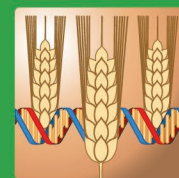




Joint FAO/IAEA Programme  
Nuclear Techniques in Food and Agriculture

# Plant Breeding & Genetics Newsletter



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## To Our Readers



*Regional Training Course on “Mutation Breeding and Efficiency Enhancing Techniques for Resistance to Banana Fusarium Wilt Race TR4 in Latin America” February 2022 (Seibersdorf, Austria)*

Dear Colleagues,

An important and high-profile event during the first half of the year was the **“Global Research Symposium in the Management of Banana Fusarium Wilt TR4”** implemented as a two-day hybrid event in Quito, Ecuador, from 24 to 25 March 2022, with technical leadership from the Plant Breeding and Genetics (PBG) Sub-program at the Joint FAO/IAEA Centre. The Symposium included three

sessions: Session 1 on “Global Experience in Foc TR4 Management”; Session 2 on “Detection, Epidemiology and Integrated Management of Foc TR4”; and Session 3 on “Genetic Resistance and Banana Fusarium Wilt TR4”. It brought together 15 international researchers working on the disease from across Australia, Asia, Africa, Europe and Latin America, who delivered research status updates on different facets of Tropical Race 4 (TR4) management in the separate sessions. The IAEA Director General, Mr Rafael

Mariano Grossi, launched the recently approved inter-regional Technical Cooperation Project (TCP) on “Strengthening Member State Capacities to Combat Banana Fusarium Wilt (TR4) through Early Detection, New Resistant Varieties and Integrated Management (INT5158)” at the start of this symposium.

Given the critical significance of the recent incursion of banana Fusarium Wilt TR4 in the Latin American region, the major region exporting banana, a series of awareness building efforts were organized under the umbrella of the World Banana Forum of the Food and Agricultural Organization at which PBG represented its work. These included a thematic session of the “Working Groups of the World Banana Forum” on 21 April, and two global webinars, namely “TR4-Resistant Banana Varieties: Development, Introduction and Evaluation” on 17 May, and the “International Conference on Banana Fusarium Wilt Disease in Africa” from 30 May to 01 June. At each of the three events, PBG presented talks on “**Efforts to Combat Banana Fusarium Wilt at the Joint FAO/IAEA Centre**”. In addition, PBG reported to the International Plant Protection Commission (IPPC) for the first time in its history on the application of plant mutation breeding for crop pest and disease management, with focus on capacity building in eight Member States. A talk on “**Induced Genetic Diversity for Resistance to Fall Army Worm in Maize**” was presented at the IPPC Capacity Development and Implementation Committee (IC) Virtual Meeting on 17 May, describing the efforts on management of Fall Army Worm in the Central African Republic.

A **two-week training course** was held at the PBG laboratory in Seibersdorf, Austria, on tissue culture, mutagenesis and screening of banana plantlets for resistance to the Fusarium Wilt TR4. This was the first training course held in the Seibersdorf laboratories since March of 2020 and was attended by 12 researchers from six countries in Latin America. In addition to mutation breeding and efficiency-enhancing techniques in banana, the course also addressed PCR-based detection of the pathogen, *Fusarium oxysporum* f.sp. *cubense* TR4. **Important protocols in tissue culture, mutagenesis and screening for TR4 resistance** derived as part of a recently completed PBG Coordinated Research Project (CRP) (D22005) was published as an open access book by Springer Nature and is accessible here.

The current newsletter features four articles on the banana Fusarium Wilt, Tropical Race 4, from page 5 to 14.

The PBG Laboratory has started a systematic exploration of **Rapid Generation Advancement (RGA)** in plant mutation breeding for seed crops, following previous single-season

successes observed in sorghum and barley. Using single-seed descent and controlled environments, a protocol is being optimized to reduce breeding time for grain crops. Early results with two genotypes of lentil, *Lens culinaris*, show a reduction of 21 days from sowing to physiological maturity, which allows an anticipated 4-5 generations of the crop per year. The laboratory also applied an in-house automated workflow to undertake a genome-wide comparative analysis of 15 rice lines, 14 of them mutants with potential resistance to the bacterial leaf blight disease.

The **new CRP (D24015)**, launched in 2022, “Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development”, held its virtual workplan meeting in April 2022 and plans to have the first Research Coordination Meeting in person in Vienna, Austria, during November of this year. The project brings together the analyses of structural DNA variations resulting from different mutagen sources, including cosmic radiation, genomic selection for drought tolerance, the establishment of genetic associations using bulk segregation analyses, MutMap or MutMap plus approaches, and functional validation of genetic associations using gene editing.

The PBG subprogram currently provides technical support to **97 TCPs** across more than a hundred Member States, of which 36 TCPs commenced in January 2022, and 61 are ongoing. With the gradual ease of Covid-19-related travel restrictions across the globe, there is rapidly increasing demand for capacity building support through expert missions, fellowships, scientific visits and training courses. With very few experts with unique experience in mutation breeding, and in the various efficiency enhancing techniques in breeding, this poses a challenge. Even so, PBG provided technical support for one regional and three national training courses during the first half of the year and is preparing the implementation of 12 regional and nine national training courses for the second half of the year.

Finally, PBG presented a stellar booth at the **Long Night of Research** in Vienna, Austria, on 12 May 2022, attracting many visitors with its examples of plant mutation breeding results, DNA extraction, the portable sequencing MinION device and other exhibits.

My sincere appreciation for all your support and efforts in induced genetic variation and plant mutation breeding. I look forward to our continuing strong collaborations to deliver important results for global food security and crop adaptation to climate change.

*Shoba Sivasankar*  
*Head, Plant Breeding and Genetics Section*

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## Staff News

### Welcome



**Mr Zhizhou Niu (China)** joined the PBGL on March 2022 as an intern. He obtained a BSc in Agriculture at the Huazhong Agricultural University, Wuhan and is currently working on his MSc in Plant Breeding at the same university. His MSc project is about using molecular technology to reduce the glucosinolate content in the seeds of *Brassica rapa* for breeding varieties with low glucosinolate content in seeds subsequently. Through this internship, he wants to learn more practical experimental skills and knowledge in mutation breeding and molecular breeding. His internship programme combines field, glasshouse and laboratory experiments for phenotyping, screening in the field and glasshouse and marker development for disease resistant lentil.



**Ms Jing Song (China)** joined the PBGL in March 2022. Jing obtained her MSc degree in ‘Agronomy and Seed Industry’, with focus on Molecular Plant Breeding, and a BSc degree from China Agricultural University in Beijing. As undergraduate, Jing spent one semester at Oklahoma State University (USA) as an exchange student. Jing already has experience in mutation breeding, tissue culture techniques and functional genetics. As undergraduate, Jing started out with functional studies on microRNA 396 in alfalfa, which involved transformation through tissue culture. In her master’s thesis project, Jing investigated the effect of the physical mutagen Atmosphere Room Temperature Plasma (ARTP) on switchgrass and the phenotype of overexpressing the gene ‘m6A demethylase FTO’ on root nodulation, again in alfalfa. Jing treated embryogenic calli of switchgrass with ARTP as a radiation source, regenerated close to 1,000 plants, and identified one particularly interesting mutant that displays increased vigor. Jing published two review articles: one on Advances in Cold Plasma Treatment Effects on Crop Seeds as first, and another on Research Progress on m6A RNA Modification in Plants as co-author.

Jing is familiar with molecular biology laboratory techniques and associated computer software and online resources. At PBG Laboratory Jing contributes toward protocol development for targeted mutagenesis using innovative CRISPR/Cas9 applications. To further broaden her skills, Jing assists in plant tissue culture projects on banana.

### Farewell



While we are sad to say farewell to Mr Florian Goessnitzer who has been with the Plant Breeding and Genetics Laboratory since August 2017, we wish him the very best in the next chapter of his career. Mr Goessnitzer has been an important part of the tissue culture R&D efforts at the lab, contributing to in vitro techniques in both coffee and banana. He has also participated in other activities of the lab working together with colleagues on ongoing research as well as laboratory and greenhouse management. Florian came to us with more than six years of knowledge in the use of state-of-the-art in vitro tissue culture, microbiological, molecular and analytical methods gained through several projects dealing with the discovery of natural products from plants and microorganisms. Farewell, Florian!

## Feature Articles

# The Banana Fusarium Wilt, Tropical Race 4, and Efforts to Combat the Disease

Shoba Sivasankar

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

The banana crop that ranks among the world's top ten staple foods is now threatened by a new race of the soil-borne pathogen, *Fusarium oxysporum*, f. sp. *cabense* (*Foc*), that causes the Fusarium Wilt disease. The combined annual production of bananas and plantains across the globe is 155 million metric tons with over 400 million people relying on bananas and plantains for food security and for income (Kema et al., 2021). About 84% of the banana crop is produced by smallholder farmers and supplied to domestic markets, while 16% of the global banana production that accounts to about 25 million metric tons is exported from tropical areas to countries in temperate zones (FAOSTAT 2020; data for 2018). Predominant in banana cultivation is the Cavendish variety which represents roughly 50% of the global production and is important both for domestic and export markets. The export banana market is almost exclusively (99%) constituted by this variety grown under monoculture conditions in large plantations.

Fusarium Wilt is considered the most important lethal disease of banana and is believed to have originated in Southeast Asia although its first report came from Australia in 1876 (Ploetz, 2006). Early reports of the disease in any given country were on damage in export plantations of the variety, Gros Michel, which was the basis of export trade in banana until the mid-twentieth century (Ploetz 2015). The disease was caused by *Fusarium oxysporum* f.sp. *cabense* (*Foc*), Race 1, and it invaded banana growing countries in Central and South America destroying plantations wreaking havoc on the export industry. The variety, Gros Michel, was susceptible to Race 1, and throughout the banana belt, production was eliminated or became increasingly difficult. The banana industry tried to hold on to Gros Michel during the first half of the twentieth century by shifting cultivation to escape the pathogen at huge socioeconomic and environmental costs. With increasing losses and reduced availability of pathogen-free soil, the banana industry turned to the Cavendish variety which was identified to be resistant to Race 1. This led to the disappearance of the disease for a while until the 1990s when Cavendish began to succumb to a new race of *F. oxysporum* f. sp. *cabense*, tropical race 4 (TR4), first in Southeast Asia and then Australia. For more than twenty years TR4 remained restricted to Southeast Asia

and the Northern Territory of Australia. However, recent reports confirm its presence in Jordan, Oman, Mozambique (2013), Lebanon, Pakistan (2015), Queensland in Australia (2015), Vietnam, Laos, Myanmar, Israel (2018), Mayotte, Indonesia, Colombia, Thailand, Turkey (2019) and Peru (2021). Although estimates are not available at the global level, figures for some countries indicate that in 2019 *Foc* TR4 affected some 15,700 ha of banana plantations in the Philippines out of a total of 440,000 ha and 70% of the plantations in Guangdong provinces and Hainan in China (Aquino et al., 2013a; Chen et al., 2013). Annual economic losses caused by *Foc* TR4 have been estimated at US \$121 million in Indonesia, US \$253 million in Taiwan, and US \$14 million in Malaysia (Aquino et al., 2013b). Currently *Foc* TR4 is reported in 27 countries across the world affecting thousands of hectares.



*FoC TR4, Peru 2021 (Photo: M. Dita)*

The *Fusarium Wilt* pathogen is soil-borne with an ability to survive for decades in the soil, thereby making it difficult to control. Surveillance, early detection, quarantine and containment are highly critical to prevent the spread of TR4. Once infected, control of the disease requires early detection and diagnosis of the presence of TR4 so that affected plants can be immediately destroyed, and on-farm restrictions can be put in place. If an infection is positive for the *Fusarium oxysporum* fungus in microscopic examination, PCR is used for an early confirmation of the disease. It confirms the identity of the fungus and determines its race identity to be TR4 or not. DNA sequencing is also done on markers of genes specific for the TR4 race. In parallel, the Vegetative Compatibility Grouping or VCG is also conducted for irrefutable confirmation of the presence of TR4.

TR4 has a wide host range and can target many cultivated bananas. Particularly sensitive are the Cavendish monoculture plantations which are central to the global production of dessert banana at present. The application of cultural and biological control options can slow down the development of epidemics, but alone do not provide effective control. The only long-term option is to deploy new varieties with effective disease resistance. The discovery of resistance to Race 1 in Cavendish during the last century helped to overcome the Race 1 epidemic, and this resistance lasted for almost half a century until the incidence of Tropical Race 4. However, the shift from Gros Michel to Cavendish in the export banana industry necessitated major adjustments in the supply chain in shipping and marketing.

Today, induced genetic resistance in Cavendish is possible by mutation induction and associated biotechnologies, and has been demonstrated by results from a coordinated research project of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture. At the same time, screening procedures are available to determine whether resistance to TR4 is prevalent in other varieties of *Musa*. A screening procedure has been developed at the IAEA laboratories to determine resistance to the fungus under controlled conditions. In addition, an early study on resistance to TR4 in 34 *Musa* cultivars under controlled conditions has reported resistance in two diploids and two polyploid groups (Chen et al. 2019). Thus, developing induced genetic resistance in Cavendish, and in parallel finding productive, consumer-acceptable varieties with resistance to TR4 are two important components of potential strategies to enhance resilience of the export-oriented banana industry as well as that of the local and regional production systems aimed at domestic markets.

The Joint FAO/IAEA Centre has a long and established track record in the application of nuclear and nuclear-derived techniques and associated biotechnologies to the genetic improvement of crop plants, including the development of

crop resistance to diseases. The history of banana research in the IAEA laboratories and relevant capacity building in Member States goes back at least two decades and addresses key techniques including cell and tissue culture, mutation induction, screening for disease and pest resistance, and related molecular and cytogenetics techniques (Jain and Swennen, 2004). In 2015, a Coordinated Research Project (CRP) was launched at NAFA-PBG specifically to address two critical diseases, the TR4 wilt in banana and the leaf rust in coffee, both capable of causing devastating damage to global production and livelihoods of those in the production chain. This CRP (D22005), *Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana*, brought together research institutions in China, Iran, Malaysia, the Philippines and South Africa, to specifically focus on building resistance to TR4 in banana. At its close in 2021, the CRP succeeded in developing important techniques for induced genetic resistance in banana and disease screening in the laboratory, green-house and field. Most importantly, China, a participant of the CRP succeeded in developing a TR4 resistant variety using induced mutagenesis (see page 8-9). Several mutant lines with potential tolerance to TR4 have also been identified by participating institutes in Iran, Malaysia and the Philippines. Protocols for key techniques in mutation breeding for banana TR4 resistance have been compiled and [published recently](#).

With the detection of TR4 in Latin America for the first time in 2019, two four-year TC projects were designed for implementation during the 2022-23 cycle and are in progress. These include *Improving the Resilience of Bananas to their Major Diseases through Mutation Breeding Techniques* (ECU5034 in Ecuador) and *Improving Banana Productivity through Mutation Breeding Techniques for Enhanced Disease Resistance* (VEN5023 in Venezuela).

Further, in late August 2021, experts and authorities of the Andean community — Bolivia, Colombia, Ecuador and Peru — reached out to the IAEA when they discovered continuing spread of the latest variation of the disease, banana *Fusarium Wilt*, Tropical Race 4 (TR4), in the region. In rapid response to this request, initial discussions with experts in the four countries of the Andean region were held during the first week of September to understand status of capacities and needs. These discussions highlighted the need for a three-pronged approach to combat the disease: (1) detection, surveillance and containment (immediate, short-term solutions); (2) epidemiology and integrated management (mid-term solutions); and (3) genetic resistance (long-term, sustainable solutions). Hence, coordinated efforts were sought within each country and in the region, involving phytopathologists, phytosanitary agents, and plant

breeders. Based on this, the draft of a new regional five-year project was developed and its workplan initially discussed at an Expert Mission in late October 2021, in La Molina, Peru, hosted by the Universidad Nacional Agraria La Molina (UNALM). It was attended by 18 participants from Bolivia, Ecuador and Peru, with Colombia participating virtually. Participant experts included phytopathologists, phytosanitary agents and plant breeders.



*Expert Mission on Banana Fusarium Wilt TR4 at the Universidad Nacional Agraria La Molina, Peru, 25-28 October 2021*

The five-year project was approved as an inter-regional project, INT5158, *Strengthening Member State Capacities to Combat Banana Fusarium Wilt TR4 through Early Detection, New Resistant Varieties and Integrated Management*. The project was officially launched by the IAEA Director General, Mr Rafael Mariano Grossi, in the first quarter of 2022 in Quito, Ecuador.

The launch of the project was accompanied by a symposium, *Global Research in the Management of Banana Fusarium Wilt TR4*, at which 19 experts from across the globe covered topics under three separate sessions, namely (1) Global Experience in *Foc* TR4 Management, (2) Detection, Epidemiology and Integrated Management of *Foc* TR4, and (3) Genetic Resistance and Banana Fusarium Wilt TR4.



*Symposium on Global Research in the Management of Banana Fusarium Wilt TR4, Ecuador, March 2022*

Full agenda of the March 2022 Symposium is on page 42- 43.

An early activity of the inter-regional project, INT5158, was the implementation of an in-person training course at the Plant Breeding and Genetics Laboratory on mutation breeding in banana and screening for TR4 resistance (14-25 February 2022). Twelve participants from Brazil, Costa Rica, Colombia, Ecuador, Peru and Venezuela participated in the training course that leveraged the historical experience of the Plant Breeding and Genetics Lab in mutation breeding of banana, and the protocols for TR4 screening in the laboratory developed as part of the recently completed CRP D22005.



*Participants of the training course at the Seibersdorf Laboratories, February 2022*

INT5158 is currently in its first year of implementation as a capacity building Technical Cooperation Project. A fundamental research project is currently in design for launch as a five year Coordinated Research Project in 2023.

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# Integrated Strategies for Improving Disease Resistance in Banana Against Fusarium Wilt

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## Project background

The banana industry is threatened by a severe disease, Fusarium wilt (FWB), which is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). *Foc* tropical race 4 (TR4) is the most virulent strain of the pathogen and can affect almost all the subgroups of dessert banana including Cavendish (AAA), the most traded fruits in the world. To date, no effective control method has been implemented to successfully manage *Foc* TR4 in susceptible cultivars. Due to its soil-borne nature, *Foc* TR4 is well adapted to survival in soil and can persist for decades in the absence of a banana host and cannot be eradicated from soil using fumigants. Chemical and biological control measures are proved to be ineffective since they do not provide long-term solutions to the problem. The most effective management practice would be to plant disease-resistant varieties. More than that, a better understanding of the factors that are responsible for disease development is needed for disease control. Hence, breeding programs for disease-resistant varieties and research on pathogenic mechanism of *Foc* TR4 have become the high priorities of our works, which are summarized in this letter.

## Collection, Preservation and Evaluation of Banana Germplasm Resources

Banana germplasm resources with high diversity are the fundamentals to breed new varieties with high resistance and fruit quality through conventional cross breeding, mutation breeding and new plant breeding techniques. The *Musa* genebank has been well-established since 2010 and currently holds more than 400 banana accessions from worldwide. The banana germplasm resources in the field nursery, in the greenhouse and in slow growth conservation are updated every two to three years, every one to two years and every six to 12 months, respectively. We further established the reliable, repeatable screening protocol for evaluation of banana genotypes for FWB resistance under greenhouse and field conditions, which comprises detailed information on planting material preparation, inoculum production, inoculation, experimental design, evaluation and data analysis.



Banana germplasm resource field nursery and germplasm bank in Guangdong province. (Photo: G. Yi)

## Radiation Mutagenesis System for Banana Disease-Resistance Breeding

Breeding and promotion of banana varieties with high resistance or tolerance to FWB is the most effective way to control the disease. Induced mutagenesis offers practical alternatives to create new varieties or novel germplasm and has become a dominant approach for breeding disease-resistant banana. We firstly established the embryogenic cell suspension cultures using immature male flowers of triploid banana (*Musa* AAA Cavendish subgroup cv. 'Baxi'), then developed the somatic embryogenesis and plantlet regeneration systems. A new banana variety, 'Zhongjiao No. 4', with improved quality and high resistance to FWB, has been created by Institution of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, through applying radiation mutagenesis approaches on embryogenic cell line of banana cultivar 'Baxi'. It has been officially released and planted on over 3, 000 hectares in Guangdong province.

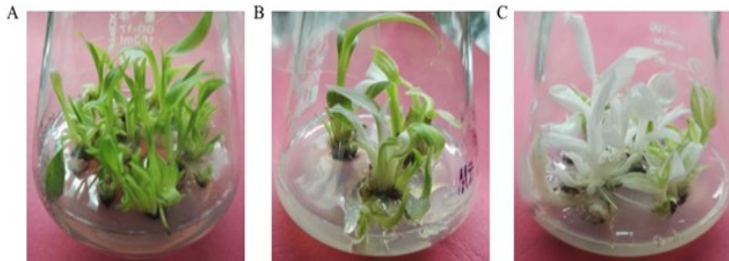


Field trials of the susceptible banana cultivar 'Baxi' and the mutant variety Zhongjiao No. 4 (ZJ4). (Photo: G. Yi.)



## Efforts Made in Cutting-Edge Technology Using Molecular Genetic Breeding System

Although disease-resistant genes were found in wild banana species, obtaining varieties with high quality and favorable flavor as Cavendish banana through conventional breeding remains a challenge. The cutting-edge breeding techniques, such as CRISPR technology, offer the opportunity to overcome the difficulties of conventional breeding by precision improvement. Firstly, we have established an efficient *Agrobacterium*-mediated genetic transformation protocol for Baxi variety using multiple bud clumps as receptor materials. This technique was awarded the Second Award of Guangdong Science and Technology Progress in 2015. Then, in 2017, we further established a CRISPR/Cas9-mediated gene-editing system for Cavendish banana. The CRISPR/Cas9 system was successfully applied to knock out the endogenous phytoene dehydrogenase gene in banana, which offer a new way for revealing gene function and disease-resistant breeding of banana.

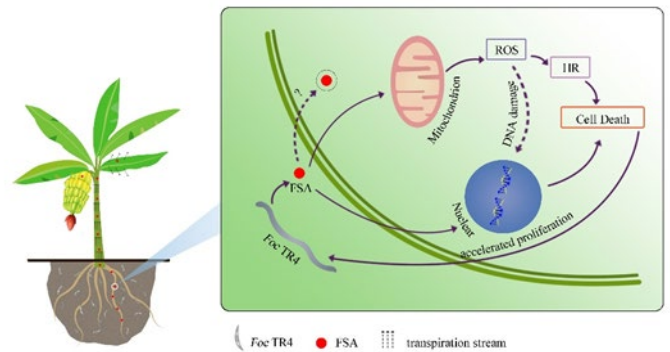


Phenotypes of CRISPR/Cas9-induced mutant banana. (A) Phenotype of non-transformed plant. (B) Phenotype of transformed plant with partial albinism. (C) Phenotype of complete albinism. (Photo: C. Hu.)

## Understanding the Pathogenic Mechanism of *Fusarium oxysporum f. sp. cubense*

Novel propositions of developing pathogen resistant plants by genome editing include altering the virulent-factor-target interaction, through editing the targeted genes. We have collected and identified the biodiversity of *Foc* strains isolated in China and other places in Asia and sequenced the genome of representative strains of *Foc* worldwide. Based on joint analysis of multi-omics, a number of virulence factors such as effector proteins and mycotoxins have been identified and systematically investigated. Among them, Fusaric acid (FSA) is a phytotoxin produced by several *Fusarium* species and other fungal pathogens. The toxicity of FSA to banana protoplasts, pseudostems and plantlets have been demonstrated, as has its accumulation in the host plants. Then we further combined cell biology, reverse genetics, RNA-Seq and disease assays to provide a comprehensive analysis of FSA function at the *Foc* TR4–

banana interface. The data reveal novel characteristics of FSA and demonstrated that FSA could act as a pioneer molecule to disturb mitochondrial functions and induce cell death, thus preparing the host for the upward invasion of *Foc* TR4



Depicting the role of (FSA) produced by *Fusarium oxysporum f. sp. cubense* tropical race 4 (*Foc* TR4) in disease development

## Future Perspectives

The scope of breeding disease-resistant banana is limited primarily due to non-availability of well characterized resistance genes. In mitigating this bottleneck several efforts aim for accessing genes from wild banana resources. More efforts should be made on the isolation and identification of disease-resistant genes based on the multi-omics analysis of various banana resources. However, transgenic bananas also faced the challenge of political, ethical and societal resistance with regulatory restrictions. In this regard, the major challenge for banana molecular breeding is to achieve gene editing without DNA delivery (DNA-free).

# Comprehensive approaches to combat Fusarium wilt of banana TR4 in China

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## Introduction

Banana is a very important cash and fruit crop in Asian and Pacific countries. It has become one of the most driving crops by market and trade in China. Currently, China is the second largest banana-producing country in the world and has a long history of banana cultivation and is also one of its centers of origin. Banana has had a great contribution in improving smallholder livelihoods especially in remote regions along the borders with Myanmar, Laos and Vietnam. In the past years, China's banana planting area has been shrinking due to disease, unfavorable weather and low price in the market. In 2017, the planting area and output dropped to 393,000 hectares and 12.5 million tons respectively. Due to factors such as Fusarium wilt of banana TR4 and cold damage, the planting area was further reduced in 2018. Now it is getting back to a more sustainable situation.

## TR4 Spread in the Greater Mekong Subregion (GMS)

The symptoms of Fusarium wilt on Cavendish cultivars were first observed in Taiwan in 1967, later the pathogen was designated as VCG 01213 (*Fusarium oxysporum* f. sp. *ubense*, Tropical Race 4: TR4) in 1989. In mainland China TR4 was first discovered in 1996 in the “Baxi” and “Guangdong No. 2” varieties in Guangdong Province. In 2001, it spread to Fujian Province and quickly it was also found in Hainan Province in 2002. Again, it spread to Guangxi Province in 2006. In 2009, TR4 was found for the first time in Mengla in Yunnan province. In GMS, extensive surveys conducted across Laos and Vietnam confirmed that TR4 is still mainly restricted to the northern regions of these countries and is limited to Cavendish cultivation (Figure 1) (Hung et al. 2017; Chittarath et al. 2017; Chittarath et al. 2022; Le Thi et al. 2022; Zheng et al. 2018).

In Thailand, within two Districts from Chiang Rai province, Phaya Mengrai and Chiang Kong, 116 and 9 TR4 infected plants were found among a total of 412,500 and 7750 plants in a 2019 survey within the FAO TCP/RAS/3619 project. In Cambodia, so far TR4 has not been detected yet within the same project period.



**Figure 1.** *Fusarium wilt of banana TR4 destroyed Cavendish banana plantation in Laos*

Presently, this devastating disease is almost everywhere in Chinese banana plantations. TR4 causes serious problems, particularly for smallholder farmers, both in banana plantations in sloping and flat lands. Fusarium wilt is a kind of vascular wilt disease. Its pathogen spores infect roots of susceptible banana cultivars and hyphae grow into the vascular system of the plant that eventually (in)directly occlude the xylem vessels, causing wilting and finally collapse of the plant. The spores can survive in the soil for a few decades and spread via the movement of people, planting materials, vehicles etc.

## TR4 Comprehensive Management

Comprehensive approaches for containment of TR4 have been adopted in China including breeding for resistant varieties, somaclonal selections, chemical mutagenesis and molecular breeding technologies to develop new resistant varieties. In the meantime, beneficial microorganisms together with cover crops and organic fertilizers are intensively applied in banana plantations to suppress TR4 (Fan et al. 2021; Li et al. 2021; Wei et al. 2020; Yuan et al. 2021). The mechanisms of tritrophic biocontrol interactions of beneficial microorganisms, TR4 and bananas linking above and below ground were also explored (He et al. 2021; Mon et al. 2021). Using resistant varieties is the best way to suppress TR4 infection. In China, several institutes such as Guangdong Academy of Agricultural Sciences (GDAAS), Guangxi Academy of Agricultural Sciences (GAAS), Yunnan Academy of Agricultural Sciences (YAAS) and

Chinese Academy of Tropical Agricultural Sciences (CATAS) use conventional breeding or somaclonal variations to develop resistant varieties to adapt to different ecological conditions. Some resistant varieties such as Nantianhuang, Baodaojiao, Guijiao No.9, Zhongjiao No.4 are already widely utilized in different locations of banana plantations. Organic fertilizer together with beneficial microorganisms are intensively applied to suppress this soil-borne disease. One successful example is from Jiangcheng Shenglong banana plantation in Yunnan province. This plantation has more than 300 hectares under banana cultivation without Fusarium wilt disease symptoms presently, although more than 200 plants had shown typical symptoms of TR4 during 2008 and 2009 (Figure 2) (Mon et al. 2021).



**Figure 2.** Banana plantation with organic fertilizer and beneficial microorganism applications in Yunnan China

The best strategy to control TR4 in Asia is a multiple stakeholder approach bringing together governmental agents, NGOs, scientists and banana farmers to work on two aspects. First is the prevention and management strategy: early warning system through diagnosis in areas where epidemics are occurring and (adjacent) areas where the pathogen is not yet found for surveillance, containment, eradication and exclusion of banana Fusarium wilt. In non-infected areas, clean starting materials using tissue culture plants are highly recommended. In the nursery process, fresh and TR4-free soil are absolutely needed in order to prevent the infection of banana plantlets before planting.

Furthermore, disinfection treatment of banana trucks is also necessary when crossing borders. For infected areas, containment measurements are extremely useful for slowing down the spread of TR4, and for this training workshops for local agricultural extension staffs and banana farmers are important. The second path is in support of research, to develop resistant cultivars by conventional or molecular breeding, cultivar substitution, clonal selection, verification of the effect of cultivar mixtures, cover plants, intercropping system, e.g., Chinese leek, pineapple and different types of beans, crop rotation, ecological intensification, and analysis

of the plant microbiome in soil (Figure 3) (Fu et al. 2017; Xue et al. 2015; Yuan et al. 2021).



**Figure 3.** Banana plantation with cover crop such as *Macrotyloma uniflorum* (Lam.) Verdc in Yunnan China

Comprehensive approaches combined with TR4 evaluation, utilization of local banana accessions and breeding new varieties adapted to local ecological conditions in Asia will be the future direction to combat TR4.

This soil-borne pathogen has recently been proven to reach the peduncle, which will have an additional threat to banana growers and traders (Figure 4) (Bai et al. 2020).

Different strategies should be implemented in TR4 absent and present regions. For countries that already have TR4, it is recommended that resistant varieties are combined with beneficial microorganisms, elicitors and cover crops for banana sustainable production in the presence of TR4.



**Figure 4.** TR4 infected peduncle in fruit setting plant in Yunnan China

## Future Perspective

Breeding resistant varieties is the most effective way to combat Fusarium wilt of banana. Replacement of Gros Michel with Cavendish to combat Fusarium wilt of banana Race 1 is an important example for the role of resistant varieties in maintaining sustainable production. However, history repeats itself with the breakdown of the resistance of Cavendish under TR4. Somaclonal selection from current varieties in TR4 infected field, and mutation breeding are good options to combat TR4. Evaluation and selection of local landraces with resistance are midterm. Gene mining from wild resistant sources to confer genetic resistance is a longer-term outlook for breeding programs. Alongside, it is important to manage the movement of people and plant materials through borders with quarantine regulations. Designing novel banana production systems with intercropping using cover crops such as beans, leeks etc. or tree crops such as coffee, could be explored in the future. The effect of these novel intercropping systems on disease incidence and soil microbial diversity are suggested to be monitored further.

## Acknowledgements

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

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Mchare bananas (Figure 1), also referred as Muraru, Mlali, or Mshale, are diploid (AA) cooking bananas prized by the people of the Northern Tanzanian highlands and its neighboring countries including Kenya and DR Congo. These bananas differ dramatically in texture and in genetic background from the more familiar East African highland triploid (AAA) cooking bananas such as Matooke. In the regions of Kilimanjaro and Arusha Mchare bananas can provide up to 30% of the caloric intake and they bring a premium price in the local markets. Importantly, due its close phylogenetic relationship with Cavendish and Gros Michel, improved Mchare diploids could be a potential donor to other bananas particularly Cavendish (AAA).



**Figure 1.** Shapes and bunch sizes of different Mchare cultivars

Mchare cultivars are susceptible to almost every major pest and pathogen of banana in Africa including *Fusarium oxysporum* f. sp. *cubense* (Foc) races 1 and Tropical Race 4 (TR4). Foc race 1 is endemic to East and Central Africa while Foc TR4 has been detected in northern Mozambique in 2014. The presence of Foc TR4 in Mozambique is a threat to the Tanzania banana industry. Being soil-borne, Foc race 1 and TR4 cannot be controlled by fungicides, therefore prevention and replacement of susceptible with resistant varieties through breeding remains essential components for effective control of this devastating disease. Following pot trials conducted under contained greenhouse conditions at the Plant Breeding and Genetics Laboratory, the susceptibility of Mchare banana to Foc TR4 was demonstrated (Figure 2).



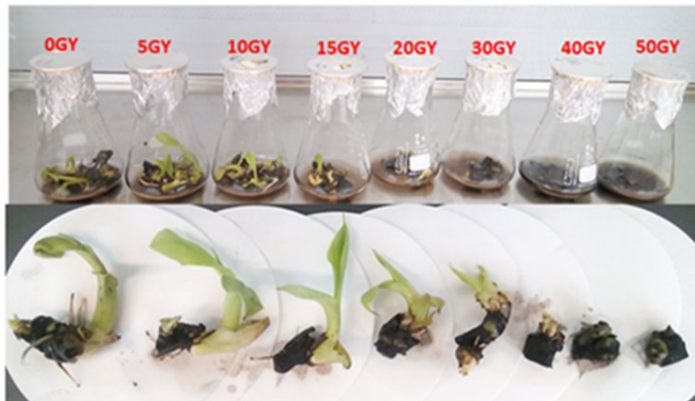
**Figure 2.** Susceptibility of Mchare to *Fusarium oxysporum* f. sp. *cubense* TR4 (inoculated plants left vs control, non-inoculated plants, right)

Conventional plant breeding is an extremely important tool in banana breeding but has its limitations. These include reduced fertility, parthenocarpy, long life cycle and large plant size of bananas. About 17 years are needed to breed improved East African highland bananas through conventional plant breeding methods.

The combined use of *in-vitro* culture and induced mutagenesis through irradiation or chemical agents is an established technique that could shorten the time required to improve bananas for Fusarium wilt resistance. Under the Peaceful Use Initiative, a breeding project was initiated aimed at improving Mchare bananas for resistance to Fusarium wilt through induced mutagenesis. The project is carried out jointly between the Laboratory, the International Institute of Tropical Agriculture (IITA), Tanzania and Stellenbosch University, South Africa. In the initial phase, two Mchare banana varieties were transferred from the field to *in vitro* conditions, rendered disease-free, and multiplied *in vitro* prior to shipping to the Plant Breeding and Genetics Laboratory. Additional germplasm was secured through the Bioversity International Musa Transit Centre, Belgium.

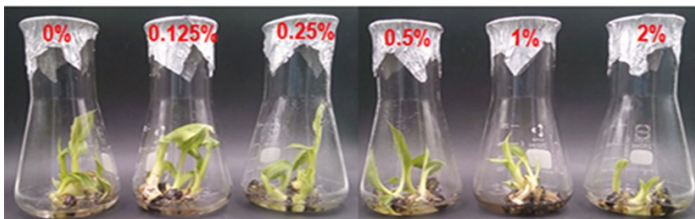
At the Laboratory, radiosensitivity experiments were carried out using shoot tip explants of Mshale and the shoots allowed to regenerate in liquid MS medium. After four weeks, fresh weight, survival rate, shoot height and numbers of leaves were assessed. Growth was observed from a dose of 5 Gy up to 30 Gy while 40 and 50 Gy were inhibitory (Figure 3). Also, dose of 5 Gy was observed to stimulate

growth compared to the control. The LD<sub>30</sub> dose was attained at 10 Gy and this dose was used for bulk mutagenesis.



**Figure 3.** Radiosensitivity experiment showing reduction in weight at increasing doses of gamma rays

Similarly, EMS toxicity test experiments were carried out using five different EMS concentrations (0.125, 0.25, 0.5, 1 and 2 %) and a control (0%). After four weeks growth in liquid medium, the fresh weight was taken and percentage of weight in relation to the control calculated. A reduction in weight and height were observed as EMS concentration increases (Fig 4). A dose of 0.125% EMS concentration stimulated growth compared to the control. The LD<sub>30</sub> dose was attained at 1.5% EMS.



**Figure 4.** EMS dose-response experiment showing decreased plant height with increasing EMS concentration

For bulk mutagenesis, 2,000 shoot tips were treated with 10 Gy of gamma-rays and another 2,000 shoot tips with 1% EMS. Currently, the 4,000 treated meristems are being multiplied to obtain circa 16,000 M<sub>1</sub>V<sub>3</sub> plantlets (Fig 5). A first batch of 1,500 M<sub>1</sub>V<sub>3</sub> plants has been shipped to IITA, Tanzania. This mutant population will be established in replicated field trials at the IITA, Tanzania for phenotyping and field-based Fusarium wilt resistance screening.



**Figure 5.** Development of Mchare mutant populations

## Forthcoming Events

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

Third Research Coordination Meeting (RCM) on Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change, D23032, Kuala Lumpur, Malaysia, 25–29 July 2022. (More information on page 19)

First Research Coordination Meeting (RCM) on Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development, D24015, Vienna, Austria, 7-11 November 2022. (More information on page 19)

Second Research Coordination Meeting (RCM) on Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops, D24014, Vienna, Austria, 14-18 November 2022. (More information in page 19).

### Other Meetings

Consultants Meeting on Enhanced Crop Adaptation to Climate Change for Food and Nutrition Security and Farmer Income, Vienna, Austria, 1-5 August 2022 (More information on page 23).

National Training Course on Improving Crop Adaptation to Drought Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods, Asmara, Eritrea- ERI5013 7- 17 June 2022 (More information on page 23)

Regional Training Course on Mutation Breeding on Vegetatively Propagated Crops- RLA5084 (*Virtual*), 20-24 June 2022 (More information on page 23)

Regional Training Course on Molecular Techniques for Crop Improvement of Mutant Populations- RER5024 (*Virtual*), 20-24 June 2022 (More information on page 23)

National Training Course on Mutation Breeding Techniques and Crop Improvement- MAU5009, Nouakchott, Mauritania, 4-8 July 2022 (More information on page 24)

Regional Meeting on Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia- RER5024, Faro, Portugal, 4-8 July 2022 (More information on page 24)

National Training Course on Polyploid Induction as a Breeding Strategy for Creole Potatoes- COL5026, Bogota, Colombia, 11-15 July 2022 (More information on page 24)

National Training Course on Introduction to Mutation Breeding in Crops, Port-au-Prince, Haiti, 11-15 July 2022 (More information on page 24)

Regional Training Course on Plant Mutation Breeding and Associated Biotechnologies in Seed Propagated Crops at National Research and Innovation Agency (BRIN), Jakarta, Indonesia, 18-29 July 2022 (More information on page 25)

National Training Course on SDS-PAGE Screening for Protein Quality in Mutant Lines- SUD5041, Gezira, Sudan, 25-29 July 2022 (More information on page 25)

National Training Course on Improving Crop Adaptation to Abiotic Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods- LIR5003, (*Virtual*), 1-12 August 2022 (More information on page 25)

Regional Training Course on Statistically Rigorous, Pre-field and Field Screening to Select Improved Mutant Lines- RAF5083, Dakar, Senegal, 8-19 August 2022 (More information on page 25)

Regional Training Course on Mutation Induction and Mutation Breeding- RAS5098, Suva, Fiji, 15-26 August 2022 (More information on page 26)

Regional Training Course on Application of Genomics, Genotyping and Marker-Assisted Selection in Mutation by Speed Breeding (MbyS)- RAS5088, (*Virtual*), 5-9 September 2022 (More information on page 26)

National Training Course on Selection for Biochemical Traits with Improved Quality and Yield in Mutant Rice and Cassava Lines-SIL5021, (*Virtual*), 5-9 September 2022 (More information on page 26)

Advance Regional Training Course on Mutation Breeding and Combined Biotechnologies, International Center for Biosaline Agriculture (ICBA), United Arab Emirates, 12-16 September 2022 (More information on page 26)

Regional Training Course on In vitro Techniques for the Application of Induced Mutations on Vegetatively Propagated Crops- RER5024. Ghent, Belgium, 12-23 September 2022 (More information on page 27)

National Training Course on Mutation Techniques, Mutation Discovery, Marker Development and Market Assisted Selection- NEP5006, Kathmandu, Nepal, 26-30 September 2022 (More information on page 27)

Midterm Project Review Meeting on Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)- RAF5083, Vienna, Austria, 26-30 September 2022 (More information on page 27)

Regional Training Course on Plant Mutation Breeding and Efficiency Enhancing Techniques to Increase Resilience to Climate Change- RAS5099, RER5024, Seibersdorf, Austria, 26 - 7 October 2022 (More information on page 28)

Regional Training Course on Mutation Breeding in Vegetatively Propagated Crops, including Micropropagation using Cell/Tissue Culture, Mutation Induction and Selection- RAF5083, Nairobi, Kenya, 24 October to 4 November 2022 (More information on page 28)

Regional Training Course on Application of Double Haploidy for Mutation by Speed Breeding (MbyS) Towards

Crop Improvement- RAS5088, Jakarta, Indonesia, 14-18 November 2022 (More information on page 28)

Regional Training Course on Methodologies for Improving Crop Resilience to Abiotic Stress Through Nuclear Techniques and Seed Systems- RAS5099, Kuwait Institute for Scientific Research (KISR), Kuwait, 20-24 November 2022 (More information on page 29)

## Past Events

### Other Meetings

Regional Training Course on Mutation Breeding and Efficiency Enhancing Techniques for Resistance to Banana Fusarium Wilt Race TR4 in Latin America, 14-25 February 2022, Seibersdorf, Austria (More information on page 29)

Regional meeting on Improving the Resilience of Crops to Climate Change through Mutation Breeding — Phase II (SAPI) - RAS5098, (*virtual meeting*) 15-16 February 2022. (More information on page 29)

Symposium on “Global Research in the Management of Fusarium Wilt, TR4”, March 2022, Quito, Ecuador (Agenda is on pages 42-43)

Scientific exchange to BOKU University in the frame of Coordination Research Project D24014, Vienna, Austria, 4 March 2022 (More information on page 30)

Regional Training Course on Double Haploidy for Mutation by Speed Breeding (MbyS) towards Crop Improvement for its RCA Regional Asia and Pacific Member States; RAS5088 (*Virtual Meeting*), 15-16 March 2022 (More information on page 30)

National Training Course on Mutants Screening for Yield, Drought and Nutritional Quality of Groundnut and Soybean – MALW5005 (*Virtual Meeting*), 11–15 April 2022. (More information on page 30)

National Training Course on on Screening Methods of Tolerance to Water Stress in Roots and Tubers in Nicaragua – NIC5011, 9-13 May 2022. (More information on page 31)

National Training course to enhance capacities on participatory plant breeding – CUB5023, (*Hybrid Meeting*), 16-20 May 2022. (More information on page 31)



## Coordinated Research Projects (CRPs)

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

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

Project Officer: S. Sivasankar

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

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

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

also revealed that the short duration, high yielding rice variety, 'Binadhan-7', plays a vital role in hunger mitigation of the northern areas of Bangladesh.

A second publication with more information on the impact assessment has been submitted and is in review.

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

Project Officer: L. Jankuloski

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

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

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

The CRP is extended for one more year and planned to be completed by the end of 2022.

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

Project Officer: L. Jankuloski, N. Warthmann

A range of diseases are responsible for hindering yield improvements in both rice and wheat. Changing climatic conditions are helping diseases to spread to new localities and exacerbating their impact. In addition to the already widespread diseases of rice, such as blast, sheath blight, false smut and bacterial leaf blight, emerging diseases like wheat blast are increasingly becoming serious threats. To minimize the impact of such diseases, environmentally friendly and cost-effective technologies are needed to help prevent and manage them. The use of mutation breeding techniques is a viable tool in the development of disease resistant germplasm and varieties. Mutations are a primary source of genetic variation in any organism, including plants. The use of mutation induction in generating new germplasm and developing new disease resistant varieties in rice and wheat is an efficient and valuable approach in crop improvement and has been very successful in rice and wheat breeding. More than 820 rice varieties and 255 wheat varieties have been developed by mutation breeding using mostly physical mutagens (<https://mvd.iaea.org>).

Progress made on planned activities since 2018 is satisfactory and even excellent in certain projects, towards the goals set during the first RCM and according to individual workplans.

Some important achievements are (1) the development of specific primers for *Magnaporthe oryzae* Triticum isolates and Recombinase Polymerase Amplification (RPA) rapid detection method of wheat blast pathogen, and (2) the identification of five blast resistant wheat mutant lines in M<sub>3</sub> generation. In addition, six wheat orthologs of rice blast susceptible genes were identified uncovering a total of 171 SNPs in M<sub>3</sub> wheat population.

In rice, three highly resistant and two moderately resistant mutant lines to Bacterial Leaf Blight (BLB) were identified in M<sub>3</sub> generation. The development of F<sub>2</sub> generation for phenotyping is in progress. Additionally, two mutant lines showing significantly lower infection of false smut disease in field hotspot (plus artificial inoculation) were identified and four rice mutant lines as putative mutants resistant to bakanae disease (*Gibberella fujikuroi*) were selected.

The network and collaboration among CRP participants are strong, and seed materials from wheat mutant populations and lines were already exchanged for wheat blast phenotyping in Bangladesh. The further collaboration will be established for genotyping of mutant lines as well as F<sub>2</sub> generations.

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

be held in July 2022 in Kuala Lumpur, Malaysia where the progress will be reviewed, and future activities will be discussed among participants.

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

Project Officer: A. Hingane, S. Sivasankar

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

Specific research objectives are (1) to generate genetic diversity in chickpea, cowpea and lentil through mutagenesis for resistance to *Helicoverpa armigera*, *Maruca vitrata* and *Stemphylium botryosum*, respectively; (2) to develop and/or refine phenotyping tools to facilitate precise and efficient selection of biotic-stress resistance in selected pulse crops; and (3) to develop genomic tools for accelerated variety development for the selected pulse crops and associated traits of interest.

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

### Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops, D24014

Project Officer: K. Bimpong, S. Sivasankar

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

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

The CRP aims to develop new genetic resources and technologies for accelerated breeding in VPCs and HTCs

through induced genetic diversity, chimera-free regeneration, and functional genomics.

The CRP 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.

## Forthcoming Events

### *Third Research Coordination Meeting (RCM)* **Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change, D23032**

*Kuala Lumpur, Malaysia. 25–29 July 2022*

Project Officer: L. Jankuloski, N. Warthmann

This CRP aims to improve disease resistance in rice and wheat through induced genetic variation and mutation breeding and the development of screening techniques for disease resistance. Specific research objectives are (1) to generate genetic diversity and develop rice lines resistant to important diseases (blast, sheath blight, bacterial blight, and false smut); (2) to develop protocols for the screening of rice mutants resistant to the target diseases; (3) to develop molecular markers for disease resistance using available mutant germplasm; and (4) to generate mutant wheat populations and develop screening methods for resistance to wheat blast.

The CRP officially started in September 2018 and had its first RCM in December 2018 in Vienna, Austria. The second RCM was held virtually in April 2021 and the third RCM is planned to be held in July 2022 in Kuala Lumpur, Malaysia.

### *First Research Coordination Meeting (RCM)* **Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development, D24015**

*Vienna, Austria. 7-11 November 2022*

Project Officer: S. Sivasankar, C. Zorrilla

CRP D24015 was approved in October 2021 and has opened calls for proposals from mid-October to mid-December. It is expected to launch in March 2022 with its first Research Coordination Meeting, likely in virtual mode.

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

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

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

### *Second Research Coordination Meeting (RCM)* **Development of Integrated Techniques for Mutation Breeding in Vegetatively Propagated and Horticultural Tree Crops, D24014**

*Vienna, Austria. 14-18 November 2022*

Project Officer: K. Bimpong, S. Sivasankar

The CRP is using both induced genetic variation and functional genomics technologies to address constraints associated with vegetatively propagated and horticultural tree crops.

The research focus is to induce genetic diversity, chimera-free regeneration, and functional genomics to accelerate breeding in cassava, olive and potato/sweet potato. In addition, the CRP also aims to develop disease-resistant and stable clones of mutants for cassava brown streak disease olive quick decline syndrome and any important diseases in potato/sweet potato. The CRP started in 2020 and had its first virtual RCM from 16 to 20 August 2021.

## Technical Cooperation Field Projects

Project Number	Country/Region	Title	Technical Officer(s)
BKF5019	Burkina Faso	Improving Food Crop Genotypes for Enhancing Yield and Adaptation to Climate Change Using Mutation Breeding and Isotopic Techniques	L. Jankuloski <i>and</i> SWMCN
BOT5019	Botswana	Improving Selected Legumes and Cereals against Biotic and Abiotic Stresses to Improve Food Production and Security	I.K. Bimpong
BUL5016	Bulgaria	Improving the Productivity and Quality of Economically Important Crops through Mutation Breeding and Biotechnology	C. Zorrilla
CAF5013	Central African Republic	Improving Productivity of Maize and Developing Resistant Armyworm Maize Varieties Using Radio-Mutagenesis Techniques	S. Sivasankar
CHI5052	Chile	Using Nuclear Techniques to Improve the Adaptation and Productivity of Forest Species Facing Climate Change	S. Sivasankar
COL5026	Colombia	Enhancing Crop Productivity of Creole Potato Using Nuclear and Related Techniques	I.K. Bimpong <i>and</i> SWMCN
CPR5024	China, People's Republic of	Enhancing the Accelerated Application of Mutant Germplasm and High-Efficiency Breeding in Crops	N. Warthmann
CUB5023	Cuba	Strengthening National Capacities for the Development of New Varieties of Crops through Induced Mutation to Improve Food Security While Minimizing the Environmental Footprint	C. Zorrilla <i>and</i> SWMCN
ERI5011	Eritrea	Developing Improved Banana and Maize Varieties through Mutagenic Nuclear Techniques	I.K. Bimpong
GHA5038	Ghana	Using Irradiated Pollen for the Development of Provitamin A Rich, Drought Tolerant and Cassava Mosaic Disease Resistant Cassava Mutants	C. Zorrilla
HON5009	Honduras	Improving Genetic Resistance of Coffee to Coffee Leaf Rust through Mutation Breeding	L. Jankuloski
INS5044	Indonesia	Using Nuclear Technology to Support the National Food Security Programme	S. Sivasankar <i>and</i> SWMCN
IRA5015	Iran, Islamic Republic of	Enhancing Capacity of National Producers to Achieve Higher Levels of Self-Sufficiency in Key Staple Crops	L. Jankuloski in collaboration with FSC <i>and</i> SWMCN
IRQ5023	Iraq	Utilizing Nuclear Technology to Improve Key Legume Crops for Climate Change Adaptation	I.K. Bimpong
IVC5040	Cote d'Ivoire	Improving Agricultural Production of Maize, Rice and Cassava through Cultivation of Induced Mutant Adaptable to Climatic Changes	I.K. Bimpong
JAM5013	Jamaica	Improving Crops by Using Experimental Mutagenesis and Diagnostic Technologies	S. Sivasankar
JAM5014	Jamaica	Establishing a Self-Contained Gamma Irradiation Facility for the Introduction of Sterile Insect Technique and Experimental Mutagenesis and Diagnostic Technologies	S. Sivasankar <i>and</i> IPC

Project Number	Country/Region	Title	Technical Officer(s)
KEN5038	Kenya	Using Nuclear Techniques to Evaluate and Improve the Impact of Mutated Forages on the Performance of Smallholder Dairy Cows	I.K. Bimpong <i>and</i> APH
KUW5005	Kuwait	Implementing Mutation Induction to Improve Barley Production under Harsh Environmental Conditions – Phase III	L. Jankuloski
MAG5026	Madagascar	Enhancing Rice and Maize Productivity through the Use of Improved Lines and Agricultural Practices to Ensure Food Security and Increase Rural Livelihoods	L. Jankuloski in collaboration with SWMCN <i>and</i> IHS
MAL5032	Malaysia	Strengthening National Capacity in Improving the Production of Rice and Fodder Crops and Authenticity of Local Honey Using Nuclear and Related Technologies	C. Zorrilla in collaboration with FSC <i>and</i> SWMCN
MLW5003	Malawi	Developing Drought Tolerant, High Yielding and Nutritious Crops to Combat the Adverse Effects of Climate Change	C. Zorrilla <i>and</i> SWMCN
NAM5017	Namibia	Improving Crops for Drought Resilience and Nutritional Quality	C. Zorrilla <i>and</i> SWMCN
NEP5006	Nepal	Enhancing Productivity of Corps and Fruit Employing Nuclear and Molecular Techniques	I.K. Bimpong
NER5024	Niger	Improving Key Staple Crops towards Food Security	S. Sivasankar
NIC5011	Nicaragua	Broadening the Genetic Variation of Vegetative Propagated Crops Using Nuclear Techniques	C. Zorrilla
PAR1005	Paraguay	Establishing National Capabilities in Irradiation Technologies for the Treatment of Patients with Burns and the Introduction of Mutation Breeding for Enhanced Quality and Productivity of Crops	S. Sivasankar collaboration with RPRT <i>and</i> ARBR
PER5034	Peru	Improving Yellow Potato and Coffee Crops through Mutation Breeding Techniques	L. Jankuloski
PRC5002	Congo, Republic of the	Developing Disease-Resistant Varieties of Cassava and Banana	I.K. Bimpong
QAT5008	Qatar	Developing Best Soil, Nutrient, Water and Plant Practices for Increased Production of Forages under Saline Conditions and Vegetables under Glasshouse Using Nuclear and Related Techniques	A. Hingane <i>and</i> SWMCN
RAF5083	Regional Africa	Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)	S. Sivasankar/I.K. Bimpong
RAS0080	Regional Asia	Promoting Self-Reliance and Sustainability of National Nuclear Institutions	S. Sivasankar in collaboration with PCG, RPRT <i>and</i> PHY
RAS5077	Regional Asia	Promoting the Application of Mutation Techniques and Related Biotechnologies for the Development of Green Crop Varieties (RCA)	I.K. Bimpong
RAS5079	Regional Asia	Improving Crop Resilience to Climate Change through Mutation Breeding in Pacific Islands	C. Zorrilla
RAS5088	Regional Asia	Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA)	S. Sivasankar/I.K. Bimpong

Project Number	Country/Region	Title	Technical Officer(s)
RER5024	Regional Europe	Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia	C. Zorrilla
RLA5084	Regional Latin America	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	S. Sivasankar in collaboration with SWMCN and APH
RWA5001	Rwanda	Improving Cassava Resilience to Drought and Waterlogging Stress through Mutation Breeding and Nutrient, Soil and Water Management Techniques	L. Jankuloski and SWMCN
SAF5016	South Africa	Promoting Mutation Breeding of Vegetables to Improve Rural Livelihoods — Phase I	N. Warthmann
SIL5021	Sierra Leone	Improving Productivity of Rice and Cassava to Contribute to Food Security	I.K. Bimpong and SWMCN
SRL5050	Sri Lanka	Supporting Genetic Improvement of Tea	S. Sivasankar
SWA5002	Eswatini, Kingdom of	Improving Adaptability of Cowpea to Climate Change through Mutation Breeding	L. Jankuloski
SYR5026	Syrian Arab Republic	Using Accelerated Mutation Breeding of Staple Crops for Enhanced Resilience to Climate Change through Speed Breeding, Phenotyping and Genotyping	L. Jankuloski
TOG5002	Togo	Improving Crop Productivity and Agricultural Practices through Radiation Induced Mutation Techniques	I.K. Bimpong and SWMCN
TUN5029	Tunisia	Developing Barley and Durum Wheat Resilience to Drought and Heat Tolerance through Mutation Breeding	A. Hingane
UGA5041	Uganda	Developing Disease Resistant High Yielding Farmer Preferred Cassava Varieties in Uganda through Induced Mutation Breeding	I.K. Bimpong/ L. Jankuloski
URT5037	Tanzania, United Rep. of	Developing Rice Varieties with Resistance to Rice Blast and Salinity Tolerant Using Mutation Breeding and Biotechnology Techniques	L. Jankuloski
YEM5015	Yemen	Enhancing Sorghum and Legume Crop Productivity through Induced Mutations with Supportive Breeding and Biotechnologies	L. Jankuloski
ZAI5029	Congo, Democratic Republic of the	Enhancing Crop Productivity of Soybean and Maize through Improved Mutant Varieties and Lines	I.K. Bimpong

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

## Forthcoming Events

### *Consultants Meeting*

#### **Enhanced Crop Adaptation to Climate Change for Food and Nutrition Security and Farmer Income**

*Vienna, Austria, 1-5 August 2022*

Project Officer: C. Zorrilla

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

Disease management requires a team with multidisciplinary capabilities to approach the problem from diverse angles. Finding ways to precisely evaluate the response of different genotypes to the pathogen for an effective determination of the resistance/susceptibility, precisely identifying the race of the pathogen; as well as finding methodologies to prevent infection and control the dispersal of the pathogen.

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

Call for proposals are expected in late 2022; and the start of the CRP in early 2023.

### *National Training Course*

#### **Improving Crop Adaptation to Drought Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods- ERI5013**

*Asmara, Eritrea, 7-17 June 2022*

Project Officer: K. Bimpong

The training objective is to enhance the understanding and research capabilities of the participants in basic principles in crop mutation breeding and basic molecular techniques for improved mutation detection and selection. The course will cover topics such as mutation breeding, concepts and methodologies, including invitro techniques of mass propagation of plantlets for vegetatively propagated crops, optimization of irradiation treatments, lab, greenhouse 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. The course is design for participants who have basic knowledge in mutation breeding techniques. Fifteen participants are expected to join the training course.

### *Regional Training Course*

#### **Mutation Breeding on Vegetatively Propagated Crops- RLA5084**

*Virtual. 20-24 June 2022*

Project Officer: C. Zorrilla, S. Sivasankar

This project aims to raise awareness among member states of the various applications of nuclear science and technology in agriculture as well as develop their human resource and institutional capacity in the use of these technologies. Many crops of relevance in this region are vegetatively propagated such as taro, cocoyam, pineapple, papaya, banana, sugar cane, among others.

Mutation breeding is not widely used in the Caribbean. A total of ten Caribbean countries participate in this project; from them, Jamaica is the only one actively applying mutation breeding to improve vegetatively propagated crops. Representatives from Guyana, Jamaica, St. Lucia, St. Vincent and the Grenadines, and Trinidad and Tobago will attend.

The purpose of this training is to develop capacities on mutation induction, radiosensitivity tests, mutation breeding and micropropagation techniques; as well as get acquainted of the challenges and opportunities of dealing with vegetatively propagated crops. The training will include lectures, case studies, audio-visual material, group discussions and invited speakers.

### *Regional Training Course*

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

*Virtual. 20-24 June 2022*

Project Officer: C. Zorrilla, S. Sivasankar

In recent years, the negative impact of climate change has become evident in agriculture. Environmental stresses seriously limit plant growth, crop yield and disease resilience. Increasing genetic diversity can play an important role in addressing the problems arising from unfavourable environmental conditions.

The main objective of this project is to improve yield and resilience to climate change of major food crops in Europe and Central Asia by inducing new genetic variation with nuclear techniques. This project includes a total of 26 countries. Some of them have experience applying mutation breeding to develop new improved varieties while others are starting to implement these techniques.

The purpose of the event is to develop capacities on molecular techniques that can be applied in a mutation breeding program; to expose participants to practical cases where QTL mapping methods and different types of populations have been applied in mutation breeding; and to develop expertise in basic data analysis related to the use of molecular techniques to identify the genomic regions that control important traits using mutant or mutant-derived populations. This training will provide theoretical (lectures) and practical demonstrations (software use) related molecular techniques (molecular markers and genomics) that can be used in conjunction with nuclear induced mutations for genetic improvement.

*National Training Course*  
**Mutation Breeding Techniques and Crop Improvement, MAU5009**

*Nouakchott in Mauritania, 4-8 July 2022*

Project Officer: K. Bimpong

The training objective is to enhance the understanding and research capabilities of the participants in basic principles in crop mutation, plant breeding schemes, selection methods for targeted stresses/traits, which will contribute to the improvement of process efficiencies for the selection of improved varieties, modalities for informal and formal seed systems for multiplication and dissemination of seed for upscaling and cultivation.

The training course will cover topics such as mutation induction, breeding schemes, early and late generation selection methods for targeted stresses, multi-location and farmer field trials, introductory 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 seed 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 design for researchers, plant breeders who are working on mutation induction of cereals (rice and sorghum). Fifteen participants are expected to join the training.

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

*Faro, Portugal, 4-8 July 2022*

Project Officer: C. Zorrilla, S. Sivasankar

The aim of the project is to strengthen the capacities in the region to develop high yielding, resilient varieties with improved nutritional quality in Europe and Central Asia by using nuclear technologies to generate new beneficial

mutations followed by mutation breeding. Application of induced mutations together with appropriate screening and biotechnological methods will contribute to the development of new improved varieties of cereals, fruits, and vegetables in the region.

This meeting is open to designated counterparts of the project RER5024. A total of 18 researchers, from 16 countries (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Georgia, Greece, Kazakhstan, Kyrgyzstan, Montenegro, North Macedonia, Poland, Portugal, Russia, Serbia, Slovakia, Slovenia, Tajikistan, Turkey, and Uzbekistan) will participate in the meeting.

The purpose of the meeting is to discuss, share experience and report on the progress made and achievements reached on mutation breeding activities conducted by Member States, as well as to identify and address encountered challenges. The workplan of the project will be reviewed, and arrangements will be made for future project activities.

This event will be hosted by the Universidade do Algarve in Faro - Portugal. Participants will have the opportunity to do technical visits to agricultural companies in the Algarve region and visit the installations of the University.

*National Training Course*  
**Polyloid Induction as a Breeding Strategy for Creole Potatoes- COL5026**

*Bogota, Colombia, 11-15 July 2022*

Project Officer: K. Bimpong

The training objective is to enhance the understanding and research capabilities of the participants in basic principles in mutation breeding in polyploids and advanced molecular techniques for improved mutation detection and selection with emphasis on Creole potatoes.

The main theme of the training will include mutation breeding, *In-vitro* techniques for mass propagation of plantlets, introductory molecular biology, molecular markers and genome variations, genomics and transcriptomics, linkage analysis, QTL mapping, genome wide association analysis, marker assisted selection in Creole potatoes and introduction to bioinformatics.

Targeted participants are 15 researchers, traditional breeders who work with creole potato as well as breeders from other groups working on mutation induction of rice.

*National Training Course*  
**Introduction to Mutation Breeding in Crops**

*Port-au-Prince, Haiti, 11-15 July 2022*

Project Officer: K. Bimpong

This is the first mutation breeding programme for the country; hence the objective of the training is to sensitive the participant on the importance of nuclear techniques in food and agriculture.



The main theme of the training course will be introductory to mutation breeding techniques, induced genetic variation, mutagenesis, preparation of radiosensitivity curves, basic breeding methods and introductory molecular biology. Participants will also be introduced to basics of molecular biology, linkage mapping, variant discoveries and marker assisted selection in plant breeding. Targeted participants are 15 researchers with no experience in mutation breeding.

#### *Regional Training Course*

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

*Jakarta, Indonesia, 18–29 July 2022*

Project Officer: L. Jankuloski

The training course will be implemented under the regional project RAS5094. The purpose of the event is to provide training in plant mutation breeding and associated biotechnologies in seed propagated crops to participants from ASEAN countries, including Pakistan, Bangladesh and Mongolia that are involved in the regional TC Project RAS5094.

The overall objective of RAS5094 is to enhance the capacities in ASEAN countries in promoting sustainable agriculture and food productivity. The expected outcome is to strengthen human capacity through training of young scientists involved in plant breeding, to improve facilities for mutation breeding and improve crop varieties.

The duration of the training course will be two weeks (ten working days) and will include theoretical and practical lectures. The training course will cover the following topics: mutation induction in seed propagated crops and radiosensitivity test, development and handling of M1 and M2 generation, selection of (putative) mutants in M2, M3 and advancing of generations, breeding of subsequent generations (M4, M5), trials/multilocation trails and data analysis (experiment design), efficiency enhancing techniques, doubled haploids, speed breeding and rapid cycling and marker assisted selection.

The event is open to qualified candidates with background in plant breeding and genetics, plant biology or related discipline from participating countries in the TC Project RAS5094.

#### *National Training Course*

### **SDS-PAGE Screening for Protein Quality in Mutant Lines- SUD5041**

*Gezira, Sudan, 25-29 July 2022*

Project Officer: C. Zorrilla

Low crop productivity and malnutrition are widespread in Sudan due to a lack of varieties with high nutritional quality and application of inadequate farming practices. The project aims to increase the productivity of sorghum,

millet and cowpeas through nuclear-based mutation induction and selection of mutant lines with high nutritional quality, specifically higher protein, fat, and mineral quality in grains. For that purpose, analytical methods to assess nutritional quality must be implemented.

The purpose of the training is to develop skills on protein isolation and quantification, as well as SDS-PAGE analysis to identify differences in protein composition between mutant and wildtype lines. This screening technique will help identify mutant lines that not only have higher total protein content but also a higher variety of proteins that will enrich the nutritional quality of seed crops such as sorghum, millet, and cowpea. This methodology will be part of the implementation of modern breeding tools for crop biofortification in the target crops.

This training will provide lectures and practical demonstrations in the laboratory. All protocols for implementing these techniques will be provided so that participants can implement these techniques in their own laboratories.

#### *National Training Course*

### **Improving Crop Adaptation to Abiotic Stresses Using Nuclear Derived Techniques and Molecular Breeding Methods- LIR5003**

*Virtual. 1-12 August 2022*

Project Officer: K. Bimpong

This is the first mutation breeding programme for Liberia, so the training is to create awareness on the importance of nuclear techniques in food and agriculture.

The main theme of the training will include mutation induction, optimization of irradiation treatments, introduction to different mutation breeding schemes, selection methods for targeted stresses/traits. Participants will also be exposed to introductory molecular biology, basics of linkage mapping, variant discoveries and marker assisted selection in plant breeding. The course is design for researchers who have no experience in mutation breeding. 15 participants are expected to join the training.

#### *Regional Training Course*

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

*Dakar, Senegal, 8-19 August 2022*

Project Officer: K. Bimpong, S. Sivasankar

The project, RAF5083 “Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)” 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, modalities for informal and formal seed systems for the multiplication and dissemination for upscaling and cultivation.

The course curriculum will include 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 1 trials generation, multilocational trials, GxE and stability analysis, and concept of mega environment and location grouping.

The training course will comprise of lectures and demonstrations. The course is design for breeders, agronomist and pathologist from Member States involved in mutation breeding programmes. Approximately 48 participants are expected to join the training.

#### *Regional Training Course*

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

*Suva, Fiji, 15-26 August 2022*

Project Officer: C. Zorrilla

Mutation breeding has been 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. Therefore, this training will cover topics related to mutation induction, radiosensitivity test, and basic principles of mutation breeding in seed and vegetatively propagated crops.

The host of this training will be the Scientific Pacific Community through its Pacific Community Centre for Pacific Crops and Trees (SPC-CePaCT). The participants will develop expertise on mutation breeding, *in vitro* and molecular techniques from the expert Dr. Suprasana Penna and with the technical support of SPC-CePaCT researchers.

The methodology will include lectures and group discussions. The course will be theoretical and practical with laboratory sessions. It will expose participants to example case studies where the methodologies covered have been applied. Participants will be receiving feedback from the expert on the implementation of mutation breeding in their research programmes.

#### *Regional Training Course*

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

*Virtual. 5-9 September 2022*

Project Officer: K. Bimpong, S. Sivasankar

The objective of the training course is to provide participants with basic and systematic knowledge, and

skills in the application of mutation breeding and bioinformatics, genomics, genotyping and marker assisted selection in MbyS. This approach, among others will be utilized to develop genetically stable lines in the Region, which would be beneficial to farmers due to the faster release of MbyS-produced varieties.

The training will be implemented under the regional project, RAS5088, and the curriculum will include mutation breeding techniques, introductory molecular biology, molecular markers and genome variations, the basics of genomics and transcriptomics, linkage analysis, QTL mapping, genome wide association analysis and introductory bioinformatics.

The training will consist of lectures, demonstrations, group discussions, and videos where necessary. The course is designed for young members of national project team in Member States involved in mutation breeding programmes, with expected participant not exceeding 25. The enhancement of capacities of the trained young scientists, could serve their countries more professionally and efficiently in their future career.

#### *National Training Course*

### **Selection for Biochemical Traits with Improved Quality and Yield in Mutant Rice and Cassava Lines-SIL5021**

*Virtual. 5-9 September 2022*

Project Officer: K. Bimpong

The purpose of the training is to provide participants with opportunities to familiarize themselves with mutation breeding, concept, and methodologies. The training will cover topics such as screening and selection methods for targeted traits such as nutritional quality (high iron, zinc, low phytate, provitamin A) using laboratory, greenhouse and field techniques, modalities for informal and formal seed systems for multiplication and dissemination for upscaling and cultivation. Approximately 15 participants are expected to join the training.

#### *Regional Training Course*

### **Mutation Breeding and Combined Biotechnologies International Center for Biosaline Agriculture (ICBA) United Arab Emirates, 12-16 September 2022**

Project Officer: K. Bimpong, S. Sivasankar

The training course will be implemented under the regional project, RAS5099 referred to as "ARASIA project", and will provide participants with knowledge, and skills in the application of mutation breeding and combined biotechnologies. The training aims to incorporate advanced technologies, especially enabling technologies, to hasten the pace and precision in developing new and improved mutant varieties.

The course curriculum will comprise of mutation breeding, concepts and methodologies, optimization of irradiation

treatments, screening protocols for biotic and abiotic stresses in the laboratory, screenhouse and field conditions, selection methods for abiotic and biotic stresses, modalities for informal and formal seed systems for multiplication and dissemination for upscaling and cultivation, molecular biology, molecular markers and genome variations, genomics and transcriptomics, linkage analysis, QTL mapping, genome wide association analysis and introduction to bioinformatics.

The course will involve lectures, demonstrations, and practical sessions on various protocols of mutation enhancing approaches and enabled biotechnological tools. The course is design for breeders, pathologist and tissue culture specialist in Member States involved in mutation breeding programmes and will be attended by approximately 20 participants from the Member States.

#### *Regional Training Course*

### **In vitro Techniques for the Application of Induced Mutations on Vegetatively Propagated Crops- RER5024**

*Ghent, Belgium, 12-23 September 2022*

Project Officer: C. Zorrilla, S. Sivasankar

The project RER 5024 is aimed at supporting the production of major food crops with higher yields, improved quality, and better resilience to climate change through mutation breeding and combined biotechnologies to contribute to food security in Europe and Central Asia. A total of 26 countries are participating in this project and the target crops for this region include cereals, fruit trees, roots and tubers, vegetables, among others.

The capacity building activities provided by the project will generate the necessary tools to strengthen the mutation breeding programmes in the region that will contribute to enhanced productivity and resilience to climate change of major food crops in the region.

The purpose of the event is to develop capacities and skills of researchers from participating member states on *in vitro* techniques and micropropagation that can be applied in a mutation breeding program; to expose participants to practical cases where the methodologies covered have been applied in mutation breeding; and to develop expertise in basic data analysis related to experimental implementation of *in vitro* techniques.

The training will be hosted by the Laboratory of Applied In Vitro Plant Biotechnology of Dr. Stefaan Werbrouck at Ghent University. It will include lectures and practical laboratory work to demonstrate the applications of the *in vitro* techniques, and data analysis.

#### *National Training Course*

### **Mutation Techniques, Mutation Discovery, Marker Development and Market Assisted Selection- NEP5006**

*Kathmandu, Nepal, 26-30 September 2022*

Project Officer: K. Bimpong

The purpose of the training course is to provide participants with opportunities to familiarize themselves with topics such as mutation breeding, concept and methodologies, linkage mapping, variant discoveries and marker assisted selection in plant breeding.

The course curriculum will include introductory molecular biology, molecular markers and genome variations, the basics of genomics and transcriptomics, linkage analysis, QTL mapping, MAS, genome wide association analysis and introduction to bioinformatics. The course is design for breeders, pathologist and tissue culture specialist involved in mutation breeding programmes. Approximately 15 participants as well as representative(s) from the IAEA are expected attend.

#### *Midterm Project Review Meeting*

### **Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)- RAF5083**

*Vienna, Austria, 26-30 September 2022*

Project Officer: K. Bimpong

The regional project in Africa mutation breeding programme works to enhance crop productivity through the application of mutation techniques and related biotechnology by providing basic infrastructure towards well-functioning tissue culture and basic molecular biology laboratories for the application of biotechnology techniques.

The project is directed towards enhancement of human capacities in both seed and vegetative crops, so as to contribute to the improvement of process efficiencies and statistical rigor in field-testing for selection of improved varieties, modalities for informal and formal seed systems for the multiplication and dissemination of seed for upscaling and cultivation, and development of capacities for the use of newly developed varieties for molecular characterization towards process efficiencies in future selection. The meeting is expected to (i) review results that have been achieved with regard to the implementation of the individual country workplans, regional project outcomes and outputs from the beginning of the project in 2020, and (ii) to discuss and finalize workplans for 2023 in terms of key achievements (such as improved research capability in the region, human capacity development, number of mutant varieties released, mutant lines with improvement for informal and formal seed systems).

Twenty-five participants in African countries from Algeria, Benin, Botswana, Burkina Faso, Cameroon, Cote

d'Ivoire, Democratic Rep. of the Congo, Egypt, Ethiopia, Ghana, Kenya, Lesotho, Libya, Madagascar, Mali, Mauritius, Morocco, Namibia, Niger, Rwanda, Senegal, Sierra Leone, Sudan, Tunisia and Zimbabwe and IAEA staff are expected to participate in the meeting.

#### *Regional Training Course*

### **Plant Mutation Breeding and Efficiency Enhancing Techniques to Increase Resilience to Climate Change- RAS5099, RER5024**

*Seibersdorf, Austria, 26 September to 7 October 2022*

Project Officer: K. Bimpong, C. Zorrilla

This will be a joint training course between Regional TC projects on (i) Developing Climate Smart Crop Production including Improvement and Enhancement of Crop Productivity, Soil and Irrigation Management, and Food Safety Using Nuclear Techniques (ARASIA) RAS5099 and (ii) Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia, RER5024.

The training objective is to enhance Member States understanding and research capabilities in basic principles in crop mutation breeding and advanced molecular techniques for improved mutation detection and selection with emphasis on seed propagated crops. The training course will consist of mutagenesis of seed crops, analysis of radiosensitivity graphs, application of accelerated breeding techniques (rapid cycling in cereals; *In-vitro* haploidy, genotyping and marker-assisted-backcross and big data and genomic tools). About 50 participants are expected to join the training.

#### *Regional Training Course*

### **Mutation Breeding in Vegetatively Propagated Crops, including Micropropagation using Cell/Tissue Culture, Mutation Induction and Selection- RAF5083**

*Nairobi, Kenya, 24 October - 4 November 2022*

Project Officer: K. Bimpong, S. Sivasankar

The training course will be implemented under the regional project, RAF5083, to provide knowledge and expertise on mutation breeding including micropropagation, pre-field and field screening to select improved mutant lines in vegetatively propagated crops. The main theme of the training course will consist of mutation induction including *In-vitro* techniques for mass propagation of plantlets on focus crops such as banana, cassava and potatoes, optimization of irradiation treatments, plant tissue culture, doubled haploids and their use in mutation breeding, introductory to protocols in the laboratory, greenhouse and field conditions for successful breeding programmes, linkage analysis, QTL mapping, genome wide association analysis and introduction to bioinformatics.

The training course will comprise of lectures, demonstrations, and practical sessions on various protocols of mutation enhancing approaches to speed up breeding

methods. The course is design for breeders, pathologist and tissue culture specialist in Member States involved in mutation breeding programmes for vegetatively propagated crops. Approximately 48 participants are expected to attend, as well as representative(s) from the IAEA.

#### *Regional Training Course*

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

*Jakarta, Indonesia, 14-18 November 2022*

Project Officer: K. Bimpong, S. Sivasankar

The objective of the training is to enhance the understanding and research capabilities of the participants in basic principles in crop mutation breeding and introductory to double haploidy for improved mutation detection and selection in crops. The training will be implemented under the regional project, RAS5088, and will provide basic knowledge through the combination of mutation induction with speed breeding methods to develop a new approach, named mutation by speed breeding (MbyS), in order to develop sound knowledge on the principles and practice of the MbyS protocols.

The course curriculum will include mutation induction, induced genetic variation, mutagenesis, preparation of radiosensitivity curves, development of mutant populations, *In-vitro* mutagenesis protocols, plant tissue culture, doubled haploids and their use in mutation breeding, screening methods for biotic and abiotic stresses, introductory to protocols in the laboratory, greenhouse and field conditions for successful breeding programmes. The training will consist of lectures, demonstrations, group discussions, and videos where necessary. The course is design for young members of the national project team in Member States involved in mutation breeding programmes, with expected number of participants not exceeding 25.

#### *Regional Training Course*

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

*KISR, Kuwait, 20-24 November 2022*

Project Officer: K. Bimpong, S. Sivasankar

The objective of the training course is to provide participants with capacities, and skills for the use of different selection methods in improving crops resilience to abiotic stress through nuclear techniques and seed systems. This training course will be implemented under the regional project, RAS5099 referred to as "ARASIA" with the main theme of the training course covering introductory to different mutation breeding scheme, selection methods for abiotic and biotic stresses, introductory to protocols in the laboratory, greenhouse and field conditions for successful breeding programmes,

modalities for informal and formal seed systems for multiplication and dissemination for upscaling and cultivation.

The training will comprise of lectures, demonstrations and practical sessions on various selection protocols for improving crop resilience to abiotic stresses. The course is design for participants who have basic knowledge in mutation breeding with 15 participants expected to join the training.

## Past Events

### *Regional Training Course*

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

*Seibersdorf, Austria, 15-16 February 2022*

Project Officer: S. Sivasankar

This Regional Training Course was implemented as part of the newly launched Inter-regional Technical Cooperation Project, INT5158, Strengthening Member State Capacities to Combat Banana Fusarium Wilt TR4 through Early Detection, New Resistant Varieties and Integrated Management. The training course was held at the Plant Breeding and Genetics Laboratories in Seibersdorf, Austria, and was attended by eleven trainees, and one international expert, from across seven countries in Latin America. The course covered, over a two-week period, lectures and hands-on training on important topics in tissue culture and mutagenesis of banana, screening for resistance to Banana Fusarium Wilt Tropical Race 4, and detection of the presence of the pathogen. Lectures provided a wide coverage of topics including epidemiology of TR4, disease infection process and symptoms, diagnosis of the disease, management options, development of mutant populations in banana, and handling mutant populations including generation advancement. The international expert on banana Fusarium wilt TR4, Mr Miguel Dita, was present at this training course and delivered lectures and practical training.



*Researchers from Latin America in training at the Seibersdorf Laboratories*

### *Regional meeting*

#### **Improving the Resilience of Crops to Climate Change through Mutation Breeding — Phase II (SAPI) - RAS5098**

*Virtual. 15-16 February 2022*

Project Officer: C. Zorrilla

The regional TC project is addressing the improvement of main crops for the Pacific Islands with support from the Joint FAO/IAEA Centre. The main objective of this project 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. An important partner in this endeavour is the Scientific Pacific Community through its Pacific Community Centre for Pacific Crops and Trees (SPC-CePaCT), a regional partner that has a very important role for the success of this project.

Participating countries include Fiji, Marshall Islands, Papua New Guinea, Samoa, and Vanuatu. Some of the crops to be improved using nuclear induced genetic variation are yam, sweet potato, banana, breadfruit, and chili.

This meeting was open to designated counterparts of the project RAS5098. The meeting objective was to identify the most important outputs and review the workplan in preparation for the implementation of the project. Lessons learned from previous project RAS5079 were also shared to implement improvements in the current project. Representatives from Fiji, Marshall Islands, Papua New Guinea, and Vanuatu participated in this meeting.

### *Meeting*

#### **Scientific Exchange with the University of Natural Resources and Life Sciences (BOKU University) in the frame of Coordination Research Project D24014**

*BOKU University, 4 March 2022*

Project Officer: K. Bimpong



*Technical Officers, - Kofi Bimpong and Cinthya Zorrilla with Prof Margit Laimer and her student during a visit to BOKU*

The Technical Officer from the section who is responsible for the CRP D24014 entitled “Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural

Tree Crops” visited the Plant Biotechnology Unit (PBU), Department of Biotechnology at BOKU University in Vienna.

The aim of the visit was to hold collaborative discussions and the progress with Dr. E. Borroto-Fernandez and Univ. Prof. Dr. Laimer who are participants of the CRP.

A second visit was made together with Ms. Cinthya Zorrilla, Technical Officer to participate in the celebration of the sixth international "Fascination of Plants Day" 2021 of the European Organisation for Plant Sciences (EPSO) which took place on 18 May 2022 under the coordination of Univ. Prof. Dr. Margit Laimer. The aim of the celebration was to improve the impact and visibility of plant science in Europe. The day also coincided with the 35th anniversary of the establishment of the Plant Biotechnology Unit (PBU), Department of Biotechnology at BOKU University, and various activities including exhibition of the PBUs scientific achievement over the period.

#### *Regional Training Course*

### **Double Haploidy for Mutation by Speed Breeding (MbyS) toward Crop Improvement for its RCA Regional Asia and Pacific Member States- RAS5088**

*Virtual. 15-16 March 2022*

Project Officer: K. Bimpong, S. Sivasankar

The training course was implemented in a virtual mode to seven Member States with 20 participants under the Regional TC project RAS5088 on Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA) in Regional Asia and Pacific. Member States that participated in the training course includes Bangladesh, Indonesia, Lao P.D.R, Malaysia, Mongolia, Philippines, Thailand.

Dr. Pena Suprasanna (invited lecturer) provided lectures on (i) Doubled Haploid (DH) technology in plant breeding, mutation induction, induced genetic variation, mutagenesis, preparation of radiosensitivity curves, (ii) *In vivo* maternal haploid induction, design and implementation of maternal haploid induction, (iii) Maternal haploid detection using anthocyanin markers and Chromosome doubling of maternal haploids (iv) Putative DH seedlings from the lab to the field and (v) Integration of marker-assisted selection in a DH-based breeding pipeline for rapid development and delivery of superior parental lines and cultivars. The course was very well received by all the participants who were highly motivated to apply the knowledge gained in their research work.

#### *National Training Course*

### **Mutants Screening for Yield, Drought and Nutritional Quality of Groundnut and Soybean – MALW5005**

*Virtual. 11-15 April 2022*

Project Officer: C. Zorrilla

The project aims to develop new drought resilient mutant soybean and groundnut varieties, using nuclear technology to contribute towards achieving food, nutrition, and income security. Currently, mutant lines of both crops, soybean and groundnut, are under screening for increased yield and drought tolerance.

This national training course had the objective to generate knowledge on current screening methodologies for yield improvement, drought tolerance and nutritional quality; get acquaintance of best practices for efficient delivery of new varieties; and diffusion of mutation breeding progress in groundnut and soybeans in Malawi.



*MLW5005 training: Group photo at a training course on soybean and groundnut breeding in Lilongwe – Malawi*

The training was held in Lilongwe at the Chitedze Research Station, and a total of 19 participants from government institutions in Malawi were trained. The participants included five females and 14 male researchers from national research institutions. The training included lectures, case studies and was complemented with field visits to demonstrate the methods of screening for biotic stresses in mutant lines and visits to seed production fields.

#### *National Training Course*

### **Screening Methods of Tolerance to Water Stress in Roots and Tubers in Nicaragua – NIC5011**

*Managua, Nicaragua, 9-13 May 2022*

Project Officer: C. Zorrilla

The project objective is to expand the genetic variability of cocoyam, taro and plantain through induction and identification of mutations, using nuclear and biotechnology techniques and searching for disease tolerance and climate change adaptation, especially to drought.

This national training course was held as part of an expert mission. The objective of the course was to develop capacities in the participants to screen for water stress tolerance in vegetatively propagated crops using simple tools to record morphological and physiological parameters such as plant temperature with Infrared imaging, chlorophyll measurement, foliar area, and soil moisture that will contribute to the selection of water-stress

tolerant mutant lines of *Colocasia esculenta* “taro” also known as “malanga”. As well, data management and basic statistical analysis was included as part of the training.



*NIC5011 training: Demonstration of data acquisition using equipment for evaluating water-stress in Colocasia esculenta “malanga” at UNA-Nicaragua*

The training was held at the Universidad Nacional Agraria in Managua. A total of 19 participants, 12 male and seven female, from several institutions including universities and national agricultural research centres participated in this training.

#### *National Training Course*

#### **Enhance capacities on participatory plant breeding - CUB5023**

*Hybrid. 16-20 May 2022*

Project Officer: K. Bimpong, C. Zorrilla

Cuba has been successfully using mutation breeding to develop new high-yielding varieties of several crops. The aim of this project is to generate mutant lines of rice,

common bean and soybean that combine tolerance to abiotic stress with good response to biofertilizer.

This training course was part of a virtual expert mission and was held in a hybrid manner at Instituto Nacional de Ciencias Agrícolas in La Habana - Cuba. Most participants joined in person, and some connected virtually. A total of 28 participants from Cuba attended this course; and 20 participants from other countries such as Argentina (3), Brazil (1), Costa Rica (2), Ecuador (1), El Salvador (1), Guatemala (1), Nicaragua (1), Panama (5), Paraguay (2), Peru (1), and Venezuela (2) also participated in this course with support from Fondo Fiduciario Perez Guerrero - UNOSSC.

Trainees got acquainted in participatory methods that contribute to farmers adoption of new mutant varieties. The training included lectures and practical demonstrations in the field. Participants also developed some action plans to implement the knowledge acquired in their respective research.



*CUB5023 training: Field day at INCA in Cuba as part of the practical application of participatory selection methodologies*

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

The Plant Breeding and Genetics Laboratory (PBGL) has been continuing its Research and Development efforts on mutation breeding and associated biotechnologies for developing resistance in banana to the Fusarium Wilt Tropical Race 4, development of coffee single-cell culture and regeneration, the development of bioinformatic tools and the advancement of mutant sorghum populations for Functional Genomics for Trait Utilization. For the first time, lentil seeds, sourced through material transfer agreement from the International Centre for Agricultural Research in Dry Areas, have been grown to flowering and seed set under rapid generation advancement conditions in the greenhouse. We anticipate continued R&D in this pulse crop as part of the CRP D22006, Enhanced Biotic-Stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-Change Adaptation.

### Accelerated Breeding Technologies

#### Deploying Rapid Generation Advancement (RGA) in Plant Mutation Breeding

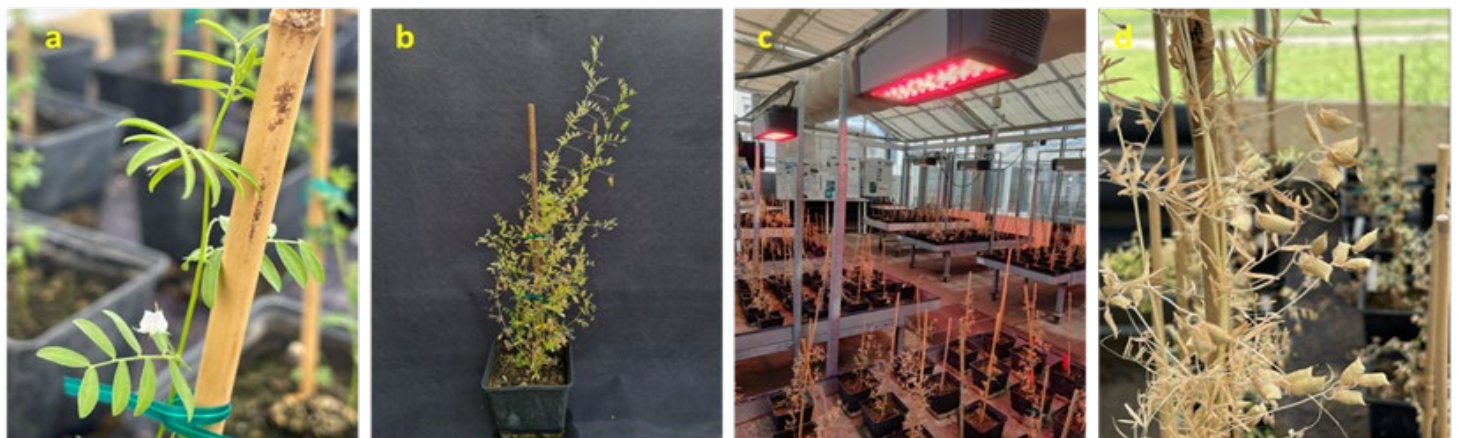
Traditional breeding methods usually take 4 to 6 years to develop genetically fixed lines and is very expensive. Hence it is imperative to use techniques that can shorten the time needed to develop breeding lines, and ultimately be able to release new varieties faster and cheaper. Combining emerging techniques, such as high-throughput phenotyping and genotyping, genomic selection (GS), Marker Assisted Selection (MAS) and Speed Breeding / Rapid Generation Advancement (RGA) with plant mutation breeding will help in rapid gene fixation for accelerated crop improvement. RGA is a breeding approach that uses single seed descent (SSD) as the breeding method in a glass / poly house which also enables to save extensive land space (~90%), labor (~50%) and shorten the time for developing breeding lines by at least 2

years compared to pedigree method. RGA can also be used for off-season nurseries to reduce the breeding cycle up to 40% or more in most species.

Plant mutation breeding can be enhanced by growing mutated plant populations under speed breeding conditions to rapidly attain advance generation lines, thereby accelerating homozygosity and the potential rate of genetic gain. Efforts are made at PBGL to optimize RGA protocols for the crops that are economically important for the Member States. Currently we are optimizing an RGA protocol for Lentil (*Lens culinaris*) while working in collaboration with the Consultative Group of International Agricultural Research (CGIAR) for securing germplasm and standardizing existing RGA protocols.

Two lentil genotypes ILL 8006 and LRIL 22-70 were grown in glass house at high density of supplemental lighting (using far-red enriched LED and blue LED lights) under an extended photoperiod of 22 h light/25.5 °C and 2 h dark/15 °C along with control in glass house without supplemental lightening in three replications of 40 pots each. Days to first flower in lentil plants grown in glass house with supplemental lightening was 28 days while it was 40 days in glass house without supplemental lightening. Days to physiological maturity was 67 days and 88 days with supplemental lightening and glass house without supplemental lightening respectively (Figure 6).

Rescuing viable immature seed will further speed up the generation turnover and will allow four to five generations per year through single seed descent (SSD) instead of only one or two in conventional greenhouse-based methods. Deploying this technology in plant mutation breeding will serve as efficient tool to accelerate plant breeding programs.



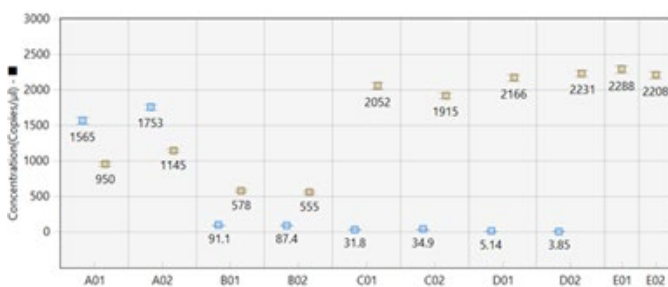
**Figure:** Lentil speed breeding protocol optimized at PBGL, Seibersdorf **a)** Flowering on 28 days after sowing **b)** Podding on 40 days after sowing **c)** Lentil plants grown under long day conditions supplemented by cool far-red enriched LED and blue LED lights in glass house **d)** Mature pods at 67 days after sowing



## Towards Cost-effective Gene-based Selection using Digital Droplet PCR

Mutation detection at the scale required for effective gene-based selection in a mutation breeding program still faces practical challenges in terms of labor, cost, limited precision and throughput. Digital droplet PCR (ddPCR) is a breakthrough technology that offers ultrasensitive and reproducible nucleic acid quantification and detection and can potentially solve some of these bottlenecks.

The PBGL set out to develop protocols for rare mutation detection in crop plants using ddPCR. As a pilot, sorghum and previously characterized SNP mutations located on chromosome 4 and validated for Marker-Assisted Selection (see PBG Newsletter No 47) are used. In reconstitution experiment comprising different ratios of mutant and wild type DNA, the mutant SNP can be reliably detected at 0.1 % (Figure 7). This result shows that pools of up to 1,000 plants can be analysed in a single PCR reaction, indicating a drastically increased throughput, reduced time and costing for several key steps for mutation detection, including tissue collection, DNA extraction and PCR.



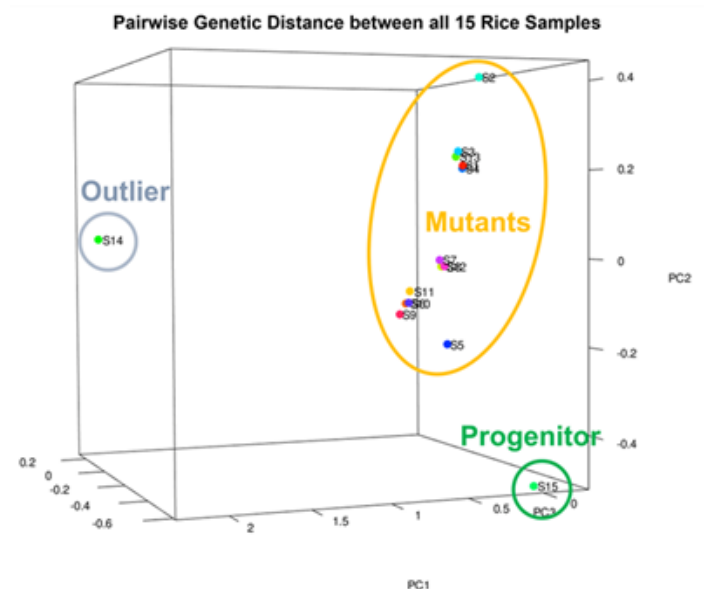
**Figure 7.** Representative plot showing number of mutant (bleu) and wild type (brown) genome copies in serial dilutions from left to right; 50% (A), 10% (B), 1% (C), 0.1% (D), and 0% mutant DNA

## Genomic Analysis of Mutant Populations (CRP D23032)

PBG Laboratory has embraced the DNA sequencing revolution and established the necessary infrastructure and analysis tools for Next Generation Sequencing (NGS). Under CRP D23032, 15 rice lines have been produced: samples S1-S14 are mutant lines in the M12 generation that were reported resistant to bacterial leaf blight (BLB), derived from the same progenitor rice line sample S15. Lines S1-S14 supposedly derive from two independent mutagenic treatments. Of interest was to determine the genomic variation caused by the mutagenesis and to leverage this information to inform further breeding.

All rice lines were grown in PBG Laboratory glass houses. DNA was isolated and sent to a sequence service provider for short read DNA sequencing on the Illumina platform (Paired-End, 150bp). We received back between 34 and 54 million read pairs per sample, which corresponds to a theoretical genome coverage of 25- to 40-fold. On this data we performed genome-wide comparative analyses: Firstly, a reference free clustering to confirm sample lineages and, secondly, read mapping to the japonica rice reference genome followed by variant calling and variant annotation. All analyses were performed with PBG Laboratory's automated snakemake analysis workflow (see PBG newsletter No 45). The workflow is available on github (<https://github.com/pbgl/>) for community use and is described in a recent PBGL Protocol: *A Software Workflow for Automated Analysis of Genome (Re-)Sequencing Projects - A LABORATORY PROTOCOL*. The analysis took less than two weeks from receipt of the sequencing data to drawing relevant conclusions which demonstrates the power of the approach.

Reference-free clustering of the raw sequence data detected confirmed that samples S1-S13 were closely related to progenitor sample S15 and to each other while sample S14 is considered an outlier (Figure 8).



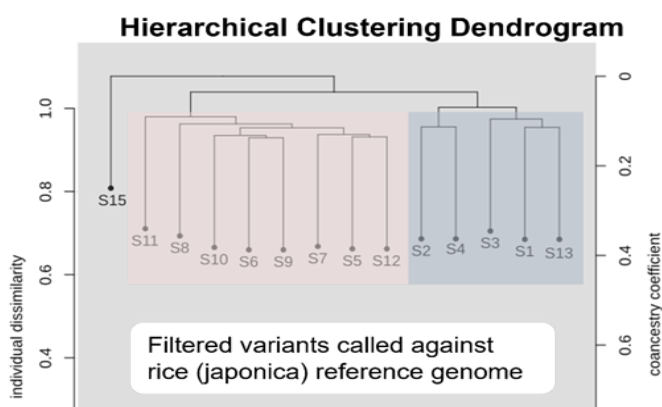
**Figure 8.** Reference-free clustering of raw sequence data of samples S1-S15. Principle component plot of a pairwise distance matrix produced by software kWIP. Sample S14 was identified as outlier, Mutants S1-S13 are similar to each other but distinct from progenitor sample S15. The mutants also cluster with each other indicating population structure. The plot was produced with R.

Variant analysis based on read mapping to the Nipponbare (japonica) reference genome revealed that sample S14 had two orders of magnitude more variants when compared to the progenitor S15 than all other samples and much more than expected from the mutagenic treatments. It can hence be excluded that S14 is a mutant derived from S15. In addition, S14 carries a large introgression on Chromosome 10 of yet another, even more divergent, variety (Figure 9).



**Figure 9.** SNP density plot for Sample S14. The genome-wide variation is beyond what is expected for the induced mutants. There appears to be an introgression on Chromosome 10 even more divergent from the Nipponbare reference genome than the rest of the genome. The plot was produced in R with R-package rMVP.

Hierarchical clustering based on homozygous variants called against the Nipponbare reference genome reveals two distinct clusters for samples S1 to S13 (Figure 10).



**Figure 10.** Dendrogram illustrating the hierarchical clustering of Samples S1-S13, and S15. S15 is the non-mutagenized progenitor sample, S1-S13 are mutants derived from S15. There is population structure that can be interpreted as the two groups reflecting two independent mutagenesis events, which confirms the experimental design. The plot was produced in R with package SNPRelate\*.

From the analysis we conclude that we probably have two independent mutagenic events. The overall genetic variation to progenitor S15 is low, between 236 and 470 homozygous SNPs and small InDels per mutant line. This number is in the expected range. Close inspection of known candidate genes for BLB-resistance (e.g., sugar transporters SWEET 11,13,14 and their promoters) showed that their sequences are not altered in these mutants and hence are not involved in the BLB resistance.

An allelism test between lines from the different clusters would reveal whether they are allelic or reflect two different resistance mechanisms. Mutations shared within a cluster are candidates for conferring the resistance. A genetic mapping study, even if small, has a good chance of identifying linked variants. For genetic mapping, at least two segregating populations should be produced, one for each cluster. Crossing to S15 will yield enough markers for such study.

### Crop Irradiation Services Provided to Member States

Coming soon!

With strong support from the MTIT Innovation Team Lead Ms Lauren Baird, MTIT intern Ms Dayanne Fernandes Cunha and NAHU Data Scientist Mr Yaroslav Pynda a portal has been developed to bring PBGL’s crop irradiation form, guide and related information online on the IAEA website for convenience and improved access to all FAO and IAEA Member States wishing to use this service.

A prototype of the Crop Irradiation Service Request Form is available online for evaluation and feedback: <https://iris.iaea.org/public/survey?cdoc=FAO00002>. Do not hesitate to contact Ms Matijevec ([m.matijevec@iaea.org](mailto:m.matijevec@iaea.org)) with any comments or suggestions.

Table 1 lists the irradiation requests that the PBGL received so far (2022-05-25); 19 requests from 11 Member States across 26 different plant species covering a total of 216 accessions/varieties treated.

**Table 1. Crop Irradiation Services**

Request Number	Country	Request Type	Crop/Species
1684	PBGL		Banana
1685	The Netherlands		Ornamental
1686	UK		<i>Euphorbia pepylus</i>
1687	Germany		<i>Brassica sp.</i>
1688	UK		Papaver Somniferum
1689	Germany		Ornamental

Request Number	Country	Request Type	Crop/Species
1690	PBGL	CRP	Lentil
1691	Hungary		Ornamental
1692	Germany		Ornamental
1693	S. Arabia/ France		<i>Solanum sisymbriifolium</i> and <i>Solanum torvum</i>
1694	UK		<i>Cannabis sativa</i>
1695	Slovenia		<i>Triticum durum</i> , <i>Triticum aestivum</i> , <i>Hordeum vulgare</i> , <i>Trifolium pratense</i> , <i>Fagopyrum esculentum</i> and <i>Fagopyrum tataricum</i>

Request Number	Country	Request Type	Crop/Species
1696	Germany		<i>Rhynchospora pubera</i> and <i>R. tenuis</i>
1697	PBGL		Coffee
1698	Philippines		Rice
1699	India		Sorghum
1700	PBGL		Sorghum
1701	Bosnia and Herzegovina	TC	<i>Phaseolus vulgaris</i>
1702	Ghana	TC	cowpea, soybean, bambara beans, tiger nut, groundnut

### Individual Training

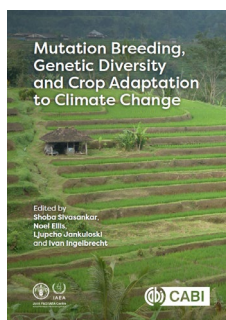
During 2022 the PBGL hosted three interns, one fellow, and two PhD students as summarized in Table 2

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

Name	Country	Status	Topic	Period
Ms Jing SONG	China	Intern	Molecular Genetics and Tissue Culture	1 year
Mr Zhizhou NIU	China	Intern	Mutation Breeding	1 year
Mr Kulasiri RANAWEERA	Sri Lanka	Fellow	Micropropagation of tea and M1 screening	9 months
Mr Michael HALL	USA	Intern	Data Science	1 year
Mr Radisras NKURUNZIZA	Uganda	PhD- Consultant	Mutation breeding for Fusarium wilt resistance in African cooking banana	1 year
Mr Hassan MDUMA	United Republic of Tanzania	PhD-Consultant	Mutation breeding for Fusarium wilt resistance in African cooking banana	1 year

## Publications

### Books



#### 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](#)

[ePub 9781789249118](#)



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

Guest Editors: F. Sarsu, B.P. Forster, S. Sivasankar

Australian Journal of Crop Science, Southern Cross Publishing, Volume 14, Number 8, 2021

DOI: 10.21475/ajcs.21.15.09.sp

[https://www.cropj.com/full\\_issue\\_IAEA\\_AJCS.pdf](https://www.cropj.com/full_issue_IAEA_AJCS.pdf)



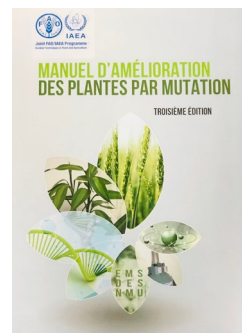
#### Manual de mejoramiento por mutaciones, Tercera edición

Editado por M.M. Spencer-Lopes, Forster, B.P., Jankuloski, L., Sub Programa de Mejoramiento de Plantas y Genética, División Conjunta FAO/OIEA de Técnicas Nucleares en Alimentación y Agricultura.

ISBN 978-92-5-133741-7

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[Manual de mejoramiento por mutaciones \(fao.org\)](#)



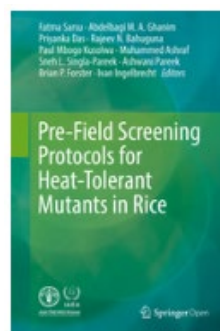
#### Manuel d'amélioration des plantes par mutation, Troisième édition

Édité par. M.M. Spencer-Lopes, B.P. Forster et L. Jankuloski, Sous-programme de Génétique et d'Amélioration des Plantes Division mixte FAO/IAEA des Techniques Nucléaires appliquées à l'Alimentation et à l'Agriculture.

ISBN 978-92-5-132932-0

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[Manuel d'amélioration des plantes par mutation \(fao.org\)](#)



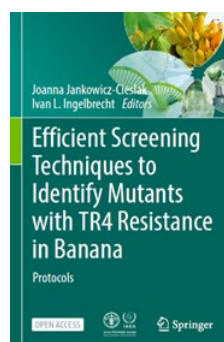
#### Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice

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

Springer, 2018

ISBN 978-3-319-77338-4

[Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice | Fatma Sarsu | Springer](#)



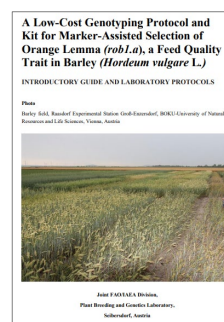
#### Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana

Editors: Joanna Jankowicz-Cieslak, Ivan L. Ingelbrech

Springer, 2022

ISBN 978-3-662-64915-2 (eBook)

[Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana | SpringerLink](#)

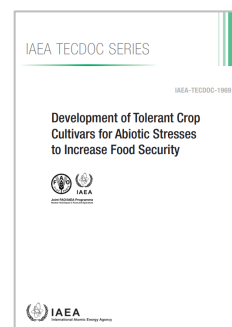


#### A Low-Cost Genotyping Protocol and Kit for Marker-Assisted Selection of Orange Lemma (*rob1.a*), a Feed Quality Trait in Barley (*Hordeum vulgare* L.).

Introductory guide and laboratory protocols. I. Ingelbrecht *et al.*, May 2021.

[Diagnostic-marker-assay-OL-barleyMay2021.pdf \(iaea.org\)](#)

### Technical Documents



#### IAEA-TECDOC-1969

#### Development of Tolerant Crop Cultivars for Abiotic Stresses to Increase Food Security

Contributors: N.K.A. Amoah, B. Manneh and I.K. Bimpong.

Salt stress (both salinity and sodicity) is the second most widespread soil problem after drought and is a serious constraint in rice production worldwide. Soil is considered saline if the electrical

conductivity is above four units of its measurement scale. Rice is most sensitive to salt stress during the early seedling (1–3 weeks) and reproductive stages, when the threshold is as low as an electrical conductivity of three units of its measurement scale. Plant breeding for crop improvement can decrease the grain yield gap in salt-prone soils, thus contributing to food security and alleviating poverty.

This publication provides information on a system for phenotyping problem soils to identify tolerant lines for use in breeding programmes that target salt stress. The standard operating procedures presented here provide comprehensive information on (a) developing a suitable phenotyping system for the two main stages of crop sensitivity to salt stress, including pre-screening of large sets of test lines in a controlled or semi-controlled environment and evaluation under field conditions, and on (b) reducing experimental error and improving repeatability of the experiment through proper stress management. The present publication is expected to be a valuable resource for plant breeders and field technicians involved in phenotyping of soils.

ISBN 978-92-0-123321-9

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

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[IAEA-TECDOC-1969](#)

## Peer-reviewed Publications

### 2021

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

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

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## Conference Abstracts and Posters

### 2021

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SARSU, F., SIVASANKAR, S., BIMPONG, I.K., JANKULOSKI, L. 2020. Induced Mutations for Food Security. Presented at the 2<sup>nd</sup> Latin American Symposium on Nuclear Applications in Agriculture, 5–6 March 2020, Ecuador.

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## News Highlights

- Tackling Cassava Brown Streak Disease in Uganda with Nuclear Techniques  
(31 May 2022)  
[Tackling Cassava Brown Streak Disease in Uganda with Nuclear Techniques | IAEA](#)
- #BiodiversityDay: How the IAEA Contributes to Bend the Curve of Biodiversity Loss  
(20 May 2022)  
[#BiodiversityDay: How the IAEA Contributes to Bend the Curve of Biodiversity Loss | IAEA](#)
- CRP Success Story: Mutation Breeding for Resistance to Striga Parasitic Weed in Cereals for Food Security (D25005)  
(18 March 2022)  
[CRP Success Story: Mutation Breeding for Resistance to Striga Parasitic Weed in Cereals for Food Security \(D25005\) \(iaea.org\)](#)
- IAEA Trains Latin American Scientists in Fighting Banana Disease  
(10 March 2022)  
[IAEA Trains Latin American Scientists in Fighting Banana Disease | IAEA](#)
- How Nuclear Science Helps Fruits and Vegetables Survive and Thrive  
(24 February 2022)  
[How Nuclear Science Helps Fruits and Vegetables Survive and Thrive | IAEA](#)
- Combating the Banana Wilt Pandemic with Nuclear Science  
(24 December 2022)  
[Combating the Banana Wilt Pandemic | IAEA](#)
- Nuclear Techniques Help Bangladeshi Experts Develop Improved Cotton Varieties in Record Time  
(23 November 2021)  
[Bangladesh Develops Improved Cotton in Record Time | IAEA](#)
- Nuclear Science Helps to Adapt to Climate Change, COP26 Participants Hear  
(6 November 2021)  
[Nuclear Science Helps to Adapt to Climate Change: COP26 | IAEA](#)
- Director General of Nuklear Malaysia: Committed to Nuclear Science and Helping Others  
(20 October 2021)  
[Director General of Nuklear Malaysia: Committed to Nuclear Science and Helping Others | IAEA](#)
- New CRP: Radiation-induced Crop Diversity and Genetic Associations for Accelerating Variety Development (D24015)  
(14 October 2021)  
[New CRP: Radiation-induced crop diversity and genetic associations for accelerating variety development \(D24015\) \(iaea.org\)](#)
- Ahead of COP26, IAEA Issues Report on the Role of Nuclear Science and Technology in Climate Change Adaptation  
(14 October 2021)  
[Ahead of COP26, IAEA Issues Report on the Role of Nuclear Science and Technology in Climate Change Adaptation | IAEA](#)
- Global Success in Plant Breeding Celebrated with New achievement Awards  
(20 September 2021)  
[Achievement Awards Celebrate Global Plant Breeding Success | IAEA](#)
- New Crop Varieties Improve Tomato and Soybean Yields in Cuba  
(24 August 2021)  
[Crop Varieties Improve Tomato and Soybean Yields in Cuba | IAEA](#)
- How Do Nuclear Techniques Contribute to Measuring and Adapting to Climate Change?  
(5 August 2021)  
[How Do Nuclear Techniques Contribute to Measuring and Adapting to Climate Change? \(iaea.org\)](#)
- Boosting Tea Plant Diversity, Quality and Resilience in Sri Lanka  
(16 June 2021)  
[Boosting Tea Plant Diversity, Quality and Resilience in Sri Lanka | IAEA](#)
- IAEA Helps Countries Halt Ecosystems Degradation  
(4 June 2021)  
[IAEA Helps Countries Halt Ecosystems Degradation | IAEA](#)



- Nuclear Techniques Help to Revive Ginger Production in Jamaica  
(11 May 2021)  
[Nuclear Techniques Help to Revive Ginger Production in Jamaica | IAEA](#)
- Cotton in Pakistan: How Nuclear Techniques are Helping the Textile Industry  
(22 January 2021)  
[Nuclear Techniques and the Textile Industry | IAEA](#)
- Call for Nominations: Recognizing Excellence in Plant Mutation Breeding and Associated Biotechnologies  
(15 March 2021)  
[Recognizing Excellence in Plant Mutation Breeding | IAEA](#)
- Improved Soil and Nutrient Management Practices Increase Rice Yields in Lao PDR  
(20 January 2021)  
[Improved Soil and Nutrient Management Practices Increase Rice Yields in Lao PDR | IAEA](#)

## Websites and Links

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



**SYMPOSIUM**  
**Global Research in the Management of**  
**Banana Fusarium Wilt TR4**

**AGENDA**

**Day 1, 24 March 2022**

**Opening Session**

- 08:00-08:40** Welcome Address and Opening Remarks  
**Mr Pedro Alava- Minister of Agriculture of Ecuador**  
**Mr Rafael Mariano Grossi, video- Director General, International Atomic Energy Agency (IAEA)**  
**Mr César Montaña- Vice Minister of Foreign Affairs**  
**Mr Qu Liang- Director, Joint FAO/IAEA Centre**  
**Mr Raul Ramirez- International Atomic Energy Agency (IAEA)**  
**Ms Shoba Sivasankar- Plant Breeding and Genetics, Joint FAO/IAEA Centre**

**Session 1 – Global experience in Foc TR4 Management**

- 08:40-09:00** Innovations and developments for Foc R4T prevention to be applied in the OIRSA region  
**Mr Carlos Urías Morales- Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA)**
- 09:00-09:30** FAO's vision on prevention of banana Fusarium wilt TR4 and its efforts in LAC  
**Mr Fazil Dusunceli/Ms Raixa Llauger/Ms Esther Peralta- Food and Agriculture Organization of the United Nations (FAO)**
- 09:30-10:00** Global Network against TR4  
**Mr Victor Prada/Mr Matheus Lima- Food and Agriculture Organization of the United Nations (FAO)**
- 10:00-10:30** Break
- 10:30-11:15** Breeding for TR4 resistance: a practical experience  
**Mr Frédéric Bakry- CIRAD**
- 11:15- 12:00** The occurrence, spread and management of Foc TR4 in Africa  
**Mr Altus Viljoen/Ms Diane Mostert- University of Stellenbosch**
- 12:00-13:00** Lunch

**Session 2 – Detection, Epidemiology and Integrated Management of Foc TR4**

- 13:00-13:45** Genes, Landscapes and People: A regional perspective to face the threat of Fusarium TR4 in Latin America and The Caribbean  
**Mr Miguel Dita- Alliance Bioversity-CIAT**
- 13:45-14:30** Productive development of plantain and banana for export and food security in Peru  
**Mr Juan Carlos Rojas- Instituto Nacional de Innovación Agraria (INIA)**
- 14:30-15:15** Staying ahead of TR4: diagnostics, control and resistance  
**Ms Elizabeth Aitken- The University of Queensland**
- 15:15-15:30** Break
- 15:30-16:15** Comprehensive management of Fusarium wilt TR4 for banana sustainable production in China  
**Mr Sijun Zheng- Alliance Bioversity-CIAT**
- 16:15-17:00** Banana Fusarium wilt TR4: Integrated Disease Management in the Philippines  
**Mr Benny M Corcolon- Tagum Agricultural Development Company, Incorporated (TADECO)**



**SYMPOSIUM**  
**Global Research in the Management of**  
**Banana Fusarium Wilt TR4**

**AGENDA**

**Day 2, 25 March 2022**

**Session 2 Continued**

**09:00-09:45** Fusarium wilt Tropical race 4 of Banana in India - Status, Characterization, Detection and Management

***Mr Raman Thangavelu- National Research Center for Banana, ICAR***

**Session 3 – Genetic resistance and banana Fusarium Wilt TR4**

**09:45-10:30** Multi-disciplinary approach for the management of Fusarium wilt TR4 in India

***Ms Uma Subbaraya- National Research Center for Banana, ICAR***

**10:30-11:00** *Break*

**11:00-11:45** Genetic improvement strategies to overcome Fusarium wilt in banana at Embrapa, Brazil

***Mr Edson Amorim- Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA)***

**11:45-13:45** *Lunch*

**13:45-14:30** Gene editing of Banana for Disease Resistance at IITA

***Ms Leena Tripathi- International Institute of Tropical Agriculture (IITA)***

**14:30-15:15** Gene-editing for TR4 resistance in Cavendish banana at QUT, Australia

***Mr James Dale- Queensland University of Technology***

**15:15-15:45** *Break*

**15:45-16:45** **Panel Discussion**

**16:45-17:00** **Closing Session**

***Ms Shoba Sivasankar- Plant Breeding and Genetics, Joint FAO/IAEA Centre***

***Mr Walther Reyes- Executive Director of INIAP***

***Mr Raul Ramirez- International Atomic Energy Agency (IAEA)***

**INT5158: Strengthening Member State Capacities to Combat Banana Fusarium Wilt (TR4) through Early Detection, New Resistant Varieties, and Integrated Management**

## Impressum

### Plant Breeding and Genetics Newsletter No. 49

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