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Laboratories

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THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

The Animal Production and Health Laboratory (APHL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides assistance to Member States (MSs) in improving productivity of livestock and preventing and controlling transboundary animal infectious diseases and zoonoses (TAZDs). Nuclear and nuclear related techniques for immunology, serology, molecular biology and genetics are the main technologies applied in the APHL and transferred to Member States' laboratories to support animal production and health.

Concerning animal health, APHL activity focuses on three major topics: 1) application of nuclear and nuclear derived technology for the development and evaluation of safer, more effective vaccines; 2) development and validation of laboratory diagnostic assays for the rapid and accurate detection of microorganisms causing animal diseases; and 3) assistance to MSs veterinary diagnostic laboratories for preparedness and rapid response to TAZDs. This is done through technical assistance such as: applied research, scientific investigations on the molecular epidemiology and spread of the infections, and preparation/distribution of harmonized protocols, laboratory reference material and reagents.

In 2019, research and development in animal health included the evaluation of performance of a new irradiated vaccine developed in APHL against avian influenza, one of the most economically important poultry diseases with zoonotic potential too. In close collaboration with Austrian and Italian laboratories, APHL has demonstrated, for the first time in chickens, the effective protection provided by an irradiated vaccine prototype against the H9N2 subtype of avian influenza virus and the higher performance of this vaccine compared to a vaccine prepared using traditional methods. These achievements open the doors for the development of novel, safer and more efficient vaccines for major transboundary animal and zoonotic diseases. The next irradiated vaccine candidate under investigation in APHL is an important viral disease of swine, the Porcine Reproductive and Respiratory Syndrome (PRRS) virus. Experiments were conducted to evaluate the immunogenicity of an irradiated candidate vaccine strain. In parallel, research was conducted to develop cost-effective in-vitro assays to evaluate the cell mediated immune response of swine to viruses and vaccines.

Concerning swine diseases, in 2019 African swine fever (ASF), a highly lethal haemorrhagic disease of domestic and wild pig endemic in several African countries, continued to spread in Europe (wild boars, mainly) and Asia, causing huge economical losses. APHL has supported several African and Asian countries to detect and characterize local isolates of ASF virus. VETLAB partners in Tanzania, Namibia, Mongolia, Lao PDR and Vietnam, collaborated with APH to study ASF virus isolates collected during outbreaks between 2015 and 2019, leading to two publications with Tanzania and Namibia. Similar support was provided to MSs facing another devastating disease, Peste des Petits Ruminants - an acute, highly contagious and often fatal viral disease of sheep, goats and small wild ruminants. Because of it social and economic impact in many countries, PPR has been selected by international organizations (FAO and OIE) for global eradication by 2030. Molecular epidemiology studies were conducted in MSs to assist them in tracking the origin and circulation of this virus.

Under animal genetics, APHL made a significant achievement by establishing a low-cost, high throughput, semi-automated genotyping protocol to support cattle breeding programs in MSs. More than 50000 markers were tested for their suitability to evaluate zebu and crossbred cattle, of which 10000 were found to be efficient and free of ascertainment bias. This new validated protocol will help developing MSs, particularly in Asia and Africa, to access highly advanced nuclear related genomic technologies and improve the efficiency of their national breeding programs. APHL also designed and developed a protocol for DNA based evaluation and characterization of indigenous pig breeds. In 2019, significant achievements were also made in successfully implementing National Action Plans on Animal Genetic Resources in various MSs. With APHL support and through IAEA technical cooperation projects, Sri Lanka completed molecular characterization of three cattle populations, while Pakistan

developed baseline genetic information on 9 indigenous cattle breeds. The technical support and guidance provided by APHL to "Marker assisted breeding program for improving sheep prolificacy in South India" was successful with increase in the number of twin births and farmers' income in small holder sheep flocks. The collaborative project on "Genomic evaluation of Eastern European cattle for development of a dual-purpose cattle breed" funded by FAO-TC project (FAO/RER3604) was also highly successful, both in terms of timely implementation and strategic information generated to guide the national breeding programs in Armenia, Georgia and Ukraine.

In addition to R&D, APHL dedicated efforts to organize and conduct capacity building activities in IAEA and FAO Member States. Through two regional, two international and six national training courses, more than 150 personnel across the world were trained in 2019 on advanced nuclear and related genomic technologies on animal health, pathogens detection, animal genetic characterization, automated sequencing and genotyping, bioinformatics analysis of pathogens and livestock genome data, artificial insemination and pregnancy diagnosis. APHL also hosted 13 fellows, 2 trainees, 2 interns and 1 scientific visitor for training on animal genetic resource characterization, animal disease detection and bioinformatics analysis of livestock and pathogens genetic/genomic data. Significant progress was made on strengthening animal genetic laboratories in at least nine countries. Notably, a fully functional sequencing/genotyping facility was established in Ouagadougou, Burkina Faso, to cater to the needs of several West African countries. All these efforts were recognized and appreciated by many Member States, most notably by Argentina, Bulgaria and Burkina Faso.

Knowledge dissemination and data sharing has been central to APHL activities, through: (a) APHL involvement and coordination of the VETLAB Network of national veterinary diagnostic laboratories, the membership of which counts 45 African and 19 Asian countries; (b) sharing of technical data and scientific information with MSs and scientific communities (24 publications in refereed high impact scientific journals, three chapters in scientific books, and 1 oral presentation in an international conference); and (c) resource mobilization to enhance capacity building and technology transfer, with financial support from USA and Japan (IAEA Peaceful Uses Initiative), from South Africa (African Renaissance Fund) and OPEC Funds for International Development.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

New animal vaccine prototypes and formulations developed using irradiation technologies

Potency of an Irradiated Vaccine Against Low Pathogenic Avian Influenza (H9N2) in Chicken

Low pathogenic avian influenza (LPAI) in chicken caused by influenza subtype H9N2 virus is the most common LPAI in chicken worldwide and causes massive economic losses to poultry economy and industry. The only control and prevention methods are through effective vaccination. Currently, commercially available vaccines are inactivated vaccines which has two major limitations: a) they do not provide optimal protection to heterologous virus infections and b) they do not induce protective immunity when administered at mucosal level as aerosols, which is easy and cost effective in large scale operations. Therefore, a study was designed to investigate if a novel irradiated H9N2 vaccine prototype could overcome above problems that exists in current chemically inactivated commercial vaccines. This study was conducted as a collaborative project with Italian veterinary institute and research organization for animal health and food safety (Istituto Zooprofilattico Sperimentale delle Venezie: IZSVe), which is an international (FAO, OIE and EU) reference centre for avian influenza. The selected H9N2 vaccine strain (A/Chicken/Saudi Arabia/13VIR362231/13) was irradiated at 60 kGy with trehalose under frozen state. Same isolate was chemically inactivated using formalin (as for traditional vaccine). Specific free pathogen (SPF) chickens at week 6 were vaccinated and boosted at week 8 according to the following schedule.

Group	Vaccine formulation	Route
1	Irradiated	Nasal-ocular
2	Formalin inactivated	Nasal-ocular
3	Irradiated	Subcutaneous
4	Irradiated with adjuvant	Subcutaneous
5	Formalin inactivated with adjuvant	Subcutaneous
6	None (Control)	

The birds were challenged with either homologous H9N2 (A/Chicken/Saudi Arabia/13VIR362231/13) or heterologous H9N2 (Iran/2002) virus at week 10 and monitored for another two weeks and sacrificed at the end of the experiment. The birds were assayed for clinical, virological and immunological parameters

Preliminary results suggest that the antigenicity of the virus was fully preserved following irradiation (based on data related to the major virus antigen, the haemagglutinin). The antibody response was high in all five vaccinated groups based on the haemagglutinin inhibition antigen. Importantly, serum antibody titer in group 1 vaccine was two logs higher for the homologous virus and one log high antibody response for the heterologous virus compared group 2 (mucosal application).

In terms of protection following challenge, following observations were made:

1. With homologous virus challenge a significant reduction in virus shedding was induced by both the irradiated vaccine (delivered through the subcutaneous route) and formalin inactivated vaccine with adjuvant (formulated resembling the existing commercially available vaccine) compared to the control group but the irradiated vaccine performed even better than the commercial vaccine.
2. With heterologous challenge, only the irradiated vaccine delivered through the subcutaneous route induced a significant virus shedding.
3. In terms of protection induced by the mucosal route, the irradiated vaccine reduced the virus shedding compared to formalin inactivated vaccine.

The preliminary results suggest irradiation technology as a promising and better alternative to chemical inactivation in producing an effective vaccine against avian influenza. Further analysis is currently done at APHL to evaluate protection and investigate the immunological mechanisms of the irradiated vaccine and further animal experiments will take place next year.

In-vitro experiments to evaluate an irradiated Porcine reproductive and respiratory syndrome (PRRS) virus as a vaccine candidate

Porcine reproductive and respiratory syndrome (PRRS) is a disease that causes heavy economic losses to the livestock industry worldwide. The currently available modified live vaccine is sub-optimal. In 2017, APHL, in collaboration with the Austrian Agency for Health and Food Safety (AGES) conducted animal experiments to evaluate irradiated-inactivated PRRS virus as a vaccine candidate (ABL report 2017). The results of the study were promising, and the immunogenicity of the irradiated vaccine was shown through the cell-mediated immune responses. However, no antibody responses were detected following vaccination. Usually, antibody responses to PRRS vaccinations become evident following challenge and the study conducted did not include infection. The absence of antibodies seen in this study could also be due to the lower antigenicity of the vaccine. Therefore, we conducted further experiments to improve the vaccine by concentrating the vaccine. In current experiments, a high pathogenic PRRSV2 field strain was cultured in MARC 145 cells and concentrated by ultracentrifugation. The concentrated virus culture was then treated with low energy electron irradiation (LEEI) or gamma irradiation (with and without trehalose as stabilizer) at a dose of 30kGy. Next, inactivation was confirmed through cell culture passaging. Virus antigenicity testing was performed through ELISA (in house developed). The results obtained by these experiments suggested the preservation of virus antigenicity following concentration through ultra-centrifugation and irradiation for both LEEI and gamma irradiation methods (*In-vitro testing of the antigenicity and safety of two newly developed irradiated vaccine candidates against highly pathogenic porcine reproductive and respiratory syndrome virus 2. p.250-253; 11th European Symposium of Porcine Health Management (ESPHM2019), May 22-24, 2019, Utrecht, Netherlands; IMM-PP-172*). Moreover, the structure of the irradiated virus remained intact when examined by electron microscope (Fig 1:). However, the augmentation of the virus concentration remained at 3-fold by ultracentrifugation. Therefore, in the second phase, we investigated another method of virus concentration; ultrafiltration, a method in which the virus is concentrated by removing low molecular weight proteins. We were able to concentrate PRRS virus cultures by 10-fold using ultrafiltration. Further experiments showed when ultrafiltrate-concentrated PRRS virus is gamma irradiated at 30kGy, it stills preserves its antigenicity. We will soon start animal trials to explore efficacy of this antigen as a vaccine in a challenge study.

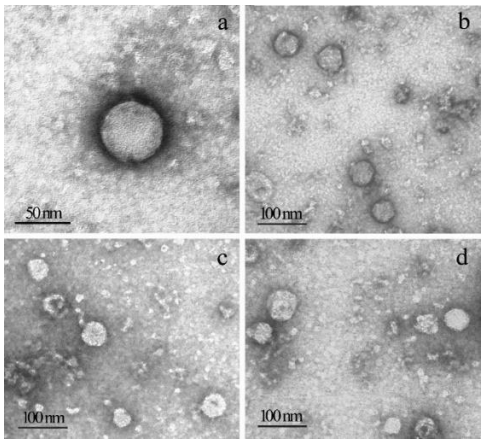


Fig 1: Electron microscopy of the ultracentrifuged virus suspensions of PRRSV2. a-b) a non-irradiated sample, c) sample irradiated by LEEI and d) a sample irradiated by γ-irradiation. -Samples were fixed in Karnovsky solution. The preparation method of "negative staining" was performed to carry out quantitative and qualitative analysis of virus particles.

Cloning swine Interluekin-4 for vaccine research and development

Interluekin-4 (IL-4) is a cytokine that participates in the regulation of the immune system at multiple levels and it acts as a growth and survival factor for lymphocytes. Although it was discovered as a B cell differentiation and stimulatory factor, its role in regulating T cell differentiation is critical during the immune response. Moreover, IL-4 in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) triggers monocytic differentiation into Dendritic cells (DCs). IL-4 also can trigger the differentiation of monocytes into macrophages. DCs are the most potent antigen presenting cells. Thus, DCs can be used to investigate the immune properties of a vaccine antigen. APHL has developed an in-vitro assay to investigate vaccine antigens using monocyte derived DCs (Mo-DCs) for bovine and same can be developed for swine if research reagents are available such as IL-4. On the other macrophages are a good target for growing virus such as African Swine Fewer (ASF). In addition, cytokines can also be used as "vaccine adjuvant", thus enhancing the immune response to a greater extent leading to protection. In order to test IL-4 as a potential vaccine adjuvant, to use it for the generation of monocyte-derived Mo-DCs to evaluate vaccine efficacy and to generate macrophages to grow virus, we produced swine recombinant IL-4. In the first step, peripheral blood mononucleocytes from swine were stimulated for higher expression of cytokines and total mRNA were harvested. Then, a reverse-transcription of the mRNA to cDNA was performed, to be then used as template for Polymerase Chain Reaction (PCR) implementation. The PCR products were subjected to gel electrophoresis to assess if the amplification of the gene of interest was successful. The amplicon was confirmed, based on the size of the band of interest. After PCR, the products were inserted into a TOPO vector and amplified in the transformed E. coli.

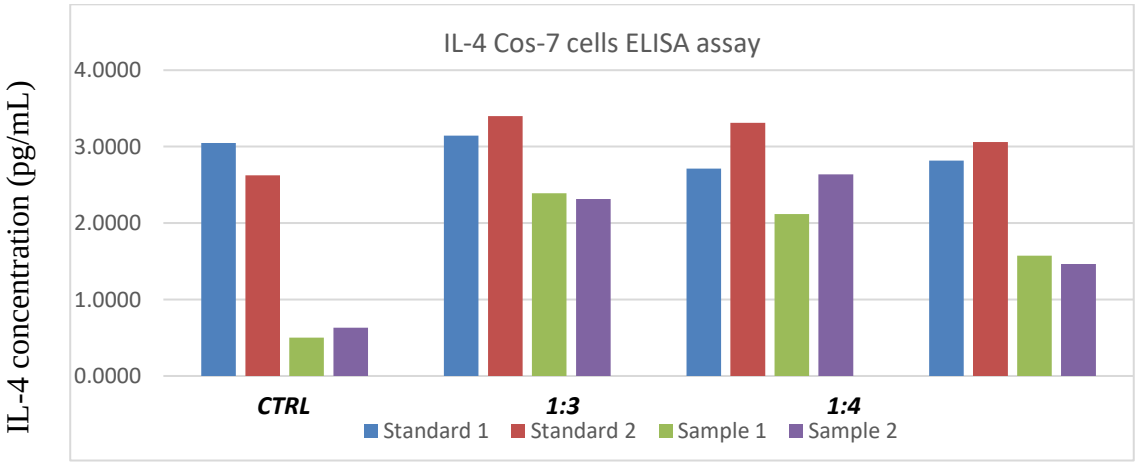


Fig.2 Concentration of swine recombinant IL-4 produced. The pcDNA-SS-IL-4 was transfected with varying ratios of DNA to transfected reagents (1:3, 1:4 and 1:6) into COS-7 cells. The supernatants and cell lysates were combined and subjected to ELISA analysis.

The sequences of inserts were determined by thermal cycle sequencing. After checking against published sequences, the coding sequence of swine IL-4 was incorporated to eukaryotic expression vectors, neomycin resistant pcDNA-SS (Vet. Immunol. Immunopathol 126 (2008) 388–391) that express a signal sequence (A kind gift from Dr Kikuya Sugiura, Osaka Prefecture University, Japan) to construct pcDNA-SS-IL-4. The pcDNA-SS-IL-4 was transfected with varying ratios of DNA to transfected reagents into COS-7 and cultured under reduced serum supplementation. Next, produced IL-4 was collected together both in the supernatant and in the cell lysates. Finally, to further confirm the functional properties of recombinant swine IL-4, a commercially available ELISA (enzyme-linked immunosorbent assay) kit to detect swine IL-4 was used. By using this powerful method for detecting and quantifying specific proteins, we assessed the presence of our target protein, thus confirming the successful cloning of Swine IL-4 (Fig.2).

Molecular epidemiology of ASF

African swine fever (ASF) is a highly lethal haemorrhagic disease of domestic and wild suids. The disease is endemic in several African countries and has recently emerged in Europe and Asia, causing huge economical losses.

In 2019, APHL has supported several African and Asian countries to detect and characterize local isolates of ASF virus. VETLAB partners in Tanzania, Namibia, Mongolia, Lao PDR and Vietnam, collaborated with APH to study ASFV isolates collected during outbreaks between 2015 and 2019, leading to two publications with Tanzania and Namibia.

Molecular epidemiology of ASF in Tanzania

In Tanzania, where ASF is endemic since 2001, they have been an increasing number of reports on the circulation of ASFV strains causing a broad range of clinical symptoms in susceptible animals. For instance, the country recorded several outbreaks, including symptomatic and asymptomatic cases between 2015 and 2017. To understand the genetic diversity of ASFV involved in those outbreaks, we analysed 35 clinical samples from four outbreaks and sequenced four genomic targets in seventeen of those samples: the partial B646L (p72), the full E183L (p54) gene, the central variable region of the B602L gene and the intergenic region between the I73R and I329L genes.

The p72 gene tree and the complete p54 (E183L) gene tree revealed that the ASFVs in samples from symptomatic pigs are of genotypes II, while those in samples from asymptomatic pigs were genotype IX viruses. The CVR profiles of the genotype II and genotype IX isolates differed between each other and from previously published Tanzanian sequences. The sequence analysis of the intergenic region between the I73R and I329L for the 2017 genotype II isolates showed the absence of one GGAATATATA motif in those isolates.

This study showed the simultaneous circulation of two different ASFV genotypes with different levels of pathogenicity in Tanzania. Since the existence of sub-clinically infected pigs may contribute to the persistence of the virus, our findings suggest continuous surveillance and characterization of ASFV isolates in disease-endemic regions. A paper describing these findings is available in the journal Transboundary and Emerging Diseases (DOI: 10.1111/tbed.13298).

African swine fever in Namibia

As for most ASF endemic countries, every year, ASF causes sporadic outbreaks throughout Namibia. To monitor the evolution of ASFV, APHL assisted the VETLAB partner in Namibia to genetically characterize isolates collected during outbreaks in 2018.

Five samples from four suspected outbreaks of African swine fever in Namibia in 2018 were sequenced and analysed. The analysis involved the following targets: The C-terminus of the B646L gene (p72 protein), the central hypervariable region (CVR) of the B602L gene, the full E183L gene (p54 protein), and the partial CD2v gene. Phylogenetic analyses of the B646L (p72) revealed that one of the samples belonged to genotype I while the remaining samples could not be assigned to any currently known genotype. In contrast, by using the E183L gene three of the samples were shown to belong to genotype Id and only two were of unknown genotype. Based on the analysis of the partial CD2v amino acid sequences of four of the samples, one of the viruses clustered with serogroup 2 while the other three did not cluster within any of the eight known serogroups. Examination of the CVR identified three variants with 8, 18 and 24 tetrameric tandem repeat sequences.

This study indicates that at least three different genetically distinct ASFV are currently present in Namibia, stressing on the need for a continuous monitoring of ASFV. A paper describing these findings has recently been accepted for publication in the journal Transboundary and Emerging Diseases (DOI: 10.1111/tbed.13399).

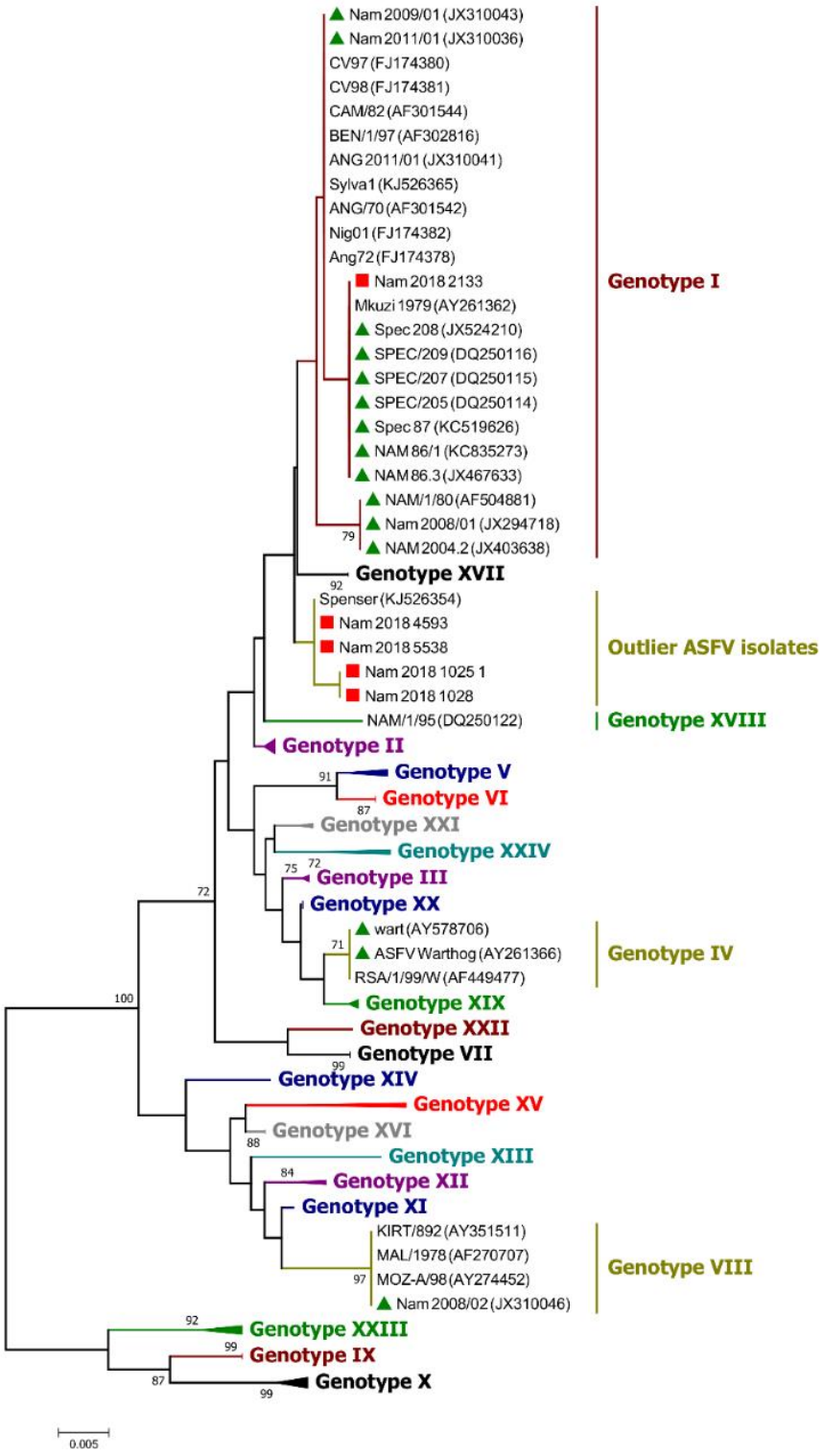


Fig.3. Neighbour-joining tree, of the partial p72 gene, depicting genetic relationships between the Namibian 2018 ASF outbreaks isolates and representatives of the 24 known ASFV genotypes.

Molecular Epidemiology of African Swine Fever in Asia

Following China, in 2018, ASF has emerged in several other countries in Asia, starting in Mongolia in January 2019 and hitting several countries in South-East Asia.

On the request of VETLAB partners APHL supported Mongolia, Lao PDR, and Vietnam, to characterize genetically their local ASFV isolates from 2019 outbreaks. The C-terminus of the B646L gene (p72 protein), the central hypervariable region (CVR) of the B602L gene, the full E183L gene (p54 protein), the partial CD2v gene and the intergenic region between the I73R and I329L genes were sequenced and analyzed for each sample. The results showed that only genotype II, Serogroup 8 ASFV isolates are circulating in those countries. Furthermore, only a single variant of the CVR with the TRS profile "BNDBNDBNAA" was found in these three countries. Likewise, the intergenic region between the I73R and the I329L genes showed 100% similarity among all genotype II ASFVs from Mongolia, Lao and Vietnam, with the insertion of an additional motif of the tandem repeat sequences (TRSs) "GGAATATATA" motif. This study shows that ASFV genotype II in Asia is highly conserved.

Molecular characterization of Peste des Petits Ruminants virus

In Niger, the small ruminant population is estimated to be greater than 12 and 16 million for sheep and goats, respectively. PPR is one of the major diseases of these animals that causes significant losses to both the local and national economy. To reduce the impact of this disease, the government of Niger has implemented an annual policy of free vaccination. However, despite this, outbreaks are still reported regularly. Previous molecular epidemiological studies conducted on PPRV samples collected in 2001 and 2013 in Niger, identified three of the four viral lineages (i.e. I, II and IV) (Tounkara et al.; 2018). This study confirms the co-circulation of lineages II and IV of PPRV in Niger from 2011 to 2017 (Figure 4). The majority of the PPRVs identified belonged to lineage IV indicating the predominance of this lineage in Niger. Interestingly, there was no detection of lineage I as described previously by Tounkara et al. (2018). This may be due to the relatively small number of samples analysed but may also support the hypothesis that lineage I has been replaced by lineage II and IV across the region (Kwiatek et al., 2007; Salami et al., 2014).

In the Democratic Republic of the Congo (DRC), the presence of PPR was officially reported in 2012 and resulted in high mortalities of small ruminants throughout the country estimated at between 75,000 and 300,000. Emergency vaccination was employed by national and international organizations in the regions considered at high risk but this has not been maintained. In 2016, further outbreaks of PPR were reported in the east of the country but, due to regional conflict, no measures were taken to control the disease or limit the movement of animals from neighbouring countries. Over 50,000 animals perished due to PPR in Nord-Kivu in 2017 according to national reporting and in 2018 further outbreaks were reported. To date, there has been no molecular characterization of the virus involved in any of the PPR outbreaks in the DRC.

Phylogenetic analysis confirmed that 10 of the samples belonged to lineage III, and 1 to lineage II. The lineage III viruses were identical to each other and were collected from the eastern part of the country close to the borders with Uganda, Burundi, Rwanda and Tanzania and in the capital city, Kinshasa, in the west of the country. Of note, however, was that the partial N gene sequences of the lineage III DRC viruses were significantly different (only 98.1% nucleotide identity) from their closest lineage III relative from Burundi suggesting that they are not directly related and that they are not the result of recent transboundary movement between the two countries. The presence of lineage III viruses in Kinshasa located in the west of the country is explained by the fact that there is regular and documented transport of goats from the east of the DRC to Kinshasa for sale or in order to improve productivity and repopulate herds in the city.

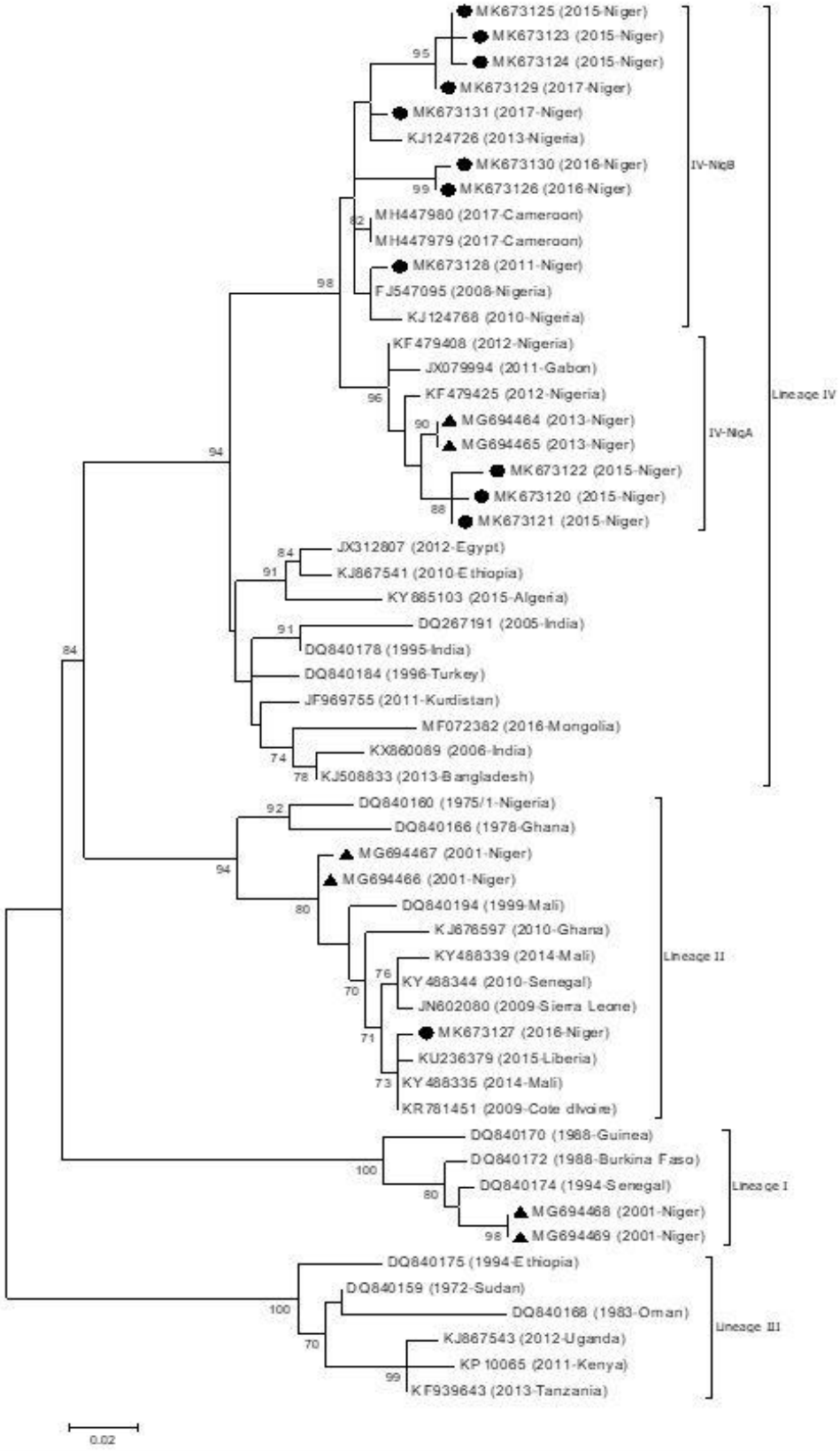


Fig. 4. ML phylogenetic tree of partial N gene sequence (219 bp) from the PPRV sampled in Niger combined with similar sequences available in GenBank. The sequences from this study are shown by filled black circles while those from previous studies (Tounkara et al., 2018) are shown with black triangles. Lineages and sub-clades are also shown.

The single lineage II virus was collected in 2016 in Goma, North Kivu in the east of the country. From the phylogenetic analysis it can be seen that this virus is most related to viruses from West Africa (e.g. Senegal, Mali, Sierra Leone). Nevertheless, and like the lineage III, there is nucleotide sequence diversity (1.4 %) between the N sequence of this lineage II virus and its nearest relative (e.g. Mali and Côte D'Ivoire). This would indicate that any shared origin of these viruses is not recent. How a lineage II virus, normally confined to Western Africa, reached eastern DRC is unclear. There is no official trading of live animals between these two regions of the continent. The identification of further lineage II viruses in DRC and the generation of full genome sequences may clarify the exact origin of this lineage II virus.

Parapoxviruses of cattle in Africa

In 2018, APHL has reported finding pseudocowpox virus (PCPV) in cattle samples submitted to the Central Veterinary Research Institute (CVRI), Zambia, on suspicion of lumpy skin disease virus infections. An HRM assay for the simultaneous detection and differentiation poxviruses of medical and veterinary importance, developed at APHL and transferred to CVRI, facilitated this detection. This assay was further transferred to other VETLAB partner laboratories: BNVL, Botswana, LNRV, Senegal and CVL, Mozambique which employed this assay to analyses skin lesion collected from diseased cattle.

The results revealed PCPV infections in Senegal and Botswana and bovine papular stomatitis virus (BPSV) infection in Mozambique. Sequencing of the full B2L gene and phylogenetic analysis confirmed these results. These results and our previous findings of PCPV in Zambia prove that parapoxvirus infections are under-documented common infections of cattle in Africa.

PCPV and BPSV produce pox diseases in cattle, causing losses in productivity, and these viruses can infect humans working in close contact with infected animals. It would be of interest to further investigate the impact of this disease on cattle productivity in African countries.

An approach to discriminated goatpox viruses based on their geographical origins.

The recent incursions of Lumpy skin disease virus (LSDV) and sheeppox virus (SPPV) in Europe, showed that Capripoxviruses could spread from their endemic geographic locations to disease-free areas. Therefore, it is of high importance to develop molecular tools to complement traditional epidemiological methods to trace the origin of viruses when outbreaks occur in disease-free areas and detect the incursion of new strains in disease-endemic ones. APHL has investigated the use of alignment-free approaches for the accurate classification of goatpox viruses (GTPVs) according to their geographical origins.

A method based on k-mer frequencies was employed to compare a comprehensive set of GPCR gene sequences of GTPVs from various geographic locations. The result showed that GTPVs could segregate based on the geographic region of origin: the African GTPVs and Asian GTPVs which further split into Western and Central Asian (WCA) GTPVs, and Southern and Eastern Asian (ESA) GTPVs.

This approach will help to determine the origin of introduction in case of GTPV appearance in disease-free regions and detect the importation of new strains in GTPV endemic areas.

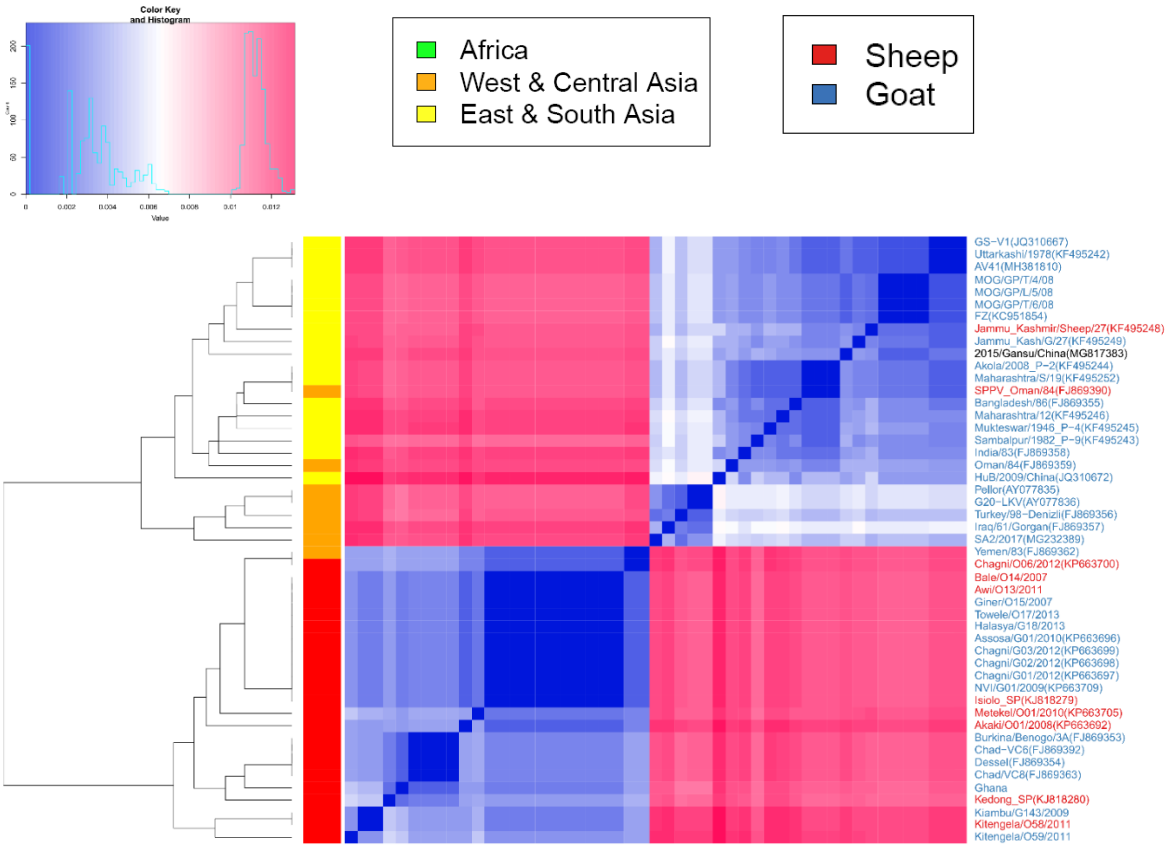


Fig.5. The heatmap for the nucleotides k-mers frequencies variations of 49 GTPVs. The Complete-linkage clustering method was used to re-order the sequences. The vertical side colors indicated where the corresponding samples came from (red for Africa, orange for West and Central Asia, yellow for East and South Asia).

Animal Genetics

Development of nuclear related genomic tools for old and new world camelids

Genomic resources and tools are scarcely available for breeding and improvement of old and new world camelids for increased productivity. In 2019, APHL in collaboration with the Institute of Wildlife, Vetmed University of Vienna, is engaged in the development of a DNA microarray tool for camelids. Whole genome camel sequence resources available with Vetmed university and International Camel Genome Consortium partners will be utilized for the design and development of a custom single nucleotide polymorphic (SNP) genotyping array. APHL will test and validate this new DNA array for use on camelids. About 4K quality control markers and ~60K SNP markers from each of dromedary, Bactrian and new world camelids have been identified. The process of design and fabrication of array is currently in progress. The new approach will not only help to construct the whole genome radiation hybrid map and reference genome assembly for dromedary, but will also lead to the development of advanced, state of the art tools for breeding and improvement of camelids.

Setting up of low-cost, high throughput genome-wide SNP genotyping protocol to support cattle breeding programs in Member States

Improvement of dairy cattle for increased milk production occurs mainly through two conventional approaches: (i) selection and breeding of superior genetic merit animals and/or (ii) cross breeding of local cattle with highly selected commercial taurine cattle. With recent advances in molecular technologies, efficiency of both these approaches can greatly be increased by testing and utilizing genome-wide DNA marker variations. However, application of these technologies in developing

member states has been constrained by several factors that includes cost of genotyping, lack of performance records, etc. To address the challenge, APHL initiated the setting up of semi-automated, low-cost genome-wide SNP typing protocol to support cattle development programs in member states. High throughput genotyping protocols were optimized by integrating automated liquid handling and Affymetrix GeneTitan systems. The semi-automated genotyping methodology is also expected to help member states in accurately estimating the level of taurine inheritance in crossbred cattle and identifying optimal genotypes that can suit the existing production system.

Validation of bovine array for genome-wide typing of Asian zebu and crossbred cattle

Commercially available 50K bovine SNP (single nucleotide polymorphism) arrays from Affymetrix (Axiom Bovine Ovicap Array) were tested for their suitability to evaluate zebu cattle and their crossbreds.

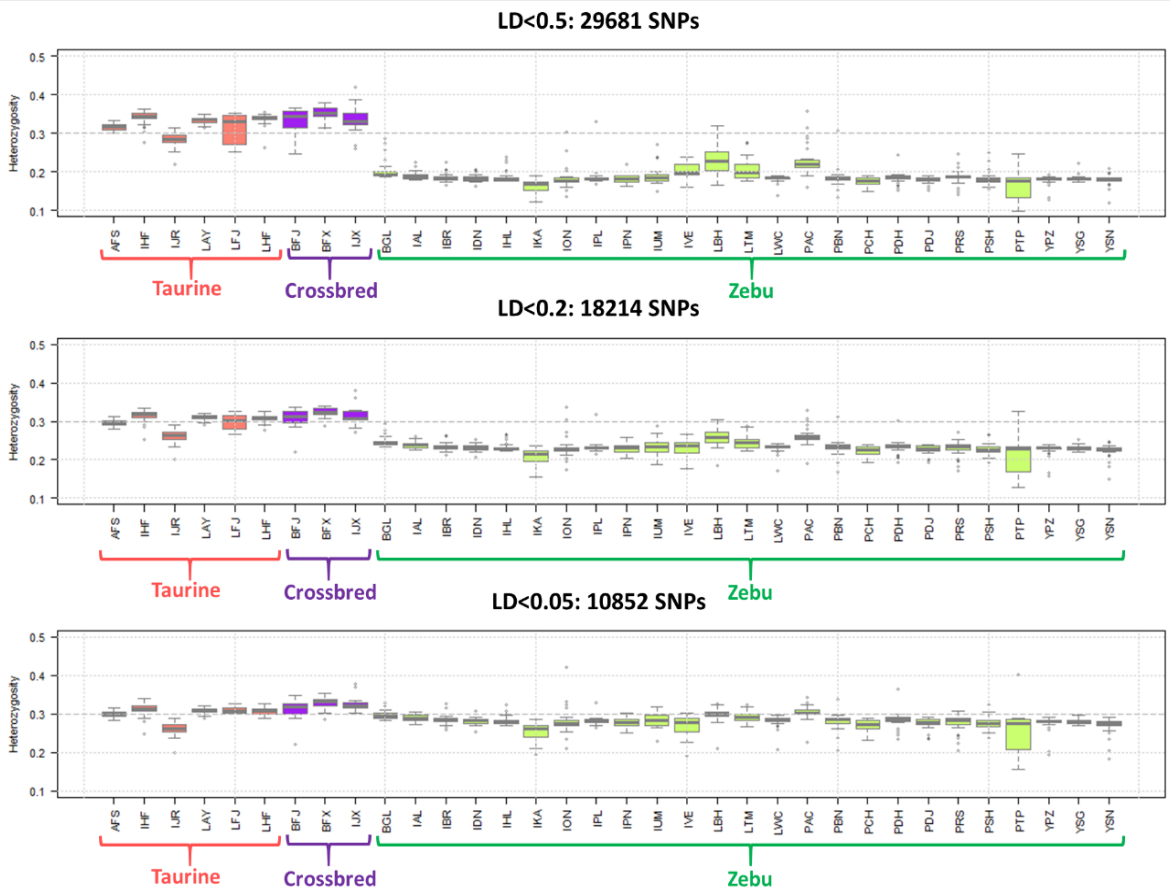


Fig.6. Heterozygosity of markers after pruning for different LD thresholds

More than 1000 zebu, taurine and crossbred cattle belonging to 34 different populations were included for testing and validation. Variability at genome-wide markers revealed significant ascertainment bias in zebu cattle. To reduce the effect of ascertainment bias, a methodology was devised to remove markers that are in linkage disequilibrium (LD) and identify those markers that pre-date zebu-taurine divergence. When the data was pruned for linkage disequilibrium, the effect of ascertainment bias got diminished linearly with decreasing LD threshold. With LD<0.1, more than 10000 markers were found to be efficient and free of ascertainment bias in Asian zebu cattle. These markers can be safely used by member states for genomic evaluation of zebu cattle and estimation of the level of taurine inheritance in crossbred cattle.

Implementing Global Plan of Action for Animal Genetic Resources (AnGR)

In continuation of Joint FAO/IAEA efforts towards implementing Global Action Plan on animal genetic resources (AnGR), APHL supported member states in at least three major strategic priority areas: characterization, sustainable use and development and capacity building in terms of establishing/strengthening laboratory infrastructure and training of personnel.

Molecular genetic characterization of Sri Lankan native cattle breeds

Technical support was provided to Sri Lanka to perform the first-ever genetic characterization of indigenous cattle breeds in the country. Three indigenous cattle breeds (Sri Lankan White cattle, Local Batu Haraka and Thawalam cattle) were evaluated and compared with five commercial taurine cattle breeds (Holstein-Friesian, Ayrshire, Jersey, Fleckvieh-Simmental and Friesian X Jersey crossbred) to understand population structure and levels of genetic admixture. High level of genetic biodiversity was observed in Sri Lankan native cattle breeds.

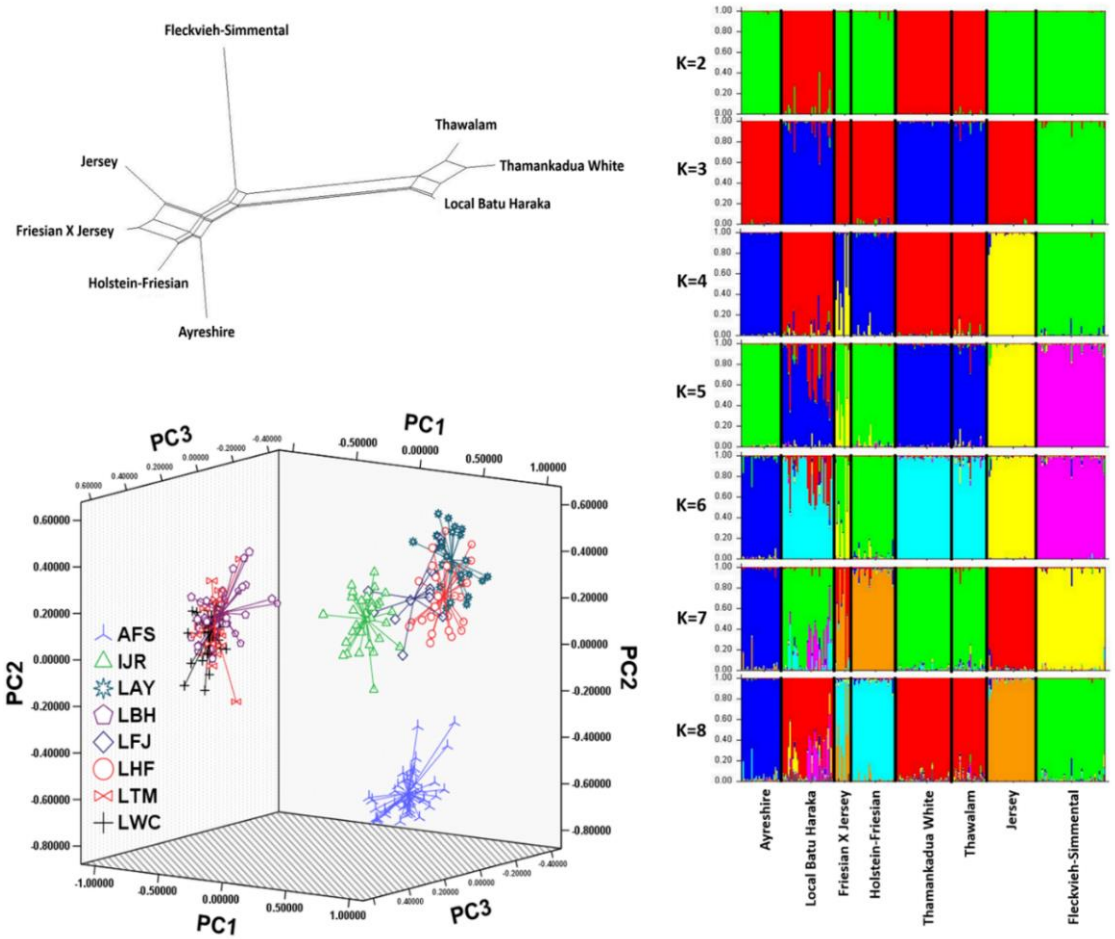


Fig.7. Genetic relationship, population structure and genetic admixture in Sri Lankan cattle breeds

Between breed differences varied from 1.5 to 2.7% indicating low genetic differentiation among them. The level of taurine admixture in Sri Lankan cattle was minimal, indicating the farmers are maintaining high level of purity in these animals. To further understand genomic regions of functional significance for production and adaptability traits, more than 15 million genomic data points (single nucleotide polymorphic data) were generated. The bioinformatics analysis of genomic data on Sri Lankan cattle is currently underway. The baseline genetic information database and genomic evaluation report will play an important role in establishing reference population for phenotype-genotype association studies and future implementation of genomic selection programs for increased productivity in Sri Lankan cattle.

Development of baseline genetic information in Pakistani cattle

APHL supported genetic characterization and establishment of baseline genetic information on native cattle breeds of Pakistan. Nine indigenous cattle populations (Achai, Bhagnari, Cholistani, Dhani, Dajal, Red Sindhi, Sahiwal, Tharparkar and Local non-descript) were evaluated using nuclear and extra nuclear DNA markers. A total of 325 samples from nine breeds were subjected to sequencing of mitochondrial DNA control region and multi locus genotyping of short tandem repeat markers. Additionally, all the samples were subjected to genome-wide typing of >50000 single nucleotide polymorphic markers to understand functional variations related to adaptability of local cattle. The data analysis is currently underway, and the results of molecular characterization will help to establish genetic admixture levels, population structure and demographic dynamics of Pakistani cattle. The genetic biodiversity information will be utilized in formulating effective strategies for the conservation and genetic improvement of Pakistani native cattle.

Genomic evaluation of Caucasian and Carpathian Brown cattle from Eastern Europe

Under the FAO technical cooperation program RER3604 “Conservation of dual-purpose cattle in Eastern Europe in Armenia, Georgia and Ukraine”, APHL provided technical support and services to implement “Genomic analysis of Caucasian and Carpathian Brown cattle”. The objective of the work was to make genomics-enabled decision on whether to manage Caucasian and Carpathian Brown cattle populations as a single genetic entity for conservation and breeding purposes. Further, genomic derived information on levels of exotic inheritance (Brown Swiss, Holstein-Friesian, Fleckvieh-Simmental, etc.) in Caucasian/Carpathian Brown cattle is expected to help formulate effective breeding strategies and develop a dual-purpose breed for increased milk and beef productivity. Under this project, APHL developed and supplied appropriate tool kits for sampling cattle in all the three member states. More than 500 hair samples were processed at APHL to extract DNA and perform state of the art genotyping technologies for genomic evaluation. Caucasian Brown cattle from Armenia and Georgia and Carpathian Brown cattle from Ukraine were compared with Brown Swiss, Holstein, Fleckvieh-Simmental, Jersey, Ayrshire, Kostroma and Ukrainian Grey cattle.

Estimates of effective population size were observed to be relatively higher (as compared to commercial European cattle) indicating possible absence of selection among breedable males and potentially very limited usage of artificial insemination technology. All the three cattle populations were closely related with low genetic differentiation among them. However, Carpathian Brown cattle (Ukraine) was found to be genetically distinct from Caucasian Brown cattle (Armenia and Georgia). Population structure analysis also revealed Carpathian Brown cattle having a distinct ancestry from that of Caucasian Brown cattle. Very limited Brown Swiss introgression was observed in Caucasian and Carpathian Brown cattle. More than 90% of cattle from these three populations had less than 25% of Brown Swiss inheritance. Genome-wide evaluation clearly showed Carpathian cattle from Ukraine to be treated as a genetically distinct entity for conservation and breeding purposes. Further, the data also indicated crossbreeding program has not reached all the regions of the three countries, with little evidence of usage of Brown Swiss bulls for genetic improvement. The results of genomic evaluation and the information generated will be utilized to improve the efficiency of conservation and genetic improvement programs targeted for development of dual-purpose Caucasian and Carpathian Brown cattle in Armenia, Georgia and Ukraine.

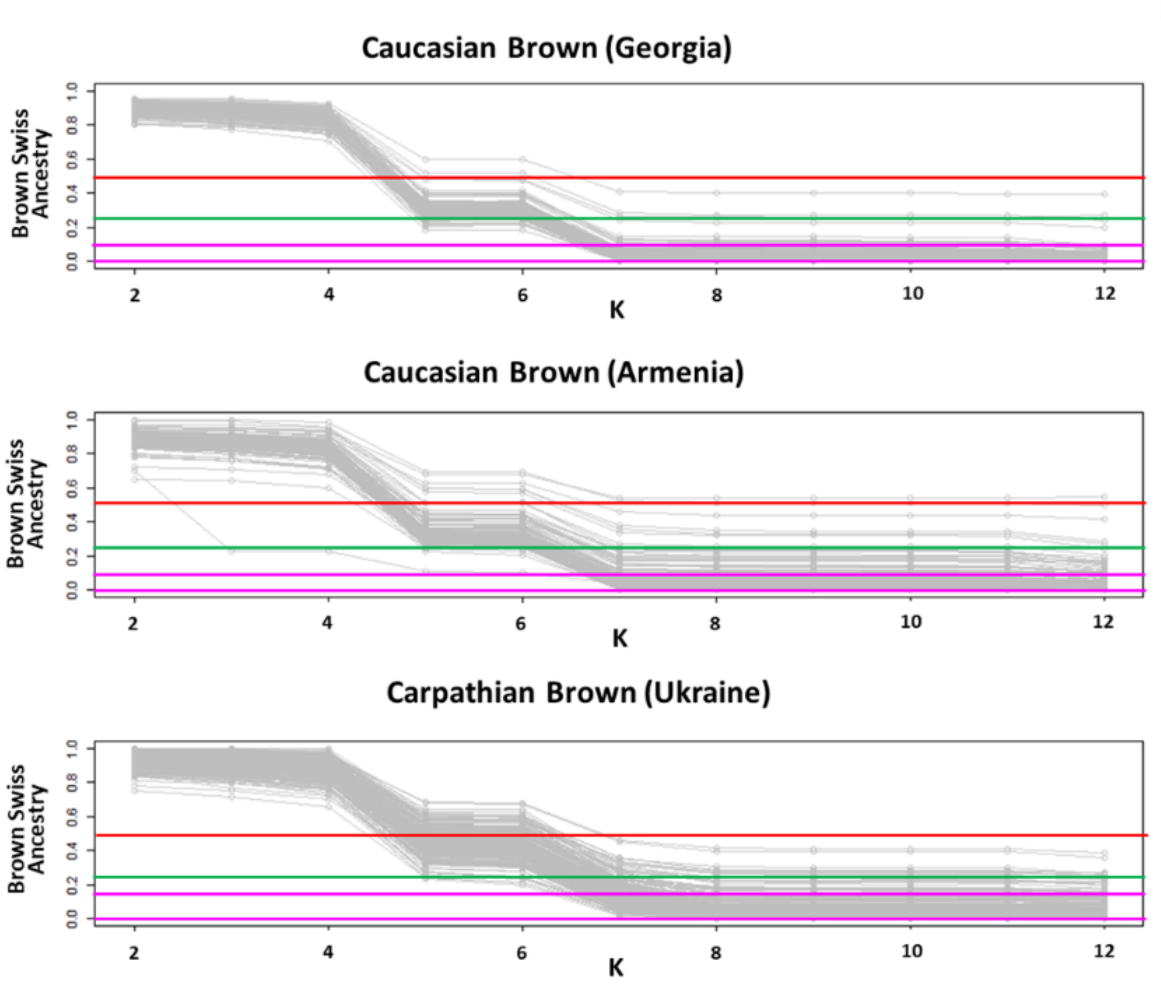


Fig.8. Extent of Brown Swiss introgression in Caucasian Brown and Carpathian Brown cattle of Armenia, Georgia and Ukraine

Strengthening laboratory infrastructure

APHL continued its efforts to improve the laboratory capacity of Member States and enable implementation of advanced DNA based technologies for efficient management of locally available animal genetic resources. Institutional and technical support were provided to nine countries (Burkina Faso, Dominican Republic, Eritrea, Indonesia, Mexico, Pakistan, Papua New Guinea, Paraguay and Sri Lanka) for establishing/strengthening molecular genetic laboratories through provision of necessary equipment and laboratory supplies under the framework of national and regional technical cooperation projects. APHL provided technical support in setting up a new sequencing facility at Unite de Genetique Animale, Institut de l'Environnement et de Recherches Agricoles, Centre de recherches environnementales et de Formation (INERA-CREAF), Ouagadougou, Burkina Faso. This new facility has attracted the attention of neighboring West African countries and is playing an active role in providing training on application of DNA marker technology for characterization and improvement of local livestock breeds.

Sustainable use and development

Artificial insemination using frozen semen technology is an important means of multiplying superior germplasm for sustainable improvement of livestock productivity. Through IAEA Technical Cooperation program, APHL provided technical support to national artificial insemination (AI) programs in Papua New Guinea and Eritrea in terms of equipment and supplies required for frozen

semen laboratories, implementation of AI under field conditions and improvement of reproductive efficiency in cows. Further, APHL supported Burkina Faso in addressing the challenges of backyard poultry production through modern genetic, breeding, nutrition and preventive healthcare interventions. Particularly, technical and scientific assistance was provided to identify optimal indigenous chicken ecotype that suit the local production system (through characterization of local chicken ecotypes) and installation of small-scale pilot hatchery units (to address the challenge of poor hatchability of chicks).

CAPACITY BUILDING

National and Regional Training Courses

During 2019, APHL implemented two regional and six national training courses, two VETLAB training courses on animal health, pathogens detection, animal genetic characterization, automated sequencing and genotyping, bioinformatics analysis of pathogens and livestock genome data, artificial insemination and pregnancy diagnosis.

***Regional Training Course* on "Genetics of Parasite Resistance in Sheep and Goats: Bioinformatics analysis of genomic data to assess population structure, genotype-phenotype association and genomic prediction".** It was organized at the Animal Production and Health Laboratory (APHL) in Seibersdorf, Austria, 1-12, July 2019. Gastro-intestinal (GI) parasitic infection is a major constraint for sheep rearing in Latin America. The training course was targeted to support ongoing national efforts in the Latin American region to apply genetic and genomic technologies for breeding and improvement of sheep with enhanced host resistance against gastro-intestinal parasites. The training course included practical training on (i) bioinformatics analysis of large sets of genomic data to assess population structure and genetic admixture (ii) analysis and interpretation of genomic data for association with phenotypes related to parasite resistance (iii) establishing gene bank of performance recorded animals and (iv) application of genomics for breeding local sheep and goat with enhanced host resistance against parasites. The objectives of the training course were successfully achieved and each of the participants was provided with a package of different software tools for analysis of genome-wide data in livestock. It is expected that the training will help the national breeding programs in Latin American countries towards controlling the gastro-intestinal parasites in sheep.

National Training Course on "Automated sequencing and genotyping for animal genetic characterization" from 19-30, August 2019 at Institut de l'Environnement et Recherches Agricoles (INERA-CREAF de Kamboinsé,), Ouagadougou, Burkina Faso. 15 participants from 3 countries attended the course.

National Training Course on "Strategies for Data Collection and Animal Sampling related to Genetic Characterization of Livestock Breeds" from 24-28, June 2019 at Papua New Guinea University of Natural Resources and Environment (PNGUNRE), Kokopo, Papua New Guinea. 19 participants from Papua New Guinea attended the course.

National Training Course on "Artificial Insemination and Pregnancy Diagnosis in Cattle" from 30 September – 11 October 2019 at Papua New Guinea University of Natural Resources and Environment (PNGUNRE), Kokopo, Papua New Guinea. 40 participants from Papua New Guinea attended the course.

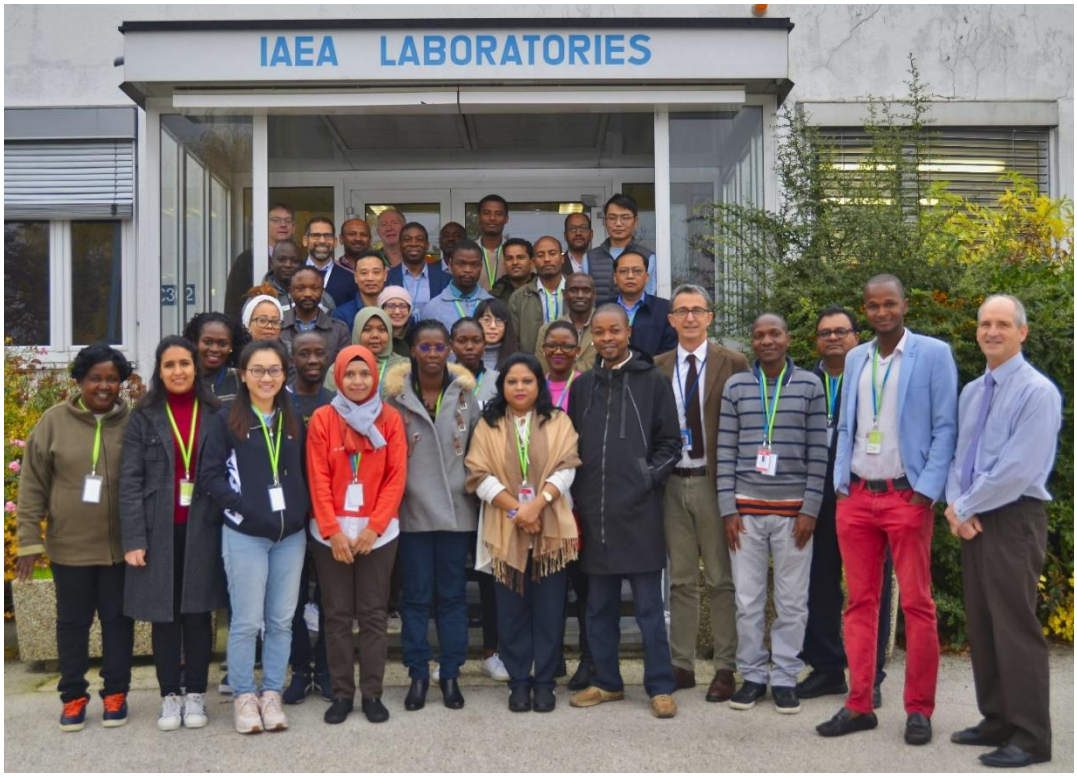
National Training Course on "Implementation of bioinformatics tools and techniques for breeding and management of Argentinian sheep" from 11-15, November 2019 at Institute of Genetics, Centro Investigación en Ciencias Veterinarias y Agronómicas (CICVyA), Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina. 10 participants from Argentina attended the course.

Regional training course on the differential diagnosis small ruminants' respiratory diseases and haemorrhagic diseases and swine, and the detection of Rift valley fever virus in vectors and sequencing protocols for ASFV, Capripoxvirus and PPRV. Senegal, 11-21 Feb 2019. The purpose of the event is to train laboratory staff of LNERV, Dakar and Mauritania on rapid nuclear-derived technologies for the detection of zoonotic pathogens; equipment maintenance and troubleshooting. Nine participants from Senegal, and Mauritania received training Besides, the participant received training on calibration and maintenance of real-time PCR detection systems.

National training course on implementation of rapid diagnostic methods for animal and zoonotic diseases, Belize. 8-12 July 2019, Belize Agricultural Health Authority (BAHA), Belize. The training was organized in the framework of the TC project BZE5007, its objective was to enhance the diagnostic capabilities of BAHA Central farm to address the threats of transboundary animal and zoonotic diseases. Three participants received training on confirmatory detection of rabies virus, and the detection Newcastle disease virus, Avian Influenza virus, Classical Swine Fever virus, brucella.

Training Course on the Detection of Multiple Pathogens for the Differential Diagnosis and Syndromic Surveillance of Transboundary Animal Diseases. This training aimed to strengthen the capacity of the VETLAB Network partner laboratories for the diagnosis and surveillance of African swine fever (ASF), peste des petits ruminants (PPR), and capripox (sheep pox, goat pox, and lumpy skin disease). ASF, PPR and capripox, have recently spread into new areas, expanding their geographical coverage to Europe and Asia. To establish suitable diagnostic methods, VETLAB partners must stay up-to-date on laboratory algorithms for differential diagnosis for each of these diseases. The training involved lectures, hand-on training on laboratory techniques, and computer sessions on bioinformatics. The lectures presented general information on the diseases, their diagnosis and control strategies, the principle of the diagnostic methods, and a short introduction to bioinformatics. The hands-on training covered serological and molecular techniques, including multi-parametric detection and gene amplification for sequencing. The computer sessions examined the comparative sequence analysis and phylogenetic reconstructions. The first week covered PPR and Capripox, and the second week entirely focused on ASF. The trainers were experts from CIRAD, the Universidad Complutense de Madrid, and the Joint FAO/IAEA Division. Twenty-six participants from VETLAB partner laboratories in twenty-three African and Asian countries attended this training course, from 04 to 15 November 2019, at the IAEA Seibersdorf Laboratories, Austria.

Training Course on Transboundary Animal Disease Diagnoses: Validation, Implementation, Monitoring and Quality Control for Molecular Assays. The purpose of this training was to strengthen laboratories' capacities in introducing, validating, and monitoring diagnostic assays for routine use. VETLAB partner laboratories are highly committed to implementing a Quality Management System (QMS) and accrediting diagnostic tests. Such accreditation increases the trust in the test results delivered by laboratories. It plays an essential role in removing technical barriers to international trade. Hence, the specific objectives of this training were to improve the participants' knowledge of the various steps of assay implementation and validation and to discuss diagnostic and epidemiological concepts of test validation and result interpretation.The training comprised lectures and the principle and critical concepts of diagnostic assays development and validation and statistical methods. Besides, the participants received practical hands-on training on assays optimization, validation, implementation and verification, and the relevant statistical approaches. The trainers were international experts from the Australian Animal Health Laboratory (Australia), Sciensano (Belgium), the Friedrich-Loeffler-Institute (Germany), and the Joint FAO/IAEA Division. Twenty-eight participants from VETLAB partner laboratories in twenty-five African and Asian countries attended this training course, from 18 to 29 November 2019, at the IAEA Seibersdorf Laboratories, Austria.



Consultants meeting on Advances in nuclear and genomic tools to improve livestock productivity-Technology gaps and new approaches for application in developing countries

The consultants meeting was held at the IAEA Headquarter, Vienna, from 14-18, October 2019. The meeting was attended by eight expert consultants, nine FAO/IAEA staff/consultants and seven professionals/students/trainees from 15 member states. The consultants meeting aimed at reviewing the current status of scientific information related to the use of genomic tools in animal genetics and breeding with a particular focus on developing countries, including smallholder, commercial and community-based breeding programs. Through specific examples, the experts demonstrated the potential of nuclear related genomic technologies in increasing the efficiency, profitability and sustainability of livestock breeding programs. The meeting participants agreed such new technologies will help livestock systems to meet the goals of feeding the increasing global human population, improve the livelihood of smallholder families and reduce environmental impact by increasing production efficiency. During the meeting, potential challenges were identified in applying genomic technologies to diverse husbandry systems, including smallholder systems. A detailed work plan, including technical procedures for implementation of a potential CRP was prepared. The proposed CRP would help the developing countries in implementing advanced genomic technologies for improved milk productivity per animal. The consultants also highlighted the need to build capacity and improve the expertise in bioinformatics analysis of data among scientists especially in developing countries and the growing role of international organizations in accomplishing this task.

Coordination meeting with Directors of Veterinary Laboratories in Africa and Asia supported by the African Renaissance Fund and the Peaceful Uses Initiative to Strengthen Animal Disease Diagnostic Capacities

The fourth coordination meeting of the VETLAB Network was held at IAEA Headquarters in Vienna, from August 19 to 23, 2019. Twenty-six directors of the VETLAB partner laboratories from Bangladesh, Botswana, Benin, Cameroon, Chad, Ethiopia, Indonesia, Jordan, Kenya, Lao, Malaysia, Mongolia,

Morocco, Mozambique, Myanmar, Namibia, Nepal, Niger, Senegal, Thailand (2), Tanzania, Tunisia, Viet Nam, Uganda, and Zambia took part in the meeting.

Representatives of the Animal Health Services (AGAH), FAO, the ERFAN (Italy), the OIE/FAO/EURL for Avian Influenza and Newcastle disease, and the FAO Reference Centre for Rabies (IZSVe), were also present. The objectives of the meeting were to: (1) update partners on the activities in 2018-2019, (2) update partners on important ongoing initiatives to support diseases diagnosis and surveillance by veterinary laboratories in Africa and Asia (FAO, IAEA and Reference laboratories), (3) discuss ways to improve laboratory diagnostics of emerging TADs and zoonotic diseases including the targeted diseases of the VETLAB CRP (4) discuss the work plan for the VETLAB PUI PPR project, (5) discuss the 2019-2020 common and individual country plans, (6) discuss and refine the VETLAB CRP work plan for 2019-2020, (7) allow experience, knowledge and information exchange between the Asian and African Laboratories.

As in previous years, the VETLAB directors gathered together with the VETLAB Research Coordination Meeting (RCM) participants.

Following the welcome address by Mr. G Viljoen, Head of the Animal Production and Health (APH) subprogram and by Mr. G. Cattoli, Head of Animal Production and Health Laboratory (APHL), IAEA staff presented the objectives of the VETLAB directors meeting and the VETLAB CRP. The meeting included four sessions. During the first session on the VETLAB network and international collaborations to strengthen laboratories capacities, IAEA staff, FAO representative, and ERFAN representatives presented various initiatives for laboratory capacity building in Africa and Asia, focusing on the main activities and achievements in 2018-2019 and the planned activities for 2020. Then followed a session on ASF, LSD (Capripox) and Equine diseases were IAEA and VETLAB partners presented on their activities on the diagnosis and surveillance of these diseases. The meeting participants stressed the importance of serological surveys of equine flu and the need to develop a real-time multiplex PCR for equine diseases by VETLAB partners under the guidance of APHL. For ASF, a set of actions to support the detection and the typing of the virus, including training, the acquisition of reagents and control were defined. .

For capripox it was suggested that APHL should further disseminate assays for differential diagnosis of poxviruses of veterinary and medical importance affecting both ruminants and camels and assays to differentiate capripoxvirus vaccines from field isolates. This session ended with a presentation by VETLAB partners on FMD and CBPP. During a third session on peste des petits ruminants, IAEA presented a newly funded VETLAB PUI project on PPR. This project intends to build member state veterinary laboratory capacities for the early diagnosis and control of peste des petits ruminants (PPR). The meeting participants approved the keys activities of this project and the proposed work-plan. The approved activities include: (1) a consultant meeting on the use of laboratory techniques to support the PPR Global Eradication Programme, (2) capacity-building activities such as trainings, technology transfer, the preparation and distribution of controls and the organization of interlaboratory testing (ILT), and PTs (3) collaborative research activities on assay validation, sequencing workflow validation, and the surveillance of respiratory diseases in domestic ruminants, camels and wildlife. Then followed presentations by the VETLAB partners and IAEA on the diagnosis and surveillance of PPR, the characterization of PPRV and the report of the 2018 proficiency testing for PPRV detection. In the fourth session zoonotic diseases were covered, IVZVe presented on Avian influenza, highlighting the potential zoonotic risk of H9N2, and on Rabies, followed by VETLAB the diagnosis and surveillance of Rabies and Avian influenza.

APHL has informed the participants on the upcoming ILT for the RITA which is a set of real-time PCR methods for subtyping influenza A viruses. The participants also discussed the possibility to accommodate more VETLAB partners in training courses and inter-laboratory testing on Rabies organised by FAO HQ and regional offices in Africa. The meeting ended with the discussions on the countries' work plans and the formulation of common work-plan. Overall, VETLAB partners still have

some challenges in the implementation of quality systems: the lack of PTs, standards and controls, the unavailability of proper maintenance and calibration services. Other major issues are the absence of proper tools for surveillance of PPR in wildlife, the limited access to new technologies and difficulties in procurement.

The meeting had successfully met its objectives. To address their main challenges, the participants have agreed on the organization of training courses, interlaboratory validation of assays, intra-network research activities, PTs. They have also committed to contribute to the VETLAB bulletin as a means of sharing information between partners. The participants agreed on the VETLAB CRP work-plan for 2019-2020.

The meeting participants recommended the Joint FAO/IAEA division to:

- Continue the capacity building of VETLAB partner laboratories to improve their ability to undertake efficiently the diagnosis and surveillance of priority and emerging diseases using validated tests which are fit-for-purpose.
- Promote the implementation of quality systems through facilitation of PTs, supply of controls and sharing of ISO 17025-compatible SOPs, and additional support, integrated in the iVetnet information platform, including equipment monitoring and maintenance.
- Promote gene-based identification of pathogens through facilitating the use of modern techniques such as multiplex assays, sequencing, next-generation sequencing, and the relevant bioinformatics.

The meeting participants strongly encouraged VETLAB partners to:

- Use multiplex technology for differential diagnosis
- Use the sequencing service facilitated through the Joint Division and make the data publicly available
- Undertake collaborative research works within the network for assay validation.
- Undertake more inter-laboratory collaborations.
  Use the VETLAB bulletin as a platform to communicate their activities, achievements, share experience, and promote their activities.

Expert missions

Expert mission to the Institut de la recherche vétérinaire of Tunisia. In the framework of the VETLAB Network, an APHL staff visited the Institut de la recherche vétérinaire of Tunisia from April 29 to May 03, 2019. The purpose of the travel was to review the infrastructure, capacities, and integration of the national laboratory with the VETLAB network and discuss selected techniques for laboratory diagnosis of transboundary animal diseases. Tunisia is facing most of the important transboundary animal diseases present in the African continent, such as peste de petits ruminants, capripox disease, foot and mouth disease, bluetongue and avian influenza. The visit of the laboratories and the discussions with scientific staff, allowed to identify the main gaps for capacity building, the opportunities that the institute can offer to other partners of the VETLAB network and the potential areas of collaborative research work with the APHL of the joint division. An action plan was defined to strengthen the capacity of the IVRT and enable it to play a leading role within the VETAB network. It was agreed to develop collaborative research work of animal poxviruses.

Expert mission to the Office National de Sécurité Sanitaire des Aliments of Morocco. In the framework of the VETLAB Network, an APHL staff visited the Institut de la recherche vétérinaire of Tunisia from March 05 to 09, 2019. The purpose of the travel was to access laboratories needs and discuss future collaborations with the responsible staff at the Division des Laboratoires, Direction des Intrants et des Laboratoires Office National de Sécurité Sanitaire des Aliments. Peste des petits ruminants, capripox disease, foot and mouth disease, bluetongue and avian influenza are some major

transboundary animal diseases impacting livestock production and trade in Morocco. The ONSSA has a network of Laboratories playing a key role for the early and rapid diagnosis and the surveillance of TADs and zoonotic animal diseases. Also, those laboratories perform the screening of horses before export. The visit of four laboratories of the ONSSA laboratories Network and the discussions enabled to identify the main areas for strengthening the laboratories capacities and the identify opportunities that these laboratories can offer to VETLAB partners. The mission has enabled the identification of areas for collaborative research work with APHL.

Fellowship and internship training

During 2019, APHL hosted 13 fellows, 2 trainees,2 interns and 1 scientific visitor.

Name	Country	Status	Duration	Topic
Menghak Phem	Cambodia	Fellow	3 months	Genetic characterization of Cambodian native cattle using DNA markers
Maphoka Mary Letseka	Lesotho	Fellow	3 months	Genomic evaluation and establishment of baseline information on local livestock
Hiracema De Jesus Inacio	Mozambique	Fellow	3 months	Genomic evaluation and establishment of baseline information on local livestock
Koffi Ganyo Somenutse	Togo	Fellow	3 months	Genomic evaluation and establishment of baseline information on local livestock
Apri Irianto	Indonesia	Fellow	3 months	Genomic evaluation and establishment of baseline information on local livestock
Betty Kenny Uranoli	Papua New Guinea	Fellow	2 months	Genomic evaluation and establishment of baseline information on local livestock
Sampath Lokugalappattige	Sri Lanka	Fellow	6 months	Genomic evaluation of indigenous zebu cattle breeds of Sri Lanka
Marcela Mora	Peru	Trainee	1 month	Real-time PCR based genotyping of DNA markers associated with parasite resistance in sheep
Boitumelo Modise	Botswana	fellow	3 months	Development and validation of multiplex qPCR assay targeting zoonotic pathogens causing abortion in domestic ruminants

Name	Country	Status	Duration	Topic
Mabusetsa J. Makalo	Lesotho	fellow	3 months	molecular diagnosis, sequencing and molecular epidemiology of transboundary animal diseases
Dadang Priyoatmojo	Indonesia	fellow	1 month	Characterization of cell-mediated immune response using flow-cytometry
Tri Handayani Suhono	Indonesia	fellow	1 month	Characterization of cell-mediated immune response using flow-cytometry
Kago Kumile	Botswana	fellow	3 months	Development and validation of nuclear-derived technologies for the early and rapid detection of transboundary animal diseases.
Kedumetse Mogwera	Botswana	fellow	3 months	Development and validation of nuclear-derived technologies for the early and rapid detection of transboundary animal diseases
Arphaphorn Dokphut	Thailand	Scientific visitor	2 weeks	Molecular and serological applications of liquid microarray technology
Yang Liu	China	Intern	3 months	Bioinformatic analysis and phylogeny of capripox viruses and ELISA test validation
Dingrong Xue	China	Intern	11 months	Molecular and bioinformatic applications for transboundary animal disease detection, prevention and control
Mihad Fath El Rahman Mahmoud Alawad	Sudan	trainee	3 weeks	Applications of flow cytometry to evaluate animal immune response

PUBLICATIONS

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VETLAB NETWOK

The Veterinary Diagnostic Laboratory (VETLAB) Network, coordinated by the Animal Production and Health Section (APH) and supported through IAEA and FAO programmatic activities as well as by South Africa through the African Renaissance Fund (ARF) and by the USA and Japan Peaceful Uses Initiative (PUI), consists of national veterinary diagnostic laboratories located in 45 African and 19 Asia and Pacific Member States. In 2019, the VETLAB Network has provided strong support to partner laboratories in Asia facing African Swine Fever (ASF) epidemics to strengthen their diagnostic capacity, preparedness and rapid response actions. Efforts concentrated on procuring reference material such as positive controls, equipment and reagents for the rapid implementation and expansion of ASF diagnosis and confirmation.

The VETLAB Network has organized the yearly interlaboratory trial for the serological and molecular detection of Peste des Petites Ruminants (PPR) virus. Thirty-one laboratories of 29 countries in Africa, Asia and Europe participated to the exercise. Countries at-risk for PPR virus introduction were also supported for their laboratory preparedness plan.

The fourth VETLAB Directors meeting took place in Vienna, Austria from 19 to 23 August 2019. Directors from Asian and African countries participated to this meeting together with experts from international reference laboratories and international organizations.

VETLAB partners attended two training courses on animal disease detection using multiplex assay platforms and laboratory test validation organized by the network with contributions of international experts from reference laboratories worldwide. Fifty-three participants attended these two events.

APH is issuing on a regular basis the VETLAB Network Bulletin in the hope of providing a forum for participating laboratories and other stakeholders to communicate and exchange knowledge/information, to showcase achievements and to share expertise within the VETLAB Network.

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. This support underpins food safety and control systems and helps to combat economic loss through food fraud - the illegal production and marketing of counterfeit and adulterated products. Activities include applied research and the development, validation, transfer and application of nuclear and related methods for testing foods. The application of these technologies and methods in Member States is supported by the development and provision of technical protocols, advice and guidance, training, and contributing to the development of international standards.

Research and development achievements in 2019 included the development and evaluation of analytical methods to test the authenticity of various food commodities and to control residues and contaminants in food. The focus was mainly on commodities that are important in international trade and for which control methodology was lacking or needed improvement. Method development encompassed both rapid screening methods and more sophisticated techniques in order to provide Member States with the options needed for their food control systems. The results of the research and development programme are made available through scientific publications, online method protocols and via laboratory networks such as the Red Analitica de Latinoamérica y el Caribe (RALACA) and those involved in technical cooperation and coordinated research projects.

In 2019 nine novel analytical methods were developed in FEPL and associated method protocols were prepared to enable further uptake of analytical methods in Member States. FEPL took part in a collaborative project to produce and characterize ten food matrix reference materials for stable isotope analysis to support accurate and reliable measurements in Member States. Rapid, cost-effective, screening methods were developed for the authenticity of argan oil and to detect adulteration of green tea with colourants using headspace gas chromatography-ion mobility spectrometry combined with multivariate statistical interpretation of results. FEPL collaborated in an interlaboratory study involving more than 30 participants in 26 countries using hand-held SCiO molecular sensor devices to test oregano authenticity. The study demonstrated the potential transferability of chemometric models built using one SCiO device to correctly identify the authenticity status of oregano samples in other laboratories. Methodology previously investigated in FEPL for the discrimination of honeys of different botanical and geographical origins by untargeted metabolomics analysis was expanded using a larger sample set of honeys covering 6 floral origins and 18 countries. The results showed that the untargeted metabolomics approach for the discrimination of honeys of different floral origins may be best suited for the use within a particular country or region with limited variation in the geography and climatic conditions. A new method developed in FEPL for measurement of δ^2H of carbon-bound-non exchangeable hydrogen was successfully demonstrated to be effective as a potential tool for detecting economically motivated C_4-sugar adulteration of pineapple juice. A method was developed for the determination of δ^{13}C in acetic acid to control the adulteration of vinegar with non-biogenic/petrochemically derived acetic acid. The method was used to validate complementary methodology applied in the Philippines to detect vinegar adulteration. A multi-class analytical method was developed and validated in collaboration with researchers in Uruguay for the detection and quantitation of 78 residues, contaminants and adulterant dyes in turmeric.

The FEPL coordinated and provided technical input to two coordinated research projects on food authenticity, involving approximately thirty countries.

The results of FEPL research were presented at seven international conferences, and the FEPL was represented in the scientific committees for two major international conferences on food safety. The FEPL contributed to international efforts to develop food fraud controls through participation in the FAO Food Fraud Workshop and as a member of the UK's Food Authenticity Methodology Working Group.

The FEPL provided capacity building for Member States through the main technical management of nine national and two regional technical cooperation projects in 2019. Human resource capability was enhanced in Member States through the training of 222 scientists, analytical chemists and laboratory personnel in 11 training courses, workshops or seminars. The FEPL hosted three fellows, two cost-free scientific personnel, one intern and two visiting scientists during the year. Technical backstopping, advice and contributions to webinars and other activities were provided to the RALACA network of food safety laboratories in Latin America and the Caribbean.

A new project, 'Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies', funded by the government of Japan under the 'peaceful Uses Initiative' commenced in 2019 with method development and training activities.

Publications by FEPL staff in 2019 comprised five papers in peer-reviewed scientific journals and eight conference papers, abstracts or reports.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head
Kelly, Simon	Food Safety Specialist
Maestroni, Britt Marianna	Food Scientist
Mihailova, Alina	Analytical Chemist
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Wafula, Caroline	Team Assistant
Permetov, Serik	Laboratory Attendant
Liang, Ying	Cost-free Expert
Jin, Shunru	Cost-free Fellow
Cesio, Veronica	Consultant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The main focus of the Food and Environmental Protection Laboratory's (FEPL) research and development activities is the development or adaptation of analytical methods to help Member States to improve their food control systems. The issues of importance to Member State governments, regulators, industry and ultimately consumers can be summarized in the three questions, "is my food safe?", "am I eating what I think I'm eating?", and "am I getting what I paid for?". The analytical methods developed in the FEPL and transferred to Member State laboratories, therefore, include methods for the detection and quantification of chemical residues and contaminants in food (e.g. pesticides, veterinary drug residues, mycotoxins), for testing various criteria related to the authenticity of foods, such as confirmation of stated geographical origin, production technique (e.g. organically produced foods), quality (e.g. extra virgin olive oil as opposed to processed olive oil) and adulteration (e.g. dilution of extra virgin olive oil with vegetable or nut oils, dilution of honey with corn syrup). Method development in the FEPL encompasses both sophisticated techniques capable of providing essential information such as the identity and amount of food contaminants present or the probable origin or production technique of a food product that allow follow-up actions to deal with the issue, and rapid screening methods that can be deployed in the field or along the food supply chain and can indicate that there might be a problem with a sample without necessarily identifying what that problem is (non-targeted analysis). A combination of these techniques provides Member States with the options needed for their food control systems.

In 2019 nine novel analytical methods and associated method protocols were developed in FEPL. Four instrument operating procedures were prepared to enable further uptake of analytical methods in Member States. Ten food matrix reference materials for stable isotope analysis were characterised in FEPL as part of an interlaboratory study; the materials will be available as USGS certified reference materials in 2020; these materials will support accurate and reliable isotopic measurements in Member States. Some of the main research activities and results are presented below.

Food traceability and authenticity

The adulteration of food, though driven by economic gain, can also present a significant risk to human health. There have been many examples of this throughout history. In recent times, notable examples include melamine in milk powder, Sudan dyes in chilli powder and methanol in counterfeit spirits. The global occurrence of such incidents may bring about negative impacts on international trade, reputational damage to companies or entire industries and at worst serious injury, or death, to consumers.

Rapid detection of food adulteration using headspace-gas chromatography-ion mobility spectrometry

Various physicochemical methods, spectrometric techniques and chromatographic methods have been reported for monitoring adulterated or contaminated food, but there is still a significant need to develop more efficient, faster, easily deployable methods to complement current techniques. Headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) is a nuclear technique that can be used for rapidly screening volatile organic components in food. Combined with optimized chemometric tools, it is a promising tool for rapid screening of foods for the presence of adulterants and/or contaminants.

The combination of gas chromatography (GC) with ion mobility spectrometry (IMS) delivers the high separation characteristics of GC and the fast response-high sensitivity of IMS. In the first step, the analytes are separated by GC on the basis of their retention in a fused-silica capillary column. In the second step, ions are generated in the ionization chamber of the IMS cell, and the charged molecules are then separated under the influence of an electrical field depending on their drift behaviour in a

nitrogen buffer gas. Recently, HS-GC-IMS has been applied in various fields, such as the quality assessment of olive oils, determination of ignitable liquids and authentication of Iberian ham. It has been demonstrated as an effective technique due to the comparatively simple system set-up, robustness, and price.

Authentication of argan oil from Morocco

In recent years, argan oil has become one of the most prized oils in the world due to its delicate hazelnut taste and multiple pharmacological properties. In addition to mono-unsaturated and saturated fatty acids, argan oil also contains diverse minor components such as polyphenols, tocopherols, sterols, squalene, and triterpene alcohols. The high content of antioxidants along with essential fatty acids are primarily responsible for its beneficial effects. Due to its extensive use in the food and cosmetic industries, both the production and price of argan oil have increased and consequently so have the economic incentives for adulteration with cheaper oils. The need has arisen, therefore, for more effective quality control screening methods aimed at detecting adulteration of argan oil.

The oil most frequently used to extend argan oil is sunflower oil because of its relatively similar fatty acid composition. Various techniques such as high-performance liquid chromatography, gas chromatography, inductively coupled optical emission spectrometry, and fluorescence spectroscopy have been applied to detect adulteration of argan oil. However, rapid and relatively low-cost screening techniques are attractive to control the quality and authenticity of vegetable oils such as argan oil. The feasibility of a rapid, simple method using Fourier-transform infrared spectroscopy with attenuated total internal reflectance (FTIR-ATR) to screen argan oil for adulterants was previously demonstrated in FEPL. However, one of the restrictions of FTIR is that it does not lend itself to automated batch analysis, which can significantly limit its application. As a result, there is still a requirement to develop an accurate, efficient and low-cost automated analytical screening method. In this study we applied HS-GC-IMS for the first time to detect the adulteration of Moroccan argan oil with sunflower oil.

Authentic samples of argan oil were obtained from a coordinated research project (D52040) counterpart in Morocco. The samples comprised argan oil from the 3 regions of Morocco (Troudante, Tiznit Sidi Ifni and Chotouka ait baha), with 10-13 samples taken from each region. Simulation of fraudulent adulteration was performed through fortification of a pooled sample of the argan oils with 5%, 10%, 20% and 30% sunflower oil. A mixture of 6 ketones was used for quality control to check the performance of the equipment during the analytical run. The determination of the headspace volatiles was performed using 6 replicates for each sample. The spectra were processed through an integrated software. Volatile organic compounds (VOC) were carefully selected through their respective 2-dimensional retention versus drift-time or 'heat maps' (Fig. 1).

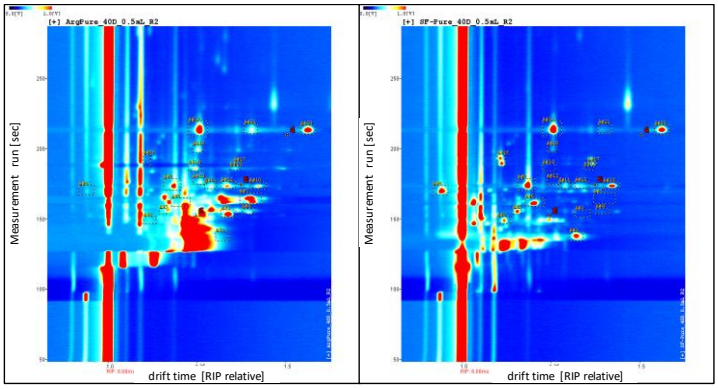


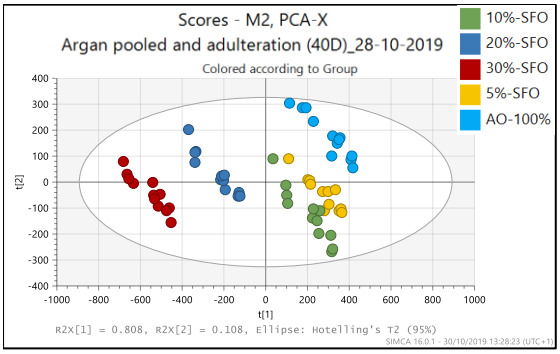
***FIG. 1**: HS-GC-IMS heat maps of pure argan oil (left) and the adulterant (sunflower oil)*

The sample results were evaluated by principal component analysis (PCA) to check for any differences and groupings. The PCA plot in Fig. 2 shows that the argan oil samples adulterated with sunflower oil at different concentrations were distinguishable from the authentic argan oil. The data was further processed by partial least square regression (PLS), showing similar discrimination.

A regression plot (Fig. 3) shows the observed versus predicted values of the level of adulteration, giving a predicted

fraction (Q2, 2 principal components) of 0.99, indicating excellent predictive power. The average prediction error was lower than 20% at 5% adulteration, falling to 4% error at 30% adulteration.

In conclusion, these preliminary results suggest that the method may be suitable as a rapid screening method to detect the adulteration of argan oil with sunflower oil. The statistical model could clearly distinguish the groups at adulteration levels between 5 and 30% and could predict, with a low prediction error, the presence of adulterant at 10% w/w. The combination of a benchtop headspace IMS device with chemometric modelling offers great promise as a reliable and rapid automated screening tool to detect adulterants in food.



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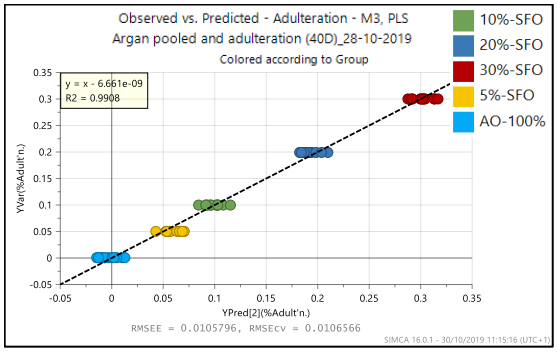

***FIG. 2**: PCA-X scores of pure argan oil and argan oil adulterated with sunflower oil*

***FIG.3**: Regression plot of observed versus predicted adulteration levels*

Analysis of green tea for detection of adulteration with food colourants

Tea (Camellia sinensis L.) is one of the most popular beverages in the world and a very important export product for many developing countries. As well as its consumption as a refreshing drink, it has been used as a natural medicine for thousands of years. The two most popular varieties are green (favoured in Asia) and black tea (favoured in the western countries). The expansion of the consumer market has increased demand for tea and has incentivised adulteration because of the potential for greater profits. The adulteration of tea is an age-old problem. Some of the most common examples are mixing tea-leaves with leaves of other plants, addition of tea-leaf debris/dust, and colour and visual enhancement of green tea with dyes such as Prussian blue or indigo which may be applied in a powder with calcium sulfate or gypsum to improve the glaze of the leaves. Several analytical techniques have been developed in FEPL and previously reported to help address these issues, including untargeted metabolomics to distinguish teas from different origins or of different varieties, and hydrogen isotope analysis for origin determination. Rapid screening methods were also developed for detection of adulteration of black tea using FTIR and for differentiation of geographic origin and type of tea by hand-held infrared spectroscopy.

To follow up on this previous work and complement those techniques, a method utilising HS-GC-IMS was developed to detect the volatile compound profile of green tea powder and to detect the presence of the potential adulterant dyes Prussian blue and indigo. Authentic samples of dried green tea leaves from different regions in China were supplied by a coordinated research project counterpart and some samples were obtained from a trusted organic tea supplier in Vienna, Austria. Simulation of fraudulent adulteration was performed through fortification of a pooled sample of the homogenized tea with 0.1%, 0.5%, 1.0%, 2.0% and 3.0% Prussian blue or indigo dye. IMS spectra were acquired for the genuine and adulterated tea samples. The processed data were further analysed using ‘Chemometrics in Excel’. PCA was applied for data exploration and detection of possible outliers. Soft independent modelling by class analogy (SIMCA) was used to construct and validate prediction models.

Using HS-GC-IMS combined with optimized chemometric methods, detection of the adulteration of green tea with these dyes was effectively demonstrated in the laboratory. The models were challenged after 3 months and could still identify the adulterated samples. Further validation of the

models with a large number of tea samples of different origins is required to investigate the robustness of the method, but it has potential as an important screening tool for the detection of adulteration of green tea with colouring agents.

Model transferability: An interlaboratory study using SCiO devices to test oregano authenticity

As an element of FEPL's research into rapid, cost-effective, portable screening methods for food authenticity, and in support of CRP D52040, 'Field-deployable Analytical Methods to Assess the Authenticity, Safety and Quality of Food', FEPL collaborated with Queen's University Belfast (QUB) in an interlaboratory study designed as part of the European Institute of Innovation and Technology's 'EIT Food' project, 'Food Fortress for raw materials and ingredients in Europe – Gaining Consumer trust through transparency of the supply chain'. The aim of the study was to investigate the transferability of methodology using a hand-held near-infrared (NIR) measurement device, the SCiO, as a screening test to determine the authenticity of the herb, oregano. Oregano is widely adulterated in the market, typically with dried leaves of other plants such as olive or myrtle. An interlaboratory study involving more than 30 participants and 34 SCiO devices around the world was undertaken. Chemometric models to detect oregano authenticity, developed using NIR spectra obtained on a single SCiO instrument at QUB, were deployed and the study investigated the ability of those chemometric models to correctly identify the authenticity status of oregano samples in the collaborating laboratories.

Six samples of oregano, authentic or adulterated, were prepared by and received in FEPL from QUB. After distribution to the participating laboratories the anonymized samples were analysed without prior knowledge of which samples were authentic and which were adulterated. SCiO instruments, samples and a measurement protocol were distributed to project participants by FEPL and the measurement data from the different laboratories were collected and processed at QUB to investigate the characteristics of the instruments and the performance of the models developed using different software packages.

***Table 1**. Comparison of 7 chemometric models for identification of adulterated oregano by 34 SCiO instruments.*

No. of correct classifications from 6 samples	M2 Predicted	M27 Predicted	M5 Predicted	M11 Predicted	M12 Predicted	M13 Predicted	M15 Predicted
6/6	2/34 (5.9%)	2/34 (5.9%)	0/34 (0%)	0/34 (0%)	0/34 (0%)	0/34 (0%)	0/34 (0%)
5/6	30/34 (88.2%)	29/34 (85.3%)	33/34 (97.1%)	32/34 (94.1%)	7/34 (20.6%)	7/34 (20.6%)	30/34 (88.2%)
Therefore, at least 5/6	32/34 (94.1%)	31/34 (91.2%)	33/34 (97.1%)	32/34 (94.1%)	7/34 (20.6%)	7/34 (20.6%)	30/34 (88.2%)

The data processing and analysis is critical to the application of the methodology. Several normalisation factors were compared: a division-based method, a subtraction-based method and piecewise direct standardization. Several different models were tested based on the normalized data. Table 1 shows the results for seven different models (the different M numbers in the top row) constructed using division-based standardization. In this case the model M5 appears to have performed best with results from 33 of the 34 instruments achieving correct results for at least 5 of the 6 oregano samples. Similar results were achieved using models constructed using the commonly applied piecewise direct standardization of data, with 100% of the instruments getting at least 5 out of 6 samples correct. In both cases the sample that was misclassified is an authentic oregano sample.

The results, therefore, look very promising, with only one false positive out of 6 samples and no false negatives, meaning that no adulterated samples are being missed by the test.

This is a good demonstration of the potential of using a cheap, portable analytical device to screen oregano samples in different locations against a chemometric model developed using authentic oregano samples in one location.

Untargeted metabolomics for the discrimination of honeys of various floral origins

In recent years, there has been a growing consumer demand for monofloral honeys that are considered more valuable than multifloral honeys due to their more desirable flavour, aroma and particular pharmacological attributes. Highly priced types of monofloral honeys (e.g. sidr, manuka, acacia) are a potential target for dilution or substitution with cheaper honeys, and thus the verification of the botanical origin of honey is an important issue from the authenticity point of view.

Detailed labelling of honey products with complete information about their botanical and geographical origin is rapidly becoming mandatory in many markets, and is already covered by legislation in areas such as the European Union. These aspects are particularly relevant in terms of both product quality and authenticity.

Conventional methods, which are commonly used for the quality control of honey, are pollen analysis (melissopalynology), physico-chemical methods (e.g. 5-hydroxymethyl furfural (HMF), enzyme activity, moisture and mono- and disaccharide analysis) and sensory evaluation. These methods are generally cumbersome and not always effective. In particular, the analysis of pollen used for the differentiation of the botanical origin is time-consuming and requires highly-skilled personnel in specialized laboratories.

Recent developments in high resolution mass spectrometry techniques and chemometrics has led to an increased interest in the application of untargeted metabolomics to the authentication of food products, including honey. The main advantage of using an untargeted metabolomic approach is that it can detect thousands of secondary metabolites, which may remain undetected if a targeted approach is used. Some of these secondary metabolites can be unique for a particular honey type, and thus may serve as authenticity markers.

Untargeted metabolomics analysis using UPLC-QTOF-MS was previously applied in FEPL for the analysis of honey, and the approach proved promising for the discrimination of honeys of different botanical and geographical origins. This follow-up study had the objective of further expanding the methodology, previously developed at FEPL, using a larger sample set of honeys. Untargeted metabolomic fingerprinting and multivariate data analysis was applied to analyse 274 monofloral honeys from 6 floral origins (manuka, acacia, clover, macadamia, orange blossom and thyme). Honey samples were obtained from markets and honey producers in 18 countries (Australia, Canada, China, Fiji, France, Germany, Greece, Hungary, Italy, Japan, Mexico, New Zealand, North Korea, Poland, Russia, Spain, United Kingdom, United States). Samples were extracted in methanol/water (1:1, v/v) and analysed on the UPLC-QTOF-MS system in positive ionisation mode.

Principle component analysis (PCA) of the data showed that most of the manuka samples were grouping separately from the rest of the samples, however a significantly large within-group variability and several outlier samples were observed in the manuka sample group (Fig. 4). Other analysed honey types also showed a high within-group variability, and thus the goodness of fit (R2) and prediction (Q2) of the PCA model were not sufficiently high to indicate good discrimination. The variability within sample groups can probably be mainly attributed to the large overall size of the sample set, the widespread locations of sampling, and geographical and climatic differences among the production countries. Supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA)

models were built to assess the discrimination between honeys of each floral origin and the other 5 floral origins either combined or individually (an example of OPLS-DA plots is shown in Fig. 5). The results of the OPLS-DA showed good discrimination between some of the analysed honey groups, e.g. manuka, clover and macadamia honeys were well separated from each other. Detailed data mining and further analysis of tentative markers for each floral origin was performed using XVar plots, S-Plots, Variable Importance in Projection (VIP) plots and Hierarchical Clustering Analysis (HCA). These analyses showed that the high variability within each honey group was a limiting factor for the selection of markers that were unique for each floral origin. Thus, the transfer of the methodology to the triple-quadrupole LC-MS system for targeted analysis of markers was not a viable option at this stage.

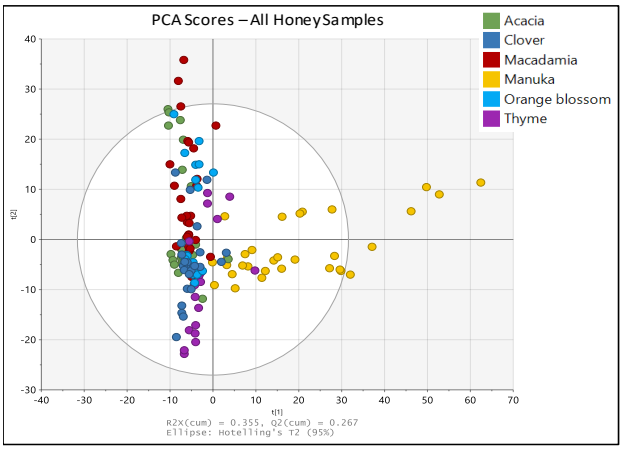

***FIG. 4**: PCA scores plot of 6 types of monofloral honeys (acacia, clover, macadamia, manuka, orange blossom and thyme) in ESI+ mode. The plot ellipse represents the 95% confidence limit for the Hotelling's T^2 (95%)*

This study investigated metabolome-wide differences of monofloral honeys obtained from the markets and producers in 18 countries. The results showed a large within-group variability, which can be attributed to the diversity of the geographical origin of the samples. The source of the samples (predominantly market) may have introduced additional variability in the dataset as the authenticity of the samples in the case of market samples could only be assumed. The authenticity of the samples is of paramount importance for the generation of robust discrimination models and unique marker identification. For the untargeted metabolomics approach to allow the identification of markers, unique for a particular type of authentic monofloral honey, the effect of the floral origin on the metabolic fingerprint of honey should overrule all other factors such as geographical origin, climatic conditions, season, processing, storage etc.

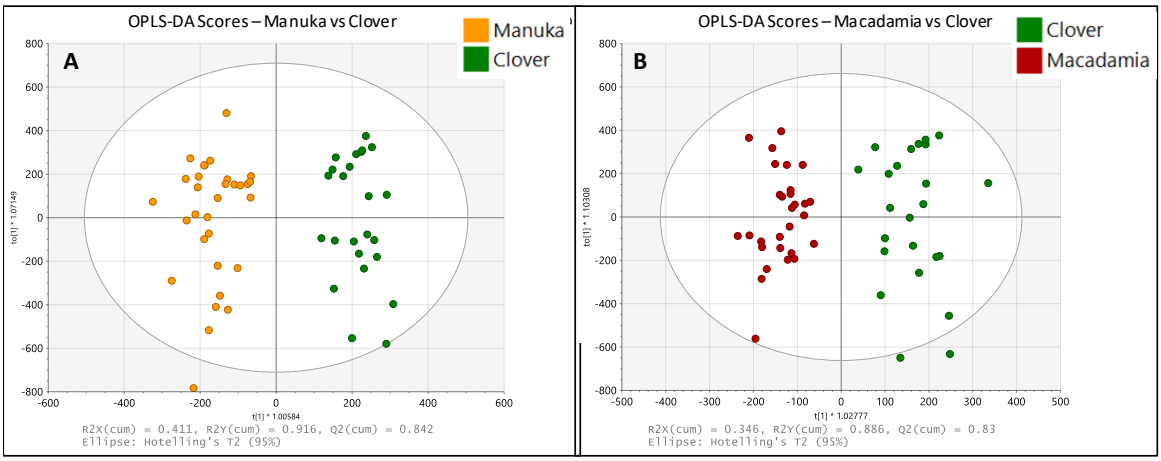

***FIG. 5**: OPLS-DA plot of clover honey vs manuka (A) and macadamia (B) honeys in ESI+ mode. The plot ellipse represents the 95% confidence limit for the Hotelling's T^2 (95%)*

The results of the study suggest that the untargeted metabolomics approach for the discrimination of honeys of different floral origins may be useful, but is best suited for the use within a particular country or region where there is little or no significant variation in the geography and climatic conditions.

Determination of C4-Plant sugar addition to pineapple juice by GC-CrAg-IRMS measurement of δ^2H of carbon-bound-non exchangeable (CBNE) hydrogen in sucrose

Differentiating between sugars produced by crassulacean acid metabolism (CAM) plants, in particular pineapple, and those derived from C_4 metabolism used to produce cheap adulterant sugar syrups (e.g. cane, maize) is challenging. This is because the overall molecular stable carbon isotope ratios of the sugars and site-specific deuterium/hydrogen ratios of the methyl group of ethanol $(D/H)_1$, measured by deuterium site-specific natural isotope fractionation nuclear magnetic resonance (^{2}H SNIF-NMR), are very similar. Currently, the only reported successful method for detecting the addition of exogenous C_4 sugars to pineapple juice is ^{13}C SNIF-NMR analysis of the methyl and methylene sites of ethanol fermented from pineapple juice sugars, with a detection limit of approximately 15% w/w of the total sugars present. However, this method is time consuming and expensive to implement due to the need to ferment the pineapple juice sugars to ethanol and the use of a high resolution, deuterium enabled, NMR.

A novel procedure for the rapid isotope analysis of the carbon-bound nonexchangeable (CBNE) hydrogen in mono and disaccharides was recently developed in FEPL. The method was targeted at the detection of the undeclared addition of exogenous sugar products in foods and beverages susceptible to economically motivated adulteration. The procedure utilizes a simple one-step reaction to substitute the exchangeable hydroxyl-hydrogens with trifluoroacetate derivatives that are sufficiently volatile to be separated by gas chromatography and measured by an isotope ratio mass spectrometer coupled to the gas-chromatograph. The derivatised sugars are converted into hydrogen gas using a high temperature chromium-silver reactor that retains carbon, oxygen and fluorine whilst releasing hydrogen for stable isotope measurement. This new procedure has advantages over existing methods in terms of ease of use, analysis time and compound-specific information. The method was initially applied in FEPL for the analysis of sugars from fruit juice and honey to demonstrate its feasibility and has now been applied to evaluate its feasibility for detecting C4-sugar addition to pineapple juice.

A crude extract of the sugars present in pineapple juice is isolated by removing pulp by centrifugation and the other major soluble-solid, citric acid, by precipitation of its insoluble calcium salt. The supernatant is lyophilised, and the sugars are derivatised with N-methyl-bis (trifluoroacetamide) (MBTFA). This process removes exchangeable hydroxyl-hydrogen atoms and replaces them with trifluoroacetatate (TFA) groups, which also makes the sugars sufficiently volatile for gas chromatography. The major sugars in pineapple juice (sucrose, glucose and fructose) are separated as their TFA derivatives by gas chromatography and then passed into a capillary furnace containing chromium metal particles and silver wool maintained at 1200 °C. The furnace retains carbon, oxygen and fluorine releasing hydrogen gas for determination of the mass distribution of the isotopologues (^{2}H^{1}H and ^{1}H^{1}H) by isotope ratio mass spectrometry and calculation of δ^2H with respect to relevant certified reference materials.

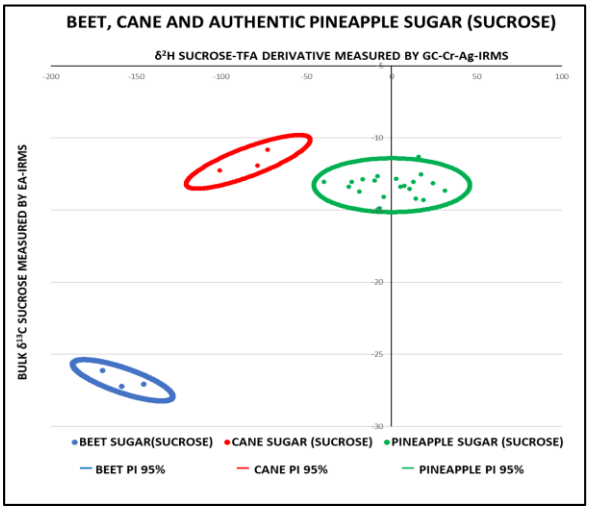

***FIG. 6**: Plot of authentic pineapple juice sucrose-TFA derivative δ^2H versus bulk sugar $\delta^{13}C$*

Authentic production samples of single strength pineapple juice and pineapple juice concentrate were obtained from a collaborating body, the SGF (Schutzgemeinschaft der Fruchtsaft- Industrie e.V, a non-profit industrial association financed by more than 650 fruit juice companies from nearly 60 countries worldwide). Pineapple concentrates were diluted to 12 °Bx (approximately 12 % w/w of sugar) with Millipore water prior to sugar extraction and analysis. Fig. 6 shows the 95% prediction intervals calculated from the bulk $\delta^{13}C$

analysis of lyophilised sugars extracted from pineapple juice and the corresponding δ^2H values of the sucrose-TFA derivative. Sucrose was selected because it is generally the highest concentration sugar present in pineapple juice and provides the highest intensity peaks for repeatable isotopic measurement. The prediction intervals may be used to assess the authenticity of retail samples with respect to the addition of exogenous C_4 and C_3 sugars e.g. cane and beet sugar (syrups) respectively. The method has been successfully demonstrated to be effective as a potential tool for detecting economically motivated C_4-sugar adulteration of pineapple juice.

Determination of $\delta^{13}C$ in acetic acid extracted from vinegar

Vinegar is an important element in Asian, European and other traditional cuisines of the world. It is one of the oldest fermentation products known, its use being recorded in Babylonian scrolls dating to around 5000 B.C. It is used, for example, for pickling and in the creation of marinades, dressings, and other sauces. Vinegar is produced from the fermentation of diluted alcohol products, yielding the organic compound acetic acid, its key ingredient, typically at 4-7% by volume. Acetic acid can be produced both synthetically and by bacterial fermentation. Synthetic acetic acid is a non-food grade material and may contain contaminants such as formic acid at significant concentrations. Today, the biological route accounts for only about ten percent of world production, but it remains important for vinegar production, as many of the world food purity laws stipulate that vinegar used in foods must be of biological origin. Vinegar is defined by the Joint FAO/WHO Food Standards Programme (1987) as "a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars, or starch and sugars by the process of double fermentation, alcoholic and acetous, and contains a specified amount of acetic acid".

Due to the high demand for vinegar as a condiment, it has been the target of adulteration with synthetic or non-biogenic acetic acid due to the price differential between natural and petrochemical sources. There is a need, therefore, for analytical methods capable of detecting the adulteration of natural vinegar with synthetic acetic acid. A method utilising ^{13}C stable isotope analysis was developed in FEPL for the differentiation of natural and non-biogenic acetic acid in vinegar to help meet this need in Member States.

The method involves the extraction of acetic acid from the vinegar by steam distillation followed by conversion of the acetic acid to calcium acetate by shaking with calcium carbonate. The aqueous solution of calcium acetate is evaporated to dryness, with the addition of acetonitrile to form a lower boiling azeotrope, and the residual calcium acetate powder is analysed by Dumas combustion in an elemental analyser. The combustion furnace is maintained at 1020°C and the reduction at 850°C. The sample is quantitatively converted, in an oxygen atmosphere, in the combustion furnace into carbon dioxide. The carbon dioxide gas is than carried to an isotope-ratio mass spectrometer by helium carrier gas. The acetic acid from natural vinegar can be differentiated from synthetic acetic acid by the differences in their $^{13}C/^{12}C$ ratio.

The method was applied in FEPL in 2019 to validate methodology used in the Philippines, where vinegar is a very important culinary commodity. As part of regional technical cooperation project RAS5081 the Philippine Nuclear Research Institute (PNRI) developed a methodology for authentication of vinegar using ^{14}C measurements, supported and verified by ^{13}C stable isotope measurements at FEPL. The measurable radioactivity of modern carbon (^{14}C) can be used to distinguish plant and fermentation-derived acetic acid from synthetic acetic acid produced from fossil fuel by-products. PNRI conducted a nation-wide survey of vinegar adulteration, testing 300 vinegar samples from major supermarkets all over the country and found that 5 out of 19 vinegar brands were adulterated with synthetic acetic acid. In the wake of the controversy ignited by PNRI's analysis results in the television news, radio and print media with reference to the proliferation of synthetic vinegar in the Philippines, their Food and Drug Administration assured the public that it will update the country's Vinegar Standard with PNRI's study results as a scientific basis.

Coordinated Research projects

In 2019, FEPL coordinated and provided technical input to two coordinated research projects (CRPs) in the fields of food authenticity and traceability.

Field deployable analytical methods to assess the authenticity, safety and quality of food

This project commenced in 2017 and outputs in 2019 include four papers published in peer reviewed journals and another three in preparation. Direct technical input from FEPL in 2019 included a successful inter-laboratory comparison exercise was carried out in collaboration with Queen's University Belfast, UK, with 30 participating labs across the world using the SCiO infrared pocket molecular sensor to compare results on authenticity of oregano herb. This CRP is attracting a lot international interest from industrial and regulatory stakeholders as global interest continues to grow in point-of-use analytical devices, e.g. UK Defra was an observer at the second research coordination meeting, and zero-cost industrial partners G.A.S. Dortmund joining the CRP in 2019.

Implementation of Nuclear Techniques for AuthentiCaTion of Foods with High-Value Labelling Claims (INTACT Food)

This project started in 2019. The first research coordination meeting (RCM) took place at the IAEA Headquarters in Vienna from the 13-17 May 2019. The participants comprised 12 contract holders (from China, Costa Rica, India, Indonesia, Jamaica, Malaysia, Morocco, Myanmar, Slovenia, Thailand and Uruguay), six agreement holders (from Denmark, Germany, Italy, Japan, New Zealand and Spain) and 5 observers representing Imprint Analytics (Austria), the Oil Crops Research Institute (China), the Tentamus Global Center for Food Fraud (Germany) and Organic Services (Germany). Workplans for the first phase of project were formulated.

RCM participants

Control of residues and contaminants in food

A multi-class analytical method for residues, contaminants and adulterants in turmeric

Herbs and spices have been used worldwide throughout human history as ingredients in food, teas and medicines due to their flavours and pharmacological, biological and antimicrobial properties. The largest spice importing trading block is the European Union, and the USA and Japan are the two largest single country importers.

Turmeric is a spice that comes from the turmeric plant (Curcuma longa L.), an economically important food and medicinal plant that grows primarily in tropical and sub-tropical regions including India, China, Taiwan, Sri Lanka, Peru, Australia and Thailand. Due to the growing demand for herbs and spices, agricultural cultivation has become increasingly intensive. The crops, before or after harvest, may be fumigated or treated to prevent pests and fungal infestation. The chemicals used may not always be authorized for use on the crops, or may be found at higher levels than the permissible maximum residue limits. In addition, it has been widely demonstrated that herbs and spices are subject to economically motivated adulteration, where dishonest producers or traders extend the product with lower value commodities. Recently, problems of adulteration of turmeric powder with other turmeric species have been reported. The adulterants used may also contain residues of pesticides not authorized for use in herbs and spices. The determination of trace amounts of pesticides and contaminants in herbs and spices is a challenging task due to the presence of natural metabolites

that are of similar physicochemical properties to those of the target analytes and at concentration levels that may be an order of magnitude, or more, higher than the target analytes.

The FEPL initiated a study on the optimization and subsequent validation of a multi residue method for the determination of selected representative pesticide residues, aflatoxins and persistent organic pollutants in turmeric. The method was an adaptation of the IAEA modified QuEChERS sample preparation technique based on an ethyl acetate extraction followed by dispersive solid-phase extraction. The clean-up step was performed using primary–secondary amine, RP-C18 and magnesium sulphate, with the amounts of the salts and adsorbents optimized for turmeric. The procedure was initially validated for 75 pesticides and contaminants at 10, 20 and 50 µg kg^{-1} in turmeric powder, with analysis by liquid chromatography- and gas-chromatography - tandem mass spectrometry (LC-MSMS and GC-MSMS).

The method was collaboratively tested with the Grupo de Análisis de Contaminantes Traza (GACT) laboratory of the Universidad de la República in Uruguay. The key method performance parameters investigated were specificity, linearity, trueness, within and inter-laboratory repeatability and reproducibility, limit of quantitation and matrix effects. Recoveries for the studied pesticides ranged from 60 to 110 %, and the RSDs were lower than 20 % for most of the evaluated pesticides. Comparable results were obtained in both laboratories (Fig. 7).

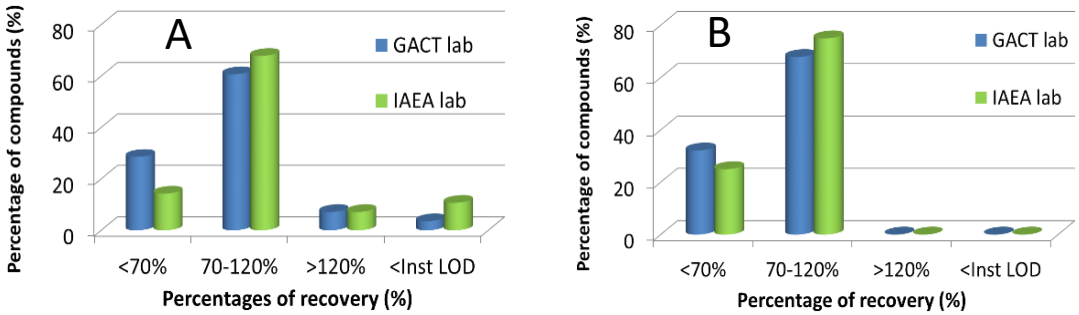

***FIG. 7**: Trueness evaluation at 10 µg/kg in IAEA FEPL and GACT laboratories by LC-MS/MS*

The method was also applied to the analysis of 21 commercial samples. One to four pesticides residues (chlorpyrifos, malathion, phorate sulfoxide and permethrin) were detected in 11 of the samples. Three of the positive samples were marketed as organic samples, demonstrating the importance of having a method available for the routine control of turmeric powder.

Since the intensity of their colour can be an important attribute of spices, affecting their market value, they may also be targets for adulteration with colourants. For turmeric, potential adulterants are yellow-orange dyes such as the Sudan dyes, which are banned for use in foods within the EU due to their carcinogenicity. The analytical method developed for residues and contaminants was, therefore, successfully expanded to include the Sudan dyes, Sudan I and Sudan IV, and butter yellow dye. The method is therefore suitable for use within food safety control programmes for a wide range of residues, contaminants and adulterants.

The results of the collaboration between GACT and FEPL were presented as a poster at the 9th International Symposium on Recent Advances in Food Analysis in Prague, Czech Republic, November 2019.

Dissemination of Research Results

The results of the research and the methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training courses, workshops, publications in the scientific literature and via the internet, public outreach events, conferences and

symposia. The 'Food Contaminant and Residue Information System' (FCRIS, http://nucleus.iaea.org/fcris/) provides useful data on food contaminants and residues and includes analytical methods databases. The methods databases for veterinary drug residues and for pesticide residues were developed in response to requests from the Codex Committees on Residues of Veterinary Drugs in Food and on Pesticide Residues.

Conferences

1st ISO-FOOD International Symposium on Isotopic and Other Techniques in Food Safety and Quality, Portorož, Slovenia, 01-03 April, 2019. The 1st ISO-FOOD symposium was designed to address the requirement for the development of new methods and techniques to verify the quality, authenticity, and safety of food. The symposium delivered a broad and interesting programme with 15 invited speakers and 31 oral and 59 poster presentations and was well attended by 126 participants from 16 countries. Mr. Simon Kelly (FEPL) gave the first scientific lecture at the symposium in the food authenticity and traceability session, on a new rapid method, developed in FEPL, using hydrogen stable isotope analysis to detect undeclared addition of sugar and sugar syrups to food.

Mr. Simon Kelly with Professor Nives Ogrinc (right) and Dr David Heath (ISO-FOOD ERA Chair, left) awarding prizes at the 1st ISO-

European Geochemical Union (EGU) General Assembly 2019, Vienna, Austria, 07-12 April 2019. The EGU General Assembly is one of the world's most well-attended and prestigious events for the geo-sciences and takes place annually at the Austria Centre Vienna. The General Assembly meeting covers a wide range of disciplines including climate science, the Earth's internal structure, volcanology, planetary exploration, and energy and resources. Nuclear techniques, especially stable isotope research, feature in many sessions. Mr. Simon Kelly (FEPL) gave a lecture focusing on the need for the development of food-matrix stable isotope reference materials. Stable isotope analysis has been used to detect economically motivated adulteration and substitution of food products since the early 1970s, but no chemically complex agricultural plant or animal-derived food materials have been offered as isotopic reference standards.

The 7th Latin American Pesticide Residue Workshop (LAPRW), Foz do Iguazu, Brazil 5-8 May 2019. LAPRW 2019 was attended by 310 delegates from 30 countries worldwide. Topics discussed at the workshop included pesticide residues in bees and bee products, pesticide residues in organic food and the environment, laboratory quality assurance/quality control/accreditation challenges, monitoring studies and risk assessment, advances in sample preparation and analytical procedures and state-of-the-art and low- and high-resolution mass spectrometry methods. Ms. Britt Maestroni (FEPL) presented a poster on preliminary studies carried out in FEPL on the use of GC-IMS profiles to optimise sample preparation for pesticide residue analysis and screen for adulteration of turmeric.

The 14th International Union of Pure and Applied Chemists (IUPAC) Congress on Crop Protection, Ghent, Belgium,19-24 May 2019. The IUPAC congress attracted around 1500 people, mainly from industry. Interesting debates held in plenary were on science-based facts and fact-based policies for crop protection; emerging food safety risks, precision agriculture and communication on agro-science to the general public. Ms. Britt Maestroni (FEPL) participated in the planning and implementation of the congress as the leader of theme 6, 'food quality and safety', in which thirty-five invited speakers gave oral presentations on the topics above. The program also included a theme 6 analytical forum to give a chance to the audience to ask experts in the field about analytical issues and challenges. The session was very successful and attracted a lot of interest from the participants.

Latin American Risk Assessment Symposium (LARAS) "Building-up a regional approach", Montivideo, Uruguay27-29 August 2019. The LARAS symposium was organized under the patronage of the Ministry of Livestock, Agriculture and Fisheries of Uruguay and the German Federal Institute of Risk Assessment (Bfr). It provided the audience with a multidisciplinary perspective on the societal and scientific challenges of risk assessment for ensuring food safety in Latin America. The symposium was attended by more than 150 participants from Uruguay and other Latin American countries. Ms. Britt Maestroni (FEPL) was an invited speaker and chaired a session on microbiological risk assessment.

Ms. Britt Maestroni (standing) in a LARAS workshop session

9th International Symposium on Recent Advances in Food Analysis (RAFA), Prague, Czech Republic, 5-8 November 2019. The RAFA programme included a wide range of food analysis-related topics, including the analysis of residues and contaminants in food; food authenticity and food fraud; QA/QC, and chemometrics and big-data handling; food forensics; novel food bioactives and supplements; and portable on-site food analysis. The symposium had approximately 850 participants from 60 countries around the world**.** Mr. Andrew Cannavan (Head, FEPL) gave an oral presentation, 'FAO/IAEA food authenticity research – some results in the field and future directions'. A poster on collaborative work with FEPL, entitled 'Multi-contaminant analysis in turmeric powder by LC-MS/MS and GC/MS/MS' was presented by Ms. N. Besil (Universidad de la República, Uruguay). FEPL also contributed to a presentation given by Mr. T. McGrath, (Queen's University Belfast, UK) on 'Model transferability: an interlaboratory study using SCIO devices to test oregano authenticity'.

Mr. Andrew Cannavan (right) with the speakers and chairs of the session 'Experiences, achievements and challenges foreseen by EU Reference Laboratories and international collaboration'

The National Institute of Standards and Technology (NIST) Food Safety Workshop and Debrief, Gaithersburg, U.S.A., 28-31 October 2019. NIST organised the Food Safety workshop to bring together experts from the food industry, government, academia, and international organizations with metrology experts to discuss challenges and possible solutions facing laboratories charged with ensuring the safety and authenticity of the global food supply. Mr. Simon Kelly (FEPL) gave an invited lecture on "The Application of Multi-Element and Multi-Isotope Analysis; A Potential Tool to Prevent Food Fraud" in the session on ''Authenticity, Fraud, and Adulteration".

CAPACITY BUILDING

The FEPL provided the main technical management for eight national and two regional technical cooperation projects in 2019. Analytical methods and technology packages were transferred and applied through training workshops held in Member States or at Seibersdorf and fellowships in, or scientific visits to, our laboratories in Seibersdorf. Human resource capability was enhanced through the training of 216 scientists, analytical chemists, laboratory personnel and food inspectors via 11 courses, workshops or seminars.

Mr. Simon Kelly led the mid-term review meeting of RCA Project RAS5081, 'Enhancing Food Safety and Supporting Regional Authentication of Foodstuffs through Implementation of Nuclear Techniques', in Hanoi, Vietnam, 16-22 November 2019. The purpose of the meeting was to review and assess the progress of project's implementation; to lead technical discussions on measures that need to be taken to ensure that the project will achieve the agreed objectives; and to guide and assist in updating the Member State participants' workplans for the second half of the project in 2020/2021.

The training activities and meetings provided a platform for interdisciplinary networking between stakeholders in the "farm-to-fork" food chain and fostered the formation of a global network.

Enhancing Food Safety and Supporting Regional Authentication of Foodstuffs through Implementation of Nuclear Techniques (RAS5081)

Two regional training courses were held in 2019 under this project.

Asia-Pacific Regional Training Course in Multivariate data analysis using the chemometrics add-in for Microsoft Excel

This course was the 3rd in a series RAS5081. Mr Simon Kelly (FEPL) collaborated with the Vice-President of the Zhejiang Academy of Agricultural Sciences, Professor Yuan Yuwei, to organise the regional training in multivariate data analysis (MDA) using the chemometrics add-in for Microsoft Excel, in Hangzhou, China from 15-19 April 2019.

The aim of the course was to provide the participants with a powerful, easily accessible and low-cost tool for basic multivariate data analysis. MDA is an essential part of complex analytical experiments and chemometrics is intensively used for processing of various nuclear data e.g. stable isotope and trace element (SITE) data for food authentication and to verify the geographical origin of food to support traceability systems. Professors Oxana Rodioniva and Alexey Pomeranstev, from the N.N. Semenov Federal Research Centre for Chemical Physics, delivered the chemometrics in Excel training to 22 participants from Bangladesh, China, Fiji, India, Indonesia, Japan, Lao P.D.R., Malaysia, Mongolia, Myanmar, Nepal, Pakistan, Philippines, R.O. Korea and Singapore, with the assistance of Dr Karyne Rogers (GNS, New Zealand). The course laid the foundation for an essential part of SITE data elaboration, stressing the importance of building reliable and robust MDA models before moving to the testing phase for food authentication methods.

Regional Training Course (RTC) on Fundamentals of Using Nuclear Techniques for Verifying Food Authenticity (Part II)

The fourth training course under this project was held at the Institute of Quality Standards and Testing Technology for Agro-Products, Beijing, China, from the 23 to 27 September 2019. The course was the second part of a fundamental introduction to the use of nuclear techniques for verifying food authenticity and covered the use of isotope ratio mass spectrometry (IRMS), inductively coupled plasma – mass spectrometry (ICP-MS), atomic absorption spectrophotometry (AAS), near infrared spectroscopy (NIRS) and multivariate statistical analysis of data (MVA). The 24 trainees were from 14 countries; Bangladesh, Cambodia, China, India, Indonesia, Republic of Korea, Malaysia, Mongolia, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand and Viet Nam. One of the other important aspects of the course was that four of the six expert trainers were from institutes within the regional project consortium and had previously attended the second "train the trainer" RTC on the use of advanced nuclear techniques for verifying food authenticity at the University of Science in Penang, Malaysia from the 19 to the 23 November 2018. The course therefore served two purposes; to further develop the knowledge and skills of the 24 regular participants and to initiate the four regional and local trainers in the self-sustainability of the consortium to provide courses in the application of nuclear techniques to detect food fraud.

Workshop on mitigation and/or remediation strategies for persistent organic pollutants (POPs)

A workshop to review preliminary project results for technical cooperation project RLA5069 and to identify possible mitigation and/or remediation strategies for persistent organic pollutants (POPs) took place from 2-5 May in Foz Do Iguazu, Brazil, organised by Britt Maestroni (FEPL). The workshop was attended by 9 participants from Argentina, Chile, Colombia, Dominican Republic, Ecuador, Guatemala, Mexico, Paraguay and Uruguay. The objective was to contribute to a better understanding of the actions and measures needed to identify mitigation and remediation strategies for POP's and the importance of technical expertise in POPs analysis. A key aim was to harmonize work in the region on monitoring levels of POPs in order to give decision makers options for targeted and effective mitigation and remediation strategies for the region.

Training Course on the Use of Nuclear Techniques to Determine Food Origin and Verify Food Authenticity

In 2019, FEPL received extrabudgetary funding from the Government of Japan under the 'Peaceful Uses Initiative' to implement a 2-year project, 'Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies'. The first training workshop under this project was held in FEPL from the 7th to the 18th of October 2019. The purpose of the training was to strengthen Member States' surveillance and research laboratory capacities in using the nuclear techniques, isotope ratio mass spectrometry (IRMS) and gas chromatography – ion mobility spectrometry (GC-IMS), to verify labelling claims related to the origin and authenticity of food products. This training was designed to underpin effective control measures to protect the public from fraud, including any associated unintended safety issues, mitigate the disruptive impact of emergencies affecting the food chain, and minimize disruption to trade in agricultural commodities.

Training course participants outside the laboratories at Seibersdorf

The training workshop was attended by 22 scientists from institutes in 16 countries; Argentina, Bangladesh, Chile, China, Costa Rica, India, Indonesia, Iraq, Jamaica, Malaysia, Morocco, Pakistan, Republic of Korea, Sri Lanka, Thailand and Vietnam. The 10-day course included theoretical lectures, hands-on laboratory sessions and discussion sessions. The first week focused on an introduction to food fraud and the application of IRMS; setting up an elemental analyser (EA) for bulk stable isotope analysis; routine coupled EA-IRMS operation; EA and IRMS fault finding and maintenance; preparation of honey protein to detect the addition of exogenous sugars, following the Association of Official Analytical Chemists methodology; detection of exogenous sugars to fruit juice, following a Codex method; calibration of the IRMS, data processing and analysis; quality control, proficiency testing and ion-source dismantling and cleaning. The second week of the training focused on stable isotope data

processing; applying stable isotopes and complementary techniques to characterise the geographical origin of food; a World Café exercise to plan a food authenticity project; an introduction to GC-IMS technology; and a second round of hands-on laboratory based training on the practical use of the GC-IMS "Flavourspec" system.

Ms. Alina Mihailova leads a practical session on detection of fruit juice adulteration

RALACA Laboratory Network

The Red de Latino America y el Caribe (RALACA) is a non-profit network of laboratories and associated institutions that brings together analytical laboratories to enhance regional capabilities for food safety and environmental sustainability. RALACA was established with assistance and guidance from FEPL. Ms. Britt Maestroni serves on the governing board of the network, as well as taking an active role in the network's activities.

Joint FAO/IAEA/RALACA Workshop on Quality Control Measures in Food Testing Laboratories

The Joint FAO/IAEA/RALACA Workshop on Quality Control Measures in Food Testing Laboratories was held on the 5th of May in Foz do Iguazu, Brazil. The workshop was attended by 45 participants from 16 countries worldwide. The objective of the workshop was to focus on analytical methods and technologies to ensure food safety. Experts in the meeting helped raise awareness of key quality concepts for the analytical testing of contaminants in food and helped strengthen the analytical capabilities of Member States in the Latin American and Caribbean region. The workshop program included lectures on food contaminant analysis and sample preparation alternatives, quality assurance/quality control measures for the development of analytical methods, instrumental techniques including gas chromatography–tandem mass spectrometry, liquid chromatography–tandem mass spectrometry and high resolution and accurate mass instrumentation, and an insight into current international guidelines/regulations for consumer protection and international trade.

RALACA third general meeting

Information sharing is key to enhancing regional opportunities. RALACA meetings are held regularly either online, through webinars, or as side events of technical meetings and/or training events. The network held its third general meeting in Brazil on 6th of May 2019 in conjunction with the 7th Latin American Pesticide Residue Workshops (5-9th May 2019). The general meeting was attended by 79 participants from 18 countries. The meeting was an important milestone in the process of gaining international recognition. Several new applications to join RALACA have been received from institutes in Latin America, indicating that the network is dynamic and continuously expanding.

Advice and Information Exchange

Staff of the FEPL were members of the scientific or organising committees of two international conferences held in 2019 or to be held in 2020; Ms. Britt Maestroni for the 14th International Union

of Pure and Applied Chemists (IUPAC) Congress on Crop Protection, Ghent, Belgium,19-24 May 2019, and Mr. Andrew Cannavan for EuroResidue IX: Current issues and emerging trends in residue control, Egmond aan Zee, The Netherlands, 18-20 May 2020. Ms. Alina Mihailova participated in a collaborative meeting on food authenticity research with Queen's University Belfast, UK, 24-30 March 2019. Mr. Simon Kelly participated in the FAO Food Fraud Workshop, an interactive workshop to develop a position paper on defining food fraud and the control system features relevant for prevention, detection and mitigation of food fraud, which was held in Rome, Italy, 3-7 November 2019.

FEPL staff regularly provide advice through participation as members of, for example, the UK's Food Authenticity Methodology Working Group, the Global Food Safety Partnership, and the Institute of Food Science and Technology.

Fellowships, Scientific Visitors and Interns

Name	Country	Status	Duration	Topic
Jin, Shunru	China	Cost-free Fellow	12 Months	Analytical methods for food control
Xu, Xiao	China	Intern	3 months	Analytical methods for food control
Liang, Ying	China	Cost-free expert	12 months	Analytical methods for food control
Adenan, Mohd Noor Hidayat	Malaysia	Fellow	1 month	Authentication of edible bird's nest using IRMS and chemometrics
Al-Saedi, Hameed Ouda	Iraq	Fellow	3 months	Multi-analyte analysis of chemical residues in honey, method validation
Karunarathna, Nuwan	Sri Lanka	Fellow	1 months	Food contaminant control using LC-MSMS

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EXTRA-BUDGETARY SUPPORT

PEACEFUL USES INITIATIVE (PUI). Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies, funded by Japan.

THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

In the Livestock Pest (LP) group of the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, a new digitalised and automated chilled adult release machine (BSI) to release adult tsetse flies was tested. Male tsetse flies that were irradiated and released through the machine mated with colony female flies in similar proportions as the non-treated colony male flies.

As part of studies to assess the interaction between endosymbionts and trypanosome infections, *Sodalis* and trypanosome prevalence was assessed in 2047 individual tsetse flies collected in nine countries. The prevalence of *Sodalis* varied from 5-95% depending on the tsetse species and location.

The prevalence of two viruses, i.e. Iflavirus and Negevirus was assessed in different natural tsetse populations. The two viruses were present in *Glossina morsitans morsitans*, *G. m. centralis*, *G. palpalis gambiensis*, *G. fuscipes fuscipes* and *G. m. submorsitans*, but the prevalence was highly variable. Only *G. pallidipes* proved to be free of the two viruses.

A new Near Infrared Pupal Sex Sorter (NIRPSS) was developed and tested. Several parameters were addressed to improve the accuracy of the sex separation. An accuracy of 91% of males was found in un-melanized pupae (assumed to be males) sorted by the NIRPSS.

In the Plant Pest (PP) group, experiments were carried out to evaluate the effect of normoxia (21% O2), hypoxia (~5% O2), and severe hypoxia (< 0.5% O2) on radiotolerance of third instar larvae of *Anastrepha fraterculus, A. ludens, Bactrocera dorsalis*, and *Ceratitis capitata*. Irradiation of third instars under hypoxia and severe hypoxia increased adult emergence and contributed to advancement of larval development of the flies only at low radiation doses that are not used as phytosanitary treatments.

Staff of the PP group continued working on the development of the SIT package for *Drosophila suzukii*. Dose-response studies for both males and females were completed and treating insects with a lower oxygen atmosphere before and during exposure to radiation mitigated some of the negative physiological effects due to the irradiation.

The PP group has also been working on the development of a genetic sexing strain for *Anastrepha fraterculus*. The strain is based on a pupal colour dimorphism (brown-black) that is resulting from a reciprocal translocation between the Y chromosome and the autosome carrying the wild type locus of the black pupae (*bp*) gene. An assessment was made of the productivity and the quality control profile of the strain IPCL-89 AF GSS. In addition, radiation studies were also carried-out. The new GSS strain will be transferred to Brazil in 2020 for pilot trial releases.

Feeding protein and a sugar mix accelerated sexual maturity of male *Bactrocera correcta* to 7 days as compared to 20 days for untreated control males. Acceleration of sexual maturity is a prerequisite to pre-treat males with the semio-chemical Methyl Eugenol (ME) before release. Early application of ME will allow the release of precocious pre-treated young male flies in a strategy of simultaneous application of the sterile insect technique and the male annihilation technique against this pest species.

In the Human Disease Vectors (HDV) group, work continued to evaluate pupae production using the FAO/IAEA rack unit and procedures and the female contamination rate after males sorting which the Fay-Morlan glass plate sorter. Results showed a high pupae production level (>2500 male pupae/tray until the second day of collection) and an estimated female contamination rate around 1%. Neither tray position nor type of tilting affected the pupae production and female contamination rate. However, the operator had a significant effect on female contamination rate.

A prototype low-cost plexiglass mass-rearing cage was developed and tested for *Aedes aegypti* and Ae. *albopictus* egg production and egg hatch in comparison to the currently used stainless-steel cage. Initial results were very encouraging. The new mass-rearing cage has the same design and dimensions but is 90% cheaper than the stainless-steel cage.

There is increasing interest expressed in several Member States to use drones for releasing mosquitoes. Staff of the HDV group developed and tested a light-weight release device (< 2 kg including the drone) with respect to the quality of released males. The releases had no negative impact on the quality of the released males as assessed with the flight ability test.

In the Genetics and Molecular Biology (GMB) group of the IPCL, a study was carried out to assess symbiotic changes of *Ceratitis capitata* during laboratory adaptation. Wild Mediterranean fruit flies were introduced at the IPCL and reared and monitored in the laboratory for 10 generations. The structure of gut microbiota communities using 16S rRNA gene sequencing was assessed and the effect of factors such as larval diet, age and sex on gut microbiota determined. Our results indicated alterations of the beta-diversity during the domestication process and significant differences in the species evenness. A similar study was conducted with *Drosophila suzukii*. The flies emerged from raspberries and were reared using an artificial carrot diet and a 23 °C rearing temperature. Results clearly showed drastic changes in the beta diversity of the samples while the Pielou's index indicated significant differences of species evenness among the samples.

Work was carried out to evaluate selectable markers for the development of *Ae. aegypti* genetic sexing strains. Wild populations were screened for the presence of thermoresistant and thermosensitive alleles. Exposure of the populations at high temperatures for a specific time interval showed that some potential thermoresistant alleles might be present in some of them but not in others, thus reflecting the diversity of the populations.

Work was likewise carried out to identify the male determining factor in major Tephritidae. It was shown that the gene maleness-on-the-Y (MoY) encodes for the male determining factor in tephritids. In experiments performed in the IPCL, the functional analysis of MoY in *Bactrocera oleae* and *Bactrocera dorsalis* was carried out by embryonic RNA interference.

In 2019, staff of the IPCL published 35 scientific papers in peer reviewed journals, either as the lead author or as a co-author.

In 2019, the IPCL hosted 6 cost-free experts (CFE) and 5 consultants (C) (of which 6 were PhD students), 11 interns, 13 fellows (F) and 7 scientific visitors (SV).

The GMB and PP groups carried out 70 fruit fly shipments to 26 institutions in 18 countries (UK, India, Spain, Argentina, Greece, Italy, Kenya, France, Pakistan, USA, Senegal, Colombia, Mauritius, Germany, Mexico, Tunisia, Czech Republic and Sweden)), and 9 shipments of preserved fruit flies to 6 institutions (in Italy, USA, South Africa, Brazil and Belgium). The LP group carried out 139 tsetse shipments of 270,407 pupae (239,744 *G. palpalis gambiensis* to Senegal) to 8 institutions in 6 countries (Senegal, France, Zimbabwe, South Africa, Belgium and USA). The HDV group carried out 10 mosquito shipments to 10 institutions (in South Africa, Ecuador, France, Italy, Serbia, Germany and Cameroon).

In 2019, the IPCL received 313 visitors from 44 countries.

STAFF

Name	Title
Vreysen, Marc	Laboratory Head
Abdalla, Adly	Molecular Biologist/Virologist
Bourtzis, Kostas	Molecular Biologist/Geneticist
Bouyer, Jeremy	Entomologist (Human Disease Vectors)
Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Livestock Pests)
Yamada, Hanano	Entomologist (Human Disease Vectors)
Nikolouli, Katerina	Geneticist (Human Disease Vectors)
De Oliviera Carvalho, Danilo	Molecular Biologist (Human Disease vectors)
Maiga, Hamidou	Medical Entomologist (Human Disease Vectors)
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Adun, Henry	Laboratory Technician
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Konczal, Anna	Laboratory Technician
Marin, Carmen	Laboratory Technician
Mohammed, Hasim	Laboratory Technician
Maxwell, Florence	Laboratory Technician
Cancio Martinez, Elena	Laboratory Technician
Dammalage, Thilakasiri	Laboratory Technician
Pillwax, Gulizar	Laboratory Technician
Bueno, Odette	Laboratory Technician
Sto. Tomas, Ulysses	Laboratory Technician
Bimbile, Severin	Laboratory Technician
Wallner, Thomas	Laboratory Technician
Duran de la Fuente, Lucia	Laboratory Technician
Kraupa, Karina	Laboratory Technician
Beckham, Stephanie	Programme Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Livestock Pests

The BSI Automated Chilled Adult Tsetse Release Machine and Mating Competitiveness of Glossina palpalis gambiensis

The sterile insect technique was successfully used to eradicate a population of the tsetse fly *Glossina austeni* from Unguja Island, Zanzibar, United Republic of Tanzania in 1994-1997. Thereafter, there were several attempts to implement the SIT in other African countries in mainland Africa i.e. Ethiopia and Senegal. However, due to the special biology of the tsetse flies, i.e. their low reproductive capacity and being hematophagous, the rearing of tsetse flies in large numbers represents a significant challenge. The establishment of large tsetse mass-rearing facilities to produce sufficient sterile males for the implementation of the SIT requires a capital investment. Therefore, smaller projects like the eradication of a tsetse fly population (*Glossina palpalis gambiensis*) from the Niayes in western Senegal adopted the strategy of procuring the sterile male pupae from abroad (the Centre international de recherche-développement sur l'elevage en zone subhumide (CIRDES) and the Insectarium Bobo Dioulasso (IBD) in Burkina Faso, the Slovak Academy of Sciences, Bratislava, Slovakia and the Insect Pest Control Laboratory, Seibersdorf, Austria).

FIG 1. (left top) BSI chilled adult tsetse release machine, (left bottom) Mr Gratian Mutika, consultant from Zimbabwe dissecting flies to assess their insemination status, (right) Ms Caroline Mirieri, consultant from Kenya, carrying out tsetse mating competitiveness studies in walk-in field cages

So far, the aerial release of sterile males has been mainly conducted using biodegradable cartons that are being dropped from a fixed-wing aircraft or a gyrocopter. The use of carton boxes is however very expensive, and therefore efforts have been made to develop devices that release the insects as chilled adults without being boxed. A new digitalised and automated chilled adult release machine (BSI) has been developed by a company in Senegal and was tested at the IPCL (Fig. 1). During the release process with this device, there will be mechanical friction and chilling of the flies before they are released. The aim of this study was therefore to assess the impact of this release machine on male fly performance with special attention to their mating competitiveness.

After calibration of the machine and evaluation of its flowability of releasing flies, the impact of the release process was assessed on *G. p. gambiensis* male's survival, flight ability, and mating competitiveness in walk-in field cages. This work was undertaken in collaboration with Mr Gratian Mutika and Ms Caroline Mirieri (two consultants hosted at the IPCL). The experimental design included both an assessment of the impact of the irradiation treatment (120 Gy) but not passed through the BSI machine, and the irradiation treatment combined with different chilling (at 5 °C) times in the

release machine, i.e. at the start of the release process, after one hour and after 2 hours, which is a realistic estimate for the duration of the entire release process. The males that were able to fly out of the fly cylinder in the flight propensity test (conducted 5 days post emergence) were used for the mating competitiveness tests in the field cages that were kept in an insect greenhouse with temperature and humidity controls under natural light. The preliminary results indicate that male flies that were irradiated and released through the machine mated with colony female flies in similar proportions as the non-treated colony male flies. Dissections of the female flies that mated also confirmed transfer of seminal fluids. The full details of this study will be published soon.

The Prevalence of the Tsetse Symbiont Sodalis glossinidius *in Natural Tsetse Populations*

Tsetse flies are harbouring four symbiont bacteria that play an important role in tsetse biology including productivity, performance and their susceptibility to trypanosome infections. The symbionts bacteria are *Sodalis*, *Wigglesworthia*, *Spiroplasma* and *Wolbachia*. *Sodalis* infections seem to be correlated with enhanced trypanosome infection and therefore it is important to assess *Sodalis* prevalence in natural tsetse population as it might provide information about the potential of developing trypanosome infections.

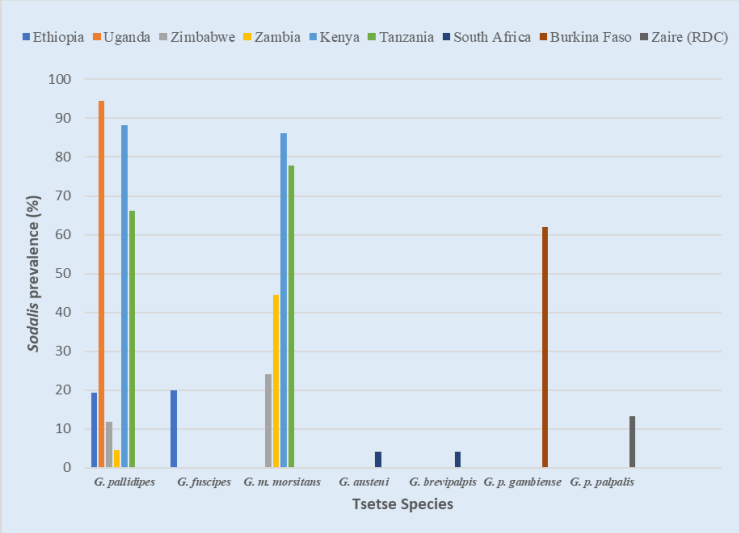

FIG. 2. Prevalence of Sodalis *in natural tsetse populations*

Therefore, Mr Mouhamadou Dieng, a consultant from Senegal was employed to assess *Sodalis* and trypanosome prevalence in natural tsetse populations. So far, 2047 individual tsetse flies from seven tsetse species collected from nine countries were analysed for the presence of *Sodalis* infections. The preliminary results indicate that the *Sodalis* prevalence varied from 5-95%. In *Glossina pallidipes*, *Sodalis* prevalence varied with sample site with the lowest prevalence of 5% in Zambia and the highest prevalence of 95% in Uganda. Similar results were observed in *G. m. morsitans* from different countries (Fig. 2).

The Impact of Iflavirus and Negevirus Infection on Tsetse Fly Performance

Insect mass-rearing often suffers from various types of stress factors such as crowding, which hampers the insect immune system and promotes the emergence of pathogenic bacteria, fungi and viruses that in many cases negatively affect the sustainability of the insect mass-rearing. Recently, two single stranded RNA viruses were detected in the tsetse fly *Glossina morsitans morsitans* of which a colony is maintained at the IPCL. The genome sequence, gene organisation and the phylogenetic analysis revealed that the two viruses belong to the Iflavirus and Negevirus taxon. The names proposed for the

two viruses are: *Glossina morsitans morsitans iflavirus* (GmmIV) and *Glossina morsitans morsitans negevirus* (GmmNV). To assess the host range of these viruses and its impact on tsetse rearing, PCR and quantitative PCR specific detection tools were developed in collaboration with Mr Mohammadreza Rezapanah, a consultant from Iran. The preliminary results indicate that the two viruses were present in *G. m. morsitans*, *G. m. centralis*, *G. p. gambiensis*, *G. f. fuscipes* and *G. m. submorsitans*, but the prevalence was highly variable (Fig. 3). Only *G. pallidipes* proved to be free of the Iflavirus and Negevirus. Preliminary results of the quantitative PCR analysis indicated that the midgut showed a high level of virus infection compared to other tissues such as salivary glands, fat bodies, and ovaries. The role of these two new viruses in the fly is enigmatic. Work is continuing to assess the impact of Iflavirus and Negavirus infections on tsetse colony performance and colonies with high and low virus prevalence are being established.

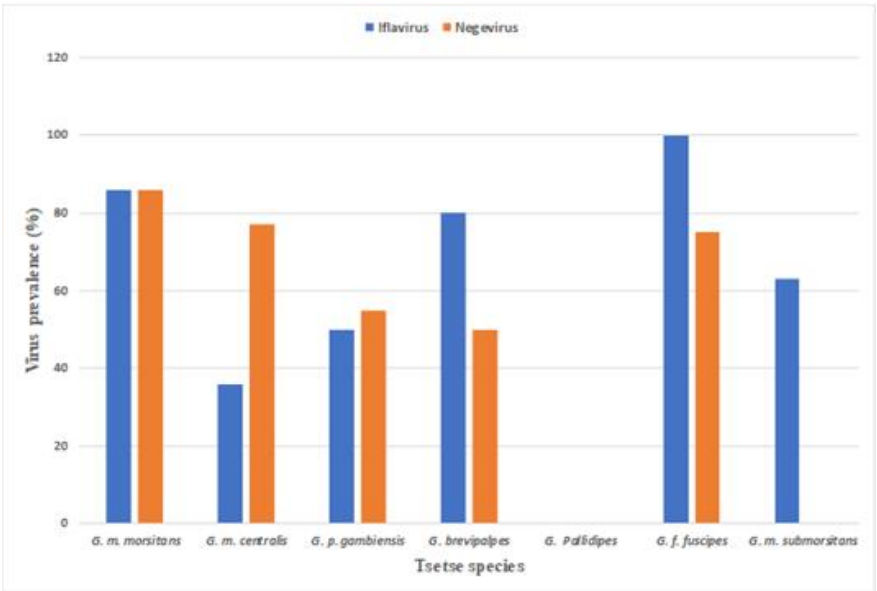

FIG. 3. (top) Mr Mohammadreza Rezapana, a consultant from Iran, assessing the prevalence of virus infections in tsetse colonies, and (bottom) the prevalence of Iflavirus and Negevirus in tsetse colonies at the IPCL

Evaluation of a Near Infrared Pupal Sex Sorter (NIRPSS) in Support of the Glossina palpalis gambiensis *Eradication Programme in Senegal.*

In view of the low reproductive ability of tsetse flies, it is necessary to retain female tsetse flies for colony maintenance and only release males for SIT implementation. One of the main limitations when implementing an SIT programme for tsetse flies is the lack of an automated and efficient system to separate the males from females in the pupal stage. To date, males and females can only be separated as adults making use of the relative protogyny, i.e. females develop and emerge 1-2 days before the males, but there is still considerable overlap in the emergence.

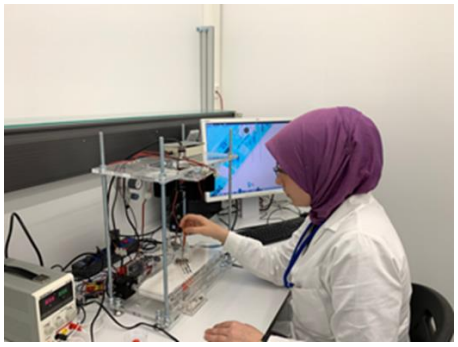

FIG. 4. Ms Sumejja Canic, an intern from Bosnia-Herzegovina sorting male and female tsetse pupae using the near infrared sex pupal sorting machine.

In addition, long distance shipments of male pupae are difficult and require chilling of the male pupae after the female emergence flush. Extended chilling of pharate adult males results often in reduced quality and increased mortality. Therefore, the availability of a system that enables the separation of the sexes several days before adult emergence would be extremely beneficial and significantly increase the quality of the shipped sterile male pupae.

Since more than 15 years, attempts have been made to separate the sexes of tsetse pupae a few days before adult emergence. Different versions of “near infrared sex sorter devices” have been developed and tested, but the results were not consistent

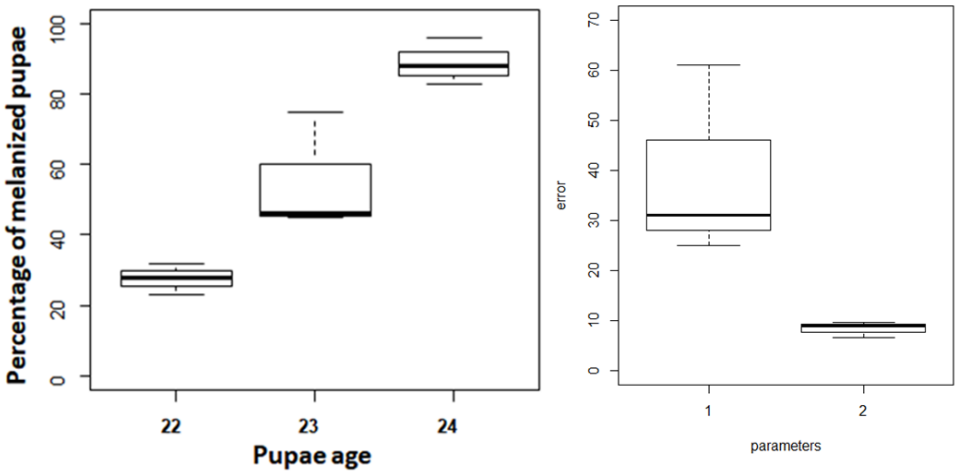

FIG. 5. (left) The proportion of melanized pupae in relation to the age of the pupae, and (right) error rate (number of females emerged from un-melanized (males) pupae) sorted by NIRPSS machine at two different parameters including pixel intensity threshold, rate of dark pixels in one frame to consider it as dark frame, and the minimum percentage of dark frames for a given pupae to consider it a melanized pupae (1) 210, 0.20, 25; (2) 225, 0.15, 20).

The discovery in 2016 that differences in wing melanization of the pupae can be made visible using near infrared was a breakthrough, as this phenomenon can be detected a few days earlier in females than males. The main problem remained the exact position of the pupae to detect the differences. A new sex separation device was recently developed that allowed the scrolling of the pupae whilst making several image frames for each pupa at different positions, making the detection of melanized wings much more accurate. The new Near Infrared Pupal Sex Sorter (NIRPSS) (Fig. 4) was developed in collaboration with Prof. Gustavo Salvador Herranz from the Cardenal Herrera University (CEU), Valencia, Spain.

As the melanization process is progressing with pupal age, the most suitable pupal age for the sorting was determined (Fig. 5). In addition, several parameters were tested to improve the accuracy of the sex separation. So far, an accuracy of 91% of males was found in un-melanized pupae (assumed to be males) sorted by the NIRPSS. It is important to note that the development rate of the pupae is affected by temperature and relative humidity. Therefore, having relative constant environmental conditions and synchronized pupal age will improve the accuracy of the NIRPSS machine in separating the sexes of the pupae. Work is continuing to further optimize the NIRPSS system and to analyse the impact of the sorting process on adult male performance.

In addition, test shipments of male *G. p. gambiensis* pupae sorted with the NIRPSS system at the IPCL and shipped to Dakar, Senegal have been initiated. Preliminary results are encouraging and indicate more than 70% operational males in the first shipments. Further optimization of this pupal sorting is undertaken.

Plant Pests

Phytosanitary Treatments under the FAO/IAEA/USDA Agreement

The FAO/IAEA/USDA agreement on “Harmonization of phytosanitary treatments for exotic fruit flies” aims to develop and validate phytosanitary treatment schedules against major fruit fly pests of the world to prevent agricultural trade barriers and ensure plant health protection. This agreement has made substantial contributions to the development and validation of cold and irradiation treatments.

Research comparing the tolerance of *Zeugodacus tau* populations to phytosanitary cold treatment was initiated (Fig. 6). Preliminary results suggest that third instar larvae of *Z. tau* from Bangladesh, China, and India respond similarly to treatments of 1.67 °C for 3 to 16 days. Interestingly, a few third instars from these *Z. tau* populations survived after either 15 or 16 days of cold treatment but failed to pupariate and did not survive to the adult stage. Cold treatments are currently not available for use on hosts of *Z. tau*. Thus, results from this research have the potential to support the application of cold treatment against *Z. tau* by FAO and IAEA Member States.

FIG. 6. Zeugodacus tau *female infesting a navel orange for phytosanitary cold treatment research*

Experiments evaluating the effect of normoxia (21% O_2), hypoxia (~5% O_2), and severe hypoxia (< 0.5% O_2) on radiotolerance of third instar larvae of *Anastrepha fraterculus*, *A. ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* were completed. Irradiation of third instars under hypoxia and severe hypoxia increased adult emergence and contributed to advancement of larval development of the flies only at low radiation doses that are not used as phytosanitary treatments. All third instars irradiated at approved phytosanitary irradiation doses in either hypoxia or severe hypoxia failed to emerge as adults and died as coarctate larvae. These results were evaluated by the International Plant Protection Convention (IPPC) Technical Panel on Phytosanitary Treatments (TPPT), which recommended to the Standard Committee (SC) the removal of the restriction from irradiation treatments under modified atmosphere against tephritid fruit flies. The SC agreed to remove the disclaimer statement regarding modified atmospheres from seven adopted irradiation treatments (PT 1, PT 2, PT 3, PT 4, PT 5, PT 7, and PT 14), and a final decision should be made in the 15 th session of the Commission on Phytosanitary Measures. The removal of restrictions from phytosanitary irradiation treatments can increase the applicability of nuclear technology in agriculture, reduce quarantine restrictions, and facilitate agricultural trade among FAO and IAEA Member States.

Drosophila suzukii

The rapid dispersal of *Drosophila suzukii* and the subsequent economic losses of the affected crops, encouraged the development of different approaches for the efficient management of this pest. The SIT can potentially be integrated into area-wide integrated pest management (AW-IPM) approaches to manage this pest under confined environment systems such as greenhouses. The staff of the IPCL have been working on the development of the SIT package for *D. suzukii*. To be able to implement the SIT for this pest, studies on irradiation/biology of this insect are required. More specifically, the optimal dose that sterilizes the males without compromising the quality and performance of the released sterile insects in the field is needed.

Recently we have completed the dose-response studies for both male and female *D. suzukii*. Treating insects with a lower oxygen atmosphere before and during exposure to radiation can mitigate some of the negative physiological effects due to the irradiation. The irradiation of pupae under oxygen-reduced environment such as hypoxia or anoxia is routinely used in the sterile insect technique of some tephritid species as it provides radiological protection. This treatment allows to have the sterile pupae already in sealed containers facilitating the shipment.

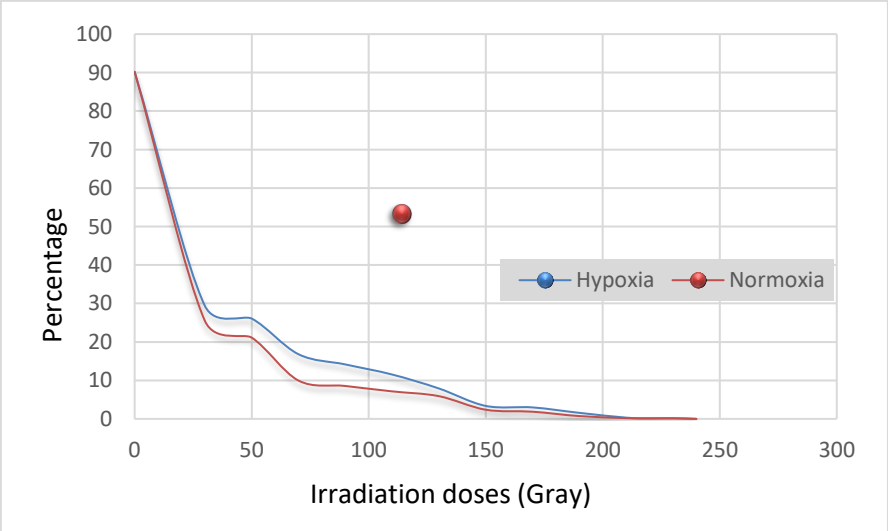

FIG. 7. The effect of different irradiation doses under hypoxia and normoxia atmosphere conditions on the egg hatch of Drosophila suzukii *in crosses of irradiated males with non-irradiated females*

Pupae of *D. suzukii* required an irradiation dose of 220 Gy to achieve >99% sterility in males expressed as reduction in egg hatch after mating with a fertile female, irrespective of the atmosphere condition. For irradiated females the same level of sterility was achieved already with a dose of 75 and 90 Gy for the normoxia and hypoxia treatments, respectively. Quality control assessments have shown that exposure to radiation doses of 170 and 220 Gy under the two atmosphere conditions did not have any effect on emergence rate and flight ability of *D. suzukii* males and females. Therefore, hypoxia conditions can be used as part of an AW-IPM programme that includes the SIT to facilitate the protocols of packing, irradiation and shipment of sterile *D. suzukii* pupae (Fig 7).

The South American fruit fly Anastrepha fraterculus

The South American fruit fly is a pest that has a major impact on the fruit industry of Brazil and other countries in South America. The SIT can be an additional component to manage this pest on an area-wide basis. Significant advances in the domestication and artificial rearing have been made for *A. fraterculus* in Peru and Brazil including an artificial rearing system that allows a rapid build-up of the colony and production of larger numbers of sterile flies that could be used to satisfy the demand of pilot-programmes against this pest. However, only bisexual strains of *A. fraterculus* are being reared in South America and it is known that sterile male and female fruit fly releases are less efficient than male-only releases in introducing sterility into the target population in the field.

The Plant Pest group of the IPCL has been working on the development of a genetic sexing strain (GSS) of *Anastrepha fraterculus* that is based on a pupal colour dimorphism (brown-black) that is resulting from a reciprocal translocation between the Y chromosome and the autosome carrying the wild type locus of the black pupae (*bp*) gene. Further screening of the colony resulted in the isolation of 4 new GSS lines (translocations), increasing to eight the number of available GSS lines of *A. fraterculus* for further evaluation.

In 2019, an assessment was made of the productivity and the quality control profile of the strain IPCL-89 AF GSS. In addition, radiation studies were also carried-out. The new GSS strain will be transferred to Brazil in 2020 for pilot trial releases under the responsibility of University of Sao Paulo and the Brazilian Agricultural Research Corporation (EMBRAPA) in collaboration with the IPCL.

The Melon Fly Zeugodacus cucurbitae

Irradiation dose response curves were developed for the GSS strain of *Zeugodacus cucurbitae*. Pupae were irradiated 24 hours before adult emergence under hypoxia or normoxia conditions. This study aimed to assess the irradiation doses needed for induction of reproductive sterilization for the application of sterile insect technique against this pest species. Male pupae needed an irradiation dose of 80 Gy under hypoxia conditions to achieve a reduction of >99% in egg hatch after mating with untreated females.

Hormones, Nutritional Supplements and/or Semio-chemicals to Accelerate the Sexual Maturation in Male Bactrocera correcta for the Combined Application of the Male Annihilation Technique (MAT) and the Sterile Insect Technique (SIT)

The effect of age, dietary protein and methoprene applications was assessed on the mating success of *Bactrocera correcta* under laboratory conditions. Emerged adult males were separated and fed or topically treated with the following four treatments:

- Feeding sugar (S)
- Feeding protein and sugar mix (PS)

- Feeding sugar and methoprene (SM)
- Feeding a protein and sugar mix plus methoprene (PSM)

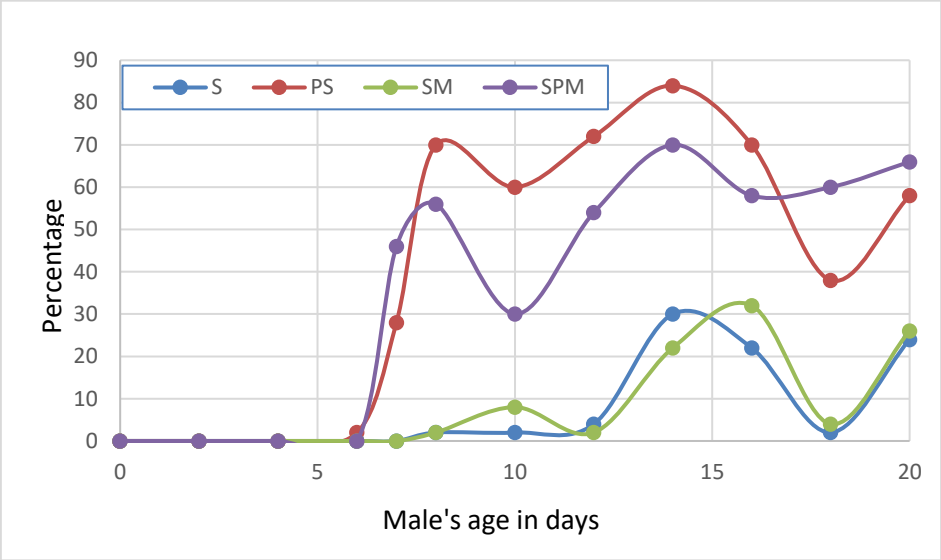

FIG. 8. The effect on mating performance of males of Bactrocera correcta *of different age treated with different nutritional supplements and/or semio-chemicals*

Preliminary results have shown that feeding protein and sugar mix accelerated sexual maturity of the males. Males treated with PS get sexual mature at 7 days whereas the untreated control males reach sexual maturity on day 20 post emergence (Fig. 8).

The purpose of this research is to elucidate the effect of the juvenile hormone treatment and protein supplement or the combination of both on the acceleration of sexual maturity and enhancement of mating competitiveness of male *B. correcta*. Acceleration of sexual maturity is a prerequisite to pre-treat males with the semio-chemical Methyl Eugenol (ME) before release. Early application of ME will allow the release of precocious pre-treated young male flies in a strategy of simultaneous application of the sterile insect technique and the male annihilation technique against this pest species.

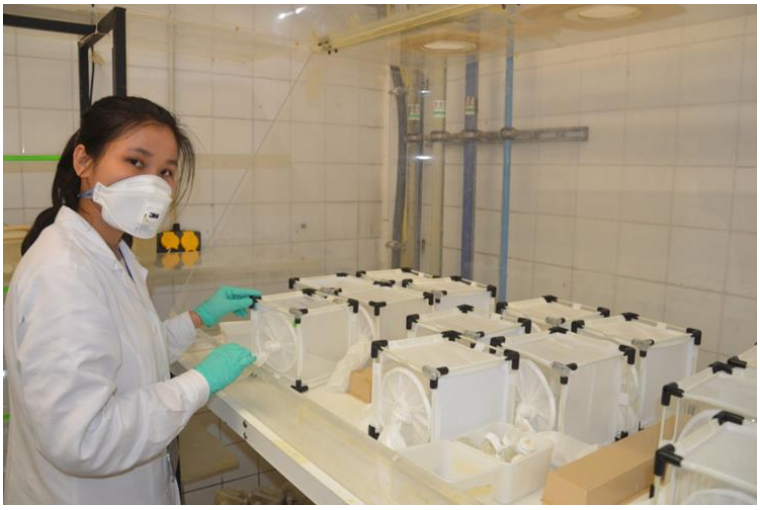

FIG. 9. Ms Le Thi Xuyen, a fellow from Vietnam preparing Bactrocera correcta *insects to assess the effects of the juvenile hormone analogue methoprene and dietary protein on sexual maturity and mating behaviour*

Human Disease Vectors

The IAEA Reference Larval Rearing Unit and Sex Separation of Aedes aegypti *with the Fay-Morlan Glass Plate Sorter*

The production of large numbers of mosquitoes of adequate biological quality and sex separation before release are key challenges when applying the sterile insect technique as part of an area-wide integrated pest management approach for mosquitoes. The IPCL of the Joint FAO/IAEA Division has developed dedicated technologies, trays and rack for rearing large number of larvae. In addition, a Fay-Morlan glass plate sorter is the most common method used to separate male from female *Aedes* mosquitoes and it is based on the sexual size dimorphism at the pupal stage. Field pilot studies prior to SIT application against *Aedes aegypti* are currently being implemented in some countries. Therefore, there is a need to fully evaluate the production and efficacy of such equipment in order to plan and reach a daily operational level allowing to sustain continuous large-scale operation activities. We evaluated pupae production using the FAO/IAEA rack unit and procedures and the female contamination rate after males sorting which the Fay-Morlan glass plate sorter.

Each tray was filled with 5 litres of osmosis water the day before the addition of larvae. Six trays per rack at different levels (from bottom to top positions in the rack that may hold up to 50 trays) were used to rear larvae at a density of 18 000 first instar larvae / tray to which 4% IAEA reference diet was added. From day 6 to 10 of development (after egg hatching), trays from each rack were tilted and their contents sorted either for each individual tray or after mixing the content of all trays from the rack. The pupae production and the female contamination rate were estimated with respect to day of collection, position of the tray, type of tilting and sorting operator.

Results showed a high pupae production level (>2500 male pupae/tray until the second day of collection) and an estimated female contamination rate around 1%. Neither tray position nor type of tilting affected the pupae production and female contamination rate. However, the operator had a significant effect on female contamination rate. This highlight the need to optimize pupae production during the early days of collection and to develop a more effective method of sex separation.

A Cost-efficient Mass-rearing Cage for Aedes albopictus

In the framework of the US grant ("Surge Expansion of Sterile Insect Technique (SIT) to Control Mosquito Populations that Transmit the Zika Virus") that was awarded in 2016 to the IPCL, a prototype low-cost plexiglass mass-rearing cage was developed and tested for *Aedes aegypti* egg production and egg hatch in comparison to the currently used stainless-steel cage. The new mass-rearing cage has the same design and dimensions but is 90% cheaper than the stainless-steel cage (Fig. 10). Initial results with *Aedes albopictus* indicate high production of fertile eggs.

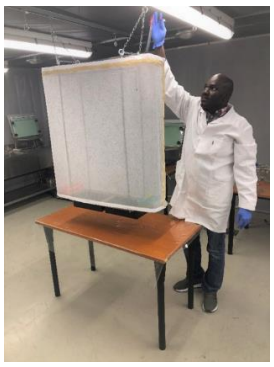

FIG. 10. Blood feeding of 12 000 female Aedes albopictus *with a collagen blood sausage in the new mass-rearing cage*

A New Aerial Release Device for Sterile Mosquitoes

Operational use of the SIT against human disease vectors continues to reveal areas where new technologies could further improve efficiency. The mosquito SIT package that is under development at the IPCL includes advances made in mass-rearing, irradiation, handling, transport and release protocols.

There is increasing interest expressed in several Member States to use drones for releasing mosquitoes, but in some countries, its use is hampered by stiff security regulations with respect to weight of the drones. Therefore, the use of lighter drones and especially, light-weight mosquito release containers is becoming imperative. We therefore developed and tested a light-weight release device (< 2 kg including the drone) with respect to the quality of released males and homogeneity of the releases at different rotation speeds. Mosquitoes were marked and irradiated as chilled adults before release through the new device.

These treatments had no negative impact on the quality of the released males as assessed with the flight ability test. Only few mosquitoes were damaged, and survival was good. The new device is now ready for testing in the open field.

Modelling the Impact of SIT and Boosted SIT on Aedes *Mosquitoes*

IPCL staff contributed to modelling the impact the SIT and its variant "boosted SIT" where sterile males are also used to transfer biocides to their female counterparts. In particular, the gains to SIT for *Aedes* control of either boosting with pyriproxyfen (BSIT) or contaminating mosquitoes at auto-dissemination stations were assessed. Thresholds in sterile male release rate and competitiveness were identified, above which mosquitoes are eliminated asymptotically.

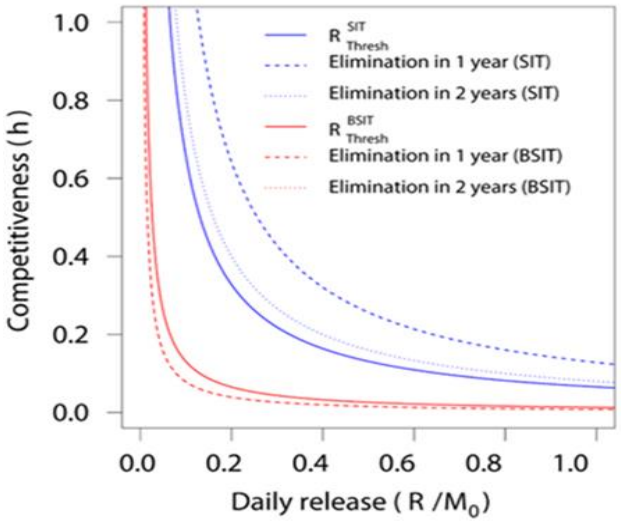

FIG. 11. Relationship between the competitiveness and the daily release rate of sterile males to obtain elimination of a local population of Aedes albopictus *in one or two years. Below a competitiveness of 0.2, the release rate (R) is increasing asymptomatically for SIT whereas boosting the sterile males with pyriproxifen (BSIT) still permits a reasonable release rate (with M0 the initial density of males). Two years (dotted) and 1-year (dashed) elimination thresholds for BSIT (red) are indistinguishable*

Both SIT and BSIT successfully eliminated target populations and boosting reduced these thresholds and aided population destabilisation, even at sub-threshold release rates. No equivalent bifurcation was observed in the auto-dissemination sub-model. Analysis suggested that BSIT could reduce by over 95% the total release required to circumvent dengue epidemics compared to SIT. We concluded that BSIT might represent a powerful new tool for the integrated management of mosquito borne diseases.

Genetics and Molecular Biology

Symbiotic Changes of Ceratitis capitata *during Laboratory Adaptation*

Several studies have proved that the microbiota profile of a *Ceratitis capitata* population adapted to laboratory conditions affects a variety of life-history traits related to SIT applications. Among other factors, the dietary environment offered in a newly established laboratory population originated from a wild niche, shapes the structure of the symbiotic communities. The "how" and "when" of these changes should undergo close monitoring that will allow identification of crucial timepoints affecting the quality of the insects during laboratory domestication. In this study, wild Mediterranean fruit flies were collected in Greece by Prof. Nikos Papadopoulos, University of Thessaly from infested bitter oranges and were introduced at the IPCL for symbiotic analysis. The flies that emerged from these fruits were divided in two sub-populations and each of them was reared either in banana or in the standard carrot diet. Both populations were adapted and monitored in the laboratory for 10 generations. We monitored the structure of gut microbiota communities using 16S rRNA gene sequencing and assessed how different factors including larval diet, age and sex can possibly affect gut microbiota. Our results indicated alterations of the beta-diversity during the domestication process and significant differences in the species evenness. The main phyla dominating all the sub-populations were Proteobacteria and Firmicutes and we showed that the symbiotic structure of the population at the genus level is significantly affected as the domestication progresses.

Symbiotic Changes of Drosophila suzukii *during Laboratory Adaptation*

Drosophila suzukii has been recently added to the SIT-agenda as a target species that needs to be urgently addressed. Following the *Ceratitis capitata* working framework discussed above, a founder wild population of *D. suzukii* was collected from infested raspberries in the area of Thorigné-Fouillard in France by Prof. Hervé Colinet, University of Rennes 1. Around 850 infested raspberries were brought to the laboratory and placed individually in plastic vials. The flies emerged from the fruits were domesticated at the IPCL using an artificial carrot diet and a 23 °C rearing temperature. Samples were collected for several generations and nine timepoints (including F0) were analysed by 16S rRNA gene sequencing. A paired-end Miseq Illumina sequencing targeting the V3-V4 region of the 16S rRNA gene was performed. Results clearly showed drastic changes in the beta diversity of the samples while the Pielou's index indicated significant differences of species evenness among the samples. Similar to *Ceratitis capitata*, an alteration of the relative abundances at the genus level was noticed.

Evaluation of Selectable Markers for the Development of Aedes aegypti *Genetic Sexing Strains*

The development of an efficient and stable genetic sexing strain that will guarantee the success of an SIT-based program requires selectable genetic markers that will be either visible or conditionally lethal mutations. These mutations are either naturally occurring in a population or can be induced using mutagenic factors. Towards our goal to identify and characterize novel selectable markers in *Aedes aegypti* mosquitoes, we screened wild populations for the presence of thermoresistant and thermosensitive alleles. Exposure of the populations at high temperatures (Fig. 12) for a specific time interval showed that some potential thermoresistant alleles might be present in some of them but not in others, thus reflecting the diversity of the populations and highlighting the need of having a closer look on them (Fig. 13). In parallel, a protocol making use of ethyl methanesulfonate (EMS) was developed and applied on an *Ae. aegypti* strain. The protocol involved several EMS concentrations applied on different mosquito adult ages and resulted in the construction of isofemale and isomale lines. These lines were screened using the thermal protocol mentioned above and are currently under investigation for the isolation of potential thermoresistant and thermosensitive alleles.

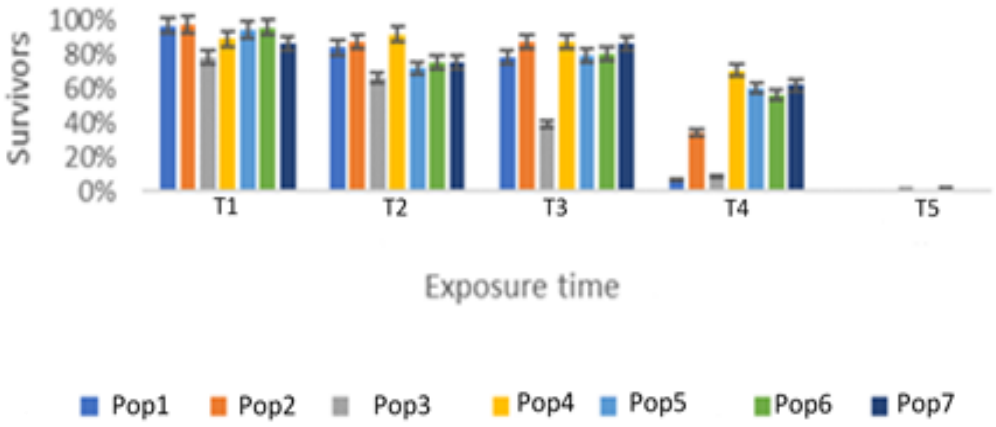

FIG. 12. Aedes aegypti *populations exposed at an elevated temperature for varying time durations*

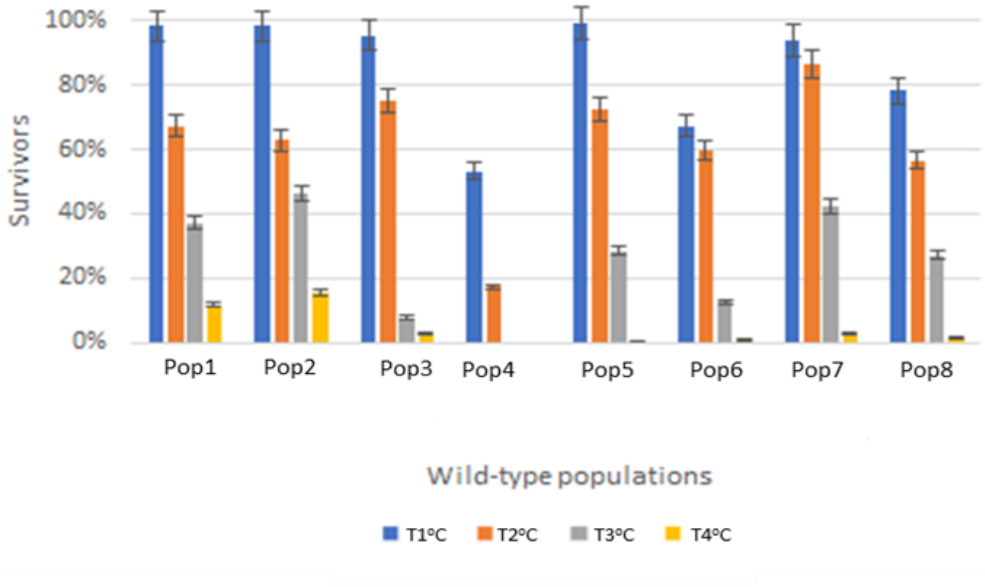

FIG. 13. Thermal exposure of different Aedes aegypti populations

Identification of the Male Determining Factor in Major Tephritid Pest Species and its Importance for SIT applications

Several studies have shown that one of the most important steps in SIT applications against insect pests and disease vectors is sex separation since the active component is the sterile males. Efficient and cost-effective sex separation can be achieved using genetic sexing strains, the development of which requires the linkage of the wild type (rescue) allele of a selectable marker (morphological or conditional lethal) to the male determining region, which in tephritids resides on the Y chromosome.

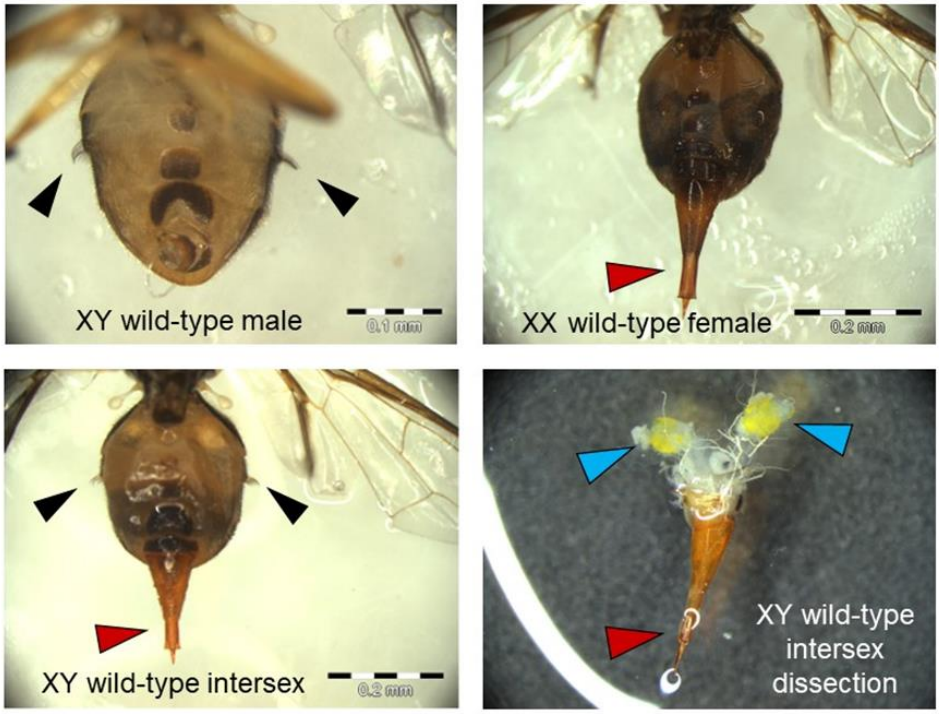

FIG. 14. Bactrocera dorsalis *male (upper left) and female (upper right) wild-type abdomens and abdomens of intersex XY flies (down left), after BdMoY RNAi silencing. XY intersexes display female-specific characteristics (ovipositor, red arrowhead) and male-specific characteristics (abdominal lateral bristles, black arrowheads). Dissection of the XY intersex abdomen (down right) reveals the presence of male-specific testes (blue arrowheads).*

In a large collaborative study, mainly between the University of Naples "Federico II", the University of Zurich, the University of Perugia, the Hebrew University of Jerusalem and the IPCL, it was shown that the gene maleness-on-the-Y (MoY) encodes for the male determining factor in tephritids. In experiments performed in the IPCL, the functional analysis of MoY in *Bactrocera oleae* and *Bactrocera dorsalis* was carried out by embryonic RNA interference. Double-stranded RNA (dsRNA) targeting of the *B. oleae* MoY (BoMoY) or *B. dorsalis* (BdMoY) resulted to the RNA silencing of MoY thus resulting to the feminization of adults. These intersexes were molecularly karyotyped as XY but displayed female-specific (ovipositor) and male-specific characteristics (abdominal lateral bristles) at the same time (Fig. 14 for *B. dorsalis* and Fig. 15 for *B. oleae*).

FIG. 15. Bactrocera oleae *male (left) and female (middle) wild-type abdomens and abdomens of intersex XY flies (right), after BoMoY RNAi silencing. XY intersexes display female-specific characteristics (ovipositor, red arrowhead) and male-specific characteristics (abdominal lateral bristles, black arrowheads).*

CAPACITY BUILDING AND SERVICES

In 2019, the IPCL hosted 6 cost-free experts (CFE) and 5 consultants (C) (of which 6 were PhD students), 11 interns, 13 fellows (F) and 7 scientific visitors (SV). 13 fellows and 4 scientific visitors were funded by the IAEA'S Department of Technical Cooperation.

Name	Country	Status	Duration	Topic
SASSU, Fabiana	Italy	CFE (PhD)	6 mth	Radiation biology and rearing of *Drosophila suzukii*
Alexandre Araujo	Brazil	Intern	11 mth	Phytosanitary treatments
ESPINAL PEREZ, Tania	Mexico	Intern	1 mth	Characterising GSS of fruit flies
NOLASCO GOMEZ, Abner	Mexico	Intern	12 mth	Characterising GSS of fruit flies
ALVAREZ, Mario	Mexico	Intern	11 mth	Characterising GSS of fruit flies
MARTINEZ BARRERA, Olga	Colombia	Intern	5 mth	Phytosanitary treatments
CARAVANTES, Luis	Guatemala	Intern	11 mth	Phytosanitary treatments
VARGAS HURTADO, Nick	Mexico	Intern	2 mth	Phytosanitary treatments
WANG, Jingjing	China	Intern	3 mth	Fruit fly rearing and quality control
LIANG, Yan	China	Intern	9 mth	Fruit fly rearing and quality control
HALLMAN, Guy	USA	C	2 weeks	Phytosanitary treatments
SIMOES DIAS DE CASTRO, Vanessa	Brazil	C	12 mth	Phytosanitary treatments
LE, Thi Xuyen	Vietnam	F	4 mth	Fruit fly rearing and quality control
GUAZELLI DELLA GIUSTINE, Paloma	Brazil	F	10 mth	Fruit fly rearing and quality control
TIENDRBEOGO, Antoine	Burkina Faso	F	2 mth	Fruit fly rearing and quality control
LAHUATTE, Paola	Ecuador	F	1 mth	Fruit fly rearing and quality control
MAZIH, Ahmed	Morocco	SV	1 wk	Fruit fly rearing and quality control
VALERIO, Federica	Italy	SV	1 wk	Rearing of olive fruit flies
DEMIRBAS, Gueler	Turkey	C	3 mth	Tsetse symbionts and pathogens
MIRIERI, Caroline	Kenya	C (PhD)	12 mth	Impact of stress factor on tsetse flies

Name	Country	Status	Duration	Topic
DIENG, Mouhamadou	Senegal	C (PhD)	12 mth	Tsetse symbionts and refractoriness to trypanosome infection
REZAPANAH, Mohammadreza	Iran	CFE	4 mth	Tsetse pathogens
TANG, Zhaoyang	China	Intern	9 mth	Detection of mosquito-borne viruses in mosquito colonies
CANIC, Sumejja	Bosnia & Herzegovina	Intern	9 mth	Impact of NIRPSS for tsetse pupae
SAWDETUO HIEN, Artisde	Burkina Faso	F	3 mth	Detection of mosquito-borne viruses in mosquito colonies
KISWENDA-SIDA DERA, Mikhailou	Burkina Faso	F	5 mth	Analysis of tsetse population genetics
MOLEFE Moyaba, Percy	South Africa	F	3 mth	Tsetse rearing
OUEDRAOGO, Gisele	Burkina Faso	F	1 mth	Detection of tsetse symbionts
DE BEER, Chantel	South Africa	SV	1 wk	Training on use of NIRPSS
NIKOLOULI, Katerina	Greece	CFE (PhD)	7 mth	*Drosophila suzukii* SIT/IIT
GOUVI, Georgia	Greece	C (PhD)	8 mth	Cytogenetics in tephritid species
SOLLAZZO, Germano	Italy	CFE (PhD)	3 mth	Temperature-sensitive lethal genes in tephritid species
GREGORIOU, Maria-Eleni	Greece	CFE	1 wk	Development of sterile male olive fruit fly
TSOUMANI, Konstantina	Greece	CFE	1 wk	Development of sterile male olive fruit fly
MORAN ACEVES, Brenda	Mexico	F	7 mth	Mosquito GSS
PUDAR, Dubravka	Serbia	F	3 mth	Compatibility studies for *Aedes albopictus* (multiple strains)
GARCIA ALBA, Marianela	Argentina	F	1 mth	Compatibility/competitiveness of *Aedes aegypti* (multiple strains)
BAKHOUM, Thierno	Senegal	F	12 mth	Handling, packing and transport of *Aedes* sterile males
NTOYI, Nonhlanhla	South Africa	F	7 mth	Assesment, management and maintenance of *Anopheles arabiensis* sexing strains

Name	Country	Status	Duration	Topic
GATO ARMAS, Rene	Cuba	SV	1 wk	New developments in mosquito SIT package
MAGALLON, Jose	Panama	SV	1 wk	Irradiation procedures for mosquitoes
LU, Deng	Singapore	SV	1 wk	Drone release system
LUTRAT, Celia	France	SV	1 wk	Development of mosquito sex sorting systems

In 2019, the Genetics and Molecular Biology (GMB) group maintained 13 species of fruit flies (140 strains/colonies/populations) and 3 species of mosquitoes (69 strains/colonies/populations in total). The Plant Pests (PP) group maintained 14 species of fruit flies (65 strains/colonies/populations), the Livestock Pests (LP) group maintained 7 tsetse species (11 strains) and the Human Disease Vectors (HDV) group maintained 3 mosquito species (8 strains).

The GMB and PP groups carried out 70 fruit fly shipments to 26 institutions in 18 countries (in UK, India, Spain, Argentina, Greece, Italy, Kenya, France, Pakistan, USA, Senegal, Colombia, Mauritius, Germany, Mexico, Tunisia, Czech Republic and Sweden), and 9 shipments of preserved fruit flies to 6 institutions (in Italy, USA, South Africa, Brazil and Belgium.). The LP group carried out 139 tsetse shipments of 270,407 pupae (239,744 *G. palpalis gambiensis* to Senegal) to 8 institutions in 6 countries (Senegal, France, Zimbabwe, South Africa, Belgium and USA The HDV group carried out 10 mosquito shipments to 10 institutions (in South Africa, Ecuador, France, Italy, Serbia, Germany and Cameroon).

In 2019, the IPCL received 313 visitors from 44 countries.

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THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

Mutation breeding has enabled the development of superior crops with higher yields, tolerance to plant pests and diseases and to abiotic stresses such as drought, salinity and heat, and has a track record of safety for food, feed and the environment. Crop mutation breeding using nuclear techniques is well positioned to help address current and future crop improvement challenges to enhance food security, increase crop resilience and improve adaptation to climate change.

Research at the Plant Breeding and Genetics Laboratory (PBGL) on transboundary pests and disease resistance continued in 2019 with focus on the parasitic weed Striga in upland rice and sorghum, the Fusarium Wilt Tropical Race 4 (TR4) disease in banana, and leaf rust in arabica coffee. These plant pests and diseases cause drastic yield losses globally, affecting the livelihood and food security of small-scale farmers and, in case of banana TR4, also agro-industries. The economic losses attributed to the parasitic weed *Striga* in Sub Saharan Africa amount to 12 billion US $ annually. Similarly, coffee leaf rust has created recent epidemics in Central and Southern America. Fusarium Wilt TR4 is now threatening Cavendish banana production globally after this disease spread to Latin America in 2019 after ravaging banana plantations in Asia and Africa.

R&D on the development/adaptation and validation of glasshouse and laboratory screening protocols for resistance to *Striga* continued under the *Striga* CRP (D25005). A total of 11 and 23 *Striga* resistant advanced mutant lines were confirmed in rice and sorghum, respectively. Laboratory-based gel and rhizotron assays were developed to investigate the *Striga* resistance mechanism in sorghum. From an initial analysis of 10 mutants, four distinct resistance mechanisms could be identified. These results offer realistic perspectives to introduce novel and durable resistance to *Striga* in sorghum through the pyramiding of different *Striga* resistance traits using Marker-Assisted Selection (MAS).

Under the coffee and banana disease CRP (D22005), a bioassay for verification and screening to Fusarium Wilt TR4 resistance in banana is being established. Several parameters were evaluated, including a method and concentration of the TR4 inoculum and symptom development. The method is based on a soil/millet seed inoculum mixture, is conducted in an incubator for containment and uses trays with 24 plants for increased throughput. Susceptibility to the Fusarium wilt TR4 could be assayed in 3-month old Cavendish banana as an inverted leaf senescence phenotype correlated with grades of browning of vascular tissue when inspected through sectioning. This protocol will next be validated using known TR4-resistant banana varieties and though the verification of candidate TR4 resistant mutant lines developed under CRP D22005.

Work on the establishment of speed breeding techniques to accelerate the mutation breeding process also continued in 2019. In vitro single cell regeneration techniques are particularly important for trees or perennial crops with a long reproductive cycle such as coffee. In 2019, an efficient method for in vitro single-cell regeneration of arabica coffee was adapted at the PBGL. The method involves somatic embryogenesis and regeneration of in vitro plantlets from leaf tissues. The method can now be combined with mutation induction techniques to accelerate mutation breeding process and avoid the production of chimeric plants, typically observed when using conventional propagules such as cuttings, seedlings or seed as source explant for mutagenesis.

To support mutant trait discovery using Next Generation Sequencing (NGS) technologies, the PBGL is establishing laboratory protocols and user-friendly NGS data analysis workflows. Identifying informative sequence variants from NGS datasets is a time-consuming step in the process of NGS-based genetic mapping and often represents a challenge for non-expert users. To facilitate NGS data analysis, publicly available software were chained together in two different variant caller pipelines; one uses the *GATK suite* of tools and the other *freebayes*. Both pipelines can be run in the cloud and are ready for use by Member States. In addition, a tool was developed to visualize mutation-induced

genome-wide sequence variations at the individual plant level which plant breeders can apply for background selection.

In 2019, the PGBL finalized the protocol book '*X-ray irradiation for mutation induction in crop plants'* for submission to the FAO, for publication. A protocol for barley genotyping based on allele-specific amplification '*A Diagnostic Marker Kit and Protocol for Marker-Assisted Selection of Orange Lemma (rob1.a) for Forage Barley Improvement'* was submitted for publication on the IAEA website as an output under the barley CRP D23030. The protocol includes a marker kit which continues to be successfully used for Marker Assisted Selection in the forage barley breeding program at BOKU, Austria. In 2019, the protocol was transferred to Kuwait transfer of the Orange Lemma trait to locally adapted varieties for animal feed. A laboratory protocol on '*Library Preparation for Medium- to High-throughput DNA Sequencing on the Illumina Sequencing Platform'* has been submitted for publication on the IAEA website. This step-by-step protocol allows cost-effective generation of sequencing libraries for large numbers of plants.

In 2019, the PBGL hosted 6 interns, 15 fellows and 10 scientific visitors for training in basic and advanced nuclear techniques for mutation induction, mutant population development and efficiency enhancing biotechnologies. The PBGL conducted one Regional Training Course on 'Mutation Breeding and Efficiency Enhancing Techniques for Crop Improvement' attended by 24 participants from 14 different Member States. The PBGL provided irradiation services for a total of 212 samples from 42 different plant species on request from 31 Member States, and these were delivered in 39 shipments. These included seven new plant species, confirming continued high demand by Member States to diversify crops using nuclear technologies. In 2019, the PBGL has published two success stories on outstanding achievements in mutation breeding for resistance to *Striga* in sorghum and Fusarium Wilt TR4 in banana.

STAFF

Name	Title
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Ali, Adel	Laboratory Technician
Morales, Laura[3]	Laboratory Technician
Islam, Tazirul[4]	Field/Greenhouse Worker
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A.5. [1]joined in February; [2]return to PBGL from Developmental Reassignment in June 2019; [3]February to May; [4]separated in September; [5] July to October; [6]Joined in April; [7]joined in May; [8]joined in October; [9]separated in February; [10]separated in May; [11]1-15 December (home-based).

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Validated glasshouse and laboratory protocols for confirmation of Striga-resistant cereal mutants and identification of underlying resistance mechanism

The parasitic weeds *Striga asiatica* and *Striga hermonthica* are major biological constraints to cereal production in most of Sub-Saharan Africa and semi-arid tropical regions of Asia. In sub-Saharan Africa, annual losses in cereals due to *Striga* infestation is estimated at 12 billion US Dollars. *Striga* is particularly challenging because it infects the roots of its host plant and remains invisible until the time when it emerges from the soil. By that time, the damage to the host plant is already inflicted. Because the damage occurs underground, conventional weed control measures cannot be applied.

The main objective of the CRP D25005 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security' is to develop laboratory, screenhouse and field screening protocols of mutant populations of sorghum and upland rice for resistance to *Striga asiatica* and *Striga hermonthica*. In addition, the CRP focuses on technologies such as rapid cycling of generation of crop plants, doubled haploid techniques and molecular markers to enhance the efficiency of mutant identification and accelerate delivery of resistant varieties.

In 2019, the PBGL continued the R&D activities on validation of developed/adapted glasshouse and laboratory screening. A pot-screening protocol was used to verify 30 and 36 putative Striga resistant rice mutants from Burkina Faso and Sudan, respectively. The putative mutants from Sudan were in M3 generations while those from Burkina Faso were in advanced M4/M5 generation. Seeds were planted in medium-size pots filled with rice clay soil and sand in 2:1 ratio with pH = 6.5. Each putative mutant was tested in a set of four pots with uniformly mixed seeds of *Striga hermonthica* and four *Striga* seed-free pots. Wild parents and known positive *Striga* resistant checks were included. Plants were maintained in a glasshouse under 25°C and natural light during June-November 2019 at the PBGL facility in Seibersdorf. Seedlings were irrigated 2-3 times a week until establishment and then continued with 1-2 watering per week or as needed. No fertilizer was applied and *Striga* plants started to emerge above the soil across the experiment in about two months. After about three months the damage due to *Striga* was scored on the pots containing *Striga* seeds as compared with the control (no *Striga* seeds). The number of *Striga* plants per pot, rice plant height and % damage (burned leaves/total leaves) were scored, and the tolerance/resistance index was calculated based on % reduction in growth due to *Striga* infection. There were significant differences in the damage due to *Striga* infection and the mutant lines were classified into susceptible, with all plants in the four pots damaged and $\leq$ 50% tolerance index, resistant with few or no *Striga* infection (no *Striga* plants) and tolerance index above 90%. In total, 4 and 7 resistant, and 2 and 3 tolerant mutants were confirmed from Sudan and Burkina Faso putative rice mutants, respectively. The lines with confirmed resistance will be further advanced to study the mechanisms of resistance and make intercrosses to test allelism and produce mapping populations to enable development of molecular markers for the verified resistance.

In the continued verification of sorghum putative mutants an additional three resistant mutants were confirmed from Burkina Faso and 10 mutants from Sudan to make a total of 23 *Striga* resistant sorghum mutants in seven farmer-preferred sorghum varieties from Burkina Faso and Sudan.

Laboratory protocols were optimized using gel-assay and rhizotron to enable the co-culture of *Striga* seeds and seedlings of resistant mutants compared to wild parents. Cleaned *Striga* seeds were settled in solidifying agar (0.8%) in plastic petri dishes. Germinated plants were placed in the center of the solidified agar and maintained in an incubator for two weeks under 28-30°C and 16/8 hours light/dark condition. After two weeks, the distance between the furthest germinated *Striga* seeds and the root was scored. The principle is that the higher the germination stimulant produced by the host-plant root, the longer the distance to *Striga* seeds stimulated to germinate by the root. Rhizotron as an alternative medium was established to provide more stable conditions for extended co-culturing of *Striga* with

host seedling in petri-dishes filled with either rockwool or fine sand. In the rhizotron, mechanisms of *Striga*-host interaction after germination can be observed and scored such as development of haustorium and attachment to the root and further development to complete *Striga* plants. Rhizotron can support Striga-host interaction for more than one month under favorable *Striga* germination conditions (28-30°C).

From the initial analysis of 10 sorghum mutants using gel-assay and rhizotron, four possible mechanisms of resistance could be differentiated. These are: (1) low germination stimulant producer; (2) inhibition of haustorium development; (3) failure of attachment (Figure 1); and (4) reduced growth of *Striga* . This indicates the great potential of pyramiding induced resistance to produce more durable resistance combining different mechanisms. Development of mapping populations is underway whereby the best three mutants with different mechanism from each country were crossed with their wild parents to develop F2 populations.

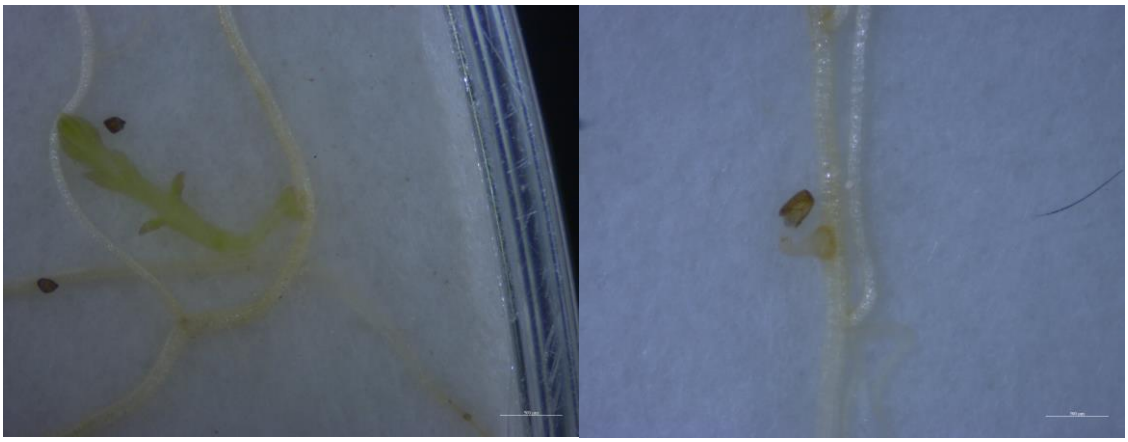

Fig. 1. Example of Rhizotron protocol showing a Striga resistant mutant (right) with germinated Striga that failed to attach to the root compared to successful attachment and growth of Striga plant (left) on wild, susceptible sorghum host plant.

Developing a Fusarium Wilt TR4 bioassay for verification and screening for TR4 resistance in Cavendish banana

The CRP D22005 on 'Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana' is focused on mutation breeding approaches to improve banana and coffee for resistance to Fusarium Wilt Tropical Race 4 (TR4) and Leaf Rust, respectively. Fusarium wilt in banana and coffee leaf rust are caused by fungal pathogens which have devastating effects on coffee and banana production in the affected countries. Coffee leaf rust has created recent epidemics in several countries in Central and Southern America while Fusarium Wilt TR4 is threatening Cavendish banana production globally.

Work on the optimization of screening/phenotyping protocol for resistance to TR4 in banana continued in 2019. *Fusarium oxysporum* TR4 is a soil-borne pathogen that infects banana roots causing obstruction of xylem vessels which results in leaf wilting and, ultimately, necrosis and plant death. An in vivo bioassay was adapted to PBGL conditions using a TR4 susceptible Cavendish banana under contained, environment-controlled conditions. The bioassay is carried out in trays containing 24 plants each for increased throughput. Two methods were used to measure TR4 symptom development: (1) a non-destructive Leaf Symptom Index (LSI) based on visual screening of leaf wilting; (2) a destructive Rhizome Discoloration Index (RDI) which assays discoloration of the inner rhizome. For these experiments TR4 susceptible banana control variety is first multiplied in vitro and hardened using previously established procedures. TR4 symptom establishment requires successful infection of

banana by the fungal pathogen and phenotypic expression of TR4 symptoms in the banana plants. Experiments were conducted to optimize the production of the TR4 inoculum, to compare two inoculation methods (liquid culture versus an inoculum in millet seed as medium) and to determine the optimal TR4 concentration for symptom development. The millet seed inoculation method proved more robust compared to inoculation using liquid cultures. The entire process from in vitro multiplication to scoring of the symptoms takes approximately 5 months. Results for millet seed inoculation method followed by LSI and RDI scoring are summarized in Figure 2. As shown in Figure 2, leaf wilting typical for TR4 infection can be visually scored and differentiated from natural leaf senescence in 3-month-old banana plants using 12 g millet seed inoculum. RDI scoring can be performed to confirm visually-screened leaf wilting as shown also in Figure 2. This optimized protocol will be validated on known TR4 resistant cultivars and through verification of putative TR4 resistant banana mutants generated under CRP D2.20.05.

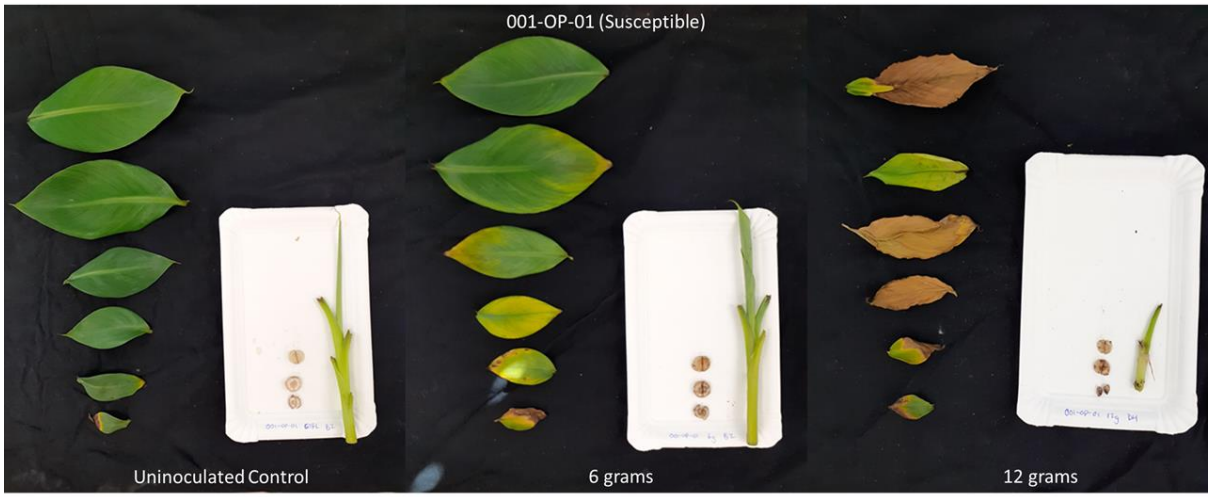

Fig. 2. Leaf symptom development of a control, TR4 susceptible non-inoculated banana plant (left) as compared to plants inoculated with 6 g (middle) and 12 g (right) millet seed inoculum. Both LSI and RDI scoring methods for the evaluation of TR4 induced symptoms in the leaves and rhizome respectively, are shown.

In vitro regeneration protocol for *Coffea arabica leaf explants*

Today, many plant species can be regenerated from individual somatic cells using in vitro tissue culture techniques. Combined with efficient mutation induction techniques, these technologies can provide solutions to avoid or reduce the presence of chimeric tissues which are produced when multicellullar tissues such as stem cuttings, seed or in vitro shoot tips are used as source explants for mutation induction. In vitro plant tissue technologies thus offer potential to shorten the mutation breeding cycle of crops such as coffee and banana as well as new opportunities for lab-based selection schemes.

In 2019, work on establishing protocols for the regeneration of coffee plants from single cells continued at the PBGL. Using somatic embryogenesis, coffee plantlets could be regenerated from leaf discs. The protocol describes the various steps from inducing embryogenic callus, through maturation and rooting to convert the somatic embryos into in vitro plantlets, as illustrated in Figure 3. So far, the protocol has been applied to three different commercial *C. arabica* varieties: Venecia, Caturra and Catuai. In a next phase, the protocol will be combined with mutagenesis techniques to determine optimal conditions for mutation induction.

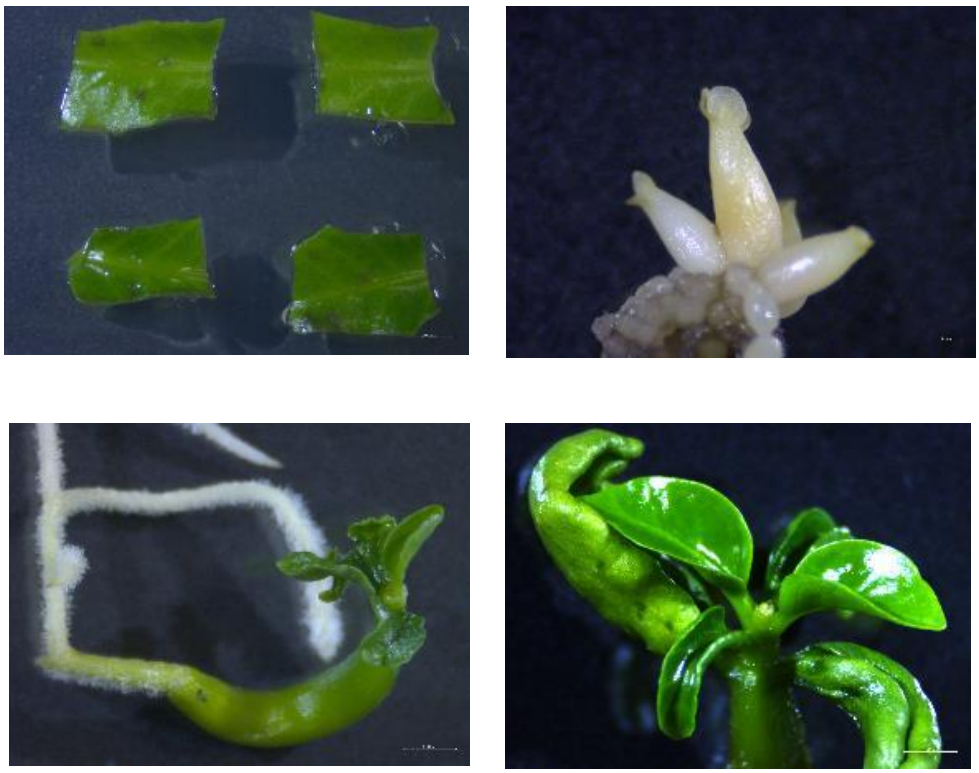

Fig. 3. Regeneration of coffee using in vitro somatic embryogenesis from leaves: leaf discs (top left); immature somatic embryos (top right); mature somatic embryo with root (bottom left); and, coffee plantlet (bottom right).

Laboratory protocol and bioinformatic tools for accelerated genetic gain

Connecting the mutant traits to their underlying induced DNA sequence variants has the potential to accelerate the breeding process by shortening the breeding cycle and enabling more precise selection. Once a causative or closely linked DNA variant for a mutant trait is identified, it can be converted into a genotyping assay for use as molecular markers for MAS applications, as shown in PBGL's results on the barley orange lemma (OL) digesitibility phenotype.

Today, Next Generation Sequencing (NGS) brings unprecedented genome-wide resolution to the plant breeder. During 2018 and 2019, PBGL has worked towards laboratory protocols and bioinformatics tools enabling mutation discovery using advanced DNA sequencing techniques.

In 2019 the laboratory protocol for the preparation of libraries for Medium- to High-throughput DNA Sequencing on the Illumina Sequencing Platform was submitted for publication on the IAEA website. This step-by-step protocol allows to cost-effectively generate sequencing libraries for large numbers of individuals and can be conducted in a standard molecular biology laboratory using standard equipment and commercially available reagents. It yields a pool of up to 864 ready-to-sequence Illumina libraries, where each sample is individually barcoded.

One way of finding the causal locus is to inspect genome-wide allele frequency plots of bulks of individuals with contrasting phenotypes. This constitutes a so-called bulk-segregant analysis. Having

the individuals sequenced separately allows for analysis in bulks as well as to inspect each individual separately. An R-module was developed to visualize wild type and mutant alleles across the genome of individual plants. Representing the sequencing data on an individual plant basis enables background selection by breeders, i.e. to select the exact individual plants that contain only the desired mutant alleles against other DNA from the mutant plant. For example, Figure 4 visualises the genomes of 30 Sorghum plants from an F2 population along with their 2 parents. Each F2 plant is a unique mosaic of the parental genomes: mutant and wildtype, yellow and blue. In this illustration, the plants are grouped by a segregating phenotype, namely, semi-dwarf and early maturing, which reveals potential co-segregation of mutations on Chromosome 4.

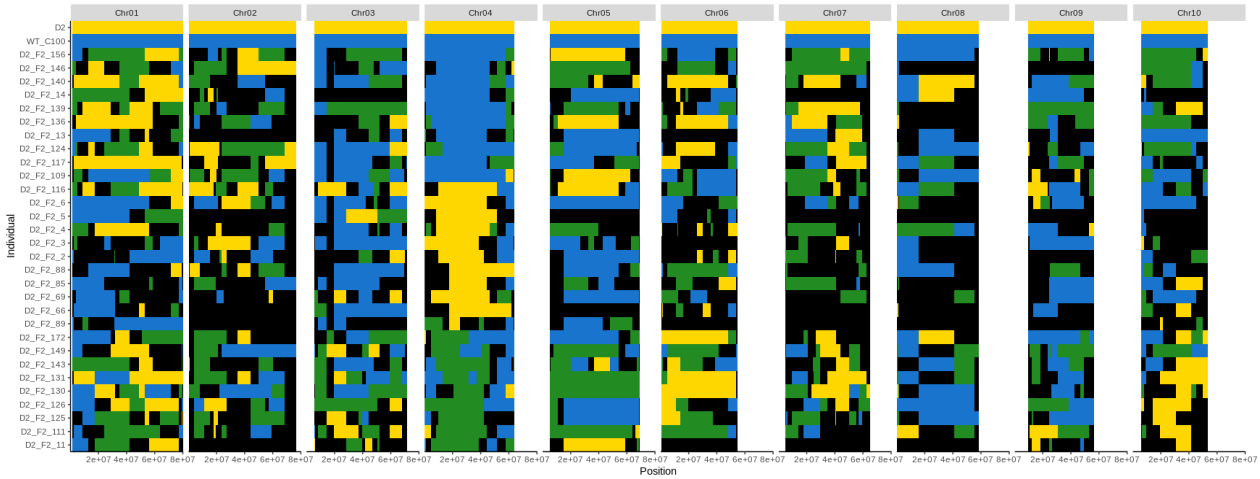

Figure 4: Example genome visualisation with vcfviewer, an R-module developed by PBGL. Depicted is whole genome sequencing data of 30 Sorghum plants from a segregating population (F2). The population is derived from a backcross of one selected mutant to its (un-mutagenised) progenitor. Columns are the positions along the 10 Chromosomes, and lines represent individuals. Mutant alleles are coloured yellow, wildtype in blue, green depicts evidence for heterozygosity at that locus, and black is missing data. The top two individuals are the parents of the cross, induced mutant (all yellow) and progenitor (wt, all blue).

PBGL also established DNA sequence data analysis workflows to extract relevant information from NGS sequence data. This is a resource-intensive step in the process of NGS-based mutation discovery. Using *snakemake* (https://snakemake.readthedocs.io), two variant calling pipelines have been developed that chain together publicly available software; one uses the *GATK suite* of tools and another *freebayes*. Both pipelines scale easily from server, cluster, grid to cloud environments and are ready for use by interested Member States. Since they can be run in the cloud, lack of suitable hardware should not be a concern.

TECHNOLOGY TRANSFER, CAPACITY BUILDING & SERVICES

A protocol and marker kit for forage barley breeding in Austria and Kuwait

In 2019, a protocol for low-cost genotyping using allele-specific amplification was submitted for online publication on the IAEA website as an output under the barley CRP D23030. The protocol describes a step-by-step procedure for genotyping of the Orange Lemma (OL) mutant allele derived from the barley genetic stock BW666. The protocol includes a marker kit with three components: the actual experimental procedure, BW666 seed as a source of the OL trait, and the primers required for conducting the genotyping assay. The marker kit continues to be used successfully for MAS in the

forage barley breeding program of BOKU, Austria and was also transferred through a fellowship to KISR, Kuwait for forage barley breeding adapted to the saline soil and irrigation conditions of Kuwait.

Regional Training Course on Mutation Breeding and Efficiency Enhancing Techniques for Crop Improvement, RAS5079, Seibersdorf, Austria, 14–24 October 2019

A nine-day training course was organized on 'Mutation Breeding Efficiency Enhancing Techniques for Crop improvement' under the Regional TC Project RAS5079 "Improving crops resilience to climate change through mutation breeding in Pacific Islands". Though organized primarily RAS5079, it developed into an inter-regional course due to demand and was delivered to a total of 23 participants from Member States globally: 11 participants from the Pacific Islands (Fiji, Marshall Islands, Palau, Papua New Guinea and Vanuatu), one fellow from Iran and an additional 11 Scientific Visitors from seven different countries (Brazzaville, Democratic Republic of the Congo, Indonesia, Namibia, Oman, Nicaragua, Palestine, and Sudan). The course covered basic and more advanced aspects of crop mutation breeding using case studies on marker-assisted selection and on the use of NGS for genetic mapping and related bioinformatics tools. In addition to the PBGL staff providing lectures and practical sessions, two external resource persons, namely, Prof R. Swennen, banana breeder from the International Institute of Tropical Agriculture, Tanzania/KUL, Belgium, and Prof S. Nishimura, Nara Institute of Science and Technology, Japan, shared their expertise in banana breeding and in the use of advanced DNA sequencing techniques for genetic mapping, respectively.

Fellowships, Scientific Visitors and Interns

In 2019, the PBGL hosted 15 fellows (F), 10 scientific visitors (SV), six interns (I) and one cost-free expert (CFE) (the fellows and scientific visitors were funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Topic	Duration
Ms Luz GOMEZ PANDO	Peru	SV	Mutation breeding in Peruvian highlands	2 days
Mr Aimé NDOFUNSU	DR Congo	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Abdallah ALIMARI	Palestine	SV	Mutation breeding and efficiency enhancing tools	10 days
Ms Yuliasti YULIASTI	Indonesia	SV	Mutation breeding and efficiency enhancing tools	10 days
Ms Lilik HARSANTI	Indonesia	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Luther CASCO HERRERA	Nicaragua	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Pio VALLECILLO RETES	Nicaragua	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Gerhard HAITEMBU	Namibia	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Kelvin KAMFWA	Zambia	SV	Mutation breeding and efficiency enhancing tools	10 days

Mr Elgailani ABDALLA	Sudan	SV	Mutation breeding and efficiency enhancing tools	10 days
Ms Wadhha AL-GHAFRI	Oman	SV	Mutation breeding and efficiency enhancing tools	10 days
Mr Sidi MENOUM	Mauritania	SV	Mutation breeding and efficiency enhancing tools	2 days
Mr H. Jhonny RABEFIRAISANA	Madagascar	F	*Striga* screening protocol, marker development	3 months
Mr Sadate AMADOU	Togo	F	Mutation induction; population development and screening; efficiency enhancing technologies	3 months
Mr Modeste PALANGA	Togo	F	Mutation induction; population development and screening; efficiency enhancing technologies	3 months
Mr Phillipe NIKIEMA	Burkina Faso	F	Mutant population development *Striga; Striga* screening protocol	6 months
Mr Solomon OTU	Ghana	F	Marker-assisted selection in barley; *in vitro* haploid rice	3 months
Mr Bawoumodom BODJONA	Togo	F	Marker-assisted selection in barley; radio-sensitivity testing cowpea	3 months
Mr Clement ANNOR	Ghana	F	Radio-sensitivity testing taro; marker-assisted selection in barley	4 months
Mr N'pagyendou LARE	Togo	F	Radio-sensitivity testing taro; marker-assisted selection in barley	3 months
Jehad RADWAN	Palestine	F	Mutation induction; population development and screening; efficiency enhancing technologies	4 months
Mr Thadey TAIRO	Tanzania	F	Mutation induction; population development and screening; efficiency enhancing technologies	5 months
Mr James ALPHONACE	Tanzania	F	Mutation induction; population development and screening; efficiency enhancing technologies	3 months
Mr Semi CAKAUNITAVUKI	Fiji	F	Radio-sensitivity testing and bulk irradiation sweet potato and yam	4 months
Mr Winda PUSPITASARI	Indonesia	CFE	Mutation induction; marker development in sorghum	3 months
Ms Eunice TEMU	Tanzania	F	Mutation induction; population development and screening;	4 months
Ms Habibah AL-MENAI	Kuwait	F	Marker-assisted selection in barley	2 months
Mr Edwin THEKKINEN	Austria	I	Molecular marker-development in barley; sesame phenotyping	2 months
Ms Samira TAJEDINI	Iran	I	Haploid in rice and sorghum; mutant population development *Striga*	5 months

Ms Yuling YUE	China	I	Drought/*Striga* screening protocols; marker development in rice and sorghum	8 months
Ms Li ZHU	China	I	Drought/*Striga* screening protocols; marker development in rice and sorghum	7 months
Mr Marcos CONDE	Spain	I	Computational analysis workflow in Snakemake	3 months
Mr Anza GHAFFAR	Pakistan	I	Allele visualisation tools using R	3 months

Crop Irradiation Services

In 2019, the PBGL received and delivered requests for irradiation of 212 accessions/varieties across 42 different plant species from 31 Member States in 39 requests . These included requests for seven new plant species. Requests were received in the context of CRPs or Technical Cooperation (TC) projects while the remaining requests were from stakeholder institutions from Member States, as summarized in the table below. The total number of irradiation requests delivered by PBGL stands at 1622 at this time.

Request No	Country	Request type	Plant
1583	Hungary		ornamental
1584	United Arab Emirates	TC	quinoa
1585	Togo	TC	maize
1586	Netherlands		ornamental
1587	Zimbabwe	TC	cowpea, sorghum
1588	Cameroon	TC	watermelon, ochra, cowpea, maize
1589	Burkina Faso	TC	cowpea
1590	DR Congo	TC	maize
1591	Netherlands		ornamental, tomato
1592	PBGL		arabidopsis
1593	Nigeria	TC	Discorea rotundata
1594	Mongolia	CRP	pea, soybean
1595	Austria		maize
1596	Mali	TC	rice
1597	USA		Capsicum annuum
1598	Malawi	TC	maize, groundnut

Request No	Country	Request type	Plant
1599	Fiji	TC	Capsicum annuum
1600	Namibia	TC	pearl millet, cowpea
1601	Ghana	CRP	taro
1602	Kenya	TC	Brachiaria, Dolichos, Mucuna pruriens
1603	Sudan		sesame, peanut, cowpea
1604	Senegal		cowpea
1605	Burkina Faso	TC	maize
1606	Germany		ornamental
1607	Mali	CRP	sorghum
1608	Fiji	TC	rice
1609	Germany		ornamental
1610	Oman	TC	date palm, lime, wheat
1611	Fiji	TC	sweet potato, yam, breadfruit
1612	PBGL		banana
1613	Namibia		bambara groundnut
1614	Germany		ornamental
1615	Ukraine		wheat
1616	Palestine	TC	cucumber, zucchini
1617	Namibia	TC	cowpea, maize
1618	Spain	TC & CRP	lentil
1619	Tanzania	TC & CRP	rice
1620	Slovenia		wheat, millet, sorghum, buckwheat, tomato, Thinopyrum intermedium
1621	Kuwait	TC	barley, Rhanterium epapposum, Calligonum polygonoides, Penisetum divisum, Farsetia aegyptia, Panicum turgidum
1622	UK		hosta

INFORMATION DISSEMINATION

PBGL success stories in 2019

In 2019, two success stories were published by the PBGL ahead of the 2020 International Year of Plant Health highlighting the successful contribution of crop mutation breeding for resistance to the parasitic weed *Striga* in cereals and the Fusarium wilt TR4 fungal disease in banana:

IAEA, FAO Help Develop Bananas Resistant to Major Fungal Disease
https://www.iaea.org/newscenter/news/iaea-fao-help-develop-bananas-resistant-to-major-fungal-disease

Nuclear Techniques Help Develop New Sorghum Lines Resistant to the Parasitic Weed *Striga*

https://www.iaea.org/newscenter/news/nuclear-techniques-help-develop-new-sorghum-lines-resistant-to-the-parasitic-weed-striga

In addition, two success stories were published by the Plant Breeding and Genetics Subprogramme highlighting the contribution of barley mutation breeding for Kuwait. The PBGL has transferred the protocol for marker assisted selection for forage barley to KISR, Kuwait:

Barley in the Desert: Kuwait Progresses in the Development of a New Variety Using Nuclear Techniques.

https://www.iaea.org/newscenter/multimedia/photoessays/barley-in-the-desert-kuwait-progresses-in-the-development-of-a-new-variety-using-nuclear-techniques

Nuclear Technology Helps Develop New Barley Variety in Kuwait
https://www.iaea.org/newscenter/news/nuclear-technology-helps-develop-new-barley-variety-in-kuwait

Using Nuclear Science in Marker-Assisted Plant Breeding – a new animated infographic by the Joint FAO/IAEA Division

A new animated infographic on 'Using Nuclear Science in Marker-Assisted Plant Breeding' explains how advanced DNA sequencing can be used for mutant trait discovery and, subsequently, for wide dissemination of useful trait through marker-assisted breeding. This video, intended for a lay audience, can be found at: https://www.iaea.org/newscenter/multimedia/videos/using-nuclear-science-in-marker-assisted-plant-breeding

PUBLICATIONS

Ingelbrecht IL, E Akgun, M Matijevic, B Hofinger, L Jarc, JB Jankowicz-Cieslak, A Ali, L Jankuloski, H Grausgruber, AMA Ghanim. A Diagnostic Marker Kit and Protocol for Marker-Assisted Selection of Orange Lemma (*rob1.a*) for Forage Barley Improvement. Submitted for online publication on the IAEA website.

Jankowicz-Cieslak JB, F Gössnitzer, S Datta, A Viljoen, I Ingelbrecht, BJ Till (2020). Induced mutations for generating bananas resistant to Fusarium Wilt Tropical Race 4. Accepted for publication in Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. (Sivasankar et al. eds). Section 4.

Oberhofer M, J Hess, M Leutgeb, F Gössnitzer, T Rattei, C Wawrosch SB Zotchev (2019). Exploring Actinobacteria Associated With Rhizosphere and Endosphere of the Native Alpine Medicinal Plant Leontopodium nivale Subspecies alpinum. Frontiers in Microbiology 10: 2531

Rabefiraisana HJ, AMA Ghanim, Alice AA Ali, B Rasoamampionona, L Jankuloski, I Ingelbrecht, NV Rakotoarisoa. Assessing the reaction of advanced *Striga asiatica*-resistant mutants (M5) of rice (*Oryza sativa*) to *Striga hermonthica. Abstract presented at the WCPP Amsterdam June 2019.*

Tramontano A, L Jarc, J Jankowicz-Cieslak, BJ Hofinger, K Gajek, M Szurman-Zubrzycka, I Szarejko, I Ingelbrecht, and BJ Till (2019). Fragmentation of Pooled PCR Products for Highly Multiplexed TILLING. G3: Genes Genomes Genetics 9 (8): 2657-2666.

Warthmann, N. Library Preparation for Medium- to High-throughput DNA Sequencing on the Illumina Sequencing Platform. Submitted for online publication on the IAEA website.

Wilson PB, JC Streich, KD Murray, SR Eichten, R Cheng, NC Aitken, K Spokas, N Warthmann, SP Gordon, JP Vogel, and Borevitz JO. (2019). Global Diversity of the Brachypodium Species Complex as a Resource for Genome-Wide Association Studies Demonstrated for Agronomic Traits in Response to Climate. Genetics, 2019, 211(1), 317-331.

THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farmers' communities to climate change through climate-smart agriculture, including soil and water conservation and optimization of 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 2019, the SWMCNL conducted a wide range of activities. It (i) developed robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture through R&D; (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 climate-smart soil and water management practices and improve remediation of radioactive contamination in agriculture; (iv) carried out isotope analyses for research and development (R&D); and (v) provided quality assurance services to Member States.

The R&D activities at the SWMCNL included novel applications of isotopic and nuclear techniques to assess soil erosion using plutonium isotopes or to identify sediment pathways across arable land, with emphasis on precision identification of sediment sources using compound specific stable isotopes (CSSI). Tests were initiated to use cosmic-ray neutron sensor technology for soil moisture assessment in the rooting zone in combination with conventional soil moisture sensors to advise on agricultural water management. Significant steps were made to develop a package of stable isotope techniques to measure water use efficiency and water stress tolerance to counteract drought effects on cassava and banana systems. Important progress was also made in the field of remediation of radioactive contamination in food and agriculture, by implementing R&D on the role of zeolite amendments to soil on radiocaesium availability for crops. All these activities are essential in supporting the implementation of the five Coordinated Research Projects (CRP) of the SWMCN Subprogramme, two of which are coordinated by the SWMCNL, i.e. CRP D1.50.17 on *'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems*' and CRP D1.50.19 on *'Monitoring and predicting radionuclide uptake and dynamics for optimizing remediation of radioactive contamination in agriculture'*.

A major component of the work of the SWMCNL is its significant contribution to training and capacity building in Member States. In 2019, the SWMCNL hosted 3 PhD students, 4 interns (including 2 MSc students) and 1 fellow, from 5 different countries, for training on the use of isotopic and nuclear techniques in support of climate-smart agriculture and remediation of radioactive contamination in agriculture.

R&D information was further communicated to Member States through 47 publications as manuals, guidelines, protocols, books, book chapters, conference papers and publications in international peer-reviewed journals, including two books and three TECDOCs on the use of isotopic and nuclear techniques for climate-smart agriculture and nuclear emergency response.

The SWMCNL analysed a total of 5701 and 300 samples for stable isotopes and fallout radionuclides, respectively. Most analyses were carried out in support of R&D activities in the SWMCNL, focusing on the design of isotope and nuclear techniques to optimize soil and water management practices. Emphasis was also put on ^{13}C-CO_2 and ^{15}N-N_2O measurements using the laboratory-based laser isotope analysers.

STAFF

Name	Title
Dercon, Gerd	Laboratory Head
Mabit, Lionel	Soil Scientist
Said Ahmed, Hami	Soil Scientist
Heiling, Maria	Senior Laboratory Technician
Resch, Christian	Senior Laboratory Technician
Weltin, Georg	Senior Laboratory Technician
Gruber, Roman	Laboratory Technician
Toloza, Arsenio	Laboratory Technician
Jagoditsch, Norbert	Technical Attendant
Mletzko, Joanna Malgorzata	Team Assistant
Eguchi, Tetsuya	Consultant
Fesenko, Sergey	Consultant
Lee Zhi Yi, Amelia	Consultant
Mirkhani, Rayehe	Consultant
Slaets, Johanna	Consultant
Van Laere, Jonas	Consultant
Vantyghem, Mathilde	Consultant
Dengra i Grau, Francesc Xavier	Intern
Ding, Yang	Intern
Jabbarimalayeri, Hoda	Intern
Willemen, Annemie	Intern

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 plot (on-farm) or 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 and crop failures worldwide. Agriculture can further accelerate climate change due to the greenhouse gas it emits. 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-smart agriculture.

Comparison of Plutonium radioisotopes ($^{239+240}$Pu) and ^{137}Cs derived soil erosion rates: a case study in an Austrian agricultural field (Grabenegg, Lower Austria)

In 2019, a study was carried out, under CRP D1.50.17 on "Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems", by the SWMCNL in Grabenegg, 100 km west from Vienna, to compare the use of Plutonium radioisotopes ($^{239+240}$Pu) and radiocaesium (^{137}Cs) to determine soil erosion rates. This study was also used to evaluate the time frame of the erosion rates, measured through these different radionuclides (FRN), as the time frame can be determined by knowing the origin of the radionuclides.

To achieve this objective, soil erosion rates were calculated along a newly investigated transect located within an agricultural field (slope inclination approximately 5%) located close to a reference site, for which the vertical distribution for the studied FRNs was determined in 2018. The vertical distribution for the studied FRNs showed a clearly exponential decrease of their content with depth and a limited lateral spatial variability with coefficients of variation below 30%, proving the sampled meadow being a good reference site for soil erosion studies using FRN techniques.

Eleven soil profiles collected along the transect were analysed for ^{137}Cs and $^{239+240}$Pu content.

The obtained datasets showed that the plutonium at the investigated agricultural site is of global fallout origin (peak of fallout at 1963) as evidenced by an activity ratio ^{238}Pu/$^{239+240}$Pu below 0.025 (data from reference site). This result was confirmed by an additional determination of the atom ratio ^{240}Pu/^{239}Pu of 0.134 ± 0.035 (n=20) at the reference site. Established ^{240}Pu/^{239}Pu values reported in the literature for mid-latitude of the northern hemisphere not affected by the Chernobyl nuclear power plant accident (1986) vary from 0.14 to 0.24, while Pu originating from Chernobyl fallout would have a ^{238}Pu/$^{239+240}$Pu ratio close to 0.50 and an atom ratio ^{240}Pu/^{239}Pu characterized by values of 0.37 to 0.41.

Based on the geographical location of the studied site and its average yearly precipitation, we could expect an activity level of ^{137}Cs around 2000 Bq m^{-2} from global fallout only. Recorded values at the

undisturbed reference site fluctuate around 8000 Bq m^{-2} thus indicating a clear Chernobyl contribution of around 70-80% of the total ^{137}Cs soil content.

To identify the ^{137}Cs origin, the $^{137}Cs/^{239+240}Pu$ activity ratio can also be used. When most of the fallout originates from Chernobyl, the ratios are significantly higher than the expected average ratio of 36, which is indicative of global fallout contribution from previous nuclear tests. In our case study, this specific ratio is reaching 140 ± 24. Our findings are in agreement with literature reporting that Northern and Southern Alpine sites are mostly affected by Chernobyl fallout and ratio values range from 90 up to 898.

If we include analytical errors of both FRN measurements, we could consider that most sampling points highlight limited soil erosion or no significant soil redistribution rates. As a matter of fact, most areal activities of ^{137}Cs and $^{239+240}Pu$ determined for the eleven soil cores are only slightly below the established reference site values (Figure 1). The model MODERN (MOdelling DEposition and Erosion rates with RadioNuclides) developed by Arata et al. (2016) was used to derive soil erosion magnitudes from established ^{137}Cs and $^{239+240}Pu$ data sets which resulted in similar mean erosion rates around 5 t ha^{-1} yr^{-1}.

As it has been clearly proven that the origin for ^{137}Cs and $^{239+240}Pu$ at the Grabenegg site is different, these findings showed that the ^{137}Cs and $^{239+240}Pu$ based methods can provide complementary information on soil erosion over time. As the estimated soil erosion rates are similar, soil erosion seems not to have significantly changed over the last 33 years (1986-2019) in comparison with the period of 1963 until 1986.

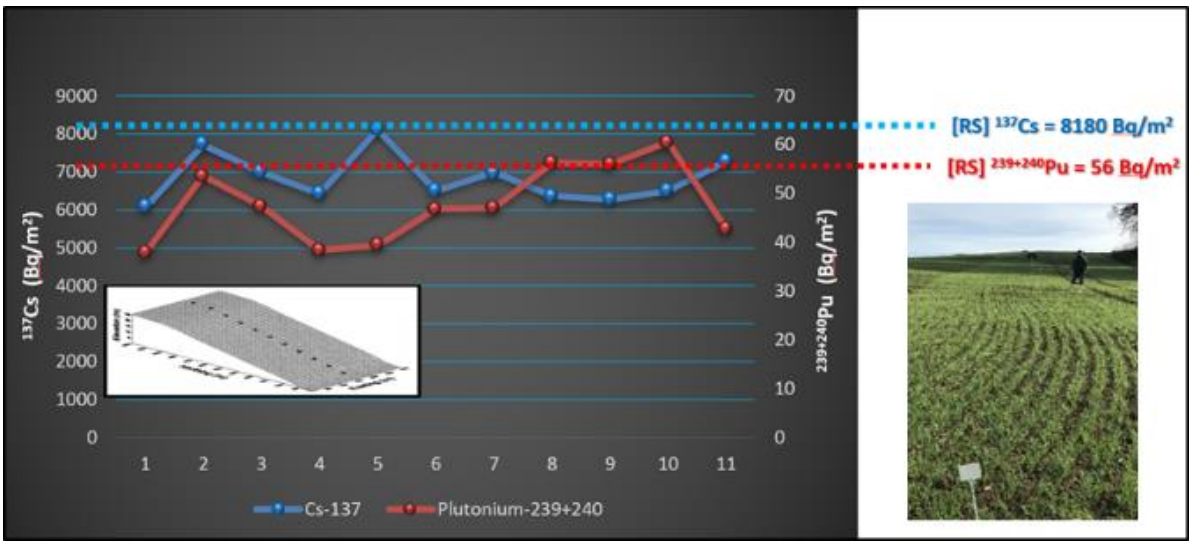

FIG. 1: *^{137}Cs and $^{239+240}Pu$ areal activities (Bq m^{-2}) along the agricultural transect compared to the reference site (RS) values.*

Tracing sediment origin in the agricultural watershed of Petzenkirchen (Lower Austria) using the Compound-Specific Stable Isotope (CSSI) technique

As part of the R&D activities performed by the SWMCNL under CRP D1.50.17, this study was implemented to validate the Compound-Specific Stable Isotope (CSSI) technique for identifying upland areas contributing to the sedimentation processes observed at the outlet of the Petzenkirchen watershed, which has a size of about 65 hectares. In the last decade, the land use of the Petzenkirchen watershed has been dominated by rotation of winter wheat and maize.

The CSSI technique based on the measurement of ^{13}C signatures of fatty acids ($\delta^{13}C$-FAs) was proposed to be used as sediment fingerprint in pinpointing the areas at risk within that agricultural site.

To identify and account for potential material contributing to the mixture collected at the outlet of the site, all fields and other possible sources within the watershed were sampled. A representative mixture of sediment produced by the watershed was collected at its outlet located at the southeast of the site. After sampling and pre-treatment of samples by the SWMCNL team, the determination of $\delta^{13}C$-FAs was performed at the National Institute of Water and Atmospheric Research (NIWA), Hamilton, New Zealand.

Under the Petzenkirchen site conditions, the most suitable FAs to be used as input in the Stable Isotope Mixing Models in R (SIMMR) were determined based on the validation technique of the mixing polygons. As expected, the $\delta^{13}C$ signature of saturated long chain FAs above 20 carbon atoms (i.e. C24:0 and C26:0) allowed the best discrimination for establishing the contribution of sources to the mixture.

Output from the SIMMR model was expressed as isotopic proportions. Using the equation proposed by Gibbs (2008), the SIMMR results were converted into soil proportions based on the specific organic carbon ($\%C_{org}$) content of the sources.

The simulated results obtained with the mixing model SIMMR highlight that the main sediments reaching the outlet of the Petzenkirchen watershed (i.e. 55%) originate from erosion processes impacting its bank. Future investigations of detailed historical land use of the watershed will help improve our understanding of the sediment budget obtained.

Impact of future precipitation patterns on GHG emissions – a stable isotope and lysimeter study

Marchfeld is one of the main food producing areas of Austria. According to regional climate model scenarios from EURO-CORDEX, this region is very vulnerable to climate change because of lower predicted rainfall. The influence of precipitation on soil organic matter mineralization, nutrient release and therefore plant production was studied in collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and the Austrian Agency for Health and Food Safety (AGES).

Dual labelled (^{13}C and ^{15}N) green manure was applied to long-term lysimeters containing two different soil types – a sandy calcaric Phaeozem and a calcic Chernozem, representative for the Pannonian area of the Marchfeld region (Figure 2). The lysimeters were irrigated according to the predicted future scenario, compared with current precipitation since 2011. N_2O and CO_2 concentrations and their corresponding isotopes were measured before and after the application of labelled green manure, simulated heavy rainfall and fertilizer application of 50 kg N ha^{-1} using off-axis Integrated Cavity Output Spectroscopy (OA-ICOS) and gas chromatography isotope ratio mass spectrometry (GC-IRMS) respectively. Nitrogen (N) and ^{15}N signatures of inorganic and organic N soil pools and plants were determined using IRMS.

Results from the lysimeter experiments showed significantly reduced plant growth under predicted future rainfall regimes by around 10%. Further, under the same future rainfall conditions a lower mineralization rate of the green manure could be observed, in particular for the calcaric Phaeozem. Based on the measurement of the N_2O fluxes and the ^{15}N signal of N_2O, the study suggests the relevance of green manure as a fertilization strategy to reduce N_2O emissions.

FIG. 2: *N_2O measurement on a lysimeter, using off-axis Integrated Cavity Output Spectroscopy (OA-ICOS).*

This study suggests that changing rainfall regimes in the future due to a changing climate may likely reduce soil CO_2 emissions from soils in the Marchfeld region. It is driven mainly by reduced plant biomass input. Further analysis is now being carried out on the datasets.

Using laser spectroscopy to evaluate the influence of soil storage on N_2O emission

To improve fertiliser use efficiency, it is essential to quantify nitrogen (N) mineralization processes including measuring gaseous emission of nitrous oxide (N_2O). Laboratory incubation experiments are conducted using disturbed soils to study these processes. After collecting and processing (sieving), soil samples are generally stored either in the fridge or in the laboratory at room temperature before being used in incubation studies.

To evaluate the influence of soil storage on N mineralization and N_2O emission, an incubation experiment was set up in the SWMCN laboratory, Seibersdorf. Fresh soil (Chernozem, 0-10 cm depth) collected from Seibersdorf was compared with the soil from the same spot either dried at room temperature or stored at 4 °C. After collection, all soils were passed through a 2 mm sieve and compacted to achieve a bulk density of 1.2 g cm^{-3}. The dried soil was rewetted 14 days before the gas measurement and the cooled soil was reconditioned at room temperature 7 days before the start of the experiment. Supplementary, fresh undisturbed soil cores were taken. All soil samples were brought to 60 % water-filled pore space (WFPS) by adding water, treated with ^{15}N labelled urea (1 atom %) at the rate of 50 mg N kg^{-1} soil, and incubated at room temperature (23 °C). The four treatments include fresh undisturbed (FU), fresh sieved (FS), fridge stored (ST), and room temperature dried (PI). The N_2O fluxes were measured for 7 weeks using off-axis integrated cavity output spectroscopy (ICOS, Los Gatos Research, California, USA). Cumulative N_2O fluxes and Keeling plot intercepts ($\delta^{15}N$ source) were calculated.

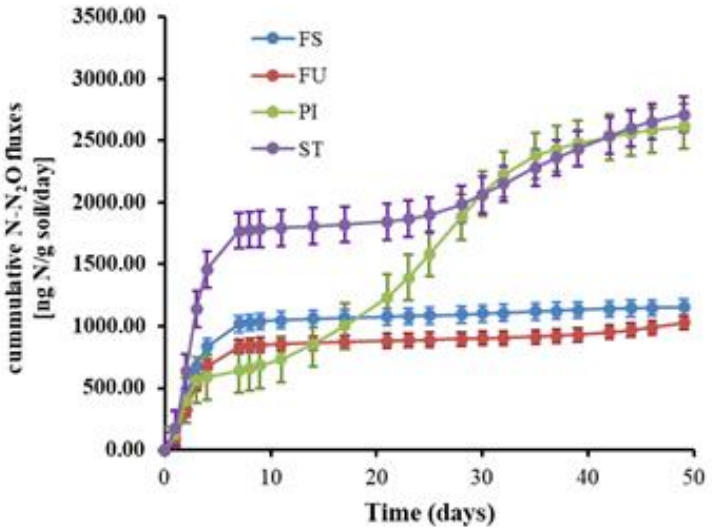

***FIG. 3**: Effect of soil storage on N_2O emission (fresh sieved (FS), fresh undisturbed (FU), room temperature dried (PI), and fridge stored (ST)).*

Soil storage had an impact on N_2O emission (Figure 3). Soil stored at 4 °C (ST) and room temperature (PI) produced the highest cumulative N_2O emissions (2.6 – 2.7 µg N g^{-1} soil; Figure 3) as well as the largest amount of N derived from fertilizer (Ndff) (1.3 – 1.6 µg N g^{-1} soil) over the 7-week period. Undisturbed (FU) and freshly sieved (FS) soils emitted at the end of the measured period significantly smaller amounts of 1.0 – 1.2 µg N g^{-1} soil (Figure 3), of which about 70% came from fertilizer, as compared to about 50-60% for the soils stored at 4 °C (ST) and room temperature (PI).

These results indicate that soil storage after collection affects microbial processes that release N from applied fertiliser and produce N_2O emission. Our results suggest using fresh soil to avoid these negative effects rather using stored soils.

Participation in the Joint Danube Survey 4

The Danube is Europe's second longest river, stretching from Germany to the Black Sea. Water quality in the Danube River Basin is regularly monitored by the national authorities of all riparian countries to evaluate contaminant sources and reduce the pollution loads to the Danube River and the Black Sea.

In 2019 the SWMCN Subprogramme participated in cooperation with the IAEA Isotope Hydrology laboratory in the Joint Danube Surveys 4 (JDS4). The survey was organized by the International Commission for the Protection of the Danube River (ICPDR). Its main purpose is to gather specific water quality data, which are not covered in standard monitoring campaigns, across the entire length of the Danube River and its major tributaries. The water sampling is implemented by the national authorities, but water analyses are performed through cooperation with external institutions in order to cover a wide range of parameters.

As no nuclear components are measured routinely along the entire Danube River, IAEA provided sampling material and performed the analysis of stable water isotopes, the isotopic compositions of nitrate as well as major ion analysis. In total 51 sites from 13 different countries across the Danube River Basin and 7 groundwater samples were sampled and analysed in IAEA laboratories. The results provide information about the origin of water and nitrate sources in the Danube watershed. The results will be presented in a workshop in 2020, contributing towards an official report of the ICPDR and the outcome will support the 2021 update of the Danube River Basin Management Plan as well as water monitoring practices across the Danube countries.

Comparing stationary Cosmic-Ray Neutron Sensor with capacitance probe for monitoring soil water content in the rooting zone of two cropped fields in Rutzendorf (Austria)

The Cosmic Ray Neutron Sensor (CRNS) can be used for measuring soil moisture content (SWC) in large areas of up to 20 hectares. In view of this important advantage, the SWMCN Subprogramme has launched a new Coordinated Research Project (CRP D1.20.14) titled *'Enhancing agricultural resilience and water security using Cosmic-Ray Neutron Sensor'* in 2019. However, a common constraint with the CRNS is that it only provides data on soil water content (SWC) from the top 30 cm of the soil depending on the SWC. The monitoring of the SWC with CRNS in the deeper rooting zone is and remains a challenge.

As part of this CRP, the SWMCNL initiated a study to compare the stationary CRNS with capacitance probe for monitoring soil water content in the rooting zone (up to 60 cm deep) of agricultural fields (Figure 4), the purpose is to study the possibility of extending the vertical footprint of the CRNS, through mathematical procedures.

This study is set up in a large bare field between two cropped fields in Rutzendorf, Marchfeld region (Lower Austria). A set of seven capacitance probes has been installed at 0, 10, 60 and 120 m from the CRNS in each direction. Additional capacitance probes were installed inside the two cropped fields.

For field calibration of the CRNS, the SWMCN Laboratory team already carried out the first sampling (135 samples). Two more sampling campaigns are planned to complete the CRNS and capacitance probe calibration.

FIG. 4: *Cosmic Ray Neutron Senor (left); one of the capacitance probes (right) installed in Rutzendorf, Austria*

How isotopes help build crop resilience to climate change in the Great Lakes Region of Africa

It is predicted that climate change will cause an increase in frequency and duration of dry spells in Central Africa, the target region of CIALCA[1]. This will lower yields of cassava and banana consumed daily by many millions of people in the highlands of Burundi, DRC and Rwanda.

[1] The Consortium for Improving Agriculture-based Livelihoods in Central Africa, in which the Joint FAO/IAEA Division is one of the official partners together with the International Institute of Tropical Agriculture and Bioversity International. For more information on CIALCA, please kindly visit www.cialca.org

To cope with problems of drought stress in cassava and banana cropping systems, stable isotope techniques based on carbon-13 or ^{13}C (related to water use efficiency) and oxygen-18 or ^{18}O (related to stomatal conductance) are being developed by the SWMCNL, in close collaboration with the International Institute of Tropical Agriculture and the University of Leuven (Belgium). Once these techniques are established and validated, they will help in decision making processes related to variety selection, choice of planting time and fertilizer application to counteract the effects of drought and cassava and banana productivity.

Drivers of water use efficiency in cassava: from greenhouse experiments to field trials

In 2018, the focus of the research activities coordinated by the SWMCNL was on how to sample leaves of cassava to assess water use efficiency based on ^{13}C and ^{18}O stable isotopes.

One year later, in 2019, the emphasis was further laid on the understanding of the drivers of water use efficiency in cassava, with emphasis on soil fertility, in particular potassium availability in the soil. Therefore, a set of experiments focussing on the application of potassium to alleviate drought stress was carried out in the SWMCN greenhouses.

Cassava plants, originating from Democratic Republic of Congo, were grown on sand substrates with nutrient solution either high or low in potassium. Water use was monitored every other day by weighing the pots and water content adjusted to field capacity. At two months after planting, a dry spell was simulated by lowering by 50% the irrigation amounts for half of the plants for 17 days. To follow the translocation of new assimilates and compare the different treatment combinations, plants were put in an airtight walk-in growth chamber. The air inside the growth chamber was enriched with ^{13}C-CO_2 so the plants assimilate the heavier carbon-13 isotope.

First results of the water use data indicate a higher water use for plants that received the nutrient solution low in potassium in the periods where all plants received the same amount of water. These results will be checked against the biomass production and $\delta^{13}C$ and $\delta^{18}O$ values of the same plants to see whether this difference in water use also leads to a difference in water use efficiency.

This potassium effect on water use, in relation to other nutrients, is now being further validated through field trials in Burundi, the Democratic Republic of Congo and Rwanda, implemented by the CIALCA team in the targeted region, in close collaboration with national agricultural research institutes (e.g. Rwandan Agricultural Board) and international organizations such as the International Fertilizer Development Centre (IDFC). In total 121 nutrient omission and planting scheduling trials were implemented to better understand how varieties, planting time and fertilizer management can help make cassava production more climate-resilient. Close to 3000 leaf samples have been taken for stable isotope and ICP-MS analysis, allowing to evaluate the role of fertilizer application in drought tolerance and water use efficiency.

Further ongoing is the ^{13}C analysis of the enriched cassava plants. With these data we expect to extract information on the translocation speed from shoot to root and compare the different treatments. Our main question is whether differences in potassium supply affect the translocation rate of assimilates towards the roots, in view of the well documented phenomenon that translocation is an additional mechanism to counteract drought effects on cassava. ^{18}O levels will also be analysed and will be used as a proxy for stomatal conductance, an important factor in water use efficiency.

FIG. 5: *Pot experiment at the SWMCNL to understand the importance of potassium in water use by cassava (left); cassava plant sampling in the Democratic Republic of Congo for ^{13}C and ^{18}O stable isotope analysis (middle), extracting plant water from cassava samples for ^{18}O stable isotope analysis (right)*

Validation of the use of isotopes and leaf temperature for assessing drought stress in banana

Under the guidance of the Soil and Water Management & Crop Nutrition (SWMCNL) and the Plant Breeding and Genetics (PBGL) Laboratories of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, a new Peaceful Uses Initiative (PUI) project, funded by the Belgian Government, has been initiated in 2019 to better understand how soil and water management and varieties can be improved for better climate change adaptation and enhanced disease resilience of banana-coffee cropping systems in Sub-Saharan Africa.

Using isotope techniques, the adaptation of these cropping systems to climate change impacts can be accelerated. They help improve banana and coffee varieties, and soil, water and crop management, but also establish recommendations for policies, enabling environments and a transformational adaptation in which farmers substitute varieties and explore alternative farming strategies.

A first data collection campaign was organized during July and August 2019 in Arusha, Tanzania. In an ongoing field-trial, two banana varieties were investigated under different watering treatments in the dry season (rainfed and optimal irrigation). Hence, the effects of drought stress could be monitored. The main purpose of the campaign was to assess the usefulness of stable isotope techniques for the evaluation of water use efficiency (WUE) and drought stress. Isotope signatures have been proven to strongly correlate with WUE. Their relationship is however not straightforward. Isotope signatures are affected by many different parameters, both environmental and plant-related. As such, the variability in isotope signals should first be explored and correlated with potential influencing factors, to distinguish their effect from the effect of drought. This will allow us to comprehend isotope signatures in banana plants and use them for the purpose of WUE evaluation.

Variability in carbon signatures (^{13}C) was investigated at field-, plant- and leaf level. Samples were taken from both varieties under the different watering treatments. Both mother plants and on-growing suckers were sampled. Within every plant, leaves of a different age were sampled. Finally, the within-leaf variability was explored by taking 6 or 10 samples per leaf (depending on the size) according to a predetermined pattern. Environmental conditions were carefully monitored with a weather station and the soil water content in every treatment was followed up daily, using time

domain reflectometry sensors. This detailed sampling, with in total 2000 samples, and their stable isotope analysis will allow to establish an innovative stable carbon isotope sampling protocol for banana.

As an additional measure for water stress and stomatal closure, leaf temperature was evaluated over the course of a day. Typically, stomata close when water supply becomes insufficient, leading to an increase in leaf temperature. Temperature was measured in a large number of plants and on both sides of the leaf, to account for the large variability. A low-cost contactless infrared thermometer was used, allowing for fast data collection.

FIG. 6: *Banana leaf sampling (top left), sample preparation (top right), and leaf temperature measurement via an infrared thermometer in the field (Bottom, Research site in Arusha, Tanzania)*

Preliminary results indicate that rainfed plants in the dry season clearly heat up more during the day than irrigated (and presumably non-stressed) plants. The difference in temperature between rainfed and irrigated mother plants becomes as large as 6°C at 14:30h. Interestingly, sucker plants, which are protected from direct sunlight by the canopy, show a lesser increase in temperature. This demonstrates the importance of canopy protection for optimal sucker development.

The leaf temperature was related to the ^{13}C data of the banana leaves, with a correlation (R^2) of about 50%, showing that the use of leaf temperature measurement with infrared thermometer can be used as a new approach for drought stress evaluation in banana.

Nuclear Emergency Preparedness in Food and Agriculture

Member States are increasingly interested in improving the capacity to respond to nuclear emergencies affecting food and agriculture due to the growing number of nuclear power plants built. Lessons learned from the Chernobyl and Fukushima Daiichi Nuclear Power Plant accidents identified critical areas for improvement and this includes data collection (sampling and analysis), data management, data visualization to make decisions swiftly, allowing food control and health authorities to respond and disseminate information to all relevant stakeholders appropriately. Further emphasis is now also put by the SWMCNL on the optimization of remediation of radioactive contamination in agriculture.

DSS4NAFA testing in Belgium

In May 2019, the SWMCNL in collaboration with the Belgian Ministry of Defense and Belgian Federal Agency for Nuclear Control (FANC) successfully performed a first testing of an SWMCNL developed IT-tool, the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA). The IT-tool was designed to strengthen Member State abilities to respond to nuclear emergencies affecting food and agriculture through optimized data management and data visualization. This testing was organized under a PUI Project titled "Global Networking for Improved Radiological and Nuclear Emergency Preparedness and Response in Food and Agriculture".

The emergency response exercise simulated a radionuclide contamination event and involved 22 civil protection personnel from the Federal Public Service (Ministry of the Interior), as well as six local coordinators from the Belgian Nuclear Research Center (SCK-CEN), the National Institute for Radioelements (IRE) and Federal Agency for the Safety of the Food Chain (FASFC).

Testing was repeated over two days – one day for the French-speaking Civil Protection group, one day for the Flemish-speaking Civil Protection group. As part of the exercise, DSS4NAFA was utilized on its desktop and mobile based interface to assign sampling tasks, digitize data input in the field, and update stakeholders on the contamination status in real-time. The principles, as well as end-user and strategic level usage of the DSS4NAFA system was explained. The IRE, FASFC, SCK-CEN personnel were appointed as local coordinators and utilised the web-based interface of DSS4NAFA to assign tasks and oversee the response exercise on a strategic level. Meanwhile, civil protection personnel utilised the mobile-based interface of DSS4NAFA to receive tasks and input in-situ measurements.

This testing and adaptation experience serve as a platform for improved adjustment and implementation of DSS4NAFA for other Member States in the future. The general feedback was constructive and points towards further customization and improvement in DSS4NAFA's user interface and system set up. A list of improvements and proposed solutions were drafted and agreed upon by all counterparts to be further implemented in 2020.

FIG. 7: *Members of the Belgian Civil Defense Force tested DSS4NAFA while undergoing training in Nuclear Emergency Response (Photo credit: Mr Jürgen Braekevelt, Belgian Ministry of Defense).*

Evaluation of the use of zeolite amendments on radiocaesium selectivity in Japanese and European soils

In the aftermath of a nuclear emergency, radioactive contamination can severely affect agricultural production and food safety. Top soil removal and potassium fertilizer application are used as major countermeasures to reduce the transfer of radioactive elements, such as radiocaesium, from soil to crop. Additionally, clay amendments, such as zeolite, known to adsorb effectively radiocaesium, are also

applied to some extent. Field studies in the areas affected by the Fukushima Nuclear Power Plant accident showed that amendment of clays can reduce the radiocaesium uptake by crops planted on contaminated soils.

To better understand the precise role of such clay amendments in radionuclides behaviour, the SWMCNL started in 2019 a collaboration with Austrian, Belgian and Japanese research institutes. Through this partnership, a set of experiments has been initiated to compare the Radiocaesium Interception Potential (RIP) of Japanese and European agricultural soils with different clay mineralogy.

RIP is a key parameter for understanding the dynamics of radiocaesium in the soil. This parameter is used in mathematical models that can assist in the prediction of radionuclide mobility in soils. Such modelling approaches may help optimize the use of remediation countermeasures.

First the selected soils are being characterised by X Ray Diffraction (XRD) analysis to identify their clay mineralogical composition. Secondly, incubation of Japanese and European soils with different clay mineralogy is being conducted for determining the RIP. As caesium competes with potassium for binding sites in soils, their content in soil solution and solid phase will both be determined with the support of a range of analytical techniques including atomic absorption spectrometry (AAS), inductively coupled plasma - mass spectrometry (ICP-MS) and sodium iodide detector, and this for different levels of potassium addition. Soils characterised by low RIP values will be further studied by considering different levels of zeolite addition.

FIG. 8: *From left to right: (i) Preparation of soils, (ii) Dialysis bags with soil samples for RIP analysis, (iii) Zeolite minerals.*

This study is in support of the new Coordinated Research Project D1.50.19 on “Monitoring and predicting radionuclide uptake and dynamics for optimizing remediation of radioactive contamination in agriculture”.

The research team expects the main outcomes of this study to contribute in the decision-making on agronomic countermeasures for radioactively contaminated soils, as well as provide primary data that can improve existing models and elucidate the role of RIP in radiocaesium behaviour in agricultural soil.

CAPACITY BUILDING

In 2019, the SWMCNL focussed on capacity building through the training and guidance of three PhD, two MSc students (through IAEA internships), two interns and one fellow from five countries in the use of nuclear and isotope techniques for climate-smart agriculture and nuclear emergency response.

ANALYTICAL SERVICES

Laboratory analyses

In 2019, 5701 samples were analysed for stable isotopes and 300 samples were measured for fallout radionuclides respectively in the SWMCN Laboratory. Most analyses (i.e. 93%) were carried out for supporting Research and Development activities at the SWMCNL focused on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. Analytical support has also been given to the Insect Pest Control Laboratory with about 180 samples analysed. An additional analytical focus of the SWMCN Laboratory was on ^{13}C-CO_2 and ^{15}N-N_2O measurements using the laboratory-based laser isotope analysers.

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 hence an important tool for external quality control. The 2019 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 SWMCN Laboratory was successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, http://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 of the two rounds 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 SWMCN Laboratory and sent to the participants together with a certificate of participation additionally to the regular WEPAL evaluation report. The participation fee for one round per year is covered by the IAEA.

In total eleven stable isotope laboratories participated in the PT-round 2019: Africa (1): Morocco; Asia (3): Pakistan and Philippines (2 labs); Europe (3): Austria, Belgium and France; Latin America (3): Argentina, Brazil and Chile, and South Pacific (1): New Zealand. All nine laboratories participating in the nitrogen analysis reported ^{15}N-data within the control limits for the enriched plant sample and seven out of nine participating laboratories in carbon analysis reported ^{13}C isotopic abundance results within the control limits.

GUIDELINES AND TECHNICAL DOCUMENTS

New open-access FAO/IAEA publication: Assessing Recent Soil Erosion Rates through the Use of Beryllium-7 (Be-7)

This open access book provides insights on how nuclear techniques can facilitate the implementation of climate-smart agricultural practices. It is the first comprehensive guideline that presents and demonstrates the unique traits of the cosmogenic fallout radioisotope beryllium-7 (Be-7) and its use as a short-term soil redistribution budgeting tool in agricultural landscapes.

While covering the fundamental and basic concepts of the approach, this book distinguishes itself from other publications by offering step-by-step guidance and easy-to-follow protocols on how to use this isotopic technique effectively with appropriate attention to tracer limitations and uncertainties. It covers experimental design considerations and clear instruction is given on data processing. As accurate laboratory measurement is crucial to ensure successful use of Be-7 to investigate soil erosion, a full chapter is devoted to its specific determination by gamma spectrometry. Further the new developments in the Be-7 technique are described. The concluding chapter highlights the potential of Be-7 method to support the implementation of soil conservation policy. More information on the open-access publication can be found at: https://link.springer.com/book/10.1007%2F978-3-030-10982-0

Use of Laser Carbon Dioxide Carbon Isotope Analysers in Agriculture (IAEA-TECDOC 1866)

Laser CO_2 carbon isotope analysis – a relatively new technology- is increasingly used to track CO_2 levels and trace the source of CO_2 emissions through isotope analysis. These measurements can be used to evaluate and select agricultural management practices that reduce its emissions. To ensure accurate measurements and data analysis, the SWMCN laboratory published a TECDOC focusing on how to create reference gases for calibration and its quality control, and how to manage data as well as to enhance accuracy and precision of ^{13}C-CO_2 measurements. This TECDOC can be downloaded from: https://www.iaea.org/publications/13479/use-of-laser-carbon-dioxide-carbon-isotope-analysers-in-agriculture. More information on this TECDOC publication can be found on: https://www.iaea.org/newscenter/news/new-iaea-publication-use-of-laser-carbon-dioxide-carbon-isotope-analysers-in-agriculture

Sample Preparation of Soil and Plant Material for Isotope Ratio Mass Spectrometry (IAEA-TECDOC 1870)

This TECDOC provides a detailed guidance on sample preparation for isotope ratio mass spectrometry (IRMS) analysis of plant and soil materials. An appropriate sample preparation is crucial to ensure the quality of stable isotope techniques: often sample volumes of harvested soil or plant material need to be reduced prior to grinding, cross-contaminations must be avoided, and the final sample must be representative and within the adequate concentration range for IRMS. The Standard Operating Procedures (SOP's) presented in this publication provide comprehensive instructions in quartering/sub-sampling, grinding and weighing samples for IRMS to determine $\delta^{15}N$ and $\delta^{13}C$ composition of plant and soil material. This TECDOC can be downloaded from: https://www.iaea.org/publications/13482/sample-preparation-of-soil-and-plant-material-for-isotope-ratio-mass-spectrometry

Guidelines for Sediment Tracing Using the Compound Specific Carbon Stable Isotope Technique (IAEA-TECDOC-1881)

With increasing attention being paid by both developing and developed countries to soil erosion and its associated sedimentation processes, this TECDOC addresses both theoretical and practical aspects of the compound-specific stable isotopes (CSSI) technique, based on the determination of $\delta^{13}C$ signatures of fatty acids (FAs) used as soil and sediment fingerprints.

This publication provides guidance in the use of the CSSI technique for identifying areas at risk and the sources of sediment within agro-ecosystems.

While covering the fundamental concepts of the CSSI technique, this comprehensive illustrated guideline distinguishes itself from others by providing step-by-step instructions for scientists, technicians and students on how to effectively use this innovative approach for effective application of climate smart agriculture and for improving area-wide soil conservation strategies in fragile agricultural landscapes.

It is important to mention that the CSSI technique using δ^{13}C-FAs is still in its infancy. We therefore encourage scientists and experts in Member States to test it under various agro-ecosystems and as well to update their knowledge about the latest development as new studies and methodological papers are regularly published in peer-reviewed soil and environmental science journals. This TECDOC can be downloaded from: https://www.iaea.org/publications/13564/guidelines-for-sediment-tracing-using-the-compound-specific-carbon-stable-isotope-technique

Data Management and Visualisation in Response to Large-Scale Nuclear Emergencies Affecting Food and Agriculture

This FAO technical guideline presents the challenges of data management, geo-visualisation and decision making in nuclear emergency preparedness and response in food and agriculture. It further elaborates how IT-Decision Support System (IT-DSS) tools and algorithms allow for improved, real-time management of large volumes of data and integrated decision-making support in a spatial and temporal context. Two case studies of such IT-DSS are presented; one by the Soil and Water Management and Crop Nutrition Laboratory of the Joint FAO/IAEA Division, and the other case study by Japanese Competent Authorities in the aftermath of the Fukushima Daiichi Nuclear Power Plant accident.http://www.fao.org/3/ca6666en/ca6666en.pdf

FAO's Global Symposium on Soil Erosion, Rome, Italy

The Global Symposium on Soil Erosion (GSER19) was held from 15 to 17 May 2019 at FAO HQ in Rome, Italy. This Symposium was jointly organized by the FAO and its Global Soil Partnership (GSP), the Intergovernmental Technical Panel on Soils (ITPS), the United Nations Convention to Combat Desertification Science-Policy Interface (UNCCD-SPI) and the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture through the SWMCN Subprogramme. Participants included scientists, practitioners, economists, policy makers, government officials, private businesses, research institutes, NGOs, civil society, farmers associations, and land users.

Based on the existing scientific knowledge on soil erosion assessment and management, the symposium addressed soil erosion prevention and control. The symposium aimed at bringing science and policy together to review the status and challenges of soil erosion control for insuring food security and ecosystem services to fulfil the planned achievement of the Sustainable Development Goals 2, 3, 6, 13 and 15.

This three-day international meeting, that comprised 20 sessions and more than 100 presentations, focused on three main themes: use of data and assessment tools for soil erosion control (theme 1), practices and policies in action to address soil erosion (theme 2) and the economics of soil erosion and soil erosion control (theme 3).

The first day of the symposium a side event on '*Soil erosion assessment: Making a difference with isotopic techniques'* was also organized by the Joint FAO/IAEA Programme to highlight the effectiveness of isotopic techniques in evaluating soil erosion magnitude and in identifying sources of sediments. This side event presented state-of-the-art isotopic tools used to investigate soil erosion as well as some recent methods development and success stories obtained in targeted African and Latin American countries (i.e. Argentina, Madagascar, Morocco and Zimbabwe). The full proceedings of the Symposium can be downloaded from: http://www.fao.org/3/ca5582en/ca5582en.pdf.

FAO EMPRES Information Sheet on the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA)

A nuclear incident often leads to disarray, and may have long-term consequences for people, trade and the economy. In April 2019, an EMPRES Information Sheet was published by the FAO on the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA), which was developed under CRP D1.50.15 on 'Response to Nuclear Emergencies Affecting Food and Agriculture'. DSS4NAFA is a cloud-based decision support system to manage large volumes of spatial

and temporal data, real-time information processing and visualization, and enhanced aid to response actions and decision-making in case of a nuclear or radiological emergency. More information can be found at: http://www.fao.org/3/ca4291en/ca4291en.pdf

Sharing our research progress and connecting with international researchers through the European Geosciences Union General Assembly 2019, Vienna, Austria

About 16,300 scientists from 113 countries came together at the European Geosciences Union (EGU) 2019 General Assembly held in Vienna, Austria on 7-12 April. The SWMCN Subprogramme's activities were reported in 17 presentations covering topics in radionuclide tracers for soil erosion investigations, area-wide soil moisture screening, climate resilient crop production, remediation of radioactive contamination of agricultural land and multi-isotope approaches to tracing pollutants. The SWMCNL's work on large scale nuclear emergency response in food and agriculture was highlighted in the EGU session on 'Geoscience problems related to massive release of radioactive materials by nuclear accidents and other human activities'. The SWMCN Section, under CRP D1.50.18 "Multiple isotope fingerprints to identify sources and transport of agro-contaminants", also hosted one EGU session on "Identification of Agro-contaminants in Surface and Groundwater Using Stable Isotope Techniques", which had 14 posters and 7 oral presentations from experts and counterparts involved in the project.

The links to all contributions from the SWMCN Subprogramme can be found in this annual report under the publication list at the end of the SWMCNL contribution.

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An Update on the ReNuAL/ReNuAL+ Project: the FAO/IAEA Agriculture & Biotechnology Laboratories

ReNuAL is the initiative to modernize the eight aging laboratories in Seibersdorf, Austria, that are managed by the IAEA's Department of Nuclear Science and Applications. These 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.

The modernization began in 2014 with the Renovation of the Nuclear Applications Laboratories (ReNuAL) project, which consists of new building construction to provide new space to some of the laboratories, the acquisition of new laboratory equipment and infrastructure upgrades. The follow-up to ReNuAL, ReNuAL Plus (ReNuAL+), began in 2017 and will provide for additional construction, targeted refurbishment of the remaining laboratories, and further equipment.

Insect Pest Control Laboratory transitioned to the new laboratory

Insect Pest Control Laboratory staff, supported by the ReNuAL Project Management Group (PMG), completed a phased transition into their modern new laboratory building in October, and the new laboratory is now operational. This approach, initially expected to take several months, was planned as a precaution to ensure that the Laboratory's precious collection of insect species would adapt well to the new building environment. Thanks in part to the laboratory staff's and PMG's detailed advance planning, the transition went smoothly and was completed in approximately half of the predicted timeframe. IPCL staff and insects alike have settled into their new environment, and the laboratory is again buzzing with ground-breaking work on how to better control invasive tsetse fly, mosquito, and fruit fly strains. With over 1700 m2 of laboratory space, the new facility will substantially increase the ability of the Joint FAO/IAEA Division to assist Member States in controlling harmful insect pests.

The new Insect Pest Control Building

The new IPCL's ecosphere room is ready for testing insect behaviour in a variety of specialized environments

Flexible Modular Laboratory named after Late-DG Amano

The 63 General Conference in September passed a resolution naming a new laboratory building nearing completion under the ReNuAL/ReNuAL+ project the "Yukiya Amano Laboratories" as a tribute to the late-Director General Amano. Formerly known as the Flexible Modular Laboratory building, the new facility will house the Animal Production and Health Laboratory, the Food and Environmental Protection Laboratory, and the Soil and Water Management and Crop Nutrition Laboratory. The three labs will begin relocating from existing facilities after the new building is ready for occupancy in the 2nd quarter of 2020.

The Yukiya Amano Laboratories Building

Introduced by Japan and "Friends of ReNuAL" co-chairs Germany and South Africa, the resolution highlighted the late-Director General's "significant contribution… to enhancing the Agency's efforts toward international peace and security and in support of the peaceful use of nuclear technologies, particularly through the motto "Atoms for Peace and Development".

Resource mobilization

The Netherlands became the most recent first-time contributor to the renovation project when it announced a generous pledge in late October, continuing a strong year for resource mobilization. The Netherlands is now among eleven Member States to announce contributions so far in 2019, joining Viet Nam, Iran and Nigeria in the group of first-time donors in that period.

In total, 39 Member States and other donors have announced pledges of approximately 38.6M in extrabudgetary resources to the project thus far. The project is now approximately €200k from meeting the €2.6 million requirement the Director General highlighted during the June Board meeting and to reaching the overall ReNuAL+ target budget.

The ReNuAL/ReNuAL+ Donor wall in Seibersdorf (November 2019)

Enhancement of the remaining laboratories

The completion of the Yukiya Amano Laboratories facility and related infrastructure in the 2nd quarter of 2020 will leave one final element of the ReNuAL/ReNuAL+ initiative to be implemented: enhancement of the four laboratories remaining in the existing building; the Dosimetry Laboratory, Nuclear Sciences and Instrumentation Laboratory, Plant Breeding and Genetics Laboratory, and Terrestrial Environment Laboratory.

The initial plan calls for reconfiguring space and improving core infrastructure in the existing NA laboratory building to enable these laboratories to keep pace with increasing Member State demand for training and services. Implementation of this project element will commence after the relocation of three other laboratories into the new Yukiya Amano Laboratories building. Currently detailed reviews of requirements and possible options for implementation are being assessed, which will lead to determining the most cost effective and efficient approach for making these 4 laboratories “fit for purpose” to meet Member States’ needs.