



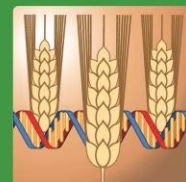
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To Our Readers

One major event this last half of 2013 was the success story of the IAEA interregional TC project INT/5/150 'Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99)'. Results have been achieved in record time; the project was started in 2009, and by mid 2013, two advanced mutant lines, resistant to the most virulent strains of wheat black stem rust (Ug99), successfully passed the national performance trials in Kenya and are ready for commercial release (see 'Success Stories' on page 17).



Wheat mutant lines resistant to races TTKSK (Sr31), TTKST (Sr24) & TTTSK (Sr36) (Photo courtesy of Prof Miriam Kinyua, Chepkoilel University College, Moi University, Eldoret, Kenya).

A Regional Training Course was organized at IRRI (The Philippines) in the framework of regional TC project RAS/5/065 'Supporting Climate-Proofing Rice Production Systems (CRiPS) Based on Nuclear Applications'. By joining efforts, the IAEA, FAO and IRRI aim to ensure that rice scientists are acquainted with the most recent plant breeding and phenotyping approaches, allowing them to address future threats to

food security stemming from climate change and other stress factors. The project also aims to act as a platform for future collaboration in rice production (see 'Past Events' page 8).

This second half of the year was also the period of 'first timers'. For the first time, Lesotho is using mutation breeding techniques in potato, sweet potato and amaranth. A fellow is being introduced to these techniques at the Plant Breeding and Genetics Laboratory (PBGL) in Seibersdorf, Austria (see 'Developments at the Plant Breeding and Genetics Laboratory, Seibersdorf' on page 21). Similarly we are supporting and nurturing the first steps of national mutation breeding programmes in Oman, Palestine, Qatar and Saudi Arabia.

Another cause for excitement is the ReNuAL project: the Seibersdorf Laboratories will be upgraded and renovated, including the FAO/IAEA Agriculture & Biotechnology Laboratories. This is a huge project with many logistical challenges, which are being met with vigour and personal investment in time and energy by our staff. Our guidance in this endeavour is 'fit-for-purpose to the Member States' in the future. IAEA Member States adopted a resolution to support this initiative, and we are now seeking financial and in-kind support. Please be reminded that these Laboratories are your laboratories, belonging to and serving the Member States (see 'Developments at the Plant Breeding and Genetics Laboratory, Seibersdorf' on page 22).

Initiation of mutation breeding in coffee: coffee is one of the world's largest export commodities and in terms of monetary value is only exceeded by crude oil. Statistical figures suggest that the income of 1 in 70 people in the world is directly or indirectly dependent upon coffee production (Cocoa Research Institute of Nigeria, CRIN). Coffee is a neglected crop in terms of plant mutation breeding, partly because no protocols for mutation induction in coffee are available. The

CRIN is interested in developing mutation breeding of coffee to combat problems in the crop, such as biennial fruiting, low yield, high caffeine content and disease resistance, especially coffee leaf rust. This is a prime example of Member States driving our adaptive R&D. Recently Guatemala and Mexico joined with a request to combat coffee leaf rust using mutation techniques (see 'News' on page 20).

Recently, we noticed a continuous increase in Member States' demands in capacity development: Training Courses (which are always over-subscribed), fellows and interns (in 2013 we had to increase the capacity of our Fellow's Room from 11 to 21. Irradiation service requests (over 80 plant samples received for irradiation in 2013) are also on the rise, for an increasing range of crop species.

The CRP D2.40.13 on Isolation and Characterization of Genes Involved in Mutagenesis of Crop Plants closed this year, but was extended without cost until a final wrap-up meeting in 2014. The objective of this CRP was to isolate and characterize genes involved in mutagenesis and find out types of mutations induced by various physical mutagens in studied crop plants; to generate mutants deficient in DNA repair pathways in crops and assess their usefulness for efficiency

enhancement of mutation induction. Major milestones have been achieved: genes encoding core proteins of the repair pathway in rice have been cloned and are being characterized. Whole genome scanning in soybean is underway for naturally mutated and selected genes. A chloroplast mutator gene and mutations caused by it have been identified in barley and three putative DNA repair genes in pea are being studied. Mutant populations of rice are being screened for reduced sensitivity to radiation. The assembly and publication of the complete sequence of the wild soybean (*Glycine soja*) genome is a major achievement. A barley EST database was established with extensive sequence alignments to arabidopsis sequences, with hyperlinks to original data sources. This database is a valuable knowledge platform on repair mechanism genes, and will go publically online in 2014, after upgrading and updating (see 'Coordinated Research Projects (CRPs) and Research Coordination Meetings (RCMs) on page 10).

Finally, please allow me to wish you a brilliant 2014 (1435 in Islamic countries, 2557 in the Buddhist Era)

Peace, Health and Prosperity to you and your loved ones.

Pierre J.L. Lagoda
Section Head



Trainees at work — Regional Training Course on GxE Testing, Seed Storage and Farmers' Participation, RAS/5/064, Los Baños, the Philippines, 8–12 July 2013 (for more details, see page 7).

Staff

**Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
Vienna International Centre, P.O. Box 100, 1400 Vienna, Austria (Phone:
+431 2600 + extension; Fax: +431 26007; Email: Official.Mail@iaea.org)**

Name	Title	Email	Extension
Qu Liang	Director	q.liang@iaea.org	21610

**Plant Breeding and Genetics Section, Wagramer Strasse 5, P.O. Box 100,
1400 Vienna, Austria (Phone: +431 2600 + extension; Fax: +431 26007;
Email: Official.Mail@iaea.org)**

Name	Title	Email	Extension
Pierre J.L. Lagoda	Head of Section	p.lagoda@iaea.org	21626
Stephan Nielen	Plant Breeder/Geneticist	s.nielen@iaea.org	21617
Vacant	Plant Breeder/Geneticist		
Fatma Sarsu	Plant Breeder/Geneticist	f.sarsu@iaea.org	21618
Katayoun Allaf	Programme Assistant	k.allaf@iaea.org	21621
Luis Mauricio Alfonso Godoy	Team Assistant	l.m.alfonzo-godoy@iaea.org	21620

**Plant Breeding and Genetics Laboratory, FAO/IAEA Agriculture and
Biotechnology Laboratories, 2444 Seibersdorf, Austria (Phone: +431 2600 +
extension)**

Name	Title	Email	Extension
Brian P. Forster	Laboratory Head	b.forster@iaea.org	28285
Bradley J. Till	Plant Breeder/Geneticist	b.till@iaea.org	28260
Adelbagi Mukhtar Ali Ghanim	Plant Breeder/Geneticist	a.mukhtar-ali-ghanim@iaea.org	28268
Mirta Matijevic	Technician	m.matijevic@iaea.org	28317
Joanna Beata Jankowicz-Cieslak	Technician	j.jankowicz@iaea.org	28275
Guenter Berthold	Technician	g.berthold@iaea.org	28418
Andreas Draganitsch	Technician	a.draganitsch@iaea.org	28418
Souleymane Bado	Technician	s.bado@iaea.org	28208
Owen Anthony Huynh	Technician	o.huynh@iaea.org	28279
Joanna Malgorzata Mletzko	Team Assistant	j.mletzko@iaea.org	28362

Forthcoming Events

Regional ARCAL Training Course on Molecular Methods for Characterization of Mutant Germplasm Derived from Native Crops and Crops of Interest in Latin America and the Caribbean, RLA/5/063, Bogotá, Colombia, 17–21 February 2014

Technical Officer: S. Nielen

The purpose of this training course is to impart knowledge and skills for the development of molecular markers and their use in mutation breeding programmes related to crops and traits of relevance to the regional project RLA/5/063. In particular, the course will include lectures and exercises on marker assisted selection; mutation detection using Targeting Induced Local Lesions in Genomes (TILLING); screening of mutant populations for tolerance to abiotic stress such as salinity; Real Time PCR for expression analysis of genes associated with stress response (salinity, high temperature); isolation and analysis of proteins involved in stress responses; basic bioinformatics tools. The Plant Breeding and Genetics Laboratory (PBGL), Seibersdorf, supports this training course by providing a test kit with DNA, enzymes and primers that will allow the practical application of the low cost TILLING protocols that have been developed and published by the PBGL (for more information on PBGL protocols, see

<http://www-naweb.iaea.org/nafa/pbg/public/manuals-pbg.html>).

Regional AFRA Training Course on Methodologies and Mechanisms for Screening against Biotic and Abiotic Stresses, RAF/5/066, Tsumeb, Namibia, 24–28 March 2014

Technical Officer: F. Sarsu

This training course will be organized by the IAEA in collaboration with the Government of Namibia, Ministry of Agriculture, Water and Forestry. It is open to candidates from AFRA project RAF/5/066 on Improving Crops Using Mutation Induction and

Biotechnology through a Farmer Participation Approach (AFRA) project partners.

The purpose of this course is to provide participants with theoretical as well as practical information on mutation induction, mutation screening and breeding for biotic and abiotic stress tolerance in crop breeding. The course will include lectures and practical sessions on:

- Mutation breeding procedures/methodologies and handling of mutated population;
- Identification, evaluation and selection of breeding lines;
- Genetics of biotic stress resistance and abiotic stress tolerance;
- Physiology of biotic stress resistance and abiotic stress tolerance;
- Screening methodologies for biotic stress resistance and abiotic stress tolerance;
- Breeding for biotic stress resistance and abiotic stress tolerance, mutation breeding, classical breeding and utilization of appropriate biotechnologies;
- Field demonstration/screening of cowpea, millet and sorghum on Mannheim Research Station in Tsumeb.

The participants should be from all participating Members States involved in the project RAF/5/066, additionally they should be actively working in mutation breeding or have basic knowledge in crop breeding. The course will enrich scientists with at least a M.Sc. degree involved in plant breeding/genetics.

Fourth Research Coordination Meeting (RCM) on Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline, D2.40.12, Venue to be finalized, 7–11 April 2014

Technical Officer: B. Till

Global demand for improved crop varieties is expected to grow to meet continuing pressures on food security due to population growth, climate change and variability, and changing diets. Inducing mutations in plants to generate novel genetic diversity is a proven method to assist breeders in their efforts. Yet, improvements can be envisioned that increase the

speed and accuracy of the process. The CRP ‘Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline’ was designed to address the major steps in the plant mutation breeding approach for both seed and vegetatively propagated crops. These include induction of mutations at a suitable density and spectrum, dissolution of chimeric sectors, phenotypic evaluation of mutant lines, and reverse-genetic approaches to identify mutations affecting protein function. More information on this CRP and a brief summary of the results achieved can be found in the CRP section of this newsletter. A final RCM is being planned to share and synthesize results, prepare for publication of CRP results, to evaluate ways to continue networking beyond the duration of the CRP, and to identify successes and failures within the project so advice can be given for future Coordinated Research Projects.

Fourth Research Coordination Meeting (RCM) on Isolation and Characterization of Genes Involved in Mutagenesis of Crop Plants, D2.40.13, Vienna, Austria, tentatively planned for April 2014

Technical Officer: P.J.L. Lagoda

The overall objective of this CRP was to understand the mechanisms of mutation induction and molecular genetic features of induced mutations in plants, in order to provide the scientific basis for developing more efficient mutation techniques, and their proper use for crop improvement. Thus the specific research objective encompassed the isolation and characterization of genes involved in mutation induction, finding out types of mutations induced by various physical mutagens in studied crop plants, to generate mutants deficient in DNA repair pathways in crops and assess their usefulness for efficiency enhancement of mutation induction.

The CRP progress was very satisfactory and is projected to be successfully concluded in 2014 by the finalization of ongoing work.

A forth, and final, RCM is projected to validate the original achievements (repair pathway genes in rice barley, pea and soybean, assembly and publication of the complete sequence of the wild soybean, *Glycine soja*, genome, barley repair related EST database) of the CRP and finalize documents on its outputs:

- 1) A barley EST database, publicly accessible to all Member States, with extensive sequence alignments to *Arabidopsis* sequences, with

hyperlinks to original data sources and to the MVDatabase.

- 2) Characterized homologs of genes involved in DNA repair and mutagenesis in crop plants.
- 3) Genetic resources that could be used for efficient mutation induction. This refers to mutant lines deficient in genes involved in mutagenesis.
- 4) Research papers and other publications.

Fourth Research Coordination Meeting (RCM) on Improving Nutritional Quality by Altering Concentrations of Enhancing Factors Using Induced Mutation and Biotechnology in Crops, D2.30.28, Cusco, Peru, 7–11 April 2014

Technical Officer: S. Nielen

The objective of the Coordinated Research Project (CRP) is to improve varieties of local crops with increased yield and crop quality, enhanced levels of micronutrient contents, other nutritional factors and market-preferred traits through induced mutation techniques and supportive biotechnologies. This will be the final RCM of the CRP, which is coming to an end, five years after its beginning in January 2009. The purpose of the meeting is to review and evaluate materials and data produced during the course of the CRP, including advanced mutant lines with improved nutritional qualities and efficient screening protocols, to develop a suitable plan for dissemination of results and conclusions, and to make suggestions for further collaborative research efforts. (More information on this CRP on page 11).

ARASIA Regional Training Course on Biotechnologies for Plant Mutation Breeding, RAS/5/058, Seibersdorf, Austria, tentatively planned for June 2014

Technical Officers: P.J.L. Lagoda and F. Sarsu
Course Director: A. Ghanim

The purpose of this training course is to provide participants with technical and practical knowledge on biotechnologies that speed up crop mutation breeding programmes.

The training course will include lectures and practical sessions on:

- 1) Induced mutations for crop improvement;
- 2) Phenotypic and genotypic screening for mutants;
- 3) Use of doubled haploids in mutation breeding;
- 4) Applications of in vitro techniques in mutation breeding;
- 5) Rapid generation cycling.

ARASIA States Parties started a mutation breeding programme under the RAS/5/048 project in 2007 with the assistance of the IAEA. This initiative has been sustained with the project RAS/5/058 to build on the progress made under the previous TC project. Capacity has to be developed in order to maximize mutation induction and breeding. The previous and also the current project produced segregating mutant lines for targeted agronomic characters. These mutant genetic stocks need to be developed, using techniques that enhance and speed up mutation breeding programmes.

The training course will include lectures, practicals, roundtable discussions, consultations on methodologies and their application in various field situations. The course is open to 20 participants and the participants should be plant breeders from Members States participating in the project RAS/5/058.

Past Events

Regional Training Course on Phenotypical Characterization of Mutants, RAS/5/058, Amman, Jordan, 19–23 May 2013

Technical Officers: P.J.L. Lagoda and F. Sarsu



Participants in Agricultural Maru Station.

The training course was open to 16 participants from ARASIA Member States: Iraq (3), Jordan (4), Qatar (1), Saudi Arabia (3), Syrian Arab Republic (2) and Yemen (3).

The objective of the course was to provide participants with advanced theoretical knowledge and practical skills of phenotypic techniques for mutation breeding and genetics involving induced mutant populations. The course covered six major topics:

- 1) Induced mutations for crop improvement;
- 2) Identification, documentation and phenotypical characterization of mutant;
- 3) Handling of mutated populations;
- 4) Experimental design for accurate phenotyping;
- 5) Principles and methods of phenotypic measurements in plant breeding;
- 6) Use of rapid phenotyping methods for selection of heritable traits to facilitate mutation breeding;
- 7) Practical protocols and methods for screening drought tolerant mutant.

Drought, disease, heat and salinity are major constraints affecting sustainable agricultural productivity in ARASIA States Parties. This training course focused on properly controlled and designed phenotypic evaluation for successful implementation of mutation breeding strategies. The course participants were brought to the Agricultural Maru Station of Ramtha Regional Research Center. The lecturers explained and showed participants phenotypical screening of the mutant wheat and barley lines for drought tolerance in the field. Practical protocols and methods for screening drought tolerant mutant were transferred to participants.

Second Research Coordination Meeting (RCM) on Approaches to Improvement of Crop Genotypes with High Water and Nutrient Use Efficiency for Water Scarce Environment, D1.50.13, Kuala Lumpur, Malaysia, 24–28 June 2013

Technical Officers: K. Sakadevan and P.J.L. Lagoda

Ten research contract holders (Bangladesh, China, Kenya, Malaysia (2), Mexico, Pakistan, Peru, Uganda and Vietnam), one technical contract holder (Peru) and one agreement holder (South Africa) participated in the meeting.

All project participants have submitted the first year project progress report and all contracts have been renewed. The second RCM was held in Malaysia from 24–28 June 2013 and all participants attended the

meeting. During the Second RCM all participants presented results obtained from the field experiments carried out during the first year and constraints to the research progress was discussed. The country work plans were revised to align with the objectives of the project.

Regional Training Course on Mutation Induction Techniques and Supportive Breeding and Biotechnologies for Wheat and Barley, RAS/5/058, Seibersdorf, Austria, 1–5 July 2013

Technical Officer: P.J.L. Lagoda
Course Director: A.M.A. Ghanim

The course was taught by staff of the Plant Breeding and Genetics Laboratory and the Plant Breeding and Genetics Section with an additional lecture on ‘Best fit soil and water management practices’ from Mr Gerd Dercon, Head of the Soil and Water Management and Crop Nutrition Laboratory. The purpose of the training course was to provide basic knowledge and skills in mutation breeding in wheat and barley. There were three main themes: 1) mutation induction, 2) mutation detection and 3) pre-breeding of mutant lines. The course was designed for plant breeders in Member States embarking on cereal mutation breeding to provide mutant variation for various desired traits, such as greater yield, salt and drought tolerance and resistance to pests and diseases, particularly in areas threatened by climate change. The training course aimed to provide knowledge and skills to participants of the TC project RAS/5/058. Methods were taught on how to induce and detect desired mutant traits and how identified mutant lines could be developed using methods that speed up plant mutation breeding, thus facilitating the development of improved mutant varieties in Member States and how to apply appropriate agronomic practices to maximize yield.

The course focused on methods pertinent to wheat and barley and participants were encouraged to bring their own seed materials for irradiation (mutation induction). The course also provided an opportunity for networking among participating countries. A major objective of the course was to provide practical training so that the techniques taught may be applied when the participants returned to their home countries.

The course was attended by 17 participants from Iraq (3), Jordan (2), Lebanon (2), Oman (1), Saudi Arabia (3), Syrian Arab Republic (3) and Yemen (3). In addition, the course was open to six in-house fellows from Democratic Republic of Congo (1), Iraq (2),

Kenya (1), Nigeria (1) and Syrian Arab Republic (1). All lectures, protocols and demonstration notes were provided to delegates in hard and/or electronic form.



Some participants and trainers.

Regional Training Course on GxE Testing, Seed Storage and Farmers’ Participation, RAS/5/065, Los Baños, the Philippines, 8–12 July 2013

Technical Officer: P.J.L. Lagoda



Course participants in Manila, the Philippines.

The course was organized as part of the IAEA’s regional technical cooperation (TC) project, ‘Supporting Climate-Proofing Rice Production Systems (CRiPS) Based on Nuclear Applications’. Held at the IRRI headquarters in the Philippines, 22 nominees from 10 participating RAS/5/065 Member State (Bangladesh, Cambodia, Indonesia, Lao, Malaysia, Mongolia, Myanmar, the Philippines, Thailand, Vietnam) and six nominees sponsored by IRRI (Bhutan, China, India and Senegal) were invited to participate in a two week training course at IRRI. The trainees included project counterparts, members of research groups and leaders of breeding programmes.

The training course offered a series of lectures informing participants about the challenges of rice production, highlighting the current understanding of useful tolerance and adaptation traits in rice crops, and underlining the fundamental role of nuclear techniques in the development of climate change tolerant crop varieties. Participants took part in hands-on exercises, using various protocols and operating specialised equipment to assess various plant stresses and to

quantify plant responses as the basis for proper selection during germplasm improvement cycles. The second training course will be carried out in 2014.



Trainees at work.

By joining efforts, the IAEA, FAO and IRRI aim to ensure that rice scientists are acquainted with the most recent plant breeding and phenotyping approaches, allowing them to address future threats to food security stemming from climate change and other stress factors. The project also aims to act as a platform for future collaboration in rice production.

Regional Training Course on Molecular Marker and Biochemical Characterization, RAF/5/066, Eldoret, Kenya, 29 July–2 August 2013

Technical Officer: F. Sarsu



Training course participants.

Under the Regional Project RAF/5/066 AFRA III on Improving Crops using Mutation Induction and Biotechnologies through a Farmer Participatory Approach, the IAEA in cooperation with the Government of Kenya, University of Eldoret organized this training course. The training course was attended by trainees from 22 African countries: Algeria (2), Benin (2), Central African Republic (1), Egypt (1), Ghana (2), Kenya (4), Lesotho (1), Madagascar (1), Morocco (1), Mauritius (1) Nigeria (1), Sierra Leone (1), South Africa (1), Democratic Rep. of the Congo (1) and Zimbabwe (1).

The purpose of the training course was to provide participants with skills and knowledge in important aspects of crop breeding including molecular marker techniques, molecular data generation by molecular

marker technologies, analysis and interpretation as well as biochemical characterization for crop improvement.

The participants were given lectures and practical exercises in the laboratory on: 1) DNA as the source of genetic information, 2) Introduction to Molecular Marker Systems, 3) Principles of the polymerase chain reaction (PCR), 4) Principles of mapping, and 5) Application of biochemical markers in mutation breeding.

The practical sections included: DNA isolation and quantification from plant tissues, polymerase chain reaction (PCR), separation of fragments/electrophoresis and population analysis of molecular data. Participants studied actively in groups and showed much enthusiasm and motivation.



Participants observing DNA isolation.

Regional (RCA) Technical Meeting to Exchange Expertise in Mutation Breeding and Best Fit Soil and Water Management Practices, RAS/5/056, Ulaanbaatar, 13–16 August 2013

Technical Officer: S. Nielen

This Technical Meeting was organized in cooperation with the Nuclear Energy Agency (NEA), Ulaanbaatar, and the Plant Science and Agriculture Research Institute (PSARI), Darkhan. The main purpose of the meeting was to discuss project activities, implementation strategies and scientific methodologies to enhance national capacities for the application of isotopic and nuclear techniques for developing and using improved crop varieties with best practice soil, water, crop and nutrient management for increasing the crop productivity. 22 participants from 15 project member countries attended the meeting. All participants gave presentations reporting on the work done and achievements made since January 2012, when the project started. Good progress in the National

project activities was noted. For example, it was reported that three new rice mutant varieties are cultivated by local farmers on more than 500 ha in the Central Highlands of Vietnam, thereby expanding new mutant rice varieties for ethnic minority people in mountainous regions and contributing for food security of poor rice growers.

As an external FNCA (Forum for Nuclear Cooperation in Asia) expert Dr Atsushi Tanaka (Japan Atomic Energy Agency) gave a lecture on ion beam irradiation and its particular advantages for mutation breeding. In the technical sessions the following four subjects have been discussed in the plenum and in working groups, which formulated summaries and respective recommendations:

- New techniques in mutation induction;
- Mutation detection using molecular techniques — experiences from the RAS/5/056 training course in Perth, Australia (2012);
- Screening for drought and salinity tolerance — experiences from the field;
- Soil, water and crop management technologies and strategies to increase productivity of improved crop germplasm.

A survey on the status of irradiation facilities for mutation induction in RAS/5/056 participating countries was made, including information on possibilities of providing irradiation service for project members.



Meeting participants in front of the Plant Science and Agricultural Research Institute, Darkhan.

A one day technical visit to the Plant Science and Agricultural Research Institute (PSARI), Darkhan, 225 km north of Ulaanbaatar included an introduction to the activities of PSARI, given by its Director, Dr N. Bayarsukh, a visit to the laboratories for field quality, soil nutrition, potato micropropagation, genebank and biofertilizer production and a visit to the experimental fields, where advanced mutant lines of the wheat and barley mutation breeding programme were demonstrated.

Interregional Training Course on Breeding Techniques for Mutant Traits with Special Reference to Ug99, INT/5/150, Seibersdorf, Austria, 25–29 November 2013

Course Director: B.P. Forster

The purpose of the training course was to focus on challenges in mutation breeding for Ug99 resistance in wheat. The course was designed for practical plant breeders in Member States embarking on mutation breeding programmes utilizing induced mutant variation for Ug99 resistance. We were delighted to welcome Professor Hermann Buerstmyr (BOKU University, Austria) to the PBGL for this training course. Hermann is an expert in breeding for disease resistance in wheat and led a discussion on the various approaches in developing genetic markers for Ug99 resistance. The course included lectures, demonstrations and practicals on various aspects of mutation induction, mutation detection and methods that can speed up the breeding of mutant traits. In addition the course provided an opportunity for networking and germplasm sharing among the participating countries. A major objective of the course was to provide practical training so that the techniques taught may be applied to breeding when the participants returned to their home countries.

There were a total of 16 participants from 16 countries from INT5/150. In addition the course was open to interested fellows present at the time in the PBGL.



Ms Emmy Chepkoech (Kenya) giving a presentation at the training course on: Development of Ug99 Resistant Wheat Varieties in Kenya.

Coordinated Research Projects (CRPs) and Research Coordination Meetings (RCMs)

Project Number	Ongoing CRPs	Scientific Secretary
D2.30.28	Improving Nutritional Quality by Altering Concentrations of Enhancing Factors Using Induced Mutation and Biotechnology in Crops	S. Nielen
D2.30.29	Climate Proofing of Food Crops: Genetic Improvement for Adaptation to High Temperatures in Drought Prone Areas and Beyond	F. Sarsu
D2.40.12	Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline	B. Till
D2.40.13	Isolation and Characterization of Genes Involved in Mutagenesis of Crop Plants	P.J.L. Lagoda
D1.50.13	Approaches to Improvement of Crop Genotypes to High Water and Nutrient Use Efficiency for Water Scarce Environment	K. Sakadevan and P.J.L. Lagoda
D2.30.30	Integrated Utilization of Cereal Mutant Varieties in Crop/Livestock Production System	B.P. Forster
CRPs in Planning Stage	Working Titles	Scientific Secretary
	Enhancement of the Efficiency of Mutation Induction by Physical and Combined Mutagenic Treatments	S. Nielen
	Efficient Screening Techniques for Mutants with Disease Resistance	B. Till

Two new CRPs, each related to one of the two projects of our subprogramme (Mutation Induction and Mutation Screening), will commence during the new biennium. The timeline for implementation of both CRPs is: Consultant meeting and project formulation in 2014; start of project in January 2015. Detailed announcements on the projects and how to participate will be published in due course.

CRP on Efficient Screening Techniques for Mutants with Disease Resistance (Working Title)

This CRP will develop screening methods for useful mutations needed for disease resistance to safeguard crop yields in an era of increased population growth and climate variation. New technologies will be exploited in developing techniques aimed at increasing the efficiency of detecting disease resistant mutants for plant breeding in Member States. Diseases and priority regions will be defined according to demands from Member States. New high-throughput methods in phenotyping and genotyping offer increased efficiencies in selecting novel disease resistance mutants for plant breeding.

This CRP builds on successes from the CRP on Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline D2.40.12, and is related to the TC project on Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99).

CRP on Enhancement of the Efficiency of Mutation Induction by Physical and Combined Mutagenic Treatments (Working Title)

The main task is to develop more efficient methods, protocols and guidelines for X or gamma ray driven mutation induction, as well as for innovative efficient irradiation treatments to increase genetic diversity for plant breeding. This CRP will therefore include research on the enhancement of mutation induction through irradiation activation of genetic elements that move within the genome (transposable elements), and cause identifiable secondary mutations.

Increased biodiversity is needed to meet the breeding goals of the 21st century to provide stable yields in an era of population growth, climate variability and rising food costs. The need to generate new genetic

biodiversity and increase productivity especially in regions affected by climate variation requires a number of approaches including striking new paths in mutation induction techniques. This CRP will contribute to uncover the full potential of mutation induction, thus making the process of crop improvement and adaptation through mutation breeding more effective.

This task is related to an ongoing CRP on Isolation and Characterization of Genes Involved in Mutagenesis of Crop Plants D2.40.13 (ends in 2014). Whilst the ongoing CRP is primarily concentrating on the spectrum of mutations in the genes and also on identifying and characterizing the genes involved in mutagenesis in crop plants, the new CRP will focus on the repetitive part of the genome, which by far represents the majority in plant genomes.

Improving Nutritional Quality by Altering Concentrations of Enhancing Factors Using Induced Mutation and Biotechnology in Crops, D2.30.28

Technical Officer: S. Nielen

This CRP aims at making available new germplasm resources with improved yield and nutrient quality. It addresses the problem that most of the major staple crops are often deficient in essential vitamins and minerals and that more than 40% of the world's population suffer from malnutrition, with respect to micronutrients like vitamin A, iron and zinc. The strategies applied to reach the project's goal comprise utilizing efficient phenotypic screens and genotypic markers to identify in mutant collections individuals exhibiting traits of interest that would be incorporated into breeding programmes. Both, the resulting genetic resources and the methodologies for identifying them constituted the main expected outputs from this CRP.

The project started beginning 2009 currently, 14 research groups from 12 countries (Bulgaria, China, Denmark, Germany, Ghana, India, Kenya, Peru, South Africa, United Kingdom, Ukraine, and the United States of America) participate in the project, thereof nine under research contracts, three under research agreements, and two under technical contracts. In course of the project new mutant germplasm collections from elite varieties of the target crops wheat, barley, sorghum, soybean, groundnut, sweet potato and tomato have been developed. Efficient phenotypic screening methods for resistant starch, increased carotenoids and increased tocopherols were developed. These include colorimetric assays for phosphate and phytate for barley and wheat flour and

for starch composition in maize and wheat and a method for HPLC analysis of some carotenoids (lutein, zeaxanthin, lycopene, beta-carotene). Various molecular marker techniques have been developed or adapted to local conditions and are being used to screen for genes affecting synthesis of resistant starch, increased carotenoids, decreased oxalate, decreased phytate and increased tocopherols. An impressive example on the use of induced mutations as an effective tool in achieving premium nutrition in staple cereals is given by a CRP Member from South Africa. Mehlo et al. (2013) reported on the analysis of their sorghum mutant lines, which revealed a mutant line with redirected accumulation of kafirin proteins in the germ, a trait associated with highly desired improved digestibility. The change in storage proteins was accompanied by high level accumulation of free lysine and other essential amino acids in the endosperm. [L. Mehlo, Z. Mbambo, S. Bado, et al. (2013) Induced protein polymorphisms and nutritional quality of gamma irradiation mutants of sorghum, *Mutation Research* 749: 66–72;

<http://dx.doi.org/10.1016/j.mrfmmm.2013.05.002>]

The final RCM, where the results of this CRP will be summarized, its outputs and outcome assessed, and manuscripts for publication finalized will be held in Cusco, Peru, 7–11 April 2014.

Climate Proofing of Food Crops: Genetic Improvement for Adaptation to High Temperatures in Drought Prone Areas and Beyond, D2.30.29

Technical Officer: F. Sarsu

This CRP has 11 research contract holders from Colombia, China, Cuba, India, Mexico, Pakistan, the Philippines, Senegal, the United Republic of Tanzania and Zimbabwe and five agreement holders from China, Japan, Spain and the United Kingdom and International Rice Research Institute (IRRI).

Climate change is now largely accepted as a real and pressing global problem. The main impacts of climate change on agriculture will most probably be experienced through higher temperatures (increase in minima and maxima), altered changes in rainfall patterns (in amount, spatial and temporal distributions), increased rates of evaporation, intensity and frequency of extreme events (floods and droughts), and a rise in sea level affecting coastal areas where large quota of cultivated land are located (intrusion of salty water).

The CRP is focused on improving the grain yields of a major cereal (rice) and a grain legume (common bean)

to high temperature stress in the face of climate change. The CRP aims to develop new high yielding mutant varieties with improved quality under low input cultivation in a range of agro-ecologies, through broadening adaptability.

This CRP is being implemented steadily in all participating countries. The M₂ generations of rice and common bean are being screened, in laboratories, screen houses and fields. Even though it is quite early to account for solid achievements, it could be noted that most efforts have been put in establishing reliable and solid screening protocols, which in turn should later ensure the selection of the most suitable mutated genotypes. The efforts made in the analysis of the genomic responses have been substantial. Thanks to the release of the common bean genome sequence early this year, a wealth of genes involved in tolerance to heat are foreseen. The major output of this CRP will be enhanced protocols and guidelines to enable improved responses to abiotic and biotic stresses under increasingly adverse and variable conditions.

The next RCM is planned to be held in Dakar, Senegal to present latest results and discuss further actions to meet project activities during the third quarter of 2014.

Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline, D2.40.12

Technical Officer: B. Till

This CRP aims at evaluating, developing and adapting technologies to enhance the efficiency of mutation induction and screening. The CRP takes a modular approach to investigate and improve the different steps involved in a typical mutation breeding pipeline. Modules include phenotyping, the development of suitably mutagenized and sized populations for both forward and reverse-genetics, dissolution of chimeric sectors, the development of TILLING reverse-genetics platforms for seed and vegetatively propagated species, and investigations into novel technologies for mutation discovery and characterization. The four target crops chosen for this CRP are banana, barley, cassava and rice. The goal is to develop modules that can be combined for the rapid creation and selection of desired mutants. This CRP has focused on methodologies to differentiate between induced and natural mutations and ways to optimize induced mutations. Seed, nodal cuttings and friable embryogenic callus have all been used as material to treat with ionizing radiation. To evaluate the density and spectrum of induced mutations, both enzymatic mismatch cleavage and next generation RESCAN sequencing has been employed to

monitor mutation induction in cassava. To date, data suggest that mutation induction in cassava is much more difficult than in banana or seed propagated crops. This recalcitrance may be one reason why there are so few mutant cassava varieties listed in the database. In rice, the major focus has been on mutation screening both at the genotypic and phenotypic level. Mutant lines of African rice are being developed in Sierra Leone. Near Infrared Reflectance Spectroscopy is being adapted for single seed evaluation of seed quality. Next generation sequencing tools for rapid recovery of induced mutations using RESCAN and exome capture methods has been developed and validated. In Vietnam, advanced mutant lines have been developed that are salinity tolerant and have improved aroma quality and iron content. These lines are currently undergoing trials to determine if they are suitable for release as new mutant varieties.

This CRP was initiated in 2008. This CRP has five research contract holders from Cuba, China, Ghana, Poland, Sierra Leone, and Vietnam and four agreement holders from Austria, Germany, and USA (2). In addition, the Plant Breeding and Genetics Laboratory is a major contributor to this CRP through direct research and development activities, and collaborative efforts with CRP participants.

Isolation and Characterization of Genes Involved in Mutagenesis of Crop Plants, D2.40.13

Technical Officer: P.J.L. Lagoda

Since 40 years ago mutation induction and chemical agents proved extremely successful to improve crop varieties, the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) through their Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture efficiently supported, both financially and scientifically, research around the world in the field of mutation induction and breeding. As a result of these research activities, many developing countries experienced growing economic benefits through mutation induction and achieved enhanced genetic diversity in many crop germplasm. In order to investigate the different parameters, a previous CRP looked into the effects of mutagens on the DNA in plants. The second component is the repair mechanisms on the genomic level. CRP D2.40.13 was created five years ago to investigate this second component of mutation induction.

The objective was to isolate and characterize genes involved in mutagenesis and find out types of mutations induced by various physical mutagens in

studied crop plants; to generate mutants deficient in DNA repair pathways in crops and assess their usefulness for efficiency enhancement of mutation induction.

Major milestones have been reached in this CRP. Genes encoding core proteins of the repair pathway in rice have been cloned and are being characterized. Whole genome scanning in soybean is underway for naturally mutated and selected genes. A chloroplast mutator gene and mutations caused by it has been identified in barley and three putative DNA repair genes in pea are being studied. Mutant populations of rice are being screened for reduced sensitivity to radiation. The assembly and publication of the complete sequence of the wild soybean (*Glycine soja*) genome is a major achievement. A barley EST database was established with extensive sequence alignments to *Arabidopsis* sequences, with hyperlinks to original data sources and to the MVDdatabase. This database is a valuable knowledge platform on repair mechanism genes, and will go publically online in 2014, after upgrading and updating.

Approaches to Improvement of Crop Genotypes to High Water and Nutrient Use Efficiency for Water Scarce Environment, D1.50.13

Technical Officers: K. Sakadevan and P.J.L. Lagoda

The overall objective of this CRP is to increase crop productivity and food security by developing and extending rapidly to farmers the improved crop varieties and soil, water, nutrient and crop management technologies that make cropping systems resilient to biotic and abiotic stresses in water scarce environment. The specific objectives are to:

- Increase the productivity of improved mutant varieties of crops tolerant to environmental stresses under existing soil and climatic conditions.
- Enhance nitrogen and water use efficiencies of crops tolerant to environmental stresses through best practice soil, water, crop and fertilizer management.

Integrated Utilization of Cereal Mutant Varieties in Crop/Livestock Production System, D2.30.30

Technical Officer: B.P. Forster

This CRP was initiated recently, in 2012, but there are already some impressive achievements. The CRP,

which is commonly known as the 'food and feed' CRP, has a general aim to maximize yields of mutant cereal varieties for both food and feed. First year reports have been received from all eight participating countries (Austria, China, Indonesia, Kuwait, the Former Yugoslav Republic of Macedonia, Malaysia, Mongolia and Peru) who are working on a range of cereal crops (barley, rice, sorghum and wheat) and a range of animals (cattle, sheep, goats, quail and guinea pigs). The CRP has three major objectives: 1) Increase yield and quality of mutant cereals by developing appropriate agronomic practices, 2) Improve farming technologies by analysing feed quality, and 3) Improve the knowledge and skill of target beneficiaries through farmer training, expert consultations and publication of results.

Major activities in year one included: 1) The identification and deployment of mutant varieties and advanced mutant lines to be studied over the five year project period, 2) The identification of test sites from which basic soil and water analyses were carried out and 3) The exchange of germplasm, bulking up of materials and dissemination of technical information. The project is supported by advice from the FAO on techniques in determining fodder quality. In addition to these basic ground preparing activities, there were some highlights from year one, these include:

- Indonesia has identified promising advanced mutant lines of sorghum that have already been used in feeding trials of cattle. The advanced mutant line CTY-33 has been taken up by farmers and has increased the range and acreage of sorghum thanks to close cooperation between researchers and farmers.
- Malaysia has identified mutant rice lines with greater digestibility and nutritional value of straw as a feed for ruminants.
- Peru has identified advanced mutant lines and varieties of barley resistant to yellow rust (a major disease in Peru).
- Kuwait has offered to host and provide funding for an international Workshop on 'Breeding for Food and Feed' to which members of the CRP would be invited.
- Barley mutant lines have been disseminated, through the PBGL, to all participants working on barley, and seeds have been sent to non-participating MSs, e.g. mutant varieties of barley have been sent from Peru for evaluation in Iceland.

Technical Cooperation Field Projects

Project Number	Country/Region	Title	Technical Officer
ALG/5/026	Algeria	Increasing the Genetic Variability for the Improvement of Strategic Crops (Wheat, Barley, Chickpeas and Dates) for Enhanced Tolerance to Biotic and Abiotic Stresses and the Development of Biotechnology Capacities	P.J.L. Lagoda
ANG/5/008	Angola	Using Nuclear Technology to Select Mutants of Cassava Resistant to the African Cassava Mosaic Virus and Various Diseases Affecting this Crop	S. Nielen
BGD/5/028	Bangladesh	Assessing Crop Mutant Varieties in Saline and Drought Prone Areas Using Nuclear Techniques	S. Nielen
BKF/5/009	Burkina Faso	Improving Voandzou and Sesame Based Cropping Systems Through the Use of Integrated Isotopic and Nuclear Techniques for Food Security and Poverty Alleviation	P.J.L. Lagoda
BOT/5/009	Botswana	Using Radiation Technology and Biotechnology to Develop Mutant Lines of Important Crops with Increased Yield and Improved Nutritional and Hygienic Qualities	A.M.A. Ghanim/S. Nielen
CAF/5/006	Central African Republic	Improving Cassava Production through High-Yielding Varieties and Sustainable Soil Fertility Management by Using Isotopic and Nuclear Techniques to Ensure Sustainable Farming	B. Till in collaboration with Soil and Water Management and Crop Nutrition Section
COL/5/024	Colombia	Supporting Mutagenesis and Functional Genomics Applied to the Improvement of Rice	B. Till/S. Nielen
ERI/5/024	Eritrea	Supporting the Livelihood of Barley Farmers through Mutation Techniques and N15 Technology to Improve Malting, Food and Feed Barley Production	A.M.A. Ghanim/S. Nielen
INS/5/039	Indonesia	Enhancing Food Crop Production Using Induced Mutation, Improved Soil and Water Management and Climate Change Adaptation	B.P. Forster/B. Till
INT/5/150	Interregional	Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99)	P.J.L. Lagoda/B.P. Forster
INT/5/152	Interregional	Supporting Mutation Breeding Impact Assessment	P.J.L. Lagoda/B.P. Forster
IVC/5/031	Cote d'Ivoire	Improving Plantain and Cassava Yields through the Use of Legume Cover Crops	P.J.L. Lagoda in collaboration with Soil and Water Management and Crop Nutrition Section
KAZ/5/003	Kazakhstan	Increasing Micronutrient Content and Bioavailability in Wheat Germplasm by Means of an Integrated Approach	F. Sarsu/S. Nielen in collaboration with Soil and Water Management and Crop Nutrition Section
KEN/5/032	Kenya	Characterising and Improving Germplasm of Selected Crops at the Molecular Level Using Nuclear and Biotechnology Techniques	F. Sarsu/P.J.L. Lagoda

Project Number	Country/Region	Title	Technical Officer
LES/5/001	Lesotho	Improving Crop Yield, Quality and Stress Tolerance for Sustainable Crop Production to Alleviate Hunger, Poverty and Environmental Degradation	S. Nielen/A.M.A. Ghanim
MAG/5/022	Madagascar	Strengthening Food Security	F. Sarsu/B.P. Forster
MAL/5/029	Malaysia	Applying Mutation Breeding and Optimized Soil, Nutrient and Water Management for Enhanced and Sustainable Rice Production	S. Nielen
MAR/5/020	Mauritius	Developing Stress Tolerant Banana and Tomato Varieties by Enhancing the National Capacity in Mutation Induction and Biotechnology	B. Till/S. Nielen
MON/5/021	Mongolia	Improving the Productivity and Sustainability of Farms Using Nuclear Techniques in Combination with Molecular Marker Technology	B.P. Forster/P.J.L. Lagoda in collaboration with Animal Production and Health Section
MOR/5/033	Morocco	Using Nuclear Techniques to Support the National Programme for the Genetic Improvement of Annual and Perennial Plants and to Develop Agricultural Production	F. Sarsu/A.M.A. Ghanim
MYA/5/020	Myanmar	Strengthening Food Security through Yield Improvement of Local Rice Varieties with Induced Mutation (Phase II)	S. Nielen/P.J.L. Lagoda
NAM/5/010	Namibia	Developing High Yielding and Drought Resistant Pearl Millet (<i>Pennisetum glaucum</i> L), Sorghum Bicolor (L) Moench, Bambara Groundnut (<i>Vigna subterranea</i>) and Cowpea (<i>Vigna unguiculata</i> (L) Walp) Following Up a Previous Project (PHASE II)	F. Sarsu/S. Nielen
NER/5/015	Niger	Improving Productivity of the Millet-Cowpea Cropping System through Development and Dissemination of Improved Varieties and New Water and Fertiliser Management Techniques	S. Nielen in collaboration with Soil and Water Management and Crop Nutrition Section
OMA/5/002	Oman	Assessing the Suitability of Sterile Insect Technique (SIT) and Related Techniques for Combating Date Palm Insect Pests	P.J.L. Lagoda in collaboration with Insect Pest Control Section
PAK/5/047	Pakistan	Developing Germplasm through TILLING in Crop Plants Using Mutation and Genomic Approaches	B. Till/S. Nielen
PAL/5/005	Palestine	Improving local Palestinian wheat and barley varieties for salt and drought resistance through mutation breeding and biotechnology	B.P. Forster/P.J.L. Lagoda
RAF/5/066	Regional Africa	Improving Crops Using Mutation Induction and Biotechnology through a Farmer Participation Approach (AFRA)	F. Sarsu/B. Till
RAS/5/056	Regional Asia	Supporting Mutation Breeding Approaches to Develop New Crop Varieties Adaptable to Climate Change	S. Nielen/P.J.L. Lagoda
RAS/5/058	Regional Asia	Supporting Mutation Breeding Approaches to Develop New Crop Varieties Adaptable to Climate Change	P.J.L. Lagoda/F. Sarsu

Project Number	Country/Region	Title	Technical Officer
RAS/5/064	Regional Asia	Enhancing Productivity of Locally-underused Crops through Dissemination of Mutated Germplasm and Evaluation of Soil, Nutrient and Water Management Practices	P.J.L. Lagoda/S. Nielen in collaboration with Soil and Water Management and Crop Nutrition Section
RAS/5/065	Regional Asia	Supporting Climate-Proofing Rice Production Systems (CRiPS) Based on Nuclear Applications	P.J.L. Lagoda/S. Nielen in collaboration with Soil and Water Management and Crop Nutrition Section
RLA/5/056	Regional Latin America	Improving Food Crops in Latin America Through Induced Mutation (ARCAL CV)	S. Nielen/B. Till
RLA/5/063	Regional Latin America	Supporting Genetic Improvement of Underutilized and Other Important Crops for Sustainable Agricultural Development in Rural Communities (ARCAL CXXVI)	S. Nielen/B. Till
SEN/5/034	Senegal	Using an Integrated Approach to Develop Sustainable Agriculture in a Context of Degrading Soil Fertility, Climate Change and Crop Diversification	F. Sarsu
SUD/5/033	Sudan	Enhancing Productivity of Major Food Crops (Sorghum, Wheat, Groundnut and Tomato) under Stress Environment Using Nuclear Techniques and Related Biotechnologies to Ensure Sustainable Food Security and Well-Being of Farmers	F. Sarsu in collaboration with Soil and Water Management and Crop Nutrition Section
UZB/5/005	Uzbekistan	Developing Mutant Cotton Breeding Lines Tolerant to Diseases, Drought and Salinity (Phase II)	S. Nielen/P.J.L. Lagoda
YEM/5/008	Yemen	Introduction of Gamma Ray Irradiation Techniques for Agriculture Purposes	F. Sarsu/S. Nielen
YEM/5/010	Yemen	Using Induced Mutations and Efficiency Enhancing Bio-Molecular Techniques for Sustainable Crop Production	S. Nielen/P.J.L. Lagoda
ZAI/5/019	Democratic Rep. of Congo	Developing Mutations, In Vitro and Molecular Techniques for Further Dissemination to Breeders and Pharmaceutical Plant Producers to Enhance the Livelihood of Target Populations	B. Till/F. Sarsu
ZAM/5/027	Zambia	Developing Maize Genotypes for Drought and Low Soil Fertility Tolerance	F. Sarsu in collaboration with Soil and Water Management and Crop Nutrition Section
ZIM/5/015	Zimbabwe	Developing Drought Tolerant and Disease/Pest Resistant Grain Legume Varieties with Enhanced Nutritional Content Using Mutation Breeding and Novel Techniques, Phase II	F. Sarsu/S. Nielen

Success Stories

Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99), INT/5/150

Technical Officer: P.J.L. Lagoda

Project Management Officer: T. Moleah

Mutation activities target rust diseases with the emphasis on Ug99 to contribute to and eventually broaden genetic base for rust resistance. Mutant lines that have resistance to Ug99, once selected, will counter the threat to global wheat and barley production, the menace of Ug99.

18 participating Member States and five national and international partner institutions collaborate in the IAEA interregional project INT/5/150 Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99), in order to produce mutant varieties resistant to wheat black stem rust race Ug99. Staff of the Plant Production and Protection Division (AGP) of the Food and Agriculture Organization of the United Nations (FAO) are invited to the coordination meetings as experts and consultants.

Globally, most commercial wheat and barley varieties are susceptible to the new race of wheat stem rust, race Ug99 (TTKST) and its mutant derivatives (TTKSK, TTTSK). Introduction of variability by irradiation of wheat seeds from a good genetic background is much simpler and faster than crossing with an exotic source and is one of the main reasons for the use of mutation techniques in breeding programmes. As the most virulent strains of Ug99 (TTKST, TTKSK, TTTSK) are endemic in Kenya, an intensive screening programme under INT/5/150 is therefore being conducted at Chepkoilel University College, Moi University to identify wheat and barley stem rust resistant mutants with good agronomic traits.

Close to 300 000 mutant lines (M_2+) have been generated by INT/5/150 participants since beginning of the project in 2009. M_2 and M_3 lines have been tested and lines exhibiting resistance have been advanced to further generations. The material is exposed to natural rust infection (Ug99), but complimented with artificial inoculation (screen-houses).

To screen for Ug99 resistance, rust spores were suspended in distilled water with a few drops of wetting agent Tween-20 and applied to the spreader lines using a sprayer with a fine nozzle at booting stage. Inoculation was done in the evening just before

or after dew formation to increase presence of free water necessary for spore formation and successful infection events.

Selected resistant mutants (M_3+) are tested for confirmation at Moi University, Kenya, where land and irrigation facilities are made available. National programmes of Member States are visiting to assist making selection as needed.

Currently 13 lines with resistance to Ug99 are available, thereof eight M_7 lines from Kenya. Four of these mutant lines have been subjected to National Performance Trials. All four lines were validated by the independent national authority. Moi University decided to release the two best performing as new varieties (2013). They are currently planted on three acres for seed multiplication (target six tonnes) to be released to farmers. First level molecular characterization and validation is going on at the FAO/IAEA PBGL (Seibersdorf, Austria).

One advanced mutant line from Uganda (M_1 produced at the PBGL) will be entered into national performance trials in Uganda. In farmer's fields this advanced mutant line showed outstanding performance: in addition to resistance to Ug99, it yields three tonnes/ha under rainfed conditions (twice the national average of: 1.2 tonnes/ha). A second mutant line from Uganda will be entered into national performance trials in Kenya.

Selected material will be repatriated to contributors for testing under local conditions for traits of interests as well as testing at rust hot spots, in order to be advanced further and eventually released as varieties.

In summary, this successful project has produced in four years:

- 13 advanced mutant lines in six Member States
- Two mutant lines passed successfully national performance trials (13 sites in Kenya, 2012) and are being released as varieties in Kenya (2013)
- Two mutant lines are in trials for release as varieties in Uganda (2014).

Two New Developed Cotton Varieties through Mutation Induction in Pakistan, PAK/5/047

Contributed by: Q. Liang

The Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, an establishment of Pakistan Atomic Energy Commission (PAEC), has achieved another milestone by releasing two new mutant cotton varieties, named 'NIAB-KIRAN' and 'NIAB-112', on

23 May 2013. This was achieved through the peaceful use of nuclear technologies, i.e. mutation induction, and conventional breeding techniques with the aim of providing improved cotton varieties to farmers for commercial cultivation in Pakistan. Of these two, 'NIAB-KIRAN' possesses early maturity, high yield potential (5500–6500 kg/ha, over 50% greater yield over the commercial standard 'MNH-786), lodging resistance (25–60% higher value in root length after 30 DAS), high tolerance to heat (no fruit abortion at lower nodes at higher temperatures (46–50 °C) tolerance to cotton leaf curl virus disease (CLCuD), superior fibre (length; 30.41 mm, fineness; 4.61 µg/inch, strength; 28.6 g/tex, uniformity index; 82.4%, maturity; 81.1% & G.O.T; 40.9%) coupled with a low short fibre index (SFI; 7.9) over other commercial cultivars used for the manufacture of quality yarn.

F₀ seed resulting from the cross between Mutant-98 (developed through pollen radiation) and NIAB-11/CE (developed through seed irradiation of F₁) was treated with 300 GY of gamma rays in 2000–2001. Selections were made in the M₂ and subsequent segregating generations; stable progenies were bulked during 2007–2008 followed by evaluations in micro, macro, and yield trials as well as in requisite national trials (NCVT, PCCT, DUS and 1.25 acre yield trials at PSC farms) in the years 2010–2011 and 2011–2012. On successful completion of these trials 'NIAB-KIRAN' was approved for commercial release in 2013.



Characteristics of an advanced mutant line of cotton.

The mutant variety 'NIAB-112' possesses the following traits: early maturity (150 days) with determinate flowering, extremely suitable for wheat-cotton-wheat rotation, higher yield potential (4500–5000 kg/ha, 44% higher yield over the standard NN-3 at PSC farm trials; Khanewal during the 2012–2013 season), high resistance to CLCuD and tolerance to heat, and good quality: enhanced lint potential; 39.0% fine quality fibre values (length; 29.0 mm, fineness; 4.74 µg/inch; strength; 33.0 g/tex).

Across of NIAB-11/CE and Mutant-999 (CLCuV resistant parents against old strain) was followed by F₁ seed treatment with 300 Gy gamma radiations in 2001–2002. Selections were made in M₂ (2003–2004)

generation. Stable progenies were bulked during 2007–2008 at the M₇ generation and this was followed by evaluations in micro, macro, and requisite national performance trials (NCVT, PCCT, DUS and 1.25 acre yield trials at PSC farms) in 2011–2012 and 2012–2013. After evaluation 'NIAB-112' was officially approved for commercial release in 2013.

Cultivation of both mutant varieties will not only help in eradicating CLCuD in Pakistan but will also help in sustaining cotton production under the changing climatic scenario including heat stress and moisture stress, while maintaining high yields and good quality (fine quality fibre for end users). After the approval of these two cotton varieties the total number of release mutant varieties from the NIAB becomes 10.



Newly developed cotton varieties in Pakistan.

Improving Crop Varieties through Use of Nuclear Techniques in Zambia, ZAM/5/026

Technical Officer: F. Sarsu

Zambia is one of Sub-Saharan Africa's most highly urbanized countries. About one-half of the country's 11.5 million people are concentrated in a few urban zones, while rural areas are under-populated. Agriculture is a major source of employment and is an increasingly important sector in the Zambian economy; however, the poor use of land resources has severely degraded the soil, making it difficult for farmers to raise agricultural production beyond subsistence levels.

The objective of the agricultural sector is to promote increased and sustainable agricultural productivity in order to ensure food security and reduction in poverty levels. Crop productivity is affected by various factors including inadequate improved seed availability and crop failure or reduced yields due to drought, biotic and abiotic factors. The development of crop varieties that are tolerant to major stress factors affecting crop production such as soil acidity, drought, pests, and diseases, is now given more prominence in agricultural research.

To address the issue of consumer preference and thereby contribute towards increased bean production and consumption in Zambia, the Department of Plant Science in collaboration with the Zambia Research

Institute (ZARI), National Institute for Scientific and Industrial Research (NISIR) and Golden Valley Agricultural Research Trust (GART), initiated character improvement of common bean through induced mutation breeding in 2000.

The overall goal of the programme was to develop bean varieties that are high yielding and possess desirable characteristics that enhance consumer preference such as seed size, seed coat colour, and tolerance to pests and diseases.



Zambian farmers evaluating common bean mutants at a field day at Golden Valley Agricultural Research Trust.

High Yielding Common Bean Varieties Tolerant to Bruchids and Acidic Soil

Common bean is a major legume crop in Zambia as it provides the bulk of the dietary protein intake in the poorer households and also accepted as a cash crop. It is grown for its mature dry seeds and immature green and yellow pods. The crop is often grown in a rotation system with cereals as the bean crop enriches soil fertility by fixing nitrogen. Nevertheless, bean production in Zambia is characterized by low yields ranging from 200–800 kg/ha which compares to the potential of 3–4 tons/ha. Apart from being low yielding, most local bean varieties are susceptible to pests and diseases.

Mutation breeding has had great success with common bean. Mutation derived common bean lines have been developed with high-yielding potential (20%–30% above parental values) especially in environments with predominantly acidic soils. Bean mutants also have different phenotypes and maturity periods which may allow them to fit into environments not adapted to their parents.

Mutants have been selected with improved nutritional composition of high iron, zinc and protein with respect to their parent varieties. Organoleptic tests showed that some mutants had improved nutritional composition compared to their respective parents. Additionally, in terms of organoleptic tests, mutation-derived of the Carioca bean variety were equally preferred as the local and released varieties despite their small seed size.

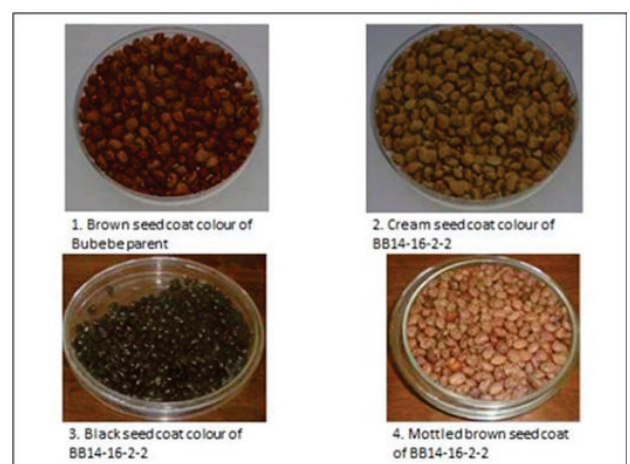
Cowpea Varieties Tolerant to Aphid and Different Seed Colour Variation for Health and Consumer Preference

Another important crop for Zambia is cowpea. Cowpea provides dietary protein and is of major importance to the livelihoods of millions of relatively poor people in less developed and drier countries of the tropics. It is also important as a nutritious livestock feed. Zambia needs cowpea that is high yielding, early maturing, resistant to aphids and storage weevils and one that is more resilient to erratic weather conditions.

Cowpea mutant varieties have been developed with high-yielding potential and altered traits for increased consumer preference such as brown mottled seed coat colour and large seed size.

Selected mutants have shorter and longer maturity periods for wider environmental adaptation and greater tolerance to insect pests such as aphids and storage pests such as bruchids. Aphids are a serious challenge to cowpea production and losses can be up to 100%. These mutants will enable production of cowpeas by small scale farmers with inadequate resources to purchase fertilizers and pesticides.

Induced mutation has resulted in variation in seed coat colour of cowpea and therefore also variation in bioactive compounds which play a major role in reducing oxidative stress and inflammation in non-malignant cells. Chronic diseases such as diabetes, cardio-vascular diseases, obesity and stunted growth can be reduced by these compounds. Yellow and darker seed coat colours are associated with higher amounts of bioactive compounds.



Bubebe cowpea variety and its mutants exhibited different seed colours and different tolerance to aphids and bruchid storage pest.

High Yielding Velvet Bean

Induced mutation produced mutants with significant variation in plant type, maturity, yield, and protein ratio compared to the parent. This enables mutants to fit into

farming systems with shorter or longer growing seasons.

Superior mutants of velvet bean varieties have been selected with significantly high biomass and seed yield of about 100% or more over that of the parent varieties. Yield had over eight ton/ha seed yield compared to about three ton/ha of the parent variety. Some of the selected mutant lines have about 2 times more crude protein than the parent and thus will provide more nutritious livestock fodder especially for dairy animals.

According to Dr Kalaluka L. Munyinda, Senior Lecturer and Team Leader in the Legume Improvement programme in the Department of Plant Science, University of Zambia, 'All these seeds especially for common beans and velvet beans are ready for commercialization and it is now up to the University to market these findings and commercialize so that the institution and the country as a whole can benefit from this mutation breeding research'. These lines have undergone evaluation by farmers and it is foreseen that they will be released for large scale cultivation in the near future.

News

Coffee Leaf Rust — A New Challenge for Mutation Breeding

Coffee is one of the world's largest export commodities and in terms of monetary value is only exceeded by crude oil. Coffee is grown in Africa, Asia and Latin America, in a tropical 'coffee belt'. Brazil is the biggest producer (54.5 million bags in 2011–12), followed by Vietnam (18.7 million bags) and Colombia (9.5 million bags). The main coffee species is *Coffea arabica* (Arabica coffee), which accounts for over 60% of world production and is grown for its high quality beans. Major Arabica coffee producers are Brazil, Colombia, Ethiopia, Central America, Mexico, India and Eastern Africa, where it is grown above 800 metres and up to 2500 meters. The second most important cultivated species is *Coffea canephora* (Robusta coffee), which is grown at low altitudes, mainly in Vietnam, Brazil and Indonesia.

Coffee Leaf Rust (CLR)

The major problem facing Arabica coffee production in the world is the disease, coffee leaf rust (CLR), caused by the fungus *Hemileia vastatrix*; the estimated worldwide annually economic damage is US\$1.5-2 billion. The current outbreak of the disease threatens to reduce yields by about 50% in the 2013/14 cropping

season in Guatemala, Columbia, Honduras, El Salvador, Nicaragua and Costa Rica.

The cause of the recent outbreak of CLR is thought to be associated with climate change (wetter and more humid conditions) and changes in farming practices (pesticide and fungicide sprays) which have wiped out organisms that help limit disease spread. Currently, disease control relies on spraying with chemicals during the rainy season; this is expensive, treatments can be washed away and can leave residues on berries. Hence there is great interest in developing disease resistant varieties.

Arabica coffee has a narrow genetic base and there is no resistance to CLR disease. The Robusta germplasm does contain resistance genes but these are difficult to transfer into Arabica coffee due to the different biology of the two species, the long duration of conventional breeding programmes and the concern of ruining the quality of Arabica coffee with introduced genetic variation from Robusta. Mutation breeding offers a solution to these problems.

Coffee cultivation has already benefitted from spontaneous mutants. Spontaneous mutants occur naturally and are very rare, but some have been selected and grown as varieties such as: 'Caturra' (a short compact mutant of 'Bourborn' grown in Brazil), 'Pacas' (a colour mutant of 'Bourborn' grown in Latin America) and 'Maragoype' (a large bean size mutant grown in Latin America)

In 2013 the IAEA was approached by Guatemala and Mexico for help in overcoming coffee leaf rust. We are currently developing mutation breeding protocols for coffee and formulating a new project, bringing together pathologists, biotechnologists and breeders to improve resistance against coffee leaf rust through induced mutation.

Information Sheets for Visitors

The PBGL produces 'Information Sheets for Visitors', these are Member State based and describe the agricultural background of the country along with the major problems facing crop production, successes in plant mutation breeding, the status of plant mutation breeding and support from the FAO/IAEA Joint Division including capacity building. These information sheets are generally compiled by fellows during their training at the PBGL for their home country. We now have a collection of 17 'Information Sheets for Visitors', those new for 2013 include Burkina Faso, Congo, Democratic Republic of, Kenya,

Lesotho, Madagascar, Nigeria, Palestine and Sudan. Others have been re-edited: Bangladesh (4th edition), Indonesia (2nd edition) and Turkey (2nd edition).



Information sheets are popular with visitors.

The current collection: Afghanistan, Bangladesh (4th edition), Burkina Faso, China, Congo Dem. Rep. of, Indonesia (2nd edition), Kenya (2nd edition), Lesotho, Madagascar, Mongolia, Nigeria, Palestine, Peru, Sudan, Thailand, Turkey (2nd edition), USA.

Developments at the Plant Breeding and Genetics Laboratory, Seibersdorf

‘Expansion’ comes close as a single word that describes many of the activities of the PBGL in 2013. At the top of the list is the ReNuAL project. ReNuAL is the acronym for ‘**R**enovation of the FAO’s and IAEA’s **N**uclear Sciences and **A**pplications **L**aboratories in Seibersdorf’, which aims to ensure that the Laboratories are fit-for-purpose and appropriately positioned to meet the evolving needs and demands of Member States with adequate infrastructure in place for the next 20–25 years. The goals are to:

- Redesign and expand the current infrastructure to ensure the efficiency and effectiveness of laboratory operations and services to better meet the current and future requirements of Member States;

- Ensure that the laboratories remain a vibrant research and training institution that continues to attract highly qualified scientists and other staff committed to advancing applied nuclear sciences to serve the needs and interests of Member States.

There has been much activity in 2013 in preparing information on future activities of the PBGL for the planning of ReNuAL. More information on the ReNuAL project is given below.

In addition to the future expansion of the PBGL as part of ReNuAL, 2013 has seen the PBGL expand its core capabilities in Research and Development, Training and Services. The refurbishments of our greenhouse facilities were completed at the end of May 2013 and this has enabled us to grow some crop plants for the very first time, such as coffee, cucumber and cotton. The coffee work aims to develop mutation induction methods for both Arabica (seed propagated) and Robusta (vegetatively propagated) coffee varieties, whereas work on cucumber aims at producing haploid embryos *via* pollination with irradiated pollen and cotton is being studied to optimize mutagenic treatments.

Compared to recent years we have been host to more individual fellows, 11 as compared to three in 2012 and nine in 2011. These fellows have come to us from nine MSs. Our fellows come with various levels of knowledge in plant mutation breeding. In the case of Lesotho, the fellow (Ms Matumelo Alice Rafiri) has undertaken the first irradiation treatments known to us for mutation induction for Lesotho crops (potato, sweet potato and amaranth); this marks the beginnings of plant mutation breeding in Lesotho. In sharp contrast we have hosted 9 visitors (fellows, interns and trainees) from Bangladesh, which is a country with well-established and vibrant programmes in plant mutation breeding.

The number of Interns and Trainees is also up on recent years and this is partly due to the PBGL hosting three training courses in 2013 on: 1) Plant mutation breeding: mutation induction, mutation detection and pre-breeding; 2) Mutation induction and supportive breeding and biotechnologies for wheat and barley; 3) Breeding techniques for mutant traits with special reference to Ug99. As a consequence of increased numbers our ‘Fellow’s Room’ has been re-designed to make more

efficient use of space, it can now accommodate 21 fellows as opposed to 11.

Also up is the number of service requests we have received for irradiation. In fact in 2013 we received a record number of plant samples for irradiation.

A new technique adapted at the PBGL for mutation induction in potato is micro-tuber production. In 2013 the PBGL received requests from Kenya, Lesotho and Morocco for potato irradiation. Tubers were sent to us from which *in vitro* cuttings were established, and these were used to produce *in vitro* micro-tubers. Micro-tubers are ideal for propagating and transporting potato germplasm and may be used as samples for mutation induction. The use of micro-tubers and other *in vitro* methods have been investigated for practical use in potato mutation breeding.

The adaptive research activities of the PBGL have expanded to include techniques that can speed up plant mutation breeding, these include methods in rapid generation cycling, doubled haploidy and marker assisted selection. These are being developed in wheat, barley and sorghum. With respect to barley, we have collaborations with BOKU University, Austria to fast track the introgression of mutant genes for improved fodder quality.

We are very pleased to increase our network of collaborations to include the University of Ljubljana, Slovenia. Prof Borut Bohanec has dynamic research programmes using X ray irradiation and we are collaborating with his group on developing X ray irradiation for both mutation induction (as an alternative to gamma irradiation) and also the use of irradiated pollen to induce haploids in cucurbit species. The efficiency of detecting haploids and subsequent doubled haploids can be greatly increased using a ploidy detector, and we are pleased to report that the PBGL has recently procured a new flow cytometer for such purposes.

ReNuAL

From Labs to Member States

The Joint FAO/IAEA Agriculture & Biotechnology Laboratories (ABL) in Seibersdorf support and implement programmatic activities in response to Member State needs in the areas of food and

agriculture. Their mandate is to assist Member States in the development and adaptation of new and existing technologies, involving isotopes, radiation and complementary techniques, to suit local requirements and environmental conditions, and to provide relevant training and analytical services.

Applied research and development are linked to coordinated research activities and technical cooperation projects, two of the IAEA's main delivery mechanisms in transferring nuclear technologies to Member States. The laboratories research and develop new and adapt existing technologies to suit local needs in Member States. This creates extraordinary opportunities for the laboratories' scientists and technicians to work with external stakeholders and Member States in meeting the often very specific challenges of both developing and developed countries. During the subsequent technology transfer process, laboratory outputs are disseminated and tested in the field and results are fed back for further improvement and validation — providing the unique feedback loop that makes this approach so effective.

Training and capacity building are crucial components of technology transfer. While most training activities are carried out and supported locally in Member States or in regional laboratories, numerous train-the-trainer workshops, courses and seminars are held at the FAO/IAEA Agriculture & Biotechnology Laboratories, involving several hundred trainees annually, with the overall goal of building sustainable capacity in Member States. While the key impetus may be the technology, thorough emphasis is placed also on the wider aspects of the problems to be studied or solved. Thus, trainees return home with comprehensive knowledge and with an extensive scientific and technical network ready to assist.

Technical and analytical support are provided to Member States through evaluation, standardization and selection of appropriate equipment and processes for each specific project and need, taking into account local conditions and infrastructure. FAO and IAEA technical staff responsible for implementing field projects has extensive experience in routine operations, maintenance and repair of the necessary processes and equipment.

The NA Laboratories in Seibersdorf

The NA laboratories in Seibersdorf — eight in total — are a unique feature in the United Nations system. Five of these laboratories are co-operatively managed by the FAO and the IAEA through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. These are:

- ❖ The **Animal Production and Health Laboratory** supports Member States in the use of radioisotopes and related technologies to map superior genes for increased animal productivity, and develops and transfers molecular and immunoassay methods for diagnosis and control of transboundary animal diseases.
- ❖ The **Food and Environmental Protection Laboratory** uses nuclear technologies to trace and authenticate food products and to detect and monitor contaminants in foods and the environment, improving Member State laboratory practices in food safety and quality to safeguard health and facilitate international trade.
- ❖ The **Insect Pest Control Laboratory** develops environmentally friendly methods of pest control for area-wide control of key insect pests, such as fruit flies, tsetse flies, moths and disease transmitting mosquitoes. It is renowned worldwide for its work on the sterile insect technique.
- ❖ The **Plant Breeding and Genetics Laboratory** focuses on mutation breeding to increase biodiversity for desired traits of crop plants and hence to accelerate the breeding of varieties with higher yield, yield stability, nutrition and improved resistance to environmental stresses such as disease, drought and salinity.
- ❖ The **Soil and Water Management and Crop Nutrition Laboratory** uses isotopic and radiation methods to measure and monitor soil, water and nutrients in cropping systems as a basis for developing strategies that ensure judicious and efficient use of resources and that minimize environmental degradation.

The three NA laboratories at Seibersdorf operated exclusively by the IAEA are:

- ❖ The **Dosimetry Laboratory**, part of the IAEA's human health programme, oversees the quality assurance aspects of the use of radiation in medicine in Member States. It provides dosimetry calibrations for national standards laboratories and conducts audits of the dose in radiotherapy and radiation protection.
- ❖ The **Nuclear Spectrometry and Applications Laboratory**, part of the IAEA's nuclear science programme, works with laboratories in Member States to enhance their use of nuclear instrumentation and analytical techniques, for example in promoting the use of various types of accelerator for materials testing and historical artefact preservation.
- ❖ The **Terrestrial Environment Laboratory**, part of the IAEA's environment programme, helps Member States to better understand and protect the terrestrial environment. To this end, the laboratory develops environmental assessment strategies and

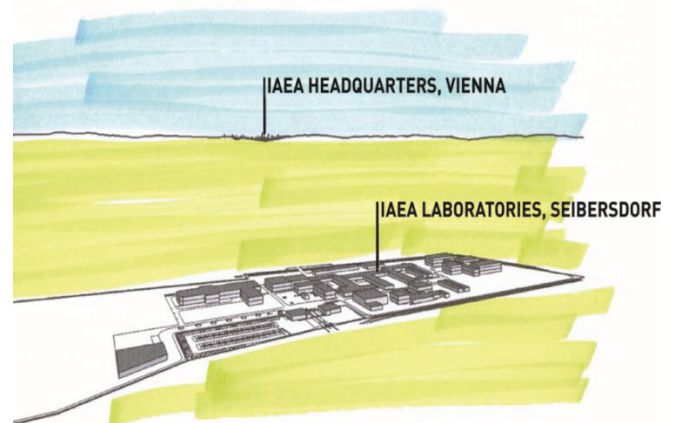
ensures the quality of analytical results by recommending methods, providing reference materials and organizing proficiency tests.

Renovation of the FAO's and IAEA's Nuclear Science and Application Laboratories in Seibersdorf

Objective: As part of the IAEA's Nuclear Sciences and Applications (NA) Laboratories in Seibersdorf, to ensure that the FAO/IAEA Agriculture & Biotechnology Laboratories (ABL) are fit-for-purpose and appropriately positioned to meet the evolving needs and demands of Member States with adequate infrastructure in place for the next 20–25 years. The goals are to:

- Redesign and expand the current infrastructure to ensure the efficiency and effectiveness of laboratory operations and services to better meet the current and future requirements of Member States;

Ensure that the laboratories remain a vibrant research and training institution that continues to attract highly qualified scientists and other staff committed to advancing applied nuclear sciences to serve the needs and interests of Member States.



The Current Situation

The NA Laboratories in Seibersdorf comprise eight laboratory groups, five of which are co-operatively supported by the FAO and the IAEA through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The laboratories support the delivery of nuclear sciences and applications through regular budget-supported activities, such as coordinated research projects (CRPs), through extrabudgetary mechanisms and through the IAEA's Technical Cooperation Programme. Currently 87 staff work in Seibersdorf, including 58 staff at the FAO/IAEA Agriculture & Biotechnology Laboratories, supported by consultants, cost-free experts, visiting scientists and

interns. The laboratories maintain a balance among applied/adaptive research and development, education and training, and scientific and technical services. Key activities in the laboratories include:

- **Applied/Adaptive Research and Development:** All laboratories are instrumental in developing and adapting proven methodologies and technologies for transfer to Member States. Research is demand driven and leads to new possibilities in the provision of services that in turn support research in Member State laboratories. Currently, over 50 CRPs are directly supported by the NA laboratories in Seibersdorf.
- **Education and Training:** The NA laboratories provide trainees and fellows from Member States with hands-on training in nuclear techniques through the TC programme and via extrabudgetary funding. The number of fellows, scientific visitors, training course and workshop participants is currently reaching 350 Member State experts per year. Most training courses are oversubscribed and there is currently little capacity to receive more.
- **Scientific and Technical Services:** The laboratories provide quality assured technical services, such as calibration and dosimetry audits, reference materials, proficiency testing and other analytical support services the demand for which continues to grow. The laboratories' services include supporting the research of Member States' scientific institutions by establishing and sharing best practices worldwide and building collaborative global scientific networks.
- **Support for the Delivery of Technical Cooperation Projects:** The NA laboratories currently provide support to almost 300 TC projects through education and training activities, scientific and technical services and technical advice.

The Need for ReNuAL

Established in 1962, more than 50 years ago, demands on the NA Laboratories in Seibersdorf have continually increased in order to meet the evolving needs and demands of Member States; as the numbers of Member States grow; and as more have recognized the value of the laboratories and sought their support. These demands are expected to further increase in the future as the issues that the laboratories are expected to address continue to evolve.

While the laboratories have seen individual upgrades and extensions during the past 51 years, these were implemented sporadically and in response to individual needs but without an overall and comprehensive concept. No concerted renovation or significant new construction has taken place during this period.

Whereas technologies and techniques used in the laboratories have changed dramatically over time, the facilities have not evolved to match the requirements for operations, training and compliance with current safety and security regulations and relevant quality management requirements.

The NA laboratories in Seibersdorf need both a quantitative and a qualitative enhancement. New investments in space and equipment is essential to secure the future of the laboratories for the benefit of Member States and to ensure that individual laboratories and support operations are fully compliant with the latest safety and security standards for laboratory research facilities.

At the 2012 IAEA General Conference, Director General Amano expressed his intention to launch a new initiative for to the modernization of the IAEA's Nuclear Sciences and Applications Laboratories at Seibersdorf, of which the FAO/IAEA Agriculture & Biotechnology Laboratories are the largest single component. This initiative was supported through the adoption of Resolution GC(56)/RES/12. In August, this year, FAO Director General da Silva warmly welcomed the invitation of Mr Amano to support this initiative and to assist in mobilising the necessary resources among Member Countries. As part of this endeavour, the current document outlines this initiative, targeted to ensure the availability of fit-for-purpose laboratories appropriately positioned to meet the needs and demands of Member States for the next 15-20 years.

Major Elements of ReNuAL

The **ReNuAL** project aims to ensure adequate infrastructure and equipment and a forward-looking approach for the FAO/IAEA Agriculture & Biotechnology Laboratories to carry out their mandate proficiently. Current projections, taking into account specific laboratory needs, space norms and training forecasts, foresee an increase in overall space of 59% compared to the current situation. Acquiring a balanced mix of key programmatic priorities and the necessary laboratory, office and training space, along with the highest priority equipment, will be crucial to the success of this project.

The current **ReNuAL** project, with strict adherence to programmatic prioritisation, incorporates the following:

- The comprehensive renovation of the current laboratory facilities to ensure adherence to applicable safety regulations and to guidelines on barrier-free access for the disabled;
- Scientific equipment and instrumentation that ensure methodological alignment with the majority of external stakeholders;

- Design, planning, contingency and project management costs commensurate with the volume of the projected construction and renovation plan.

Budgetary Target and Timeline

The **ReNuAL** concept anticipates a tentative preliminary budget of around €31 million over the next four years, and envisages completion by 2017. It foresees financing through a combination of regular budget and extrabudgetary funding. To this end, the IAEA has allocated approximately €2.6 million of Regular Budget funds each year over the next four years, i.e. a total of around €11 million, while the remaining investments will be generated from a variety of extrabudgetary sources. Efforts will also be made to attract private sector support.

Fit-for-Purpose Laboratories

The **ReNuAL** project will ensure that the laboratories will:

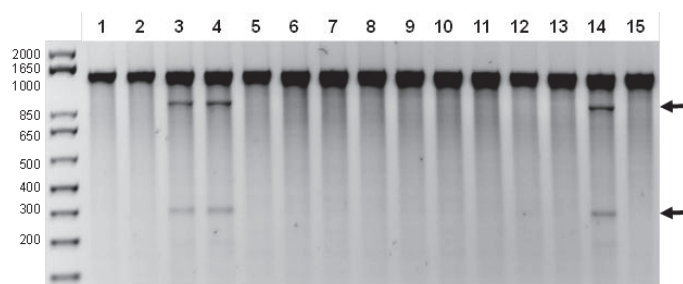
- Serve as a hub for growing networks of Member State laboratories in the respective thematic areas as a means to enhance their sustainability;
- Address emerging issues, for example, the impact of and adaption to climate change, new transboundary animal diseases, rapidly growing issues in the field of cancer;
- Foster the development of new nuclear applications, products and services;
- Increase capacity-building activities by providing hands-on training in both conversant and new areas;
- Institutionalize a systematic approach to quality assurance through modern facilities capable of accreditation to international standards, where relevant, and contribute to improving the quality of Member States' laboratories within the framework of respective agricultural, food, environmental, health and safety standards.

Detecting Mutation Events at the Molecular Level

Heritable changes in the sequence of genomic DNA are the cause of many of the mutant traits selected by breeders. While rapid and accurate ways of screening plants at the morphological level (phenotyping) remain crucial for success, evaluations at the molecular level, such as DNA based screening (genotyping) can be advantageous. The development of markers, for example, allows rapid introgression of desired traits into new genetic backgrounds without genotype by environment effects that can make phenotypic analysis difficult. Knowledge of the mutations and genes that

cause novel traits also adds much to our basic understanding of crop biology. Further, knowing genes that control traits allows for more direct approaches for precision breeding such as reverse-genetics and targeted mutagenesis. Great advances have been made in recent years in terms of technologies to evaluate genome sequences. However, accurate discovery of rare induced mutations in large plant genomes such as wheat remains challenging. Unlike natural populations where alleles are fixed, early generations of mutant populations have many new and varied nucleotide variations that segregate. Two plants from two different lines showing the same trait likely have completely different mutations that are responsible for the observed phenotype. This means that a traditional approach for marker development cannot be easily applied before the development of mapping populations. Whole genome sequencing can be a means to speed up the process of marker discovery, but many plant genomes are prohibitively large and extant methods too expensive for routine application. As part of CRP D2.40.12, Enhancing the Efficiency of Mutagenesis through an Integrated Biotechnology Pipeline, the PBGL is evaluating different sequencing strategies that rely on reduced representation libraries. The aim is to reduce the number of nucleotides sequenced in a genome to an amount where multiple plants can be sequenced in parallel using a bench top sequencer. Several approaches are being evaluated including restriction phased libraries and exome capture. Data are still being collected and evaluated. While cutting-edge high throughput approaches promise to greatly increase the efficiency of breeding, many such methods remain cost-prohibitive for developing Member States. For this reason, the PBGL has been actively developing and adapting low-cost 'do-it-yourself' approaches for molecular characterization of plants. There are two main reasons for this. First, even when considering out-sourcing high throughput screening to a modern facility, scientists need to have first-hand knowledge of basic molecular biology and in most cases need to provide highly purified nucleic acid samples. Secondly, application of low-cost methods can provide very useful information in the design and interpretation of mutation-based experiments. To address this, the PBGL has developed a suite of low-cost protocols that begins with tissue storage and ends with enzymatic mismatch cleavage for the discovery of SNP and small indel natural and induced mutations. The methods avoid the use of toxic organic chemicals and do not require liquid nitrogen or -80C freezers. The methods were adapted in 2012 and validated in early 2013. A description of this work was published in: Do-it-yourself molecular biology for plant breeding: low-cost tools for developing countries Huynh, O.A., Hofinger, B.J., Beshir, M.M., Jankowicz-Cieslak, J., Guo, H., Forster, B.P., Till, B.J.. Plant

Genetics and Breeding Technologies; Plant Diseases and Resistance Mechanisms: Proceedings, 18–20 February 2013, Vienna, Austria. Medimond — Monduzzi Editore International Proceedings Division, Pianoro, Italy, 2013, p. 33–36. A training course manual was developed that incorporates these methods for use in a 1 week training course. This manual was used for the June, July and November training courses held at the PBGL. To date, more than 50 scientists have been trained on these methods. The PBGL continues its work on developing and adapting low-cost methodologies for molecular characterization. Most recently, we have been working on methods to validate the production of doubled haploid (DH) plants. Doubled haploidy is a powerful breeding approach because all mutations in DH plants are homozygous and all nucleotide variation is fixed. This means that recessive traits are immediately observable and true breeding in DH plants. The process of making DH plants can be inefficient and it is important that putative DH plants are indeed homozygous and not heterozygous contaminants. As part of CRP D2.40.12, and in collaboration with CRP agreement holder Jochen Kumlehn's group from IPK Gatersleben, Germany, the PBGL set out to adapt an enzymatic mismatch cleavage screening approach for the validation of doubled haploid plants. Assays clearly show that while F₁ material is heterozygous in target genes, putative doubled haploids are not. The parental origin of genic regions can also easily be determined (see figure below). This work was published recently in the journal *Plant Methods* (see publications below). In a direct comparison with a standard SSR molecular marker screening approach, enzymatic mismatch cleavage was successful for 42% of gene targets compared to only 9% success with SSRs.



Agarose gel evaluation of doubled haploid production in barley by enzymatic mismatch cleavage.

Enzymatic mismatch cleavage was carried out to evaluate homozygosity in putative barley doubled haploid lines. A gene fragment was PCR amplified and digested with a crude celery juice extract (CJE) containing single-strand specific nuclease activity followed by agarose gel analysis. The top band in lanes 1–15 represents undigested PCR product. The cleavage

products present in heterozygous samples are marked with arrows. Parental lines Golden Promise (GP) and HOR1606 are homozygous for this gene region (lanes 1&2 respectively). A synthetic mixture of parental DNA and also the F₁ sample from crossing of the two parents show cleavage fragments resulting from a heterozygous SNP (lanes 3&4). Doubled haploid plants (lanes 5–13) are homozygous. Mixtures of genomic DNA from a DH plant and GP show cleavage products while mixture of the same material with HOR1606 does not, indicating the DH harbours the GP allele (lanes 14&15). This figure and text legend was reproduced from: Hofinger, et al., 2013, Validation of doubled haploid plants by enzymatic mismatch cleavage. *Plant Methods* 11/2013 9(1):43.

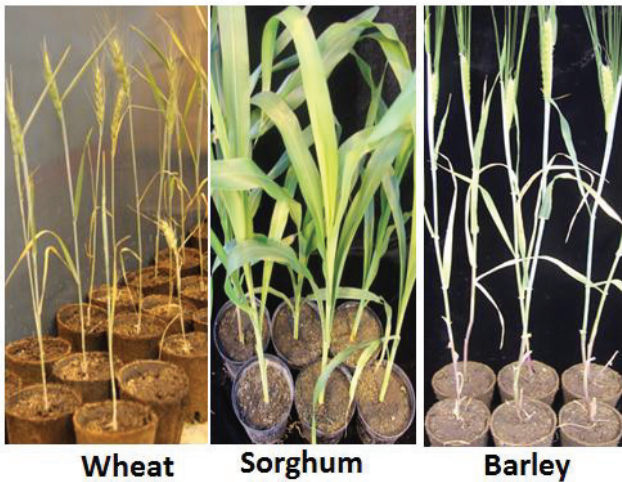
Accelerated Techniques in Plant Mutation Breeding

Although plant mutation breeding is faster than conventional breeding, techniques are available that can speed up the time from mutation induction to mutant variety release. Advances in high-throughput phenotyping and genotyping, and tissue culture provide several opportunities to make efficiency gains and accelerate the delivery of mutant varieties. The PBGL is carrying out adaptive research to integrate methods in rapid generation cycling, doubled haploidy and marker-assisted selection to speed up the breeding of mutant varieties. Encouraging results were made in shortening generation cycles of some major cereal crops, wheat, barley and sorghum. This was achieved by growing plants in small pots with low levels of watering and exposure to continuous lighting in a glasshouse. Ten wheat and seven sorghum varieties from Kenya and Sudan were used in these trials along with barley mutant lines. At the grain milk-ripe stage (15–20 days after pollination) immature embryos were rescued and cultured. Cultured embryos germinate immediately and shorten the time to the next generation as they circumvent the normal physiological maturity of the seeds, which can take several weeks. The use of embryo culture shortens the generation time to an average of 48 days in wheat, 60 in sorghum and 40 days in barley. In theory, this enables the production of 6–8 generations a year, which is enough to reach sufficient homozygosity to advance a mutant line for evaluation trials. Moreover, the plantlets produced from cultured embryos provide ideal materials from which DNA can be extracted for genotyping, thus only plantlets with desired genotypes may be selected and advanced.

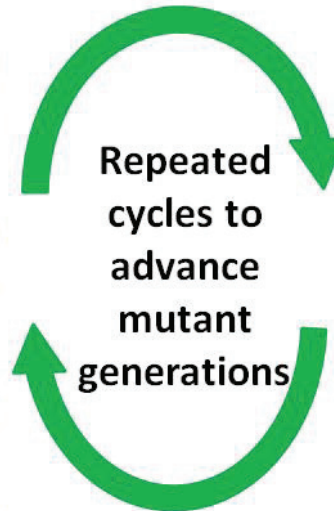
NORMAL AND ACCELERATED GENERATION TIMES AND THE RESPECTIVE NUMBER OF GENERATIONS PER YEAR FOR WHEAT, BARLEY AND SORGHUM

Plant Species	Normal Generation Time	Generations/Year ¹	Accelerated Generation Time ²	Generations/Year
Wheat (Spring)	4–5 months	1–2	45 days	7
Barley	4–5 months	1–2	40 days	8
Sorghum	5–6 months	1–2	60 days	6

¹In many countries only one generation per year is possible during the normal cropping season, sometimes two crosses can be done when additional facilities, such as greenhouses or irrigation are available. ²These are averages for 5–10 varieties from each crop species.



Shortening the crop cycle by growing plants in small pots, with sparse watering and continuous light



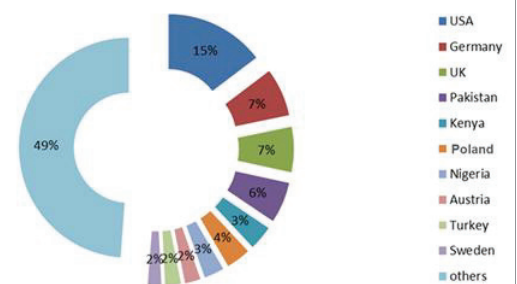
Gaining time by the removal and culture of immature embryos and subsequent sowing in small pots.

Rapid generation cycling techniques to accelerate mutant line development.

Irradiation Services

The PBGL received a total of 45 requests from Member States for plant irradiation services in 2013. The total number of irradiation requests since records began is 1354. At the time of writing we have received over 80 plant samples for irradiation in 2013. This is a record; previous highs were 59 samples in 1997 and 64 in 2009. Some MSs are interested in irradiation of just one species whereas others request irradiation treatments for many species. The PBGL is performing more irradiation service requests for more MSs on more crop species than ever.

Pie chart showing Member States plant irradiation service requests as a percentage of all requests since 1977, the top ten requesting countries are indicated (others account for 2% or less of total requests).



Human Capacity Development

Tables of trainees, fellows, interns, scientific visitors and consultants at the PBGL in 2013 are given below taking these together, the PBGL hosted 79 people from 35 countries.

Fellowship Training – Group Training at PBGL in 2013

Regional training course on Plant Mutation Breeding: Mutation Induction, Mutation Detection and Pre-Breeding, 3–14 June 2013.

This training course was primarily organised for Indonesia and Bangladesh participants from TC projects INS/5/039 (Enhancing Food Crop Production using Mutation, Improved Soil and Water Management and Climate Change Adaptation) and BGD/5/028 (Assessing Crop Mutant Varieties in Saline and Drought Prone Areas Using Nuclear Techniques), respectively.

Course participants: Mr Peter Jerem-James (Austria), Mr Mohammad Abul Kalam Azad (Bangladesh), Ms Hosne-Ara Begum (Bangladesh), Ms Shamsun Nahar Begum (Bangladesh), Mr Md Abul Kashem (Bangladesh), Mr Mohammad Ibrahim Khalil (Bangladesh), Mr Mohammad Lutfar Rahman Mollah (Bangladesh), Mr Lekgari Aatshwaelwe Lekgari (Botswana), Mr Odireleng Molosiwa (Botswana), Mr Molla Fentie (Ethiopia), Mr Wijaya Murti Indriatama (Indonesia), Ms Nurlina Kasim (Indonesia), Mr Muhamad Samaullah (Indonesia), Mr Rinaldi Sjahril (Indonesia), Mr Untung Susanto (Indonesia), Ms Sasanti Widiarsih (Indonesia), Mr Aziz Abdelkarim Salameh (Palestine). The course was open to other participants including fellows at the PBGL at the time.

Regional Training Course on Mutation Induction Techniques and Supportive Breeding and Biotechnologies for Wheat and Barley (RAS/5058-003), 1–5 July 2013.

This training course was organized for participants in the TC project RAS/5058 Supporting mutation

breeding approaches to develop new crop varieties adaptable to climate change.

Course participants: Mr Dheyab Hashim (Iraq), Mr Hatem Jasim (Iraq), Mr Azeez Majeed (Iraq), Mr Awad Al-Kaabnh (Jordan), Ms Siham Allouzi (Jordan), Mr Salah El Hajj Hassan (Lebanon), Ms Faten Raad (Lebanon), Ms Al-Ghaliya Humaid Khamis Al-Mamari (Oman), Mr Mohammed Almuwalld (Saudi Arabia), Mr Abdullah Al-Salman (Saudi Arabia), Mr Abdulmajeed Jamal Khashoggi (Saudi Arabia), Mr Soultan Al Yahya (Syrian Arab Republic), Mr Imad Alnabulsi (Syrian Arab Republic), Mr Imad Zein (Syrian Arab Republic), Mr Ahmed Saeed Binguoid (Yemen), Mr Ahmed Malek (Yemen), Mr Nabil Thabet (Yemen). The course was also open to fellows at the PBGL at the time.

Interregional Training Course on Breeding Techniques for Mutant Traits with Special Reference to Ug99 (INT/5/150-004), 25–29 November 2013.

This course was organized for participants of the TC project INT/5/150 Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99).

Course participants: Ms Chafika Djenadi (Algeria), Ms Boryana Dyulgerova (Bulgaria), Mr Luxiang Liu (China), Mr Mohammed Ayaad (Egypt), Mr Bikram Das (India), Mr Ali Eskandari (Islamic Republic of Iran), Ms Siham Allouzi (Jordan), Ms Makarim Mohammed Bashir Younus (Iraq), Ms Chepkoech Emmy (Kenya), Mr Ahmad Elbitar (Lebanon), Mr Najeeb Alharbi (Saudi Arabia), Mr Eben Von Well (South Africa), Mr Modather Abdalla (Sudan), Ms Amina Sshoab (Syrian Arab Republic), Mr Youssef Trifa (Tunisia), Mr William Wamala Wagoire (Uganda). The course was also open to fellows at the PBGL at the time.

Fellowship Training – Individual Training

Name	Country	Area of Training	Period
Ms Mayada Beshir	Sudan	Mutation induction and detection using molecular techniques such as TILLING	Nov. 2012–Feb. 2013
**Mr Amos Ego	Kenya	Induced mutation, molecular screening and validation of Ug99 resistant wheat mutants	Feb. 2013–Oct. 2013
Mr Mohammed Elsidig Ahmed	Sudan	Induced mutations, sorghum mutation screening and associated biotechnologies	June 2013–Sept. 2013
***Mr. Md. Rafiqul Islam	Bangladesh	Mutation induction in vegetable crops and haploid production using irradiated pollen	June 2013–Nov. 2013
*Ms Matumelo Alice Rafiri	Lesotho	Mutation induction and detection in potato, sweet potato and amaranth	Aug. 2013–Jan. 2014
*Mr Hery Lalao Lwysset Randrianarivony	Madagascar	Mutation induction and detection in rice	Oct. 2013–Jan. 2014
*Mr Abderrahmane Hannachi	Algeria	Mutation induction and detection in barley	Nov. 2013–Jan. 2014

Name	Country	Area of Training	Period
*Ms Kaoutar El Achouri	Morocco	Mutation induction and detection in potato	Nov. 2013–Feb. 2014
*Ms Munkhbat Tungalag	Mongolia	Mutation induction and genotypic detection methods in wheat	Nov. 2013– Dec. 2013
*Mr Sukhbaatar Purevjav	Mongolia	Mutation induction and detection in forage crops	Nov. 2013–Dec. 2014
*Ms Babita Dussoruth	Mauritius	Mutation induction and detection in banana	Nov. 2013–Feb. 2014

*Participated in the Inter-regional Training Course on Breeding Techniques for Mutant Traits with Special Reference to Ug99 (INT/5/150-004), 25–29 November 2013.

**Participated in the Regional Training Course on Plant Mutation Breeding: Mutation Induction, Mutation Detection and Pre-breeding, 3–14 June 2013; and Regional Training Course on Mutation Induction Techniques and Supportive Breeding and Biotechnologies for Wheat and Barley (RAS/5/058-003), 1–5 July 2013.

***Participated in the Regional Training Course on Plant Mutation Breeding: Mutation Induction, Mutation Detection and Pre-breeding, 3–14 June 2013; Regional Training Course on Mutation Induction Techniques and Supportive Breeding and Biotechnologies for Wheat and Barley (RAS/5/058-003), 1–5 July 2013; and Interregional Training Course on Breeding Techniques for Mutant Traits with Special Reference to Ug99 (INT/5/150-004), 25–29 November 2013.

Scientific visitors at the PBGL in 2013

Name	Country	Areas of Training	Period
Mr Mohammad Jouhar	Syria	Molecular screening of mutations (TILLING and Ecotilling)	May 2013
Ms Brunhilde Beltoisine Rabealaina	Madagascar	Training in plant mutation breeding	Oct. 2013
Mr Innocent Zinga	Central African Republic	Discussions and training in plant mutation breeding	Oct. 2013
*Mr Dheyaa Yousuf	Iraq	Training in plant mutation breeding	July 2013
*Mr Jalal Naji Mamoori	Iraq	Training in plant mutation breeding	July 2013
*Mr Wessam Akel	Syrian Arab Republic	Training in plant mutation breeding	July 2013
*Mr Lukanda Tshilenge	Democratic Republic of Congo	Training in plant mutation breeding	July 2013
Mr Mouad Chentouf	Morocco	Mutation induction and screening	Oct. 2013
Mr Shamduth Panday Beni Madhu	Mauritius	Mutation induction and screening for disease resistance	Nov. 2013
Ms Cecilia Brunner	Austria	Mutation induction and screening	Sept. 2013
**Mr Sibu Simon	Austria	Training in plant mutation breeding	Nov. 2013
Ms Likyelesh Gugsu	Ethiopia	Training in plant mutation breeding, genetic markers and DH screening in tef.	Dec. 2013
**Ms Anna Sochacka	Poland	Training in plant mutation breeding techniques	Nov.–Dec. 2013

*Participated in the Regional Training Course on Mutation Induction Techniques and Supportive Breeding and Biotechnologies for Wheat and Barley (RAS/5058/003), 1–5 July 2013. ,**Participated in the Inter-regional training course on Breeding Techniques for Mutant Traits with Special Reference to Ug99 (INT/5/150-004), 25–29 November 2013.

Consultants at the PBGL in 2013

Name	Country	Topic	Period
*Mr Tao Lan	China	Mutation induction and identification in rice	July 2013–June 2014

Conferences

Vienna International Plant Conferences

Various members of the PBGS and PBGL participated in the Vienna International Plant Conferences in February 2013, specifically the 'Plant Genetics & Breeding Technologies' and 'Plant Disease & Resistance Mechanism' conferences. Each of these conferences had over 250 participants and a significant number of our collaborators were in attendance. In addition to giving two oral presentations and five posters, many individual and small group meetings were possible and collaborations were initiated and reinforced, such as developing protocols for X ray mutagenesis, mutation detection and the deployment of techniques to increase the efficiency of plant mutation breeding.

Oral presentations

- Wheat Black Stem Rust (race Ug99): Mutation Assisted Breeding Meets the Challenge (Pierre J.L. Lagoda, et al.).
- Do-it-yourself Molecular Biology for Plant Breeding: Low-cost Tools for Developing Countries (Owen A. Huynh, Bernhard J. Hofinger, Mayada M. Beshir, Joanna Jankowicz-Cieslak, Huijun Guo, Brian P. Forster and Bradley J. Till).

Poster presentations

- Rapid introgression of mutant traits for fodder quality in barley (Farzaneh Taassob-Shirazi, Brian P. Forster, Biguang Huang, Heinrich Grausgruber and Jerome Franckowiak).
- Impact of induced mutations in plant breeding (Mirta Matijevec, Souleymane Bado, Pierre J.L. Lagoda, and Brian P. Forster).
- Doubled haploid production in spring wheats of hot irrigated environments (Abdelbagi M. Ali, H.M. Elamein, I.S.A. Tahir, M. Baum and Brian P. Forster).
- Resurgence of X rays in plant mutation breeding (S. Bado, K. Kozak-Stankiewicz, H. Sekander, N. Alhajaj, A.M.A. Ghanim, B.P. Forster and M. Laimer).
- Application of Soft X ray and Near-Infrared Reflectance Spectroscopy for rapid phenotyping of mutant seed. (J. Jankowicz-Cieslak, L. Razafinirina, J. Rabefiraisana, N. Rakotoarisoa, G. Seballos, B.P. Forster, B.J. Till and J. Vollmann).

Second International Conference of Cereal Biotechnology and Breeding, Budapest, Hungary, 5–7 November 2013

Oral presentation

- Induced mutations for increased disease resistance: an example of resistance to race Ug99 in spring wheat. (Ego K. Amos, Miriam G. Kinyua, Oliver K. Kiplagat, Abdelbagi M. Ali, Souleymane Bado, Joanna Jankowicz-Cieslak, Owen Huynh, Isabelle Henry, Luca Comai, Pierre J.L. Lagoda, Brian P. Forster, Bradley J. Till).

Professional Networking

In an effort to enhance scientific networking and increase the quality of services provided to Member States, the PBGL and Plant Breeding and Genetics Section developed a LinkedIn profile (<http://at.linkedin.com/pub/iaea-plant-breeding-and-genetics/31/4b6/aa3>). Our connections are growing to plant science professionals. We have also created a LinkedIn discussion group called IM PLANTS (Induced Mutations in Plants). We hope that this can serve as a real-time resource where experts can share views and advice on topics surrounding induced mutations and plant breeding. You are welcome to join our network.

Manuals, Guidelines and Protocols

In order to facilitate the generation, screening and up-take of mutants, the PBGL is involved in the development of simple and user-friendly protocols that can be used by breeders in developing countries. These include a recent update to the laboratory manual 'Molecular Characterization of Mutant Germplasm', where proper application of molecular markers for mutant populations has been added, a range of low cost methods for DNA extraction and mutation discovery, a protocol for salt tolerance screening, crossing barley plants, and X ray irradiation of crop seed. These are available on our website <http://www-naweb.iaea.org/nafa/Pbg/public/manuals-pbg.html>.

Positive Control Kits for Low Cost DNA Extraction

The PBGL has recently developed low-cost methods for plant tissue collection and storage that obviate the need for liquid nitrogen, -80C freezers. DNA extraction

protocols have also been developed that are non-toxic and avoid the use of organic phase separation. The cost of DNA extraction is greatly reduced compared to commercial kits. To assist Member States in using these methods, the PBGL has developed a positive control kit and detailed protocols that are distributed upon request. This is a new development and kits have already been distributed to counterparts in Mauritius and Botswana.

Positive Control Kits for Low Cost Mutation Discovery

The PBGL continues to provide assistance to Member States for low-cost mutation discovery through production and distribution of positive control kits that contain DNA with known mutations or polymorphisms, gene-specific primers, and other materials for mutation discovery by agarose gel or polyacrylamide gel electrophoresis. A suite of protocols have been developed in 2013 for a variety of different gel types to accompany the positive control kit. Recently, the positive control kit for mutation discovery has been distributed to Iran, Mauritius, India and China.

Publications

Staff Publications in the Field of Plant Breeding and Genetics

Journal Publications/Book Chapters and Published Abstracts

LAGODA, P.J.L. 2.1 Use of Tissue Culture and Mutation Induction to Improve Banana Production for Smallholders in Sri Lanka. In: Chapter 2: Case Studies in the Crop Sector of Biotechnologies at Work for Smallholders: Case Studies from Developing Countries in Crops, Livestock and Fish (Edited by J. Ruane, J.D. Dargie, C. Mba, P. Boettcher, H.P.S. Makkar, D.M. Bartley and A. Sonnino), Food and Agriculture Organization of the United Nations (FAO); ISBN 978-5-107877-8 (2013) pp. 8–17.

CZYCZYŁO-MYSZA, I., MARCIŃSKA, I., JANKOWICZ-CIEŚLAK, J., DUBERT, F. The effect of ionizing radiation on vernalization, growth and development of winter wheat. *Acta biologica Cracoviensia. Series botanica.* 01/2013; 55(1):1-6. DOI:DOI: 10.2478/abcsb-2013-0004 pp.1-6 (2013).

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HUYNH, O.A., HOFINGER, B., JANKOWICZ-CIESLAK, J., GUO, H., BESHIR, M., FORSTER, B.P., TILL, B.J. Do-it-yourself molecular biology for plant breeding: low-cost tools for developing countries. International Conference on Molecular Mapping and Marker Assisted Selection.

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