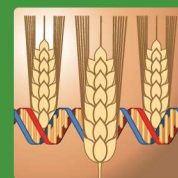




Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Plant Breeding & Genetics Newsletter



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To Our Readers



New CRP (D24014) on vegetatively propagated crops (e.g. cassava and other roots/tubers) and horticultural tree crops (e.g. olive) starts in 2021.

Dear Colleagues,

Plant Breeding and Genetics (PBG) continued our technical support to Member States through Coordinated Research Projects (CRPs) and Technical Cooperation Projects (TCPs) as best as possible during the first half of 2021, even as the pandemic situation remains mostly unabated.

Of particular mention is the ongoing preparation for the recognition of major achievements in plant mutation breeding and related biotechnologies through the **Outstanding Achievement Awards, Women in Plant Mutation Breeding Awards and Young Scientists Awards**. A total of 76 nominations were received from 41 Member States, relative to 42 nominations from 29 Member States received in 2014 when awards were first

given in this technical area by the Joint FAO/IAEA Centre. The Awards will be officially announced during the 65th regular session of the IAEA General Conference, to be held at IAEA Headquarters in Vienna, Austria, from 20 to 24 September 2021.

The **new CRP** on ‘Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops (D24014)’ is preparing to hold its first Research Coordination Meeting in August 2021. Call for proposals for the CRP has resulted in contracts awarded to 10 institutions including seven with a focus on cassava and other roots/tuber crops, and three on olive. While the new CRP is in its launch year in 2021, **two previous CRPs are coming to a close** during the year, namely, ‘Mutation Breeding for

Resistance to *Striga* Parasitic Weeds in Cereals for Food Security (D25005)' and 'Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding (D23031)'. Both have delivered strong research outputs during the duration that they have been in implementation.

Key achievements of the **CRP on *Striga* resistance (D25005)**, as presented at its last Research Coordination Meeting (RCM) in May 2021, include the identification of confirmed mutants of rice, maize and sorghum with resistance to *Striga asiatica* or *Striga hermonthica*. Several of these are in the process of being tested in farmers' fields in a participatory selection modality. In addition, the CRP team succeeded in the development or adaptation of (1) field- and greenhouse-based screening protocols for resistance; (2) laboratory protocols for analysing resistance mechanisms; and (3) protocols for haploidy induction and doubled haploid generation. The **CRP on Drought Tolerance (D23031)**, which held its final RCM in April 2021, succeeded in (1) the application of streamlined screening procedures for drought tolerance at critical developmental stages; (2) testing and confirmation of putative drought-tolerant mutant lines of rice and sorghum in field drought environments; and (3) identification or confirmation of secondary markers for drought tolerance. Research and development (R&D) activities implemented by contract holders in **ongoing CRPs** remain on track in most instances, although in a few cases, delays imposed by the pandemic were experienced.

PBG continued to provide technical support to the **implementation of 47 Technical Cooperation Projects (TCPs)**, of which 35 began in 2020, and to the **design of 35 new TCPs** planned for the 2022–23 cycle. The year 2021 has seen to date the release of the **first cotton mutant variety in Bangladesh**, CBD Tula M1, and that of the **soybean mutant variety, Cuvin 22, and the tomato mutant variety, Giron 50, in Cuba**. In June 2021, a virtual training course was offered over a two-week duration on 'Plant Mutation Breeding and Efficiency Enhancing Techniques for Climate-Change Adaptation', jointly to participants of two regional TCPs, RAF5083 and RER5024. Virtual meetings and increasingly frequent email communications are enabling implementation of those planned activities of TCPs that can be implemented in this mode. Other important capacity building activities, namely training through fellowships and scientific visits, remain pending. However, preparations for these are in place should travel restrictions ease during the second half of the year.

R&D efforts at the PBG Laboratory recently completed the validation of **KASP marker assays** for three single nucleotide polymorphisms (SNPs) that were identified to determine a gamma ray induced early maturity, semi-dwarf trait in sorghum. The laboratory also successfully evaluated

an **exome-capture kit** for tetraploid *Coffea arabica* that includes baits against 275 000 exon regions. The preparation of the kit had been previously commissioned from Arbor BioSciences, Michigan, USA. Exome capture facilitates genome-wide exon sequencing offering the possibility for variant analysis in germplasm collections including mutant populations. The kit will be used at the PBG Laboratory to characterize an existing population of coffee mutants.

Another important highlight from the laboratory during the first half of this year is the adaptation of methodologies for **digital droplet PCR (ddPCR)**, which allows highly sensitive detection and quantitation of rare nucleic acid variants. Its successful use in human diagnostics for rare mutant detection has been reported. We plan to apply this technique to a recently developed large mutant population of sorghum consisting of approximately 5000 M2 families.

On the publications front, I am happy to announce the availability of French and Spanish translations of the *Manual on Mutation Breeding*, third edition, at www.fao.org/publications/card/en/c/I9285EN. Upcoming major publications during the second half of 2021 include the book, '*Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change*', resulting from the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, and the special issue of the Australian Journal of Crop Science, '*Crop Adaptation to Climate Change: High-Temperature Stress*', resulting from a recently completed CRP under the same name.

Finally, I would like to note the inclusion in this Newsletter of two feature articles on the use of heavy ion beam in an endeavour to help familiarize the research community on the applications and results of ion beam mutagenesis in crop improvement.

Before closing, with much regret, I note the passing of Udda Lundqvist, one of the pioneers of barley mutation breeding, on the 26th of December 2020. Udda was a passionate scientist who, together with colleagues, has developed more than 10 000 barley mutants currently preserved in Nordgen's seed collection, and a number of improved varieties with new traits. Her commitment to barley research and achievements will continue to inspire the next generation of plant researchers.

My sincere appreciation for all your support and continued efforts during these difficult times! I look forward to our continuing strong collaborations to deliver important results for global food security and climate-change adaptation.

Shoba Sivasankar
Head
Plant Breeding and Genetics Section

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¹ Separated in May 2021

² Separated in December 2020

³ Joined in February 2021

Staff News

Awards and Recognition

The Plant Breeding and Genetics (PBG) team members greatly appreciate receiving the IAEA Director General's Recognition Award for Exceptional Service from Mr Qu Liang, Director of the Joint FAO/IAEA Centre of Nuclear Applications in Food and Agriculture. This Award recognized all Agency staff for the co-ordinated Agency-wide response to the COVID-19 pandemic. The PBG team stepped up to the multiple challenges of the pandemic including work-from-home, virtual events and travel restrictions to ensure the seamless implementation of projects. At the PBG Laboratory, the commitment of staff members ensured that operations and services continued, and important germplasm resources were maintained.

Another important recognition this year has been the 25-year Service Anniversary of Ms Katayoun Entekhabi, Programme Assistant at PBG. We greatly appreciate her continuing commitment and hard work for the seamless functioning of the PBG office and diligent implementation of PBG events.



Ms Fatma Sarsu (Turkey)

The first half of this year marks the end of Ms Fatma Sarsu's current tenure with the IAEA. Ms Sarsu has been an exceptional contributor to the outputs of PBG during her nine years with us. Her sincere dedication, hard work, commitment, and pleasant demeanour is recognized and appreciated by colleagues in NAFA, TC and in Member States. Ms Sarsu successfully led several Technical Cooperation Projects and two Coordinated Research Projects, delivering important outputs in the form of improved crop varieties, advanced mutant lines, technical protocols and one special issue on crop drought tolerance currently in preparation. Her support especially to the TC projects in Africa has resulted in adding new and improved mutant crop varieties to the region. She was also responsible for the curation of the FAO/IAEA Mutant Variety Database, which led to the tracking and addition of several missing records to the database. We will miss her ready smile and consistent can-do attitude.



Ms Ayca Eda Akgun (Turkey) joined the Plant Breeding and Genetics Laboratory (PBGL) in February 2021 as a Laboratory Attendant. Ms Akgun obtained a BSc in Genetics and Bioengineering at Istanbul Bilgi University, Turkey (2017) and MSc in Evolutionary Systems Biology at the

University of Vienna, Austria (2020). In 2017 she was an Intern at the PBGL for six months where she worked on the molecular characterization of Orange Lemma mutants and marker development in barley. Parallel to her studies, she worked at a pharmaceutical company and other institutes. She has experience in population genetics, developmental biology and metabolomics. Ms Akgun is covering for Mr Adel Ali during his Development Reassignment. Welcome back Eda!



Mr Andrew Gennett (USA)

We are sad to bid farewell to Andrew Gennett who joined the PBGL in January 2019 as a Junior Professional Officer (JPO) Plant Pathology/Weed Science. During his two years' stay at the PBGL he contributed significantly to the work of the *Striga* CRP D25005 in verifying *Striga* resistant/tolerant rice mutants from Burkina Faso and Sudan, and in optimizing screening protocols for resistance to Fusarium wilt TR4 disease in banana. In addition, he participated in R&D activities of the pilot project of molecular screening for mutants (FGTU) in sorghum in the field and glasshouse. We greatly appreciate his contributions and wish him all the best in pursuing a master's degree in Spain.

Forthcoming Events

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

First Research Coordination Meeting (RCM) on Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops, D24014, (*Virtual Meeting*), 16–20 August 2021. (More information on Page 8)

Second Research Coordination Meeting (RCM) on Enhanced Biotic-stress Tolerance of Pulses Towards

Sustainable Intensification of Cropping Systems for Climate-change Adaptation, D22006, (*Virtual Meeting*), 6–10 September 2021. (More information on Page 8)

Consultants Meeting

Consultants Meeting on Radiation-induced Crop Genetic Diversity and Functional Genomics to Accelerate Variety Development for Tolerance to Climate Extremes, (*Virtual Meeting*), 2–6 August 2021. (More information on Page 9)

Past Events

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

Third Research Coordination Meeting (RCM) on Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding, D23031, (*Virtual Meeting*), 12–16 April 2021. (More information on Page 9)

Second Research Coordination Meeting (RCM) on Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change, D23032, (*Virtual Meeting*), 26–30 April 2021. (More information on Page 9)

Final Research Coordination Meeting (RCM) on Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security, D25005, (*Virtual Meeting*) 17–12 May 2021. (More information on Page 10)

Other Meetings

First Project Coordination Meeting on Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia, RER5024, (*Virtual Meeting*), 8–12 February 2021. (More information on Page 16)

Training Course on Plant Mutation Breeding and Efficiency Enhancing Techniques for Climate Change Adaptation, RER5024 and RAF5083, Seibersdorf, Vienna, Austria, 14–25 June 2021. (More information on Page 16)

First Project Coordination Meeting on Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA), RAS5088, (*Virtual Meeting*), 21–25 June 2021. (More information on Page 16)

Coordinated Research Projects (CRPs)

Project Number	Ongoing CRPs	Project Officers
D25005	Mutation Breeding for Resistance to <i>Striga</i> Parasitic Weeds in Cereals for Food Security (2016–2022)	A.M.A. Ghanim L. Jankuloski
D23031	Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding (2017–2021)	F. Sarsu A.M.A. Ghanim
D23032	Disease Resistance in Rice and Wheat for Better Adaptation to Climate Change (2018–2023)	L. Jankuloski I.K. Bimpong
D22006	Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation (2019–2024)	S. Sivasankar N. Warthmann
D24014 (New)	Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops (2021–2025)	I.K. Bimpong S. Sivasankar
D20003	Impact Assessment of Mutant Crop Varieties Developed by BINA, Bangladesh (Single-contract project)	S. Sivasankar
D20004	Impact Assessment of Cotton Mutant Varieties Developed by NIAB, Pakistan (Single-contract project)	L. Jankuloski

Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security, D25005

Project Officers: A.M.A. Ghanim; L. Jankuloski

Striga is a serious parasite in cereal crops and cowpea in sub-Saharan Africa, infesting more than 50 million ha, affecting nearly 300 million people and causing crop losses in the range of 20 to 100% in infested fields of maize, millet, rice and sorghum with enormous economic losses. The main objective of the *Striga* CRP D25005 is to develop laboratory, screenhouse and field screening protocols of mutant populations of sorghum and upland rice for resistance to *Striga asiatica* and *S. hermonthica*. In addition, the CRP addressed technologies such as rapid cycling of crop plants, doubled haploid techniques and molecular markers to enhance the efficiency of mutant identification and to accelerate the delivery of resistant varieties.

The CRP is being implemented during 2016 to 2021, so this is the final year of the project. Field, screenhouse and laboratory screening protocols for mutant populations of cereals, mainly upland rice, maize, and sorghum are completed and submitted for compilation into a CRP book of protocols. The book includes three field and screenhouse screenings, three laboratory screenings, one rapid cycling of generation in sorghum and two *in vitro* doubled haploid in rice protocols.

Substantial achievements were produced in terms of planned protocols, development of new germplasm of resistant mutants in rice, maize, and sorghum for *Striga hermonthica*.

and *Striga asiatica* in farmer-preferred varieties from participating *Striga*-prone Member States in Africa. At least a few of them were advanced for multi-locational trials to be released in Burkina Faso and Madagascar. Furthermore, for the first time through this CRP the scope of *Striga* resistance mechanism is widened. Results in sorghum mutants from Burkina Faso showed resistance in terms of low *Striga* germination stimulant producer, *Striga* germination inhibitor, hypersensitive reactions, necrosis leading to early death of *Striga* plants. Details of the achievements will be published in the CRP book and peer-reviewed publication. See more highlights about achievements of the CRP in other sections of this newsletter.

Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding, D23031

Project Officers: F. Sarsu; A.M.A. Ghanim

The CRP officially started in October 2017 and will be completed by the end of 2021. The third and final RCM was held virtually from 12 to 16 April 2021.

This CRP focuses on improving rice and sorghum to drought tolerance for current and future climate change scenarios. These two crops are essential staples in the diets of millions of impoverished and vulnerable populations, and therefore any attempt in increasing their yields under drought stress could have a major and positive impact in terms of food security and income generation.

The main objective of the CRP is to improve drought resilience of rice and sorghum germplasm through induced

mutations and the development/adaptation of screening techniques for sustainable food security. Since mutation breeding involves the screening of large mutant populations, effective protocols are required to reduce the cost and labour of selecting the rare, useful variants. The goal is to simplify the identification of drought tolerant lines of rice and sorghum in breeding populations in glasshouse and controlled-environment growth chambers using a screening method at the flowering stage as this is the development stage most vulnerable to drought stress. Improving grain yield under drought through selection on secondary traits such as root architecture, leaf water potential, panicle water potential, osmotic adjustment, and relative water content did not yield the expected results to improve grain yield under drought. Breeders and physiologists practiced selection for secondary traits as low selection efficiency for direct selection of grain yield under drought stress. These physiological traits rather could be used as the confirmation tools after selection in M₃ stage based on phenotyping in the field at M₃ stage. Similarly, at the molecular level, initial efforts in rice were devoted to mapping of QTLs for secondary drought-related traits such as root morphology and osmotic adjustment.

Following extensive and very enthusiastic discussions during the final RCM, below deliverables/achievements were highlighted:

1. Robust greenhouse, screenhouse and field-based screening protocols developed under the CRP which allow plant breeders to screen for enhanced tolerance to drought stress in rice and sorghum in a plant mutation breeding programme. Two critical heat sensitive stages in the life cycle of the crops were targeted - seedling and reproductive stages (before/after flowering) with screening based on simple phenotypic responses. The protocols were optimized and tested for efficient screening of mutant lines for drought tolerance in rice and sorghum which has been specifically adapted for a mutation breeding programme.
2. Secondary traits were also tested for physiological and biochemical indicators associated with the phenotyping screening protocols. The drought tolerant known as potent mutant lines were subjected to biochemical assays such as antioxidant enzyme activity [superoxide dismutase (SOD), catalase activity (CAT), and ascorbate peroxidase (APX)], electrolyte leakage, SPAD value, grain yield loss, and measurement of drought stress indicators (proline and methylglyoxal) at reproductive stage under both, control and drought stress conditions. Under drought stress, it was observed that antioxidant enzymes such as superoxide dismutase (SOD), catalase activity (CAT), and ascorbate peroxidase (APX) were more expressed as compared to the wild type.
3. Furthermore, the candidate drought tolerant mutant lines were also tested in hot spot areas in the field in Bangladesh, China, Malaysia, Pakistan, and Vietnam to evaluate their performance under field conditions and drought stressed growing environments. Drought tolerant rice and sorghum mutant germplasm were identified for current and future climate change scenarios. The identified mutant lines will be released to farmers.
4. Genetic and molecular confirmation to validate drought tolerance explored in rice and sorghum as given below:
 - Screening for stay green specific SSR markers to identify association between drought responsive genes in sorghum;
 - Transcription analysis for seedling stage of rice, expression profiling of DREBs, TPS and GLY genes in gamma induced mutant lines;
 - Genetic mapping of selected drought tolerant rice lines by Mutmap analysis;
 - Whole genome sequencing for drought tolerant mutants to develop molecular markers.
5. Capacity building in efficient mutation breeding and screening techniques for drought tolerance in rice and sorghum.
6. Scientific publications, guidelines, brochures, and protocols generated from the findings of the research activities.

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

Project Officers: L. Jankuloski; I.K. Bimpong

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 Research Coordination Meeting (RCM) from 10–14 December 2018 in Vienna, Austria. The second RCM was held virtually from 26–30 April 2021. The third RCM is planned to be held in April 2022 in China.

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

Project Officers: S. Sivasankar; N. Warthmann

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 pod 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 is planned to be held virtually in September 2021.

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.

Good progress has been made during its first year (2020) despite the difficulties of the pandemic situation. The CRP is planned to be completed in 2021 with a full report of the results achieved.

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

Project Officer: L. Jankuloski

A new individual CRP on Impact Assessment of Cotton Mutant Varieties Developed by NIAB, Pakistan, was initiated this year.

The overall objective of this single-contract CRP is to evaluate the economic impact of the cotton mutant varieties developed by Nuclear Institute for Agriculture and Biology.

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 suggest policy recommendations for promotion of mutant cotton varieties.

The CRP is planned to be completed by the end of 2021.

Forthcoming Events

First Research Coordination Meeting (RCM)

Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops, D24014
Virtual Meeting. 16–20 August 2021

Project Officer: K. Bimpong

This project builds upon previous initiatives to address the bottlenecks in vegetatively propagated and horticultural tree crops through mutation breeding. It is intended to enhance the current effort by (1) developing some *in vitro* mutation induction techniques that can lead to the development of chimera-free regenerants and stable mutant lines of cassava, potato/sweet potato and olive; and (2) screening techniques and functional genomic tools for specific disease resistance in cassava, potato/sweet potato and olive.

The project participants include three researchers from Austria, Belgium and Spain as technical contract holders with olive crop as their focus, four researchers from Ghana, Kenya, Malaysia and Uganda as research contracts holders with cassava as their target crop, three researchers from Chile, Colombia and Peru also as research contracts holders with potato/sweet potato as their focus crop, and three researchers from India, Sweden and USA as research agreements holders serving as advisors for the CRP.

The Coordination Meeting has been re-scheduled from 21–25 June 2021 to 16–20 August 2021 as a result of the COVID-19 pandemic. The purpose of the meeting is to review and design the technical work plan for the participating researchers for the year and for the entire project duration. This meeting will be held virtually and open to designated researchers of the CRP D24014 and the PBG Technical Officers.

Second Research Coordination Meeting (RCM)

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

Virtual Meeting. 6–10 September 2021

Project Officer: S. Sivasankar

The CRP plans to hold its second RCM during 6–10 September 2021 with primary focus on presenting and discussing research results to date, trouble-shooting technical issues in implementation, and planning the next steps including course corrections in research implementation where necessary. This RCM allows the three separate crop teams (cowpea, chickpea and lentil) to

come together immediately after three informal crop meetings in August to ensure effective implementation, with finer coordination where necessary, to achieve intended objectives during the subsequent duration of the CRP. This second RCM will be followed by the final RCM in 2023, and is anticipated to bring participants together in Vienna, Austria.

Consultants Meeting

Radiation-induced Crop Genetic Diversity and Functional Genomics to Accelerate Variety Development for Tolerance to Climate Extremes

Virtual Meeting. 2–6 August 2021

Project Officer: S. Sivasankar

Climate change increasingly threatens agricultural biodiversity and crop productivity. Farmers, especially smallholders in developing countries, continue to wrestle with crop losses resulting from droughts, flooding, pests and diseases. Radiation-induced genetic diversity, coupled with mutation breeding, remains the most efficient and productive avenue to develop resilient crop varieties for cultivation by farmers. Current advances in mutation induction, genomics and big data recently prompted Member States to come together with IAEA support in two regional Mutation Breeding Networks in the Asia Pacific and Latin America for further technology development and exchange. Use of new mutagen sources including ion beam, e-beam and cosmic rays or space breeding are becoming increasingly relevant for accelerated variety development along with functional genomics for marker-assisted breeding and gene editing.

The general objective 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 within the framework of the Mutation Breeding Network to facilitate the accelerated development of crop varieties resilient to the impact of climate change.

The planned Consultants' Meeting will define a Concept Note to address this general Objective, which is anticipated to result in an open call for proposals in late 2021. The new CRP will start in early 2022.

Past Events

Third Research Coordination Meeting (RCM)

Improving Crops Resilience to Drought in Rice and Sorghum through Mutation Breeding, D23031

Virtual Meeting. 12–16 April 2021

Project Officer: F. Sarsu

Twelve participants from 10 Member States (Bangladesh, China (2), India (2), Indonesia, Japan, Mali, Malaysia, Pakistan, Sudan, and Viet Nam) plus four observers from Pakistan, Malaysia (2), Food and Agriculture Organization of the United Nations (FAO) and Technical Officers from the Plant Breeding and Genetics Subprogramme participated

in the meeting. Ms Shoba Sivasankar, Head, Plant Breeding and Genetics Section delivered the opening remarks and gave a presentation on the Plant Breeding and Genetics Subprogramme. In the first two days of the meeting, participants presented their research activities and achievements. During the RCM, the progress of individual projects and data including mutant lines with improved drought tolerance were reviewed and evaluated.

The participants also presented their excellent results obtained under this CRP and discussed how to transform these outputs into guidelines and protocols for efficient mutation breeding applications to contribute to global food security.

For detailed information on the project achievements, see Coordinated Research Projects (CRPs) on page 6.

Second Research Coordination Meeting (RCM)

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

Virtual Meeting. 26–30 April 2021

Project Officer: L. Jankuloski

Diseases are among the major obstacles hindering yield improvements, both in rice and wheat. Changing climatic conditions increase the spread of diseases to new destinations and exacerbate their impact. In addition to the already widely spread diseases of rice such as blast, sheath blight, false and bacterial leaf blight, threats of emerging diseases are becoming more serious such as in the case of false smut of rice and blast of wheat.

The objectives of this Coordinated Research Project (CRP) are to improve disease resistance in rice and wheat through induced mutation/mutation breeding, to develop protocols for screening rice resistant mutants to diseases and to develop molecular markers for disease resistance using available mutant germplasm.

The meeting objectives were to assess the progress and consolidate the activities among participating contract holders.

The second Research Coordination Meeting (RCM) was attended by nine participants from Bangladesh, Brazil, China, India, Indonesia, Malaysia, Pakistan, Tanzania, USA, and two observers from FAO.

Each participant presented the progress and achievements made within the CRP, particularly during the period after the last RCM in December 2018.

In the first two days of the meeting, contract holders of the CRP made presentations highlighting their progress since December 2018 and their work plans for next years, followed by discussions. During the third and fourth day, the Project Officer had individual meetings with all participants discussing their work plans for the next years. The fifth day, CRP progress and work plan activities for the next years were summarized.

It was generally agreed that progress made by participants on planned activities during 2018–2020 was satisfactory and even excellent in certain projects, toward the goals set during the first RCM and according to their individual work plans. Specific recommendations were made for those producing mutant population and development of F₂ generations.

Here are some important achievements:

- Developed specific primers for *Magnaporthe Oryzae Triticum* isolates and RPA (Recombinase Polymerase Amplification) rapid detection method of wheat blast pathogen;
- Identified five blast resistant wheat mutant lines in M₃ generation;
- Identified six wheat orthologs of rice blast susceptible genes and uncovered a total of 171 SNPs in M₃ wheat population;
- Identified three highly resistant and two moderately resistant mutant lines to BLB in rice M₃ generation;
- Developed M₂ wheat mutant population for screening in hot-spot areas in Bangladesh;
- Developed two different crosses between rice mutant lines resistant to BLB and susceptible genotypes. Developed F₁ generation and F₂ generation is in progress for phenotyping,
- Developed four different crosses between rice blast resistant mutant lines and susceptible genotypes. Developed F₁ generations and F₂ generations are in progress for phenotyping;
- Developed M₂ population that is being subjected to artificial infections and phenotyping for rice blast resistance;
- Identified two mutant lines showing significantly lower infection of false smut disease in field hotspot (plus artificial inoculation);
- Selected four rice mutant lines as putative mutants resistant to bakanae disease (*Gibberella fujikuroi*).

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 F₂ generations. PBGL will be involved in providing services for sequencing and bioinformatics analysis.

The third RCM will be held in April 2022 in China where the progress will be reviewed, and future activities will be discussed among participants.

**Final Research Coordination Meeting (RCM)
Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security, D25005**

Virtual Meeting. 17–21 May 2021

Project Officer: A.M.A. Ghanim

The parasitic weeds *Striga* are major biological constraints to cereal production in most of sub-Saharan Africa and semi-

arid tropical regions of Asia. The main objective of the CRP D25005 is to develop laboratory, greenhouse and field screening protocols of mutant populations of sorghum and upland rice for resistance to *Striga asiatica* and *S. hermonthica*. In addition, the CRP focuses on speed breeding technologies such as rapid cycling, doubled haploid techniques and genomics to enhance efficiency of mutant identification and accelerate delivery of resistant varieties.

The final RCM was originally planned for the fourth quarter of 2020. After revision during the CCRA meeting in October 2019, it was decided to hold the meeting in the second quarter of 2021. The RCM was organized virtually from 17 to 21 May 2021 with the objective to share the progress and challenges in the remaining final portion of the CRP, revising outputs, achievements and prepare for the closure of the CRP. The meeting was attended by six research contract holders (Burkina Faso, Iran, Kenya, Madagascar, Sudan and Turkey), two technical contract holders (Japan and USA), and two agreement holders (The Netherlands and USA), besides the staff of the Plant Breeding and Genetics Subprogramme.

The meeting was opened by the Head of the Plant Breeding and Genetics Section, Ms Shoba Sivasankar, with an introduction to the Plant Breeding and Genetics Subprogramme. During the first two days of the meeting, the participants provided an update on the progress made during the reporting period and a summary of the overall achievements. Thereafter, individual virtual meetings were held with the CRP participants to discuss the final reports and the way forward towards closure of the project. Substantial achievements were presented by the project contract and agreement holders based on the projects' objectives and targeted outputs:

1. Three field and greenhouse screening protocols were developed and submitted for publication by Burkina Faso, Madagascar and Sudan;
2. Three laboratory protocols were developed by Japan and USA for analyzing mechanisms of resistance by histological, gel and rhizotron assays;
3. A protocol on Next Generation Sequencing (NGS) applications was developed by Japan for the discovery of causative mutation and marker development for resistance to *Striga*;
4. Doubled haploid protocols were developed by PBGL, Iran and Turkey;
5. The PBGL contributed to the protocols on greenhouse screening, doubled haploid in rice, rapid cycling in sorghum and development of mapping materials for NGS-based discovery of causative mutations for marker development.

The meeting was closed on 21 May with a summary of the meeting report and closing remarks by the Section Head. The meeting concluded that the CRP has excellently met the objectives and achieved the expected outputs including a draft book of protocols for screening and efficiency enhancing molecular and *in vitro* technologies, verified resistant germplasm with prospective of releasing resistant

varieties in some of the participating countries; strong capacity building in the participating Member States in breeding for resistance of *Striga* in cereals and building of extensive networks between national programmes from the developing Member States and advanced laboratories of leading world experts in the field of *Striga* parasitic weeds.

Technical Cooperation Field Projects

Project Number	Country/Region	Title	Technical Officer(s)
AZB5002	Azerbaijan	Developing Mutant Cotton Breeding Lines Tolerant to Diseases, Drought and Salinity through Mutation Breeding	F. Sarsu
BKF5019	Burkina Faso	Improving Food Crop Genotypes for Enhancing Yield and Adaptation to Climate Change Using Mutation Breeding and Isotopic Techniques	L. Jankuloski (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
BOT5019	Botswana	Improving Selected Legumes and Cereals against Biotic and Abiotic Stresses to Improve Food Production and Security	I.K. Bimpong
BUL5015	Bulgaria	Increasing Productivity and Quality of Basic Food Crops	F. Sarsu
BUL5016	Bulgaria	Improving the Productivity and Quality of Economically Important Crops through Mutation Breeding and Biotechnology	F. Sarsu
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>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
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	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
ERI5011	Eritrea	Developing Improved Banana and Maize Varieties through Mutagenic Nuclear Techniques	A.M.A. Ghanim
GHA5038	Ghana	Using Irradiated Pollen for the Development of Provitamin A Rich, Drought Tolerant and Cassava Mosaic Disease Resistant Cassava Mutants	F. Sarsu
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>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
IRA5015	Iran, Islamic Republic of	Enhancing Capacity of National Producers to Achieve Higher Levels of Self-Sufficiency in Key Staple Crops	L. Jankuloski (<i>Participating</i>) in collaboration with FEP (<i>Leading</i>) and SWMCN (<i>Participating</i>)
IRQ5023	Iraq	Utilizing Nuclear Technology to Improve Key Legume Crops for Climate Change Adaptation	I.K. Bimpong

Project Number	Country/Region	Title	Technical Officer(s)
IVC5039	Cote d'Ivoire	Improving Maize Production in Savannah Areas with Severe Pedoclimatic Degradation in the North of Cote d'Ivoire through the Cultivation of Induced Mutants Adapted to these Areas	K. Bimpong in collaboration with APH (<i>Participating</i>)
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>Participating</i>) in collaboration with IPC (<i>Leading</i>)
KAZ5004	Kazakhstan	Developing Drought Tolerant and Disease Resistant Wheat Varieties with Enhanced Nutritional Content Using Mutation Breeding	F. Sarsu
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>Leading</i>) in collaboration with APH (<i>Participating</i>)
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 (<i>Participating</i>) in collaboration with SWMCN (<i>Leading</i>) and IHS (<i>Participating</i>)
MAL5032	Malaysia	Strengthening National Capacity in Improving the Production of Rice and Fodder Crops and Authenticity of Local Honey Using Nuclear and Related Technologies	F. Sarsu (<i>Leading</i>) in collaboration with FEP (<i>Participating</i>) and SWMCN (<i>Participating</i>)
MAR5023	Mauritius	Improving Landraces of Crucifers (Cauliflower and Cabbage) and Carrot through the Use of Nuclear Techniques for Mutation Breeding and Biotechnology	F. Sarsu
MLW5003	Malawi	Developing Drought Tolerant, High Yielding and Nutritious Crops to Combat the Adverse Effects of Climate Change	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
NAM5016	Namibia	Developing Drought Tolerant Mutant Crop Varieties with Enhanced Nutritional Content	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
NAM5017	Namibia	Improving Crops for Drought Resilience and Nutritional Quality	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
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

Project Number	Country/Region	Title	Technical Officer(s)
NHE5001	Vanuatu	Enhancing the Productivity and Quality of Crops through the Application of Mutation Breeding Techniques	L. Jankuloski
NIC5011	Nicaragua	Broadening the Genetic Variation of Vegetative Propagated Crops Using Nuclear Techniques	F. Sarsu
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 (<i>Participating</i>) in collaboration with RPRT (<i>Leading</i>) and ARBR (<i>Participating</i>)
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.M.A. Ghanim (<i>Participating</i>) in collaboration with SWMCN (<i>Leading</i>)
RAF5083	Regional Africa	Enhancing Crop Productivity through Climate Smart Crop Varieties with Improved Resource Use Efficiency (AFRA)	S. Sivasankar
RAS0080	Regional Asia	Promoting Self-Reliance and Sustainability of National Nuclear Institutions	S. Sivasankar (<i>Participating</i>) in collaboration with PCG (<i>Leading</i>), RPRT (<i>Participating</i>) and PHY (<i>Participating</i>)
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	F. Sarsu
RAS5088	Regional Asia	Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA)	S. Sivasankar
RER5024	Regional Europe	Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia	F. Sarsu
RLA5068	Regional Latin America	Improving Yield and Commercial Potential of Crops of Economic Importance (ARCAL CL)	F. Sarsu
RLA5084	Regional Latin America	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	S. Sivasankar (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>) and APH (<i>Participating</i>)
RWA5001	Rwanda	Improving Cassava Resilience to Drought and Waterlogging Stress through Mutation Breeding and Nutrient, Soil and Water Management Techniques	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
SAF5016	South Africa	Promoting Mutation Breeding of Vegetables to Improve Rural Livelihoods — Phase I	N. Warthmann

Project Number	Country/Region	Title	Technical Officer(s)
SIL5020	Sierra Leone	Enhancing the Concurrent Selection and Evaluation of Biofortified and Bio-enriched Varieties Derived from Mutant Rice, Cassava and other Crops	I.K. Bimpong
SIL5021	Sierra Leone	Improving Productivity of Rice and Cassava to Contribute to Food Security	I.K. Bimpong (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
SRL5050	Sri Lanka	Supporting Genetic Improvement of Tea	S. Sivasankar
SUD5037	Sudan	Applying Nuclear Techniques to Improve Crop Productivity and Livelihood of Small-scale Farmers in Drought Prone Areas	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)
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 (<i>Participating</i>) in collaboration with SWMCN (<i>Leading</i>)
TUN5029	Tunisia	Developing Barley and durum Wheat Resilience to Drought and Heat Tolerance through Mutation Breeding	F. Sarsu
UGA5041	Uganda	Developing Disease Resistant High Yielding Farmer Preferred Cassava Varieties in Uganda through Induced Mutation Breeding	L. Jankuloski
URT5032	Tanzania, United Rep. of	Developing Maize Cultivars for Improved Yield and Resistance to Viral Disease	F. Sarsu
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
ZAM5031	Zambia	Improving the Yield of Selected Crops to Combat Climate Change	F. Sarsu (<i>Leading</i>) in collaboration with SWMCN (<i>Participating</i>)

APH: Animal Production and Health, NAFA; FEP: 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

Past Events

First Project Coordination Meeting

Enhancing Productivity and Resilience to Climate Change on Major Food Crops in Europe and Central Asia, RER5024

Virtual Meeting. 8–12 February 2021

Project Officer: F. Sarsu

The regional TC project is addressing the improvement of the main crops (legumes, cereals, and other important food crops) in Europe and Central Asia with technical support from the Joint FAO/IAEA Centre. Nuclear technology is used to generate new beneficial mutations followed by mutation breeding to develop improved lines and varieties. The overall objective of the project is to support 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.

This meeting was open to designated counterparts of the TC project RER5024. The main objectives of this first coordination meeting were (1) to review the work plan of this new regional TC project RER5024 (2020–2024); (2) to establish national work plans of the participating countries to be implemented during 2020–2024; and (3) to provide technical feedback to strengthen crop improvement efforts in participating countries. The meeting was held virtually and participants from 22 countries (Albania, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Georgia, Greece, Kazakhstan, Kyrgyzstan, Moldova, Montenegro, North Macedonia, Poland, Portugal, Russia, Serbia, Slovakia, Slovenia, Tajikistan, Turkey and Uzbekistan) attended the meeting.

The participating project coordinators (PCs) presented their project work plans and arrangements for the future project activities. Technical work plans of the individual projects for the coming year and the project duration were discussed and feedback was provided by the Technical Officer. The Technical Officer also discussed the individual and regional needs for 2021–2023.

Training Course

Plant Mutation Breeding and Efficiency Enhancing Techniques for Climate Change Adaptation, RER5024 and RAF5083

Seibersdorf, Vienna, Austria. 14–25 June 2021

Project Officers: F. Sarsu and S. Sivasankar

Course Director: I. Ingelbrecht

This was a joint training course between the regional TC projects on (1) Enhancing Productivity and Resilience to Climate Change of Major Food Crops in Europe and Central Asia, RER5024; and (2) Enhancing Crop

Productivity through Climate Smart Crop Varieties with Improved Resource Efficiency (AFRA) RAF5083. The objective of the training course was to strengthen Member States' understanding and research laboratory capacities in basic principles of crop mutation breeding and advanced molecular techniques for improved mutation detection and selection with a focus on seed propagated crops. These capabilities are intended to mainstream applications of molecular genetics, genotyping and bioinformatics tools for improved resilience to climate change. The course consisted of theoretical sessions and exercises on (1) Scientific background of mutation induction and its application to crop breeding (induced mutation, radio sensitivity test, mutant population development; (2) Marker-assisted breeding and molecular marker application in mutation breeding; (3) Principles of genetic mapping (recombination, linkage data and segregation analysis); and (4) Next Generation Sequencing (NGS) and its applications for mutation breeding.

First Project Coordination Meeting

Enhancing Crop Productivity and Quality through Mutation by Speed Breeding (RCA), RAS5088

Virtual Meeting. 21–25 June 2021

Project Officer: S. Sivasankar

Plant mutation breeding has played important roles in the past 60 years to breed new mutant varieties, elite lines and mutant germplasm. However, the long breeding circle restricts the release of new varieties. Newly developed speed breeding techniques, such as double haploidy, marker-assisted selection and rapid generation advance, among others, are being utilized to develop genetically stable improved lines, but the technique has not yet been widely used in the Asia and Pacific Region. The current project aims to combine mutation induction with speed breeding methods in a regional Technical Cooperation project for the Asia and Pacific region, with technical support from the Joint FAO/IAEA Centre. The general objective of the project is to improve food security in the Asia Pacific region through the faster release of mutant crop varieties with improved productivity and quality, with the anticipated outcome of established mutation by speed breeding approach in the RCA GPs including relevant technologies in speed breeding.

The meeting was open to designated counterparts of the regional TC project, RAS5088. The meeting objectives were to: (1) provide an overview of the objective, expected outcome and outputs, and workplan of the project; (2) develop national workplans; and (3) provide technical feedback on mutation breeding plans, especially on speed breeding technologies. The national project coordinators presented and finalized their workplans in discussion with the Technical Officer.

Feature Articles

Ion Beam Mutagenesis for Crop Improvement

Tomoko Abe

Ion beam Breeding Team, Beam Mutagenesis Group, RIKEN Nishina Center for Accelerator-Based Science, Wako, Japan

Introduction

Ion beams have been used to develop approximately 70 new crop varieties in Japan in the last two decades, and beneficial mutants have been identified in a variety of species. These mutants include aromatic rice with high fertility, lettuce with low polyphenol oxidase, mandarin oranges with delayed coloring and prolonged harvest, a medicinal plant with high productivity of the medicinal ingredient, peanuts without major allergens, cherry blossoming without cold vernalization, microalgae with high oil productivity, moss with increased ease of culturing and high adsorption of heavy metals, and large marine plankton.

Linear Energy transfer (LET) is an important parameter in radiation biology. LET is the amount of energy given by an ionizing particle to the target per unit path length along its track, and higher LET means larger biological effect in general. X rays and gamma-rays have low LET of 0.2–2 keV/ μm and produce secondary radicals throughout the irradiated cell nucleus and cause single-strand breaks in DNAs. Ion beams generated by particle accelerators have much higher LET that is widely variable depending on the ion species and the velocity. When a high LET is required a heavier, highly charged ion is selected with a low velocity. For example, an LET of 23 keV/ μm is available from 135-AMeV carbon (C) ions and 640 keV/ μm from 90-AMeV iron (Fe) ions at our irradiation facility in RIKEN Nishina Center.

High-LET ion beams break both DNA double-strands and histones, causing cluster damages. Such cluster damages can only be repaired incompletely by cells, thus inducing mutations. In X rays and gamma rays, the only physical factor that affects mutagenesis is the dose, but in ion beams, not only the dose but also the LET are crucial. Effective LETs for crop breeding are between 23 and 70 keV/ μm because of the high mutation rates achieved by this range. These mutations are mostly small but are sufficient to disrupt a gene. On the other hand, LETs of 290 to 800 keV/ μm are good for microbe breeding.

Advantage of Ion Beams over other Mutagens

A fast ion densely deposits its energy in a localized region along its path and breaks DNA only within its nanometer-diameter track. Because ions have low impacts on the whole genome and induce small number of genetic mutations, the mutants can survive and be developed into new varieties. Thus, ion beams efficiently induce mutations at low doses that do not reduce viability or severely inhibit growth. A short irradiation period between seconds to a few minutes is enough to induce mutations. When the optimal LET for mutation induction is selected, the mutation rate would be comparable to chemical mutagens known to produce a high mutation rate. Table 1 shows new varieties of various crops recently developed in Japan using ion beams. We have succeeded in selecting useful mutants from M_2 populations of 100 to 1000 plants.

Table 1. New Varieties Developed in Various Crops Using Ion Beam Irradiation in Japan

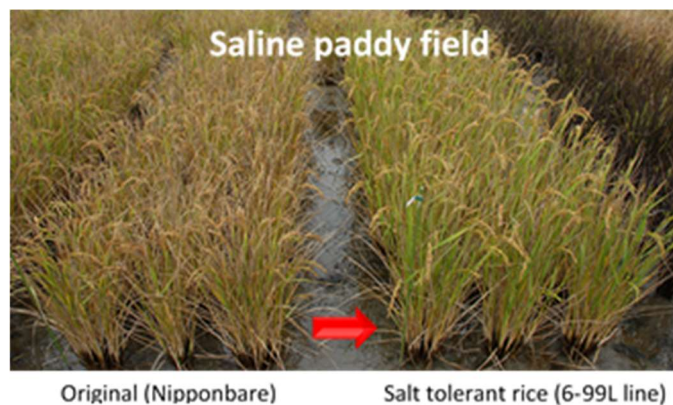
Plant New Variety	Breeding Goal	Treated Material	No. Mutants/Selection Population	Accelerator Facility	Reference
Lettuce 'Fortuna'	High yield	IVC (leaf)	4/98 M_2 plants	W-MAST	Personal communication
Onion 'Smile ball'	Tearless and non-pungent	Dry seed	18/197 M_2 bulbs	RIBF	Kato <i>et al.</i> , Sci.Rep.6,23779 (2016)
Tomato 'Lucina'	Parthenocarpy	Dry seed	16/400 M_1 plants	W-MAST	Personal communication
Edible Chrysanthemum 'Kiku Meigetsu'	Late flowering	IVC (floral petal)	43/859 regenerated plants	RIBF	Endo <i>et al.</i> , Bull. Agricul. Res. Yamagata pref. 8,93 (2016)
Japanese millet 'Nebariko 2 go'	Short culm	Dry seed	17/906 M_2 plants	RIBF	Nakajo <i>et al.</i> , Millet Newslet.24,12 (2009)
Rice 'Koshihikari-kan 1 go'	Low cadmium uptake	Dry seed	3/2592 M_2 plants	TIARA	Ishikawa. <i>et al.</i> , Proc. Natl. Acad. Sci. USA 109, 19166 (2012)
Sake Yeast 'Saitama G Yeast'	Good flavor and fermentability	Yeast	2/260 cerulenin resistant strains	RIBF	Yokobori <i>et al.</i> , RIEKEN APR 45, H-23 (2012)

IVC: *In Vitro Cultured* Tissues, RIBF: RI Beam Factory (RIKEN), W-MAST: the Wakasawan Energy Research Center Multipurpose Accelerator System with Synchrotron and Tandem, TIARA: The Takasaki Ion Accelerators for Advanced Radiation Application (QST)

Induced Mutagenesis at the RIKEN RI Beam Factory

The RIKEN RI Beam Factory (RIBF) is a heavy ion accelerator complex consisting of four ring cyclotrons and two injectors and can accelerate ions from hydrogen to uranium to high energies. Although nuclear physics is the primary subject of the RIBF, the ion beams are also used in ion beam mutagenesis for breeding since the beam energy is high enough to irradiate biological samples in the air. The ion beam accelerated by the RIKEN Ring Cyclotron (RRC) is transported to the E5B beam line used exclusively for biology experiments. We have developed an automatic irradiation system, which consists of a range shifter and an automatic sample changer to cope with numerous requests with various biological samples (dry seeds, callus, tissue cultured materials, seedlings, cuttings etc.). The ion beam passes through aluminum plate absorbers in the range shifter, and the decreasing velocity of the beam results in increasing the LET. Therefore, the LET of the ion beam incident on the samples can be adjusted using the range shifter, for example, between 23 and 290 keV/ μm of C ion and 640 and 3762 keV/ μm of Fe ion. We irradiated *Arabidopsis* seeds with ion beams and investigated albino mutants in the M₂ generation. Different LETs 23–640 keV/ μm were achieved by selecting the ion species and using the range shifter. The highest mutation rate was observed at 30 keV/ μm . It was comparable to that of chemical mutagens. The LET with a high mutation rate, designated as LET_{max}, was 30 keV/ μm in *Arabidopsis*, 23–39 keV/ μm in buckwheat, 23–50 keV/ μm in rice and 50–70 keV/ μm in wheat. By contrast, when LET was 290 keV/ μm , the mutation rate was low, and the survival rate was greatly reduced. A whole-genome analysis with high-throughput sequencing is a powerful tool used to characterize the nature of induced mutations. We have been using whole genome sequencing to analyze gene mutations in *Arabidopsis* and rice. C ions with LET_{max} mainly induced single nucleotide variants (SNVs) and small insertions and deletions (InDels), while the number of large deletions and chromosomal rearrangements was low. However, 290-keV/ μm Argon (Ar) ions showed a different mutation spectrum: SNVs and number of small InDels was low, while the number of large deletions ($\geq 100\text{bp}$) and chromosomal rearrangements was high. Number of mutated genes induced by C ion and Ar ion irradiation is less than 10, relatively small, and often only 1 mutation is found near the mapped location. Thus, irradiation with these ions can efficiently generate knockout mutants of a target gene and can be applied to reverse genetics. We compared the mutation type in the causative gene of *Arabidopsis* mutants induced by ion beam irradiation with three representative LETs at 23, 30 and 290 keV/ μm . The most mutations irradiated with C ion at 23–30 keV/ μm were small deletions (<100bp). Irradiation with 290-keV/ μm C and Ar ions reduced proportion of small deletions and increased that of large deletion and

chromosomal rearrangements. This result showed that the biological effect was dependent on LET rather than ion species. High-yielding, long-grain mutant lines and salt-tolerant lines were obtained as useful lines of rice. ‘Koshihikarigan 1go’ is a new variety with low cadmium uptake developed from irradiation with C ion at 76 keV/ μm in TIARA. The causative genes of these rice mutants were found to be new and not yet reported in relevance to the traits long grain, salt tolerance and cadmium uptake.

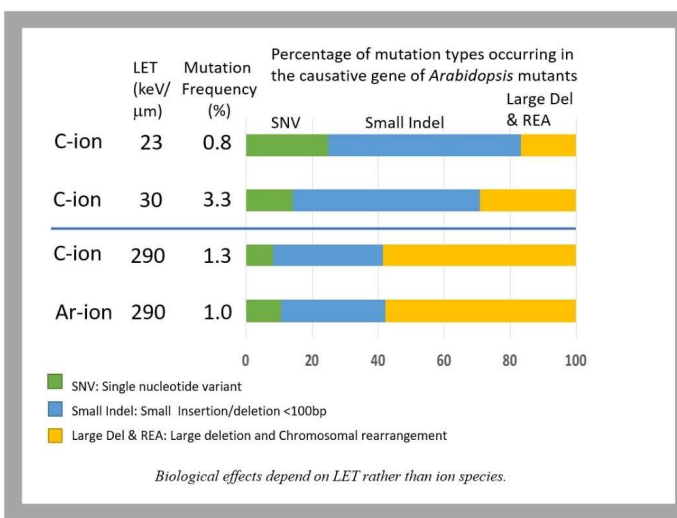
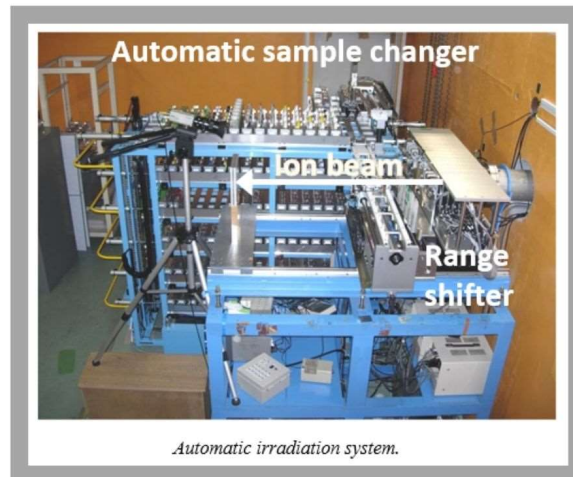
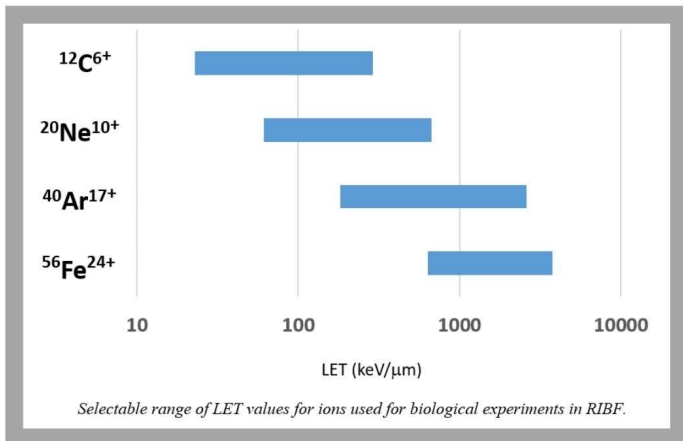


Salt tolerant mutant in saline paddy field.

Irradiation Services by RIKEN to FAO/IAEA Member States

Ion beams are now being increasingly used in plant mutation breeding although accelerator facilities remain limited across the world. The ion beam breeding team at RIKEN Nishina Center routinely uses this mutagen source for crop improvement and has also been supporting plant breeders in the region by providing ion beam mutagenesis as a service.

The RIKEN Nishina Center provides the following services to user parties with a collaboration research agreement. RIKEN undertakes the execution of irradiation experiments and support for developing mutagenesis techniques for users' samples, including the investigation of optimal exposure conditions. Irradiation experiments are conducted about five times a year in February, March-April, June-July, September, and December. Researchers in a user party prepare the samples and export them to Japan after obtaining an import / export permit and phytosanitary inspection in their own country. RIKEN receives the imported samples, stores them, conducts irradiation experiments, and exports the irradiated samples. Irradiated samples received by each country are evaluated for survival rate and initial growth rate which are reported back to RIKEN where the information is used to estimate optimum irradiation conditions. Desired mutants are selected by researchers in their own countries where priority given to seed samples subjected to irradiation conditions expected to produce a high mutation rate. Results of the irradiation experiment must be reported in the RIKEN Accelerator Progress Report.



Future Perspectives

In addition to the development of useful crop variants that can be deployed for cultivation, LETmax mutants have become more and more useful and important in modern genetic studies, enabling the discovery of genes that control important traits in crops. Since 2012, we have succeeded in identifying 14 new genes in rice, wheat, buckwheat, etc. One of them, the causative gene for high yielding, long grain trait in rice, *LINI*, can be a target gene for genome editing.

Because heavier ions such as Ar and Fe are expected to be more effective in breeding polyploidy crops and microbes, heavier ion beams with higher energy is required in future research. We thus built a new beam line ‘Wide AppliCable to Mutagenesis Experiment (WAKAME)’ to increase available nuclides with higher LETs and longer ranges.

The ion beams are accelerated by three cyclotrons, AVF, RRC and IRC, to 160 MeV/A and sent to the E5B beam line. As a result, a peculiar LET with a high mutation rate even with Ar ion has been discovered. We plan to develop a unique technology for mutagenesis using these heavier ion beams. Proton beams have relatively low LET, but there are many proton accelerator facilities for cancer therapy. Heavy-ion accelerator facilities that have been used for breeding purposes are RIBF, TIARA, W-MAST, Heavy Ion Medical Accelerator in Chiba (HIMAC) in Japan, Institute of Modern Physics (IMP) in China, and the Laboratori Nazionali del Sud (LNS) in Italy. Further in Asia, large accelerator facilities that can be used for mutation breeding are under construction in China and Korea. We intend to spread the unique technique globally through international collaboration.

Development of Early-flowering Durum Wheat Suitable for the Climate Conditions of the Hokuriku Region of Japan Through Conventional Breeding Aided by Heavy Ion Beam Mutagenesis

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Project Background

Durum wheat (*Triticum turgidum* ssp. *durum*) is a tetraploid species with the genome constitution AABB derived from two wild diploid ancestral species: The A genome from *T. urartu* and the B genome from *Aegilops speltoides* or another species classified in the Sitopsis section. Bread wheat (*Triticum aestivum*) is a hexaploid species with the genome constitution AABBDD that was derived from the crossing between durum wheat and *Ae. tauschii*, D genome donor of bread wheat.

Early maturity is one of the important properties of bread wheat in East Asia, including Japan, to avoid the rainy season from middle of June to July for harvesting. Therefore, the main target trait of breeding is early flowering/maturing. In the Hokuriku region located in the Japan-Seaside of Honshu, wheat plants must be both snow resistant and early maturing because of a lot of snow in the winter season.

Durum wheat is an important crop for making pasta in Japan: annual domestic demand for durum wheat is about 0.3 million tons. However, durum wheat cultivars are usually late-heading and not suitable for cultivation in Japan because of the rainy season. All known foreign cultivars of durum wheat show pre-harvest sprouting when exposed to prolonged rainfall before harvest. Furthermore, durum wheat cultivars are susceptible to Fusarium Head Blight disease. Because of these disadvantages, durum wheat has not been cultivated in Japan. Recently, one durum wheat cultivar ‘Setodure’ was developed by the National Agriculture and Food Organization. However, ‘Setodure’ is still susceptible to Fusarium Head Blight and was therefore released in a limited manner in the Seto-uchi region which receives little rain.

To develop durum wheat cultivars suitable for wide regions in Japan, especially in the Hokuriku region, we tried ‘the integrated breeding system’ which combines conventional pedigree breeding method and heavy ion beam mutagenesis.

Project Strategy

The durum wheat cultivar suitable for Japanese climate conditions should have the following agricultural traits for protection from the rainy season: early flowering/maturing, short culm length, lack of pre-harvest sprouting, and resistance to Fusarium Head Blight. In the present breeding project of durum wheat cultivars, we attempted to introduce reduced or no pre-harvest sprouting and Fusarium Head

Blight resistance from ‘Fuku-komugi’, an elite bread wheat cultivar developed by the bread wheat breeding programme of Fukui Prefectural University. To identify the genetic resources for short culm length, we examined agronomic characters of 80 foreign durum wheat strains collected by the NBRP (National Bio-Resource Project)-KOMUGI, wheat genetic resource protection project of the Japan Government. As a result, we identified the durum wheat strain N120 derived from Greece which shows short culm length with normal fertility in Japanese climate conditions. In our breeding programme, we used N120 as a donor for the short culm length trait. For the genetic source of early-flowering/maturing in durum wheat, we focused on identifying mutant plants of early-flowering/heading in durum wheat using heavy ion beam mutagenesis.

Preliminary Study for Heavy Ion Beam Mutagenesis in Wheat by Using Diploid Einkorn Wheat

The hexaploid and tetraploid wheat genome contains triplicated and duplicated homeologous genes, respectively, which were derived from each ancestral diploid species. This polyploidy feature increases the difficulty of screening for mutants in polyploid wheat. As the first step to develop a heavy ion beam mutagenesis system in wheat, we chose to use cultivated diploid einkorn wheat (*T. monococcum*) with A^m genome, similar to the A genome in bread wheat for making a large-scale mutant panel. We spent more than 10 years on this preliminary study as described below.

Dry seeds of the diploid einkorn wheat strain KU104-1 were irradiated with 20, 30, 40, 50 or 60 Gy of ¹²C⁶⁺ ions at LET of 30 or 50 keV/μm to determine the optimal conditions for generating viable mutants, using the E5B beam line of Ring Cyclotron (RRC) in the RIKEN RI-beam factory (RIBF). The irradiated seeds (called M₁ seeds) were sown in the experimental field of Fukui Prefectural University. About 150 seeds were sown for each LET-Dose (Gy) combination. At flowering time, the spikes of M₁ plants were bagged and harvested self-pollinated seeds from each spike were used to produce next generation (M₂) lines. About 70–120 M₂ lines for each LET-Gy combination were sown in the field. Ten seeds of each M₂ line were examined. The frequency of lines with albino plant(s) among the ten plants was determined in order to assess the comparative mutation rates of the different irradiation conditions. The frequency of albino mutations in the M₂ generation differed between the high- and low-LET treatments. A relatively higher rate of albino

mutations was found after 50 keV/ μm treatments than 30 keV/ μm treatments except for seeds given 60 Gy. The highest rate was about 4% for LET50–30 Gy combination and the second was about 3.5% for LET50–50Gy combination.

Furthermore, we also examined the effect of dose (Gy) and LET on plant viability and mutation rate by using einkorn wheat strain DV92 with 10, 15, 20, 30, 40, or 50 Gy of $^{12}\text{C}^{6+}$ ions at 50, 70, or 80 keV/ μm LET. The germination rate was examined using the irradiated seeds (M_1 seeds) of each LET-Gy combination. The germination rate was not affected by ion beam irradiation. The M_1 seedlings were planted in the field and the survival ratio was observed at the heading stage. The survival ratio was reduced to less than 80% when LET of ion beam was 70 or 80 keV/ μm . The harvested seeds from each individual M_1 plant were used to produce the next generation (M_2) lines. The M_2 lines for each LET-Gy combination were sown in the field; ten seeds of each M_2 line were examined. The frequency of lines with albino plant(s) among the ten plants was determined to assess the comparative mutation ratio of the different irradiation conditions. The frequency of albino plants in the M_2 generation was different for different LET-Gy combinations. The highest ratio was observed for the LET50–50 Gy treatment condition. The data of survival ratio and appearance rate of albino plants indicated that LET50–50 Gy treatment was the optimal condition of mutagenesis for einkorn wheat.

As a result of this 15-year effort, we have now developed a large-scale mutant panel of diploid einkorn wheat. This mutant panel stocks approximately 10,000 M_2 lines of einkorn wheat strains KU104-1, KU104-2 and DV92. Strain KU104-1 is a late-heading type and DV92 shows moderate earliness. KU104-2 is an X ray induced early flowering mutant derived from KU104-1. The mutant panel is very useful for identification and investigation of wheat genes. We continue to screen the mutant panel for mutations affecting reproductive growth, especially for flowering-time mutants. For example, we have identified the non-flowering mutation, *maintained vegetative phase (mvp)*, which is a deletion mutant of the flowering promoter gene *VRN1*, and *extra-early flowering (exe)* mutants which are deletion mutants of clock-related genes.

Heavy Ion Beam Mutagenesis System for Durum Wheat

As with einkorn wheat, we first examined the effects of the LET-Dose (Gy) combination of ion beam irradiation using Carbon (C) and Argon (Ar) as nuclei on viability rates in M_1 generation. Dry seeds of the durum wheat cultivar ‘Langdon (Ldn)’ were irradiated with 15, 30, or 50 Gy of $^{12}\text{C}^{6+}$ ions at LET values of 30, 50, or 70 keV/ μm , or 2.5, 5.0, 7.5, 10, or 20 Gy of $^{40}\text{Ar}^{17+}$ ions (290 keV/ μm) to determine the optimal conditions for mutant generation by using the RRC in the RIBF. The germination rate was tested for 150 seeds at each LET-Gy combination. The germination rate was not affected

by heavy ion beam irradiation conditions. Surprisingly, seeds treated with Ar ion beam with high dose also showed normal germination rate.

The M_1 seedlings were planted in the experimental field of Fukui Prefectural University. The viability rate was reduced to less than 80% when LET was 70 keV/ μm for C ion beam. As for Ar ion beam, the viability rate was reduced to less than 60% with more than 7.5 Gy, and all plants died when dose was 10 and 20 Gy. Considering that the LET-Gy condition with 80% viability is optimal for mutant generation, the condition with LET70–15 Gy is the best.



Field screening of durum wheat mutant lines.

The harvested seeds from each individual M_1 plant were used to produce the next generation (M_2) lines. We planted 77–134 M_2 lines (1068 lines in total) for each LET-Gy combination in the field; ten seeds of each M_2 line were examined. The frequency of lines with albino plant(s) among the ten plants was determined to assess the comparative mutation ratio of the different irradiation conditions. The frequency of albino plants in the M_2 generation was different for different LET-Gy combinations. The highest ratio (>2.0%) was observed for the LET50–30 Gy treatment condition.

The frequency of albino plants in the M_2 generation together with the data of survival ratio in the M_1 generation suggests that the optimal condition of mutagenesis is around the LET50–30 Gy - LET70–15 Gy of C ion beam treatment for durum wheat.

Early-flowering Durum Wheat Mutant

In the mutant screening in 2016, we observed moderately early flowering mutations that were earlier by seven days or less and extra early-flowering mutations that were earlier by more than one week, as compared with the wild-type. Extra early-flowering mutants were mainly obtained under harsher treatment condition of 5.0 Gy with Ar. However, these extra early-flowering mutants showed dwarf phenotype and/or decreased seed set rate (fertility), indicating that they are not useful for breeding. We observed some useful early-flowering mutations that were around 5–6 days earlier than the wild-type Ldn plant. The percentages of the M_2 lines

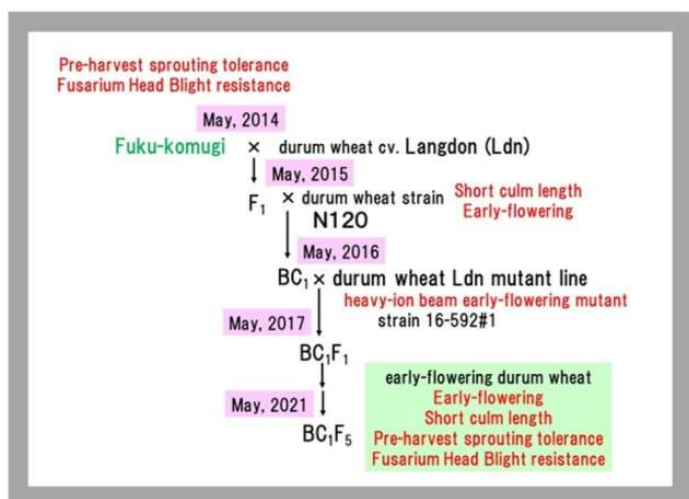
segregating for the early-flowering trait were as follows: about 4% for LET50–30Gy, about 2% for LET50–50Gy, about 8% for LET70–15Gy, about 4% for LET70–30Gy. Among these early-flowering mutants, mutant plant 16–592#1 screened from 91 individuals treated by LET70–30Gy irradiation condition was used for the present breeding program. This mutant was used as a donor for the early-flowering trait because growth vigor and fertility did not differ from the wild type.



Early-flowering/maturing mutant (left) compared with wild-type durum wheat (right).

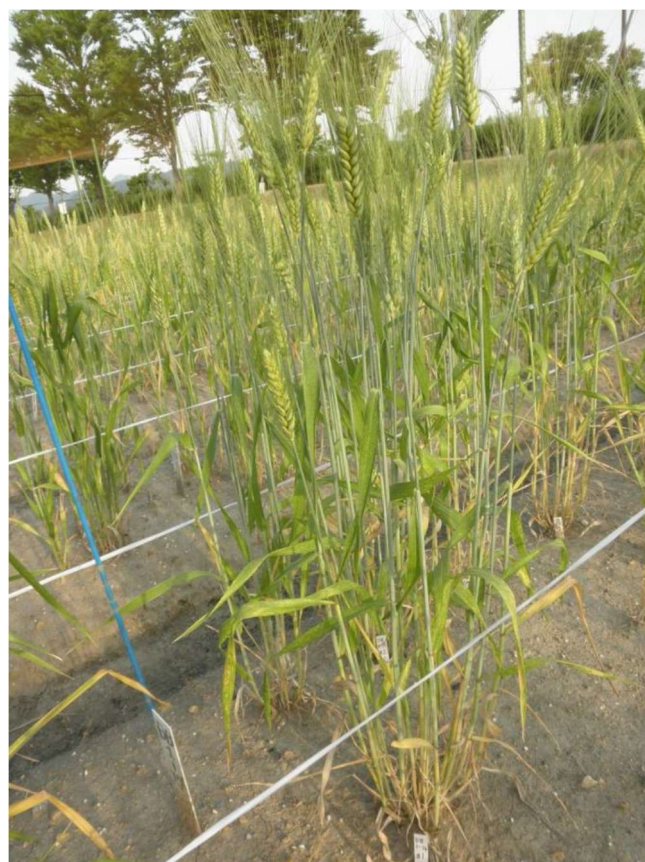
Development of Early-flowering Durum Wheat Cultivar Using the Integrated Breeding System

The F₁ lines of bread wheat cv. Fuku-komugi and durum wheat cv. Langdon (Ldn) were grown in the experimental field of Fukui Prefectural University during the 2014/2015 season. The F₁ plants were crossed with durum wheat strain N120, and the BC₁ plants were grown for 2015/2016 season. Among the segregating BC₁ plants, one plant with short culm length and high fertility was selected and crossed with an early flowering/maturing Ldn mutant plant, 16–592#1. The chromosome number of hexaploid bread wheat is 2n=42, but that of tetraploid durum is 2n=28. In the above crossing, durum wheat was backcrossed to bread wheat Fuku-komugi three times, and we were able to obtain tetraploid plants with a stable number of chromosomes.



Breeding scheme of early-flowering durum wheat cultivar.

The backcrossed progenies were planted in the field and individual plants with suitable agronomic traits were selected.



The selected candidate of durum wheat cultivar.

We obtained BC₁F₄ plants with the following characters: early flowering/maturing, short culm length, absence of pre-harvest sprouting, and resistance to Fusarium Head Blight. The selected line is a candidate of durum wheat cultivar suitable for the climate conditions of the Hokuriku region including Fukui. We will examine their yield performance and will also conduct quality tests for durum semolina.

Integrated Approaches for Improving Drought Tolerance in Rice and Sorghum

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Introduction

The need to produce 50% more food in order to feed the ever-increasing global population by 2050 is a generational challenge with which the international development community continues to grapple (FAO, 2017). To underscore the enormity of the hurdles to achieve the universal food security and nutrition as committed to in the Agenda 2030, about two billion people do not have regular access to safe and nutritious food (FAO *et al.*, 2020). Yet, climate change, i.e. the rise of the average temperature of the earth's climate system which is caused by humans, is evident through the increasing frequencies and greater intensities of erratic extreme weather events, such as dry spells with low precipitation and the resultant droughts, flooding etc. The negative impacts of droughts on the biological processes of crops include reduced growth rates and development which culminate inevitably in significant losses in yields, especially in the developing tropical countries of the world (IPCC, 2018; Cammarano *et al.*, 2019). Climate change is therefore exacerbating food insecurity and malnutrition (IPPC, 2019) and by implication, making the achievement of zero hunger by 2030 much more difficult.

Considering these scenarios, improving drought tolerance in food crops to increase the efficiency of water use and to enhance agricultural productivity under rain-fed conditions is the top priority for agriculture policy in most countries. Breeding for hardy, input use-efficient 'smart crop varieties' that being inter alia drought tolerant and produce stable or more yields would constitute part of the solution to the anticipated abiotic stresses arising in water scarce regions in the developing and underdeveloped countries. Among the cereal crops, rice and sorghum contribute a substantial part of the food requirement of a large population worldwide. Rice, being the staple crop consumed by 50% of the population, contributes up to 80% of the calorific need (Khush, 2005). Significant yield losses are reported due to frequent drought stress, with the vegetative and reproductive stages being the most vulnerable (Bouman *et al.*, 2007). Under moisture stress conditions, 20–80% of yield loss is reported in rice depending upon whether the vegetative or reproductive growth stage is affected. In addition, grain quality and nutritional parameters are also severely affected during drought stress. Among the ecotypes, low land and upland rice are the two main types being cultivated in paddy

fields and rainfed conditions respectively. In order to breed for drought tolerant rice cultivars, there is a strong need to assess the globally available germplasm resources, creating genetic variability for drought responsive traits and incorporating them in breeding programmes.

Sorghum is widely cultivated in arid and semi-arid regions covering an area of 44 million ha, with production estimates of 62.5 million tonnes. Major sorghum producing countries are the USA, India, Mexico, Nigeria, Sudan, Ethiopia and Australia, contributing about 77% of the world production (Rakshit *et al.*, 2014). In order to harness the yield potential, several genetic improvement programmes have been undertaken utilizing historical landraces, germplasm lines and exotic genotypes in the hybridization process. Among the five genetic races grown, Indian sorghum is dominated by durra type, and the USA and African countries by kafir and guinea types, respectively. Although sorghum can tolerate moisture stress, post-flowering drought during reproductive stage can severely affect grain yield, seed size and its nutritional qualities (Badigannavar *et al.*, 2018).

Looking to the importance of breeding crops for stable performance under moisture stress conditions, the Plant Breeding and Genetic Section launched a Coordinated Research Project (CRP) in 2017, 'Improving resilience to drought in rice and sorghum through mutation breeding' – CRP D23031. The objective was to improve grain yields of rice and sorghum under the negative effects of drought stress. Rice and sorghum being essential staples in the diets of millions of impoverished and vulnerable populations, any attempt in stabilizing or increasing their yields under drought stress can have a major and positive impact on local and national food security. The approaches were to identify and/or apply i) morpho-physiological, ii) gene expression, iii) physiological and iv) biochemical markers indicative of tolerance to drought. Fourteen scientists/breeders from eleven participating countries contributed to this research project and continue to develop screening protocols for assessing drought response in populations developed through induced genetic variation. The initial results suggest the isolation of drought tolerant rice and sorghum mutants upon exposure to pre and post flowering moisture stress conditions (FIG. 1 and FIG. 2). By employing precise phenotyping and efficient selection of drought responsive traits, it is envisaged that stress-tolerant varieties can be

developed. This review emphasizes various mechanisms and methods for breeding rice and sorghum under drought stress conditions in rainfed areas.

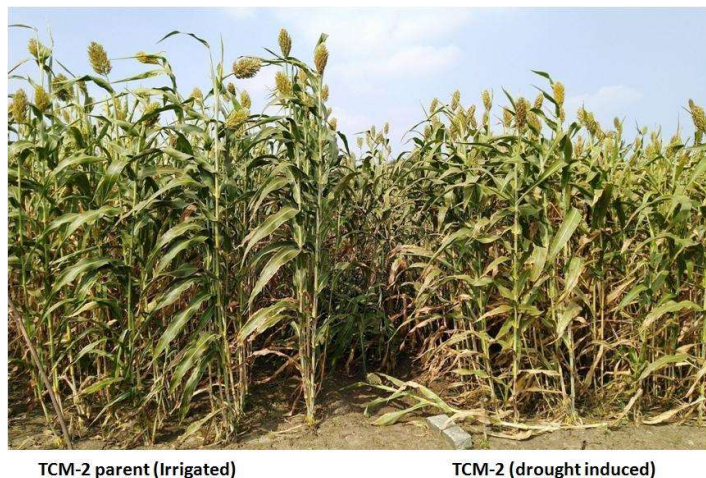


FIG. 1. Sorghum mutants under well-watered and drought stress situations under deep black soils of Southern India.

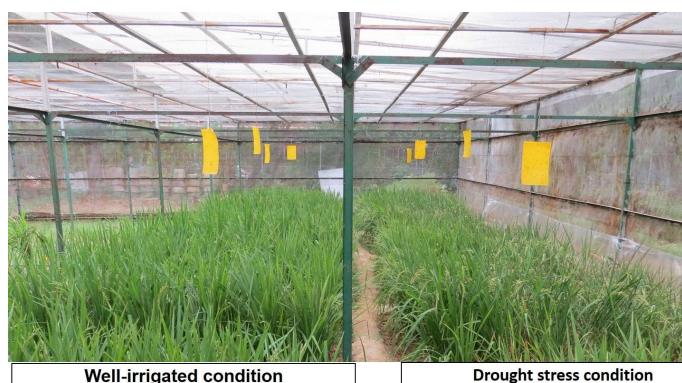


FIG. 2. Representative picture from the well-irrigated condition and drought stress condition rice plants showing the screen house (Open rain out shelter) at Jawaharlal Nehru University, New Delhi, India.

Genetic Mechanisms for Drought Tolerance in Rice and Sorghum

A leading counterpart of the project at the Jawaharlal Nehru University studies stress triggers on various physiological changes in plants viz., reduction in pigments, photosynthetic rate, transpiration, stomatal conductance, relative water content, and water use efficiency that ultimately results in growth reduction. These physiological parameters, in addition to yield components, are used as a criterion for improving drought tolerance in crops. Plants have evolved multiple mechanisms at the morphological, physiological, cellular and molecular levels to adapt to or overcome drought stress conditions. Broadly speaking, plants can tolerate drought by four basic strategies, viz., drought avoidance, drought tolerance, drought escape and drought recovery. Stress tolerance is a complex polygenic character manifested by several physiological and molecular mechanisms operative at both cellular and whole plant-levels. During the past decade, significant progress has been made in achieving drought avoidance and drought tolerance

by exploiting genetic variations for drought responsive traits.

Rice is cultivated both in anaerobic (wetland) and aerobic (upland) environments. Upland rice requires 50% or less water relative to wetland rice. During the last decade, rice cultivation has been rapidly moving away from the water-intensive puddled conditions of the wetland to less water requiring practices such as direct seeding, alternate wetting and drying, and upland cultivation. Upland rice varieties with deeper root system take up more water from deeper soil profiles and maintain high leaf water potential during drought stress which is an important strategy for drought tolerance. High-yielding rice varieties which are bred for the wetland ecosystem generally perform poorly in aerobic soils. Drought stress at vegetative growth, especially at booting stage in rice, flowering and terminal period can reduce fertility in spikelet and grain filling resulting in lower yields. In order to reduce water usage in rice production and to maintain rice productivity in water limiting environments, there is a need to accelerate the process of genetic improvement of rice for aerobic cultivation (Venuprasad *et al.*, 2011).

Sorghum is a C4 plant with an extensive and fibrous root system enabling it to draw moisture from deep layers of the soil. It requires less moisture for growth compared to other major cereal crops. Sorghum has the capacity to survive dry periods and resume growth upon receipt of rain (House 1985). Due to this, sorghum is grown on marginal and poorly nourished soils and environments under rainfed conditions, meeting the basic requirement of grains and fodder for poor farmers. Pre- and post-flowering drought stress in sorghum can significantly reduce spikelet fertility, carbohydrate accumulation and seed formation. 'Staygreen'- a trait characterized by delayed leaf senescence during the grain filling stage - has a key role in combating moisture stress. A well-balanced supply and demand of water for efficient production of biomass is evident in sorghum. Recent studies have indicated the presence of three loci responsible for the staygreen trait in sorghum viz., *stg1*, *stg2*, *stg3* (Hausmann *et al.*, 2002). In addition, leaf/stem waxiness and leaf rolling mechanisms are also operative in sorghum as drought avoidance mechanisms (Harris *et al.*, 2007).

Selection Strategies for Enhanced Drought Tolerance in Rice and Sorghum

The genetic variation in drought-related traits is a manifestation of quantitatively inherited morphological and physiological traits whose effects on grain yield and biomass may vary depending upon the interaction with the environment. Based on the inheritance and expression of these traits under stress conditions, selection is initiated. Understanding the inheritance of the traits, especially whether additive or dominance variation is prevailing, is most important to adopt the best breeding schemes. Traits with additive variations are amenable for selection and high narrow sense heritability would also show good response to

selection. Therefore, the breeder needs to focus on such traits for breeding for drought tolerance in crop plants. Of the several mechanisms reported to circumvent drought stress in rice and sorghum, drought escape (related to shorter maturity durations), drought avoidance (maintenance of higher leaf water potential, LWP), and drought tolerance (related to greater osmotic adjustment, OA) are important and have been well characterized (Reddy *et al.*, 2009). However, LWP and OA does not correlate well enough with grain yield in field conditions to merit selection based on them. Empirical screening based on imposing drought at various growth stages and measuring plant morphological and yield responses remains the most effective approach.

Tolerance to drought stress in rice and sorghum is a complex trait that depends on various environmental and physiological factors. Therefore, a comprehensive understanding of the plant morpho-physiological, molecular, and biochemical responses to water deficit may provide a means to identify and confer tolerance in terms of agronomic, molecular, and genetic aspects. For example, important morpho-physiological traits such as root length, tiller number, plant height, harvest index, flag length/width, filled/unfilled spikelet, and panicle number are most affected by drought stress. Further, in response to water deficit, osmotic adjustment is a biochemical mechanism that helps plants to acclimate to dry soil. One mechanism for osmotic adjustment is the accumulation of compatible solutes such as proline and glycine betaine, and storage sugar solutes such as mannitol, trehalose, fructan, sorbitol, and inositol/ononitol which are low-molecular-weight and highly soluble compounds that are usually nontoxic even at high cytosolic concentrations. Generally, they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, and stabilization of membranes and native structures of enzymes and proteins (Jewell *et al.*, 2010). The induction of antioxidant enzyme activities is a natural adaptation strategy to overcome oxidative stress of plants. An increase in certain antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), and superoxide dismutase (SOD) has been observed in response to stress. An increased antioxidant activity is generally believed to be associated with drought tolerance (Foyer and Noctor 2003; Mittler *et al.*, 2011). However, to know the health of the plant under stress conditions, various parameters are used such as photosynthetic efficiency/activity and chlorophyll content. These can be assessed non-destructively using instruments such as the HandyPEA (Plant Efficiency Analyzer, Hansatech Ltd., UK) and SPAD (Model SPAD-502; Spectrum Technologies Inc. Plainfield, IL). Chlorophyll-a (Chl a) fluorescence measurements are widely used to monitor abiotic stress such as drought, salinity, cold, high temperature and even ion stress. Chlorophylla fluorescence (ChlF) measurements, including studies of induction kinetics in dark adapted samples followed by OJIP analysis, have been commonly used for studies of plant reaction to abiotic stresses. Measurement of

the chlorophyll content in a plant is one of the most frequently used method for evaluating the severity of drought stress in rice and sorghum where higher content of chlorophyll is generally associated with higher tolerance to stress (Kalaji *et al.*, 2016 and Wungrampha *et al.*, 2019).

Finally, a category of genes activated by abiotic stresses comprise regulatory proteins that further regulate stress signal transduction and modulate gene expression and, thus, probably function in the stress response. A well-studied example are the dehydration responsive element binding proteins (DREB 1F, DREB 2A, and DREB2B) which suggest a role for transcriptional regulatory mechanisms in stress signal transduction pathways. Transcript abundance of the DREB 1F, DREB 2A and 2B genes has been extensively applied in enhancing abiotic stress in plants (Shinozaki and Yamaguchi-Shinozaki, 2007).

Recent advances in molecular genetics, genomics, and marker-assisted selection (MAS) offer new opportunities to meet the challenges of identifying and characterizing effective drought-responsive traits in rice and sorghum. Important genes and/or quantitative trait loci (QTLs) governing the rice plant's tolerance to drought have already been tagged with molecular markers. Such informative QTLs are key to incorporate (through marker assisted selection) abiotic stress tolerance in popular high-yielding crop varieties that typically are susceptible to abiotic stresses (Serraj *et al.*, 2009). There has been good success in the use of conventional breeding to develop drought-tolerant varieties. Breeding for hardy, input use-efficient 'smart cereal varieties', which are not only drought-tolerant but also produce sustained yields, would constitute part of the solution to the envisaged threat to food security posed by abiotic stresses arising from climate change.

Induced mutation has been hugely successful in rice and sorghum breeding for modifying stress related traits in field crops. So far, according to the FAO/IAEA mutant variety database, 18 sorghum mutant varieties and 851 rice cultivars have been developed by mutation breeding mostly using gamma ray irradiation. Specifically, a rice mutant, Nagina 22 (N22), which is deep-rooted and tolerant to drought and high temperatures has been released in India. The mutant shows reduced accumulation of reactive oxygen species in the leaf under 40°C ambient temperatures (Poli *et al.*, 2013). In addition, two drought tolerant sorghum mutant varieties, viz., Samurai-2 and Pahat have been recommended for dry-season cultivation in drought prone areas of the eastern part of Indonesia. These are not only drought tolerant but also early maturing, and high yielding (Human *et al.*, 2020).

Conclusions

Past and current plant breeding efforts have met the challenge posed by drought with some success. With the knowledge generated by these efforts and the application of functional genomic techniques, marker-aided selection and speed breeding, it is becoming increasingly possible to identify and integrate genomic strategies and solutions with

conventional breeding and physiological screening. Plants have developed several mechanisms to tolerate prolonged periods of drought among which reduction in the loss of water through stomatal adjustment, increased water capture through optimum root architecture, and the accumulation of osmolytes such as sugar are of particular note (Kapoor *et al.*, 2020). Selection and breeding for traits contributing to drought tolerance by tapping primary and secondary gene pools of plant species, or by creating wide genetic variation through mutation breeding are viable options for inducing drought tolerance in high yielding backgrounds.

During the final research coordination meeting of the FAO/IAEA Coordinated Research Project on Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding, it was emphasized that the project has achieved tangible outputs especially in the development of effective screening protocols for drought tolerance. These protocols will be used by plant breeders who need practical and rapid screening to process large mutant populations, including segregating populations, advanced generations and germplasm collections of rice and sorghum. The project also evaluated and identified secondary traits as physiological and biochemical indicators for tolerance. Transcription analysis and expression profiling of DREBs, TPS and GLY genes in gamma induced mutant lines have yielded promising early results, and genetic mapping of selected drought tolerant rice lines by Mutmap analysis is in progress. Results of the CRP are planned to be published as a book (Springer Nature) shortly.

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Developments at the Plant Breeding and Genetics Laboratory (PBGL)

During this reporting period, the second pilot project on genetic mapping and marker development initiated in 2018 was successfully completed. This project focused on the discovery of gamma-ray induced sequence variations that are linked with early maturity semi-dwarf mutant trait in African sorghum. Field experiments conducted during the 2020 season in Seibersdorf, Austria showed that the semi-dwarf early maturity trait can be transferred to unrelated tall, African landraces. Closely linked markers were identified on chromosome 4 next to the centromere and a KASP™ marker useful for Marker-Assisted Breeding has been developed. Under the CRP D25005, different *Striga* resistance mechanisms were identified in advanced gamma-ray induced sorghum mutants opening perspectives for widening resistance to this parasitic weed which is affecting cereal production in most of Sub Saharan Africa and parts of Asia. Under the overall umbrella of FGTU an exome capture kit for arabica coffee was successfully tested using an in-house bioinformatics workflow. The exome capture kit is commercially available for genome-wide mutation discovery and breeding and genetics studies in coffee. During this period, the PBGL initiated a new project for predictive mutation breeding. The approach entails adapting innovative methods for the detection of rare variants in the field of medical diagnostics to crop mutation breeding. A pilot project on sorghum was initiated and two training sessions were provided to PBGL staff by a field application specialist from BioRad Laboratories, Germany. Finally, the PBGL staff greatly appreciated receiving the Recognition Award for Exceptional Service from NAFA Director, Mr Qu Liang, in view of their dedicated service during the COVID-19 lockdown period. Mr Liang thanked all the PBGL staff for their commitment and support during this challenging period which allowed the PBGL Laboratory to continue its operations and services.



Mr Qu Liang, Director NAFA, presenting the Recognition Award for Exceptional Service to PBGL staff.

Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security

Under the *Striga* CRP D25005, the PBGL conducted further experiments for identification of the mechanism of resistance in eight, gamma-induced, sorghum mutants with verified resistance to *Striga hermonthica* from Burkina Faso. These mutants were induced in three farmer-preferred varieties of sorghum (Sa-P, GK-P, and IC-P) each had two, three and three mutants, respectively. Seeds from each of the mutants and parents were surface sterilized and germinated in petri-dishes on moist filter paper and placed in an incubator under 28°C at 16/8 hr light/dark regime. Extended gel and mini-rhizotron assays were conducted to assess *Striga* germination rate, post germination attachment and establishment of parasitism on resistant mutants and wild parents following protocols adapted at the PBGL.

For the extended gel assay, the relative germination percentage of resistant versus wild type plants was determined: three replicates each with three plates were assigned to each genotype (see FIG. 1). Data were compiled and statistically analysed using Duncan's Multiple Range Test at 0.001 probability. The extended gel assay experiments indicated that three of the mutants (one from each parent) have low rate of *Striga* germination due to either low stimulant production or the presence of a germination inhibitor.

The mini-rhizotron assay is based on growing seven-day old sorghum seedling in a petri-dish (90 cm Ø) filled with fine sand and covered with filter paper. The seedling was gently placed on the surface and surrounded by pre-conditioned *Striga* seeds. Mini-rhizotrons were incubated at 28°C under a 16/8 hours light/dark regime. The cultures were inspected weekly for up to one month for *Striga* seed germination, haustoria development and attachment, necrosis, and growth rate of *Striga* plants. The results confirmed the low germination rate of *Striga* and/or presence of germination inhibitor observed in the gel assay. In addition, some mutants showed a hypersensitive reaction and necrosis killing the *Striga* parasite or limiting its growth rate compared to the wild parents (see FIG. 2). Seven of the eight mutants had a significantly lower number *Striga* plants attached to the host root compared to the wild parent at 30 days.

Genomic DNA from three sorghum mutants and parents together with bulks of sensitive siblings is currently being sequenced and will be analysed for discovery of the possible causative mutations for the resistance to *Striga hermonthica*. Details on the methods and results of this research will be published in peer reviewed journal articles.

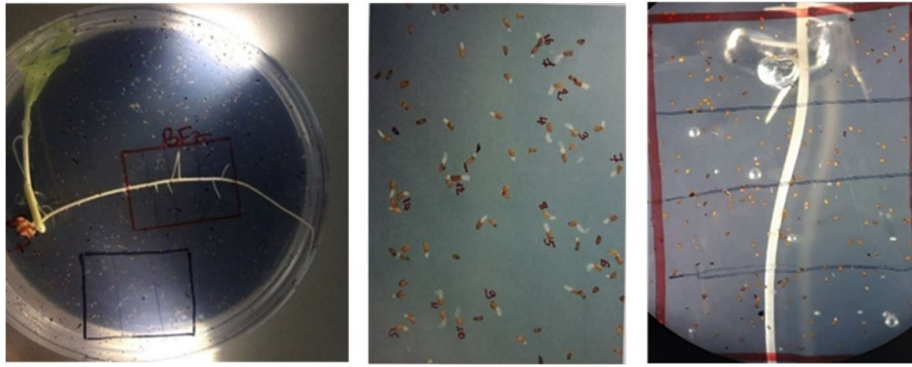


FIG. 1. Representative extended gel-assay for assessment of induced germination rate of *Striga* seeds among mutants and parents. From left to right: whole plate showing the position of select areas for data collection, and the two selected areas magnified for counting germinating *Striga* seeds.

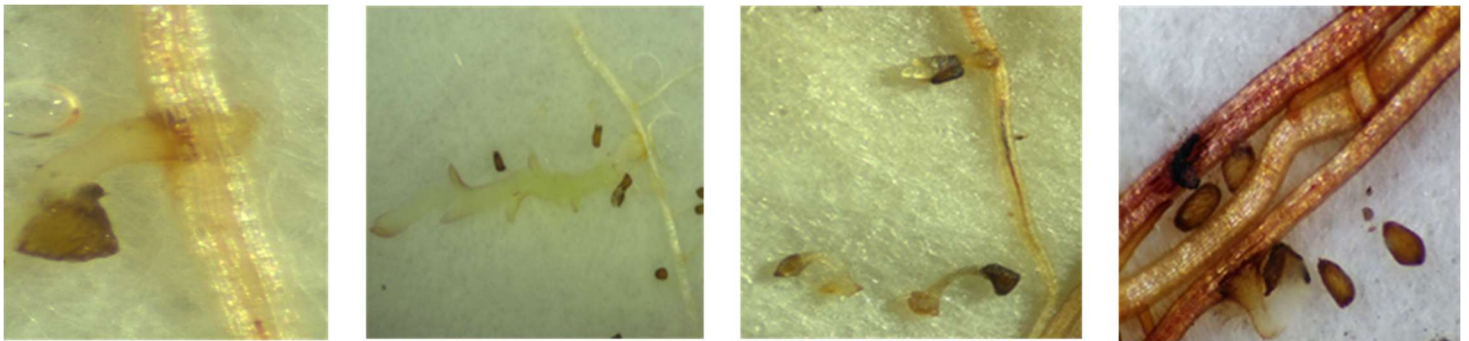


FIG. 2. Representative mini-rhizotron assays showing from left to right: early normal attachment; healthy *Striga*; *Striga* necrosis; hypersensitive response.

A New Molecular Marker for a Gamma-ray Induced Early-maturing Semi-dwarf Trait for Accelerated Breeding of African Sorghum

An early-maturing semi-dwarf trait was induced by gamma irradiation of a farmer-preferred sorghum variety (Wad Ahmed) from Sudan. The early maturity trait is useful for drought escape in areas prone to terminal drought while the semi-dwarf trait is useful in preventing crop lodging in high-input agriculture production, beside its suitability for combine harvesting in large-scale mechanized agricultural systems. Previous genetic studies showed that the mutation is controlled by a single recessive gene.

Previously, mapping populations were developed by crossing the mutants with wild parent and F_2 populations were planted in the field and the glasshouse at the PBGL. Comparing genome sequence of the contrasting bulks with short versus tall phenotypes of the mutant and wild parents enabled to position the possible causative mutation on chromosome 4, near the centromere. In addition, seven sequence polymorphisms were identified in that region between the mutant versus wild type parent. These SNPs were analyzed *in silico* and through PCR at the PBGL for their potential use as markers for accelerated breeding. Overall, three SNPs were found to be consistent among sibling mutants (D2 and D3) compared to the wild parent producing robust PCR amplicons of the expected size

across all genotypes tested. Next, a fluorescence based KASP™ assay was commissioned for the three SNPs for further validation and marker-trait association studies at the PBGL.

The KASP™ assays clearly separated individuals from F_2 populations generated by backcrossing the mutant to its wild parent Wad Ahmed showing the homozygous mutants, heterozygous and homozygous wild parent phenotype/genotype alleles in the expected ratios (see FIG. 3). Genotyping the F_2 individuals from two populations (D2 and D3) revealed percent of crossing over between the marker and the trait in two populations in the range of 3–5%. This indicates that the KASP™ markers are closely linked to the mutant trait (3–5 cM) and therefore have the potential to be useful for accelerated transfer of the early maturing/semi-dwarf mutant trait to improve tall varieties and landraces from Member States through Marker-Assisted Backcrossing (MAB).

The three KASP™ assays were next validated on crosses involving the mutants and three tall, locally adapted sorghum land races from Africa. The markers were perfectly linked with the dwarf allele in the segregating F_2 populations with the heterozygous individuals clearly separated from the homozygous wild type, as required in a backcrossing breeding process (See FIG. 4 and FIG. 5).

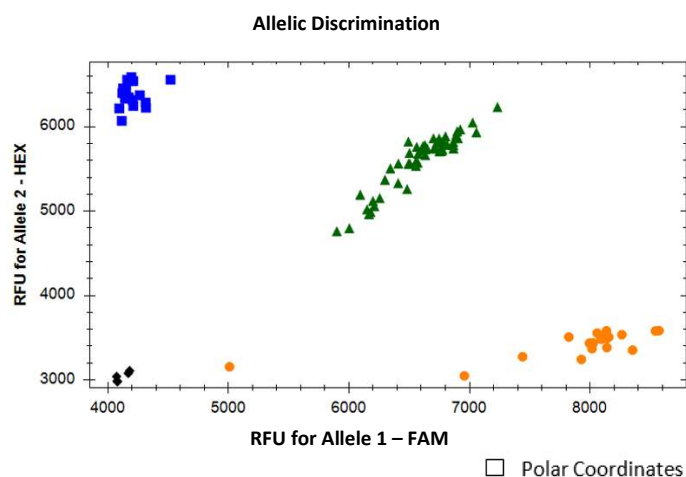


FIG. 3. Representative genotyping image of F2 population (D3 X wild parent) by KASP marker for SNP 37 showing marker-trait association with clustering of homozygous individuals for mutant allele (blue), heterozygous (green) and homozygous wild type individuals (orange).

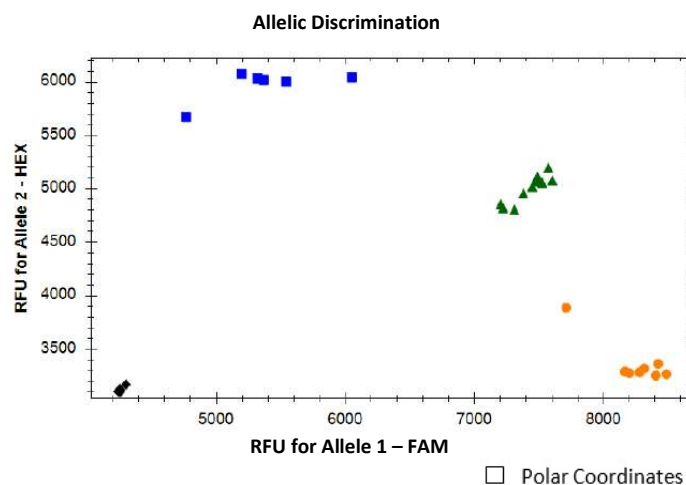


FIG. 4. Representative validation of the KASP markers in one of the three tested landraces whereby the tested F2 individuals are separated by the marker into the three expected groups: homozygous mutant (blue); heterozygous (green) and homozygous wild type (orange).



FIG. 5. Picture showing the tall African sorghum land races with the early maturing-semi-dwarf mutant in the centre used for introgression of the early maturity/semi-dwarf trait and the marker-trait association studies.

Coffee Exome Capture

Coffee production is under threat. Abiotic stresses and biotic pressures are mounting and breeding new and improved varieties will be key to ensure sustainable coffee production into the future.

Any breeding is an iterative process that relies on shuffling existing genetic diversity into novel combinations, select superior individuals, and from those eventually create new varieties. Coffee being a tree, the breeding process is naturally slow requiring about three years for the first fruits and seeds to form. Also, there isn't much genetic diversity in the currently cultivated coffee varieties to begin with because coffee, particularly the tetraploid arabica coffee, went through several serious genetic bottlenecks. That means, when existing varieties are crossed with each other, there isn't much novelty to be gained.

PBGL is addressing the coffee breeding challenges on several fronts: Most importantly, we generate new diversity in coffee through mutagenesis. Every new mutation has the potential of bringing desirable traits to the coffee gene pool. For mutagenesis we use irradiation as well as chemicals and, either way, this process is rather quick and cheap. We can induce thousands of mutations in a matter of days. What is not cheap at all, however, is raising all these plants and identifying and selecting the superior individuals. As said, growing coffee needs time and space and proper selection requires to test every individual for desired traits at the respective stage in the life of the plant. Depending on the trait, these can be very meticulous and expensive phenotyping assays, such as for example in case of quality traits of the roasted coffee bean. It is therefore desirable to have methods in hand to somehow make informed decisions ahead of time, particularly about promising mutations and which mutant plants are worth keeping and phenotyping. Genomics can guide such decisions.

At PBGL we are developing genomic tools to aid and accelerate breeding processes. Next Generation Sequencing is a widely used method for detecting new mutations, and the idea is to use DNA sequencing on mutant coffee populations to identify promising mutations and plants for further selection and breeding. While the price for sequencing service continues to come down, sequencing the entire genomes of thousands of coffee plants is still prohibitively expensive at present. We have hence decided to develop a so-called 'Exome Capture Kit'. Sequence capture is a complexity reduction technique that allows the researcher to physically select desired fragments from a genome prior to sequencing. The technique uses oligonucleotide baits with which DNA molecules, that are complementary to the target sequences, are fished out from a sequencing library. Non-target DNA is washed away and hence subsequently not sequenced, greatly reducing the sequencing cost.

We procured the designing and production of the exome capture kit from Arbor BioSciences, Michigan, USA (FIG. 6). The kit consists of baits against around 275,000 exonic regions in the tetraploid *Coffea arabica* genome. We evaluated the kit in-house at PBGL on pools of coffee DNA samples and found it to perform very well (FIG. 7). Gene sequences are significantly enriched, while the background noise is low.



FIG. 6. Exome Capture Kit for tetraploid *Coffea arabica*. We developed the kit in 2020 with Arbor BioSciences (Ann Arbor MI, USA). The bait design is based on a publicly available *Coffea arabica* genome assembly and annotation (NCBI, isolate: CCC135-36, cultivar: Caturra red, assembly: Cara_1.0 by Johns Hopkins University).

The exome capture approach offers a cost-effective compromise for genome wide characterisation of coffee plants, and the kit will be of great value to the coffee research community. Using the kit reduces the sequencing cost per sample by 8-fold while providing sufficient informative data for most breeding applications. At PBGL we will use the kit for characterising our mutant populations and identify promising mutations in candidate genes. Obvious additional use cases are: To quantify existing genetic variations in the global coffee germplasm collections, the straight-forward detection of hybrids and uncovering their pedigrees, to confirm successful crosses, and to use the kit directly for genotyping in genetic mapping experiments. The kit can be purchased from Arbor BioScience, MI, US as a regular product.

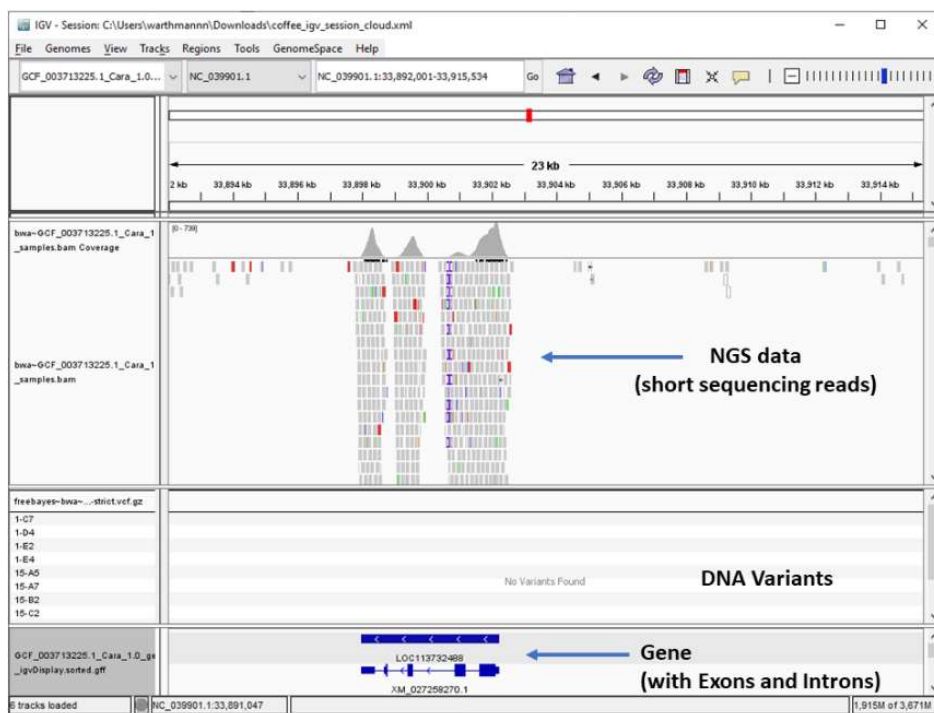


FIG.7. Evaluating the Exome Capture kit in a pilot study at PBGL. The screenshot shows sequencing reads aligned to the genome, zoomed in on a random gene. Clearly visible is the enrichment of sequencing reads around exons of the gene. Using the kit reduces the cost for characterizing a coffee plant genome by 8-fold. (Visualization of the data with open source software Integrated Genome Viewer, IGV).

Introductory Guide and Protocols for Low-cost Genotyping and Marker-Assisted Selection of Orange Lemma (*rob1.a*), a Feed Quality Trait in Barley (*Hordeum vulgare* L.) is Publicly Available

Previously, a low-cost and breeder-friendly marker kit was developed for a reduced lignin trait in barley, called Orange

Lemma, for use in MAB experiments. The accompanying introductory guide and protocols is now publicly available. The publication provides laboratory and greenhouse protocols for the development and application of marker assay for SNPs using allele-specific PCR as applied to barley Orange Lemma trait. In addition, the publication introduces methods for plant genotyping, provides

guidance on the use of a candidate gene approach for mutation discovery and for chi-square ‘goodness of fit’ data analysis.

Adapting Innovative Techniques for Rare Mutation Detection in Human Diagnostics to Crop Mutation Breeding

Digital droplet PCR (ddPCR) is an emerging technology that offers highly sensitive and reproducible nucleic acid quantification and detection. This technology is applied in human disease diagnostics for the precise detection of rare somatic variants and for copy number variations. Other applications include fast and simple detection of genome editing events. In 2020 the PBGL initiated a pilot project in sorghum intended to adapt ddPCR methods and protocols applied for rare variant detection in human diagnostics to crop mutation breeding. The overall goal of this project is to develop cost-effective methods for predictive mutation breeding based on knowledge of trait control genes. Target traits to develop the protocols include tolerance to the *Striga* parasitic weed, feed quality and haploid inducers. During this reporting period, the PBGL has expanded a sorghum gamma-ray mutant population which now comprises ca 5,000 M₂ families. In addition, an expert from BioRad Laboratories, Germany, provided a virtual and practical training on ddPCR on site to PBGL staff. The two-day hands-on training was focused on two main applications: the detection of copy number variation and rare mutation detection (FIG. 8). In addition to the training, potential strategies, and approaches for adapting existing protocols in the field of human disease diagnostics to crop mutation breeding were discussed.



FIG. 8. Training of PBGL staff on digital droplet PCR by a Field Application Specialist from BioRad Laboratories, Germany.

Individual Training

Table 2. Individual Training Activities at the PBGL

Name	Country	Status	Topic
Ms Susu ALKIERS	Austria	Intern	Plant breeding and genetics
Mr Anibal E. MORALES ZAMBRANA	USA	Intern	Data science
Ms Faith LUVAI	Kenya	Intern	Plant molecular biology; <i>in vitro</i> tissue culture
Mr Phillipe NIKIEMA	Burkina Faso	Fellow	Screening protocols for resistance to the parasitic weed <i>Striga</i>

Crop Irradiation Services Provided to Member States

Due to the COVID-19 pandemic with related travel and shipment restrictions imposed by governments worldwide, a reduced number of requests were received by the PBGL in 2020. After the COVID-19 lockdown earlier in the year, the number of requests for irradiation has steadily increased from November 2020 onwards. At this time of writing (18 May 2021), the PBGL has received 12 requests from 11 Member States across 12 different plant species covering a total of 100 accessions/varieties as summarized in Table 1.

Table 1. Crop Irradiation Services

Request Number	Country	Request Type	Species
1647	Namibia	TC	Groundnut
1648	Slovakia		Wheat
1649	Central African Republic	TC	Maize
1650	Germany		Ornamental
1651	Hungary		Ornamental
1652	Serbia	TC	Soybean
1653	Kyrgyzstan	TC	Barley, Wheat
1654	Poland		<i>Brassica napus</i>
1655	Croatia	TC	<i>Phaseolus vulgaris</i>
1656	Nigeria		Yam
1657	Greece	TC	Chickpea, Sesame
1658	Germany		Ornamental

Individual Training Activities

Table 2 summarizes PBGL’s training activities during this reporting period.

New CRP: Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops (D24014)

Isaac Kofi Bimpong, IAEA Department of Nuclear Sciences and Applications



Vegetatively propagated crops (VPCs) and horticultural tree crops (HTCs) play an important role in food security and income and represent significant agricultural opportunities in most countries of the globe. However, due to limited genetic diversity, their improvement has been very slow as they cannot be easily self or cross-pollinated to produce seed or expand variation.

Climate change further worsens this situation by causing serious production losses from factors such as intensifying and transboundary spread of pests and pathogens. To meet the rising demands for food and nutrition without adverse environmental footprints, it is imperative to develop efficient methodologies and protocols capable of overcoming these limitations associated with plant mutation breeding in VPCs and HTCs.

The IAEA, in cooperation with the Food and Agriculture Organization of the United Nations (FAO), is launching this new Coordinated Research Project (CRP) with a time frame of five years to develop novel genetic resources, methodologies and tools for accelerated breeding for productivity improvement in VPCs (root and tuber crops) and HTCs (olive) by using mutation induction and associated biotechnologies.

The CRP will provide outcomes that can guide National Agricultural Research Systems (NARS) in Member States to accelerate the development of new varieties of VPCs and 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.

CRP Overall Objective

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.

Specific Research Objectives

1. To develop or refine protocols for tissue culture, chimera-free generation, and retention of induced mutation in cassava, potato, and olive crop.
2. To generate induced genetic diversity in cassava and olive through physical mutagenesis for tolerance to cassava brown streak virus, and olive quick decline syndrome, respectively or a major disease in potato/sweet potato.
3. To develop functional genomics tools and methodologies for the discovery of molecular markers and candidate genes in cassava, potato, and olive crop.

Impact

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.

Relevance

Genetic improvement of these important crops will help to directly meet some of the Sustainable Development Goals (SDGs), particularly those related to health and nutrition, and the reduction of poverty and hunger. For instance, more than 240 million tons of roots and tuber crops covering around 23 million hectares are produced annually in sub-Saharan Africa. There is a high demand from Member States to improve VPCs and HTCs as reflected in many technical cooperation projects (TCPs).

The use of mutagens such as gamma or X rays is particularly appealing for VPCs and HTCs, since unlike chemical mutagens they create structural mutations which involve many genes, increasing the overall probability of success in this CRP.

In Memoriam



Udda (Ulrike) Lundqvist (1928–2020)

Much to our regret we inform you that Udda Lundqvist, one of the pioneers of barley mutation research, passed away on 26 December 2020 at an age of 92.

Udda Lundqvist was born in Germany in 1928, the daughter of the botanist and geneticist Fritz von Wettstein, who was then a professor in Göttingen. Her mother Else also studied botany. Within the von Wettstein Austrian family, several generations were prominent researchers in the natural sciences, something that influenced Udda's choice of profession. She maintained an interest in natural sciences throughout her life.

Udda moved to Sweden in 1949 for further university studies. In Sweden, the geneticist Åke Gustafsson, with a background at Lund University, was about to build up a large group for mutation research in both agricultural plants and forest trees where Udda was included. At the same time, she got a job at the Swedish Seed Association in Svalöv, first as an experimental technician and later as a research leader. Although Udda Lundqvist studied science at Lund University in parallel with her initial research work at the Swedish Seed Association, she had not completed her education. Late in her career, however, she was convinced to write her dissertation. Three months before retirement, in 1992, Udda Lundqvist defended her dissertation at Swedish University of Agricultural Sciences (SLU) on the now classic dissertation: 'Mutation research in barley'. The dissertation described the great Swedish mutation work in barley for more than 60 years and included no less than 12 of her own articles.

From the very beginning, barley research, including mutation induction and characterization of mutations was

the focus of her work, which has remained so throughout her career. Barley is an important crop for agriculture for the whole of Sweden and the mutation programme provided opportunities to produce lines and varieties with improved traits. Especially during the 1960s to 1980s, a number of interesting varieties were introduced with new properties created by mutation breeding as a result of Udda Lundqvist's work. The variety 'Pallas' (1958), with high yield and straw strength, became very widespread in cultivation around Europe. 'Mari' (1960), was another barley mutant variety with a short duration, insensitive, and it was the first two-row barley variety that could be grown in northern Sweden.

Together with her colleagues, Udda has produced more than 10 000 mutations that are preserved in NordGen's seed collection, associated with database description and characterization. They are a completely unique collection that is in demand by researchers around the world. Useful mutations in barley include a wide range of economically important characters such as: disease resistance, low- and high-temperature tolerance, photo- and thermo-period adaptation, earliness, grain weight and -size, protein content, improved amino acid composition, good brewing properties, improved straw morphology and anatomy in relation to superior lodging resistance. Udda was able to laboriously over the years build up knowledge about the individual genes and their variants and where on the barley chromosomes the different genes were located. In this post-genomic era, Udda's mutants have been in great demand by researchers from around the world. She published 145 scientific articles about her work, the latest only a couple of months before she passed.

Udda was an advisor to several international institutions such as the Max Planck Institute, the Nordic Gene Bank, The Scottish Crop Institute, and the German Gene Bank, IPK Gatersleben. A street in Svalöv has been named Udda Lundqvist's Road as a tribute to Udda and Swedish mutation breeding at the Swedish Seed Association.

Udda's work has been appreciated worldwide and she has been a frequent speaker at international conferences. She was the only person to attend all meetings of the International Barley Genetics Symposium. Udda has also attended Symposiums on Plant Mutation Breeding at the IAEA in Vienna. The last she attended was in 2018 as a Keynote Speaker with a presentation on 'Ninety Years of Scandinavian Barley Mutation Genetics, Research and Breeding'.

Udda Lundqvist received several awards for her work including the Nilsson-Ehle Medal from the Royal Swedish Academy of Forestry and Agriculture and was recognized for her lifelong work with the Lifetime Achievement Award from the International Barley Genetics Symposium.

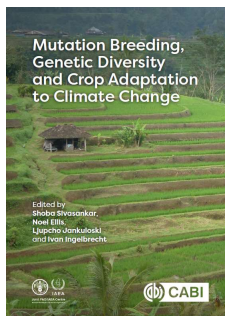
International Atomic Energy Agency (IAEA) and Food and Agriculture Organization (FAO) honored Udda with the Achievement Award in Plant Mutation Breeding in 2014 during the 50 years anniversary of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

Udda Lundqvist was a passionate scientist and lived for her work. She was very friendly, cheerful, and was always willing to share her ideas and information. She gave much

to her work and her achievements will continue to be the source of inspiration for the next generation of researchers. Her life commitment to the barley mutation/research programme and resulting accomplishments are an inspiration to all of us as we strive to meet the UN Sustainable Development Goals. The PBG Team is deeply thankful and feel privileged to have known such a great co-worker, scientist, and expert.

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
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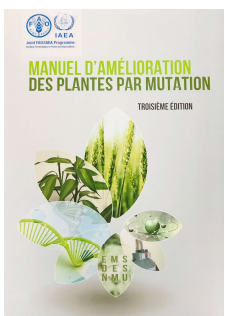
Manual de mejoramiento por mutaciones, Tercera edición

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.

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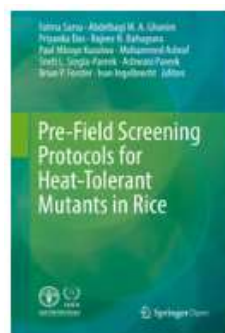
Manuel d'amélioration des plantes par mutation, Troisième édition

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.

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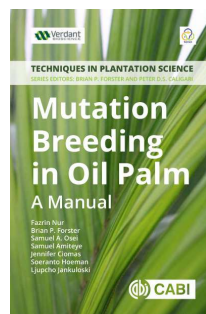


Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice

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](https://www.springer.com/9783319773384)



Mutation Breeding in Oil Palm: A Manual

F. Nur, B.P. Forster, S.A. Osei, S. Amiteye, J. Coimas, S. Hoeman, L. Jankuloski.

CABI, 2018

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

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Peer-reviewed Publications

2021

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

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<https://revistas.usfq.edu.ec/index.php/avances/issue/view/153>

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NIKIÈMA, M.P., OUÉDRAOGO, N., TRAORÉ, H., SAWADOGO, M., JANKULOSKI, L., GHANIM, A.M.A., YONLI, D. (2020). Sorghum Mutation Breeding for Tolerance to Water Deficit Under Climate Change.

Journal of Plant Breeding and Crop Science, 12(3), 192–199.

AHUMADA-FLORES, S., GÓMEZ PANDO, L.R., PARRA COTA, F.I., DE LA CRUZTORRES, E., SARSU, F., DE LOS SANTOS VILLALOBOS, S. (2020) Technical Note: Gamma Irradiation Induces Changes of Phenotypic and Agronomic Traits in Wheat (*Triticum turgidum* ssp. durum), Applied Radiation and Isotopes. <https://doi.org/10.1016/j.apradiso.2020.109490>

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JANKOWICZ-CIESLAK, J., INGELBRECHT, I., TILL, B.J. Mutation detection using Low Coverage Copy Number Variation. In: Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana. (*submitted*)

CÉSPEDES, R., ARRIETA, N., BARQUERO, M., ABDELNOUR, A., NIELEN, S. and INGELBRECHT, I. (2020) Determination of Radiosensitivity of *Coffea arabica* var. Venecia Seeds to Gamma-ray Irradiation. Chapter 7, Section 3. Mutation Induction Techniques for Enhancing Genetic Variation. Proceedings of the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology. (*in press*)

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Conference Abstracts and Posters

2021

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

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

WARTHMAN, N., GHANIM, A.M.A, ALI, A., and INGELBRECHT, I. (2020). Mutation Breeding Creates Desired Traits for African Sorghum –Semi-Dwarf and Early Maturing. PE0810. Plant and Animal Genome XXVIII, 11-15 January 2020, San Diego, USA.

WARTHMAN, N., GHANIM, A.M.A., ALI, A. and INGELBRECHT, I. (2020). Mutation Breeding Creates Desired Traits for African Sorghum – Semi-Dwarf and Early Maturing. International Symposium of the Society for Plant Breeding (GPZ), Digital Breeding, 11–13 February 2020, Tulln – Austria.

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News Highlights

- 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](#)
- Call for Nominations: Recognizing Excellence in Plant Mutation Breeding and Associated Biotechnologies (**15 March 2021**)
[Recognizing Excellence in Plant Mutation Breeding | IAEA](#)
- Cotton in Pakistan: How Nuclear Techniques are Helping the Textile Industry (**22 January 2021**)
[Nuclear Techniques and the Textile Industry | 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](#)
- Climate Change and Coffee: Combatting Coffee Rust through Nuclear Techniques (**21 December 2020**)
[Climate Change and Coffee: Combatting Coffee Rust | IAEA](#)
- New CRP: Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops (D24014) (**16 December 2020**)
[Development of Integrated Techniques for Induced Genetic Diversity and Improvement of Vegetatively Propagated and Horticultural Tree Crops \(D24014\)](#)
- New IAEA Publications Highlight Technical Cooperation Achievements in Asia and the Pacific (**2 December 2020**)
<https://www.iaea.org/newscenter/news/new-iaea-publications-highlight-technical-cooperation-achievements-in-asia-and-the-pacific>
- World Food Day 2020: IAEA & FAO's Joint Work Benefits Farmers and Increases Food Security Worldwide (**16 October 2020**)
<https://www.iaea.org/newscenter/news/world-food-day-2020-iaea-faos-joint-work-benefits-farmers-and-increases-food-security-worldwide>
- Homegrown Soybeans are Making a Comeback in Indonesia Thanks to New Varieties Developed Using Irradiation (**5 October 2020**)
<https://www.iaea.org/newscenter/news/homegrown-soybeans-are-making-a-comeback-in-indonesia-thanks-to-new-varieties-developed-using-irradiation>
- Asian Cooperative Agreement Convenes 49th Annual Meeting to Discuss Regional Priorities, Challenges and Solutions (**23 September 2020**)
<https://www.iaea.org/newscenter/news/asian-cooperative-agreement-convenes-49th-annual-meeting-to-discuss-regional-priorities-challenges-and-solutions>
- Double the Yield, Double the Harvest: Zanzibar Improves Rice Production Using Nuclear Techniques (**7 August 2020**)
<https://www.iaea.org/newscenter/multimedia/photoessays/double-the-yield-double-the-harvest-zanzibar-improves-rice-production-using-nuclear-techniques>
- Tastier and More Nutritious Vegetables: Bulgaria Improves Food Quality with IAEA Support (**27 July 2020**)
<https://www.iaea.org/newscenter/news/tastier-and-more-nutritious-vegetables-bulgaria-improves-food-quality-with-iaea-support>

- Nuclear Technology Helps to Develop Heat-Tolerant Tomato Varieties in Mauritius (**15 July 2020**)
<https://www.iaea.org/newscenter/news/nuclear-technology-helps-to-develop-heat-tolerant-tomato-varieties-in-mauritius>
- World Environment Day 2020: How the IAEA Contributes to Soil, Plant and Animal Biodiversity (**4 June 2020**)
<https://www.iaea.org/newscenter/news/world-environment-day-2020-how-the-iaea-contributes-to-soil-plant-and-animal-biodiversity>
- Drought Tolerant Crops: Zambia, IAEA and FAO Help Zambia Improve Production and Farmers' Income (**1 June 2020**)
<https://www.iaea.org/newscenter/news/drought-tolerant-crops-zambia-iaea-and-fao-help-zambia-improve-production-and-farmers-income>
- Malaysia's Nuclear Agency and Partners Bring Improved Rice Variety to Country's Farmers (**6 April 2020**)
<https://www.iaea.org/newscenter/news/malysias-nuclear-agency-and-partners-bring-improved-rice-variety-to-countrys-farmers>
- IAEA and FAO Help Zanzibar Grow More Rice (**12 March 2020**)
<https://www.iaea.org/newscenter/news/iaea-and-fao-help-zanzibar-grow-more-rice>
- Fighting Two Major Cassava Diseases in Burundi with Nuclear Technology (**18 February 2020**)
<https://www.iaea.org/newscenter/news/fighting-two-major-cassava-diseases-in-burundi-with-nuclear-technology>
- Climate-Proof Crops: Capacity Building to Develop Resilient Crop Varieties in Small Island Developing States (**6 November 2019**)
<https://www.iaea.org/newscenter/news/climate-proof-crops-capacity-building-to-develop-resilient-crop-varieties-in-small-island-developing-states>
- Ethiopian Plant Breeders Turn to a Nuclear Technique to Help Teff Farmers Adapt to Climate Change (**5 November 2019**)
<https://www.iaea.org/newscenter/news/ethiopian-plant-breeders-turn-to-a-nuclear-technique-to-help-teff-farmers-adapt-to-climate-change>
- Targeting Hunger with Nuclear Techniques (**1 November 2019**)
<https://www.iaea.org/newscenter/multimedia/videos/targeting-hunger-with-nuclear-techniques>
- From Field to Table: Nuclear Techniques Toward Zero Hunger (**16 October 2019**)
<https://www.iaea.org/newscenter/news/from-field-to-table-nuclear-techniques-toward-zero-hunger>
- IAEA, FAO Help Develop Bananas Resistant to Major Fungal Disease (**30 September 2019**)
<https://www.iaea.org/newscenter/news/iaea-fao-help-develop-bananas-resistant-to-major-fungal-disease>
- Nuclear Techniques Help Develop New Sorghum Lines Resistant to the Parasitic Weed *Striga* (**5 September 2019**)
<https://www.iaea.org/newscenter/news/nuclear-techniques-help-develop-new-sorghum-lines-resistant-to-the-parasitic-weed-striga>
- Accelerating Growth: IAEA Launches Plant Mutation Breeding Network for Asia and the Pacific (**16 August 2019**)
<https://www.iaea.org/newscenter/news/accelerating-growth-iaea-launches-plant-mutation-breeding-network-for-asia-and-the-pacific>
- From Lab to Field: Indonesian Scientists Develop New Crops for Farmers Using Nuclear Science (**24 April 2019**)
<https://www.iaea.org/newscenter/news/from-lab-to-field-indonesian-scientists-develop-new-crops-for-farmers-using-nuclear-science?fbclid=IwAR0AiQtFLTCQ5tKuIgf9UfdnBIAX-ExoXksMQyFmTnD4XFIwxhKrTTxLbt8>
- How Nuclear Techniques Help Feed China (**4 April 2019**)
<https://www.iaea.org/newscenter/news/how-nuclear-techniques-help-feed-china>

- Drought-tolerant Crops to Contribute to Food Security in Namibia (**13 March 2019**)
<https://www.iaea.org/newscenter/news/drought-tolerant-crops-to-contribute-to-food-security-in-namibia>
- New CRP: Enhanced Biotic-stress Tolerance of Pulses Towards Sustainable Intensification of Cropping Systems for Climate-change Adaptation (D22006) (**27 February 2019**)
<https://www.iaea.org/newscenter/news/new-crp-enhanced-biotic-stress-tolerance-of-pulses-towards-sustainable-intensification-of-cropping-systems-for-climate-change-adaptation-d22006>
- Sierra Leone to Tackle Hidden Hunger with Better Crops Through Nuclear Technology (**26 February 2019**)
<https://www.iaea.org/newscenter/news/sierra-leone-to-tackle-hidden-hunger-with-better-crops-through-nuclear-technology>
- Barley in the Desert: Kuwait Progresses in the Development of a New Variety Using Nuclear Techniques (**18 February 2019**)
<https://www.iaea.org/newscenter/multimedia/photoessays/barley-in-the-desert-kuwait-progresses-in-the-development-of-a-new-variety-using-nuclear-techniques>
- Nuclear Technology Helps Develop New Barley Variety in Kuwait (**18 February 2019**)
<https://www.iaea.org/newscenter/news/nuclear-technology-helps-develop-new-barley-variety-in-kuwait>
- Bangladesh's Crop Scientists Find an Ally to Better Cope with Climate Change (**14 February 2019**)
<https://www.iaea.org/newscenter/news/bangladeshs-crop-scientists-find-an-ally-to-better-cope-with-climate-change>
- Using Nuclear Science in Marker-Assisted Plant Breeding (**22 January 2019**)
<https://www.iaea.org/newscenter/multimedia/videos/using-nuclear-science-in-marker-assisted-plant-breeding>

Websites and Links

- Plant Breeding and Genetics Section:
<http://www-naweb.iaea.org/nafa/pbg/index.html>
- InfoGraphic on Mutation Breeding:
<http://www-naweb.iaea.org/nafa/resources-nafa/Plant-Mutation-breeding.mp4>
- Mutant Variety Database:
<http://mvd.iaea.org>
- Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture:
<http://www-naweb.iaea.org/nafa/index.html>
<http://www-naweb.iaea.org/nafa/news/index.html>
- Joint FAO/IAEA Centre Publications
<http://www-naweb.iaea.org/nafa/resources-nafa/publications.html>
- Food and Agriculture Organization of the United Nations (FAO):
<http://www.fao.org/about/en/>
- FAO Agriculture and Consumer Protection Department:
http://www.fao.org/ag/portal/index_en/en/

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