**To Our Readers**

Dear Colleagues,

It is with this curious mixture of elation and a little heaviness of heart that I write my final “To our Readers” section of the Plant Breeding and Genetics (PBG) biannual newsletter prior to my retirement in December 2023.

The PBG team continued to deliver steadily and strongly over the last six months and through 2023 in research and development (R&D), and technology transfer, to Member States in support of crop improvement for food and nutrition security and farmers’ income under climate change. We continued to support 56 active Technical Cooperation Projects (TCPs), including national and regional, across more than a hundred Member States while we finalized design for 28 new TCPs due to start in 2024. A total of thirty-one training courses were coordinated and delivered during the year on different topics in crop improvement and related biotechnologies and were attended by 290 women and 352 men researchers from counterpart institutions across various countries.

The current issue of our newsletter provides an update on the seeds returned from the International Space Station (ISS), specifically, on the initial germination tests on one of the two species of seeds, *Arabidopsis thaliana* (page 34). The space experiment included three different seed treatments, with seeds...
placed inside the ISS for five months, outside the ISS for five weeks, and retained on Earth as control. All three seed treatments germinated in our laboratory with similar germination percentages, all close to one hundred percent and grew out into well-formed seedlings. This *astrobiology experiment* falls under the FAO/IAEA Coordinated Research Project (CRP), D24015, which focuses on advanced technologies in radiation-induced genetic variation for crop improvement (page 17). This CRP launched in 2022 addresses, as part of its objectives, the effects of different types of radiation, including cosmic, on DNA structural variation and plant biology. Of the 15 project components, four address space-induced mutations, including our own feasibility study at the ISS. First report from one of these projects, *Molecular Analysis of Genetic Imprints Across Rice Genome and Enhancement of Germplasm Induced by Circumlunar Space Flight of Chang'e 5*, indicates extensive genomic variation from deep space flight. GC to AT mutations were higher, DNA methylation modification was different, RNA methylation modification was significant, and differentially methylated regions were enriched for genes functioning in stress response in rice from the circumlunar deep space flight, compared to earth control. Many different plant and grain type mutants were recovered, and both mutation frequency and biological effects were higher in the deep space samples compared to other space mutagenic methods. On the topic of *astrobiology*, I further note our successful organization of a virtual event, “*Astrobiology and Space Breeding for Food Security under Climate Change*”, at the World Food Forum on 18 October 2023, (page 15).

Support to Member States for management of the banana *Fusarium wilt* disease continues, both on R&D and technology transfer. The CRP, *An Integrative Approach to Enhance Disease Resistance Against Fusarium Wilt (Foc TR4) in Banana – Phase II*, launched in early 2023, continues research on genetic resistance, disease detection and surveillance, and biological control involving beneficial microbes. As part of the inter-regional TCP, INT5158, in addition to the global workshop on Disease Detection and Surveillance and the regional coordination meeting reported in the first half of the year, PBG within the context of the IAEA Technical Cooperation Programme coordinated a study tour in October 2023 for researchers from Latin America and the Caribbean to Brisbane and Queensland, Australia, on *How Australia is managing TR4*. Further, a two-week regional training course on tissue culture, mutation breeding, resistance screening and disease detection was offered in October-November to a team of plant breeders and national plant protection officers in our laboratories at Seibersdorf, Austria.

Results from our CRPs are highly significant, specifically from CRP D22006 on pulses, and from CRP D24014 on vegetatively propagated crops and horticultural tree crops, (pages 16 and 17). *Research at our laboratory* reports important outputs in this issue, especially in relation to the Peaceful Uses Initiative (PUI) project, *Enhancing Climate Change Adaptation and Disease Resilience in Banana-Coffee Cropping Systems in East Africa* (page 34). The first field screening of about 5,000 *in vitro* Mchare banana plants in a hot spot field in Tanzania is in progress with dual scoring for symptoms. The Loop-Mediated Isothermal Amplification (LAMP) Assay for rapid diagnosis of the disease has been validated and optimized at the lab. A protocol is in development for high-throughput screening in coffee for resistance to the leaf rust disease. Beyond the PUI project, a new protocol has also been developed for amplicon-sequencing with Oxford Nanopore long reads, and the digital droplet PCR method was successfully applied for the detection of rare mutations in genes involved in *Striga* resistance in sorghum.

I am happy to announce that the *first IAEA Collaborating Center for PBG in Africa* was established this year at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission following a signing ceremony on 29 September during the 67th General Conference of the IAEA. Under the agreed workplan, BNARI will share capabilities and expertise on *in vitro* multiplication and provide irradiation services to other countries in the region, while performing collaborative research on tissue culture and mutation breeding with PBG. The extension agreement for the IAEA Collaborating Centre at the Malaysia Nuclear Agency to facilitate extensive use of the gamma greenhouse facility, including provision of services to plant breeders across Southeast Asia, was also signed during the 67th General Conference.

It is also my pleasure to announce that in line with the *International Year of Millets 2023*, our next CRP to be launched in 2024 focuses on improvement of pearl millet, finger millet and proso millet. The call for proposals to participate in this CRP, D24016, *Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation*, is now open (page 39). Also, of note is that PBG will coordinate the second workshop on Plant Mutation Breeding at the Plant and Animal Genome Conference (PAG) 31 in San Diego, USA, in January 2024 (page 15). Further, this newsletter reintroduces summary information and pictures of recently released mutant varieties, a practice that was in place a few decades ago during the time of one of my predecessors, and that was significantly appreciated by our readers as I learned recently.

Finally, this newsletter also introduces our efforts towards onboarding genome editing for plant breeding research at PBG. The intention is to have the technology in our laboratory for research purposes, (1) should a Member State request capacity building on it, and (2) for our in-house research to validate genetic associations in mutant plant phenotypes. Three feature articles in this issue address the topic, including the perspective of PBG, and results of leading researchers in the field on application of the technology to seed crops and to vegetative crops (page 6).

And now, to say goodbye…

These last five plus years at the Joint FAO/IAEA Centre have been a bit of an eye-opener, one way or the other, at every turn. The emergence of the first hybrid corn and the publication of the first reports on radiation-induced mutations in barley, maize, oats and wheat by Dr LJ Studler both during the decade of 1920 holds a significant meaning. The early 1900s witnessed the testing of multiple approaches to enhance crop genetic variation for productivity gains. While heterosis remains a boon for productivity enhancements in cross-pollinated crops, the improvement of self-pollinated and clonally propagated crop remains stilled by limitations in genetic variation. Interestingly, these latter also come under the category loosely named, ‘under-utilized’, ‘under-explored’, or ‘orphan’ crops. Radiation-induced genetic variation emerged as a strong avenue for enhancing these crops in many developing countries of the world, significantly contributing to global food security over the last few decades. Equally, it has been used for the improvement of major seed crops in many of the same countries, and rice mutant varieties lead among the crop species in the Joint FAO/IAEA Mutant Variety Database. Induction of novel genetic variation at the start of a
breeding pipeline significantly broadens the possibilities to select traits of interest. Following that start, effectiveness, efficiencies and outcomes are determined by the precision of selections, techniques that accelerate the pace of breeding, and stakeholder platforms that allow scaling up of quality seeds of newly developed varieties. However, success in realizing fast, precise and uniform gains in crop productivity remains highly variable between countries and is determined by the level and sophistication of existing technical and infrastructure capacities.

In recent years, PBG endeavored to (1) increase statistical rigor in design and analysis of breeding experiments for efficient selections, (2) incorporate speed breeding techniques and rapid generation advancement in breeding pipelines for faster variety development, (3) expand molecular breeding tools beyond TILLING under the broader umbrella of Functional Genomics for Trait Utilization (FGTU) to keep pace with advancing and cost-effective genome-level sequencing methods, (4) address crop improvement from a holistic perspective by coupling mutation breeding for disease resistance with detection and surveillance methods, and with biological control using beneficial microbes, (5) explore forms of radiation for mutagenesis, including cosmic radiation, beyond the traditionally used gamma- and x-rays, (6) evaluate seed system models to scale up newly released varieties, and (7) pilot a mutation breeding network to facilitate wider exchange of knowledge and expertise for crop improvement.

It has been my humble pleasure to serve you in my role at PBG, and to have made many of your acquaintance directly or indirectly. I am grateful for your collaboration over these years. I have been fortunate to be able to stand on the shoulders of giants who led PBG before me and this helped – to see further beyond, and to strategize and implement on your behalf. I want to note that this would not have been possible had it not been for the grace of my teachers and mentors through life who allowed me to imbibe some of their critical thinking, work ethics, integrity, grounding, courage, compassion, and other old-school values.

I wish you and PBG the very best in your efforts and contributions to the global march towards food security.

I am retiring from the Joint FAO/IAEA Centre, but my journey continues. The simple words on a wall-hanging in my undergraduate classroom comes to mind ... from Robert Frost’s “Stopping by Woods on a Snowy Evening”

The woods are lovely, dark and deep,
But I have promises to keep
And miles to go before I sleep
And miles to go before I sleep

Yours sincerely,
Shoba

Shoba Sivasankar
Head, Plant Breeding and Genetics Section
# Staff

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## Plant Breeding and Genetics Section

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## Plant Breeding and Genetics Laboratory

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Welcome

Ms Velina Bojkova joined the Plant Breeding and Genetics Section as a Programme Assistant. She completed her master’s degree in English studies and economics in Germany, including a semester abroad in London. Before joining PBG, Velina had the opportunity to gather experience in the Department of Nuclear Safety and Security, namely from the Division of Radiation, Transport and Waste Safety, Incident and Emergency Centre, Division of Nuclear Security and Division of Nuclear Installation Safety.

Farewell

In this newsletter, we bid farewell to Sobhana (Shoba) Sivasankar who led Plant Breeding and Genetics (PBG) at the Joint FAO/IAEA Centre as Section Head for the last five years and four months. Shoba retired in December 2023 at the standard UN retirement age, after strong contributions to the PBG program during her short tenure leading a technical team for R&D and technology transfer to more than a hundred Member States/Countries of the IAEA and the FAO. In her role as Section Head, she provided strategic direction to onboard, strengthen and transfer next-generation concepts and technologies in crop improvement towards national, regional and global food security under climate change. Concepts, technologies and practical applications onboarded/strengthened during her five plus years at the IAEA headquarters in Vienna, Austria, include functional genomics for trait utilization, cell/tissue culture and induced genetic variation for improvement of vegetative and tree crops, seed systems for upsaling of improved crop varieties, microbes in biological control of plant diseases, astrobiology and space breeding, gene editing, and the emerging concept of PlantLabs for plant disease detection.

Shoba came to us with over 25 years of experience leading international agricultural R&D programs in both the public and private sectors, with focus on crop improvement and climate-smart solutions for crop production. Formerly, as Director of two CGIAR mega-Programs, she drove their exceptional performance solutions for crop production. Formerly, as Director of two private sectors, with focus on crop improvement and climate-smart international agricultural R&D programs in both the public and private sectors, she led Plant Breeding and Genetics (PBG) at the Joint FAO/IAEA Centre as Section Head for the last five years and four months. Shoba retired in December 2023 at the standard UN retirement age, after strong contributions to the PBG program during her short tenure leading a technical team for R&D and technology transfer to more than a hundred Member States/Countries of the IAEA and the FAO. In her role as Section Head, she provided strategic direction to onboard, strengthen and transfer next-generation concepts and technologies in crop improvement towards national, regional and global food security under climate change. Concepts, technologies and practical applications onboarded/strengthened during her five plus years at the IAEA headquarters in Vienna, Austria, include functional genomics for trait utilization, cell/tissue culture and induced genetic variation for improvement of vegetative and tree crops, seed systems for upsaling of improved crop varieties, microbes in biological control of plant diseases, astrobiology and space breeding, gene editing, and the emerging concept of PlantLabs for plant disease detection.

high-throughput plant phenotyping platform at DuPont Pioneer, (c) the diversity networks and mentoring program at DuPont Pioneer, (d) the first Farming Systems Research Station in Kerala, India, and (e) the agricultural extension office in Kottanad, Kerala. Shoba is inventor in over 120 published patent applications and is author/editor of many peer-reviewed publications/books. She has a Ph D from the University of Guelph, Canada, and MBA from the University of Iowa, USA.

Shoba leaves us in search of newer pastures to apply, to the extent possible, her vision, creativity, drive and entrepreneurship for global good.

Ms Emma Ramirez joined PBGL for a year-long internship, funded by Argonne National Laboratory (USA), to support the Banana Fusarium Wilt disease project. She came to us fresh out of university with a passion for agricultural sustainability and a B.S. in genetics and genomics from UC Davis. Her time at the lab began in plant pathology where she helped run phenotyping experiments and develop screening procedures for *Foc* TR4 in banana. Emma also trained in implementing molecular diagnostic methods to target the fungal pathogen’s DNA in planta through the validation of a LAMP PCR assay. She expanded her efforts in supporting another staple crop, sorghum, where she gained experience with next generation library preparations and amplicon sequencing of sequences known to affect *Striga* resistance. She participated in the design and development of digital droplet PCR assays for rare mutation detection in putative sorghum mutant lines. Her training and efforts in the lab translated into three protocols and training material for use by Member States. She leaves with a solid understanding of *in vitro* tissue culture, molecular biology, molecular marker development and analysis.

Mr Sharath Chandran U.S. (India) joined the PBG Laboratory in August 2023 as a consultant for three months. Sharath holds a BSc in Agricultural Sciences from Kerala Agricultural University, India, and a MSc in Plant Pathology from Acharya N.G. Ranga Agricultural University, India. Later, he joined the Legumes Pathology Division at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, where he worked as a Senior Research Fellow for five years. There, Sharath focused on assessing the impact of climate change on the major diseases of chickpea and pigeonpea using artificially simulated environments.

At the PBGL, Sharath was tasked with developing a protocol for screening the mutant lentil population against the Stemphylium Blight disease. Given his previous experience working with multiple crop pathogens, Sharath was able to quickly design the experiments required to standardize a screening protocol, and he later compiled the results of his experiments in an elaborate report, including the necessary guidelines and recommendations.

Sharath plans to continue his education by pursuing a PhD in the field of translational phytopathology. He is passionate about mycology and plant protection, hopes to develop sustainable and eco-friendly solutions for crop disease management.
**Feature Articles**

**Gene Editing in Research: Functional Genomics, Disease Diagnostics**

Shoba Sivasankar  
Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria

### Introduction

Mutations and genetic recombination are the bases of evolution and contributed to the empirical selection and domestication of crop plants for more than 10,000 years. After the rediscovery of Mendel’s laws of inheritance in 1900, scientific and systematic plant breeding applied increasingly refined technologies to the development and selection of new genetic variation for the continuous improvement of several crop species. In 1901, the Dutch biologist Hugo de Vries, one of the three who rediscovered Mendel’s laws, coined the term “mutation” for the sudden heritable variation he observed in his experimental material, the evening primrose. While observations of such sudden heritable changes were reported at least three centuries earlier, purposeful application of mutations in plant breeding started only in the early twentieth century. Similarly, while the scientific identification of hybrids in plants happened as early as the 1700s, the application of hybridization in scientific plant breeding took off only in the early twentieth century. It is interesting that the development and commercialization of the first maize hybrid, and the first discovery of radiation-induced mutagenesis in plants, including maize, both occurred during the decade of 1920-30. The years since then have seen increasing sophistication in plant breeding tools and technologies in conventional breeding, hybridization, mutation breeding, quantitative genetics, molecular breeding, transgenic technologies, genomic selection, and most recently, gene editing. Together, over the last hundred plus years, these have enabled continuously increasing crop productivity, i.e., the weight of consumable plant product per unit area under cultivation, aiming for food sufficiency on the planet. They also enabled the stabilization of productivity under such pressures of climate change as drought, warming temperatures, soil salinization, flooding, and increasing incidence of diseases and pests. These improvements have not, however, been the same across all parts of the world or across all crops critical to nutrition and diet diversity. Tested and proven new innovations relevant to the improvement of crops, and their evolving modifications, are crucial for research and where possible, for the development of improved crop varieties. They provide new and additional leverage to the plant breeder’s toolkit in the quest towards global food sufficiency.

Gene editing, as a recent technology with important applications in plant breeding research, is addressed in three feature articles in the current newsletter. The present article is written in the context of our work on mutation breeding at Plant Breeding and Genetics and is followed by two further articles that describe the application of the technology in vegetative and seed crops by global experts in the field. I further note here that the recent Issue Paper from the Food and Agricultural Organization of the United Nations (FAO), *Gene Editing and AgriFood Systems*, provides a very good discussion of the technology, addressing its application in plant and animal breeding together with considerations in governance and regulation. At the Joint FAO/IAEA Centre, gene editing using the CRISPR (clustered regularly interspaced short palindromic repeats)-associated protein (Cas) system is being onboarded in Plant Breeding and Genetics for (1) fundamental research to validate mutations for establishing genetic associations, and (2) capacity building, should a Member State request technical support for the technology. Eventual exploration of the CRISPR-Cas system for field diagnostics of plant viral diseases is also a future consideration once this application matures in academic research.

**Gene Editing and CRISPR-Cas**

Gene editing has facilitated the introduction of site-specific changes in the existing genetic structure of an organism. Prior to the discovery of CRISPR-Cas9, other sequence-specific nucleases were in use for gene editing including meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). The programmable DNA endonuclease, CRISPR-Cas9, and its potential for gene editing was first published in 2012 (Jinek et al., 2012). Awarded the Nobel Prize in Chemistry in 2020, the CRISPR-Cas gene editing system works by producing DNA double-strand breaks by a sequence-specific nuclease at the targeted site in the genome thereby facilitating the possibility of site-specific mutations and integrations. Beyond the earlier known Cas proteins, Cas9 and Cas12a, current research continues to identify newer CRISPR-Cas systems from bacteria and archaea, and the type I CRISPR-Cas10 was shown to induce small indels as well as bi-directional long-range deletions in tomato (Osakabe et al., 2020). The Type VI CRISPR-Cas13 was identified to be a unique system that recognizes and cleaves single-stranded RNA and was shown to knock down transcript levels of three endogenous genes in rice protoplasts (Abudayyeh et al., 2017). Other advances made in this field since 2012 include base editing, DNA-free delivery methods of CRISPR-Cas reagents to cells, multiplex editing, high-throughput editing, etc.

Several instances of gene editing in plants have been reported in scientific literature in the years since 2012, and these have covered a few different species. The majority of these sought to address resistance to diseases or tolerance to abiotic stresses, while some addressed food and feed quality. Three gene-edited plants have been commercialized so far, the first introduction being that of soybean with improved oil quality in 2019 in the USA where the edit was accomplished with TALENs, targeting the fatty acid desaturase 2 (*FAD2*) genes (Demorest et al., 2016). In 2021, tomato plants edited with CRISPR-Cas9 for higher amounts of gamma-aminobutyric acid (GABA) for better health were released for growing in Japan (Nonaka et al., 2017). In 2023, mustard greens edited with the CRISPR technology for reduced bitterness became available as a food product in the USA under the name, Conscious Greens. At the present time, for successful deployment as a new crop variety, the plant species and the variety to be edited should be amenable to transformation, and the end product should...
not contain foreign DNA such as the Cas9 gene and selection markers.

**Established genetic association is a pre-requisite for editing**

The central requirement for gene editing is that information is known about the gene or sequence to be edited to obtain the desired trait or characteristic in the edited organism. Established genetic associations between a given trait and its causal gene(s) are known for only the smallest fraction of the genes in a genome. The genomics database of the National Center for Biotechnology Information reports a total of 27,562 protein-coding genes in the model plant species, *Arabidopsis thaliana*, 34,301 in maize, 28,240 in sorghum, and 28,849 in rice. Of these tens of thousands total number of genes, established genetic associations are available for perhaps less than a hundred. In this context, it is important to note that the function of the FAD2 genes that led to the gene-edited soybean product was first reported as early as 1994 by associating the oil quality trait, namely, fatty acid composition, in mutant Arabidopsis with the cloned gene (Okuley et al., 1994). Since the 1980s, mutant populations of Arabidopsis have been rich resources for establishing plant gene functions related to traits of interest, and for delineating biochemical pathways. Chemical and physical mutagenesis, transposon insertions, T-DNA-tagging and activation tagging have all played a role. In the case of the gene-edited tomato with higher GABA levels, information of the plant glutamate decarboxylase gene with a calmodulin-binding domain with postulated role in GABA synthesis was first reported in the nineties, this time in petunia (Baum et al., 1993).

Thus, the established function of the gene(s) to be edited and association with the trait sought in the edited plant constitute crucial information needed prior to initiation of a gene editing project for product development, i.e., for the development of a new crop variety. Conversely, the CRISPR-Cas system can be used for functional validation of gene(s) and the establishment of the aforementioned genetic associations. In the words of Dr Jennifer Doudna, Nobel Laureate, in the Foreword to the FAO Issue Paper on gene editing, “The benefit of CRISPR extends beyond the development of products. As a research tool it can be used to conduct genetic screens, unlocking new biological pathways and expanding our knowledge of the genome and the functional impact of mutations, all of which provide us with new options for future applications”.

**CRISPR-Cas in functional genomics research**

The large number of farmer-preferred mutant crop phenotypes resulting from radiation-induced mutagenesis reported from counterpart institutions in Member States of the Joint FAO/IAEA Centre offers a rich resource for the establishment of such genetic associations. Usable traits for the farmer such as short duration, short stature, disease resistance, salt tolerance, seed color (as with black seed in soybean), increased pod number etc., have been selected in crops of importance to individual Member States, mainly for direct deployment to variety development. Identification of the gene(s) responsible for such simple traits through whole genome analysis of bulk segregants followed by validation of the genetic association by gene editing will yield novel information for all forms of precision breeding, either existing currently or newly evolving in the future. A note of caution is that, with recalcitrance in transformation for many crop species and varieties, such validation experiments will need to be designed and inferences made using orthologous genes in transformable model plant species.

The time required to develop and deploy a new crop variety through gene editing in seed crops can be as short as 4-5 years, provided that: (1) the gene(s) to be edited is known, with established evidence for association with the target trait, (2) the crop variety to be edited is transformable, (3) the regulatory environment of the country is positive with regard to the technology, and (4) there exists the required technical and infrastructure capacity in the country. Further, gene editing at present is most successful in the case of simple traits governed by one or a few genes, such as early maturity, short duration etc., and not for complex traits such as yield, or drought tolerance that are governed by large numbers of genes. When the above four provisions do not exist, as is the case possibly for more than 90% of public sector breeding programs across the globe, and when the target trait is complex, mutation breeding is a very good alternative for the induction of random mutations in the genome of the crop variety to be improved followed by precise selection for the desired trait. Mutation breeding has been used for almost the same length of time as conventional plant breeding and has been heavily deployed for crop improvement during the almost 100 years since Dr Lewis John Stadler first reported radiation-induced mutations in plants from the University of Missouri, USA, in 1928. Results are evident in the 3400 plus varieties voluntarily submitted into the FAO/IAEA Mutant Variety Database by breeders in FAO/IAEA Member States. Beyond variety development, the genomic era now offers the opportunity to establish genetic associations in mutant plants for deployment in all forms of precision breeding, as mentioned earlier. On the topic of time taken for crop variety development, I want to note here that ion beam radiation that produces double-strand DNA breaks much the same as the Cas9 nuclease, coupled with photo-insensitivity, a Green-Revolution breeding target, resulted in the development of new rice varieties recently in a period of 4.5 years from the date of seed irradiation to the date of variety registration and release in Bangladesh.

**CRISPR-Cas in disease detection**

Before closing, I want to discuss the potential application of CRISPR-Cas systems in the diagnosis of diseases (Aman et al., 2020). Increasing insights into the CRISPR-Cas system and the discovery of new variants of the Cas endonuclease have led to the utilization of specific attributes in disease diagnosis. Some CRISPR-Cas systems have been shown to exhibit nonspecific catalytic activity after sequence recognition and cleavage of the target, resulting in the degradation of single-strand DNA (ssDNA) and single-strand RNA (ssRNA) sequences in the vicinity enabling the release and detection of quenched-fluorescence signals in reporter sequences. Thus, Cas13a was deployed in the *in vitro* nucleic acid detection platform, Specific High Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK), for real-time detection of attomolar concentrations of genomic fragments of the Zika virus, the Dengue virus and pathogenic bacteria (Gootenberg et al., 2017). Subsequent to SHERLOCK, a few other detection platforms have been described, starting with DNA endonuclease-targeted CRISPR trans reporter (DETECTR) using Cas12a, and heating unextracted diagnostic samples to obliterate nucleases (HUDSON) using Cas13 (Chertow, 2018, and the articles discussed). While the previously described detection methods used pre-amplification of the target sequence with different PCR methods prior to detection by the CRISPR-Cas system, a recent Cas13a-based method with improved sensitivity and specificity.
without a pre-amplification step was described to detect SARS-CoV-2 (Fozoumi et al., 2021). The detection process took 30 minutes and was coupled with mobile phone microscopy to enable point-of-care screening.

In plants, CRISPR-based diagnostic methods were first described in 2021. The endonuclease, Cas12a, was used in the highly specific detection of the tomato yellow leaf curl virus and the tomato leaf curl New Delhi virus (Mahas et al., 2021). Also, in an inexpensive, field-deployable multiplex detection of five different apple viruses, namely, apple necrotic mosaic virus, apple stem pitting virus, apple stem grooving virus, apple chlorotic leaf spot virus and apple scar skin viroid (Jiao et al., 2021). Rapid, low-cost, highly specific, sensitive, field-based methods are critical in the early detection and management of plant diseases, and the prevention of their spread. CRISPR-Cas-based diagnostic methods are still in research but offer great potential especially with the possibility of multiplexing and without a need for pre-amplification.

**Summary**

Innovations are pre-requisite for removing existing bottlenecks in interventions, and for achieving step changes where the need exists. Gene editing is a recent innovation that continues to be explored in plant sciences for crop improvement. Its application in the development of new crop varieties for cultivation is dependent on a few distinct factors, and its application for research purposes can help in the validation of genetic associations for precision breeding. Emerging research opens new possibilities in the application of CRISPR-Cas in low-cost, field-based disease diagnosis.

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**GEd for improvement of banana**

**Jaindra Nath Tripathi, Valentine Otang Ntui, Leena Tripathi**

*International Institute of Tropical Agriculture, Nairobi, Kenya*

In East African countries like Burundi, Rwanda, and Uganda, bananas hold significant importance by providing 30–60% of the daily per-person calorie intake, with Uganda exhibiting the highest consumption rates (Dale et al., 2017). East Africa is the largest region in banana cultivation and consumption, with a remarkable annual per-person consumption ranging from 220 to 460 kilograms (Ainemhabazi et al., 2015). Despite its prominence, a substantial yield gap exists in African bananas and plantains production, ranking among the lowest in the world (FAOSTAT, 2021). This yield disparity can be attributed to the vulnerability of these crops to climate change, erratic weather, and a myriad of bacterial, fungal, and viral diseases (Tripathi et al., 2020). Considering the pivotal role of bananas as a fundamental food source in Africa, it becomes imperative to prioritize genetic enhancement strategies to bolster the crop’s resilience against biological and environmental stressors.

One promising avenue for achieving this goal involves the deployment of precise genetic tools such as GEd (GEd). Among all the GEd techniques, the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas associate protein (Cas)
(CRISPR/Cas) has been most commonly used to manipulate plant genomes. The CRISPR/Cas creates double-stranded breaks, which are then repaired by the cell’s own natural mechanism of homology-directed repair (HDR) or non-homologous end joining (NHEJ). The CRISPR/Cas tool comprising the Cas nuclease and the gRNA (guide RNA) is simple, easy to adapt, and can edit multiple genes simultaneously (multiplexing) with high efficiency (Ntui et al., 2020). Several CRISPR/Cas variants with different editing strategies, such as Cas9, Cas12a (Cpf1), Cas13, CRISPR activation (CRISPRa), CRISPR interference (CRISPRi), base editing, and prime editing have been developed for editing in plants (Tripathi et al. 2020). These advanced tools can facilitate the production of superior banana cultivars with enhanced yields and resistance to diseases and pests.

**Improving Banana through GEd**

Banana production is grappling with a multitude of challenges, both biotic and abiotic, leading to significant yield losses. To address these constraints effectively, there is a pressing need to explore cutting-edge scientific innovations, particularly in the realm of new breeding technologies like GEd. While genome-edited bananas have not yet made their way into commercial markets, researchers are actively engaged in developing banana varieties with improved traits using genome-editing techniques.

In various research laboratories, GEd technologies have been successfully employed for bananas. For instance, Kaur et al. (2018) demonstrated GEd in the "Rasthali" cultivar (AAB genome) by targeting the *Phytoene desaturase* (PDS) gene. They utilized a single gRNA to introduce mutations in the PDS gene, resulting in albino phenotypes, albeit with an efficiency of 59%. The PDS gene is involved in the carotene biosynthetic pathway, and its mutation leads to the development of albino plants, which are easily recognizable. Similarly, Naim et al. (2018) reported successful editing of the PDS gene in "Cavendish Williams" (AAA genome) with a remarkable efficiency of 100%, achieved using polycistronic gRNAs.

Researchers at the International Institute of Tropical Agriculture (IITA) have established a robust GEd tool for bananas and plantains, targeting the PDS gene (Ntui et al., 2020). By delivering a CRISPR/Cas9 construct containing two gRNAs into embryonic cells of both the "Sukali Ndiizi" banana cultivar and the "Gonja Manjaya" plantain cultivar, they produced mutants displaying albino phenotypes, indicating disruptions in the function of the PDS gene. Sequence analysis confirmed the presence of indels (insertions/deletions) at the targeted sites, demonstrating a remarkable mutation efficiency of 100%.

It is worth noting that knocking out the PDS gene can have adverse effects on plant development, often leading to plant death due to the inability to photosynthesize. To mitigate these negative impacts, Zorrilla-Fontanesi et al. (2020) modified the *RP43/CHAOS39* gene, responsible for encoding the chloroplast signal recognition particle (cpSRP) machinery, to serve as a visual marker. The CHAOS39-modified banana plants exhibited pale-green phenotypes but grew normally. However, it is essential to exercise caution when using cpSRP43/CHAOS39 as a visible marker, as pale green phenotypes can also result from other factors.

These advancements in CRISPR/Cas-based GEd have opened up new horizons for accelerating banana improvement. With well-annotated reference genome sequences, a well-established CRISPR/Cas9-editing system, and a developed banana transformation and regeneration process, researchers are poised to generate disease-resistant banana varieties by precisely editing the plant's endogenous genes (Tripathi et al., 2019a; 2020; 2022). An exciting application is the development of varieties resistant to diseases like banana Xanthomonas wilt (BXW) by editing genes that render the plant susceptible to the disease.

For instance, the *Downy mildew resistance 6 (DMR6)* gene, a susceptibility gene encoding 2-oxoglutarate Fe (II)-dependent oxygenase (2OGO), which is upregulated during pathogen infection, has been targeted for editing (Tripathi et al., 2021). DMR6 and its paralog, DMR6-Like Oxygenase1 (DLO1), play a pivotal role in suppressing plant immunity and are overexpressed during pathogen infection (Zeilmaker et al., 2015). By delivering CRISPR/Cas9 reagents targeting *MusaDMR6* editing into the embryonic cell suspensions of "Sukali Ndiizi," researchers at IITA generated mutants exhibiting enhanced resistance to BXW, as confirmed in greenhouse evaluations (Figure 1). These banana mutants showed no detrimental effects on plant growth, although further testing in field conditions will be necessary to assess overall agronomic performance.

**Figure 1:** Genome-edited bananas with edited *MusaDMR6* gene showing resistance to bacterial wilt disease. A) The edited banana plants displayed no symptoms following challenged with the deadly bacterial pathogen *Xanthomonas campestris pv. musacearum*. B) Non-edited control plants exhibiting wilting symptoms within 35 days of inoculation.

To combat the *Banana Streak Virus* (BSV), scientists at IITA have explored the feasibility of deactivating the integrated endogenous *Banana Streak Virus* (eBSV) by disrupting the integrated viral sequences within the B genome of the preferred plantain cultivar "Gonja Manjaya" (Tripathi et al., 2019b). Through targeted genetic modifications, these researchers were successful in impeding the formation of functional episomal BSV proteins. Consequently, 75% of the mutants exhibited no symptoms, starkly contrasting to the non-edited plants, especially under stress conditions in controlled greenhouse experiments. This breakthrough validates the inactivation of eBSV, and its implications are far-reaching. The study has the potential to yield virus-free banana germplasm, enhancing the B genome and its derived genotypes for the development of improved hybrid bananas with global dissemination potential (Tripathi et al., 2019b).

Furthermore, CRISPR/Cas9 technology has been deployed for banana improvement for enhancing micronutrients. Kaur et al. (2020) used CRISPR/Cas9 to increase β-carotene content in the "Grand Naine" cultivar by editing the *lycopene epsilon-cyclase (LCYe)* gene. This resulted in enhanced β-carotene accumulation, up to 6-fold higher (~24 μg/g), in the fruit pulp compared to unedited plants. Additionally, the *gibberellin 20ox2 (MaGA20ox2)* gene was edited in the "Gros Michel" banana cultivar, resulting in reduced plant height (Shao et al., 2020). Similarly, the *aminocyclopropane-1-carboxylase oxidase (MaACO1)* gene was edited in *M. acuminate* (AAA group, cv. Brazilian), leading to plants with shorter heights and delayed ripening (Hu et al., 2021).
In addition to CRISPR/Cas9 technology, CRISPR/Cas activation (CRISPRa) comes out as a complementary tool that can enhance classical breeding efforts by activating the expression of endogenous genes of bananas. By utilizing a modified version of the Cas9 enzyme, known as dead Cas9 (dCas9), it becomes possible to upregulate the expression of single or multiple endogenous genes without introducing double-stranded breaks in the target genes. Researchers at IITA are making significant progress in their endeavors to confer resistance against BXW disease by upregulating the expression of multiple endogenous defense genes in the susceptible banana cultivars.

Researchers have pioneered an innovative genome-editing method for bananas utilizing the Cas-CLOVER system (Tripathi et al., 2023), resulting in complete albino plantlets (Figure 2). The Cas-CLOVER system is characterized by its dual-guided mechanism, wherein it induces double-strand cuts through dimerization with the Clo051 nuclease (Madison et al., 2022). This system combines a nuclease-inactivated Cas9 (dCas9) protein with the Clo051 endonuclease, acting as a binding agent on the intended genomic site of any organism. In contrast to the single-guide RNA (gRNA) approach commonly used in CRISPR, the Cas-CLOVER endonuclease system employs two gRNAs alongside the Clo051 nuclease activity, which necessitates dimerization of the subunits associated with each gRNA. This dual gRNA approach renders the Cas-CLOVER GEd system exceptionally precise and target-specific. The Clo051 endonuclease initiates a double-stranded break only when both gRNAs guide it to the correct location within the plant's genome. The dual gRNA localization of Clo051 is effective within a flexible optimal spacer length of 11–31 nucleotides, providing ample room for design flexibility. This technique ensures a more specific DNA cleavage process while minimizing the risk of off-target effects. Additionally, it results in larger, sticky-ended overhangs compared to the smaller blunt-ended deletions typically observed with CRISPR/Cas9. The Cas-CLOVER gene editing technology in bananas represents a groundbreaking advancement in precision GEd, with a notable advantage of minimal detectable off-target mutations.

One of the major challenges in modern agriculture today is ensuring food security for our rapidly growing global population, which is projected to exceed 10 billion soon (United Nations, World Population Division). This pressing issue necessitates an urgent increase in food production, particularly for staple crops. In the African continent, bananas hold a critical role in food security and income generation. Investing in precision genetics for bananas presents a promising avenue for addressing these challenges, offering the potential to feed more people per unit area of production compared to cereal crops (Tripathi et al., 2019a).

GEd technology can be harnessed to enhance disease resistance, yield potential, and nutritional content in bananas. Furthermore, it allows for the refinement of specific plant traits, such as reducing plant height, through precise genetic modification. To overcome regulatory hurdles related tobiosafety, one approach is to integrate edited parent or diploid progenitor banana varieties into breeding programs for further enhancement. By segregating transgenes, the final products are transgene-free.

Notable progress has already been made in applying CRISPR-Cas9 protein-gRNA ribonucleoproteins (RNPs) to embryogenic cells in banana and other clonally propagated crops. These RNPs edit target sites upon delivery and are subsequently degraded by the plant's own proteases (Tripathi et al., 2019a). This foreign DNA-free approach is instrumental in developing disease-resistant banana varieties by targeting multiple genes simultaneously. In many countries, plants edited without foreign DNA face less stringent regulatory approval processes, making their commercialization more favorable compared to transgenic crops (Razzaq et al., 2019). Wu et al. (2020) established a PEG-mediated banana protoplast transformation system that successfully delivered CRISPR/Cas9 and CRISPR/Cas12a plasmids and CRISPR/Cas9 ribonucleoproteins (RNPs) targeting the PDS gene into banana cultivar "Cavendish." However, it's worth noting that the editing efficiency using CRISPR/Cas9-RNPs was lower than that of the CRISPR/Cas9 plasmid.

In conclusion, the development of disease-resistant and high-yielding banana varieties is paramount for ensuring food security and the economic well-being of smallholder farmers. The advent of CRISPR/Cas-based GEd technology has opened up new possibilities for precisely modifying the genes of bananas. This technology holds great potential for creating disease-resistant banana varieties by introducing specific mutations into genes that render the plant susceptible to pathogens.

Integrating GEd technology with conventional breeding programs and employing foreign DNA-free approaches can further unlock the potential for banana improvement. By combining the advantages of GEd with ongoing breeding efforts, banana researchers can develop disease-resistant, high-yielding banana varieties with enhanced nutritional profiles.

**Acknowledgments**

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**References**

Mutation breeding using mainly radiation treatment has been quite an effective tool in crop improvement in developing countries across the world (Ahloowalia et al., 2004). More than three thousand varieties in annual and tree crops as well as ornamental plants have been released. This constitutes remarkable impact on ensuring food security.

Forward breeding has been the driving force behind increasing crop yields. Discovery of heterosis, a term used to describe the improved performance of a hybrid as compared to its inbred parents, qualitatively increased the rate of yield improvement in the early phase of hybrid breeding (Sivasankar et al., 2012). As abiotic and biotic stresses contribute to the gap between the potential and the harvested grain yield, breeding for stress tolerance has been an important facet of crop improvement (Duvick, 2005).

Indispensable as forward breeding is in crop improvement, particularly for complex traits and in stressful environments, it is a resource-intensive and time-consuming process. Further,

Genome Editing in Cereal Crops

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Summary

Spontaneous and induced mutations in plant genomes contribute to genetic diversity, which underpins continued crop improvement through crossing and selection. When the gene sequence responsible for a plant trait is known, gene editing assumes significance in the speedy development of new varieties. Once a mutation is identified, it can be fine mapped through established genetic methods to a specific gene. Recent advances have helped streamline gene editing directly in elite cultivars. In contrast to the conventional approach of trait introgression from an exotic donor parent, editing of major-effect allelic variants directly in elite cultivars saves time and resources, and eliminates yield drag resulting from the residual donor genes that continue to persist at the end of backcrossing.

Introduction
introduction of a trait through crossing and backcrossing results in yield drag, which is caused by the unadapted genes from the donor parent that continue to persist in the improved cultivar.

Relative to conventional breeding gene editing reduces time to product development and eliminates yield drag

The number of donor genes still present after \( m \) backcrosses, assuming no selection and no suppression of recombination, is \( n \times d^{*(1/2)} \) where \( d \) is the fraction of loci that differ between the donor and the elite line and \( n \) is the total number of genes (Dhugga, 2018). As an example, bread wheat has \( \sim 110K \) genes (Consortium et al., 2018). If a wild, donor accession differs from the recurrent parent at 30% of the loci, after four backcrosses more than a thousand genes from the donor parent would continue to persist in the converted variety.

In the subsequent sections, I present the advantages gene editing offers over conventional breeding for the simply inherited traits.

The field of gene editing has progressed through several phases starting with oligo-mediated editing in the 1980s (Carroll, 2017). The main hurdle in its adoption was the very low frequency of the edited events (Zhu et al., 2000; Zhu et al., 1999). Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9), together referred to as CRISPR-Cas9, has revolutionized the field of gene editing because of its ease of use, specificity, and high success rate (Wang and Doudna, 2023).

CRISPR-Cas9-mediated gene editing has been widely used to mutate genes through spontaneous non-homologous end-joining (NHEJ), which follows the double-strand break at the site directed by the guide-RNA. The alternative scenario, referred to as homology-directed repair (HDR), entails template-mediated nucleotide changes or insertion of a gene or a DNA fragment at a precise location in the genome (Wang and Doudna, 2023).

Many examples of gene editing in crop plants are listed in recent reviews (Schenke and Cai, 2020; Tiwari et al., 2021). Most of what I discuss in the subsequent sections is related to accelerating improvement by directly introducing high-value traits into elite lines (Gao et al., 2020). Further, useful alleles identified through established genetic methods from radiation or chemical mutagenesis could be reproduced directly in elite crop varieties by gene editing.

Most of the reports on gene editing thus far have been from experimental lines, which are older accessions, obviously because of the difficulty in transforming elite varieties (Schenke and Cai, 2020; Tiwari et al., 2021). Editing a gene directly in elite lines eliminates the need for backcrossing. After self-pollinating or outcrossing the edited plant to the non-edited plants of the same genetic makeup accompanied by simultaneous screening for unintended changes in the genome with highly sensitive molecular tools, no elements of the vector backbone remain in the edited plants (Zastrow-Hayes et al., 2015). This reduces the time to market the improved variety by approximately two-thirds. Savings in field resources, which constitute one of the most expensive components of varietal development, is proportional to the time saved; only 2-3 generations are needed for the gene edited plants to commercialize as compared to 5-6 for forward breeding.

Bottlenecks in gene editing in elite varieties are being overcome

Efficient transformation of crop varieties is essential for making gene editing cost-effective (Biswal et al., 2023). The hurdle of transforming elite lines directly in maize and wheat has recently been overcome by including cell morphogenesis genes in the transformation vector (Debernardi et al., 2020; Lowe et al., 2018a; Lowe et al., 2018b; Lowe et al., 2016).

Under a partnership with Corteva Agriscience, we at CIMMYT have successfully transformed elite lines of tropical maize with nearly perfect efficiency. Similarly, we have used the GIF/GRF-containing vector to transform elite wheat varieties (Biswal et al., 2023; Debernardi et al., 2020).

Examples of traits in crop plants that can be improved by gene editing

Disease resistance and grain biofortification are two of the areas where gene editing could assist in expediting crop improvement. Rusts affect wheat crop more than any other disease, resulting in the loss of one-fifth of the crop every year (Oerke, 2006). Resistance against fungal diseases in wheat like powdery mildew and rusts can be significantly improved by editing single or a few genes.

Host resistance, which is attributed to resistant (R) genes, results from a hypersensitive response of the host, which kills the infected cell and thus limits the spread of the pathogen (Gill et al., 2015). This type of resistance tends to break down with time, however, requiring the introduction of new sources of resistance, again necessitating backcrossing to the recipient line. Non-host resistance, in contrast, involves metabolic or transport proteins. It allows the pathogen to grow at a slow rate but without significantly effecting grain yield. It is also referred to as durable resistance or adult plant resistance (APR). Further, the APR genes confer resistance against a broad spectrum of fungal pathogens (Moore et al., 2015). Because of its durability, CIMMYT breeders prefer APR to host resistance and have integrated it into their breeding program.

Three APR loci, \( Lr34, Lr46, \) and \( Lr67 \) are known in wheat and genes for two \( (Lr34 \text{ and } Lr67) \) have been isolated. Whereas \( Lr34 \) encodes an ATP-binding cassette (ABC) transporter, \( Lr67 \) encodes a hexose transporter (Krattinger et al., 2009; Moore et al., 2015). Just to highlight the durability of resistance conferred by these genes, \( Lr34 \) has not broken down for over the 100 years it has been available to the farmers (Moore et al., 2015). In both \( Lr34 \) and \( Lr67 \), mutations in the transmembrane domains of the respective proteins apparently make them nonfunctional. Loss of function of the mutated protein has been demonstrated for \( Lr67 \) in a heterologous system but not for the \( Lr34 \) protein, probably because its substrate is not known. ABC family of transporters facilitates the transport of a wide variety of substrates and is also referred to as multidrug resistance (MDR) protein family in bacteria. However, like \( Lr67 \), the causal mutations occur in two of the transmembrane domains in \( Lr34 \) as well, one of which, a tyrosine to histidine change, is expected to attenuate, if not destroy, its function.

These nonfunctional transporters might normally be involved in the transport of plant metabolites. However, they most likely confer resistance against fungal pathogens by blocking the transport of toxins or effectors into the plant cells. The mutations are partially dominant, which can be explained by the dimerization of the encoded proteins. Assuming the mutant and the wildtype genes express at the same level in a heterozygote, three-fourth of
the dimers would be defective. Many transporters are known to function as dimers (Feng and Frommer, 2015).

At least for Lr67, as the mutant protein is nonfunctional, an exact replication of the mutation is not necessary. A simple inactivation via NHEJ knockout should phenocopy the spontaneous mutant (Moore et al., 2015). Same logic could be applied for Lr34.

Although homeoalleles for each of the isolated APR genes are present, genetic alteration of a single homeolog conferred resistance (Krattinger et al., 2009; Moore et al., 2015). With gene editing, we have obtained knockouts for all three alleles of Lr67 in an elite CIMMYT line, Reedling (Figure 1). It is possible now to test them individually and in combinations to determine whether they further augment resistance. Gene editing thus provides additional tools to explore further strengthening of resistance against fungal pathogens.

CIMMYT identified a strong QTL that provides qualitative resistance against MLN (Murithi et al., 2021). The QTL, which was completely recessive, was present in an exotic line, which is unrelated to the African germplasm. As forward breeding to introgress this QTL continued, we also fine mapped it and are currently in the process of identifying the causal gene through gene editing. Once identified, this gene could be directly knocked out in the susceptible elite lines.

Editing herbicide tolerance into commercial varieties directly could help reduce drudgery for women in Africa where they manually remove weeds from the crops (Sun et al., 2016; Svitashev et al., 2015). Affordability of herbicides by the smallholder farmers remains a concern, however. Further, small land holdings would make it a challenge to apply the herbicide without impacting neighboring fields.

Editing herbicide tolerance into commercial varieties directly could help reduce drudgery for women in Africa where they manually remove weeds from the crops (Sun et al., 2016; Svitashev et al., 2015). Affordability of herbicides by the smallholder farmers remains a concern, however. Further, small land holdings would make it a challenge to apply the herbicide without impacting neighboring fields.

Grain biofortification is another area where gene editing can assist in expending product development. A lack of micronutrients and vitamins can cause developmental defects in children (Wen et al., 2022). Shukla et al. demonstrated that it was possible to reduce grain phytic acid in maize by gene editing, targeting the enzyme that phosphorylates inositol (Shukla et al., 2009). The same approach could be used to reduce phytic acid in the released commercial varieties, particularly targeted for increased iron and zinc contents (Wen et al., 2022). Similarly, provitamin-A in the grains of maize and other cereals could be improved by knocking out the genes that divert the substrate to other reactions as well as the ones that oxidize beta-carotene (Sestili et al., 2019).

Dough from wheat flour turns dark because of polyphenol oxidase (PPO) activity (Dhugga, 2022). Similarly, peeled potatoes turn brown if left exposed to air. Gene editing has been used to knock out a PPO gene in potato, which reduced browning (González et al., 2020). We are using a similar approach in wheat to prolong dough longevity.

Prospects of gene editing in crop improvement: NHEJ and HDR

Replacement of specific nucleotides, promoter swapping, and allele insertion have been successfully demonstrated in crop plants (Shi et al., 2017; Svitashev et al., 2015). Low frequency of the edited events, which would be lower still in crops where transformation is a challenge, and extensive screening required to identify the targeted changes would limit the use of these approaches to high-value traits, however (Shukla et al., 2009; Zhu et al., 2000; Zhu et al., 1999). These were the hurdles that kept the prior gene editing technologies from wide adoption (Carroll, 2017; Shukla et al., 2009; Zhu et al., 2000; Zhu et al., 1999). As has been the case thus far, gene editing via NHEJ would continue to dominate trait improvement in commercial germplasm followed by limited use of HDR for the traits that justify investment of additional resources.

Acknowledgements

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References


Announcements

First IAEA Collaborating Centre in Africa for Plant Breeding and Genetics

A new IAEA Collaborating Centre with PBG was established in September 2023 with the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission. This Collaborating Centre will provide an important platform to support the IAEA's efforts to enhance research and development on mutation breeding in the region, by sharing capabilities and experience on *in vitro* multiplication, performing collaborative research, supporting capacity building, as well as providing services to other Member States in the region. The BNARI was recognized as a new Collaborating Centre in a ceremony that took place on 29 September 2023 at the Vienna International Centre as part of a Side Event at the IAEA 67th General Conference.

World Food Forum Side Event on Astrobiology and Space Breeding

The Joint FAO/IAEA Centre organized a virtual side event on “Astrobiology and Space Breeding for Food Security under Climate Change” on 18 October 2023, at the Youth Forum of the World Food Forum Flagship Event in Rome, Italy. This panel discussion addressed induced genetic variation in plants, including by space conditions, to breed for adaptation to climate change on earth and to develop plant ideotypes for space or vertical farming. Panelists included Section Head of PBG, Sr Scientists of the Chinese Academy of Agricultural Sciences, CEO of Space Cargo Unlimited, Ph D Student at University of Colorado, USA, and Assoc Professor at University of Agricultural Sciences, Dharwad, India. Moderators were Ms Emma Ramirez, intern at PBG Laboratory, and Ms Nicola Ulm, student at American International School, Vienna, Austria.

PBG at the Preparatory Committee for the 2026 Review Conference of the Parties to the Treaty on the Non-Proliferation of Nuclear Weapons (NPT)

PBG was well-represented at the Preparatory Committee for the 2026 Review Conference of the Parties to the Treaty on the Non-Proliferation of Nuclear Weapons (NPT) at the end of July, offering delegates a close-up view of coffee and banana trees, as well as showcasing the new 3D virtual tour of PBGL facilities (coffee dome, greenhouses and labs) on our iPad. PBG team members from the Section and Lab took turns greeting passers-by, which included several ambassadors and other high-level visitors, informing each guest how our important work contributes to the Agency’s mandate for the peaceful use of nuclear technology.

Mutation Breeding Workshop and Participation at the Plant and Animal Genome (PAG 31)

*San Diego, United States of America, 12-17 January 2024*

Project Officers: I. K. Bimpong and N. Warthmann

The Plant Breeding and Genetics Subprogram of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture is organizing its second workshop under theme “plant breeding” at the upcoming Plant and Animal Genome Conference (PAG 31). This workshop’s presentations will focus on the latest advances in combining genomics, bioinformatics and biosystems as well as mutation breeding to support various areas of food and agricultural research, as well as the use of artificial intelligence with speed breeding in diverse crops. It will provide a forum for the global plant mutation breeding community to share information and resources, align methodologies, and build collaborative projects in order to take full advantage of the genomics revolution. The invited speakers include distinguished academic, industrial, and government researchers and experts, namely, Michael J. Thomson (Texas A&M University, USA), Chengdao Li (Murdoch University, Australia), Kenneth McNally (IRRI -CGIAR, The Philippines), Suresh D. Pillai (Texas A&M University, USA), Lee Hickey (UQ and QUAAFI, Australia) and Manish K. Pandey (ICRISAT-CGIAR, India).
Coordinated Research Projects (CRPs)

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

Project Officers: A. Hingane, S. Sivasankar

The CRP aims to boost the productivity of three important pulse crops, namely chickpea, cowpea, and lentil, which collectively account for 40% of global pulse production. Induced genetic variation and genomics technologies are leveraged to enhance the resistance of chickpea to the pod borer, *Helicoverpa armigera*, cowpea to the pod borer, *Maruca vitrata*, and lentil to the disease, *Stemphylium blight*.

The CRP has facilitated important partnerships with the National Agricultural Research Systems of Member States as well as with CGIAR centres. Significant advancements have been made by most participants. Results from the CGIAR centre, International Crops Research Institute for Semi-Arid Tropics, and from the Bhabha Atomic Research Centre, India, and from the University of Namibia were reported in the previous Newsletter. Initiated in 2019, the project has yielded several mutant populations in the past four years to enhance target traits in the three target pulse crops. Moreover, standardized screening protocols are being developed for the target pests and diseases. Promising mutants, displaying tolerance to pod borer in chickpea and cowpea, as well as resistance to *Stemphylium blight* in lentil, have been identified. These mutants are being advanced for further assessment and development into genetic resources, ultimately culminating in the release of novel crop varieties.

Researchers from the Nuclear Institute for Agriculture & Biology (NIAB), Pakistan, evaluated pod-borer resistance in the high-yielding chickpea mutant lines CM8/15, DCH21-14, CM286/15, CM269/15 and CM137/15, that had previously been selected for yield from a multilocational trial of eighteen promising mutants. Evaluation included a laboratory bioassay with detached leaf and pod, analysis of variations in malic and oxalic acid levels as potential markers for resistance and testing under natural field conditions during the 2022-23 *Rabi* season. The five promising mutants exhibited superior performance for both yield and pod-borer resistance and have been identified for inclusion in National Uniform Yield Trials for eventual release as new varieties.

At the Bidhan Chandra Krishi Viswavidyalaya in India, mass production of the *Stemphylium* blight inoculum was successfully standardised. Field trials were conducted with M6 lentil mutants at the pre-flowering stage maintaining the necessary humidity and scoring for disease severity 7 and 10 days after inoculation using foliar spot count and coverage area. Progenies of the mutant RM216 exhibited tolerance (Figure 1) with disease severity score of 01, relative to a score of 08 for WBL77 and 10 for Ranjan and L4727 on a scale of 01 to 10. Expression of a cystine-rich kinase gene was repressed in the mutant relative to the parent, WBL77. Crosses have been initiated between the identified tolerant mutant RM216 and its sensitive parent, WBL77, to develop a mapping population for identifying genes responsible for tolerance to *Stemphylium blight*. A second tolerant mutant, RM560, with a disease severity score of 01 was identified from a M3 population of about 1500 families and over 150,000 individual plants derived from the variety Ranjan.

**Figure 1.** Screening of lentil M6 mutants for resistance to *Stemphylium* blight in field conditions: Left, 7 days after inoculation; top right, 15 days after inoculation; bottom right, tolerant mutant line RM216 with no disease symptoms.
CRP D24014: Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops

Project Officers: I.K. Bimpong, S. Sivasankar

The CRP aims to develop new genetic resources and technologies for accelerated breeding in vegetatively propagated crops (VPCs) and horticultural tree crops (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.

Good progress has been made by participating research teams of the project since its start in 2021. The research team at the University of Ghent in Belgium has standardized protocol(s) for generating large-scale in vitro shoots as explants in the olive crop for large-scale mutagenesis programs, which has solved the problem of low success rates during various in vitro stages. The CRP is now testing several slow-release nanoparticles of plant growth regulators and auxin beads to regulate the induction of adventitious shoot or root meristems in vitro and for root regeneration. At Centro IFAPA "Alameda del Obispo", Junta de Andalucía in Spain, twelve olive cultivars with earliness have been identified and subjected to mutation induction.

At the Bhabha Atomic Research Centre in India, the research team has developed a micro-tuber induction technique for potato that will be utilized for proton beam irradiation. At the Universidad Distrital Francisco José de Caldas in Colombia, advanced mutant clones of creole potatoes with high shelf-life and dormancy have been developed. These are being evaluated currently for their physiological characteristics and for genetic associations using GWAS and GBS. The team is also conducting semi-commercial trials at three locations prior to release as varieties.

The issue of the low success rates typically attained during various in vitro stages in cassava is being addressed through the CRP. Project participants in Uganda and Ghana have standardized single-cell regeneration protocols for large-scale generation of in vitro plantlets. These protocols are ready for dissemination to interested Member States to enable acceleration of variety development, and facilitate basic research, in cassava and potentially other VPCs. Through the support of the CRP, two MSc students have graduated, and three MSc and two PhD students are in various stages of their studies.

On the use of different radiation sources as mutagens, the CRP participant from South China Agricultural University reported on genetic imprints and germplasm enhancement in rice after the circumlunar space flight in Chang’e 5. Mutation frequency in the M1 ranged from $7.97 \times 10^4$ to $1.9 \times 10^3$ with mutations distributed across all chromosomes, and GC content decreased as did methylation rate, relative to earth-based control. A variety of phenotypes including increased tillering, dwarf stature, long grains, wide grains, and salt tolerance were observed in the M2 generation. Results from the Institut de biologie moléculaire des plantes of CNRS, France, reported similar structural variants in wild type Arabidopsis plants with ionizing (proton beam) and non-ionizing (UV-A and UV-B) radiations, but higher DNA methylation changes with proton beam. The National Center of Space Mutagenesis for Crop Improvement at the Chinese Academy of Agricultural Sciences reported characterization and gene mapping for plant height and early heading in wheat mutant lines induced by space flight, as well as transcriptome variation and phenotypes resulting from C-ion beam irradiation in wheat. At Kongju National University in the Republic of Korea, irradiation dosage for proton beam on Brassica rapa subsp. trilocularis were standardized and a new mutant population was developed.

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

Project Officers: C. Zorrilla, S. Sivasankar

CRP D23033 integrates banana breeding using induced genetic variation with identification of candidate genes using functional genomics tools, improvement of molecular detection methods and the identification of microbes with enhanced antagonistic and growth promoting activity.

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. This CRP
CRP D23033 started in March 2023 and had its first virtual Research Coordination Meeting (RCM) from 15 to 19 May 2023. A total of 12 institutions from Asia, Africa and Latin America were selected to participate through research contracts and 1 institution through an agreement. Detailed workplans for 2023 and a general strategy were discussed during the meeting. Collaboration between the participating institutions was encouraged and initial steps established.

CRP D24016 aims to expand results obtained by a previous CRP that focused on screening methods by including new aspects such as candidate gene identification and mutagenesis of beneficial microbes.

CRP E43041: Application of Nuclear Techniques to Improve and Evaluate Nutritional and Health Benefits of Underutilized Crops

Project Officers: K. Bimpong, V. Owino

Malnutrition as a result of nutrient deficiencies, especially for micronutrients (e.g., iron, zinc, calcium, vitamin A, thiamine, riboflavin) and essential amino acids, is associated with almost 50% of mortality among children under 5 years of age, especially in Low- and Middle-Income Countries.

For example, iron deficiency affects at least 30% (2 billion) of the world’s population; it is associated with anaemia, decreased work capacity, impaired immune and endocrine function, and impaired physical and cognitive development in children. Underutilized crops can be used to enhance nutrition security as they have higher concentration of nutrients, including protein, vitamins, and minerals.

Mutation breeding is a routine method in plant breeding and has contributed to the development of new varieties with desired traits. However, nutritional benefits of most nutrient-enhanced underutilized crop varieties have not been evaluated adequately since the focus of mutation induction has been on enhancing crop yield. This CRP aims to fill this gap by generating robust evidence on the nutritional value and health efficacy of underutilized crops among vulnerable groups in Asia and Pacific, Africa, Latin America, and Europe. This approved CRP is an innovative cross-disciplinary approach to improving and evaluating the nutritional benefits of underutilized crops between NAFA-Plant Breeding and Genetics (PBG) and NAHU- Nutritional and Health-Related Environmental Studies (NAHRES).

CRP D24016: Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation

Project Officers: F. Sarsu, A. Hingane

CRP D24016 was approved in September 2023 and is open to receive proposals from October to December 2023. The project is expected to launch with its first coordination meeting in May 2024. The CRP aims to develop novel genetic stocks of key dryland millets using mutation breeding and to use biotechnologies to accelerate the development of new varieties for food and nutrition security and climate-change adaptation. Specific objectives are (1) to generate genetic diversity in selected millets with improved nutrition and quality traits, and improved resilience to biotic/abiotic stress through induced mutation for better adaptation to climate change; (2) to develop/adopt phenotyping tools for precise screening/selection of mutant lines with the desired traits in selected millet crops; (3) to develop genomic tools for delivery of novel induced variation to accelerate genetic gain in millet improvement.

Forthcoming Events

First Research Coordination Meeting (RCM)
Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation (D24016)
Vienna, Austria, 6-10 May 2024
Project Officers: F. Sarsu, A. Hingane

Past Events

Fourth Research Coordination Meeting (RCM)
Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation (D22006)
Vienna, Austria, 4-8 December 2023
Project Officers: A. Hingane, S. Sivasankar

The CRP aims to boost the productivity of three important pulse crops, namely chickpea, cowpea, and lentil which collectively account for 40% of global pulse production. The approach leverages mutation induction and genomics technologies to enhance the resistance of chickpea to the pod borer Helicoverpa armigera, cowpea to the pod borer Maruca vitrata, and lentil to combat 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, the third RCM also took place virtually from 17 to 19 October 2022, and the fourth RCM was held in December 2023 in Vienna, Austria.

Consultancy Meeting
Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation
Virtual Meeting, 31 July-4 August 2023
Project Officers: F. Sarsu, A. Hingane

This Consultancy meeting aimed to discuss and develop a concept note towards a call for the next CRP at NAFA-PBG. Agriculture is facing the challenge of feeding the ever-growing world population that is projected to reach ten billion by 2050. While improving crop yield and productivity can address this challenge, the increasing effects of global warming and climate change seriously threaten agricultural productivity.

Millets, hardy dryland crops, that can grow with minimal inputs in Asian and African countries, are gaining popularity due to their nutritional qualities and resilience to climate change. They present an ideal solution for countries to increase self-sufficiency and reduce reliance on imported cereal grains. Millets are rich in dietary fibres, proteins, minerals (iron, potassium, magnesium, and...
phosphorous) and vitamins. Additionally, they exhibit exceptional efficiency in water and nitrogen use, which enables them to flourish in water-deficit conditions in rain-fed regions. Due to their nutritional superiority and resilience to harsh growing conditions, small millets can supplement staple cereal crops. They are rich in micro- and macro-nutrients, proteins, essential amino acids, dietary fibre, and resistant starch.

A concept was approved, and a call for proposals is open, with focus on improving grain yield under prevalent biotic and abiotic stresses in pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana* (L.) Gaertn.) and proso millet (*Panicum miliaceum* L.) under climate change. These are essential millets in the diets of millions of impoverished and vulnerable people.

**Third Research Coordination Meeting (RCM)**

**Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops (D24014)**

*Vienna, Austria, 10-14 July 2023*

**Project Officer:** I. K. Bimpong

The CRP aims to 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 includes thirteen 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 brings 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.

The third RCM for CRP D24014 assessed progress and discussed 2023-2024 workplan activities. Good progress has been made, following individual work plans, besides, CRP participants have established an effective collaboration. Standardised/optimised screening protocols have been developed and shared among participants to enable precise and efficient development of new genetic resources and technologies for accelerated breeding in VPCs and HTCs through induced genetic diversity, chimera-free regeneration, and functional genomics.
Since the last update of the Mutant Variety Database (MVD) in 2022, a total of 31 new records were published as of October 2023. The new records include varieties of Acalypha, barley, *Brassica napus*, bread grass, chrysanthemum, groundnut, lentil, mung bean, potato, rice, sesame and wheat. The new varieties were reported by Bangladesh, China, India, Iran, Kenya, Philippines, Sierra Leone, Thailand, Turkey and Yemen.

A new form to register mutant varieties in the MVD has been established for a simpler and more precise collection of relevant information of new mutant varieties published in the database.

The MVD currently holds 3433 mutant and mutant-derived varieties registered. To submit a new variety, please write to MVD.Contact-Point@iaea.org for more information.

With the present PBG Newsletter, we aim to reinstate pages displaying recent mutant varieties by plant breeder colleagues in Member States, a practise that used to be in place some time ago. Towards this we request colleagues to send information on recently released varieties, ideally through the MVD submission form, including pictures. We will attempt to capture this information to the extent that our page limit allows. We hope that this will also encourage timely and increased submissions to the MVD.

**NEWLY RELEASED MUTANT VARIETIES FROM MEMBER STATES**

**Rice** var BINA dham 25  
Released 2022, Bangladesh  
Key characteristics: Yield (5-7.6 ton/ha); Short duration (138-148 days); Photo-insensitive

**Mungbean** var BINA moog12  
Released 2022, Bangladesh  
Key characteristics: Tolerance to submergence; Short duration (125-130 days); Yield (3.5 tons/ha in submerged field)

**Wheat** var Hangmai106  
Released 2022, China  
Breeder: Luxiang Liu  
Key characteristics: Yield; Resistance to lodging, powdery mildew, leaf rust, stripe rust

**Wheat** var Hangmai818  
Released 2021, China  
Breeder: Luxiang Liu  
Key characteristics: Yield

**Rice** var Saphart 1  
Released 2022, Lao PDR  
Breeder: Chanthakhone Boualaphanh, Siviengkhek Phommalath, Laer Homsengchan, Sonthaya, Kongchay Sithichack  
Key characteristics: Early maturity; Short stature; Resistance to lodging; Yield; Good eating quality

**Rice** var Houykhod 2  
Released 2022, Lao PDR  
Breeder: Chanthakhone Boualaphanh, Siviengkhek Phommalath, Laer Homsengchan, Sonthaya, Kongchay Sithichack  
Key characteristics: Non-glutinous; Moderate resistance to blast disease and flooding; Good for processing rice noodle

**Hibiscus rosa** var *Hibiscus rosa sinensis* “Peach Beauty”  
Released 2021, Malaysia  
Breeder: Shuhaimi Shamsudin, Shakinah Salleh  
Key characteristics: Petal color peach; Centre color deep red

**Rice** var IS21 (NMR152)  
Released 2021, Malaysia  
Key characteristics: Yield (8 tons/ha); Tolerance to drought and submergence
Cowpea var ShR4P1
Released 2018, Namibia
Breeder: Lydia Horn
Key characteristics: Improved over traditional variety; Semi-erect growth habit; Medium duration (70-85 days); Grain color yellow; Average grain yield 1-2 tons/ha

Cowpea var NkR8P9
Released 2018, Namibia
Breeder: Lydia Horn
Key characteristics: Improved over traditional variety; Semi-erect growth habit; Medium duration (70-85 days); Grain color brown; Average grain yield 1-2 tons/ha

Cowpea var BrR4P11
Released 2018, Namibia
Breeder: Lydia Horn
Key characteristics: Improved over traditional variety; Semi-erect growth habit; Medium duration (70-85 days); Grain color red; Average grain yield 1-2 tons/ha

Cowpea var NkR1P3
Released 2018, Namibia
Breeder: Lydia Horn
Key characteristics: Improved over traditional variety; Semi-erect growth habit; Medium duration (70-85 days); Grain color white; Average grain yield 1-2 tons/ha

Cowpea var ShR10P10
Released 2018, Namibia
Breeder: Lydia Horn
Key characteristics: Improved over traditional variety; Semi-erect growth habit; Medium duration (70-85 days); Grain color yellow; Average grain yield 1-2 tons/ha

Sorghum var NAMSO-01 (L7P9-3)
Released 2023, Namibia
Breeder: Wanga Athon
Key characteristics: Improved over traditional variety; Early flowering (days to 50% flowering 56-65 days); Medium plant height; Grain color: white; Average yield 2-3 t/ha

Sorghum var NAMSO-02 (L7P9-4)
Released 2023, Namibia
Breeder: Wanga Athon
Key characteristics: Improved over traditional variety; Early flowering (days to 50% flowering 56-65 days); Medium plant height; Grain color: white; Average yield 1.5-2.5 t/ha

Sorghum var NAMSO-03 (L7P9-13)
Released 2023, Namibia
Breeder: Wanga Athon
Key characteristics: Improved over traditional variety; Late (days to 50% panicle emergence 76-85 days); Medium plant height; Grain color brown; Average yield 2-4 t/ha

Sorghum var N NAMSO-02 (L7P9-4)
Released 2023, Namibia
Breeder: Wanga Athon
Key characteristics: Improved over traditional variety; Early flowering (days to 50% flowering 56-65 days); Medium plant height; Grain color: white; Average yield 2-3 t/ha
**Castorbean var NIAB Spineless**  
Released 2023, Pakistan  
Breeder: Wanga Athon  
Key characteristics: Improved over traditional variety; Late (days to 50% panicle emergence 76-85 days); Medium plant height; Grain color red; Average yield 1.5-3 t/ha

**Mungbean var NIAB PRI Mung**  
Released 2023, Pakistan  
Breeder: Jawad Asghar, NIAB, Faisalabad  
Key characteristics: Early maturity; Semi-determinate growth habit; Short stature; High yield potential; Heat tolerant

**Rice var NIAB HT-39**  
Released 2023, Pakistan  
Key characteristics: High yield (3.8 tons/ha compared to 3.5 tons/ha for the check variety)

**Rice var NIAB HT-18**  
Released 2023, Pakistan  
Key characteristics: High yield (4.4 tons/ha compared to 3.8 tons/ha for the check variety)

**Rice var NIAB-195**  
Released 2023, Pakistan  
Breeder: Muhammad Arif  
Key characteristics: High yield; Early maturity; Heat tolerant; Short stature; Resistant to BLB

**Rice var NUCOS1**  
Released 2022, Sierra Leone  
Breeder: Alieu Mohamed Bah  
Key characteristics: Moderate resistance to bacterial leaf blight, sheath blight, stem borer, brown plant hopper; Higher yield (2.5t/ha over 2.1t/ha for check variety)

**Sesame var NIAB Millennium**  
Released 2022, Pakistan  
Breeder: Jawad Asghar, NIAB, Faisalabad  
Key characteristics: High yield (2.2 tons/ha compared to 1.2 tons/ha for the check variety)

**Cotton var NIAB-512**  
Released 2022, Pakistan  
Key characteristics: High yield (3.8 tons/ha compared to 3.2 tons/ha for the check variety)

**Sorghum var NAMSO-04 (L3P15-16)**  
Released 2023, Namibia  
Breeder: Wanga Athon  
Key characteristics: Improved over traditional variety; Late (days to 50% panicle emergence 76-85 days); Medium plant height; Grain color red; Average yield 1.5-3 t/ha

**Mungbean var NIAB PRI Mung**  
Released 2023, Pakistan  
Breeder: Jawad Asghar, NIAB, Faisalabad  
Key characteristics: Early maturity; Semi-determinate growth habit; Short stature; High yield potential; Heat tolerant

**Rice var NIAB-9**  
Released 2023, Pakistan  
Breeder: Muhammad Arif  
Key characteristics: High yielding; Early maturity

**Rice var NUCOS1**  
Released 2022, Sierra Leone  
Breeder: Alieu Mohamed Bah  
Key characteristics: Moderate resistance to bacterial leaf blight, sheath blight, stem borer, brown plant hopper; Higher yield (2.5t/ha over 2.1t/ha for check variety)

**Groundnut var LDT3**  
Released 2023, Vietnam  
Breeder: Le Duc Thao, Nguyen Van Manh, Pham Thi Bao Chung, Le Thi Anh Hong  
Key characteristics: Black seed coat (parent has yellow seed coat); Higher carotenoid, omega 3, omega 6 content (39, 30 and 12% higher than parent); Shorter duration (90-100 days); High yield (2.46-3.18 tons/ha)
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Forthcoming Events

Regional Meeting

Promoting Sustainable Agricultural and Food Productivity in the Association of Southeast Asian Nations Region - RAS5094

Hanoi, Vietnam, 18-22 March 2024
Project Officer: I.K. Bimpong

Project Objective: Project RAS5094 is aimed at supporting the transfer of knowledge and expertise among ASEAN countries through meetings, workshops, and trainings with participation of scientists and experts in the region. ASEAN countries will collaborate in a regional project to enhance crop production through national crop improvement programmes by using mutation breeding and related technologies.

Course Objective: This event will focus on the planning of a technical workplan, technical visits, discussion of proposed training activities according to the needs of the region, exchange of knowledge and progress on mutation breeding among participants, and future actions to be taken to increase the impact of the project in the availability of improved varieties for sustainable food production and strengthening/establishing collaborations among participating Member States.

National Training Course

Development Mutation induction and Selection in Rice - LIR5003

Monrovia, Liberia, 1-12 August 2024
Project Officer: I.K. Bimpong

As this is the first mutation breeding programme for Liberia, the goal is to continue to raise awareness of the importance of nuclear techniques in food and agriculture. This event is a follow-up to last year’s virtual training. The main topics of the training are mutation induction, breeding schemes, early and late generation selection methods for targeted stresses, introduction to protocols in the laboratory, greenhouse, and field conditions for successful implementation of breeding programmes, informal and formal seed systems for the multiplication and dissemination of seeds for upscaling and cultivation. The course will include lectures, demonstrations, and practical sessions on various protocols on mutation breeding and selection methods. The course is designed for researchers and plant breeders who are working on mutation induction of cereals (rice and sorghum). Fifteen participants are expected to attend the training.

National Training Course

Improving Crop Adaptation to Drought Stresses using Nuclear-Derived Techniques, and Molecular-Breeding Method - ERIS011

Asmara, Eritrea, 9-20 October 2024
Project Officer: I.K. Bimpong

Course Objective: Enhance the understanding and research capabilities of the participants in basic principles in mutation breeding and molecular techniques for improved mutation detection and selection. The course will cover topics such as mutation breeding, concepts, and methodologies, including in vitro techniques of mass propagation of plantlets for vegetatively propagated crops, optimization of irradiation treatments, laboratory, screenhouse and field-based screening protocols in developing new and improved mutant varieties/lines for improving crop resilience to drought stress. Other courses such as selection of clone/s for registration and release as variety, seed systems and introductory molecular biology and techniques for improved mutation detection and selection will be part of the training. The course will include lectures, demonstrations, and practical sessions on various protocols on mutation breeding and selection methods. It is designed for participants with basic knowledge of mutation breeding techniques. Fifteen participants are expected to attend the training course.

Past Events

National Training Course

Mutation breeding and efficiency enhancing biotechnologies for sustainable agriculture and food security in Qatar - QAT5008

Doha, Qatar, 3-14 December 2023
Project Officer: F. Sarsu

Project Objective: Enhance the sustainability of natural resources with smart agricultural practices.

Course Objective: The course had the dual objective of equipping national training participants with both theoretical knowledge and practical expertise in the realm of mutant variety development. It delved into the fundamental aspects of crop mutation breeding, highlighted the different sources of irradiation and explained techniques for optimizing doses for mutation induction in seeds and vegetatively propagated fruit trees, especially the date palm. In addition, the course also emphasized the creation of genetically stable mutant lines through mutation breeding and associated biotechnologies. The ultimate goal was to enable participants to engineer crop varieties resilient to abiotic stresses, which would play a pivotal role in bolstering food security in Qatar.

The course was attended by twenty participants (twelve women and eight men researchers) from different organizations across the country and was hosted by the Agriculture Research Department.

Regional Training Course

Statistically Rigorous, Pre-field and Field Screening to Select Improved Mutant Lines to Release for Farmers - RAS5099

National Center for Agricultural Research and Extension (NCARE), Jordan, November 26-30, 2023
Project Officer: I. K. Bimpong

Project Objective: Project RAS5099 “Developing Climate Smart Crop Production including Improvement and Enhancement of Crop Productivity, Soil and Irrigation Management, and Food Safety Using Nuclear Techniques (ARASIA)” aims to strengthen participant researcher capacity in technologies involved in plant breeding, specifically for related enabling techniques and statistical rigor in field-testing for the selection of improved varieties.
The course curriculum includes breeding informatics in plant breeding, use of software to intermate parents, genealogy management, generation advancement, basics of experiment design and early generation testing, barcoding of plots/plants using any good software, digital data recording using field book, introductory R Language (Installing R and R studio, vectors and matrices, factors, data frame, plotting), stage I trials generation, multilocalational trials, GxE and stability analysis, and concept of mega environment and location grouping. The training course comprised of lectures and demonstrations. The course was designed for breeders, agronomists and other crop specialists from Member States involved in mutation breeding programmes.

Twenty participants (six women and fourteen men researchers) attended the course.

Final Coordination Meeting
Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia - RER5024
Tbilisi, Georgia, 20-24 November 2023
Project Officer: F. Sarsu

Project Objective: Support the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and combined biotechnologies, in order to contribute to food security in Europe and Central Asia.

Course Objective: Review the results achieved under the project and explore the potential for a future one. During the meeting, the progress and achievements made in each participating Member State during the project cycle were assessed. The event consisted of presentations on the progress of project implementation and achievements in each country, exchange of knowledge and information, and discussions on the project outputs and future prospects.

The meeting saw participation of sixteen National Project Coordinators from Europe and Central Asia, comprising six women and ten men. The Ministry of Environmental Protection and Agriculture of Georgia (MEPA) had the honour of hosting the event.

Regional Training Course
Experimental Design and Data Analysis for the Advancement of Mutant Populations - RAS5094 and RAS5098
Faisalabad, Pakistan, 20-24 November 2023
Project Officer: Cinthya Zorrilla

The aim of this training was to strengthen the agricultural data analysis skills for breeders, agronomists, biologists, and plant pathologists involved in projects RAS5094 and RAS5098.

Project RAS5094 aims to support the transfer of knowledge and expertise among ASEAN countries through meetings, workshops, and trainings with participation of scientists and experts in the region. ASEAN countries will collaborate in a regional project to enhance crop production through crop improvement programmes by using mutation breeding and related technologies.

Regional Training Course
Accelerated Breeding Techniques for the Development of Crop Tolerance to Abiotic Stress - RER5024
Ankara, Türkiye, 6-17 November 2023
Project Officer: F. Sarsu

Project Objective: Support the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and combined biotechnologies, in order to contribute to food security in Europe and Central Asia.

Course Objective: Enhance capacity building in mutation breeding and related biotechnologies, in order to develop genetically stable lines, shorten breeding cycles and produce crop varieties tolerant to abiotic stresses with the purpose of contributing to food security. The training included accelerated breeding techniques, such as double haploidy, embryo culture, rapid cycling methods and shuttle breeding, in order to develop better performing mutant crop varieties and phenotyping/genotyping selection methods for abiotic stress tolerance.

Twenty-two researchers (twelve women and ten men) from various countries in Europe and Central Asia participated in the training.

This course provided theoretical and practical sessions (software use) related to experimental design for early and advanced generation testing, data recording, and statistical analysis of field experiments for mutant populations advancement, multilocalational trials, GxE, and stability analysis using open-source software, as well as case examples of the analysis of different kinds of variables.
event, which was graciously hosted by the Field Crops Central Research Institute in Ankara, Türkiye.

**National Training Course**

**National Training Course on Improving Coffee Resistance to Rust through Mutation Breeding Techniques and Biotechnologies - PER5034**

Pucallpa, Peru, 30 October-10 November 2023

Project Officer: F. Sarsu

Project Objective: Improve food security through increased productivity of yellow potatoes and coffee.

Course Objective: Provide lectures and practical sessions covering a certain range of topics, including assessing resistance to rust in coffee lines, identifying disease symptoms and providing protocols for the evaluation of the aggressiveness of rust isolates. The course also covered microscopic analysis of rust spore viability and inoculation techniques, which encompassed various methods for inoculating both attached and detached leaves and leaf discs. In this training, participants learned how to conduct germination tests and prepare infected leaf samples for microscopic observation, which, using both bright field and fluorescence techniques, focused on the identification of fungal structures and early host responses. Additionally, the course covered the assessment of reaction types based on a qualitative scale.

The training was attended by eighteen participants (twelve women and six men researchers) from different organizations across the country and was hosted by the Pucallpa Agrarian Experimental Station of the National Institute of Agrarian Innovation (INIA).

**Regional Training Course**

**Mutation Breeding and Efficiency Enhancing Techniques for Resistance to Banana Fusarium Wilt Race TR4 in Latin America - INT5158**

Vienna, Austria, 23 October-3 November 2023

Project Officers: Cinthya Zorrilla, Shoba Sivasankar

Project Objective: Project INT5158 is aimed at developing capacities for disease management against Banana Fusarium Wilt (Foc TR4) and the development of new resistant banana varieties using mutation breeding and combined biotechnologies to contribute to the prevention of the spread of this disease and reduce farmers’ losses.

Ms Anupama Hingane as Course Director coordinated the training at the Plant Breeding and Genetics Laboratory located in Seibersdorf. A total of eight participants (one woman and seven men researchers) from eight countries, namely, Brazil, Colombia, Ecuador, Mexico, Nicaragua, Paraguay, Saint Lucia, and Venezuela, attended.

The training included basic concepts of mutation induction, radio-sensitivity testing, banana tissue culture methods, and hands-on practical sessions on screening methods for resistance to Foc TR4 under greenhouse conditions, identification of symptoms in comparison to other sources of stress; and basic molecular biology methods for detection of Foc TR4.

This training was offered for phytopathologists and breeders from National Phytosanitary Protection Organizations (NPPOs) and National Agricultural Research Institutions (NARIs) and provided a general overview of activities that can be applied to combat Foc TR4 in Latin America and the Caribbean region.

**Workshop**

**Study Tour on “How Australia is Managing Tropical Race 4 (TR4)” - INT5158**

Cairns and Brisbane, Australia, 16-25 October 2023

Project Officers: Cinthya Zorrilla, Shoba Sivasankar

Project Objective: Project INT5158 is aimed at developing capacities for disease management against Banana Fusarium Wilt (Foc TR4) and the development of new resistant banana varieties using mutation breeding and combined biotechnologies to contribute to the prevention of the spread of this disease and reduce farmers’ losses.

The Study Tour was led by Professor Elizabeth Aitken from the University of Queensland and Ms. Rosie Godwin from the Australian Banana Growers Council. A total of nine participants (five women and four men researchers) from five countries, namely, Brazil, Colombia, Ecuador, Peru, and Venezuela, participated in this event.

This is the first interregional activity to exchange experiences between Australia, which has more experience in managing and controlling this disease, and Latin America and the Caribbean region, where the disease has recently been introduced. The Study Tour included topics on biosecurity and prevention measures including herbicides/insecticides use, containment, monitoring and eradication, a visit to a banana farm for demonstration of biosecurity procedures, as well as other management methods such as biocontrol. Additionally, specialists from the University of Queensland shared their research work in detection methods, mapping of resistance genes, breeding and genetic engineering in banana.

This Study Tour was offered to phytopathologists and breeders from National Phytosanitary Protection Organizations (NPPOs) and National Agricultural Research Institutions (NARIs) from countries in the Latin America and the Caribbean region that have officially reported the disease and those that are at high risk based on their geographic location.
Participants of the Study Tour to Australia for INT5158

Mid-Term Project Coordination Meeting
Enhancing Crop Productivity and Quality through Mutation by Speed Breeding - RAS5088
Beijing, China, 16-20 October 2023
Project Officer: F. Sarsu

Project Objective: The project combines mutation induction with speed breeding methods under the term, mutation by speed breeding (MbyS), and is extended to Government Parties (GPs) through training courses, expert missions or technical meetings.

Meeting Objective: The meeting aimed to assess progress made in implementation of the project to achieve expected outcomes, progress in individual countries, project results and technological advancements. A significant portion of the event was dedicated to strategizing regional activities for 2024, and project activities for optimal implementation by the end of December 2024.

The meeting, hosted by the Chinese Academy of Agricultural Sciences (CAAS) was attended by sixteen National Project Coordinators, including six women and ten men.

Participants at the training course for RER5024

National Training Course
Mutation Breeding - MLW5005
Lilongwe, Malawi, 25-29 September 2023
Project Officer: Cinthya Zorrilla

Project Objective: This project aims at developing new drought resilient mutant soybean and groundnut varieties using nuclear technology, to contribute towards achieving food, nutrition, and income security of the nation, as highlighted as one of the major areas of cooperation with the IAEA in the Country Programme Framework (CPF) for the period of 2016–2021. Mutagenized populations of groundnut, pigeon pea and soybean have been generated.

The training course on Plant Mutation Breeding and Associated Biotechnologies was comprised of lectures, practical sessions, case examples, and discussions. The topics covered included mutation induction, radiosensitivity test, the process of advancing mutant populations, and biotechnological techniques associated with mutation breeding. The training also included a field visit to the site where the new M1 lines of groundnuts are growing.

A total of seventeen participants attended the course, eight women and nine men researchers.

The training was held at the Chitedze Research Station, with participation of breeders and technicians from other Research Stations and Institutions in the country, who were interested in learning more about the potential of mutation breeding.

National Training Course
National Training Course on Mutation Breeding and Biotechnologies for Climate Smart Crops - SWA5003

Regional Training Course
Molecular Markers and TILLING Applications for Crop Improvement - RER5024
Katowice, Poland, 25 September-6 October 2023
Project Officer: F. Sarsu

Project Objective: Support the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and combined biotechnologies, in order to contribute to food security in Europe and Central Asia.

Course Objective: Foster capacity development in genotypic screening for desired mutations in genes of interest, gene expression analysis (from qPCR to RNA-Seq) and NGS Analysis, as well as the basics of informatics and the use of DNA markers, DNA sequencing and targeted induced local lesions in genomes (TILLING) in applications related to the improvement of mutant populations which have been generated using nuclear techniques and chemical agents.

The training was attended by fifteen participants (nine women and six men researchers) from various countries in Europe and Central Asia. It was hosted by the University of Silesia in Katowice, Poland.
**Malkerns, Eswatini, 18-22 September 2023**  
**Project Officer: F. Sarsu**

Project Objective: Develop improved cowpea varieties that are high-yielding and drought-tolerant contributing to food security in the country.

Course Objective: Enhance capacities to improve cowpea productivity using mutation breeding and biotechnologies, with the purpose of developing climate-smart varieties. The training program covered both phenotypic and genotypic selection of mutant lines. It also included data collection, analysis, and interpretation, as well as the application of biotechnologies in developing crop varieties tolerant to both biotic and abiotic stresses. The course emphasized the development of genetically stable lines/varieties which can contribute to food security.

The event was attended by eleven participants, five women and six men researchers, who represented various organizations, including universities and research institutes under the Ministry of Agriculture. The training was hosted by the Department of Agricultural Research and Specialist Services.

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**Participants at the National Training Course for SWA5003**

**Advanced Regional Training Course**  
**Mutation Breeding and Combined Biotechnologies - RAS5099**  
**Sidi-Thabet, Tunisia, 14-15 September 2023**  
**Project Officer: I. K. Bimpong**

Project Objective: Contribute to the establishment of climate-smart agricultural practices across the ARASIA region for better productivity with minimum use of resources and fewer gas emissions with optimal food safety through improving the productivity and quality of economically important ARASIA crops adaptable to climatic changes through mutation breeding and biotechnology.

Course Objective: Capacity building in the application of DNA-based molecular approaches to crop improvement (molecular markers and sequencing methodologies), principles of mutation breeding programs and case studies in seed crops.

The training course was attended by twenty-one participants (ten women and eleven men researchers) at Tunisia’s National Center for Nuclear Sciences and Technologies (CNSTN).

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**Participants of the National Training Course for SWA5003**

**Regional Training Course**  
**Banana breeding for resistance to Fusarium wilt (Foc TR4) using tissue culture and nuclear techniques - INT5158**  
**Cruz das Almas, Brazil, 28 August-1 September 2023**  
**Project Officers: Cinthya Zorrilla, Shoba Sivasankar**

Project Objective: Support the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and combined biotechnologies, in order to contribute to food security in Europe and Central Asia.

Course Objective: Develop capacities in mutation breeding and molecular techniques in crop breeding, specifically in cereal mutation breeding programs. Develop and understand the use of molecular markers in seed crops such as wheat, barley and maize, including concepts, applications and data analysis, as well as molecular techniques in applied crop improvement, methods for detection of mutations in candidate genes and principles of RT-PCR, genotyping using markers (especially SNP based KASP markers) and application of MAS to crop improvement programs.

The training was attended by seventeen participants (ten women and seven men researchers) from various research institutes across Türkiye and was hosted by the Field Crops Central Research Institute, under the General Directorate of Agricultural Research and Policies.

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**Participants of the training course practicing DNA extraction**
Project Objective: Project INT5158 aims to develop capacities for disease management against *Foc* TR4 and the development of new resistant banana varieties using mutation breeding and combined biotechnologies to contribute to the prevention of the spread of this disease and reduce farmers’ losses.

Course Objective: The purpose of this training was to develop capacities in breeding methods used to develop resistance to *Fusarium oxysporum* f.sp. *cubense*, Tropical Race 4 (Foc TR4), with special emphasis on tissue culture from apical and floral meristems, embryogenic cell suspensions in banana, preparation of plant material for irradiation with nuclear sources, screening methods for resistance to Fusarium wilt, including preparation and multiplication of inoculum, and molecular techniques for detection of mutations in radio-induced mutants and somaclones.

A total of ten participants (five women and five men researchers) from Colombia, Costa Rica, Mexico, Peru, Venezuela, and Brazil who have initiated, or will soon be initiating efforts to conduct mutation breeding, participated in this event. The training was held at the Cassava and Fruits Research Station of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Expertise gained in the implementation of mutation breeding as part of a long-standing banana and plantains breeding programme was shared with participants during the training.

The training was attended by twenty-two participants (twelve women and ten men researchers) from various countries in the Asia and the Pacific region. It was hosted by the Research Centre for Nuclear Radiation Processing Technology under the National Research and Innovation Agency (BRIN) in Indonesia.

**Workshop**

**Screening for anthracnose resistance in Chili (Capsicum spp) and Yam (Dioscorea spp) in Fiji - RAS5098**

**Suva, Fiji, 7-18 August 2023**

**Project Officer: Cinthya Zorrilla**

**Project Objective:** The project addresses the improvement of main crops for the Pacific Islands. Its main objective is to contribute to food security in the region by building capacities in mutation breeding and generating new mutant varieties with increased productivity and better adaptation to biotic/abiotic stress. Mutation breeding was only 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.

The workshop was conducted in Fiji and was hosted by the Pacific Community through its Pacific Community Centre for Pacific Crops and Trees (SPC-CePaCT). One of the main traits of interest for our Fiji counterparts is breeding for resistance to anthracnose disease in Capsicum and yam varieties. In this workshop, participants developed expertise on isolation, culturing, and inoculum preparation of *Colletotrichum* species, as well as application of screening methods to select for resistant mutant lines of Capsicum and yam.

A total of eleven participants (eight women and three men researchers) from SPC-CePaCT as well as Dobuilevu, Sigatoka and Koronivia Research Stations of the Ministry of Agriculture participated in the training. Technical experts coordinating the workshop were Dr. Paul Taylor from the University of Melbourne, phytopathologist, and Ms Orarat Mongkolporn, breeder, from Kasetsart University. The workshop included lectures, practical demonstrations, and field visits.

**National Training Course**

**Phenotypic and Genotypic Selection of Mutant Population in Improvement of Namibia Staple Food Crops - NAM5020**

Project Objective: The project combines mutation induction with speed breeding methods under the term, mutation by speed breeding (MbyS), and is extended to Government Parties (GPs) through regional training courses, expert missions or technical meetings.

Course Objective: Participants in the training enhanced their knowledge, experience, and skills in establishing national plant breeding programs. They were trained in the application of speed breeding techniques such as double haploidy, marker assisted selection, artificial growth environments for rapid generation advancement, development of genetically stable lines and breeding new crop varieties with tolerance to abiotic stresses.
**Tsumeb, Namibia, 24-28 July 2023**  
**Project Officer: F. Sarsu**

Project Objective: Improve farmers’ income and livelihood by developing high yielding and drought tolerant crop varieties through mutation breeding and associated biotechnologies in Namibia.

Course Objective: The training aimed to assist Namibian breeders/researchers in the practical application of phenotypic and genotypic data analyses in induced mutation breeding programs for maize, pearl millet, sorghum, cowpea and groundnut. It covered important traits and marker-assisted selection (MAS) in breeding programs, as well as the planning and process for release/registration of new crop varieties.

The training was attended by twenty-one participants (eleven women and ten men researchers) from various research institutes across the country and was hosted by the Mannheim Research Institute in Tsumeb, Namibia.

**National Training Course**  
**Mutation induction and crop improvement in rice for adaptation to extreme climatic conditions - MLI5013**  
**Bamako, Mali, 17-28 July 2023**  
**Project Officer: I. K. Bimpong**

Project Objective: Improve rice productivity through the development of improved varieties and efficient water and nutrient management that will increase producers' resilience to climate change, which poses enormous and unpredictable risks.

Course Objective: Capacity building for the implementation of a mutation breeding program for seed and vegetatively propagated crops, accelerated breeding techniques such as rapid cycling in cereals and in vitro induction of haploidy, as well as an introduction to molecular breeding techniques and seed systems.

The training course was attended by fifteen participants (three women and twelve men researchers) and was held at the Institute d’Economie Rurale (IER) in Bamako, Mali.

**National Training Course**  
**Improving Cassava Resilience to Drought and Waterlogging Stress through Mutation Breeding - RWA5001**  
**Kigali, Rwanda, 17-22 July 2023**  
**Project Officer: F. Sarsu**

Project Objective: Enhance the human and infrastructural capacities to improve cassava through mutation breeding and combined biotechnologies, in order to contribute to food security and improved livelihoods of smallholder cassava farmers in Rwanda. This is the first mutation breeding program in the country.

Course Objective: Provide a basic understanding of the theory and application of induced mutations in cassava breeding. Course participants were trained to prepare explants for irradiation, to screen for tolerance to biotic/abiotic stress in laboratory, greenhouse and field conditions, and the implementation of breeding programmes from design to variety registration.

The training, hosted by the Rwanda Agriculture and Animal Resource Development Board (RAB), was attended by eight participants (one woman and seven men researchers) from both RAB and the University of Rwanda.

**Practical session in cassava fields during the Training Course for RWA5001**

**National Training Course**  
**Nutritional Quality Assessment Techniques - MALW5005**  
**Lilongwe, Malawi, 3-7 July 2023**  
**Project Officer: Cinthya Zorrilla**

Project Objective: The project aims at developing new drought resilient mutant soybean and groundnut varieties, using nuclear technology to contribute towards achieving food, nutrition, and income security of the nation, as highlighted in the Country Programme Framework (CPF) for 2016–2021 as one of the major areas of cooperation with the IAEA. Mutagenized populations of groundnut, pigeon pea and soybean have been generated and will be subjected to selection for drought tolerance and improved nutritional quality.

Course Objective: This training provided a basic understanding of the types of biochemical analyses used for nutritional quality evaluation including important grain traits such as proteins, fats, and fatty acids, as well as an overview of the equipment and materials needed for measuring relevant nutritional parameters such as total proteins, total oils, and oleic acids. A total of twenty-four participants were trained including twelve women and twelve men researchers.
The training was held at the Chitedze Research Station, with participation of breeders, food scientists and technicians involved in mutation breeding as well as other breeding groups interested in this topic.

National Training Course
Application of Marker Assisted Mutation Breeding and Basic Bioinformatics for Improvement of Namibia Staple Food Crops - NAMS020
Windhoek, Namibia, 19-23 June 2023
Project Officer: F. Sarsu

Project Objective: Improve farmers’ incomes and livelihoods by developing high yielding, drought tolerant crop varieties through mutation breeding and associated biotechnologies in Namibia.

Course Objective: The aim of the course was to provide protocols for DNA analysis, in order to detect mutations in the induced mutation breeding program, targeting sorghum, cowpea and groundnut. This includes genotyping using SSR markers, PCR and PAGE gel techniques. Furthermore, guidance on data handling and the application of marker-assisted selection (MAS) in crop improvement programs will be provided. A work plan will also be prepared for the integration of MAS into the Namibian Breeding Program, aimed at improving Namibia’s staple food crops.

The training saw participation from twenty-six attendees (sixteen women and ten men researchers) from various research institutes and was hosted by the Ministry of Agriculture and Land Reform in Windhoek, Namibia.

National Training course
Mutation induction and crop improvement in cowpea and sorghum for adaptation to extreme climatic conditions - BOT5024
Gaborone, Botswana, 19-23 June 2023
Project Officer: I. K. Bimpong

Project Objective: Focus on using nuclear related technology to develop (1) an improved variety of the two crops (cowpea and sorghum) to enhance yields which are currently very low; and (2) climate smart agricultural practices, to improve soil fertility for enhanced cowpea and sorghum production in a sustainable manner.

Course Objective: Capacity building for mutation breeding programs (seed and vegetatively propagated crops), field and lab-based selection methods for biochemical traits (drought, low and high temperature etc.), introduction to molecular breeding techniques and seed systems.

The training course was attended by thirty-eight participants (twelve women and twenty-six men researchers) at the National Agricultural Research and Development Institute (NARDI) in Gaborone, Botswana.

National Training Course
Mutation induction, selection in the two crops (Sweet potato and Sorghum) for adaptation to extreme climatic conditions - LESS012
Maseru, Lesotho, 19-23 June 2023
Project Officer: I. K. Bimpong

Project Objective: Exploit the diversity of potato and sorghum crops in order to identify novel genotypes with improved yields and adaptation to prevailing drought and resistance to transboundary plant diseases, such as potato late blight and cyst nematode.

Course Objective: Development of capacity to undertake mutation breeding programs, strategies for selecting simple and few (monogenic) as well as complex (polygenic) traits. Experimental designs and exposure to case studies using the approaches discussed.

The training course was attended by twenty-one participants (eleven women and ten men) in Maseru, Lesotho.

National Training Course
Mutation Breeding Techniques and Crop Improvement - MAU5009
Kaédi, Mauritania, 12-19 June 2023
Project Officer: I. K. Bimpong

Project Objective: Develop rice and sorghum varieties through mutation breeding that are tolerant to abiotic and biotic stresses, especially drought, and have improved quality.

Course Objective: Develop capacity for implementing mutation breeding programs, accelerated breeding techniques such as rapid cycling in cereals, and an introduction to molecular breeding techniques and seed systems.

The training course was attended by twenty participants (one woman and nineteen men researchers) and was held at the National Centre of Agricultural Research and Development, (CNRADA) in Kaédi, Mauritania.
Developments at the Plant Breeding and Genetics Laboratory (PBGL)

Update: Seeds returned from space show high viability

To test the mutagenic effects of evolutionary stress in space, including cosmic radiation, PBG sent seeds of *Arabidopsis thaliana* and *Sorghum bicolor* to the International Space Station (ISS) in November 2022 as a feasibility study under the CRP D24015. The goal is to assess the types of changes on genome and plant biology that result from exposure to microgravity, cosmic radiation and extreme temperatures in space, and to determine if the mutagenic effects of space, “the ultimate stressor”, is different from mutagenesis on earth for facilitating crop adaptation to climate change. Samples of the two plant species were exposed to two separate treatments, one inside the ISS, with exposure to microgravity alone, and the second outside the ISS with exposure to microgravity, extreme temperatures and unshielded cosmic radiation.

As a first step after their return, germination tests conducted in *Arabidopsis* seeds at PBGL revealed almost 100 % germination and the production of true leaves after five weeks under extreme conditions outside the ISS. Seeds from the same seed batch that remained on earth served as control. A random subset of the germinated plants was transplanted to soil to obtain M2 plants for subsequent genomic analyses. All plants showed normal morphology and reproductive behavior and produced normal siliques.

PUI Project: Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa

A. Field screening of Mchare banana mutant population for resistance to Fusarium wilt

Recent results are presented from the Peaceful Uses Initiative (PUI) project on breeding for Fusarium Wilt (FW) resistance in cooking type Mchare (AA) bananas, one of the parents of the Cavendish bananas through induced mutagenesis (gamma-ray and EMS). The project continues to be implemented at the PBGL in cooperation with the International Institute of Tropical Agriculture (IITA), Tanzania, and Stellenbosch University, South Africa. Mchare are susceptible to FW caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), race 1 and TR4.

Previously in May 2022, a mutant population of ca 5,000 *in vitro* plants was established and shipped from PBGL, Austria, to IITA, Tanzania, to be hardened and planted in a hot spot field for resistance screening to *Foc* race 1. The field is at the Tanzania Agricultural Research Institute (TARI) in Maruku, a leading center for banana research in Tanzania.

The field was divided into 3 blocks of 50 x 30 m (figure 1) and three-month old plants were planted with spacing of 1 x 1 m. Each block carried 1000 mutants in a Complete Randomized Design, with Grand Naine (Cavendish, AAA) planted as resistant control and Sukari Ndizi (AAB) as susceptible control. Each plant was planted in a planting hole of 30x30 cm and in addition to the natural inoculum, every planting hole was filled with infested soil containing 100 g of *Foc* race 1 VCG 0124 inoculated millet seeds to maintain high inoculum pressure (Figure 2).

The disease was evaluated by scoring external symptoms, mainly the yellowing of leaves, which appeared 3 months after planting and inoculation. Disease development in the field was scored on a weekly basis using the rating scale for the Leaf Discoloration Index (LDI) ranging from 1 to 5, with 1 indicating absence of leaf symptoms, and 5 with all leaves yellow and wilted.

As internal disease scoring is more reliable than external scoring (Viljoen et al., 2017), a few plants were selected and cut open to evaluate internal symptoms using the Rhizome Discoloration Index (RDI) with 1 to 6 rating, where 1 has no discoloration of the inner rhizome and 6 shows discoloration of the entire inner rhizome (Viljoen et al., 2017) (Figure 3). Scores from external symptoms showed an increase of disease incidence from 10% in week 9 after inoculation to 44% in week 33 after inoculation. So far, over 50% of plants (1500) still remain in the field without any external or internal disease symptoms.

More external disease symptoms are expected to be seen in weeks 38-40 during flowering. Subsequently, plants that remain in the field without any disease symptoms will be considered as resistant. Internal disease symptoms – including discoloration of the inner rhizome – will also be evaluated at the end of the experiment.
These candidate lines are being backed up \textit{in vitro} and multiplied for second-batch field screening and genome-wide profiling for trait discovery and functional analyses.

**B. Advanced and Rapid Diagnostic Techniques for Foc TR4 - Loop-Mediated Isothermal Amplification (LAMP Assay)**

The PBG Lab has validated and optimized a reliable and rapid \textit{in planta} diagnostic method for the banana Fusarium Wilt TR4, including low-cost portable real-time colorimetric devices for use in the field, and real-time fluorescent q-PCR under laboratory conditions. Loop-mediated isothermal amplification is a highly specific amplification technique. Unlike conventional PCR, LAMP uses four to six primers for isothermal amplification of specific DNA sequences, allowing results to be analyzed in only 30 minutes.

Rhizome samples from 20 infected banana plants from TR4 inoculation were collected and scored for disease symptoms on a scale of 1-6 on the rhizome discoloration index (RDI). Each RDI score was equally represented during testing of the assay. Utilizing real-time fluorescent q-PCR a standard curve was prepared for quantifying the presence of \textit{Foc} TR4 DNA \textit{in planta}. Sensitivity of the assay was validated, with a limit of detection confirmed at 4 pg/µL. A constant positive read for the presence of pathogen DNA was observed once the RDI passed a score of 2 (few internal symptoms). We hope to use the LAMP assay’s sensitivity to quantify the Rhizome Discoloration Index, assigning a range of amount of fungal DNA to each symptomatic stage of infection.

**Rhizome Discoloration Index (RDI)**

<table>
<thead>
<tr>
<th>RDI</th>
<th>Disease Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No internal spots</td>
</tr>
<tr>
<td>2</td>
<td>Few internal spots</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1/3 of the inner rhizome discolored</td>
</tr>
<tr>
<td>4</td>
<td>1/3-2/3 of the inner rhizome discolored</td>
</tr>
<tr>
<td>5</td>
<td>&gt;2/3 of the inner rhizome discolored</td>
</tr>
<tr>
<td>6</td>
<td>Entire inner rhizome discolored</td>
</tr>
</tbody>
</table>

**C. Protocol development for high-throughput screening of coffee (Coffea arabica) mutant population for resistance to coffee leaf rust disease (CLR)**

Following the Coordinated Research Project, D22005, ‘Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana’, the PUI project ‘Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa’, addresses as part of its focus, the development of screening techniques for resistance to the leaf rust disease in coffee. The PBGL is developing robust and high-throughput, low-cost methods to screen large mutant populations for resistance to coffee leaf rust (CLR). These assays are being conducted on greenhouse grown M\textsubscript{2} populations developed at PBGL, using uredospores (fungal inoculum) in collaboration with the National Agricultural Research Institute, Honduras. Two inoculation methods such as spore suspension and spore powder methods are being optimised using leaf discs (Figure 1A). After inoculation, the assessment integrates visual observation and photographic imaging to monitor disease progress. Our preliminary results with spore powder method indicate good spore germination and infection of leaf discs, exhibiting symptoms under \textit{in vitro} conditions (Figure 1B). The selected mutants are also being tested with spore suspensions for optimal infection and disease progression.

For many laboratories, the method of choice for targeted mutation discovery in candidate genes or to analyze genetic variation in specific genomic regions is Sanger-sequencing of amplicons (PCR products), which are typically outsourced to service providers. While straightforward, the process quickly becomes time consuming and expensive for longer genomic regions, i.e., beyond 2-3 kb.

A novel, cost effective alternative is 3rd generation, long-read sequencing with Oxford Nanopore Technology (ONT). Using their hand-held ‘MinION’ sequencing device and ‘Flongle’ flow cells, such amplicon sequencing is easily performed in-house. The entire protocol, from amplicon(s) to sequencing result(s), takes less than two days with only a few hours hands-on time. While already cost-effective for sequencing one amplicon, the sequencing capacity of a ‘Flongle’ flow cell of several hundred thousand reads is sufficient to simultaneously sequence several amplicons from several samples at the same time. A recent PBGL experiment is shown in Figure 1: Sequencing 4.7 kb PCR amplicons from 3 barley varieties. The amplicon covers a CAD gene locus, and novel SNPs, including one with high impact, was readily discovered.

PBGL has streamlined protocols and established Oxford Nanopore long-read sequencing. Sequencing and analysis are performed with standard laptops running Linux. Amplicon sequencing with long reads is now part of PBGL’s molecular biology training portfolio and the first Member State researchers have been trained in 2023 during their fellowships at PBGL.

Protocol Development: Rare Mutation Detection in Sorghum using ddPCR

Detection of SNPs in genes of interest, whether induced or endogenous, is a powerful tool to explore gene function and to identify desired mutations for plant breeding and biotechnological applications. However, the detection of rare mutations usually fails due to the interference of predominantly surrounded wild-type DNA. In recent years, digital droplet PCR (ddPCR) has emerged as a leading technology that offers highly sensitive amplification and detection of nucleic acids based on water-oil emulsion droplet partitioning, providing ultrafast throughput for screening mutant plant populations to identify and isolate targeted variants of interest.

The PBG Lab has successfully designed and optimized SNP based ddPCR assays in sorghum recently, targeting four candidate genes that contribute towards resistance against the parasitic weed Striga hermonthica that causes up to 100% yield losses and affects over 60% of cultivable farmlands and livelihoods in Africa. DNA from mutant sorghum lines was pooled together to test the robustness and sensitivity of the four assays. Experiments displayed the assay’s sensitivity and yielded a lower limit (0.001%) of detection (LOD) reporting the application and validation of ddPCR-based analyses for the detection of SNP markers in large mutant populations (Figures 1 and 2). In addition to mutation detection, due to the precise partitioning technology and qualitative discrimination, the duplex ddPCR assay can decipher homozygous from heterozygous mutations with superior levels of precision and sensitivity in individual mutant lines, offering potential for large scale genome-informed breeding applications.

Efforts are underway to develop methods for detecting copy number variant distribution and zygotically estimated for breeding applications. An important advantage of the ddPCR technology relates to the fast generation of results, with time required from extraction of DNA to completed evaluation of PCR results in 8-10 hours, which provides diagnostic molecular results within a single day.

PBGL will use the optimized assays to begin screening our large-scale mutation populations of sorghum for breeding cultivars that can withstand Striga infection, while venturing into other applications of ddPCR.

Figure 1: Three typical droplet plots of the wild type (WT: upper left panel), mutant (upper right panel), and pooled samples carrying WT + individual rare mutants (LOD 0.01 & 0.001 %) down panel.

Figure 2: Quantification of mutation ratio in mutant (blue) and WT (brown) genomes in serially diluted samples.
Table 1. Crop Irradiation Services

<table>
<thead>
<tr>
<th>Request Number</th>
<th>Country</th>
<th>Request Type</th>
<th>Crop/Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1743</td>
<td>The Netherlands</td>
<td></td>
<td>ornamental</td>
</tr>
<tr>
<td>1744</td>
<td>Germany</td>
<td></td>
<td>ornamental</td>
</tr>
<tr>
<td>1745</td>
<td>Poland</td>
<td>TC</td>
<td>buckwheat</td>
</tr>
<tr>
<td>1746</td>
<td>Namibia</td>
<td>TC</td>
<td>Bambara groundnut</td>
</tr>
<tr>
<td>1747</td>
<td>UAE</td>
<td>TC</td>
<td>proso millet</td>
</tr>
<tr>
<td>1748</td>
<td>Germany</td>
<td>TC</td>
<td>beans, cowpea, maize</td>
</tr>
<tr>
<td>1749</td>
<td>Cameroon</td>
<td>TC</td>
<td></td>
</tr>
<tr>
<td>1750</td>
<td>Germany</td>
<td></td>
<td>ornamental</td>
</tr>
<tr>
<td>1751</td>
<td>Cambodia</td>
<td>TC</td>
<td>rice</td>
</tr>
<tr>
<td>1752</td>
<td>Austria</td>
<td>CRP</td>
<td>cassava</td>
</tr>
</tbody>
</table>

Individual Training

Table 2. Individual Training Activities at the PBGL

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Status</th>
<th>Topic</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Radisras NKURUNZIZA</td>
<td>Uganda</td>
<td>PhD Consultant</td>
<td>Coffee mutation breeding</td>
<td>1 year</td>
</tr>
<tr>
<td>Mr Hassan MDUMA</td>
<td>United Republic of Tanzania</td>
<td>PhD Consultant</td>
<td>Mutation breeding of African cooking banana for Fusarium Wilt resistance</td>
<td>1 year</td>
</tr>
<tr>
<td>Ms Emma RAMIREZ</td>
<td>USA</td>
<td>Intern</td>
<td>Banana Fusarium Wilt disease</td>
<td>1 year</td>
</tr>
<tr>
<td>Mr Yonis Alberto MORALES REYES</td>
<td>Honduras</td>
<td>Fellow</td>
<td>Genetics of qualitative and quantitative (mutant) traits; intro Next Generation Sequencing</td>
<td>6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Molecular Bio Lab procedures and NGS data analysis</td>
<td></td>
</tr>
<tr>
<td>Ms Tonika MALEMA</td>
<td>Malawi</td>
<td>Fellow</td>
<td>Mutation breeding, mutation induction, radiosensitivity, screening methods</td>
<td>2 months</td>
</tr>
<tr>
<td>Mr Gerhard Shangeshapwako HAITEMBU</td>
<td>Namibia</td>
<td>Fellow</td>
<td>Mutation breeding, mutation induction, development &amp; handling of mutant populations, detection &amp; selection of mutant plants</td>
<td>3 months</td>
</tr>
<tr>
<td>Ms Aneera Devi PURMESSUR MAYEPUTH</td>
<td>Mauritius</td>
<td>Fellow</td>
<td>Radiosensitivity, DNA extractions &amp; QC, PCR, KASP marker, Genetic/genomics/NGS, advanced mutation breeding</td>
<td>3 months</td>
</tr>
<tr>
<td>Mr Raymond MASSAQOI</td>
<td>Sierra Leone</td>
<td>Fellow</td>
<td>Irradiation and methods for radiosensitivity assay of rice and cassava, selection of mutant lines</td>
<td>3 months</td>
</tr>
<tr>
<td>Mr Mohammad RAHEMI</td>
<td>Iran</td>
<td>Fellow</td>
<td>DNA extractions &amp; QC, PCR, KASP marker, Genetic/genomics/NGS</td>
<td>4 months</td>
</tr>
</tbody>
</table>

Table 1 lists the irradiation requests that the PBGL received to date in the second half of 2023 (2023-09-25). A total of 22 requests were received from 18 Member States across 28 different plant species covering 92 accessions/varieties.
<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Title</th>
<th>Experience Description</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Joel Larhlheu PILON</td>
<td>Papua New Guinea</td>
<td>Fellow</td>
<td>Mutation breeding of vegetatively propagated crops using banana as a model, include tissue culture, mutation induction and radiosensitivity testing</td>
<td>2 months</td>
</tr>
<tr>
<td>Mr Ellis VAEGA</td>
<td>Samoa</td>
<td>Fellow</td>
<td>Hands-on experience in routine laboratory, greenhouse, and field activities related to mutation breeding, including mutation induction, radio-sensitivity testing, and basic screening methods in seed crops</td>
<td>2 months</td>
</tr>
<tr>
<td>Mr Khamphouvanh SENAMOUNTY</td>
<td>Lao PDR</td>
<td>Fellow</td>
<td>Hands-on experience in routine laboratory, greenhouse, and field activities related to mutation breeding, including mutation induction, radio-sensitivity testing, and basic screening methods in seed crops</td>
<td>2 months</td>
</tr>
<tr>
<td>Ms Nhebsae OSIN</td>
<td>Cambodia</td>
<td>Fellow</td>
<td>Hands-on experience in routine laboratory, greenhouse, and field activities related to mutation breeding, including mutation induction, radio-sensitivity testing, and basic screening methods in seed crops</td>
<td>2 months</td>
</tr>
<tr>
<td>Mr Mokhtar BARAKET</td>
<td>Tunisia</td>
<td>Fellow</td>
<td>Evaluation of resistance to Stemphylium blight in lentil mutant lines and application of standardized resistance screening protocol for lentil</td>
<td>3 months</td>
</tr>
<tr>
<td>Ms Katrina MALABANAN-BAUAN</td>
<td>Philippines</td>
<td>Scientific Visitor</td>
<td>Learn about enabling technologies such as Rapid Generation Advancement and molecular tools in plant mutation breeding, and to facilitate exchange of information</td>
<td>5 days</td>
</tr>
<tr>
<td>Mr Mark Ian CALAYUGAN</td>
<td>Philippines</td>
<td>Scientific Visitor</td>
<td>Learn about enabling technologies such as Rapid Generation Advancement and molecular tools in plant mutation breeding and to facilitate exchange of information</td>
<td>5 days</td>
</tr>
<tr>
<td>Mr Charles GRANT</td>
<td>Jamaica</td>
<td>Scientific Visitor</td>
<td>Gamma Cell Use for Mutation Breeding</td>
<td>5 days</td>
</tr>
<tr>
<td>Ms Sherine HUNTLEY JONES</td>
<td>Jamaica</td>
<td>Scientific Visitor</td>
<td>Gamma Cell Use for Mutation Breeding</td>
<td>5 days</td>
</tr>
<tr>
<td>Ms Charah WILSON</td>
<td>Jamaica</td>
<td>Scientific Visitor</td>
<td>Gamma Cell Use for Mutation Breeding</td>
<td>5 days</td>
</tr>
<tr>
<td>Mr Ryan FRANCIS</td>
<td>Jamaica</td>
<td>Scientific Visitor</td>
<td>Gamma Cell Use for Mutation Breeding</td>
<td>5 days</td>
</tr>
</tbody>
</table>
NEW CRP: Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation (D24016)

Fatma Sarsu, Anupama J. Hingane, IAEA Department of Nuclear Sciences and Applications

The IAEA, through the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, is launching a new Coordinated Research Project (CRP) focused on Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation, spanning a five-year timeframe from 2024 to 2028.

Millets, hardy dryland crops that can grow with minimal inputs in Asian and African countries, are gaining popularity due to their nutritional qualities and resilience to climate change. They present an ideal solution for countries to increase self-sufficiency and reduce reliance on imported cereal grains. Millets are rich in dietary fibres, proteins, minerals (iron, potassium, magnesium, and phosphorous) and vitamins. Additionally, millets exhibit exceptional efficiency in water and nitrogen use, enabling them to flourish in water-deficit conditions in rain-fed regions. They require minimal irrigation and nitrogen fertilizers to achieve better yields. Thanks to their nutritional superiority and climate resilience features, small millets can supplement staple cereal crops. They are rich in micro- and macro-nutrients, proteins, essential amino acids, dietary fibre, and resistant starch.

This CRP will focus on improving the grain yield of pearl millet (Pennisetum glaucum), finger millet (Eleusine coracana (L.) Gaertn.) and proso millet (Panicum miliaceum L.) to several biotic and abiotic stresses in the face of climate change. Increasing the yield of these millets for climate change adaptation could have a major positive impact on food security, improved health and income generation for millions of impoverished and vulnerable people.

Pearl millet (Pennisetum glaucum) is the major millet, accounting for approximately 50 per cent of total millet production. However, blast, also known as leaf spot disease, a seed and wind-borne disease caused by Pyricularia grisea Sacc., has emerged as a severe threat in recent years across major pearl-millet-growing regions. Another significant disease affecting pearl millet is downy mildew caused by Sclerospora graminicola. This is a systemic and devastating disease that can completely destroy infected plants and lead to up to 100 per cent grain loss in highly susceptible cultivars. Achieving stable and durable resistance to these diseases is challenging due to temporal changes in the virulence of the pathogen, which can lead to a rapid breakdown of resistance.

Finger millet (Eleusine coracana (L.) Gaertn.) blast disease is caused by the fungus Magnaporthe oryzae which is a global pathogen occurring in all areas where the crop is grown. Finger millet blast affects all growth stages of its host, from the seedling stage through grain formation and can cause over 90 per cent yield losses. Drought can occur at any stage of finger millet development; however, the most damaging droughts for finger millet are those that occur at the seedling stage just after germination and at the flowering stage.

Proso millet (Panicum miliaceum L.) is cultivated in many countries because of its short growing cycle (60-90 days), minimal input requirements and resilience to biotic and abiotic stresses. However, it does have certain challenges, including small seeds, seed shattering and a shallow root system which makes it susceptible to lodging. The most important selling point of the crop is its high protein content. Therefore, development of gene specific markers is critical to facilitate rapid screening of proso millet lines for high protein content. Additionally, conducting transcriptomics, proteomics and metabolomics studies will enhance our understanding of protein accumulation in proso millet in relation to yield. Mutation in proso millet can lead to the development of new varieties that do not exist in the germplasm, with characteristics such as non-shattering, deep root system, uniform maturity of panicles and bold grains.

A.1.1. Objective of the CRP

To develop novel genetic stocks of key dryland millets using mutation breeding and biotechnologies to accelerate the development of new varieties for food and nutrition security and climate-change adaptation.

A.1.2. Specific objectives

1. To generate genetic diversity in selected millets with improved nutrition and quality traits, and improved resilience to biotic/abiotic stress through induced mutation for better adaptation to climate change,
2. To develop/adapt phenotyping tools for precise screening/selection of mutant lines with the desired traits in selected millet crops,
3. To develop genomic tools for delivery of novel induced variation to accelerate genetic gain in millet improvement.

How to join this CRP

Up to ten research contracts are expected to be awarded and five no-cost agreement holders from advanced laboratories and research institutes with recognized expertise in the targeted technologies will be invited to share their experience with the contract holders and contribute to the development and validation of the planned technical packages. Additionally, two technical contracts are foreseen to be awarded for services in advanced areas, such as functional genomics, the establishment of genetic association for traits of interest, genomic selection, and gene editing technologies. Coordination and technical management will be handled by the scientific secretary in the Plant Breeding and Genetics Section with the involvement of the Plant Breeding and Genetics Laboratory.

Please submit your Proposal for Research Contract or Agreement by email, no later than 15 December 2023, to the IAEA’s Research Contracts Administration Section using the appropriate template on the CRA web portal. Same form can be used for the research contract and the technical contract.

For further information related to this CRP, potential applicants should use the contact form under the CRP page.
**Publications**

**Books**

- **Mutation Breeding in Coffee with Special Reference to Leaf Rust**
  Editors: Ivan L.W. Ingelbrecht, Maria do Céu Lavado da Silva, Joanna Jankowicz-Cieslak
  Springer, 2023
  ISBN 978-3-662-67273-0 (eBook)

- **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)

- **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
Manual de mejoramiento por mutaciones, Tercera edición
Editado por M.M. Spencer-Lopes, Forster, B.P., Jankulski, L., Sub
Programma de Mejoramiento de Plantas y Genética, División Conjunta
FAO/OIEA de Técnicas Nucleares en Alimentación y Agricultura.Manu
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Manuel d’amélioration des plantes par mutation, Troisième édition
Édité par. M.M. Spencer-Lopes, B.P. Forster et L. Jankulski, Sous-
programme de Genétique et d’Amélioration des Plantes
Division mixte FAO/OIAE des Techniques Nucléaires appliquées à
l’Alimentation et à l’Agriculture.
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., Balhuguna, R.N., Kusolwa, P.M.,
Ashraf, M., Singla-Pareek, S.L., Pareek, A., Forster, B.P., Ingelbrecht,
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Sarsu | Springer

Technical Documents
A Low-Cost Genotyping Protocol and Kit for Marker-Assisted
Selection of Orange Lemma (rob.l.a), a Feed Quality Trait in Barley
(Hordeum vulgare L.). Introductory guide and laboratory protocols. I.
Ingelbrecht et al., May 2021.

IAEA-TECDOC-1969
Development of Tolerant Crop Cultivars for Abiotic Stresses to
Increase Food Security (IAEA-TEC-DOC-1969)
ISBN 978-92-0-123322-1 (PDF)

Peer-reviewed Publications
2023
KENZHEBAYEVA S., ATABAYEVA S., SARSU F., ABEKOVA A,
SHOINBEKOVA S., OMIRBEKOVA N., DOKTYRBAY G.,
BEISENOVA A., SHAVRUKOV Y. (2023). Organ-specific expression of
genes involved in iron homeostasis in wheat mutant lines with
increased grain iron and zinc content. PeerJ 10:e13515
https://doi.org/10.7717/peerj.13515

BESHIR M. M., MOHAMED M. S., SARSU F., HASSAN O. B.,
ABDALLAH A. E., OMER R. A., SULIMAN S., AHMED N. E.,
Drought Tolerance under Gezira Irrigated Conditions, Sch J Agric Vet
Sci, ISSN 2348-8883 (Print) (saspublishers.com)

WANGA M. A., SHIMELIS H., MASHILO J., HORN L. N., SARSU
F., 2023. Responses of elite sorghum (Sorghum bicolor [L.] Moench) lines developed via gamma-radiation for grain yield, component traits and
drought tolerance. Reproduction and Breeding, Volume 3, Issue 4,
2023, Pages 184–196, ISSN 2667-0712, Responses of elite sorghum
(Sorghum bicolor [L.] Moench) lines developed via gamma-radiation
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SIVASANKAR S. and LE HUY HAM (2023) Impact of induced
genetic variation and genomics technologies on rice and soybean
the Use of Agricultural Biotechnologies to Meet the Needs of
Smallholders in Developing Countries. Rome, FAO
SIVASANKAR S. (2023) Crop improvement through induced genetic
variation and mutation breeding: Challenges and Opportunities. In

Penna and Jain eds. 2023. Mutation Breeding for Sustainable Food
Production and Climate Resilience. Springer Nature, pp 293-300
Mutation Breeding for Sustainable Food Production and Climate
Resilience | SpringerLink
SARSU F., PENNA S., NIKALJE G.C. (2023) Strategies for screening
Mutation Breeding for Sustainable Food Production and Climate
Resilience. Springer Nature, pp 151-176 Mutation Breeding for
Sustainable Food Production and Climate Resilience | SpringerLink

Field Screening of Elite Cassava (Manihot esculenta) Mutant Lines for
their Response to Mosaic and Brown Streak Viruses. In: Journal of
Experimental Agriculture International. Volume 45, Issue 9, Page 205-
215, 2023; Article no.JEAI.104503, ISSN: 2457-0591

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S. (2023) Virulence and Molecular Detection of Cassava Mosaic and
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BOREVITZ, J., WARTHMANN, N. (2023) Cost-conscious generation of
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Coffee with Special Reference to Leaf Rust. Springer Nature.

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., INGELBRECHT,
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GHANIM, A.M.A., BADO, S., DADA, K. (2023) Physical mutagenesis of
Arabica coffee seeds and seedlings. In: Mutation Breeding in Coffee
with Special Reference to Leaf Rust. Springer Nature.

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., INGELBRECHT,
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L. var Venecia using EMS. In: Mutation Breeding in Coffee with
Special Reference to Leaf Rust. Springer Nature.

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INGELBRECHT, I.L.W. (2023) Use of open-source tools for imaging
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BOHRA, A., KILIAN, B., SIVASANKAR, S., CACCAMO, M., MBA,
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Conference Abstracts and Posters

2023

SUBRAMANIAN N, PILLAI S, ROONEY W, BAGAVATHIANNAN M, SIVASANKAR S (2023) Utilization of electron-beam mutagenesis for sorghum crop improvement. Global Sorghum Conference 2023, June 5-9, Montpellier, France


SIVASANKAR S (2023) The role of induced genetic variation in crop improvement towards food and nutrition security – a global context. Invited Keynote Lecture at the International Conference on Food and Nutrition Security (iFANS-2023) January 6-9, 2023, Mohali, India.

JAWDAT D., MOSTAFA O., JANKULOSKI L., SARSU F., MALEK M., MIR A.N. (2023) Mutation breeding, an affordable crop improvement strategy in challenging times: Barley mutation breeding projects in Syria and the urge for acceleration. The 3rd International Barley Mutant Conference- 8-10 October 2023, - Kurashiki, Japan

KIRYAKOV I., PETKOVA M., AZIZ S., MASHEVA V., SARSU F., TOMLEKOVA N. (2023) Phytopathology and molecular investigation of resistances to bacterial and fungal pathogens in common bean mutant and breeding lines. 2-4 November 2023, Antalya, Türkiye.


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News Highlights

- NEW CRP: Accelerated Genetic Improvement of Key Dryland Millets for Climate Change Adaptation (D24016) | IAEA (31 October)
- Africa's First IAEA Collaborating Centre for Plant Breeding and Genetics (23 October)
- IAEA and FAO Announce Winners of Seeds in Space Comic Book Contest | IAEA (11 August)
- Crop Seeds Return from Space in IAEA/FAO Project to Help Feed a Warming World (15 April)
- IAEA and FAO Engage Youth in STEM with First-of-a-Kind Space-Themed Event (28 March)
- Seeds in Space: ‘Cosmic crops’ for food security and climate change adaptation (27 March)
- Cosmic crops poised for harvest on Earth (27 March)
- Creating ‘cosmic crops’ for food security and climate change adaptation (27 March)
- IAEA and FAO Launch ‘Seeds in Space’ Youth Comic Book Competition (16 March)
- Climate Change Is Launching a Mutant Seed Space Race (5 March)
- Seeds Undergo Radiation in Space to Explore Biology and Genetics for Enhanced Food Security (11 January)

Websites and Links

- Plant Breeding and Genetics Section: [https://www.iaea.org/topics/plant-breeding](https://www.iaea.org/topics/plant-breeding)
- Mutant Variety Database: [http://mvd.iaea.org](http://mvd.iaea.org)
- Plant Breeding Publications: [Plant breeding publications | IAEA](https://www.iaea.org/topics/plant-breeding)

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