

Joint FAO/IAEA Programme Nuclear Techniques in Food and Agriculture

# Plant Breeding & Genetics Newsletter

https://www.iaea.org/topics/plant-breeding https://www.fao.org/agriculture/fao-iaea-nuclear-techniques/en/

ISSN 1564-2569

#### No. 50, January 2023

## Contents

To Our Readers	1
Staff	4
Feature articles- Space Induced Mutagenesis and Breeding	Plant 6
Forthcoming Events	13

Past Events	13
Coordinated Research Projects	15
Technical Cooperation Field Projects	s 19
Highlights from Technical Cooper Projects	ration 23

Developments at the Plant Breeding and Genetics Laboratory	35
Publications	42
News and Highlights	44

## **To Our Readers**



The Joint FAO/IAEA Centre launched plant seeds to the International Space Station on 07 November 2022 to study the effects of cosmic radiation and microgravity on plant biology and genetics. Design: M. Godoy

Dear Colleagues,

It is with great excitement that I open this section of the Plant Breeding and Genetics (PBG) Newsletter No. 50 with the announcement of <u>our ground-breaking research</u> in astrobotany, aimed at exploring the effects of microgravity and cosmic radiation in space at low earth orbit (LEO) on DNA structural variation and plant biology. For the first time, the International Atomic Energy Agency (IAEA), in collaboration with the Food and Agricultural Organization of the United Nations (FAO), has sent plant seeds to space to study the generation of novel genetic diversity from exposure to harsh space environments for better adaptation to climate change on earth. Early hours of the dawn of Monday, 7 November 2022, saw the launch of our Feasibility Study on Seed Irradiation in Space for Induced Genetic Diversity and Plant Mutation Breeding loaded in the Cygnus Capsule carried by the Northrop Grumman Antares Rocket taking off from the Wallops Flight Facility of the National Aeronautics and Space Administration (NASA). This feasibility study exposes two batches of seeds of the model plant, Arabidopsis thaliana, and of two accessions of sorghum (Sorghum bicolor) to two distinct environments at the International Space Station (ISS). The first environment is the interior of the ISS, and the second is the exterior of the ISS enabled by the Nanoracks' External Platform (NREP). The interior environment is characterized mainly by microgravity, while the exterior is characterized by a combination of microgravity, cosmic radiation and extreme temperatures. Once returned to earth, the seeds will be assessed for viability, plant growth biology and DNA structural variation by whole genome sequencing. Results will be compared to samples of the same seeds that have been retained on earth. This feasibility study is mediated as a service provided by Voyager Space and its operating company Nanoracks.

Highlighting this very first venture of the Joint FAO/IAEA Centre into space mutation breeding and astrobotany research, the PBG Newsletter No. 50 features four articles on space-induced plant mutagenesis and breeding on pages 6 to 12. The research is part of the new Coordinated Research Project (CRP), D24015, that started in 2022. It is focused on front-end technologies (as opposed to crop-trait focus), and addresses: (1) evaluation of the nature of DNA structural variations resulting from electron beam, heavy ion beam, proton beam, gamma-rays, X-rays, microgravity, and cosmic radiation in combination with microgravity; (2) tools for determining genetic associations for a few selected simple traits in diploid crops, and validation through functional genomics, mainly, gene editing; and (3) application of genomics prediction for a complex trait to mutant population structures.

Our technical support continues in earnest for the interregional Technical Cooperation Project (TCP) on "Strengthening Member State Capacities to Combat Banana Fusarium Wilt (TR4) through Early Detection, New Resistant Varieties and Integrated Management (INT5158)" that was launched in February 2022. An important training activity was undertaken during the second half of the year on Detection and Diagnosis of Fusarium Tropical Race 4 (TR4). The training was held in the laboratories of the National Phytosanitary Reference Centre (CNRF) of the National Food Health, Safety and Quality Service (SENASICA) in Mexico during 10-14 October and was attended by 12 participants from ten Latin American countries. Given the continuing significance of research and development efforts critical to combat banana Fusarium Wilt TR4, the next CRP at PBG, D23033, was approved on An Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana – Phase II and is due to start in April 2023. A call for proposals for participation in the CRP is now open (page 17).

In other important news, a visit to the National Centre for Electron Beam Research at the Texas A&M University in November 2022 combined discussions on (1) the inclusion of plant mutation breeding as part of the focus of the Centre's next extension as a Collaborating Centre of the IAEA, (2) discussions with plant breeders and phytopathologists, and (3) collaborating opportunities with the Norman Borlaug Institute for International Agriculture. Further, an expert mission in October 2022 to the University of the Philippines Los Baños and the Philippines Nuclear Research Institute as part of the TCP, PHI5036, had a major focus on onboarding the **Breeding Management System** (**BMS**). The BMS, developed by the Integrated Breeding Platform of the CGIAR to enable digitization towards modernizing crop breeding, is planned to be rolled out for the first time through PHI5036, and the roll-out process could be modelled for other interested Member States.

In another highlight from TCPs technically supported by PBG, a dual fellowship of the project SRL5050 achieved tremendous success in establishing direct somatic embryogenesis from tea leaves (page 23). The project, SRL5050, aims at reducing the time required for cultivar development in the tea crop and at developing novel genetic diversity in the tea germplasm in Sri Lanka. Integration of embryo culture techniques into the breeding process and the application of induced mutagenesis can achieve this aim. The Fellowships at the PBG laboratory succeeded in the modification of existing protocols for direct embryogenesis to obtain somatic embryos in tea leaves within a week of culturing. Mutagenesis of these somatic embryos using gamma rays is in process, while simultaneous efforts are being directed to the culturing of single cells from friable callus towards the development of chimera-free regenerants from induced mutagenesis.

Research and development efforts at the PBG laboratory during the recent months has succeeded in achieving important milestones. The Laboratory has recently optimized screening conditions for resistance to the disease, Stemphylium blight in lentil that is caused by the fungal pathogen, Stemphylium botryosum. Further, in vitro somatic embryogenesis was integrated with temporary immersion systems and mutation induction to accelerate the production of chimera-free mutant plantlets in arabica coffee. The laboratory has also concluded the development of a bioinformatic protocol package based on Next Generation Sequencing for forward genetic mapping of induced mutations linked to traits of interest. In the present newsletter, the laboratory also reports on mutation-assisted breeding of African cooking banana for resistance to Fusarium wilt race 1.

The PBG subprogram currently provides technical support to **72 active TCPs** across more than a hundred Member States, along with several ongoing projects from earlier years of start. Of the 72 active TCPs, 36 commenced in January 2022. Further, PBG is also supporting the design of 25 new TCPs for planned start in the 2024-25 biennium. The second half of 2022 was extremely productive in the organization and delivery of 33 training courses at the national and regional levels that provided in-person or virtual training to a total of 317 women and 387 men.

In this issue, I would also like to announce the publication of the Chinese translation of the Manual on Mutation Breeding. Even as the different translations are completed for the Manual first published in 2018, we are preparing for the next edition.

I want to take this opportunity to wish Mr Qu Liang, Director of the Joint Centre, all the very best in his future endeavors upon his retirement in October 2022. For 17 years Director Qu Liang was a strong and stalwart leader for the Joint Centre, steering it from troubled waters to a strong presence for global R&D and capacity building in the field of Food and Agriculture. We will miss his able guidance and deep experience. At the same time, I welcome the new leadership of Mr Thanawat Tiensin and Ms Dongxin Feng.

I conclude with my sincere appreciation for your tremendous support and our joint efforts in induced genetic variation and mutation breeding for crop improvement. The strength of our continuing collaborations can deliver important outcomes towards global food and nutrition security under climate change.



Shoba Sivasankar Head, Plant Breeding and Genetics Section



Director Qu Liang with part of the PBG team at the Joint FAO/IAEA Centre's farewell party in Vienna, Austria in October 2022

## Staff

### Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture

Name	Title	Email	Extension	Location
Thanawat TIENSIN	Director of the Animal Production and Health Section Officer in Charge of the Joint FAO/IAEA Centre	Thanawat.Tiensin@fao.org	(+39)065 05 3371	Italy/FAO
Dongxin FENG	Acting Director of the Joint FAO/IAEA Centre Programme Coordinator of the Joint FAO/IAEA Centre	D.Feng@iaea.org	21610	Vienna

#### **Plant Breeding and Genetics Subprogramme**

Name	Title	Email	Extension	Location
Shoba SIVASANKAR	Section Head	S.Sivasankar@iaea.org	21626	Vienna
Isaac Kofi BIMPONG	Plant Breeder/Geneticist	I.Bimpong@iaea.org	21617	Vienna
Ljupcho JANKULOSKI	Plant Breeder/Geneticist	L.Jankuloski@iaea.org	21623	Vienna
Cinthya ZORRILLA	Plant Breeder/Geneticist	C.Zorrilla@iaea.org	21618	Vienna
Katayoun ENTEKHABI	Programme Assistant	K.Entekhabi@iaea.org	21621	Vienna
Luis Mauricio ALFONZO GODOY	Team Assistant	L.M.Alfonzo-Godoy@iaea.org	21620	Vienna
Ivan INGELBRECHT	Laboratory Head	I.Ingelbrecht@iaea.org	28285	Seibersdorf
Anupama HINGANE	Plant Breeder/Geneticist	A.Hingane@iaea.org	28268	Seibersdorf
Norman WARTHMANN	Molecular Geneticist	N.Warthmann@iaea.org	28260	Seibersdorf
Samira TAJEDINI	Consultant	S.Tajedini@iaea.org		Seibersdorf
Hassan MDUMA	Consultant	H.Mduma@iaea.org		Seibersdorf
Radisras NKURUNZIZA	Consultant	R.Nkurunziza@iaea.org		Seibersdorf
Mirta MATIJEVIC	Technician	M.Matijevic@iaea.org	28317	Seibersdorf
Joanna Beata JANKOWICZ- CIESLAK	Technician	J.Jankowicz@iaea.org	28275	Seibersdorf
Adel ALI	Technician	A.Ali@iaea.org	28427	Seibersdorf
Karynne ABEL	Team Assistant	K.Abel@iaea.org	28750	Seibersdorf
Zhizhou NIU	Intern	Z.Niu@iaea.org		Seibersdorf
Jing SONG	Intern	J.Song@iaea.org		Seibersdorf
Emma RAMIREZ	Intern	E.Ramirez@iaea.org		Seibersdorf

Plant Breeding and Genetics Section

Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture Wagramer Strasse 5, P.O. Box 100, 1400 Vienna, Austria Phone: +431 2600 + extension; Fax: +431 26007

Plant Breeding and Genetics Laboratory

FAO/IAEA Agriculture & Biotechnology Laboratories, 2444 Seibersdorf, Austria Phone: +431 2600 + extension; Fax: +431 26007

## Staff News

#### Welcome



Ms Karynne Abel (USA) joined the PBG and SWMCN Labs in September 2022 as Team Assistant to both. She studied philosophy and Spanish, some law, teaching English as a second language, German, and sex education in the United States (Louisiana

State University), Spain (Universidad de Granada) and Austria. Karynne brings extensive administrative, paralegal and teaching experience, having most recently worked in Publishing at the IAEA. A Louisiana native and environmentalist, Karynne loves the great outdoors, gardening, writing, and traveling. She happily lives in Vienna since 2010.



Ms Emma Ramirez (USA) joined the PBGL in October 2022 as a US-funded intern on a Banana Fusarium Wilt disease project. Born and raised in California, Emma is a recent graduate of the University of California Davis, where she studied genetics and genomics. At UC Davis, Emma also

worked as a Laboratory Assistant researching the gene and regulatory networks required for seed development, and she interned at the student farm in the organic plant breeding programme. These experiences inspired Emma's passion for food security and sustainable agriculture. In her free time she enjoys playing chess, watercolour painting, and spending time with friends and family. Welcome Emma!

#### **Farewell**



Funded by ARGONNE National Labs (USA), Michael joined PBGL as a data science intern from October 2021 through September 2022. Hailing from Houston, Texas, Michael's academic background is in economics and statistics, with a MSc degree in Statistics and Data Science from the College of Business, U Texas, San Antonio, a BSc in

Applied Statistics with a Minor in Mathematics from U Houston Downtown, and a BBA in Economics from Sam Houston State University. Michael was completely new to plant biology, genetics, bioinformatics, and not least to food security and all its challenges but he clearly enjoyed analyzing data. Once he understood the biological questions, he started to make valuable contributions to several of PBGL's analysis protocols. In the process, he learned quite a bit about modern genomics and its applications. We thank Michael for his patient and diligent work and wish him good luck for the future. We are further happy to hear, inspired by his work at PBGL, Michael is now applying to biologyrelated jobs!

## **Feature Articles**

## Space-Induced Genetic Variation and its Possible Role for Crop Improvement under Climate Change

#### Shoba Sivasankar

Plant Breeding and Genetics Sub-Programme, Joint FAO/IAEA Centre of Nuclear Applications in Food and Agriculture, Vienna, Austria

Spontaneous mutations are the bases of evolution, and together with chromosomal crossovers, have driven the genetic variation implicit in crop domestication and crop improvement for centuries. With induced mutations, the process of crop improvement can be hastened. The Joint FAO/IAEA Centre has continued to support Member States over the last fifty plus years in the use of induced mutations for crop breeding and improvement through technology development and capacity building. With increasing challenges to crop production and food security from climate change, induced mutations assume critical significance as they generate novel genetic variation for crop improvement.

Gamma- and x-rays have been the predominant agents used to induce genetic variation for plant mutation breeding since the 1930s. Currently, heavy ion beam, electron beam, proton beam and cosmic radiation are coming into increasing use. Information remains sparse, however, on standard procedures for inducing mutagenesis in plant seeds with some of these sources, the nature of DNA structural variations generated, and advantages, if any, of a particular source in developing specific plant traits. A new Coordinated Research Project (CRP) of the Joint Centre, D24015, aims to conduct a comparative assessment of DNA structural variations and plant biology induced by different mutagens. As part of this CRP, a Feasibility Study on Seed Irradiation in Space for Induced Genetic Diversity and Plant Mutation Breeding was launched to the International Space Station (ISS) in November 2022.

By definition, "outer space" begins 100 km above the Earth's sea level, at the so-called Kármán line, the boundary between aeronautics and astronautics as accepted by the Fédération Aéronautique Internationale. The two physical phenomena that differ significantly in outer space relative to Earth's surface are gravitation and radiation. Space radiation includes photons emitted by the sun, and energetic particles from the solar wind and galactic cosmic rays (GCR). The electromagnetic radiation from the sun includes spectral categories ranging from gamma rays to radio waves. The non-electromagnetic radiation environment in interstellar space is dominated by GCRs, which consist of 95% protons, 4% helium and about 1% heavier nuclei,

electrons and positrons. The critical difference among the various possible Earth orbits, and other locales, for many astrobiology experiments is the radiation information. The ISS in Low Earth Orbit or LEO is at an altitude of 330-435 km from the earth's surface, at an inclination of 51.6°, with an orbital period of 91-93 minutes around the earth, and experiences mainly electrons and protons as the predominant particle radiation sources. At high inclination LEO at altitudes of 400-2000 km, the radiation sources include electrons, protons, GCRs and solar energetic particles (SEPs).



The Cygnus Spacecraft. Photo: NASA

The unique conditions of microgravity and radiation in Earth Orbit have motivated a series of biological experiments since the beginning of space exploration in the late 1950s. Microgravity has been the focus of most experiments and its effects on plant growth were studied in the ISS or in simulated space environments on Earth in Space Labs such as the NASA Space Life Sciences Laboratory. These fundamental biology experiments attempted to understand the ability to grow plants productively under microgravity during space missions, and results show effects on cell proliferation and growth, gene expression and epigenetics. Most recently, experiments have been initiated with the aim of utilizing the effects of microgravity on DNA to select for superior plant material with adaptability to harsh growing conditions. In Jan 2021, a payload of 320 cuttings of grapevine were returned to earth from the ISS where they were held for ten months in the internal environment of the

ISS. In this experiment, researchers at the space science company, Space Cargo Unlimited, are investigating the effects of microgravity on grapevine genome and physiological performance. With a similar research purpose, the company, Front Range Biosciences sent tissue cultures of hemp and tobacco to the ISS in 2020.

Astrobiology experiments investigating the effects of space radiation on organisms are fewer, and most have focused on survivability of plant seeds and microorganisms. The first exposure of microorganisms to space radiation was conducted on sounding rockets in 1965 at 150 km, then extended to the Gemini 9 and 12 missions in 1966 at 300 km, and finally as the Apollo 16 mission was flying back to earth from the moon in 1974. Since then, a series of biological experiments were conducted to prove that life can survive in the extremely harsh conditions of space, using bacteria, algae, lichens and to a limited extent, plant seeds. These experiments were implemented on specific platforms such as the Long Duration Exposure Facility (LDEF), the EUropean REtrievable Carrier (EURECA), the space station MIR, the research programme Bioinformatics Methodology for Pathway Analysis (BioPAN), the Externally mounted payloads for 1st utilization phase, EXPOSE, on the ISS, the TANPOPO experiment at the Japanese Experiment Module (JEM) on the ISS, the Organism/Organic Exposure to Orbital Stresses (O/OREOS), and its Space Environment Survivability of Living Organisms (SESLO) payload.

Radiation in outer space has been used for mutation induction and crop improvement at least in China, and many improved crop mutant varieties have been released in the last fifteen years from space-induced mutagenesis. These were developed by exposure of seeds to space in orbiting satellite(s), high-altitude balloons or simulated cosmic radiation on earth, and used the combination of radiation and microgravity unique to outer space to induce mutations.

Scientific information on the mutagenic effects of the space environment at the genomic and physiological levels of crop plants is limited in published literature. However, it has been reported that in wheat seeds sent to space in the recoverable satellite, Shijian-8, the comprehensive factors of the space environment induced the most frequent mutations, indicating a synergistic effect between radiation and microgravity, while the individual effect of microgravity was much lower than that of radiation (Guo et al., 2010). Also reported is the discovery of pathways and genes involved in mutants with tolerance to salinity generated from spaceflight mutagenesis (Xiong et al., 2017). Other fundamental physiological investigations were undertaken by researchers in the EU, the USA and the former USSR prior to the genomic era and also more recently. These studies have used the ISS or the Svet Greenhouse onboard the Russian Space Station Mir. Almost all these studies used

the internal space environment of the ISS or Mir. The few studies that explored external exposure of plant seeds in space were part of research projects approved to participate in the European Space Agency's exposure facility, EXPOSE, mounted on the balcony of the European Columbus module in 2008 (EXPOSE-E) or on the external URM-D platform of the Russian Zvezda module in 2009 (Rabbow et al., 2009).

Currently, there is increasing interest to understand the effect of the space environment in producing mutations in plant genomes and in modifying plant physiology, to improve the ability of plants to withstand adverse growth conditions on earth. Rapid advances in the field are foreseen with continuing interest to explore plant biology in space both for feeding astronauts and for generating mutations with space exposure to breed resilient crop varieties on earth.



Seeds were carried by the Northrop Grumman's 18th Cygnus Cargo Resupply Spacecraft, on 07 Nov 2022. Photo: NASA

The Joint FAO/IAEA Centre has ventured into the field of astrobiology and space breeding for the first time in November 2022 with its feasibility study under CRP, D24015. Seeds of the plant species, *Arabidopsis thaliana* and *Sorghum bicolor*, will be hosted both within and outside the ISS for a limited time. The seeds were part of the cargo of the CRS2 NG-18 mission that launched from NASA's Wallops Flight Facility on 7 November 2022. This feasibility study sets the Joint Centre on a systematic exploration to understand the effects of cosmic radiation and microgravity on induced genetic variation, and to utilize these for developing crops that can withstand harsh growing conditions on earth as those imposed by climate change.

Part of this feature article is presented in the IAEA Nuclear Technology Review 2022.

#### References

Guo H et al. (2010) Acta Agronomica Sinica, 36(5) Rabbow et al. (2009) Orig Life Evol Biosph 39:581-598 Xiong H et al. (2017) Sci Rep 7(1):2731

## Advances in Space-Induced Mutation Breeding in China

#### Luxiang Liu

Deputy Director General of the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), President of the Chinese Society of Nuclear Agricultural Sciences, Beijing, China

It is estimated that food production needs to increase by 50% worldwide over the next 30 years under the increase of extreme climatic events caused by global warming and the expanding of the world's population (Hatfield and Beres, 2019). The development of elite crop varieties through induced mutations constitutes an important and effective strategy to ensure future food security (Pathirana, 2011). Apart from the traditional mutation induction techniques such as gamma-ray irradiation and chemicals, a new approach, space-induced mutagenesis, is increasingly used for crop improvement in China. Significant progress has been achieved in the optimization of techniques, gene identification and the discovery of molecular mechanisms associated with mutant lines, development of elite crop resources and new varieties, and the establishment of national research network and platforms.

#### Optimization of the techniques for spaceinduced mutagenesis

In September 2006, the plant breeding satellite, Shijian 8, was launched and recovered to conduct basic and applied research in space breeding. To efficiently develop new resources and varieties for improved performance through space-induced mutagenesis, ground-based simulation techniques and high-throughput mutant gene screening approaches were also established and optimized. A diverse technical platform for simulation of space environment was established by high energy and heavy ion beam (<sup>7</sup>Li, <sup>12</sup>C) irradiations, mixed particle field, and magnetic-free space treatment using high-energy accelerators and irradiation facilities. Since 2016, the irradiation accuracy of <sup>12</sup>C ion beam has been highly improved, and the irradiation target room was optimized for automatic sample replacement and significantly increase the amount available for irradiation.



*Figure 1*. *TILLING platform for the identification of mutations induced in genes.* 

Further, a TILLING platform for mutant gene screening (Figure 1) and a double haploids-based *in vitro* mutagenesis technique were established to develop new wheat varieties.

## Gene mapping and the study of molecular mechanisms driving mutant traits

Through space-induced mutagenesis and ground-based simulation techniques, crop mutants involved in many trait variations were identified, such as leaf color, plant architecture, grain size and resistance to biotic or abiotic stresses. Using these mutants, researchers explored genes associated with important agronomic traits, identified the mechanisms of phenotypic variation, evaluated molecular features of space-induced mutation, and mapped the dwarfing gene from maize mutant *sil1*.

The mechanisms behind phenotypic variations in the leaf color mutant *eal1* in maize, as well as the albino mutant *mta* (Shi et al., 2017) and the salinity-tolerant mutant *st1* in wheat (Xiong et al., 2017) were also explored. A transcriptome sequence variation analysis of the space-induced mutant, *st1* of wheat, was conducted, which detected a higher number of SNPs compared to InDels, and more transitions than transversions between the wildtype and mutant (Xiong et al., 2017). Retrotransposon insertion was identified in two dwarf maize mutants, indicating that spaceflight may promote transposition of the LTR retrotransposon (Li et al., 2020)



*Figure 2.* Study of the molecular mechanisms of the salinity-tolerant wheat mutant, st1, induced by spaceflight

# Development of new mutant germplasm and crop varieties

Based on established space-induced or simulated mutation techniques, eight mutant populations were constructed for various crops including wheat, maize, rice, soybean, and cotton. The main associated traits in wheat, for example, include biotic and abiotic stress resistance, plant height, tiller number, spike length and thousand-grain weight, etc. From these mutant resources, hundreds of elite mutant lines with improved important agronomic traits, such as high anther culture frequency, high yield, fine quality, and strong disease or stress resistance were developed and used for crop breeding. For instance, by using a high anther culture frequency and high yield potential wheat mutant line SPLM2, several wheat mutant varieties such as Hangmai 2566 and Hangmai 501 have been developed. A diseaseresistant soybean mutant line has been used for development of the varieties Suinong 86 and Suidou 5.



Figure 3. Wheat germplasm developed by induced mutagenesis

By space-induced mutation breeding, China has officially released more than 260 mutant varieties in rice, wheat, maize, soybean, cotton, sesame, sweet pepper, tomato, etc. Notably, 54 mutant varieties including 21 in wheat, 15 in rice and 7 in maize have been officially released since 2016.



*Figure 4.* Field production of, and national award for, the wheat mutant variety, Luyuan 502, developed through a combination of space mutagenesis and cross breeding.

Among these, the wheat mutant variety, Luyuan 502, (Figure 4), which was officially released nationally in 2011 represents the second leading variety and has been cultivated in more than 7.7 million hectares in total in China. It has a grain yield of 12.18 t/ha in large-scale production area, which is a 12% higher yield increase over existing checks, and improved tolerance to drought and major diseases.

These numbers reflect the significant benefits and socialeconomic impacts to farmers, and the achievement was awarded the 1st Prize of Shandong Award for Progress in Science and Technology in 2018 and the 2nd Prize of the National Science and Technology Progress Award 2019.

#### National network and platforms for spaceinduced mutagenesis research

Under the support from The National Key Research and Development Program of China and RCA/TC/CRP projects of the IAEA, national research working team on spaceinduced mutagenesis was formed from across various scientific research institutes, colleges and universities in China. This enabled us to train a large number of young scientists to promote the advancement of the space breeding technology.

Three irradiation platforms for simulation of space-induced mutagenesis were established, including the tandem accelerator-based <sup>7</sup>Li ion beam irradiation, the cyclotron-based <sup>12</sup>C ion beam irradiation, and the atmospheric and room temperature plasma (ARTP) mutation system. These platforms provide basic facilities for mutation induction and breeding.

The laboratory *Wheat Mutation Breeding Team of Institute* of Crop Sciences, Chinese Academy of Agricultural Sciences, has been authorized as an IAEA Collaborating Center for Nuclear Techniques in Plant Mutation Breeding since 2019.

The Asia and Oceania Association of Plant Mutagenesis (AOAPM) have been established, which made it possible for outstanding scientists and their teams to participate in the association and promote the development of plant mutation breeding.

The contributions made by this team in recent years led to outstanding achievement award for plant mutation breeding by the FAO and IAEA through the Joint FAO /IAEA Centre in 2021

The importance of space-induced mutagenesis and groundbased simulation techniques for enhancing genetic diversity and the development of elite crop germplasms and novel varieties will continue to grow in the future.

The integration of new technologies, such as high throughput genomic sequencing for genotyping analysis, phenomics and the precise determination of phenotypes, and pyramiding of target genes regulating important traits will bring significant benefits to space breeding technology designed for crop improvement.

#### References

Hatfield J.L., and Beres B.L. (2019). Front Plant Sci 10:1603.
Li C. et al. (2020). Plant Cell Rep. 39:393-408.
Pathirana R. (2011). Cab Reviews Perspectives in Agriculture Veterinary Science Nutrition & Natural Resources 6:1-20.
Shi K. et al. (2017). PLoS ONE. 12 (5): e0177992.
Xiong H. et al. (2017). Sci Rep 7:2731.

## Enhancement of Germplasm and Genetic Diversity in Rice via Space Mutagenesis in China

#### Tao Guo

National Engineering Research Centre of Plant Space-induced Breeding, South China Agricultural University, Guangzhou, Guangdong, China

#### Introduction

Exposure of plant seeds to cosmic radiation in space, combined with microgravity and other factors in low earth orbit and deep space can introduce mutations across the genome. Compared with traditional radiation mutagenesis in plants, it is possible that space radiation can introduce more DNA lesions thus widening the genetic base for breeding selections. With the steady advancement of space exploration projects in China, space mutagenesis combined with modern phenotyping and genotyping methods have played an important role in enhancing the diversity of germplasm and the development and cultivation of new rice varieties in recent decades.

#### **Space-Induced Mutagenesis in Rice**

The space environment is extreme and complex, and is characterized by radiation, microgravity, vacuum, extreme temperature, day-night disorder and others (Tapan et al. 2021). Space radiation is mainly produced by solar cosmic rays (SCRs) and galactic cosmic rays (GCRs). SCRs and GCRs include high proportions of protons, electron and alpha-particles, and less than 1% heavy ions of charge Z>2 (HZE particles) with high linear energy transfer (LET). The radiation of high LET can penetrate the spacecraft cabin and excite many secondary particles in the spacecraft cabin, including X-rays, gamma rays, protons, HZE and other radiation factors with low dose (Ding et al. 2014). Long term continuous exposure to low-dose composite radiation from different radiation sources could produce considerable mutagenic effects and introduce DNA mutations from single nucleotide polymorphisms (SNP) to large DNA fragments insertion or deletion (In/Del) (Figure 1). Therefore, high LET radiation has been considered the main reason for the variation of organisms in space (Satoshi et al. 2020).



Figure. 1 High LET space radiation causes DNA mutation

# Application of Space Mutagenesis in Rice Breeding

Many germplasm resources have been created using space mutagenesis, and nearly 100 rice varieties have been released. In 1987, the Institute of Genetics of the Chinese Academy of Sciences cooperated with Guangxi Agricultural University to breed a new rice hybrid between indica and japonica subspecies with high seed-setting rate and full seeds (Jiang et al. 1996). Xie et al. bred the restoration lines, Hang 1 and Hang 2, by using space-based mutagenesis technology and developed a series of super-hybrid rice varieties that were widely applied in south China (Xie et al. 2004). Wang et al. obtained space-induced materials such as Hanghui 1173, Hanghui 1179, and the highly rice blastresistant H4 and bred more than 50 rice varieties, including Huahang 1 (Wang et al. 2004). Xu et al. created a multi-tiller dwarf mutant R955 by space mutation, which has application value in cultivating multi-spike rice varieties (Xu et al. 2003). Yan et al. screened the indica rice variety, Zhe9248, carried by the recoverable satellite for disease resistance over many generations, and bred the mutant, Zhe101, that is resistant to rice blast and bacterial blight (Yan et al. 2004). Guo et al. recovered a batch of mutants with different amylose content after space mutagenesis of the indica rice varieties Xianxiaozhan and Shengbashi Miao (Guo et al. 2007). Huang et al. successfully selected a twoline male sterile line, Hang 17S, with low amylose content and low threshold temperature for sterility from the space mutation offspring of Peiai 64S, significantly improving the grain quality of the wild type (Huang et al. 2018).

In recent years, the development of modern high-throughput instruments and the combination with molecular marker assisted selection technology have enabled a space mutation breeding pipeline which conducted targeted screening of mutation populations in successive generations (Chen et al. 2019).

New mutant germplasm can be directly selected for new varieties or indirectly cultivated as a donor parent of superior genes. Luo et al. conducted comparative analysis of rice space induced mutants through genome sequencing and found more frequency of space mutagenesis than gamma-ray radiation (Luo et al. 2014). Recently, researchers from South China Agricultural University screened some mutants from the mutagenesis offspring derived from the space flight of Chang'e 5 lunar exploration vehicle (Figure 2).



*Figure 2.* Mutants obtained by space mutagenesis, WT on the extreme left and mutants on the right

#### **Future Perspectives**

The study of mutagenic effects in plant species in the space environment is a key subject involving multiple disciplines such as space biology, genetics, mutagenesis, and breeding. Although many studies have confirmed the mutagenic effects of the space environment, and a series of varieties have been selected through space mutation, there is still a lack of research on the molecular characteristics and genetic mechanisms of mutations induced by the space environment, especially in a major food crop such as rice.

The following aspects are still worthy of in-depth discussion: 1) Analysis of the synergistic mutagenic effects of microgravity and space radiation; 2) Single-factor analysis and ground simulation of space radiation mutagenic factors; 3) Construction of single-cell mutation map of space radiation-induced mutations and genetic network research; 4) High-efficiency identification and rapid fixation of spaceinduced variations. The development of single-cell sequencing, high-throughput sequencing, and high-throughput detection technologies provides favorable conditions for studying the effects of space-induced mutagenesis and accelerating the utilization of genetic variation at the whole genome level.

#### References

Chen Z, et al. (2019) Journal of South China Agricultural University (40):195-202. doi: 10.7671/j.issn.1001-411X.201905073

Ding N, et al. (2014) Rendiconti Lincei (25):59-63. doi: 10.1007/s12210-014-0288-y

Guo T, et al. (2007) Journal of South China Agricultural University 28(1):6-9. doi:10.3969/j.issn.1001-411X.2007.01.002

Huang M, et al. (2018) Journal of South China Agricultural University 39(2):34-39. doi:10.7671/j.issn.1001-411X.2018.02.006

Jiang X. (1996) Journal of Space Science (16):77-82. doi: CNKI:SUN:KJKB.0.1996-S1-013

Luo W L, et al. (2014) Molecular Breeding 34(3):805-815. doi: 10.1007/s11032-014-0076-5

Satoshi F, et al. (2020) BioMed Research International (1–3):1-25. doi: 10.1155/2020/4703286

Tapan K, et al. (2021) Frontiers in plant science (12):771985. doi: 2021,12:771985. 10.3389/fpls.2021.771985

Wang H, et al. (2004) Journal of South China Agricultural University (4):1-5. doi: CNKI:SUN:HNNB.0.2004-04-001

Xie H, et al. (2004) Chinese Agricultural Sciences (11):1688-92. doi: CNKI: SUN:ZNYK.0.2004-11-00H

Xu J, et al. (2003) Acta Agriculturae Nucleatae Sinica 17(2):90-94. doi: CNKI: SUN:HNXB.0.2003-02-001

Yan W, et al. (2004) Chinese Journal of Rice Science 18(5): 415-419. doi:10.3321/j.issn:1001-7216.2004.05.007

## From Space Breeding to Space Agriculture

#### Prof. Maozhi Ren

Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, China

Prof. Maozhi Ren focuses on space breeding and space agriculture at the Institute of Urban Agriculture of the Chinese Academy of Agricultural Sciences. He is the chief designer of the Biological Experiments Load (BEL) of the lunar rover "Chang'e-4" and Director of Space Biology Laboratory, Joint Research Center for Deep Space Exploration, Ministry of Education, China.



Prof. Maozhi Ren at the Xichang Satellite Launch Center

The unique space environment provides a combination of stressors (e.g., cosmic radiation and microgravity) that organisms have never experienced on Earth. Space breeding is taking crop seeds or tube seedlings to space, using the special environment of space to induce mutations in the seed. Prof. Ren's group has already obtained mutant seeds of cotton from the Chinese Space Station. At present, these have been sown for phenotypic and genotypic investigation.

With the funding from CRP D24015 of the IAEA, his group will mainly focus on those mutants with drought tolerance and will try to clone candidate genes and uncover the underlying molecular mechanism. In the future, his group plans to send more seeds to the Chinese Space Station for space breeding.

During interstellar exploration and colonization, supplying food to outer space bases from Earth is expensive and unsustainable. Therefore, establishing an efficient space farming system based on space crops and achieving food self-sufficiency is crucial for long-term missions and human interstellar immigration.

On January 3, 2019, China's Chang'e-4 probe, carrying a biological experiment payload, successfully landed on the Von Kármán Crater in the South Pole-Aitken Basin on the far side of the moon. In this biological experiment payload, in addition to water, soil and air, Prof. Maozhi Ren and his colleagues deliberately selected three important crops [a food crop, potato (*Solanum tuberosum*); a fibre crop, cotton (*Gossypium hirsutum*); and an oil crop, rape (*Brassica napus*)] and three model organisms [Arabidopsis (*Arabidopsis thaliana*) seeds, fruit fly (*Drosophila melanogaster*) eggs, and yeast (*Saccharomyces cerevisiae*)] to construct a Lunar Micro Ecosystem simultaneously comprising producers, consumers and decomposers.

Under the challenges of space radiation and microgravity, on January 5, it was found that a *G. hirsutum* seed within the payload successfully germinated, developed and grew green radicles. It marked the first time a plant has been grown on the moon. This breakthrough achievement has been widely reported by authoritative media such as *Nature* and *Science*, reflecting the enthusiasm for space agriculture.

Prof. Maozhi Ren is now focusing on the development of functional plants through synthetic biology and genetic engineering for interstellar agriculture.



The lunar microecosystem (LME) carried by the Chang'e-4 lunar rover. In the LME, Arabidopsis, cotton, potato, and rape ac as producers of oxygen through photosynthesis for all organisms to use; the fruit fly (consumer) and yeast (decomposer) use oxygen to produce carbon dioxide for plant photosynthesis. Yeast can decompose wastes of plants and fruit flies to grow, and it can also be used as food for fruit flies.

## **Forthcoming Events**

# Research Coordination Meetings (RCMs) of FAO/IAEA Coordinated Research Projects (CRPs)

Second Research Coordination Meeting (RCM) for CRP D24015, Radiation-Induced Crop Diversity and Genetic Associations for Accelerating Variety Development, Virtual, 6-10 March 2023

First Research Coordination Meeting (RCM) for CRP D23033, Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana – Phase II, Vienna, Austria, 17-21 April 2023

#### **Other Meetings**

National Training Course on Mutation Techniques, Mutation Discovery, Marker Development and Marker Assisted Selection (MAS)- NEP5006, Kathmandu, Nepal, 13-17 March 2023

Regional Training Course on Application of Genomics, Genotyping and Marker-Assisted Selection in Mutation by Speed Breeding (MbyS) (*virtual*)- RAS5088, Q1 2023

National Training Course on Introductory to Mutation Breeding in Crops- HAI0007, Port-au-Prince, Haiti, Q2 2023

Regional Training Course on Methodologies for Improving Crop Resilience to Abiotic Stress Through Nuclear Techniques and Seed Systems- RAS5099, Sakha Kafr Elsheikh, Egypt, Q3 2023

Advance Regional Training Course on Mutation Breeding and Combined Biotechnologies- RAS5099, Sakha Kafr Elsheikh, Egypt, Q3 2023

## **Past Events**

# Research Coordination Meeting (RCMs) of FAO/IAEA Coordinated Research Projects (CRPs)

Third Research Coordination Meeting (RCM) for CRP D22006, Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation (virtual), 17-19 October 2022

First Research Coordination Meeting (RCM) for CRP D24015, Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development (virtual) 8-12 August 2022

Third Research Coordination Meeting (RCM) for CRP D23032, Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change, Kuala Lumpur, Malaysia, 25–29 July 2022

#### **Other Meetings**

National Training Course on Improving Crop Adaptation to Abiotic Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods-BDI5005, Bujumbura, Burundi, Q4 2022

Workshop on Mutation Induction and Mutation Breeding-CHI5054, Santiago, Chile, 12 December 2022

Regional Training Course on Application of Double Haploidy for Mutation by Speed Breeding (MbyS) Towards Crop Improvement-RAS5088, Jakarta, Indonesia, 5-9 December 2022

National Training Course on Nutritional Quality Screening Using NIR Spectroscopy- CUB5023, La Habana, Cuba, 5-9 December 2022 Workshop on Applications of Nuclear Technologies in Forest Conservation and Improvement- CHI5052, Concepcion, Chile, 29 November 2022

National Training Course on Seed Production and Multiplication of Mutant Lines of Crucifiers and Carrot-MAR5029, Reduit, Mauritius, 28 November- 2 December 2022

National Training Course on Plant Breeding Using Nuclear Technology-PAR5012, San Lorenzo, Paraguay, 28 November- 2 December 2022

Regional Training Course on Field Experimental Design and Data Analysis for the Advancement of Mutant Populations-RER5024 (*virtual*), 21 November – 2 December 2022

National Training Course on Molecular Methods for Identification of Mutations-NIC5011, Managua, Nicaragua, 7-11 November 2022

National Training Course on Molecular Techniques for Crop Improvement of Seed-Crops-GHA5039, Accra, Ghana, 7-11 November 2022

Vegetatively Propagated Crops - MAK5010, Skopje, North Macedonia, 31 October – 4 November 2022

National Training Course on Selection Methods and Regional Training Course on Mutation Breeding in Vegetatively Propagated Crops, Including Micropropagation Using Cell/Tissue Culture, Mutation Induction and Selection- RAF5083, Dakar, Senegal, 24 October-4 November 2022

National Training Course on Selection for Biochemical Traits with Improved Quality and Yield in Mutant Rice and Cassava Lines- SIL5021, Njala, Sierra Leone, 17-21 October 2022

Regional Training Course on Detection and Diagnostics of Fusarium Tropical Race 4 (*Foc* R4T)- INT5158, Mexico City, Mexico, 10-14 October 2022

National Training Course on Mutation Breeding for Salinity Tolerance in Rice- URT5037, Morogoro, United Republic of Tanzania, 3-7 October 2022

National Training Course on Molecular Techniques for Crop Improvement of Vegetatively Propagated Crops, GHA5039- Accra, Ghana, 3-7 October 2022

Regional Training Course on Plant Mutation Breeding and Efficiency Enhancing Techniques to Increase Resilience to Climate Change- RAS5099 and RLA5084, (*virtual*), 26 September-7 October 2022

Regional Training Course on Mutation Induction and Mutation Breeding- RAS5098, Suva, Fiji, 26 September-7 October 2022

National Training Course on Evaluation of Grain Nutritional Quality- MAL5032 (*virtual*), 26-30 September 2022

National Training Course on SDS-PAGE Screening for Protein Quality in Mutant Lines- SUD5041, Khartoum, North Sudan, 25-29 September 2022

National Training Course on Mutation Breeding Techniques and Crop Improvement- MAU5009, Kaedi, Mauritania, 28 August -1 September 2022

Regional Training Course on Statistically Rigorous, Pre-Field and Field Screening to Select Improved Mutant Lines- RAF5083, Dakar, Senegal, 8-19 August 2022

More information from Forthcoming and Past Events can be found from pages 25 to 34

National Training Course on Mutation Breeding in Maize Crops and Developing Resistance to the Fall Army Worm- CAF5013, Bangui, Central African Republic, 9-12 August 2022

National Training Course on Improving Crop Adaptation to Abiotic Stresses Using Nuclear-Derived Techniques, and Molecular-Breeding Method-LIR5003, Monrovia, Liberia, 1-12 August 2022

Regional Training Course on Molecular Techniques for Crop Improvement of Mutant Populations- RER5024 (*virtual*), 1-5 August 2022

Regional Training Course on Plant Mutation Breeding and Associated Biotechnologies in Seed Propagated Crops at National Research and Innovation Agency (BRIN), Jakarta, Indonesia, 18-29 July 2022

National Training Course on Polyploid Induction as a Breeding Strategy for Creole Potatoes- COL5026, Bogota, Colombia, 11-15 July 2022

Mid-Term Regional Meeting on Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia- RER5024, Faro, Portugal, 4-8 July 2022

Regional Training Course on Mutation Breeding on Vegetatively Propagated Crops- RLA5084 (virtual), 20- 24 June 2022

National Training Course on Improving Crop Adaptation to Drought Stresses Using Nuclear-Derived Techniques, and Molecular-Breeding Method-ERI5013, Asmara, Eritrea, 7-17 June 2022

## Coordinated Research Projects (CRPs)

Project Number	Ongoing CRPs	Project Officers
D20003	Impact Assessment of Mutant Crop Varieties Developed by BINA, Bangladesh (Single-contract project) (2019–2021)	S. Sivasankar
D20004	Impact Assessment of Cotton Mutant Varieties Developed by NIAB, Pakistan (Single-contract project) (2020–2022)	L. Jankuloski
D23032	Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change (2018–2023)	L. Jankuloski N. Warthmann
D22006	Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation (2019–2024)	A. Hingane S. Sivasankar
D24014	Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops (2021–2025)	I.K. Bimpong S. Sivasankar
D24015	Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development (2022-2027)	S. Sivasankar
D23033	Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana – Phase II	C. Zorrilla S. Sivasankar

## CRP D20003: Impact Assessment of Mutant Crop Varieties Developed by BINA, Bangladesh

#### Project Officer: S. Sivasankar

This is a single-contract CRP with the overall objective of estimating the economic impacts of promising mutant rice varieties developed by the Bangladesh Institute of Nuclear Agriculture (BINA).

The specific objectives are (1) to estimate the aggregate economic benefits associated with BINA-developed mutant rice varieties; (2) to determine the annual production and profitability trends of BINA-developed mutant rice varieties by location and variety; (3) to assess the adoption level of BINA-developed mutant rice varieties in all areas; (4) to identify farmers' preferences and constraints for cultivation of mutant rice varieties; and (5) to suggest policy guidelines/recommendations.

A first paper, Comparative Profitability and Impact of BINA Developed Aman Mutant Rice 'Binadhan-7' with Non-Mutant in Bangladesh has been published based on the results. On average, the total cost of production was US \$696 per hectare, with 29% for fixed costs and 71% for variable cost. The average net return for the cultivation specifically of the mutant rice variety, 'Binadhan-7', ranged from US \$394 per hectare in the Jashore region to US \$828 in the Dinajpur region. The average benefit cost ratio for Binadhan-7 production on total cost basis was 1.90 relative to 1.43 for farmers growing other varieties in the study region. Short duration was ranked as the highest preferred trait by farmers and high yield was ranked fifth among the five trait options in the survey. High cost of labour was identified as the main constraint in cultivation. The study also revealed that the short duration, high yielding rice variety, 'Binadhan-7', plays a vital role in hunger mitigation of the northern areas of Bangladesh.

A second publication with more information on impact assessment is expected soon.

CRP D20004: Impact Assessment of Cotton Mutant Varieties Developed by NIAB, Pakistan

#### Project Officer: L. Jankuloski

An individual CRP on Impact Assessment of Cotton Mutant Varieties Developed by the Nuclear Institute for Agriculture and Biology (NIAB), Pakistan, was initiated in 2020.

The overall objective of this single-contract CRP is to evaluate the economic impact of the cotton mutant varieties developed by NIAB.

The specific objectives of this CRP are (1) to evaluate economic returns from mutant cotton varieties; (2) to assess adoption of mutant cotton varieties in different cotton growing areas of the country; (3) to identify the constraints to adoption of mutant cotton varieties in Pakistan; (4) to assess and determine annual production of NIAB cotton mutant varieties; (5) to evaluate the total area covered with cotton mutant varieties, their yield and economic benefits in total and of each mutant variety; and (6) to provide policy recommendations for promotion of mutant cotton varieties.

The CRP was extended for one more year and will complete at the end of 2022.

# CRP D23032: Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change

#### Project Officer: L. Jankuloski, N. Warthmann

Changing climatic conditions are helping diseases to spread to new localities and exacerbating their impact. In addition to the already widespread diseases of rice, such as blast, sheath blight, false smut and bacterial leaf blight, emerging diseases like wheat blast are increasingly becoming serious threats. To minimize the impact of such diseases, mutation breeding is a viable technique.

Progress made on planned activities since 2018 is satisfactory and even excellent in certain projects. Some important achievements are (1) the development of specific primers for *Magnaporthe oryzae* Triticum isolates and Recombinase Polymerase Amplification (RPA) rapid detection method of wheat blast pathogen, and (2) the identification of five blast resistant wheat mutant lines in  $M_3$ generation. In addition, six wheat orthologs of rice blast susceptible genes were identified uncovering a total of 171 SNPs in  $M_3$  wheat population.

In rice, three highly resistant and two moderately resistant mutant lines to Bacterial Leaf Blight (BLB) were identified in  $M_3$  generation. The development of F2 generation for phenotyping is in progress. Additionally, two mutant lines showing significantly lower infection of false smut disease in field hotspot (plus artificial inoculation) were identified and four rice mutant lines as putative mutants resistant to bakanae disease (*Gibberella fujikuroi*) were selected. Collaboration among CRP participants are strong, and seed materials from wheat mutant populations and lines were exchanged for blast phenotyping in Bangladesh.

The CRP officially started in September 2018 and had its first Research Coordination Meeting (RCM) from 10 to 14 December 2018 in Vienna, Austria. The second RCM was held virtually from 26 to 30 April 2021. The third RCM was held in July 2022 in Kuala Lumpur, Malaysia.

#### CRP D22006: Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation

#### Project Officer: A. Hingane, S. Sivasankar

In this CRP, induced genetic variation and genomics technologies will be used for productivity improvement of three pulses, namely, chickpea, cowpea and lentil, which together account for 40% of global pulses' production. The research focus is on enhancing the tolerance of (1) chickpea to the pod borer, *Helicoverpa armigera*; (2) cowpea to the bod borer, *Maruca vitrata*, and (3) lentil to the disease, *Stemphylium* blight.

Specific research objectives are (1) to generate genetic diversity in chickpea, cowpea and lentil through mutagenesis for resistance to *Helicoverpa armigera*, *Maruca vitrata* and *Stemphylium botryosum*, respectively;

(2) to develop and/or refine phenotyping tools to facilitate precise and efficient selection of biotic-stress resistance in selected pulse crops; and (3) to develop genomic tools for accelerated variety development for the selected pulse crops and associated traits of interest.

The CRP which started in 2019 had its first RCM in September 2019 in Vienna, Austria. The second RCM took place virtually from 6 to 10 September 2021 (more information on page 12), and the third RCM was held virtually in November 2022.

CRP D24014: Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops

#### Project Officer: K. Bimpong, S. Sivasankar

The CRP will provide outcomes that can guide National Agricultural Research Systems (NARS) in Member States to accelerate the development of new varieties of vegetatively propagated crops (VPCs) and horticultural tree crops (HTCs) through the use of efficient state-of-the-art technology packages.

The CRP aims to develop new genetic resources and technologies for accelerated breeding in VPCs and HTCs through induced genetic diversity, chimera-free regeneration, and functional genomics. It will lead to the generation of (a) stable mutant clones that are free-of-chimeras and characterized at the genetic and molecular levels for traits of interest; and (b) publication of protocols for phenotyping and genomic analyses for Member States.

The CRP includes 13 participating countries from Member States where the crops are grown extensively, some advanced institutions and the CGIAR research centres with the respective mandates. Each country will bring together researchers covering the fields of micropropagation, advanced functional genomics for variant discovery and the use of nuclear techniques to induce genetic diversity at the cell or tissue level in selected VPCs and HTCs to address the research objectives.

CRP D24015: Radiation-Induced Crop Diversity and Genetic Associations for Accelerating Variety Development

Project Officer: S. Sivasankar, C. Zorrilla

CRP D24015 was approved in October 2021 and was launched in April 2022 with its first Research Coordination Meeting.

Breeding with induced genetic diversity has remained a highly effective avenue for the improvement of both simple and complex crop traits in Member States of the FAO/IAEA. Mutation breeding has mainly relied on gamma rays, but most recently the ion beam, electron beam, proton beam and space irradiation (cosmic rays) are coming into increasing use at least in some Member States, though the effect of these different sources on the plant genome remain to be assessed systematically. Newer genomic technologies that establish genetic associations for marker and candidate gene discovery also remain yet to be applied to mutation breeding for increased precision and breeding efficiency.

Mutant populations generated from induced genetic variation are traditionally used directly as source germplasm for breeding and variety development. However, they can also render themselves to the establishment of genetic associations for marker-assisted breeding and gene editing. Theoretically, mutant populations can also be used for genomic predictions for increased efficiency of the breeding process.

The overall objective of this CRP is to strengthen the ability of the Agency and its Member States to develop and test emerging technologies in mutation induction, genomics and big data to facilitate the accelerated development of crop varieties for food security and climate-change adaptation.

#### CRP D23033: Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana – Phase II

#### Project Officer: C. Zorrilla, S. Sivasankar

The CRP D23033 was approved in September 2022 and has opened calls for proposals from mid-October to mid-December. This project is expected to launch with its first Research Coordination Meeting in April 2023.

This CRP is aimed at improving disease resistance in banana and developing microbes with enhanced beneficial activities through induced mutagenesis for the management of Fusarium wilt (*Foc* TR4) disease. Specific research objectives are (1) to generate induced genetic diversity in bananas using physical mutagenesis for developing resistance; (2) to generate functional genomics tools and methodologies for understanding the mechanisms of disease resistance using available resistant germplasm that will contribute to markers development and gene editing; (3) to develop rapid and reliable diagnostic protocols for field detection of the pathogen; and (4) to develop protocols for physical mutagenesis of microbes for enhanced biocontrol and plant growth promotion activities, and evaluation against the disease.

#### **Forthcoming Events**

#### Research Coordination Meeting (RCM)

Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development, D24015

6-10 March 2023

First Research Coordination Meeting (RCM) Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (FoC TR4) in Banana – Phase II D23033

Vienna, Austria, 17-21 April 2023

#### Past Events

Third Research Coordination Meeting (RCM) Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change, D23032 Kuala Lumpur, Malaysia, 25-29 July 2022 Project officer: L. Jankuloski

The meeting aimed to assess progress, consolidate activities among participating research teams and discuss further activities.

The 3rd RCM was hosted by the Malaysian Nuclear Agency (Nuclear Malaysia) Ministry of Science, Technology & Innovation (MOSTI) in Kuala Lumpur, Malaysia. The RCM was attended in person by 7 participants from 7 Member States. Participants from India, China (2) and USA attended virtually. Participants presented progress and achievements during the period since December 2018.

Progress on planned activities was satisfactory and even excellent in certain projects, despite delays and postponed activities during the COVID-19 pandemic period. Specific recommendations were made for those producing mutant populations, developing F2 generations, and for genotyping F2 populations. Important achievements are:

-Developed and published protocol for rapid detection of wheat blast isolates

-Developed and deployed two (2) genome-specific primers from wheat and rice blast isolates in diagnostic PCR and LAMP (Loop-mediated Isothermal Amplification) method for specific detection of wheat blast

-Identified five blast resistant wheat mutant lines in M4

-Identified functional mutant alleles. Uncovered a total of 171 single nucleotide polymorphisms (SNPs) in a M2 wheat population, including 81 in exon and promoter regions

-Developed F1 (cross between blast resistant mutant line and susceptible parent), and F2 development is in progress

-Identified 3 highly resistant and 2 moderately resistant mutant lines for bacterial leaf blight (BLB) in rice

-Developed one F2 population to map gene(s) resistant to BLB (two different crosses between rice mutant lines resistant to BLB and susceptible parent/genotypes)

-Developed three (3) F2 populations to map rice blast resistant gene(s)

-In progress are the development of one F2 population to map BLB resistant gene(s), and two F2 populations to map blast resistant gene(s)

-All participants have identified new mutant plants/lines with resistance to rice blast, rice BLB, wheat blast, or other rice diseases, which are at M3 or advanced generations

Seeds from wheat mutant lines and populations were exchanged for blast phenotyping in hot spot areas in Bangladesh. Future activities include publication of the results, and collaborations for genotyping.



**D23032** Participants from the Third RCM on Disease Resistance in Wheat for Better Adaptation to Climate Change

#### Third Research Coordination Meeting (RCM) Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation- D22006 Virtual. 17-19 October 2022

Project officer: A. Hingane, S. Sivasankar

The recently held third RCM for CRP D22006 reviewed progress to date and discussed workplan activities for 2023.

The progress made so far is excellent and in line with individual work plans. Good collaboration has been established among CRP participants, and screening protocols have been standardised or optimized and shared to facilitate precise and efficient selection of tolerant mutants. Putative chickpea and cowpea mutants with tolerance to the pod borer and lentil mutants with tolerance to *Stemphylium* blight were identified. These are in evaluation and advancement to develop new genomic resources and release as new varieties. Several mutant populations were generated and phenotyped for target traits in all three crops.

The team at the Bhabha Atomic Research Centre, India, has identified three cowpea mutant lines, currently at M5, with tolerance to the pod borer, *Maruca vitrata*. These are planned for further evaluation in State and National trials towards variety release.

The research team at the University of Zambia has identified important mutant traits such as raised peduncles (Fig. 1a) and unique pod orientation (Fig. 1b).



*Fig. 1a*. Cowpea mutant with long peduncle (Photo: Kelvin Kamfwa)



*Fig. 1b.* Cowpea mutant with unique pod orientation (Photo: Kelvin Kamfwa)

These mutant lines, currently in M3, are tolerant to the cowpea pod borer, and the raised peduncles and unique pod orientation appear to contribute to this tolerance.

In chickpea, the project participants from the International Crop Research Institute for the Semi-Arid Tropics in India have developed a dense genetic map and QTL analysis for component traits in the resistance to the chickpea pod borer (*Helicoverpa armigera*). Nine main-effect QTLs were identified that explain up to 42.49% PVE identified.

In lentil, CRP participants at the Spanish Council of Scientific Research (CSIC) have standardized screening protocols under controlled conditions for resistance to *Stemphylium* blight using mycelium as opposed to existing protocols with spore inoculum. The team identified putative M2 mutants that can be made available to interested researchers across the globe. Further, in support of ongoing research activities under this CRP, the PBG Laboratory has developed a Rapid Generation Advancement (RGA) technique for lentil that is ready for deployment to interested Member States, towards faster generation advancement for fundamental research and variety development.

# **Technical Cooperation Field Projects**

Project Number	Country/Region	Title	Technical Officer(s)
BDI5005	Bangladesh	Enhancing Productivity of Staple Crops Using Nuclear- derived Technologies	I.K. Bimpong with SWMCN
BKF5024	Burkina Faso	Improving Food Crops through Mutation Breeding and Best Soil and Nutrient Management to Ensure Food Security	A. Hingane with SWMCN
BOL1012	Bolivia	Strengthening National Capacities on Irradiation Technology	C. Zorrilla with NAPC
BOT5019	Botswana	Improving Selected Legumes and Cereals against Biotic and Abiotic Stresses to Improve Food Production and Security	I.K. Bimpong
BOT5024	Botswana	Improving Selected Legumes and Cereals against Biotic and Abiotic Stresses for Enhanced Food Production and Security	I.K. Bimpong with SWMCN
BUL5016	Bulgaria	Improving the Productivity and Quality of Economically Important Crops through Mutation Breeding and Biotechnology	C. Zorrilla
CAF5013	Central African Republic	Improving Productivity of Maize and Developing Resistant Armyworm Maize Varieties Using Radio-Mutagenesis Techniques	S. Sivasankar
CAF5015	Central African Republic	Improving Productivity of Maize and Developing Resistance to Fall Armyworm Using Radiation-Induced Novel Genetic Diversity — Phase II	S. Sivasankar
CHI5052	Chile	Using Nuclear Techniques to Improve the Adaptation and Productivity of Forest Species Facing Climate Change	C. Zorrilla
CHI5054	Chile	Use of Irradiation and Isotopic Techniques to Improve Native and Agricultural Plant Genetics	C. Zorrilla
COL5026	Colombia	Enhancing Crop Productivity of Creole Potato Using Nuclear and Related Techniques	I.K. Bimpong with SWMCN
CPR5024	China, People's Republic of	Enhancing the Accelerated Application of Mutant Germplasm and High-Efficiency Breeding in Crops	N. Warthmann
CUB5023	Cuba	Strengthening National Capacities for the Development of New Varieties of Crops through Induced Mutation to Improve Food Security While Minimizing the Environmental Footprint	C. Zorrilla with SWMCN
ECU5034	Ecuador	Improving the Resilience of Bananas to their Major Diseases through Mutation Breeding Techniques	S. Sivasankar
ERI5011	Eritrea	Developing Improved Banana and Maize Varieties through Mutagenic Nuclear Techniques	I.K. Bimpong
ERI5013	Eritrea	Improving Food Crop Varieties through Mutation Breeding and Related Technologies	I.K. Bimpong
GHA5038	Ghana	Using Irradiated Pollen for the Development of Provitamin A Rich, Drought Tolerant and Cassava Mosaic Disease Resistant Cassava Mutants	C. Zorrilla
GHA5039	Ghana	Mainstreaming Nuclear Based Climate Smart Agriculture Technologies into Sustainable Production	C. Zorrilla with IPC <i>and</i> SWMCN
HAI0007	Haiti	Building National Capacity in Nuclear Technology Applications	I.K. Bimpong with NAPC
HON5009	Honduras	Improving Genetic Resistance of Coffee-to-Coffee Leaf Rust through Mutation Breeding	L. Jankuloski

Project Number	Country/Region	Title	Technical Officer(s)
INS5044	Indonesia	Using Nuclear Technology to Support the National Food Security Programme	S. Sivasankar with SWMCN
IRA5015	Iran, Islamic Republic of	Enhancing Capacity of National Producers to Achieve Higher Levels of Self-Sufficiency in Key Staple Crops	L. Jankuloski with FSC <i>and</i> SWMCN
IRQ5023	Iraq	Utilizing Nuclear Technology to Improve Key Legume Crops for Climate Change Adaptation	I.K. Bimpong
IVC5040	Cote d'Ivoire	Improving Agricultural Production of Maize, Rice and Cassava through Cultivation of Induced Mutant Adaptable to Climatic Changes	I.K. Bimpong
JAM5013	Jamaica	Improving Crops by Using Experimental Mutagenesis and Diagnostic Technologies	S. Sivasankar
JAM5014	Jamaica	Establishing a Self-Contained Gamma Irradiation Facility for the Introduction of Sterile Insect Technique and Experimental Mutagenesis and Diagnostic Technologies	S. Sivasankar with IPC
KAM5007	Cambodia	Improving Cotton for Enhanced Resilience to Climate Change	L. Jankuloski
KEN5038	Kenya	Using Nuclear Techniques to Evaluate and Improve the Impact of Mutated Forages on the Performance of Smallholder Dairy Cows	I.K. Bimpong with APH
KUW5005	Kuwait	Implementing Mutation Induction to Improve Barley Production under Harsh Environmental Conditions – Phase III	L. Jankuloski
KUW5006	Kuwait	Improving Barley and Sorghum Production Under Harsh Environmental Conditions Using Mutation Breeding Techniques	L. Jankuloski
LAO5006	Lao PDR	Enhancing Crop Production with Climate Smart Agricultural Practices and Improved Crop Varieties	L. Jankuloski with SWMCN
LES5012	Lesotho	Improving Productivity of Potato and Sorghum through Mutation Breeding and Best Soil, Nutrient and Water Management Practices	I.K. Bimpong
LIR5003	Liberia	Enhancing Rice Productivity Using Induced Mutation and Speed Breeding for Food Self–Sufficiency and Income Generation	I.K. Bimpong
MAG5026	Madagascar	Enhancing Rice and Maize Productivity through the Use of Improved Lines and Agricultural Practices to Ensure Food Security and Increase Rural Livelihoods	L. Jankuloski with SWMCN <i>and</i> IHS
MAK5010	Macedonia	Improving Dry Bean Productivity and Tolerance to Diseases and Drought by Use of Nuclear Techniques	C. Zorrilla
MAL5032	Malaysia	Strengthening National Capacity in Improving the Production of Rice and Fodder Crops and Authenticity of Local Honey Using Nuclear and Related Technologies	C. Zorrilla with FSC <i>and</i> SWMCN
MAR5029	Mauritius	Improving Landraces of Crucifers (Cauliflower and Cabbage) and Carrot through Mutation Breeding and Biotechnology — Phase II	C. Zorrilla
MAU5009	Mauritania	Improving Rice and Sorghum through the Application of Nuclear Techniques	I.K. Bimpong
MHL5003	Marshall Islands	Enhancing Food Productivity by Promoting New Mutant Varieties and Traditional Varieties	C. Zorrilla
MLI5031	Mali	Improving Rice Productivity through Mutation Breeding and Better Soil, Nutrient and Water Management Practices	I.K. Bimpong with SWMCN
MLW5005	Malawi	Developing Drought Tolerant, High Yielding and Nutritious Crops to Combat the Adverse Effects of Climate Change	C. Zorrilla

Project Number	Country/Region	Title	Technical Officer(s)
NAM5017	Namibia	Improving Crops for Drought Resilience and Nutritional Quality	C. Zorrilla with SWMCN
NAM5020	Namibia	Enhancing Staple Crop Yields, Quality, and Drought Tolerance through Broadening Genetic Variation and Better Soil and Water Management Technologies	C. Zorrilla with SWMCN
NEP5006	Nepal	Enhancing Productivity of Corps and Fruit Employing Nuclear and Molecular Techniques	I.K. Bimpong
NER5024	Niger	Improving Key Staple Crops towards Food Security	S. Sivasankar
NER5025	Niger	Improving Food and Biological Hazard Detection, Food Preservation and Mutation Breeding	I.K. Bimpong with FSC
NHE5001	Vanuatu	Enhancing the Productivity and Quality of Crops through the Application of Mutation Breeding Techniques	L. Jankuloski
NIC5011	Nicaragua	Broadening the Genetic Variation of Vegetative Propagated Crops Using Nuclear Techniques	C. Zorrilla
PAK5053	Pakistan	Strengthening and Enhancing National Capabilities for the Development of Climate Smart Crops, Improvement in Animal Productivity and Management of Soil, Water, and Nutrient Resources Using Nuclear and Related Techniques	L. Jankuloski with SWMCN
PAL5011	Palestine	Enhancing Food Security via Nuclear Based Approaches	L. Jankuloski with SWMCN
PAR1005	Paraguay	Establishing National Capabilities in Irradiation Technologies for the Treatment of Patients with Burns and the Introduction of Mutation Breeding for Enhanced Quality and Productivity of Crops	S. Sivasankar with NAPC
PAR5012	Paraguay	Evaluation of Varieties and Advanced Mutant Lines against Biotic and Abiotic Stress Conditions to Mitigate the Effects of Climate Change in Crops	C. Zorrilla
PER5034	Peru	Improving Yellow Potato and Coffee Crops through Mutation Breeding Techniques	L. Jankuloski
PHI5036	Philippines	Providing an Innovative Platform for Germplasm Utilization for Rainfed and Irrigated Lowland Rice Ecosystems — Phase	S. Sivasankar
QAT5008	Qatar	Developing Best Soil, Nutrient, Water and Plant Practices for Increased Production of Forages under Saline Conditions and Vegetables under Glasshouse Using Nuclear and Related Techniques	L. Jankuloski with SWMCN
RAF5083	Regional Africa	Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)	S. Sivasankar/I.K. Bimpong
RAS5077	Regional Asia	Promoting the Application of Mutation Techniques and Related Biotechnologies for the Development of Green Crop Varieties (RCA)	I.K. Bimpong
RAS5088	Regional Asia	Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA)	S. Sivasankar/I.K. Bimpong
RAS5094	Regional Asia	Promoting Sustainable Agricultural and Food Productivity in the Association of Southeast Asian Nations Region	S. Sivasankar/ L. Jankuloski with FSC <i>and</i> SWMCN
RAS5098	Regional Asia	Improving the Resilience of Crops to Climate Change through Mutation Breeding — Phase II (SAPI)	C. Zorrilla
RAS5099	Regional Asia	Developing Climate Smart Crop Production including Improvement and Enhancement of Crop Productivity, Soil and Irrigation Management, and Food Safety Using Nuclear Techniques (ARASIA)	I.K. Bimpong with SWMCN

Project Number	Country/Region	Title	Technical Officer(s)
RER5024	Regional Europe	Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia	C. Zorrilla
RLA5084	Regional Latin America	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	S. Sivasankar with SWMCN and APH
RWA5001	Rwanda	Improving Cassava Resilience to Drought and Waterlogging Stress through Mutation Breeding and Nutrient, Soil and Water Management Techniques	L. Jankuloski with SWMCN
SAF5016	South Africa	Promoting Mutation Breeding of Vegetables to Improve Rural Livelihoods — Phase I	N. Warthmann
SIL5021	Sierra Leone	Improving Productivity of Rice and Cassava to Contribute to Food Security	I.K. Bimpong with SWMCN
SRL5050	Sri Lanka	Supporting Genetic Improvement of Tea	S. Sivasankar
SUD5041	Sudan	Enhancing Productivity and Quality of High Value Crops through Improved Varieties and Best Soil, Nutrient and Water Management Practices	C. Zorrilla with SWMCN
SWA5002	Eswatini, Kingdom of	Improving Adaptability of Cowpea to Climate Change through Mutation Breeding	L. Jankuloski
SYR5026	Syrian Arab Republic	Using Accelerated Mutation Breeding of Staple Crops for Enhanced Resilience to Climate Change through Speed Breeding, Phenotyping and Genotyping	L. Jankuloski
TOG5004	Togo	Improving Crop Productivity and Agricultural Practices through Radiation Induced Mutation Techniques	I.K. Bimpong with SWMCN
TUN5031	Tunisia	Developing Cereal and Legume Mutants for Improving Food Security and Farmers' Resilience to Climate Change	A. Hingane
UGA5043	Uganda	Improving Cassava and Rice Disease Resistance through Mutation Breeding Techniques	I.K. Bimpong
URT5037	Tanzania, United Rep. of	Developing Rice Varieties with Resistance to Rice Blast and Salinity Tolerant Using Mutation Breeding and Biotechnology Techniques	L. Jankuloski
VEN5023	Venezuela	Improving Banana Productivity through Mutation Breeding Techniques for Enhanced Disease Resistance	S. Sivasankar
YEM5015	Yemen	Enhancing Sorghum and Legume Crop Productivity through Induced Mutations with Supportive Breeding and Biotechnologies	L. Jankuloski
YEM5016	Yemen	Enhancing Sorghum and Legume Crop Productivity through Induced Mutations with Supportive Breeding and Biotechnologies — Phase II	I.K. Bimpong
ZAI5029	Congo, Democratic Republic of the	Enhancing Crop Productivity of Soybean and Maize through Improved Mutant Varieties and Lines	I.K. Bimpong
COD5030	DR Congo	Improving Staple Crop Productivity and Quality through Biotechnology and Nuclear Techniques — Phase IV	I.K. Bimpong
INT5158	Interregional	Strengthening Member State Capacities to Combat Banana Fusarium Wilt (TR4) through Early Detection, New Resistant Varieties, and Integrated Management	S. Sivasankar

APH: Animal Production and Health, NAFA; FSC: Food and Environmental Protection, NAFA; IPC: Insect Pest Control, NAFA; SWMCN: Soil and Water Management and Crop Nutrition, NAFA; RPRT: Radioisotope Products and Radiation Technology, NAPC; ARBR: Applied Radiation Biology and Radiotherapy, NAHU; PHY: Physics, NAPC; PCG: Programme Coordinator, NA; IHS: Isotope Hydrology Section, NAPC

## **Highlights from Technical Cooperation Projects**

# FAO/IAEA training facilitates development of tissue culture protocols for tea

#### Kulasiri Ranaweera, Mihirangani Jayaratne and Mahasen Ranatunga

Tissue culture technologies are important in supplementing the development of new tea cultivars with disease resistance, drought tolerance and enhanced metabolites for quality and health benefits. Tissue culture techniques have proven advantageous over conventional measures to shorten the time for cultivar development. Additionally, micropropagation offers an alternative vegetative propagation technique for year-round production of plants of new tea cultivars. Currently, conventional vegetative propagation alone is unable to fulfill the requirement for annual planting material of tea plantations in Sri Lanka. Recent figures confirmed that annual requirement cannot be met by conventional means and huge deficit of tea plants remain unfulfilled. Hence micropropagation offers a lucrative alternative approach to conventional bulk propagation of tea in times to come.

"Conventional tea cultivar development takes 20-25 years" said Mahasen Ranatunga, the Head of the Plant Breeding Division at the Tea Research Institute of Sri Lanka (TRI). "We have trimmed the length of tea cultivar development by around 6 years by integrating embryo culture techniques and we strongly believe that lots of further developments are possible with tissue culture techniques". It is high time to accommodate nuclear techniques in tea crop improvement coupled with tissue culture to speed up the conventional tea breeding programme.

# Sri Lanka's first mutation breeding programme in tea

The IAEA, in partnership with the Food and Agriculture Organization of the United Nations (FAO), has been working with the TRI to support genetic improvement in tea using nuclear techniques under a Technical Cooperation Project (TC Project, SRL5050). The first phase of the project commenced in 2020 and will continue till the end of 2023. TRI receives financial and technical assistance from IAEA to upgrade laboratory facilities to induce mutations using *in vitro* and *ex vitro* materials and screen mutants for traits of interest. Scientists from TRI are being trained on mutation breeding and tissue culture techniques at the IAEA Plant Breeding and Genetics Laboratory in Seibersdorf, Austria.

"Developing tissue culture protocols is a prerequisite for single cell induced mutagenesis, which we believe is successful in tea" said Shoba Sivasankar, Head of the IAEA's Section on Plant Breeding and Genetics at the Joint

FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture. Her team at Seibersdorf has been successful in developing a regeneration protocol for single cells isolated from coffee leaves for mutagenesis. "Single cell mutagenesis ensures significant reduction of chimeras in mutants that can subsequently be regenerated into plantlets" explained Sivasankar.



TRI scientist working with tea plants

#### Direct and indirect somatic embryogenesis in tea

"We have been attempting the development of direct and indirect somatic embryogenesis protocols from vegetative parts of tea, however, success has been very low until recently" said fellow Kulasiri Ranaweera, Research Officer, Plant Breeding Division of TRI, Sri Lanka who has been training at the Seibersdorf lab since April 2022. "We studied the optimized protocol for coffee at the lab and commenced working on tea with some modifications" Ranaweera further explained. With these modifications, tremendous success has been achieved in direct somatic embryogenesis from tea leaves. Somatic embryos have been obtained within a week after culturing, and mutagenesis using gamma rays has already commenced. Now somatic embryos are being monitored. Ranaweera expects to develop regeneration protocol for somatic embryos simultaneously which could be a remarkable steppingstone that would lay the foundations for commercial micropropagation of tea.



TRI scientist culturing tea leaves for inducing somatic embryos (A); Somatic embryos observed after one week of culturing tea leaves (B.

Ranaweera's attempt on indirect somatic embryogenesis is also showing promising results and so far, he has been successful in obtaining embryogenic callus from tea leaves. "We will start culturing single cells once friable calli are obtained and developing a regeneration protocol as has been done for coffee" he further said. Ranaweera's training holds strong scope for single-cell induced mutagenesis of tea with the aim of developing new tea cultivars with favorable traits.

#### Training in radiosensitivity studies

Another training at the Plant Breeding and Genetics laboratory was hands on experience in radiosensitivity studies and mutation breeding, using the seeds of chickpea and sorghum as examples.

Mihirangani Jayarathne, Technical Officer attached to the Plant Breeding Division of TRI, Sri Lanka returned to the country after completion three months of fellowship. "Exposure to mutation breeding techniques, especially to radiation sensitivity studies, enhance the ability and confidence to conduct irradiation work on tea in Sri Lanka" Jayarathne said.



TRI scientist working with gamma treated chickpea seeding at the PBG Lab, Seibersdorf

The fellow witnessed successful mutation breeding activities during the training period and is expected to help fine tune ongoing tea breeding activities.

Outputs of the above fellowships clearly demonstrate that tissue culture combined with induced mutagenesis can be productively applied for the development of new tea cultivars much faster than conventional methods.

#### **Forthcoming Events**

#### National Training Course

#### Mutation Techniques, Mutation Discovery, Marker Development and Marker Assisted Selection (MAS) - NEP5006

#### *Kathmandu, Nepal,* 13-17 March 2023 Project Officer: K. Bimpong

Course Objective: Familiarization with concepts and methodologies in mutation breeding, linkage mapping, variant discovery and marker-assisted selection.

Course curriculum will include introductory molecular biology, molecular markers and genome variations, the basics of genomics and transcriptomics, linkage analysis, QTL mapping, MAS, genome wide association analysis and introduction to bioinformatics. The course is designed for breeders, pathologists and tissue culture specialists involved in mutation breeding programmes. It is expected that 15 participants will join the training.

#### **Regional Training Course**

#### Application of Genomics, Genotyping and Marker-Assisted Selection in Mutation by Speed Breeding (MbyS) - RAS5088 Virtual. Q1 2023

Project Officer: K. Bimpong, S. Sivasankar

Course Objective : Training in the application of mutation breeding, genotyping, genomics, bioinformatics, and marker-assisted selection.

Course curriculum will include mutation breeding techniques, introductory molecular biology, molecular marker discovery, basics of genomics and transcriptomics, linkage analysis, QTL mapping, genome wide association analysis and introductory bioinformatics. The training will include lectures, demonstrations, group discussions, and videos, as necessary. The course is designed for early career scientists in the project teams of participating Member States, and participant number is expected to be within 25.

#### National Training Course

Introductory to Mutation Breeding in Crops - HAI0007

#### Port-au-Prince, Haiti, Q2 2023

#### Project Officer: K. Bimpong

Course Objective: As this is the first mutation breeding project for the country, training objective is to provide awareness on the application of nuclear techniques in food and agriculture.

Course curriculum will include introductory mutation breeding techniques, induced genetic variation, mutagenesis, preparation of radiosensitivity curves, basic breeding methods and introductory molecular biology. Participants will also be introduced to the application of linkage mapping, variant discovery and marker-assisted selection. Participation is expected from 15 researchers with no prior experience in mutation breeding.

#### Regional Training Course

Methodologies for Improving Crop Resilience to Abiotic Stress Through Nuclear Techniques and Seed Systems - RAS5099

#### Rice Research and Training Center (RRTC), Sakha Kafr Elsheikh, Egypt, Q3 2023

Project Officer: K. Bimpong, S. Sivasankar

Course Objective: Capacity building in the use of different selection methods to improve crop resilience to abiotic stress, and an overview of seed system modalities.

Course curriculum will include mutation breeding schemes, introduction to selection methods for abiotic and biotic stresses in the laboratory, greenhouse and field, discussion of modalities of informal and formal seed systems for multiplication and dissemination of seeds. The course is designed for participants with basic knowledge in mutation breeding and 15 participants are expected.

#### Advance Regional Training Course

#### Mutation Breeding and Combined Biotechnologies -RAS5099

Rice Research and Training Center (RRTC), Sakha Kafr Elsheikh, Egypt, Q3 2023

Project Officer: K. Bimpong, S. Sivasankar

Course Objective: Capacity building in advanced technologies, especially enabling technologies, to hasten the pace and precision of developing new and improved mutant varieties.

Course curriculum will include concepts and methodologies in molecular biology, molecular markers and genome variations, genomics and transcriptomics, linkage analysis, QTL mapping, genome wide association analysis and introduction to bioinformatics. The course is designed for breeders, pathologists and tissue culture specialists in Member States involved in mutation breeding programmes. It is expected to be attended by approximately 20 participants.

#### **Past Events**

#### National Training Course

#### Molecular Techniques for Crop Improvement of Vegetatively Propagated Crops - MAK5010

*Skopje, North Macedonia,* 31 October - 4 November 2022 Project Officer: C. Zorrilla

Project Objective for MAK5010: Expand the genetic variability in common bean (*Phaseolus vulgaris*), the most cultivated legume crop of **North Macedonia** using induced mutations with gamma ray irradiation and select

mutant lines with better agronomic performance and tolerance to biotic and abiotic stresses.

Training Course Objective: Capacity development for implementing a mutation breeding program, analysis of basic data on mutation induction, biotechnological methods applied in a mutation breeding program, exposure to practical cases where the methodologies covered have been applied.

A total of 20 participants (7 men; 13 women) attended the training held at the Faculty of Agricultural Sciences and Food at Ss. Cyril and Methodius University.

#### National Training Course

#### Molecular Methods for Identification of Mutations-NIC5011

*Managua, Nicaragua*, 7-11 November 2022 Project Officer: C. Zorrilla, K. Bimpong

Project Objective for NIC5011: Expand the genetic variability of cocoyam, taro and plantain in **Nicaragua** through induction and identification of mutations, using nuclear and biotechnology techniques towards disease tolerance and climate change adaptation. Specific focus is on drought and soil-borne diseases.

Training Course Objective: Capacity building in molecular techniques applied in crop improvement, methods for detection of mutations in candidate genes, and principles of RT-PCR. Lectures and practical sessions for demonstration and data analysis were included.

The training was held at the Universidad Nacional Agraria in Managua (UNA), with participation of Nicaraguan Institute of Agricultural Technology (INTA), and Institute of Agricultural Protection and Health (IPSA).

#### National Training Course

# Molecular Techniques for Crop Improvement of Seed Crops - GHA5039

*Accra, Ghana,* 7-11 November 2022 Project Officer: C. Zorrilla

Project Objective for GHA5039: Mainstreaming nuclear technologies for sustainable production in **Ghana**, with emphasis on building capacity for crop improvement through mutation breeding, soil, water, and nutrient management, and technology transfer to smallholder farmers. Using cassava and sweet potato (vegetatively propagated) and tomato (seed propagated) as focus crops, mutation breeding is used to breed new varieties resistant to cassava mosaic virus disease and sweet potato weevil.

Training Course Objective: Develop capacities in molecular techniques in crop breeding, including concepts, applications, and data analysis in respect to seed crops. Course topics focused on developing an understanding on the use of molecular markers in mutation breeding of seed crops such as tomato, maize, and sorghum. The course included lectures and sessions for data analysis and interpretation of results. Additionally, case studies of successful uses of QTL analysis and marker assisted selection in target crops were presented.

Participants included 9 female and 7 male researchers from the Ghana Atomic Energy Centre, Universities, and other National Research Centres.

#### Regional Training Course

# Field Experimental Design and Data Analysis for the Advancement of Mutant Populations - RER5024

*Virtual.* 21 November - 2 December 2022 Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for RER5024: This regional project for **Europe** is aimed at supporting the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and associated biotechnologies to contribute to food security in Europe and Central Asia.

Training Course Objective: Capacity development in statistical analysis and experimental design for crop improvement with emphasis on mutant population advancement, and expertise in the use of open-source software for statistical analysis of phenotypic data. The training is divided between two weeks: the first week focused on statistical analysis, experimental design and applications used for mutant populations advancement; and the second week focused on an update of statistical methods for data analysis using open-source software. The course will include lectures, case examples and practical sessions for data analysis and interpretation of results.

#### National Training Course

Seed Production and Multiplication of Mutant Lines of Crucifers and Carrot - MAR5029

*Reduit, Mauritius,* 28 November - 2 December 2022 Project Officer: C. Zorrilla

Project Objective for MAR5029: Improving the characteristics of landraces of crucifers and carrot to provide the farming community of **Mauritius** with high quality and yielding new mutant varieties for reduced dependence on imported planting material.

Training Course Objective: Capacity building in seed production and processing to obtain viable, disease-free, high-quality seeds of carrot and crucifer mutants, among other crops. The training is part of an expert mission held at the Food and Agricultural Research and Extension Institute (FAREI).

#### National Training Course

Plant Breeding Using Nuclear Technology - PAR5012 San Lorenzo, Paraguay, 28 November - 2 December 2022 Project Officer: C. Zorrilla

Project Objective for PAR5012: Conduct morphological and physiological evaluations in hot spots in **Paraguay** for

drought, fungi, and virus to select for new varieties with improved agronomic performance, physiological response, and nutritional composition in crops including soybeans, beans and Stevia for registration as commercial varieties.

Course Objective: Capacity development in mutation breeding with topics including mutation induction, dosimetry, radiosensitivity, screening for biotic and abiotic stress and selection for improved nutritional quality.

This training course was part of an expert mission at the Multidisciplinary Centre of Technology Research (CEMIT) at Universidad Nacional de Asuncion. Participating institutions included Research Centres and Institutes at Universidad Nacional de Asuncion, Public and Private Universities in Paraguay, Ministry of Agriculture, Instituto Paraguayo de Tecnologia Agraria, production cooperatives, and IICA.

#### Workshop

#### Applications of Nuclear Technologies in Forest Conservation and Improvement - CHI5052 Concepcion, Chile, 29 November 2022

Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for CHI5052: Generate improved genetic material through mutagenesis for reforestation in **Chile** using both native and exotic species better adapted to climate change, specifically, drought and resistance to pests and diseases.

Course Objective: Discuss the application of nuclear techniques for conservation and genetic improvement of forest trees, share experiences with specialists from the Nuclear Research National Institute in Mexico (ININ), and get acquainted of the progress made by the National Forest Institute of Chile (INFOR) and the Chilean Nuclear Energy Commission (CCHEN) in the application of these techniques to forest species. The event also presented the new Molecular Biology Laboratory installed at INFOR with FAO/IAEA support.

Participants included researchers from INFOR, CCHEN, ININ, academia, public institutions, and representatives of the private forest sector.

#### Regional Training Course

#### Application of Double Haploidy for Mutation by Speed Breeding (MbyS) Towards Crop Improvement - RAS5088

#### National Research and Innovation Agency (BRIN), Jakarta, Indonesia, 5-9 December 2022

Project Officer: K. Bimpong, S. Sivasankar

Project Objective for RAS5088 : The objective of this regional project is to improve food security in the **Asia Pacific** region through faster release of mutant varieties with improved crop productivity and quality.

Course Objective : Enhance research capabilities in basic principles of crop mutation breeding, doubled haploidy and other speed breeding methods, and techniques in mutation detection. Curriculum included mutation induction, induced genetic variation, mutagenesis, preparation of radiosensitivity curves, development of mutant populations, In-vitro mutagenesis protocols, plant tissue culture, doubled haploids in mutation breeding, screening methods for biotic and abiotic stresses.

#### National Training Course Nutritional Quality Screening Using NIR Spectroscopy - CUB5023 La Habana, Cuba, 5-9 December 2022

Project Officer: Z. Zorrilla, K. Bimpong

Project Objective for CUB5023: Generate improved mutant lines of rice, common bean and soybean in **Cuba** that combine tolerance to abiotic stress with good response to biofertilizer.

Course Objective: Develop capacities in methodologies to analyse nutritional compounds of interest in mutant lines or varieties improved by mutation breeding using NIR spectroscopy. Curriculum included practical demonstration of calibration, support for analysis of NIRSbased nutritional quality data and model development, and exchange experiences in designing validation trials for nutritional quality evaluations in field experiments.

This training course was part of an expert mission at Instituto Nacional de Ciencias Agricolas (INCA) in La Habana - Cuba.

#### Workshop

#### Mutation Induction and Mutation Breeding -CHI5054

#### *Santiago, Chile,* 12 December 2022 Project Officer: C. Zorrilla

Project Objective for CHI5054: Conduct two pilot studies using nuclear techniques in plant breeding on agricultural and native plant species of **Chile**, with one example of a seed propagated crop and one for vegetative propagation.

Course Objective: Capacity building in application of nuclear techniques to induce new genetic diversity, towards the generation of improved varieties in seed and vegetatively propagated crops.

The workshop was part of an expert mission at the Chilean Nuclear Energy Commission (CCHEN), and participants included researchers from CCHEN, CEAF and PUCV, as well as others in the academy and the agricultural sector.

#### National Training Course

Improving Crop Adaptation to Abiotic Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods - BDI5005 Bujumbura, Burundi, Q4 2022

#### Project Officer: K. Bimpong

Project Objective for BDI5005: The objective of this project is to enhance the production of cassava, sweet potato and rice in **Burundi** using mutation breeding approach and best soil, nutrient, and water management practices.

Course Objective: Enhance the understanding of basic principles in mutation breeding and introduction to molecular techniques for mutation detection and selection. The training course will include mutagenesis of plant material, radiosensitivity analysis, introductory lectures on accelerated breeding techniques such as *in- vitro* haploidy induction, genotyping and marker-assisted-backcross.

The course is geared for early career researchers in the national project team and 15 participants are expected.

#### National Training Course

Improving Crop Adaptation to Drought Stresses Using Nuclear-Derived Techniques, and Molecular-Breeding Method - ERI5013

#### National Agricultural Research Institute (NARI), Asmara, Eritrea, 7-17 June 2022

Project Officer: K. Bimpong

Project objective for ERI5013: Integrate radiation-induced mutagenesis to develop crops with improved yields and quality in **Eritrea**.

Course Objective: Develop capacities in the basic principles of mutation breeding and basic molecular techniques for improved mutation detection and selection.



**ERI5013** Course participants measuring the height of some irradiated maize varieties

This national training course was held as part of an expert mission at the National Agricultural Research Institute (NARI) in Asmara. A total of 23 participants (18 men and five women researchers) participated.

#### Regional Training Course

Mutation Breeding on Vegetatively Propagated Crops - RLA5084 Virtual. 20-24 June 2022

#### Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for RLA5084: To raise awareness among Member States in **the Caribbean** on the various applications of nuclear science and technology in agriculture and to develop their human resource and institutional capacity in the use of these technologies towards the Region's effort to improve agricultural productivity of participating Member States. Targets for crop improvement include the vegetatively propagated crops taro, cocoyam, pineapple, papaya, banana, sugar cane, among others.

Course Objective: Develop capacities in mutation induction, radiosensitivity tests, mutation breeding and micropropagation techniques; and to get acquainted with the challenges and opportunities of dealing with vegetatively propagated crops.

The training was attended by 7 female and 2 male researchers from Guyana, Jamaica, St. Lucia, St. Vincent and the Grenadines, and Trinidad and Tobago.

#### Mid-Term Regional Meeting

Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia - RER5024

*Faro, Portugal,* 4-8 July 2022 Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for RER5024: Strengthen capacities of plant mutation breeding researchers in Member States of **Europe and Central Asia** to develop high yielding, resilient varieties with improved nutritional quality.

Event Objective: Report on progress and achievements, and to identify and address challenges encountered. The workplan of the project was revised and updated and plans on future project activities were discussed.

A total of 15 counterparts, from 14 countries (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Georgia, Greece, Kyrgyzstan, North Macedonia, Poland, Portugal, Russia, Serbia, Slovakia, Slovenia, and Turkey) participated in the meeting.

This event was hosted by the Universidade do Algarve in Faro, Portugal. Participants had the opportunity for technical visits facilities of the University and to agricultural companies in the Algarve region.

#### National Training Course

#### Polyploid Induction as a Breeding Strategy for Creole Potatoes - COL5026

## Universidad Nacional de Colombia, Bogota, Colombia, 11- 15 July 2022

#### Project Officer: K. Bimpong

Project Objective for COL5026: Generate mutant clones of creole (yellow) potato in **Colombia** that combine dormancy period with high yield potential.

Course Objective: Training in mutation breeding for polyploid species, crossbreeding between elite materials and desirable traits for dormancy and yield, and the use of genomics to improve mutation detection and selection.

A total of 28 participants (17 men and 11 women researchers), participated in the training held at the Universidad Distrital Francisco José de Caldas (UDFJC) in Bogota. The training course was held as part of an expert mission.

#### Regional Training Course

Plant Mutation Breeding and Associated Biotechnologies in Seed Propagated Crops at National Research and Innovation Agency (BRIN) - RAS5094

*Jakarta, Indonesia,* 18-29 July 2022 Project Officer: L. Jankuloski

Project Objective for RAS5094: Enhance the capacities in **ASEAN countries** in promoting sustainable agriculture and food productivity. On the plant breeding front, the aim is to strengthen human capacity and infrastructure facilities for mutation breeding to develop improved crop varieties.

Course Objective: Training in plant mutation breeding and associated biotechnologies in seed propagated crops to participants from ASEAN countries.

The two-week training course was attended by 14 trainees from 10 Member States, and was organized by BRIN, Indonesia. Beside theoretical and practical lectures, trainees visited irradiation facilities at BRIN, Nuclear Facilities at Serpong, Banten, Center for Agricultural Biotechnology (BIOGEN), In-vivo Gene bank (Kebun Raya Bogor) and Bogor Agricultural University (IPB), Bogor, West Java. Technical visits were organized to experimental fields, with different mutant generations, where practical screening of mutant plants/lines was demonstrated.



RAS5094 Regional training course participants in the field

#### Regional Training Course

#### Molecular Techniques for Crop Improvement of Mutant Populations - RER5024

Virtual. 1-5 August 2022

Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for RER5024: Improve yield and resilience to climate change of major food crops in **Europe and Central Asia** by inducing new genetic variation with nuclear techniques. This project includes a total of 26 countries.

Course Objective: To develop capacities in molecular techniques for application in a mutation breeding program; to expose participants to practical cases where QTL mapping methods and different types of populations have been applied in mutation breeding; and to develop expertise in basic data analysis related to the use of molecular techniques to identify genomic regions that control important traits using mutant or mutant-derived populations.

A total of 18 female and 8 male participants from 13 countries attended this training.

#### Consultants Meeting

Radiation-induced Crop Genetic Diversity and Integrated Breeding of Major Traits to Accelerate Variety Development for Food Security and Climate-Smart Agriculture

Vienna, Austria, 8-12 August 2022 Project Officer: C. Zorrilla, S. Sivasankar

The purpose of the event was to discuss and develop a Concept Note towards a call for the next CRP at NAFA PBG. The topic was on combating the banana Fusarium Wilt Tropical Race 4 disease through radiation-induced genetic resistance of the plant and radiation-induced genetic diversity of beneficial microbes.

Climate change increases the risk of disease outbreaks due to increased temperatures as well as higher frequency and unpredictability of environmental stresses worldwide. Farmers growing vegetatively propagated crops in tropical regions are especially vulnerable to these threatens. Therefore, generating new genetic variation through nuclear techniques together with mutation breeding and the development of adequate pathogen management tools can help reduce the spread of diseases.

Disease management requires a multidisciplinary approach to address the problem from diverse angles. Finding ways to precisely evaluate the response of different genotypes to the pathogen for an effective determination of the resistance/susceptibility, precisely identifying the race of the pathogen; as well as identifying methodologies to prevent infection and control the dispersal of the pathogen. The event was conducted in hybrid mode with in-person and virtual participation of experts. The concept was approved and a call for proposals is open from October 2022 to December 2022. The CRP is expected to start in March-April 2023.



Consultants and PBG staff at the Vienna International Centre, Vienna, Austria

#### National Training Course

Improving Crop Adaptation to Abiotic Stresses Using Nuclear-Derived Techniques, and Molecular-Breeding Method - LIR5003 Virtual. 1-12 August 2022

Project Officer: K. Bimpong

Project Objective for LIR5003: To enhance rice productivity using mutation breeding and associated biotechnologies for crop improvement in **Liberia** towards food security and income generation. This is the first mutation breeding programme for Liberia.

Course Objective: Basic introduction to range of topics including mutation induction methods, radiosensitivity testing, dosimetry, generation advancement of mutant lines/populations, testing of advanced mutant lines, basics of experiment design. Participants were also introduced to the concepts of accelerated breeding techniques (rapid generation advancement, *in vitro* haploidy induction), molecular markers, marker assisted selection and use of existing bioinformatic platforms for accessing/sharing information, and data management and analysis.

The two-week course, implemented in virtual mode, was attended by 21 participants (15 men and six women researchers) and delivered by two international experts.

#### National Training Course

#### Mutation Breeding in Maize Crops and Developing Resistance to the Fall Army Worm - CAF5013

University of Bangui, Bangui, Central African Republic, 9-12 August 2022

Project Officer: S. Sivasankar

Project Objective for CAF5013: Improving productivity of maize and developing maize varieties resistant to the fall army worm using radiation-induced mutagenesis in the **Central African Republic**.

Course Objective: Training in introductory biology, mutation induction, calculations of radiosensitive curves, generation advancements of mutant lines, field techniques and selection methods in mutation breeding, introductory molecular biology, molecular markers and applications of molecular markers to mutation breeding, basics of QTL mapping and marker assisted selection.

The training course was held as part of an expert mission by Isaac Kofi Bimpong, Technical Officer from the PBG Subprogram and was held at the University of Bangui. It was attended by a total of 18 participants (13 men and five women researchers), from the university and national agricultural research centres.



**CAF5013** Course participants at Bangui, Central African Republic

#### Regional Training Course

#### Statistically Rigorous, Pre-Field and Field Screening to Select Improved Mutant Lines - RAF5083

Univerisite Cheik Anta Diop (UCAD), Dakar, Senegal, 8-19 August 2022

Project Officer: K. Bimpong, S. Sivasankar

Project Objective for RAF5083: Support AFRA States in developing and disseminating new and improved mutant crop varieties with improved yield, quality, and tolerance to biotic and abiotic stresses in Africa.

Course Objective: Hands-on training on important topics in experimental design, mixed model approach for data analysis, exploratory data analysis, split-plot design and analysis, breeding informatics, data collection and analysis of environmental trial data, principal component analysis, multiple comparisons and contrast, correlation analysis.

The two-week training course was held at the Université Cheikh Anta Diop (UCAD), Dakar, Sénégal and was attended by 28 participants from across 18 countries in Africa (13 men and 15 women researchers). The international expert, Mr Thomas Odong, delivered lectures and practical training in research Methods and biometry/applied statistics.



**RAF5083** Researchers from 18 African Countries attended the training at the UCAD, Dakar, Senegal

#### National Training Course

#### Mutation Breeding Techniques and Crop Improvement - MAU5009

National Centre of Agricultural Research and Development (CNRADA), Kaide, Mauritania, 28 August - 1 September 2022

Project Officer: K. Bimpong

Project Objective: Develop improved rice and sorghum varieties with higher yields and adaptation to drought conditions in **Mauritania** using induced genetic variation and mutation breeding.

Course Objective: Enhance research capabilities in mutation breeding principles, including selection methods

for targeted stresses/traits, process efficiencies for the selection of improved varieties for registration and release, and models for multiplication and dissemination of seeds.

This training was part of an expert mission and was held at the CNRADA, Ministry of Rural Development, Kaide in Mauritania. A total of 20 participants attended, including 18 men and two women researchers.



**MAU5009** Course participants observing mutant rice seedlings in the screenhouse

#### National Training Course SDS-PAGE Screening for Protein Quality in Mutant Lines - SUD5041

*Khartoum, North Sudan,* 25-29 September 2022 Project Officer: C. Zorrilla

Project Objective: Increase the productivity of sorghum, millet and cowpeas through nuclear-based mutation induction and breeding in **Sudan** for nutritional quality, specifically higher protein, fat, and minerals in grains.

Course Objective: Capacity building in protein isolation and quantification, and SDS-PAGE analysis to identify differences in protein composition between mutant and wildtype lines.



**SUD5041** Participants of the training course at the Biotechnology and Biosafety Research Center (BBRC) of the Agricultural Research Corporation, Sudan

This one-week training included lectures and practical demonstrations in the laboratory. It was attended by 28 participants, including 12 women and 16 men researchers

from Universities, National Research Centres and Research Stations.

#### National Training Course

#### **Evaluation of Grain Nutritional Quality - MAL5032** *Virtual.* 26-30 September 2022

Project Officer: C. Zorrilla, S. Sivasankar

Project Objective for MAL5032: Strengthen **Malaysia**'s national capacity and technical capabilities in mutation breeding of fodder crops towards generating high-quality animal feed, and in molecular marker techniques for the selection and identification of rice and Napier grass mutants.

Course Objective: Capacity development in methodologies and equipment used for grain nutritional quality evaluation in the mutation breeding programme in Malaysia. Topics addressed the analysis of grain physicochemical and nutritional parameters including protein, fats, ashes, carbohydrates, etc in rice and other seed crops.

Lectures and demonstration sessions were provided by experts from the National Agri-Food Biotechnology Institute (NABI). Participants included 12 female and 3 male researchers from Malaysian Nuclear Agency, National University of Malaysia, and Universiti Teknologi MARA.

#### Regional Training Course Mutation Induction and Mutation Breeding-RAS5098

*Suva, Fiji,* 26 September - 7 October 2022 Project Officer: C. Zorrilla

Project Objective for RAS5098: This regional project addresses mutation breeding, recently introduced to the **Pacific Islands**, as a tool to obtain new improved varieties adapted to abiotic and biotic stress challenges. Even though they share similar environmental conditions, there are particularities in each country's crop and trait priorities.

Course Objective: Introductory techniques in mutation breeding in seed and vegetative crops, *in vitro* and molecular techniques and selection for target traits.



**RAS5098** Training group at the Pacific Community Centre for Pacific Crops and Trees (SPC-CePaCT)

The two-week training was hosted by the Pacific Community through its Pacific Community Centre for Pacific Crops and Trees (SPC-CePaCT) and was delivered by Dr. Suprasana Penna with technical support of SPC-CePaCT researchers.

A total of 18 participants attended, including 12 female and 6 male researchers. Trainees were from Fiji (8), Marshall Islands (2), Papua New Guinea (4), Samoa (3) and Vanuatu (2).

#### Regional Training Course

#### Plant Mutation Breeding and Efficiency Enhancing Techniques to Increase Resilience to Climate Change – RER5024 and RAS5099

Virtual. 26 September - 7 October 2022

Project Officer: K. Bimpong, C. Zorrilla, S. Sivasankar

Project Objective for RER5024 and RAS5099: This training course was delivered as part of a joint training course between two regional projects, one for **Europe and Central Asia** and the other for the **Middle East**. They are RER5024, Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia, and RAS5099, Developing Climate Smart Crop Production including Improvement and Enhancement of Crop Productivity, Soil and Irrigation Management, and Food Safety Using Nuclear Techniques.

Course Objective: Training in basic principles of mutation breeding with emphasis on seed crops, mutant population development, rapid cycling and *in vitro* haploidy induction, molecular breeding, genomics, next generation sequencing and bioinformatics for mutant trait discovery and marker assisted breeding. The training also included, for the first time, an introduction to genome editing.

The two-week training was attended by 31 participants (18 males and 13 females) and was coordinated by the PBG Laboratory.

#### National Training Course **Molecular Techniques for Crop Improvement of Vegetatively Propagated Crops - GHA5039** *Accra, Ghana,* 3-7 October 2022 Project Officer: C. Zorrilla

Project Objective for GHA5039: Mainstreaming nuclear technologies into sustainable production with emphasis on building capacity for crop improvement through mutation breeding, and soil, water, and nutrient management. Focus for mutation breeding is on cassava and sweet potato for resistance to cassava mosaic virus disease and sweet potato weevil. Tomato is also a target crop.



**GHA5039** Participants of the training course at the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Accra, Ghana

Course Objective: Develop capacities on molecular techniques applied to crop breeding, including concepts, applications, and data analysis in vegetatively propagated crops using cassava and sweet potato as a model.

Participants included 9 female and 7 male researchers from the Ghana Atomic Energy Centre, Universities, and other National Research Centres.

#### National Training Course

# Mutation Breeding for Salinity Tolerance in Rice - URT5037

*Tanzania Agriculture Research Institute, Dakawa, Tanzania,* 3-7 October 2022 Project Officer: L. Jankuloski

Project Objective for URT5037: Strengthen human capacity in plant mutation breeding in **Tanzania** and develop improved rice varieties with resistance to the blast disease and tolerance to salinity.

Course Objective: Capacity building in mutation induction in rice, development of mutant populations, screening techniques for identification of mutant plants, handling of  $M_1, M_2$  and other mutant generations, screening for salinity tolerance and efficiency enchanting technologies in breeding.



**URT5037** Demonstration of screening for tolerance to salinity in the filed

The training hosted by the Tanzanian Agriculture Research Institute (TARI) in Dakawa, Tanzania was attended by 39 participants from 7 different agriculture research institutes and universities in Tanzania. The training was covered by invited lecturer, Dr Abdelbagi Ghanim.

#### Regional Training Course

Detection and Diagnostics of Fusarium Tropical Race 4 (Foc R4T) - INT5158 Mexico City, Mexico, 10-14 October 2022 Project Officer: S. Sivasankar, C. Zorrilla

Project Objective for INR5158: Develop capacities for the management of the disease banana Fusarium Wilt, *Foc* TR4, and develop genetic variability in banana for resistance. Current focus is the Latin American region, although designed as an inter-regional project

Course Objective: Develop capabilities for the detection and diagnosis, in the field and lab, of *Fusarium oxysporum* f.sp. *cubense* Tropical race 4 (Foc R4T). The training provided lectures and practical sessions on identification of disease symptoms relative to other stress; protocols and strategies for management of suspected samples; evaluation of morphology and reproductive structures of the pathogen; molecular diagnostics; and genomic analysis of *Foc* TR4.

The training was hosted by the laboratories of the National Phytosanitary Reference Centre (CNRF) of the National Food Health, Safety and Quality Service (SENASICA) in Mexico. A total of 12 participants, 5 female and 7 males, from 10 Latin American countries attended this training.





#### National Training Course

Selection for Biochemical Traits With Improved Quality and Yield in Mutant Rice and Cassava Lines -SIL5021

*Njala University, Njala, Sierra Leone,* 17-21 October 2022 Project Officer: K. Bimpong

Project Objective: Develop and improve rice and cassava productivity in **Sierra Leone** through mutation breeding

and better soil and nutrient management in saline environments.

Course Objective: Capacity building in mutation breeding and screening for target traits, yield and nutritional quality (high iron, zinc, low phytate, provitamin A).

This national training course was held as part of an expert mission and hosted by the Njala University. A total of 20 participants including 13 male and seven female researchers participated from the University and National Agricultural Research Centres.



*SIL5021* Participants at the training course at Njala, Sierra Leone

#### Regional Training Course

Mutation Breeding in Vegetatively Propagated Crops, Including Micropropagation Using Cell/Tissue Culture, Mutation Induction and Selection - RAF5083

Universite Cheik Anta Diop (UCAD), Dhakar, Senegal, 24 October - 4 November 2022

Project Officer: K. Bimpong, S. Sivasankar

Project Objective for RAF5083: Support AFRA States in developing and disseminating new and improved mutant crop varieties in **Africa** with improved yield, quality, and tolerance to biotic and abiotic stresses.

Course Objective: Capacity building in micropropagation, and screening methods to select improved mutant lines in vegetatively propagated crops. Topics included mutation induction, optimization of irradiation treatments, plant tissue culture, and doubled haploidy.



**RAF5083** Participants of the regional training course at UCAD, Dakar, Sénégal

The two-week training course held at the UCAD, Dakar, Senegal was attended by 36 participants (21 males and 15 females) from across 18 countries in Africa. Lectures were provided by Dr. Elegba, invited lecturer.

## Developments at the Plant Breeding and Genetics Laboratory (PBGL)

#### **Upcoming Events**

PBG will host a workshop "Plant Mutation Breeding" at the **International Plant and Animal Genome Conference** (PAG 30, January 13-18, 2023). PAG is the most significant annual conference on the genetics and genomics of plant and animal research and breeding, bringing together over 3,000 leading scientists.

Our workshop is scheduled for **Tuesday, January 17 at 1:30 PM**, in Pacific H-I (2nd Floor) with five slots for 20minute presentations plus discussion. We will fill the two hours with recent science from modern plant breeding with a particular angle on induced, novel genetic variation: the essence of mutation breeding. We will cover technical aspects such as mutagenesis, phenotyping, genomics, and speed breeding, as well as the utility of mutation breeding in generating important crop traits towards reaching the millennium development goals, achieving food security and planetary health, and securing and improving the livelihoods of farmers. We are hoping to establish this workshop at PAG as a yearly activity, which would serve as a networking hub to support our capacity building for Member States.

#### **Plant Health**

# Mutation assisted breeding of African cooking banana for Fusarium wilt race 1 resistance

Mchare bananas are diploid (AA) cooking bananas, cultivated in the Northern Tanzanian highlands, Kenya and DR Congo where they provide up to 30% of the caloric intake. They are also one of the parents of the export bananas Cavendish. Mchare cultivars are susceptible to Fusarium wilt disease (FW), caused by *Fusarium oxysporum* f. sp. cubense (Foc), race 1 and the more recently described strain Tropical Race 4 (TR4). In Africa, Foc race 1 is found throughout East and Central Africa

while Foc TR4 is currently limited to Mozambique. Being soil-borne, Foc race 1 and TR4 cannot be controlled by fungicides, therefore prevention and replacement of susceptible with resistant varieties through breeding remains one of the best options for effective control of this devastating disease.

About 17 years are needed to develop improved East African highland bananas through conventional plant breeding methods. The combined use of *in vitro* culture and induced mutagenesis through irradiation or chemical agents is an established technique that can significantly shorten the banana breeding cycle while preserving the favourable traits/genetic background.

A research project was set up at the FAO/IAEA PBG Laboratory in cooperation with the International Institute of Tropical Agriculture (IITA), Tanzania, and Stellenbosch University, South Africa, to develop Mchare bananas resistant to FW through induced mutagenesis (gamma-ray and EMS) (see Feature article p. 13 PBG Newsletter No 49). In May 2022, a mutant population comprising ca 5,000 *in vitro* plants was shipped from PBGL, Austria, to IITA, Tanzania, where they were hardened and prepared for planting in a 2.5 ha hot spot field for resistance screening to Foc race 1 (see Fig 1).

The PBGL initiated experiments for greenhouse-based screening for FW resistance in cooking banana. This research complements earlier studies on screening of dessert bananas for resistance to FW TR4 under environmentally controlled conditions in growth chambers carried out in context of CRP D22005 (see Newsletter No 45).



Figure 1. Mchare mutant population at different stages (in vitro-left, hardening-middle), ready for transplanting to the field (right).

Different banana varieties and FW strains (Foc race 1 and TR4, and garlic Fo f. sp. *sepae*) are being compared. Plants are infected using a liquid inoculum (Garcia-Bastidas et al., 2019). Symptom development is monitored based on (1) visual inspection of wilting and discolouration of leaves; (2) discolouration of the rhizome using a Rhizome Discolouration Index (Viljoen et al., 2017); and (3) plant

height. Results for Mchare cv Huti Green are shown in Fig 2. Results so far suggest that, under the PBGL greenhouse conditions, both Foc race 1 and TR4 resulted in typical banana FW symptoms. Thus, with appropriate experimental design, the inoculation technique is suitable for larger greenhouse evaluation of Mchare mutants.



*Figure 2.* Mchare cv Huti Green, symptom development in leaves (top) and rhizome/pseudostem (bottom). From left to right: three plants each infected with garlic Fo f. sp. sepae; banana Foc Race 1; banana Foc TR4. White bar indicates height of 50 cm.

# Standardising Lentil Stemphylium blight Disease Screening Conditions for Greenhouse

Lentil (Lens *culinaris* Medik.) is one of the most nutritious and protein-rich legumes grown mainly in Canada, India, USA, and Australia, and it accounts for over 70% of global production. Since the past decade, production and productivity of lentil have stagnated in traditional base of South Asia, mainly because of yield losses from various biotic and abiotic stresses (FAOSTAT). *Stemphylium* blight (SB) disease can reduce the crop yield by up to 62-100 percent.

SB is caused by the fungal pathogen *Stemphylium botryosum* Wallr., a devastating lentil disease in several lentil-producing countries, including Bangladesh, India, Nepal, Syria, Australia, USA, and Canada. The disease initially appears as small, light beige to brown lesions on leaves and leaflets during flowering. This results in large scale and rapid defoliation of lentil plants, causing a reduction in photosynthetic capacity at the flowering and pod-filling stage, affecting grain quantity and quality.

Screening germplasm and elite lines have shown variation in reaction to this disease and allowed selection for tolerance. This has been partially successful in developing tolerant varieties; however, true resistance to this pathogen is still missing in the germplasm, and mutation breeding appears to be a strong option to find novel sources of resistance to widen the genetic base of disease resistance.

Efforts are being made at the PBGL to develop mutant lines resistant to *Stemphylium* blight disease in lentil (Fig 1).

Towards this goal, research to standardize lentil *Stemphylium* blight screening conditions for the greenhouse is in progress.



*Fig. 1. PBGL team examining sub-culture of Stemphilium after 10 days of incubation* 

Currently, this screening protocol is standardized in a growth chamber, which will be further upscaled in a glass house to screen larger numbers of mutants. Mutant population and its wild parent were planted under rapid generation advancement (RGA) conditions, reported in the last newsletter (No. 48), to accelerate plant growth. After three weeks, young lentil plants were transferred to the growth chamber for acclimatization before inoculation.

This study uses the Canadian field isolate SB-19 of *S. botryosum* received from our counterpart Ms. Sabine Banniza from University of Saskatchewan, Canada .for inoculation. As reported in similar previous studies about the difficulty in obtaining desired spore density for

screening many plant populations, we have opted for the mycelial suspension method as reported by Adobor et. al, 2020. A handheld spray was used to inoculate each plant with 1ml of mycelial suspension. After inoculation, plants were dried for two hours and then incubated in the dark for 48 hours under maximum RH at 26°C (Fig xx). These plants will now be incubated for 20 days at 26°C with 70% RH under a 14h/8h light-dark period, and disease infection will be recorded 14 and 28 days after inoculation. The severity of Stemphylium blight will be assessed visually using a semi-quantitative 0-9 rating scale given by Hashemi et al., 2005.

Based on the severity of the disease compared to the susceptible parent in the growth chamber and variation among the mutant population, we plan to upscale this protocol in greenhouse conditions. The mutant population to be screened will be grown under supplemental LED lighting and extended photoperiod to enable rapid growth of plants. Integrating emerging technologies like RGA and Marker Assisted Selection will enhance the speed and accuracy of developing disease-resistant mutants of lentil and will be deployed in ongoing R&D for the determination of genetic associations at PBGL.



Figures(s). Optimising Lentil Stemphilium blight screening protocol at PBGL

A) S. botryosum culture after 10 days of incubation B) S. botrysum subculture incubated at  $26^{\circ}C$  for 10 days under constant agitation (170rpm) C) Mycelim growth of S. botryosum after 10 days of incubation.



**D**) Harvesting of S. botryosum mycelium using a vacuumfiltering method **E**) Dried mycelium under sterile conditions **F**) Grinding of the mycelium to fine powder with a mortar and pestle



*G*) Lentil mutant population grown under extended day length conditions to accelerate plant growth *H*) Inoculation of mutant population with hand-held sprayer *I*) Mutant population screened for Stemphilium blight resistance in growth chamber.

#### **Plant Breeding Technologies**

Integrating in vitro somatic embryogenesis with temporary immersion systems and mutation induction for accelerated, chimera-free production of mutant arabica coffee

Coffee is one of the most valuable cash crops worldwide providing employment and income to millions of people, especially in Latin America and parts of Africa and Asia. Coffee leaf rust caused by the fungus *Hemileia vastatrix* is the most significant disease in arabica coffee causing significant yield losses. As coffee is a perennial, breeding programmes take many years to produce new varieties.

Advanced plant *in vitro* techniques based on single-cell regeneration enable mass production of seedlings that are identical to the mother plant. Such *in vitro* cell cultures are also suitable targets for physical and chemical mutation induction. The unicellular origin of regenerated plantlets is expected to produce chimera-free plants. In case of perennial crops or trees such as coffee, this would significantly increase speed and throughput of the mutation breeding process compared to mutagenesis of seed.

Indirect somatic embryogenesis (SE) refers to the production of somatic embryos and plants with an intermediate callus phase. Direct SE techniques refers to the production of SE directly from explants in the absence of a callus phase. Both direct and indirect SE are expected to produce chimera-free plants, a key bottleneck for mutation-assisted breeding of perennial crops such as coffee.

PBGL previously reported *in vitro* regeneration of arabica coffee through indirect SE (Newsletter No 44). In this report, several critical steps of the experimental procedures are reported and illustrated in Fig xx, including the friable callus and the suspension culture used for mutagenesis treatments. Further, the use of a temporary immersion system has been integrated to scale up SE, as would be required when embarking on a mutation-assisted breeding program. Temporary immersion system are widely used in plant cell culture for mass propagation, including coffee (Ducos J-P et al., 2007).



**Fig 1a.** Coffea arabica in vitro regeneration and radiation treatment. (a) production of friable embryogenic callus, indicated with arrow; (b) suspension culture; (c) sample for radiation treatment; (d) transfer of culture to RITA system; (e) somatic embryos with close up (f); (g) in vitro plantlets.

To evaluate the potential of direct SE for mutation breeding of arabica coffee, the PBGL is working in partnership with Prof Werbrouck, Applied In Vitro Plant biotechnology Laboratory, Ghent University, Belgium under the PUI 'Initiative 'Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa' and following the protocols by Murvanidze, et al. (2021). These experiments so far show that direct SE of arabica coffee induces embryogenic masses two weeks after culture initiation. At four weeks, somatic embryos are visible and after six weeks the embryos germinate (Fig. 1a, 1b). Radiosensitivity experiments have been initiated to determine the optimal timing and doses of the irradiation treatment using coffee leaf discs as propagules. This is a significant time gain compared to indirect SE methods while indirect SE has the potential advantage of generating highly synchronized cell cultures



Figure 1b. Direct somatic embryogenesis in arabica coffee. A) Leaf disc on the first day of inoculation. B) Pro-embryogenic mass at two weeks after inoculation. C) Round embryogenic structures at four weeks after inoculation. D) Somatic embryo development six weeks after inoculation.

Efficient Microspore Embryogenesis and Doubled Haploidy for Accelerated Rice Mutation Breeding

Doubled haploid (DH) technology facilitates the rapid production of homozygous plants and thus has the potential to increase the efficiency of mutant selection. For many crops, in vitro tissue culture technologies are used to develop DH plants. Androgenesis is one of two main methods of haploid induction forcing cultured male gametophytes into the sporophytic pathway and the most widely used for in vitro haploid induction in plants. Anther and isolated microspore culture are the two main forms of androgenesis-based haploid induction, but there is also an intermediate method of androgenesis, called shedmicrospore culture. Under CRP D25005 efficient protocols were developed for the regeneration of haploid rice plants from anthers at the PBGL in partnership with the Agriculture Biotechnology Research Institute, Iran(see Newsletter No 45). Here the relevant in vitro steps are illustrated along with results from ploidy determination and cytological studies, illustrated in Fig 1 and 2. Detailed information on the technologies developed and potential applications for rice mutation breeding can be found in a recently published article (Tajedini. et al. 2022).

#### Plant Breeding & Genetics Newsletter, No. 50, January 2023



**Fig. 1** The regeneration steps in another culture of rice Hashemi mutant cultivar. **a** globular embryos (red arrows) (bar = 0.5 mm). **b** heart shape embryos (red arrows) (bar = 0.5 mm). **c** differentiating callus showing green spots (bar = 2 cm). **d** regenerated green plantlets (bar = 2 cm)



**Fig. 2** Ploidy determination and chromosome counting of regenerated haploid plantlets (a) and control diploid plants of rice Hashemi mutant cultivar (b)

# IAEA PhD program on mutation breeding of ornamentals

Ornamental plants play an important role in the economies of many countries. Orchids and roses are two of the most prized flowers on both the international and local markets, including in Ghana. Both crops are predominantly propagated through vegetative means by Ghanaian growers.

Conventional breeding of orchids and roses is challenging. Further, Phalaenopsis orchids have a long juvenile growth period, and many varieties are polyploids. Induced mutagenesis provides an attractive and proven alternative to create new diversity in many ornamental plants, including orchids. Under the IAEA Technical Cooperation Project RAF0055, improved in vitro protocols are being integrated with physical mutagenesis techniques to increase genetic diversity in orchids and roses. The project is a collaborative effort between the Nuclear Agriculture Research Institute and the West African Centre for Crop Improvement of University of Ghana, and the Ghent University (UGent), Belgium. Using novel plant hormones, in vitro protocols are being optimized at UGent. At the PGB Laboratory the PhD consultant optimized mutation induction of different propagules of orchids and roses using X- and gamma-ray irradiation during the period June-September 2022.

Radiosensitivity tests were conducted for in vitro propagules and mature plants of roses and Phalaenopsis, respectively. Effect of the radiation treatment on flowering of two Phalaenopsis varieties is illustrated (Fig 1). The irradiated *in vitro* rose nodal cuttings and mature orchid plants were shipped to Ghana for further phenotypic evaluation. Target traits include flower color and shape, growth habit and others of commercial interest to growers.



**Figure 1** Effects of irradiation on flowering time of Eve and Warsaw Phalaenopsis orchids three months after treatment: A and D control plants flowering; B and C irradiated plants three months after radiation treatment

#### **Genomics and Bioinformatics** Fast forward Genetics- PBGL's Bulk Segregant Analysis Software

Bulk Segregant Analysis (BSA) combined with Next Generation Sequencing (NGS) is a fast and effective method to genetically map DNA mutations linked to a phenotype, hence 'fast-forward genetics'. Such phenotype/genotype association knowledge is indispensable for Marker Assisted Selection (MAS). PBGL provides software and training to conduct such mapping projects.

NGS-supported BSA has been used to map Mendelian traits as well as QTLs and thus is an appropriate approach for genetic mapping of induced mutations. Prerequisite is a sufficiently large segregating population. In BSA, individuals are phenotyped for the trait of interest and assigned to one of two bulks (pools) of contrasting phenotype. These two bulks are then subjected to NGS.



**Figure 1.** QTLseqR output depicting the G prime values along all 10 chromosomes of a bulk segregant analysis in Sorghum bicolor. The analysis has used an F2 derived from a backcross of a semi-dwarf mutant to its progenitor. The strong signal in the middle of Chromosome 4 marks the likely location of the mutation causing the segregating recessive semi-dwarf phenotype.



Figure 2. QTLseqR output plotting the G prime values only for Chromosome 4 reveals the peak of the signal to reside at around 35 MB. The three peaks observed may be caused by multiple loci contributing to the phenotype or could be due to inconsistencies in the reference genome, which is highly repetitive around the middle of Chromosome 4, or by structural variants such as a large inversion event.

With whole genome sequencing of the bulks and a reference genome, marker density is saturated and allele frequencies in the bulks are directly observed. Several approaches and statistics have been put forward to leverage the allele frequency signal and underlying recombination events to determine the causal locus. Two such statistics, DeltaSNPindex and G', have been implemented in the statistics software R by Mansfeld and Grumet (2018): QTLseqR . We have adapted this software to read VCF output from our preferred variant calling software, freebayes, and provide the modified QTLseqR along with detailed instructions on installation and use on our PBGL github page (https://github.com/pbgl/QTLseqr). We also

provide case studies in the form of step-by-step tutorials with functional R code and real data. One of the case studies is our in-house mapping of a gamma-ray induced semi-dwarf mutation/trait in sorghum

#### **Data Science Internship at PBGL**

The efficiency of plant mutation breeding can be greatly enhanced through genomics approaches. Analyzing the associated data is still a bottleneck and there aren't nearly enough plant breeders with the necessary computer skills.

The PBGL is directly addressing this shortage. With generous support from ARGONNE National Labs (USA), PBGL was able to host Michael Hall, a statistics graduate from University of Texas, Austin, for a 12-months data science internship (Oct 2021-Nov 2022). Michael received hands-on training in genetics and computer programming, contributing to our ongoing software development.

If you are a recent graduate in bioinformatics, computer science, statistics, or engineering with experience in computer programming we would like to hear from you. You could be our next intern and assist in genome data analysis and learn more about plant mutation breeding.

#### Crop Irradiation Services Provided to Member States

Table 1 lists the irradiation requests received as of 2022-10-03 at PBGL. A total of 34 requests from 17 Member States across 33 different plant species covering 273 accessions/varieties/samples were received and treated.

CRP and TC: Irradiation for CRP and TC projects respectively. PUI: Irradiation treatments for the EB-funded Peaceful Use Initiative 'Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa'

#### **Table 1. Crop Irradiation Services**

Request Number	Country	Request Type	<b>Crop/Species</b>
1647	Namibia	TC	Groundnut
1684	PBGL	PUI	Cooking Banana
1685	The Netherlands		Ornamental
1686	UK		Euphorbia peplus
1687	Germany		Brassica sp.
1688	UK		Papaver sominferum
1689	Germany		Ornamental
1690	PBGL	CRP	Lentil
1691	Hungary		Ornamental
1692	Germany		Ornamental
1693	Saudi Arabia/ France		Solanum sisymbriifolium and Solanum torvum
1694	UK		Cannabis sativa

Plant Breeding & Genetics Newsletter, No. 50, Januar	y 2023
--	--------

Request Number	Country	Request Type	Crop/Species
1695	Slovenia		Triticum durum, Triticum aestivum, Hordeum vulgare, Trifolium pratense, Fagopyrum esculentum and Fagopyrum tataricum
1696	Germany		Rhynchospora pubera and R. tenuis
1697	PBGL	PUI	Coffee
1698	Philippines		Rice
1699	India		Sorghum
1700	PBGL		Sorghum
1701	Bosnia and Herzegovina	TC	Phaseolus vulgaris
1702	Ghana		Cowpea, Soybean, Bambara Beans, Tiger Nut, Groundnut
1703	Ghana	TC	Orchids

Request Number	Country	Request Type	<b>Crop/Species</b>
1704	UK		Cannabis sativa
1705	Burkina Faso	TC	Bambara Groundnut
1706	Nigeria	CRP	Cowpea
1707	Mauritania	TC	Sorghum
1708	DR Congo	TC	Phaseolus vulgaris
1709	Germany		Ornamental
1710	PBGL	PUI	Coffee
1711	Kuwait	TC	Barley, Sorghum
1712	PBGL		Sorghum
1713	Malawi	TC	Groundnut, Pigeon Pea, Soybean,
1714	PBGL	CRP	Chickpea
1715	Bosnia and Herzegovina	TC	Wheat
1716	PBGL	PUI	Coffee
1717	PBGL	PUI	Coffee

#### Individual Training

During 2022 the PBGL hosted four interns, five fellows, and three PhD students as summarized in **Table 2**. In addition, PBGL hosted 9 scientific visitors for periods between 1 day and 2 weeks.

#### Table 2. Individual Training Activities at the PBGL

Name	Country	Status	Торіс	Period
Ms Mihirangani Madushika JAYARATHNE	Sri Lanka	Fellow	Coffee single-cell mutagenesis, mutation induction	3 months
Mr Kulasari RANAWEERA	Sri Lanka	Fellow	Coffee and tea single cell mutagenesis	9 months
Mr Behnam KHIABANI NASERIAN	Iran	Fellow	MABC/bio informatics for mutant discovery	4 months
Ms Alina CAMACHO VILLALOBOS	Peru	Fellow	Coffee seeds and single cell mutagenesis	3 months
Ms Jing SONG	China	Intern	Molecular genetics and genome editing	1 year
Mr Zhizhou NIU	China	Intern	Lentil mutation breeding	1 year
Mr Michael Hall	USA	Intern	Data science	1 year
Mr Radisras NKURUNZIZA	Uganda	PHD-Consultant	Coffee mutation breeding	1 year
Mr Hassan MDUMA	United Republic of Tanzania	PHD-Consultant	Mutation breeding of African cooking banana for Fusarium Wilt resistance	3 months
Ms Sonia MEJRI EP MEJRI	Tunisia	Fellow	Lentil disease resistance	3 months
Ms Abigail TWENEBOAH ASARE	Ghana	PHD-Consultant	Ornamental mutation breeding	4 months
Ms Emma Ramirez	USA	Intern	Banana Fusarium Wilt disease	1 year

## **Publications**

#### **Books**



## Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana

Editors: Joanna Jankowicz- Cieslak, Ivan L. Ingelbrech Springer, 2022 ISBN 978-3-662-64915-2 (eBook) Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana | SpringerLink

#### Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change Edited by S. Sivasankar, T.H.N. Ellis, L. Jankuloski, I. Ingelbrecht. CABI, 2021

ePDF 9781789249101

## Crop Adaptation to Climate Change: High-Temperature Stress in Drought-Prone Areas

Guest Editors: F. Sarsu, B.P. Forster, S. Sivasankar Australian Journal of Crop Science, Southern Cross Publishing, Volume 14, Number 8, 2021 DOI: 10.21475/ajcs.21.15.09.sp https://www.cropj.com/full\_issue\_IAEA\_AJCS.pdf

#### Manual de mejoramiento por mutaciones, Tercera edición

Editado por M.M. Spencer-Lopes, Forster, B.P., Jankuloski, L., Sub Programma de Mejoramiento de Plantas y Genética, División Conjunta FAO/OIEA de Técnicas Nucleares en Alimentación y Agricultura.Manu ISBN 978-92-5-133741-7 © FAO, 2021

Manual de mejoramiento por mutaciones (fao.org)

Manuel d'amélioration des plantes par mutation, Troisième édition Édité par. M.M. Spencer-Lopes, B.P. Forster et L. Jankuloski, Sousprogramme de Génétique et d'Amélioration des Plantes Division mixte FAO/IAEA des Techniques Nucléaires appliquées à

l'Alimentation et à l'Agriculture. ISBN 978-92-5-132932-0 © FAO, 2020 Manuel d'amélioration des plantes par mutation (fao.org)

#### Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice

Sarsu, F., Ghanim, A.M.A., Das, P., Bahuguna, R.N., Kusolwa, P.M., Ashraf, M., Singla-Pareek, S.L., Pareek, A., Forster, B.P., Ingelbrecht, I.L. Springer, 2018

ISBN 978-3-319-77338-4

Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice | Fatma Sarsu | Springer

#### **Technical Documents**

A Low-Cost Genotyping Protocol and Kit for Marker-Assisted Selection of Orange Lemma (rob1.a), a Feed Quality Trait in Barley (Hordeum vulgare L.). Introductory guide and laboratory protocols. I. Ingelbrecht et al., May 2021. https://www.iaea.org/sites/default/files/21/06/nafa-pbg-manual-

diagnostic-marker-assay-ol-barleymay2021.pdf

#### IAEA-TECDOC-1969

**Development of Tolerant Crop Cultivars for Abiotic Stresses to Increase Food Security (<u>IAEA-TEC DOC-1969</u>)** Contributors: N.K.A. Amoah, B. Manneh and I.K. Bimpong. ISBN 978-92-0-123321-9

ISBN 978-92-0-123221-2 (PDF)

#### **Peer-reviewed Publications**

#### 2022

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., TILL, B.J., INGELBRECHT, I. (2022) Physical Mutagenesis and Population Development in Musa spp. In: Efficient Screening Techniques to Identify Mutants with TR 4 Resistance in Banana. Springer Nature. JANKOWICZ-CIESLAK, J., INGELBRECHT, I., TILL, B.J. (2022) Mutation Detection Using Low Coverage Copy Number Variation. In: Efficient Screening Techniques to Identify Mutants with TR 4 Resistance in Banana. Springer Nature.

#### 2021

BOHRA, A., KILIAN, B., SIVASANKAR, S., CACCAMO, M., MBA, C., MCCOUCH, S.R., VARSHNEY, R.K. (2021) Reap the crop wild relatives for breeding future crops. Trends in Biotechnology Oct 8; S0167-7799(21)00197-9.

RAHMAN, M.H., SULTANA, R., SARKAR, M.M.A., ISLAM, S., AZAD, M.A.K., SIVASANKAR, S. (2021) Comparative Profitability and Impact of BINA Developed Aman Mutant Rice Binadhan-7 with Non-Mutant Variety in Bangladesh. Asian Research Journal of Agriculture 14(3): 11-25.

HUSSAIN, M., JANKULOSKI, L., HABIB-UR-RAHMAN, M., MALEK, M., KAMRUL ISLAM, Md., RAHEEMI, M.R., DANA, J., MYAT LWIN, K., AHMAD, F., RIZWAN, M., MOHYUDDIN TALHA, G., ASIF, M., ALI, S. (2021) Improving Sustainable Cotton Production Through Enhanced Resilience to Climate Change Using Mutation Breeding. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

SOFKOVA-BOBCHEVA, S., PANTCHEV, I., KIRYAKOV, I., CHAVDAROV, P., MUHOVSKI, Y., SARSU, F., TOMLEKOVA, N. (2021) Induced Mutagenesis for Improvement of Bean (*Phaseolus vulgaris* L.) Production in Bulgaria. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

JANKULOVSKA, M., IVANOVSKA, S., JANKULOSKI, L., MARKOSKI, M., KUZMANOVSKA, B., BOSHEV, D. (2021) Evaluation of Advanced Wheat Mutant Lines for Food and Feed Quality. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

CÉSPEDES, R., ARRIETA, N., BARQUERO, M., ABDELNOUR, A., NIELEN, S., INGELBRECHT, I. (2021) Determination of Radiosensitivity of *Coffea arabica* var. Venecia Seeds to Gamma-ray Irradiation. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

RABEFIRAISANA, H.J., GHANIM, A.M.A., ANDRIANJAKA, A., RASOAMAMPIONONA, B., JANKULOSKI, L., RAZAFINDRASOA, M.A., RAVELONJANAHARY, N.H., RAKOTOARISOA, N.V. (2021) Impact of Mulch-Based Cropping Systems Using Green Mulch and Residues on the Performance of Advanced Mutants Lines of Maize (*Zea mays* (L.)) Under Infested Field with the Parasitic Weed *Striga asiatica* (L.) Kuntze in Madagascar. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., DATTA, S., VILJOEN, A., INGELBRECHT, I., TILL, B.J. Induced Mutations for

Generating Bananas Resistant to Fusarium Wilt Tropical Race 4. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

THUDI, M., PALAKURTHI, R. SCHNABLE, J.C., CHITIKINENI, A., DREISIGACKER, S., MACE, E., SRIVASTAVA, R.K., SATYAVATHI, C.T., ODENY, D., TIWARI, V.K., LAM, H.M., HONG, Y.B., SINGH, V.K., LI, G., XU, Y., CHEN, X., KAILA, S., NGUYEN, H., SIVASANKAR, S., JACKSON, S.A., CLOSE, T.J., SHUBO, W., VARSHNEY, R.K. (2021) Genomic Resources in Plant Breeding for Sustainable Agriculture. Journal of Plant Physiology (TSI), 257. pp. 1–18. ISSN 0176-1617.

https://doi.org/10.1016/j.jplph.2020.153351

MATOVA, P.M., KAMUTANDO, C.N., SARSU, F., MAGOROKOSHO, C., LABUSCHAGNE, M. (2021) Determining the Optimum Gamma Irradiation Dose for Developing Novel Maize Genotypes. Journal of Crop Improvement. https://doi.org/10.1080/15427528.2020.1850591

SARSU, F., BIMPONG, I.K., JANKULOSKI, L. (2021). Contribution of Induced Mutation in Crops to Global Food Security. ACI Avences en Ciencias e Ingenierías, 12(22), 2–11. https://revistas.usfq.edu.ec/index.php/avances/issue/view/153

#### **Conference Abstracts and Posters**

#### 2022

DHANASEKAR P, SOUFRAMANIEN J, DHOLE VJ, HINGANE AJ, SIVASANKAR S (2022) Physical mutagenesis for induction of resistance against *Maruca* pod borer in cowpea [*Vigna unguiculata* (L.) Walp]. Submitted to the International Conference on Pulses: "Smart Crops for Agricultural Sustainability and Nutritional Security" February 10-12, 2023, New Delhi.

#### 2021

SAIF, A.A., AL-SHAMIRI, A., ALSAGIR, M., JANKULOSKI, L. (2021) Evaluation and Selection Disease Resistant Wheat Mutants to Yellow Rust Disease *Puccinia striiformis f. sp.triticii* Using Gamma-Irradiation Technique in Yemen. 15th Arab Conference on the Peaceful Use of Atomic Energy. 12–16 December 2021. Aswan, Egypt.

TILL, B.J., HAWLICZEK, A., JANKOWICZ-CIEŚLAK, J., GUPTA, P., HOWARD-TILL, R., OSORIO, C.E., BOLIBOK-BRĄGOSZEWSKA, H. (2021) High-Throughput Discovery of Induced and Natural Mutations in Plants. Application Genetics of Plants – Challenges of the 21st Century, 22–24 September 2021, Warsaw, Poland.

AZIZ, S., KANTOGLU, Y., TOMLEKOVA, N., STAYKOVA, T., GANEVA, D., SARSU, F. (2021) Molecular Characterization of Tomato Genotypes (*Solanum lycopersicum* L.) by Simple Sequence Repeat (SSR) Markers. Presented at the 5th Balkan Scientific Conference on Biology (BalkanBio'2021), Plovdiv, Bulgaria: Zenodo. http://doi.org/10.5281/zenodo.4650460

# **News Highlights**

- Nuclear Science Helps Bangladeshi Farmers Resist Climate Change (17 November 2022)
- IAEA and FAO Send Seeds to International Space Station to Develop Crops Able to Adapt to Climate Change on Earth (7 November 2022)
- New CRP: An Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana Phase II (D23033) (12 October 2022)
- Nuclear Explained Developing New Plant Varieties for Food Security, Biodiversity and Sustainable Ecosystems (5 August 2022)
- Tackling Cassava Brown Streak Disease in Uganda with Nuclear Techniques (31 May 2022)
- #BiodiversityDay: How the IAEA Contributes to Bend the Curve of Biodiversity Loss (20 May 2022)
- CRP Success Story: Mutation Breeding for Resistance to Striga Parasitic Weed in Cereals for Food Security (D25005) (18 March 2022)
- IAEA Trains Latin American Scientists in Fighting Banana Disease (10 March 2022)
- Mow Nuclear Science Helps Fruits and Vegetables Survive and Thrive (24 February 2022)

## Websites and Links

- Plant Breeding and Genetics Section: <u>https://www.iaea.org/topics/plant-breeding</u>
- InfoGraphic on Mutation Breeding: <u>https://www.iaea.org/newscenter/multimedia/videos/using-nuclear-science-to-boost-plant-biodiversity</u>
- Mutant Variety Database: <u>http://mvd.iaea.org</u>
- Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture: <u>https://www.iaea.org/about/organizational-structure/department-of-nuclear-sciences-and-applications/joint-fao/iaea-centre-of-nuclear-techniques-in-food-and-agriculture</u>
- Plant Breeding Publications: <u>Plant breeding publications | IAEA</u>
- Food and Agriculture Organization of the United Nations (FAO): Home | Food and Agriculture Organization of the United Nations (fao.org)

#### Impressum

#### Plant Breeding and Genetics Newsletter No. 50

The Plant Breeding and Genetics Newsletter is prepared twice per year by the Plant Breeding and Genetics Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture & Biotechnology Laboratories, Seibersdorf.

> International Atomic Energy Agency Vienna International Centre, PO Box 100, 1400 Vienna, Austria Printed by the IAEA in Austria, January 2023

> > 23-00134

#### Disclaimer

This newsletter has not been edited by the editorial staff of the IAEA. The views expressed remain the responsibility of the contributors and do not necessarily represent the views of the IAEA or its Member States. The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.