Opening Session

Expanding the Boundaries of Gene Variation for Crop Improvement

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Abstract

Directed and undirected mutagenesis continues to offer unique opportunities for crop improvement. Mutations also occur naturally and different forms are present in each strain of plants within and among species. Modifying genes affect the expression of all mutants and examples exist where the deleterious features of a mutant can be significantly changed by selection. New technologies, including those associated with genomics such as re-sequencing, TILLING, and RNA interference, allow the detection of gene variation at an unprecedented frequency. Knowledge of genes that affect recombination among homoeologous chromosomes may lead to inducible methods regulating the exchange among chromosomes in a polyploid species. Forward and reverse genetic methods are readily available in many species, including model plant species. There are an estimated one million sites in the japonica rice genome tagged via Tos17, Ac/Ds, T-DNA, and other insertion elements. Site-specific mutagenesis and gene replacement methods may replace the need for transgenic technology in some cases. Transcriptome modification occurs via mutagen treatment, aneuploidy, and uniparental chromosome loss, and sometimes results in a mutant phenotype. The boundaries of gene variation appear to be more expansive as plant genetics knowledge and technologies increase.

Introduction

Mutations reflect alternatives (exceptions) to the normal state of a gene or chromosome structure. William Bateson[1] said: "Treasure your exceptions". Indeed, these exceptions provide the variation for selection of new and useful types of plants as well as the basis for evolution. Mutations are as natural as nature itself and have led to many positive outcomes (*see* Plant Mutation Reports and Plant Breeding & Genetics Newsletters; www.-naweb.iaea.org/nafa/index.html, and www.fao.org/waicent/VAOINFO/Agricult?Default.htm).

Eighty years ago, L.J. Stadler [2, 3] demonstrated the induction of mutations in barley and maize by using x-rays and radium. In 1937, A.H. Sturtevant[4] said "Mutations are accidents, and accidents happen." These comments reflect the period of biology when we could only draw on naturally-occurring mutations or technology that led to "undirected mutagenesis." But now, "directed mutagenesis" methods are increasingly common where mutations can be planned. Genome sequence information is often required for the new directed mutagenesis applications. Fortunately, for today's plant scientists, at least 23 plant species' genomes either have been, or are currently being sequenced [5, 6]. The use of model species, such as *Arabidopsis* [7], is also leading to the more rapid development of new mutagenesis techniques.

In 1995, R. Phillips co-organized a meeting on non-Mendelian inheritance in Japan with K. Oono and, together with M. Matzke, wrote a report for The Plant Cell called "Treasure Your Exceptions" [8]. The report reviewed discussions on homology-dependent gene silencing, paramutation, epimutations, parental imprinting, somaclonal variation, uniparental genome loss, recombination systems, and other interesting findings that expanded the boundaries of our understanding of gene variation. These boundaries have been further expanded as the field of mutagenesis has transitioned from "Treasure your exceptions" to "Detect and create your exceptions".

Now it is clear that the mutagenesis research field includes many directed and undirected approaches. Several interesting aspects of ways that gene variation can be detected or modified are briefly highlighted in this report, including *de novo* variation, altering mutant phenotypes through selection, TILLING (Targeted Induced Local Lesions IN Genomes), resequencing, RNAi (RNA interference), mismatch site-specific mutagenesis, homoeologous recombination, forward and reverse genetics via transposable elements, gene replacement, gene addition, and transcriptome modification by mutagenic treatment, aneuploidy, and uniparental chromosome loss.

De novo variation

De novo variation occurs via many pathways. This is variation not present in the parents, but in the progenies, and can be due to naturally occurring point mutations, intragenic recombination, unequal crossing over, transposable elements including the Mutator system, DNA methylation, paramutation, gene amplification, and other means [9]. The variation that is still present in long-term selection experiments may not be due to the variation present in the starting materials but rather the result of *de novo* variation occurring in generations subsequent to the initial cross.

Altering mutant phenotype through selection

Expression of a gene can be modified through selection. R. Phillips' first exposure to this idea was from H.K. Hayes (personal communication) relative to a maize mutation that has defective tissue between the veins of older leaves resulting in holes and tears, called *ragged*. Hayes had crossed the dominant *Rg1* plants to normal and had continuously selected for modifier genes to the point that the plants were of normal phenotype.

An example important in human nutrition is the selection for hard endosperm in the *opaque2* genotype. This mutation causes an obvious phenotypic visual change in the appearance of the kernel. The endosperm has considerable soft starch making the kernel opaque to light transmitted through the kernel. The *opaque2* genotype was found to cause an increase in the content of lysine and tryptophan, two essential amino acids deficient in maize. Although the nutritional value was obvious, the soft endosperm caused the kernels to crack leading to insect and fungal infections. The mutation also resulted in reduced yield. Researchers recognized that the kernel phenotype could be altered via selection for *o2* modifiers while constantly selecting for the high lysine and tryptophan phenotype. Several generations of selection for hard endosperm in the *opaque2* genotype led to maize lines with good yield and high nutrition [10]. This "Quality Protein Maize" is being grown on nine million acres worldwide.

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Recognizing gene variation via new technologies

Plant species, especially polyploids, possess in their genomes considerable variation for specific genes. These often are not recognized because recessive alleles may need to be present in each chromosome (homologous and homoeologous) in order to detect the phenotype. TILLING (Targeted Induced Local Lesions IN Genomes) is a reverse genetic, non-transgenic approach to finding new alleles by DNA assay techniques recognizing changes in the DNA sequence of a targeted gene. In wheat, 246 alleles of the waxy genes were identified by TILLING each homoeologue in 1,920 allohexaploid and allotetraploid individuals [11]. These alleles encode waxy enzymes ranging in activity from near wild type to null. They represent more genetic diversity for a trait in wheat than had been described in the previous 25 years.

Resequencing

DNA resequencing involves sequencing an individual's DNA for a specific region and comparing it to a reference sequence that is already available in order to detect mutations. Resequencing the genome of many individuals allows determination of the relationship between sequence variation and specific phenotypes. This substantially increases the ability to detect gene variation.

In rice, genome-wide SNP (Single Nucleotide Polymorphism) discovery was attempted across the unique sequence fraction of the Nipponbare rice genome. Twenty diverse varieties were selected for resequencing based on geographic representation, diversity, usage, and traits by a group of rice researchers in the OryzaSNP Consortium [12] (www.oryzasnp.org). Overall, the consortium (McNally and Leung, personal communication) found 2.6 SNPs per kb (146,576 genic variants and 112,623 intergenic variants). QTL mapping studies are often restricted due to the absence of known polymorphic sites between parental lines. Having a large number of SNPs reduces this problem by providing information on nucleotide variation between cultivars. Each SNP reflects gene variation.

Directed mutagenesis through regulation of gene expression: RNA interference is a RNA-guided regulation of gene expression utilizing double-stranded ribonucleic acid complementary to the genes for which expression is to be inhibited (Wikipedia). Although a relatively new discovery, RNAi has already been demonstrated to be useful in generating variation for important traits. Root-knot nematode resistance has been produced in *Arabidopsis* [13], delayed senescence in wheat [14], gossypol reduction in cotton seed [15], and cytoplasmic male sterility in tobacco and tomato [16].

Site-specific mutagenesis

An interesting example of site-specific mutagenesis is the use of oligonucleotides with mismatches to the specific gene to be mutated. The company, Cibus LLC, is expected to soon release herbicide resistant sorghum [17]. The company estimates a development cost of about 3-5 million US dollars compared to 30-40 million US dollars for transgenics due to avoiding regulatory hurdles. Such a method of directed mutagenesis is considered to be a non-transgenic approach. This more inexpensive approach may facilitate the generation of new traits in minor crops.

Homoeologous recombination

The *Ph1* locus controls the pairing of the sets of chromosomes in wheat. In crosses with wild relatives, this locus unfortunately prevents the pairing of wheat and the chromosomes of wild relatives making it difficult to introgress new genes into wheat. The ability to alter the control exerted by *Ph1* would enable wheat breeders to access a much greater range of genetic diversity. A *cdc2* gene complex is thought to be the *Ph1* locus responsible for the pairing of homoeologous chromosomes in wheat [18]. Wheat has three genomes that are similar but vary in genetic constitution. Recombination between these similar chromosomes can lead to new variation. Control of recombination in crosses of wheat with wild relatives may be possible through the use of okadaic acid, a phophatase inhibitor, and lead to more variation.

Forward and reverse genetics via transposable element insertions

The introduction or activation of transposable elements of various sorts provides the possibility of altering genes to generate phenotypically detectable mutants (forward genetics). The presence of such elements in a gene allows one to correlate these changes in a given genetic sequence with a specific phenotype (reverse genetics). Fortunately, many genetic stocks have been produced in a variety of organisms to make this a robust technology. For example, in rice there are about 50,000 lines with Tos17 insertions produced at the National Institute of Agrobiological resources (http://tos.nias.affrc.go.jp). In addition, about 150,000 lines of rice possess Ac/Ds, enhancer traps, T-DNA, and activation tags which have been produced by researchers in Korea, Australia, China, Taiwan, France, Singapore, Netherlands, and the U.S. Perhaps a total of a million tagged sites are available in japonica rice [19].

Gene targeting

Targeting specific genes for modification is becoming more and more common. Zinc-finger nucleases can be targeted to specific genes causing a double-stranded break which disables the gene [20, 21]. Zinc finger nucleases are comprised of a DNA-recognition domain and a cleavage domain. The double-strand breaks at specific locations may disable the targeted allele or even lead to a modified sequence upon repair. The potential exists to insert a gene at the double-strand break.

Gene additions

Advances in biotechnology have allowed the addition of genes to plants from almost any source. The generation of transgenic plants has led to 12 years of commercialization of new biotech crops that provide insect resistance, herbicide tolerance, and many other traits and have been grown on over 1.7 billion acres [22]. In India, the eggplant crop is sprayed 80 times a season (nearly every day) in some regions, farmers in other regions spray an average of 40 times, and the most common frequency is more than 20 sprayings (U. Barwale, personal communication). Not only is the consumer exposed to pesticides by such extensive spraying, but also the farmer is more subject to pesticide poisoning. Most of the unintentional chemical poisonings in the developing world are due to pesticides [23]. India has been able to cut pesticide treatment of cotton in half by growing varieties containing introduced Bt (*Bacillus thuringensis*) insecticidal protein, thereby leading to a safer environment for both producers and consumers [22].

Transcriptome modification

By mutagenesis: A recent paper by Batista, *et al.* [24] reports that Gamma-ray mutagenesis in rice induces extensive transcriptome changes. By microarray analysis, over 11,000 genes showed changes in gene expression in the M_1 generation following gamma irradiation compared to the control. A Gamma-ray-induced semi-dwarf mutant (*Estrella A*), produced in 1988 and subsequently selfed for more than 10 generations, had 51 genes still showing differential expression. Thus, mutants derived from mutagenesis may result in broader boundaries of gene variation (expression) than anticipated.

By aneuploidy: Individuals with other than an exact multiple of the basic chromosome number are called aneuploids and those with only a portion of the chromosome altered in dosage are termed segmental aneuploids. The expectation would be that a gene altered in dosage via aneuploidy would have a corresponding change in gene expression, and that only genes in the aneuploid regions would show changes in expression. Neither of these conclusions is always true. Studies on the human Down's syndrome indicate that many of the genes are on chromosome

21 but several genes with altered gene expression are not, and these may contribute to the syndrome of phenotypic effects [25]. Birchler and Veitia [26] have reviewed many aspects of dosage effects, or lack thereof.

A segmental aneuploid of maize (trisomic for 90% of the short arm of chromosome 5 and monosomic for a small region of the short arm of chromosome 6) deriving from an interchange heterozygote had been utilized in a male-sterility system [27]. Makarevitch, *et al.* [28] determined that only about 40% of the expressed genes in the trisomic region showed the expected 1.5 fold change in gene expression while 60% were not altered in gene expression. Eighty-six genes not in the aneuploid region were found to be altered in expression. The aneuploid condition in the B73 background was found to have leaf outgrowths called knots in later generations (Phillips, personal communication). There are several knotted-like homeobox genes in maize. Out of the nine knotted-like genes tested, only *knox10* located in the chromosome 5 trisomic region was ectopically expressed [28]. Thus, in some cases, the occurrence of developmental phenotypes may be related to unusual expression patterns induced by changes in chromosome constitution.

By uniparental chromosome loss: Crossing wheat with maize followed by embryo rescue [29] led to an efficient means of producing haploid wheat plants [30]. In contrast, crossing oat with maize yields haploids of oat only about two-thirds of the time, and yields plants with the haploid oat chromosome constitution plus one or more maize chromosomes about one-third of the time [31]. The latter plants are termed oat-maize additions (OMAs). Because maize has 10 pairs of chromosomes, there are 10 possible oat-maize addition lines (**Table 1**). We have recovered all 10 OMAs and have several of them in various oat and maize genetic backgrounds (**Table 2**).

The OMA materials have many uses [32]. The principal use is for mapping maize DNA sequences. One of the powerful aspects for mapping is that no polymorphisms are required; the PCR test is plus/minus depending on whether the sequence is represented on the particular chromosome in an OMA. If there is a related sequence in the oat genome that also is amplified by the PCR, then the maize sequence can be mapped to chromosome if the oat and maize bands are distinguishable. If the maize sequence is part of a gene family, then a PCR band will appear with more than one OMA reflecting the location of gene family members on different chromosomes. Other uses of the OMAs include: chromosome sorting, chromosome pairing studies, comparing repeated sequences on non-homologous chromosomes, checking for chimeric BACs, centromere isolation, and searching for corn traits (such as C4 photosynthesis) in oat.

The phenotypes of the OMAs are generally not dramatically different from the oat parent, although the genotype of the parents often makes a difference. However, OMA chromosome 3 from maize Seneca 60 has a crooked panicle and a *liguleless 3* phenotype where the upper ligules are abnormal. Muehlbauer, *et al.* [33] showed that the phenotype was due to the ectopic expression of *Lg3*. We hypothesize that genes such as *lg3* may be expressed when interacting genes on other chromosomes are not present. In this case, *rs2 (rough sheath 2)* on chromosome 1 would be absent in OMA 3 and therefore cannot regulate the expression of *lg3* in the addition line. Another interesting case of a mutant appearing in an addition line is the disease lesion mimic phenotype associated with OMA chromosome 6 with the Seneca 60 or B73 chromosome.

The OMAs have been irradiated with Gamma-rays to break the maize chromosome and derive "Radiation Hybrids (RH)" that possess only a part of the maize chromosome, either due to creating a deficient maize chromosome or via a translocation between the maize chromosome and one of the oat chromosomes. In most cases, the translocation event is the more desirable due to higher transmission frequencies [34]. Interestingly, several RH lines were derived from OMA2 and OMA9 that had little more than the maize centromere present and may be useful for maize centromere isolation. RHs with all tested chromosome markers present except for those in deleted terminal segments (either by terminal deletions of the maize chromosome or the presence of only one maize segment translocated to an oat chromosome) are shown in **Table 3**. These stocks should be useful for the deletion mapping of mutations. A complete listing of over 600 RHs can be found at http://agronomy.cfans.umn.edu/Maize_Genomics.html.

Gene-expression microarray analyses of three independent OMA 5 events in the B73 background indicated that at least 17% of the maize chromosome 5 genes expressed in maize B73 seedlings also are expressed in OMA 5 seedlings [35]. All three independent OMA5s expressed the same set of genes. Those expressed were not associated with a specific genome location, predicted function, or methylation state; expression levels in the OMA seedlings were intermediate to that found in B73 maize seedlings (Cabral, personal communication).

Table 1. Available oat-maize addition lines in various maize genetic backgrounds

	Oat-Maize Addition Line										
Maize Chromosome Donor	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**B**
Seneca 60	1	11	2	6	3	3	3	2	9	1*	
B73	1	1		3	11	3		1	1	1	
Mo17		8	1*	1	8	3	1*			2	
A188				1			1				
bz1-mum9		1						1			
B73 w/Black Mexican Sweet B Chrom											2

*OMAs for which no seed was produced, but limited DNA of the original plant is available.

Table 2. Parental backgrounds of oat-maize addition lines

Oat Background	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park
Chromosome	**1**					**2**					**3**					**4**				
Maize Donor																				
Seneca 60	x					x		x					x	x		x				
B73			x			x										x		x		
Mo17						x							x			x				
A188																		x		
bz1-mum9						x														
B73 w/Bck Mex SweetBs																				

Oat Background	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park
Chromosome	**5**					**6**					**7**					**8**				
Maize Donor																				
Seneca 60	x	x				x	x				x				x					x
B73			x					x										x		
Mo17	x		x			x		x												
A188											x									
bz1-mum9																x				
B73 w/Bck Mex SweetBs																				

Oat Background	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park	Starter	F_1 Hybrid	Sun II	Preakness	GAF Park
Chromosome	**9**					**10**					**B**				
Maize Donor															
Seneca 60	x				x										
B73	x					x									
Mo17						x									
A188															
bz1-mum9															
B73 w/Bck Mex SweetBs											x				

Table 3. Radiation hybrids with terminal deletions or translocations per chromosome

Chromosome 1

IBM2 Map Site	0.00	10.50	32.08	68.71	85.20	97.97	114.40	124.70	143.50	160.60	170.00	198.32	226.40	257.40	279.13	301.37	326.70	358.40	386.40	405.00	417.00	445.10	457.00	464.08	483.83	503.30	521.41	548.30	587.00	598.60	636.08	653.40	662.18	697.22	718.50	747.90	769.40	787.49	800.70	836.70	858.39	874.30	886.10	923.01	933.09	985.11	1007.60	1023.30	1051.10	1073.46	1103.00	1120.30	1128.00	*
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																																(1)

Chromosome 2

IBM2 Map Site	0.93	28.10	47.40	50.90	59.90	92.80	114.89	122.40	143.10	159.86	164.80	179.40	194.45	197.20	227.10	243.30	244.70	269.52	273.70	295.10	310.20	316.70	322.19	339.30	342.40	347.00	357.50	364.50	368.80	369.18	379.20	394.52	398.54	401.50	453.80	453.80	475.10	478.70	480.70	498.30	523.50	525.01	565.90	591.50	600.70	625.31	654.80	674.67	632.40	712.10	*
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Chromosome 3

IBM2 Map Site	5.60	7.10	11.20	28.20	38.00	67.20	77.00	103.30	129.40	165.00	177.40	181.70	189.00	193.10	208.60	259.40	192.54	210.40	276.61	227.80	228.50	213.60	234.40	244.70	266.00	195.13	254.60	262.90	269.10	270.88	279.30	280.40	290.60	306.10	313.40	346.80	398.40	401.20	445.00	473.10	491.40	511.50	538.20	544.40	568.30	579.50	617.50	633.80	702.20	738.70	752.10	757.00	806.90	828.90	*
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Chromosome 4

IBM2 Map Site	9.00	29.30	52.30	62.20	85.70	105.80	138.80	148.20	168.10	203.70	211.90	231.30	249.10	263.30	276.30	276.30	292.90	310.50	328.90	352.60	365.60	384.50	393.80	416.40	435.70	447.70	488.30	497.90	508.30	518.30	539.00	546.00	565.90	574.30	604.40	619.60	621.30	637.20	648.30	663.10	679.70	*
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Chromosome 5

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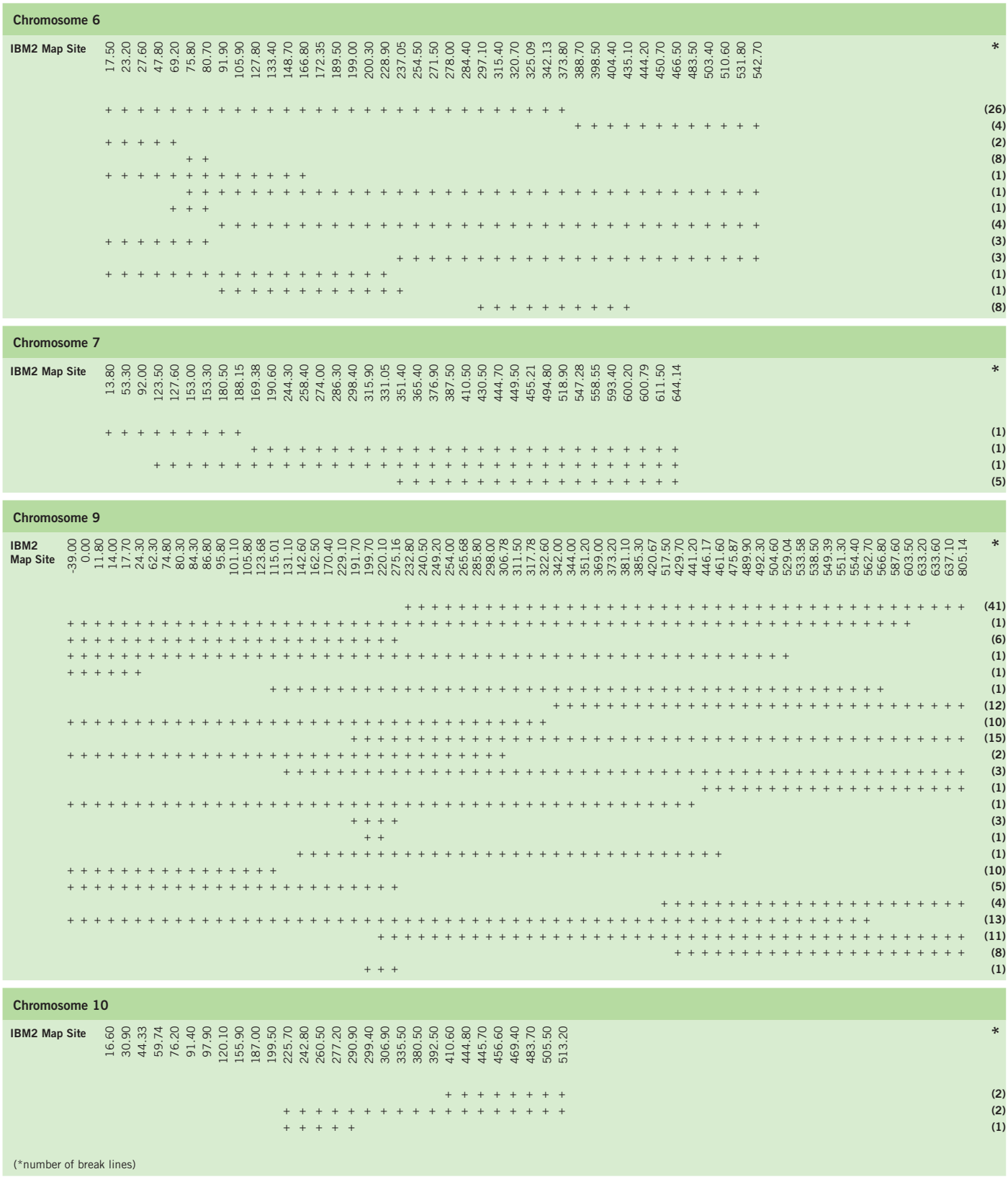

Conclusions

As with most fields of study, new information and new technologies allow more opportunities for the creation of novel products with various uses. The ability to detect inherent variation has expanded greatly, allowing the detection of more variants within the genome than previously expected. With genome sequence information and techniques for modifying specific genes, the field of mutagenesis is having a renaissance. Crop improvement will benefit since it depends on gene variation, both natural and induced. Increased food production via the Green Revolution in wheat and rice depended in large part on semi-dwarf mutations. A

current example of gene variation benefiting crop improvement is the naturally occurring *submergence 1* mutation that [36] allows rice to be flooded for up to two weeks with little effect on yield. Understanding the enormous variety of gene interactions in plant species will promote genomic manipulations resulting in interesting variation. Continued research and education on mutagenesis will allow us to realize the ever-increasing potential of gene variation for crop improvement.

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Networking and Fostering of Cooperation in Plant Mutation Genetics and Breeding: Role of the Joint FAO/IAEA Division

P J L Lagoda

Abstract

Over the past 50 years, the use of induced mutations (through irradiation and chemical agents) has played a major role in the development of superior crop varieties translating into a tremendous economic impact on agriculture and food production that is currently valued in billions of US dollars and millions of cultivated hectares. For the past 40 years, the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations have through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, sponsored extensive research and development activities in their Member States on mutation induction to enhance the genetic diversity in the germplasm of food and industrial crops and these efforts have resulted in the official release of over 2,700 new crop varieties in some 170 species to farmers, including rice, wheat, barley, apples, citrus, sugar cane, banana, and others (FAO/IAEA Mutant Variety Database, MVD, http://www-mvd.iaea.org/MVD/default.htm). These represent the information submitted voluntarily by FAO and IAEA Member States but one must be aware that thus many more mutants are not registered.

There is no difference between artificially produced induced mutants and spontaneous mutants found in nature. As in traditional cross-breeding, induced mutants are passed through several generations of selfing or clonal propagation, usually through *in vitro* techniques. This is exactly what happens in nature (through evolution) and leads to the fixation of the mutation events. All plant breeders do is mimic nature in this regard. It should also be noted that in most cases, the induced mutants are merely "raw materials," that in order for their potential to be realized, they must be integrated into established breeding schemes. Thus, mutation induction has proven to be a workable, sustainable, highly-efficient, environmentally acceptable, flexible, unregulated, non-hazardous and a low-cost technology in the breeder's toolbox to enhance crop improvement.

With increasing recognition of the roles of radiation in altering genomes and phenotypes and of isotopes as detection systems in molecular biology, demands from countries and their institutions for support in various applications of "modern biotechnology" increased dramatically over the last 20 years. Hence support for both R&D (through IAEA Research Contract activities) and for training and capacity building through fellowships, expert services and provision of equipment (through the IAEA Technical Cooperation Programme) in molecular and genomic approaches to solving agricultural constraints have increasingly become part of the technological packages - combining mutation induction and efficiency enhancing bio-molecular technologies - fostered by the Agency in recent years.

The IAEA Programme in Food and Agriculture is planned, implemented and co-financed with FAO and is known as the Joint FAO/IAEA Programme. As such it contributes to "Biotechnology in Food and Agriculture" which is an FAO corporate Priority Activity for Interdisciplinary Action (PAIA). Moreover, its activities – particularly in crop improvement are conducted in close collaboration with the relevant International Agricultural Research Centres of the CGIAR with which it has a number of Memoranda of Understanding on biotechnology and other applications.

Biotechnology, defined as any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use (CBD, 1992), has been at the heart of the IAEA Programme in Food Agriculture since it was established as a joint programme with FAO more than 40 years ago.

The IAEA serves as the global focal point for nuclear cooperation, mobilizing peaceful applications of nuclear science and technology for critical needs in developing countries, including fighting hunger, disease, poverty and pollution of the environment, and thereby contributing to the sustainable development of goals of its Member States.

It should be emphasized that the IAEA does not operate a separate Biotechnology Programme, but rather fosters the integration of modern and conventional bio-molecular technology approaches only where these are considered appropriate for the furthering of nuclear applications (e.g. TILLING).

The IAEA currently coordinates research networks through Coordinated Research Projects (CRPs) and supports human and institutional capacity building Technical Cooperation Projects (TCPs) for integrating plant tissue culture techniques, advanced molecular methods and induced mutations within the framework of national plant breeding and conservation programmes to characterize plant genetic resources, widen plant genetic diversity, and identifies and introduces agronomically and commercially useful traits.

What are nuclear techniques?

Everything in the universe including soil, plants and animals that we use for agriculture and carbohydrates, proteins and fats in the food we eat is made up of around 100 elements. These elements consist of atoms with a nucleus composed of neutrons and protons surrounded by electrons. However, not all atoms of an element have the same number of neutrons in their nucleus i.e. they exist in different isotopic forms- some are heavier than others, some are stable while yet others undergo decay and emit energy as radiation.

Applications of nuclear techniques in food and agriculture make use of isotopes to measure and track with great accuracy and precision what is happening to agriculturally important processes and compounds, and to manipulate these for greater productivity. They also make use of sealed sources containing radiation-emitting isotopes to mimic nature in changing the genetic make-up of plants, insects and micro-organisms and produce better crops, sterile insects for controlling pests and increasing the shelf-life and safety of certain foods.

Nuclear techniques, combined with the application of modern biotechnology, are essential for providing a more efficient means, both for understanding the processes that underpin the production and transformation of biophysical resources into food and agricultural products,

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and directly or indirectly, for manipulating these processes to increase crop and livestock productivity while conserving and sustainably using natural resources and improving food quality and safety. The effective transfer of existing nuclear techniques to developing countries and the development of new and safe biotechnologies combined with nuclear techniques can greatly enhance the prospects for sustainably improving agricultural productivity today and in the future.

Mutation induction and breeding

The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits, which limit their productivity or enhance their quality value. The global impact of mutation-derived varieties on food production and quality enhancement is difficult to monitor, even in a five-year window, given that normally the release of a new variety takes 10 to 15 years. Looking back on the past 70 years, close to 3,000 varieties have been released worldwide that have been derived either as direct mutants or from their progenies. Induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties. Part of this success might be rightfully claimed by the Agency, either directly or indirectly through Technical Cooperation Projects (TCPs) and Coordinated Research Projects (CRPs), fellowship training, organized scientific visits and expert missions.

Officially released mutation-derived varieties include many important crops such as rice, wheat, cotton, rapeseed, sunflower, sesame, grapefruit and banana. Among these, some have made a major economic impact and include rice varieties in Australia, China, India, Pakistan, Thailand and Vietnam, cotton in Pakistan, Japanese pear in Japan, grapefruit in the USA, barley varieties in Europe, durum wheat in Italy, sunflower in the USA, sorghum in Mali and wheat varieties in the North-Western Frontier Province in Pakistan; groundnut and pulse crops in India, peppermint in the USA, and ornamentals in India, the Netherlands and Germany.

In several mutation-derived varieties, the changed traits have resulted in a synergistic effect on increasing the yield and quality of the crop, improving agronomic inputs, crop rotation, and consumer acceptance.

The economic value of a new variety can be assessed through several parameters. These include area planted to the variety and percentage of the area under the crop in the region, increased yield, enhanced quality, reduced use of pesticides and fungicides (e.g. in varieties resistant to diseases and insect pests). But to make a long and complicated story short, a review of the socio-economic impact of mutant varieties has been recently published by the PBG section, reporting on millions of hectares cultivated and an additional value of billions of dollars created.

Many mutants have made a transnational impact on increasing yield and quality of several seed propagated crops. Induced mutations will continue to play an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of environmentally sustainable agriculture. Future research on induced mutations will also be important in the functional genomics of many food crops.

The Agency has addressed the problems of drought, salinity and stress tolerance to improve nutrition provided by the plants and to their resistance to specific environmental and geographical problems. Up to 80% of plant yield can be lost because of drought and salinity. Problems are particularly severe in developing countries in arid and semi-arid regions, with both devastating short-term effects on the livelihoods of poor people and long-term effects on food security, and are likely to increase in the future as competition for water increases. The integration of mutation induction and efficiency enhancing bio-molecular technologies into plant breeding and adoption of advanced selection methods can lead to the official release and wide uptake by farming communities of new varieties of basic food and industrial crops that are higher yielding, have better quality, are more nutritious, which are better adapted to climate change and variability.

With the integration of molecular genetic information and techniques, mutation breeding is in the mainstream of progress to develop novel varieties. Mutation induction combined with bio-molecular technologies such as plant tissue culture and molecular markers plays a very important role in crop improvement. Mutation induction is an integral part of the newest technology package in the forefront of modern and efficient methods in reverse genetics and breeding: TILLING (targeting induced local lesions in genomes), e.g. breeding hexaploid wheat for quality traits (starch). Mutation induction is producing mutation grids for gene discovery and gene function analyses (e.g. *Arabidopsis*, rice and barley), an invaluable resource for genomics, reverse and forward genetics.

- There have been more than 2,700 officially released mutant varieties from 170 different plant species in more than 60 countries throughout the world.
- Over 1,000 mutant varieties of major staple crops enhance rural income, improve human nutrition and contribute to environmentally sustainable food security in Asia. Vast numbers of induced mutant varieties are developed with the Agency's assistance, including support on mutant germplasm exchange and dissemination in Asia and around the world.
- Worldwide, more than 60% of all mutant varieties were officially released after the year 1985, in the era of biotechnology in plant breeding. The integration of mutation techniques and efficiency-enhancing bio-molecular techniques that permit rapid selection of the most beneficial mutants has pushed the use of mutation induction to new and higher levels of applicability.
- In vegetatively propagated crops, where genetic variation is difficult to obtain due to limited sexual reproduction due to sterility and polyploidy, mutation induction is a tool of choice to be promoted. Mutation induction allows for escaping the deadlock of sterility and parthenocarpy by creating useful variants.

In recent years there has been increased interest in understanding the genome. This goes in parallel with the explosion of fundamental and strategic research to understand gene structure and function, especially in crop and model plants. The IAEA Plant Breeding and Genetics section and laboratory unit are adapting the TILLING strategy to the peculiarities of tropical orphan crops. In addition to the work on the relatively more studied crop, rice, the Joint Programme has made significant progress in the development of protocols, i.e. simplifying procedures and exploring low cost options, facilitating the use of TILLING to routinely query the genomes of the scantily studied polyploid and vegetatively propagated crops that are important to the food security and livelihoods of Member States such as cassava and bananas, thus creating an invaluable resource for reverse genetics and breeding for the global community. The widespread routine adoption of TILLING, for instance, will significantly reduce the costs and time invested in the development of superior crop varieties.

Nuclear Applications in Food and Agriculture as exemplified by the activities of the Joint FAO/IAEA Programme

On 1 October, 1964, the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) created the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture with the first arrangements concluded by directors general of both Organizations. The goal was to bring the talents and resources of both organizations into assisting their Member States in applying nuclear techniques for providing people with more, better and safer food and other agricultural products, while sustaining the natural resources base.

Over four decades, Joint Division activities have evolved to respond to the ever-changing landscape of agriculture and nuclear technology and the expectations of national and international organizations for cooperation in nuclear research and technology transfer. Throughout this process, the Division has successfully remained at the forefront of assisting countries in fostering the uses of nuclear science and technology where these really add value. Today, the Joint Division strives to mobilize commitment and action to meeting the World Food Summit and Millennium Development Goals of reducing hunger, poverty and environmental degradation through sustainable agriculture and rural development.

An important part of this Programme is the FAO/IAEA Agriculture & Biotechnology Laboratory, set up to provide applied research, services and training to member countries. The arrangements on the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture were revised in 1997 and signed by the Directors General of FAO and IAEA in December 2001.

The Joint FAO/IAEA Programme includes three interdependent components:

- The Joint FAO/IAEA Division in Vienna, which provides normative and technology transfer support, coordinates research networks, policy advice and public information activities to Member States.
- The FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, which provides applied research, services and training to member countries, and serves as reference centre.
- Food and agriculture projects under the IAEA Technical Cooperation Programme, which manages the implementation of operational activities in member countries.

The Joint Programme continues to be a successful model of cooperation within the UN System, providing necessary assistance to the needs of Member States in the peaceful application of nuclear techniques in food and agriculture.

High priority activities of the Joint FAO/IAEA Programme focus on three thematic areas, i.e. (i) productivity enhancement; (ii) plant, animal and consumer protection; and (iii) the conservation and sustainable use of natural resources. The Joint FAO/IAEA Programme, which includes the FAO/IAEA Agriculture and Biotechnology Laboratory, continues to contribute to different programme chapters (Crop Production Systems Management, Pest of Animals and Plants, as well as Nutrition and Consumer Protection) by integrating policy advice, capacity building, R&D, as well as normative and operational technical support to the application in Member States of nuclear techniques.

In general, nuclear techniques are essential to providing unique support for these programme chapters, and are the only solution in certain areas. The necessity for nuclear applications lies first in their capacity to bring about changes into the genetic make-up of plants, and to offer great potential to increase the biodiversity of crop plants. Furthermore, the need for nuclear applications also lies in their unique sensitivity and specificity as markers. They can be used to measure – with a greater accuracy than is possible by any other conventional method – basic, and yet strategically essential processes which take place within and between soils, plants, and animals. Finally, radiation can be effectively applied for sanitary and phytosanitary purposes in support of food safety and can facilitate international agricultural trade, as well as specialized applications such as the sterile insect technique, a process whose leadership lies with the Joint FAO/IAEA Programme.

Crop Production Systems Management focuses on the enhancement and sustainability of crop production systems and the conservation and use of plant genetic resources together with sustainable seed production.

By using mutation induction, isotope tracer and radio-nuclide fallout techniques, the Joint FAO/IAEA Programme provides unique support to this programme chapter:

- Using the mutation induction techniques, an abundance of plant mutants have been created, which not only increase biodiversity, but are also used by modern biotechnology, and provide breeding material for conventional plant breeding, thus directly contributing to the conservation and use of plant genetic resources.
- Through mutation breeding, member countries may directly develop new high-yielding cultivars with good agronomic characteristics such as disease resistant, well-adapted and high value-added traits, which is difficult or impossible to attain through conventional plant breeding from any germplasm source including local landraces. This helps to enhance crop production for food security, increase farmer income and conserve biodiversity.
- Isotope tracer techniques characterize agriculturally important processes between water, soils and plants. The use of isotope techniques contributes to the improvement of crop water productivity, enhancement of soil fertility and minimization of land and water degradation, thus making cropping systems more productive and sustainable.
- Spatial and temporal distribution of fallout and naturally occurring radio-nuclides provide a reliable means of measuring soil erosion and sedimentation on a landscape scale and contribute to better soil and water conservation.

Diseases and Pests of Animals and Plants highlights the control of transboundary pest and disease threats to crop and livestock with focus on off-farm technical interventions for pest and disease control at global, regional and national levels.

The sterile insect technique (SIT) and isotope and related biotechnological methods (RIA, ELISA, PCR and molecular markers), when appropriately integrated with other methodologies, provide substantial added value to national and international efforts to enhance livestock productivity and protect human health and the environment through more effective feed and genetic resource utilization, breeding management and suppression or eradication of both trade and poverty related transboundary animal diseases (TADs) and plant pests. This also includes the production of guidelines and manuals, databases, policy advice and standard-setting, training materials and e-learning modules, early detection methods and quality-assured data from national serological and molecular surveys in support of the diagnostic, surveillance and analytical aspects of the programme. The Joint FAO/IAEA Programme contributes specifically in the following ways:

- Improves livestock productivity using gene-based technologies to optimise reproduction and breeding and nutrition strategies; e.g. isotopic tracing to optimise nutrition elements, radio immuno tracers and markers to optimise artificial insemination/birth frequency.
- Develops and applies nuclear and nuclear-related molecular technologies for early, rapid and sensitive diagnosis and cost-effective characterization of animal and human pathogens (such as HPAI), and the development of stable isotopic applications for the movement/origin tracing of animals and animal products.
- Develops and integrates the application of the sterile insect technique against key insect pests of agricultural and environmental importance.
- Facilitates country access to molecular tools and insect pest population genetics to develop improved insect pest control methods and to determine the origin of pest outbreaks.
- Provides support to national plant health services within biosecurity approaches for pests of national and regional quarantine importance, as well as off-farm technical interventions to prevent, eradicate, contain or suppress invasive, alien and emerging major insect pests.
- Collaborates with the International Plant Protection Convention Secretariat on the development and revision of standards on beneficial insects, fruit fly free and low prevalence areas and systems approaches to facilitate international trade of horticultural products.

Nutrition and Consumer Protection focuses on promoting and monitoring the production, processing, distribution and consumption of nutritionally adequate and safe food for all. The programme promotes the establishment of food control and quality assurance systems, compatible with international standards, in particular those of the Codex Alimentarius Commission, and contributes to building national capacities in food quality and safety.

Food irradiation is one of the few technologies which address food quality and safety by virtue of its ability to control spoilage and foodborne pathogenic micro-organisms and insect pests without significantly affecting sensory or other attributes. In addition, nuclear analytical methods such as electron capture gas chromatography, X-ray fluorescence and RIA coupled with the use of isotopically-labelled compounds are essential components of the armoury used by food control organizations for analysing food samples (e.g. for pesticides and veterinary drug residues), for compliance with Codex standards, as well as for improving sampling and analytical methods. Through the use of these nuclear techniques, the Joint FAO/IAEA Programme provides unique support to FAO and other international bodies in their efforts to enhance food quality and safety, protect consumer health and facilitate international trade in foodstuffs. The following are examples of the Joint FAO/IAEA Programme's work:

The development and use of nuclear-related methods of analysis for the determination, monitoring and control of pesticide and veterinary drug residues in foods, as well as in the finalization of Codex Guidelines for the Use of Mass Spectrometry and for the Estimation of Uncertainty of Results.

- Joint FAO/IAEA Programme assistance in the development and application of Codex standards to ensure food safety, which has also led to an increased use of food irradiation for over 60 different types of foodstuffs (spices, grains, chicken, beef, seafood, fruits and vegetables) in over 60 countries, resulting in the annual treatment of 500,000 metric tons of foods in over 180 gamma radiation facilities.

Collaboration with the International Plant Protection Convention Secretariat, expanding the use of irradiation for quarantine purposes, including in the identification of 12 specific phytosanitary treatments and one generic dose (fruit flies) recommended for adoption and subsequent inclusion into the IPPC Guidelines for the Use of Irradiation for Phytosanitary Purposes.

- The Joint FAO/IAEA Programme also looks forward to its continued collaboration with FAO in assisting its Member States to effectively prepare and respond to nuclear emergencies affecting food and agriculture, especially through the application of the jointly developed Codex Guideline Levels for Radio-nuclides in Foods.

All major activities of the Joint Programme are within the 'public goods' area, both in developing and developed countries and address urgent needs and requirements from FAO and IAEA Member States. In addition, many constraints to agricultural development related to the above thematic areas, especially animal and crop pests and diseases, are transboundary in nature and require an area-wide approach to be managed successfully. Regional collaboration is therefore necessary and collaboration between international organizations is best positioned to coordinate these activities. In that respect, the Joint FAO/IAEA Programme has also made significant contributions that need to be highlighted:

- Tens of millions of hectares of higher-yielding or more disease-resistant crops developed through induced mutations and released to poor farmers.
- Millions of tons of valuable topsoil and thousands of tons of plant nutrients, as well as water for crop and livestock production are saved from land degradation, soil erosion and water wastage through soil conservation measures and efficient land and water management.
- Thousands of plant mutants produced by Joint Programme not only increased biodiversity, but also provided breeding material for conventional plant breeding, thus directly contributing to the conservation and use of plant genetic resources.
- Control of major livestock disease vector and plant pest populations through the integrated application of the sterile insect technique and biological control agents.
- Near eradication of the fatal cattle disease rinderpest, aided by the widespread use of immunoassay technology developed and transferred to diagnose and monitor vaccination against the disease, has helped millions of poor livestock producers worldwide. In Africa alone, this brings benefits of 1 billion US dollars annually.
- The development of animal disease diagnostic tools (and those of zoonotic nature) to ensure the sensitive, rapid and quality assured detection of harmful pathogens.
- Elaboration of international standards on pre-harvest and harvest pest control, including the irradiation of foods and agricultural products to kill pathogens and insect pests. More than 50 countries are using food irradiation to ensure the safety and quality of food, for reducing post-harvest food losses and to satisfy international plant quarantine regulations.
- Eradication of tsetse fly in Zanzibar, screwworm in Libya, Mediterranean fruit fly in Chile, California, Mexico, and parts of Argentina and Peru, representing hundreds of millions US dollars in economic, trade-related and environmental benefits.
- Policy advice is provided through expert support, country programme framework, steering committees, guidelines and international legislation.

Over the past decade, the Joint Programme annually contributes to capacity building through over 50 training courses and workshops, 350 fellowships and scientific visits, and has over 500 national institutions participating in R&D networks. Through the regular budget, the Joint Programme organizes symposia, conferences, consultants meetings, interregional training courses and workshops, provides normative and policy advice, disseminates information through databases, e-learning modules and Web pages, and assists Member States through a network of coordinated research projects (CRP) and research coordination meetings (RCM) to address specific practical problems related to a range of areas.

Through IAEA-Technical Cooperation (TC) funding, the Joint FAO/IAEA Programme provides technical support to more than 250 IAEA-TC projects every year, as well as capacity building and technology transfer (expert advice, training, and assisting with the procurement of experts and equipment) to Member States through these technical cooperation projects.

Approximately 400-500 institutions and experimental stations in Member Countries cooperate in 30-40 Coordinated Research Projects per year organized by Joint FAO/IAEA Programme.

The FAO/IAEA Agriculture & Biotechnology Laboratory (ABL) is unique within the UN system in that it provides hands-on training and gives participants the opportunity to accelerate capacity building in their respective countries. The training programme is developed based on the demand for expanding expertise in developing countries.

The IAEA is the only organization within the UN family that has the mandate to promote the peaceful use of nuclear techniques. In some of the agricultural areas, nuclear techniques are an essential component, and when properly integrated with other conventional technologies, provide substantial added value to national and international efforts for sustainable agricultural development while at the same time creating strong synergies. The Joint FAO/IAEA Programme is the only international body that can provide technology development and transfer, capacity building and services in this area to the Member States and is in this respect unique.