. Additionally, the workshop brought together not only researchers and academics, but also stakeholders, distributers and producer associations. In accordance with the framework goals of FoodIntegrity, the opportunity to have all the relevant actors together, including legislators, involved in food authenticity issues, was a mark of success. Mr Simon Kelly presented the opening lecture on 'The application of nuclear techniques in food authentication and traceability' covering an overview of the work of the Joint FAO/IAEA Division in promoting the use of nuclear and related techniques to detect food adulteration in developing countries and some relevant examples in the fight against food fraud and verifying labelling claims such as organic, halal and country of origin.

A Food Integrity Network has been established under the project, which has the primary objective of sharing intelligence on incidence of food fraud and forming stakeholder groups with shared interests in detection, methodology, open innovation and knowledge transfer in relation to food authentication. A set of position papers are currently being prepared for publication in refereed journals covering 'The use of stable isotope data to determine food authenticity in court cases'; 'What are the scientific challenges/advantages of moving from targeted to non-targeted analytical screening methods?'; 'Multivariate statistics: considerations and confidences in food authenticity'; 'Best practice when constructing reference databases for food authentication studies'; 'The use of proton-nuclear magnetic resonance spectrometry to tackle food fraud issues' and 'The future of next generation sequencing in food authenticity testing. Other activities include the formation of expert committees on the authentication of different food types and the preparation of e-learning materials and educational videos.

Authent-Net

'Authent-Net' is a two-year EU Horizon 2020 research and innovation project in which the FEPL is a partner.

It is acknowledged that historically anti-food fraud capability within Europe has not been consolidated and lacks the coordination and support structures available to those working in food safety. There are various initiatives underway to redress this imbalance, e.g. DG Santé's Food Fraud network, DG Research's FoodIntegrity project, as well as numerous national programmes and industry initiatives.

One pivotal area that still needs to be addressed is bringing together national research funding bodies to facilitate the development of transnational research programmes. 'Authent-Net' will address this need by mobilising and coordinating relevant research budget holders in order to facilitate the eventual development of a transnational European funding vehicle that will allow EU Member States to jointly fund anti-fraud research. 'Authent-Net' comprises a core group of 19 participants from 10 member states, 1 NGO and the USA, who are either national research funding bodies; experts in food authenticity and/or experts in transnational funding mechanisms. The FEPL provides expertise in food authenticity and a link to researchers and funding bodies both within and outside Europe.

'Authent-Net' will bring together relevant EU Member States' R&D budget holders to coordinate inter-disciplinary research effort and build a cohesive and sustainable network, and develop a high level research and innovation strategy for transnational research on food authenticity. A coherent research strategy will enable integration of FAO/IAEA research projects with European projects to provide synergism and leveraging of European research to the benefit of IAEA and FAO member countries worldwide.

Dissemination of Research Results

The methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training workshops, publications in the scientific literature and via the internet, public outreach events and conferences and symposia. The 'Food Contaminant and Residue Information System' (FCRIS, nucleus.iaea.org/fcris/) provides a wealth of useful data on food contaminants and residues and 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.

European Development Days, Belgium, Brussels, 15-16 June 2016

The FEPL was represented in an IAEA delegation composed of representatives of the Director General's Office for Coordination, the Department of Nuclear Sciences and Applications (NA) and the Department of Technical Cooperation (TC), which attended the tenth edition of the European Development Days (EDDs) - Europe's leading forum on development and international cooperation. The EDDs, organized yearly by the European Commission, brought together a global audience of development experts and practitioners, political leaders, civil society and the private sector as well as major multilateral and bilateral development agencies. This was the first time the IAEA took part in the event. Mr Aiman Abrahim, from the FEPL, represented NA at the IAEA exhibition stand.

Visitors to the IAEA exhibition at the 10th edition of the European Development Days in Brussels

The IAEA showcased three projects, one of which focussed on supporting food safety controls in Chile, conducted through TC and the FEPL. At the stand, around 200 visitors were able to examine various aspects of the work of the FAO/IAEA Agriculture & Biotechnology Laboratories. Mr. Abrahim demonstrated how to analyse the protein and fat content of various food products using a handheld molecular sensor and learned about the potential applications of such technology to food authenticity testing. This event provided an opportunity to build awareness of the role of IAEA in nuclear sciences and applications and of the Joint FAO/IAEA Division in the fields of food and agriculture.

The Long Night of Research, 22 April 2016, VIC

On 22 April the IAEA, along with other VIC-based organizations, took part in the biennial Long Night of Research (Lange Nacht der Forschung), an Austria-wide event coordinated by several Austrian government ministries that aims to spark interest in science and research. This was the seventh year of the Long Night of Research, and the first time IAEA was involved. The VIC was one of 253 exhibit locations across the country. The event gave visitors a chance to learn about the variety of nuclear applications in various fields. The exhibition had more than 1000 external visitors and several hundred staff members of the VIC-based organizations.

From 17:00 until midnight, a number of stations in the VIC Rotunda to showcased the Agency`s science and research to the general public, staff members and their families and friends. IAEA scientists hosted more than a dozen exhibition booths, including displays by the five laboratories of the Joint FAO/IAEA Division.

The FEPL exhibition booth focused on testing for food authenticity, posing the question 'is your food what you think it is?'. Four FEPL staff members manned the booth for the entire evening, providing information to the visitors and giving hands-on demonstrations of hand-held and bench-top spectrophotometric instruments for which applications are being developed in FEPL to provide screening tests for the authenticity of foods, or detection of adulteration.

Visitors were invited to inspect (visually and by smell) twelve olive oil samples prepared in FEPL, only one of which was a genuine extra-virgin olive oil, and to choose which they thought was the authentic sample. They could then test the samples with a hand-held infra-red spectrophotometer connected to a smart phone or tablet, to match the spectrum with a spectral library of the authentic oil, and confirm the result by testing on another bench-top infra-red spectrophotometer. Similar hands-on demonstrations were available for honey samples of different floral/geographical origin

and for milk powder adulterated with melamine. The demonstrations were very successful, with queues for the hands-on testing constantly from the opening of the event until almost midnight. There was a broad spectrum of visitors, including students, university professors, scientists, school children and other interested members of the public, and a keen interest in the subject. The hands-on demonstrations fostered discussion around food authenticity, and in many cases there was healthy competition between participants, for example to find out who had picked the correct, authentic sample of olive oil.

Hands-on demonstration of authenticity testing for honey, olive oil and milk powder at the FEPL booth

In addition to the demonstrations, visitors were informed about some of the other areas of FEPL's work in food safety, traceability, authenticity and contaminant control through a rolling video display, which included presentations by FEPL staff members, animated graphics and slide shows.

The participation of IAEA in The Long Night of Research provided a unique opportunity to showcase the peaceful uses of nuclear energy and nuclear applications.

Conferences

- **The third Food Integrity Conference**, Prague, Czech Republic, 6-7 April 2016. The conference focused on the latest research outputs, developments and strategies in the field of food integrity - safety, quality, authenticity and traceability, from the 'FoodIntegrity' project consortium and beyond. Almost 250 scientists from 37 countries participated in the conference. There were 43 lectures and 111 posters presented. Sessions covered tools for food integrity assessment, knowledge and methodological gaps in current research topics such as non-targeted analysis, complex foods, transparency along the food chain and screening methods. There was also a demonstration on the European 'knowledge base' on analytical methodology and databases for food authenticity and how it can be exploited by stakeholders. In addition, there was a series of workshops and oral presentation sessions covering food crime, occurrence, motivations and mitigations; industrial perspectives for strategies applied for assuring food authenticity; potential citizen science approaches to food integrity and a session on the authenticity of herbs and spices. FEPL played an active role in the consortium and work package meetings and continue to play the role of adding insight into activities in the international dimension on food authenticity to the European 'FoodIntegrity' project consortium.

- **EuroResidue VIII International Conference on Residues of Veterinary Drugs in Food**, Egmond aan Zee, The Netherlands, 23-25 May 2016. The EuroResidue conferences, held every four years, focus on the control of residues of veterinary drugs in food and the environment. The conferences cover aspects such as analytical techniques, pharmacological and toxicological studies, anti-microbial resistance and regulation of veterinary drugs. The eighth EuroResidue conference had more than 330 participants from 58 countries worldwide. The programme included sessions on: antibiotics, residues and resistance; residues and the environment; new techniques and confirmatory analysis; alternative matrices; and broad screening. Approximately 170 posters were presented throughout the duration of the event. The conference attendees included a number of participants in the technical cooperation project RAS5078, 'Enhancing Food Safety Laboratory Capabilities and Establishing a Network in Asia to Control Veterinary

Drug Residues and Related Chemical Contaminants'. Mr Andrew Cannavan, Head of the FEPL, participated as Chair of the Scientific Committee for the conference and gave an oral presentation entitled 'Global perspectives on antimicrobial resistance in the food chain' in the first session, 'Antibiotics, residues and resistance'.

- **11th European Pesticide Residue Workshop**, Limassol, Cyprus, 24-27 May 2016. The European Pesticide Residue Workshop (EPRW) – hosted every second year by a different European member state – is the premier European meeting for the presentation and discussion of the latest concepts and developments in the field of pesticide residues in food and drink. Its objective is to provide a platform for the exchange of information and experience in the field and to bring together people from each of the relevant sectors. The 11th EPRW had more than 250 participants from the around the world. The topics covered included the development and application of pesticide residue analytical methods; toxicology and risk assessment; regulatory issues and monitoring; and a special themed day on sampling, sample preparation and sample processing. Ms Britt Maestroni gave an oral presentation on a study carried out in the FEPL comparing two approaches for the robustness testing of an analytical method. The opportunity was taken during the conference to discuss with RALACA representatives several issues related to the use of GC-MS/MS instrumentation, such as the use of analyte protectants, calibration accuracy, ion ratios and sample preparation for conazole herbicides.

- **International Symposium and Annual Meeting, 'Integrated Management of Agricultural Environment for Food Security'**, Busan, Republic of Korea, 4-9 July 2016. The Korean Society for Environmental Agriculture's International Symposium and Annual Meeting had approximately 350 participants and comprised opening and plenary sessions, as well as parallel sessions and workshops. Mr Andrew Cannavan gave an invited oral presentation on 'Food Authenticity – Fighting Food Crime' in the plenary session of the symposium. Mr Cannavan also gave a lecture entitled 'The Joint FAO/IAEA Division – Food and Environmental Protection Activities' to approximately 30 overseas students at Pukyong National University (PKNU) in the KOICA-PKNU International Graduate Program of Fisheries Science.

- **2nd International Max Rubner Conference on Food Metabolomics**, Karlsruhe, Germany, 10-12 October 2016. The second international Max Rubner Conference focused on food quality, food safety and the effect of food and nutrition on human health, which are of great interest to consumers, policy-makers and the food industry. The conference discussed the current status of metabolomics in food sciences and the advantages and limitations of the analytical methods used, the current status of metabolomics databases, and results of recent applications in food quality, food safety and nutrition, through presentations and plenary meetings. Topics included: the use of food metabolomics in the characterisation of food; metabolomics databases; metabolite profiling: a tool to assess safety and quality of crops; metabolomics approaches to detect food spoilage; and metabolomics as a tool to assess food authenticity. The conference was attended by more than 200 participants from more than 15 countries. Ms Zora Jandrić presented current applied research done in the FEPL on applying a non-targeted approach for the discrimination of Sri Lankan teas using UPLC-QToF MS and multivariate data analysis. The research aimed to provide analytical methodology for food authenticity and safety to support FAO and IAEA Member States in ensuring sustainable food systems.

- **ISOFOOD workshop: 'Isotopic techniques in food characterisation'**, Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia, December 7-9, 2016. The exploratory workshop was dedicated to the discussion of new directions and emerging topics in the field of isotope ratio mass spectrometry (IRMS) and its application to food science. The aim was to bring together experts from various backgrounds, including public-sector research institutes, universities, the private sector and other stake-holders interested in the study of isotopic techniques for the origin and authenticity characterisation of food. The workshop was organized as a series of plenary lectures, oral and poster communications designed to create a

platform for sharing of knowledge, experiences, good practices and cooperation among experts and aimed to define the state-of-the-art in instrumentation, methodologies and applications in isotope ratio mass spectrometry in this field. Mr Simon Kelly was invited to give a lecture on organic and halal food authentication, which was well-received as a new and exciting area of research to substantiate some of the increasing plethora of food production labelling claims that add retail value to food and are susceptible to fraud. Other topics discussed included food matrix stable isotope reference materials; flavours & fragrance authentication; non-traditional isotopes in food science; advances in stable isotope analyses and statistical evaluation and modelling.

Special issue of 'Food Control'

A special issue of the Elsevier journal, 'Food Control', focusing on selected manuscripts prepared from presentations at the FAO/IAEA Symposium 'Food Safety and Quality: Applications of Nuclear and Related Techniques', Vienna, 10-13 November 2014, and from follow-up work discussed at the symposium and completed over the subsequent year, is due to be published in February 2017 . Twenty-nine manuscripts on various aspects of food safety and control were accepted through the journal's stringent peer review process. The special issue will be available online with free access for one year from the publication date, and a limited number of hard copies will be available from the Food and Environmental Protection Subprogramme for developing country scientists upon request.

Food Authenticity Methodology Working Group

The 20th meeting of the UK Department for Environment Food and Rural Affairs (Defra), Food Authenticity Methodology Working Group (AMWG), took place at Nobel House, London on 27 April 2016. Mr Simon Kelly participates in Defra's AMWG, which is a peer review committee advising on the science and methodology used by Defra and which provides a quality assurance function to ensure methods being developed are fit for purpose. The group is comprised of representatives from the UK National Competent authorities (Defra and Food Standards Agency), food industry, enforcement bodies, consumer organisations and academia to ensure balance and focus. Mr Kelly's role on the working group is primarily to advise on stable isotope methodology and to provide general input into the review of analytical methods; quality control procedures; standard operating procedures; sampling protocols; direction of research requirements and intelligence on food fraud and international collaboration. The working group gave rise to a number of useful discussion documents and position papers relating to food authenticity sampling protocols; standard operating procedures; project review procedures and a number of potential mutually beneficial collaboration opportunities.

CAPACITY BUILDING

The FEPL provided technical management for twenty national and five regional TCPs in 2016. Analytical methods and technology packages were transferred and applied through the TCPs and through training workshops held in Member States and in Seibersdorf. More than 180 scientists, analytical chemists, laboratory personnel and food inspectors from more than 50 countries were trained through these activities. Each of the workshops was designed with an individual focus, but all were within the framework of food safety and quality and included the protection of the integrity of the food supply chain as a holistic process, involving multiple stakeholders and requiring the application and integration of different analytical methods and technologies. The workshops provided a forum for interdisciplinary networking between stakeholders in the 'farm-to-fork' food chain and fostered the formation of a global network.

Developing Indicators to Determine the Effect of Pesticides, Heavy Metals and Emerging Contaminants on Continental Aquatic Ecosystems Important to Agriculture and Agroindustry

A mid-term project coordination meeting and workshop was held under TCP RLA7019 in Panama, 6-8 June 2016. The project aims to provide risk maps to local authorities and other stakeholders for monitoring the impact of agricultural production on water resources in Latin America and the Caribbean. The project started in 2014 and has 11 participating countries. The meeting, which had 25 participants, collated and organized the outputs achieved to date by each Member State. Ms Britt Maestroni gave introductory remarks focusing on the role of analytical laboratories in the efficient control of water resources affected by intensive agricultural production, and a presentation on technical issues encountered in implementing the project, focusing on the concept of quality data as an essential input to risk assessment models. Four working groups were organised to consolidate progress in the areas of biological monitoring, chemical monitoring, modelling and communication. The results of the group discussion were used to adjust the project work plan. Panama provides an example of optimal national coordination and integration. The presence of the Ministry of Agriculture at the meeting was an indication of its importance and motivated the representative of each participating Member State to implement better communication and promote increased collaboration among national institutions.

Food Authenticity, Safety and Traceability (FAST) awareness raising training, Vienna, Austria, 5-9 September 2016

A one-week training course was organised as a group scientific visit to raise awareness of issues and analytical methodologies to address current challenges in food authenticity, safety and traceability (FAST). The training course was attended by laboratory managers and senior researchers from Iraq, Kuwait, Libya, the Marshall Islands and Syria. The course content was delivered through 12 lectures covering veterinary drug and pesticide residue analysis; persistent organic pollutants; naturally occurring contaminants; food authentication using stable isotope and metabolite analysis; food traceability; food irradiation detection; and analytical method development, validation and quality control. Lectures were delivered by staff from the Food and Environmental Protection Section and the FEPL and included four World Café interactive sessions to help the delegates identify and design proposals to address the major FAST issues in their respective Member States. The course also included a one-day seminar at the FEPL facilities in Seibersdorf to cover the specifics of setting up and maintaining gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry and isotope ratio-mass spectrometry facilities and infrastructure. The course was very well-received by the delegates and achieved an overall 'excellent' rating from feedback questionnaires. The intention is to make the course more widely available in the future through the IAEA's Department of Technical Cooperation.

Trainees at the Food Authenticity, Safety and Traceability (FAST) awareness raising course receive their certificates

Summer school, 'Food Safety and Food Security in Europe: Feeding the City', Brescia, Italy, 4 July 2016

The second annual summer school on 'Food Safety and Food Security in Europe: Feeding the City' was organized under the patronage of the University of Brescia. It provided students with a multidisciplinary perspective on the societal and scientific challenges of food security and of food safety in an urban context. Ms Maestroni presented an introductory lecture on food safety.

Protection of the integrity of the food supply is of utmost importance in terms of food security; food safety and quality; consumer protection; and international trade. Techniques to maintain and assure the quality and safety of food are necessary throughout the food production and supply chain. The need for methods to monitor and verify food safety and quality and ensure food traceability is evidenced by the ever growing list of food product recalls due to contamination or food fraud. The introductory lecture highlighted the central role played by the analytical laboratory in providing end product testing and advice in the context of food control systems. A workshop was held in the afternoon to discuss various issues, including urban and peri-urban horticulture; street food; distribution and retailing; the role of local government; and environmental issues. The lecture and the workshop were attended by twenty-five university students from Italy.

The lecture was well received and met the expectations of both students and organizers.

Providing Assistance on Food Safety and Risk Assessment in Bulgaria

In response to a request from the Bulgarian Nuclear Regulatory Agency, Ms Britt Maestroni undertook a technical mission on 24-25 October 2016, funded under TCP BUL5014, to give a presentation on risk assessment and food safety, to provide expertise on the implementation of technical requirements for food safety in Bulgaria and to identify gaps and opportunities for improvement in food contaminant analysis to target food safety in Bulgaria.

Ms Maestroni visited the Central Laboratory for Veterinary Expertise and Ecology (CLVEE), which belongs to the Risk Assessment Centre of the Bulgarian Food Safety Agency located in Sofia, and provided advice and guidance on issues critical to maintaining or increasing laboratory capacity, including the purchase of consumables and reagents; replacement of analytical equipment and identification of new equipment needed; training and retention of staff; and sustainable funding. A discussion on possible collaboration with the FEPL and the Joint FAO/IAEA Division focused on food traceability and contaminants in matrices such as honey and milk.

Ms Maestroni also gave a presentation on risk assessment and food safety at the 9th Scientific Conference held by the Bulgarian focal point of the European Food Safety Authority (EFSA) in Hissar. The presentation helped to introduce the main technical themes of the conference, which included the safety of the food chain, epizoology and epidemiology. The presentation was well received.

Regional Latin America/Caribbean meeting on emerging contaminants

The FEPL together with the IAEA Technical Cooperation Department organized a regional meeting on emerging contaminants in Montevideo, Uruguay from 14-18 March 2016. The meeting was held in the context of the current regional project, TCP RLA7019, which aims at providing a technical framework for monitoring continental aquatic systems in Latin American and to provide government authorities with an early warning tool for environmental sustainability and food safety management.

The meeting was attended by 36 participants from eight Latin American countries. The purpose was to raise awareness of emerging contaminants and their associated emerging risks, in order to develop the capability to evaluate their potential threat to consumers and the environment. Emerging contaminants can be broadly defined as any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects. It has become easier to identify these emerging contaminants with the development of

novel analytical capabilities such as accurate mass and high resolution mass spectrometry that can be used for untargeted analysis. The meeting addressed current analytical methodology and instrumentation with a special focus on chemical contamination. Aspects discussed related to current knowledge; quality assurance and quality control; sampling and current regulations; harmonisation of analytical capabilities; and methodology. Working groups were organized to discuss different areas of work and to train on a risk screening tool for chemical pollutants in water, 'HERWE' (Human and Ecological Risk Screening Tool for Chemical Pollutants in Wastewater Effluents). The participants were also provided with a manual on sampling and analytical tools.

The meeting was appreciated by all participants.

The RALACA Laboratory Network

The 'Red Analitica de Latinoamérica y el Caribe' (RALACA) is a non-profit network of laboratories and associated institutions in Latin America and Caribbean countries that aims to enhance regional capabilities for food safety and environmental sustainability (red-ralaca.net). The network was initiated and established with FEPL assistance in March 2012, with nine laboratories initially involved. Today RALACA encompasses more than 50 laboratories and institutions in 21 countries.

As part of the project to implement sustainable technology transfer to developing countries, the activities in 2016 concentrated on the implementation of three management meetings by the board (which includes a representative of the FEPL) and the committees of RALACA. The meetings, each of which had fifteen participants, were held as side events on the occasion of conferences and training events in Mexico, Uruguay and Cyprus. A RALACA laboratory in Ecuador prepared a guide on the use of passive samplers and shared it with the network. Another guide on risk assessment was prepared by experts from Colombia and was used as a preparatory training document for a workshop that was held in Panama in 2016. A one-hour webinar via webex on biomonitoring was held in June and was attended online by 15 participants from the RALACA network. Two manuals were also prepared for publication using an external publisher.

INFOSAN meeting, 'New science for food safety: supporting food chain transparency for improved health'

The International Food Safety Authority Network (INFOSAN), a WHO/FAO initiative, was set up in response to the increased likelihood of international incidents involving contaminated food as a consequence of the rapid globalization of food production and trade.

In recent years, several new important scientific developments have emerged with significant future implications for food production and food safety – and with direct impact upon the future food chain transparency and food safety solutions. The potential linkage between authorities related to food safety and food production regulatory action, and science institutions in the region would enable a faster and more consistent introduction of new international science-based developments in this area. Likewise, the internationally adopted (WHO and FAO) food safety risk analysis principles suggests independent scientific advice as the basis for food safety risk management and food control. The rapid rise of food science and applied technologies in the Asia region opens new possibilities for regional collaboration in support of scientific and regulatory development. The recent creation of the ASEAN Risk Assessment Centre (ARAC) is one example of such developments.

In this context, INFOSAN convened a meeting on regional perspectives on food science and developments in Asia. The meeting, 'New science for food safety: supporting food chain transparency for improved health', was co-organised by Nanyang Technological University Food Technology Centre (NAFTEC), FAO and WHO and held in Singapore, 7-10 November 2016. The meeting had more than 160 participants from 30 countries.

The meeting focused on four inter-related major subjects where new scientific developments are already resulting in major changes to food science and food regulatory systems in Europe and the

Americas: foodborne disease burden; risk assessment and sustainability; next generation (DNA) sequencing – linking data for faster and better decisions; and novel food technology – hindering fraud and helping health. Mr Andrew Cannavan, Head of the FEPL, gave an invited presentation on 'International Efforts to Combat Food Fraud' and participated as a member of an expert panel and in break-out sessions to discuss various aspects of food control.

The direct interaction between regulatory authorities and science/research agencies and institutions has developed rapidly in both the EU and the US systems. It was clear from the presentations and discussions at the INFOSAN meeting that this modality should also be encouraged and promoted in Asia, with support from the relevant international organizations in this area, including WHO and FAO.

Webinar on pesticide residues analysis

A webinar on 'Practical Experiences with Agilent's GC/MS/MS Pesticide Analyzer' was held on 21 July 2016, Sep Science webinar with 50 participants from 25 countries. Ms Britt Maestroni emphasized the development and validation of a multi-residue method for pesticides in potato in order to highlight practical aspects of method optimization, including sample preparation using a modified QuEChERS strategy and effective method development with Curated Pesticide MRM Database and RTL tools.

Fellowships, Scientific Visitors and Interns

The FEPL hosted a total of three interns, three fellows, two scientific visitors and one visiting scientist during 2016.

In June 2016 Ms Hanna Zakala completed a one-year internship in FEPL. During her time in FEPL, Hanna worked mainly on methods for food authenticity testing, concentrating on untargeted metabolomics profiling using liquid chromatography/high resolution mass spectrometry. She gained experience in sample preparation, sample analysis by UPLC-QToF MS and data analysis, and contributed to FEPL outputs in this field. Hanna returned to Ukraine in June, and we wish her all the best for the future.

In November, Ms Valeria Avossa completed a one-year internship in FEPL. Valeria gained experience in a number of techniques related to food authenticity, traceability and contaminant control during her internship, notably in a collaborative project with the Joint FAO/IAEA Division's Animal Production and Health Laboratory on DNA sequencing techniques for the genetic traceability of fish species. Valeria left FEPL on completion of her internship to perform further research and study for a PhD in Italy. We wish her all the best and hope for future collaboration and interaction in projects of shared interest.

Mr Sharif Shawky completed a two–month internship in FEPL in August. Sharif, an undergraduate student of environmental science and sustainability at Colorado State University, USA, gained experience in various analytical techniques for food contaminant control and traceability during his internship.

In October, FEPL welcomed three Malaysian scientists for 2-month fellowships under TCP MAL5030, Strengthening National Technical Capability in Food Traceability of Edible Birds Nest through the Application of Nuclear and Related Technologies. Ms Syahidah Almal Binti Muhammad, from the Analytical Biochemistry Research Centre, University Sains Malaysia, and Ms Salmah Moosa, from the Malaysian Nuclear Agency, undertook training on metabolomics using UPLC QToF MS and related screening methods using vibrational spectroscopy for authentication of edible bird's nest under the tutelage of Ms Zora Jandrić. Mr Mohd Noor Hidayat Adenan, also from the Malaysian Nuclear Agency, undertook training in authentication of edible birds nest by isotope ratio mass spectrometry under the supervision of Mr Simon Kelly.

In May, FEPL welcomed a Syrian scientist for a three-day scientific visit under TCP SYR5023, Enhancing Analytical Capacities of Major Pesticide Residues. Mr Iyad Ghanem from the Atomic Energy Commission of Syria actively participated in a round table discussion on possible options for pesticide analysis, including sample preparation for the analysis of pesticides in food of vegetal origin. In addition, Mr Ghanem observed the implementation of a modified QuEChERS method for analysis of organophosphates in potato.

In November, the FEPL welcomed a Panamanian scientist for a two-week scientific visit under TCP PAN5024, Developing Analytical Capabilities for the Detection of Chemical Contaminants in Food and the Quality of Agrochemicals. Ms Brenda Checa, Head of the Pesticide Residue and Formulation Control Laboratories of the Ministry of Agriculture of Panama, actively participated in laboratory work on the optimization of analyte protectants for the GC-MS/MS analysis of selected pesticides in fresh vegetables. This project is of great importance since it will contribute to the pesticides surveillance programs that are being implemented in Panama for food safety.

In February, the FEPL welcomed a scientist from LVA in Klosterneuburg, Austria, for five weekly meetings as part of a scientific collaboration on the use and deployment of GC-MS/MS. Ms Celine Lesueur actively contributed to optimizing GC-MS/MS experimental conditions for the analysis of 350 pesticides currently used in agricultural production.

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THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

In the Livestock Pest group of the Insect Pest Control Laboratory (IPCL), work continued on the potential of developing tsetse strains that are refractory to the transmission of trypanosomes by assessing the role of microbiota. Culture-dependent and independent methods indicated that there were great temporal differences in the density of cultivable gut microbiota in tsetse flies.

In addition, work continued on the molecular identification of tsetse species, in view of the difficulties experienced with morphological characterization. Using different molecular markers, i.e. a nuclear marker (internal transcribed spacer 1 (ITS1)), microsatellite markers, mitochondrial markers (COI, COII, 12S and 16S rRNA) and Wolbachia 16S rRNA, it was possible to distinguish between different tsetse species, both from colonized and wild populations.

In the Plant Pest group, cold treatment research with the South American cucurbit fruit fly *Anastrepha grandis* indicated that a cold treatment for phytosanitary security at 1°C would require at least 14 days. In another study, no significant differences could be found in cold tolerance of different populations of the Mediterranean fruit fly *Ceratitis capitata* from Argentina, Australia and Spain.

Work continued on developing various aspects of the combined use of the male annihilation technique (using a male attractant [methyl eugenol (ME)] and insecticide in baits) and the sterile insect technique (SIT) as a component of area-wide integrated pest management (AW-IPM) programmes for *Bactrocera dorsalis*. The two techniques could be applied simultaneously if the sterile males had a lower tendency to be attracted to the ME-insecticide baits. One issue that is under investigation at the IPCL is the delivery mechanism of ME in large mass-rearing facilities, i.e. feeding versus aromatherapy. Preliminary results showed that ME-aromatherapy enhances the mating competitiveness of *B. dorsalis* males.

Irradiation studies using X-rays or gamma rays showed different dose responses with the two types of radiation depending on the species (*Bactrocera tryoni, Anastrepha fraterculus* and *Anastrepha ludens*), and work continued to characterize a genetic sexing strain (GSS) of *A. fraterculus* that is based on a colour dimorphism of the pupae, i.e. brown males and black females. Significant progress was made with the rearing of *Drosophila suzukii* and specifically with the development of better oviposition systems. The quality of cryopreserved samples of the VIENNA 8 GSS of the Mediterranean fruit fly was assessed under semi mass-rearing conditions and all production parameters and quality control indices were comparable to those of the non-cryopreserved VIENNA 8 strain.

In the Human Disease Vectors group of IPCL, research focussed on the development of suitable adult *Anopheles arabiensis* holding cages to enable adequate egg production. Results indicated that the initial number of pupae loaded into the cages cannot exceed a threshold of 15 000, but pupae can be added daily to the mass-rearing cage to produce 1 million eggs per cage. In addition, a new protocol was developed for larvae rearing that reduced the quantity of water per unit by ¼, the daily work by 2 days and the larval food by 33%, and led to an optimal synchronization of the pupation. *Aedes aegypti* egg production was improved by using a 3:1 female:male adult ratio in the adult holding cages. This resulted in the production of 650 000 eggs/cage after the second week of the rearing cycle.

Research continued on the effects of various handling procedures on the quality of the mosquitoes. Male mosquito survival was not affected by chilling, packing of canisters or canister shapes, or by compacting males within the canister. Immobile male mosquitoes were not capable of producing heat metabolically when maintained compacted within a release cassette. Work was also started on

the development of a system capable of releasing sterile male mosquitoes by air from an unmanned aerial vehicle (UAV) platform.

In the Genetics and Molecular Biology group of IPCL, work was carried out to assist unravelling the *Ceratitis FAR* species complex. Evidence exists that supports the presence of at least four well-defined species within this complex (*C. fasciventris*, *C. rosa, C. anonae* and, recently, *C. quilicii*). In collaboration with Greek colleagues, mitotic nucleus and polytene chromosome maps were developed for *C. fasciventris*, which is only the second member of the *Ceratitis* genus with photographic polytene chromosome maps. The complete mitochondrial genome of *C. fasciventris* was also constructed, which will facilitate genotyping and phylogenetic studies in tephritids.

The group also contributed to work on the *A. fraterculus* species complex and the *Wolbachia* status of colonies representing different morphotypes of the complex. Analysis showed that all colonies were infected and colonies from Brazil (Piracicaba, Parnamirim, Vacaria), Argentina (Tucuman), Mexico and Peru were single infected with the same *Wolbachia* strain, while the colony from Colombia was single infected with a different strain. In addition, polytene chromosome maps of the *A. fraterculus* Af. sp.1 member have been constructed, which is the first polytene chromosome map for a member of the *A. fraterculus* complex and only the second map available for the *Anastrepha* genus.

All VIENNA GSS from mass-rearing facilities around the world were re-introduced in the IPCL and screened with the 'tsl' test. In addition, their cytogenetic profile was evaluated through polytene chromosome analysis. This analysis verified the stability of the sexing character of these strains and provided some interesting findings, such as important differences in hatch, pupation and emergence rates. Results of such an analysis can show whether specific rearing practices are beneficial or harmful for the VIENNA GSS, recognize putative deleterious effects upon emergence and help to select the most appropriate option to overcome them.

Symbiotic communities and especially gut symbionts are important for the fitness and behaviour of insects and their overall performance. Stresses such as heat shock, irradiation and the presence of *Wolbachia* might affect the symbiotic communities in general. Gut samples were collected from both VIENNA 8 D53+ and VIENNA 8 D53+/56S2 flies of different ages and findings suggested that the gut symbiotic communities of the VIENNA 8 D53+ and VIENNA 8 D53+/56S2 strains were different and differentially affected by the stresses applied. The heat shock treatment had a more severe effect than the irradiation stress with respect to the structure of the gut symbiotic communities.

In 2016, the IPCL hosted eight cost-free experts, 12 consultants, 12 interns, 13 fellows and four scientific visitors (the latter two categories funded by the IAEA's Department of Technical Cooperation). The Plant Pest and the Genetics and Molecular Biology groups delivered 41 shipments of live fruit fly insects to 13 different institutions in Canada, Czech Republic, France, Greece, Italy, Mauritius, Senegal, South Africa, Spain, Sweden and the UK. The Livestock Pest group delivered 67 shipments of live tsetse insects, including 59 shipments to Senegal of 156 000 *G. palpalis gambiensis* pupae, as well as to six different institutions in Belgium, Germany, Senegal, Uganda and Zimbabwe. The Human Disease Vectors group sent four shipments of live mosquitoes to three institutions in Germany, the UK and USA.

STAFF

Name	Title
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Bourtzis, Kostas	Molecular Biologist/Geneticist
Gilles, Jeremie	Entomologist (Human Health Pests)
Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Livestock Pests)
Targovska, Asya	Senior Laboratory Technician
Haq, Ihsan Ul	Research Assistant
Adun, Henry	Laboratory Technician
Ahmad, Sohel	Laboratory Technician
Ali, Adel	Laboratory Technician
Marin, Carmen	Laboratory Technician
Mohammed, Hasim	Laboratory Technician
Maxwell, Florence	Laboratory Technician
Cancio Martinez, Elena	Laboratory Technician
Dammalage, Thilakasiri	Laboratory Attendant
Gembinsky, Keke	Laboratory Attendant
Lapiz, Edgardo	Laboratory Attendant
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Beckham, Stephanie	Team Assistant
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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Livestock Pests

Identification of cultivable tsetse gut microbiota and their effects on fly performance

Control of the tsetse vector remains the primary strategy for the control of African trypanosomoses, and the SIT has potential as an additional tool within area-wide integrated pest management (AW-IPM) approaches. The irradiation procedure used to sexually sterilize tsetse males for the SIT potentially damages the gut epithelia and the beneficial gut-inhabiting microbiota. These effects may reduce the quality (sexual performance and competitiveness) of the sterile males; low-quality sterile males could compromise the success of AW-IPM programmes with an SIT component and increase its operational costs. Another valid concern is that, although the non-viable matings between the sterile males and virgin wild females result in a time-dependent decline in the target tsetse populations, the sterile males are capable of transmitting trypanosomes, which would be disastrous if millions of sterile males are released into areas with active parasite circulation. This has so far been mitigated by mixing trypanocidal drugs with the blood meals that the sterile males receive before release. However, it would be extremely beneficial if we could reduce the vectorial capacity of the sterile males to transmit trypanosomes, while maintaining or improving the quality of the sterile males. A potential strategy to achieve this is to exploit the beneficial traits conferred by the gut microbiota to their insect hosts.

Culture-dependent and independent methods (Fig. 1) can be used to identify cultivable gut microbiota and assess their potential to enhance the quality of the sterile males. The work is based

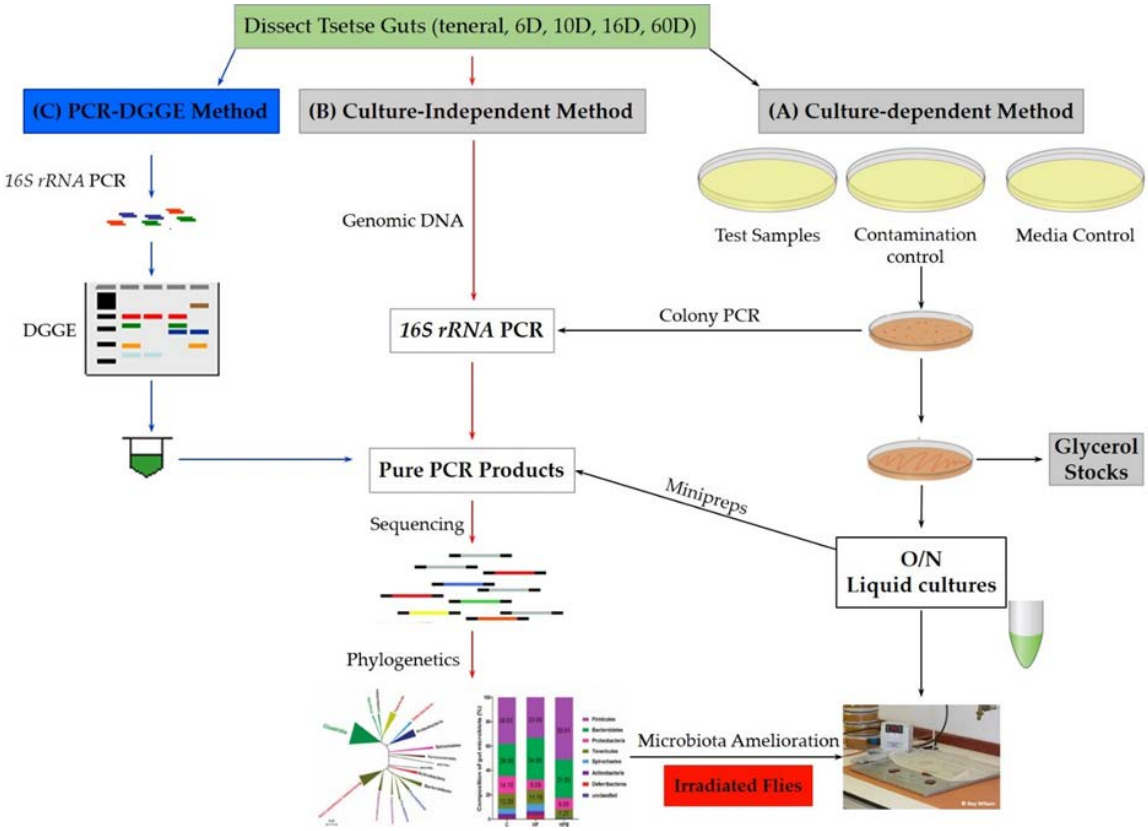

FIG. 1: Flow chart of an experimental set-up for the identification of tsetse gut microbiota and easements of their potential to improve the quality of sterile males used in SIT programs. The primary approach is the use of a culture-dependent approach (A), which is supplemented with culture-independent approaches (B and C)

on the sequencing of the 16S rRNA gene of a bacterial isolate from the guts of *Glossina fuscipes fuscipes*, a major trypanosome vector responsible for ≈90% of all reported cases of sleeping sickness in Sub-Saharan Africa.

Preliminary results obtained from the analysis of the 16S rRNA gene sequencing showed age-dependent variations in the densities of cultivable gut microbiota. There was no cultivable gut microbiota in freshly larviposited third-instar larvae and in teneral flies. The densities of cultivable gut microbiota increased at 6 days post emergence, followed by a sharp decrease in 10-day-old flies and finally by a significant increase in older flies (16 and 60 days old). So far, the identified gut microbiota belonged to five major bacterial species, i.e. *Microbacteria* spp., *Serratia* spp., *Klebsiella* spp., *Sphingobacteria* spp. and *Acinetobacter* spp. These bacterial species have been reported in other insects, some of which are documented to confer beneficial traits in their insect hosts. For instance, although many *Serratia* spp. are harmful to insects, *Serratia marcescens* (identified in the current study) is one of the cultivable mosquito microbiota, and confers anti-plasmodium functions (reduced parasite loads) in Anopheline mosquitoes. Studies have also shown that *Klebsiella* improves sexual performance of irradiated fruit fly males. On the other hand, supplementing blood meals with *Acinetobacter* isolates resulted in increased susceptibility of mosquitoes to infections by the Japanese encephalitis virus.

Molecular identification of tsetse species

The objective of this study was to develop quick, cheap and easily applied tools to identify tsetse species in Africa for SIT application. The main reason for developing these tools is that SIT application is species-specific and therefore it is of paramount interest to correctly identify the targeted species to ensure adequate mating compatibility of the target species in the field and the released flies. Identification of tsetse species using morphological characteristics is difficult, especially for closely related species and only skilled taxonomists are sometimes able to identify them. In addition, some species that have been misidentified in the past need to be correctly identified. The large DNA library of the IPCL database needs confirmation in terms of species identity for research work.

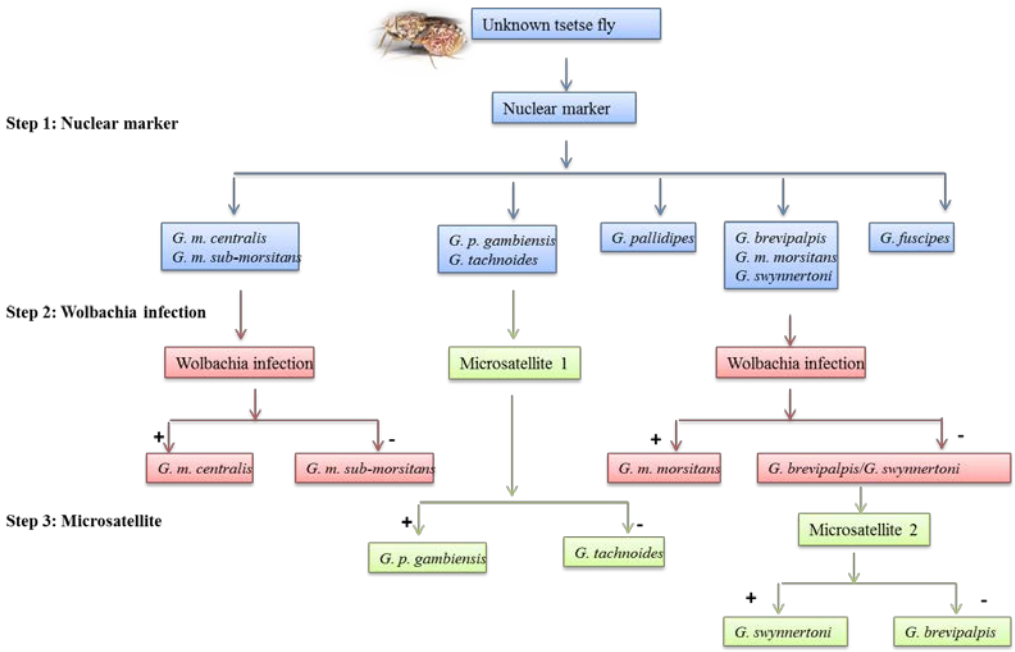

FIG. 2: Schematic representation of a step-by-step approach using molecular tools for tsetse species identification

In this study, eight specimens from each of the nine IPCL colonized *Glossina* species (*G. pallidipes, G. m. morsitans, G. m. centralis, G. swynnertoni, G. m. sub-morsitans, G. p. gambiensis, G. f. fuscipes, G. tachinoides* and *G. brevipalpis*) were screened using different molecular markers, i.e. a nuclear marker (internal transcribed spacer 1 (ITS1)), microsatellite markers, mitochondrial markers (COI, COII, 12S and 16S rRNA) and *Wolbachia* 16S rRNA. Tsetse species from wild populations were also included in the study. Application of these molecular tools has been successful in distinguishing different tsetse species both from colonized and wild populations (Fig. 2).

In addition to the above-mentioned methods, sequencing of the tsetse species mitochondrion (mtDNA) genome is an additional step to identifying variable regions that can be used to distinguish the species and the sub-species from different geographical locations. The variable regions can be used to design microsatellite markers for identification of a specific species, sub-species or even haplotypes within a species. In this study, the mtDNA of the *G. m. centralis* and *G. brevipalpis* was sequenced using the HiSeq system and about 15kb sequence of each of these species was obtained. The mtDNA sequence of each of the two species was used to 'fish' the mtDNA sequences of other tsetse species from the total genome sequence. The genome sequence of the mtDNA of each species was confirmed by Sanger sequencing. These species include *G. pallidipes, G. m. morsitans, G. m. centralis, G. p. gambiensis, G. f. fuscipes, G. austeni* and *G. brevipalpis*. This was followed by a comparative analysis of the mtDNA of seven species and identification of variable regions that can be used to design microsatellite markers for the different species. Fig. 3 shows an example of the variable regions.

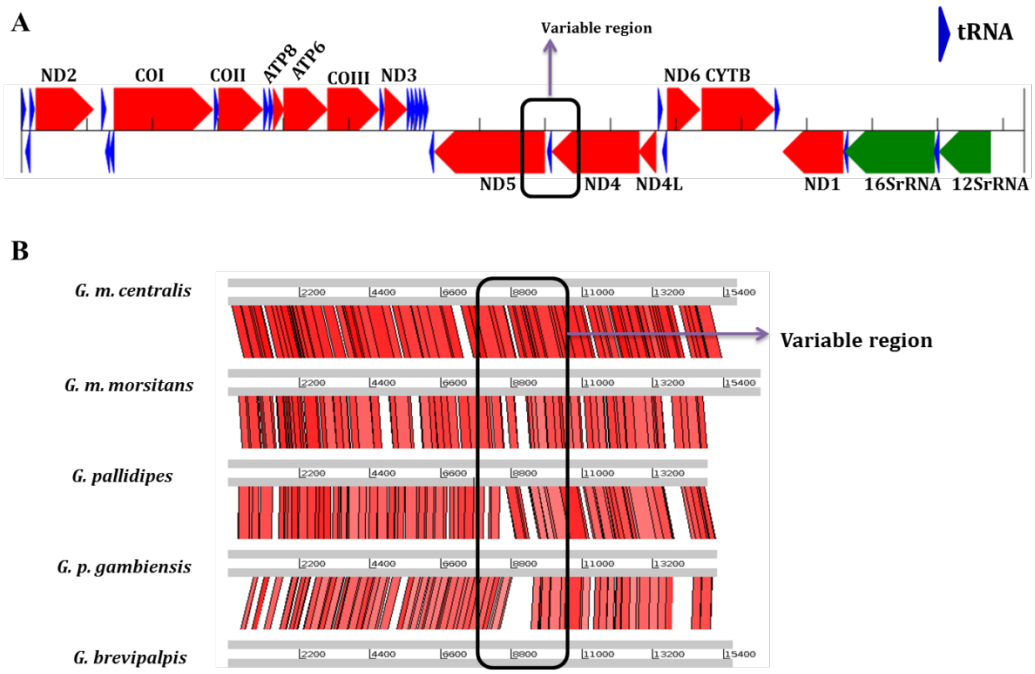

FIG 3: Mitochondrial genome analysis of seven tsetse species. (A) The organization of 13 protein coding genes, the tRNAs and the rRNAs. (B) An example of a variable region among the tsetse species

Impact of salivary gland hypertrophy virus infection on the performance of Glossina fuscipes fuscipes

Several tsetse species are infected with the salivary gland hypertrophy virus (SGHV). The virus infection was demonstrated to have severe negative impact on fly productivity and mortality of *G. pallidipes*. This negative impact was not reported so far in other tsetse species. However, the *G. f. fuscipes* colony maintained at the Institute of Zoology of the Slovak Academy of Sciences, Bratislava, Slovakia collapsed and the size of the colony maintained at the National Institute for Controlling and Eradication of Tsetse and Trypanosomosis, Addis Ababa, Ethiopia (where the fuscipes colonies are

maintained together with the virus-infected pallidipes) prompted us to analyse the impact of the SGHV infection on the performance of *G. f. fuscipes*. Injection of *G. f. fuscipes* teneral flies with the SGHV showed a reduced life span of the adults and it reduced the flies' productivity (Fig. 4). These results indicate the need to implement measures to prevent the spread of SGHV in tsetse mass-rearing facilities holding more than one tsetse species.

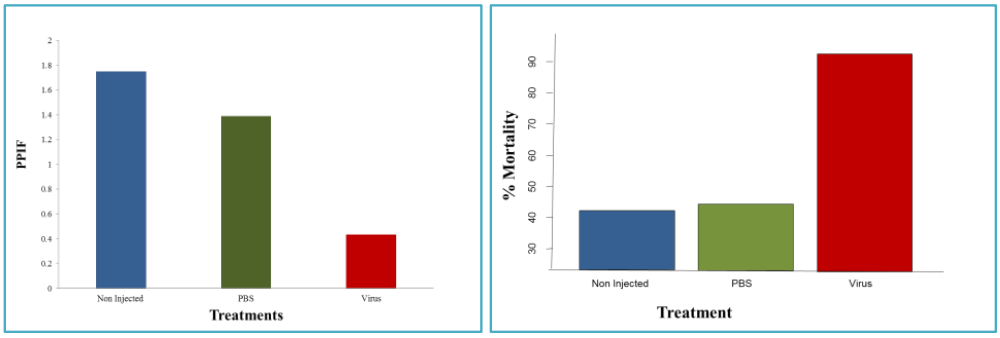

FIG 4: Impact of SGHV infection on the productivity and mortality of the tsetse fly G. f. fuscipes

Plant Pests

Phytosanitary treatments under the FAO/IAEA/USDA agreement

Research under the FAO/IAEA/USDA agreement on 'Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies' continued to concentrate on cold phytosanitary treatments, taking advantage of the tephritid resources at the IPCL to develop broadly applicable treatments. However research was also done with vapour heat and irradiation treatments. The results of phytosanitary treatment research at the IPCL guide phytosanitary treatment scheduling at the national and international levels among Member States of the FAO and IAEA as well as the International Plant Protection Convention (IPPC). Collaboration with the IPPC through the Technical Panel on Phytosanitary Treatments (TPPT) and the IPPC-liaison organization, the Phytosanitary Measures Research Group, leverages cooperative international efforts toward the development of broadly applicable phytosanitary treatments.

Cold treatment research with *A. grandis* was terminated and an article published indicating that a dose for phytosanitary security at 1°C would be at least 14 days. Research continues to substantiate a dose of 18 days at 1°C against *Bactrocera* (*Zeugodacus*) *tau*.

The study comparing populations of *C. capitata* from Argentina, Australia and Spain to determine if they differ in cold tolerance was terminated with the general conclusion that differences were not significant enough to prevent the development of broadly applicable cold treatments against that pest species. Those findings were announced during the 2016 meeting of the TPPT (www.ippc.int/en/news/phytosanitary-treatments-on-fruit-fly-can-now-move-forward//) and are being prepared for publication. A similar conclusion was published in 2016 for *B. dorsalis* based on research previously done at the IPCL.

Results of studies supporting several generic phytosanitary irradiation treatments arising from a recently concluded Coordinated Research Project on generic phytosanitary irradiation doses have been published in a special issue of Florida Entomologist (see: journals.fcla.edu/flaent/issue/view/4278). These are being submitted to the IPPC and national plant protection organizations for consideration as commercial treatments to disinfest commodities in international trade of regulated pests.

Populations of *B. dorsalis* from subtropical China, Kenya and Thailand were found to not be significantly different in tolerance to the vapour heat phytosanitary treatment, aiding in the

development of broadly applicable phytosanitary heat treatments. The results are being prepared for publication.

Zucchini infested by *A. grandis* via oviposition and developed to the late 3rd instar (the most radio-tolerant stage) was subjected to a phytosanitary irradiation dose range of 30-36 Gy with no adults emerging from a total of 182 3rd instars tested. This research demonstrates that *A. grandis* is not more radio-tolerant than other species of the genus and supports a dose of 70 Gy for the entire genus.

Combined application of the male annihilation technique (MAT) and the sterile insect technique (SIT) for the management of Bactrocera dorsalis

The Oriental fruit fly, *B. dorsalis* is mainly native to South East Asia and has invaded sub-Saharan Africa, the Pacific Islands and the USA. It causes economic losses to horticultural production and interferes with the trade of fresh horticultural products. The male annihilation technique (MAT) is based on the use of methyl eugenol, a powerful male attractant that occurs naturally in many plants, which attracts the males to baits impregnated with insecticides. This lure-and-kill technique can be used to suppress the male population. Using MAT as a stand-alone technique is probably not sufficient to suppress the population to an acceptable level, as the few remaining males can still inseminate many females. Therefore, it is suggested to integrate the MAT with the SIT as a component of AW-IPM programmes. Application of MAT and SIT has so far been sequential, i.e. first MAT was applied to suppress the wild population followed by the release of sterile males. However, the two techniques could be applied simultaneously if the sterile males are not attracted or showed a lower tendency to be attracted to the ME-insecticide baits. *B. dorsalis* males that had fed once on ME showed reduced frequency to visit ME baits; therefore, it is proposed to expose the sterile males to ME before releasing them in the target area. Feeding ME to mass-reared sterile males is not practicable with the current holding protocols in mass-rearing and release facilities. This study was designed to assess the possibility of ME application by aromatherapy (Fig. 5) and its effect on male mating competitiveness. The preliminary results showed that ME-aromatherapy enhances the mating competitiveness of *B. dorsalis* males.

FIG. 5: (left) Methyl eugenol feeding system, (right) methyl eugenol aromatherapy

The use of X-rays and gamma rays to induce reproductive sterility in some fruit fly species

Irradiation studies were carried out with the fruit fly species *B. tryoni*, *A. fraterculus* and *A. ludens* by exposing adult males to X-rays in a RadSource RS-2400 irradiator (Rad Source Technologies, USA) or to gamma rays in a ^{60}Co gamma irradiator (Gamma Cell 220, Nordion, Canada). The treated males were allowed to mate with non-irradiated females. Untreated female *B. tryoni* that had mated with males treated with X-rays or gamma rays showed similar levels of sterility for the same dose. However, untreated *A. fraterculus* females mated with males irradiated with gamma rays had a slightly higher egg hatch (0.67%) as compared with females that had mated with males exposed to X-rays (0.25%). Untreated female *A. ludens* that had mated with males irradiated with gamma rays (60-80 Gy) showed similar egg hatch (0.47% and 0.48%, respectively). Likewise, untreated females mated with males irradiated with 60-80 Gy of X-rays showed 0.24% and 0.08% egg hatch,

respectively. However, untreated females mated with males irradiated with 40 Gy using gamma and X-rays showed a much higher egg hatch (0.88% and 0.2% respectively).

South American fruit fly

Anastrepha fraterculus is a pest that has a major impact on the economy in South America because it attacks several fruit commodities of economic importance in the region. It not only causes direct fruit damage, but trade of food commodities is impeded between infested and non-infested countries due to phytosanitary regulations. Governments and farmers use integrated pest management approaches to manage *A. fraterculus* populations and the SIT could be an additional component to control this pest on an area-wide basis. The SIT relies on the production and release of sterile males in the target area, but has not been implemented yet to control *A. fraterculus*. To facilitate the efficient implementation of the SIT against this pest, Mr Salvador Meza (Mexico) and Ms Silvana Caravantes (Guatemala) have been working on a genetic sexing strain (GSS) that is based on a pupal colour dimorphism (brown-black) (Fig. 6). The sexing mechanism was developed by the induction of a reciprocal translocation between the Y chromosome and the autosome carrying the wild type locus of the black pupae (*bp*) gene. The GSS was constructed from a laboratory population with aff1-morphotype, which implies that the SIT with male-only releases could in theory be applied in a large target area that extends from southern Brazil to central Argentina. Several GSS lines are under evaluation to determine their production and quality control profile.

FIG. 6: Phenotype of the A. fraterculus *genetic sexing strain, brown pupae and light normal body colour adult male; black pupae and black body adult female (photos by Salvador Meza).*

Drosophila suzukii

As reported previously, the IPCL has been collaborating as an active counterpart in the project SUZUKILL (suzukill.univ-rennes1.fr/). One of the objectives of this project is to develop alternative and innovative approaches for the biological control of this pest, and the IPCL has engaged itself to assist with the development of the SIT to and assess the feasibility of its use in greenhouses. Good progress has been achieved with the development of an oviposition system that uses a combination of different synthetic netting and a synthetic larval substrate. Combination of both systems has allowed the production of a large number of eggs and pupae (Fig. 7). Further work is ongoing to fine-tune the system for use into an optimal economic mass-rearing system. Production of a larger number of pupae will also allow further work on irradiation dose response curves and irradiation protocols.

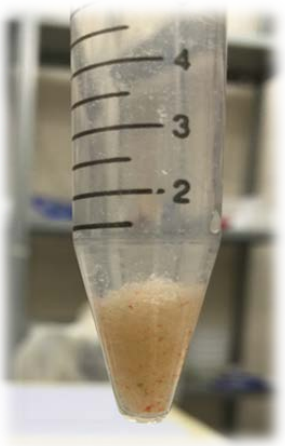

FIG. 7: Sample of DSW eggs collected on an artificial substrate

Evaluation of cryopreserved Mediterranean fruit fly VIENNA-8 strain under semi mass-rearing conditions

Most AW-IPM programmes that incorporate the SIT for the management of the Mediterranean fruit fly *Ceratitis capitata* are using the VIENNA 8 GSS that was developed at the IPCL. The VIENNA 8 is a GSS that carries a *white pupa* (*wp*) and a *temperature sensitive lethal* (*tsl*) mutation. These mutations

can be used to separate female pupae (white colour) from male pupae (wild type; brown colour) and the elimination of the females from the production line by exposing the eggs to 34°C for 24 h (as the *tsl* mutation will kill all female embryos). Rearing of the VIENNA-8 strain has allowed the release of only males, which has significantly increased the cost-effectiveness and biological efficiency of the SIT component. However, rearing of a strain for many generations under mass-rearing conditions often adversely affects its fitness traits, which can be mitigated by refreshing the strain with wild flies. Refreshing a GSS using wild flies whilst preserving the mutations is complex and takes several months to complete. Although colony management protocols are in place and adhered to in most mass-rearing facilities, cryopreservation is an alternative strategy to preserve the parent lines and that would allow quick refreshment of the colony when needed.

We tested the quality of cryopreserved samples of the VIENNA 8 strain when reared under semi mass-rearing conditions. The cryopreserved strain was similar to the normal VIENNA 8 strain in terms of production parameters and quality control indices (including mating behaviour in field cage test).

Evaluating different sources of protein for larval rearing of Mediterranean fruit fly

Brewer's yeast is commonly used as a source of protein in the larval diet of the Mediterranean fruit fly, but it is also the most expensive ingredient. It would therefore be desirable to find alternative cheaper sources of protein. We evaluated larval diets that contained proteins originating from plants and from bacterial biomass (Enterobacteriaceae spp.) and compared it with the standard diet containing brewer's yeast. The diet containing bacterial biomass was better and the larvae developed faster as compared to the control diet with the brewer's yeast, whereas the diets containing proteins from plants were less efficient as compared to the control diet. Studies are continuing to assess other cheap ingredients that could replace yeast as the/a source of protein in the larval medium used for mass rearing fruit flies.

Human Disease Vectors

The work of the Human Disease Vectors group of the IPCL has focussed on support to several pilot suppression trials using the SIT, or the combined SIT/IIT (incompatible insect technique) approach to reduce mosquito vector populations in selected field sites. The development of release and trapping methods is ongoing and progressing in the frame of current Coordinated Research Projects, while mass-rearing technologies are being transferred to our most advanced Technical Coordination Projects in Member States.

Improvements in mass-rearing

Optimization of *Anopheles arabiensis* egg production for mass-rearing the malaria vector: effects of cage volume, blood meal source and adult population density on female fecundity

Improving rearing methods while minimizing related costs to produce the maximum possible number of eggs is one of the goals for the efficient production of sterile male adults for timely releases required for a successful area-wide integrated vector control program with an SIT component. To continually produce large numbers of eggs (millions of eggs/day) that could fill several tray-rack larval rearing units on an operational scale, there is a need to fully evaluate the productivity of the mass-production cage for *An. arabiensis* and to quantify how operational parameters affect egg production. We assessed whether egg production would be affected by the size of the adult holding cages, the source of the blood meal, the total number of pupae that could be loaded into the cages and by adding additional pupae to the cage daily. Results demonstrated that it is possible to obtain sufficiently high *An. arabiensis* egg production using the currently available *Anopheles* mass-rearing cages, both large and small versions, when feeding females with either bovine or porcine blood. Egg productivity was significantly reduced when the initial number of pupae loaded into the cages exceeded a threshold of 15 000. However, the addition of further pupae

(total of 30 000) daily to the mass-rearing cage was useful to increase egg production to 1 million eggs while reducing production costs, space and handling time. Results from this study should be incorporated into existing mass-rearing guidelines and taken into consideration when mass-rearing *An. arabiensis* to make the most efficient use of available resources and effectively manage adult rearing cages to meet high productivity goals.

Improvement of *Anopheles* larvae mass-rearing using the larval rearing unit (LRU)

In order to optimize the productivity of the larval rearing unit (LRU), a new protocol has been developed. This reduced the quantity of water per LRU by ¼, the daily work by 2 days, and the larval food by 33% and leading to an optimal synchronization of the pupation. Optimal larval density per tray still needs to be assessed.

Improving *Aedes aegypti* egg production by altering male:female adult ratios in mass-rearing cages

Experiments were carried out to assess productivity of the mass-rearing cages (dimensions of 100x10x100 cm) (Fig. 8) for *Ae. aegypti*. A female to male ratio of 3:1 (12 000 females: 4000 males) was tested and females were blood fed twice per week. A 10% sucrose solution was supplied *ad libitum* and eggs were collected twice per week. Around 650 000 eggs/cage were harvested following the second week of the rearing cycle using the mass-rearing cages.

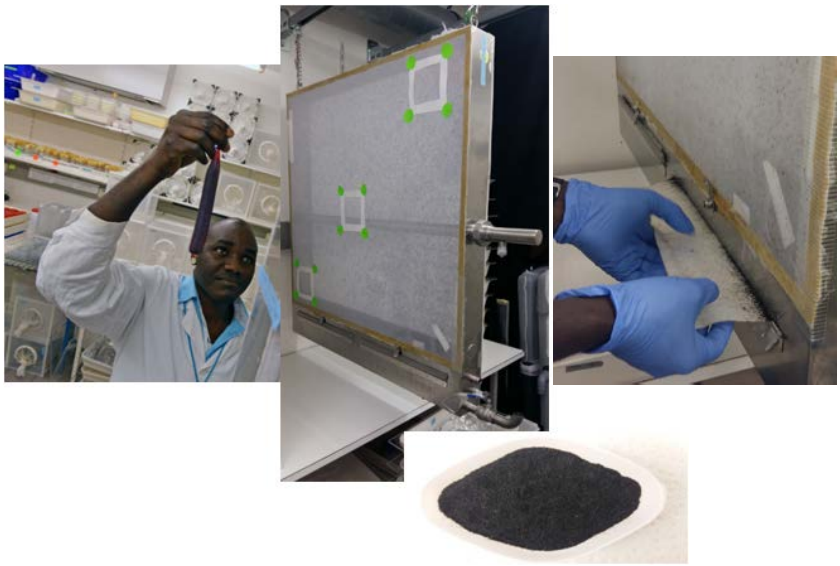

FIG 8: Mass-rearing cages containing males and females (Aedes aegypti*) were fed with fresh pig blood using sausage skins. Egg papers were collected twice per week and kept for maturation and drying under laboratory conditions for 4-7 days*

This improvement will allow for the mass production of sterile males at levels required for operational programs, including the SIT. However, further investigation is needed in order to improve the efficiency of egg collection from the cages.

Effect on *Aedes aegypti* egg collection frequency on mass rearing cage egg yield

In order to find the most cost-effective egg collection in mass-rearing cages , 300 females and 100 males (ratio 3:1) were reared in Bugdorm 30x30x30 cm insect cages. Females were fed on two consecutive days and egg were collected either daily, or twice or once per week. No difference in mean number of eggs per female was seen between daily collections and twice per week, but more eggs were collected when egg papers were collected only once per week, suggesting that pre-existing eggs could stimulate egg laying and result in a higher egg yield per cage.

Viral screening of IPCL mosquito colony

To ensure adequate biosafety for the staff handling and working with mosquito colonies at the IPCL, it is important to assess any potential viral load in the colonies, especially when new colonies are developed. There are two main methods that can be used for the viral screening: RNA extraction and RT-qPCR, a process which uses fluorescence to identify levels of viral gene expression in mosquito samples. Both *A. aegypti* and *Aedes albopictus* were screened for dengue virus (DENV1 and 2), Zika

virus, chikungunya virus and West Nile virus and it was shown that all the examined samples were negative for these pathogens.

Post irradiation handling, transport and release of male mosquitoes

Optimal conditions for the handling and transport of sterile male mosquitoes, prior to their release, are critical for the SIT to be successful. During the last two years, research has been addressing this as part of a Coordinated Research Project entitled 'Mosquito handling, transport, release and male trapping methods'.

The effect of chilling male mosquitoes at different temperatures and for various lengths of time was assessed with regard to survival. Subsequently, a suitable temperature range was determined within which male mosquitoes can be maintained immobile during transport without detriment to their survival. In anticipation of a dosed aerial release approach for the release of adult sterile male mosquitoes, several parameters have been assessed including calculating the weights and volumes of various densities of males to determine a suitable batch size. Following this, different shapes and sizes of release cassettes were produced, based on the same internal volume and tested to assess the effect on mosquito survival. There was no significant impact on mosquito survival from the process of chilling, packing the canisters or between different canister shapes. Compacting males within the canister was also found not to have a significant effect upon mosquito survival when compared to canisters where compaction was not imposed. Preliminary studies have also led us to conclude that immobile male mosquitoes are not capable of producing heat metabolically when maintained compacted within a release cassette.

It is crucial to monitor sterile males after release and to be able to distinguish them from wild males and females when collected in traps. A standardised protocol for fluorescent dust marking has therefore been developed. An optimal dust weight has been determined with no significant effect on male longevity. The presence of the mark lasted for upwards of 30 days and the immobilisation of dusted males did not reduce the presence of dust.

Fighting future threats using autonomous aerial robotics

In mid-2016, the IPCL, together with the American humanitarian organization WeRobotics and the UAV platform developer Vayu, joined forces and entered a call by USAID for funding under the Combating Zika and Future Threats Grand Challenge. Our concept involved developing a system capable of releasing sterile male mosquitoes aerially from a UAV platform. Currently, there is no aerial platform available capable of releasing sterile male mosquitoes and this is one of the major bottlenecks preventing the SIT from reaching operational level,one which has been overcome with other insect pests. We aim to develop a system that is compatible with different UAV platforms to ensure that our release mechanism can be used worldwide.

Genetics and Molecular Biology

The combined SIT/IIT approach as a tool for the population suppression of Aedes *mosquito species transmitting dengue, chikungunya, Zika and yellow fever viral pathogens*

Aedes aegypti and *Ae. albopictus* are important vectors of human pathogenic viruses, including dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV). The diseases associated with these viruses are still a major human health problem in over 100 countries, and have an enormous economic impact. There are no efficient, safe and inexpensive drugs and/or vaccines to control these diseases, and the extensive use of insecticides to control the vectors has resulted in resistance to all major groups of insecticides, while it is almost impossible to eliminate all larval breeding sites throughout urban and

suburban areas. So, there is an urgent need for novel, sustainable and environment-friendly approaches for controlling populations of *Aedes* mosquitoes, such as the SIT. A critical step for mosquito SIT is the separation of males from females (for male-only releases) since elimination of female mosquitoes prior to male releases is essential because females transmit the diseases. In the absence of 100% efficient sex separation methods (or genetic sexing strains), we have proposed the integration of the SIT with the incompatible insect technique (IIT), which is based on the symbiont *Wolbachia* that is known to (a) induce cytoplasmic incompatibility (CI; expressed as embryonic mortality in crosses between infected males and females who lack the *Wolbachia* strain present in males) and (b) to provide protection against some major human pathogens, including DENV, CHIKV, ZIKV and yellow fever virus. The idea behind the combined SIT/IIT approach is that the application of IIT alone has the risk of releasing a few fertile *Wolbachia*-infected females that could result in population replacement instead of population suppression. Application of the SIT alone can be effective, nevertheless in the absence of a perfect sex separation method (currently the sex separation is being performed with Fay-Morlan separators as is the case with other interventions, e.g. transgenic methods), all such suppression approaches have the risk of releasing a small proportion of females. It is also known that in most insect species, including *Ae. aegypti* and *Ae.*

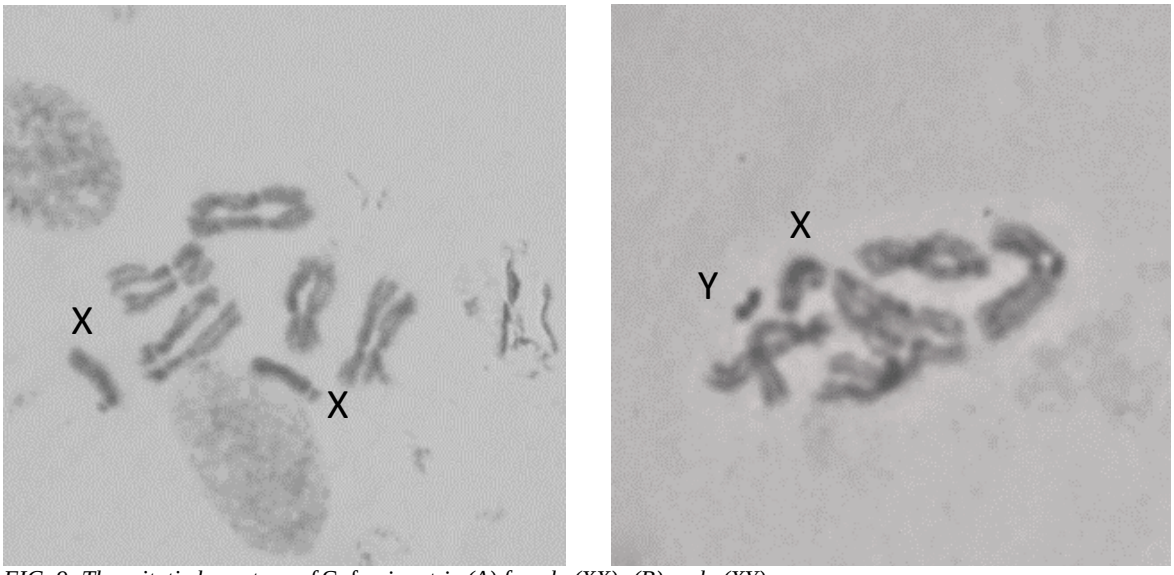

FIG. 9: The mitotic karyotype of C. fasciventris.*(A) female (XX); (B) male (XY)*

albopictus, females can be completely sterilized with irradiation doses that are much lower than those required for the complete sterilization of males. By combining the two approaches, and using a *Wolbachia*-infected *Aedes* mosquito line in which the symbiont provides protection against pathogens like DENV, CHIKV and ZIKV, we can eliminate both risks because even if a few females are released, these females would be unable (or would have significantly reduced ability) to transmit the pathogens due to the presence of *Wolbachia* and they would also be completely sterile, due to the irradiation dose applied. Therefore, no females can become established and breed with the wild population. The combined SIT/IIT approach therefore represents a bio-safe and bio-secure approach for mosquito population control. The proof-of-concept of this approach has been tested at the IPCL, in collaboration with Prof. Zhiyong Xi, and there are ongoing and small-scale open field trials for its validation planned in Brazil, China, Mexico and Thailand.

Contribution to the resolution of the Ceratitis FAR *complex*

The *Ceratitis FAR* species complex is an African complex of economically important agricultural insect pests, belonging to the Tephritidae family. In recent years, coordinated research has provided evidence that support the presence of at least four well-defined species within it (*C. fasciventris*, *C.*

rosa, *C. anonae* and, recently, *C. quilicii*). Data from different research fields accumulatively supported this notion. Cytogenetic and genetic data in general are valuable for the fine resolution of complex species and additional evidence can be provided by nuclear and mitochondrial markers. In this respect, the mitotic nucleus and polytene chromosome maps have been constructed for the first member of the complex, *C. fasciventris* (Figs. 9 and 10), in collaboration with Profs. Antigone Zacharopoulou, Penelope Mavragani-Tsipidou and Elena Drosopoulou. This is only the second member of the *Ceratitis* genus with photographic polytene chromosome maps, following the model species of the Tephritidae family, *C. capitata*. Furthermore, the complete mitochondrial genome of *C. fasciventris* has also been constructed (from the same colony), which can also facilitate genotyping and phylogenetic studies in tephritids (Fig. 12). These two tools can act as reference for further insight in the *Ceratitis FAR* complex, with analysis of more colonies from its entities, aiming to provide diagnostic markers and evidence that can be coupled with the recent taxonomic changes suggested. Species delimitation in the *Ceratitis FAR* species complex is a prerequisite for the development and application of the sterile insect technique, which is a species-specific population control method.

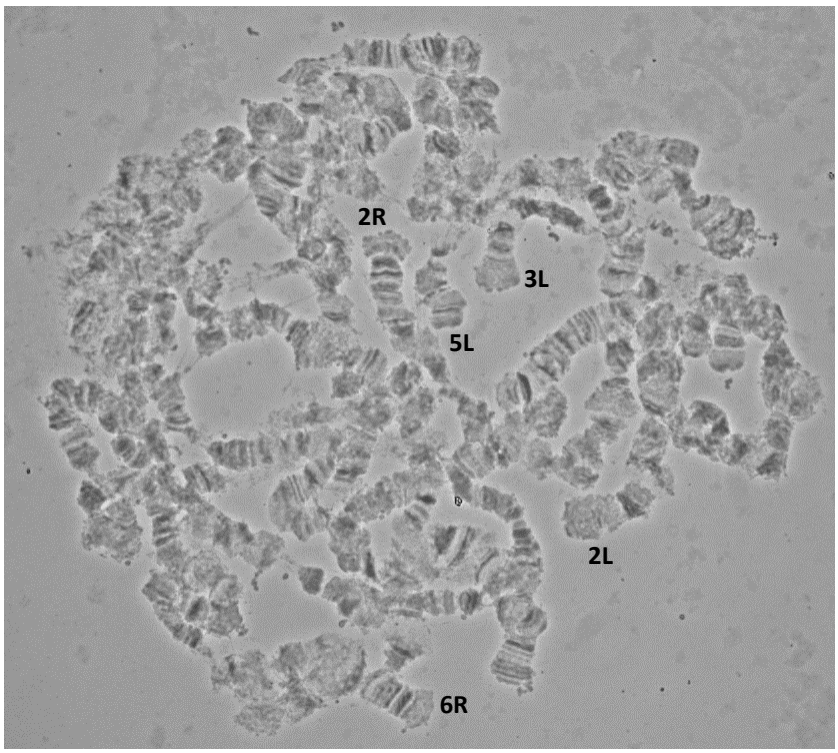

FIG. 10: The polytene complement of C. fasciventris*. The tips of 5 of the 10 polytene arms are marked*

Contribution to the resolution of the Anastrepha fraterculus *species complex*

The *A. fraterculus* complex has a Latin America distribution and is considered a major agricultural pest in the area. Lines of evidence from many different research fields have unravelled at least seven distinct taxa in the complex that could represent an equal number of discrete species. The understanding that speciation can be driven through a variety of forces that are not mutually exclusive (including chromosomal, geographic and symbiotic factors) leads to the need for additional tools to collaboratively address speciation in this complex of species. The documented presence of the reproductive symbiont *Wolbachia*, a bacterium known to be involved in speciation and restriction in gene flow and with the tendency to 'masking' the mitochondrial-derived phylogenetic signal, in all natural populations studied so far suggests the need for alternative experimental approaches to clarify the relationships among the members of this species complex. Species delimitation in the *A. fraterculus* species complex is a prerequisite for the development and application of the SIT and we have been contributing to this goal in different ways: (a) in order to understand the effect of *Wolbachia* on the restriction of gene flow among the different morphotypes of the complex, the *Wolbachia* status of colonies representing different morphotypes of the complex has been studied. Analysis shows that all colonies are 100% infected. Based on MLST analysis, colonies from Brazil (Piracicaba, Parnamirim, Vacaria), Argentina (Tucuman), Mexico and

Peru were single infected with the same *Wolbachia* strain, while the colony from Colombia was single infected with a different strain. Screening for additional reproductive parasites, such as *Spiroplasma*, *Arsenophonus*, *Cardinium* and *Rickettsia* was negative for all *A. fraterculus* colonies. To shed further insight, colonies from Piracicaba, Parnamirim, Vacaria and Peru were treated with tetracycline that effectively removed *Wolbachia* (Fig. 11), thus creating *Wolbachia*-free lines. These lines are currently being used in IPCL in mating compatibility and competitiveness experiments; (b) the complete mitochondrial genomes of the different morphotypes are currently being constructed, using Sanger sequencing and next generation sequencing approaches and (c) in collaboration with Profs. Antigone Zacharopoulou, Penelope Mavragani-Tsipidou and Elena Drosopoulou, and Drs María Cecilia Giardini, Silvia B. Lanzavecchia and Jorge L. Cladera, the polytene chromosome maps of the *A. fraterculus* Af. sp.1 member have been constructed (Fig. 12). This is the first polytene chromosome map for a member of the *A. fraterculus* complex and only the second map available for the *Anastrepha* genus, following the one of *A. ludens*. The existence of workable polytene chromosome maps can: i) support phylogenetic studies within and outside the *A. fraterculus* complex, ii) reveal chromosomal rearrangements that have either facilitated or accompanied speciation in the complex, iii) contribute to the development and characterization of genetic sexing strains (following the *C. capitata* and *A. ludens* paradigm) and iv) support on-going genome projects in the complex through in situ hybridization and mapping of selected sequences.

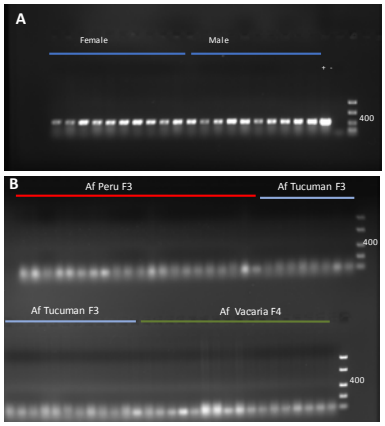

FIG. 11: Screening for Wolbachia *through PCR amplification of a* Wolbachia*-specific 438 bp amplicon of the 16s rRNA gene, using the wspecF and wspecR primer pair (A) 20 individuals from the* A. fraterculus *colony derived from Colombia, where the expected PCR product is amplified. This fragment was sequenced and verified as* Wolbachia*-specific; (B) absence of PCR amplification of the 438 bp fragment in the colonies treated with tetracycline.*

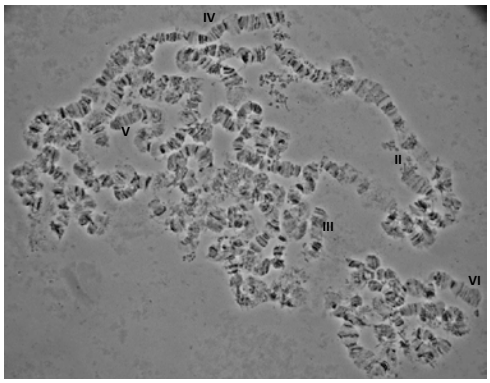

FIG. 12: A polytene chromosome nucleus of the A. fraterculus *Af. sp.1 member of the complex. For details see Gariou-Papalexiou et al., 2016 (PLoS One).*

Laboratory evaluation of the Mediterranean fruit fly VIENNA genetic sexing strains used in mass-rearing facilities worldwide

The GSS of the Mediterranean fruit fly are considered as a model for SIT applications and they are currently used in all mass-rearing facilities worldwide that have projects with an SIT component against this pest. These strains were developed through classical genetics, by linking the wild type alleles of the *temperature sensitive lethal* (*tsl*) and *white pupae* (*wp*) loci with the part of the Y chromosome that is responsible for the male determination (maleness factor). The last generation of these strains, the VIENNA 7 and the VIENNA 8, are those currently utilized in all facilities. The incorporation of the D53 inversion in the VIENNA 8 strains minimizes the risk of recombination events that could destroy the 'sexing' character of the strain. Although deriving from the same parental strains, the long-standing isolation among some of them and the different rearing practices in the facilities may have led to accumulation of differences that can affect their performance and effectiveness in the field. Minor changes cannot be easily recognized and deleterious effects of laboratory domestication cannot be detected upon emergence unless universalized, routine, easy to

apply Quality Control protocols are used. The IPCL has been using two protocols to ensure the stability and quality of these strains: the 'tsl' test and the '40 ml pupae' test. All VIENNA GSS from the mass-rearing facilities were re-introduced in the IPCL and were screened with the 'tsl' test. At the same time, their cytogenetic profile was evaluated through polytene chromosome analysis.

This analysis verified the stability of the sexing character of these strains and provided some interesting findings, such as important differences in hatch, pupation and emergence rates, both under standard (25°C) and elevated (31-35°C) temperatures (Fig. 13). As an example, the VIENNA 8 D53+ strain derived from Israel exhibited very high hatch, pupation and adult emergence rates (Fig. 13). Such findings need to be followed up and support the need to apply these protocols in the same way in all mass-rearing facilities. Comparison of the results can show whether specific rearing practices are beneficial or harmful for the VIENNA GSS, recognize putative deleterious effects upon emergence, understand their nature and help to select the most appropriate option to overcome them.

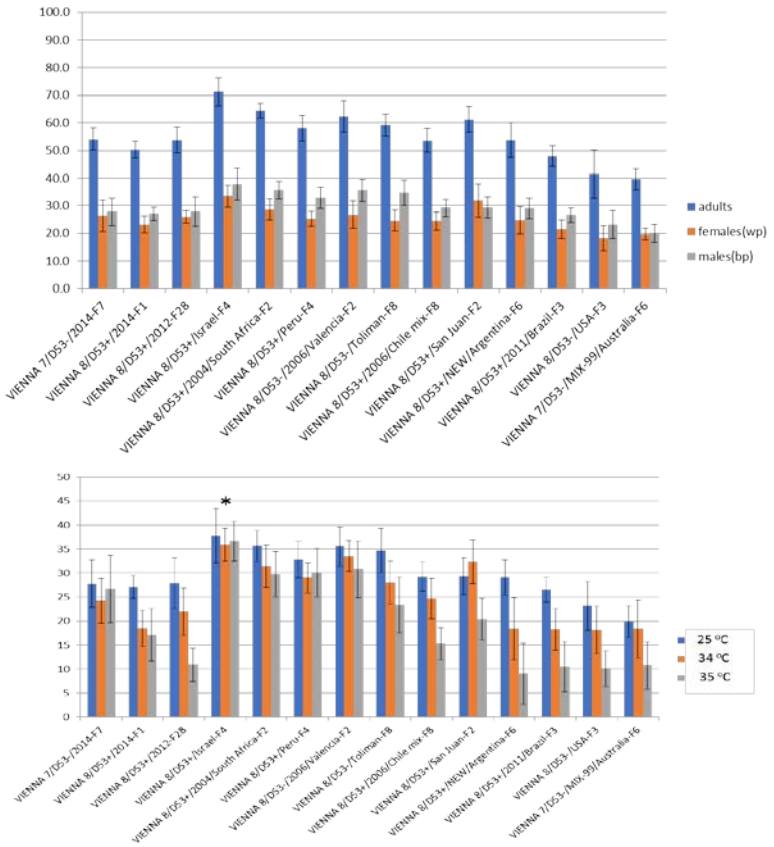

FIG. 13: (top) Adult emergence rates (per 100 laid eggs) at 25°C. Bars represent the mean of 9 replicas (100 eggs each) and standard deviation bars are also shown; (bottom) Male emergence (per 100 laid eggs) at 25, 34 and 35°C. Bars represent the mean of 9 replicas (starting from 100 laid eggs each) and standard deviation bars are also shown. In both top and bottom, note the increased recovery rates of the VIENNA 8 D53+ from Israel, marked with an asterisk (*)

The challenge of laboratory adaptation of SIT targeted insect pest populations – monitoring changes at the genetic and symbiotic level

Adaptation of natural populations to laboratory conditions can bring several genetic changes, due to intense selective pressure and low founding population size in the first generations that may result in high inbreeding, genetic drift and bottleneck effects. Although such changes are documented for some Tephritid species, there are no studies up to now that follow laboratory domestication both at the genetic and symbiotic levels. The monitoring of these changes can be beneficial for the SIT in two different aspects: first, to follow up the genetic and symbiotic profile of strains used (or to be used) in SIT applications and, second, to record the profile of the natural populations that are introduced in the laboratory and are being used as 'wildish' material in experiments that aim to measure the mating competitiveness of the laboratory strains that are of SIT importance. Knowledge of the genetic and symbiotic changes can be used along with the established quality control protocols that are currently applied. Using Mediterranean fruit fly as a model, the laboratory adaptation of a natural population from Greece was followed for 12 generations after its domestication using two different larvae oviposition substrates and larvae diets. After its

introduction in the IPCL, this population was split in two and was given either perfumed domes for oviposition, followed by feeding of larvae on artificial carrot diet or bananas both for oviposition and larvae feeding.

Gut samples were collected for selected generations (F0-F3, F6 and F10) from 3rd instar larvae and adults of different ages (1 day, 5-10 and 15-20 days old) and sex (males and females separately). In collaboration with Dr George Tsiamis, analysis of the gut symbiotic communities was performed with next generation sequencing of the 16S rRNA gene and revealed a higher gut symbiotic diversity of the wild population, in respect to previous studies in long-established medfly laboratory populations. Our data indicate that the degree of domestication, the oviposition and larvae feeding substrates, the developmental stage and, to some extent, age, are important parameters that influence the structuring of the gut symbiotic communities (Fig. 14). Such changes may be important both for the improvement of fitness and competitiveness of laboratory reared strains and for the interpretation of the results derived from mating competitiveness experiments among laboratory and wildish populations.

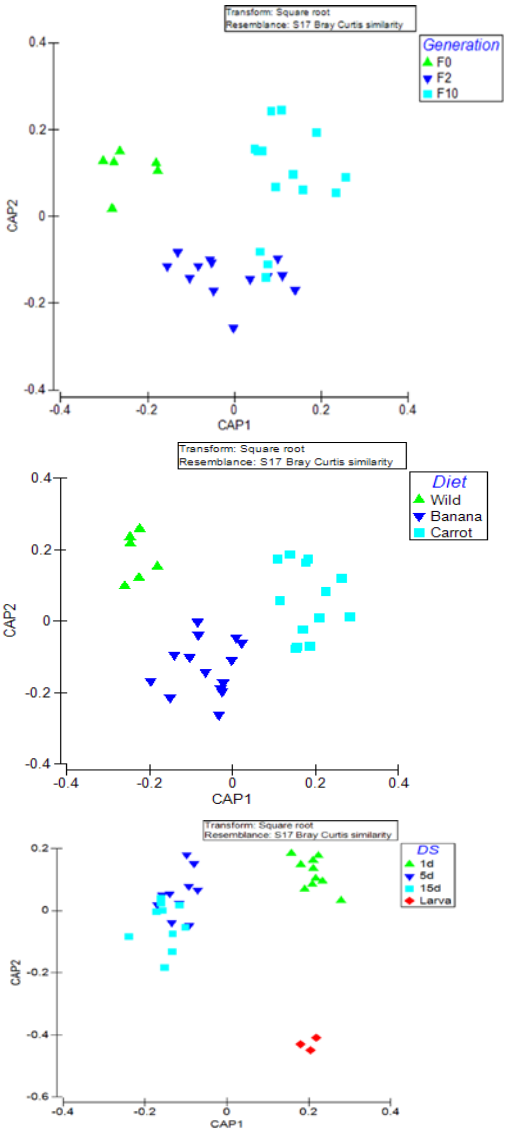

FIG. 14: (upper) Canonical analysis of principal coordinates (CAP) addressing the importance of the degree of adaptation (generations in the lab) in the structuring of the gut symbiotic communities, (middle) CAP addressing the importance of the oviposition and larvae feeding substrate in the structuring of the gut symbiotic communities, (lower) CAP addressing the importance of the developmental stage (3rd instar larvae vs adults) and the age (1 day vs 5-10 days vs 15-20 days old adults) in the structuring of the gut symbiotic communities

Effect of the hot bath-irradiation treatment and Wolbachia *on the gut symbiotic communities of the VIENNA 8 D53+ strain*

The VIENNA 8 D53+ is the most widely used Mediterranean fruit fly *Ceratitis capitata* GSS in mass-rearing facilities that are involved in SIT applications. It belongs to the last generation of Mediterranean fruit fly GSS and the strain carries both the T(Y;5)52A translocation (responsible for the sexing character of the strain) and the D53 inversion (that improves the stability of the strain through restricting genetic recombination). Males of this strain 'undergo' two specific stresses before release in the field. The first stress is the 'hot bath' (HB) treatment, which is applied to 24 h old eggs for at least 24 h and that kills all female embryos, as these are sensitive to elevated temperatures. In mass-rearing facilities, the HB treatment is applied by putting the eggs in a water bath under constant oxygenation. Later, at the pupal stage (two days before emergence), these males are irradiated and then released in the field. A similar approach, which can be combined with the SIT is the IIT that is based on reproductive symbionts such as *Wolbachia*. In this respect, the VIENNA 8 D53+/56S2 strain

has been constructed, which is in principle the VIENNA 8 D53+ strain that also harbours the wCer2 *Wolbachia* strain.

Symbiotic communities and especially gut symbionts are important for the fitness and behaviour of insects and their overall performance. Introducing a symbiotic factor such as *Wolbachia* may affect the symbiotic communities in general, and the heat shock treatment and irradiation stresses may also interfere with them. To address these concerns, gut samples were collected from both VIENNA 8 D53+ and VIENNA 8 D53+/56S2 flies of different ages. Four different samples, males (M) and females (F) were collected: NT (no stress applied), HB (only heat shock, applied), I (only irradiation applied) and HBI (both heat shock and irradiation applied), with (W) and without (NW) *Wolbachia*. Since the HB treatment eliminates female embryos, the only samples collected for females are the untreated control groups and only irradiated groups. Samples were analysed using next generation sequencing of the 16S rRNA gene. Analysis provided some interesting findings, such as a) the gut symbiotic communities of the VIENNA 8 D53+ and VIENNA 8 D53+/56S2 strains were different and differentially affected by the stresses applied and b) the HB treatment seems to have a more severe effect than the irradiation stress with respect to the structure of the gut symbiotic communities (Fig. 15).

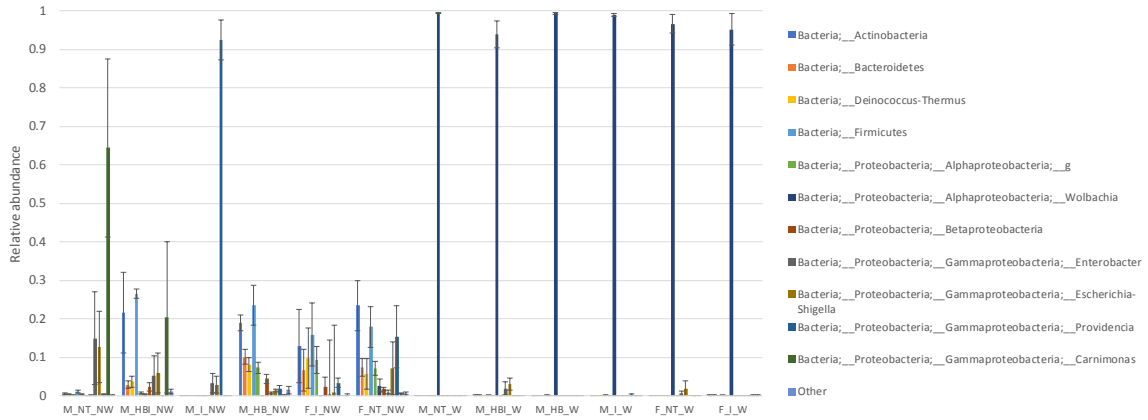

FIG. 15: Relative abundance of symbiotic communities of VIENNA 8

CAPACITY BUILDING AND SERVICES

In 2016, the IPCL hosted eight cost-free experts (CFE), 12 consultants (C), 12 interns, 13 fellows (F) and four scientific visitors (SV) (the latter two categories funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
ABDELAZIZ ABBAS, Ramadan	Egypt	Intern	4 mth	Hybridization of tsetse
PROTOLIPAC, Katharina	Serbia	Intern	10½ mth	Tsetse fly rearing
AGUIAR MASET, Bruno	Brazil	Intern	1¼ mth	Post harvest treatment of fruit flies
JIANG, Fan	China	Intern	12 mth	Fruit fly endosymbionts
KALANTAROW, Inessa	Israel	Intern	3 mth	Endosymbionts tsetse flies

Name	Country	Status	Duration	Topic
DIEUDONNE, Diloma	Burkina Faso	Intern	6 mth	Mosquito rearing
CARAVANTES, Silvana	Guatemala	Intern	11½ mth	Fruit fly rearing
JUAREZ, Jose Guillermo	Guatemala	Intern	5½ mth	Radiation studies mosquitoes
KRUPA, Frank	Austria	Intern	4 mth	Virus work mosquitoes
KOSKINIOTI, Panagiota	Greece	Intern	4 mth	Microinjections mosquitoes
COUTINO MORENO, David	Mexico	Intern	4 mth	Post harvest treatment of fruit flies
WANG, Lincong	China	Intern	9 mth	Post harvest treatment of fruit flies
YAMADA, Hanano	US	C	6 mth	Mosquito mass-rearing
MEZA, Salvadore	Mexico	C	6¾ mth	Characterisation of fruit fly GSS on mass rearing conditions
LEES, Rosemary	UK	C	5 mth	Mosquito mass-rearing
WADAKA, Mamai	Cameroon	C	11¾ mth	Developing mass rearing tools for mosquitoes
CARVALHO, Danilo	Brazil	C	12 mth	Development of GSS for mosquitoes
MAIGA, Hamidou	Burkina Faso	CFE	11½ mth	Developing mass rearing tools for mosquitoes
TARET, Gustavo	Argentina	CFE	10½ mth	Developing mass rearing tools for *Drosophila suzukii*
BIMBILE, Severin	Burkina Faso	C	10 mth	Developing mass rearing tools for mosquitoes
CULBERT, Nicole	UK	C	12 mth	Quality control mosquitoes
ZACHAROPOULOU, Antigone	Greece	C	3 wks	Cytogenetics fruit flies
DEVESCOVI, Francisco	Mexico	C	1½ mo	Rearing fruit flies
DEMIRBAS, Güler	Turkey	C	12 mth	Endosymbionts tsetse flies
AVGOUSTINOS, Antonios	Greece	CFE	6 mths	-Fruit flies

Name	Country	Status	Duration	Topic
BALESTRINO, Fabrizio	Italy	C	7½ mth	Mosquito rearing
KARIITHI, Henry	Kenya	CFE	5 mth	Tsetse flies
SASSU, Fabiana	Italy	CFE	5¾ mth	Fruit flies
NIKOLOULI, Katerina	Greece	CFE	5 mth	Fruit flies
HALLMAN, Guy	USA	CFE	12 mth	Fruit flies
LYRAKIS, Emmanouil	Greece	C	2½ mth	Fruit flies
RAS, Erica	Netherlands	CFE	12 mth	Olive fly endosymbionts
NOMAN, Kheder	Sudan	F	11 d	Mosquitoes
VENTER, Cornelius Johannes	South Africa	F	2 mth	Mosquitoes
SABAWE, Amel	Sudan	F	3 mth	Mosquitoes
CHITAMBO, Andrew	Zimbabwe	F	10 d	Irradiation/dosimetry
BHOYROO, Reena Devi	Mauritius	F	10 d	Fruit flies GSS
SYLLA, Mamadou	Burkina Faso	F	10 d	Irradiation/dosimetry
SAWADOGO, Sibire Laurent	Burkina Faso	F	10 d	Irradiation/dosimetry
TOE, Ange Irenee	Burkina Faso	F	10 d	Irradiation/dosimetry
PAGABELEGUEM, Soumaila	Burkina Faso	F	10 d	Irradiation/dosimetry
PODA, Aristide Belangta	Burkina Faso	F	10 d	Irradiation/dosimetry
DEMBELE, Seribe	Burkina Faso	F	10 d	Irradiation/dosimetry
JAVIER, Abigaile Mia	Philippines	F	2 mth	Mosquitoes
MUKARAKATE, Trymore	Zimbabwe	F	10 d	Irradiation/dosimetry
AHMED, Fayez Tag Elsir Ali	Sudan	SV	5 d	Mosquitoes
AGEEP, Tellal Babiker	Sudan	SV	5 d	Mosquitoes
MOHLOBOLI, Maleoa Christina	Lesotho	SV	2 d	Mosquitoes
HAPUGODA, Menaka Dilani	Sri Lanka	SV	5 d	Mosquitoes

In 2016, the Plant Pest group maintained 99 different fruit fly species and strains and the Genetics and Molecular Biology group maintained 11 different species and 191 mutant strains. In total, the two groups delivered 41 shipments of live fruit fly insects to 13 different institutions in Canada, Czech Republic, France, Greece, Italy, Mauritius, Senegal, South Africa, Spain, Sweden and the UK. Seven shipments of preserved fruit flies were sent to seven institutions in Australia, Belgium, Peru, South Korea and the USA.

The Livestock Pest group delivered 67 shipments of live tsetse insects (of which 59 shipments to Senegal of 156 000 *G. palpalis gambiensis* pupae and to 6 different institutions in Belgium, Germany, Senegal, Uganda, Zimbabwe. Seven shipments of DNA samples were shipped to three institutions in China, Germany and South Korea.

The Human Disease Vectors group maintained three *An. arabiensis* strains, four *Ae. aegypti* and five *Ae. albopictus* strains, and two different strains of *Ae. albopictus* (China) carrying different type of *Wolbachia* infection. The group sent four shipments of live mosquitoes to three institutions in Germany, the UK and USA.

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THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

Nuclear techniques are powerful tools to induce genetic variation in plants; they have enabled the development of superior varieties with higher yields, tolerance to plant diseases and greater resilience to climate change worldwide.

The Plant Breeding and Genetics Laboratory (PBGL) assists plant breeders in Member States to develop superior crop varieties using nuclear techniques such as gamma and X-rays and ion beam. These efforts are supported by latest advances in genomics and in *in vitro* tissue culture to enhance the efficiency and broaden the scope of crop mutation breeding.

In 2016 the PBGL focussed on following activities: (i) optimize and streamline methods to identify natural or induced variation in genes encoding desirable traits, intended to facilitate genotypic selection of specific traits (reverse genetics); (ii) initiate pilot projects in sorghum and barley to develop molecular markers and protocols facilitating rapid introgression of desirable mutant traits into elite germplasm; (iii) further R&D on mutation discovery using whole genome sequencing approaches to aid mutant induction and detection protocols; (iv) human and institutional capacity building of Member States in the design and implementation of crop mutation breeding, including efficiency-enhancing *in vitro* tissue culture and genomics tools; and (v) provide technical backstopping and mutation induction services to Member States where appropriate facilities are not available.

Mutant selection is a key step in crop mutation breeding, typically involving multi-location field trials for phenotyping and mutant line development. Methods to uncover nucleotide variation in desirable traits could enhance the efficiency of mutation breeding through selection for mutations in specific, predetermined genes. In 2016, PBGL further optimized protocols and processes to advance such 'genotypic selection'. Using amplicon-based sequencing, sequence variations have been successfully identified in herbicide tolerance and starch biosynthesis genes in cassava and in drought tolerance related genes in barley. The methods are reliable and high throughput, allowing the screening of populations comprising several thousand individuals. These methods could be applied to priority traits and crops of Member States for which appropriate genetic and genomics resources are available.

PBGL also initiated a programme for the development of molecular markers for important mutant traits to facilitate their wider utilization by Member States. The initiative started with pilot examples in food security crops, such as sorghum, to establish the protocols and will then gradually expand to other priority crops and traits of Member States. The sorghum project focuses on semi-dwarfism and early maturing for higher yield and to enhance stay-green at maturity useful for tolerance to terminal drought. Preliminary high-throughput sequencing data reveal the presence of large deletions on different chromosomes. Crosses have been initiated to create segregating populations for fine mapping, linkage analysis and subsequent marker development. In barley, a candidate gene approach is followed to develop markers linked to a reduced lignin content trait useful as animal feed. Different point mutations have been identified that are likely candidates for the reduced lignin content phenotype. This project supports the coordinated research project (CRP) D2.30.30 on 'Integrated Utilization of Cereal Mutant Varieties in Crop/Livestock Production Systems for Climate-Smart Agriculture'.

The PBGL continued its R&D on mutation discovery using whole genome sequencing. In rice and tomato, the data show widespread occurrence of point mutations and small insertion/deletions (InDels) in gamma and X-ray irradiated mutants, in addition to large deletions and other structural variants. In an officially released mutant banana variety, a large copy number variation was identified, reducing the ploidy level from three to two over a large portion of one chromosome. This

data provides insights into the type and distribution of mutations induced by gamma and X-rays and can aid the development of protocols for mutation induction and detection.

Since 2014, the PBGL has pioneered mutation induction techniques in coffee that led to the formulation of CRP D2.20.05 on 'Efficient Screening Techniques to Identify Mutants with Disease Resistance in Coffee and Banana'. In 2016, the PBGL continued coffee mutation induction experiments and produced a training manual on 'Training Course on Mutation Induction in Coffee', to share protocols and experiences with participants of the Workshop 'Coffee Mutation Induction' held at the PBGL in October 2016.

Further, a protocol book 'Biotechnologies for Plant Mutation Breeding' and 'Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley' have been published in 2016, the former as an output of CRP D2.40.12 'Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline'.

STAFF

Name	Title
Ingelbrecht, Ivan	Laboratory Head
Till, Bradley John[1]	Plant Breeder/Geneticist
Ghanim, Abdelbagi Mukhtar Ali	Plant Breeder
Matijevic, Mirta	Laboratory Technician
Jankowick-Cieslak, Joanna Beata	Laboratory Technician
Hofinger, Bernhard	Laboratory Technician
Berthold, Guenter	Field/Greenhouse Worker
Draganitsch, Andreas[2]	Laboratory Technician
Bado, Souleymane[3]	Laboratory Technician
Mletzko, Joanna Malgorzata	Team Assistant

[1] Separated in December 2016; [2] Retired in May 2016; [3] Separated in March 2016

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

PBGL's R&D focuses on two main areas: mutation induction and mutant selection or screening. Special attention is paid to develop methods and protocols that are tailored to meet the often very specific needs of Member States both in terms of climatic and environmental requirements and of locally available infrastructure.

Mutation induction

Dose optimization for mutation induction in coffee

Coffee, a perennial tropical crop, can be grown from seed or from cloned plants in the form of cuttings, grafts or tissue cultured plants. Arabica coffee is most commonly grown from seeds while canephora (formerly robusta) is mostly grown vegetatively from cuttings and other propagules.

Improving coffee through conventional breeding is seriously limited by the lack of genetic variation. Mutation breeding provides great potential to induce the novel genetic variation needed for coffee improvement. The PBGL has established and validated protocols for mutation induction in numerous seed crops and some vegetatively propagated crops using X-ray and gamma irradiation. Recently the PBGL adapted mutation induction protocols to coffee seeds and vegetative propagules (cuttings and seedlings). During the reporting period the protocols have been completed and compiled in a training manual to be used by Member States in their coffee mutation breeding programme. The process of developing the optimum dose (LD_{50} and LD_{30}) involved series of optimization experiments investigating the patterns of germination and growth rate reduction with increasing dose rate. We used a dose range of 0, 50, 100, 150, 200 and 400 Gy for *Coffea arabica* seeds and 0, 5, 10, 15, 20 and 30 Gy for seedlings and cuttings of both *C. arabica* and *C. canephora*. The protocol for seed treatment follows the general procedure of sorting clean viable seeds, moisture equilibration, irradiation treatments, planting the treated material in suitable containers, such as petri-dishes, transferring to soil in trays or pots and incubating at appropriate environmental condition. Germination and growth rate is recorded after 30 days and plotted relative to the untreated seeds over the series of doses. From the plotted graphs, doses for LD_{50}, GR_{50} (LD=lethal dose; GR, growth reduction) and LD_{30}, GR_{30} are estimated and used for the bulk treatments (Fig. 1). The same was done for vegetative propagules (cuttings, seedling, embryo, etc.) (Fig. 2) Detailed procedures of dose optimization in coffee seeds and vegetative propagules are described in the manual 'Training Course on Mutation Induction in Coffee'.

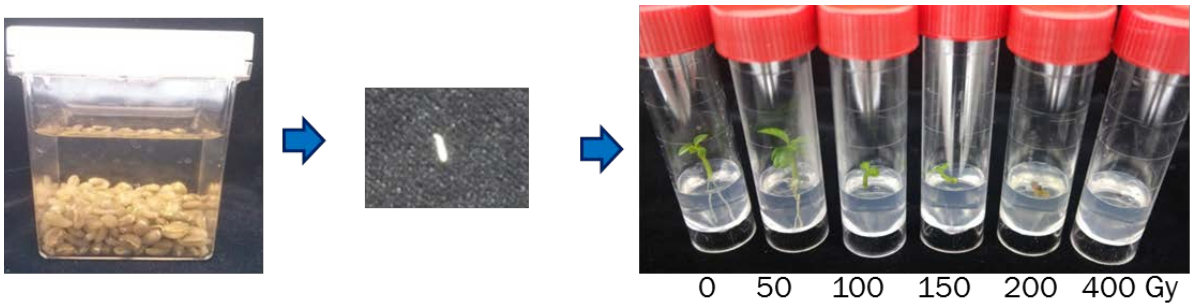


FIG. 1: In vitro *propagation of coffee embryos excised from seeds and treated with different doses of gamma ray (0, 50, 100, 150, 200, 400 Gy) for dose optimization. From left to right; soaked treated seeds, excised embryo, embryos germinated on MS medium 4 weeks after irradiation*

FIG. 2: From left to right: Coffee seedlings collected 58 days after germination for treatment with low doses (0-30 Gy) of gamma ray; gamma cell; seedlings 14 days after treatment; seedlings 28 days after treatment

Mutant selection

Optimizing protocols for gene-target based selection (reverse genetics)

Knowledge of nucleotide variation in genes encoding desirable traits in a mutant population would substantially accelerate mutation breeding programs. Indeed, genotypic selection could serve as a pre-field screen to narrow down the number of candidate mutants for further phenotypic screening. In addition, knowledge of nucleotide variation in germplasm core collections or elite germplasm prior to mutagenesis can guide innovative approaches for mutation breeding programs.

Advances in high-throughput sequencing technologies enable the rapid evaluation of nucleotide diversity in large germplasm collections. Crop mutation breeding typically involves the screening of large mutant populations comprising many thousands of individuals. Projects of this scale are often too expensive for widespread adoption by Member States and require a high level of technical expertise. Therefore, strategies and protocols are required to reduce cost and enhance throughput.

In addition to increasing DNA sequencing throughput using high-throughput sequencing, sample pooling strategies can further reduce cost provided mutations with low-frequency can still be detected. Such pooling strategies can be efficiently integrated with high-throughput sequencing platforms.

In 2016, PBGL developed the necessary in house expertise and set up cost-effective platforms for fast and precise mutation selection based on screening for sequence variations in genes of interest to plant breeders (reverse genetics). Specifically, the PBGL further improved methods to facilitate high-throughput discovery of natural and induced variations present in preselected genes in large populations (>1,500 individuals) in seed (barley) and vegetatively (cassava) propagated crops.

The work on cassava focused on the discovery of natural polymorphisms in genes underlying desirable traits, while the work on barley aimed at the discovery of single-nucleotide polymorphisms (SNPs) induced through chemical mutagenesis. The work on cassava was carried out in collaboration with Dr H. Ceballos (International Center for Tropical Agriculture, CIAT, Colombia) and with financial support from the Colombian grant agency, COLCIENCIAS.

Dr Ceballos provided the PBGL with 1,728 DNA samples prepared from a core collection of cassava cultivars and landraces, with as objective to identify sequence variants in genes involved in starch biosynthesis and herbicide tolerance, two key traits for cassava improvement. A total of 93 primer pairs were designed. Computational selection and recovery of variants was performed with a bioinformatics pipeline that was set up in PBGL in 2016 with assistance from Mr P. Gupta (University of Hyderabad, India). Using the optimized procedure, over 7,000 SNPs and InDel variants were identified. The optimized pipeline for genotypic selection is summarized in Fig. 3.

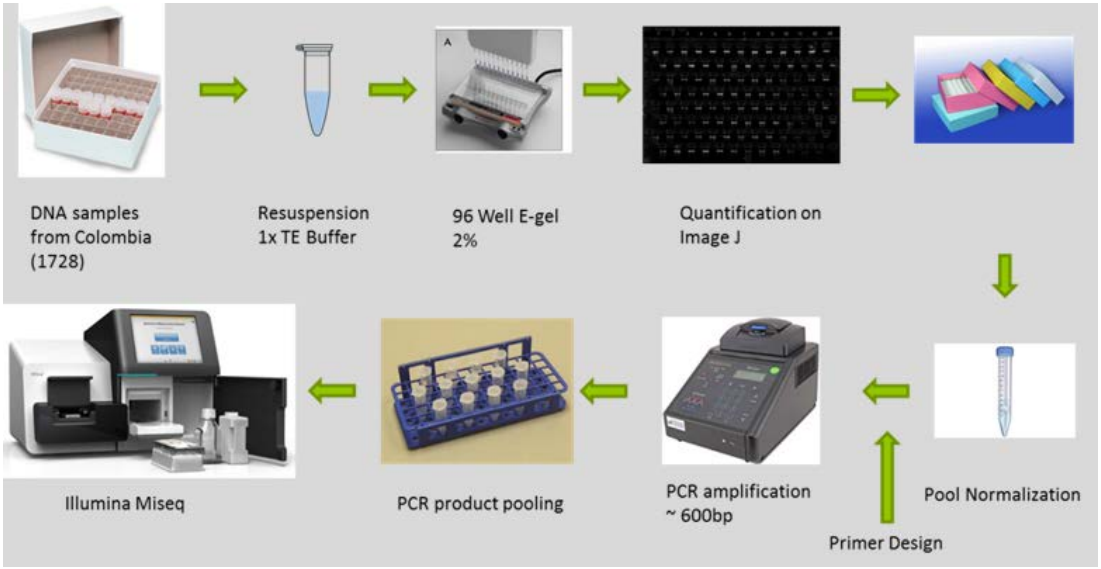


FIG. 3: Pipeline for the discovery of SNPs and small InDels developed at the PBGL illustrating the various steps in the process: genomic DNA extraction, sample pooling, PCR amplification, DNA sequencing and bioinformatics analysis for identification of sequence variants

These methods were further enhanced and validated in a project aimed at identifying SNPs in a large mutant barley population. This work was carried out with assistance from Ms K. Gajek (Silesia University, Poland) who provided a barley mutant population comprising ~4,000 individuals. This

population had been previously developed within CRP D2.40.12 on 'Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline' and had been screened for induced mutations in drought tolerance related genes using traditional TILLING approaches. Optimization included the preparation of libraries for high-throughput sequencing to increase the size of the amplicons (Fig. 4). Using the optimized procedure, both the population size, i.e. the number of accessions that can be screened, as well as the size of the amplicons could be significantly increased.

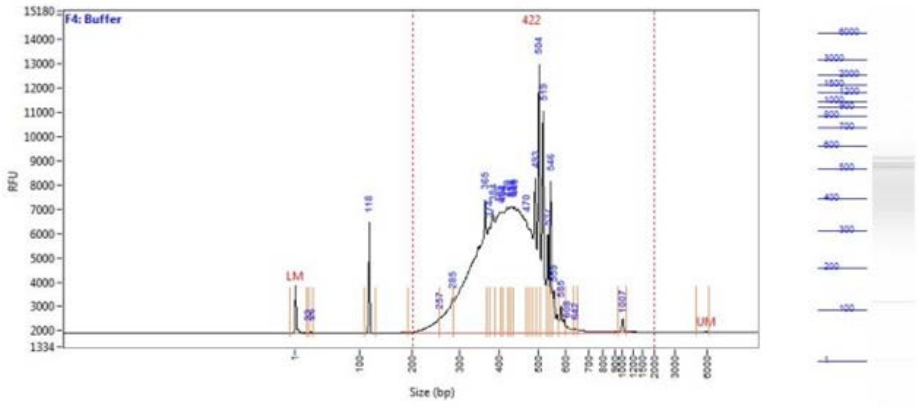


FIG. 4: Validation of barley libraries of sonicated amplicons. For library quantification Fragment Analyzer was used (protocol: High Sensitivity NGS Fragment Analysis Kit (1bp-6000bp)). The analysis was performed by Ms K. Gajek (Silesia University, Poland), a PhD student and participant of the CRP D2.40.12

This reverse genetics approach for genotypic selection of mutations can be adapted to other crops and traits. We are now evaluating options to apply this strategy to the ongoing CRPs D2.20.05 on 'Efficient Screening Techniques to Identify Mutants with Disease Resistance in Coffee and Banana' and D2.50.05 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security'.

Marker development and trait genetics

The PBGL has initiated a programme for development of molecular markers for important mutant traits to facilitate their wider utilization by Member States. The initiative started with pilot examples in food security crops, such as sorghum, to establish the protocols and will gradually expand to other priority crops and traits. The approach is expected to enable wider utilization of available useful mutant germplasm and is a first step towards establishing an integrated molecular breeding platform to support priority crops and traits of Member States.

Marker development for a gamma-induced semi-dwarf and early maturing trait in sorghum

A semi-dwarf and early maturing mutant trait in sorghum was chosen in the pilot phase of marker development. The mutation was induced by gamma irradiation in a tall farmer-preferred sorghum variety, Wad Ahmed, from Sudan. The trait is recessive and assumed to be controlled by a single gene. The mutant is useful as an agronomically important trait for semi-dwarf plant height that reduces loss in yield due to lodging, enhances response to fertilizer application, facilitates mechanized combine harvesting in large farming systems and is a critical trait in hybrid sorghum breeding. The mutant is also associated with early maturity and enhances stay-green at maturity, which is useful for tolerance to terminal drought and in forage sorghum production. Based on the effect of similar mutations in other cereals, the mutant is expected to improve yield, maximize crop potential and secure production in terminal drought prone areas. Since the semi-dwarf trait is recessive, development of a functional marker will facilitate rapid introgression of the mutant trait widely into farmer-preferred open pollinated varieties and inbred lines for hybrid production.

In 2016, six fairly homogenous M_6 lines were planted together with the wild Wad Ahmed parent in the PBGL field (Fig. 5).

FIG. 5: Mutant sorghum lines with wild parent in the field of PBGL, Seibersdorf, Austria used for the training of fellows from Member States on mutant line development, crossing, phenotyping and development of molecular markers. The six M_6 lines showing varying degrees of dwarfism are shown on the right.

The material was phenotyped for the mutant traits plant height and flowering (Table 1) and associated agronomic characteristic, such as biomass, etc. Sample DNA was collected from the parental and mutant lines. The D2 line was sequenced along with the parental line using the MiSeq Illumina platform at the PBGL. Preliminary bioinformatics analysis suggests the presence of large deletions on different chromosomes as well as numerous putative smaller sequence variants.

Genotype	Plant height	Days to flowering
Wild parent	121	116
D1	87	108
D2	74	106
D3	89	108
D4	88	106
D5	81	102
D6	94	105

Table 1. Comparison of plant height and days to flowering for sorghum mutant lines and parent

Bulked segregant analysis in combination with whole genome sequencing has recently been successfully used to identify causative sequence variants in soybean fast neutron mutants. We envisage following a similar approach and have initiated inter-crossing to produce segregating populations for linkage analysis, allelism tests and subsequent marker development.

The availability of a marker would allow reducing the time for introgression of the trait in a backcross breeding program as it would obviate the need for selfing after each backcross to enable identification of individuals carrying the recessive gene to be further backcrossed to the recurrent parent. Thus, in a typical backcross scheme of 8–10 generations of crossing and selfing, the number of generations can be reduced by half to 4–5 generations. Furthermore, combining marker selection

with PBGL's rapid cycling cultivation protocol in sorghum (allowing four cycles per year) introgression can be achieved in ~2 years, a significant gain in time.

This project may link with the ongoing CRP D2.30.30 on 'Integrated Utilization of Cereal Mutant Varieties in Crop/livestock Production Systems for Climate-smart Agriculture' and the planned CRP on drought..The material might also be useful in the context of CRP D2.50.05 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security'.

Marker development for a reduced lignin mutant trait in barley

The orange lemma mutation (*rob1*) in barley results in reduced lignin content and has applications for animal feed due to its higher digestibility. Under CRP D2.30.30 on 'Integrated Utilization of Cereal Mutant Varieties in Crop/livestock Production Systems for Climate-smart Agriculture', *in vitro* studies using the Hohenheim gas test and the RUSITEC (rumen simulating technique) have been carried out, which confirmed the higher digestibility of this mutant. The Austrian CRP partner, Prof. Grausgruber (BOKU) is now introducing this trait into locally adapted germplasm.

The *rob1* mutation appeared for the first time in a barley accession from the Krasnodar region in Russia as a spontaneous mutation. Later, the orange lemma trait was induced by various mutagens (EMS, ethylene oxide, ethylene imine, neutrons, X-rays) in Swedish barley varieties. The trait is recessive and located on chromosome 6HS.

PBGL searched for genes involved in the lignin biosynthesis pathway for a candidate gene approach. We have identified the *cad2* gene encoding the cinnamyl-alcohol dehydrogenase 2 (CAD2) protein in the lignin biosynthesis pathway as the most likely candidate because the *cad2* gene (i) is located on the short arm of chromosome 6 that coincides with the position of the *rob1* mutation, and (ii) is homologous to genes responsible for an orange lemma phenotype in other cereals, including rice, maize and sorghum.

In collaboration with Prof. Grausgruber, we have assembled a collection of 14 orange lemma barley mutants (spontaneous and induced) sourced from different germplasm collections, designed PCR primers to amplify the complete coding sequence of the *cad2* gene, isolated genomic DNA from the mutant and parental plants, and carried out PCR amplification and Sanger sequencing of the *cad2* genes.

From these experiments, the complete *cad2* coding sequence was derived for all 14 orange lemma mutants. All of the accessions showed mutations in the *cad2* coding sequence predicted to impair CAD2 protein function (Fig. 6). This is strong evidence for the assumed role of the *cad2* gene for the orange lemma trait. Nine accessions showed the same SNP in exon 1, which is predicted to result in a non-functional CAD2 protein. The five induced barley mutants of Swedish origin showed distinct and unique point mutations in the *cad2* gene resulting in amino acid substitutions predicted by Protein Variation Effect Analyzer (PROVEAN) software to have deleterious effects on the protein function.

FIG. 6: Structure of the barley cad2 gene showing the four exons in blue. The mutations identified in orange lemma barley mutants and predicted to impair cad2 gene expression are indicated with triangles

We have initiated crosses between accessions carrying the different SNPs and their parental lines for linkage analysis. In parallel, we are developing different molecular marker systems to enable marker assisted breeding for this trait. The markers will be validated on the material derived from these crosses and the most suitable type of DNA marker will be identified. The objective is to develop allele-specific markers for the orange lemma trait in barley that are high-throughput, applicable across a range of populations and that can be applied at low cost in a standard biotechnology laboratory to enable wider utilization by Member States.

Mutation discovery

The majority of officially released mutant crop varieties are produced from plant materials treated with ionizing radiation or with chemical compounds. It is well established that the chemical mutagen EMS favours GC to AT transitions. Much less is known about the effects of physical mutagens such as gamma or X-ray irradiation at the DNA level.

PBGL continued experiments for mutation discovery in both seed (rice, tomato) and vegetatively (banana) propagated crops. In the case of tomato the material was derived from earlier mutagenesis experiments conducted in the context of technical cooperation project (TCP) MAR5020 (Mauritius).

FIG. 7: Tomato mutants three days after heat stress recovery. Mutant showing tolerance to heat stress maintains vigour (b); non-irradiated parent shows a recovery phenotype producing new leaves (b); mutant susceptible to heat stress (c)

The major objective of this TC project was to develop tomato mutants that are tolerant to heat stress. Heat stress especially affects plants at the flowering and fruiting stages. At PBGL, we have developed a seedling and mature stage heat stress screening approach, which has enabled the identification of a heat stress tolerant tomato mutant (Fig. 7).

In 2009 a mutant rice population was created at the PBGL with the use of gamma and X-ray, and candidate mutants expressing different phenotypes (plant height, early flowering, seed morphology) were selected (Fig. 8). In the context of this project a protocol for near-infrared reflectance spectroscopy (NIRS) was developed and published in 'Biotechnologies for Plant Mutation Breeding' (www.springer.com/book/9783319450193).

FIG. 8: Photo left: Mutant rice line with reduced stature (left) compared to control (right); Photo right: mutant rice line showing altered grain size and colour

During 2016, selected mutant lines showing different phenotypes were used to develop mutation discovery protocols using whole genome sequencing. Samples (two biological replicates of each mutant line and of non-mutated plants) were sequenced on an Illumina HiSeq 2500 platform. Bioinformatics analysis was carried out to identify induced mutations and map the mutation spectrum in these rice mutant lines. The analysis revealed a high number of high-quality variants (>1 million per sample). Precise filtering steps of known natural mutations were undertaken as well as background changes to remove background sequence variations that may not be linked to mutation

induction. This resulted in 1000s of unique SNPs and InDels as well as fewer structural changes (Fig. 9).

A first conclusion from these experiments is that the number of SNPs resulting from gamma and X-ray irradiation is similar to those induced with chemical mutagens. Also, this result shows that genetics and phenotyping need to be integrated with sequence analysis to uncover any causative mutation(s) for the observed phenotypes and for validation studies, in line with the strategy followed for sorghum. Analysis of whole genome sequencing to uncover lesions causative for the observed mutant phenotypes is ongoing.

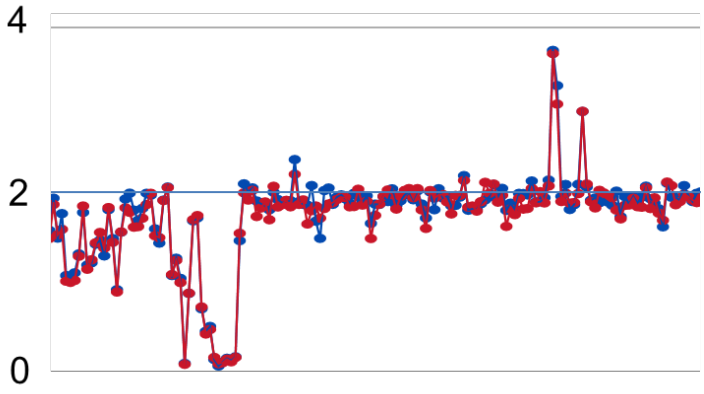


FIG. 9: Identification of a large deletion in mutant rice. Two biological replicates were compared to the control and graphically displayed with the use of JMP software. Numbers represent ploidy levels: regions on the 2 axis are diploid whereas a drop to ploidy level 0 represents a deletion. Each dot represents a 25kb region.

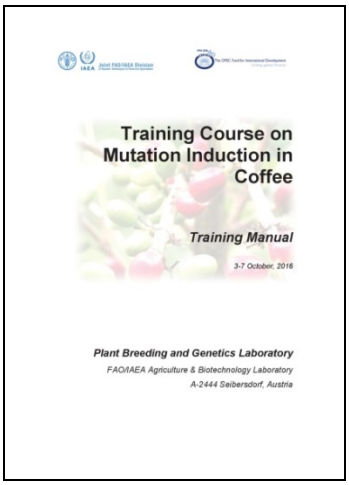
The research on mutation detection in banana supports the CRP D2.20.05 'Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana'. In 2016, the PBGL identified a large copy number variation that reduces the ploidy level from three to two over a large part of a chromosome using low coverage whole genome sequencing.

In the context of this CRP, one of the counterparts has identified five mutant banana lines that show field resistance to the fungal pathogen *Fusarium oxysporum* TR4 during hot spot field screening. These candidate resistant mutant lines have been acquired by the PBGL and are currently maintained *in vitro* together with control banana varieties (both resistant and susceptible accessions). These materials will be multiplied *in vitro* for confirmation of the resistance phenotype and for genetic stability studies under greenhouse conditions.

CAPACITY BUILDING AND SERVICES

Group and individual training

Training course on mutation induction in coffee



PBGL organized the 'Training Course on Mutation Induction in Coffee' as part of the project, funded by the OPEC Fund for International Development (OFID), to establish a global research and development network with a core in Latin America to use mutation breeding to help coffee producing countries respond to coffee leaf rust. The training course brought together experts from seven Latin American countries involved in coffee improvement and interested in incorporating mutation breeding in their breeding programs or research (Costa Rica, El Salvador, Guatemala, Honduras, Jamaica, Nicaragua, Panama, Mexico and Peru). The workshop focused on mutation induction in coffee and molecular methods for mutant detection. PBGL has pioneered the work on coffee mutation breeding since 2014 and since then the number of coffee producers and breeders interested in joining the coffee mutation breeding network has steadily increased. The workshop was extensively covered in a press release with interviews and newspaper articles disseminated through print and social media.

Ad hoc group training

The PBGL hosted the following group training courses in 2016:

- 'Next Generation Sequencing for the Discovery of Gamma Induced Mutations in Rice', 8-16 February, covering genomic DNA fragmentation, library preparation and next generation sequencing. Participants from Madagascar, Thailand and Sierra Leone.
- 'EMS Mutagenesis of Barley', 12-14 April, covering hands-on experiments of an SOP for the treatment of seeds with EMS and optimized post-treatment handling. Participants from Pakistan and Thailand.
- 'Low-Cost Purification of Single-Strand-Specific Nucleases for Mutation Discovery', 25-26 April, covering extraction of enzyme from mung bean and low-cost protocols for bench-top enzyme purification and mutation discovery. Participants from Indonesia, Pakistan and Thailand.

Fellowships, Scientific Visitors and Interns

The PBGL hosted four interns, one cost-free expert (CFE), 16 fellows, and two scientific visitors (SV) (the latter two categories funded by IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
ANWAR, Yassier	Indonesia	SV	1 mth	Mutation induction in barley, screening and accelerated breeding
JOUHAR, Mohammed	Syrian Arab Republic	SV	1 mth	Development of low cost disease diagnostic
DATTA, Sneha	India	Intern	10 mth	Plant mutation detection
KAFURI, Lina[2]	Colombia	Intern	2 mth	Discovery of natural mutations in cassava
TELLO, Daniel[2]	Colombia	Intern	2 mth	Discovery of natural mutations in cassava
GUPTA, Prateek	India	CFE	2 mth	Plant mutation detection
JARC, Luka	Slovenia	Intern	3 mth	Plant mutation detection
RABEFIRAISANA, Harimialimalala Jhonny	Madagascar	Fellow	2 mth	Mutation detection in maize and rice

[2] Supported by COLCIENCIAS, Colombia.

Name	Country	Status	Duration	Topic
KAEWCHEENCHAI, Reunreudee	Thailand	Fellow	2 mth	Mutation detection in rice
NZOUMBOU-BOKO, Romaric	Central African Republic	Fellow	2 mth	Mutation induction in cassava
KASSA, Geralde Gado Yamba	Central African Republic	Fellow	2 mth	Mutation detection in cassava
SESAY, Junatsu V.	Sierra Leone	Fellow	3 mth	Mutation induction in cassava
AZAM, Farooq	Pakistan	Fellow	3 mth	Mutation detection in wheat
BACHIRI, Hamid	Algeria	Fellow	3 mth	Mutation detection for drought tolerance in wheat
PURUPUNYAVANICH , Vichai	Thailand	Fellow	4 mth	Mutation detection for salt tolerance in rice
MOSAZGHI, Zeremariam G.	Eritrea	Fellow	3 mth	Mutation detection for drought tolerance in barley
UBALUS, Alfred	Nigeria	Fellow	2 mth	Mutation induction
AKER, Dina	Palestine	Fellow	2 mth	Doubled haploids in wheat
HASSAN, Omar	Sudan	Fellow	4 mth	Mutation in sorghum and millet for abiotic stress and Striga resistance
MHENI, Nafeti	Tanzania	Fellow	3 mth	Doubled haploids in wheat
KUMARARATHNA, Munasingha J.P.	Sri Lanka	Fellow	3 mth	Mutation induction in mung bean
LAHLOUH, Ala	Palestine	Fellow	2 mth	Doubled haploids in wheat
EL MOCTAR, Cheikh Ahmed	Mauritania	Fellow	4 mth	Mutation induction in rice

Irradiation services

In 2016, the PBGL received a total of 54 requests for plant irradiation from 38 Member States, covering 37 plant species. Of these, 32 requests were received in the context of CRPs, TCPs or fellowships (F) with the remaining 22 requests from stakeholder institutions from Member States, as

summarized in below table. In many cases, PBGL carried out a radio-sensitivity test to determine the optimal irradiation dose for mutation induction. The total number of irradiation requests now stands at 1494.

To streamline international germplasm exchange in the context of the CRPs and TCPs, the list of crops registered for import into Austria from non-EU countries has now been expanded from four to 22 crops, in compliance with EU regulations and following consultations with the Austrian Agency for Health and Food Safety (AGES).

Request no.	Country	Request type	Crop
1441	Cambodia	TCP	Cassava
1442	Tanzania, United Rep. of	CRP	Maize, barley
1443	Uzbekistan		Paulownia
1444	Germany		Ornamentals
1445	Côte d'Ivoire	TCP	Maize
1446	Sri Lanka	TCP	Onion
1447	Nepal	TCP	Rice
1448	Germany		*Salvia hispanica* (chia)
1449	Burkina Faso	TCP	Rice
1450	Oman	TCP	Date palm, banana
1451	Cambodia	TCP	Cassava
1452	Sudan	TCP	Pearl millet, sorghum, groundnut
1453	Sierra Leone	TCP	Cassava, cowpea, maize, soybean
1454	Czech Republic		Barley
1455	Mongolia	TCP	Wheat, oat, rye, barley, soybean, pea, flax
1456	UK/India		Watermelon
1457	Germany		Ornamentals
1458	Niger	TCP	Sesame
1459	Spain		Clementine
1460	Burkina Faso	TCP	Rice, cowpea

Request no.	Country	Request type	Crop
1461	Sri Lanka	TCP	Mung bean, soybean, millet, cowpea, chilli, onion, sorghum, horse gram
1462	Bulgaria	TCP	Wheat
1463	Eritrea	TCP	Barley
1464	Mauritania	TCP	Rice
1465	Germany		Ornamentals
1466	Libya	TCP	Barley
1467	Hungary		Ornamentals
1468	Italy		Strawberry
1469	Iraq		Cowpea
1470	Austria		Wheat
1471	Germany		Ornamentals
1472	Spain		*Marachantia polymorpha* (common liverwort)
1473	Namibia	TCP	Maize
1474	Tanzania, United Rep. of	F	Wheat, barley
1476	Mozambique	TCP	Sorghum, pearl millet
1477	Romania	TCP	Pea
1478	Palestine	F	Durum wheat, barley
1479	Cameroon	TCP	Maize
1480	Tanzania, United Rep. of	TCP	Sorghum, rice
1481	Netherlands		Dahlia
1482	Guatemala	CRP	*Coffea arabica*
1483	Honduras	CRP	*Coffea arabica*
1484	Nigeria		Cowpea

Request no.	Country	Request type	Crop
1485	Costa Rica	CRP	*Coffea arabica*
1486	El Salvador	CRP	*Coffea arabica*
1487	Burundi	TC	Cassava
1488	UK		Hosta
1489	Sri Lanka	F	Finger millet, *Zea mays*, mung bean
1490	Germany		*Boechera divaricarpa* (spreading-pod rockcress)
1491	Germany		Ornamentals
1492	Czech Republic		Wheat
1493	Austria		*Cannabis sativa*
1494	Germany		Sunflower

The PBGL has developed kits to assist Member States in optimizing protocols in their own laboratories for their own species. Each kit contains a detailed protocol along with the material needed to successfully complete the protocol. The full list of available kits can be found at www-naweb.iaea.org/nafa/pbg/public/manuals-pbg.html.

The following kits were distributed to the following countries in 2016:

- Low cost DNA extraction kits distributed to Costa Rica, Iran, Pakistan.
- Low cost enzyme extraction for mutation discovery: Costa Rica, Indonesia, Iran, Pakistan, Thailand.

Guidelines and information dissemination

The National Geographic Channel explores how nuclear applications in agriculture can help curb the effects of global warming

The work of the Joint FAO/IAEA Division, including that of the PBGL, was broadcast in December 2016 on the National Geographic Channel in Belgium, France and the Netherlands. The 24-minute episode 'Nuclear Lifeline' highlights how nuclear techniques can help advance crop resilience to global warming and climate change. In Belgium, the episode and trailer reached 27% of the population, with more than 1 million views on the online trailers. The pre- and the post-test of the Nuclear Lifeline project showed a significant and positive impact both on knowledge and opinion on nuclear technologies, with 97% of the viewers considering the program successful. It is available in Dutch and French; see: www.nuclearlifeline.be/episode-2/#detail-episode-2 (click 'FR' in top right-hand corner of the screen to reach the French version, select 'Episode 2', scroll down and start film); an English version is planned for 2017 (same link).

Long Night of Research

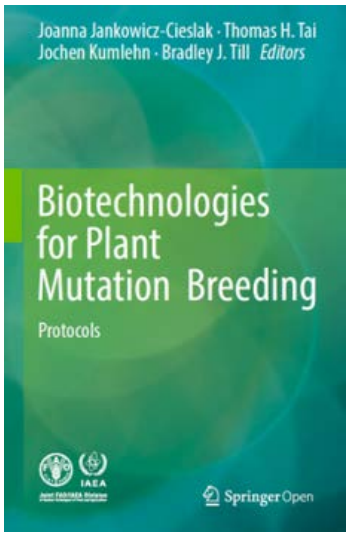
The Plant Breeding and Genetics team, along with the Joint FAO/IAEA Division and other VIC-based organizations, took part in the 'Lange Nacht der Forschung', an Austria-wide event aimed at sparking interest in science and research. The PBGL prepared a live display illustrating the different steps in mutation breeding and showcasing the contribution of mutation breeding to food security and climate-smart agriculture. Hundreds of visitors participated in the quiz prepared by our team as a fun approach to familiarize the public with the methods and benefits of crop mutation breeding.

The PBGL team in action at the Long Night of Research

Celebration of the International Year of Pulses

The PBG Subprogramme joined forces with the Soil and Water Management & Crop Nutrition team of the Joint FAO/IAEA Division to celebrate the International Year of Pulses at the IAEA headquarters on 20 September 2016. The event helped raise awareness of the nutritional benefits of pulses and their important role for sustainable food production. The event highlighted the work supporting Member States on the contribution of pulses to food security and to mitigating the effects of climate change. A wide variety of mutant pulses were displayed and eight dishes were served to visitors. See also www.iaea.org/newscenter/multimedia/videos/pulses-celebrating-a-powerful-superfood.

New protocols books



The book entitled 'Biotechnologies for Plant Mutation Breeding', published in 2017, contains 19 protocols in the area of plant mutation induction and chimera dissociation, phenotypic and genotypic screening, and an introduction on mutagenesis for crop breeding and functional genomics. The book was a result of CRP D2.40.12 on 'Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline'. It is freely available at: link.springer.com/book/10.1007%2F978-3-319-45021-6.

The book entitled 'Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley' offers effective, low-cost and user-friendly protocols for the pre-field selection of salt-tolerant mutants in cereal crops. It presents simple methods for measuring soil salinity, including soil sampling and the analysis of water-soluble salts, and describes a screening test for salt tolerance in rice, wheat and barley seedlings, which uses hydroponics. It is free to download at link.springer.com/book/10.1007%2F978-3-319-26590-2

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THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) 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 by protecting soil and water resources and optimizing soil, water and nutrient management practices. The SWMCNL also helps Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well in remediating the impact of these events on soil and agricultural water resources.

In 2016, the SWMCNL conducted a wide range of activities: (i) it developed robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture; (ii) supported the improvement of nuclear emergency preparedness and response in food and agriculture, (iii) trained technical staff and scientists from Member States in the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices; (iv) conducted isotope analyses for research and development, and for IAEA projects where analytical facilities are not locally available; and (v) provided quality assurance services to Member States.

The research and development activities at the SWMCNL included improvements in the use of compound-specific stable isotope and energy dispersive X-ray fluorescence analysis to identify sediment pathways and areas prone to land degradation. New mathematical modelling techniques, using a wide range of fallout radionuclides, were developed. The use of plutonium radioisotopes was initiated to test their application for assessing long-term erosion, in particular in fragile upland environments. Nitrogen-15 and carbon-13 isotope analysis in greenhouse gases, soil organic carbon and nitrate in water was improved; and the cosmic-ray soil moisture neutron probe for area-wide soil moisture assessment was further adapted for upland agro-ecosystems. More emphasis was put on how to use isotope and nuclear data for improving soil and water management practices. Further important progress was made in nuclear emergency preparedness and response in food and agriculture through enhanced data collection, management and visualization. These activities are essential in supporting the implementation of the seven Coordinated Research Projects (CRP) of the SWMCN Subprogramme, two of which are coordinated by the SWMCNL.

A second major component of the work of the SWMCNL is its significant contribution to training and capacity building in Member States. The SWMCNL hosted 51 fellows and interns from 27 countries, covering in total 157 man-months of training on the use of isotopic and nuclear techniques to improve nitrogen and agricultural water management as well as soil conservation in support of climate-smart agriculture.

Two IAEA publications, *Supporting Sampling and Sample Preparation Tools for Isotope and Nuclear Analysis* (IAEA-TECDOC-1783) and *Cosmic Ray Neutron Sensing: Use, Calibration, and Validation for Soil Moisture Estimation* (IAEA-TECDOC-1809), were published. These provide guidance for scientists, technicians and students on sampling procedures and tools for isotope and nuclear analysis for soil and water management at scales ranging from field to area-wide level.

Information was further communicated to Member States through 33 publications as book chapters, conference papers and publications in international peer-reviewed journals.

The SWMCNL analysed a total of 5850 and 220 samples for stable isotopes and fallout radionuclides, respectively. Most analyses were carried out in support of research and development activities in the SWMCNL focusing on the design of isotope and nuclear techniques to improve soil and water management practices. About 36% of all stable isotope analyses were performed to support the research and development activities of the Plant Breeding and Genetics, Insect Pest Control and Food and Environmental Protection Laboratories of the Joint FAO/IAEA Division.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farming communities to climate change 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) or at the area-wide level.

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

Climate-Smart Agriculture

Climate change is a major threat to global food security. Changes in weather patterns, with increasing severity of storms, floods, droughts and extreme temperatures, impact sustainable agricultural production. These increasingly amplify soil erosion, land degradation, greenhouse gas emission and crop failures worldwide. The need to sustain agricultural production in these challenging conditions has never been greater. Consequently, there is an increasing demand from Member States for technical assistance and training in developing soil and water management packages for climate change mitigation and adaptation.

First version of an innovative conversion model (i.e. MODERN) for assessing soil redistribution magnitudes from fallout radionuclides inventories

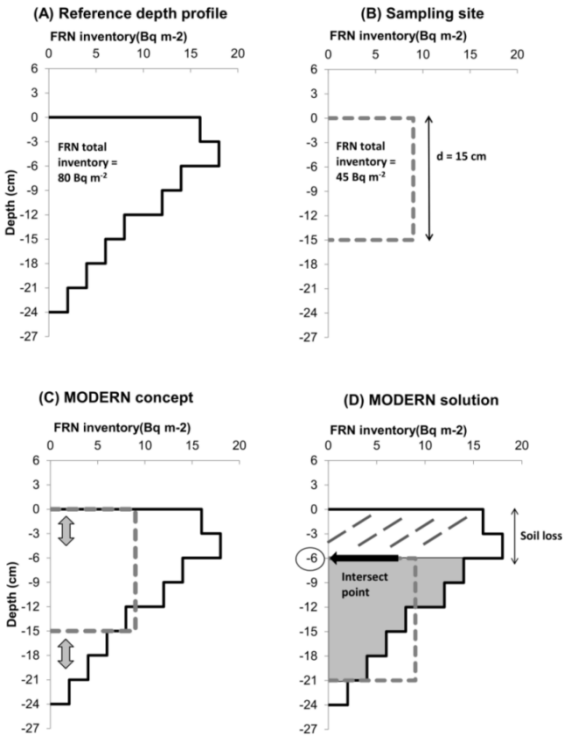


FIG. 1: Concept of the conversion model MODERN (Modelling Deposition and Erosion rates with Radionuclides)

The SWMCNL recently developed, in collaboration with the Department of Environmental Sciences (University of Basel, Switzerland), a new conversion model, called MODERN (Modelling Deposition and Erosion rates with Radionuclides), to derive soil redistribution rates from fallout radionuclide (FRN) inventories. MODERN is based on the comparison between the depth profile of the FRN distribution at a reference site and the total FRN inventory at the sampling site. To estimate soil losses or gains, MODERN aligns the total inventory of the sampling site to the depth profile of the reference site (Fig. 1).

The uniqueness of MODERN, as compared to classical FRN conversion models, can be summarised as following: (a) it does not make any assumptions on the depth profile of the FRN, but accurately describes the soil profile shape of any selected FRN at the reference sites, (b) it accurately estimates the soil redistribution rates, (c) it allows adaptation of the depth profile by simulating the behaviour of the selected FRN under different agro-environmental conditions (e.g. ploughing activities, erosion and sedimentation processes) and (d) it is the only conversion model that can be used for caesium-137 (^{137}Cs), lead-210 (^{210}Pb), beryllium-7 (^{7}Be) and the new soil tracer plutonium-239+240 ($^{239+240}$Pu).

MODERN is based on a unique algorithm to convert FRN inventories into both erosion and deposition rates, whereas common conversion models (e.g. the Profile Distribution Model, the Diffusion and Migration Model) are specifically developed to quantify rates of only one redistribution process (i.e. erosion or deposition). MODERN was developed in the Matlab™ environment, and a forthcoming release of the code in open source programming codes (i.e. R) is planned. The code is transparent, easily adaptable and its preliminary version is freely available at: modern.umweltgeo.unibas.ch.

This research supports the CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'.

A statistical approach for selecting best discriminant fatty acids to establish soil source contribution to sediment mixture (sub-watershed of Mistelbach, Austria)

The innovative study conducted in the sub-catchment of Mistelbach for testing and validating the use of compound-specific stable isotope (CSSI) techniques to determine the origin of the eroded soil was complemented by a step-by-step statistical approach to study agro-environmental source-sink interaction, using fatty acids (FAs) as soil/sediment fingerprints. The study site consists of one sediment mixture (M) and four different contributing sources that includes three agricultural fields (S1, S2, S3) and one grassed waterway (S4). The $\delta^{13}C$ values of the bulk soil carbon and of four different FAs (i.e. C_{16}, $C_{18,}$ C_{22}, C_{24}) were determined in the soil sources and the sediment mixture.

Prior to applying statistical tests, exploratory data set analysis were performed through a series of bi-scatter plots of all possible combinations of $\delta^{13}C$ FAs, including the bulk soil carbon $\delta^{13}C$ for allowing visual inspection and to obtain an overall qualitative description of the variables. This preliminary simple data comparison already illustrated that bulk soil carbon $\delta^{13}C$ is a strong discriminant among the other FAs. The results of one-way analysis of variance, through Tukey's multiple comparisons of means with 95% confidence level, provide information about the differences in $\delta^{13}C$ among sources and FAs. For example, the bulk soil carbon $\delta^{13}C$ values showed the highest significant difference between the four sources: S4 vs. S1 ($P<0.0001$), S3 vs. S1 ($P<0.001$), S3 vs. S2 ($P<0.001$), S2 vs. S4 ($P<0.001$), S3 vs S4 ($P<0.001$), and S2 vs. S1 ($P<0.001$). S3 vs. S1 and S4 vs. S2 did not differ with $\delta^{13}C$ of FAs C_{16} ($p=0.10$) and C_{18} ($p=0.10$), respectively. The $\delta^{13}C$ values of lignoceric acid (C_{24}) showed significant differences for all sources ($p<0.001$) while $\delta^{13}C$ of behenic acid (i.e. C_{22}) did not exhibit a significant difference between S1 and S2 ($P=0.80$).

After the one-way analysis of variance, a correlation analysis was performed to establish the dependencies between the different biomarkers. This analysis revealed that the highest significant linear dependencies are between $\delta^{13}C_{16}$ and $\delta^{13}C_{18}$ ($r=0.86$; $p<0.01$), $\delta^{13}C_{18}$ and $\delta^{13}C_{24}$ ($r=0.79$; $p<0.01$), and $\delta^{13}C_{16}$ and $\delta^{13}C_{24}$ ($r=0.77$; $p<0.01$). Among the variables, the bulk soil carbon $\delta^{13}C$ was found to be the least correlated parameter, highlighting that it is the most reliable discriminator for determining the sediment origins in the mixture.

Based on the results obtained from the above steps, only behenic (C_{22}) and lignoceric (C_{24}) acids as well as the bulk soil carbon $\delta^{13}C$ pass our multivariate statistical approach. Principal component analysis (Fig. 2b) confirmed these findings as well as the previous FAs fingerprint selection based on the mixing polygon tests (Fig. 2a). Using different mixing models, our next investigation will focus on deriving a better understanding of the soil redistribution and its linkages with the historical land uses of the four agricultural sources of the study area.

This research was conducted to support the CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'.

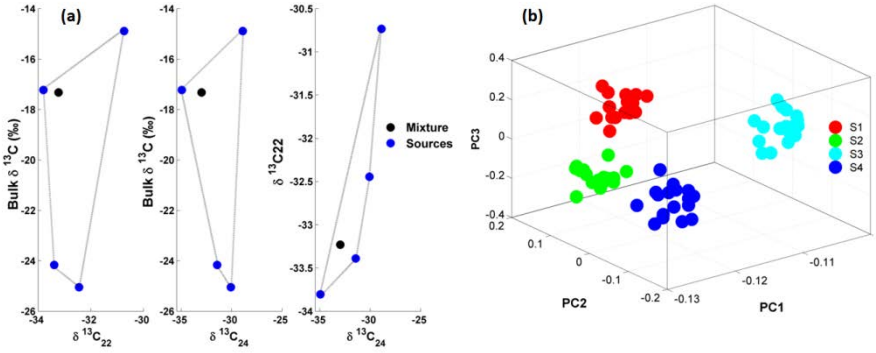


FIG. 2: (a) Mixing polygon of selected FAs contributing to the mixture; (b) Principal component analysis of the data set

Using artificial soil sediment mixtures for calibrating fingerprinting techniques at the catchment scale

Soil erosion and related sediment transportation and deposition are key environmental problems in central Argentina. Certain land use practices, such as intensive grazing, are considered particularly harmful in causing erosion and sediment mobilization. The studied sub-catchment Estancia Grande (630 hectares), 23 km northeast of San Luis, is characterized by erosive loess soils. Sediment source fingerprinting techniques were tested to identify critical hot spots of land degradation, based on the concentration of 43 elements determined by energy-dispersive X-ray fluorescence (EDXRF). To validate these fingerprinting techniques, with the support of the SWMCNL created artificial mixtures using the most representative sediment sources of the studied catchment. The artificial mixtures were also measured as regular samples by EDXRF. Using known proportions of these sources in the mixture, we tested which algorithms and measured elements would allow re-establishing the source sediment proportion of the artificial mixture. Elements including Ca, Fe, Na, P and V were identified as the most effective fingerprints for our studied catchment. These fingerprints could be linked mainly to land management practice, such as cattle grazing and feedlot cattle, and to geomorphological units in the landscape such as gullies.

These preliminary results will contribute to a much wider research project including additional fingerprinting approaches such as CSSI, in addition to soil erosion quantification techniques through the use of FRNs.

This research was conducted with the assistance of Ms Romina Torres Astorga (Institute of Applied Mathematics San Luis [IMASL], National University of San Luis, Argentina) during her ICTP/IAEA sandwich training at the SWMCNL to support CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'.

New on-going investigation using plutonium (Pu) isotopes as soil tracer

As less than 30% of the deposited global fallout of ^{137}Cs is still present today due to its radioactive decay (half-life of 30 years), alternative anthropogenic isotopic soil tracers need to be considered for investigating the magnitude of erosion and sedimentation processes. Recently, a new artificial radioisotope tracer, Plutonium (Pu), primarily originating from past aerial nuclear weapons tests, has raised the attention of the scientific community. One of the main advantages of anthropogenic Pu isotopes over ^{137}Cs is their long half-life (i.e. ^{239}Pu = 24 110 years and ^{240}Pu = 6561 years) that ensures long-term availability as tracers for agro-environmental and other purposes.

To date, the use of Pu in soil redistribution research in agroecosystems is still in its infancy, with only a few of studies having been conducted. However, these studies have clearly demonstrated the potential of Pu isotopes.

A preliminary ^{137}Cs fallout baseline in an undisturbed reference site has already been established at Grabenegg, the experimental research station of the Austrian Agency for Health and Food Safety (AGES), with values of 7890 ± 1510 Bq m^{-2}; CV = 19.2 %; AE = 11.8% at 90% confidence level; n=9).

The SWMCNL team initiated new activities to reinforce the ^{137}Cs information gained on this reference site and to test $^{239+240}Pu$ versus ^{137}Cs. Depth distribution of these radioisotopes and their spatial variability will be tested in the coming months. Pu isotopic determinations of Grabenegg soil samples are currently being performed using alpha spectrometry analytical facilities at the Centre National de l'Energie, des Sciences et de Techniques Nucléaires (CNESTEN) in Morocco. This collaboration will also allow us to obtain key information using Pu ratios for determining and quantifying the ^{137}Cs that originates from Chernobyl and from past nuclear bomb tests. This additional information will increase precision of derived soil erosion rate estimates when using the ^{137}Cs conversion model.

In comparing the reference site spatial heterogeneity and depth distribution of $^{239+240}Pu$ to ^{137}Cs, the main objective of the first investigation, to test Pu isotopes as potential tracers of soil and sediment redistribution under Austrian agro-climatic conditions, will be achieved. Further work involving a new sampling campaign to quantify soil redistribution through a multi-fallout radionuclides approach – including ^{137}Cs, $^{210}Pb_{ex}$ and $^{239+240}Pu$ determination – will be scheduled along a typical transect of an adjacent agricultural field.

This research was conducted to support the CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'.

Assessing soil conservation efficiency of traditional agricultural practices by fallout radionuclides: Example in the highlands of Madagascar

Soil degradation induced by human activity is a major concern in Madagascar. More than 30% of the island's total soil area is degraded.

In collaboration with the SWMCNL, the Institut National des Sciences et Techniques Nucléaires (Madagascar-INSTN) located in Antananarivo (Madagascar) tested both ^{137}Cs and ^{210}Pb methods in a study area located 40 km east of Antananarivo in the Madagascar highlands. To evaluate the effectiveness of traditional Malagasy soil conservation strategies for controlling soil erosion processes, two adjacent cultivated fields were selected, i.e. an unprotected field and a terraced field, as well as an undisturbed reference site in the vicinity of these agricultural fields. Soil samples were collected along transects and ^{137}Cs and $^{210}Pb_{ex}$ analyses were performed using a high resolution and low background N-type HPGe gamma detector. The improved mass balance model (MBM2) conversion was employed to estimate the soil redistribution rates. The sloped field showed downward movement of soil particles. At the terraced field, soil is retained and redistributed within the plot limiting off-site sediment delivery (Fig. 3). In this field with conservation measures, ^{137}Cs and $^{210}Pb_{ex}$ inventories reached 145 Bq m^{-2} to 280 Bq m^{-2} and 2141 Bq m^{-2} to 4253 Bq m^{-2}, respectively. At the unprotected field, the ^{137}Cs and $^{210}Pb_{ex}$ inventories values ranged from 110 Bq m^{-2} to 280 Bq m^{-2} and 2026 Bq m^{-2} to 4110 Bq m^{-2}, respectively. The net soil erosion rates determined for the unprotected field were 7.4 t ha^{-1} yr^{-1} and 5.9 t ha^{-1} yr^{-1} for ^{137}Cs and $^{210}Pb_{ex}$ methods, respectively. In contrast, at the terraced field, the net soil erosion rates reached only 3.4 t ha^{-1} yr^{-1} and 3.8 t ha^{-1} yr^{-1} for ^{137}Cs and $^{210}Pb_{ex}$ methods, respectively.

Moreover, FRNs timeframe discrimination highlights that at the unprotected field, erosion increased for the last 50 years (from ^{137}Cs data) compared to the last 100 years (from $^{210}Pb_{ex}$ data). On the other hand, it decreased at the terraced field. The results demonstrate that soil terracing reduces soil erosion as well as sediment delivery and, therefore, provides an efficient solution to protect the soil resources of the Malagasy highlands.

This study was performed within the CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'.

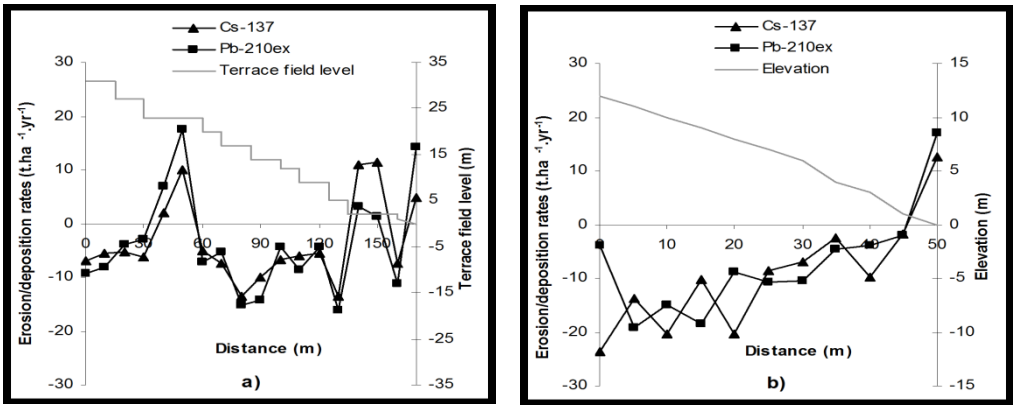


FIG. 3: Soil redistribution rates along a) the terraced field and b) the unprotected field

Increasing precision and accuracy of laser isotope analyser data

Isotope analysis with laser spectroscopy is an emerging technology that is growing in scientific demand, because the technology allows for real-time, *in situ* measurements of carbon-13 and nitrogen-15 of CO_2, CH_4 and N_2O, and is increasingly being used to monitor fluxes of these greenhouse gases. However, despite its potential to readily provide data to researchers, methods of calibration and data correction still need to be developed and refined. Furthermore, standard reference gases are currently not available and researchers must make their own gases to ensure proper calibration and data correction. To improve the quality of data and allow for comparison of data between studies, it is essential that standard CO_2, CH_4 and N_2O gases are accessible.

The SWMCNL is now developing methods to make CO_2, CH_4 and N_2O gas standards on a universal gas mixing line that can both evacuate gas bottles and fill them with desired gas mixtures (Fig. 4). These gases are being isotopically labelled at natural isotope abundance levels as well as at depleted and enriched isotope abundance levels so that they can be used in both natural abundance and tracer isotope studies. Furthermore, the gas mixtures will be produced at ambient and elevated concentration levels, which can be measured in natural environments and experiments. In addition to filling gas bottles to create larger volumes of standard gases, our universal gas mixing line can be used to produce mixed gases in small volume multi-layer foil gas sampling bags.

FIG. 4: Gas mixing line (left) linked to a vacuum pump, pressure gauge, CO_2 and synthetic air gas tanks for mixing of gases into desired containers, such as this 1L gas cylinder, to make standard reference gases for laser isotope analyzers; ^{13}C - CO_2 laser isotope analyzer (right) for measuring emissions of CO_2 from soils

With use of standard CO_2, CH_4 and N_2O gases in laser isotope analysis studies, confidence and accuracy in reported data and in comparisons across studies will be improved. Once the methods for making gas standards for laser isotope analysers have been finalized, we plan to develop standard operating procedures for FAO and IAEA Member States to use these to produce their own gas standards.

This research was conducted under CRP D1.50.16 on 'Minimizing Farming Impacts on Climate Change by Enhancing Carbon and Nitrogen Capture and Storage in Agro-Ecosystems'.

Quantitative isotopic tracing using homogeneously carbon-13 labelled plant material

Carbon-13 and nitrogen-15 labelled plant material is increasingly being used to trace the fate of plant-derived C and N into the atmosphere, soil, water and organisms, including investigations into the potential of soils to store greenhouse gases belowground. However, accurate quantitative tracing of plant-derived C and N in such studies is only possible if plant material is labelled both homogeneously and in sufficient quantities. The SWMCNL has developed a method that achieves these two requirements for ^{13}C labelling by monitoring $^{13}CO_2$ labelling of plants in a walk-in growth chamber with laser spectroscopy (Fig. 5). This approach enables the creation of homogeneously labelled material at the intra-plant, inter-plant and metabolic levels, which can be used for quantitative tracing (Fig. 6).

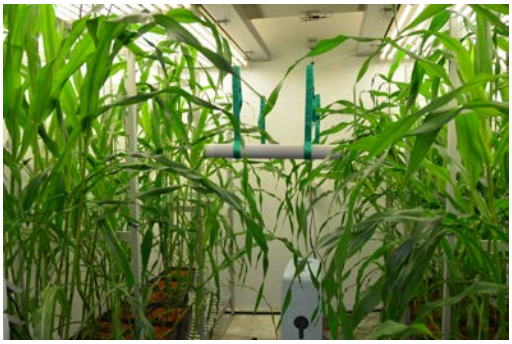


FIG. 5: Maize plants grown in a growth chamber with controlled $^{13}CO_2$ labelling at time of harvest

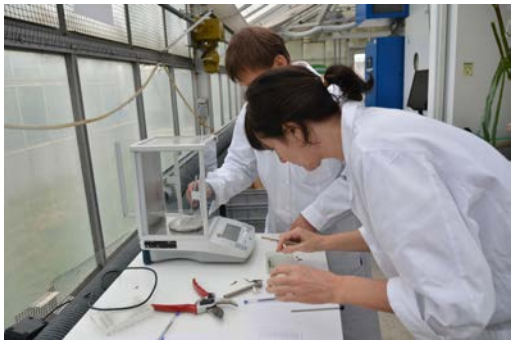


FIG. 6: SWMCNL researchers prepare samples of maize leaf tissue to check for homogeneity of ^{13}C labelling throughout the labelled plants

Initial labelling trials focused on maize because of its global importance as a crop and due to its potential to produce relatively large amounts of biomass, yielding one kilogram of dry plant material with each labelling. With successful ^{13}C labelling of maize plants, the SWMCNL is now attempting to label other agricultural plants, such as soybeans, to open more research avenues to better understand carbon dynamics. Additionally, we are attempting to create homogeneously labelled ^{13}C and ^{15}N plant material by supplying both $^{13}CO_2$- and ^{15}N-labelled hydroponic nutrient solutions during plant production. Dual labelling of plants is advantageous when studying agricultural greenhouse gas emissions, as it allows researchers to simultaneously account for plant-derived CO_2 as well as other greenhouse gasses, such as N_2O.

In addition to homogenous ^{13}C and ^{15}N labelling of plant material, we plan to also produce heterogeneously labelled plant material in which only labile, easily decomposable material is labelled. By performing incubation and field decomposition experiments using both types of labelled plant material, researchers will be able to investigate the forms of plant material that contribute the most to greenhouse gas emissions and, conversely, store them belowground. Furthermore, a comparison between the two types of plant material should allow us to elucidate the error propagation that can occur from using heterogeneously labelled materials and its effects on the accuracy of estimating sequestration rates, emission rates and residence times.

The method developed at the SWMCNL for producing large amounts of homogeneous ^{13}C labelled plant material opens up new research pathways and assessment methods in the field of soil carbon dynamics and agricultural greenhouse gas emissions. Further development of homogenous ^{15}N labelled plant material will also help with research in the field of soil nitrogen dynamics and agricultural greenhouse gas emissions, as will the production of additional ^{13}C and ^{15}N labelled agricultural plants. This plant material will enable IAEA and FAO Member States to accurately

quantify carbon storage and reduction of atmospheric greenhouse gas levels of various agricultural systems as well as assessing the efficacy of different agricultural practices under local conditions, both via *in situ* and incubation experiments.

This research was conducted under CRP D1.50.12 on 'Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-Based Cropping Systems in Sub-Saharan Africa' and CRP D1.50.16 on 'Minimizing Farming Impacts on Climate Change by Enhancing Carbon and Nitrogen Capture and Storage in Agro-Ecosystems'.

Evaluating the effectiveness of mulch application to store carbon belowground: Short-term effects of mulch application on soluble soil and microbial C and N in agricultural soils with low and high organic matter

Agricultural soils have the potential to contain a large pool of carbon and, depending on the farming techniques applied, can either effectively store carbon belowground or further release carbon in the form of CO_2, into the atmosphere. Farming techniques, such as mulch application, are frequently proposed to increase carbon content belowground and improve soil quality and can be used in efforts to reduce greenhouse gas levels, such as in the '*4 per 1000 Initiative: Soils for Food Security and Climate*'. To test the effectiveness of mulch application to store carbon belowground in the short-term and improve soil nutrient quality, we maintained agricultural soils with low and high organic carbon content in FAO/IAEA greenhouse mesocosms with controlled moisture for four years. During this time, maize and soybean were grown yearly in rotation and mulch was removed or applied to soils once plant material was harvested. After four years, we measured the effects of mulch application on soluble soil and microbial carbon and nitrogen in the mesocosms and compared the effects of mulch application versus no mulch on soils with low and high organic matter. Mulching is expected to increase soil carbon and nitrogen contents and should therefore have a greater effect on soils with low organic matter than on soils with high organic matter.

In soils with low organic carbon content, and hence an expected higher potential to increase soil carbon, mulch application did not increase soluble soil or microbial carbon or nitrogen compared to the treatments without mulch application. However, mulch application significantly increased the $\delta^{13}C$ of both microbial and soluble soil carbon in these soils by 1‰, indicating a shift in belowground processes, such as increased decomposition coupled with increased carbon inputs. In soils with more organic content and lower potential to increase soil carbon, mulch application decreased microbial carbon by 0.01 mg C g^{-1} soil and increased soluble soil nitrogen by 0.01 mg N g^{-1} soil (Fig. 7). Soluble soil carbon also decreased by 0.04 mg C g^{-1} soil and microbial nitrogen increased with mulch application by 0.006 mg N g^{-1} soil, but only in the 5-15 cm soil layer. Mulch application only decreased $\delta^{13}C$ of soluble soil carbon by 1.5‰, indicating a decrease in decomposition. Contrary to our initial expectations, mulch did not increase soil carbon content and only increased nitrogen content in soils that already had relatively high organic matter. These results suggest that mulch application (with only soil

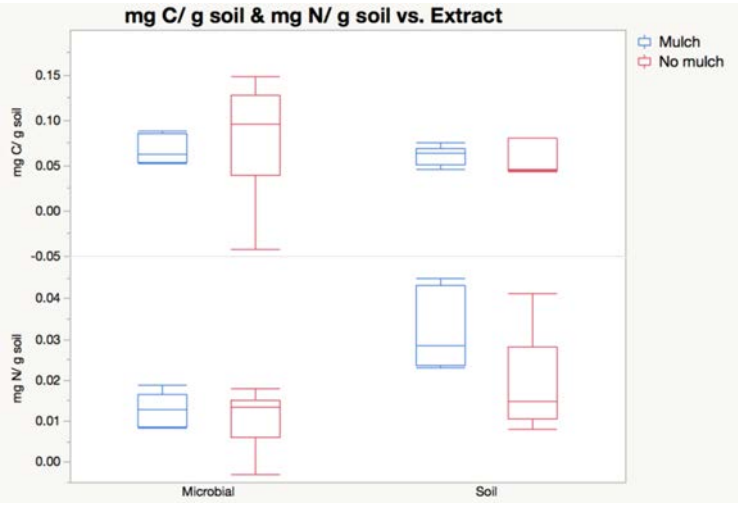


FIG. 7: Microbial and soluble soil carbon and nitrogen (mg C g^{-1} soil or mg N g^{-1} soil) extracted after four years in mulch and no-mulch experimental mesocosm treatments

surface disturbance) may not play a significant role in increasing soil carbon content and overall soil quality, at least within a 4-year period.

This research was conducted under CRP D1.50.12 on 'Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-Based Cropping Systems in Sub-Saharan Africa' and CRP D1.50.16 on 'Minimizing Farming Impacts on Climate Change by Enhancing Carbon and Nitrogen Capture and Storage in Agro-Ecosystems'.

Determining isotopic signatures of dissolved nitrate using bacterial denitrification and laser spectroscopy

Quantifying the nitrogen-15 and oxygen-18 isotopic signatures of nitrate in aqueous samples enables the identification of sources of contamination by organic and inorganic nitrogen fertilizers. This information can assist in remediating nitrate contaminated water and developing improved agricultural management practices. The SWMCNL has established a standard operating procedure (SOP) detailing a method based on bacterial denitrification and laser spectroscopy to determine the nitrogen-15 and oxygen-18 isotopic signatures of dissolved nitrate.

Preliminary studies include growing batches of healthy *Pseudomonas aureofaciens* bacteria, needed to fully denitrify the water sample and convert the dissolved nitrate into N_2O, whose nitrogen-15 and oxygen-18 signatures is then measured by laser isotope analysis. Ideal conditions for growth and complete denitrification of the *Pseudomonas aureofaciens* bacteria were determined by varying (1) availability of oxygen during the growth phase and (2) duration of autoclave time for growth medium.

Availability of oxygen during the growth phase

In testing the growth potential of *Pseudomonas aureofaciens* in aerobic and anaerobic conditions, breathable plastic covers were used to create an aerobic environment while capped bottles were used for anaerobic conditions (Fig. 8). The denitrification progress was evaluated using the sulphanilamide and NED indicator test, which is complete when the solution no longer turns magenta due to NO_2^- in the medium. Optical density was measured as an indicator for bacteria growth.

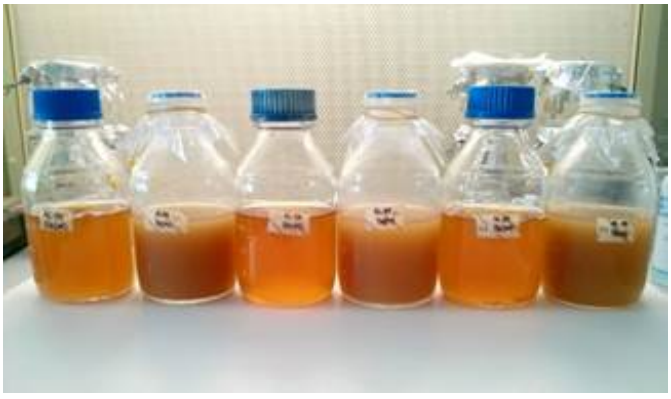


FIG. 8: Aerobic (bottles with plastic covers) growth was faster than anaerobic growth (bottles with blue caps), leading to greater yields as evidenced through higher optical density values and pallet size

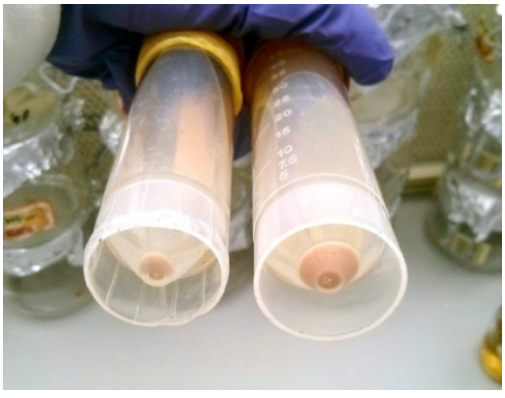


FIG. 9: Pallet bacteria yield of aerobic growth bottle (right) and anaerobic growth bottle (left)

By measuring levels of opacity in the growth medium, it was observed that the aerobic method resulted in increased bacterial growth compared to the anaerobic method. Yield of the aerobically grown *Pseudomonas aureofaciens* was on average twice that of the anaerobically grown bacteria (Fig. 9). In addition, sulphanilamide and NED indicators showed that the aerobically grown bacteria were unable to completely denitrify all dissolved nitrate.

Duration of autoclave time for growth medium

The second parameter tested was the autoclave time of the *Psuedomonas aureofaciens* growth medium. The medium was autoclaved for 30, 60 and 90 minutes, after which *Pseudomonas aureofaciens* was inoculated and left to grow in anaerobic conditions. Nitrate was completely consumed for all treatments at day 5. Using autoclave times of 30 and 60 minutes, the growth medium produced higher bacterial yields than 90-minute autoclaved medium. However, the effect of autoclave time of growth medium on bacterial growth and yield was not significant. Hence, the lowest autoclave time was recommended in the SOP.

The results of the research thus far have been written as Part I of a three-part SOP. Further work in 2017 includes validation of the denitrification method through further testing with laboratory nitrate standards and the use of laser spectroscopy for measuring the nitrogen-15 and oxygen-18 signatures in N_2O (originating from nitrate in aqueous samples).

This research was conducted under CRP D1.20.12 on 'Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-Livestock Production Systems' and CRP D1.20.13 on 'Landscape Salinity, and Water Management for Improving Agricultural Productivity'.

Comparisons of in-situ *soil moisture data with soil moisture data generated via a cosmic ray neutron sensor*

Previous and current work focused on the application of the cosmic ray neutron sensor (CRNS) for area-wide soil water content (SWC) monitoring. The CRNS is a technology capable of monitoring SWC on a large spatial scale (radius of ≈250 m). Ongoing research involving the stationary version of the CRNS has taken place mainly at an agricultural study site located near Petzenkirchen, Austria (100 km west of Vienna, Fig. 10a). Stemming from research beginning in December of 2013, this work has involved the development of a generalized protocol for the use, calibration, and validation of the technology. Additionally, published work conducted at the Petzenkirchen site since the end of 2013 (see reference list) as well as work conducted during the growing season of 2016 showed that the CRNS generates reliable SWC data in comparison with traditional *in-situ* soil sampling estimates of SWC in addition to *in-situ* time domain reflectometry, time domain transmissivity, and a mobile 'backpack' version of the CRNS.

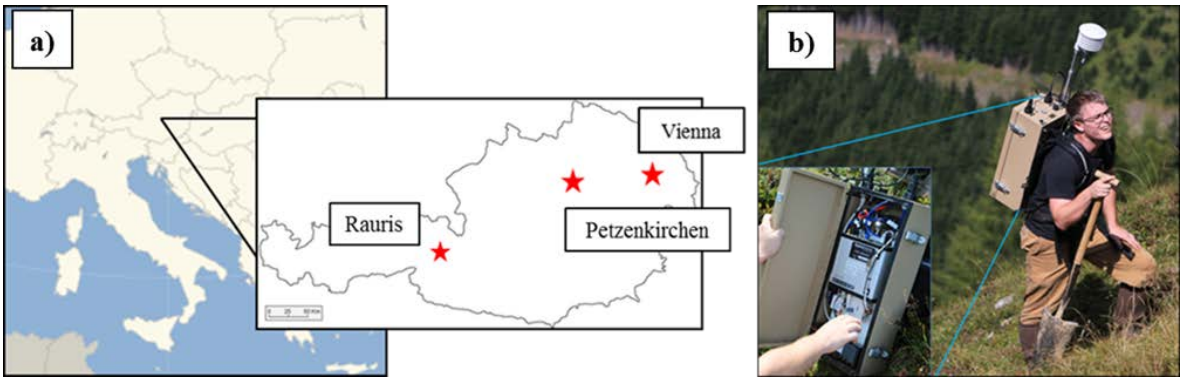


FIG. 10: a) Location of the stationary CRNS at Petzenkirchen Austria (48.154°N, 15.1483°E). b) Mobile backpack CRNS, photo taken near Rauris, Austria in a grazed alpine setting; photo credit Dale Pulker

Comparisons of this nature serve the purpose of method validation. However, much of the work of the SWMCNL as well as of many academic institutions has been performed under mid to low elevation agricultural environments that often lack significant heterogeneous topography, hydrology and biology. Recent interest in the behaviour and applicability of the CRNS in higher altitude alpine environments led to the initiation of CRP D1.50.17 on 'Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems' (2017 – 2021). A new mobile 'backpack' version of the standard stationary CRNS device was tested in the

Austrian Alps near the village of Rauris (Fig. 10, a and b). The mobile backpack CRNS is intended to preserve the inherent advantage of the CRNS features (large spatial scale) with additional applications in difficult terrain and remote locations. CRNS backpack soil moisture sensing was conducted across three different field sites representing diverse elevations within the Rauris valley. These sites were chosen for their management styles and uses by the local agricultural community (i.e. cattle grazing at ≈1700 m and ≈1400 m and hay production at ≈900 m). The mobile CRNS was calibrated to local environmental conditions following the standard methodologies and procedures regarding proper field calibration of a mobile or stationary CRNS device. These methods primarily entail the sampling of soil and biomass within the footprint of the instrument to quantify environmental variables that can contribute error to the CRNS signal, such as clay content, green biomass, and atmospheric water vapour. Additionally, *in-situ* soil samples and time domain reflectance measurements were taken to determine soil moisture within the instrument footprint for data validation. The effect of altitude on the accuracy and footprint radius of the backpack CRNS device has yet to be fully understood; yet preliminary results indicate an increase in cosmic ray intensity at higher altitudes (Fig. 11a) and an overestimation of SWC by the CRNS backpack when compared to *in-situ* soil sampling estimates of SWC (Fig. 11b). It is important to note that additional data needs to be collected before any conclusions can be drawn. Field work is set to resume in the spring of 2017.

The SWMCNL is also in the process of developing a protocol guideline to supplement our ongoing research with the CRNS technology at high altitudes. This guideline will be designed to serve as a tool for Member States to reference when applying the CRNS within mountainous or high altitude environments. Details will be given regarding calibration, validation, and data processing. Additional discussion on the possible applications of SWC information at high altitudes will be included. Field work is also here set to resume in the spring of 2017.

This research has been conducted under CRP D1.20.13 on 'Landscape Salinity, and Water Management for Improving Agricultural Productivity'.

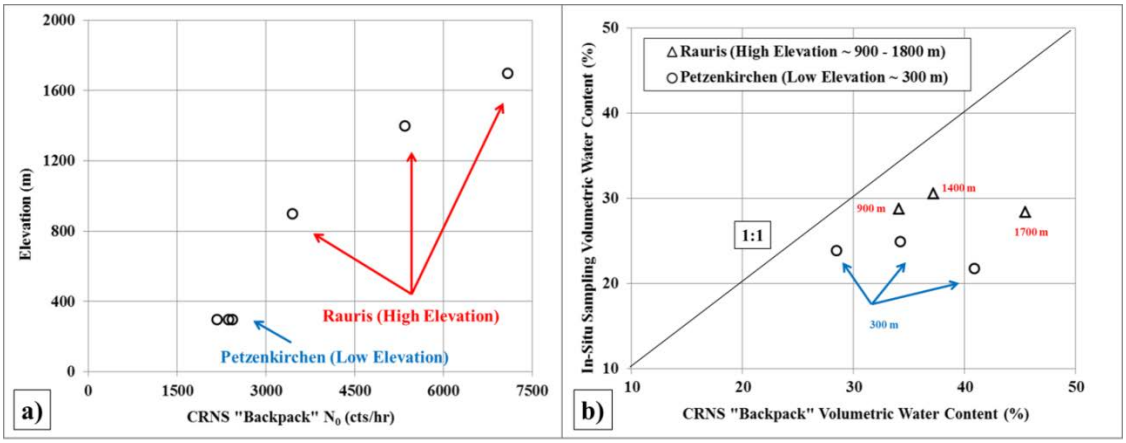


FIG. 11: a) CRNS 'backpack' cosmic ray neutron counts per hour (cts/hr) is compared to elevation across three field sites located at different elevations near Rauris, Austria, and a low elevation site near Petzenkirchen, Austria. b) Comparison of SWC values determined via the CRNS 'backpack' and traditional in-situ *sampling of SWC across all four field study sites (high and low elevations)*

This research was conducted under CRP D1.20.13 on 'Landscape Salinity, and Water Management for Improving Agricultural Productivity'.

Protocol development for the estimation of crop biomass water equivalent for use in the cosmic-ray neutron sensor calibration process in measuring area-wide soil moisture

Much of the CRNS work of the SWMCNL has gone into the development of protocols and guidelines on its proper use. This includes a detailed calibration process that is ultimately the key to the

successful implementation of the CRNS technology within any particular environment (see previous section). However, further details are needed regarding the incorporation of biomass water equivalent into the calibration process. The CRNS technology measures soil moisture through the detection of cosmic rays in the atmosphere near the soil surface. These rays have a great affinity to be absorbed by hydrogen atoms. As such, the CRNS can produce data that is highly correlated with SWC present in the soil. However, water within growing green biomass can introduce a false signal to CRNS data that must be quantified and accounted for. In 2016, research and development activities were initiated to detail three different methods for quantifying biomass and ultimately biomass water equivalent for use in the CRNS calibration process, through exploring and linking traditional *in-situ* destructive sampling of biomass (tailored for the CRNS footprint), satellite based remote sensing data, and the stationary CRNS[3]. These three techniques are applicable to the proper use of the CRNS technology, particularly in agricultural environments where homogeneous vegetation is the norm. Future work on this subject will include field based research and will explore the aforementioned techniques in biomass estimation for incorporation into the calibration process for both a stationary and mobile 'backpack' CRNS.

This research was conducted under CRP D1.20.13 on *'*Landscape Salinity, and Water Management for Improving Agricultural Productivity'.

The mitigation of soil water evaporation in agricultural areas via the application of mulched biomass cover

The SWMCNL has begun investigating methods to determine appropriate management strategies aimed at reducing or minimizing soil evaporation. Specifically, experiments have begun exploring the optimization of mulch biomass application techniques in and around existing crop vegetation in an attempt to discern its capacity to reduce water loss from the surface. An experiment has been conducted at the Seibersdorf laboratories comparing ground cover type (e.g. growing biomass and mulch) and dynamics between mulched and non-mulched maize. Another experiment utilized the mulching experiment which is ongoing since 2013 near Grabenegg, Austria in which multiple plots of maize crops with mulched and non-mulched areas were compared. Initial results indicate that the type of mulch used is important due mainly to the nitrogen composition of the plant from which the mulch originated (high nitrogen resulting in fast biodegradation).

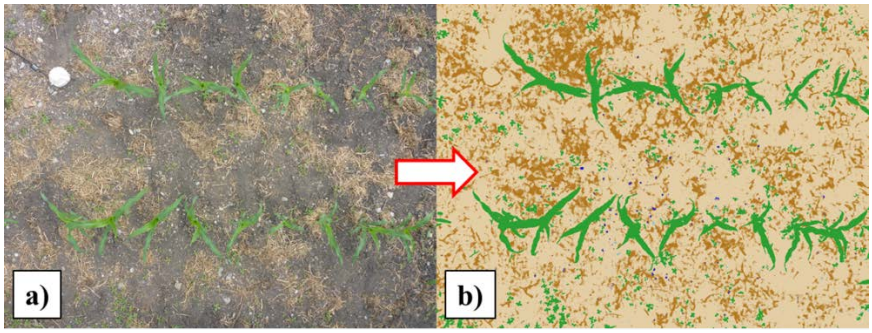


FIG. 12: a) Unprocessed raw image of immature maize plants recently planted (image taken with a standard hand-held digital camera mounted on a tripod). b) Processed version of the image in Fig. 12a; computer software classification was performed to determine percentages of bare ground, living and dead plant matter and rocks or stones

Ground cover imaging testing was carried out primarily via tripod-mounted camera equipment and image processing software. Additionally, a protocol is in development that will detail precisely how to set up experiments to observe the effect of mulch on soil water content and evaporation. An

[3] Our mobile CRNS device is not capable of estimating biomass itself due to its lack of a specialized detector capable of measuring both "thermalized" and "fast" neutrons (cosmic rays become neutrons by the time they reach the surface of the Earth), the ratio of which is key to the estimation of biomass via a CRNS alone.

example of the determination of ground cover, whether it is green biomass, mulch or bare soil is given in Fig. 12. Research in 2017 will use previously implemented ground cover imaging tools, along with in-situ soil moisture sensing technology and isotopic techniques (oxygen-18 and/or deuterium) to determine the impact of mulch on soil moisture and soil evaporation.

This research has been conducted under CRP D1.20.13 on 'Landscape Salinity, and Water Management for Improving Agricultural Productivity' and CRP D1.20.12 on 'Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-Livestock Production Systems'.

Nuclear Emergency Preparedness in Food and Agriculture

Based on past experience, there is a critical need to improve nuclear emergency preparedness in food and agriculture, including the collection (sampling and analysis), management and visualization of appropriate data from affected areas, for timely dissemination and communication to stakeholders. Member States are accordingly requesting technical assistance in their endeavours to improve nuclear emergency preparedness and response in food and agriculture.

Response to nuclear emergencies affecting food and agriculture - an online food safety information system for nuclear and radiological emergencies

The CRP D1.50.15 on 'Response to Nuclear Emergencies Affecting Food and Agriculture', aims to develop and assess systems of innovative data collection (including soil and foodstuff sampling and analysis), management and geo-visualization platforms that can be used for both routine monitoring and emergency response to nuclear and radiological incidents that could affect food and agriculture.

To date, standard operating procedures (SOPs) are being compiled and prepared, in particular for large-scale sampling and radionuclide concentration analysis of soil and foodstuffs in case of a nuclear or radiological emergency affecting food and agriculture. Few, if any, emergency tools or protocols focus on the food production environment, for example radioactivity concentrations in soils. In 2016, the Operational Intervention Levels for Soils (OIL for Soils) concept was proposed, it is an optimization tool developed at the SWMCNL to facilitate agricultural decision making and to improve nuclear emergency preparedness and response capabilities. OILs for Soils – pre-determined reference levels of air dose rates linked to radionuclide concentrations in soils – can be used to trigger response actions particularly important for agricultural and food protection. Key considerations in the development of the OILs for Soils are: (1) establishing a pragmatic sampling approach to prioritize and optimize available resources and data requirements for decision making in agricultural sites: (2) creating a system that is adaptable to the needs of different countries, and; (3) developing a framework to calculate default values of OILs for Soils for application during an emergency.

Further, an advanced prototype of the online information system to support decision-making in food safety in case of a nuclear emergency is now available, called DSS4NAFA, and being further developed. The Information Technology Advisory Group (ITAG) of the IAEA has approved the development of the system, and an independent review of the system has been started by KPMG in close collaboration with the IAEA - MTIT department to ensure sustainable implementation and information security. Major efforts are being made to integrate the data management and visualization part of the information system, and to establish the algorithm for decision support with regards to the implementation of food restrictions. Significant progress has been made as well to link this system with existing data exchange platforms of the IAEA, such as the Unified System for Information Exchange on Incidents and Emergencies (USIE) and International Radiation Monitoring Information System (IRMIS) managed by IEC.

CAPACITY BUILDING AND SERVICES

Training on 'Nitrogen and Soil Water Management in Agro-Ecosystems', 25 July-19 August 2016, Seibersdorf, Austria

The SWNCNL hosted a four-week group fellowship training in Seibersdorf. During the first two weeks, and as part of the 2016 International Year of Pulses, the training focused on the use of ^{15}N for improving nitrogen management in agro-ecosystems and specifically on how to assess the capacity of grain legume crops (pulses) to capture nitrogen from the atmosphere. The contribution by Ms Rebecca Hood, Austrian Institute of Technology, and the WebEx video lecture by Mr Walter Palme on unmanned aerial vehicle (UAV)-based multispectral analytical tools for assessing nitrogen and water status in crops were much appreciated. During the last two weeks of the course, agricultural water management was addressed, in particular the use of soil moisture sensors and data interpretation. Nineteen fellows from ten countries participated during the first two weeks of the course; and ten during the last two weeks.

Regional training course on 'The use of short-lived fallout radionuclides (e.g. ^{7}Be) for evaluating soil erosion/sedimentation magnitudes and the effectiveness of soil conservation', 17-28 October 2016, Seibersdorf, Austria

This two-week training course was carried under RAF5075 on 'Enhancing Regional Capacities for Assessing Soil Erosion and the Efficiency of Agricultural Soil Conservation Strategies through Fallout Radionuclides' and was attended by 24 fellows from 11 African Member States (i.e. Algeria, Benin, Côte d'Ivoire, Egypt, Madagascar, Morocco, Senegal, Sudan, Tunisia, Uganda and Zimbabwe), one intern from Malaysia, one fellow from Argentina under the ICTP/IAEA Sandwich Training Educational Programme and two Iranian participants funded through the national IRA5013 project on 'Investigating the Effects of Deforestation and Afforestation on Soil Redistribution'. The course included lectures, guided exercises and discussions on how to use short-lived FRN such as ^{7}Be (and to some extent ^{137}Cs) to investigate short soil redistribution time scale processes and to assess the effectiveness of soil conservation strategies in agro-ecosystems.

Technical Workshop on 'Remediation of Radioactive Contamination in Agriculture', 17-18 October 2016, Vienna, Austria

A Technical Workshop on 'Remediation of Radioactive Contamination in Agriculture' was co-organised by the Joint FAO/IAEA Division and the National Agriculture and Food Research Organization of Japan (NARO) and held at the IAEA headquarters, Vienna, Austria from 17-18 October 2016. Over 100 experts from around the world participated in the event. Presentations and discussions focused on research results and practical experience from Japan and from countries affected by the Chernobyl NPP accident. The event was a great success in promoting and sharing knowledge and experience related to remediation of radioactive contamination in food and agriculture. Copies of the presentations are available online at www-naweb.iaea.org/nafa/news/2016-FAO-IAEA-NARO.html.

Analytical services

A total of 5850 samples were analysed at the SWMCNL during 2016 for stable isotopes and 220 samples were measured for fallout radionuclides. Most analyses were carried out in support of research and development activities at the SWMCNL focusing on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. Analytical support was provided as well to the Plant Breeding and Genetics Laboratory with about 300 samples, to the Insect Pest Control Laboratory with about 540 samples and to the Food and Environmental Protection Laboratory with about 480 samples.

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

The 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 important tool for external quality control.

The 2016 Proficiency Test (PT) on ^{15}N and ^{13}C isotopic abundance in plant materials, organized by the University of Wageningen, the Netherlands and funded by the SWMCNL, was successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, www.wepal.nl) is accredited for the organization of Inter-laboratory Studies by the Dutch Accreditation Council.

Every year, one ^{15}N-enriched plant test sample is included in one round of the WEPAL IPE (International Plant-Analytical Exchange) programme. A special evaluation report for IAEA participants on the analytical performance in stable isotope analysis is issued by the SWMCNL and sent to the participants together with a certificate of participation additionally to the regular WEPAL evaluation report.

In total, fourteen stable isotope laboratories participated in the PT-round 2016: Africa (2): Kenya and Morocco; Asia (3): Pakistan and Philippines (2 labs); Europe (4): Austria, Belgium, France and Germany; Latin America (4): Argentina, Brazil, Chile and Uruguay); and South Pacific (1): New Zealand.

Eleven out of thirteen laboratories participating in the nitrogen analysis reported ^{15}N-data within the control limits for the enriched plant sample and seven out of eleven participating laboratories in carbon analysis reported ^{13}C isotopic abundance results within the control limits.

GUIDELINES AND PUBLISHED INFORMATION

New FAO/IAEA Publication: 'Cosmic Ray Neutron Sensing: Use, Calibration, and Validation for Soil Moisture Estimation'

This FAO/IAEA publication (IAEA-TECDOC-1809) provides guidelines for scientists, technicians, and students in Member States on the use of the recently developed Cosmic Ray Neutron Sensor (CRNS) to measure water content in the topsoil over wide areas, covering up to 30 hectares. The CRNS is a device that monitors soil water content in a non-invasive, non-hazardous, and continuous way. It fills the gap in measuring soil moisture over large areas for better agricultural water management. The technique is applicable to a number of disciplines, including ecology, agronomy, atmospheric science, and remote sensing which require a robust, readily deployable field instrument for automatic monitoring of near surface and area wide moisture conditions. It can be found at: http://www-pub.iaea.org/books/IAEABooks/11097/Cosmic-Ray-Neutron-Sensing-Use-Calibration-and-Validation-for-Soil-Moisture-Estimation.

New FAO/IAEA Publication: 'Supporting sampling and sample preparation tools for isotope and nuclear analysis'

This FAO/IAEA publication (IAEA-TECDOC-1783) was developed to provide illustrated, step by step, comprehensive guidance for sampling and processing of soil, water and plant materials. It aims to assists scientists, technicians and students in Member States in implementing procedures and tools to take and prepare samples for isotope and nuclear analyses in their efforts to develop climate-smart agricultural practices for improved soil, water and nutrient management and to prepare and respond to nuclear emergencies in food and agriculture. The TECDOC includes the following modules: (i) Particulate organic matter separation, (ii) Method for the purification of inorganic phosphate in soil and sediment samples prior to analysis of the $\delta^{18}O$ isotopic abundance in phosphate, (iii) Extraction of water from soil and plant samples for $^{18}O/^{16}O$ and D/H isotope ratio

measurements, (iv) How to perform precise soil and sediment sampling? One solution: The Fine Increment Soil Collector and (v) Guidelines for measuring bulk density of soil. It can be found at: www-pub.iaea.org/MTCD/Publications/PDF/TE-1783_web.pdf.

Using Nuclear Science to Manage Nitrogen – a new animated infographic video by the Joint FAO/IAEA Division

A new animated infographic video on 'Managing Nitrogen' provides the lay audience with a comprehensible introduction to the importance of nitrogen (N) for crop production and the many uses of nitrogen-15 towards sustainable, climate-smart agriculture. It can be found at: www.youtube.com/watch?v=wC2f8hMd3-Y&feature=youtu.be**Error! Hyperlink reference not valid..** You will find the full list of all the animated infographic videos produced by the Joint FAO/IAEA Division at: www.youtube.com/playlist?list=PLzp5NgJ2-dK7malfX4U8aqEiO1wXmeQrv.

National Geographic Channel explores how nuclear technology helps climate change adaptation

The work of the Joint FAO/IAEA Division, including that of the SWMCNL, on climate change adaptation was shown in December 2016 on the National Geographic Channel in Belgium, France and the Netherlands for a wide audience. The 24-minute documentary highlights how nuclear techniques can help the agricultural sector to assess and curtail the worldwide challenge of climate change. It is currently available in Dutch and French; see: www.nuclearlifeline.be/episode-2/#detail-episode-2 (click 'FR' in top right-hand corner of the screen to reach the French version, select 'Episode 2', scroll down and start film); an English version is planned for 2017 (same link).

Long Night of Research, 22 April 2016, Vienna, Austria

More than 1300 visitors visited the IAEA Headquarters in Vienna on 22 April 2016 for the *Long Night of Research*. The large rotunda of the Vienna International Centre was transformed for one night into an interactive exhibition space to showcase nuclear sciences and applications for peace and development. IAEA scientists highlighted more than a dozen scientific fields, with special attention to the smallest, youngest and very junior 'scientists' under us.

The SWMCN Subprogramme presented the use of innovative and state-of-the-art stable isotope techniques for monitoring greenhouse gases from agriculture. Visitors to the *Long Night of Research* also learned, through a keynote speech given by the Head of the SWMCNL, why soil management matters to climate change.

World Soil Day: Madagascar Combats Soil Erosion with Tradition and Nuclear Science

An age-old agricultural method is helping to combat soil degradation and protect a source of food and income for the population in Madagascar. Using radioisotopic techniques, Malagasy scientists, working in cooperation with the SWMCNL, found that traditional terrace farming can reduce soil erosion and run-off on mountainous land by up to 40%.

The full story, which was published on World Soil Day on 5th December 2016, is available at: www.iaea.org/newscenter/news/world-soil-day-madagascar-combats-soil-erosion-with-tradition-and-nuclear-science.

Sharing our research progress and connecting with international researchers through the European Geosciences Union General Assembly 2016, Vienna, Austria

The European Geosciences Union (EGU) 2016 Programme Committee organized a very successful 2016 EGU General Assembly, with 4863 oral, 10 320 poster and 947 PICO (i.e. Presenting Interactive COntent™) presentations. Approximately 13 650 scientists from 109 countries attended this event in Vienna and its Division of Soil System Sciences had more than 1400 scientific contributions, of which the SWMCN Subprogramme activities were reported in 16 presentations (oral, poster and PICO)

covering soil erosion, soil conservation, climate change, carbon and nitrogen cycling (see list of publications below).

Contribution to the IAEA Nuclear Technology Review 2016

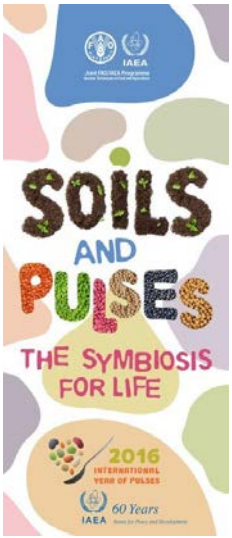
The SWMCNL contributed to the IAEA's Nuclear Technology Review 2016 (see pp. 80-84) by reporting on research and development progress in conjointly using fallout radionuclides and compound-specific stable isotope techniques for optimising soil conservation strategies. For more information, see: www.iaea.org/sites/default/files/16/08/ntr2016.pdf

SWMCNL success stories in 2016

Eight success stories were published by the SWMCN Subprogramme highlighting examples of country impacts derived through improving soil and water management and crop nutrition across Africa and Asia, and enhancing nuclear emergency preparedness and response in food and agriculture. Four stories were prepared with the support of the SWMCNL, i.e. (i) Madagascar Combats Soil Erosion with Tradition and Nuclear Science, (ii) Morocco – Erosion in Moroccan watersheds can be reduced up to 60 percent through the use of isotopic techniques, (iii) Iraq - Nuclear Technology to Improve Crop Productivity and Adapt to Climate Change, and (iv) Food and agriculture: responding to nuclear emergencies. These stories can be downloaded at:

- www.iaea.org/newscenter/news/world-soil-day-madagascar-combats-soil-erosion-with-tradition-and-nuclear-science;
- www-naweb.iaea.org/nafa/resources-nafa/IAEA-success-Stories-3.pdf;
- www.iaea.org/newscenter/news/iraq-uses-nuclear-technology-to-improve-crop-productivity-and-adapt-to-climate-change.
- www-naweb.iaea.org/nafa/resources-nafa/food-agriculture-brochure.pdf

Celebration of 2016 International Year of Pulses



The SWMCN and the Plant Breeding and Genetics Subprogrammes of the Joint FAO/IAEA Division celebrated the International Year of Pulses on 20 September at the IAEA headquarters to help raise public awareness of the nutritional benefits and the role of pulses in sustainable food production. The event, *Enhancing Pulses for Food Security using Nuclear Applications*, highlighted the contribution of pulses towards food security and mitigating the effects of climate change. A variety of pulses and pulse plants were displayed with their nitrogen-fixing roots and nodules visible. The event also highlighted the roles of nuclear applications related to pulses, including the use of stable isotope of nitrogen to identify pulses and legumes with high nitrogen fixing ability and to quantify the amount of nitrogen fixed. Eight delicious pulse recipes were also served. More information can be found at www.iaea.org/newscenter/multimedia/videos/pulses-celebrating-a-powerful-superfood.

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AN UPDATE ON THE RENUAL PROJECT FOR THE FAO/IAEA AGRICULTURE & BIOTECHNOLOGY LABORATORIES

ReNuAL is an initiative to modernize aging laboratories in Seibersdorf, Austria that are managed by the IAEA's Department of Nuclear Science and Applications. These eight laboratories, five of which belong to the Joint FAO/IAEA Division, strengthen Member States' capacities to use nuclear and related techniques in food and agriculture, human health, the environment and scientific instrumentation.

Construction Progress

Construction cranes and heavy vehicles dominate the ReNuAL construction site in Seibersdorf. Work on the new laboratory buildings began in July 2016 following the completion of the site's new high-voltage electrical network that will power the new buildings. Already, the foundation and the exterior structure of the Insect Pest Control Laboratory (IPCL) have been completed. The next stage of construction, which has already commenced, is focused on the interior. The building remains on schedule for completion by December 2017.

The new IPCL facility remains on schedule for completion in 2017

The original strategy for ReNuAL foresaw three wings of a second building, the Flexible Modular Laboratory (FML), the third wing planned for the Terrestrial Environment Laboratory (TEL). Since then, however, demands on the Animal Production and Health Laboratory (APHL) have grown particularly rapidly. In 2015, the Agency responded to an outbreak of the Ebola virus in West Africa. In 2016, the Agency's activities extended to support Member States to cope with the outbreak of the Zika virus in Latin America and the outbreak of lumpy skin disease in Eastern Europe. These animal and zoonotic disease outbreaks resulted in increasing demands by Member State for support from the APHL. The APHL is therefore now planned to be the third wing of the FML.

Work on the foundation of the FML also began in December. The full construction of two of the three planned laboratory wings of the FML is currently planned for completion in mid-2018.

Key Fundraising Goal Reached

ReNuAL officially commenced in January 2014 with the goal of raising €20.6 million in extrabudgetary contributions to fund the overall project budget of €31 million. This target was reached in mid-2016. At the June 2016 meeting of the Board of Governors, New Zealand and Canada pledged further funds to the project to close the remaining extrabudgetary funding gap.

ReNuAL has a strong base of support among Member States. The extrabudgetary funding target was reached with bilateral contributions from 26 Member States and a collective contribution made by AFRA (the African Regional Cooperative Agreement for Research, Development and Training Related to Nuclear Science and Technology).

Regular budget funds of €10.4 million have been allotted to reach the full €31 million budget for the project.

During the 60th General Conference, Director General Yukiya Amano lauded the success of the ReNuAL project thus far at a side event for Member States, and discussed the future of the project. He was joined by South African Ambassador Tebogo Joseph Seokolo and Thorsten Herdan, Director General of the Energy Policy Department of the Federal Ministry of Economic Affairs and Energy of Germany, both co-chairing the Friends of ReNuAL, and by several high-level dignitaries from contributing Member States.

Contributing Member States' representatives at the 60th IAEA General Conference in September 2016

Each of the contributing Member States will be recognised in the new laboratories as part of a commemorative wall. Symbolic bricks engraved with contributing states' names were presented at the General Conference event.

Bricks recognise contributing Member States

Director General Amano described the importance of ReNuAL: "ReNuAL will lead to a significant enhancement in the high-quality scientific support we can offer our Member States in improving the well-being and prosperity of their people," he said. "Much important work still lies ahead of us. In the coming years, we will also work to ensure the smooth operation and long-term sustainability of the laboratories."

Paving the Way Forward

To keep ReNuAL within the €31 million budget following cost increases during the design process, some construction components were postponed to be addressed as follow-up to ReNuAL. These include the remaining finishings and furnishings for the new IPCL; the construction of the third wing of the FML; a bunker to house a linear accelerator (LINAC) for the Dosimetry Laboratory; and some remaining unfunded equipment needs.

Member States are already demonstrating their readiness to support these continuing resource mobilization efforts with new contributions. More than €4 million have recently been pledged or provided by several Member States: Germany, Indonesia, Kazakhstan, Oman, Qatar, the United Kingdom and the United States of America; in addition, Ambassador Sadiq Marafi, Permanent Representative of the State of Kuwait, made a personal contribution. The available funds are expected to be sufficient to achieve full completion of both the IPCL and the Dosimetry Bunker. The next funding priority is to raise extrabudgetary resources for the completion of the third wing of the FML.

ReNuAL and ReNuAL+ will deliver significant enhancements for all five FAO/IAEA Agriculture & Biotechnology Laboratories in Seibersdorf to ensure that they continue to be able to respond to Member States' growing and evolving needs. The successful conclusion of these projects will significantly enhance the Joint FAO/IAEA Division's capacities to assist Member States in their efforts to achieve the Sustainable Development Goals through the peaceful uses of nuclear science and technology in food and agriculture. Accordingly, completing ReNuAL on time and within budget is a key priority. In parallel, immediate ReNuAL+ fundraising efforts will focus on securing the funds needed for the third wing of FML in a timely manner to achieve maximum cost efficiency.

Perspective of the IPCL building currently under construction